Supporting Information

Design and Combinatorial Development of Shield-1 Peptide Mimetics Binding to Destabilized FKBP12

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Abbreviations

Ac: acetyl; Abu: α-aminobutyric acid; Amb: aminomethylbenzoic acid; Bn: benzyl; Boc: *tert*-butoxycarbonyl; Bu: butyl; Cbz: carboxybenzyl; Comp: compound; DD: destabilizing domain; DIPEA: *N*,*N*-diisopropylethylamine; DMF: *N*,*N*-dimethylformamide; DMSO: Dimethylsulfoxide; EDTA: ethylenediaminetetraacetic acid; FKBP FK506-binding protein; Fmoc: 9-fluorenylmethyloxycarbonyl; FT-ICR: fourier-transform ion cyclotron resonance; HATU: 1-[Bis(dimethylamino)methylene]-1N-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HMBA: hydroxymethylbenzoic acid; HPLC: high-performance liquid chromatography; HRMS: high resolution mass spectrometry; LCMS: liquid chromatography mass spectrometry; MBP: maltose-binding protein; Me: methyl; MeIm: *N*-methyl-imidazole; MSNT: 1-(2-Mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole; NEM: *N*-ethylmorpholine; NHS: *N*-hydroxysuccinimide; NSB: non-specific binding; OBOC: one-bead-one-compound; PEGA: polyethylene glycol dimethyl acrylamide; Ph: phenyl; Pip: piperidine; PS: polystyrene; Py: pyridine; PyBOP: benzotriazole-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate; ROX: 5(6)-rhodamine-X; rt: room temperature; Shld1: Shield-1; SPPS: solid phase peptide synthesis; SPS: solid phase synthesis; TBTU: *N*-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methyl methanaminium tetrafluoroborate; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TIPS: triisopropylsilane.

General Methods

All purchased chemicals were used without further purification. The 2-Chlorotrityl chloride PS resin, MSNT, HMBA, TBTU, PyBop, MeIm, *N*-Fmoc-, and *N*-Boc protected amino acids were purchased from Bachem AG, Chem-Impex International, Inc., or Fluka. NEM, DIPEA, TIPS, TFA, 5(6)-ROX N-succinimidyl ester were purchased from Sigma-Aldrich. $PEGA_{800}$, $PEGA_{1900}$, and the fluorescenct MicroParticle Matrix encoded $PEGA_{1900}$ resins were produced be free-radical polymerization as previous described. [1, 2] The solid phase synthesis was carried out in TELOS® filtration SPE columns fitted with Teflon tubing and valves, which allowed suction to be applied to the SPE columns on various resins. All solvents were HPLC-grade. HPLC analysis was performed on an analytical Agilent 1100 HPLC using a 100 mm XBrigde C18 column. A linear gradient of acetonitrile in water with 0.1 % TFA was used, running from 0 % to 90 % acetonitrile, 1 mL/min. over 10 min. The detection was performed by measurement of absorbance of UV-light at 215, 230, and 254 nm. LCMS analysis was performed on a Dionex ultimate 3000 equipped with an acclaim RSIC 120 C18 column (2.2 µM 120Å 2.1 x 100 mm) and a microTOF-QIII MS unit. A linear gradient of acetonitrile in water with 0.1 % TFA was used, running from 5 % to 90 % acetonitrile, 5 mL/min. over 13 min. The detection was performed by measurement of absorbance of UV-light at 214, 225, 250, and 275 nm. HRMS analysis was performed on a Bruker SolariX XR instrument equipped with a ParaCell™ ICR cell, a MALDI and an ESI source. NMR spectra were recorded on a Bruker ADVANCE III 500 MHz CRYO probe instrument. Chemical shifts are reported in

ppm relative to residual solvent signals ($CHCl_3$, 7.26 ppm; DMSO-d_6, 2.50 ppm) for ^1H NMR spectra, relative to the central solvent resonance ($CDCl_3$, 77.0 ppm; DMSO-d_6, 39.5 ppm) for ^{13}C NMR. All new compounds were characterized by ^1H-NMR, ^{13}C-NMR, g-COSY, HSQC, and HRMS. Only the chemical shifts of the major keto-amide rotamer is reported for compounds containing tertiary amides.

General procedures for solid phase synthesis

Resin preparation

The PEGA resin was stored at 5 °C and swelled in EtOH/H_2O (2:8, v/v). Before usage, the resin was washed in the following order with MeOH, H_2O, DMF, MeOH, and CH_2Cl_2, six times of each solvent. The resin was then dried and stored at 5 °C. The Trityl-PS resin was stored dry at 5 °C and was used with no further preparation.

Loading of 2-chloro Trityl chlroride resin

In a flame dried glass vial under N_2 atmosphere was a mixture of the amino acid (4 equiv.) and DIPEA (9 equiv.) in dry CH_2Cl_2 added to the PS-Trityl resin. After shaking the mixture for 16 hours at ambient temperature, the resin was washed five times with CH_2Cl_2. A mixture of a CH_2Cl_2/MeOH/DIPEA (17:2:1, v/v/v) was hereafter added to the resin and shaken for 30 min to cap any unreacted trityl chloride. The capping solution was removed by filtration and the resin was washed with CH_2Cl_2 and DMF.

TBTU Coupling

TBTU (2.88 equiv.) was added to a mixture of an acid (3 equiv.) and NEM (4 equiv.) in DMF. The mixture was pre-activated for 5 min. before being added to the beads, which were pre-swelled in DMF. The mixture was shaken for 2 h at rt., followed by washing with CH_2Cl_2 and DMF. If the subsequent coupling was a MSNT coupling, the resin beads were washed additionally with 3 X CH_2Cl_2 and one time with dry CH_2Cl_2.

MSNT Coupling

MeIm (2.25 equiv.) was added to a suspension of an acid (3 equiv.) in dry CH_2Cl_2. Upon dissolution of the acid, MSNT (3 equiv.) was added to the mixture. The solution was then added to the pre-swelled resin in dry CH_2Cl_2. The reaction was proceeded for 1 h and was then washed with dry CH_2Cl_2. The procedure was then repeated one additional time to ensure full esterification, followed by removal of the reaction mixture by filtration and washing of the resin using CH_2Cl_2 and DMF.

HATU coupling

HATU (2.88 equiv.) was added to a mixture of an acid (3 equiv.) and DIPEA (4 eq.) in DMF. The mixture was pre-activated for 5 min. before being added to the beads, which were pre-swelled in DMF. The mixture was shaken for 2 h at rt., followed by washing with CH_2Cl_2 and DMF.

Acetylation

A mixture of DMF/Ac_2O/Py (8:1:1, v/v/v) was added to a resin containing free amine groups that was swelled in and shaken for 30 min. The acetylation mixture was hereafter removed by filtration and the resin was washed with CH_2Cl_2 and DMF.

Cleavage of Fmoc-protecting group

20% piperidine in DMF was added to the resin. The mixture was shaken for 3 min before the solution was drained. More piperidine in DMF was added, and the deprotection procedure was allowed to proceed for an additional 17 min. The resin was then washed with CH_2Cl_2, MeOH, and DMF.

Diazo transfer[3]

Imidazole-1-sulfonyl azide hydrochloride (3 equiv.), K_2CO_3 (4.5 equiv.), and $CuSO_4.5H_2O$ (0.01 equiv.) was added to the PEGA-resin containing a free primary amine, swelled in H_2O. The mixture was gently shaken for 12 h at rt, whereupon the azido resin was washed with H_2O, MeOH, DMF, and CH_2Cl_2.

CuAAC Reaction

Procedure **A**: The alkyne (3 equiv.), $CuSO_4.5H_2O$ (0.4 equiv.), and Ascorbic acid (10 equiv.) was added to the N_2-purged azido resin, which was swelled in *t*-BuOH/H_2O (2:1, v/v). The mixture was shaken for 12 h at rt, whereupon the resin was washed with H_2O, 0.1M HCl (aq.), MeOH, DMF, and CH_2Cl_2. If the substrate was linked via an acid labile linker, the washing with 0.1M HCl (aq.) was not carried out.

Procedure **B**:[4] The alkyne (3 equiv.), 2,6-lutidine (10 equiv.), and DIPEA (10 equiv.) was added to the N_2-purged azido resin, which was swelled in DMF. This was followed by the addition of a solution of ascorbic acid (50mM, 4 equiv.) in DMF, and a solution of CuBr (70mM, 1.5 equiv.) in N_2-purged MeCN. The reaction was shaken under N_2-atmosphere from 6-14 h, whereupon the resin was washed with H_2O, 0.1M HCl (aq.), MeOH, DMF, and CH_2Cl_2. If the substrate was linked via an acid labile linker, the washing with 0.1M HCl (aq.) was not carried out.

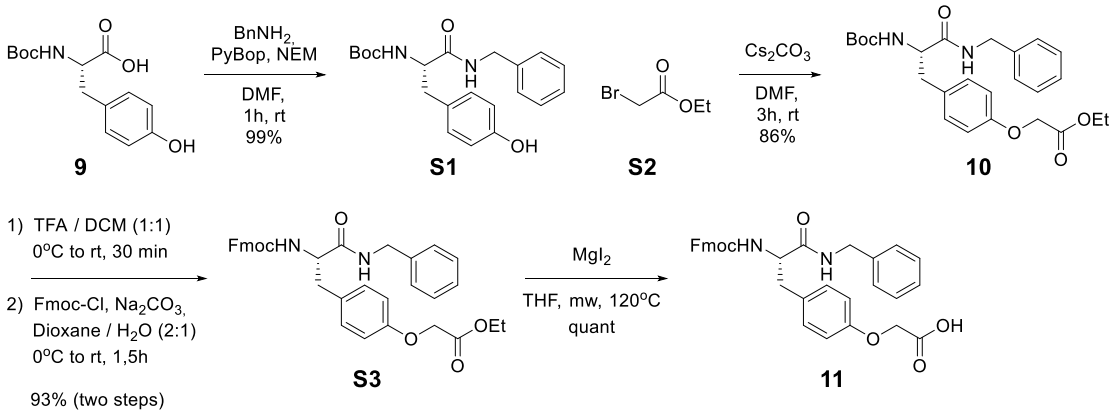
Peptide cleavage from acid labile linkers

For the Rink-amide linker and the Trityl linker, the resin was treated with a solution of TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) and (19:80:1, v/v/v), respectively. The resin was shaken for 2-3 h at rt (10-15 min for test cleavages), the solution was filtered off, and the resin was washed with MeCN, and CH_2Cl_2. The collected organic solvent was evaporated under reduced pressure and the residue was either purified by flash column chromatography, or directly analyzed by HPLC and LCMS.

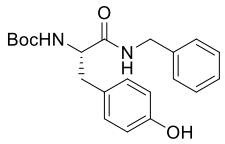
Peptide cleavage from the HMBA linker

For test cleavages 70 µL 0.1 M NaOH was added to 1-3 mg of functionalized resin. The mixture was shaken for 10-30 min before being added 69 µL 0.1 M HCl. The aqueous solution was then filtered and collected, and the remaining beads were washed additionally with 150 µL MeCN.

Experimental Methods

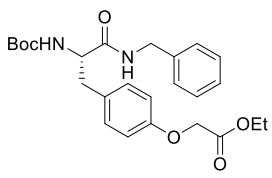


tert-Butyl (_S_)-(1-(benzylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (S1)



In a round bottom flask was PyBop (3.89 g, 7.47 mmol) added to a solution of N^{α}-Boc-L-tyrosine (2.00 g, 7.11 mmol) and 4-Ethylmorpholine (2.25 mL, 17.8 mmol) in dry DMF (30 mL). The mixture was stirred at room temperature for 5 min before being added benzylamine (855 µL, 7.82 mmol). After 1 h of stirring, the mixture was partitioned between EtOAc and H_2O. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed two times with sat. $NaHCO_3$, two times with sat. citric acid, and two times with brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.33) to obtain the title product in 2.61 g (99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.15 (s, 1H), 8.34 (t, J = 6.0 Hz, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.22 (d, J = 7.2 Hz, 1H), 7.16 (d, J = 6.9 Hz, 2H), 7.02 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.4 Hz, 1H), 6.64 (d, J = 8.5 Hz, 2H), 4.32 – 4.21 (m, 2H), 4.14 – 4.07 (m, 1H), 2.83 (dd, J = 13.7, 5.3 Hz, 1H), 2.66 (dd, J = 13.7, 9.5 Hz, 1H), 1.33 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 171.8, 155.7, 155.2, 139.3, 130.1, 128.1, 128.1, 127.0, 126.6, 114.8, 77.9, 56.2, 41.9, 36.8, 28.2. HRMS calc. for $C_{21}H_{27}N_2O_4$: 371.1965 $[M+H]^+$ found 371.1965.

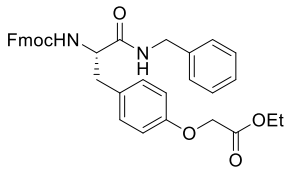
Ethyl (_S_)-2-(4-(3-(benzylamino)-2-((_tert_-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)acetate (10)



In a flame dried round bottom flask was Cs_2CO_3 (880 mg, 2.70 mmol) added to a solution of **S1** (500 mg, 1.35 mmol) in dry DMF (15 mL) under N_2-atmosphere. The solution was stirred for 15 min before being added ethyl bromoacetate (225 µL, 2.03 mmol). The mixture was stirred for 3 h before being partitioned between H_2O

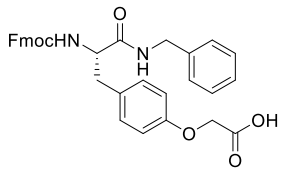
and CH_2Cl_2. The organic phase was isolated, and the remaining aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed five times with water, two times with brine, dried with Na_2SO_4, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.28) to afford the title product in 530 mg (86%) as a colorless wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.22 (m, 3H), 7.15 – 7.07 (m, 4H), 6.82 – 6.78 (m, 2H), 6.07 (t, *J* = 5.9 Hz, 1H), 5.01 (bs, 1H), 4.58 (s, 2H), 4.36 (d, *J* = 5.8 Hz, 2H), 4.27 (q, *J* = 7.1 Hz, 3H), 3.08 – 2.96 (m, 2H), 1.39 (s, 9H), 1.32 – 1.28 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 168.9, 156.9, 155.4, 137.7, 130.5, 129.8, 128.6, 127.7, 127.5, 114.9, 80.3, 65.5, 61.4, 56.1, 43.5, 37.6, 28.3, 14.2. HRMS calc. for $C_{25}H_{32}N_2NaO_6$: 479.2147 $[M+Na]^+$ found 479.2151.

Ethyl (*S*)-2-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(benzylamino)-3-oxopropyl)phenoxy)acetate (**S3**)



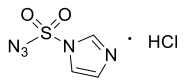
In a flame dried round bottom flask was **10** (200 mg, 438 µmol) treated with a mixture of CH_2Cl_2/TFA (1:1, v/v) at 0°C. The mixture was allowed to reach rt and after 1 h of stirring, the volatiles were removed under reduced pressure. The residue was redissolved in a dioxane/H_2O mixture (2:1, v/v), cooled to 0°C, and added Na_2CO_3 (116 mg, 1.10 mmol). After 5 min of stirring Fmoc-Cl (147 mg, 570 µmol) was added to the solution. The mixture was allowed to reach rt and after 2 h of stirring it was partitioned between water and CH_2Cl_2. The organic phase was isolated, and the remaining aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.25) to afford the title product in 235 mg (93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 6.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 – 7.21 (m, 5H), 7.14 – 7.02 (m, 4H), 6.78 (d, *J* = 8.0 Hz, 2H), 6.00 (s, 1H), 5.40 (s, 1H), 4.56 (s, 2H), 4.47 – 4.30 (m, 5H), 4.27 (q, *J* = 7.1 Hz, 2H), 4.17 (t, *J* = 6.7 Hz, 1H), 3.13 – 2.92 (m, 2H), 1.30 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 169.0, 157.1, 156.1, 143.8, 141.4, 137.6, 130.6, 129.6, 128.8, 127.9, 127.9, 127.7, 127.2, 125.1, 120.1, 115.1, 67.1, 65.6, 61.5, 56.6, 47.3, 43.7, 38.0, 14.3.

(*S*)-2-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(benzylamino)-3-oxopropyl)phenoxy)acetic acid (**11**)

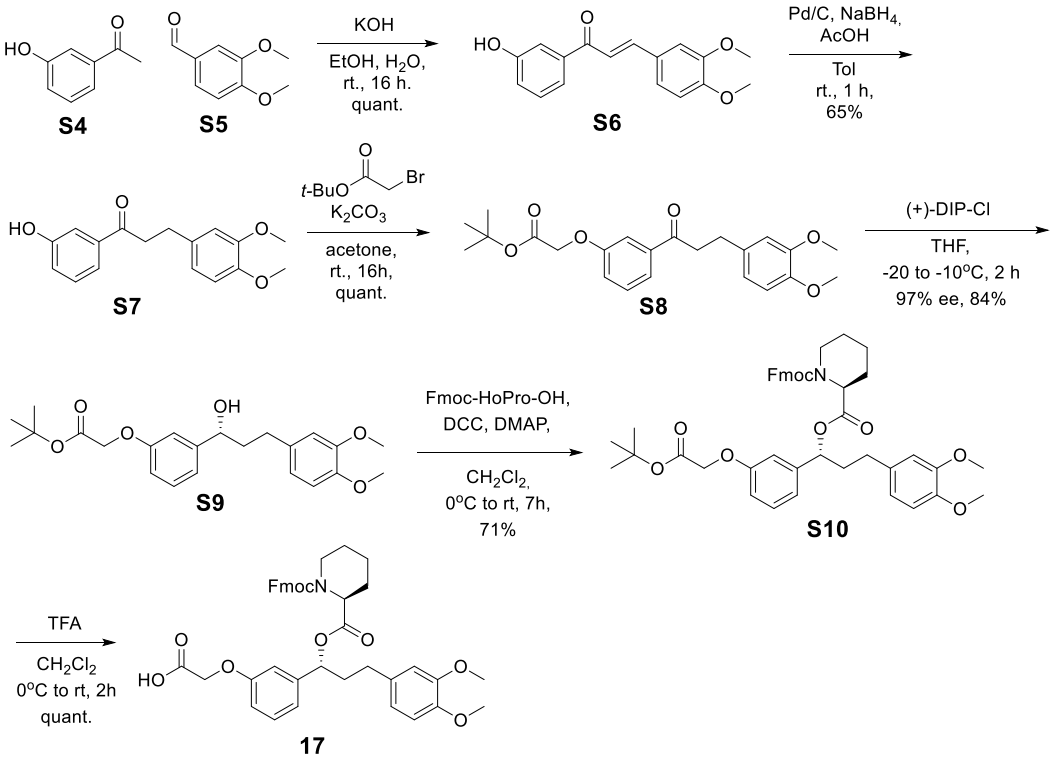


MgI_2 (192 mg, 691 µmol) was added to a solution of **S3** (40 mg, 69.1µmol) in dry THF (600 µL) under N_2-atmosphere. The suspension was heated in a sealed reactor by microwave irradiation at 120 °C for 30 min. Upon completion, $Na_2S_2O_3$ (aq., 7.0 µL 0.1 M) was added to the reaction mixture. The resulting homogeneous solution was partitioned between EtOAc and HCl (aq., 0.1 M). The organic phase was isolated, and the remaining aqueous phase was extracted three times with EtOAc. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo* to afford the title product in quantitative yield. ^{1}H NMR (500, 1H), 7.41 (td, *J* = 7.4, 2.9 Hz, 2H), 7.33 – 7.26 (m, 4H), 7.23 – 7.17 (m, 5H), 6.79 (d, *J* = 8.6 Hz, 2H), 4.59 (s, MHz, DMSO-d_6) δ 8.48 (t, *J* = 5.9 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.67 (t, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 8.6 Hz 2H), 4.33 – 4.10 (m, 6H), 2.93 (dd, *J* = 13.6, 4.9 Hz, 1H), 2.77 (dd, *J* = 13.7, 10.0 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 171.4, 170.2, 156.3, 155.8, 143.8, 143.7, 140.6, 139.2, 130.2, 128.2, 127.6, 127.1, 127.0, 126.7, 125.3, 120.1, 114.0, 65.6, 64.5, 56.5, 46.6, 42.1, 36.7. LCMS calc. for $C_{33}H_{31}N_2O_6$: 551.2 $[M+Na]^+$ found 551.2.

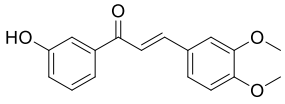
Imidazole-1-sulfonyl azide hydrochloride [5]



In a flame dried round bottom flask was sulfuryl chloride (16.1 mL, 200 mmol) added drop-wise to a suspension of NaN_3 (13g, 200 mmol) in dry MeCN (200 mL) at 0°C under N_2-atmosphere. The mixture was allowed to reach rt and after 16 h of stirring, imidazole (25.9 g, 380 mmol) was added in small portions at 0°C. After 3 h of stirring at rt, the mixture was partitioned between EtOAc and H_2O. The organic phase was isolated, and the remaining aqueous phase was extracted two times with EtOAc. The combined organic phases were washed with H_2O, sat. $NaHCO_3$ (aq.), dried with Na_2SO_4, and filtered. EtOH (75 mL) was treated with AcCl (21.3 mL, 300 mmol) at 0°C, and the resulting solution was added drop-wise to the filtrate under stirring at 0°C. The chilled mixture was filtered and the filter cake was washed with three times with EtOAc (100 mL) to give the title product in 29.8 g (71%) as white needles. ^{1}H NMR (500 MHz, D_2O) δ 9.21 (d, *J* = 1.4 Hz, 1H), 7.99 (d, *J* = 2.1 Hz, 1H), 7.57 (dd, *J* = 2.1, 1.1 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ 137.7, 124.9, 119.7.

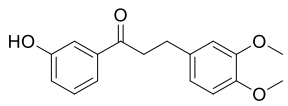


(*E*)-3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)Prop-2-en-1-one (**S6**) [6]



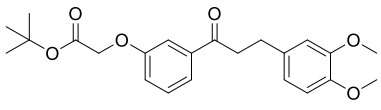
3,4-Dimethoxybenzaldehyde (6.00 g, 36,1 mmol) and 1-(3-hydroxyphenyl)ethan-1-one (4,92 g, 36,1 mmol) were dissolved in EtOH (3 mL) and cooled to 0 °C. The partly dissolved suspension was treated with an aqueous KOH solution (15 mL, 144 mmol), which was allowed to reach room temperature, and stirred for 16 h. The solution was then cooled to 0 °C and added 50 mL of an ice cooled 2 M HCl solution. The formed yellow solid was redissolved in EtOAc, the organic phase was isolated, and remaining aqueous phase was extracted three times using EtOAc. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo* to afford the title product in quantitative yield yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 15.6 Hz, 1H), 7.64 (dd, *J* = 2.6, 1.6 Hz, 1H), 7.57 (ddd, *J* = 7.8, 1.6, 0.9 Hz, 1H), 7.40 – 7.35 (m, 2H), 7.23 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.15 (d, *J* = 2.0 Hz, 1H), 7.11 (ddd, *J* = 8.0, 2.6, 0.9 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.33 (s, 1H), 3.94 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.9, 156.5, 151.7, 149.4, 145.8, 140.0, 130.0, 127.9, 123.5, 121.0, 120.3, 120.0, 115.3, 111.3, 110.3, 56.2, 56.1. HRMS calc. for $C_{17}H_{17}O_4$: 285.1121 $[M+H]^+$ found 285.1124.

3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)Propan-1-one (**S7**) [6, 7]



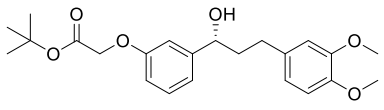
In a flame dried round bottom flask was **S6** (1,00 g, 3,52 mmol) dissolved in toluene (30 mL) at 90 °C for 2 h under N_2-atmosphere. The solution was then cooled to an ambient temperature, at which **S6** precipitated as a fine powder. Pd/C (187 mg, 175 µmol), AcOH (403 µL, 7.03 mmol), and $NaBH_4$ (14.1 mmol, 532 mg) was added in the given order. The mixture was stirred for 30 min before two additional equivalents of AcOH (403 µL, 7.03 mmol) were added. After an additional 15 min of stirring, the solution was cooled to 0 °C and quench by addition HCl (0,1 M). The mixture was filtered through celite and washed with EtOAc. The organic phase was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography using eluent CH_2Cl_2/EtOAc (9:1, v/v, R_f = 0,4) to obtain the title product in 650 mg (65%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (ddd, *J* = 7.9, 1.5, 1.0 Hz, 1H), 7.48 (dd, *J* = 2.6, 1.5 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.06 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H), 6.82 – 6.76 (m, 3H), 5.50 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.26 (t, *J* = 7.6 Hz, 2H), 3.01 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.6, 156.2, 149.0, 147.6, 138.5, 133.9, 130.1, 120.8, 120.5, 120.3, 114.7, 112.0, 111.5, 56.1, 56.0, 41.0, 30.0. HRMS calc. for $C_{17}H_{19}O_4$: 287.1278 $[M+H]^+$ found 287.1234.

***Tert*-butyl 2-(3-(3-(3,4-dimethoxyphenyl)propanoyl)phenoxy)acetate** (**S8**) [6]



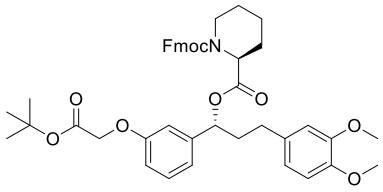
In a flame dried round bottom flask was K_2CO_3 (483 mg, 3.49 mmol) added to a solution of **S7** (500 mg, 1.75 mmol) in acetone (10 mL) under N_2-atmosphere. The solution was stirred for 15 min prior to addeding *tert*-butyl 2-bromoacetate (284 µL, 1,92 mmol). The mixture was stirred for 16 h before being partitioned between H_2O and CH_2Cl_2. The organic phase was isolated, and remaining aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent EtOAc/Hep (3:7, v/v, R_f = 0.28) to afford the title product in 679 mg (97%) as a colorless wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (ddd, *J* = 7.7, 1.5, 1.0 Hz, 1H), 7.46 (dd, *J* = 2.7, 1.5 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.12 (ddd, *J* = 8.2, 2.7, 1.0 Hz, 1H), 6.81 – 6.76 (m, 3H), 4.56 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.25 (t, *J* = 7.8 Hz, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.0, 167.8, 158.3, 149.1, 147.6, 138.4, 133.9, 129.9, 121.6, 120.3, 120.2, 113.2, 112.0, 111.5, 82.8, 65.8, 56.1, 56.0, 40.9, 30.0, 28.2. LCMS calc. for $C_{23}H_{28}NaO_6$: 423.2 $[M+Na]^+$ found 423.2.

***tert*-butyl (*R*)-2-(3-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenoxy)acetate (S9)** [6]



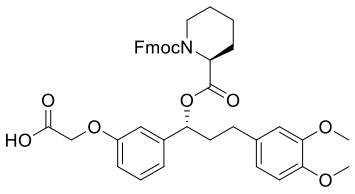
(+)-Ipc_2BCl (4.63 mL, 7.49 mmol, 58% in hexane) was added dropwise to a solution of **S8** (1.00 g, 2.50 mmol) in dry THF (4 mL), in a flame dried round bottom flask under N_2-atmosphere at -20 °C. The mixture was stirred at 1 h at the specified temperature before being allowed to reach an ambient temperature at which it was stirred for an additional hour. The solution was diluted with 10 mL CH_2Cl_2 and quenched with sat. NH_4Cl. The organic phase was isolated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4, filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.27) to afford the title product in quantitative yield as a colorless oil. 97% ee by chiral HPLC, 30% i-PrOH/Heptane, retention time 37.1 min for *S*-enantiomer and 47.1 min for *R*-enantiomer. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (t, *J* = 7.9 Hz, 1H), 6.97 – 6.95 (m, 1H), 6.93 (dd, *J* = 2.7, 1.5 Hz, 1H), 6.82 – 6.77 (m, 2H), 6.74 – 6.70 (m, 2H), 4.66 (dd, *J* = 7.9, 5.2 Hz, 1H), 4.52 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 2.73 – 2.58 (m, 2H), 2.13 – 1.95 (m, 2H), 1.84 (bs, 1H), 1.48 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 158.3, 149.0, 147.3, 146.6, 134.5, 129.7, 120.3, 119.2, 113.7, 112.4, 111.9, 111.4, 82.5, 73.9, 65.8, 56.1, 56.0, 40.7, 31.7, 28.2. LCMS calc. for $C_{23}H_{30}NaO_6$: 425.2 $[M+Na]^+$ found 425.2.

1-((9*H*-fluoren-9-yl)methyl) 2-((*R*)-1-(3-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl) (*S*)-piperidine-1,2-dicarboxylate (S10) [8]



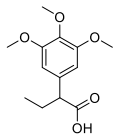
In a flame dried round bottom flask was DCC (254 mg, 1.23 mmol) added to a solution of **S9** (450 mg, 1.12 mmol), *N*-Fmoc-L-pipecolic acid (433 mg, 1.23 mmol), and DMAP (15 mg, 123 µmol) in 10 mL dry CH_2Cl_2 at 0°C. The mixture was stirred at 0°C for 1 h before being allowed to reach rt and continued stirred for 6 h. The mixture was filtered through celite and washed using EtOAc. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography using eluent EtOAc/Hep (3:7, v/v, R_f = 0.19) to afford the title product in 774 mg (94 %), as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 7.6, 3.1 Hz, 2H), 7.62 – 7.56 (m, 2H), 7.50 – 7.15 (m, 5H), 6.96 – 6.92 (m, 1H), 6.89 (s, 1H), 6.83 – 6.71 (m, 2H), 6.67 – 6.55 (m, 2H), 5.76 (q, *J* = 7.5 Hz, 1H), 5.02 (d, *J* = 5.5 Hz, 1H), 4.51 – 4.26 (m, 5H), 4.17 – 4.05 (m, 1H), 3.85 – 3.82 (m, 6H), 3.18 – 3.08 (m, 1H), 2.60 – 2.39 (m, 2H), 2.36 – 2.27 (m, 1H), 2.24 – 2.14 (m, 1H), 2.07 – 1.97 (m, 1H), 1.78 – 1.66 (m, 4H), 1.46 (s, 9H), 1.30 – 1.22 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 170.7, 158.1, 156.8, 149.1, 147.6, 144.0, 143.8, 141.4, 133.5, 129.9, 127.9, 127.2, 125.2, 120.3, 120.1, 119.7, 115.9, 111.8, 111.5, 110.0, 76.6, 68.4, 65.5, 56.1, 56.1, 54.7, 47.2, 42.0, 38.3, 31.6, 27.3, 24.9, 20.9.

2-(3-((*R*)-1-(((*S*)-1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (**17**) [8]

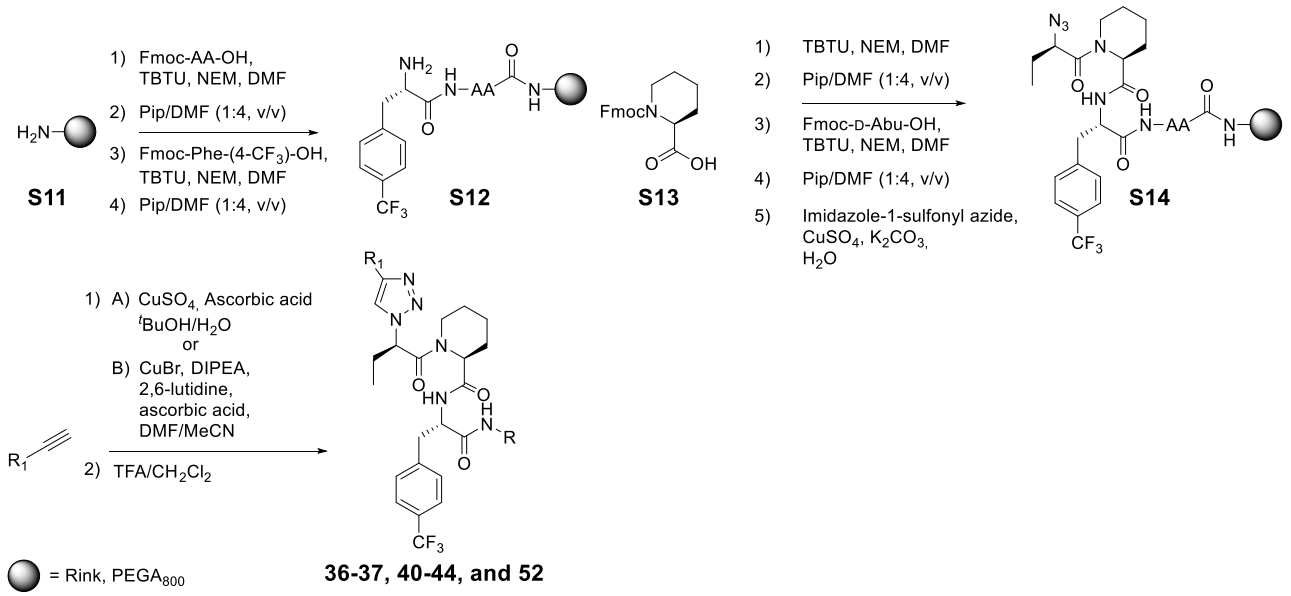


In a round bottom flask was 1.2 mL TFA added dropwise to a stirring solution of **S10** (170 mg, 231 µmol) in CH_2Cl_2 at 0°C. The mixture was allowed to reach rt and after 2 hours of stirring, it was further diluted with 20 mL of toluene. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using eluent EtOAc/Hep/AcOH (73:24:3, v/v/v, R_f = 0.26) to afford the title product in quantitative yield as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.7, 2.5 Hz, 2H), 7.56 – 7.51 (m, 2H), 7.41 – 7.35 (m, 2H), 7.31 – 7.19 (m, 3H), 6.91 – 6.85 (m, 2H), 6.80 – 6.77 (m, 2H), 6.71 – 6.66 (m, 2H), 5.67 (dd, *J* = 8.8, 4.8 Hz, 1H), 5.05 – 4.99 (m, 1H), 4.70 – 4.58 (m, 2H), 4.44 – 4.36 (m, 2H), 4.23 (t, *J* = 7.1 Hz, 1H), 4.05 (d, *J* = 13.6 Hz, 1H), 3.86 – 3.85 (m, 6H), 3.21 (td, *J* = 13.1, 3.2 Hz, 1H), 2.71 – 2.64 (m, 1H), 2.62 – 2.54 (m, 1H), 2.38 – 2.32 (m, 1H), 2.25 – 2.18 (m, 1H), 2.10 – 2.01 (m, 1H), 1.85 – 1.76 (m, 2H), 1.74 – 1.67 (m, 1H), 1.50 – 1.40 (m, 1H), 1.36 – 1.28 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 170.7, 158.1, 156.8, 149.1, 147.6, 144.0, 143.8, 141.4, 133.5, 129.9, 127.9, 127.2, 125.2, 120.3, 120.1, 119.7, 115.9, 111.8, 111.5, 110.0, 76.6, 68.4, 65.5, 56.1, 56.1, 54.7, 47.2, 42.0, 38.3, 31.6, 27.3, 24.9, 20.9. HRMS calc. for $C_{40}H_{41}NNaO_9$: 702.2674 $[M+H]^+$ found 702.2670

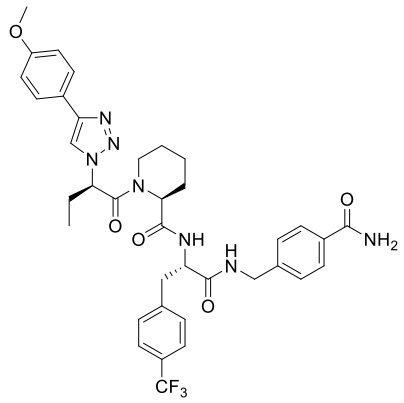
2-(3,4,5-trimethoxyphenyl)butanoic acid (**S11**) [9]



In a flame dried round bottom flask under N_2-atmosphere was 6.96 mL of a 1 M THF solution of sodium bis(trimethylsilyl)amide (6.96 mmol, 2.25 equiv.) added to a solution of 3,4,5-trimethoxyphenylacetic acid (700 mg, 3.09 mmol) in 6 mL dry THF at 0°C. After 1 h of stirring, iodoethane (299 µL, 3.71 mmol) was added dropwise to the solution. The mixture was allowed to reach rt and after 11 h of stirring it was further diluted with 20 mL EtOAc, cooled to 0°C, and quenched by addition of 1 M HCl (aq.). The layers were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried with Na_2SO_4, filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent Hep/EtOAc/AcOH (60:38:2, R_f = 0.35) to afford the title product in 618 mg (79%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.53 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.38 (t, *J* = 7.7 Hz, 1H), 2.13 – 2.04 (m, 1H), 1.80 (dt, *J* = 13.6, 7.4 Hz, 1H), 0.93 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.9, 153.4, 137.5, 134.1, 105.2, 61.0, 56.3, 53.6, 26.5, 12.3. HRMS calc. for $C_{13}H_{18}NaO_5$: 277.1046 $[M+Na]^+$ found 277.1048.

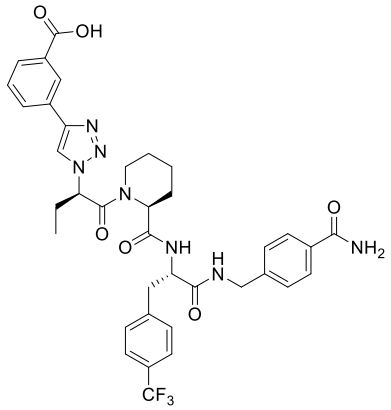


(*S*)-*N*-((*S*)-1-((4-carbamoylbenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**36**)



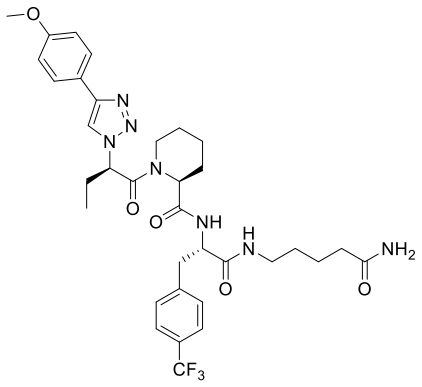
Following **general procedures for SPPS** and using procedure **A** for the CuAAC reaction, **36** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using $TFA/CH_2Cl_2/H_2O$ (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent $MeOH/CH_2Cl_2$ (4:96, v/v, R_f = 0.31) to obtain the title product in quantitative yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.81 – 7.77 (m, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.26 – 7.23 (m, 2H), 7.12 (t, *J* = 6.1 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.95 – 6.91 (m, 2H), 6.36 (s, 2H), 6.16 (s, 1H), 5.32 (t, *J* = 7.2 Hz, 1H), 5.08 – 5.04 (m, 1H), 5.00 (ddd, *J* = 10.9, 9.0, 5.6 Hz, 1H), 4.45 (dd, *J* = 15.3, 6.5 Hz, 1H), 4.22 (dd, *J* = 15.3, 5.2 Hz, 1H), 3.81 (s, 3H), 3.55 – 3.49 (m, 1H), 3.42 (dd, *J* = 14.3, 5.6 Hz, 1H), 3.18 (dd, *J* = 14.4, 10.8 Hz, 1H), 2.30 – 2.20 (m, 2H), 2.18 – 2.10 (m, 1H), 2.07 – 1.97 (m, 1H), 1.52 – 1.28 (m, 4H), 0.92 (t, *J* = 7.3 Hz, 3H), 0.86 – 0.80 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 170.0, 169.5, 167.7, 160.1, 148.3, 142.1, 142.1, 132.2, 129.7, 128.93 (q, *J* = 32.3 Hz), 127.6, 127.5, 127.2, 125.3 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 271.8 Hz), 122.6, 118.4, 114.5, 61.2, 55.5, 54.1, 54.0, 43.6, 43.0, 37.3, 26.1, 25.3, 24.8, 19.7, 10.3. HRMS calc. for $C_{37}H_{41}F_3N_7O_5$: 686.3272 $[M+H]^+$ found 686.3265.

3-(1-((*R*)-1-((*S*)-2-(((*S*)-1-((4-carbamoylbenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**37**)



Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **37** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2/AcOH (5:94:1, v/v/v, R_f = 0.26) to obtain the title product in 53 mg (72%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.11 (bs, 1H), 8.74 (s, 1H), 8.56 – 8.45 (m, 2H), 8.32 (d, *J* = 8.9 Hz, 1H), 8.14 – 8.08 (m, 2H), 7.99 – 7.87 (m, 4H), 7.77 (d, *J* = 7.9 Hz, 2H), 7.57 – 7.51 (m, 2H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 2H), 5.87 (dd, *J* = 8.9, 5.7 Hz, 1H), 5.02 – 4.99 (m, 1H), 4.71 – 4.64 (m, 1H), 4.28 (d, *J* = 5.6 Hz, 2H), 3.84 – 3.76 (m, 1H), 3.11 (dd, *J* = 13.7, 5.3 Hz, 1H), 2.94 (dd, *J* = 13.6, 9.6 Hz, 1H), 2.83 (td, *J* = 13.4, 3.0 Hz, 1H), 2.15 – 1.90 (m, 3H), 1.51 – 1.32 (m, 4H), 1.12 – 1.01 (m, 1H), 0.83 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 170.6, 169.8, 167.6, 167.5, 167.1, 145.6, 142.7, 142.4, 132.8, 131.2, 130.1, 130.0, 129.2, 127.4, 127.1 (q, *J* = 31.4 Hz), 126.8, 125.9, 124.9 (q, *J* = 3.5 Hz), 124.3 (q, *J* = 271.9 Hz), 121.8, 43.0, 41.8, 37.1, 26.6, 25.8, 24.8, 19.7, 10.1. HRMS calc. for $C_{37}H_{39}F_3N_7O_6$: 734.2908 $[M+H]^+$ found 734.2900.

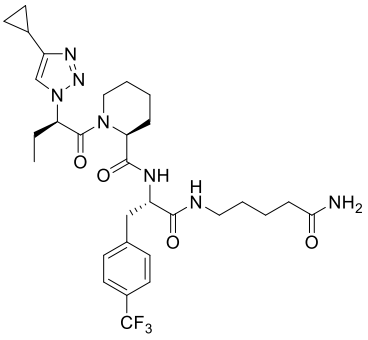
(*S*)-*N*-((*S*)-1-((5-amino-5-oxopentyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**40**)



Following **general procedures for SPPS** and using procedure **A** for the CuAAC reaction, **40** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (5:95, v/v, R_f = 0.23) to obtain the title product in 49 mg (71%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 – 7.74 (m, 2H), 7.43 (d, *J* = 6.3 Hz, 2H), 7.26 (d, *J* = 1.2 Hz, 2H), 7.02 – 6.93 (m, 2H), 6.72 – 6.59 (m, 1H), 6.19 – 5.98 (m, 2H), 5.54 – 5.35 (m, 1H), 5.26 – 4.88 (m, 2H), 3.84 (s, 3H), 3.57 – 3.39 (m, 2H),

3.17 (s, 3H), 2.41 – 2.11 (m, 6H), 1.69 – 1.28 (m, 8H), 1.05 – 0.94 (m, 3H), 0.79 (d, *J* = 31.1 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 170.3, 167.9, 160.2, 142.2, 129.7, 129.0 (q, *J* = 32.4 Hz), 127.2, 125.8, 125.4 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 271.9 Hz) 122.8, 114.6, 61.5, 55.5, 54.3, 54.2, 43.7, 38.9, 37.2, 28.4, 26.2, 25.3, 24.9, 22.4, 19.7, 10.4. HRMS calc. for $C_{34}H_{43}F_3N_7O_5$: 686.3272 $[M+H]^+$ found 686.3265.

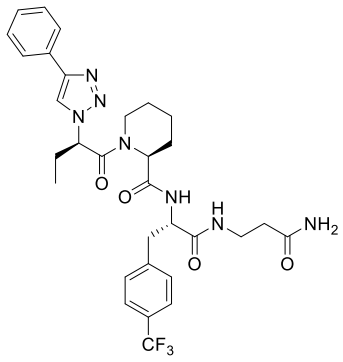
(*S*)-*N*-((*S*)-1-((5-amino-5-oxopentyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**41**)



Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **41** was cleaved off the $PEGA_{800}$ resin (300 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (5:95, v/v, R_f = 0.25) to obtain the title product in 58 mg (78%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.43 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 6.61 (t, *J* = 5.7 Hz, 1H), 6.14 (bs, 1H), 5.68 (bs, 1H), 5.25 (t, *J* = 7.9 Hz, 1H), 5.08 – 5.04 (m, 1H), 4.91 (ddd, *J* = 11.4, 8.9, 4.9 Hz, 1H), 3.48 (dd, *J* = 14.5, 4.9 Hz, 1H), 3.41 (dt, *J* = 13.9, 3.6 Hz, 1H), 3.27 – 3.10 (m, 3H), 2.31 – 2.10 (m, 5H), 2.08 – 1.97 (m, 2H), 1.55 (h, *J* = 7.6 Hz, 6H), 1.35 – 1.26 (m, 2H), 1.03 – 0.98 (m, 2H), 0.97 – 0.88 (m, 5H), 0.81 – 0.67 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.8, 170.8, 169.9, 167.7, 151.2, 142.4, 129.7, 128.9 (q, *J* = 32.4 Hz), 125.3 (q, *J* = 3.8 Hz), 124.3 (q, *J* = 271.8 Hz) 119.8, 61.1, 54.2, 54.1, 43.5, 38.8, 37.0, 28.5, 26.2, 25.3, 24.8, 22.6, 19.7, 10.4, 8.3, 8.2, 7.0. HRMS calc. for $C_{30}H_{41}F_3N_7O_4$: 620.3167 $[M+H]^+$ found 620.3162.

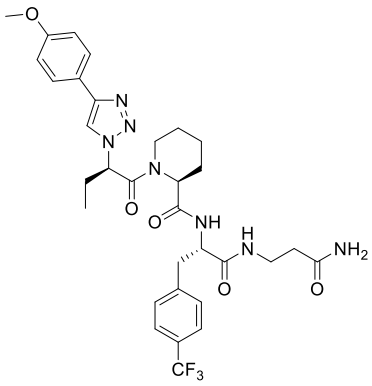
(*S*)-*N*-((*S*)-1-((3-Amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**42**)

Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **42** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using



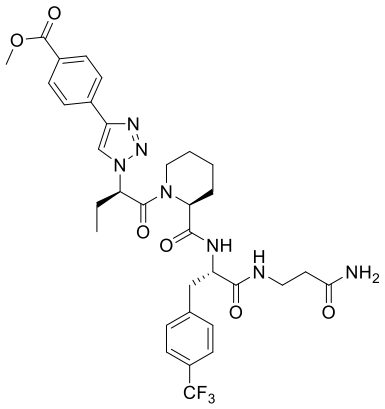
eluent MeOH/CH_2Cl_2 (4:96, v/v, R_f = 0.26) to obtain the title product in quantitative yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.47 – 7.40 (m, 4H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.89 (t, *J* = 6.1 Hz, 1H), 5.86 – 5.81 (m, 1H), 5.70 – 5.63 (m, 1H), 5.46 (t, *J* = 7.3 Hz, 1H), 5.14 – 5.10 (m, 1H), 4.86 (td, *J* = 9.4, 5.8 Hz, 1H), 3.61 – 3.55 (m, 1H), 3.53 – 3.45 (m, 1H), 3.43 – 3.36 (m, 1H), 3.23 (dd, *J* = 14.3, 5.8 Hz, 1H), 3.00 (dd, *J* = 14.3, 10.2 Hz, 1H), 2.43 – 2.21 (m, 6H), 2.20 – 2.10 (m, 1H), 1.57 – 1.37 (m, 3H), 1.04 – 0.94 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 169.9, 167.7, 141.8, 130.2, 129.7, 129.1, 129.1 (q, *J* = 32.2 Hz) 128.8, 125.9, 125.4 (q, *J* =3.3 Hz), 119.3, 61.4, 53.9, 53.8, 43.7, 37.5, 35.7, 26.5, 25.4, 25.1, 19.9, 10.4. HRMS calc. for $C_{31}H_{37}F_3N_7O_4$: 628.2854 $[M+H]^+$ found 628.2849.

(*S*)-*N*-((*S*)-1-((3-amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**43**)



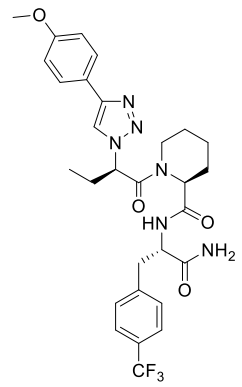
Following **general procedures for SPPS** and using procedure **A** for the CuAAC reaction, **43** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (5:95, v/v, R_f = 0.31) to obtain the title product in 47 mg (80%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.40 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H), 7.09 – 7.05 (m, 1H), 7.02 (d, *J* = 8.6 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 2H), 6.02 – 5.93 (m, 2H), 5.44 (t, *J* = 7.3 Hz, 1H), 5.13 – 5.09 (m, 1H), 4.93 – 4.87 (m, 1H), 3.83 (s, 3H), 3.60 – 3.54 (m, 1H), 3.54 – 3.46 (m, 1H), 3.43 – 3.35 (m, 1H), 3.21 (dd, *J* = 14.3, 5.6 Hz, 1H), 2.96 (dd, *J* = 14.3, 10.3 Hz, 1H), 2.41 – 2.08 (m, 6H), 1.55 – 1.31 (m, 4H), 0.96 (q, *J* = 6.4, 5.6 Hz, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 170.9, 169.9, 167.7, 160.0, 148.5, 141.8, 129.7, 129.0 (q, *J* = 32.4 Hz), 127.3, 125.3 (q, *J* = 3.6 Hz), 124.2 (q, *J* = 271.6 Hz), 122.8, 118.4, 114.5, 61.5, 55.5, 53.8, 53.7, 43.6, 37.6, 35.7, 26.5, 25.4, 25.0, 19.9, 10.4. HRMS calc. for $C_{32}H_{39}F_3N_7O_5$: 658.2959 $[M+H]^+$ found 658.2956.

Methyl 4-(1-((*R*)-1-((*S*)-2-(((*S*)-1-((3-amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoate (**44**)



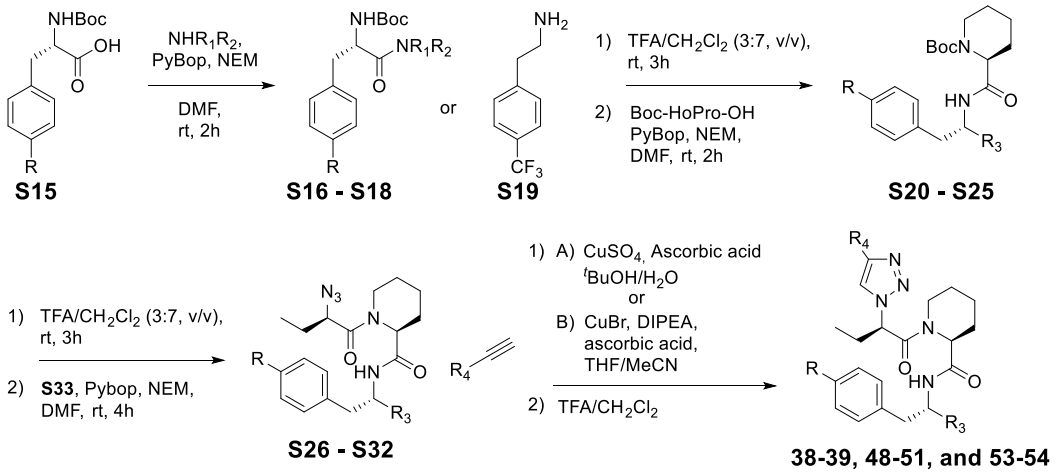
Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **44** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (4:96, v/v, R_f = 0.27) to obtain the title product in 67 mg (97%) as a light brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 2H), 8.02 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.22 (t, *J* = 5.8 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.15 (s, 1H), 6.02 (s, 1H), 5.57 (t, *J* = 7.4 Hz, 1H), 5.15 – 5.12 (m, 1H), 4.90 (td, *J* = 9.0, 5.9 Hz, 1H), 3.94 (s, 3H), 3.69 – 3.63 (m, 1H), 3.51 – 3.39 (m, 2H), 3.10 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.85 (dd, *J* = 14.1, 9.6 Hz, 1H), 2.44 – 2.22 (m, 5H), 2.21 – 2.11 (m, 1H), 1.58 – 1.53 (m, 1H), 1.52 – 1.33 (m, 3H), 1.17 – 1.04 (m, 1H), 0.98 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 169.7, 167.6, 166.8, 147.3, 141.3, 134.5, 130.2, 129.8, 129.6, 128.9 (q, *J* = 32.4 Hz), 125.6, 125.2 (q, *J* = 3.2, 2.6 Hz), 124.1 (q, J = 271.9 Hz), 123.0, 120.2, 61.5, 53.5, 53.5, 52.2, 43.6, 37.9, 35.4, 26.5, 25.3, 25.0, 19.8, 10.3. HRMS calc. for $C_{33}H_{39}F_3N_7O_6$: 686.2908 $[M+H]^+$ found 686.2902.

(*S*)-*N*-((*S*)-1-Amino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**52**)

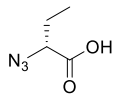


Following **general procedures for SPPS** and using procedure **A** for the CuAAC reaction, **52** was cleaved off the $PEGA_{800}$ resin (250 mg, 0.4 mmol/g) bearing a Rink-Amide linker using TFA/CH_2Cl_2/H_2O (50:48:2, v/v/v) for 3h at room temperature. The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (4:96, v/v, R_f = 0.32) to obtain the title product in 28 mg (48%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.84 – 7.80 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.20 (m, 2H), 6.99 – 6.95 (m, 2H), 6.22 (s, 1H), 5.66 (s, 1H), 5.37 (t, *J* = 7.2 Hz, 1H), 5.16 – 5.13 (m, 1H), 4.85 (ddd, *J* = 11.2, 8.9, 5.2 Hz, 1H), 3.83 (s, 3H), 3.47 (dt, *J* = 13.9, 3.6 Hz, 1H), 3.31 (dd, *J* = 14.6, 5.2 Hz, 1H), 3.16 (dd, *J* = 14.6, 11.3 Hz, 1H), 2.36 – 2.27 (m, 2H), 2.17 – 2.08 (m,

1H), 2.07 – 2.00 (m, 1H), 1.79 (s, 1H), 1.55 – 1.48 (m, 1H), 1.47 – 1.39 (m, 1H), 1.37 – 1.29 (m, 2H), 0.99 (t, *J* = 7.3 Hz, 3H), 0.94 – 0.85 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9, 167.5, 160.1, 148.4, 142.1, 129.7, 129.0 (q, *J* = 32.3 Hz), 127.2, 124,3 (q, *J* = 271.6 Hz), 125.4 (q, *J* = 3.7 Hz), 122.7, 118.2, 114.6, 61.1, 55.5, 54.0, 53.4, 43.6, 36.4, 26.4, 25.3, 25.1, 19.9, 10.4. HRMS calc. for $C_{29}H_{34}F_3N_6O_4$: 587.2588 $[M+H]^+$ found 587.2587.

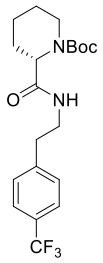


(*R*)-2-azidobutanoic acid (**S33**) [10]



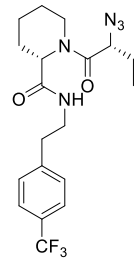
In a round bottom flask was imidazole-1-sulfonyl azide hydrochloride (732 mg, 3.49 mmol) added to a solution of L-2-aminobutyric acid (300 mg, 2.91 mmol), K_2CO_3 (1.09 g, 7.86 mmol), and $CuSO_4 \cdot 5H_2O$ (7.30 mg, 29.1 µmol) in MeOH (15 mL). After 16 h of stirring, the mixture was concentrated and the residue was partioned between CH_2Cl_2 and HCl (aq, 1M). The organic phase was isolated, and the remaining aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography using eluent EtOAc/Hep/HCOOH (29:70:1, v/v/v, R_f = 0.31) to obtain the title product in 300 mg (80%), as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (dd, *J* = 8.0, 5.3 Hz, 1H), 2.01 – 1.81 (m, 2H), 1.07 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.2, 63.2, 25.0, 10.3.

***tert*-Butyl (*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidine-1-carboxylate** (**S20**)



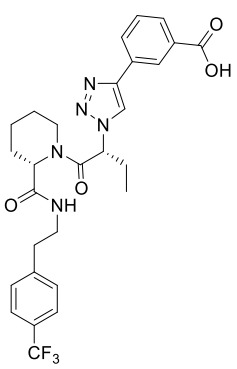
General procedure C: In a round bottom flask was PyBop (1.06 g, 2.04 mmol) added to a solution of *N*-Boc-L-Pipecolic acid (467 mg, 2.04 mmol) and 4-Ethylmorpholine (585 µL, 4.628 mmol) in dry DMF (8 mL). The mixture was stirred at room temperature for 5 minutes before being added 2-(4-CF_3-phenyl)-ethylamine (350 mg, 1.85 mmol). After 2 h of stirring, the mixture was partitioned between EtOAc and H_2O. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed two times with sat. $NaHCO_3$, two times with sat. citric acid, and two times with brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.29) to obtain the title product in 646 mg (87 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.9 Hz, 2H), 7.31 (d, *J* = 7.9 Hz, 2H), 6.10 (bs, 1H), 4.74 – 4.63 (m, 1H), 4.07 – 3.80 (m, 1H), 3.65 – 3.49 (m, 2H), 2.95 – 2.83 (m, 2H), 2.60 – 2.46 (m, 1H), 2.33 – 2.24 (m, 1H), 1.65 – 1.60 (m, 1H), 1.55 – 1.32 (m, 13H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 143.0, 129.3, 129.1 (q, *J* = 32.3 Hz), 125.7 (q, *J* = 3.8 Hz), 124.3(q, *J* = 271.8 Hz), 80.8, 54.2, 42.4, 40.2, 35.7, 28.4, 25.5, 25.0, 20.7. HRMS calc. for $C_{20}H_{27}F_3N_2NaO_3$: 423.1866 $[M+Na]^+$ found 423.1867.

(*S*)-1-((*R*)-2-Azidobutanoyl)-*N*-(4-(trifluoromethyl)phenethyl)piperidine-2-carboxamide (**S26**)



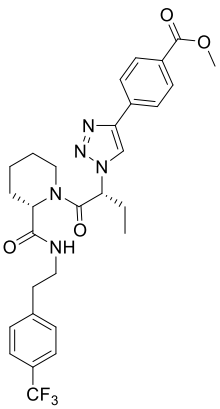
General procedure D: In a round bottom flask was TFA (2 mL) added to a solution of **S20** (200 mg, 500 µmol) in CH_2Cl_2 (8 mL) at 0°C. The mixture was allowed to reach rt and stirred for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and the residue was was redissolved in dry DMF (2 mL). The amine solution was then added to a mixture of (*R*)-2-azidobutanoic acid (129 mg, 1.00 mmol),, NEM (221 µL, 1.75 mmol), and PyBop (481 mg, 924 µmol) in dry DMF under N_2-atmosphere. After 2 h of stirring, the mixture was partitioned between EtOAc and H_2O. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed two times with sat. $NaHCO_3$, two times with sat. citric acid, and two times with brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.40) to obtain the title product in 135 mg (66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9 Hz, 2H), 7.31 (d, *J* = 7.9 Hz, 2H), 6.19 (t, *J* = 6.2 Hz, 1H), 5.09 – 5.04 (m, 1H), 3.80 (t, *J* = 7.1 Hz, 1H), 3.64 – 3.56 (m, 2H), 3.51 – 3.43 (m, 1H), 2.95 – 2.80 (m, 3H), 2.27 – 2.17 (m, 1H), 1.87 (p, *J* = 7.3 Hz, 2H), 1.78 – 1.63 (m, 3H), 1.50 – 1.33 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 170.4, 143.0, 129.2, 129.0 (q, *J* = 32.6 Hz), 125.5 (q, *J* = 3.8 Hz), 124.4 (q, *J* = 271.9 Hz), 60.6, 52.6, 43.9, 40.1, 35.6, 25.6, 25.3, 24.6, 20.3, 10.9. HRMS calc. for $C_{19}H_{24}F_3N_5NaO_2$: 434.1774 $[M+H]^+$ found 434.1774.

3-(1-((*R*)-1-oxo-1-((*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidin-1-yl)butan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**38**)



General procedure F: In a round bottom flask was a 70 mM solution of CuBr (417 µL, 29.2 µmol) in degassed MeCN added to a solution of azide **S26** (30 mg, 73.0 µmol3-ethynylbenzoic acid (21.3 mg, 146 µmol), ascorbic acid (57.8 mg, 291 µmol), and DIPEA (2.54 µL, 14.6 µmol) in degassed THF under N_2-atmosphere. The reaction mixture was stirred for 16 h at room temperature, and the volatiles were subsequently removed *in vacou*. The residue was purified by flash column chromatography using eluent CH_2Cl_2/MeOH (97:3, v/v, R_f = 0.25) to obtain the title product in 41.1 mg (77%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (t, *J* = 1.8 Hz, 1H), 8.45 (s, 1H), 8.20 (dt, *J* = 7.8, 1.5 Hz, 1H), 8.06 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.50 (t, *J* = 5.9 Hz, 1H), 5.66 (dd, *J* = 8.3, 6.4 Hz, 1H), 5.25 – 5.22 (m, 1H), 3.84 – 3.78 (m, 1H), 3.61 – 3.51 (m, 1H), 3.35 (ddt, *J* = 13.6, 8.2, 5.7 Hz, 1H), 2.84 – 2.71 (m, 3H), 2.39 – 2.24 (m, 2H), 2.23 – 2.13 (m, 1H), 1.71 – 1.65 (m, 2H), 1.61 – 1.43 (m, 3H), 0.99 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 169.4, 167.8, 147.4, 143.0, 130.7, 130.6, 130.4, 130.0, 129.3, 129.3, 128.7 (q, *J* = 32.4 Hz), 127.5, 125.4 (q, *J* = 3.8 Hz), 124.3 (q, *J* = 272.0 Hz), 120.0, 61.5, 53.5, 44.2, 40.3, 35.3, 26.7, 25.7, 25.6, 20.2, 10.4. HRMS calc. for $C_{28}H_{31}F_3N_5O_4$: 558.2323 $[M+H]^+$ found 558.2319.

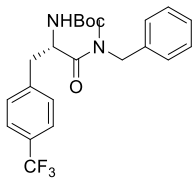
Methyl 4-(1-((*R*)-1-oxo-1-((*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidin-1-yl)butan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoate (**39**)



General procedure E: In a round bottom flask was $CuSO_4 \cdot 5H_2O$ (5.8 mg, 29.2 µmol) added to a solution of **S26** (30 mg, 73.0 µmol), methyl 4-ethynylbenzoate (23.4 mg, 146 µmol), and ascorbic acid (129 mg, 730 µmol), in a N_2-purged mixture of THF/H_2O (1:1, v/v, 4 mL) under N_2-atmosphere. After 16 h of stirring, the reaction mixture was partitioned between EtOAc and H_2O. The organic phase was isolated, and the remaining aqueous phase was extracted three times with EtOAc. The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography using eluent EtOAc/Hep (7:3, v/v, R_f = 0.35) to obtain the title product in 36 mg (91%), as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 – 8.08 (m, 3H), 7.95 – 7.92 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.33 (t, *J* = 5.9 Hz, 1H), 5.54 (t, *J* = 7.3 Hz, 1H), 5.18 – 5.16 (m, 1H), 3.94 (s, 3H), 3.71 – 3.58 (m, 2H), 3.45 – 3.37 (m, 1H), 2.91 – 2.77 (m, 2H), 2.69 – 2.62 (m, 1H), 2.38 – 2.23 (m, 2H), 2.20 – 2.09 (m, 1H), 1.69 – 1.60 (m, 2H), 1.53 – 1.38 (m, 3H), 1.00 (t, *J* = 7.4 Hz,

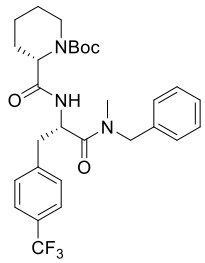
3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 167.4, 166.8, 147.3, 143.0, 134.6, 130.5, 130.1, 129.3, 128.8 (q, *J* = 32.6 Hz), 125.6, 125.4 (q, *J* = 4.0 Hz), 124.3 (q, *J* = 271.8 Hz), 120.0, 61.3, 53.3, 52.3, 44.0, 40.1, 35.4, 26.7, 25.6, 25.6, 20.3, 10.5. HRMS calc. for $C_{29}H_{33}F_3N_5O_4$: 572.2479 $[M+H]^+$ found 572.2475.

***tert*-Butyl (*S*)-(1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamate (S16)**[11]



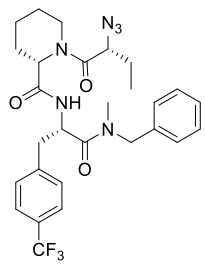
Following **General procedure C**, PyBop (547 mg, 1.05 mmol), N^{α}-Boc-L-(4-CF_3)-phenylalanine (350 mg, 1.05 mmol), NEM (332 µL, 2.63 mmol), and *N*-methylbenzylamine (149 µL, 1.16 µmol) in dry DMF was reacted for 4 h at room temperature to give the title product in 389 mg (85%) as a white solid after flash column chromatography using eluent EtOAc/Hep (3:7, v/v, R_f = 0.27). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.9 Hz, 2H), 7.32 – 7.26 (m, 5H), 7.12 – 7.08 (m, 2H), 5.40 (d, *J* = 8.8 Hz, 1H), 4.89 (td, *J* = 8.4, 6.1 Hz, 1H), 4.66 (d, *J* = 14.5 Hz, 1H), 4.36 (d, *J* = 14.5 Hz, 1H), 3.12 – 2.98 (m, 2H), 2.72 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 155.2, 140.6, 136.4, 130.1, 129.3 (q, *J* = 32.3 Hz), 128.8, 128.3, 127.8, 125.4 (q, *J* = 4.2 Hz), 124.3 (q, *J* = 271.6 Hz), 80.1, 51.4, 51.3, 39.7, 34.8, 28.4. Spectroscopic data was in accordance with those previous reported. HRMS calc. for $C_{23}H_{28}F_3N_2O_3$: 437.2047 $[M+H]^+$ found 437.2047.

***tert*-Butyl (*S*)-2-(((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate (S21)**



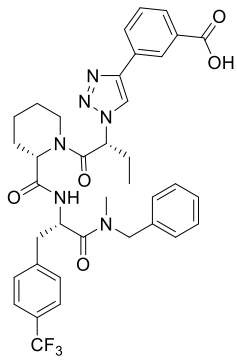
Following **general procedure D**, Boc protected **S16** (359 mg, 823 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were removed *in vacou*, co-evaporated with toluene, and followed by the reaction of PyBop (471 mg, 906 µmol), *N*-Boc-L-Pipecolic acid (208 mg, 906 µmol), and NEM (260 µL, 2.06 mmol) in dry DMF for 2 h under N_2-atmosphere. The title product was obtained in quantitative yield as a white solid after flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.30). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 – 7.47 (m, 2H), 7.33 – 7.29 (m, 5H), 7.14 – 7.11 (m, 2H), 5.30 (q, *J* = 7.6 Hz, 1H), 4.84 – 4.59 (m, 2H), 4.38 – 4.20 (m, 1H), 4.01 – 3.79 (m, 1H), 3.18 (dd, *J* = 13.4, 7.9 Hz, 1H), 3.11 – 3.03 (m, 1H), 2.78 (s, 3H), 2.65 – 2.42 (m, 1H), 2.28 – 2.20 (m, 1H), 1.64 – 1.27 (m, 14H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 170.7, 155.9, 140.4, 136.4, 130.1, 129.5 (q, *J* = 32.0 Hz) 129.1, 128.8, 128.4, 125.5 (q, *J* = 3.4 Hz), 124.2 (q, *J* = 272.1 Hz), 80.9, 54.3, 51.5, 49.7, 42.6, 39.3, 34.8, 28.5, 25.7, 24.9, 20.6. HRMS calc. for $C_{29}H_{37}F_3N_3O_4$: 548.2731 $[M+H]^+$ found 548.2728.

(*S*)-1-((*R*)-2-Azidobutanoyl)-*N*-((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (**S27**)



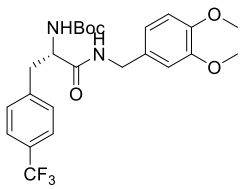
Following **general procedure D**, Boc protected **S21** (180 mg, 329 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were removed *in vacou*, co-evaporated with toluene, and followed by the reaction of PyBop (342 mg, 657 µmol), (*R*)-2-azidobutanoic acid (84.9 mg, 657 µmol), and NEM (145 µL, 1.15 mmol) in dry DMF for 3.5 h under N_2-atmosphere. The title product was obtained in 99 mg (54%), as a yellow solid after flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.35). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.0 Hz, 2H), 7.33 – 7.26 (m, 5H), 7.11 (dd, *J* = 7.2, 2.1 Hz, 2H), 6.96 (d, *J* = 8.3 Hz, 1H), 5.27 – 5.20 (m, 1H), 5.17 – 5.13 (m, 1H), 4.76 (d, *J* = 14.5 Hz, 1H), 4.28 (d, *J* = 14.5 Hz, 1H), 3.86 (t, *J* = 7.0 Hz, 1H), 3.63 – 3.57 (m, 1H), 3.15 (dd, *J* = 13.5, 7.7 Hz, 1H), 3.05 – 3.00 (m, 1H), 2.88 (td, *J* = 13.3, 2.7 Hz, 1H), 2.79 (s, 3H), 2.22 – 2.17 (m, 1H), 1.91 – 1.83 (m, 2H), 1.67 – 1.35 (m, 5H), 1.09 – 1.03 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 170.8, 170.0, 140.4, 136.3, 130.1, 129.4 (q, *J* = 32.7 Hz), 128.8, 128.4, 127.8, 125.4 (q, *J* = 4.2 Hz), 124.2 (q, *J* = 273 Hz), 60.8, 52.7, 51.6, 49.8, 43.7, 38.7, 34.9, 25.9, 25.5, 24.6, 20.3, 10.9. HRMS calc. for $C_{28}H_{34}F_3N_6O_3$: 559.2639 $[M+H]^+$ found 559.2636.

3-(1-((*R*)-1-((*S*)-2-(((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**48**)



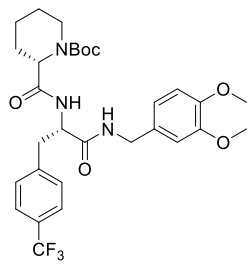
Following **general procedure F**, the reaction of CuBr in degassed MeCN (70 mM, 307 µL, 21.5 µmol), azide **S28** (30 mg, 53.7 µmol), 3-ethynylbenzoic acid (15.7 mg, 107 µmol), ascorbic acid (37.9 mg, 215 µmol), and DIPEA (1.88 µL, 10.8 µmol) in degassed THF under N_2-atmosphere were reacted for 16 h at room temperature to give the title product in 32 mg (85%) as a white solid after flash column chromatography using eluent EtOAc/Hep/AcOH (140:57:3, v/v/v, R_f = 0.33). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.49 (s, 1H), 8.23 (d, *J* = 7.1 Hz, 1H), 8.07 (d, *J* = 8.6 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.29 – 7.27 (m, 1H), 7.24 – 7.20 (m, 2H), 7.05 (d, *J* = 7.8 Hz, 2H), 6.97 – 6.94 (m, 2H), 5.72 – 5.64 (m, 2H), 5.24 – 5.14 (m, 2H), 4.65 (d, *J* = 14.5 Hz, 1H), 4.25 (d, *J* = 14.5 Hz, 1H), 3.92 – 3.86 (m, 1H), 2.94 – 2.88 (m, 1H), 2.76 (dd, *J* = 13.4, 7.1 Hz, 1H), 2.68 (s, 3H), 2.48 – 2.39 (m, 1H), 2.32 – 2.22 (m, 2H), 1.67 – 1.52 (m, 4H), 1.49 – 1.32 (m, 1H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 169.7, 169.5, 168.0, 147.5, 140.1, 136.1, 130.8, 130.7, 130.1, 130.0, 129.8, 128.9, 128.7, 128.2, 127.8, 126.5, 125.4 (q, *J* = 3.5 Hz), 124.2 (q, *J* = 272.0 Hz) 119.9, 61.7, 53.2, 51.4, 49.7, 43.9, 38.8, 34.7, 27.0, 25.9, 25.4, 20.2, 10.4. HRMS calc. for $C_{37}H_{40}F_3N_6O_5$: 705.3007 $[M+H]^+$ found 705.3000.

***tert*-Butyl (*S*)-(1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamate** (**S17**)



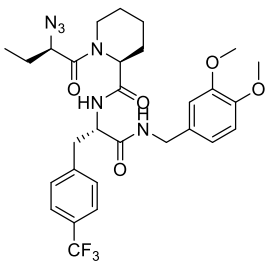
Following **General procedure C**, PyBop (547 mg, 1.05 mmol), N^{α}-Boc-L-(4-CF_3)-phenylalanine (350 mg, 1.05 mmol), NEM (332 µL, 2.63 mmol), 3,4-Dimethoxybenzylamine (263 mg, 1.58 mmol) in dry DMF was reacted for 2 h at room temperature to give the title product in 426 mg (84%) as a white solid after flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.33). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 6.61 (d, *J* = 7.2 Hz, 1H), 6.10 (t, *J* = 5.7 Hz, 1H), 5.02 (s, 1H), 4.39 – 4.24 (m, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.16 (dd, *J* = 13.7, 7.3 Hz, 1H), 3.09 (dd, *J* = 13.7, 6.7 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 155.5, 149.2, 148.7, 141.0, 130.2, 129.9, 129.7 (q, *J* = 32.9 Hz), 125.6 (q, *J* = 3.7 Hz), 124.3 (q, *J* = 272.3 Hz), 120.1, 111.3, 111.3, 80.7, 56.1, 56.0, 55.9, 43.6, 38.5, 28.3. HRMS calc. for $C_{24}H_{29}F_3N_2NaO_5$: 505.1921 $[M+Na]^+$ found 505.1919.

***tert*-butyl (*S*)-2-(((*S*)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate** (**S22**)



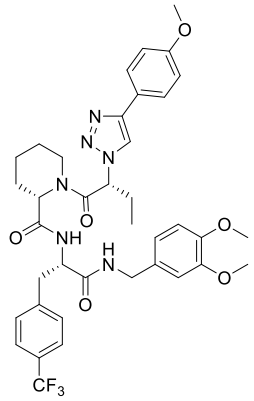
Following **general procedure D**, Boc protected **S17** (218 mg, 452 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were removed *in vacou*, co-evaporated with toluene, and followed by the reaction of PyBop (259 mg, 497 µmol), *N*-Boc-L-Pipecolic acid (114 mg, 497 µmol), and NEM (143 µL, 1.13 mmol) in dry DMF for 2 h under N_2-atmosphere. The title product was obtained in 264 mg (99%), as a white solid after flash column chromatography using eluent EtOAc/Hep (6:4, v/v, R_f = 0.39). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 2H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.64 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.48 (d, *J* = 8.2 Hz, 1H), 6.28 (bs, 1H), 4.73 (q, *J* = 7.5 Hz, 1H), 4.65 – 4.62 (m, 1H), 4.36 (dd, *J* = 14.5, 6.0 Hz, 1H), 4.25 (dd, *J* = 14.5, 5.4 Hz, 1H), 3.90 – 3.85 (m, 4H), 3.85 (s, 3H), 3.23 (dd, *J* = 14.0, 6.9 Hz, 1H), 3.13 (dd, *J* = 14.0, 7.6 Hz, 1H), 2.37 – 2.26 (m, 1H), 2.18 – 2.11 (m, 1H), 1.60 – 1.53 (m, 1H), 1.52 – 1.30 (m, 13H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7, 170.0, 155.7, 149.2, 148.7, 140.9, 130.2, 129.8, 129.6 (q, *J* = 32.3 Hz), 125.7 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 272.3 Hz), 120.1, 111.3, 81.1, 56.0, 56.0, 54.9, 54.0, 43.6, 42.1, 37.8, 28.4, 25.4, 24.7, 20.4. HRMS calc. for $C_{30}H_{39}F_3N_3O_6$: 594.2785 $[M+H]^+$ found 594.2785.

(*S*)-1-((*R*)-2-azidobutanoyl)-*N*-((*S*)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (S28)



In a round bottom flask was TFA (3 mL) added to a solution of **S22** (200 mg, 337 µmol) in CH_2Cl_2 (7 mL) at 0°C. The mixture was allowed to reach rt and stirred for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and the residue was was redissolved in dry DMF (2 mL). The amine solution was then added to a mixture of (*R*)-2-azidobutanoic acid (65.3 mg, 505 µmol), DIPEA (147 µL, 842 µmol), and HATU (192 mg, 505 µmol) in dry DMF under N_2-atmosphere. After 2 h of stirring, the mixture was partitioned between EtOAc and H_2O. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed two times with sat. $NaHCO_3$, two times with sat. citric acid, and two times with brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography using eluent EtOAc/Hep (7:3, v/v, R_f = 0.41) to obtain the title product as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (q, *J* = 6.0, 5.0 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.50 – 7.42 (m, 2H), 6.88 – 6.82 (m, 2H), 6.74 – 6.67 (m, 1H), 4.97 – 4.94 (m, 1H), 4.75 – 4.69 (m, 1H), 4.25 – 4.19 (m, 2H), 4.11 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.73 – 3.71 (m, 6H), 3.56 – 3.49 (m, 1H), 3.17 – 3.07 (m, 1H), 3.04 – 2.91 (m, 1H), 2.84 – 2.78 (m, 1H), 2.13 – 2.03 (m, 1H), 1.80 – 1.27 (m, 6H), 1.00 – 0.92 (m, 4H); ^{13}C NMR (126 MHz, DMSO) δ 174.7, 170.5, 169.8, 148.7, 147.8, 142.9, 131.6, 130.1, 127.1 (q, *J* = 31.4 Hz), 124.9 (q, *J* = 3.9 Hz), 124.4 (q, *J* = 271.7 Hz), 119.3, 111.7, 111.3, 59.3, 55.6, 55.4, 55.4, 53.6, 52.2, 42.6, 41.9, 37.1, 26.5, 24.9, 24.4, 19.8, 10.5. HRMS calc. for $C_{29}H_{36}F_3N_6O_5$: 605.2694 $[M+H]^+$ found 605.2694.

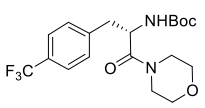
(*S*)-*N*-((*S*)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**49**)



Following **general procedure E**, 4-ethynylanisole (35.0 mg, 265 µmol), ascorbic acid (233 mg, 1.32 mmol), $CuSO_4$ (10.5 mg, 52.9 µmol), and azide **S29** (80 mg, 132 µmol) were reacted for 16 h at rt to give the title product as a white solid (28.3 mg, 29%) after flash column chromatography using eluent EtOAc/Hep (7:3, v/v, R_f = 0.37). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.28 – 7.23 (m, 3H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 1.8 Hz, 1H), 6.66 – 6.61 (m, 2H), 6.45 (t, *J* = 5.7 Hz, 1H), 5.28 (t, *J* = 7.1 Hz, 1H), 5.10 – 5.07 (m, 1H), 4.95 – 4.88 (m, 1H), 4.39 (dd, *J* = 14.6, 6.2 Hz, 1H), 4.22 (dd, *J* = 14.6, 5.1 Hz, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.49 – 3.41 (m, 2H), 3.21 (dd, *J* = 14.4, 10.7 Hz, 1H), 2.32 – 2.26 (m, 1H), 2.21 – 2.05 (m, 2H),

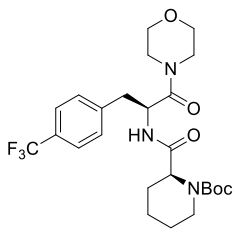
2.03 – 1.93 (m, 1H), 1.52 – 1.38 (m, 2H), 1.37 – 1.28 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H), 0.87 – 0.78 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3, 169.8, 167.4, 160.2, 149.1, 148.4, 148.4, 142.2, 130.4, 129.7, 129.0 (q, *J* = 32.3 Hz), 127.2, 125.3 (q, *J* = 3.7 Hz), 124.2 (q, *J* = 272 Hz), 122.6, 120.0, 118.1, 114.6, 111.2, 111.1, 61.2, 56.0, 55.9, 55.5, 54.1, 54.1, 43.5, 43.5, 37.3, 26.2, 25.2, 24.9, 19.7, 10.3. HRMS calc. for $C_{30}H_{39}F_3N_3O_6$: 737.3269 $[M+H]^+$ found 737.3272.

***tert*-Butyl (*S*)-(1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamate** (**S18**)



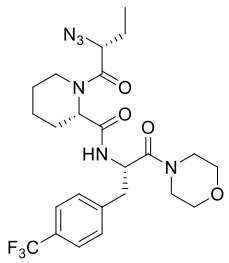
Following **General procedure C**, PyBop (547 mg, 1.05 mmol), N^{α}-Boc-L-(4-CF_3)-phenylalanine (350 mg, 1.05 mmol), NEM (332 µL, 2.63 mmol), and morpholine (101 µL, 1.16 mmol) in dry DMF was reacted for 4 h at room temperature to give the title product in 414 mg (98%) as a white solid after flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.38). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 5.35 (d, *J* = 8.9 Hz, 1H), 4.82 (td, *J* = 8.4, 6.2 Hz, 1H), 3.66 – 3.56 (m, 2H), 3.55 – 3.48 (m, 3H), 3.41 (ddd, *J* = 13.2, 6.5, 3.0 Hz, 1H), 3.17 (ddd, *J* = 11.5, 6.4, 2.9 Hz, 1H), 3.12 – 2.98 (m, 3H), 1.40 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9, 155.1, 140.7, 130.1, 129.6 (q, *J* = 32.7 Hz), 125.5 (q, *J* = 3.7 Hz), 124,2 (q, *J* = 272.5 Hz), 80.2, 66.7, 66.4, 50.7, 46.2, 42.5, 39.9, 28.4. HRMS calc. for $C_{19}H_{26}F_3N_2O_4$: 403.1839 $[M+H]^+$ found 403.1839.

***tert*-butyl (*S*)-2-(((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate** (**S23**)



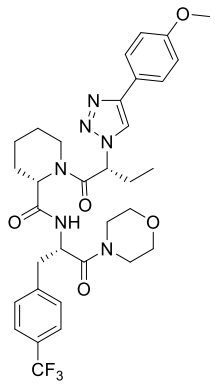
Following **general procedure D**, Boc protected **S18** (404 mg, 926 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and followed by the reaction of PyBop (462 mg, 889 µmol), *N*-Boc-L-Pipecolic acid (213 mg, 930 µmol), and NEM (267 µL, 2.11 mmol) in dry DMF for 2 h under N_2-atmosphere. The title product was obtained in 452 mg (95%) as a white solid after flash column chromatography using eluent EtOAc/Hep (7:3, v/v, R_f = 0.35). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 6.86 (bs, 1H), 5.25 – 5.15 (m, 1H), 4.79 – 4.68 (m, 1H), 3.95 – 3.82 (m, 1H), 3.66 – 3.40 (m, 6H), 3.28 – 3.00 (m, 4H), 2.56 – 2.41 (m, 1H), 2.22 – 2.15 (m, 1H), 1.69 – 1.23 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 169.3, 155.9, 140.4, 130.1, 129.2 (q, *J* = 32.6 Hz), 125.6 (q, *J* = 3.4 Hz), 124.2 (q, *J* = 272.0 Hz), 81.0, 66.7, 66.4, 54.3, 49.0, 46.2, 42.6, 41.2, 39.3, 28.5, 25.6, 24.9, 20.5. HRMS calc. for $C_{25}H_{34}F_3N_3NaO_5$: 536.2343 $[M+Na]^+$ found 536.2341**.**

(*S*)-1-((*R*)-2-azidobutanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (S29)



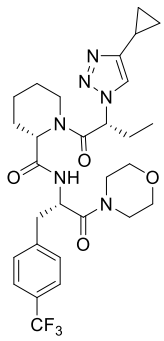
Following **general procedure D**, Boc protected **S23** (230 mg, 448 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and followed by the reaction of PyBop (466 mg, 896 µmol), (*R*)-2-azidobutanoic acid (116 mg, 896 µmol), and NEM (198 µL, 1.57 mmol) in dry DMF for 3.5 h under N_2-atmosphere. The title product was obtained in 196 mg (83%) after flash column chromatography using eluent EtOAc/Hep (8:2, v/v, R_f = 0.39).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 6.82 (d, *J* = 8.2 Hz, 1H), 5.18 – 5.08 (m, 2H), 3.81 (q, *J* = 6.8 Hz, 1H), 3.68 – 3.50 (m, 7H), 3.49 – 3.36 (m, 1H), 3.30 – 3.16 (m, 1H), 3.11 (dd, *J* = 13.6, 7.2 Hz, 1H), 3.03 (dd, *J* = 13.2, 6.1 Hz, 1H), 2.82 – 2.74 (m, 1H), 2.24 – 2.14 (m, 1H), 1.87 (q, *J* = 7.4 Hz, 2H), 1.72 – 1.57 (m, 4H), 1.55 – 1.44 (m, 1H), 1.08 – 1.03 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 169.9, 169.2, 140.5, 130.1, 129.6 (q, *J* = 32.4 Hz), 125.5 (q, *J* = 5.0 Hz), 124.2 (q, *J* = 272 Hz), 66.7, 66.4, 60.7, 52.7, 49.2, 46.2, 43.7, 42.6, 39.0, 25.8, 25.5, 24.6, 20.3, 10.9. HRMS calc. for $C_{24}H_{32}F_3N_6O_4$: 525.2432 $[M+H]^+$ found 525.2432.

(*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (**50**)



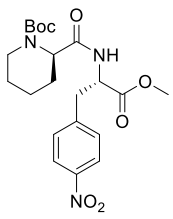
Following **general procedure E**, 4-ethynylanisole (15.1 mg, 114 µmol), ascorbic acid (101 mg, 572 µmol), $CuSO_4$ (4.5 mg, 22.9 µmol), and azide **S27** (30 mg, 57.2 µmol) were reacted for 16 h at rt to give the title product as a white solid (16 mg, 43%) after flash column chromatography using eluent CH_2Cl_2/MeOH (97:3, v/v, R_f = 0.33). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (bs, 1H), 7.88 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 7.7 Hz, 2H), 7.02 – 6.90 (m, 4H), 6.80 (d, *J* = 8.1 Hz, 1H), 5.59 (t, *J* = 7.6 Hz, 1H), 5.18 – 5.14 (m, 1H), 5.05 (q, *J* = 7.5 Hz, 1H), 3.86 – 3.81 (m, 4H), 3.63 – 3.56 (m, 2H), 3.52 – 3.45 (m, 3H), 3.37 – 3.29 (m, 1H), 3.17 – 3.11 (m, 1H), 3.05 – 2.99 (m, 1H), 2.73 – 2.68 (m, 2H), 2.39 – 2.32 (m, 1H), 2.30 – 2.08 (m, 3H), 1.64 – 1.29 (m, 5H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.3, 167.8, 159.9, 140.3, 130.0, 129.4 (q, *J* = 32.6 Hz) 127.4, 125.4 (q, *J* = 3.2 Hz), 124.1 (q, *J* = 272.2 Hz) 123.1, 114.3, 66.7, 66.3, 61.5, 55.4, 52.9, 48.9, 46.1, 43.7, 42.6, 39.1, 26.7, 25.3, 25.3, 20.2, 10.3. HRMS calc. for $C_{33}H_{40}F_3N_6O_5$: 657.3007 $[M+H]^+$ found 657.3002**.**

(*S*)-1-((*R*)-2-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)butanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (**51**)



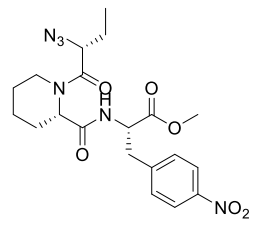
General procedure F: In a round bottom flask was a solution of CuBr (70 mM, 327 µL, 22.9 µmol) in degassed MeCN added to a solution of azide **S27** (30 mg, 57.2 µmol), cyclopropylacetylene (9.67 µL, 114 µmol), ascorbic acid (40.3 mg, 229 µmol), and DIPEA (2.00 µL, 11.5 µmol) in degassed THF under N_2-atmosphere. The reaction mixture was stirred for 16 h at room temperature, and the volatiles were subsequently removed *in vacou*. The residue was purified by flash column chromatography using eluent CH_2Cl_2/MeOH (97:3, v/v, R_f = 0.25) to obtain the title product in 18.7 mg (55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 – 7.49 (m, 2H), 7.32 – 7.21 (m, 2H), 6.85 (bs, 1H), 5.77 – 5.49 (m, 1H), 5.29 – 5.06 (m, 2H), 3.85 – 3.41 (m, 7H), 3.36 – 2.97 (m, 4H), 2.51 – 2.18 (m, 2H), 1.91 – 1.84 (m, 1H), 1.73 – 1.17 (m, 9H), 1.12 – 0.74 (m, 5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 169.4, 167.6, 140.6, 130.2, 129.5 (q, *J* = 32.6 Hz), 125.5 (q, *J* = 3.9 Hz), 124.1 (q, *J* = 272.2 Hz), 66.8, 66.4, 60.5, 53.1, 49.2, 46.2, 43.8, 42.7, 38.9, 26.4, 25.6, 25.3, 20.2, 14.3, 10.6, 7.5. HRMS calc. for $C_{29}H_{38}F_3N_6O_4$: 591.2901 $[M+H]^+$ found 591.2900.

***tert*-Butyl (*R*)-2-(((*S*)-1-methoxy-3-(4-nitrophenyl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate** (**S24**)



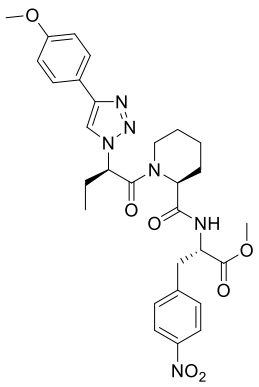
Following **General procedure C**, PyBop (1.04 g, 1.99 mmol), *N*-Boc-L-Pipecolic acid (456 mg, 1.99 mmol), NEM (881 µL, 6.96 mmol), 4-Nitro-L-phenylalanine methyl ester hydrochloride and (600 mg, 2.19 mmol) in dry DMF was reacted for 2 h at room temperature to give the title product in 854 mg (98 %) as a white solid after flash column chromatography using eluent EtOAc/Hep (4:6, v/v, R_f = 0.31). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.6 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 2H), 6.71 – 6.44 (m, 1H), 5.00 – 4.85 (m, 1H), 4.77 – 4.63 (m, 1H), 4.15 – 3.83 (m, 1H), 3.75 (s, 3H), 3.34 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.15 (dd, *J* = 14.0, 6.9 Hz, 1H), 2.62 – 2.33 (m, 1H), 2.20 (d, *J* = 13.3 Hz, 1H), 1.65 – 1.31 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 171.3, 156.0, 147.3, 144.0, 130.4, 123.8, 81.0, 53.9, 52.9, 52.8, 42.4, 38.2, 28.4, 25.5, 24.9, 20.5. HRMS calc. for $C_{21}H_{29}N_3NaO_7$: 458.1898 $[M+Na]^+$ found 458.1898.

Methyl (*S*)-2-((*S*)-1-((*R*)-2-azidobutanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanoate (**S30**)



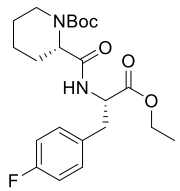
Following **general procedure D**, Boc protected **S24** (150 mg, 345 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and followed by the reaction of PyBop (179 mg, 345 µmol), (*R*)-2-azidobutanoic acid (44.4 mg, 345 µmol), and NEM (109 µL, 862 µmol) in dry DMF for 3.5 h under N_2-atmosphere. The title product was obtained in 95 mg (62%), as a slight yellow solid after flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.27). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.7 Hz, 2H), 7.28 – 7.25 (m, 2H), 6.56 (d, *J* = 7.9 Hz, 1H), 5.01 – 4.98 (m, 1H), 4.80 (td, *J* = 7.7, 5.5 Hz, 1H), 3.72 (t, *J* = 7.1 Hz, 1H), 3.69 (s, 3H), 3.55 – 3.48 (m, 1H), 3.27 (dd, *J* = 14.0, 5.5 Hz, 1H), 3.04 (dd, *J* = 14.0, 7.7 Hz, 1H), 2.77 (td, *J* = 13.3, 2.7 Hz, 1H), 2.12 – 2.07 (m, 1H), 1.82 (p, *J* = 7.0 Hz, 2H), 1.64 – 1.56 (m, 3H), 1.49 – 1.39 (m, 1H), 1.38 – 1.28 (m, 1H), 0.98 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 170.6, 170.4, 147.2, 144.0, 130.2, 123.7, 60.3, 52.8, 52.7, 52.4, 43.6, 37.8, 25.4, 25.3, 24.5, 20.0, 10.7. HRMS calc. for $C_{20}H_{26}N_6NaO_6$: 469.1806 $[M+H]^+$ found 469.1805.

methyl (S)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanoate (**53**)



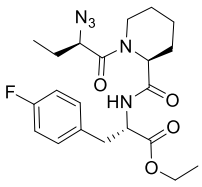
Following **general procedure F**, the reaction of CuBr in degassed MeCN (70 mM, 128 µL, 8.96 µmol), azide **S30** (20 mg, 44.8 µmol), 4-ethynylanisole (11.9 mg, 89.6 µmol), ascorbic acid (3.16 mg, 17.9 µmol), and DIPEA (1.56 µL, 8.96 µmol) in degassed THF under N_2-atmosphere were reacted for 16 h at room temperature to give the title product in 21 mg (76%) as a white solid after flash column chromatography using eluent EtOAc/Hep (6:4, v/v, R_f = 0.24).^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 – 7.99 (m, 2H), 7.98 (s, 1H), 7.81 – 7.77 (m, 2H), 7.11 (d, *J* = 8.7 Hz, 2H), 6.98 – 6.94 (m, 2H), 6.65 (d, *J* = 8.0 Hz, 1H), 5.53 (t, *J* = 7.3 Hz, 1H), 5.22 – 5.18 (m, 1H), 4.83 (td, *J* = 8.2, 5.8 Hz, 1H), 3.86 (s, 3H), 3.80 – 3.75 (m, 1H), 3.66 (s, 3H), 3.13 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.99 (dd, *J* = 14.1, 8.5 Hz, 1H), 2.48 – 2.42 (m, 1H), 2.33 – 2.26 (m, 2H), 2.21 – 2.13 (m, 1H), 1.66 – 1.60 (m, 1H), 1.57 – 1.27 (m, 4H), 0.98 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 169.8, 167.8, 160.0, 148.2, 147.1, 144.3, 130.2, 127.2, 123.6, 123.0, 117.8, 114.4, 61.2, 55.5, 53.1, 52.8, 52.8, 44.0, 37.8, 26.5, 25.4, 25.3, 20.3, 10.4. HRMS calc. for $C_{29}H_{35}N_6O_7$: 579.2562 $[M+H]^+$ found 579.2559.

***tert*-Butyl (*S*)-2-(((*S*)-1-ethoxy-3-(4-fluorophenyl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate** (**S25**)



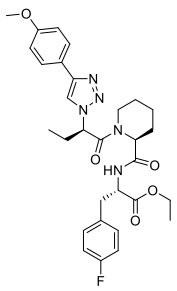
Following **General procedure C**, PyBop (575 mg, 1.10 mmol), *N*-Boc-L-Pipecolic acid (253 mg, 1.10 mmol), NEM (489 µL, 3.86 mmol), 4-fluoro-L-phenylalanine ethyl ester hydrochloride and (300 mg, 1.215 mmol) in dry DMF was reacted for 3 h at room temperature to give the title product in quantitative yield as a thick colorless syrup after flash column chromatography using eluent EtOAc/Hep (3:7, v/v, R_f = 0.29). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 – 7.06 (m, 2H), 6.99 – 6.94 (m, 2H), 6.60 – 6.33 (m, 1H), 4.93 – 4.60 (m, 2H), 4.23 – 4.12 (m, 2H), 4.08 – 3.81 (m, 1H), 3.19 (dd, *J* = 14.1, 5.9 Hz, 1H), 3.02 (dd, *J* = 14.1, 6.9 Hz, 1H), 2.57 – 2.34 (m, 1H), 2.23 (d, *J* = 13.3 Hz, 1H), 1.62 – 1.31 (m, 14H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 171.0, 162.1 (d, *J* = 245.3 Hz), 154.7, 131.9, 130.9 (d, *J* = 8.1 Hz), 115.5 (d, *J* = 21.2 Hz)., 80.8, 61.7, 55.5, 53.2, 42.4, 37.5, 28.4, 25.6, 24.9, 20.6, 14.3. HRMS calc. for $C_{22}H_{31}FN_2NaO_5$: 445.2109 $[M+Na]^+$ found 445.2111.

Ethyl (*S*)-2-((*S*)-1-((*R*)-2-azidobutanoyl)piperidine-2-carboxamido)-3-(4-fluorophenyl)propanoate (**S31**)

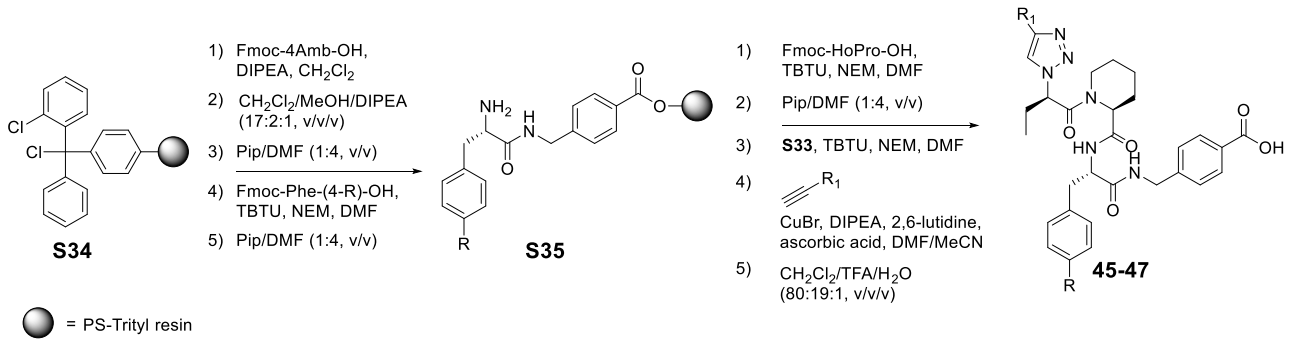


Following **general procedure D**, Boc protected **S25** (150 mg, 355 µmol) was treated with TFA/CH_2Cl_2 (3:7, v/v) for 3 h. The volatiles were then removed under reduced pressure, co-evaporated twice with toluene (30 mL), and followed by the reaction of PyBop (268 mg, 515 µmol), (*R*)-2-azidobutanoic acid (46.4 mg, 533 µmol), and NEM (112 µL, 888 µmol) in dry DMF for 3.5 h under N_2-atmosphere. The title product was obtained in 114 mg (70%) after flash column chromatography using eluent EtOAc/Hep (7:13, v/v, R_f = 0.27). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 – 7.07 (m, 2H), 7.00 – 6.95 (m, 2H), 6.50 (d, *J* = 7.9 Hz, 1H), 5.11 (d, *J* = 5.8 Hz, 1H), 4.78 (td, *J* = 7.9, 5.7 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.80 (t, *J* = 7.0 Hz, 1H), 3.58 – 3.52 (m, 1H), 3.19 (dd, *J* = 14.2, 5.7 Hz, 1H), 2.97 (dd, *J* = 14.2, 7.8 Hz, 1H), 2.78 (td, *J* = 13.3, 2.6 Hz, 1H), 2.22 – 2.16 (m, 1H), 1.87 (p, *J* = 7.3 Hz, 2H), 1.69 – 1.61 (m, 3H), 1.51 – 1.34 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.05 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 170.5, 170.2, 162.1 (d, *J* = 245.4 Hz), 132.1 (d, *J* = 3.4 Hz), 131,0 (d, *J* = 7.9 Hz), 115.5 (d, *J* = 21.3 Hz), 61.8, 60.6, 53.3, 52.6, 43.7, 37.3, 25.6, 24.7, 20.3, 14.3, 10.9. HRMS calc. for $C_{21}H_{28}FN_5NaO_4$: 456.2018 $[M+Na]^+$ found 456.2017.

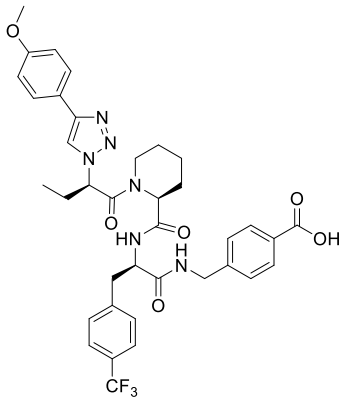
Ethyl (*S*)-3-(4-fluorophenyl)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)propanoate (**54**)



Following **general procedure F**, the reaction of CuBr in degassed MeCN (70 mM, 249 µL, 17.4 µmol), azide **S31** (20 mg, 43.6 µmol), 4-ethynylanisole (11.5 mg, 87.2 µmol), ascorbic acid (6.14 mg, 34.9 µmol), and DIPEA (1.52 µL, 8.71 µmol) in degassed THF under N_2-atmosphere were reacted for 16 h at room temperature to give the title product in quantitative yield as a colorless solid after flash column chromatography using eluent EtOAc/Hep (1:1, v/v, R_f = 0.27). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.83 – 7.79 (m, 2H), 6.97 – 6.93 (m, 2H), 6.88 – 6.82 (m, 4H), 6.38 (d, *J* = 8.1 Hz, 1H), 5.57 (t, *J* = 7.4 Hz, 1H), 5.19 – 5.16 (m, 1H), 4.77 – 4.71 (m, 1H), 4.13 – 4.06 (m, 2H), 3.79 – 3.73 (m, 1H), 2.98 (dd, *J* = 14.1, 6.0 Hz, 1H), 2.73 (dd, *J* = 14.2, 8.5 Hz, 1H), 2.45 – 2.37 (m, 1H), 2.31 – 2.10 (m, 3H), 1.64 – 1.34 (m, 5H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 169.4, 167.8, 161.90 (d, *J* = 245.3 Hz), 159.9, 148.3, 132.0 (d, *J* = 3.3 Hz), 130.7 (d, *J* = 8.1 Hz), 127.2, 123.2, 117.7, 115.3 (d, *J* = 21.3 Hz), 114.3, 61.7, 61.3, 55.5, 53.1, 53.0, 43.8, 37.3, 26.8, 25.5, 25.4, 20.2, 14.2, 10.4. HRMS calc. for $C_{30}H_{37}FN_5O_5$: 566.2773 $[M+H]^+$ found 566.2769.

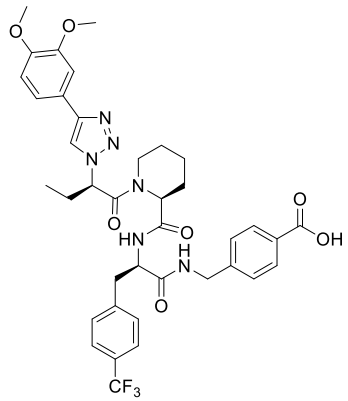


4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-Triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanamido)methyl)benzoic acid (**45**)



Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **45** was cleaved off the Trityl-PS resin (75 mg, 2 mmol/g) for 2h at room temperature using CH_2Cl_2/TFA/H_2O (80:19:1, v/v/v). The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (7:93, v/v, R_f = 0.31) to obtain the title product in 51.1 mg (47%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 7.9 Hz, 2H), 7.48 – 7.43 (m, 3H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.9 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 2H), 6.85 (t, *J* = 5.8 Hz, 1H), 5.29 (t, *J* = 7.1 Hz, 1H), 5.15 – 5.12 (m, 1H), 5.10 – 5.04 (m, 1H), 4.56 (dd, *J* = 15.4, 6.5 Hz, 1H), 4.29 (dd, *J* = 15.4, 5.1 Hz, 1H), 3.84 (s, 3H), 3.55 – 3.46 (m, 2H), 3.27 (dd, *J* = 14.5, 11.2 Hz, 1H), 2.36 – 2.29 (m, 1H), 2.25 – 2.14 (m, 2H), 2.03 (dp, *J* = 14.1, 7.2 Hz, 1H), 1.54 – 1.42 (m, 2H), 1.39 – 1.31 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H), 0.90 – 0.78 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 170.2, 167.7, 160.2, 148.5, 143.9, 142.2, 130.4, 129.7, 129.0 (q, *J* = 32.2 Hz), 128.3 127.6, 127.3, 125.4 (q, *J* = 3.6 Hz), 124.3 (q, *J* = 269.4 Hz) 118.2, 114.6, 61.1, 55.5, 54.3, 54.0, 43.6, 43.3, 37.1, 26.1, 25.2, 24.8, 19.7, 10.4. HRMS calc. for $C_{37}H_{40}F_3N_6O_6$: 721.2956 $[M+H]^+$ found 721.2955.

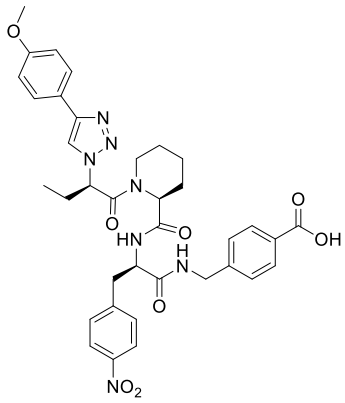
4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanamido)methyl)benzoic acid (**46**)



Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **46** was cleaved off the Trityl-PS resin (75 mg, 2 mmol/g) for 2h at room temperature using CH_2Cl_2/TFA/H_2O (80:19:1, v/v/v). The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (7:93, v/v, R_f = 0.22) to obtain the title product in 63.6 mg (57%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.76 (d, *J* = 7.9 Hz, 2H), 7.48 – 7.43 (m, 3H), 7.40 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.13 (d, *J* = 7.9 Hz, 2H), 6.92 – 6.86 (m, 2H), 5.37 (t, *J* = 7.2 Hz, 1H), 5.16 – 5.13 (m, 1H), 5.08 – 5.01 (m, 1H), 4.52 (dd, *J* = 15.4, 6.4 Hz, 1H), 4.29 (dd, *J* = 15.3, 5.1 Hz, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.54 (dt, *J* = 13.8, 3.6 Hz, 1H), 3.44 (dd, *J* = 14.4, 5.7 Hz, 1H), 3.18 (dd, *J* = 14.3, 10.8 Hz, 1H), 2.34 – 2.14 (m, 3H), 2.06 (dp, *J* = 14.4, 7.4 Hz, 1H), 1.55 – 1.32 (m,

4H), 0.98 – 0.90 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 170.3, 169.8, 167.8, 149.7, 149.6, 148.6, 143.7, 142.0, 130.4, 129.7, 129.1 (q, *J* = 32.4 Hz), 128.4, 127.5, 125.4 (q, *J* = 3.8 Hz), 124.3 (q, *J* = 272 Hz), 122.9, 118.6, 118.4, 111.6, 109.1, 61.3, 56.2, 56.1, 54.1, 54.0, 43.7, 43.3, 37.2, 26.3, 25.3, 24.9, 19.8, 10.4. HRMS calc. for $C_{38}H_{42}F_3N_6O_7$: 751.3062 $[M+H]^+$ found 751.3058.

4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanamido)methyl)benzoic acid (**47**)



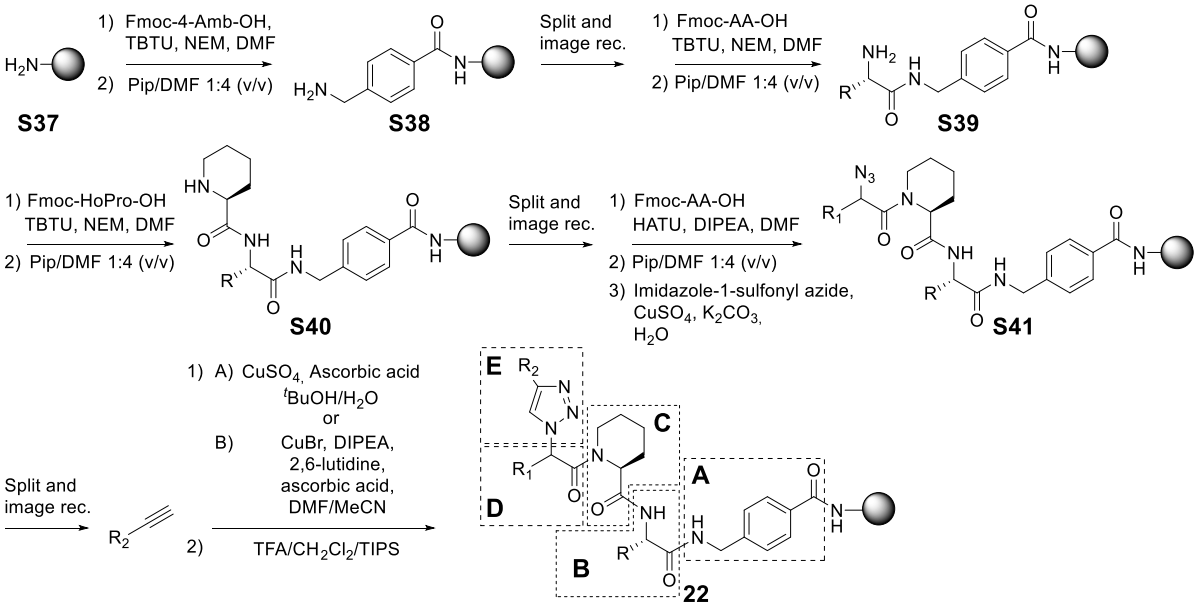
Following **general procedures for SPPS** and using procedure **B** for the CuAAC reaction, **47** was cleaved off the Trityl-PS resin (75 mg, 2 mmol/g) for 2h at room temperature using CH_2Cl_2/TFA/H_2O (80:19:1, v/v/v). The cleavage mixture was filtered off and the resin was washed with MeCN, and CH_2Cl_2. The volatiles were removed *in vacou* and the residue was purified by flash column chromatography using eluent MeOH/CH_2Cl_2 (7:93, v/v, R_f = 0.27) to obtain the title product in 62.0 mg (59%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.7 Hz, 2H), 7.92 (s, 1H), 7.80 – 7.74 (m, 4H), 7.64 (d, *J* = 9.1 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.01 – 6.94 (m, 3H), 5.31 (t, *J* = 7.1 Hz, 1H), 5.15 – 5.12 (m, 1H), 5.09 – 5.02 (m, 1H), 4.56 (dd, *J* = 15.4, 6.7 Hz, 1H), 4.29 (dd, *J* = 15.4, 5.1 Hz, 1H), 3.84 (s, 3H), 3.59 – 3.52 (m, 2H), 3.36 (dd, *J* = 14.6, 10.8 Hz, 1H), 2.44 – 2.30 (m, 2H), 2.23 – 2.13 (m, 1H), 2.03 (dp, *J* = 14.1, 7.1 Hz, 1H), 1.57 – 1.47 (m, 2H), 1.45 – 1.34 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H), 0.93 – 0.85 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 170.4, 170.1, 167.7, 160.2, 148.6, 146.8, 146.0, 143.8, 130.4, 130.2, 128.4, 127.6, 127.2, 123.6, 122.4, 118.3, 114.6, 61.2, 55.5, 54.4, 54.0, 43.7, 43.3, 37.1, 26.1, 25.2, 24.8, 19.9. HRMS calc. for $C_{36}H_{40}N_7O_8$: 698.2933 $[M+H]^+$ found 698.2928.

Modelling of Peptide Mimetics

The PDB-file of the crystal structure by Hatada *et al*.[12] of SLF* bound to FKPB from Protein Data Bank (id: 1BL4) was retrieved and the structure was repaired using the modeling program Molecular Operating Environment (MOE, CCG). The coordinates of the protein were fixed and the structure was saved as a starting point for all subsequent calculations of ligand interactions. The SLF* ligand was removed and replaced with different peptide based analogs, soaked in a droplet of water. Maintaining the fixed coordinates of the protein, the structures of the ligands were placed in the binding site maintaining the orientation of the core pipecolic amide in order to fill the deep pocked with a short aliphatic amino acid side chain. The ligand was allowed to relax by energy minimization and molecular dynamics (MD, 350 to 230 K, ~ 1 ns) in the binding site. The deep hydrophobic pocket binding the ethyl group in Shld1 was preferred for size of ligand and the groove binding to pipecolic amide was preferred with respect to ligand ring size and substitution pattern. For each analog, the binding interaction over the entire binding site was evaluated and for ligands presenting a good fit to the binding site, the fixation of protein residues in direct contact with the ligand were released and calculation was continued at 298 K in order to optimize the local interactions. The final structures were energy minimized. Particular care was taken to optimize the interaction of the three peripheral aromatic rings. The best structures displayed a variety of key interaction residues that were selected for the design of a combinatorial library shown in **Table S1**. The library was synthesized on encoded beads and screened as follows.

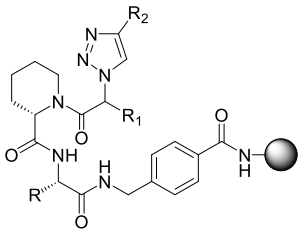
Library Screening

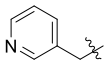
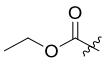
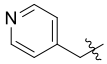
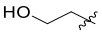
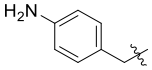
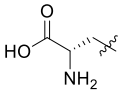
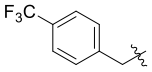
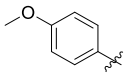
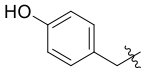
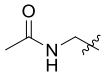
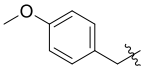
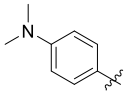
Encoded bead library synthesis

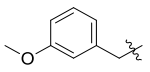
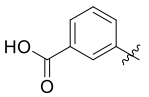
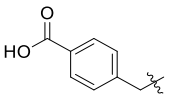
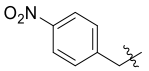
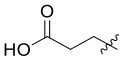
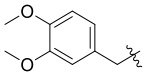
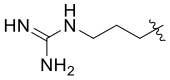


The encoded bead library was synthesized on $PEGA_{1900}$ beads (500-550 µm), optically encoded with 10 µm tentagel microparticles, which were labeled with the ATOTA fluorophore.[13] The synthesis of the encoded beads were carried out as previously described.[2] Following the general procedures for solid phase synthesis, the synthesis was carried out on approx. 4500 beads, using the split-and-mix approach. Each peptide coupling was repeated once to ensure full amidation. To minimize any movement and delocalization of the interior microbeads, and hereby loss of structural information, all swelling and washing with CH_2Cl_2 was excluded from the standard procedures. Upon each split, the beads were swelled in water and individually decoded by passing them through a MPM-decoder. Three images were taken by three orthogonally positioned cameras with laser excitation of the bead at 477 nm. The vectors that connect them were calculated using software programs ImToCoord and H2SCompare, hence correlating a specific modification to a given bead.[13] After the decoding, the beads were collected and divided into a number 1 mL syringes equipped with a Teflon® filter, equal to the number of modifications in the split. Three splits with 16, 4, and 14 different building blocks respectively for the R, R_1, and R_2 was carried out giving 896 different compounds. After the final deprotection step, the beads were carefully washed with H_2O, DMF, MeOH, H_2O and assay buffer, prior to the screening of the library.

Table S1. Building blocks



#	R	R_1	R_2
1		 (R)	
2		 (R)	
3		 (R)	
4		 (S,S)	
5			
6			
7			
8			
9			
10			
11			

12			
13			
14			
15			
16			

MBP-DD production and purification

E. coli containing pOPINM plasmid with the MBP-DD insert, was grown until OD 0.75 at 37 °C. The promotor was activated with IPTG and temperature was reduced to 20 °C for protein production o/n. E coli was pelleted and total proteins was extracted using Bugbuster (Novagen) according to the manufacture. Total protein was diluted in column buffer (20mM Tris-HCl pH 7.4, 200mM NaCl, 1mM EDTA and 1mM DTT) and run through an MBP trap beads column (GE healthcare) using an Aktar. The MBP-DD protein was eluted by adding column buffer with maltose (200mM). Protein concentration was measured using a nanodrop.

Rhodamine-X labeling of MBP-DD

250 µL MBP-DD (17.5 µM, 4.38 nmol) in buffer (20mM Tris-HCl, 200 mM NaCl, 1mM EDTA, 1mM DTT, 20 mM maltose, pH 7.4), was cooled to 0°C. Rhodamine-X®-OSu (1,5 mM in DMSO, 8.76 µL, 13.1 nmol) was diluted in the same buffer (10 µL) as above and added immediately to the protein. After 1 hour at 0°C, the excess NHS-ester was quenched by addition of ethanolamine (5µL, 150 mM), whereupon the solution was allowed to reach room temperature for 1 hour. The mixture was loaded onto a gel filtration column (Sephadex G-25, 7 mL, 4 cm) and eluted with the same degassed and freshly prepared buffer as above. The fractions containing labeled protein was gathered and the final concentration was measured on a NanoDrop™ 2000 spectrophotometer after treatment with Pierce™ 660nm protein assay reagent, to be 7.9 µM. The degree of labeling was analyzed by MALDI-TOF.

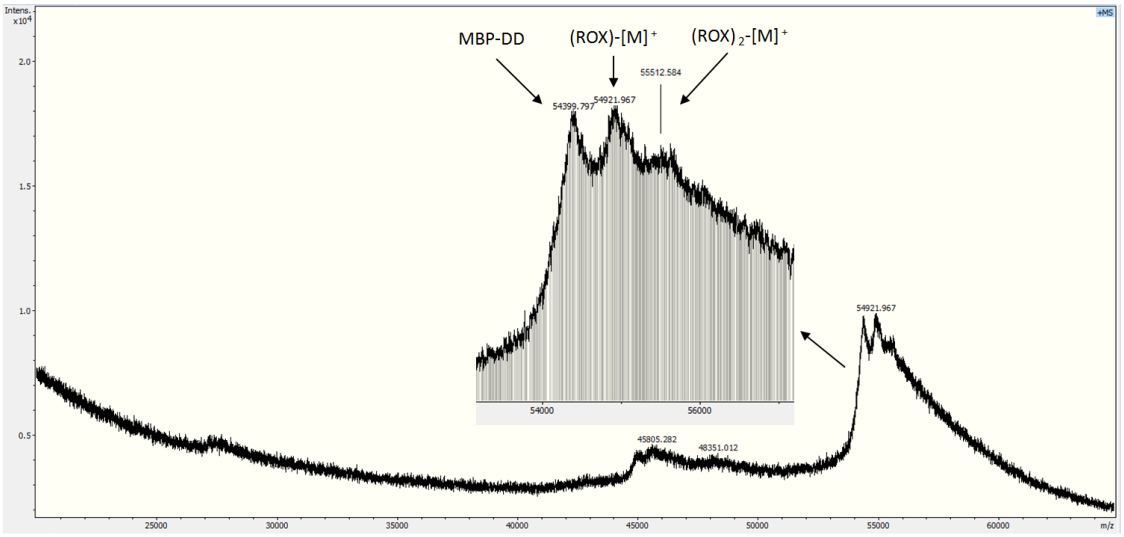
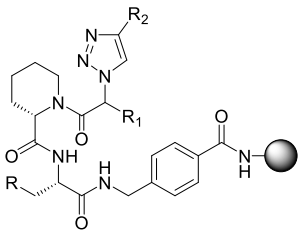


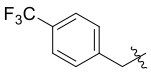
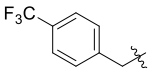
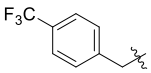
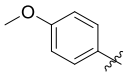
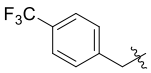
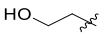
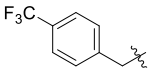
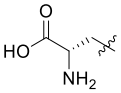
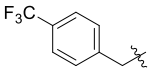
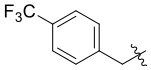
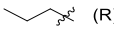
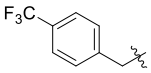
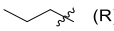
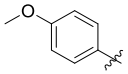
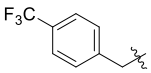
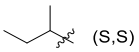
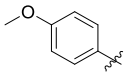
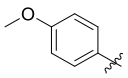
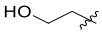
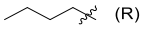
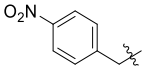
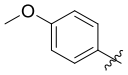
Figure S1: MALDI spectra of His_6-MBP-DD protein labeled with the rhodamine-X fluorophore after size exclusion purification.

Library screening

In a 5 mL syringe fitted with a Teflon® filter, was the solid supported and deprotected library beads washed and swelled in freshly prepared assay buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 20 mM maltose, pH 7,4). MBP-DD-(ROX) (12.2 µM, 123 µL) was mixed with Shld1 solution in assay buffer (12.2 µM, 123 µL) and incubated for 30 min. The solution was then further diluted to 1 µM by addition of 1.25 mL assay buffer and added to the preswelled library beads. The mixture was carefully shaken for two days, whereupon the protein solution was removed by filtration and the library beads were then washed 5 times with water. 17 Of these beads were then manually sorted based on the observed fluorescence intensity under an Olympus IZ73 fluorescence microscope (ex. 560/14 em. 605/52). The micro-particle code of the isolated beads were then recorded and the structures were decoded against the split recordings, as described in the library synthesis, to obtain the structures correlating to the individual beads.

Table S2. Hit structures



#	Comp.	R	R_1	R_2
1	**S42**			
2	**S43**			
3	**S44**			
4	**S45**			
5	**S46**			
6	**S47**			
7	**S48**			
8	**S49**			
9	**S50**			
10	**S51**			
11	**S52**			
12	**S53**			
13	**S54**			
14	**S55**			
15	**S56**			
16	**S57**			

Assay protocols

Well based on-bead assay

The selected library hits were resynthesized on a $PEGA_{1900}$ resin (125-200 µm) following the general procedures for SPS. The beads were swollen in water and sorted by a modified COMPAS nematode sorter[14] into a transparent flat bottom 96 well plate (TC 96 standard F, Sarstedt) with three beads per well. The 96 well plate was left without a lid on for one day to evaporate the water droplets collected in each well during the sorting. In an Eppendorf tube was MBP-DD-(ROX) solution (7.9 µM, 56.7 µL) mixed with a solution of Shld1 (7.9 µM, 56.7 µL) in assay buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 20 mM maltose, pH 7.4). The mixture was incubated for 30 min before being diluted to a final maximum concentration of 3 µM, and titrated in a 2:1 dilution series. The dilution series of the Shld1 stabilized protein solution was then added to the wells containing beads with the synthesized ligands and incubated for 16 hours at room temperature.

The fluorescence values of the control tripeptide Ac-Ala-Phe-Gly was used as a measure of the NSB, and was subtracted from the values obtained from the binding studies of the solid supported SLF*, and the values obtained with the hit compounds from the library screen.

The fluorescence intensity was measured on an Olympus IZ73 fluorescence microscope (ex. 560/14 em. 605/52), by determining the difference in fluorescence intensity between the bead and the surrounding media.

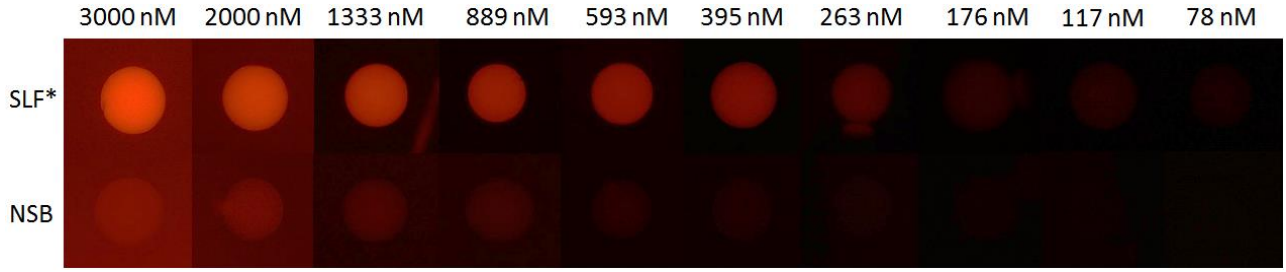
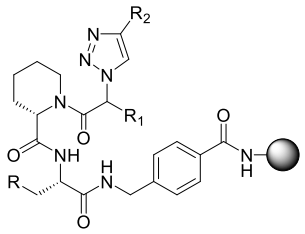
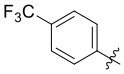
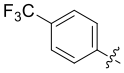
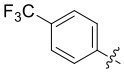
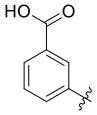
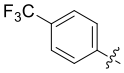
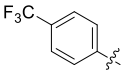
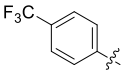
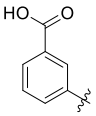
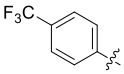
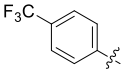
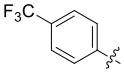
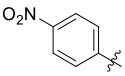
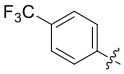
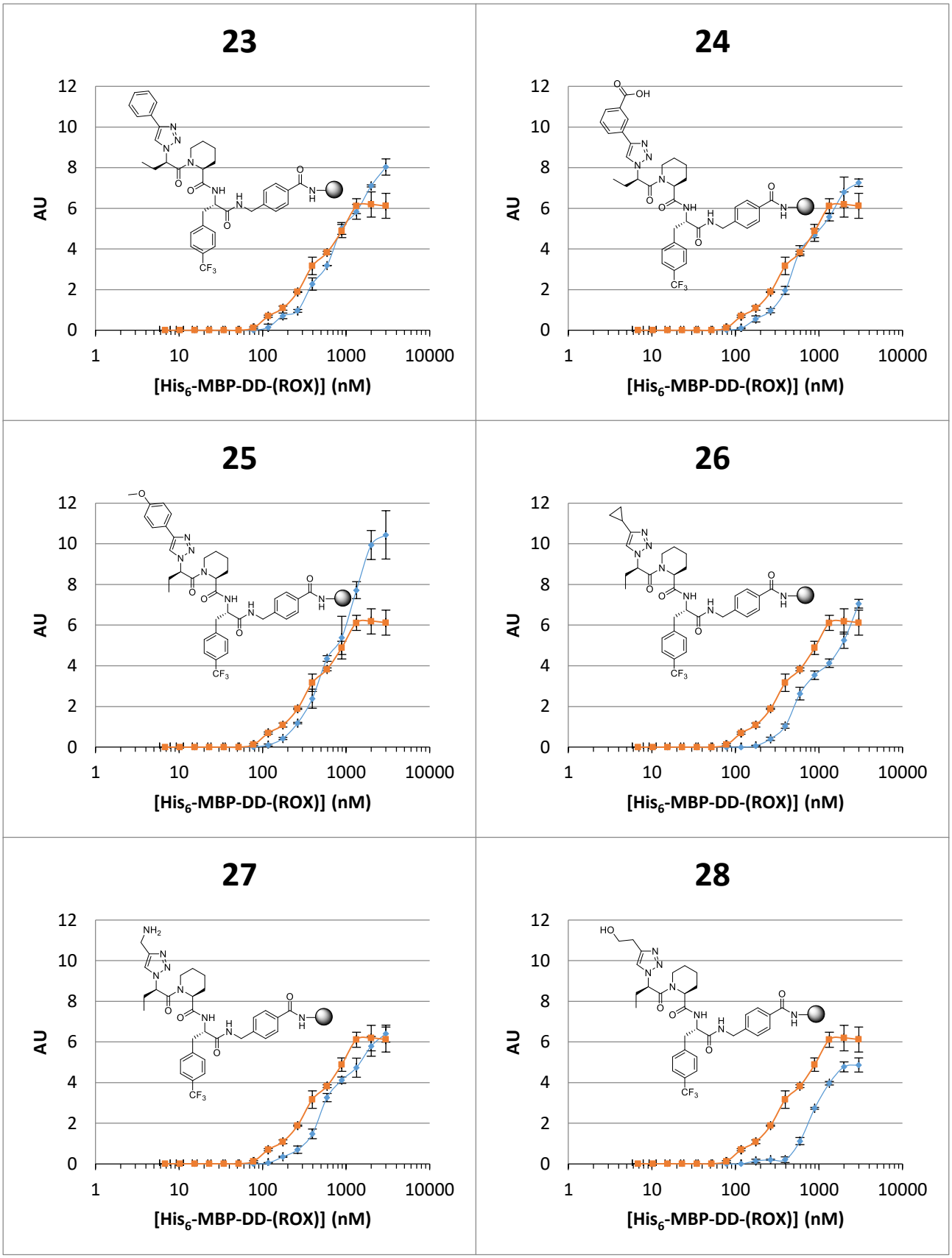


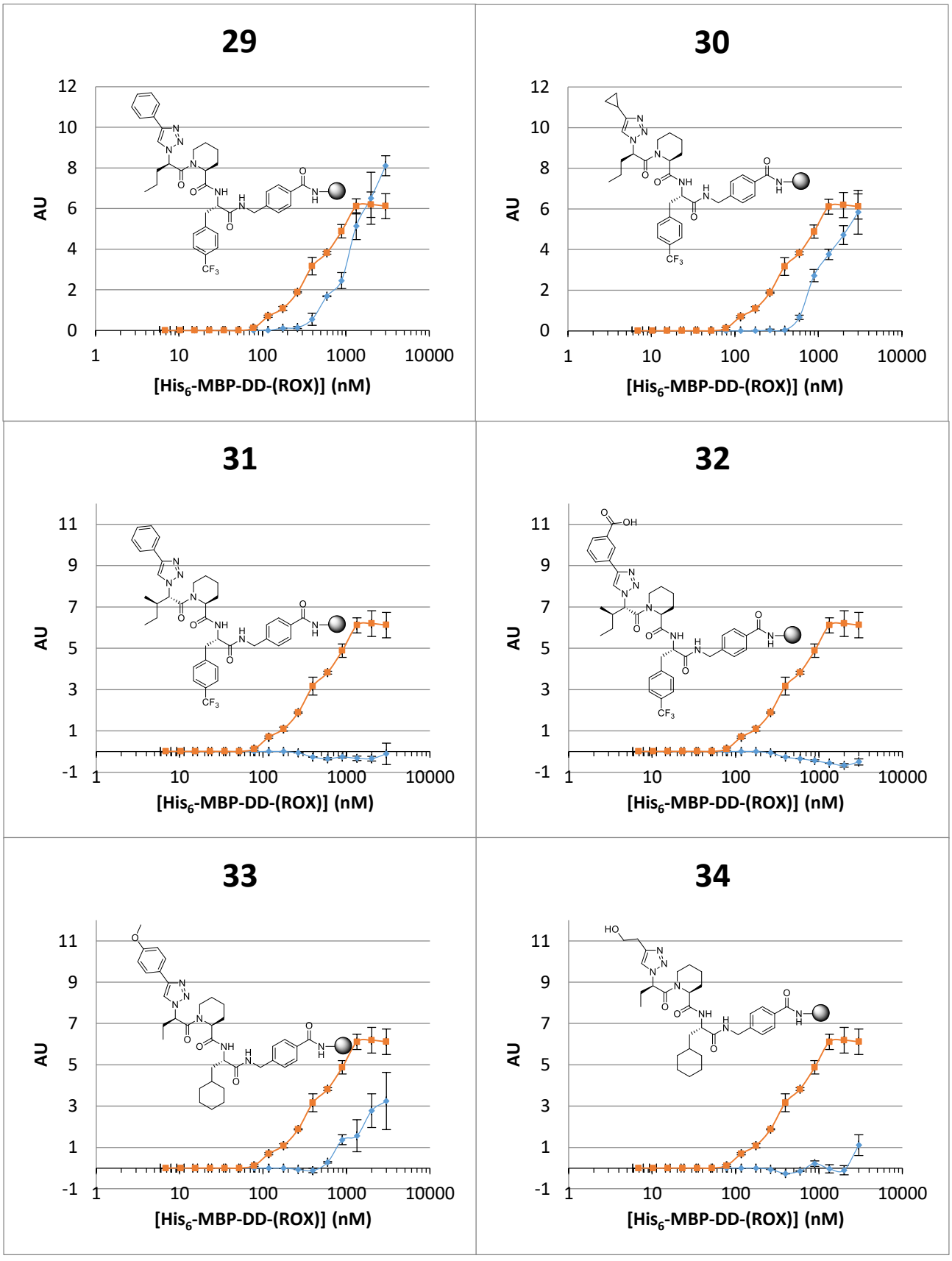
Table S3. Resynthesized compounds



#	Comp.	R	R_1	R_2	Purity[a]	#	Comp.	R	R_1	R_2	Purity[a]
1	**23**				>95%	8	**30**				94%
2	**24**				93%	9	**31**				ND
3	**25**				>95%	10	**32**				89%
4	**26**				88%	11	**33**				94%
5	**27**				>95%	12	**34**				93%
6	**28**				92%	13	**35**				94%
7	**29**				89%	14	**18**				>95%

[a] The purity is measured by HPLC analysis using UV absorption at 254 nm.


23
24
25
26
27
28
AU
0
2
4
6
8
10
12
1
10
100
1000
10000
[His6-MBP-DD-(ROX)] (nM)


29
30
31
32
33
34
AU
[His6-MBP-DD-(ROX)] (nM)
1
10
100
1000
10000

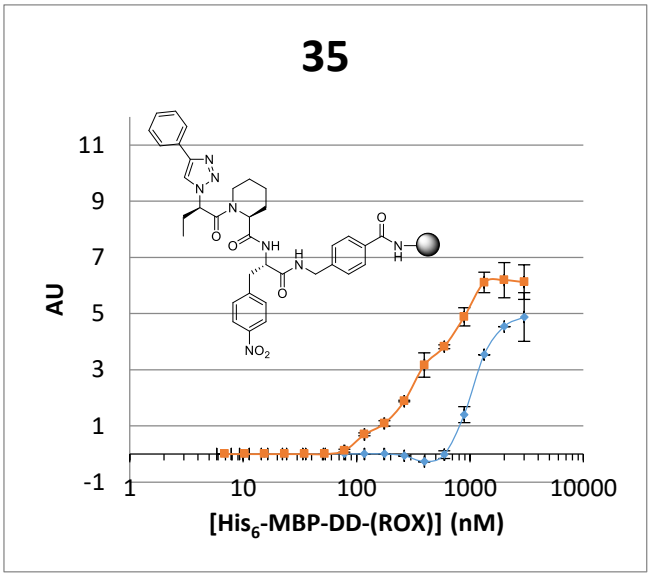
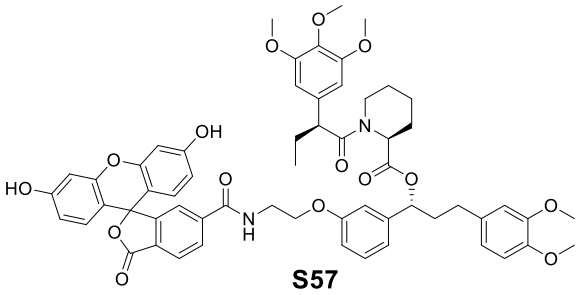


Figure S2 Binding curves (blue) obtained from resynthesized peptide mimetics on solid support. The binding curve of the solid-supported SLF* (**18** orange) is represented in each graph as a comparison to the PM. The binding study was carried out on a $PEGA_{1900}$ resin (125-200 µm) and the fluorescence intensity was measured under a fluorescence microscope after incubation with the His_6-MBP-DD-(ROX) for 16 hours. The assay was carried out in a 96-well plate format with three beads per well. The fluorescence intensity of a NSB-peptide (Ac-Ala-Phe-Gly) was subtracted from the observed fluorescence intensity of the peptide mimetics.

Fluorescence polarization competitive binding assay



The fluorescence polarization assay was prepared as previously described.[15] A 1:1 serial dilution of the competitive inhibitor was performed in DMSO at 200 times the final concentration. The fluorescent probe (**S57**) was diluted from a DMSO stock in a HEPES buffer (20 mM HEPES, 0.01% Triton-X, pH 8, final DMSO conc. 11%) to give a final concentration of 80 nM. Each sample in the competitive inhibitor dilution series was in a 1:10 ratio combined with the solution of the fluorescent probe and the combined mixture was further diluted by a factor of 10 to give two times the times the final concentration of **S57** and competitive inhibitor. Equal volumes of ligand mixture and a MBP-DD solution in HEPES buffer (30µL, 34 nM) were combined. The samples were then transferred to a black, flat bottom 384-well NBS microplates (No.: 3820, Corning Life Science) and the fluorescence anisotropy was after 30 min of incubation measured on a Safire2 plate-reader (Tecan, Mannedorf, Switzerland). Subsequently, the data was processed to determine the fraction of bound probe (F_{SB}) using equation SE1, with A_{OBS} being the observed anisotropy, and A_F and A_B is the anisotropies of the free and bound probe respectively.

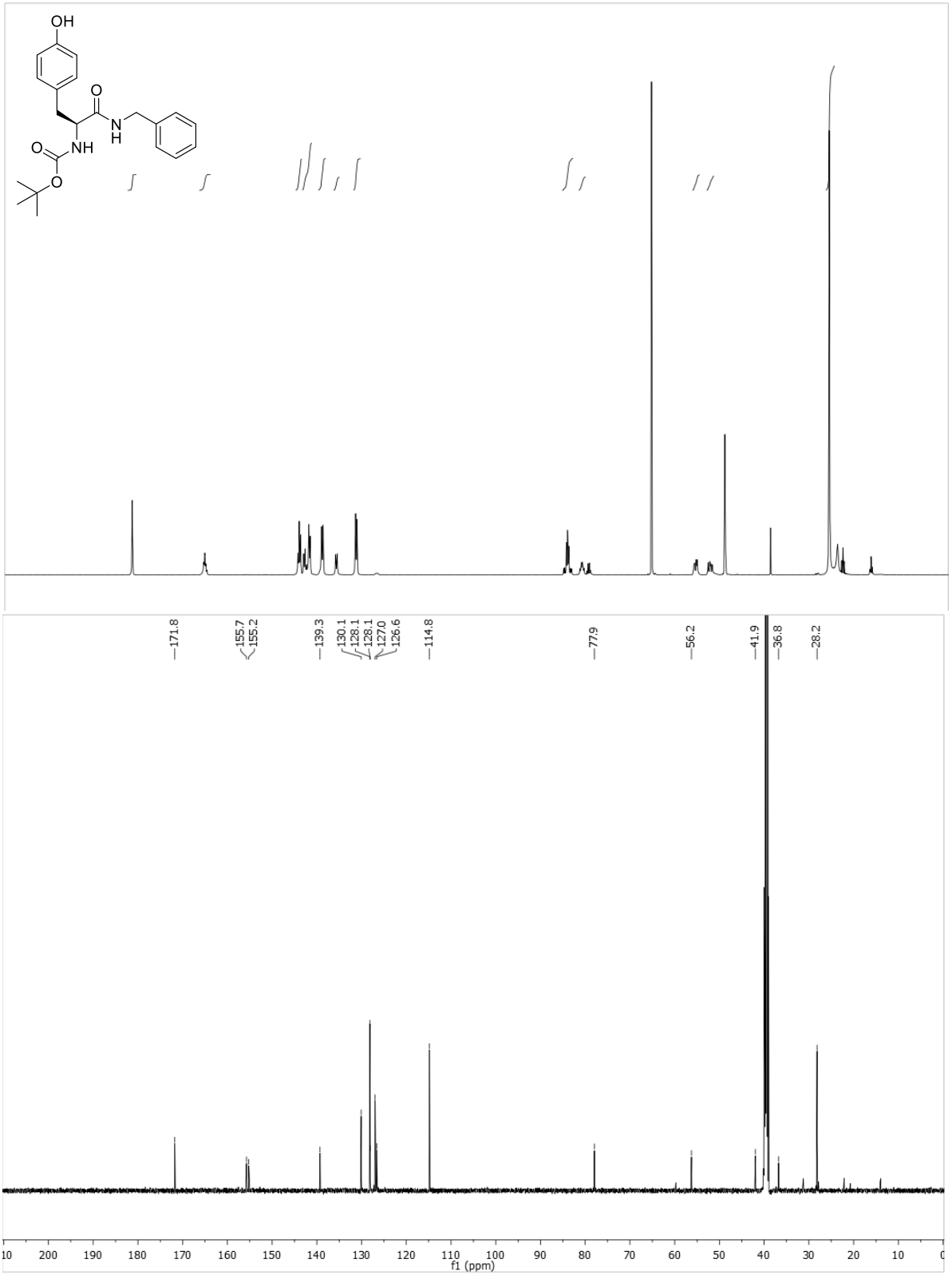
$$F_{SB} = \frac{A_{OBS} - A_F}{(A_B - A_{OBS}) + A_{OBS} - A_F} \quad \text{(SE1)}$$

The competitive binding curves were analyzed using Prism 7.0 (GraphPad, La Jolla, CA, USA) and the data were fitted to a four parameter logistic curve to deduce the IC_{50}. The error bars represent the standard deviation of experiments performed in triplicates. The K_i values were determined using equation SE2, with f_0 being the fraction of bound probe over the fully bound species.

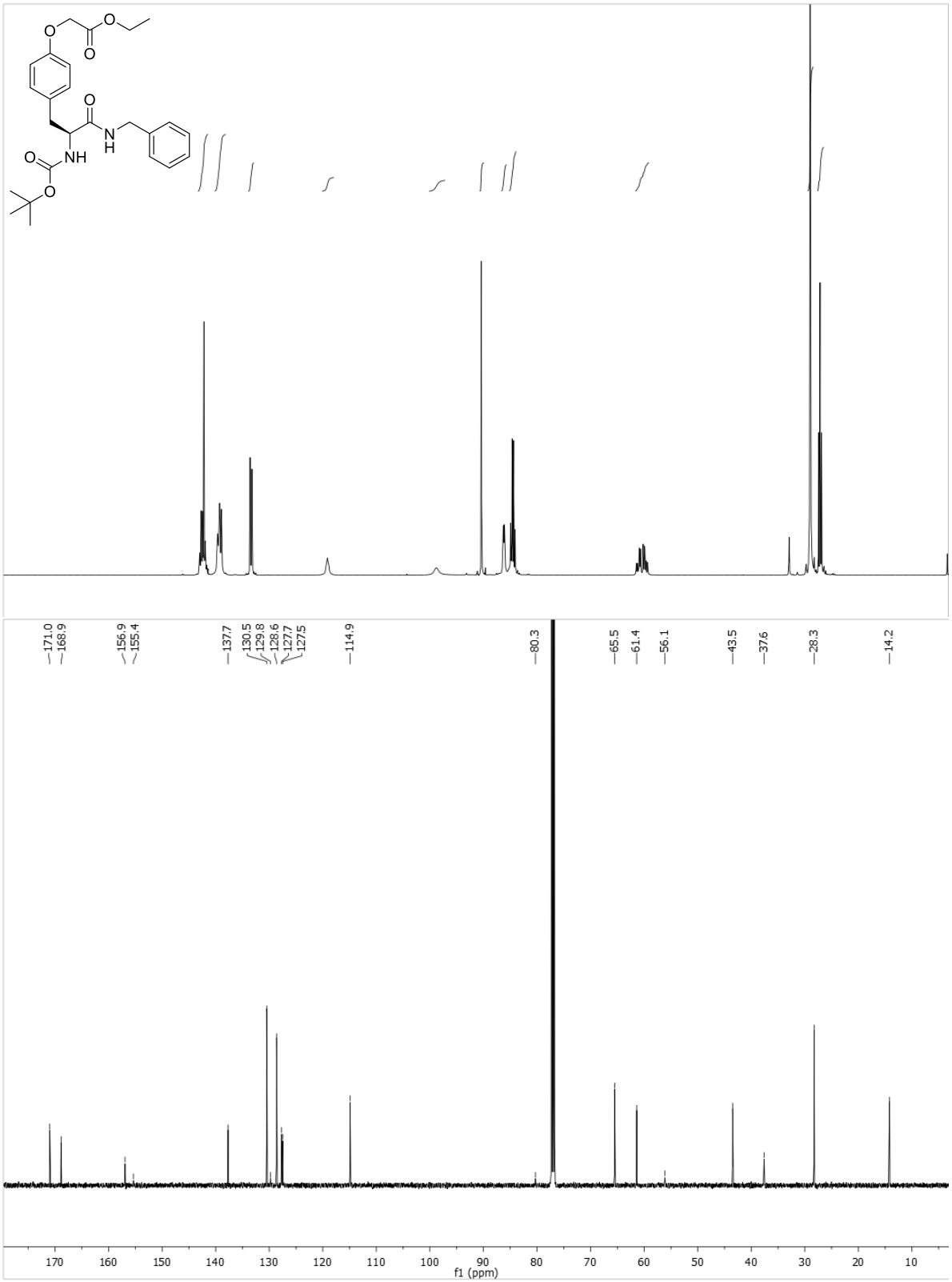
$$K_i = \frac{IC_{50}}{1 + \frac{[L_T](f_0+2)}{2K_d(f_0+1)} + f_o} + K_d \frac{f_0}{f_0+2} \quad \text{(SE2)}$$

^{1}H and ^{13}C-NMR

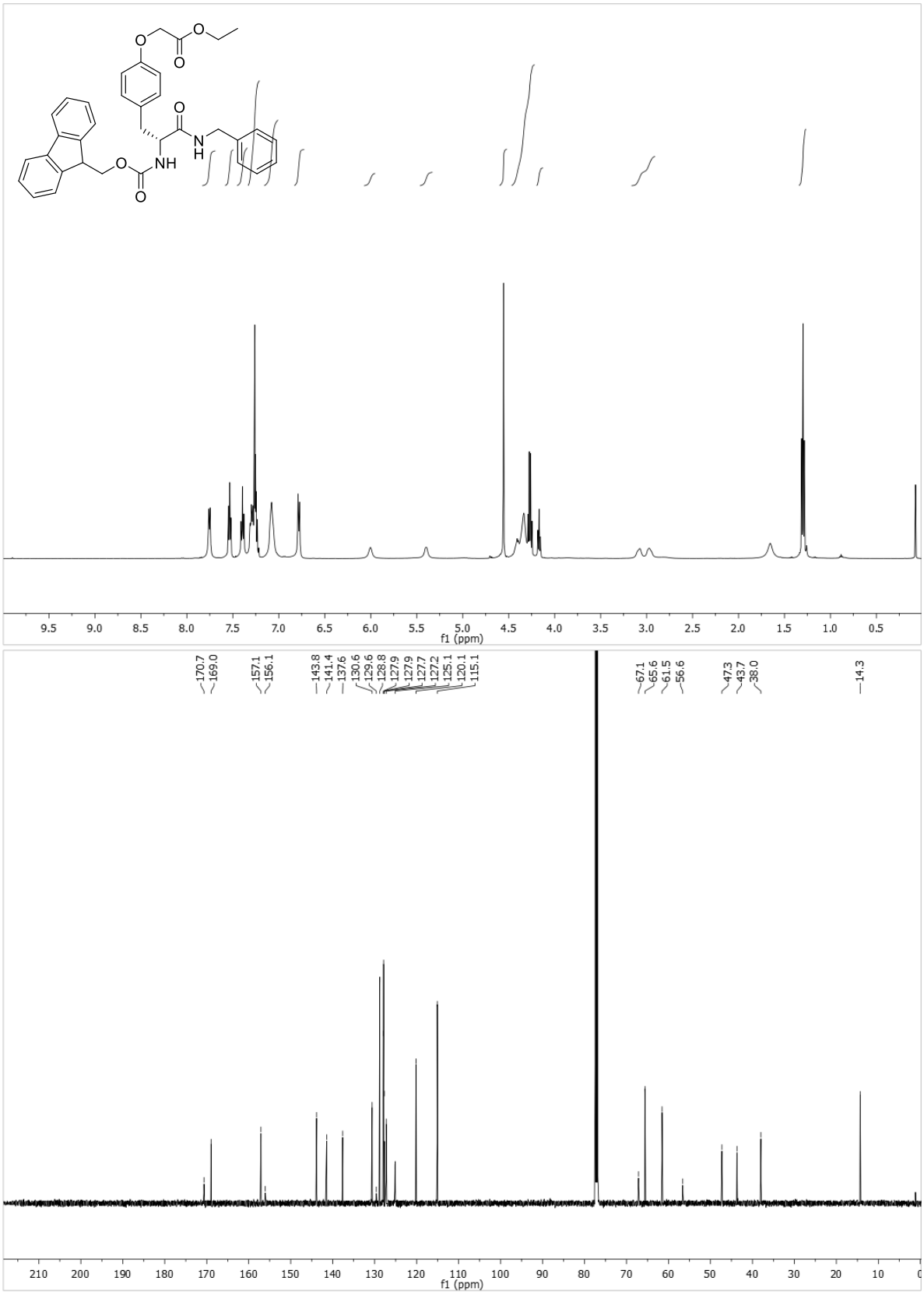
***Tert*-butyl (*S*)-(1-(benzylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (S1)**



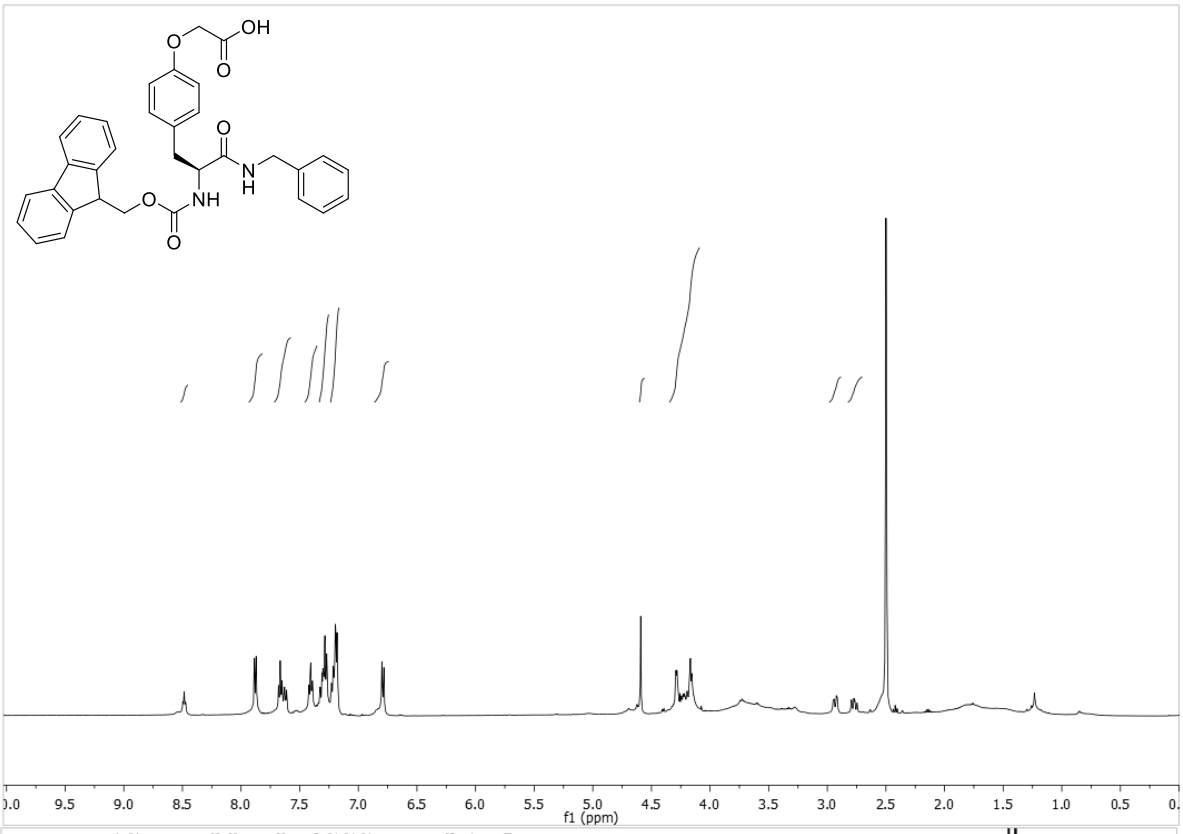
Ethyl (*S*)-2-(4-(3-(benzylamino)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)acetate (10)

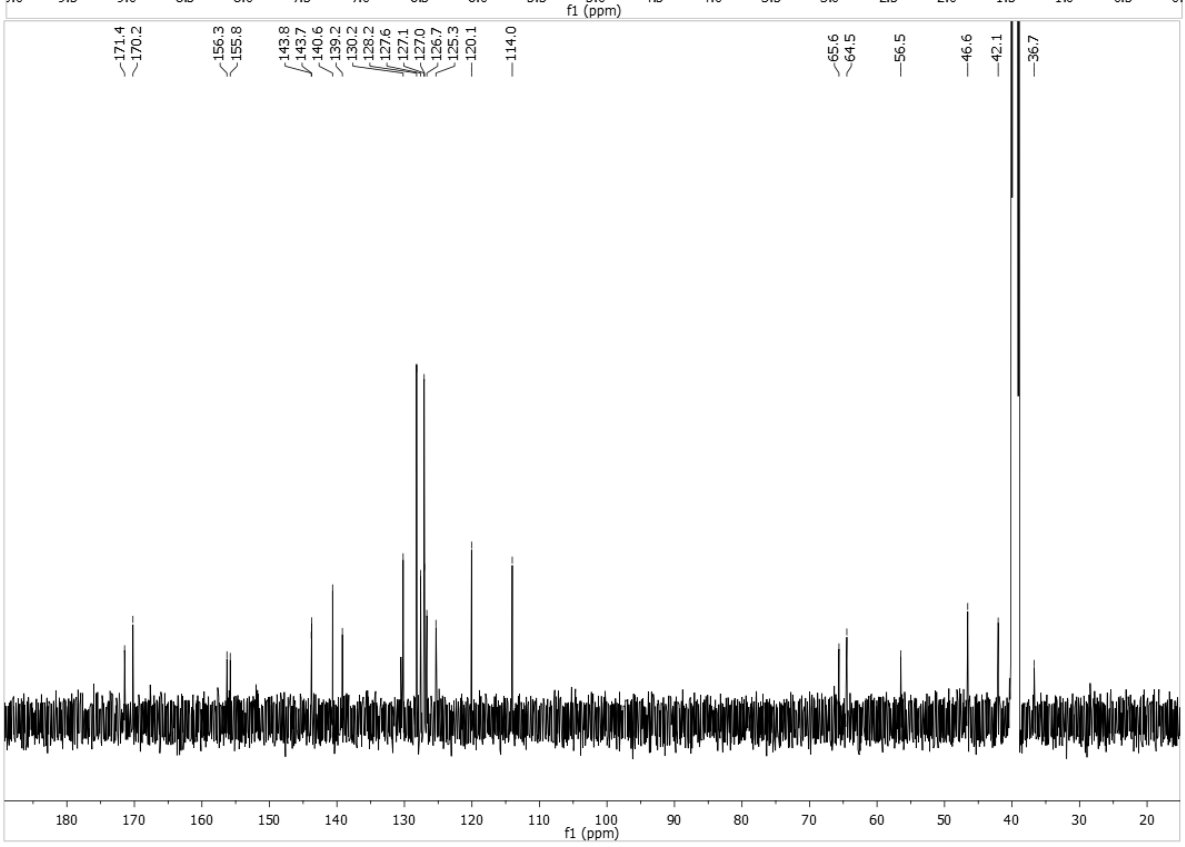


Ethyl (*S*)-2-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(benzylamino)-3-oxopropyl)phenoxy)acetate (S3)

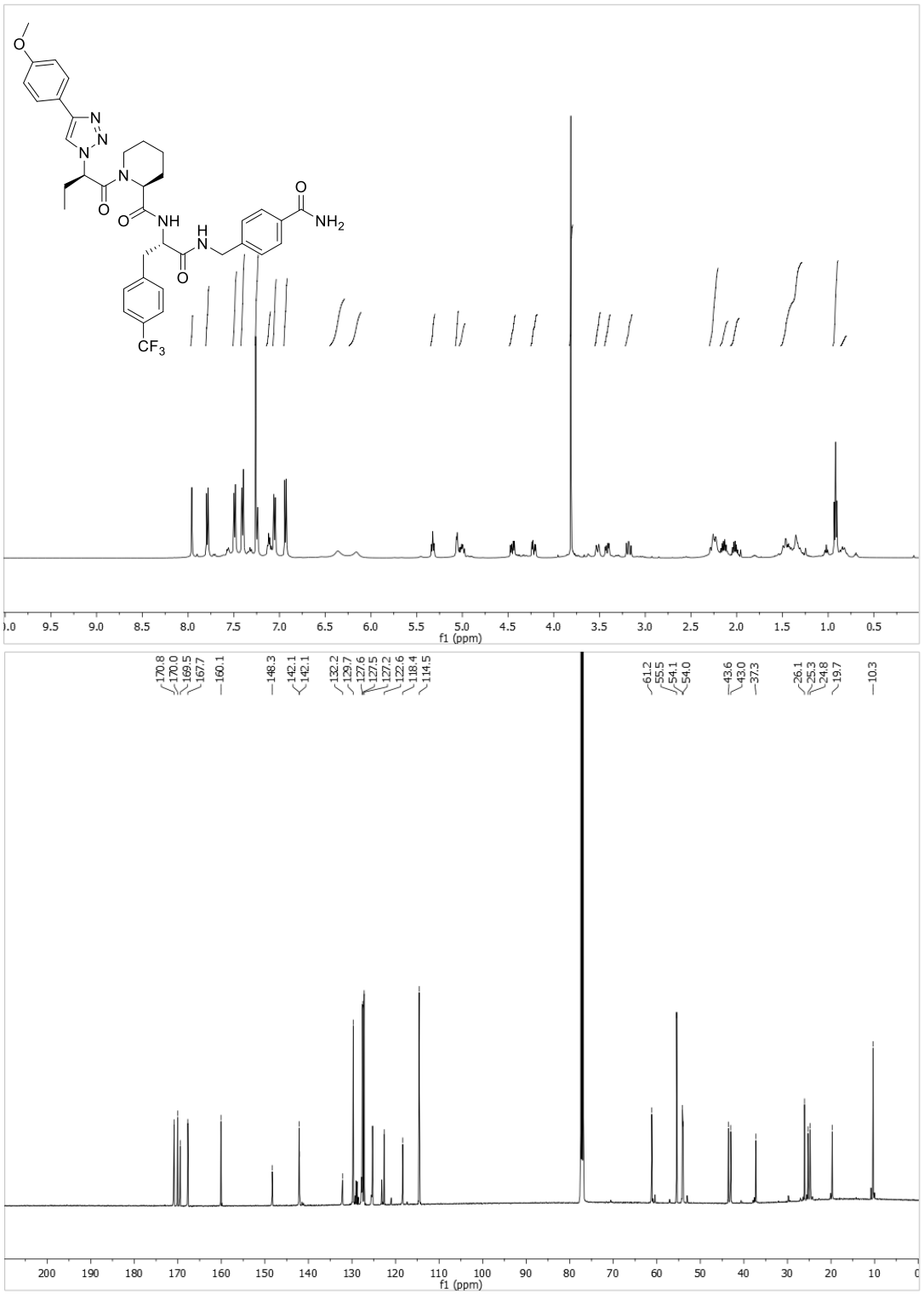


(*S*)-2-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(benzylamino)-3-oxopropyl)phenoxy)acetic acid (11)

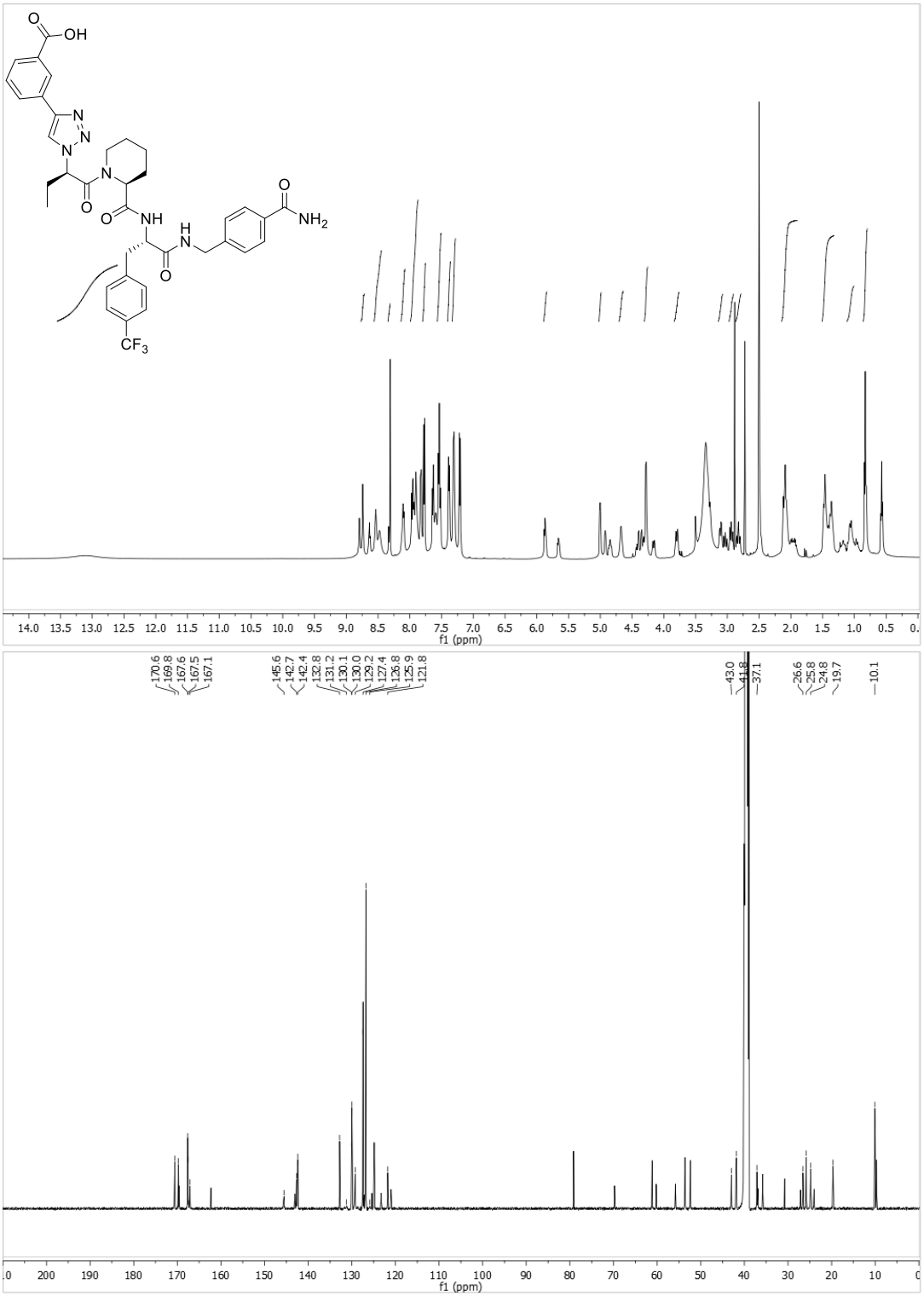




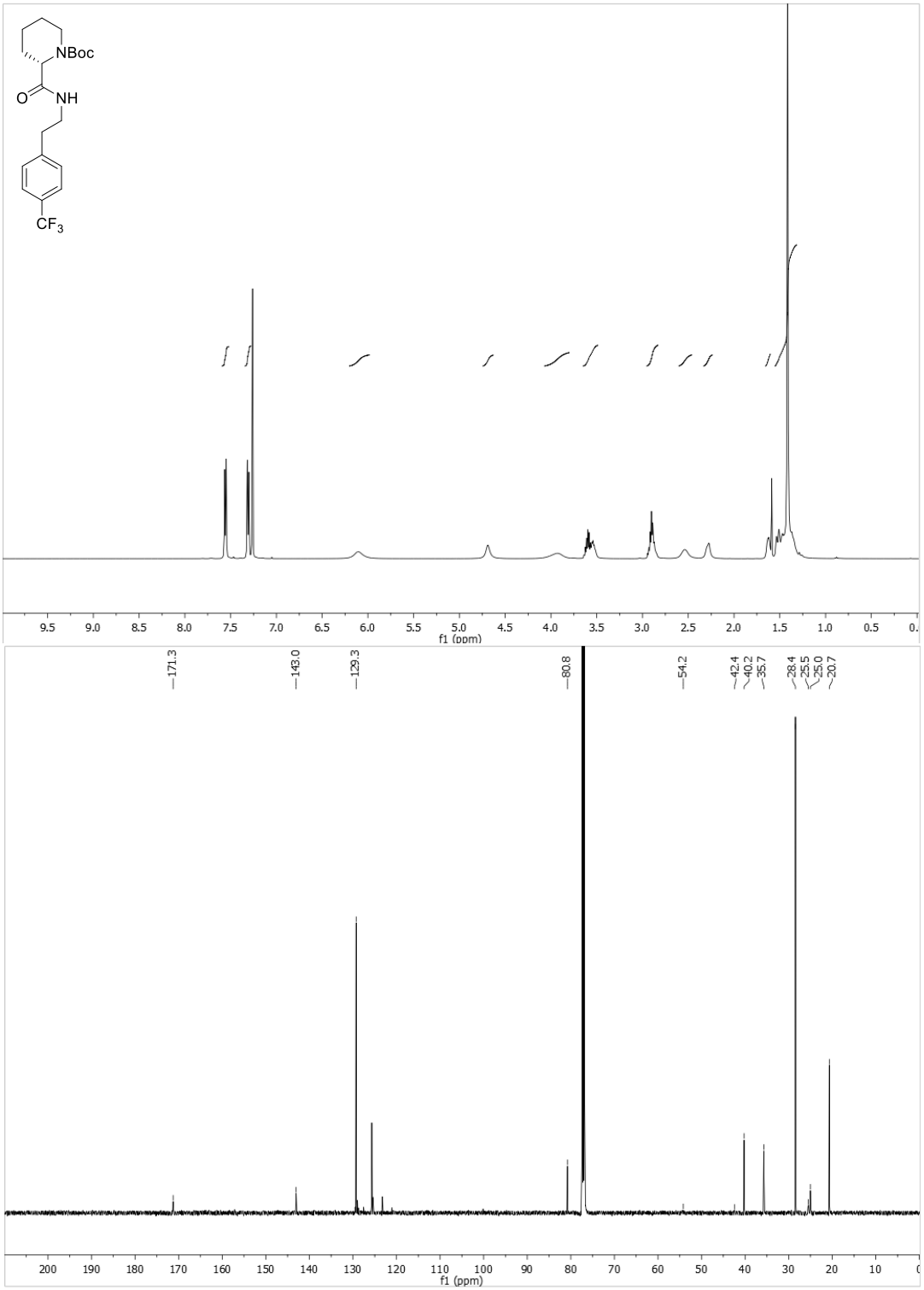
(*S*)-*N*-((*S*)-1-((4-carbamoylbenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**36**)



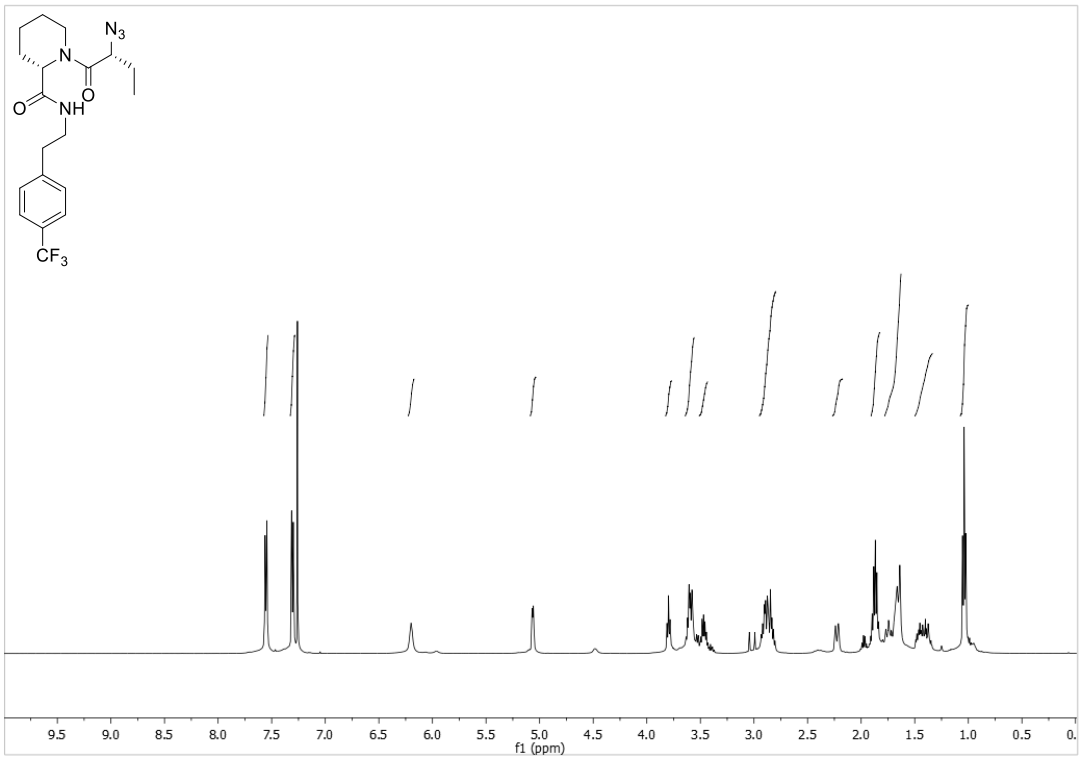
3-(1-((*R*)-1-((*S*)-2-(((*S*)-1-((4-carbamoylbenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**37**)

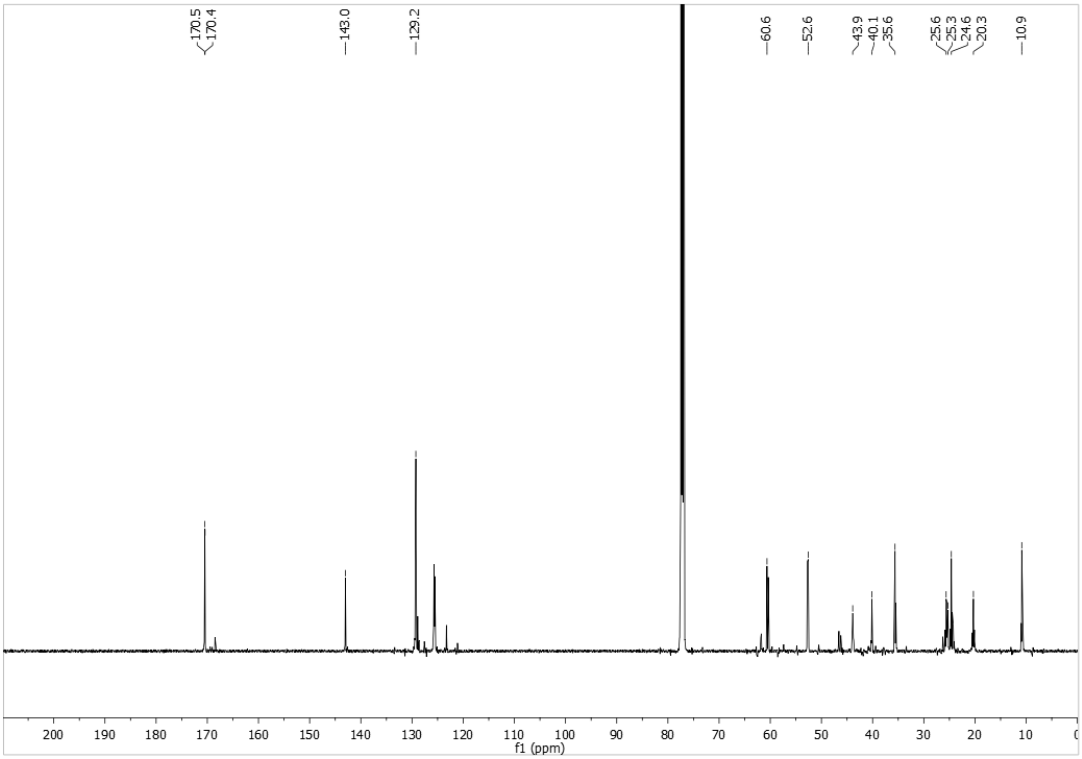


***tert*-Butyl (*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidine-1-carboxylate (S20)**

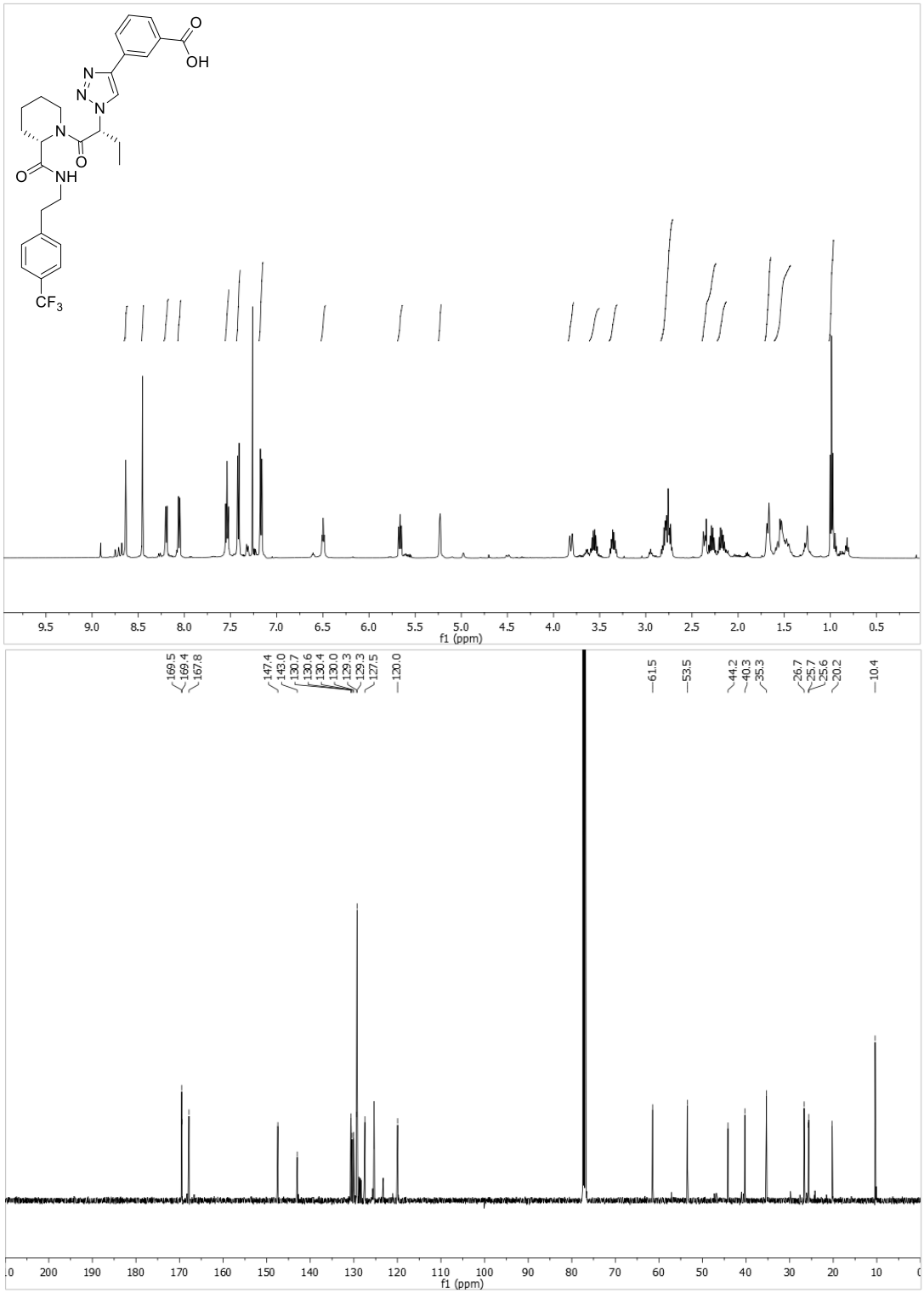


(*S*)-1-((*R*)-2-Azidobutanoyl)-*N*-(4-(trifluoromethyl)phenethyl)piperidine-2-carboxamide (S26)

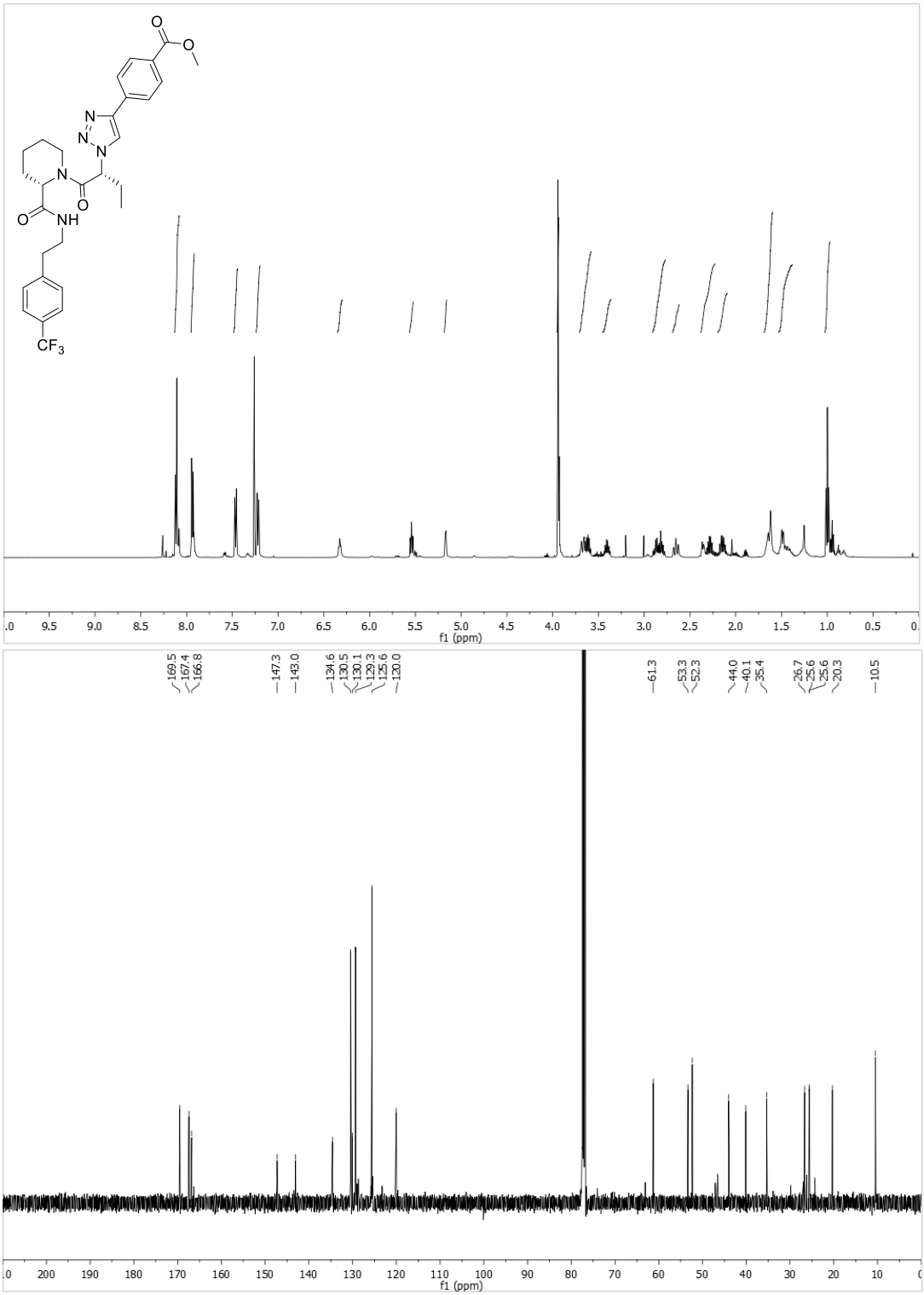




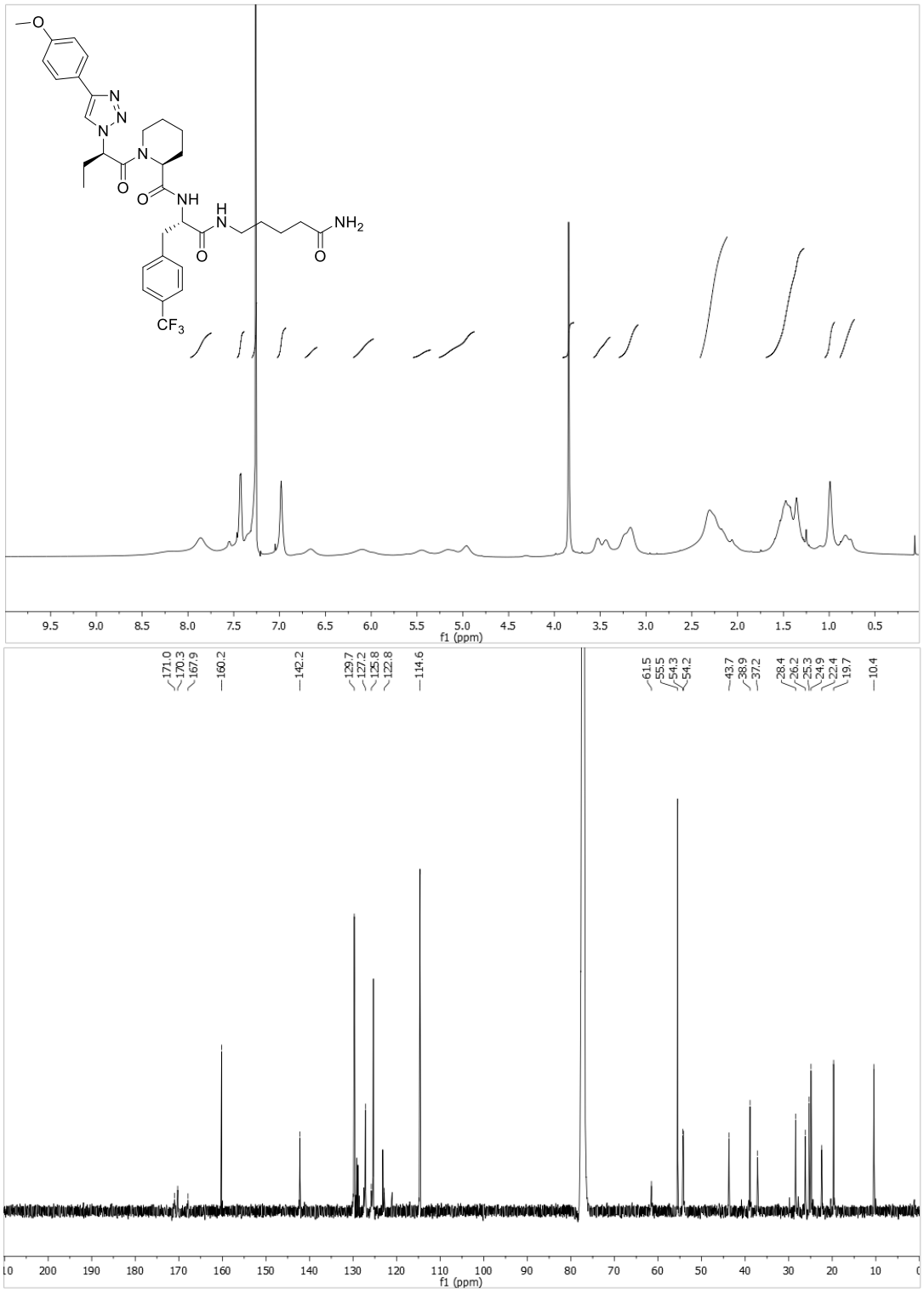
3-(1-((*R*)-1-oxo-1-((*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidin-1-yl)butan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**38**)



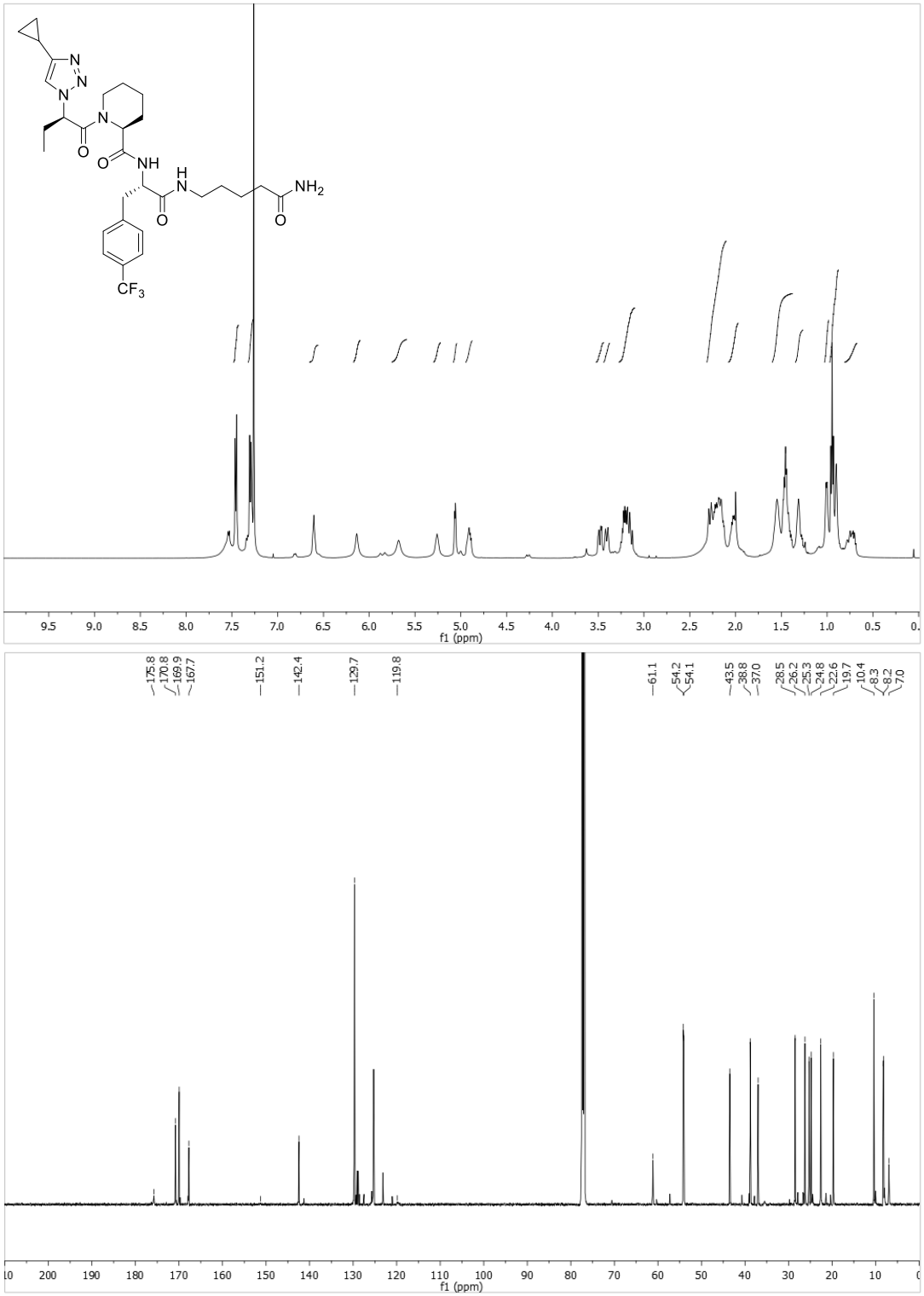
Methyl 4-(1-((*R*)-1-oxo-1-((*S*)-2-((4-(trifluoromethyl)phenethyl)carbamoyl)piperidin-1-yl)butan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoate (**39**)



(*S*)-*N*-((*S*)-1-((5-amino-5-oxopentyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**40**)

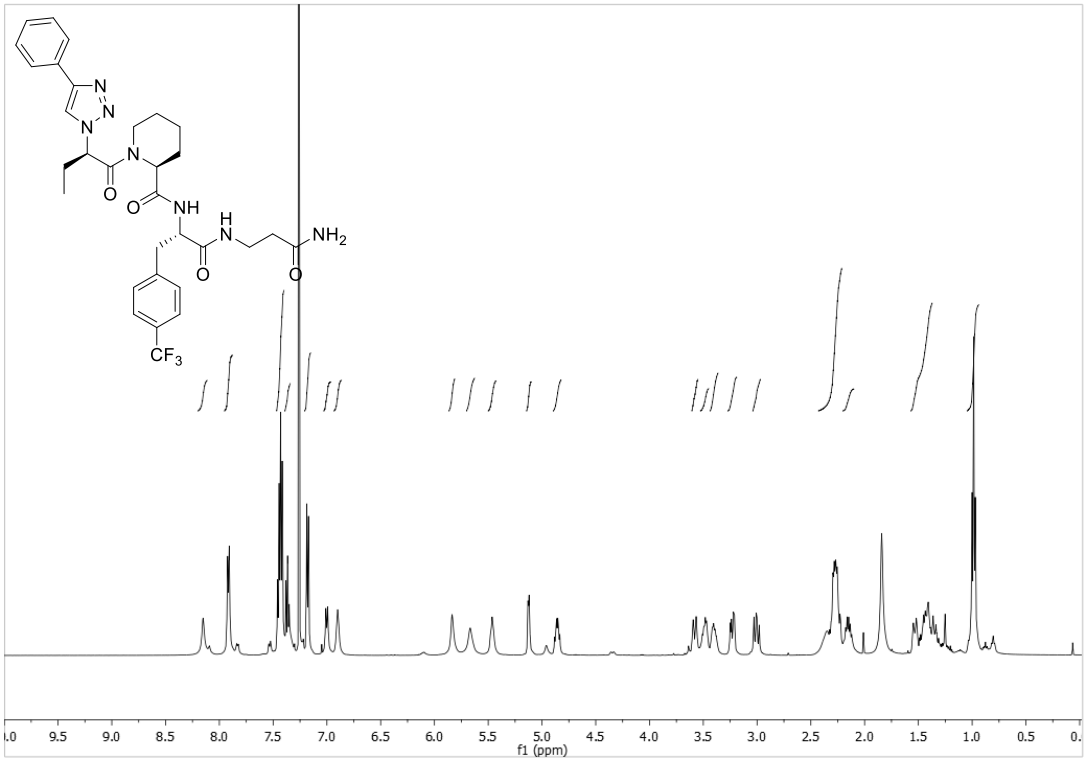


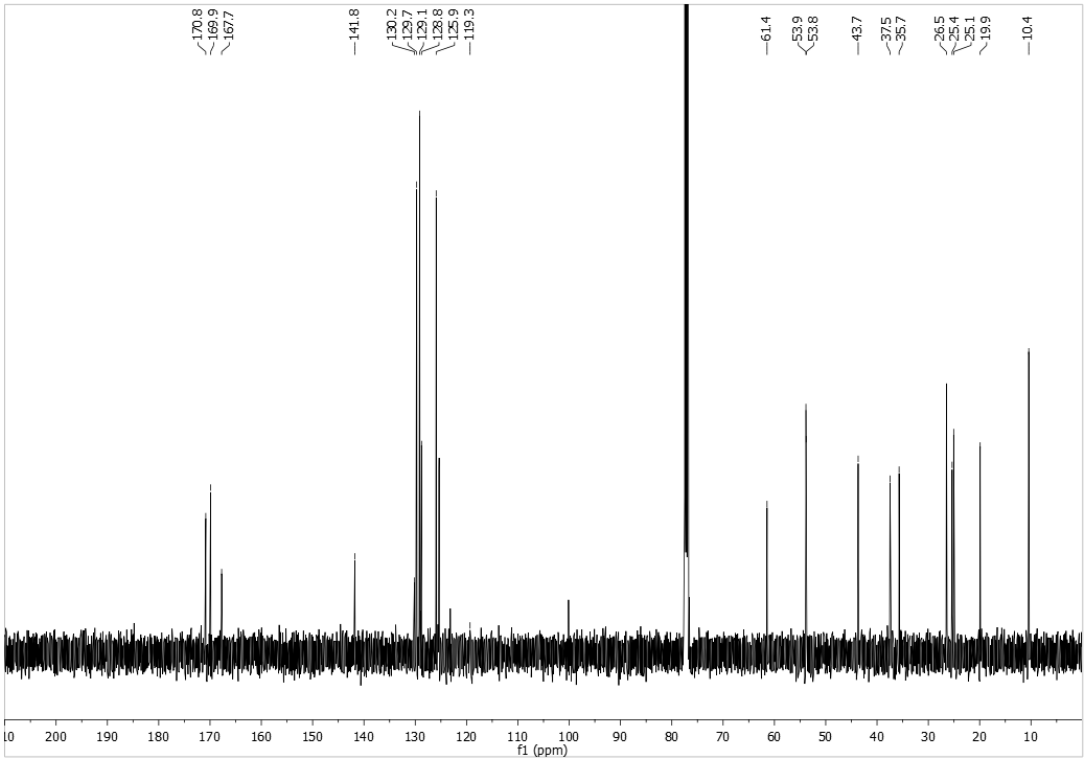
(*S*)-*N*-((*S*)-1-((5-amino-5-oxopentyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (41)



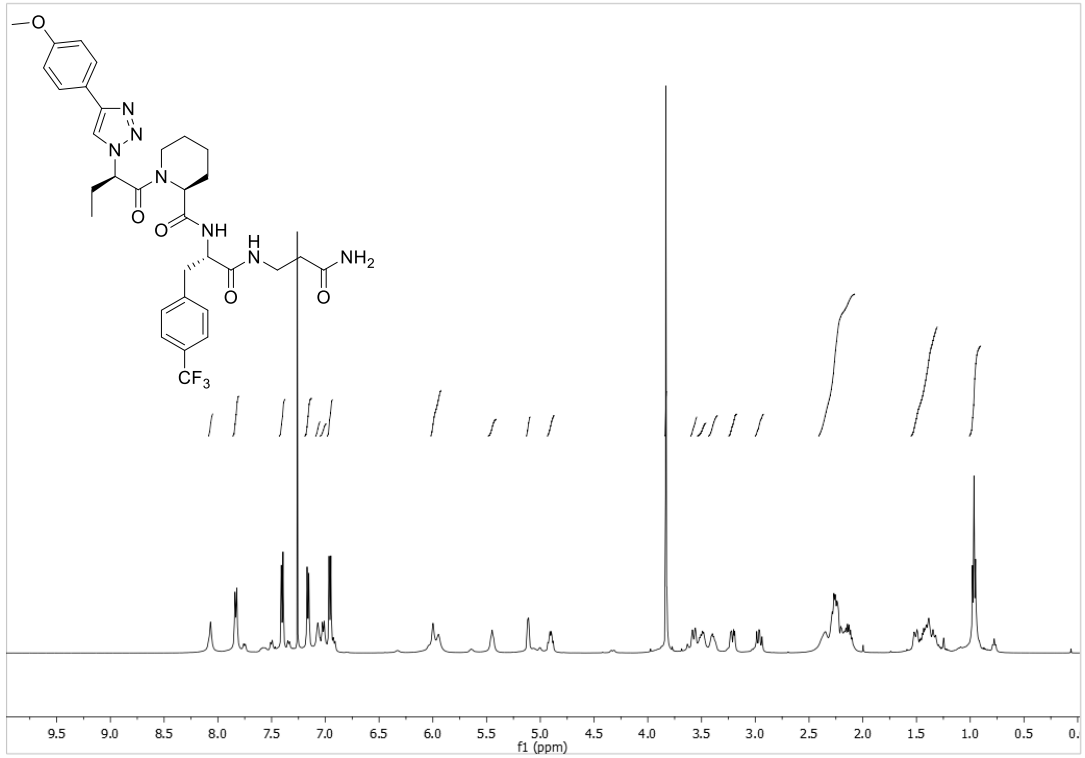
(*S*)-*N*-((*S*)-1-((3-Amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**42**)

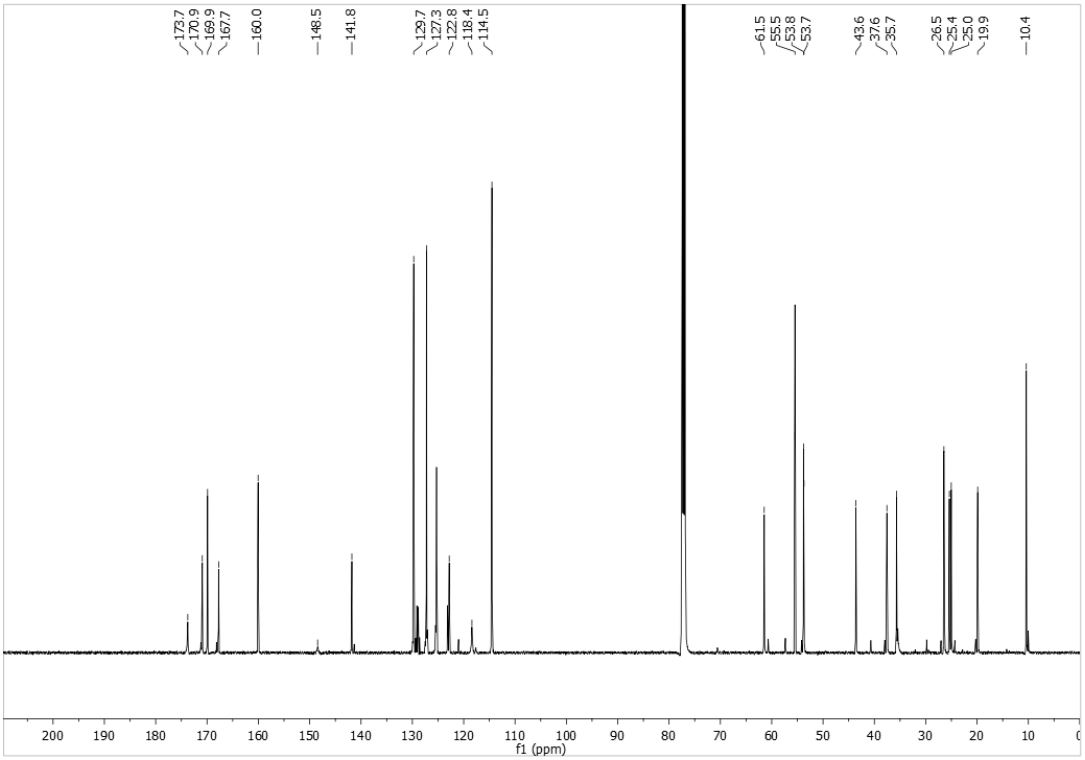
.



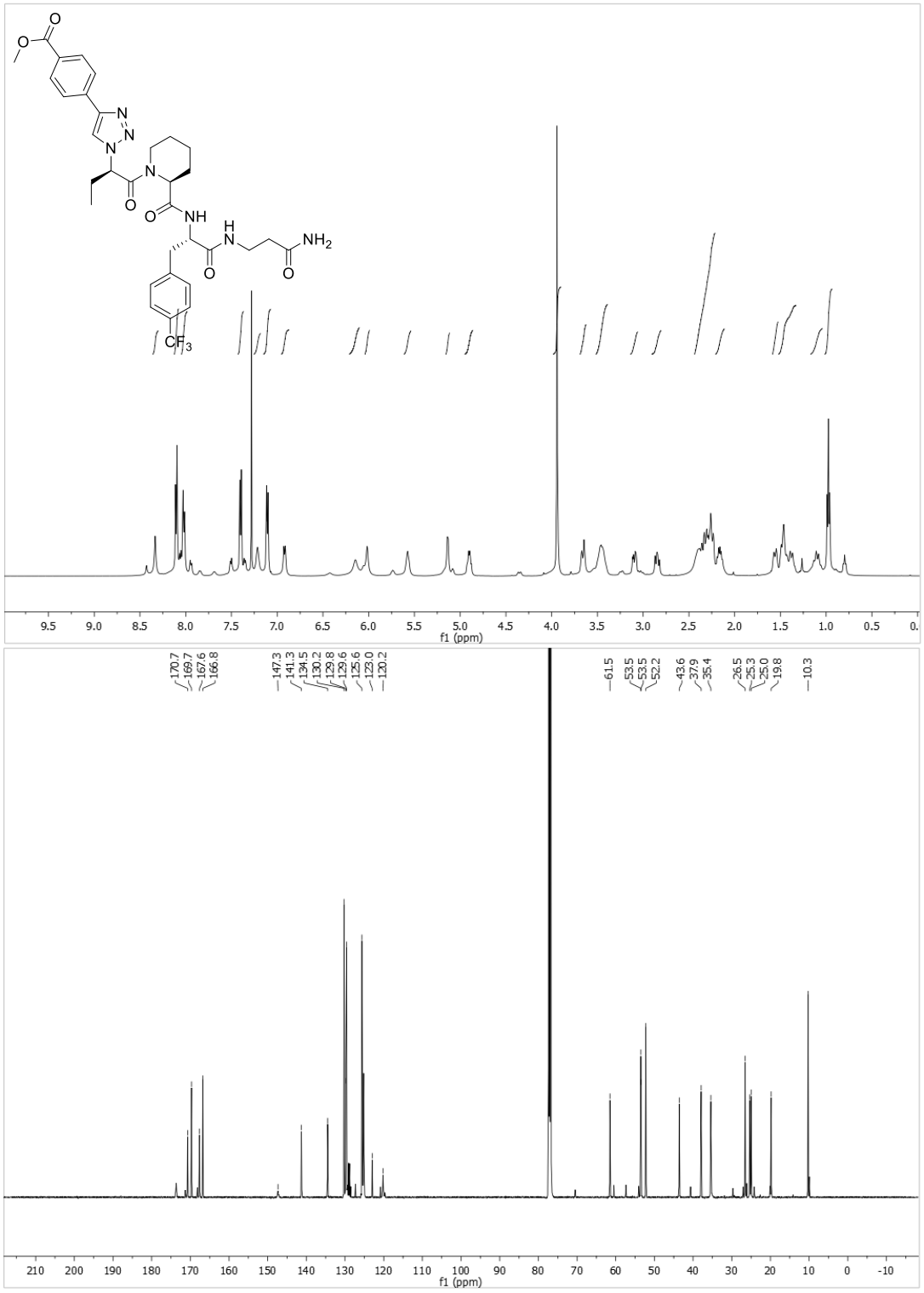


(*S*)-*N*-((*S*)-1-((3-amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (43)

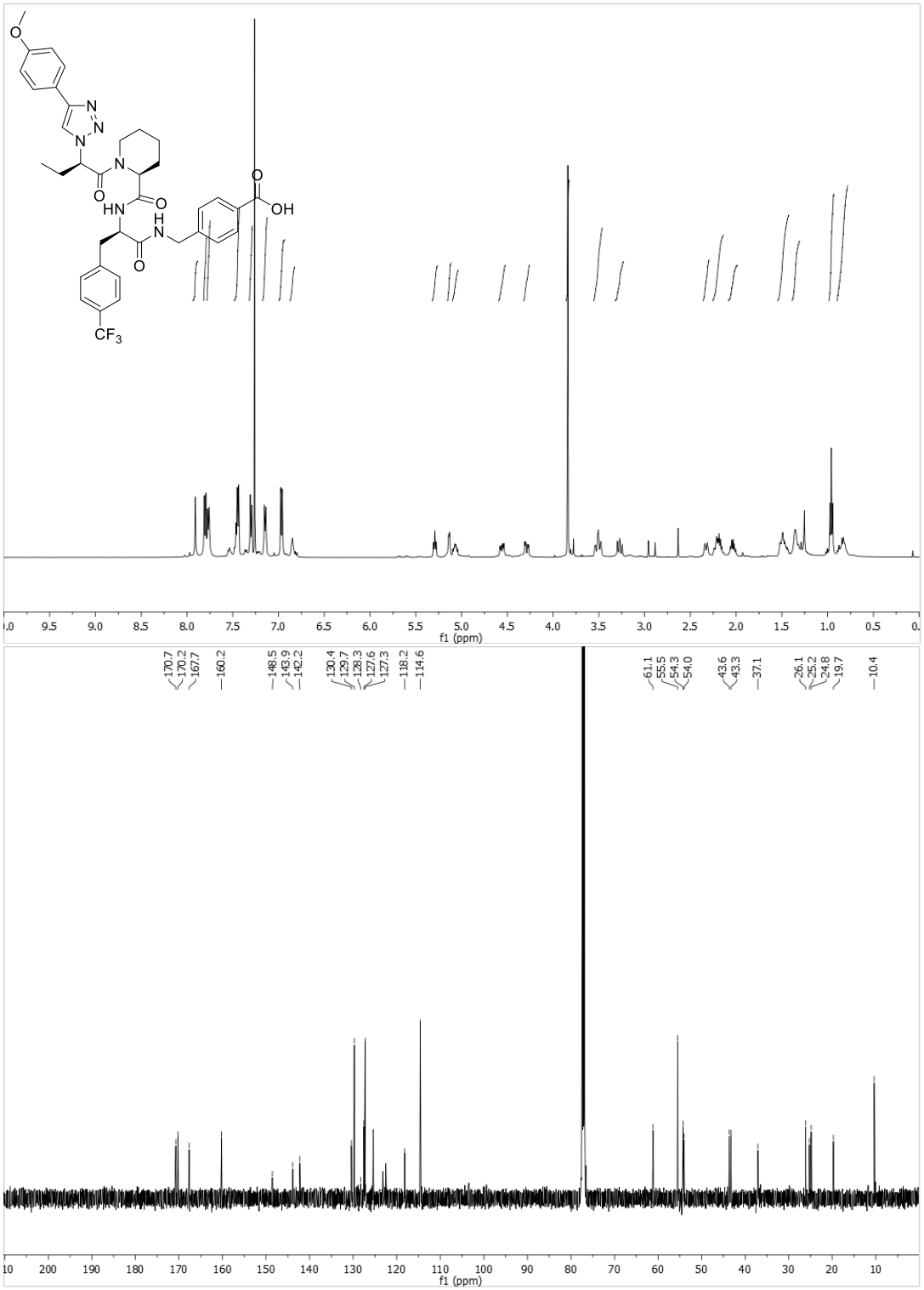




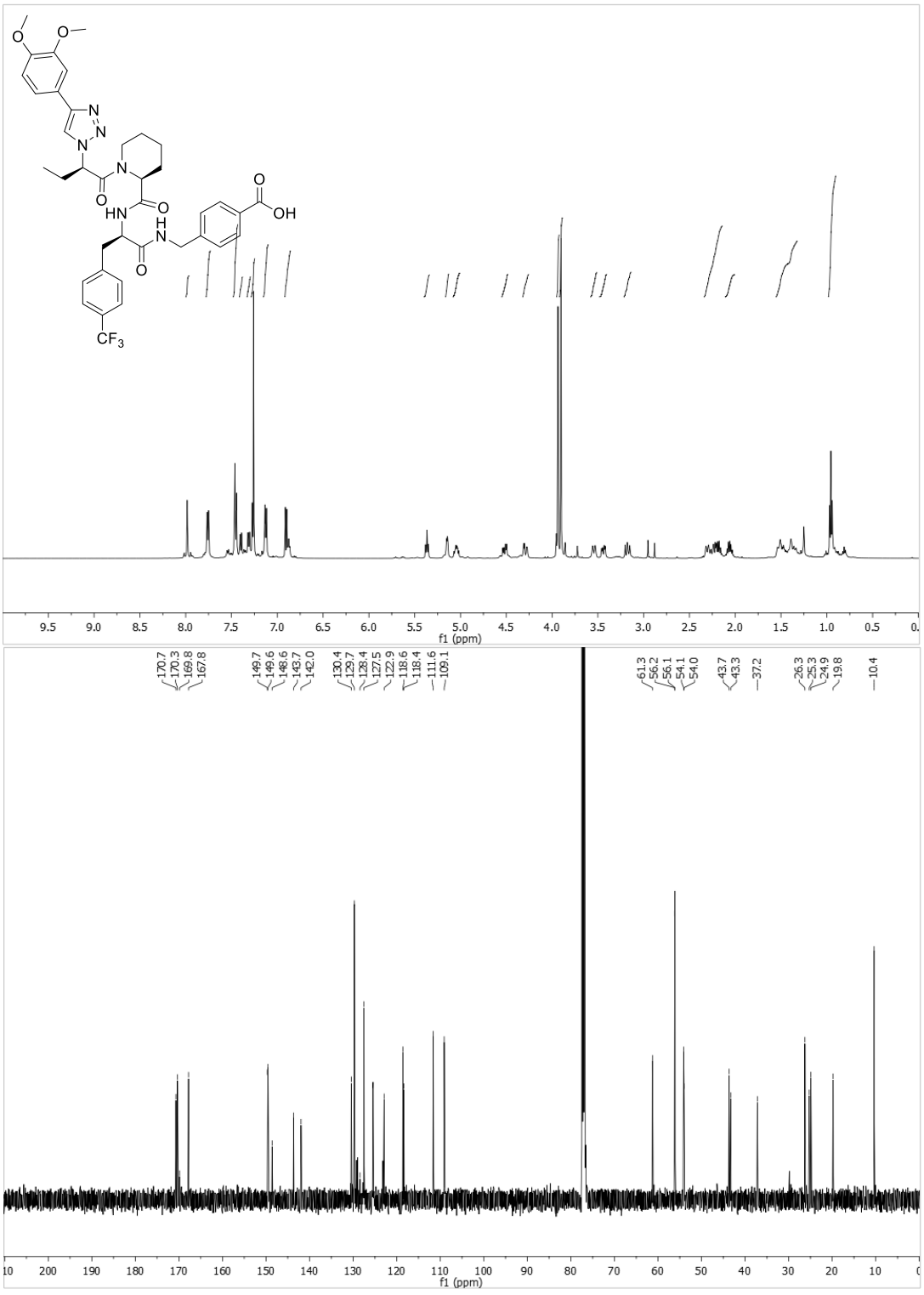
Methyl 4-(1-((*R*)-1-((*S*)-2-(((*S*)-1-((3-amino-3-oxopropyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoate (**44**)



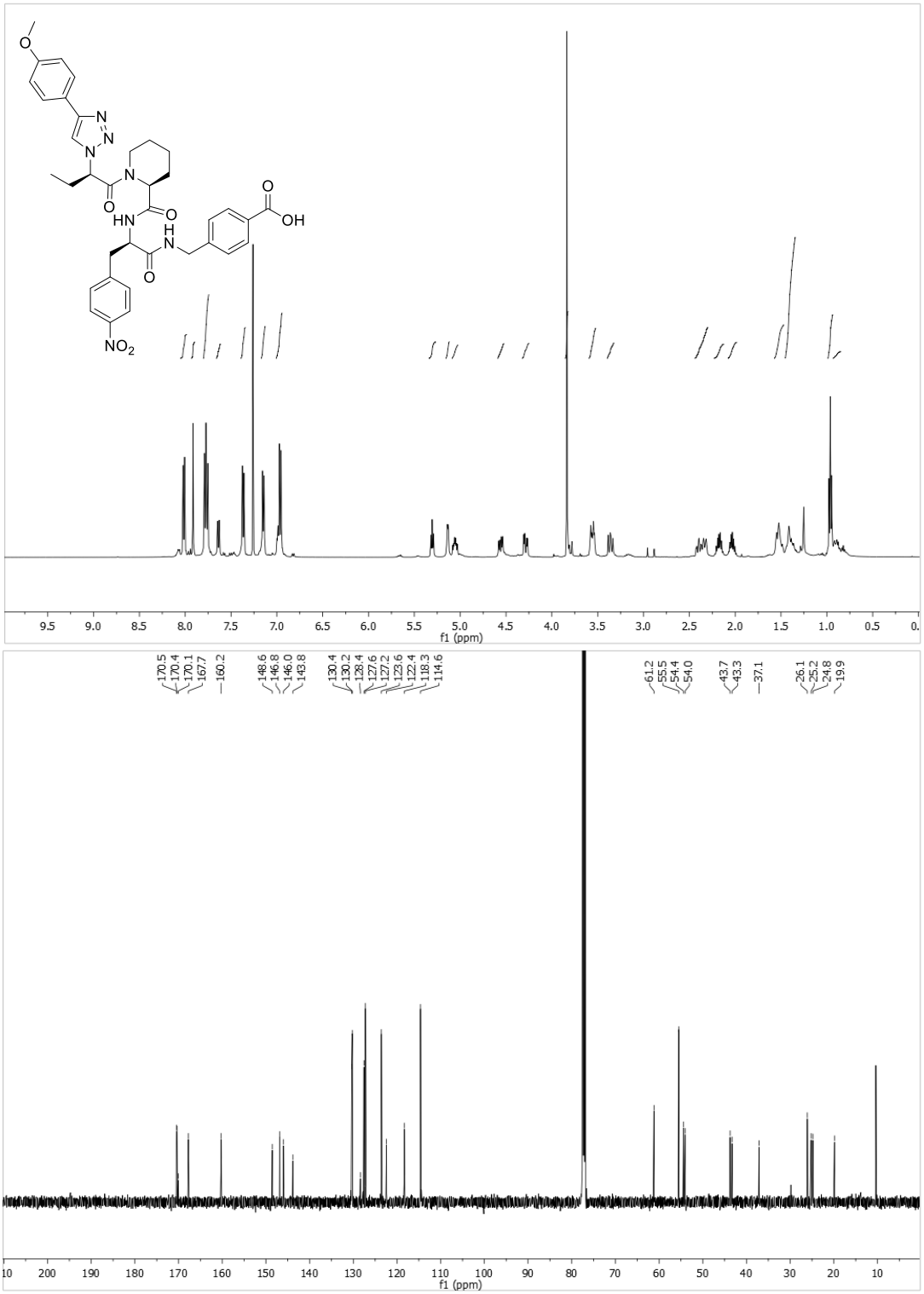
4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanamido)methyl)benzoic acid (45)



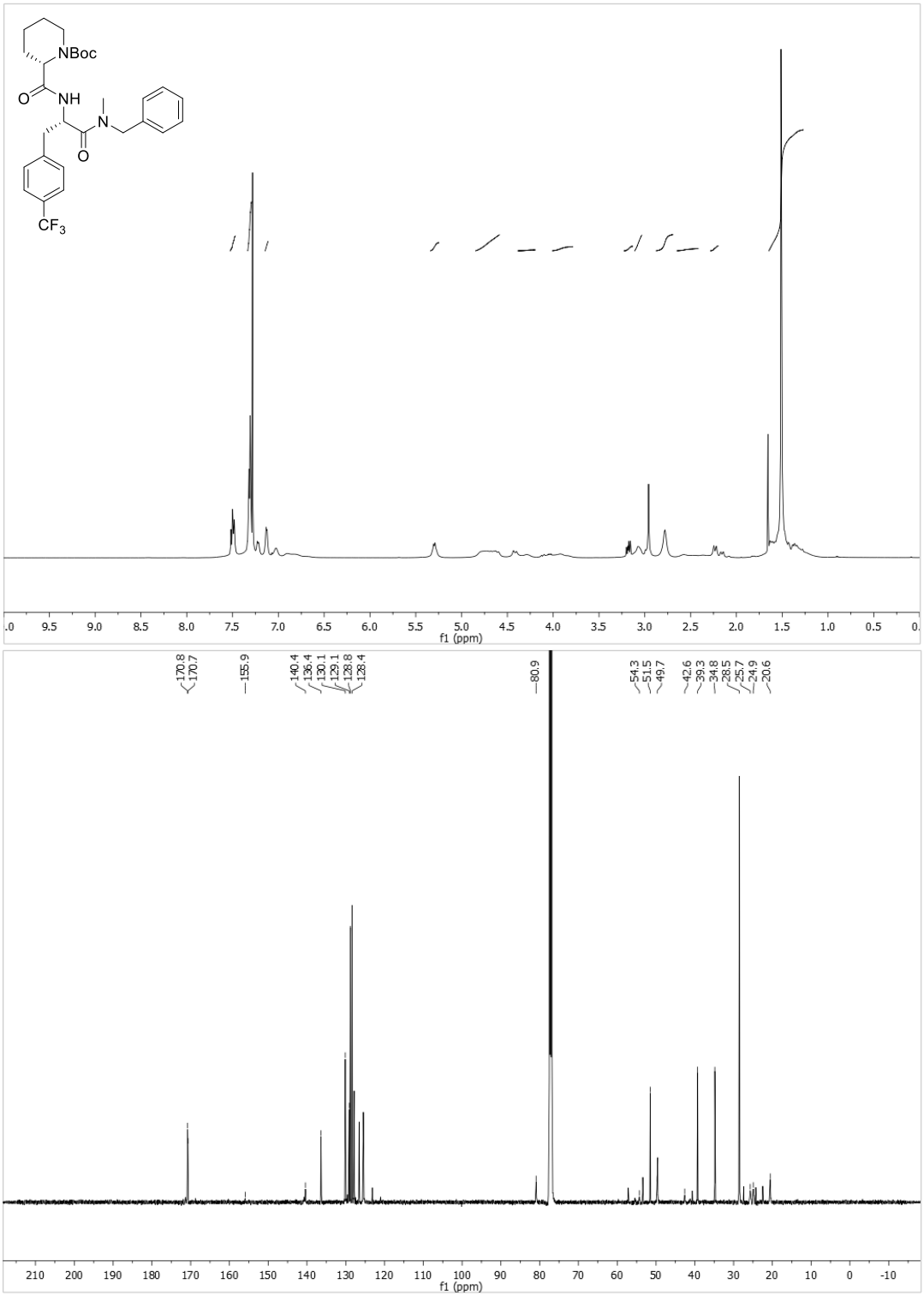
4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-(trifluoromethyl)phenyl)propanamido)methyl)benzoic acid (**46**)



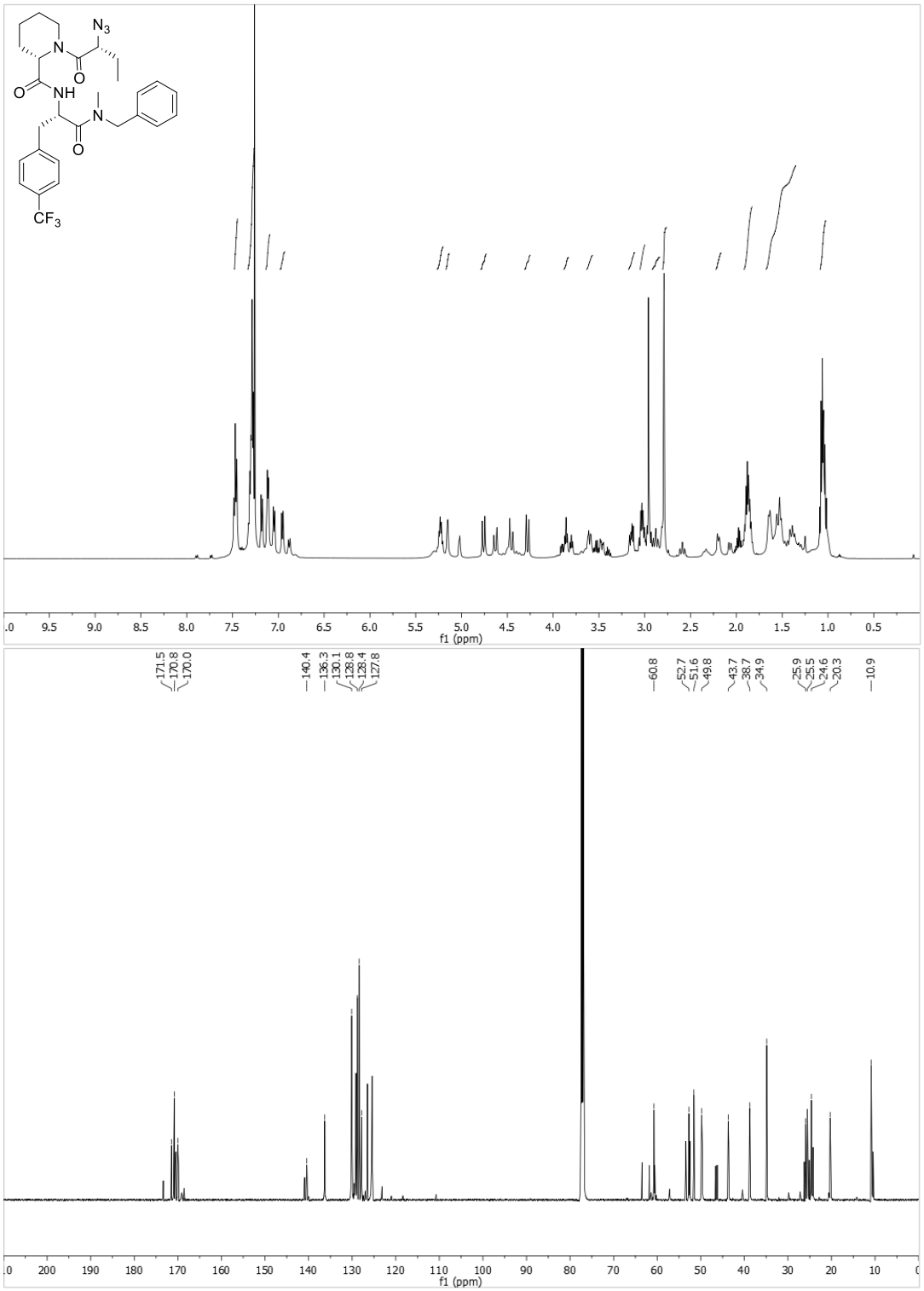
4-(((*R*)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanamido)methyl)benzoic acid (**47**)



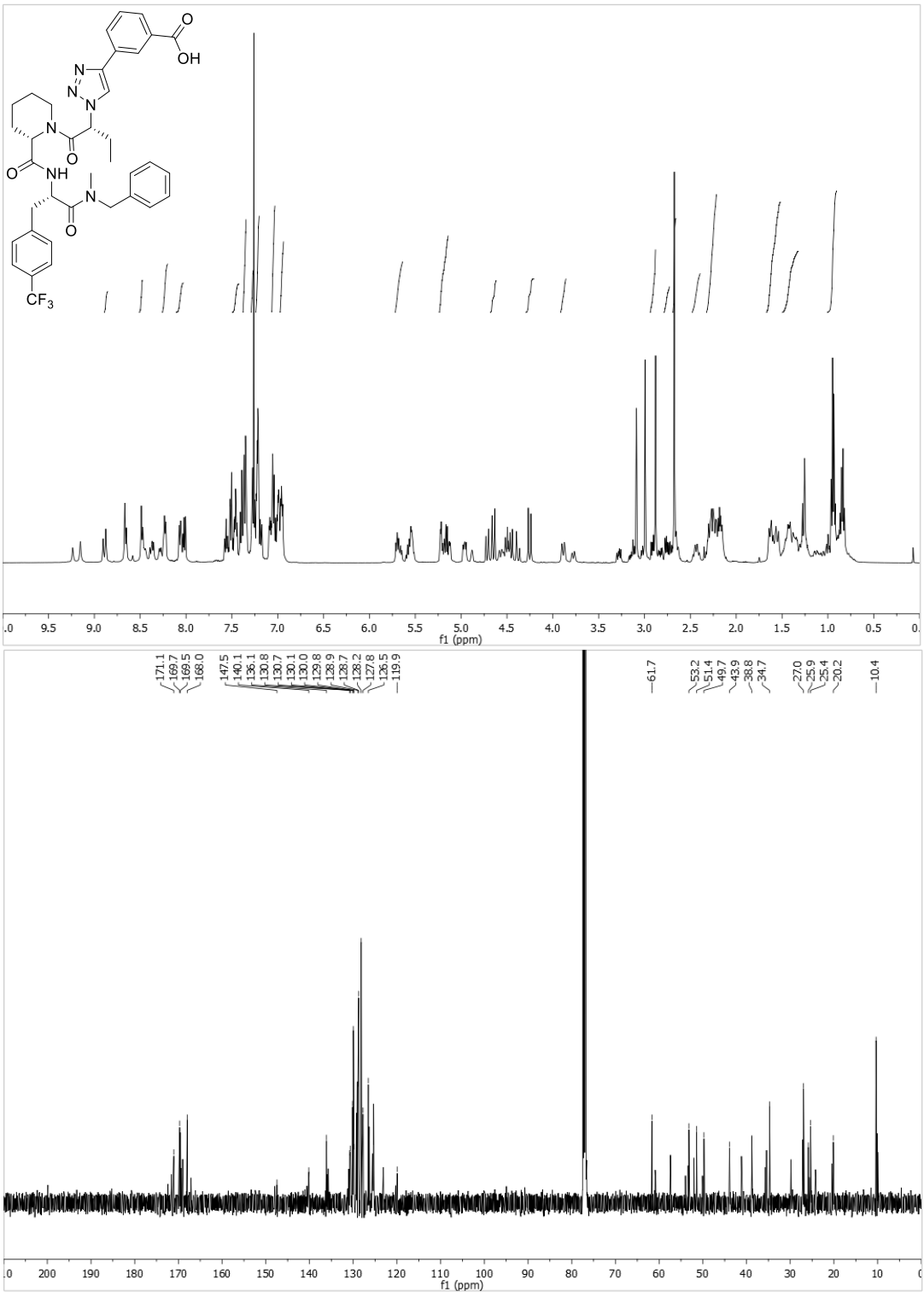
***tert*-Butyl (*S*)-2-(((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate (S21)**



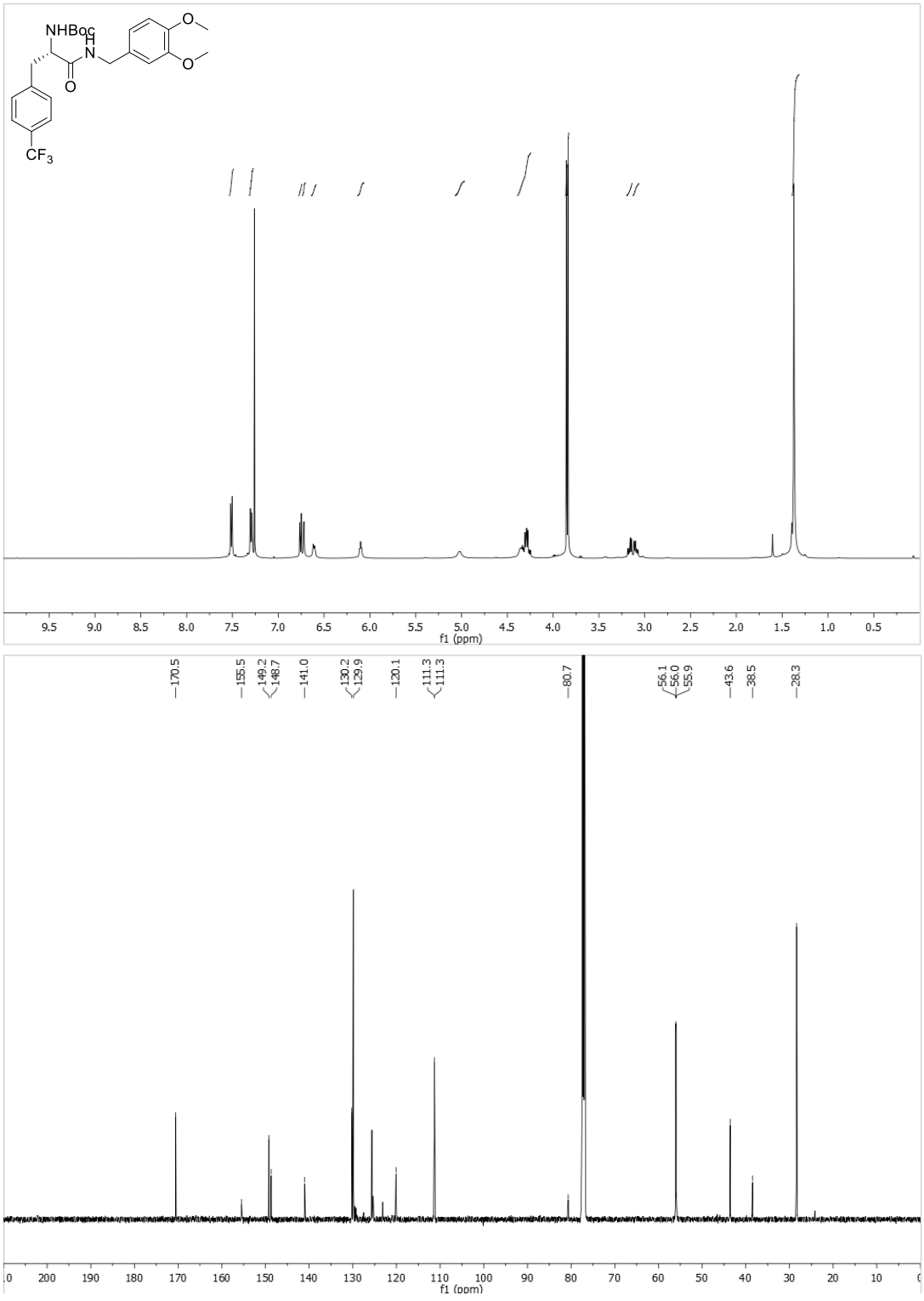
(*S*)-1-((*R*)-2-Azidobutanoyl)-*N*-((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (**S27**)



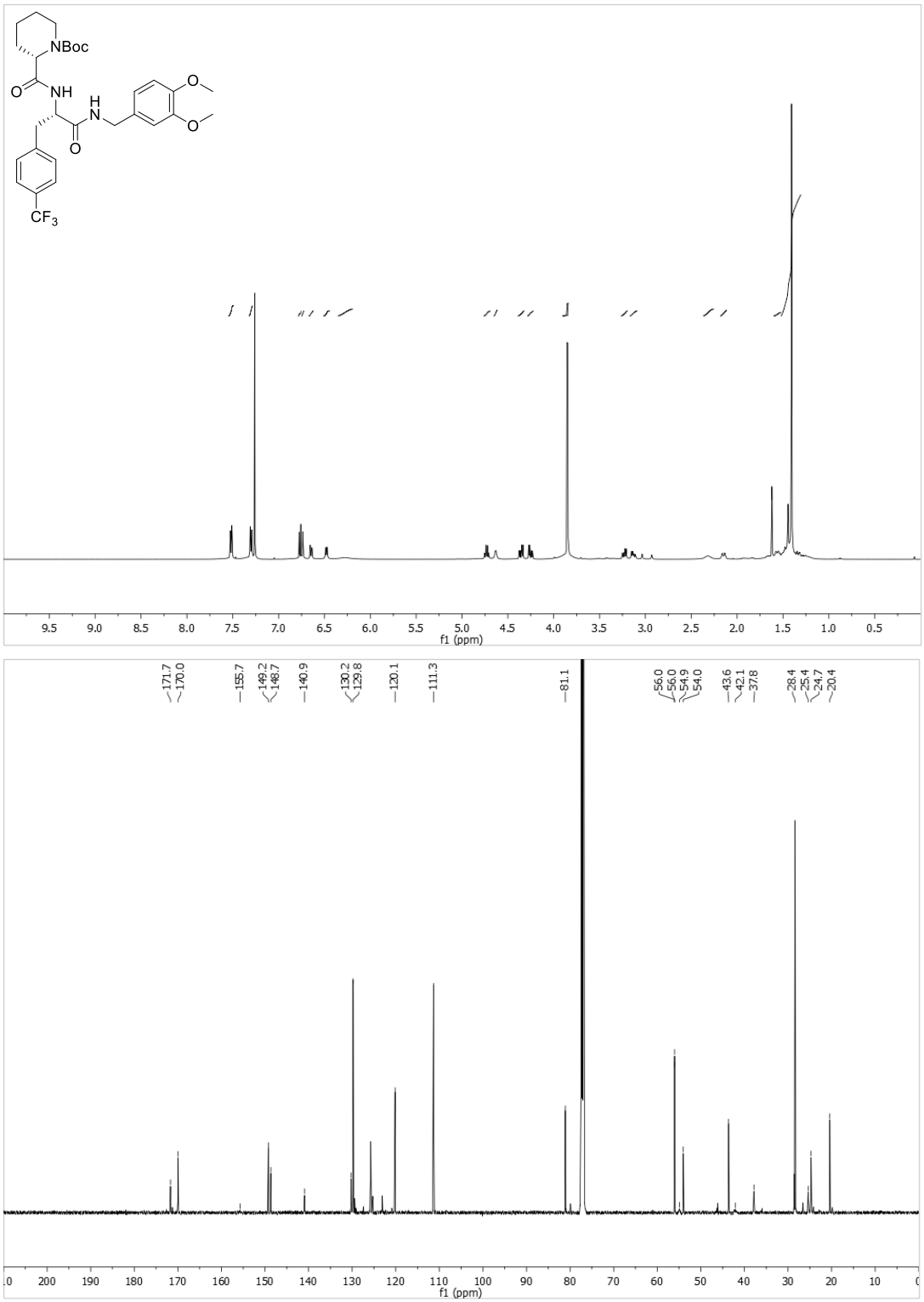
3-(1-((*R*)-1-((*S*)-2-(((*S*)-1-(benzyl(methyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidin-1-yl)-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (**48**)



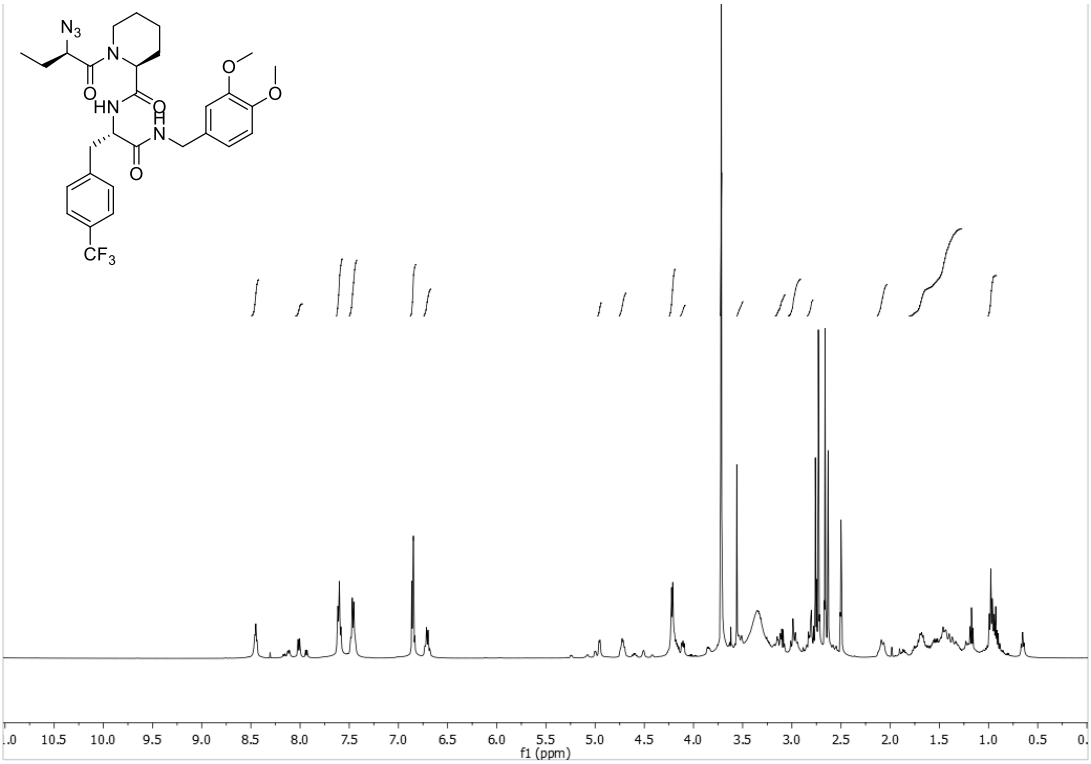
***tert*-Butyl (*S*)-(1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamate (S17)**

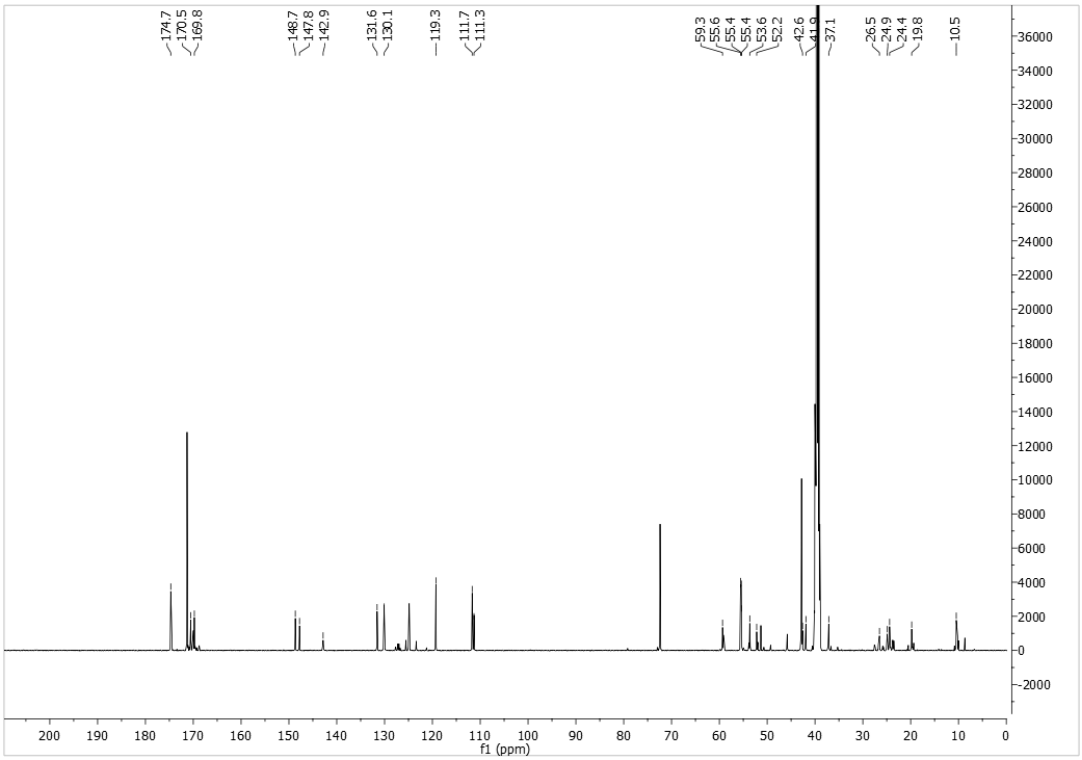


***tert*-butyl (*S*)-2-(((*S*)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate (S22)**

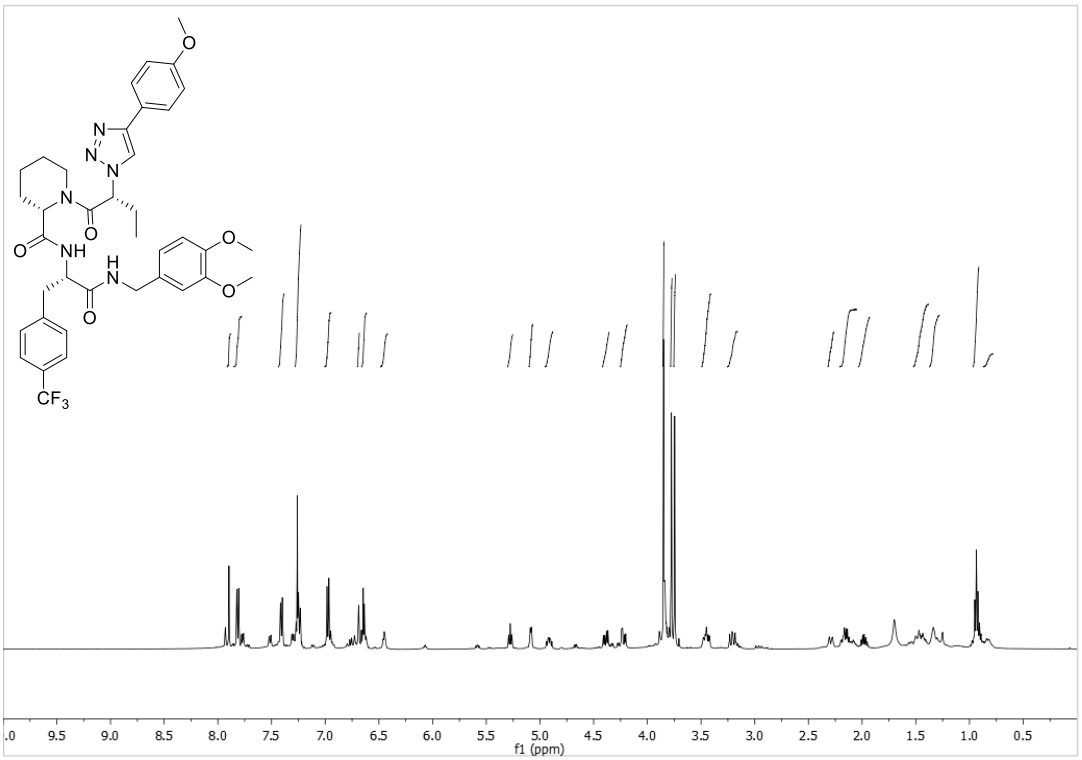


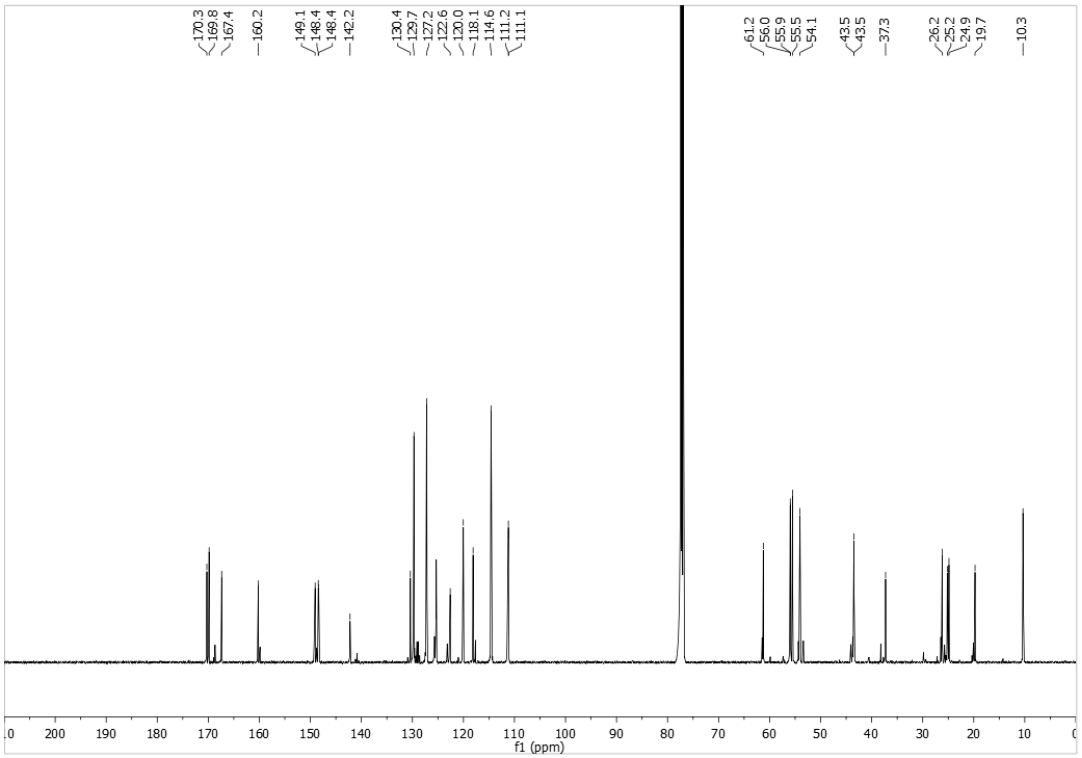
(*S*)-1-((*R*)-2-azidobutanoyl)-*N*-((*S*)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (S28)



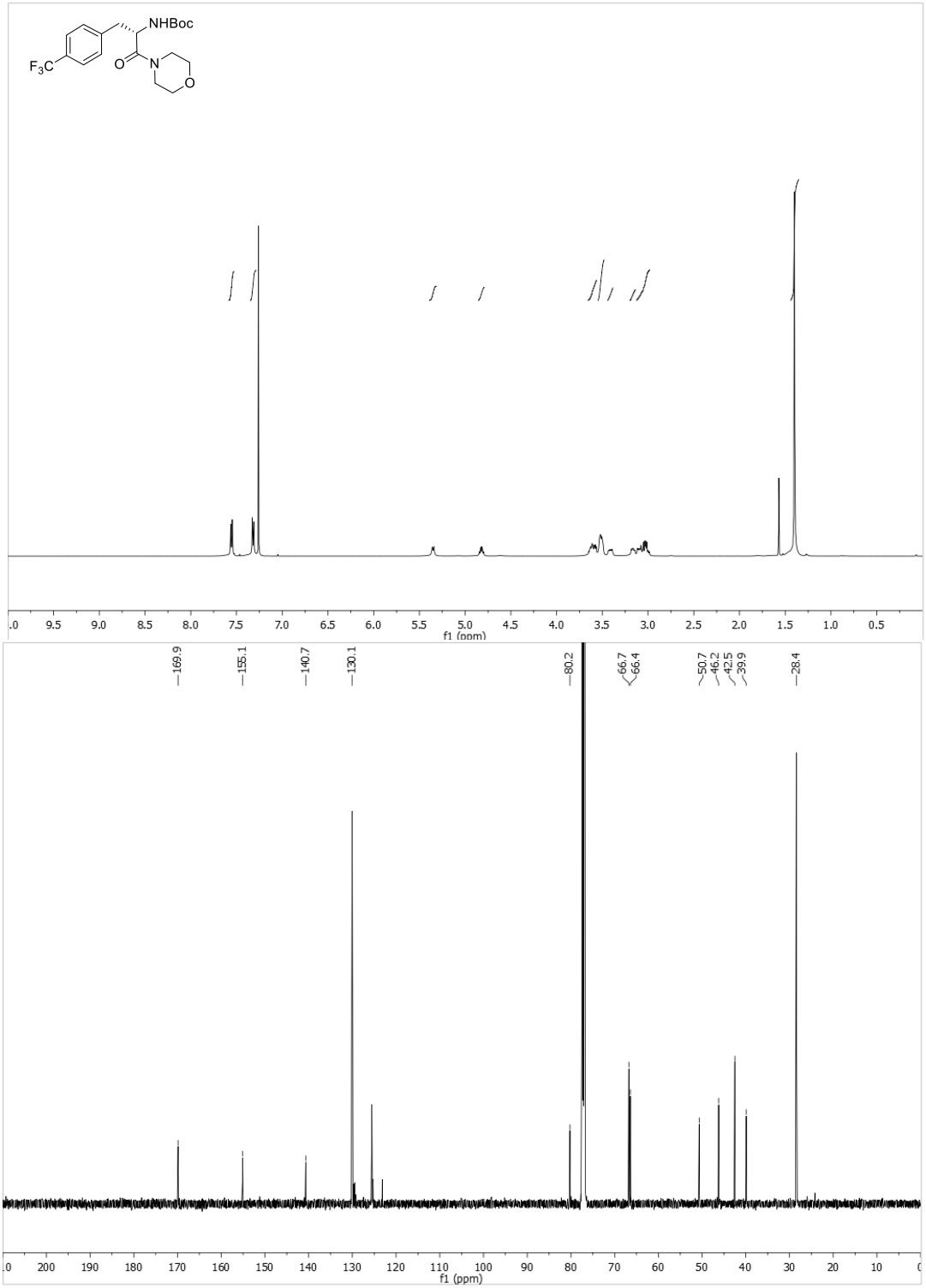


(S)-N-((S)-1-((3,4-dimethoxybenzyl)amino)-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((R)-2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (**49**)

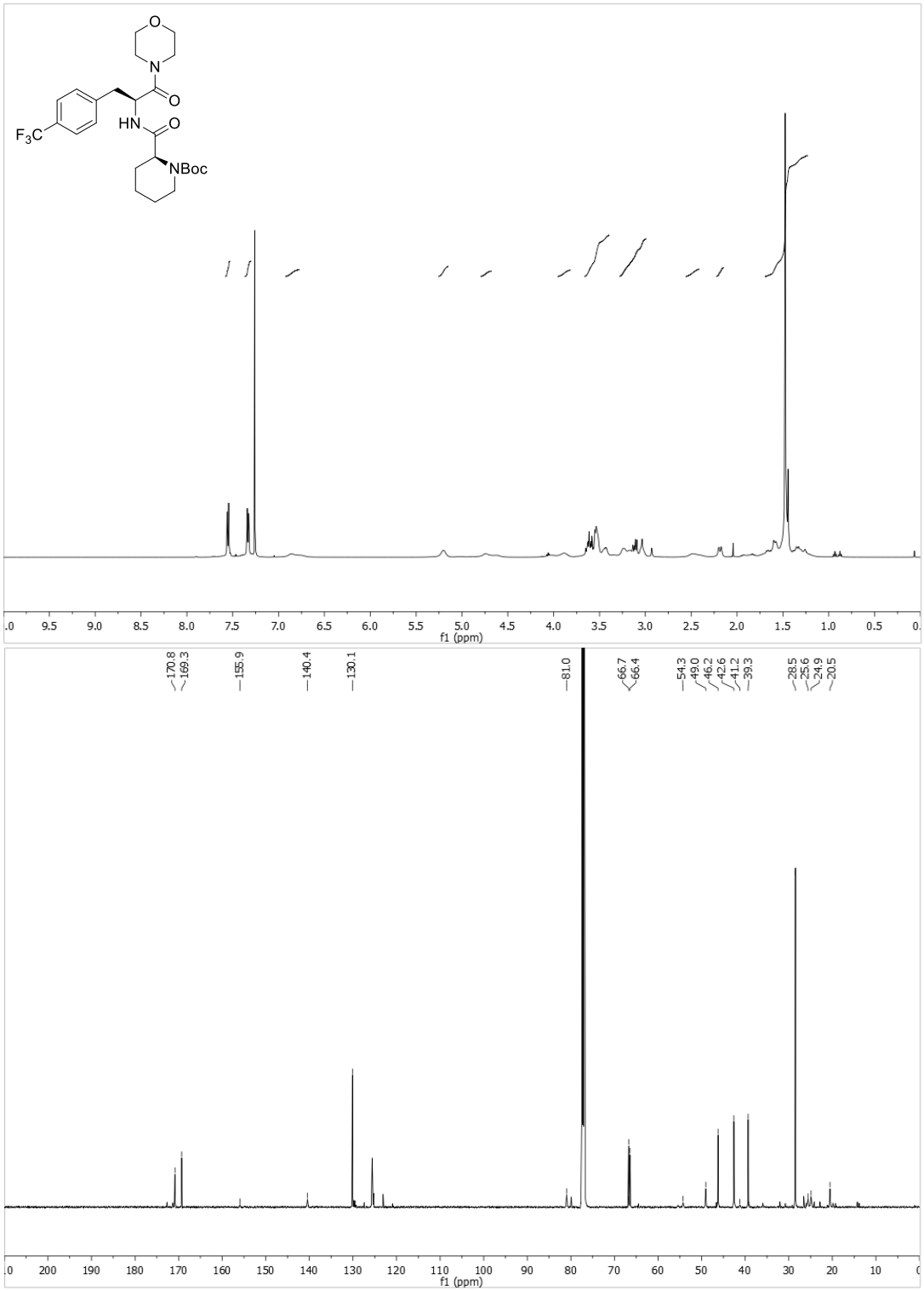




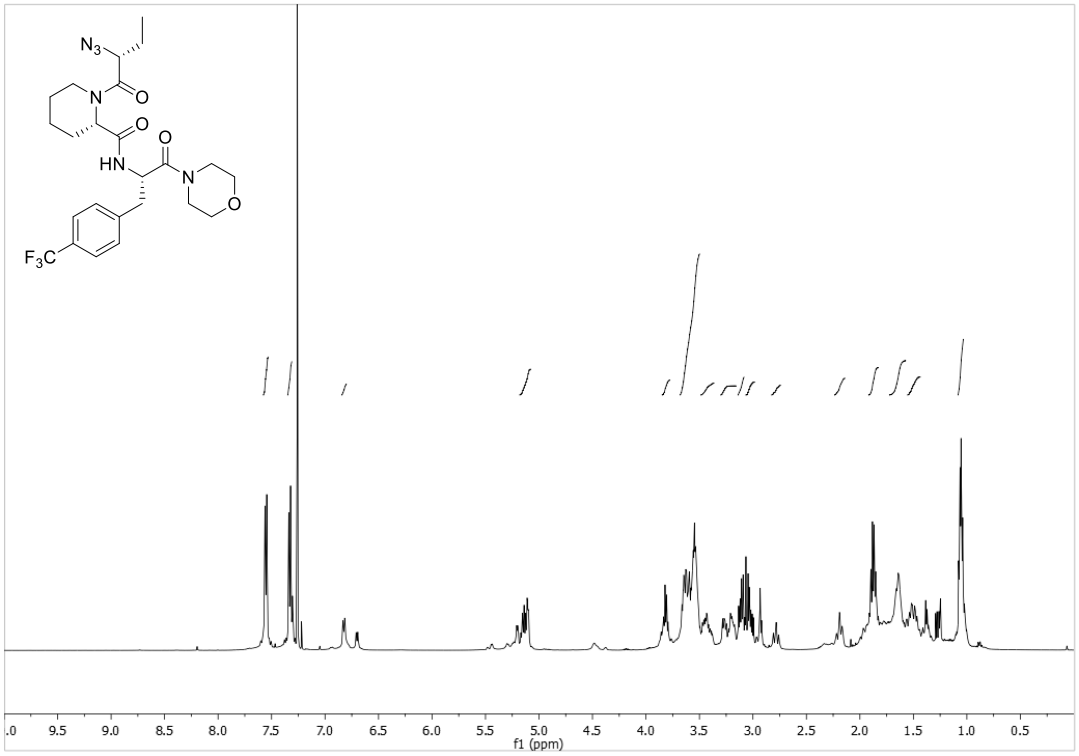
***tert*-Butyl (*S*)-(1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamate (S18)**

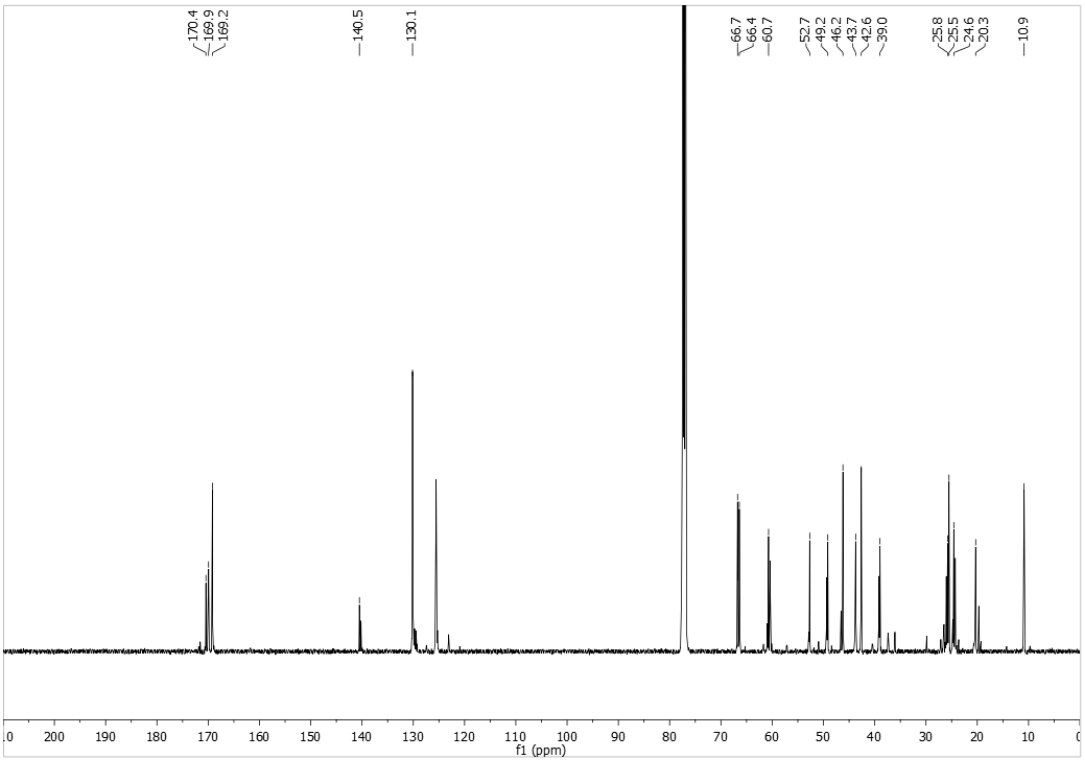


***tert*-butyl (*S*)-2-(((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)piperidine-1-carboxylate (S23)**

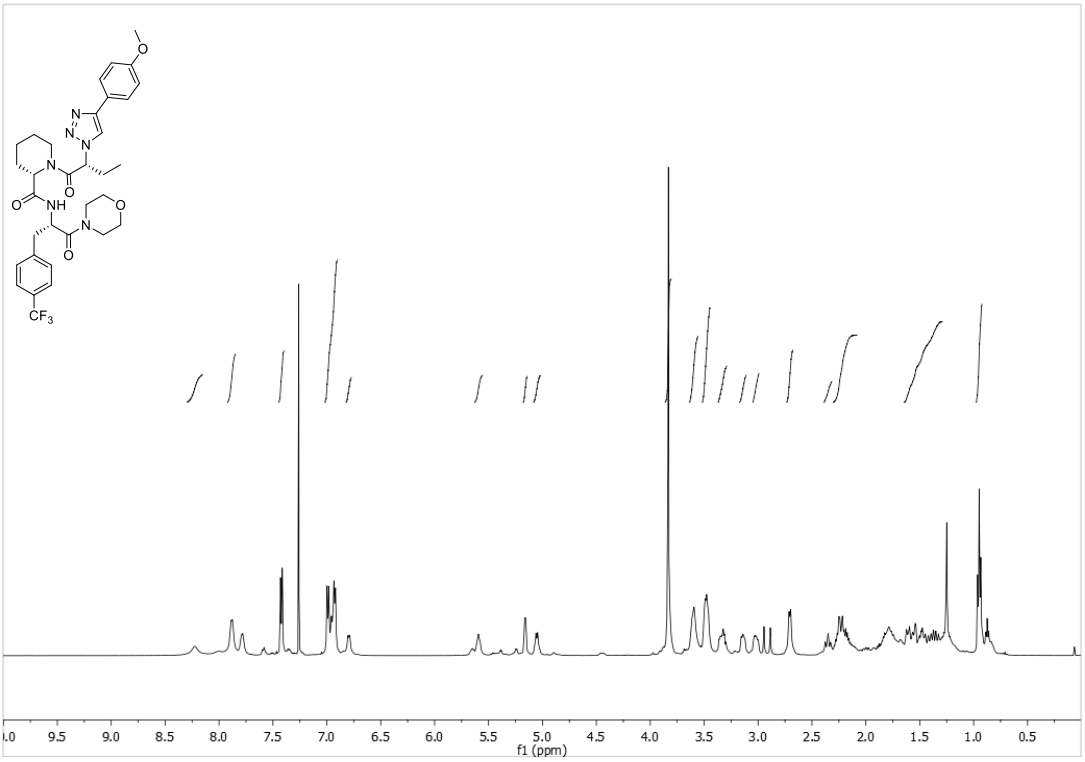


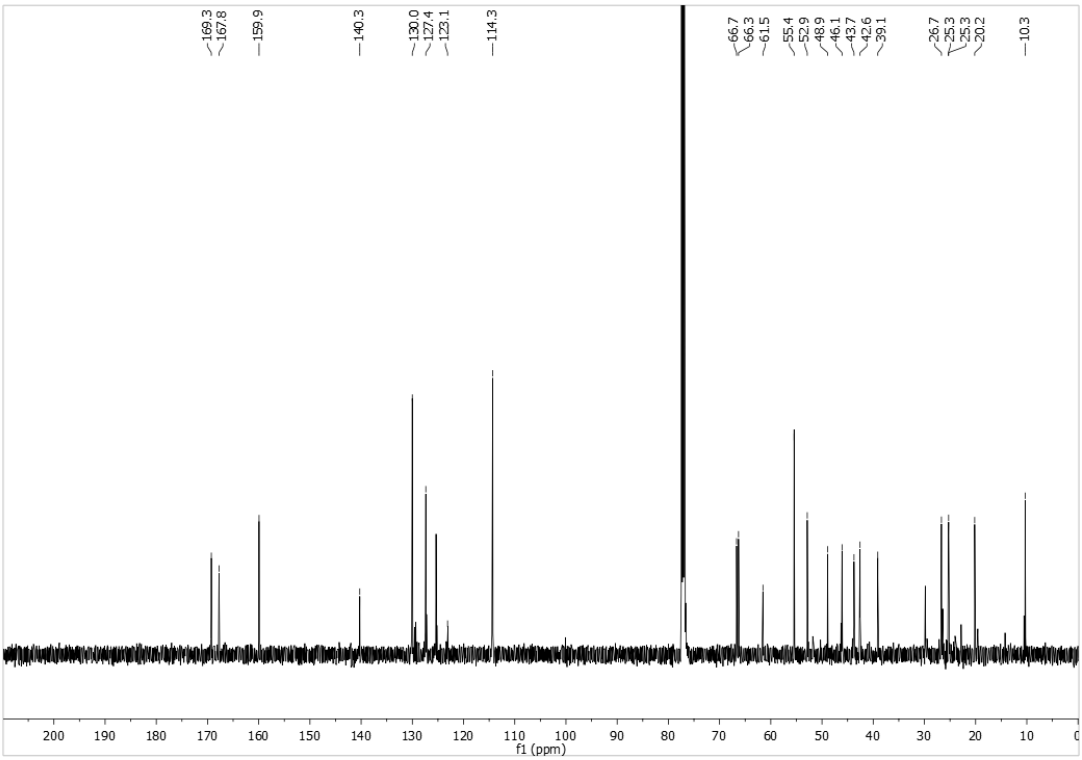
(*S*)-1-((*R*)-2-azidobutanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (S29)



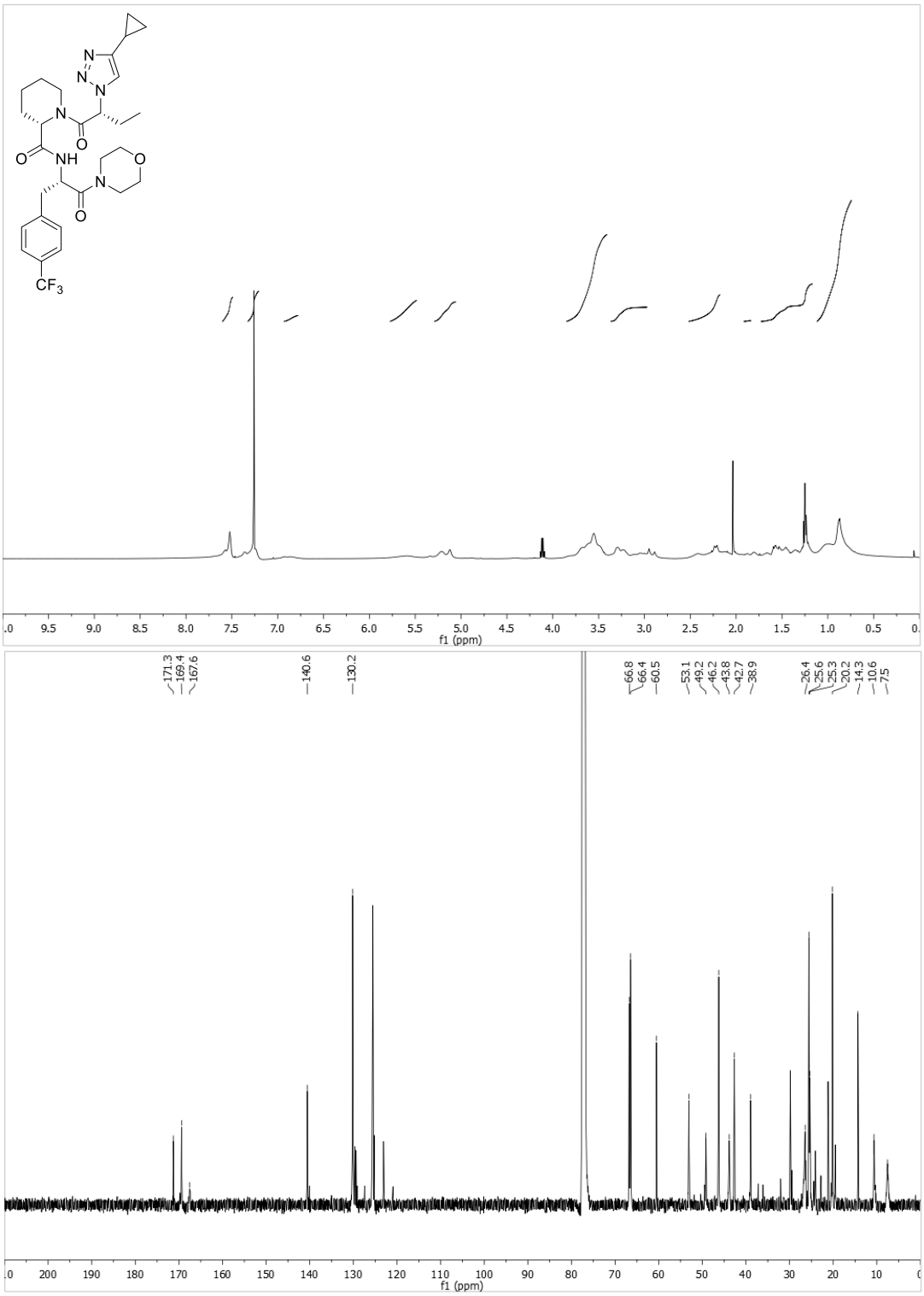


(*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (50)

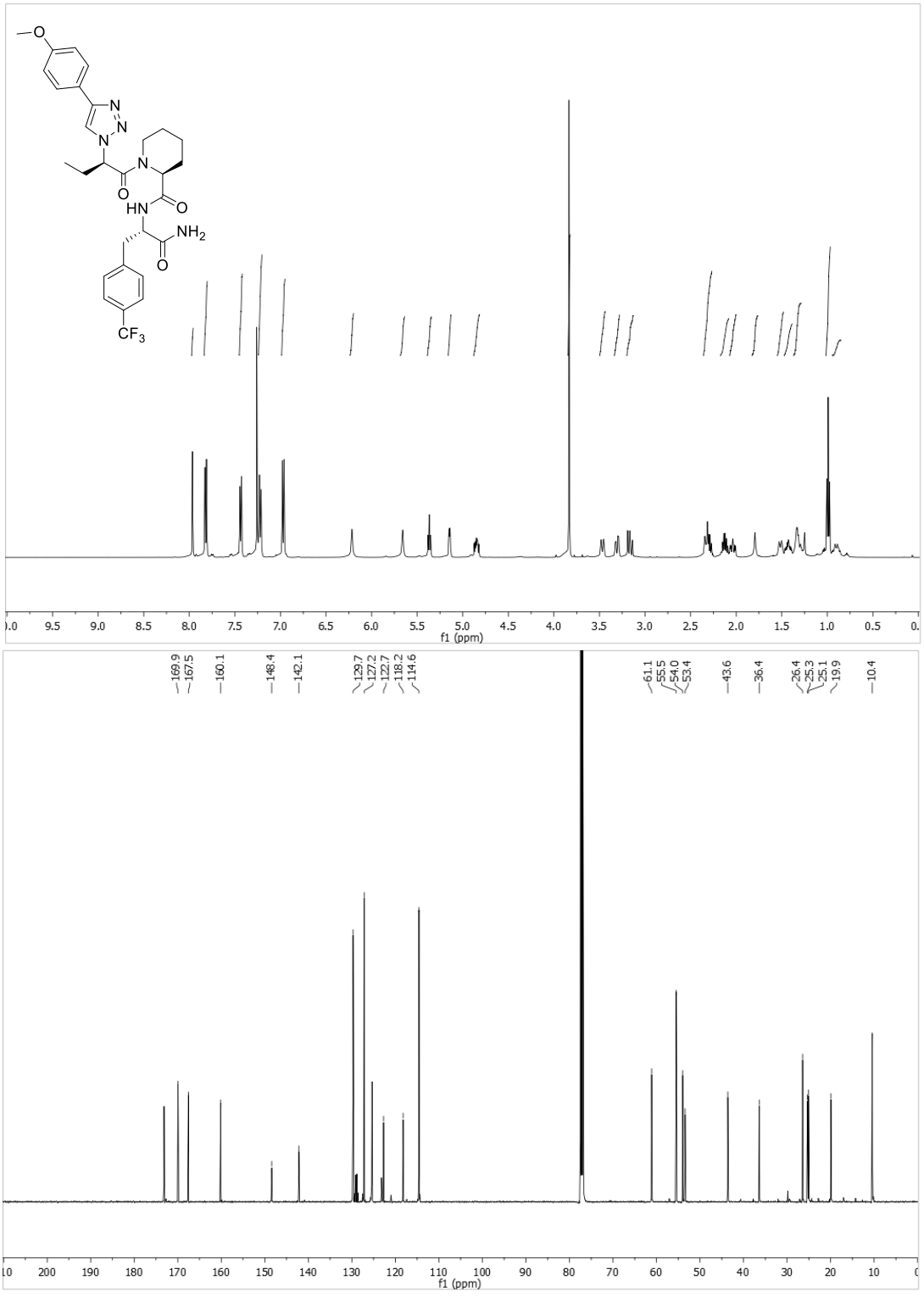




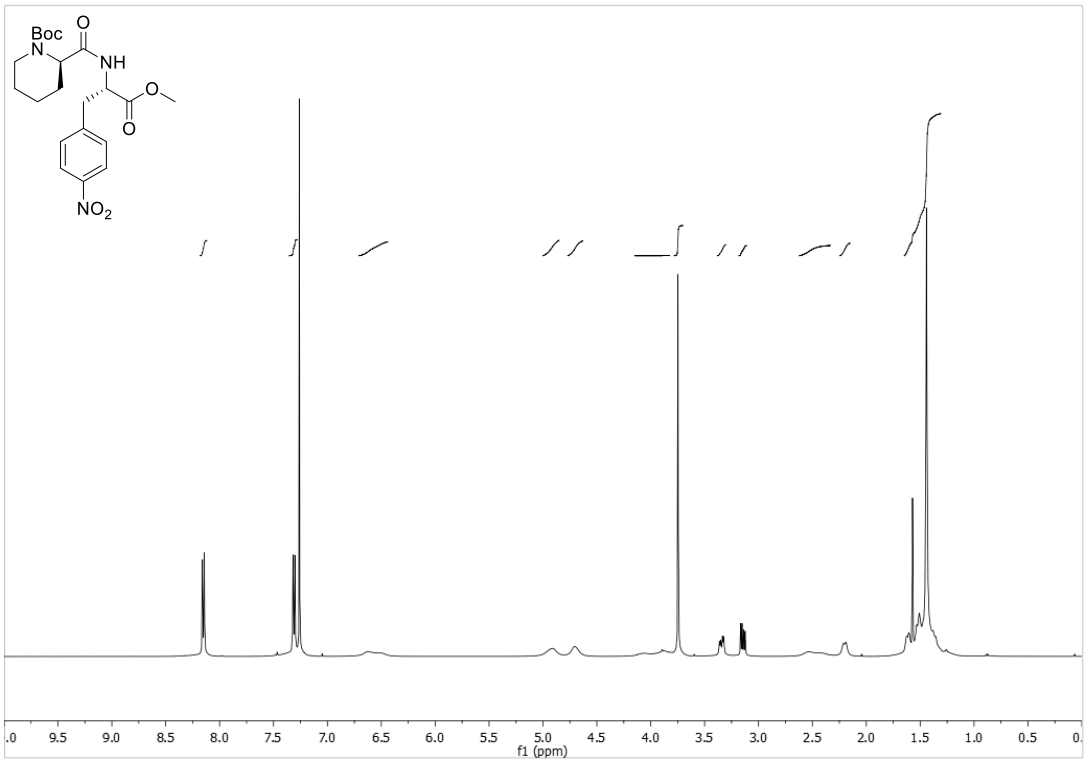
(*S*)-1-((*R*)-2-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)butanoyl)-*N*-((*S*)-1-morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)piperidine-2-carboxamide (**51**)

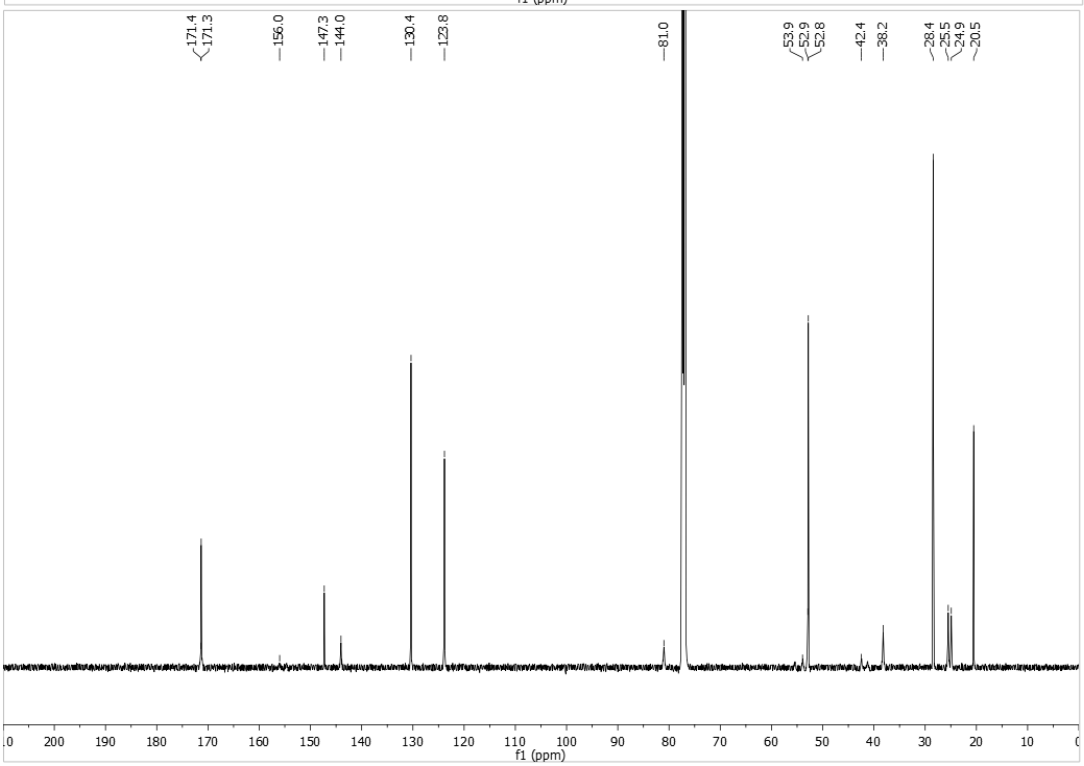


(*S*)-*N*-((*S*)-1-Amino-1-oxo-3-(4-(trifluoromethyl)phenyl)propan-2-yl)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamide (52)

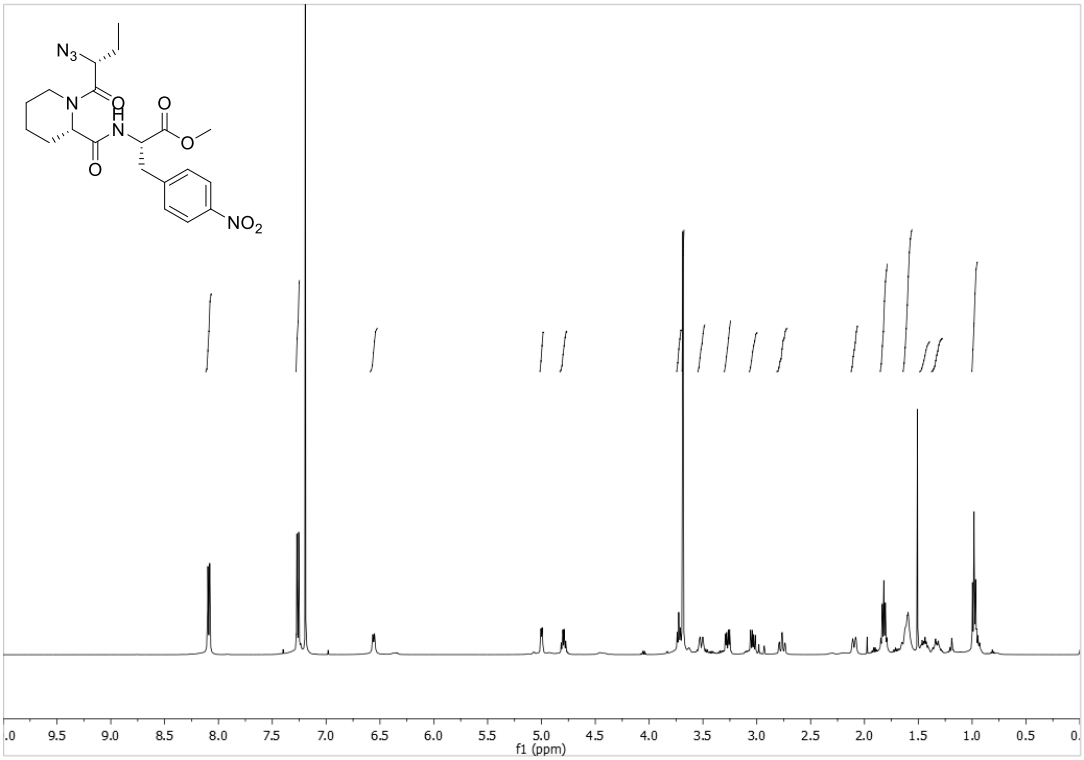


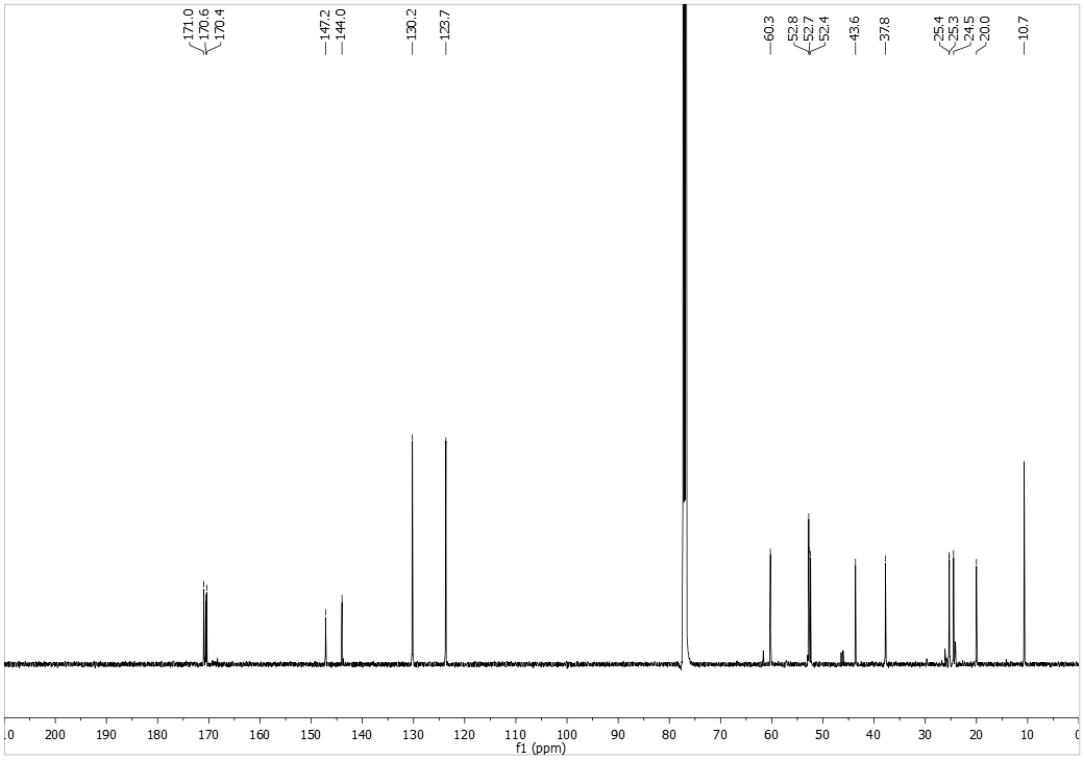
***tert*-Butyl (*R*)-2-(((*S*)-1-methoxy-3-(4-nitrophenyl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate (S24)**



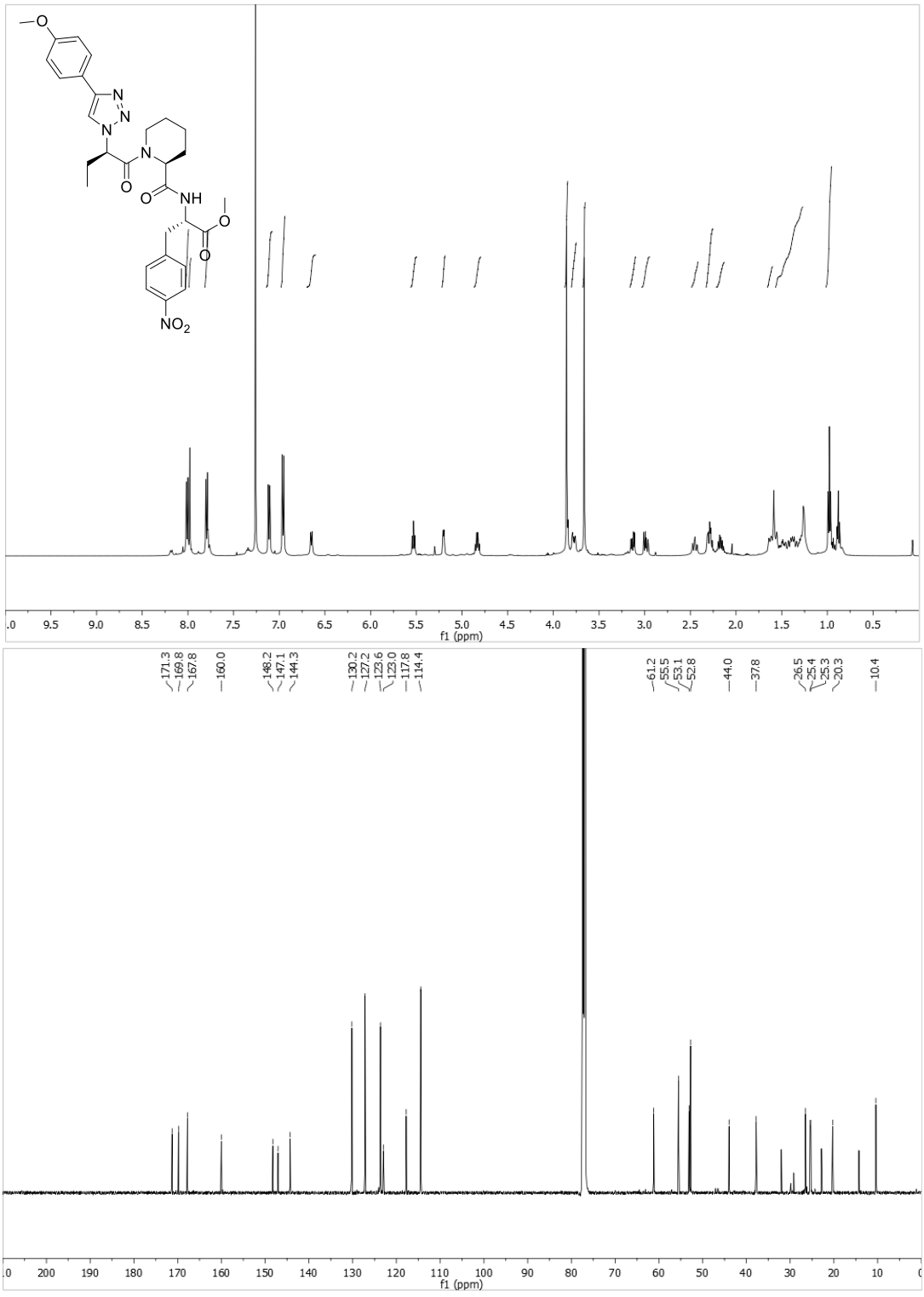


Methyl (*S*)-2-((*S*)-1-((R)-2-azidobutanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanoate (S30)

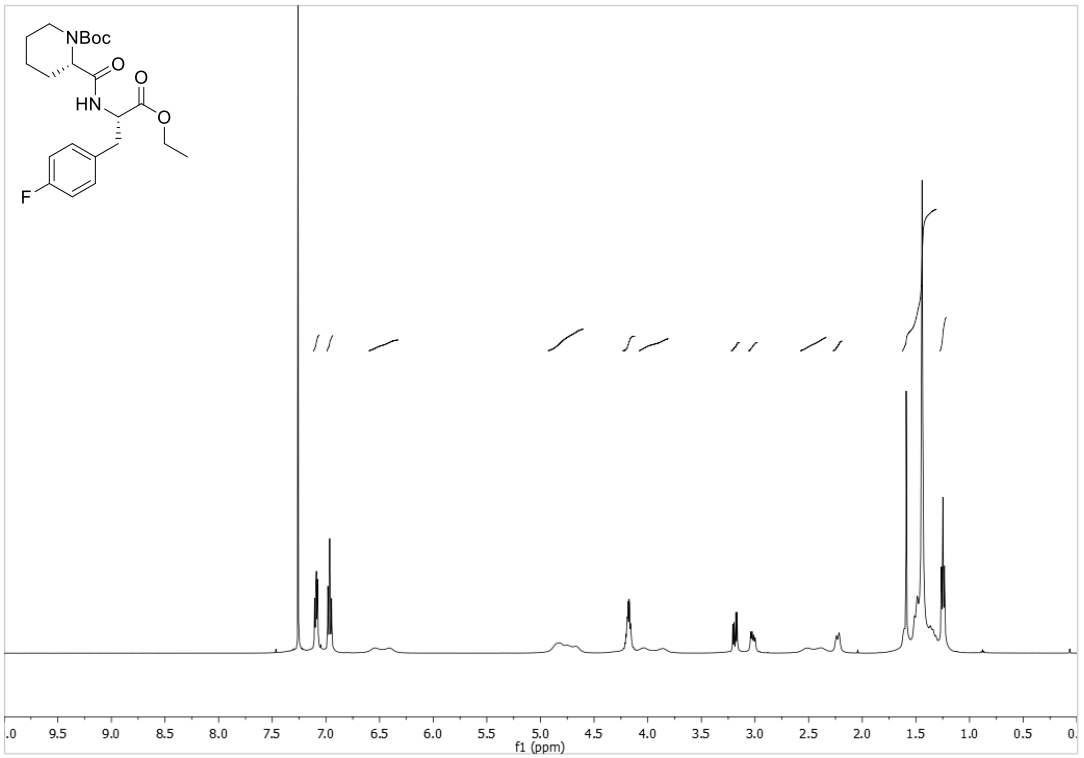


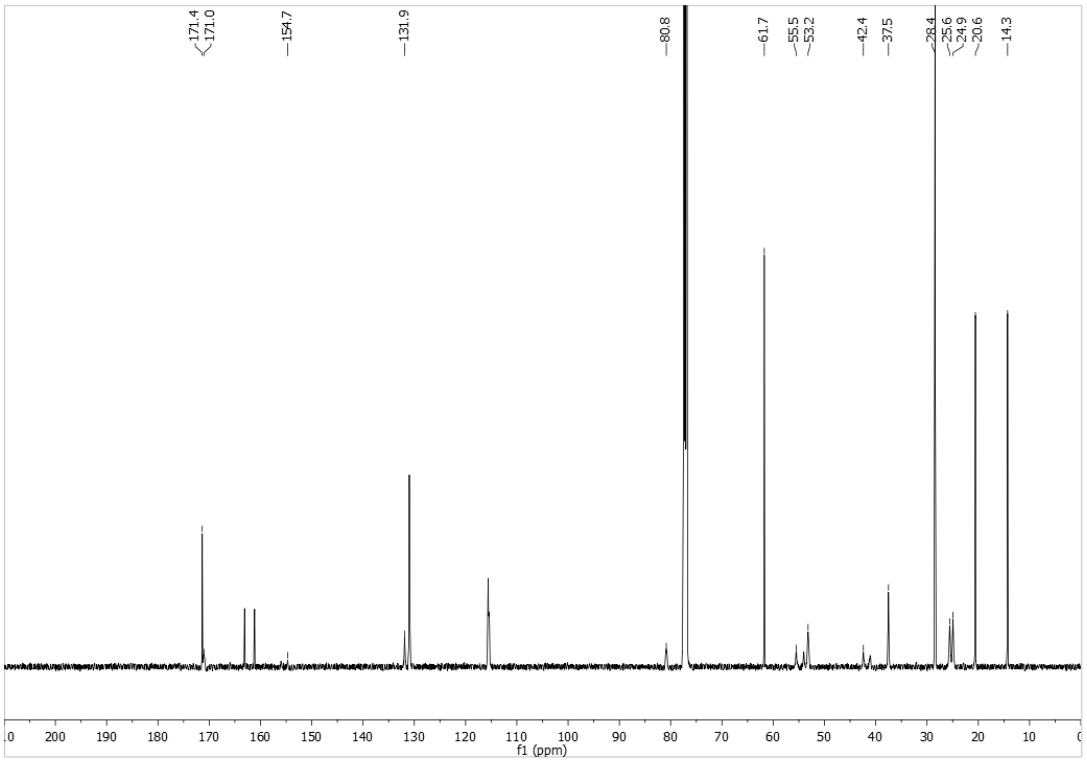


methyl (*S*)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)-3-(4-nitrophenyl)propanoate (53)

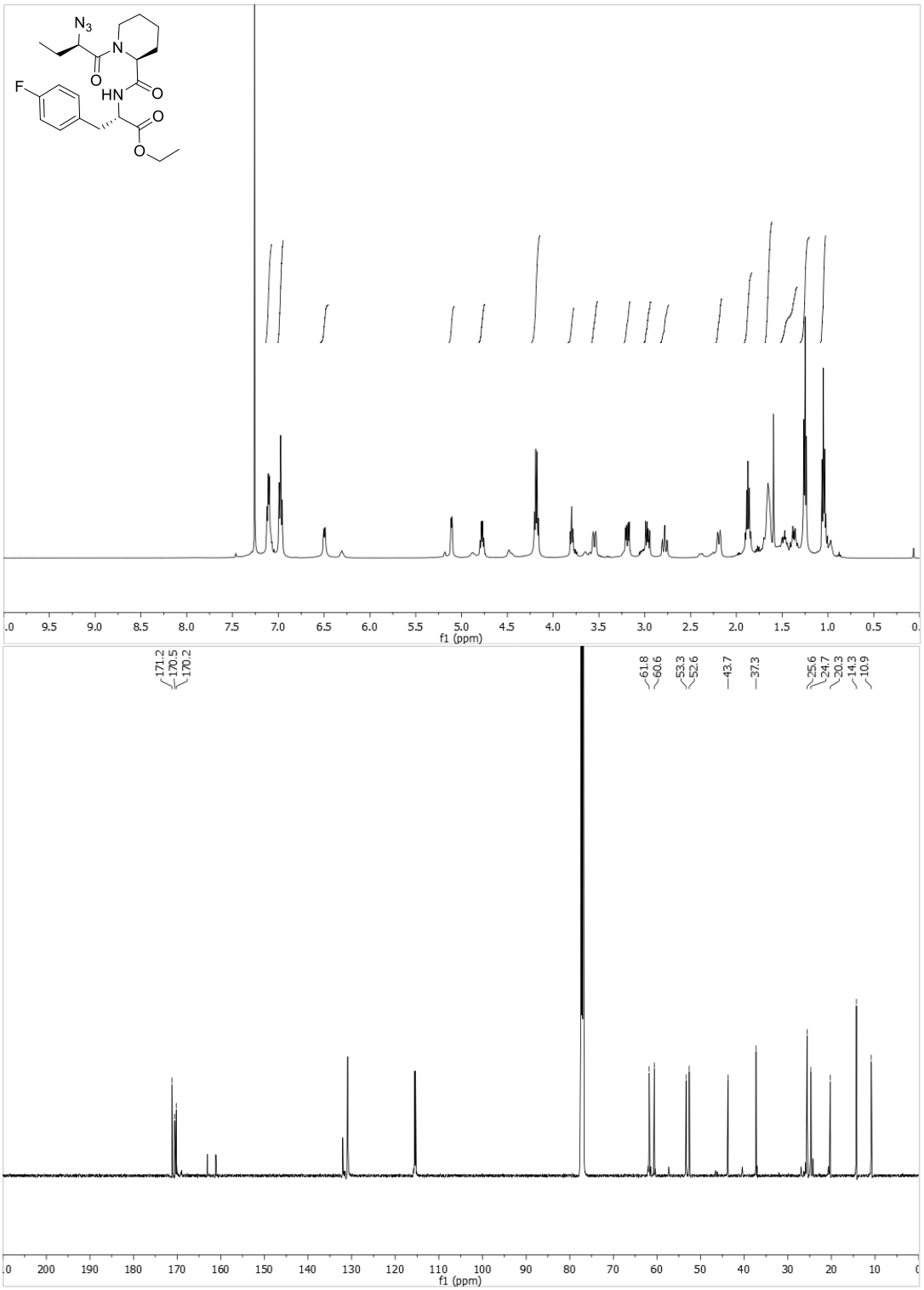


***tert*-Butyl (*S*)-2-(((*S*)-1-ethoxy-3-(4-fluorophenyl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate (S25)**

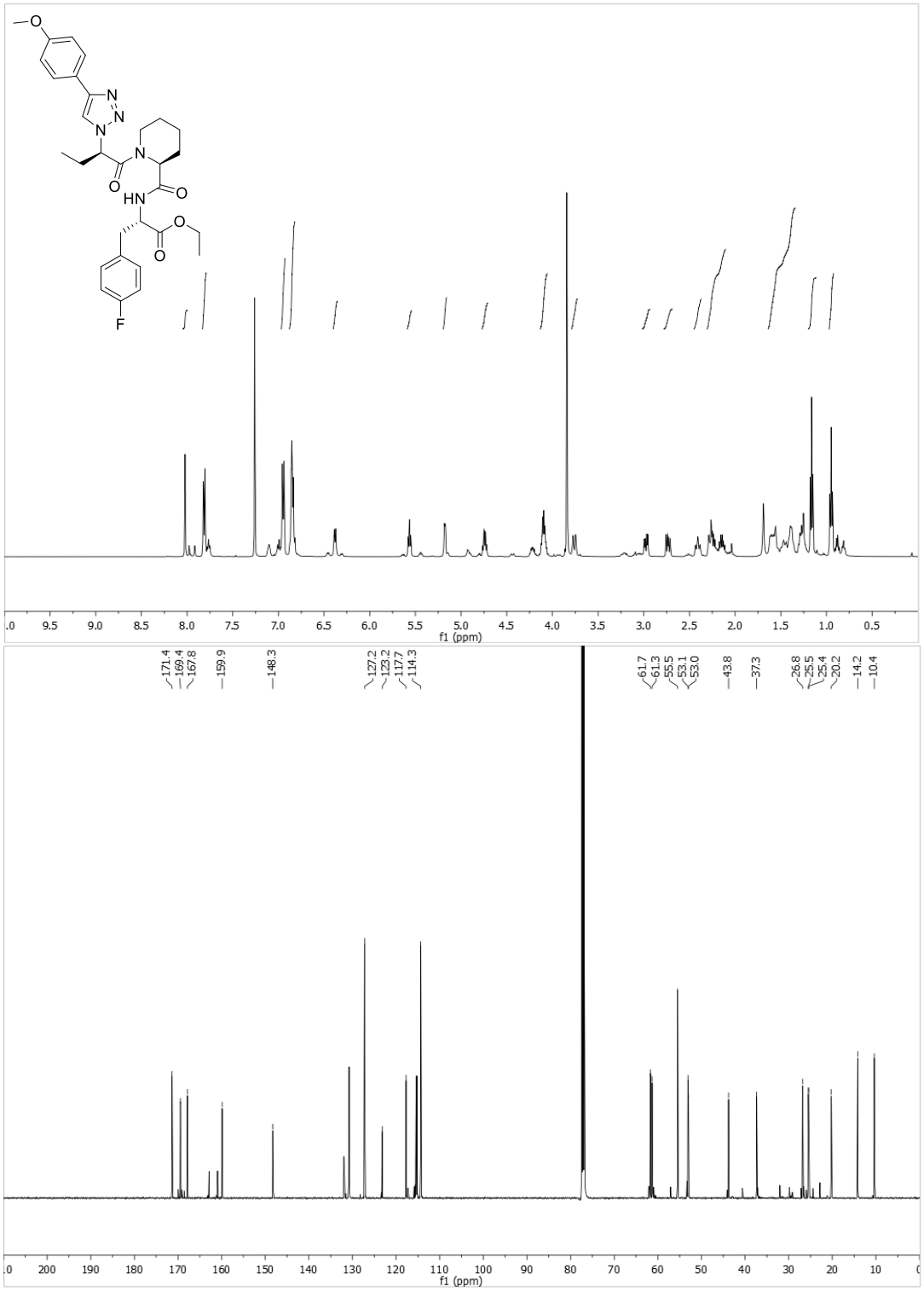




Ethyl (*S*)-2-((*S*)-1-((*R*)-2-azidobutanoyl)piperidine-2-carboxamido)-3-(4-fluorophenyl)propanoate (S31)



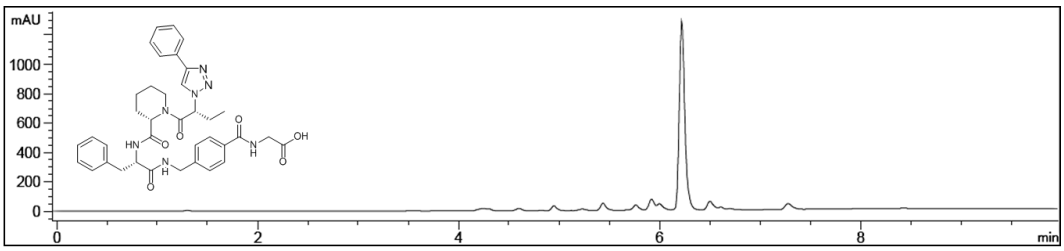
Ethyl (*S*)-3-(4-fluorophenyl)-2-((*S*)-1-((*R*)-2-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperidine-2-carboxamido)propanoate (**54**)



Crude HPLC and MS of solid-supported ligands

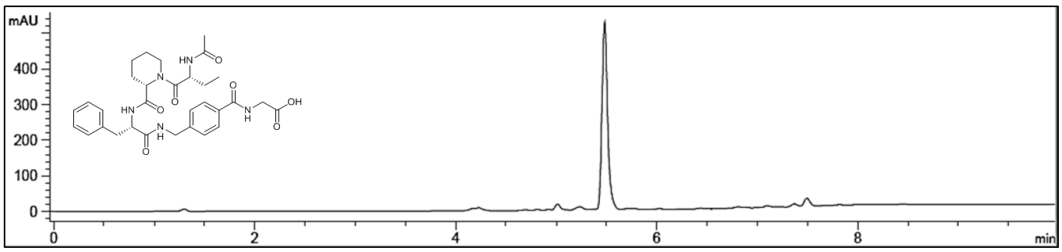
6: (254 nm) RT = 6.22 min

HRMS calc. for $C_{37}H_{42}N_7O_6$: 680.3191 $[M+H]^+$ found 680.3198.



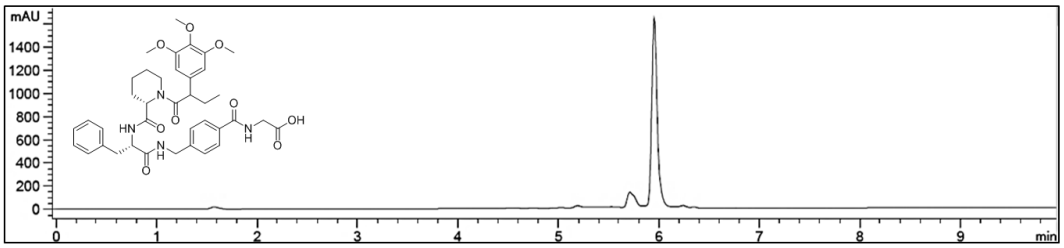
7: (254 nm) RT = 5.48 min

HRMS calc. for $C_{31}H_{40}N_5O_7$: 594.2922 $[M+H]^+$ found 594.2927.



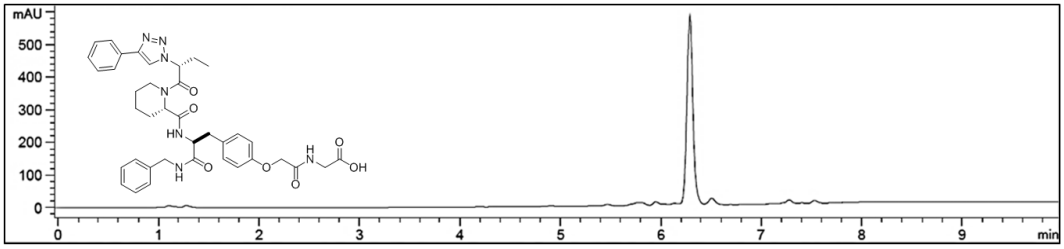
8: (254 nm) RT = 5.98 min

HRMS calc. for $C_{38}H_{47}N_4O_9$: 703.3338 $[M+H]^+$ found 703.3340.



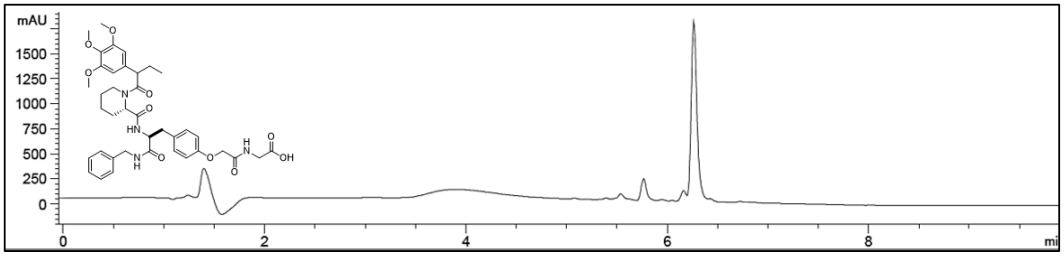
15: (254 nm) RT = 6.29 min

HRMS calc. for $C_{38}H_{44}N_7O_7$: 710.3297 $[M+H]^+$ found 710.3294.



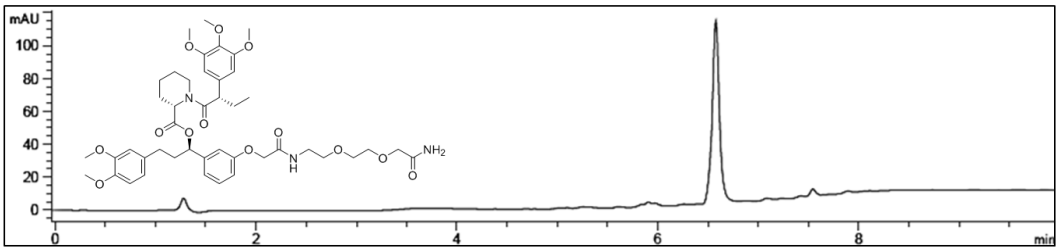
16: (254 nm) RT = 6.23 min

HRMS calc. for $C_{39}H_{49}N_4O_{10}$: 733.3443 $[M+H]^+$ found 733.3461.



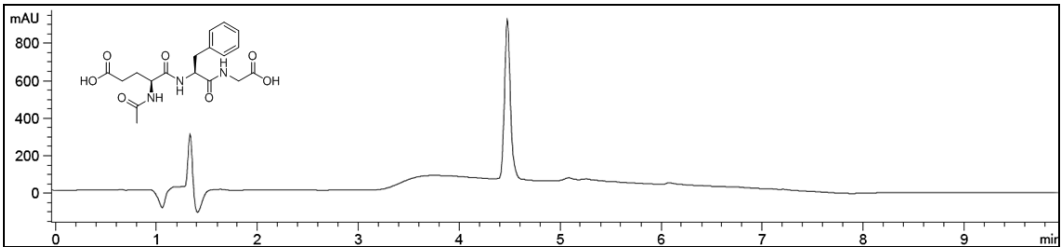
18: (254 nm) RT = 6.62 min

HRMS calc. for $C_{44}H_{60}N_3O_{13}$: 838.4121 $[M+H]^+$ found 838.3996.



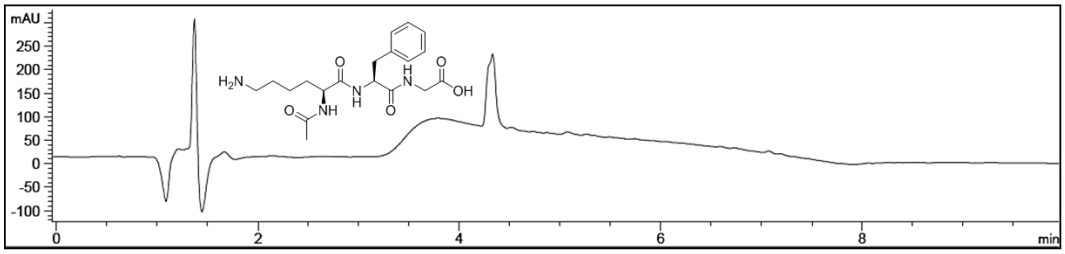
19: (215 nm) RT = 4.42 min

HRMS calc. for $C_{18}H_{24}N_3O_7$: 394.1609 $[M+H]^+$ found 394.1612.



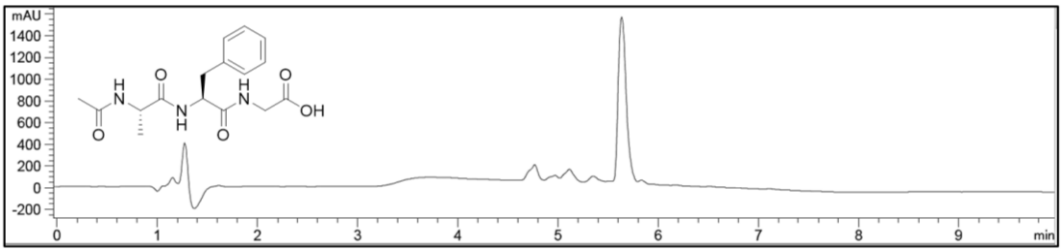
20: (215 nm) RT = 4.44 min

HRMS calc. for $C_{19}H_{29}N_4O_5$: 393.2132 $[M+H]^+$ found 393.2140.



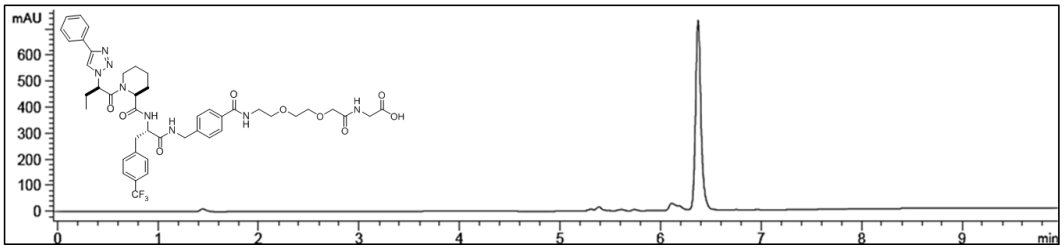
21: 2.38 (215 nm) RT = 5.64 min

HRMS calc. for $C_{16}H_{22}N_3O_5$: 336.1554 $[M+H]^+$ found 336.1558



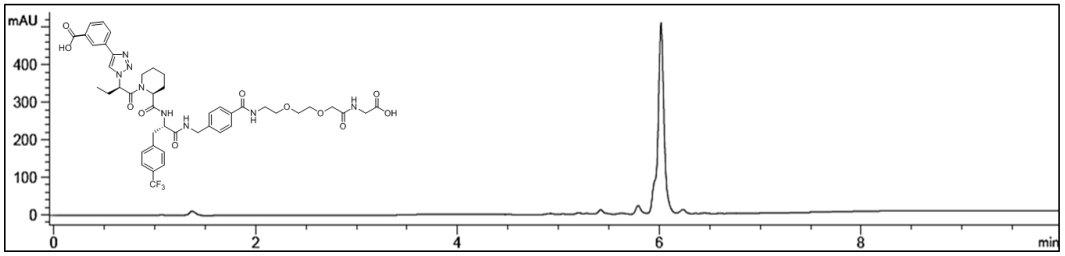
23 (254 nm) RT = 6.37 min

HRMS calc. for $C_{44}H_{52}F_3N_8O_9$: 893.3804 $[M+H]^+$ found 893.3830.



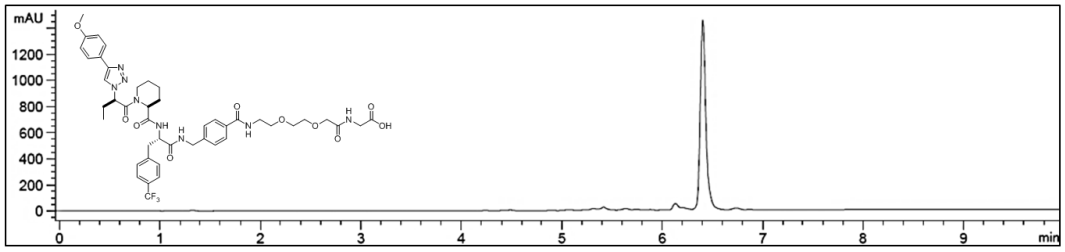
24: (254 nm) RT = 6.02 min

HRMS calc. for $C_{45}H_{52}F_3N_8O_{11}$: 937.3702 $[M+H]^+$ found 937.3695.



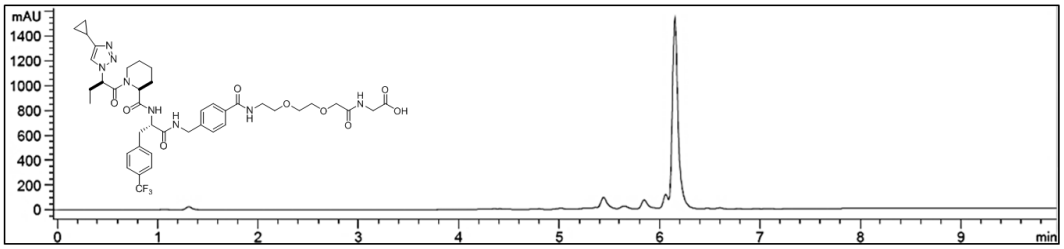
25: (254 nm) RT = 6.40 min

HRMS calc. for $C_{45}H_{53}F_3N_8NaO_{10}$: 945.3729 $[M+Na]^+$ found 945.3711.



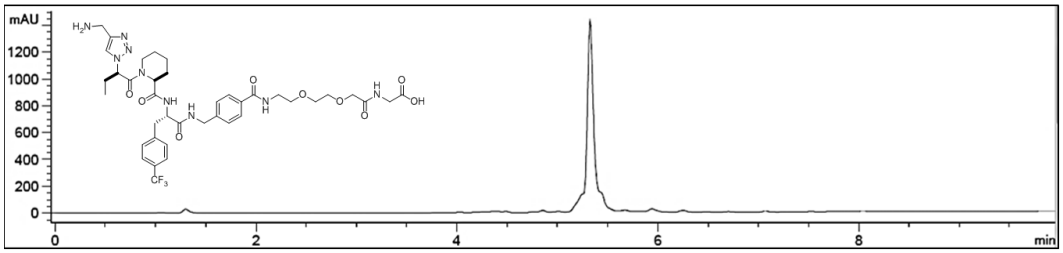
26: (254 nm) RT = 6.29 min

HRMS calc. for $C_{41}H_{52}F_3N_8O_9$: 857.3804 $[M+H]^+$ found 857.3796.



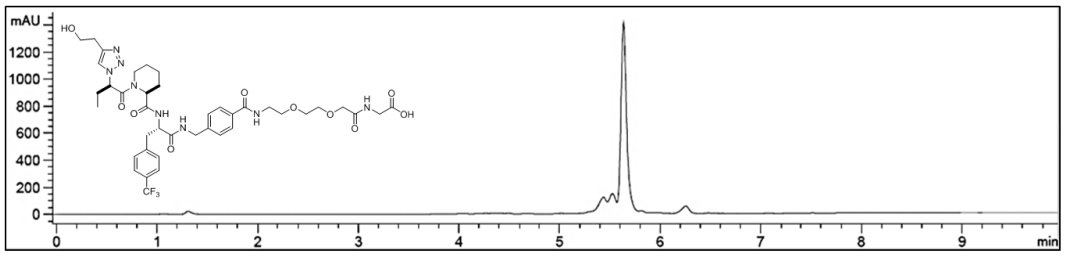
27: (254 nm) RT = 5.33 min

HRMS calc. for $C_{39}H_{52}F_3N_8O_9$: 846.3756 $[M+H]^+$ found 846.3762.



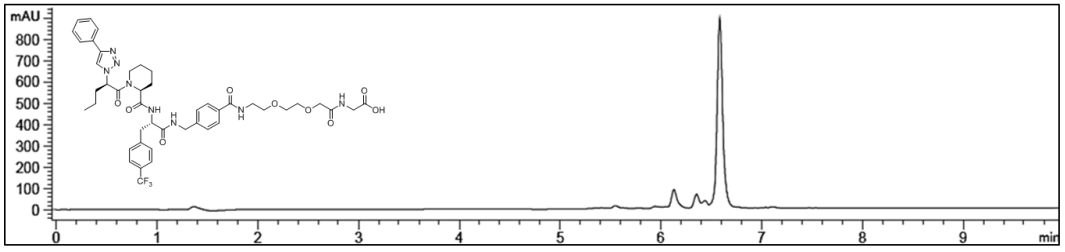
28: (254 nm) RT = 5.64 min

HRMS calc. for $C_{40}H_{52}F_3N_8O_{10}$: 861.3753 $[M+H]^+$ found 861.3741.



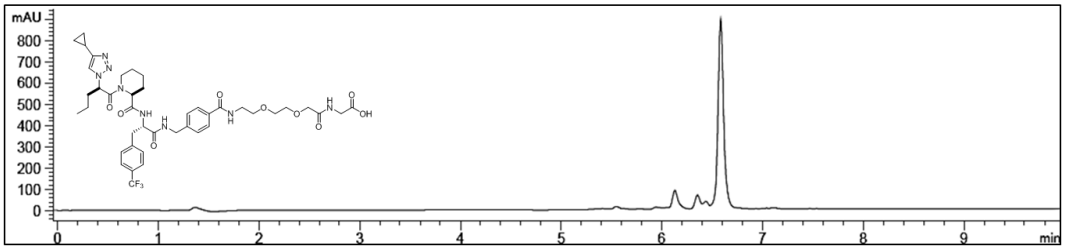
29: (254 nm) RT = 6.58 min

HRMS calc. for $C_{45}H_{54}F_3N_8O_9$: 907.3960 $[M+H]^+$ found 907.3946.



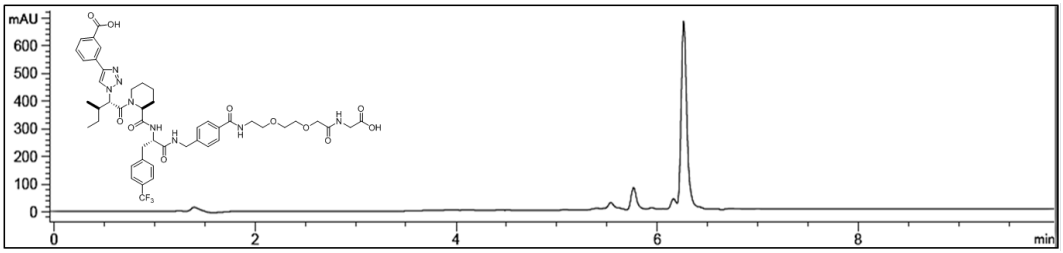
30: (254 nm) RT = 6.27 min

HRMS calc. for $C_{42}H_{54}F_3N_8O_9$: 871.3960 $[M+H]^+$ found 871.3949.



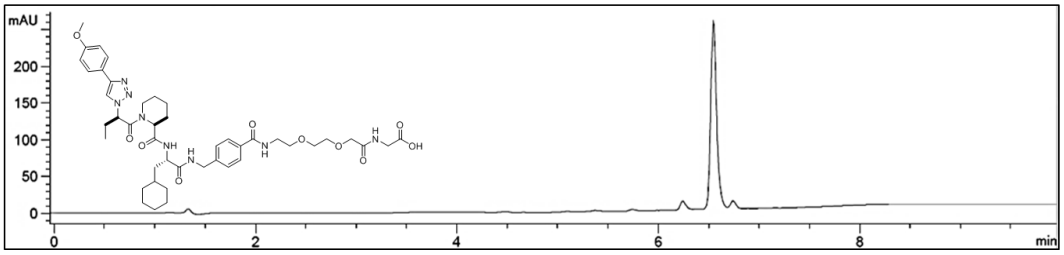
32: (254 nm) RT = 6.25 min

HRMS calc. for $C_{47}H_{55}F_3N_8O_{11}$: 964.3942 $[M+H]^+$ found 964.4005.



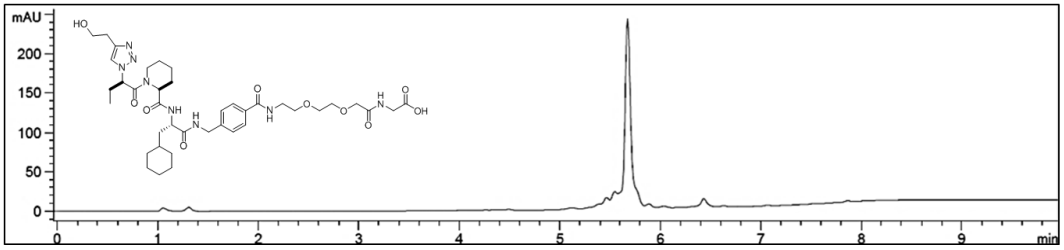
33 (254 nm) RT = 6.55 min

HRMS calc. for $C_{44}H_{61}N_8O_{10}$: 861.4505 $[M+H]^+$ found 861.4508.



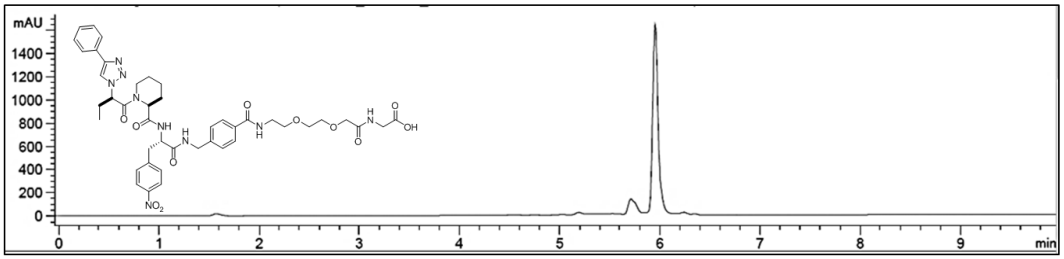
34 (254 nm) RT = 5.68 min

HRMS calc. for $C_{39}H_{59}N_8O_{10}$: 799.4349 $[M+H]^+$ found 799.4387.

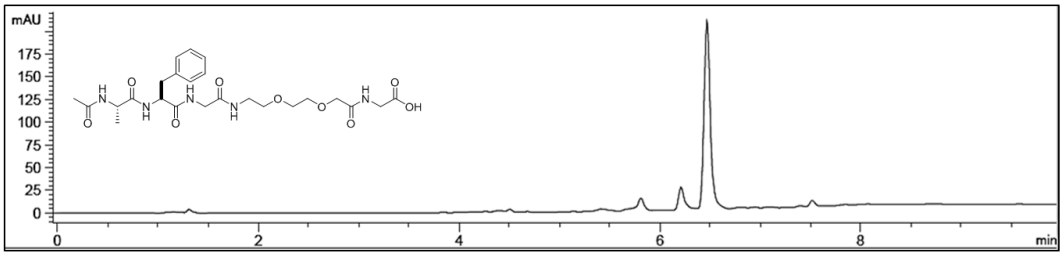


35: (254 nm) RT = 5.95 min

HRMS calc. for $C_{43}H_{52}N_9O_{11}$: 870.3781 $[M+H]^+$ found 870.3770.



NSB2 Ac-Ala-Phe-Gly (254 nm) 6.49 min



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