SUPPORTING INFORMATION

Cathepsin B Inhibitors: Combining Dipeptide Nitriles with an Occluding Loop Recognition Element by Click Chemistry

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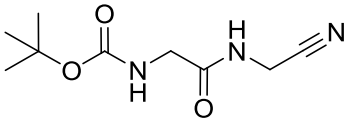
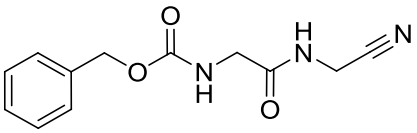
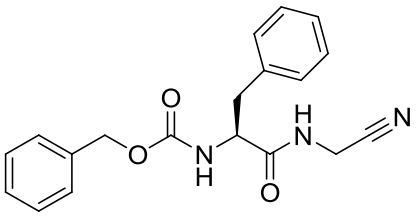
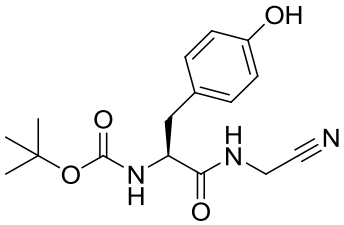
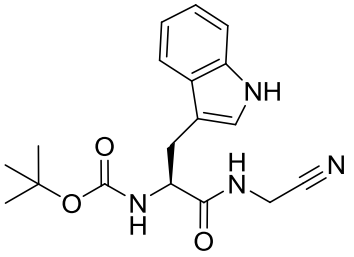
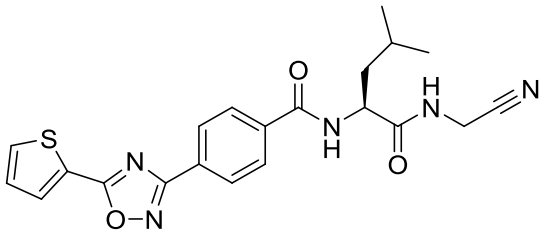
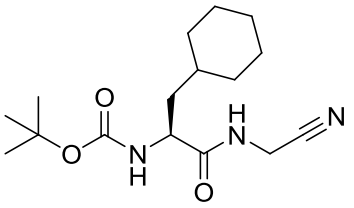
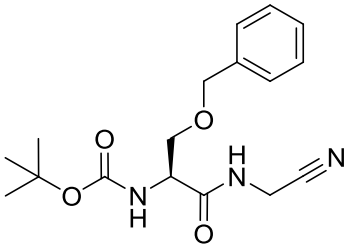
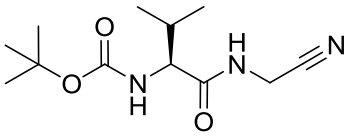
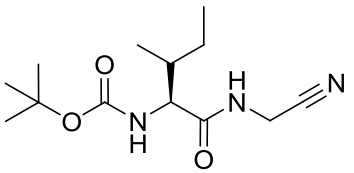
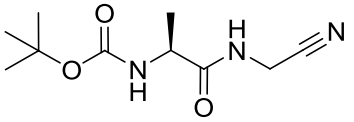
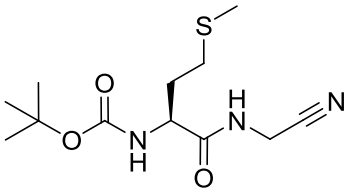
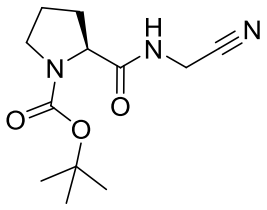
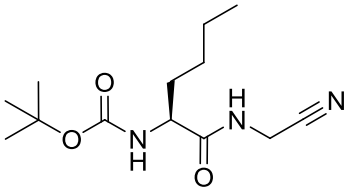
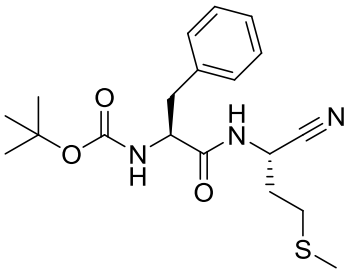
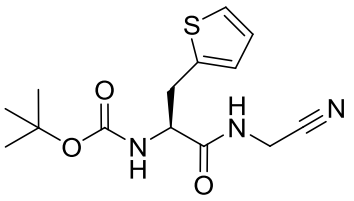
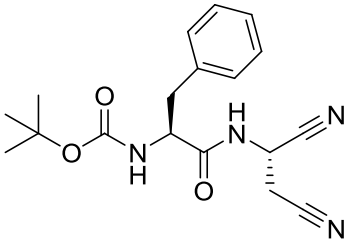
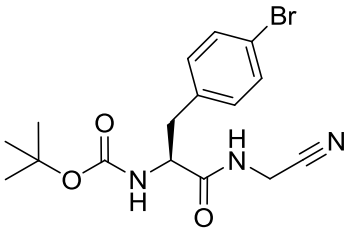
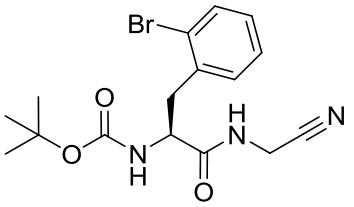
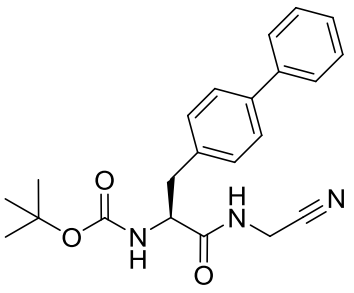
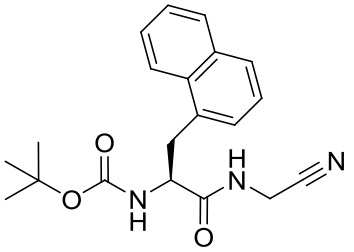
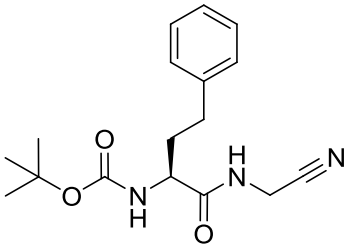
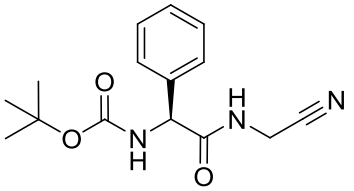
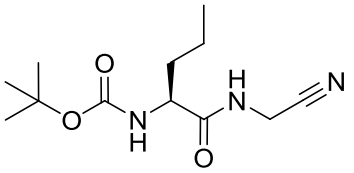
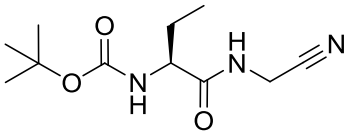
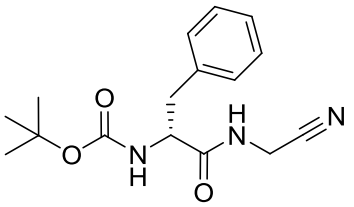
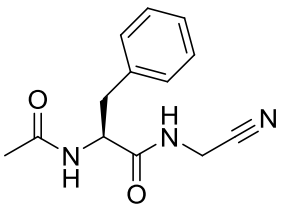
Table S1. Kinetic Evaluation of Dipeptide Nitrile Inhibitors at Human Cathepsin B

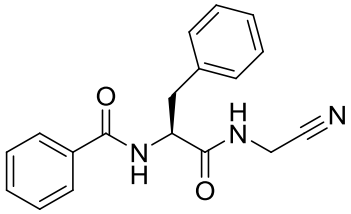
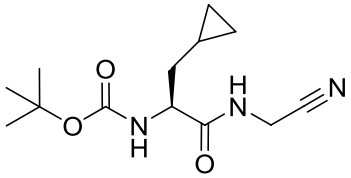
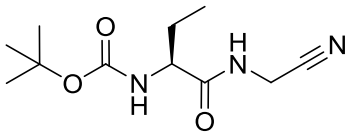
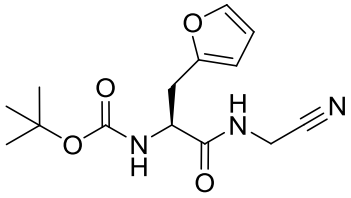
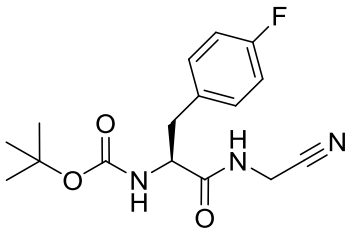
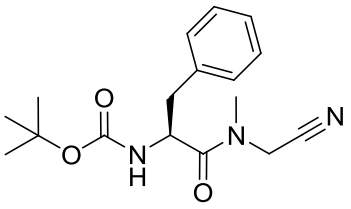
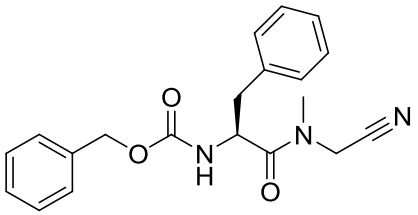
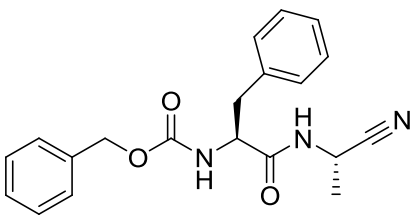
Values without standard errors refer to duplicate experiments with a single inhibitor concentration of 50 µM. IC_{50} values were calculated by using the equation $IC_{50} = [I]/(v_0/v - 1)$, where v and v_0 are the rates in the presence and absence of the inhibitor, respectively, and [I] is the inhibitor concentration. Values with standard errors refer to duplicate measurements in the presence of five different inhibitor concentrations. IC_{50} values were determined by nonlinear regression using equation $v = v_0/(1+[I]/IC_{50})$. Standard error of the mean (SEM) values refer to this nonlinear regression. K_i values ± SEM were calculated from IC_{50} values by applying the equation $K_i = IC_{50}/(1+[S]/K_m)$, where [S] is the substrate concentration.

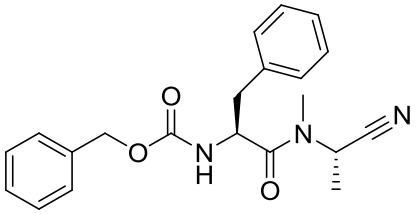
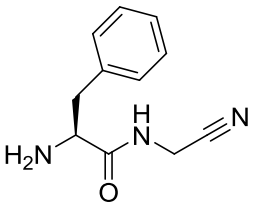
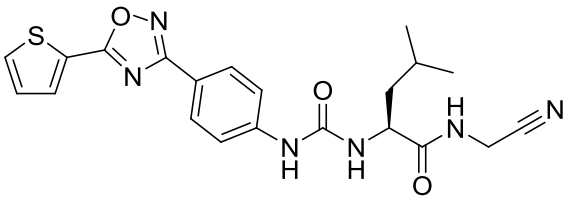
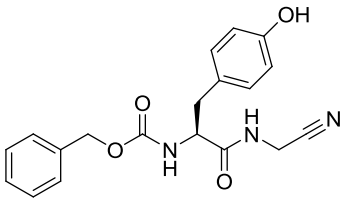
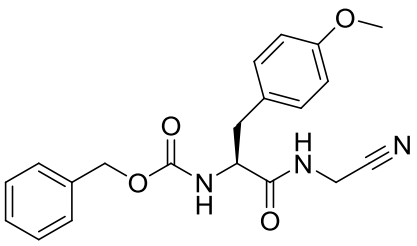
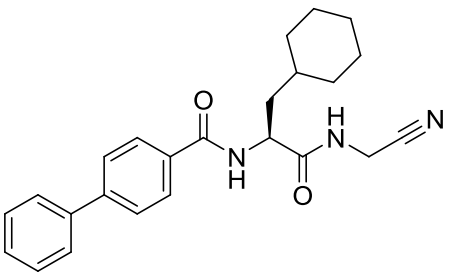
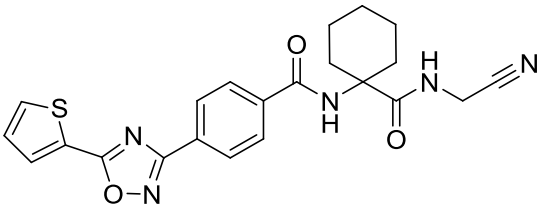
	compound	[I] (µM)	K_i (µM)
51		50	>500
52		50	>500
53			14.6 ± 0.8
54		50	22
55		50	25

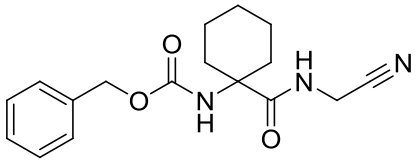
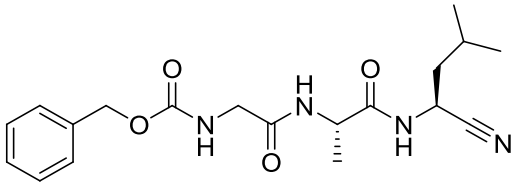
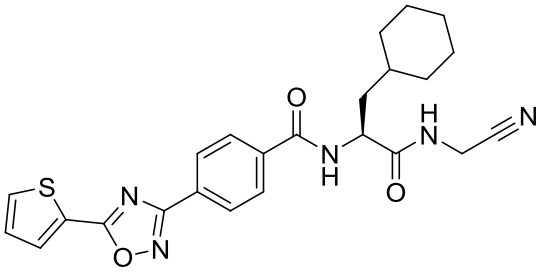
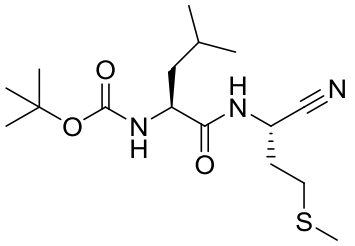
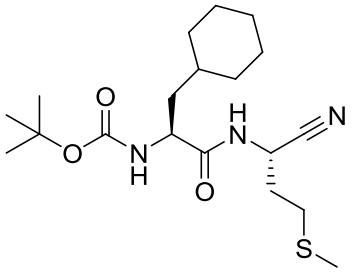
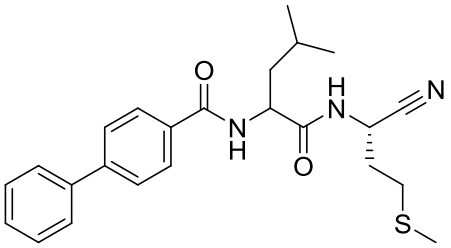
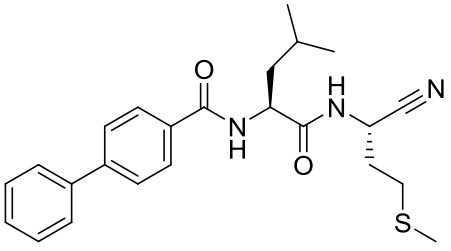
56			2.89 ± 0.13
57		50	48
58		50	190
59		50	68
60		50	79
61		50	280
62		50	61
63		50	>500

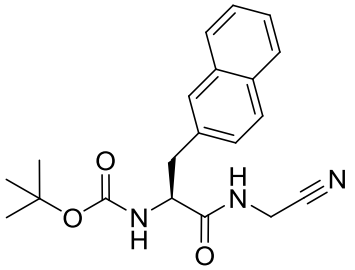
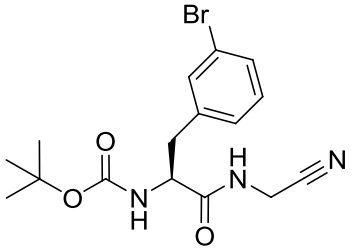
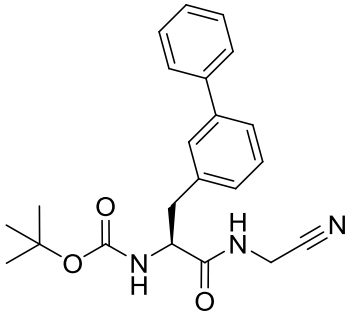
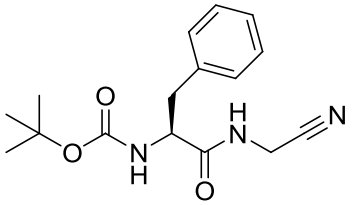
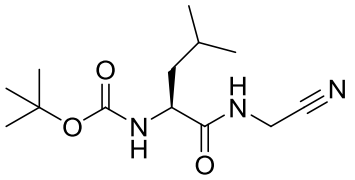
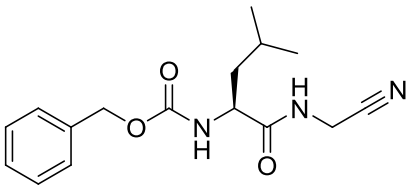
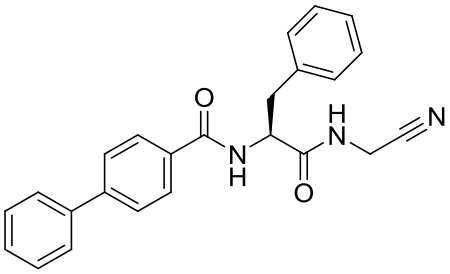
64		50	52
65			13.4 ± 1.7
66		50	36
67		50	72
68		50	52
69		50	>500
70		50	370

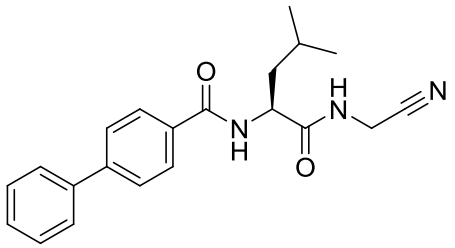
71		50	240
72		50	52
73		50	>500
74		50	27
75		50	43
76		50	>500
77		50	64

78			6.79 ± 0.44
79		50	48
80		50	70
81		50	29
82		50	80
83		50	340
84		50	470
85		50	34

86		50	200
87		50	>500
88		10	31
89			12.2 ± 2.0
90		50	21
91			0.76 ± 0.06
92		5	9.9

93		50	53
94		50	59
95			4.94 ± 1.31
96		50	36
97			16.6 ± 0.8
98			0.80 ± 0.04
99			0.33 ± 0.03

1			3.46 ± 0.19
2			5.17 ± 0.37
3			5.35 ± 0.21
4		50	37
5		50	94
6		50	32
7			3.92 ± 0.97

8		1.79 ± 0.14

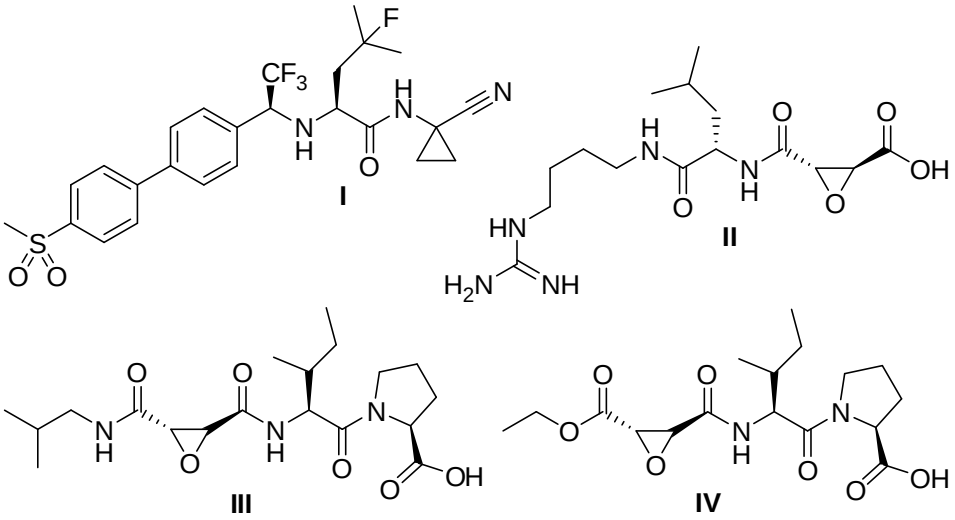


Figure S1. Structures of odanacatib (**I**) and the irreversible cathepsin B inhibitors E-64 (**II**), CA-074 (**III**) and CA-030 (**IV**).

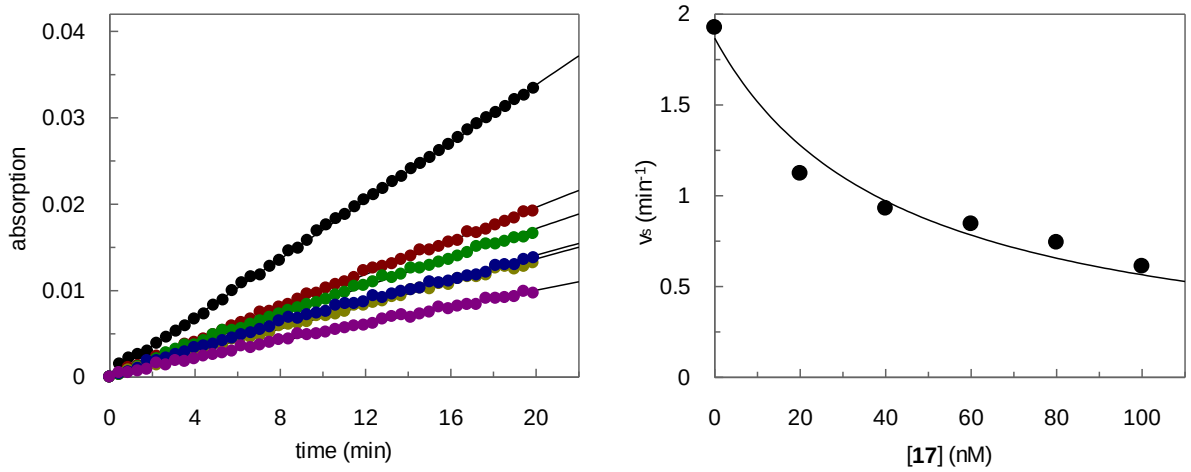
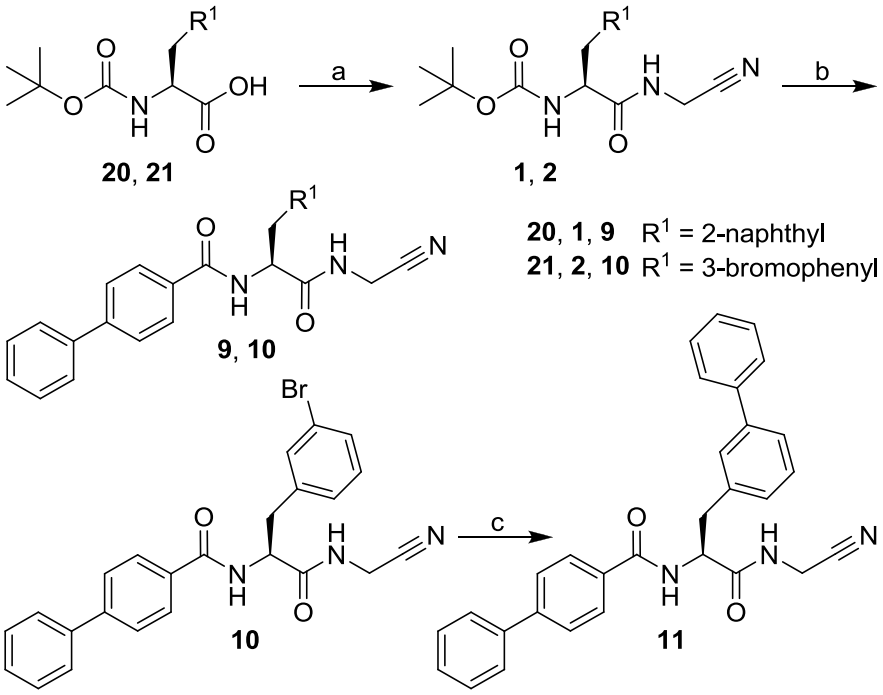


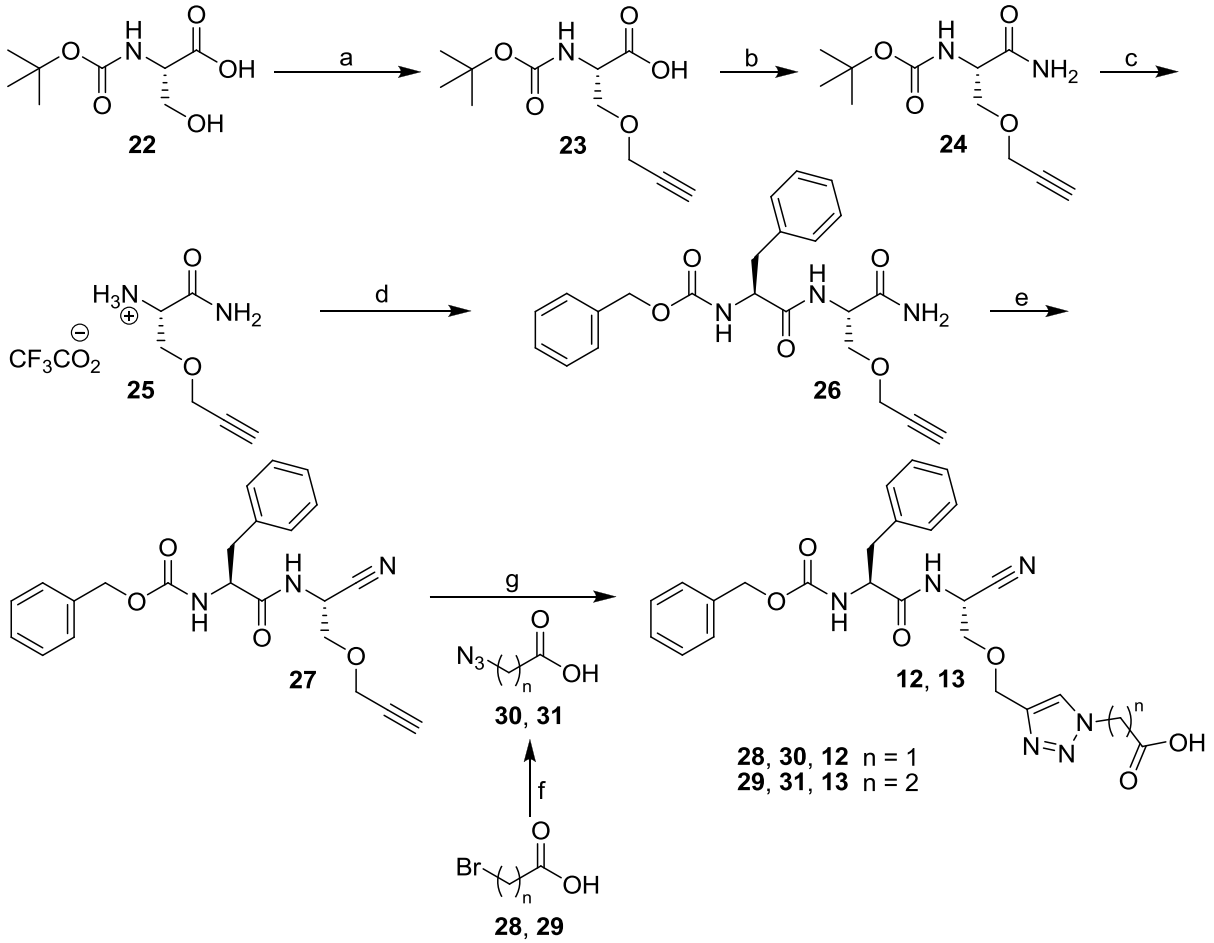
Figure S2. Inhibition of human cathepsin B by **17**. Left: Monitoring of the cathepsin B-catalyzed hydrolysis of Cbz-Arg-Arg-pNA at pH 4.5 in the presence of increasing inhibitor concentrations (•, 0 µM; •, 20 µM; •, 40 µM; •, 60 µM; •, 80 µM; •, 100 µM). Right: Plot of the steady-state rates v_s of hydrolysis of Cbz-Phe-Arg-pNA versus increasing concentrations of **17**. Non-linear regression gave an IC_{50} value of 43.3 ± 6.4 nM and an inhibition constant of K_i = 27.3 ± 4.0 nM.

Scheme S1. Synthesis of Dipeptide Nitriles 9-11[a]



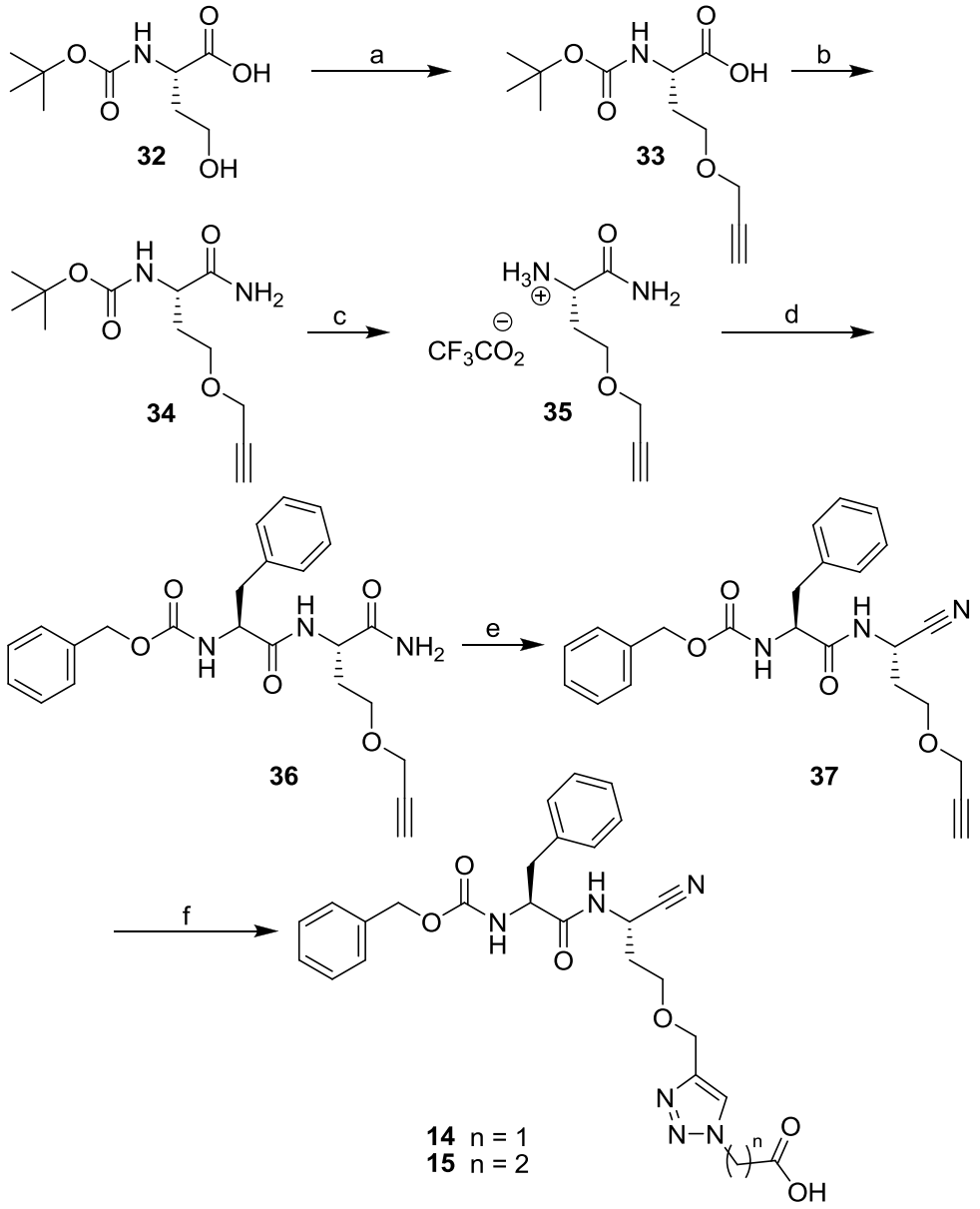
[a] Reagents and conditions: (a) 1. *N*-methylmorpholine, $ClCO_2i$-Bu, THF, -25 °C, 2. $H_2NCH_2CN \times 0.5\ H_2SO_4$, 2N NaOH, -25 °C to rt; (b) 1. methanesulfonic acid, THF, 0 °C to rt, 2. biphenyl-4-carboxylic acid, DIPEA, HATU, CH_2Cl_2, rt; (c) phenylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, dimethoxyethane, H_2O, microwave, 70 °C.

Scheme S2. Synthesis of Dipeptide Nitriles 12 and 13[a]



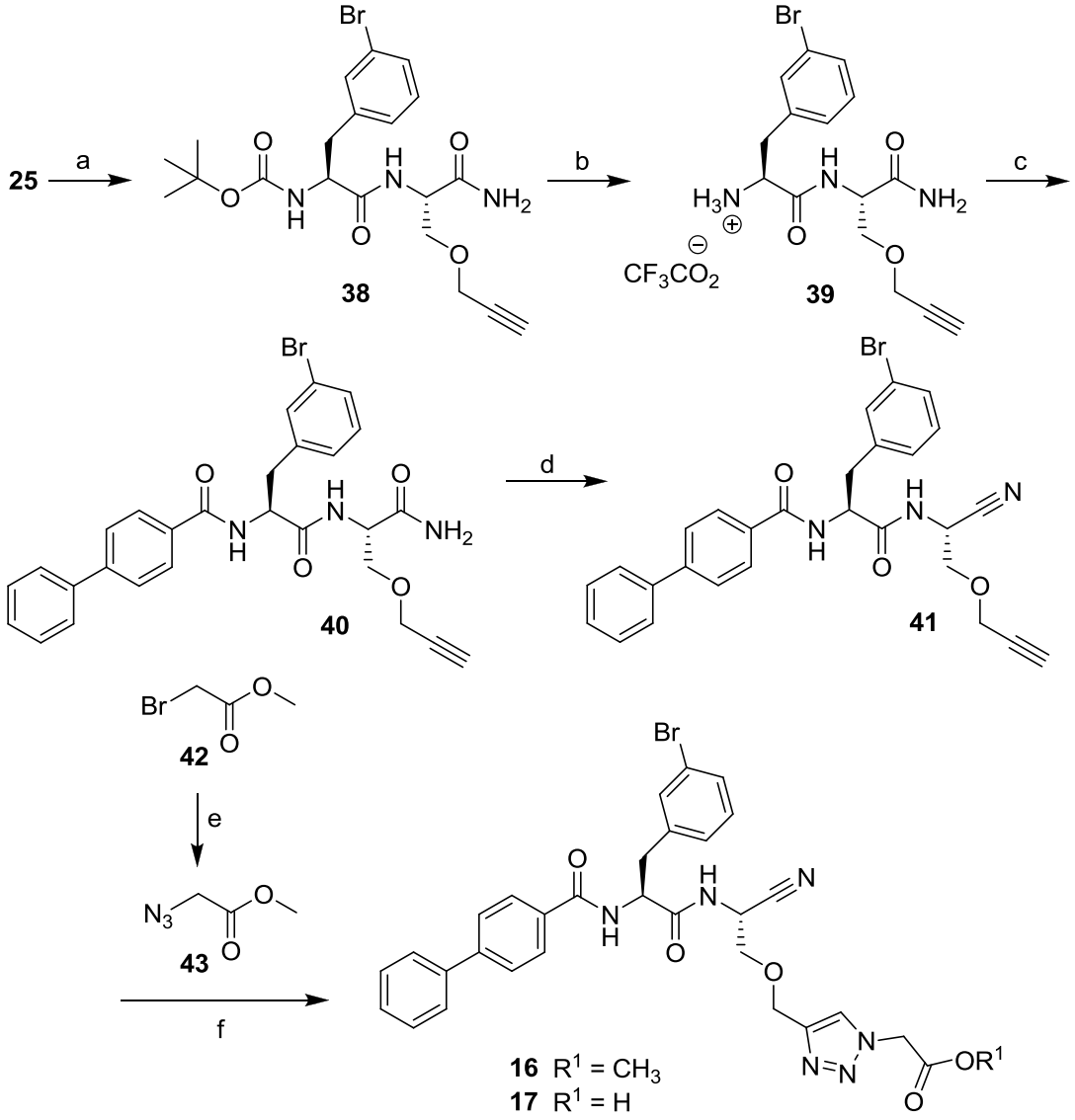
[a] Reagents and conditions: (a) NaH, propargyl bromide, DMF, 0 °C to rt; (b) 1. *N*-methylmorpholine, $ClCO_2$*i*-Bu, THF, -25 °C, 2. aqueous ammonia (25%), -25 °C to rt; (c) trifluoroacetic acid, CH_2Cl_2, 0 °C to rt; (d) Z-Phe-OH, DIPEA, HATU, CH_2Cl_2, rt; (e) cyanuric chloride, DMF, rt; (f) NaN_3, H_2O, rt; g) **30** or **31** $CuSO_4 \times 5\ H_2O$, sodium ascorbate, DMSO, H_2O, rt.

Scheme S3. Synthesis of Dipeptide Nitriles 14 and 15[a]



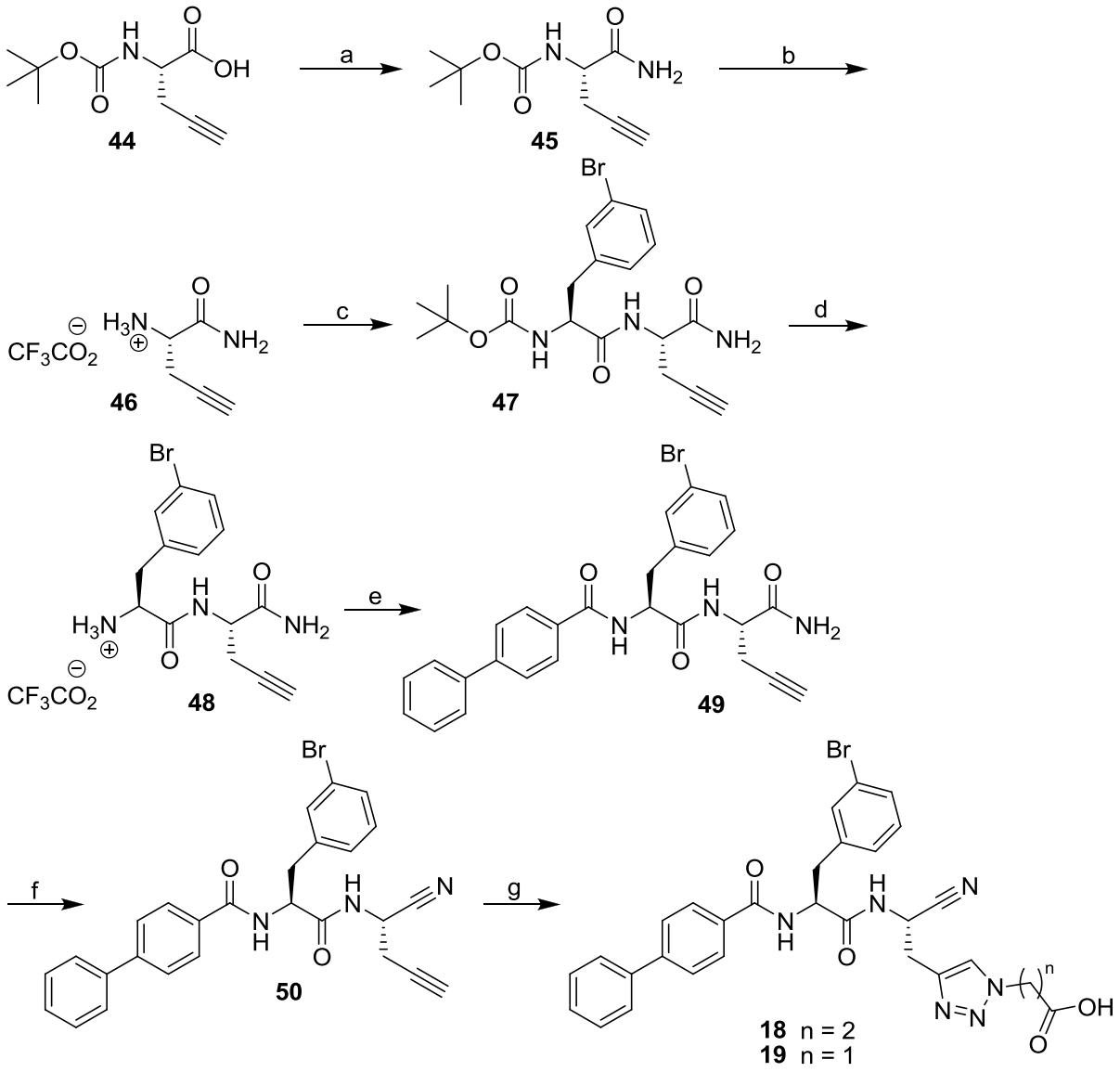
[a] Reagents and conditions: (a) NaH, propargyl bromide, THF, DMSO, -40 °C to rt; (b) 1. *N*-methylmorpholine, $ClCO_2$*i*-Bu, THF, -25 °C, 2. aqueous ammonia (25%), -25 °C to rt; (c) trifluoroacetic acid, CH_2Cl_2, 0 °C to rt; (d) Z-Phe-OH, DIPEA, HATU, CH_2Cl_2, rt; (e) cyanuric chloride, DMF, rt; (f) **30** or **31**, $CuSO_4 \times 5\ H_2O$, sodium ascorbate, DMSO, H_2O, rt.

Scheme S4. Synthesis of Dipeptide Nitriles 16 and 17[a]



[a] Reagents and conditions: (a) **21**, DIPEA, HATU, CH_2Cl_2, rt; (b) trifluoroacetic acid, CH_2Cl_2, 0 °C to rt; (c) biphenyl-4-carboxylic acid, DIPEA, HATU, CH_2Cl_2, rt; (d) cyanuric chloride, DMF, rt; (e) NaN_3, DMF, rt; (f) **43** or **30**, $CuSO_4 \times 5\ H_2O$, sodium ascorbate, DMSO, H_2O, rt.

Scheme S5. Synthesis of Dipeptide Nitriles 18 and 19[a]



[a] Reagents and conditions: (a) 1. *N*-methylmorpholine, $ClCO_2i$-Bu, THF, -25 °C, 2. aqueous ammonia (25%), -25 °C to rt; (b) trifluoroacetic acid, CH_2Cl_2, 0 °C to rt; (c) **21**, DIPEA, HATU, CH_2Cl_2, rt; (d) trifluoroacetic acid, CH_2Cl_2, 0 °C to rt; (e) biphenyl-4-carboxylic acid, DIPEA, HATU, CH_2Cl_2, rt; (f) cyanuric chloride, DMF, rt; (g) **31** or **30**, $CuSO_4 \times 5\ H_2O$, sodium ascorbate, DMSO, H_2O, rt.

General Methods and Materials

Reagents and solvents were obtained from abcr GmbH (Karlsruhe, Germany), Acros (Geel, Belgium), Alfa Aesar (Karlsruhe, Germany), Bachem AG (Bubendorf, Switzerland), Calbiochem (Darmstadt, Germany), Enzo Life Sciences (Lörrach, Germany), Fluorochem Ltd (Hadfield, United Kingdom), Merck (Darmstadt, Germany), Sigma-Aldrich (Steinheim, Germany), and TCI Deutschland GmbH (Eschborn, Germany). Preparative column chromatography was performed on silica gel 60, 0.060–0.200 mm (Acros Organics). Mass spectra were recorded on an API 2000 mass spectrometer (electron spray ion source, Applied Biosystems, Darmstadt, Germany) coupled with an Agilent 1100 HPLC system using a Phenomenex Luna HPLC C18 column (50 × 2.00 mm, particle size 3 μm). The purity of the tested compounds was determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC-MS/MS, HPLC Agilent 1100) using the procedure as follows: dissolving of the compounds at a concentration of 1.0 mg/mL in MeOH. Then, 10 μL of the substance solution was injected into a Phenomenex Luna C18 HPLC column (50 × 2.00 mm, particle size 3 μm) and elution performed with a gradient of water/MeOH containing 2 mM ammonium acetate from 90:10 in 10 min up to 0:100 to 20 min at a flow rate of 250 μL/min. UV absorption was detected from 220 to 400 nm using a diode array detector. All tested compounds possessed a purity of not less than 95%. ^{1}H and broadband, proton decoupled ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer with DMSO-d_6 as solvent. NMR spectra were recorded at room temperature. Chemical shifts are given in parts per million (ppm) relative to the remaining protons of the deuterated solvent used as internal standard. Coupling constants *J* are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Melting points were determined on a Büchi 510 oil bath apparatus and were uncorrected. Compounds **1-8** and **51-99** were prepared as described.[1-5]

Synthetic Procedures

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-2-naphthylalanyl-glycine nitrile (1). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-2'-naphthylalanine (**20**, 0.32 g, 1.00 mmol) was dissolved in dry THF (10 mL) and cooled at -25°C. To the stirred solution, *N*-methylmorpholine (0.10 g, 1.00 mmol) and isobutyl chloroformate (0.14 g, 1.00 mmol) were added consecutively, followed by aminoacetonitrile monosulfate (0.15 g, 1.00 mmol) dissolved in 2 N NaOH (0.50 mL, 1.00 mmol). The mixture was allowed to warm up to rt within 30 min and stirred overnight at rt. After evaporation of the solvent, the residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (2 × 30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether (1:1) to obtain **1** as a colorless solid (0.19 g, 54%); mp 144–146 °C, lit.[4] mp 147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.24 (s, 9H), 2.93 (dd, 1H, J = 13.6 Hz, J = 10.1 Hz), 3.14 (dd, 1H, J = 13.7 Hz, J = 4.6 Hz), 4.13 (d, 2H, J = 5.7 Hz), 4.24-4.29 (m, 1H), 7.08 (d, 1H, J = 8.5 Hz), 7.41-7.49 (m, 3H), 7.72 (s, 1H), 7.81 (t, 2H, J = 6.8 Hz), 7.85 (d, 1H, J = 7.6 Hz), 8.65 (t, 1H, J = 5.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.28, 28.18, 37.51, 55.66, 78.29, 117.60, 125.53, 126.06, 127.48, 127.57, 127.57, 127.62, 127.85, 131.98, 133.09, 135.70, 155.38, 172.44; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 354 ($[M + H]^+$), 97% purity.

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanyl-glycine nitrile (2). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanine (**21**, 0.34 g, 1.00 mmol) was coupled with aminoacetonitrile monosulfate (0.15 g, 1.00 mmol) following the procedure noted above. The crude product was recrystallized from ethyl acetate to obtain **2** as a colorless solid (0.18 g, 47%); mp 135–136 °C, lit.[4] mp 139 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29 (s, 9H), 2.73 (dd, 1H, J = 13.6 Hz, J = 10.7 Hz), 2.97 (dd, 1H, J = 13.6 Hz, J = 4.1 Hz), 4.12-4.17 (m, 3H), 7.09 (d, 1H, J = 8.9 Hz), 7.21-7.26 (m, 2H), 7.38 (d, 1H, J = 7.3 Hz), 7.47 (s, 1H), 8.64 (t, 1H, J = 5.4 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.28, 28.21, 36.80, 55.43, 78.36, 117.60, 121.49, 128.47, 129.29, 130.31, 132.05, 141.01, 155.41, 172.20; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 382 and 384 ($[M + H]^+$), 98% purity.

(*S*)-*N*-(4-Phenylbenzoyl)-2-naphthylalanyl-glycine nitrile (9). Compound **1** (0.19 g, 0.54 mmol) was dissolved in dry THF (10 mL). Under ice cooling, methanesulfonic acid (0.31 g, 3.24 mmol) was added. The resulting mixture was stirred at rt overnight. The solvent was evaporated and the crude product, 2-naphthylalanyl-glycine nitrile methanesulfonate, was used without further purification. Biphenyl-4-carboxylic acid (0.10 g, 0.50 mmol) was dissolved in dry CH_2Cl_2 (15 mL). DIPEA (0.52 g, 4.00 mmol) and HATU (0.19 g, 0.50 mmol) were added and stirred for 10 min at rt. 2-Naphthylalanyl-glycine nitrile methanesulfonate (0.17 g, 0.50 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate (30 mL). The organic layer was washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL), sat. $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate to obtain **9** as a colorless solid (0.15 g, 64% over two steps); mp 217–218 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.20 (dd, 1H), 4.14-4.21 (m, 2H), 4.82-4.85 (m, 1H), 7.38-7.48 (m, 5H), 7.51 (d, 1H, J = 7.1 Hz), 7.69-7.73 (m, 4H), 7.79-7.83 (m, 4H), 7.89 (d, 2H, J = 7.1 Hz), 8.79-8.82 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.41, 37.21, 54.89, 117.68, 125.59, 126.14, 126.52, 127.00, 127.50, 127.57, 127.61, 127.69, 127.88, 128.21, 128.30, 129.16, 131.97, 132.75, 133.12, 135.99, 139.27, 143.06, 166.18, 172.16; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 434 ($[M + H]^+$), 98% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{28}H_{23}N_3O_2$: 434.1863, found: 434.1852.

(*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-glycine nitrile (10). Compound **2** (0.21 g, 0.54 mmol), dissolved in dry THF (7.3 mL) was deprotected with methanesulfonic acid (0.31 g, 3.24 mmol) and the crude product, 3-bromophenylalanyl-glycine nitrile methanesulfonate, was coupled with biphenyl-4-carboxylic acid following the procedure noted above. The crude product was recrystallized from ethyl acetate to obtain **10** as a colorless solid (0.17 g, 68% over two steps); mp 216–217 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.02 (dd, 1H, J = 13.7 Hz, J = 10.9 Hz), 3.14 (dd, 1H, J = 13.9 Hz, J = 4.1 Hz), 4.16-4.19 (m, 2H), 4.70-4.74 (m, 1H), 7.22 (t, 1H, J = 7.7 Hz), 7.33-7.37 (m, 2H), 7.38-7.41 (m, 1H), 7.47-7.50 (m, 2H), 7.58 (t, 1H, J = 1.6 Hz), 7.71-7.73 (m, 2H), 7.75-7.76 (m, 2H), 7.89-7.92 (m, 2H), 8.75 (d, 1H, J = 8.5 Hz), 8.80 (t, 1H, J = 5.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.36, 36.48, 54.57, 117.63, 121.54, 126.52, 127.00, 128.20, 128.28, 128.38, 129.15, 129.36, 130.36, 132.07, 132.72, 139.25, 141.20, 143.08, 166.22, 171.91; LC-MS (ESI) (90% H_2O to 100% MeOH in

10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 462 and 464 ($[M + H]^+$), 98% purity. QTOF: HRMS (ESI): m/z $[M - H]^-$ calcd. for $C_{24}H_{20}BrN_3O_2$: 460.0666 and 462.0646, found: 460.0666 and 462.0647. HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{24}H_{20}BrN_3O_2$: 462.0812 and 464.0791, found: 462.0803 and 464.0789.

(*S*)-*N*-(4-Phenylbenzoyl)-3-biphenylalanyl-glycine nitrile (11). Compound **10** (46.0 mg, 0.10 mmol), phenylboronic acid (18.0 mg, 0.15 mmol), and K_2CO_3 (30.0 mg, 0.22 mmol) were dissolved in dimethoxyethane (5 mL) and H_2O (2 mL). $Pd(PPh_3)_4$ (12.0 mg, 0.01 mmol) was added. The resulting mixture was placed into a microwave reactor (sealed tube) and irradiated for 60 min at 40 W and 70 °C. The reaction mixture was transferred into a round-bottom flask and evaporated. The residue was suspended in H_2O (20 mL). The aqueous suspension was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 10% $KHSO_4$ (2 × 20 mL), H_2O (30 mL), sat. $NaHCO_3$ (2 × 20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether (1:1) to obtain **11** as a colorless solid (27.0 mg, 59%); mp 215–216 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.11 (dd, 1H, *J* = 11.4 Hz, *J* = 9.1 Hz), 3.23 (dd, 1H, *J* = 11.4 Hz, *J* = 3.5 Hz), 4.15-4.23 (m, 2H), 4.77-4.81 (m, 1H), 7.31-7.36 (m, 3H), 7.38-7.43 (m, 3H), 7.45-7.49 (m, 3H), 7.58 (d, 2H, *J* = 6.0 Hz), 7.65 (s, 1H), 7.70-7.74 (m, 4H), 7.93 (d, 2H, *J* = 7.0 Hz), 8.79 (d, 1H, *J* = 6.9 Hz), 8.82 (t, 1H, *J* = 4.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.40, 36.97, 54.86, 117.69, 124.87, 126.49, 126.79, 127.00, 127.49, 127.81, 128.21, 128.33, 128.38, 128.86, 128.96, 129.16, 132.77, 138.98, 139.28, 140.15, 140.37, 143.08, 166.20, 172.20; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 460 ($[M + H]^+$), 97% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{30}H_{25}N_3O_2$: 460.2020, found: 460.2015.

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-*O*-(propynyl)-serine amide (24). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-serine (**22**, 2.05 g, 10.0 mmol) was dissolved dry DMF (30 mL). Under ice cooling, sodium hydride (60%, 0.97 g, 24.0 mmol) was added slowly and stirred for 30 min. Then, the reaction mixture was treated with propargyl bromide (80%, 2.09 g, 14.0 mmol) and stirred for 4 h at rt. After evaporation of the solvent, the residue was suspended in 10% $KHSO_4$ (50 mL). The aqueous suspension was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product, (*S*)-*N*-(*tert*-

butyloxycarbonyl)-*O*-(propynyl)-serine (**23**), was used without further purification as brown oil. Compound **23** (1.46 g, 6.00 mmol) was dissolved in dry THF (30 mL) and cooled at -25 °C. To the stirred solution, *N*-methylmorpholine (0.61 g, 6.00 mmol) and isobutyl chloroformate (0.82 g, 6.00 mmol) were added consecutively. Concd aqueous ammonia solution (25%, 2.25 mL, 30.0 mmol) was given to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. It was allowed to warm to rt within 30 min and stirred for 6 h at rt. After evaporation of the solvent the resulting aqueous residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 1:1 to obtain **24** as a colorless oil (0.81 g, 33% over two steps); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (s, 9H), 3.41 (t, 1H, J = 2.4 Hz), 3.53 (dd, 1H, J = 9.6 Hz, J = 6.8 Hz), 3.59 (dd, 1H, J = 9.6 Hz, J = 4.9 Hz), 4.04-4.08 (m, 1H), 4.12 (t, 2H, J = 2.1 Hz), 6.65 (d, 1H, J = 8.2 Hz), 7.06 (s, 1H), 7.29 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.28, 54.15, 57.73, 69.59, 77.45, 78.34, 80.13, 155.23, 171.72; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 243 ($[M + H]^+$), 100% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-serine amide (26). Compound **24** (0.48 g, 2.00 mmol) was dissolved in trifluoroacetic acid/CH_2Cl_2 (1:1, 30 mL) and stirred for 3 h at 0 °C. The solvent was evaporated under reduced pressure to obtain (*S*)-*O*-(propynyl)-serine amide trifluoroacetate (**25**) as an oily residue. Compound **25** (0.51 g, 2.00 mmol) was dissolved in dry THF (15 mL) and cooled at -25 °C. To the stirred solution, *N*-methylmorpholine (0.20 g, 2.00 mmol) and isobutyl chloroformate (0.27 g, 2.00 mmol) were added consecutively. (*S*)-*N*-(Benzyloxycarbonyl)-phenylalanine (0.60 g, 2.00 mmol) was dissolved in 2N NaOH (5.00 mL, 10.0 mmol) and the resulting solution was given to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. It was allowed to warm to rt within 30 min and stirred overnight at rt. After evaporation of the solvent, the resulting aqueous residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was recrystallized from ethyl acetate to obtain **26** as a colorless solid (0.18 g, 21%). mp 198–200 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.73 (dd, 1H, J = 13.7 Hz, J = 10.9 Hz), 3.03 (dd, 1H, J = 13.9 Hz, J = 3.8 Hz), 3.43 (t, 1H, J = 2.4 Hz), 3.60 (dd,

1H, *J* = 9.6 Hz, *J* = 5.2 Hz), 3.66 (dd, 1H, *J* = 9.8 Hz, *J* = 5.7 Hz), 4.15 (s, 2H), 4.29–4.33 (m, 1H), 4.38–4.42 (m, 1H), 4.94 (s, 2H), 7.13 (s, 1H), 7.17–7.33 (m, 11H), 7.50 (d, 1H, *J* = 8.6 Hz), 8.06 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.48, 52.57, 56.26, 57.91, 65.36, 69.54, 77.58, 80.08, 126.34, 127.50, 127.77, 128.14, 128.41, 129.35, 137.12, 138.19, 155.97, 171.10, 171.56; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 424 ($[M + H]^+$), 100% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-serine nitrile (27). Compound **26** (0.42 g, 1.00 mmol) was dissolved in dry DMF (15 mL). Cyanuric chloride (0.18 g, 1.00 mmol) was added and the mixture was stirred for 14 h at rt. The solvent was evaporated under reduced pressure. The resulting solid was treated with 10% $NaHCO_3$ (20 mL) and stirred for 10 min at rt. The aqueous suspension was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude colorless solid **27** was used without further purification (0.36 g, 89%); mp 130–131 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.76 (dd, 1H, *J* = 13.6 Hz, *J* = 10.4 Hz), 2.96 (dd, 1H, *J* = 13.7 Hz, *J* = 4.6 Hz), 3.52 (t, 1H, *J* = 2.4 Hz), 3.68 (d, 2H, *J* = 5.7 Hz), 4.22-4.31 (m, 3H), 4.99-5.03 (m, 1H), 4.91-4.97 (m, 2H), 7.18–7.33 (m, 10H), 7.56 (d, 1H, *J* = 8.5 Hz), 8.94 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.37, 40.53, 55.97, 58.16, 65.42, 67.94, 78.18, 79.60, 117.97, 126.49, 127.58, 127.82, 128.20, 128.41, 129.34, 137.07, 137.76, 155.94, 171.93; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 406 ($[M + H]^+$), 82% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (12). Bromoacetic acid (**28**, 0.69 g, 5.00 mmol) was dissolved in H_2O (10 mL). Sodium azide (0.65 g, 10.00 mmol) was added and it was stirred overnight. The reaction mixture was acidified with concd HCl to pH = 1 and extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated at 25 °C to obtain azidoacetic acid (**30**) as a colorless oil, which was used without further purification (0.51 g, quantitative yield). Compound **27** (0.20 g, 0.50 mmol) and 2-azidoacetic acid (**30**, 51.0 mg, 0.50 mmol) were dissolved in DMSO/H_2O (2:1, 15 mL). Sodium ascorbate (0.10 g, 0.50 mmol) and copper(II) sulfate pentahydrate (62.0 mg, 0.25 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 10% $KHSO_4$ solution (50 mL) was added and the aqueous suspension was extracted with ethyl

acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/MeOH/acetic acid (89:10:1) to obtain **12** as colorless solid (90.0 mg, 36%). mp 183–184 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.76 (dd, 1H, *J* = 13.6 Hz, *J* = 10.4 Hz), 2.95 (dd, 1H, *J* = 13.9 Hz, *J* = 4.4 Hz), 3.70 (d, 2H, *J* = 6.0 Hz), 4.26-4.31 (m, 1H), 4.66 (s, 2H), 4.90-4.97 (m, 2H), 4.98-5.02 (m, 1H), 5.25 (s, 2H), 7.18-7.33 (m, 10H), 7.58 (d, 1H, *J* = 8.2 Hz), 8.10 (s, 1H), 8.98 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.38, 40.66, 50.71, 56.02, 63.97, 65.45, 68.40, 118.10, 125.74, 126.51, 127.61, 127.85, 128.23, 128.44, 129.37, 137.09, 137.81, 143.27, 156.00, 168.69, 171.97; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 507 ($[M + H]^+$), 95% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{25}H_{26}N_6O_6$: 507.1987, found: 507.1988.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(2-carboxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (13). 3-Bromopropionic acid (**29**, 0.76 g, 5.00 mmol) was dissolved in H_2O (10 mL). Sodium azide (0.65 g, 10.00 mmol) was added and stirred overnight. The reaction mixture was acidified with concd HCl to pH = 1 and extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated at 25 °C to obtain 3-azidopropanoic acid (**31**) as light yellow oil, which was used without further purification (0.58 g, quantitative yield). Compound **27** (0.13 g, 0.32 mmol) and 3-azidopropanoic acid (**31**, 37.0 mg, 0.32 mmol) were dissolved in DMSO/H_2O (2:1, 12 mL). Sodium ascorbate (63.0 mg, 0.32 mmol) and copper(II) sulfate pentahydrate (40.0 mg, 0.16 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 10% $KHSO_4$ solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/acetic acid (99:1) to obtain **13** as a colorless solid (61.0 mg, 36%). mp 80–82 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.75 (dd, 1H, *J* = 13.6 Hz, *J* = 10.4 Hz), 2.85 (t, 2H, *J* = 13.6 Hz), 2.95 (dd, 1H, *J* = 13.7 Hz, *J* = 4.3 Hz), 3.68 (d, 2H, *J* = 5.7 Hz), 4.25-4.30 (m, 1H), 4.53 (t, 2H, *J* = 6.8 Hz), 4.63 (s, 2H), 4.90-4.96 (m, 2H), 4.97-5.01 (m, 1H), 7.18-7.33 (m, 10H), 7.63 (d, 1H, *J* = 7.9 Hz), 8.10 (s, 1H), 8.99 (d, 1H, *J* = 7.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 34.44, 37.37, 40.63, 45.63, 56.01, 63.96, 65.43, 68.31, 118.07, 124.49, 126.48, 127.58, 127.82, 128.21, 128.41, 129.34, 137.08, 137.79, 143.20, 155.98, 171.94; LC-MS (ESI) (90%

H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 521 ($[M + H]^+$), 97% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{26}H_{28}N_6O_6$: 521.2143, found: 521.2167.

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-*O*-(propynyl)-homoserine amide (34). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-homoserine (**32**, 1.21 g, 5.50 mmol) was dissolved dry THF/DMSO (1:4, 25 mL). Under cooling to -50 °C, sodium hydride (60%, 0.75 g, 18.7 mmol) was added slowly and it was stirred for 30 min. Then, the reaction mixture was treated with propargyl bromide (80%, 1.64 g, 10.0 mmol) and stirred for 60 min at -40 °C. It was allowed to warm to rt within 60 min and stirred for 20 h at rt. After evaporation of the solvent, the residue was suspended in 10% $KHSO_4$ (50 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated to obtain (*S*)-*N*-(*tert*-butyloxycarbonyl)-*O*-(propynyl)-homoserine (**33**) as brown oil without further purification. The crude product (1.42 g, 5.50 mmol) was dissolved in dry THF (40 mL) and cooled at -25 °C. To the stirred solution, *N*-methylmorpholine (0.56 g, 5.50 mmol) and isobutyl chloroformate (0.75 g, 5.50 mmol) were added consecutively. Concd aqueous ammonia solution (25%, 2.05 mL, 27.5 mmol) was given to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. It was allowed to warm to rt within 30 min and stirred for 18 h at rt. After evaporation of the solvent, the resulting oily residue was suspended in ethyl acetate (70 mL). The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2:1 to obtain **34** as a colorless oil (0.94 g, 67% over two steps); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (s, 9H), 1.65-1.72 (m, 1H), 1.83-1.89 (m, 1H), 3.37 (s, 1H), 3.43 (t, 2H, *J* = 6.2 Hz), 3.88-3.93 (m, 1H), 4.08 (d, 2H, *J* = 1.3 Hz), 6.74 (d, 1H, *J* = 7.9 Hz), 6.92 (s, 1H), 7.19 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.33, 31.84, 51.54, 57.55, 66.27, 77.10, 78.13, 80.51, 155.42, 174.03; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 257 ($[M + H]^+$), 100% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-homoserine amide (36). Compound **34** (0.90 g, 3.50 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and cooled in an ice bath. Trifluoroacetic acid (20 mL) was added and the reaction mixture was stirred for 2 h. The solvent was evaporated under reduced pressure to obtain (*S*)-*O*-(propynyl)-homoserine amide

trifluoroacecate (**35**) as an oily residue. (*S*)-*N*-(Benzyloxycarbonyl)-phenylalanine (1.05 g, 3.50 mmol) was dissolved in dry CH_2Cl_2 (20 mL). DIPEA (2.26 g, 17.5 mmol) and HATU (1.33 g, 3.50 mmol) were added and it was stirred for 10 min at rt. Compound **35** (0.95 g, 3.50 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate. The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate to obtain **36** as a light yellow solid (1.11 g, 72% over two steps); mp 140–141 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.71-1.78 (m, 1H), 1.88-1.98 (m, 1H), 2.75 (dd, 1H, *J* = 13.9 Hz, *J* = 10.7 Hz), 3.02 (dd, 1H, *J* = 13.9 Hz, *J* = 4.1 Hz), 3.36 (t, 1H, *J* = 2.4 Hz), 3.39-3.48 (m, 2H), 4.06 (t, 2H, *J* = 2.7 Hz), 4.24-4.29 (m, 2H), 4.94 (s, 2H), 7.03 (s, 1H), 7.23-7.34 (m, 11H), 7.49 (d, 1H, *J* = 8.5 Hz), 8.00 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 32.04, 37.36, 49.91, 56.36, 57.57, 65.39, 66.15, 77.11, 80.51, 126.37, 127.57, 127.80, 128.17, 128.42, 129.32, 137.11, 138.19, 156.00, 171.44, 173.19; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 438 ($[M + H]^+$), 91% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-homoserine nitrile (37). Compound **36** (0.66 g, 1.50 mmol) was dissolved in dry DMF (20 mL) and cyanuric chloride (0.28 g, 1.50 mmol) was added. The reaction mixture was stirred overnight at rt. Then, H_2O (30 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/ petroleum ether 3:1 to obtain **37** as colorless solid (0.47 g, 75%). mp = 141–142 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.95-2.01 (m, 1H), 2.03-2.09 (m, 1H), 2.79 (dd, 1H, *J* = 13.3 Hz, *J* = 10.4 Hz), 2.97 (dd, 1H, *J* = 13.7 Hz, *J* = 4.6 Hz), 3.41 (t, 1H, *J* = 1.9 Hz), 3.47-3.56 (m, 2H), 4.06-4.15 (m, 2H), 4.19-4.23 (m, 1H), 4.74-4.79 (m, 1H), 4.92-4.98 (m, 2H), 7.20-7.34 (m, 10H), 7.60 (d, 1H, *J* = 8.2 Hz), 8.78 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.69, 37.36, 37.54, 56.15, 57.70, 64.81, 65.45, 77.43, 80.18, 119.01, 126.50, 127.61, 127.83, 128.24, 128.41, 129.29, 137.06, 137.75, 155.97, 171.68; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 420 ($[M + H]^+$), 83% purity.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-homoserine nitrile (14). Compound **37** (0.21 g, 0.50 mmol) and azidoacetic acid (**30**, 51.0 mg, 0.50 mmol) were dissolved in DMSO/H_2O (2:1, 30 mL). Sodium ascorbate (0.10 g, 0.50 mmol) and copper(II) sulfate pentahydrate (62.0 mg, 0.25 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 2N HCl solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/MeOH/acetic acid (89:10:1) to obtain **14** as colorless solid (0.18 g, 69%). mp 159–160 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.95-2.07 (m, 2H), 2.78 (dd, 1H, *J* = 13.6 Hz, *J* = 10.1 Hz), 2.97 (dd, 1H, *J* = 13.7 Hz, *J* = 4.9 Hz), 3.49-3.53 (m, 2H), 4.19-4.24 (m, 1H), 4.50 (s, 2H), 4.74-4.79 (m, 1H), 4.91-4.98 (m, 2H), 5.09 (s, 2H), 7.18-7.32 (m, 10H), 7.62 (d, 1H, *J* = 8.2 Hz), 8.01 (s, 1H), 8.81 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.82, 37.58, 40.61, 51.46, 56.21, 63.70, 65.12, 65.48, 119.12, 125.32, 126.52, 127.63, 127.83, 128.26, 128.42, 129.32, 137.07, 137.79, 143.64, 156.00, 168.61, 171.74; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 521 ($[M + H]^+$), 94% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{26}H_{28}N_6O_6$: 521.2143, found: 521.2172.

(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(2-carboxyethyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-homoserine nitrile (15). Compound **37** (0.21 g, 0.50 mmol) and 3-azidopropanoic acid (**31**, 58.0 mg, 0.50 mmol) were dissolved in DMSO/H_2O (2:1, 30 mL). Sodium ascorbate (0.10 g, 0.50 mmol) and copper(II) sulfate pentahydrate (62.0 mg, 0.25 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 2N HCl solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/MeOH/acetic acid (94:5:1) to obtain **15** as a colorless resin (81.0 mg, 30%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.93-2.07 (m, 2H), 2.78 (dd, 1H, *J* = 13.6 Hz, *J* = 10.1 Hz), 2.86 (t, 2H, ^3J = 6.6 Hz), 2.96 (dd, 1H, *J* = 13.6 Hz, *J* = 4.4 Hz), 3.50 (t, 2H, *J* = 5.8 Hz), 4.19-4.23 (m, 1H), 4.48 (s, 2H), 4.52 (t, 2H, *J* = 6.8 Hz), 4.75-4.79 (m, 1H), 4.91-4.98 (m, 2H), 7.19-7.32 (m, 10H), 7.61 (d, 1H, *J* = 8.6 Hz), 8.04 (s, 1H), 8.79 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.81, 34.29, 37.25, 37.57, 45.50, 56.18, 63.68, 65.10, 65.47, 119.08, 124.16, 126.51, 127.62, 127.83, 128.25, 128.41, 129.30, 137.05, 137.76, 143.77,

155.99, 171.71, 171.89; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 535 ($[M + H]^+$), 97% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{27}H_{30}BrN_6O_6$: 535.2305, found: 535.2314.

(*S,S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanyl-*O*-(propynyl)-serine amide (38). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanine (**21**, 0.59 g, 1.70 mmol) was dissolved in dry CH_2Cl_2 (20 mL). DIPEA (1.10 g, 8.5 mmol) and HATU (0.65 g, 1.70 mmol) were added and it was stirred for 10 min at rt. (*S*)-*O*-(Propynyl)-serine amide trifluoroacetate (**25**, 0.44 g, 1.70 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate. The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether (2:1) to obtain **38** as a colorless solid (0.80 g, 99%); mp 140–141 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29 (s, 9H), 2.69-2.72 (m, 1H), 2.99-3.01 (m, 1H), 3.42 (t, 1H, *J* = 2.2 Hz), 3.59 (dd, 1H, *J* = 9.6 Hz, *J* = 5.1 Hz), 3.67 (dd, 1H, *J* = 9.8 Hz, *J* = 5.5 Hz), 4.15 (s, 2H), 4.17-4.21 (m, 1H), 4.36-4.40 (m, 1H), 7.01 (d, 1H, *J* = 8.5 Hz), 7.14 (s, 1H), 7.22 (t, 1H, *J* = 7.6 Hz), 7.27 (d, 1H, *J* = 7.6 Hz), 7.36 (t, 2H, *J* = 9.1 Hz), 7.50 (s, 1H), 7.96 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.23, 36.94, 52.51, 55.64, 57.93, 69.56, 77.59, 78.36, 80.05, 121.44, 128.55, 129.17, 130.22, 132.10, 141.27, 155.43, 171.11, 171.43; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 468 and 470 ($[M + H]^+$), 91% purity.

(*S,S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-(propynyl)-serine amide (40). Compound **38** (0.68 g, 1.45 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and cooled at 0 °C. Trifluoroacetic acid (20 mL) was added and the reaction mixture was stirred for 2 h. The solvent was evaporated under reduced pressure to obtain (*S*)-3-bromophenylalanyl-*O*-(propynyl)-serine amide trifluoroacetate (**39**) as an oily residue. Biphenyl-4-carboxylic acid (0.29 g, 1.45 mmol) was dissolved in dry CH_2Cl_2 (20 mL). DIPEA (0.94 g, 7.25 mmol) and HATU (0.55 g, 1.45 mmol) were added and stirred for 10 min at rt. Compound **39** (0.70 g, 1.45 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate. The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was

dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether (2:1) to obtain **40** as a colorless solid (0.40 g, 50% over two steps); mp 229–230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.98 (dd, 1H, *J* = 11.4 Hz, *J* = 9.4 Hz), 3.16 (dd, 1H, *J* = 11.5 Hz, *J* = 3.1 Hz), 3.44 (t, 1H, *J* = 2.0 Hz), 3.63 (dd, 1H, *J* = 8.1 Hz, *J* = 4.2 Hz), 3.69 (dd, 1H, *J* = 8.0 Hz, *J* = 4.9 Hz), 4.14-4.20 (m, 2H), 4.41-4.44 (m, 1H), 4.77-4.81 (m, 1H), 7.16 (s, 1H), 7.21 (t, 1H, *J* = 6.6 Hz), 7.34-7.41 (m, 4H), 7.48 (t, 2H, *J* = 6.4 Hz), 7.65 (s, 1H), 7.71-7.75 (m, 4H), 7.87 (d, 2H, *J* = 6.9 Hz), 8.24 (d, 1H, *J* = 6.9 Hz), 8.66 (d, 1H, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.05, 53.05, 55.14, 58.28, 69.92, 77.98, 80.42, 121.85, 126.91, 127.34, 128.54, 128.87, 129.49, 129.59, 130.60, 132.48, 133.26, 139.61, 141.85, 143.37, 166.60, 171.48, 171.64; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 548 and 550 ($[M + H]^+$), 91% purity.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-(propynyl)-serine nitrile (41). Compound **40** (0.38 g, 0.70 mmol) was dissolved in dry DMF (20 mL) and cyanuric chloride (0.13 g, 0.70 mmol) was added. The reaction mixture was stirred overnight at rt. Then, H_2O (30 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/ petroleum ether 2:1 to obtain **41** as yellow oil (0.31 g, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.01 (dd, 1H, *J* = 11.4 Hz, *J* = 9.2 Hz), 3.10 (dd, 1H, *J* = 11.4 Hz, *J* = 3.4 Hz), 3.53 (t, 1H, *J* = 2.0 Hz), 3.70-3.74 (m, 2H), 4.27-4.33 (m, 2H), 4.74-4.78 (m, 1H), 5.05-5.08 (m, 1H), 7.22 (t, 1H, *J* = 6.5 Hz), 7.36-7.41 (m, 3H), 7.48 (t, 2H, *J* = 6.4 Hz), 7.63 (s, 1H), 7.71 (d, 2H, *J* = 6.0 Hz), 7.75 (d, 2H, *J* = 7.1 Hz), 7.89 (d, 2H, *J* = 7.0 Hz), 8.72 (d, 1H, *J* = 7.1 Hz), 9.06 (d, 1H, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.54, 40.60, 54.50, 58.18, 67.99, 78.21, 79.59, 118.03, 121.51, 126.53, 126.98, 128.23, 128.47, 129.13, 129.37, 130.30, 132.08, 132.70, 139.22, 141.06, 143.06, 166.19, 171.60; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 530 and 532 ($[M + H]^+$), 95% purity.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-((1-(methoxycarbonylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (16). Methyl bromoacetate (**42**, 0.31 g, 2.00 mmol) was dissolved in dry DMF (5 mL). Sodium azide (0.13 g, 2.00 mmol) was added and it was stirred overnight. The reaction mixture was diluted with H_2O (30 mL) and extracted with

diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated at 25 °C to obtain methyl azidoacetate (**43**) as an oily product which was used without further purification. Compounds **41** (0.11 g, 0.20 mmol) and **43** (23.0 mg, 0.20 mmol) were suspended in DMSO/H_2O (3:1, 20 mL). Sodium ascorbate (40.0 mg, 0.20 mmol) and copper(II) sulfate pentahydrate (25.0 mg, 0.10 mmol) were added and the reaction mixture was stirred overnight at rt. Then, H_2O (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/petroleum ether (4:1) to obtain **16** as a colorless solid (78.0 mg, 60%). mp 164–165 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.98-3.03 (m, 1H), 3.09 (dd, 1H, *J* = 13.4 Hz, *J* = 3.7 Hz), 3.69 (s, 3H), 3.74 (d, 2H, *J* = 5.7 Hz), 4.70 (s, 2H), 4.73-4.78 (m, 1H), 5.04-5.07 (m, 1H), 5.40 (s, 2H), 7.22 (t, 1H, *J* = 7.7 Hz), 7.36 (d, 2H, *J* = 7.6 Hz), 7.40 (d, 1H, *J* = 7.3 Hz), 7.48 (t, 2H, *J* = 7.6 Hz), 7.62 (s, 1H), 7.71-7.75 (m, 4H), 7.89 (d, 2H, *J* = 7.6 Hz), 8.14 (s, 1H), 8.72 (d, 1H, *J* = 8.5 Hz), 9.07 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.59, 40.74, 50.42, 52.66, 54.58, 63.97, 68.47, 118.13, 121.56, 125.79, 126.57, 127.02, 128.27, 128.50, 129.18, 129.41, 130.35, 132.12, 132.74, 139.26, 141.09, 143.11, 143.48, 166.29, 167.82, 171.62; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 645 and 647 ($[M + H]^+$), 99% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{31}H_{29}BrN_6O_5$: 645.1456 and 647.1436, found: 645.1447 and 647.1424.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (17). Compound **41** (0.13 g, 0.25 mmol) and azidoacetic acid (**30**, 25.0 mg, 0.25 mmol) were suspended in DMSO/H_2O (4:1, 25 mL). Sodium ascorbate (50.0 mg, 0.25 mmol) and copper(II) sulfate pentahydrate (25.0 mg, 0.13 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 10% $KHSO_4$ solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using CH_2Cl_2/MeOH/acetic acid (89:10:1) to obtain **17** as a colorless solid (90.0 mg, 57%). mp 190–191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.01 (dd, 1H, *J* = 13.6 Hz, *J* = 11.0 Hz), 3.09 (dd, 1H, *J* = 13.6 Hz, *J* = 4.1 Hz), 3.73 (d, 2H, *J* = 5.7 Hz), 4.64-4.69 (m, 2H), 4.73-4.78 (m, 1H), 4.94 (s, 2H), 5.02-

5.06 (m, 1H), 7.21 (t, 1H, *J* = 7.9 Hz), 7.35-7.41 (m, 3H), 7.46-7.49 (m, 2H), 7.62 (t, 1H, *J* = 1.6 Hz), 7.70-7.75 (m, 4H), 7.88-7.91 (m, 2H), 8.03 (s, 1H), 8.77 (d, 1H, *J* = 8.5 Hz), 9.09 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.57, 40.74, 52.56, 54.61, 64.08, 68.38, 118.15, 121.52, 125.53, 126.54, 127.00, 128.28, 128.50, 129.15, 129.37, 130.32, 132.11, 132.74, 139.27, 141.13, 142.79, 143.06, 166.23, 168.51, 171.62; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 631 and 633 ($[M + H]^+$), 98% purity. QTOF: HRMS (ESI): m/z $[M + H]^+$ calcd. for $C_{30}H_{27}BrN_6O_5$: 631.1299 and 633.1279, found: 631.1299 and 633.1276.

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-propargylglycine amide (45). (*S*)-*N*-(*tert*-Butyloxycarbonyl)-propargylglycine (**44**, 1.07 g, 5.00 mmol) was dissolved in dry THF (20 mL) and cooled at -25 °C. To the stirred solution, *N*-methylmorpholine (0.51 g, 5.00 mmol) and isobutyl chloroformate (0.68 g, 5.00 mmol) were added consecutively. Concd aqueous ammonia solution (25%, 1.87 mL, 25.0 mmol) was given to the reaction mixture when the precipitation of *N*-methylmorpholine hydrochloride occurred. It was allowed to warm to rt within 30 min and stirred overnight at rt. After evaporation of the solvent the resulting aqueous residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude colorless solid **45** was used without further purification (0.88 g, 83%); mp 109–110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38 (s, 9H), 2.38-2.44 (m, 1H), 2.50-2.55 (m, 1H), 2.78 (s, 1H), 4.00-4.04 (m, 1H), 6.73 (d, 1H, *J* = 8.2 Hz), 7.07 (s, 1H), 7.31 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.07, 28.29, 52.99, 72.76, 78.36, 81.05, 155.18, 172.20; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 213 $[M + H]^+$; 83% purity.

(*S*,*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanyl-propargylglycine amide (47). Compound **45** (0.17 g, 0.80 mmol) was dissolved in trifluoroacetic acid/CH_2Cl_2 (1:1, 20 mL) and stirred for 3 h at 0 °C. The solvent was evaporated under reduced pressure to obtain (*S*)-propargylglycine amide trifluoroacetate (**46**) as an oily residue. (*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanine (**21**, 0.28 g, 0.80 mmol) was dissolved in dry CH_2Cl_2 (20 mL). DIPEA (0.52 g, 4.00 mmol) and HATU (0.30 g, 0.80 mmol) were added and it was stirred for 10 min at rt. Compound **46** (0.18 g, 0.80 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was

evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate. The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by recrystallization using ethyl acetate to obtain **47** as a light yellow solid (0.33 g, 94% over two steps); mp 152–154 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29 (s, 9H), 2.52-2.55 (m, 2H), 2.71 (dd, 1H, *J* = 13.6 Hz, *J* = 11.0 Hz), 2.83 (t, 1H, *J* = 2.5 Hz), 2.99 (dd, 1H, *J* = 13.7 Hz, *J* = 3.6 Hz), 4.15-4.20 (m, 1H), 4.31 (q, 1H, *J* = 6.7 Hz), 7.03 (d, 1H, *J* = 8.5 Hz), 7.18 (s, 1H), 7.20-7.23 (m, 1H), 7.26-7.28 (m, 1H), 7.36-7.39 (m, 2H), 7.49 (s, 1H), 8.01 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.96, 28.22, 37.03, 51.36, 55.75, 73.16, 78.38, 80.53, 121.45, 128.52, 129.18, 130.23, 132.08, 141.20, 155.40, 171.39, 171.46; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 438 and 440 $[M + H]^+$; 93% purity.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-propargylglycine amide (49). Compound **47** (0.30 g, 0.68 mmol) was dissolved in trifluoroacetic acid/CH_2Cl_2 (1:1, 30 mL) and stirred for 3 h at 0 °C. The solvent was evaporated under reduced pressure to obtain (*S*,*S*)-3-bromophenylalanyl-propargylglycine amide trifluoroacetate (**48**) as an oily residue. Biphenyl-4-carboxylic acid (0.13 g, 0.68 mmol) was dissolved in dry CH_2Cl_2 (20 mL). DIPEA (0.44 g, 3.40 mmol) and HATU (0.26 g, 0.68 mmol) were added and it was stirred for 10 min at rt. Compound **48** (0.31 g, 0.68 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate. The organic layer was washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by recrystallization using ethyl acetate to obtain **49** as a colorless solid (0.23 g, 65% over two steps); mp 226–227 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.53-2.63 (m, 2H), 2.83 (t, 1H, *J* = 2.5 Hz), 3.01 (dd, 1H, *J* = 13.7 Hz, *J* = 10.9 Hz), 3.16 (dd, 1H, *J* = 13.7 Hz, *J* = 3.9 Hz), 4.33-4.37 (m, 1H), 4.74-4.79 (m, 1H), 7.19 (s, 1H), 7.21 (t, 1H, *J* = 7.7 Hz), 7.34-7.36 (m, 1H), 7.37-7.41 (m, 2H), 7.45 (s, 1H), 7.46-7.49 (m, 2H), 7.64 (t, 1H, *J* = 1.7 Hz), 7.70-7.75 (m, 4H), 7.88-7.89 (m, 2H), 8.32 (d, 1H, *J* = 7.9 Hz), 8.71 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.92, 36.72, 51.66, 54.94, 73.14, 80.65, 121.49, 126.54, 126.99, 128.18, 128.22, 128.50, 129.14, 129.25, 130.27, 132.12, 132.93, 139.27, 141.45, 143.02, 166.25, 171.22, 171.54; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 518 and 520 $[M + H]^+$; 95% purity.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-propargylglycine nitrile (50). Compound **49** (0.38 g, 0.70 mmol) was dissolved in dry DMF (15 mL) and cyanuric chloride (0.08 g, 0.41 mmol) was added. The reaction mixture was stirred overnight at rt. Then, H_2O (30 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/ petroleum ether 1:1 to obtain **50** as colorless solid (0.15 g, 75%); mp 211–212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.77-2.79 (m, 2H), 2.99-3.04 (m, 1H), 3.10-3.14 (m, 2H), 4.73-4.78 (m, 1H), 4.91 (q, 1H, *J* = 7.2 Hz), 7.22 (t, 1H, *J* = 7.7 Hz), 7.35-7.41 (m, 3H), 7.48 (t, 2H, *J* = 7.7 Hz), 7.61 (s, 1H), 7.71-7.76 (m, 4H), 7.90 (d, 2H, *J* = 8.2 Hz), 8.72 (d, 1H, *J* = 8.2 Hz), 9.05 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.24, 36.62, 54.50, 74.86, 78.65, 118.31, 121.54, 126.54, 126.99, 128.20, 128.26, 128.44, 129.14, 129.39, 130.35, 132.06, 132.73, 139.25, 141.06, 143.08, 166.19, 171.51, one carbon signal is obscured by the DMSO signal; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 500 and 502 $[M + H]^+$; 98% purity.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-2-((1-(2-(carboxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-glycine nitrile (18). Compound **50** (60.0 mg, 0.12 mmol) and 3-azidopropanoic acid (**31**, 14.0 mg, 0.12 mmol) were suspended in DMSO/H_2O (4:1, 25 mL). Sodium ascorbate (24.0 mg, 0.12 mmol) and copper(II) sulfate pentahydrate (15.0 mg, 0.06 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 10% $KHSO_4$ solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/MeOH/acetic acid (89:10:1) to obtain **18** as a colorless solid (26.0 mg, 35%). mp 170-172 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.83 (t, 2H, *J* = 7.0 Hz), 3.03 (dd, 1H, *J* = 13.7 Hz, *J* = 10.6 Hz), 3.09-3.16 (m, 3H), 3.23-3.27 (m, 1H), 4.51 (t, 2H, *J* = 6.8 Hz), 4.67-4.72 (m, 1H), 5.02 (q, 1H, *J* = 7.7 Hz), 7.22 (t, 1H, *J* = 7.9 Hz), 7.34-7.41 (m, 3H), 7.47-7.50 (m, 2H), 7.59 (t, 1H, *J* = 1.6 Hz), 7.71-7.76 (m, 4H), 7.91-7.93 (m, 2H), 8.01 (s, 1H), 8.79 (d, 1H, *J* = 8.2 Hz), 9.04 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.35, 34.60, 36.40, 45.71, 54.77, 118.82, 121.56, 123.82, 126.55, 127.01, 128.21, 128.31, 128.43, 129.15, 129.40, 130.37, 132.08, 132.68, 139.27, 140.97, 141.12, 143.12, 166.31, 171.43, 171.96; LC-MS (ESI) (90% H_2O to 100% MeOH in

10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 614 ($[M + H]^+$), 97% purity. QTOF: HRMS (ESI): m/z $[M - H]^-$ calcd. for $C_{30}H_{27}N_6O_4$: 613.1204 and 615.1189, found: 613.1239 and 615.1189.

(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-2-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-glycine nitrile (19). Compound **50** (60.0 mg, 0.12 mmol) and azidoacetic acid (**30**, 12.0 mg, 0.12 mmol) were suspended in DMSO/H_2O (4:1, 25 mL). Sodium ascorbate (24.0 mg, 0.12 mmol) and copper(II) sulfate pentahydrate (15.0 mg, 0.06 mmol) were added and the reaction mixture was stirred overnight at rt. Then, 10% $KHSO_4$ solution (50 mL) was added and the aqueous suspension was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (20 mL), H_2O (20 mL) and brine (20 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate/MeOH/acetic acid (89:10:1) to obtain **19** as a colorless solid (71.0 mg, 98%). mp 164-166 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.00 (dd, 1H, *J* = 13.4 Hz, *J* = 10.9 Hz), 3.10-3.15 (m, 2H), 3.22-3.26 (m, 1H), 4.72 (s, 3H), 5.00 (q, 1H, *J* = 7.7 Hz), 7.20 (t, 1H, *J* = 7.9 Hz), 7.34-7.36 (m, 2H), 7.39 (t, 1H, *J* = 7.3 Hz), 7.48 (t, 2H, *J* = 7.7 Hz), 7.59 (s, 1H), 7.71-7.75 (m, 4H), 7.87 (s, 1H), 7.93 (d, 2H, *J* = 8.5 Hz), 8.97 (d, 1H, *J* = 8.2 Hz), 9.17 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.35, 36.38, 53.75, 54.87, 119.04, 121.51, 124.75, 126.52, 127.00, 128.17, 128.36, 128.48, 129.15, 129.32, 130.32, 132.09, 132.76, 139.31, 140.25, 141.28, 143.02, 166.29, 168.41, 171.45, one carbon signal is obscured by the DMSO signal; LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), m/z = 601 and 603 ($[M + H]^+$), 97% purity. QTOF: HRMS (ESI): m/z $[M - H]^-$ calcd. for $C_{29}H_{25}BrN_6O_4$: 599.1048 and 601.1032, found: 599.1098 and 601.1080.

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-cyclohexylalanyl-glycine nitrile (57). *(S)*-*N*-(*tert*-Butyloxycarbonyl)-cyclohexylalanine (0.95 g, 3.50 mmol) was dissolved in dry THF (10 mL) and cooled at -25°C. To the stirred solution, NMM (0.35 g, 3.50 mmol) and isobutyl chloroformate (0.48 g, 3.50 mmol) were added consecutively. Aminoacetonitrile monosulfate (0.54 g, 3.50 mmol) was dissolved in 2 N NaOH (1.75 mL, 3.50 mmol) and given to the reaction mixture. It was allowed to warm up to rt within 30 min and stirred overnight at rt. After evaporation of the solvent, the residue was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (2 × 30 mL), H_2O (30 mL) and brine (30 mL). The solvent was dried (Na_2SO_4) and

evaporated. The crude product was recrystallized from ethyl acetate to obtain **57** as a white solid (0.35 g, 32%); mp 82–83 °C, lit.[4] mp 81–83 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.78–0.92 (m, 2H), 1.08–1.18 (m, 3H), 1.23–1.29 (m, 1H), 1.37 (s, 9H), 1.41 (t, *J* = 7.1 Hz, 2H), 1.58–1.71 (m, 5H), 3.98 (q, *J* = 7.7 Hz, 1H), 4.09 (dd, *J* = 5.6 Hz, *J* = 2.7 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 1H), 8.50 (t, *J* = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.78, 25.97, 26.17, 27.20, 28.30, 31.83, 33.24, 33.70, 51.93, 78.22, 117.68, 155.53, 173.48, one carbon signal is obscured by the DMSO signal; LC-MS(ESI) (90% H_2O to 100% MeOH in 20 min, then 100% MeOH over 10 min, DAD 220-400 nm), m/z = 308 ([M – H]$^-$), 100% purity. M = 309.40 g/mol

(*S*)-*N*-(4-Phenylbenzoyl)-cyclohexylalanyl-glycine nitrile (91). Compound **57** (0.31 g, 1.00 mmol) was dissolved in dry THF (8 mL). Under ice cooling, methanesulfonic acid (0.58 g, 6.00 mmol) was added. The resulting mixture was stirred at rt overnight. The solvent was evaporated and the crude product, cyclohexylalanyl-glycine nitrile methanesulfonate, was used without further purification. Biphenyl-4-carboxylic acid (52.0 mg g, 0.25 mmol) was dissolved in dry CH_2Cl_2 (10 mL). DIPEA (97.0 mg, 0.75 mmol) and HATU (95.0 mg, 0.25 mmol) were added and stirred for 10 min at rt. Cyclohexylalany-glycine nitrile methanesulfonate (76.0 mg, 0.25 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and added to the reaction mixture. It was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was suspended in ethyl acetate (20 mL). The organic layer was washed with 10% $KHSO_4$ (15 mL), H_2O (15 mL), sat. $NaHCO_3$ (15 mL), H_2O (15 mL) and brine (15 mL). The solvent was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using ethyl acetate to obtain **91** as a white solid (59.0 mg, 61%); mp 179–180 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84-0.99 (m, 2H), 1.08-1.19 (m, 3H), 1.31-1.39 (m, 1H), 1.58-1.74 (m, 7H, C_6H_{11}, CHCH_2), 4.12 (d, 2H, *J* = 5.4 Hz, CH_2CN), 4.53-4.57 (m, 1H, CH), 7.39-7.42 (m, 1H, H_{arom}), 7.48-7.51 (m, 2H, H_{arom}), 7.72-7.74 (m, 2H, H_{arom}), 7.77 (d, 2H, *J* = 8.6 Hz, H_{arom}), 8.01 (d, 2H, *J* = 8.2 Hz, H_{arom}), 8.58 (d, 1H, *J* = 7.9 Hz, N*H*CH), 8.65 (t, 1H, *J* = 5.7 Hz, N*H*CH_2CN); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.73, 25.90, 26.17, 27.29, 31.72, 33.32, 33.89 (C_6H_{11}, CH_2CN), 38.63 (CHCH_2), 51.02 (*C*HCH_2), 117.71 (CN), 126.51, 126.99, 128.17, 128.42, 129.14, 132.87, 139.34, 143.05 (C_{arom}), 166.20 (*C*ONHCH), 173.18 (CH*C*O); LC-MS(ESI) (90% H_2O to 100% MeOH in 20 min, then 100% MeOH over 10 min, DAD 220-400 nm), m/z = 390 ([M + H]$^+$), 96% purity. M = 389.49 g/mol

Enzyme Inhibition Assays

Human cathepsins B and L were assayed photometrically on a Cary 50 Bio, Varian, at 405 nm. Peptidic substrates were purchased from Bachem AG (Bubendorf, Switzerland). The reactions were followed at 37 °C over 20 min. Human cathepsins K and S were assayed fluorimetrically on a Monaco Safas spectrofluorometer flx or on a PlateReader FLUOstar Optima, BMG Labtech. When using the substrates Cbz-Leu-Arg-AMC or Cbz-Phe-Arg-AMC, the wavelength for excitation was 360 nm and for emission was 440 nm. In case of the substrate Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH, 320 nm and 420 nm were used as excitation and emission wavelengths. The 10 mM inhibitor solutions were prepared in DMSO. The final concentration of DMSO in all assays was 2%. Progress curves were analyzed by linear regression.

Cathepsin B inhibition assay at pH 6.0 with Cbz-Arg-Arg-pNA.[2] Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, 0.01% Brij 35. A stock solution of human isolated cathepsin B (Calbiochem, Darmstadt, Germany) of 0.47 mg/mL in 20 mM sodium acetate buffer pH 5.0, 1 mM EDTA was diluted 1:500 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. A 100 mM stock solution of the chromogenic substrate Cbz-Arg-Arg-pNA was prepared with DMSO. The final concentration of the substrate was 500 μM (0.45 K_m). Assays were performed with a final concentration of 19 ng/mL of cathepsin B. Into a cuvette containing 960 μL assay buffer, inhibitor solution and DMSO in a total volume of 15 μL, and 5 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 μL of the cathepsin B solution. The reactions were followed at 37 °C over 20 min.

Cathepsin B inhibition assay at pH 4.5 with Cbz-Arg-Arg-pNA. Assay buffer was 50 mM sodium acetate buffer pH 4.5, 50 mM NaCl, 2.5 mM EDTA, 0.005% Brij 35. A stock solution of human isolated cathepsin B (Calbiochem, Darmstadt, Germany) of 0.47 mg/mL in 20 mM sodium acetate buffer pH 5.0, 1 mM EDTA was diluted 1:500 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. A 100 mM stock solution of the chromogenic substrate Cbz-Arg-Arg-pNA was prepared with DMSO. In inhibition assays, the final concentration of the substrate was 500 μM (0.59 K_m). Assays were performed with a final concentration of 56 ng/mL of cathepsin B. Into a cuvette containing 920 μL assay buffer, inhibitor solution and DMSO in a total volume of 15 μL, and 5 μL of the substrate solution

were added and thoroughly mixed. The reaction was initiated by adding 60 µL of the cathepsin B solution. The reactions were followed at 37 °C over 20 min. A K_m value of 853 ± 72 µM was obtained in triplicate measurements with 21 different substrate concentrations between 20 µM and 5000 µM.

Cathepsin B inhibition assay at pH 4.5 with Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH. Assay buffer was 50 mM sodium acetate buffer pH 4.5, 50 mM NaCl, 2.5 mM EDTA, 0.005% Brij 35. A stock solution of human isolated cathepsin B (Calbiochem, Darmstadt, Germany) of 0.47 mg/mL in 20 mM sodium acetate buffer pH 5.0, 1 mM EDTA was diluted 1:1000 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. A 2 mM stock solution of the substrate Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH was prepared with DMSO. In inhibition assays, the final concentration of the substrate was 10 µM (2.67 K_m). Assays were performed with a final concentration of 2.35 ng/mL of cathepsin B. Into a cuvette containing 195 µL assay buffer, inhibitor solution and DMSO in a total volume of 3 µL, and 1 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 1 µL of the cathepsin B solution. The reactions were followed at 37 °C over 10 min. A K_m value of 3.74 ± 0.51 µM was obtained in triplicate measurements with 12 different substrate concentrations between 1 µM and 40 µM.

Cathepsin S inhibition assay.[6] Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, and 0.01% Brij 35. A stock solution of human recombinant cathepsin S (Enzo Life Sciences, Lörrach, Germany) of 70 µg/mL in 100 mM MES buffer, pH 6.5, 1 mM EDTA, 50 mM L-cysteine, 10 mM DTT, 0.5% Triton X-100 and 30% glycerol was diluted 1:100 with a 50 mM sodium phosphate buffer pH 6.5, 50 mM NaCl, 2 mM EDTA, 0.01% Triton X-100 and 5 mM DTT and incubated for 60 min at 37 °C. A 10 mM stock solution of the fluorogenic substrate Cbz-Phe-Arg-AMC was prepared in DMSO. The assay was performed with a final substrate concentration of 40 µM (= 0.74 K_m) and a final concentration of 42 ng/mL of cathepsin S. Into a cuvette containing 920 µL assay buffer, inhibitor solution and DMSO in a total volume of 16 µL, and 4 µL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 60 µL of the cathepsin S solution. All measurements were done with a Monaco Safas spectrofluorometer flx, except for experiments with compounds **14** and **15**, which were performed on a PlateReader FLUOstar Optima with a total volume of 200 µL. The reactions were followed at 25 °C over 20 min.

Cathepsin L inhibition assay.[2] Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, and 0.01% Brij 35. A stock solution of human isolated cathepsin L (Enzo Life Sciences, Lörrach, Germany) of 135 μg/mL in 20 mM malonate buffer pH 5.5, 400 mM NaCl, and 1 mM EDTA was diluted 1:100 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. A 10 mM stock solution of the chromogenic substrate Cbz-Phe-Arg-pNA was prepared with DMSO. The final concentration of the substrate was 100 μM (= 5.88 K_m). Assays were performed with a final concentration of 54 ng/mL of cathepsin L. Into a cuvette containing 940 μL assay buffer, inhibitor solution and DMSO in a total volume of 10 μL, and 10 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 40 μL of the cathepsin L solution. The reactions were followed at 37 °C over 20 min

Cathepsin K inhibition assay.[2] A human recombinant cathepsin K (Enzo Life Sciences, Lörrach, Germany) stock solution of 23 μg/mL in 50 mM sodium acetate pH 5.5, 50 mM NaCl, 0.5 mM EDTA, 5 mM DTT was diluted 1:100 with assay buffer (100 mM sodium citrate pH 5.0, 100 mM NaCl, 1 mM EDTA, 0.01% CHAPS) containing 5 mM DTT and incubated for 30 min at 37 ºC. A 10 mM stock solution of the fluorogenic substrate Cbz-Leu-Arg-AMC was prepared with DMSO. The final concentration of the substrate was 40 μM (= 13.3 K_m). Assays were performed with a final concentration of 5 ng/mL of cathepsin K. Into a cuvette containing 960 μL assay buffer, inhibitor solution and DMSO in a total volume of 16 μL, and 4 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 μL of the cathepsin K solution. All measurements were done with a Monaco Safas spectrofluorometer flx, except for experiments with compounds **14** and **15**, which were performed on a PlateReader FLUOstar Optima with a total volume of 200 μL. The reactions were followed at 25 °C over 20 min.

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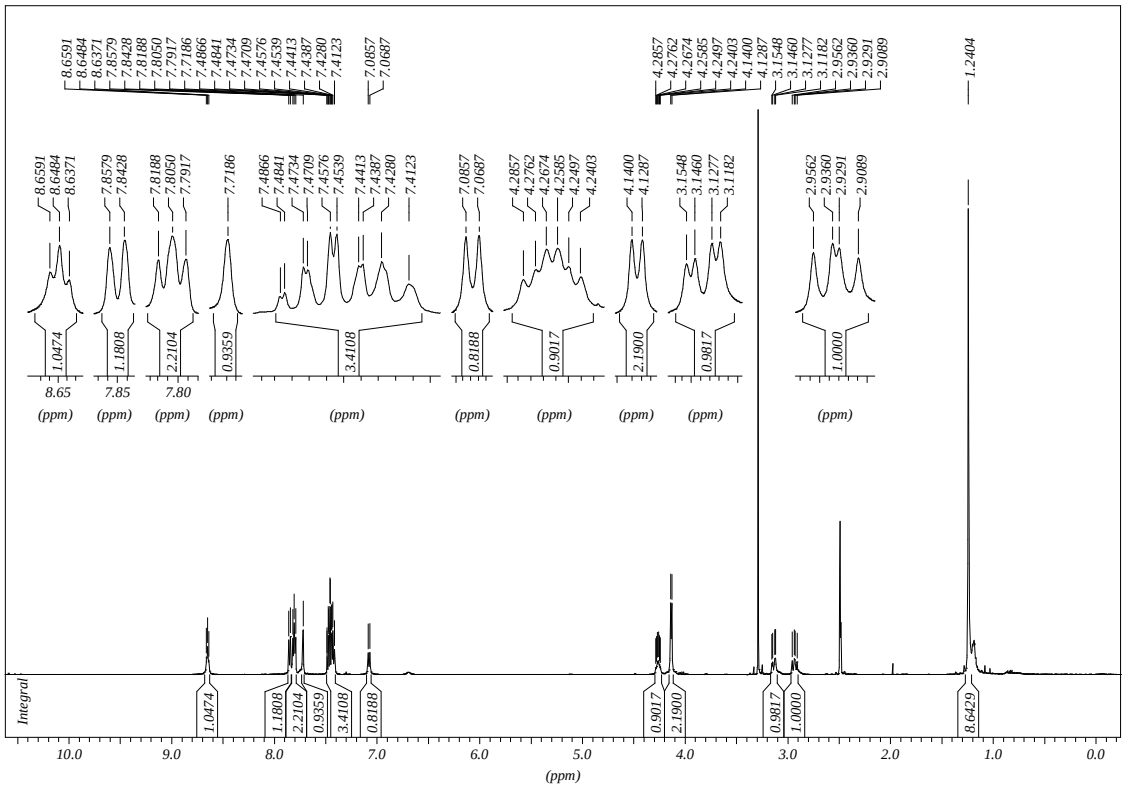
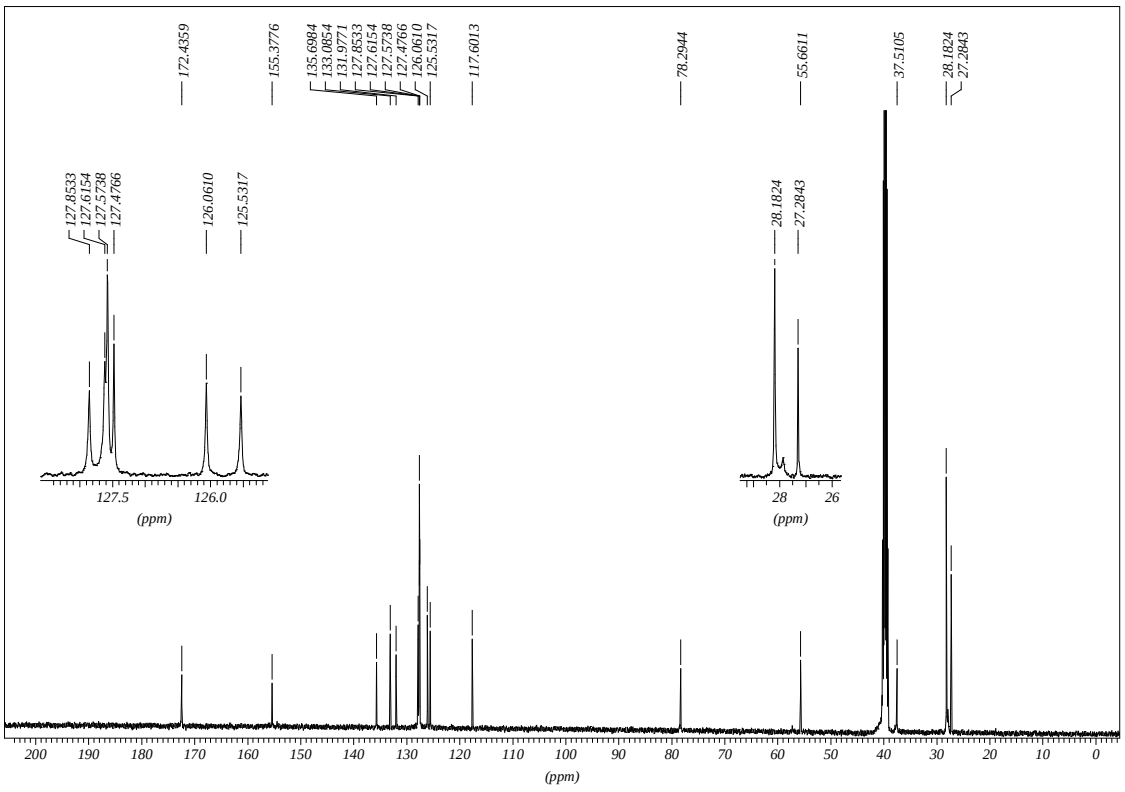
^{1}H and ^{13}C NMR Spectra

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-2-naphthylalanyl-glycine nitrile (1)





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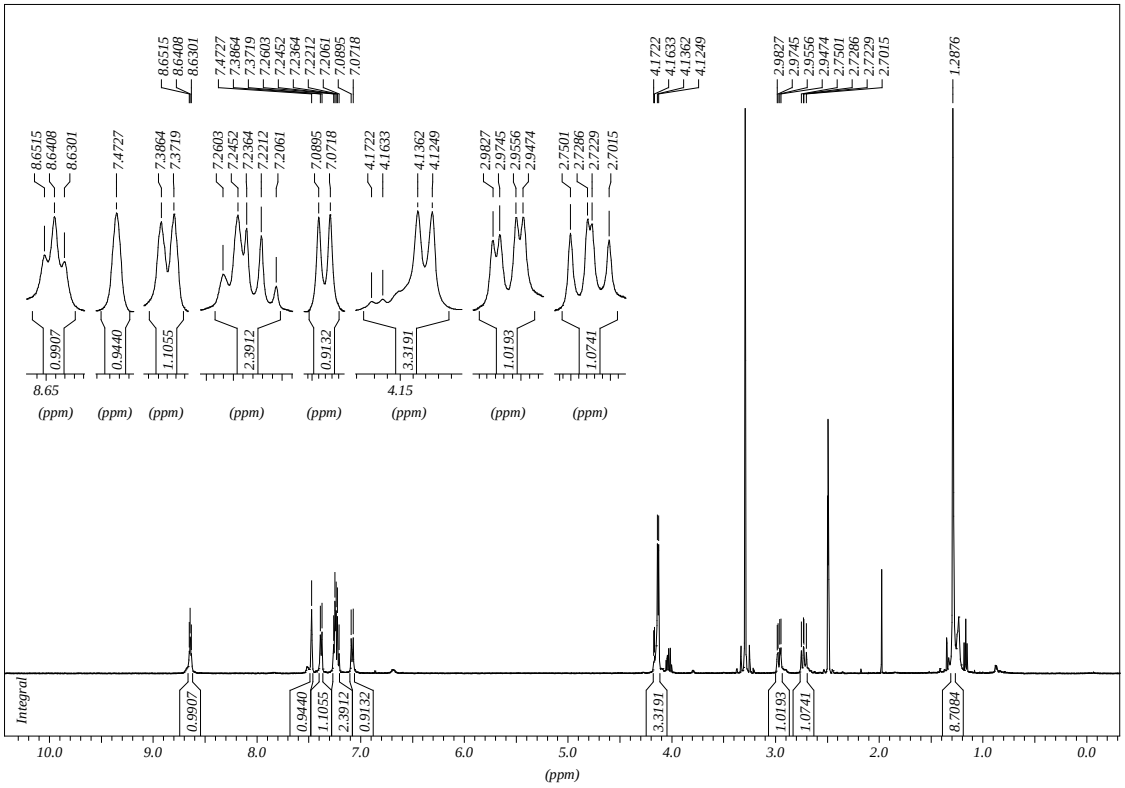

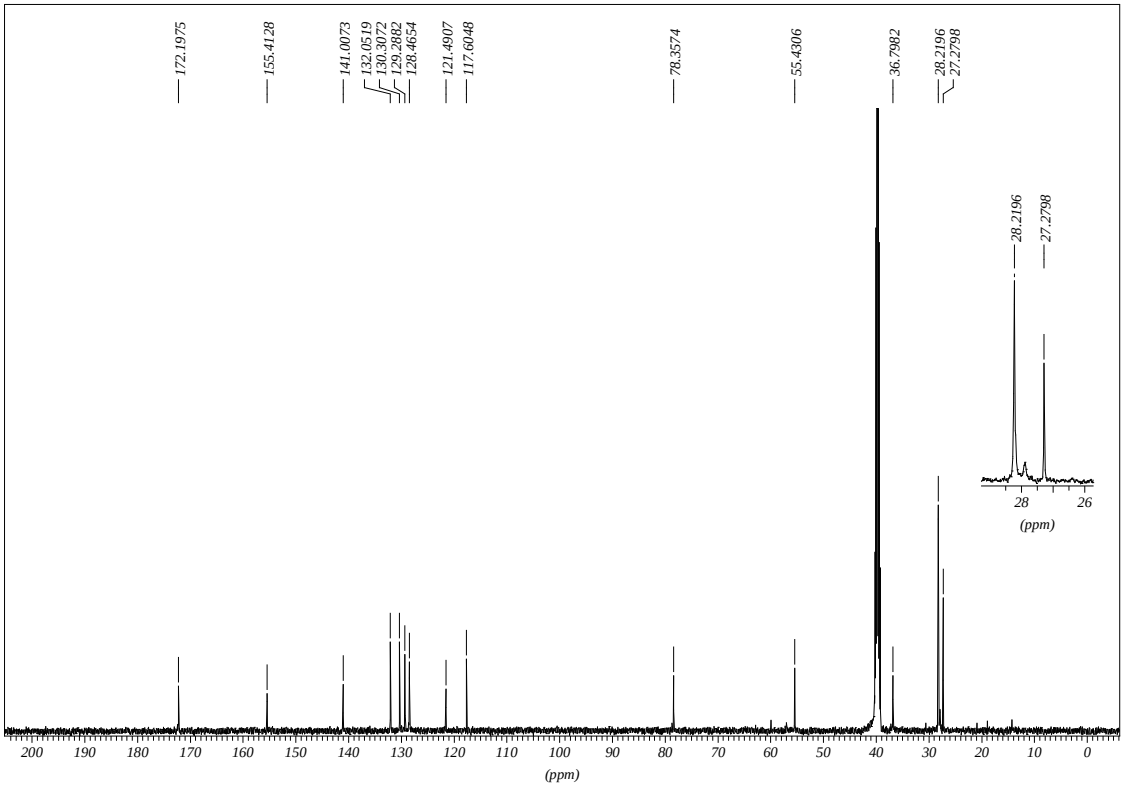

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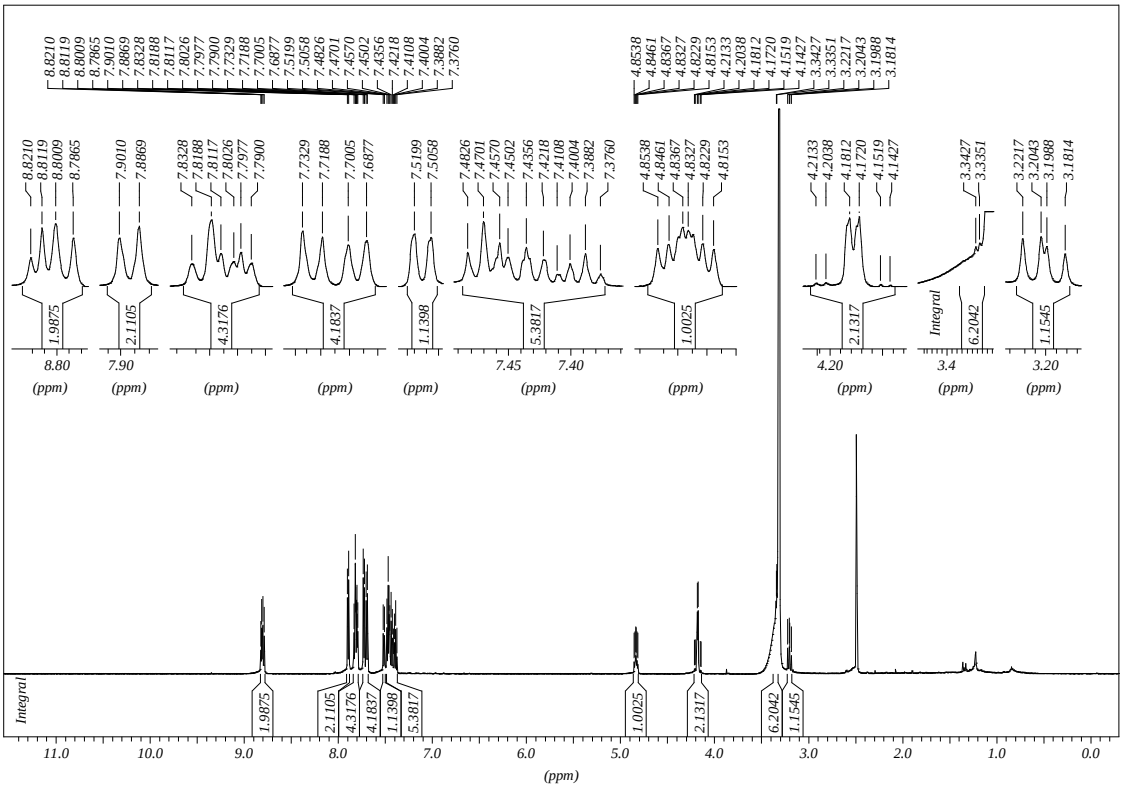

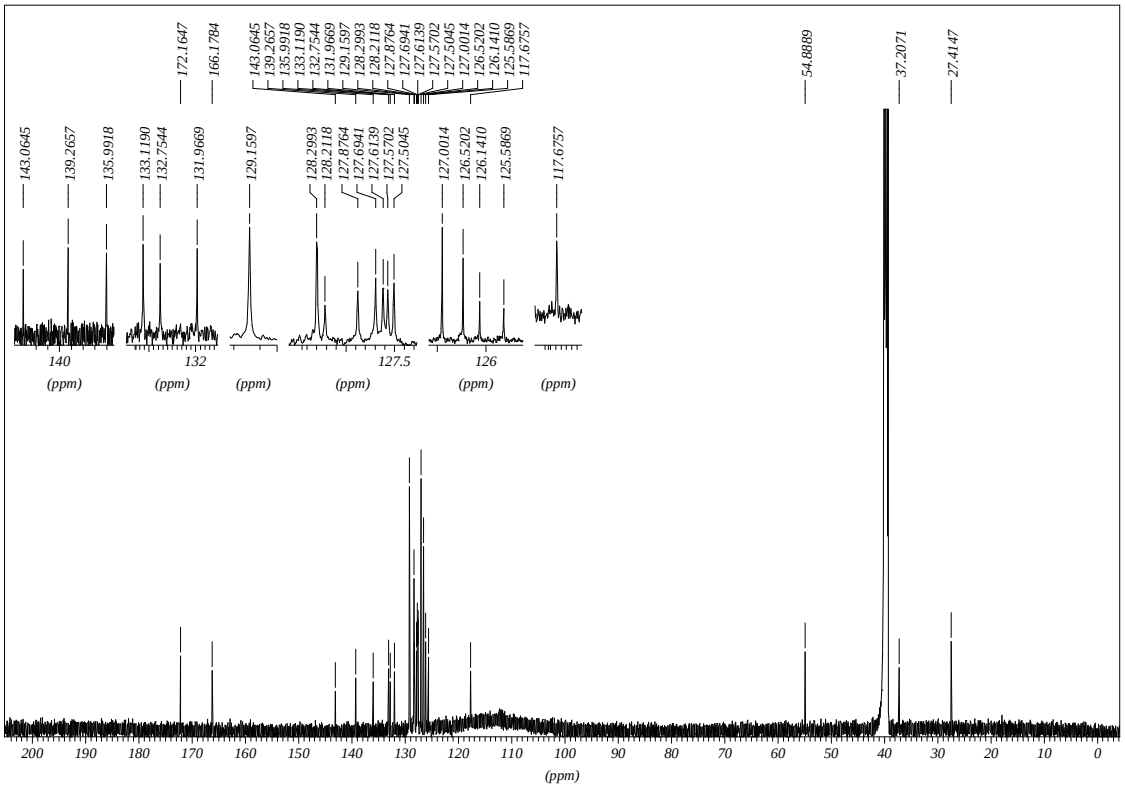

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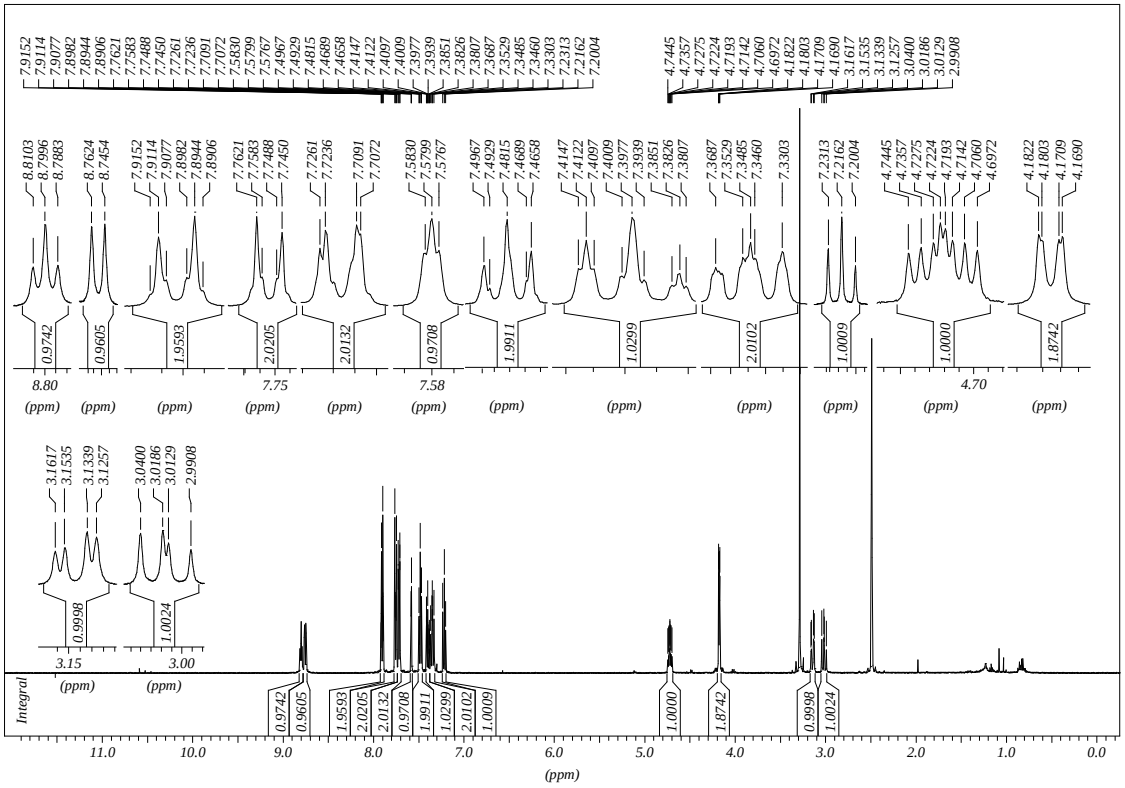

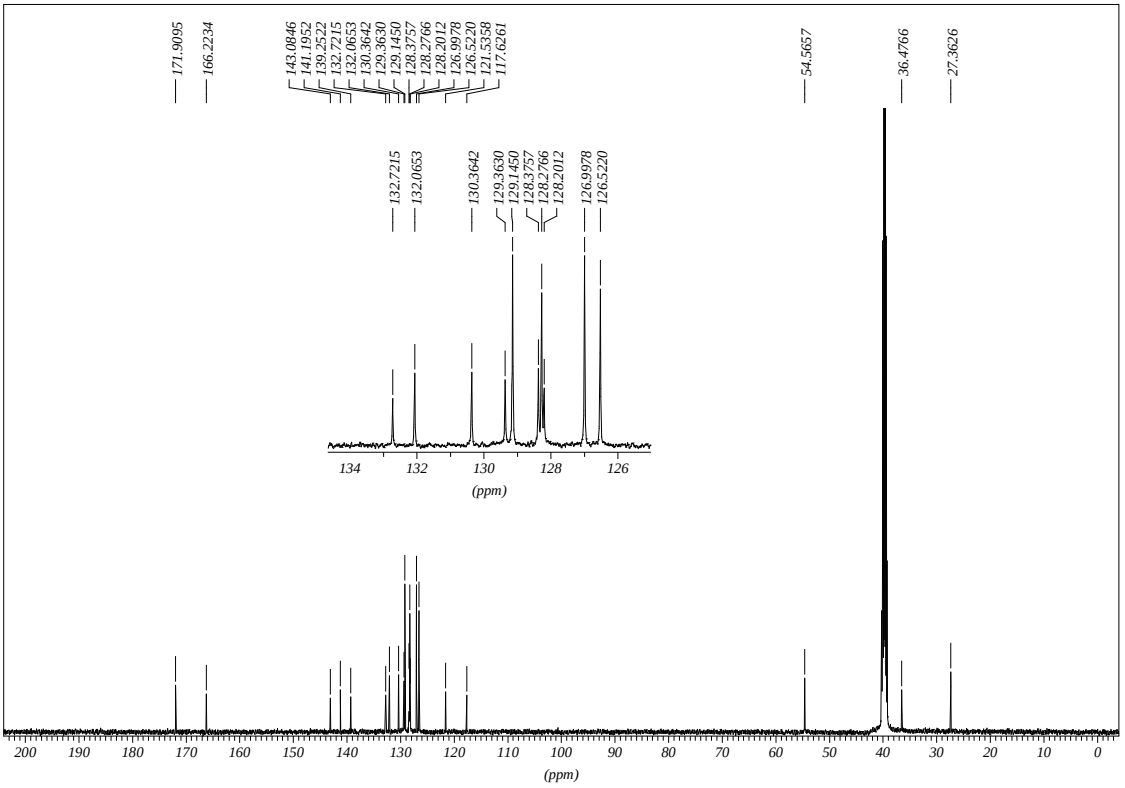

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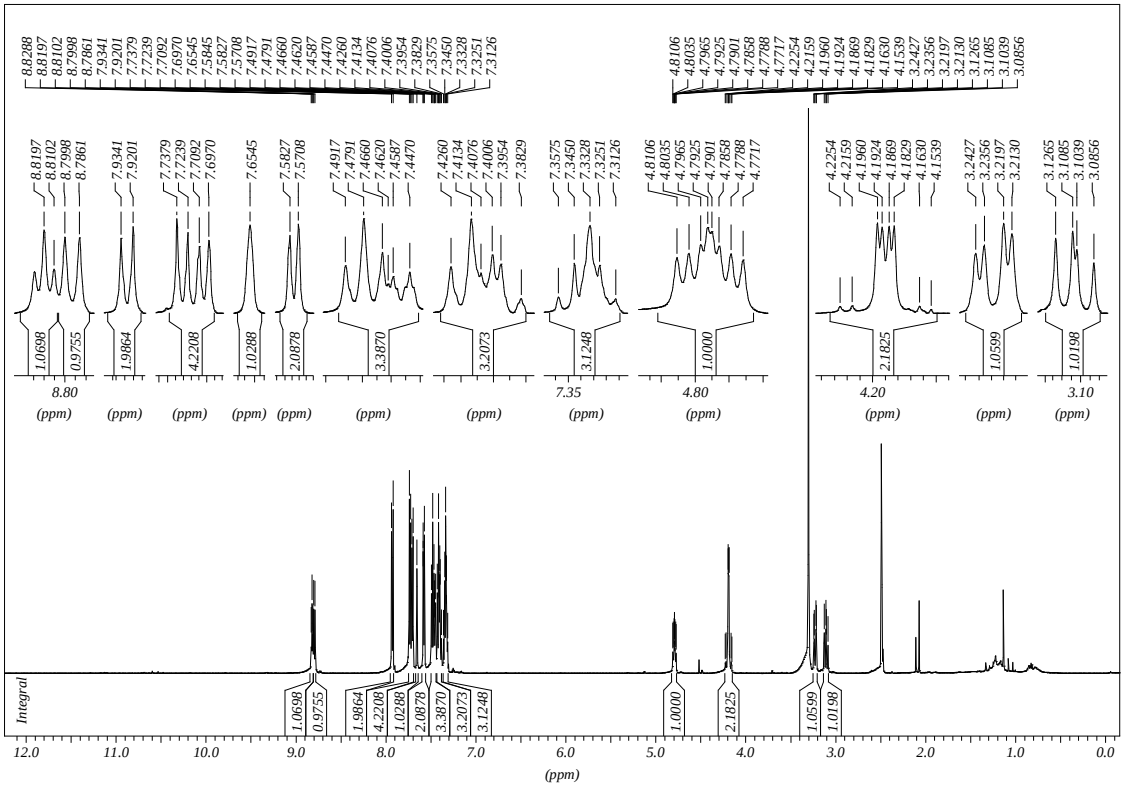

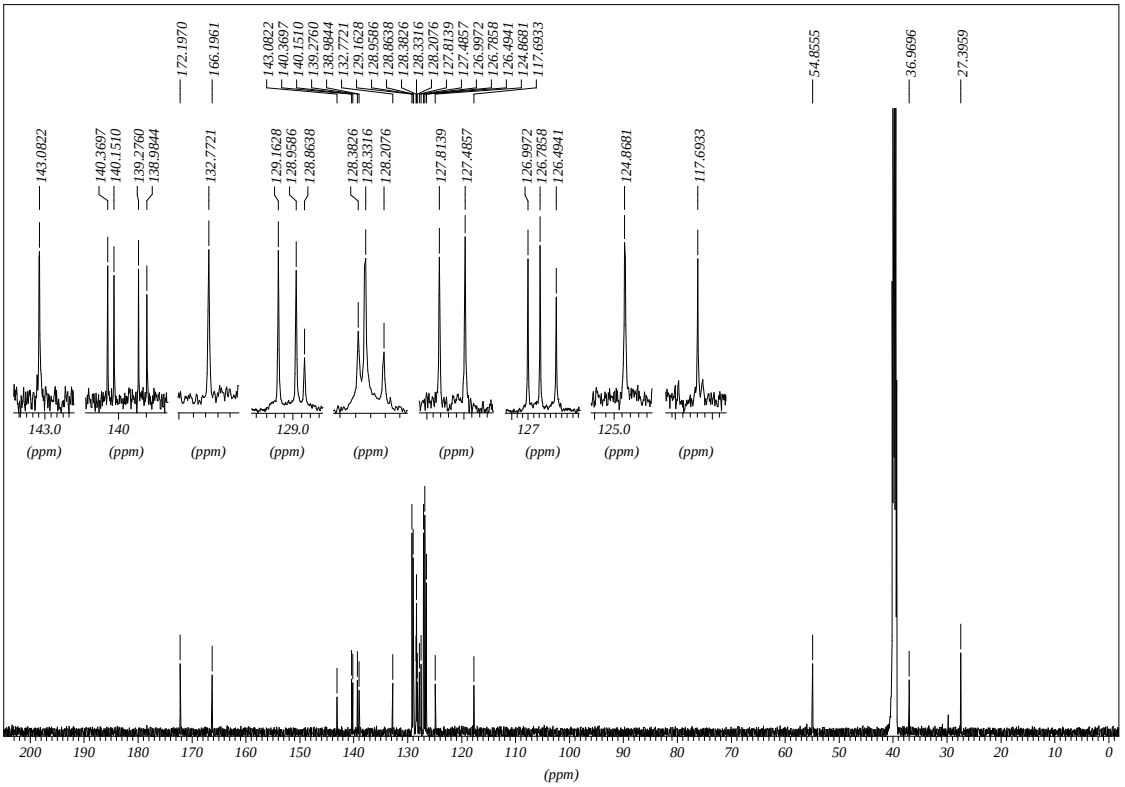

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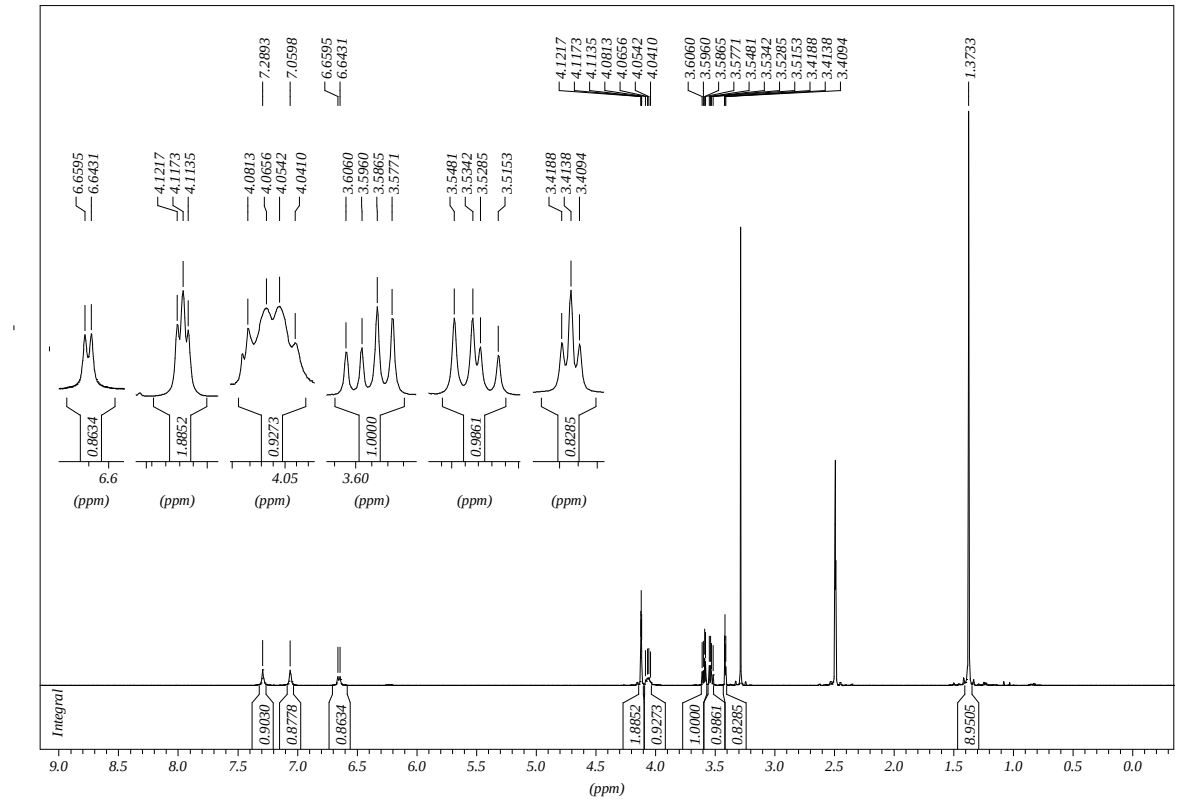

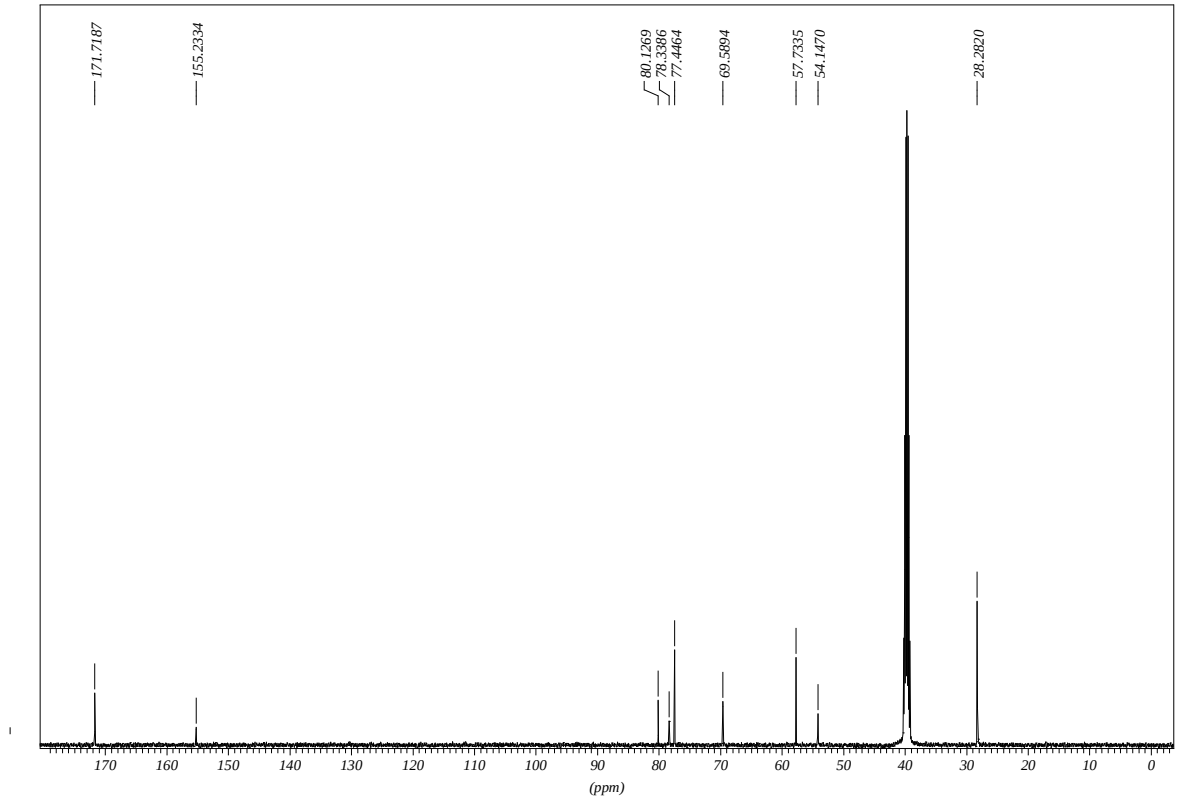

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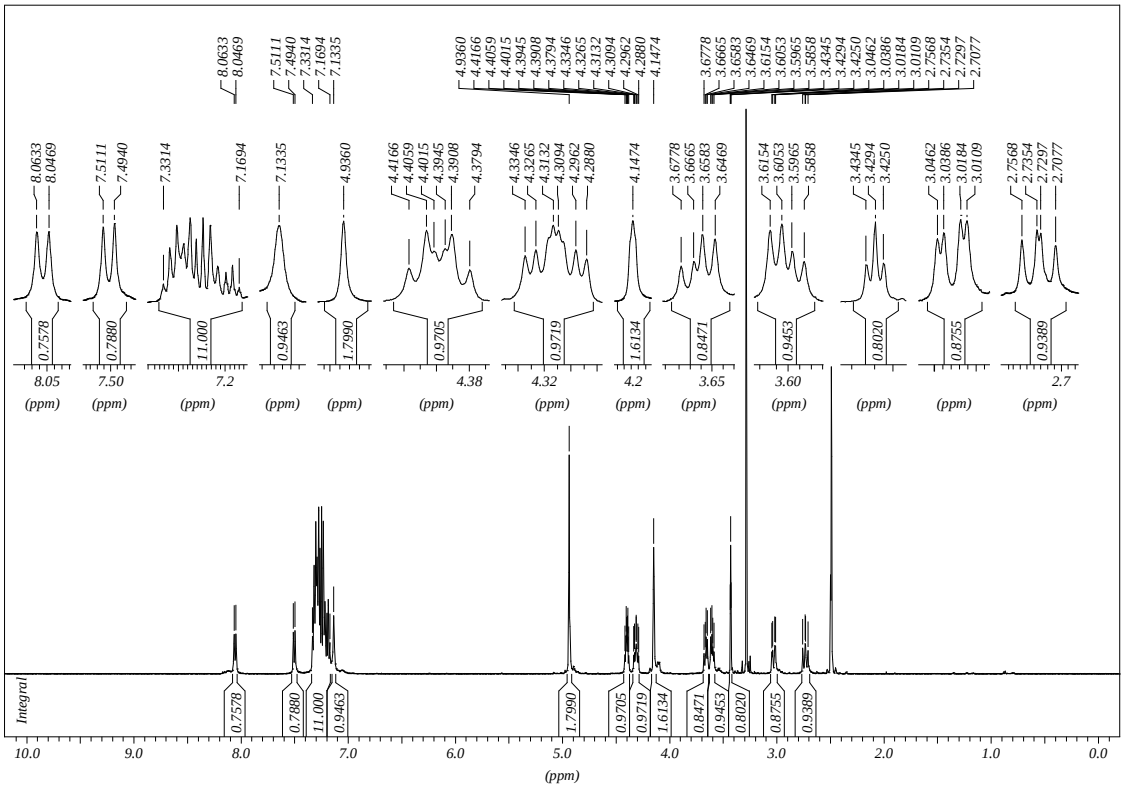

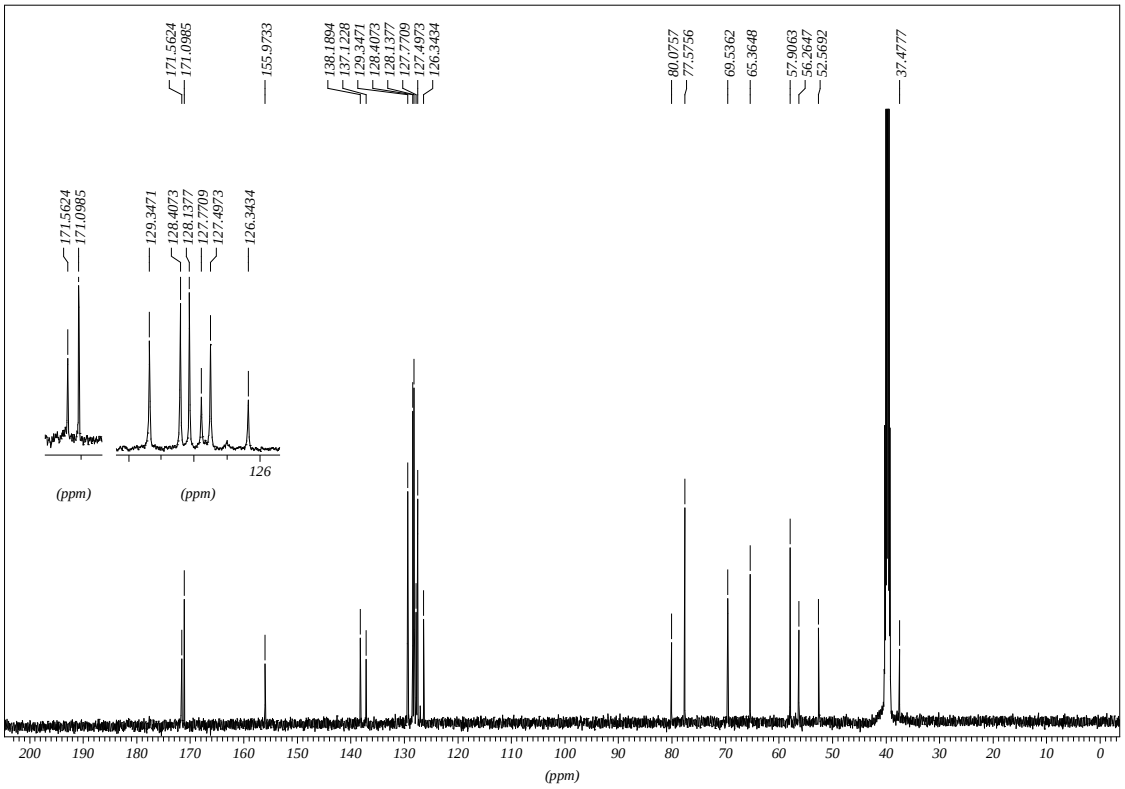

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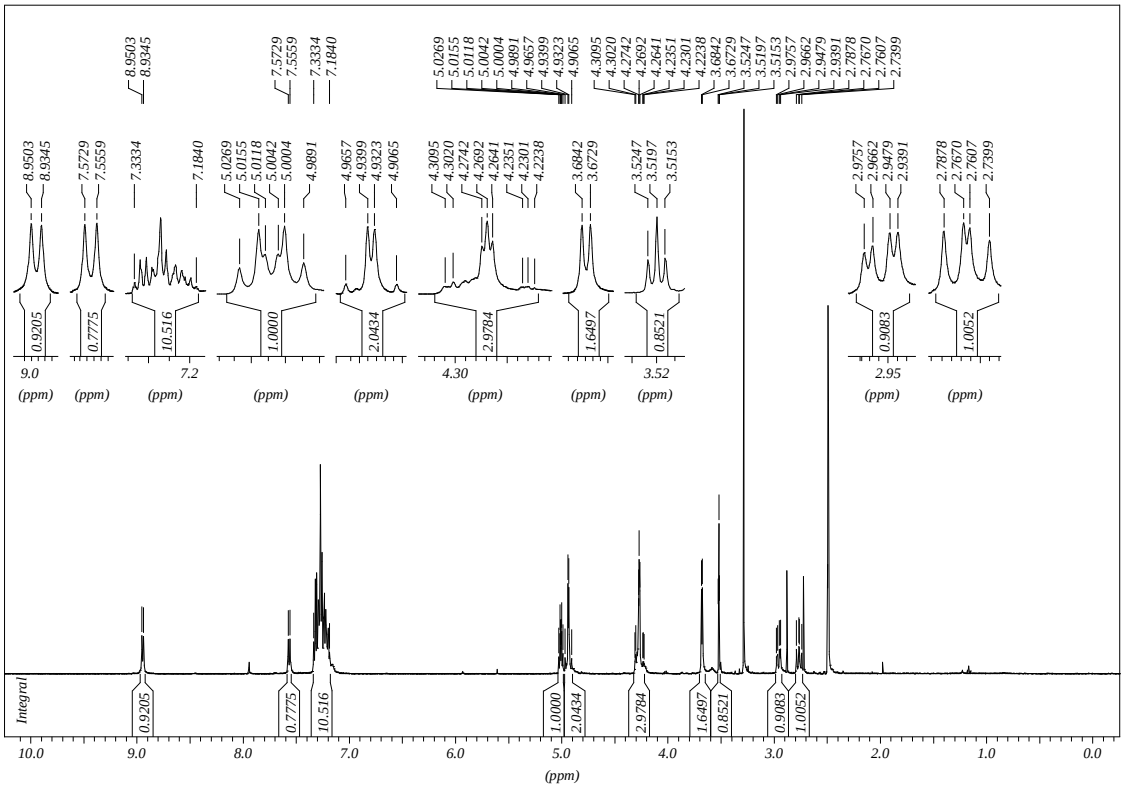

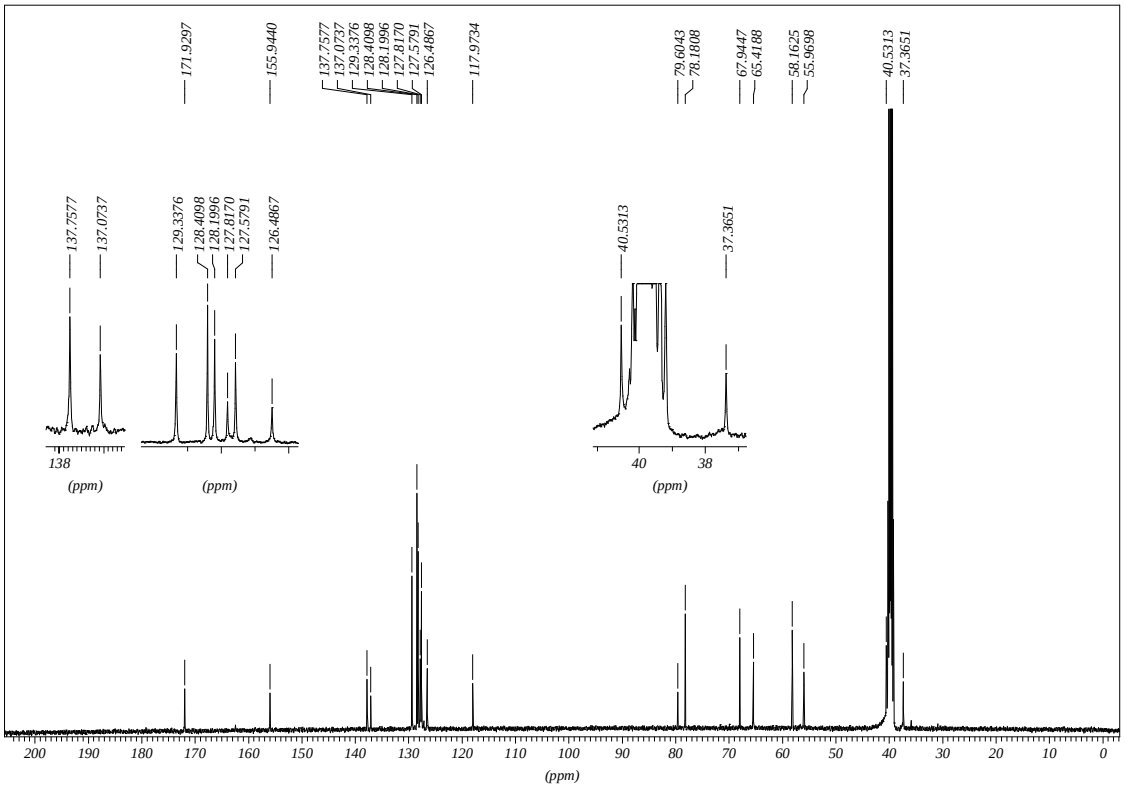

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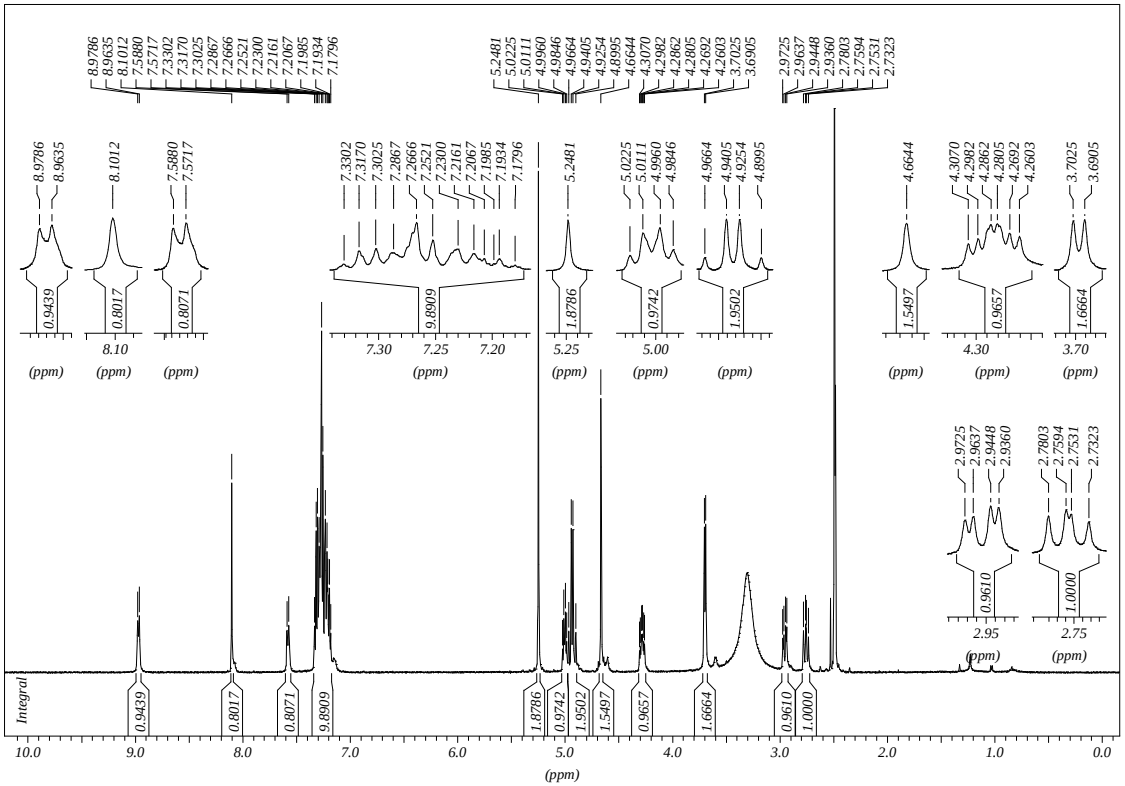
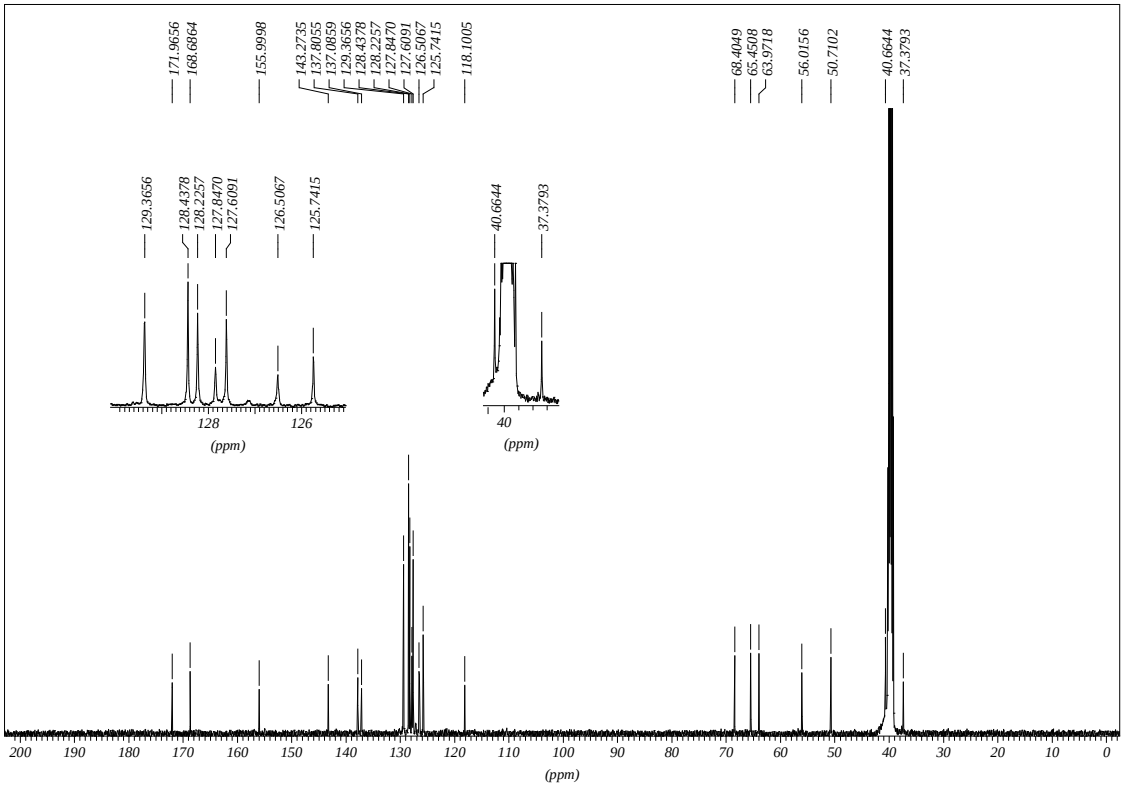
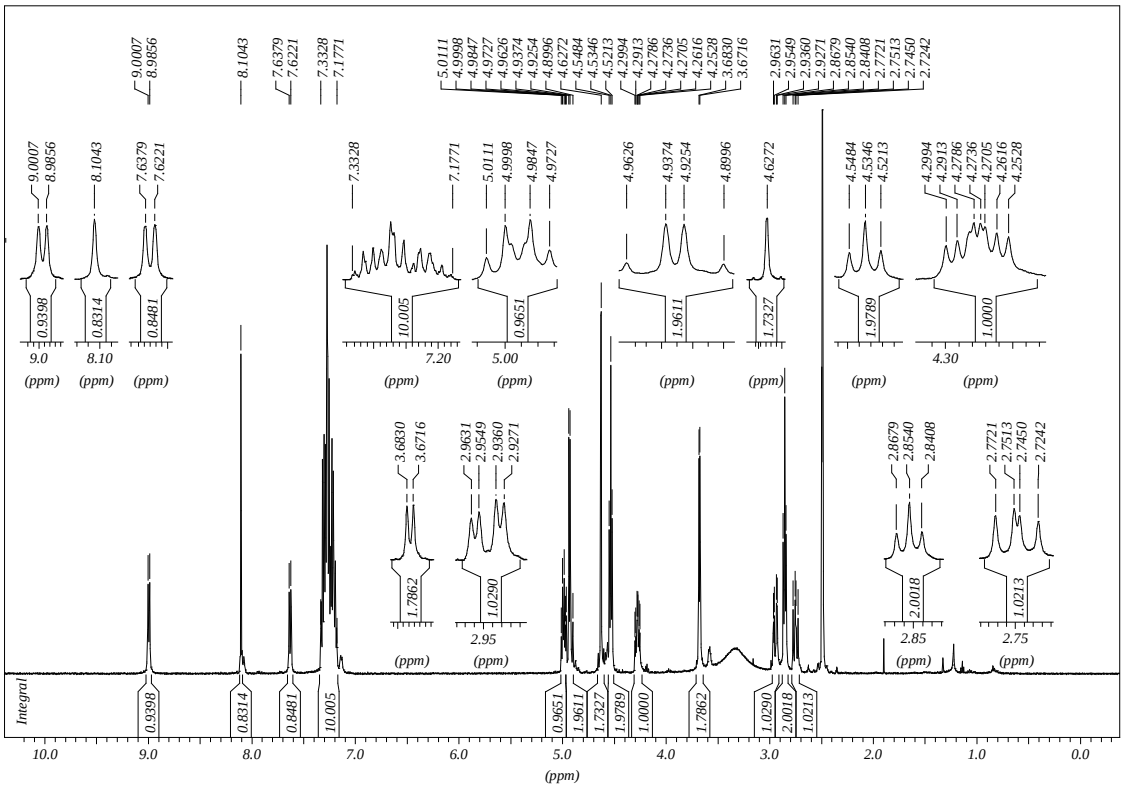
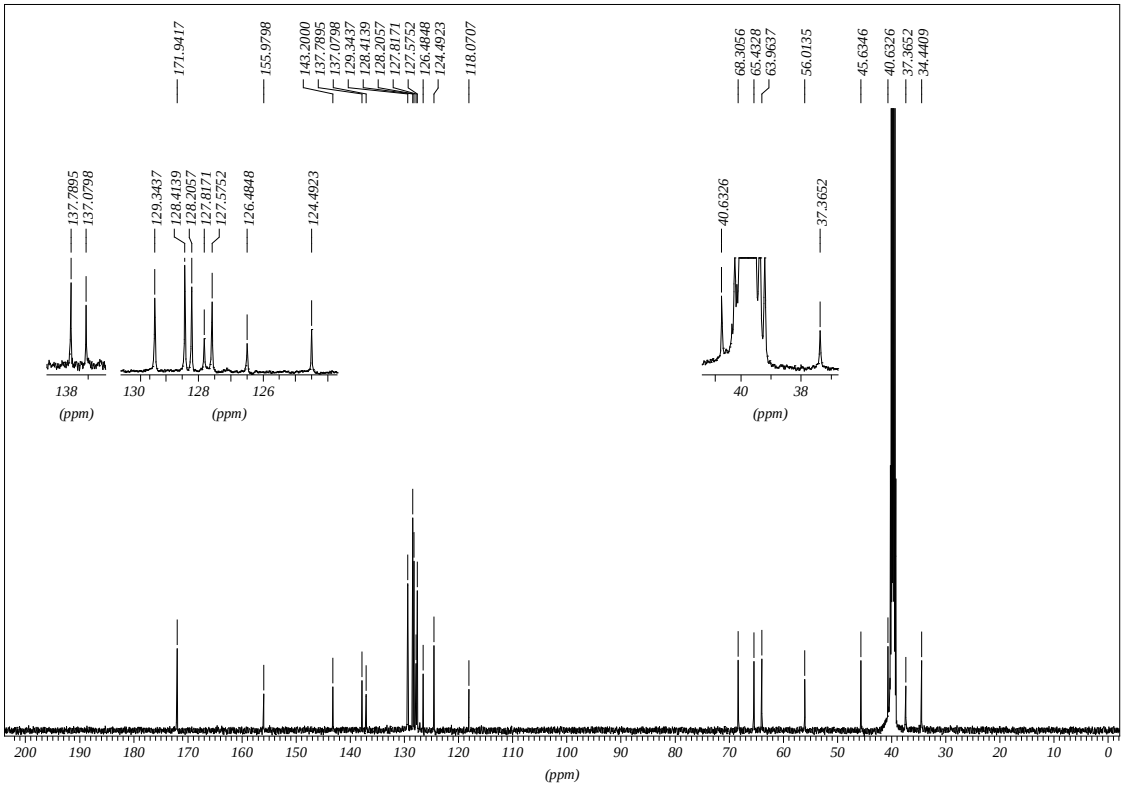


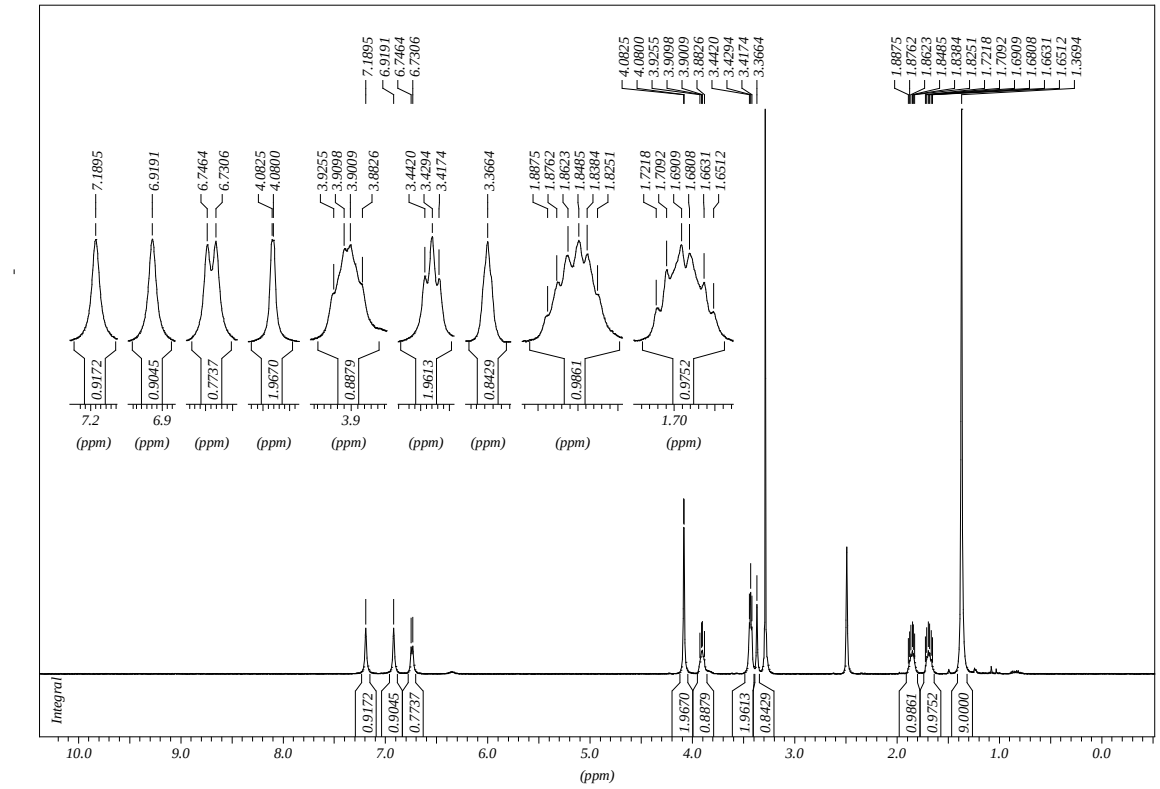


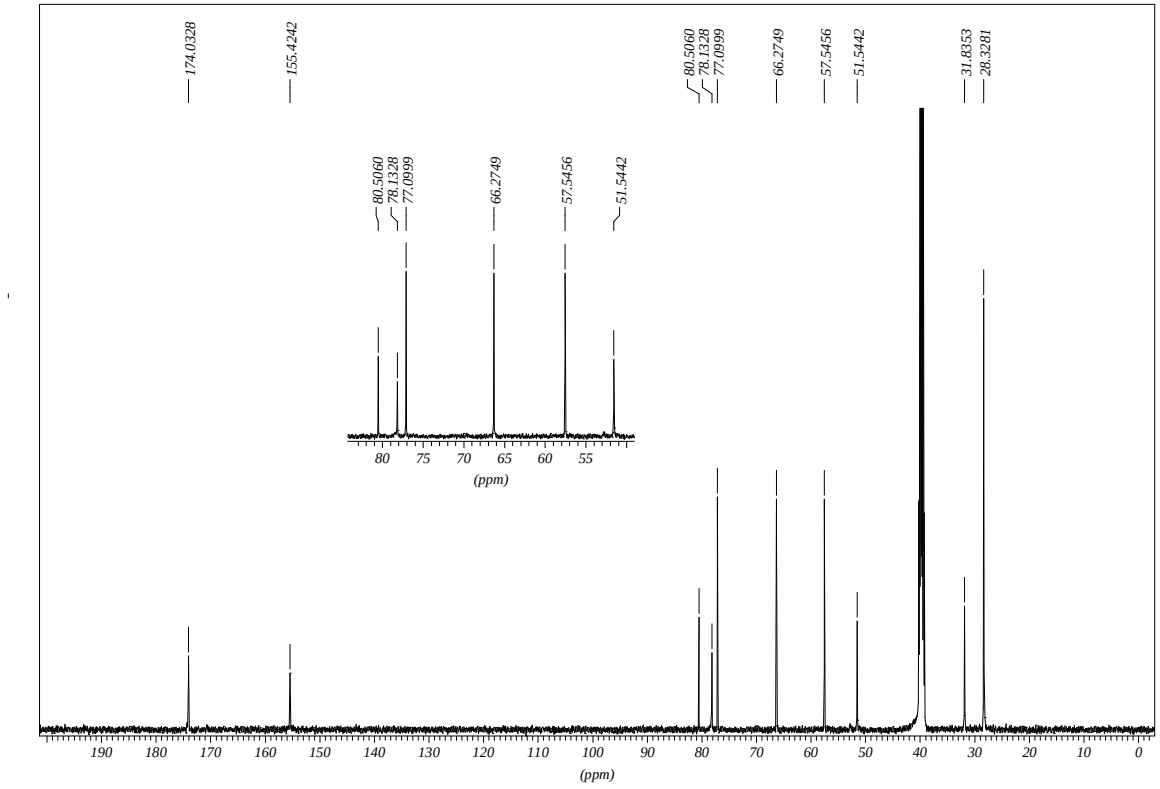
(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(2-carboxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (13)



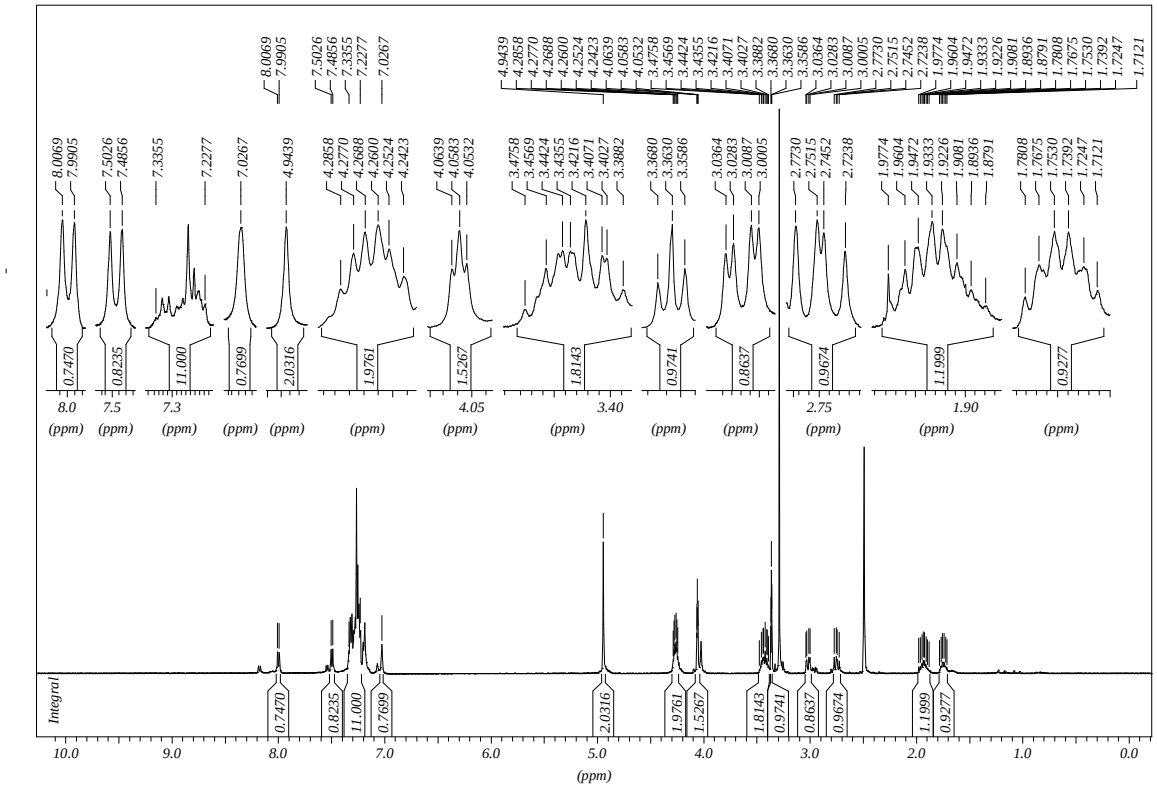


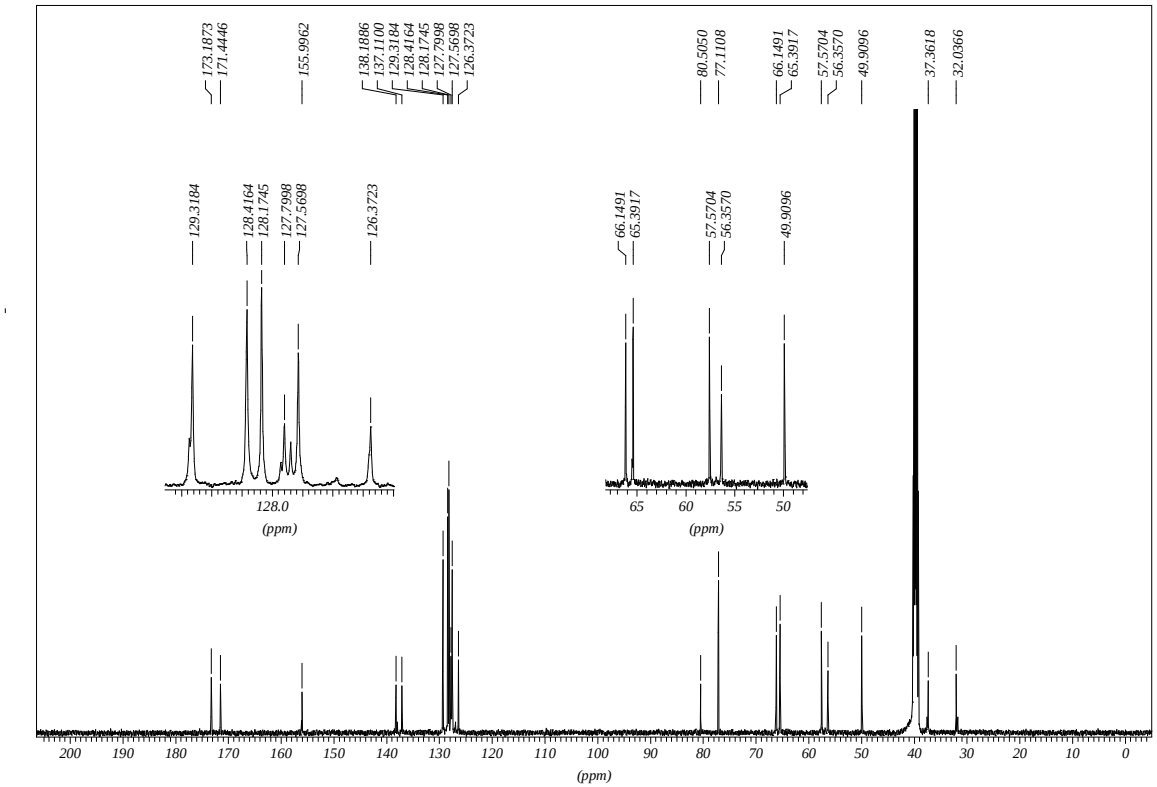
(*S*)-*N*-(*tert*-Butyloxycarbonyl)-*O*-(propynyl)-homoserine amide (34)



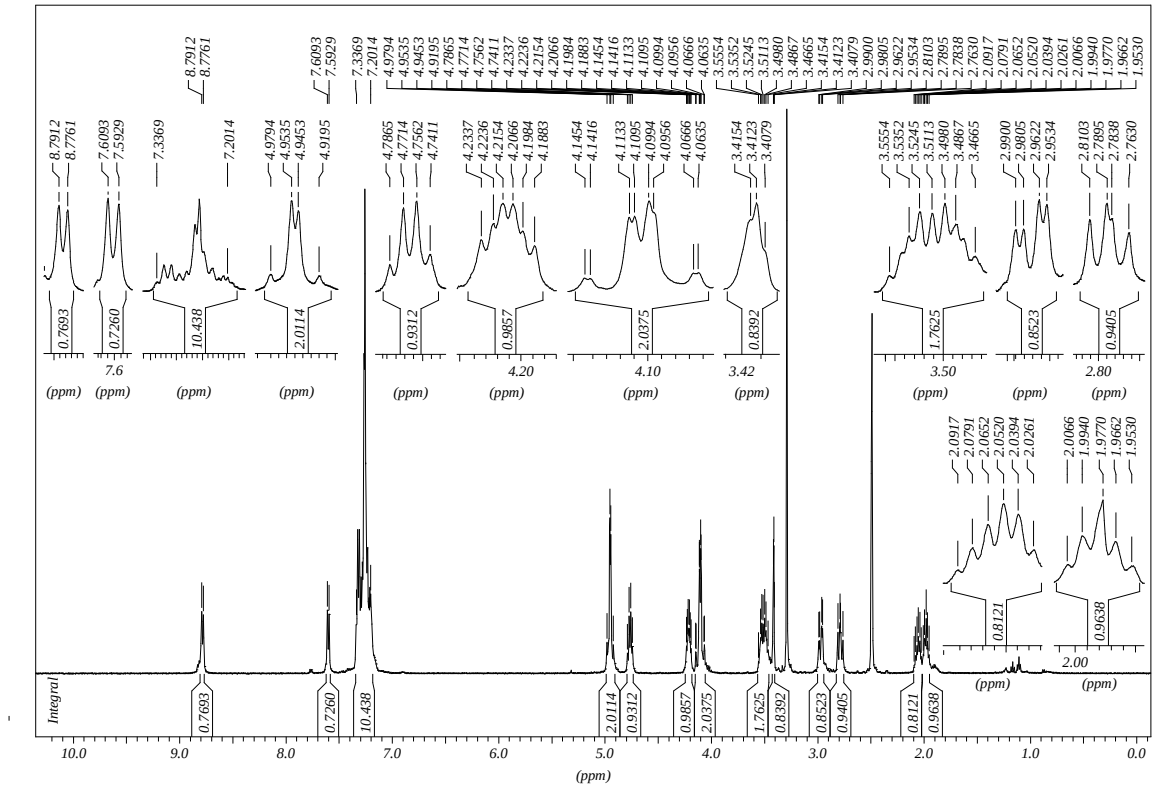


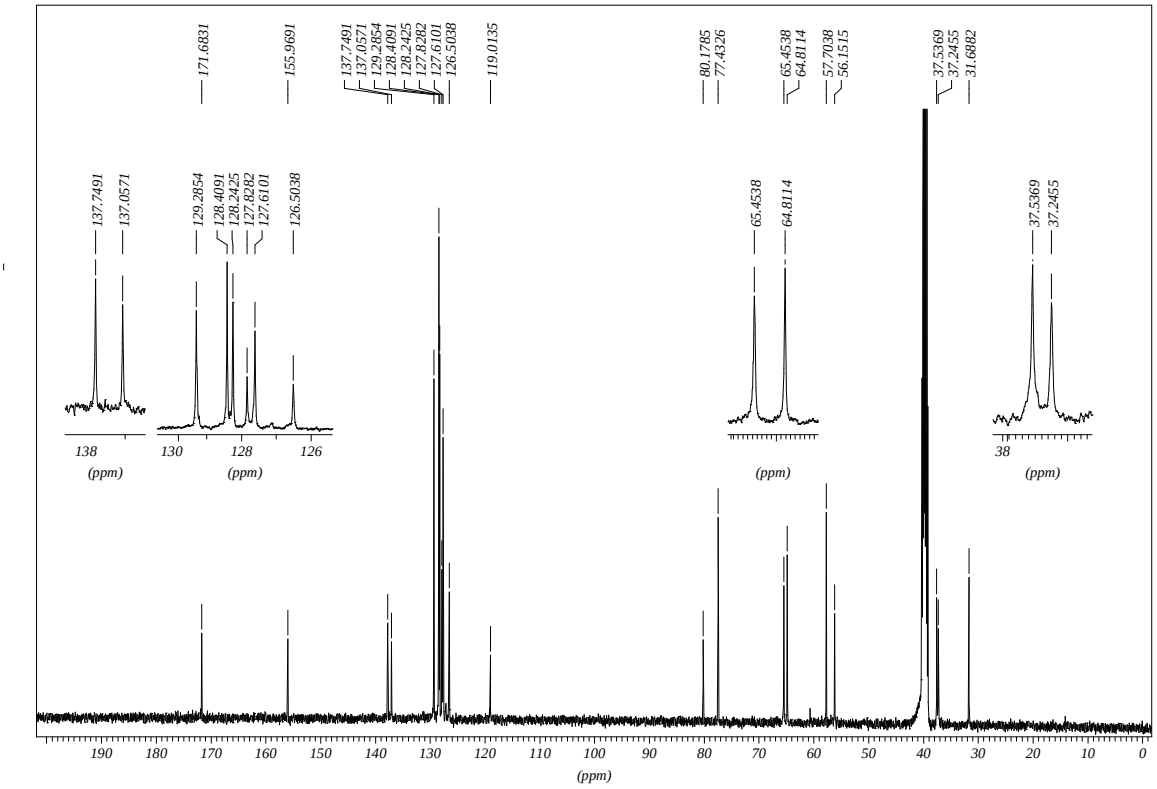
(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-homoserine amide (36)



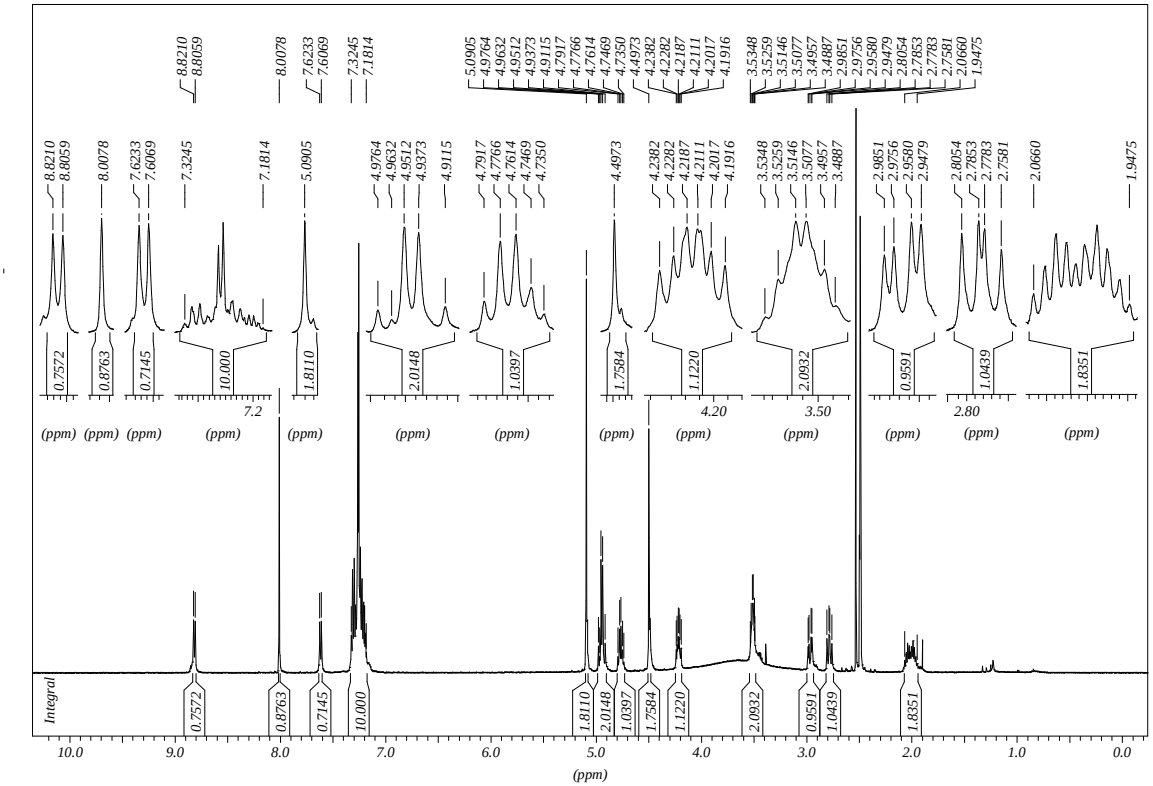


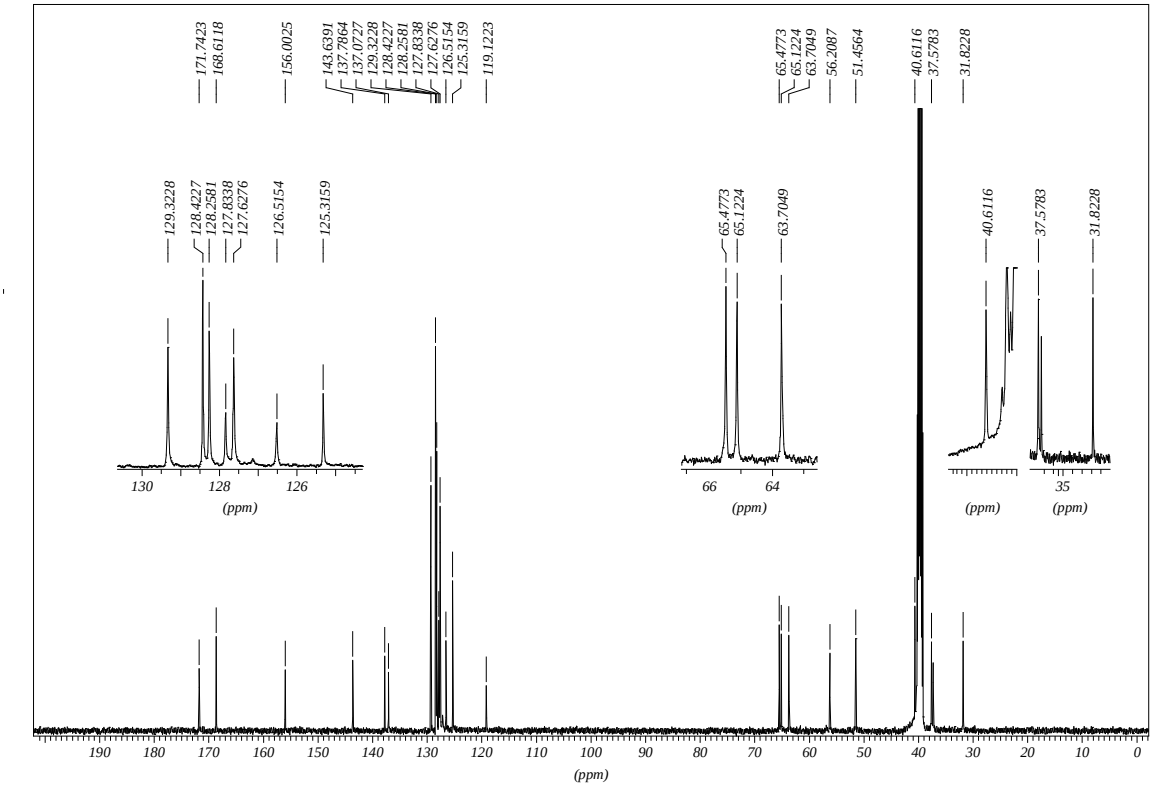
(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-(propynyl)-homoserine nitrile (37)



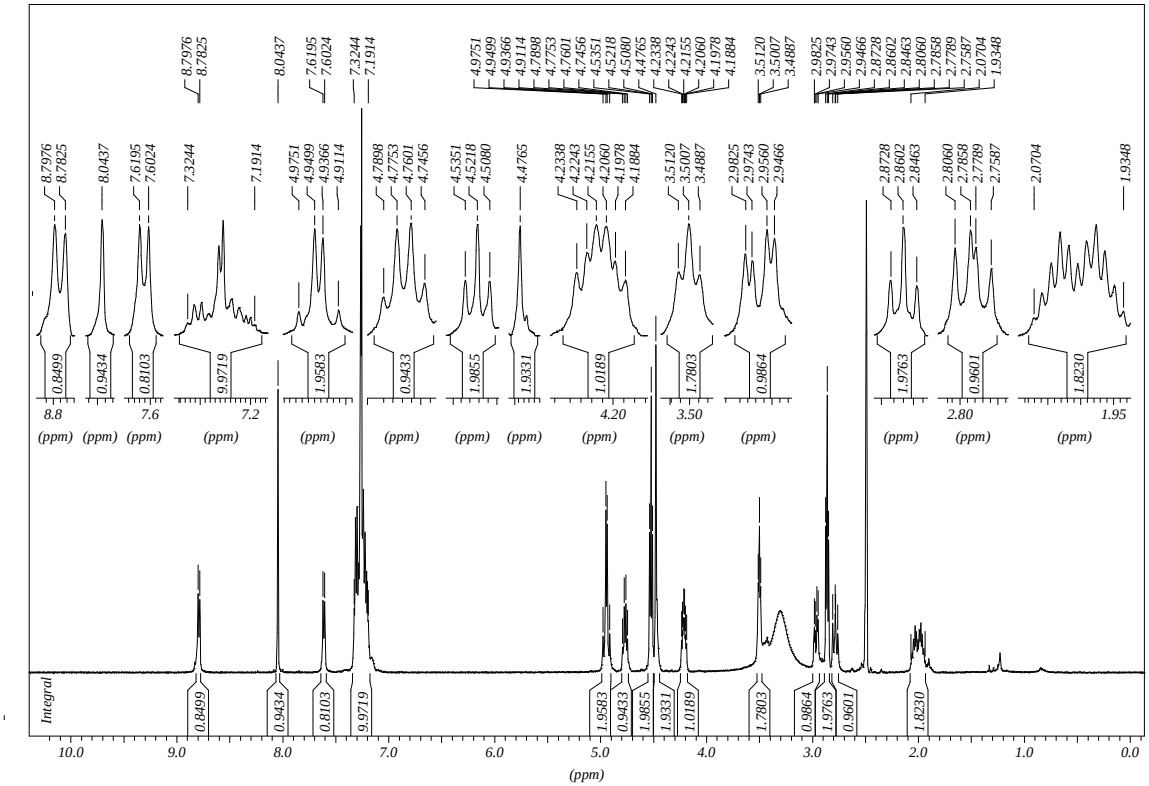


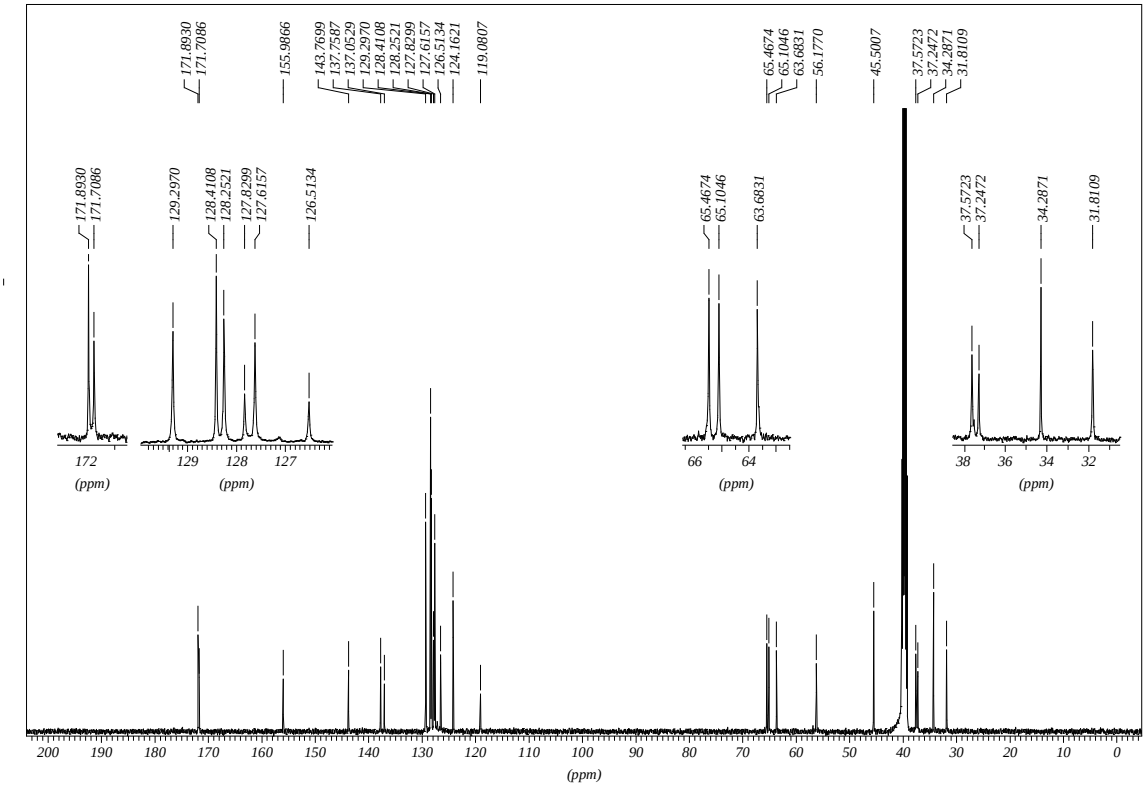
(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-homoserine nitrile (14)



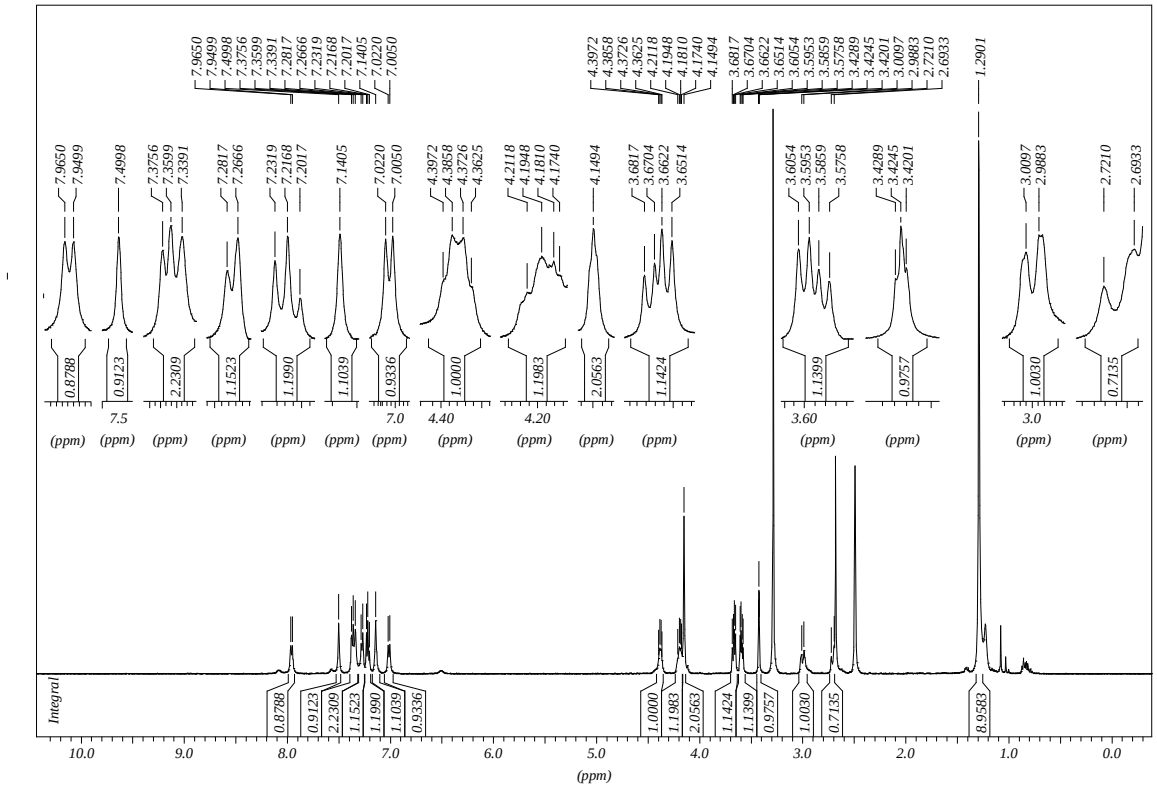


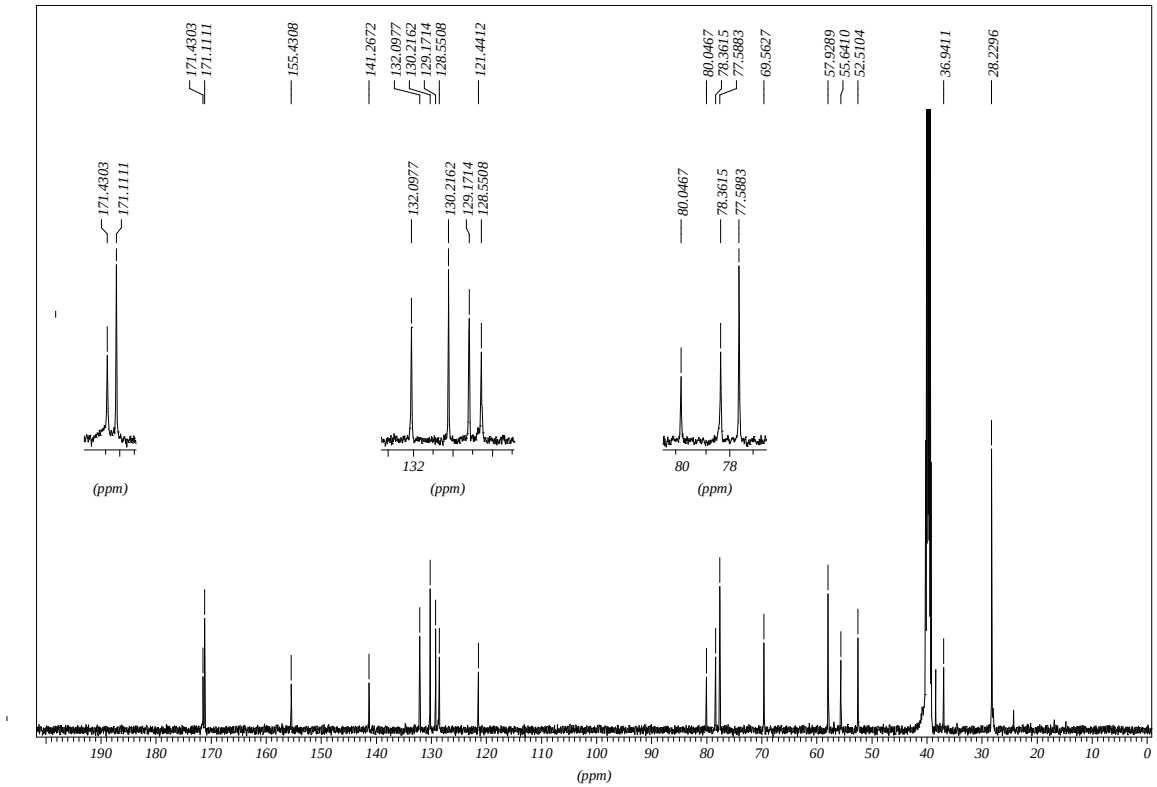
(*S*,*S*)-*N*-(Benzyloxycarbonyl)-phenylalanyl-*O*-((1-(2-carboxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-homoserine nitrile (15)



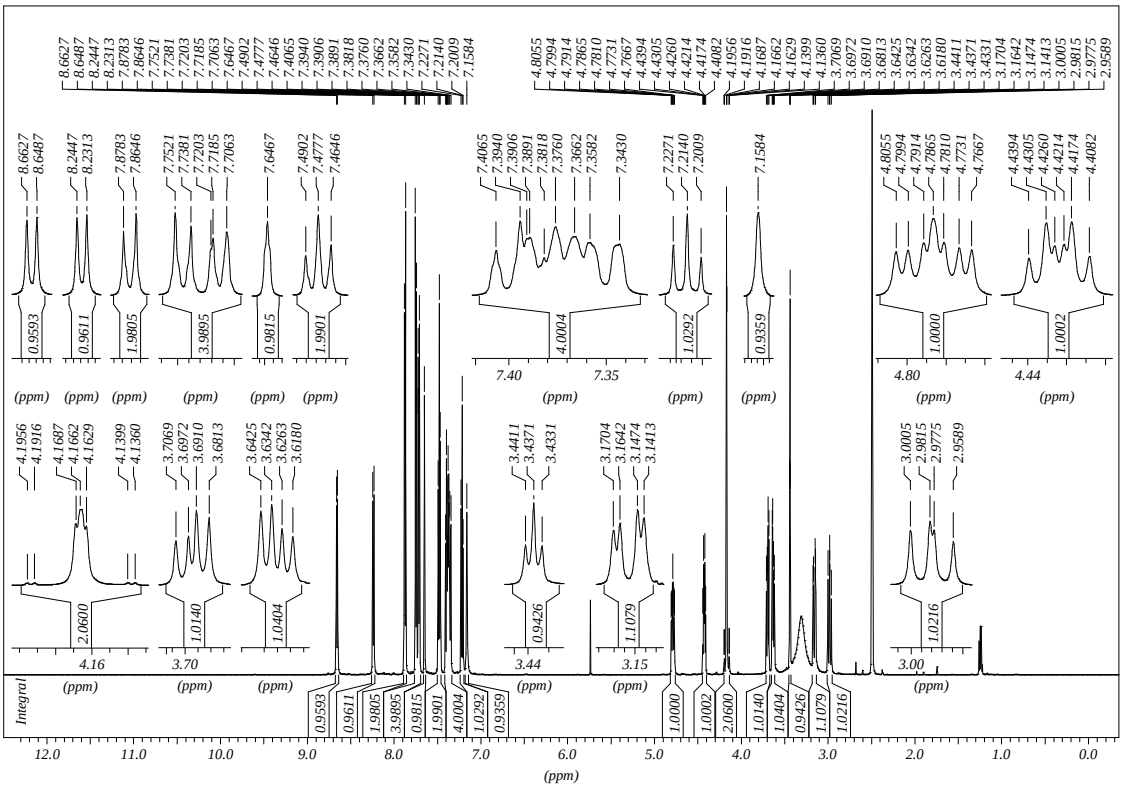


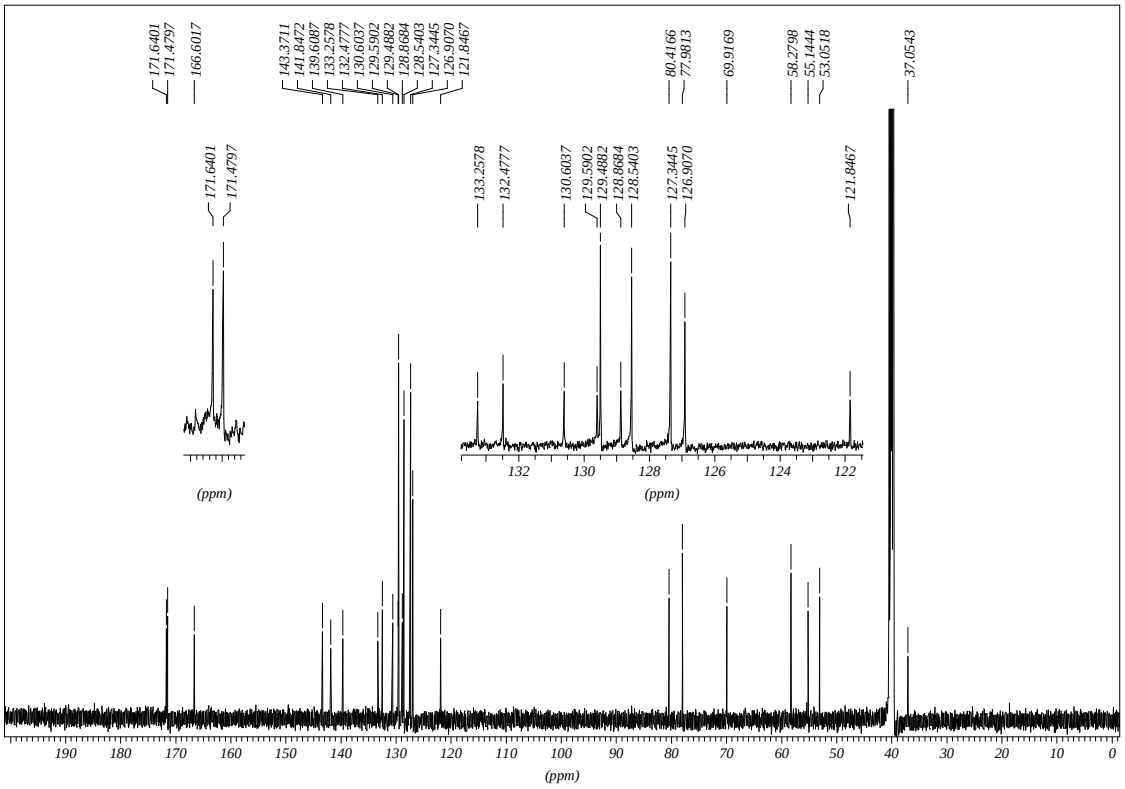
(*S*,*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanyl-*O*-(propynyl)-serine amide (38)



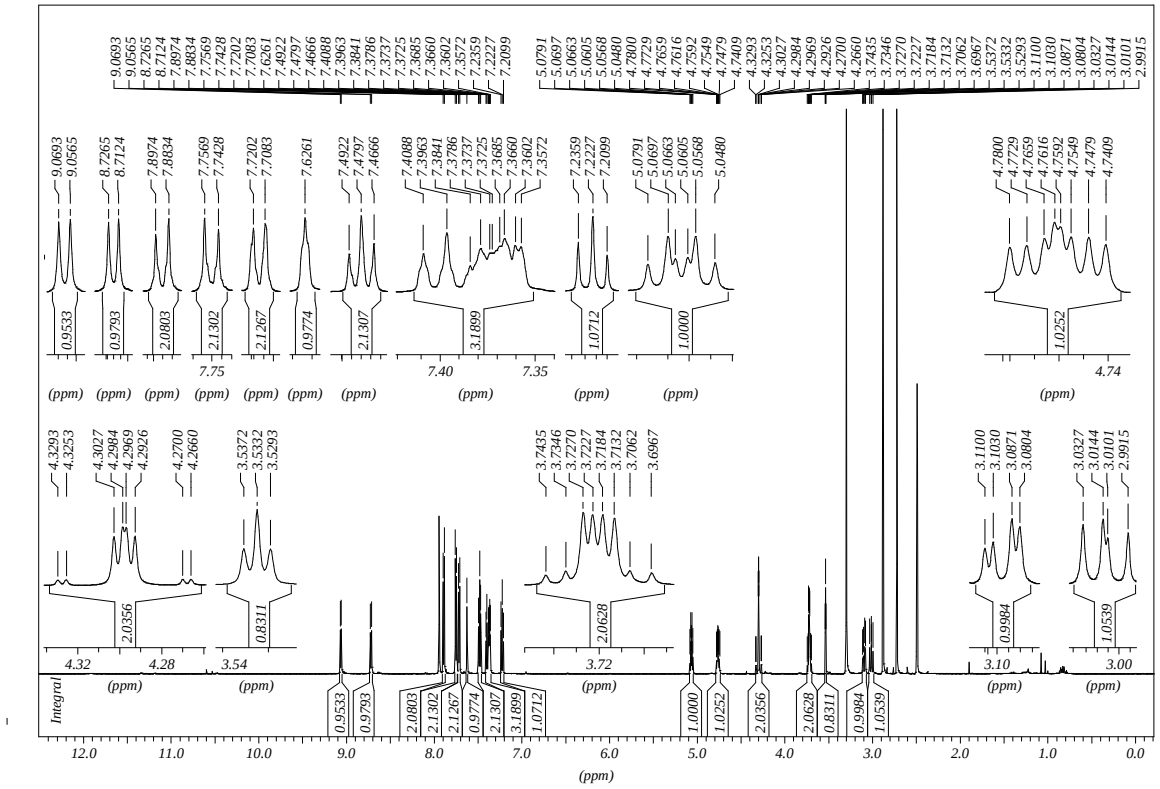


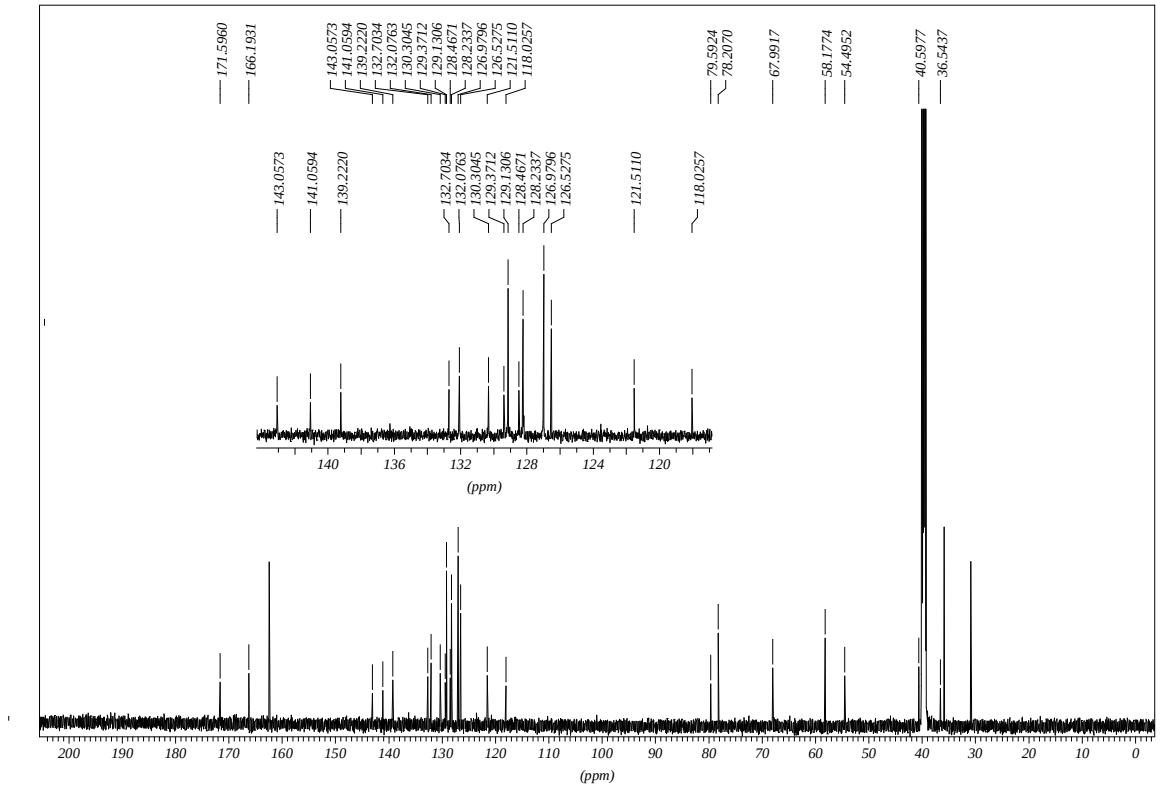
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-(propynyl)-serine amide (40)



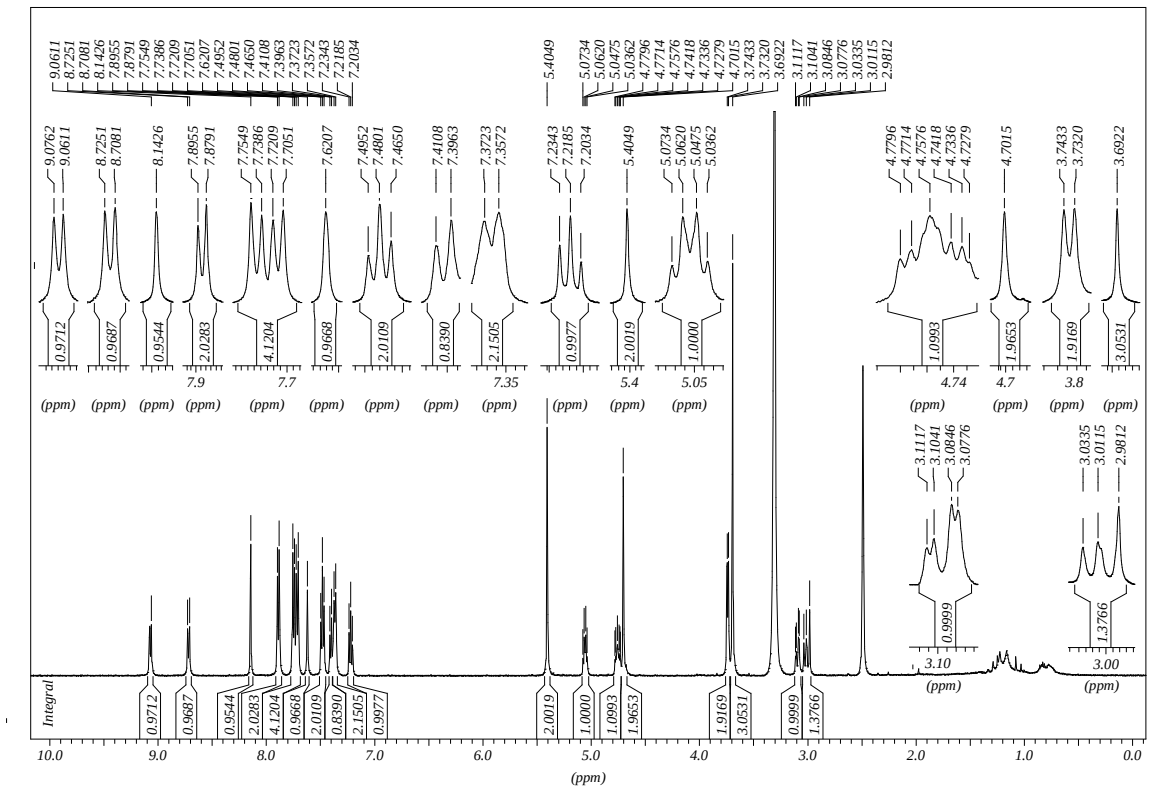


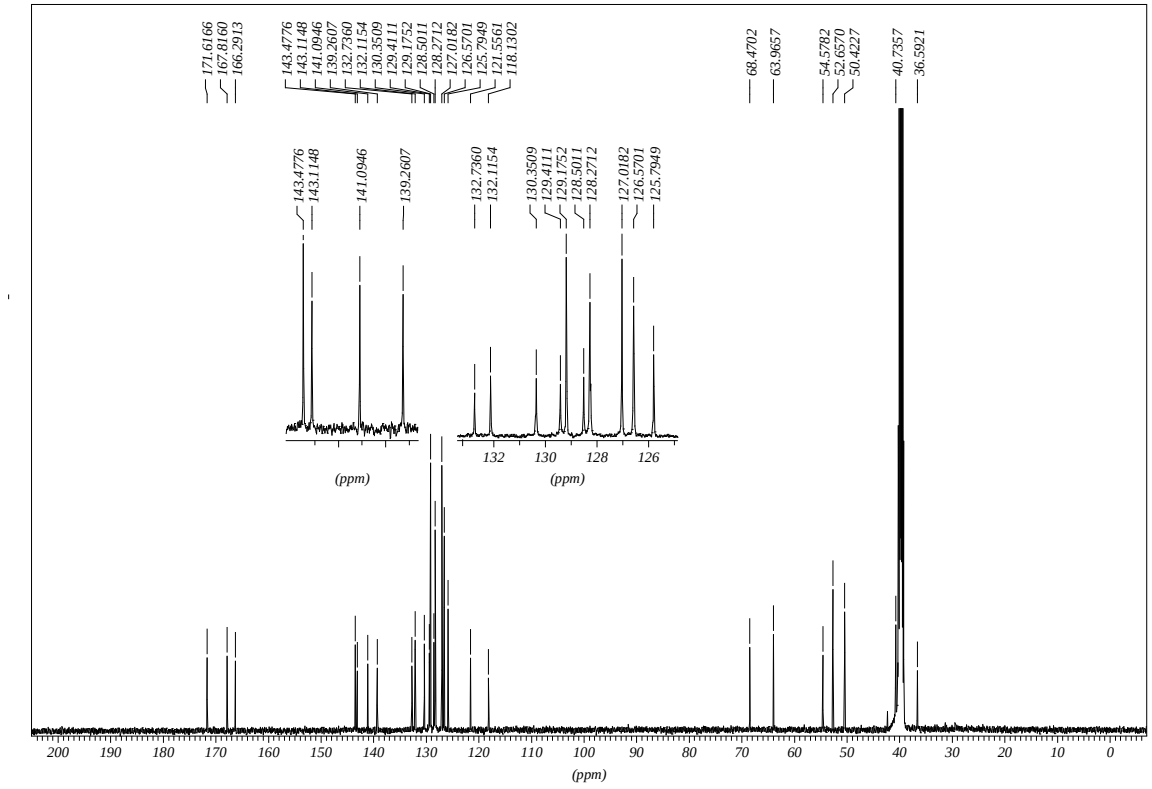
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-(propynyl)-serine nitrile (41)



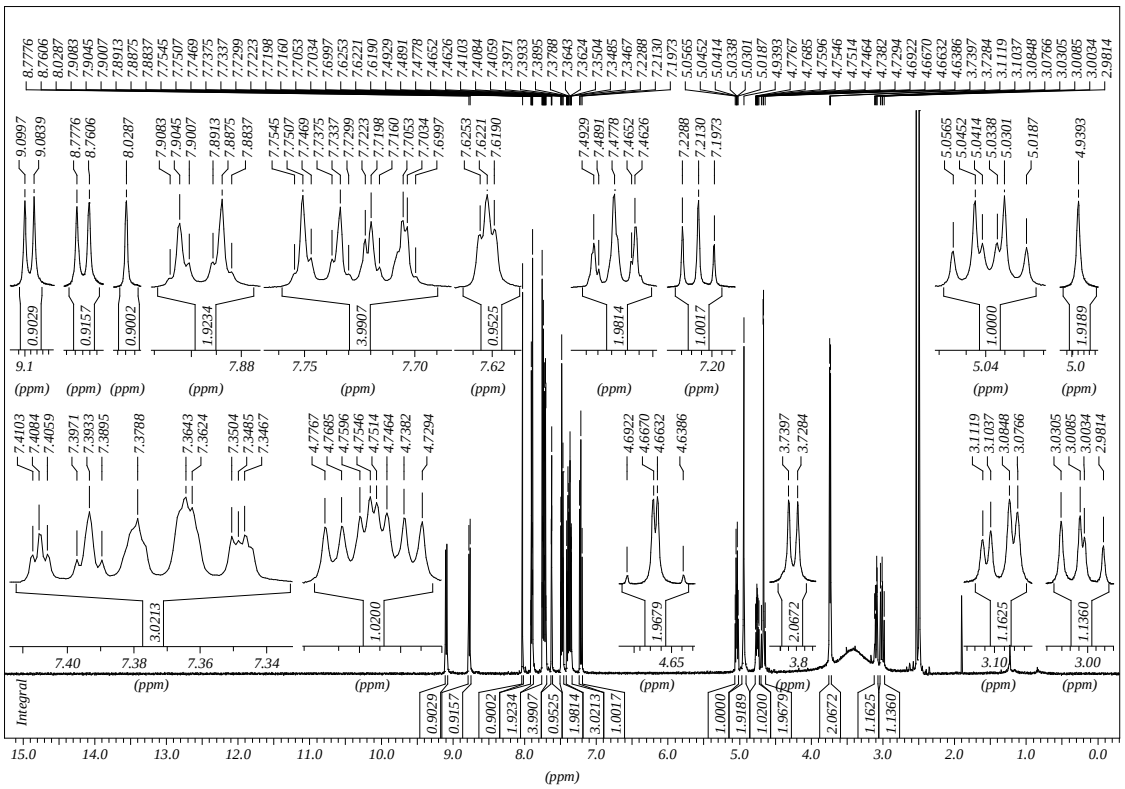


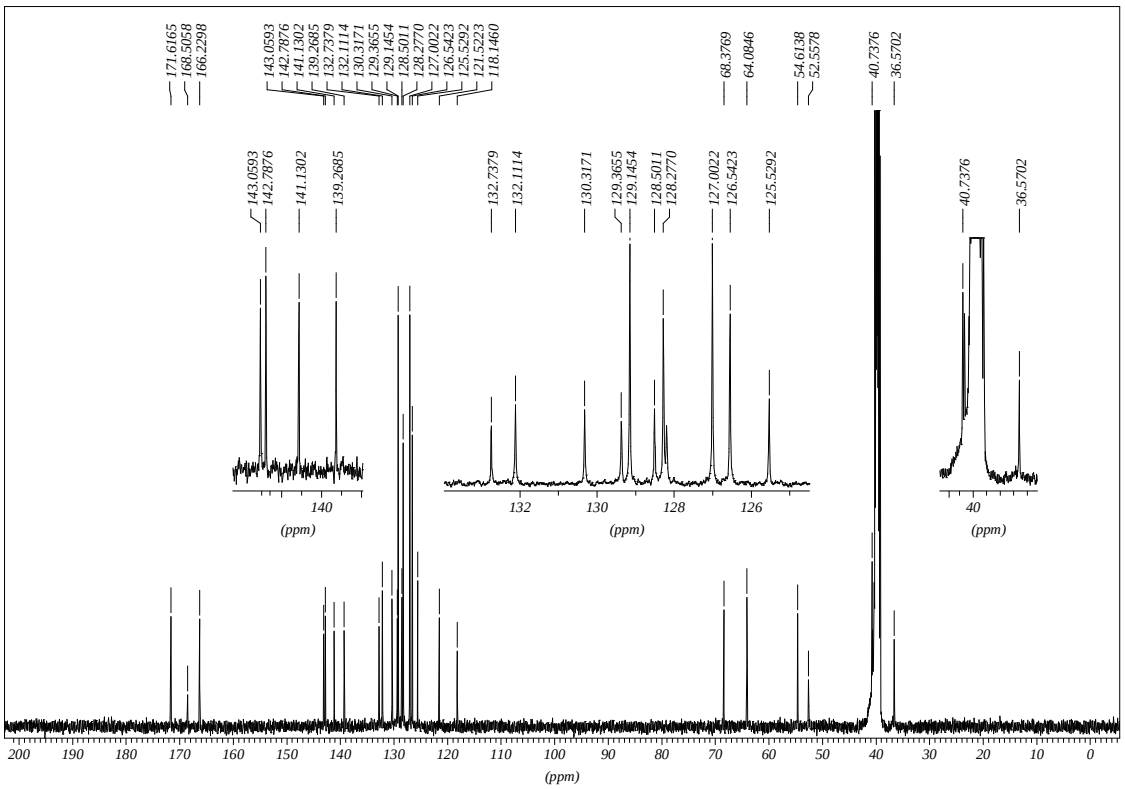
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-((1-(methoxycarbonylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-serine nitrile (16)



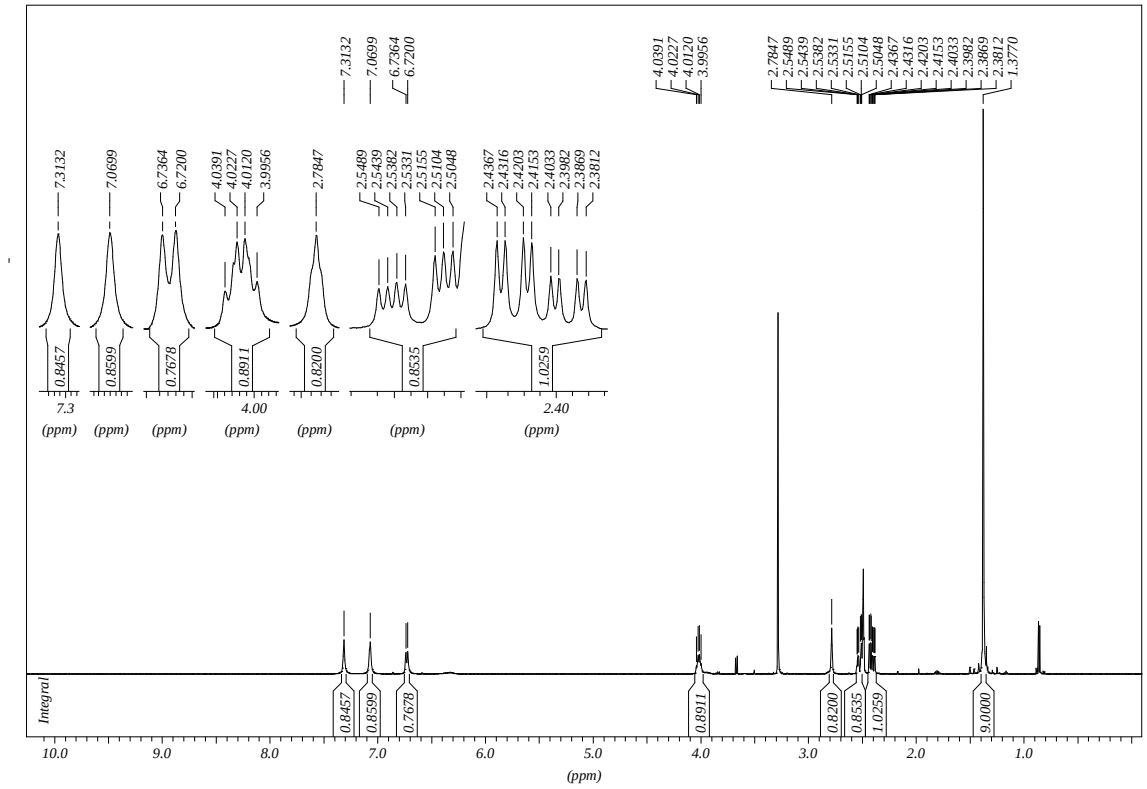


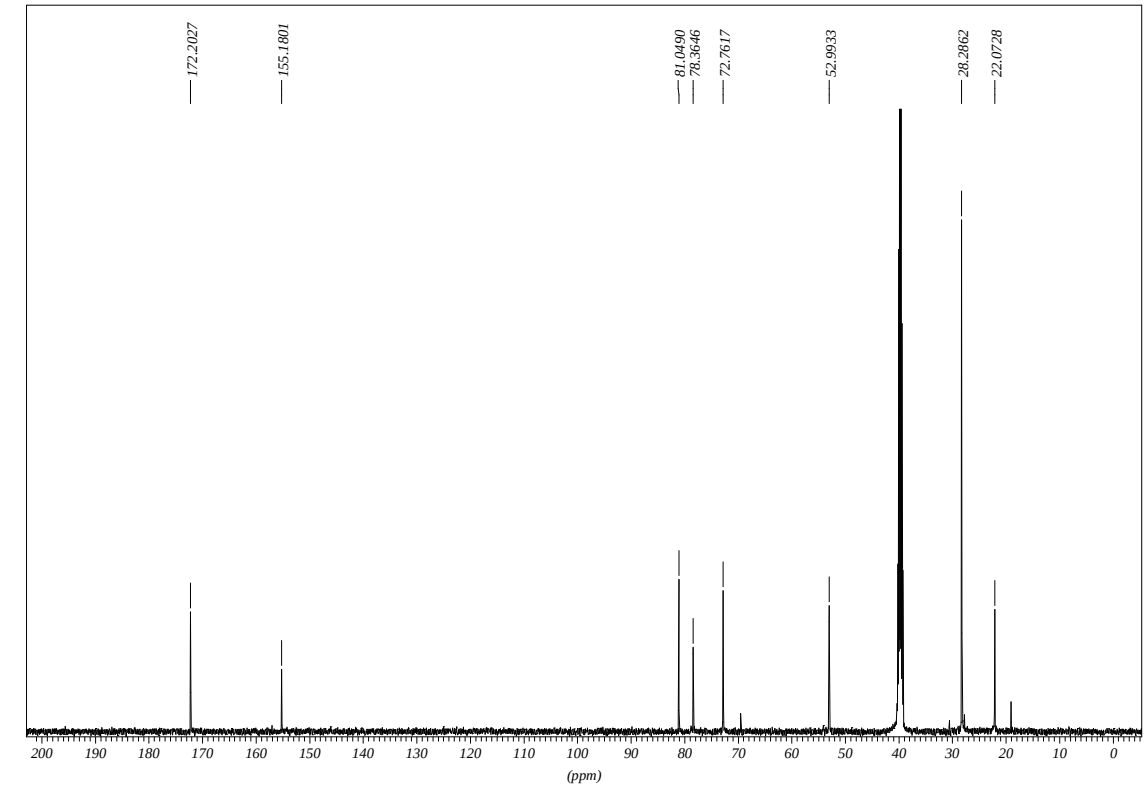
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-*O*-(methyl-1H-1,2,3-triazol-1-yl)acetic acid)-serine nitrile (17)



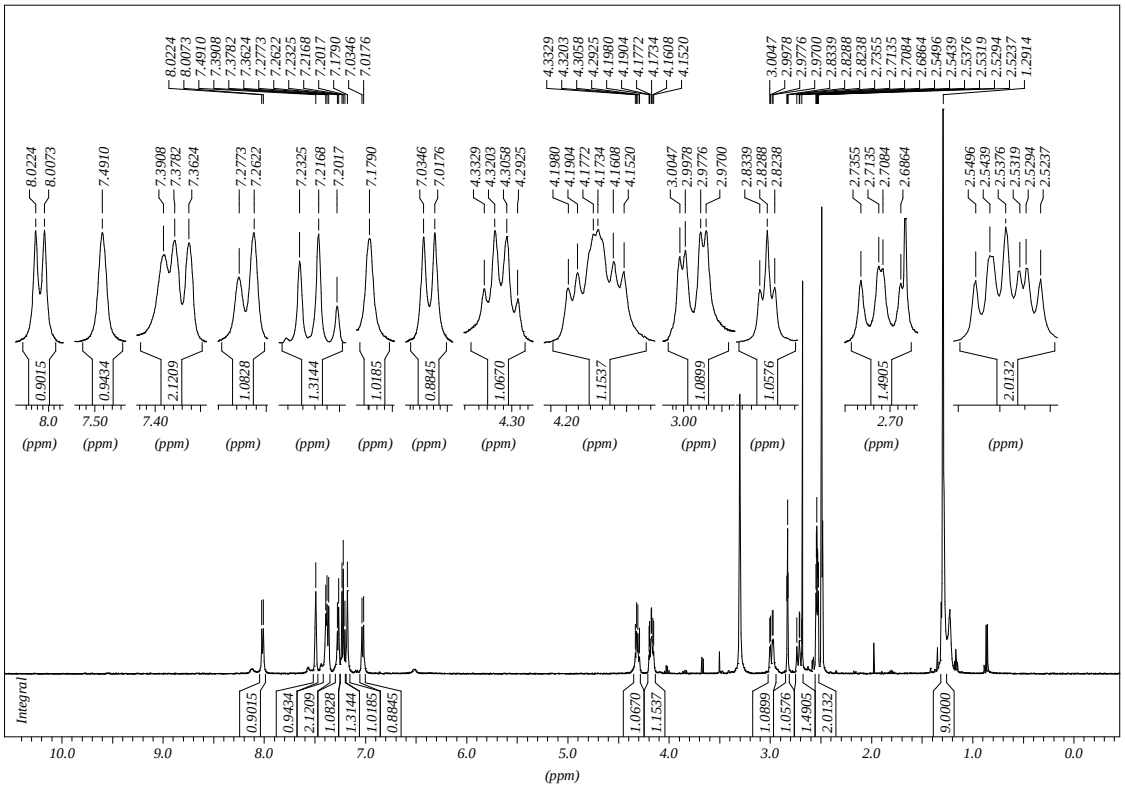


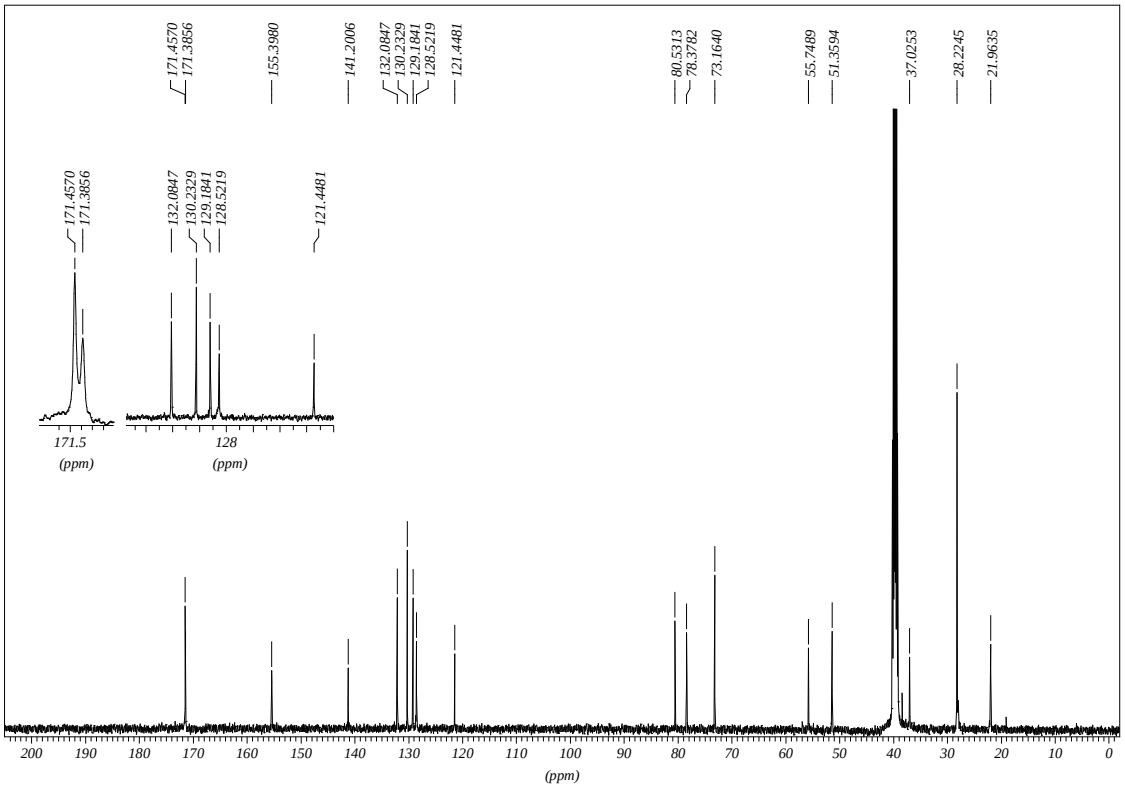
(*S*)-*N*-(*tert*-Butyloxycarbonyl)-propargylglycine amide (45)



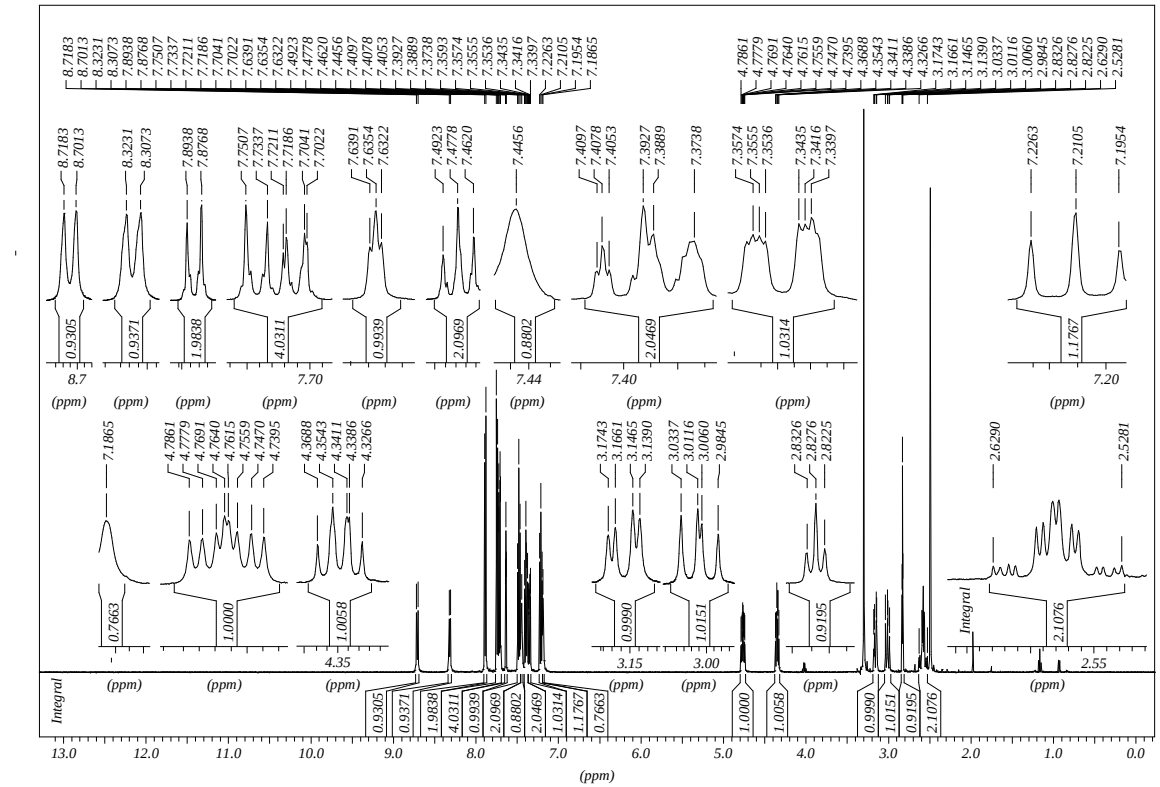


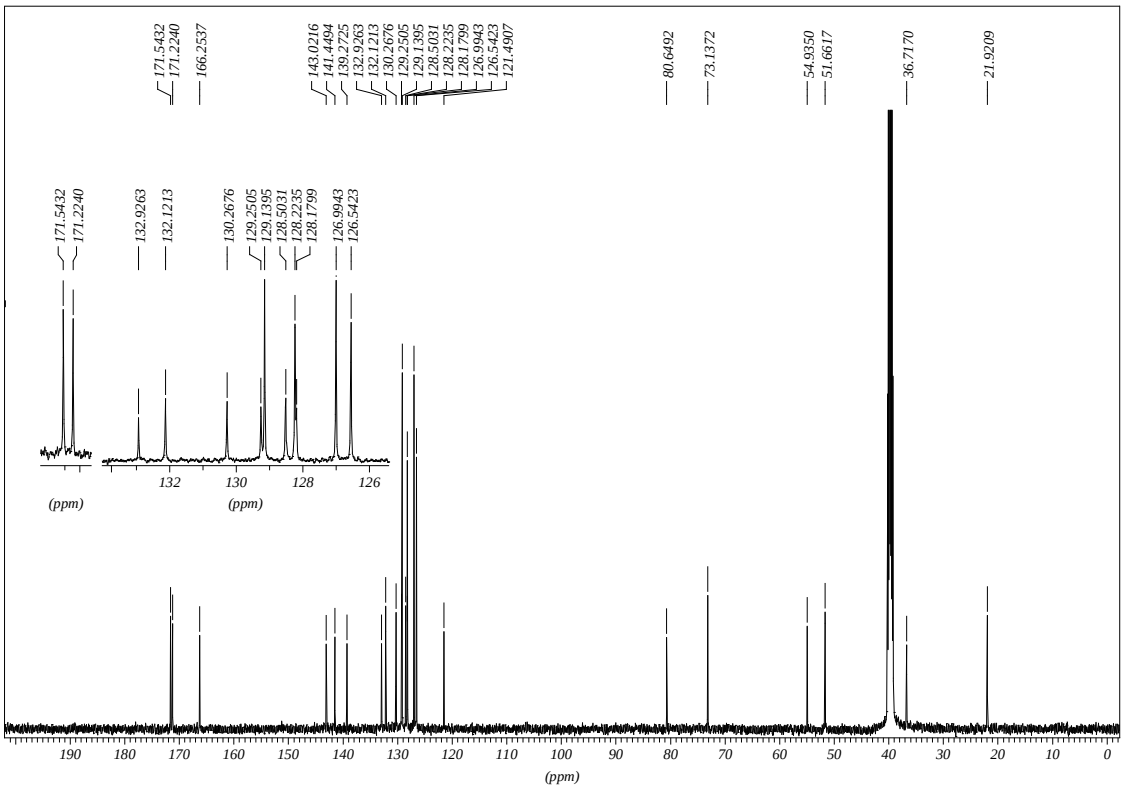
(*S*,*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-bromophenylalanyl-propargylglycine amide (47)



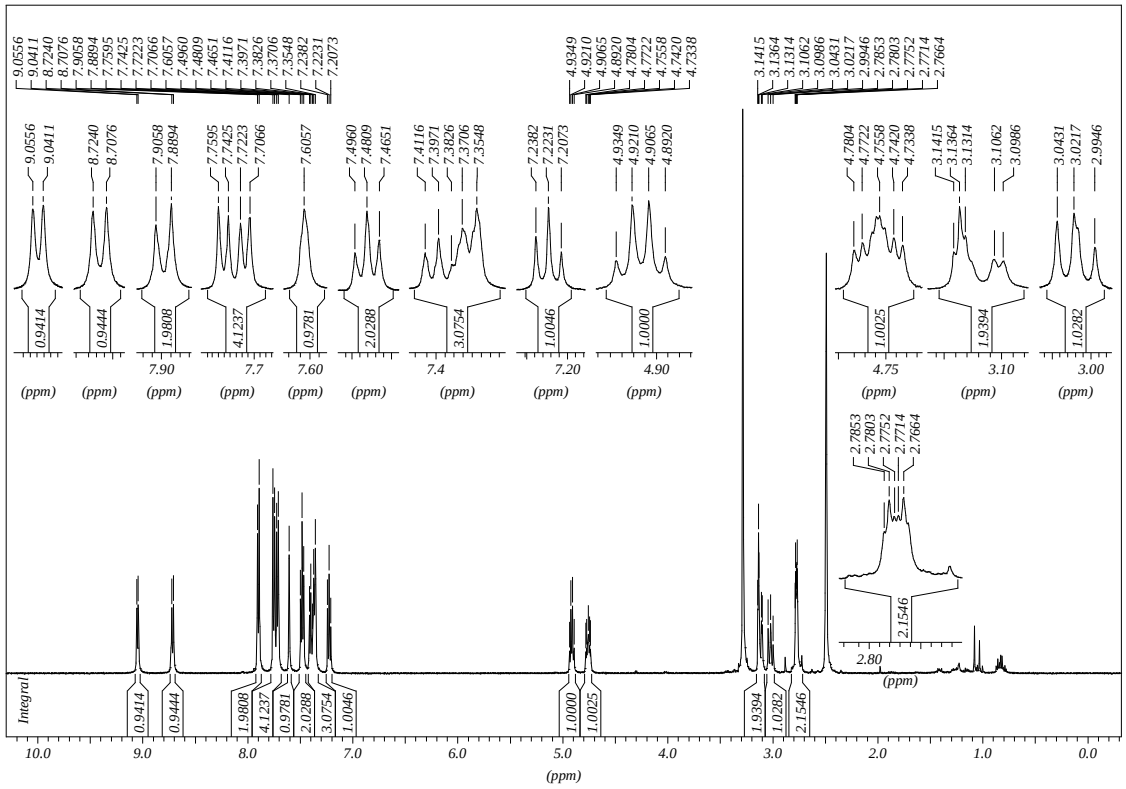


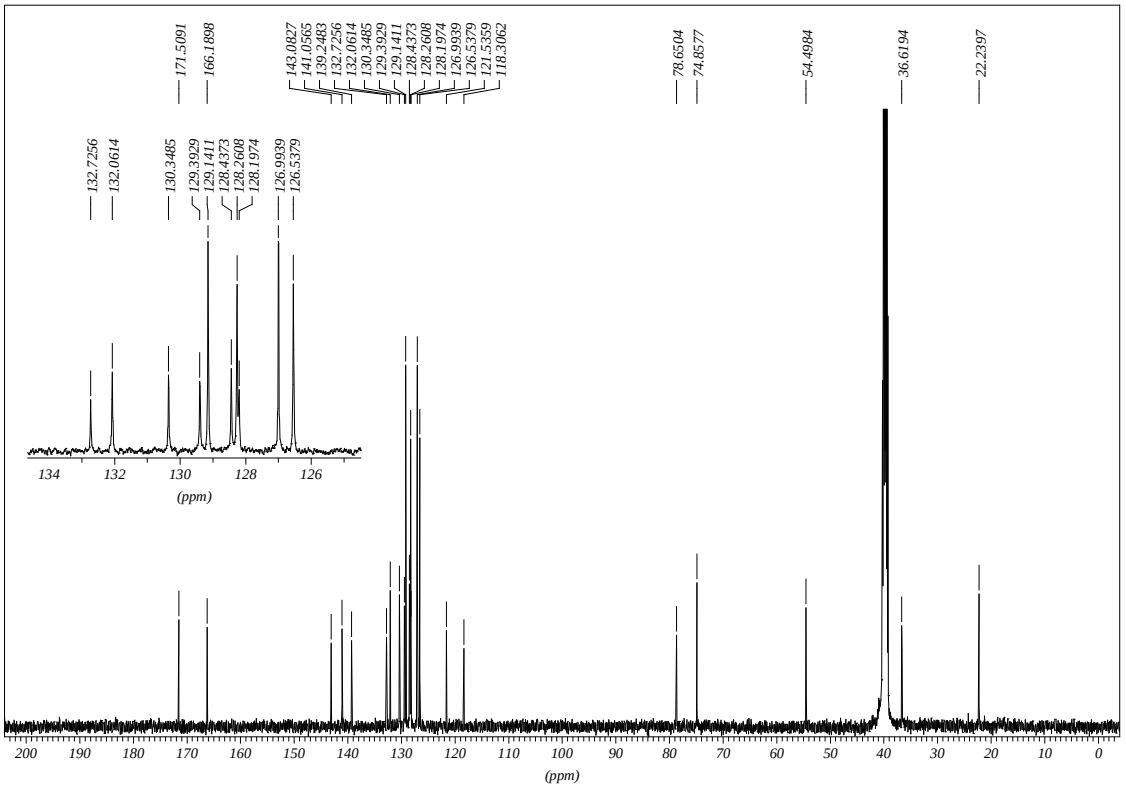
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-propargylglycine amide (49)



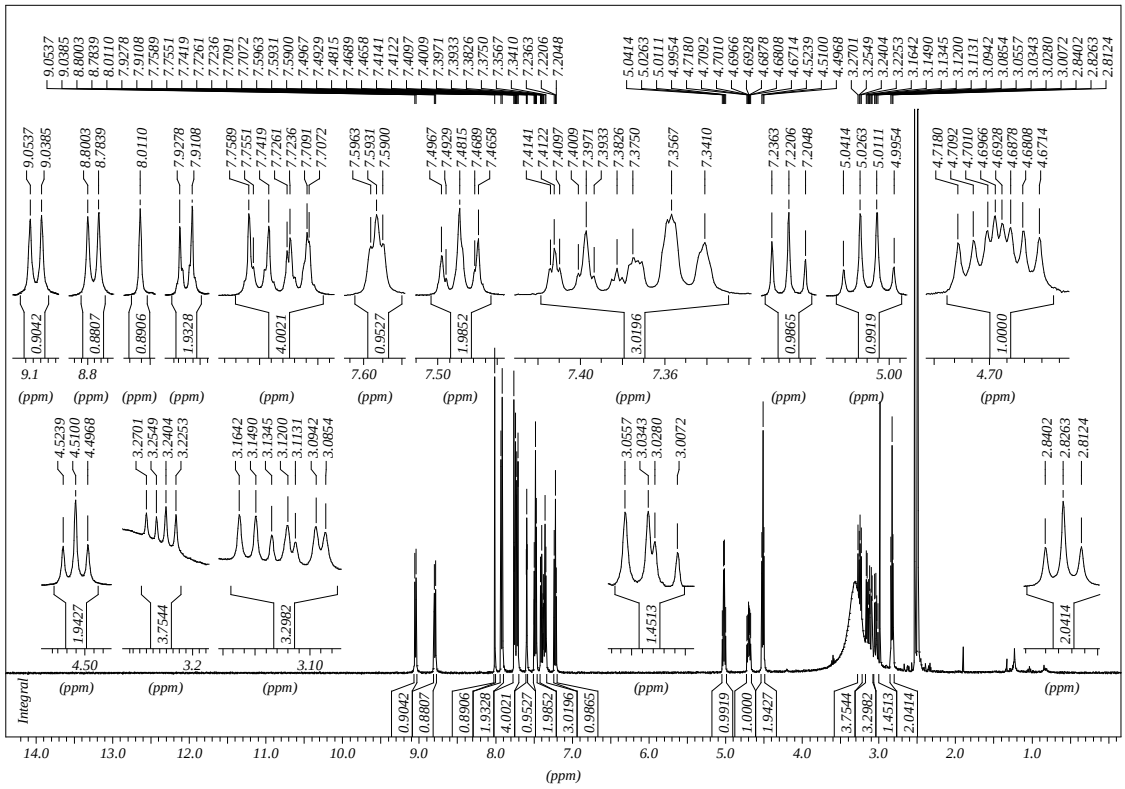


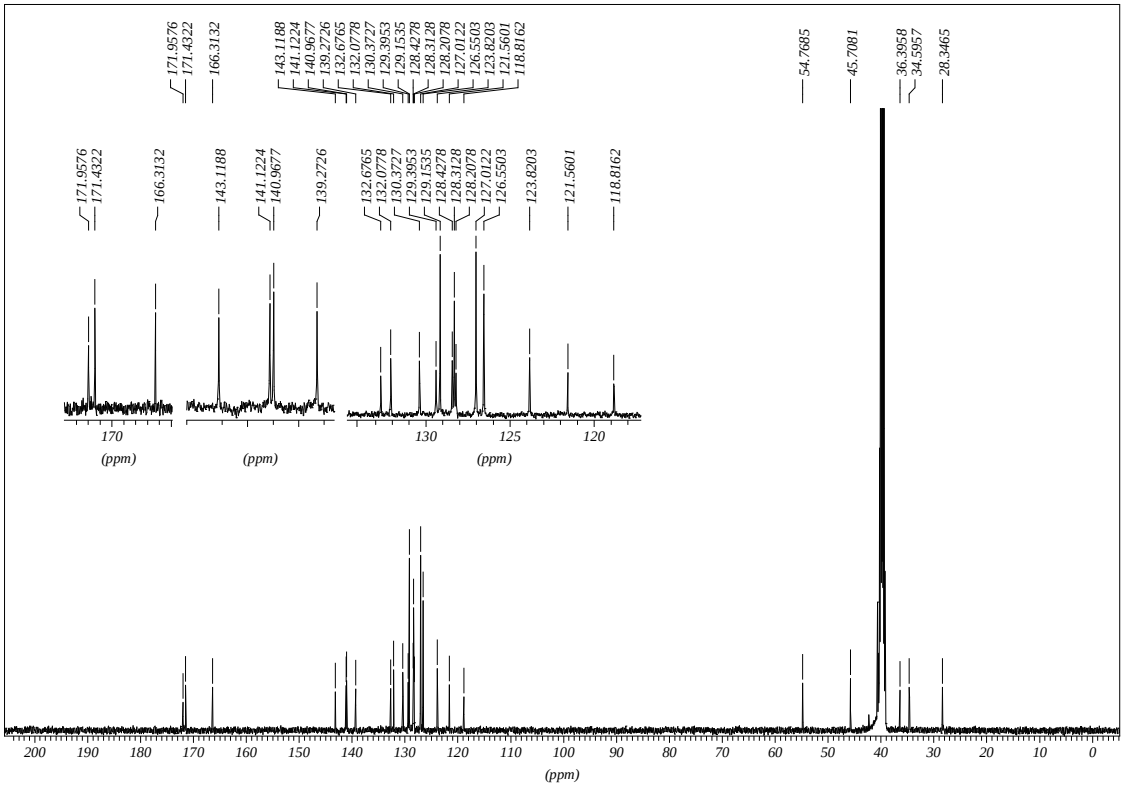
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-propargylglycine nitrile (50)



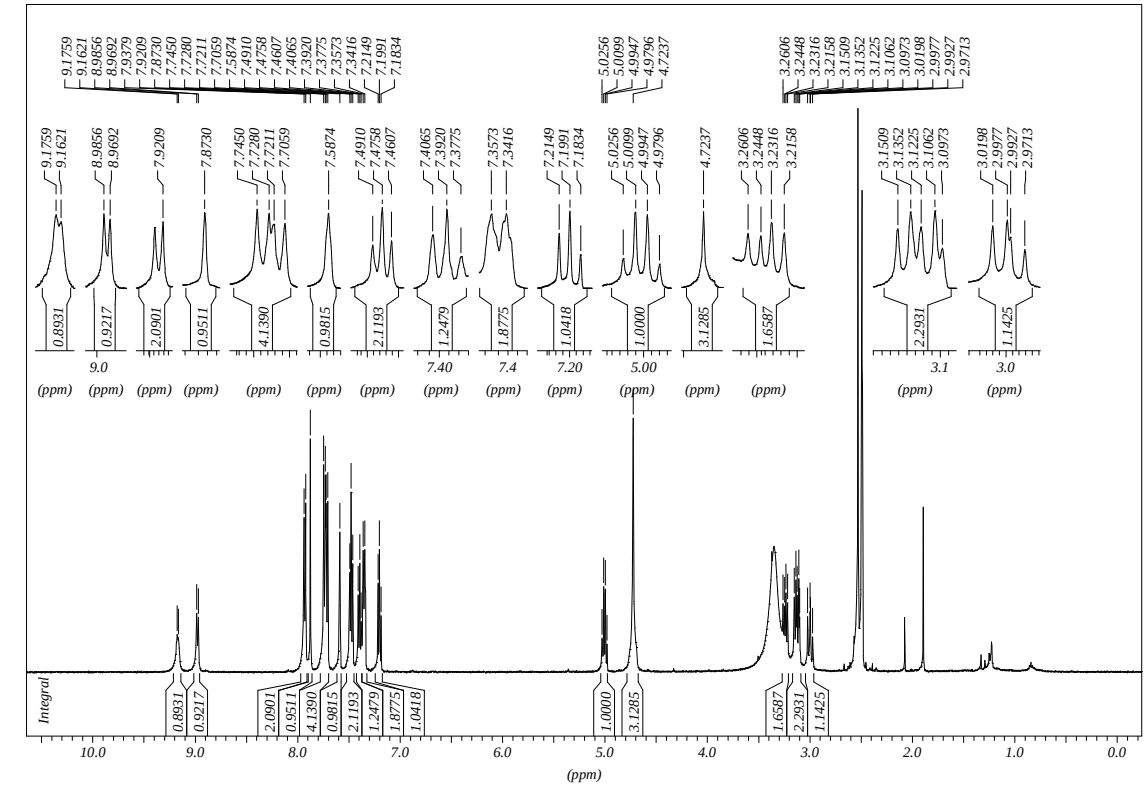


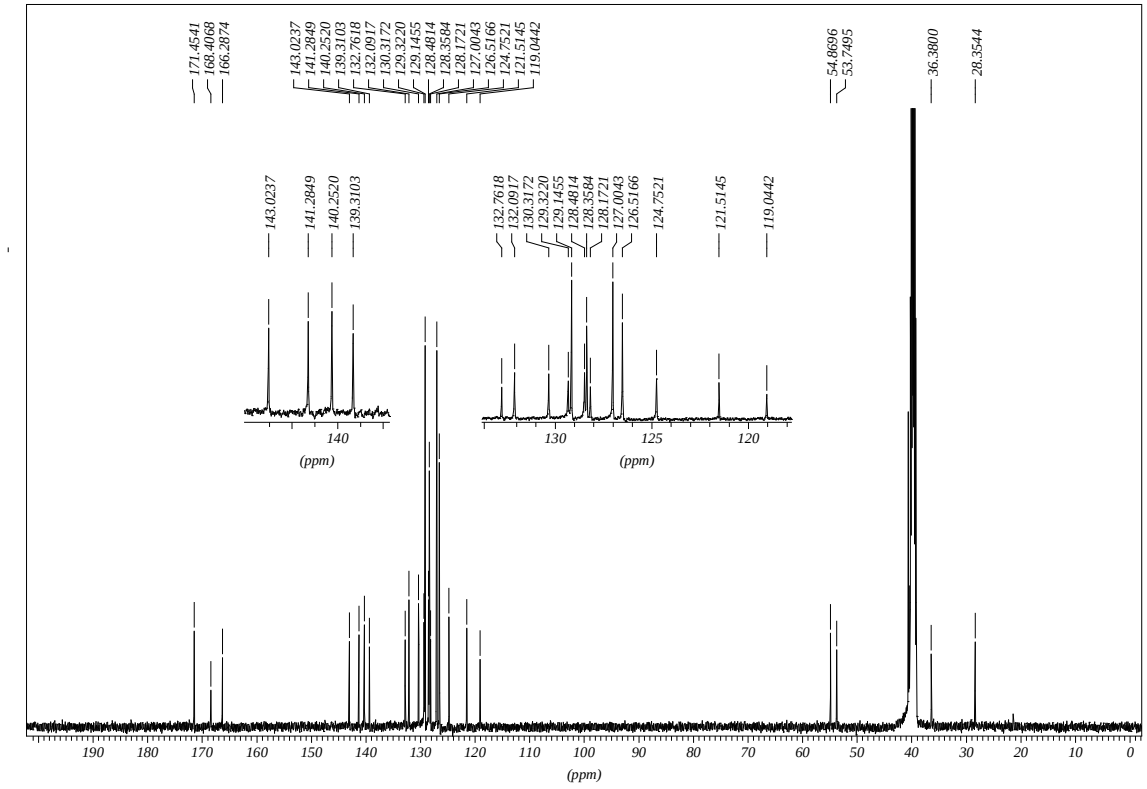
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-2-((1-(2-(carboxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-glycine nitrile (18).



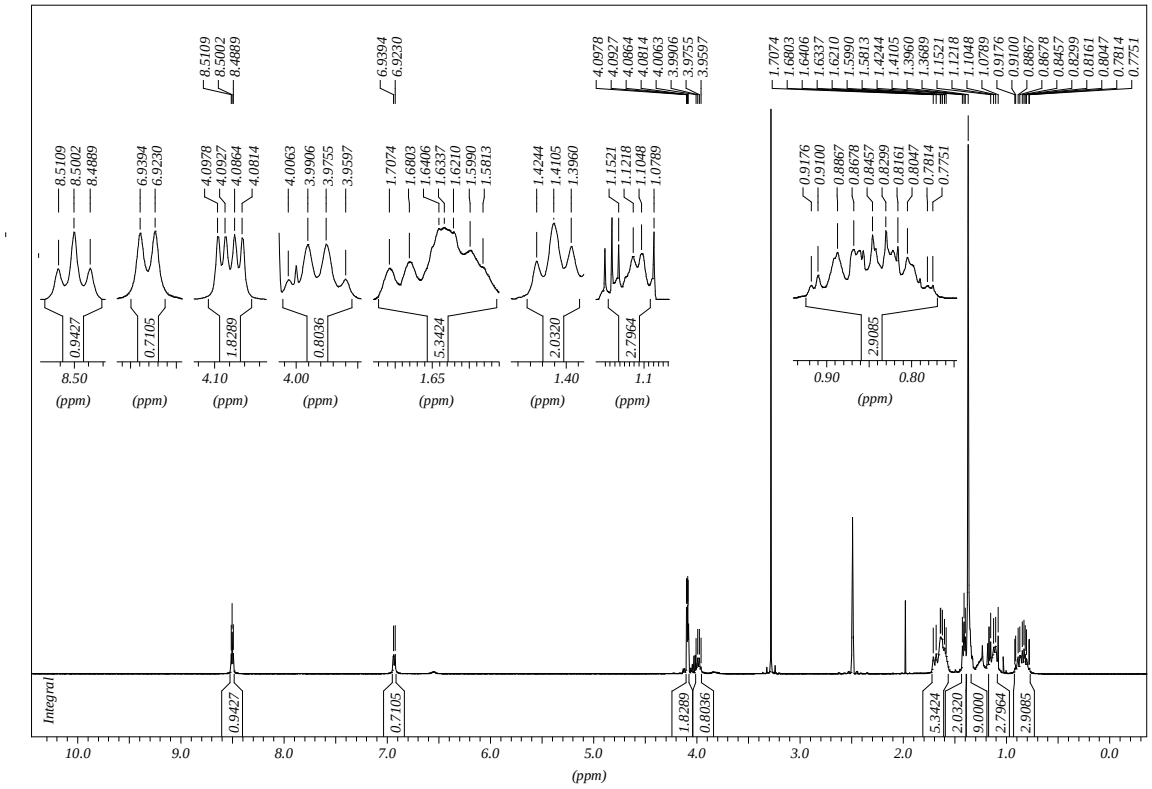


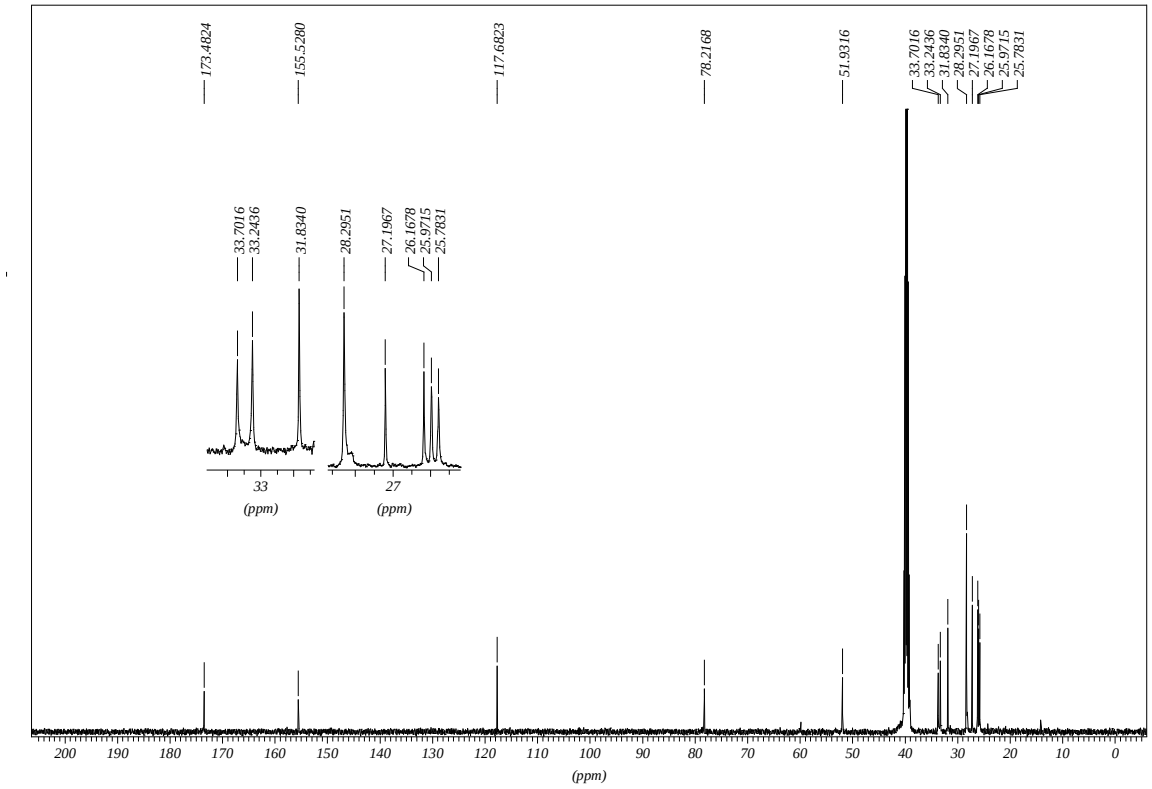
(*S*,*S*)-*N*-(4-Phenylbenzoyl)-3-bromophenylalanyl-2-((1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-glycine nitrile (19).





(*S*)-*N*-(*tert*-Butyloxycarbonyl)-cyclohexylalanyl-glycine nitrile (57)





(*S*)-*N*-(4-Phenylbenzoyl)-cyclohexylalanyl-glycine nitrile (91)