The A³-Mannich Coupling Reaction via Chiral Propargylglycine Ni(II) Complex: An Approach for Synthesizing Enantiomerically Enriched Unnatural α-Amino Acids

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EXPERIMENTAL SECTION

General information

All amines, aldehydes were obtained from commercial sources and used without further purification. The initial **2** complexes were prepared following literature protocols[1]. TLC analyses were performed on glass plates coated with silica gel 60 F254. Column chromatography was performed on silica gel (60×120 mesh) on a glass column.

Instrumentation

Melting points (mp) were determined by «Electrothermal». ^{1}H and ^{13}C NMR spectra («Mercury-300 Varian» 300 MHz respectively) were recorded using TMS as an internal standard (0 ppm). Elemental analyses were done by elemental analyzer EURO EA 3000. The enantiomeric purity of the amino acids was determined by HPLC («Waters Alliance 2695 HPLC System») on the chiral phase Diaspher-110- Chirasel-E-PA 6:0 mkm 4.0*250 mm, and a mixture of 20% MeOH and 80% 0:1 M aqueous solution $NaH_2PO_4*2H_2O$ was used as the eluent. The optical rotation was measured on a Perkin Elmer-341 polarimeter, The X-Ray was done by Enraf-Nonius CAD4, LCMS analysis was done by Shimadzu LCMS 2020 with prominence-I LC-2030C 3D. The CD analyses was done my Chirascan™ V100._

General Procedures

A. Genral procedure of Mannich reaction with paraformaldehyde

A 2 g (0.0037 mol) sample of Ni^{II}-(*S*)-BPB-(*S*)-PGly complex, 0.336 g (0.011 mol) of paraformaldehyde, and 0.060 g (0.00037 mol) of $FeCl_3$ were added to a round-bottom flask connected to a reflux condenser and supplied with an argon gas flow. The mixture was dissolved in 20 ml toluene for 15-20 minutes. Then, 0.07 g (0.00037 mol) of CuI and (0.0074 mol) of amine mixed with a small amount of toluene were added. The reaction mixture was stirred under heating conditions at a temperature of 50-60°C. The course of the reaction was monitored by TLC (SiO_2, $CHCl_3/CH_3COCH_3$ = 3/1) until the traces of the initial complex were eliminated. Based on TLC data, the reaction took 0.5-3 hours.

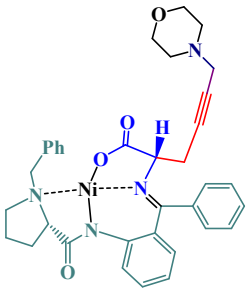
After completion of the reaction, the mixture was poured into distilled water (50 mL) under stirring for 15 min and extracted with methylene chloride (3 × 30 mL). The combined organic layers were washed with distilled water (2 × 20 mL), dried over anhydrous $MgSO_4$ for 30 min, and filtered. The solvent was removed under reduced pressure, and the residue was crystallized from acetone (20 mL) to afford the pure product.

B. General procedure for isolation of amino acids

Complexes **3a–d** were dissolved in MeOH (50 mL) and slowly added to 2 M HCl (50 mL). The reaction mixture was heated at 50 °C until the characteristic red color of the metal complex disappeared. The solution was then concentrated under reduced

pressure, diluted with water (50 mL), and the precipitated (S)-BPB·HCl was collected by filtration. The optically active α-amino acids **4a–d** were subsequently isolated from the aqueous layer by ion-exchange chromatography on Dowex-50 (H^+ form) resin, using 5% aqueous NH_4OH as the eluent. The eluates were concentrated under reduced pressure, and the amino acids were purified by recrystallization from a water/EtOH mixture (1:2 v/v).

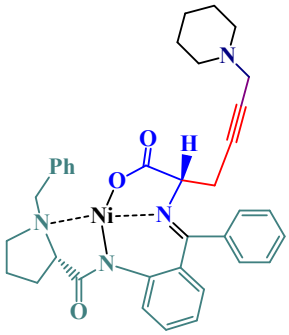
Complex 3a.



Method A. Yield 79% (1.85 g), Mp +213-214^{0}C, the reaction duration is 1.5 hour. $[\alpha]_D^{20}$ +2503^0 (c 0.15, CH_3OH): Anal Calc. for: $C_{35}H_{36}N_4NiO_4$ (635.38); C 66.16; H 5.71; N 8.82; Found: 66.56; H 5.99; N 9.21: MS, *m/z*: 635.15

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01-2.15 (2H, m, γ, δ-Ha); 2.39-2.57 (6H, m); 2.67-2.82 (2H, m); 3.39-3.42 (2H, m); 3.46 (1H, dd, J=10.7, 6.1,α-H-Pro); 3.55-3.70 (7H, m); 3.98 (1H, dd, J=7.3,3.0); 4.44 (1H, d, J=12.7, CH_2Ph); 6.63-6.69 (2H, m, H-3,4 C_6H_4); 7.01-7.06 (1H, m, C_6H_5); 7.16 (1H, ddd, J=8.6, 5.5, 3.1, H-5 C_6H_4); 7.18-7.28 m (3H, Ar); 7.32-7.39 (2H, m,H-3.3, Ph); 7.44-7.55 (3H, m, Ar); 8.00-8.05 (2H, m,H-2.2, Ph); 8,25 (1H, br,d, J=8.6,H-6, C_6H_4).^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6 (δ-CH_2, Pro); 24.2 ($CH_2C{\equiv}C$); 30.8 (β-CH_2 Pro); 48.2 (CH_2-morpholin); 52.5 (α-CH_2, NCH_2 morpholin); 57.1 (δ-CH_2 Pro); 63.2 (CH_2 Ph); 66.8 (2C, OCH_2 morpholin,); 67.9 (NCH,CH_2); 70.2 (α -CH_2 Pro); 80.0 (2C, C≡C); 120.7 (CH); 123.8 (CH); 126.1; 126.8 (CH); 127.8 (CH); 128.9 (2CH); 129.0 (CH); 129.1 (CH); 129.2 (CH); 130.0 (CH); 131.7 (2CH); 132.5 (CH); 133.2; 133.5 (CH); 134.1; 142.9; 178.7; 180.4:

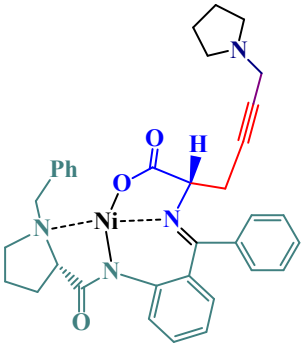
Complex 3b.



Method A. Yield 51% (1.2 g), Mp +186^{0}C, the reaction duration is 3 hour. $[\alpha]_D^{20}$ +2278.67^0 (c 0.15, CH_3OH): Anal Calc. for: $C_{35}H_{38}N_4NiO_3$ (633.41); C 68.26; H 6.05; N 8.85; Found: 68.70; H 6.39; N 9.20: MS, *m/z*: 633.20

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16-1.46 (9H, m), 1.45-1.68 (5H, m), 1.98-2.22 (3H, m), 2.35-2.80 (9H, m, γ, δ-Ha), 3.46 (4H, t, *J* = 8.4 Hz, α-H-Pro), 3.62 (3H, t, *J* = 10.3 Hz), 3.92-4.05 (1H, m), 4.43 (1H, d, *J* = 12.7 Hz), 6.54-6.73 (2H, m, H-3,4 C_6H_4), 7.03 (1H, d, *J* = 6.5 Hz, C_6H_5), 7.10-7.27 (3H, m, H-5 C_6H_4, Ar), 7.35 (2H, t, *J* = 7.6 Hz, Ph), 7.44-7.59 (3H, m, Ar), 8.03 (2H, d, *J* = 7.1 Hz, Ph), 8.25 (1H, d, *J* = 8.7 Hz, H-6, C_6H_4).^{13}C NMR (75 MHz, $CDCl_3$) δ 23.55 (δ-CH_2, Pro), 23.61 (CH_2-piperidine), 24.36 ($CH_2C{\equiv}C$), 25.42 (CH_2-piperidine), 29.39 (CH_2-piperidine), 29.72 (CH_2-piperidine), 30.87 (β-CH_2 Pro), 48.24 (CH_2-piperidine), 53.18 (α-CH_2, NCH_2 piperidine), 57.15 (δ-CH_2, Pro), 63.18 (CH_2, Ph), 68.00 (NCH,CH_2), 70.27 (α-CH_2, Pro), 76.83, 79.58 (≡C), 80.21 (C≡), 120.69, 123.78, 126.14, 126.82, 127.73, 128.90, 128.97, 129.09, 129.14, 129.95, 131.64, 132.49, 133.24, 133.47, 134.09, 142.85, 171.82 (C-N), 178.69, 180.46 (C=O):

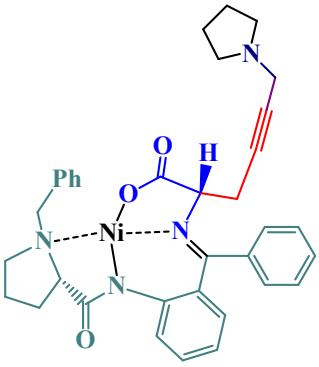
Complex 3c



Method A. Yield 67% (1.54 g), Mp +213-214^{0}C, the reaction duration is 0.5 hour. $[\alpha]_D^{20}$ +2218.89^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{35}H_{36}N_4NiO_3$ (619.38) C 67.87; H 5.86; N 9.05; Found: C 70.13; H 5.99; N 9.49. MS, *m/z*: 619.15:

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 (4H, s, CH_2-pyrrolidine); 1.97-2.24 (2H, m); 2.36-2.91 (9H, m); 3.37-3.76 (6H, m); 3.97 (1H, dd, J = 7.0, 2.8 Hz, CH); 4.43 (1H, d, J = 12.7 Hz, CH_2Ph); 6.56-6.75 (2H m, H-3,4 C_6H_4); 7.01 (1H, d, J = 7.1 Hz, C_6H_5); 7.19 (3H, dt, J = 14.1, 11.3 Hz, Ph); 7.35 (1H, t, J = 7.5 Hz, H-5 C_6H_4); 7.48 (3H,d, J = 11.0 Hz, Ar); 8.03 (2H, d, J = 7.5 Hz, Ph); 8.25 (1H, d, J = 8.7 Hz, H-6, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.54 (δ-CH_2, Pro); 23.65; 23.795 (CH_2-pyrolidine); 23.91; 24.22 (CH_2C=C); 30.83 (β-CH_2 Pro); 43.89; 52.69 (CH_2-pyrolidine); 57.12 (δ-CH_2 Pro); 63.01; 63.12 (CH_2 Ph); 68.049, 70.24 (α -CH2 Pro); 79.02 (≡C); 80.62 (C≡); 120.63; 123.73; 126.15; 126.18; 126.79; 127.74; 128.89; 129.03; 129.09; 129.24; 129.90; 131.65; 132.45; 133.22; 133.45; 134.15; 142.91; 171.76 (C-N) 178.69; 180.46 (C=O):

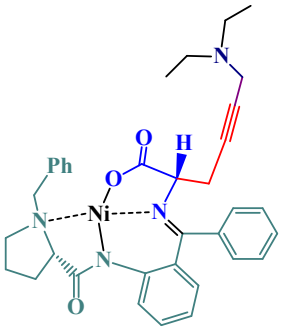
Complex 3d.



Method A. Yield 53% (1.21 g), Mp +179-180^{0}C, the reaction duration is 1.5 hour. $[\alpha]_D^{20}$ +2192.45^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{37}H_{40}N_4NiO_3$ (646,25) C 68.64; H 6.23; N 8.65; Found: C 68.90; H 6.51; N 9.99. MS, *m/z*: 647.15

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 1.41 (4H, m, azepam). 1.4-1.66 (9H, m, azepam), 1.96-2.28 (4H, m, azepam, CH_2-Pro), 2.32-2.78 (9H, m, azepam, CH_2-Pro), 3.35-3.70 (6H, m, azepam, CH-Pro), 3.95 (1H, dd, J = 7.3, 3.1 Hz, CH), 4.42 (1H, d, J = 12.7 Hz, CH_2Ph), 6.56-6.69 (2H, m, H-3,4 C_6H_4), 6.95-7.20 (3H, m, Ph and C_6H_5), 7.33 (2H, t, J = 7.6 Hz, H-5 C_6H_4), 7.41-7.59 (3H, m, Ar), 8.00 (2H, d, J = 7.0 Hz, Ph), 8.24 (1H, d, J = 8.6 Hz, H-6, C_6H_4). 13C NMR (75 MHz, $CDCl_3$) δ 23.44 (δ-CH_2 ,Pro), 24.19 (CH_2-azepam), 26.60 (CH_2-azepam), 27.85 (CH_2-azepam), 30.78 (β-CH_2 Pro), 48.83, 55.30, 57.01 (δ-CH_2 Pro), 63.06 (CH_2 Ph), 67.96, 70.16 (α -CH_2 Pro), 120.55, 123.69, 126.07, 126.73, 127.67, 128.80, 128.87, 129.02, 129.82, 131.58, 132.36, 133.37, 133.13, 134.12, 142.85, 171.66, 178.69 (C-N), 180.34 (C=O):

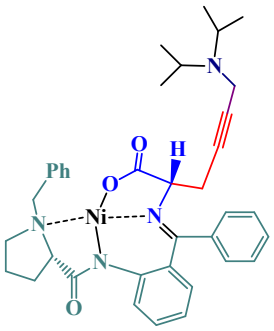
Complex 3e.



Method A. Yield 53% (1.21 g), Mp +215-216^{0}C, the reaction duration is 3 hour. $[\alpha]_D^{20}$ +2543^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{35}H_{38}N_4NiO_3$ (621.39); C 67.65; H 6.16; N 9.02; Found: C 67.95; H 6.64; N 9.49. MS, *m/z*: 621.20

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06-1.10 (6H, t, J = 7.0 Hz, CH_3); 2.03-2.20 (2H, m, CH_2-Pro); 2.44-2.81 (8H, m, CH_2-CH_3*2, CH_2-C≡, and CH_2-Pro); 3.42-3.72 (6H, m, CH_2-Bz, CH-Pro); 3.97 (1H, dd, J = 7.2, 3.7 Hz, CH); 4.44 (1H, d, J = 12.7 Hz, CH_2-Bz); 6.62-6.70 (2H, m, H-5,6 C_6H_4); 7.02 (1H, d, J = 7.1 Hz, H-4 C_6H_4); 7.13-7.26 (3H, m, Ar); 7.36 (2H, t, J = 7.6 Hz, Ph); 7.44 -7.59 (3H, m, Ar); 8.03 (2H, d, J = 7.2 Hz, Ph); 8.25 (1H, d, J = 8.6 Hz, H-3 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.35 (CH_3); 23.52 (δ-CH_2, Pro); 24.32 (CH_2C≡C); 30.85 (β-CH_2 Pro); 41.88 (CH_2-CH_3), 47.21 (CH_2-CH_3), 57.05 (δ-CH_2 Pro); 63.13 (CH_2 Bz); 68.09, 70.23 (α-CH_2 Pro); 79.47 (2C, C≡C); 120.65 (CH); 123.74 (CH); 126.15 (CH); 126.83 (CH); 127.71 (CH); 128.88 (2CH); 129.02 (CH); 129.11 (CH); 129.90 (CH); 131.63 (CH); 132.47 (2CH); 133.18, 133.45; 134.10 (CH); 142.85; 171.74 (C=N); 178.64; 180.40 (C=O).

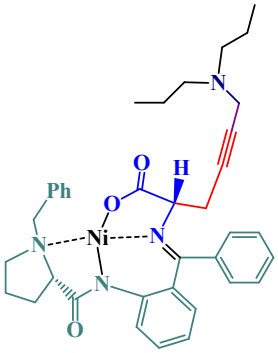
Complex 3f.



Method A. Yield 67% (1.65 g), Mp +125-126^{0}C, the reaction duration is 1.5 hour. $[\alpha]_D^{20}$ +1486.13^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{37}H_{42}N_4NiO_3$ (649,45) C 68.43; H 6.52; N 8.63; Found: C 68.79; H 6.95; N 8.89. MS, *m/z*: 649.15

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04-1.49 (22H, m, CH_3,), 2.01-2.23 (4H, m, CH_2-Pro), 2.63 (5H, ddd, *J* = 32.9, 21.6, 9.5 Hz, CH_2-CH_3*2, CH_2-Pro), 3.48 (2H, dd, *J* = 10.9, 5.9 Hz, CH-Pro), 3.62 (3H, d, *J* = 12.6 Hz, CH_2-C≡), 3.72 (2H, s,), 3.93 (1H, t, *J* = 5.8 Hz, CH), 4.43 (1H, d, *J* = 12.7 Hz, CH_2-Bz), 6.60-6.72 (2H, m, H-5,6 C_6H_4), 7.00 (1H, d, *J* = 8.1 Hz, H-4 C_6H_4), 7.19 (3H, dd, *J* = 16.8, 5.2 Hz, Ar), 7.36 (2H, t, *J* = 7.6 Hz, Ph), 7.43-7.59 (3H, m, Ar), 8.05 (2H, d, *J* = 7.1 Hz, Ph), 8.23 (1H, d, *J* = 8.7 Hz, H-3 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.64 and 19.73 (CH_3), 23.78 (δ-CH_2, Pro), 25.06(CH_2C≡C), 29.74, 30.92 (β-CH_2 Pro), 35.21, 50.37, 57.25 (CH_2-Pro), 63.26 (CH_2-Bz), 68.06 (CH), 70.31 (CH), 120.76 (CH); 123.83 (CH); 126.07, 126.93, 127.67 (CH); 128.95 (CH); 129.01 (CH); 129.10 (CH), 129.18, 129.98 (CH), 131.61 (CH), 132.61 (CH);, 133.29, 133.48, 133.94 (CH); 142.85, 171.79 (C=O), 178.30 (C=N), 180.49(C=O).

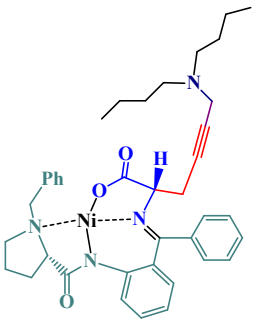
Complex 3g.



Method A. Yield 41% (0.99 g), Mp +88^{0}C, the reaction duration is 2.5 hour. $[\alpha]_D^{20}$ +2000^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{37}H_{42}N_4NiO_3$ (649,45) C 68.43; H 6.52; N 8.63; Found: C 69.25; H 6.52; N 8.99. MS, *m/z*: 649.25

^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (6H, t, *J* = 7.3 Hz, CH_3*2), 1.48 (5H, dd, *J* = 15.0, 7.5 Hz,), 1.95-2.22 (4H, m, CH_2-CH_3*2,), 2.39-2.62 (7H, m, CH_2-CH_3*2, CH_2-C≡, CH_2-Pro), 2.61-2.85 (2H, m,), 3.46 (1H, dd, *J* = 10.8, 6.0 Hz, CH_2-Pro), 3.54-3.74 (5H, m, CH_2-Bz, CH-Pro), 3.96 (1H, dd, *J* = 7.3, 3.4 Hz, CH), 4.43 (1H, d, *J* = 12.7 Hz, CH_2-Bz), 6.66 (2H, dd, *J* = 4.5, 1.9 Hz, H-3,4 C_6H_4), 7.01 (1H, d, *J* = 6.6 Hz, C_6H_5), 7.10-7.27 (4H, m, H-5 C_6H_4, Ar), 7.36 (2H, t, *J* = 7.6 Hz, Ph), 7.42-7.57 (3H, m, Ar), 8.03 (2H, d, *J* = 7.1 Hz, Ph), 8.24 (1H, d, *J* = 8.6 Hz, H-3 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.81 (CH_3), 20.26 (CH_3), 23.58 (δ-CH_2, Pro), 24.44 (CH_2C≡C), 30.90 (β-CH_2 Pro), 42.95, 55.68, 57.11 (CH), 63.19 (CH_2 Ph), 68.13, 70.29 (α-CH_2 Pro), 76.66, 77.08 (C, C≡C), 77.51 (C, C≡C), 120.72, 123.84, 126.17, 126.89, 127.69, 128.93, 129.06, 129.16, 129.95, 131.65, 132.52, 133.23, 133.46, 134.11, 142.89, 171.77 (C=N), 178.60, 180.43 (C=O).

Complex 3h.

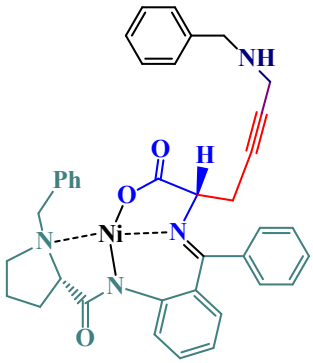


Method A. Yield 26% (0.65 g), Mp +91^{0}C, the reaction duration is 2 hour. $[\alpha]_D^{20}$ +501^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{39}H_{46}N_4NiO_3$ (677,50) C 69.14; H 6.84; N 8.27; Found: C 69.51; H 7.30; N 8.65. MS, *m/z*: 677.25

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (6H, t, *J* = 7.2 Hz, CH_3*2), 1.26 (7H, t, *J* = 7.2 Hz,), 2.43 (6H, dd, *J* = 15.4, 8.1 Hz, CH_2-CH_3*2, CH_2-C≡, CH_2-Pro), 2.72 (3H, d, *J* = 17.2 Hz,), 3.40-3.71 (7H, m, CH_2-Bz, CH-Pro, CH_2-Pro), 3.94-4.01 (1H, m, CH), 4.44 (1H, d, *J* = 12.6 Hz, CH_2-Bz), 6.60-6.69 (2H, m, H-3,4 C_6H_4), 7.02 (1H, d, *J* = 7.3 Hz, C_6H_5), 7.09-7.26

(4H, m, Ar), 7.35 (3H, t, J = 7.5 Hz, Ph), 7.40-7.59 (4H , m, Ar), 8.03 (2H, d, J = 7.5 Hz, Ph), 8.26 (1H, d, J = 8.7 Hz, H-3 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.05 (CH_3), 20.65 (CH_3), 23.47 (δ-CH_2, Pro), 24.24 (CH_2C≡C), 29.69, 30.90 (β-CH_2 Pro), 43.22, 53.55, 57.03 (CH), 63.13 (CH_2 Ph), 68.17, 70.28 (α-CH_2 Pro), 79.06 (C, C≡C), 80.48 (C, C≡C), 120.65, 123.80, 126.24, 126.85, 127.76, 128.89, 128.96, 129.11, 129.89, 131.67, 132.43, 133.20, 133.44, 134.20, 142.91, 171.71 (C=N), 178.79, 180.41 (C=O).

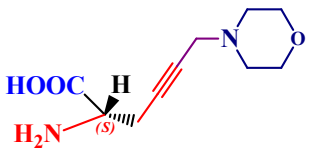
Complex 3i.



Method A. Yield 31% (0.75 g), Mp +150-152^{0}C, the reaction duration is 3 hour. $[\alpha]_D^{20}$ +1745.88^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{38}H_{36}N_4NiO_3$ (655,41), C 69.64; H 5.54; N 8.55; Found: C 69.99; H 5.94; N 8.93.

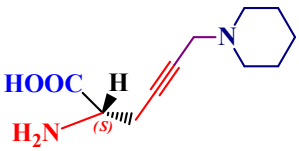
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (3, dt, J = 21.2, 9.8 Hz, γ, δ-Ha), 2.19-2.54 (3H, m,), 2.69 (3H, dd, J = 34.3, 9.5 Hz, CH_2-C≡, and CH_2-Pro), 3.40 (1H, dd, J = 10.7, 6.1 Hz, α-H-Pro), 3.59 (6H, dd, J = 25.0, 12.5 Hz,), 3.93-4.05 (1H, m, CH), 4.41 (1H, d, J = 12.6 Hz, CH_2Ph), 6.63 (2H, d, J = 2.8 Hz, H-3,4 C_6H_4), 6.99 (1H, d, J = 6.9 Hz, C_6H_5), 7.17 (8H, dd, J = 25.9, 8.7 Hz, H-5 C_6H_4, Ar), 7.36 (3H, d, J = 7.3 Hz, Ph), 7.49 (4H, dt, J = 13.5, 6.8 Hz, Ar), 8.03 (2H, d, J = 7.5 Hz, Ph), 8.25 (1H, d, J = 8.6 Hz, H-6, C_6H_4).^{13}C NMR (75 MHz, $CDCl_3$) δ 23.45 (δ-CH_2, Pro), 24.35 (CH_2C≡C), 30.24, 30.89 (β-CH2 Pro), 31.58(C≡C-CH_2), 43.11 (NH-CH_2), 57.19 (δ-CH_2, Pro), 63.13 (CH_2 ,Ph), 68.14 (NCH,CH_2), 70.32 (α-CH_2, Pro), 79.87 (≡C), 80.38 (C≡), 120.65, 123.80, 126.23, 126.81, 127.82, 128.40, 128.89, 129.12, 129.26, 129.95, 131.66, 132.45, 133.29, 133.48, 134.11, 137.86, 142.94, 171.83 (C=N), 178.59, 180.53 (C=O).

(*S*)-2-amino-6-morpholinohex-4-ynoic acid-4a.



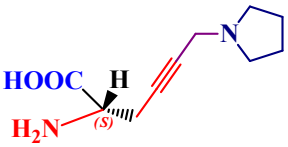
Method B. Yield 33%, Mp +190^{0}C, $[\alpha]_D^{20}$=+12^0 (C=0.1,6N HCl), Anal Calcu. for: $C_{10}H_{16}N_2O_3$ (212,25) C 56.59; H 7.60; N 13.20; Found: C 56.99; H 7.98; N 13.56. ^{1}H NMR (300 MHz, DMSO+CF_3COOD) δ 2.89 – 2.99 (2H, m, CH_2≡C); 3.15 – 3.52 (4H, m, CH_2*2-morph); 3.74 – 3.94 (4H, m, CH_2*2-morph); 4.06-4.13 (3H, dd, J = 11.9, 6.7 Hz, CH_2-N and CH).^{13}C NMR (75 MHz, DMSO+ CF_3COOD) δ 20.33 (CH_2-C≡); 50.19 CH_2-morph); 50.45 CH_2-morph); 63.30; 73.21 (≡C), 83.80 (C≡); 113.11 CH_2-morph); 120.76 CH_2-morph); 169.00 (C=O).

(*S*)-2-amino-6-(piperidin-1-yl)hex-4-ynoic acid-4b



Method B. Yield 45%, Mp +192^{0}C, $[\alpha]_D^{20}$=+7.4^0 (C=0.1,6N HCl), Anal Calcu. for: $C_{11}H_{18}N_2O_2$ (210,27): C 62.83; H 8.63; N 13.32; Found: C 63.13; H 8.88; N 13.82. ^{1}H NMR (300 MHz, CD_3OD) δ 1.45-1.57 (2H m, CH_2-piperidine), 1.66 (4H, dt, J = 11.0, 5.6 Hz, CH_2-piperidine), 2.60 (4H, s, CH_2-C≡, CH_2-piperidine), 2.78-2.88 (2H, m, CH_2-C≡, CH_2-piperidine), 3.26 (2H, t, J = 2.2 Hz, CH_2-N), 3.66 (1H, dd, J = 6.8, 4.9 Hz, CH). ^{13}C NMR (75 MHz, CD_3OD) δ 22.39 (CH_2-C≡), 24.55, 26.36, 48.40 (CH_2), 54.53, 54.80 (CH), 79.70 (C≡), 80.78 (≡C), 172.74 (C=O).

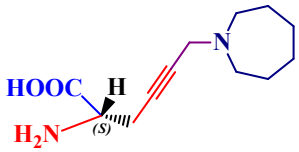
(*S*)-2-amino-6-(pyrrolidin-1-yl)hex-4-ynoic acid-4c



Method B. Yield 31%, Mp +230-235^{0}C, $[\alpha]_D^{20}$=+19.5^0 (C=0.1,6N HCl), Anal Calcu. for: $C_{10}H_{16}N_2O_2$ (196,25): C 61.20; H 8.22; N 14.27; Found: C 61.70; H 8.75; N 14.76.

^{1}H NMR (300 MHz, DMSO) δ 2.01 (4H, s, CH_2); 2.86-2.99 (2H, m, CH_2-C); 3.02-3.79 (4H, m, CH_2); 3.99-4.19 (3H, m, CH_2-N and CH). ^{13}C NMR (75 MHz, DMSO) δ 20.25 (CH_2-C≡); 23.01 (CH_2); 42.58 (CH_2-N); 50.54 (CH_2); 52.22 (CH); 74.43 (≡C); 82.34 (C≡);168.95 (C=O); 52.22 (CH); 74.43 (≡C); 82.34 (C≡); 168.95 (C=O).

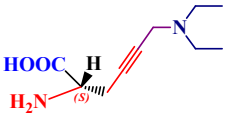
(*S*)-2-amino-6-(azepan-1-yl)hex-4-ynoic acid-4d



Method B. Yield 53%, Mp +198-200^{0}C, $[\alpha]_D^{20}$=+19^0 (C=0.1, 6N HCl), Anal Calcu. for: $C_{12}H_{20}N_2O_2$ (224,30): C 64.26; H 8.99; N 12.49; Found: C 64.72; H 9.45; N 12.92.

^{1}H NMR (300 MHz, DMSO) δ 1.68-1.87 (4H ,d, J = 58.6 Hz, N-C_6H_8); 2.82-3.58 (6H, m,CH_2*2, N-C_6H_8); 3.92-4.18 (3H, m, CH_2-N and CH). ^{13}C NMR (75 MHz, DMSO) δ 20.26 (CH_2-C≡); 23.28 (CH_2*2); 25.51(CH_2*2); 46.40 (CH_2-N); 50.55 (CH_2*2); 53.61 (CH); 74.02 (≡C); 82.97 (C≡); 168.93 (C=O).

(*S*)-2-amino-6-(diethylamino)hex-4-ynoic acid-4e

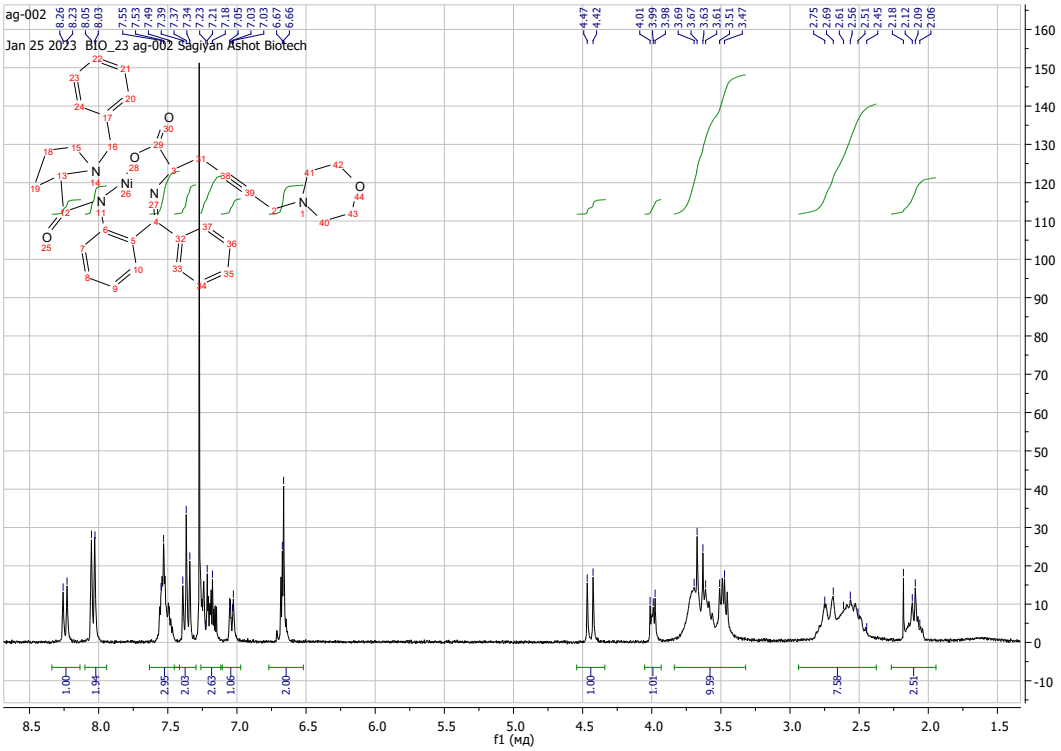


Method B. Yield 20%, Mp +219-220^0 C, $[\alpha]_D^{20}$=+18^0 (C=0.1,6N HCl), Anal Calcu. for: $C_{10}H_{18}N_2O_2$ (198,26): C 60.58; H 9.15; N 14.13; Found: C 60.89; H 9.65; N 14.59.

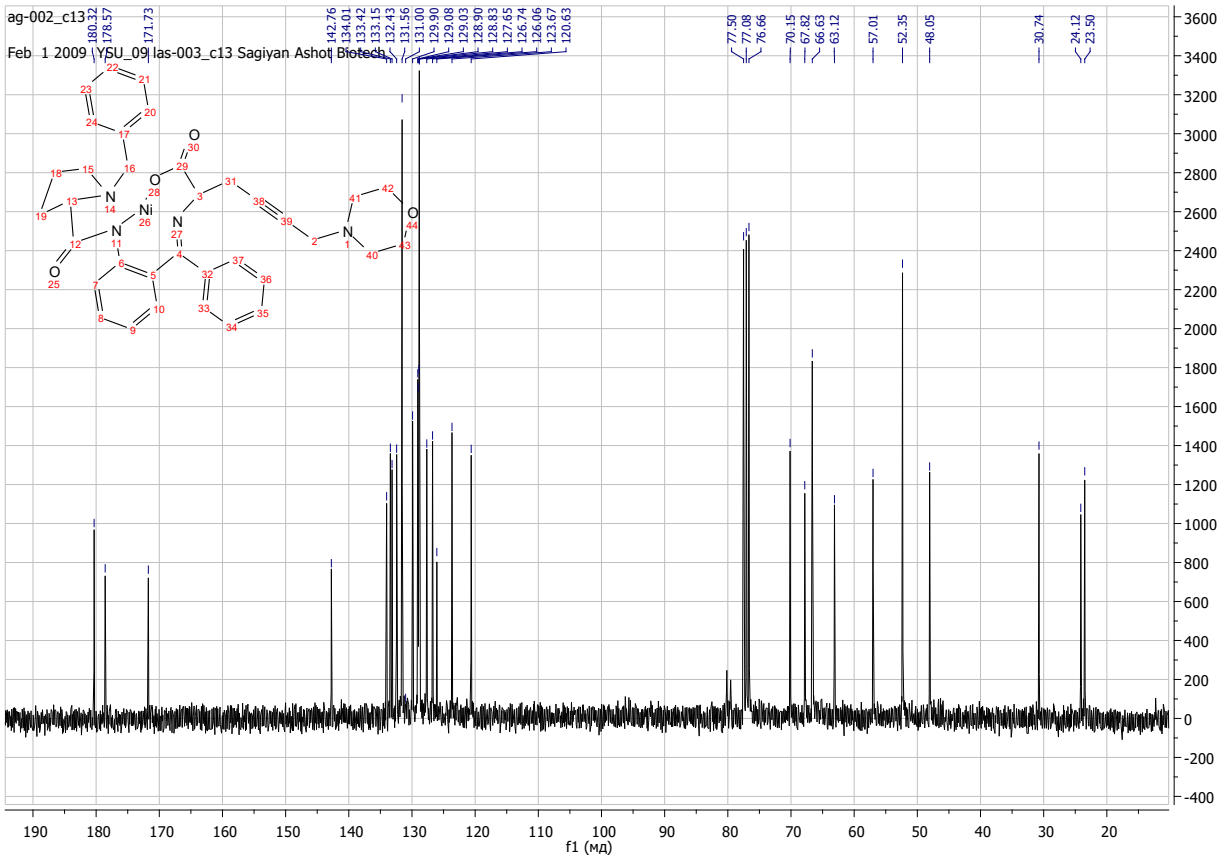
^{1}H NMR (300 MHz, DMSO+CF_3COOD) δ 1.24-1.29 (6H, t, J=6.8 Hz, CH_3*2); 2.82-3.37 (6H, m, CH_2*3); 4.06 (3H, d, J=22.0 Hz, CH_2-N and CH). ^{13}C NMR (75 MHz, DMSO+CF_3COOD) δ 8.81 (CH_3); 20.31 (CH_2-C≡); 40.47 (CH_2-N); 46.88 (N-CH_2); 50.54 (CH); 72.89 (≡C); 83.40 (C≡); 169.01 (C=O).

NMR spectra

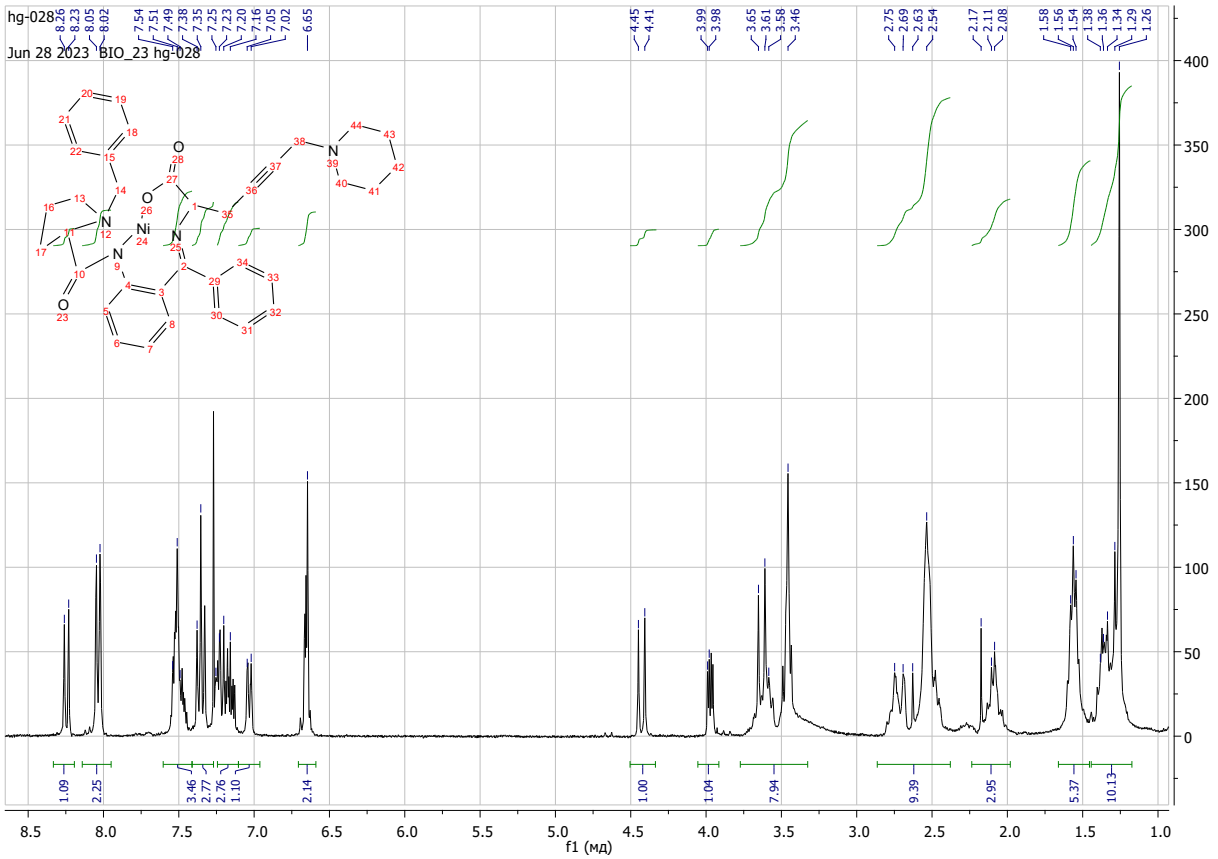
^{1}H NMR Complex 3a



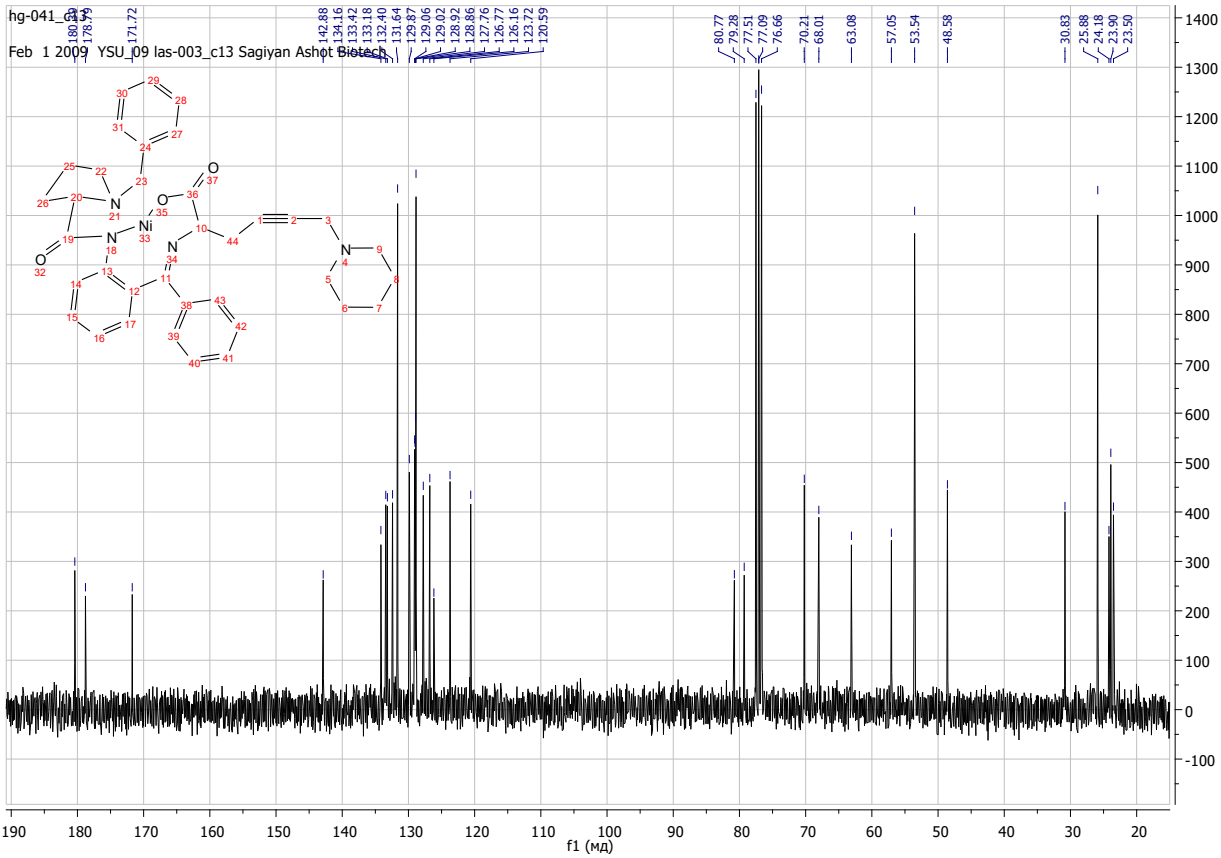
^{13}C NMR Complex 3a



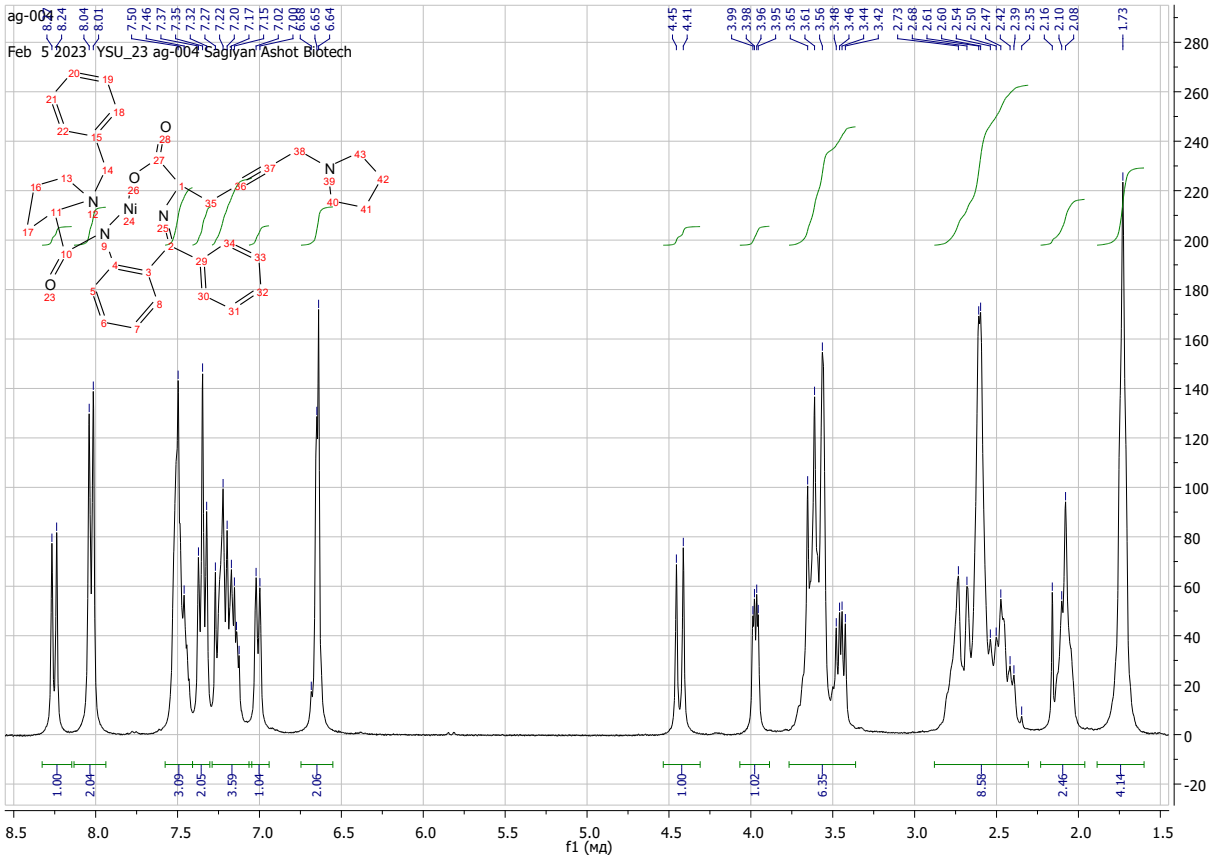
^{1}H NMR Complex 3b



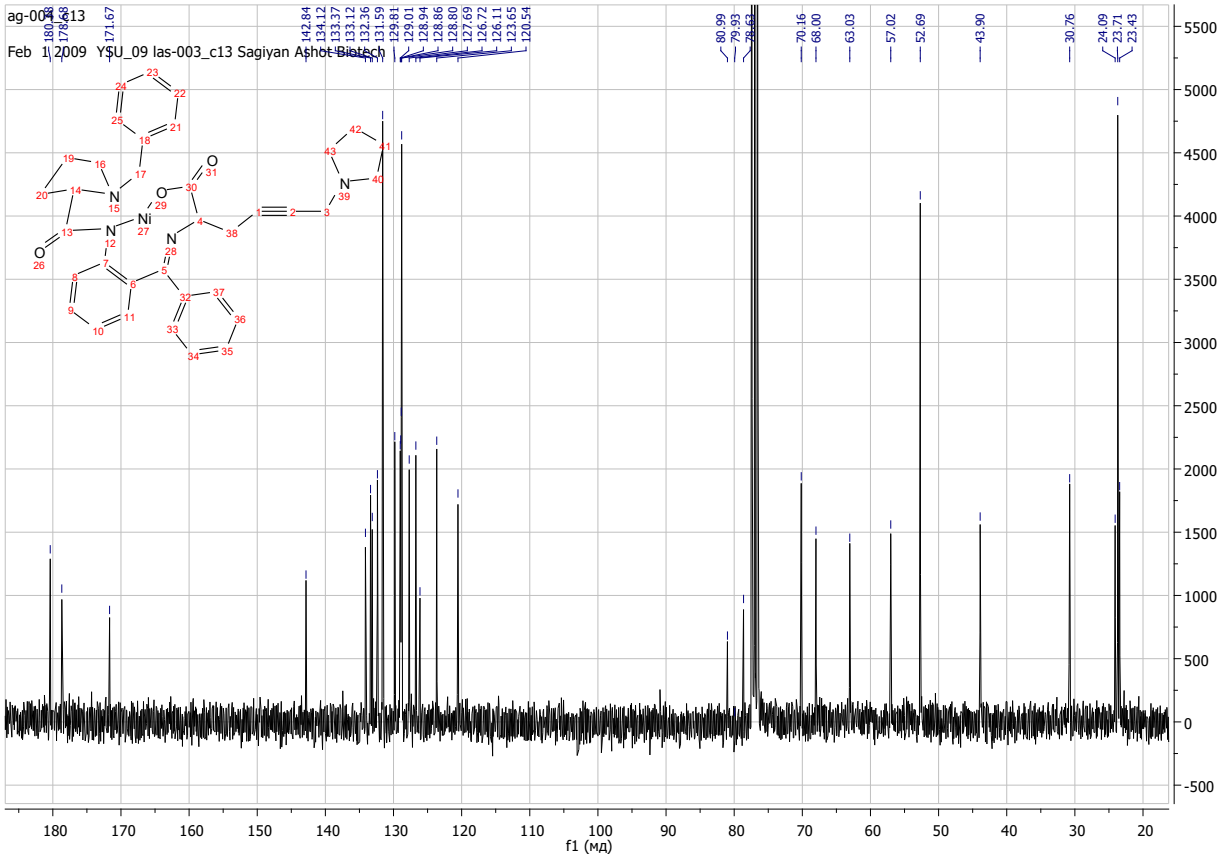
^{13}C NMR Complex 3b



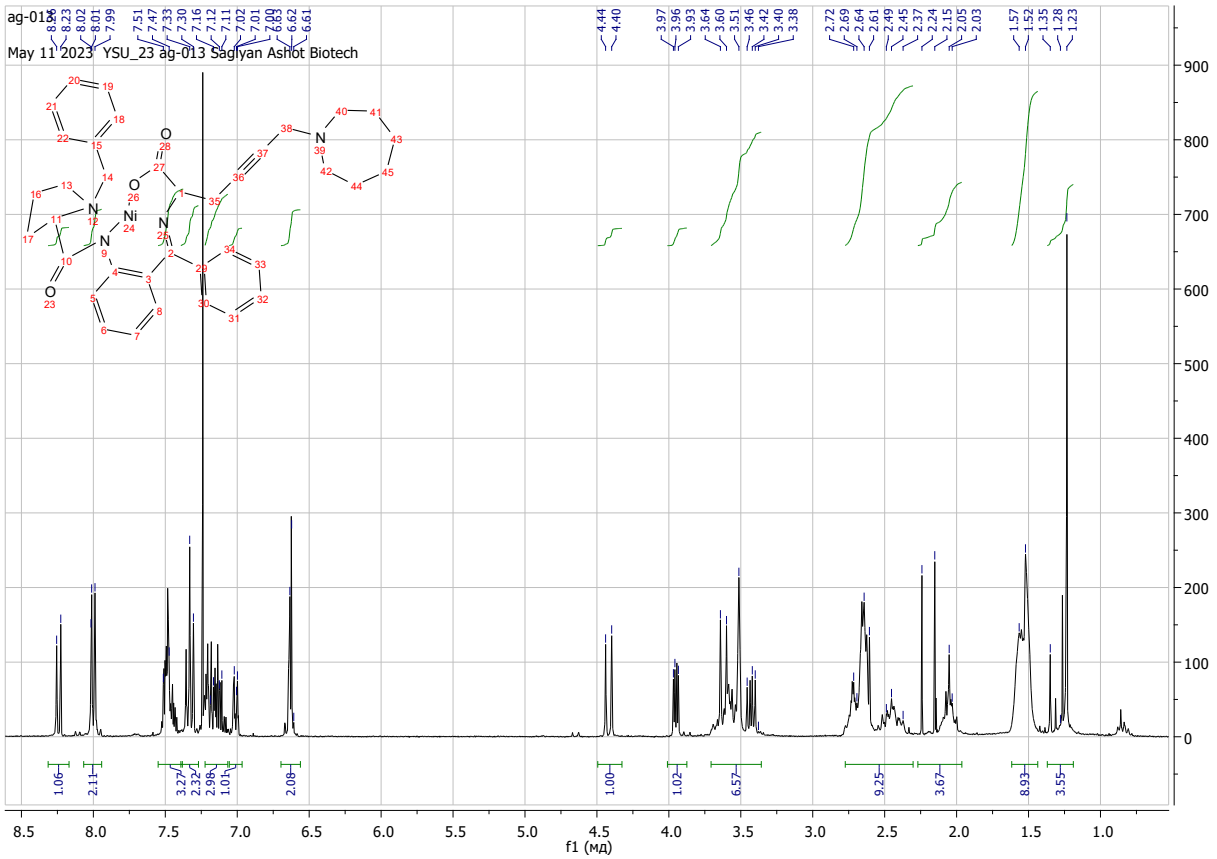
^{1}H NMR Complex 3c



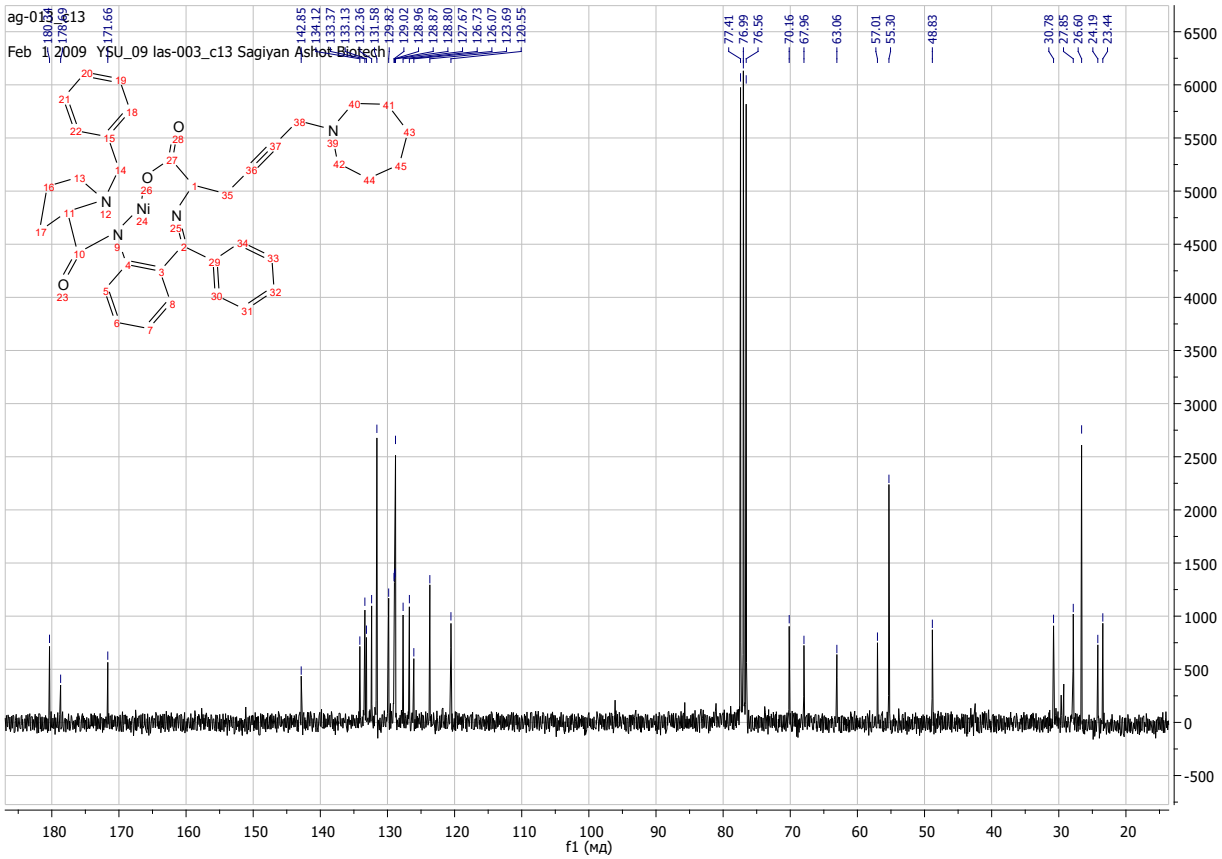
^{13}C NMR Complex 3c



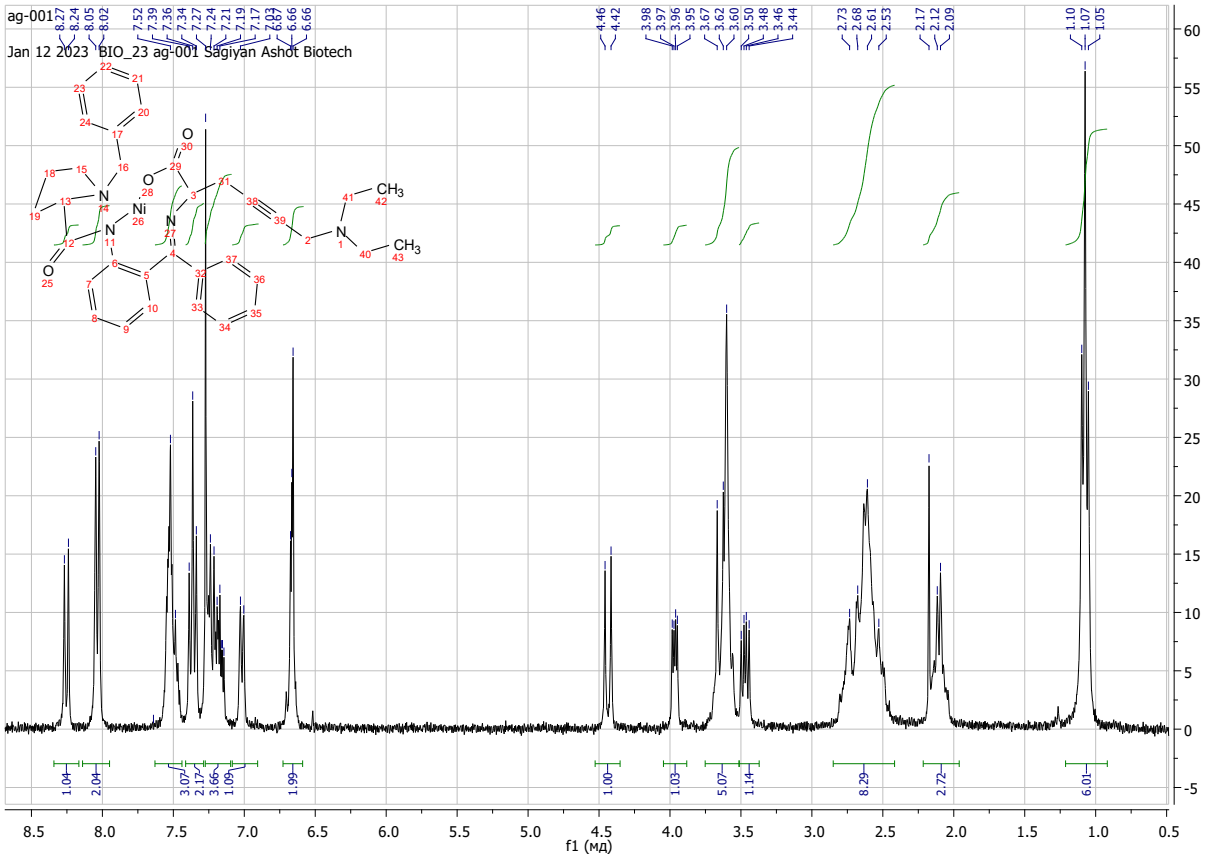
^{1}H NMR Complex 3d



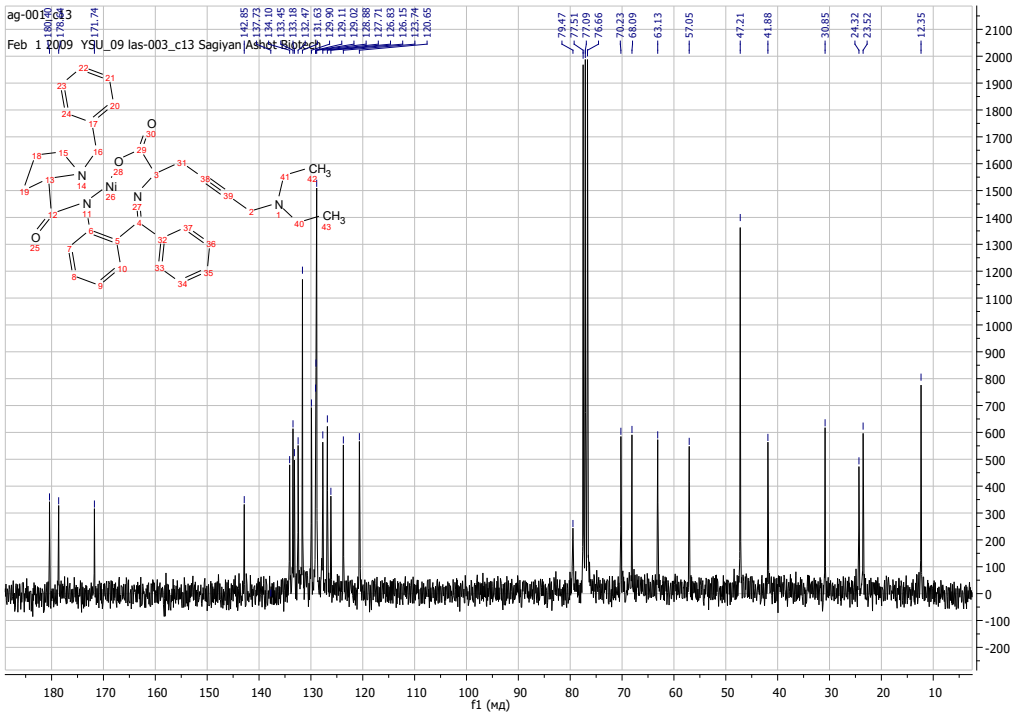
^{13}C NMR Complex 3d



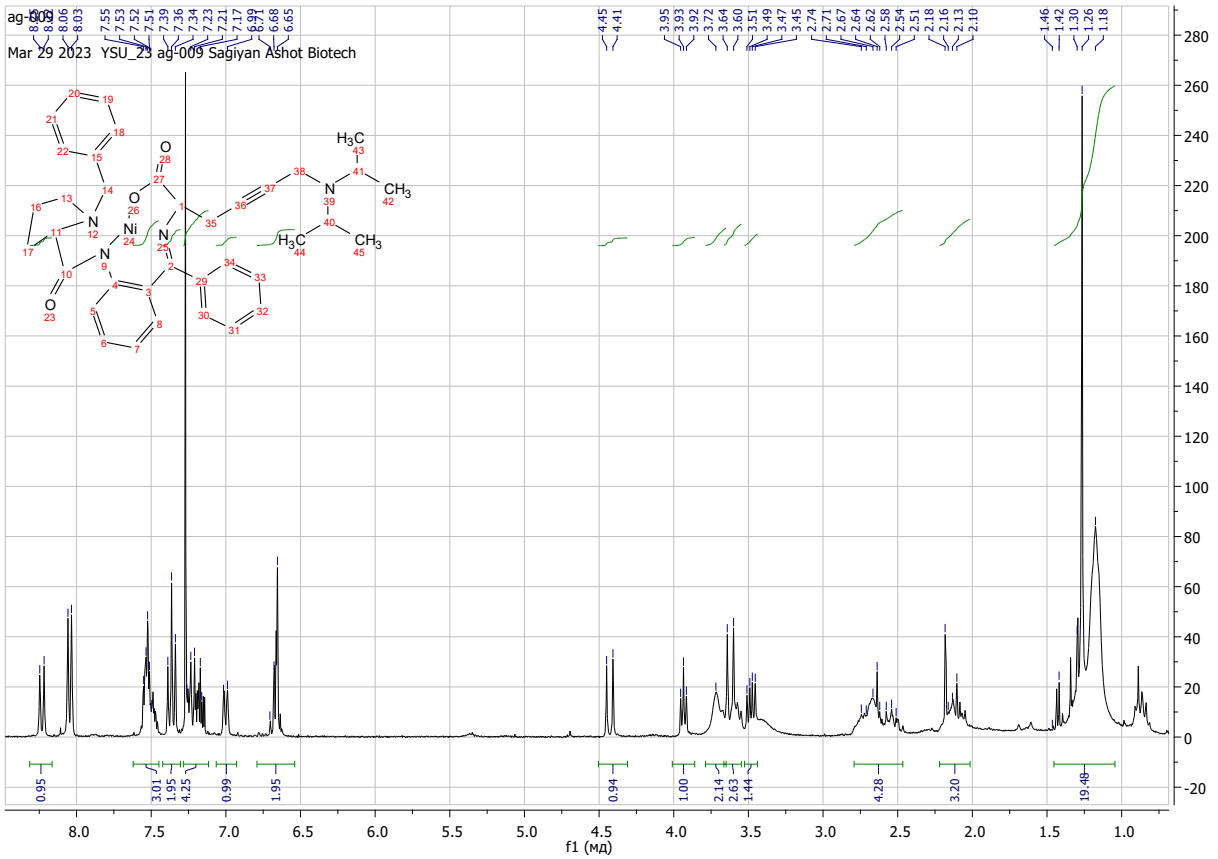
^{1}H NMR Complex 3e



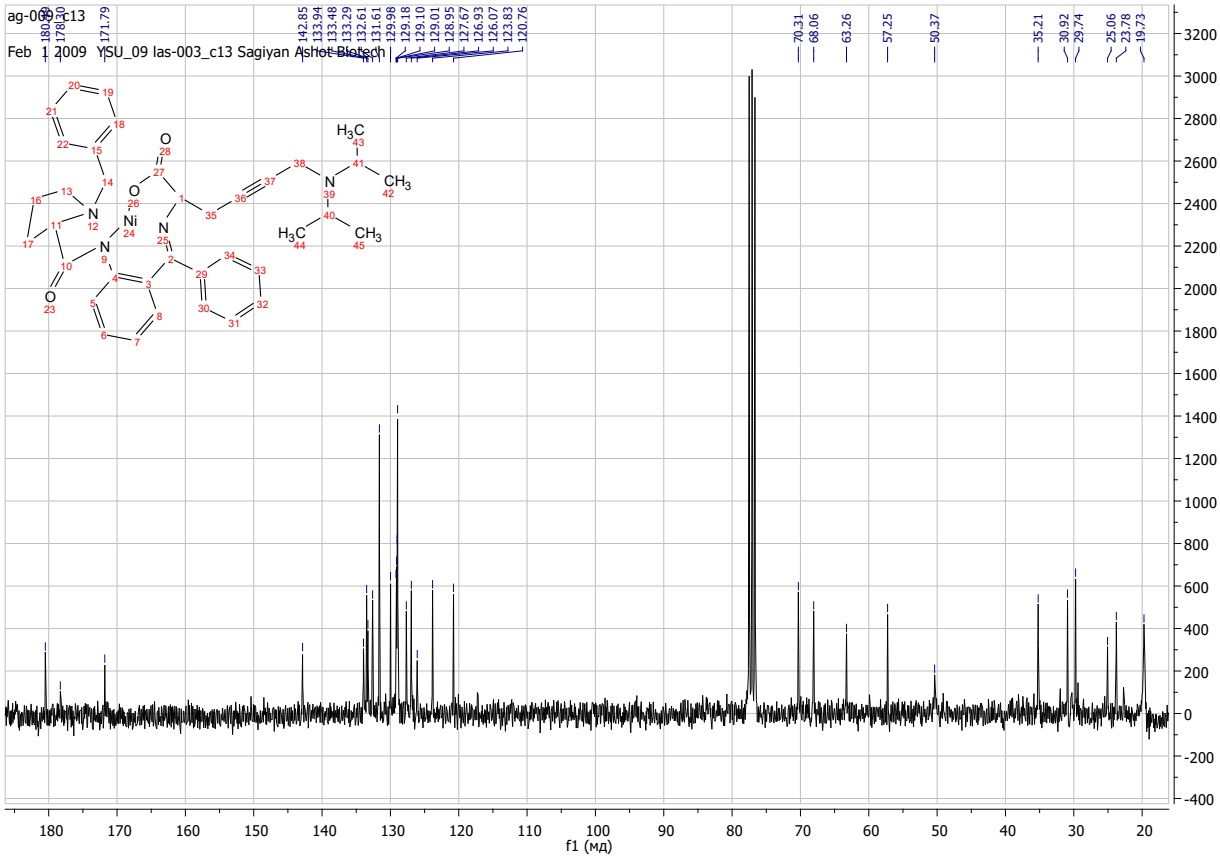
^{13}C NMR Complex 3e



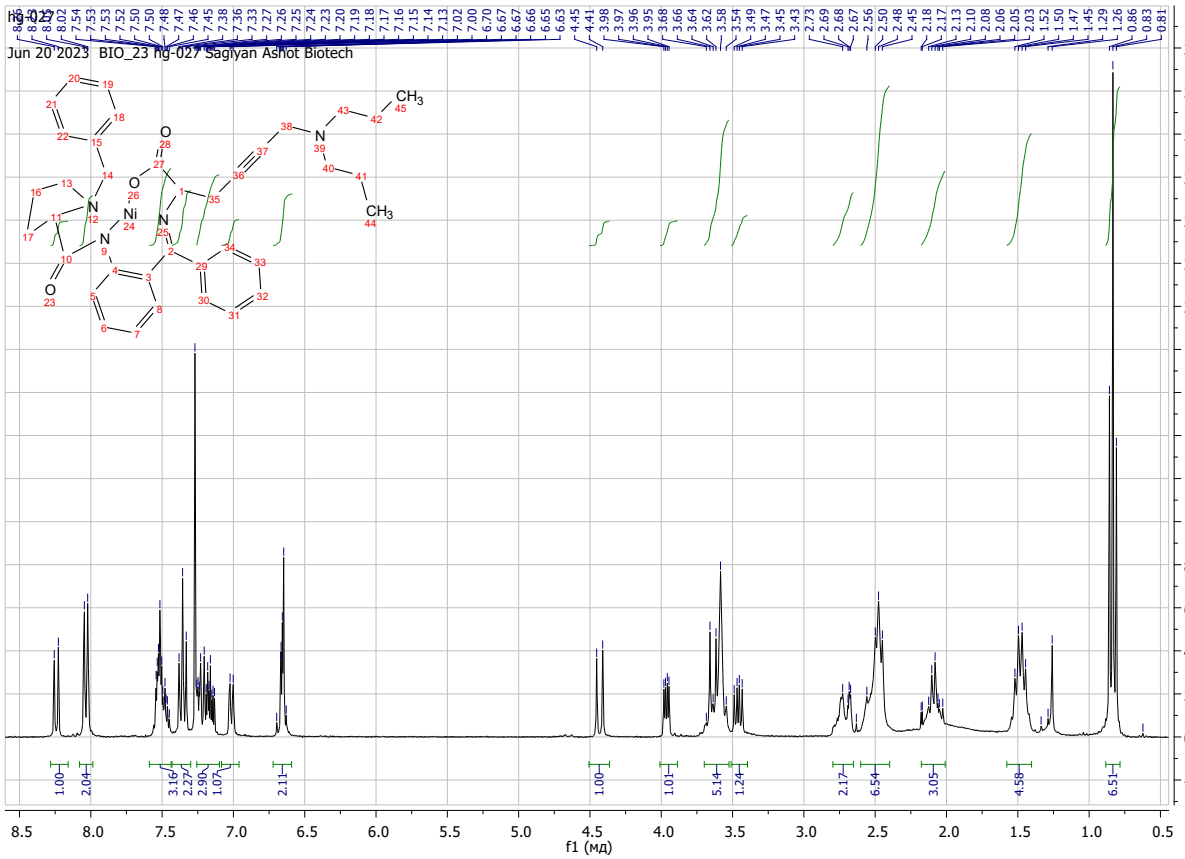
^{1}H NMR Complex 3f



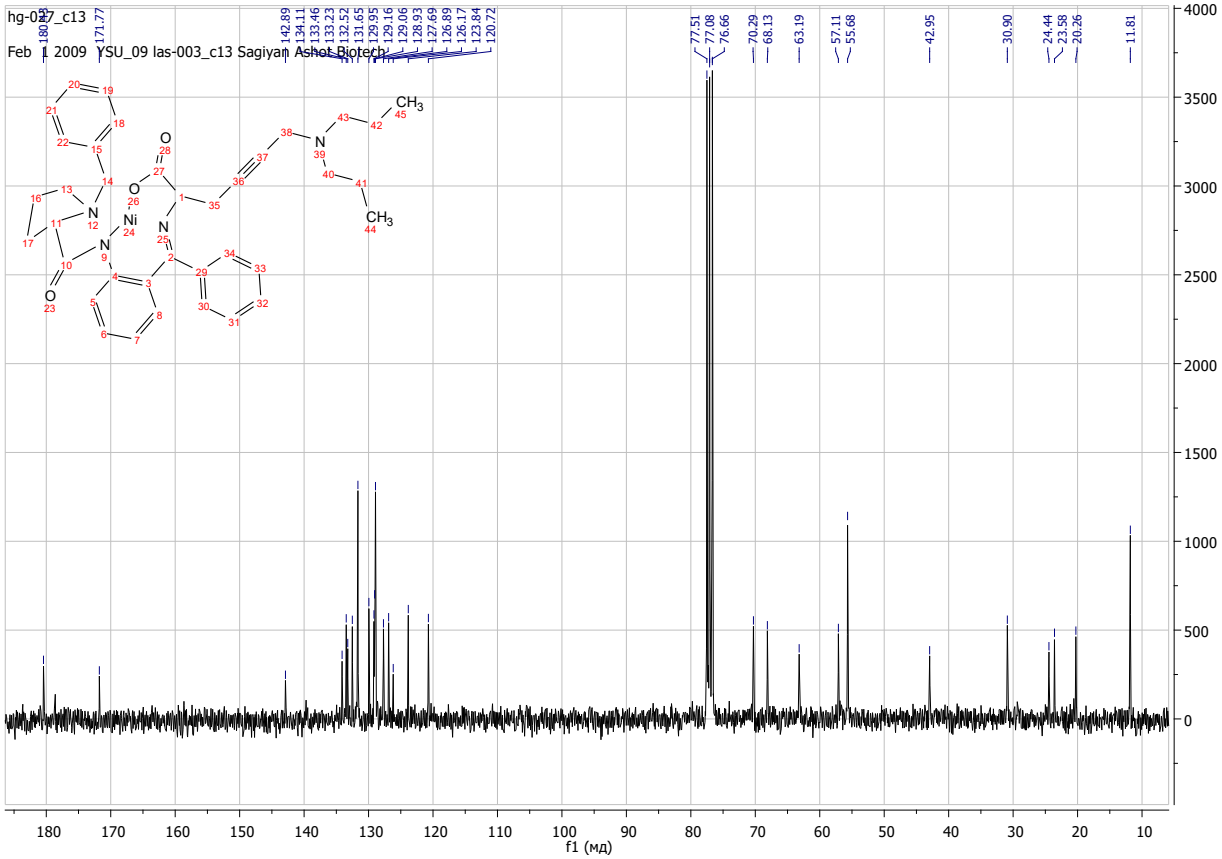
^{13}C NMR Complex 3f



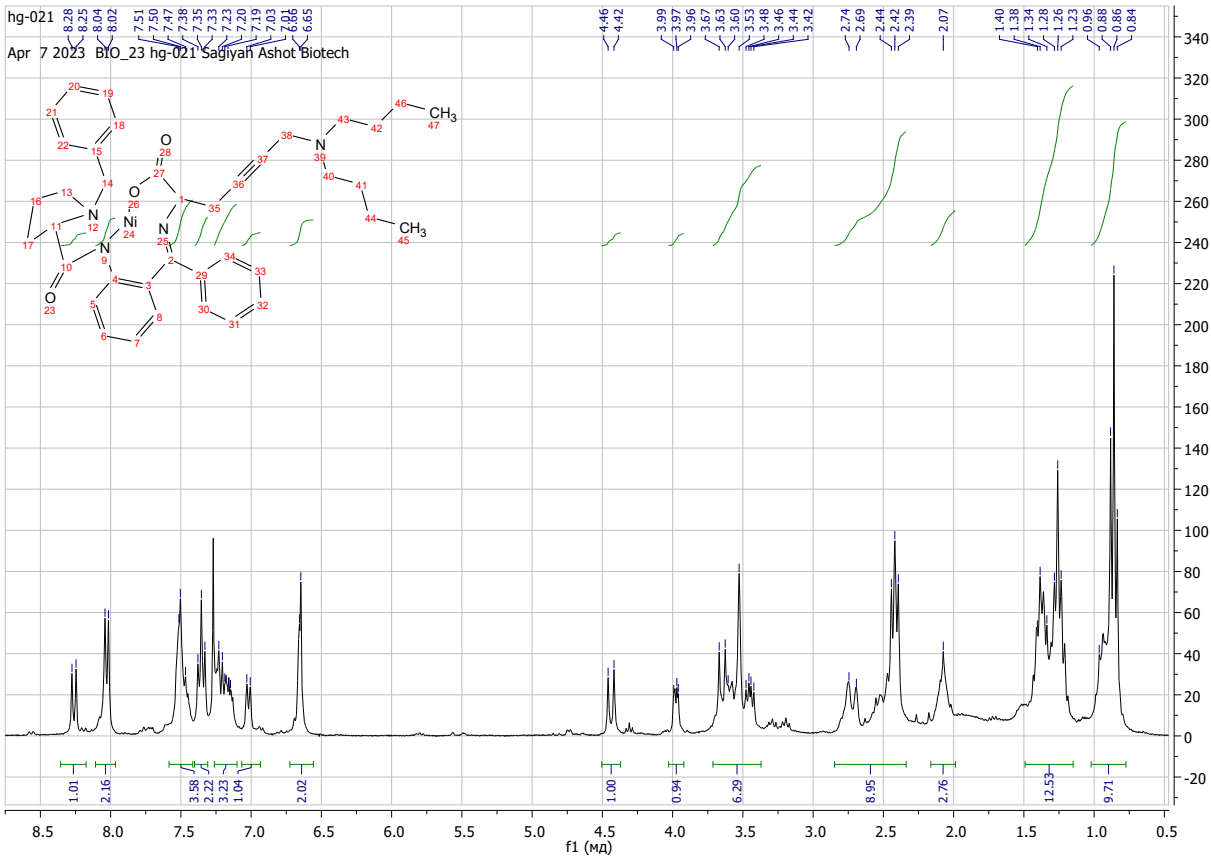
^{1}H NMR Complex 3g



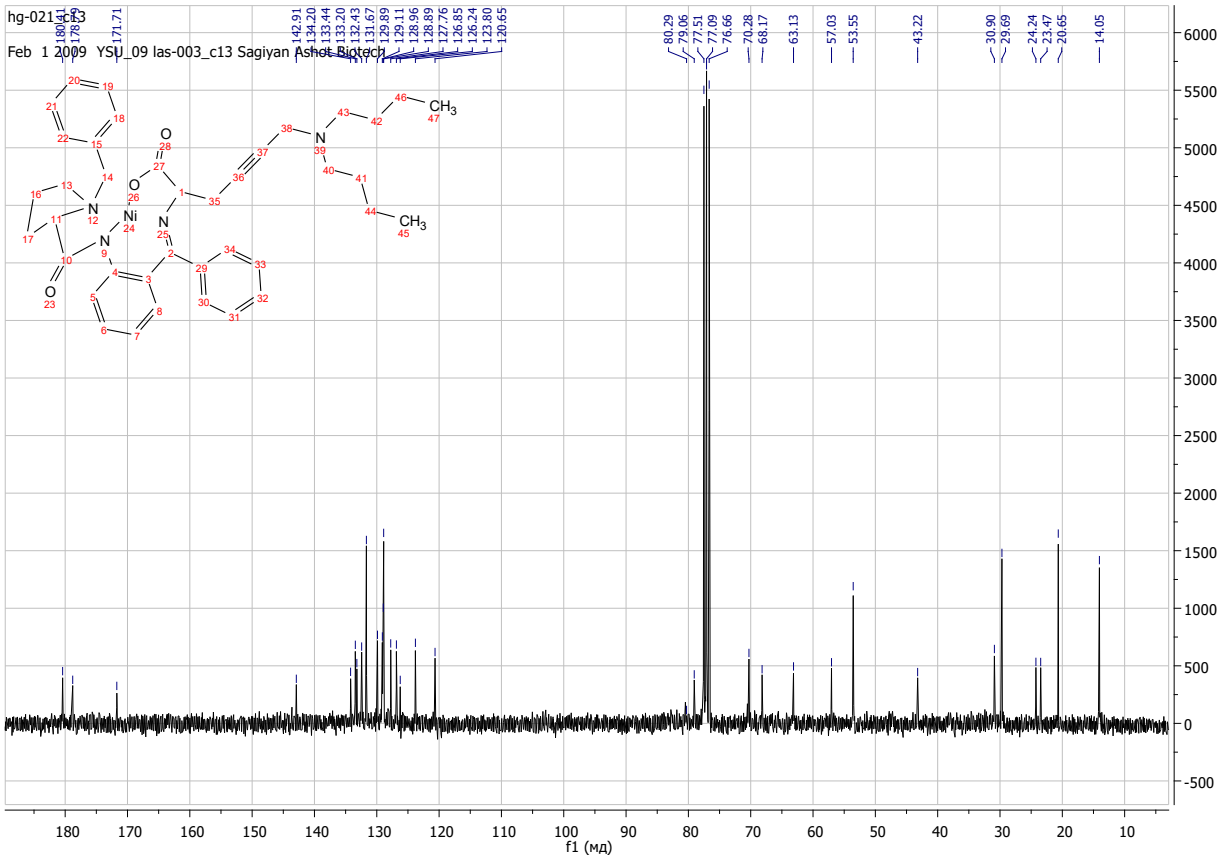
^{13}C NMR Complex 3g



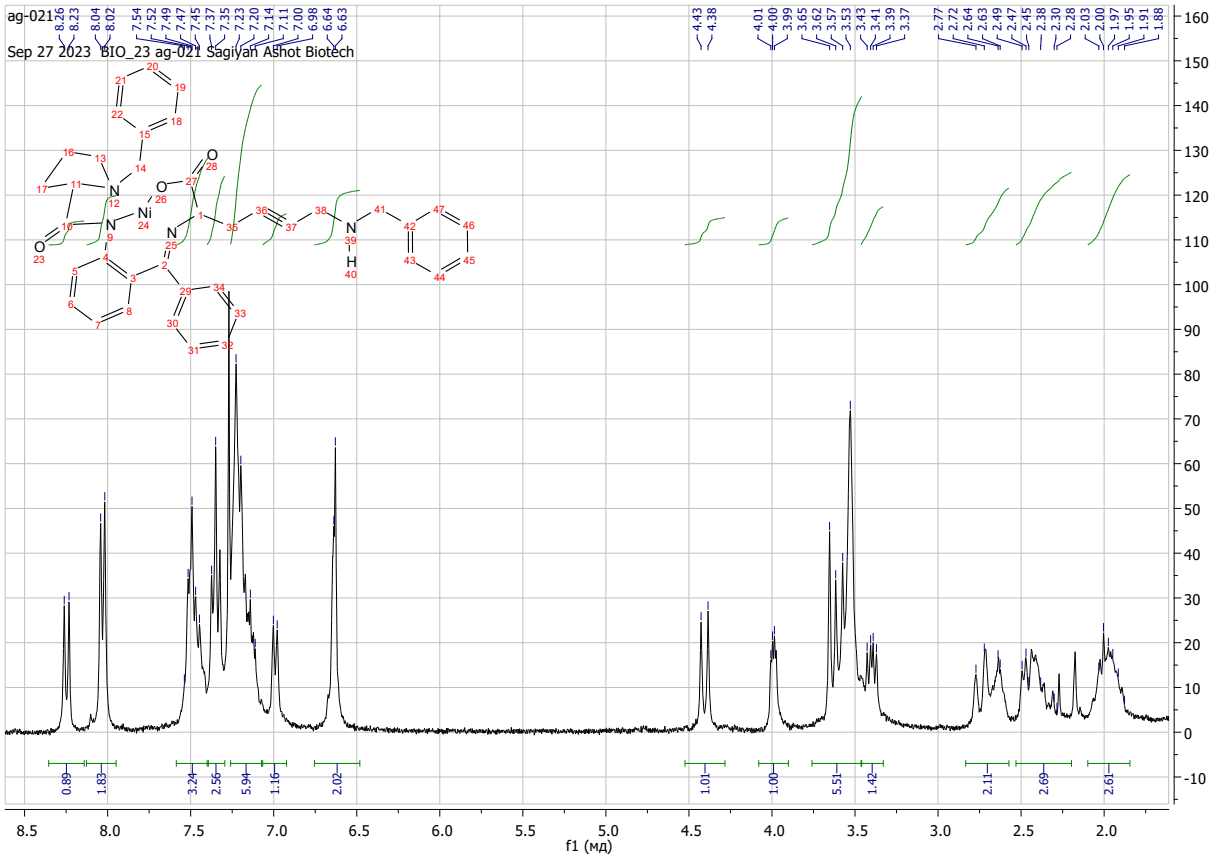
^{1}H NMR Complex 3h



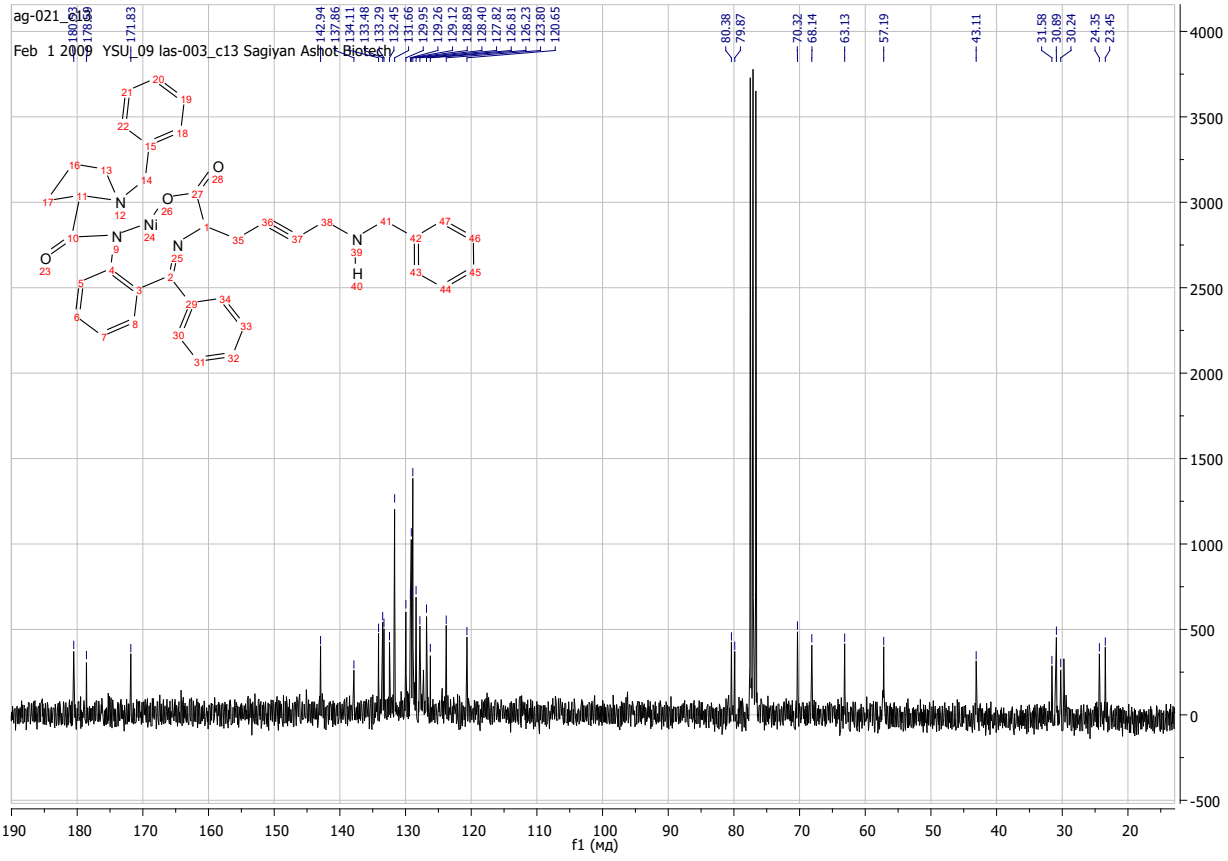
^{13}C NMR Complex 3h



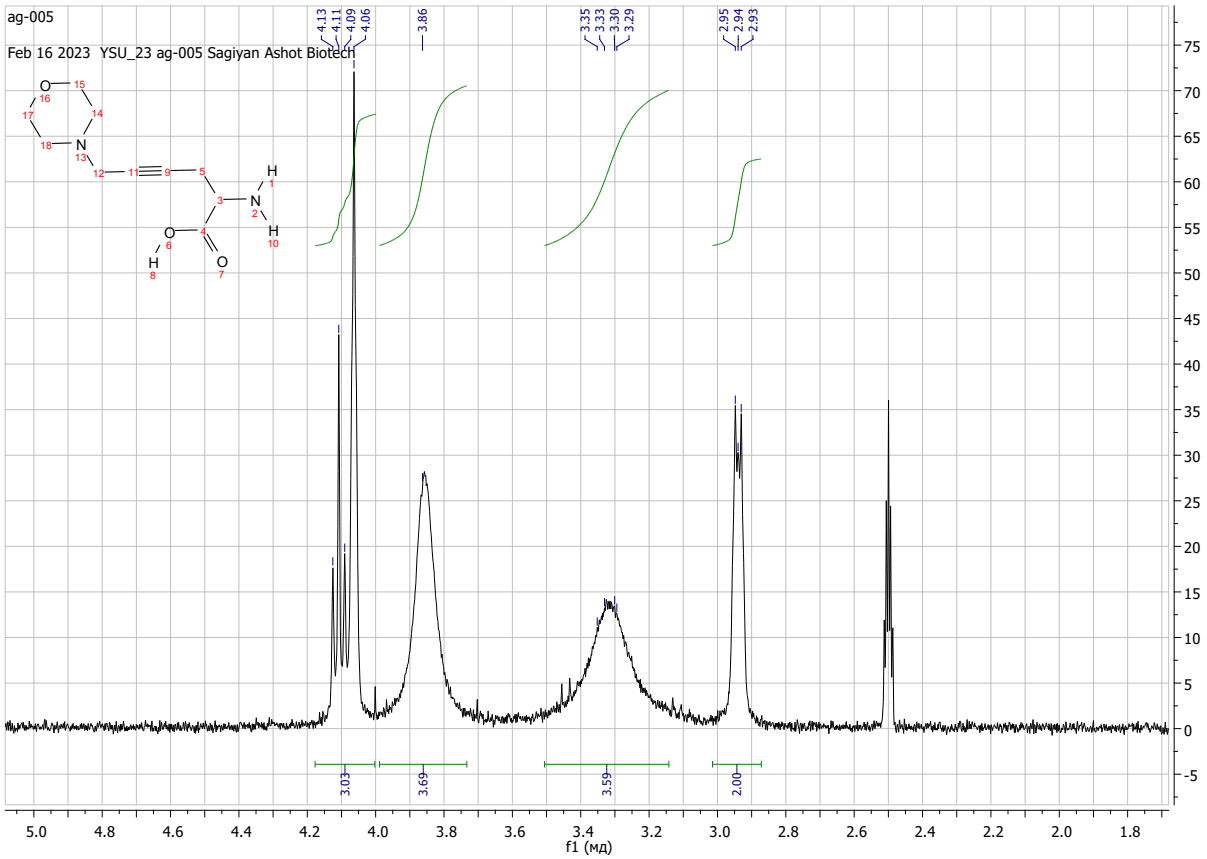
^{1}H NMR Complex 3i



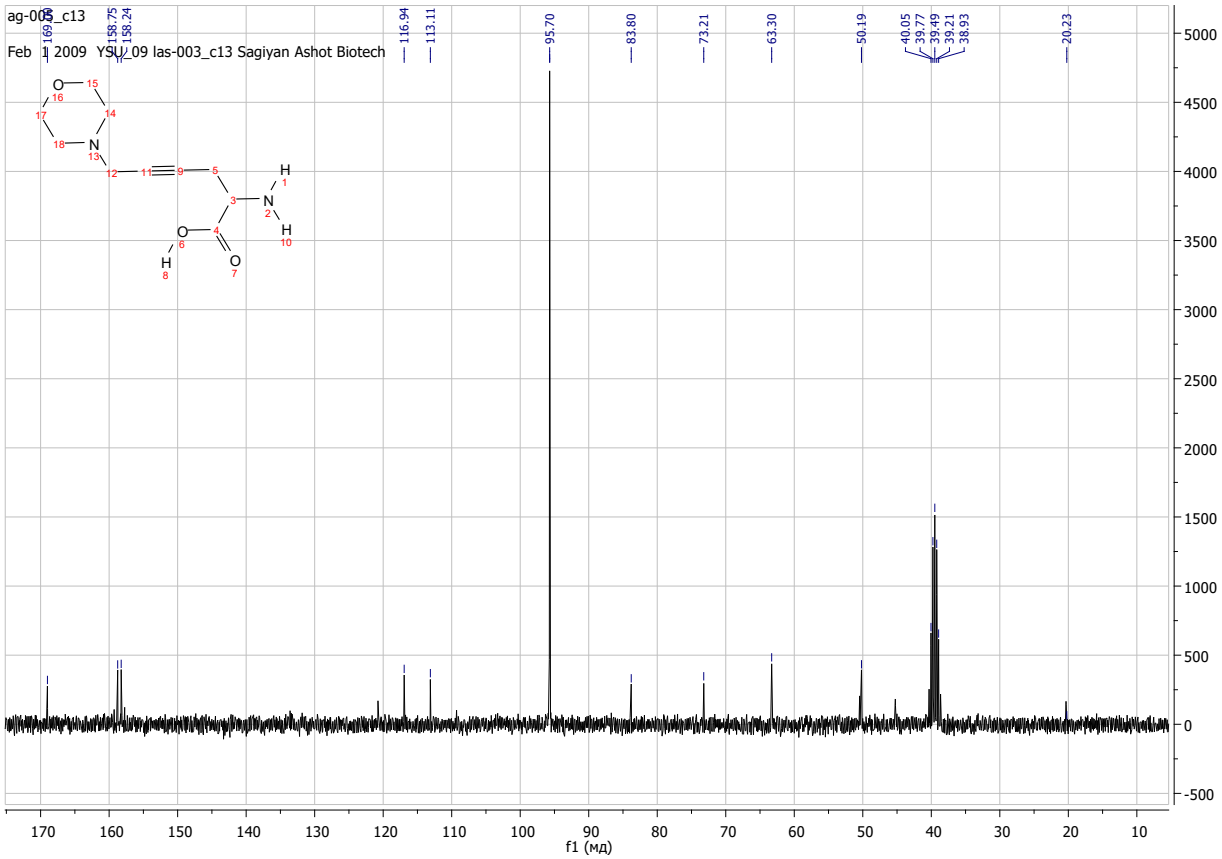
^{13}C NMR Complex 3i



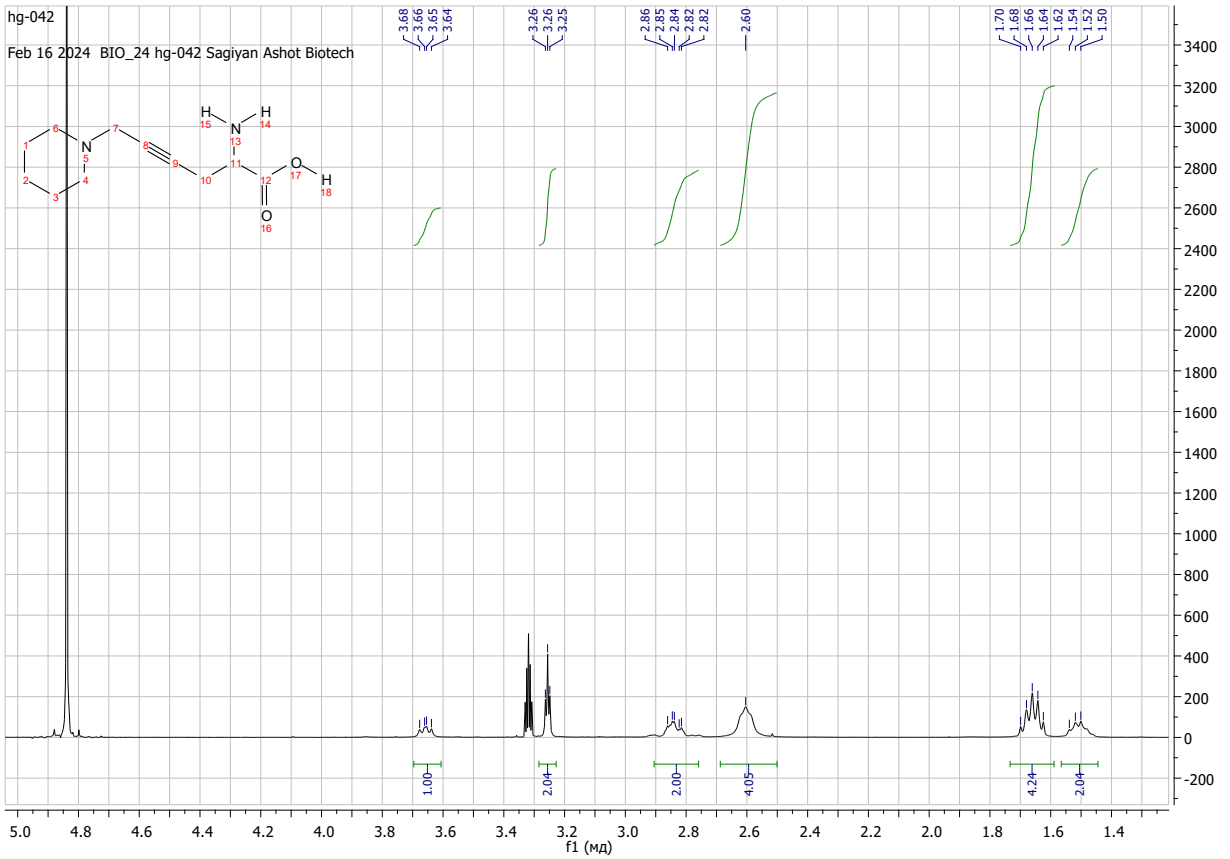
^{1}H NMR (*S*)-2-amino-6-morpholinohex-4-ynoic acid-4a.



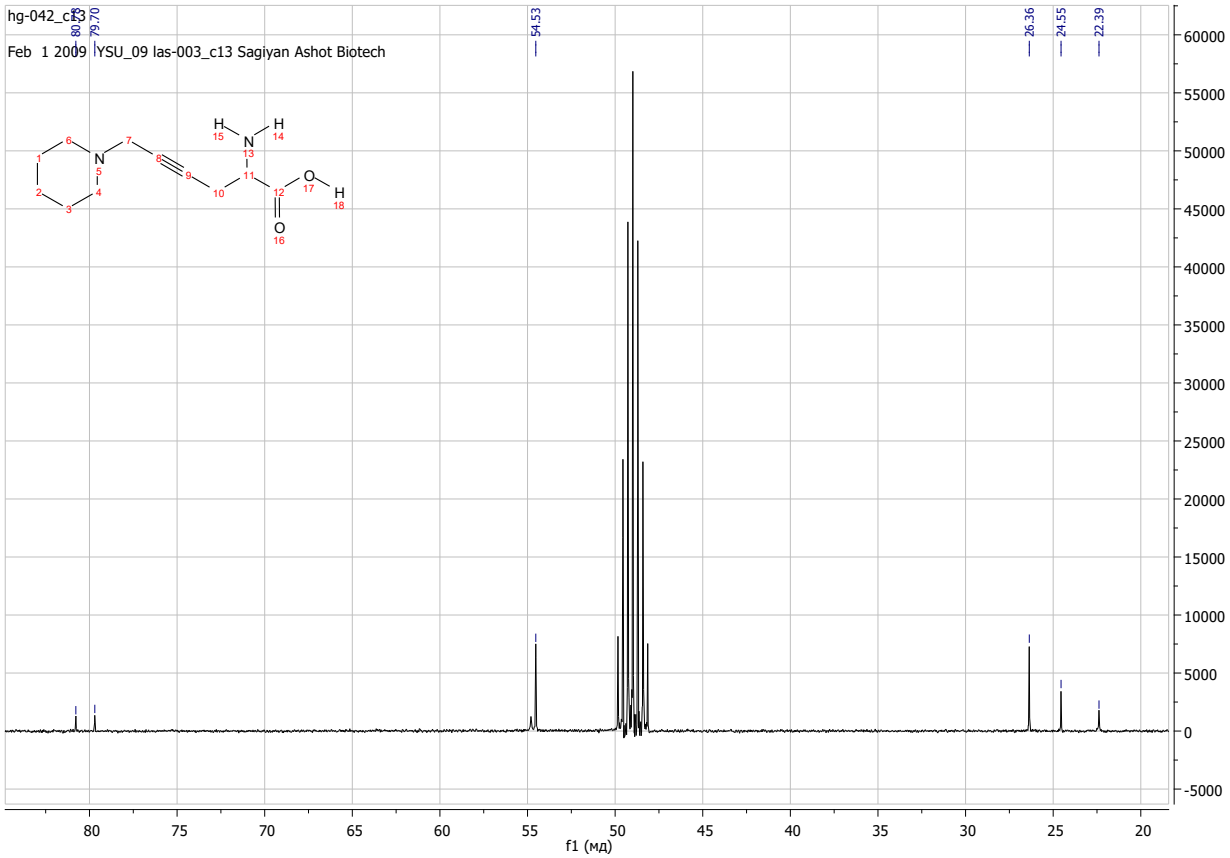
^{13}C NMR (*S*)-2-amino-6-morpholinohex-4-ynoic acid-4a



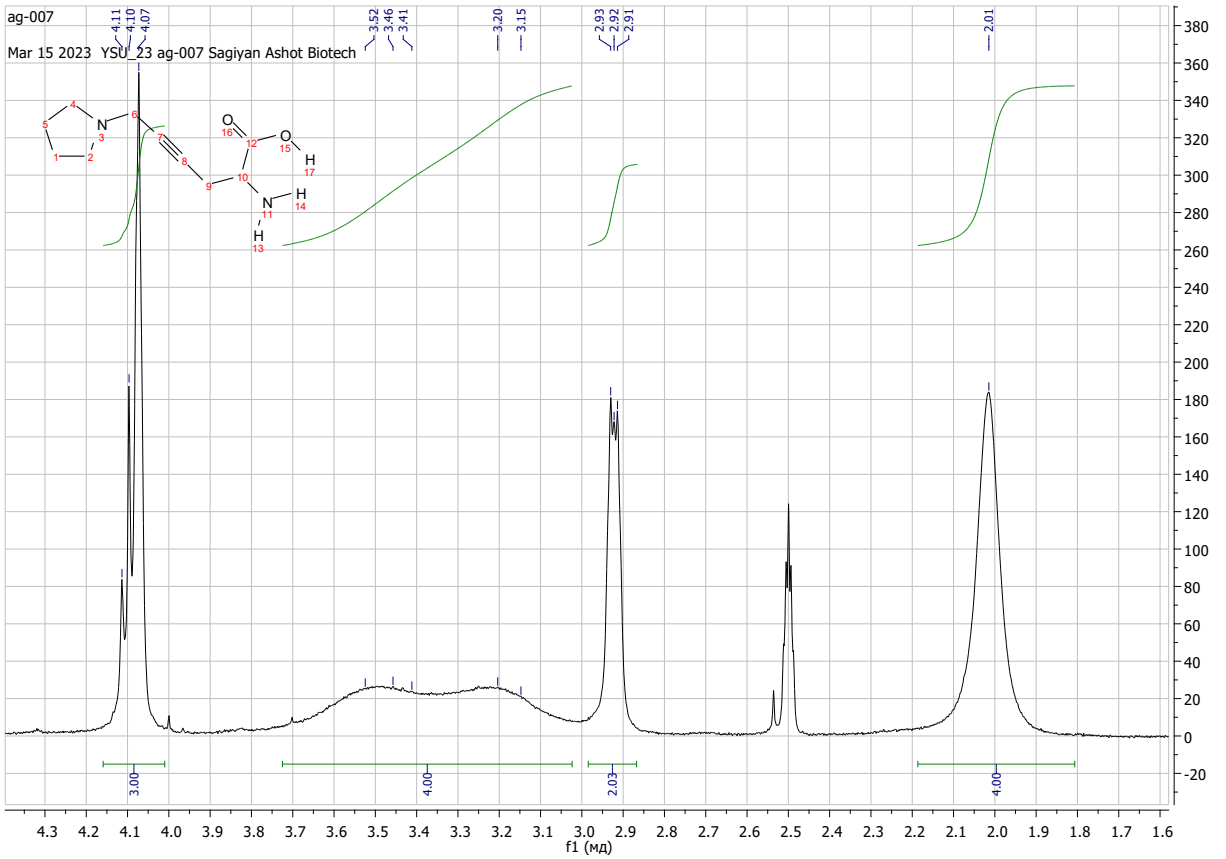
^{1}H NMR (*S*)-2-amino-6-(piperidin-1-yl)hex-4-ynoic acid-4b



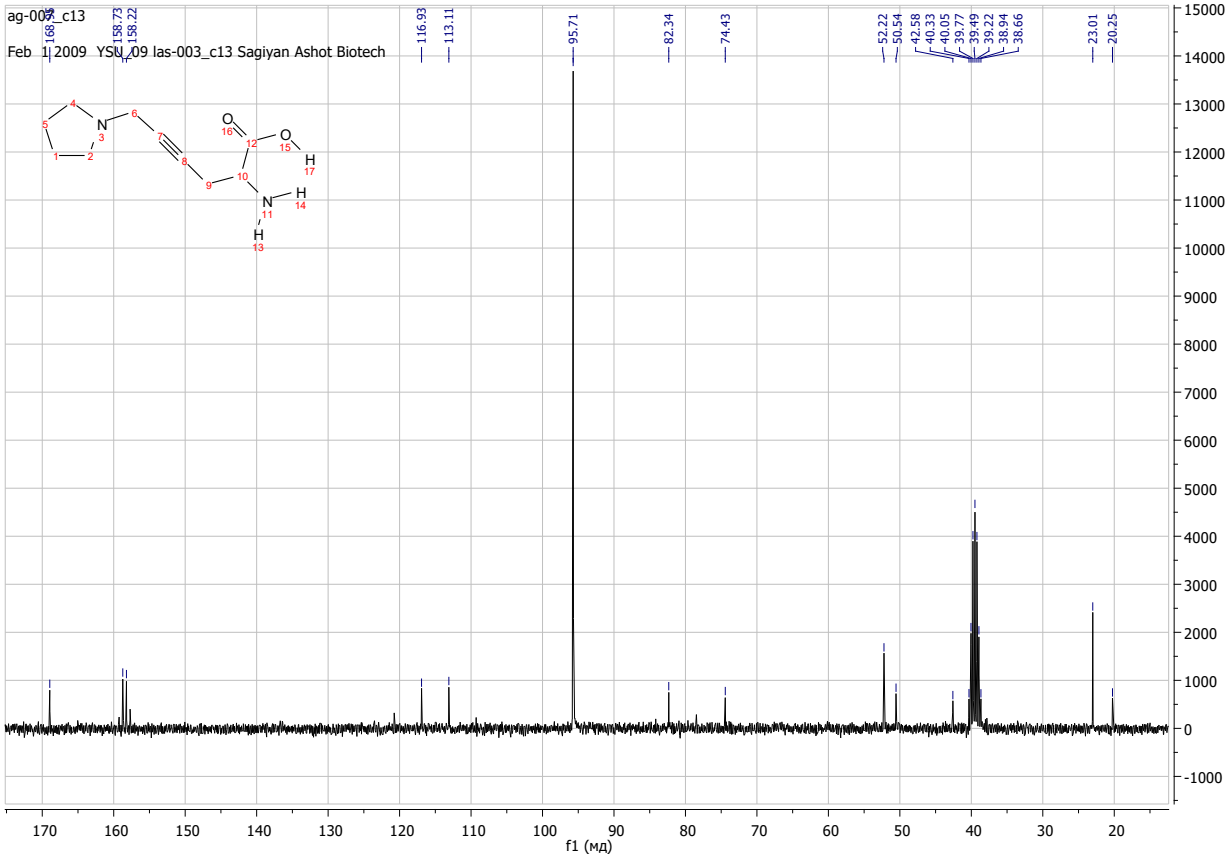
^{13}C NMR (*S*)-2-amino-6-(piperidin-1-yl)hex-4-ynoic acid-4b



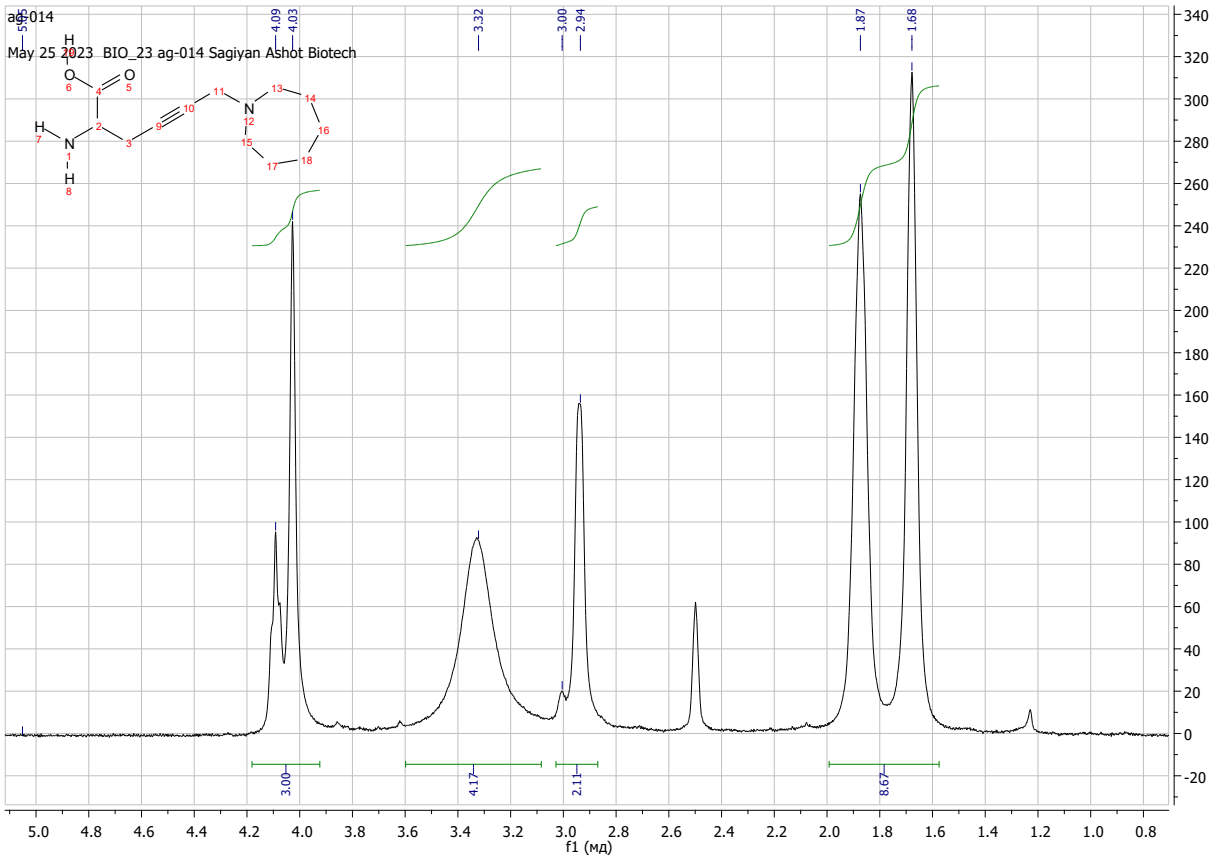
^{1}H NMR (*S*)-2-amino-6-(pyrrolidin-1-yl)hex-4-ynoic acid-4c



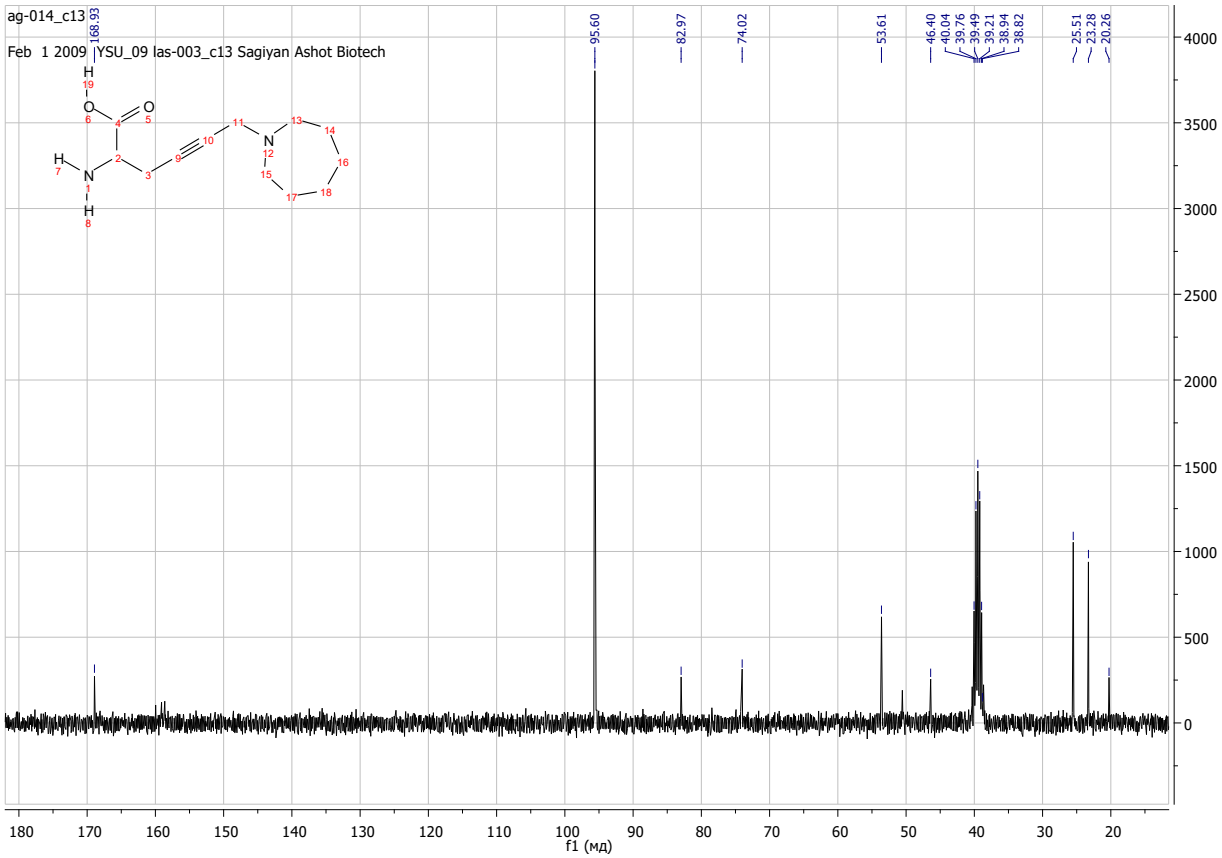
^{13}C NMR (*S*)-2-amino-6-(pyrrolidin-1-yl)hex-4-ynoic acid-4c



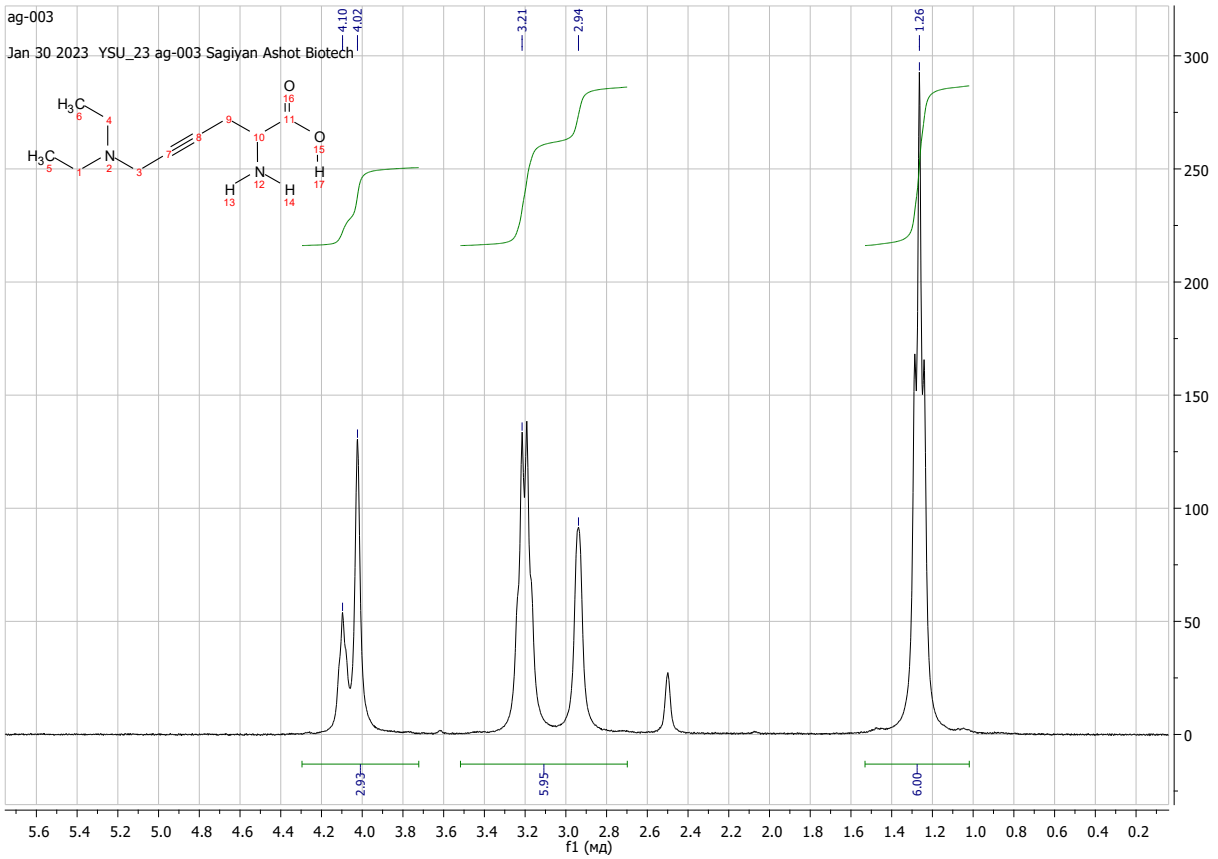
^{1}H NMR (*S*)-2-amino-6-(azepan-1-yl)hex-4-ynoic acid-4d



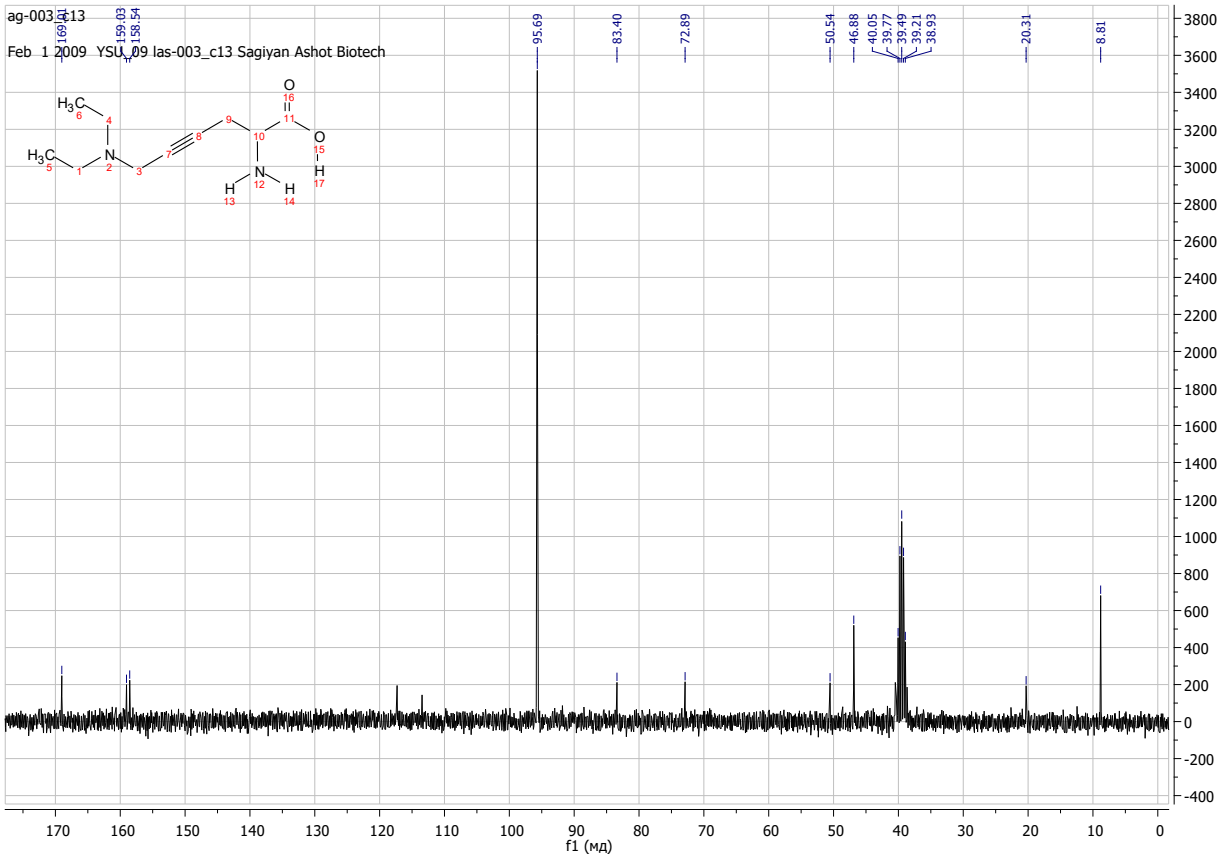
^{13}C NMR (*S*)-2-amino-6-(azepan-1-yl)hex-4-ynoic acid-4d



^{1}H NMR (*S*)-2-amino-6-(diethylamino)hex-4-ynoic acid-4e



^{13}C NMR (*S*)-2-amino-6-(diethylamino)hex-4-ynoic acid-4e



HPLC analysis of (*S, R*)-2-amino-6-morpholinohex-4-ynoic acid-4a

<Sample Information>

Sample Name	: S,R-morpholine-16.05.24		
Sample ID	:		
Data Filename	: S,R-morpholine-16.05.24.lcd		
Method Filename	: Chiral_29.11.23.lcm		
Batch Filename	:		
Vial #	: 1-1	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/16/2024 2:17:13 PM	Acquired by	: System Administrator
Date Processed	: 5/16/2024 2:37:14 PM	Processed by	: System Administrator

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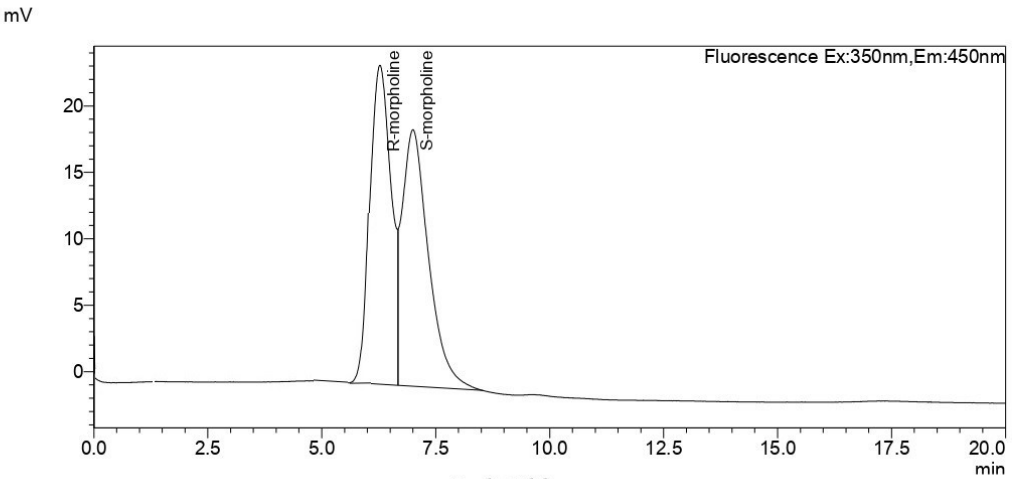

Name	Ret. time	Area	Area %
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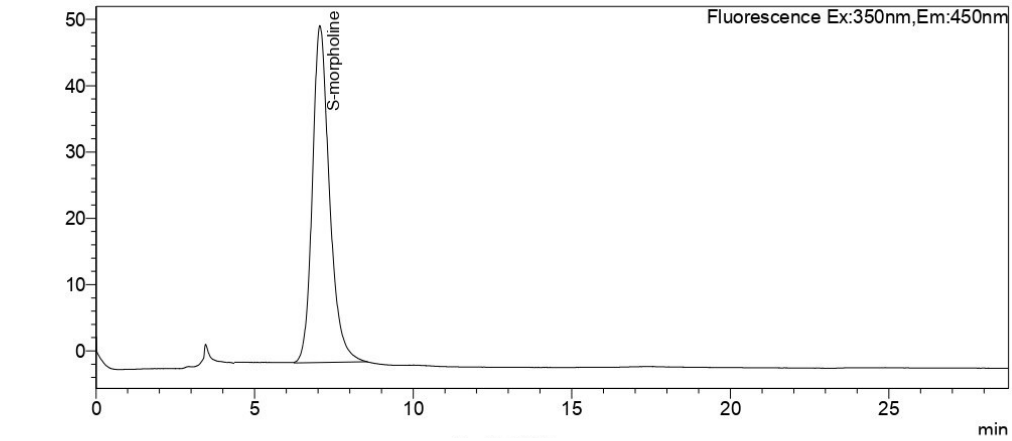
S-**4a**	6.277	763861	54.7

HPLC analysis of 4a

<Chromatogram>



Name	Ret. time	Area	Area %
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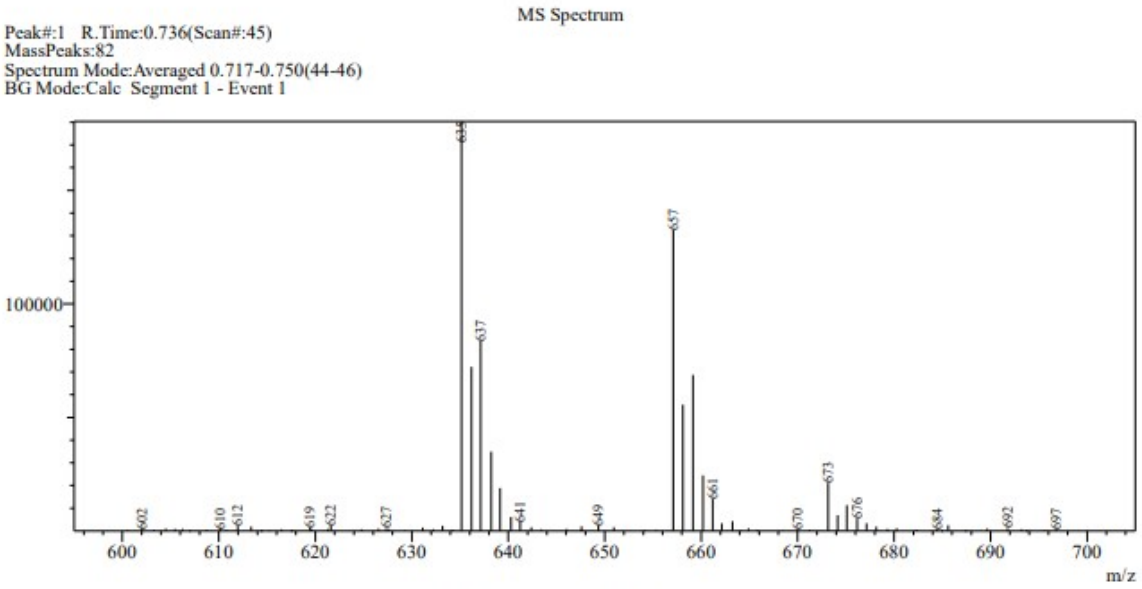
MS spectra

3a



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
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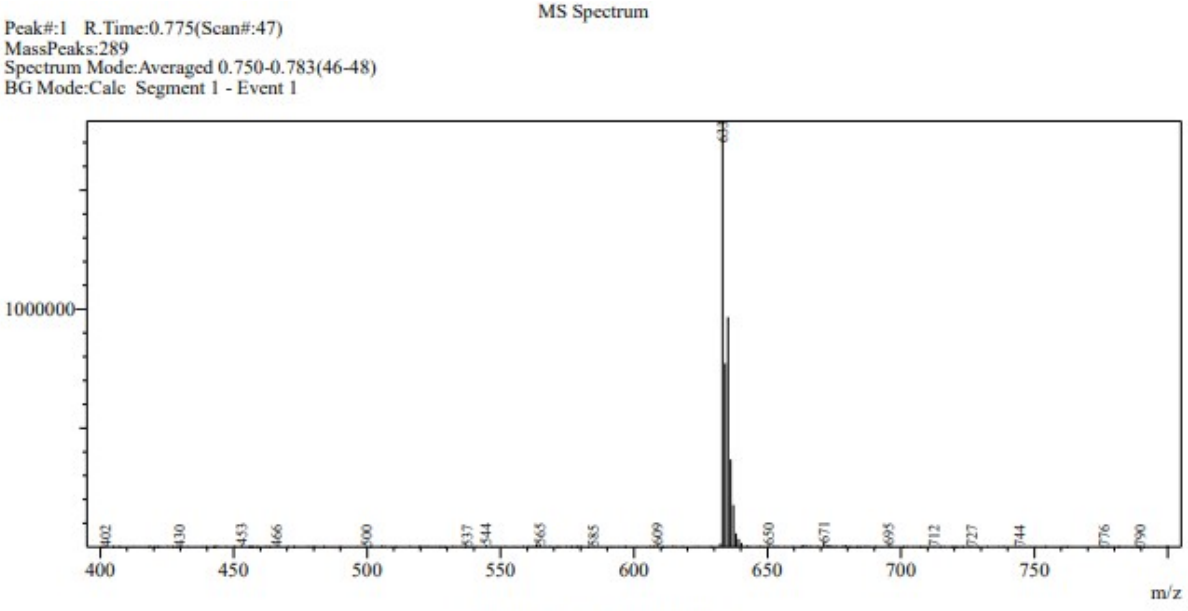
Total		12352309	

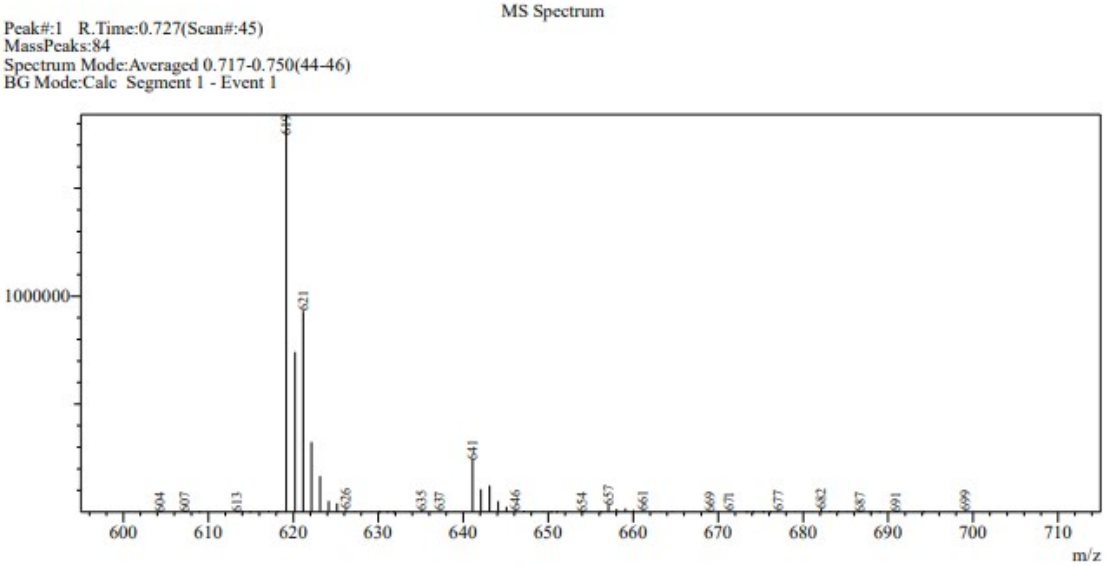
3b



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.775	112529139	633.20
Total		112529139	

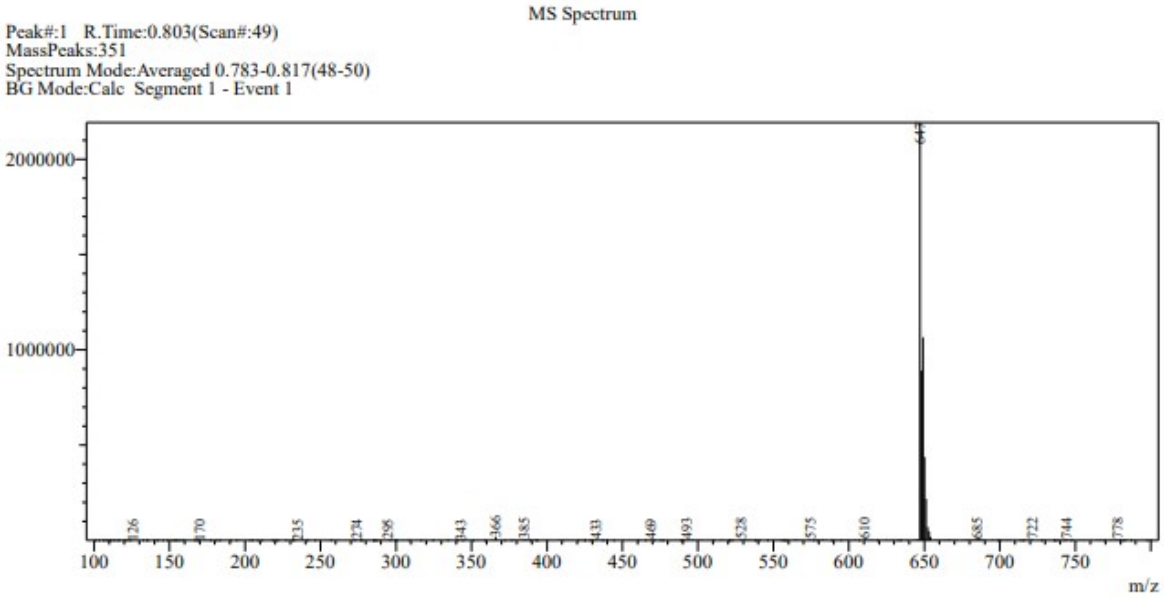
3c



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.727	73823633	619.15
Total		73823633	

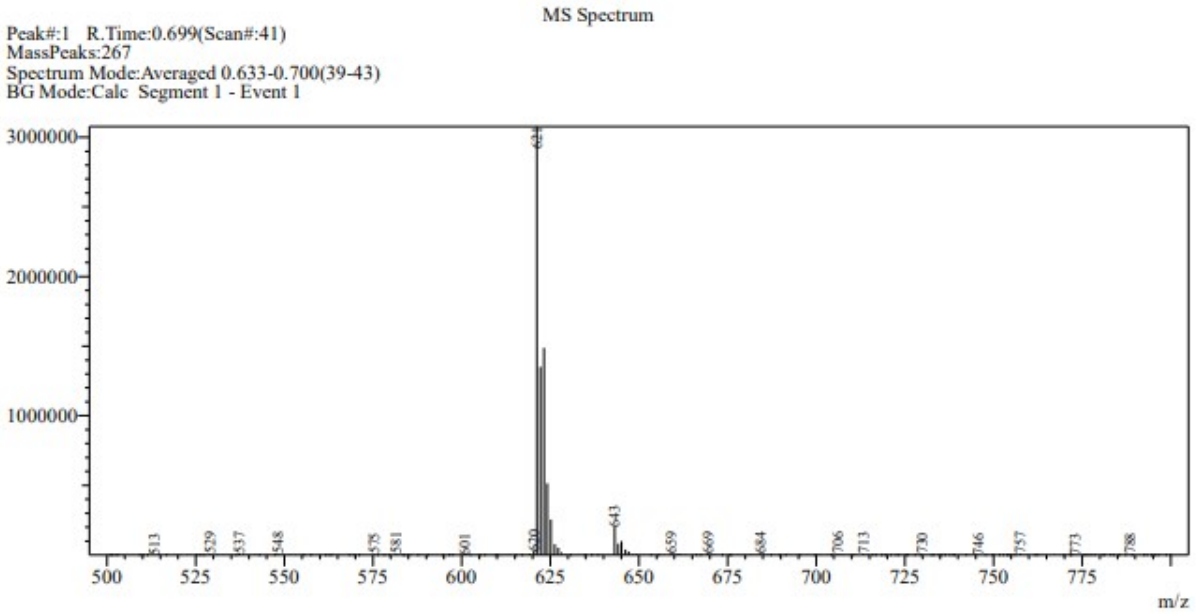
3d



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.803	118913257	647.15
Total		118913257	

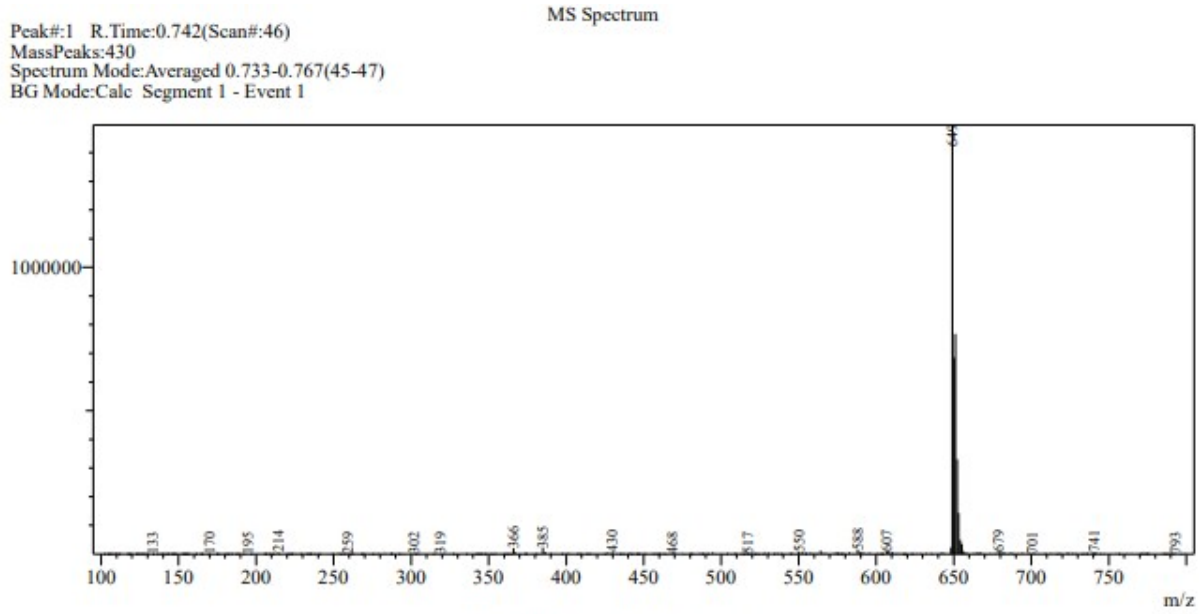
3e



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.699	123776772	621.20
Total		123776772	

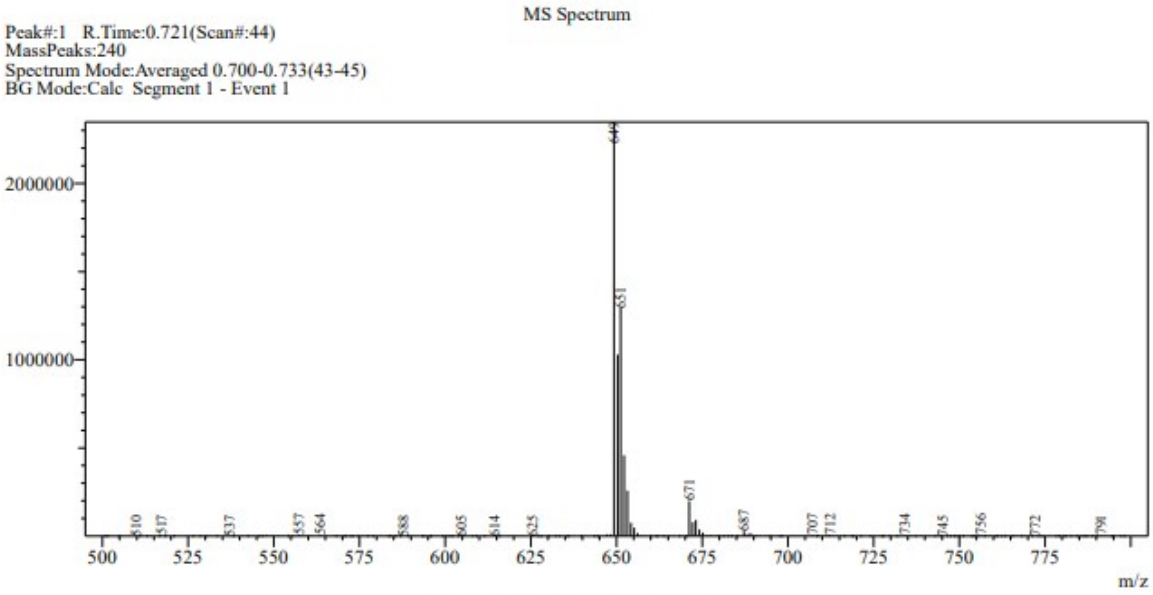
3f



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.742	85404788	649.15
Total		85404788	

3g



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.721	91648455	649.25
Total		91648455	

X-Ray study

The diffraction measurements of crystals of compounds **3a**, **3c** and **3e** were carried out at temperature of 100K on a XtaLAB Synergy-S diffractometer using Mo-Kα radiation, ω-scans rotation. A multi-scan absorption correction for all samples was performed using CrysAlisPro 1.171.43.92a (Rigaku Oxford Diffraction, 2023) using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. The structures were solved by direct method and refined using the software package SHELXTL. In all structural models, all hydrogen atoms were positioned geometrically and refined using riding model. All non-hydrogen atoms were refined anisotropically by full-matrix least squares methods. Crystallographic and experimental data and depositions numbers are listed in table 1.

Table 1. Crystallographic and experimental data

Crystal Data			
Compound	3a	3c	3e
Depositions Number	2355404	2355346	2355349
Formula	$C_{35}H_{36}N_4O_4Ni$	$C_{35}H_{36}N_4O_3Ni$	$C_{35}H_{38}N_4O_3Ni$
Formula Weight	635.39	619.39	621.40
Crystal System	Orthorhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a, b, c [Å]	7.9906(1), 13.1337(1), 28.5419(3)	9.8518(2), 9.0638(1), 16.7122(3)	8.0669(1), 11.4585(1), 32.7459(4)
α, β, γ [deg.]	90, 90, 90	90, 90.598(1), 90	90, 90, 90
V [Å^3]	2995.36(5)	1492.23(4)	3026.85(6)
Z	4	2	4
D(calc) [g/cm^3]	1.409	1.378	1.364
μ(MoKα) [mm^{-1}], T_{min}, T_{max}	0.695 0.62623, 1.00000	0.693, 0.43958, 1.00000	0.683, 0.66803, 1.00000
F(000)	1336	652	1312
Crystal Size [mm]	0.30×0.15×0.10	0.30×0.16×0.10	0.32×0.24×0.10
Data Collection			
Temperature (K)	100	100	100
Radiation [Å]	MoKα 0.71073	MoKα 0.71073	MoKα 0.71073
θ_{min}, θ_{max} [Deg]	2.1, 33.0	2.1, 33.6	2.2, 33.7
Dataset	-11≤h≤11; -20≤k≤19; -40≤l≤42	-14≤h≤15; -13≤k≤13; -25≤l≤25	-12≤h≤12; -17≤k≤15; -50≤l≤51
Tot., Uniq. Data, R(int)	106683, 10298, 0.027	54508, 10491, 0.047	113657, 11103, 0.022
Observed data [I > 2.0 σ(I)]	9974	9696	10858
Refinement			
Nref, Npar	10298, 397	10491, 388	11103, 390
R, wR2, S	0.0219, 0.0570, 1.02	0.0320, 0.0805, 1.04	0.0220, 0.0595, 1.06

The full crystallographic data in CIF format are available at: https://www.ccdc.cam.ac.uk/about-us/ (free of charge), deposition number are CCDC 2355404, 2355346, 2355349.

Molecular docking

The structures of the compounds were build using ChemBioOffice 2010 (ChemBio3D Ultra 12.0). Minimization of the ligand free energy was performed using the MM2 force field and the truncated Newton-Raphson method. The crystallographic structure of collagenase G (PDB ID: 2Y50) was used in analysis. Water molecules were removed, and polar hydrogens were added according to software producer's suggested protocol of macromolecule preparation. Docking of ligands to the enzyme was performed using AutoGrid 4 and AutoDock Vina software[2]. Ligands were ranked based on an energy-dependent score function, and protein-ligand interactions were modeled on a grid to speed up calculations. Interaction modes were identified as well as bond types and lengths were determined.

Determination of collagenase activity

Collagenase activity was determined by a method based on the measurement of free amino groups that are released during substrate hydrolysis[3]. The reaction mixture consisted of 0.05 M HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) with pH 7.2, 10 mg/ml gelatin and 0.025 mg/ml collagenase, the reaction was carried out at 37 ^{o}C.

To determine the concentration of amino groups, the ortho-phthalaldehyde (OPA) reagent was used consisting of 0.2 M borate buffer with pH 9.7, 0.1667 mg/ml OPA and 1.18 mM mercaptoethanol. Aliquots of 50 μL were taken every 30 minutes, and the reaction was stopped by adding 10 μL of 30% trichloroacetic acid. Then 1.5 mL of OPA reagent and 1.5 mL of water were added to the resulting sample, and the A340 value was recorded after 5-minute incubation at a temperature of 27 ^{o}C.

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[1] Saghyan AS, Yedoyan JA, Mkrtchyan AF, Hovsepyan GTs, Tsaturyan AO, Langer P (2014) Asymmetric synthesis of new enantiomerically enriched α-substituted analogues of (S) – propargylglycine. Chemical Journal of Armenia, 67: 67-74..

[2] Trott O., Olson A.J. (2010) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem., 31(2), 455–461. DOI: 10.1002/jcc.21334.

[3] Sargsyan A., Hakobyan H., Mardiyan Z., Jamharyan S., Dadayan A., Sargsyan T., Hovhannisyan N. (2023) Modeling, synthesis and in vitro screening of unusual amino acids and

as protease inhibitors. J. Chem. Technol. Metall., 58(3), 615–620. DOI: 10.59957/jctm.v58i3.93