Synthesis of Enantiomerically Enriched β-N-Amino (S)-α-Alanine Analogs via Sequential Reactions on a Chiral Ni(II) Complex

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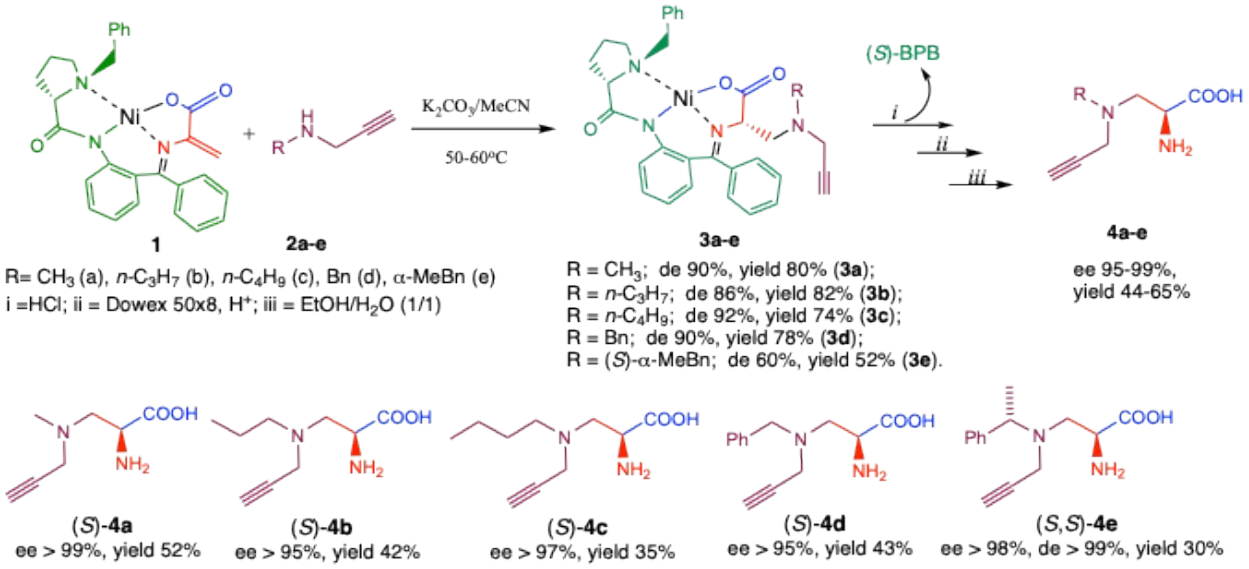
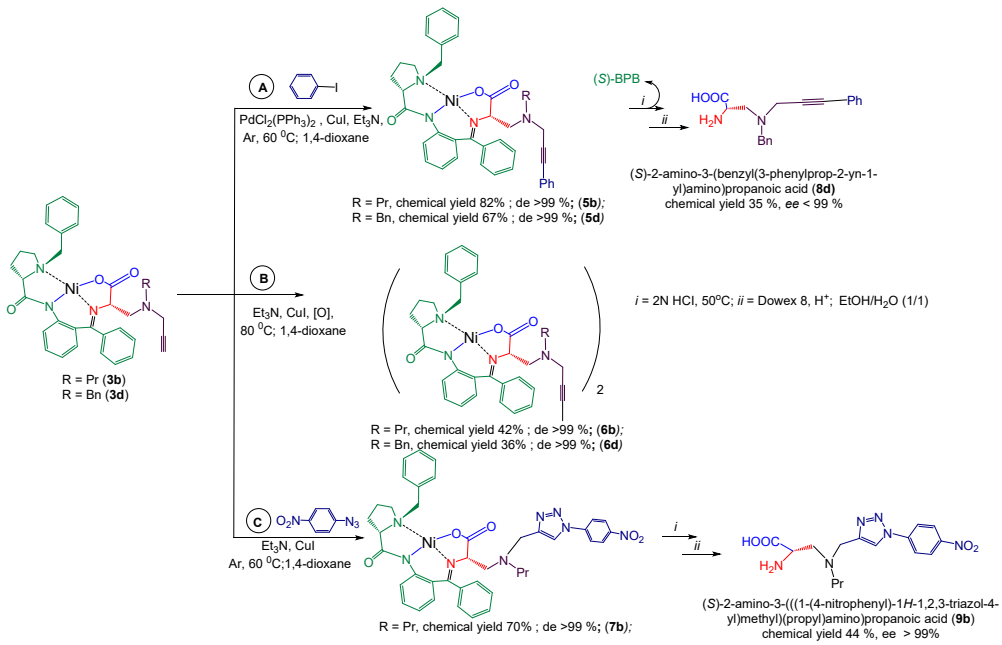
Schemes



Scheme 1. The addition of the propargylamines **2a-e** to the C=C bond of **1**.



Scheme 2. Cross-coupling and [3+2]-cycloaddition reactions of complexes **3b** and **3d**.

EXPERIMENTAL SECTION

Materials

All initial reagents were obtained from commercial sources and used without further purification. The initial **2** complexes were prepared following literature protocols. 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. 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. LCMS analysis was done byShimadzu LCMS 2020 with prominence-I LC-2030C 3D. The CD analyses were carried out with Chirascan™ V100.

General procedure for nucleophilic Michael addition to complex 1.

1.5 g (2.85mmol) of complex **1**, 1.97g (14.25 mmol) of K_2CO_3, and 8.55 mmol of a nucleophile were dissolved in 20 mL of acetonitrile. The reaction mixture was stirred at 50-60°C. The progress

of the reaction was monitored by TLC on silica, using a 3:1 mixture of ethyl acetate and chloroform. Upon completion of the reaction, as confirmed by TLC monitoring (disappearance of the starting complex and appearance of the product), the reaction mixture was filtered to remove potassium carbonate and the filtrate was concentrated under reduced pressure to remove acetonitrile. The resulting residue was extracted with chloroform–water (3 × 30 mL). The extraction was continued until the aqueous phase reached a neutral pH. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude products were purified by column chromatography on silica gel, eluting with a 2:1 mixture of acetone and chloroform, to afford complexes **3a,b** and **3d,e**. Complex **3c** was recrystallized from acetone.

General procedure for Sonogashira reaction

Iodobenzene (2.5 mL, 12.2 mmol) and 5 mL of triethylamine were placed in a pressure tube under an argon atmosphere, followed by the addition of $PdCl_2(PPh_3)_2$ (0.1 g, 0.15 mmol) and copper iodide (0.058 g, 0.3 mmol) under argon. Then, 1,4-dioxane (5 mL) and complexes **3b (1.85 g,** 3.05 mmol**)** or **3d** (**2.0 g,** 3.05 mmol**)** were added to the reaction vessel. The reaction mixture was stirred at 60 °C. and was monitored by thin-layer chromatography (TLC) on silica gel using ethyl acetate/chloroform (3:1, v/v) as the eluent. Upon completion of the reaction, as indicated by TLC monitoring (disappearance of the starting complex and appearance of the product), the mixture was cooled to room temperature and diluted with ethyl acetate (30 mL). The organic layer was separated and washed successively with water (3 × 20 mL) and brine (20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product. A small portion of the products was purified by column chromatography for the characterization of complexes **5b** and **5d**.

General procedure for Glaser reaction

Complex **3b** (1.85 g, 3.05 mmol) or **3d** (2.00 g, 3.05 mmol) and copper(I) iodide (0.58 g, 3.05 mmol) were added to a mixture of 1,4-dioxane (5 mL) and triethylamine (5 mL). The reaction mixture was stirred at 80 °C, and the progress of the reaction was monitored by thin-layer chromatography (TLC) on silica gel using ethyl acetate/chloroform (3:1, v/v) as the eluent. Upon completion of the reaction, as indicated by TLC monitoring (disappearance of the starting complex and appearance of the product), the mixture was cooled to room temperature and diluted with ethyl acetate (30 mL). The organic layer was separated and washed successively with water (3 × 20 mL)

and brine (20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product. A small portion of the crude residue was purified by column chromatography on silica gel (ethyl acetate/chloroform, 3:1, v/v) to yield the analytically pure compounds 6b and 6d for characterization.

General procedure for [2+3]-cycloaddition reaction

1-Azido-4-nitrobenzene (1.08 g, 6.5 mmol) was dissolved in 5 mL of 1,4-dioxane in a round-bottom flask under an argon atmosphere at room temperature. Copper iodide (0.062 g, 0.3 mmol) was added to the solution, and the mixture was stirred for 5 minutes. Then triethylamine (1.14 mL, 8.2 mmol) was added, and stirring was continued for 30 minutes. After that, complex **3b** (2.0 g, 3.3 mmol) was added, and the reaction mixture was stirred at 60°C and the progress was monitored by TLC gel using ethyl acetate/chloroform (3:1, v/v) as the eluent. Upon completion of the reaction, as indicated by TLC monitoring (disappearance of the starting complex and appearance of the product), the mixture was cooled to room temperature and diluted with ethyl acetate (30 mL). The organic layer was separated and washed successively with water (3 × 20 mL) and brine (20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product. The filtrate was concentrated to dryness under reduced pressure, and the residue was recrystallized from acetone to yield a pure product. A small portion (0.1 g) was used for the characterization of complex **7b**.

General procedure for isolation of amino acids

Diastereomeric mixtures of complexes **3a-e**, **5b**, **5d**, **6b**, **6d,** and **7b** (before chromatography) were suspended in CH_3OH and then were added slowly to a vigorously stirred 4M aqueous HCI at 50-60°C.

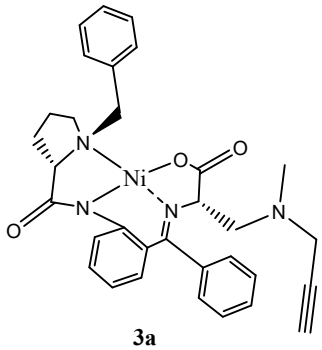
After decomposition of the complexes (*ca*. 20 min), the target amino acids were demineralized using cation-exchange resin Dowex 50-8 (H^+) and then recrystallized from an aqueous ethanol. The reported chemical yields correspond to the isolated products obtained after recrystallization. The structure, chemical, and optical purities of the target amino acids **4a-e, 8d,** and **10b** were established by physicochemical methods - NMR, HPLC, CD, $[\alpha]^D$.

Complex 3a

To 1.5 g (2.94mmol) of Ni^{II}-(*S*)-BPB-ΔAla complex, in 20ml of CH_3CN were added 2.02g (14.7 mmol) of K_2CO_3 and 8.82 mmol (0.61g) N-methylpropargylamine. Stir the reaction mixture under

heating conditions at a temperature of 50^0C. We followed the course of the reaction by the SiO_2 method (SiO_2, eluent: $CH_3COOC_2H_5$) until the traces of the starting material complex were eliminated. Based on spec data, the reaction took 2-3 hours. After the completion of the reaction, the reaction mixture filtered to get rid of potassium, then distilled to get rid of acetonitrile. Next dissolved in chloroform and extracted with distilled water, extraction was done to neutral *ph*, the organic layer was vacuum distilled to dryness and purified via silica gel column (SiO2, $CH_3COOC_2H_5$ /CH_3COCH_3 (4:1)).

Complex 3a.



3a

Yield 80% (1.36g), Mp +180-182^{0}C, $[\alpha]_D^{20}$ +1253.3^0 (c 0.34, CH_3OH): Anal Calc. for: $C_{32}H_{32}N_4NiO_3$ (579,31): C 66.34; H 5.57; N 9.67; Found: 66.76; H 5.96; N 9.99: MS, m/z: 579.00

^{1}H NMR (300 MHz, $CDCl_3$) δ=2.05-2.22 (2H, m, γ-H_a, δ-H_a Pro), 2.17 (1H, t, J=2.3, ≡CH), 2.41 (3H, s, CH_3), 2.51-2.65 (1H_a, m, β-H Pro), 2.77-2.85 (1H_b Pro, m), 2.97 (1H, dd, J=14.0, 4.9, $\underline{CH_2}$CH), 3.09 (1H, dd, J=14.0, 4.6, $\underline{CH_2}$CH), 3.48-3.59 (4H, m), 3.59 (1H, d, J=12.6, $\underline{CH_2}$Ph), 3.76-3.94 (1H, m, γ-H_b Por), 4.02 (1H, dd, J=4.9, 4.6, $\underline{CH}CH_2$), 4.44 (1H, d, J=12.6, $\underline{CH_2}$Ph), 6.65-6.72 (2H, m, C_6H_4), 7.00-7.04 (m, Ar), 7.11-7.25 (1H, m, Ar), 7.25-7.33 (1H, m, Ar), 7.38 (1H, t, J=7.6, Ar), 7.43-7.60 (1H, m, orto-Ph), 8.01-8.30 (1H, m, C_6H_4).

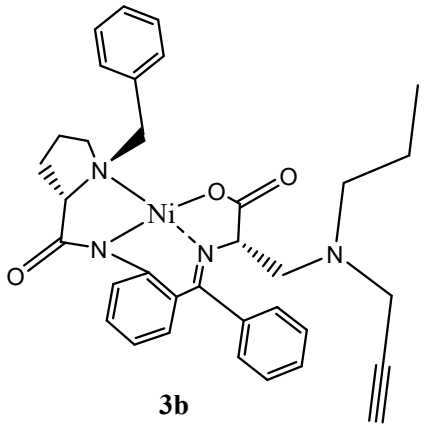
^{13}C NMR (75 MHz, $CDCl_3$) δ=23.2 (CH_3), 30.8 (γ- CH_2 Pro), 43.8(β- CH_2 Pro), 47.6 ($\underline{CH_2}$C≡CH), 57.1(δ- CH_2 Pro), 59.8 ($\underline{CH_2}$CH), 63.05 ($\underline{CH_2}$Ph), 70.4 (α-CH Pro), 70.7 $\underline{CH}CH_2$), 73.1 (≡CH), 78.7 ($\underline{C}$≡CH), 120.6 (CH), 123.5 (CH), 126.5, 127.2 (CH), 128.48 (2CH), 128.69 (CH), 128.72 (2CH), 128.8 (CH), 129.57 (CH), 131.47 (2CH), 132.08 (CH), 133.28 (CH),133.31(CH), 133.92, 142.46, 170.85, 178.47, 180.34:

Complex 3b

To 1.5 g (2.94mmol) of Ni^{II}-(*S*)-BPB-ΔAla complex, in 20ml of CH_3CN were added 2.02g (14.7 mmol) of K_2CO_3 and 8.82 mmol (0.857g) N-propylpropargylamine. Stir the reaction mixture under heating conditions at a temperature of 50^0C. We followed the course of the reaction by the SiO_2 method (SiO_2, eluent: $CH_3COOC_2H_5$) until the traces of the starting material complex were eliminated. Based on spec data, the reaction took 6 hours. After the completion of the reaction, the reaction mixture filtered to get rid of potassium, then distilled to get rid of acetonitrile. Next

dissolved in chloroform and extracted with distilled water, extraction was done to neutral *ph*, the organic layer was vacuum distilled to dryness and purified via silica gel column (SiO2, $CH_3COOC_2H_5$ /CH_3COCH_3 (4:1)).

Complex 3b.



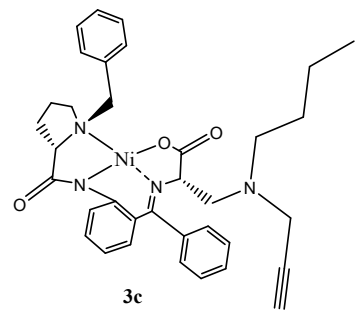
3b

Yield 82% (1.46g), Mp +204-207^{0}C, $[\alpha]_D^{20}$ +2145.238 (c 0.42, CH_3OH): Anal Calc. for: $C_{34}H_{36}N_4NiO_3$ (607,37): C 67.23; H 5.97; N 9.22; Found: 67.72; H 6.26; N 9.72: MS, m/z: 607.00

^{1}H NMR (300 MHz, $CDCl_3$) δ=0.82 (3H, t, J=7.3, CH_3), 1.37-1.55(2H, m, $\underline{CH_2}CH_3$), 2.01-2.11 (1H, m, δ-H_a Pro), 2.11 (1H, t, J=2.3, ≡ CH), 2.12-2.21 ((1H, m, γ- H_a Pro), 2.45-2.50 (2H, m, $N\underline{CH_2}C_2H_5$), 2.48-2.58 (1H, m, β-H_a Pro), 2.66-2.77 (1H, m, β- H_b Pro), 3.13 (1H, dd, J= 14.2, 5.3, $N\underline{CH_2}CH$), 3.15 (1H, dd, J=14.2, 5.3, $N\underline{CH_2}CH$), 3.38 (1H, dd, J= 17.4, 2.3, $\underline{CH_2}C{\equiv}CH$), 3.43 (1H, dd, J= 17.4, 2.3, $\underline{CH_2}C{\equiv}CH$), 3.46 (1H, dd, J=11.0, 5.7, α-H Pro), 3.48-3.56 (1H, m, δ-H_b Pro), 3.52 (1H, d, J=12.6, $\underline{CH_2}Ph$), 3.73-3.90 (1H, m, γ-H_b Pro), 3.96 (1H, t, J= 5.3, $\underline{CH}CH_2$), 4.41 (1H, d, J= 12.6, $\underline{CH_2}Ph$), 6.59-6.68 (2H, m, C_6H_4), 6.99-7.03 (1H, m, C_6H_5), 7.11 (1H, ddd, J=8.6, 6.0, 2.7, C_6H_4), 7.12-7.18 (1H, m, H-4 Ph), 7.19-7.25 (1H, m, Ar), 7.29-7.35 (2H, m, Ar), 7.39-7.51 (3H, m, Ar), 8.05-8.09 (2H, m, orto-Ph), 8.16 (1H, dd, J= 8.6, 1.0, C_6H_4).

^{13}C NMR (75 MHz, $CDCl_3$) δ 11.8(CH_3), 20.8 ($\underline{CH_2}CH_3$), 23.7 (γ- CH_2 Pro), 30.9(β- CH_2 Pro), 44.5 ($\underline{CH_2}C{\equiv}CH$), 57.3(δ- CH_2 Pro), 57.5 ($\underline{CH_2}CH$), 58.0 ($N\underline{CH_2}C_2H_5$), 63.1($\underline{CH_2}Ph$), 70.4 (α-CH Pro), 71.0 ($\underline{CH}CH_2$), 73.1 (≡CH), 79.2 ($\underline{C}{\equiv}CH$), 120.6 (CH), 123.6 (CH), 126.5, 127.4 (CH), 128.77 (2CH), 128.81(CH), 128.9(2CH), 129.6(CH), 131.6(2CH), 132.2(CH),133.4(CH), 134.0, 142.6, 170.9, 178.4, 180.4.

Complex 3c



3c

To 1.5 g (2.94mmol) of Ni^{II}-(*S*)-BPB-ΔAla complex, in 20ml of CH_3CN were added 2.02g (14.7 mmol) of K_2CO_3 and 8.82 mmol (0.98g) N-buthylpropargylamine. Stir the reaction mixture under heating conditions at a temperature of 50^{0}C. We followed the course of the reaction by the SiO_2 method (SiO_2, eluent: $CH_3COOC_2H_5$) until the traces of the starting material complex were eliminated. Based on spec data, the reaction took 20 hours. After the completion of the reaction, the reaction

mixture filtered to get rid of potassium, then distilled to get rid of acetonitrile. Next dissolved in chloroform and extracted with distilled water, extraction was done to neutral *ph*, the organic layer was vacuum distilled to dryness and crystallized from acetone.

Complex 3c

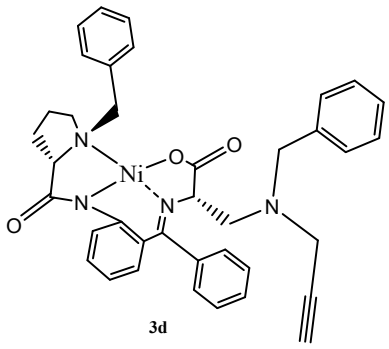
Yield 74% (1.36g), Mp +194-197^{0}C, $[\alpha]_D^{20}$ +2036.92 (c 0.52, CH_3OH): Anal Calc. for: $C_{35}H_{38}N_4NiO_3$ (621,39): C 67.65; H 6.16; N 9.02; Found: 67.98; H 6.57; N 9.51: MS, m/z: 621.05 ^{1}H NMR (300 MHz, $CDCl_3$) δ=0.82 (3H, t, J=7.3, CH_3), 1.18-1.30 (2H, m, $\underline{CH_2}CH_3$), 1.34-1.50 (2H, m, $\underline{CH_2}C_2H_5$), 2.01 -2.09 (1H, m, δ-H_a Pro), 2.11 (1H, t, J= 2.3, ≡ CH), 2.11-2.20 (1H, m, γ-H_a Pro), 2.45-5.59 (1H, m, β- H_a Pro), 2.51 (2H, t, J=7.6, $\underline{CH_2}C_3H_7$), 2.66-2.78 (1H, m, β- H_b Pro), 3.15 (2H, dd, J= 5.3, $\underline{CH_2}$CH), 3.37 (1H, dd, J= 17.5, 2.3, $\underline{CH_2}$C≡CH), 3.42 (1H, dd, J= 17.5, 2.3, $\underline{CH_2}$C≡CH), 3.46 (1H, dd, J= 11.0, 5.8, α-H Pro), 3.52 (1H, d, J= 12.6, $\underline{CH_2}$Ph), 3.50-3.57 (1H, m, δ-H_b Pro), 3.67-3.91 (1H, m, γ-H_b Pro), 3.96 (1H, t, J= 5.3, $\underline{CH}$), 4.41 (1H, d, J= 12.6, $\underline{CH_2}$Ph), 6.59-6.67 (2H, m, C_6H_4), 6.99-7.03 (1H, m, C_6H_5), 7.11 (1H, ddd, J=8.7, 5.9, 2.9, C_6H_4), 7.13-7.18 (1H, m, p- Ph), 7.20-7.26 (1H, m, C_6H_5), 7.29-7.35 (2H Ar), 7.39-7.51 (3H, m, Ar), 8.05-8.09 (2H, m, orto-Ph), 8.19 (1H, ddd, J= 8.7, 1.0, 0.6 C_6H_4).

^{13}C NMR (75 MHz, $CDCl_3$) δ=14.0(CH_3), 20.5 (CH_2), 23.7 (γ- CH_2 Pro), 29.7 (CH_2), 30.9(β- CH_2 Pro), 44.4 (CH_2), 57.2(δ- CH_2 Pro), 57.7 (CH_2), 63.1($\underline{CH_2}$Ph), 70.4 (CH), 70.9 (α-CH Pro), 73.0 (≡CH), 79.2 ($\underline{C}$≡CH), 120.6, 123.6, 126.4, 127.4, 128.76 (2C), 128.82, 128.6(3C), 129.6, 131.6(2CH), 132.2,133.4, 134.0, 142.6, 170.8, 178.3, 180.4.

Complex 3d

To 1.5 g (2.94mmol) of Ni^{II}-(*S*)-BPB-ΔAla complex, in 20ml of CH_3CN were added 2.02g (14.7 mmol) of K_2CO_3 and 8.82 mmol (1.28g) N-propargylbenzylamine. Stir the reaction mixture under heating conditions at a temperature of 50^{0}C. We followed the course of the reaction by the SiO_2 method (SiO_2, eluent: $CH_3COOC_2H_5$) until the traces of the starting material complex were eliminated. Based on spec data, the reaction took 10 hours. After the completion of the reaction, the reaction mixture filtered to get rid of potassium, then distilled to get rid of acetonitrile. Next dissolved in chloroform and extracted with distilled water, extraction was done to neutral *ph*, the organic layer was vacuum distilled to dryness and purified via silica gel column (SiO2, $CH_3COOC_2H_5$ /CH_3COCH_3 (4:1)).

Complex 3d



3d

Yield 78% (1.49g), Mp +163-165^{0}C, $[\alpha]_D^{20}$ +2147.7 (c 0.22, CH_3OH): Anal Calc. for: $C_{38}H_{36}N_4NiO_3$ (655,41): C 69.64; H 5.54; N 8.55; Found: 70.19; H 5.96; N 8.94: MS, m/z: 655.00

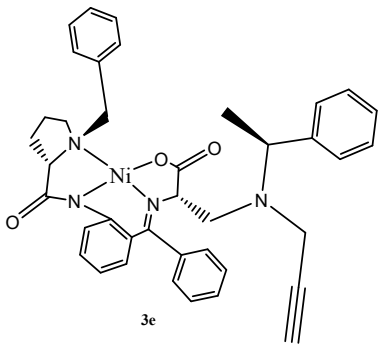
^{1}H NMR (300 MHz, $CDCl_3$) δ=1.85-1.96 (1H, m, γ-H_a Pro), 1.98 -2.08 (1H, m, δ-H_a Pro), 2.20 (1H, t, J=2.3, ≡ CH), 2.28-2.55 (2H, m, β- CH_2 Pro), 3.04 (1H, dd, J=13.8, 5.1, $\underline{CH_2}$CH), 3.16(1H, dd, J=13.8, 5.3, $\underline{CH_2}$CH), 3.37-3.51 (6H, m), 3.66 (1H, d, J=13.2, $\underline{CH_2}$Ph), 3.83 (1H, d, J=13.2, $\underline{CH_2}$Ph), 3.96 (1H, dd, J= 5.3, 5.1, CH), 4.41 (1H, d, J= 12.6, $\underline{CH_2}$Ph), 6.49(1H, b.d. J= 7.6, C_6H_5), 6.56(1H, dd, J=8.2, 1.8, C_6H_4), 6.65 (1H, ddd, J=8.2, 6.9, 1.2, C_6H_4), 7.10-7.21 (6H, m, Ar), 7.31-7.37 (3H, m, Ar), 7.40-7.49 (4H, m, Ar), 8.05-8.10 (2H, m, Ph), 8.28 (1H, dd, J=8.7, 1.2, C_6H_4).

^{13}C NMR (75 MHz, $CDCl_3$) δ= 23.8 (γ- CH_2 Pro), 30.7(β- CH_2 Pro), 43.6 (CH_2), 56.6(CH_2), 57.4 (δ-CH_2 Pro), 60.1(CH_2), 63.2 (CH_2Ph), 70.1 (CH), 70.3 (α-CH Pro), 73.9 (≡CH), 78.7 ($\underline{C}$≡CH), 120.6, 123.6, 126.3, 127.4 , 127.5, 128.6 (2CH), 128.7, 128.81, 128.84, 128.9(2CH), 129.4(2CH), 129.6, 131.6(2CH), 132.3,133.50, 133.54, 133.0, 138,1,142.8, 171.0, 178.4, 180.5:

Complex 3e

To 1.5 g (2.94mmol) of Ni^{II}-(*S*)-BPB-ΔAla complex, in 20ml of CH_3CN were added 2.02g (14.7 mmol) of K_2CO_3 and 8.82 mmol (1.404g) N-(1-phenylethyl)prop-2-yn-1-amine. Stir the reaction mixture under heating conditions at a temperature of 50^{0}C. We followed the course of the reaction by the SiO_2 method (SiO_2, eluent: $CH_3COOC_2H_5$) until the traces of the starting material complex were eliminated. Based on spec data, the reaction took 40 hours. After the completion of the reaction, the reaction mixture filtered to get rid of potassium, then distilled to get rid of acetonitrile. Next dissolved in chloroform and extracted with distilled water, extraction was done to neutral *ph*, the organic layer was vacuum distilled to dryness and purified via silica gel column (SiO_2, $CH_3COOC_2H_5$ /CH_3COCH_3 (4:1)).

Complex 3e



3e

Yield 52% (1.02g), Mp +185-188^{0}C, $[\alpha]_D^{20}$ +692.85 (c 0.84, CH_3OH): Anal Calc. for: $C_{39}H_{38}N_4NiO_3$ (669,44): C 69.97; H 5.72; N 8.37;Found: 70.45; H 6.26; N 6.20: MS, m/z: 669.55

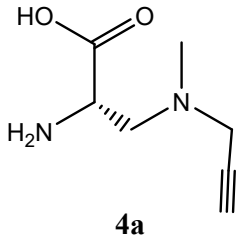
^{1}H NMR (300 MHz, $CDCl_3$) δ=Mixture of two dyastereomers 80 and 20% `1.29 (2.4H, d, J 6.6) & 1.44 (0.6H, d, J=6.6, CH3), 1.85-2.13 (3H, m), 2.19 (0.8H, t, J=2.3) & 2.21 (0.2H, t, J=2.3, CH Acetylen), 2.24-2.7 (2H, m), 2.60 (0.8H, dd, J=18.0, 2.3, CH2-Acetylen), 3.01-3.17 (2H, m) 3.29 (0.8H, dd, J=13.7, 3.7, C<u>H</u>$_2$-CH), 3.36-3.45 (2.8H, m), 3.41 (1H, dd, J=10.9, 5.6, a-CH Prol.), 3.59 (0.8H, ddd, J=13.7, 3.6, 1.2, C<u>H</u>$_2$-CH), 3.67-3.82 (1H, m), 3.88 (0.8H, q, J=6.6, C<u>H</u>-CH_3), 4.11 (1H, dd, J=8.5, 3.7, C<u>H</u>$_2$-Acetylen), 4.36 (0.8H, d, J=12.6) & 4,44 (0.8H, J=12.6, C<u>H</u>$_2$-Ph), 6.51 (0.2H, b. dd, J=8.2, 1.7, Ar), 6.66 (0.2H, ddd, J=8.2, 6.9, 1.2, Ar) & 6.68-6.75 (1.8H, m, H-3,4 C_6H_4), 7.01-7.07 (0.8H, m, Ar), 7.12-7.24 (5H, m, Ar), 7.25-7.39 (5H, m, Ar), 7.43-7.57 (3H, m, Ar), 8.06-8.14 (2H, m, H-2,2' Ph), 8.31 (0.2H, b.dd, J=8.7, 1.2, Ar) & 8.36 (0.8H, dt, J=8.7, 0.7, H-6 C_6H_4).

^{13}C NMR (75 MHz, $CDCl_3$) δ=

	Major Izomer (A)	Minor Izomer (B)
CH_3	21.0	22.4
g-CH_2 Prol.	23.7	23.9
b-CH_2 Prol.	30.3	30.7
NCH_2	39.3	40.8
NCH_2	55.1	57.0
d-CH_2 Prol.	57.3	57.3
NCH_2	61.3	62.3
<u>C</u>H_2-Ph	63.98	64.0
NCH	69.6	70.1
a-CH Prol.	70.1	70.3
CH -Acetylen	73.6	73.5
C -Acetylen	78.5	79.6

Aromatic Carbons: 120.4 – 134.1 many peaks, 142.6 (B) & 142.8 (A), 144.2 (A) & 145.0 (B), 166.6 (B) & 170.9 (A), 177.9 (A) & 178.4 (B), 180.1 (A) & 182.8 (B).

(*S*)-3-(N-methyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4a



4a

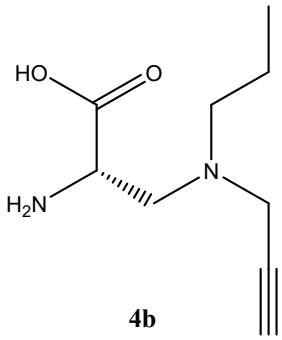
Yield 52%, Mp +210-212^{0}C, $[\alpha]_D^{20}$=+19.79^0 (C=0.1, CH_3OH), Anal. Calcu. for: $C_7H_{12}N_2O_2$ (156,18): C 53.83; H 7.74; N 17.94; Found: C 54.12; H 7.98; N 18.42.

^{1}H NMR (300 MHz, CD_3OD) δ=2.4 (3H, s, CH_3), 2.65 (1H, t, J=2.3, ≡ CH), 2.73 (1H, dd, J=13.5, 10.4, <u>CH$_2$</u>CH), 3.00 (1H, dd, J=13.5, 3.9, <u>CH$_2$</u>CH), 3.42

(1H, dd, J=17.8, 2.3, $\underline{CH_2}C{\equiv}CH$), 3.44 (1H, dd, J=17.8, 2.3, $CH_2C{\equiv}CH$), 3.66 (1H, dd, J=10.4, 3.9, NCH):

^{13}C NMR (75 MHz, CD_3OD) δ 41.5, 46.8, 54.2, 57.1, 74.9(≡CH), 78.9 (C ≡), 173.1(CO):

(*S*)-3-(N-(prop-2-ynyl)-N-propylamino)-2-aminopropanoic acid-4-ynoic acid-4b



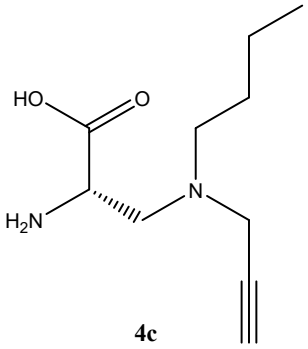
4b

Yield 42%, Mp +138-140^{0}C, $[\alpha]_D^{20}$=-28,5^0 (C=0.2 CH_3OH), Anal. Calcu. for: $C_9H_{16}N_2O_2$ (184.24):

^1H NMR: (300 MHz, CD_3OD) δ=0.90 (3H, t, *J* 7.3, CH_3), 1.42-1.51 (2H, m, CH_2CH_3), 2.41-2.56 (2H, m, $CH_2C_2H_5$), 2.62 (1H, t, *J* 2.4, acetylene-H), 2.68 (1H, dd ^2J=12.8, ^3J=10.5, $C\underline{H_2}CH$), 2.94 (1H, dd ^2J=12.8, ^3J=3.1, $C\underline{H_2}CH$);3.40 (1H, dd ^2J=17.9, ^4J=2.4, CH_2-Acetylen), 3.47 (1H, dd ^2J=17.9, ^4J=2.4, CH_2-Acetylen), 3.55 (1H, b., CH), 7.45 (3H, very b., NH2 & COOH).

^{13}C NMR Spectrum:11.4 (CH_3); 19.8 (CH_2); 41.0 (NCH_2); 51.9 (NCH_2); 53.9 (NCH_2);54.6 (CH); 74.2 & 78.3 (Acetylen); 170.7 (CO).

(*S*)-3-(N-butyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4c



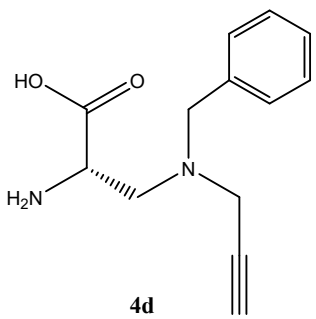
4c

Yield 35%, Mp +160-162^{0}C, $[\alpha]_D^{20}$=-37.42^0 (C=0.326 CH_3OH), Anal. Calcu. for: $C_{10}H_{18}N_2O_2$ (198,26): C 60.58; H 9.15; N 14.13; Found: C 61.05; H 9.58; N 14.59.

^1H NMR (300 MHz, CD_3OD) δ=0.96 (3H, t, J =7.3, CH_3), 1.31-1.43 (2H, m, $\underline{CH_2}CH_3$), 1.43-1.55 (2H, m, $\underline{CH_2}C_2H_5$), 2.53-2.71 (2H, m, $\underline{CH_2}C_3H_7$), 2.61 (1H, t, J=2.4, ≡CH), 2.74 (1H, dd, J=13.9, 10.4, $C\underline{H_2}CH$), 3.10 (1H, dd, J=13.9, 4.0, $C\underline{H_2}CH$), 3.46 (1H, dd, J=17.7, 2.4, $\underline{CH_2}C{\equiv}CH$), 3.49 (1H, dd, J=17.7, 2.4, $\underline{CH_2}C{\equiv}CH$), 3.64 (1H, dd, J= 10.4, 4.0, $\underline{CH}NH_2$).

^{13}C NMR (75 MHz, CD_3OD) δ 14.3 (CH_3), 21.5(CH_2), 30.6(CH_2), 42.6(NCH_2), 54.42(NCH_2), 54.44(NCH_2), 55.8 (CH), 74.7 (≡CH), 78.9(≡C), 173.0(CO)

(*S*)-3-(N-benzyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4d



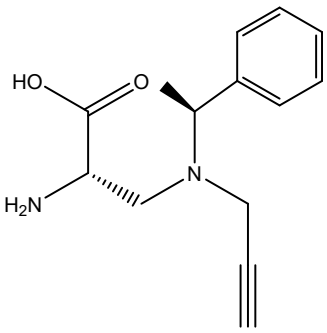
4d

Yield 43%, Mp +202-205^{0}C, $[\alpha]_D^{20}$=-15.5^0 (C=0.4 CH_3OH), Anal. Calcu. for: $C_{13}H_{16}N_2O_2$ (232,28): C 67.22; H 6.94; N 12.06; Found: C 67.71; H 7.48; N 12.56.

^{1}H NMR (300 MHz, CD_3OD) δ=2.69 (1H, t, J=2.4, ≡CH), 2.88 (1H, dd, J=13.9, 10.0, $\underline{CH_2}CH$), 3.22 (1H, dd, J=13.9, 4.0, $\underline{CH_2}CH$), 3.69 (1H, dd, J=10.0, 4.0, $CH_2\underline{CH}$), 3.73 (1H, d, J=12.9, CH_2Ar), 3.83 (1H, d, J=12.9, CH_2Ar), 7.25-7.37 (3H, m, m,p-C_6H_5), 7.39-7.44 (2H, m, o- C_6H_5), 3.30 (1H, dd, J=17.4, 2.4, $\underline{CH_2}C\equiv CH$), 3.34 (1H, dd, J=17.4, 2.4, CH2 C≡ CH):

^{13}C NMR (75 MHz, CD_3OD) δ 58.72(CH_2), 55.5(CH_2),54.32(CH),42.194(CH_2), 78.58 (C ≡), 75.34(≡ CH), 128.57(Ar), 129.467(2Ar), 134.78(2Ar), 139.09(Ar), 172.83(CO).

(*S*)-2-amino-3-(((*S*)-1-phenylethyl)(prop-2-yn-1-yl)amino)propanoic acid-4e



4e

Yield 30%, Mp +185-187^{0}C, $[\alpha]_D^{20}$=-57.78^0 (C=0.09 CH_3OH), Anal. Calcu. for: $C_{14}H_{18}N_2O_2$ (246,3): C 68.27; H 7.37; N 11.37; Found: C 68.79; H 7.88; N 11.89.

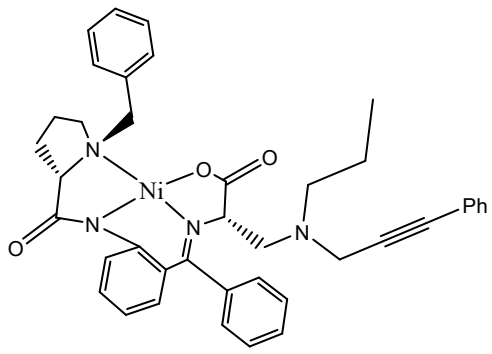
^{1}H NMR (300 MHz, DMSO-d_6/CF_3COOD) δ=1.36 (3H, d, J=6.6, CH_3), 2.86 (1H, dd, J=13.6, 9.5, N$\underline{CH_2}CH$), 3.01 (1H, dd, J=13.6, 4.5, N$\underline{CH_2}CH$), 3.19 (1H, t, J=2.3, ≡CH), 3.28 (1H, dd, J=18.0, 2.3, $\underline{CH_2}C\equiv CH$), 3.46 (1H, dd, J=18.0, 2.3 $\underline{CH_2}C\equiv CH$), 3.91 (1H, q, J= 6.6, $\underline{CH}CH_3$), 4.06 (1H, dd, J=9.5, 4.5, $NCH_2\underline{CH}$), 7.24-7.45 (5H, m, C_6H_5).

^{13}C NMR (75 MHz, CD_3OD) δ 20.1(CH_3), 38.85(CH_2),51.3,52.9,60.7, 75.7($\underline{CH}\equiv C$), 79.3($CH\equiv\underline{C}$), 126.9(CH, Ph), 127.5(2CH, Ph), 128.3(2CH, Ph), 143.4($C_{i,}$ Ph), 168.49(COOH). Isomer>15%

Complex 5b

To a mixture of iodobenzene (1.1 mL, 9.87 mmol) and 5 mL of triethylamine, $PdCl_2(PPh_3)_2$ (0.115 g, 0.16 mmol) and copper iodide (0.062 g, 0.32 mmol) were added in a pressure tube under an argon atmosphere. Subsequently, a mixture of 1,4-dioxane (5 mL) and complex **3b** (2.0g, 3.29 mmol) was added to the reaction vessel. The reaction mixture was heated at 50 °C with stirring. The progress of the reaction was monitored by TLC on silica using a 3:1 ethyl acetate/chloroform mixture as a mobile phase. Based on TLC data, the reaction took 5 hours. Upon completion, the reaction mixture was extracted with ethyl acetate and distilled water. The organic phase was dried over anhydrous $MgSO_4$ to remove residual moisture and filtered through filter paper. The filtrate was concentrated to dryness under reduced pressure and purified by column chromatography.

Complex 5b



5b

Yield 82% (1.85g), Mp 103^{0}C, $[\alpha]_D^{20}$ +1937.83^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{40}H_{40}N_4NiO_3$ (683.46) C 70.29; H 5.90; N 8.20; Found: C 70.55; H 6.33; N 8.28: MS, *m*/*z*: 683.10.

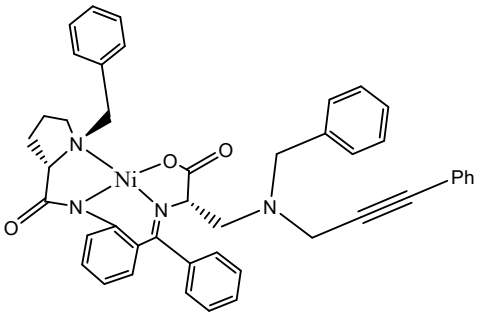
^{1}H NMR: (400 MHz, $CDCl_3$) δ=0.86 (3H, t, J=7.3, CH_3), 1.43–1.62 (2H, m, $CH_3\underline{CH_2}$), 2.00–2.16 (2H, m, 1H γ-Pro, 1H δ-Pro), 2.47– 2.63 (3H, m, 1H β-Pro, 2H $CH_3CH_2\underline{CH_2}$), 2.66–2.79 (1H, m, β-Pro), 3.08 – 3.29 (2H, m, $CH\underline{CH_2}$), 3.47 (1H, dd, J=11.0, 5.8, γ-Pro), 3.50 – 3.59 (2H, m, 1H, $CC\underline{CH_2}$, 1H δ-Pro), 3.64 (1H, d, J = 17.6, $N\underline{CH_2}Ph$), 3.78 (1H, d, J = 17.5, $N\underline{CH_2}Ph$), 3.82–3.94 (1H, m, CH α-Pro), 4.01 (1H, t, J = 5.1, $CH_2\underline{CH}$), 4.44 (1H, d, J = 12.6, $CC\underline{CH_2}$), 6.59 – 6.70 (2H, m, Ar), 7.05 (1H, d, J = 7.7, Ar), 7.09 – 7.21 (2H, m, Ar), 7.21 – 7.28 (2H, m, Ar), 7.28 – 7.52 (10H, m, Ar), 8.19 (1H, d, J = 8.6, Ar), 8.09 (2H, d, J = 7.4, Ar).

^{13}C NMR: (101 MHz, $CDCl_3$) δ=11.9(CH_3), 21.0($CH_3\underline{CH_2}$), 23.7(γ-CH_2 Pro), 30.9(β-CH_2 Pro), 45.6($N\underline{CH_2}CC$), 57.3($N\underline{CH_2}CH$), 57.3(δ- CH_2 Pro), 58.4($N\underline{CH_2}CH$), 63.2(CH_2Ph), 70.5(α-CH Pro), 71.3($\underline{CH}CH_2$), 85.0($C\equiv\underline{C}CH_2$), 85.6($\underline{C}\equiv CCH_2$), 120.7(Ar), 123.2(Ar), 123.7(Ar), 126.5(Ar), 127.4(Ar), 128.2(Ar), 128.4(Ar), 128.8(Ar), 128.9(Ar), 129.7(Ar), 131.6(Ar), 131.7(Ar), 132.3(Ar), 133.5(Ar), 133.5(Ar), 134.0(Ar), 142.7(Ar), 171.0(C=N), 178.6(O-C=O), 180.6(N-C=O).

Complex 5d

To a mixture of iodobenzene (1.7 mL, 15.2 mmol) and 5 mL of triethylamine, $PdCl_2(PPh_3)_2$ (0.107 g, 0.15 mmol) and copper iodide (0.058 g, 0.3 mmol) were added in a pressure tube under an argon atmosphere. Subsequently, a mixture of 1,4-dioxane (5 mL) and complex **3d** (2.0g, 3.05 mmol) was added to the reaction vessel. The reaction mixture was heated at 60 °C with stirring. The progress of the reaction was monitored by TLC on silica using a 3:1 ethyl acetate/chloroform mixture as a mobile phase. Based on TLC data, the reaction took 6 hours. Upon completion, the reaction mixture was extracted with ethyl acetate and distilled water. The organic phase was dried over anhydrous $MgSO_4$ to remove residual moisture and filtered through filter paper. The filtrate was concentrated to dryness under reduced pressure and purified by column chromatography.

Complex 5d



5d

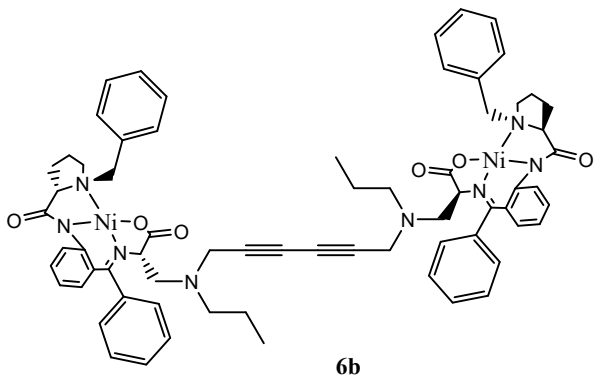
Yield 67% (1.5g), Mp 114^{0}C, $[\alpha]_D^{20}$ +1857.24^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{44}H_{40}N_4NiO_3$ (731.51) C 72.24; H 5.51; N 7.66; Found: C 72.44; H 5.83; N 7.97: MS, *m*/*z*: 730.25.

^{1}H NMR:(400 MHz, $CDCl_3$) δ=1.84-1.92 (1H, m, γ-H_a Pro.), 1.98-2.06 (1H, m, δ-H_a Pro.), 2.29-2.40 (1H, m) & 2.45-2.54 (1H, m, β-CH_2 Pro.), 3.11 (1H, dd, ^{2}J 13.8, ^{3}J 5.1, NC$\underline{H}_2$CH), 3.23 (1H, dd, ^{2}J 13.8, ^{3}J 5.3, NC$\underline{H}_2$CH), 3.32-3.45 (1H, m, γ-H_b Pro.), 3.43 (1H, dd, ^{3}J 11.0, ^{3}J 5.5, α-CH Pro.), 3.44-3.50 (1H, m, δ-H_b Pro.), 3.49 (1H, d, ^{2}J 12.6, C$\underline{H}_2$Ph), 3.53 (1H, d, ^{2}J 17.6, C$\underline{H}_2$CC-Ph), 3.69 (1H, d, ^{2}J 17.6, C$\underline{H}_2$CC-Ph), 3.75 (1H, d, ^{2}J 13.3, NC$\underline{H}_2$Ph), 3.90 (1H, d, ^{2}J 13.3, NC$\underline{H}_2$Ph), 4.00 (1H, dd, ^{3}J 5.3, ^{3}J 5.1, CH_2C$\underline{H}$), 4.41 (1H, d, ^{2}J 12.6, C$\underline{H}_2$Ph), 6.54 (1H, m, Ar), 6.57 (1H, dd, J 8.2 & 1.7, H-3 C_6H_4), 6.64 (1H, ddd, J 8.2, 6.9 & 1.1, H-4 C_6H_4), 7.12-7.21 (6H, m, Ar), 7.24-7.28 (1H, m, Ar), 7.30-7.36 (5H, m, Ar), 7.40-7.49 (6H, m, Ar), 8.06-8.10 (2H, m, orto-Ph), 8.29 (1H, dd, J 8.7 & 1.1, H-6 C_6H_4).

^{13}C NMR: 23.7 (γ-CH_2 Pro.), 30.7 (β-CH_2 Pro.), 44.4 (CH_2), 56.9 (CH_2), 57.4 (δ-CH_2 Pro.), 60.3 (CH_2), 63.1 (N$\underline{C}H_2$Ph), 70.2 (CH), 70.3 (α-CH Pro.), 84.5 & 82.2 (Acet.), 120.5 (Ar), 123.2 (Ar), 123.6 (Ar), 126.3 (Ar), 127.3 (Ar), 127.5 (Ar), 128.2 (Ar), 128.4 (Ar), 128.5 (Ar), 128.7 (Ar), 128.78 (Ar), 128.81 (Ar), 128.9 (Ar), 129.4 (Ar), 129.6 (Ar), 131.6 (Ar), 131.8 (Ar), 132.3 (Ar), 133.51 (Ar), 13.52 (Ar), 134.0 (Ar), 138.3 (Ar), 142.8 (Ar), 171.0 (Ar), 178.5 (Ar), 180.5 (Ar).

Complex 6b

To a mixture of 1,4-dioxane (5 mL) and triethylamine (5 mL), copper iodide (0.62 g, 3.29 mmol) and complex **3b** (2.0 g, 3.29 mmol) were added. The reaction mixture was stirred at 80 °C, and the progress of the reaction was monitored by TLC on silica using a 3:1 ethyl-acetate/chloroform mixture as a mobile phase. Based on TLC data, the reaction took 12 hours. Upon completion, the reaction mixture was extracted with ethyl acetate and distilled water. The organic phase was dried over anhydrous $MgSO_4$ to remove residual moisture and filtered through filter paper. The filtrate was concentrated to dryness under reduced pressure and purified by column chromatography.



6b

Complex 6b

Yield 42% (1.7g), Mp 127 ^{0}C, $[\alpha]_D^{20}$ +3764^0 (c=0.15, CH_3OH). Anal Calc. for:

$C_{68}H_{70}N_8Ni_2O_6$ (1212.72) C 67.35; H 5.82; N 9.24; Found: C 67.58; H 5.94; N 9.28: MS, *m*/*z*: 606.95.

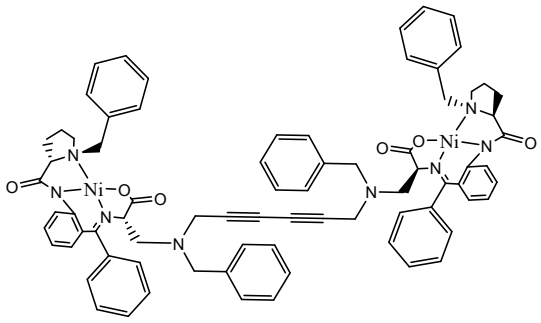
^{1}H NMR: (400 MHz, $CDCl_3$) δ=0.84 (6H, t, *J* = 7.4, CH_3), 1.36 – 1.60 (4H, m, $CH_3\underline{CH_2}$), 1.99–2.10 (m, 2H, γ-Pro), 2.10–2.21 (2H, m, δ-Pro), 2.44–2.61 (6H, m, 2H, β-Pro, 4H $CH_3CH_2\underline{CH_2}$), 2.61–2.77 (2H, m, β-Pro), 3.04–3.20 (4H, m, $\underline{CH_2}CH$), 3.35–3.66 (10H, m, 2H δ-Pro, 2H γ-Pro, 2H $CC\underline{CH_2}$, 4H $N\underline{CH_2}Ph$), 3.73–3.90 (2H, m, CH α-Pro), 3.96 (2H, t, *J* = 5.2, $CH_2\underline{CH}$), 4.41 (2H, d, *J* = 12.6, $CC\underline{CH_2}$), 6.59 – 6.69 (4H, m, Ar), 6.99 (2H, dd, *J* = 7.1, 1.9, Ar), 7.07–7.26 (6H, m, Ar), 7.33 (4H, t, *J* = 7.6, Ar), 7.38–7.55 (6H, m, Ar), 8.04–8.13 (4H, m, Ar), 8.18 (2H, d, *J* = 8.7, Ar).

^{13}C NMR: (101 MHz, $CDCl_3$) δ=11.8(CH_3), 21.0($CH_3\underline{CH_2}$), 23.7(γ-CH_2 Pro), 30.9(β-CH_2 Pro), 45.3($N\underline{CH_2}CC$), 57.3($N\underline{CH_2}CH$), 57.3(δ- CH_2 Pro), 58.3($N\underline{CH_2}CH_2$), 63.2(CH_2Ph), 69.8(α-CH Pro), 70.4($\underline{CH}CH_2$), 70.9($\underline{C}{\equiv}CCH_2$), 74.2 $C{\equiv}\underline{C}CH_2$), 120.7(Ar), 123.6(Ar), 126.4(Ar), 127.4(Ar), 128.7(Ar), 128.9(Ar), 128.9(Ar), 129.8(Ar), 131.6(Ar), 132.3(Ar), 133.5(Ar), 133.5(Ar), 133.9(Ar), 142.7(Ar), 171.0(C=N), 178.3(O-C=O), 180.5(N-C=O).

Complex 6d

To a mixture of 1,4-dioxane (5 mL) and triethylamine (5 mL), copper iodide (0.58 g, 3.05 mmol) and complex **3d** (2.0 g, 3.05 mmol) were added. The reaction mixture was stirred at 80 °C, and the progress of the reaction was monitored by TLC on silica using a 3:1 ethyl-acetate/chloroform mixture as a mobile phase. Based on TLC data, the reaction took 18 hours. Upon completion, the reaction mixture was extracted with ethyl acetate and distilled water. The organic phase was dried over anhydrous $MgSO_4$ to remove residual moisture and filtered through filter paper. The filtrate was concentrated to dryness under reduced pressure and purified by column chromatography.

Complex 6d



6d

Yield 36% (1.45g), Mp 158^{0}C, $[\alpha]_D^{20}$ +1998.72^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{76}H_{70}N_8Ni_2O_6$ (1308.81) C 69.74; H 5.39; N 8.56; Found: C 70.04; H 5.66; N 8.96: MS, *m*/*z*: 654.95.

^{1}H NMR: ($CDCl_3$): δ=1.86-1.97 (1H, m, γ-H_a Pro); 1.98-2.05 (1H, m, δ-H_a Pro); 2.28-2.54 (2H, m, β-

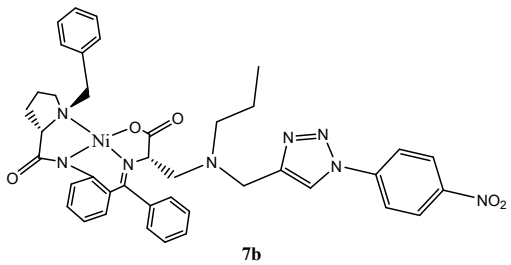
CH_2 Pro), 3.01 (1H, dd, J=13.8, 5.2, $\underline{CH_2}CH$), 3.14 (1H, dd, J=13.8, 5.0, $\underline{CH_2}CH$), 3.31-3.58 (6H, m), 3.62-3.72 (1H, m, $\underline{CH_2}Ph$), 3.87 (1H, d, J= 13.2, $\underline{CH_2}Ph$), 3.96 (1H, t, J= 5.1, CH), 4.40 (1H, t, J=12.6, $\underline{CH_2}Ph$), 6.4 (1H, d, J= 7.6, C_6H_5), 6.56 (1H, dd, J=8.2, 1.7, C_6H_4), 6.64 (1H, ddd, J=8.1, 6.9, 1.2, C_6H_4), 7.08-7.20 (6H, m, Ar), 7.30-7.39 (3H, m, Ar), 7.40-7.50 (4H, m, Ar), 8.05-8.11 (2H, m, Ar), 8.28 (1H, dd, J=8.7, 1.1, Ar).

^{13}C NMR: ($CDCl_3$) δ =23.8 (γ- CH_2 Pro), 30.7(β- CH_2 Pro), 44.4 (CH_2), 56.6(CH_2), 57.5 (δ-CH_2 Pro), 60.4(CH_2), 63.2(CH_2Ph), 70.0 (CH), 70.2 (α-CH Pro), 70.5 ($\underline{C}\equiv CH$), 73.9 (≡CH), 120.6 (Ar), 123.6 (Ar), 126.2 (Ar), 127.2 (Ar), 127.6 (Ar), 128.6 (Ar), 128.6 (Ar), 128.8 (Ar), 128.9 (Ar), 129.3 (Ar), 129.7 (Ar), 131.6 (Ar), 132.3 (Ar), 133.5 (Ar), 133.6 (Ar), 133.9 (Ar), 138,0 (Ar), 142.9 (Ar), 171.1 (Ar), 178.4 (Ar), 180.6 (Ar).

Complex 7b

1-Azido-4-nitrobenzene (1.08 g, 6.5 mmol) was dissolved in 5 mL of 1,4-dioxane in a round-bottom flask under an argon atmosphere at room temperature. Copper iodide (0.062 g, 0.3 mmol) was added to the solution, and the mixture was stirred for 5 minutes. Triethylamine (1.14 mL, 8.2 mmol) was then added, and stirring was continued for 30 minutes. Subsequently complex **3b** (2.0 g, 3.29 mmol) was added and the reaction mixture was stirred at 60 °C. The progress of the reaction was monitored by TLC on silica using a 3:1 ethyl-acetate/chloroform mixture as a mobile phase. After completion, the reaction mixture was extracted with ethyl acetate and distilled water. The organic layer was dried over anhydrous $MgSO_4$ to remove any residual water, and then filtered through filter paper. The filtrate was concentrated to dryness under reduced pressure, and the residue was crystallized from acetone to yield the purified product.

Complex 7b



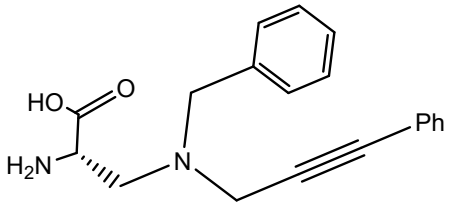
7b

Yield 70% (1.78g), Mp 132^{0}C, $[\alpha]_D^{20}$ +2017.9^0 (c=0.15, CH_3OH). Anal Calc. for: $C_{40}H_{40}N_8NiO_5$ (771.49) C 62.27; H 5.23; N 14.52; Found: C 62.58; H 5.63; N 14.89: MS, *m*/*z*: 771.10.

^1H NMR ($CDCl_3$) δ=0.76 (3H, t, J=7.3, CH_3), 1.29-1.46 (2H, m, $\underline{CH_2}CH_3$), 1.85-1.97 (1H, m, δ-H_a Pro), 1.97-2.09 (1H, m, γ- H_a Pro), 2.27-2.53 (3H, m, $N\underline{CH_2}C_2H_5$, β- H_a Pro), 2.58-2.72 (1H, m, β- H_b Pro), 2.92 (1H, dd, J= 13.5, 4.8, $N\underline{CH_2}CH$), 3.23

(1H, dd, J= 10.6, 6.3, N$\underline{CH_2}$CH), 3.36-3.55 (4H, m), 3.71-3.86 (2H, m), 3.90 (1H, dd, J=8.1, 4.7, $\underline{CH}CH_2$), 4.33(1H, d, J= 12.6, $\underline{CH_2}$Ph), 6.54-6.66(2H, m, Ar), 6.82-6.91 (1H, m, Ar), 7.07 (1H, ddd, J=8.7, 6.6, 2.1, Ar), 7.11-7.21 (1H, m, Ar), 7.21-7.28 (1H, m, Ar), 7.28-7.37 (2H, m, Ar), 7.40-7.56 (3H, m, Ar), 7.88-7.97 (2H, m, Ar), 7.97-8.10 (3H, m, Ar), 8.27 (1H, s, Ar), 8.29-8.38 (2H, m, Ar).

^{13}C NMR ($CDCl_3$) δ=11.7 (CH_3), 19.4 ($\underline{CH_2}CH_3$), 23.8 (γ- CH_2 Pro), 30.8(β- CH_2 Pro), 50.1, 57.1(δ-CH_2 Pro), 57.3 ($\underline{CH_2}$CH), 58.5 (N$\underline{CH_2}C_2H_5$), 63.3($\underline{CH_2}$Ph), 69.9 (α-CH Pro), 70.3 ($\underline{CH}CH_2$), 120.3 (CH), 120.9 (CH), 121.8, 124.0 (CH), 125.5, 126.2, 127.6, 128.1, 128.9, 129.0, 129.9, 131.6, 132.2, 133.3, 133.6, 141.2, 142.4, 147.0, 148.0, 170.5, 178.5, 180.4.



8d

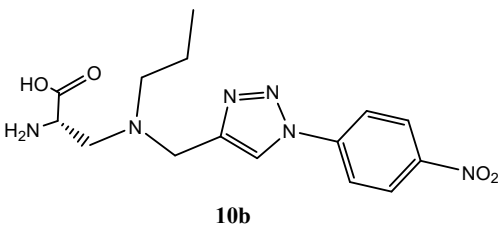
(*S*)-2-amino-3-(benzyl(3-phenylprop-2-yn-1-yl)amino)propanoic acid-8d

Yield 31% (0.3g), Mp 192^{0}C, $[\alpha]_D^{20}$ (c=0.2, 6N HCl). Anal Calc. for: $C_{19}H_{20}N_2O_2$ (308.37) C 74.00; H 6.54 N 9.08; Found: C 74.24; H 6.89; N 9.36:

^{1}H NMR (400 MHz, CD_3OD_SPE) δ=2.96 (1H_a, dd, *J*=13.9, 10.0, N$\underline{CH_2}$CH), 3.23–3.29 (1H_b, m, N$\underline{CH_2}$CH), 3.48–3.61 (2H, m, N$\underline{CH_2}$CC), 3.73–3.92 (3H, m, 2H N$\underline{CH_2}$Ph, 1H $\underline{CH}NH_2$), 4.79 (3H, br.s., 2H NH_2, 1H COOH), 7.32–7.40 (5H, m, Ar), 7.41–7.52 (5H, m, Ar).

^{13}C NMR (101 MHz, CD_3OD_SPE) δ 40.9(N$\underline{CH_2}$CC), 52.2($\underline{CH}NH_2$), 53.5(N$\underline{CH_2}$CH), 56.9(N$\underline{CH_2}$Ph), 82.4(C≡C), 85.1(C≡C), 122.1(Ar), 126.7(Ar), 127.5(Ar), 128.1(Ar), 128.4(Ar), 128.5(Ar), 130.7(Ar), 137.0(Ar), 171.2(C=O).

(*S*)-2-amino-3-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)(propyl)amino)propanoic acid-9b



10b

Yield 44% (0.5g), Mp 197^{0}C, $[\alpha]_D^{20}$ +5.94^0 (c=0.2, 6N HCl). Anal Calc. for: $C_{15}H_{20}N_6O_4$ (348.36) C 51.72; H 5.79; N 24.12; Found: C 51.97; H 6.13; N 24.31: MS, *m*/*z*: 349.0.

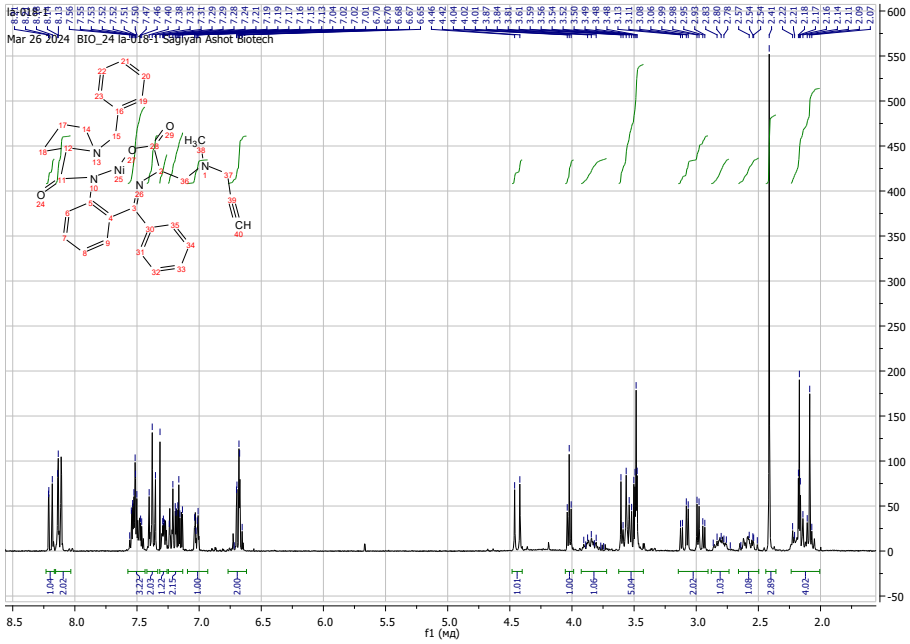
^{1}H NMR (400 MHz, DMSO+CCl_4) δ=0.90 (3H, t, J=7.3, CH_3), 1.53–1.66 (2H, m, $\underline{CH_2}CH_3$), 2.51–2.64 (2H, m, $\underline{CH_2}CH_2CH_3$), 2.91–3.06 (2H, m,

N<u>CH$_2$</u>CH), 3.88–4.10 (3H, m, 2H N<u>CH$_2$</u>Ar, 1H <u>CH</u>NH$_2$), 8.25 (2H, d, J=8.9, Ar), 8.41 (2H, d, J=9.0, Ar), 8.49–8.95 (3H, m, NH$_2$, COOH), 9.02 (s, 1H, Ar).

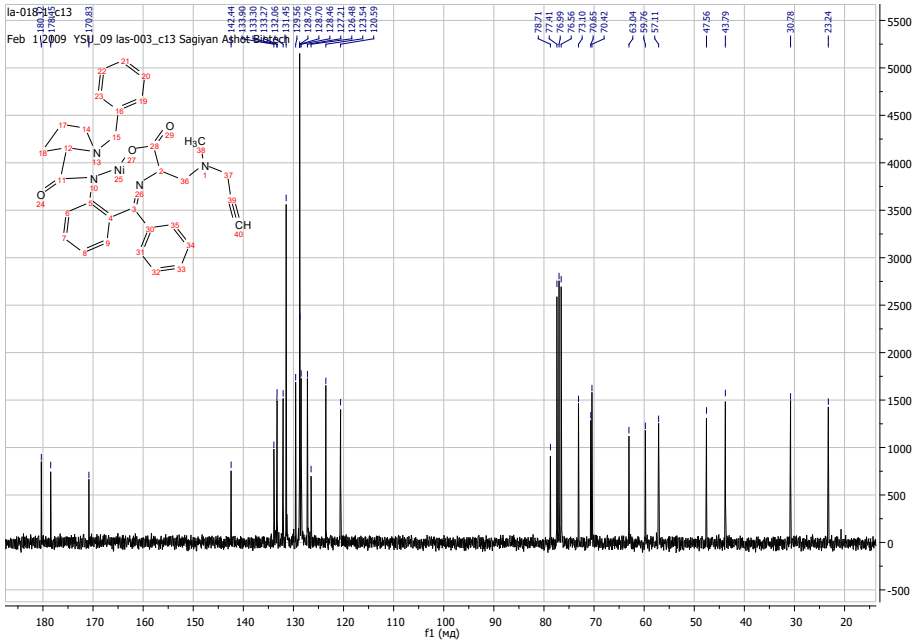
^{13}C NMR: (101 MHz, DMSO+CCl$_4$) δ=11.2 (CH$_3$), 18.9 (<u>CH$_2$</u>CH$_3$), 47.5 (N<u>CH$_2$</u>CH), 50.4 (CHNH$_2$), 52.9 (N<u>CH$_2$</u>C), 55.1 (N<u>CH$_2$</u>CH$_2$), 120.0 (Ar), 122.5 (Ar), 124.9 (Ar), 141.0 (Ar), 144.4 (Ar), 146.3 (Ar), 169.4 (C=O).

NMR Spectra

^{1}H NMR Complex 3a



^{13}C NMR Complex 3a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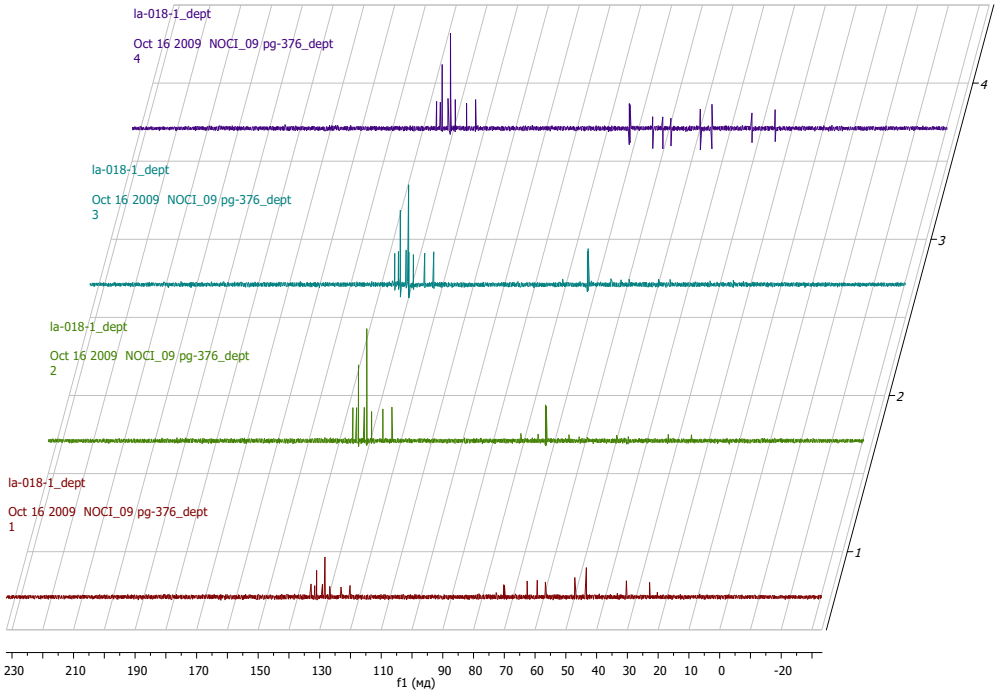
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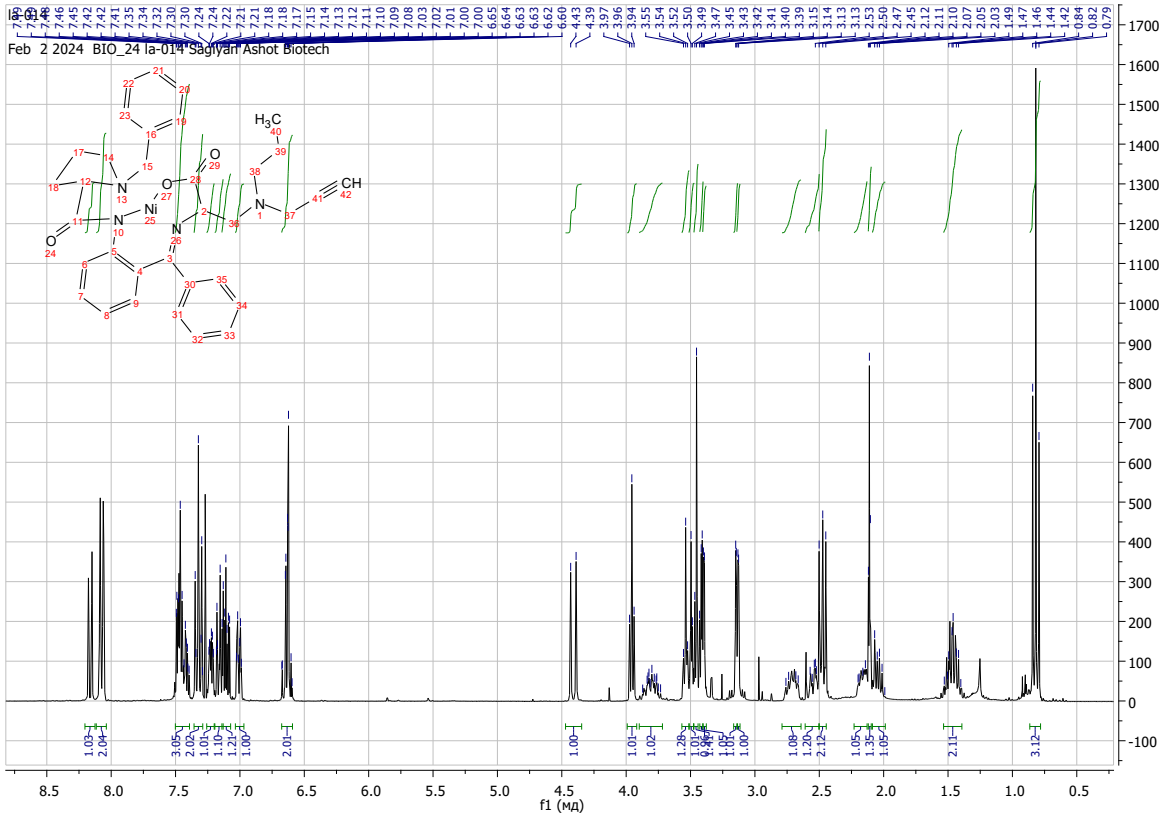
f1 (мд)

^{1}H NMR Complex 3b



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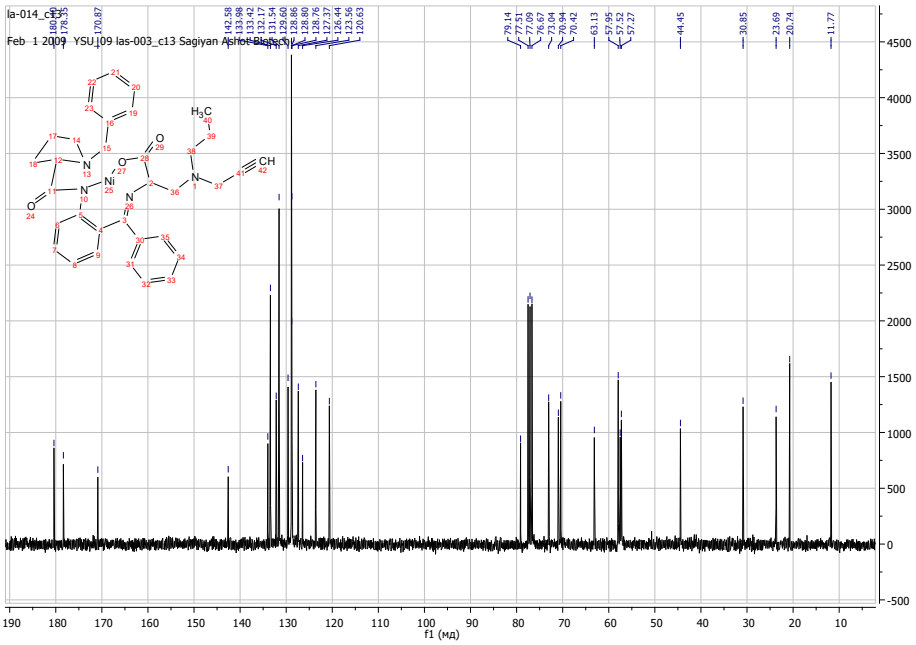

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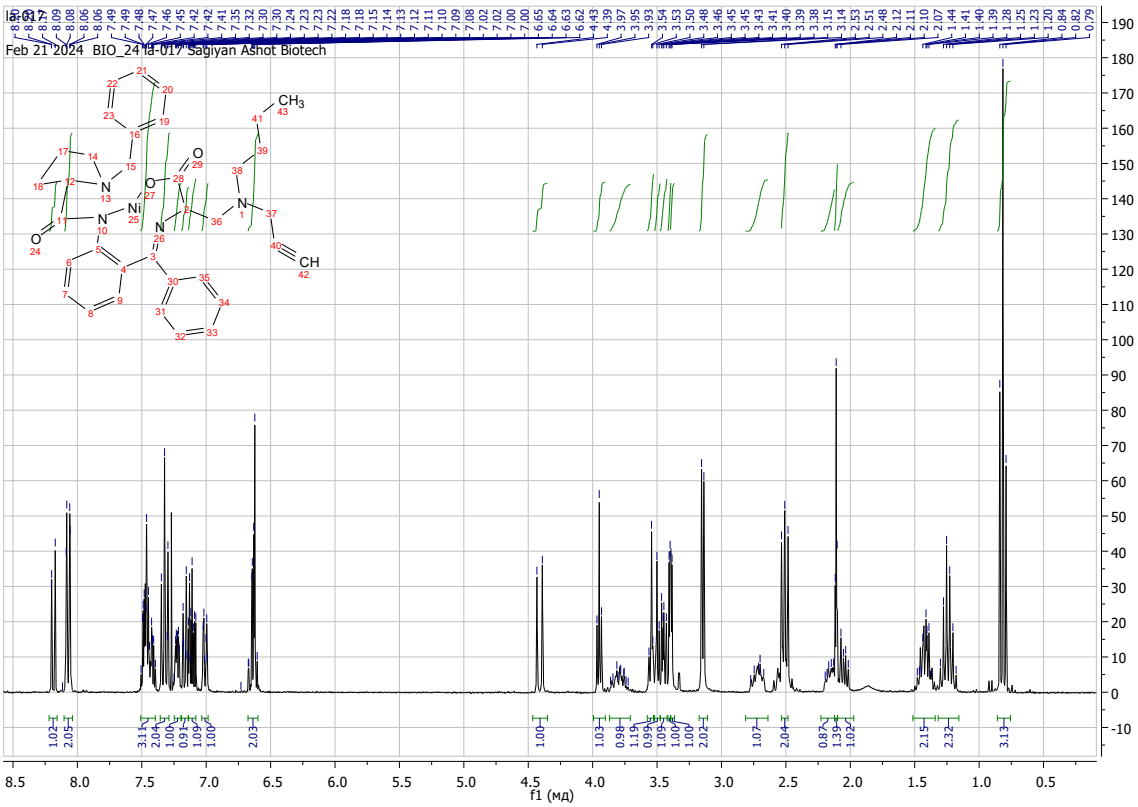

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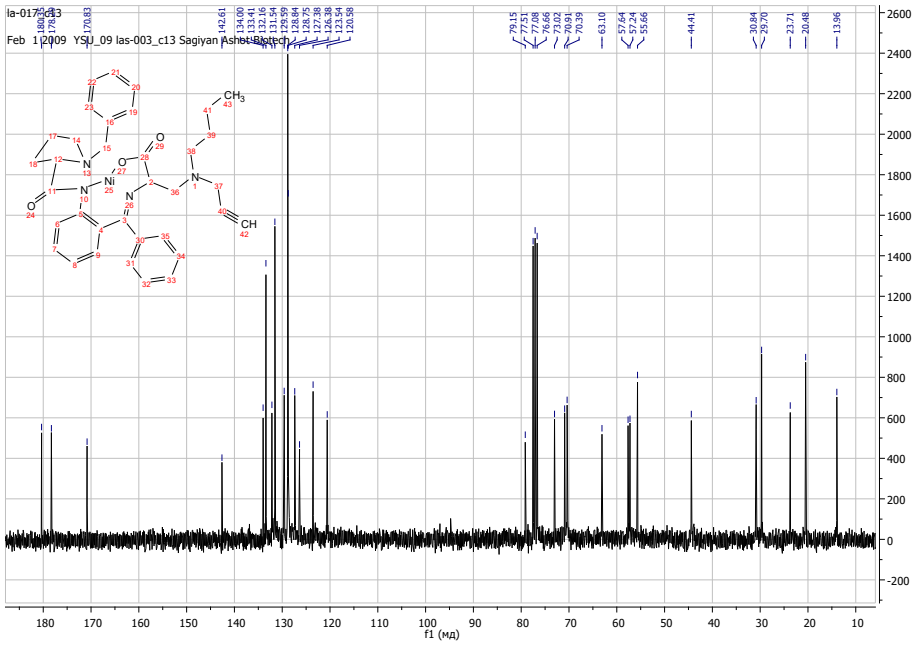
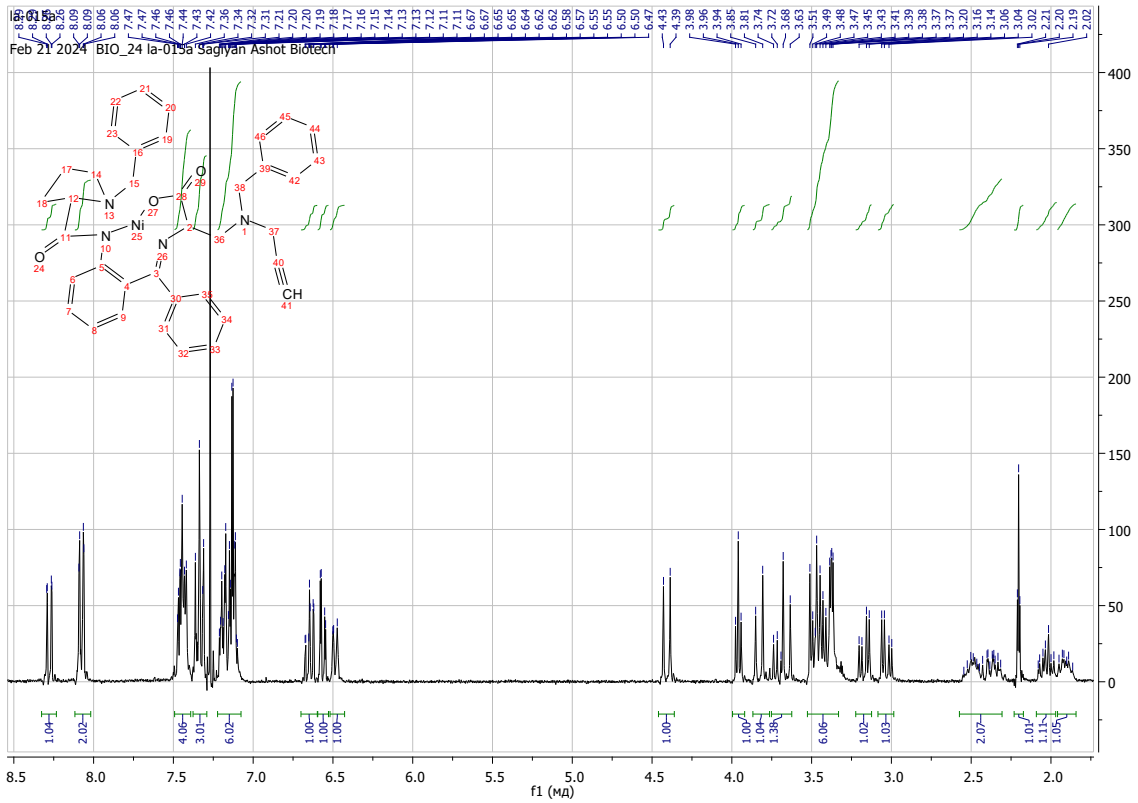


^{1}H NMR Complex 3d



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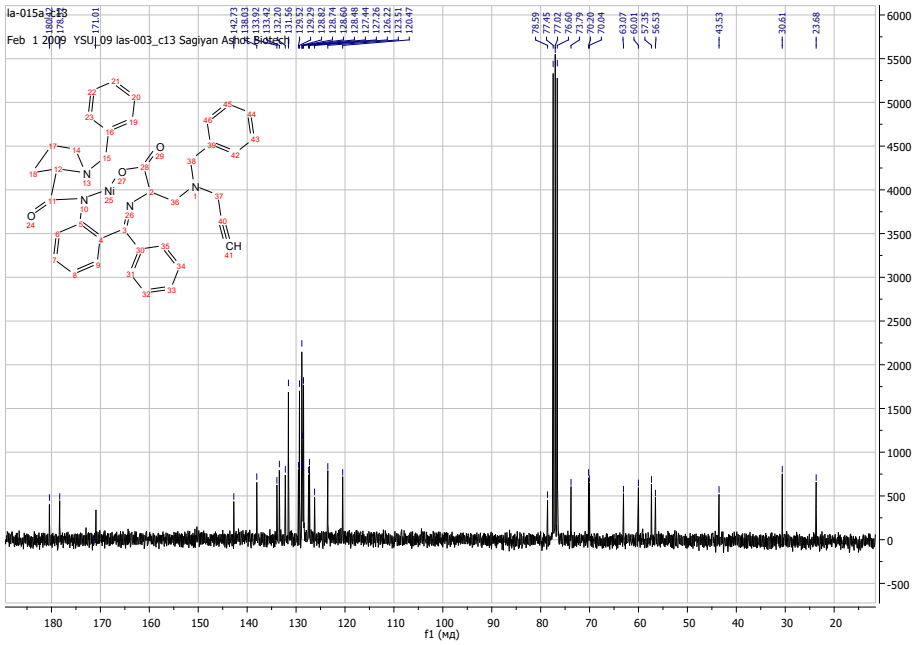

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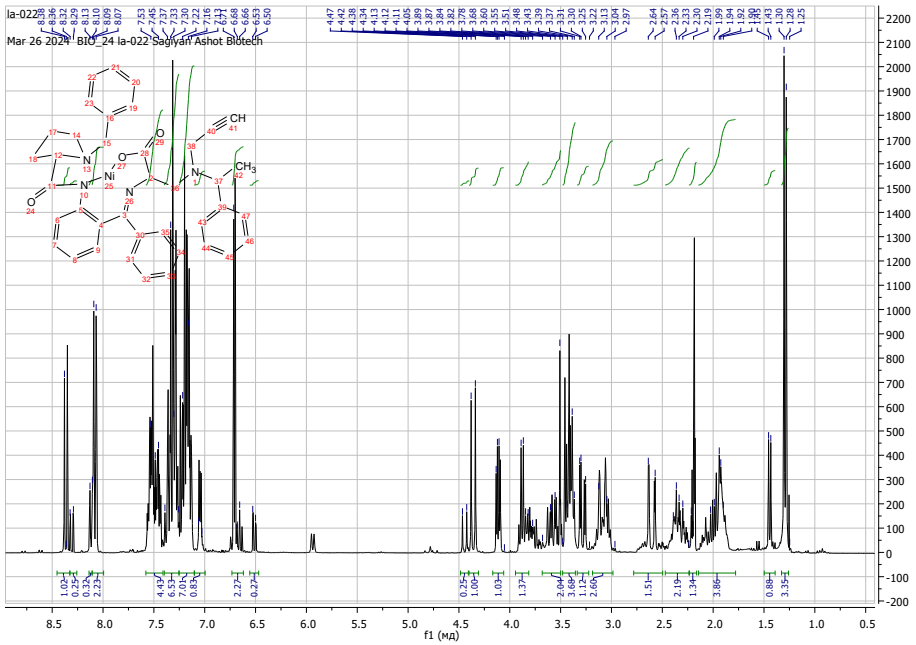
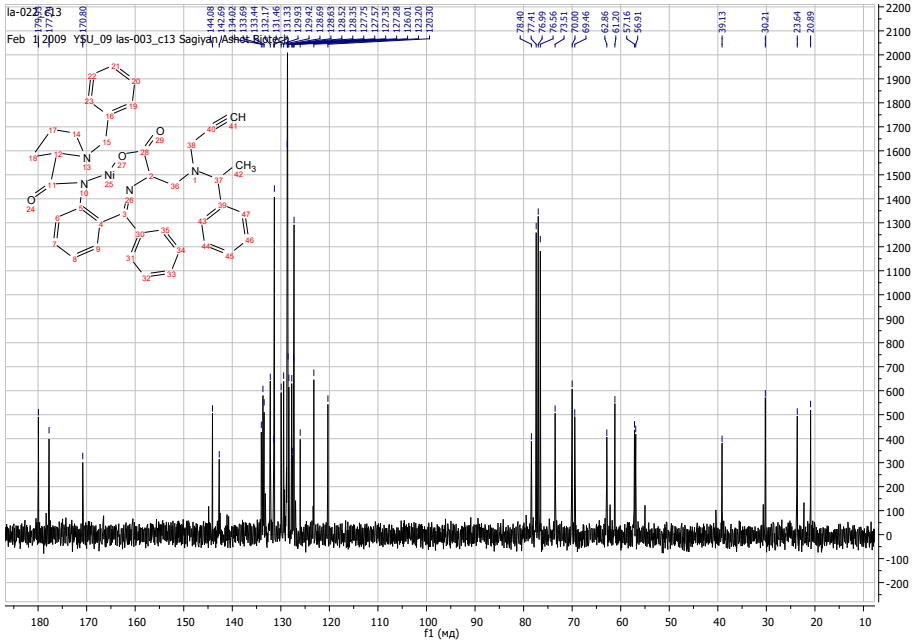
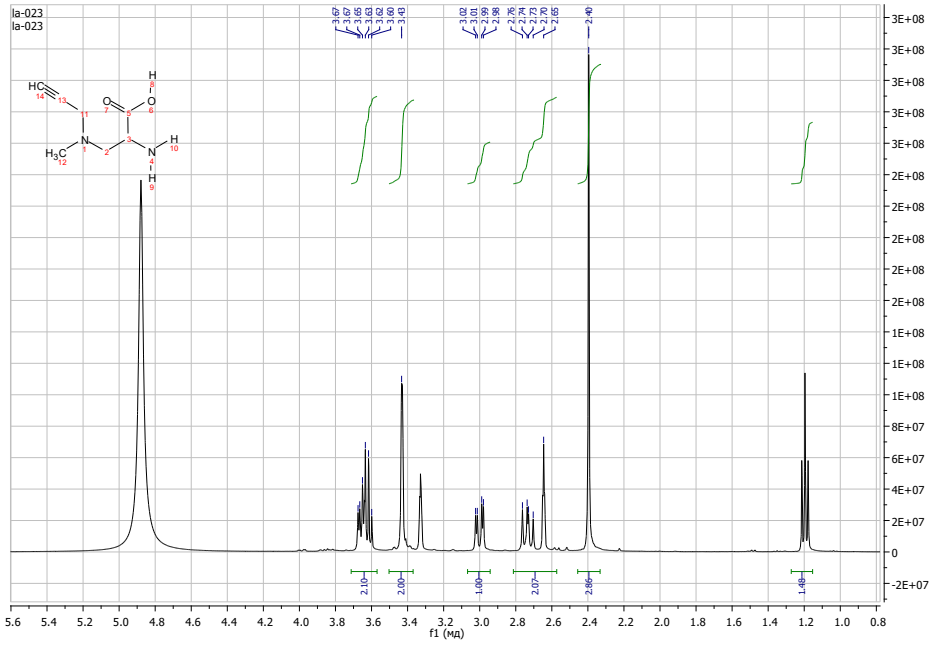


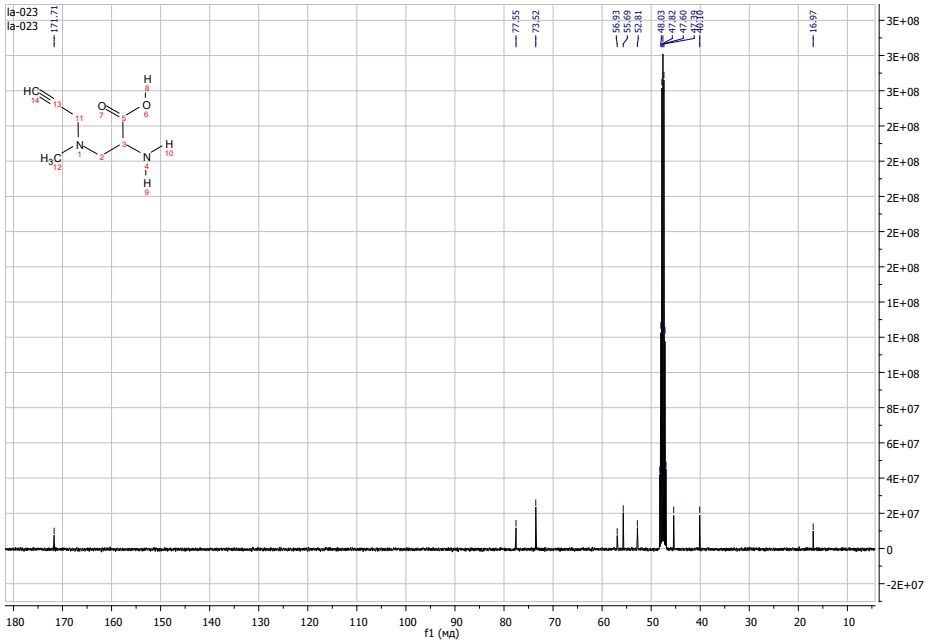
^{13}C NMR Complex 3e



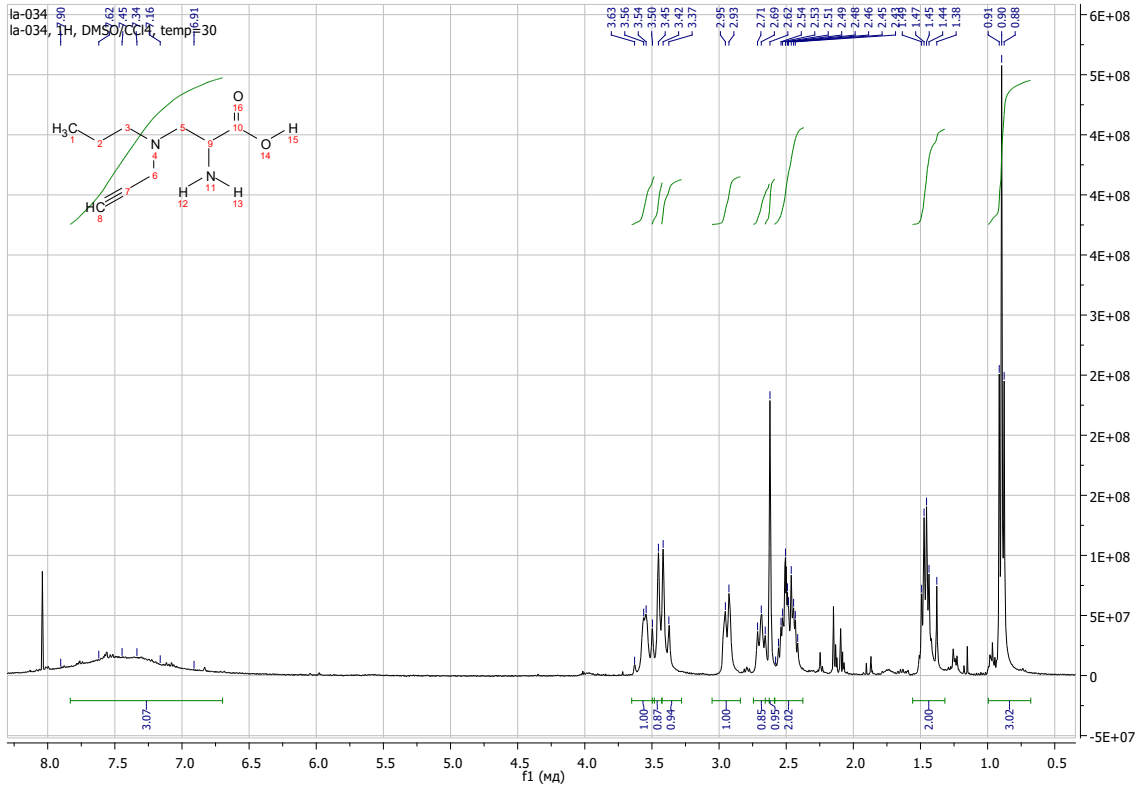
^{1}H NMR (*S*)-3-(N-methyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4a



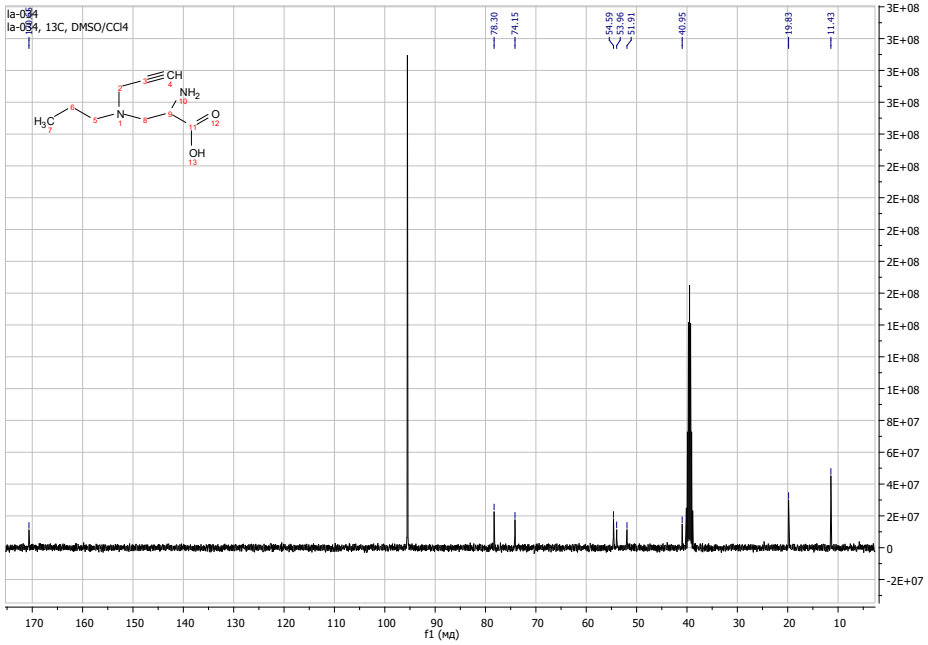
^{13}C NMR (*S*)-3-(N-methyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4a



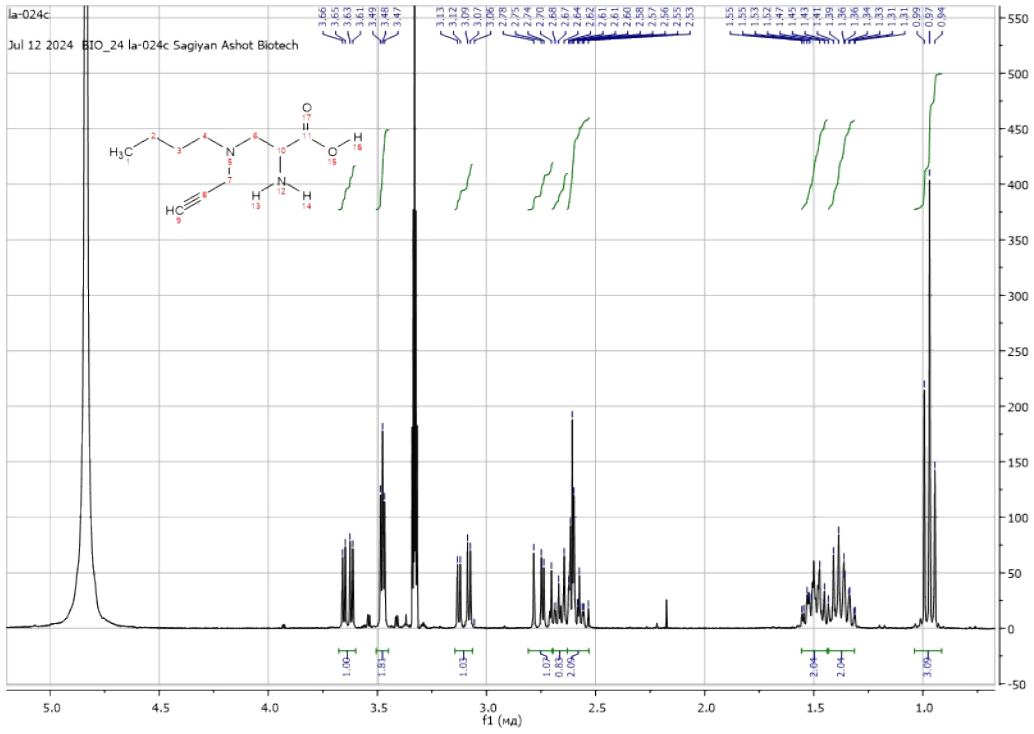
^{1}H NMR (*S*)-3-(N-(prop-2-ynyl)-N-propylamino)-2-aminopropanoic acid-4-ynoic acid-4b



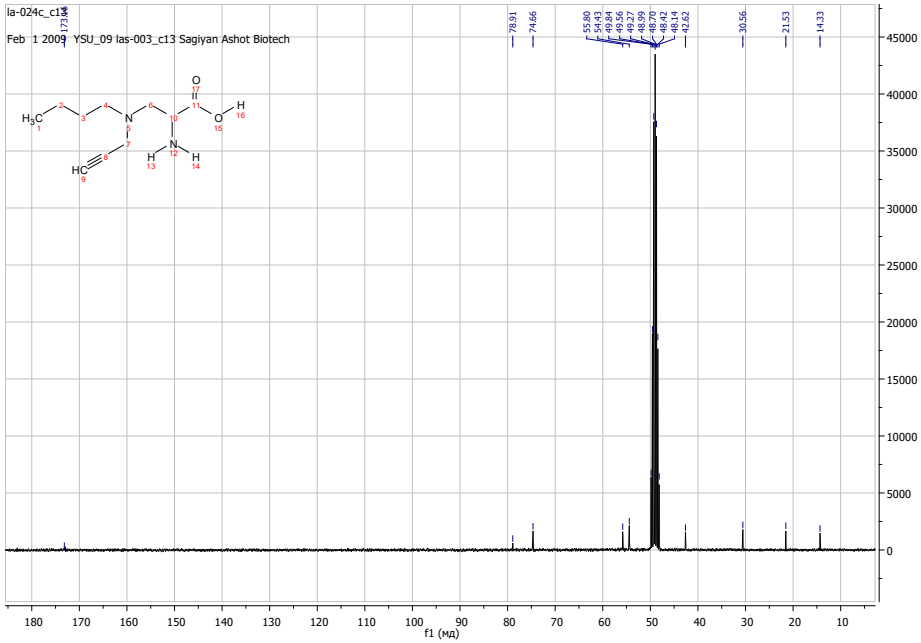
^{13}C NMR (*S*)-3-(N-(prop-2-ynyl)-N-propylamino)-2-aminopropanoic acid-4-ynoic acid-4b



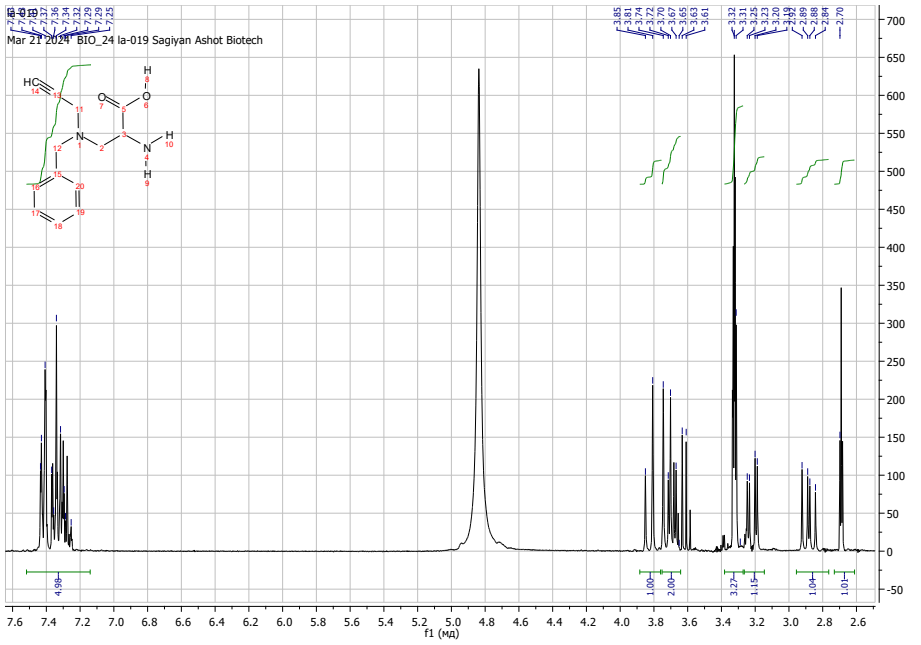
^{1}H NMR (*S*)-3-(N-butyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid -4c.



^{13}C NMR (*S*)-3-(N-butyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid -4c



^{1}H NMR (*S*)-3-(N-benzyl-N-(prop-2-ynyl)amino)-2-aminopropanoic acid-4d



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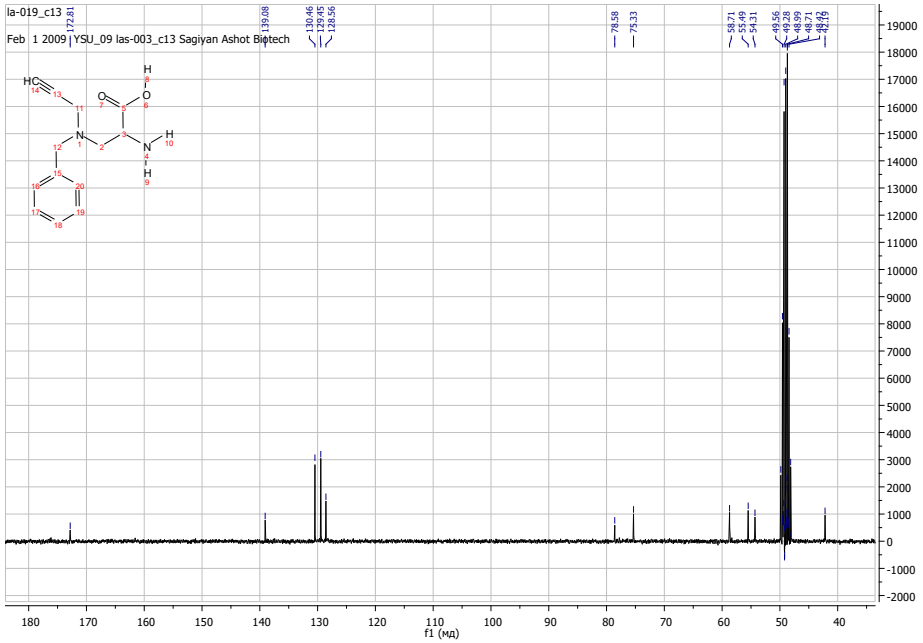
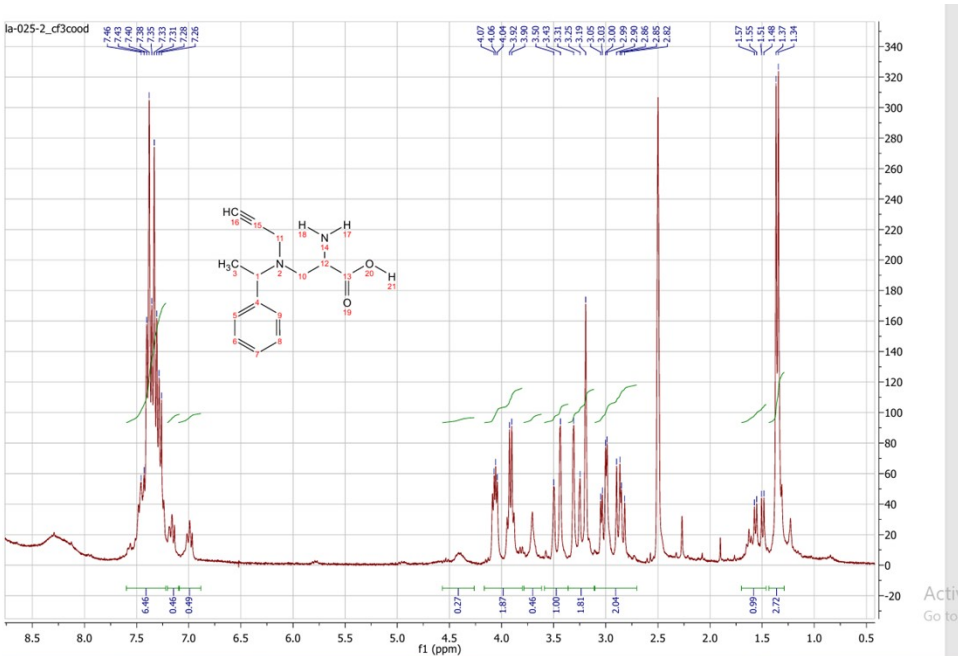
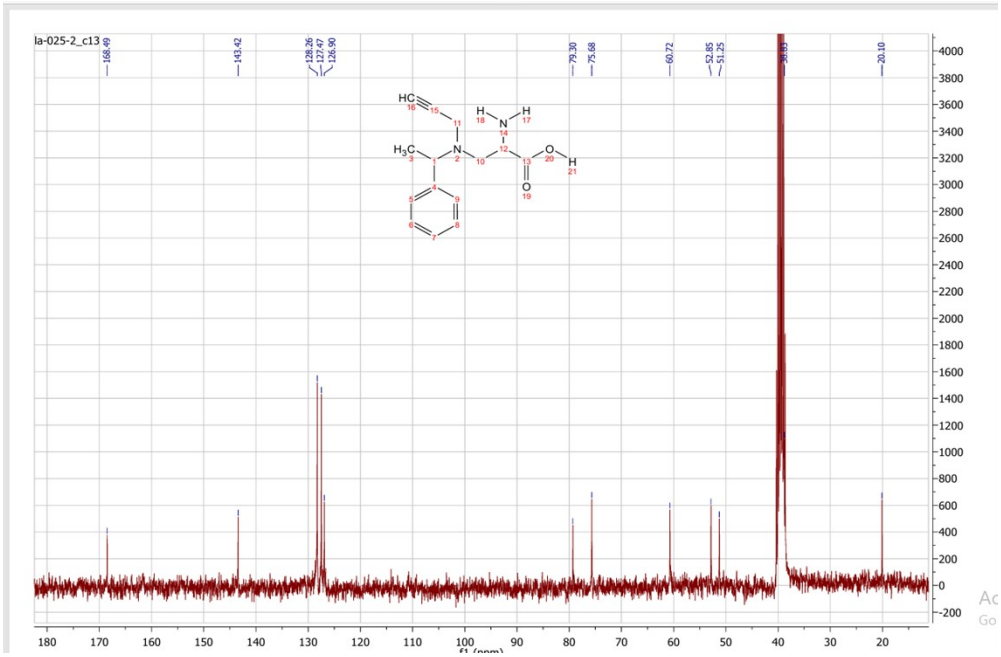


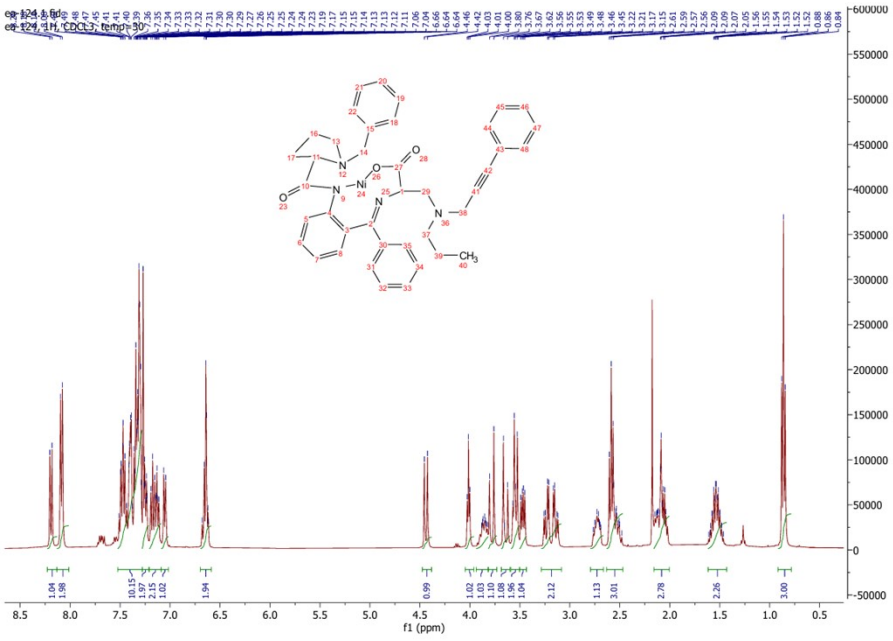
^{1}H NMR (*S*)-2-amino-3-(((*S*)-1-phenylethyl)(prop-2-yn-1-yl)amino)propanoic acid -4e



^{13}C NMR (*S*)-2-amino-3-(((*S*)-1-phenylethyl)(prop-2-yn-1-yl)amino)propanoic acid -4e



^{1}H NMR Complex 5b



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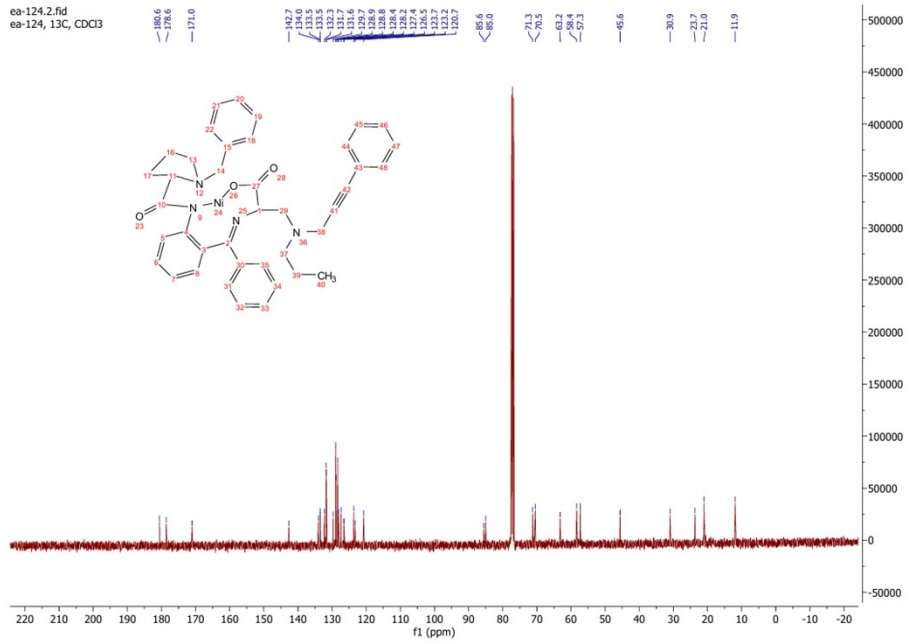
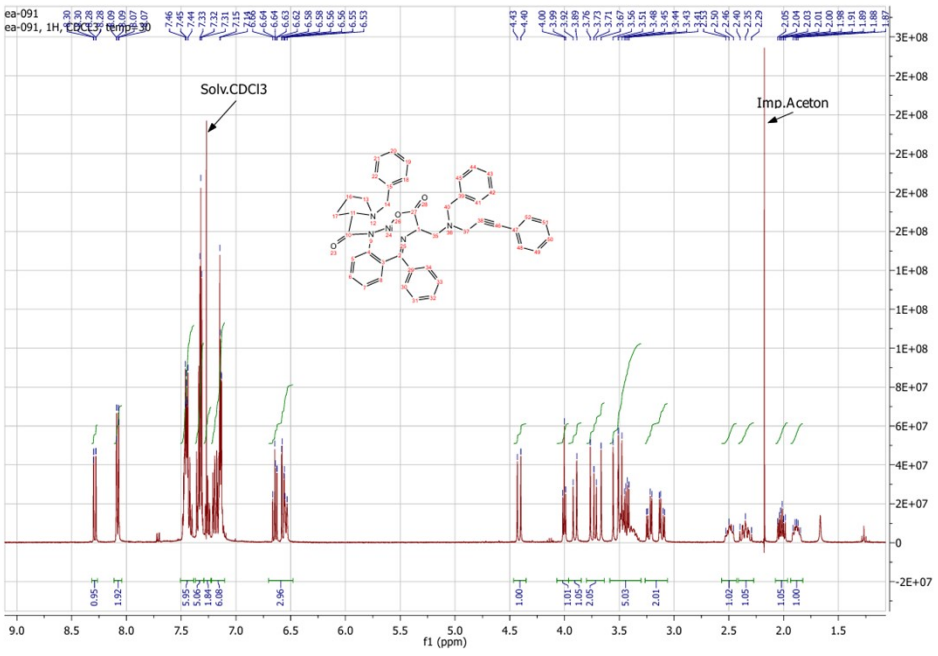


^{1}H NMR Complex 5d



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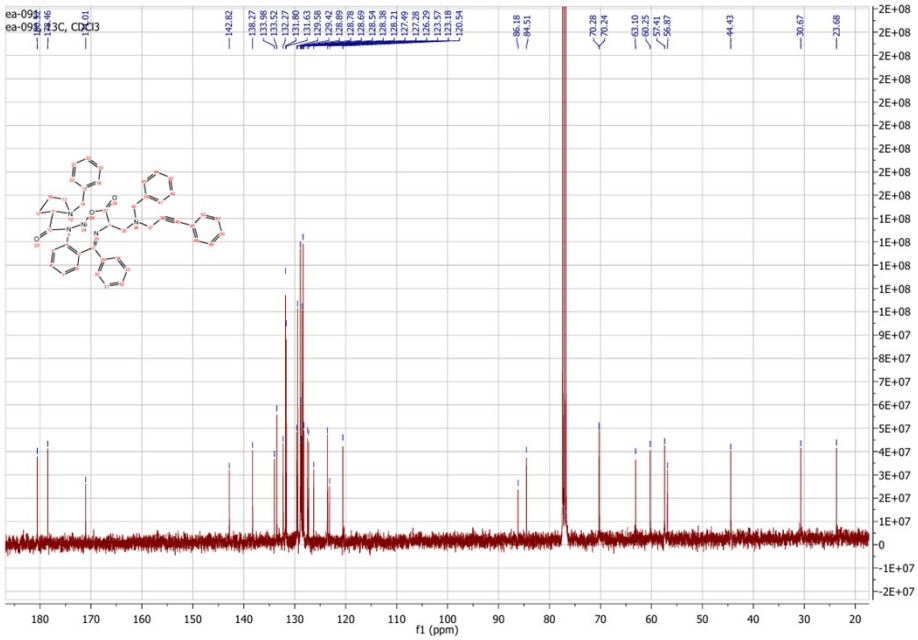
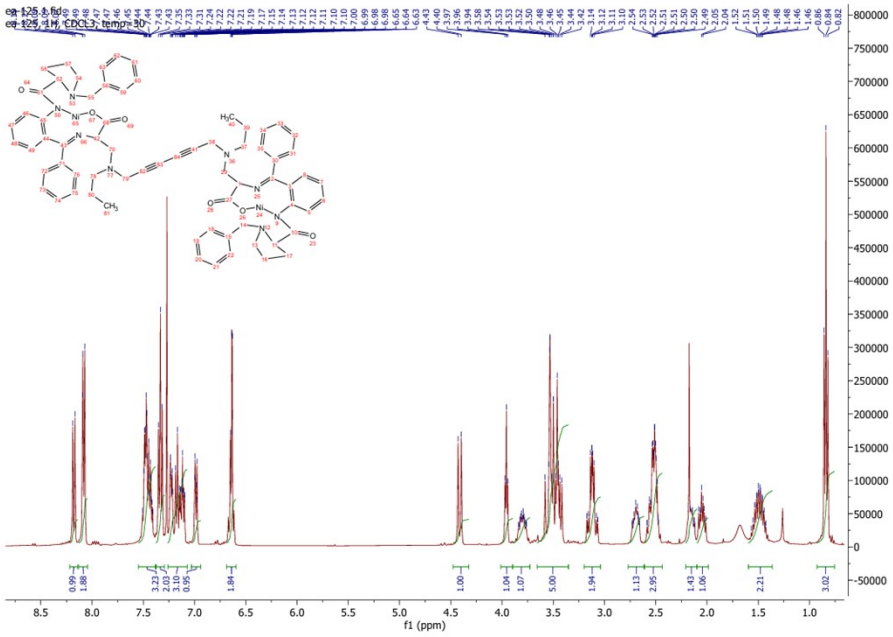


^{1}H NMR Complex 6b



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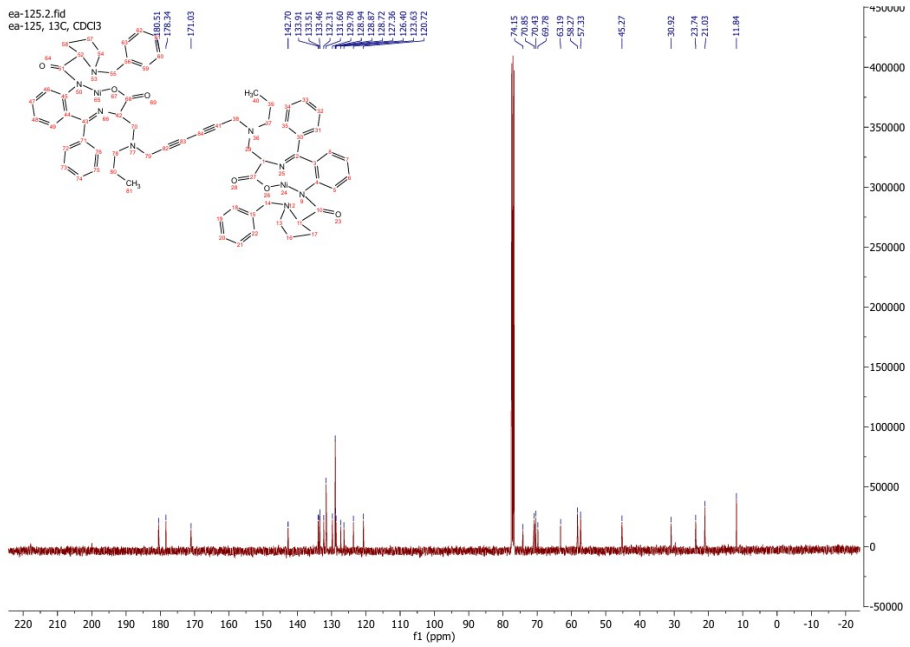
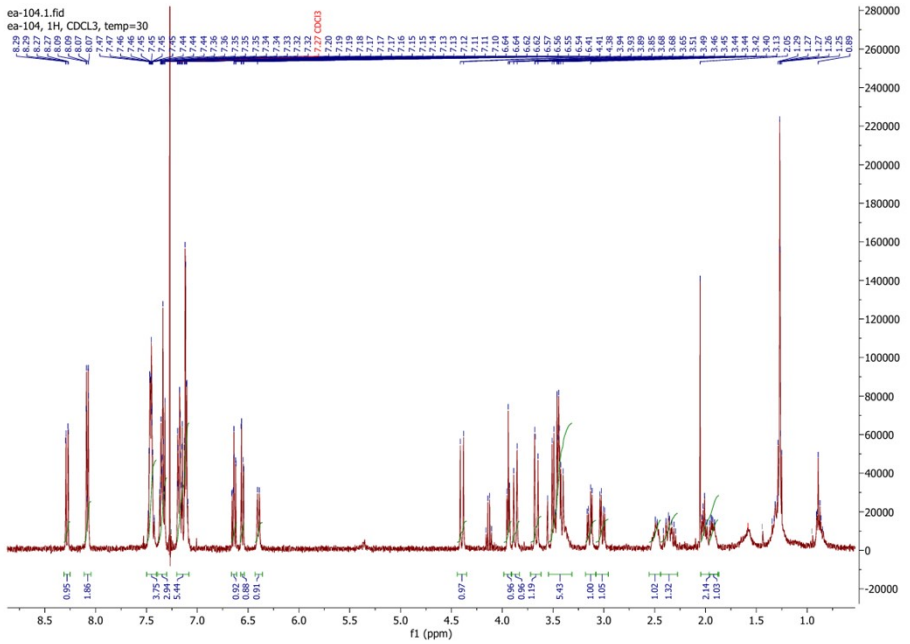


^{1}H NMR Complex 6d



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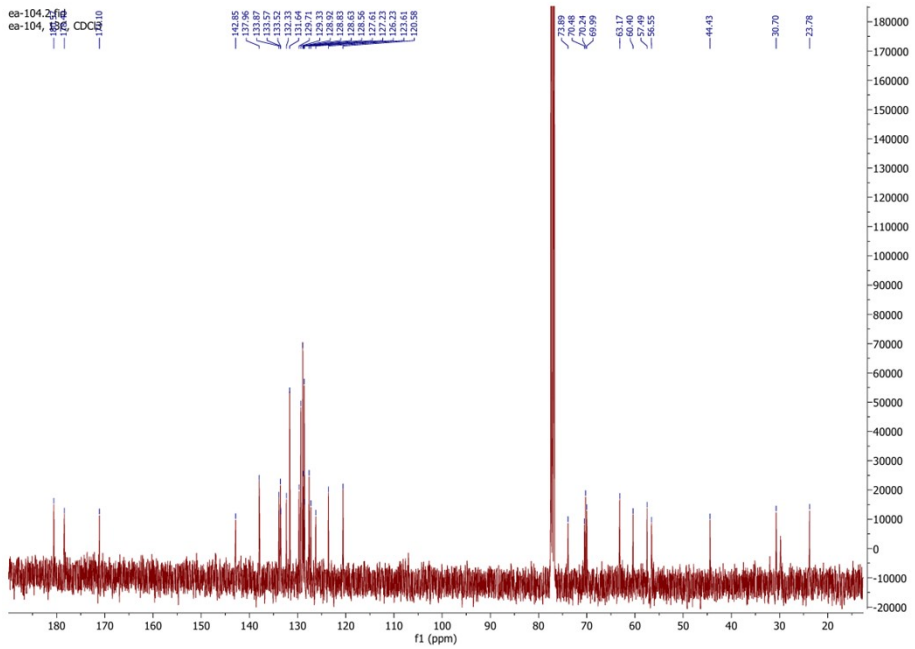
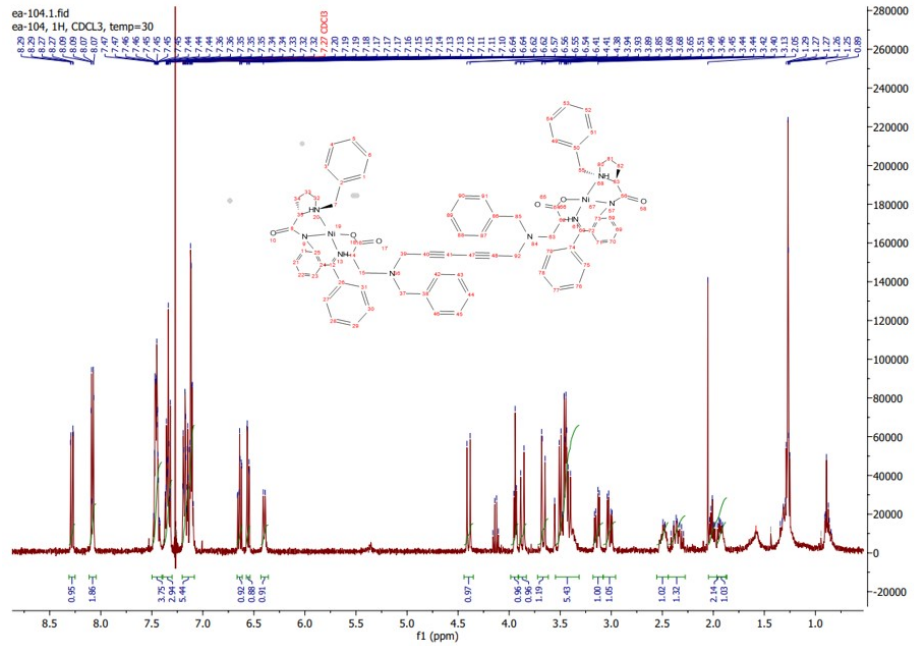


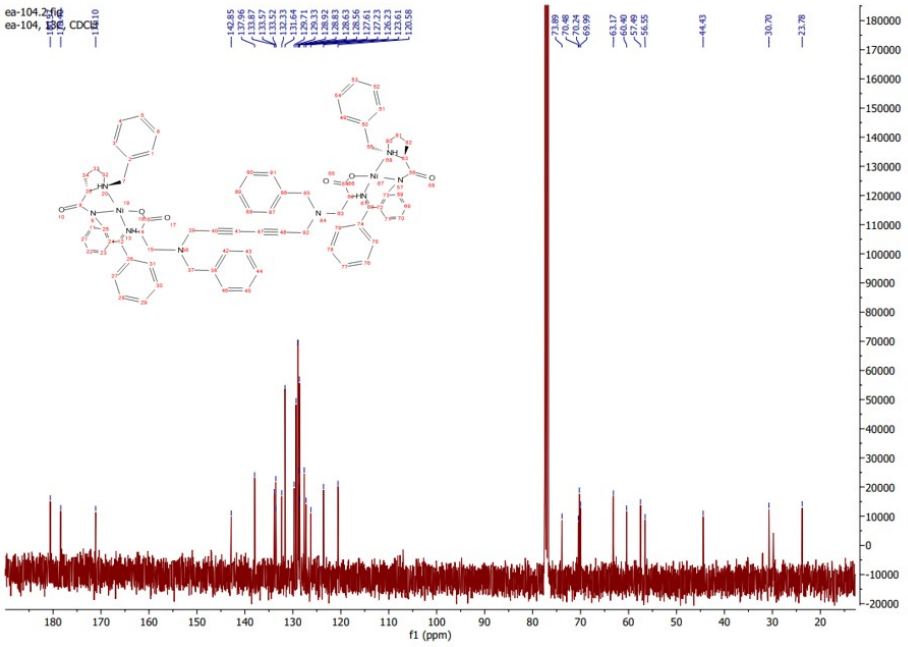
^{1}H NMR Complex 6b

^{13}C NMR Complex 6b

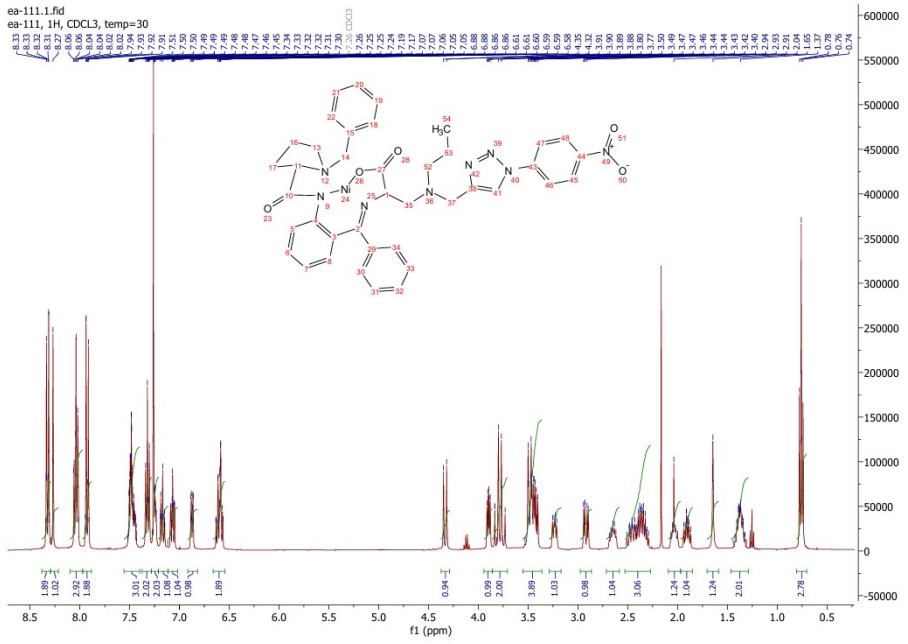
^{1}H NMR Complex 6d



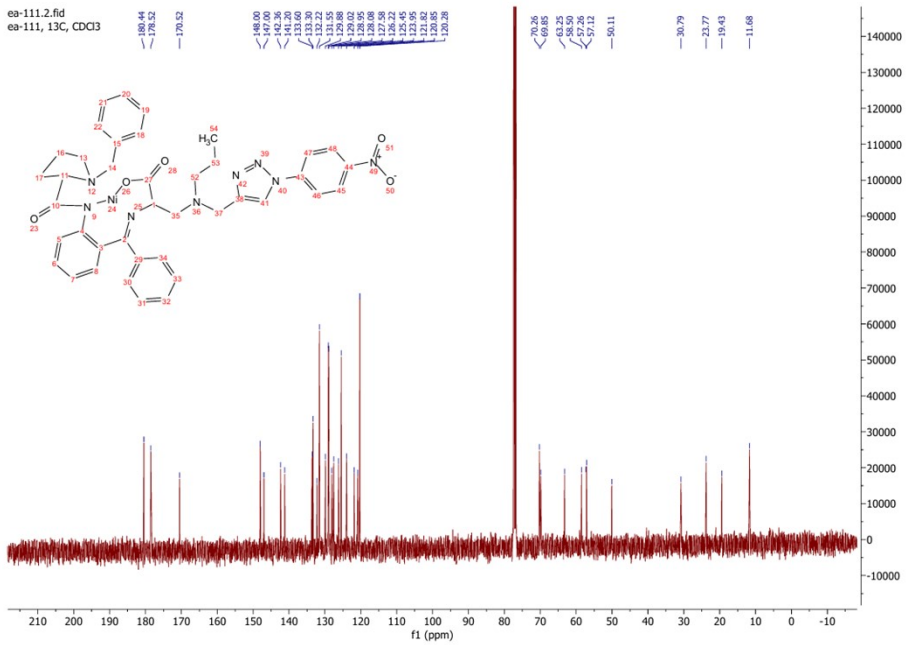
^{13}C NMR Complex 6d



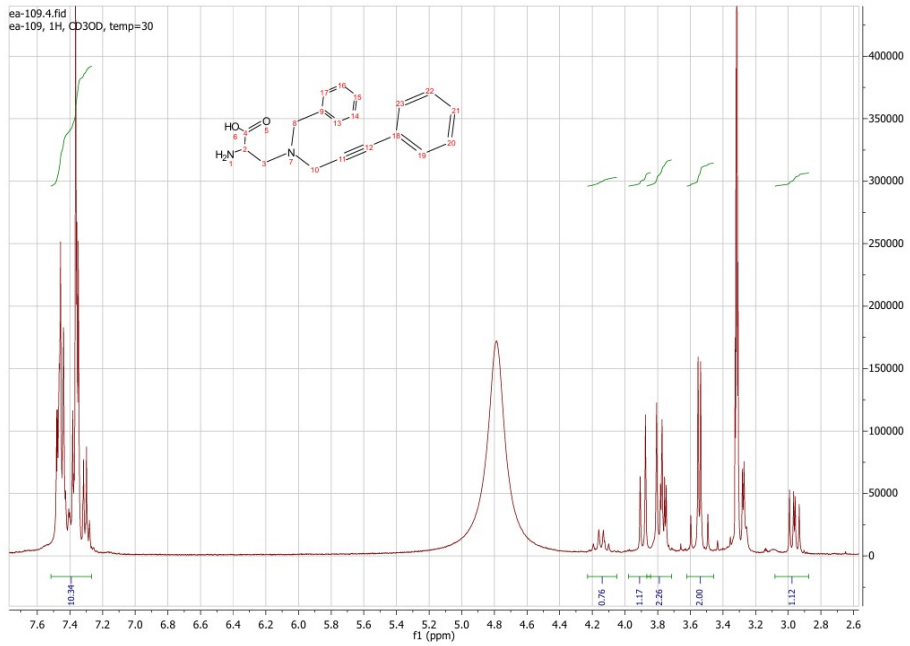
^{1}H NMR Complex 7b



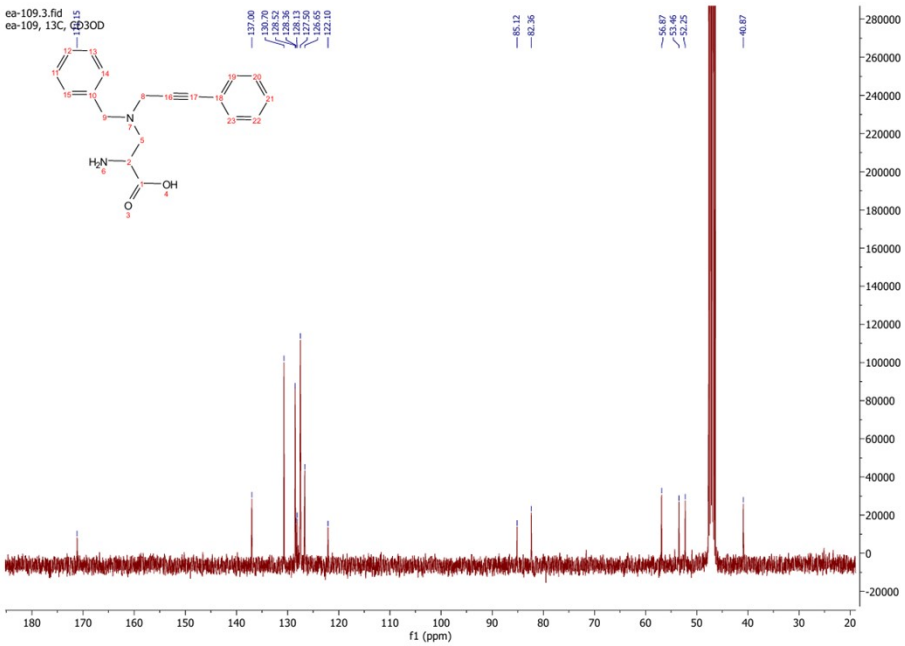
^{13}C NMR Complex 7b



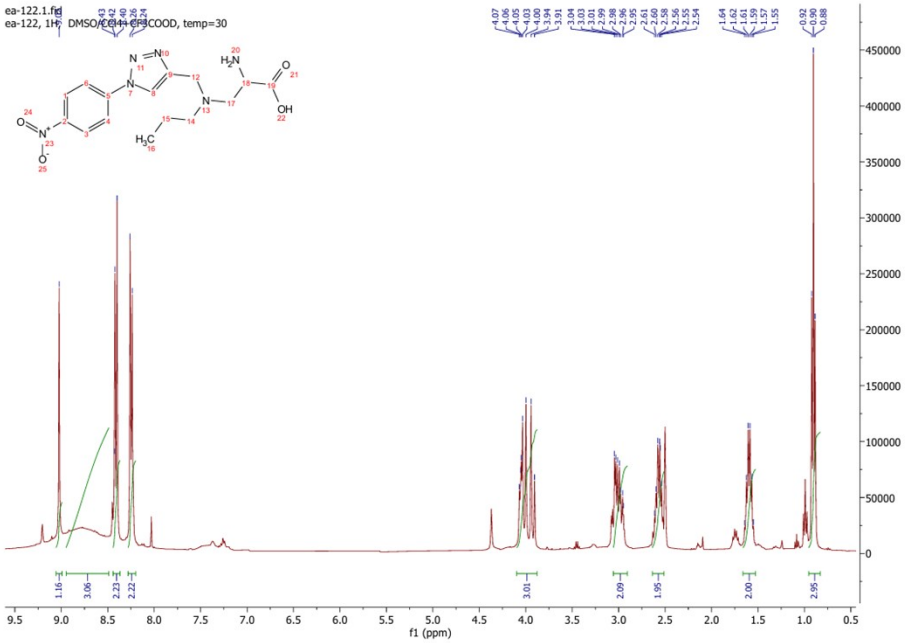
^{1}H NMR (*S*)-2-amino-3-(benzyl(3-phenylprop-2-yn-1-yl)amino)propanoic acid-8d



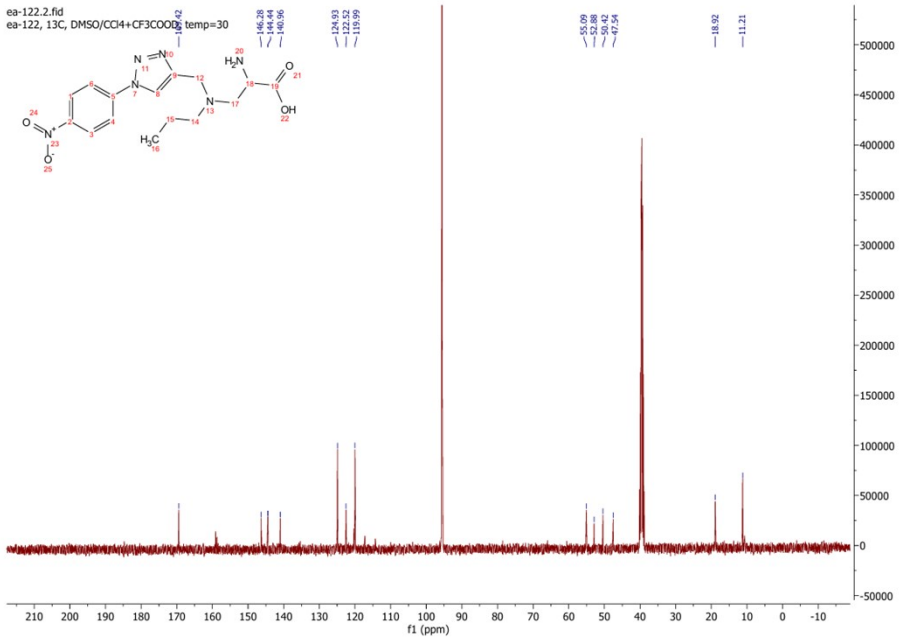
^{13}C NMR (*S*)-2-amino-3-(benzyl(3-phenylprop-2-yn-1-yl)amino)propanoic acid-8d



^{1}H NMR (*S*)-2-amino-3-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl) (propyl)amino) propanoic acid-9b



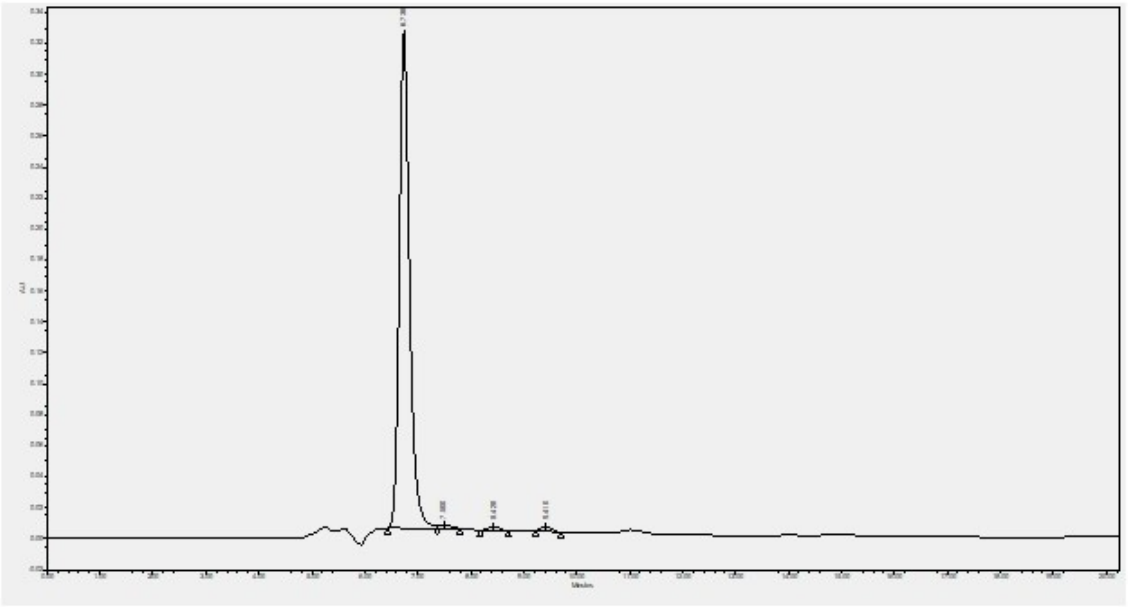
^{13}C NMR (*S*)-2-amino-3-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl) (propyl)amino) propanoic acid-10b



HPLC analysis

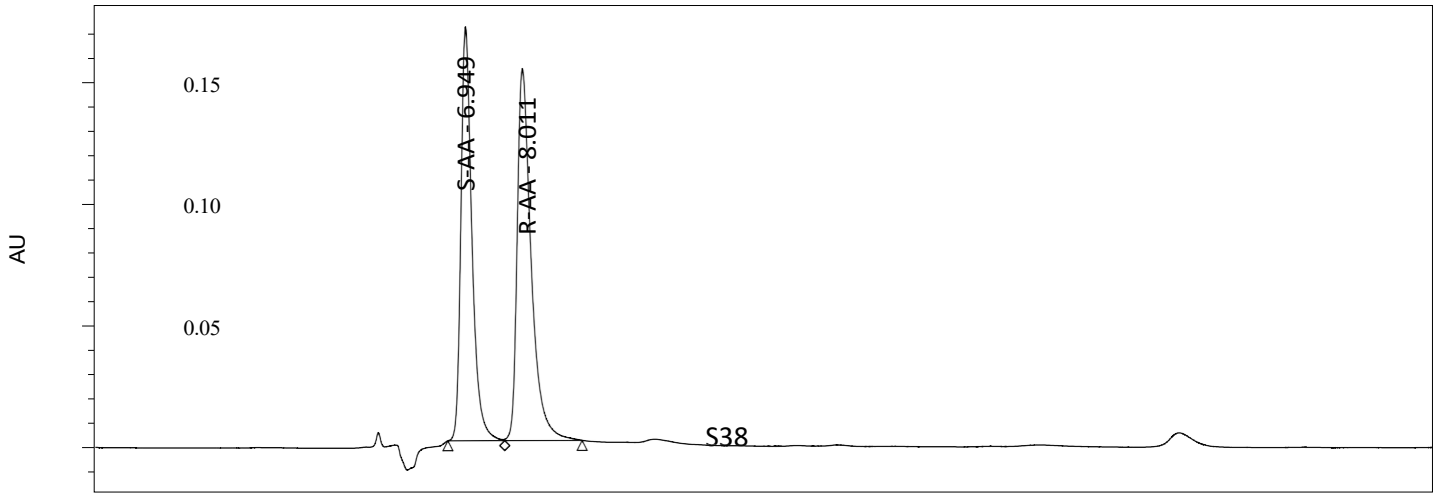
HPLC analysis: The separation of chiral amino acids was carried out on the Shimadzu Nexera X2 UHPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an RF-20A fluorescence detector. The derivatized chiral amino acids with OPA were separated on an analytical column Nautilus-E, 5 µm (250mm. × 4.6 mm) (BioChemMak ST, Moscow, Russia). The mobile phase consisted of 0.1% Perchloric acid in water and methanol (90:10 v/v). Analysis was carried out at a flow rate of 0.5 mL/min, and the column temperature was set to 40ºC. The fluorescence excitation and emission wavelengths were 350 and 450 nm, respectively, and the injection volume was 10 µl.

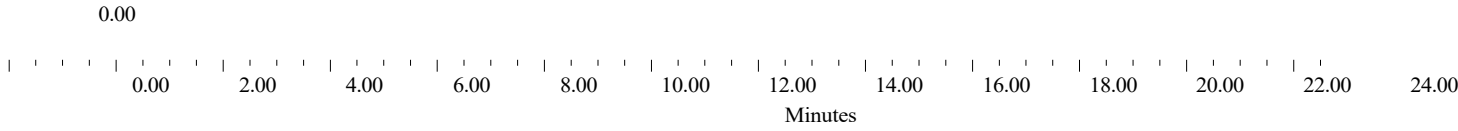
HPLC analysis of **4a** amino acid



	Name	Retention Time	Area	% Area
1	(*S*)-**4a**	6.738	4313864	99.23
2	(*R*)-**4a**	7.500	33410	0.77

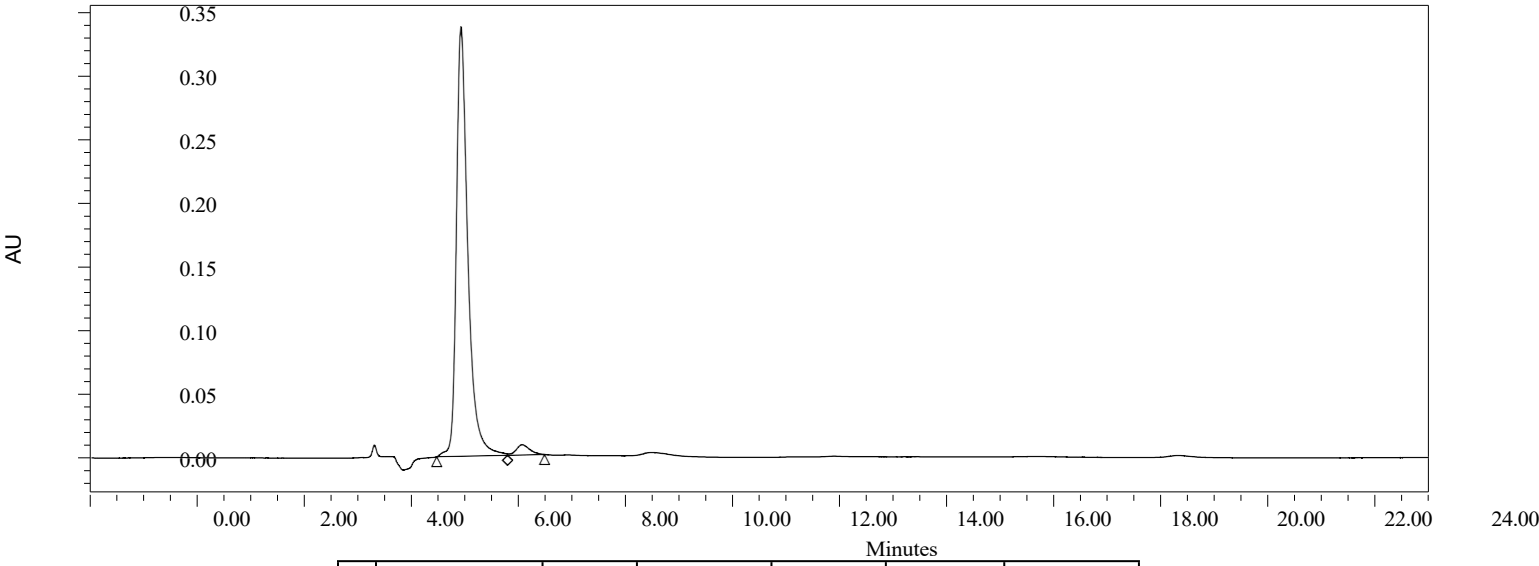
HPLC analysis of (*S*, *R*) **-4b amino acid**





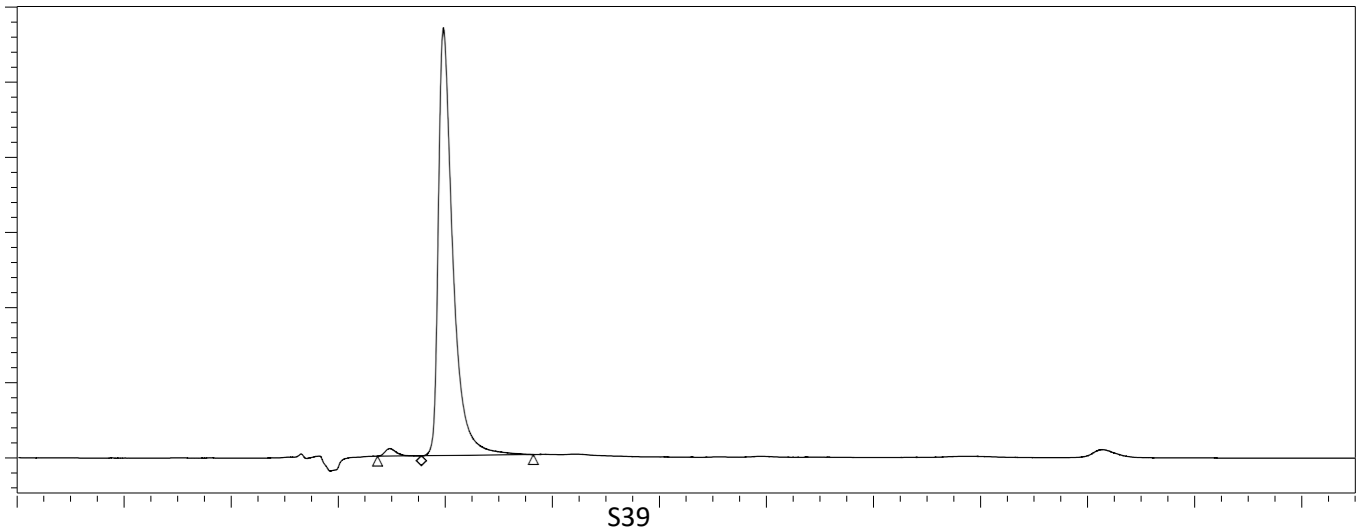
	Peak Name	RT	Area	% Area	Height	% Height
1	*S*-**4b**	6.949	2427838	46.67	169763	52.67
2	*R*-**4b**	8.011	2773955	53.33	152580	47.33

HPLC analysis of **4b** amino acid



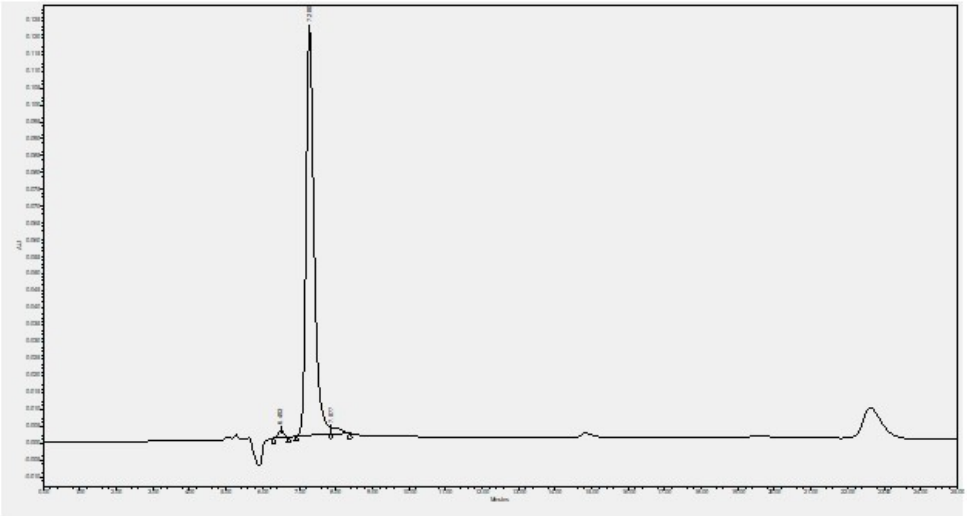
	Peak Name	RT	Area	% Area	Height	% Height
1	*S*-**4b**	6.940	5006444	97.25	336735	97.67
2	*R*-**4b**	8.084	141773	2.75	8049	2.33

HPLC analysis of (*R*) - **4b** amino acid



	Peak Name	RT	Area	% Area	Height	% Height
1	S-**4b**	6.966	71043	1.31	4893	1.69
2	R-**4b**	7.974	5334550	98.69	283868	98.31

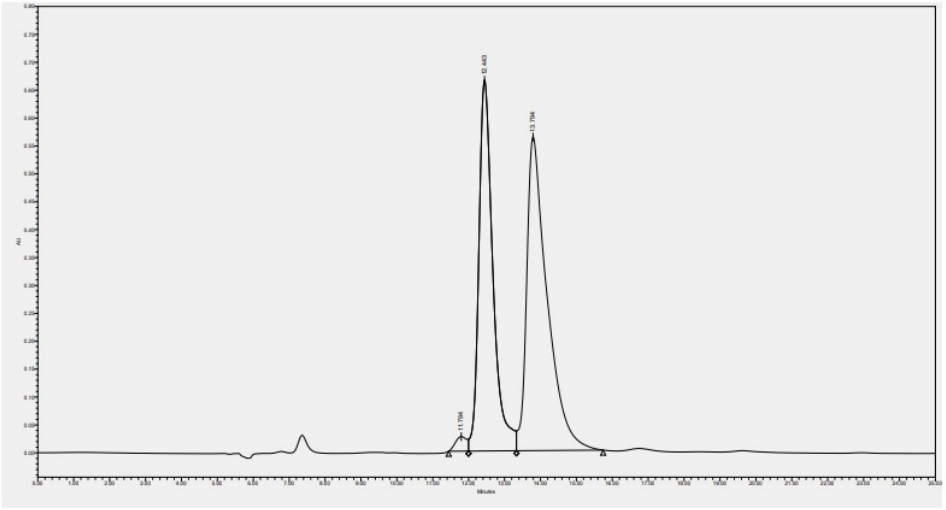
HPLC analysis of **4c** amino acid



	Peak Name	RT	Area	% Area
1	*S*-**4c**	7.280	1841429	98.28
2	*R*-**4c**	7.877	32197	1.72

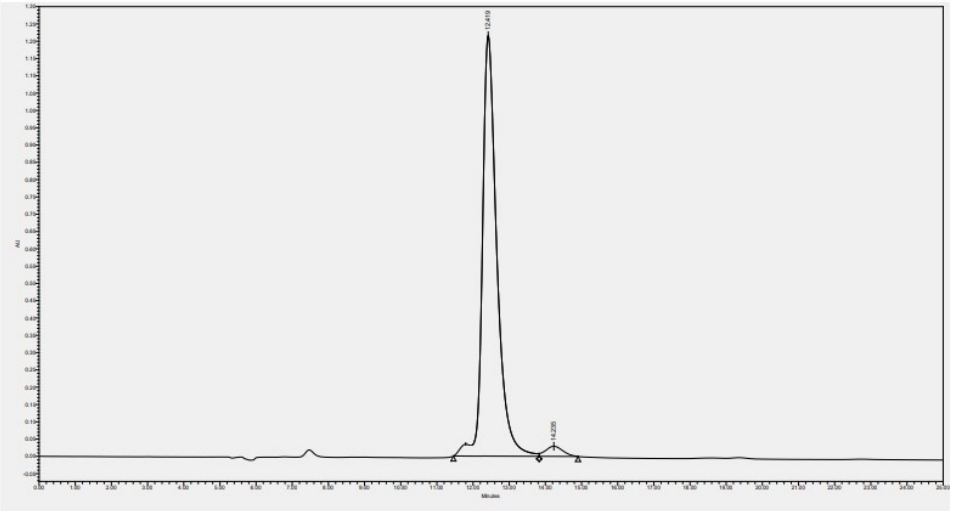
HPLC analysis of **4d**

Analysis of (*S*, *R*) – **4d**



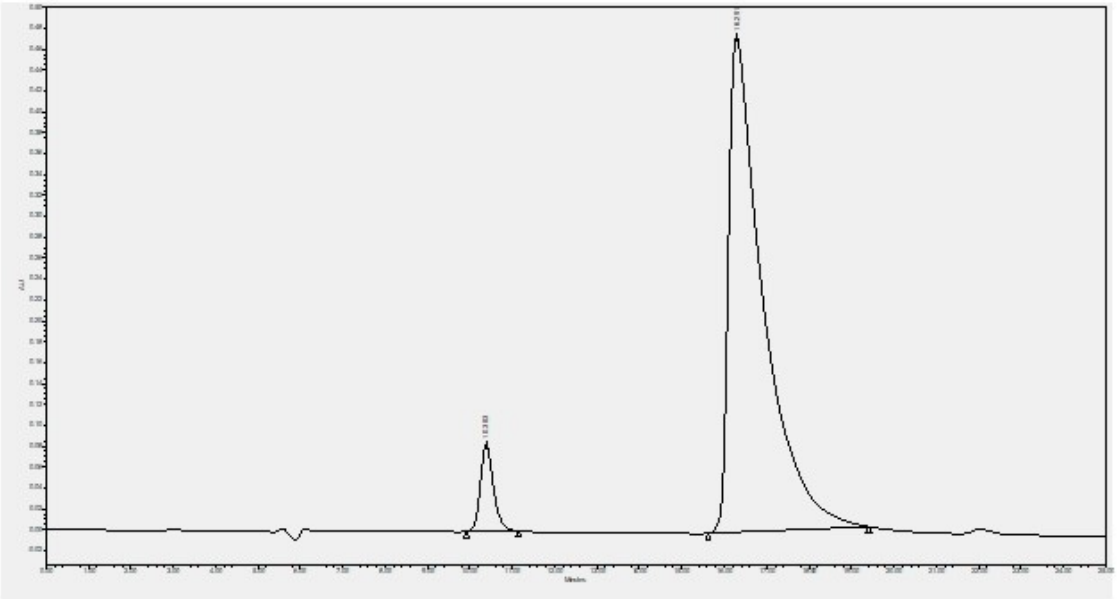
	Name	Retention Time	Area	% Area	Height
1	(*S*)-**4d**	12.443	18141619	44.90	664720
2	(*R*)-**4d**	13.794	22259282	55.01	562387

HPLC analysis of **4d** amino acid



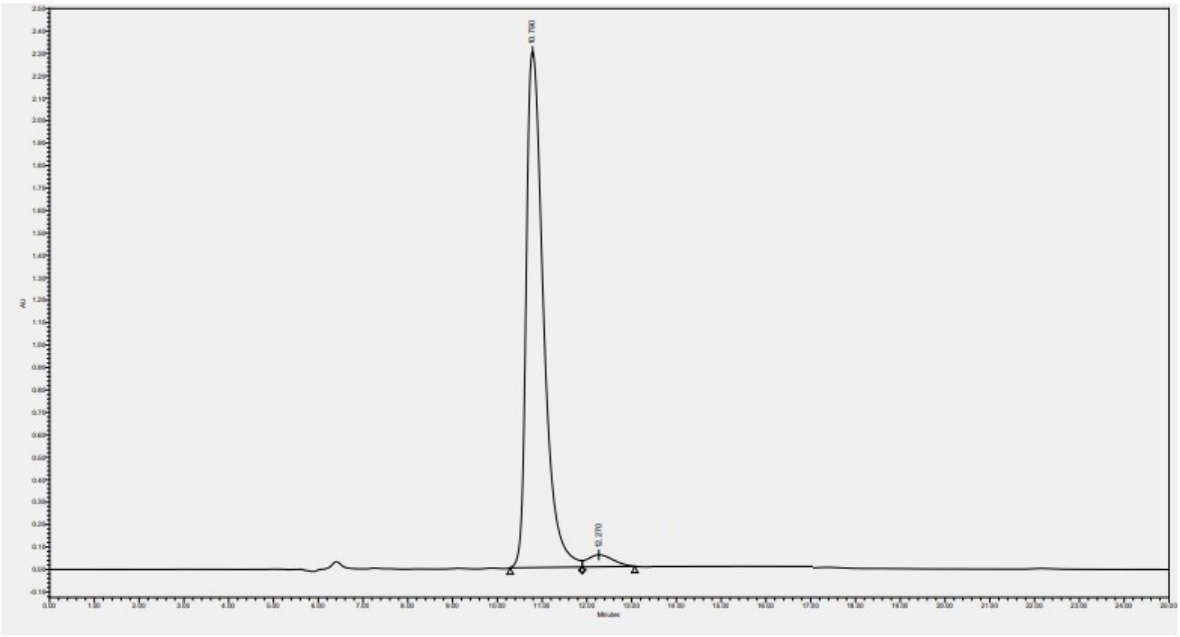
	Name	Retention Time	Area	% Area	Height
1	(*S*)-**4d**	12.419	33040276	97.19	1215538
2	(*R*)-**4d**	14.235	974820	2.81	29377

Analysis of (*R*) – 4d



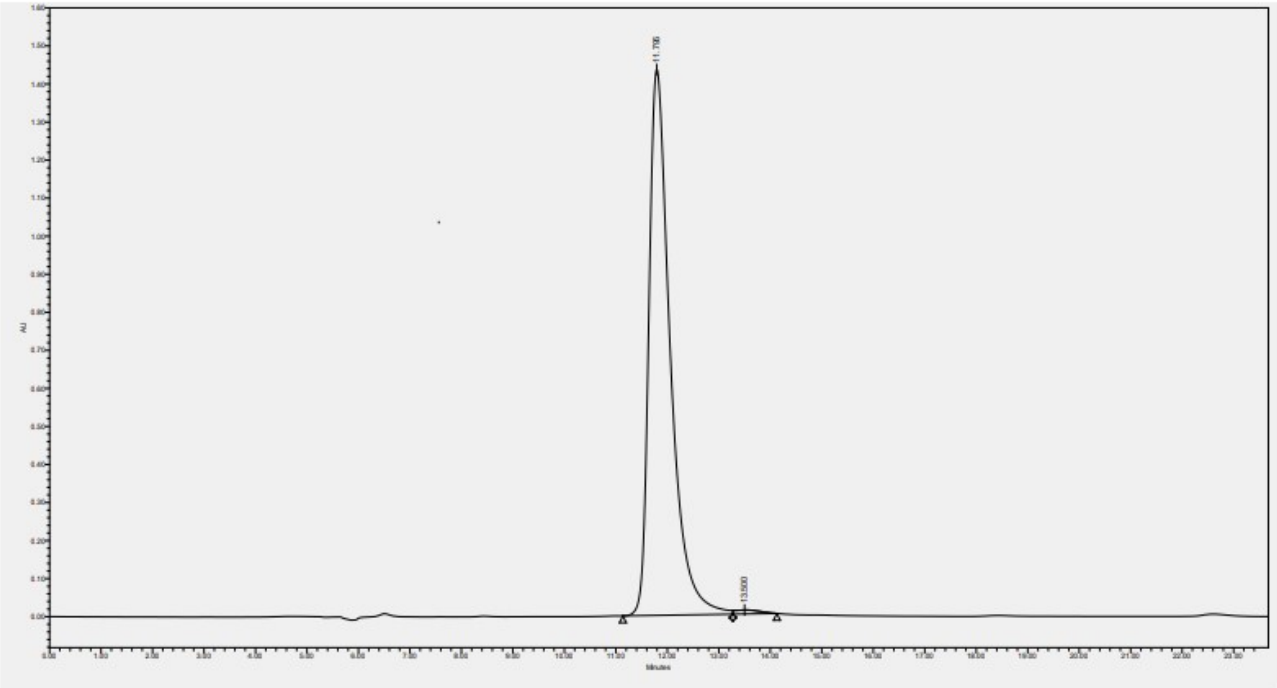
	Name	Retention Time	Area	% Area	Height
1		10.383	1797716	6.25	82907
2		16.291	26949765	93.75	473890

HPLC analysis of **4e** amino acid



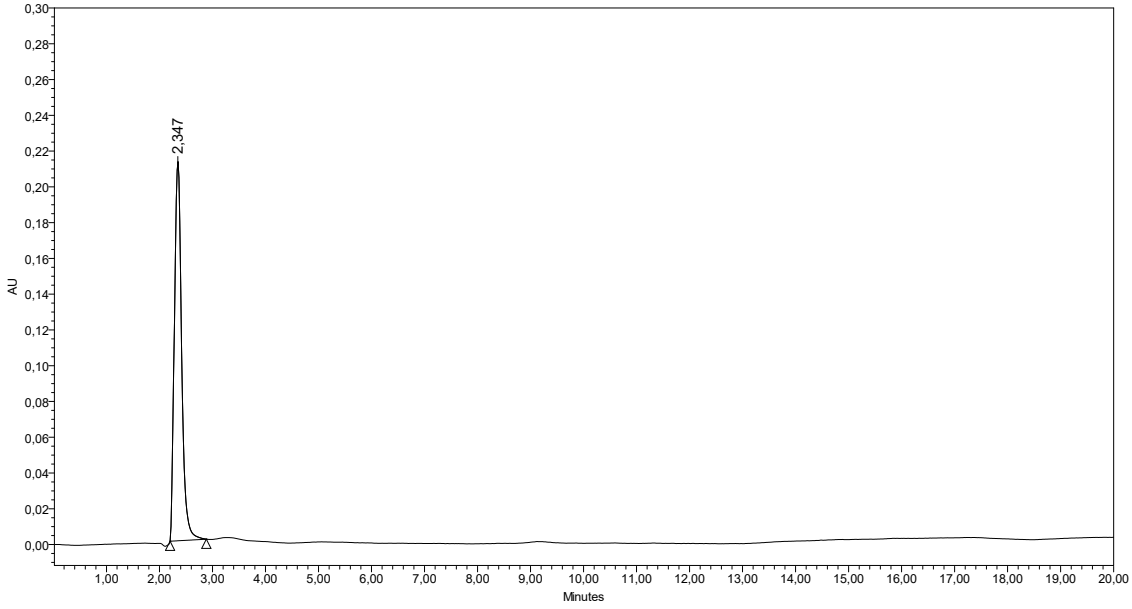
	Name	Retention Time	Area	% Area	Height
1	(*S*)-**4e**	10.790	61525992	98.96	1328982
2	(*R*)-**4e**	12.270	2099990	1.04	27598

HPLC analysis of **8d**



	Name	Retention Time	Area	% Area	Height
1	(*S*)-**8d**	11.795	41344682	99.15	1424828
2	(*R*)-**8d**	13.500	352664	0.85	10451

HPLC analysis of **9b** amino acid

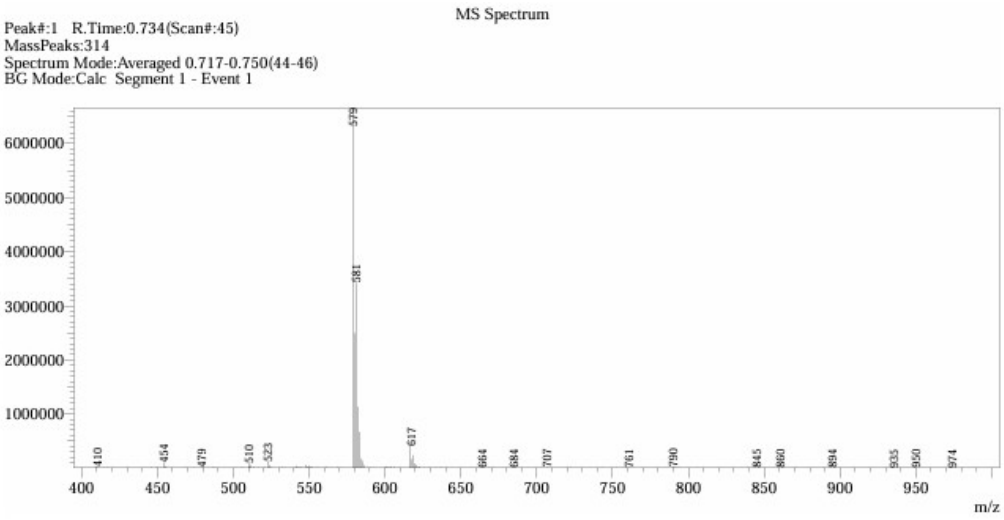


	Name	Retention Time	Area	% Area	Height
1	(*S*)- **9b**	2,347	2024939	100,00	212387

MS spectra

LC-MS: Compounds were analyzed using a Prominence I LC-2030C 3D Plus (Shimadzu Corporation, Kyoto, Japan) LC system connected to a simple quadrupole MS system (LC-MS-2020, Shimadzu Corporation, Kyoto, Japan) equipped with electrospray ionization (ESI) source. The mobile phase contained 0.1 % formic acid in water and 0.1 % formic acid in methanol (20/80 v/v) at a flow rate of 0.2 mL/min. The mass spectrometer was operated in the positive electrospray ionization mode. The interface settings were as follows. Nebulizing-gas flow rate: 1.5 L/min; drying-gas flow rate: 15.0 L/min; interface temperature: 350 °C; heat block temperature: 200 °C, DL temperature: 250 °C. All the peak analyses for the LC-MS were performed using LabSolutions ver. 5.99 SP2 (Shimadzu, Japan).

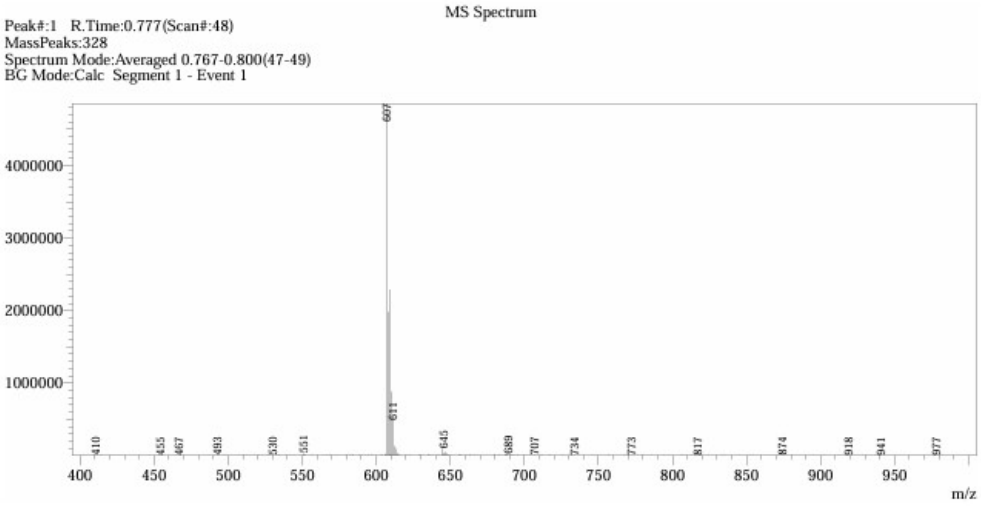
Complex 3a



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.734	415843864	579.00
Total		415843864	

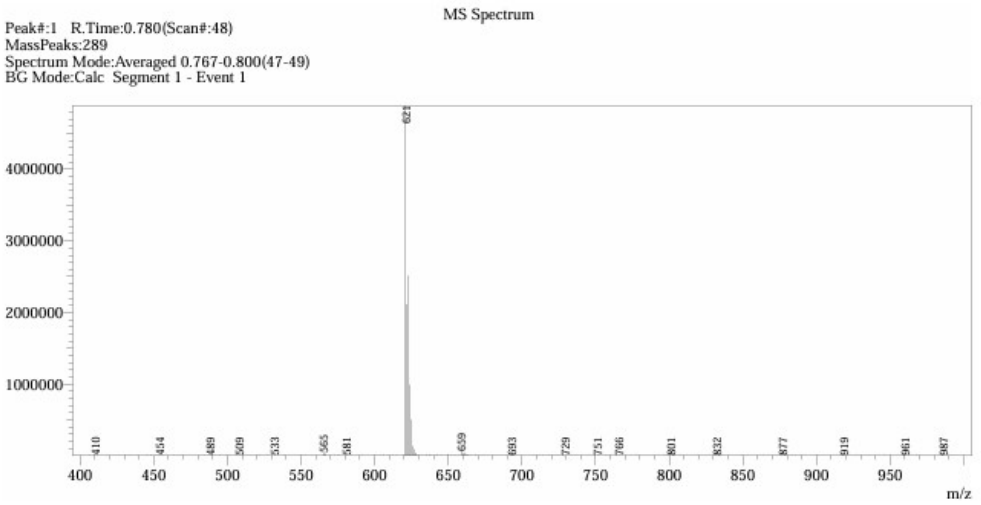
Complex 3b



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.777	258117293	607.00
Total		258117293	

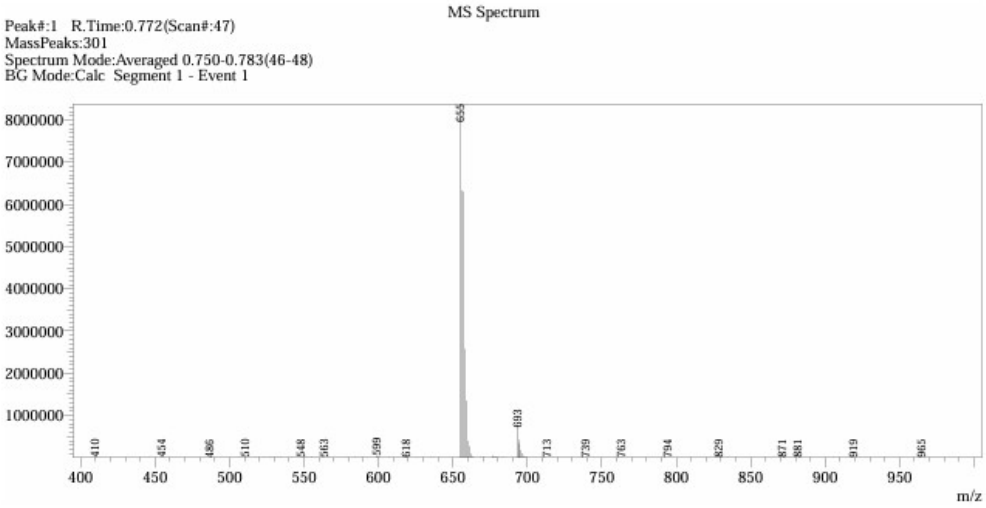
Complex 3c



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.780	243011398	621.05
Total		243011398	

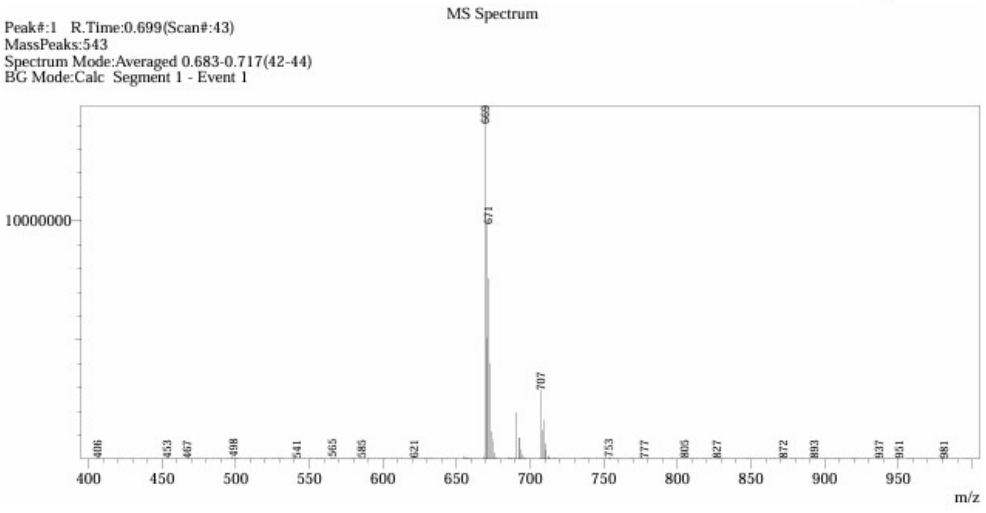
Complex 3d



MASS Peak Table TIC

Peak#	Ret. Time	Area	Base Peak m/z
1	0.772	729885448	655.00
Total		729885448	

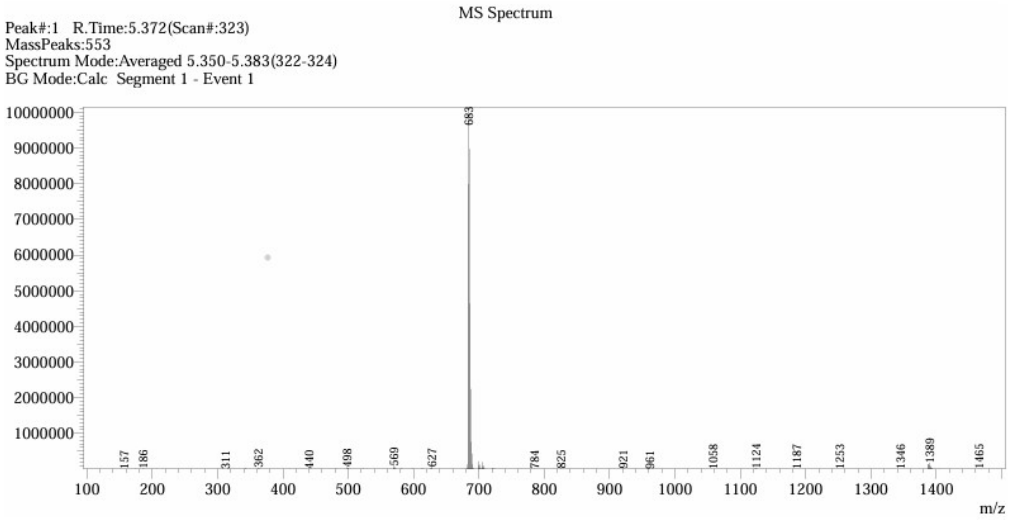
Complex 3e



MASS Peak Table TIC

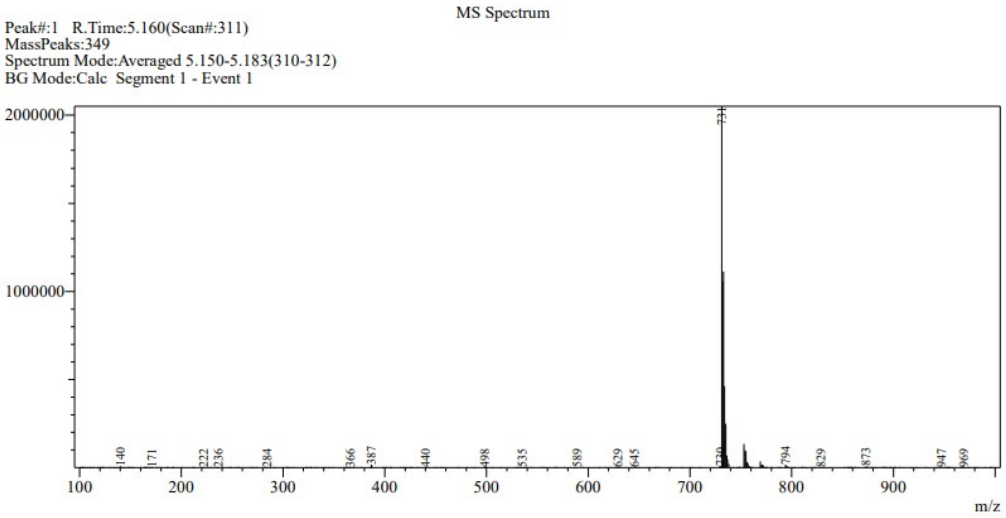
Peak#	Ret. Time	Area	Base Peak m/z
1	0.699	2946790490	669.55
Total		2946790490	

Complex 5b



Peak#	Ret. Time	Area	Base Peak m/z
1	5.372	1343977224	683.10
Total		1343977224	

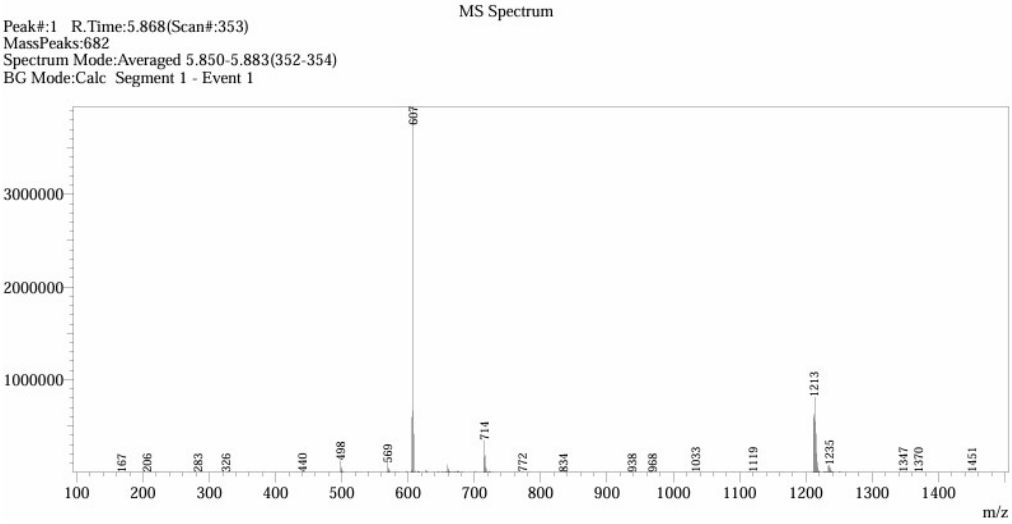
Complex 5d



MASS Peak Table TIC

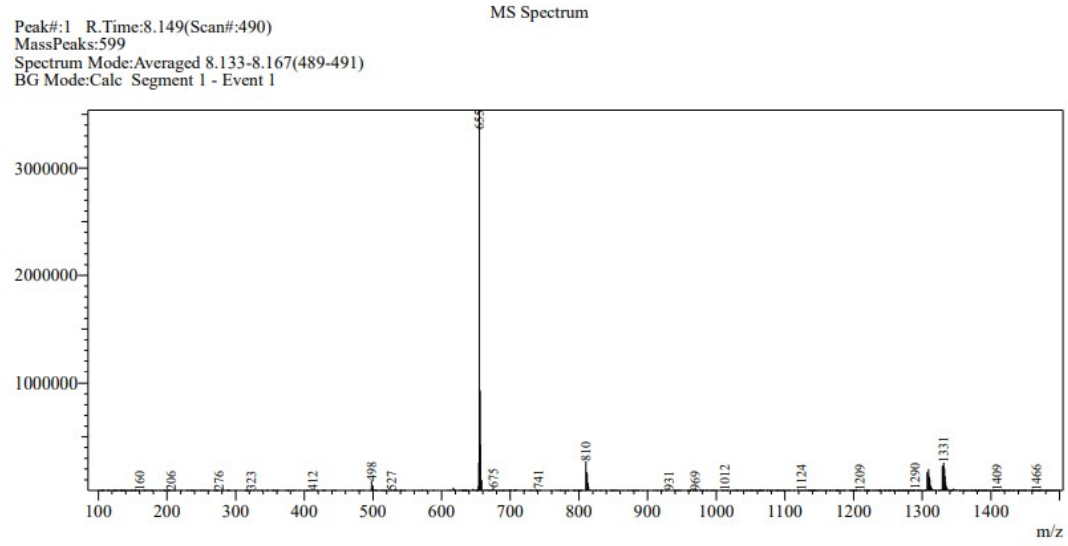
Peak#	Ret. Time	Area	Base Peak m/z
1	5.160	80257284	731.15
Total		80257284	

Complex 6b



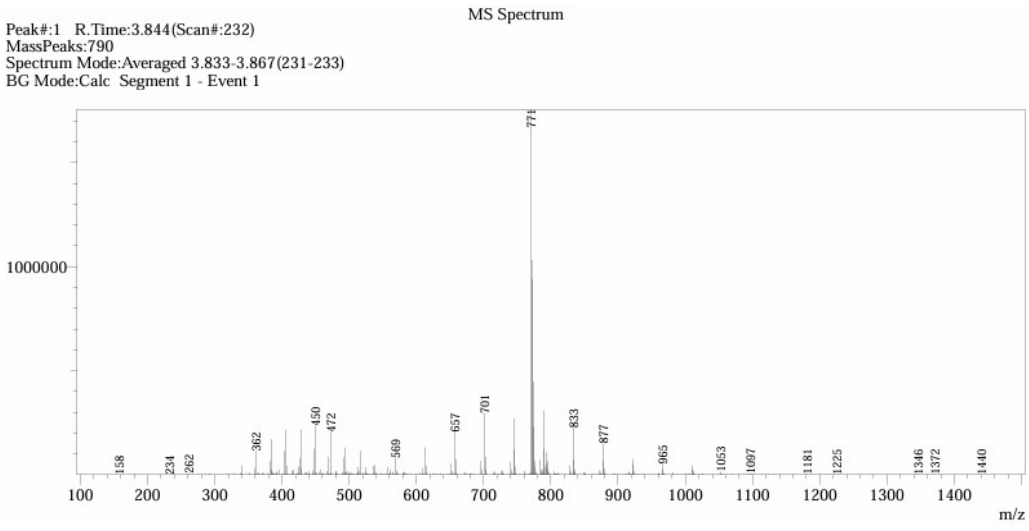
Peak#	Ret. Time	Area	Base Peak m/z
1	5.868	246951506	606.95
Total		246951506	

Complex 6d



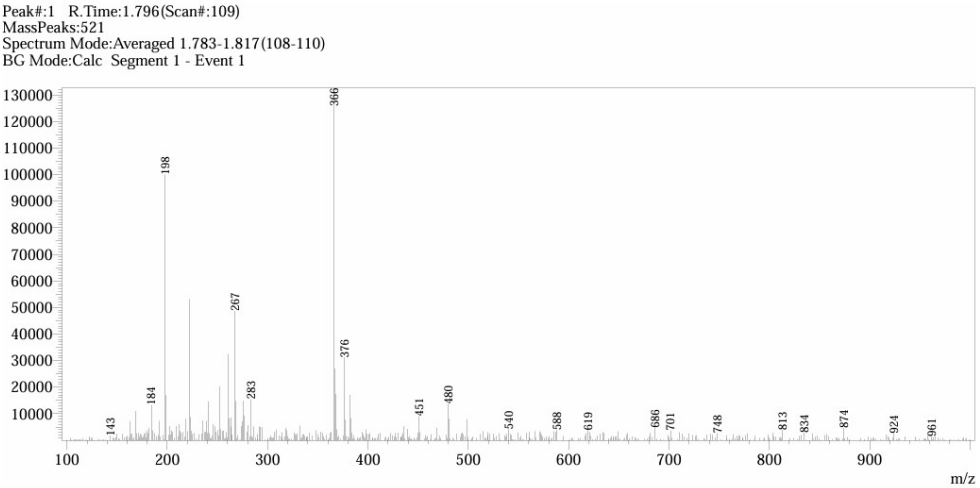
Peak#	Ret. Time	Area	Base Peak m/z
1	8.149	196814344	654.95
Total		196814344	

Complex 7b



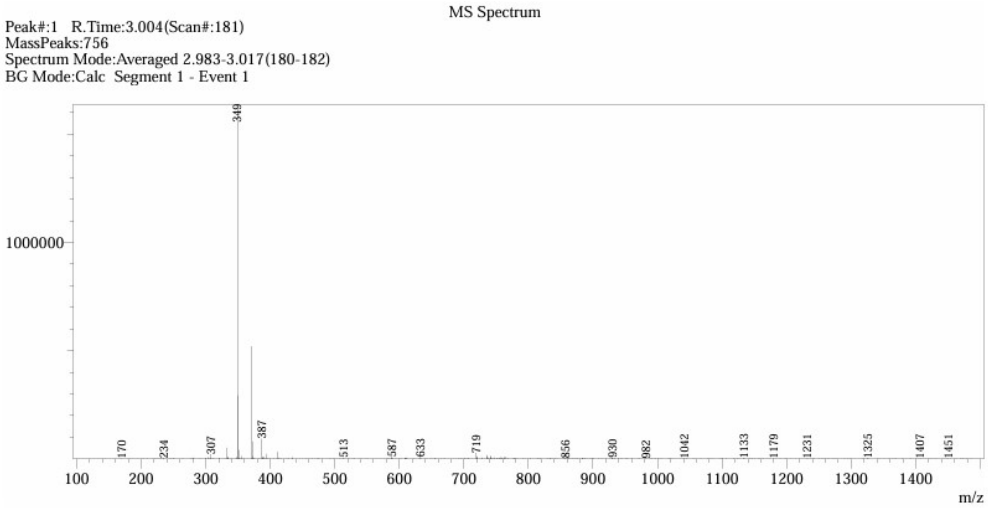
Peak#	Ret. Time	Area	Base Peak m/z
1	3.844	242772729	771.10
Total		242772729	

(*S*)-2-amino-3-(benzyl(3-phenylprop-2-yn-1-yl)amino)propanoic acid-8d



Peak#	Ret. Time	Area	Base Peak m/z
1	1.796	22163772	366.05
2	2.161	99505758	309.00
3	2.552	19961415	195.00
Total		141630945	

(*S*)-2-amino-3-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)(propyl)amino)propanoic acid 9b



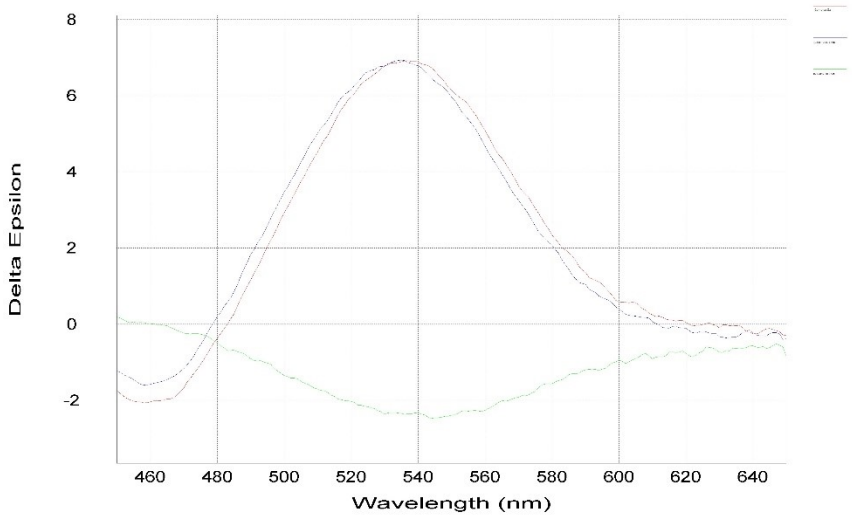
Peak#	Ret. Time	Area	Base Peak m/z
1	3.004	52843061	349.00
Total		52843061	

Circular Dichroism

The CD analyzes was done by Chirascan™ V100. As a standard, CD curves of (*S*,*S*)- and (*S*,*R*)-diastereomers were produced using the (*S*,*R*)-diastereomer of the alanine complex as the initial alkyne substrate, following a similar procedure.

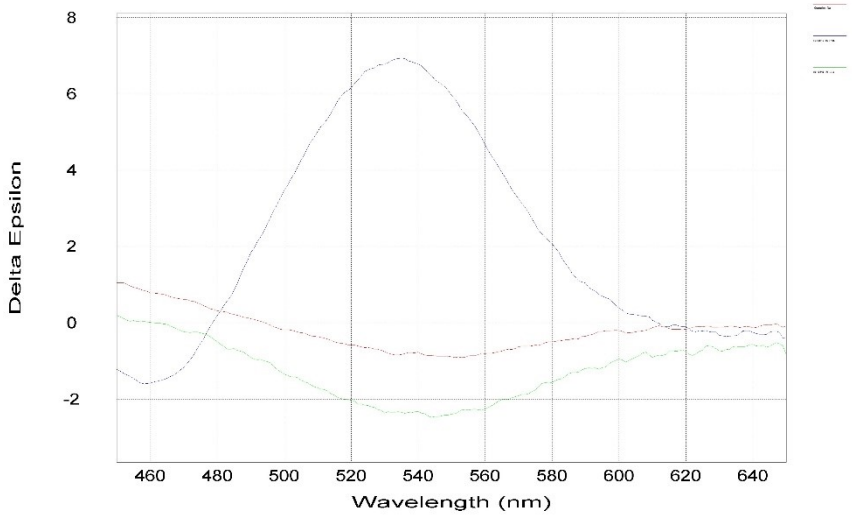
Complex 3a *S*-*S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -2.07 (458); 6.91 (537).



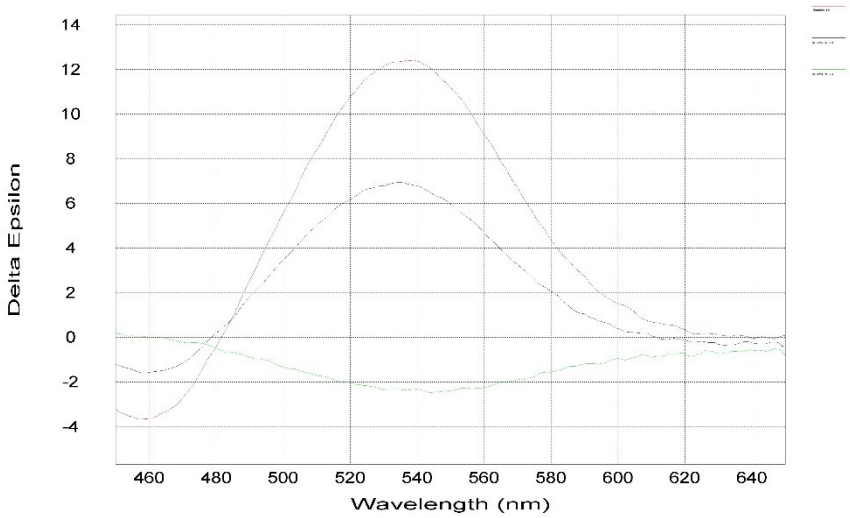
Complex 3a *S*-*R*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): 1.05 (449); -0.91 (553).



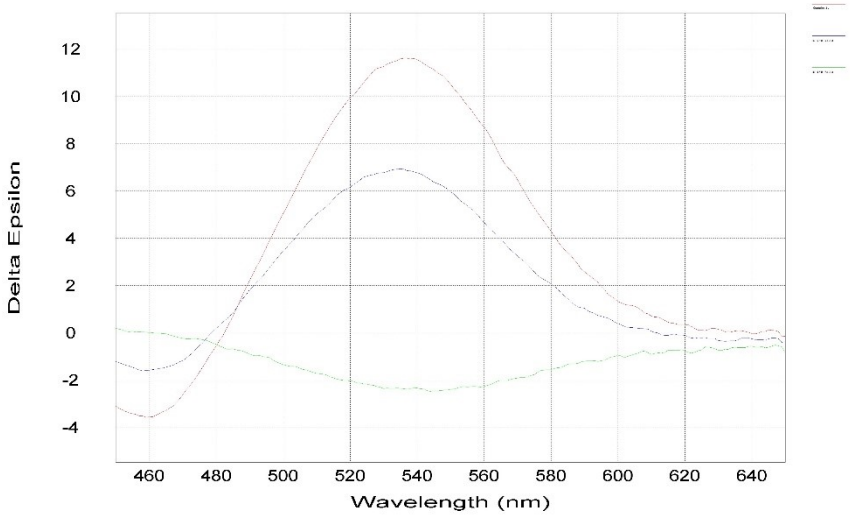
Complex 3b *S-S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -3.67 (458); 12.4 (537).



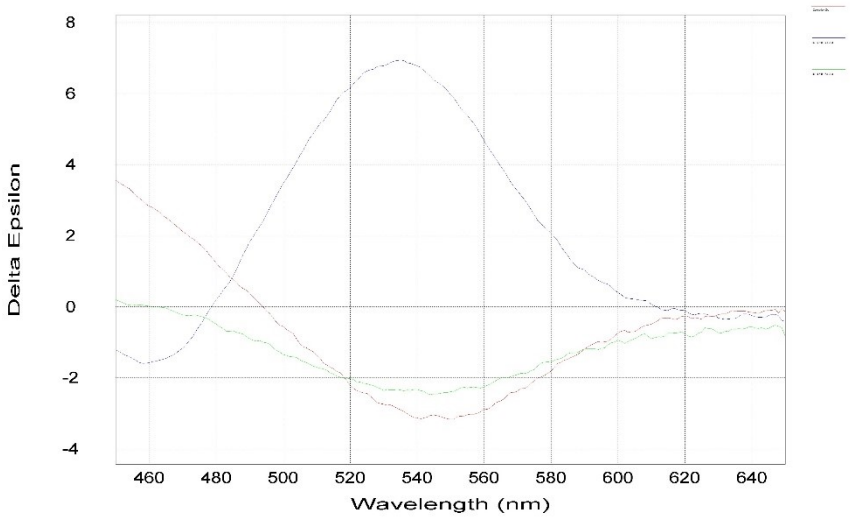
Complex 3c *S-S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -3.55 (460); 11.61 (536).



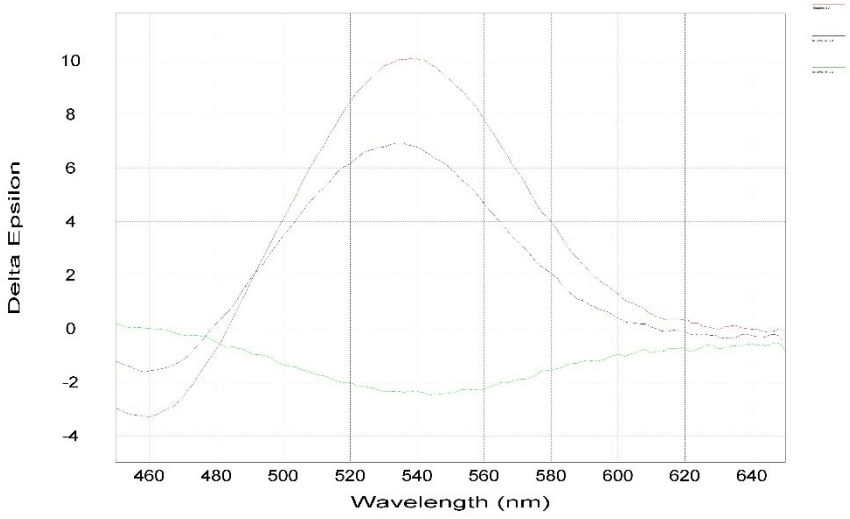
Complex 3c *S-R*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): 3.48 (451); -3.17 (541).



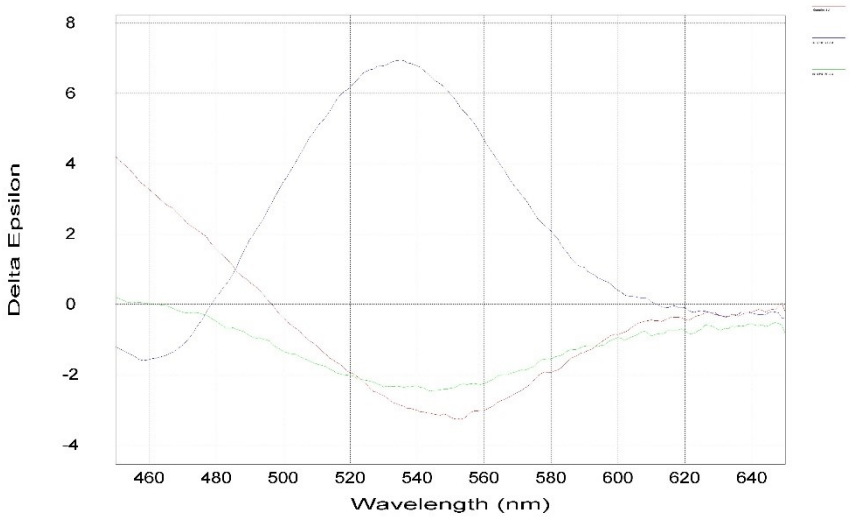
Complex 3d *S-S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -3.31 (460); +10.07 (540).



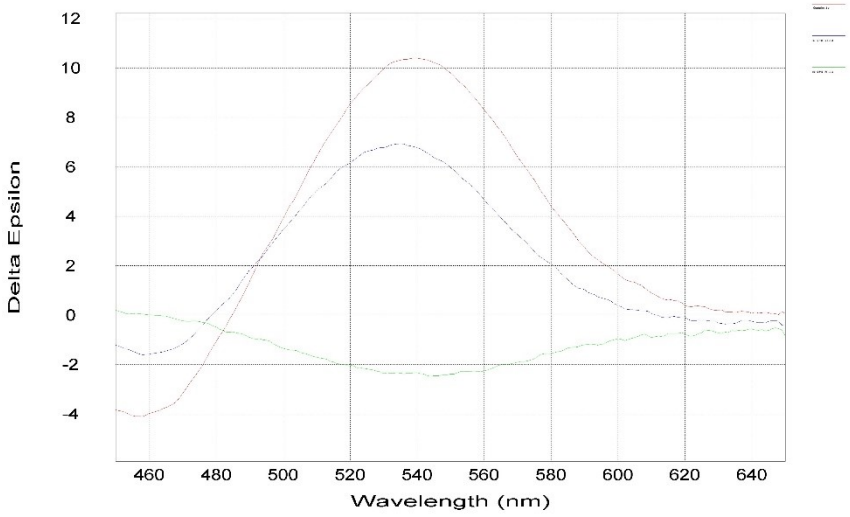
Complex 3d *S-R*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): 4.0 (452); -3.28 (553).



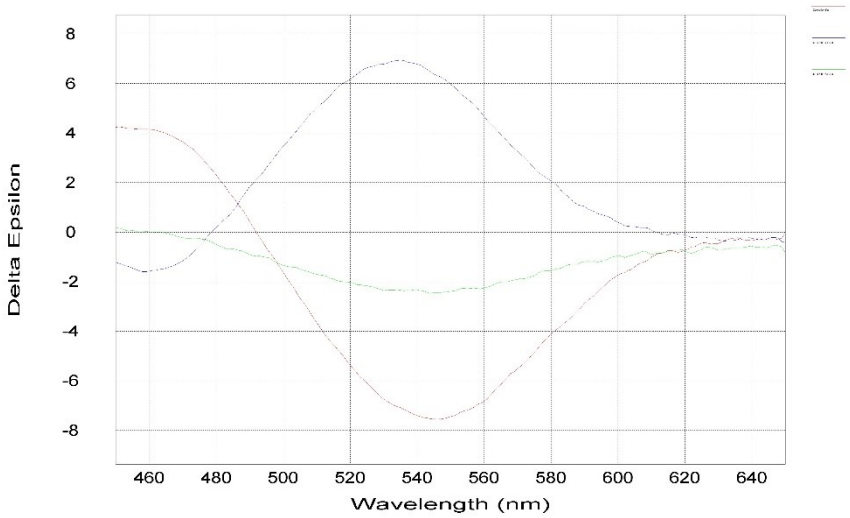
Complex 3e *S-S-S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -4.10 (458); +10.40 (540).



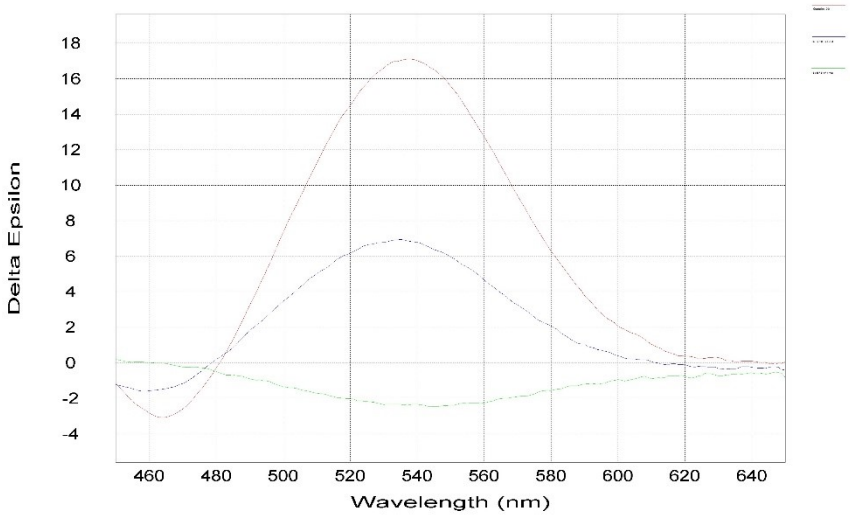
Complex 3e *S-R-S*

C:0.5mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): 4.17 (455); -7.56 (546).



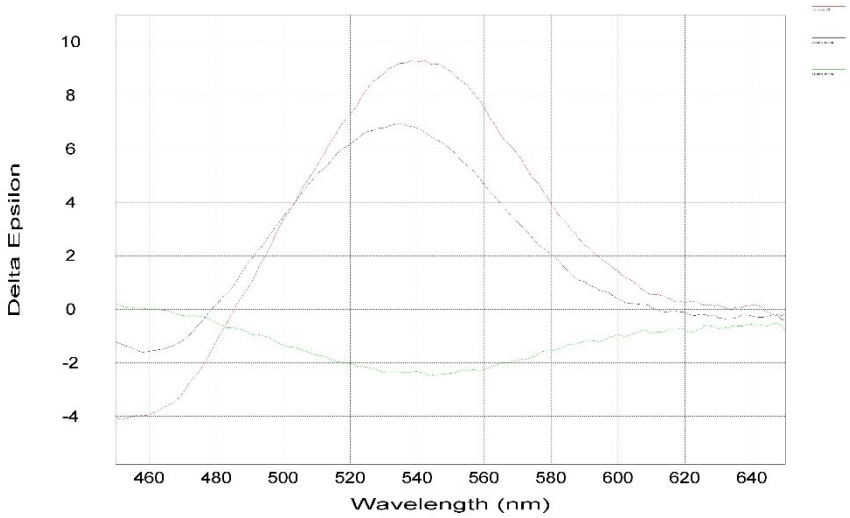
Complex 5b

C:0.4mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -3.08 (464); 17.09 (537).



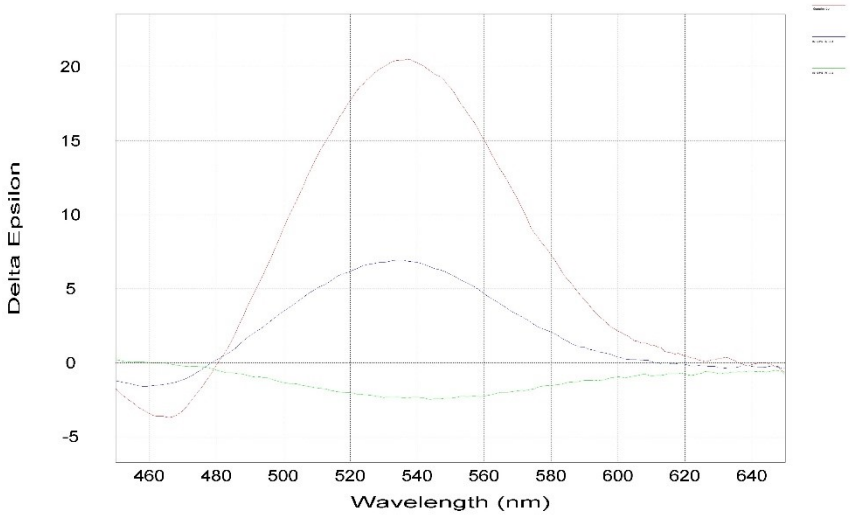
Complex 5d

C:0.4mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -4.07 (453); 9.28 (538).



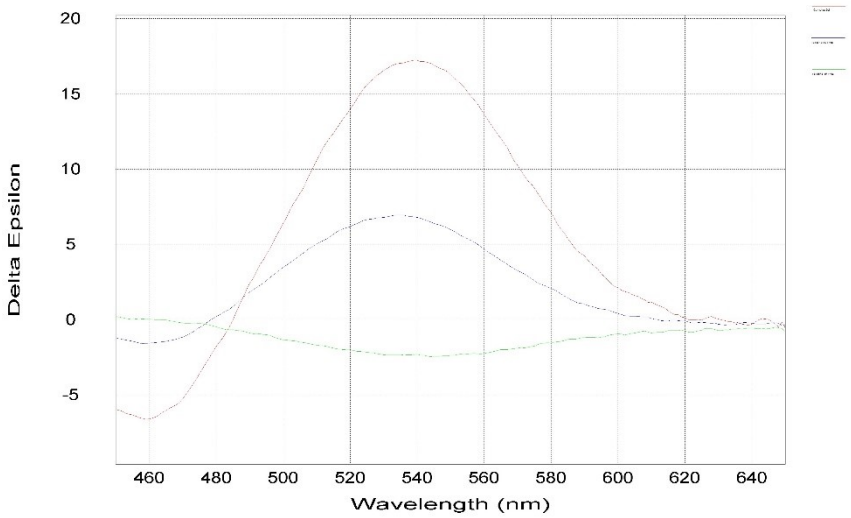
Complex 6b

C:0.4mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -3.7 (466); 20.51 (537).



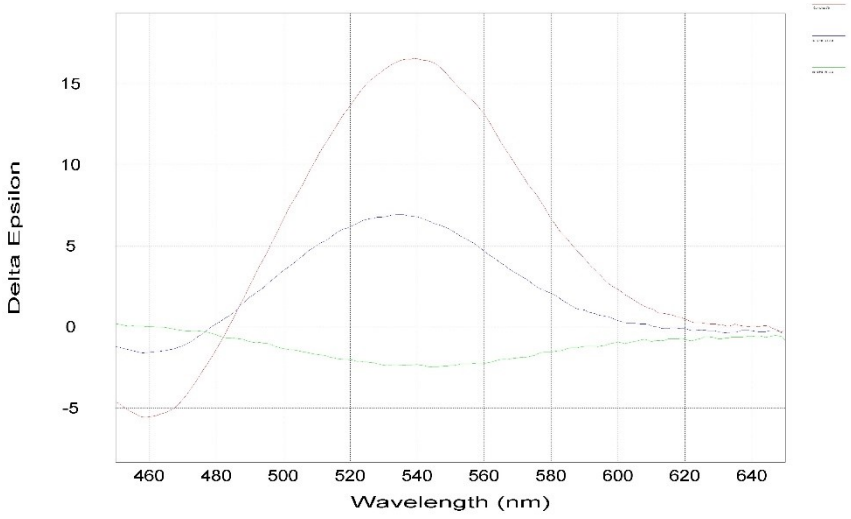
Complex 6d

C:0.4mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -6.63 (459); 17.21 (540).



Complex 7b

C:0.4mg/ml. $\Delta\varepsilon_{max}$ (λ_{max}): -5.58 (459); 16.56 (539).



Molecular docking

The structures of the compounds were built 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 for macromolecule preparation. Docking of ligands to the enzymes was performed using AutoGrid 4 and AutoDock Vina 1.2.3 softwares [J. Eberhardt, D. Santos-Martins, A. F. Tillack, and S. Forli AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings, J. Chem. Inf. Model. (2021) DOI 10.1021/acs.jcim.1c00203]. 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 [Sargsyan, A. S., Karapetyan, L. T., Mkhitaryan, A. V., Stepanyan, L. A., Sargsyan, T. H., Danghyan, Yu. M., Sargsyan, A. V., Oganezova, G. G., Hovhannisyan, N. A. (2024). Modeling, synthesis and in vitro testing of peptides based on unusual amino acids as potential antibacterial agents. Biomeditsinskaya Khimiya, 70(6), 413-420.https://doi.org/10.18097/PBMC20247006413]. 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.

X-Ray study

The diffraction measurements of crystal of compound **3b** were carried out at temperature of 100K on a XtaLAB Synergy-S diffractometer using Mo-Kα radiation, ω-scans rotation. The structure was solved and the space group $P2_12_12_1$ determined by the ShelXT (a) structure solution program using dual and refined by Least Squares using version 2018/3 of ShelXL 2018/3 (b). All non-hydrogen atoms were refined anisotropically by full-matrix least squares methods. The hydrogen atoms were positioned geometrically and refined using riding model. Crystallographic and experimental data are listed in table. The full crystallographic data in CIF format available free of charge via internet at: www.ccdc.cam.ac.uk/structures, deposition number: CCDC 2486969.

Table. Crystallographic and experimental data

Crystal Data	
Formula	$C_{34}H_{36}N_4O_3Ni$
Formula Weight	607.38
Crystal System	orthorhombic
Space group	$P2_12_12_1$
a, b, c [Å]	7.54522(7), 16.10301(15), 24.9287(2)
α, β, γ [deg.]	90, 90, 90
V [Å^3]	3028.86(5)
Z	4
D(calc) [g/cm^3]	1.332
μ(MoKα) [mm^{-1}]	0.681
F(000)	1280
Crystal Size [mm]	0.30×0.12×0.10
Data Collection	
Temperature (K)	100
Radiation [Å]	MoKα 0.71073
θ_{min}, θ_{max} [Deg]	2.1, 32.2
Dataset	-10≤h≤10; -23≤k≤22; -34≤l≤36
Tot., Uniq. Data, R(int)	98490, 9681, 0.028
Observed data [I > 2.0 σ(I)]	9354
Refinement	
Nref, Npar	9681, 380
R, wR2, S	0.0222, 0.0571, 1.04
Min. and Max. resd. dens. [e/Å^3]	0.23, 0.35
Flack x	0.010(2)

a. G.M. Sheldrick, SHELXT-Integrated Space-Group and Crystal-Structure Determination. Acta Cryst., (2015), A71, 3-8.

b. G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Cryst., (2015), C71, 3-8.