# Supporting Information

## Indium Mediated Allylation in Peptide and Protein Functionalization

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### General Procedures and Materials

Unless otherwise noted, all reagents were of analytical grade, obtained from commercial sources and used without further purification. Experiments involving moisture and/or air sensitive components were performed in oven-dried glassware under a positive pressure of nitrogen using freshly distilled solvents. Commercial grade solvents and reagents were used without further purification. Hexane, ethyl acetate were fractionally distilled. Analytical thin layer chromatography (TLC) was performed on Merck 0.25 mm silica gel 60-$F_{254}$ glass plates with visualization by ultraviolet (UV) irradiation at (254 nm) on Spectroline Model ENF-24061/F 254 nm. Further visualization was possible by staining with acidic solution of ceric molybdate, Ninhydrin and Iodine.

Purifications by flash column chromatography were performed using silica gel 60 (230-400 mesh) from Merck KGaA Co., Germany. Columns were typically packed as slurry and equilibrated with the appropriate solvent system prior to use. Chromatography solvents were of analytical grade and either Ethyl acetate : Hexane or Dichloromethane : Methanol solvent systems were used. Eluting system was determined by TLC analysis. All organic solvents were removed using a rotary evaporator under reduced pressure. Water used in biological procedures was distilled and deionized using Millipore purification system (Bedford, MA, USA). Dipeptide (Ser-Ala-OMe, **1a**) and tetrapeptide (Ser-Phe-Leu-Glu(OMe)-OMe**, 1b**) were prepared by standard solution synthesis. Myoglobin (M 1882) from horse heart and (+)-Biotin-X-hydrazide (B3770) were purchased from Sigma and used without further purification. Allylic bromide substrates

**4, 5, 7, 9, 11** and **13** were purchased from Sigma and used as obtained. Rest of the allylic bromide reagents were prepared in house. Rhodamine B base was purchased from Sigma.

**Instrumentation and Sample Analysis**

**IR.** Infrared spectra were recorded from thin film samples on a sodium chloride plate using a Shimadzu IR Prestige-21 FT-IR Spectrometer (Japan). Solid samples were analyzed as a KBr pressed-disk. The oil samples were examined under neat conditons.

**UV/Vis and CD Spectra.** UV-VIS spectroscopic measurement was conducted on a Infinite Tecan M1000 benchtop spectrophotometer. Circular dichroism spectra were obtained in a J-810 spectropolarimeter (JASCO, Tokyo, Japan). The data were processed using Microsoft Excel and Prizm software.

**Optical Rotation.** Optical rotation was measured using a JASCO P-1030 Polarimeter (Japan) equipped with a sodium vapor lamp at 589 nm. Concentration is denoted as *c* and was calculated as grams per milliliters (g / 100 mL) whereas the solvent was indicated in parentheses (*c*, solvent).

**Nuclear Magnetic Resonance Spectroscopy.** Proton nuclear magnetic resonance ($^{1}$H NMR) and carbon nuclear magnetic resonance ($^{13}$C NMR) spectroscopy were performed on a Bruker Avance DPX 300 and Bruker AMX 400 and 500 NMR and Varian 300 NMR spectrometers as noted. Chemical shifts are reported as δ in units of parts per million (ppm) downfield from tetramethylsilane (δ 0.00), using the residual solvent signal as an internal standard: chloroform-*d*, (δ 7.26, singlet); methanol-$d_4$, (δ 3.31, septet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplets) and b (broad). Coupling constants (*J*) are recorded in Hertz (Hz). The number of protons (n) for a given resonance based on spectral integration values is indicated by nH. $^{13}$C NMR chemical shifts are reported as δ in units of parts per million (ppm) downfield from tetramethylsilane (δ 0.00), using the residual solvent signal as an internal standard: chloroform-*d*, (δ 77.20, triplet); methanol-$d_4$, (δ 49.0, septet).

**Mass Spectroscopy.** Peptide mass spectrometry (ESI, electrospray ionization) spectra was recorded on a Thermo Finnigan LCQ Deca XP Max (San Jose, CA) ultra high sensitivity quadruple ion trap mass spectrometer fitted with Surveyor LC Auto Sampler

and MS Pump. Acquisition mass range was typically *m/z* 500-2000. Data were recorded and processed using X-Calibur software (Thermo Scientific, MA, USA). High Resolution Mass Spectrometry (HRMS) (ESI) spectra were recorded on a Q-Tof Premier mass spectrometer (Micromass, MS Technologies, UK) fitted with Acquity Ultra Performance Auto sampler and LC (Waters, Manchester, UK). Acquisition mass range was typically *m/z* 100-1000. Calibration was achieved by using the multiple-charged ion peaks of sodium formate. Data was processed using MassLynx™ software (Waters, Manchester, UK). MS and HRMS were reported in units of mass to charge ratio (*m/z*).

**High Performance Liquid Chromatography.** Peptide purification was performed on an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA). Chromatographic separation of **1a** and **1b** were achieved on 19 mm x 280 mm Atlantis C18 dC OBC$^{TM}$ 10 μm (Waters, Manchester, UK) reversed-phase column at room temperature. Sample volumes of 500 μl (250 mg) were typically injected onto the column manually. Eluent A was water containing 0.1% TFA. Eluent B was acetonitrile containing 0.1% TFA. A gradient from 0% B to 50% B was run in 30 minutes. HPLC was controlled by MassLynx software (Waters, Manchester, UK). UV detection was performed at 214 nm. The flow rate was 20 ml/min. Upon purification, the solvent was removed by freeze drying.

**Protein Purification and Mass Spectrometry.** Separation of proteins was performed on a HP1100 HPLC system (Hewlett Packard, Palo-Alto, CA) employing a 1 mm x 150 mm column packed with POROS R1/H (Perspective Biosystems, Foster City, CA). The column was kept at 80 °C. Sample volumes of 3-25 μl were typically injected onto the column using a CTC PAL autosampler (CTC, Zwingen, Switzerland) fitted with a Valco model C6UW HPLC valve (Valco, Houston, TX) and a 25 μl injection loop. HPLC was controlled by MassLynx software (Waters, Manchester, UK). UV detection was performed at 214 nm. Eluent A was water containing 0.05% TFA. Eluent B was a 1:9 mixture of water : acetonitrile containing 0.045% TFA. A gradient from 20% B to 90% B was run in 12 minutes. The flow rate was typically 80 μl/min. The total flow from the LC system was introduced into the UV detection cell prior to introduction in the ESI source. The HPLC system was controlled and the signal from the UV detector was processed

using MassLynx™ software (Waters, Manchester, UK). Mass spectrometry was carried out using a Q-Tof (Waters, Manchester, UK) quadrupole time-of-flight hybrid tandem mass spectrometer equipped with a Waters Z-type electrospray ionization source. Acquisition mass range was typically *m/z* 500-2000. Data were recorded and processed using OpenLynx™ software and MaxEnt option (Waters, Manchester, UK). Calibration of the 500-2000 *m/z* scale was achieved by using the multiple-charged ion peaks of horse heart myoglobin (MW 16951.5 Da).

Trypsin digested protein fragments were analyzed using Vydak C18 reversed phase column (1 mm X 150 mm) with acetonitrile : water (1:9) isocratic mobile phase (80 µl/min) containing 0.05% trifluoroacetic acid at 40 ºC. Sample analysis for all HPLC experiments was achieved with an inline diode array detector (DAD).

**Gel Analyses.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was accomplished on a Mini-Protean apparatus (Bio-Rad, USA) with 12% gradient polyacrylamide gels under reducing conditions following the general protocol of Laemmli.[1] All electrophoresis protein samples were mixed with SDS loading buffer in the presence of dithiothreitol (DTT) and heated to 100 ºC for 10 min to ensure reduction of disulfide bonds and complete denaturation of protein. Commercially available markers (Bio-Rad, USA) were applied to at least one lane of each gel for calculation of apparent molecular weights. Visualization of protein bands was accomplished by staining with Coomassie Brilliant Blue R-250 (Bio-Rad, USA). Biotinylated proteins were detected with Avidin-Alkaline Phosphatase Conjugate (Avidin-AP) using Western Blotting Detection System (GE Healthcare, USA).

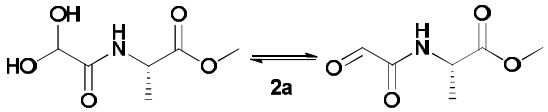
**Experimental section**

**Syntheses of Starting Material 1a and 2a.** According to previous reported procedures.[2]

**Representative experimental procedure for periodate oxidation of peptides:** To a solution of peptide **1a** (0.12 g, 0.64 mmol) in 25 mM sodium phosphate buffer (pH 7.0) (0.2 mL) was added a solution of sodium metaperiodate (0.27 g, 1.28 mmol) in the same buffer (0.8 mL) and stirred in the dark till the LC-ESI-MS analysis revealed complete conversion of starting material to product. After the completion of reaction, excess periodate was neutralized by sodium sulfite (0.8 g, 0.64 mmol) solution in the same buffer (0.4 mL) till (pH 7.0). The neutralized reaction was loaded onto a solid phase

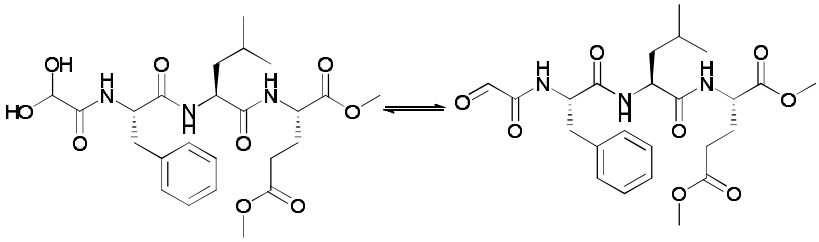
extraction cartridge (OASIS HLB, Waters) equilibrated with water and after an initial wash with water eluted with methanol. After evaporating the solvent in vacuo the product aldehyde was freeze dried to yield **2a** as a transparent gum (100 mg, quantitative) and carried forward to next step.

**(*S*)-2-(2-Oxo-acetylamino)-propionic acid methyl ester/ (*S*)-2-(2,2-Dihydroxy-acetylamino)-propionic acid methyl ester (2a).**




$R_f$ = 0.5 (dichloromethane – methanol, 93:7). $^1$H NMR (400 MHz, $CD_3OD$) δ 1.42 (d, 3H, *J* = 7.7 Hz), 3.74 (s, 3H), 4.44-4.50 (m, 1H) ppm. $^{13}$C NMR (75 MHz, $CD_3OD$) δ 16.0, 48.0, 51.4, 93.6, 170.1, 172.8 ppm. IR (neat): *v* 1725, 1688, 1437 cm$^{-1}$. LRMS (ESI) m/z calculated for $C_6H_{10}NO_4$ $[M+H]^+$ 160.05 found 160.94. HRMS (ESI) *m/z* calculated for $C_6H_{10}NO_4$ $[M+H]^+$ 161.0610 found 161.0613. $[\alpha]^{20}_d$ -6.0 (c = 1.1 g/100 mL, $CH_2Cl_2$).

**(*S*)-2-{(*S*)-4-Methyl-2-[(*S*)-2-(2-oxo-acetylamino)-3-phenyl-propionylamino]-pentanoylamino}-pentanedioic acid dimethyl ester/ (*S*)-2-{(*S*)-2-[(*S*)-2-(2,2-Dihydroxy-acetylamino)-3-phenyl-propionylamino]-4-methyl-pentanoylamino}-pentanedioic acid dimethyl esterpentanedioate (2b).**

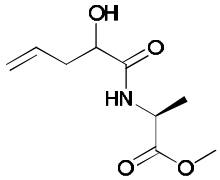



A mixture of the aldehyde and hydrated form. Obtained as yellow oil (0.030 g, quantitative). $R_f$ = 0.25 (ethyl acetate – methanol, 4:1). $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87-0.91 (m, 6H), 1.43-1.48 (m, 2H), 1.48-1.67 (m, 1H), 1.97-2.04 (m, 1H), 2.18-2.23 (m, 1H), 2.35-2.47 (m, 2H), 3.04-3.15 (m, 2H), 3.67-3.68 (s, 3H), 3.71-3.76 (s, 3H), 4.42-4.49 (m, 1H), 4.53-4.60 (m, 1H), 4.72-4.83 (m, 1H), 6.69-6.93 (m, 2H), 7.18-7.29 (m, 6H), 9.24 (s, 1H) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$) δ 22.0, 22.2, 22.6, 22.7, 24.6, 27.0, 29.9, 30.0, 37.9, 38.0, 38.1, 40.8, 41.1, 41.2, 51.6, 51.9, 52.0, 52.5, 54.2, 54.3, 54.5, 54.7, 93.4, 93.6, 127.1, 127.2, 127.3, 128.6, 128.7, 128.8, 129.2, 129.3, 135.6, 135.9, 136.0,

159.7, 169.4, 169.6, 169.7, 170.4, 170.5, 171.4, 171.6, 171.7, 171.9, 172, 173.3, 173.6, 187.3 ppm. IR (neat): *v* 3424, 2999, 1736, 1663, 1534, 1437, 1028 $cm^{-1}$. LRMS (ESI) m/z calculated for $C_{24}H_{34}N_3O_8$ $[M+H]^+$ 492.23 found 492.67. HRMS (ESI) *m/z* calculated for $C_{24}H_{34}N_3O_8$ $[M+H]^+$ 492.2346 found 492.2360. $[\alpha]^{20}_d$ -11.0 (c = 0.60 g/100 mL, $CH_2Cl_2$).

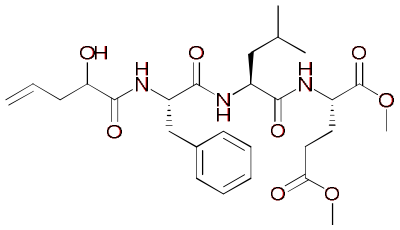
**Representative experimental procedure for Indium mediate allylation reaction of peptides:** To a suspension of the peptide aldehyde **2a** (0.1 g, 0.63 mmol) in 0.025 M sodium phosphate buffer, pH 7.0, (3.0 mL) was indium powder (0.15 g, 1.26 mmol) and cooled to 0 °C followed by the addition of allyl bromide (0.16 mL, 1.89 mmol). The mixture was allowed to run at room temperature for 16 h. White suspension had formed during the reaction. The reaction was filtered through a filter paper to get rid of the unreacted metal. The aqueous suspension was extracted with diethyl ether (3 X 10 mL). The combined organic phase was dried over $MgSO_4$. After filtration and solvent evaporation, the crude was purified by flash chromatography using 0-70% ethyl acetate in hexane as the eluent.

**(*S*)-2-(2-Hydroxy-pent-4-enoylamino)-propionic acid methyl ester (3a).**




Pale transparent gum (0.071 g, 56% yield). Rf = 0.17 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis revealed that the compound is in a diastereomeric ratio of 50:50. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.40 (dd, *J* = 3.0, 7.2 Hz, 3H), 2.35-2.48 (m, 1H), 2.57-2.65 (m, 1H), 3.73 (s, 3H), 4.13-4.18 (m, 1H), 4.52-4.62 (m, 1H), 5.12-5.20 (m, 2H), 5.73-5.88 (m, 1H), 7.20-7.23 (m, 1H) ppm. $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 18.1, 18.3, 38.9, 39.0, 47.6, 52.4, 52.5, 70.7, 70.8, 119.0, 119.3, 133.2, 172.9, 173.0, 173.2, 173.4 ppm. FTIR (neat): *v* 3077, 2955, 1770, 1455, 1301, 1259, 1225, 1159 $cm^{-1}$. LCMS calculated for $C_9H_{15}NO_4$ $[M+H]^+$ 202.10 found 202.4. HRMS calculated for $C_9H_{15}NO_4$ $[M+H]^+$ 202.1079 found 202.1083.

**(*S*)-2-{(*S*)-2-[(*S*)-2-(2-Hydroxy-pent-4-enoylamino)-3-phenyl-propionylamino]-4-methyl-pentanoylamino}-pentanedioic acid dimethyl ester (3b).**

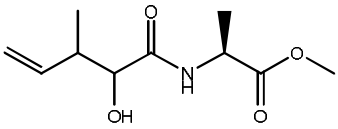


Yellow oil (0.17 g, 53% yield). $^{13}$C NMR analysis showed that the compound is in a diastereomeric ratio of 65:35. Rf = 0.475 (dichloromethane – methanol, 93:7). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88-0.92 (m, 7H), 1.25-1.27 (m, 3H), 1.46-1.72 (m, 4H), 1.95-2.61 (m, 7H), 3.01-3.19 (m, 2H), 3.68 (s, 3H), 3.75 (s, 3H), 4.12-4.14 (m, 1H), 4.39-4.47 (m, 1H), 4.52-4.59 (m, 1H), 4.66-4.77 (m, 1H), 5.07-5.16 (m, 2H), 5.52-5.78 (m, 1H), 6.74-6.83 (m, 1H), 7.18-7.32 (m, 7H) ppm.

$^{13}$C NMR (100 MHz, $CDCl_3$)

δ 22.0, 22.1, 22.7, 22.8, 24.6, 26.9, 27.0, 29.6, 30.0, 37.5, 37.7, 38.7, 38.9, 40.7, 40.8, 51.7, 51.9, 52.0, 52.1, 52.5, 53.4, 53.9, 54.4, 68.8, 70.8, 70.9, 119.2, 119.4, 127.0, 127.1, 128.7, 129.2, 132.9, 136.2, 170.8, 171.0, 171.9, 173.2, 173.3, 173.5, 173.6 ppm. FTIR (neat) *v* 3700, 3273, 3081, 2957, 1738, 1635, 1539, 1436, 1265, 1211, 1174, 715 cm$^{-1}$. LCMS calculated for $C_{27}H_{39}N_3O_8$ $[M+H]^+$ 534.61 found 534.07. HRMS calculated for $C_{27}H_{39}N_3O_8$ $[M+H]^+$ 534.2815 found 534.2816.

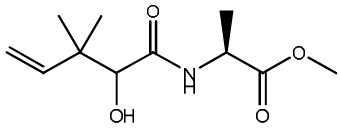
**(*S*)-2-(2-Hydroxy-3-methyl-pent-4-enoylamino)-propionic acid methyl ester (6).**



Pale transparent gum (0.068 g, 50% yield). Rf = 0.35 (hexane – ethyl acetate, 1:1). $^{13}$C NMR analysis showed that the compound is in a diastereomeric ratio of 23:24:25:28. $^{1}$H NMR ($CDCl_3$, 300 MHz) δ 0.97 (dd, *J* = 7.0, 10.4 Hz, 1.5H), 1.14 (dd, 1.4H, *J* = 0.8, 7.0 Hz), 1.39-1.44 (m, 3H), 2.75-2.82 (m, 1H), 3.0-3.5 (m, 1H), 3.74-3.75 (m, 3H), 4.02-4.14 (m, 1H), 4.55-4.63 (m, 1H), 5.09-5.18 (m, 2H), 5.73-5.96 (m, 1H), 7.00-7.28 (m, 1H) ppm. $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 11.7, 11.9, 15.4, 15.8, 18.1, 18.2, 18.3, 18.4, 40.6, 40.7, 41.2, 41.3, 47.6, 47.7, 47.7, 52.4, 52.5, 52.5, 52.5, 74.2, 74.3, 74.9, 75.1, 115.8, 116.0, 116.7, 117.0, 137.6, 137.7, 140.0, 140.1, 172.2, 172.3, 172.5, 172.6, 173.0, 173.1,

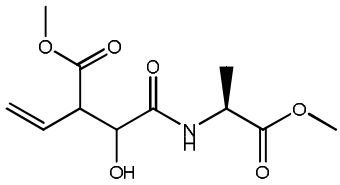
173.3, 173.4 ppm. FTIR (KBr, neat): *v* 3059, 2985, 1745, 1662, 1524, 1450, 1440, 1423, 1395, 1343, 1264, 1217, 1155, 1058, 1017, 924, 736 $cm^{-1}$. GCMS calculated for $C_{10}H_{17}NO_4$ [M] 215.25 found 215.98. HRMS calculated for $C_{10}H_{17}NO_4$ $[M+H]^+$ 216.1236 found 216.1236.

**(*S*)-2-(2-Hydroxy-3,3-dimethyl-pent-4-enoylamino)-propionic acid methyl ester (8).**



Pale transparent gum (0.065 g, 45% yield). *Rf* = 0.41 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 51:49. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.09 (s, 6H), 1.40 (dd, *J* = 4.0, 7.1 Hz, 3H), 3.20-3.22 (m, 0.4H), 3.47 (bs, 0.4H), 3.74, 3.80 (s, 3H), 3.81 (s, 1H), 4.54-4.58 (m, 1H), 5.09-5.18 (m, 2H), δ 5.87-5.95 (m, 1H), 6.79-6.90 (m, 1H) ppm. $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 18.2, 18.3, 22.3, 22.4, 22.5, 22.7, 41.4, 41.6, 47.8, 52.4, 52.5, 76.7, 77.0, 114.2, 114.4, 144.5, 144.7, 171.4, 171.7, 173.1, 173.3 ppm. FTIR (neat): *v* 2968, 1742, 1668, 1520, 1455, 1266, 1217, 1159, 738, 704 $cm^{-1}$. LCMS calculated for $C_{11}H_{19}NO_4$ $[M+H]^+$ 230.27 found 229.99. HRMS calculated for $C_{11}H_{19}NO_4$ $[M+H]^+$ 230.1392 found 230.1392.

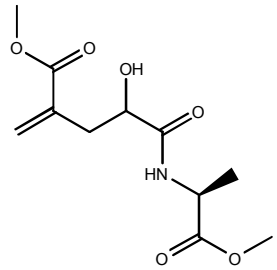
**2-[Hydroxy-((*S*)-1-methoxycarbonyl-ethylcarbamoyl)-methyl]-but-3-enoic acid methyl ester (10).**



Yellow oil (0.055 g, 34% yield). Rf = 0.23 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 50:35:1:4. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.39 (dd, *J* = 7.5, 12.6 Hz, 3H), δ 3.71 (s, 1.9H), δ 3.72 (s, 1.7H), 3.73 (s, 3H), 4.17-4.20 (m, 0.7H), 4.29-4.32 (m, 0.7H), 4.53-4.59 (m, 1.1H), 5.25-5.31 (m, 2H), 5.80-6.0 (m, 0.93H), 7.35 (s, 1H) ppm. $^{13}C$ NMR ($CDCl_3$, 50 MHz) δ 18.0, 18.1, 18.3, 47.6, 47.8, 47.9, 52.0, 52.2, 52.3, 52.4, 52.5, 71.9, 72.0, 72.5, 72.6, 120.0, 120.1, 120.9, 129.6, 129.7, 131.4, 170.3, 171.3, 171.4, 172.9, 173.1, 173.6 ppm. FTIR (neat) *v* 2956, 1734, 1663, 1533, 1457, 1437, 1217, 1162, 1101, 1061, 996, 931 $cm^{-1}$. LCMS

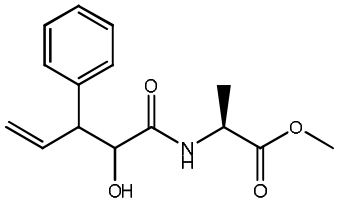
Calculated for $C_{11}H_{17}NO_6$ $[M+H]^+$ 260.26 found 260.92. HRMS Calculated for $C_{11}H_{17}NO_6$ $[M+H]^+$ 260.1134 found 260.1134.

**4-Hydroxy-4-((*S*)-1-methoxycarbonyl-ethylcarbamoyl)-2-methylene-butyricacid methyl ester (12).**




Yellow oil (0.031 g, 19% yield). Rf = 0.23 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 65:35. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.41 (dd, *J* = 7.2, 11.8 Hz, 3H), 2.64-2.71 (m, 1H), 2.91-2.96 (m, 1H), 3.74 (s, 3.3H), 3.82 (s, 2.5H), 4.24-4.25 (m, 1H), 4.43 (bs, 0.4H), 4.54-4.60 (m, 1.4H), 5.81-5.82 (m, 1H), 6.28-6.29 (m, 1H), 7.30-7.4 (m, 1H) ppm. $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 18.2, 18.5, 37.3, 47.6, 47.7, 52.4, 52.5, 52.6, 52.7, 71.9, 72.2, 129.7, 130.0, 135.8, 135.9, 169.7, 169.9, 172.3, 173.0. FTIR (neat) *v* 3150, 2955, 1722, 1658, 1652, 1527, 1454, 1439, 1339, 1312, 1213, 1155, 1091, 1060, 955, 735 $cm^{-1}$. LCMS calculated for $C_{11}H_{17}NO_6$ $[M+H]^+$ 260.26 found 260.94. HRMS calculated for $C_{11}H_{17}NO_6$ $[M+H]^+$ 260.1134 found 260.1134.

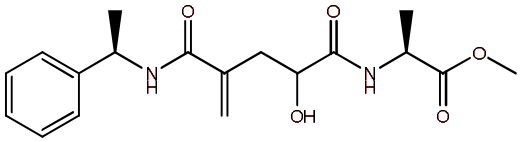
**(*S*)-2-(2-Hydroxy-3-phenyl-pent-4-enoylamino)-propionic acid methyl ester (14).**




Pale transparent gum (0.044 g, 25% yield). Rf = 0.28 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 45:44:6:5. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.94 (d, *J* = 7.0 Hz, 1H), 1.23-1.31 (m, 2H), 3.57, 3.61, 3.66 (s, 3H), 3.80-3.86 (m, 1H), 4.26-4.40 (m, 2H), 5.09-5.17 (m, 2H), 6.06-6.22 (m, 1H), 6.72-6.78 (m, 1H), 7.13-7.27 (m, 5H) ppm. $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 18.1, 18.3, 18.4, 18.5, 47.4, 47.8, 52.5, 52.6, 52.7, 52.9, 53.1, 53.2, 74.9, 75.0, 75.5, 75.7, 117.1, 117.4, 118.6, 118.8, 127.0, 127.1, 127.2, 127.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8,

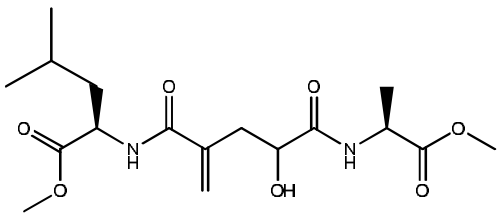
129.1, 129.4, 135.0, 137.5, 137.9, 138.5, 138.6, 140.8, 171.7, 171.8, 171.8, 9, 172.0, 172.0, 172.9, 173.1, 173.4 ppm. FTIR (neat) *v* 3077, 2955, 1770, 1455, 1301, 1259, 1225, 1159 $cm^{-1}$. LCMS calculated for $C_{15}H_{19}NO_4$ $[M+H]^+$ 278.32 found 278.30. HRMS calculated for $C_{15}H_{19}NO_4$ $[M+H]^+$ 278.1392 found 278.1392.

**(*S*)-methyl-2-(2-hydroxy-4-((*R*)-1-phenylethylcarbamoyl)pent-4-enamido) propanoate (16).**



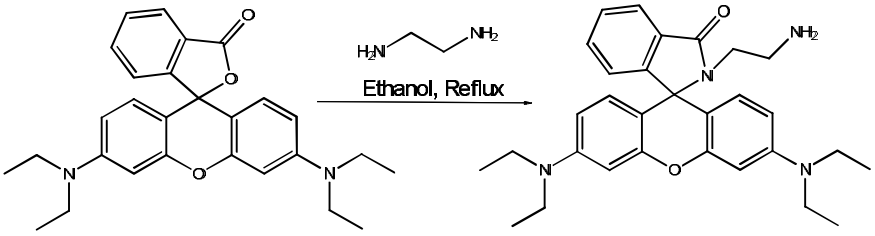
White solid. (0.80 g, 37% yield). Rf = 0.13 (hexane – ethyl acetate, 1:1). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 63:37. $^{1}H$ NMR ($CDCl_3$, 400 MHz) δ 1.33 (d, *J* = 7.2 Hz, 1H), 1.41 (d, *J* = 7.2 Hz, 2H), 1.53 (d, *J* = 6.9 Hz, 3H), 2.61-2.69 (m, 1H), 2.75-2.81 (m, 1H), 3.71 (s, 3H), 4.14-4.17 (m, 1H), 4.49-4.57 (m, 1H), 5.08-5.15 (m, 1H), 5.50 (d, *J* = 8.3 Hz, 1H), 5.66 (d, *J* = 5.5 Hz, 1H), 6.61-6.70 (m, 1H), 6.88-6.92 (m, 1H), 7.25-7.34 (m, 5H), 7.45-7.53 (m, 1H) ppm. $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 18.2, 18.5, 21.6, 21.7, 37.3, 37.4, 47.6, 47.7, 49.5, 49.6, 52.4, 52.5, 72.5, 72.8, 122.2, 122.3, 126.1, 126.1, 127.6, 128.8, 140.8, 140.9, 142.7, 170.3, 170.5, 172.9, 173.0, 173.0, 173.2 ppm; FTIR (neat) *v* 3400, 2900, 1741, 1654, 1608, 1529, 1452, 1436, 1232, 1159, 700 $cm^{-1}$. LCMS calculated for $C_{18}H_{24}N_2O_5$ $[M+H]^+$ 349.17 found 349.06. HRMS calculated for $C_{18}H_{24}N_2O_5$ $[M+H]^+$ 349.1763 found 349.1764.

**(*R*)-2-[4-Hydroxy-4-((S)-1-methoxycarbonyl-ethylcarbamoyl)-2-methylene-butyrylamino]-4-methyl-pentanoic acid methyl ester (18).**



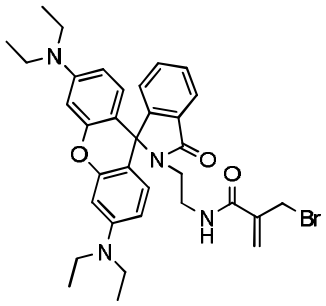
White solid (0.10 g, 46% yield). $^{13}C$ NMR analysis showed that the compound is in a diastereomeric ratio of 60:40. *Rf* = 0.13 (hexane – ethyl acetate, 1/1). $^{1}H$ NMR ($CDCl_3$, 400 MHz) δ 0.94-0.96(m, 6H), 1.39 (dd, *J* = 7.2, 23.4 Hz, 3H), 1.57-1.72 (m, 3H), 2.52-2.93 (m, 2H), 3.71, 3.72, 3.74, 3.75 (s, 6H), 4.16-4.25 (m, 1H), 4.56-4.62 (m, 2H), 5.52 (d, *J* = 16 Hz, 1H), 5.66 (d, *J* = 8 Hz, 1H), 6.80-6.82 (m, 0.3H), 7.12-7.14 (m, 0.5H),

7.57-7.64 (m, 1H) ppm. $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 18.0, 18.7, 22.0, 22.1, 22.9, 23.0, 25.2, 37.7, 37.2, 38.0, 47.6, 47.7, 51.4, 51.5, 52.5, 52.6, 52.7, 52.8, 72.2, 73.0, 122.5, 122.6, 140.5, 140.7, 142.4, 171.0, 171.9, 172.7, 172.9, 173.5, 173.6, 173.7, 173.9 ppm. FTIR (KBr, neat): *v* 2956, 1734, 1663, 1533, 1457, 1437, 1217, 1162, 1101, 1061, 996, 931 cm$^{-1}$. LCMS calculated for $C_{17}H_{28}N_2O_7$ $[M + H]^+$: 373.41 found 373.01. HRMS calculated for $C_{17}H_{28}N_2O_7$ $[M + H]^+$: 373.1975 found 373.1975.

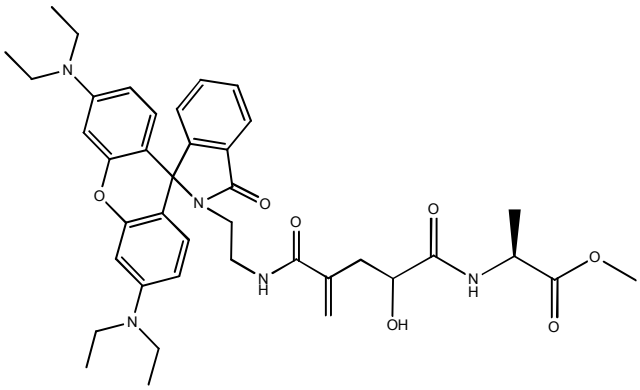



To a solution of rhodamine B (1.2g, 2.5 mmol) in ethanol (30 mL) in a round bottom flask was added ethylenediamine (0.22 mL, 3.25 mmol) dropwise at room temperature with vigorous stirring. The stirred mixture was then heated to reflux overnight while the solution became clear. Upon reaction completion as judged from LCMS analysis, the reaction was cooled and solvent evaporated under reduced pressure. 1 M HCl (50 mL) was added to the residue to remove any unreacted amine followed by slow addition of 1M NaOH with stirring until the solution pH was around 9-10. The aqueous phase was extracted with dichloromethane (3 X 50 mL), the combined organic phase washed with water (30 mL), dried over $Na_2SO_4$, filtered and the solvent evaporated under reduced pressure. The crude product thus obtained was purified by flash silica gel column chromatography (dichloromethane – methanol, 95:5) to give desired product as a pink foam (0.9 g, 75% yield). $^1$H NMR (300 MHz, $CDCl_3$): δ 1.14 (t, *J* = 7.2 Hz, 12H), 2.67 (t, *J* = 5.4 Hz, 2H), 3.24-3.34 (m, 10H), 6.25 (dd, *J* = 2.6, 8.9 Hz, 2H), 6.35 (d, *J* = 2.3 Hz, 2H), 6.40 (s, 1H), 6.42 (s, 1H), 7.04-7.06 (m, 1H), 7.40-7.43 (m, 2H), 7.83-7.86 (m, 1H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$) δ 12.7, 31.0, 41.0, 41.7, 44.4, 53.6, 65.9, 97.9, 104.7, 108.5, 123.0, 124.0, 128.3, 128.6, 1 30.6, 132.9, 149.0, 153.4, 153.7, 169.7 ppm. FTIR (neat): *v* 3400, 2970, 1680, 1633, 1614, 1514, 1467, 1427, 1357, 1265, 1232, 1118, 680 cm$^{-1}$. LCMS calculated for

$C_{30}H_{36}N_4O_2$ $[M+H]^+$ 484.63 found 484.42. HRMS calculated for $C_{30}H_{36}N_4O_2$ $[M+H]^+$ 485.2917 found 485.2917.



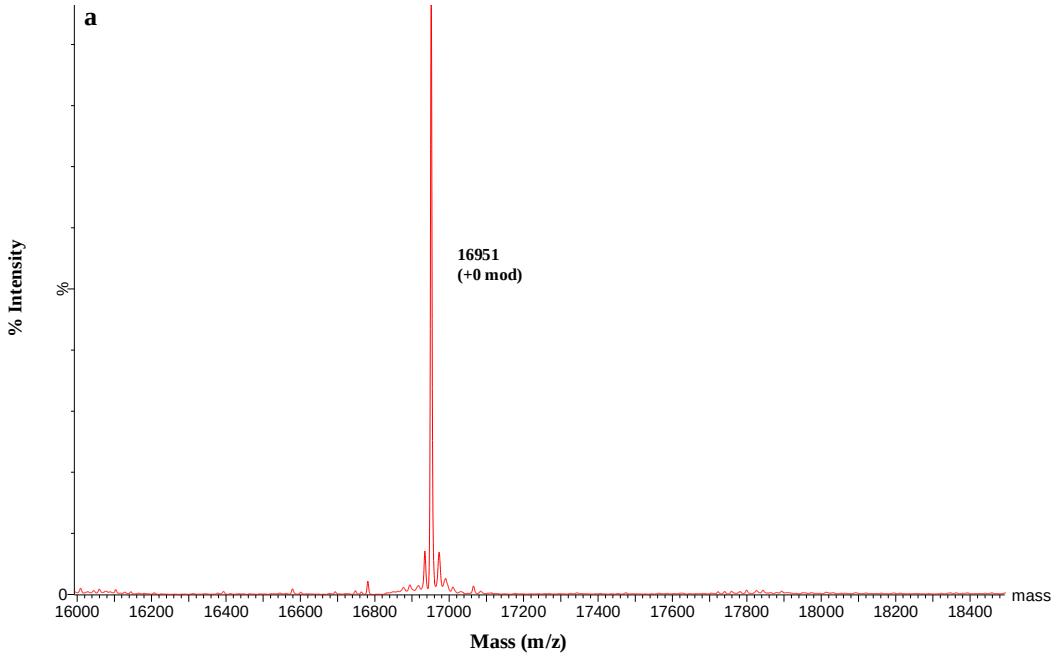
**(19).** To a solution of 2-bromomethyl-acrylic acid (0.29 g, 1.77 mmol) in dry dichloromethane (5 mL) at 0 °C under $N_2$ was added thionyl chloride (0.4 mL, 5.34 mmol) and heated to reflux for 2 h. After removing the excess thionyl chloride in vacuo the formed acyl chloride was redissolved in dry dichloromethane (10 mL) and cooled to 0 °C in an ice bath. To this stirring solution was added a solution of *N*,*N*-dimethylamino pyridine (0.02 g, 0.18 mmol) and rhodamine amine (0.77 g, 1.6 mmol) in dry dichloromethane (10 mL). The reaction was stirred for 18 h while the temperature was allowed to rise to room temperature. Upon reaction completion the solvent was evaporated under reduced pressure. The residue was redissolved in ethyl acetate (50 mL) and washed successively with water (20 mL), sat. $NaHCO_3$ (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered and solvent evaporated in vacuo under reduced pressure. The crude product thus obtained was purified by flash silica gel column chromatography. Obtained as a purple solid (0.82 g, 78%). Rf = 0.23 (hexane – ethyl acetate, 3:7). $^1$H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, *J* = 7.0 Hz, 12H), 3.04-3.09 (m , 2H), 3.33 (q, *J* = 7.0 Hz, 10H), 4.31 (s, 2H), 5.71 (s, 1H), 5.94 (s, 1H), 6.26 (dd, *J* = 2.6, 8.8 Hz, 2H), 6.37 (d, *J* = 2.3 Hz, 2H), 6.41 (s, 1H), 6.44 (s, 1H), 7.06-7.09 (m, 1H), 7.43-7.46 (m, 2H), 7.79 (brs, 1H), 7.89-7.92 (m, 1H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$)
δ 12.7, 39.9, 41.4, 43.7, 44.6, 66.1, 98.0, 104.7, 108.6, 122.1, 123.1, 124.1, 128.4, 128.6, 130.4, 133.1, 140.8, 149.1, 153.4, 154.0, 166.0, 170.5 ppm. FTIR (neat): *v* 3444, 2970, 2927, 2378, 1666, 1631, 1614, 1546, 1514, 1467, 1427, 1398, 1355, 1267, 1118, 949 $cm^{-1}$. LCMS calculated for $C_{34}H_{39}BrN_4O_3$ $[M+H]^+$ 633.60 found 633.10. HRMS calculated for $C_{34}H_{39}BrN_4O_3$ $[M+H]^+$ 631.2284 found 631.2284.

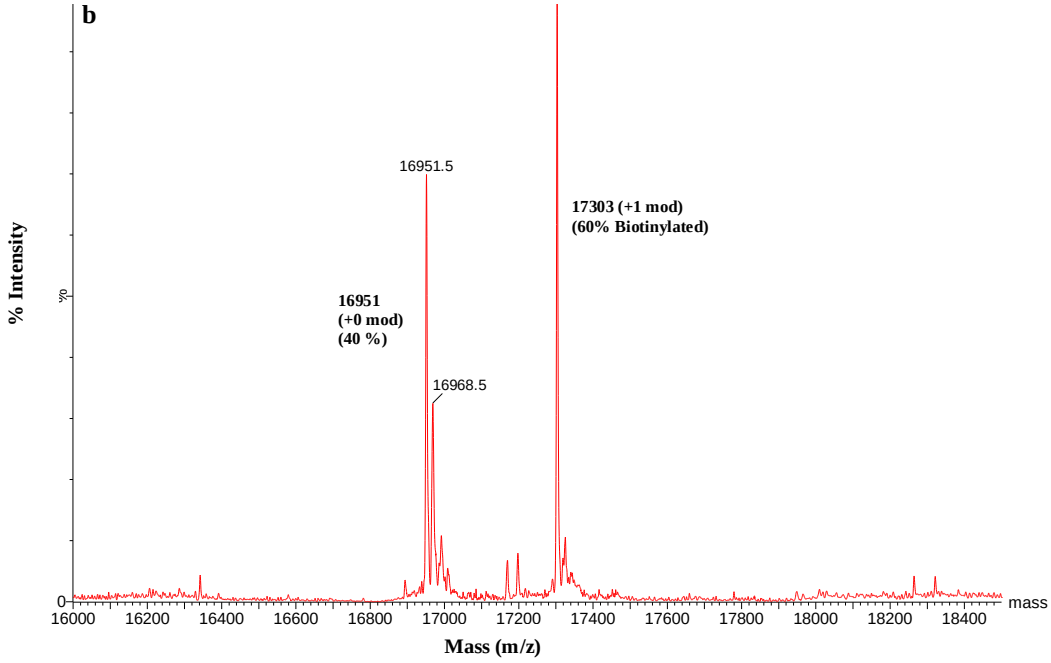


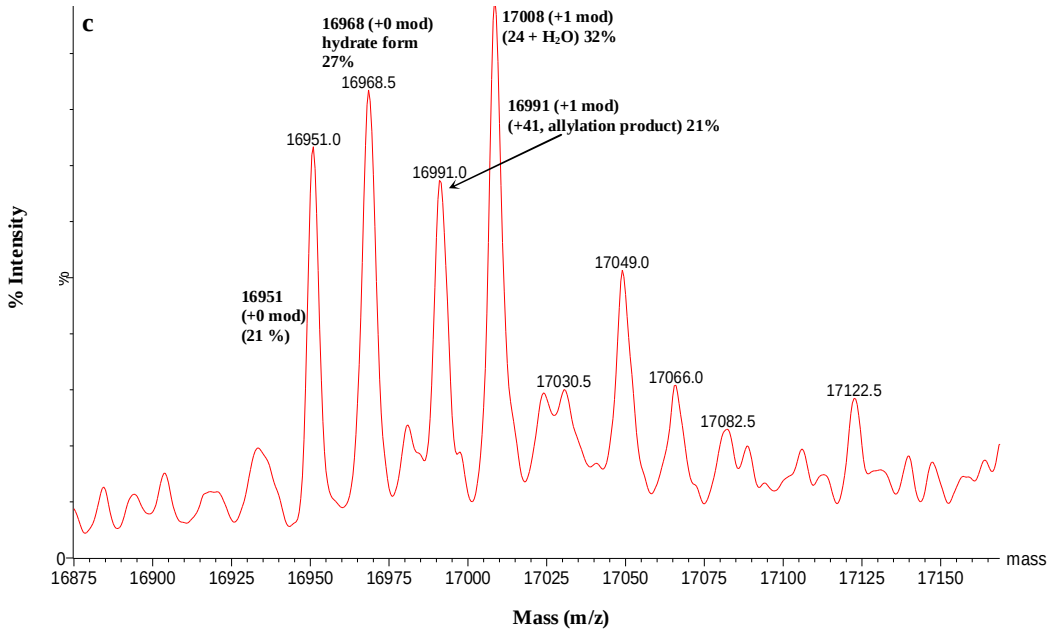
**(20).** Obtained as a purple gum (0.32 g, 72% yield). *Rf* = 0.12 (hexane – ethyl acetate, 3:7). $^{1}$H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, *J* = 7.0 Hz, 12H), 1.28-1.41 (m, 3H), 2.57-2.68 (m, 1H), 2.74-2.81 (m, 1H), 2.99-3.04 (m , 2H), 3.30-3.37 (m, 10H), 3.66, 3.71, 3.75, 3.76, 3.77 (s, 3H), 4.13-4.18 (m, 1H), 4.50-4.63 (m, 1H), 5.54, 5.57 (s, 1H), 5.78, 5.87 (s, 1H), 6.26-6.30 (m, 2H), 6.38-6.44 (m, 4H), 7.08-7.10 (m, 1H), 7.45-7.48 (m, 2H), 7.88-7.91 (m, 1H), 8.02-8.05 (m, 1H) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$) δ 12.6, 18.1, 18.6, 37.3, 37.4, 41.6, 44.3, 44.6, 47.5, 47.6, 52.2, 52.3, 66.0, 72.5, 72.9, 97.7, 104.4, 108.3, 122.7, 122.8, 122.9, 123.9, 128.3, 130.0, 130.2, 133.0, 140.2, 149.0, 153.2, 153.7, 170.8, 170.9, 171.17, 172.9, 173.0, 173.1 ppm. FTIR (neat): *v* 3725, 3316, 2969, 2925, 2171, 2157, 1712, 1669, 1633, 1613, 1513, 1467, 1427, 1375, 1355, 1328, 1304, 1265, 1217, 1153, 1117, 1091, 1016, 819, 788, 758, 733, 701, 670, 576, 537. LCMS calculated for $C_{40}H_{49}N_5O_7$ $[M+H]^+$ 712.85 found 712.43. HRMS calculated for $C_{40}H_{49}N_5O_7$ $[M+H]^+$ 712.3710 found 712.3710.

**General Procedure for Protein Modification.** To a solution of myoglobin (120 µL of 250 µM solution in 25 mM sodium phosphate buffer, pH 6.5) and sodium phosphate buffer (180 µL, 25 mM, pH 6.5) in a 1.6 mL eppendorf tube was added a solution of pyridoxal 5´-phosphate (**21**, 300 µL of a 20 mM solution in 25 mM phosphate buffer, pH adjusted to 6.5 with 2 M NaOH). After brief agitation for proper mixing, the mixture was incubated at 37 ºC for 18 h without further agitation. The PLP was removed from the reaction mixture via spin concentration (Nanosep centrifugal filter 10,000 MWCO (PALL)) eluting with water. An aliquot of this purified protein (100 µL) was treated with biotinamidohexanoic acid hydrazide (**22**, 80 µL, 25 mM solution in 16% EtOH-water

system) and water (20 µL). The reaction was allowed to sit without agitation for another 18 h (Scheme **2**). After freeze drying the reaction mixture was analyzed by LC-ESI-MS (Poros R1/H column), SDS-PAGE and Western Blotting. LC-ESI-MS (**23**, *m/z* 17303), gel analysis and western blotting confirmed biotinylation and hence generation of *N*-terminal aldehyde (**Figure S1**).




b
% Intensity
%
16951.5
17303 (+1 mod)
(60% Biotinylated)
16951
(+0 mod)
(40 %)
16968.5
0
16000
16200
16400
16600
16800
17000
17200
17400
17600
17800
18000
18200
18400
mass
Mass (m/z)


c
% Intensity
%
16968 (+0 mod)
hydrate form
27%
16968.5
17008 (+1 mod)
(24 + H2O) 32%
16991 (+1 mod)
(+41, allylation product) 21%
16951.0
16991.0
17049.0
16951
(+0 mod)
(21 %)
17030.5
17066.0
17122.5
17082.5
0
16875
16900
16925
16950
16975
17000
17025
17050
17075
17100
17125
17150
mass
Mass (m/z)

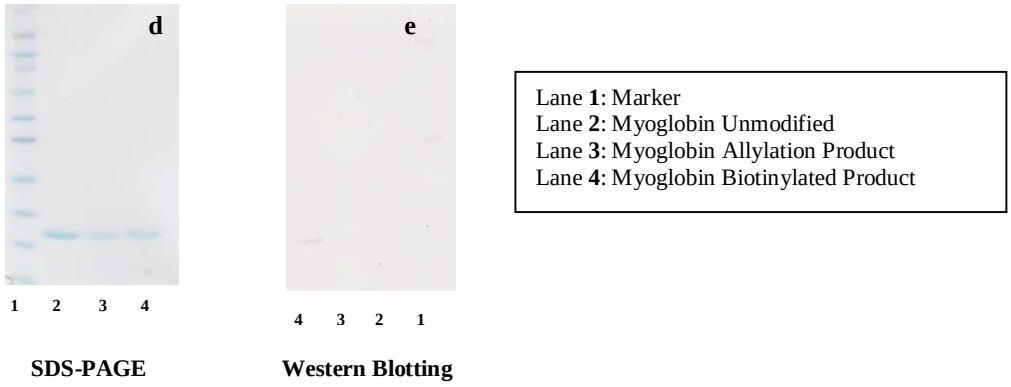
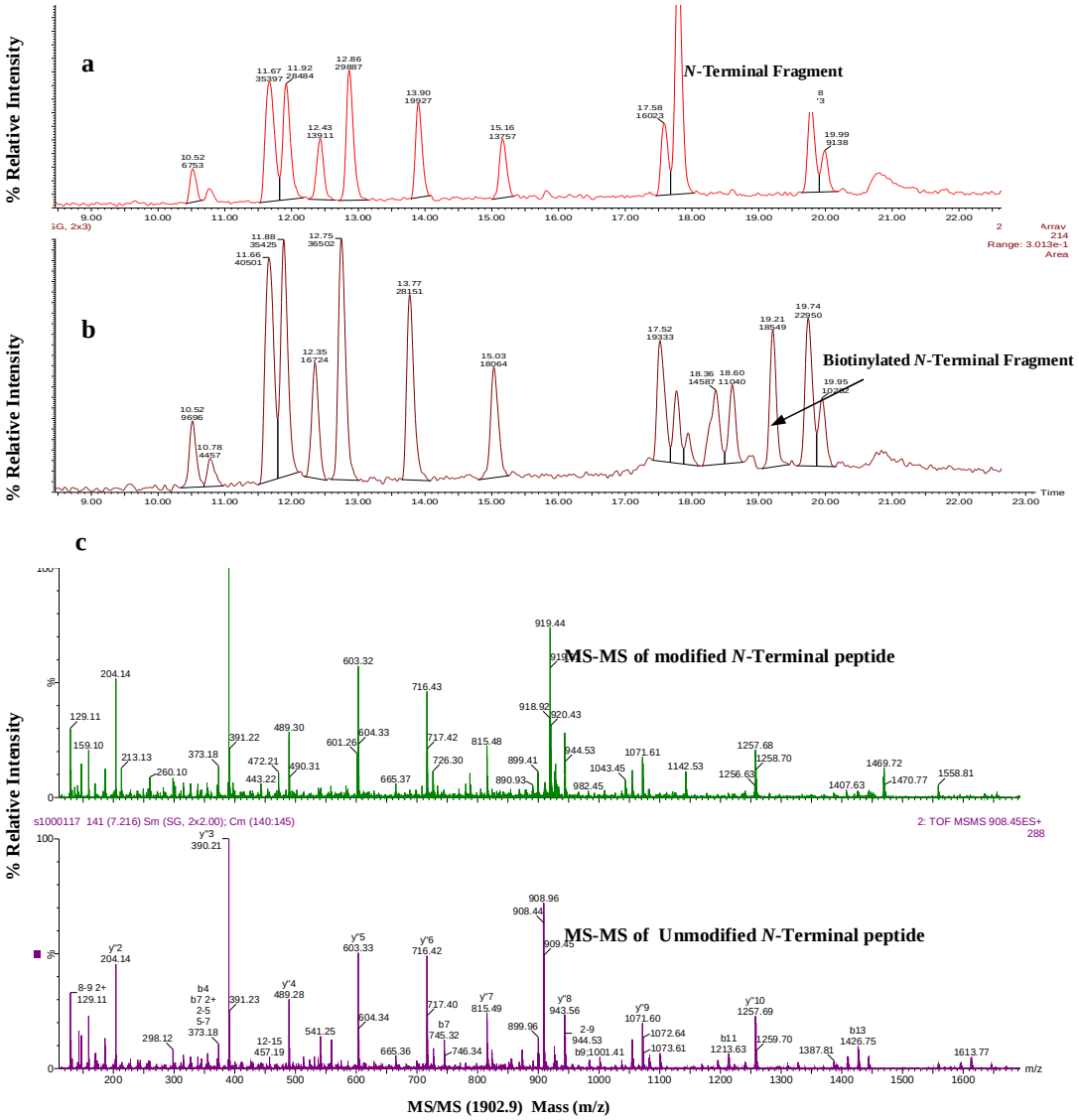



**Figure S1:** LC-ESI-MS and Western Blotting analysis of modified horse heart Myoglobin. **a)** Unmodified Myoglobin. **b)** Biotinylated Myglobin **23**. **c)** Allylation modified myoglobin **24**. **d)** SDS-PAGE analysis of modified products upon coomassie staining. **e)** Western blot analysis of the biotinylated product using Avidin-AP.

**General Procedure for Protein Functionalization by Indium-mediated Allylation reaction.** After preparing the aldehyde containing myoglobin by the procedure described above, an aliquot of the purified mixture (100 µL, 37.5 µM final protein concentration) was added to a pre-vortexed suspension of allylbromide (120 µL, 2.09 mM in *t*-BuOH), Indium (0.001 g, .008µmol) and water (80 µL); after brief vortex mixing and sonication the reaction mixture was incubated on a laboratory rotisserie for 20 h at room temperature. The excess reagents were removed by spin concentration (Nanosep centrifugal filter 10,000 MWCO (PALL)) and the reaction product freeze dried before subjecting to LC-ESI-MS (Poros R1/H column) analysis. LC-ESI-MS confirmed 54% conversion to desired allylation product **24** (*m/z* 16099) (**Figure S1, c**).

**General Procedure for Protein Control Reaction.** In the control reaction the aldehyde generation reaction was inhibited by performing the reaction without PLP under identical conditions. After spin concentration (Nanosep centrifugal filter 10,000 MWCO (PALL)) eluting with water, an aliquot of the mixture (100 µL, 37.5 µM final protein concentration) was added to a pre-vortexed suspension of allylbromide (120 µL, 2.09 mM in *t*-BuOH), Indium (0.001 g, .008µmol) and water (80 µL); after brief vortex mixing and sonication the reaction mixture was incubated on a laboratory rotisserie for 20 h at room temperature. Upon spin concentration (Nanosep centrifugal filter 10,000 MWCO (PALL)) and freeze drying the reaction mixture was analyzed by LC-ESI-MS (Poros R1/H column) revealing only unmodified myoglobin.

**Procedure for Trypsin Digestion of Myoglobin.** Proteolytic protein digests by trypsin were performed by incubating the desalted and concentrated protein (40 µg) with 8M urea (5 µL in 0.4M $NH_4HCO_3$), water (44 µL), tris (1 µL, pH 10) and trypsin (Promega, 1 µL, 1 µg/µL) at 37 ºC overnight. The resulting peptide fragments were then analyzed by LC-ESI-MS using Vydac C18 Column (1 X 150 mm), at 40 ºC and at a flow rate of 0.08 mL/min with eluent containing 90% acetonitrile (with 0.045% trifluoroacetic acid) and 0.05% trifluoroacetic acid (**Figure S4**).



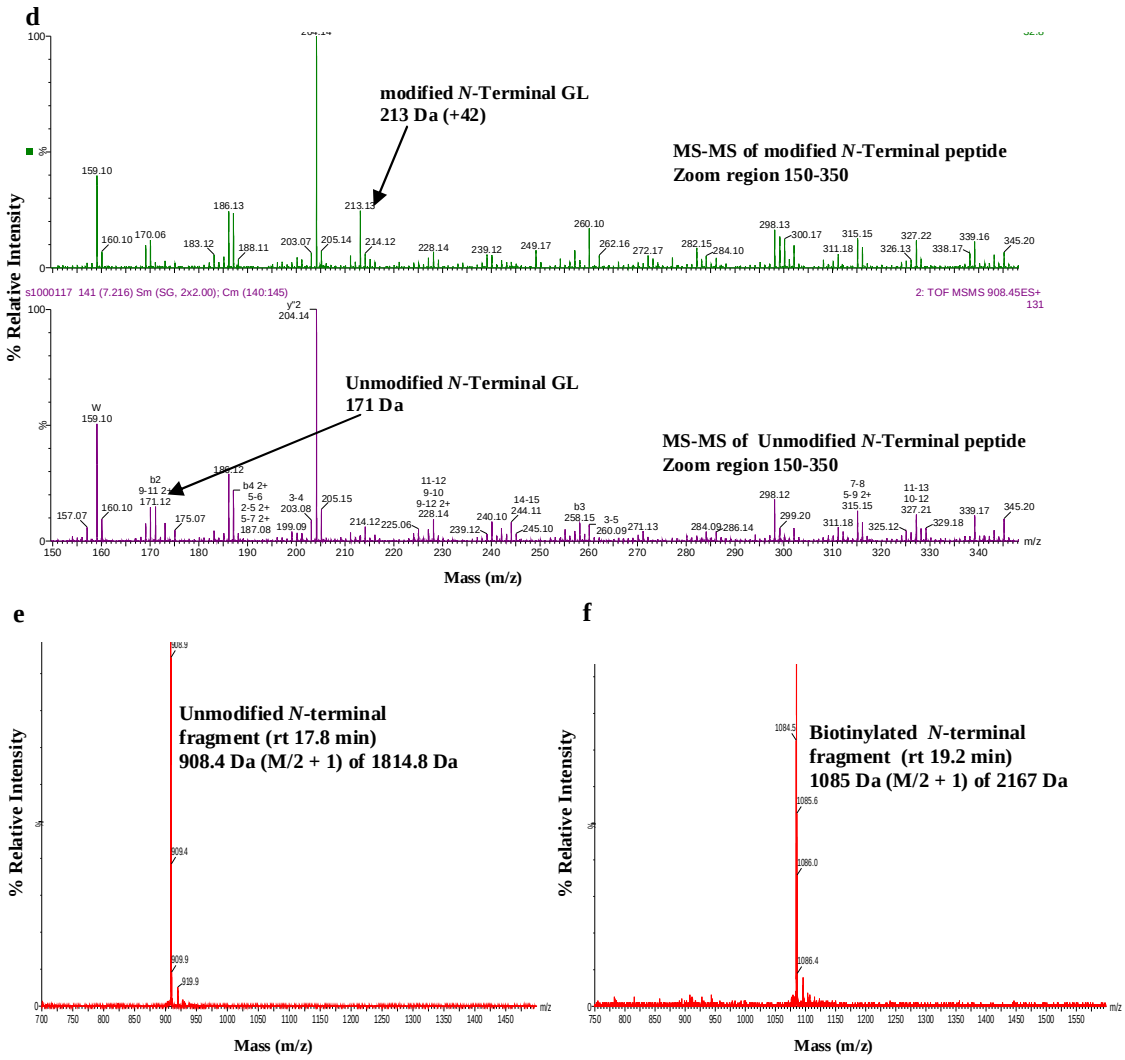



**Figure S2.** HPLC-ESI-MS analysis of trypsin digest of myoglobin. **a)** Unmodified protein, the *N*-terminal fragment (rt 17.8 min) **(residues 1-16)** GLSDGEWQQVLNVWGK (expected mass = 1815, observed mass = 1814.9). **b)** Biotinylated protein **23** (rt 19.2 min) **(residues 1-16)** (expected mass + 352 = 2167, observed mass = 2167.0). 352 is the expected mass increase for myoglobin after modification with biotinamidohexanoic acid hydrazide to form the hydrazone. **c)** MS-MS of Allylation modified and unmodified *N*-terminal peptide **24**. **d)** MS-MS of *N*-terminal fragment **(zoom region 150-350)** GL (expected mass = 171, observed mass = 213 (+42)). 42 is the expected mass increase for myoglobin after modification with allyl bromide though indium mediated allylation. **e)** MS of unmodified protein, *N*-terminal fragment **(residues 1-16)** (expected mass = 1815, observed mass = 908.4, (M/2 + 1)). **f)** MS of Biotinylated protein, *N*-terminal fragment **(residues 1-16)** (expected mass + 352 = 2167, observed mass = 1085, (M/2 + 1)).

**Reconstitution of Modified Horse Heart Myoglobin.** A 500 µL of aliquot of spin concentrated (Nanosep centrifugal filter, 10,000 MWCO (PALL)) allylation modified myoglobin (30 nmol) was diluted with 1900 µL of 100 mM $Na_2HPO_4$ (pH 7.0). A solution of chlorohemin (20 µL, 1.0 mg/mL, 30 nmol) in 100 mM NaOH was then added and the reaction mixture incubated on a laboratory rotisserie for 20 h at room temperature. After filtration the modified, reconstituted sample was analyzed by UV-Vis spectrum that revealed a strong absorbance at $\lambda_{max}$ = 409 nm. This 409 nm absorbance is characteristic of the heme moiety bound to myoglobin.[3] The obtained UV-Vis spectrum was in good agreement with data obtained from a sample of unmodified myoglobin (**Figure S3**).

**UV-Vis Spectroscopy.** The modified reconstituted sample was analyzed by UV-Vis spectrum that revealed a strong absorbance at $\lambda_{max}$ = 409 nm. This 409 nm absorbance is characteristic of the heme moiety bound to myoglobin.[3] The obtained UV-Vis spectrum was in good agreement with data obtained from a sample of unmodified myoglobin (**Figure S3**).

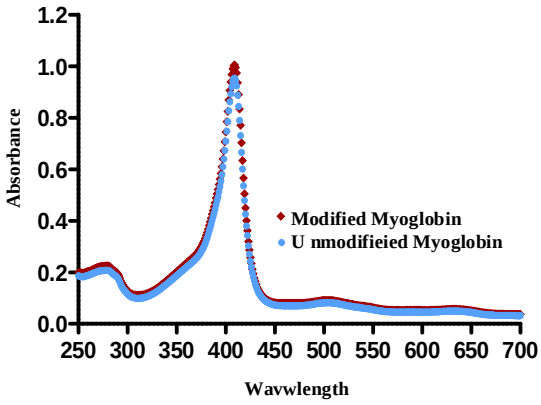



**Figure S3.** UV-Vis spectra of the modified reconstituted and unmodified myoglobin.

**Circular Dichroism Spectroscopy.** Myoglobin samples (3 µM) were prepared in 100 mM sodium phosphate buffer (pH 7.0). Circular dichroism spectra were recorded with Jasco Model J-810 spectrophotometer. Protein solutions were placed in a cylindrical, water-jacketed quartz cell (1-cm path length, volume 3.0 mL, 25 ºC), and the average of three scans from 190 to 250 nm was collected. The ellipticity values were plotted against wavelength using prizm software. The ultraviolet CD spectra exhibited by the modified

reconstituted sample suggested that the secondary structure of the modified protein remained essentially unperturbed relative to that of unmodified myoglobin (**Figure S4**).

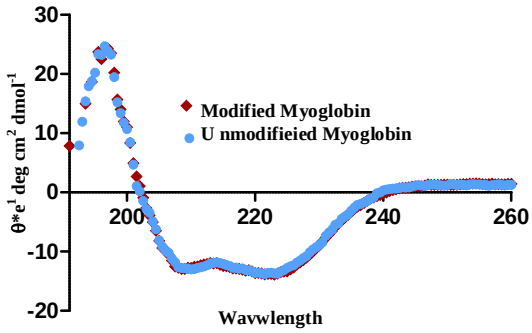



**Figure S4.** CD spectra of the modified reconstituted and unmodified myoglobin.

**Allyl Bromide Quantity Screening.** Samples of purified aldehyde containing myoglobin, (100 µL, 37.5 µM, final protein concentration in reaction) were treated with different concentrations of pre-vortexed suspension of Allyl Bromide **4**, (120 µL, X mM, in *t*-BuOH), Indium (0.001 g, .008µmol) and (80 µL) of $H_2O$. After brief vortex mixing and sonication the reaction mixture was incubated on a laboratory rotisserie for 20 h at room temperature. Upon spin concentration and freeze drying the reaction mixtures were analyzed by LC-ESI-MS (Poros R1/H column). 53% conversion to allylation product is achieved with as low as (67 equivalent) of allyl bromide (Table S1, entry 3).

**Table S1.** Equivalent of allyl bromide screening

| Entry | Equivalent of Allyl Bromide | % Conversion[b] | Remarks |
|---|---|---|---|
| 1 | 200 | 53 | |
| 2 | 100 | 53 | |
| 3 | 67 | 53 | |
| 4 | 50 | 35 | Complex mixture |

[b] Based on LC-ESI-MS peak integrations.

**Solvent Studies.** Samples of purified aldehyde containing myoglobin, (100 µL, 37.5 µM) were added to pre-vortexed suspension of allyl bromide (120 µL, 2.09 mM, in *t*-BuOH), Indium (0.001 g, .008µmol) and (80 µL) different solvent system. After brief vortex

mixing and sonication the reaction mixture was incubated on a laboratory rotisserie for 20 h at room temperature. Upon spin concentration and freeze drying the reaction mixtures were analyzed by ESI-LCMS (Poros R1/H column). As seen in Table S1, highest conversion to allylation modified product is achieved in water:*tert*-butyl alcohol (6:4) system (Table S2, Entry 7).

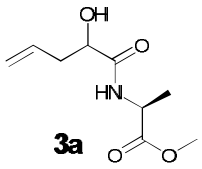
**Table S2.** Solvent study for the Allylation reaction.

| Entry | Solvent | Equivalent of Allyl Bromide | % Conversion[b] |
|---|---|---|---|
| 1 | 25 mM NaPi (pH 6.5) | 200 | 0 |
| 2 | $H_2O$ | 200 | 0 |
| 3 | *t*-BuOH:25 mM NaPi (pH 6.5) (1:1) | 200 | trace |
| 4 | *t*-BuOH:$H_2O$ (1:1) | 200 | 53 |
| 5 | *t*-BuOH:$H_2O$ (1:1) | 100 | 53 |
| 6 | *t*-BuOH:$H_2O$ (1:1) | 67 | 53 |
| 7 | *t*-BuOH:$H_2O$ (4:6) | 67 | 53 |
| 8 | *t*-BuOH:$H_2O$ (3:7) | 67 | 0 |
| 10 | *t*-BuOH:$H_2O$ (1:4) | 67 | 0 |
| 11 | Ethylene glycol:$H_2O$ (1:4) | 67 | 0 |
| 12 | Ethylene glycol:$H_2O$ (1:1) | 67 | 0 |
| 13 | DMF:$H_2O$ (1:1) | 67 | 0 |

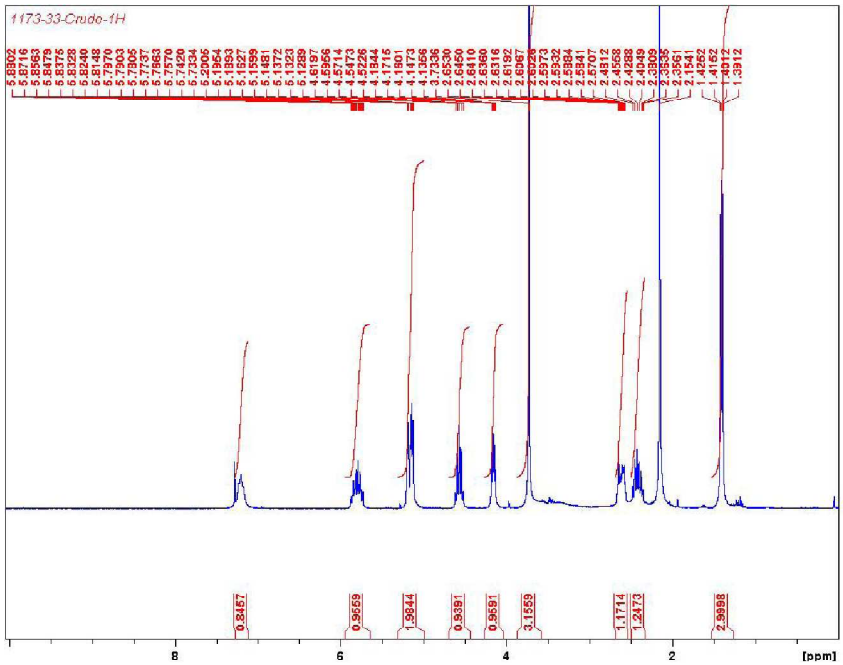
[b] Based on LC-ESI-MS peak integrations.

**References:**

1. Laemmli, U. K. *Nature* **1970**, *227*, 680.
2. Alam, J.; Keller, T. H.; Loh, T. P. *J. Am. Chem. Soc.* **2010,** *132*, 9546-9548.
3. Makinen, M. W.; Churg, A. K. In *Iron Porphyrins*; Lever, A. B. P.; Gray, H. B., Eds.; Addison-Wesley: Reading, 1983; Vol. 1, pp 141-236.


OH
O
HN
O
O

**3a**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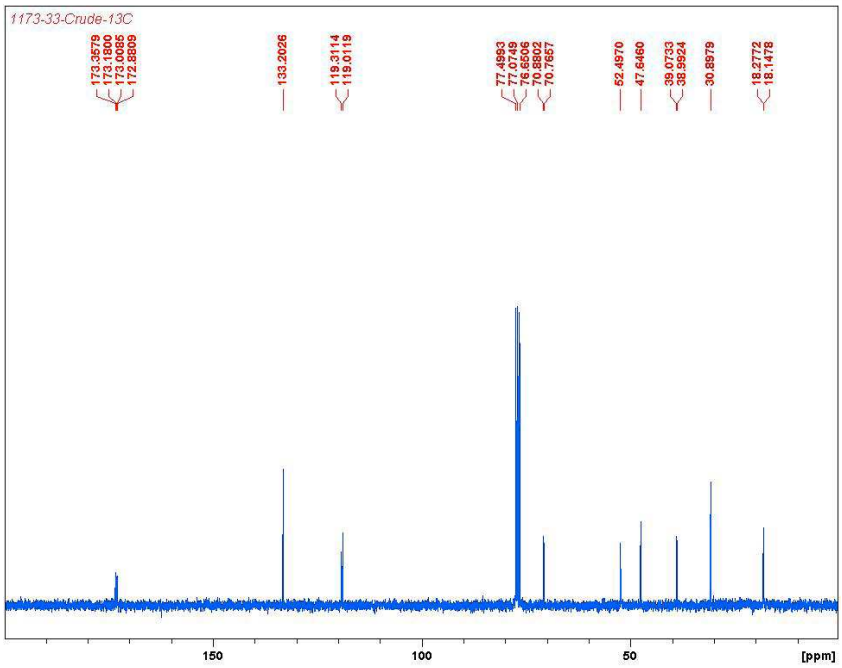
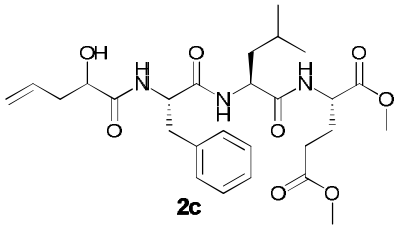
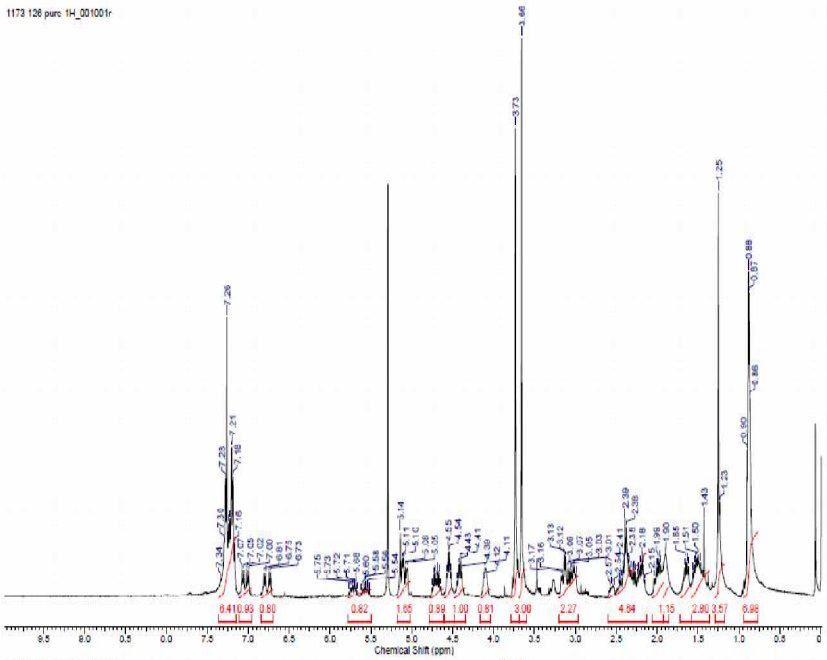
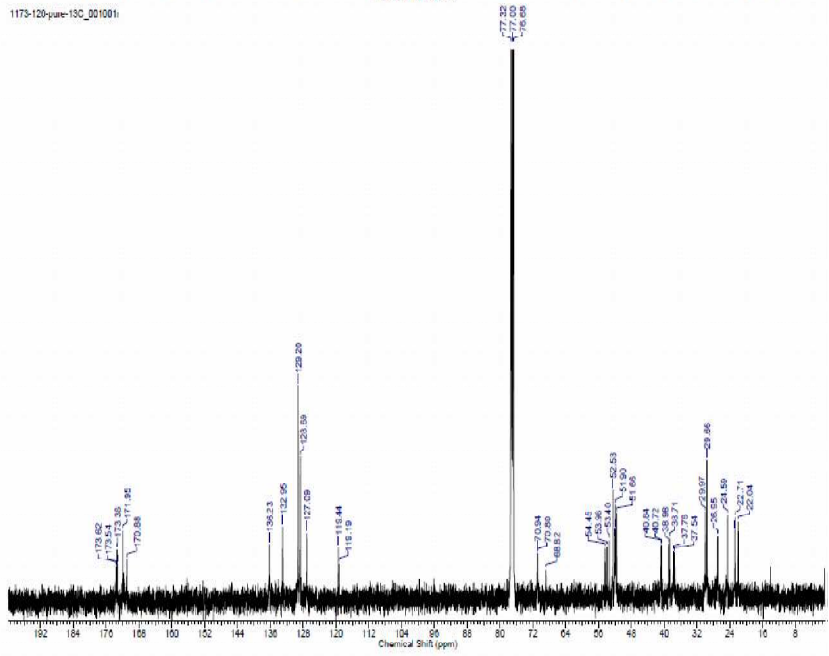
0.8457
0.9559
1.9844
0.8391
0.9591
3.1559
1.1714
1.2473
2.9998
8
6
4
2
[ppm]

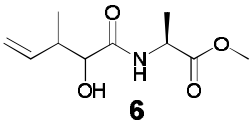

1173-33-Crude-13C
173.3579
173.1800
173.0085
172.8809
133.2026
119.3114
119.0119
77.4893
77.0749
76.6606
76.4562
70.7657
52.4970
47.6460
39.0733
38.9924
30.8979
18.2772
18.1478
150
100
50
[ppm]



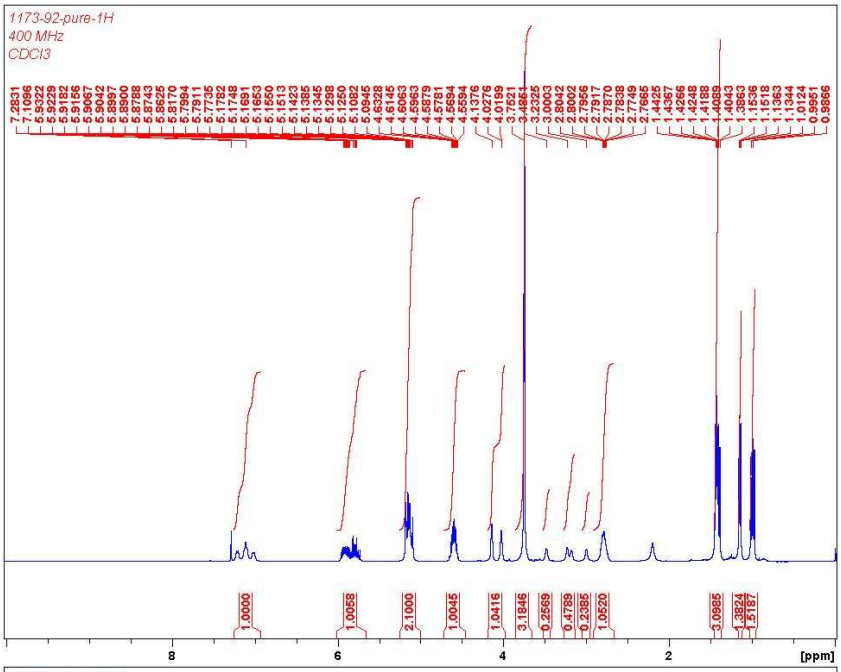
**2c**







**6**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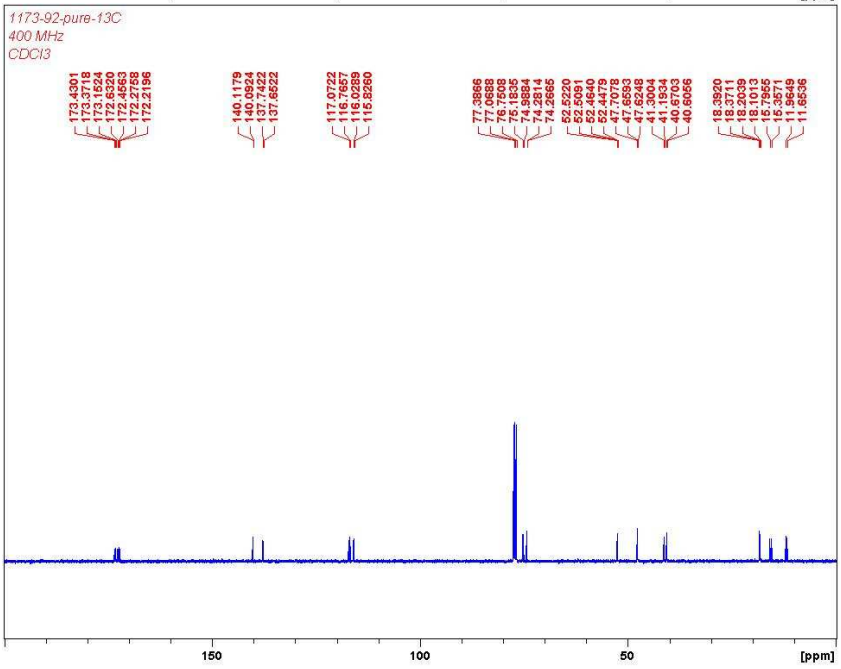
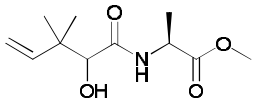
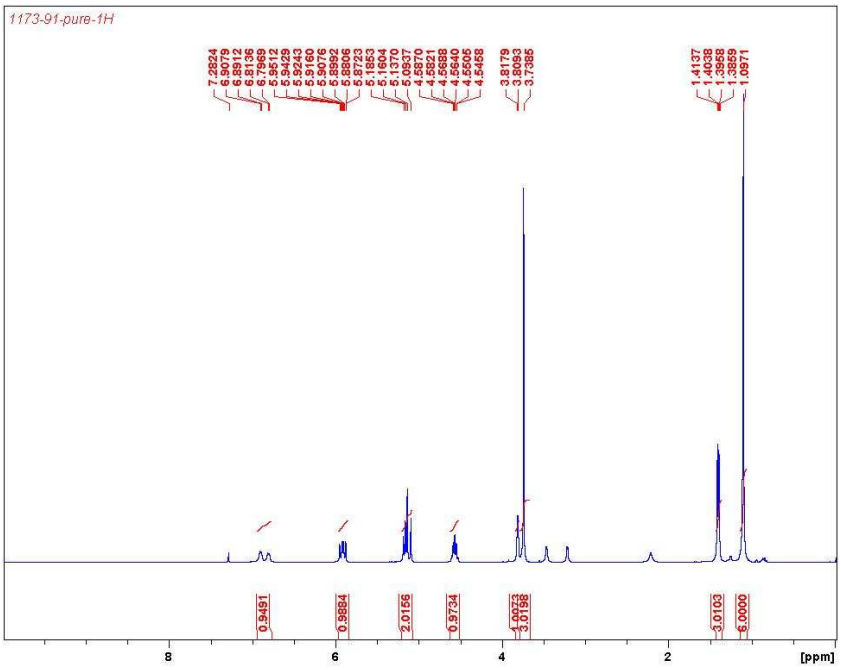
400 MHz
CDCl3
1.0000
1.0058
2.1000
1.0045
1.0416
3.1846
0.2565
0.4789
0.2385
1.0520
3.0985
1.3824
1.5187
8
6
4
2
[ppm]


1173-92-pure-13C
400 MHz
CDCl3
150
100
50
[ppm]



**8**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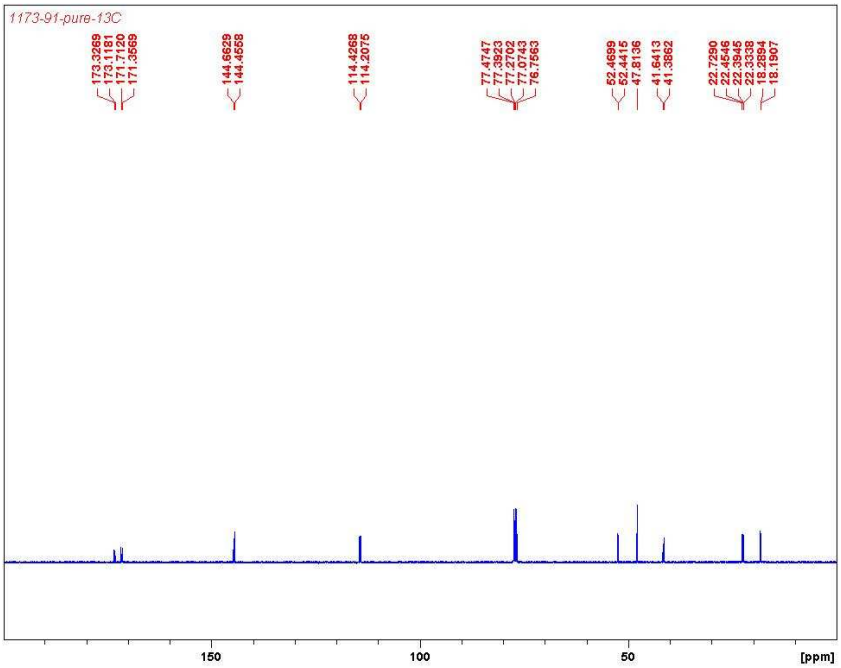
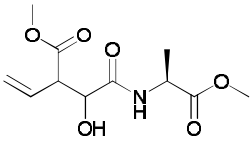
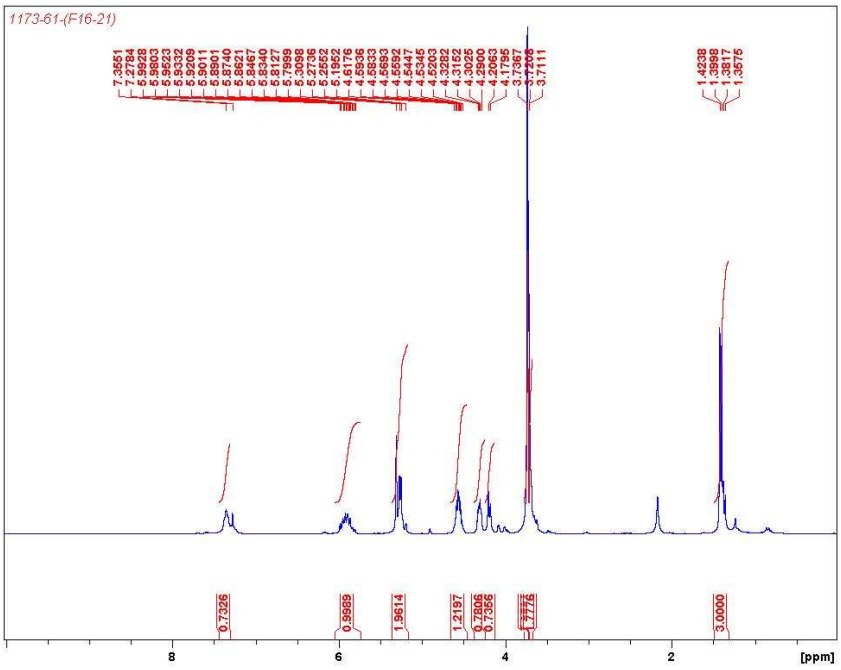
6.8912
6.8136
6.7969
5.9429
5.9243
5.9076
5.8992
5.8806
5.8723
5.1853
5.1604
5.1370
5.0937
4.5870
4.5821
4.5688
4.5640
4.5505
4.5468
3.8179
3.8093
3.7385
1.4137
1.4038
1.3968
1.3859
1.0971
0.9491
0.9884
2.0156
0.9734
1.0073
3.0198
3.0103
6.0000
8
6
4
2
[ppm]


1173-91-pure-13C
173.3269
173.1181
171.7120
171.3569
144.6629
144.4558
114.4098
114.2075
77.4747
77.3923
77.2702
77.0743
76.7563
52.4699
52.4415
47.8136
41.6413
41.3862
22.7290
22.4545
22.3945
22.3338
18.2894
18.1907
150
100
50
[ppm]



**10**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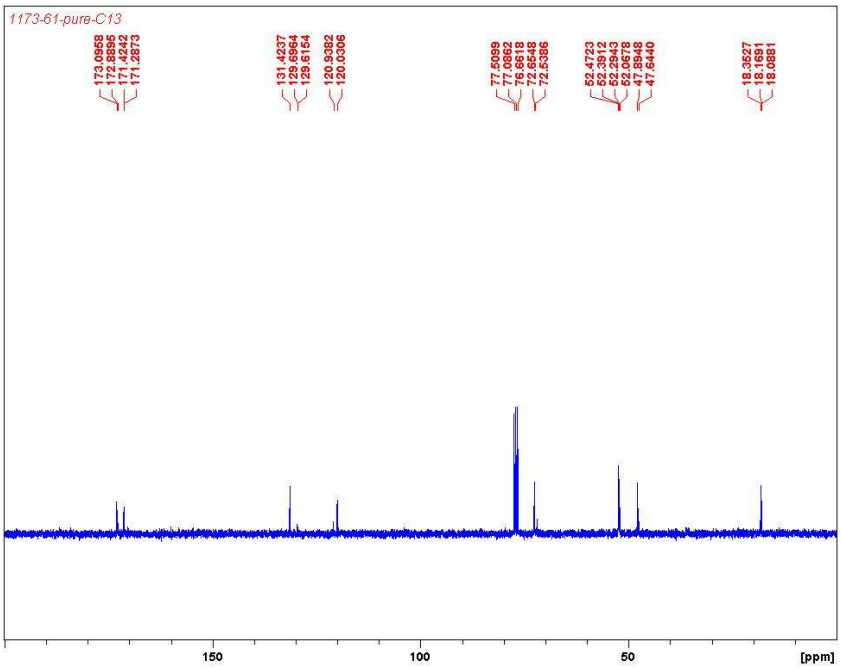
7.2784
5.9923
5.9623
5.9523
5.9332
5.9209
5.9011
5.8901
5.8740
5.8621
5.8467
5.8340
5.8127
5.7999
5.3098
5.2736
5.2655
5.1952
4.6176
4.5936
4.5833
4.5693
4.5592
4.5447
4.5345
4.5203
4.3282
4.3152
4.3025
4.2900
4.2763
4.1795
3.7367
3.7208
3.7111
1.4238
1.3998
1.3817
1.3575
0.7326
0.9689
1.9614
1.2197
0.7806
0.7356
1.7775
3.0000
8
6
4
2
[ppm]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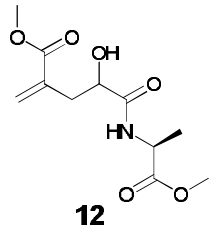
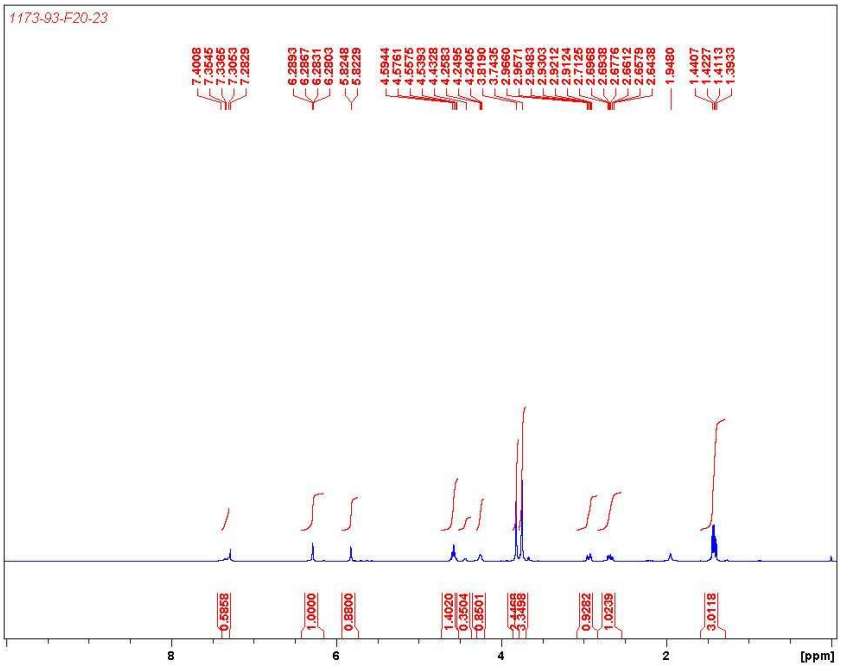
172.8895
171.4242
171.2873
131.4237
129.6964
129.6154
120.9382
120.0306
77.5099
77.0862
76.6618
72.6548
72.5386
52.4723
52.3912
52.2943
52.0678
47.8948
47.6440
18.3627
18.1691
18.0881
150
100
50
[ppm]



**12**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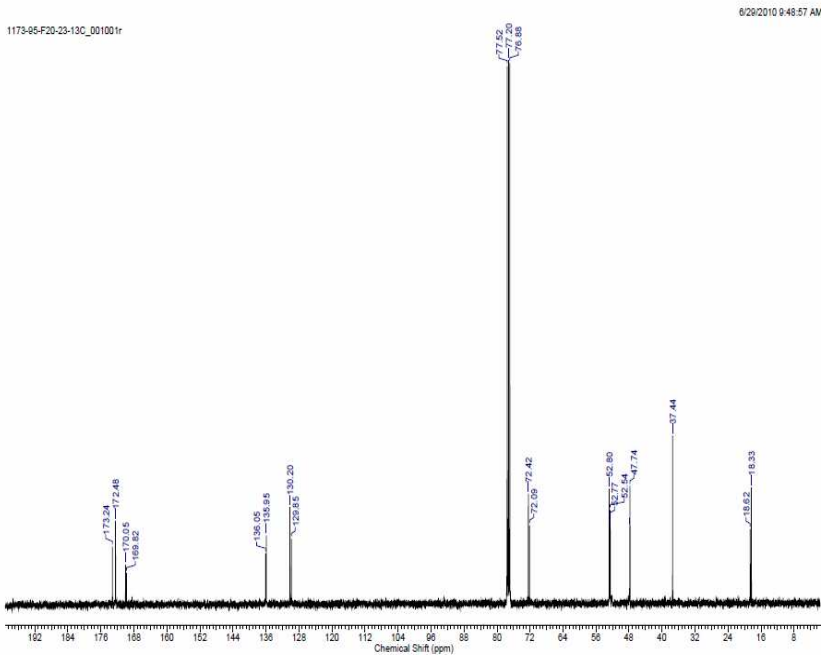
7.3545
7.3365
7.3053
7.2829
6.2893
6.2867
6.2831
6.2803
5.8248
5.8229
4.5944
4.5761
4.5576
4.5393
4.4328
4.2583
4.2495
4.2405
3.8190
3.7435
3.6660
2.9571
2.9483
2.9303
2.9212
2.9124
2.7125
2.6938
2.6933
2.6776
2.6612
2.6579
2.6438
1.9480
1.4407
1.4227
1.4113
1.3933
0.5858
1.0000
0.8800
1.4020
0.3504
0.8501
2.4166
3.3498
0.3282
1.0239
3.0118
8
6
4
2
[ppm]


6/29/2010 9:48:57 AM
1173-95-F20-23-13C_001001r
77.52
77.20
76.88
173.24
172.48
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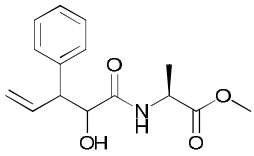
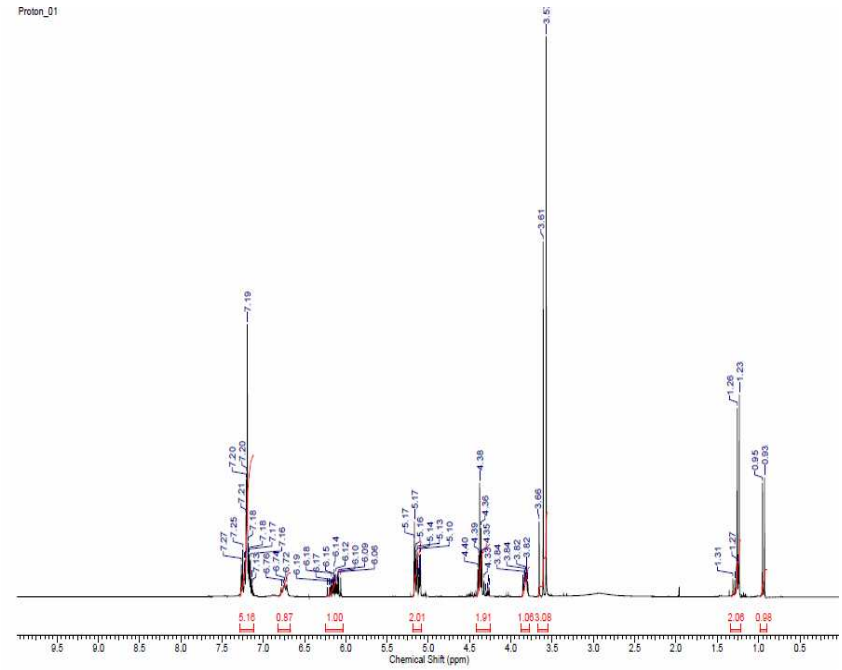
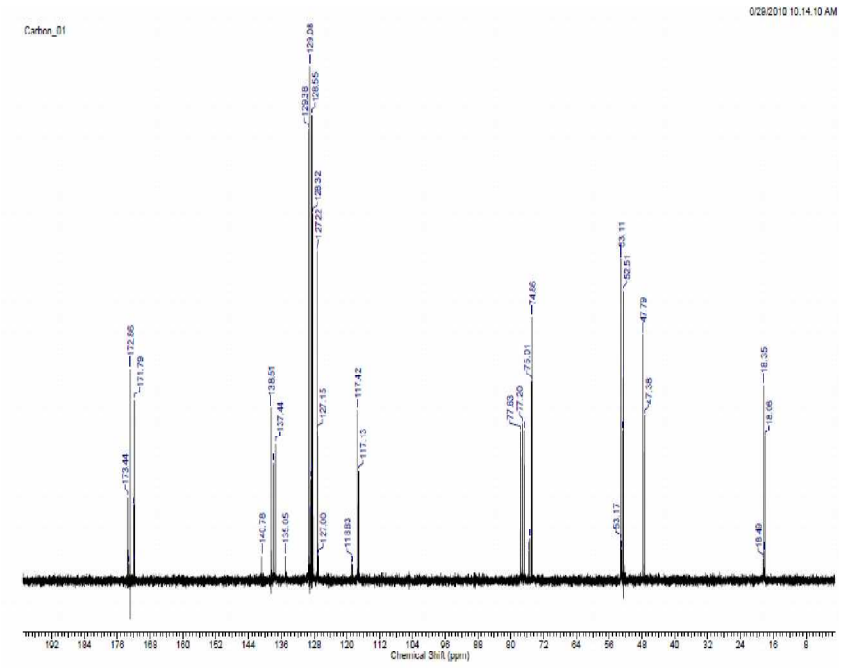
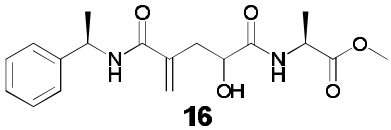
169.82
136.05
135.85
130.20
129.85
72.42
72.09
52.80
52.37
52.04
47.74
37.44
18.33
18.02
Chemical Shift (ppm)



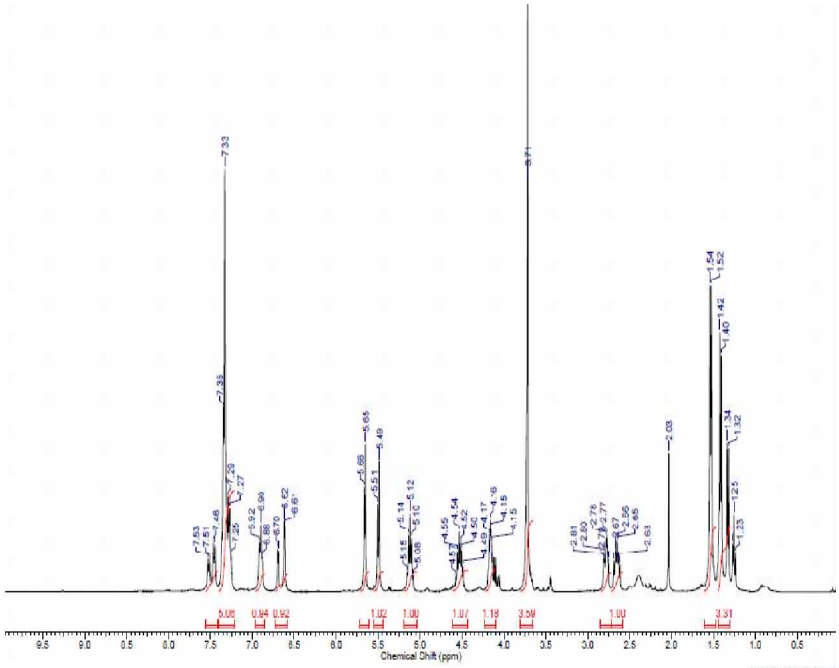
**14**

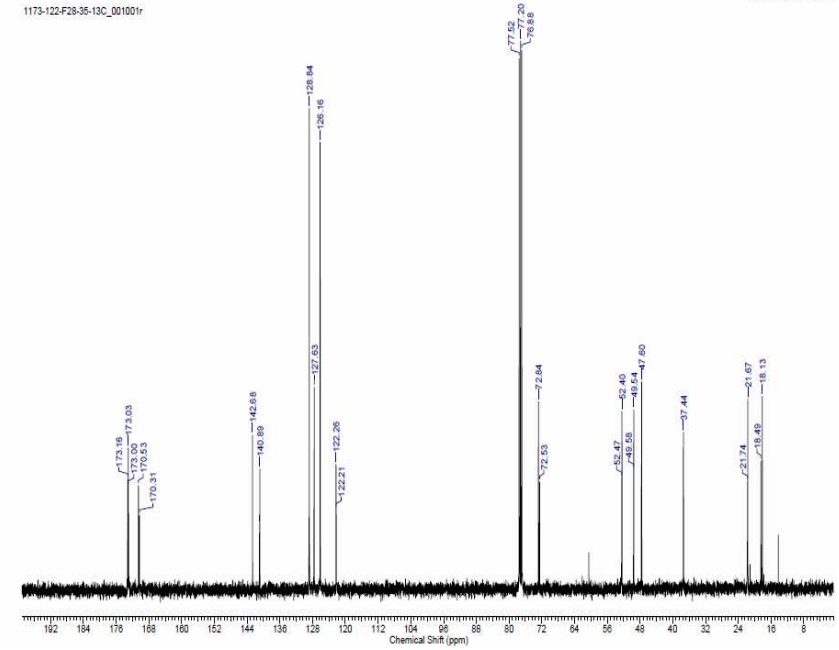




**16**


Chemical Shift (ppm)
6/29/2010 3:39:13 PM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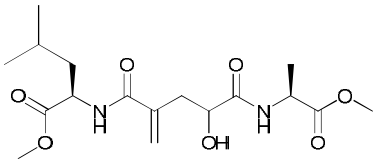
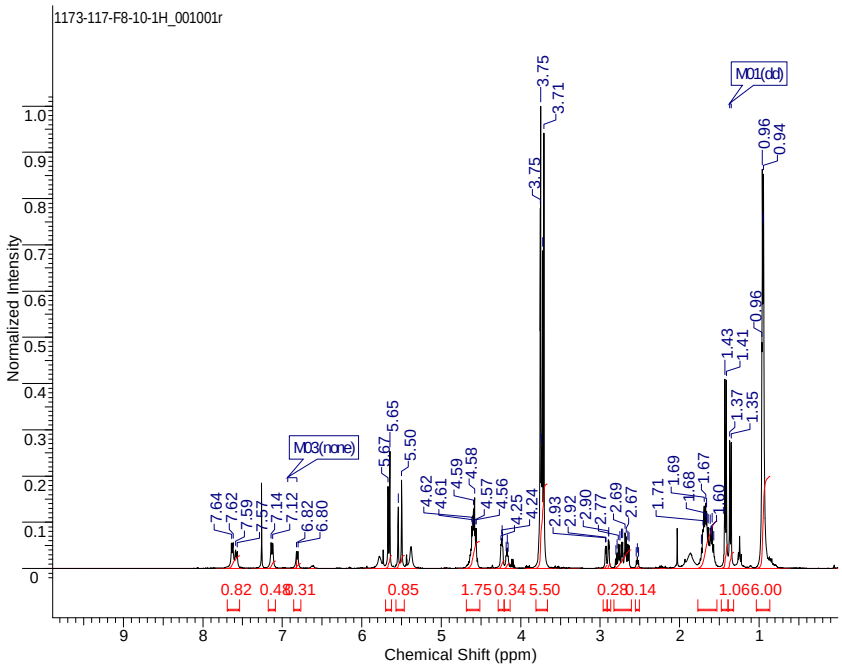
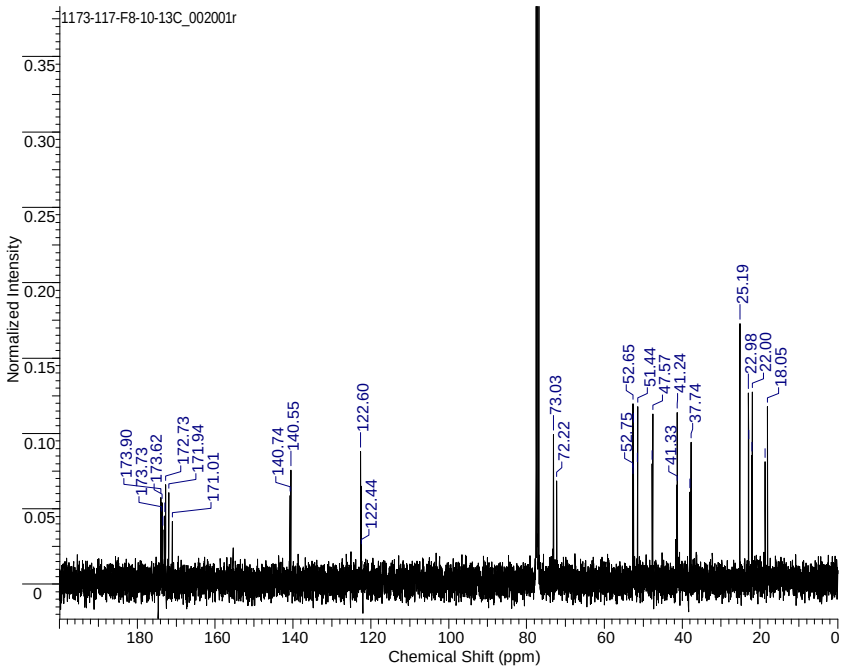
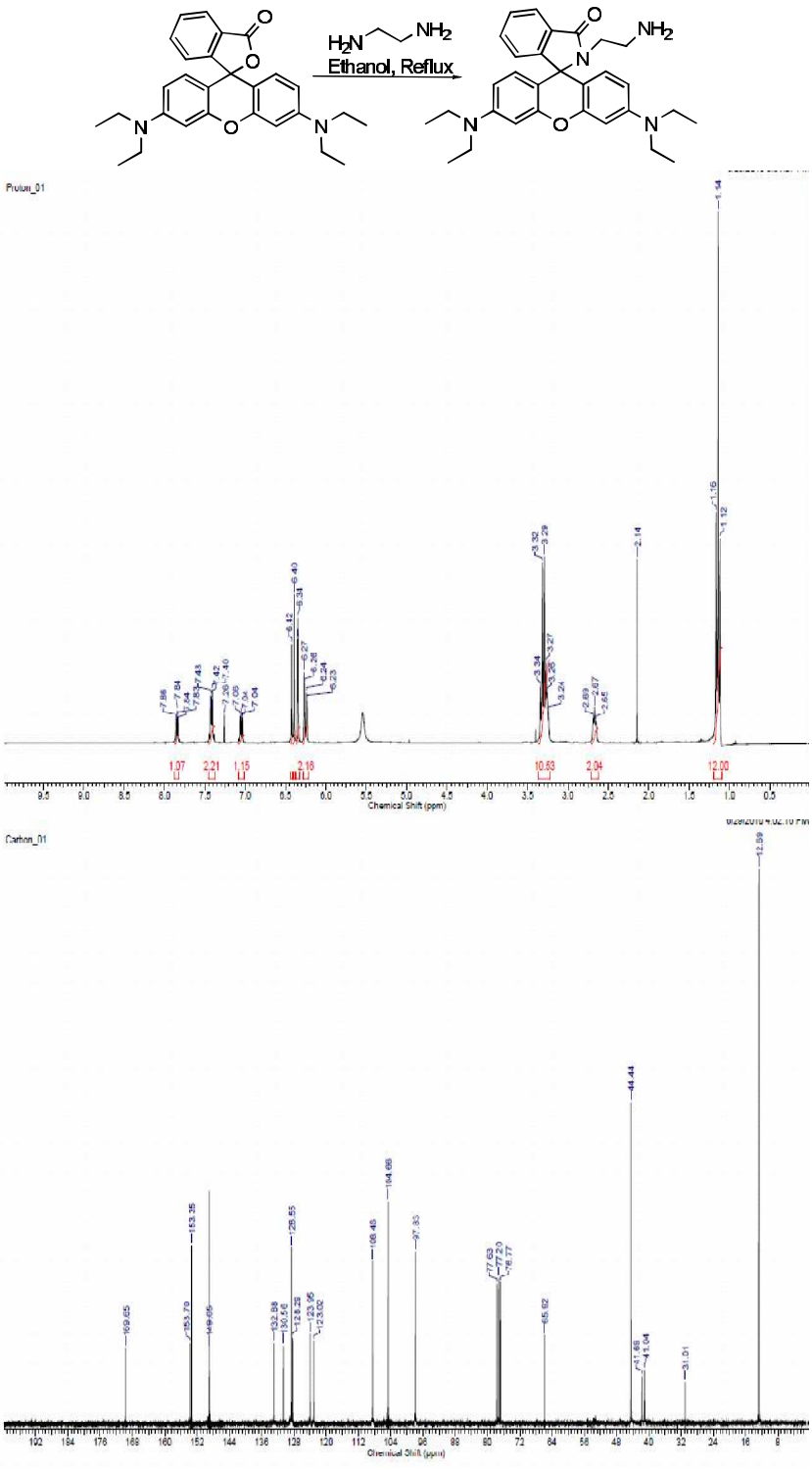
Chemical Shift (ppm)

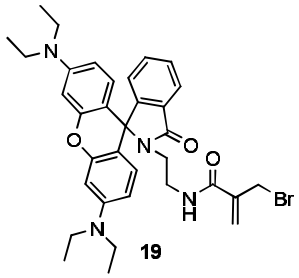


**18**

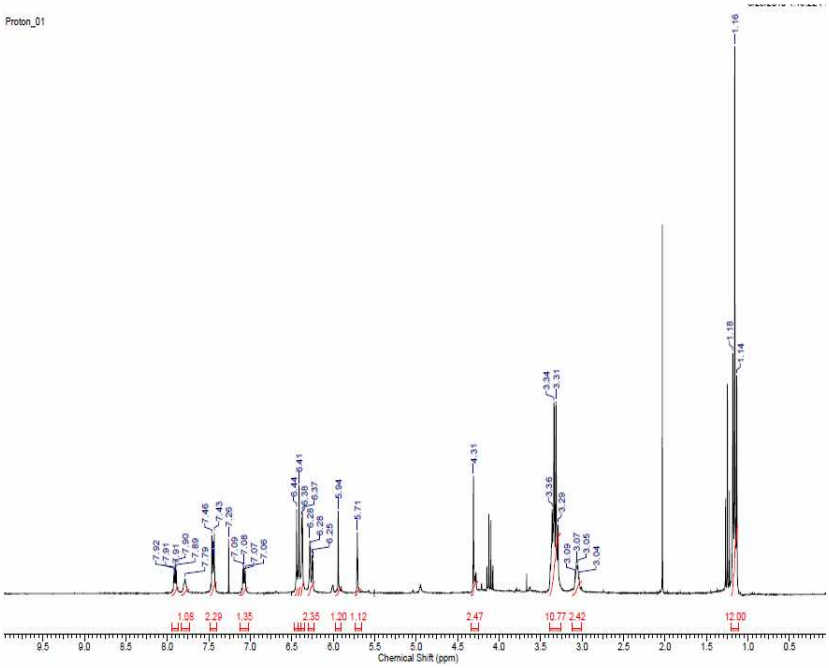

1173-117-F8-10-1H_001001r
M01(dd)
M03(none)
Normalized Intensity
Chemical Shift (ppm)


1173-117-F8-10-13C_002001r
Normalized Intensity
Chemical Shift (ppm)


Ethanol, Reflux
Proton_01
Chemical Shift (ppm)
Carbon_01


N
O
N
O
O
N
H
Br
N

**19**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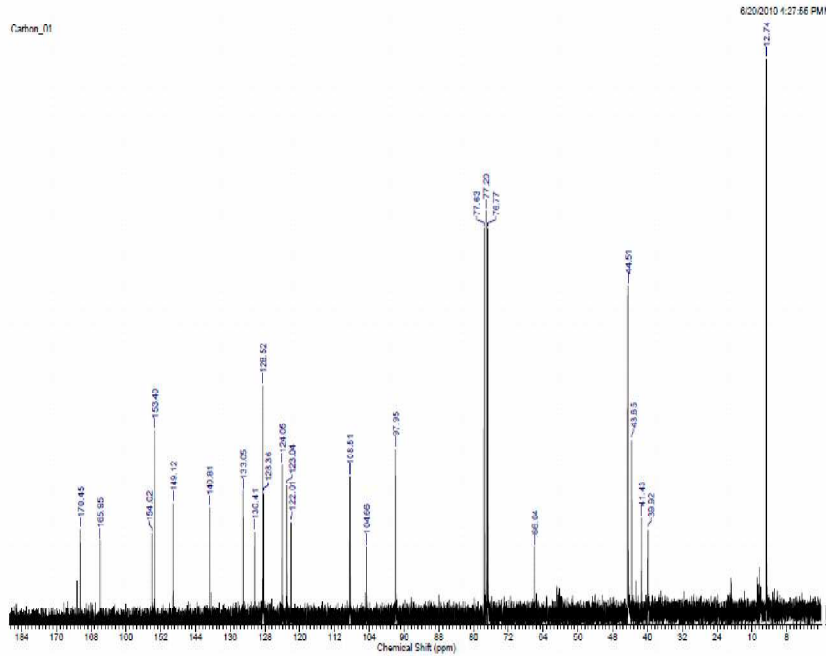
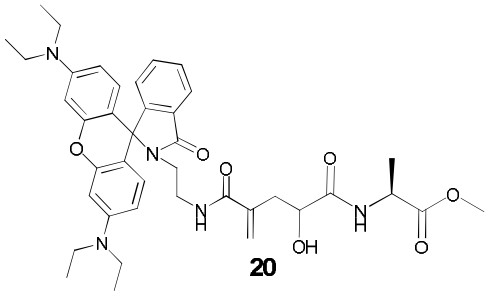
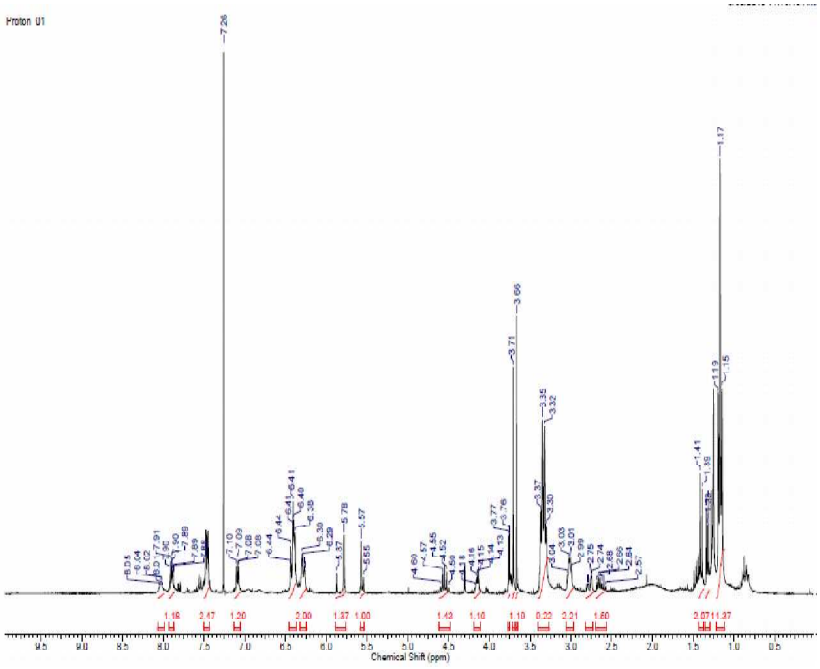
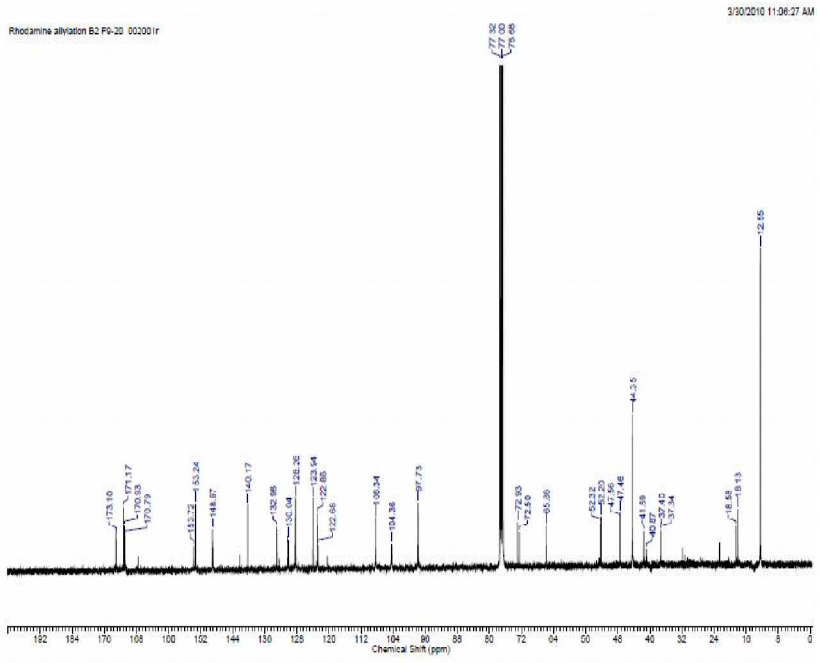
Chemical Shift (ppm)


Carbon_01
Chemical Shift (ppm)



**20**


Proton_01
Chemical Shift (ppm)


3/30/2010 11:06:27 AM
Rhodamine allylation B2 P9-20 00200 1r
Chemical Shift (ppm)