Electronic Supplementary Information

Enabling Wittig reaction on site-specific protein modification

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General information

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Reactions involving moisture-sensitive components were performed in oven-dried glassware. Petroleum ether and ethyl acetate were fractionally distilled. Reactions were monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} plates (0.25 mm), visualized by ultraviolet light. Further visualization was possible by staining with ninhydrin. Purifications by flash column chromatography were performed on silica gel (200-300 mesh).

Hexapeptide (Ser-Leu-Lys-Phe-Tyr-Gln, **4a**) and pentapeptide (Ser-Val-Thr-Arg-Ala**, 5a**) were purchased from GL Biochem (Shanghai) Ltd, and used as obtained. IL-8 (8-79) was commercially available from Genscript. Myoglobin (M 1882) from horse heart and PLP (pyridoxal-5-phosphate) were purchased from Sigma and used without further purification.

^{1}H NMR spectra were obtained on Bruker AVANCE III 400 (400 MHz) spectrometer at ambient temperature. ^{13}C NMR spectra were obtained with proton decoupling on a Bruker AVANCE III 400 (100 MHz) spectrometer and were reported in ppm with residual solvent for internal standard. Data were reported as follows: chemical shift on the δ scale (using either TMS or residual proton solvent as internal standard), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) in hertz, and integration. High resolution mass spectra were obtained on a Bruker APEX IV FT-MS (7.0 T) or Waters Xevo G2 Q-TOF mass spectrometer, using ESI with MeOH:H_2O (1:1) as the carrier solvent. Nominal and exact m/z values were reported in Daltons. Optical rotations were measured on Rudolph Research and Analytical Autopol III Automatic Polarimetre with a halogen lamp at 589 nm with a path length

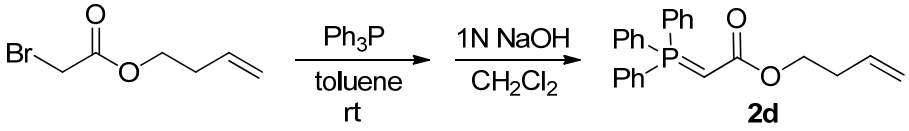
of 1 dm. Concentration was symbolized as *c* and was calculated as grams per milliliters (g/100 mL) whereas the solvent was specified in parentheses (*c*, solvent).

Peptide analyst was analyzed by electrospray ionization MS (ESI-MS) in positive ion mode on LCQ (ThermoFisher, San Jose, CA, USA) equipped with Agilent 1100 HPLC using a Agilent ZORBAX Eclipse XDB-C18 (5μ, ID 4.6 mm×250 mm) column. The flow rate was 1.0 mL/min using a gradient from 90% Solvent A (99.9 % H_2O, 0.1 % formic acid) to 90% Solvent B (99.9 % acetonitrile, 0.1% formic acid) within 25 min. 16.7% of the eluent was introduced to the mass spectrometer. High purity nitrogen (99.9%) and helium (99.99%) were used for MS analysis.

Protein Purification and Mass Spectrometry. Mass spectrometry was carried out using a Q-Tof Micro mass spectrometer equipped with a Waters Z-type electrospray ionization source (Waters, Manchester, UK). Sample was diluted to about 10 pmol/μL and introduced to MS with syringe pump at a flow rate of 500 nL/min. Acquisition mass range was typically *m/z* 500-2000. Data were recorded and processed using OpenLynx™ software and MaxEnt1 option (Waters, Manchester, UK). Calibration of the 500-2000 *m/z* scale was achieved by a multi-point calibration using selected fragment ions that resulted from the CID of Glu-fibrinopeptide B (+2 ion, m/z 785.8, Sigma-Aldrich, US)

Chemical synthesis

Ylides **2a**,[1] **2b**,[2] **2c**,[3] **2e**,[4] **2f**,[5] **2h**,[6] and the protected dipeptide**1**[7] were prepared according to the method described in the related references. Ylide **2g** was commerarially available.



Scheme 1: Synthetic scheme for **2d**

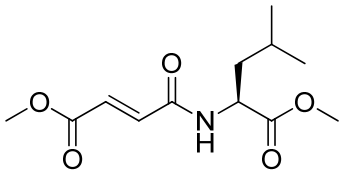
3'-Butyen-1'-yl 2-(triphenylphosphoranylidene)-acetate (2d)

To a solution of but-3-en-1-yl 2-bromoacetate[8] (193.0 mg, 1 mmol) in toluene (5 mL) was added Ph_3P (162.0 mg, 1 mmol). After stirring for 12 h, solvent was evaporated and the residue was dissolved in H_2O (10 mL). The aqueous layer was neutralized by 1N NaOH until pH = 8 and exacted by CH_2Cl_2 (10 mL) for 3 times. The combined organic layer was washed by brine, dried over Na_2SO_4. The solvent was removed under reduced pressure to yield product **2d** (160.0 mg, 43% yield) as red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70-7.42 (m, 15H), 5.65 (br, 1H), 4.98-4.89 (m, 2H), 3.97. (t, *J* = 6.8 Hz, 2H), 2.93 (br, 1H), 2.16 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.09, 135.47, 133.10, 133.00, 132.19, 132.09, 131.96, 131.93, 128.80, 128.67, 128.59, 128.47, 115.98, 61.46, 33.91, 30.17 (d, 124 Hz, 1C). HRMS (ESI) *m/z* calculated for $C_{24}H_{24}O_2P[M+H]^+$: 375.1508,found: 375.1505.

General experimental procedures for Wittig reaction on dipeptide 1

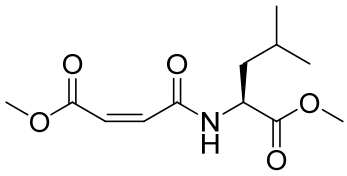
To a solution of the protected peptide **1** (34.7 mg, 0.1 mmol) in H_2O (5 mL) was added $NaIO_4$ (42.0 mg, 0.2 mmol, 2.0 eq.). The mixture was stirred at room temperature. After stirring for 0.5h, ylide (2.0 eq.) and *t*-BuOH (5 mL) were added. The reaction was completed within 1.5 h (monitored by TLC). The mixture was concentrated under reduced pressure, and diluted with H_2O

(10 mL), extracted with EtOAc (3×15 mL). The combined organic layer was washed with brine (30mL), dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography to give the desired product.



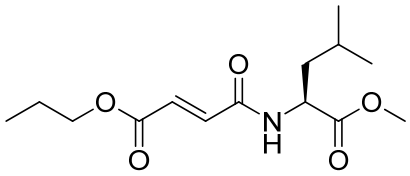
(*S,E*)-2-(4-Methoxy-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3a-1)

Colorless oil (5.0 mg, 19% yield). R_f= 0.41 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, *J* = 15.6 Hz, 1H), 6.85 (d, *J* = 15.6 Hz, 1H), 6.75 (br, 1H), 4.74 (td, *J* = 8.4, 5.2 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 3H),1.90-1.58 (m, 3H), 0.96 (d, *J* = 3.2 Hz,3H), 0.94 (d, *J* = 3.2 Hz,3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.31, 166.14, 163.38, 136.18, 130.51, 52.53, 52.29, 51.10, 41.60, 24.94, 22.79, 21.96. HRMS (ESI) *m/z* calculated for $C_{12}H_{20}NO_5$ $[M+H]^+$: 258.1336, found: 258.1338. $[\alpha]^{21}_D$ –42.1 (*c* = 1.47 g/100 mL, CH_3OH).



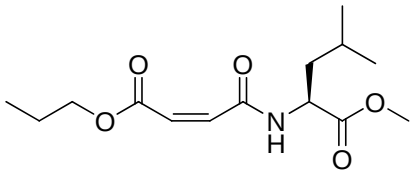
(*S,Z*)-2-(4-Methoxy-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3a-2)

Colorless oil (15.0 mg, 58% yield). R_f= 0.19 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 3.6 Hz, 1H), 6.33 (d, *J* = 12.8 Hz, 1H), 6.17 (d, *J* = 12.8 Hz, 1H), 4.68-4.62 (m, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 1.76–1.63 (m, 3H), 0.97 (d, *J* = 2.0 Hz,3H), 0.95 (d, *J* = 2.0 Hz,3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 173.14, 166.60, 163.66, 137.73, 125.72, 52.54, 52.29, 51.25, 41.24, 24.90, 22.78, 22.00. HRMS (ESI) *m/z* calculated for $C_{12}H_{20}NO_5$ $[M+H]^+$: 258.1336, found: 258.1337. $[\alpha]^{21}_D$ –28.8 (*c* = 0.93 g/100 mL, CH_3OH).



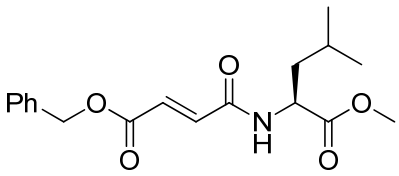
(*S*,*E*)-2-(4-Oxo-4-propoxybut-2-enamido)-4-methylpentanoic acid methyl ester (3b-1)

Colorless oil (5.0 mg, 18% yield). R_f= 0.56 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, *J* = 15.4 Hz, 1H), 6.84 (d, *J* = 15.4 Hz, 1H), 6.27 (d, *J* = 8.4 Hz, 1H), 4.75 (td, *J* = 8.8, 5.2 Hz, 1H), 4.16 (t, *J* = 6.8 Hz, 2H), 3.76 (s, 3H), 1.75–1.55 (m, 5H), 0.99-0.94 (m, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 173.25, 165.66, 163.43, 135.76, 131.12, 66.91, 52.54, 51.11, 41.73, 24.97, 22.81, 22.02, 21.98, 10.40. HRMS (ESI) *m/z* calculated for $C_{14}H_{24}NO_5$ $[M+H]^+$: 286.1649, found: 286.1652. $[\alpha]^{21}_D$ –33.5 (*c* = 0.87 g/100 mL, CH_3OH).



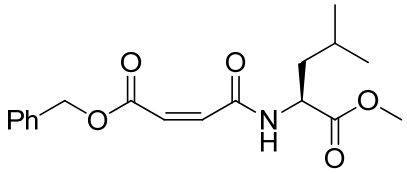
(*S*,*Z*)-2-(4-Oxo-4-propoxybut-2-enamido)-4-methylpentanoic acid methyl ester (3b-2)

Colorless oil (16.0 mg, 56% yield). R_f= 0.30 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, *J* = 6.8 Hz, 1H), 6.32 (d, *J* = 13.2 Hz, 1H), 6.18 (d, *J* = 13.2 Hz, 1H),4.64 (td, *J* = 8.0, 5.6 Hz, 1H), 4.16 (t, *J* = 6.8 Hz, 2H), 3.74 (s, 3H), 1.76–1.63 (m, 6H), 0.99-0.94 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.14, 166.33, 163.64, 137.95, 126.08, 67.32, 52.26, 51.31, 41.24, 24.93, 22.80, 22.03, 21.80, 10.33. HRMS (ESI) *m/z* calculated for $C_{14}H_{24}NO_5$ $[M+H]^+$: 286.1649, found: 286.1645. $[\alpha]^{21}_D$ –25.5 (*c* = 2.0 g/100 mL, CH_3OH).



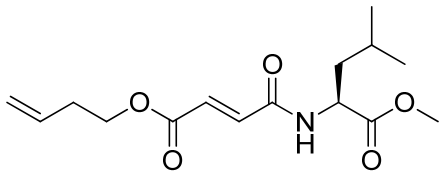
(*S*,*E*)-2-(4-(Benzyloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3c-1)

Colorless oil (10.0 mg, 30% yield). R_f= 0.41 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.34 (m, 5H), 6.96 (d, *J* = 15.4 Hz, 1H), 6.87 (d, *J* = 15.6 Hz, 1H), 6.20 (d, *J* = 8.4 Hz, 1H), 5.23 (s, 2H), 4.73 (td, *J* = 8.5, 5.2 Hz, 1H), 3.75 (s, 3H), 1.73–1.55 (m, 3H), 0.95 (d, *J* = 5.6 Hz, 3H), 0.94 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.24, 165.33, 163.28, 136.24, 135.41, 130.75, 128.70, 128.53, 128.34, 67.06, 52.55, 51.10, 41.70, 24.95, 22.80, 22.01. HRMS (ESI) *m/z* calculated for $C_{18}H_{24}NO_5$ $[M+H]^+$: 334.1649, found: 334.1651. $[\alpha]^{21}_D$ –32.4 (*c* = 0.93 g/100 mL, CH_3OH).



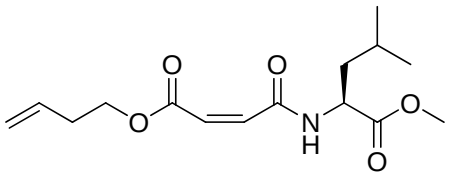
(*S*,*Z*)-2-(4-(Benzyloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3c-2)

Colorless oil (20.0 mg, 60% yield). R_f= 0.22 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 7.2 Hz, 1H), 7.38–7.35 (m, 5H), 6.34 (d, *J* = 13.2 Hz, 1H), 6.20 (d, *J* = 13.2 Hz, 1H), 5.23 (s, 2H), 4.64 (td, *J* = 8.4, 6.0 Hz, 1H), 3.74 (s, 3H), 1.75–1.62 (m, 3H), 0.96 (d, *J* = 4.8 Hz, 3H), 0.95 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.13, 165.97, 163.60, 138.23, 134.97, 128.72, 128.66, 128.51, 125.72, 67.47, 52.31, 51.30, 41.28, 24.93, 22.82, 22.05. HRMS (ESI) *m/z* calculated for $C_{18}H_{24}NO_5$ $[M+H]^+$: 334.1649, found: 334.1643. $[\alpha]^{21}_D$ –28.9 (*c* =2.27 g/100 mL, CH_3OH).



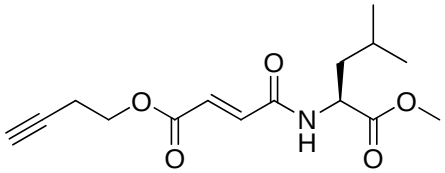
(*S*,*E*)-2-(4-(But-3-en-1-yloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3d-1)

Colorless oil (9.0 mg, 30% yield). R_f= 0.52 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, *J* = 15.6 Hz, 1H), 6.83 (d, *J* = 15.6 Hz, 1H), 6.35 (d, *J* = 8.0 Hz, 1H), 5.79 (ddt, *J* = 17.2, 10.4, 6.8 Hz, 1H), 5.16–5.08 (m, 2H), 4.74 (td, *J* = 8.8, 5.2 Hz, 1H), 4.25 (t, *J* = 6.4 Hz, 2H), 3.76 (s, 3H), 2.47–2.41 (m, 2H), 1.72–1.57 (m, 3H), 0.95 (d, *J* = 4.8 Hz, 3H), 0.94 (d, *J* = 4.8 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 173.26, 165.53, 163.38, 135.94, 133.66, 130.91, 117.61, 64.34, 52.54, 51.10, 41.70, 32.99, 24.96, 22.81, 22.01. HRMS (ESI) *m/z* calculated for $C_{15}H_{24}NO_5$ $[M+H]^+$: 298.1649, found: 298.1651. $[\alpha]^{21}_D$ –29.7 (*c* = 0.93 g/100 mL, CH_3OH).



(*S,Z*)-2-(4-(But-3-en-1-yloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3d-2)

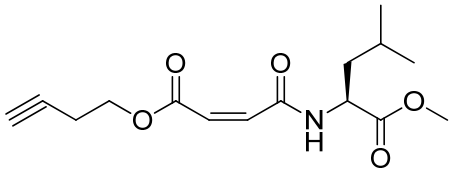
Colorless oil (16.0 mg, 54% yield). R_f= 0.33 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 6.8 Hz, 1H), 6.32 (d, *J* = 13.2 Hz, 1H), 6.17 (d, *J* = 12.8 Hz, 1H), 5.79 (ddt, *J* = 17.2, 10.4, 6.8 Hz, 1H), 5.16–5.09 (m, 2H), 4.64 (td, *J* = 8.4, 5.2 Hz, 1H), 4.26 (t, *J* = 6.8 Hz, 2H), 3.74 (s, 3H), 2.47–2.42 (m , 2H), 1.74–1.65 (m, 3H), 0.96 (d, *J* =4.4 Hz, 3H), 0.95 (d, *J* =4.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.12, 166.16, 163.60, 137.92, 133.47, 125.94, 117.66, 64.70, 52.26, 51.28, 41.24, 32.78, 24.91, 22.79, 22.02. HRMS (ESI) *m/z* calculated for $C_{14}H_{24}NO_5$ $[M+H]^+$: 298.1649, found: 298.1654.$[\alpha]^{21}_D$ –27.0 (*c* = 1.87 g/100 mL, CH_3OH).



(*S,E*)-2-(4-(But-3-yn-1-yloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester

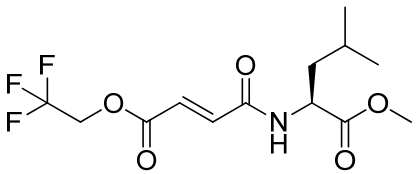
(3e-1)

Colorless oil (7.0 mg, 24% yield). R_f= 0.52 (petroleum ether/ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 15.2 Hz, 1H), 6.85 (d, *J* = 15.2 Hz, 1H), 6.36 (d, *J* = 8 Hz, 1H), 4.74 (td, *J* = 8.4, 5.2 Hz, 1H), 4.31 (t, *J* = 6.8 Hz, 2H), 3.76 (s, 3H), 2.59 (td, *J* = 6.8, 2.4 Hz, 2H), 2.02 (t, *J* = 2.4 Hz, 1H), 1.74–1.56 (m, 3H), 0.96 (d, *J* = 4.4 Hz, 3H), 0.95 (d, *J* = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.19, 165.18, 163.20, 136.29, 130.59, 79.73, 70.25, 62.91, 52.58, 51.14, 41.78, 24.99, 22.81, 22.05, 19.01. HRMS (ESI) *m/z* calculated for $C_{15}H_{22}NO_5$ $[M+H]^+$: 296.1493, found: 296.1492. $[\alpha]^{21}_D$ –30.7 (*c* =1.07 g/100 mL, CH_3OH).



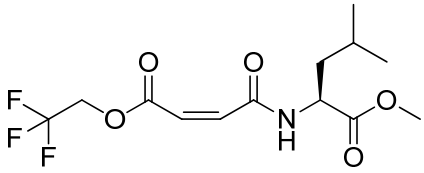
(*S*,*Z*)-2-(4-(But-3-yn-1-yloxy)-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3e-2)

Colorless oil (18.0 mg, 61% yield). R_f= 0.30 (petroleum ether/ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 6.8Hz, 1H), 6.35 (d, *J* = 13.2 Hz, 1H), 6.20 (d, *J* = 13.2 Hz, 1H), 4.64 (td, *J* = 8.0, 5.5 Hz, 1H), 4.31 (t, *J* = 6.8 Hz, 2H), 3.75 (s, 3H), 2.60 (td, *J* = 6.8, 2.7 Hz, 2H), 2.03 (br, 1H), 1.75–1.63 (m, 3H), 0.96 (d, *J* = 3.6 Hz, 3H), 0.95 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.14, 165.86, 163.54, 138.21, 125.58, 79.55, 70.34, 63.23, 52.33, 51.30, 41.33, 24.95, 22.82, 22.07, 18.84. HRMS (ESI) *m/z* calculated for $C_{15}H_{22}NO_5$ $[M+H]^+$: 296.1493, found: 296.1500. $[\alpha]^{21}_D$ –26.3 (*c* =2.33 g/100 mL, CH_3OH).



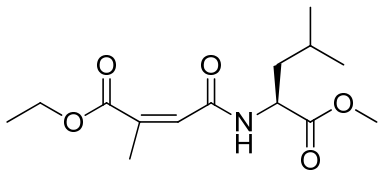
(*S*,*E*)-2-(4-Oxo-4-(2,2,2-trifluoroethoxy)but-2-enamido)-4-methylpentanoic acid methyl ester (3f-1)

Colorless oil (12.0 mg, 38% yield). R_f= 0.48 (petroleum ether/ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, *J* = 15.2 Hz, 1H), 6.89 (d, *J* = 15.2 Hz, 1H), 6.43 (d, *J* = 8.0 Hz, 1H), 4.75 (td, *J* = 8.4, 5.2 Hz, 1H), 4.58 (q, *J* = 8.0 Hz, 2H), 3.77 (s, 3H), 1.75–1.57 (m, 3H), 0.96 (d, *J* = 5.6 Hz, 3H), 0.95 (d, *J* = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.11, 163.73, 162.66, 137.84, 129.01, 60.92 (q, *J* = 36 Hz, 1C), 52.62, 51.24, 41.83, 25.02, 22.80, 22.07. HRMS (ESI) *m/z* calculated for $C_{13}H_{19}NO_2F_3$ $[M+H]^+$: 326.1210, found: 326.1220. $[\alpha]^{21}_D$ –26.6 (*c* = 0.67 g/100 mL, CH_3OH).



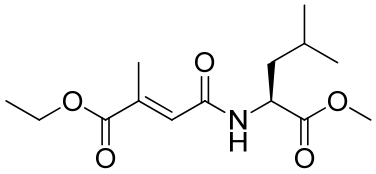
(*S*,*Z*)-2-(4-Oxo-4-(2,2,2-trifluoroethoxy)but-2-enamido)-4-methylpentanoic acid methyl ester (3f-2)

Colorless oil (17.0 mg, 54% yield). R_f= 0.26 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.2 Hz, 1H), 6.45 (d, *J* = 12.8 Hz, 1H), 6.23 (d, *J* = 12.4Hz, 1H), 4.70-4.65 (m, 1H), 4.62-4.53 (m, 2H), 3.75 (s, 3H), 1.74–1.60 (m, 3H), 0.96 (br, 3H), 0.96 (br, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.12, 164.27, 163.29, 138.51, 124.31, 61.08 (q, *J* = 36 Hz, 1C), 52.41, 51.20, 41.53, 24.92, 22.76, 22.08. HRMS (ESI) *m/z* calculated for $C_{13}H_{19}NO_2F_3$ $[M+H]^+$: 326.1210, found: 326.1218. $[\alpha]^{21}_D$ –22.2 (*c* = 0.6 g/100 mL, CH_3OH).



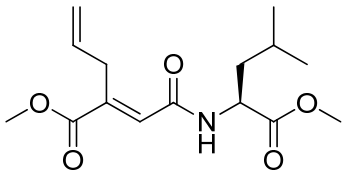
(*S*,*Z*)-2-(4-Ethoxy-3-methyl-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3g-1)

Colorless oil (15.0 mg, 53% yield). R_f= 0.52 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (d, *J* = 1.6 Hz, 1H), 6.20 (d, *J* = 8.1 Hz, 1H), 4.71 (td, *J* = 8.4, 4.8 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 3.75 (s, 3H), 2.27 (d, *J* = 1.2 Hz, 3H), 2.27–1.53 (m, 3H), 1.32 (t, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 4.4 Hz, 3H), 0.95 (d, *J* = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.45, 167.51, 165.21, 140.55, 128.97, 61.51, 52.43, 50.69, 41.62, 24.95, 22.80, 21.97, 14.18, 13.97. HRMS (ESI) *m/z* calculated for $C_{14}H_{24}NO_5$ $[M+H]^+$: 286.1649, found: 286.1637. $[\alpha]^{21}_D$ –37.3 (*c* = 1.93 g/100 mL, CH_3OH).



(*S*,*E*)-2-(4-Ethoxy-3-methyl-4-oxobut-2-enamido)-4-methylpentanoic acid methyl ester (3g-2)

Colorless oil (4.0 mg, 14% yield). R_f= 0.19 (petroleum ether/ ethyl acetate, 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (d, *J* = 7.6 Hz, 1H), 5.92 (q, 1.6 Hz, 1H), 4.67 (td, *J* = 8.4, 5.2 Hz, 1H), 4.32-4.24 (m, 2H), 3.75 (s, 3H), 2.04 (d, *J* = 1.6 Hz, 3H), 1.70–1.54 (m, 3H), 1.28 (t, *J* = 7.2 Hz, 3H), 0.95 (d, *J* = 4.4 Hz, 3H), 0.93(d, *J* = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.45, 168.98, 164.12, 140.68, 125.76, 61.53, 52.36, 50.82, 41.83, 24.92, 22.81, 22.20, 20.86, 14.04. HRMS (ESI) *m/z* calculated for $C_{14}H_{23}NO_5$ $[M+Na]^+$:308.1474, found: 308.1476. $[\alpha]^{21}_D$ –25.5 (*c* = 0.2 g/100 mL, CH_3OH).

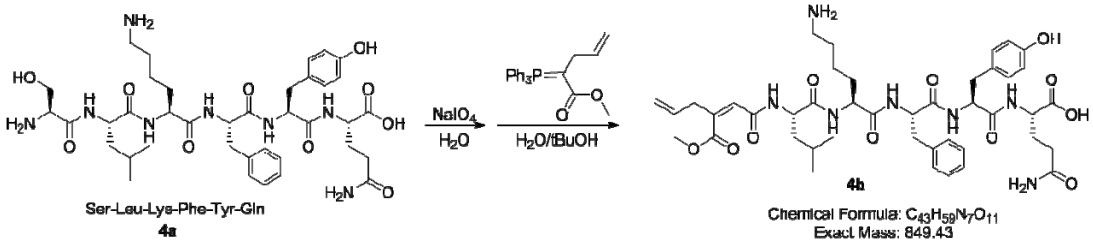


(*S*,*E*)-2-(2-((1-Methoxy-4-methyl-1-oxopentan-2-yl)amino)-2-oxoethylidene)pent-4-enoic acid methyl ester (3h)

Colorless oil (20.0 mg, 67% yield). R_f= 0.35 (petroleum ether/ethyl acetate, 3/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.27 (d, *J* =8.0 Hz, 1H), 5.92 (ddt, *J* = 16.4, 10.8, 6.0 Hz, 1H), 5.13-5.04 (m, 2H), 4.78 (td, *J* = 8.4, 4.8 Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 3.56 (d, *J* = 6.0 Hz, 2H), 1.72–1.54 (m, 3H), 0.96 (d, *J* = 2.8 Hz, 3H), 0.95 (d, *J* =3.2 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 173.54, 167.60, 164.98, 141.28, 135.30, 130.60, 116.66, 52.83, 52.75, 51.04, 41.84, 31.85, 25.24, 23.06, 22.28. HRMS (ESI) *m/z* calculated for $C_{15}H_{24}NO_5$ $[M+H]^+$: 298.1654, found: 298.1647. $[\alpha]^{21}_D$ –39.8(*c* =1.33 g/100 mL, CH_3OH).

Modification of peptides by Wittig reaction

To a solution of peptide **4a** or **5a** (10 mM) in H_2O (0.5 mL) was added $NaIO_4$ (2.1 mg, 10 mM, 2.0 eq.). The mixture was stirred at room temperature. After 0.5 h, ylide **2h** (5.6 mg, 15 mM, 3.0 eq.) and *t*-BuOH (0.5 mL) was added, and the mixture was stirred at 37 ^{o}C. The reaction was finished within 0.5 h which was followed byLC-MS/MS analysis.



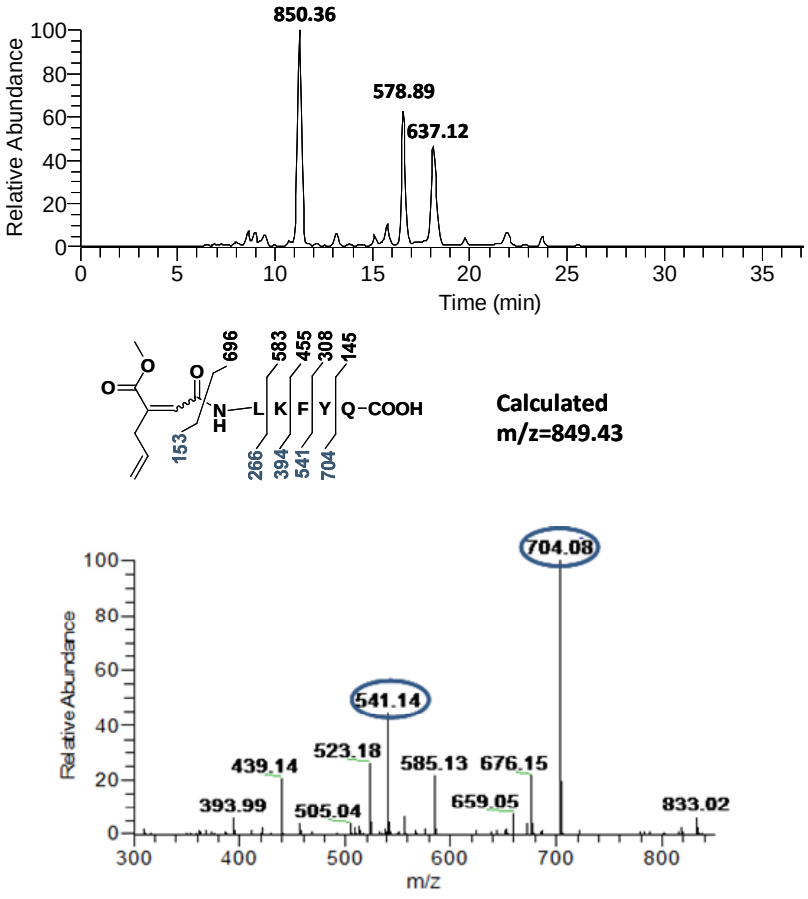
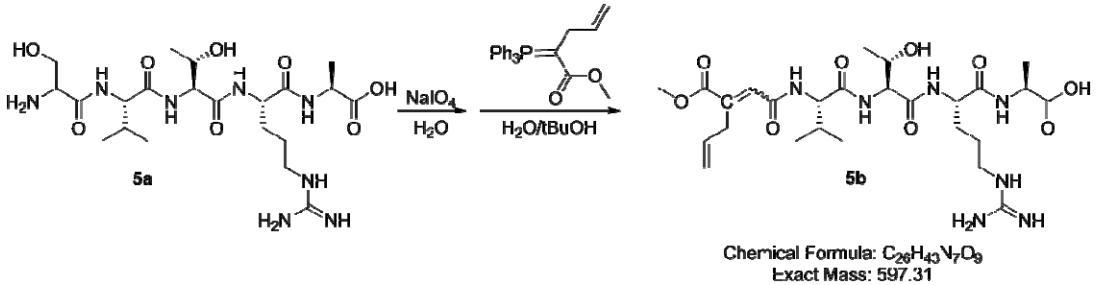


Figure S1: Modification of peptide **4a. a)** BPI chromatogram for modified peptide **4b**. The peak of $[M+H]^+$ for **4b** was 850.36. The other two peaks at 578.89 and 637.12 which were present in the functionalizations of both **4a** and **5a**, were not generated by peptides. **b)**The MS/MS spectra for the peak at 850.36. The peaks marked in blue were the identical fragmentation patterns that were consistent with modified peptide **4b.**



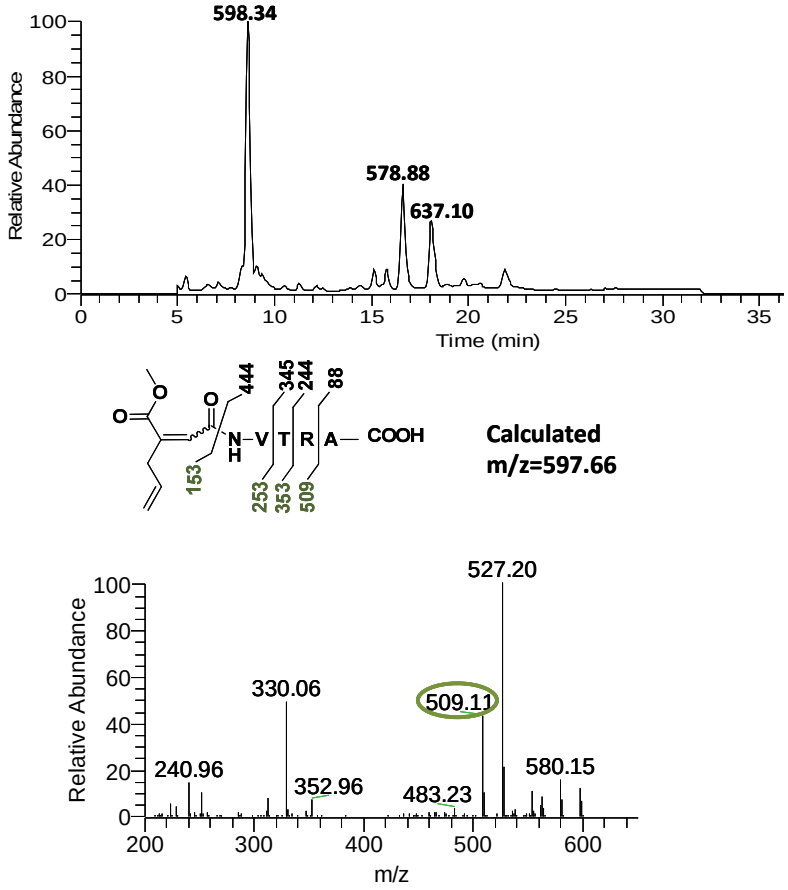


Figure S2: Modification of peptide **5a. a)** BPI chromatogram for modified peptide **5b**. The peak of $[M+H]^+$ for **5b** was 589.34. **b)** The MS/MS spectra for the peak at 589.34. The peak marked in green was the identical fragmentation pattern that was consistent with modified peptide **5b.**

Modification of IL-8 (8-79) by Wittig reaction

To a solution of IL-8 (5.0 μg, 0.60 nmol) in H_2O (16 μL) was added $NaIO_4$ (1.2 nmol, 0.25 μg). The mixture was allowed to take place at room temperature for 0.5 h, and ylide **2h** (30 nmol, 11.0 μg, 50 eq.) and *t*-BuOH (4 μL) was added. The mixture was incubated at 37 °C for 0.5 h which was followed by MALDI-TOF analysis.

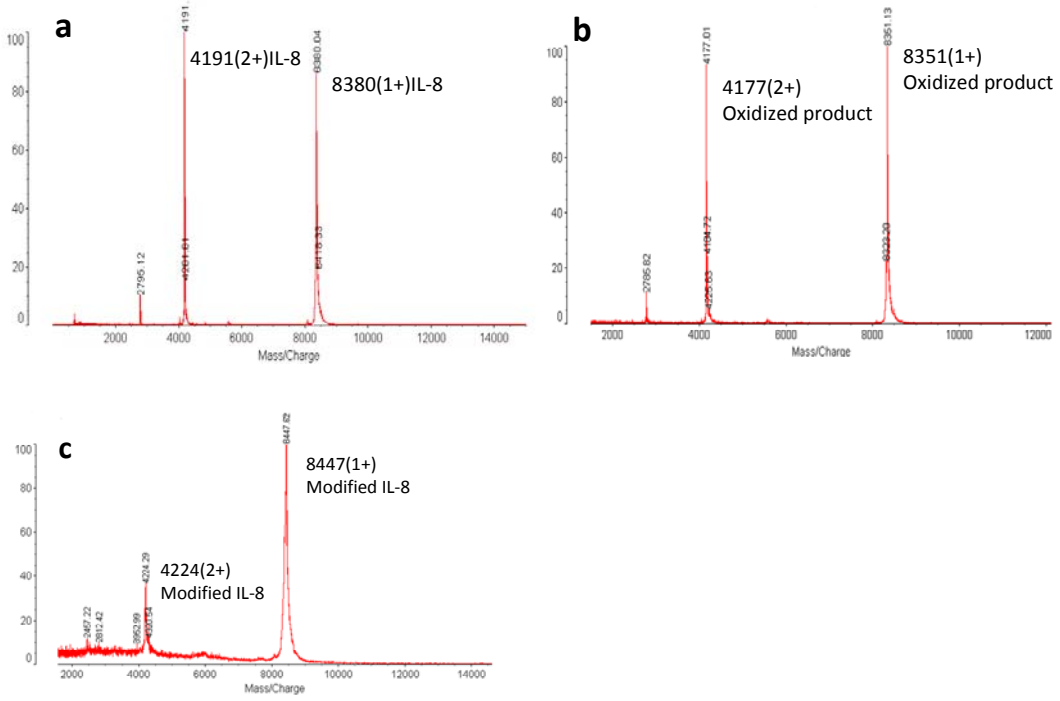
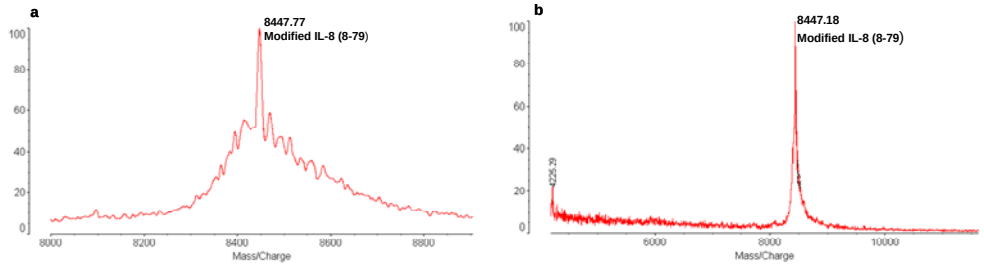


Figure S3: MALDI-TOF analysisof IL-8 modification through Wittig reaction: (a) unmodified IL-8; (b) the oxidized product of IL-8; (c) modified IL-8 by Wittig reaction.

Solvent Studies. The oxidized IL-8 (8-79) (5.0 μg, 0.60 nmol) containing aldehyde at N-terminus was treated with ylide **2h** (30 nmol, 11.0 μg, 50eq.), different solvent systems were screened. After proper mixing, the mixture was incubated at 37 ^{o}C for 1.5 h followed by MALDI-TOF analysis. The reaction proceeded very well at neutral and basic conditions, and yielded the similar result. However, if the reaction was performed at acidic conditions, no reaction occurred.



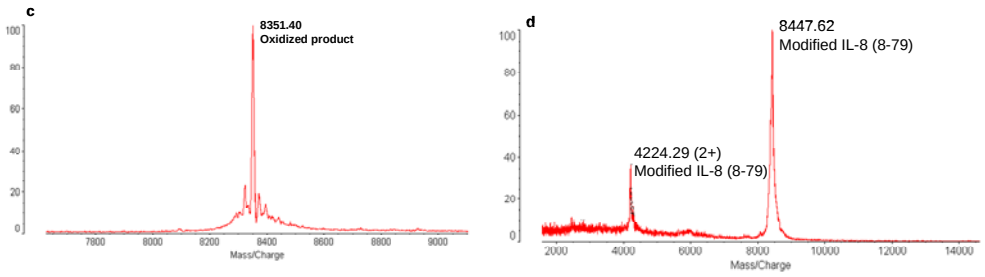


Figure S4. MALDI-TOF analysis of IL-8 modification through Wittig reaction at a) phosphate buffer pH = 7.4; b) phosphate buffer pH = 8, or glycine buffer/NaOH pH = 9, or glycine buffer/NaOH pH = 10; c) phosphate buffer pH＜6; d) H_2O.

Modification of myoglobin by Wittig reaction

To a solution of myoglobin (120 μL of 250 μM solution in 25 mM sodium phosphate buffer, pH 6.5) and phosphate buffer (180 μL, 25 mM, pH 6.5) in a 1.6 mL eppendorf tube was added a solution of pyridoxal 5′-phosphate (PLP) (300 μL of 20 mM solution in 2 mM phosphate buffer, pH adjusted to 6.5 with 1M NaOH). After brief agitation for proper mixing, the mixture was incubated at 37 ºC without further agitation for 18 h. The PLP was removed from the reaction mixture via spin concentration (Microcon® centrifugal filter 10,000MWCO (Millipore, MA)) eluting with water. A aliquot of this purified protein (25 μL, 60 μM) was diluted with H_2O (50 μL), ylide **2h** (28 μg, 50 eq.) and *t*-BuOH (25 μL) were added. The mixture was allowed to stand at 37 °C for 0.5 h without agitation. The reaction mixture was diluted with water (400 μL), and small molecules were removed via spin concentration (Microcon® centrifugal filter 10,000MWCO (Millipore, MA)). The residue was analyzed by ESI-MS.

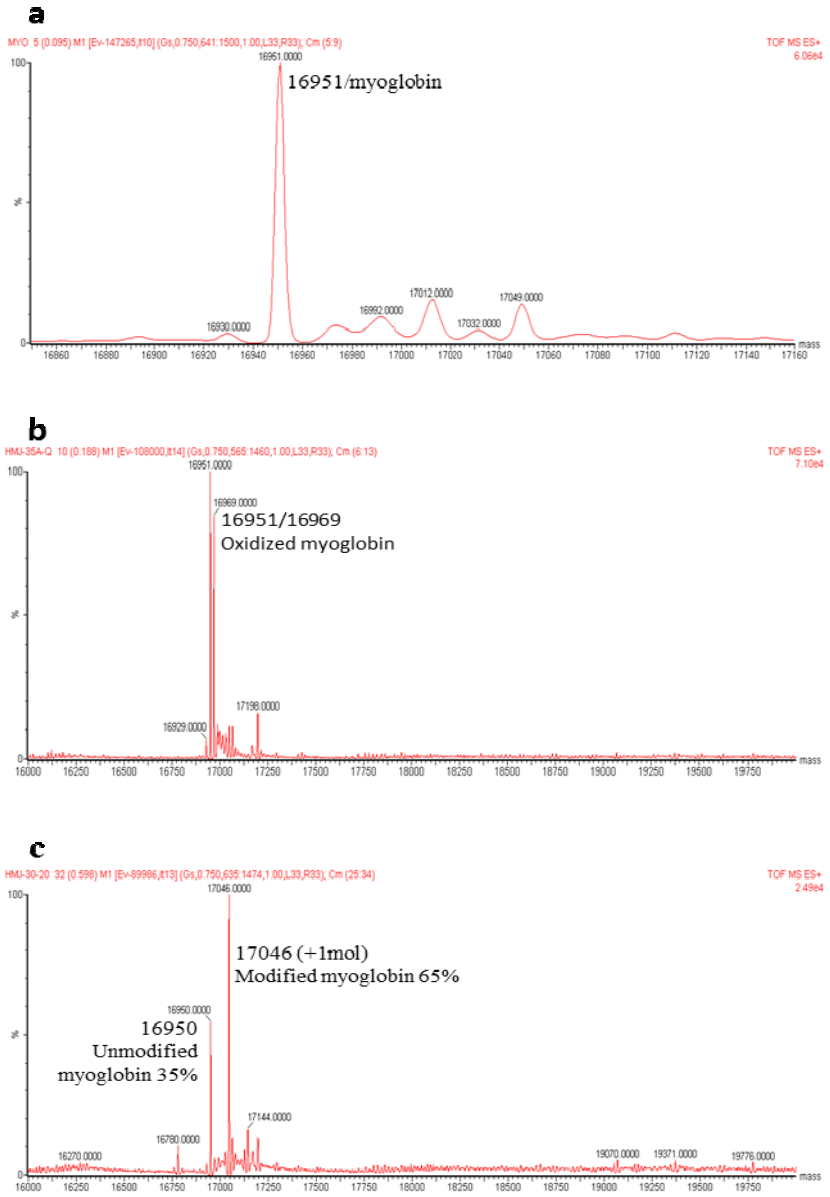


Figure S5: ESI-MS analysis of a) myoglobin; b) myoglobin after oxidation; c) myoglobin after modification by Wittig reaction.

Procedure for Trypsin Digestion of Myoglobin

Tryptic digest was performed by incubating the concentrated protein (20 μg, 40μL) with DTT (10 mM, 10 μL in 200 mM NH_4HCO_3) at 56 ^oC for 30 min. Then, the residue was added iodoacetamide (20 mM) to alkylate for 1h at room temperature. The mixture was added trypsin solution (1 μg) (Roche). The resulting solution was briefly vortexed, and then incubated at 37 °C

overnight. The peptide fragments were analyzed by MALDI-TOF-TOF (Brukerultraflex).

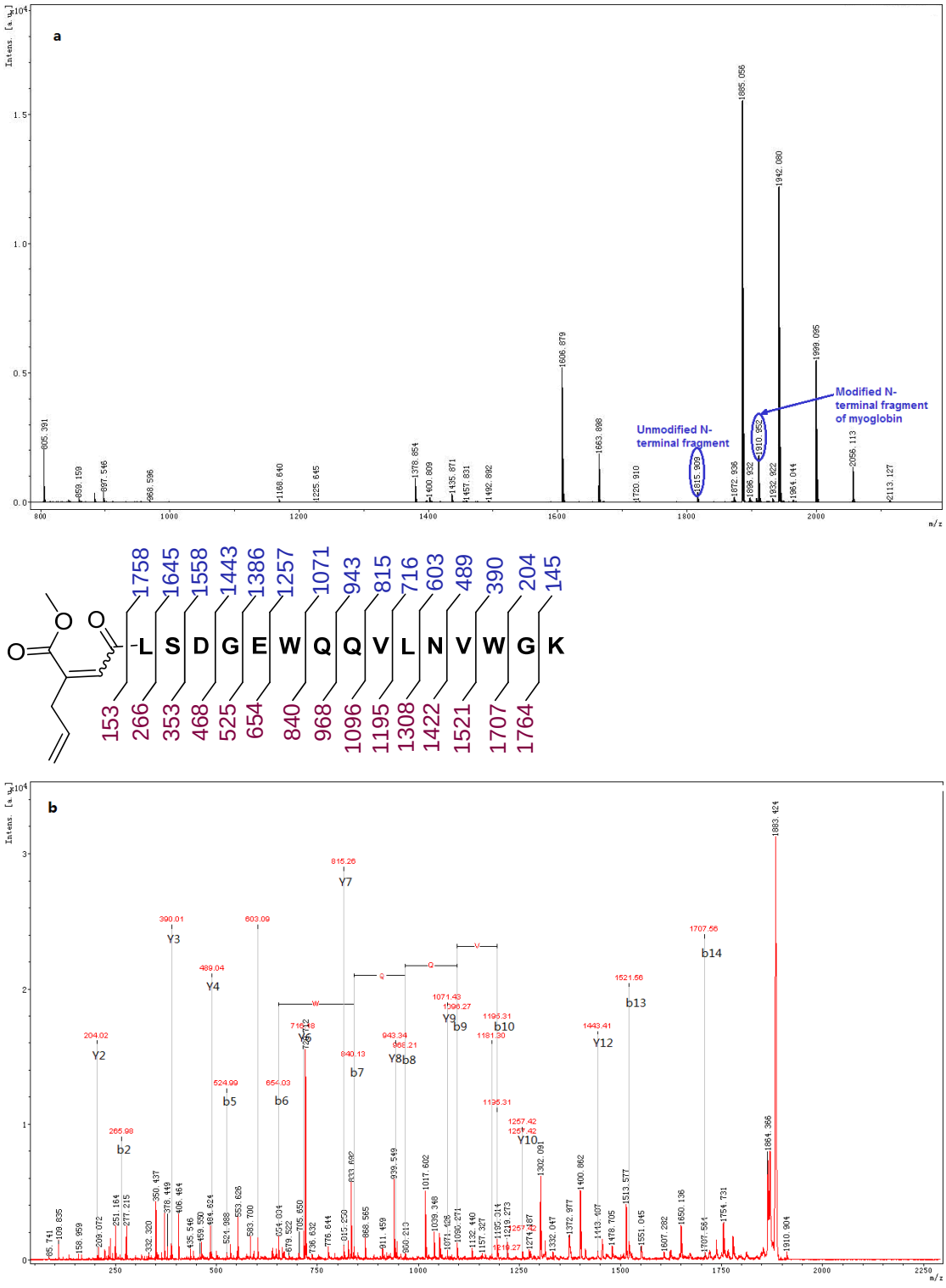


Figure S6: MALDI-TOF-TOF analysis of trypsin digestion of myoglobin. a) MALDI-TOF analysis of trypsin digest of myoglobin: the N-terminal fragment of unmodified myoglobin (residues 1-16) GLSDGEWQQVLNVWGK (expected mass = 1814.9, observed mass =1815.9);

the N-terminal peptide of modified myoglobin byWittig reaction (residues 1-16) (expected mass = 1910.2, observed mass = 1910.9). b) MALDI-TOF-TOF analysis of Wittig reaction modified N-terminal fragment (residues 1-16).

UV-Vis Spectroscopy. The myoglobin modified by Wittig reaction was analyzed by UV-Vis spectra. The strong absorbance at λ_{max} = 410 nm revealed that the heme moiety bound to myoglobin. The UV-Vis spectra obtained from the modified and unmodified samples were in good agreement with each other.

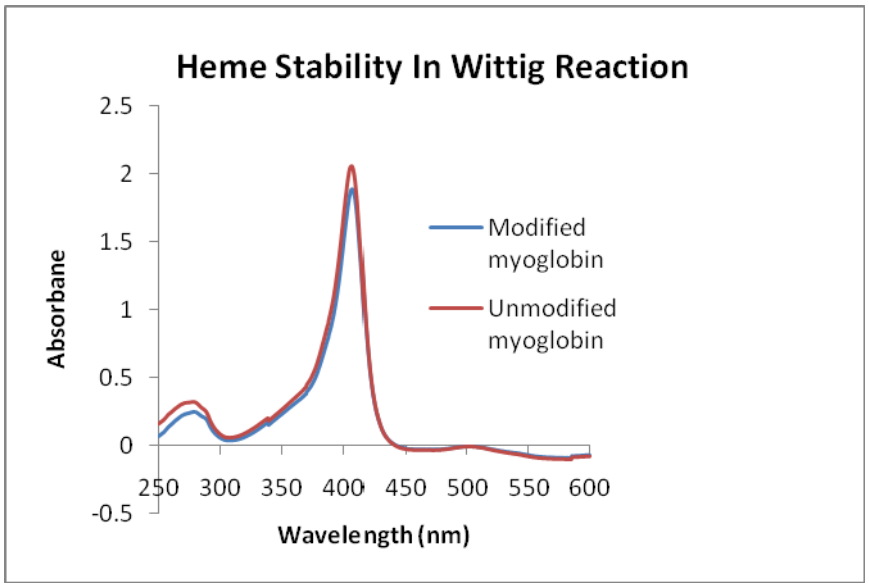


Figure S7: The UV-Vis spectra of the myoglobin before and after modification

Circular Dichroism Spectroscopy. A solution of myoglobin sample (15 μM) in H_2O (100 μL) was detected. Circular dichroism spectra were recorded with Pistar π-180 from Applied Photophysics Ltd spectrophotometer. Sample solutions were placed in quartz cell, and the average of three scans from 190 to 260 nm was reported. The ellipticity values were plotted against wavelength using Pro-Data Pistar software. The ultraviolet CD spectrum presented by the modified myoglobin demonstrated that the secondary structure of the modified protein was undamaged through the functionlization of Wittig reaction.

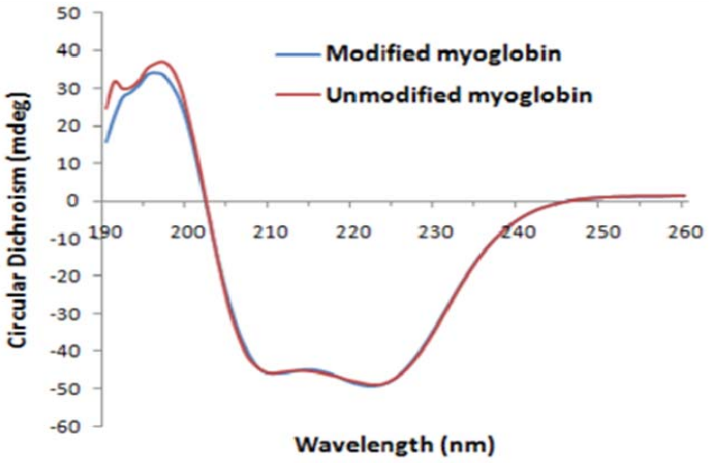


Figure S8: The CD spectra of the myoglobin before and after modification

Evaluation of the function of modified myoglobin for storing and releasing oxygen

The modified myoglobin by Wittig reaction and a control sample of myoglobin were dissolved in 50 mM sodium phosphate buffer (100 μL, pH 7.4), making the concentration of proteins to be 80 μM. The visible spectra (450 nm-700 nm) of two samples were analyzed directly (Figure S9a). Sodium hydrosulfite (1.14 M, 20 μL) was added to the solution. After brief agitation, the mixture was incubated at 37 °C for 20 min. The visible spectrum of the residue was analyzed (Figure S9b). The spectrum showed that the modified myoglobin could release oxygen at reductive conditions. Small molecules were removed via spin concentration (Microcon® centrifugal filter 10,000MWCO (Millipore, MA)), and the visible spectrum was analyzed (Figure S9c), which indicated that modified myoglobin could store oxygen in the presence of oxygen. The identical traces demonstrated that the function of myoglobin was not influenced after the modification by Wittig reaction. The color changes were also collected at each case, providing a good evidence for the conclusion.

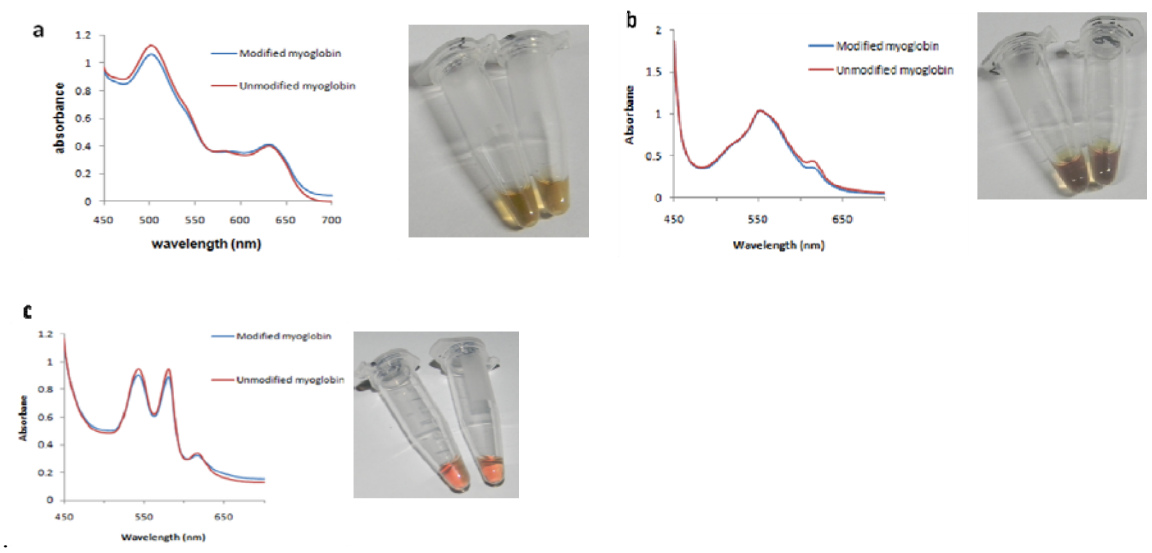


Figure S9: UV-Vis spectra and the color changes of a) myoglobin and modified myoglobin; (b) releasing and (c) storing oxygen function of the modified and unmodified myoglobin.

References

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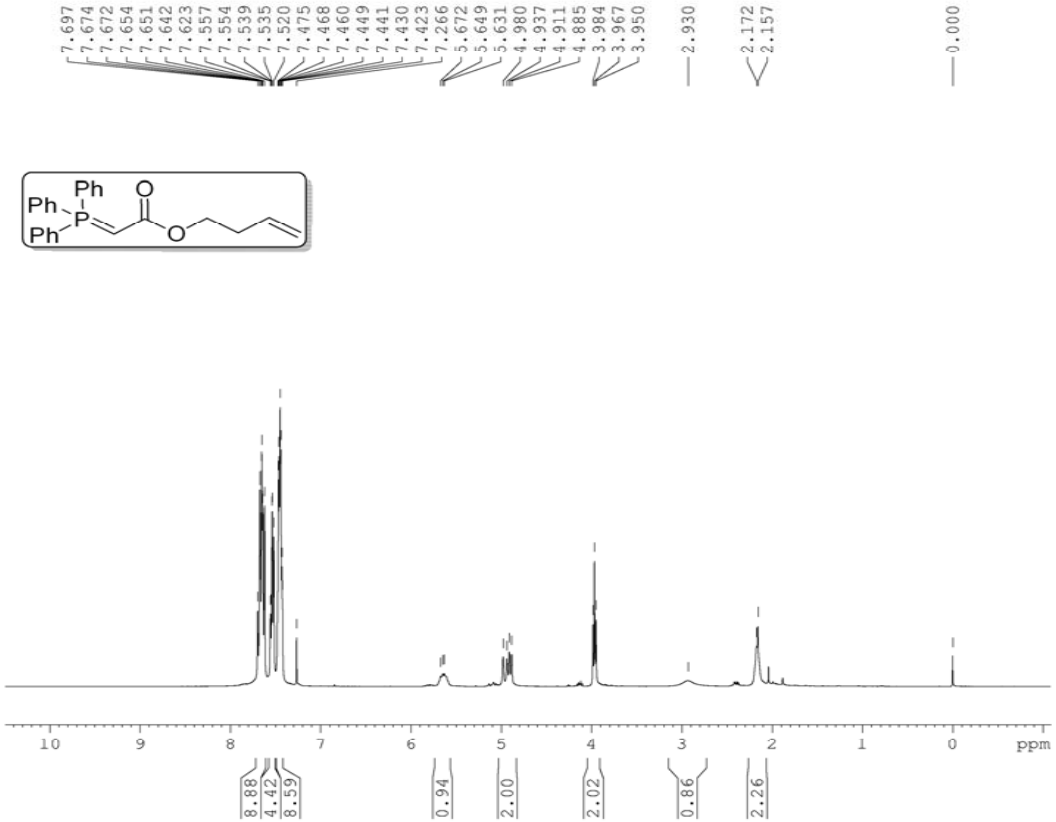
^{1}H and ^{13}C NMR spectra of compounds

^{1}H NMR of **2d**



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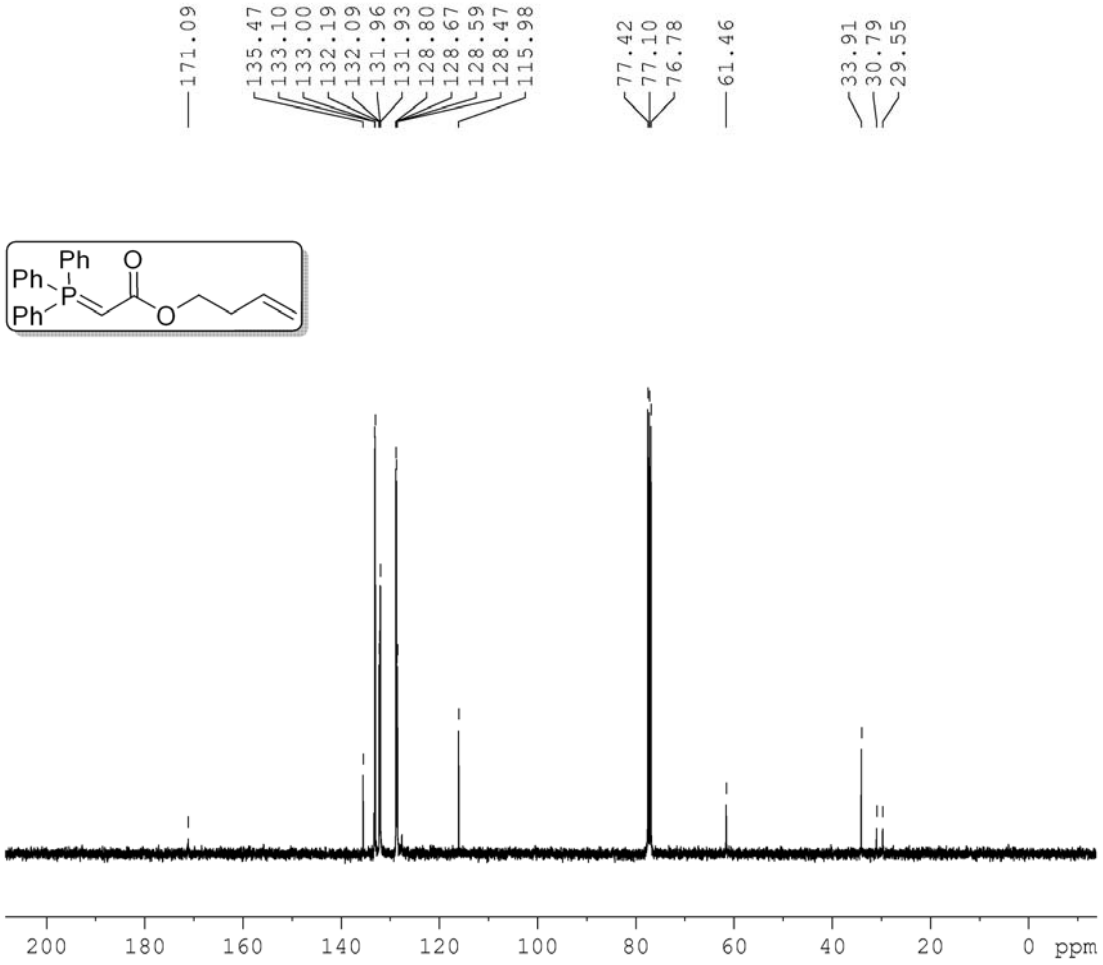
^{13}C NMR of **2d**



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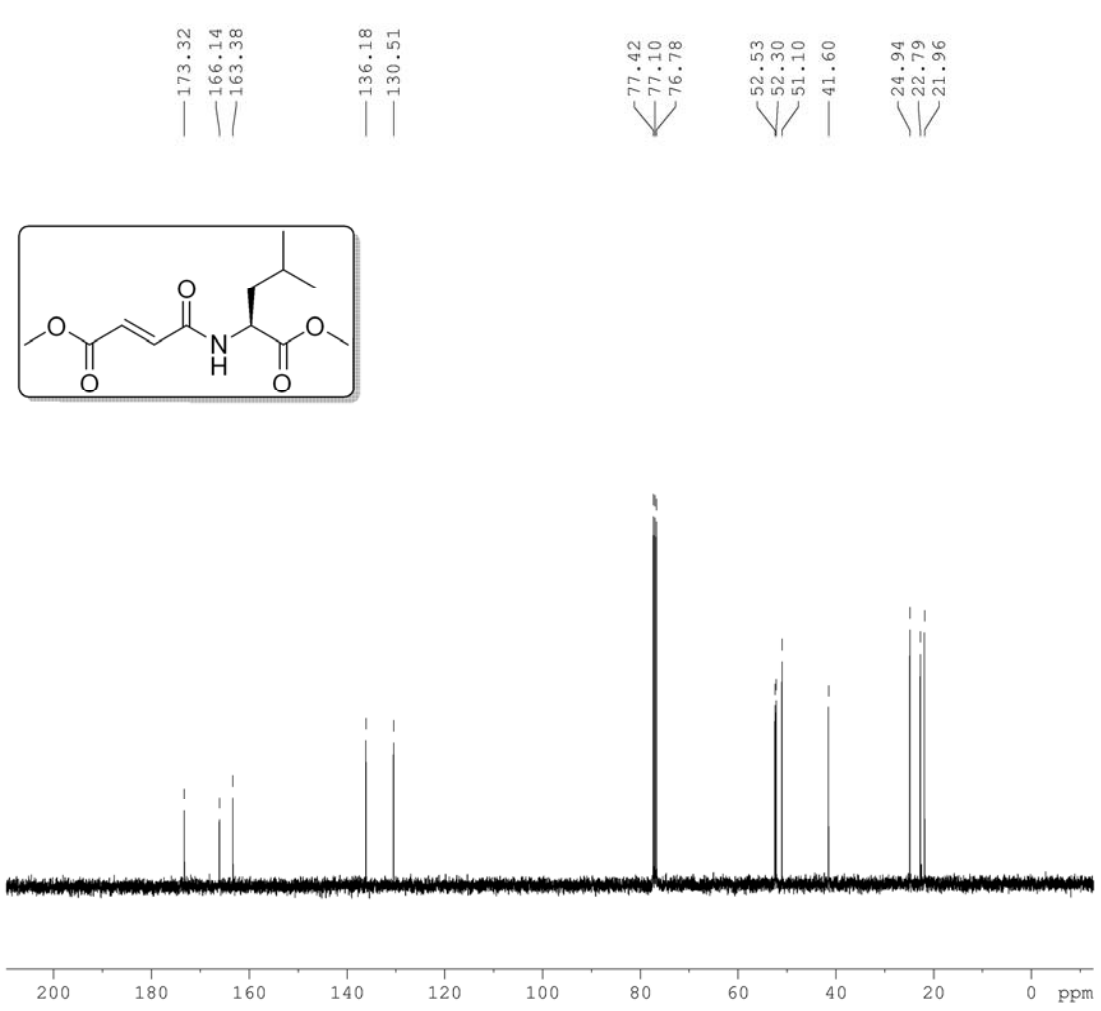
^{13}C NMR of **3a-1**



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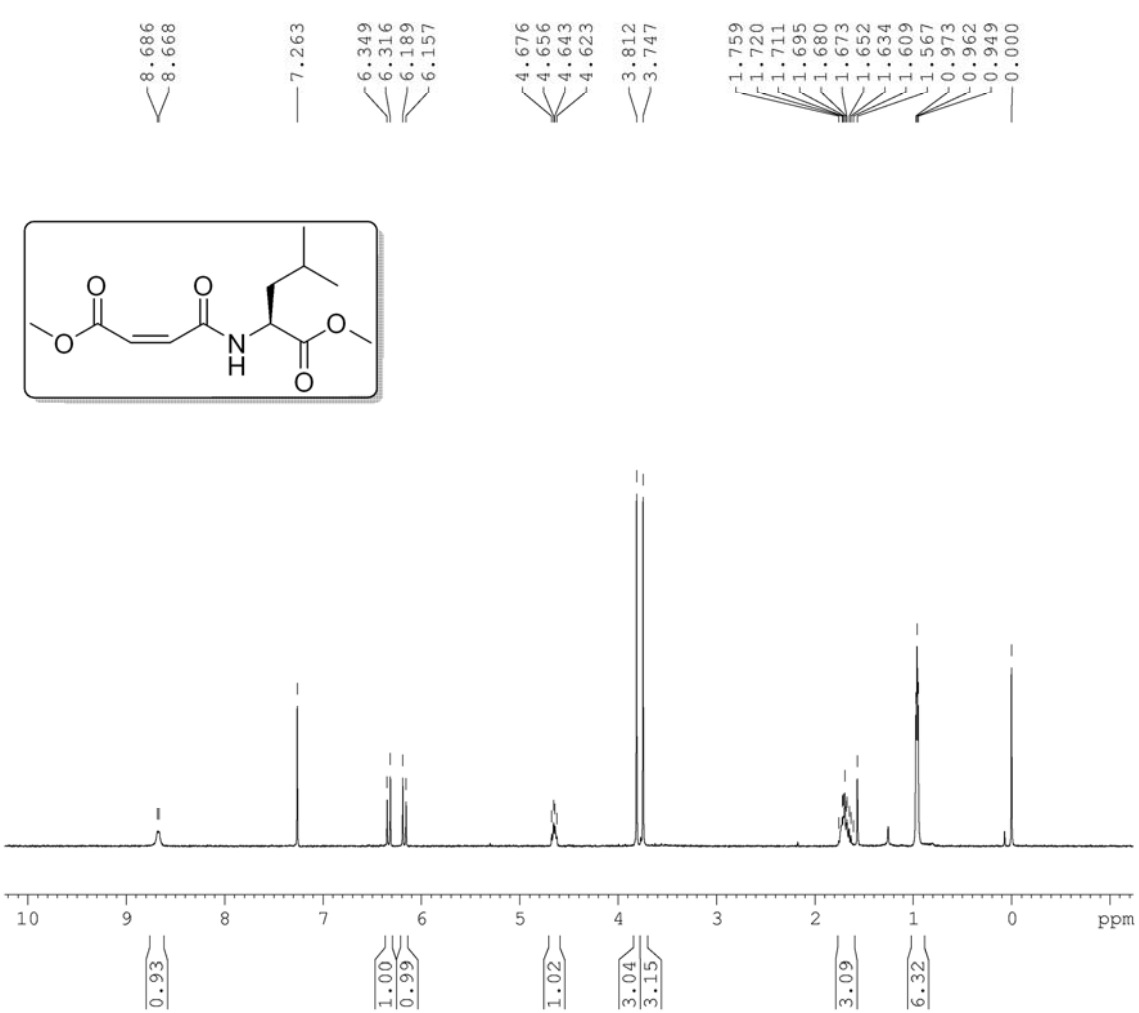
^{1}H NMR of **3a-2**



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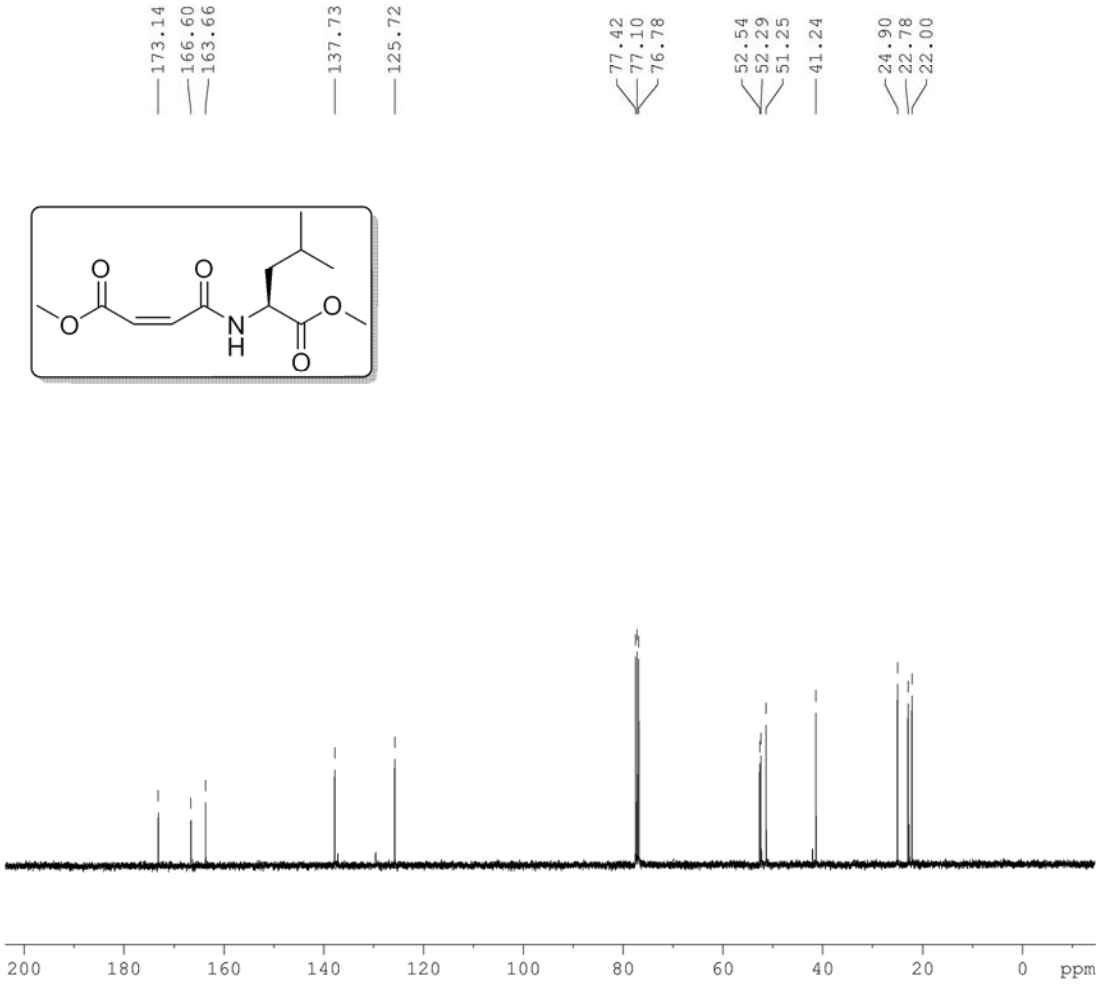
^{13}C NMR of **3a-2**



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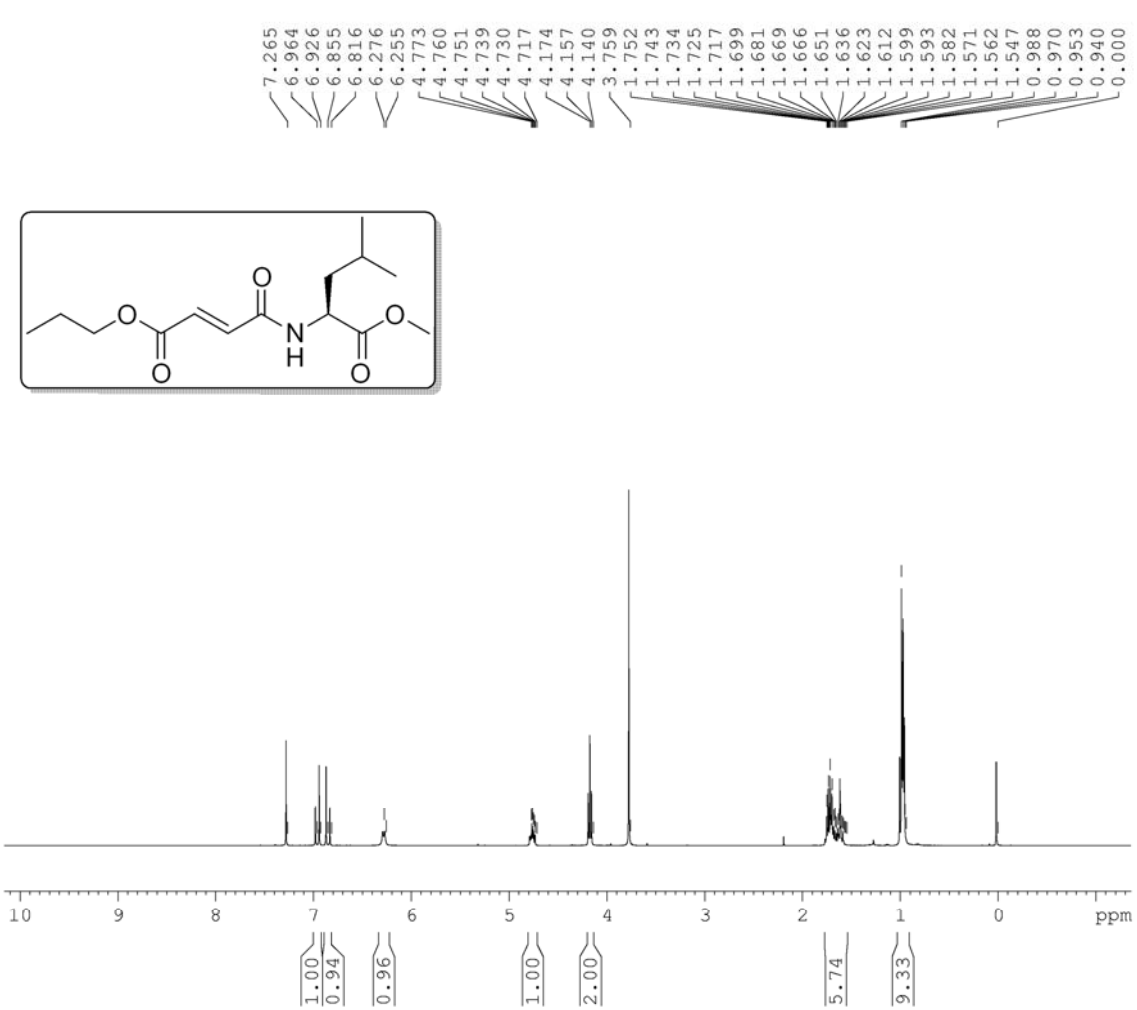
EXPNO	2
PROCNO	1
Date_	20110701
Time	16.19
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDC13
NS	80
DS	4
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	298.5 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127644 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

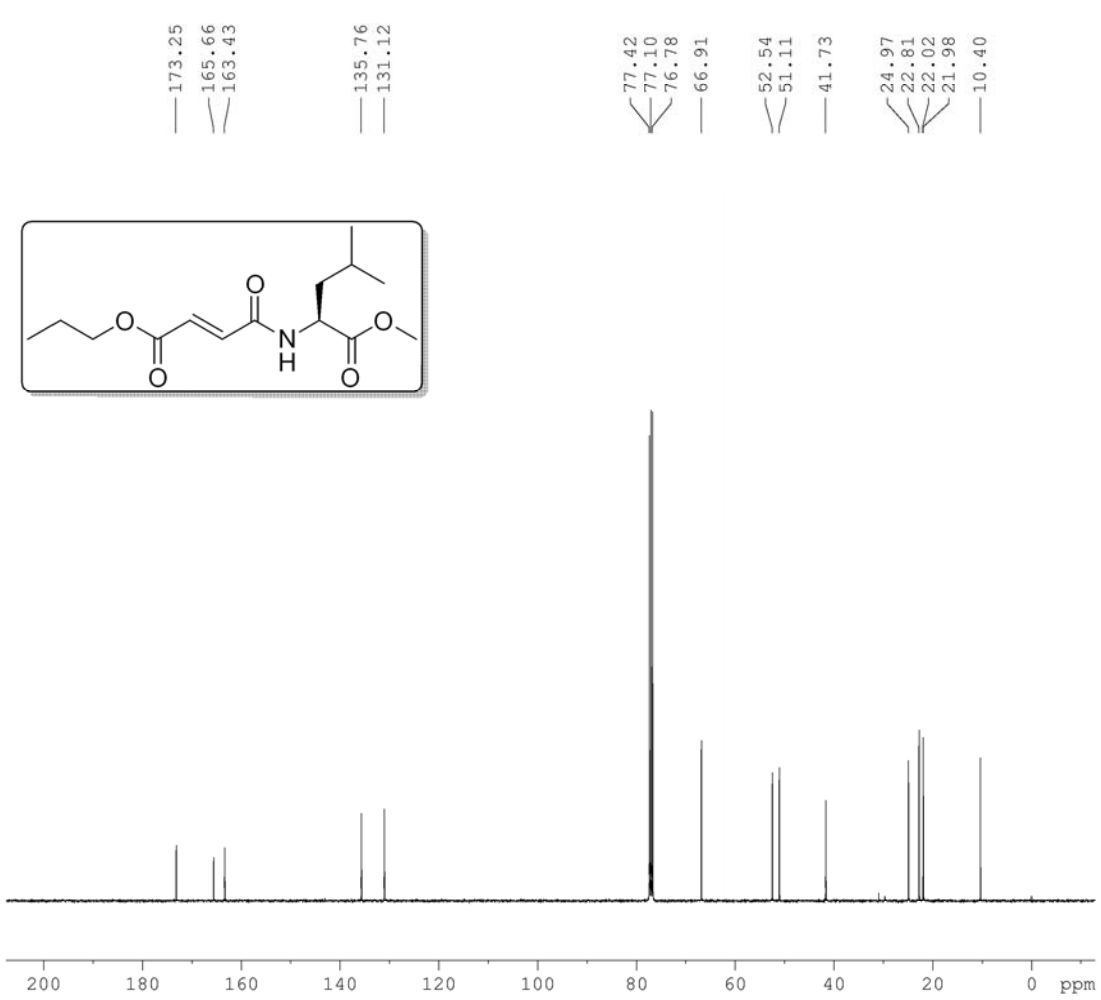
^{1}H NMR of **3b-1**



BRUKER

NAME	HMJ-c-44a-1
EXPNO	1
PROCNO	1
Date_	20110603
Time	16.00
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	183.47
DW	60.800 usec
DE	6.50 usec
TE	298.2 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

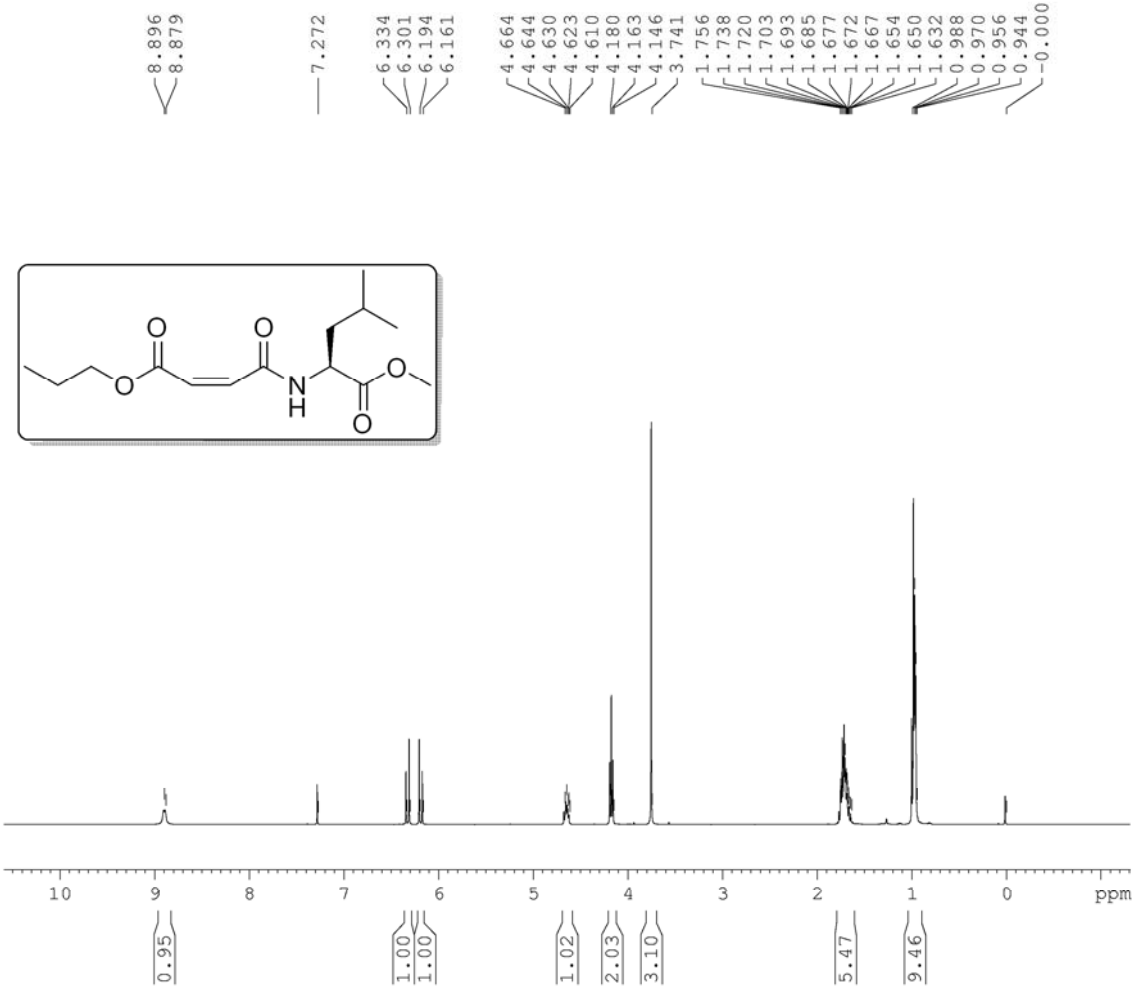
^{13}C NMR of **3b-1**



BRUKER

NAME	HMJ-c-44a-1
EXPNO	2
PROCNO	1
Date_	20110615
Time	11.15
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	2585
DS	2
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	299.9 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

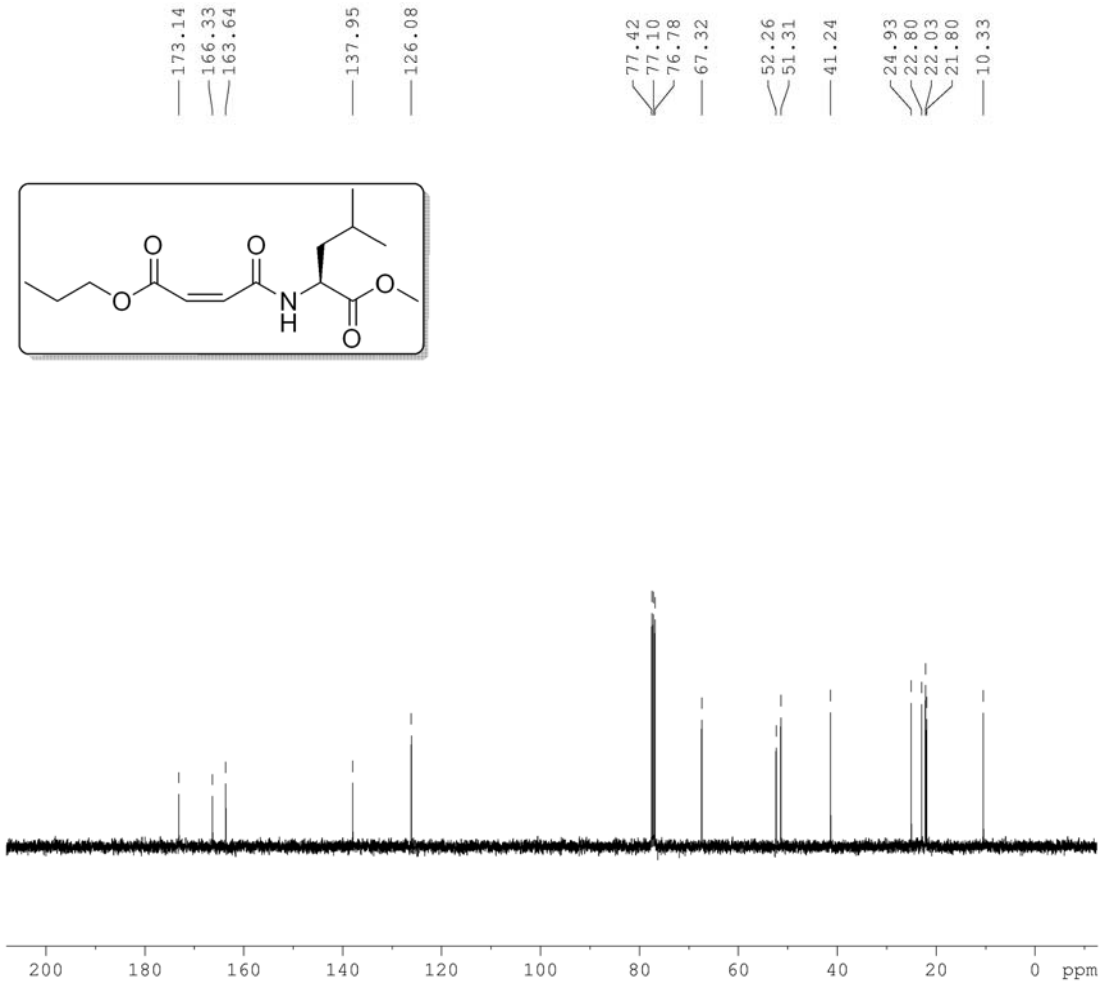
^{1}H NMR of **3b-2**



BRUKER

NAME	HMJ-c-44a-2
EXPNO	1
PROCNO	1
Date_	20110603
Time	15.56
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	119.06
DW	60.800 usec
DE	6.50 usec
TE	298.2 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

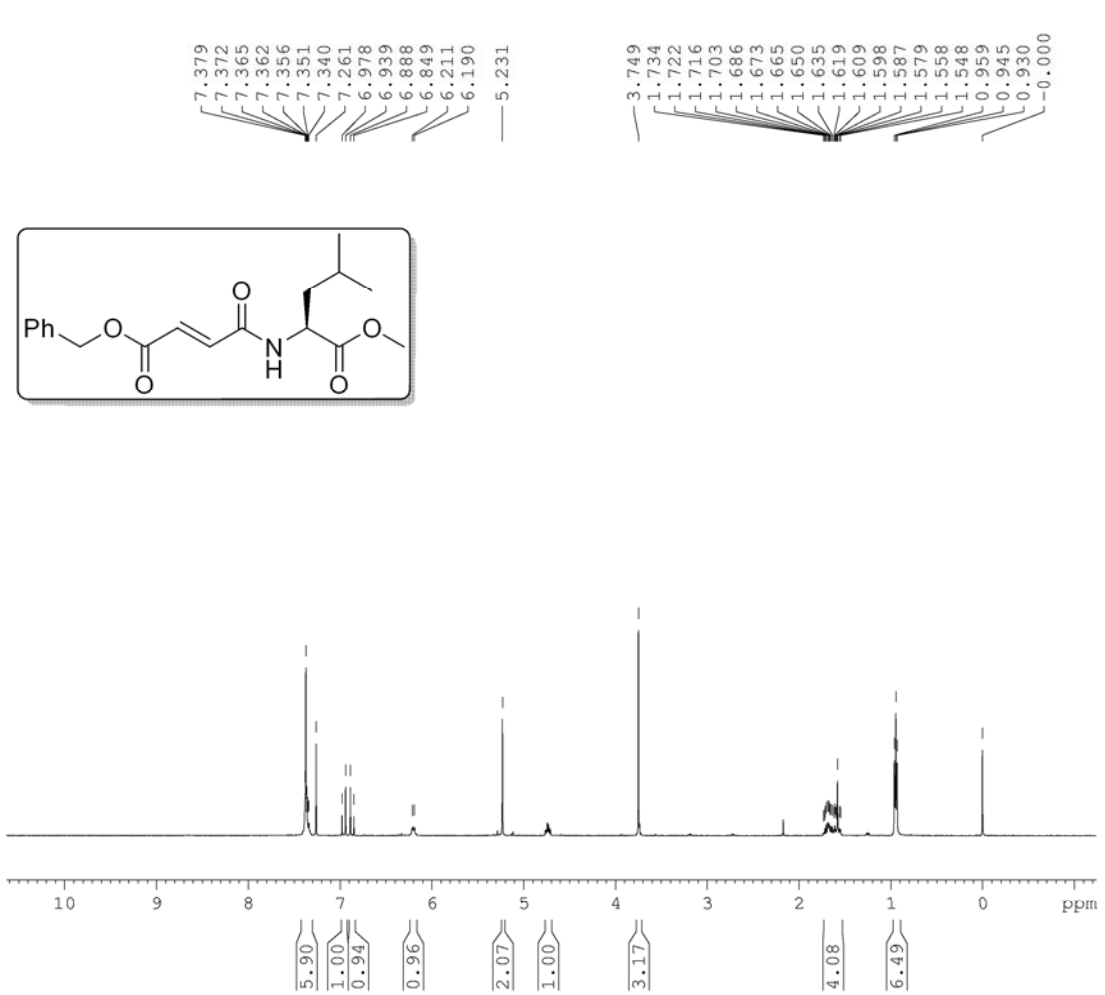
^{13}C NMR of **3b-2**



BRUKER

NAME	HMJ-c-44a-2
EXPNO	2
PROCNO	1
Date_	20110610
Time	17.45
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDC13
NS	24
DS	2
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	183.47
DW	20.800 usec
DE	6.50 usec
TE	300.4 K
D1	1.00000000 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127633 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

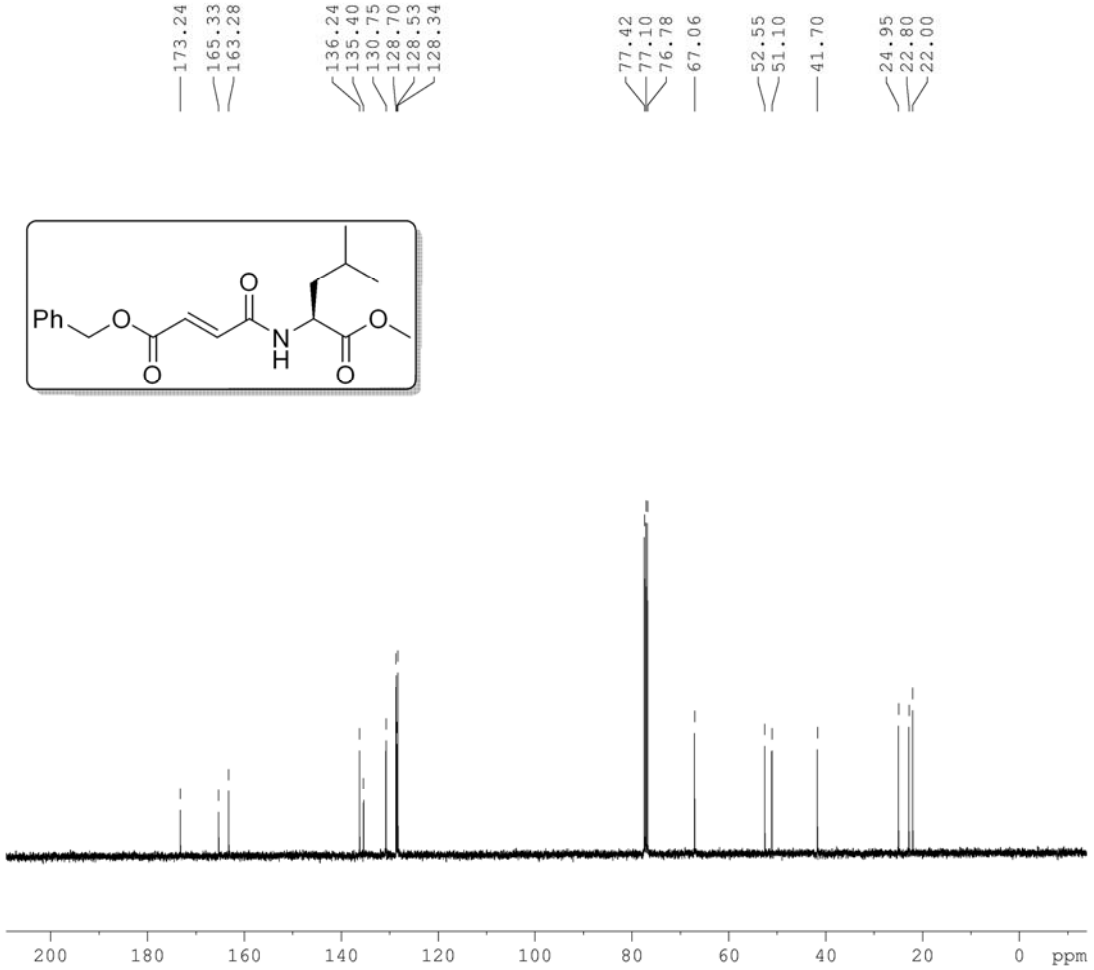
^{1}H NMR of **3c-1**



BRUKER

NAME	HMJ-c-43a-1
EXPNO	1
PROCNO	1
Date_	20110601
Time	16.24
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	183.47
DW	60.800 usec
DE	6.50 usec
TE	298.4 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300092 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

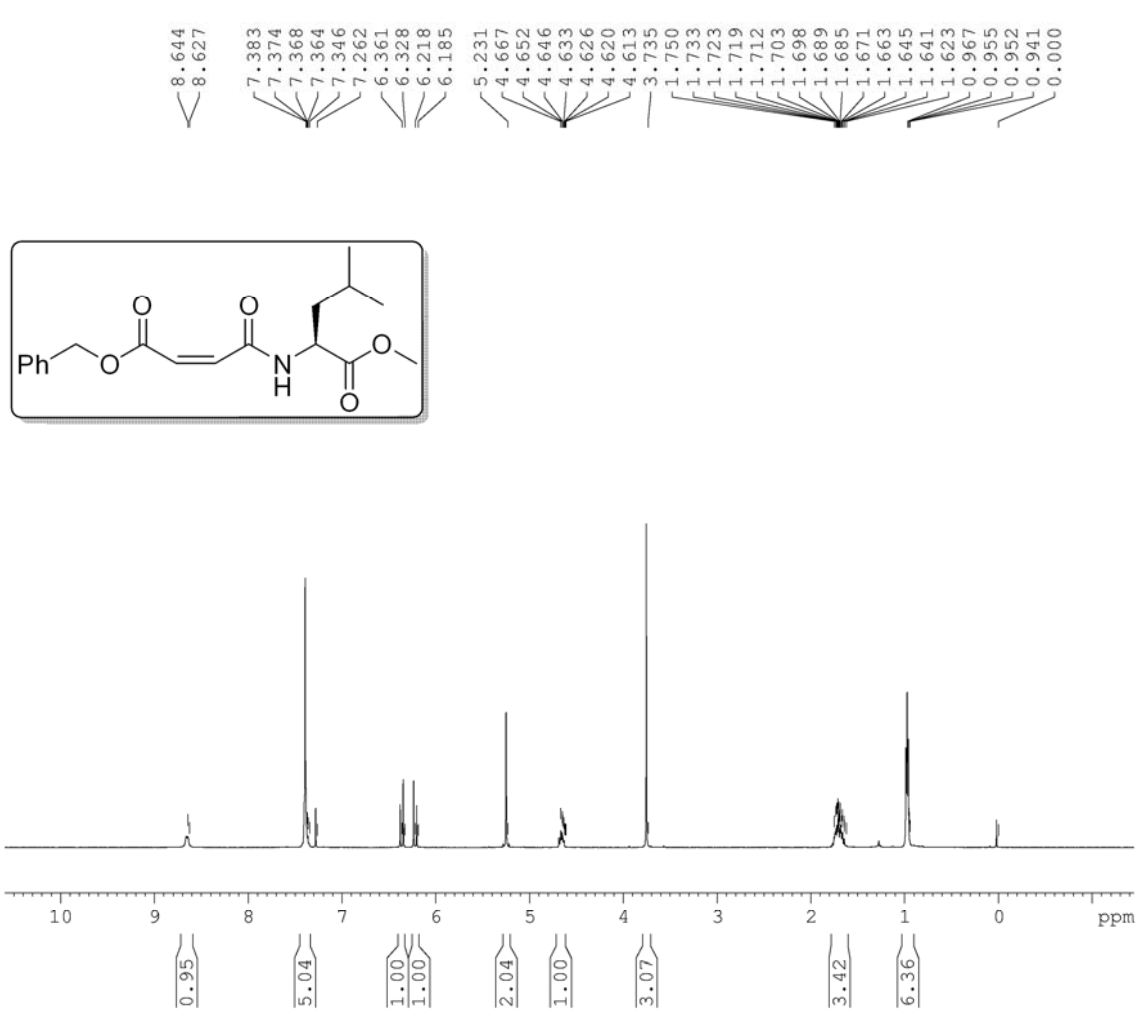
^{13}C NMR of **3c-1**



BRUKER

NAME	HMJ-c-43a-1
EXPNO	2
PROCNO	1
Date_	20110701
Time	16.28
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	272
DS	4
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	299.2 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127625 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

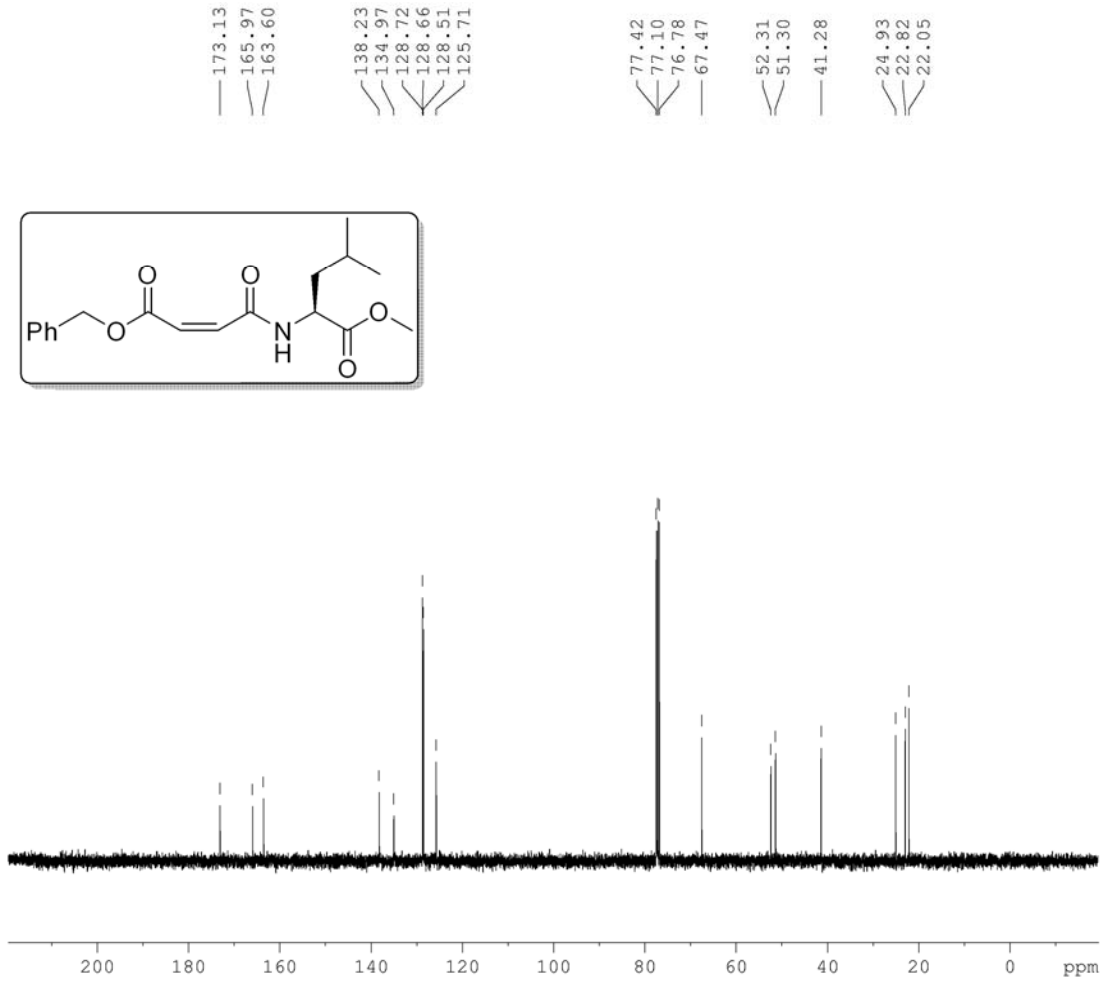
^{1}H NMR of **3c-2**



BRUKER

NAME	HMJ-c-44b-2
EXPNO	1
PROCNO	1
Date_	20110603
Time	15.48
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	133.41
DW	60.800 usec
DE	6.50 usec
TE	298.2 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

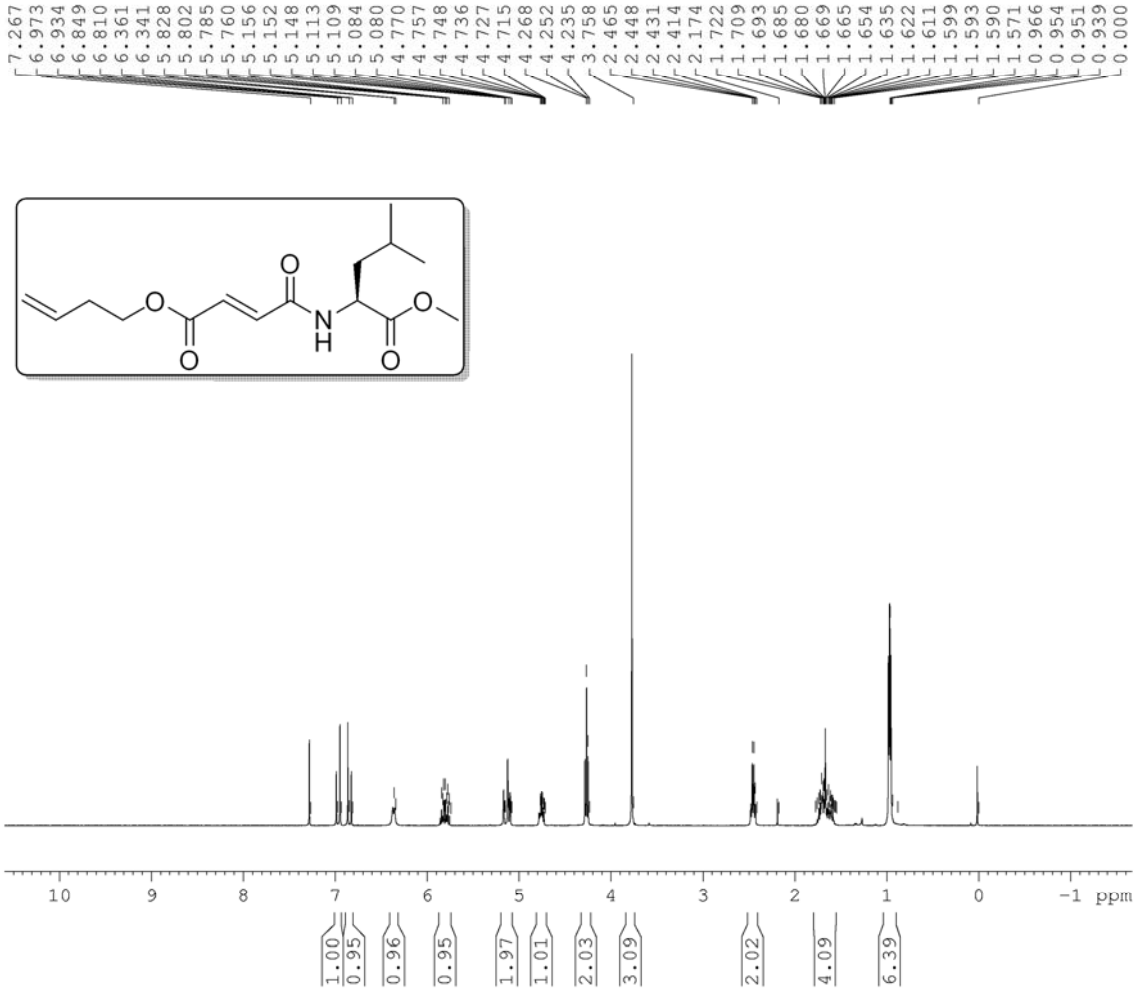
^{13}C NMR of **3c-2**



BRUKER

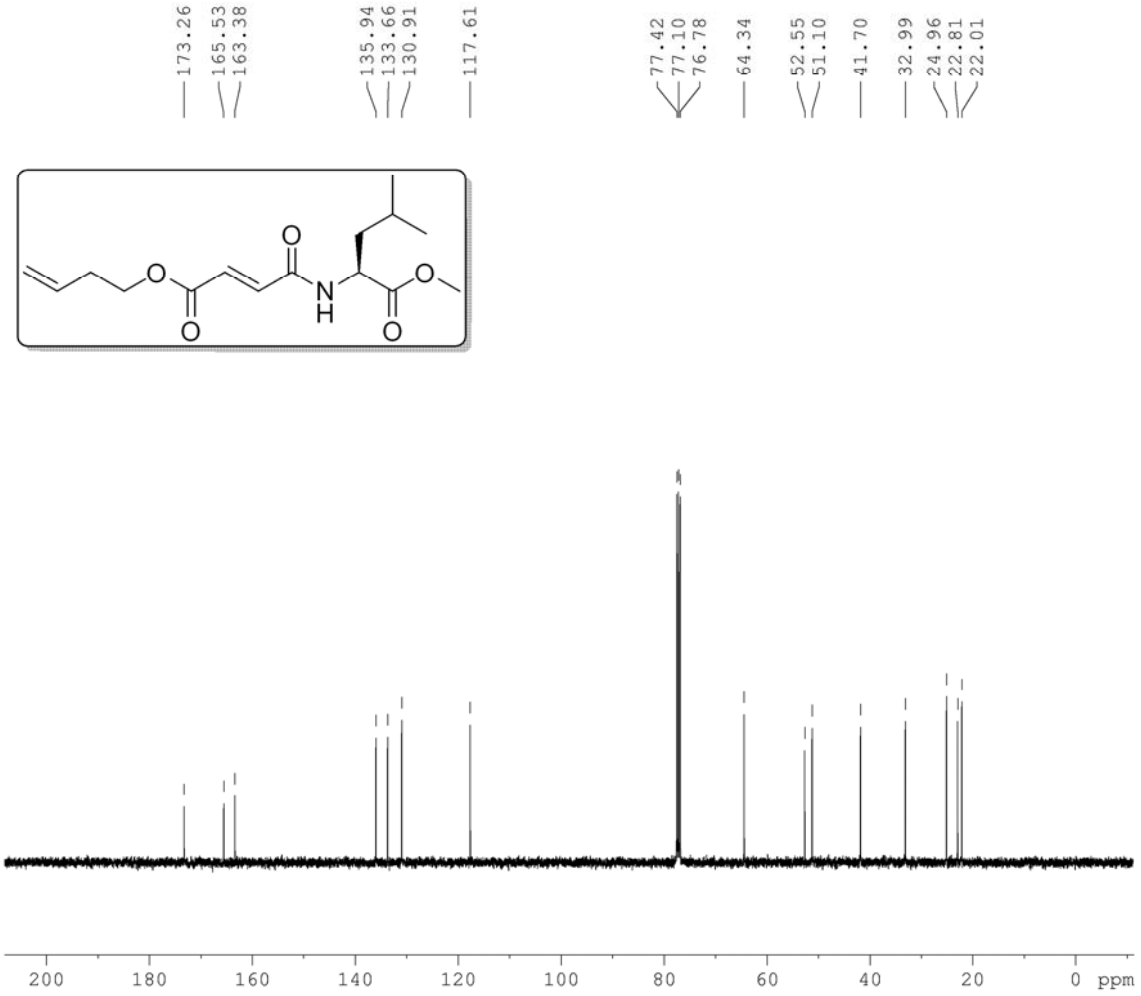
NAME	HMJ-c-44b-2
EXPNO	2
PROCNO	1
Date_	20110604
Time	16.32
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDC13
NS	30
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	183.47
DW	20.800 usec
DE	6.50 usec
TE	299.3 K
D1	2.00000000 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127636 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{1}H NMR of **3d-1**



NAME	HMJ-D-2a-1
EXPNO	1
PROCNO	1
Date_	20110614
Time	20.05
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	148.12
DW	60.800 usec
DE	6.50 usec
TE	298.4 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

^{13}C NMR of **3d-1**

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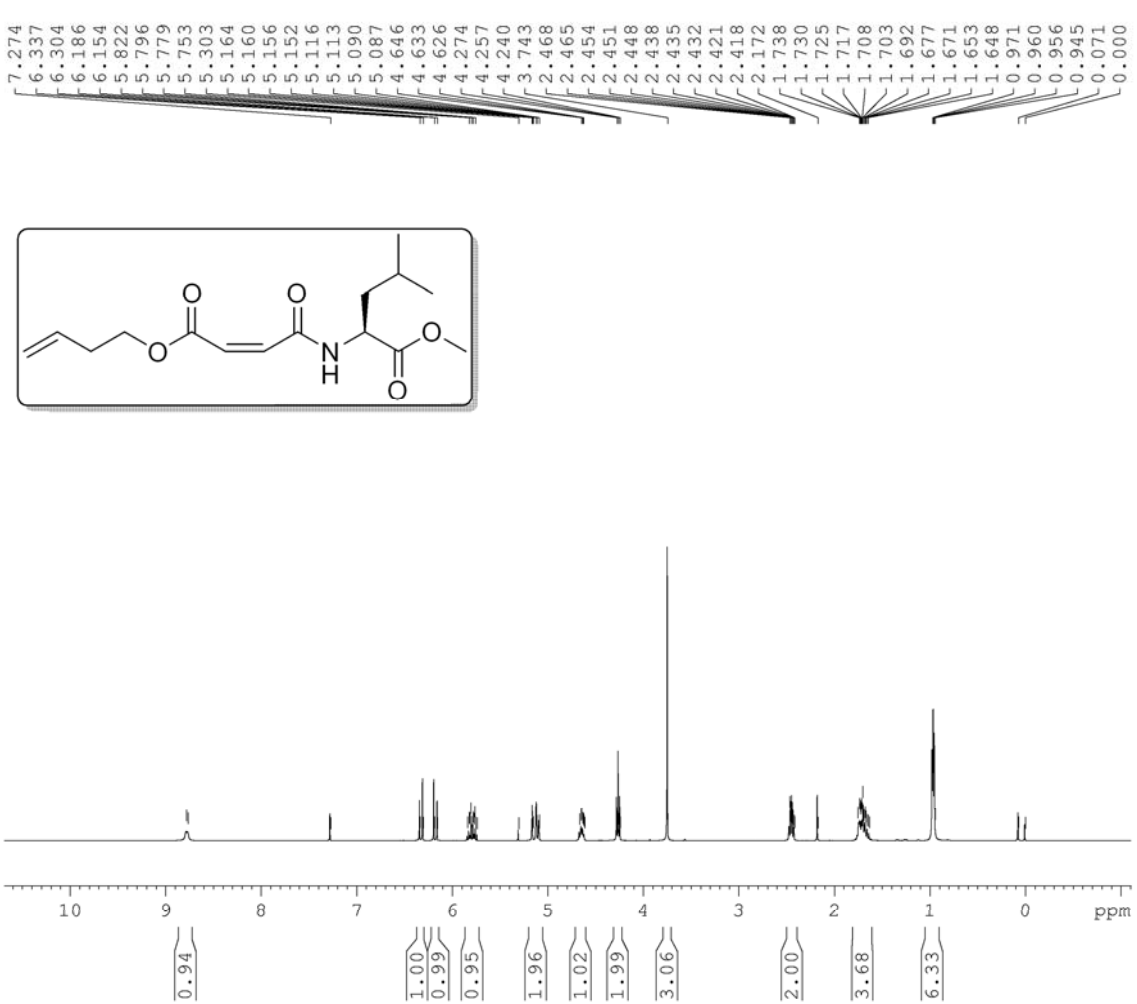
NAME            HMJ-D-2a-1
EXPNO                    2
PROCNO                   1
Date_             20110617
Time                 11.08
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      198
DS                        4
SWH               28409.092 Hz
FIDRES             0.433488 Hz
AQ                1.1534836 sec
RG                   183.47
DW                   17.600 usec
DE                     6.50 usec
TE                    300.2 K
D1               1.20000005 sec
D11              0.03000000 sec

======== CHANNEL f1 ========
NUC1                    13C
P1                     9.00 usec
SI                    32768
SF             100.6127615 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

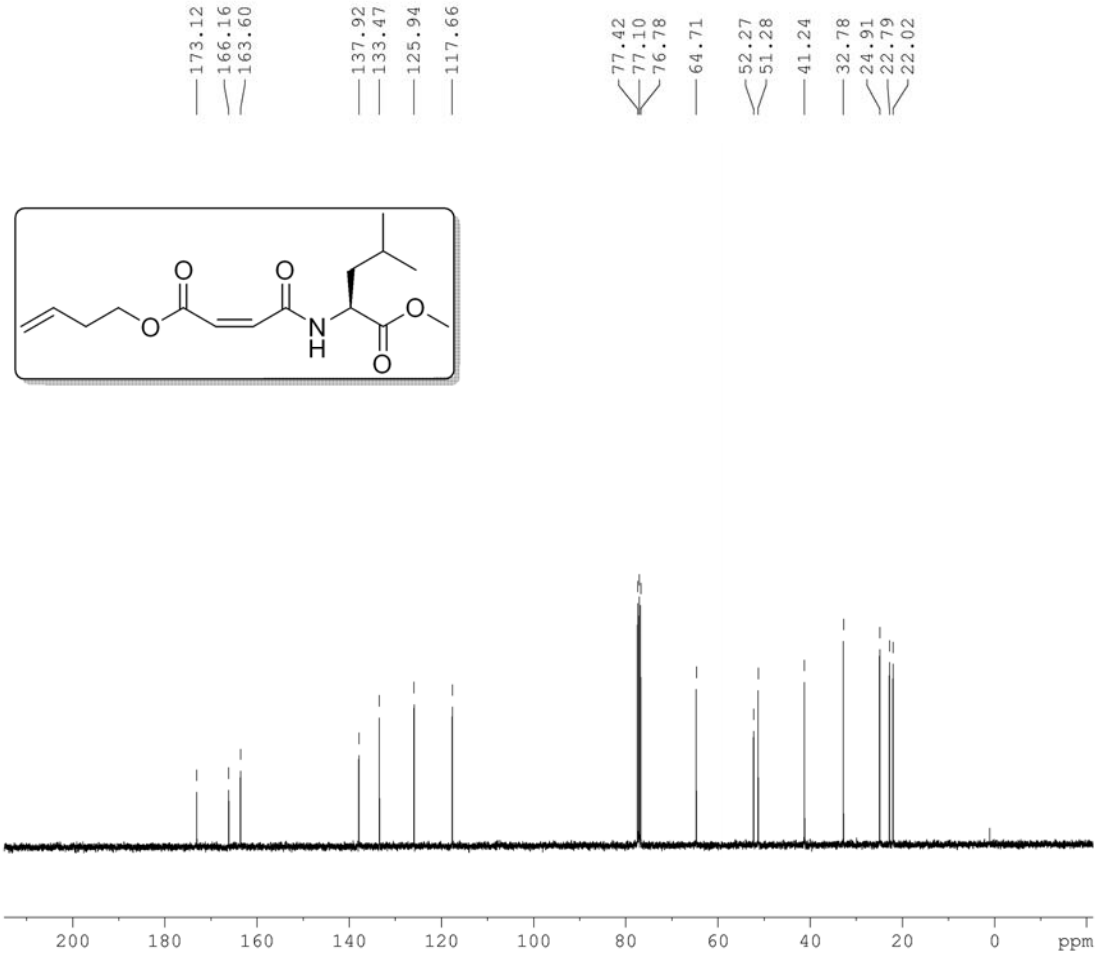
^{1}H NMR of **3d-2**



BRUKER

NAME	HMJ-D-2a-2
EXPNO	1
PROCNO	1
Date_	20110614
Time	20.14
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDC13
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	91.92
DW	60.800 usec
DE	6.50 usec
TE	298.3 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

^{13}C NMR of **3d-2**



BRUKER

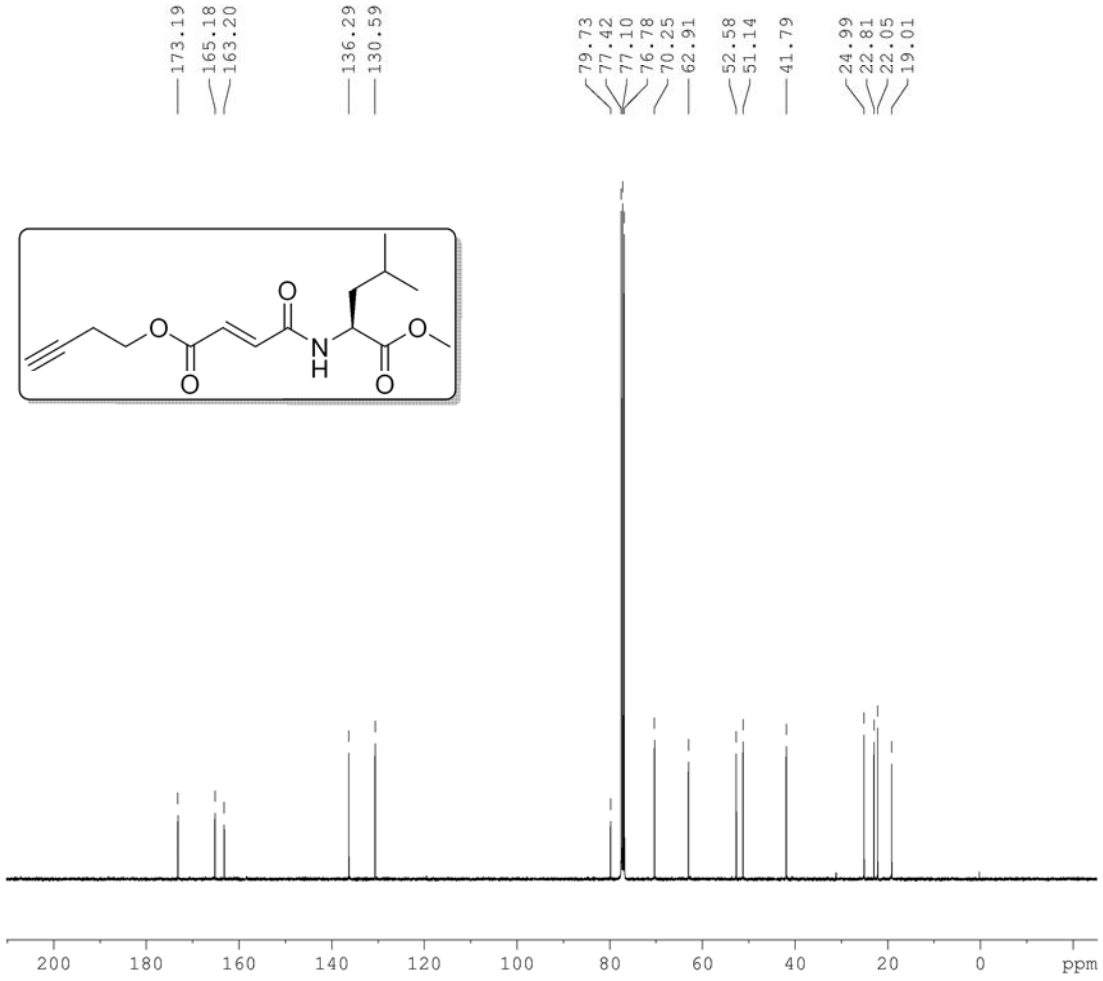
NAME	HMJ-D-2a-2
EXPNO	2
PROCNO	1
Date_	20110620
Time	11.10
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	140
DS	2
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	300.4 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127643 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{1}H NMR of **3e-1**

BRUKER

NAME	hmj-d-5b-1
EXPNO	1
PROCNO	1
Date_	20110621
Time	18.51
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	148.12
DW	60.800 usec
DE	6.50 usec
TE	299.1 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300064 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

^{13}C NMR of **3e-1**



BRUKER

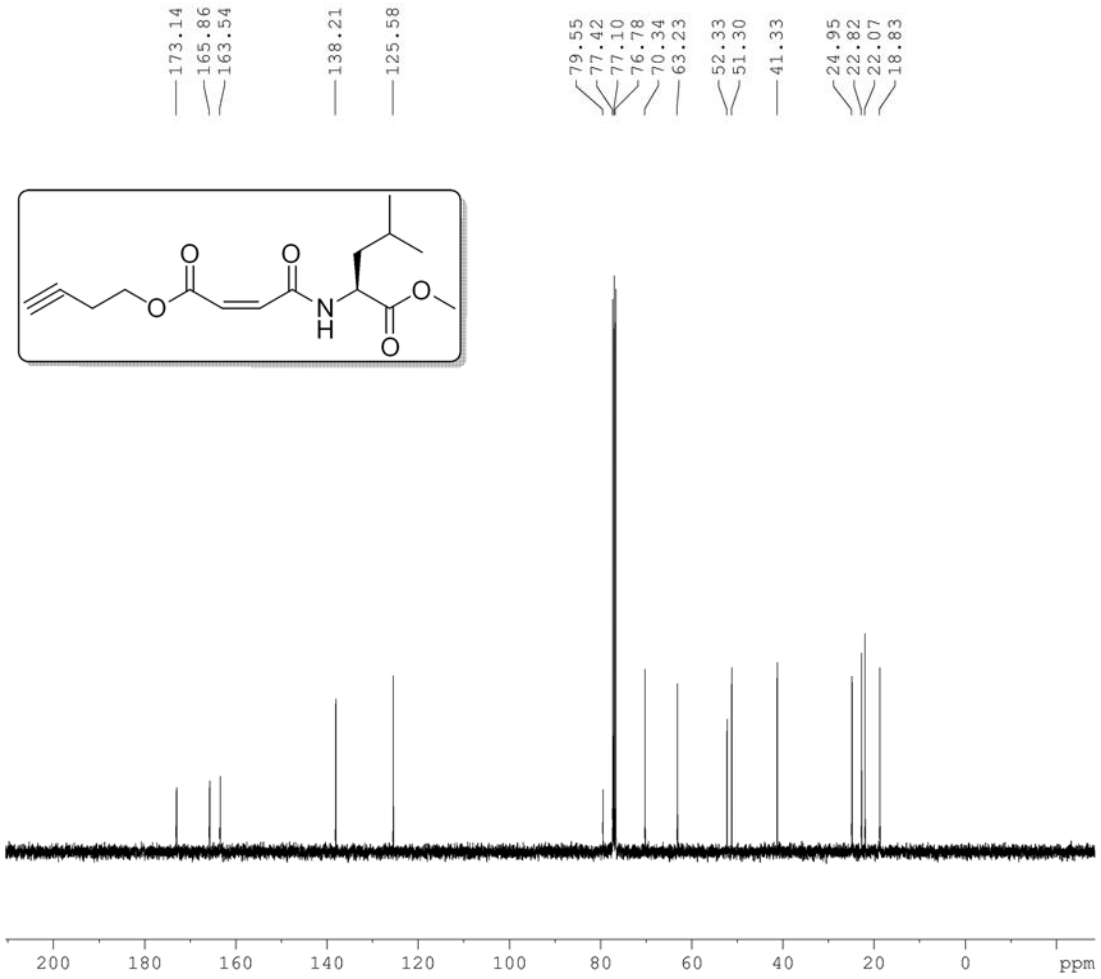
NAME	hmj-d-5b-1
EXPNO	2
PROCNO	1
Date_	20110622
Time	11.10
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	4384
DS	2
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	300.3 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127601 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{1}H NMR of **3e-2**

BRUKER

NAME	HMJ-D-5b-2
EXPNO	1
PROCNO	1
Date_	20110620
Time	21.52
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	112.54
DW	60.800 usec
DE	6.50 usec
TE	673.1 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.00 usec
SI	65536
SF	400.1300048 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

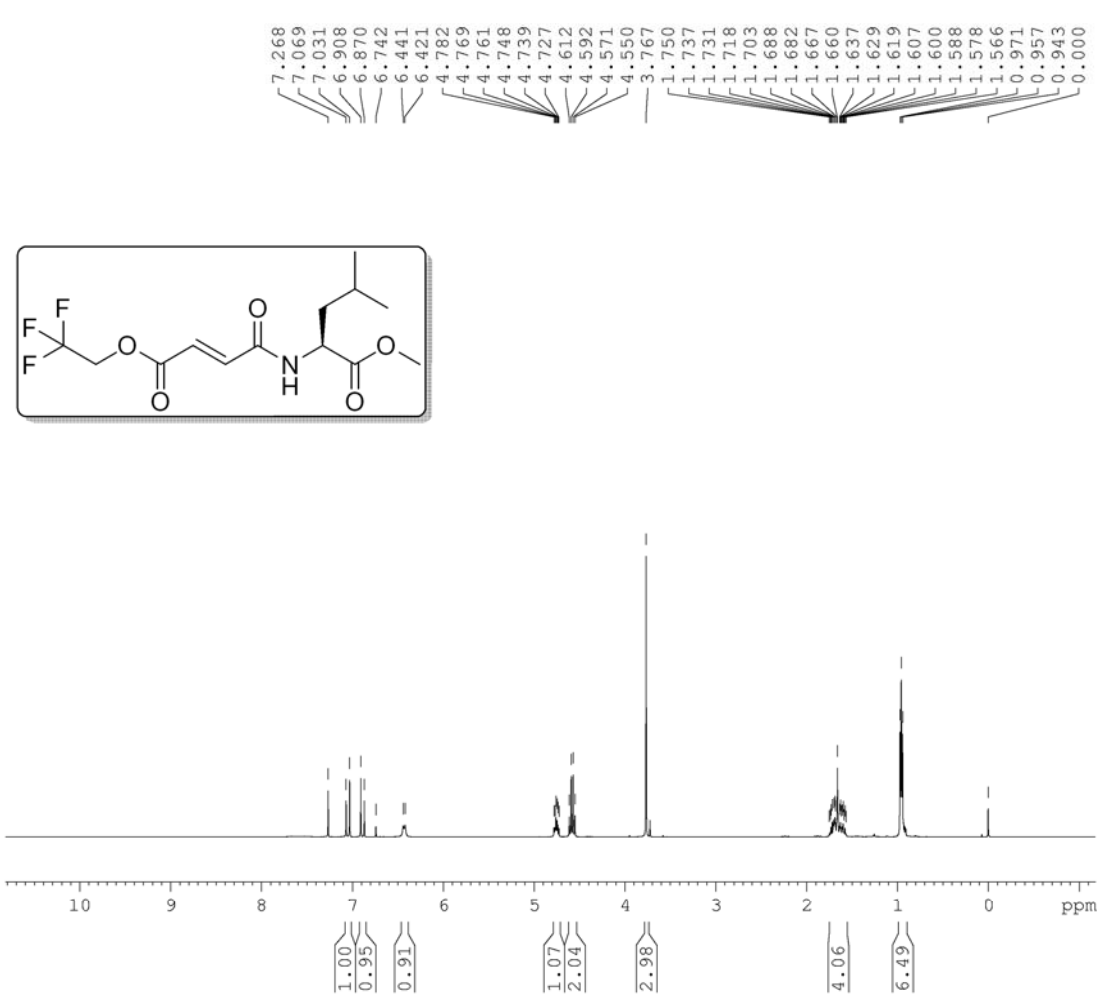
^{13}C NMR of **3e-2**



BRUKER

NAME	HMJ-D-5b-2
EXPNO	2
PROCNO	1
Date_	20110621
Time	18.58
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDC13
NS	117
DS	2
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	300.6 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

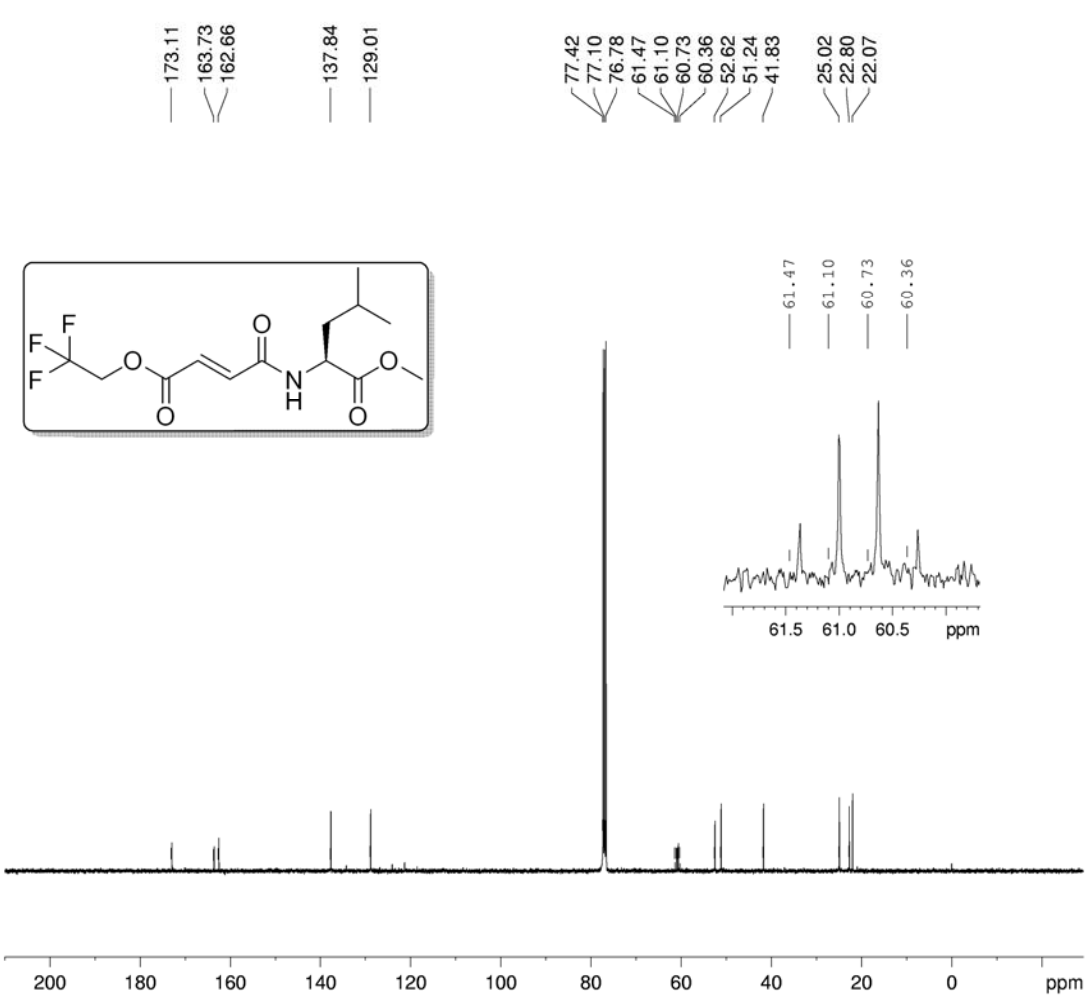
^{1}H NMR of **3f-1**



BRUKER

NAME	HMJ-D-8a-1
EXPNO	1
PROCNO	1
Date_	20110624
Time	11.31
INSTRUM	spect
PROBHD	5 mm PADUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	287
DW	60.800 usec
DE	6.50 usec
TE	296.8 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.00 usec
PL1	0.00 dB
PL1W	9.46981144 W
SFO1	400.1544711 MHz
SI	32768
SF	400.1520053 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

^{13}C NMR of **3f-1**




```
NAME            HMJ-D-7a-1
EXPNO                   22
PROCNO                   1
Date_             20120320
Time                 18.26
INSTRUM              spect
PROBHD    5 mm PADUL 13C
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                    3124
DS                       4
SWH              28409.092 Hz
FIDRES           0.433488 Hz
AQ               1.1534836 sec
RG                    2050
DW                  17.600 usec
DE                   6.50 usec
TE                  307.0 K
D1              1.20000005 sec
D11             0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                    9.29 usec
PL1                   0.00 dB
PL1W          35.66878891 W
SFO1         100.6293680 MHz

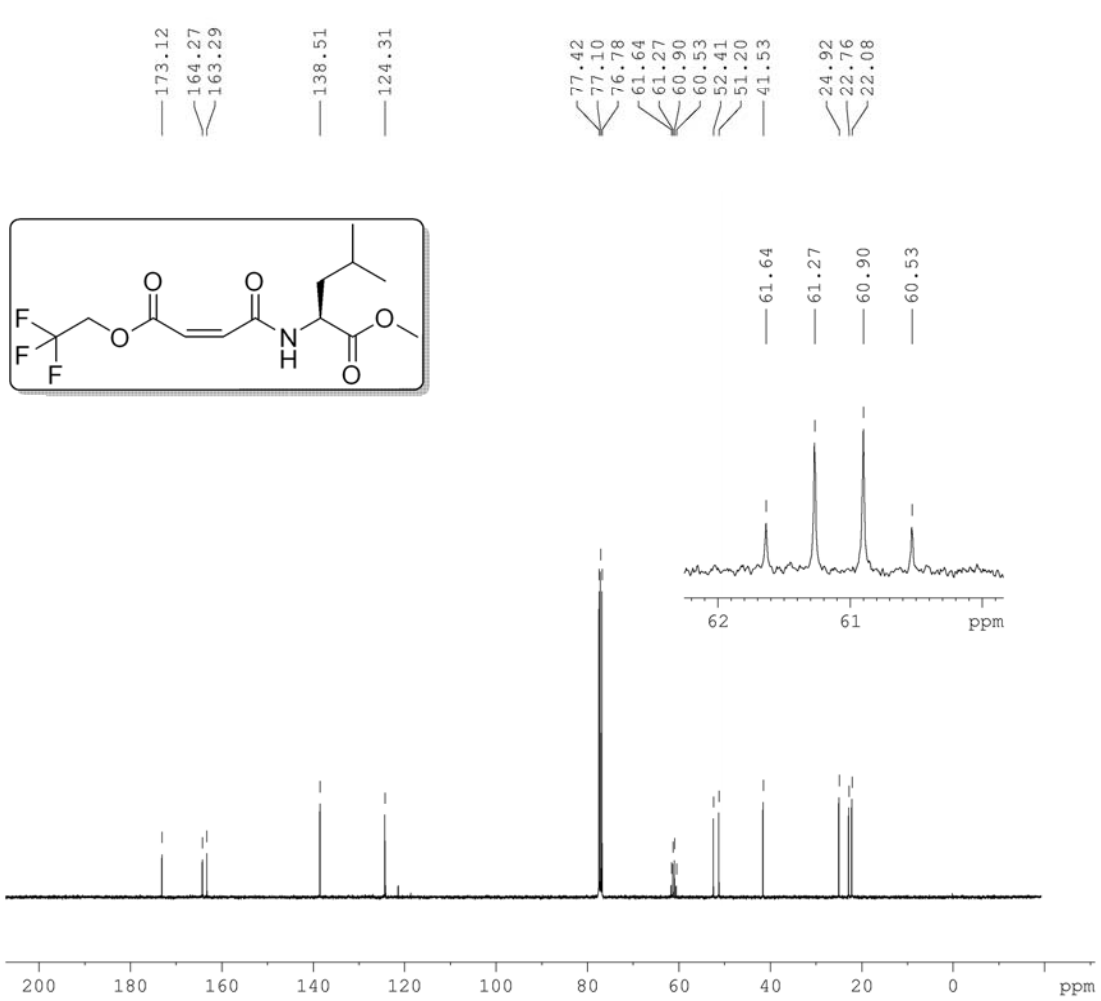
======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                60.00 usec
PL2                   0.00 dB
PL12                 12.64 dB
PL13                 13.05 dB
PL2W          9.46981144 W
PL12W         0.51563370 W
PL13W         0.46918198 W
SFO2         400.1536006 MHz
SI                   32768
SF           100.6183000 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

^{1}H NMR of **3f-2**

BRUKER

NAME	HMJ-D-7a-2
EXPNO	1
PROCNO	1
Date_	20110624
Time	11.15
INSTRUM	spect
PROBHD	5 mm PADUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	161
DW	60.800 usec
DE	6.50 usec
TE	295.4 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.00 usec
PL1	0.00 dB
PL1W	9.46981144 W
SFO1	400.1544711 MHz
SI	32768
SF	400.1520033 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

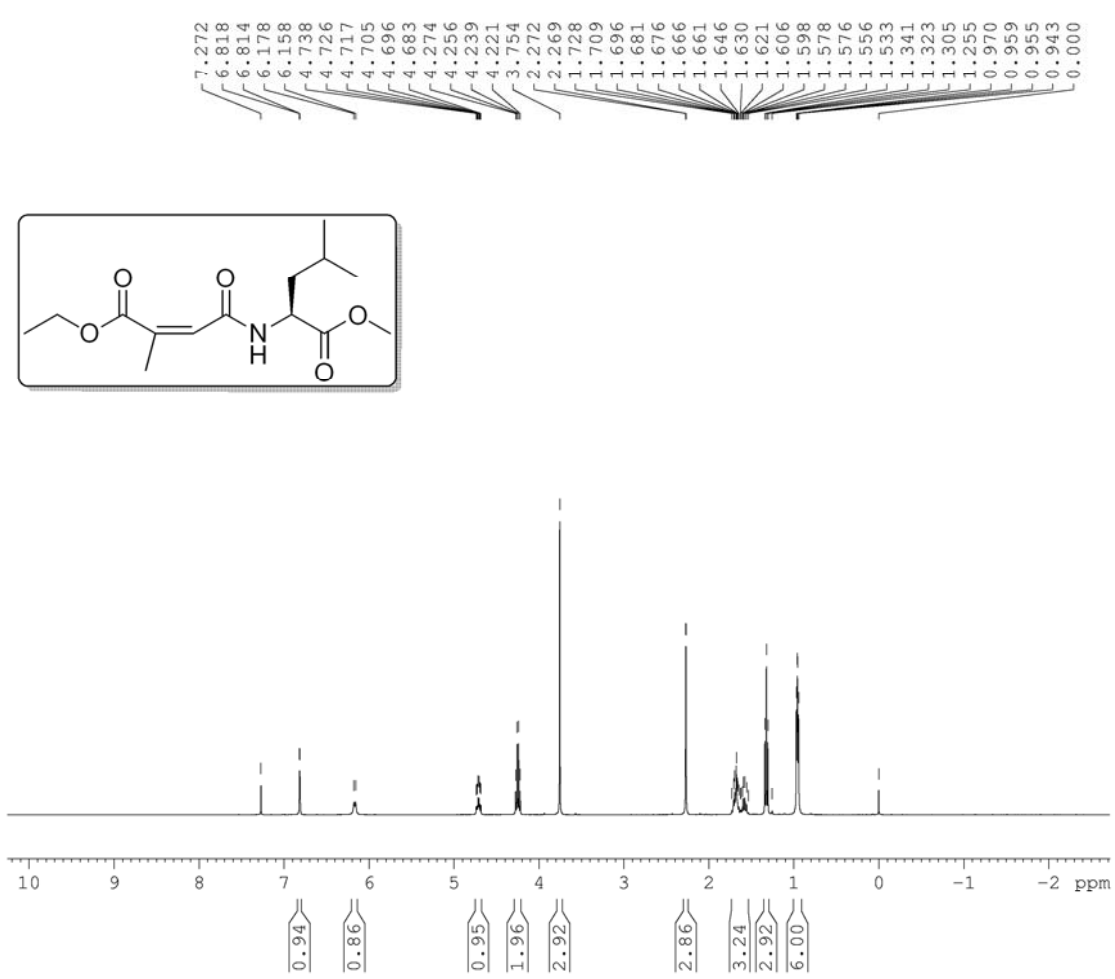
^{13}C NMR of **3f-2**



BRUKER

NAME	HMJ-D-7a-2
EXPNO	2
PROCNO	1
Date_	20110624
Time	16.23
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	825
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	161.46
DW	20.800 usec
DE	6.50 usec
TE	673.1 K
D1	2.00000000 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127603 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

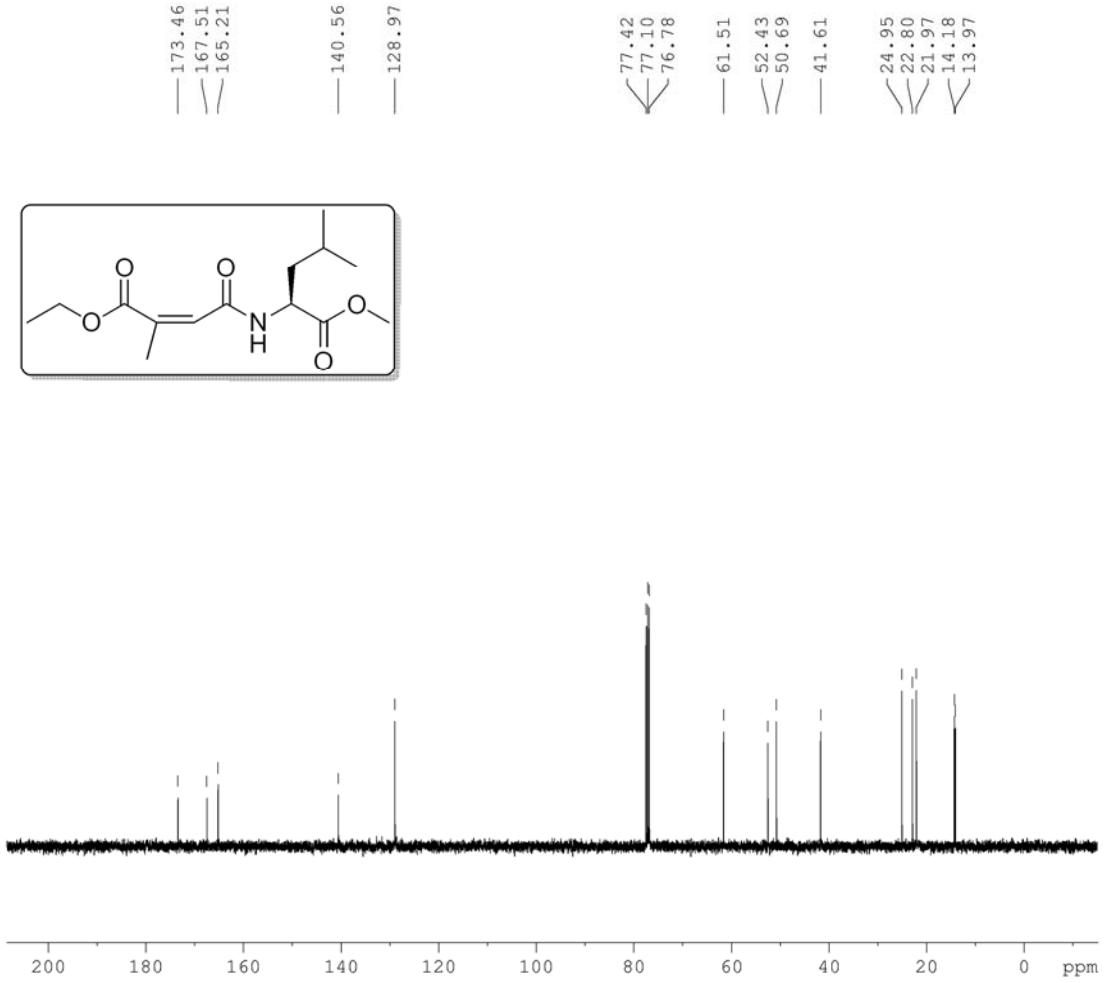
^{1}H NMR of **3g-1**



BRUKER

NAME	HMJ-c-43b-1
EXPNO	1
PROCNO	1
Date_	20110601
Time	16.33
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	119.06
DW	60.800 usec
DE	6.50 usec
TE	298.4 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300045 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

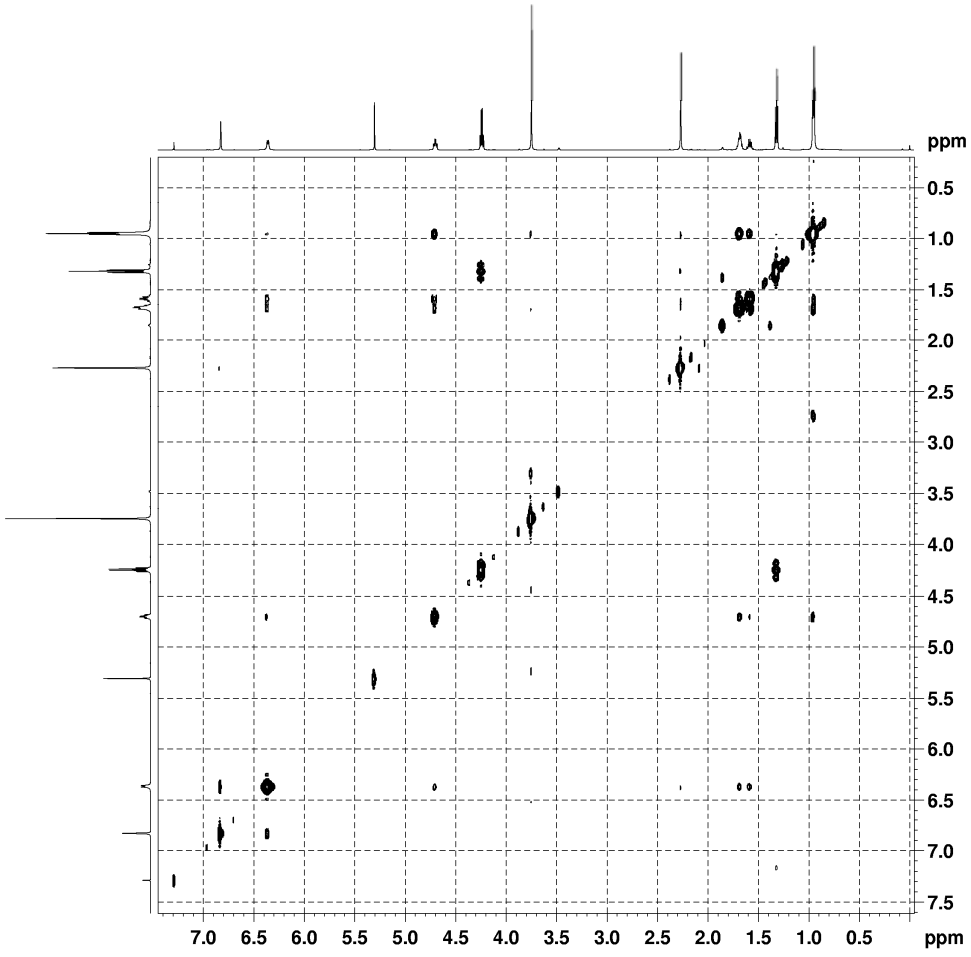
^{13}C NMRof **3g-1**



BRUKER

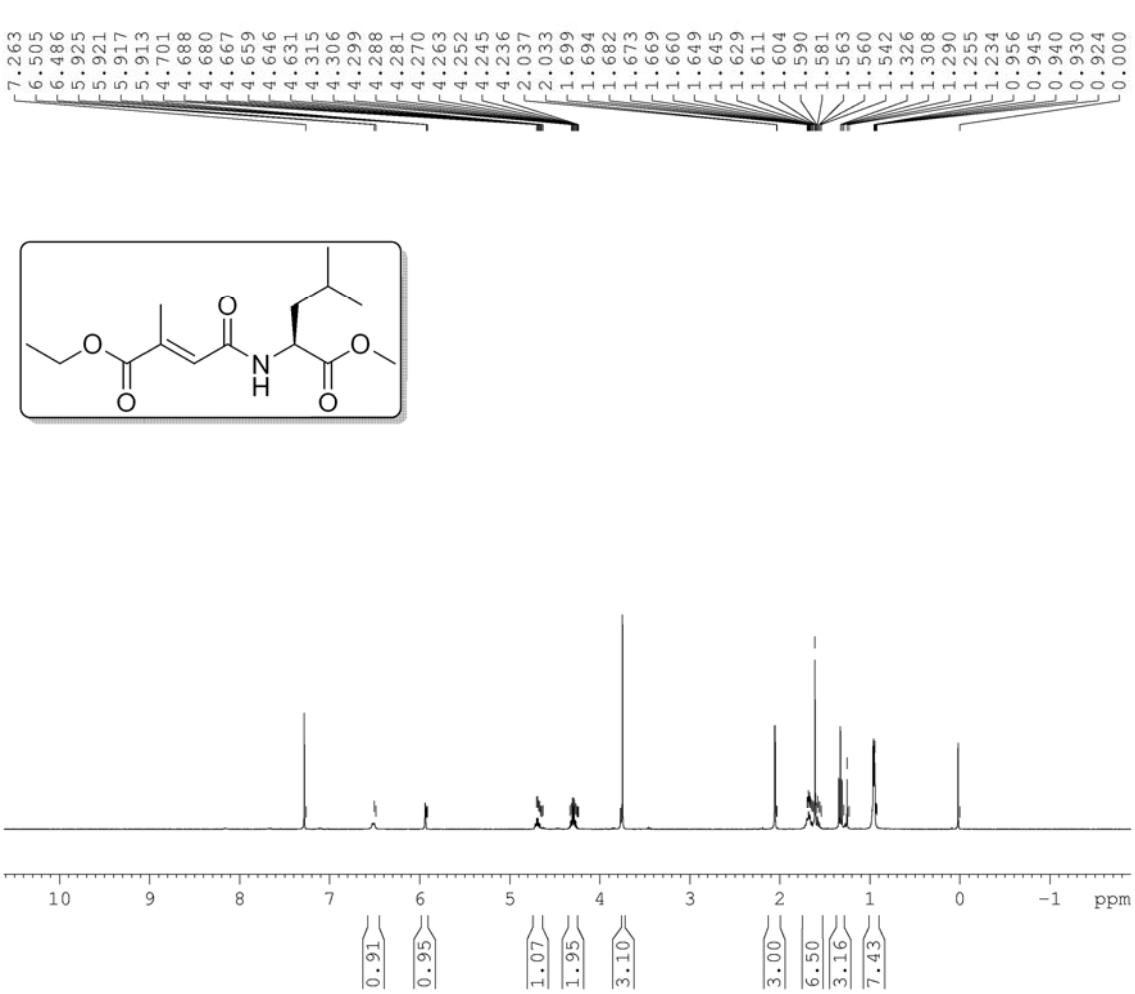
NAME	HMJ-c-46a-1
EXPNO	2
PROCNO	1
Date_	20110610
Time	17.51
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDC13
NS	40
DS	2
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	183.47
DW	20.800 usec
DE	6.50 usec
TE	300.5 K
D1	1.00000000 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127630 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

NOESY of **3g-1**



NAME	HMJ-c-43b-1
EXPNO	2
PROCNO	1
Date_	20120508
Time	17.10
INSTRUM	spect
PROBHD	5 mm CPTCI 1H-
PULPROG	noesygpphpp
TD	2048
SOLVENT	CDCl3
NS	16
DS	32
SWH	5376.344 Hz
FIDRES	2.625168 Hz
AQ	0.1905140 sec
RG	32
DW	93.000 usec
DE	10.00 usec
TE	300.4 K
D0	0.00007479 sec
D1	2.00000000 sec
D8	0.69999999 sec
D11	0.03000000 sec
D12	0.00002000 sec
D16	0.00020000 sec
IN0	0.00018600 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.30 usec
P2	28.60 usec
P17	2500.00 usec
ND0	1
TD	256
SFO1	600.1322 MHz
FIDRES	21.001366 Hz
SW	8.959 ppm
FnMODE	States-TPPI
SI	2048
SF	600.1299967 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00
SI	1024
MC2	States-TPPI
SF	600.1299966 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz

^{1}H NMR of **3g-2**



BRUKER

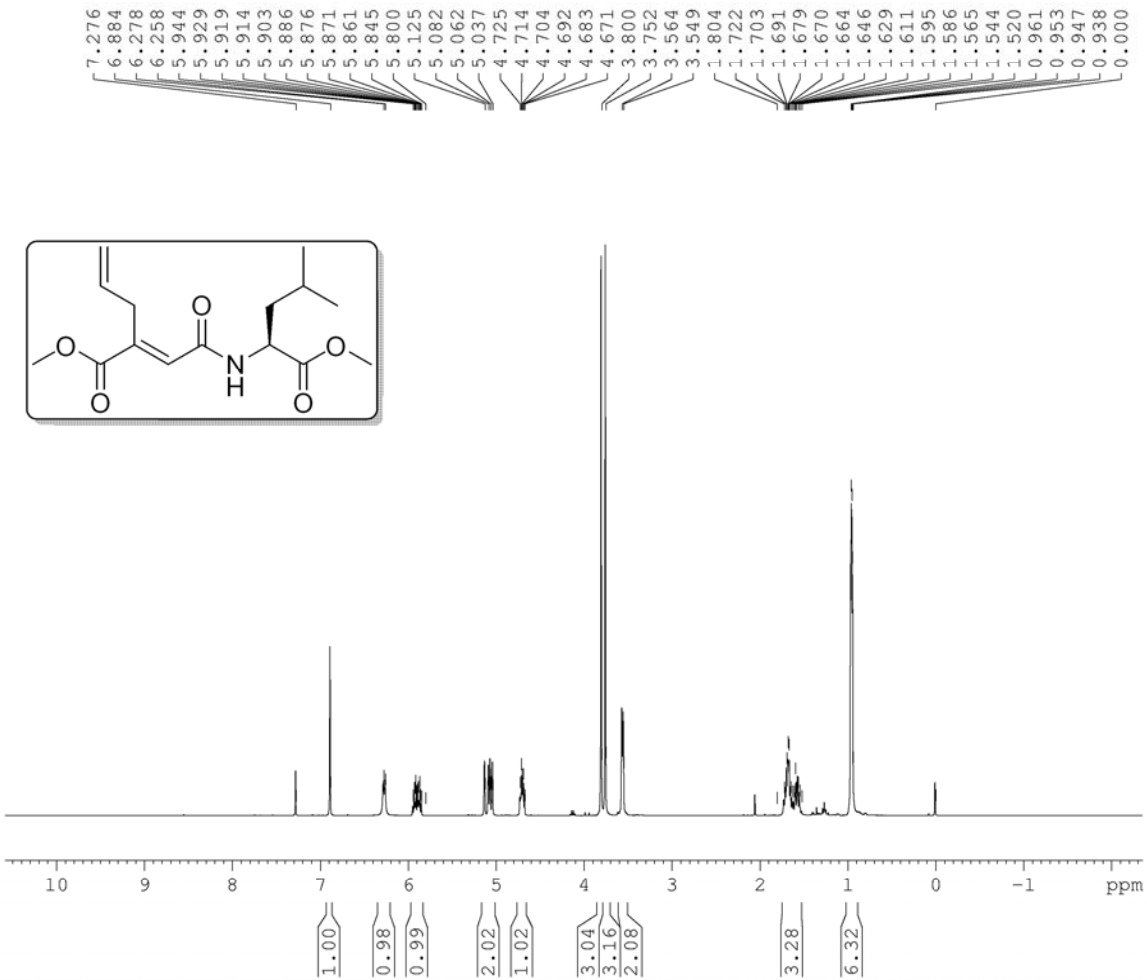
NAME	HMJ-C-46a-2
EXPNO	1
PROCNO	1
Date_	20110607
Time	19.04
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	0
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	183.47
DW	60.800 usec
DE	6.50 usec
TE	298.6 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

^{13}C NMRof **3g-2**

BRUKER

NAME	HMJ-C-46a-2	
EXPNO	2	
PROCNO	1	
Date_	20110620	
Time	11.18	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	12647	
DS	2	
SWH	28409.092	Hz
FIDRES	0.433488	Hz
AQ	1.1534836	sec
RG	183.47	
DW	17.600	usec
DE	6.50	usec
TE	299.8	K
D1	1.20000005	sec
D11	0.03000000	sec
======== CHANNEL f1 ========		
NUC1	13C	
P1	9.00	usec
SI	32768	
SF	100.6127594	MHz
WDW	EM	
SSB	0	
LB	3.00	Hz
GB	0	
PC	1.40	

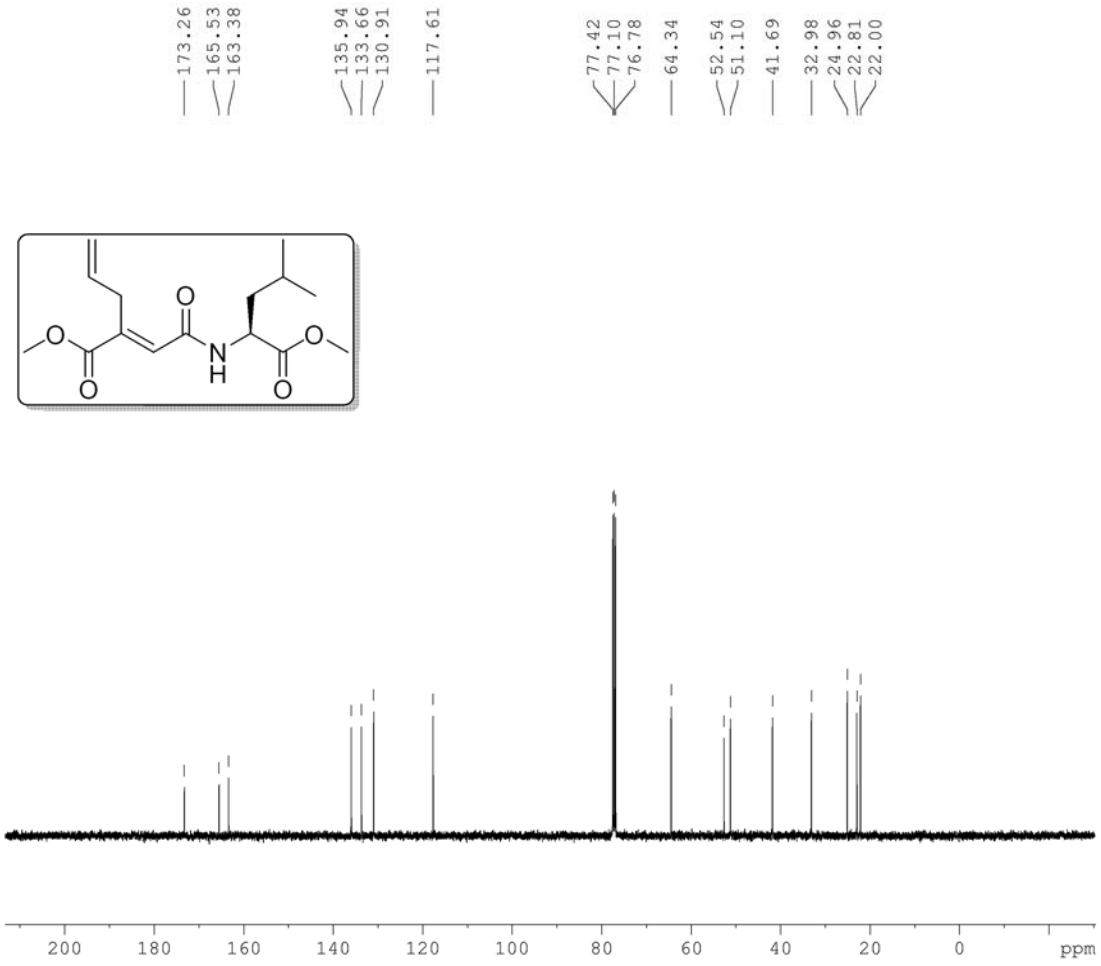
^{1}H NMR of **3h**



BRUKER

NAME	hmj-E-19a
EXPNO	11
PROCNO	1
Date_	20120207
Time	22.01
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	91.92
DW	60.800 usec
DE	6.50 usec
TE	293.8 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

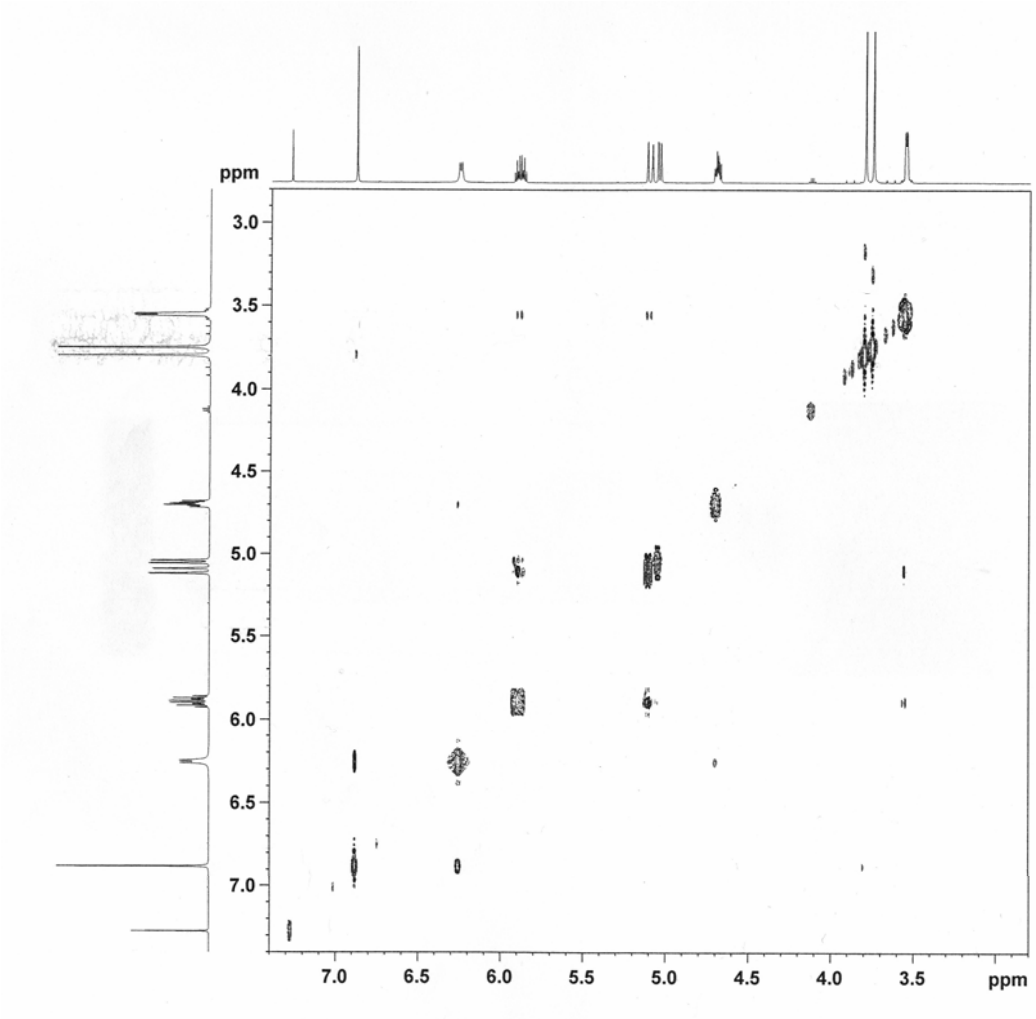
^{13}C NMR of **3h**



BRUKER

NAME	HMJ-D-2a-1
EXPNO	2
PROCNO	1
Date_	20110617
Time	11.08
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	198
DS	4
SWH	28409.092 Hz
FIDRES	0.433488 Hz
AQ	1.1534836 sec
RG	183.47
DW	17.600 usec
DE	6.50 usec
TE	300.2 K
D1	1.20000005 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127616 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

NOESY of **3h**



BRUKER

```
Current Data Parameters
NAME            hmj-E-19a
EXPNO                   4
PROCNO                  1

F2 - Acquisition Parameters
Date_            20120209
Time                11.57
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG      noesygpphpp
TD                   2048
SOLVENT             CDCl3
NS                     16
DS                     32
SWH              5617.978 Hz
FIDRES           2.743153 Hz
AQ               0.1823220 sec
RG                    45.2
DW                 89.000 usec
DE                 10.00 usec
TE                 300.4 K
D0               0.00007028 sec
D1               2.00000000 sec
D8               0.60000002 sec
D11              0.03000000 sec
D12              0.00002000 sec
D16              0.00020000 sec
IN0              0.00017800 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                 14.70 usec
P2                 29.40 usec
P17              2500.00 usec
PLW1          3.16230011 W
PLW10         0.67361999 W
SFO1         600.1321947 MHz

====== GRADIENT CHANNEL =====
GPNAM1        SMSQ10.100
GPZ1               40.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1           600.1322 MHz
FIDRES         21.947042 Hz
SW                9.362 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                   2048
SF           600.1300070 MHz
WDW                  QSINE
SSB                     2
LB         0 Hz
GB         0
PC                   1.00

F1 - Processing parameters
SI                   1024
MC2          States-TPPI
SF           600.1300064 MHz
WDW
SSB                     2
LB         0 Hz
GB         0
```