Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Synthesis of α, β-unsaturated γ-amino esters with unprecedented high (*E*)-stereoselectivity and their conformational analysis in peptides.

Sachitanand M. Mali, Anupam Bandyopadhyay, Sandip V. Jadhav, Mothukuri Ganesh Kumar and Hosahudya N. Gopi*

hn.gopi@iiserpune.ac.in

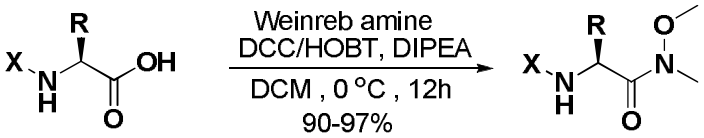
Table of Content

General Information.

All amino acids, Weinreb amine hydrochloride salt , DCC , LAH , DIPEA , PPh_3 were purchased from Aldrich. The solvents THF, DCM, toluene were purchased from Merck. THF and DIPEA was dried over sodium and distilled prior to use. Ethyl bromoacetate, di-tert-butyl dicarbonate , Fmoc-OSu were purchased from Spectrochem and used without further purification. Column chromatography was performed on Merck silica gel (100-200 mesh). ^{1}H NMR spectra were recorded on Jeol 400 MHz and ^{13}C NMR on100 MHz spectrometer using residual solvent as internal standard ($CDCl_3$ δ_H, 7.24 ppm, δ_c 77.0 ppm). The chemical shifts (δ) were reported in ppm and coupling constant (*J*) in *Hz*. Specific rotations were recorded using methanol and DMF (Rudolph Analytical Research). Mass spectra were obtained from MALDI-TOF/TOF (Applied Biosystem).

General Procedure for the Synthesis of Boc/Fmoc-amino Weinreb Amide .

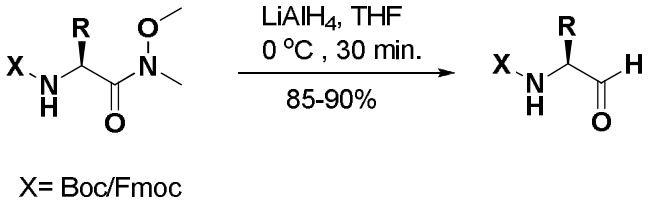
In a typical experimental procedure, protected amino acid (20 mmol) was dissolved in a DCM and to this solution hydrochloride salt of weinreb amide (30 mmol) was added. The reaction mixture was then cooled at 0 °C. This reaction mixture was treated with DIPEA, DCC and HOBt. The progress of the reaction was monitored by TLC. After the completion of reaction(12 h) DCM was evaporated and residue was diluted with 150 mL of ethyl acetate and washed with 5% HCl (50 mL) , 10% Na_2CO_3 (50 mL) fallowed by brine soultion. The organic layer was then dried over the anhydrous Na_2SO_4 and the product was concentrated under reduced pressure. The pure N-protected amino acid Weinreb amide was isolated after the column chromatography using EtOAc/ pet.ether (60-80 °C) solvent system.



X= Boc/Fmoc

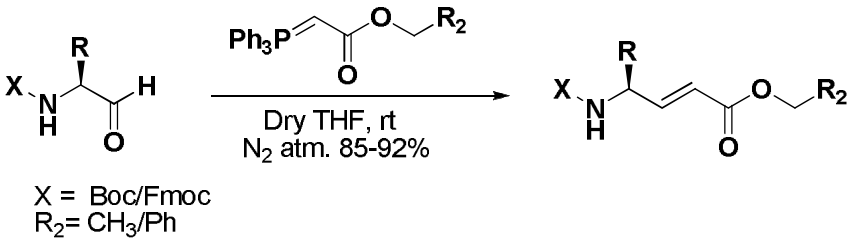
General Procedure for Synthesis of Synthesis of Boc/Fmoc Amino Aldehyde.

The *N*-Protected Weinreb amide (20 mmol) was dissolved in 130 mL of dry THF under N_2 atmosphere, cooled to 0 °C, and then $LiAlH_4$ (22 mmol) was added slowly during 10 min. Reaction mixture was stirred for another 20 min. After completion, the reaction was quenched with 5% HCl (5 % by volume in water) very slowly in ice cool condition (*p*H 3). THF was evaporated from the reaction mixture and the *N*-protected amino aldehyde was extracted with ethyl acetate (3 × 80 mL). Combined organic layer was washed with brine (40 mL) and dried over anhydrous Na_2SO_4. Organic layer was concentrated under reduced pressure to get oily product and immediately used for next step without purification.



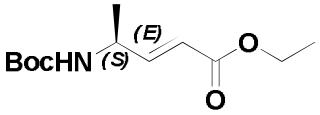
General Procedure for Synthesis of Boc/Fmoc Vinylogous Amino Ester.

The N-protected amino aldehyde (10 mmol) was dissolved in dry THF (40 mL) under the N_2 atmosphere. To this solution Wittig ylide (11.5 mmol) was added. The progress of reaction was monitored by TLC. After the completion of reaction (8h) the THF was evaporated and product was purified by coloumn chromatography using 5:95 ethyl acetate /pet ether solvent system.

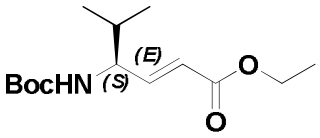


Spectroscopic Data for N-Protected vinylogous Amino Esters

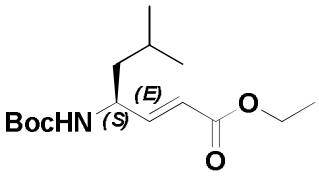
(*S,E*)-ethyl 4-(tert-butoxycarbonylamino)pent-2-enoate : Colourless Oil (Yield 2.25g, 93%); $[\alpha]_D^{25}$= -20.8 (c = 1 MeOH) UV= 216nm, t_R = 5.59min **^{1}H NMR**(400 MHz, $CDCl_3$) δ 6.876-6.827 (dd, 1H vinylic β proton), 5.898-5.859 (d, 1H vinylic α proton) , 4.5 (br, NH), 4.38 (br, α proton), 4.198-4.144 (q, *J*= 7.3 Hz, OCH_2), 1.432 (s, 9H, $C(CH_3)_3$ Boc), 1.265-1.247 (m, 6H, $(CH_3)_2$); **^{13}C NMR** (100 MHz $CDCl_3$) 166.472, 154.974, 120.201, 79.851, 60.534, 47.080, 28.431, 20.422, 14.301; **MALDI.TOF/TOF** m/z Calcd. for $C_{12}H_{21}NO_4$ (M+Na) 266.1368 Observed.266.1365.



(*S,E*)-ethyl 4-(tert-butoxycarbonylamino)-5-methylhex-2-enoate : Colourless solid (yield 2.43g, 90%); $[\alpha]_D^{25}$= -3.40 (c = 1 MeOH); mp= 59 ^{0}C UV= 216nm, t_R = 8.02min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 6.855-6.816 (dd,1H vinylic β proton) 5.924-5.880 (d, *J*=15.6 Hz, 1H, vinylic α proton), 4.55 (d, 1H, NH), 4.187-4.168 (q, *J* =6.88 Hz, 2H, OCH_2), 1.862-1.84 (m, 1H, γ proton), 1.429 (s, 9H, $C(CH_3)_3$ Boc), 1.292-1.256 (t, *J* =7.32 Hz, 3H, CH_3), 0.932-0.885 (q, *J*=6.4 Hz, $C(CH_3)_2$); **^{13}C NMR** (100 MHz, $CDCl_3$); 166.2437, 155.3170, 147.3461, 121.4025, 79.6125, 60.3812, 56.6151, 32.1875, 28.2974, 18.8105, 17.9428, 14.1862 **MALDI. TOF/TOF** Calcd. for $C_{14}H_{25}NO_4$ 294.1681 Observed 294.1686;

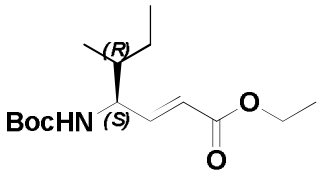


(*S,E*)-ethyl 4-(tert-butoxycarbonylamino)-6-methylhept-2-enoate: Colorless crystalline soild (2.70g, 95%); $[\alpha]_D^{25}$= -25.50 (c = 1 MeOH); mp = 55 ^{0}C, UV= 218nm, t_R = 11.5min. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.854-6.811(dd, *J*=16 Hz, *J*=5.5 Hz, 1H, C*H*=CHCO$_2$Et), 5.936-5.904 (d, *J*=16 Hz, 1H, CH=C*H*CO$_2$Et), 4.451 (br, 1H, NH), 4.334 (br, 1H, C*H*-CH=CH), 4.215-4.172 (q, *J*=7 Hz, 2H, -OC*H*$_2$), 1.726-1.671 (m, 1H, C*H*-$(CH_3)_2$), 1.446 (s, 9H, -$(CH_3)_3$ Boc), 1.400-1.372 (t, *J*=7 Hz, 2H, C*H*$_2$CH-$(CH_3)_2$), 1.304-1.276 (t, *J*=7 Hz, 3H, -OCH$_2$C*H*$_3$), 0.945-0.932 (d, *J*=6.5 Hz, 6H, CH-$(CH_3)_2$); **^{13}C NMR** (100MHz, $CDCl_3$) δ 166.4153, 155.0501, 148.8907, 120.3537, 79.6506, 60.4098, 49.7597, 43.7815, 28.3164, 24.6742, 22.6815, 22.1476, 14.2053; **MALDI.TOF/TOF** m/z Calcd. For $C_{15}H_{27}NO_4$ [M+Na$^+$] 308.1838, Observed. 308.1840.

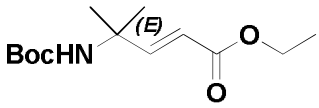


(*4S,5R,E*)-ethyl 4-(tert-butoxycarbonylamino)-5-methylhept-2-enoate: Colourless solid,(yield 2.62.g, 92%); $[\alpha]_D^{25}$= -11.20 (c = 1 MeOH); mp = 62 ^{0}C, UV= 216nm, t_R = 10.41min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 6.858-6.819 (d, 1H vinylic β proton), 5.917-5.878 (d, *J*=14.36 Hz, 1H, vinylic α proton), 4.568 (br, 1H, NH), 4.255 (br, 1H, α proton), 4.197-4.161 (q, *J*= 6.88 Hz, 2H, OCH_3), 1.655-1.595 (b, 2H, CH_2), 1.421 (s, 9H, $C(CH_3)_3$, Boc), 1.284-1.248 (t, *J*= 6.88 Hz, 3H, CH_3) , 0.9152-0.8659 (m, 6H, $(CH_3)_2$); **^{13} CNMR** (100MHz, $CDCl_3$) δ 166.310, 155.317, 147.155, 121.612, 79.651, 60.448,

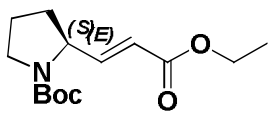
55.757, 39.043, 28.402, 25.322, 15.321, 14.281, 11.679; **MALDI.TOF/TOF** m/z Calcd. for $C_{15}H_{27}NO_4$ (M+Na) 308.1838 Observed. 308.1837



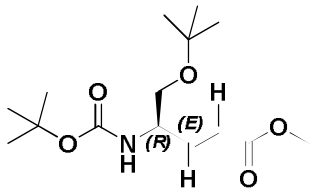
(*E*)-ethyl 4-(tert-butoxycarbonylamino)-4-methylpent-2-enoate: Colourless solid (yield 1.92g, 75%); mp = 58 ^{0}C, UV= 221nm, t_R = 6.30min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.020-6.980 (d, *J*= 16.04 Hz, 1H, vinylic β proton), 5.860-5.820 (d, *J*= 16.04 Hz, 1H, vinylic α proton), 4.710 (br, 1H, NH), 4.216-4.164 (q, *J*= 6.88Hz , 2H, OCH_2), 1.428 (s, 9H, $C(CH_3)_3$, Boc), 1.408 (s, 6H, $C(CH_3)_2$), 1.306-1.270(t, *J*= 6.88 Hz, 3H, CH_3); **^{13}CNMR** (100 MHz, $CDCl_3$) 166.787, 154.201, 153.629, 118.523, 79.479, 60.410, 52.963, 29.747, 28.421, 27.411, 14.310; **MALDI.TOF/TOF** m/z Calcd. for $C_{13}H_{23}NO_4$ (M+Na) 280.1525 Observed. 280.1526.



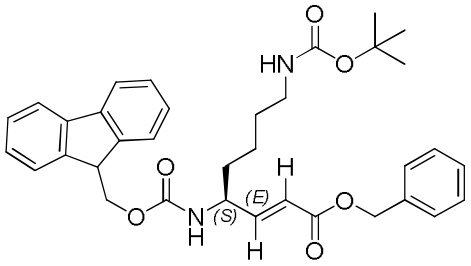
(*S,E*)-tert-butyl2-(3-ethoxy-3-oxoprop-1-enyl)pyrrolidine-1-carboxylate: Colourless oil (yield,2.23g, 83%) $[\alpha]_D^{25}$= -72 (c = 1 MeOH); UV= 214nm, t_R = 7.5min. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.836-6.783 (dd, *J*= 15.6 Hz, 1H, CH vinylic β proton), 5.838-5.800 (d, *J*= 15.2 Hz, 1H, CH vinylic α proton), 4.514&4.373 (br, 1H CH γ proton), 4.213-4.179 (q, *J*= 6.4 Hz, 2H OCH_2), 3.446-3.431 (t, *J*= 6 Hz, 2H, CH_2), 2.146-2.056 & 1.892-1.821 (m, 4H, β & γ CH_2), 1.421(s, 9H $C(CH_3)_3$ Boc) 1.313-1.279 (t, *J*= 6.8 Hz, 3H, CH_3); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 172.107, 154.306, 148.538, 120.478, 79.631, 60.324, 57.845, 46.232, 31.720, 28.393, 22.910, 14.234; **MALDI.TOF/TOF** m/z Calcd. for $C_{14}H_{23}NO_4$ (M+Na) 292.1525 Observed 292.1520.



(*R,E*)-ethyl 5-tert-butoxy-4-(tert-butoxycarbonylamino)pent-2-enoate: Coluorless oil(2.55g, 81%); $[\alpha]_D^{25}$= +4.4 (c = 1, MeOH); UV= 211nm, t_R = 10.03min. **^{1}HNMR** (400 MHz, $CDCl_3$), δ6.918-6.866 (dd, *J*= 15.6 Hz, 1H, CH vinylic β proton), 5.948-5.909 (d, *J*= 15.6 Hz, 1H, CH vinylic α proton), 5.012 (br, 1H, NH), 4.355 (br 1H, CH γ proton), 4.191-4.137 (q, *J*= 7.2 Hz, 2H, OCH_2), 3.478-3.445 (d, *J*= 4.4 Hz, 2H, CH_2), 1.423 (s, 9H, $C(CH_3)_3$, Boc) , 1.135 (s, 9H $C(CH_3)_3$, OtBu); **^{13}CNMR**(100 MHz, $CDCl_3$) δ 166.377, 155.336, 146.917, 121.708, 79.736, 73.415, 63.232, 60.400, 51.762, 28.404, 27.391, 14.272;**MALDI.TOF/TOF** m/z Calcd for $C_{16}H_{29}NO_5$ (M+Na) 338.1943 Observed.338.1904 .

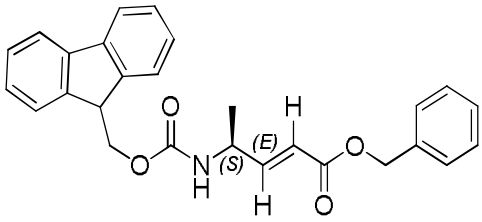


(*S,E*)-benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-8(tertbutoxycarbonylamino)oct 2-enoate: White solid, (4.55g, 78%); $[\alpha]_D^{25}$= -9.30(c = 1 MeOH); mp = 107 ^{0}C, UV= 211nm, 264nm, 289nm, t_R = 8.05min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.773-7.755 (d, *J* = 7.2 Hz, 2H, aromatic Fmoc-), 7.603-7.585 (d, *J* = 7.2 Hz, 2H, aromatic Fmoc-) , 7.391-7.300 (m, 9H aromatic Fmoc & Benzylic), 6.909-6.858 (dd, *J* = 15.6 Hz 1H, vinylic β proton), 5.967-5.928 (d, *J*= 15.6 Hz, 1H, vinylic α proton), 5.186 (s, 2H, benzylic), 4.460-4.429 (t, *J* = 6.4 Hz 1H, CH Fmoc-), 4.975 (br, 2H ,CH_2), 4.225-4.193 (m, 1H, γ proton), 3.121-3.106 (t, *J*= 6 Hz, 2H, CH_2), 1.620-1.543 (m, 4H, β CH_2 δCH_2), 1.392-1.356 (m, 2H, γ CH_2), 1.438 (s, 9H, $C(CH_3)_3$, Boc); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 166.10, 155.89, 148.57, 143.86, 141.42, 135.89, 128.69, 128.47, 127.82, 127.18, 125.08, 120.75, 120.08, 79.35, 67.23, 66.75, 66.51, 52.02, 47.33, 40.01, 33.90, 31.05, 29.87, 29.80, 28.50, 22.78; **MALDI.TOF/TOF** m/z Calcd. For $C_{35}H_{40}N_2O_6$ (M+ Na) = 607.2784 Observed. 607.2787.

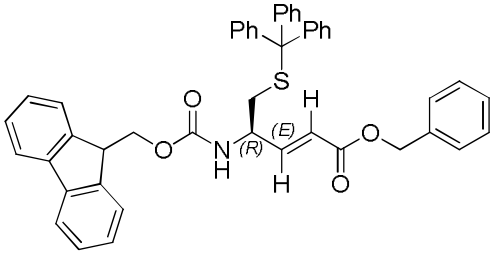


(*S,E*)-benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)pent-2-enoate : White solid (4g, 94%); $[\alpha]_D^{25}$= - 16.70 (c = 1, MeOH); mp = 116 ^{0}C, UV= 210nm, 264nm, 289nm, t_R = 8.15min. **^{1}HNMR**

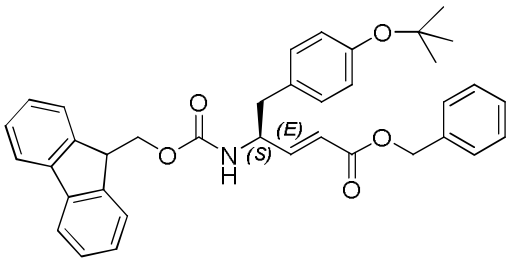
(400 MHz, CDCl3) δ 7.762-7.743 (d, *J*= 7.6 Hz, 2H aromatic Fmoc-), 7.583-7.565 (d, *J*= 7.2 Hz, 2H, aromatic Fmoc-) 7.416-7.300 (m, 9H, aromatic Fmoc & benzylic), 6.941-6.890 (dd, *J*= 16Hz, 1H vinylic β proton), 5.955-5.916 (d, *J*= 15.6 Hz, 1H vinylic α proton), 5.178 (s, 1H benzylic), 4.782-4.762 (d, *J*= 8Hz, 1H, NH), 4.507-4.490 (d, *J*= 6.8 Hz, 2H, OCH_2), 4.445-4.429 (t, *J*= 6.4 Hz, 1H, CH Fmoc), 4.215-4.182 (m, 1H γ proton), 1.292-1.275 (d, *J*= 6.8 Hz, 3H, CH_3); **^{13}CNMR** (100 MHz, $CDCl_3$) 166.151, 155.548, 149.436, 143.858, 141.427, 135.887,133.846, 128.764, 128.697, 128.468, 127.181, 125.064, 124.950, 120.240, 120.097, 66.777, 66.519, 47.659, 47.306, 31.059, 20.322; **MALDI.TOF/TOF** m/z Calcd. For $C_{27}H_{25}NO_4$ (M+Na) 450.1681 Obeserved.450.1641.



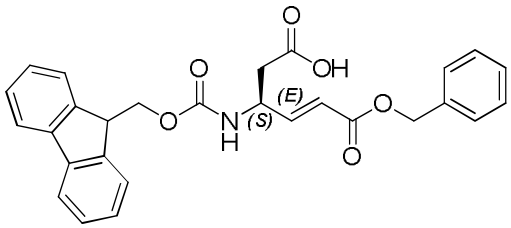
(*R*,*E*)-benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(tritylthio)pent-2-enoate: Coluorless solid (5.6g, 80%); $[\alpha]_D^{25}$= +6.70 (c = 1, MeOH); UV= 212nm,264nm, 289nm, t_R = 7.12min. **^{1}HMNR** δ 7.747-7.732 (d, *J*= 6.4 Hz, 2H, aromatic Fmoc), 7.573-7.555 (d, *J*= 7.2 Hz, 2H, aromatic Fmoc), 7.388-7.347 (t, *J*= 7.2 Hz, 2H, aromatic Fmoc) 7.334-7.186 (m, 17H, aromatic Ph), 6.717-6.69 (dd, *J*= 15.6 Hz, 1H, vinylic β proton), 5.820-5.782 (d, *J*= 15.2 Hz, 1H, vinylic α proton), 5.140 (s, 1H, benzylic), 4.850-4.805 (t, 1H, CH Fmoc), 4.407-4.391 (d, *J*= 6.4 Hz, 2H, OCH_2), 4.222-4.160 (m, 1H, γ proton), 2.449-2.436 (d, *J*= 5.2 Hz, 2H, βCH_2); **^{13}CNMR** (100MHz , $CDCl_3$) 165.836, 155.548, 146.823, 144.382, 143.848, 141.427, 135.849, 129.632, 128.201, 127.086, 125.093, 121.594, 120.116, 98.509, 82.081, 68.064, 67.425, 66.586, 65.356, 51.525, 50.453, 47.325, 35.988, 34.358, 33.318, 31.068, 30.448, 25.719; **MALDI TOF/TOF** m/z Calcd. for $C_{46}H_{39}NO_4S$ (M+Na) 724.2497 Observed.724.2491.



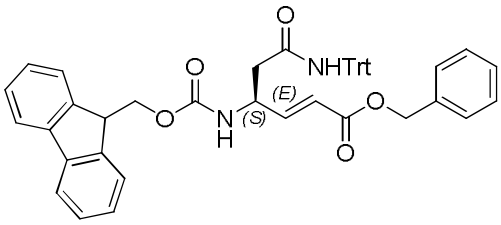
(*S,E*)-benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(4-tert-butoxyphenyl)pent-2-enoate : Colourless solid (5.3g, 93%); $[\alpha]_D^{25}$= -28.6 (c = 1, MeOH); UV= 210nm, 264nm, 289nm, t_R = 8.57min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.775-7.757 (d, *J*= 7.2 Hz, 2H, aromatic Fmoc), 7.560-7.529 (t, *J*= 6.4 Hz, 2H, aromatic Fmoc-), 7.415-7.397 (d, *J*= 7.2 Hz, 2H, aromatic Fmoc-), 7.385-7.289 (m, 11H, aromatic proton), 6.996-6.913 (dd, 1H, vinylic β proton), 5.914-5.875 (d, *J*= 15.6 Hz, 1H, vinylic α proton), 5.186 (s, 2H, CH_2 benzylic), 4.790-4.769 (d, *J*= 8.4 Hz, 1H, CH), 4.453-4.353 (m, 1H, CH γ proton), 4.200-4.167 (t, *J*= 6.4 Hz, 2H, OCH_2), 2.885-2.850 (t, *J*= 7.2 Hz, 2H, β CH_2), 1.327 (s, 9H, $C(CH_3)_3$ tBu); **^{13}CNMR** (100 MHz, $CDCl_3$) 164.110, 155.596, 151.295, 147.844, 144.544, 143.801, 141.388, 132.931, 132.235,131.892, 128.564, 128.344, 127.791, 127.620, 127.153, 126.724, 124.340, 124.054, 120.106, 68.055, 66.443, 65.242, 47.278, 33.957, 31.040, 28.894, 25.691; **MALDI.TOF/TOF** m/z Calcd. for $C_{37}H_{37}NO_5$ (M+ Na) 598.2569 Obsrved.598.2574.



(*S,E*)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-6-(benzyloxy)-6-oxohex-4-enoic acid: Colourless solid (3.76g, 80%); $[\alpha]_D^{25}$= - 16.60 (c = 1, MeOH), mp = 146 ^{0}C, UV= 213nm, 263nm, 289nm, t_R = 18.09min. **^{1}HNMR** (400 MHz, $CDCl_3$) δ 7.760-7.742 (d, *J*= 7.2Hz, 2H aromatic Fmoc-), 7.583-7.564 (d, *J*= 7.6 Hz, 2H, aromatic Fmoc-) 7.395-7.297 (m, 9H, aromatic), 6.993-6.941 (dd, *J*= 15.6 Hz, 1H, vinylic β proton), 6.038-6.001 (d, *J*= 14.8 Hz, 1H vinylic α proton), 5.568-5.545 (d, *J*= 9.2 Hz, 1H, NH), 5.185 (s, 2H, benzylic), 4.768(b, 1H,Fmoc), 4.439-4.423(d, *J*= 6.4 Hz, 2H, -OCH_2), 4.223-4.188 (m, 1H, CH γ proton), 2.764-2.725(m, *J*= 4.8 Hz, 2H, βCH_2); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 174.599, 166.027, 155.710, 146.366, 143.725,141.398, 135.677, 132.988, 128.707,127.200, 125.103, 121.947, 120.106, 67.130, 66.758, 48.317, 47.230, 37.943, 31.059); **MALDI.TOF/TOF** m/z Calcd. for $C_{28}H_{25}NO_6$ (M+ Na) 494.1580 Observed.494.1555.



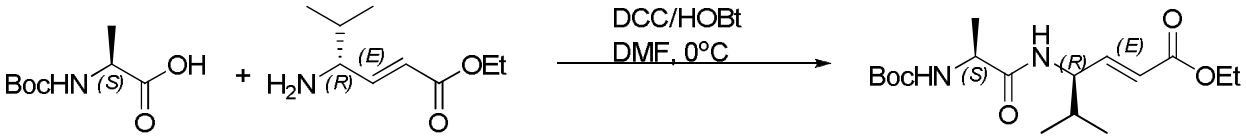
(*S,E*)-benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-6-amino-6-oxohex-2-enoate: Colourless solid (6.4g, 90%), $[\alpha]_D$ ^{25}= - 3.7 (c = 1, MeOH); UV= 275nm, 293nm, 306nm, t_R = 8.55min. **^{1}HNMR**(400 MHz, $CDCl_3$) δ 7.773-7.755 (d, *J*= 7.2 Hz, 2H, aromatic Fmoc-), 7.591-7.572 (d, *J*= 7.6 Hz, 2H, aromatic Fmoc-), 7.391-7.142 (m, 24H, aromatic protons), 7.049-6.999 (dd, *J*= 15.6Hz, 1H, vinylic β proton), 6.450-6.429 (b, 1H, NH amide) , 6.052-6.013 (d, *J*= 15.6 Hz, 1H, vinylic α proton), 5.23 (s, 2H, CH_2 benzylic), 4.695 (b, 1H, NH Boc), 4.390-4.301 (m, *J*= 7.2 Hz, 1H, CH γ proton), 4.190-4.154 (t, *J*= 6.8 Hz, 1H, Fmoc), 2.688 (b, 2H, β CH_2); **^{13}CNMR** (100 MHz, $CDCl_3$) 178.756, 169.469, 165.932, 155.929, 147.005, 144.192, 141.350, 135.849, 128.726, 128.173, 127.372, 125.265, 120.049, 71.077, 67.111, 66.567, 49.528, 47.211, 40.107, 33.948, 25.671; **MALDI TOF/TOF** m/z Calcd. for $C_{47}H_{40}N_2O_5$ (M+Na) 735.2835 Observed 735.2889.



Synthesis of dipeptide Boc-Ala-(D)dgVal-OEt

Boc-Ala-OH(0.129g 0.68 mmol) and NH_2-dgDVal-OEt(0.185g, 0.68 mmol) were dissolved in dissolved in DMF (1.5 ml). The reaction mixture was cooled at 0 ^{o}C. Then DCC (0.141g, 0.68 mmol) , HOBt (0.092g, 0.68 mmol) were added together. The reaction mixture was then allowed to stir for further 12 h. After the completion of reaction, the reaction mixture was diluted with ethyl acetate and DCU generated in the reaction mixture was filtered and the filtrate was then washed with 5 % HCl, 10% Na_2CO_3 and dried over anhydrous Na_2SO_4. The organic layer was then concentrated under reduced pressure. The dipeptide was purified using ethyl acetate/pet ether solvent system (1:3). The pure

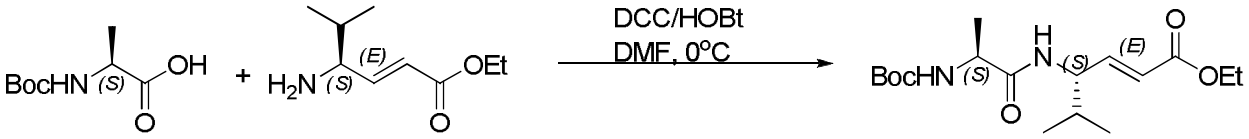
dipeptide BocAla-dgDVOEt obtained as colourless oil. Yield 74.85% (0.250g). $[\alpha]_D^{25}$= +2.7 (c = 1 MeOH); **^{1}HNMR** (400 MHz, $CDCl_3$) δ 6.893-6.842 (dd, *J*= 15.6 Hz, 1H, CH vinylic β proton), 6.60 (br, 1H, NH), 5.891-5.852(d, *J*= 15.6 Hz, 1H, vinylic α proton), 5.037-5.020 (d, *J*= 6.8 Hz, 1H, NH Boc), 4.529-4.476 (m, CH γ proton Val), 4.202-4.150 (q, *J*= 6.8 Hz, 2H, OCH_2), 1.953-1.871(m, CH α proton Ala), 1.457(s, 9H, $C(CH_3)_3$ Boc), 1.380-1.363(d, *J*= 6.8Hz ,3H, CH_3 Ala), 1.293-1.257(t, *J*= 7.2 Hz , 3H, CH_3), 0.955-0.907(dd, *J*= 6.8Hz 6H, $(CH_3)_2$); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 172.355, 166.282, 155.975, 146.745, 121.603, 80.585, 60.505, 55.070, 53.516, 50.189, 32.092, 28.354, 19.011, 17.943, 14.291; **MALDI TOF/TOF** m/z Calcd. for $C_{17}H_{30}N_2O_5$ (M+Na).365.2052 observed.365.2050.



Dipeptide D1

Synthesis of Boc-Ala-dgVal-OEt

Same protocol described above was used for the synthesis of dipeptide BocAla-dgVal-OEt. 93% (0.392g); $[\alpha]_D^{25}$= -50.8 (c = 1, MeOH); **^{1}HNMR**(400 MHz, $CDCl_3$) δ 6.891-6.338(dd, *J*= 15.6 Hz, 1H, CH vinylic β proton), 6.637(br, 1H, NH), 5.919-5.879 (d, *J*= 16 Hz, 1H, CH vinylic α proton), 5.042-5.024 (d, *J*= 7.2 Hz, 1H, NH Boc), 4.527-4.477 (m, 1H, CH α proton Val), 4.205-4.152 (q, *J*= 7.2 Hz, 2H, OCH_2), 1.915-1.851 (m, 1H, CH α proton), 1.444 (s, 9H, $C(CH_3)_3$) Boc), 1.368-1.350 (d *J*= 7.2 Hz, 3H, CH_3 Ala), 1.293-1.254 (t, *J*= 8Hz, 3H, CH_3), 0.929-0.894 (t, *J*= 7.2 Hz , 6H, $(CH_3)_2$ Val); **^{13}CNMR** (100 MHz, $CDCl_3$) δ 172.336, 166.272, 155.965, 146.745, 121.746, 80.451, 60.515, 55.061, 53.507, 32.159, 28.364, 21.118, 19.011, 17.895, 14.281;**MALDI.TOF/TOF** m/z Calcd. for $C_{17}H_{30}N_2O_5$ (M+Na) 365.2052 Observed 365.2057.



Synthesis of homo-dipeptide Boc-dgL-dgL-OEt

(*S,E*)-4-(tert-butoxycarbonylamino)-6-methylhept-2-enoic acid(Boc-dgL-OH): Boc-dgL-OEt, **4C** (1.86g 6.8 mmol) was dissolved in 4 mL of ethanol followed by 10 mL of 1N NaOH was added slowly to the solution. The reaction mixture was then stirred for about 8h. The progress of reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The aqueous layer was diluted with water (50 mL) and then acidified (~pH 3.0) with 5% HCl and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was then washed with brine and dried over anhydrous Na_2SO_4. Product was concentrated under reduced pressure to get 1.67g (95%) of oily Boc-dgL-OH.

(*S,E*)-ethyl 4-amino-6-methylhept-2-enoate(H_2NdgL-OEt): The solution of Boc-dgL-OEt (1.95g, 7.2 mmol) in 5mL of DCM was cooled to 0 ^oC followed by 5 mL of neat TFA was added. The reaction mixture was stirred for about 1.5 h at the same temperature. The progress of the reaction was monitored by TLC. After completion of the reaction (1.5 h), the solvent was evaporated under reduced pressure. The residue was then treated with saturated Na_2CO_3 solution in cold condition. This aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to 4 mL.

The solution of H_2N-dgL-OEt in ethyl acetate (4 mL) was added to the ice-cold solution of Boc-dgL-OH (1.67g, 6.5 mmol) in DMF (4 ml). The reaction mixture was then treated with DCC (1.34g, 6.5 mmol) followed by HOBt (0.884g, 6.5 mmol). The reaction mixture was stirred for about 12 h at room temperature and the progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with ethyl acetate (100 mL) and DCU formed in reaction was filtered. This filtrate was washed with brine (3 x 50 mL), 5% HCl (3 x50 mL), 10% Na_2CO_3(3 x 50 mL), brine (30 mL) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography using ethyl acetate/ pet ether to get 1.2 g (40%) of the pure dipeptide D4.

^{1}H NMR (400 MHz, $CDCl_3$); δ 6.858-6.805 (dd, *J*=15.6 Hz, 1H vinylic β CH=CH-CO_2Et), 6.709-6.656 (dd, *J*=15.2 Hz, 1H vinylic β CH=CH-CONH), 5.924-5.885(d, *J*= 15.6 Hz, 2H vinylic α CH=CH-CO), 5.655(br, 1H NH amide), 4.773-4.737(m, *J*=7.2 Hz, 1H γ proton), 4.547(br, 1H NH Boc), 4.297(m, 1H, γ proton), 4.205-4.151(q, *J*=7.2 Hz, 2H, OCH_2CH_3), 1.696-1.630(m, *J*=6.8 Hz, 2H, CH_3CHCH_3), 1.464-1.43(t, *J*=6.8 Hz, 2H, CH-CH_2-CH), 1.439(s, 9H, $(CH_3)_3$, Boc), 1.398-1.363(t, *J*=6.8 Hz, 2H CH-CH2-CH), 1.294-1.258(t, *J*=6.8 Hz, 3H, OCH_2CH_3), 0.934-0.918(d, *J*=6.4 Hz 12H, $(CH_3)_4$); **^{13}CNMR** (100 MHz, $CDCl_3$) 166.434, 164.975, 147.985, 145.296, 122.728, 120.973, 60.572, 49.950, 48.492, 44.020, 43.543, 28.459, 24.703, 22.796, 14.301; **MALDI.TOF/TOF**; m/z Calcd. for $C_{23}H_{40}N_2O_5$ (M+K) 463.2574 Observed 463.1999.

Crystal structure analysis of Boc-dgV-OEt: Crystals of peptide were grown by slow evaporation from a solution of EtOAc and Hexane. A single crystal (0.50× 0.35 × 0.20 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 200K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 55.84), for a total of 3650 independent reflections. Space group P2(1), 2(1), 2(1), a = 9.978(4), b = 10.083(3), c = 16.904(6), V= 1700.7(10)Å^3, Orthorhombic P, Z=4 for chemical formula $C_{14}H_{25}NO_4$, with one molecule in asymmetric unit; ρ Calcd = 1.060gcm^{-3}, μ= 0.077mm^{-1}, F(000)= 592, R_{int}= 0.0268. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0581 (wR2= 0.1589) 1978 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 178 variables, S = 1.011. The largest difference peak and hole were 0.344 and -0.160eÅ^3, respectively.

Crystal structure analysis of Boc-dgL-OEt: Crystals of peptide were grown by slow evaporation from a solution of EtOAc and Hexane. A single crystal (0.45× 0.34 × 0.24 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 296K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 48.56), for a total of 2788 independent reflections. Space group P2(1), a = 10.340(3), b = 9.733(3), c = 18.073(5), β =

106.331(5), V= 1700.7(10)Å^3, Monoclinic P, Z=2 for chemical formula $C_{30}H_{54}N_2O_8$, with two molecule in asymmetric unit; ρ Calcd = 1.086gcm^{-3}, μ= 0.078mm^{-1}, F(000)= 624, R_{int}= 0.0277. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0341 (wR2= 0.0804) 2413 observed reflections ($F_0 \geq 4\sigma$ (|F$_0$|)) and 373 variables, S = 0.985. The largest difference peak and hole were 0.110 and - 0.130 eÅ^3, respectively.

Crystal structure analysis of Boc-dgU-OEt: Crystals of peptide were grown by slow evaporation from a solution of EtOAc and Hexane. A single crystal (0.45× 0.30 × 0.23 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 296K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 60.56), for a total of 4448 independent reflections. Space group P 21/c, a = 10.669(2), b = 9.092(2), c = 15.882(4), β = 90.923(5), V= 1540.3(6)Å^3, Monoclinic P, Z=4 for chemical formula C_{30} $H_{54}N_2O_8$, with one molecule in asymmetric unit; ρ Calcd = 1.114gcm^{-3}, μ= 0.082mm^{-1}, F(000)= 564, R_{int}= 0.0579. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0651 (wR2= 0.1879) 1713 observed reflections ($F_0 \geq 4\sigma$ (|F$_0$|)) and 169 variables, S = 1.031. The largest difference peak and hole were 0.303 and -0.283eÅ^3, respectively.

Crystal structure analysis of Boc-dgI-OEt: Crystals of peptide were grown by slow evaporation from a solution of EtOAc and Hexane. A single crystal (0.54× 0.43 × 0.32 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 200K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 59.20), for a total of 3650 independent reflections. Space group P2(1), 2(1), 2(1), a = 10.153(3), b = 10.273(3), c = 16.191(5), V= 1688.8(9)Å^3, Orthorhombic P, Z=4 for chemical formula $C_{15}H_{27}NO_4$, with one molecule in asymmetric unit; ρ calcd = 1.122gcm^{-3}, μ= 0.080mm^{-1}, F(000)= 624, R_{int}= 0.0265. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0378 (wR2= 0.0912) 3750 observed reflections ($F_0 \geq 4\sigma$ (|F$_0$|)) and 188 variables, S = 1.023. The largest difference peak and hole were 0.185 and - -0.190eÅ^3, respectively.

Crystal structure analysis of Boc-Ala-dgVal-OEt : Crystals of peptide were grown by slow evaporation from a solution of ethylacetate. A single crystal (0.25× 0.24 × 0.20 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100K temperature on a

Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 56.56), for a total of 25408 independent reflections. Space group P1, a = 13.3376(19), b = 14.030(2), c = 17.956(3), α = 88.064(5), β = 70.670(4), γ = 73.874(5), V= 3039.6(7)Å^3, Triclinic P, Z= 1 for chemical formula C_{102} H_{180} N_{12} O_{30}, with six molecule in asymmetric unit; ρ Calcd = 1.122gcm^{-3}, μ= 0.082mm^{-1}, F(000)= 1116, R_{int}= 0.0718. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0881 (wR2= 0.1946) 12742 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 1339 variables, S = 0.990. The largest difference peak and hole were 0.587and -0.334eÅ^3, respectively.

Crystal structure analysis of Boc-dgLeu-dgLeu-OEt: Crystals of peptide were grown by slow evaporation from a solution of EtOAc. A single crystal (0.35× 0.25 × 0.11 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 200K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 58.26), for a total of 5271 independent reflections. Space group P1, a = 5.053(2), b = 9.812(5), c = 13.498(6), α= 73.640(9), β= 84.653(9), γ= 78.566(9), V= 628.8(5)Å^3, Triclinic P, Z=1 for chemical formula C_{23} H_{40} N_2 O_5, with one molecule in asymmetric unit; ρ calcd = 1.121gcm^{-3}, μ= 0.078mm^{-1}, F(000)= 232, R_{int}= 0.0613. The structure was obtained by direct methods using SHELXS-97.[1]The final R value was 0.0663 (wR2= 0.1509) 3557 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 305 variables, S = 1.036. The largest difference peak and hole were 0.575 and -0.373eÅ^3, respectively.

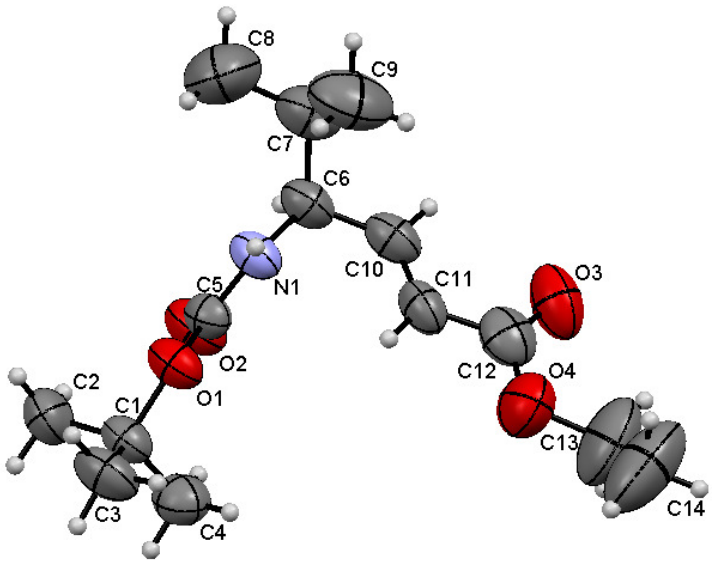


Figure 1: ORTEP diagram of Boc-dgV-OEt. All H-atoms are not labeled for clarity

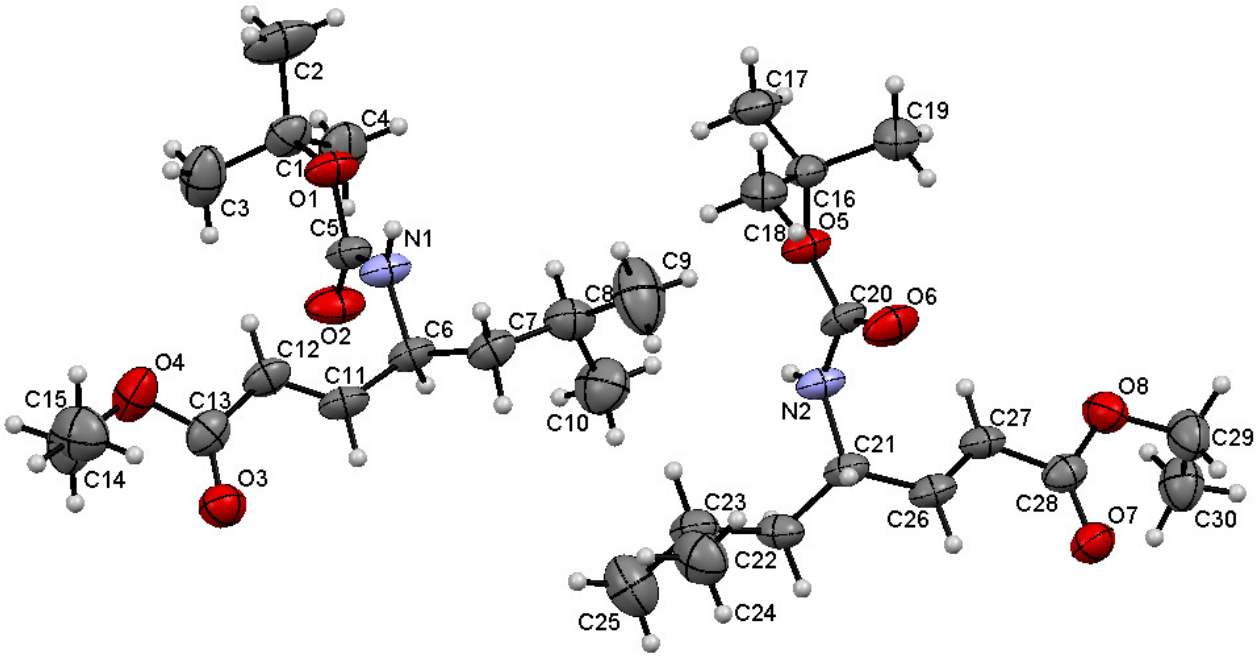


Figure 2: ORTEP diagram of Boc-dgL-OEt. All H-atoms are not labeled for clarity. Two molecules are appeared in the asymmetric unit.

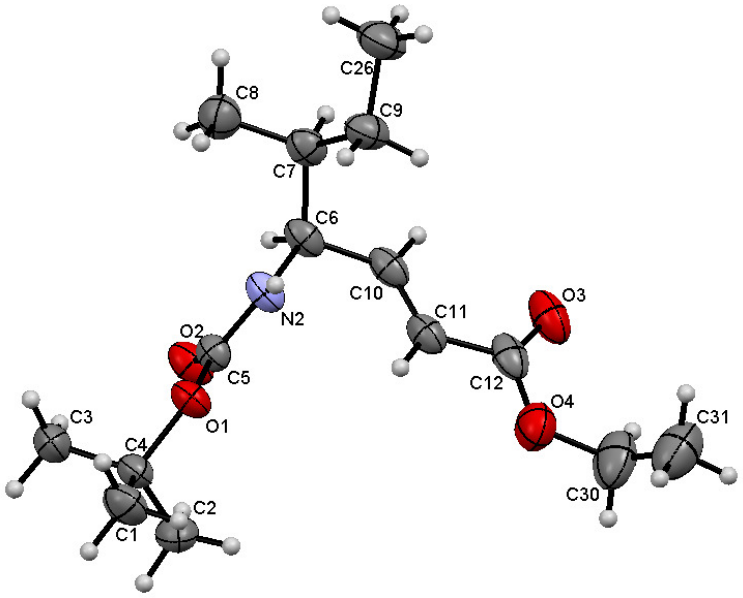


Figure 3: ORTEP diagram of Boc-dgI-OEt. All H-atoms are not labeled for clarity

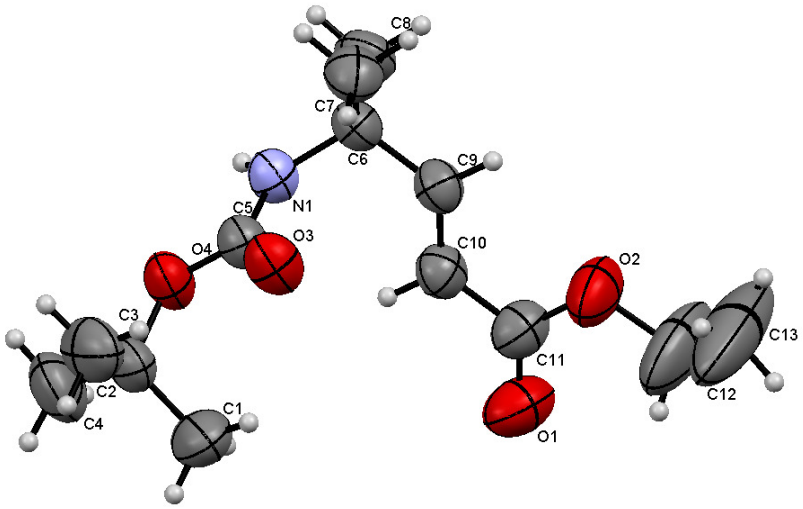


Figure 4: ORTEP diagram of Boc-dgI-OEt. All H-atoms are not labeled for clarity

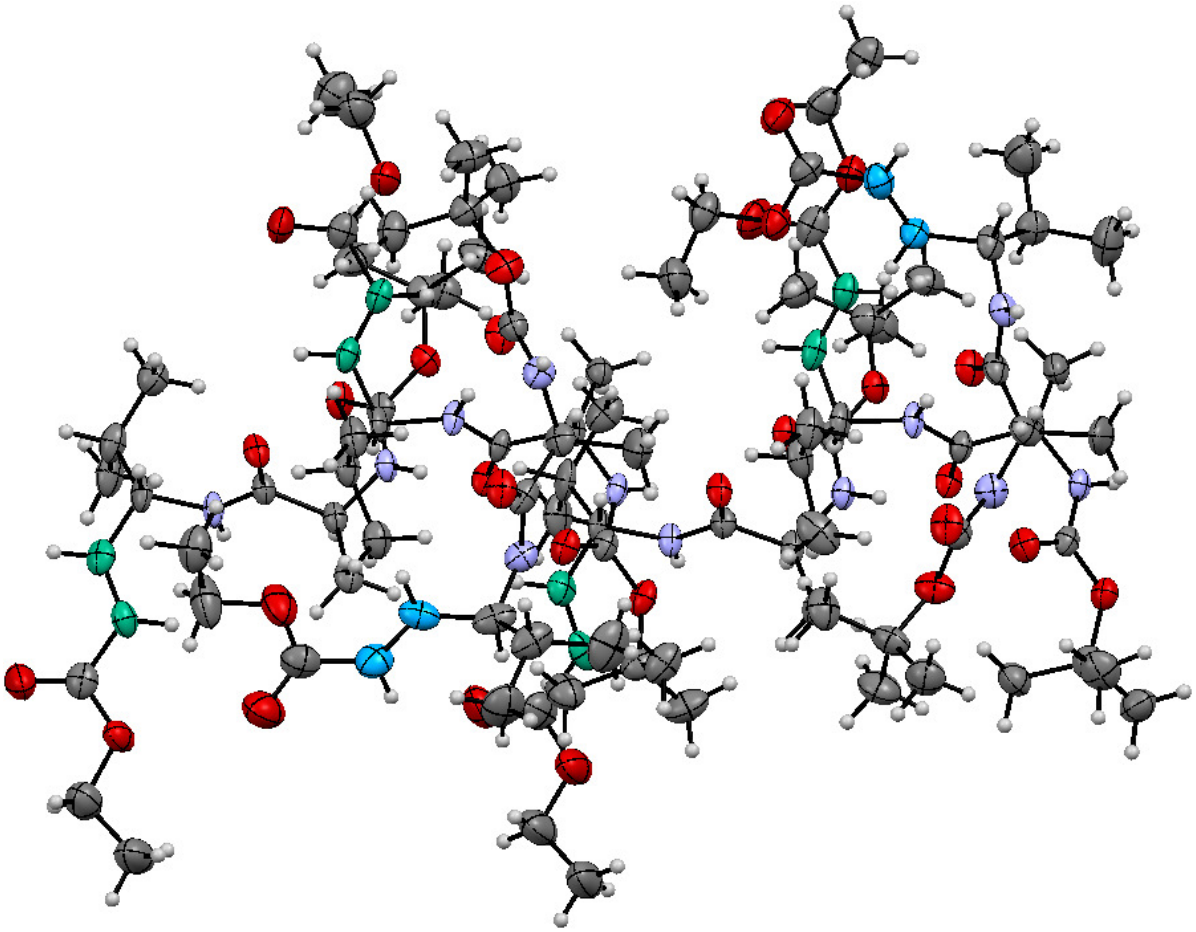
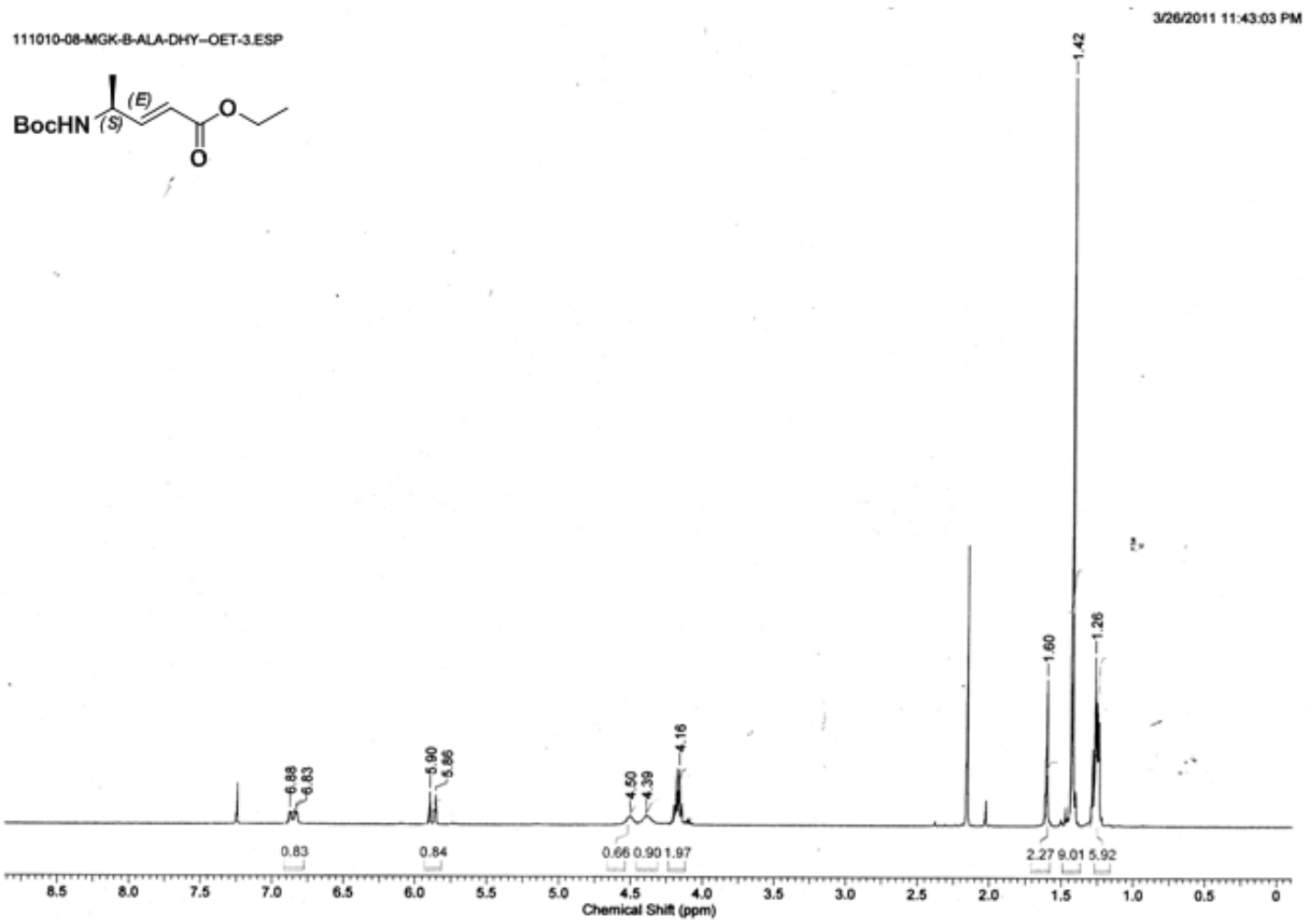


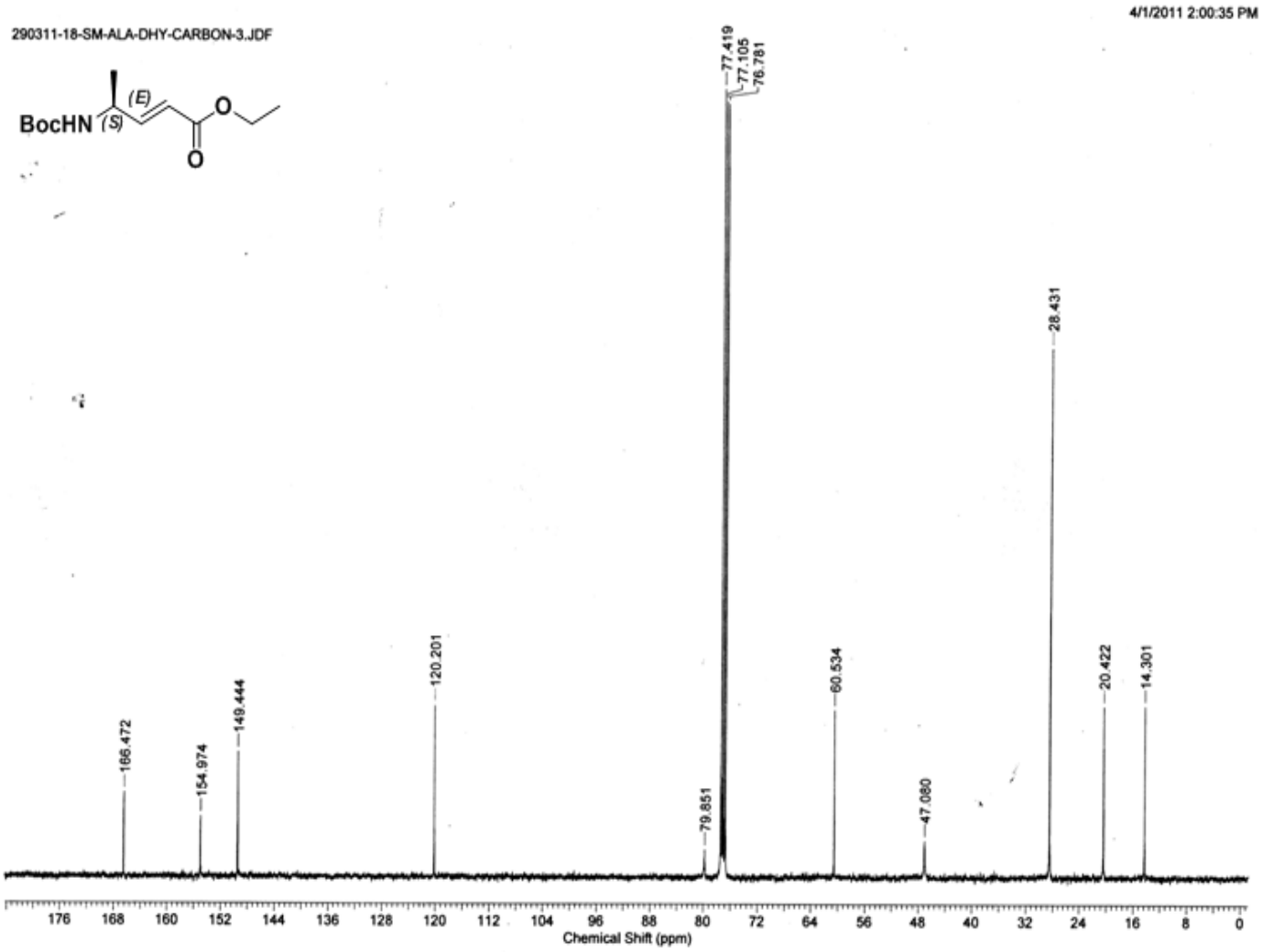
Figure 5: ORTEP diagram of Boc-Ala-dgV-OEt. Six molecules are appeared in the asymmetric unit. The double bonds are highlighted in different colors.

References

1. SHELXS-97: G. M. Sheldrick, *Acta Crystallogr. Sect A*, 1990, **46**, 467 -473, b) G. M. Sheldrick, SHELXL-97, Universität Göttingen (Germany) **1997**

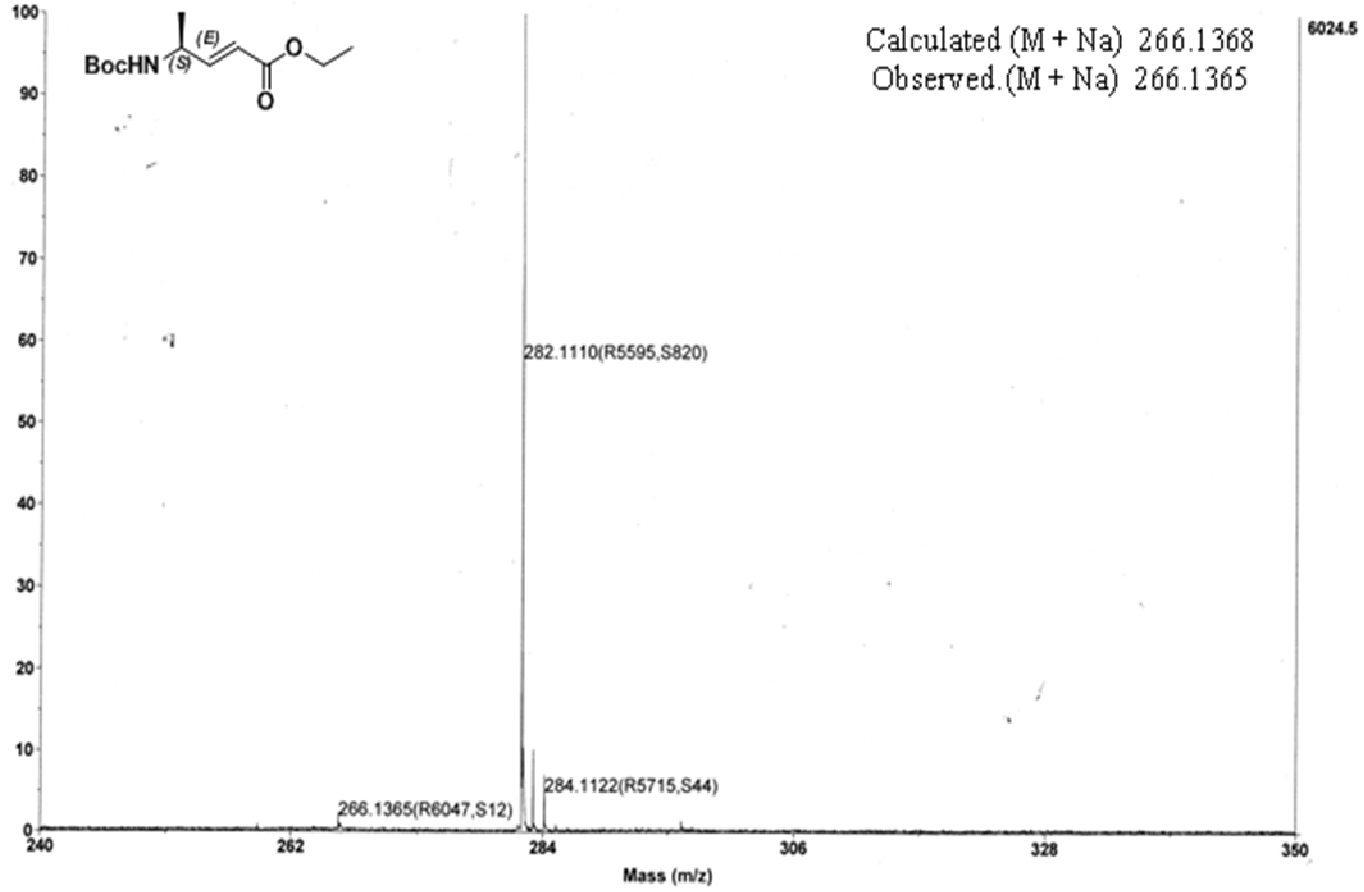
^{1}H, ^{13}C, and Mass Spectra of all Compounds



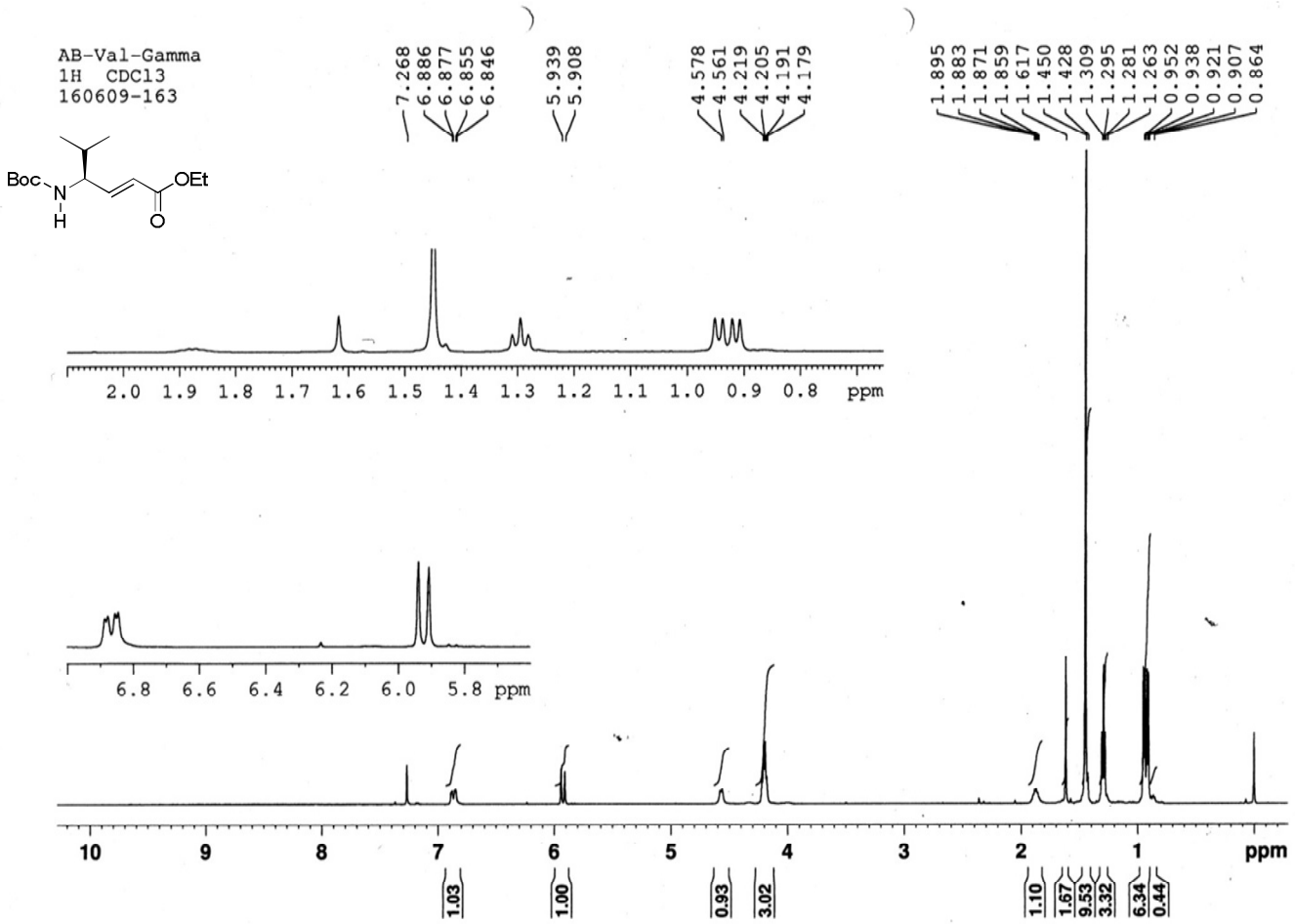


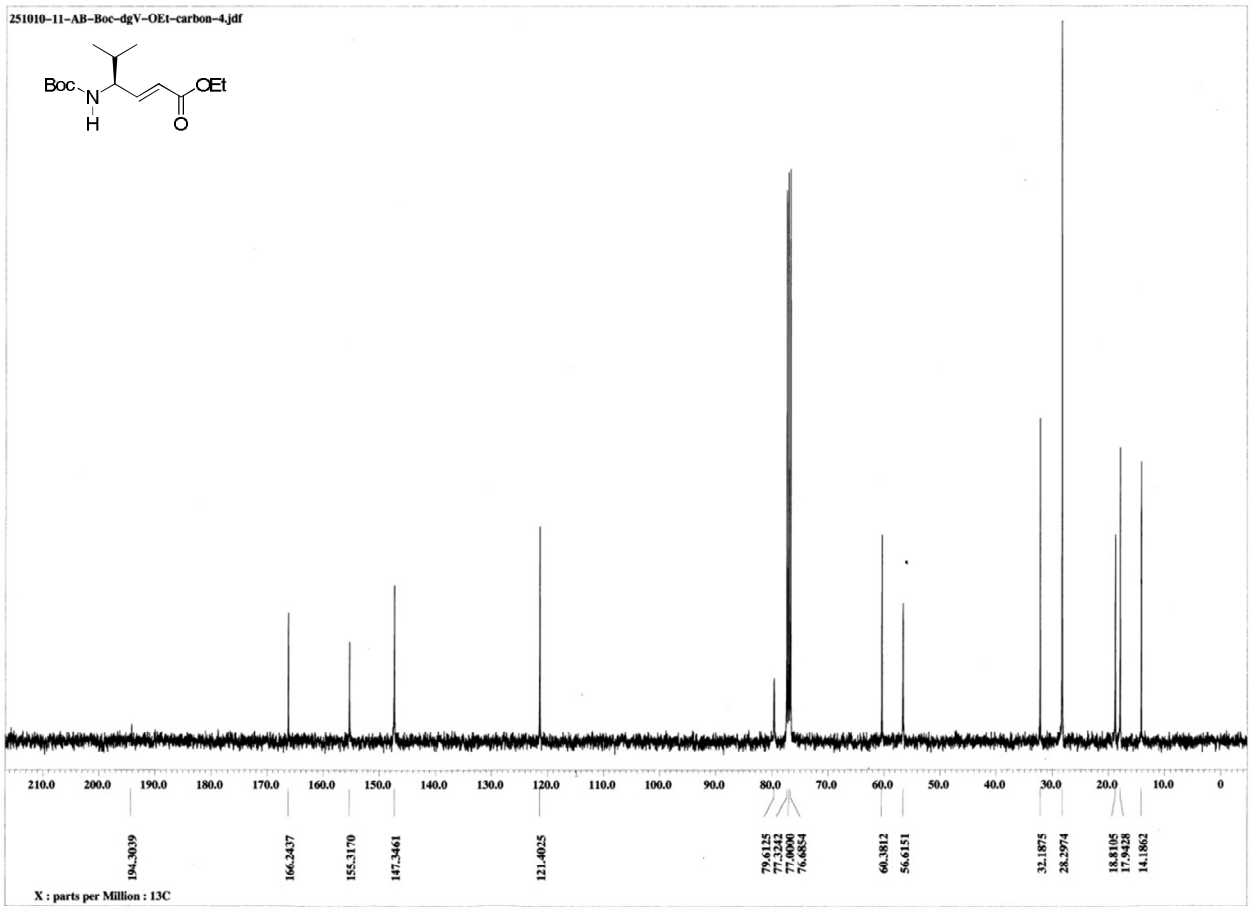
Spectrum Report

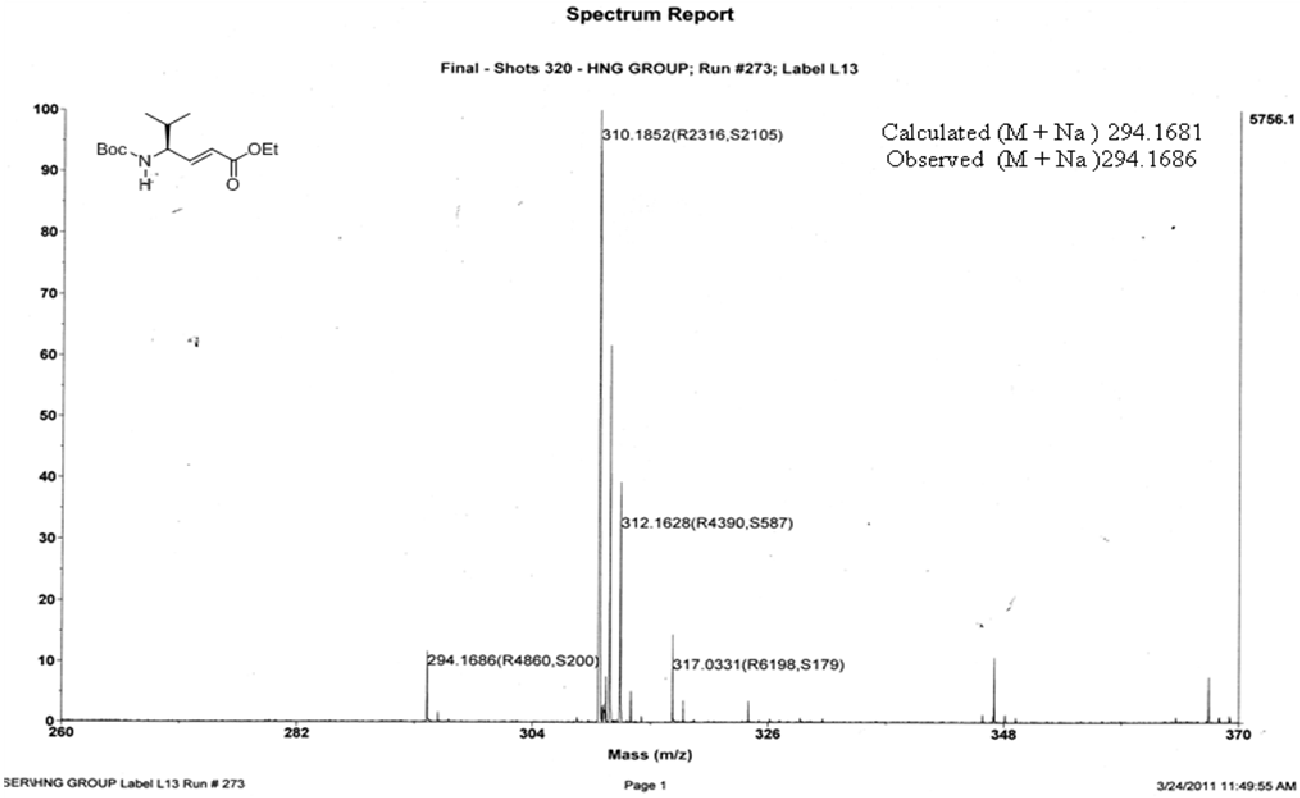
Final - Shots 320 - HNG GROUP; Run #273; Label G10

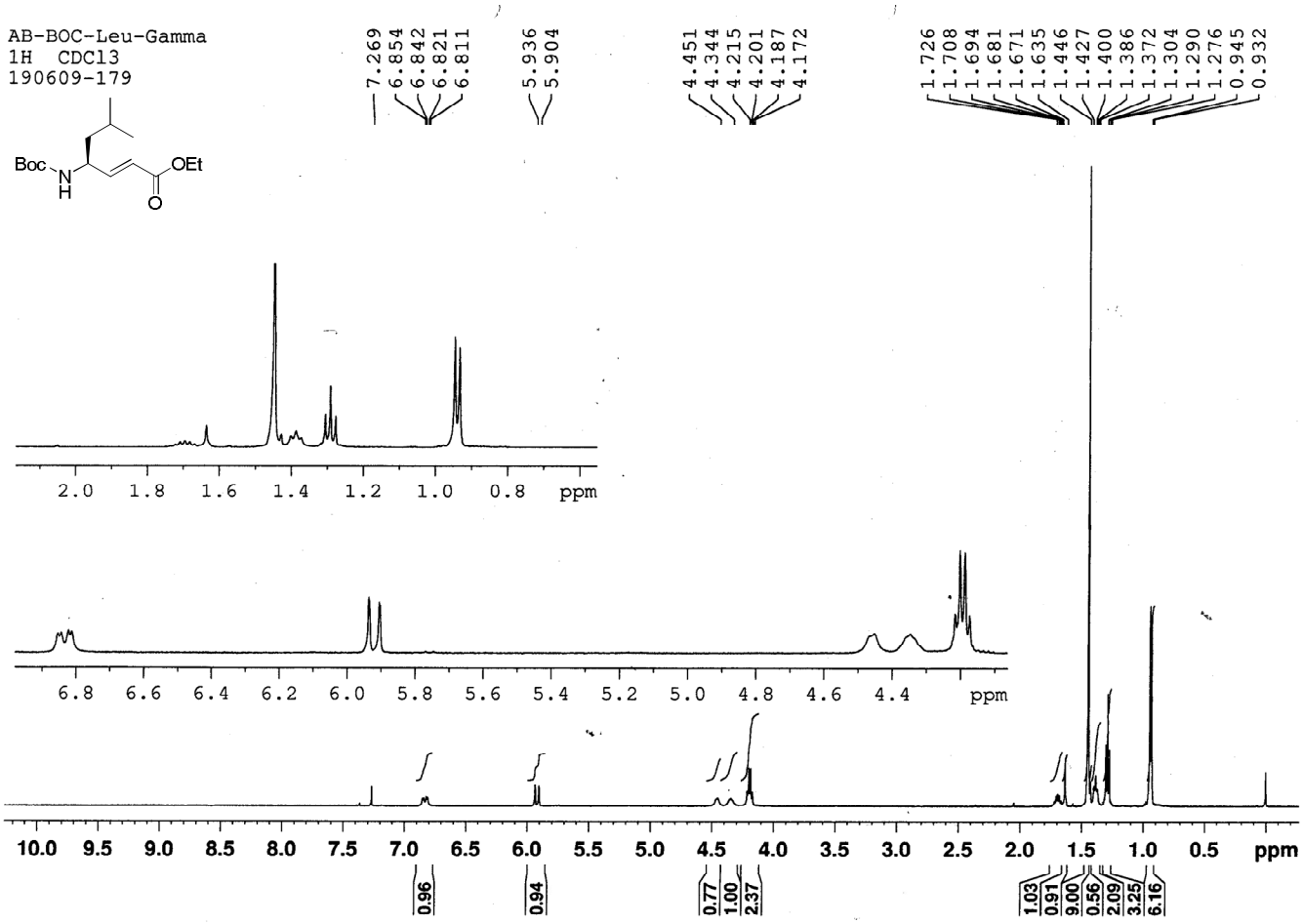


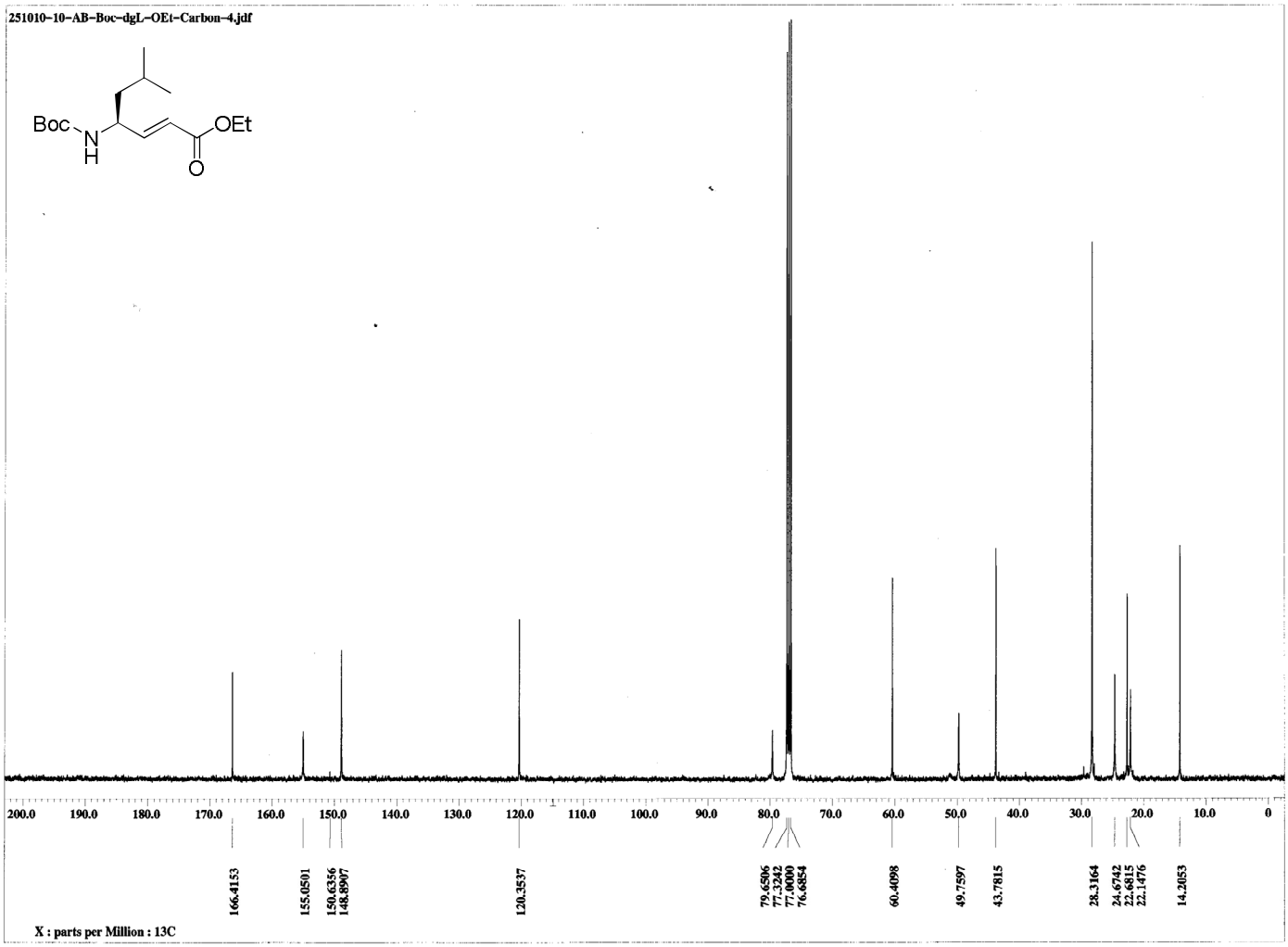
ER\HNG GROUP Label G10 Run # 273 Page 1 3/25/2011 1:34:22 PM





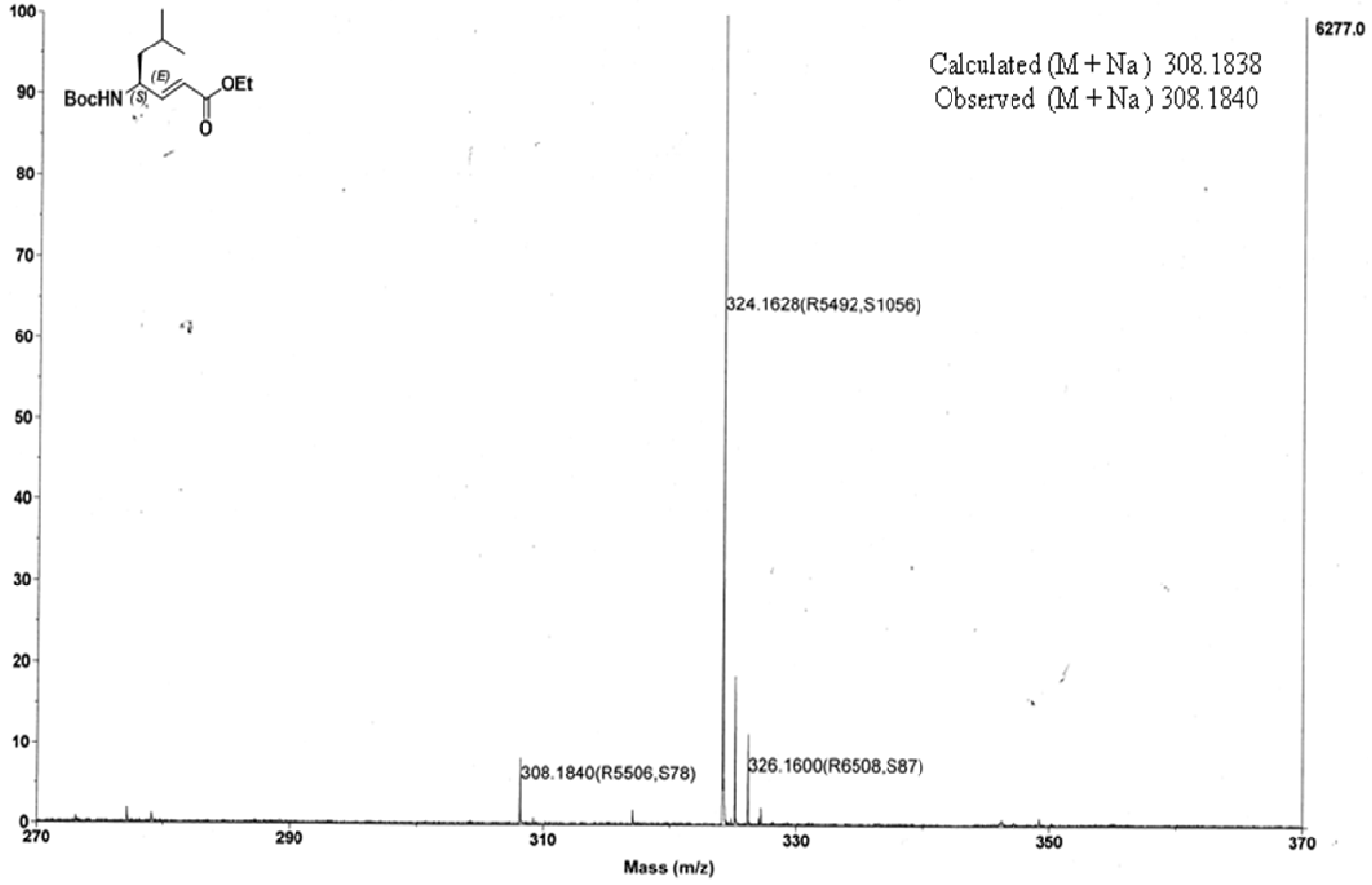


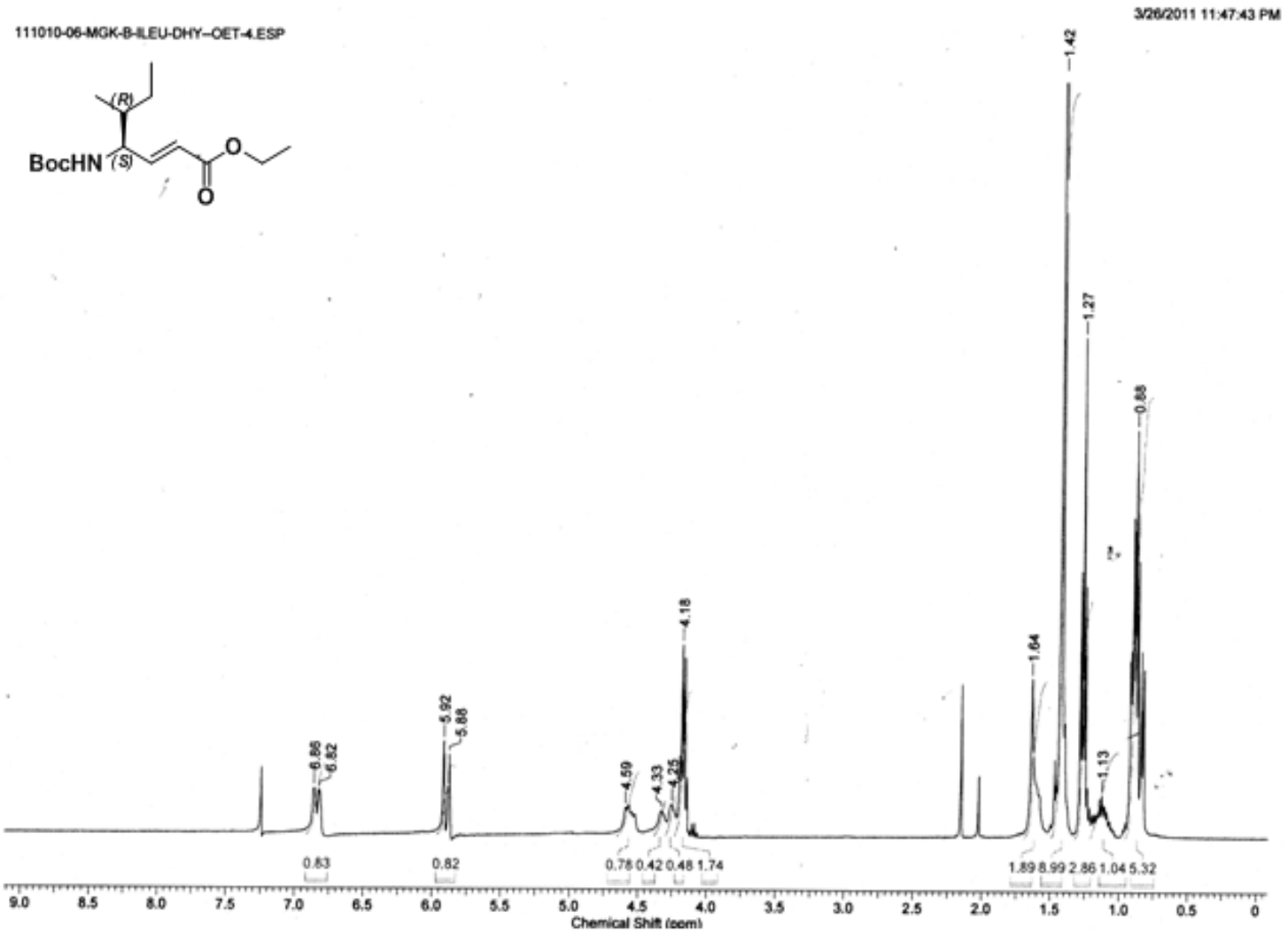


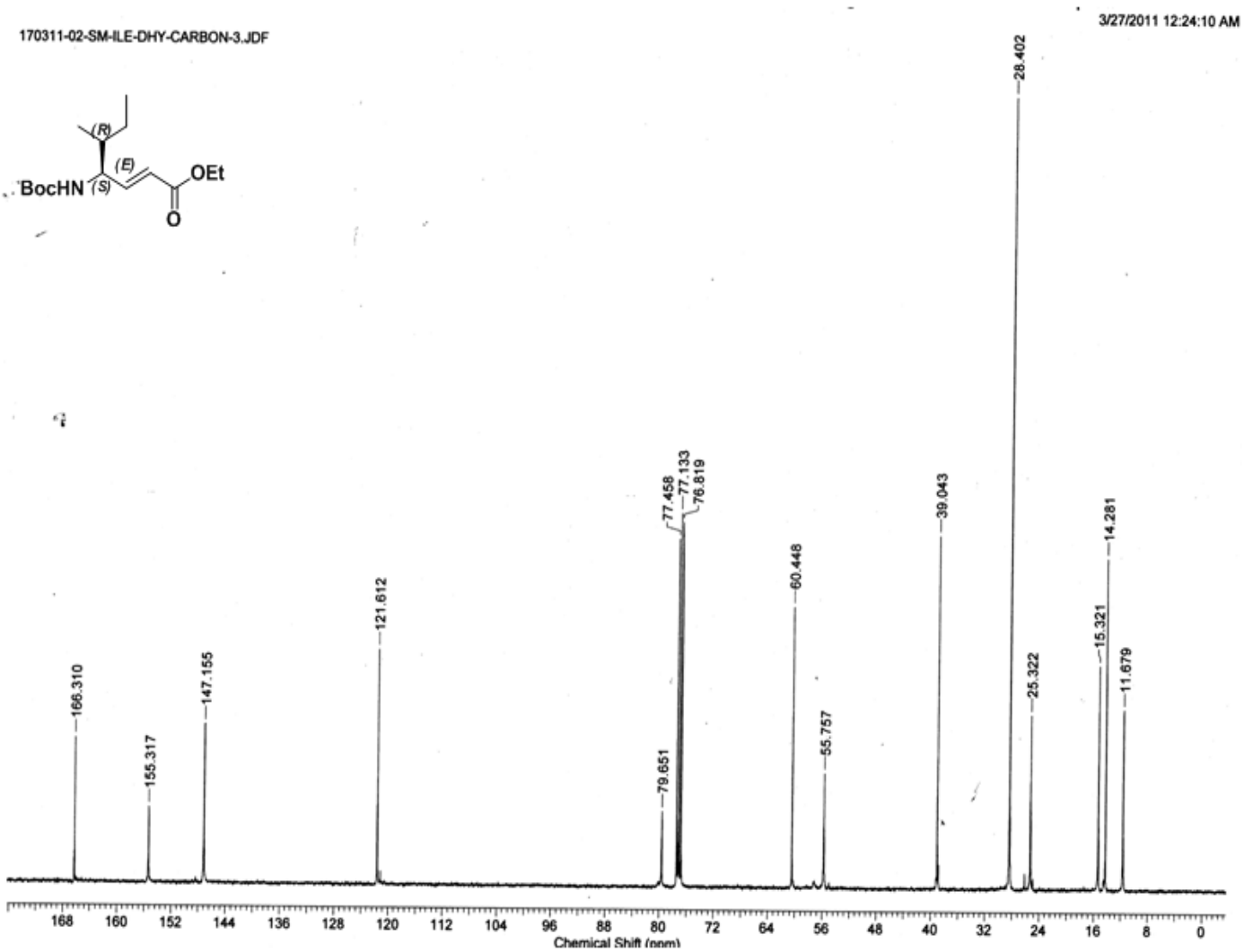


Spectrum Report

Final - Shots 320 - HNG GROUP; Run #273; Label L15

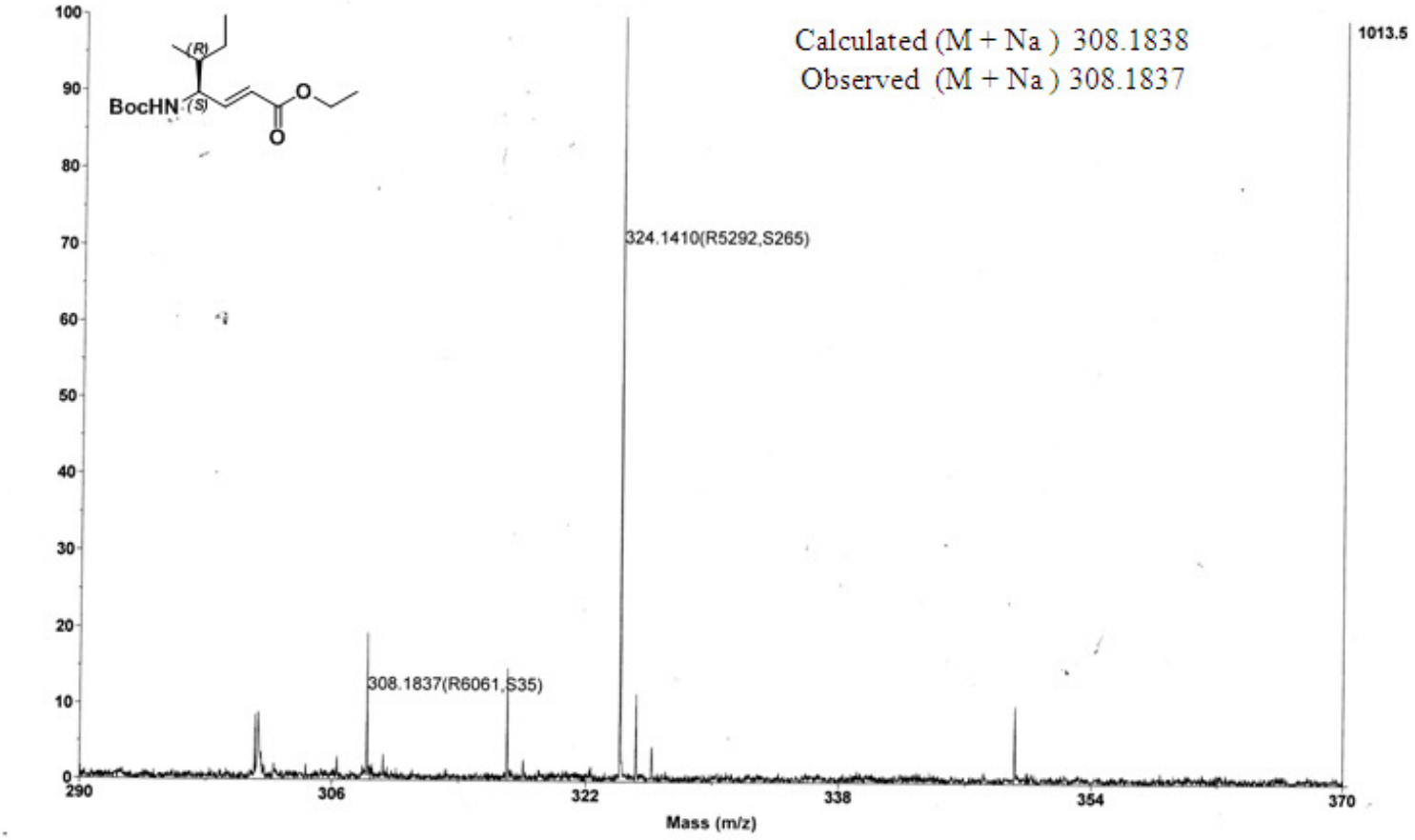






Spectrum Report

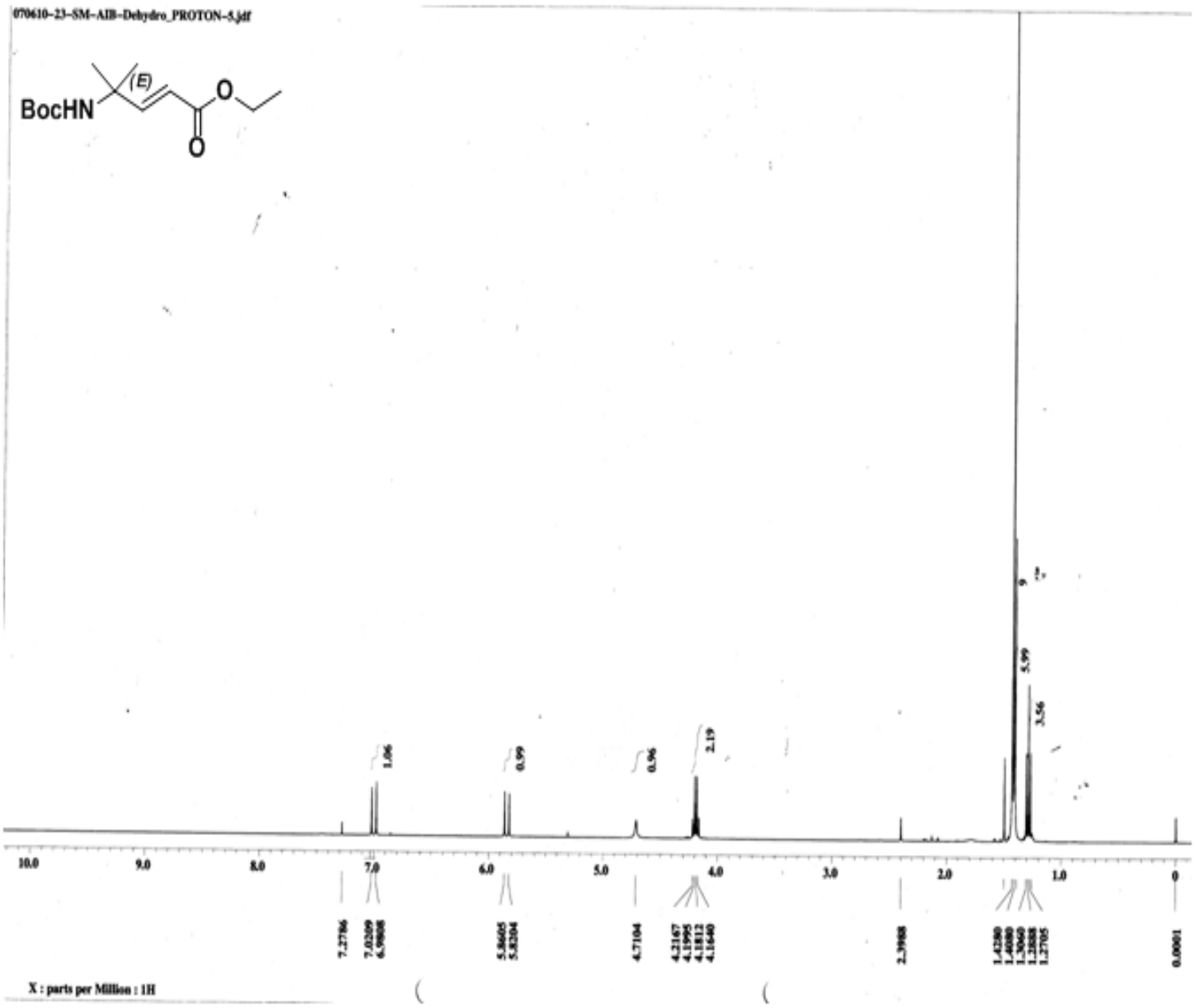
Final - Shots 320 - HNG GROUP; Run #273; Label L17

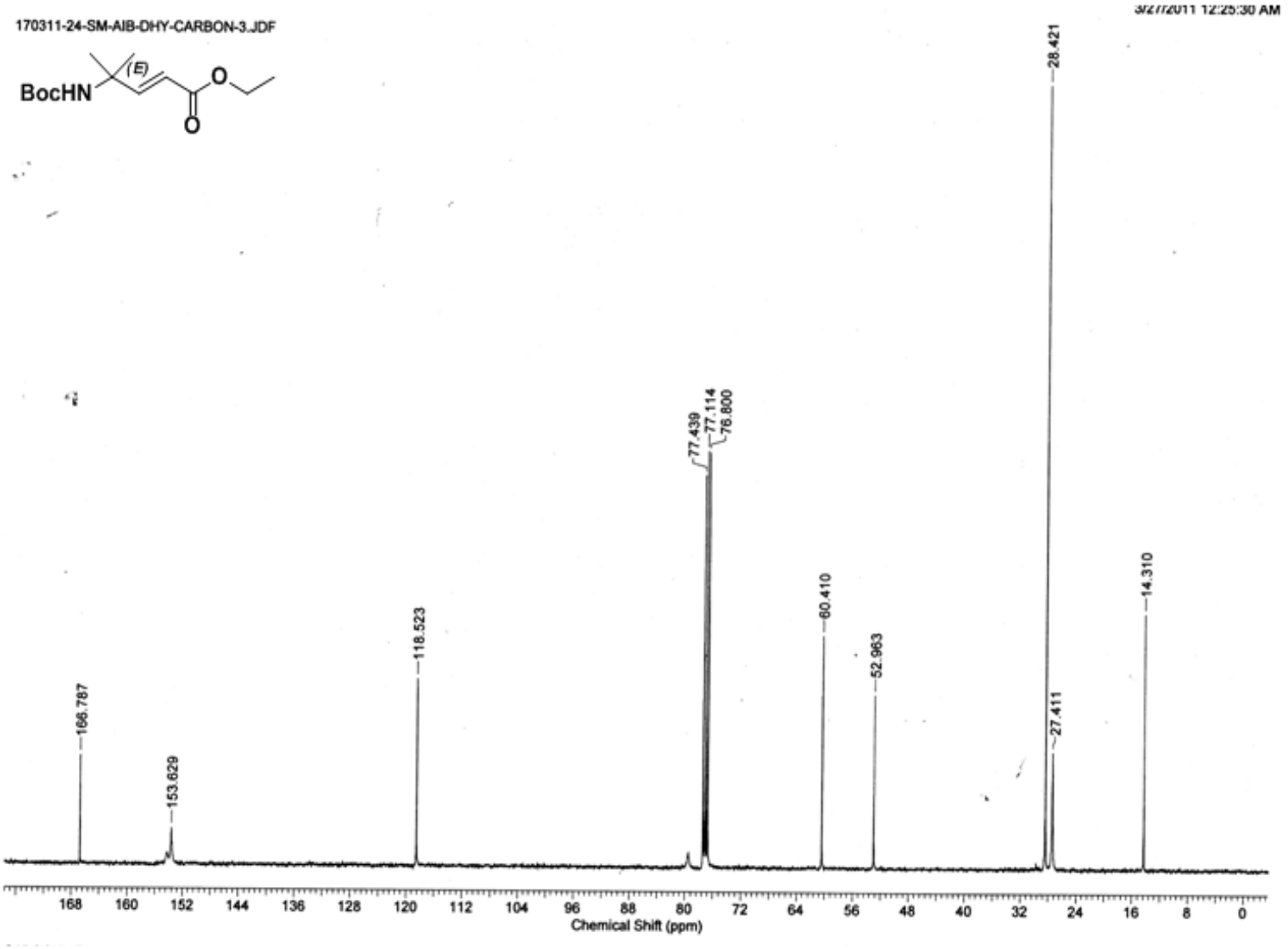


IISER\HNG GROUP Label L17 Run # 273

Page 1

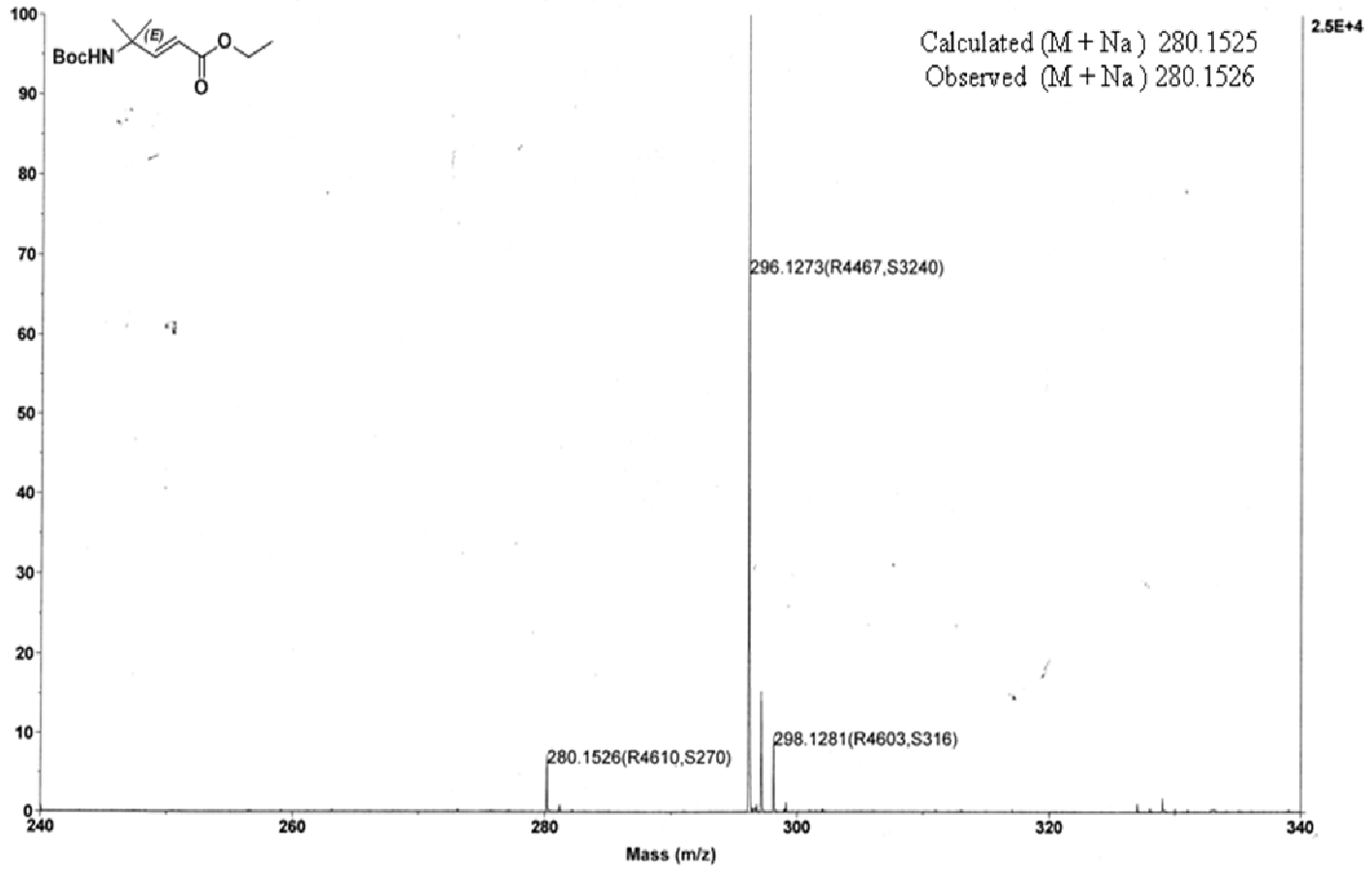
3/24/2011 12:11:32 PM



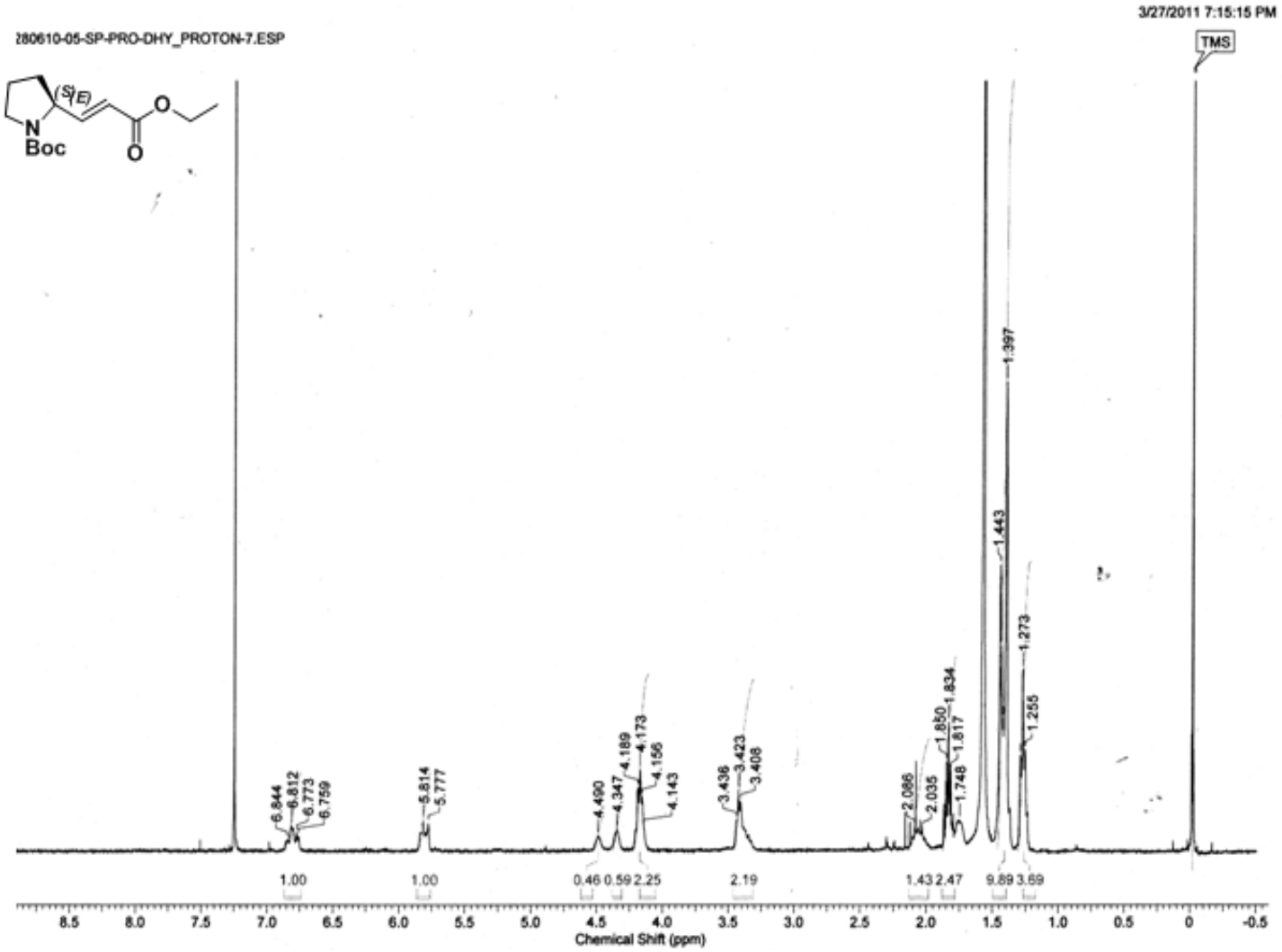


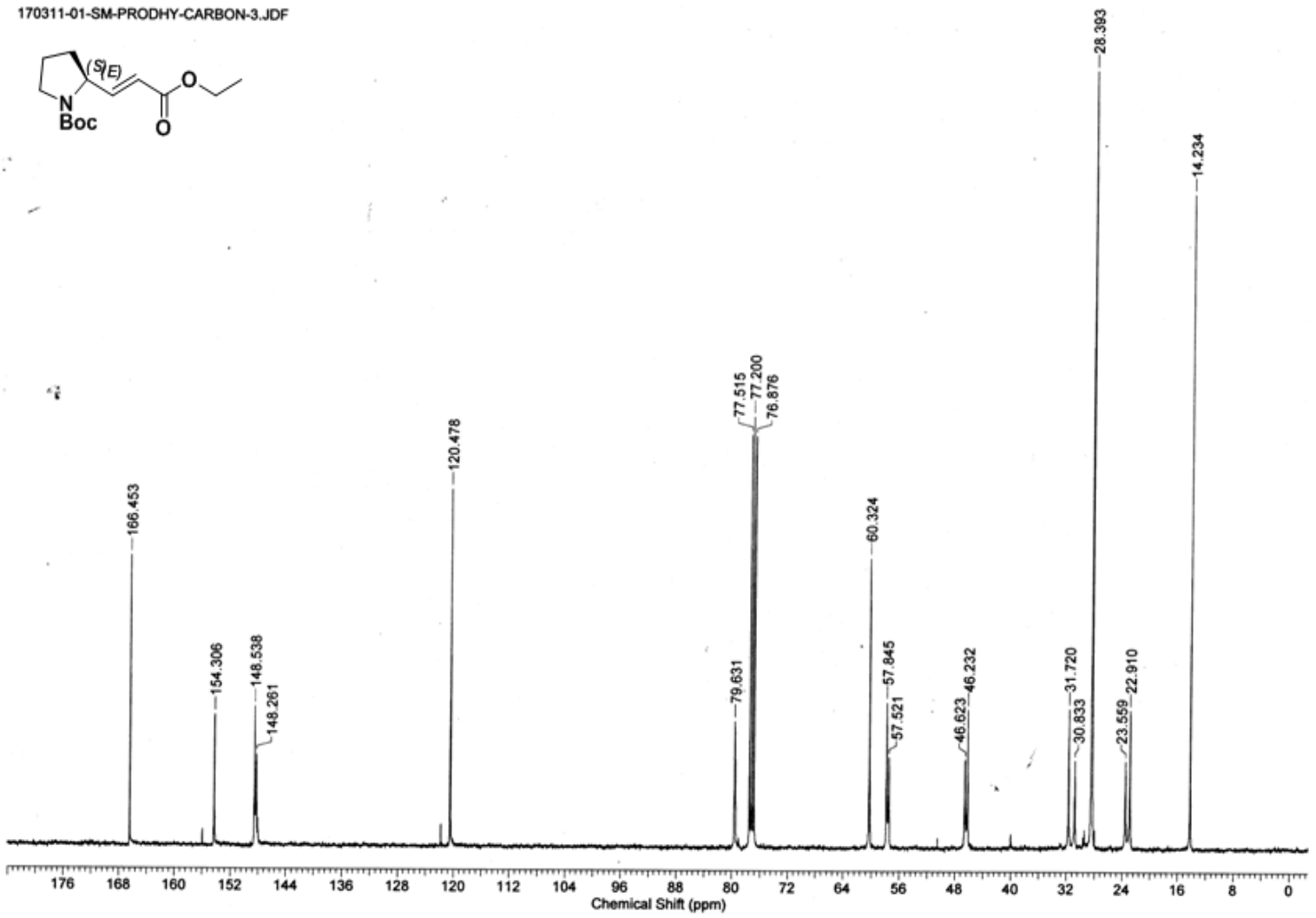
Spectrum Report

Final - Shots 320 - HNG GROUP; Run #273; Label G12



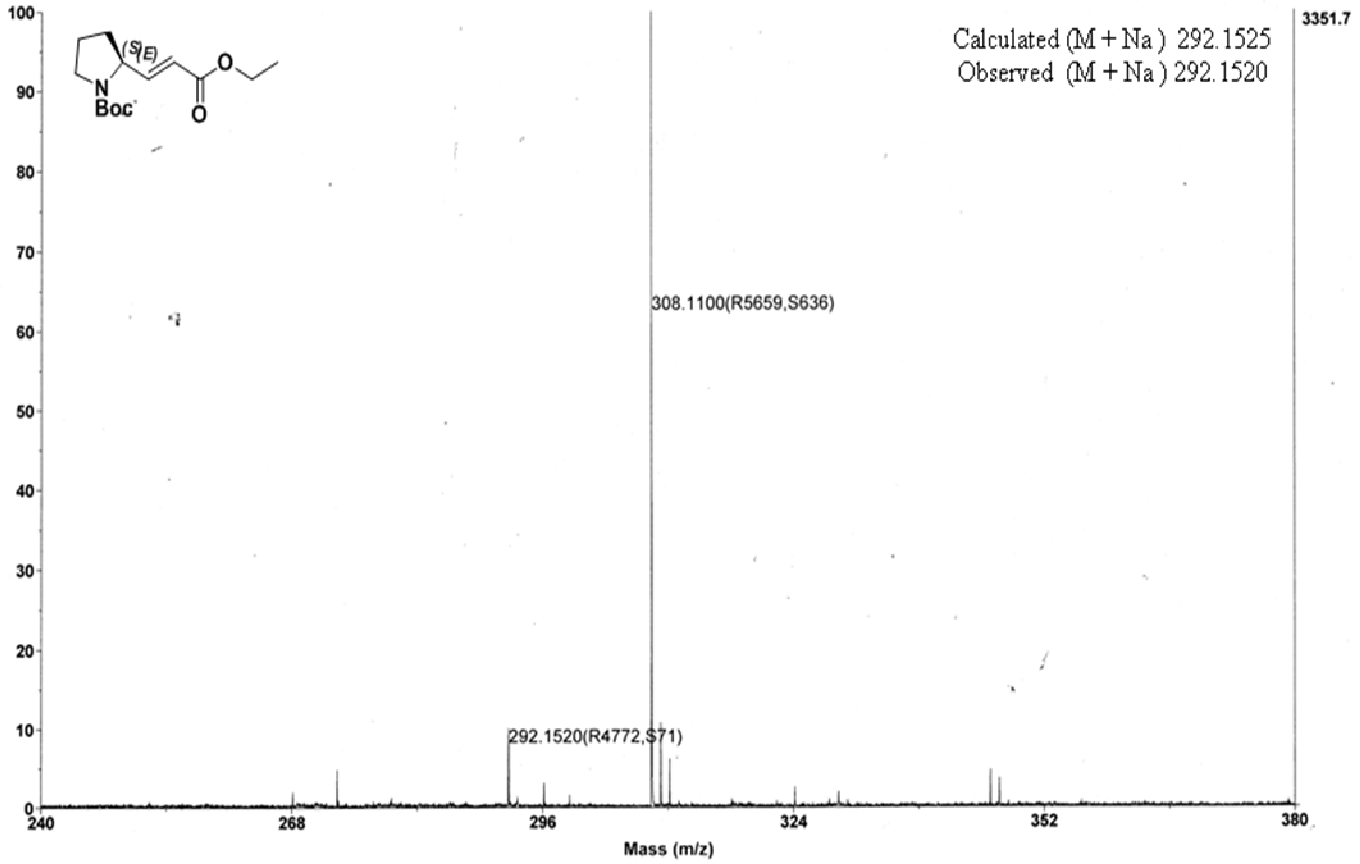
IISER\HNG GROUP Label G12 Run # 273 Page 1 3/25/2011 1:36:23 PM



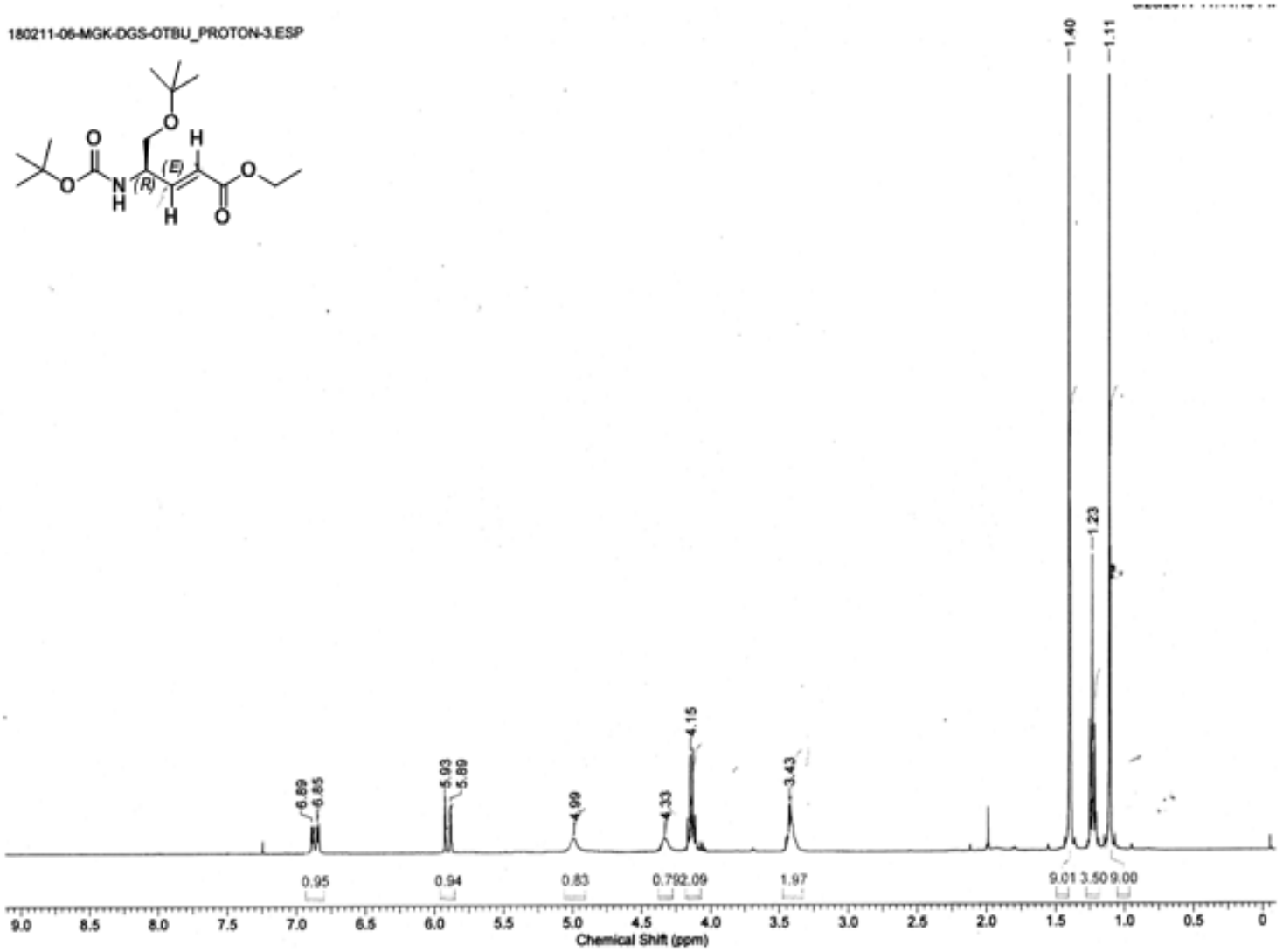


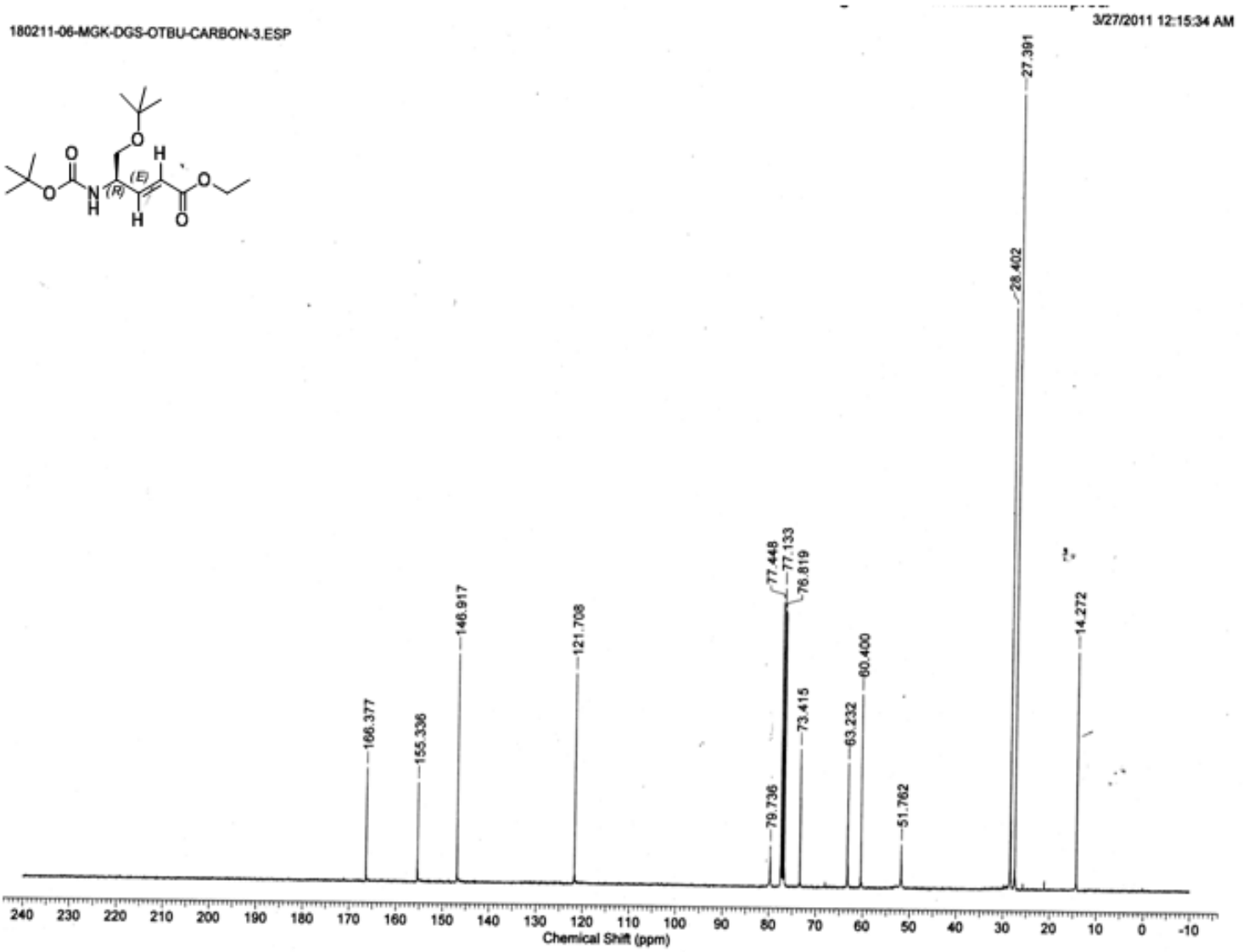
Spectrum Report

Final - Shots 320 - HNG GROUP; Run #273; Label L21



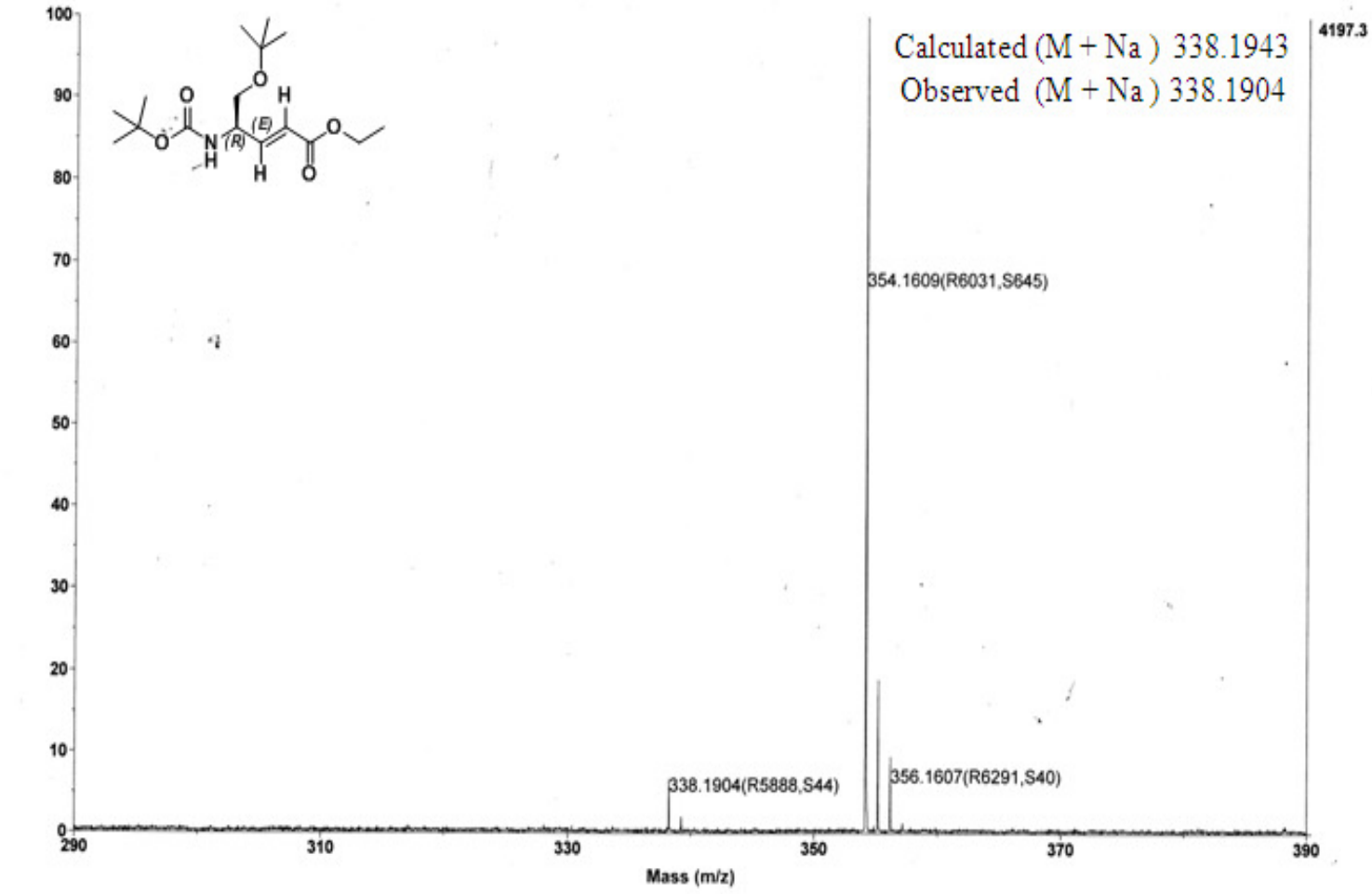
IISER\HNG GROUP Label L21 Run # 273 Page 1 3/24/2011 12:14:05 PM

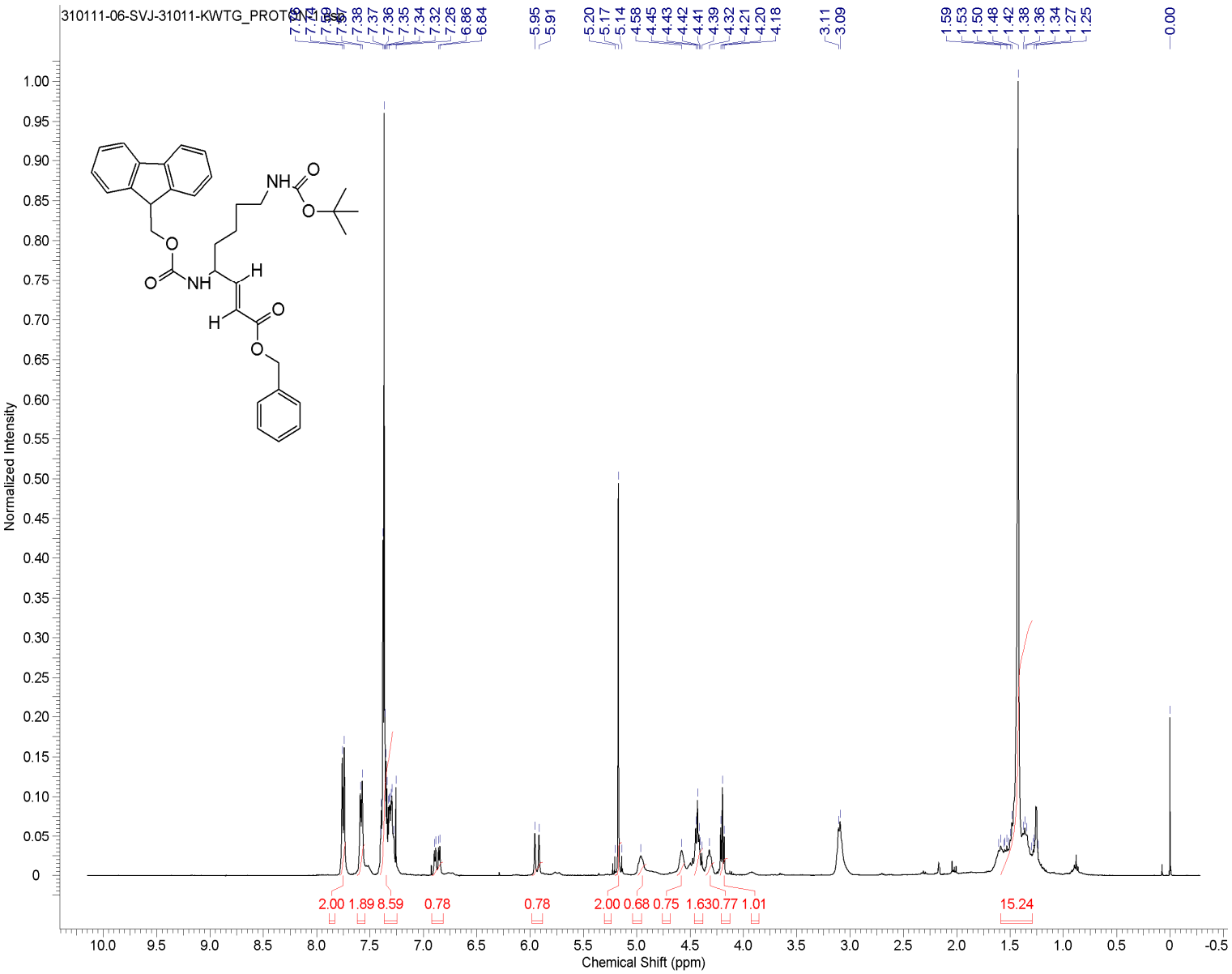


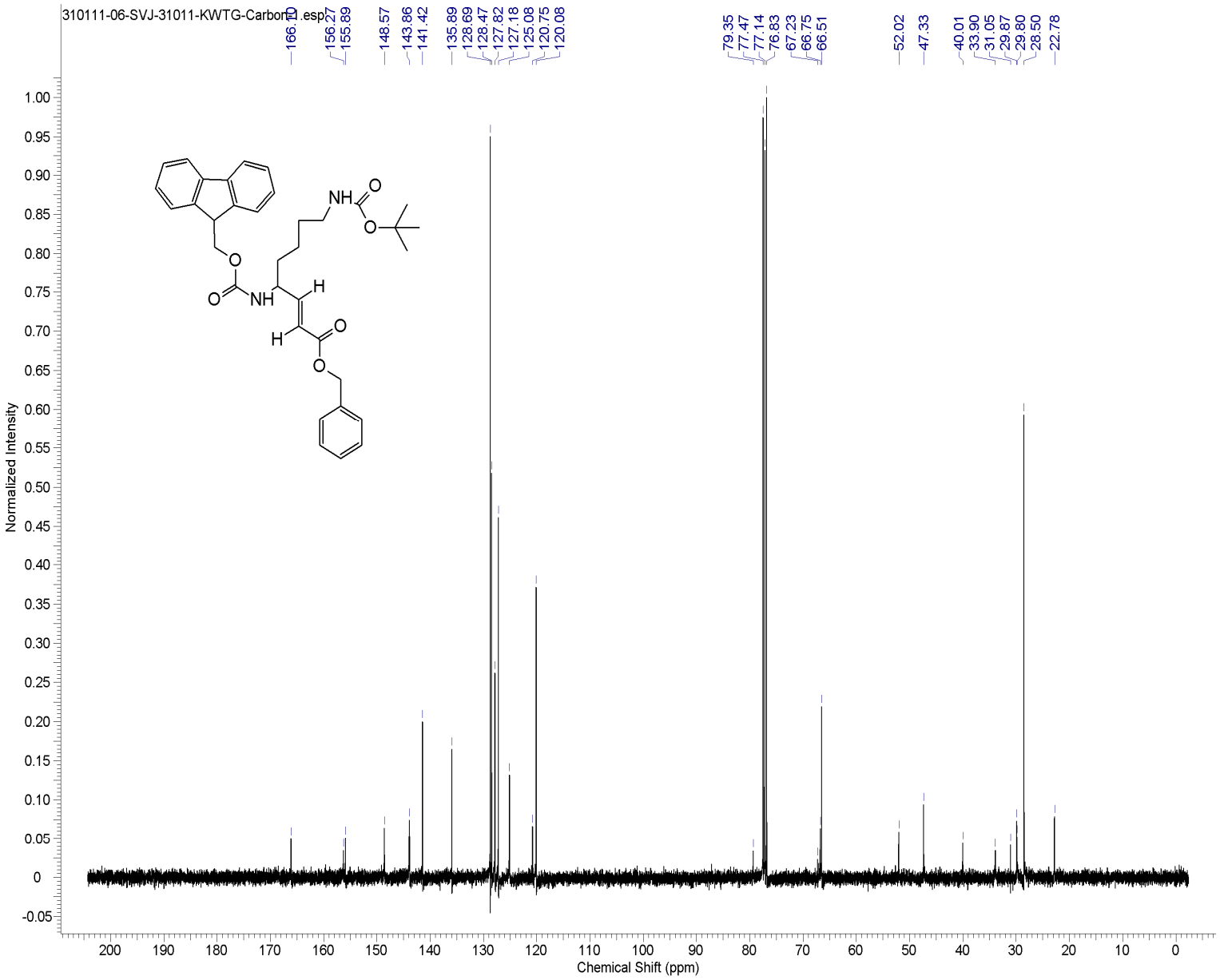


Spectrum Report

Final - Shots 320 - HNG GROUP; Run #274; Label H22

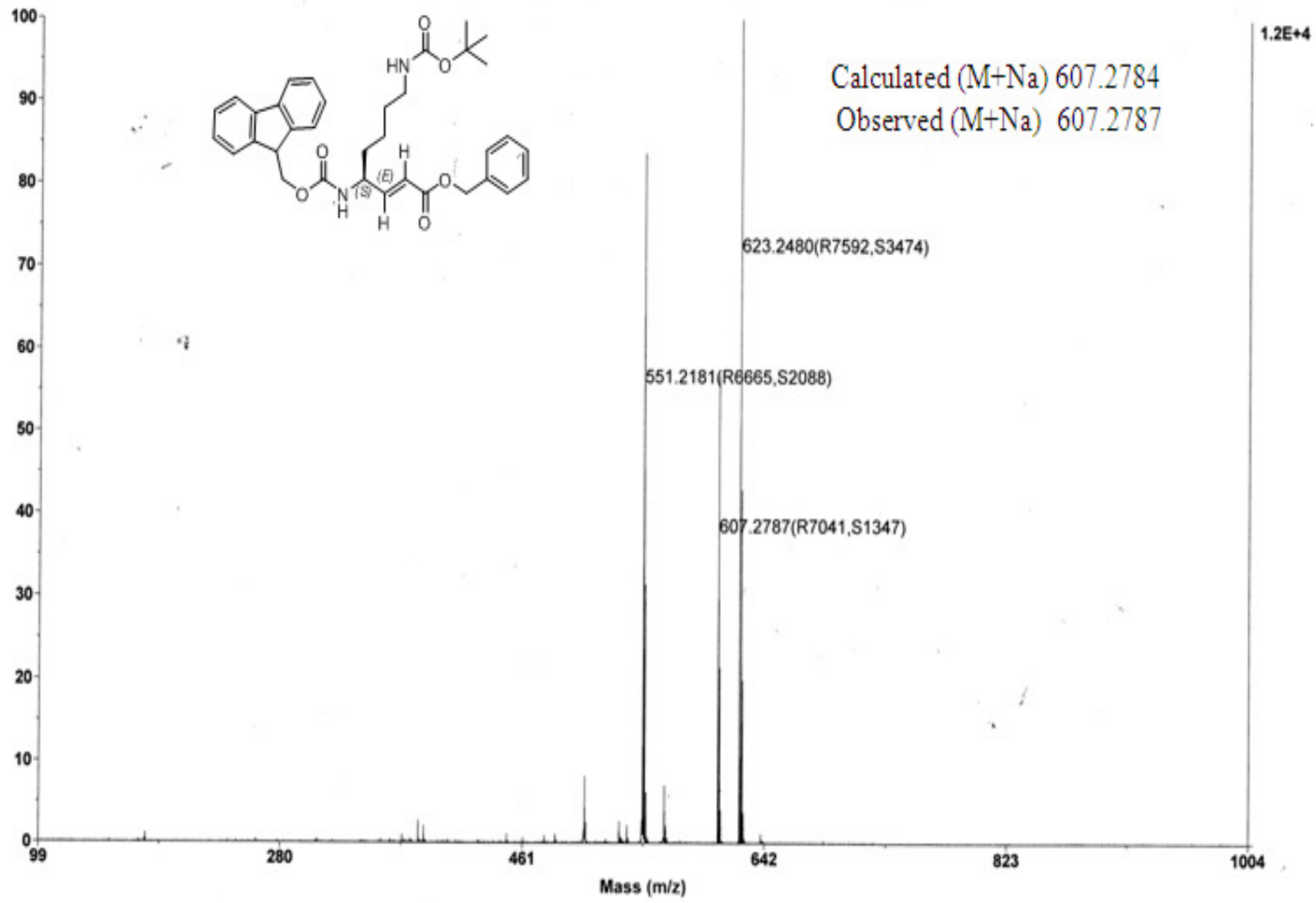






Spectrum Report

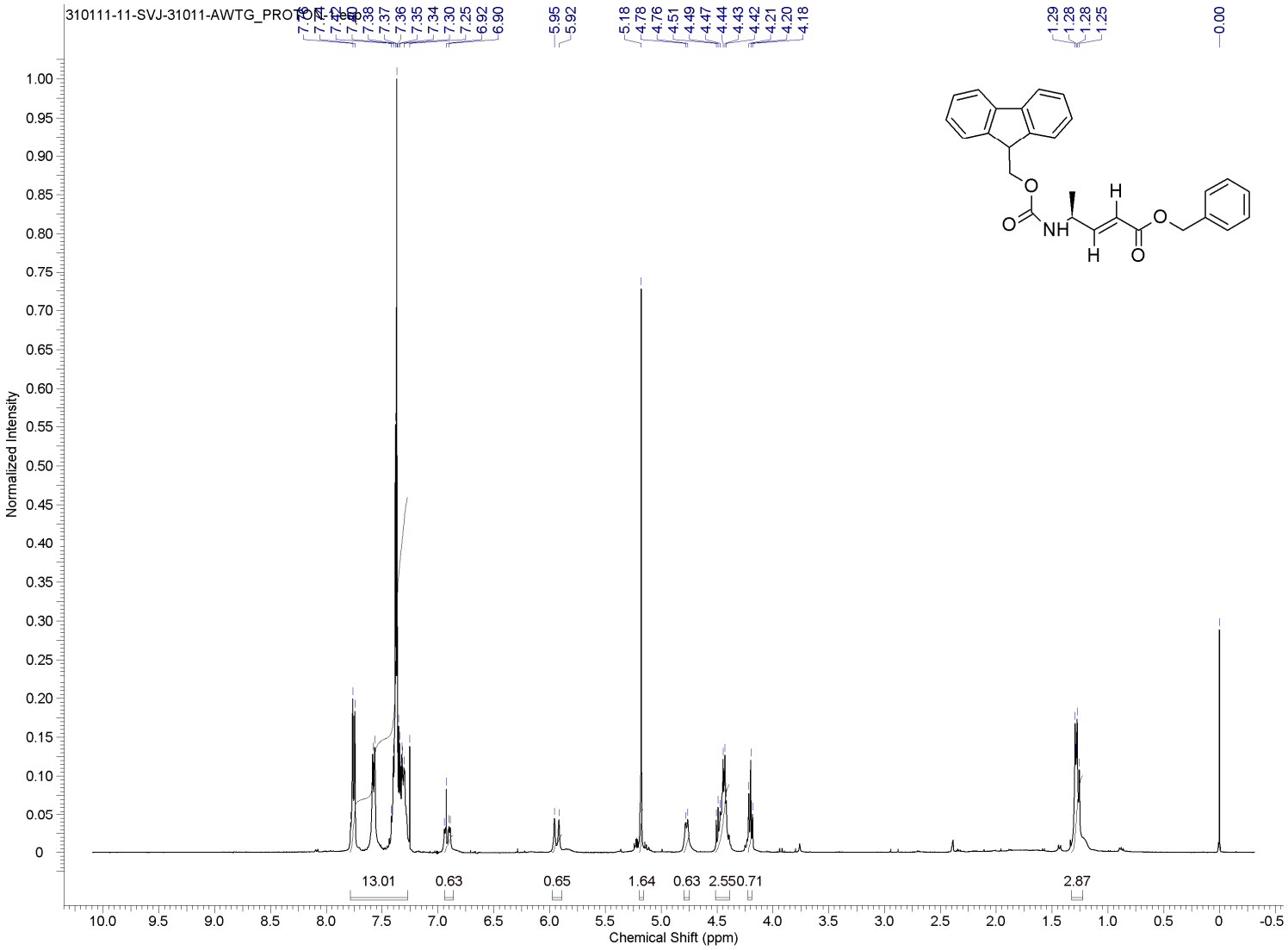
Final - Shots 400 - HNG GROUP; Run #221; Label E15

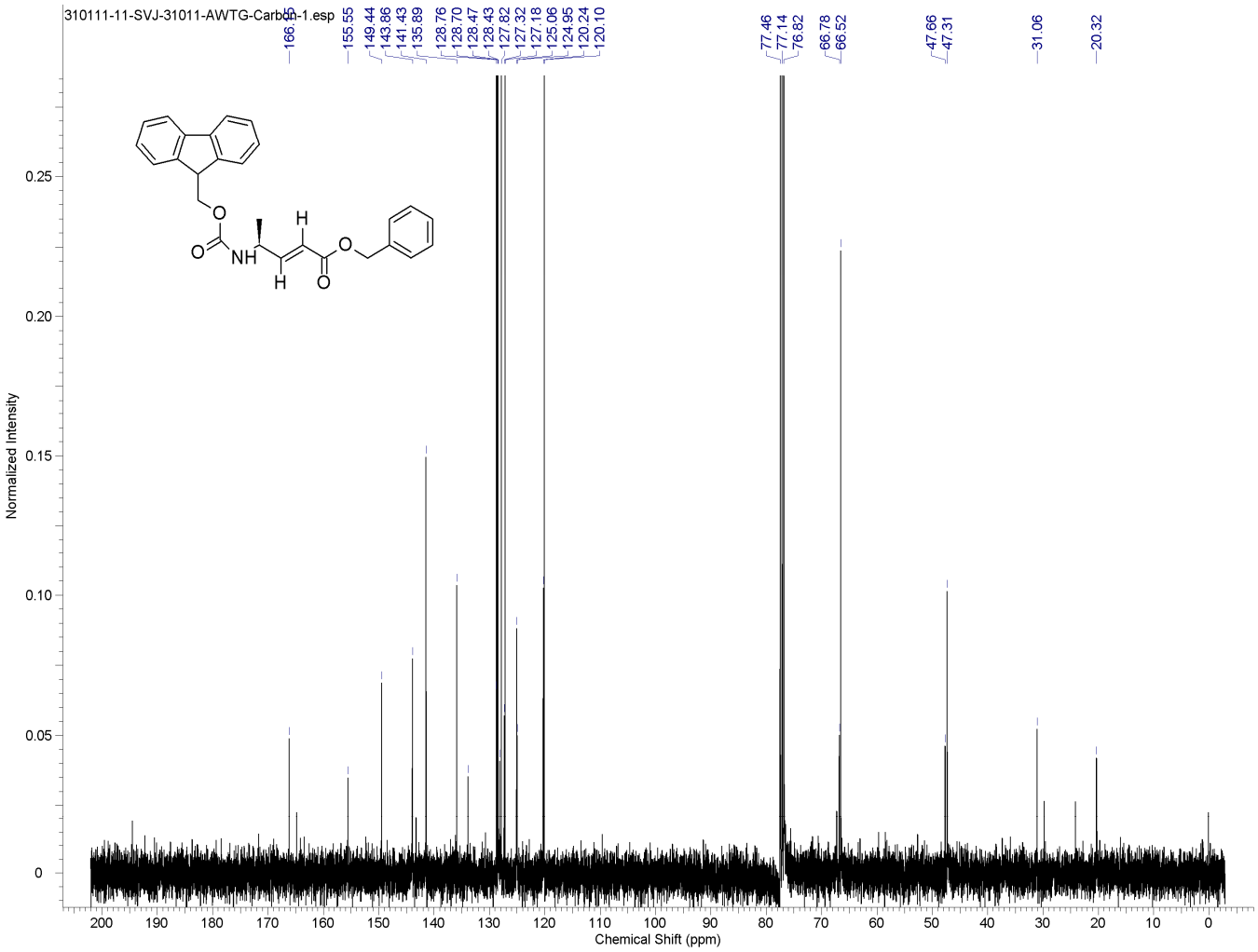


IISER\HNG GROUP Label E15 Run # 221

Page 1

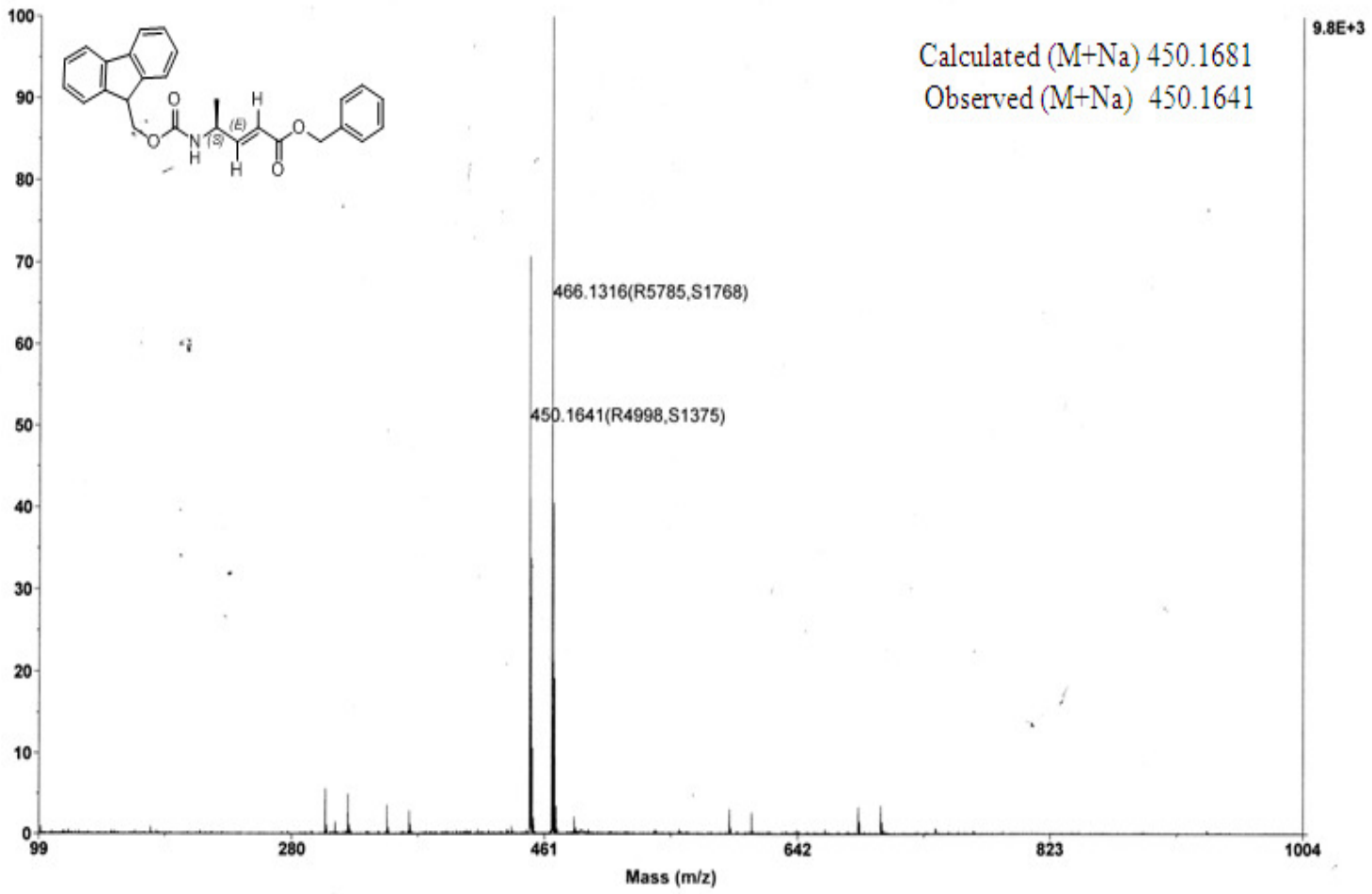
3/28/2011 5:49:59 PM

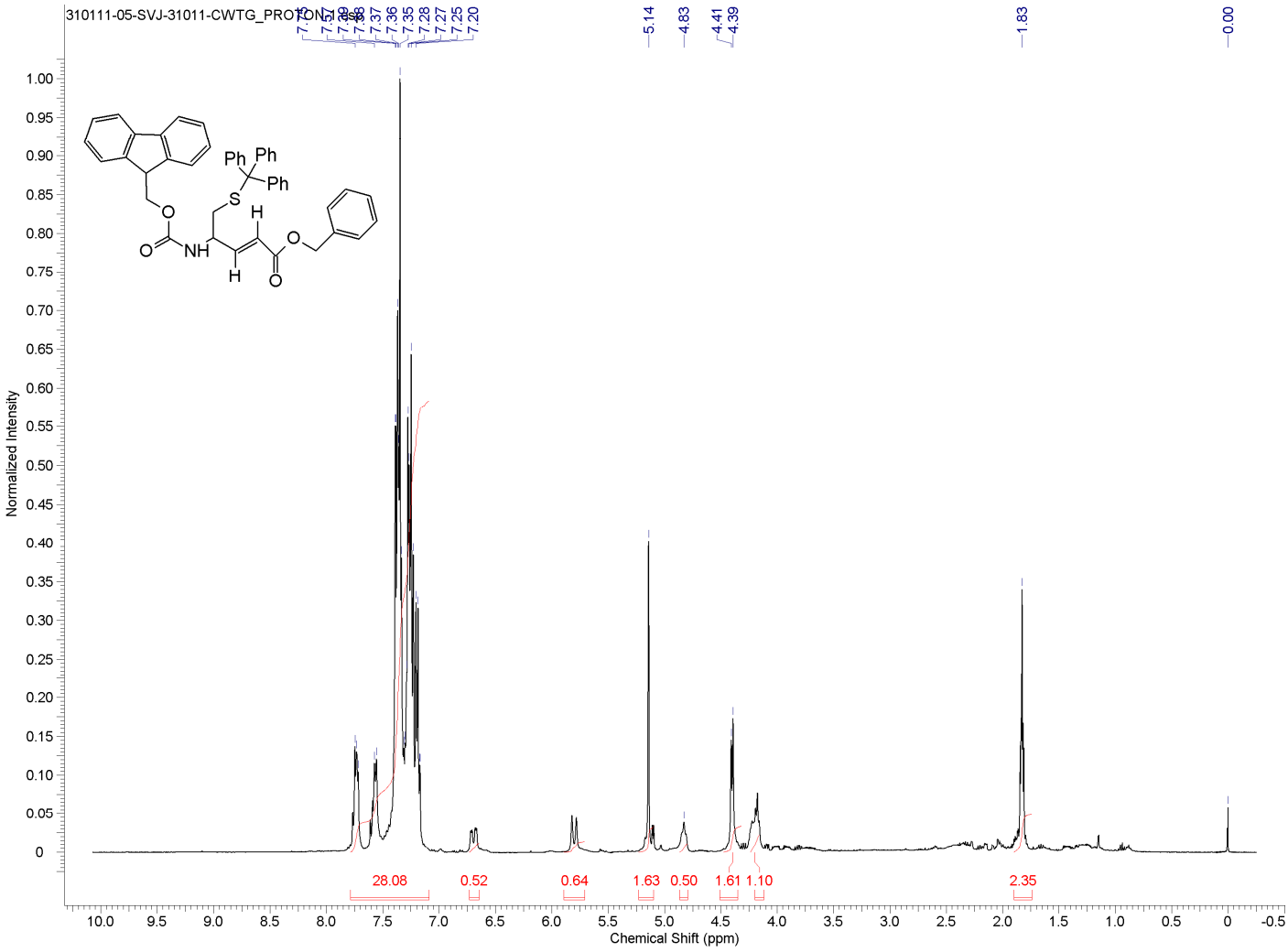

310111-11-SVJ-31011-AWTG_PROTON-1.esp
7.74 7.72 7.70 7.68 7.38 7.37 7.36 7.35 7.34 7.30 7.25 6.92 6.90
5.95 5.92
5.18 4.78 4.76 4.51 4.49 4.47 4.44 4.43 4.42 4.21 4.20 4.18
1.29 1.28 1.28 1.25
0.00
Normalized Intensity
13.01 0.63 0.65 1.64 0.63 2.55 0.71 2.87
Chemical Shift (ppm)

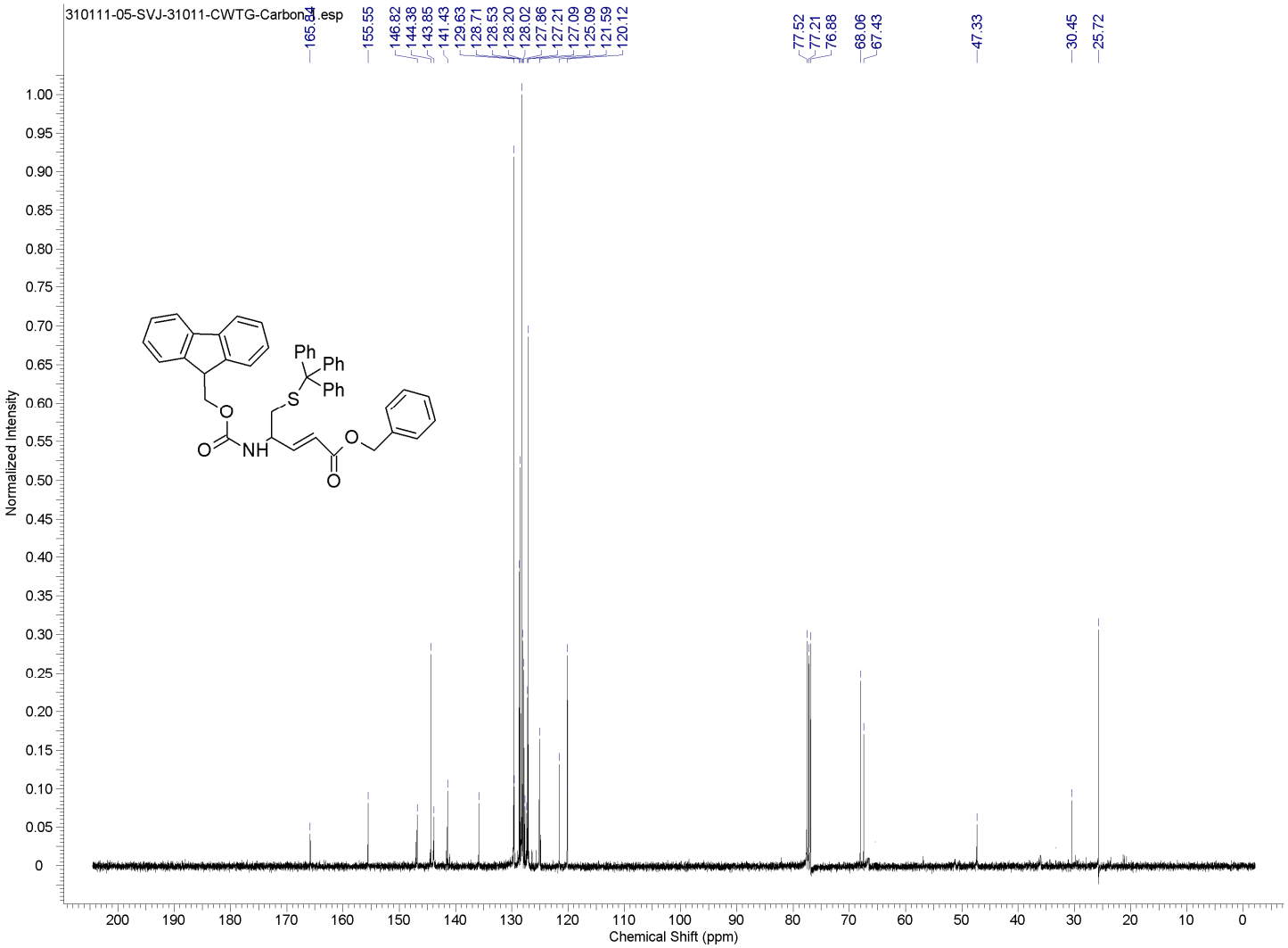


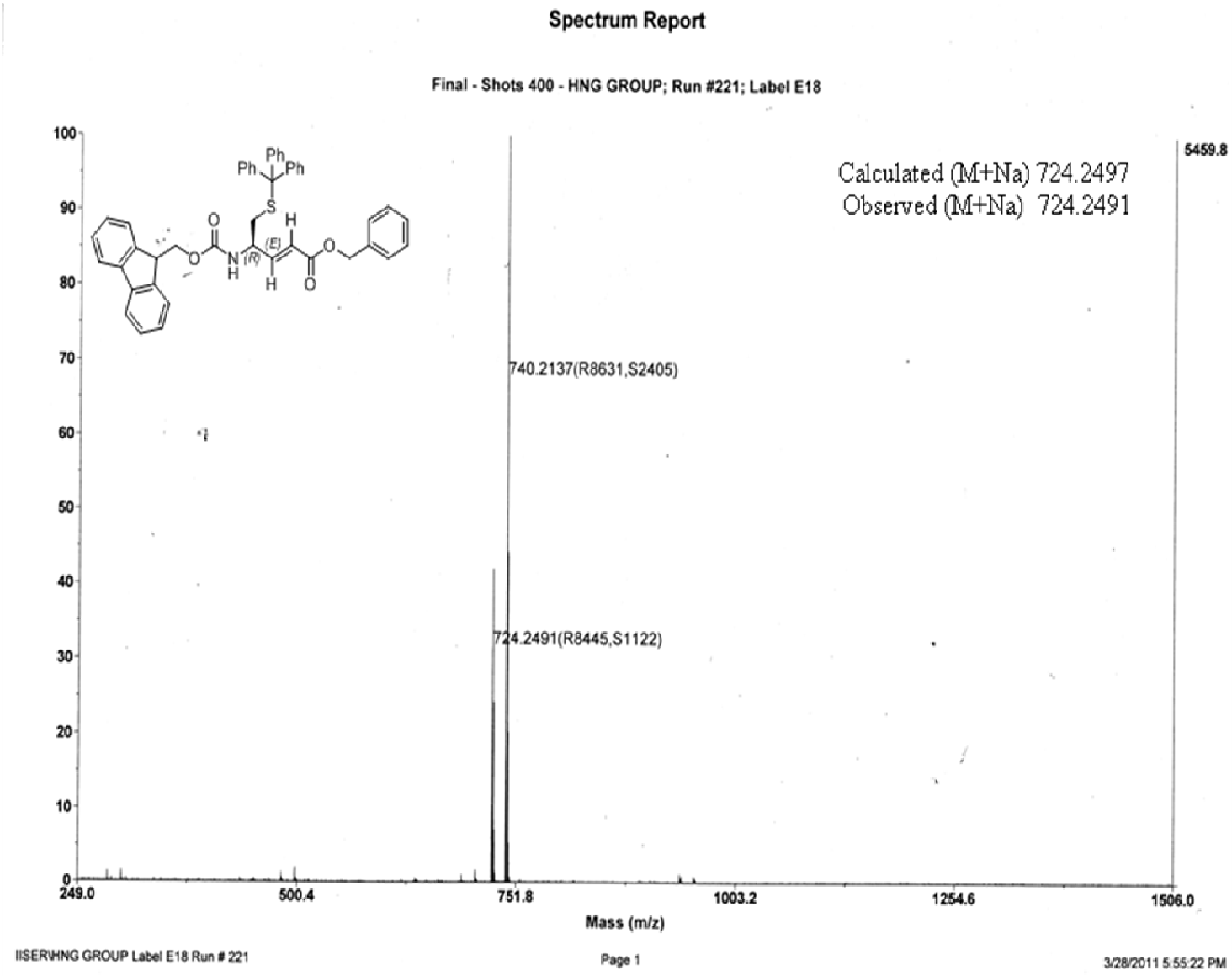
Spectrum Report

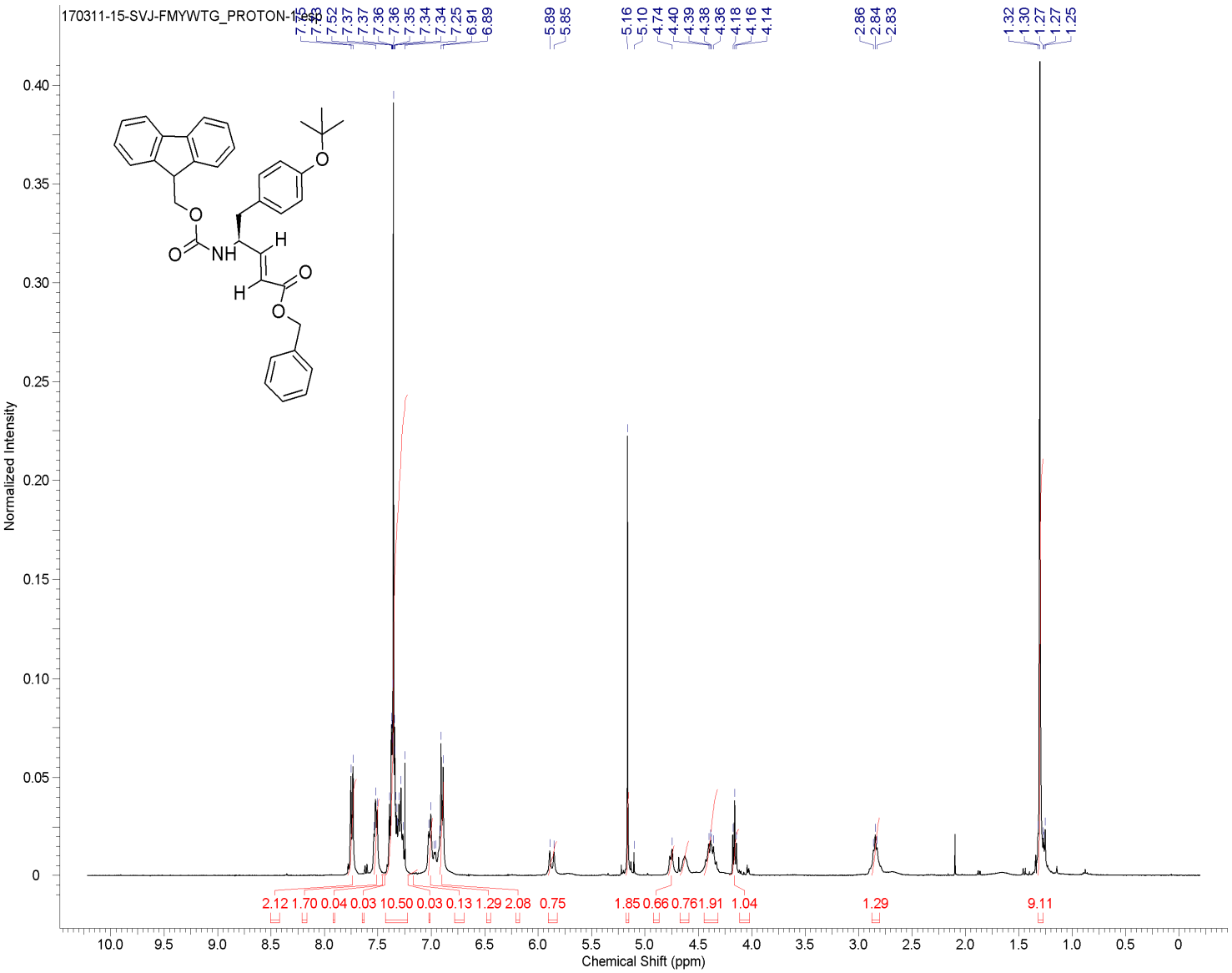
Final - Shots 400 - HNG GROUP; Run #221; Label E7

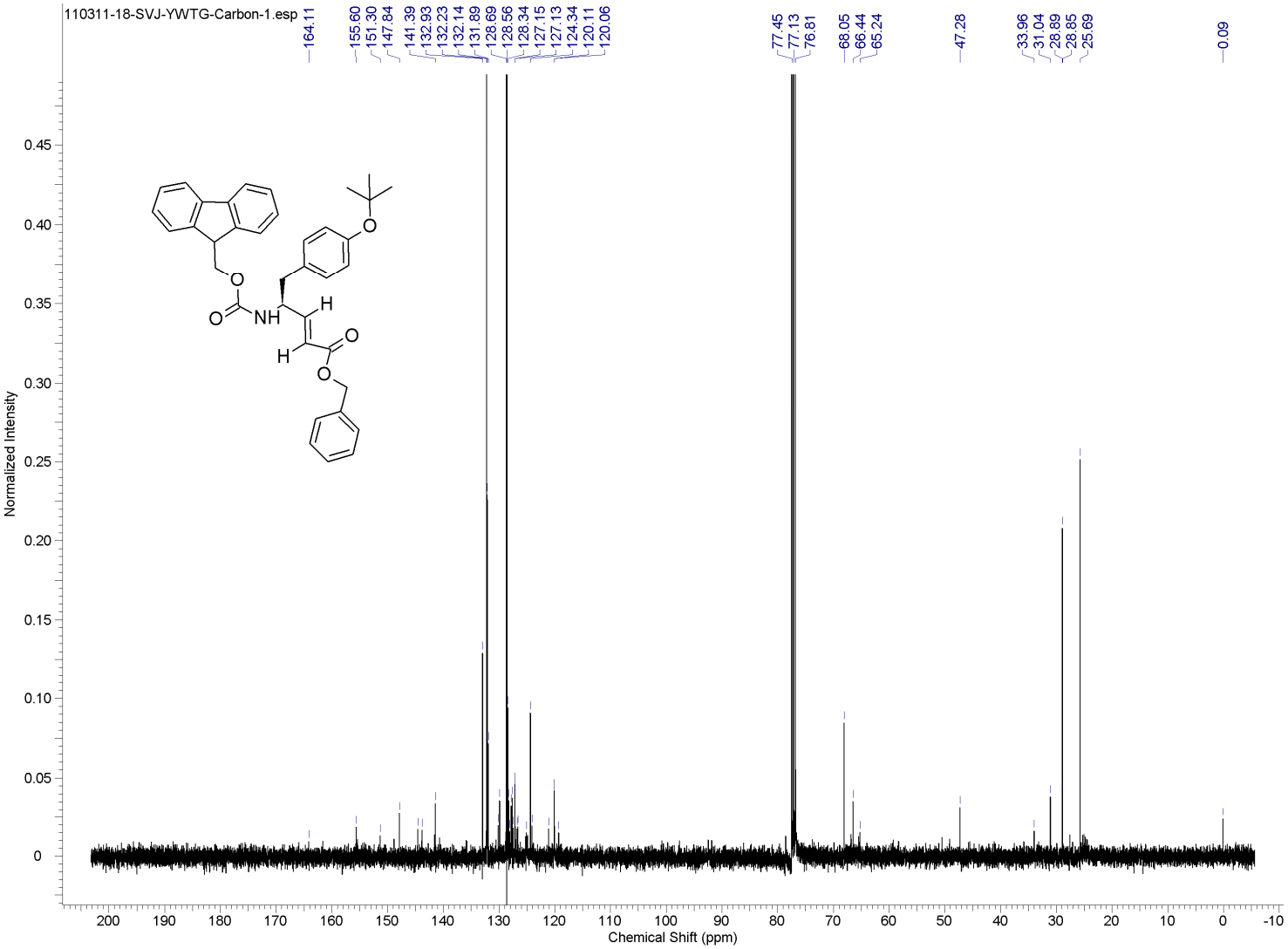



310111-05-SVJ-31011-CWTG_PROTON_1.esp
7.76
7.74
7.57
7.56
7.39
7.38
7.37
7.36
7.35
7.28
7.27
7.25
7.20
5.14
4.83
4.41
4.39
1.83
0.00
Ph
Ph
Ph
S
H
O
NH
H
O
O
Normalized Intensity
Chemical Shift (ppm)
28.08
0.52
0.64
1.63
0.50
1.61
1.10
2.35



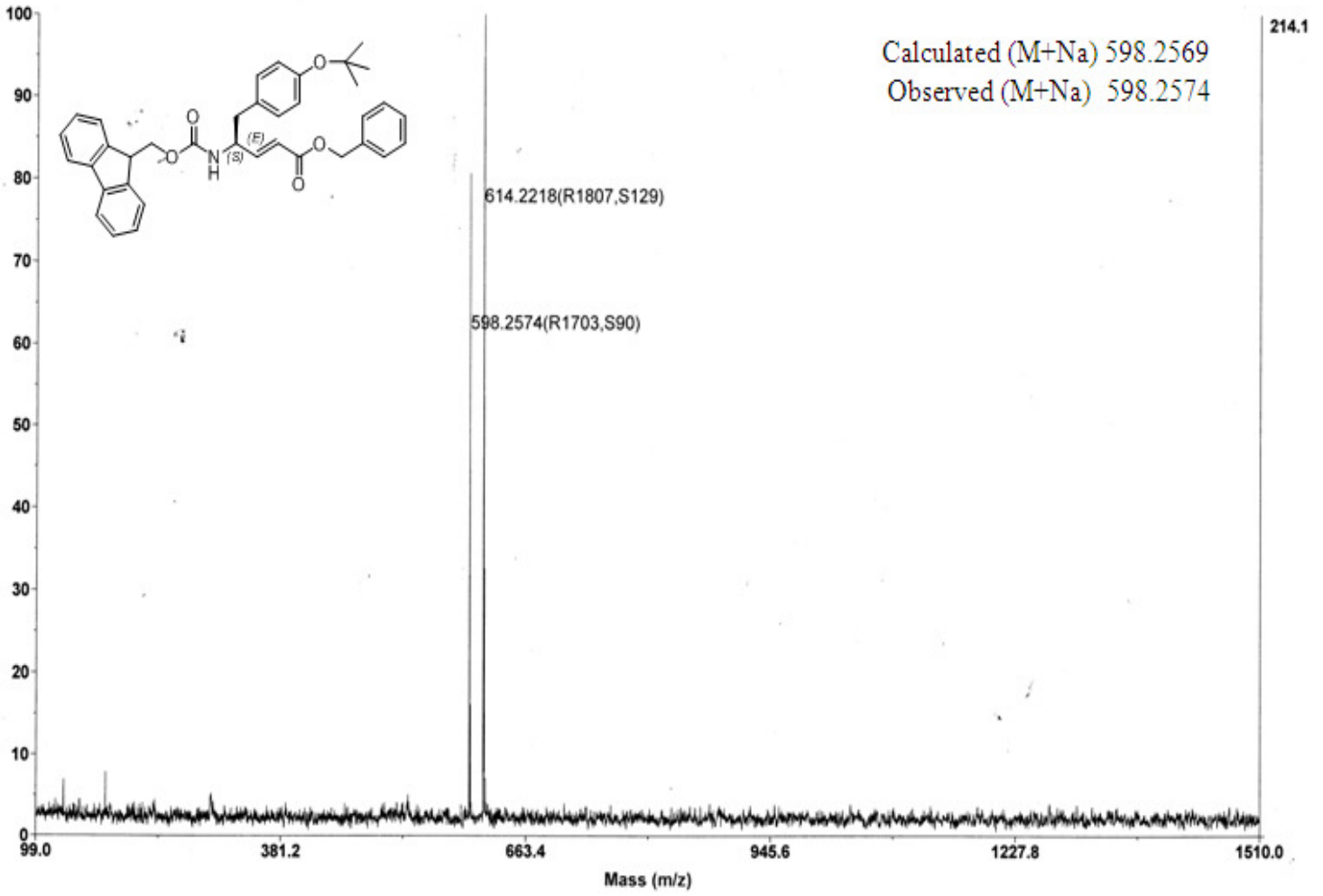



170311-15-SVJ-FMYWTG_PROTON-1.esp
7.76 7.73 7.52 7.37 7.37 7.36 7.36 7.35 7.34 7.34 7.25 6.91 6.89
5.89 5.85
5.16 5.10 4.74 4.40 4.39 4.38 4.36 4.18 4.16 4.14
2.86 2.84 2.83
1.32 1.30 1.27 1.27 1.25
Normalized Intensity
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
2.12 1.70 0.04 0.03 10.50 0.03 0.13 1.29 2.08 0.75
1.85 0.66 0.76 1.91 1.04
1.29
9.11
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)

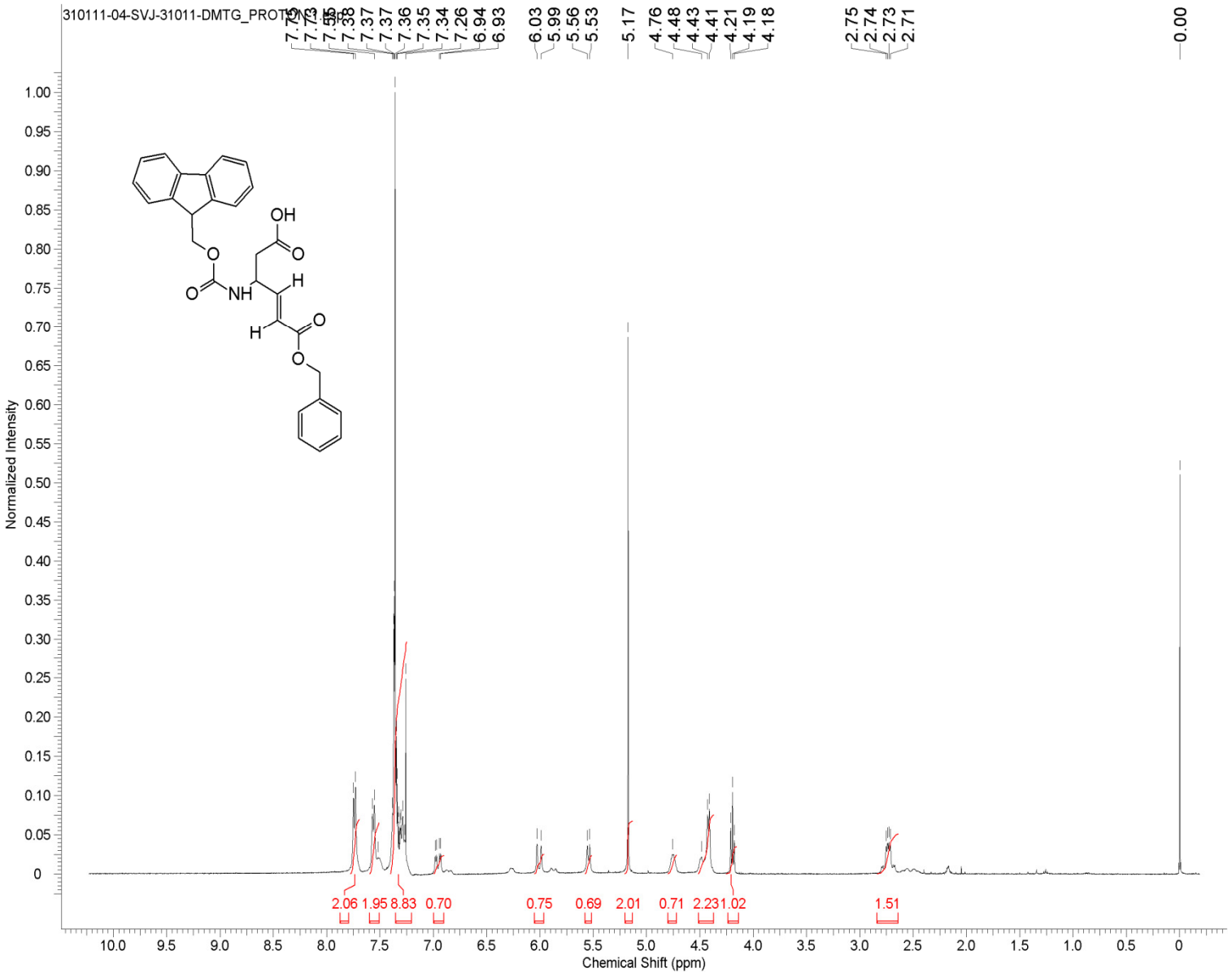


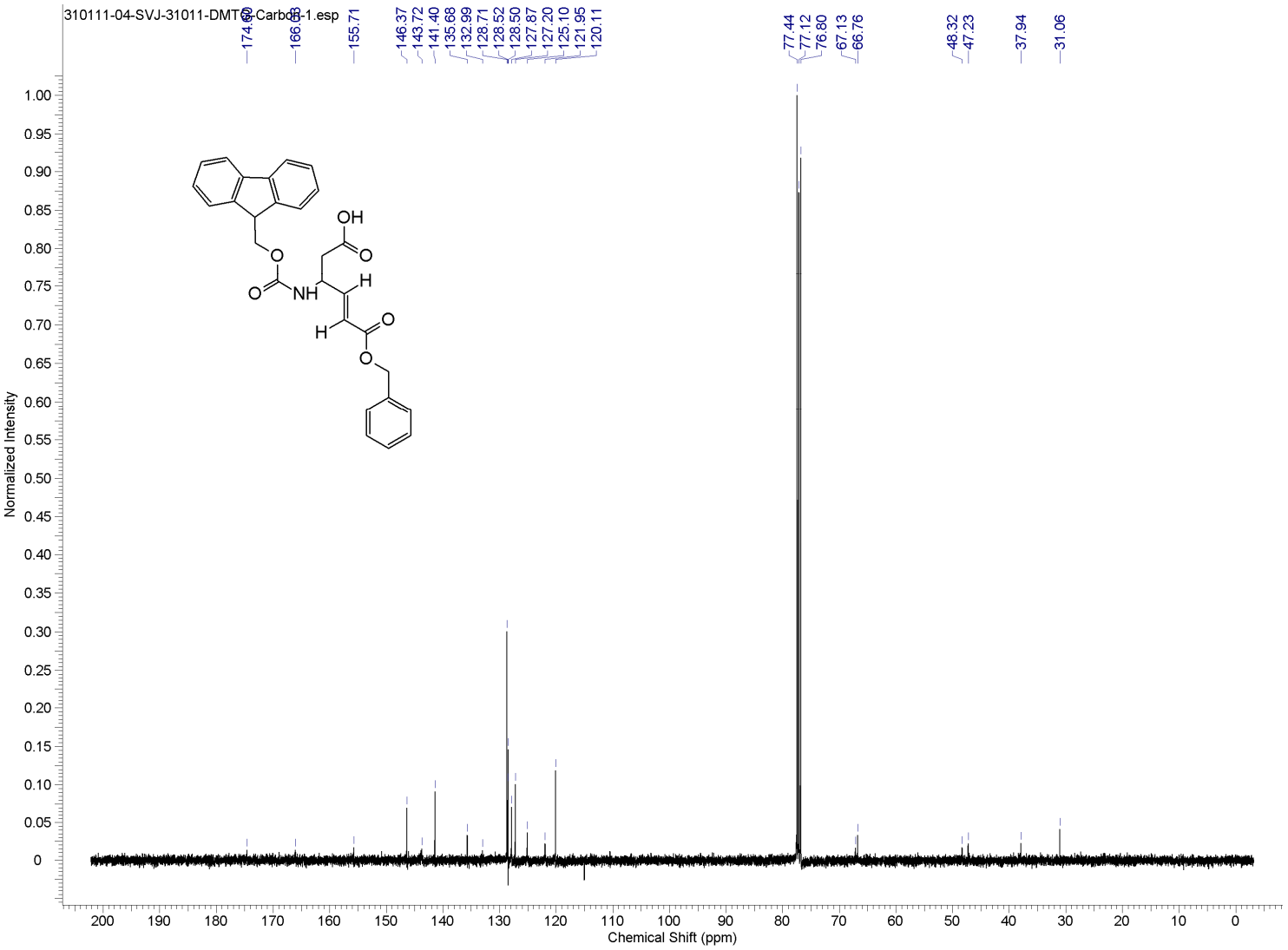
Spectrum Report

Final - Shots 800 - HNG GROUP; Run #266; Label P2



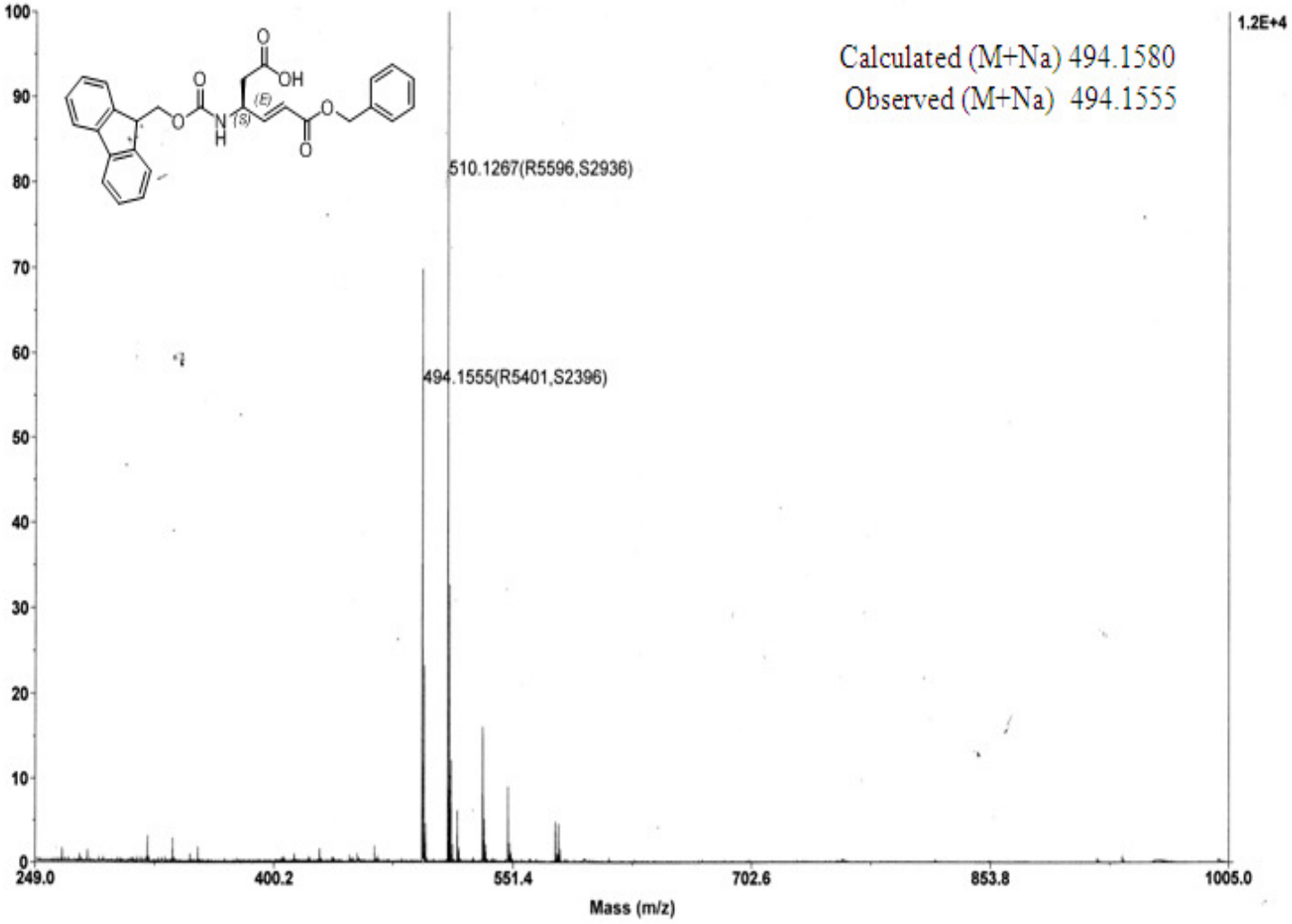
IISER\HNG GROUP Label P2 Run # 266 Page 1 3/28/2011 5:46:13 PM

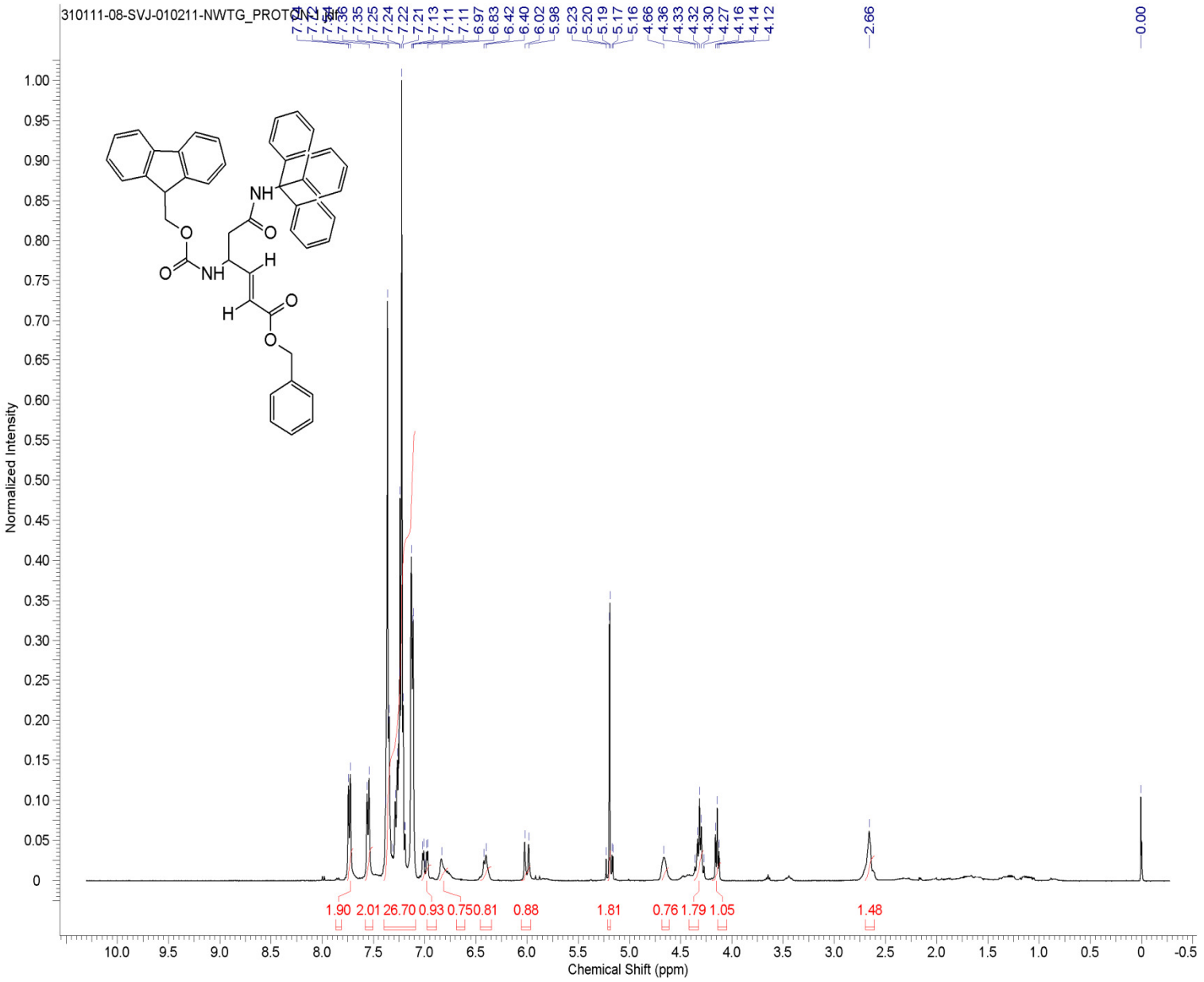


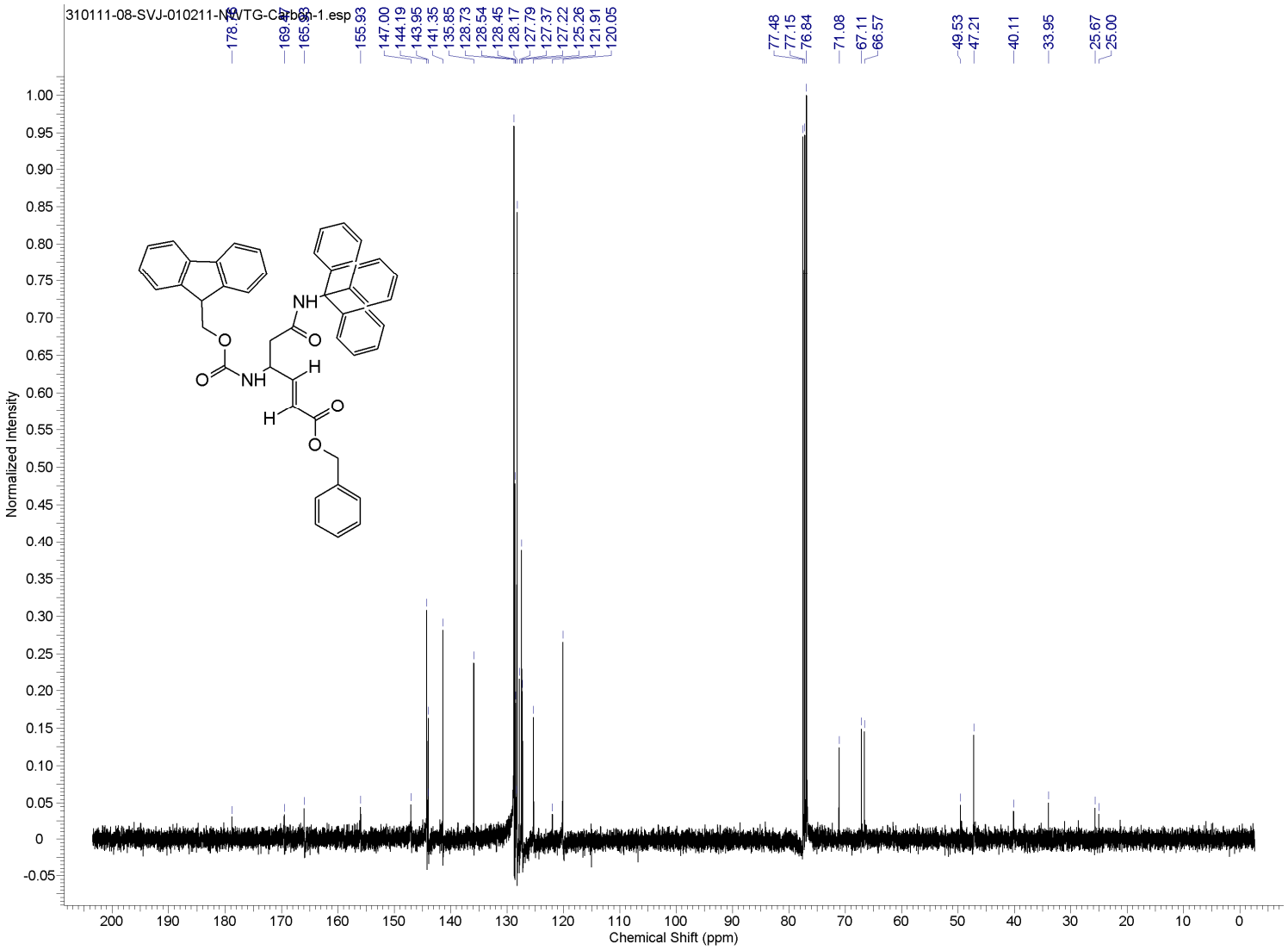


Spectrum Report

Final - Shots 400 - HNG GROUP; Run #221; Label I10

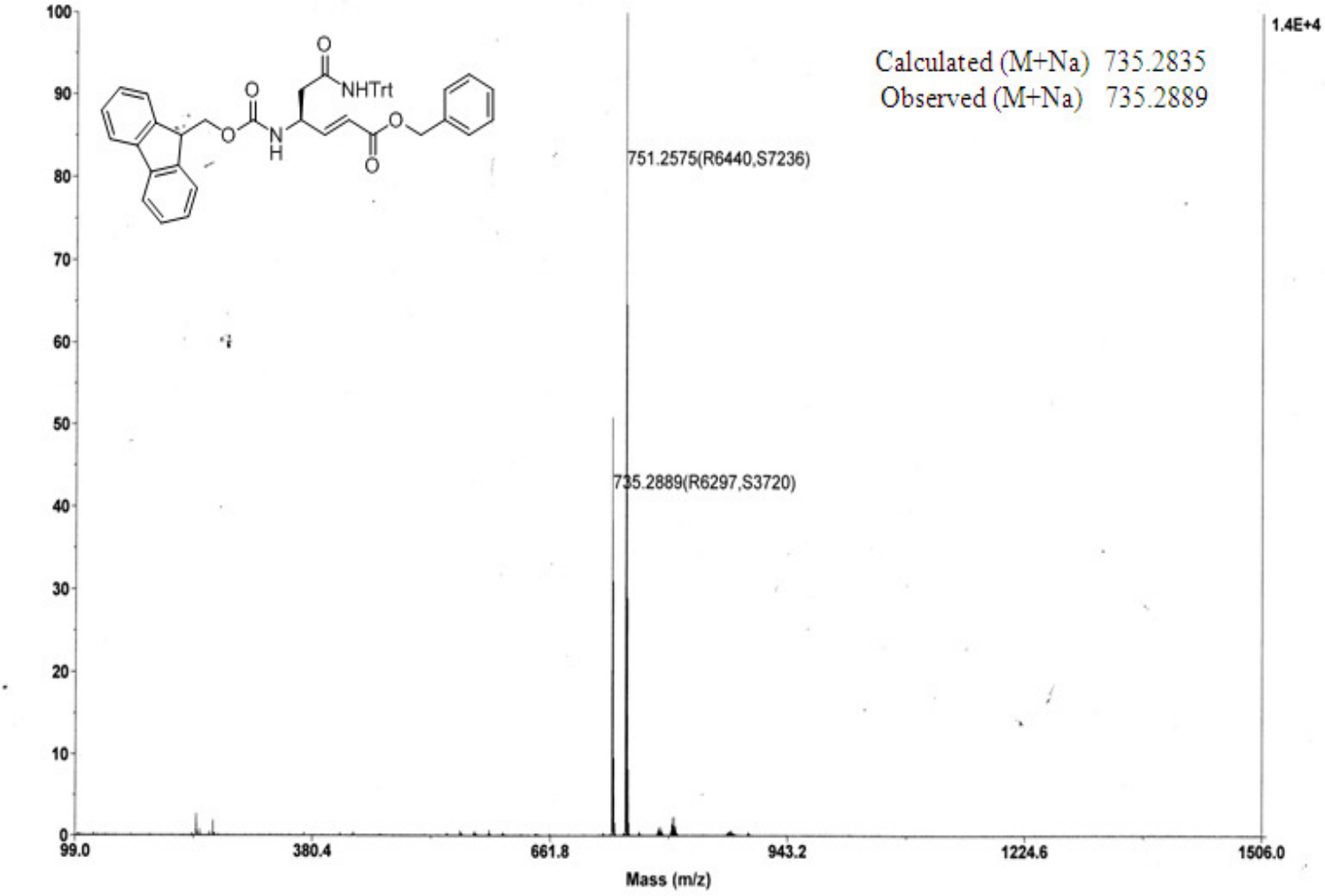


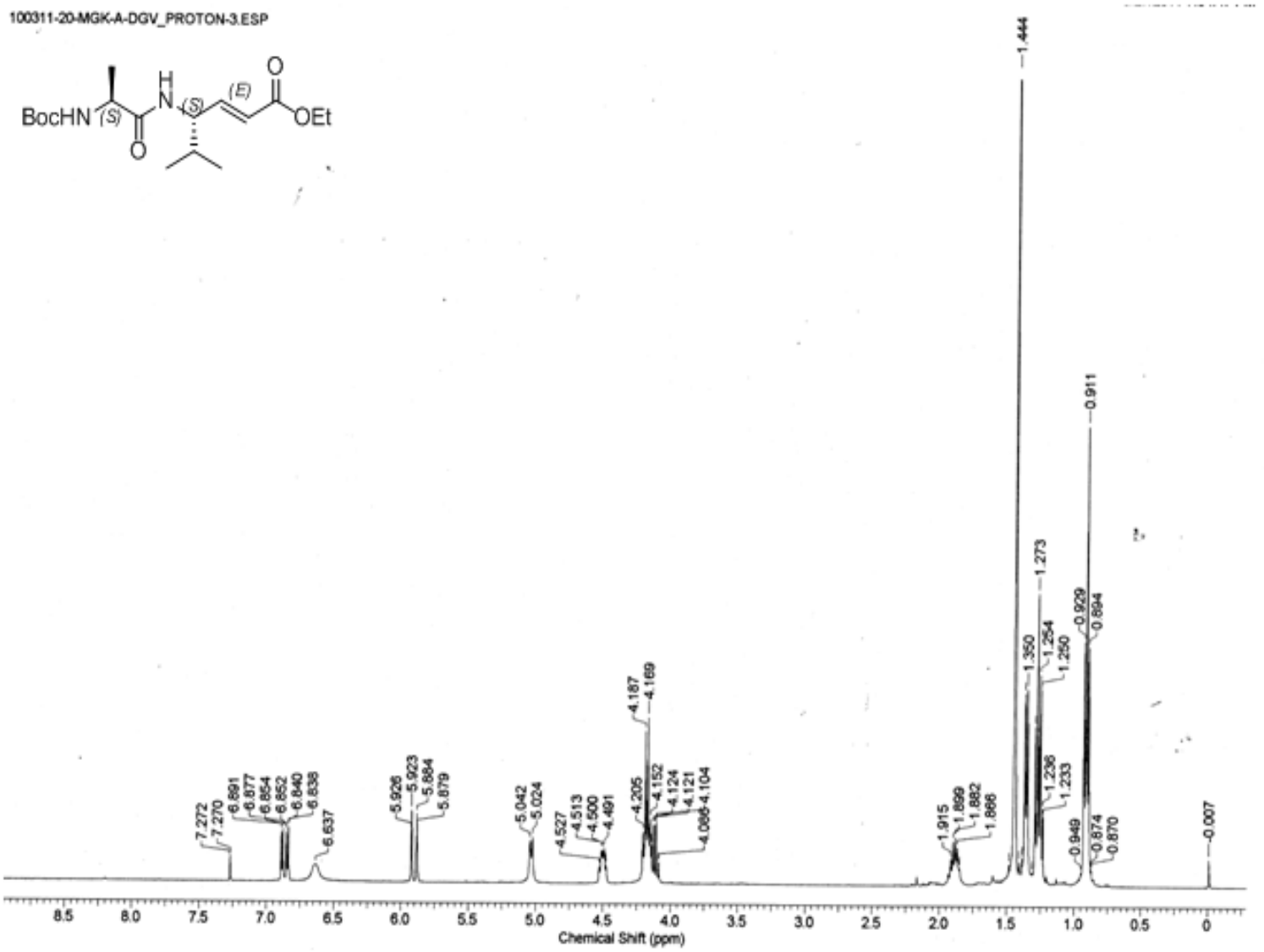


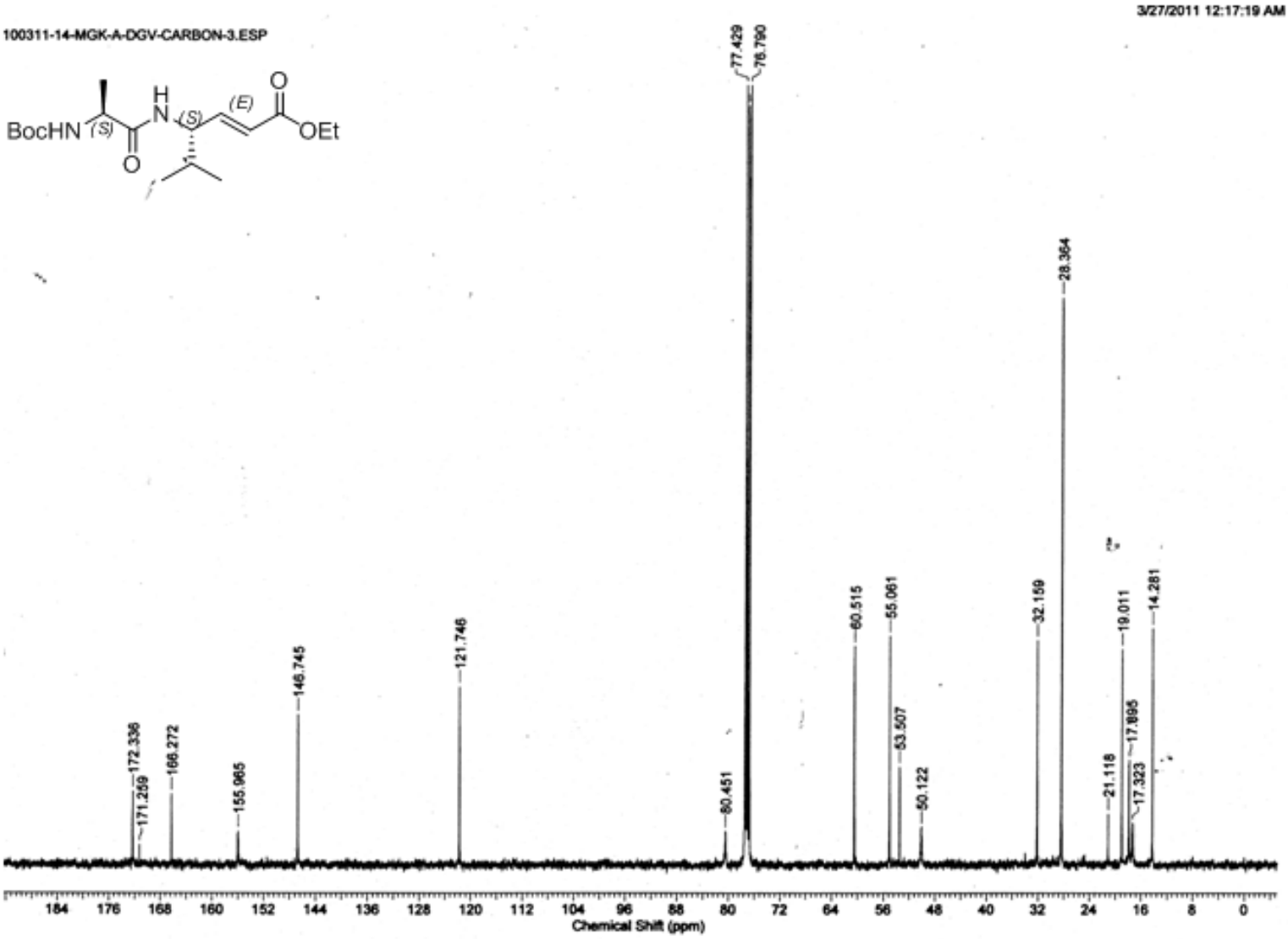


Spectrum Report

Final - Shots 400 - HNG GROUP; Run #222; Label A4

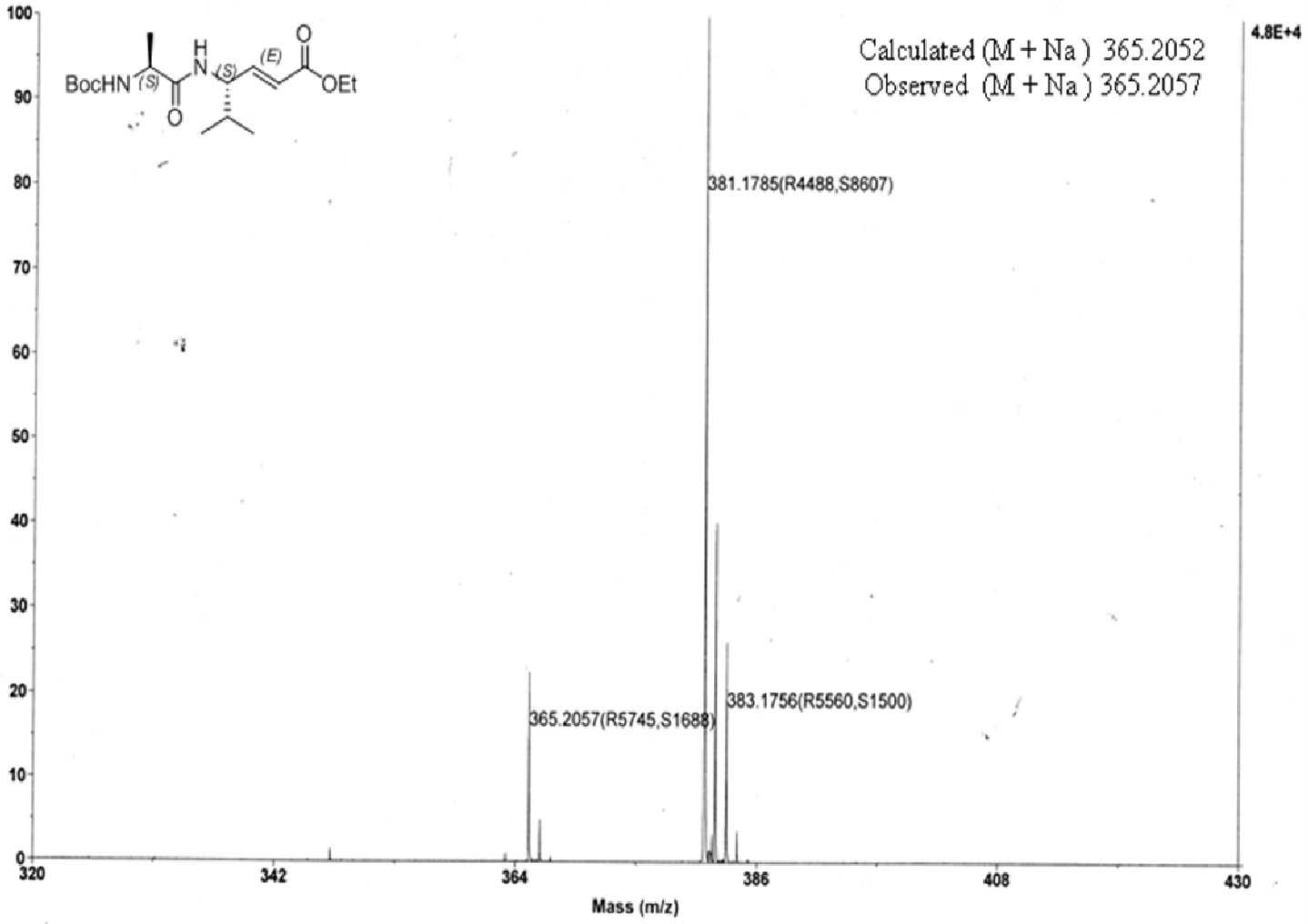


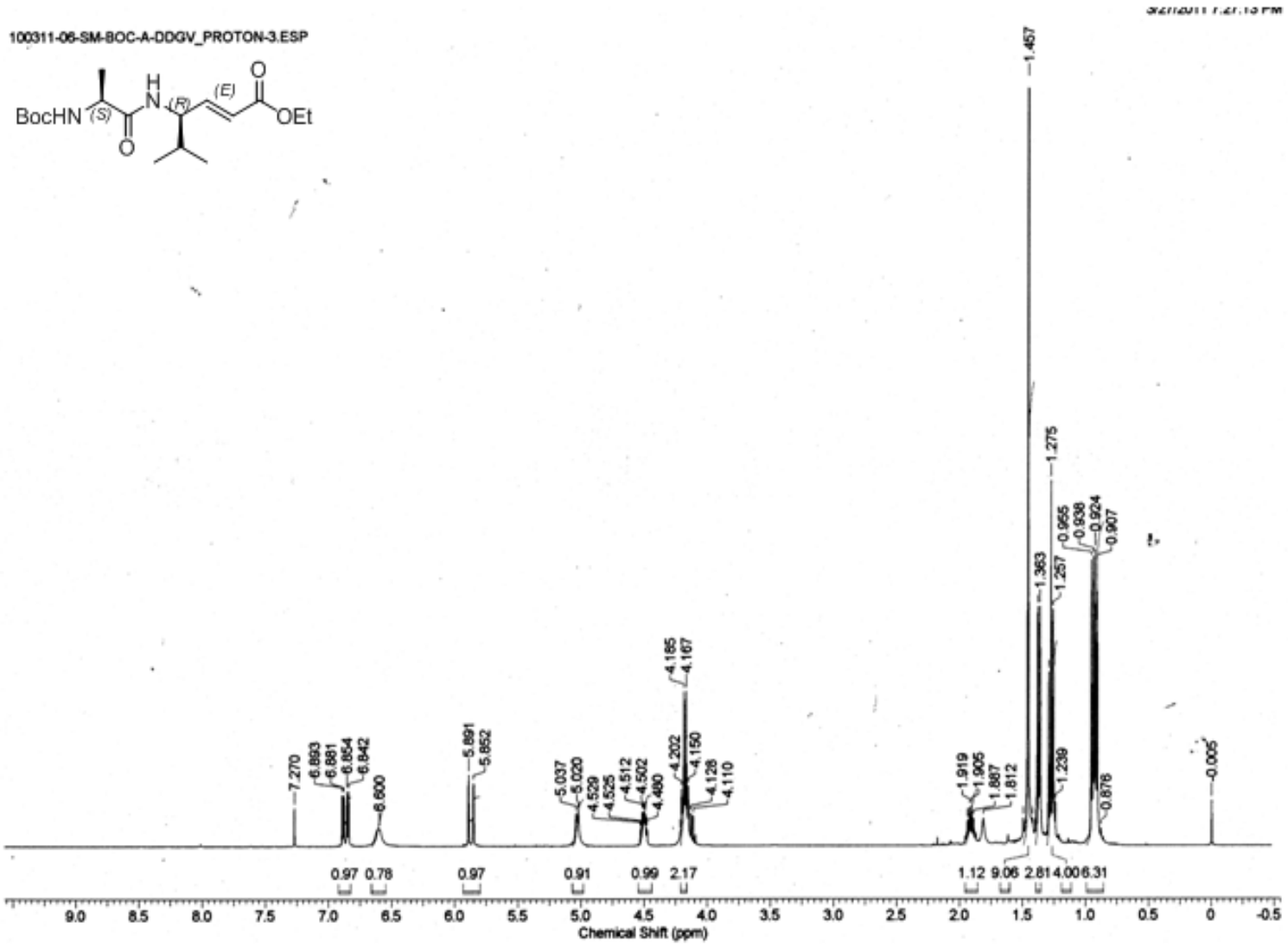


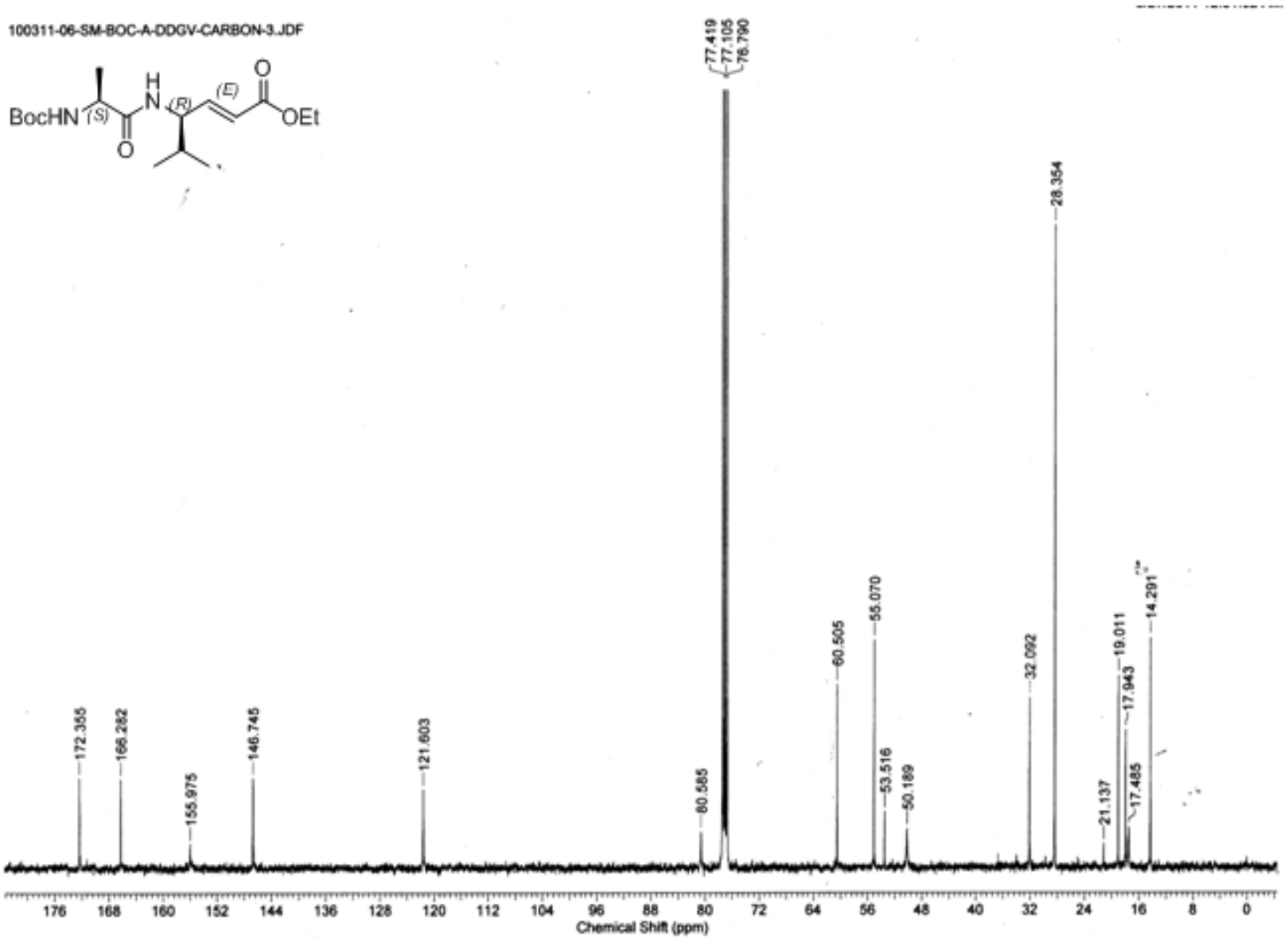


Spectrum Report

Final - Shots 320 - HNG GROUP; Run #273; Label G13

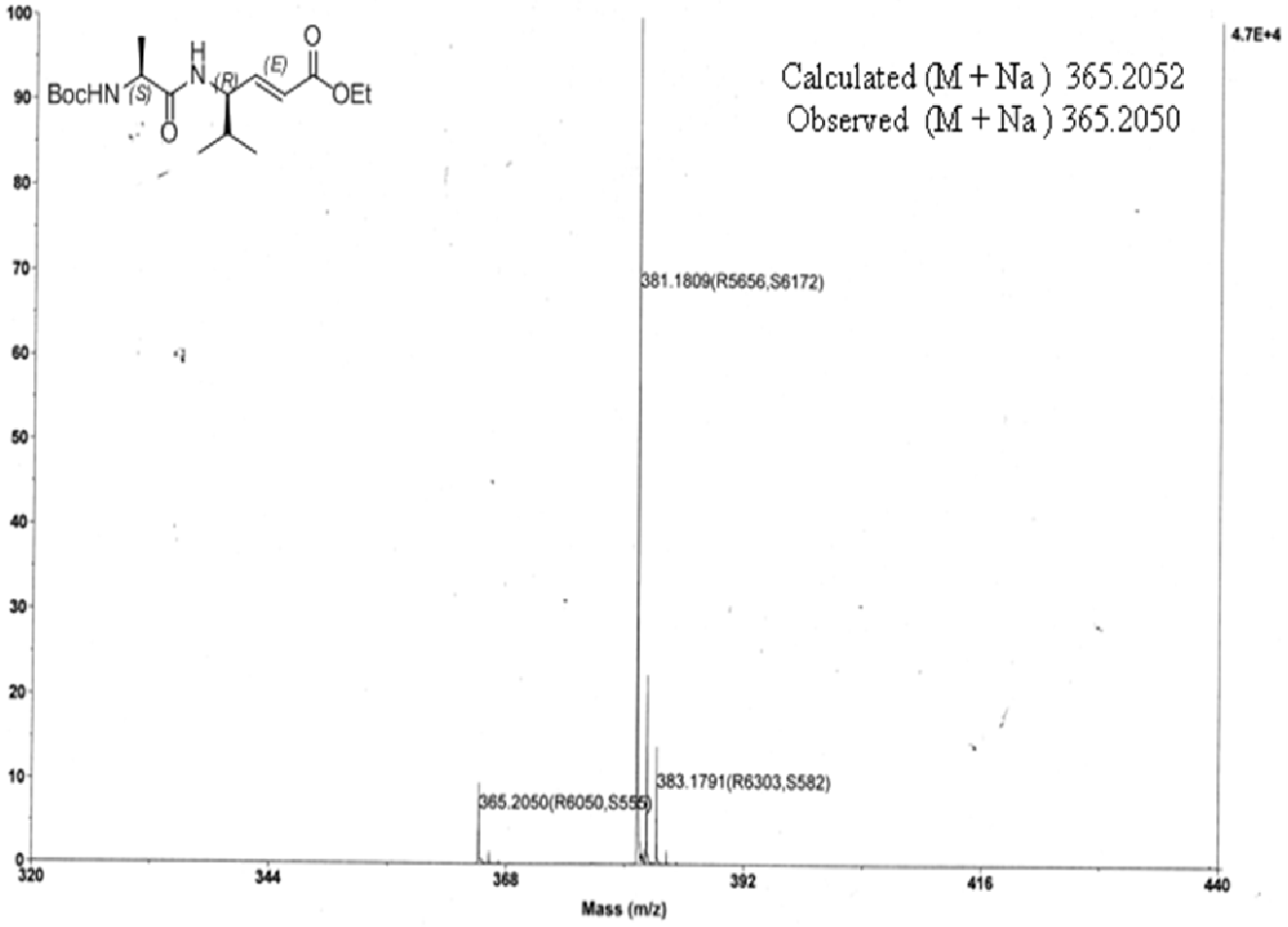


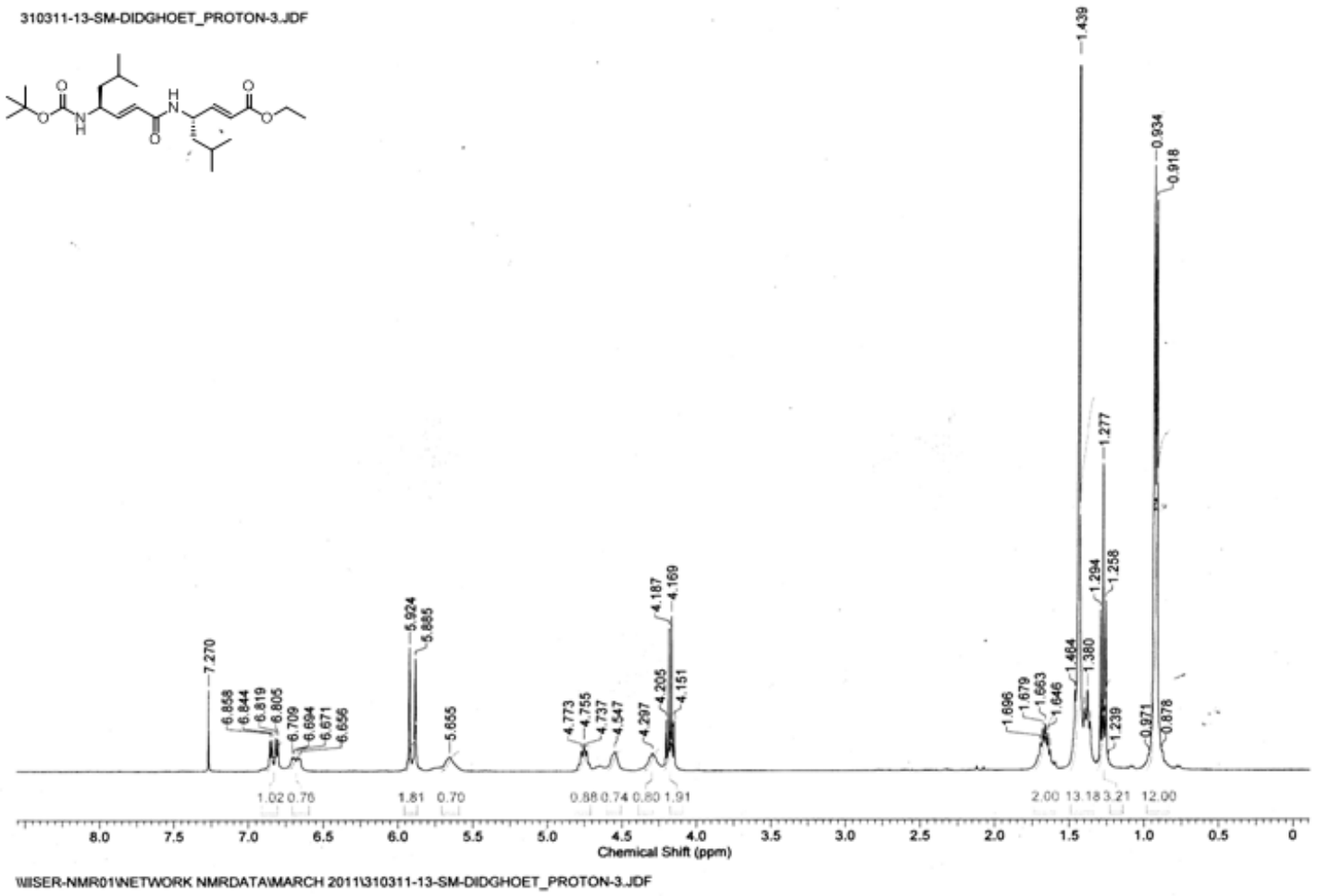


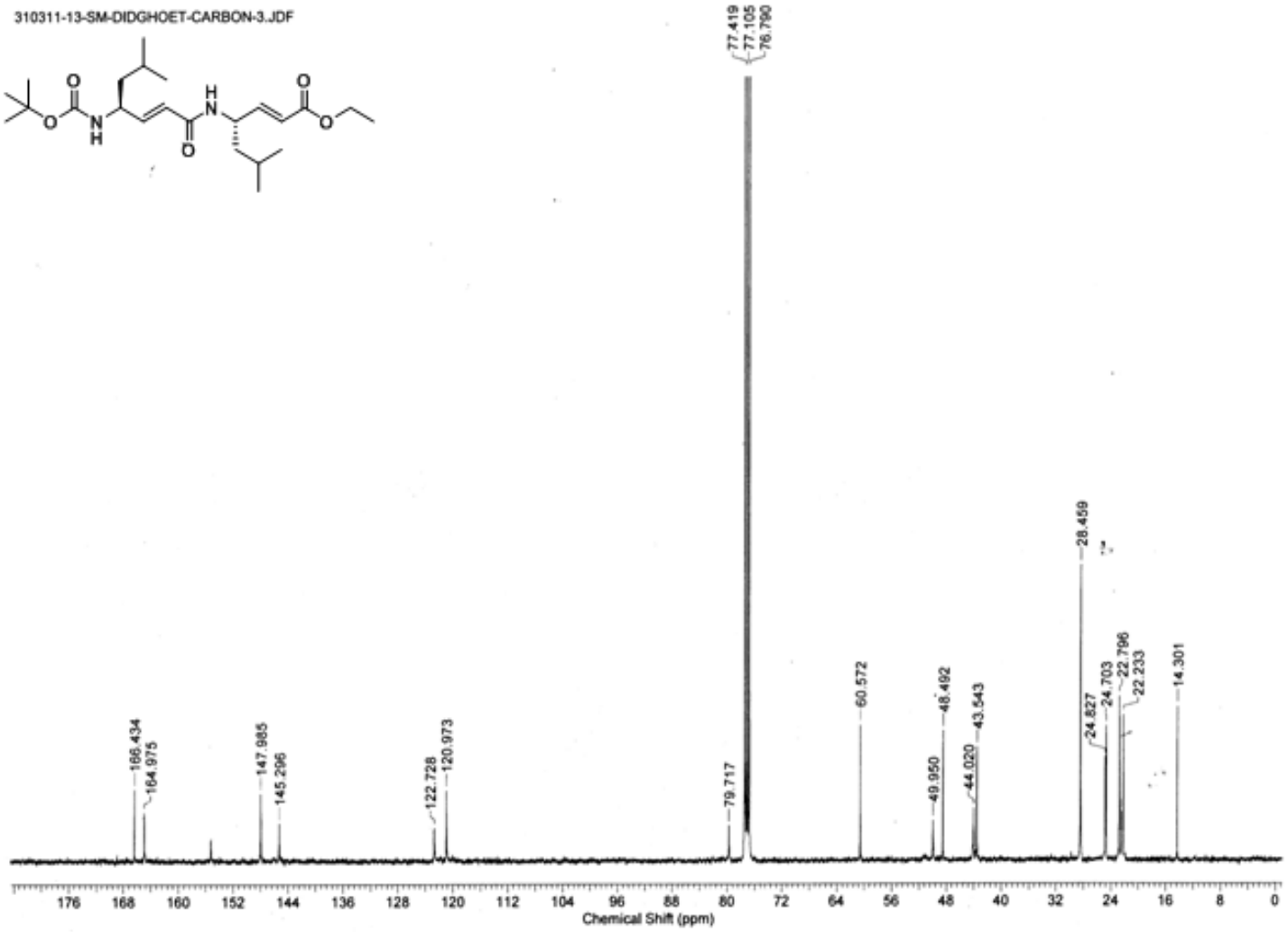


Spectrum Report

Final - Shots 320 - HNG GROUP; Run #273; Label G16



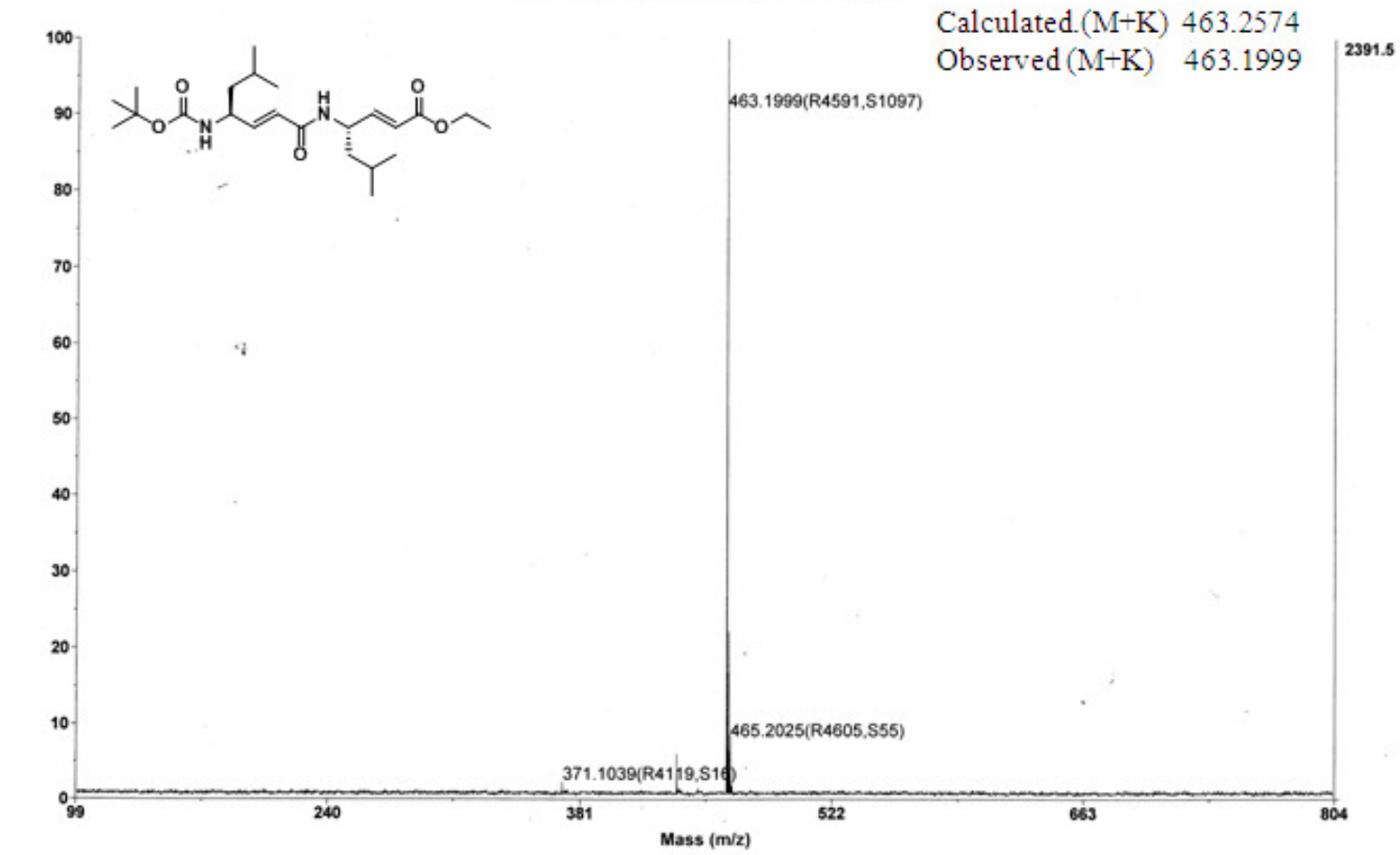




Spectrum Report

SM-1

Final - Shots 320 - IISER; Run #207; Label O12



iiser pune\IISER Label O12 Run # 207 Page 1 4/19/2011 6:18:37 PM