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# Supporting Information



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A Practical Solid Phase Synthesis of Glu7-Phalloidin and Entry into Fluorescent F-Actin Binding Reagents

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**General**. Fmoc-protected amino acids and 2-chlorotrityl chloride polystyrene resin were purchased from NovaBiochem and were used without further purification. All other reagents were purchased from Aldrich and used without further purification. Hexanes, ethyl acetate, dichloromethane, and methanol were purchased from EMD, N, N-dimethylformamide was purchased from Acros, and all were used without purification unless noted. Solvents were dried over molecular sieves under argon overnight. Thin layer chromatography (TLC) was performed on Fischer silica gel 60 $F_{254}$ (250µm). Column chromatography was performed using Acros silica gel (4nm pore size). 500MHz Varian NMR used to record 1D $^{1}H$ and $^{13}C$ spectra; additionally, a Protasis microflow probe was used to record 1D and 2D spectra for Glu$^{7}$-phalloidin and rhodamine-Glu$^{7}$-phalloidin. A Thermo LCQ Classic ESI-MS with an HP 1100 LC system and a Sedex evaporative light scattering detector were used to determine purity and identity of peptide intermediates. Peptides were purified on a Shimadzu LC-8A preparative HPLC system equipped with a Higgins Analytical C18 column, 250 x 20 mm.

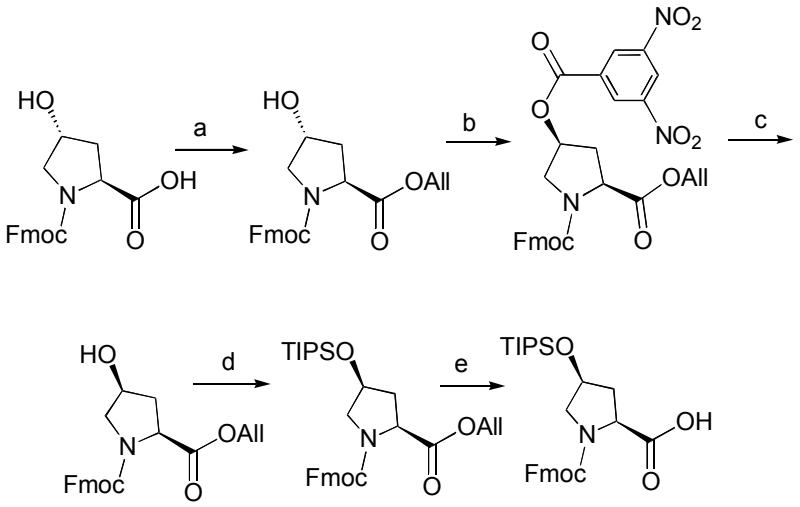



Figure S1. Synthesis of protected *cis*-hydroxyproline monomer. Reagents used for each step are a) neat allyl bromide, N,N-diispropylethylamine, reflux for 2 hours (95%) b) $PPh_3$, diisopropylazodicarboxylate (DIAD), 3,5-dinitrobezoic acid, 1:1 THF:toluene, 0°C to rt, overnight (90%) c) $NaN_3$ , 1:1 MeOH:THF, rt, 2 hrs (92%) d) TIPS-OTf, N-methylmorpholine (90%) e) $Pd(PPh_3)_4$, $PhSiH_3$ (80%).

**Fmoc-*trans*-Hyp-OAll ester** . In a 250 mL round bottomed flask equipped with a magnetic stir bar, Fmoc-trans-Hyp-OH (5 g, 14.1 mmol) was suspended in neat allyl bromide (61.6 mL, 707.4 mmol). To this suspension, N,N-diisopropylethylamine (DIPEA; 11.5 mL, 69.3 mmol) was added. The mixture was heated to reflux, and a small amount of DMF was added to aid dissolution. After two hours refluxing, the reaction was judged complete by TLC in 3% MeOH in dichloromethane. The reaction mixture was allowed to cool to room temperature, was diluted with ethyl acetate, and then washed with brine (50 mL x 3). The crude product was diluted slightly with 3% methanol in dichloromethane, and was purified by isocratic column chromatography in the same solvent system. 5.47 g pure product was recovered (98%). $^{1}H$-NMR ($CDCl_3$) $\delta$ 2.13 (p, 1H, $J$ = 6.2 Hz), 2.34 (m, 3H), 3.56 (d, 1H, $J$ = 11.5Hz), 3.75 (dd, 1H, $J$ = 4.5Hz, $J$ = 11.4Hz), 4.22 (d, 1H), 4.35 (m, 1H), 4.60 (d, 2H), 4.66 (d, 2H), 5.27 (ddd, 1H, $J$ = 13.9Hz, $J$ = 23.4Hz, $J$ = 29.4Hz), 5.88 (dddd, 1H, $J$ = 5.7Hz, $J$ = 10.9Hz, $J$ = 16.4Hz, $J$ = 22.9Hz), 7.31 (t, 2H, $J$ = 7.5 Hz), 7.40 (t, 2H, $J$ = 7.4 Hz) , 7.61 (m, 2H), 7.77 (t, 2H, $J$ = 4.7 Hz). $^{13}C$-NMR ($CDCl_3$) $\delta$ 172.3, 154.9, 144.2, 143.7, 141.4, 131.6, 127.2, 125.1, 120.1, 118.7, 69.44, 67.7,

66.0, 57.8, 54.8, 47.3, 38.5. ESI-MS: calculated mass for $C_{23}H_{23}NO_5$+H: 394.4. observed m/z: 394.0.

**Fmoc-*cis*-Hyp-OAll 3,5-dinitrobenzoate ester.** Fmoc-*trans*-Hyp-OAll (4.53 g, 11.5 mmol) was dissolved in 100 mL 1:1 dry toluene/dry THF, transferred to a flame-dried 250mL round bottomed flask equipped with a stir bar and septum, and cooled to 0˚C under positive argon pressure. 3,5-dinitrobenzoic acid (4.89 g, 23.0 mmol) and triphenylphosphine (6.04 g, 23.0 mmol) were added to the reaction mixture and stirred until dissolved. DIAD (4.54 mL, 23.0 mmol) was added in one portion, and the reaction mixture was allowed to stir overnight under positive argon pressure, slowly warming to room temperature. After 22 h the reaction was judged complete by TLC in 3:2 hexanes/ethyl acetate. The reaction mixture was evaporated, and the residue taken up into ethyl acetate, washed with 10% $NaHCO_3$ (30 mL x 1), brine (30 mL x 2), dried over $MgSO_4$, filtered, and evaporated onto silica. The product was isolated by column chromatography using 5:2 to 3:2 hexanes/ethyl acetate, yielding 5.93 g of yellow foam (88%). $^1$H-NMR ($CDCl_3$) δ 2.55 (m, 1H), 2.65 (m, 1H), 3.90 (m, 2H), 4.23 (dt, 1H, *J* = 28.8 Hz, *J* = 6.8 Hz), 4.48-4.74 (m, 3H), 4.83 (d, 1H, *J* = 5.9Hz), 5.15 (t, 1H, *J* = 10.7Hz), 5.30 (t, 1H, *J* = 20.0Hz), 5.68 (d, 1H, *J* = 19.5Hz), 5.68 (d, 1H), 5.88 (dddd, 1H, *J* = 5.8Hz, *J* = 10.8Hz, *J* = 22.9Hz, *J* = 27.6Hz), 7.31 (m, 2H), 7.40 (t, 2H, *J* = 7.3Hz), 7.58 (m, 2H), 7.76 (d, 2H, *J* = 7.3Hz), 9.09 (d, 2H, *J* = 15.6Hz), 9.23 (m, 1H). $^{13}$C-NMR ($CDCl_3$) δ 171.3, 163.0, 154.9, 148.8, 144.1, 131.6, 130.9, 129.7, 127.8, 127.2, 125.0, 122.7, 120.1, 118.7, 75.5, 67.9, 66.2, 58.1, 53.2, 47.3, 35.5. ESI-MS: mass calculated for $C_{30}H_{25}N_3O_{10}$+H: 588.5; m/z observed= 588.0.

**Fmoc-*cis*-Hyp-OAll.** Fmoc-*cis*-Hyp-OAll 3,5-dinitrobenzoate ester (5.6 g, 9.5 mmol) was dissolved in 250 mL 1/1 MeOH:THF in a 500 mL round bottom flask. Sodium azide (4.96 g, 76.3 mmol) was added, and the reaction was stirred at room temperature for 2 h at which point the reaction was judged complete by TLC in 3/2 hexanes:ethyl acetate. The reaction mixture was evaporated onto silica and the product was isolated by column chromatography using 3:2 hexanes/ethyl acetate, yielding 3.8 g of a clear, colorless oil (85%). $^1$H-NMR ($CDCl_3$) δ 2.19 (t, 1H, *J* = 14.8Hz), 2.38 (m, 1H), 3.65 (t, 1H), 3.75 (m, 1H), 4.1-4.6 (m, 6H), 4.71 (d, 2H), 5.30 (m, 2H), 5.90 (dddd, 1H, *J* = 5.8Hz, *J* = 10.8Hz,
*J* = 22.9Hz, *J* = 27.6Hz), 7.32 (m, 2H), 7.42 (t, 2H, *J* = 7.4Hz), 7.58 (m, 2H), 7.78 (d, 2H, *J* = 7.5Hz). $^{13}$C-NMR ($CDCl_3$) δ 171.3, 154.8, 144.2, 143.8, 141.3, 131.9, 131.8, 127.7, 127.0, 125.3, 125.1, 119.9, 118.6, 118.3, 70.8, 70.0, 67.5, 65.8, 57.9, 57.8, 55.5, 55.1, 47.3, 47.2, 40.0, 39.0, 31.6, 22.7, 17.9, 14.1, 12.0. ESI-MS: calculated mass for $C_{23}H_{23}NO_5$+H: 394.4, observed m/z: 394.2.

**Fmoc-*cis*-Hyp(OTIPS)-OAll ester**. Fmoc-cis-Hyp-OAll (60 mg, 0.15 mmol) was evaporated from toluene three times and taken up into 2 mL dry DCM in a flame-dried 25mL pear-shaped flask with stir bar. N-methylmorpholine (117 µL, 1.1 mmol) and triisopropylsilyl triflate (0.16 mL, 0.6 mmol) were added, and the reaction mixture was stirred under positive argon pressure. After 45 minutes, the reaction was judged complete by TLC in 3:2 hexanes/ethyl acetate. The reaction mixture was diluted with 50 mL ethyl acetate and the organic layer was washed with 0.1 M HCl (100 mL x 1) followed by brine (100 mL x 2). The organic layer was dried over $MgSO_4$, filtered, and evaporated to a yellow goo. The mixture was separated by column chromatography in 3:2 hexanes/ethyl acetate, yielding 70mg of pure yellow goo (84%). $^1$H-NMR ($CDCl_3$) δ 7.76 (t, 2H, *J* = 7.7Hz), 7.59 (m, 2H), 7.40 (q, 2H, *J* = 7.1Hz), 7.30 (m, 2H), 5.90 (dddd, 1H, *J* = 5.8Hz, *J* = 10.8Hz, *J* = 22.9Hz, *J* = 27.6Hz), 5.30 (dd, 1H, *J* = 17.2Hz, *J* = 23.7Hz), 5.19 (t, 1H, *J* = 9.6Hz), 4.10-4.68 (m, 6H), 3.73 (m, 1H), 3.51 (d, 1H, *J* = 10.9Hz), 2.38 (tdd, 1H, *J* = 4.6Hz, *J* = 9.0Hz, *J* = 13.3Hz), 2.26 (t, 1H, *J* = 13.0Hz), 1.06 (d, 21H). $^{13}$C-NMR ($CDCl_3$) δ 171.3, 154.8, 144.2, 141.3, 131.9, 127.7, 125.3, 120.0, 118.6, 70.8, 70.0, 67.5, 65.8, 57.9, 55.5, 47.3, 40.1, 38.9, 31.6, 22.7, 17.9, 14.2, 11.9. ESI-MS: calculated mass for $C_{32}H_{43}NO_5Si$+H: 550.8;

observed m/z: 550.0, 572.2 ($M+Na^+$).

**Fmoc-cis-Hyp(OTIPS)-OH**. Fmoc-cis-Hyp(OTIPS)-OAll (1.48 g, 2.7 mmol) was dissolved in 33 mL of THF in a 50mL pear-shaped flask, capped with a septum, and argon was bubbled through this solution for 30 min. Phenylsilane (1.33 mL, 10.8 mmol) and $Pd(PPh_3)_4$ (0.31 g, 0.27 mmol) were added, and the solution was agitated with bubbling argon for 1.5 hours. The reaction was judged complete by TLC in 3:2 hexanes/ethyl acetate. $H_2O$ (20 mL) was added to quench the reaction, and then ethyl acetate (20 mL) was added. The organic layer was washed with 0.1M HCl (1 x 50 mL) followed by brine (1 x 50 mL). The organic layer was then dried over $MgSO_4$, filtered, and evaporated onto silica, and purified by 2 successive columns, first 3:2 hexanes/ethyl acetate and then diethyl ether. The final product was isolated as 1.1 g of clear light brown oil (80%). $^1$H-NMR (CDCl3) $\delta$ 7.76 (bs, 2H), 7.59 (bs, 2H), 7.40 (bs, 2H), 7.31 (t, 2H, *J* = 7.5Hz), 4.20-4.55 (m, 5H), 3.43-3.70 (m, 2H), 2.28-2.41 (m, 2H), 7.08 (s, 21H). $^{13}$C-NMR ($CDCl_3$) $\delta$ 176.7, 176.3, 171.5, 163.4, 155.3, 154.8, 144.2, 143.9, 141.4, 127.8, 127.2, 125.3, 125.2, 120.0, 70.8, 70.1, 67.9, 67.7, 60.6, 58.0, 57.7, 55.4, 54.9, 53.6, 47.3, 39.9, 38.7, 21.2, 18.0, 14.3, 12.0. ESI-MS: mass calculated for $C_{29}H_{39}NO_5Si$+H: 510.1; m/z observed: 509.9 (M+H), 532.2($M+Na^+$), 554.3($M+2Na^+$), 1063.3($2M+2Na^+$), 1085.4($2M+3Na^+$).

**Solid Phase Peptide Synthesis Methods:**

**Note about LCMS data collection:** To obtain LCMS data for each step of SPPS, ~5mg resin was treated with 1% TFA/DCM for 20 minutes. The suspension was then filtered, and the beads were washed 3 x 200 μL 1%TFA/DCM. The combined filtrate was evaporated down, and then re-dissolved in 100 μL of either acetonitrile or methanol to make up the LCMS sample. The cleavage conditions also remove some of the side chain protecting groups, dependant on the peptide's sequence. Any spectrum in which the only products detected were the desired peptide in various states of protection were declared pure.

**Loading of Resin**. The 2-chlorotrityl chloride resin and Fmoc-Glu-OAll were stored under vacuum over $P_2O_5$. 200 mg of dried resin were placed in a flame-dried 50 mL pear-shaped flask. Fmoc-Glu-OAll (8.3 mg, 0.02 mmol) was dissolved in 3 mL dry dichloromethane. DIPEA (0.015 mL, 0.80 mmol) was added to the solution, and this mixture was added to the resin. The reaction was stirred at room temperature under argon for 1.5h. The resin was transferred to a synthesis vial, drained, and washed 3 x 3 mL 17:2:1 DCM/MeOH/DIPEA to cap unreacted positions. The resin was then rinsed with DMF x 3, DCM x 3, and dried under vacuum dessication. The amino acid loading of the resin was determined by the Fmoc deprotection method (absorbance measured at 290nm, $\varepsilon$ = 8300). The loading determined by this method was found to be 0.11 mmol/g.

**Standard Peptide Coupling Procedure**. The dried resin was swelled in DMF in a fritted reaction vial equipped with stopcock for 30 minutes. The resin was drained and treated with a solution of 20% piperidine in DMF for 20 minutes to remove the N-terminal Fmoc group. The resin was then drained and rinsed with DMF x 3, DCM x 3, DMF x 3. Next, a solution amino acid (5 eq. relative to loading), HBTU (4.9 eq. relative to loading), and DIPEA (10 eq. relative to loading) in minimum DMF was added to the resin. The reaction vial was capped, and the mixture was agitated until the reaction was judged complete by the bromophenol blue test; typical reaction times varied from 40-60 minutes. After the reaction was judged complete, the vial was drained and the beads were washed with DMF x 3, DCM x 3, DMF x 3. The final sequence was Fmoc-Ala-D-Thr(tBu)-Cys(Trt)-*cis*-Hyp(OTIPS)-Ala-Trp-Glu-OAll.

**Deallylation of linear peptide**. 200 mg resin was loaded into a fritted tube and attached to an Ar manifold to allow bubbling of Ar through the reaction. The resin was taken up in 20 mL 37:2:1 chloroform/ acetic acid/N-methylmorpholine. Argon was bubbled through the solution to degass the solvent. After 45 minutes, 46.2 mg $Pd(PPh_3)_4$ (2 eq.) was added to the solution, and the reaction bubbled slowly with argon for 3 hours at room temperature. After 3h the resin was drained and rinsed thoroughly with DMF x 3. Next the resin was washed with a solution of 0.05% sodium diethyldithiocarbamate and 5% DIPEA in DMF to remove residual palladium. Then the resin was washed with 3 x DCM, and allowed to dry under air. The beads were then swelled in DMF, and treated with 1% DBU in DMF for 10 minutes with agitation. Beads were then rinsed 3 x DMF, 3 x DCM, and dried under flowing air. The peptide sequence at this stage is NH2-Ala-D-Thr(tBu)-Cys-*cis*-Hyp(OTIPS)-Ala-Trp-Glu-OH. ESI-MS: calculated mass for $C_{57}H_{70}N_8O_{12}S$+H (M+H-TIPS): m/z = 1091.5; observed mass: m/z = 1091.5. Calculated mass for $C_{66}H_{90}N_8O_{12}SSi$+H (M+H): 1247.6 observed m/z = 1247.8.

**Backbone cyclization.** The Fmoc-deprotected resin was swelled in dry DMF for 30 minutes. DIPEA (4 eq) and diphenylphosphoryl azide (DPPA, 2 eq) were taken up in dry DMF, and the solution was added to the drained resin. The reaction mixture was agitated overnight at 8°C, then drained and rinsed with DMF x 3, DCM x 3. ESI-MS: calculated mass for $C_{66}H_{88}N_8O_{11}SSi$+H: m/z = 1229.6; observed m/z = 1229.3.

**Thioether formation.** 200 mg dry, cyclized resin is taken up in 12.6 mL of a 2 mg/mL solution of iodine in DMF and the reaction was agitated for 2.5 hours at room temperature. The beads were then drained and washed with DMF x 3, DCM x 3, DMF x 3. The peptide sequence at this stage is Fmoc-Ala-D-Thr(tBu)-Cys-*cis*-Hyp(OTIPS)-Ala-Trp-Glu-OH. ESI-MS: calculated mass for $C_{47}H_{72}N_8O_{11}SSi$ + H: m/z = 985.3; observed m/z = 985.3.

**Cleavage and Deprotection.** 250 mg resin was treated with 10 mL 1% TFA in DCM for 20 min at room temperature with gentle agitation. After 20 min, the beads were filtered and rinsed with 1% TFA in DCM (2 x 1 mL). The rinses and filtrate were combined and evaporated to dryness. This residue was then treated with 4 mL 1:1 TFA/DCM for 20 min, and evaporated to dryness, evaporating 3 x from heptane to remove all trace TFA. The product was then transferred to a polypropylene eppendorf tube and treated with 4 mL 50% HF-pyridine in THF for 20 minutes. The reaction was then quenched by extraction with 10 mL of a 5% solution of triethylamine in methyl tert-butyl ether, producing a bilayer. The bottom layer was then purified by preparative HPLC and the isolated product was lyophilized to give a brown solid (9.3 mg, 12 μmol) in an average overall yield of 50% over 19 steps. ESI-MS: calculated mass ($M+Na^+$): 795.2743; Observed mass ($M+Na^+$): 795.2735. Error = 0.9 ppm. Molecular formula: $C_{34}H_{44}N_8O_{11}SNa$.

**Fluorophore conjugation.** 4.6 mg (6.02 μmol) of $Glu^7$-phalloidin was dissolved in 100 μL dry DMF under nitrogen. 1.5 mg EDC (7.7 μmol) and 0.88 mg NHS (7.6 μmol) were to the flask and the reaction was stirred overnight. The formation of the the NHS ester was followed by LC/MS. After NHS formation was complete, 6.6 μmol tetramethylrhodamine-cadaverene (Invitrogen) and 5 μL DIPEA (6.8 mg, 50 μmol) were added, and the reaction was allowed to stand at rt for 1.5 h. The resulting mixture was purified directly by HPLC (10% to 60% acetonitrile/water, 0.1% TFA). 2.1 mg 1.7 μmol (28%). Calculated mass:1269.5397; Observed mass: 1269.5414. Error = 1.3 ppm. Molecular formula: $C_{64}H_{77}N_{12}O_{14}S$

## Bioassays.

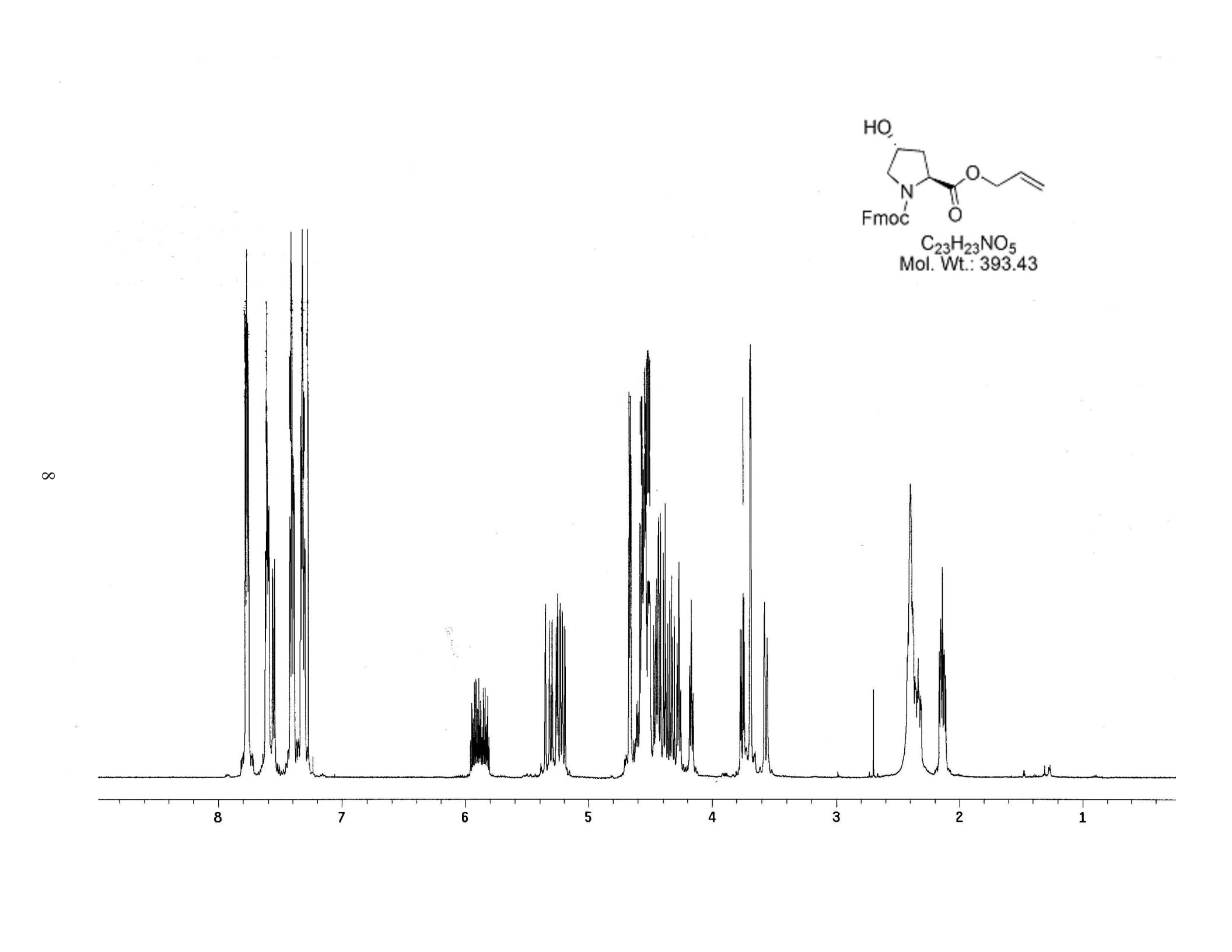
**Competitive Actin Binding.** Glass slides were washed in 60°C 1M HCl for 2 hours, and then rinsed with copious Milli-Q water, finishing with a 95% ethanol rinse. The plates were either stored under sterile conditions or used immediately. The glass slides were seeded with BS-C-1 cells, and the cells were allowed to grow for 3-4 days in cell growth media. The slides were removed from the growth media and fixed in 4% formaldehyde in CBS for 20 min. Slides are then rinsed 3 x TBS, and then permablized in 0.5% TX – TBS for 10 minutes. The slides were then washed 3 x 5 minutes in 0.1% TX – TBS, and then treated with AbDIL(0.1% TX – TBS with 2% BSA and 0.1% $NaN_3$) for 10 minutes. Plates were then rinsed with AbDIL, and treated with either TR-phalloidin (1ug/mL in AbDIL) or rhodamine-Glu$^7$-phalloidin (1ug/mL in AbDIL) for 20 minutes. The slides are then rinsed with TBS, mounted, and examined under an inverted fluorescence microscope. In order to test the specificity of rhodamine-Glu7-phalloidin, slides were prepared as described above, and were incubated with phalloidin (5 mg/mL in AbDIL for 12 h at 4°C) prior to staining with either rhodamine-Glu$^7$-phalloidin or TR-phalloidin.

Table S1. H-NMR assignment for Glu$^7$-phalloidin (recorded at 500MHz in $d_6$-DMSO at 25°C using a flow probe)

| Residue | N-H | $H_\alpha$ | Others |
|---|---|---|---|
| Ala$^1$ | 7.23 (J=8.2) | 4.52 (dd, *J*=7.7, 7.3) | 1.23 (3) |
| D-Thr$^2$ | 8.52 (J=8.23) | 3.99 (dd, *J*=7.7, 7.3) | 4.23 (1), 1.05 (3) |
| Cys$^3$ | 7.64 (J=5.49) | 4.70 (d, *J*=5.94) | 3.44 (2) |
| Hyp$^4$ | - | 4.35 (d, *J*=4.5) | 2.28(2); 1.80(1); 3.74(1); 4.14(1); 5.61(1) |
| Ala$^5$ | 7.53 (J=8.23) | 3.89 (t, *J*=7.31) | 0.64 (3) |
| Trp$^6$ | 7.09 (J=7.32) | 4.77 (d, 5.03) | 3.08 (2) |
| Trp$^6$ indole | 11.23 | - | 6.97; 7.09; 7.23; 7.72 |
| Glu$^7$ | 8.33 (J=5.03) | 3.84 (d, *J*=7.31) | 2.01 (2); 2.37 (2) |

Table S2. H-NMR assignment for tetramethylrhodamine-cadaverine-Glu$^{7}$-phalloidin (recorded at 600MHz in $d_6$-DMSO at 25°C)

| Residue | N-H | $H_\alpha$ | Others |
|---|---|---|---|
| Ala$^{1}$ | 7.24 | 4.53 | 1.23 (3) |
| D-Thr$^{2}$ | 8.83 | 3.28 | 1.34 (1) 1.57 (3) |
| Cys$^{3}$ | 7.61 | 4.71 | 3.44 (2) |
| Hyp$^{4}$ | - | 4.35 | 2.28 (2), 3.73 (1), 3.98 (1), 4.16 (1); 5.32 (1) |
| Ala$^{5}$ | 7.45 | 3.93 | 0.66 (3) |
| Trp$^{6}$ | 7.09 | 4.76 | 3.09 (2), 6.98 (1), 7.09 (1), 7.24 (1), 7.77 (1). |
| Glu$^{7}$ | 8.49 | 3.83 | 2.18 (2), 2.01(2) |
| cadaverine chain | 7.90<br>7.20 | | 3.09 (4), 1.46 (2), 1.34 (2), 1.04 (2) |
| | | | 8.49 (1), 8.23 (1), 7.33 (1), 6.53 (4), 1.17 (12), 0.84 (2) |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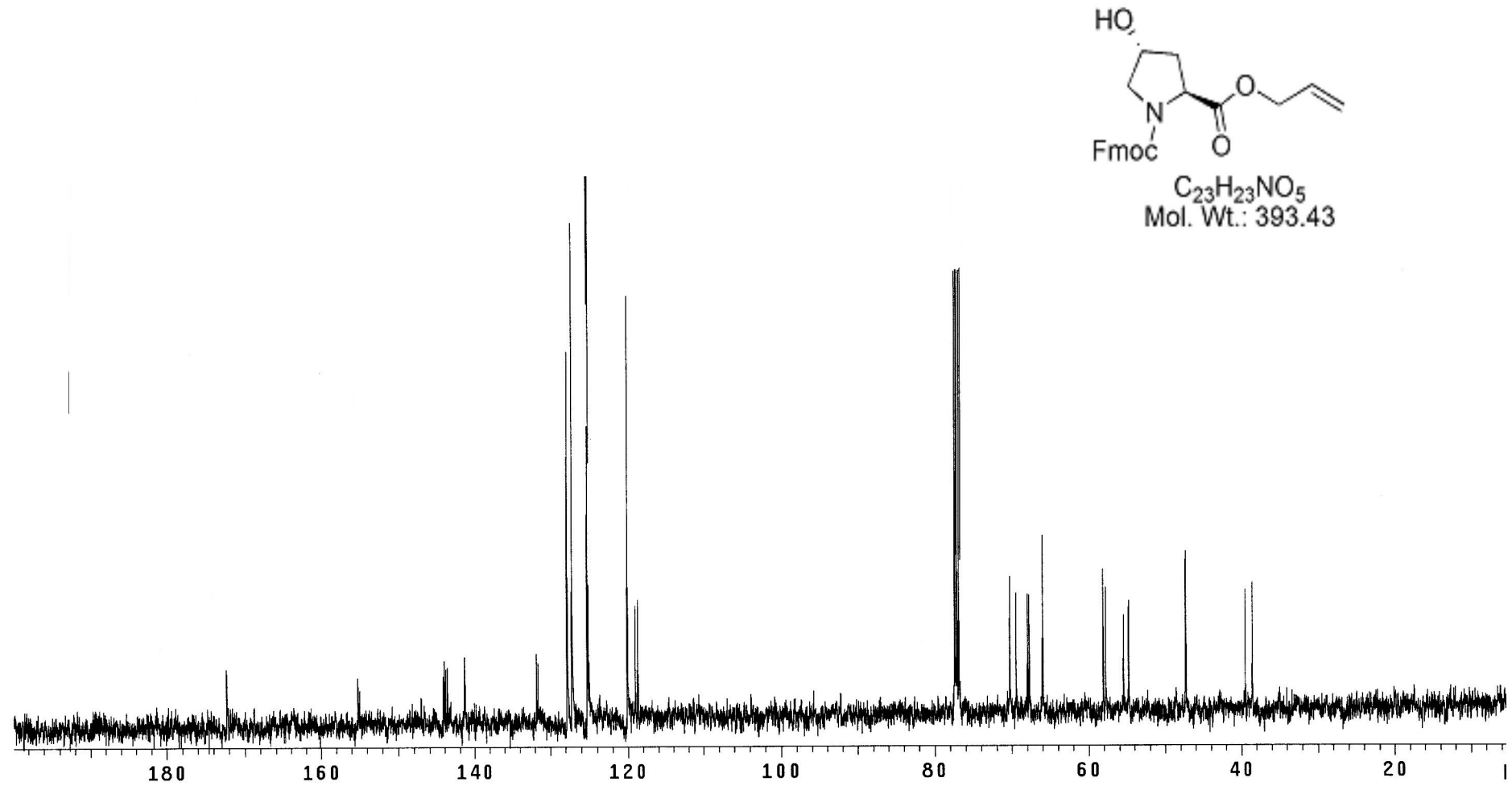
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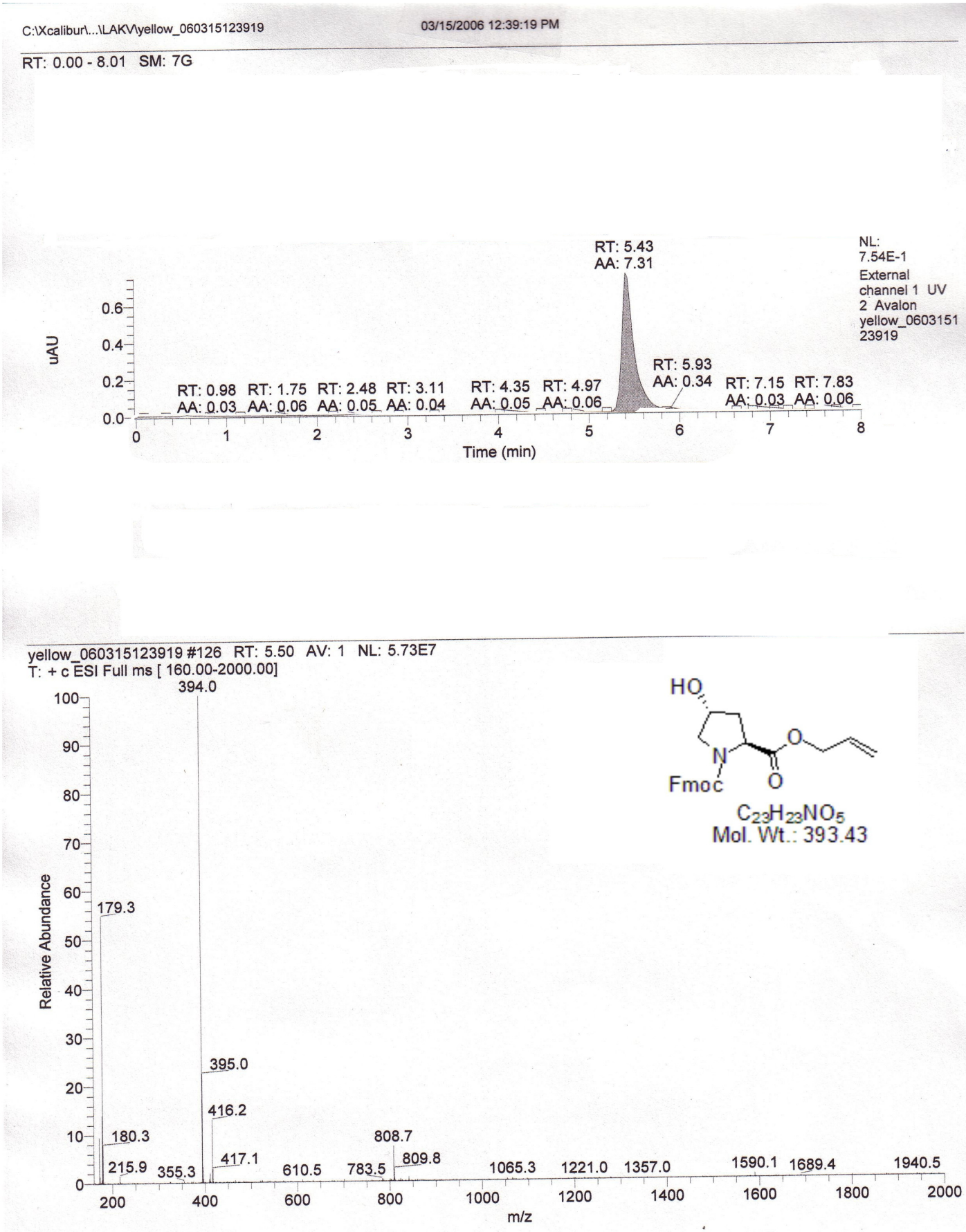
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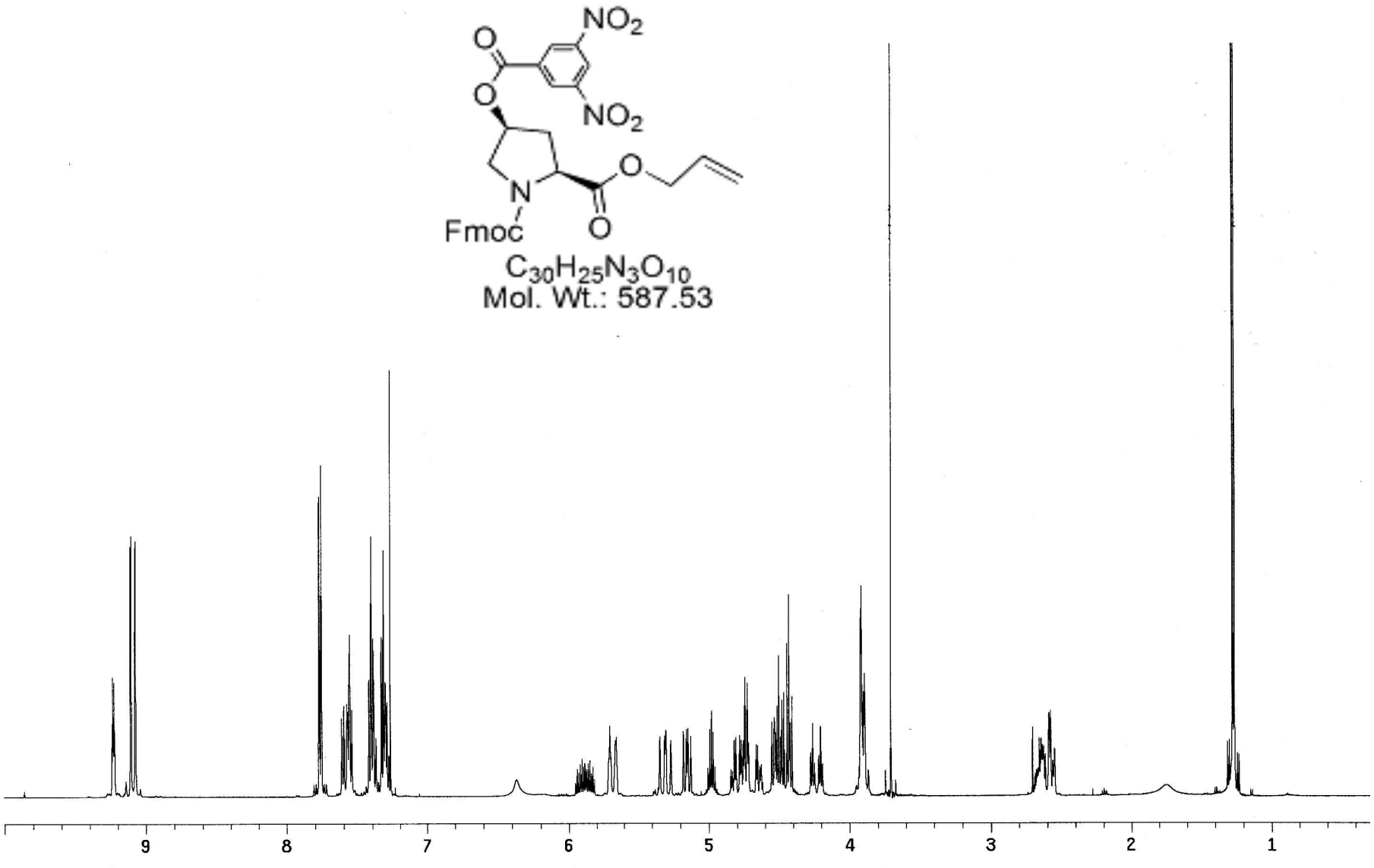
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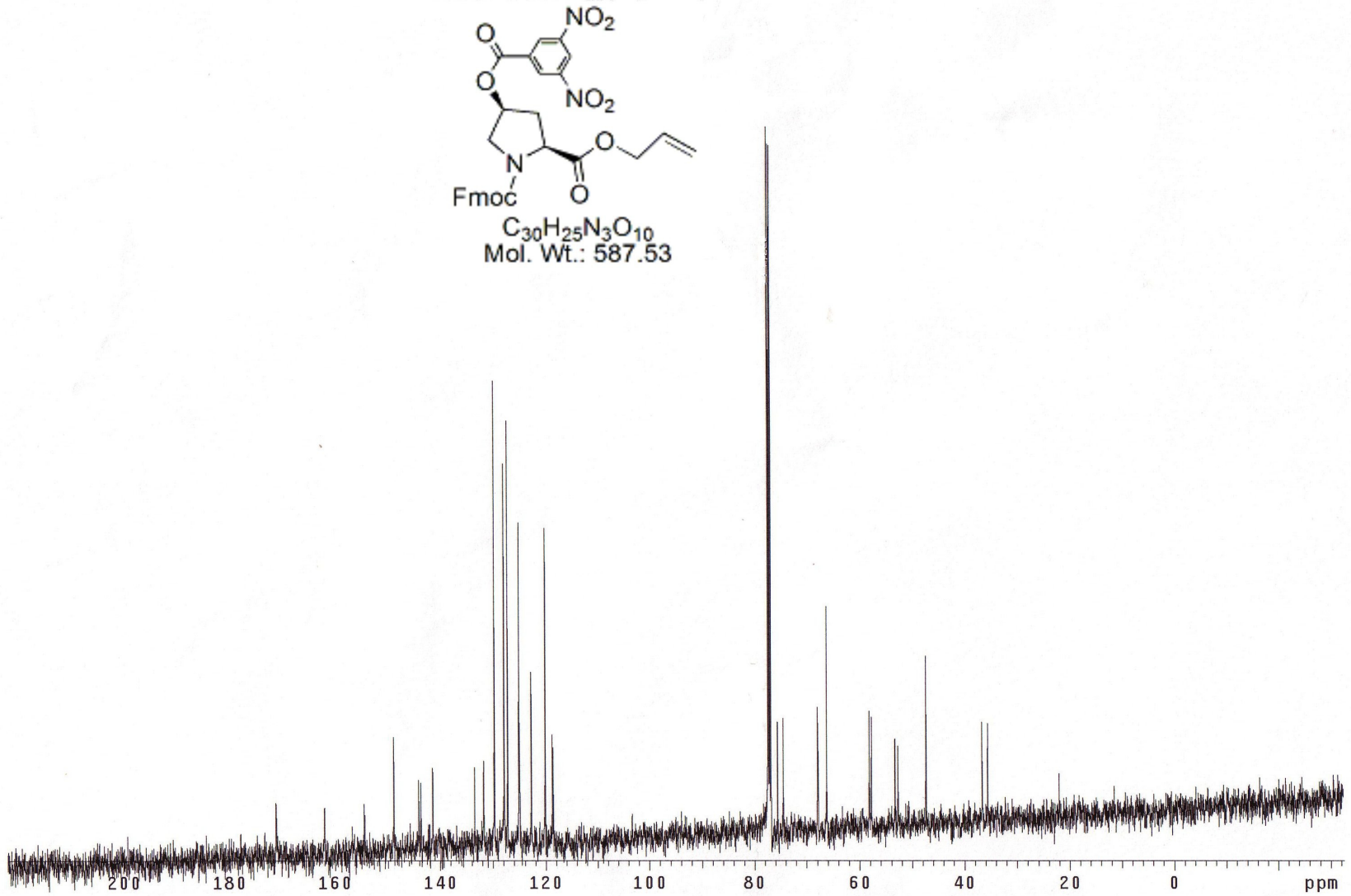
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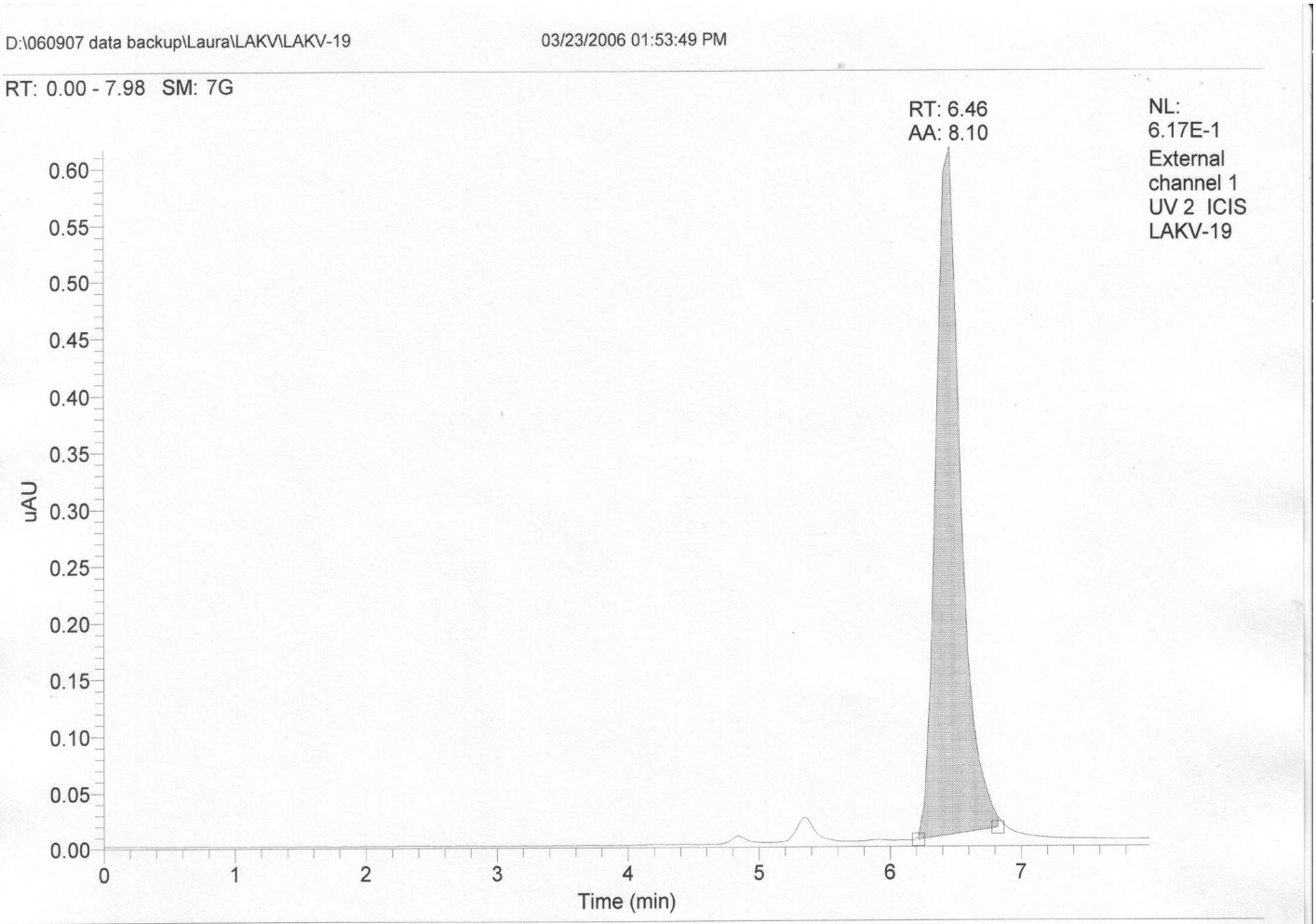
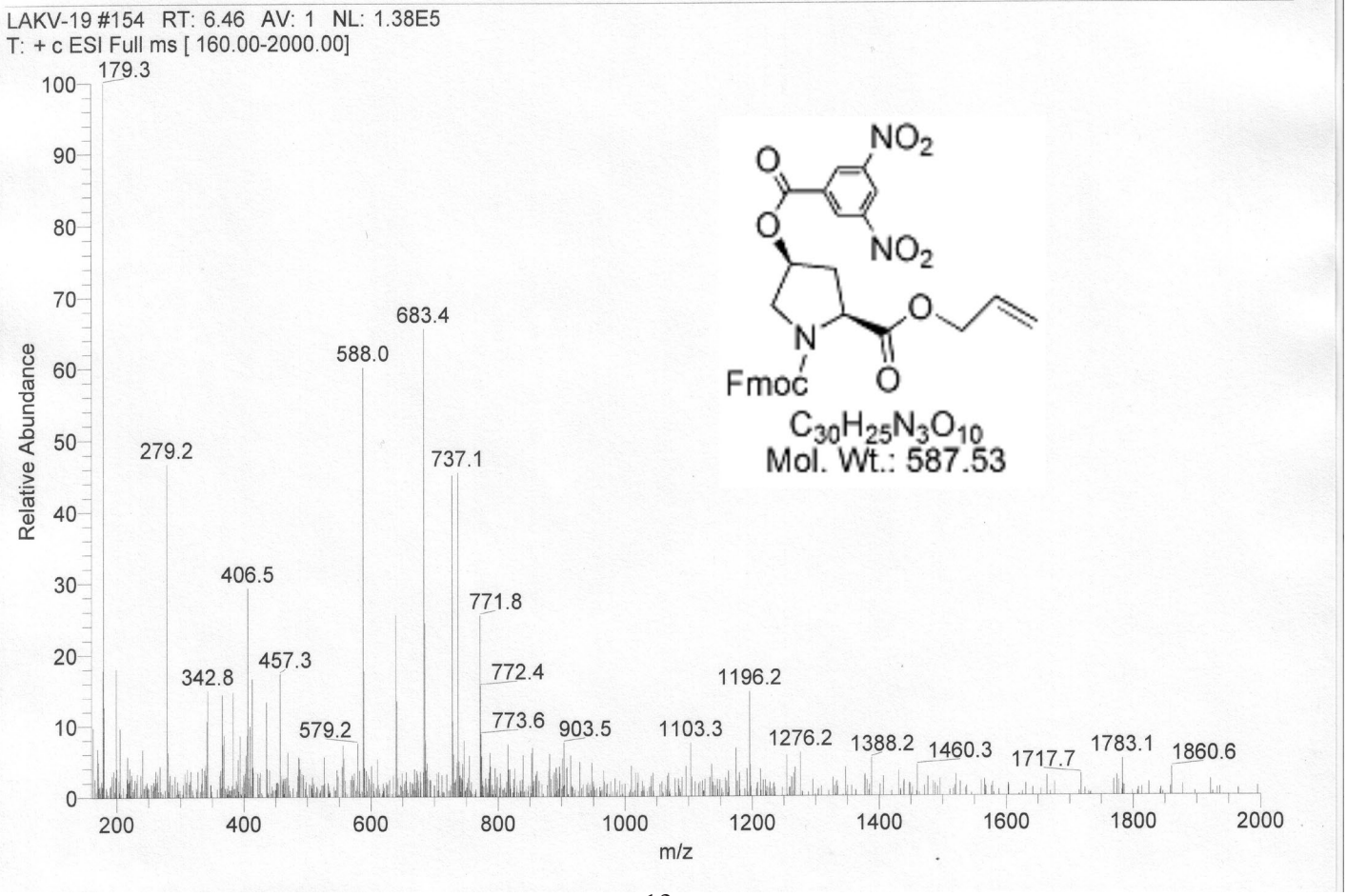
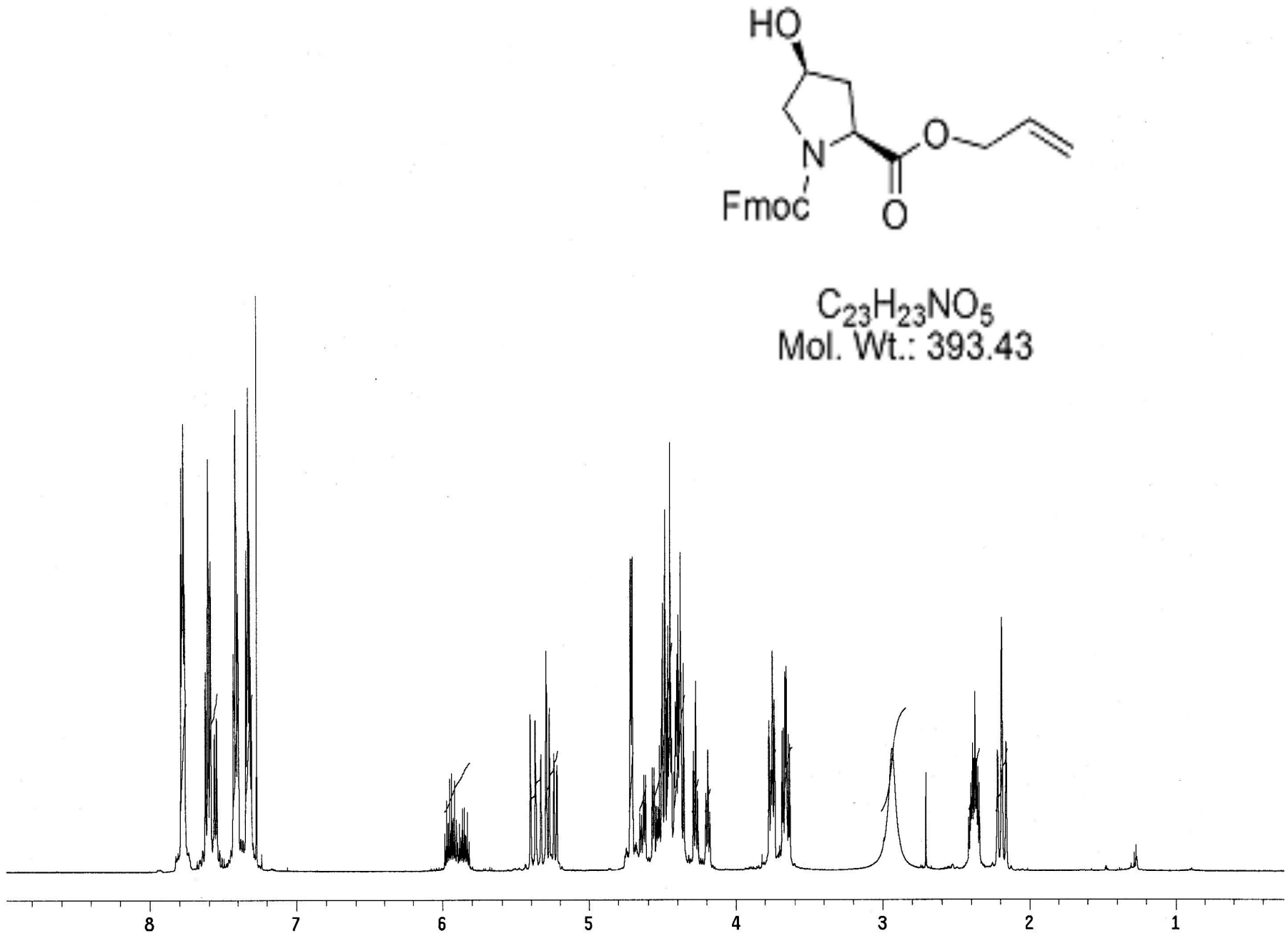
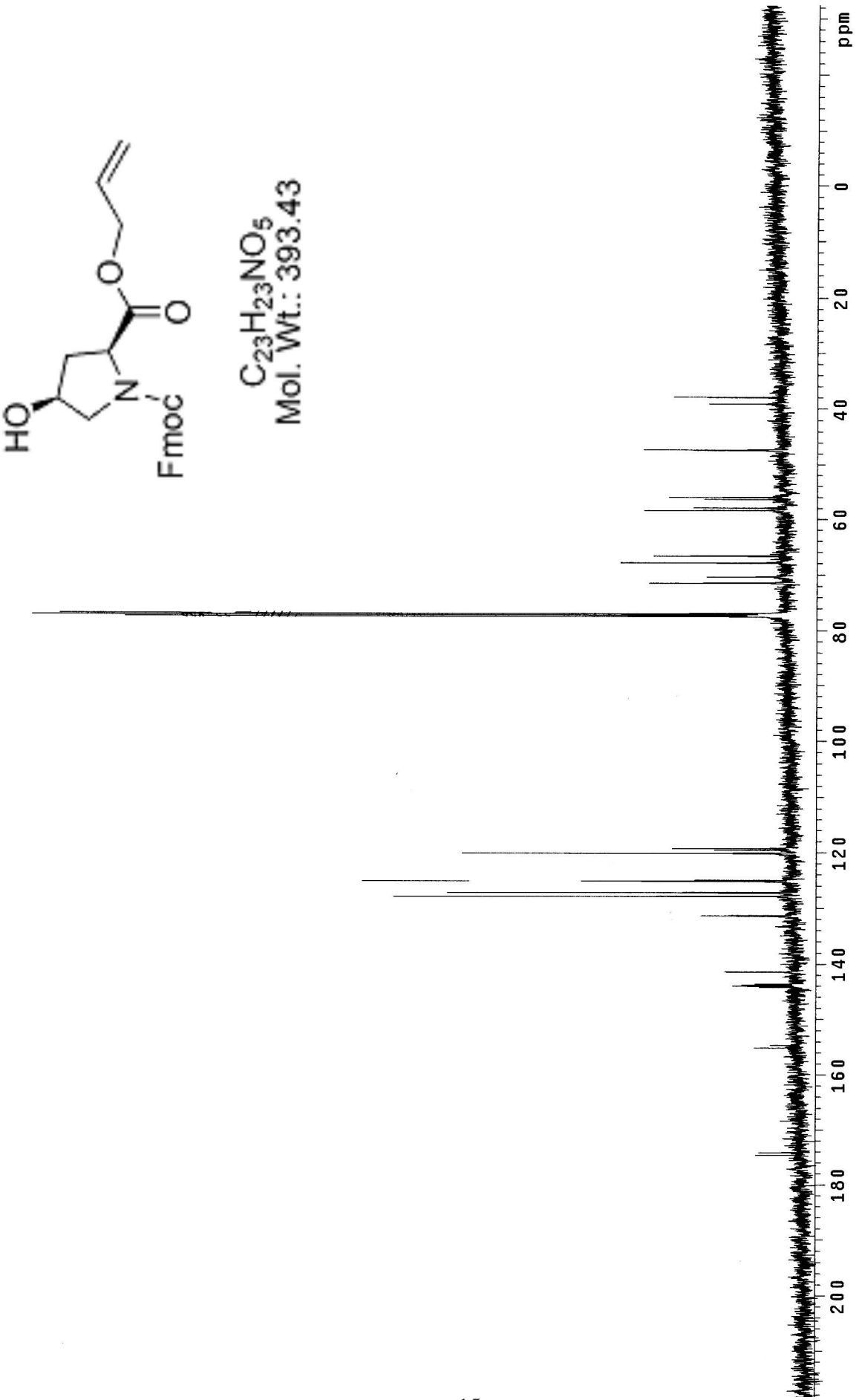
0
ppm

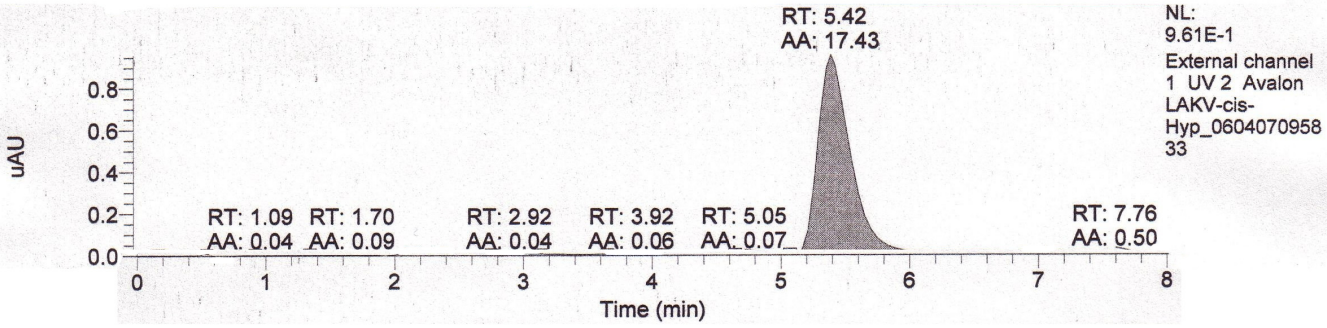

D:\060907 data backup\Laura\LAKV\LAKV-19
03/23/2006 01:53:49 PM
RT: 0.00 - 7.98 SM: 7G
RT: 6.46
AA: 8.10
NL:
6.17E-1
External
channel 1
UV 2 ICIS
LAKV-19
uAU
Time (min)


LAKV-19 #154 RT: 6.46 AV: 1 NL: 1.38E5
T: + c ESI Full ms [ 160.00-2000.00]
Relative Abundance
m/z
179.3
683.4
588.0
279.2
737.1
406.5
771.8
457.3
342.8
772.4
1196.2
579.2
773.6
903.5
1103.3
1276.2
1388.2
1460.3
1717.7
1783.1
1860.6
Fmoc
C30H25N3O10
Mol. Wt.: 587.53

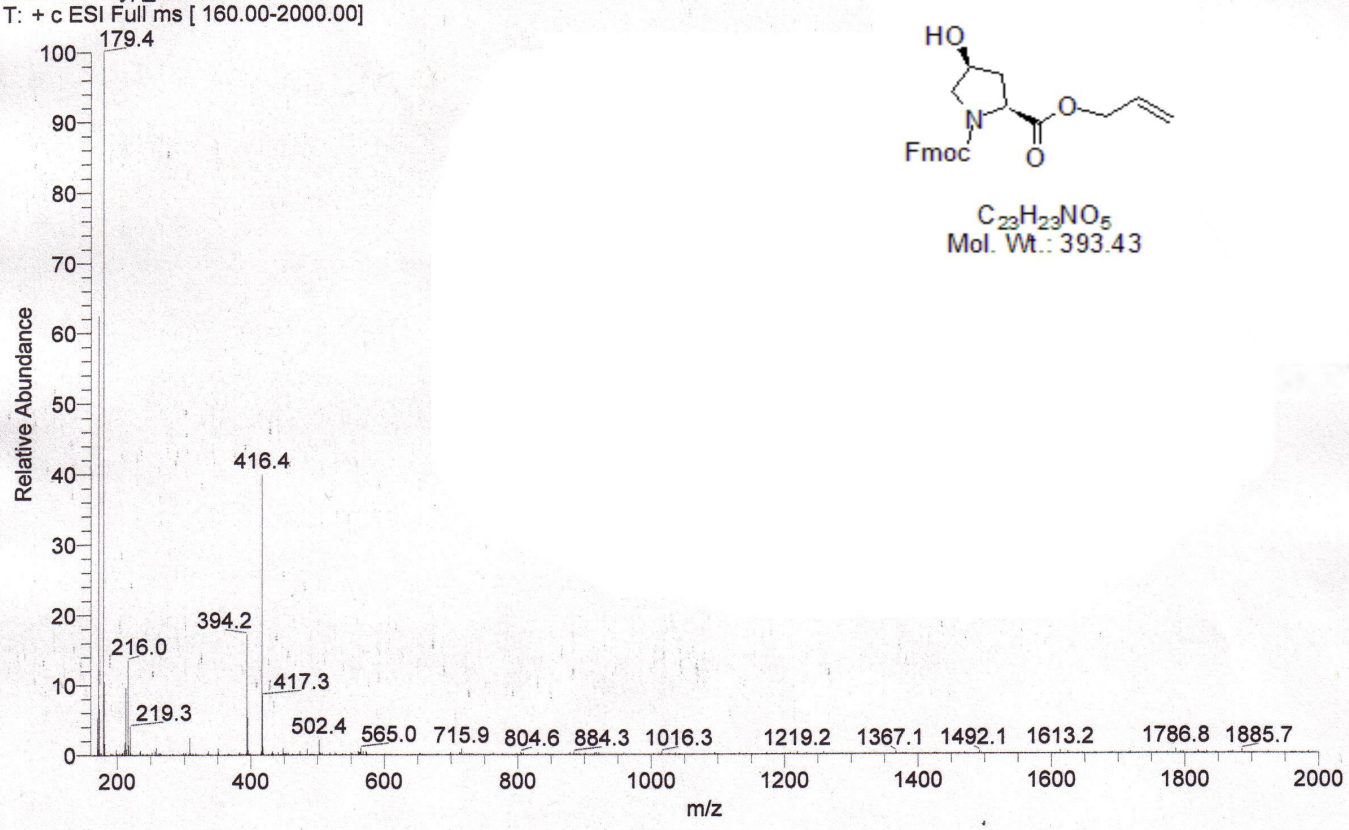

HO
N
Fmoc
O
O
$C_{23}H_{23}NO_5$
Mol. Wt.: 393.43
8
7
6
5
4
3
2
1


HO
N
Fmoc
O
O
O
$C_{23}H_{23}NO_5$
Mol. Wt.: 393.43
ppm
200
180
160
140
120
100
80
60
40
20
0

RT: 0.00 - 8.01 SM: 7G



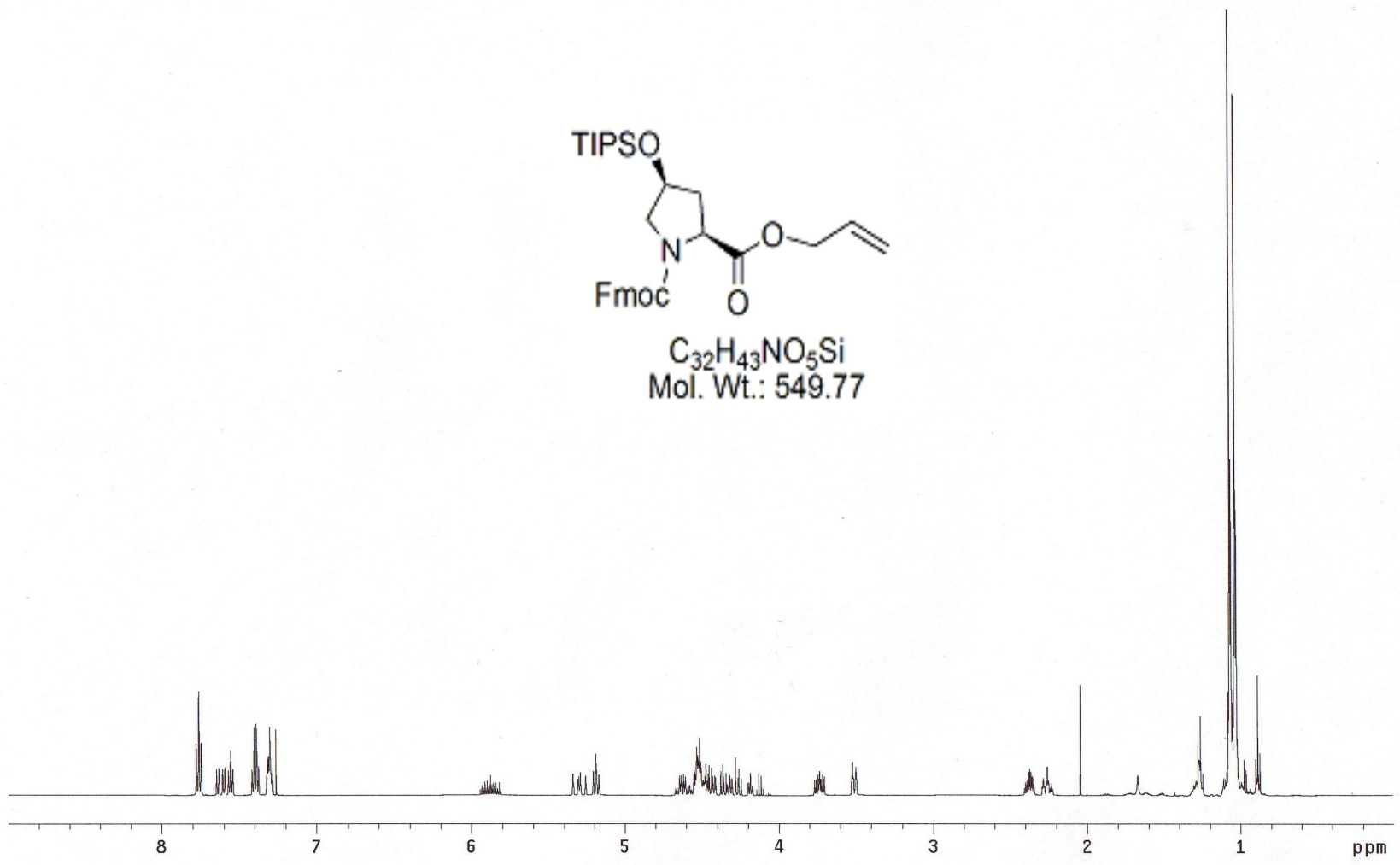

LAKV-cis-Hyp_060407095833 #117 RT: 5.45 AV: 1 NL: 1.53E6
T: + c ESI Full ms [ 160.00-2000.00]

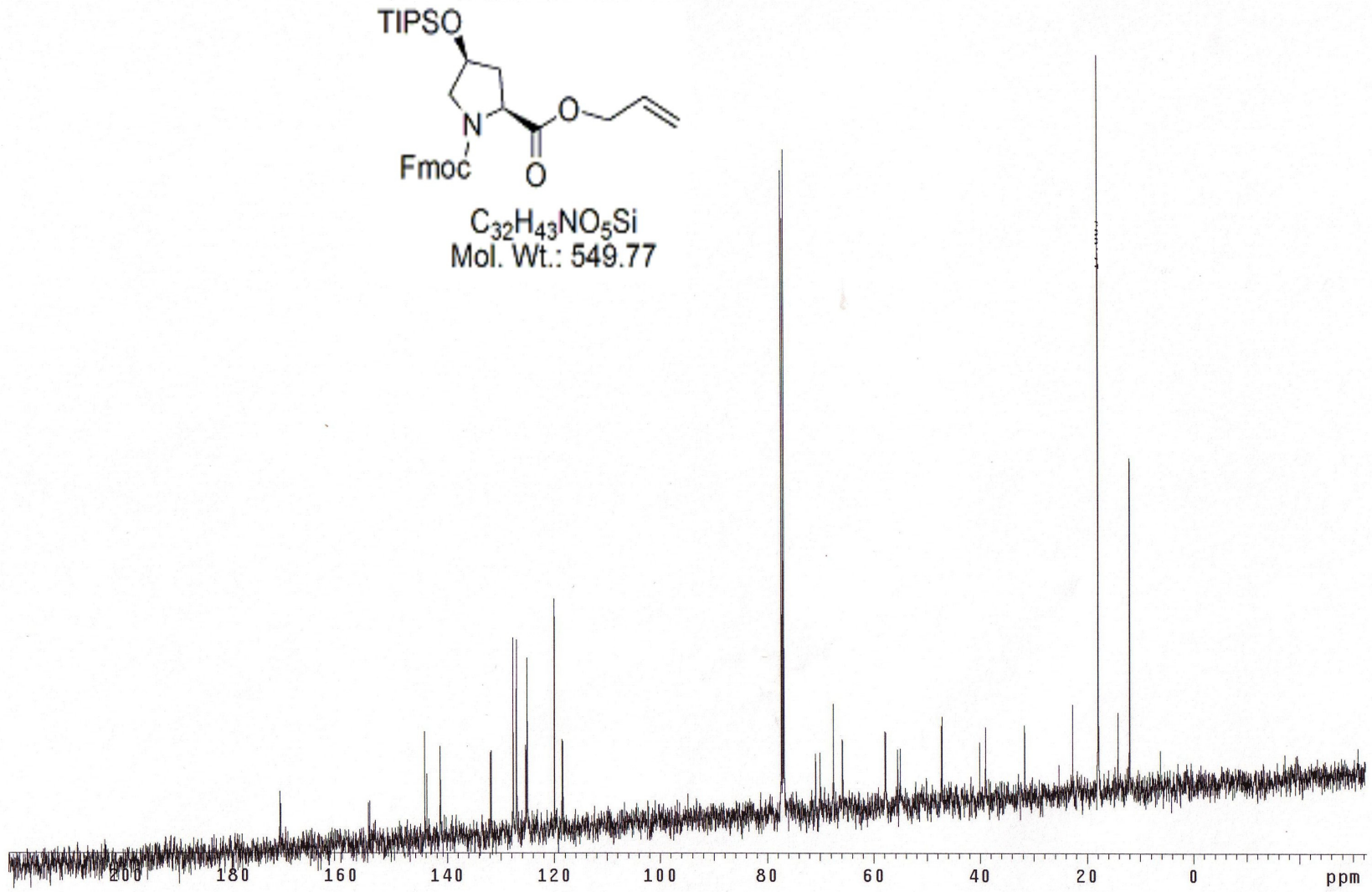


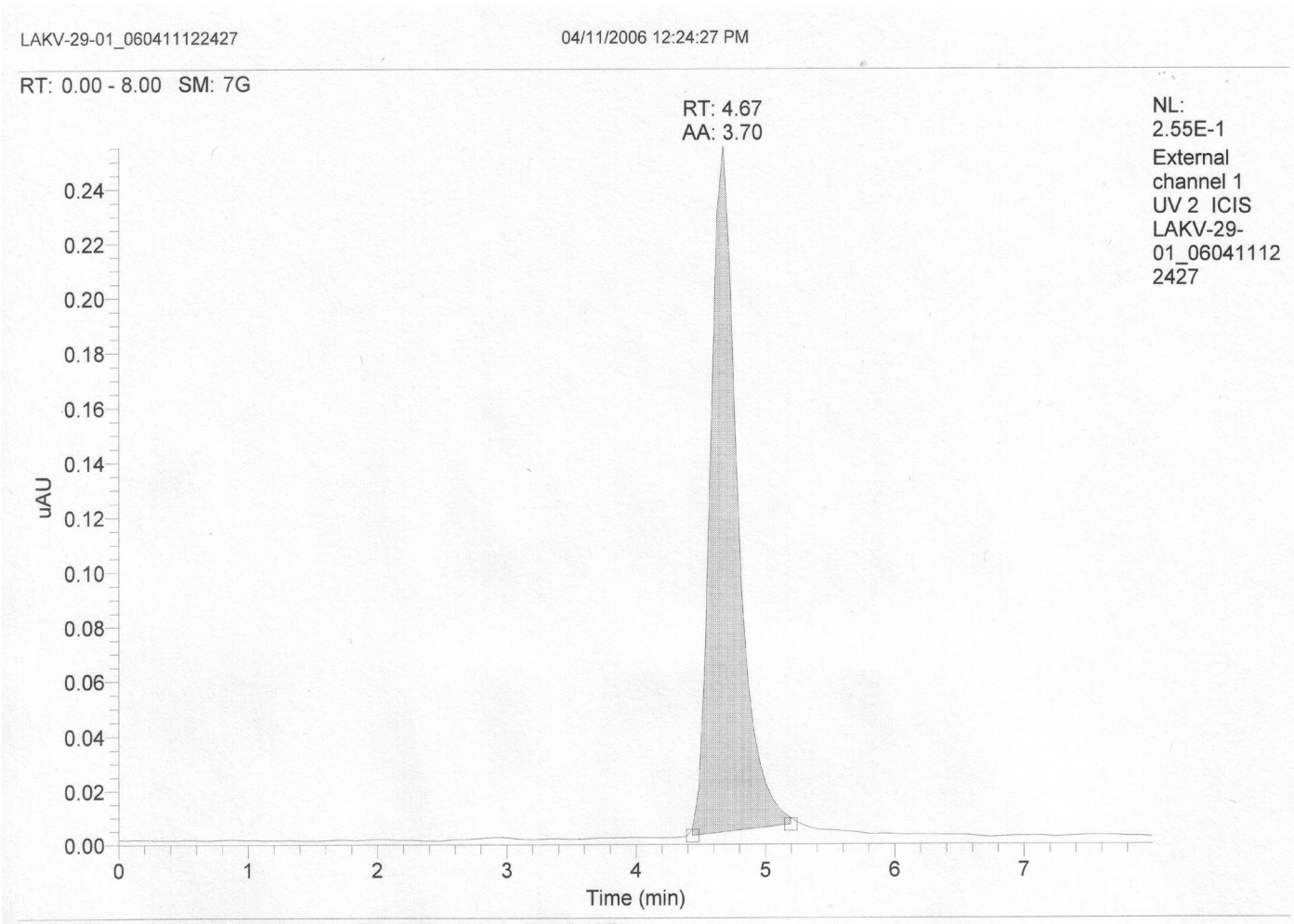


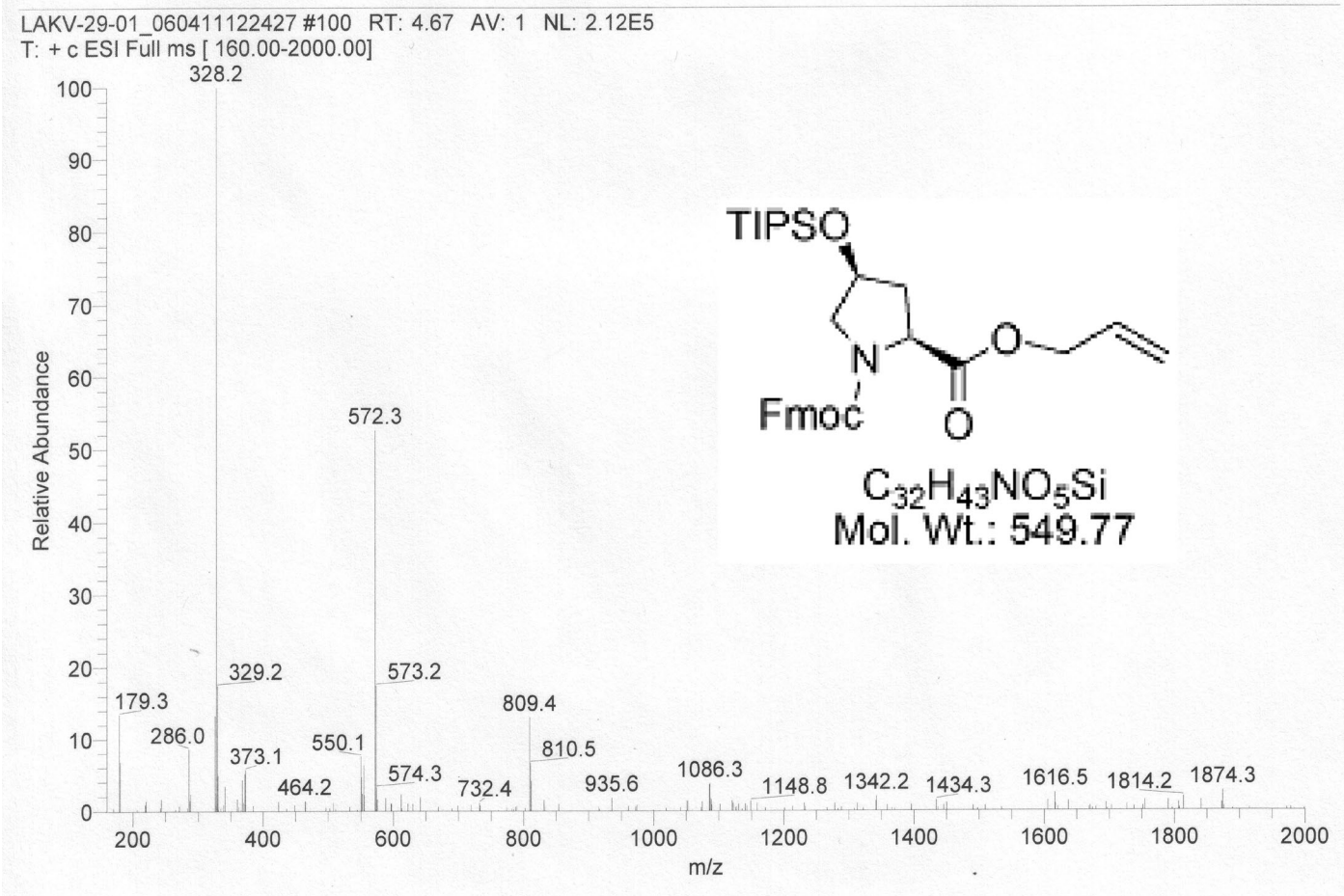


$C_{23}H_{23}NO_5$
Mol. Wt.: 393.43


TIPSO
N
Fmoc
O
O
O
C32H43NO5Si
Mol. Wt.: 549.77
8
7
6
5
4
3
2
1
ppm


TIPSO
N
Fmoc
O
O
O
C32H43NO5Si
Mol. Wt.: 549.77
200
180
160
140
120
100
80
60
40
20
0
ppm

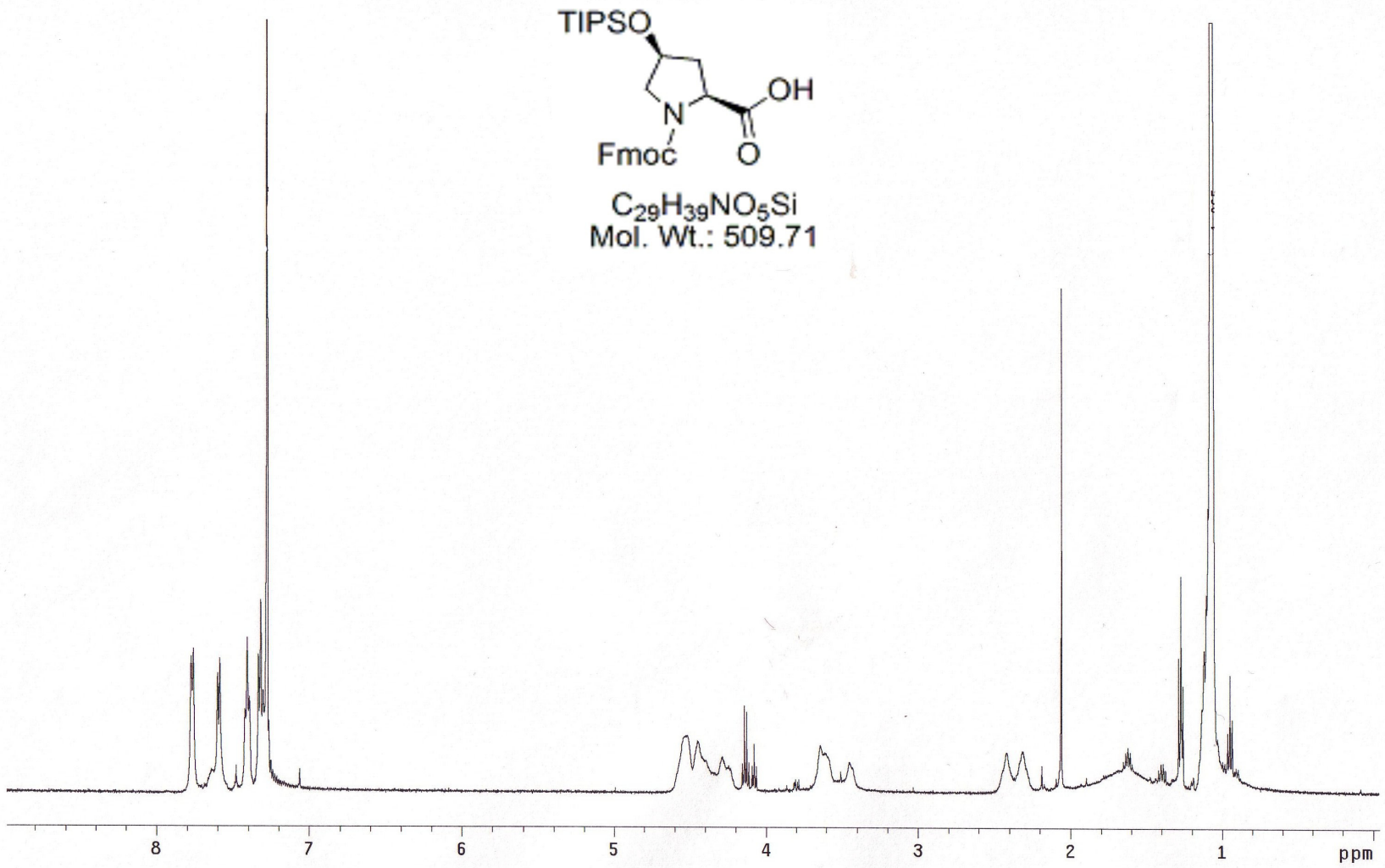


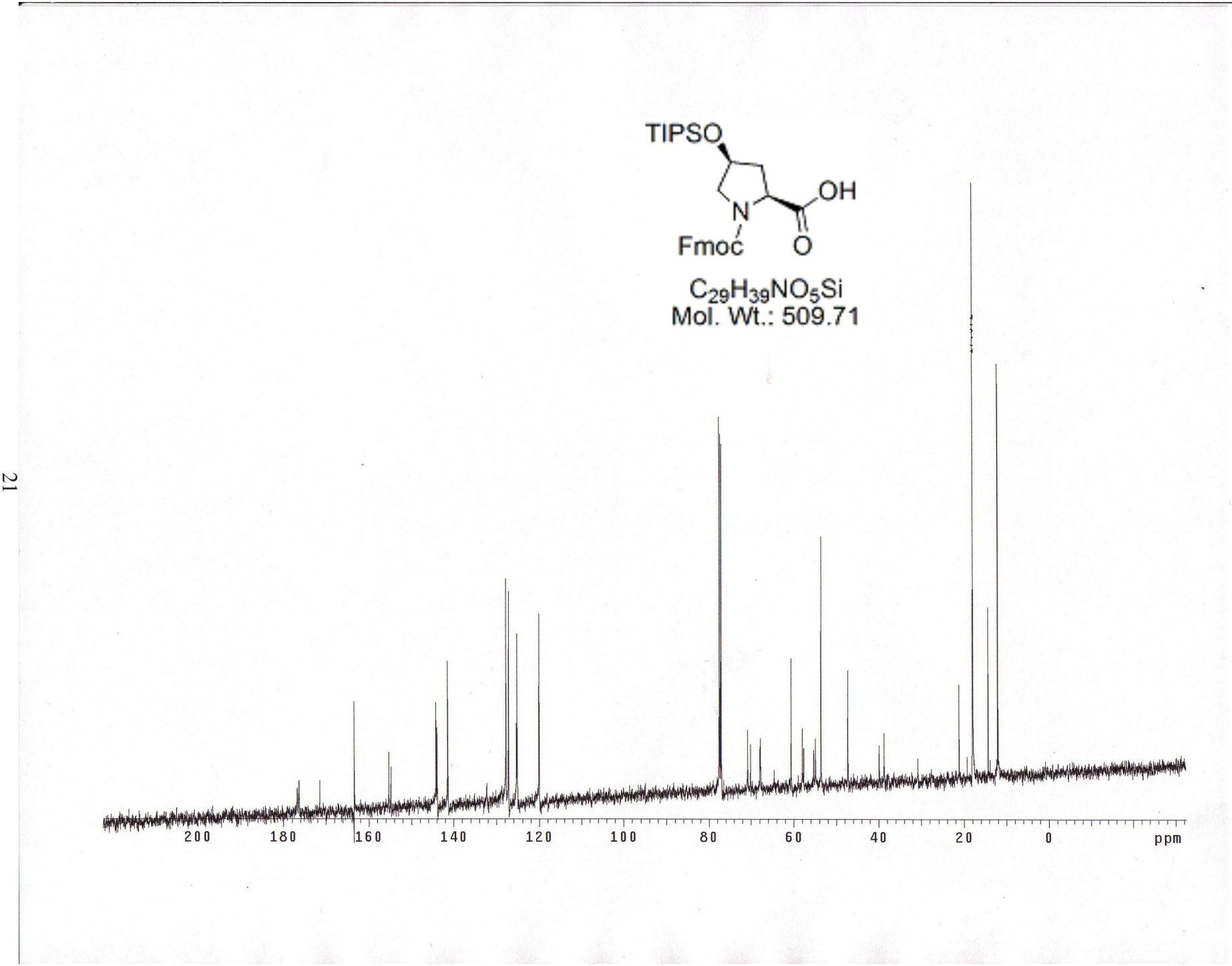



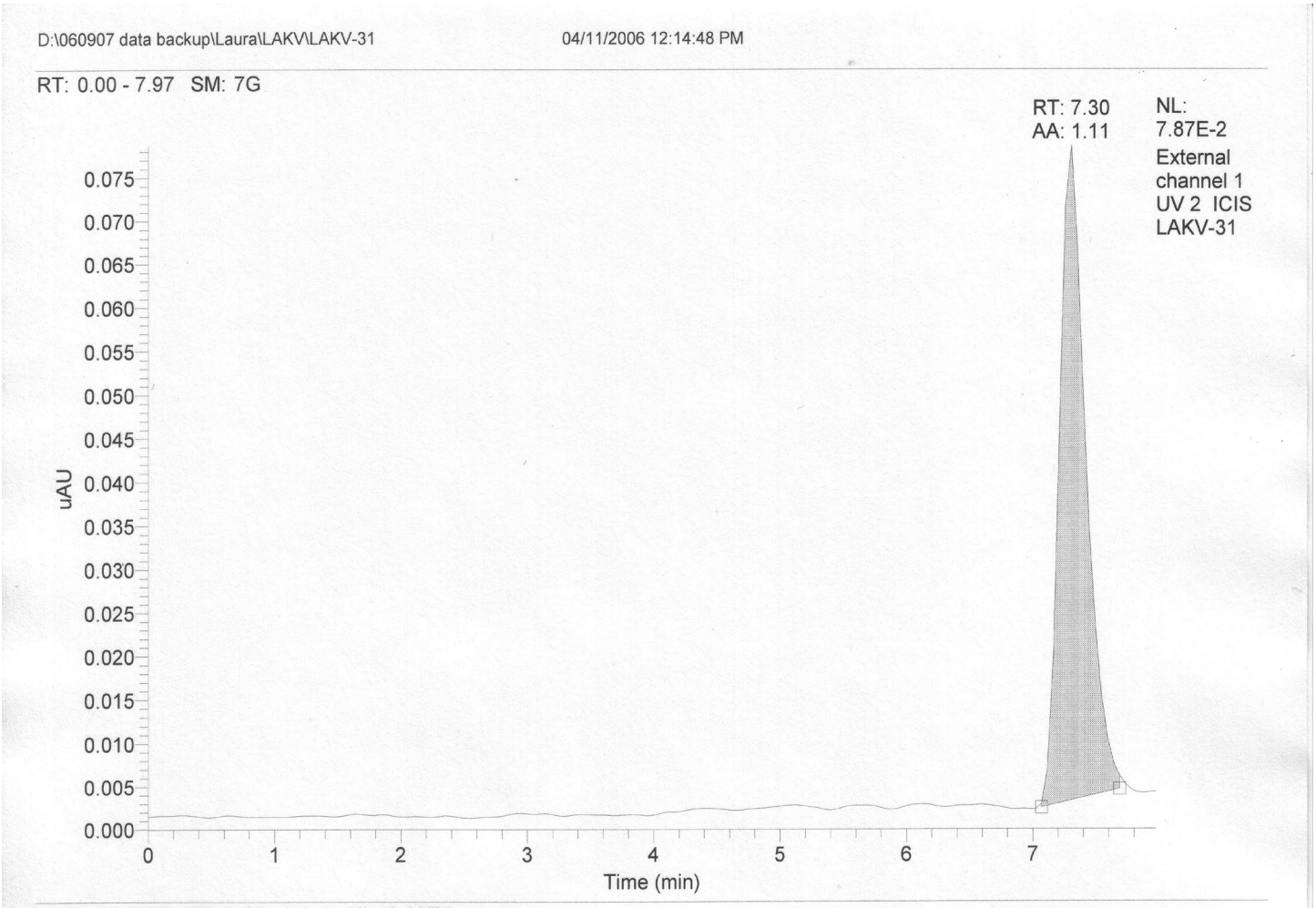


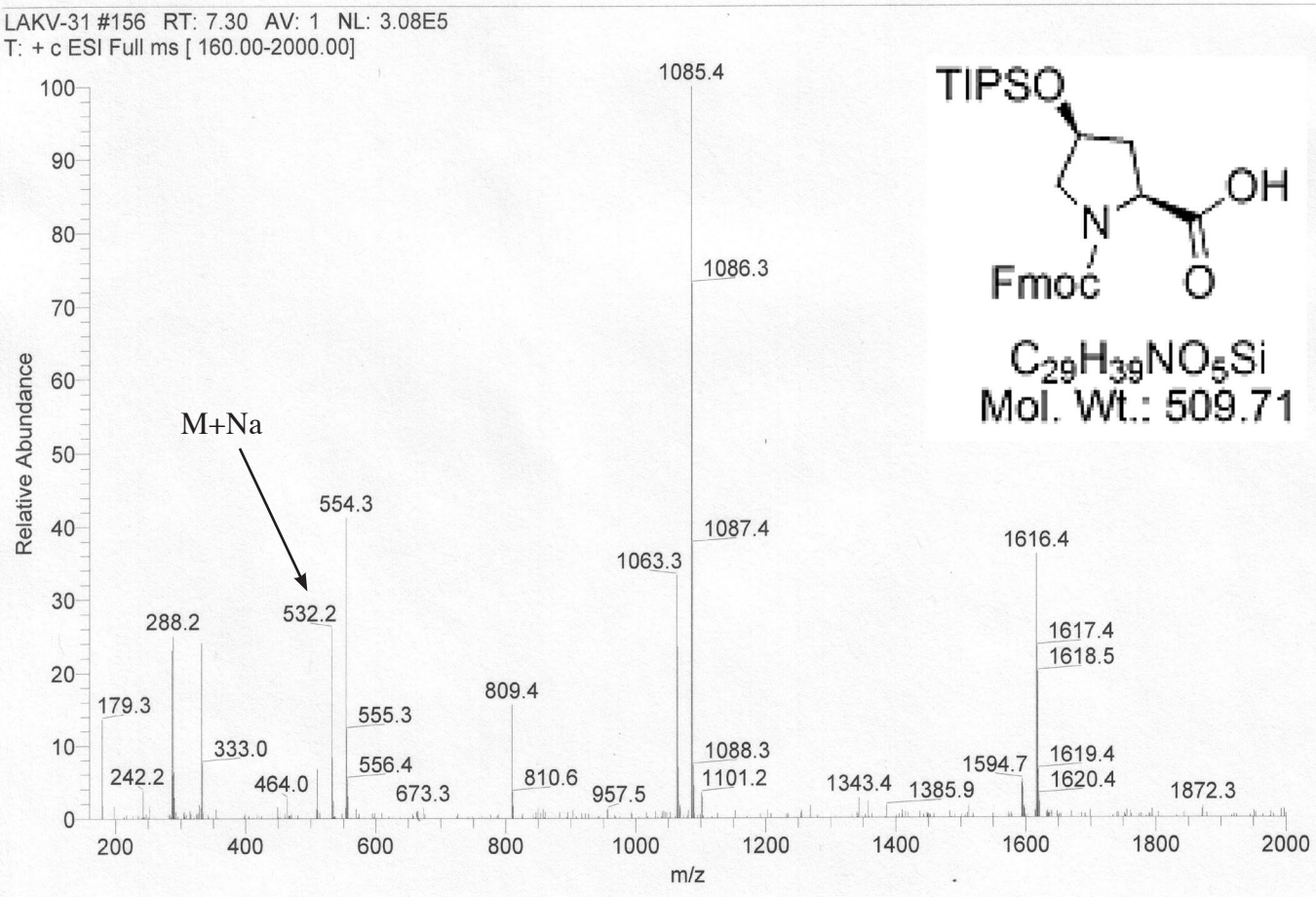


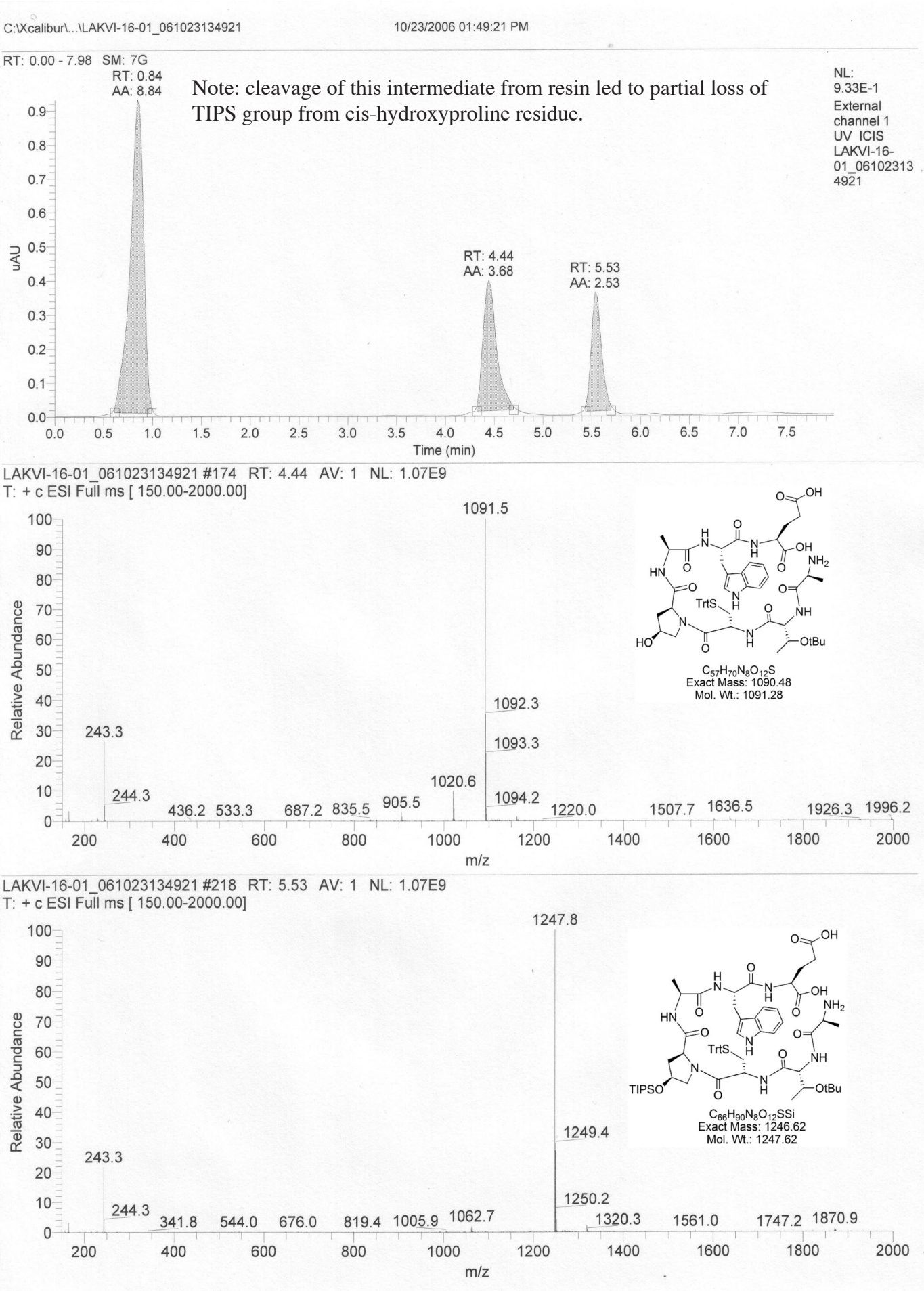
$C_{32}H_{43}NO_5Si$
Mol. Wt.: 549.77

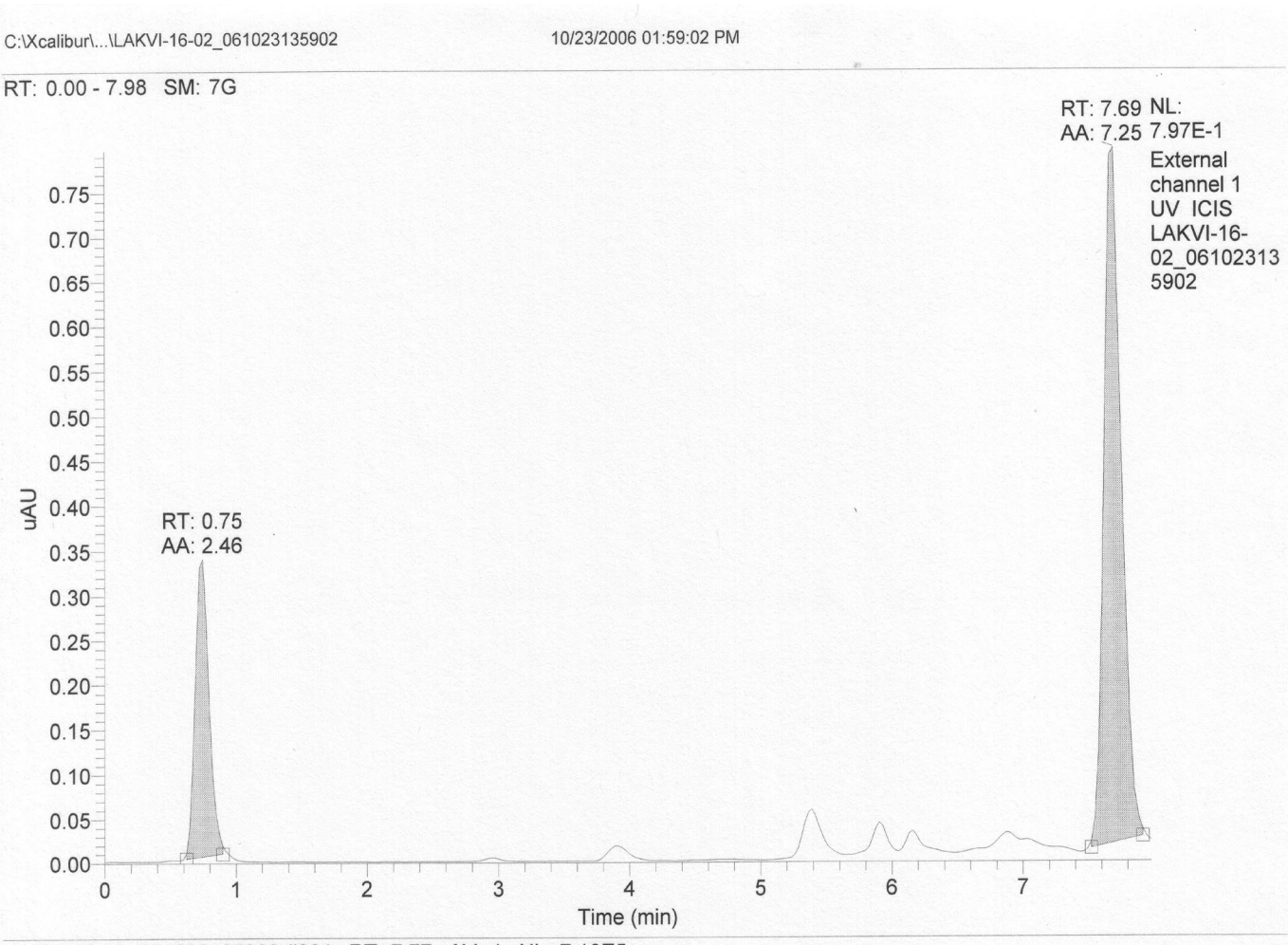

TIPSO
OH
N
Fmoc
O
C29H39NO5Si
Mol. Wt.: 509.71
8
7
6
5
4
3
2
1
ppm

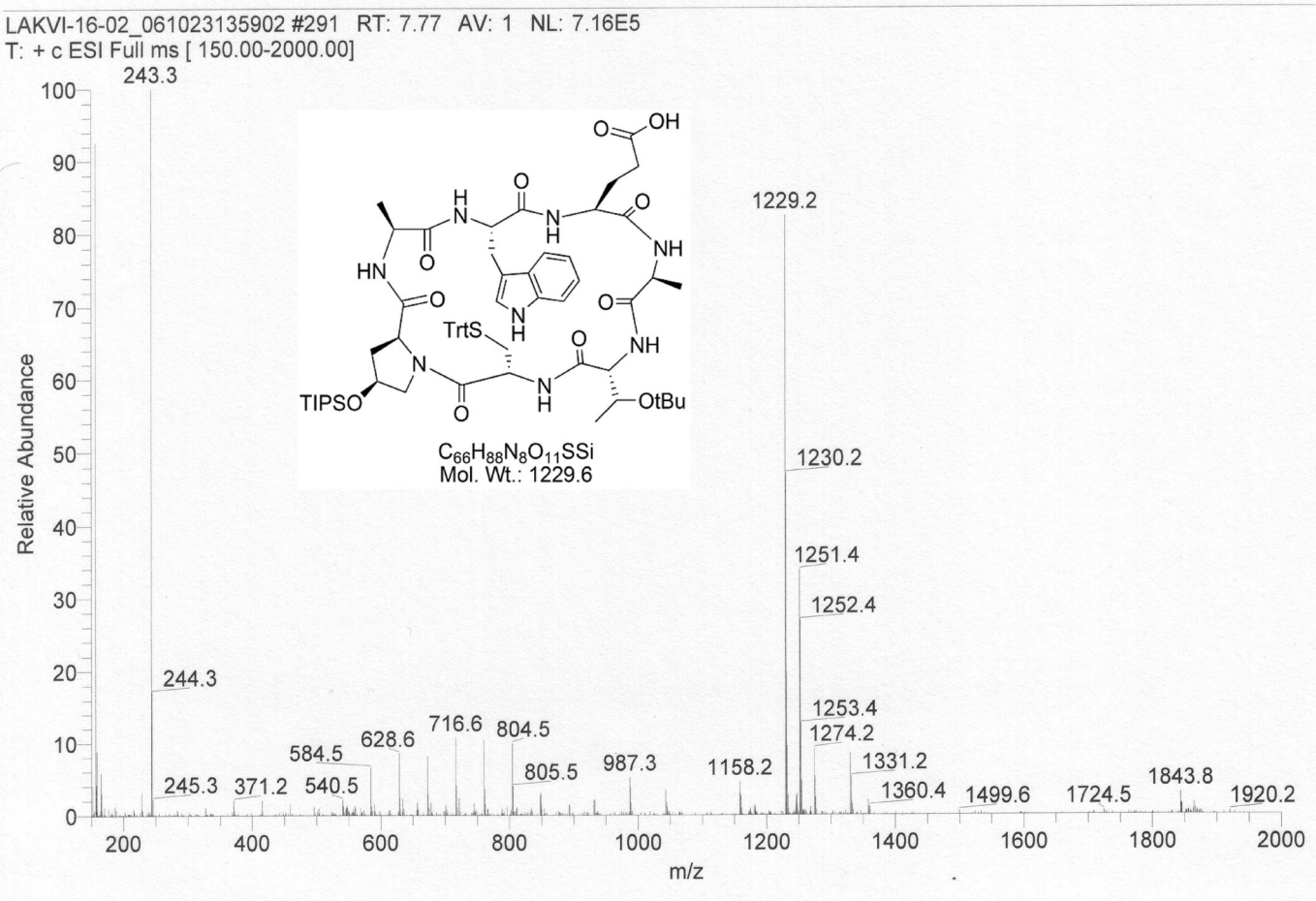

TIPSO
OH
N
Fmoc
O
C29H39NO5Si
Mol. Wt.: 509.71
200
180
160
140
120
100
80
60
40
20
0
ppm

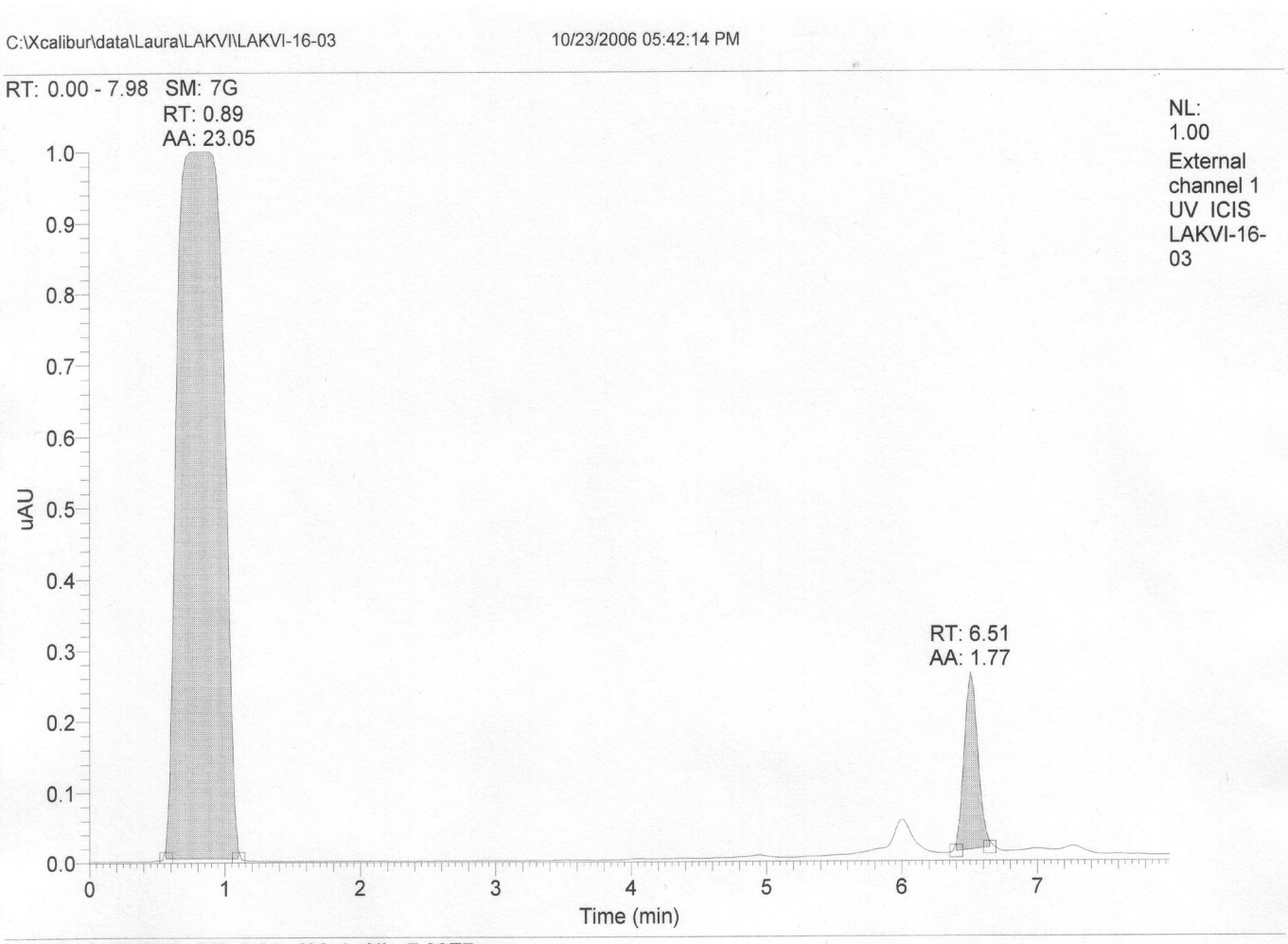

D:\060907 data backup\Laura\LAKV\LAKV-31
04/11/2006 12:14:48 PM
RT: 0.00 - 7.97 SM: 7G
RT: 7.30
AA: 1.11
NL:
7.87E-2
External
channel 1
UV 2 ICIS
LAKV-31
uAU
Time (min)

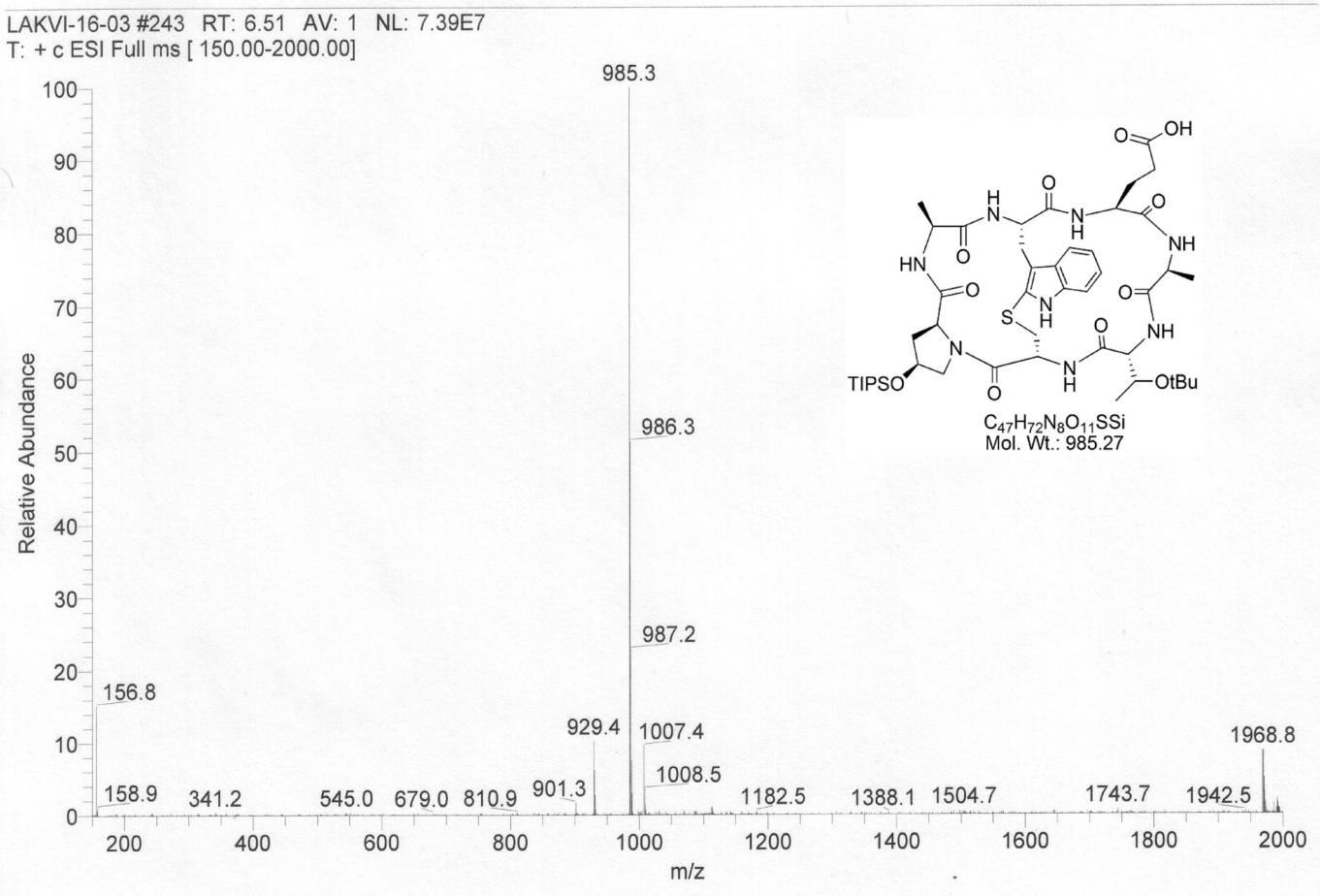

LAKV-31 #156 RT: 7.30 AV: 1 NL: 3.08E5
T: + c ESI Full ms [ 160.00-2000.00]
Relative Abundance
m/z
M+Na
1085.4
1086.3
1087.4
1063.3
554.3
532.2
288.2
179.3
242.2
333.0
464.0
555.3
556.4
673.3
809.4
810.6
957.5
1088.3
1101.2
1343.4
1385.9
1594.7
1616.4
1617.4
1618.5
1619.4
1620.4
1872.3
TIPSO
OH
N
Fmoc
O
C29H39NO5Si
Mol. Wt.: 509.71

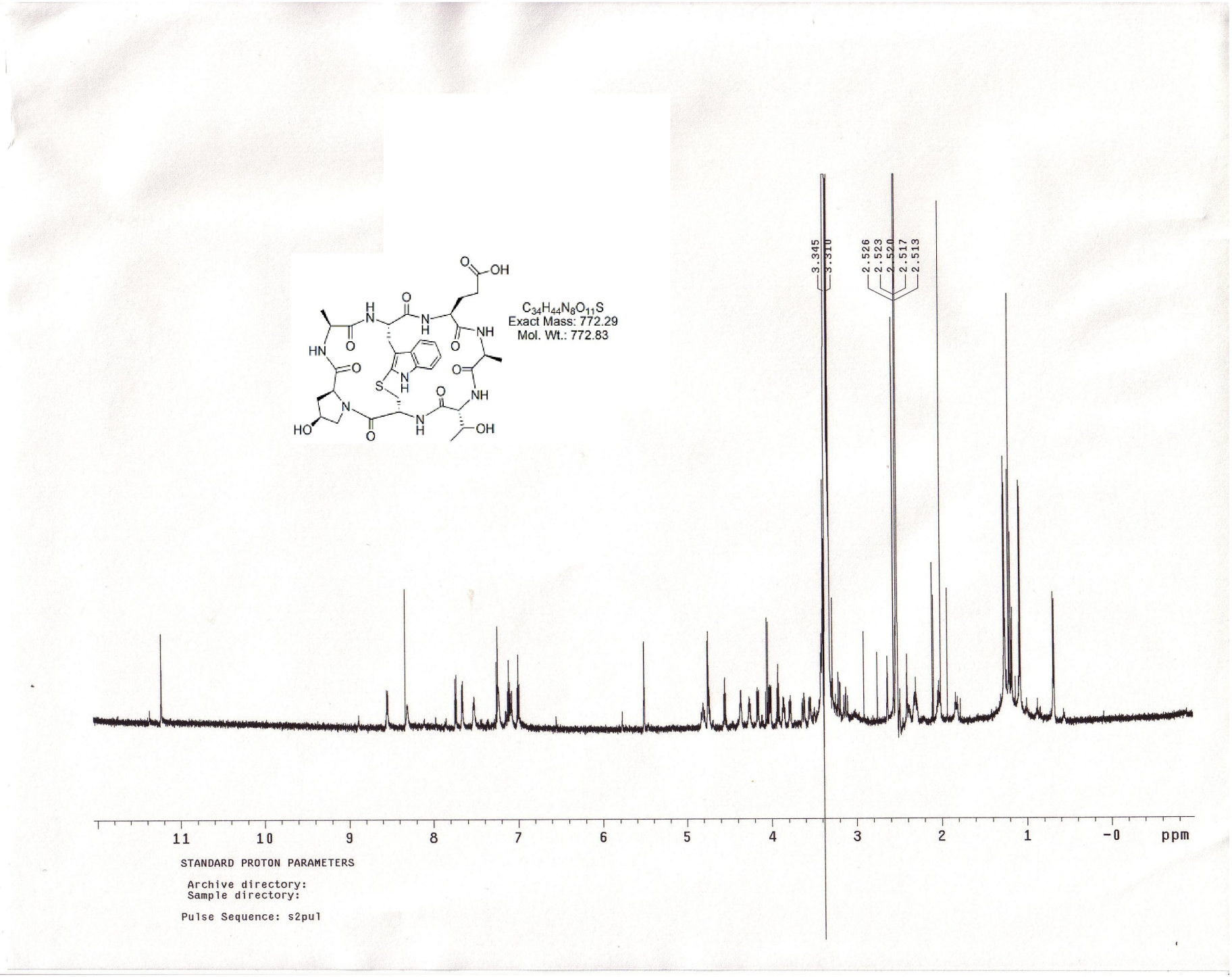

C:\Xcalibur\...\LAKVI-16-01_061023134921 10/23/2006 01:49:21 PM
RT: 0.00 - 7.98 SM: 7G
RT: 0.84
AA: 8.84
Note: cleavage of this intermediate from resin led to partial loss of TIPS group from cis-hydroxyproline residue.
NL:
9.33E-1
External
channel 1
UV ICIS
LAKVI-16-
01_06102313
4921
RT: 4.44
AA: 3.68
RT: 5.53
AA: 2.53
uAU
Time (min)
LAKVI-16-01_061023134921 #174 RT: 4.44 AV: 1 NL: 1.07E9
T: + c ESI Full ms [ 150.00-2000.00]
1091.5
1092.3
1093.3
1020.6
1094.2
243.3
244.3
436.2
533.3
687.2
835.5
905.5
1220.0
1507.7
1636.5
1926.3
1996.2
Relative Abundance
m/z
TrtS
OtBu
HO
C57H70N8O12S
Exact Mass: 1090.48
Mol. Wt.: 1091.28
LAKVI-16-01_061023134921 #218 RT: 5.53 AV: 1 NL: 1.07E9
T: + c ESI Full ms [ 150.00-2000.00]
1247.8
1249.4
1250.2
243.3
244.3
341.8
544.0
676.0
819.4
1005.9
1062.7
1320.3
1561.0
1747.2
1870.9
Relative Abundance
m/z
TrtS
OtBu
TIPSO
C66H90N8O12SSi
Exact Mass: 1246.62
Mol. Wt.: 1247.62

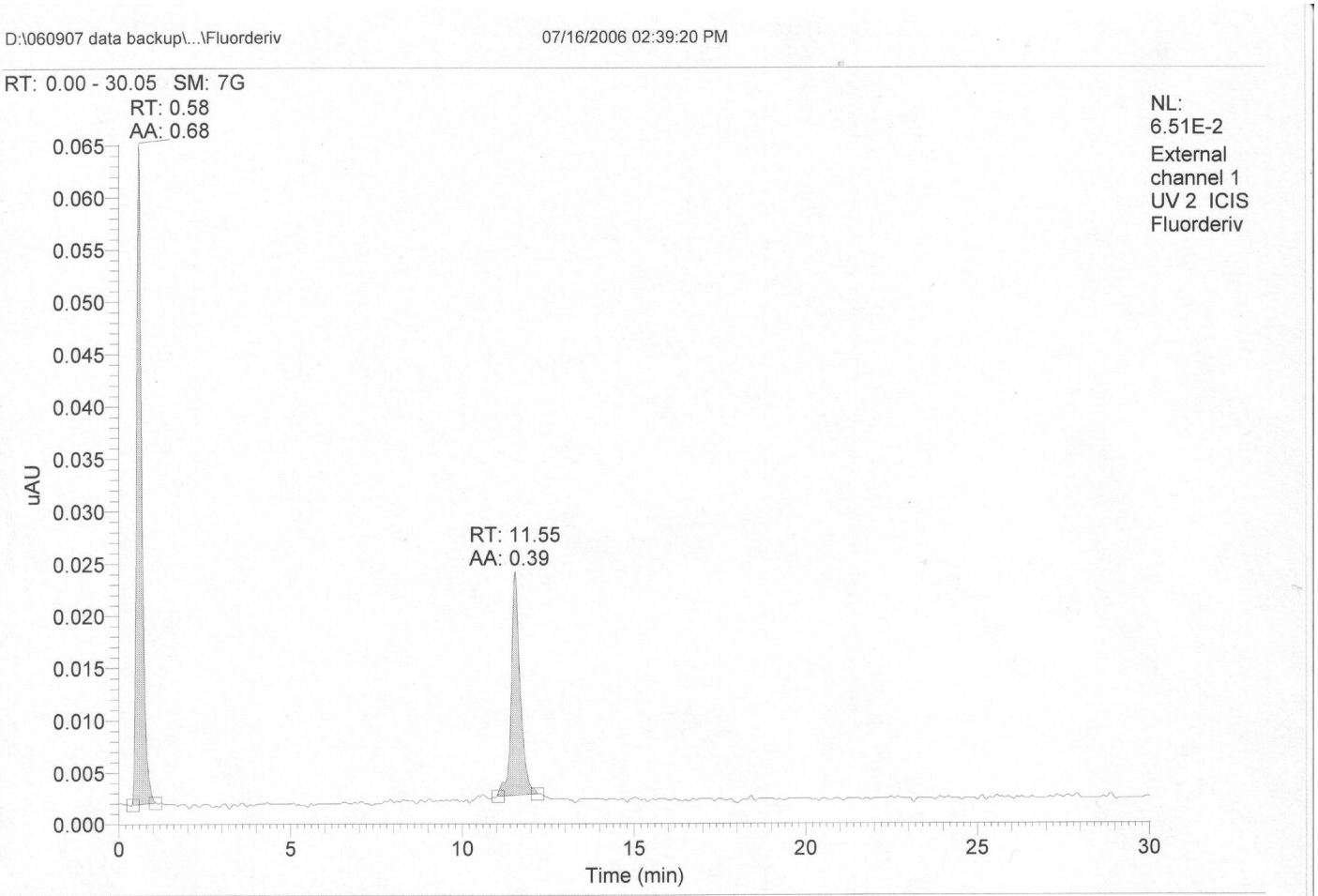

C:\Xcalibur\...\LAKVI-16-02_061023135902
10/23/2006 01:59:02 PM
RT: 0.00 - 7.98 SM: 7G
RT: 7.69 NL:
AA: 7.25 7.97E-1
External
channel 1
UV ICIS
LAKVI-16-
02_06102313
5902
RT: 0.75
AA: 2.46
uAU
Time (min)

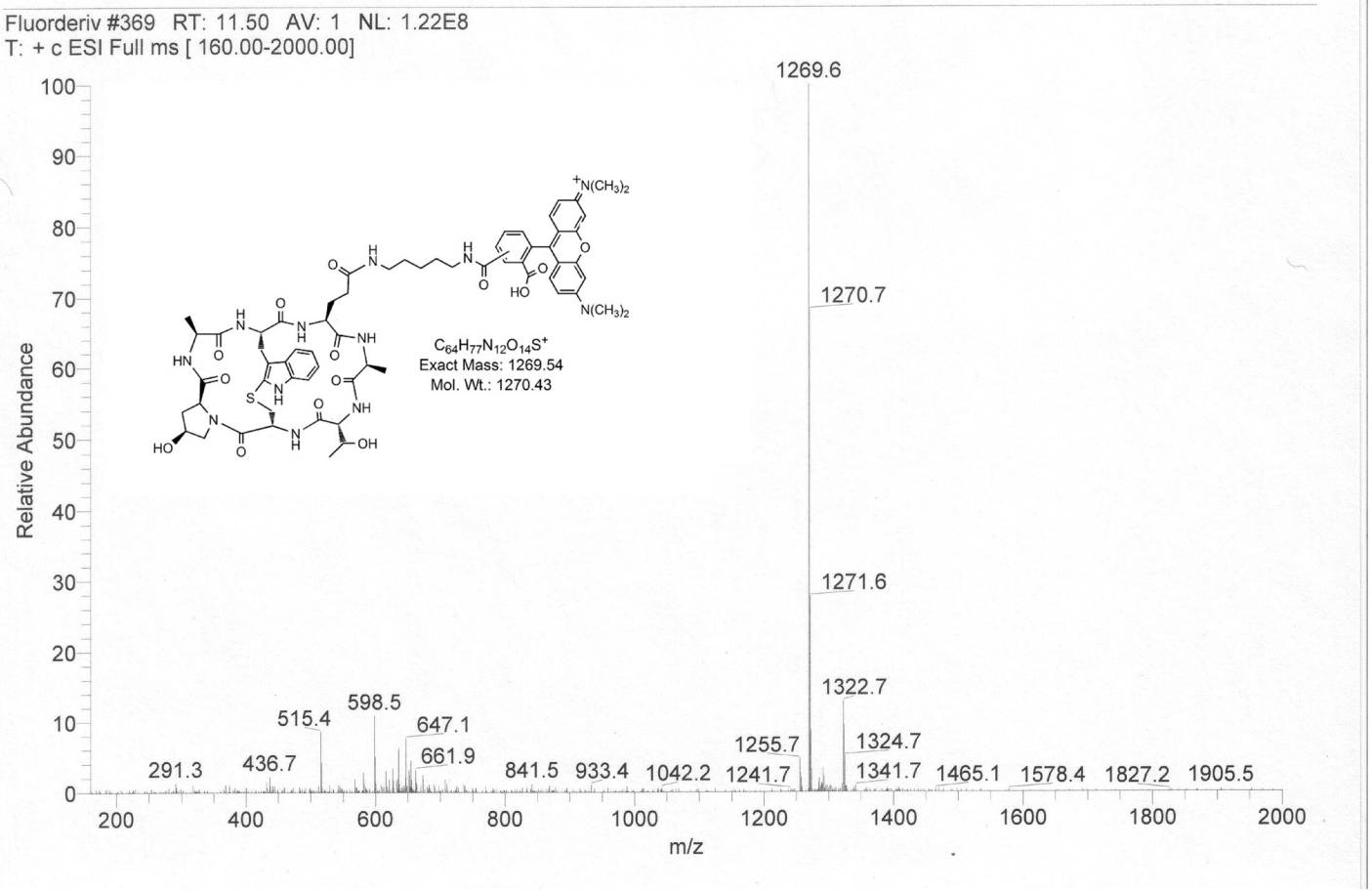

LAKVI-16-02_061023135902 #291 RT: 7.77 AV: 1 NL: 7.16E5
T: + c ESI Full ms [ 150.00-2000.00]
TrtS
TIPSO
OtBu
C66H88N8O11SSi
Mol. Wt.: 1229.6
Relative Abundance
m/z


C:\Xcalibur\data\Laura\LAKVI\LAKVI-16-03
10/23/2006 05:42:14 PM
RT: 0.00 - 7.98 SM: 7G
RT: 0.89
AA: 23.05
NL:
1.00
External
channel 1
UV ICIS
LAKVI-16-
03
RT: 6.51
AA: 1.77
uAU
Time (min)


LAKVI-16-03 #243 RT: 6.51 AV: 1 NL: 7.39E7
T: + c ESI Full ms [ 150.00-2000.00]
985.3
986.3
987.2
156.8
929.4
1007.4
1008.5
1968.8
158.9
341.2
545.0
679.0
810.9
901.3
1182.5
1388.1
1504.7
1743.7
1942.5
Relative Abundance
m/z
TIPSO
OtBu
C47H72N8O11SSi
Mol. Wt.: 985.27


C34H44N8O11S
Exact Mass: 772.29
Mol. Wt.: 772.83
3.345
3.310
2.526
2.523
2.520
2.517
2.513
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
STANDARD PROTON PARAMETERS
Archive directory:
Sample directory:
Pulse Sequence: s2pul


D:\060907 data backup\...\Fluorderiv
07/16/2006 02:39:20 PM
RT: 0.00 - 30.05 SM: 7G
RT: 0.58
AA: 0.68
NL:
6.51E-2
External
channel 1
UV 2 ICIS
Fluorderiv
RT: 11.55
AA: 0.39
uAU
Time (min)

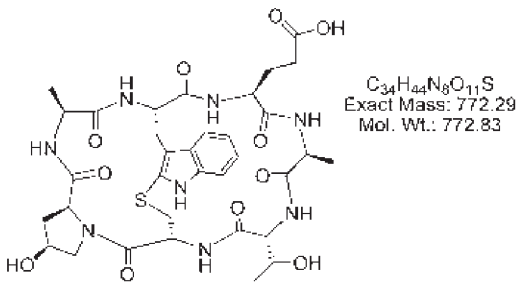

Fluorderiv #369 RT: 11.50 AV: 1 NL: 1.22E8
T: + c ESI Full ms [ 160.00-2000.00]
C64H77N12O14S+
Exact Mass: 1269.54
Mol. Wt.: 1270.43
Relative Abundance
m/z
1269.6
1270.7
1271.6
1322.7
1324.7
1255.7
1341.7
1465.1
1578.4
1827.2
1905.5
598.5
515.4
647.1
661.9
291.3
436.7
841.5
933.4
1042.2
1241.7

Lokey Schuresko (UCSC) ~~2-1~~ 1-2 ACN/CHCA/MIX1A

[redacted]

Ref. #2

2.0E+4

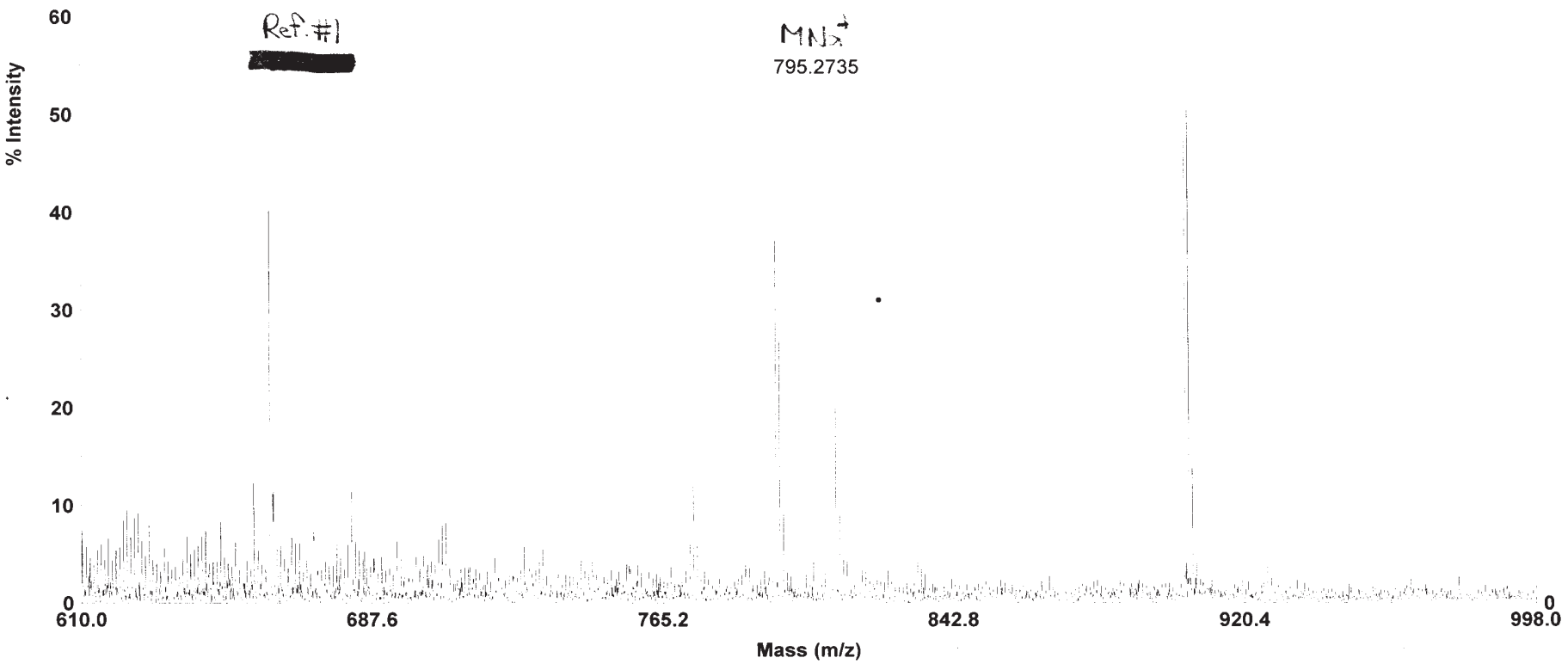


$C_{34}H_{44}N_8O_{11}S$
Exact Mass: 772.29
Mol. Wt.: 772.83

Ref. #1

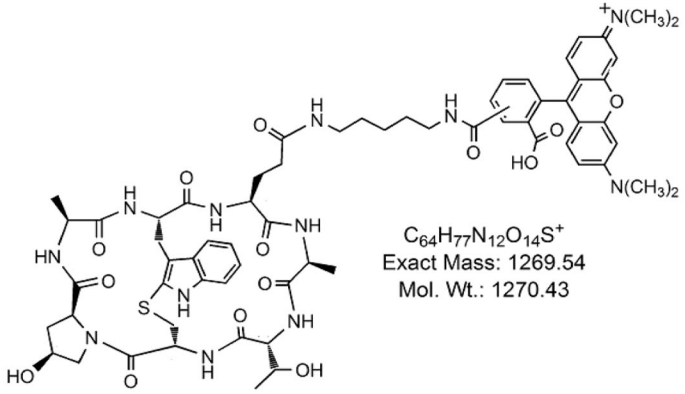
[redacted]

MNa+
795.2735

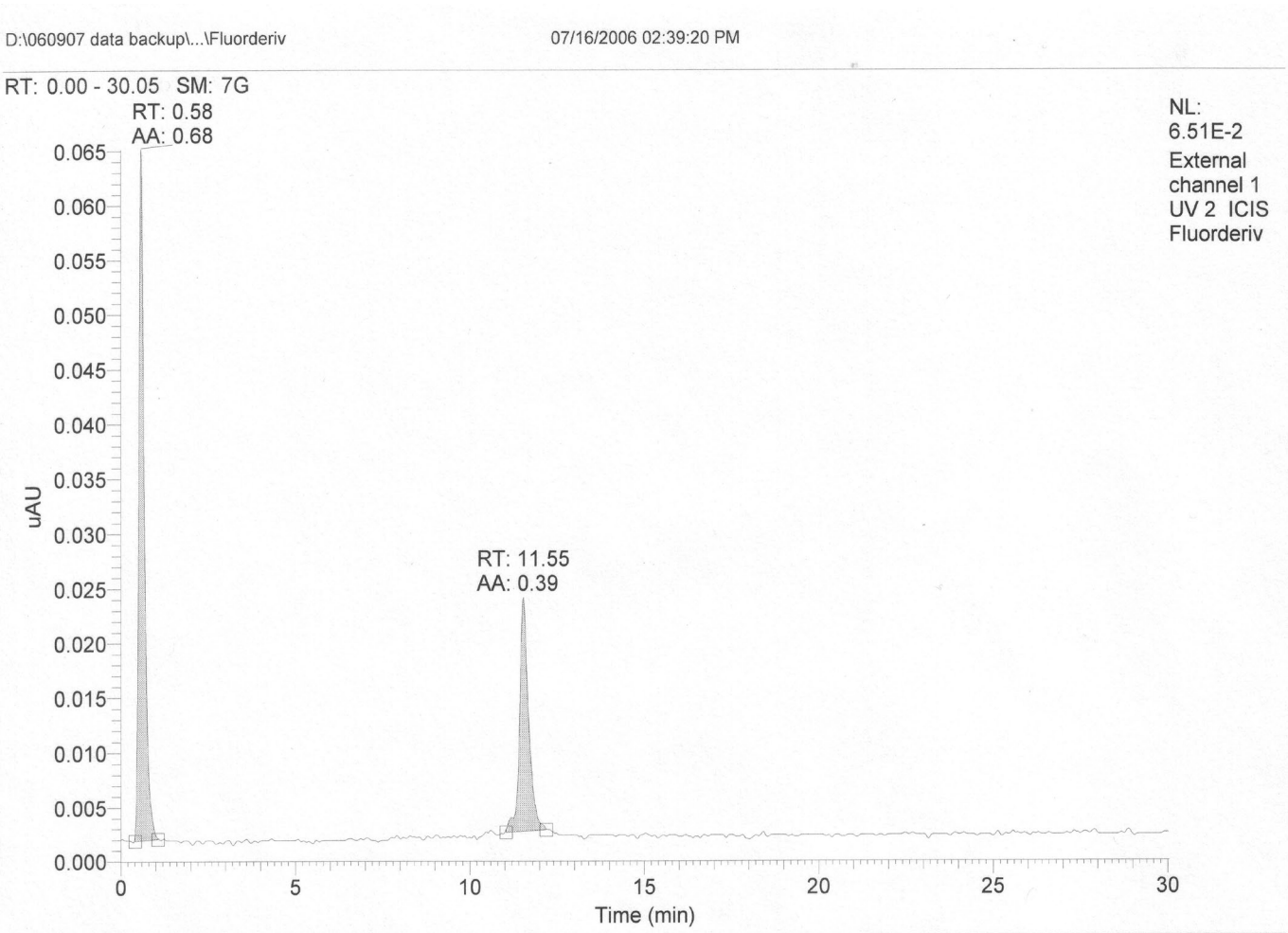


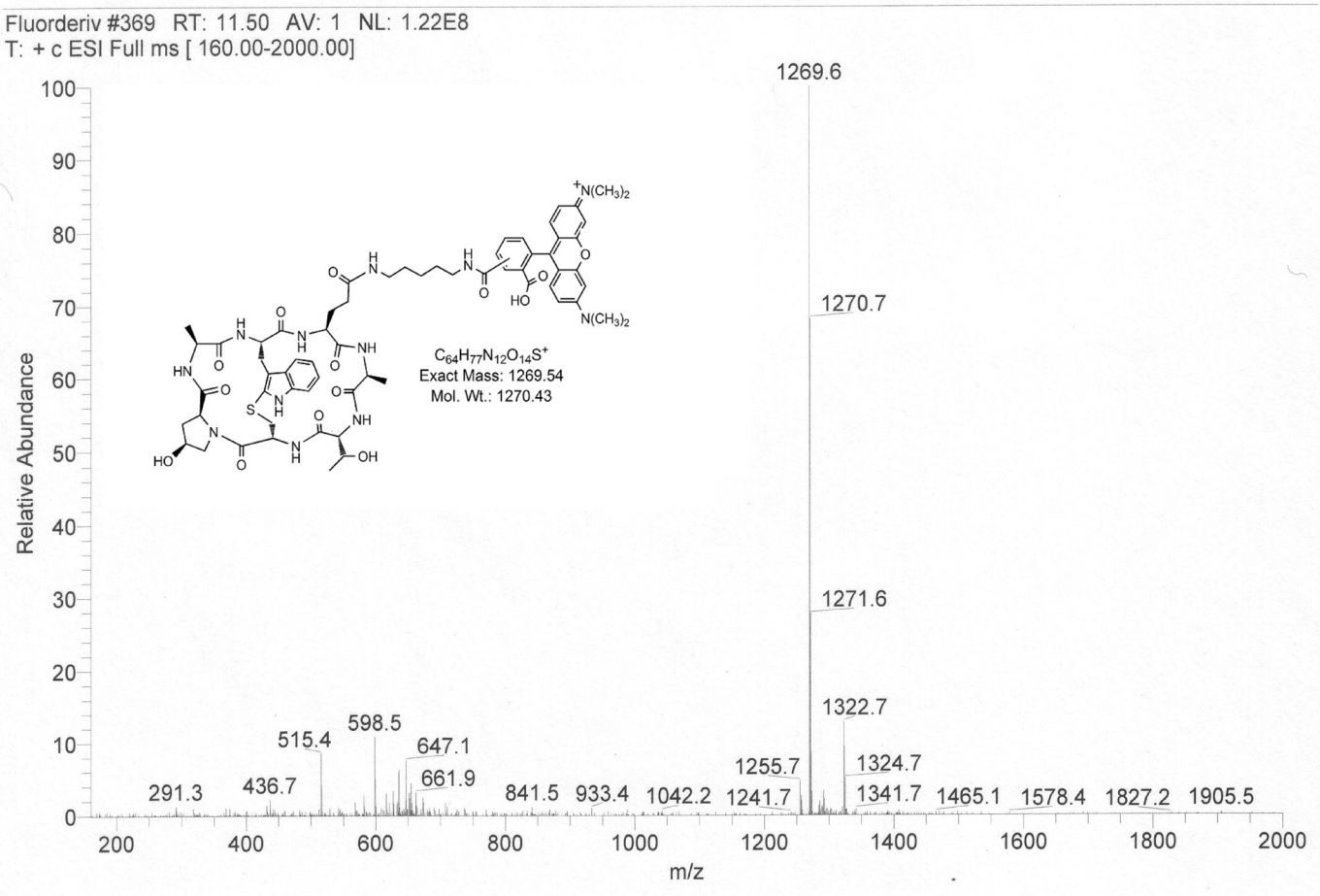

Lokey Schuresko (UCSC) 2-1 ACN/CHCA/MIX1A
D:\...\8210602p.dat
Acquired: 10:15:00, August 21, 2006

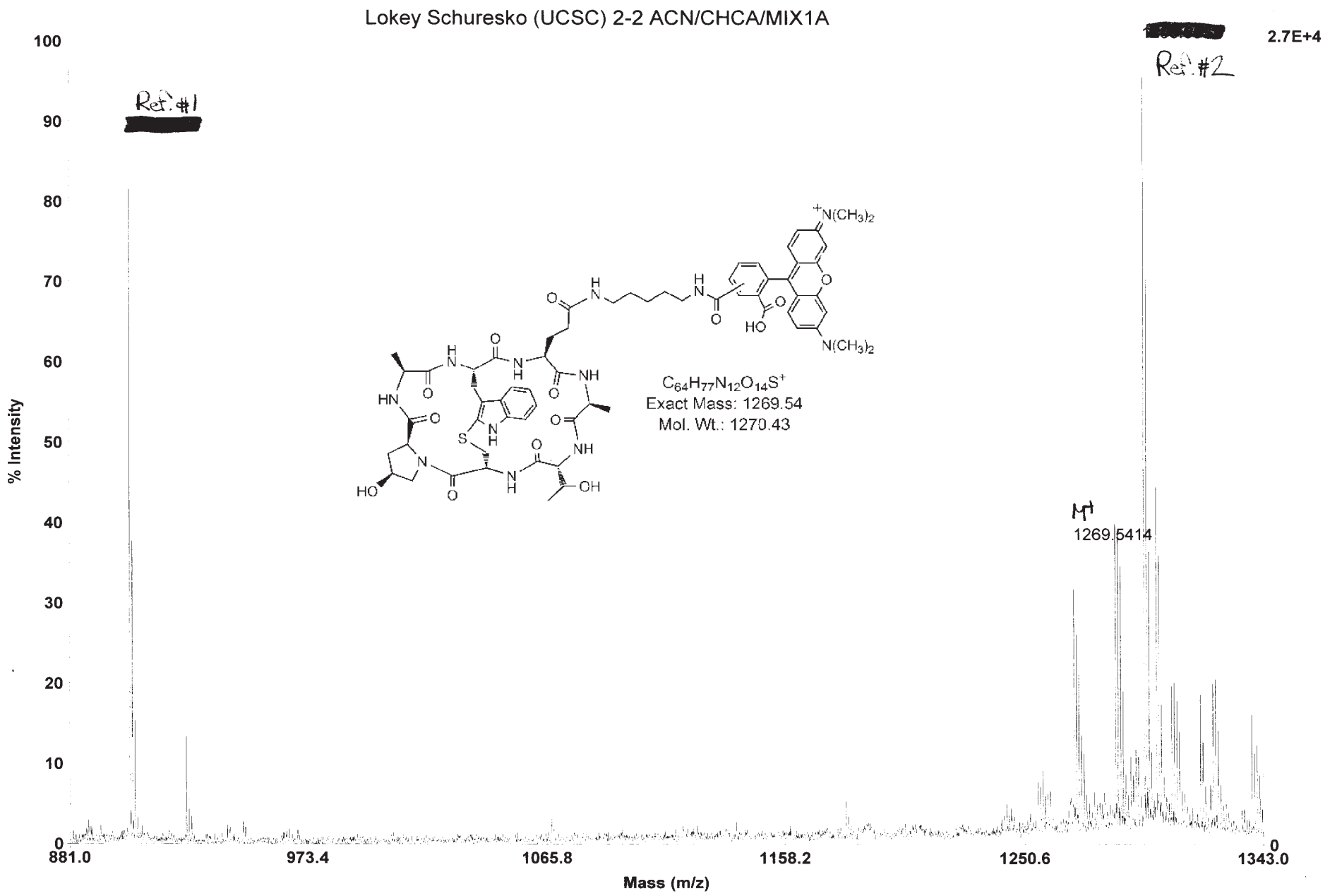
27 Nov 2006



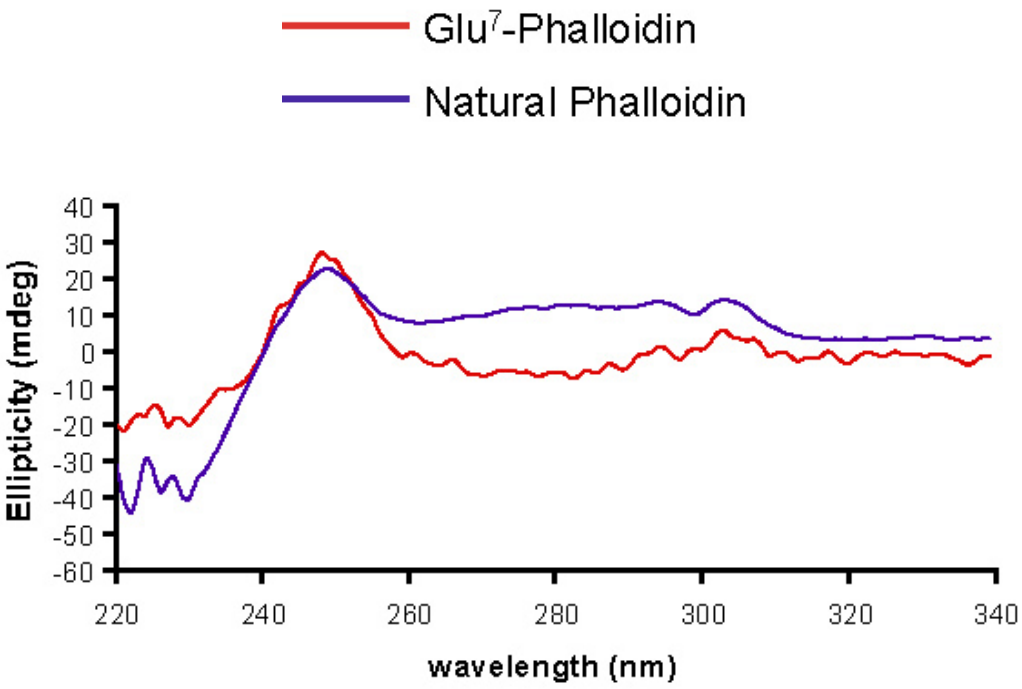
$C_{64}H_{77}N_{12}O_{14}S^+$
Exact Mass: 1269.54
Mol. Wt.: 1270.43


D:\060907 data backup\...\Fluorderiv
07/16/2006 02:39:20 PM
RT: 0.00 - 30.05 SM: 7G
RT: 0.58
AA: 0.68
NL:
6.51E-2
External
channel 1
UV 2 ICIS
Fluorderiv
RT: 11.55
AA: 0.39
uAU
Time (min)


Fluorderiv #369 RT: 11.50 AV: 1 NL: 1.22E8
T: + c ESI Full ms [ 160.00-2000.00]
Relative Abundance
m/z
1269.6
1270.7
1271.6
1322.7
1324.7
1341.7
1465.1
1578.4
1827.2
1905.5
1255.7
1241.7
1042.2
933.4
841.5
661.9
647.1
598.5
515.4
436.7
291.3
C64H77N12O14S+
Exact Mass: 1269.54
Mol. Wt.: 1270.43




Lokey Schuresko (UCSC) 2-2 ACN/CHCA/MIX1A
D:\...\8210604p.dat
Acquired: 11:55:00, August 21, 2006




CD spectra for natural phalloidin and synthetic Glu7-phalloidin in methanol (0.3 mM).