Supporting Information

A chemically accessible Hsp 90 inhibitor that does not cause induction of the heat shock response

Yen Chin Koay[a], Jeanette R. McConnell[a], Yao Wang[a], Seong Jong Kim[a], Laura Buckton[a], Flora Mansour[a] and Shelli R. McAlpine[a]*
[a]*School of Chemistry, The University of New South Wales, Kensington, NSW 2052 Australia.*
*Corresponding author Email: s.mcalpine@unsw.edu.au

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General Remarks

All chemicals were purchased from commercial suppliers (Chem-Impex International, Peptide International and GL-Biochem) and used without further purification. All moisture sensitive reactions were performed under nitrogen gas and was monitored by thin-layer chromatography (TLC) and liquid-chromatography mass spectrometry (LC/MS). TLC was performed on aluminium silica gel sheets 250 μm Whatman® (4861-820) using UV light (̀λ = 254 nm) as visualizing method. The developing agents for TLC include potassium permanganate (general purpose) and ninhydrin (for amine groups detection).

SiliCycle SiliaFlash silica gel (60 Å, particle size 40-63 μm) were used for flash chromatography. ^{1}H and ^{13}C NMR spectra were obtained and recorded at 25ºC on Bruker Avance III 500 MHz and 600 MHz. Multiplicity of NMR signals were represented by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, dd = doublet of doublet.

High-resolution mass spectrometry (HRMS) analyses were recorded on a Thermo LTQ Orbitrap XL ESI/APCI with UPLC system at the Bioanalytical Mass Spectrometry Facility in Mark Wainwright Analytical Centre at the University of New South Wales.

LC/MS analyses were performed on Shimadzu Prominence High performance LCMS 2010EV system (Water Symmetry® C18 column, 3.5 μm, 4.65x75 mm) connected to a Shimadzu LCMS 2010EV mass spectrometer. The mobile phase consist of DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B) at a flow rate of 0.5 mL/min, starting at 70% solvent A, 30% solvent B.

Semi-preparative HPLC for purification was performed on Shimadzu Prominence High performance LCMS 2010EV system (Phenomenex® Jupiter C18 column, 4μm, 250x10 mm). The mobile phase was prepared by DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution were as follow: flow rate 2 mL/min; initial 70% solvent A, 30% solvent B hold for 35 min; at 35 min 100% solvent B hold for 18 min; at 53 min 70% solvent A, 30% solvent B hold for 7 min.

Synthesis: Experimental Procedures

General solid phase peptide synthesis

Stepwise solid phase peptide synthesis (SPPS) was performed in a polypropylene solid-phase extraction cartridge fitted with a 20 μM polyethylene frit purchased from Applied Separations (Allentown, PA) and pre-loaded 2-chlorotrityl resins with an approximately 0.5 mmol/g loading scale were used. The resin was weight, transferred to the cartridge and was swelled in DMF for 30 minutes prior to peptide coupling in the corresponding sequence.

General peptide coupling

Couplings were performed in DMF solution at 0.2 M, consisting Fmoc protected amino acid (3.0 equivalents) and 1-hydroxybenzotriazole (3.0 equivalents) mixed with the resin. Diisopropylcarbodiimide was then added to activate the coupling process. Coupling was allowed to shake for a minimum of 2 hours on a shaker (Labquake tube shaker, Thermo Fisher Scientific) and checked via ninhydrin test to confirm competition. Upon completion, the coupling reaction solution was drained, and the resin was subjected to Fmoc removal. (Note: For the peptide coupling between Fmoc and *N*-methyl amino terminus, 1-hydroxybenzotriazole was replaced by 1-hydroxy-7-azabenzotriazole and the coupling process was allowed to run overnight).

General Fmoc removal

After the peptide coupling process was completed, removal of Fmoc protecting group was performed according to the following steps: DMF wash (3 × 1 minute), 20% Piperidine/DMF (1 × 5 minutes), 20% Piperidine/DMF(1 × 10 minutes), DMF wash (2 × 1 minute), IPA wash (1 × 1 minute), DMF was (1 × 1 minute), IPA (1 × 1 minute), DMF (3 × 1 minute). The complete removal of Fmoc group was verified via ninhydrin test. The resin was then ready for the next amino acid coupling.

General *N*-terminal solid phase amine deprotection

Once the desired length of amino acid residue has been generated, the peptide-bound resin was treated for the final removal of the Fmoc group: DMF wash (3 × 1 minute), 20% Piperidine/DMF (1 × 5 minutes), 20% Piperidine/DMF (1 × 10 minutes), DMF wash (3 × 1 minute), IPA wash (3 × 1 minute), MeOH (3 × 1 minute). Upon the completion of Fmoc group removal via ninhydrin test, the peptide- bound resin was then dried *in vacuo* overnight.

Resin cleavage of linear peptide

The eventual cleavage of linear pentapeptide from resin was performed by swelling the resin in a mixed solution of 1:1 (v/v) TFE/CH_2Cl_2 (10 millilitres/gram of dried resin) and was allow to stir for 24 hours. The suspension was then filtered through a Büchner filter, and the resin was washed repeatedly with additional CH_2Cl_2 to fully extract the cleaved peptide. The filtrate was then evaporated and dried *in vacuo* overnight. The dried solid was eventually re-dissolved in CH_2Cl_2, co-evaporated with CH_2Cl_2 several times to remove the entrapped TFE residue completely and was dried *in vacuo* overnight.

Macrocyclization procedure (syringe pump)

Macrocyclization of the double deprotected linear pentapeptide using a combination of three coupling agents (DMTMM, HATU, and TBTU) at 0.8 equivalent each, together with DIPEA (8.0 equivalents) in 75% of a calculated volume of anhydrous CH_2Cl_2 to generate a 0.001 M overall concentration. The crude and dry double deprotected linear peptide (DDLP) was dissolved in the remaining amount of CH_2Cl_2. The DDLP solution was then added to the bulk solution drop-wise using a syringe pump, over 2 hours. After the addition of all DDLP, the reaction was stirred overnight and was monitored via LCMS and generally complete in 1-2 hours. Upon completion, the crude product was subjected to acid-base wash to remove excess DIPEA and coupling agents. The resulting crude product was first purified by flash column chromatography, followed by reversed-phase HPLC, using a gradient of acetonitrile and deionized water with 0.1% TFA to afford the final pure compounds.

Synthesis of biotinylated compounds

Boc Removal

The Boc protecting group on tagged compounds were removed using a mixture of TFA/CH_2Cl_2 (1:4, 0.1 M overall concentration) and anisole (2.0 equivalents). The reaction was run at room temperature under open atmosphere and monitored via TLC and LC/MS every 30 min. Upon completion the reaction solution was evaporated with addition of CH_2Cl_2 to remove excess TFA and the then concentrated *in vacuo*.

Peg-Biotin attachment

After the removal of the Boc groups on compound **1**-tag and compound **7**-tag compounds, the free amines at the lysine residues of the tagged compounds were then biotinylated using *N*-hydroxysuccinimydyl-d-biotin-15-amino-4,7,10,13-tetraoxapentadecylate (NHS-dPEG$_4$-biotin, 1.4 equivalents), and base DIPEA (8.0 equivalents) in CH_2Cl_2 (0.1 M) under nitrogen gas. The reaction mixture was then stirred and monitored by LC/MS. Upon completion, the crude mixture was purified via RP-HPLC to yield the biotinylated compounds.

Solubility Determination

1 mg of each SM145, compound **1** and **7** were added in three separate vials and stirred in 1.0 mL of mixture ethanol: Milli-Q water (8:2), and were allowed to stir for 30 minutes until the solution turned clear. This was followed the by subsequent adding of 1 mg of each compound to the respective vial. The compounds were then stirred for another half an hour until it turned clear. The process was repeated until the clear solution

turned into a white gel. The observations indicated that the solution containing SM145 became saturated and no longer soluble when 5.2 mg of SM145 were added, whereas addition of 2 mg of compound **1** turned the solution into a white gel. Solution containing compound **7** remained clear and turned into a white gel only when 10.0 mg of compound **7** was added into the solution, whereas the solution containing compound **5** and **6** turned into a gel solution after the addition of 1.5 mg and 1.9 mg of each compound to the respective vial.

Experimental methods for compound 1

Dipeptide Resin-O-Leu-*N*-Me-Val-Fmoc was synthesized following "**General peptide coupling**" procedure, by using 1.00 g (0.5 mmol, 1.0 equivalent) of Resin-O-Leu-NH_2, 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-*N*-Me-Val-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound dipeptide.

Resin-O-Leu-*N*-Me-Val-NH_2

The dipeptide Resin-O-Leu-*N*-Me-Val-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-Fmoc

Following "**General peptide coupling**" procedure for SPPS, tripeptide Resin-O-Leu-*N*-Me-Val-D-Leu-Fmoc was synthesized using Resin-O-Leu-*N*-Me-Val-NH_2 synthesized from the previous peptide coupling, together with 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-OH, 0.20 g of HOAt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tripeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2

The tripeptide Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-Fmoc

Tetrapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-Fmoc was synthesized following "**General peptide coupling"** procedure for SPPS, which Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2 synthesized from the previous peptide coupling, together with 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tetrapeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-NH_2

The tetrapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-Fmoc

Pentapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-Fmoc was synthesized following "**General peptide coupling"** procedure for SPPS, using Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-NH_2 synthesized from the previous peptide coupling, together with 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-D-Ala-NH$_2$

The pentapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl- D-Ala-NH$_2$ was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

HO-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-NH$_2$

The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-NH$_2$ was generated following the **"Resin cleavage of linear peptide"** procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH_2Cl_2. The resin containing solution was filtered and dried *in vacuo* to yield the DDLP as a white solid (325 mg, overall 89%).

LC/MS (ESI): *m/z* called for $C_{42}H_{57}N_5O_6$ (M+1) = 728.43, found 728.15.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 9.4 Hz, 2H), 7.30-7.05 (m, 14H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.87 (br, 1H), 6.62 (d, *J* = 7.4 Hz, 1H), 6.59 (s, *br*, 1H), 6.35 (s, *br*, 1H), 5.83 (s, *br*, 1H), 5.08 (t, *J* = 10.9 Hz, 1H), 4.94-4.90 (m, 1H), 4.69 (d, *J* = 10.9 Hz, 1H), 4.55 (d, *J* = 11.8 Hz, 1H), 4.33 (s, *br*, 1H), 3.98 (s, *br*, 1H), 3.62 (s, *br*, 1H), 3.51 (t, *J* = 12.1 Hz, 1H), 3.06 (s, 3H), 2.93-2.90 (m. 1H), 2.29-2.24 (m. 1H), 2.15-2.11 (m. 1H), 1.53 (m, 2H), 1.40 (s, *br*, 1H), 1.19 (s, 1H), 0.99-0.61 (m, 18H).

***cyclo*-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala (Compound 1)**

Compound **1** was synthesized using 0.33 g of the DDLP generated (0.44 mmol, 1.0 equivalent), 0.11 g of TBTU (0.36 mmol, 0.80 equivalent), 0.14 g of HATU (0.36 mmol, 0.80 equivalent), 0.10 g of DMTMM (0.36 mmol, 0.80 equivalent), 0.62 mL of DIPEA (3.6 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (446 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) $HCl_{(aq)}$. The organic layer was then re-extracted with a saturated of $NaHCO_3$ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **1** as white solid (151 mg, 39%).

R_f: 0.74 (EtOAc:Hex = 0.75:0.25)

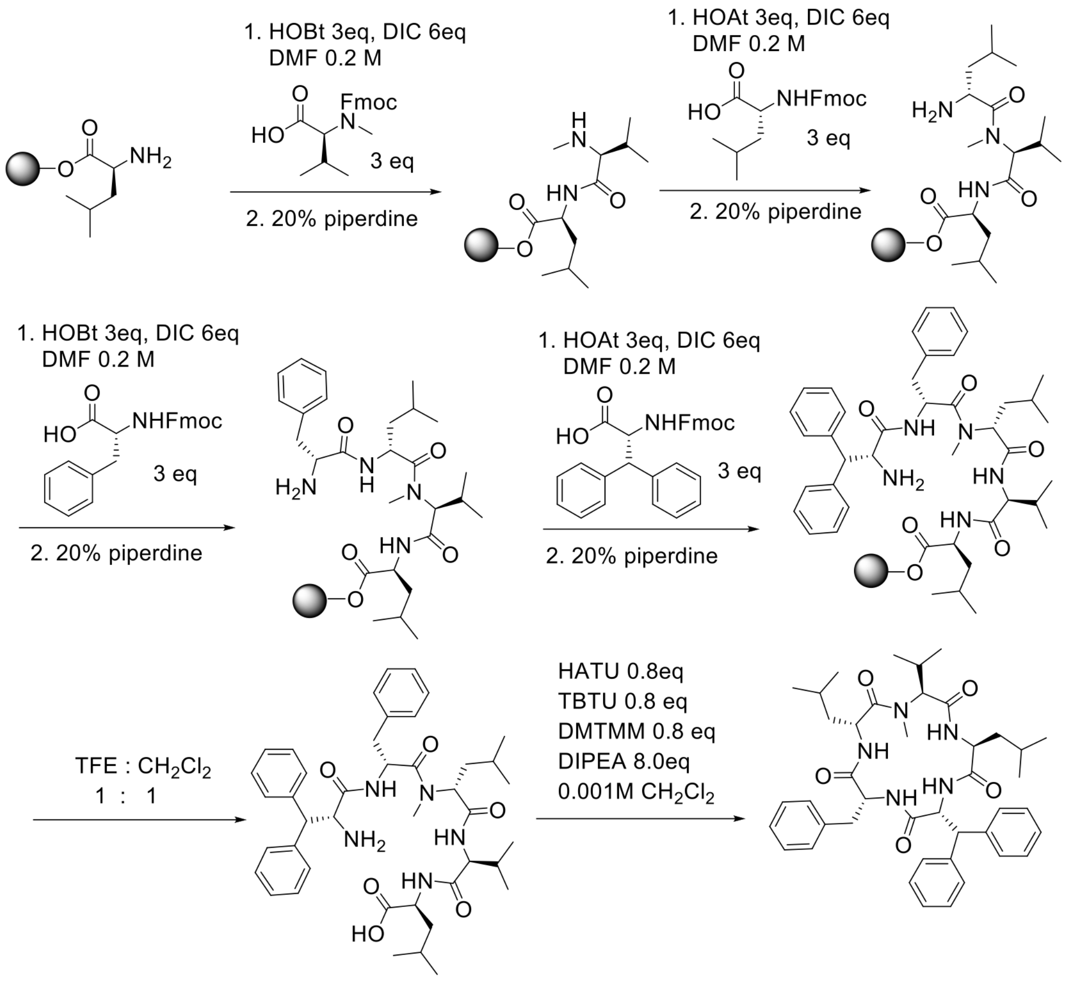
LC/MS: *m/z* called for $C_{42}H_{55}N_5O_5$ (M+1) = 710.42, found 710.10.

HRMS (ESI-TOF): M+Na$^+$, found 732.4102 $C_{42}H_{55}N_5O_5$ requires 709.4203.

^{1}H NMR (500 MHz, DMSO): δ 8.22 (m, N$\underline{H}$), 8.07 (m, N$\underline{H}$), 7.87 (d, *J* = 7.4 Hz, N$\underline{H}$), 7.72 (m, N$\underline{H}$), 7.29-7.14 (m, 13H), 6.92 (m, 2H), 5.16 (m, 1H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.43 (dd, *J* = 7.0, 15.3 Hz, 1H), 4.33 (d, *J* = 11.9 Hz, 1H), 4.15 (dd, *J* = 7.2, 15.6 Hz, 1H), 3.46 (dd, *J* = 7.4, 14.9 Hz, 1H), 2.77 (m, 1H), 2.70 (m, 1H), 2.62 (s, 3H), 2.16 (m, 1H), 1.61 (m, 1H), 1.46 (m, 1H), 1.37 (m, 3H), 1.22 (m, 1H), 0.92-0.63 (m, 18H).

^{13}C NMR (125 MHz, DMSO): δ 170.71, 170.22, 169.91, 169.80, 168.82, 141.78, 141.11, 137.38, 129.32, 129.03, 128.80, 128.74, 128.59, 128.47, 128.38, 128.12, 127.10, 126.91, 126.76, 126.60, 63.17, 58.14, 57.35, 56.44, 53.75, 51.90, 48.10, 40.87, 37.96, 37.83, 30.21, 25.23, 24.59, 24.50, 23.11, 22.81, 22.54, 18.86, 18.14.

Scheme 1: Synthesis of compound 1



Experimental methods for compound 1_TagII

Dipeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Fmoc was synthesized following "**General peptide coupling"** procedure, by using 1.00 g (0.5 mmol, 1.0 equivalent) of Resin-O- D-Phe-NH_2, 0.69 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala -OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound dipeptide.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-NH_2

The dipeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-Fmoc

Following "**General peptide coupling"** procedure for SPPS, tripeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-Fmoc was synthesized using Resin-O-D-Phe-3,3-Diphenyl-D-Ala-NH_2 synthesized from the previous peptide coupling, together with 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-Lys(Boc)-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tripeptide.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2

The tripeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-Fmoc

Tetrapeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-Fmoc was synthesized following "**General peptide coupling"** procedure for SPPS, which Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2 synthesized from the previous peptide coupling, together with 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-*N*-Me-Val-OH, 0.20 g of HOAt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tetrapeptide.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2

The tetrapeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu-Fmoc

Pentapeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu-Fmoc was synthesized following "**General peptide coupling"** procedure for SPPS, using Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2 synthesized from the previous peptide coupling, together with 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide.

Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu-NH_2

The pentapeptide Resin-O-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

HO-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu-NH_2

The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-NH_2 underwent cleavage following the **"Resin cleavage of linear peptide"** procedure for SPPS, using a mixed solution of 6 mL of TFE and 6 mL of CH_2Cl_2. The resin containing solution was filtered and dried *in vacuo* to yield the DDLP as a white solid (180 mg, overall 42%).

LC/MS (ESI): *m/z* called for $C_{47}H_{66}N_6O_8$ (M+1) = 843.49, found 843.20.

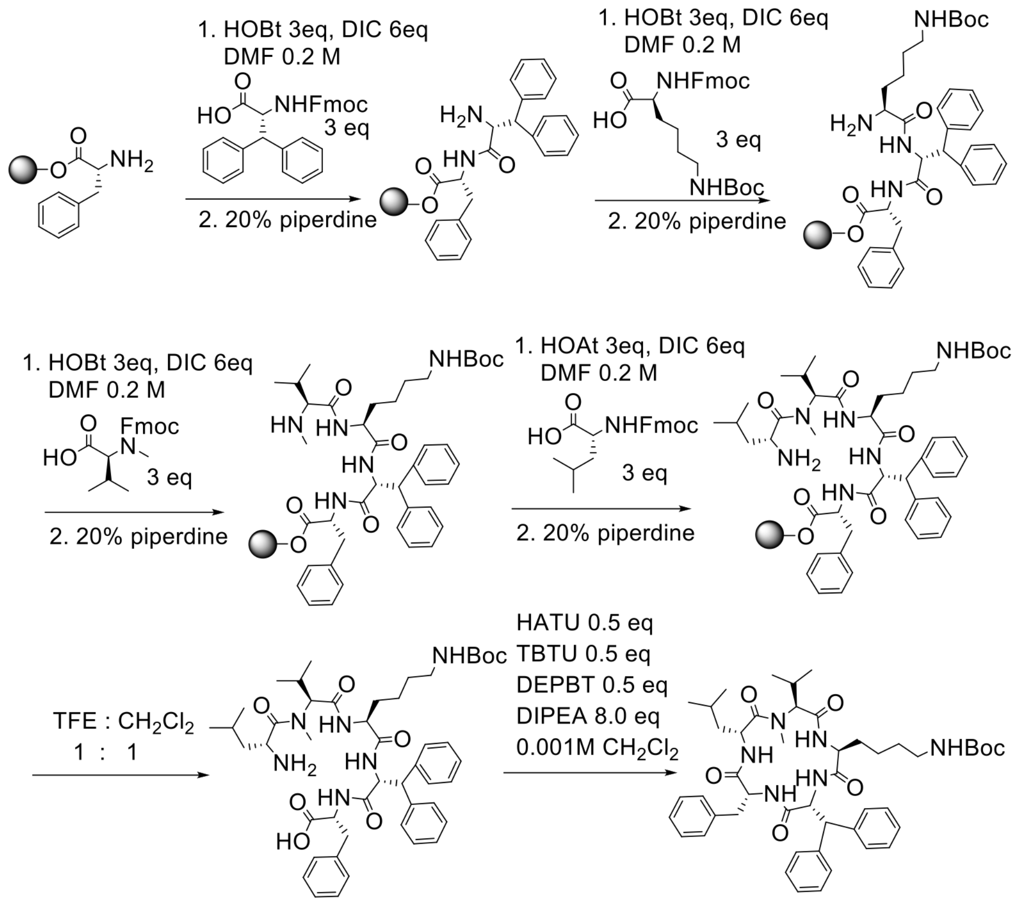
^{1}H NMR (300 MHz, DMSO) δ 8.70-7.62 (m, 3H), 7.45-7.02 (m, 15H), 6.80-6.68 (m, 1H), 4.72-4.58 (d, J = 11.4 Hz, 1H), 4.41-4.17 (m, 4H), 4.08-3.98 (m, 2H), 3.03-2.92 (m, 1H), 2.91-2.84 (s, 3H), 2.82-2.63 (m, 4H), 2.33-2.03 (m, 1H), 1.88-1.71 (br, 1H), 1.68-1.53 (m, 1H), 1.52-1.31 (m, 11H), 1.27-1.21 (s, 1H), 1.19-0.99 (m, 4H), 0.94-0.69 (m, 12H).

***cyclo*-D-Phe-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-D-Leu (Compound 1_TagII)**

Macrocyclization of 0.15 g DDLP compound **1** (0.18 mmol, 1.0 equivalent) was achieved using 0.029 g of TBTU (0.089 mmol, 0.50 equivalent), 0.023 g of HATU (0.089 mmol, 0.50 equivalent), 0.025 g of DMTMM (0.089 mmol, 0.50 equivalent), 0.37 mL of DIPEA (2.14 mmol, 12.0 equivalents) in anhydrous CH_2Cl_2 (178 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The reaction was stirred overnight and the reaction was monitored via TLC and LC/MS. Upon completion, the reaction mixture was

subjected to acid-base wash to afford crude product, which was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **1**_TagII as white solid (19.4 mg, 13.2%).
LC/MS (ESI): *m/z* called for $C_{47}H_{64}N_6O_7$ (M+1) = 825.48, found 825.20.
^{1}H NMR (300 MHz, CDCl3) δ 7.39-7.13 (m, 15H), 7.12-7.04 (d, *J* = 6.6 Hz, 1H), 6.96-6.83 (m, 2H), 6.68-6.60 (br, 1H), 6.60-5.95 (d, *J* = 6.0 Hz, 1H), 5.10-5.00 (t, *J* = 8.9 Hz, 1H), 4.81-4.74 (d, *J* = 9.3 Hz, 1H), 4.71-4.64 (d, *J* = 9.3 Hz, 1H), 4.57-4.48 (m, 1H), 4.39-4.25 (m, 1H), 4.10-4.00 (m, 1H), 3.20-2.99 (m, 4H), 2.97-2.92 (s, 3H), 2.37-2.20 (m, 1H), 1.70-1.54 (m, 1H), 1.49-1.44 (m, 2H), 1.43-1.36 (s, 9H), 1.35-1.17 (m, 6H), 1.15-1.02 (d, *J* = 7.3 Hz, 3H), 0.95-0.72 (d, *J* = 6.5 Hz, 9H).

Scheme 2: Synthesis of compound 1_TagII



Synthesis of biotinylated compound 1_TagII

Boc removal

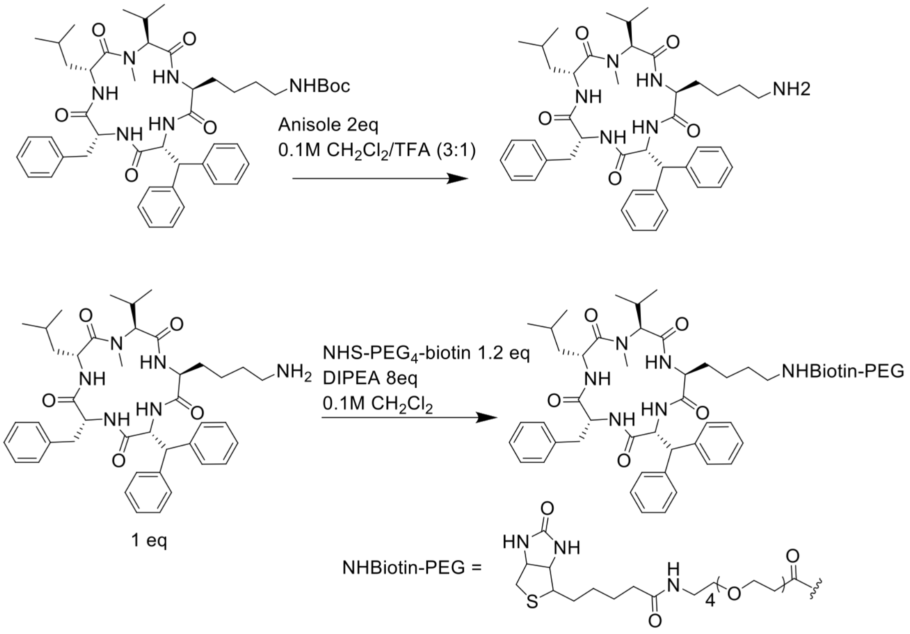
The Boc protecting group of compound **1** was removed following **"Boc removal"** procedure, utilizing a mixture of TFA/CH_2Cl_2 (1:4, 0.1 M) and anisole (2.0 equivalents) to generate free amines at lysine residue. The free amine was taken to the subsequent biotinylation reaction without purification.
LC/MS (ESI): *m/z* called for $C_{42}H_{56}N_6O_5$ (M+1) = 725.43, found 725.05.

Peg-Biotin attachment

The biotinylated compound **1** was afforded by using **"Peg-Biotin attachment"** procedure with 19.4 mg (0.027 mmol, 1.0 equivalent) of deprotected compound **1**_TagII, 22.0 mg (0.037 mmol, 1.4 equivalents) of NHS-PEG$_4$-Biotin, 27 µL (0.16 mmol, 8.0 equivalents) of DIPEA in 268 µL of CH_2Cl_2. The reaction mixture

was stirred for 4 hours and monitored via LC/MS. Upon completion, the crude product was purified using preparative HPLC to generate pure biotinylated compound in 24.9% yield as white solid.
R_f: 0.78 (EtOAc:MeOH = 0.60:0.40)
LC/MS (ESI): *m/z* called for $C_{63}H_{91}N_9O_{12}S$ (M+1) = 1198.65, found 1198.10.
HRMS (ESI-TOF): M+Na$^+$, found 1220.6414 $C_{63}H_{91}N_9O_{12}S$ requires 1197.6508.
^{1}H NMR (600 MHz, MeOD) δ 7.30-7.26 (m, 13H), 7.01 (d, *J* = 7.1 Hz, 2H), 5.21 (d, *J* = 11.3 Hz, 1H), 4.72-4.61 (m, 1H), 4.48-4.33 (m, 2H), 4.14-4.09 (m, 1H), 3.82-3.77 (m, 1H), 3.74 (t, *J* = 6.2 Hz, 2H), 3.66-3.62 (m, 12H), 3.57-3.55 (m, 3H), 3.39-3.38 (m, 2H), 3.25-3.16 (m, 1H), 3.11-2.94 (m, 5H), 2.84 (s, 3H), 2.67-2.62 (m, 2H), 2.45 (t, *J* = 6.2 Hz, 2H), 2.32-2.24 (m, 4H), 1.80-1.58 (m, 8H), 1.50-1.30 (m, 4H), 1.24-1.16 (m, 1H), 1.11-1.05 (m, 2H), 1.00-0.98 (m, 3H), 0.86-0.81 (m, 8H).
^{13}C NMR (150 MHz, MeOD): δ 174.60, 172.59, 172.43, 172.33, 171.27, 170.69, 170.01, 164.69, 140.68, 140.34, 136.63, 128.73, 128.72, 128.54, 128.30, 128.24, 128.19, 128.14, 128.01, 127.67, 126.89, 126.82, 126.65, 126.40, 126.13, 70.56, 70.19, 70.17, 70.13, 70.07, 69.96, 69.86, 69.20, 66.92, 64.15, 63.92, 62.64, 62.16, 58.03, 57.37, 56.40, 55.63, 53.96, 53.18, 53.16, 48.17, 39.74, 39.01, 39.82, 36.27, 28.38, 26.74, 25.47, 25.29, 25.14, 25.07, 23.99, 23.04, 22.13, 20.88, 18.76, 17.85.

Scheme 3: Synthesis of biotinylated compound 1_TagII



Experimental methods for compound 2

HO- Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2

Following "**General peptide coupling**" procedure for SPPS and the similar peptide coupling sequence as mentioned for compound **1**, except that the forth peptide coupling was substituted by Fmoc- D-3-(2-Pyridyl)Ala-OH. The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2 was generated through the cleavage of the following **"Resin cleavage of linear peptide"** procedure (270 mg, overall 75%).

LC/MS (ESI): *m/z* called for $C_{41}H_{56}N_6O_6$ (M+1) = 729.43, found 729.05.

cyclo-Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala (Compound 2)

Compound **2** was synthesized using 0.14 g of the DDLP generated (0.19 mmol, 1.0 equivalent), 0.05 g of TBTU (0.15 mmol, 0.80 equivalent), 0.06 g of HATU (0.15 mmol, 0.80 equivalent), 0.04 g of DMTMM (0.15 mmol, 0.80 equivalent), 0.27 mL of DIPEA (1.54 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (192 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **2** as white solid (72 mg, 29%).

R_f: 0.69 (EtOAc:MeOH = 0.90:0.10)

LC/MS (ESI): *m/z* called for $C_{41}H_{54}N_6O_5$ (M+1) = 711.42, found 711.

^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, br, 1H), 8.02 (s, br, 1H), 7.74 (s, br, 1H), 7.45-7.08 (m, 14H), 6.89 (s, br, 1H), 5.22-4.64 (m, 3H), 4.60-4.39 (m, 1H), 3.74-3.20 (m, 1H), 3.03-2.81 (m, 3H), 2.45-2.29 (m, 1H), 2.29-2.06 (m, 1H), 1.76-1.47 (s, br, 3H), 1.46-1.07 (m, 5H), 1.05-0.15 (m, 18H).

Experimental methods for compound 3

HO- Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2

Following "**General peptide coupling"** procedure for SPPS and the similar peptide coupling sequence as mentioned for compound **1**, except that the forth peptide coupling was substituted by Fmoc- D-3-(3-Pyridyl)Ala-OH. The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2 was generated through the cleavage of the following **"Resin cleavage of linear peptide"** procedure (278 mg, 76%).

LC/MS (ESI): *m/z* called for $C_{41}H_{56}N_6O_6$ (M+1) = 729.43, found 729.05.

cyclo-Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala (Compound 3)

Compound **3** was synthesized using 0.28 g of the DDLP generated (0.38 mmol, 1.0 equivalent), 0.10 g of TBTU (0.30 mmol, 0.80 equivalent), 0.12 g of HATU (0.30 mmol, 0.80 equivalent), 0.08 g of DMTMM (0.30 mmol, 0.80 equivalent), 0.27 mL of DIPEA (3.04 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (380 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **3** as white solid (110 mg, 31%).

R_f: 0.69 (EtOAc:MeOH = 0.90:0.10)

LC/MS (ESI): *m/z* called for: $C_{41}H_{54}N_6O_5$ (M+1) = 711.42, found 711.10.

^1H NMR (300 MHz,$CDCl_3$)) δ 8.76 (s, 1H), 8.51 (s, 2H), 8.07 (S,1H), 7.41-7.35 (m, 2H), 7.18 (m, 6H), 7.13-7.05 (m, 2H), 5.30 (s, 2H), 5.02 (s, 1H), 4.8-4.76 (m, 2H), 4.54 (d, *J* =10.8 Hz, 1H), 3.51-3.30 (m, 4H), 2.97 (s, 1H), 2.8 (s, 2H), 0.98 (s, 1H),0.93 (m, 6H), 0.89 (m, 5H), 0.80 (d, *J* =6.29 Hz, 4H), 0.74 (m, 7H).

Experimental methods for compound 4

HO- Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2

Following "**General peptide coupling"** procedure for SPPS and the similar peptide coupling sequence as mentioned for compound **1**, except that the forth peptide coupling was substituted by Fmoc- D-3-(4-Pyridyl)Ala-OH. The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2 was generated through the cleavage of the following **"Resin cleavage of linear peptide"** procedure (350 mg, overall 96%).

LC/MS (ESI): *m/z* called for $C_{41}H_{56}N_6O_6$ (M+1) = 729.43, found 729.20.

***cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala (Compound 4)**

Compound **4** was synthesized through macrocyclization using 0.35 g of the DDLP generated (0.50 mmol, 1.0 equivalent), 0.12 g of TBTU (0.40 mmol, 0.80 equivalent), 0.15 g of HATU (0.40 mmol, 0.80 equivalent), 0.11 g of DMTMM (0.40 mmol, 0.80 equivalent), 0.67 mL of DIPEA (4 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (484 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)**" procedure. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **4** as white solid (103 mg, 30%).

R_f: 0.75 (EtOAc)

LC/MS (ESI): *m/z* called for $C_{41}H_{54}N_6O_5$ (M+1) = 711.42, found 711.20.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (*br*, 1H), 8.27 (d, *J* = 5.4 Hz, 1H), 7.85 (d, *J* = 9.2 Hz, N$\underline{H}$), 7.58 (*br*, N$\underline{H}$), 7.39-7.15 (m, 10H), 6.90 (*br*, N$\underline{H}$), 6.69 (d, *J* = 3.5 Hz, 2H), 6.51 (*br*, N$\underline{H}$), 5.27 (t, *J* = 10.7 Hz, 1H), 5.01 (m, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.59 (m, 1H), 4.39 (m, 1H), 3.73 (m, 2H), 3.11 (s, 3H), 3.02 (d, *J* = 9.8 Hz, 1H), 2.82 (*br*, 1H), 2.35 (m, 1H), 1.62 (m, 4H), 1.03-0.71 (m, 18H).

Experimental methods for compound 5

HO-Leu-*N*-Me-Val-D-Leu-D-Trp(Boc)-3,3-Diphenyl-D-Ala-NH_2

Following "**General peptide coupling**" procedure for SPPS and the similar peptide coupling sequence as mentioned for compound **1**, except that the forth peptide coupling was substituted by Fmoc- D-Trp(Boc)-OH. The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu- D-Trp(Boc)-3,3-Diphenyl-D-Ala-NH_2 was generated through the cleavage of the following **"Resin cleavage of linear peptide"** procedure (360 mg, overall 83%).

LC/MS (ESI): *m/z* called for $C_{49}H_{66}N_6O_8$ (M+1) = 867.49, found 867.15.

***cyclo*-Leu-*N*-Me-Val-D-Leu-D-Trp(Boc)-3,3-Diphenyl-D-Ala (Compound 5)**

Compound **5** was synthesized using 0.36 g of the DDLP generated (0.40 mmol, 1.0 equivalent), 0.11 g of TBTU (0.33 mmol, 0.80 equivalent), 0.13 g of HATU (0.33 mmol, 0.80 equivalent), 0.09 g of DMTMM (0.33 mmol, 0.80 equivalent), 0.59 mL of DIPEA (3 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (420 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)**" procedure. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC and "**Boc Removal**" procedure to remove the Boc protecting group, yielded compound **5** as white solid (94.5 mg, 30%).

R_f: 0.50 (Hex:EtOAc = 0.30:0.70)

LC/MS (ESI): *m/z* called for $C_{44}H_{56}N_6O_5$ (M+1) = 749.43, found 749.10.

^{1}H NMR (500 MHz, DMSO): δ 10.79 (d, *J* = 2.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.84 (m, 2H), 7.51 (d, *J* = 9.5 Hz, 1H), 7.34-6.99 (m, 14H), 6.76 (s, 1H), 5.17 (m, 1H), 4.70 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 11.5 Hz, 1H), 4.43 (dd, *J* = 7.3, 15.2 Hz, 1H), 4.27 (d, *J* = 11.8 Hz, 1H), 4.13 (m, 1H), 3.53 (dd, *J* = 7.4, 14.9 Hz, 1H), 3.08 (m, 1H), 2.94 (m, 1H), 2.63 (s, 3H), 2.16 (m, 1H), 1.50 (m, 2H), 1.38 (m, 1H), 1.26 (m, 4H), 0.90-0.63 (m, 18H).

Experimental methods for compound 6

HO-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala-NH_2

Following "**General peptide coupling**" procedure for SPPS and the similar peptide coupling sequence as mentioned for compound **1**, except that the forth peptide coupling was substituted by Fmoc- D-Tyr(Me)-OH. The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala-NH_2 was generated through the cleavage of the following **"Resin cleavage of linear peptide"** procedure (500 mg, overall 100%).

LC/MS (ESI): *m/z* called for $C_{43}H_{59}N_5O_7$ (M+1) = 758.44, found 758.55.

***cyclo*-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala (Compound 6)**

Compound **6** was synthesized using 0.50 g of the DDLP generated (0.70 mmol, 1.0 equivalent), 0.13 g of TBTU (0.50 mmol, 0.80 equivalent), 0.15 g of HATU (0.50 mmol, 0.80 equivalent), 0.11 g of DMTMM (0.50 mmol, 0.80 equivalent), 0.70 mL of DIPEA (5 mmol, 8.0 equivalents) in anhydrous CH_2Cl_2 (660 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **6** as white solid (160 mg, 32%).
R_f: 0.38 (Hex:EtOAc = 0.25:0.75)
LC/MS (ESI): *m/z* called for $C_{43}H_{57}N_5O_6$ (M+1) = 740.43, found 740.10.
^{1}H NMR (600 MHz, DMSO): δ 7.88 (m, 3N$\underline{H}$), 7.47 (d, *J* = 9.5 Hz, N$\underline{H}$), 7.29-7.18 (m, 10H), 7.00 (m, 4H), 5.18 (t, *J* = 10.7 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.41 (dd, *J* = 7.2, 15.1 Hz, 1H), 4.30 (d, *J* = 11.9 Hz, 1H), 4.11 (dd, *J* = 7.1, 14.7 Hz, 1H), 3.73 (s, 3H), 3.51 (dd, *J* = 7.2, 14.5 Hz, 1H), 2.68 (m, 1H), 2.64 (s, 3H), 2.19 (m, 1H), 1.59 (m, 1H), 1.47 (m, 1H), 1.39-1.30 (m, 3H), 0.93-0.62 (m, 18H).

Experimental methods for compound 7

Dipeptide Resin-O-Leu-*N*-Me-Val-Fmoc was synthesized following "**General peptide coupling"** procedure, by using 1.00 g (0.5 mmol, 1.0 equivalent) of Resin-O-Leu-NH_2, 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-*N*-Me-Val-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound dipeptide.

Resin-O-Leu-*N*-Me-Val-NH_2

The dipeptide Resin-O-Leu-*N*-Me-Val-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-Fmoc

Following "**General peptide coupling"** procedure for SPPS, tripeptide Resin-O-Leu-*N*-Me-Val-D-Leu-Fmoc was synthesized using Resin-O-Leu-*N*-Me-Val-NH_2 synthesized from the previous peptide coupling, together with 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-OH, 0.20 g of HOAt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tripeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2

The tripeptide Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-Fmoc

Tetrapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-Fmoc was synthesized following "**General peptide coupling"** procedure for SPPS, which Resin-O-Leu-*N*-Me-Val-D-Leu-NH_2 synthesized from the previous peptide coupling, together with 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tetrapeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-NH_2

The tetrapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Fmoc

Pentapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Fmoc was synthesized following "**General peptide coupling**" procedure for SPPS, using Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-NH$_2$ synthesized from the previous peptide coupling, together with 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide.

Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH$_2$

The pentapeptide Resin-O-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl- D-Ala-NH$_2$ was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

HO-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH$_2$

The double deprotected linear pentapeptide (DDLP) HO-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH$_2$ was generated following the **"Resin cleavage of linear peptide"** procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 7 mL of TFE and 7 mL of CH$_2$Cl$_2$. The resin containing solution was filtered and dried *in vacuo* to yield the DDLP as a pale yellow solid (372 mg, overall 93%).

LC/MS (ESI): *m/z* called for $C_{39}H_{54}N_6O_6S$ (M+1) = 735.38, found 735.15

^{1}H NMR (500 MHz, CDCl$_3$) δ 8.66 (s, 1H), 8.32 (s, 1H), 8.17 (d, *J* = 7.9 Hz, 1H). 7.49-7.16 (m, 10H), 6.90 (s, 1H), 6.87 (s, 1H), 5.35-5.31 (s, *br*, 1H), 4.88-4.74 (m, 3H), 4.55 (s, 1H), 3.15 (s, *br*, 1H), 3.05 (s, 3H), 2.34-2.30 (m, 1H), 1.68-1.51 (m. 5H), 1.36-1.26 (m, 5H), 1.07-1.06 (d, *J* = 5.8 Hz, 1H), 0.92-0.84 (m, 17H).

***cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala (Compound 7)**

Compound **7** was synthesized using 0.37 g of the DDLP generated (0.50 mmol, 1.0 equivalent), 0.13 g of TBTU (0.40 mmol, 0.80 equivalent), 0.15 g of HATU (0.40 mmol, 0.80 equivalent), 0.11 g of DMTMM (0.40 mmol, 0.80 equivalent), 0.70 mL of DIPEA (4 mmol, 8.0 equivalents) in anhydrous CH$_2$Cl$_2$ (505 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl$_{(aq)}$. The organic layer was then re-extracted with a saturated of NaHCO$_3$ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound **7** as white solid (151 mg, 40.5%).

R$_f$: 0.30 (100% EtOAc)

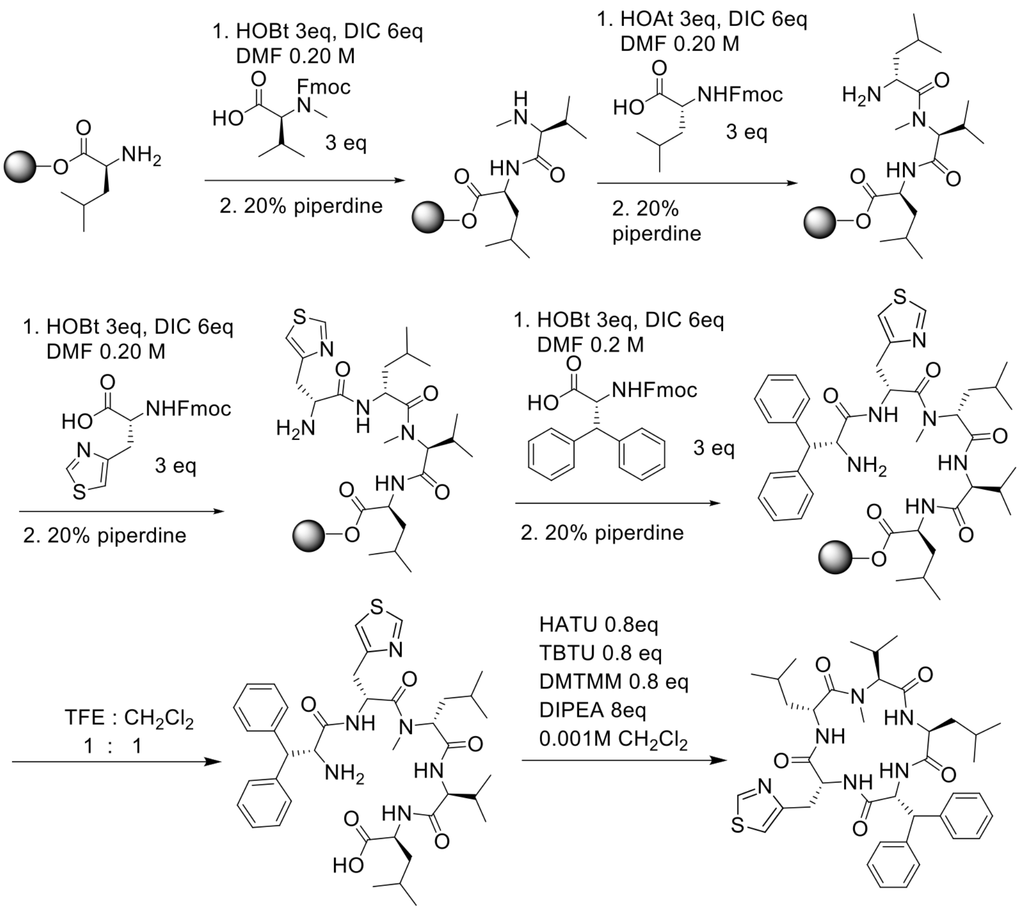
LC/MS: *m/z* called for $C_{39}H_{52}N_6O_5S$ (M+1) = 717.37, found 717.05.

HRMS (ESI-TOF): M+Na$^+$, found 739.3608 $C_{39}H_{52}N_6O_5S$ requires 716.3720.

^{1}H NMR (500 MHz, DMSO): δ 9.11 (s, 1H), 8.35 (d, *J* = 8.7 Hz, N$\underline{H}$), 7.95-7.85 (m, 2N$\underline{H}$), 7.65 (d, *J* = 9.8 Hz, N$\underline{H}$), 7.40-7.25 (m, 5H), 7.25-7.15 (m, 4H), 7.15-7.10 (m, 1H), 7.05 (s, 1H), 5.25 (m, 1H), 4.70 (d, *J* = 10.7 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 1H), 4.45 (dd, *J* = 7.8, 15.1 Hz, 1H), 4.35 (d, *J* = 12.2 Hz, 1H), 4.30 (dd, *J* = 6.5, 15.1 Hz, 1H), 2.87 (m, 2H), 2.60 (s, 3H), 2.14 (m, 1H), 1.51 (m, 2H), 1.39 (m, 1H), 1.25 (m, 4H), 0.90-0.60 (m, 18H).

^{13}C NMR (125 MHz, DMSO): δ 170.74, 169.95, 169.69, 169.46, 168.68, 154.49, 152.72, 141.63, 141.30, 128.88, 128.62, 128.56, 128.47, 127.20, 126.85, 116.28, 63.07, 56.93, 54.77, 53.78, 51.80, 47.83, 41.09, 37.97, 32.90, 30.19, 25.35, 24.49, 24.31, 23.57, 22.91, 22.64, 22.52, 21.93, 19.59, 18.85.

Scheme 4: Synthesis of compound 7



Experimental methods for compound 7_TagII

Dipeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-Fmoc was synthesized following "**General peptide coupling"** procedure, by using 1.00 g (0.5 mmol, 1.0 equivalent) of Resin-O-D-Leu-NH_2, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound dipeptide.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-NH_2

The dipeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Fmoc

Following "**General peptide coupling"** procedure for SPPS, tripeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Fmoc was synthesized using Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-NH_2 synthesized from the previous peptide coupling, together with 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tripeptide.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH_2

The tripeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH_2 was synthesized following the

"General Fmoc removal" procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-Fmoc

Tetrapeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-Fmoc was synthesized following "**General peptide coupling**" procedure for SPPS, which Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH_2 synthesized from the previous peptide coupling, together with 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-Lys(Boc)-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound tetrapeptide.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2

The tetrapeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-Fmoc

Pentapeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-Fmoc was synthesized following "**General peptide coupling**" procedure for SPPS, using Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-NH_2 synthesized from the previous peptide coupling, together with 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-*N*-Me-Val-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. The coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide.

Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2

The pentapeptide Resin-O-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2 was synthesized following the **"General Fmoc removal"** procedure for SPPS. A positive ninhydrin was used to confirm the completion removal of Fmoc group.

HO-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2

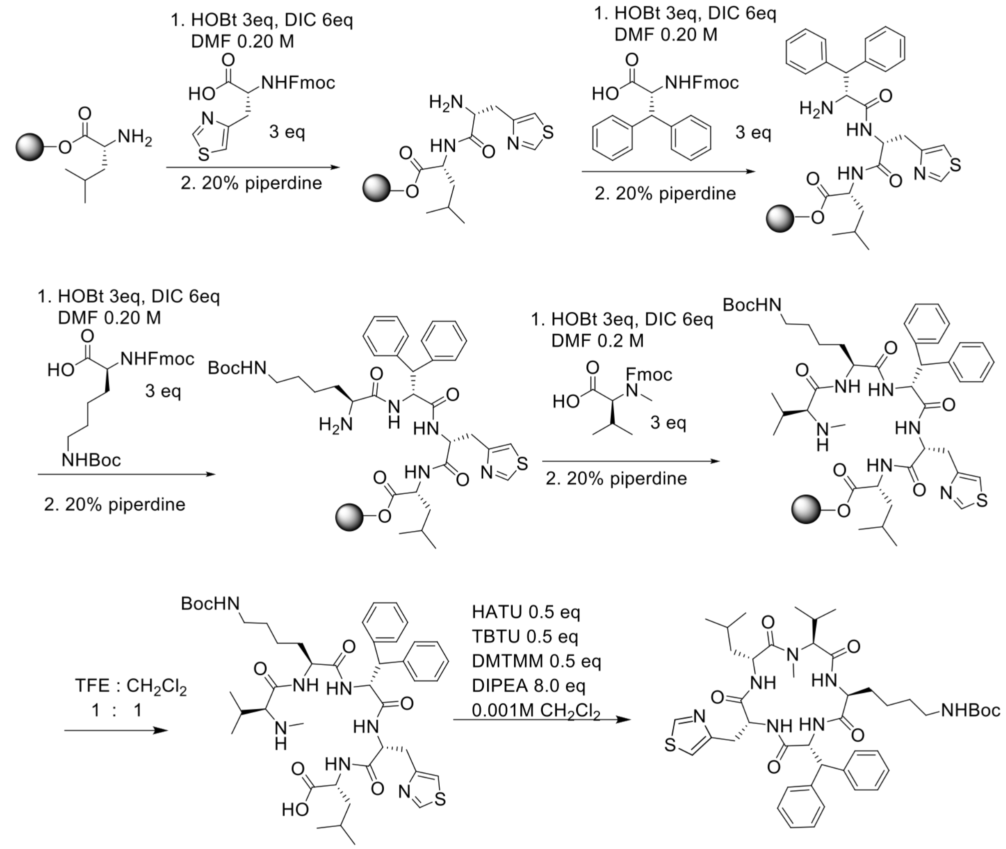
The double deprotected linear pentapeptide (DDLP) HO-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val-NH_2 underwent cleavage following the **"Resin cleavage of linear peptide"** procedure for SPPS, using a mixed solution of 6 mL of TFE and 6 mL of CH_2Cl_2. The resin containing solution was filtered and dried *in vacuo* to yield the DDLP as a white solid (140 mg, overall 33%).
LC/MS (ESI): *m/z* called for $C_{44}H_{63}N_7O_8S$ (M+1) = 850.46, found 850.10.
^{1}H NMR (300 MHz, DMSO) δ 8.93-8.86 (s, 1H), 8.45-8.21 (dd, J = 7.87, 9.70 Hz, 2H), 8.02-7.73 (dd, J = 7.40, 8.63, 2H). 7.37-7.02 (m, 11H), 6.69-6.61 (t, J = 4.60 Hz, 1H), 5.42-5.27 (t, J = 8.63 Hz, 1H), 4.51-4.41 (m, 1H), 4.34-4.27 (d, J = 11.82 Hz, 1H), 4.25-4.20 (m, 1H), 4.13-4.06 (m, 1H), 3.14-3.04 (dd, J = 4.60, 5.91 Hz, 1H), 2.92-2.82 (m, 1H), 2.81-2.75 (d, J = 5.91 Hz, 1H), 2.74-2.68 (m. 2H), 2.24-2.17 (s, 3H), 1.92-1.76 (m, 1H), 1.53-1.32 (m, 13H), 1.14-1.00 (m, 2H), 0.92-0.75 (m, 16H).

cyclo-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-Lys(Boc)-*N*-Me-Val (Compound 7_TagII)

Macrocyclization of 0.11 g DDLP compound **7** (0.13 mmol, 1.0 equivalent) was achieved using 0.021 g of TBTU (0.065 mmol, 0.50 equivalent), 0.017 g of HATU (0.065 mmol, 0.50 equivalent), 0.019 g of DMTMM (0.065 mmol, 0.50 equivalent), 0.27 mL of DIPEA (1.55 mmol, 12.0 equivalents) in anhydrous CH_2Cl_2 (129 mL, 0.001M) following "**Macrocyclization procedure (syringe pump)"** procedure. The reaction was stirred overnight and the reaction was monitored via TLC and LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash to afford crude product, which was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield

compound **7**_TagII as white solid (23 mg, 21.4%).
LC/MS (ESI): *m/z* called for $C_{44}H_{61}N_7O_7S$ (M+1) = 832.44, found 832.20.
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.14 (m, 15H), 5.30-5.20 (m, 1H), 4.90-4.45 (m, 1H), 4.03 (d, *J* = 9.8 Hz, 1H), 3.80-3.77 (m, 2H), 3.67-3.64 (m, 12H), 3.59-3.58 (m, 2H), 3.43 (s, *br*, 1H), 3.26-3.03 (m, 6H), 3.00-2.80 (m, 11H), 2.57-2.48 (m, 4H), 2.41-2.28 (m, 3H), 2.03 (s, 1H), 1.83-1.64 (m, 5H), 1.56-1.35 (m, 5H), 0.96-0.77 (m, 12H).

Scheme 5: Synthesis of compound 7_TagII



Synthesis of biotinylated compound 7_TagII

Boc removal

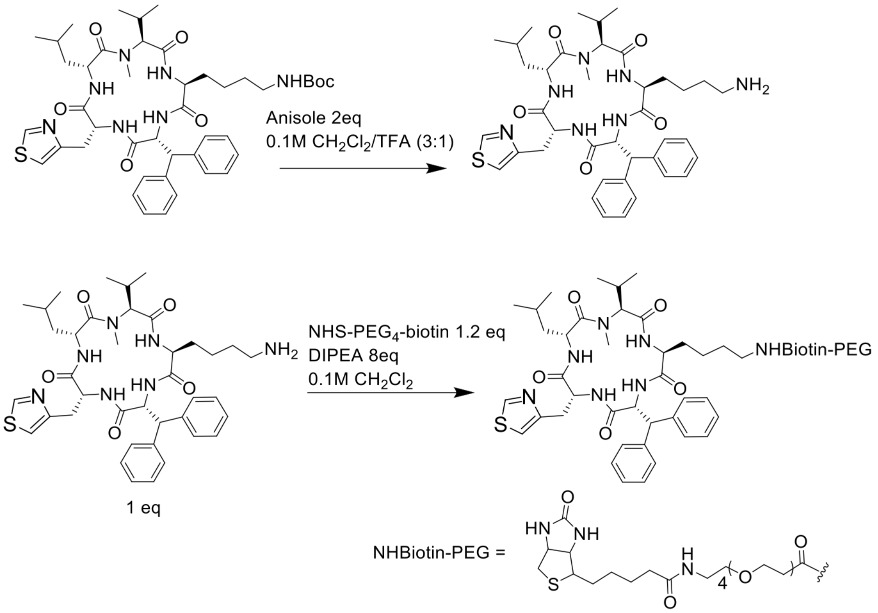
The Boc protecting group of compound **7**_TagII was removed following **"Boc removal"** procedure, utilizing a mixture of TFA/CH_2Cl_2 (1:4, 0.1 M) and anisole (2.0 equivalents) to generate free amines at lysine residue. The free amine was taken to the subsequent biotinylation reaction without purification.
LC/MS (ESI): *m/z* called for $C_{39}H_{53}N_7O_5S$ (M+1) = 732.38, found 732.00.

Peg-Biotin attachment

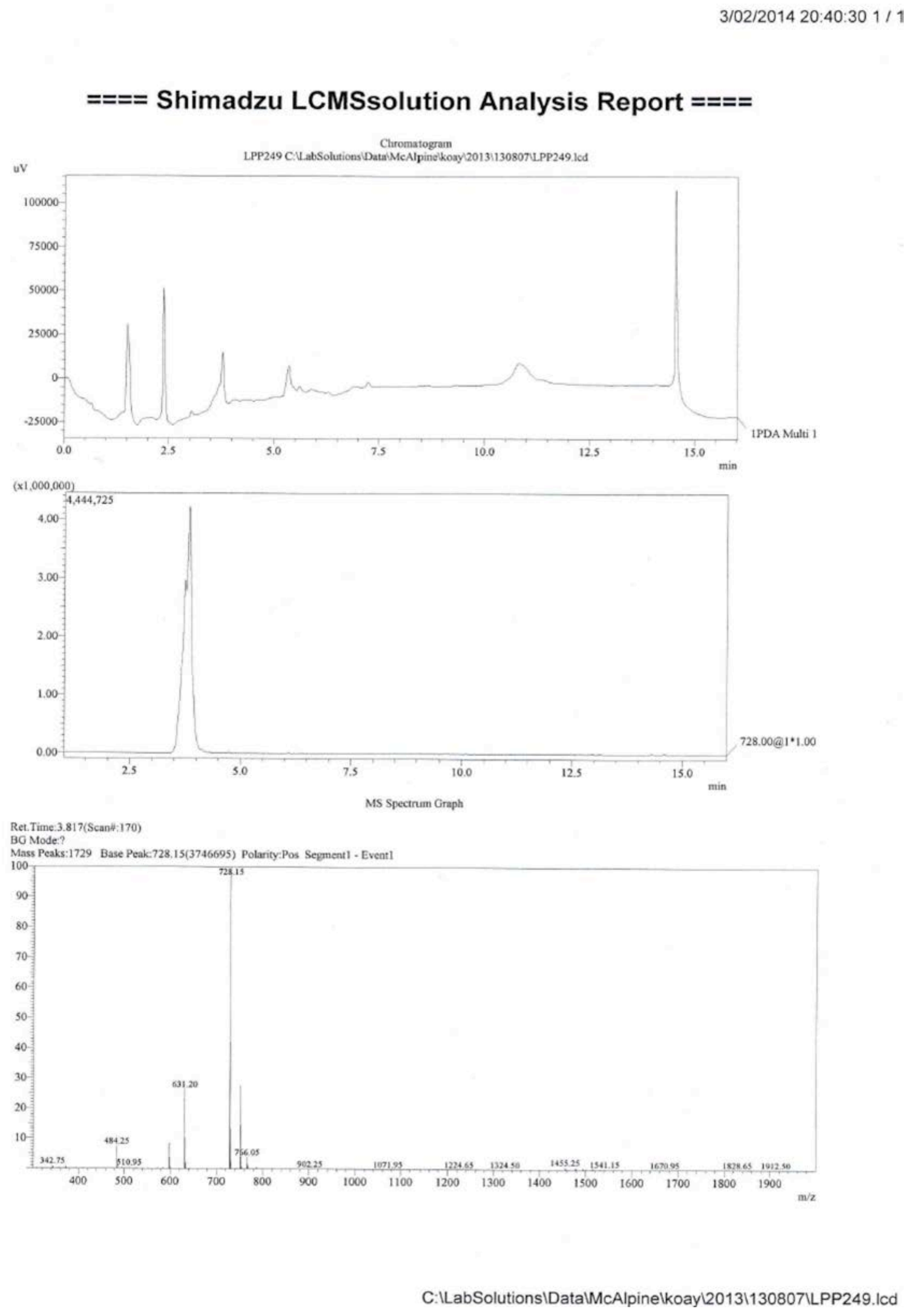
The biotinylated compound **7**_TagII was afforded by using **"Peg-Biotin attachment"** procedure, utilizing 23 mg (0.031 mmol, 1.0 equivalent) of deprotected compound **7**_TagII, 25.9 mg (0.044 mmol, 1.4 equivalents) of NHS-PEG$_4$-Biotin, 43.8 μL (0.25 mmol, 8.0 equivalents) of DIPEA in 314 μL of CH_2Cl_2. The reaction mixture was stirred for 4 hours and monitored via LC/MS. Upon completion, the crude product was purified using preparative HPLC to generate pure biotinylated compound in 33.1 % yield (12 mg) as white solid.
R_f: 0.71 (EtOAc:MeOH = 0.60:0.40)
LC/MS (ESI): *m/z* called for $C_{60}H_{88}N_{10}O_{12}S_2$ (M+1) = 1205.60, found 1205.05.
HRMS (ESI-TOF): M+Na$^+$, found 1227.5926 $C_{60}H_{88}N_{10}O_{12}S_2$ requires 1204.6025.
^{1}H NMR (600 MHz, MeOD): δ 9.34 (m, 1H), 7.28-7.04 (m, 11H), 5.19-5.16 (m, 1H), 4.54-4.48 (m, 2H),

4.43-4.41 (m, 1H), 4.25-4.19 (m, 2H), 4.07 (s, 1H), 4.02 (s, 1H), 3.26 (t, J = 6.4 Hz, 3H), 3.53-3.49 (m, 14H), 3.45-3.42 (m, 3H), 3.28-3.24 (m, 3H), 2.99-2.95 (m, 2H), 2.82 (s, 1H), 2.75 (s, 3H), 2.35-2.32 (m, 3H), 2.17-2.11 (m, 4H), 1.82-1.76 (m, 1H), 1.68-1.40 (m, 12H), 1.29-1.19 (m, 5H), 0.82-0.72 (m, 12H).
^{13}C NMR (150 MHz, MeOD): δ 174.77, 172.44, 171.59, 170.93, 170.16, 170.03, 161.91, 156.12, 148.31, 140.75, 140.63, 128.47, 128.39, 128.34, 128.26, 128.23, 128.13, 128.09, 127.90, 127.76, 126.91, 126.62, 118.49, 70.56, 70.18, 70.16, 70.11, 70.06, 69.95, 69.93, 69.87, 69.19, 66.91, 63.71, 62.91, 58.03, 56.95, 56.86, 52.68, 48.45, 48.16, 39.01, 38.86, 36.25, 35.31, 34.91, 29.92, 29.09, 28.74, 28.45, 28.39, 28.11, 26.74, 25.47, 25.30, 25.06, 24.34, 23.13, 22.01, 21.17, 21.14, 18.75, 18.72, 17.84.

<u>Scheme 6: Synthesis of biotinylated compound 7_TagII</u>



Compound 1: LCMS of DDLP HO-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-NH_2



3/02/2014 20:40:30 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

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MS Spectrum Graph

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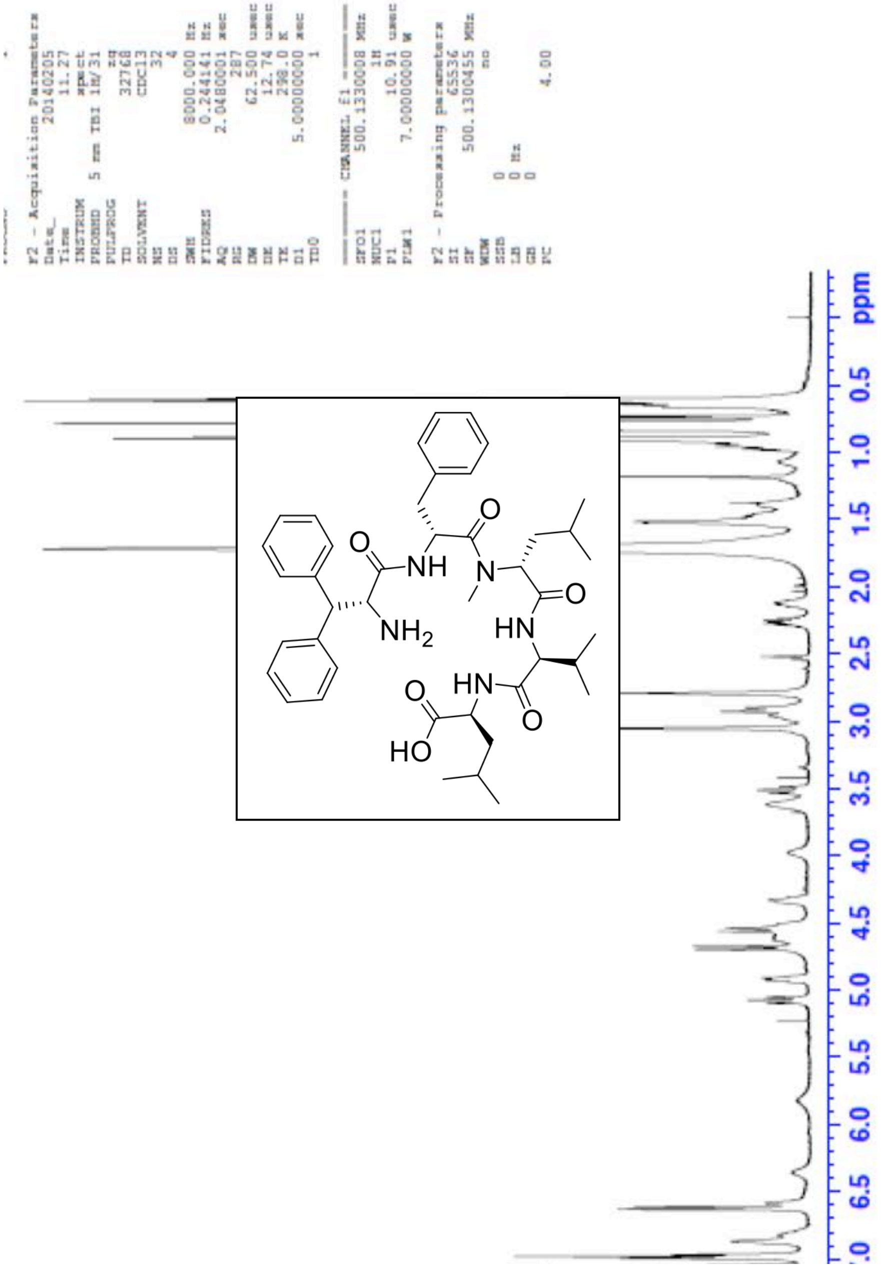
Mass Peaks:1729 Base Peak:728.15(3746695) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\130807\LPP249.lcd

Supporting Information

Compound 1: ^{1}HNMR of DDLP HO-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala-NH$_2$



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DS                    4
SWH            8000.000 Hz
FIDRES         0.244141 Hz
AQ            2.0480001 sec
RG                  287
DW               62.500 usec
DE                12.74 usec
TE                298.0 K
D1           5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
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NUC1                 1H
P1                10.91 usec
PLW1         7.00000000 W

F2 - Processing parameters
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WDW                  no
SSB      0
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GB       0
PC                 4.00
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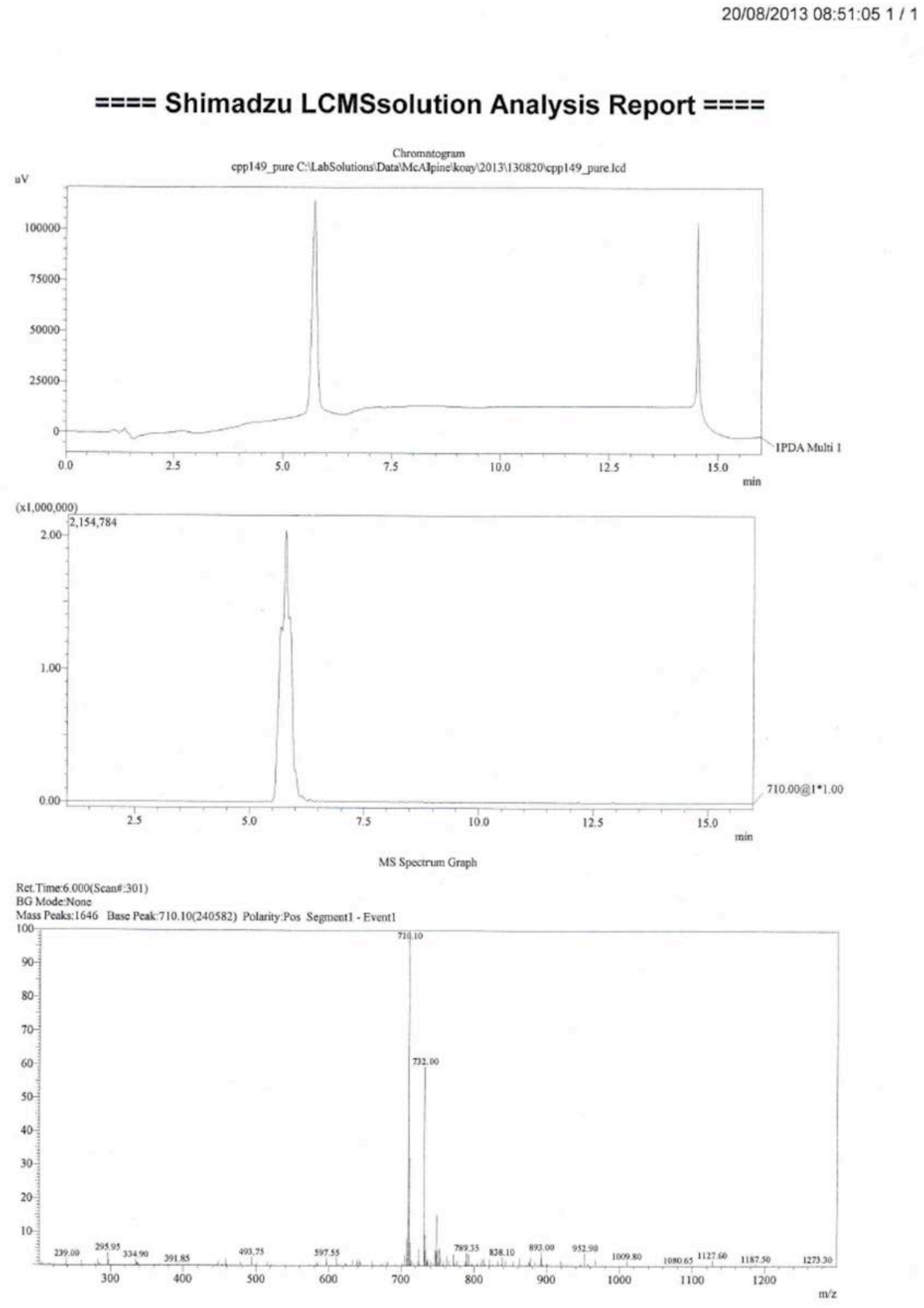
Compound 1: LCMS of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala



20/08/2013 08:51:05 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
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MS Spectrum Graph

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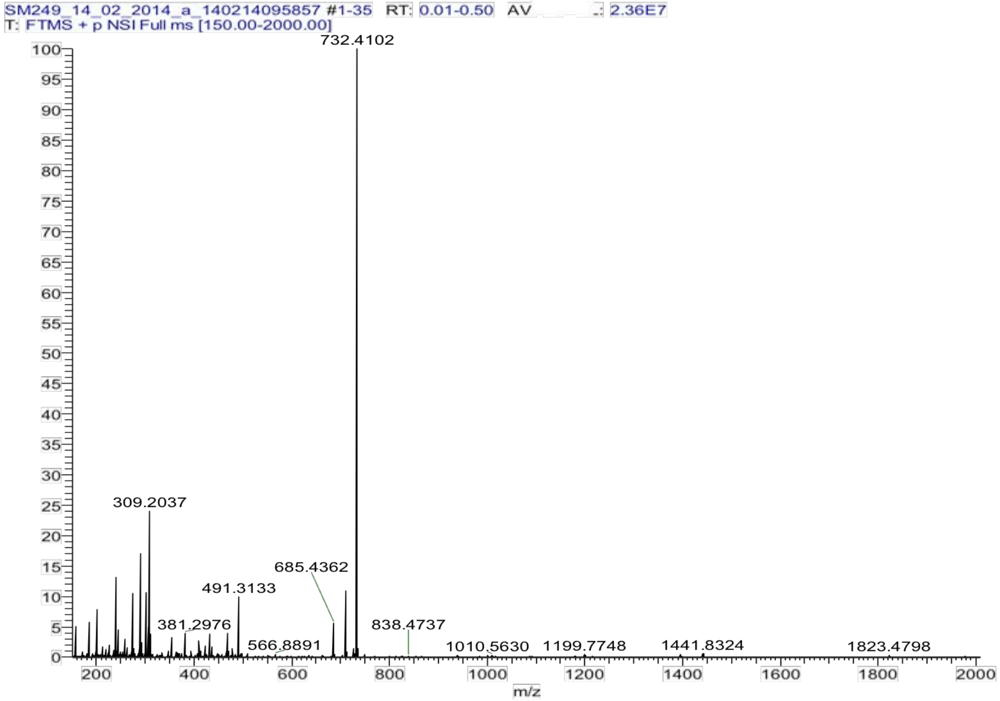
BG Mode:None
Mass Peaks:1646 Base Peak:710.10(240582) Polarity:Pos Segment1 - Event1

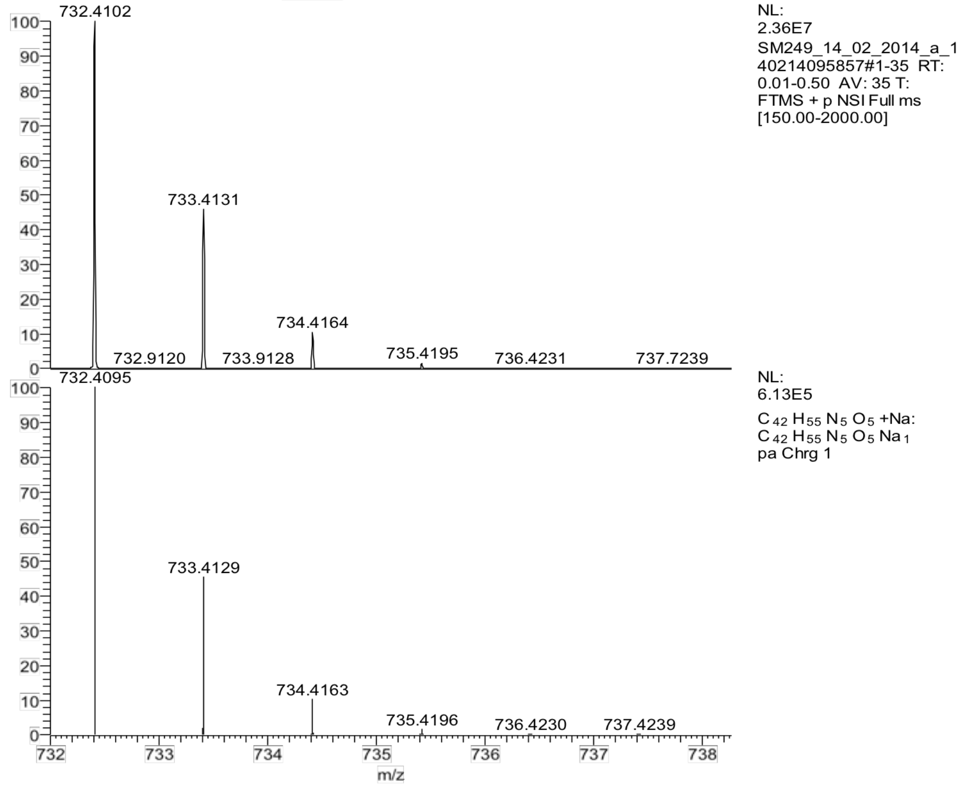
Compound 1: HRMS of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala

MS Data from Orbitrap

Full Spectrum

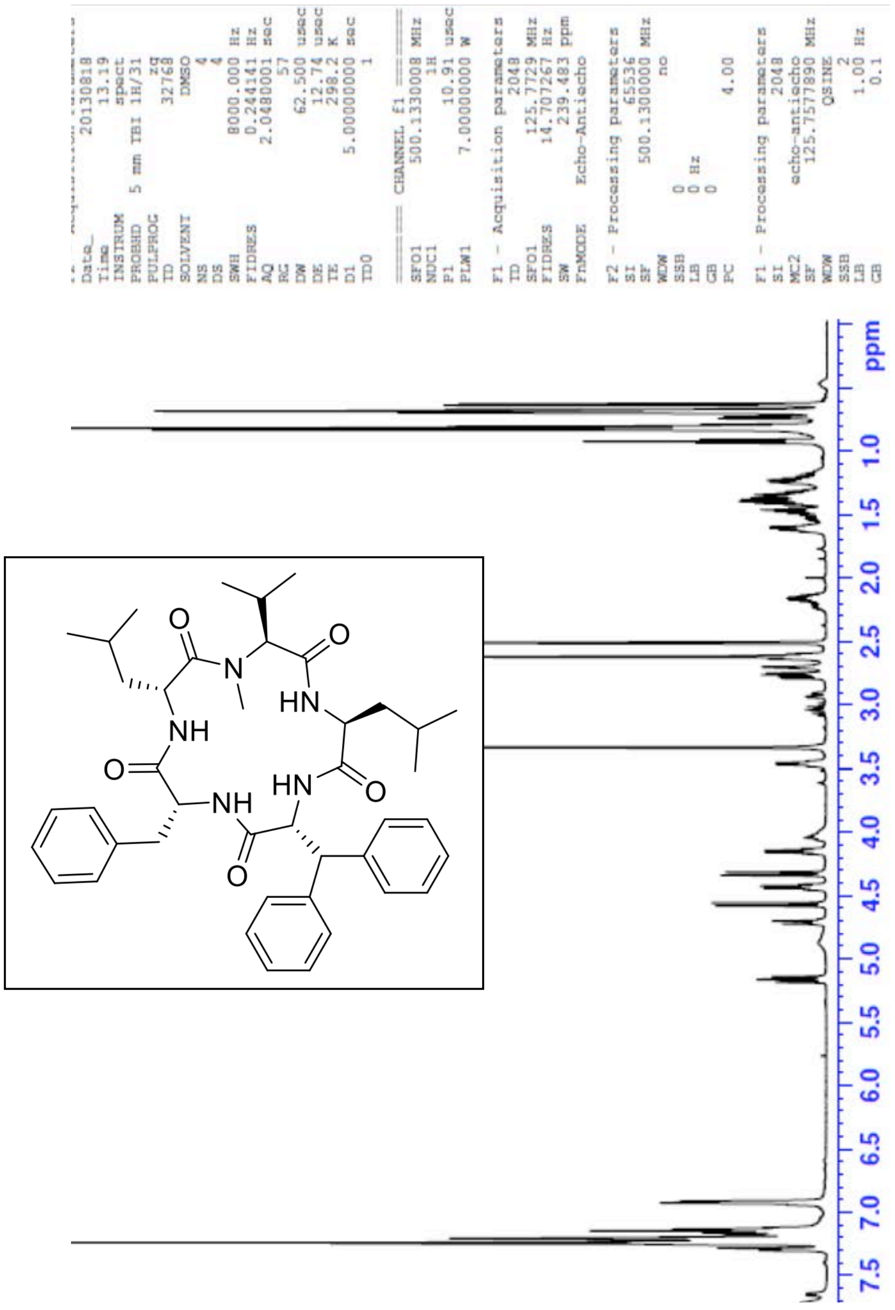


Zoomed Spectrum



Supporting Information

Compound 1: ^{1}HNMR of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala



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TD                32768
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DS                    4
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FIDRES         0.244141 Hz
AQ            2.0480001 sec
RG                   57
DW               62.500 usec
DE                12.74 usec
TE                298.2 K
D1           5.00000000 sec
TD0                   1

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P1                10.91 usec
PLW1         7.00000000 W

F1 - Acquisition parameters
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FIDRES        14.707267 Hz
SW              239.483 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
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SF          500.1300000 MHz
WDW                  no
SSB      0
LB       0 Hz
GB       0
PC                 4.00

F1 - Processing parameters
SI                 2048
MC2        echo-antiecho
SF          125.7577890 MHz
WDW               QSINE
SSB                   2
LB                 1.00 Hz
GB                  0.1
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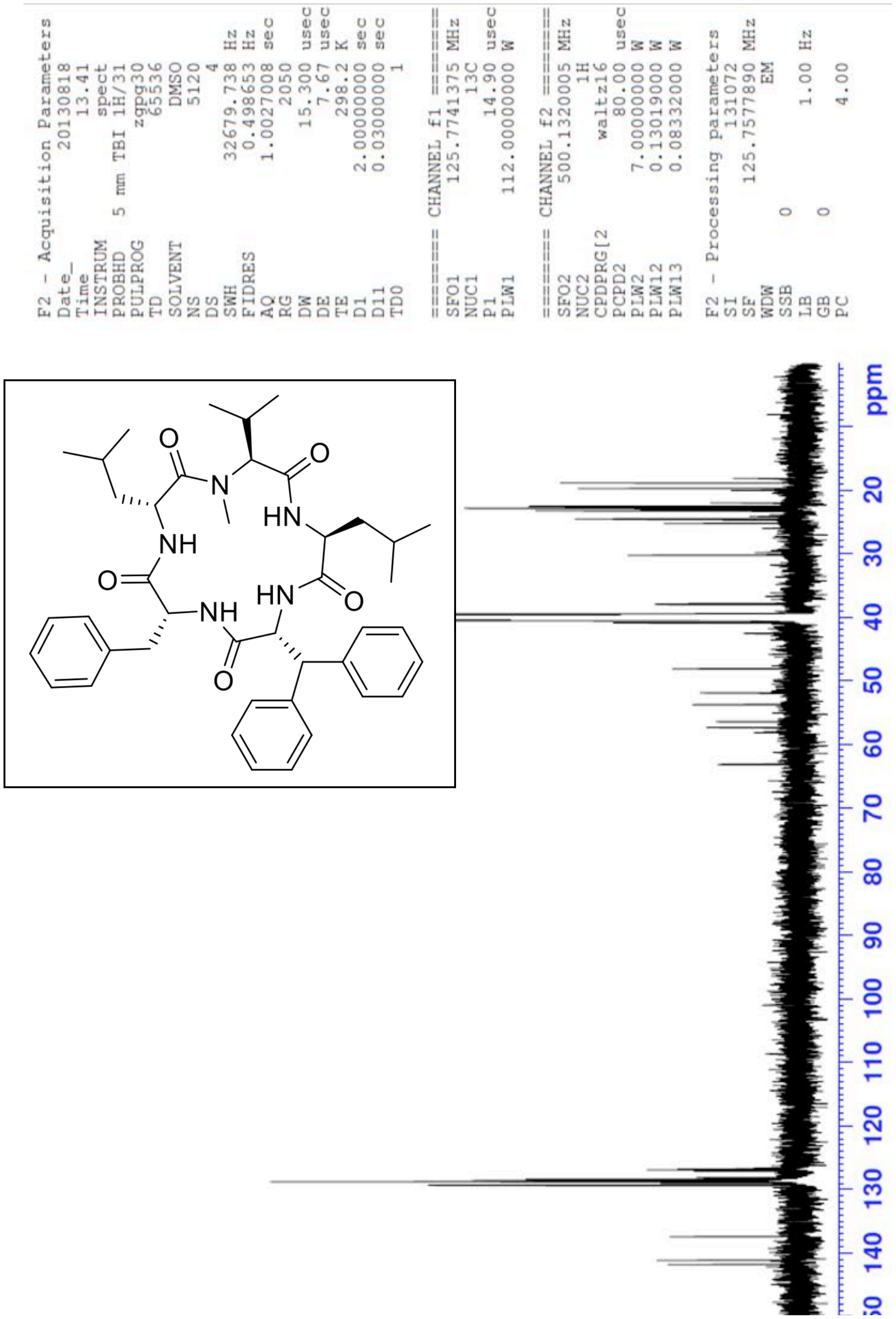
Supporting Information

Compound 1: ^{13}CNMR of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala



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DS                    4
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FIDRES         0.498653 Hz
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RG                 2050
DW               15.300 usec
DE                 7.67 usec
TE                298.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.7741375 MHz
NUC1                13C
P1                14.90 usec
PLW1       112.00000000 W

======== CHANNEL f2 ========
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NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         7.00000000 W
PLW12        0.13019000 W
PLW13        0.08332000 W

F2 - Processing parameters
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SF          125.7577890 MHz
WDW                  EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 4.00
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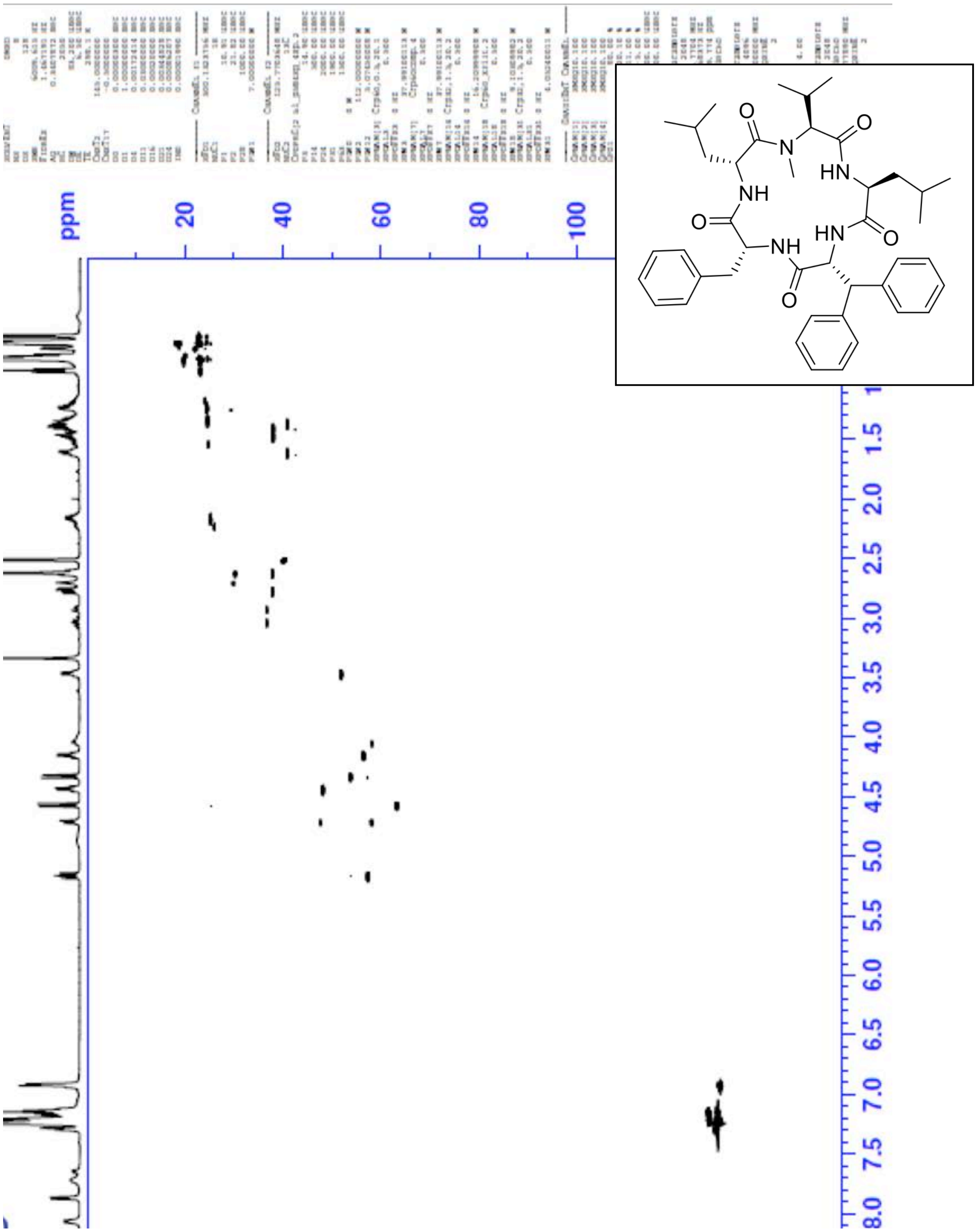
Supporting Information

Compound 1: ^{1}H-^{13}C HSQC of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala

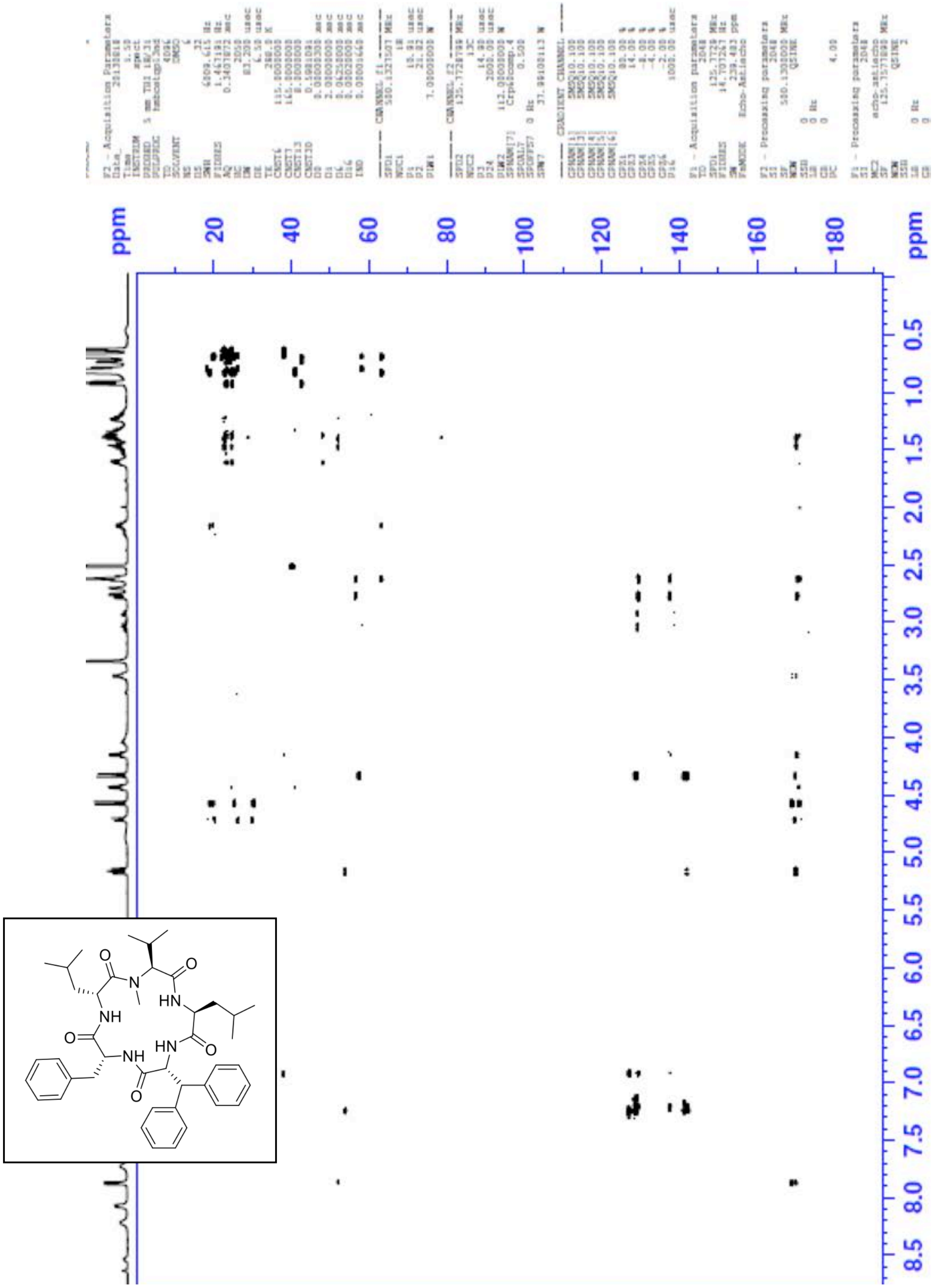


Supporting Information

Compound 1: ^{1}H-^{13}C HMBC of cyclo-Leu-N-Me-Val-D-Leu-D-Phe-3,3-Diphenyl-D-Ala

Supp ortin g Infor matio n

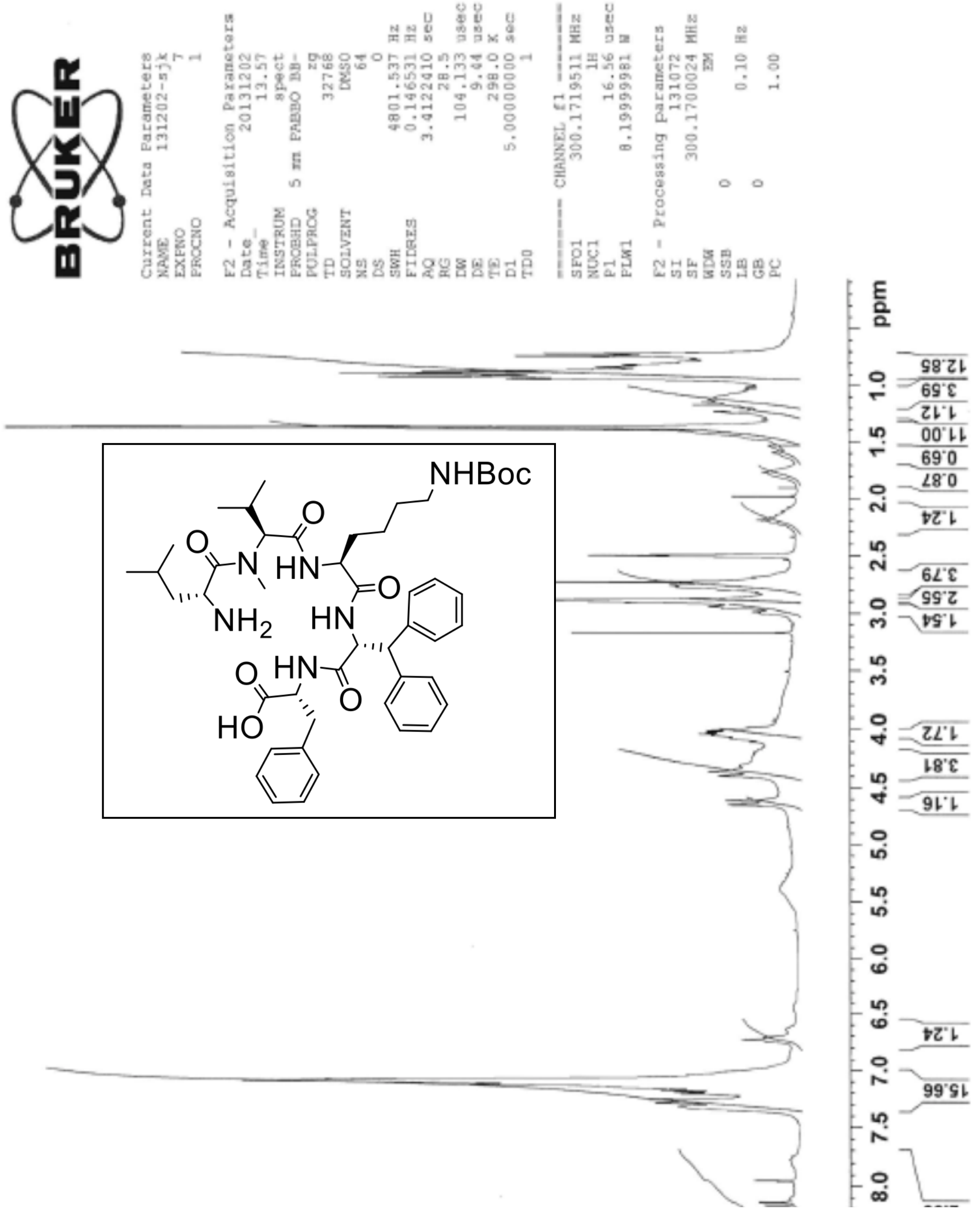
Com



pound 1-tag: LCMS of DDLP 1-TagII

Supporting Information

Compound 1-tag: ^{1}HNMR DDLP 1-TagII



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EXPNO                  7
PROCNO                 1

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PULPROG               zg
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DS                     0
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FIDRES          0.146531 Hz
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RG                  28.5
DW               104.133 usec
DE                  9.44 usec
TE                 298.0 K
D1            5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
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NUC1                  1H
P1                 16.56 usec
PLW1         8.19999981 W

F2 - Processing parameters
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WDW                   EM
SSB                    0
LB                  0.10 Hz
GB                     0
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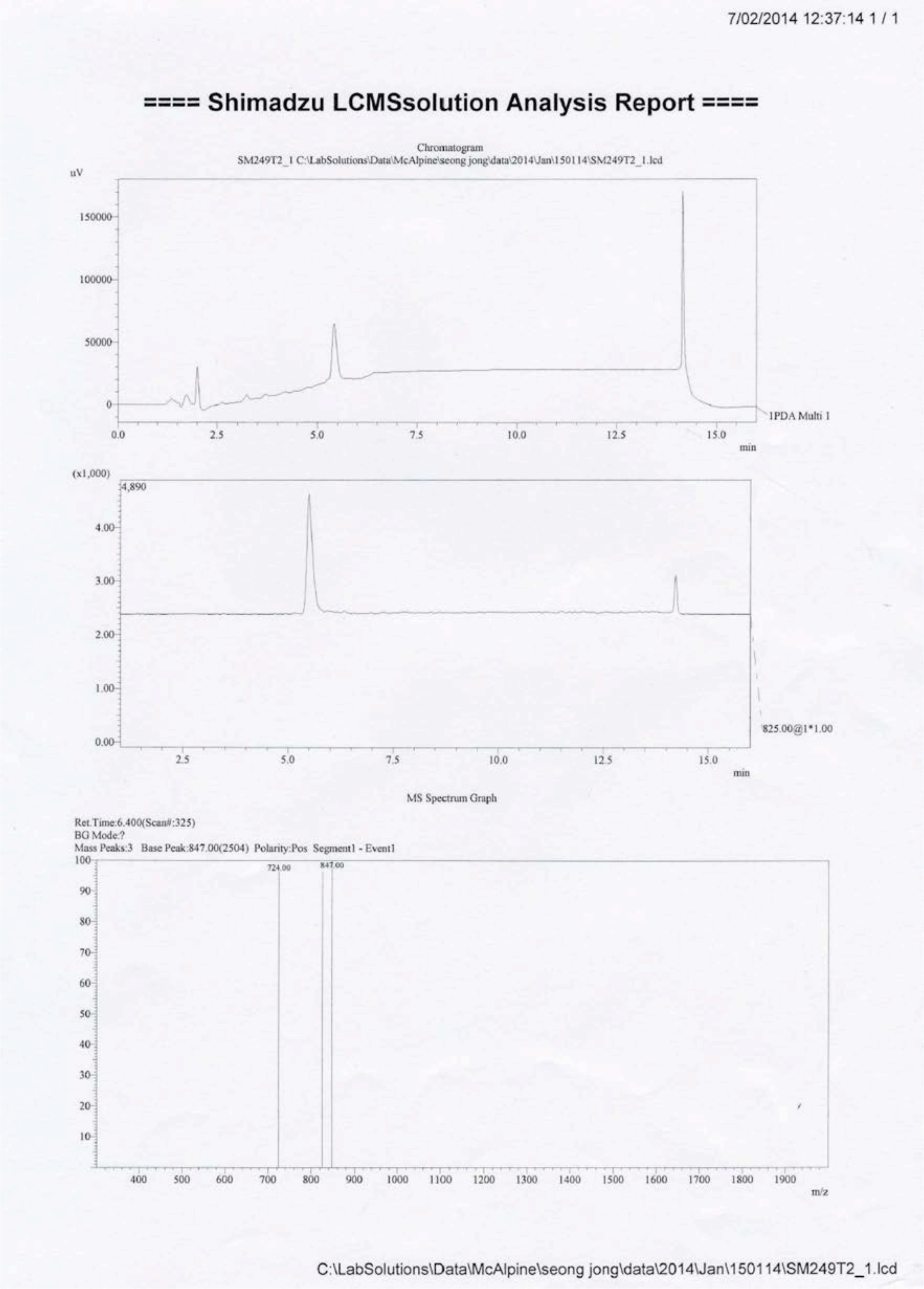
Compound 1-tag: LCMS of Macrocycle 1-TagII



7/02/2014 12:37:14 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

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MS Spectrum Graph

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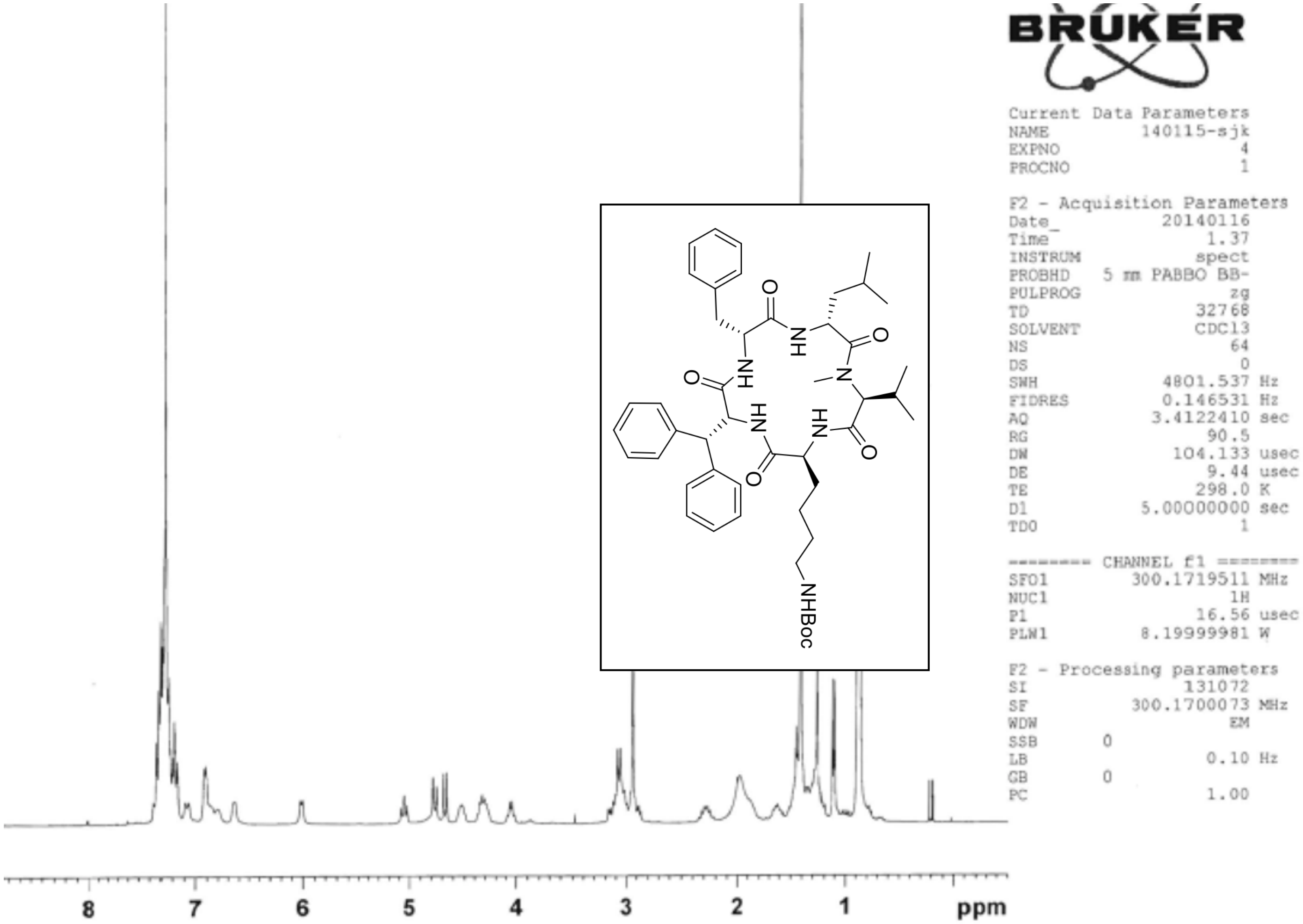
C:\LabSolutions\Data\McAlpine\seong jong\data\2014\Jan\150114\SM249T2_1.lcd

Compound 1-tag: ^{1}HNMR Macrocycle 1-TagII



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PROCNO                 1

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PULPROG               zg
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DW               104.133 usec
DE                  9.44 usec
TE                 298.0 K
D1           5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
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NUC1                  1H
P1                 16.56 usec
PLW1          8.19999981 W

F2 - Processing parameters
SI                131072
SF           300.1700073 MHz
WDW                   EM
SSB      0
LB                  0.10 Hz
GB       0
PC                  1.00
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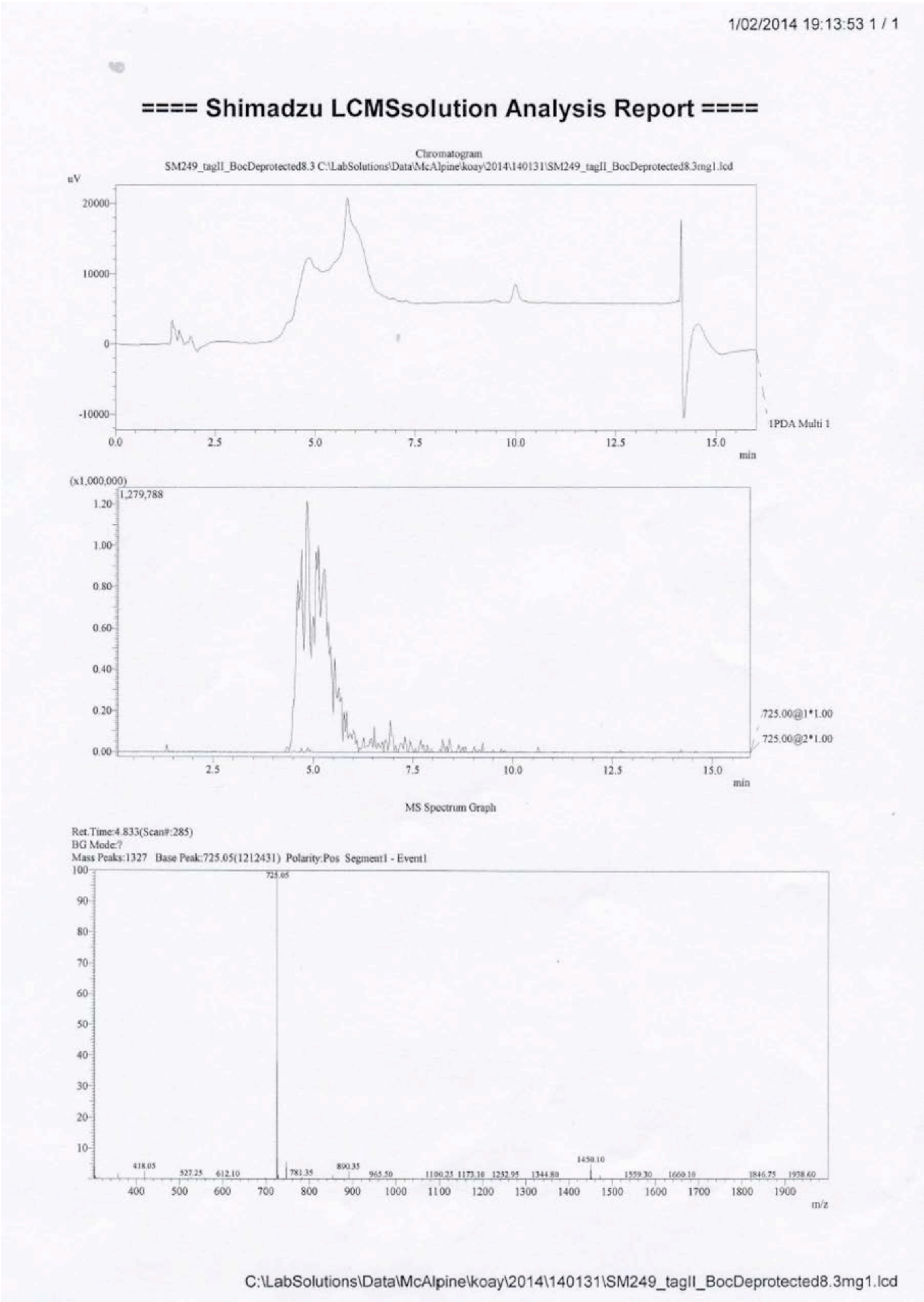
Compound 1-tag: LCMS of Boc-deprotected Macrocycle 1-TagII



1/02/2014 19:13:53 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

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MS Spectrum Graph

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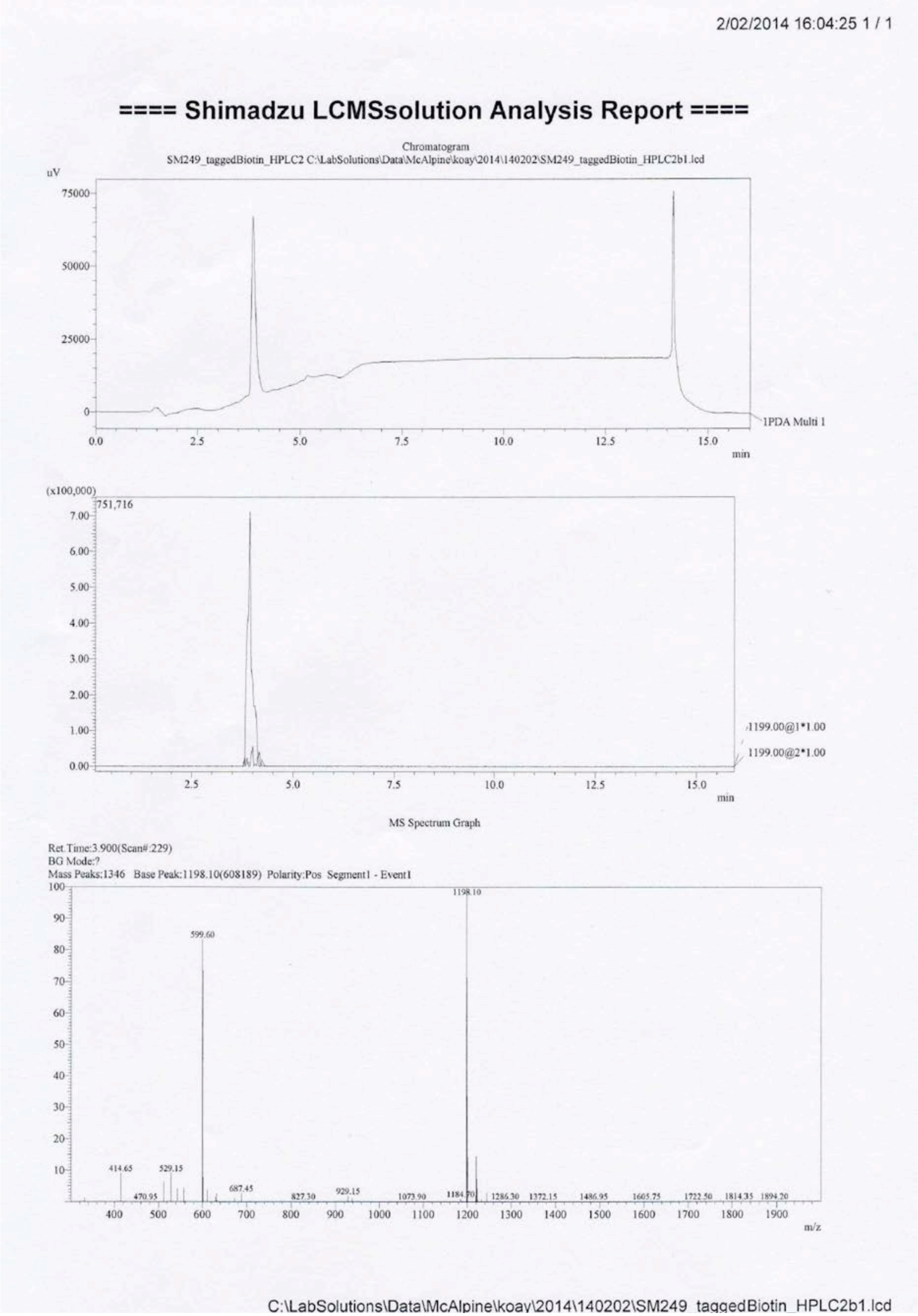
Compound 1-tag: LCMS of Macrocycle 1-Biotinylated tag



2/02/2014 16:04:25 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

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MS Spectrum Graph

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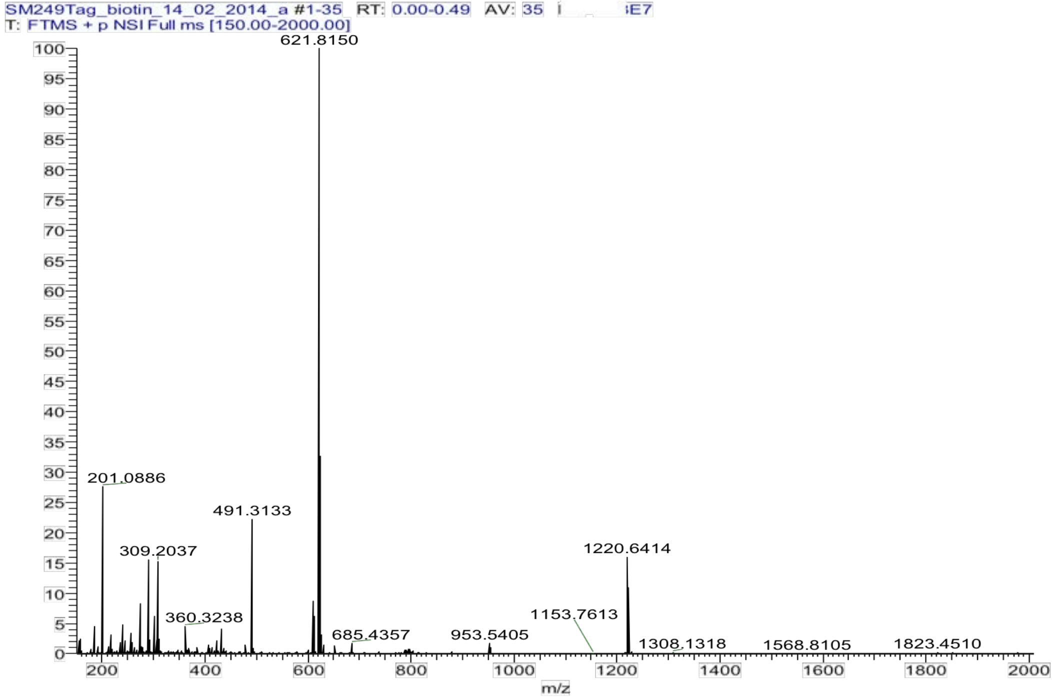
BG Mode:?
Mass Peaks:1346 Base Peak:1198.10(608189) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2014\140202\SM249_taggedBiotin_HPLC2b1.lcd

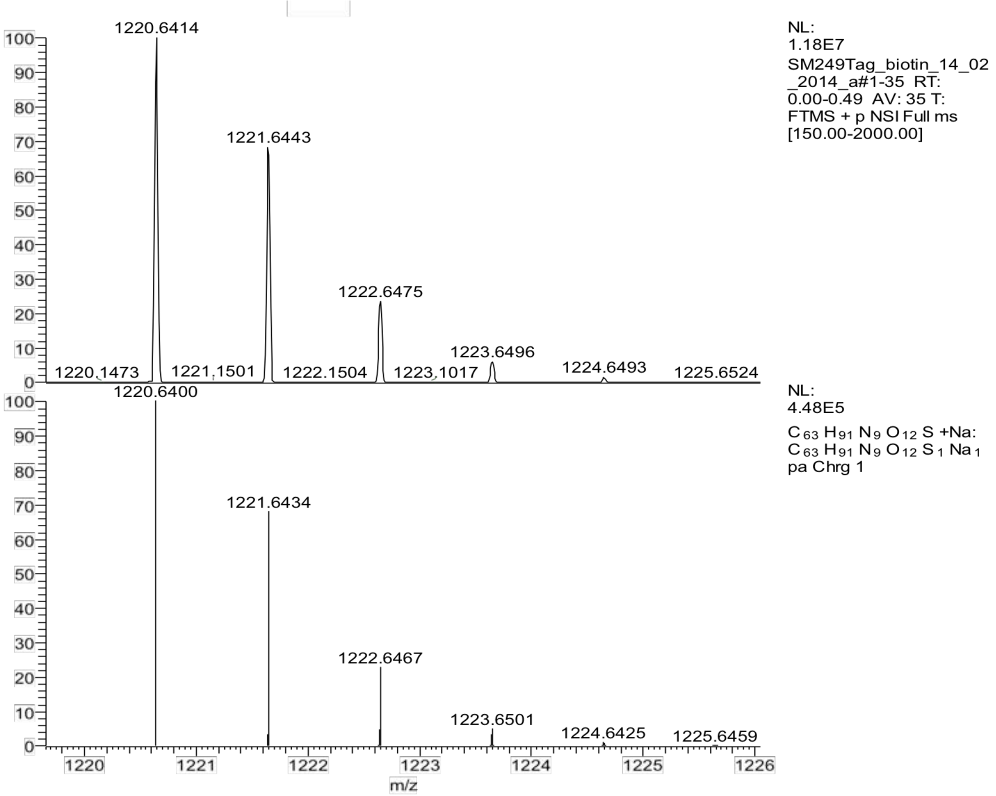
Compound 1-tag: HRMS of Macrocycle 1-Biotinylated tag

MS Data from Orbitrap

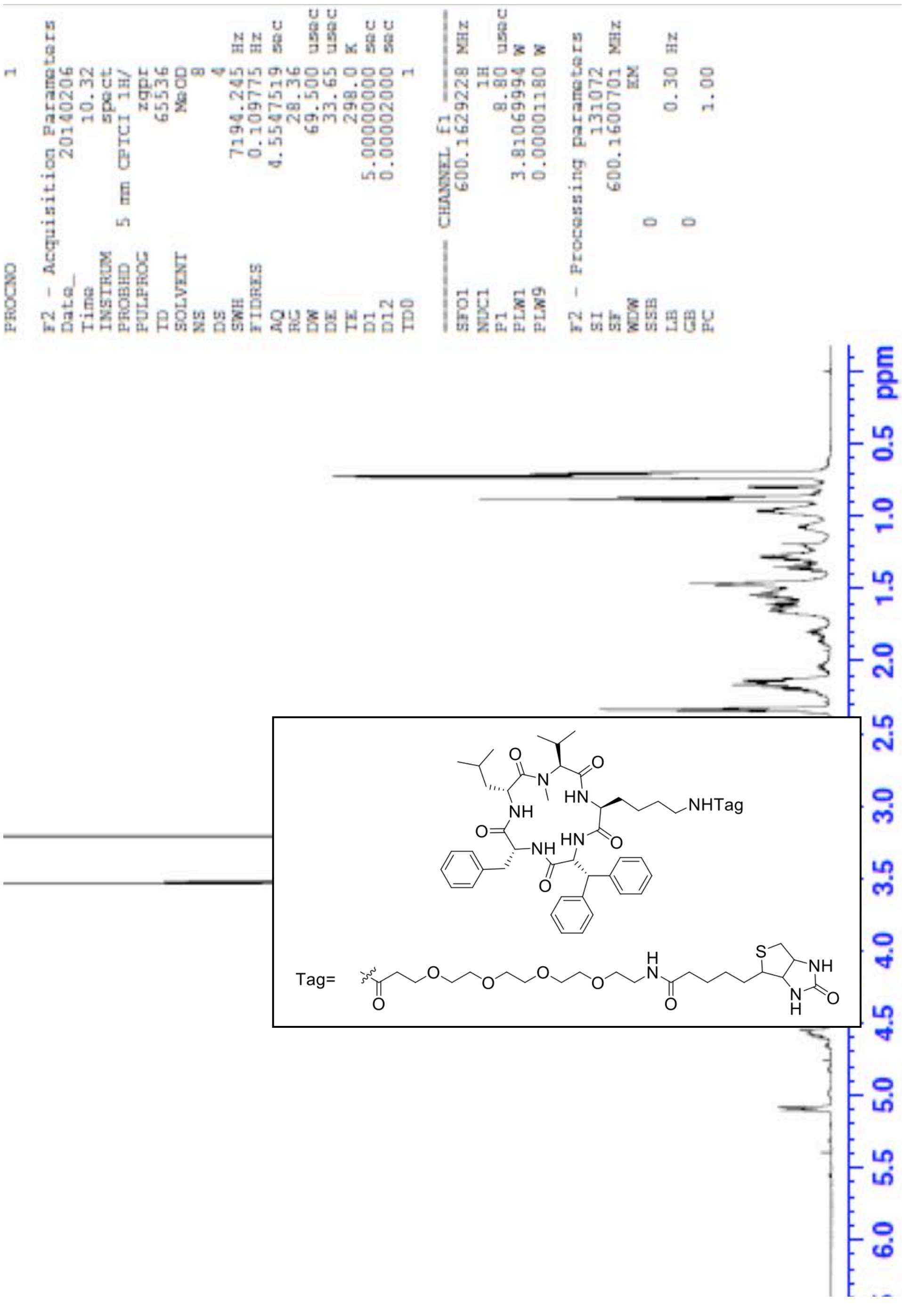
Full Spectrum



Zoomed Spectrum



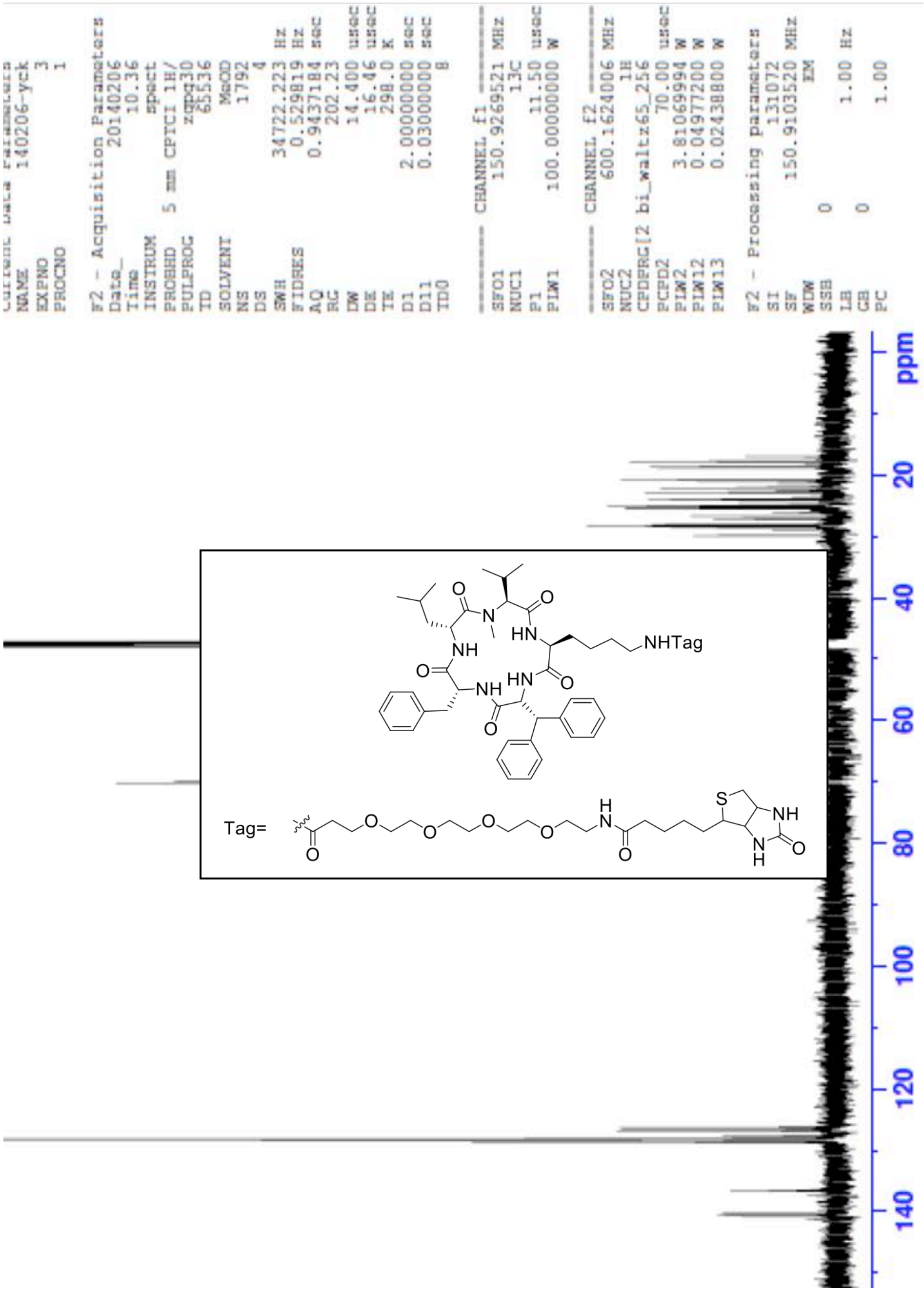
Compound 1-tag: ^{1}HNMR Macrocycle 1-Biotinylated tag



```
PROCNO                   1

F2 - Acquisition Parameters
Date_             20140206
Time                 10.32
INSTRUM              spect
PROBHD   5 mm CPTCI 1H/
PULPROG               zgpr
TD                   65536
SOLVENT               MeOD
NS                       8
DS                       4
SWH               7194.245 Hz
FIDRES            0.109775 Hz
AQ               4.5547519 sec
RG                   28.36
DW                  69.500 usec
DE                   33.65 usec
TE                   298.0 K
D1              5.00000000 sec
D12             0.00002000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          600.1629228 MHz
NUC1                    1H
P1                    8.80 usec
PLW1            3.81069994 W
PLW9            0.00001180 W

F2 - Processing parameters
SI                  131072
SF             600.1600701 MHz
WDW                     EM
SSB       0
LB        0           0.30 Hz
GB        0
PC                    1.00
```

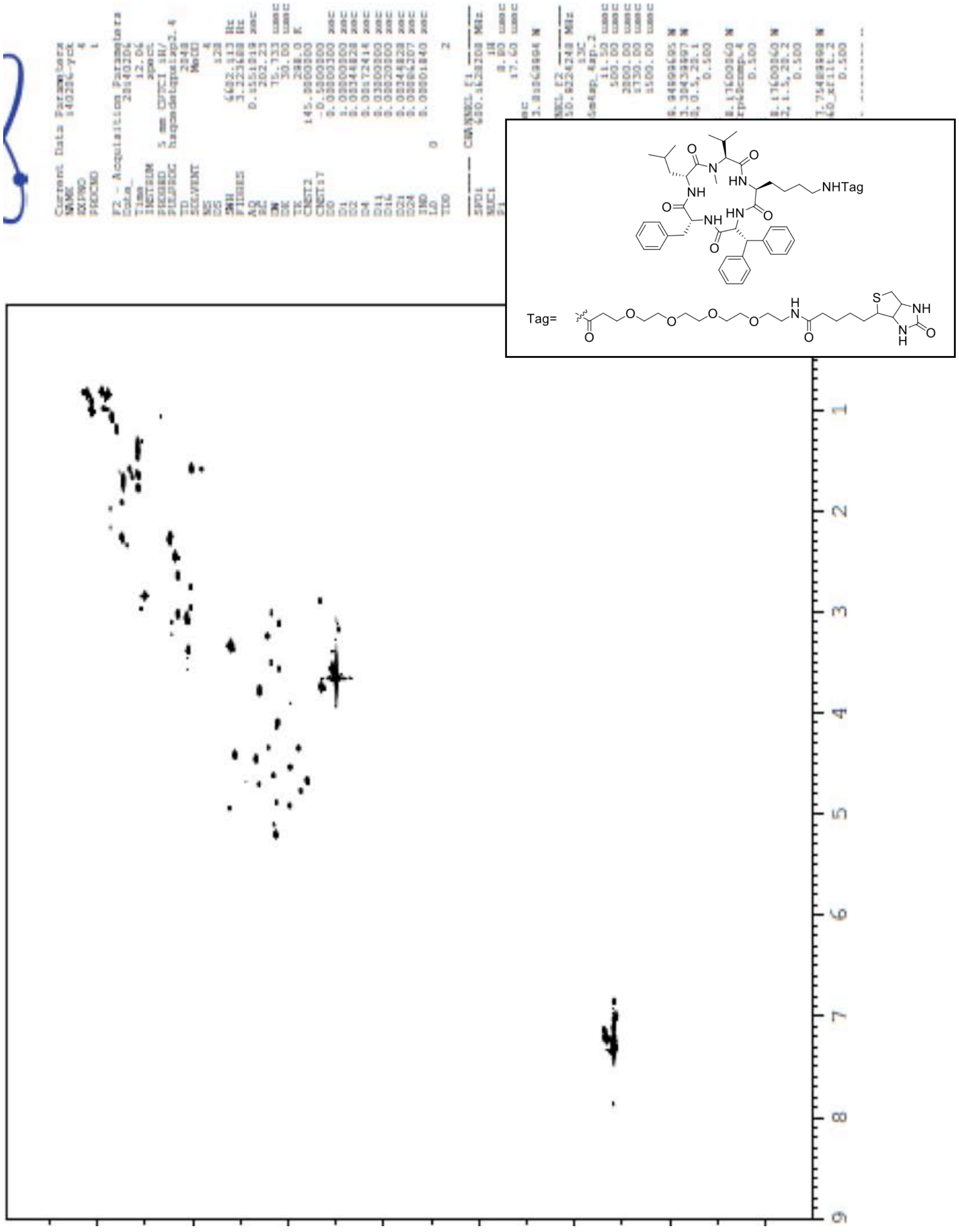
Supp ortin

Compound 1-tag: ^{13}CNMR Macrocycle 1-Biotinylated tag

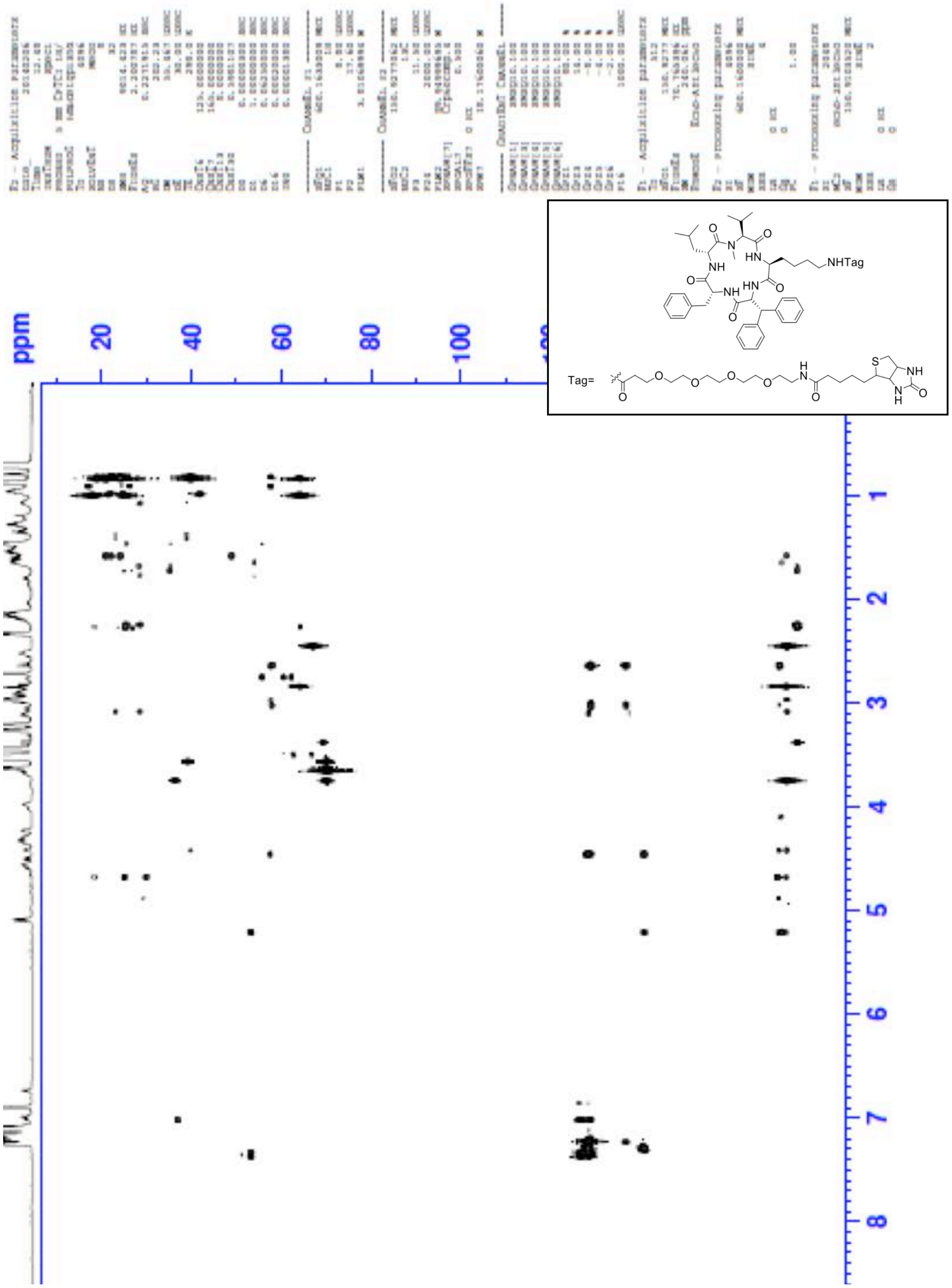


```
Current Data Parameters
NAME        140206-yck
EXPNO                3
PROCNO               1

F2 - Acquisition Parameters
Date_         20140206
Time             10.36
INSTRUM          spect
PROBHD   5 mm CPTCI 1H/
PULPROG        zgpg30
TD               65536
SOLVENT           MeOD
NS                1792
DS                   4
SWH          34722.223 Hz
FIDRES        0.529819 Hz
AQ           0.9437184 sec
RG              202.23
DW              14.400 usec
DE               16.46 usec
TE               298.0 K
D1          2.00000000 sec
D11         0.03000000 sec
TD0                  8

======== CHANNEL f1 ========
SFO1       150.9269521 MHz
NUC1               13C
P1               11.50 usec
PLW1      100.00000000 W

======== CHANNEL f2 ========
SFO2       600.1624006 MHz
NUC2                1H
CPDPRG[2 bi_waltz65_256
PCPD2            70.00 usec
PLW2        3.81069994 W
PLW12       0.04977200 W
PLW13       0.02438800 W

F2 - Processing parameters
SI              131072
SF         150.9103520 MHz
WDW                 EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.00
```

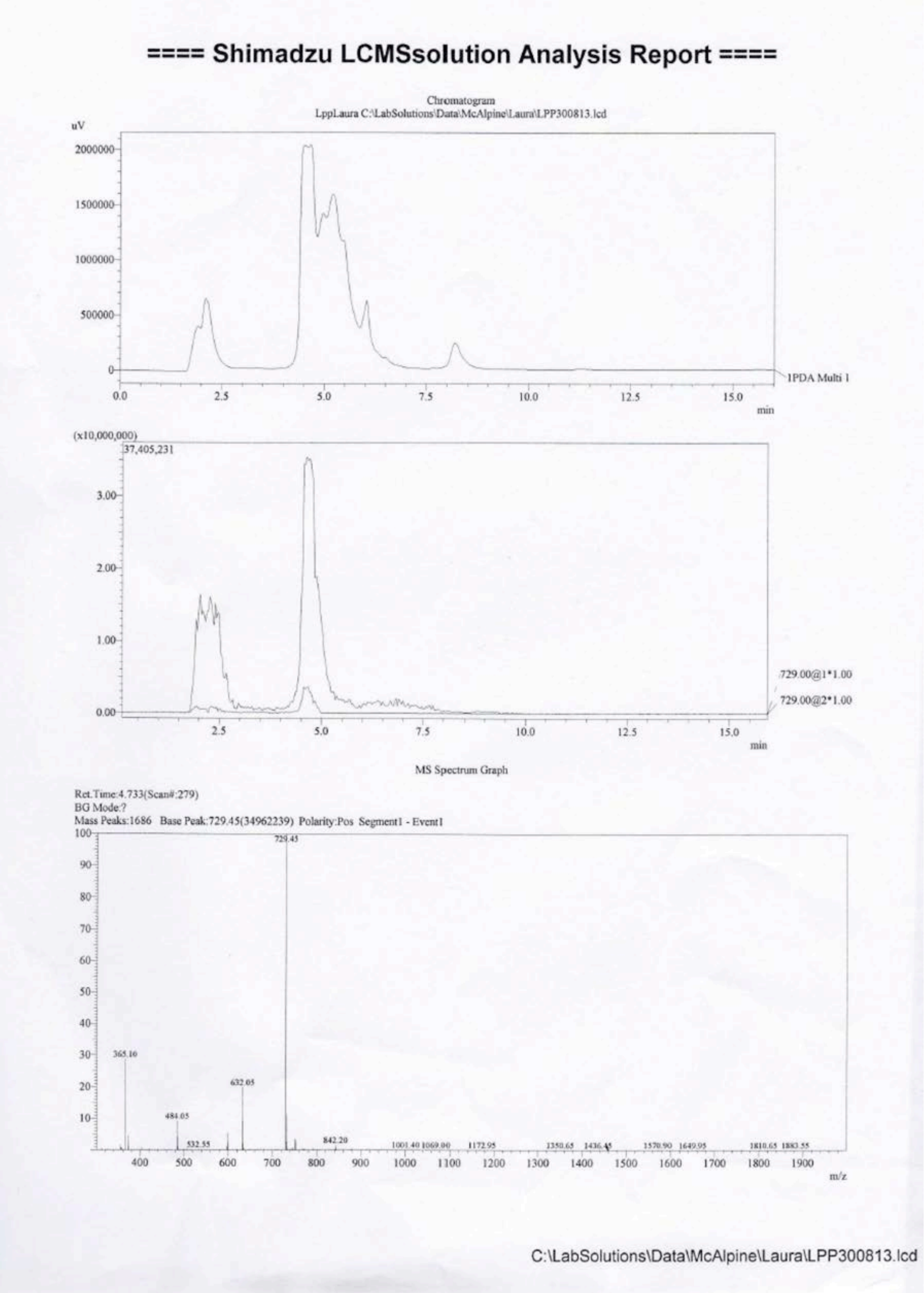
Compound 1-tag: ^{1}H-^{13}C HSQC Macrocycle 1-Biotinylated tag



Compound 1-tag: ^{1}H- ^{13}C HMBC Macrocycle 1-Biotinylated tag



Compound 2: LCMS of DDLP HO-Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2



==== Shimadzu LCMSsolution Analysis Report ====

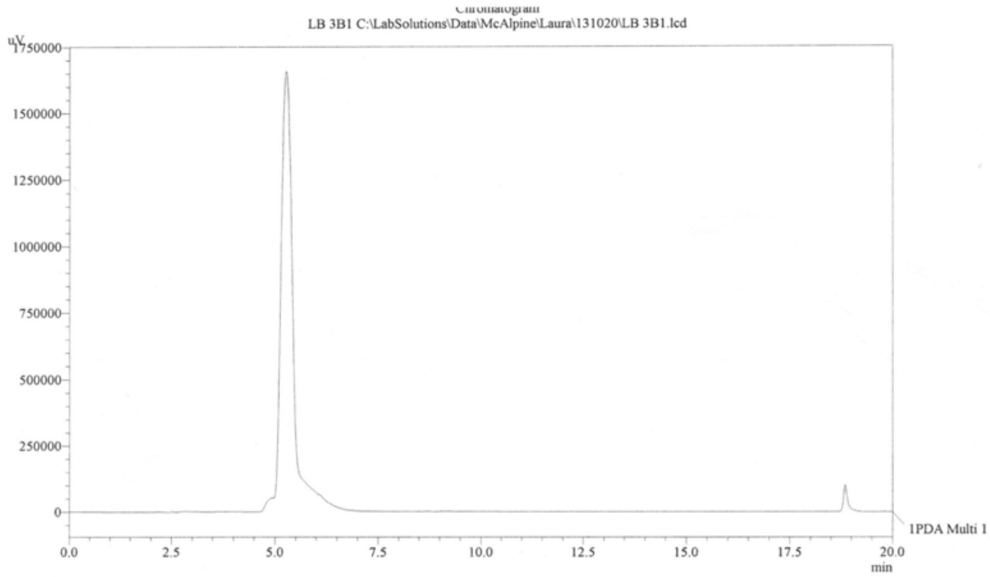
Chromatogram
LppLaura C:\LabSolutions\Data\McAlpine\Laura\LPP300813.lcd

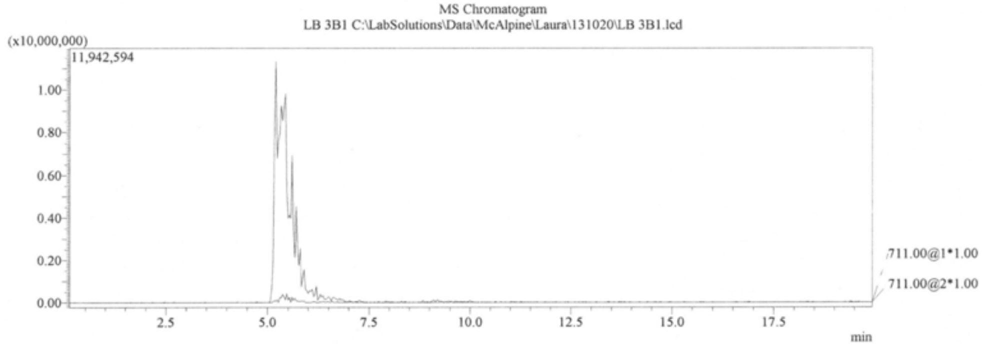
MS Spectrum Graph

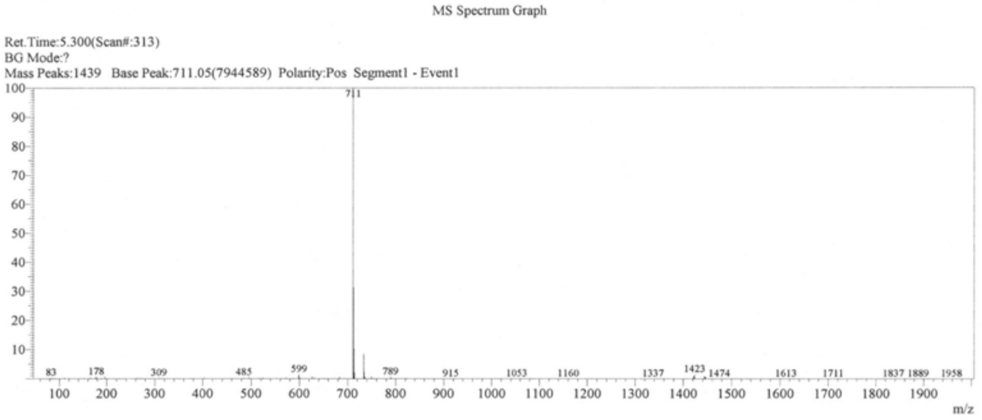
Ret.Time:4.733(Scan#:279)
BG Mode:?
Mass Peaks:1686 Base Peak:729.45(34962239) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\Laura\LPP300813.lcd

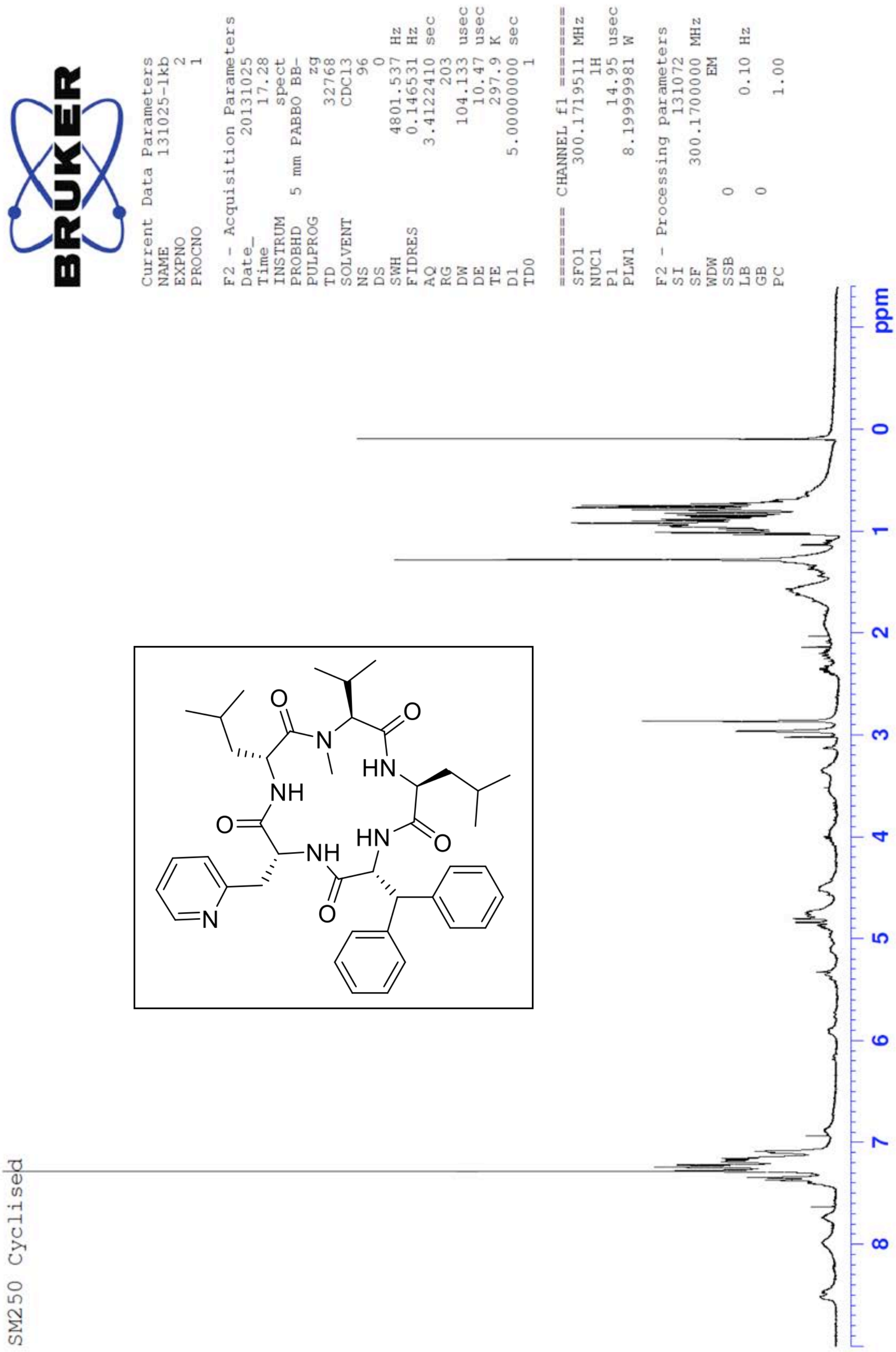
Compound 2: LCMS of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala





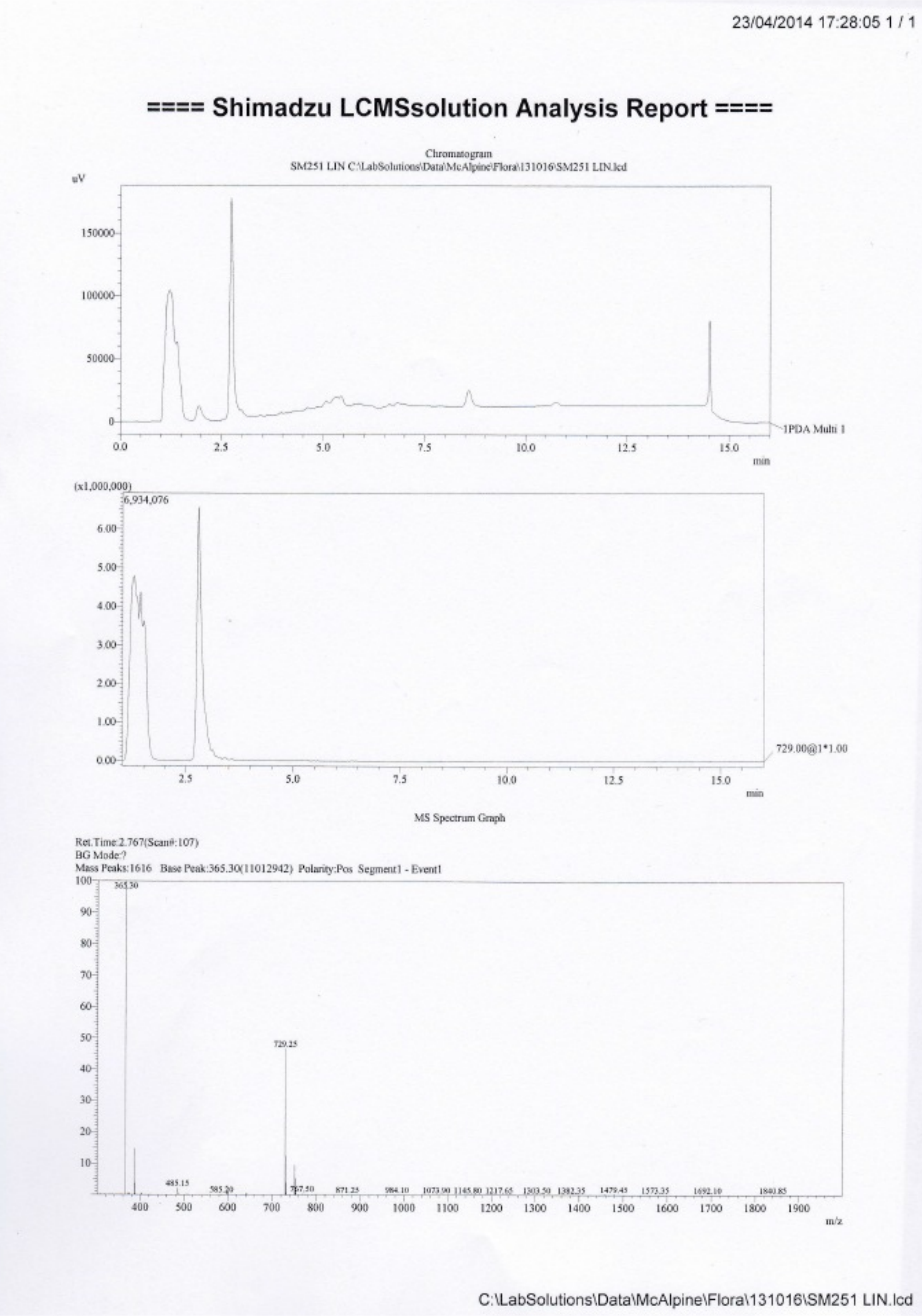


Compound 2: ^{1}HNMR of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(2-Pyridyl)Ala-3,3-Diphenyl-D-Ala



```
Current Data Parameters
NAME          131025-1kb
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131025
Time               17.28
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 32768
SOLVENT            CDC13
NS                    96
DS                     0
SWH             4801.537 Hz
FIDRES          0.146531 Hz
AQ             3.4122410 sec
RG                   203
DW               104.133 usec
DE                 10.47 usec
TE                 297.9 K
D1            5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         300.1719511 MHz
NUC1                  1H
P1                 14.95 usec
PLW1          8.19999981 W

F2 - Processing parameters
SI                131072
SF           300.1700000 MHz
WDW                   EM
SSB                    0
LB                  0.10 Hz
GB                     0
PC                  1.00
```

Compound 3: LCMS of DDLP HO-Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2



23/04/2014 17:28:05 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
SM251 LIN C:\LabSolutions\Data\McAlpine\Flora\131016\SM251 LIN.lcd

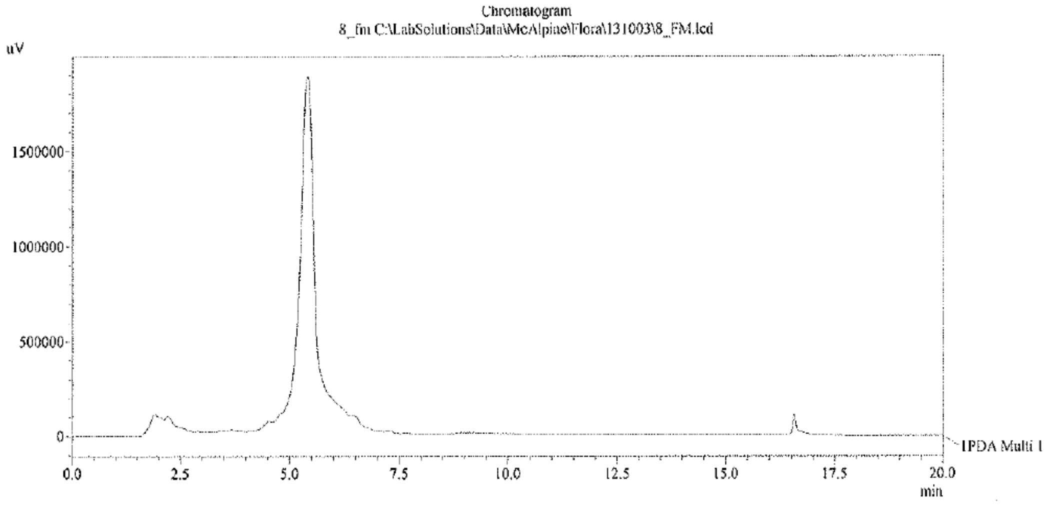
MS Spectrum Graph

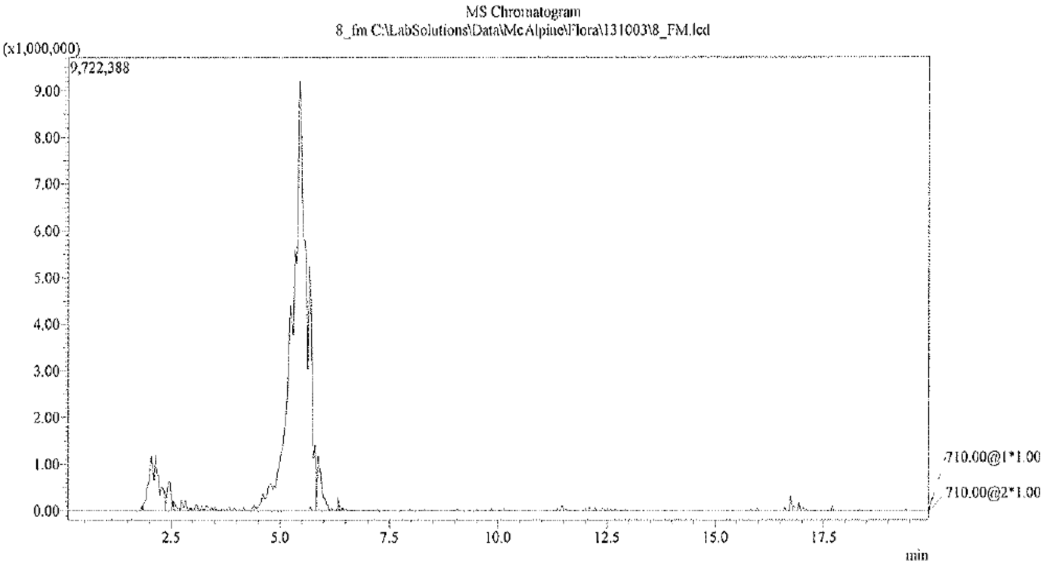
Ret.Time:2.767(Scan#:107)
BG Mode:?
Mass Peaks:1616 Base Peak:365.30(11012942) Polarity:Pos Segment1 - Event1

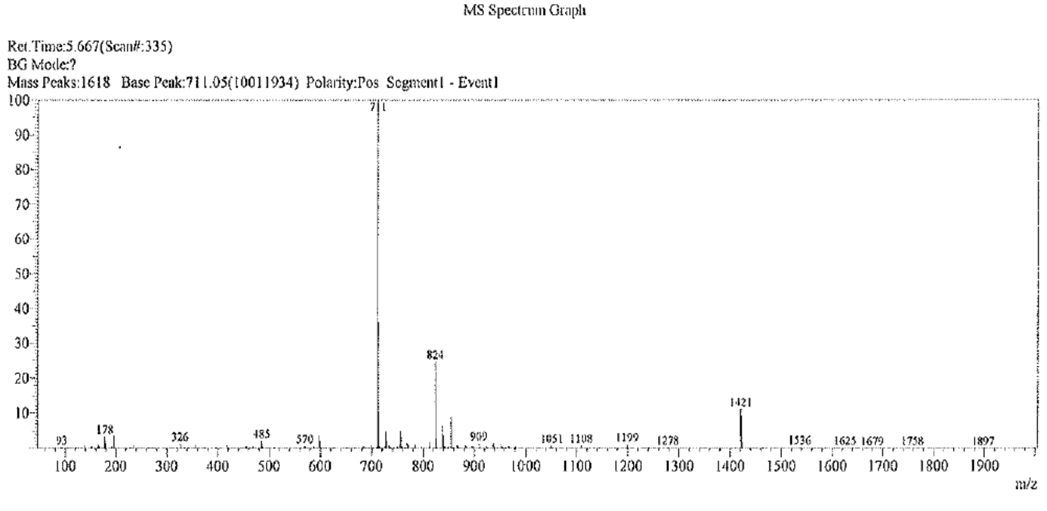
C:\LabSolutions\Data\McAlpine\Flora\131016\SM251 LIN.lcd

Supporting Information

Compound 3: LCMS of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala

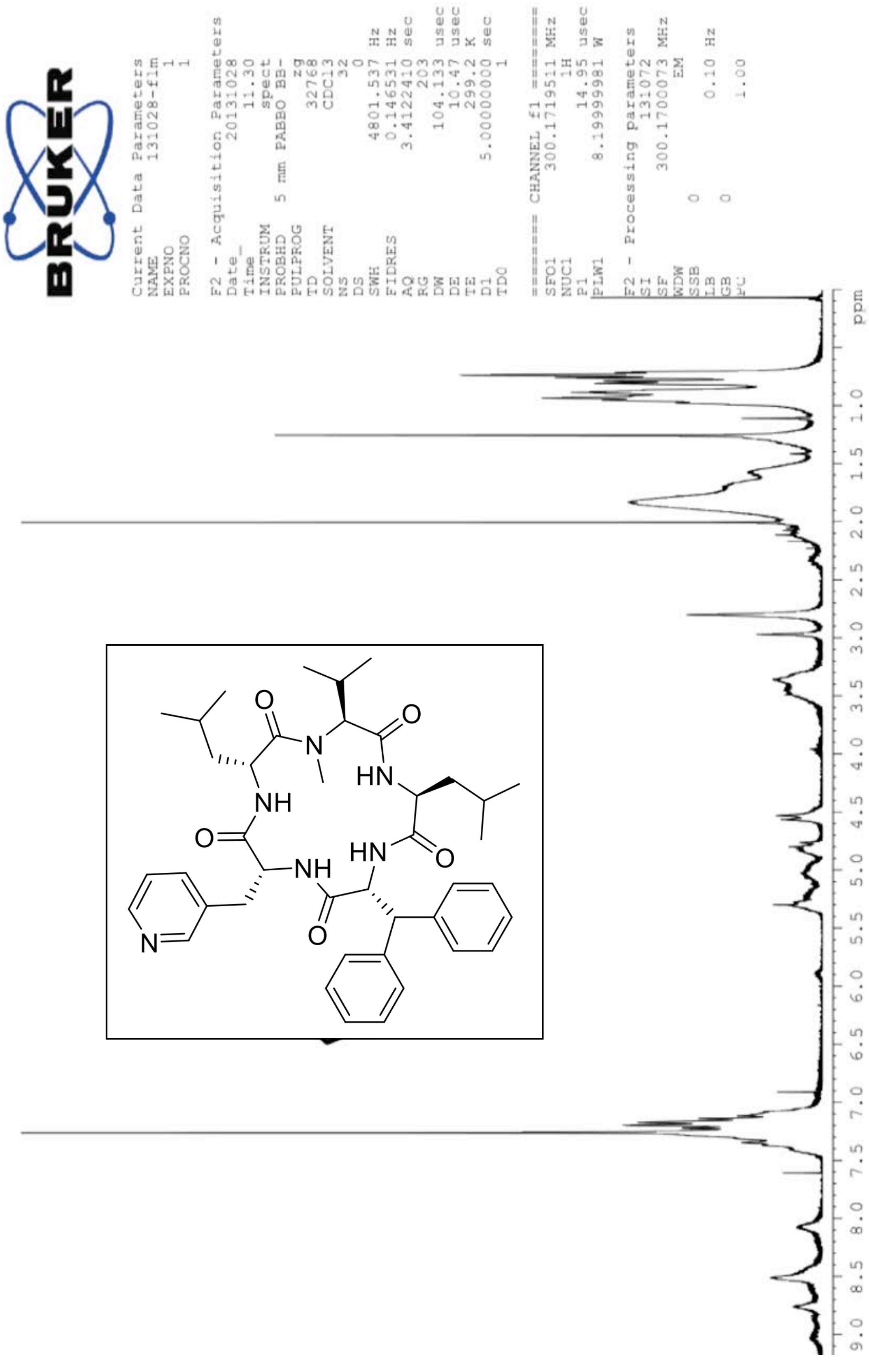






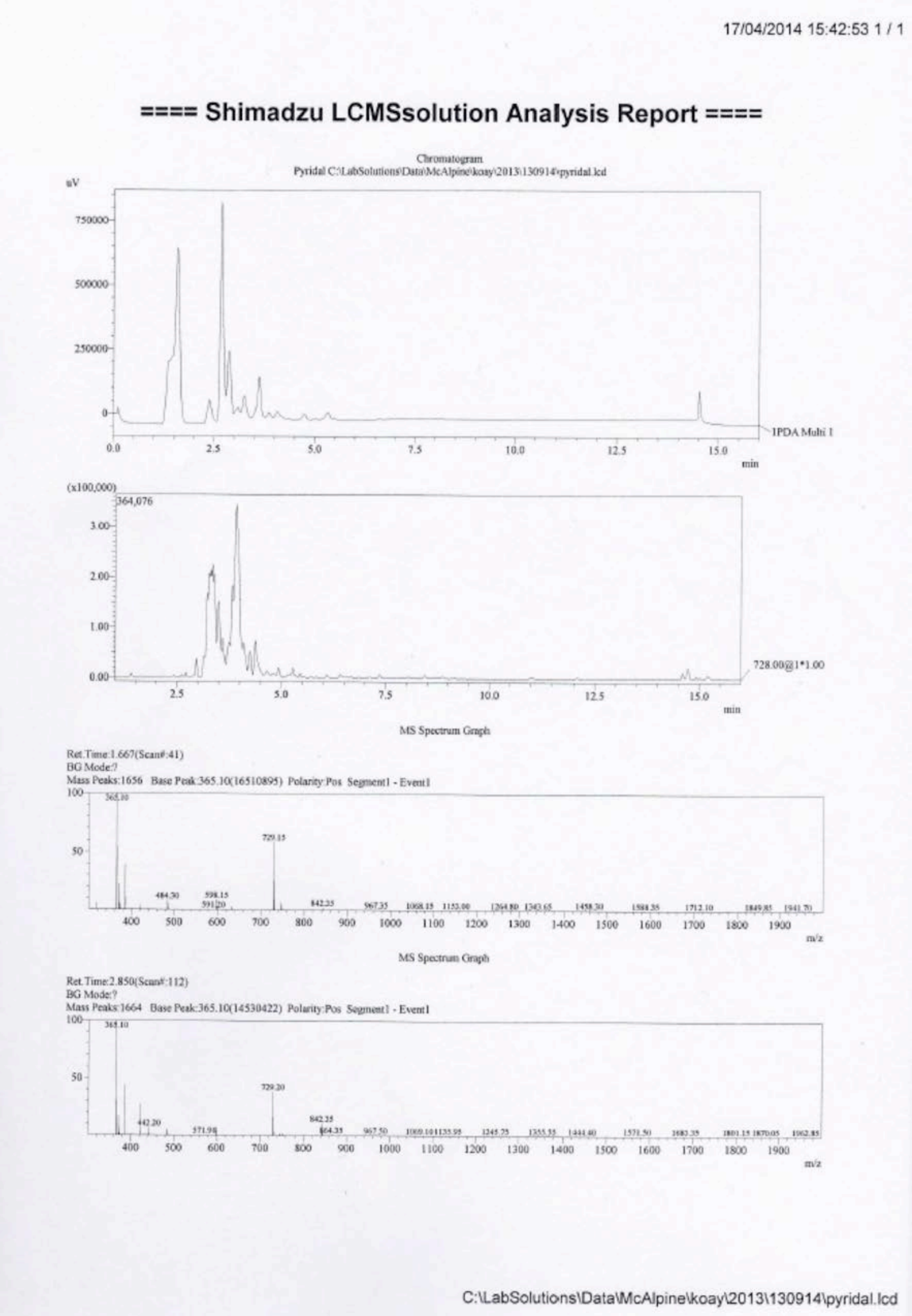
Supporting Information

Compound 3: ^{1}HNMR of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(3-Pyridyl)Ala-3,3-Diphenyl-D-Ala



```
Current Data Parameters
NAME        131028-f1m
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_         20131028
Time             11.30
INSTRUM          spect
PROBHD   5 mm PABBO BB-
PULPROG             zg
TD               32768
SOLVENT          CDCl3
NS                  32
DS                   0
SWH           4801.537 Hz
FIDRES        0.146531 Hz
AQ           3.4122410 sec
RG                 203
DW             104.133 usec
DE               10.47 usec
TE               299.2 K
D1          5.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1      300.1719511 MHz
NUC1               1H
P1              14.95 usec
PLW1       8.19999981 W

F2 - Processing parameters
SI              131072
SF         300.1700073 MHz
WDW                 EM
SSB                  0
LB                0.10 Hz
GB                   0
PC                1.00
```

Compound 4: LCMS of DDLP HO-Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala-NH_2



17/04/2014 15:42:53 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
Pyridal C:\LabSolutions\Data\McAlpine\koay\2013\130914\pyridal.lcd

MS Spectrum Graph

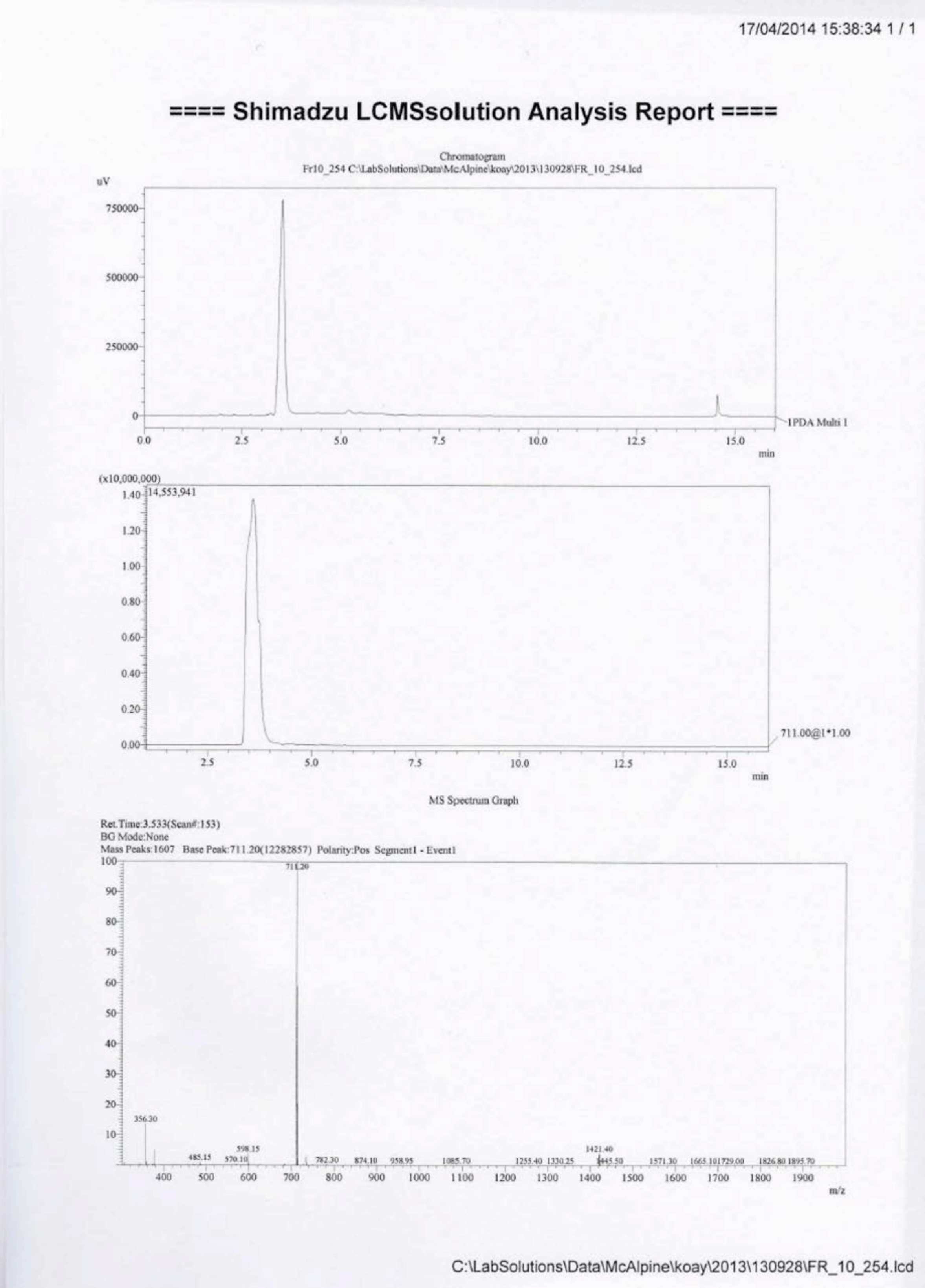
Ret.Time:1.667(Scan#:41)
BG Mode:?
Mass Peaks:1656 Base Peak:365.10(16510895) Polarity:Pos Segment1 - Event1

MS Spectrum Graph

Ret.Time:2.850(Scan#:112)
BG Mode:?
Mass Peaks:1664 Base Peak:365.10(14530422) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\130914\pyridal.lcd

Compound 4: LCMS of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala



17/04/2014 15:38:34 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

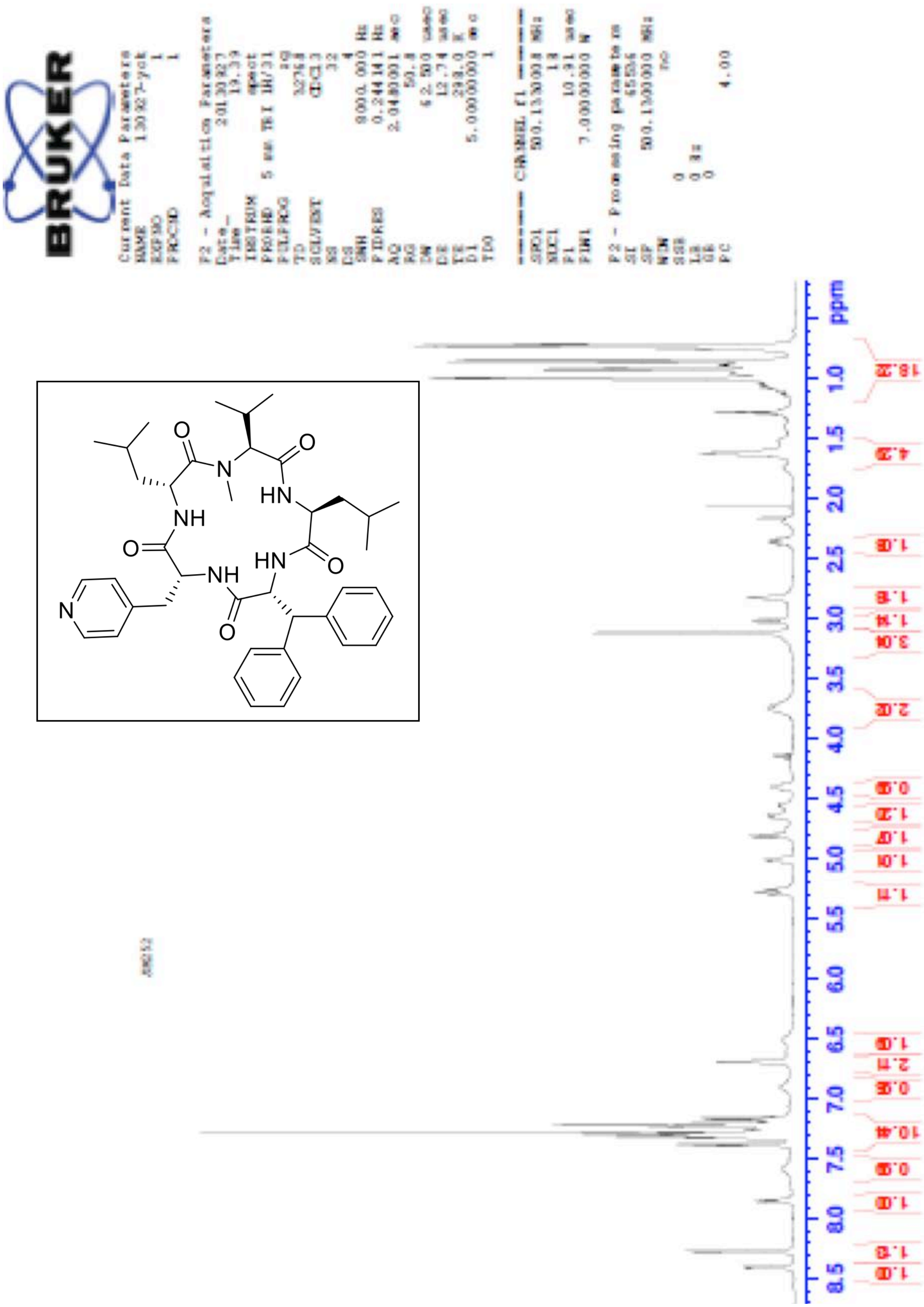
Chromatogram
Fr10_254 C:\LabSolutions\Data\McAlpine\koay\2013\130928\FR_10_254.lcd

MS Spectrum Graph

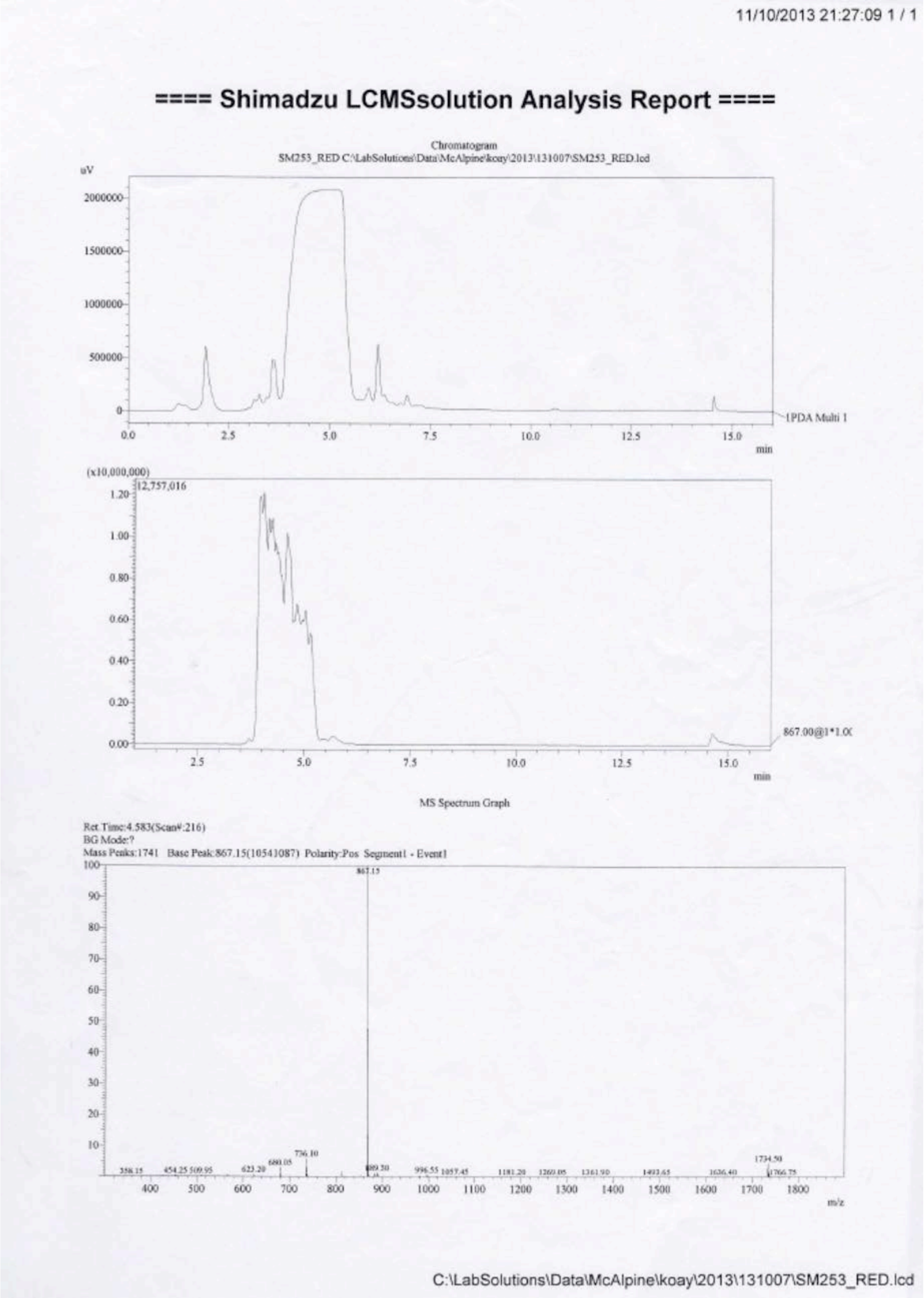
Ret.Time:3.533(Scan#:153)
BG Mode:None
Mass Peaks:1607 Base Peak:711.20(12282857) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\130928\FR_10_254.lcd

Compound 4: ^{1}HNMR of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-3-(4-Pyridyl)Ala-3,3-Diphenyl-D-Ala



Compound 5: LCMS of DDLP HO-Leu-*N*-Me-Val-D-Leu-D-Trp(Boc)-3,3-Diphenyl-D-Ala



11/10/2013 21:27:09 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

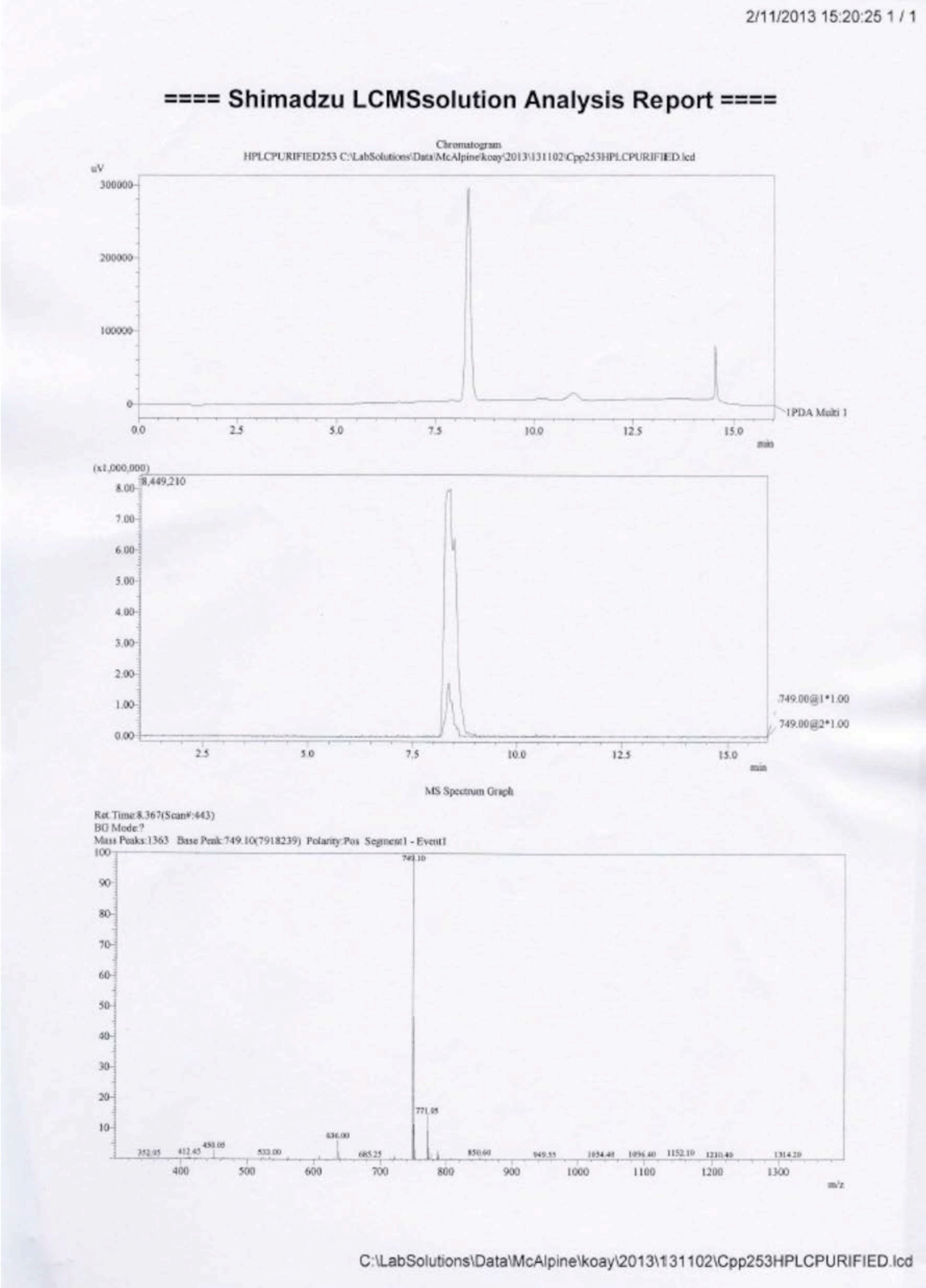
Chromatogram
SM253_RED C:\LabSolutions\Data\McAlpine\koay\2013\131007\SM253_RED.lcd

MS Spectrum Graph

Ret.Time:4.583(Scan#:216)
BG Mode:?
Mass Peaks:1741 Base Peak:867.15(10541087) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\131007\SM253_RED.lcd

Compound 5: LCMS of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-Trp(Boc)-3,3-Diphenyl-D-Ala



2/11/2013 15:20:25 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

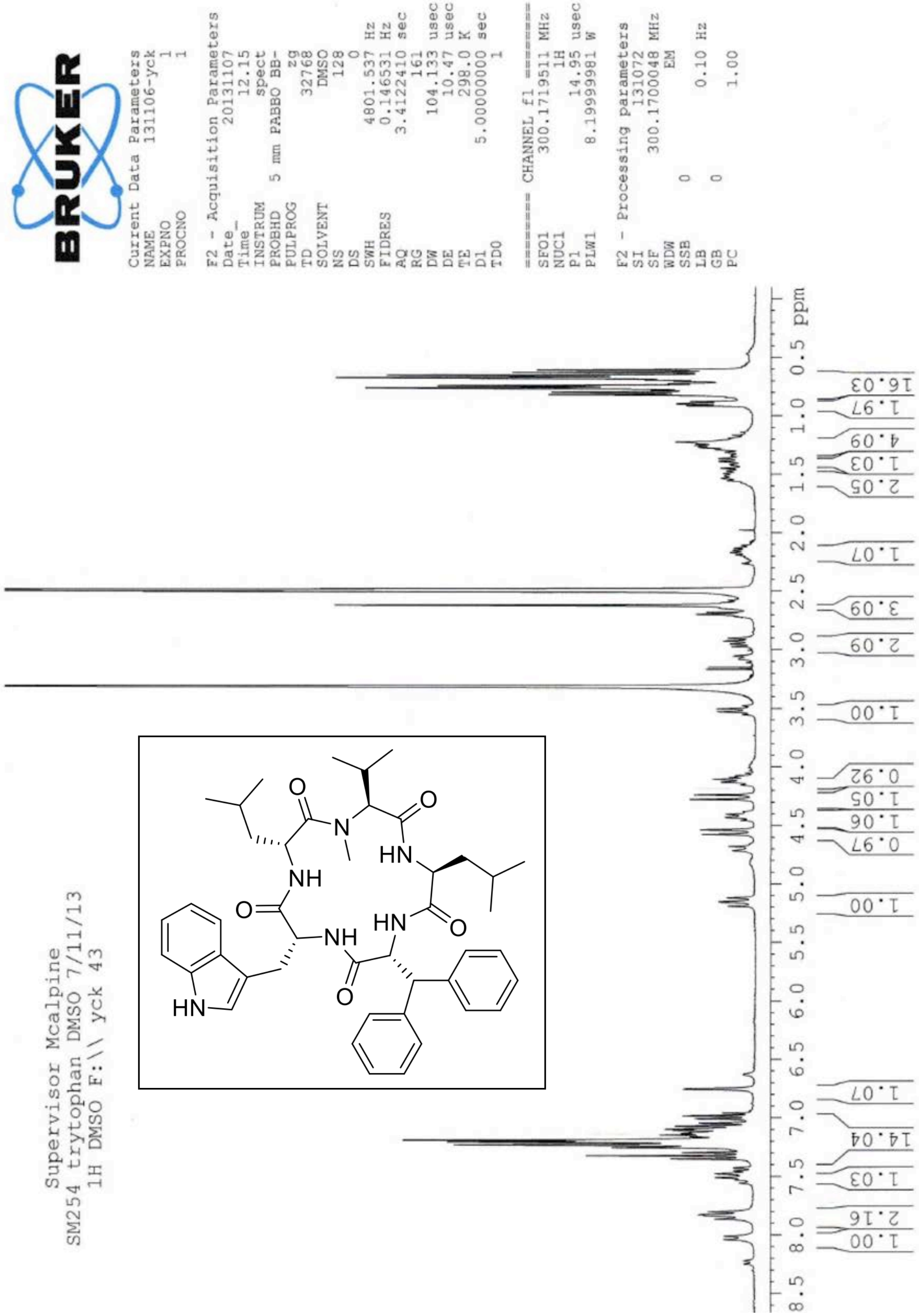
Chromatogram
HPLCPURIFIED253 C:\LabSolutions\Data\McAlpine\koay\2013\131102\Cpp253HPLCPURIFIED.lcd

MS Spectrum Graph

Ret.Time:8.367(Scan#:443)
BG Mode:?
Mass Peaks:1363 Base Peak:749.10(7918239) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\131102\Cpp253HPLCPURIFIED.lcd

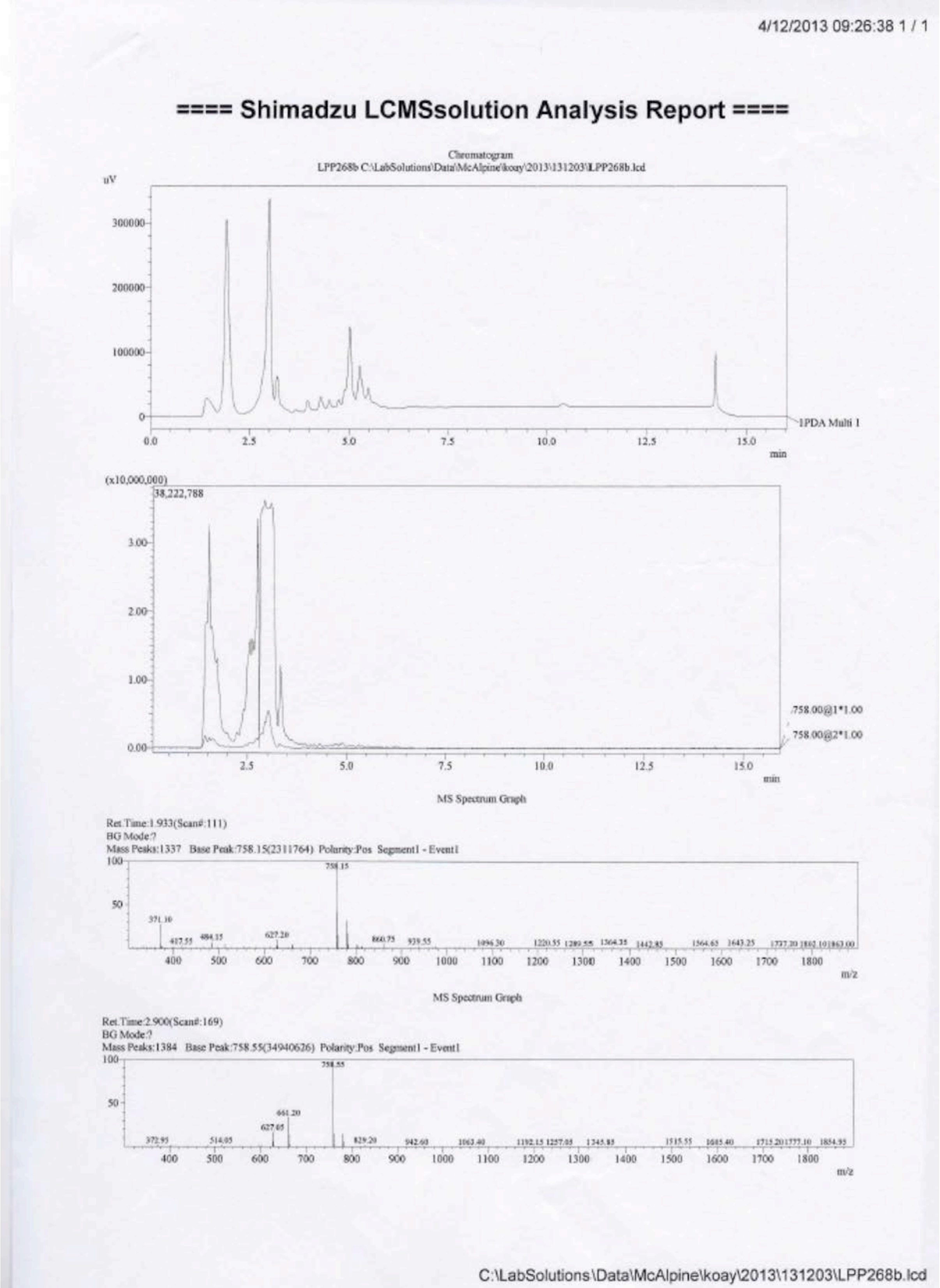
Compound 5: ^{1}HNMR of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-Trp(Boc)-3,3-Diphenyl-D-Ala



```
Current Data Parameters
NAME        131106-yck
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_         20131107
Time_            12.15
INSTRUM          spect
PROBHD   5 mm PABBO BB-
PULPROG             zg
TD               32768
SOLVENT           DMSO
NS                 128
DS                   0
SWH           4801.537 Hz
FIDRES        0.146531 Hz
AQ           3.4122410 sec
RG                 161
DW             104.133 usec
DE               10.47 usec
TE               298.0 K
D1          5.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1       300.1719511 MHz
NUC1                1H
P1               14.95 usec
PLW1        8.19999981 W

F2 - Processing parameters
SI              131072
SF         300.1700048 MHz
WDW                 EM
SSB                  0
LB                0.10 Hz
GB                   0
PC                1.00
```

Supervisor Mcalpine
SM254 trytophan DMSO 7/11/13
1H DMSO F:\\ yck 43

Compound 6: LCMS of DDLP HO-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala-NH$_2$



4/12/2013 09:26:38 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
LPP268b C:\LabSolutions\Data\McAlpine\koay\2013\131203\LPP268b.lcd

MS Spectrum Graph

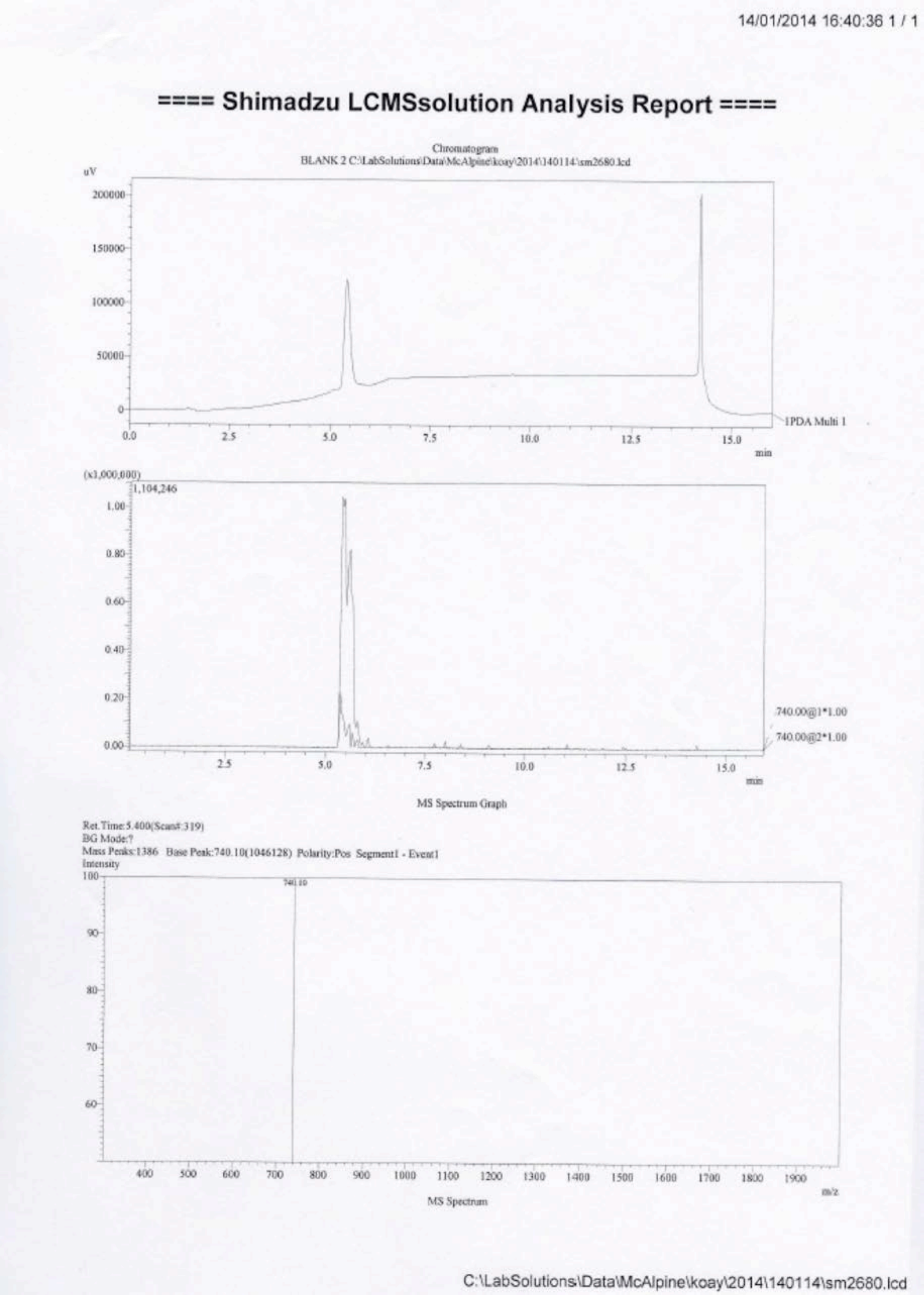
Ret.Time:1.933(Scan#:111)
BG Mode:?
Mass Peaks:1337 Base Peak:758.15(2311764) Polarity:Pos Segment1 - Event1

MS Spectrum Graph

Ret.Time:2.900(Scan#:169)
BG Mode:?
Mass Peaks:1384 Base Peak:758.55(34940626) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\131203\LPP268b.lcd

Compound 6: LCMS of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala



14/01/2014 16:40:36 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
BLANK 2 C:\LabSolutions\Data\McAlpine\koay\2014\140114\sm2680.lcd

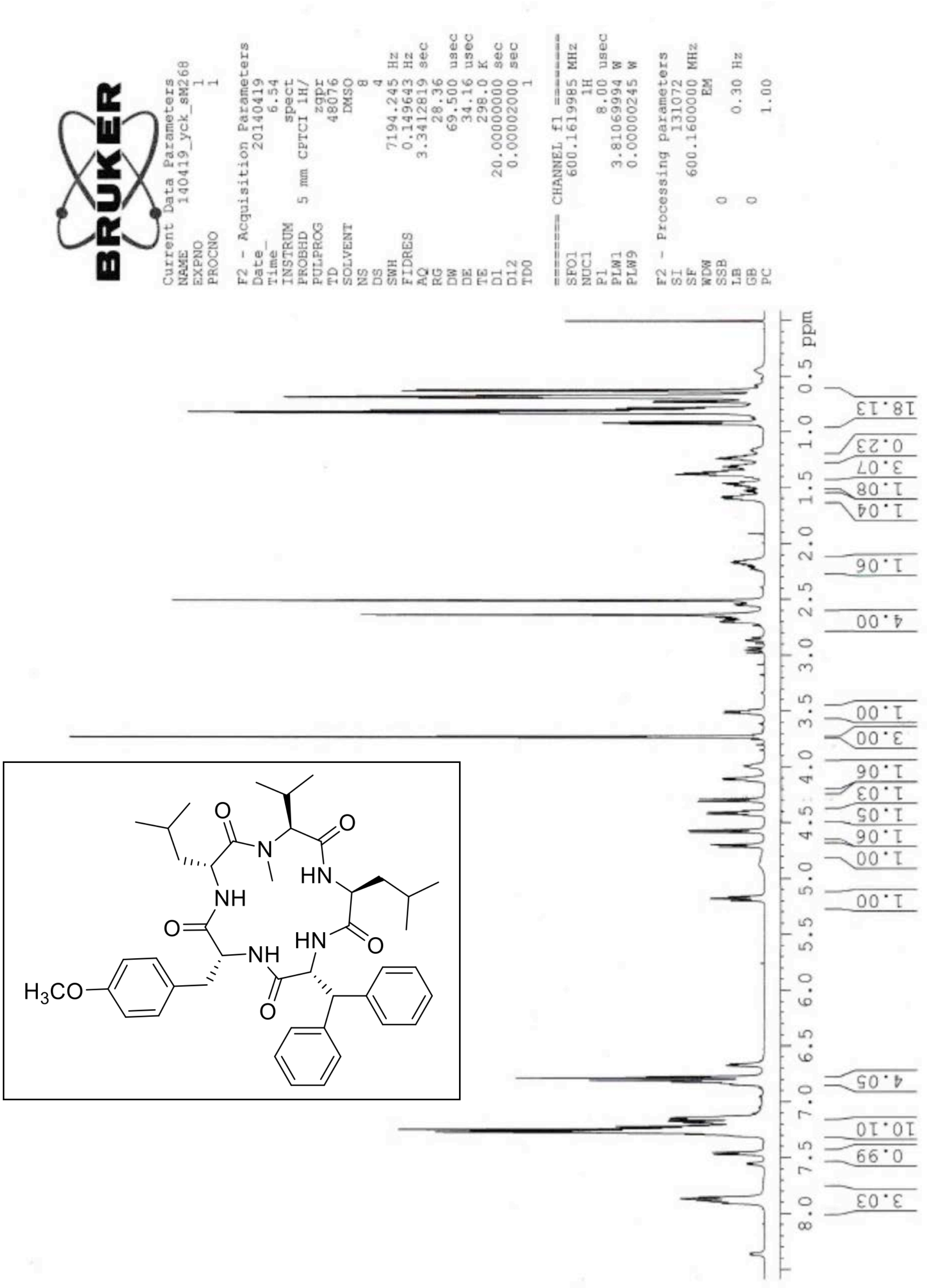
MS Spectrum Graph

Ret.Time:5.400(Scan#:319)
BG Mode:?
Mass Peaks:1386 Base Peak:740.10(1046128) Polarity:Pos Segment1 - Event1

MS Spectrum

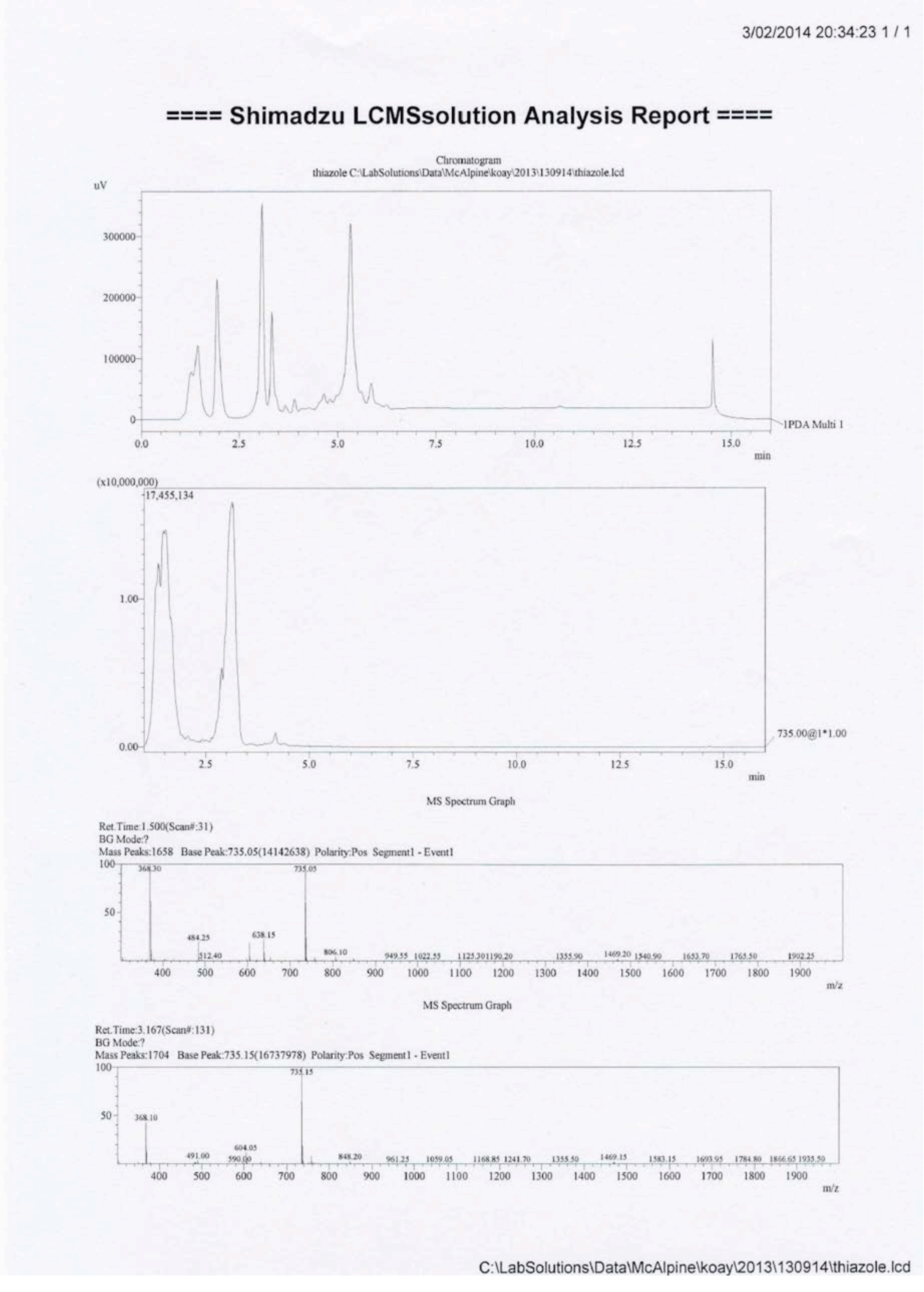
C:\LabSolutions\Data\McAlpine\koay\2014\140114\sm2680.lcd

Compound 6: ^{1}HNMR of *cyclo*-Leu-*N*-Me-Val-D-Leu-D-Tyr(Me)-3,3-Diphenyl-D-Ala



```
Current Data Parameters
NAME          140419_yck_sM268
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20140419
Time                  6.54
INSTRUM              spect
PROBHD   5 mm CPTCI 1H/
PULPROG               zgpr
TD                   48076
SOLVENT               DMSO
NS                       8
DS                       4
SWH               7194.245 Hz
FIDRES            0.149643 Hz
AQ               3.3412819 sec
RG                   28.36
DW                  69.500 usec
DE                   34.16 usec
TE                   298.0 K
D1             20.00000000 sec
D12             0.00002000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1        600.1619985 MHz
NUC1                    1H
P1                    8.00 usec
PLW1            3.81069994 W
PLW9            0.00000245 W

F2 - Processing parameters
SI                  131072
SF             600.1600000 MHz
WDW                     EM
SSB      0
LB       0            0.30 Hz
GB       0
PC                    1.00
```

Compound 7: LCMS of DDLP HO-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH_2



3/02/2014 20:34:23 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
thiazole C:\LabSolutions\Data\McAlpine\koay\2013\130914\thiazole.lcd

MS Spectrum Graph

Ret.Time:1.500(Scan#:31)
BG Mode:?
Mass Peaks:1658 Base Peak:735.05(14142638) Polarity:Pos Segment1 - Event1

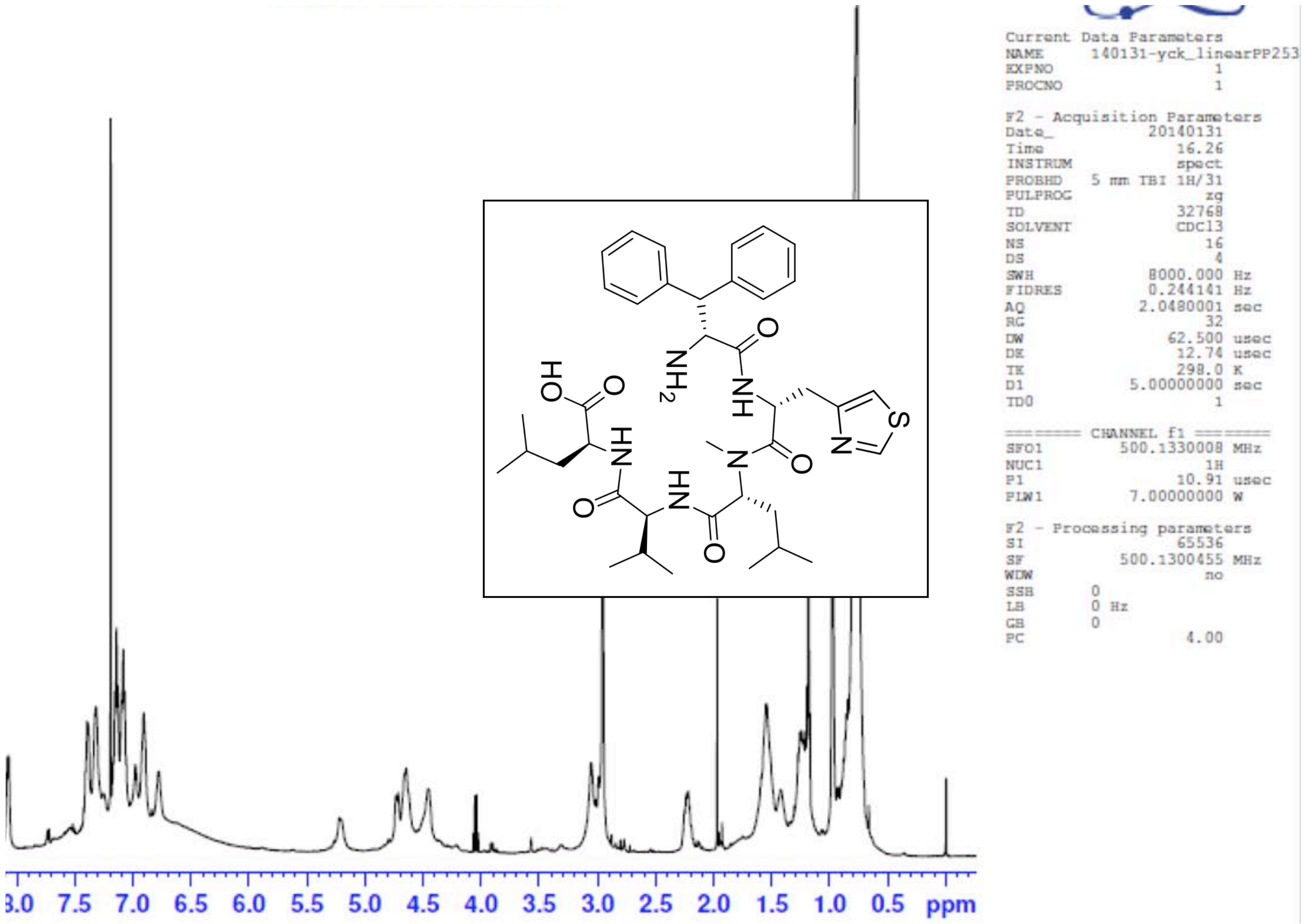
MS Spectrum Graph

Ret.Time:3.167(Scan#:131)
BG Mode:?
Mass Peaks:1704 Base Peak:735.15(16737978) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2013\130914\thiazole.lcd

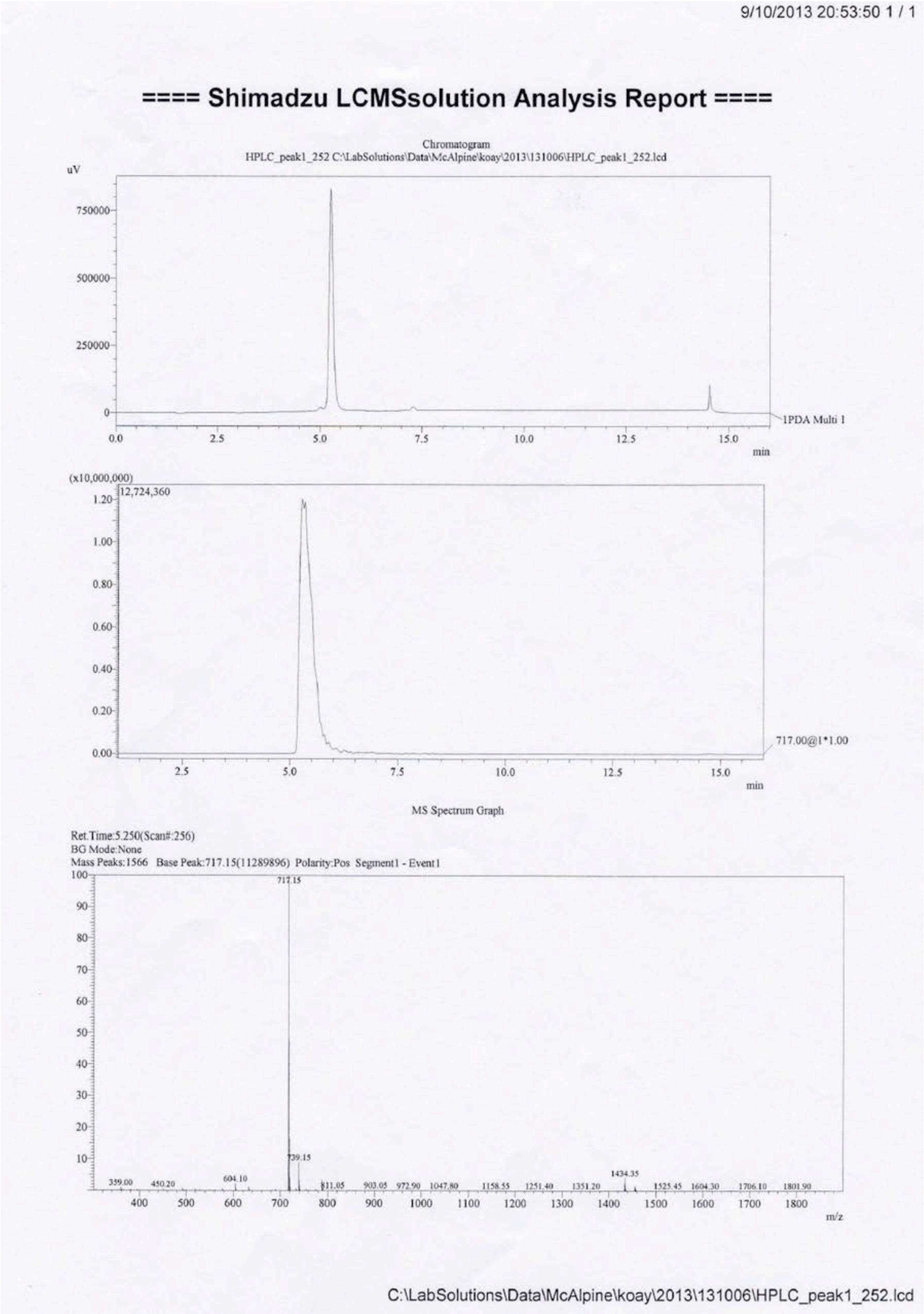
Supporting Information

Compound 7: ^{1}HNMR of DDLP HO-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala-NH_2



```
Current Data Parameters
NAME       140131-yck_linearPP253
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20140131
Time                16.26
INSTRUM             spect
PROBHD    5 mm TBI 1H/31
PULPROG                zg
TD                  32768
SOLVENT             CDCl3
NS                     16
DS                      4
SWH              8000.000 Hz
FIDRES           0.244141 Hz
AQ              2.0480001 sec
RG                     32
DW                 62.500 usec
DE                  12.74 usec
TE                  298.0 K
D1             5.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          500.1330008 MHz
NUC1                   1H
P1                  10.91 usec
PLW1           7.00000000 W

F2 - Processing parameters
SI                  65536
SF            500.1300455 MHz
WDW                    no
SSB       0
LB        0 Hz
GB        0
PC                   4.00
```

Compound 7: LCMS of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3- Diphenyl-D-Ala



9/10/2013 20:53:50 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
HPLC_peak1_252 C:\LabSolutions\Data\McAlpine\koay\2013\131006\HPLC_peak1_252.lcd

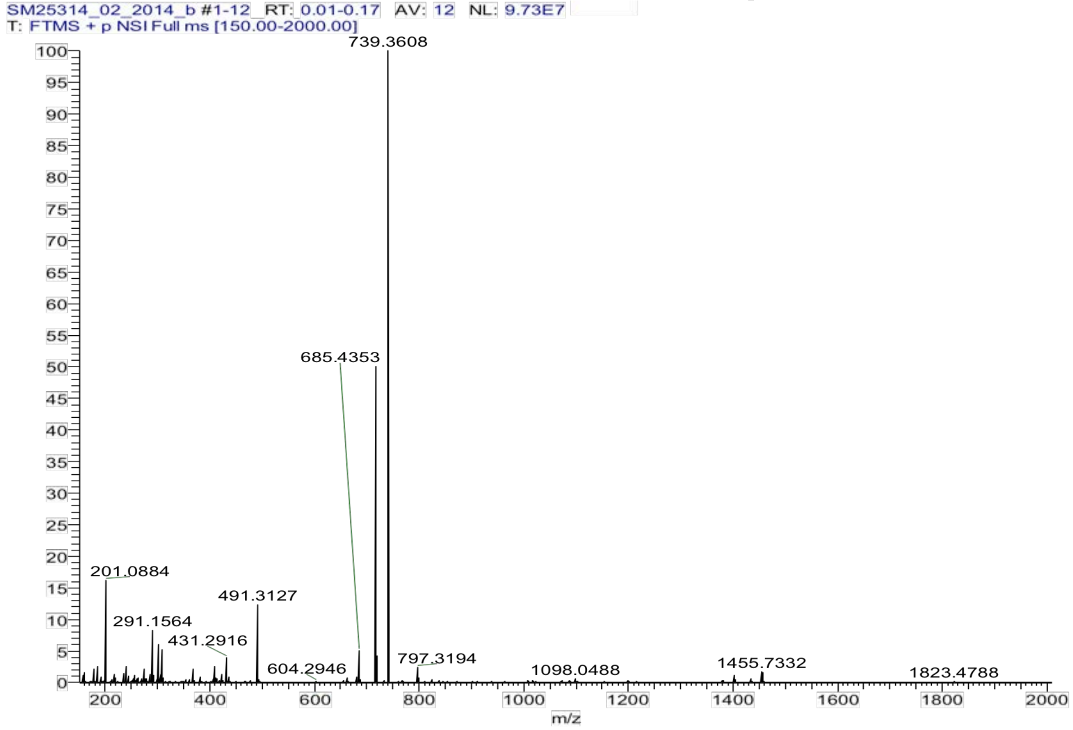
MS Spectrum Graph

Ret.Time:5.250(Scan#:256)
BG Mode:None
Mass Peaks:1566 Base Peak:717.15(11289896) Polarity:Pos Segment1 - Event1

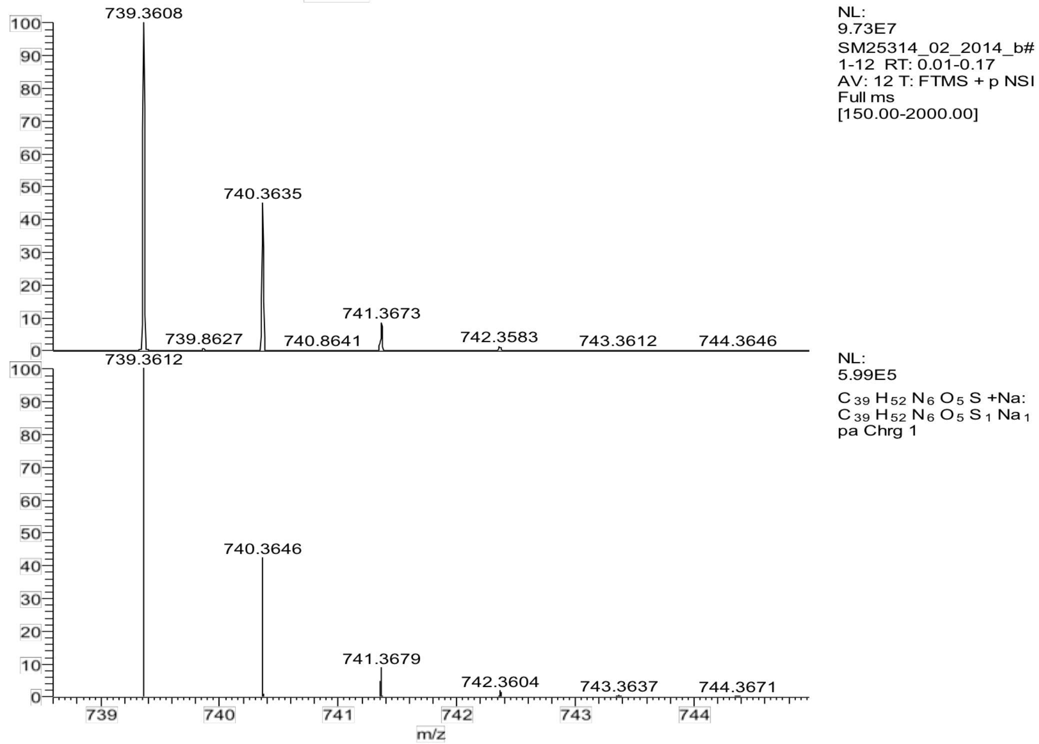
C:\LabSolutions\Data\McAlpine\koay\2013\131006\HPLC_peak1_252.lcd

Compound 7: HRMS of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3- Diphenyl-D-Ala

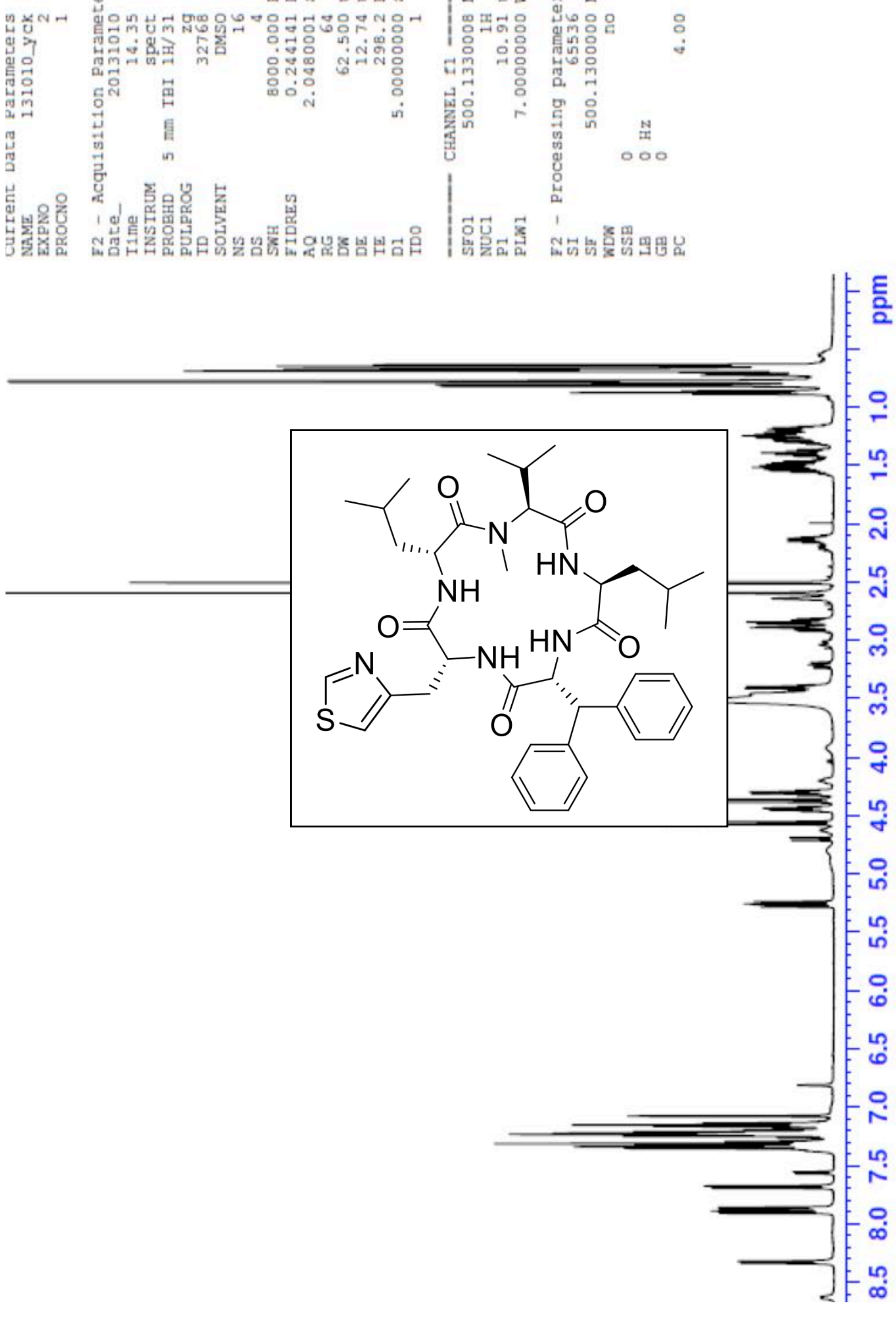
MS Data from Orbitrap



Zoomed Spectrum



Compound 7: ^{1}HNMR of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala



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PROCNO               1

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PULPROG             zg
TD               32768
SOLVENT           DMSO
NS                  16
DS                   4
SWH           8000.000 Hz
FIDRES        0.244141 Hz
AQ           2.0480001 sec
RG                  64
DW              62.500 usec
DE               12.74 usec
TE               298.2 K
D1          5.00000000 sec
TD0                  1

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NUC1               1H
P1              10.91 usec
PLW1       7.00000000 W

F2 - Processing parameters
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WDW                 no
SSB      0
LB       0 Hz
GB       0
PC                4.00
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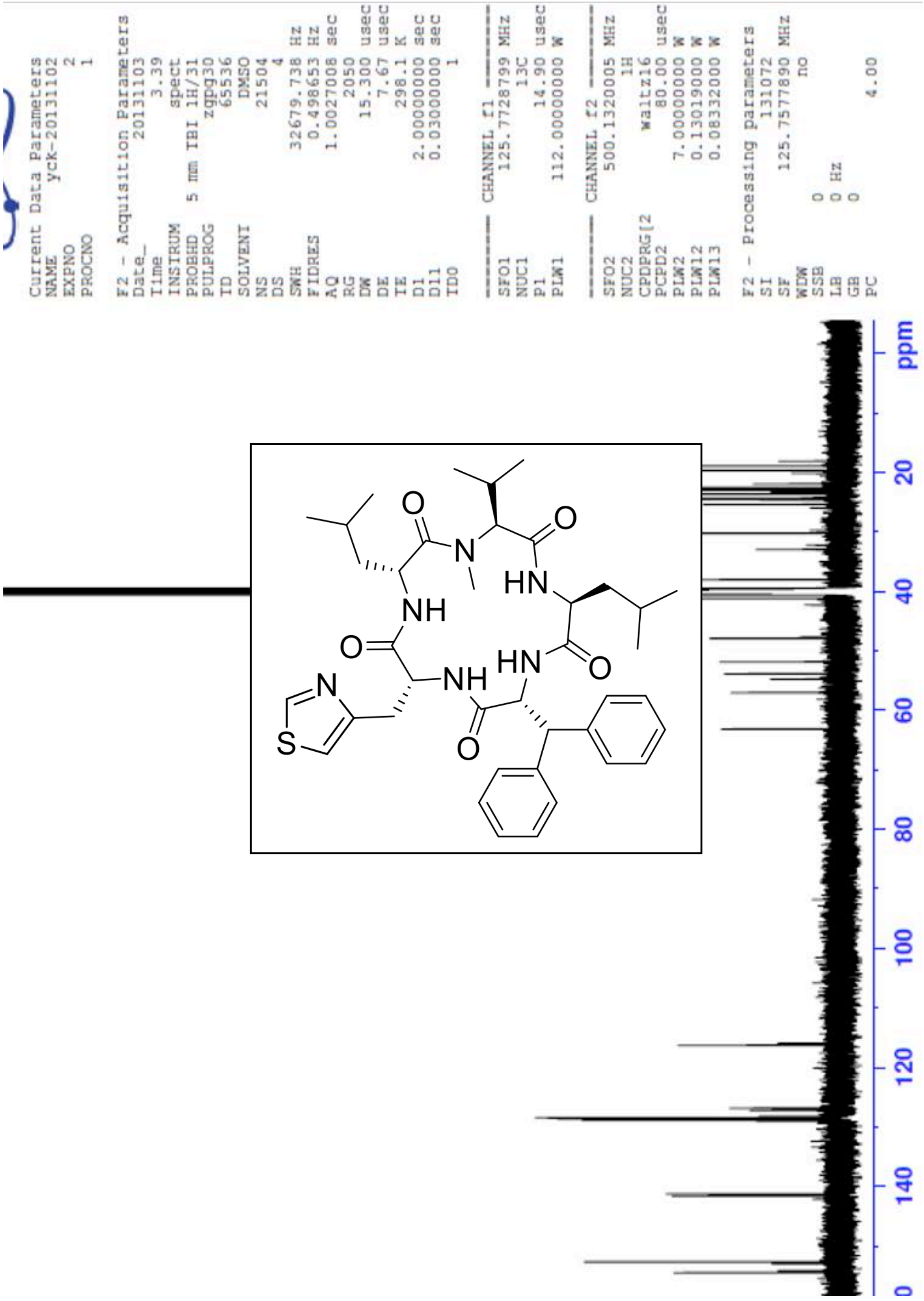
Supporting Information

Compound 7: ^{13}CNMR of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala



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PROCNO                 1

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NS                 21504
DS                     4
SWH            32679.738 Hz
FIDRES          0.498653 Hz
AQ             1.0027008 sec
RG                  2050
DW                15.300 usec
DE                  7.67 usec
TE                 298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
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NUC1                 13C
P1                 14.90 usec
PLW1        112.00000000 W

======== CHANNEL f2 ========
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NUC2                  1H
CPDPRG[2         waltz16
PCPD2              80.00 usec
PLW2          7.00000000 W
PLW12         0.13019000 W
PLW13         0.08332000 W

F2 - Processing parameters
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SF           125.7577890 MHz
WDW                   no
SSB      0
LB       0 Hz
GB       0
PC                  4.00
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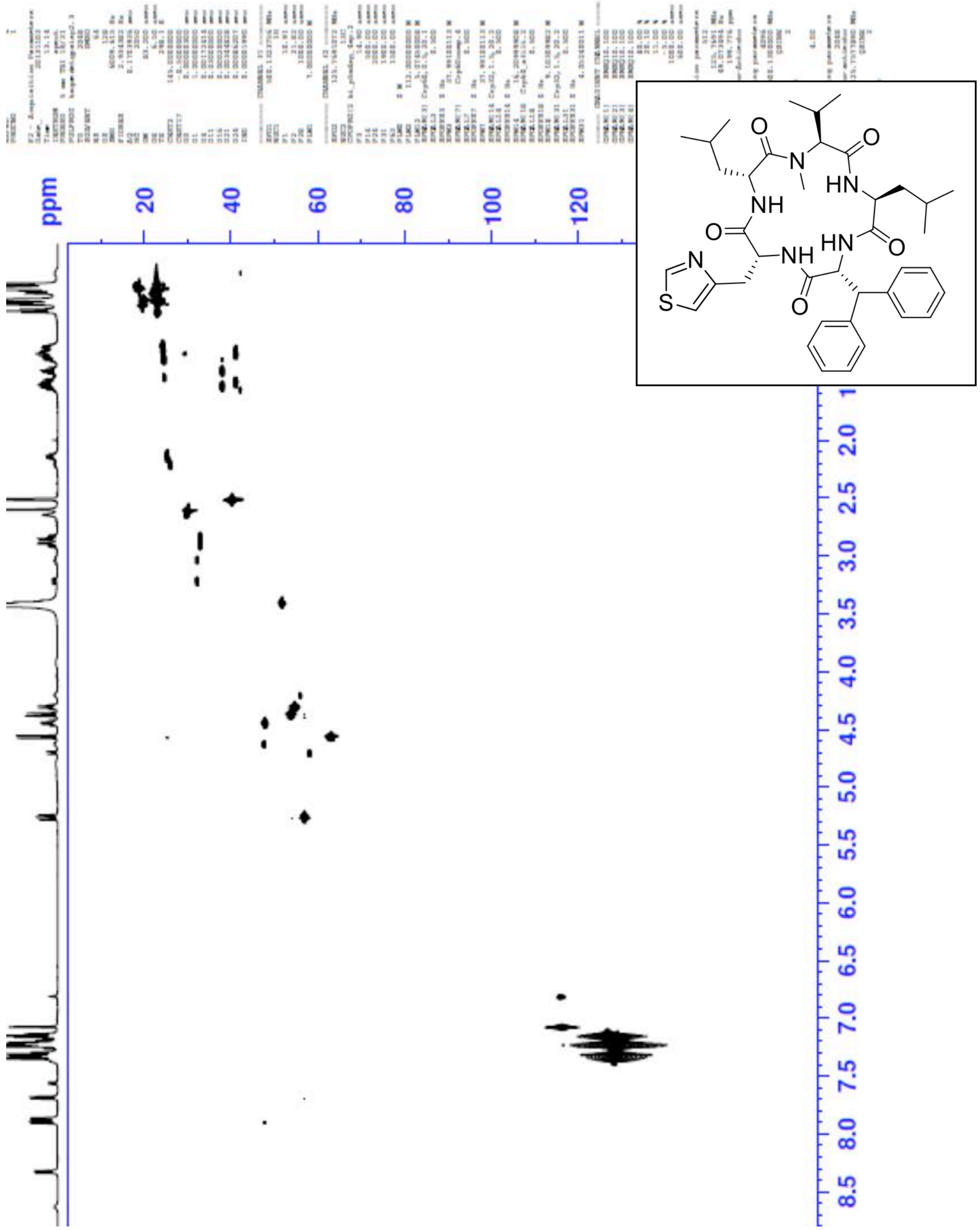
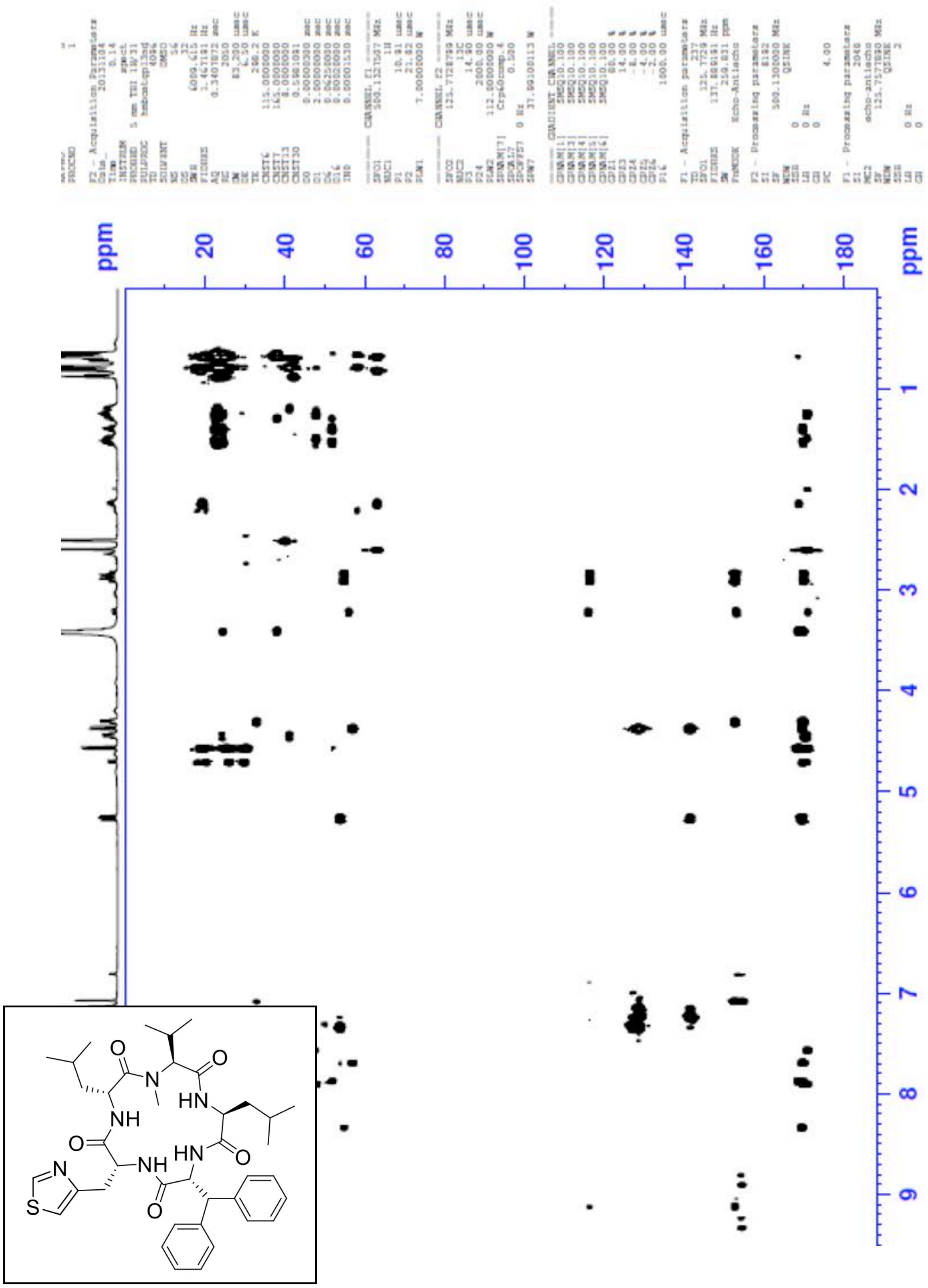
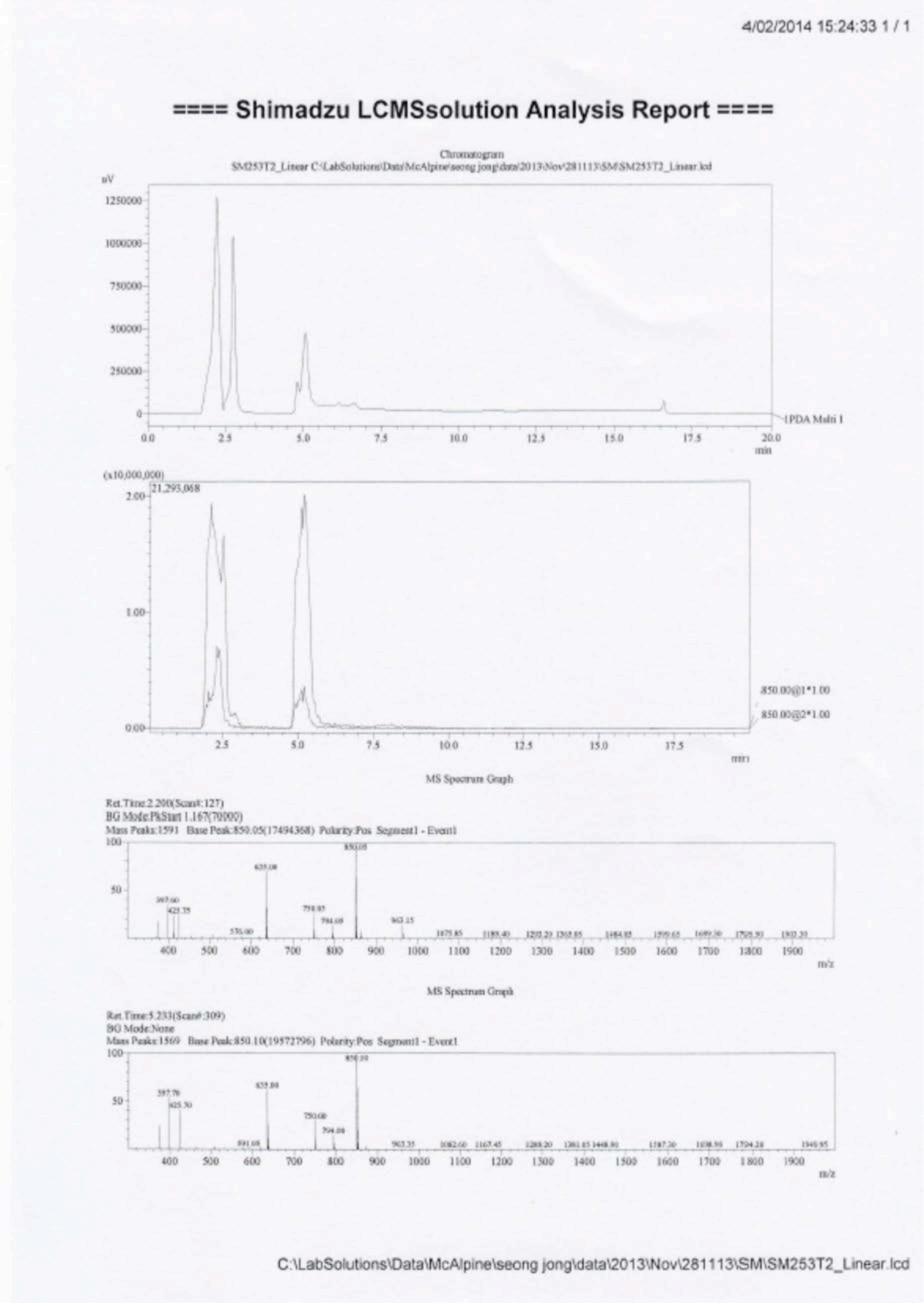
Compound 7: ^{1}H-^{13}C HSQC of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala



Compound 7: ^{1}H-^{13}C HMBC of cyclo-Leu-N-Me-Val-D-Leu-D-3-(4-Thiazoyl)Ala-3,3-Diphenyl-D-Ala



Compound 7-tag: LCMS of DDLP 7-TagII



4/02/2014 15:24:33 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
SM253T2_Linear C:\LabSolutions\Data\McAlpine\seong jong\data\2013\Nov\281113\SM\SM253T2_Linear.lcd

MS Spectrum Graph

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Mass Peaks:1591 Base Peak:850.05(17494368) Polarity:Pos Segment1 - Event1

MS Spectrum Graph

Ret.Time:5.233(Scan#:309)
BG Mode:None
Mass Peaks:1569 Base Peak:850.10(19572796) Polarity:Pos Segment1 - Event1

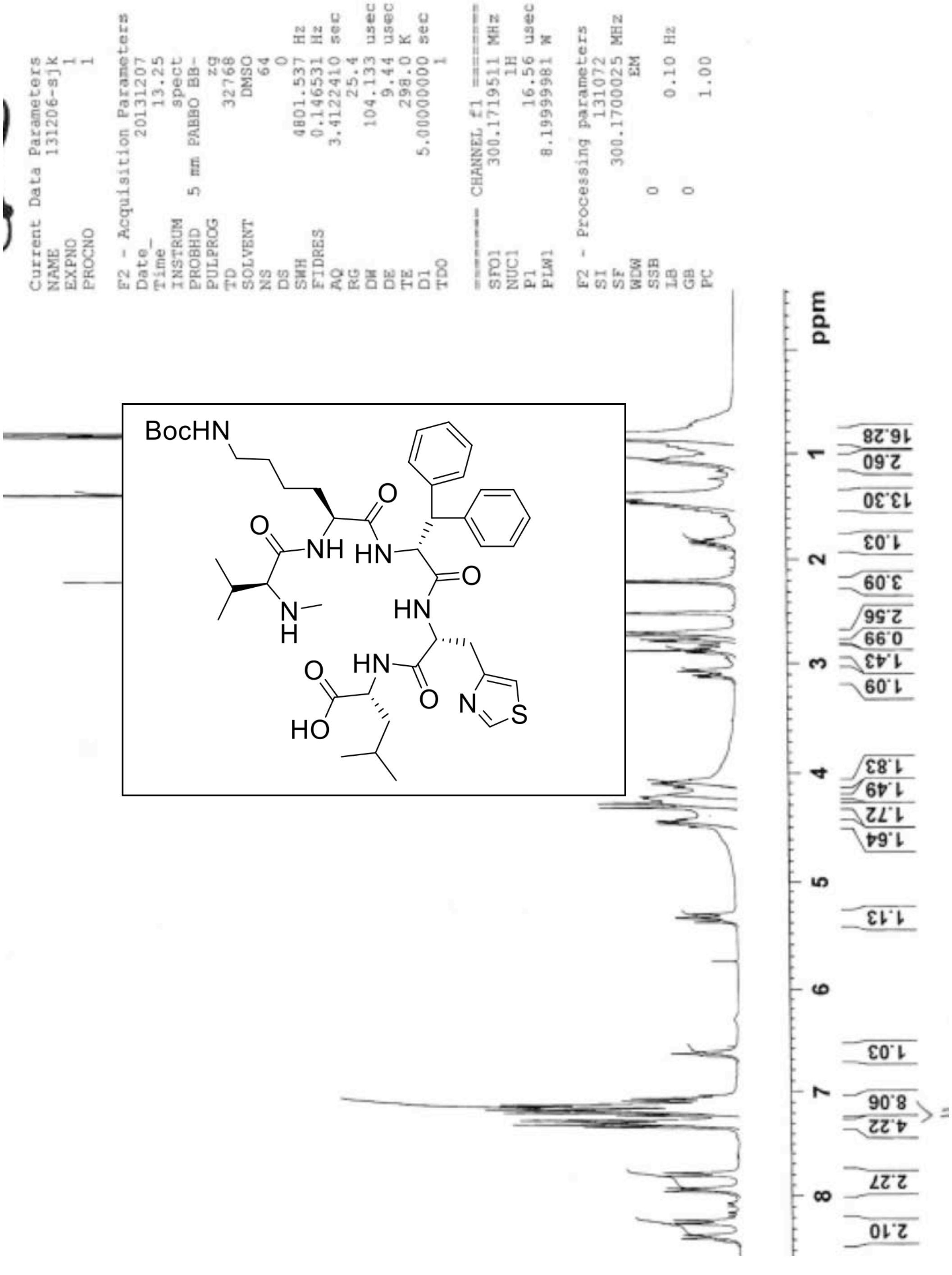
C:\LabSolutions\Data\McAlpine\seong jong\data\2013\Nov\281113\SM\SM253T2_Linear.lcd

Supporting Information

Compound 7-tag: ^{1}HNMR DDLP 7-TagII

Supporting Information

Compound 7-tag: LCMS of Macrocycle 7-TagII



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EXPNO                  1
PROCNO                 1

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Time               13.25
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PULPROG               zg
TD                 32768
SOLVENT             DMSO
NS                    64
DS                     0
SWH             4801.537 Hz
FIDRES          0.146531 Hz
AQ             3.4122410 sec
RG                  25.4
DW               104.133 usec
DE                  9.44 usec
TE                 298.0 K
D1            5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         300.1719511 MHz
NUC1                  1H
P1                 16.56 usec
PLW1          8.19999981 W

F2 - Processing parameters
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WDW                   EM
SSB      0
LB                  0.10 Hz
GB       0
PC                  1.00
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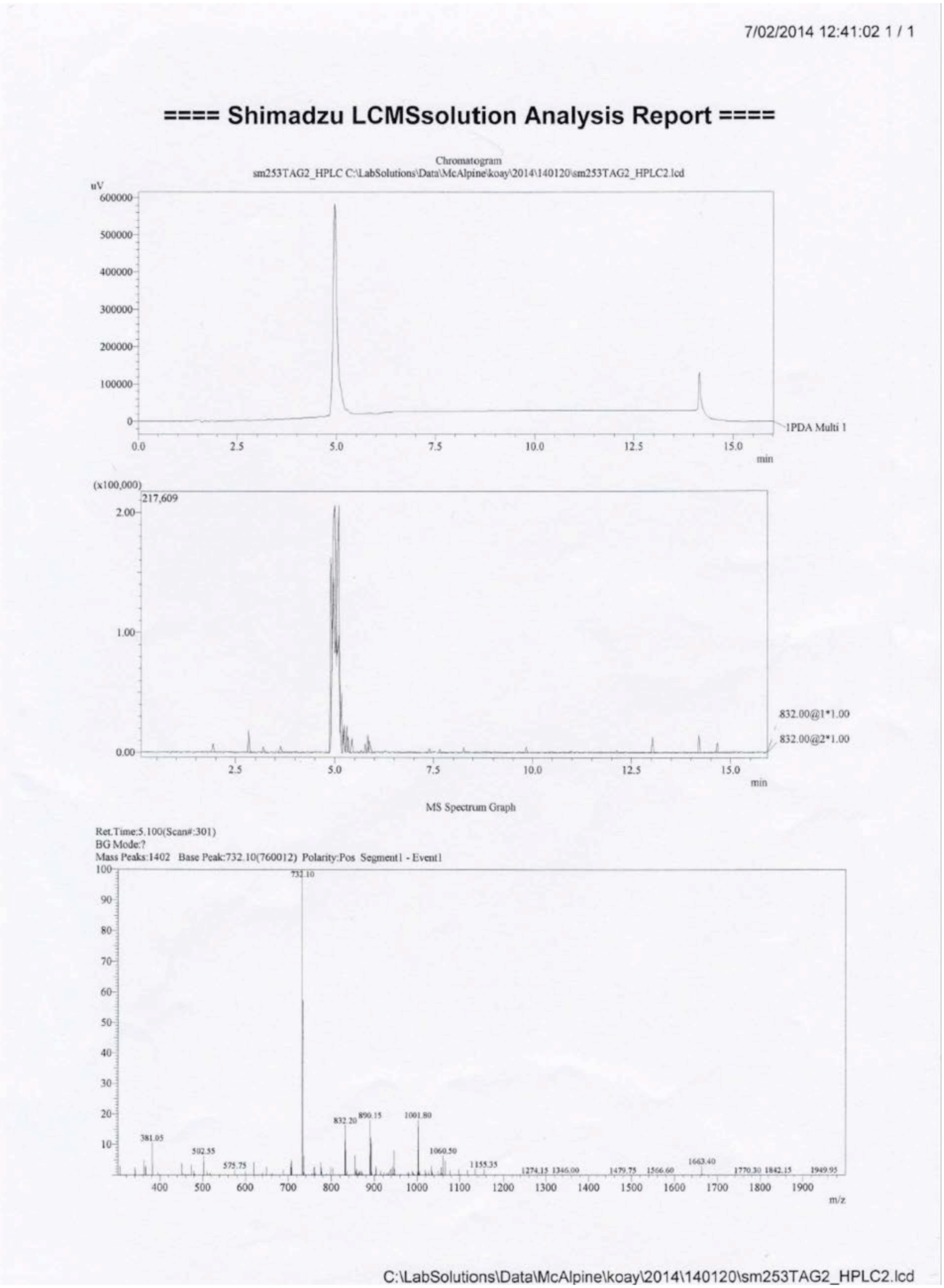

7/02/2014 12:41:02 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

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MS Spectrum Graph

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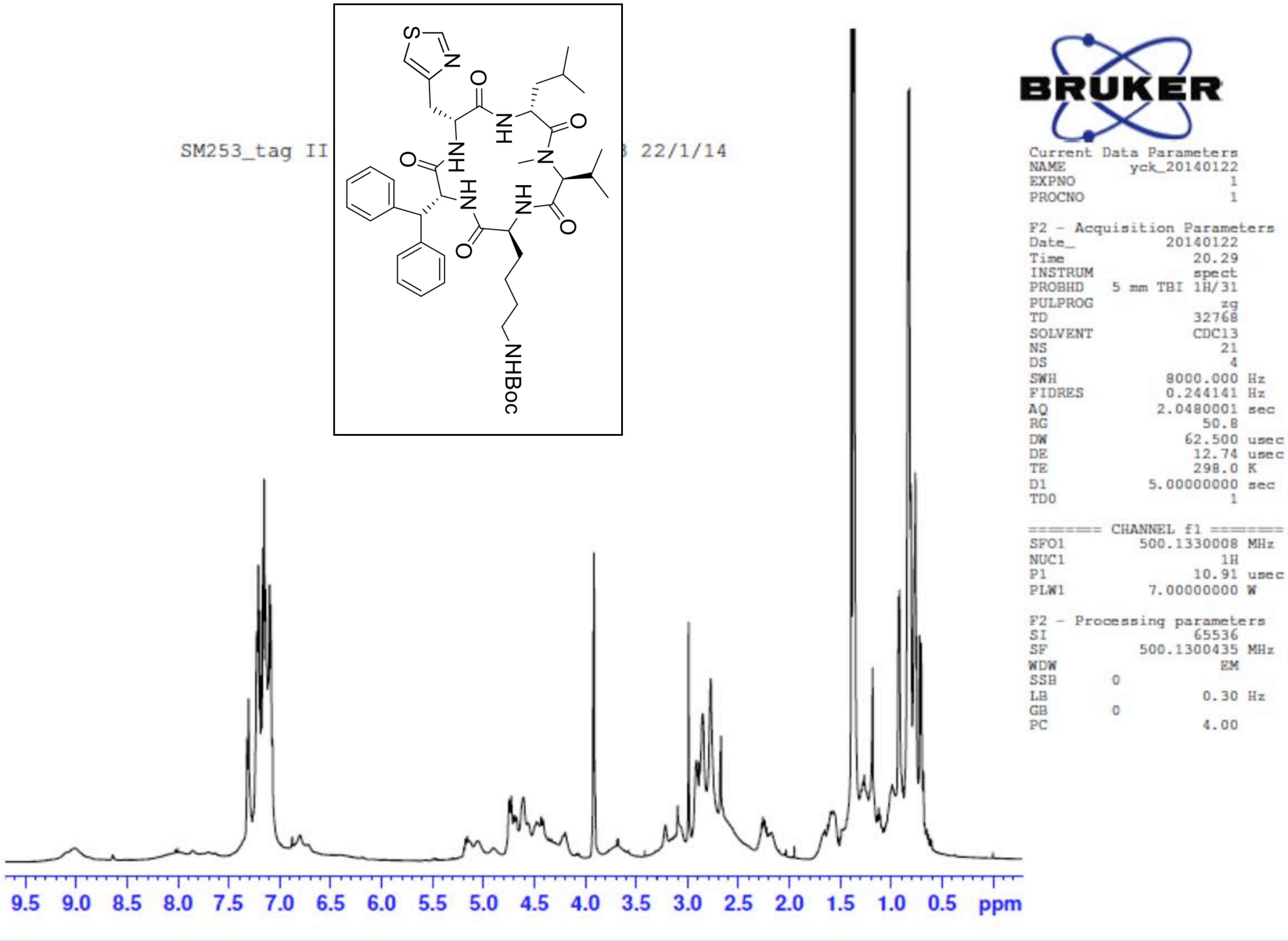
BG Mode:?
Mass Peaks:1402 Base Peak:732.10(760012) Polarity:Pos Segment1 - Event1

C:\LabSolutions\Data\McAlpine\koay\2014\140120\sm253TAG2_HPLC2.lcd

Supporting Information

Compound 7-tag: ^{1}HNMR Macrocycle 7-TagII



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Current Data Parameters
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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140122
Time               20.29
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PULPROG               zg
TD                 32768
SOLVENT            CDC13
NS                    21
DS                     4
SWH             8000.000 Hz
FIDRES          0.244141 Hz
AQ             2.0480001 sec
RG                  50.8
DW                62.500 usec
DE                 12.74 usec
TE                 298.0 K
D1            5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
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NUC1                  1H
P1                 10.91 usec
PLW1          7.00000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300435 MHz
WDW                   EM
SSB      0
LB                  0.30 Hz
GB       0
PC                  4.00
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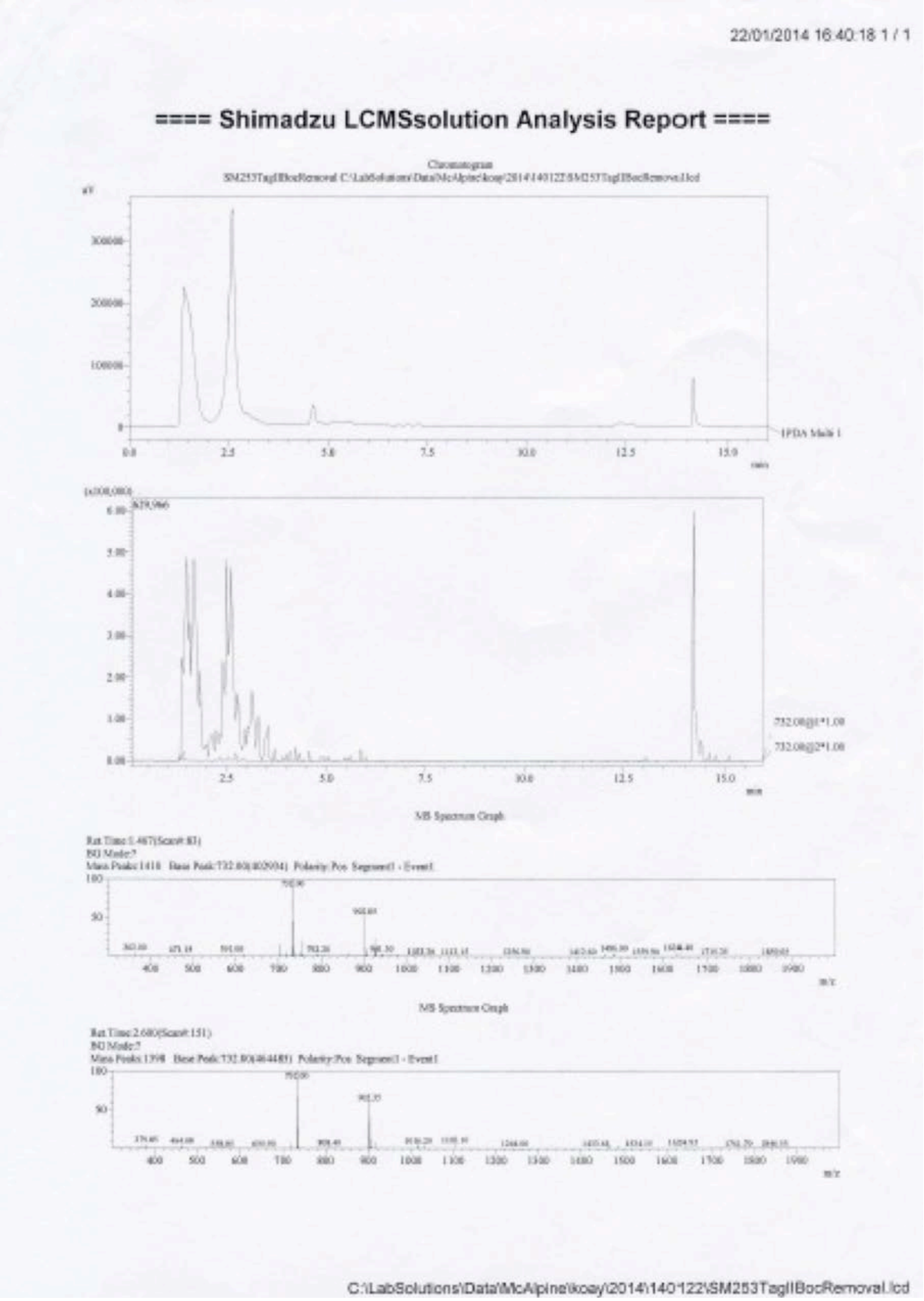
Supporting Information

Compound 7-tag: LCMS of Boc-deprotected Macrocycle 7-TagII



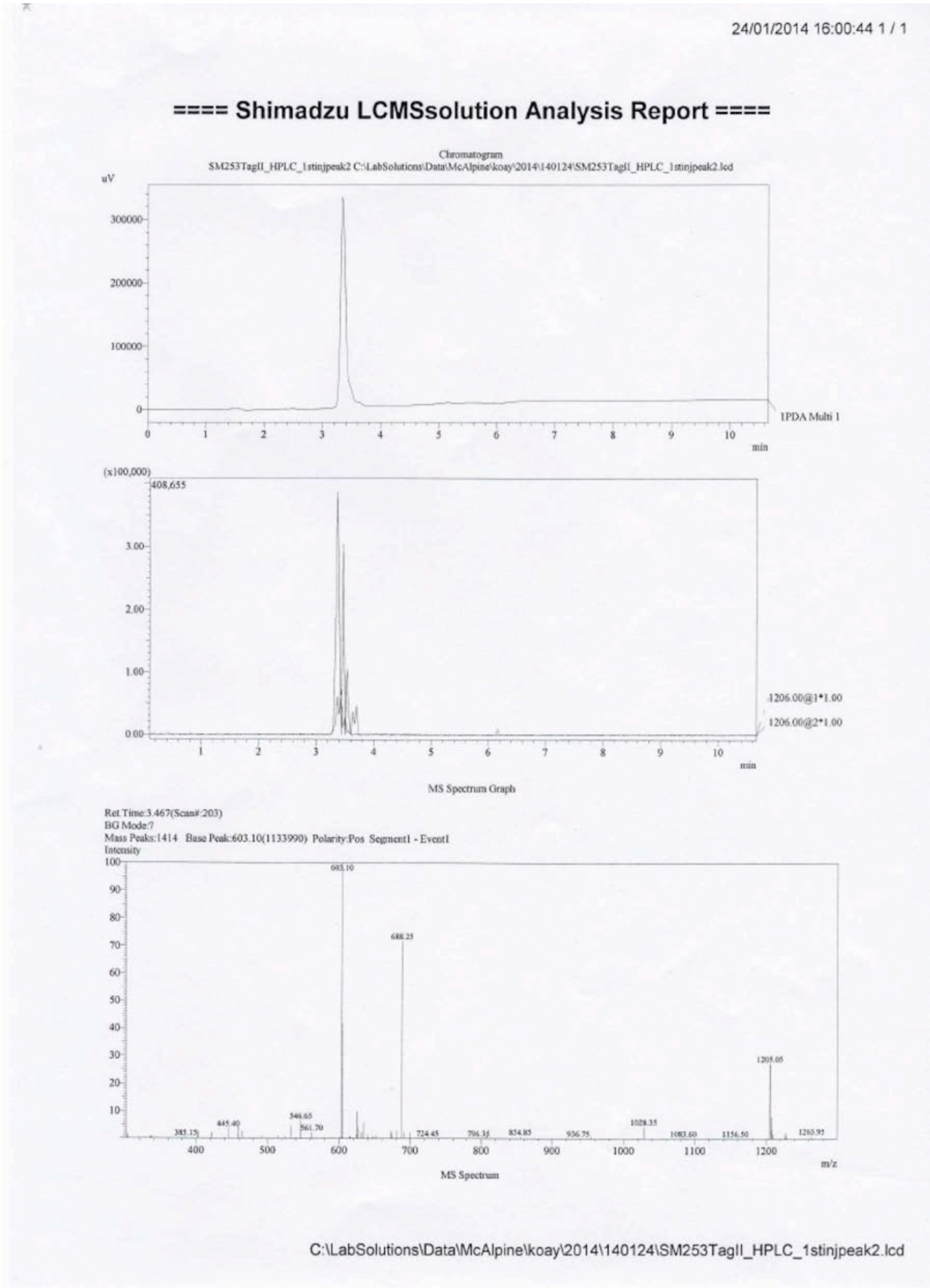
22/01/2014 16:40:18 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

C:\LabSolutions\Data\McAlpine\koay\2014\140122\SM253TagIIBocRemoval.lcd

Supporting Information

Compound 7-tag: LCMS of Macrocycle 7-Biotinylated tag



24/01/2014 16:00:44 1 / 1

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
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MS Spectrum Graph

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BG Mode:?
Mass Peaks:1414 Base Peak:603.10(1133990) Polarity:Pos Segment1 - Event1

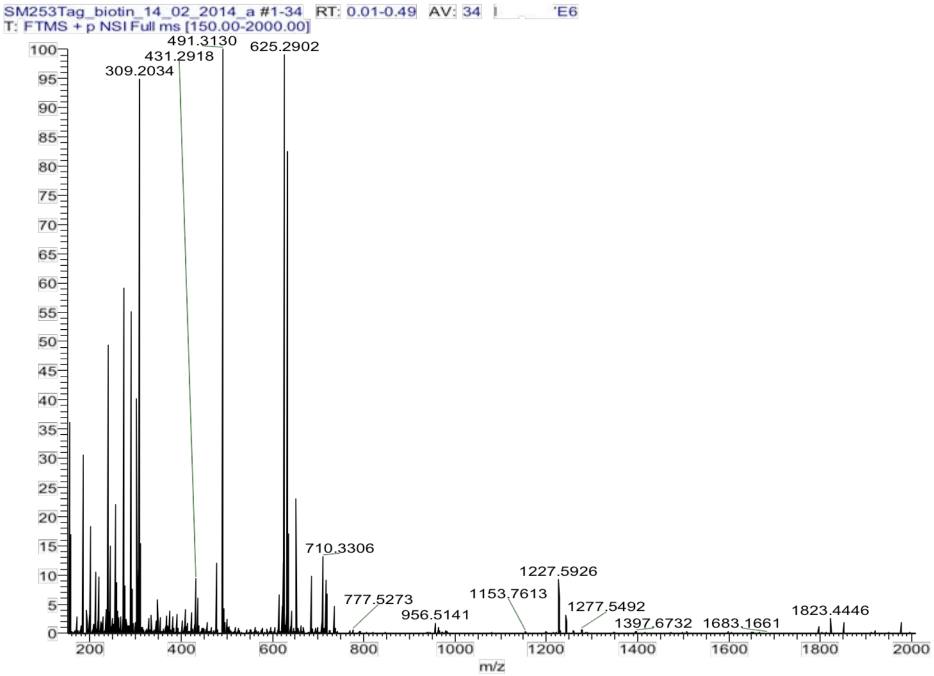
MS Spectrum

C:\LabSolutions\Data\McAlpine\koay\2014\140124\SM253TagII_HPLC_1stinjpeak2.lcd

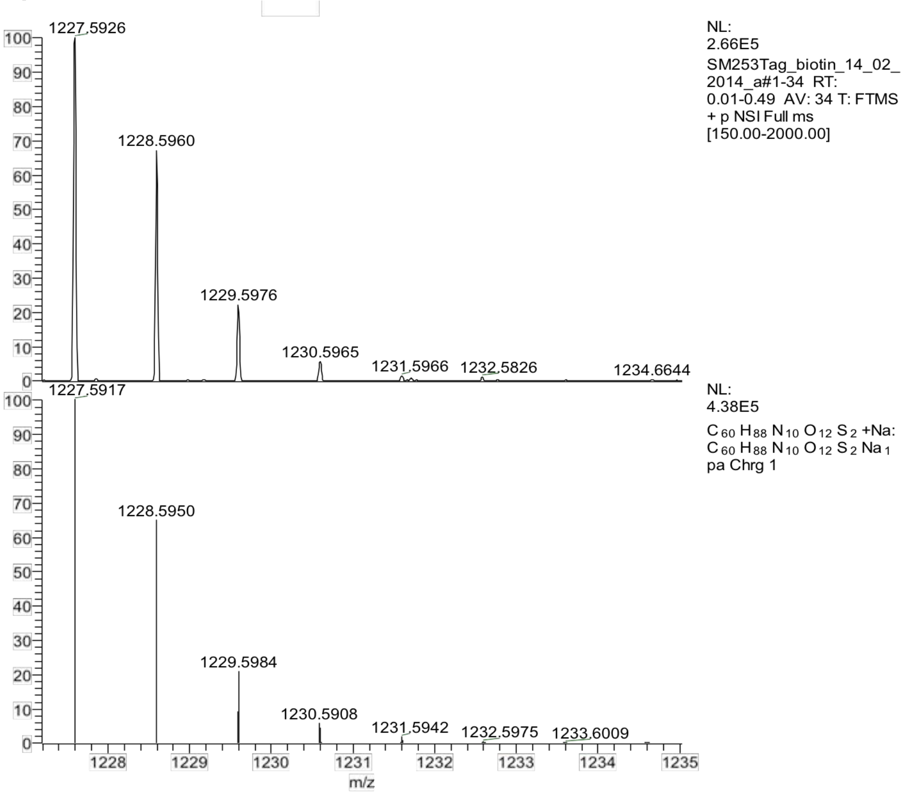
Compound 7-tag: HRMS of Macrocycle 7-Biotinylated tag

MS Data from Orbitrap

Full Spectrum

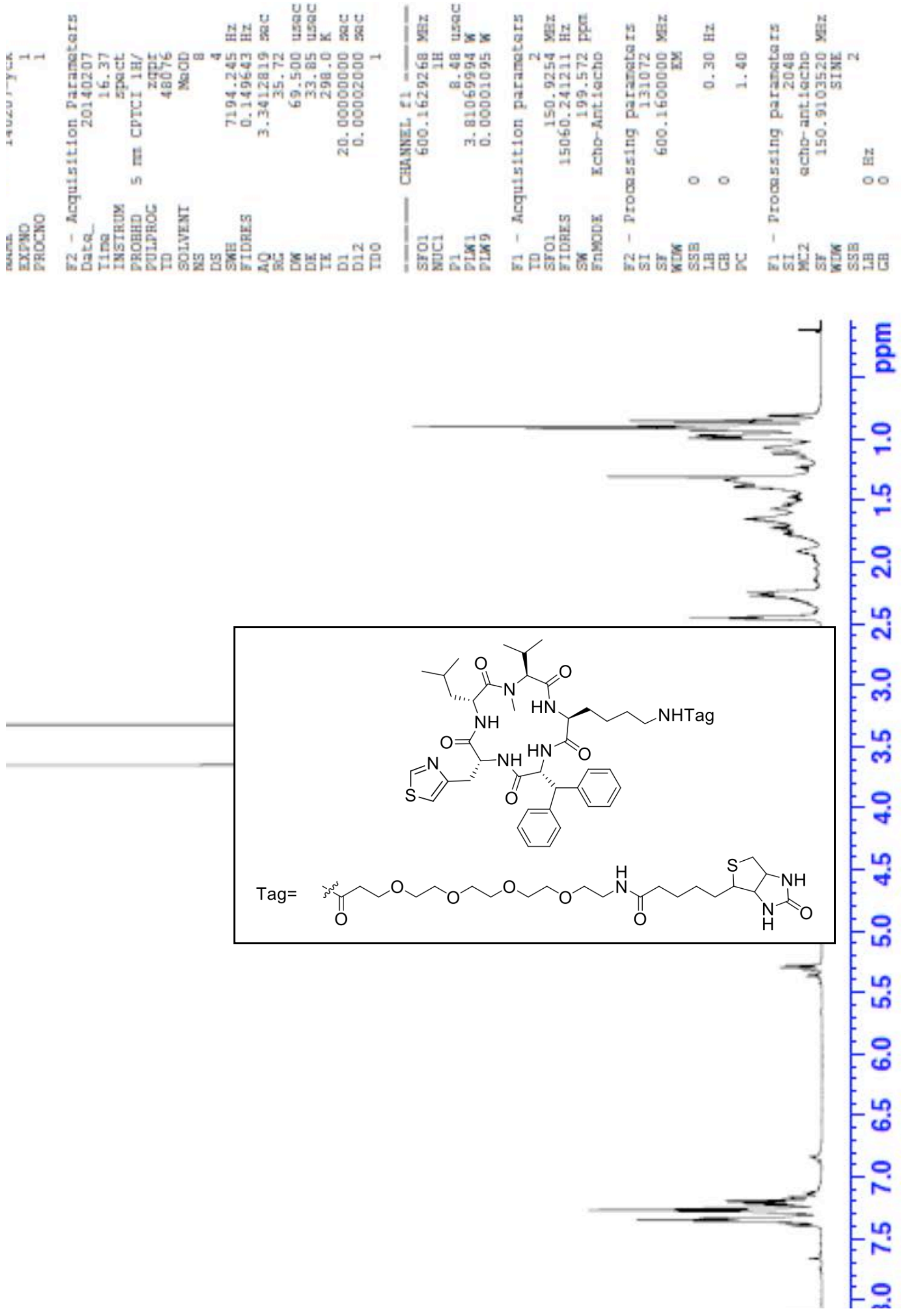


Zoomed Spectrum



Supporting Information

Compound 7-tag: ^{1}HNMR Macrocycle 7-Biotinylated tag



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EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
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Time                16.37
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PULPROG              zgpr
TD                  48076
SOLVENT              MeOD
NS                      8
DS                      4
SWH              7194.245 Hz
FIDRES           0.149643 Hz
AQ              3.3412819 sec
RG                  35.72
DW                 69.500 usec
DE                  33.85 usec
TE                  298.0 K
D1            20.00000000 sec
D12            0.00002000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1        600.1629268 MHz
NUC1                 1H
P1                   8.48 usec
PLW1          3.81069994 W
PLW9          0.00001095 W

F1 - Acquisition parameters
TD                      2
SFO1             150.9254 MHz
FIDRES       15060.241211 Hz
SW                199.572 ppm
FnMODE       Echo-Antiecho

F2 - Processing parameters
SI                 131072
SF            600.1600000 MHz
WDW                    EM
SSB      0
LB                   0.30 Hz
GB       0
PC                   1.40

F1 - Processing parameters
SI                   2048
MC2          echo-antiecho
SF            150.9103520 MHz
WDW                  SINE
SSB                     2
LB                  0 Hz
GB                  0
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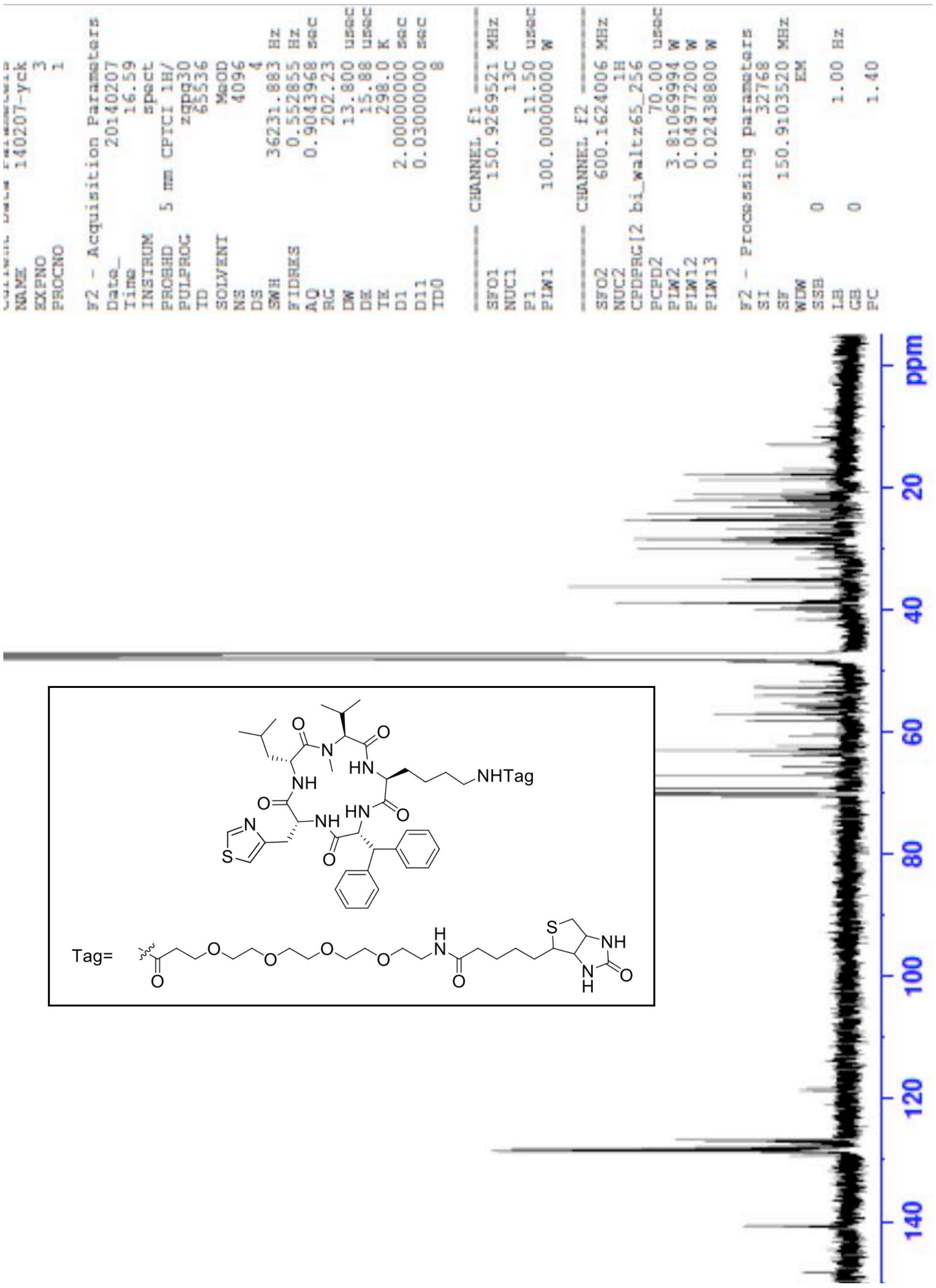
Compound 7-tag: ^{13}CNMR Macrocycle 7-Biotinylated tag



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PROCNO                    1

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PULPROG             zgpg30
TD                   65536
SOLVENT               MeOD
NS                    4096
DS                       4
SWH              36231.883 Hz
FIDRES            0.552855 Hz
AQ               0.9043968 sec
RG                  202.23
DW                  13.800 usec
DE                   15.88 usec
TE                   298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                      8

======== CHANNEL f1 ========
SFO1           150.9269521 MHz
NUC1                   13C
P1                   11.50 usec
PLW1       100.00000000 W

======== CHANNEL f2 ========
SFO2           600.1624006 MHz
NUC2                    1H
CPDPRG[2 bi_waltz65_256
PCPD2                70.00 usec
PLW2            3.81069994 W
PLW12           0.04977200 W
PLW13           0.02438800 W

F2 - Processing parameters
SI                   32768
SF             150.9103520 MHz
WDW                     EM
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GB        0
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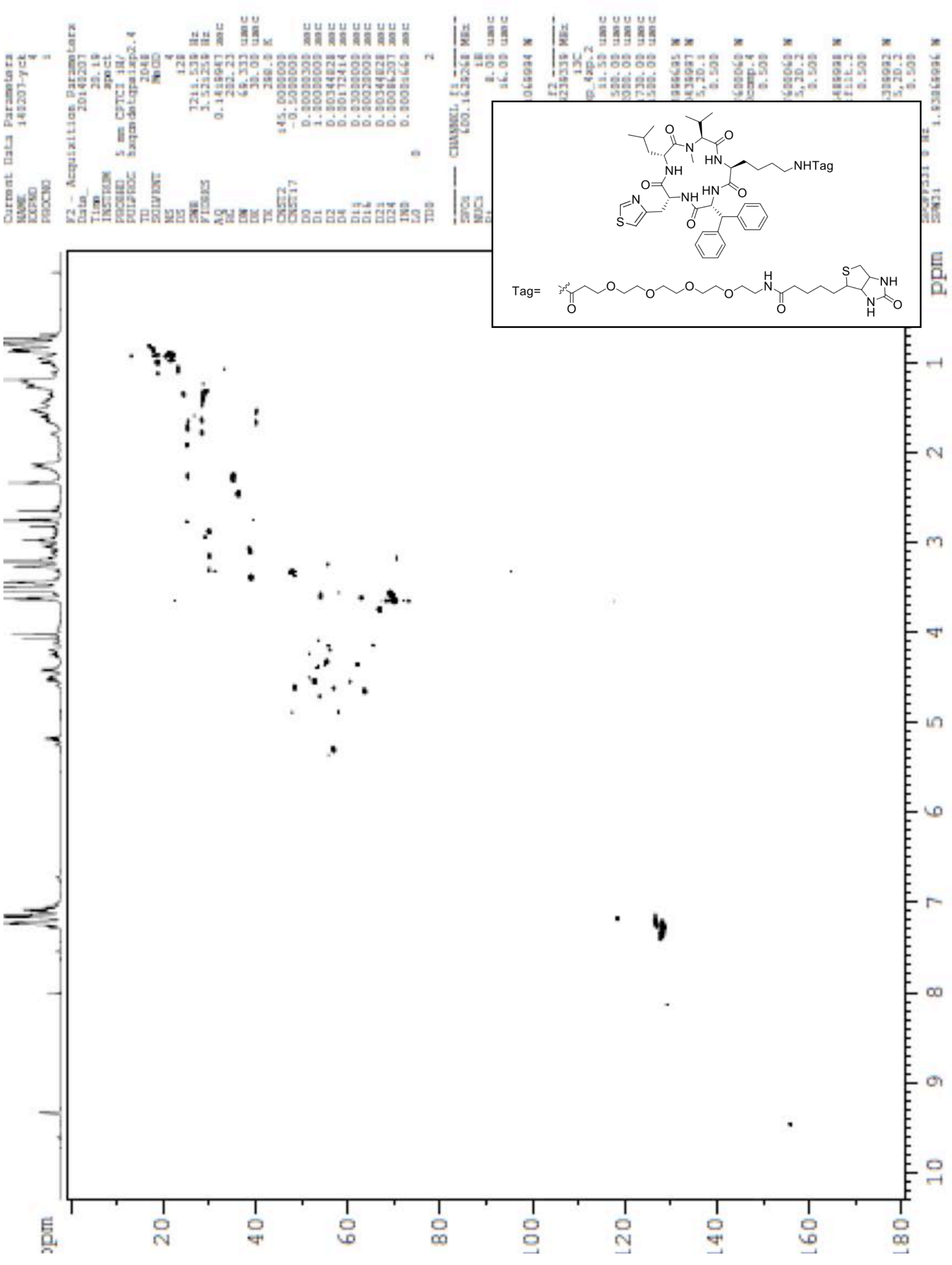
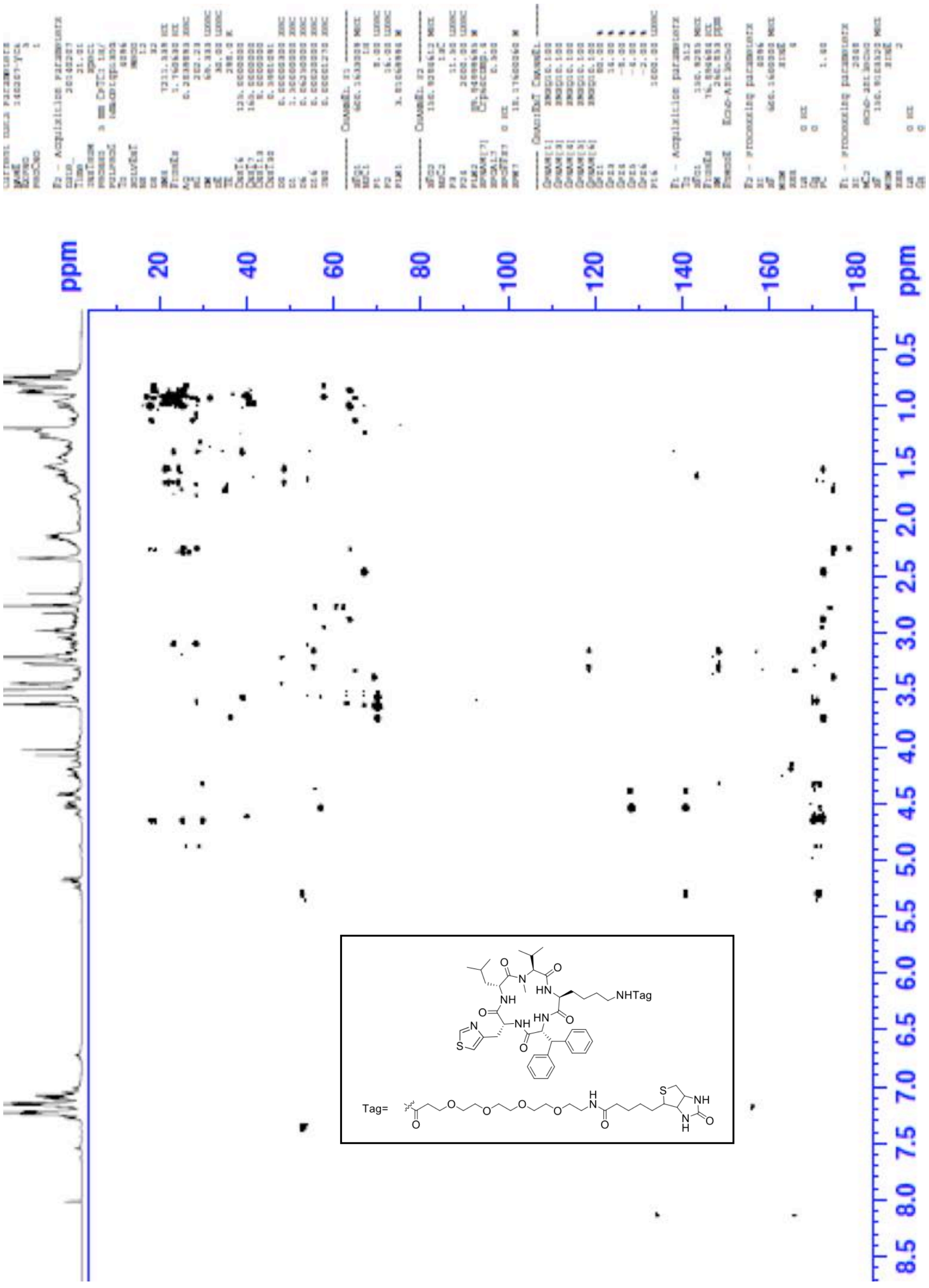
Compound 7-tag: ^{1}H- ^{13}C HSQC Macrocycle 7-Biotinylated tag



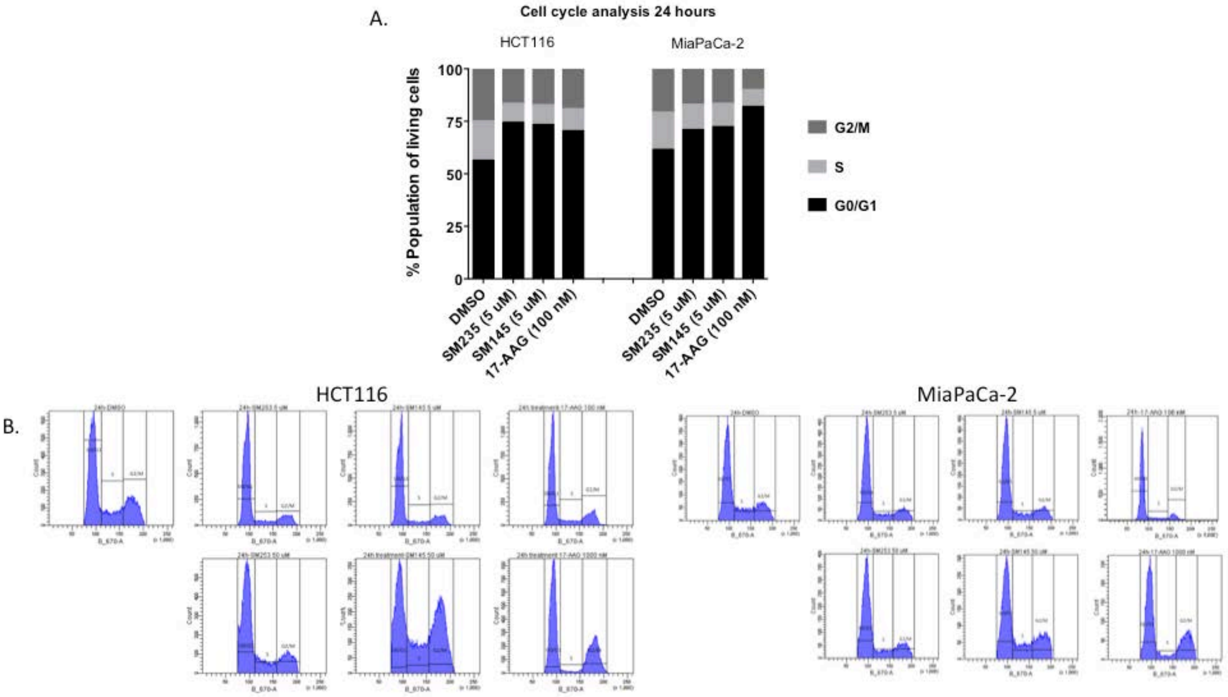
Compound 7-tag: ^{1}H- ^{13}C HMBC Macrocycle 7-Biotinylated tag



Biology: Methodology and Results

Cell-cycle analysis

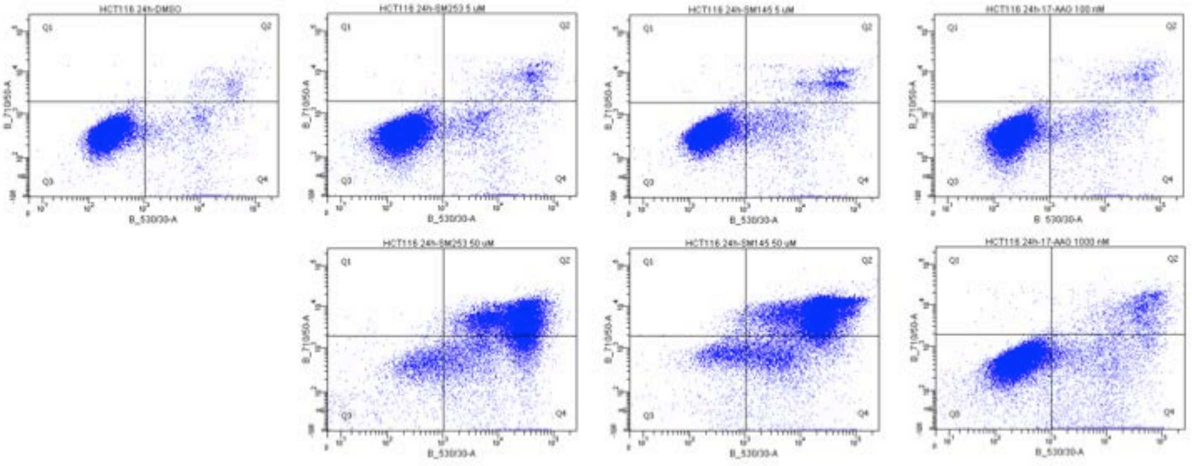
Cells were seeded in 6-well plates at a density of 3 × 10^5 cells per well. After 24 hour-incubation at 37^0C, cells were treated with compound **7**, SM145, or 17-AAG with indicated concentrations for another 24 hours, and then fixed at -20^0C with 75% ethanol overnight. Fixed cells were stained with propidium iodide (Invitrogen) for cell cycle analysis using a BD LSRFortessa flow cytometer.



Supplemental Figure S1: Analysis od cell cycle in HCT116 and MiaPaCa-2 cells. A) Percentage of cells in each stage of the cell cycle for HCT116 and MiaPaCa-2 cells after 24 hors with indicated treatement. B) Raw flow cytometer data for cell cycle analysis.

Apoptosis analysis

Cells were seeded in 6-well plates at a density of 3 × 10^5 cells per well and incubated at 37^0C for 24 hours before indicated treatments. Treated cells were harvested, collected in phosphate buffered saline and stained with Annexin V-FITC (Biolegend) and 7AAD (Biolegend) for apoptosis analysis using BD LSRFortessa flow cytometer.



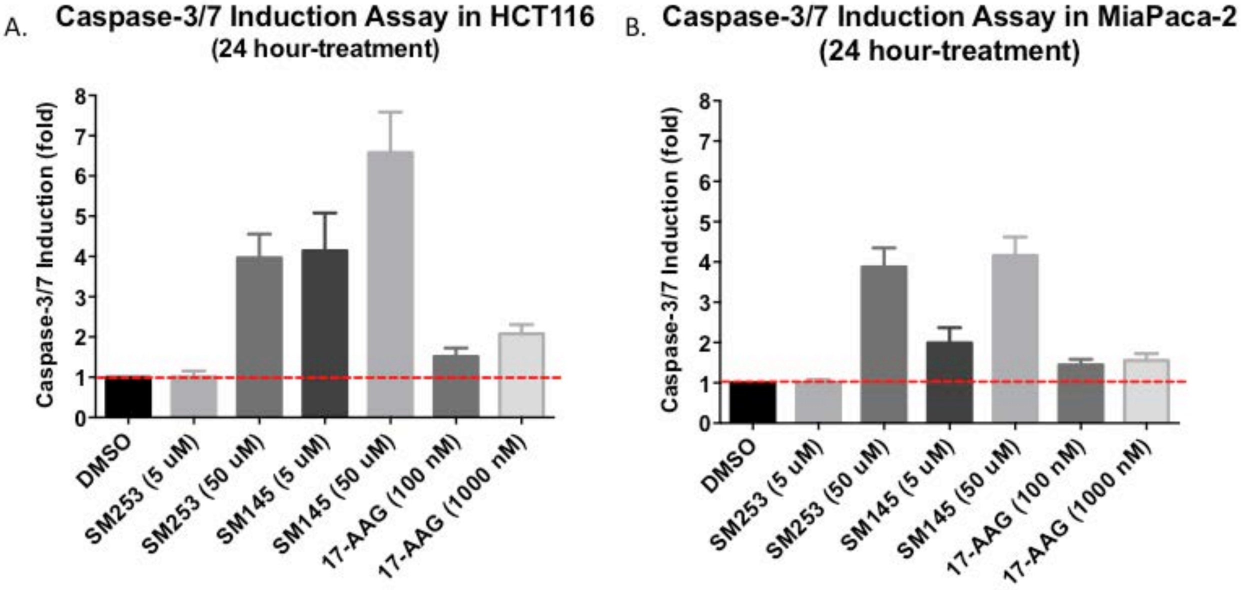
24 h treatment in HCT116

DMSO	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	93.05	3.9	2.75	0.25
Standard Deviatio	3.156474827	1.537313674	2.829016319	0.173205081
SM253 (5 uM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	87.1	9.575	3.15	0.175
Standard Deviatio	3.67151195	5.248094892	1.736855396	0.095742711
SM253 (50 uM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	24.06	25.08	49.8	1.02
Standard Deviatio	1.494322589	14.93810564	15.24335921	0.715541753
SM145 (5 uM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	89.425	6.125	4.2	0.25
Standard Deviatio	2.670049937	1.8080837	1.119523708	0.129099445
SM145 (50 uM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	24.66666667	16.03333333	57.03333333	2.333333333
Standard Deviatio	1.497776129	7.542325017	10.90168183	1.247330483
17-AAG (100 nM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	91.375	5.5	2.9	0.225
Standard Deviatio	1.105667219	0.883176087	0.391578004	0.15
17-AAG (1000 nM)	Annexin V - / 7AAD -	Annexin V + / 7AAD -	Annexin V + / 7AAD +	Annexin V - / 7AAD +
Mean	75.26666667	15.5	8.4	0.833333333
Standard Deviatio	1.18462371	1.452583905	0.529150262	0.550757055

Supplemental Figure S2: Annexin V and 7AAD determination of apoptosis in HCT116. Raw flow cytometer data for annexin V and 7AAD staining.

Caspase-3/7 induction assay

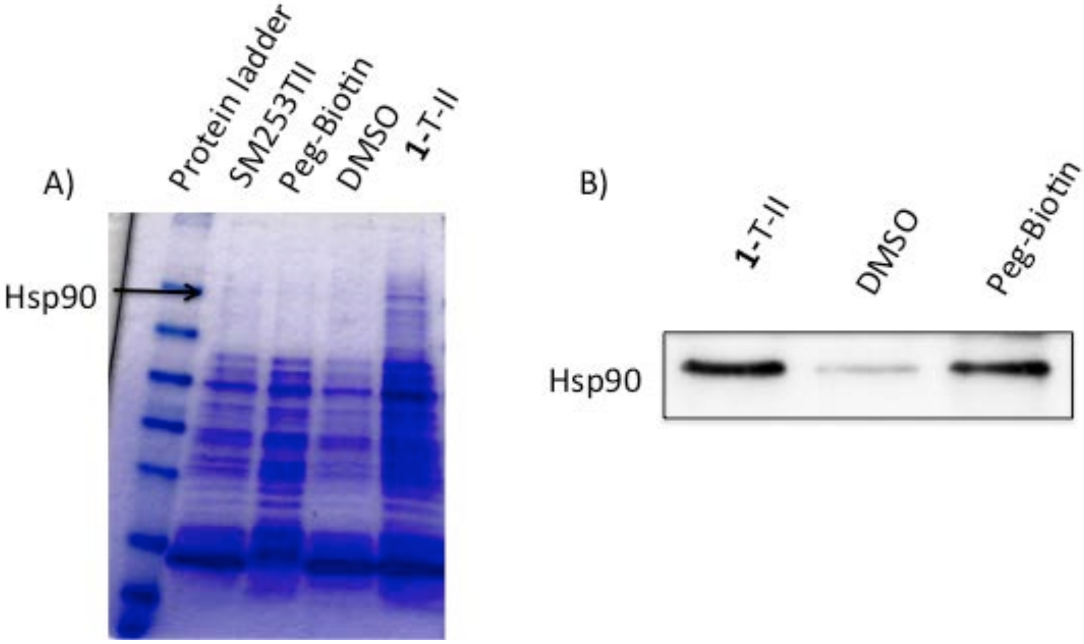
Cells were seeded at 2000 cells per well in 96-well plate, incubated at 37^{0}C for 24 hours, and then exposed to compound **7**, SM145, or 17-AAG with indicated concentrations for another 24 hours. 50 µL of Caspase-Glo 3/7 reagent (Promega) was then added into each well. After incubating for 1 hour at room temperature, 100 µL of each reaction mixture was removed and added to a white 96 well plate (Greiner Bio-One). The luminescence was measured using a luminometer (Berthold Orion Microplate Luminometer).



Supplemental Figure S3: Induction of Caspase3/7 activity. (SM253) compound **7**, SM145, and 17-AAG cause apoptosis in both A) HCT 116 and B) MiaPaCa-2 cells.

In vitro pulldown assays with 7-T-II (SM253) and 1-T-II in HCT-116 and MiaPaCa-2 cell lysates

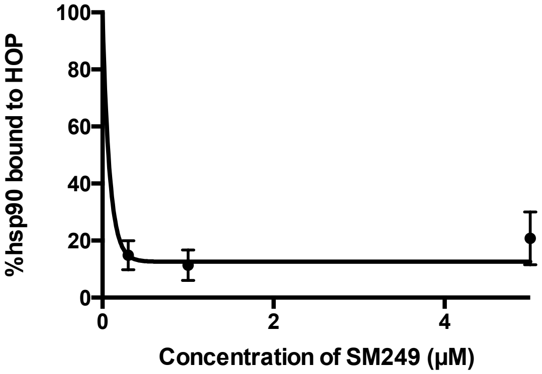
Assay was performed as previously reported with modifications. REF To prepare the lysates, HCT 116 or MiaPaCa-2 cells were grown to 90% confluence and lysed at 4°C for 24 hours in lysis buffer (50mM Tris-HCl, 150mM NaCl, 2mM $MgCl_2$, 1% (v/v) Triton X-100, pH 7.5), containing protease and phosphatase inhibitors (2µg/mL aprotinin, 0.1mM leupeptin, 1µM pepstatin A, 1mM phenylmethylsulfonamide, and 130µL of phosphatase inhibitor cocktail 2; Sigma Aldrich). Cellular debis was pelleted by centrifugation, and the concentration of soluble protein was measured by bicinchoninic acid (BCA) assay (Thermo Scientific Pierce, Rockford, Illinois, USA). Using a standard batch purification method, 5mg/ml of crude cell lysate from HCT 116 colon cancer or MiaPaCa-2 pancreatic cancer cells was incubated with biotinylated compound **7** tagged II (previously dissolved in DMSO) that had been pre-incubated with NeutrAvadin agarose (Thermo Scientific Pierce) resin for 24 hours at 4°C. The resin bound compound **7** tagged II mixture was incubated in the cell lysate for 48 hours at 4°C rocking. The resin was washed 4 times with wash buffer (20mM Tris-HCl, 300mM NaCl, 1% Triton X-100, pH 7.4) 1 time for 30 minutes, and 3 times for 15 minutes at 4°C with rocking. Proteins were eluted from the beads with sample buffer (62.5mM Tris-HCl, 2% (w/v) sodium dodecyl sulfate, 10% (v/v) glycerol, 0.1% (w/v) bromophenol blue, 100mM dithiothreitol), and the proteins were resolved on a 4-20% tris-glycine gel followed with staining with coomassie brilliant blue R-250 (Bio-Rad) or transfer to a PVDF membrane. The bands from the coomassie stained gel were excised and the protein sequences analyzed by LC/MS/MS (liquid chromatography/ tandem mass spectroscopy) at the Bioanalytical Mass Spectrometry Facility at UNSW. The PVDF membrane was blocked with 5% milk in Tris buffered Saline with 0.1% Tween-20 (TBST) for 30 minutes and then probed with a polyclonal rabbit anti hsp90 antibody (ADI-SPA-836, Enzo) for 12 hours. The primary antibody was removed and the membrane washed for 15 minutes in TBST, the secondary horseradish peroxidase (HRP) conjugated antibody was then added for 45 minutes. The membrane was washed in TBST and the bands were visualized with the LAS4000 chemiluminescence imager.



Supplemental Figure S4: *In vitro* pulldown assays with 7-T-II (SM253) and 1-T-II in HCT-116 and MiaPaCa-2 cell lysates. A) Proteins visualized on a 4-20% Tri-glycine gel stained with coomassie-R250. B) Validation of hsp90 as a target for compound **1_**TagII via western blot.

Binding Assay

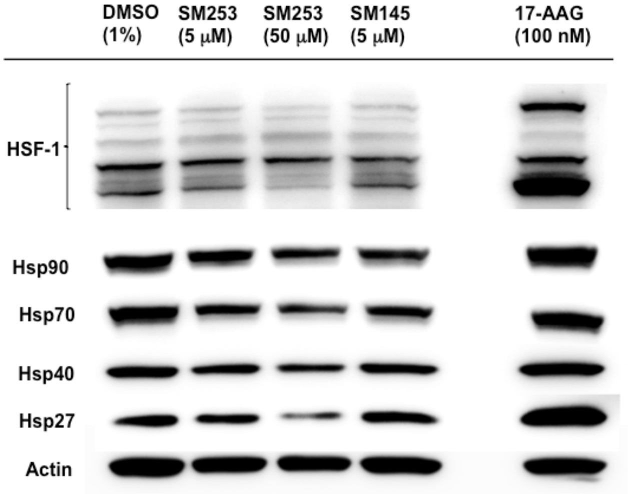
The binding affinity between Hsp90 and HOP completed using 200 nM (final concentration) of human native protein Hsp90 and 100 nM (final concentration) of human recombinant his-tagged HOP. Experiments were carried out with concentrations ranging from 0-5µM of compound **1**. Protein pull-down was completed using Talon-Metal Affinity Resin (Clontech, cat. no. 635501), followed by three washes of the beads in binding buffer and finally boiling the beads with 5x Laemmli sample buffer. Samples were analyzed using 4-20% SDS-PAGE gel, followed by standard Western blot protocol to detect Hsp90 and HOP. The respective ratio of Hsp90 to its co-chaperone or client proteins were analyzed via Image J and transformed to a percent of Hsp90 bound to co-chaperone or client proteins. Each experiment was completed with n=3.



Supplemental Figure S5: Binding of hsp90 with HOP after increasing concentrations of compound **1**.

Cell Lysate Analysis

Assay was performed as previously reported with modifications. REF HCT-116 or MiaPaCa-2 cells were seeded into 6 well dishes at 300,000 cells per well and allowed to adhere for 24 hours. The cells were treated with compound **7** (5 µM and 50 µM), or SM145 (5 µM), or 17-AAG (100 nM) (DMSO concentration always 1%) for 24 hours and then harvested and lysed in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM $MgCl_2$, 1% (v/v) Triton X-100, pH 7.5) containing protease and phosphatase inhibitors (2 µg/mL aprotinin, 0.1 mM leupeptin, 1 µM pepstatin A, 1 mM phenylmethylsulfonamide, and 130 µL of phosphatase inhibitor cocktail 2; Sigma Aldrich). Cellular debis was pelleted by centrifugation, and the concentration of soluble protein was measured by bicinchoninic acid (BCA) assay (Thermo Scientific Pierce, Rockford, Illinois, USA).. 30-100 µg of protein lysate was boiled with 5x Laemmli sample buffer at 100°C for 15 minutes. Samples were analyzed using 4-20% SDS-PAGE gel, followed by standard Western blot protocol using PVDF membranes. The membranes were probed with primary antibodies and then with secondary HRP conjugated antibodies to visualize the results. The respective amount of target protein was determines via Image J. The band intensity was normalized to actin, and then compared to the DMSO control. Fold changes were reported. Each experiment was completed with n = 3.



Supplemental Figure S6: Protein level analysis for 17-AAG in MiaPaCa-2 cells.

Firefly luciferase refolding assay

Assay was performed as reported previously with modifications. REF Luciferase (Novus cat. no. NB810-74573) was diluted to 100 μg/mL and denatured at 41°C for 30 minutes. After denaturing, 1 μL of luciferase was added to 20 μL of rabbit reticulocyte lysate (Promega cat. no. L4960) that had been pre-incubated at 30°C with DMSO, compound **7** (5 μM) or SM145 (5 μM) for 5 hours. After a 2 hours incubation of the luciferase in the lysate, 5 μL was removed and combined with 50 μL of Bright Glo Luciferase Assay System (Promega cat. no. E2610) and read on an illuminometer (Manufacturer). Compounds were tested for direct inhibition of luciferase activity by combining 0.5 μL of 100 μg/mL non-denatured luciferase with the compounds, adding 50 μL Bright Glo Luciferase Assay System and then read on illuminometer. Percent Luciferase activity was determined using the luminescence of DMSO after 2 hours as 100%, and all other samples compared to this value. Each experiment was completed with n = 3.

Cell culture

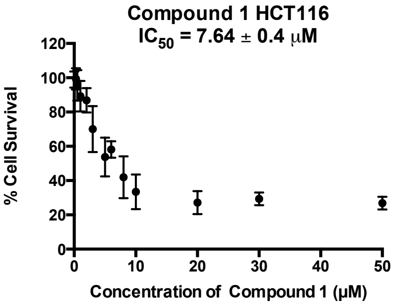
HCT 116 cell line (human colorectal carcinoma) and MiaPaCa-2 (human pancreas carcinoma) was obtained from ECACC. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen/Life Technologies). Cells were propagated in a humidified chamber at 37^{0}C with 5% CO_2. When cells were passaged or harvested, they were rinsed with phosphate buffered saline, incubated for two minutes with 0.25% trypsin-EDTA, and then collected in DMEM to quench the trypsin.

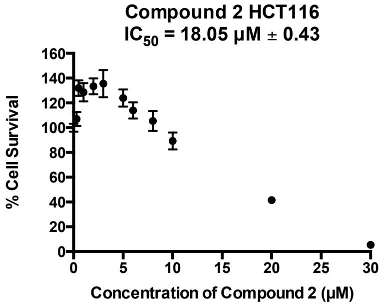
Cytotoxicity Assay

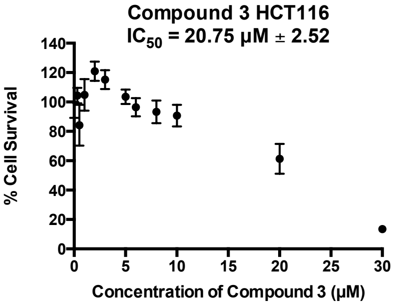
HCT 116 or MiaPaCa-2 cells were seeded into 96 well plates at 2000 cells per well and allowed to adhere overnight. The cells were treated with varying concentrations of compound **1** and **7**
(300 nM – 30 μM) (DMSO concentration always 1%) for 72 hours. After 72 hours the media was

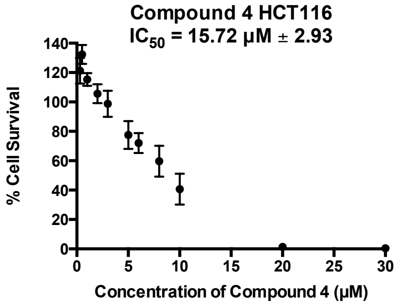
removed and replaced with 100 µL of DMEM with 10 µL of Cell Counting Kit 8 reagent (Dojindo). The cells were then left in the incubator at 37^{0}C with 5% CO_2 for 2-4 hours and then the absorbance was read according to the manufactures protocol using a Chromate plate reader.

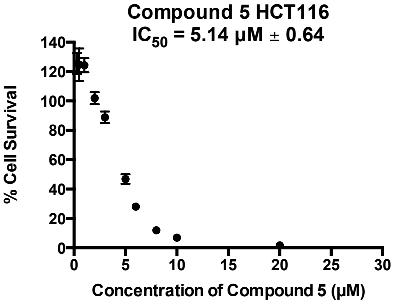
HCT116

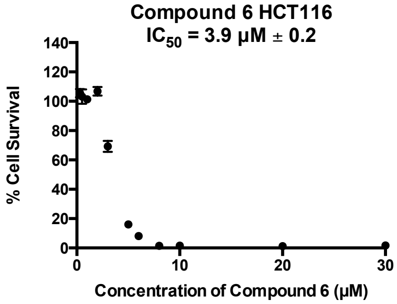

Compound 1 HCT116
IC_{50} = 7.64 ± 0.4 µM

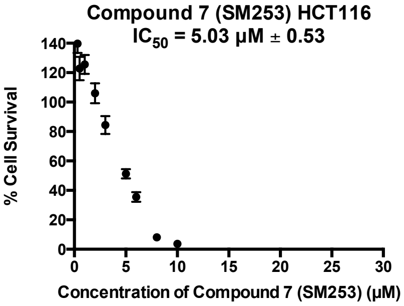

Compound 2 HCT116
IC_{50} = 18.05 µM ± 0.43

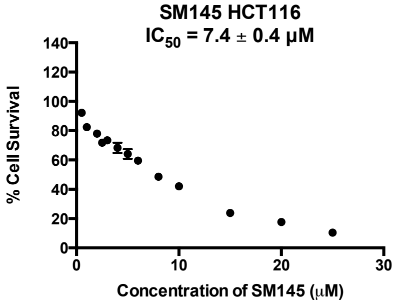

Compound 3 HCT116
IC_{50} = 20.75 µM ± 2.52


Compound 4 HCT116
IC_{50} = 15.72 µM ± 2.93

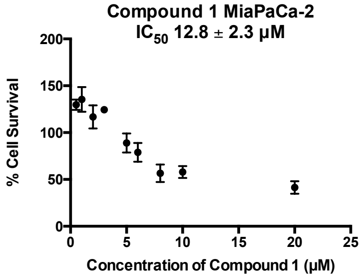

Compound 5 HCT116
IC_{50} = 5.14 µM ± 0.64

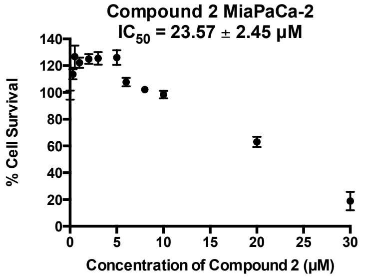

Compound 6 HCT116
IC_{50} = 3.9 µM ± 0.2

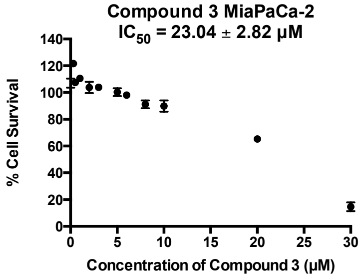

Compound 7 (SM253) HCT116
IC_{50} = 5.03 µM ± 0.53

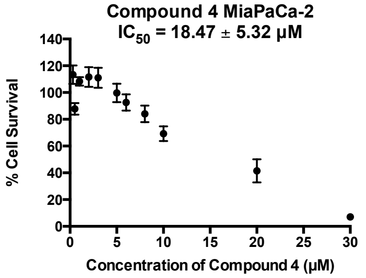

SM145 HCT116
IC_{50} = 7.4 ± 0.4 µM

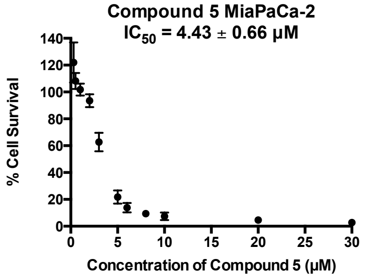
MiaPaCa-2

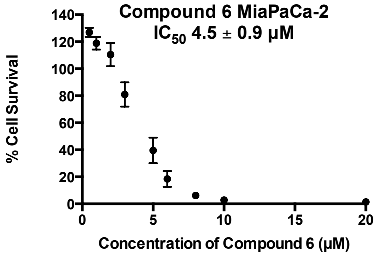

Compound 1 MiaPaCa-2
IC_{50} 12.8 ± 2.3 μM

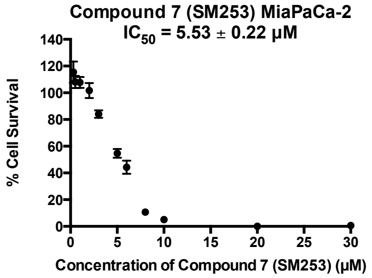

Compound 2 MiaPaCa-2
IC_{50} = 23.57 ± 2.45 μM

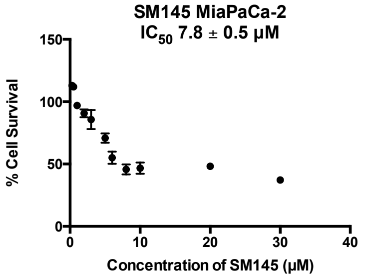

Compound 3 MiaPaCa-2
IC_{50} = 23.04 ± 2.82 μM


Compound 4 MiaPaCa-2
IC_{50} = 18.47 ± 5.32 μM

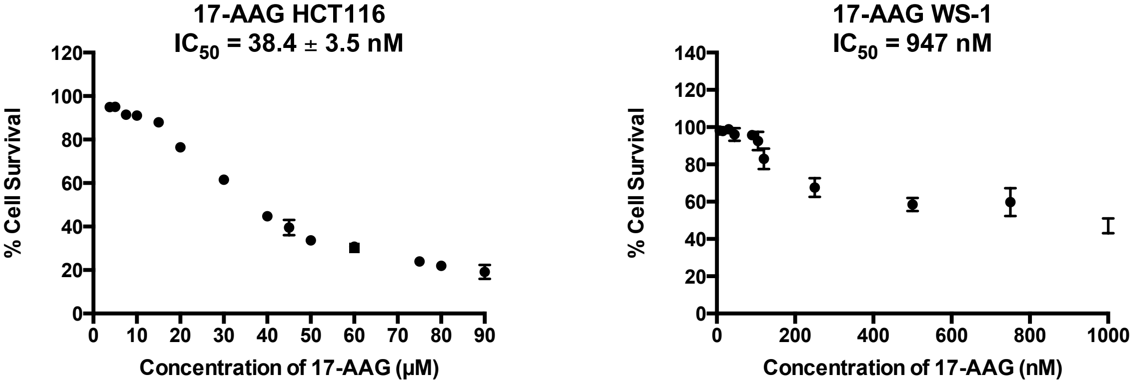

Compound 5 MiaPaCa-2
IC_{50} = 4.43 ± 0.66 μM


Compound 6 MiaPaCa-2
IC_{50} 4.5 ± 0.9 μM


Compound 7 (SM253) MiaPaCa-2
IC_{50} = 5.53 ± 0.22 μM

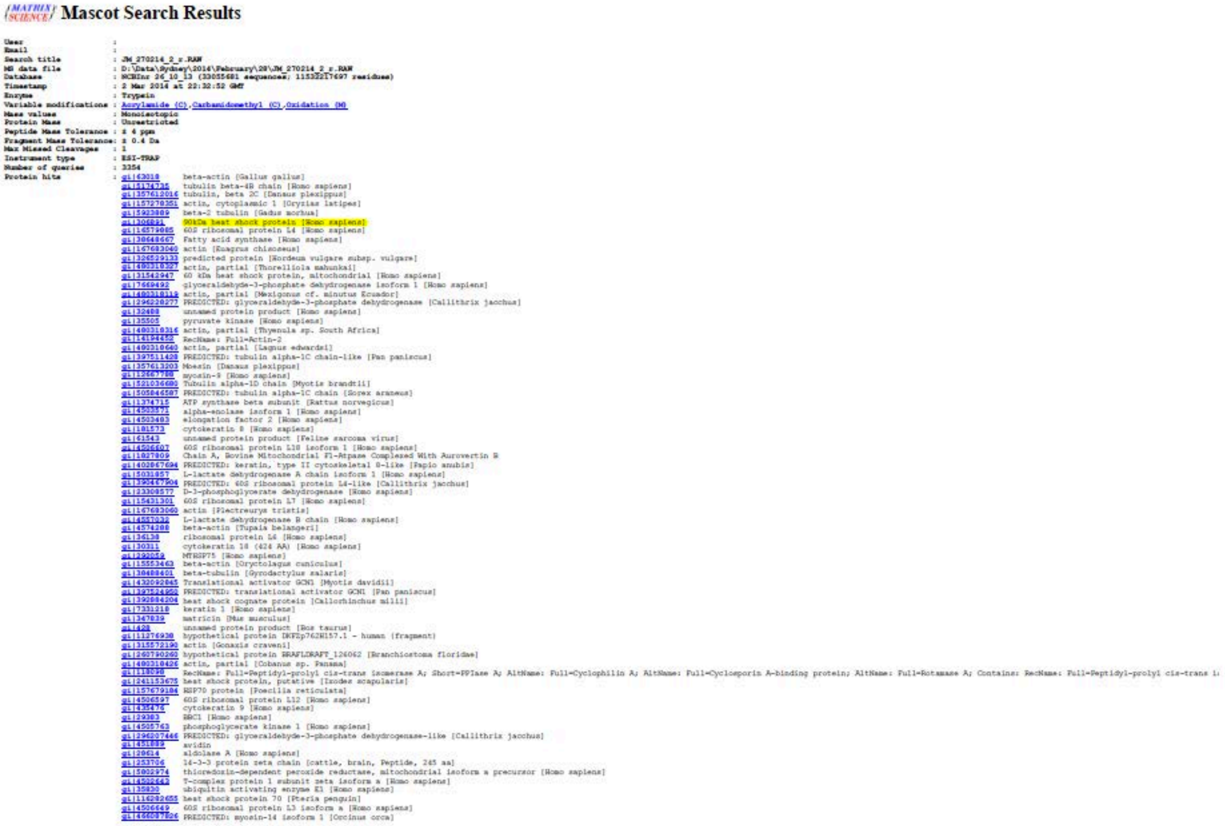

SM145 MiaPaCa-2
IC_{50} 7.8 ± 0.5 μM

17-AAG



Supplemental Figure S7: IC_{50} curves for compound **1**, **2**, **3**, **4**, **5**, **6**, **7** and SM145 in HCT-116 and MiaPaCa-2, and 17-AAG in HCT-116.

Supporting Information



Mascot Search Results

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Timestamp                : 2 Mar 2014 at 22:32:52 GMT
Enzyme                   : Trypsin
Variable modifications   : Acrylamide (C),Carbamidomethyl (C),Oxidation (M)
Mass values              : Monoisotopic
Protein Mass             : Unrestricted
Peptide Mass Tolerance   : ± 4 ppm
Fragment Mass Tolerance: ± 0.4 Da
Max Missed Cleavages     : 1
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                           gi|5923889   beta-2 tubulin [Gadus morhua]
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                           gi|38648667  Fatty acid synthase [Homo sapiens]
                           gi|167683040 actin [Euagrus chisoseus]
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                           gi|521036680 Tubulin alpha-1D chain [Myotis brandtii]
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                           gi|181573    cytokeratin 8 [Homo sapiens]
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                           gi|15431301  60S ribosomal protein L7 [Homo sapiens]
                           gi|167683060 actin [Plectreurys tristis]
                           gi|4557032   L-lactate dehydrogenase B chain [Homo sapiens]
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                           gi|30311     cytokeratin 18 (424 AA) [Homo sapiens]
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                           gi|15553463  beta-actin [Oryctolagus cuniculus]
                           gi|30488401  beta-tubulin [Gyrodactylus salaris]
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                           gi|241153675 heat shock protein, putative [Ixodes scapularis]
                           gi|157679184 HSP70 protein [Poecilia reticulata]
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                           gi|28614     aldolase A [Homo sapiens]
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                           gi|4502643   T-complex protein 1 subunit zeta isoform a [Homo sapiens]
                           gi|35830     ubiquitin activating enzyme E1 [Homo sapiens]
                           gi|116282655 heat shock protein 70 [Pteria penguin]
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gi|13606056   DNA dependent protein kinase catalytic subunit [Homo sapiens]
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gi|204595       histone (H1d) [Rattus norvegicus]
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gi|466001414 60S ribosomal protein L10 [Chelonia mydas]
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gi|531171       Csa-19 [Homo sapiens]
gi|100913206 ATP-dependent RNA helicase A [Homo sapiens]
gi|197099610 C-1-tetrahydrofolate synthase, cytoplasmic [Pongo abelii]
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gi|431920762 60S ribosomal protein L13a [Pteropus alecto]
gi|256427061 heat shock protein 90 [Odontesthes bonariensis]
gi|306553       ribosmal protein small subunit [Homo sapiens]
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gi|315451369 actin [Dinophysis caudata]
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gi|444021       sub2.3 [Homo sapiens]
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gi|619788       L21 ribosomal protein [Homo sapiens]
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gi|198570	ribosomal protein [Mus musculus]
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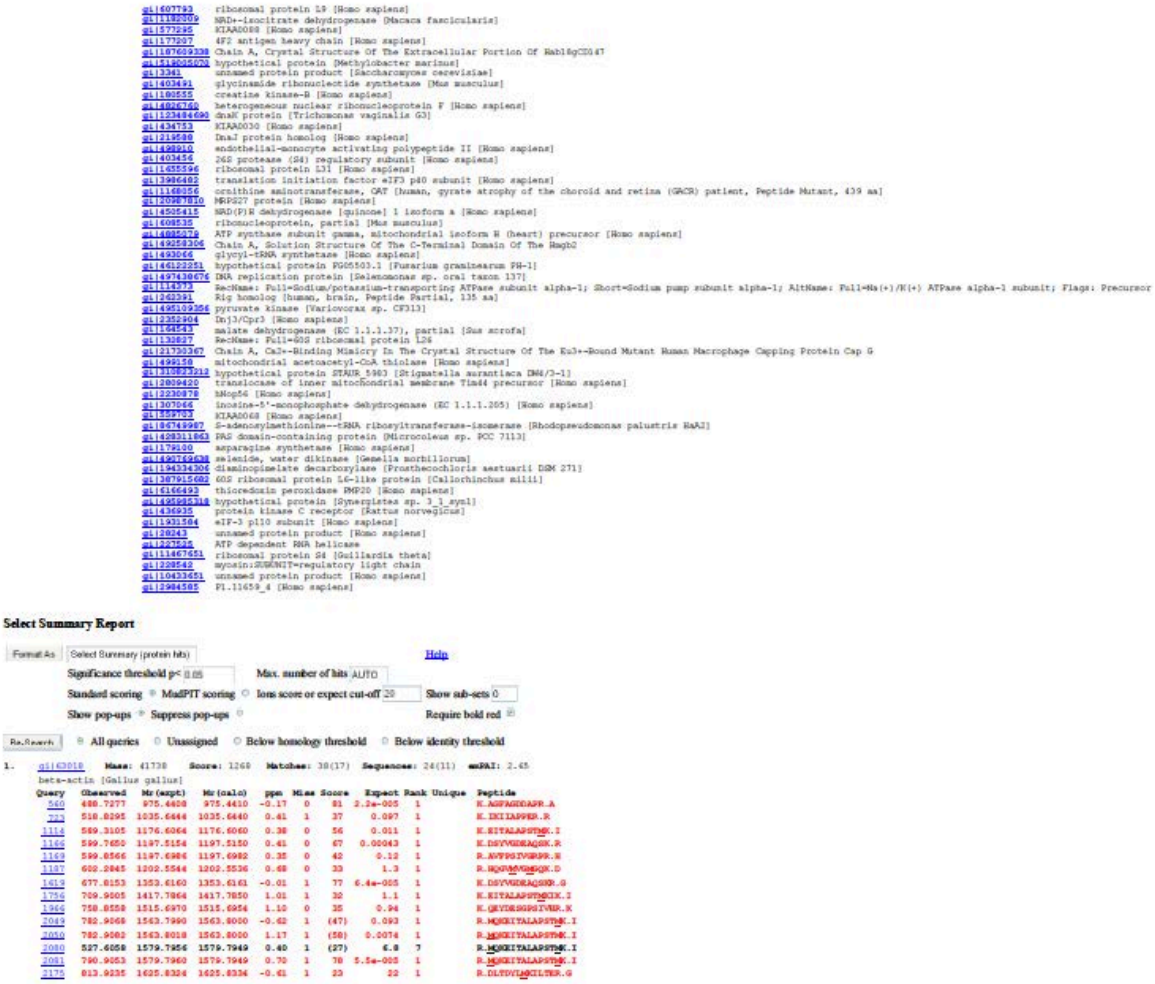
gi|2500241	RecName: Full=60S ribosomal protein L11
gi|123368	RecName: Full=High mobility group protein B1; AltName: Full=High mobility group protein 1; Short=HMG-1
gi|13489087	leukocyte elastase inhibitor [Homo sapiens]
gi|34039	unnamed protein product [Homo sapiens]
gi|225632	casein alphaS1
gi|432376	rhodanese [Homo sapiens]
gi|337518	ribosomal protein, partial [Homo sapiens]
gi|1232079	huMCM5 [Homo sapiens]
gi|5031755	heterogeneous nuclear ribonucleoprotein R isoform 2 [Homo sapiens]
gi|189238	neuroleukin [Homo sapiens]
gi|293294	synexin [Mus musculus]
gi|35655	unnamed protein product [Homo sapiens]
gi|2769254	NIPSNAP2 protein [Homo sapiens]
gi|32532	unnamed protein product [Homo sapiens]
gi|3037013	Gry-rbp [Homo sapiens]
gi|116222251	beta tubulin, partial [Leucocryptos marina]
gi|4506707	40S ribosomal protein S25 [Homo sapiens]
gi|340546069	ribosomal protein L7a [Ichthyophis bannanicus]
gi|29935	ATP-citrate (pro-S-)-lyase [Homo sapiens]
gi|5453832	hypoxia up-regulated protein 1 precursor [Homo sapiens]
gi|5031595	actin-related protein 2/3 complex subunit 4 isoform a [Homo sapiens]
gi|445615	ribosomal protein L27
gi|57002	pyrimidine binding protein 1 [Rattus norvegicus]
gi|4505775	phosphate carrier protein, mitochondrial isoform b precursor [Homo sapiens]
gi|524892341	PREDICTED: 14-3-3 protein epsilon-like [Aplysia californica]
gi|260137	annexin V=CaBP33 isoform [cattle, brain, Peptide, 320 aa]
gi|1421609	Chain A, X-ray Structure Of Nm23 Human Nucleoside Diphosphate Kinase B Complexed With Gdp At 2 Angstroms Resolution
gi|162705	vacuolar H+-ATPase A subunit [Bos taurus]
gi|529768044	DNA replication licensing factor MCM6 [Camelus ferus]
gi|3095186	cargo selection protein TIP47 [Homo sapiens]
gi|11321601	6-phosphofructokinase type C isoform 1 [Homo sapiens]
gi|34258	talin [Mus musculus]
gi|404105	alpha-catenin [Homo sapiens]
gi|4506675	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 precursor [Homo sapiens]
gi|448295	TLS protein
gi|1703105	RecName: Full=Actin, cytoplasmic; AltName: Full=Actin, macronuclear
gi|90	synthetase fragment (AA 67) [Bos taurus]
gi|63781	ribosomal protein S17 (AA 7-135) [Gallus gallus]
gi|35570	unnamed protein product [Homo sapiens]
gi|3098148	ASMTL [Homo sapiens]
gi|326630480	crustacean hyperglycemic hormone-like peptide precursor [Procambarus clarkii]
gi|5031635	cofilin-1 [Homo sapiens]
gi|515634	ubiquinol-cytochrome c reductase core I protein [Homo sapiens]
gi|183082	glutamine:fructose-6-phosphate amidotransferase [Homo sapiens]
gi|4503481	elongation factor 1-gamma [Homo sapiens]
gi|132949	RecName: Full=Putative 60S ribosomal protein L32'
gi|30130	colligin [Homo sapiens]
gi|490477228	signal peptidase [Prevotella buccalis]
gi|640350	Chain A, Crystal Structures Of Medium Chain Acyl-Coa Dehydrogenase From Pig Liver Mitochondria With And Without Substrate
gi|302416755	CTD kinase subunit alpha [Verticillium alfalfae VaMs.102]
gi|1002369	coatomer protein [Homo sapiens]
gi|167537852	hypothetical protein [Monosiga brevicollis MX1]
gi|123976082	hypothetical protein [Trichomonas vaginalis G3]
gi|1871210	T-complex protein 1, Beta subunit (TCP-1-BETA) [Homo sapiens]
gi|14010879	26S proteasome non-ATPase regulatory subunit 1 [Rattus norvegicus]
gi|4929561	CGI-46 protein [Homo sapiens]
gi|187281	M4 protein [Homo sapiens]
gi|501299522	acyl-coa dehydrogenase, partial [Riptortus pedestris]
gi|179832	calnexin [Homo sapiens]



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gi|607793     ribosomal protein L9 [Homo sapiens]
gi|1182009    NAD+-isocitrate dehydrogenase [Macaca fascicularis]
gi|577295     KIAA0088 [Homo sapiens]
gi|177207     4F2 antigen heavy chain [Homo sapiens]
gi|187609338 Chain A, Crystal Structure Of The Extracellular Portion Of Hab18gCD147
gi|519005070 hypothetical protein [Methylobacter marinus]
gi|3341       unnamed protein product [Saccharomyces cerevisiae]
gi|403491     glycinamide ribonucleotide synthetase [Mus musculus]
gi|180555     creatine kinase-B [Homo sapiens]
gi|4826760    heterogeneous nuclear ribonucleoprotein F [Homo sapiens]
gi|123484690 dnaK protein [Trichomonas vaginalis G3]
gi|434753     KIAA0030 [Homo sapiens]
gi|219588     DnaJ protein homolog [Homo sapiens]
gi|498910     endothelial-monocyte activating polypeptide II [Homo sapiens]
gi|403456     26S protease (S4) regulatory subunit [Homo sapiens]
gi|1655596    ribosomal protein L31 [Homo sapiens]
gi|3986482    translation initiation factor eIF3 p40 subunit [Homo sapiens]
gi|1168056    ornithine aminotransferase, OAT [human, gyrate atrophy of the choroid and retina (GACR) patient, Peptide Mutant, 439 aa]
gi|20987810   MRPS27 protein [Homo sapiens]
gi|4505415    NAD(P)H dehydrogenase [quinone] 1 isoform a [Homo sapiens]
gi|608535     ribonucleoprotein, partial [Mus musculus]
gi|4885079    ATP synthase subunit gamma, mitochondrial isoform H (heart) precursor [Homo sapiens]
gi|49258306   Chain A, Solution Structure Of The C-Terminal Domain Of The Hmgb2
gi|493066     glycyl-tRNA synthetase [Homo sapiens]
gi|46122251   hypothetical protein FG05503.1 [Fusarium graminearum PH-1]
gi|497430676 DNA replication protein [Selenomonas sp. oral taxon 137]
gi|114373     RecName: Full=Sodium/potassium-transporting ATPase subunit alpha-1; Short=Sodium pump subunit alpha-1; AltName: Full=Na(+)/K(+) ATPase alpha-1 subunit; Flags: Precursor
gi|262391     Rig homolog [human, brain, Peptide Partial, 135 aa]
gi|495109356 pyruvate kinase [Variovorax sp. CF313]
gi|2352904    Dnj3/Cpr3 [Homo sapiens]
gi|164543     malate dehydrogenase (EC 1.1.1.37), partial [Sus scrofa]
gi|132827     RecName: Full=60S ribosomal protein L26
gi|21730367   Chain A, Ca2+-Binding Mimicry In The Crystal Structure Of The Eu3+-Bound Mutant Human Macrophage Capping Protein Cap G
gi|499158     mitochondrial acetoacetyl-CoA thiolase [Homo sapiens]
gi|310823212 hypothetical protein STAUR_5983 [Stigmatella aurantiaca DW4/3-1]
gi|2809420    translocase of inner mitochondrial membrane Tim44 precursor [Homo sapiens]
gi|2230878    hNop56 [Homo sapiens]
gi|307066     inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205) [Homo sapiens]
gi|559703     KIAA0068 [Homo sapiens]
gi|86749987   S-adenosylmethionine--tRNA ribosyltransferase-isomerase [Rhodopseudomonas palustris HaA2]
gi|428311863 PAS domain-containing protein [Microcoleus sp. PCC 7113]
gi|179100     asparagine synthetase [Homo sapiens]
gi|490769638 selenide, water dikinase [Gemella morbillorum]
gi|194334306 diaminopimelate decarboxylase [Prosthecochloris aestuarii DSM 271]
gi|387915682 60S ribosomal protein L6-like protein [Callorhinchus milii]
gi|6166493    thioredoxin peroxidase PMP20 [Homo sapiens]
gi|495985318 hypothetical protein [Synergistes sp. 3_1_syn1]
gi|436935     protein kinase C receptor [Rattus norvegicus]
gi|1931584    eIF-3 p110 subunit [Homo sapiens]
gi|28243      unnamed protein product [Homo sapiens]
gi|227525     ATP dependent RNA helicase
gi|11467651   ribosomal protein S4 [Guillardia theta]
gi|228542     myosin:SUBUNIT=regulatory light chain
gi|10433651   unnamed protein product [Homo sapiens]
gi|2984585    P1.11659_4 [Homo sapiens]
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Select Summary Report

Format As | Select Summary (protein hits) | Help

Significance threshold p< 0.05 | Max. number of hits AUTO

Standard scoring ● MudPIT scoring ○ | Ions score or expect cut-off 20 | Show sub-sets 0

Show pop-ups ● Suppress pop-ups ○ | Require bold red ☑

Re-Search | ● All queries ○ Unassigned ○ Below homology threshold ○ Below identity threshold

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1.    gi|63018      Mass: 41738     Score: 1268    Matches: 38(17)    Sequences: 24(11)    emPAI: 2.45
      beta-actin [Gallus gallus]
      Query   Observed    Mr(expt)    Mr(calc)    ppm   Miss Score    Expect Rank Unique  Peptide
        560   488.7277    975.4408    975.4410   -0.17    0     81  2.2e-005    1          K.AGFAGDDAPR.A
        723   518.8295   1035.6444   1035.6440    0.41    1     37     0.097    1          K.IKIIAPPER.R
       1114   589.3105   1176.6064   1176.6060    0.38    0     56     0.011    1          K.EITALAPSTMK.I
       1166   599.7650   1197.5154   1197.5150    0.41    0     67   0.00043    1          K.DSYVGDEAQSK.R
       1169   599.8566   1197.6986   1197.6982    0.35    0     42      0.12    1          R.AVFPSIVGRPR.H
       1187   602.2845   1202.5544   1202.5536    0.68    0     33       1.3    1          R.HQGVMVGMGQK.D
       1619   677.8153   1353.6160   1353.6161   -0.01    1     77  6.4e-005    1          K.DSYVGDEAQSKR.G
       1756   709.9005   1417.7864   1417.7850    1.01    1     32       1.1    1          K.EITALAPSTMKIK.I
       1966   758.8558   1515.6970   1515.6954    1.10    0     35      0.94    1          K.QEYDESGPSIVHR.K
       2049   782.9068   1563.7990   1563.8000   -0.62    1   (47)     0.093    1          R.MQKEITALAPSTMK.I
       2050   782.9082   1563.8018   1563.8000    1.17    1   (58)    0.0074    1          R.MQKEITALAPSTMK.I
       2080   527.6058   1579.7956   1579.7949    0.40    1   (27)       6.8    7          R.MQKEITALAPSTMK.I
       2081   790.9053   1579.7960   1579.7949    0.70    1     78  5.5e-005    1          R.MQKEITALAPSTMK.I
       2175   813.9235   1625.8324   1625.8334   -0.61    1     23        22    1          R.DLTDYLMKILTER.G
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Supporting Information

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2180   815.4156  1628.8166  1628.8150   0.50  1   40      0.37  1   R.GYSFTTTAEREIVR.D
2207   820.4222  1638.8298  1638.8287   0.71  1   39      0.43  1   R.IDLAGRDLTDYLMK.I
2419   872.9466  1743.8786  1743.8792  -0.29  1   31       3.9  1   K.ILTERGYSFTTTAER.E
2501   895.9501  1789.8856  1789.8846   0.57  0   87  9.7e-006  1   K.SYELPDGQVITIGNER.F 2500 2502 2503
2779   652.0265  1953.0577  1953.0571   0.29  0  (35)     0.83  3   R.VAPEEHPVLLTEAPLNPK.A
2780   977.5366  1953.0586  1953.0571   0.79  0   52     0.015  1   R.VAPEEHPVLLTEAPLNPK.A
2947  1047.5350  2093.0554  2093.0542   0.62  1   34       1.5  1   K.SYELPDGQVITIGNERFR.C
2997   719.3839  2155.1299  2155.1287   0.55  1   31       2.2  1   K.AGFAGDDAPRAVFPSIVGRPR.H
3040  1116.0360  2230.0574  2230.0576  -0.07  0   96  8.8e-007  1   K.DLYANTVLSGGTTMYPGIADR.M
3079   765.7514  2294.2324  2294.2382  -2.56  1   35      0.53  1   R.VAPEEHPVLLTEAPLNPKANR.E
3097   789.6957  2366.0653  2366.0631   0.91  1  (33)      1.2  1   R.HQGVMVGMGQKDSYVGDEAQSK.R
3102   596.5211  2382.0553  2382.0580  -1.15  1  (20)      2.9  1   R.HQGVMVGMGQKDSYVGDEAQSK.R
3103   795.0269  2382.0589  2382.0580   0.35  1   34      0.71  1   R.HQGVMVGMGQKDSYVGDEAQSK.R
3104  1192.0380  2382.0614  2382.0580   1.44  1  (25)      6.4  3   R.HQGVMVGMGQKDSYVGDEAQSK.R
3178   850.7294  2549.1664  2549.1665  -0.06  0  (23)       13  2   K.LCYVALDFEQEMATAASSSSLEK.S
3187  1283.5830  2565.1514  2565.1614  -3.89  0   91  1.5e-006  1   K.LCYVALDFEQEMATAASSSSLEK.S
3188   856.0613  2565.1621  2565.1614   0.25  0  (61)   0.0017  1   K.LCYVALDFEQEMATAASSSSLEK.S
3215   878.7571  2633.2495  2633.2465   1.11  1   84  1.5e-005  1   K.DLYANTVLSGGTTMYPGIADRMQK.E
3315  1061.8780  3182.6122  3182.6071   1.61  0  (50)    0.028  1   R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L
3318  1067.2080  3198.6022  3198.6020   0.06  0   55    0.0097  1   R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L 3317

Proteins matching the same set of peptides:
gi|113271      Mass: 41821   Score: 1268   Matches: 38(17)   Sequences: 24(11)
RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta actin
gi|809561      Mass: 40992   Score: 1268   Matches: 38(17)   Sequences: 24(11)
gamma-actin [Mus musculus]
gi|4501885      Mass: 41710   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, cytoplasmic 1 [Homo sapiens]
gi|4501887      Mass: 41766   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, cytoplasmic 2 [Homo sapiens]
gi|14250401      Mass: 40978   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, beta, partial [Homo sapiens]
gi|16924319      Mass: 40477   Score: 1268   Matches: 38(17)   Sequences: 24(11)
Unknown (protein for IMAGE:3538275), partial [Homo sapiens]
gi|33415846      Mass: 41754   Score: 1268   Matches: 38(17)   Sequences: 24(11)
cytoplasmic actin type 4 [Pelophylax lessonae]
gi|37690410      Mass: 41460   Score: 1268   Matches: 38(17)   Sequences: 24(11)
beta-actin [Passer domesticus]
gi|45269029      Mass: 44763   Score: 1268   Matches: 38(17)   Sequences: 24(11)
cytoskeletal beta actin, partial [Sus scrofa]
gi|45361511      Mass: 41738   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, cytoplasmic 2 [Xenopus (Silurana) tropicalis]
gi|47498068      Mass: 41726   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, cytoplasmic 1 [Xenopus (Silurana) tropicalis]
gi|54696726      Mass: 41823   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, beta [synthetic construct]
gi|56119084      Mass: 41809   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin, cytoplasmic type 5 [Gallus gallus]
gi|60389477      Mass: 41775   Score: 1268   Matches: 38(17)   Sequences: 24(11)
RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-actin; Contains: RecName: Full=Actin, cytoplasmic 1, N-terminally processed
gi|60653037      Mass: 41879   Score: 1268   Matches: 38(17)   Sequences: 24(11)
actin gamma 1 [synthetic construct]
gi|62897409      Mass: 41696   Score: 1268   Matches: 38(17)   Sequences: 24(11)
beta actin variant [Homo sapiens]
gi|62897671      Mass: 41694   Score: 1268   Matches: 38(17)   Sequences: 24(11)
beta actin variant [Homo sapiens]
gi|66735458      Mass: 41738   Score: 1268   Matches: 38(17)   Sequences: 24(11)
beta-actin [Phascolarctos cinereus]
gi|74191564      Mass: 41724   Score: 1268   Matches: 38(17)   Sequences: 24(11)
unnamed protein product [Mus musculus]
gi|74191566      Mass: 41784   Score: 1268   Matches: 38(17)   Sequences: 24(11)
unnamed protein product [Mus musculus]
gi|74213524      Mass: 41724   Score: 1268   Matches: 38(17)   Sequences: 24(11)
unnamed protein product [Mus musculus]
gi|82195535      Mass: 41752   Score: 1268   Matches: 38(17)   Sequences: 24(11)
RecName: Full=Actin, cytoplasmic 2; AltName: Full=Cytoplasmic actin type 5; AltName: Full=Gamma-actin; Contains: RecName: Full=Actin, cytoplasmic 2, N-terminally processed
gi|82213456      Mass: 41724   Score: 1268   Matches: 38(17)   Sequences: 24(11)
RecName: Full=Actin, cytoplasmic 2; AltName: Full=Gamma-actin; Contains: RecName: Full=Actin, cytoplasmic 2, N-terminally processed
gi|118419977      Mass: 40552   Score: 1268   Matches: 38(17)   Sequences: 24(11)
beta-actin, partial [Eubalaena glacialis]
gi|126272476      Mass: 41827   Score: 1268   Matches: 38(17)   Sequences: 24(11)
PREDICTED: actin, cytoplasmic type 5-like [Monodelphis domestica]
gi|146386601      Mass: 41718   Score: 1268   Matches: 38(17)   Sequences: 24(11)
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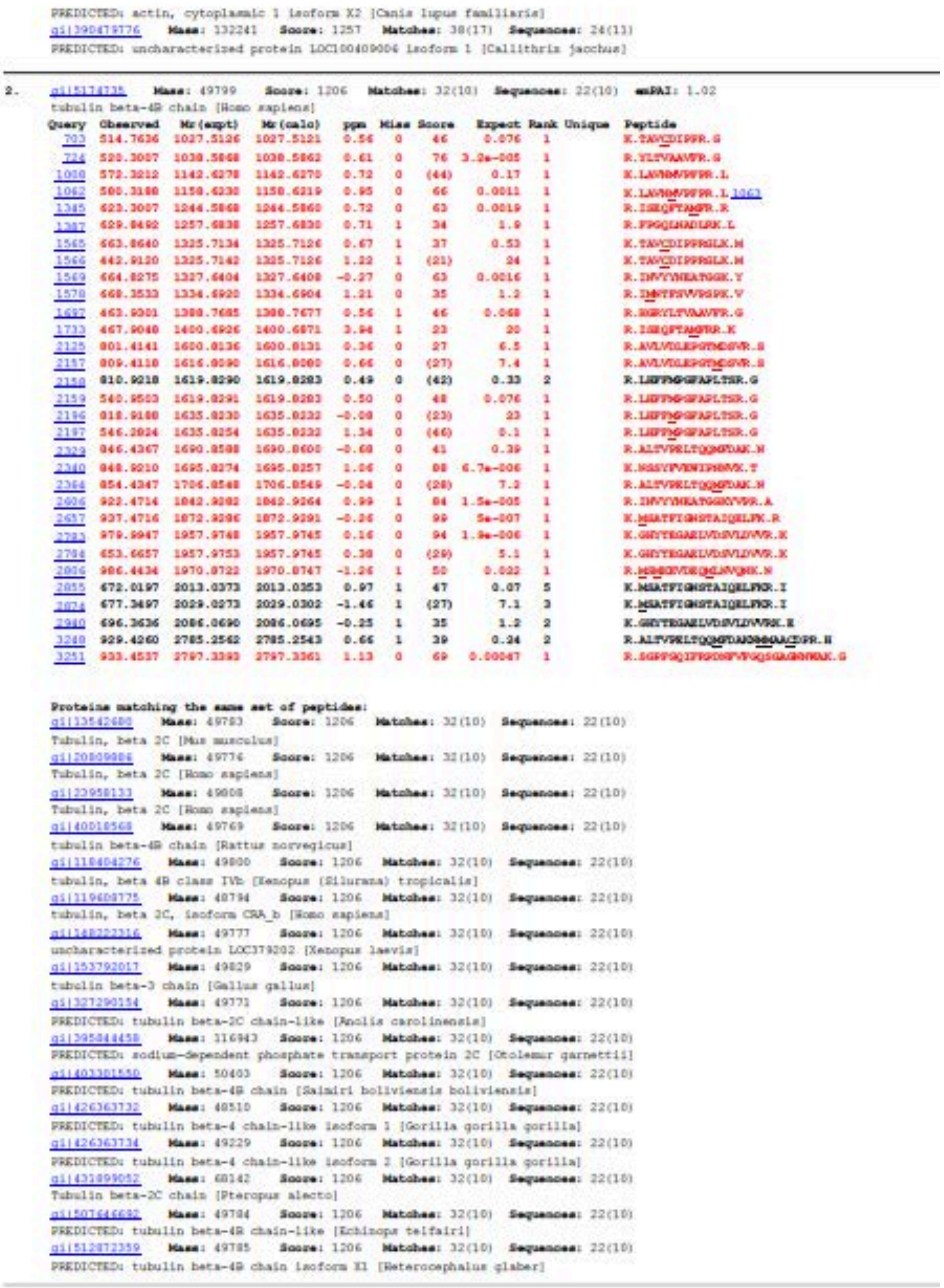

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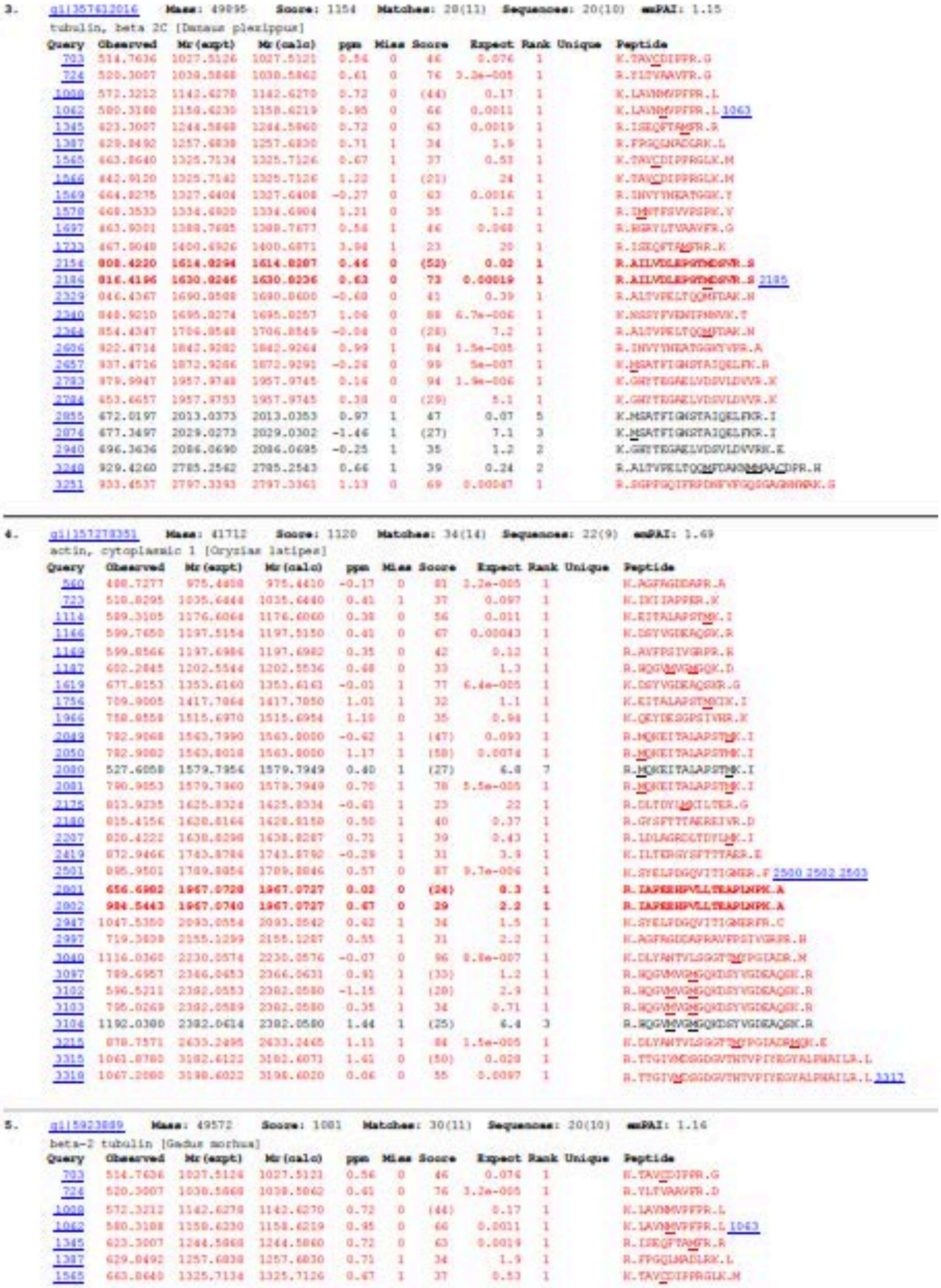
PREDICTED: actin, cytoplasmic 1 isoform X2 [Canis lupus familiaris]
gi|390479776     Mass: 132241    Score: 1257    Matches: 38(17)    Sequences: 24(11)
PREDICTED: uncharacterized protein LOC100409006 isoform 1 [Callithrix jacchus]

2.  gi|5174735     Mass: 49799    Score: 1206    Matches: 32(10)    Sequences: 22(10)    emPAI: 1.02
    tubulin beta-4B chain [Homo sapiens]
    Query  Observed   Mr(expt)   Mr(calc)    ppm  Miss Score   Expect Rank Unique  Peptide
     703   514.7636  1027.5126  1027.5121   0.56    0    46    0.076    1          K.TAVCDIPPR.G
     724   520.3007  1038.5868  1038.5862   0.61    0    76  3.2e-005   1          R.YLTVAAVFR.G
    1008   572.3212  1142.6278  1142.6270   0.72    0   (44)    0.17    1          K.LAVNMVPFPR.L
    1062   580.3188  1158.6230  1158.6219   0.95    0    66   0.0011    1          K.LAVNMVPFPR.L 1063
    1345   623.3007  1244.5868  1244.5860   0.72    0    63   0.0019    1          R.ISEQFTAMFR.R
    1387   629.8492  1257.6838  1257.6830   0.71    1    34      1.9    1          R.FPGQLNADLRK.L
    1565   663.8640  1325.7134  1325.7126   0.67    1    37     0.53    1          K.TAVCDIPPRGLK.M
    1566   442.9120  1325.7142  1325.7126   1.22    1   (21)      24    1          K.TAVCDIPPRGLK.M
    1569   664.8275  1327.6404  1327.6408  -0.27    0    63   0.0016    1          R.INVYYNEATGGK.Y
    1578   668.3533  1334.6920  1334.6904   1.21    0    35      1.2    1          R.IMNTFSVVPSPK.V
    1697   463.9301  1388.7685  1388.7677   0.56    1    46    0.068    1          R.HGRYLTVAAVFR.G
    1733   467.9048  1400.6926  1400.6871   3.94    1    23       20    1          R.ISEQFTAMFRR.K
    2125   801.4141  1600.8136  1600.8131   0.36    0    27      6.5    1          R.AVLVDLEPGTMDSVR.S
    2157   809.4118  1616.8090  1616.8080   0.66    0   (27)     7.4    1          R.AVLVDLEPGTMDSVR.S
    2158   810.9218  1619.8290  1619.8283   0.49    0   (42)    0.33    2          R.LHFFMPGFAPLTSR.G
    2159   540.9503  1619.8291  1619.8283   0.50    0    48    0.076    1          R.LHFFMPGFAPLTSR.G
    2196   818.9188  1635.8230  1635.8232  -0.08    0   (23)      23    1          R.LHFFMPGFAPLTSR.G
    2197   546.2824  1635.8254  1635.8232   1.34    0   (46)     0.1    1          R.LHFFMPGFAPLTSR.G
    2329   846.4367  1690.8588  1690.8600  -0.68    0    41     0.39    1          R.ALTVPELTQQMFDAK.N
    2340   848.9210  1695.8274  1695.8257   1.06    0    88  6.7e-006   1          K.NSSYFVEWIPNNVK.T
    2364   854.4347  1706.8548  1706.8549  -0.04    0   (28)     7.2    1          R.ALTVPELTQQMFDAK.N
    2606   922.4714  1842.9282  1842.9264   0.99    1    84  1.5e-005   1          R.INVYYNEATGGKYVPR.A
    2657   937.4716  1872.9286  1872.9291  -0.26    0    99    5e-007   1          K.MSATFIGNSTAIQELFK.R
    2783   979.9947  1957.9748  1957.9745   0.16    0    94  1.9e-006   1          K.GHYTEGAELVDSVLDVVR.K
    2784   653.6657  1957.9753  1957.9745   0.38    0   (29)     5.1    1          K.GHYTEGAELVDSVLDVVR.K
    2806   986.4434  1970.8722  1970.8747  -1.26    1    50    0.022    1          R.MSMKEVDEQMLNVQNK.N
    2855   672.0197  2013.0373  2013.0353   0.97    1    47     0.07    5          K.MSATFIGNSTAIQELFKR.I
    2874   677.3497  2029.0273  2029.0302  -1.46    1   (27)     7.1    3          K.MSATFIGNSTAIQELFKR.I
    2940   696.3636  2086.0690  2086.0695  -0.25    1    35      1.2    2          K.GHYTEGAELVDSVLDVVRK.E
    3240   929.4260  2785.2562  2785.2543   0.66    1    39     0.24    2          R.ALTVPELTQQMFDAKNMMAACDPR.H
    3251   933.4537  2797.3393  2797.3361   1.13    0    69  0.00047    1          R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G

    Proteins matching the same set of peptides:
    gi|13542680     Mass: 49783    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    Tubulin, beta 2C [Mus musculus]
    gi|20809886     Mass: 49776    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    Tubulin, beta 2C [Homo sapiens]
    gi|23958133     Mass: 49808    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    Tubulin, beta 2C [Homo sapiens]
    gi|40018568     Mass: 49769    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    tubulin beta-4B chain [Rattus norvegicus]
    gi|118404276     Mass: 49800    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    tubulin, beta 4B class IVb [Xenopus (Silurana) tropicalis]
    gi|119608775     Mass: 48794    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    tubulin, beta 2C, isoform CRA_b [Homo sapiens]
    gi|148222316     Mass: 49777    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    uncharacterized protein LOC379202 [Xenopus laevis]
    gi|153792017     Mass: 49829    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    tubulin beta-3 chain [Gallus gallus]
    gi|327290154     Mass: 49771    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-2C chain-like [Anolis carolinensis]
    gi|395844458     Mass: 116943    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: sodium-dependent phosphate transport protein 2C [Otolemur garnettii]
    gi|403301550     Mass: 50403    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-4B chain [Saimiri boliviensis boliviensis]
    gi|426363732     Mass: 48510    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-4 chain-like isoform 1 [Gorilla gorilla gorilla]
    gi|426363734     Mass: 49229    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-4 chain-like isoform 2 [Gorilla gorilla gorilla]
    gi|431899052     Mass: 68142    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    Tubulin beta-2C chain [Pteropus alecto]
    gi|507646692     Mass: 49784    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-4B chain-like [Echinops telfairi]
    gi|512872359     Mass: 49785    Score: 1206    Matches: 32(10)    Sequences: 22(10)
    PREDICTED: tubulin beta-4B chain isoform X1 [Heterocephalus glaber]
```



3. gi|357612016 **Mass:** 49895 **Score:** 1154 **Matches:** 28(11) **Sequences:** 20(10) **emPAI:** 1.15

tubulin, beta 2C [Danaus plexippus]

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
703	514.7636	1027.5126	1027.5121	0.56	0	46	0.076	1		K.TAVCDIPPR.G
724	520.3007	1038.5868	1038.5862	0.61	0	76	3.2e-005	1		R.YLTVAAVFR.G
1008	572.3212	1142.6278	1142.6270	0.72	0	(44)	0.17	1		K.LAVNMVPFPR.L
1062	580.3188	1158.6230	1158.6219	0.95	0	66	0.0011	1		K.LAVNMVPFPR.L 1063
1345	623.3007	1244.5868	1244.5860	0.72	0	63	0.0019	1		R.ISEQFTAMFR.R
1387	629.8492	1257.6838	1257.6830	0.71	1	34	1.9	1		R.FPGQLNADLRK.L
1565	663.8640	1325.7134	1325.7126	0.67	1	37	0.53	1		K.TAVCDIPPRGLK.M
1566	442.9120	1325.7142	1325.7126	1.22	1	(21)	24	1		K.TAVCDIPPRGLK.M
1569	664.8275	1327.6404	1327.6408	-0.27	0	63	0.0016	1		R.INVYYNEATGGK.Y
1578	668.3533	1334.6920	1334.6904	1.21	0	35	1.2	1		R.IMNTFSVVPSPK.V
1697	463.9001	1388.7685	1388.7677	0.56	1	46	0.068	1		R.HGRYLTVAAVFR.G
1733	467.9048	1400.6926	1400.6871	3.94	1	23	20	1		R.ISEQFTAMFRR.K
2154	**808.4220**	**1614.8294**	**1614.8287**	**0.46**	**0**	**(52)**	**0.02**	**1**		**R.AILVDLEPGTMDSVR.S**
2186	**816.4196**	**1630.8246**	**1630.8236**	**0.63**	**0**	**73**	**0.00019**	**1**		**R.AILVDLEPGTMDSVR.S** 2185
2329	846.4367	1690.8588	1690.8600	-0.68	0	41	0.39	1		R.ALTVPELTQQMFDAK.N
2340	848.9210	1695.8274	1695.8257	1.06	0	88	6.7e-006	1		K.NSSYFVEWIPNNVK.T
2364	854.4347	1706.8548	1706.8549	-0.04	0	(28)	7.2	1		R.ALTVPELTQQMFDAK.N
2606	922.4714	1842.9282	1842.9264	0.99	1	84	1.5e-005	1		R.INVYYNEATGGKYVPR.A
2657	937.4716	1872.9286	1872.9291	-0.26	0	99	5e-007	1		K.MSATFIGNSTAIQELFK.R
2783	979.9947	1957.9748	1957.9745	0.16	0	94	1.9e-006	1		K.GHYTEGAELVDSVLDVVR.K
2784	653.6657	1957.9753	1957.9745	0.38	0	(29)	5.1	1		K.GHYTEGAELVDSVLDVVR.K
2855	672.0197	2013.0373	2013.0353	0.97	1	47	0.07	5		K.MSATFIGNSTAIQELFKR.I
2874	677.3497	2029.0273	2029.0302	-1.46	1	(27)	7.1	3		K.MSATFIGNSTAIQELFKR.I
2940	696.3636	2086.0690	2086.0695	-0.25	1	35	1.2	2		K.GHYTEGAELVDSVLDVVRK.E
3240	929.4260	2785.2562	2785.2543	0.66	1	39	0.24	2		R.ALTVPELTQQMFDAKNMMAACDPR.H
3251	933.4537	2797.3393	2797.3361	1.13	0	69	0.00047	1		R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G

4. gi|157278351 **Mass:** 41712 **Score:** 1120 **Matches:** 34(14) **Sequences:** 22(9) **emPAI:** 1.69

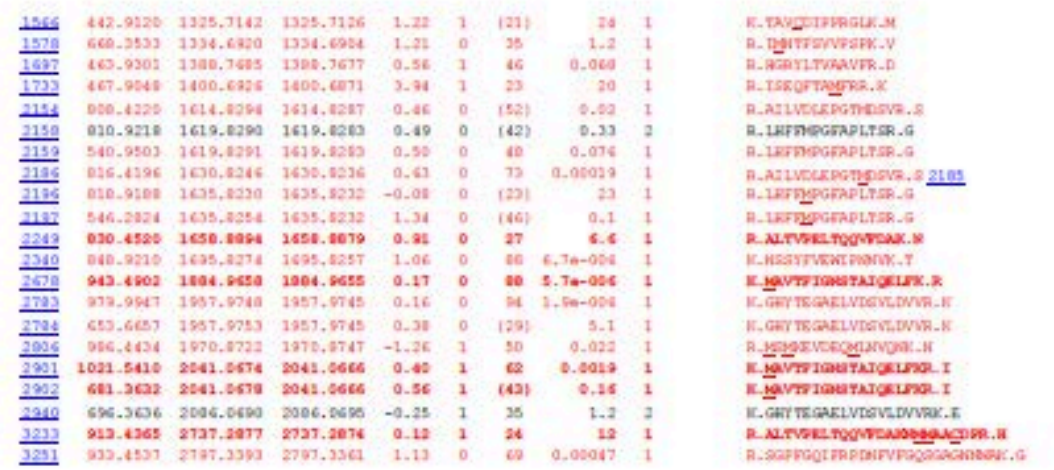
actin, cytoplasmic 1 [Oryzias latipes]

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
560	488.7277	975.4408	975.4410	-0.17	0	81	2.2e-005	1		K.AGFAGDDAPR.A
723	518.8295	1035.6444	1035.6440	0.41	1	37	0.097	1		K.IKIIAPPER.K
1114	589.3105	1176.6064	1176.6060	0.38	0	56	0.011	1		K.EITALAPSTMK.I
1166	599.7650	1197.5154	1197.5150	0.41	0	67	0.00043	1		K.DSYVGDEAQSK.R
1169	599.8566	1197.6986	1197.6982	0.35	0	42	0.12	1		R.AVFPSIVGRPR.H
1187	602.2845	1202.5544	1202.5536	0.68	0	33	1.3	1		R.HQGVMVGMGQK.D
1619	677.8153	1353.6160	1353.6161	-0.01	1	77	6.4e-005	1		K.DSYVGDEAQSKR.G
1756	709.9005	1417.7864	1417.7850	1.01	1	32	1.1	1		K.EITALAPSTMKIK.I
1966	758.8558	1515.6970	1515.6954	1.10	0	35	0.98	1		K.QEYDESGPSIVHR.K
2049	782.9068	1563.7990	1563.8000	-0.62	1	(47)	0.093	1		R.MQKEITALAPSTMK.I
2050	782.9082	1563.8018	1563.8000	1.17	1	(58)	0.0074	1		R.MQKEITALAPSTMK.I
2080	527.6058	1579.7956	1579.7949	0.40	1	(27)	6.8	7		R.MQKEITALAPSTMK.I
2081	790.9053	1579.7960	1579.7949	0.70	1	78	5.5e-005	1		R.MQKEITALAPSTMK.I
2175	813.9235	1625.8324	1625.8334	-0.61	1	23	22	1		R.DLTDYLMKILTER.G
2180	815.4156	1628.8166	1628.8158	0.50	1	40	0.37	1		R.GYSFTTTAEREIVR.D
2207	820.4222	1638.8298	1638.8287	0.71	1	39	0.43	1		R.IDLAGRDLTDYLMK.I
2419	872.9466	1743.8786	1743.8792	-0.29	1	31	3.9	1		K.ILTERGYSFTTTAER.E
2501	895.9501	1789.8856	1789.8846	0.57	0	87	9.7e-006	1		K.SYELPDGQVITIGNER.F 2500 2502 2503
2801	**656.6982**	**1967.0728**	**1967.0727**	**0.03**	**0**	**(24)**	**0.3**	**1**		**R.IAPEEHPVLLTEAPLNPK.A**
2802	**984.5443**	**1967.0740**	**1967.0727**	**0.67**	**0**	**29**	**2.2**	**1**		**R.IAPEEHPVLLTEAPLNPK.A**
2947	1047.5350	2093.0554	2093.0542	0.62	1	34	1.5	1		K.SYELPDGQVITIGNERFR.C
2997	719.3839	2155.1299	2155.1287	0.55	1	31	2.2	1		K.AGFAGDDAPRAVFPSIVGRPR.H
3040	1116.0360	2230.0574	2230.0576	-0.07	0	96	8.8e-007	1		K.DLYANTVLSGGTTMYPGIADR.M
3097	789.6957	2366.0653	2366.0631	0.91	1	(33)	1.2	1		R.HQGVMVGMGQKDSYVGDEAQSK.R
3102	596.5211	2382.0553	2382.0580	-1.15	1	(20)	2.9	1		R.HQGVMVGMGQKDSYVGDEAQSK.R
3103	795.0269	2382.0589	2382.0580	0.35	1	34	0.71	1		R.HQGVMVGMGQKDSYVGDEAQSK.R
3104	1192.0380	2382.0614	2382.0580	1.44	1	(25)	6.4	3		R.HQGVMVGMGQKDSYVGDEAQSK.R
3215	878.7571	2633.2495	2633.2465	1.11	1	84	1.5e-005	1		K.DLYANTVLSGGTTMYPGIADRMQK.E
3315	1061.8780	3182.6122	3182.6071	1.61	0	(50)	0.028	1		R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L
3318	1067.2080	3198.6022	3198.6020	0.06	0	55	0.0097	1		R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L 3317

5. gi|5923889 **Mass:** 49572 **Score:** 1081 **Matches:** 30(11) **Sequences:** 20(10) **emPAI:** 1.16

beta-2 tubulin [Gadus morhua]

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
703	514.7636	1027.5126	1027.5121	0.56	0	46	0.076	1		K.TAVCDIPPR.G
724	520.3007	1038.5868	1038.5862	0.61	0	76	3.2e-005	1		R.YLTVAAVFR.D
1008	572.3212	1142.6278	1142.6270	0.72	0	(44)	0.17	1		K.LAVNMVPFPR.L
1062	580.3188	1158.6230	1158.6219	0.95	0	66	0.0011	1		K.LAVNMVPFPR.L 1063
1345	623.3007	1244.5868	1244.5860	0.72	0	63	0.0019	1		R.ISEQFTAMFR.R
1387	629.8492	1257.6838	1257.6830	0.71	1	34	1.9	1		R.FPGQLNADLRK.L
1565	663.8640	1325.7134	1325.7126	0.67	1	37	0.53	1		K.TAVCDIPPRGLK.M



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1566   442.9120  1325.7142  1325.7126   1.22  1  (21)       24  1   K.TAVCDIPPRGLK.M
1578   668.3533  1334.6920  1334.6904   1.21  0   35       1.2  1   R.IMNTFSVVPSPK.V
1697   463.9301  1388.7685  1388.7677   0.56  1   46     0.060  1   R.HGRYLTVAAVFR.G
1733   467.9048  1400.6926  1400.6871   3.94  1   23        20  1   R.ISEQFTAMFRR.K
2154   808.4220  1614.8294  1614.8287   0.46  0  (52)     0.02  1   R.AILVDLEPGTMDSVR.S
2158   810.9218  1619.8290  1619.8283   0.49  0  (42)     0.33  2   R.LHFFMPGFAPLTSR.G
2159   540.9503  1619.8291  1619.8283   0.50  0   40     0.076  1   R.LHFFMPGFAPLTSR.G
2186   816.4196  1630.8246  1630.8236   0.63  0   73   0.00019  1   R.AILVDLEPGTMDSVR.S 2185
2196   818.9188  1635.8230  1635.8232  -0.08  0  (23)       23  1   R.LHFFMPGFAPLTSR.G
2187   546.2824  1635.8254  1635.8232   1.34  0  (46)      0.1  1   R.LHFFMPGFAPLTSR.G
2249   830.4520  1658.8894  1658.8879   0.91  0   27       6.6  1   R.ALTVPELTQQVFDAK.N
2340   848.9210  1695.8274  1695.8257   1.06  0   86  6.7e-006  1   K.NSSYFVEWIPNNVK.T
2678   943.4902  1884.9658  1884.9655   0.17  0   80  5.7e-006  1   K.MAVTFIGNSTAIQELFK.R
2783   979.9947  1957.9748  1957.9745   0.16  0   94  1.9e-006  1   K.GHYTEGAELVDSVLDVVR.K
2784   653.6657  1957.9753  1957.9745   0.38  0  (29)      5.1  1   K.GHYTEGAELVDSVLDVVR.K
2806   986.4434  1970.8722  1970.8747  -1.26  1   50     0.022  1   R.MSMKEVDEQMLNVQNK.N
2901  1021.5410  2041.0674  2041.0666   0.40  1   62    0.0019  1   K.MAVTFIGNSTAIQELFKR.I
2902   681.3632  2041.0678  2041.0666   0.56  1  (43)     0.16  1   K.MAVTFIGNSTAIQELFKR.I
2940   696.3636  2086.0690  2086.0695  -0.25  1   35       1.2  2   K.GHYTEGAELVDSVLDVVRK.E
3233   913.4365  2737.2877  2737.2874   0.12  1   24        12  1   R.ALTVPELTQQVFDAKNMMAACDPR.H
3251   933.4537  2797.3393  2797.3361   1.13  0   69   0.00047  1   R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G
```
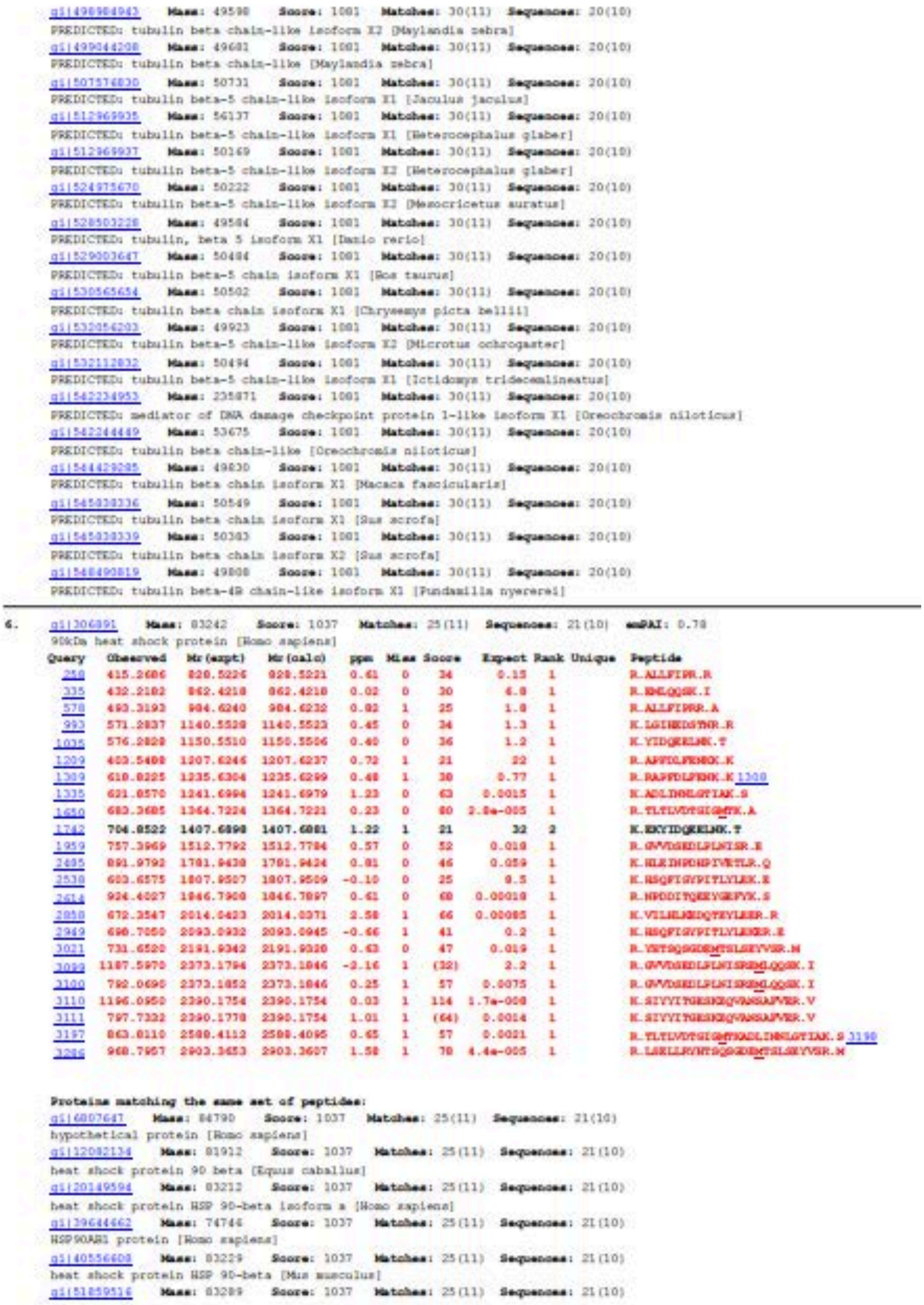

```
Proteins matching the same set of peptides:
gi|7106439     Mass: 49639   Score: 1001   Matches: 30(11)   Sequences: 20(10)
tubulin beta-5 chain [Mus musculus]
gi|12846758    Mass: 49608   Score: 1001   Matches: 30(11)   Sequences: 20(10)
unnamed protein product [Mus musculus]
gi|30480747    Mass: 49641   Score: 1001   Matches: 30(11)   Sequences: 20(10)
tubulin, beta 5 [Danio rerio]
gi|55742495    Mass: 49696   Score: 1001   Matches: 30(11)   Sequences: 20(10)
tubulin beta chain [Xenopus (Silurana) tropicalis]
gi|74141821    Mass: 49667   Score: 1001   Matches: 30(11)   Sequences: 20(10)
unnamed protein product [Mus musculus]
gi|74223737    Mass: 49652   Score: 1001   Matches: 30(11)   Sequences: 20(10)
unnamed protein product [Mus musculus]
gi|148691289    Mass: 49910   Score: 1001   Matches: 30(11)   Sequences: 20(10)
tubulin, beta 5 [Mus musculus]
gi|221045918    Mass: 46537   Score: 1001   Matches: 30(11)   Sequences: 20(10)
unnamed protein product [Homo sapiens]
gi|291395962    Mass: 49695   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin, beta 5-like [Oryctolagus cuniculus]
gi|332245916    Mass: 53454   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 3 [Nomascus leucogenys]
gi|332245918    Mass: 44790   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 4 [Nomascus leucogenys]
gi|346644703    Mass: 49710   Score: 1001   Matches: 30(11)   Sequences: 20(10)
beta tubulin [Cricetulus griseus]
gi|410905733    Mass: 49655   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Takifugu rubripes]
gi|410958666    Mass: 50209   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 2 [Felis catus]
gi|426250622    Mass: 50494   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain isoform 2 [Ovis aries]
gi|426352283    Mass: 50452   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 4 [Gorilla gorilla gorilla]
gi|432882729    Mass: 49667   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Oryzias latipes]
gi|432908810    Mass: 49641   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Oryzias latipes]
gi|441594011    Mass: 50221   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain [Nomascus leucogenys]
gi|449266665    Mass: 44408   Score: 1001   Matches: 30(11)   Sequences: 20(10)
Tubulin beta-7 chain [Columba livia]
gi|470614409    Mass: 48145   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Tursiops truncatus]
gi|471417858    Mass: 50538   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 1 [Trichechus manatus latirostris]
gi|478500263    Mass: 50453   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform 1 [Ceratotherium simum simum]
gi|488527589    Mass: 53861   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: LOW QUALITY PROTEIN: tubulin beta chain [Dasypus novemcinctus]
gi|498984939    Mass: 49655   Score: 1001   Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like isoform X1 [Maylandia zebra]
```



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gi|498984943    Mass: 49598    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like isoform X2 [Maylandia zebra]
gi|499044208    Mass: 49681    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Maylandia zebra]
gi|507574830    Mass: 50731    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X1 [Jaculus jaculus]
gi|512969935    Mass: 56137    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X1 [Heterocephalus glaber]
gi|512969937    Mass: 50169    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X2 [Heterocephalus glaber]
gi|524975670    Mass: 50222    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X2 [Mesocricetus auratus]
gi|528503228    Mass: 49584    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin, beta 5 isoform X1 [Danio rerio]
gi|529003647    Mass: 50484    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain isoform X1 [Bos taurus]
gi|530565654    Mass: 50502    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform X1 [Chrysemys picta bellii]
gi|532056203    Mass: 49923    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X2 [Microtus ochrogaster]
gi|532112832    Mass: 50494    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-5 chain-like isoform X1 [Ictidomys tridecemlineatus]
gi|542234953    Mass: 235871   Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: mediator of DNA damage checkpoint protein 1-like isoform X1 [Oreochromis niloticus]
gi|542244449    Mass: 53675    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain-like [Oreochromis niloticus]
gi|544429285    Mass: 49830    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform X1 [Macaca fascicularis]
gi|545838336    Mass: 50549    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform X1 [Sus scrofa]
gi|545838339    Mass: 50383    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta chain isoform X2 [Sus scrofa]
gi|548490819    Mass: 49808    Score: 1001    Matches: 30(11)   Sequences: 20(10)
PREDICTED: tubulin beta-4B chain-like isoform X1 [Pundamilia nyererei]
```

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6.   gi|306891     Mass: 83242    Score: 1037   Matches: 25(11)   Sequences: 21(10)   emPAI: 0.78
     90kDa heat shock protein [Homo sapiens]
    Query   Observed   Mr(expt)   Mr(calc)    ppm  Miss Score    Expect Rank Unique   Peptide
     258    415.2686   828.5226   828.5221   0.61    0    34      0.15    1           R.ALLFIPR.R
     335    432.2182   862.4218   862.4218   0.02    0    30       6.8    1           R.EMLQQSK.I
     578    493.3193   984.6240   984.6232   0.82    1    25       1.8    1           R.ALLFIPRR.A
     993    571.2837  1140.5528  1140.5523   0.45    0    34       1.3    1           K.LGIHEDSTNR.R
    1035    576.2828  1150.5510  1150.5506   0.40    0    36       1.2    1           K.YIDQEELNK.T
    1209    403.5488  1207.6246  1207.6237   0.72    1    21        22    1           R.APFDLFENKK.K
    1309    618.8225  1235.6304  1235.6299   0.48    1    30      0.77    1           R.RAPFDLFENK.K 1308
    1335    621.8570  1241.6994  1241.6979   1.23    0    63    0.0015    1           K.ADLINNLGTIAK.S
    1650    683.3685  1364.7224  1364.7221   0.23    0    80  2.8e-005    1           R.TLTLVDTGIGMTK.A
    1742    704.8522  1407.6898  1407.6881   1.22    1    21        32    2           K.EKYIDQEELNK.T
    1959    757.3969  1512.7792  1512.7784   0.57    0    52     0.018    1           R.GVVDSEDLPLNISR.E
    2485    891.9792  1781.9438  1781.9424   0.81    0    46     0.059    1           K.HLEINPDHPIVETLR.Q
    2538    603.6575  1807.9507  1807.9509  -0.10    0    25       8.5    1           K.HSQFIGYPITLYLEK.E
    2614    924.4027  1846.7908  1846.7897   0.61    0    68   0.00018    1           R.NPDDITQEEYGEFYK.S
    2858    672.3547  2014.0423  2014.0371   2.58    1    66   0.00085    1           K.VILHLKEDQTEYLEER.R
    2949    698.7050  2093.0932  2093.0945  -0.66    1    41       0.2    1           K.HSQFIGYPITLYLEKER.E
    3021    731.6520  2191.9342  2191.9328   0.63    0    47     0.019    1           R.YHTSQSGDEMTSLSEYVSR.M
    3099   1187.5970  2373.1794  2373.1846  -2.16    1  (32)       2.2    1           R.GVVDSEDLPLNISREMLQQSK.I
    3100    792.0690  2373.1852  2373.1846   0.25    1    57    0.0075    1           R.GVVDSEDLPLNISREMLQQSK.I
    3110   1196.0950  2390.1754  2390.1754   0.03    1   114  1.7e-008    1           K.SIYYITGESKEQVANSAFVER.V
    3111    797.7322  2390.1778  2390.1754   1.01    1  (64)    0.0014    1           K.SIYYITGESKEQVANSAFVER.V
    3197    863.8110  2588.4112  2588.4095   0.65    1    57    0.0021    1           R.TLTLVDTGIGMTKADLINNLGTIAK.S 3198
    3286    968.7957  2903.3653  2903.3607   1.58    1    78  4.4e-005    1           R.LSELLRYHTSQSGDEMTSLSEYVSR.M

     Proteins matching the same set of peptides:
     gi|6807647     Mass: 84790    Score: 1037   Matches: 25(11)   Sequences: 21(10)
     hypothetical protein [Homo sapiens]
     gi|12082134    Mass: 81912    Score: 1037   Matches: 25(11)   Sequences: 21(10)
     heat shock protein 90 beta [Equus caballus]
     gi|20149594    Mass: 83212    Score: 1037   Matches: 25(11)   Sequences: 21(10)
     heat shock protein HSP 90-beta isoform a [Homo sapiens]
     gi|39644662    Mass: 74746    Score: 1037   Matches: 25(11)   Sequences: 21(10)
     HSP90AB1 protein [Homo sapiens]
     gi|40556608    Mass: 83229    Score: 1037   Matches: 25(11)   Sequences: 21(10)
     heat shock protein HSP 90-beta [Mus musculus]
     gi|51859516    Mass: 83289    Score: 1037   Matches: 25(11)   Sequences: 21(10)
```

```
Heat shock protein 90kDa alpha (cytosolic), class B member 1 [Rattus norvegicus]
gi|74147026     Mass: 83171    Score: 1037    Matches: 25(11)    Sequences: 21(10)
unnamed protein product [Mus musculus]
gi|90075818     Mass: 83113    Score: 1037    Matches: 25(11)    Sequences: 21(10)
unnamed protein product [Macaca fascicularis]
gi|91234898     Mass: 83230    Score: 1037    Matches: 25(11)    Sequences: 21(10)
84 kDa heat shock protein [Rattus norvegicus]
gi|126352614     Mass: 83185     Score: 1037    Matches: 25(11)    Sequences: 21(10)
heat shock protein HSP 90-beta [Equus caballus]
gi|194378142     Mass: 82119     Score: 1037    Matches: 25(11)    Sequences: 21(10)
unnamed protein product [Homo sapiens]
gi|194386896     Mass: 79145     Score: 1037    Matches: 25(11)    Sequences: 21(10)
unnamed protein product [Homo sapiens]
gi|197100267     Mass: 83186     Score: 1037    Matches: 25(11)    Sequences: 21(10)
heat shock protein HSP 90-beta [Pongo abelii]
gi|291396282     Mass: 83415     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock 90kDa protein 1, beta [Oryctolagus cuniculus]
gi|346986428     Mass: 83201     Score: 1037    Matches: 25(11)    Sequences: 21(10)
heat shock 90kD protein 1, beta [Sus scrofa]
gi|355748592     Mass: 83136     Score: 1037    Matches: 25(11)    Sequences: 21(10)
hypothetical protein EGM_13637 [Macaca fascicularis]
gi|397526725     Mass: 82242     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock cognate protein HSP 90-beta-like isoform 2 [Pan paniscus]
gi|410959298     Mass: 83215     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta [Felis catus]
gi|431822408     Mass: 82269     Score: 1037    Matches: 25(11)    Sequences: 21(10)
heat shock protein HSP 90-beta isoform c [Homo sapiens]
gi|431838325     Mass: 84145     Score: 1037    Matches: 25(11)    Sequences: 21(10)
Heat shock protein HSP 90-beta [Pteropus alecto]
gi|444725038     Mass: 89569     Score: 1037    Matches: 25(11)    Sequences: 21(10)
Heat shock protein HSP 90-beta [Tupaia chinensis]
gi|478499606     Mass: 84161     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform 1 [Ceratotherium simum simum]
gi|478499608     Mass: 83245     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform 2 [Ceratotherium simum simum]
gi|504157887     Mass: 83245     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Ochotona princeps]
gi|504157889     Mass: 82302     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Ochotona princeps]
gi|505791410     Mass: 83215     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Sorex araneus]
gi|505791413     Mass: 82272     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Sorex araneus]
gi|507924642     Mass: 83243     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Condylura cristata]
gi|507924644     Mass: 83322     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Condylura cristata]
gi|524941111     Mass: 83261     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Mesocricetus auratus]
gi|524941115     Mass: 82318     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Mesocricetus auratus]
gi|532033960     Mass: 83157     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Microtus ochrogaster]
gi|532065639     Mass: 83289     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta [Ictidomys tridecemlineatus]
gi|537261579     Mass: 80464     Score: 1037    Matches: 25(11)    Sequences: 21(10)
heat shock protein HSP 90-beta-like protein [Cricetulus griseus]
gi|544427662     Mass: 87537     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: uncharacterized LOC101926380 isoform X1 [Macaca fascicularis]
gi|545519269     Mass: 84340     Score: 1037    Matches: 25(11)    Sequences: 21(10)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Canis lupus familiaris]
```

Supplemental Figure S8: LC/MS/MS sequencing results from compound **7**_TagII lysate pulldown in HCT-116 cell lysates.

Mascot Search Results

```
User                   :
Email                  :
Search title           : JM_270214_1_r.RAW
MS data file           : D:\Data\Sydney\2014\February\28\JM_270214_1_r.RAW
Database               : NCBInr 26_10_13 (33055681 sequences; 11532217697 residues)
Timestamp              : 2 Mar 2014 at 22:57:45 GMT
Enzyme                 : Trypsin
Variable modifications : Acrylamide (C),Carbamidomethyl (C),Oxidation (M)
Mass values            : Monoisotopic
Protein Mass           : Unrestricted
Peptide Mass Tolerance : ± 4 ppm
Fragment Mass Tolerance: ± 0.4 Da
Max Missed Cleavages   : 1
Instrument type        : ESI-TRAP
Number of queries      : 2928
Protein hits           : gi|12667788  myosin-9 [Homo sapiens]
                         gi|113271    RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta actin
                         gi|530653524 PREDICTED: myosin-9-like [Chrysemys picta bellii]
                         gi|5174735   tubulin beta-4B chain [Homo sapiens]
                         gi|7106439   tubulin beta-5 chain [Mus musculus]
                         gi|16579885  60S ribosomal protein L4 [Homo sapiens]
                         gi|306891    90kDa heat shock protein [Homo sapiens]
                         gi|7669492   glyceraldehyde-3-phosphate dehydrogenase isoform 1 [Homo sapiens]
                         gi|296228277 PREDICTED: glyceraldehyde-3-phosphate dehydrogenase [Callithrix jacchus]
                         gi|480318327 actin, partial [Thorelliola mahunkai]
                         gi|348506289 PREDICTED: heat shock protein HSP 90-alpha-like [Oreochromis niloticus]
                         gi|38648667  Fatty acid synthase [Homo sapiens]
                         gi|32488     unnamed protein product [Homo sapiens]
                         gi|480318316 actin, partial [Thyenula sp. South Africa]
                         gi|1374715   ATP synthase beta subunit [Rattus norvegicus]
                         gi|480318469 actin, partial [Euryattus sp. Tualapa]
                         gi|4506667   60S acidic ribosomal protein P0 [Homo sapiens]
                         gi|181573    cytokeratin 8 [Homo sapiens]
                         gi|426254043 PREDICTED: LOW QUALITY PROTEIN: tubulin beta-6 chain [Ovis aries]
                         gi|4503483   elongation factor 2 [Homo sapiens]
                         gi|397511428 PREDICTED: tubulin alpha-1C chain-like [Pan paniscus]
                         gi|390352752 PREDICTED: tubulin beta-4 chain-like isoform 3 [Strongylocentrotus purpuratus]
                         gi|35903     ribosomal protein L7 [Homo sapiens]
                         gi|16753227  60S ribosomal protein L6 [Homo sapiens]
                         gi|1827809   Chain A, Bovine Mitochondrial F1-Atpase Complexed With Aurovertin B
                         gi|337930    scar protein [Homo sapiens]
                         gi|521036680 Tubulin alpha-1D chain [Myotis brandtii]
                         gi|432092845 Translational activator GCN1 [Myotis davidii]
                         gi|4506607   60S ribosomal protein L18 isoform 1 [Homo sapiens]
                         gi|505846589 PREDICTED: tubulin alpha-1B chain isoform X1 [Sorex araneus]
                         gi|390467904 PREDICTED: 60S ribosomal protein L4-like [Callithrix jacchus]
                         gi|291389091 PREDICTED: mCG18413-like [Oryctolagus cuniculus]
                         gi|27807325  myosin-10 [Bos taurus]
                         gi|61543     unnamed protein product [Feline sarcoma virus]
                         gi|4574288   beta-actin [Tupaia belangeri]
                         gi|4502643   T-complex protein 1 subunit zeta isoform a [Homo sapiens]
                         gi|306553    ribosmal protein small subunit [Homo sapiens]
                         gi|4506663   60S ribosomal protein L8 [Homo sapiens]
                         gi|100913206 ATP-dependent RNA helicase A [Homo sapiens]
                         gi|480318426 actin, partial [Cobanus sp. Panama]
                         gi|451889    avidin
                         gi|5031857   L-lactate dehydrogenase A chain isoform 1 [Homo sapiens]
                         gi|291395669 PREDICTED: histone cluster 1, H2bc-like [Oryctolagus cuniculus]
                         gi|31542947  60 kDa heat shock protein, mitochondrial [Homo sapiens]
                         gi|260790260 hypothetical protein BRAFLDRAFT_126062 [Branchiostoma floridae]
                         gi|23308577  D-3-phosphoglycerate dehydrogenase [Homo sapiens]
                         gi|15055539  40S ribosomal protein S2 [Homo sapiens]
                         gi|4557032   L-lactate dehydrogenase B chain [Homo sapiens]
                         gi|33438760  myosin heavy chain [Homo sapiens]
                         gi|36796     t-complex polypeptide 1 [Homo sapiens]
                         gi|7513316   ribosomal protein L14 - human
                         gi|337516    ribosomal protein s6 [Homo sapiens]
                         gi|344238180 Histone H2A type 1 [Cricetulus griseus]
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gi|4506597   60S ribosomal protein L12 [Homo sapiens]
gi|34228     unnamed protein product [Homo sapiens]
gi|55982450  actin [Leptosphaeria biglobosa 'canadensis' group]
gi|531171    Csa-19 [Homo sapiens]
gi|13592053  60S ribosomal protein L10 [Rattus norvegicus]
gi|704416    elongation factor Tu [Homo sapiens]
gi|4885375   histone H1.2 [Homo sapiens]
gi|730529    RecName: Full=60S ribosomal protein L13
gi|29383     BBC1 [Homo sapiens]
gi|465985766 60S ribosomal protein L3-like protein [Chelonia mydas]
gi|544482    aldehyde dehydrogenase 6 [Homo sapiens]
gi|4506619   60S ribosomal protein L24 [Homo sapiens]
gi|198643    ribosomal protein L19 [Mus musculus]
gi|28317     unnamed protein product [Homo sapiens]
gi|13606056  DNA dependent protein kinase catalytic subunit [Homo sapiens]
gi|431921648 Keratin, type II cytoskeletal 1 [Pteropus alecto]
gi|52741     J1 protein [Mus musculus]
gi|550019    ribosomal protein L28 [Homo sapiens]
gi|392601408 beta-tubulin, partial [Teleopsis dalmanni]
gi|57143     ribosomal protein S9 [Rattus norvegicus]
gi|375314779 keratin 1 [Homo sapiens]
gi|6755372   40S ribosomal protein S3 [Mus musculus]
gi|309319    heat shock protein 70 cognate [Mus musculus]
gi|4506661   60S ribosomal protein L7a [Homo sapiens]
gi|11415026  60S ribosomal protein L18a [Homo sapiens]
gi|292435    ribosomal protein L26 [Homo sapiens]
gi|189498    pyrroline-5-carboxylate reductase [Homo sapiens]
gi|431920762 60S ribosomal protein L13a [Pteropus alecto]
gi|181402    epidermal cytokeratin 2 [Homo sapiens]
gi|63333543  beta-tubulin, partial [Basidiobolus microsporus]
gi|6005860   60S ribosomal protein L35 [Homo sapiens]
gi|11321601  6-phosphofructokinase type C isoform 1 [Homo sapiens]
gi|404015    ribosomal protein L23a, partial [Homo sapiens]
gi|537140561 importin subunit beta-1 [Cricetulus griseus]
gi|4506753   ruvB-like 1 [Homo sapiens]
gi|4506685   40S ribosomal protein S13 [Homo sapiens]
gi|132983    RecName: Full=60S ribosomal protein L5-A
gi|662841    heat shock protein 27 [Homo sapiens]
gi|514749793 PREDICTED: actin-1-like isoform X1 [Setaria italica]
gi|347839    matricin [Mus musculus]
gi|1666234   actin [Pisum sativum]
gi|10863945  X-ray repair cross-complementing protein 5 [Homo sapiens]
gi|4506743   40S ribosomal protein S8 [Homo sapiens]
gi|194399199 beta-actin [Odocoileus virginianus]
gi|435476    cytokeratin 9 [Homo sapiens]
gi|323361175 beta-tubulin [Phytophthora sp. 1 EEO-2011]
gi|5031753   heterogeneous nuclear ribonucleoprotein H [Homo sapiens]
gi|198578    ribosomal protein [Mus musculus]
gi|156382552 predicted protein [Nematostella vectensis]
gi|2230878   hNop56 [Homo sapiens]
gi|1263008   aldehyde dehydrogenase [Homo sapiens]
gi|494066    Chain A, Three-Dimensional Structure Of Class Pi Glutathione S-Transferase From Human Placenta In Complex With S-Hexylglutathione At 2.8 Angstroms Resolution
gi|30311     cytokeratin 18 (424 AA) [Homo sapiens]
gi|984249    protein kinase [Sus scrofa]
gi|392341350 PREDICTED: glyceraldehyde-3-phosphate dehydrogenase-like [Rattus norvegicus]
gi|31092     unnamed protein product [Homo sapiens]
gi|187281    M4 protein [Homo sapiens]
gi|115206    RecName: Full=C-1-tetrahydrofolate synthase, cytoplasmic; Short=C1-THF synthase; Includes: RecName: Full=Methylenetetrahydrofolate dehydrogenase; Includes: RecName:
gi|2865466   heat shock protein 75 [Homo sapiens]
gi|1002369   coatomer protein [Homo sapiens]
gi|703093    serine hydroxymethyltransferase, partial [Homo sapiens]
gi|1244508   assembly protein 50 [Homo sapiens]
gi|50815     unnamed protein product [Mus musculus]
gi|755746    p85Mcm protein [Homo sapiens]
gi|162811    kappa-casein precursor [Bos taurus]
gi|4506617   60S ribosomal protein L17 isoform a [Homo sapiens]
gi|225632    casein alphaS1
gi|603225    p67 [Homo sapiens]
gi|414587    ribosomal protein L10 [Homo sapiens]
gi|15146358  glyceraldehyde 3-phosphate dehydrogenase [Pagrus major]
gi|4506635   60S ribosomal protein L32 [Homo sapiens]
gi|225008742 AtpD [Rhizobium sp. MITP02]
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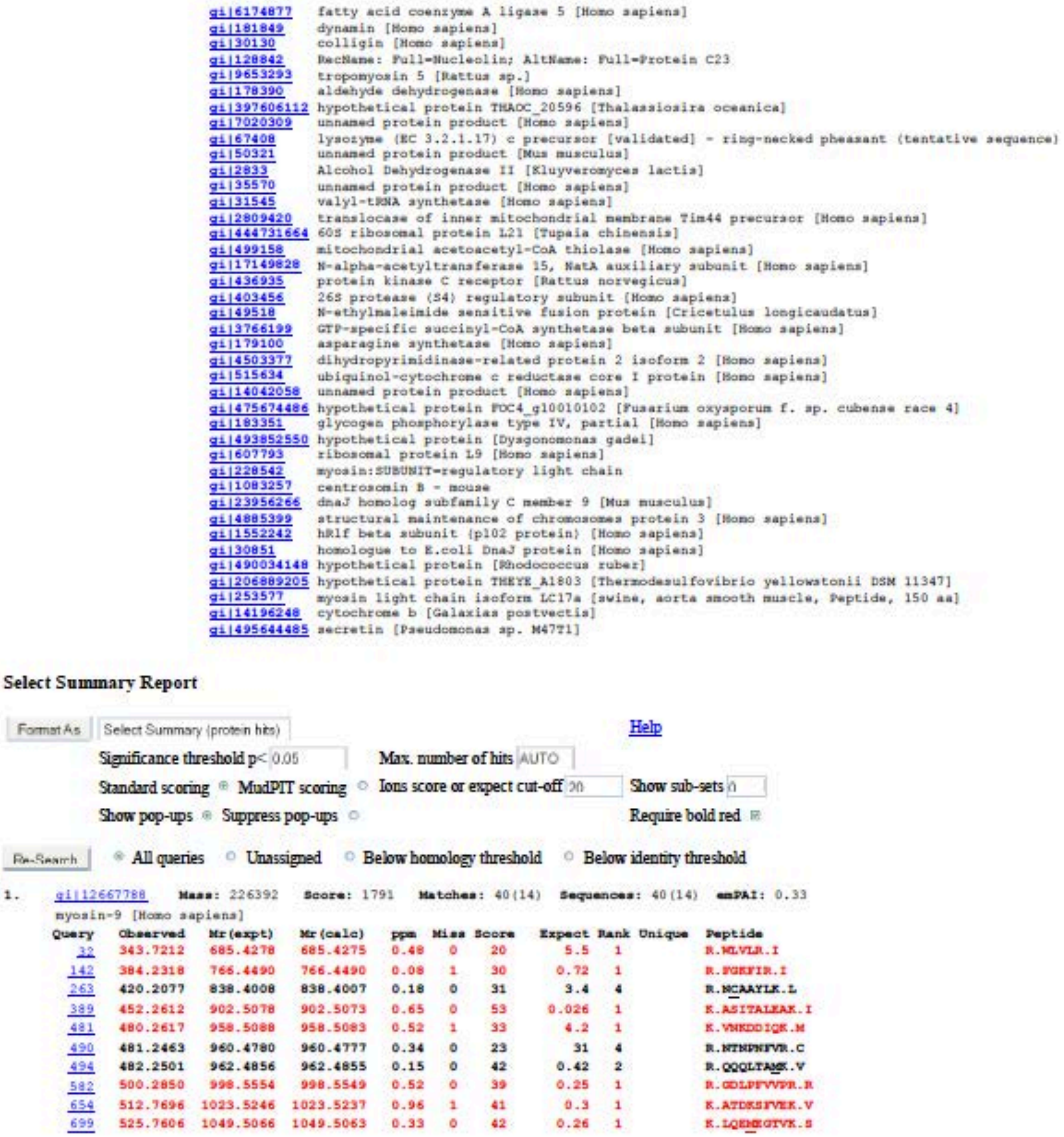
gi|468860736	heat shock protein 90 [Cellana toreuma]
gi|337518	ribosomal protein, partial [Homo sapiens]
gi|306875	C protein [Homo sapiens]
gi|34234	laminin-binding protein [Homo sapiens]
gi|619788	L21 ribosomal protein [Homo sapiens]
gi|5802974	thioredoxin-dependent peroxide reductase, mitochondrial isoform a precursor [Homo sapiens]
gi|445615	ribosomal protein L27
gi|1911441	DAM6 protein [human, testis, Peptide, 319 aa]
gi|136429	RecName: Full=Trypsin; Flags: Precursor
gi|351715766	Heat shock cognate 71 kDa protein [Heterocephalus glaber]
gi|28935	ATP-citrate (pro-S-)-lyase [Homo sapiens]
gi|1706000	RecName: Full=Coatomer subunit gamma-1; AltName: Full=Gamma-1-coat protein; Short=Gamma-1-COP
gi|116222251	beta tubulin, partial [Leucocryptos marina]
gi|4929561	CGI-46 protein [Homo sapiens]
gi|307066	inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205) [Homo sapiens]
gi|4826760	heterogeneous nuclear ribonucleoprotein F [Homo sapiens]
gi|2500241	RecName: Full=60S ribosomal protein L11
gi|86610100	F0F1 ATP synthase subunit alpha [Synechococcus sp. JA-2-3B'a(2-13)]
gi|399219045	unnamed protein product [Babesia microti strain RI]
gi|4503841	X-ray repair cross-complementing protein 6 [Homo sapiens]
gi|528763911	Clathrin heavy chain 1 (CLH-17) isoform 5-like protein [Camelus ferus]
gi|4506691	40S ribosomal protein S16 [Homo sapiens]
gi|5174449	histone H1x [Homo sapiens]
gi|443925834	dynein heavy chain [Rhizoctonia solani AG-1 IA]
gi|7243085	KIAA1352 protein [Homo sapiens]
gi|299029	pre-mRNA binding K protein, hnRNP K [Xenopus laevis, Peptide, 396 aa]
gi|34039	unnamed protein product [Homo sapiens]
gi|262391	Rig homolog [human, brain, Peptide Partial, 135 aa]
gi|1655596	ribosomal protein L31 [Homo sapiens]
gi|67969044	unnamed protein product [Macaca fascicularis]
gi|195350828	GM11458 [Drosophila sechellia]
gi|730633	RecName: Full=40S ribosomal protein S14
gi|5730027	KH domain-containing, RNA-binding, signal transduction-associated protein 1 isoform 1 [Homo sapiens]
gi|1232079	huMCM5 [Homo sapiens]
gi|940536	P1 Cdc21 protein [Homo sapiens]
gi|2580550	dead box, X isoform [Homo sapiens]
gi|2914385	Chain C, Human Pcna
gi|4506707	40S ribosomal protein S25 [Homo sapiens]
gi|7705855	estradiol 17-beta-dehydrogenase 12 [Homo sapiens]
gi|1228049	multifunctional protein CAD [Homo sapiens]
gi|127276	RecName: Full=Phosphate carrier protein, mitochondrial; AltName: Full=Phosphate transport protein; Short=PTP; AltName: Full=Solute carrier family 25 member 3; Flags:
gi|541040124	tubulin beta-1 chain [Ascaris suum]
gi|433888	coproporphyrinogen oxidase [Homo sapiens]
gi|5911903	hypothetical protein [Homo sapiens]
gi|189754	pyruvate dehydrogenase beta subunit [Homo sapiens]
gi|133972	RecName: Full=40S ribosomal protein S5; Contains: RecName: Full=40S ribosomal protein S5, N-terminally processed
gi|4506901	serine/arginine-rich splicing factor 3 [Homo sapiens]
gi|3088342	ribosomal protein S23 [Homo sapiens]
gi|220618	DNA topoisomerase I [Mus musculus]
gi|2258465	succinyl-CoA synthetase alpha subunit [Sus scrofa]
gi|395828418	PREDICTED: thioredoxin-dependent peroxide reductase, mitochondrial [Otolemur garnettii]
gi|1217668	arginyl-tRNA synthetase [Homo sapiens]
gi|302683018	hypothetical protein SCHCODRAFT_82385 [Schizophyllum commune H4-8]
gi|1182009	NAD+-isocitrate dehydrogenase [Macaca fascicularis]
gi|7959733	PRO0992 [Homo sapiens]
gi|793843	ribosomal protein L29 [Homo sapiens]
gi|1304314	pyrroline 5-carboxylate synthetase [Homo sapiens]
gi|5453832	hypoxia up-regulated protein 1 precursor [Homo sapiens]
gi|171692263	hypothetical protein [Podospora anserina S mat+]
gi|387100	Tum-P91A antigen, partial [Mus musculus]
gi|56676330	heterochromatin protein 1-binding protein 3 [Homo sapiens]
gi|51263	p68 RNA helicase [Mus musculus]
gi|76257839	beta-tubulin [Pterosperma cristatum]
gi|403491	glycinamide ribonucleotide synthetase [Mus musculus]
gi|123976082	hypothetical protein [Trichomonas vaginalis G3]
gi|4507353	TATA-binding protein-associated factor 2N isoform 2 [Homo sapiens]
gi|336262795	hypothetical protein SMAC_06647 [Sordaria macrospora k-hell]
gi|4506491	replication factor C subunit 4 [Homo sapiens]
gi|50360	unnamed protein product [Mus musculus]
gi|4506703	40S ribosomal protein S24 isoform c [Homo sapiens]
gi|180928	core protein II precursor [Homo sapiens]
gi|1403050	phosphoenolpyruvate carboxykinase (GTP) [Homo sapiens]



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gi|6174877   fatty acid coenzyme A ligase 5 [Homo sapiens]
gi|181849    dynamin [Homo sapiens]
gi|30130     colligin [Homo sapiens]
gi|128842    RecName: Full=Nucleolin; AltName: Full=Protein C23
gi|9653293   tropomyosin 5 [Rattus sp.]
gi|178390    aldehyde dehydrogenase [Homo sapiens]
gi|397606112 hypothetical protein THAOC_20596 [Thalassiosira oceanica]
gi|7020309   unnamed protein product [Homo sapiens]
gi|67408     lysozyme (EC 3.2.1.17) c precursor [validated] - ring-necked pheasant (tentative sequence)
gi|50321     unnamed protein product [Mus musculus]
gi|2833      Alcohol Dehydrogenase II [Kluyveromyces lactis]
gi|35570     unnamed protein product [Homo sapiens]
gi|31545     valyl-tRNA synthetase [Homo sapiens]
gi|2809420   translocase of inner mitochondrial membrane Tim44 precursor [Homo sapiens]
gi|444731664 60S ribosomal protein L21 [Tupaia chinensis]
gi|499158    mitochondrial acetoacetyl-CoA thiolase [Homo sapiens]
gi|17149828  N-alpha-acetyltransferase 15, NatA auxiliary subunit [Homo sapiens]
gi|436935    protein kinase C receptor [Rattus norvegicus]
gi|403456    26S protease (S4) regulatory subunit [Homo sapiens]
gi|49518     N-ethylmaleimide sensitive fusion protein [Cricetulus longicaudatus]
gi|3766199   GTP-specific succinyl-CoA synthetase beta subunit [Homo sapiens]
gi|179100    asparagine synthetase [Homo sapiens]
gi|4503377   dihydropyrimidinase-related protein 2 isoform 2 [Homo sapiens]
gi|515634    ubiquinol-cytochrome c reductase core I protein [Homo sapiens]
gi|14042058  unnamed protein product [Homo sapiens]
gi|475674486 hypothetical protein FOC4_g10010102 [Fusarium oxysporum f. sp. cubense race 4]
gi|183351    glycogen phosphorylase type IV, partial [Homo sapiens]
gi|493852550 hypothetical protein [Dysgonomonas gadei]
gi|607793    ribosomal protein L9 [Homo sapiens]
gi|228542    myosin:SUBUNIT=regulatory light chain
gi|1083257   centrosomin B - mouse
gi|23956266  dnaJ homolog subfamily C member 9 [Mus musculus]
gi|4885399   structural maintenance of chromosomes protein 3 [Homo sapiens]
gi|1552242   hRlf beta subunit (p102 protein) [Homo sapiens]
gi|30851     homologue to E.coli DnaJ protein [Homo sapiens]
gi|490034148 hypothetical protein [Rhodococcus ruber]
gi|206889205 hypothetical protein THEYE_A1803 [Thermodesulfovibrio yellowstonii DSM 11347]
gi|253577    myosin light chain isoform LC17a [swine, aorta smooth muscle, Peptide, 150 aa]
gi|14196248  cytochrome b [Galaxias postvectis]
gi|495644485 secretin [Pseudomonas sp. M47T1]
```

Select Summary Report

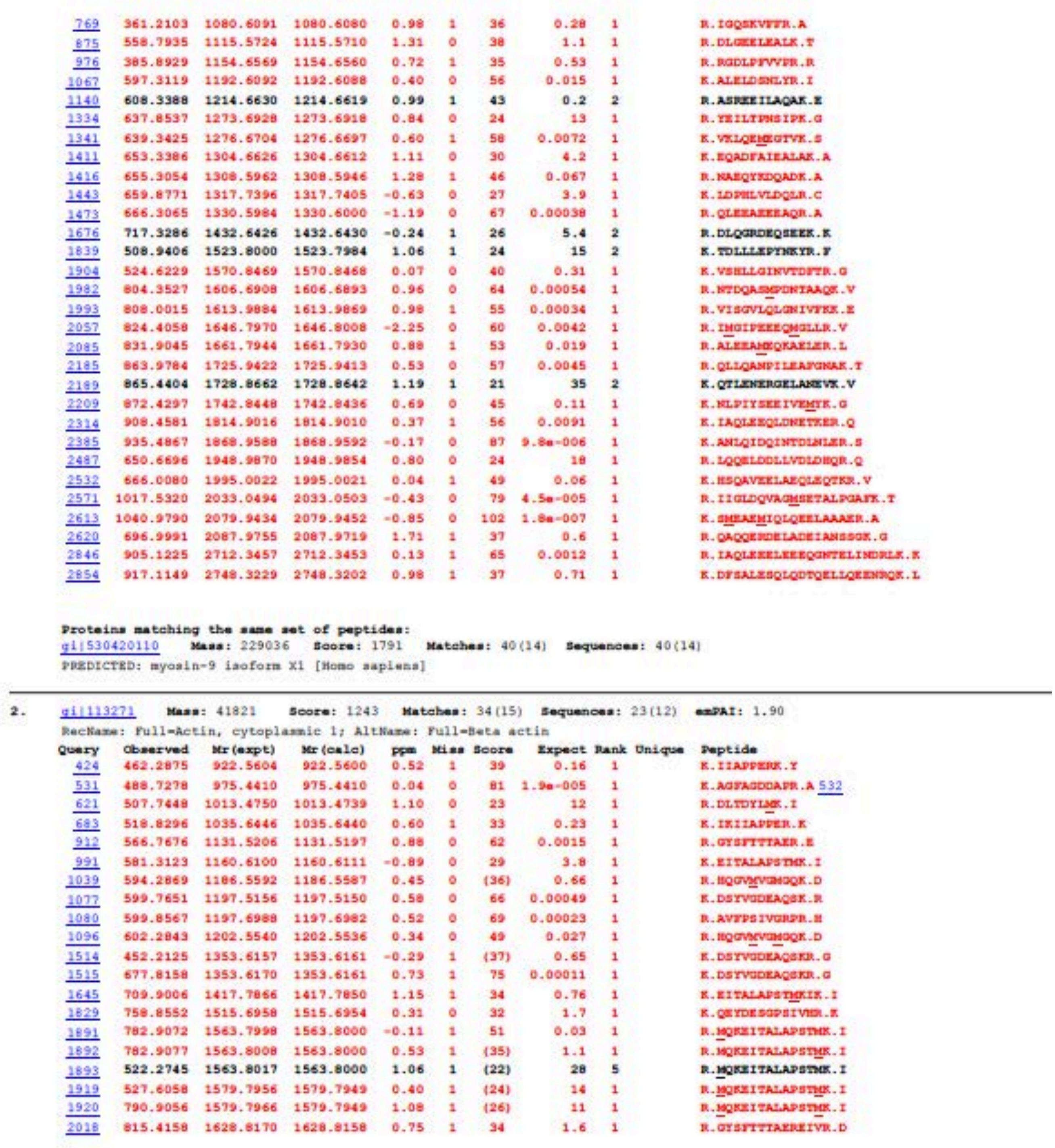
Significance threshold p< 0.05 Max. number of hits AUTO

Standard scoring ● MudPIT scoring ○ Ions score or expect cut-off 20 Show sub-sets 0

Show pop-ups ● Suppress pop-ups ○ Require bold red ☑

● All queries ○ Unassigned ○ Below homology threshold ○ Below identity threshold

```
1.    gi|12667788     Mass: 226392   Score: 1791   Matches: 40(14)   Sequences: 40(14)   emPAI: 0.33
      myosin-9 [Homo sapiens]
      Query   Observed    Mr(expt)    Mr(calc)    ppm  Miss Score   Expect Rank Unique   Peptide
         32   343.7212    685.4278    685.4275   0.48    0    20      5.5    1          R.MLVLR.I
        142   384.2318    766.4490    766.4490   0.08    1    30     0.72    1          R.FGKFIR.I
        263   420.2077    838.4008    838.4007   0.18    0    31      3.4    4          R.NCAAYLK.L
        389   452.2612    902.5078    902.5073   0.65    0    53    0.026    1          K.ASITALEAK.I
        481   480.2617    958.5088    958.5083   0.52    1    33      4.2    1          K.VNKDDIQK.M
        490   481.2463    960.4780    960.4777   0.34    0    23       31    4          R.NTNPNFVR.C
        494   482.2501    962.4856    962.4855   0.15    0    42     0.42    2          R.QQQLTAMK.V
        582   500.2850    998.5554    998.5549   0.52    0    39     0.25    1          R.GDLPFVVPR.R
        654   512.7696   1023.5246   1023.5237   0.96    1    41      0.3    1          K.ATDKSFVEK.V
        699   525.7606   1049.5066   1049.5063   0.33    0    42     0.26    1          K.LQEMEGTVK.S
```



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   769   361.2103  1080.6091  1080.6080   0.98   1    36      0.28   1          R.IGQSKVFFR.A
   875   558.7935  1115.5724  1115.5710   1.31   0    38       1.1   1          R.DLGEELEALK.T
   976   385.8929  1154.6569  1154.6560   0.72   1    35      0.53   1          R.RGDLPFVVPR.R
  1067   597.3119  1192.6092  1192.6088   0.40   0    56     0.015   1          K.ALELDSNLYR.I
  1140   608.3388  1214.6630  1214.6619   0.99   1    43       0.2   2          R.ASREEILAQAK.E
  1334   637.8537  1273.6928  1273.6918   0.84   0    24        13   1          R.YEILTPNSIPK.G
  1341   639.3425  1276.6704  1276.6697   0.60   1    58    0.0072   1          K.VKLQEMEGTVK.S
  1411   653.3386  1304.6626  1304.6612   1.11   0    30       4.2   1          K.EQADFAIEALAK.A
  1416   655.3054  1308.5962  1308.5946   1.28   1    46     0.067   1          R.NAEQYKDQADK.A
  1443   659.8771  1317.7396  1317.7405  -0.63   0    27       3.9   1          K.LDPHLVLDQLR.C
  1473   666.3065  1330.5984  1330.6000  -1.19   0    67   0.00038   1          R.QLEEAEEEAQR.A
  1676   717.3286  1432.6426  1432.6430  -0.24   1    26       5.4   2          R.DLQGRDEQSEEK.K
  1839   508.9406  1523.8000  1523.7984   1.06   1    24        15   2          K.TDLLLEPYNKYR.F
  1904   524.6229  1570.8469  1570.8468   0.07   0    40      0.31   1          K.VSHLLGINVTDFTR.G
  1982   804.3527  1606.6908  1606.6893   0.96   0    64   0.00054   1          R.NTDQASMPDNTAAQK.V
  1993   808.0015  1613.9884  1613.9869   0.98   1    55   0.00034   1          R.VISGVLQLGNIVFKK.E
  2057   824.4058  1646.7970  1646.8008  -2.25   0    60    0.0042   1          R.IMGIPEEEQMGLLR.V
  2085   831.9045  1661.7944  1661.7930   0.88   1    53     0.019   1          R.ALEEAMEQKAELER.L
  2185   863.9784  1725.9422  1725.9413   0.53   0    57    0.0045   1          R.QLLQANPILEAFGNAK.T
  2189   865.4404  1728.8662  1728.8642   1.19   1    21        35   2          K.QTLENERGELANEVK.V
  2209   872.4297  1742.8448  1742.8436   0.69   0    45      0.11   1          K.NLPIYSEEIVEMYK.G
  2314   908.4581  1814.9016  1814.9010   0.37   1    56    0.0091   1          K.IAQLEEQLDNETKER.Q
  2385   935.4867  1868.9588  1868.9592  -0.17   0    87  9.8e-006   1          K.ANLQIDQINTDLNLER.S
  2487   650.6696  1948.9870  1948.9854   0.80   0    24        18   1          R.LQQELDDLLVDLDHQR.Q
  2532   666.0080  1995.0022  1995.0021   0.04   1    49      0.06   1          K.HSQAVEELAEQLEQTKR.V
  2571  1017.5320  2033.0494  2033.0503  -0.43   0    79  4.5e-005   1          R.IIGLDQVAGMSETALPGAFK.T
  2613  1040.9790  2079.9434  2079.9452  -0.85   0   102  1.8e-007   1          K.SMEAEMIQLQEELAAAER.A
  2620   696.9991  2087.9755  2087.9719   1.71   1    37       0.6   1          R.QAQQERDELADEIANSSGK.G
  2846   905.1225  2712.3457  2712.3453   0.13   1    65    0.0012   1          R.IAQLEEELEEEQGNTELINDRLK.K
  2854   917.1149  2748.3229  2748.3202   0.98   1    37      0.71   1          K.DFSALESQLQDTQELLQEENRQK.L

Proteins matching the same set of peptides:
gi|530420110     Mass: 229036   Score: 1791   Matches: 40(14)   Sequences: 40(14)
PREDICTED: myosin-9 isoform X1 [Homo sapiens]
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2.    gi|113271     Mass: 41821     Score: 1243    Matches: 34(15)   Sequences: 23(12)   emPAI: 1.90
      RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta actin
     Query   Observed   Mr(expt)   Mr(calc)     ppm  Miss Score    Expect Rank Unique  Peptide
       424   462.2875   922.5604   922.5600    0.52   1    39      0.16   1          K.IIAPPERK.Y
       531   488.7278   975.4410   975.4410    0.04   0    81  1.9e-005   1          K.AGFAGDDAPR.A 532
       621   507.7448  1013.4750  1013.4739    1.10   0    23        12   1          R.DLTDYLMK.I
       683   518.8296  1035.6446  1035.6440    0.60   1    33      0.23   1          K.IKIIAPPER.K
       912   566.7676  1131.5206  1131.5197    0.88   0    62    0.0015   1          R.GYSFTTTAER.E
       991   581.3123  1160.6100  1160.6111   -0.89   0    29       3.8   1          K.EITALAPSTMK.I
      1039   594.2869  1186.5592  1186.5587    0.45   0  (36)      0.66   1          R.HQGVMVGMGQK.D
      1077   599.7651  1197.5156  1197.5150    0.58   0    66   0.00049   1          K.DSYVGDEAQSK.R
      1080   599.8567  1197.6988  1197.6982    0.52   0    69   0.00023   1          R.AVFPSIVGRPR.H
      1096   602.2843  1202.5540  1202.5536    0.34   0    49     0.027   1          R.HQGVMVGMGQK.D
      1514   452.2125  1353.6157  1353.6161   -0.29   1  (37)      0.65   1          K.DSYVGDEAQSKR.G
      1515   677.8158  1353.6170  1353.6161    0.73   1    75   0.00011   1          K.DSYVGDEAQSKR.G
      1645   709.9006  1417.7866  1417.7850    1.15   1    34      0.76   1          K.EITALAPSTMKIK.I
      1829   758.8552  1515.6958  1515.6954    0.31   0    32       1.7   1          K.QEYDESGPSIVHR.K
      1891   782.9072  1563.7998  1563.8000   -0.11   1    51      0.03   1          R.MQKEITALAPSTMK.I
      1892   782.9077  1563.8008  1563.8000    0.53   1  (35)       1.1   1          R.MQKEITALAPSTMK.I
      1893   522.2745  1563.8017  1563.8000    1.06   1  (22)        28   5          R.MQKEITALAPSTMK.I
      1919   527.6058  1579.7956  1579.7949    0.40   1  (24)        14   1          R.MQKEITALAPSTMK.I
      1920   790.9056  1579.7966  1579.7949    1.08   1  (26)        11   1          R.MQKEITALAPSTMK.I
      2018   815.4158  1628.8170  1628.8158    0.75   1    34       1.6   1          R.GYSFTTTAEREIVR.D
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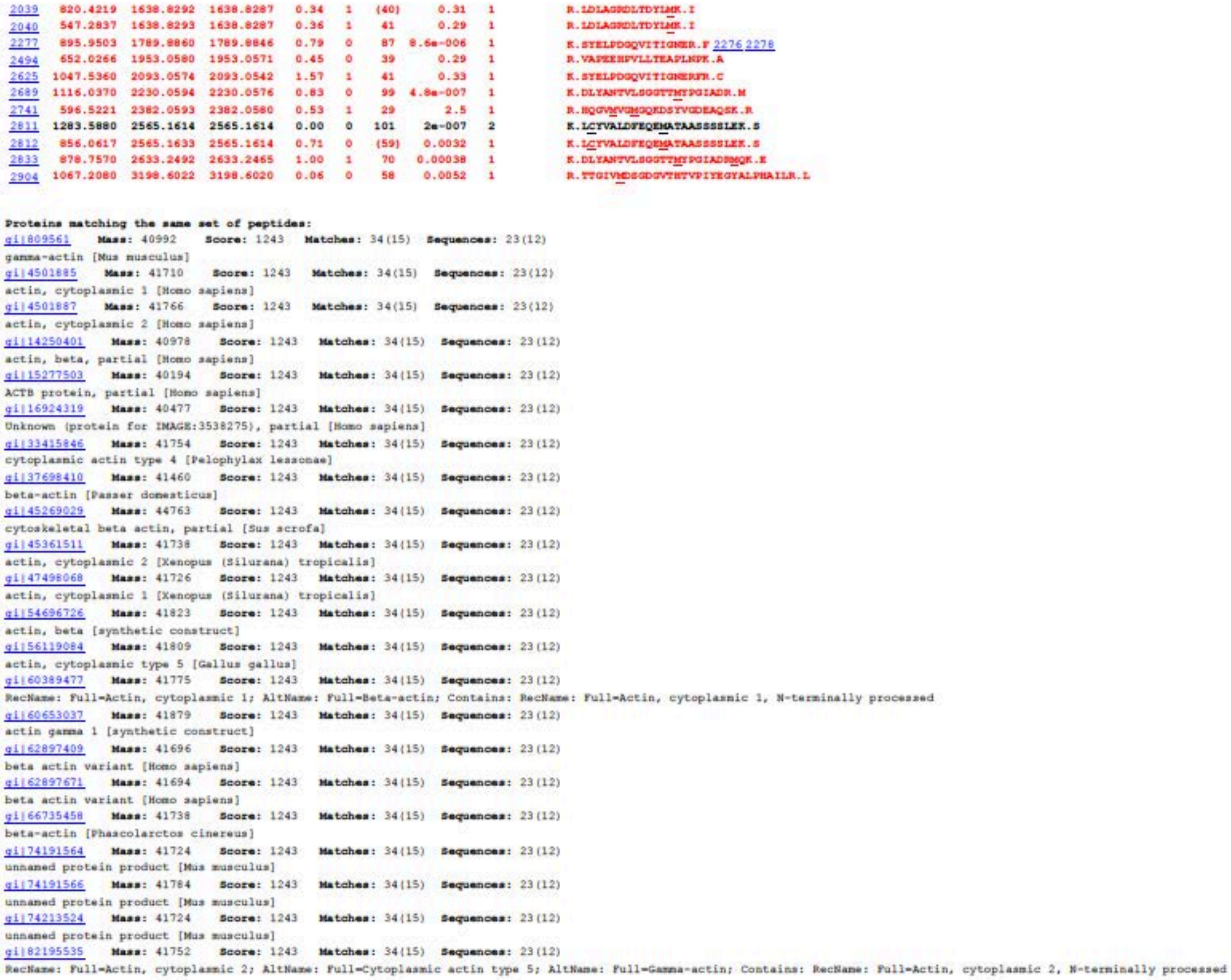

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2039   820.4219  1638.8292  1638.8287  0.34  1  (40)      0.31  1     R.LDLAGRDLTDYLMK.I
2040   547.2837  1638.8293  1638.8287  0.36  1   41       0.29  1     R.LDLAGRDLTDYLMK.I
2277   895.9503  1789.8860  1789.8846  0.79  0   87   8.6e-006  1     K.SYELPDGQVITIGNER.F 2276 2278
2494   652.0266  1953.0580  1953.0571  0.45  0   39       0.29  1     R.VAPEEHPVLLTEAPLNPK.A
2625  1047.5360  2093.0574  2093.0542  1.57  1   41       0.33  1     K.SYELPDGQVITIGNERFR.C
2689  1116.0370  2230.0594  2230.0576  0.83  0   99   4.8e-007  1     K.DLYANTVLSGGTTMYPGIADR.M
2741   596.5221  2382.0593  2382.0580  0.53  1   29        2.5  1     R.HQGVMVGMGQKDSYVGDEAQSK.R
2811  1283.5880  2565.1614  2565.1614  0.00  0  101     2e-007  2     K.LCYVALDFEQEMATAASSSSLEK.S
2812   856.0617  2565.1633  2565.1614  0.71  0  (59)    0.0032  1     K.LCYVALDFEQEMATAASSSSLEK.S
2833   878.7570  2633.2492  2633.2465  1.00  1   70    0.00038  1     K.DLYANTVLSGGTTMYPGIADRMQK.E
2904  1067.2080  3198.6022  3198.6020  0.06  0   58     0.0052  1     R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L

Proteins matching the same set of peptides:
gi|809561     Mass: 40992    Score: 1243   Matches: 34(15)  Sequences: 23(12)
gamma-actin [Mus musculus]
gi|4501885     Mass: 41710    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, cytoplasmic 1 [Homo sapiens]
gi|4501887     Mass: 41766    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, cytoplasmic 2 [Homo sapiens]
gi|14250401     Mass: 40978    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, beta, partial [Homo sapiens]
gi|15277503     Mass: 40194    Score: 1243   Matches: 34(15)  Sequences: 23(12)
ACTB protein, partial [Homo sapiens]
gi|16924319     Mass: 40477    Score: 1243   Matches: 34(15)  Sequences: 23(12)
Unknown (protein for IMAGE:3538275), partial [Homo sapiens]
gi|33415846     Mass: 41754    Score: 1243   Matches: 34(15)  Sequences: 23(12)
cytoplasmic actin type 4 [Pelophylax lessonae]
gi|37698410     Mass: 41460    Score: 1243   Matches: 34(15)  Sequences: 23(12)
beta-actin [Passer domesticus]
gi|45269029     Mass: 44763    Score: 1243   Matches: 34(15)  Sequences: 23(12)
cytoskeletal beta actin, partial [Sus scrofa]
gi|45361511     Mass: 41738    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, cytoplasmic 2 [Xenopus (Silurana) tropicalis]
gi|47498068     Mass: 41726    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, cytoplasmic 1 [Xenopus (Silurana) tropicalis]
gi|54696726     Mass: 41823    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, beta [synthetic construct]
gi|56119084     Mass: 41809    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin, cytoplasmic type 5 [Gallus gallus]
gi|60389477     Mass: 41775    Score: 1243   Matches: 34(15)  Sequences: 23(12)
RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-actin; Contains: RecName: Full=Actin, cytoplasmic 1, N-terminally processed
gi|60653037     Mass: 41879    Score: 1243   Matches: 34(15)  Sequences: 23(12)
actin gamma 1 [synthetic construct]
gi|62897409     Mass: 41696    Score: 1243   Matches: 34(15)  Sequences: 23(12)
beta actin variant [Homo sapiens]
gi|62897671     Mass: 41694    Score: 1243   Matches: 34(15)  Sequences: 23(12)
beta actin variant [Homo sapiens]
gi|66735458     Mass: 41738    Score: 1243   Matches: 34(15)  Sequences: 23(12)
beta-actin [Phascolarctos cinereus]
gi|74191564     Mass: 41724    Score: 1243   Matches: 34(15)  Sequences: 23(12)
unnamed protein product [Mus musculus]
gi|74191566     Mass: 41784    Score: 1243   Matches: 34(15)  Sequences: 23(12)
unnamed protein product [Mus musculus]
gi|74213524     Mass: 41724    Score: 1243   Matches: 34(15)  Sequences: 23(12)
unnamed protein product [Mus musculus]
gi|82195535     Mass: 41752    Score: 1243   Matches: 34(15)  Sequences: 23(12)
RecName: Full=Actin, cytoplasmic 2; AltName: Full=Cytoplasmic actin type 5; AltName: Full=Gamma-actin; Contains: RecName: Full=Actin, cytoplasmic 2, N-terminally processed
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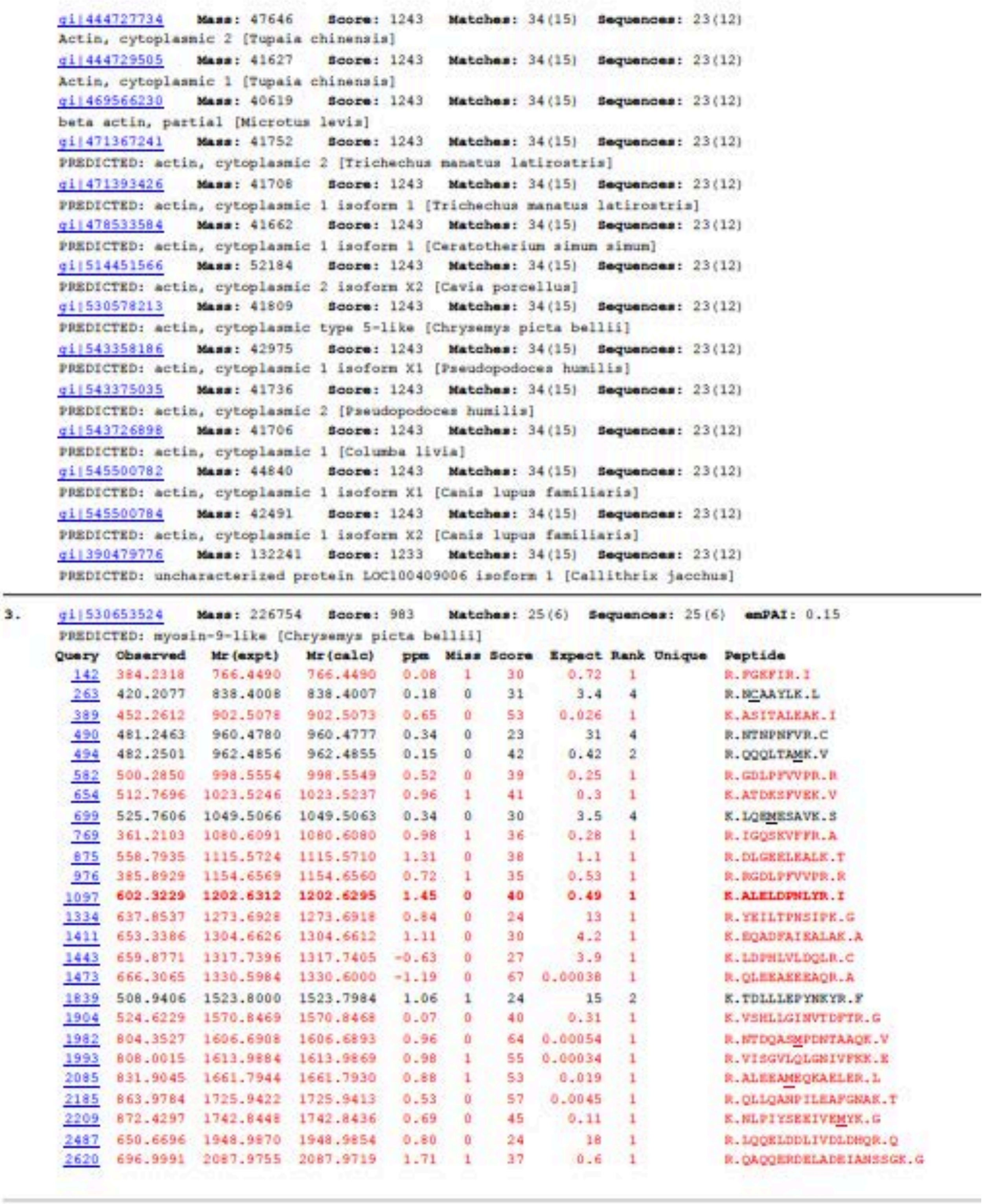

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gi|444727734      Mass: 47646     Score: 1243    Matches: 34(15)   Sequences: 23(12)
Actin, cytoplasmic 2 [Tupaia chinensis]
gi|444729505      Mass: 41627     Score: 1243    Matches: 34(15)   Sequences: 23(12)
Actin, cytoplasmic 1 [Tupaia chinensis]
gi|469566230      Mass: 40619     Score: 1243    Matches: 34(15)   Sequences: 23(12)
beta actin, partial [Microtus levis]
gi|471367241      Mass: 41752     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2 [Trichechus manatus latirostris]
gi|471393426      Mass: 41708     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform 1 [Trichechus manatus latirostris]
gi|478533584      Mass: 41662     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform 1 [Ceratotherium simum simum]
gi|514451566      Mass: 52184     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2 isoform X2 [Cavia porcellus]
gi|530578213      Mass: 41809     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic type 5-like [Chrysemys picta bellii]
gi|543358186      Mass: 42975     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform X1 [Pseudopodoces humilis]
gi|543375035      Mass: 41736     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2 [Pseudopodoces humilis]
gi|543726898      Mass: 41706     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 [Columba livia]
gi|545500782      Mass: 44840     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform X1 [Canis lupus familiaris]
gi|545500784      Mass: 42491     Score: 1243    Matches: 34(15)   Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform X2 [Canis lupus familiaris]
gi|390479776      Mass: 132241    Score: 1233    Matches: 34(15)   Sequences: 23(12)
PREDICTED: uncharacterized protein LOC100409006 isoform 1 [Callithrix jacchus]
```

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3.    gi|530653524      Mass: 226754    Score: 983     Matches: 25(6)   Sequences: 25(6)   emPAI: 0.15
      PREDICTED: myosin-9-like [Chrysemys picta bellii]
     Query  Observed   Mr(expt)   Mr(calc)    ppm  Miss Score   Expect Rank Unique   Peptide
       142  384.2318   766.4490   766.4490   0.08    1    30     0.72    1          R.FGKFIR.I
       263  420.2077   838.4008   838.4007   0.18    0    31      3.4    4          R.NCAAYLK.L
       389  452.2612   902.5078   902.5073   0.65    0    53    0.026    1          K.ASITALEAK.I
       490  481.2463   960.4780   960.4777   0.34    0    23       31    4          R.NTNPNFVR.C
       494  482.2501   962.4856   962.4855   0.15    0    42     0.42    2          R.QQQLTAMK.V
       582  500.2850   998.5554   998.5549   0.52    0    39     0.25    1          R.GDLPFVVPR.R
       654  512.7696  1023.5246  1023.5237   0.96    1    41      0.3    1          K.ATDKSFVEK.V
       699  525.7606  1049.5066  1049.5063   0.34    0    30      3.5    4          K.LQEMESAVK.S
       769  361.2103  1080.6091  1080.6080   0.98    1    36     0.28    1          R.IGQSKVFFR.A
       875  558.7935  1115.5724  1115.5710   1.31    0    38      1.1    1          R.DLGEELEALK.T
       976  385.8929  1154.6569  1154.6560   0.72    1    35     0.53    1          R.RGDLPFVVPR.R
      1097  602.3229  1202.6312  1202.6295   1.45    0    40     0.49    1          K.ALELDPNLYR.I
      1334  637.8537  1273.6928  1273.6918   0.84    0    24       13    1          R.YEILTPNSIPK.G
      1411  653.3386  1304.6626  1304.6612   1.11    0    30      4.2    1          K.EQADFAIEALAK.A
      1443  659.8771  1317.7396  1317.7405  -0.63    0    27      3.9    1          K.LDPHLVLDQLR.C
      1473  666.3065  1330.5984  1330.6000  -1.19    0    67  0.00038    1          R.QLEEAEEEAQR.A
      1839  508.9406  1523.8000  1523.7984   1.06    1    24       15    2          K.TDLLLEPYNKYR.F
      1904  524.6229  1570.8469  1570.8468   0.07    0    40     0.31    1          K.VSHLLGINVTDFTR.G
      1982  804.3527  1606.6908  1606.6893   0.96    0    64  0.00054    1          R.NTDQASMPDNTAAQK.V
      1993  808.0015  1613.9884  1613.9869   0.98    1    55  0.00034    1          R.VISGVLQLGNIVFKK.E
      2085  831.9045  1661.7944  1661.7930   0.88    1    53    0.019    1          R.ALEEAMEQKAELER.L
      2185  863.9784  1725.9422  1725.9413   0.53    0    57   0.0045    1          R.QLLQANPILEAFGNAK.T
      2209  872.4297  1742.8448  1742.8436   0.69    0    45     0.11    1          K.NLPIYSEEIVEMYK.G
      2487  650.6696  1948.9870  1948.9854   0.80    0    24       18    1          R.LQQELDDLIVDLDHQR.Q
      2620  696.9991  2087.9755  2087.9719   1.71    1    37      0.6    1          R.QAQQERDELADEIANSSGK.G
```

4. gi|5174735 **Mass:** 49799 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9) **emPAI:** 1.16

tubulin beta-4B chain [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
665	514.7631	1027.5116	1027.5121	-0.41	0	42	0.17	1		K.TAVCDIPPR.G 664 666
684	520.3006	1038.5866	1038.5862	0.42	0	63	0.00061	1		R.YLTVAAVFR.G
942	572.3214	1142.6282	1142.6270	1.07	0	(36)	1.2	1		K.LAVNMVPFPR.L
988	580.3186	1158.6226	1158.6219	0.60	0	62	0.0031	1		K.LAVNMVPFPR.L
1265	626.8622	1251.7098	1251.7088	0.85	1	25	3.7	1		R.YLTVAAVFRGR.M
1467	664.8286	1327.6426	1327.6408	1.39	0	66	0.00073	1		R.INVYYNEATGGK.Y
1734	731.8458	1461.6770	1461.6769	0.07	0	71	0.00021	1		K.EVDEQMLNVQNK.N
1997	809.4114	1616.8082	1616.8080	0.16	0	41	0.34	1		R.AVLVDLEPGTMDSVR.S
2000	540.9503	1619.8291	1619.8283	0.50	0	(28)	7.8	1		R.LHFFMPGFAPLTSR.G
2001	810.9221	1619.8296	1619.8283	0.86	0	38	0.65	1		R.LHFFMPGFAPLTSR.G
2029	546.2820	1635.8242	1635.8232	0.61	0	(34)	2.2	1		R.LHFFMPGFAPLTSR.G
2030	818.9205	1635.8264	1635.8232	2.00	0	(29)	5.3	1		R.LHFFMPGFAPLTSR.G
2133	846.4379	1690.8612	1690.8600	0.74	0	37	0.84	1		R.ALTVPELTQQMFDAK.N
2144	848.9205	1695.8264	1695.8257	0.47	0	46	0.12	1		K.NSSYFVEWIPNNVK.T
2395	937.4710	1872.9274	1872.9291	-0.90	0	73	0.00018	3		K.MSATFIGNSTAIQELFK.R
2500	653.6655	1957.9747	1957.9745	0.07	0	(33)	2.2	1		K.GHYTEGAELVDSVLDVVR.K
2501	979.9952	1957.9758	1957.9745	0.67	0	102	3e-007	1		K.GHYTEGAELVDSVLDVVR.K
2513	657.9659	1970.8759	1970.8747	0.58	1	54	0.0079	1		R.MSMKEVDEQMLNVQNK.N
2551	672.0196	2013.0370	2013.0353	0.82	1	(23)	16	5		K.MSATFIGNSTAIQELFKR.I
2566	1015.5220	2029.0294	2029.0302	-0.39	1	91	2.9e-006	1		K.MSATFIGNSTAIQELFKR.I
2617	696.3635	2086.0687	2086.0695	-0.39	1	33	1.9	1		K.GHYTEGAELVDSVLDVVRK.E
2859	929.4256	2785.2550	2785.2543	0.23	1	22	13	3		R.ALTVPELTQQMFDAKNMMAACDPR.H
2912	1109.8470	3326.5192	3326.5218	-0.78	0	75	6.4e-005	1		K.EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK.I

Proteins matching the same set of peptides:

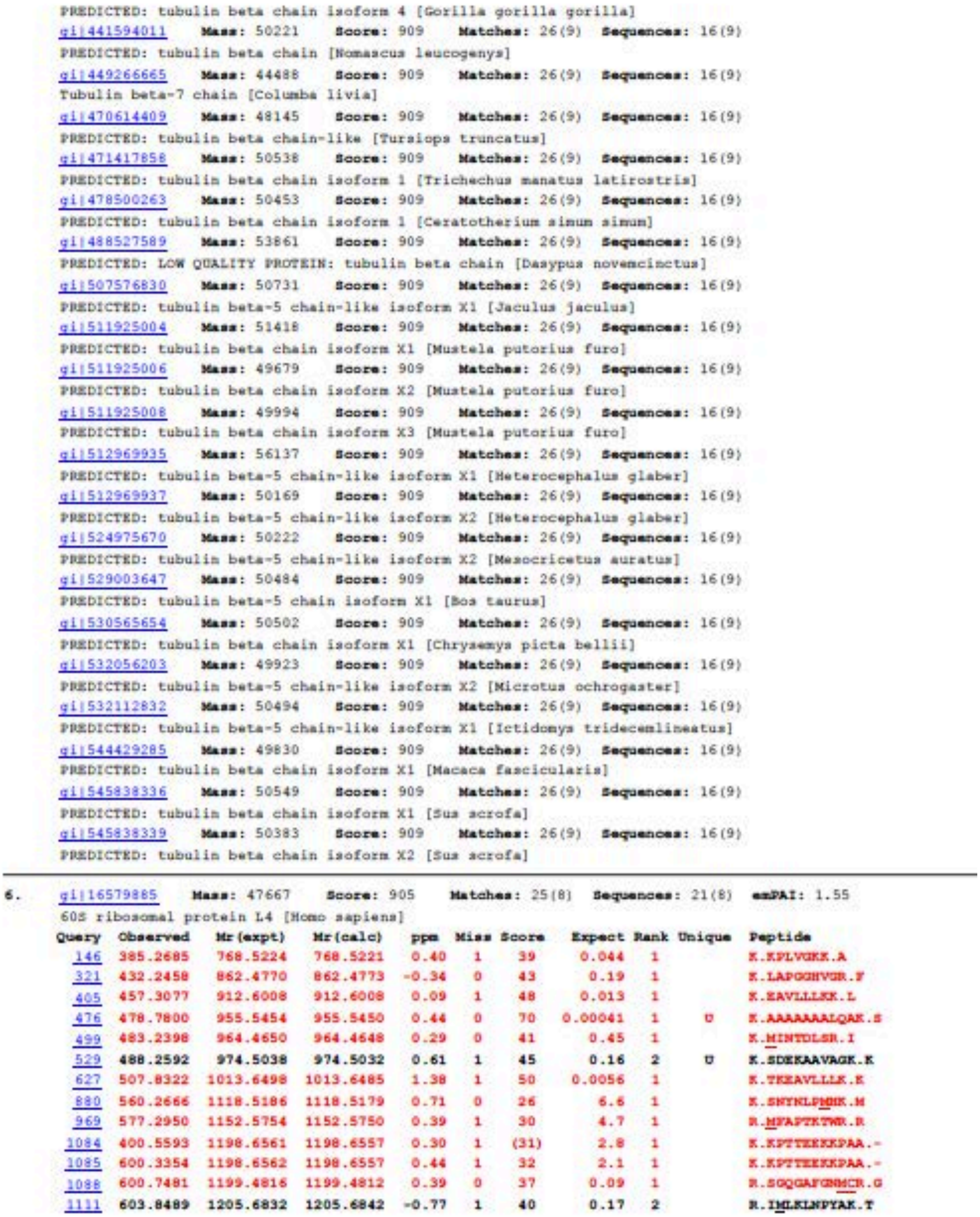
gi|13542680 **Mass:** 49783 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
Tubulin, beta 2C [Mus musculus]
gi|20809886 **Mass:** 49776 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
Tubulin, beta 2C [Homo sapiens]
gi|23958133 **Mass:** 49808 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
Tubulin, beta 2C [Homo sapiens]
gi|40018568 **Mass:** 49769 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
tubulin beta-4B chain [Rattus norvegicus]
gi|118404276 **Mass:** 49800 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
tubulin, beta 4B class IVb [Xenopus (Silurana) tropicalis]
gi|119608775 **Mass:** 48794 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
tubulin, beta 2C, isoform CRA_b [Homo sapiens]
gi|148222316 **Mass:** 49777 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
uncharacterized protein LOC379202 [Xenopus laevis]
gi|153792017 **Mass:** 49829 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
tubulin beta-3 chain [Gallus gallus]
gi|327290154 **Mass:** 49771 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
PREDICTED: tubulin beta-2C chain-like [Anolis carolinensis]
gi|346644707 **Mass:** 49716 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
tubulin beta-2C chain [Cricetulus griseus]
gi|395844458 **Mass:** 116943 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
PREDICTED: sodium-dependent phosphate transport protein 2C [Otolemur garnettii]
gi|403301550 **Mass:** 50403 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
PREDICTED: tubulin beta-4B chain [Saimiri boliviensis boliviensis]
gi|426363732 **Mass:** 48510 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
PREDICTED: tubulin beta-4 chain-like isoform 1 [Gorilla gorilla gorilla]
gi|426363734 **Mass:** 49229 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
PREDICTED: tubulin beta-4 chain-like isoform 2 [Gorilla gorilla gorilla]
gi|431899052 **Mass:** 68142 **Score:** 940 **Matches:** 25(9) **Sequences:** 17(9)
Tubulin beta-2C chain [Pteropus alecto]

```
gi|82213656     Mass: 41724    Score: 1243    Matches: 34(15)   Sequences: 23(12)
RecName: Full=Actin, cytoplasmic 2; AltName: Full=Gamma-actin; Contains: RecName: Full=Actin, cytoplasmic 2, N-terminally processed
gi|118419977     Mass: 40552     Score: 1243    Matches: 34(15)    Sequences: 23(12)
beta-actin, partial [Eubalaena glacialis]
gi|126272476     Mass: 41827     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic type 5-like [Monodelphis domestica]
gi|146386601     Mass: 41718     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Chain A, Structure Of Oxidized Beta-Actin
gi|148231177     Mass: 41740     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic 1 [Xenopus laevis]
gi|148744172     Mass: 41680     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Actin, beta [Bos taurus]
gi|151176139     Mass: 41711     Score: 1243    Matches: 34(15)    Sequences: 23(12)
beta-actin [Anas platyrhynchos]
gi|168177284     Mass: 41579     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Chain A, Model Of Actin-Fimbrin Abd2 Complex
gi|197099682     Mass: 41709     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic 1 [Pongo abelii]
gi|207298859     Mass: 41809     Score: 1243    Matches: 34(15)    Sequences: 23(12)
beta-actin [Acipenser transmontanus]
gi|224061779     Mass: 41825     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic type 5-like [Taeniopygia guttata]
gi|288541396     Mass: 41823     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic type 5 [Xenopus laevis]
gi|293342999     Mass: 41767     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2-like [Rattus norvegicus]
gi|297273827     Mass: 41744     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2-like isoform 4 [Macaca mulatta]
gi|301762186     Mass: 41720     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1-like [Ailuropoda melanoleuca]
gi|301792411     Mass: 68102     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2-like [Ailuropoda melanoleuca]
gi|307938303     Mass: 41740     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic 1 [Canis lupus familiaris]
gi|313507212     Mass: 41664     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Chain A, The Structure Of Crystalline Profilin-Beta-Actin
gi|343960006     Mass: 41634     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic 1 [Pan troglodytes]
gi|344289654     Mass: 42429     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1-like [Loxodonta africana]
gi|347360906     Mass: 41711     Score: 1243    Matches: 34(15)    Sequences: 23(12)
actin, cytoplasmic 1 [Cricetulus griseus]
gi|390459127     Mass: 41708     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 isoform 1 [Callithrix jacchus]
gi|395501273     Mass: 41925     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic type 5-like [Sarcophilus harrisii]
gi|395845536     Mass: 41709     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1 [Otolemur garnettii]
gi|397479373     Mass: 68315     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 1-like [Pan paniscus]
gi|407726065     Mass: 41971     Score: 1243    Matches: 34(15)    Sequences: 23(12)
beta actin [Cynops pyrrhogaster]
gi|410984335     Mass: 49757     Score: 1243    Matches: 34(15)    Sequences: 23(12)
PREDICTED: actin, cytoplasmic 2-like [Felis catus]
gi|431908661     Mass: 41750     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Actin, cytoplasmic 2 [Pteropus alecto]
gi|432119092     Mass: 41707     Score: 1243    Matches: 34(15)    Sequences: 23(12)
Actin, cytoplasmic 2 [Myotis davidii]
```

```
gi|512872359     Mass: 49785     Score: 940     Matches: 25(9)  Sequences: 17(9)
PREDICTED: tubulin beta-4B chain isoform X1 [Heterocephalus glaber]
```

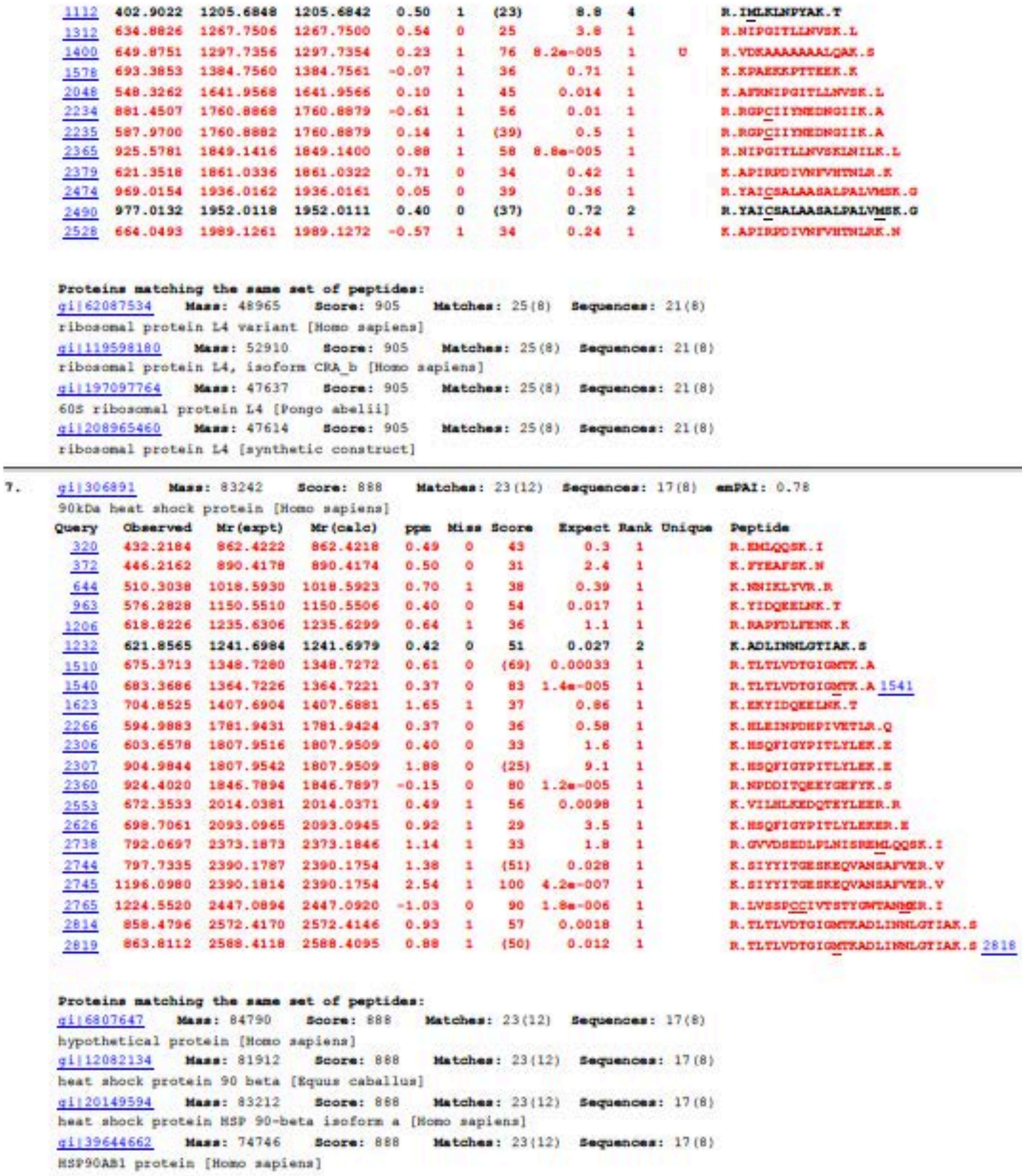
```
5.    gi|7106439     Mass: 49639     Score: 909     Matches: 26(9)   Sequences: 16(9)   emPAI: 1.16
      tubulin beta-5 chain [Mus musculus]
     Query   Observed   Mr(expt)   Mr(calc)    ppm  Miss Score   Expect Rank Unique  Peptide
       665   514.7631  1027.5116  1027.5121  -0.41    0    42      0.17    1          K.TAVCDIPPR.G 664 666
       684   520.3006  1038.5866  1038.5862   0.42    0    63   0.00061    1          R.YLTVAAVFR.G
       942   572.3214  1142.6282  1142.6270   1.07    0  (36)       1.2    1          K.LAVNMVPFPR.L
       988   580.3186  1158.6226  1158.6219   0.60    0    62    0.0031    1          K.LAVNMVPFPR.L
      1265   626.8622  1251.7098  1251.7088   0.85    1    25       3.7    1          R.YLTVAAVFRGR.M
      1734   731.8458  1461.6770  1461.6769   0.07    0    71   0.00021    1          K.EVDEQMLNVQNK.N
      1994   808.4222  1614.8298  1614.8287   0.71    0  (48)     0.059    1          R.AILVDLEPGTMDSVR.S
      2000   540.9503  1619.8291  1619.8283   0.50    0  (28)       7.8    1          R.LHFFMPGFAPLTSR.G
      2001   810.9221  1619.8296  1619.8283   0.86    0    38      0.65    1          R.LHFFMPGFAPLTSR.G
      2022   816.4194  1630.8242  1630.8236   0.38    0    76  9.1e-005    1          R.AILVDLEPGTMDSVR.S
      2029   546.2820  1635.8242  1635.8232   0.61    0  (34)       2.2    1          R.LHFFMPGFAPLTSR.G
      2030   818.9205  1635.8264  1635.8232   2.00    0  (29)       5.3    1          R.LHFFMPGFAPLTSR.G
      2079   830.4523  1658.8900  1658.8879   1.27    0    44      0.13    1          R.ALTVPELTQQVFDAK.N 2080
      2144   848.9205  1695.8264  1695.8257   0.47    0    46      0.12    1          K.NSSYFVEWIPNNVK.T
      2409   943.4889  1884.9632  1884.9655  -1.21    0    95     1e-006   1          K.MAVTFIGNSTAIQELFK.R
      2500   653.6655  1957.9747  1957.9745   0.07    0  (33)       2.2    1          K.GHYTEGAELVDSVLDVVR.K
      2501   979.9952  1957.9758  1957.9745   0.67    0   102    3e-007    1          K.GHYTEGAELVDSVLDVVR.K
      2513   657.9659  1970.8759  1970.8747   0.58    1    54    0.0079    1          R.MSMKEVDEQMLNVQNK.N
      2580  1021.5410  2041.0674  2041.0666   0.40    1  (34)       1.3    1          K.MAVTFIGNSTAIQELFKR.I
      2581   681.3633  2041.0681  2041.0666   0.70    1    36      0.66    1          K.MAVTFIGNSTAIQELFKR.I
      2617   696.3635  2086.0687  2086.0695  -0.39    1    33       1.9    1          K.GHYTEGAELVDSVLDVVRK.E
      2852   913.4367  2737.2883  2737.2874   0.34    1    47      0.06    1          R.ALTVPELTQQVFDAKNMMAACDPR.H
      2912  1109.8470  3326.5192  3326.5218  -0.78    0    75  6.4e-005    1          K.EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK.I

      Proteins matching the same set of peptides:
      gi|12846758     Mass: 49608     Score: 909     Matches: 26(9)   Sequences: 16(9)
      unnamed protein product [Mus musculus]
      gi|55742495     Mass: 49696     Score: 909     Matches: 26(9)   Sequences: 16(9)
      tubulin beta chain [Xenopus (Silurana) tropicalis]
      gi|74141821     Mass: 49667     Score: 909     Matches: 26(9)   Sequences: 16(9)
      unnamed protein product [Mus musculus]
      gi|74204140     Mass: 49616     Score: 909     Matches: 26(9)   Sequences: 16(9)
      unnamed protein product [Mus musculus]
      gi|148691289     Mass: 49910     Score: 909     Matches: 26(9)   Sequences: 16(9)
      tubulin, beta 5 [Mus musculus]
      gi|221045918     Mass: 46537     Score: 909     Matches: 26(9)   Sequences: 16(9)
      unnamed protein product [Homo sapiens]
      gi|291395962     Mass: 49695     Score: 909     Matches: 26(9)   Sequences: 16(9)
      PREDICTED: tubulin, beta 5-like [Oryctolagus cuniculus]
      gi|332245916     Mass: 53454     Score: 909     Matches: 26(9)   Sequences: 16(9)
      PREDICTED: tubulin beta chain isoform 3 [Nomascus leucogenys]
      gi|332245918     Mass: 44790     Score: 909     Matches: 26(9)   Sequences: 16(9)
      PREDICTED: tubulin beta chain isoform 4 [Nomascus leucogenys]
      gi|346644703     Mass: 49710     Score: 909     Matches: 26(9)   Sequences: 16(9)
      beta tubulin [Cricetulus griseus]
      gi|355561503     Mass: 49540     Score: 909     Matches: 26(9)   Sequences: 16(9)
      Tubulin beta-5 chain [Macaca mulatta]
      gi|410958666     Mass: 50209     Score: 909     Matches: 26(9)   Sequences: 16(9)
      PREDICTED: tubulin beta chain isoform 2 [Felis catus]
      gi|426250622     Mass: 50494     Score: 909     Matches: 26(9)   Sequences: 16(9)
      PREDICTED: tubulin beta-5 chain isoform 2 [Ovis aries]
      gi|426352283     Mass: 50452     Score: 909     Matches: 26(9)   Sequences: 16(9)
```



```
    PREDICTED: tubulin beta chain isoform 4 [Gorilla gorilla gorilla]
    gi|441594011    Mass: 50221    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain [Nomascus leucogenys]
    gi|449266665    Mass: 44488    Score: 909    Matches: 26(9)  Sequences: 16(9)
    Tubulin beta-7 chain [Columba livia]
    gi|470614409    Mass: 48145    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain-like [Tursiops truncatus]
    gi|471417858    Mass: 50538    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform 1 [Trichechus manatus latirostris]
    gi|478500263    Mass: 50453    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform 1 [Ceratotherium simum simum]
    gi|488527589    Mass: 53861    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: LOW QUALITY PROTEIN: tubulin beta chain [Dasypus novemcinctus]
    gi|507576830    Mass: 50731    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X1 [Jaculus jaculus]
    gi|511925004    Mass: 51418    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X1 [Mustela putorius furo]
    gi|511925006    Mass: 49679    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X2 [Mustela putorius furo]
    gi|511925008    Mass: 49994    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X3 [Mustela putorius furo]
    gi|512969935    Mass: 56137    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X1 [Heterocephalus glaber]
    gi|512969937    Mass: 50169    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X2 [Heterocephalus glaber]
    gi|524975670    Mass: 50222    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X2 [Mesocricetus auratus]
    gi|529003647    Mass: 50484    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain isoform X1 [Bos taurus]
    gi|530565654    Mass: 50502    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X1 [Chrysemys picta bellii]
    gi|532056203    Mass: 49923    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X2 [Microtus ochrogaster]
    gi|532112832    Mass: 50494    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta-5 chain-like isoform X1 [Ictidomys tridecemlineatus]
    gi|544429285    Mass: 49830    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X1 [Macaca fascicularis]
    gi|545838336    Mass: 50549    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X1 [Sus scrofa]
    gi|545838339    Mass: 50383    Score: 909    Matches: 26(9)  Sequences: 16(9)
    PREDICTED: tubulin beta chain isoform X2 [Sus scrofa]

6.  gi|16579885    Mass: 47667    Score: 905    Matches: 25(8)  Sequences: 21(8)  emPAI: 1.55
    60S ribosomal protein L4 [Homo sapiens]
   Query  Observed  Mr(expt)   Mr(calc)   ppm   Miss Score  Expect  Rank Unique  Peptide
    146   385.2685  768.5224   768.5221   0.40   1    39    0.044    1          K.KPLVGKK.A
    321   432.2458  862.4770   862.4773  -0.34   0    43     0.19    1          K.LAPGGHVGR.F
    405   457.3077  912.6008   912.6008   0.09   1    48    0.013    1          K.EAVLLLKK.L
    476   478.7800  955.5454   955.5450   0.44   0    70  0.00041    1     U    K.AAAAAAALQAK.S
    499   483.2398  964.4650   964.4648   0.29   0    41     0.45    1          K.MINTDLSR.I
    529   488.2592  974.5038   974.5032   0.61   1    45     0.16    2     U    K.SDEKAAVAGK.K
    627   507.8322 1013.6498  1013.6485   1.38   1    50   0.0056    1          K.TKEAVLLLK.K
    880   560.2666 1118.5186  1118.5179   0.71   0    26      6.6    1          K.SNYNLPMHK.M
    969   577.2950 1152.5754  1152.5750   0.39   1    30      4.7    1          R.MFAPTKTWR.R
   1084   400.5593 1198.6561  1198.6557   0.30   1   (31)     2.8    1          K.KPTTEEKKPAA.-
   1085   600.3354 1198.6562  1198.6557   0.44   1    32      2.1    1          K.KPTTEEKKPAA.-
   1088   600.7481 1199.4816  1199.4812   0.39   0    37     0.09    1          R.SGQGAFGNMCR.G
   1111   603.8489 1205.6832  1205.6842  -0.77   1    40     0.17    2          R.IMLKLNPYAK.T
```



```
 1112  402.9022 1205.6848 1205.6842  0.50  1  (23)      8.8  4         R.IMLKLNPYAK.T
 1312  634.8826 1267.7506 1267.7500  0.54  0   25       3.8  1         R.NIPGITLLNVSK.L
 1400  649.8751 1297.7356 1297.7354  0.23  1   76  8.2e-005  1      U  R.VDKAAAAAAALQAK.S
 1578  693.3853 1384.7560 1384.7561 -0.07  1   36      0.71  1         K.KPAEKKPTTEEK.K
 2048  548.3262 1641.9568 1641.9566  0.10  1   45     0.014  1         K.AFRNIPGITLLNVSK.L
 2234  881.4507 1760.8868 1760.8879 -0.61  1   56      0.01  1         R.RGPCIIYNEDNGIIK.A
 2235  587.9700 1760.8882 1760.8879  0.14  1  (39)      0.5  1         R.RGPCIIYNEDNGIIK.A
 2365  925.5781 1849.1416 1849.1400  0.88  1   58  8.8e-005  1         R.NIPGITLLNVSKLNILK.L
 2379  621.3518 1861.0336 1861.0322  0.71  0   34      0.42  1         K.APIRPDIVNFVHTNLR.K
 2474  969.0154 1936.0162 1936.0161  0.05  0   39      0.36  1         R.YAICSALAASALPALVMSK.G
 2490  977.0132 1952.0118 1952.0111  0.40  0  (37)     0.72  2         R.YAICSALAASALPALVMSK.G
 2528  664.0493 1989.1261 1989.1272 -0.57  1   34      0.24  1         K.APIRPDIVNFVHTNLRK.N


Proteins matching the same set of peptides:
gi|62087534    Mass: 48965    Score: 905    Matches: 25(8)  Sequences: 21(8)
ribosomal protein L4 variant [Homo sapiens]
gi|119598180    Mass: 52910    Score: 905    Matches: 25(8)  Sequences: 21(8)
ribosomal protein L4, isoform CRA_b [Homo sapiens]
gi|197097764    Mass: 47637    Score: 905    Matches: 25(8)  Sequences: 21(8)
60S ribosomal protein L4 [Pongo abelii]
gi|208965460    Mass: 47614    Score: 905    Matches: 25(8)  Sequences: 21(8)
ribosomal protein L4 [synthetic construct]
________________________________________________________________________________________

7.   gi|306891    Mass: 83242    Score: 888    Matches: 23(12)  Sequences: 17(8)  emPAI: 0.78
     90kDa heat shock protein [Homo sapiens]
     Query  Observed   Mr(expt)   Mr(calc)   ppm  Miss Score   Expect Rank Unique  Peptide
       320  432.2184   862.4222   862.4218  0.49    0    43      0.3    1          R.EMLQQSK.I
       372  446.2162   890.4178   890.4174  0.50    0    31      2.4    1          K.FYEAFSK.N
       644  510.3038  1018.5930  1018.5923  0.70    1    38     0.39    1          K.NNIKLYVR.R
       963  576.2828  1150.5510  1150.5506  0.40    0    54    0.017    1          K.YIDQEELNK.T
      1206  618.8226  1235.6306  1235.6299  0.64    1    36      1.1    1          R.RAPFDLFENK.K
      1232  621.8565  1241.6984  1241.6979  0.42    0    51    0.027    2          K.ADLINNLGTIAK.S
      1510  675.3713  1348.7280  1348.7272  0.61    0  (69)  0.00033    1          R.TLTLVDTGIGMTK.A
      1540  683.3686  1364.7226  1364.7221  0.37    0    83  1.4e-005   1          R.TLTLVDTGIGMTK.A 1541
      1623  704.8525  1407.6904  1407.6881  1.65    1    37     0.86    1          K.EKYIDQEELNK.T
      2266  594.9883  1781.9431  1781.9424  0.37    0    36     0.58    1          K.HLEINPDHPIVETLR.Q
      2306  603.6578  1807.9516  1807.9509  0.40    0    33      1.6    1          K.HSQFIGYPITLYLEK.E
      2307  904.9844  1807.9542  1807.9509  1.88    0  (25)      9.1    1          K.HSQFIGYPITLYLEK.E
      2360  924.4020  1846.7894  1846.7897 -0.15    0    80  1.2e-005   1          R.NPDDITQEEYGEFYK.S
      2553  672.3533  2014.0381  2014.0371  0.49    1    56   0.0098    1          K.VILHLKEDQTEYLEER.R
      2626  698.7061  2093.0965  2093.0945  0.92    1    29      3.5    1          K.HSQFIGYPITLYLEKER.E
      2738  792.0697  2373.1873  2373.1846  1.14    1    33      1.8    1          R.GVVDSEDLPLNISREMLQQSK.I
      2744  797.7335  2390.1787  2390.1754  1.38    1  (51)    0.028    1          K.SIYYITGESKEQVANSAFVER.V
      2745 1196.0980  2390.1814  2390.1754  2.54    1   100  4.2e-007   1          K.SIYYITGESKEQVANSAFVER.V
      2765 1224.5520  2447.0894  2447.0920 -1.03    0    90  1.8e-006   1          R.LVSSPCCIVTSTYGWTANMER.I
      2814  858.4796  2572.4170  2572.4146  0.93    1    57   0.0018    1          R.TLTLVDTGIGMTKADLINNLGTIAK.S
      2819  863.8112  2588.4118  2588.4095  0.88    1  (50)    0.012    1          R.TLTLVDTGIGMTKADLINNLGTIAK.S 2818


     Proteins matching the same set of peptides:
     gi|6807647    Mass: 84790    Score: 888    Matches: 23(12)  Sequences: 17(8)
     hypothetical protein [Homo sapiens]
     gi|12082134    Mass: 81912    Score: 888    Matches: 23(12)  Sequences: 17(8)
     heat shock protein 90 beta [Equus caballus]
     gi|20149594    Mass: 83212    Score: 888    Matches: 23(12)  Sequences: 17(8)
     heat shock protein HSP 90-beta isoform a [Homo sapiens]
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     HSP90AB1 protein [Homo sapiens]
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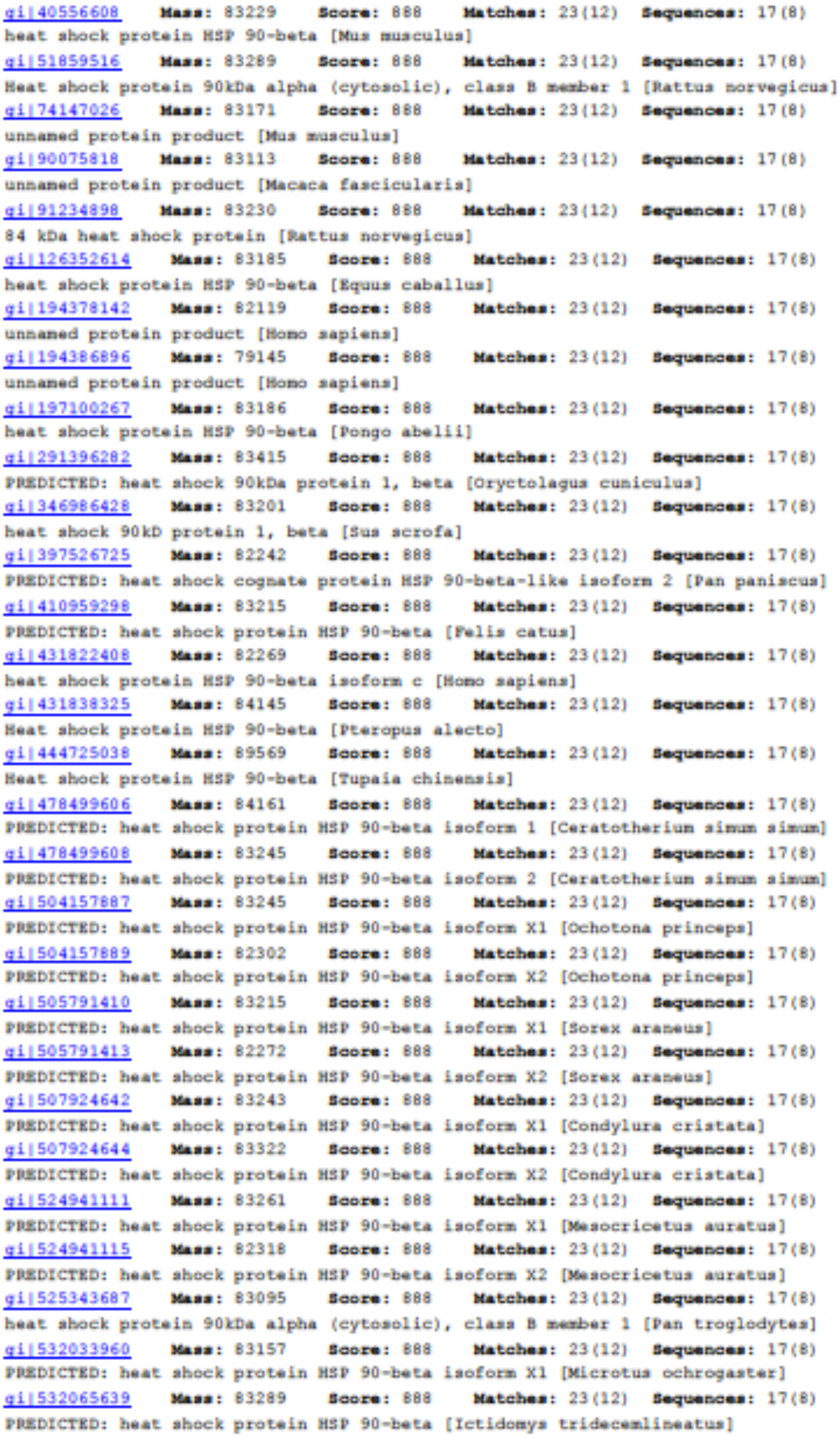

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Heat shock protein 90kDa alpha (cytosolic), class B member 1 [Rattus norvegicus]
gi|74147026     Mass: 83171    Score: 888    Matches: 23(12)   Sequences: 17(8)
unnamed protein product [Mus musculus]
gi|90075818     Mass: 83113    Score: 888    Matches: 23(12)   Sequences: 17(8)
unnamed protein product [Macaca fascicularis]
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84 kDa heat shock protein [Rattus norvegicus]
gi|126352614     Mass: 83185    Score: 888    Matches: 23(12)   Sequences: 17(8)
heat shock protein HSP 90-beta [Equus caballus]
gi|194378142     Mass: 82119    Score: 888    Matches: 23(12)   Sequences: 17(8)
unnamed protein product [Homo sapiens]
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unnamed protein product [Homo sapiens]
gi|197100267     Mass: 83186    Score: 888    Matches: 23(12)   Sequences: 17(8)
heat shock protein HSP 90-beta [Pongo abelii]
gi|291396282     Mass: 83415    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock 90kDa protein 1, beta [Oryctolagus cuniculus]
gi|346986428     Mass: 83201    Score: 888    Matches: 23(12)   Sequences: 17(8)
heat shock 90kD protein 1, beta [Sus scrofa]
gi|397526725     Mass: 82242    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock cognate protein HSP 90-beta-like isoform 2 [Pan paniscus]
gi|410959298     Mass: 83215    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta [Felis catus]
gi|431822408     Mass: 82269    Score: 888    Matches: 23(12)   Sequences: 17(8)
heat shock protein HSP 90-beta isoform c [Homo sapiens]
gi|431838325     Mass: 84145    Score: 888    Matches: 23(12)   Sequences: 17(8)
Heat shock protein HSP 90-beta [Pteropus alecto]
gi|444725038     Mass: 89569    Score: 888    Matches: 23(12)   Sequences: 17(8)
Heat shock protein HSP 90-beta [Tupaia chinensis]
gi|478499606     Mass: 84161    Score: 888    Matches: 23(12)   Sequences: 17(8)
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gi|478499608     Mass: 83245    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform 2 [Ceratotherium simum simum]
gi|504157887     Mass: 83245    Score: 888    Matches: 23(12)   Sequences: 17(8)
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gi|504157889     Mass: 82302    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Ochotona princeps]
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gi|505791413     Mass: 82272    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Sorex araneus]
gi|507924642     Mass: 83243    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Condylura cristata]
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PREDICTED: heat shock protein HSP 90-beta isoform X2 [Condylura cristata]
gi|524941111     Mass: 83261    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Mesocricetus auratus]
gi|524941115     Mass: 82318    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Mesocricetus auratus]
gi|525343687     Mass: 83095    Score: 888    Matches: 23(12)   Sequences: 17(8)
heat shock protein 90kDa alpha (cytosolic), class B member 1 [Pan troglodytes]
gi|532033960     Mass: 83157    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X1 [Microtus ochrogaster]
gi|532065639     Mass: 83289    Score: 888    Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta [Ictidomys tridecemlineatus]
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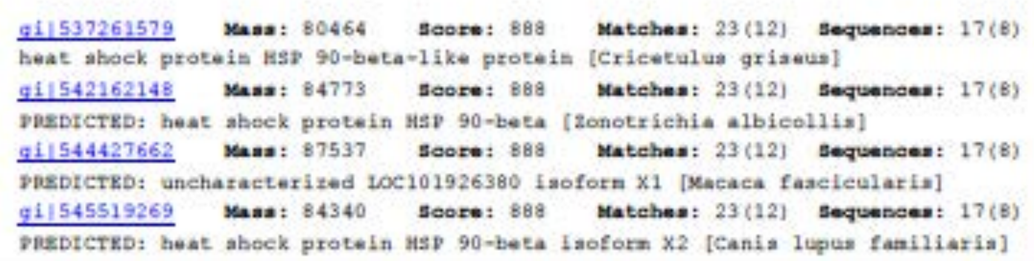

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heat shock protein HSP 90-beta-like protein [Cricetulus griseus]
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PREDICTED: heat shock protein HSP 90-beta [Zonotrichia albicollis]
gi|544427662     Mass: 87537     Score: 888     Matches: 23(12)   Sequences: 17(8)
PREDICTED: uncharacterized LOC101926380 isoform X1 [Macaca fascicularis]
gi|545519269     Mass: 84340     Score: 888     Matches: 23(12)   Sequences: 17(8)
PREDICTED: heat shock protein HSP 90-beta isoform X2 [Canis lupus familiaris]
```

Supplemental Figure S9: LC/MS/MS sequencing results from compound **1_**TagII lysate pulldown in HCT-116 cell lysates.

List of abbreviations

μM	Micromolar
CTC	Chlorotrityl chloride
nM	Nanomolar
HOAt	1-Hydroxybenzotriazole
HOBt	1-Hydroxybenzotriazole
DIC	*N,N'*-Diisopropylcarbodiimide
Fmoc	9-Fluorenylmethyl chloroformate
IPA	Isopropyl alcohol
TFE	*2,2,2*-Trifluroethanol
v/v	Volume to volume
ºC	Degree Celcius
Boc	*tert*-butoxycarbonyl
TBTU	O-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluroborate
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexaflurophosphate
DMTMM	4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium chloride
DIPEA	*N,N*-Diisopropylethylamine
TFA	Trifluroacetic acid
PEG	Polyethylene glycol
Lys	Lysine
Phe	Phenylalanine
Tyr	Tyrosine
Trp	Tryptophan
Val	Valine
DDLP	Double deprotected linear pentapeptide