Supplementary Information

Optimizing PK properties of cyclic peptides: the effect of side chain substitutions on permeability and clearance

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Contents

Synthesis

General. 2-chlorotrityl-chloride resin was purchased from Aapptec (1.6 meq/g). All Fmoc-protected amino acids, and all reagents were purchased from Aapptec. A standard ion trap electrospray LCMS system (Thermo-Finnegan LCQ Classic) equipped with both a photodiode array detector and an evaporative light scattering detector was used to determine the identity, purity and ratios of peptide products. Peptides were purified on a preparative HPLC system (Shimazdu LC-8A) equipped with a C18 10 μ column, 250x20 mm.

Abbreviations. ACN, acetonitrile; DMF, N,N-dimethylformamide; DIPEA, diisopropylethylamine, DCM, dichloromethane; BME, 2-mercaptoethanol; NMP, *N*-Methyl-2-pyrrolidone; THF, tetrahydrofuran; EtOH, ethanol; DMSO; dimethyl sulfoxide; DIAD, Diisopropyl azodicarboxylate; DBU, 1,8- Diazabicyclo[5.4.0]undec-7-ene; HBTU, N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)-uronium hexafluoro-phosphate; TFA, trifluoroacetic acid; LCMS, liquid chromatography mass spectrometry; ELSD, evaporative light scattering detector; SPPS, solid phase peptide synthesis.

Loading of Fmoc-tyrosine-allyl ester: 1110 mg of the ester was taken into a round bottom flask and dried with P_2O_5 in a vacuum desiccator overnight. The ester was then dissolved is dry DCM and 3 g of $CsCO_3$ was added and stirred for 30 min, and the resin was added and gently agitated for 3 h. After 3 h, the resin and solution were transferred to a polypropylene synthesis vial containing a fritted disk and fitted with a Teflon stopcock. The resin was drained and rinsed with DCM (3x30 mL), DMF (3x30 mL), and again with DCM (3x30 mL). The resin was dried thoroughly, and a 10 mg aliquot was taken for use in determining the loading value. The remaining resin was treated with a solution of DCM, methanol and DIPEA (17:2:1 respectively, 2x30 min.), in order to cap any unreacted 2- chlorotrityl chloride linker.

Determination of resin loading. 10 mg of resin was added to a 2 mL polypropylene tube and 0.8 mL of DMF was added and allowed to sit for 10 minutes. Next, 0.2 mL of piperidine was added, and the mixture was agitated for an additional 20 min. After cleavage of the N-terminal Fmoc group was complete, 10 µL of the solution was pipetted into a quartz cuvette and diluted

with 0.990 mL of DMF. The absorbance at λ = 301 nm was determined to be 0.312. The piperidine-dibenzofulvene adduct that is formed upon fmoc deprotection has a molar extinction coefficient of ε = 7800 L•mol^{-1}•cm^{-1}; thus, using Beer's Law the loading value was determined to be 0.4 mmol/g.

General SPPS procedures. All linear peptide intermediates were synthesized on a Protein Technologies Prelude peptide synthesizer, using advanced programming mode and 60 mL reaction vessels. All syntheses were performed on a 0.2 mmol scale. All fmoc deprotections were performed by immersing the resin in 20% piperidine in DMF (v/v) for 20 minutes while agitating by bubbling nitrogen through the suspension. All coupling reactions were performed by sequential addition of 6 eq of DIPEA (diluted to 0.5 mL with DMF), 4 eq of Fmoc-protected amino acid (dissolved in 1.5 mL of DMF), and 3.8 eq of HBTU (dissolved in 1.5 mL of DMF). All coupling reactions were agitated by bubbling nitrogen through the suspension for 2.5 h. Between each coupling reaction the resin was washed with 3x5 mL DMF, 3x5 mL of DCM, and 3x5 mL DMF, and dried under nitrogen for 1 min.

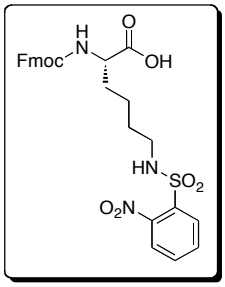
On-resin cyclization. To the resin-bound protected peptide (N-fmoc, C-allyl ester) a solution of 1 eq $Pd(PPh_3)_4$ dissolved in 10% piperidine/THF (v/v) was added. The solution was agitated at rt for 3 h. The reaction vessel was then drained and 10 mL of a chelating solution consisting of 5% sodium diethyldithiocarbamate (w/v) and 5% DIPEA in DMF (v/v) was added. The reaction vessel was agitated for 10 min. The resin was washed with 3x5 mL DMF, 3x5 mL of DCM, and 3x5 mL DMF, and dried under nitrogen for 1 min. The resin was then treated with 5 eq HOAt, 5

eq HATU and 8 eq DIPEA in minimal DMF and agitated for 3 h. The complete cyclized peptide was washed 3x5 mL DMF, 3x5 mL of DCM, and 3x5 mL DMF, and dried under nitrogen for 1 min.

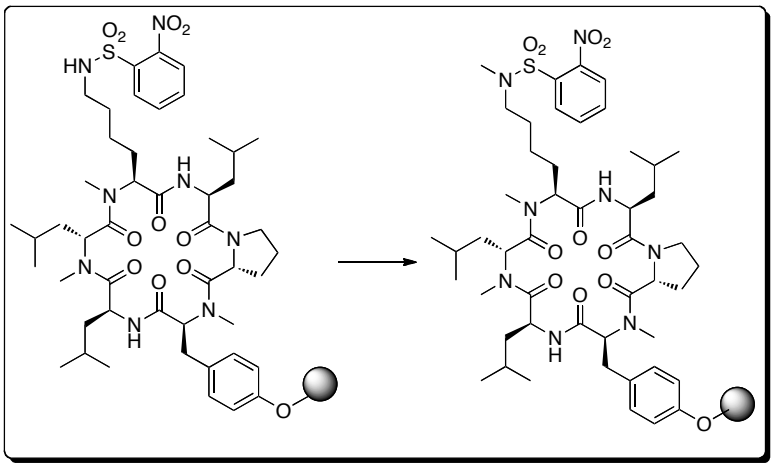
Conformation-directed on-resin N-methylation. Peptide-bound resin (0.5 g, 0.2 mmol) was added to a polypropylene reaction vessel, 8 mL of 1.0 M LiO*t*-Bu THF was added via syringe driven hydrophilic filter and agitated at RT for 45 minutes. The reaction vessel was drained and 8 mL of 10% CH_3I in DMSO (v/v) was immediately added and agitated at RT for 45 minutes. The resin was washed with 3x5 mL DMSO, 3x5 mL DMF, 3x5 mL DCM.

Resin cleavage. All cleavages were performed by immersing the resin in 1% TFA in DCM (v/v) for 20 minutes under an atmosphere of nitrogen at rt. After cleavage the suspension was filtered into a flask and the solvent was evaporated under a stream of nitrogen.

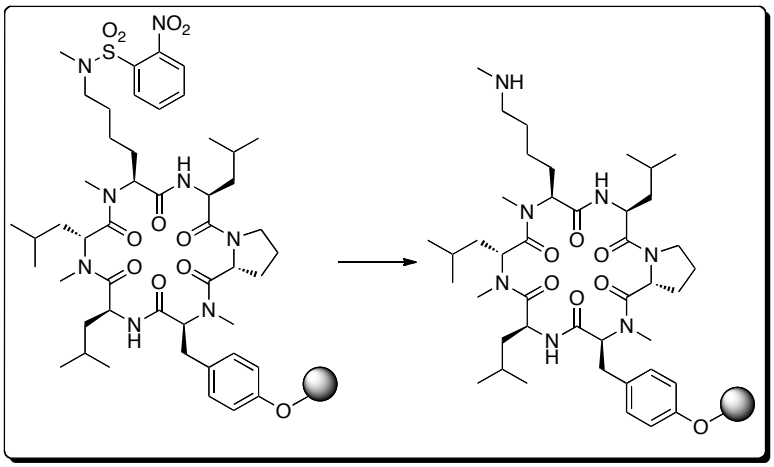
Side chain deprotection. Crude peptide was taken up in a solution of 1:1 TFA:DCM and stirred at room temperature for 2 h. Solvent was then removed *in vacuo.*



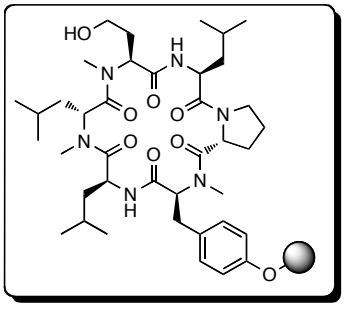
Fmoc-Lys(oNBS)-OH. Fmoc-L-Lys(NH_2)-OH (500 mg, 1.36 mmol) was taken into a round-bottom flask and 40 mL of ACN was added. To the suspension triethylamine (607 µL, 4.35 mmol) was added. The suspension was stirred and pure water was added dropwise until all solids were dissolved. 2-Nitrobenzenesulfonyl chloride (390 mg, 1.76 mmol) was dissolved in minimal ACN and added dropwise to the solution. The reaction was stirred for 30 min. The solvent was then removed *in vacuo* and the resulting oil was taken up in ethyl acetate (50 mL), washed 3x30 mL saturated $NaHCO_3$ and finally with 50 mL brine. The organic layers were combined and the solvent removed resulting in a off white solid, which was analytically pure by LCMS and taken on without further purification.



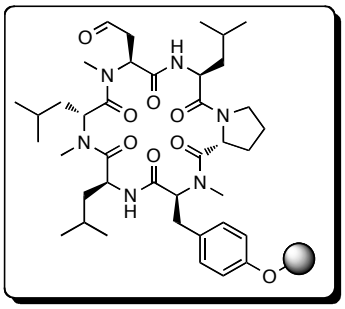
N-methylation of ε-nitrogen of Lys[3]. Resin-bound peptide (350 mg, 0.14 mmol) was taken up into a 6 mL polypropylene cartridge with a polyethylene frit. The resin was immersed in 5 mL of THF. Separately triphenylphosphine (114 mg, 0.7 mmol) was dissolved in minimal THF and methanol (60 µL, 1.4 mmol) was added. This solution was then added to the resin. DIAD (138 µL, 0.7 mmol) was added dropwise with agitation. The reaction was agitated at RT for 15 minutes, and then washed with 3x5 mL of THF, 3x5 mL DMF, and 3x5 mL DCM.



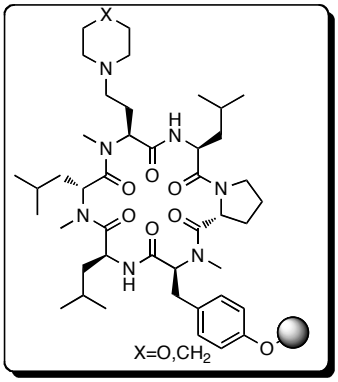
Deprotection of ε-nitrogen of Lys[3]: Resin-bound peptide (350 mg, 0.14 mmol) was placed in a 6 mL polypropylene cartridge with a polyethylene frit. The resin was immersed in 5 mL of NMP, BME (99 µL, 1.4 mmol) and DBU (105 µL, 0.7 mmol) were added. The reaction was agitated at RT for 30 minutes. The resin was washed with 3x5 mL NMP and 3x5 mL DCM.



On-resin reduction of Asp(OMe) side chain. A 250 mL round-bottom flask was charged with the peptide-bound resin (1g, 0.4 mmol) which was submersed in 20 mL of 1:1 EtOH:THF. To the suspension $NaBH_4$ (75.6 mg, 2 mmol) dissolved in minimal 1:1 EtOH:THF. The suspension was stirred magnetically at RT for 24 h. The reaction was quenched with 30 mL of methanol and the resin was filtered and washed with 3x30 mL THF.



On-resin oxidation of homoserine side chain. A 250 mL round-bottom flask was charged with the peptide-bound resin (1g, 0.4 mmol) which was submersed in 20 mL of DMSO. To the suspension was added 2-iodoxybenzoic acid (448 mg, 1.6 mmol) dissolved in minimal DMSO. The suspension was stirred at RT for 4-18 h. The resin was filtered and washed 3x30 mL DMSO and 3x30 DCM.



On-resin reductive amination. Peptide-bound resin (0.5 g, 0.2 mmol) was added to a polypropylene reaction vessel and swelled in 3 mL of DMF. To the suspension the 2 molar eq of secondary amine (piperidine or morpholine) was added. Following brief mixing, $NaBH_3CN$ (25 mg, 0.4 mmol) dissolved in minimal DMF was added and the reaction was agitated at RT for 3 hrs. The reaction was then quenched with methanol and the resin was filtered, washed with 3x5 mL of DMF and 3x5 mL of DCM.

In vitro metabolism studies

RRCK cell permeability. Cell permeability was determined using RRCK cells (Pfizer, Inc. Groton, CT)[1]. RRCK cells were generated in house as a subclone of Madin-Darby Canine Kidney wild-type (MDCK-WT) cells that displayed low expression of endogenous p-glycoprotein (~ 1–2% of MDCK-WT cells, based on mRNA level). Cells were cultured in minimal essential medium α with supplements and passaged when 70–80% confluent. Cell monolayer flux studies were conducted five days after seeding in 24-well transwell inserts (RRCK in 1.0 μm pore size (Becton Dickinson, Cowley, UK) at 4.2 x 10^4 cells/cm^2. Donor and acceptor solutions were prepared from HBSS containing HEPES at 20 mM, pH 7.4. Stock solutions of test compounds, prepared at 10 mM in DMSO, were used to prepare donor solutions of 2 μM compound in 0.05% (v/v) DMSO. Apparent permeability (P_{app}) was determined in apical to basolateral (AB) direction in triplicate by incubating with compound for 2 h at 37 °C. Samples of the medium were analyzed by liquid-chromatography tandem mass spectrometry (LC-MS/MS). P_{app} values were calculated according to the equation $P_{app} = (Q/t) \times 1/C_0 \times 1/A$, where Q is the sampled concentration in the acceptor compartment, t is the incubation time, C_0 is the initial concentration in the donor compartment and A is the area of the filter of the transwell plate.

Caco-2 permeability. Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Cell culture medium (Dulbecco's Modified Eagles Medium with 20% fetal bovine serum, 1% Non-essential amino acids, 1% Glutamax-1 and 0.08% gentamycin) and Hank's balanced salt solution (HBSS) with 25 mM D-glucose monohydrate, 1.25 mM $CaCl_2$ and 0.5 mM $MgCl_2$; pH 7.4 or pH 6.5) obtained from Invitrogen (Grand Island, NY). Both protease

inhibitor and albumin from bovine serum were obtained from Sigma Aldrich. Transwell permeable support 24-well cell culture plates (PET membrane, 0.33 cm^2 growth area, 1.0 μm pore size) were obtained from BD Biosciences (Franklin Lakes, NJ). Transepithelial electrical resistance (TEER) was measured using EVOM from World Precision Instruments. Caco-2 cells were cultured at 37 °C with cell culture medium in an atmosphere of 10% CO_2 and 90% relative humidity incubator. The cells were passaged upon reaching approximately 90% confluence from T-flasks using 0.25% trypsin-EDTA. Caco-2 cells were seeded onto polycarbonate membranes with 60,000 cells/well. The individual feeding tray wells received 1.0 mL of cell culture medium. The cell culture medium was changed bi-weekly. Caco-2 cells monolayers were used for experimentation 21-days post seeding. An apical→basolateral (A→B) assay was performed to assess the P_{app} of test compounds in the absorptive transport direction ($P_{app,A\rightarrow B}$). Transport was determined under conditions in which HBSS (pH 6.5 with protease inhibitor) was used in A compartment and HBSS [pH 7.4 with 0.4% bovine serum albumin (BSA) and protease inhibitor] was used in B compartment. Protease inhibitor was added to both pH 6.5 and 7.4 HBSS at a ratio of 400 μL per 100 mL of buffer. These buffers are referred as "modified 6.5 HBSS" and "modified 7.4 HBSS". Starting compound solutions were made from 10mM DMSO stock solution diluted to 10 μM in modified pH 6.5 HBSS. Silanized glass tubes were used for stock solutions to limit non-specific binding.

Experiments were initiated by removing the cell culture medium from the apical and basolateral sides of the cell monolayers. Then 0.3 mL of modified pH 6.5 HBSS was placed in the A compartment and 1.0 mL of modified pH 7.4 HBSS was placed in the B compartment, and the monolayers were preincubated for 15 min at 37°C . After 15 min, HBSS was removed from both compartments. To conduct an A→B assay, 0.3 mL of compound solution in modified pH

6.5 HBSS was added to the A compartment and 1.0 mL of modified pH 7.4 HBSS was added to the B compartment. Experiments were carried out in triplicate. Monolayers were incubated for 2 hours 30 min at 37°C with continuous agitation (60 rpm), then the basolateral samples were collected. The assay samples collected were analyzed by LC-MS/MS. TEER was measured across the cell membranes at the beginning and the end of experiments to indicate the integrity of the Caco-2 monolayers (TEER >350Ω.cm^2). The TEER (Ω.cm^2) values of the cell monolayers were calculated according to the following equation: Resistance of unit area = resistance (Ω) x effective membrane area (cm^2). Absorptive transport were represented as permeability values (P_{app} x 10^{-6} cm/sec), calculated using the equation:

$$P_{app} = \frac{1}{Area * C_D(0)} * \frac{dM_r}{dt}$$

where Area is the surface area of the cell monolayer (0.33 cm^2), $C_D(0)$ is the initial concentration of compound applied to the donor chamber, t is time, M_r is the mass of compound in the receiver compartment, and dM_r/dt is the flux of the compound across the cell monolayer.

Liver microsomal stability. Microsomal stability was determined upon incubation of test compounds (1 μM) with rat or human liver microsomes (cytochrome P450 (CYP) concentration = 0.25 μM) in the presence of 0.1 M potassium phosphate buffer (pH 7.4) at 37 °C. The total incubation volume was 0.6 mL. Stock solutions of test compounds were prepared in acetonitrile/DMSO (97.5%/2.5%). The final concentration of acetonitrile and DMSO in the incubation media was 0.975% and 0.025% (v/v), respectively. The reaction mixture was prewarmed at 37 °C for 5 min before addition of NADPH (1.3 mM) co-factor. Aliquots (50 μL) of the reaction mixture at 0, 5, 10, 15, 30 and 60 min (time period associated with reaction linearity) were added to acetonitrile (200 μL), and the samples were centrifuged at 2500 *g* for 10

min prior to LC-MS/MS analysis for substrate disappearance (half-life determination). For control experiments, NADPH was omitted from the incubations. In vitro liver microsomal half-lives ($T_{1/2}$) were scaled to hepatic intrinsic clearance (CL_{int}) using the well-stirred model as described in detail by Ito and Houston.[2]

For metabolite identification studies, rat and human liver microsomes (CYP concentration = 0.5 μM) were incubated with test compound (10 μM) in 0.1 M potassium phosphate buffer (pH 7.4) for 60 min at 37 °C. Enzymatic reactions were initiated by the addition of NADPH (1 mM) or uridine-diphosphate-glucuronic acid (UDPGA, 5 mM) to study the contribution of CYP and uridine glucuronosyl transferase (UGT) enzymes, respectively. Reactions were terminated by the addition of ice-cold acetonitrile (4 mL). The solutions were centrifuged (3,000 x *g*, 15 min) and the supernatants were dried under a steady nitrogen stream. The residue was reconstituted with 25% aqueous acetonitrile (250 μL) and analyzed for metabolite formation by LC-MS/MS.

The HPLC system consisted of an Accela quaternary solvent delivery pump and autoinjector, a Surveyor PDA Plus photodiode array detector (Thermo Electron Corporation, Waltham, MA). Chromatography was performed on a Phenomenex Synergy RP column, 150 × 4.6 mm, 5 μm (Phenomenex, Torrance, CA). LC analysis was performed at a constant flow rate of 1 mL/min using a binary solvent system: solvent A, 5 mM ammonium formate buffer (pH ~ 3.0) with 0.1% formic acid and solvent B, acetonitrile. The initial HPLC gradient system was held at 5% solvent B for 3 min and linearly increased to 80% solvent B in 35 min, followed by a return to initial conditions for column re-equilibration. Post-column flow passed through the PDA detector to provide UV (λ= 258 nm) detection prior to being split to the mass spectrometer such that mobile phase was introduced into the electrospray source at a rate of 50 μL/min. The LC

system was interfaced to a Thermo Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Xcalibur software version 2.0 (Thermo Fisher Scientific, Bremen, Germany) was used to control the HPLC/MS system. Mass spectroscopy analyses were carried out in the positive ion mode using full-scan MS with a mass range of 100-1000 Da. Full scan data and data-dependent MS/MS acquisition on the two most intense ions were collected at 15,000 resolution. All experimental data were acquired using external calibration prior to data acquisition (mass accuracy obtained under the current experimental conditions was < 3 ppm).

Brush border membrane vesicle stability. Brush border membrane vesicles (BBMVs) were prepared from combined duodenum, jejunum, and upper ileum of Wistar-Hanover rats by a Ca^{2+} precipitation method.[3] The enzymatic reaction was performed as follows: stock solutions of test compounds (final concentration of 1.0 μM) were diluted with purified BBMV (final protein concentration 0.5 mg/ml) in 0.1 M potassium phosphate buffer (pH 7.4), with and without 1.3 mM NADPH. The solution was incubated at 37 °C and sampled at time 0, 2, 5, 10, 30, 60, and 120 min. The enzymatic reaction was stopped by transferring 50 μl of sample to 200 μl ice-cold acetonitrile, then centrifuged (3000 *g*, 10 min) before analysis of substrate disappearance by LC-MS/MS.

Rat intestinal S-9 stability. All animal care and in vivo procedures conducted were in accordance with guidelines of the Pfizer Animal Care and Use Committee. Sprague-Dawley rat intestinal S9 and microsomes, prepared without PMSF and EDTA, were purchased from BD Biosciences. S9 and microsomes were diluted to 0.5 mg/ml protein in 0.1 M potassium phosphate buffer (pH 7.4), with and without NADPH (1.3 mM). Stock solution was added to the pre-warmed (37 °C) mixture. The solution was incubated at 37 °C and sampled at time 0, 2, 5,

10, 30, 60, and 120 min. The enzymatic reaction was stopped by transferring 50 µl of sample to 200 µl ice-cold acetonitrile, then centrifuged (3000 *g*, 10 min) before analysis of substrate disappearance by LC-MS/MS.

In vivo pharmacokinetic studies.

Rat studies were done at Pfizer Global Research and Development (PGRD, Groton, CT) and BioDuro, Pharmaceutical Product Development Inc. (Beijing, PRC); animal care and in vivo procedures were conducted according to guidelines from the Pfizer Animal Care and Use Committee and the BioDuro Institutional Animal Care and Use Committee, respectively. Jugular vein-cannulated male Wistar-Hannover rats (230–250 g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA) or Vital River (Beijing, China). During the pharmacokinetic studies animals were housed individually. All orally dosed animals were fasted overnight before dosing and fed after collection of the 4 h blood samples, with access to food and water *ad libitium*. For intravenously dosed animals access to food and water was provided *ad libitium*. Test compounds **1**, **2**, **3**, **9** and **10** were administered intravenously (i.v.) via the tail vein of rats (n = 3) over 30 s at doses of ~ 0.4–1.5 mg/kg, respectively, and serial blood samples were collected before dosing and at 0.03, 0.083, 0.25, 0.5, 1.0, 2.0, 4.0, 7.0 and 24 h after dosing via the jugular vein cannula. The vehicle used in i.v. dosing were 10% self-emulsifying drug delivery system (SEDDS):90% sterile dextrose (compound **1**), 1:1 propylene glycol: 20 mM sodium phosphate (pH 7.4) (compounds **2**, **3** and **10**) and 10% propylene glycol: 90% 20 mM sodium phosphate buffer (pH 7.4) (compound **9**). Test compounds were also administered orally (p.o.) to rats (n = 3) in the same formulations, with the exception of compound **1** dosed in 10% self-emulsifying drug delivery system (SEDDS): 90% water. Blood samples were taken prior to administration and then serially at 0.083, 0.25, 0.50, 1.0, 2.0, 4.0, 7.0 and 24 h after dosing.

Blood samples from various pharmacokinetic studies were collected into tubes containing K2EDTA and stored on until centrifugation. Blood samples were centrifuged at 3000 *g* for 10 min at 4 °C to obtain plasma. All plasma samples were kept frozen until analysis. Aliquots of plasma (50 μL) were transferred to 96-well plates, and methanol/acetonitrile (50%/50%, v/v, 200 μL) containing an internal standard (5 ng/mL terfenadine) was added to each well. Samples were vortexed for 1 minute, and centrifuged at 3000 *g* for 15 minutes.. Supernatant was removed and mixed with an equal volume of water containing formic acid (0.1%). These samples were analyzed by LC-MS/MS, and concentrations of analyte in plasma were determined by interpolation from a standard curve.

Pharmacokinetic parameter generation. A plasma concentration versus time profile was generated for each animal. Standard non-compartmental pharmacokinetic analysis was performed using WinNonlin Professional version 4.1 (Pharsight Corporation, Mountain View, CA, USA) or Watson LIMS 7.4 (Thermo Scientific, Waltham, MA) to determine plasma clearance (CL_p) and volume of distribution at steady state (Vd_{ss}). CL_p was calculated as the i.v. dose divided by the area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$). $AUC_{0-\infty}$ was calculated by the linear trapezoid rule. The terminal slope of the ln(concentration) versus time plot was calculated by linear least-squares regression and $T_{1/2}$ was calculated as 0.693 divided by the absolute value of the slope. The maximum plasma concentration (C_{max}) observed after p.o. dosing and the time at which it was observed (T_{max}) were determined directly from the individual plasma concentration versus time curves. The absolute bioavailability of the oral dose (F) was calculated using the following equation: $F = (AUC_{0-\infty}^{p.o.}/AUC_{0-\infty}^{i.v.}) \times (dose^{i.v.}/dose^{p.o.})$.

LC-MS/MS quantitation. Plasma samples from the pharmacokinetic studies were prepared for analysis by precipitating proteins with four volumes of methanol/acetonitrile (50/50, v/v) containing terfenadine as an internal standard. The samples were vortexed, and the precipitated proteins were removed by centrifugation. The resulting supernatants were transferred to a new 96-well plate, mixed with an equal volume of water containing formic acid (0.1%), and 10 μL were injected into the LC-MS/MS for analysis. The HPLC system consisted of two Shimadzu LC20AD pumps (Columbia, MD) and CTC PAL autosampler (Leap Technologies, Switzerland). The column used was a Kinetix C18 (30 x 3.0 mm, 100A) (Phenomenex, Torrance, CA). The column temperature was at room temperature. Mobile phase A consisted of 0.1 % formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The flow rate was 0.8 mL/min. The initial mobile phase composition was 20% B and remained at 20% B for 0.4 min to equilibrate the column. The column was ramped to 95% B over 0.5 min and held at 95% B for an additional 1.0 min. The mobile phase was returned to initial conditions in 0.01 min. Total analysis time was 2.5 min. A switching valve was used to divert eluents between 0 and 0.5 min to waste. The HPLC was interfaced to a Sciex API 4000 mass spectrometer (Applied Biosystems, Foster City, CA), and was equipped with a TurboIonspray ionization source operating in positive mode. Ultrahigh purity nitrogen gas was used as the nebulizing and turbo gas. The temperature of turbo gas was set at 500 °C. Curtain gas, Gas 1, Gas 2 and CAD gas were set at 35, 90, 90 and 9, respectively. The ion spray voltage was set at 5000 v. Multiple reaction monitoring (MRM) was utilized to monitor **1** (m/z 755.6→99.8), **2** (m/z 729.5→711.4), **3** (m/z 743.5→485.2), **9** (m/z 757.7→388.2), **10** (m/z 770.3→388.2) and the internal standard, terfenadine (m/z 472.4→436.4). LC-MS analysis was done using Analyst Software 1.4 (Applied

Biosystems, Life Technologies Corporation, Carlsbad, CA) and standard curve regression by Watson LIMS 7.4.

References

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LCMS – CMPD **1**

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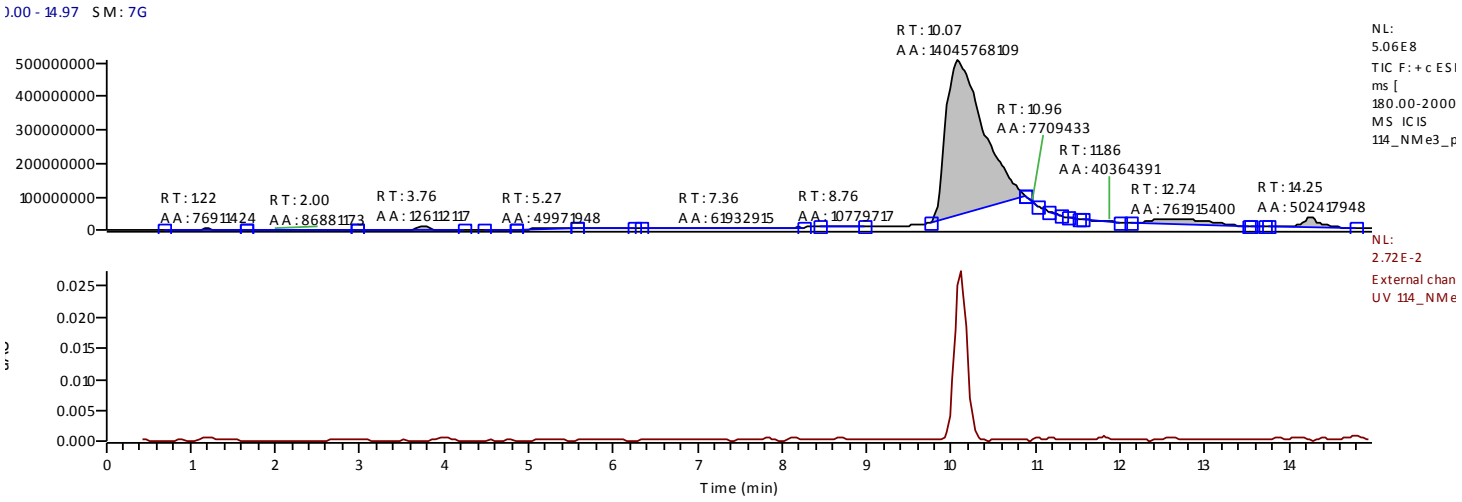

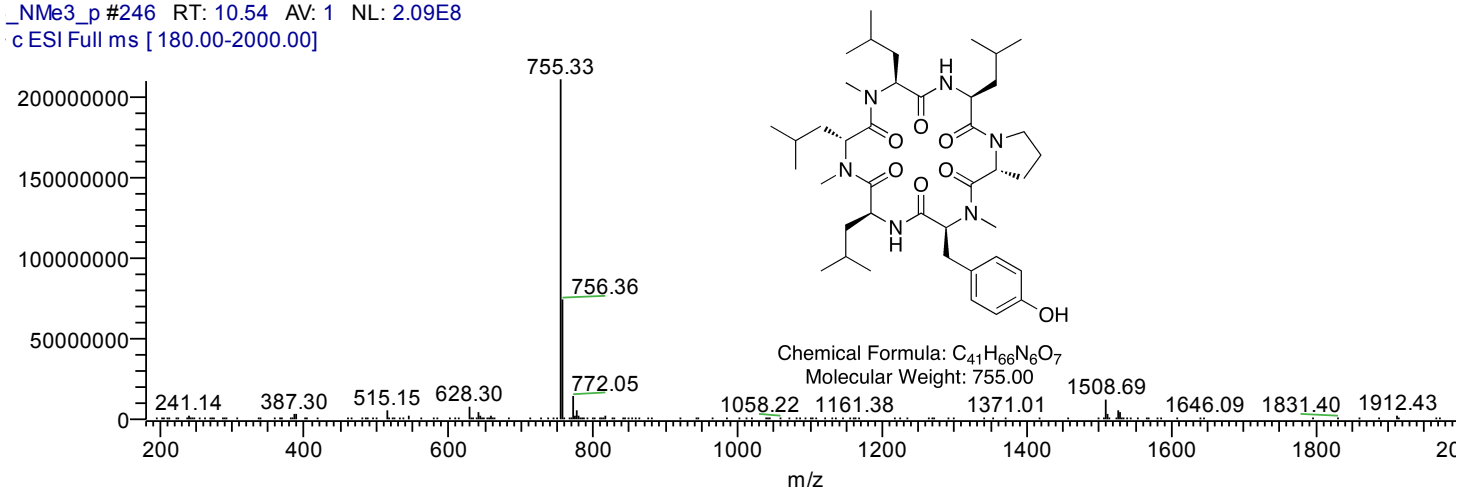

Chemical Formula: $C_{41}H_{66}N_6O_7$
Molecular Weight: 755.00

LCMS – CMPD **2**

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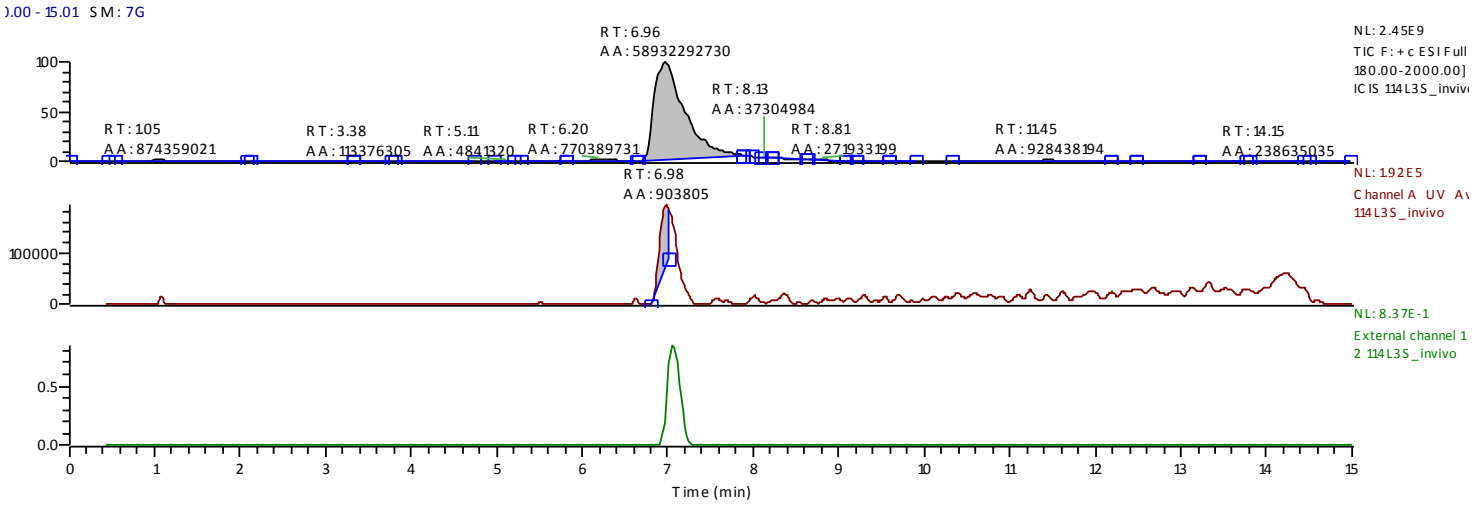
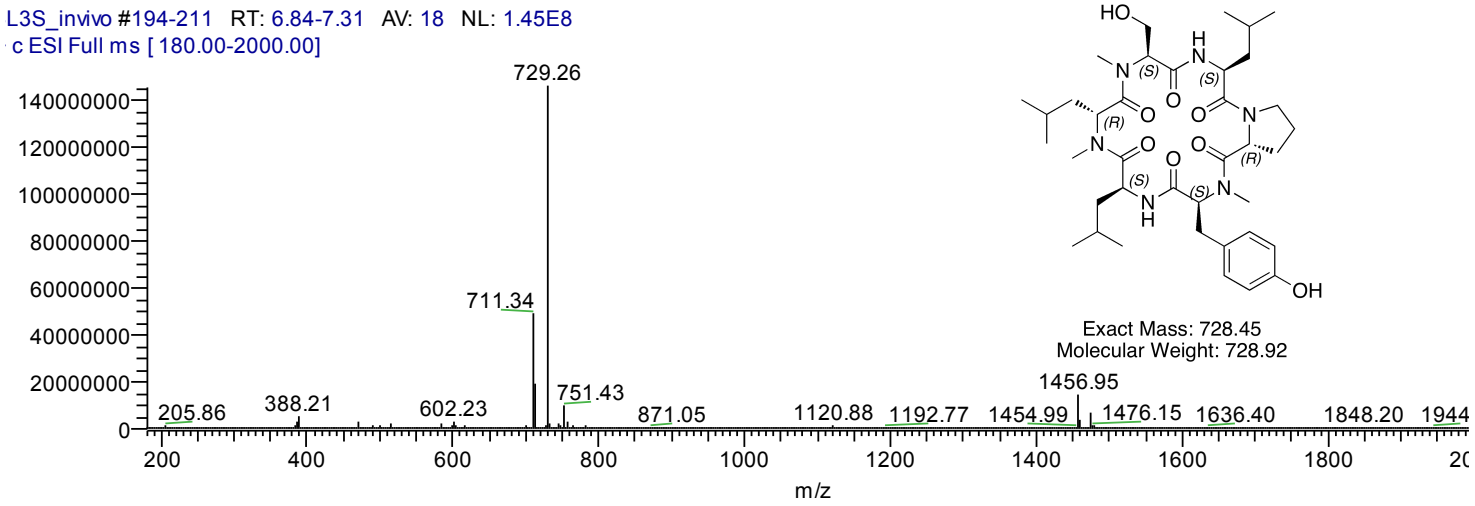
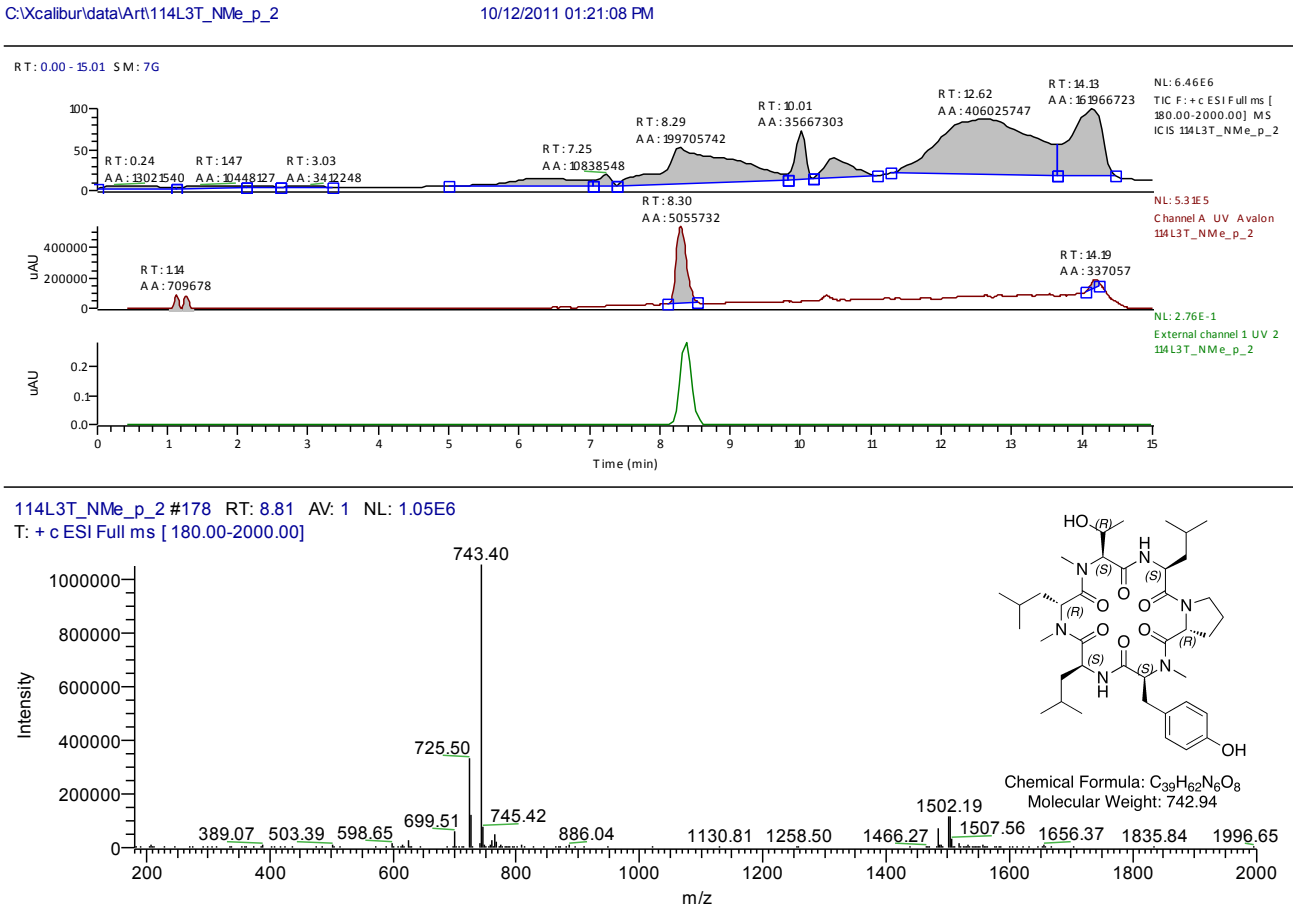


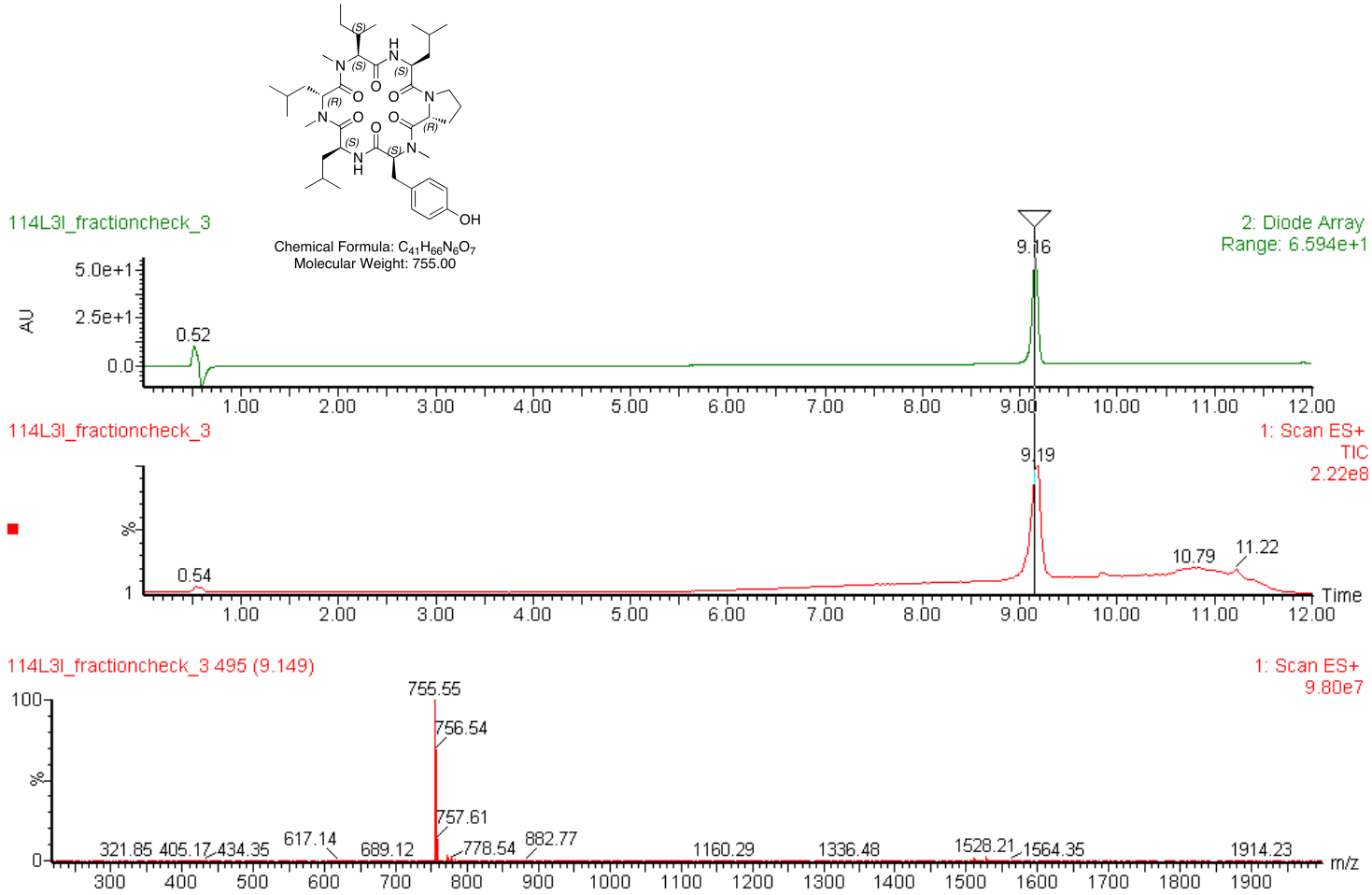


Exact Mass: 728.45
Molecular Weight: 728.92

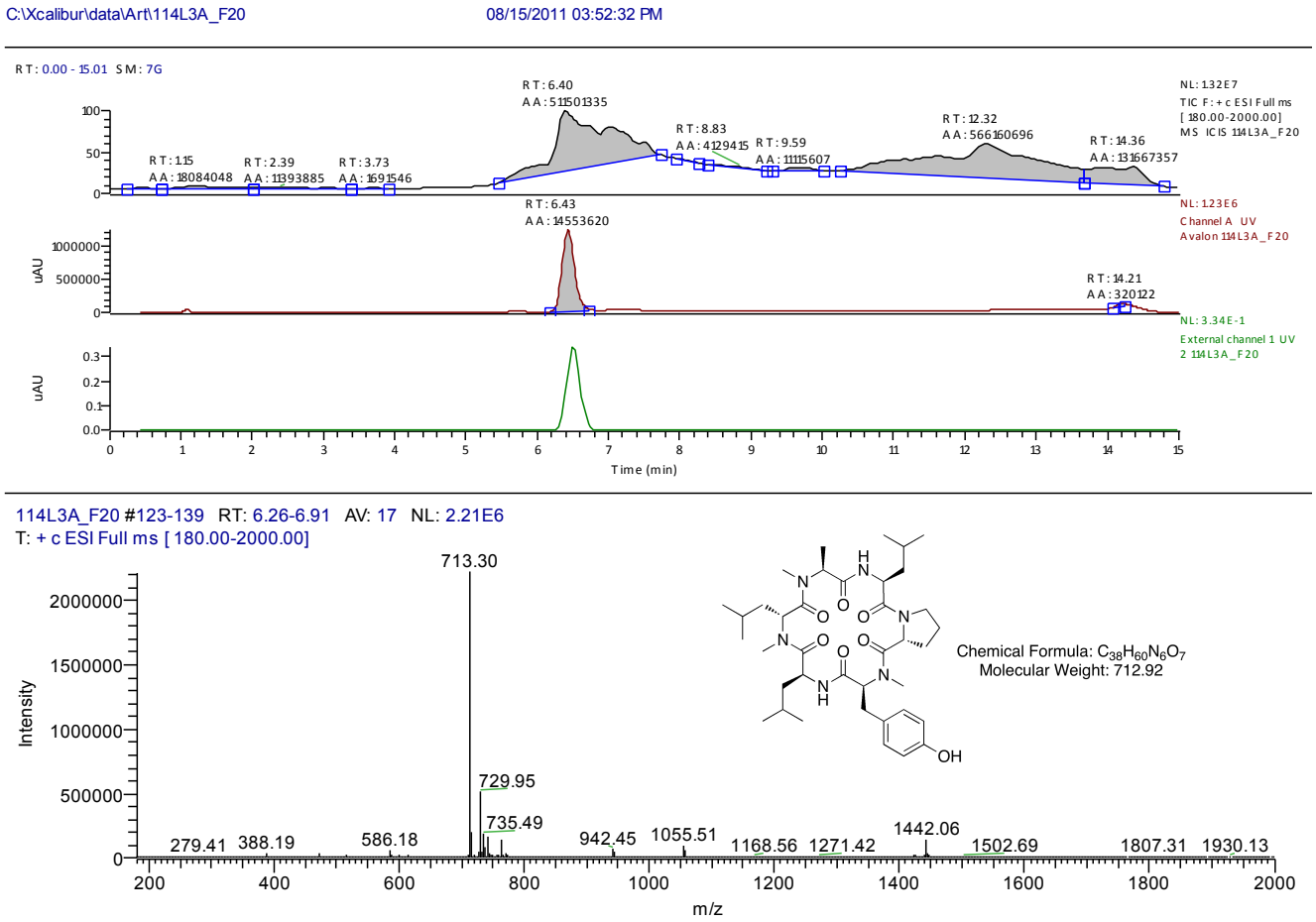
LCMS – CMPD **3**



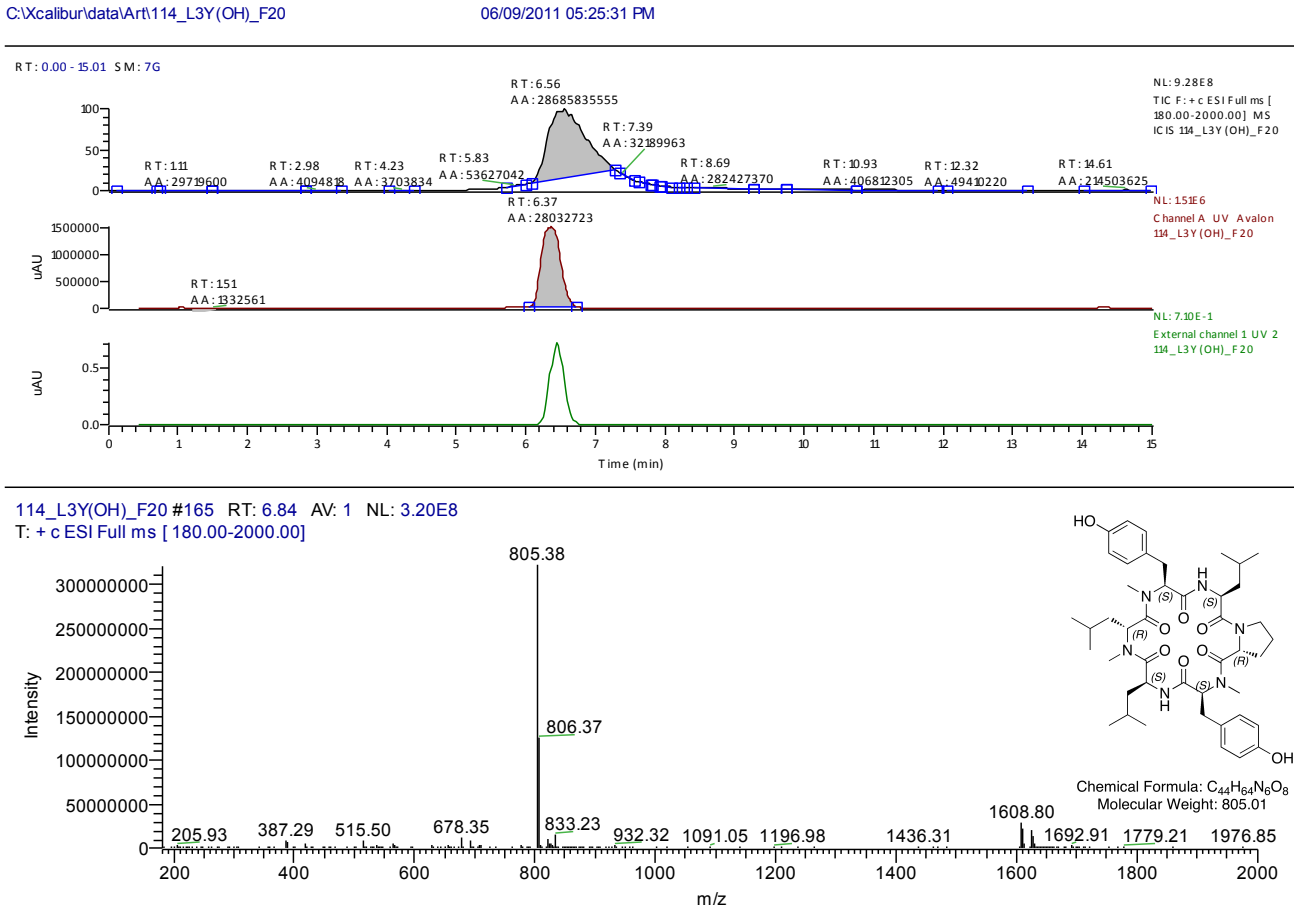
LCMS – CMPD **4**



LCMS – CMPD **5**



LCMS – CMPD **6**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RT: 6.56
AA: 28685835555
RT: 7.39
AA: 32189963
RT: 1.11
AA: 29719600
RT: 2.98
AA: 4094818
RT: 4.23
AA: 3703834
RT: 5.83
AA: 53627042
RT: 8.69
AA: 282427370
RT: 10.93
AA: 40681230
RT: 12.32
AA: 49410220
RT: 14.61
AA: 214503625
NL: 9.28E8
TIC F: + c ESI Full ms [180.00-2000.00] MS IC IS 114_L3Y(OH)_F20
RT: 6.37
AA: 28032723
RT: 1.51
AA: 1332561
NL: 1.51E6
Channel A UV Avalon 114_L3Y(OH)_F20
NL: 7.10E-1
External channel 1 UV 2 114_L3Y(OH)_F20
uAU
Time (min)

114_L3Y(OH)_F20 #165 RT: 6.84 AV: 1 NL: 3.20E8
T: + c ESI Full ms [180.00-2000.00]
805.38
806.37
833.23
205.93
387.29
515.50
678.35
932.32
1091.05
1196.98
1436.31
1608.80
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1976.85
Intensity
m/z
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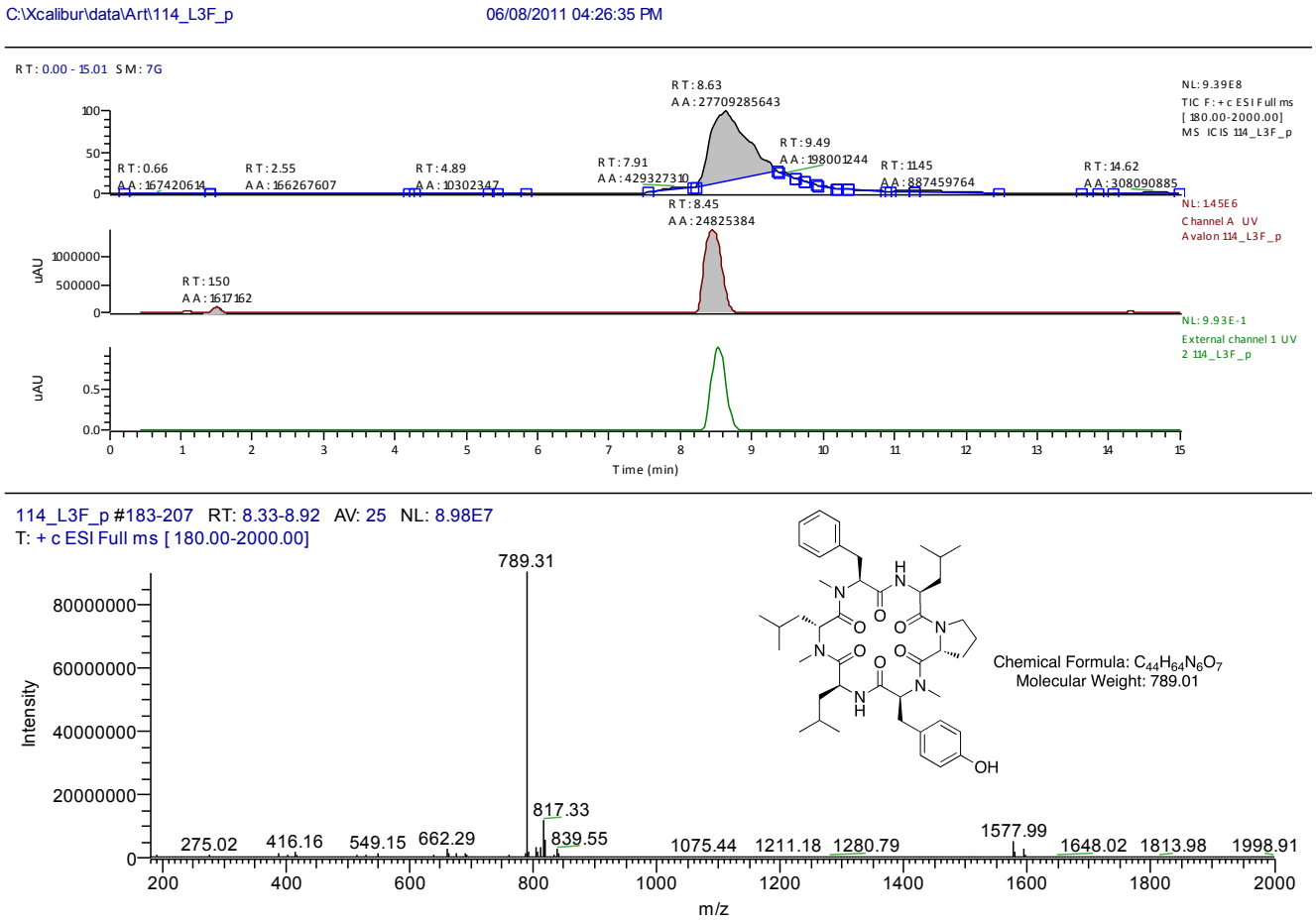
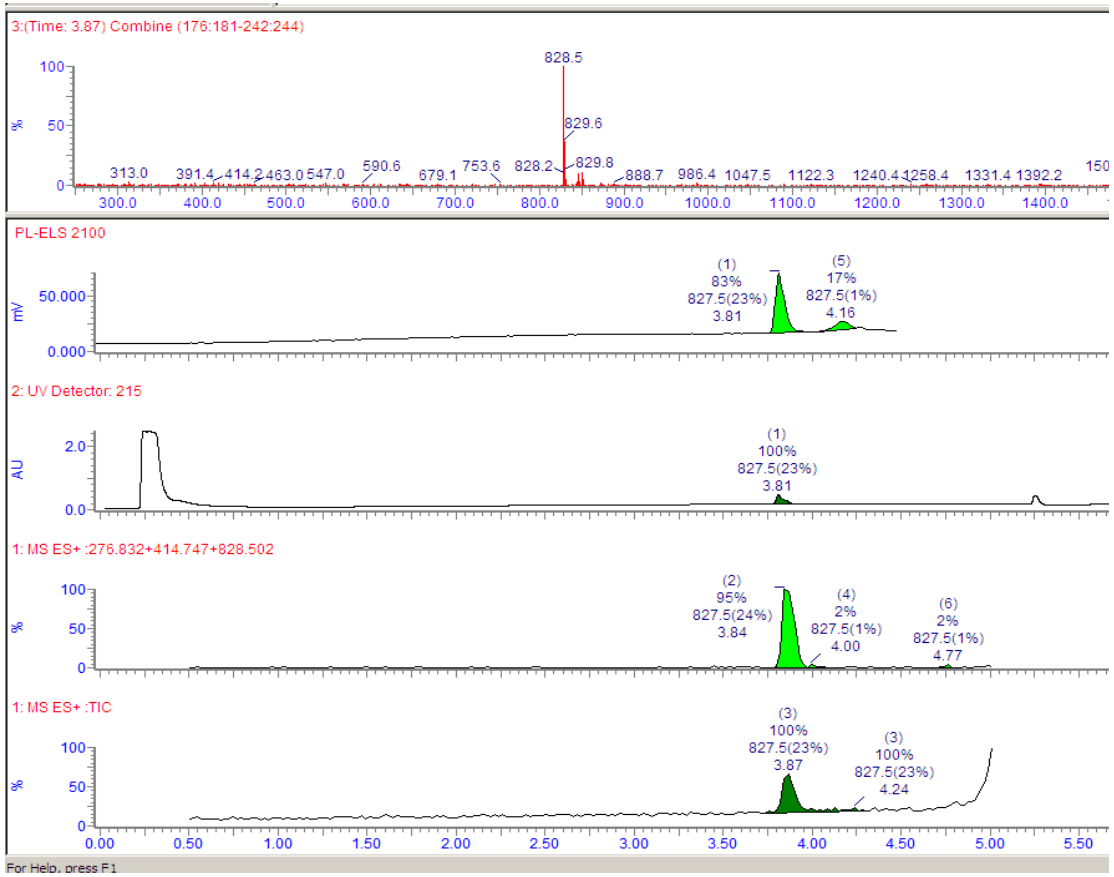
Molecular Weight: 805.01

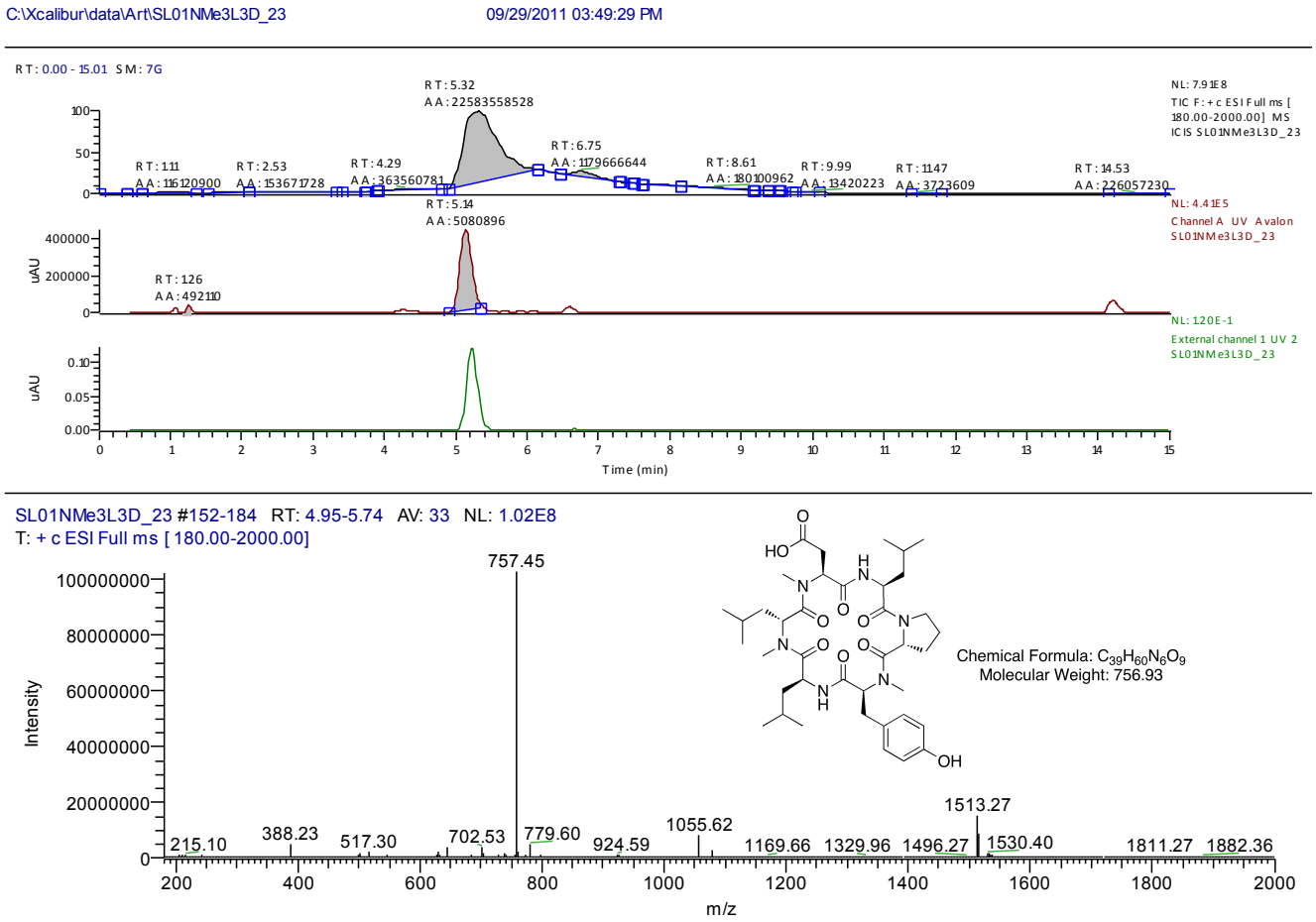
LCMS – CMPD **7**



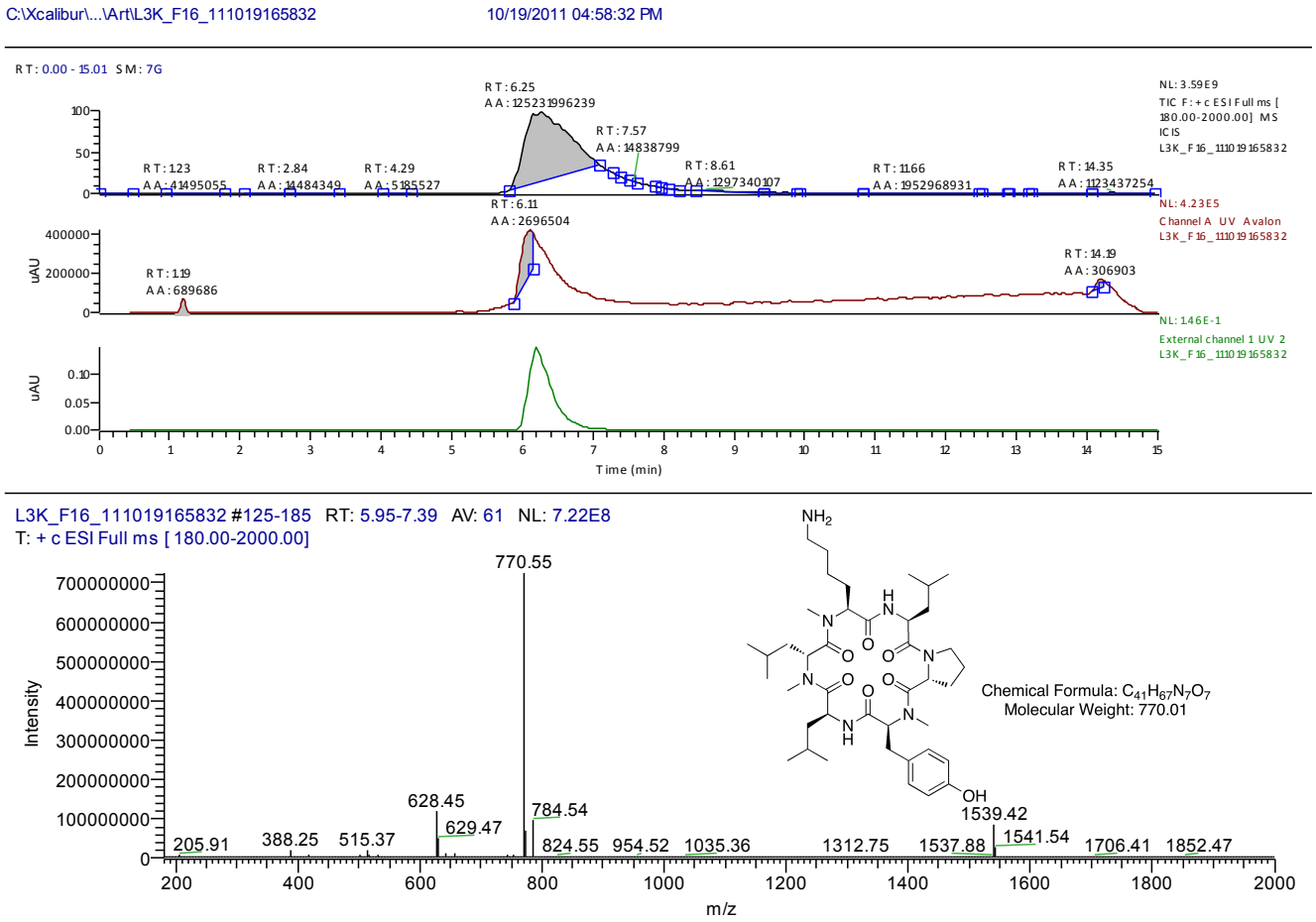
LCMS – CMPD **8**



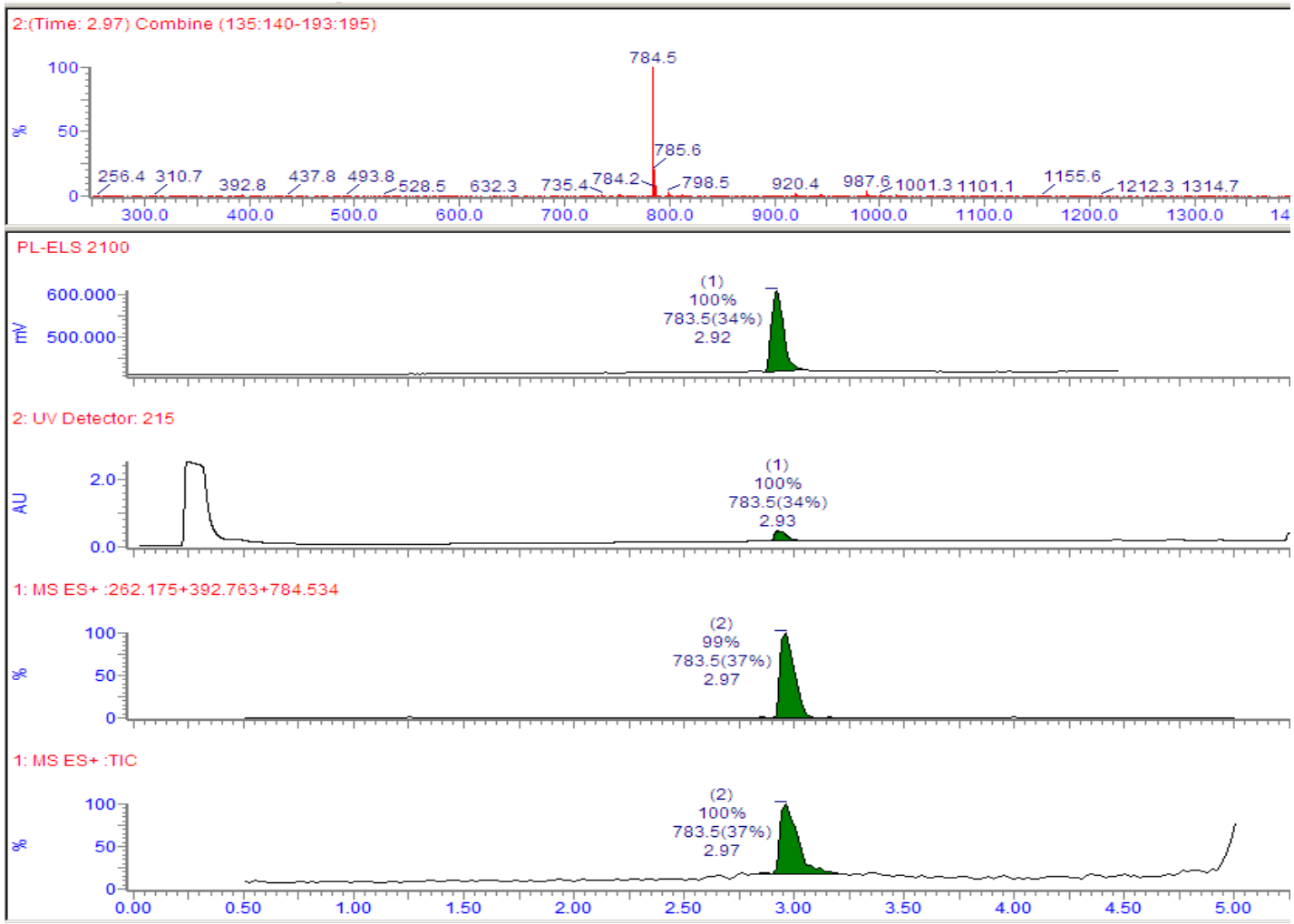
LCMS – CMPD **9**



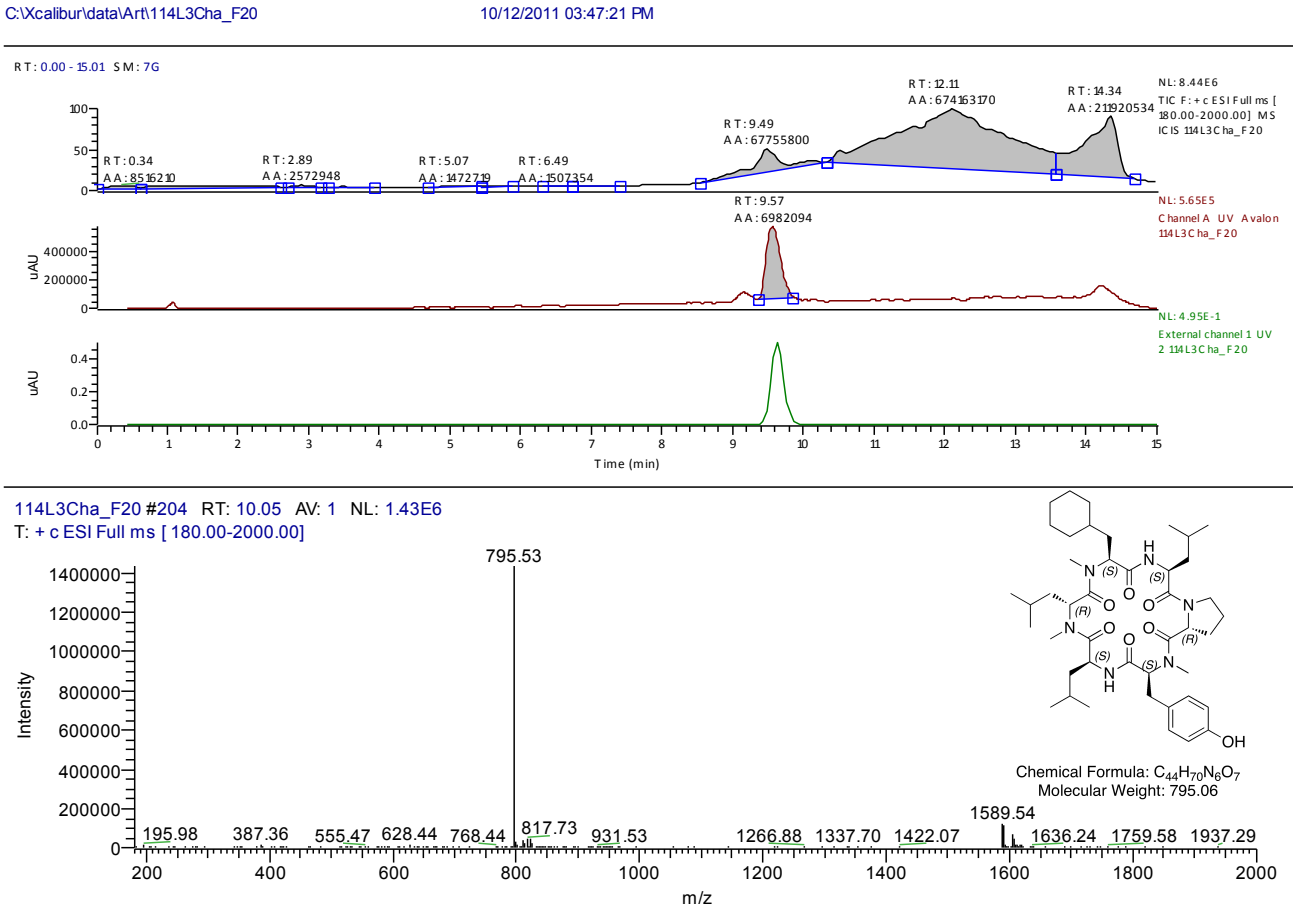
LCMS – CMPD **10**



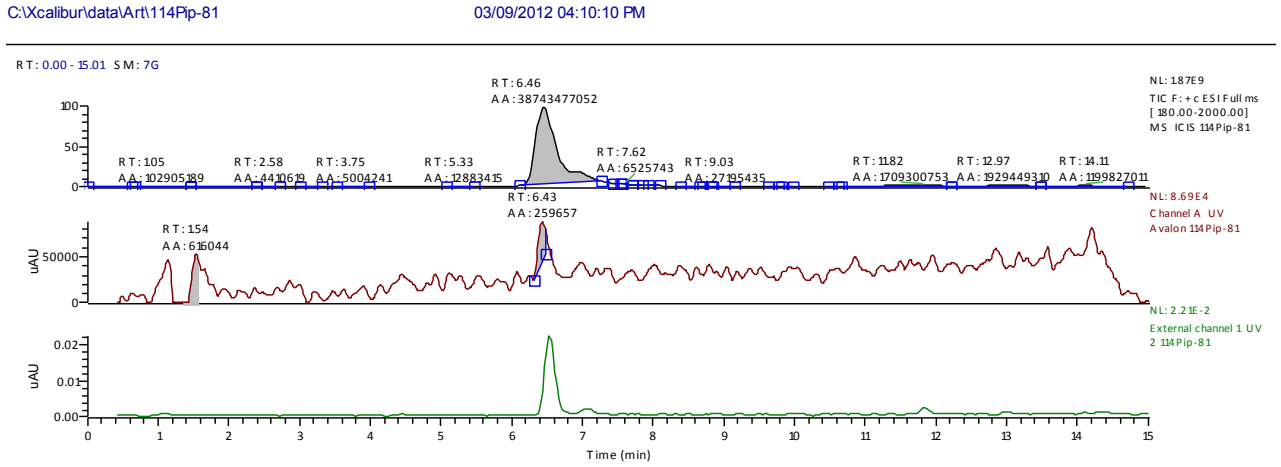
LCMS – CMPD **11**

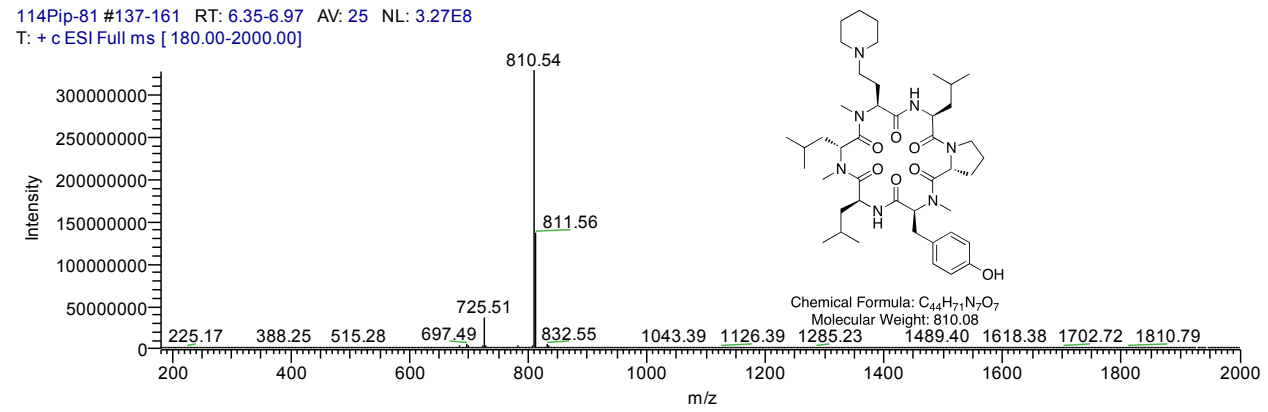


LCMS – CMPD **12**

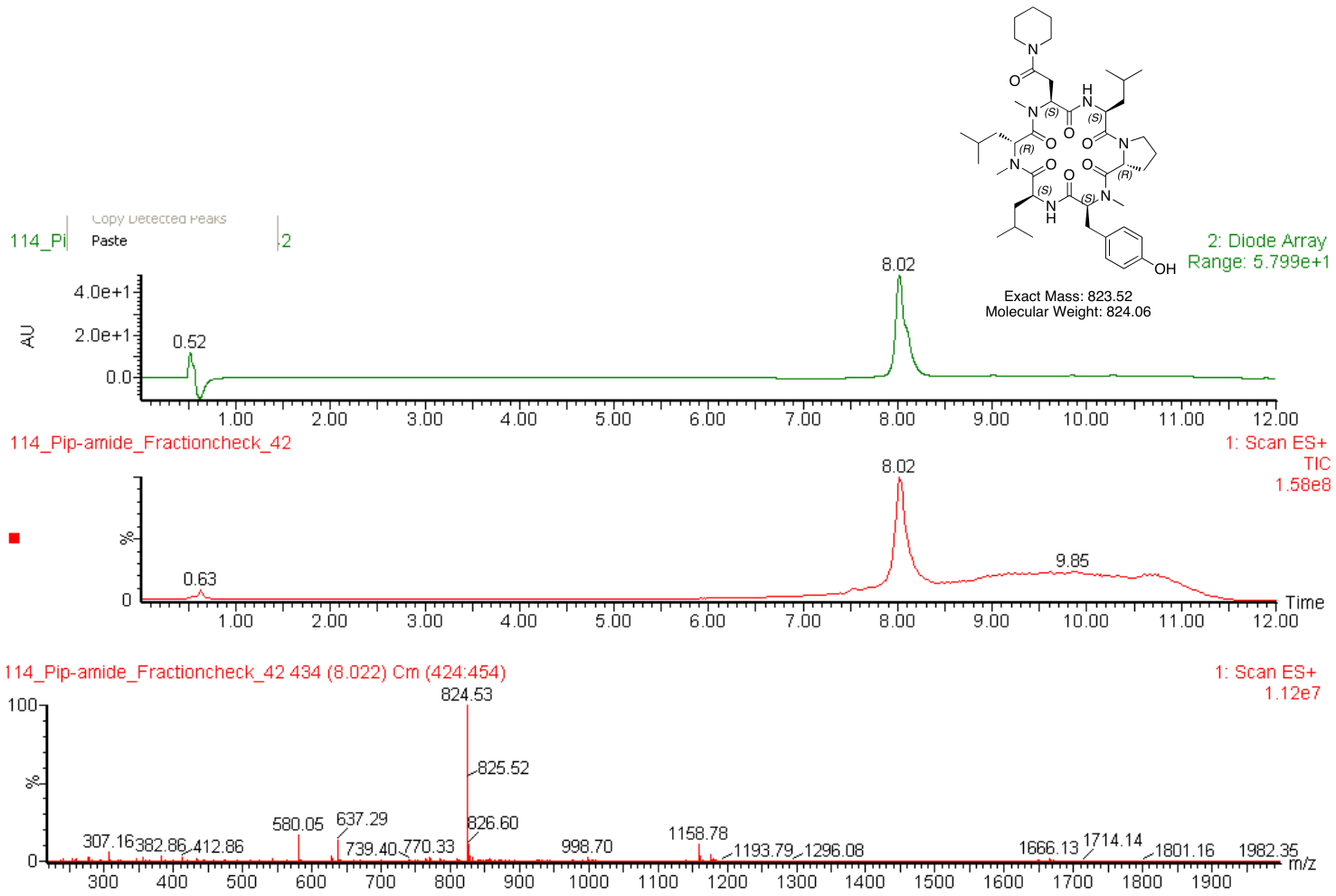


LCMS – CMPD **13**

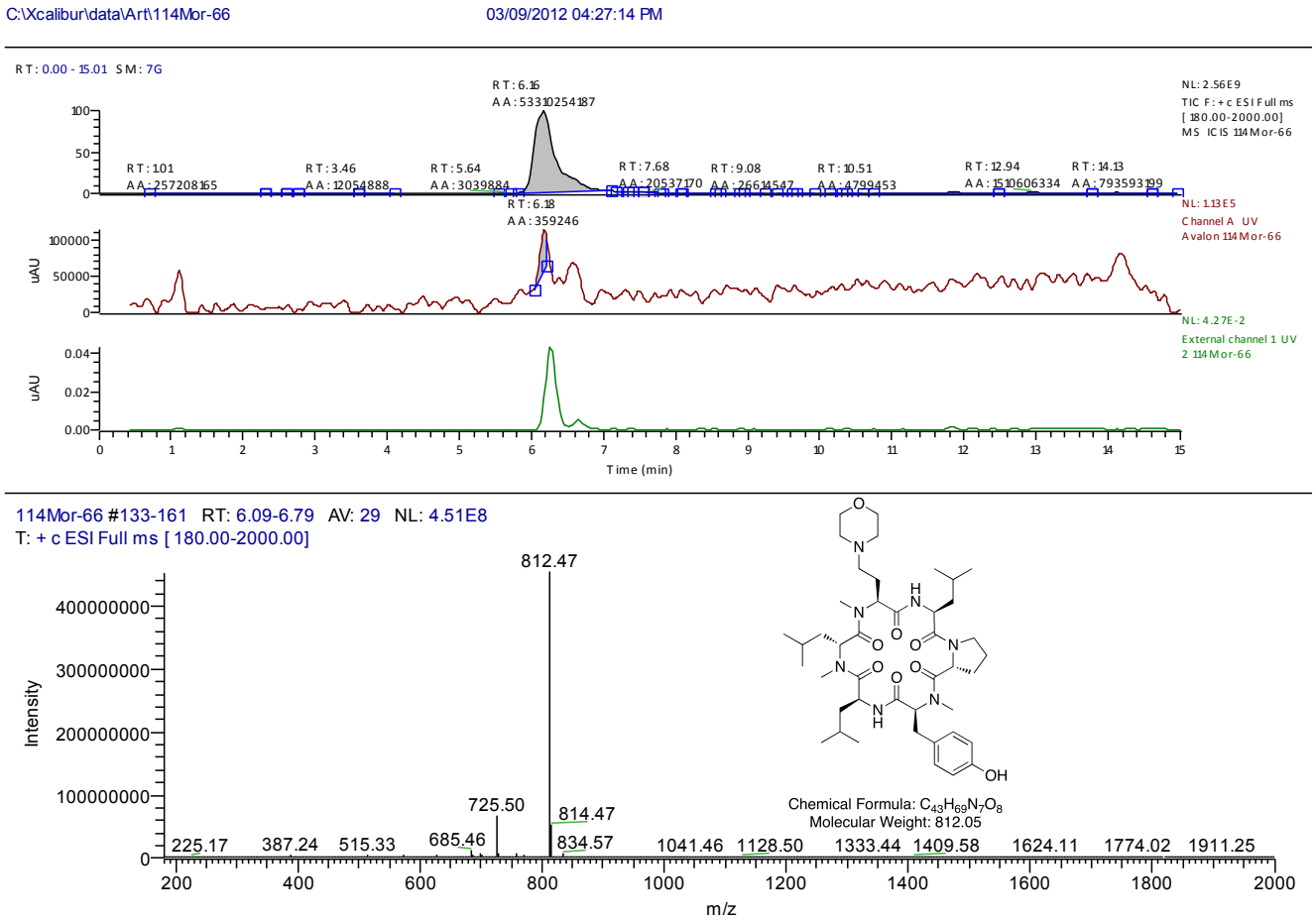




LCMS – CMPD **14**



LCMS – CMPD **15**



LCMS – CMPD **16**