SUPPLEMENTAL INFORMATION FOR:

An autoinducer analog reveals an alternative mode of ligand binding for the LasR quorum-sensing receptor

Jon E. Paczkowski[1], Amelia R. McCready[1], Jian-Ping Cong[1,2], Zhijie Li[3], Philip D. Jeffrey[1], Chari D. Smith[1], Brad R. Henke[4], Frederick M. Hughson[1], and Bonnie L. Bassler[1,2,*]

[1]Princeton University, Department of Molecular Biology, Princeton, NJ 08544, [2]Howard Hughes Medical Institute, Chevy Chase, MD 20815, [3]North Carolina State University, Department of Molecular and Structural Biochemistry, Raleigh, NC 27695, [4]Opti-Mol Consulting, LLC, Cary, NC 27513

[*]To whom correspondence should be addressed: Prof. Bonnie L. Bassler, Department of Molecular Biology, Princeton University, 329 Lewis Thomas Laboratory, Princeton, NJ 08544. Email: bbassler@princeton.edu

Supplemental Information contents:

References

SUPPLEMENTAL INFORMATION

Supplemental Figure 1. *Synthesis of novel LasR ligand analogs.* Synthetic routes for A) BB0231, BB0232, and BB0233 and B) BB0020, BB0126, BB0272, and BB0273. The scheme for the synthesis of BB0221 is not shown because it is only one step from the intermediate we show in the scheme and procedures for attaching the lactone head group are known. See Supplemental Methods for details. Reagents and conditions for panel A: (a) $(Boc)_2O$, Et_3N, dioxane, rt (i.e., room temperature); (b) *N,O*-dimethylhydroxylamine·HCl, Et_3N, PYBOP, DCM, rt; (c) vinylmagnesium bromide, THF, -78 °C; (d) Grubb's 2nd-gen catalyst, DCM, rt; (e) $(Boc)_2O$, DMAP, CH_3CN, rt; (f) NaH, trimethyloxosulfonium iodide, DMSO, 50 °C; (g) TFA, DCM, rt; (h) $CeCl_3 \cdot 7H_2O$, NaI, CH_3CN, rt; (i) $Li(OtBu)_3AlH$, EtOH, -78 °C to rt; (j) HCl/EtOAc, rt; (k) $LiBH_4$, THF, -78 °C to rt; (l) dodecanoyl chloride, Et_3N, DCM, rt. Reagents and conditions for panel B: (a) K_2CO_3, DMF, 100 °C; (b) *m*-CPBA, $CHCl_3$ rt; (c) $LiOH \cdot H_2O$, THF/H_2O, rt; (d) one of three amide coupling conditions were used: (i) EDCI, HOBt, DIPEA, DMF, rt; (BB0272, BB0273) (ii) isopropyl chloroformate, Et_3N, THF, 0 °C to rt; (BB0126) (iii) oxalyl chloride, cat. DMF, DIPEA, DCM, rt. (BB0020).

Supplemental Figure 2. *Electron density for ligands in the LasR LBD and LasR LBD T75V/Y93F/A127W structures.* A simulated annealing omit map, contoured at 1σ, shows the electron density (gray) around A) mBTL (red), B) BB0020 (green), c) BB0126 (blue) in the LasR LBD, and D) BB0126 (blue) in the LasR LBD T75V/Y93F/A127W.

Supplemental Figure 3. *The sulfonyl group on the BB0221 ligand drives enhanced potency with LasR mutants*. A) Dose response analyses using the *E. coli lasB-lux* reporter strain expressing WT LasR (black), LasR T75V (red), LasR Y93F (blue), and LasR A127W (purple) to BB0221. B) As in panel A with LasR T75V/Y93F (orange), LasR Y93F/A127W (green), LasR T75V/A127W (magenta), and LasR T75V/Y93F/A127W (cyan). Dose response data are depicted as curve fits with the raw data plotted as individual points. Error bars represent SEM, *n*=3.

Supplemental Figure 4. *$3OC_{12}HSL$ and synthetic agonists exhibit reduced potency with LasR R61A.* A) Dose response analyses using the *E. coli lasB-lux* reporter strain expressing WT LasR (black) and LasR R61A (brown) to $3OC_{12}HSL$, B) mBTL, C) BB0020, D) BB0126, E) BB0221, F) BB0231, G) BB0232, H) BB0233, I) BB0272, and J) BB0273. Dose response data are depicted as curve fits with the raw data plotted as individual points. Error bars represent SEM, *n*=3.

Supplemental Figure 5. *Comparison of the CviR LBD:CL and LasR LBD:mBTL binding pockets.* A) Structures of CL and mBTL. B) Overlay of the crystal structures of CviR LBD:CL (CviR: blue; CL: gold, PDB: 3QP5[62]) and LasR LBD:mBTL (LasR: green; mBTL: red). C) Docking of CL (gold) in the LBD of LasR compared to crystal structure of the LasR LBD containing mBTL (red).

Supplemental Table 1. Primers used in this study

Name	Sequence	Description
oARM203	cggttttcttgagctggaacgc	LasR forward primer
oARM204	aacggccataatggccgctac	LasR reverse primer
oJP779	acagtgactgaccactgggtcgacccgcgc	lasRT75V forward
oJP780	gcgcgggtcgacccagtggtcagtcactgt	lasRT75V reverse
oJP819	gctgaggctcagccagccgagttcgccg	lasRA127W forward
oJP820	cggcgaactcggctggctgagcctcagc	lasRA127W reverse
oJP933	gtcgtaatgctcggcccaggcggccggg	lasRR61A forward
oJP934	cccggccgcctgggccgagcattacgac	lasRR61A reverse
oARM449	cgcgtctggaagatggacggttcccag	lasRY93F forward
oARM450	ctgggaaccgtccatcttccagacgcg	lasRY93F reverse
oARM455	taattaagcttccgaactggaaaagtggctatgtcgcc	lasR pEXG2 upstream forward HindIII
oARM456	tattagtcgacgctcgccgacctgagaggcaaga	lasR pEXG2 downstream reverse SalI
oARM470	taattggatccccgaactggaaaagtggctatgtcgcc	pUCP18 lasR upstream forward BamHI
oARM471	tattagaattcgctcgccgacctgagaggcaaga	pUCP18 lasR downstream reverse EcoRI
oARM472	ctcgactaacccagatgccg	pUCP18 lasR overlapping forward
oARM473	ttggagcgaacgacctacac	pUCP18 lasR overlapping reverse

Supplemental Table 2. Strains used in this study

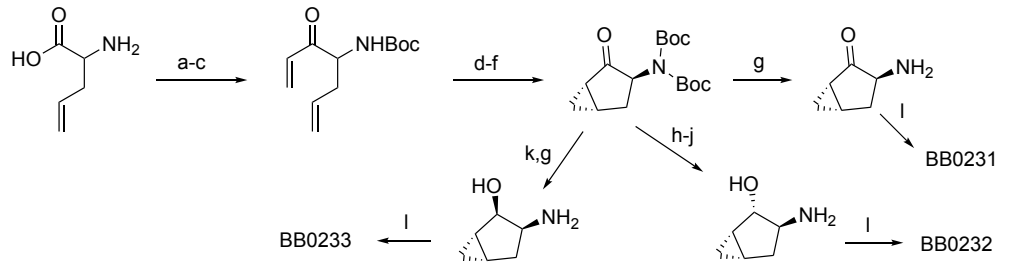
Name	Description	Source
UCBPP-PA14	Wild type, generous gift from Dr. George O'Toole	(Kukavica-Ibruli *et al*., 2008)
SM51	*P. aeruginosa* Δ*lasI*	(Mukherjee *et al*., 2017)
JP113	*E. coli* pBad-A-*lasR* PA14 pCS26-*lasB-lux*	(Paczkowski *et al*., 2017)
AM33	*E. coli* pBad-A-*lasR*-Y93F PA14 pCS26-*lasB-lux*	This study
JP148	*E. coli* pBad-A-*lasR*-T75V PA14 pCS26-*lasB-lux*	This study
JP149	*E. coli* pBad-A-*lasR*-T75V/Y93F PA14 pCS26-*lasB-lux*	This study
JP150	*E. coli* pBad-A-*lasR*-A127W PA14 pCS26-*lasB-lux*	This study
JP151	*E. coli* pBad-A-*lasR*-Y93F/A127W PA14 pCS26-*lasB-lux*	This study
JP152	*E. coli* pBad-A-*lasR*-T75V/A127W PA14 pCS26-*lasB-lux*	This study
JP153	*E. coli* pBad-A-*lasR*-T75V/Y93F/A127W PA14 pCS26-*lasB-lux*	This study
JP154	*P. aeruginosa* Δ*lasI* LasR-T75V/Y93F/A127W	This study
JP156	*E. coli* pBad-A-*lasR*-R61A PA14 pCS26-*lasB-lux*	This study
JP128	*E. coli* DH-IBP-6xHis-*lasR:LBD* (1-170)	(Paczkowski *et al*., 2017)
JP155	*E. coli* DH-IBP-6xHis-*lasR:LBD*-T75V/Y93F/A127W (1-170)	This study
SM10l*pir*	*E. coli thi thr leu tonA lacY supE recA*::RP4-2-Tc::Mu	(Simon et al., 1983)
pEXG2	Allelic exchange vector with pBR origin, gentamicin resistance, *sacB,* generous gift from Dr. Joseph Mougous	(Borlee et al., 2010)
pUCP18	*E. coli-Pseudomonas* Amp^r shuttle vector	(Schweizer., 1991)
BL21 (DE3)	*E. coli* B F– *dcm ompT hsdS(r – m –) gal* λ(DE3)	Agilent
One Shot Top10	*E. coli* F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara- leu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Thermo-Fisher

Supplemental Table 3. R^2 values for nonlinear regression analysis.

	Compound Class									
	I	I	I	II	II	I	II	II	III	III
LasR	$3OC_{12}HSL$	mBTL	BB0020	BB0126	BB0221	BB0231	BB0232	BB0233	BB0272	BB0273
WT	0.9	0.9	0.8	0.7	0.5	0.7	0.7	0.8	0.5	0.6
T75V	0.7	0.8	0.8	0.5	0.5	0.5	0.8	0.7	NR	NR
Y93F	0.8	0.9	0.8	0.5	0.4	0.8	0.7	0.8	NR	NR
A127W	0.8	0.9	0.8	0.5	0.5	0.7	NR	NR	NR	NR
T75V/Y93F	0.9	0.8	0.8	0.6	0.4	0.7	0.6	0.8	NR	NR
T75V/A127W	0.9	0.9	0.8	0.7	0.6	0.6	NR	NR	0.6	0.5
Y93F/A127W	0.7	0.8	0.8	0.6	0.4	0.7	NR	NR	NR	0.5
T75V/Y93F/A127W	0.9	0.9	0.9	0.7	0.7	0.6	NR	NR	0.6	0.7
R61A	0.8	0.8	0.8	0.6	0.5	0.7	0.5	0.5	NR	NR
NR = non-responsive										

Supplemental Figure 1

A



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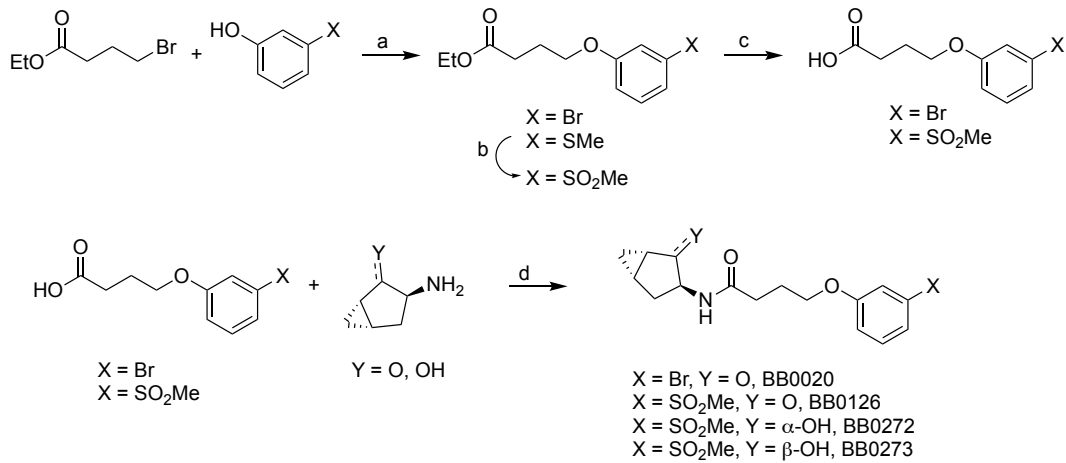
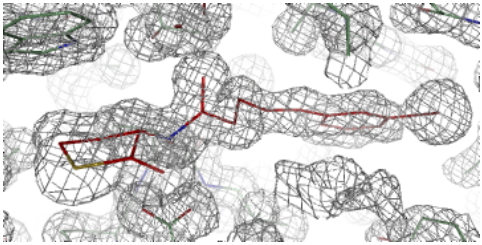


Supplemental Figure 2

A



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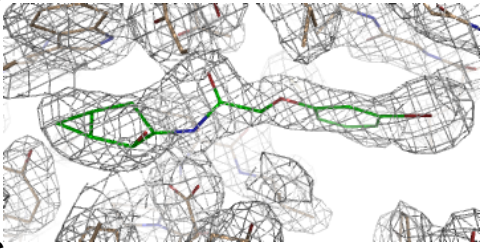

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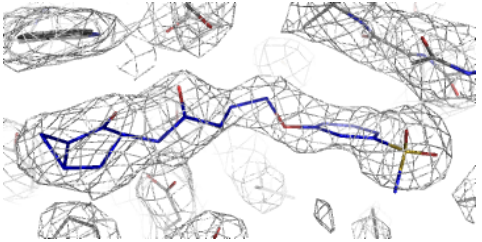

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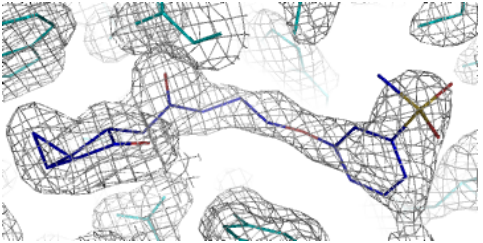
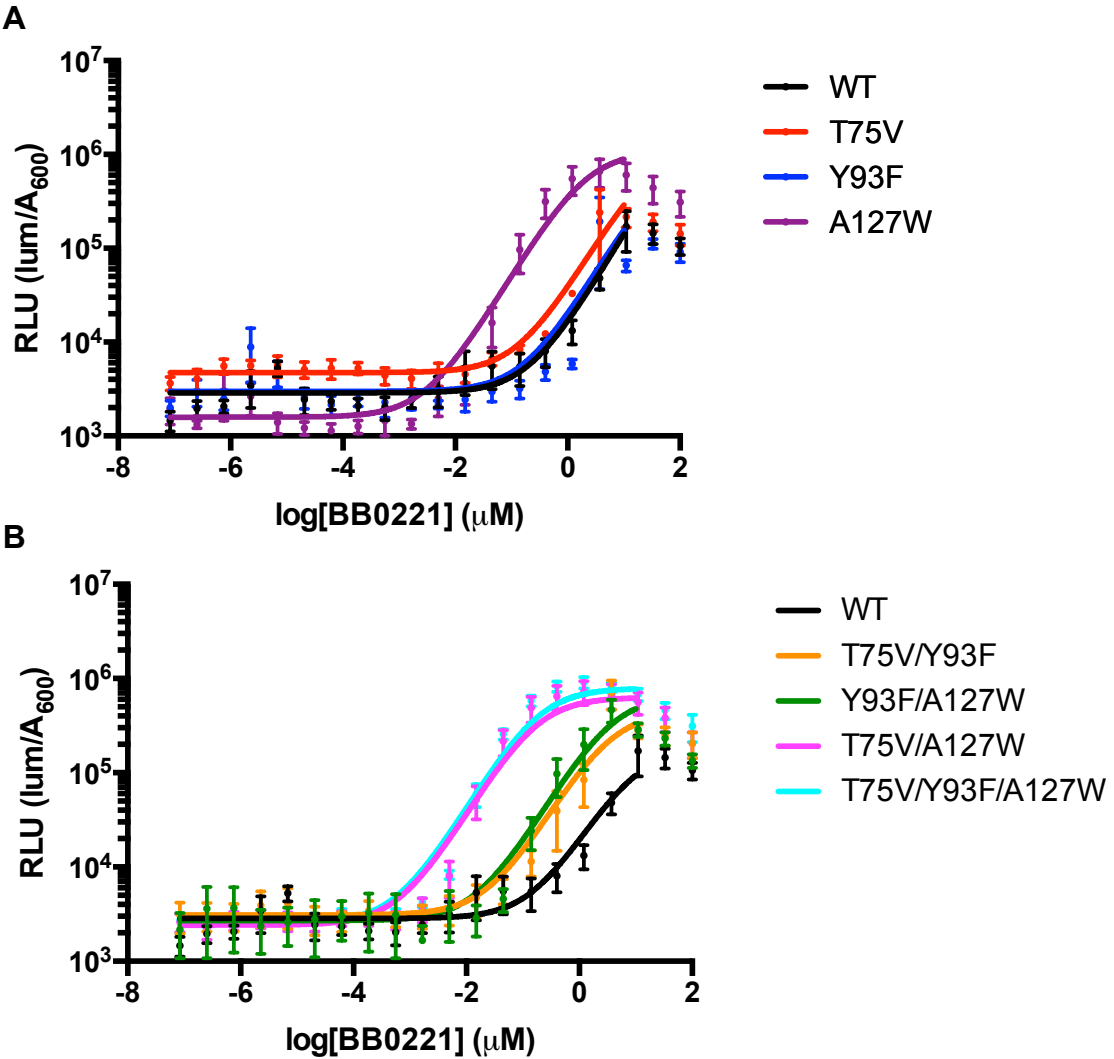
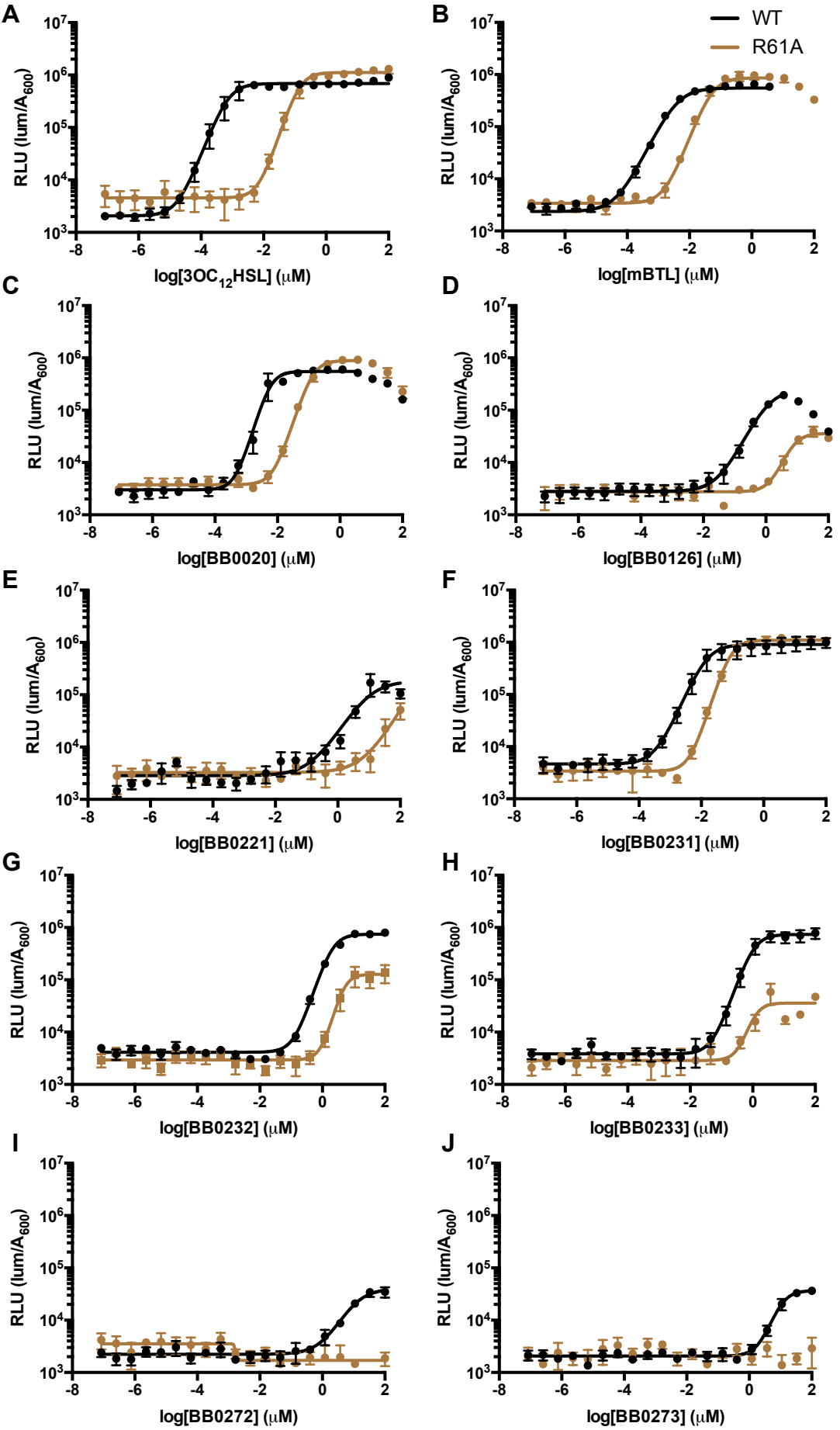


Supplemental Figure 3

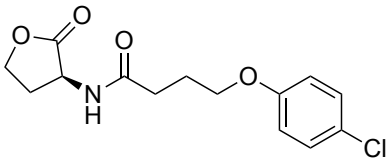


Supplemental Figure 4

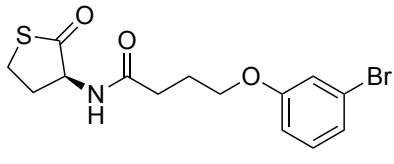


Supplemental Figure 5

A

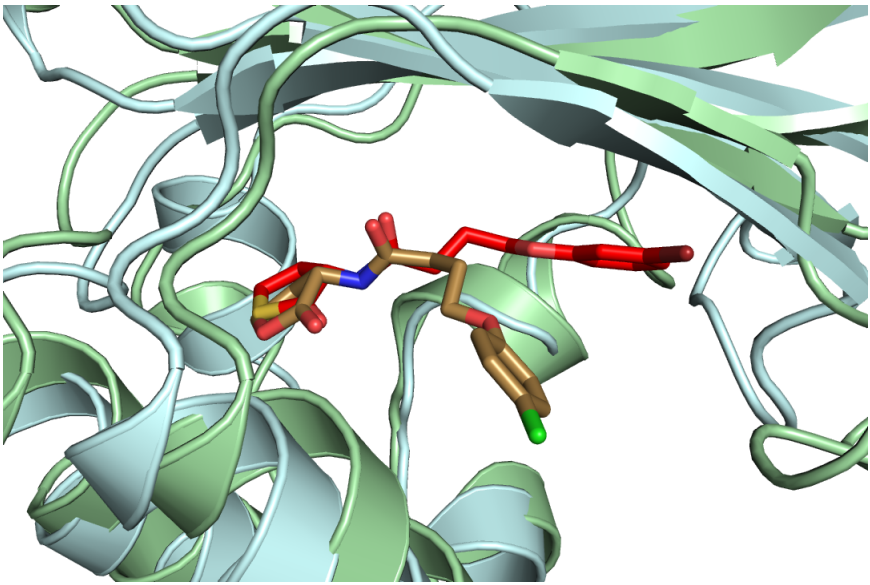


CL



mBTL

B



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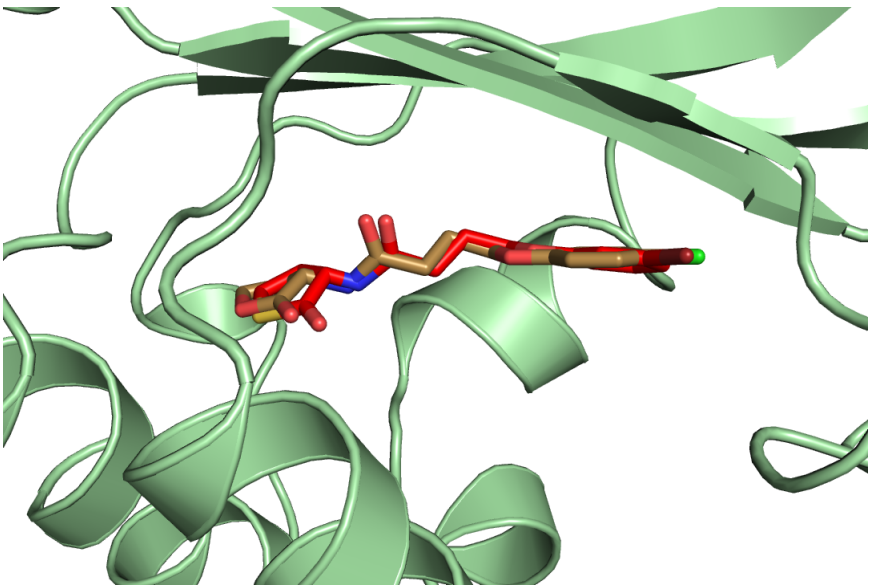


SUPPLEMENTAL METHODS

Abbreviations:

As used herein the symbols and conventions used in these processes are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry. Specifically, the following abbreviations may be used:

g (grams);	mg (milligrams);
L (liters);	mL (milliliters);
µL (microliters);	psi (pounds per square inch);
M (molar);	mM (millimolar);
µM (micromolar)	MHz (megahertz);
mol (moles);	mmol (millimoles);
rt (room temperature);	hr (hours);
min (minutes);	TLC (thin layer chromatography);
mp (melting point);	RP (reverse phase);
T_r (retention time);	TFA (trifluoroacetic acid);
Et_3N (triethylamine);	THF (tetrahydrofuran);
TFAA (trifluoroacetic anhydride);	$CDCl_3$ (deuterated chloroform);
CD_3OD (deuterated methanol);	DMSO (dimethylsulfoxide);
SiO_2 (silica);	atm (atmosphere);
EtOAc (ethyl acetate);	CHCl3 (chloroform);
HCl (hydrochloric acid);	Ac (acetyl);
DMF (N,N-dimethylformamide);	Me (methyl);
Cs_2CO_3 (cesium carbonate);	EtOH (ethanol);
MeOH (methanol);	p-TsOH (p-toluenesulfonic acid);
DCM (dichloromethane);	DCE (dichloroethane);
K_2CO_3 (potassium carbonate);	Na_2CO_3 (sodium carbonate);
$NaHCO_3$ (sodium bicarbonate);	ACN (acetonitrile);

PE (petroleum ether); Hex (hexanes);

H_2SO_4 (sulfuric acid); $Na_2S_2O_3$ (sodium thiosulfate)

Et_3N (triethylamine); Na2SO4 (sodium sulfate);

MTBE (methyl tert-butyl ether); Boc (tert-butoxycarbonyl);

DIPEA (diisopropylethylamine); IPA (isopropanol);

DMAP (dimethylaminopyridine) EDCI (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide)

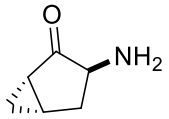
HOBt (hydroxybenzotriazole) NMP (N-methyl-2-pyrrolidone)

HOSu (N-hydroxysuccinimide) *m*-CPBA (m-chloroperoxybenzoic acid)

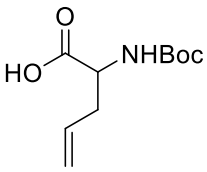
PYBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate)

Unless otherwise indicated, all temperatures are expressed in °C (degrees Centigrade). All reactions were conducted at room temperature unless otherwise noted. ^{1}H-NMR spectra were recorded on a Varian VXR-400, or a Varian Unity-400 at 400 MHz field strength. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants (*J*) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). The mass spec was run on a Sciex API 100 using electrospray ionization (ESI). The LCMS was run using a C-18 reverse phase column (2.1 ID, 3.5 micron, 50 mm). The column conditions were 98% H_2O with 0.05%TFA and 2% MeOH to 100% MeOH over 5.5 minutes. Analytical thin layer chromatography was used to verify the purity as well as to follow the progress of reaction(s). Unless otherwise indicated, all final products were at least 95% pure as judged by HPLC-MS.

rel-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one



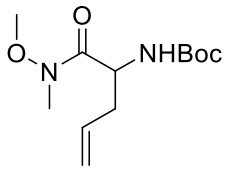
Step 1: 2-(*tert*-butoxycarbonylamino)pent-4-enoic acid



To a suspension of 2-aminopent-4-enoic acid (35.0 g, 304 mmol, 1.00 eq) in dioxane (1.20 L) and H_2O (30 mL) was added *tert*-butoxycarbonyl *tert*-butyl carbonate (139.33 g,

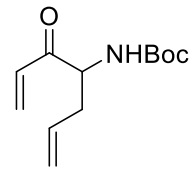
638.4 mmol, 147 mL, 2.10 eq), and Et_3N (76.9 g, 760 mmol, 105 mL, 2.50 eq). The mixture was stirred at rt for 12 hr. The reaction mixture was concentrated, and the residue was diluted with EtOAc (400 mL) and extracted with $NaHCO_3$ (500 mL); the organic layer was discarded. The aqueous layer was washed with EtOAc (2 x 400 mL). The aqueous was acidified to pH=3 with 2N H_2SO_4 and then extracted with EtOAc (3 x 500 mL). The combined organic layer was concentrated to give 2-(*tert*-butoxycarbonylamino)pent-4-enoic acid (116 g, 538.9 mmol, 88% yield) as a white solid. ^{1}HNMR: (400 MHz, $CDCl_3$) δ 9.06 (s, br, 1H), 5.77 (m, 1H), 5.20 (m, 2H), 5.09 (m, 1H), 4.43 (m,1H), 2.65 (m, 2H), 1.47(s, 9H).

Step 2: *tert*-butyl *N*-[1-[methoxy(methyl)carbamoyl]but-3-enyl]carbamate



To a solution of 2-(*tert*-butoxycarbonylamino)pent-4-enoic acid (60 g, 278.7 mmol, 1.00 eq) in DCM (600 mL) was added Et_3N (56.4 g, 557.5 mmol, 77.3 mL, 2.00 eq) and PYBOP (174.0 g, 334.5 mmol, 1.20 eq). The mixture was stirred at rt for 1 hr, then *N*-methoxymethanamine hydrochloride (29.91 g, 306.63 mmol, 1.10 eq) was added. The final mixture was stirred at rt for 11 hr. The reaction mixture was diluted with DCM (300 mL), washed with sub-saturated aqueous citric acid (3 x 400 mL) followed by brine (400 mL), dried over Na_2SO_4 and concentrated to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 30:1 to 8:1) to give *tert*-butyl N-[1-[methoxy(methyl)carbamoyl]but-3-enyl]carbamate (57.40 g, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (m, 1H), 5.16 (m, 3H), 4.76 (s, br, 1H), 3.78 (s, 3H), 3.21 (s, 3H), 2.49 (m, 1H), 2.37 (m, 1H), 1.43 (s, 9H).

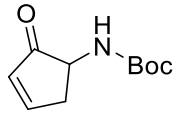
Step 3: *tert*-butyl *N*-(1-allyl-2-oxo-but-3-enyl)carbamate



To a solution of vinylmagnesiumbromide (1 M, 420 mL, 3.0 eq) in THF (600 mL) was added *tert*-butyl *N*-[1-[methoxy(methyl)carbamoyl]but-3-enyl]carbamate (36.0 g, 139.4 mmol, 1.00 eq) at -78 °C. The mixture was allowed to warm to rt and the reaction was stirred 12 hr. The reaction mixture was diluted with EtOAc (1200 mL) and poured into 1N HCl (1500 mL) at 0 °C. The layers were separated, and the organic layer was washed with 1N HCl (1000 mL), $NaHCO_3$ (1000 mL) and brine (500 mL), dried over Na_2SO_4 and concentrated to give the crude product, *tert*-butyl N-(1-allyl-2-oxo-but-3-enyl)carbamate (108.0 g, crude), which was obtained as a yellow oil. The crude product was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (dd, *J* = 10.3, 17.3, 1H), 6.39 (dd, *J* = 1.3,

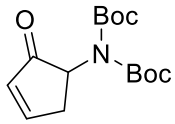
17.4, 1H), 5.89 (d, *J* = 10.2, 1H), 5.66 (tdd, *J* = 7.2, 10.7, 16.3, 1H), 5.34 (m, 1H), 5.15 (m, 2H), 4.69 (q, *J* = 6.1, 1H), 2.63 (m, 1H), 2.39 (m, 1H), 1.44 (s, 9H).

Step 4: *tert*-butyl *N*-(2-oxocyclopent-3-en-1-yl)carbamate



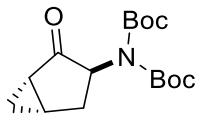
To a stirred solution of *tert*-butyl *N*-(1-allyl-2-oxo-but-3-enyl)carbamate (16.0 g, 71.0 mmol, 1.00 eq) in DCM (500 mL) was added benzylidene-[1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene]-dichloro-ruthenium tricyclohexylphosphane (0.7 g) under N_2. The reaction mixture was stirred at rt for 24 hr, then additional benzylidene-[1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene]-dichloro-ruthenium tricyclohexylphosphane (0.15 g) was added. The reaction mixture was stirred for 12 hr, then a third batch of benzylidene-[1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene]-dichlororuthenium tricyclohexylphosphane (0.15 g) was added. The reaction mixture was stirred under N_2 at rt for 12 hr. The reaction mixture was then concentrated to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 20:1 to 6:1) to give *tert*-butyl *N*-(2-oxocyclopent-3-en-1-yl)carbamate (26.0 g, 131.8 mmol, 93% yield) as a pale solid. ^{1}HNMR: (400 MHz, $CDCl_3$) δ 7.62 (t, *J* = 3.2, 1H), 6.19 (m, 1H), 5.08 (s, br, 1H), 3.92 (s, br, 1H), 3.12 (d, *J* = 15.2, 1H), 2.59 (m, 1H), 1.38 (s, 9H).

Step 5: *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-(2-oxocyclopent-3-en-1-yl)carbamate



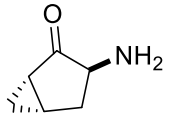
To a solution of *tert*-butyl *N*-(2-oxocyclopent-3-en-1-yl)carbamate (26.0 g, 131.8 mmol, 1.00 eq) in acetonitrile (400 mL) was added DMAP (19.3 g, 158.2 mmol, 1.20 eq) and *tert*-butoxycarbonyl *tert*-butyl carbonate (57.5 g, 263.7 mmol, 60.5 mL, 2.00 eq). The resulting reaction mixture was stirred at rt for 12 hr. The reaction mixture was concentrated to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 30:1 to 10:1) to give *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-(2-oxocyclopent-3-en-1-yl)carbamate (23.0 g, 77.3 mmol, 58% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (td, *J* = 2.8, 6.0, 1H), 6.28 (td, *J* = 2.0, 6.2, 1H), 4.77 (dd, *J* = 3.7, 7.1, 1H), 3.01 (m, 1H), 2.77 (m, 1H), 1.49 (s, 18H).

Step 6: *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[(*rel*-1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl] carbamate



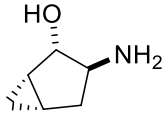
NaH (480 mg, 12.0 mmol, 60% in oil, 2.4 eq) was washed with hexane (3 x 50 mL) and then added into a solution of trimethyloxosulfonium iodide (2.66 g, 12.0 mmol, 2.4 eq) in DMSO (15 mL). The mixture was stirred at rt for 1 hr, then the mixture was added into a solution of *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-(2-oxocyclopent-3-en-1-yl)carbamate (1.50 g, 5.04 mmol, 1.00 eq) in DMSO (20 mL) at 50 °C. The mixture was stirred at 50 °C for 5 hr. The reaction mixture was diluted with EtOAc (60 mL) and washed with brine (3 x 60 mL). The organic layer was separated and dried over Na_2SO_4 and concentrated to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 35:1 to 20:1) to give *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[(*rel*-1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (900 mg, 2.60 mmol, 51% yield, 90% purity) as a white solid. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 4.59 (t, *J* = 8.7, 1H), 2.37 (dd, *J* = 2.8, 8.6, 2H), 2.11 (m, 1H), 1.90 (ddd, *J* = 3.3, 5.1, 9.0, 1H), 1.44 (s, 18H), 1.19 (m, 1H), 0.86 (m, 1H).

Step 7: *rel*-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one

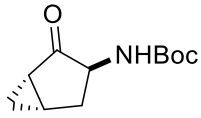


To a solution of *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-(1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (5.70 g, 18.3 mmol, 1.00 eq) in DCM (70 mL) was added TFA (23 g, 202 mmol, 15 mL, 11.0 eq) . The reaction mixture was stirred at rt for 6 hr. The reaction mixture was concentrated to give a residue then was washed with MTBE (20 mL) to afford *rel*-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one (2.88 g, 66% yield, TFA salt) as a pale yellow solid. ^{1}H NMR: (400 MHz, CD_3OD) δ = 3.82 (tdd, *J* = 0.9, 8.5, 9.5, 1H), 2.57 (dd, *J* = 8.3, 12.7, 1H), 2.28 (qd, *J* = 5.2, 7.8, 1H), 2.09 (dddd, *J* = 0.7, 5.2, 9.6, 12.8, 1H), 1.95 (m, 1H), 1.39 (m, 2H).

rel-(1*S*,2*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol



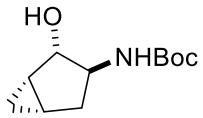
Step 1: *tert*-butyl *N*-[(r*el*-1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate



To a solution of *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (5.0 g, 16.1 mmol, 1.00 eq) in acetonitrile (120 mL) was added NaI (2.41 g, 16.1 mmol, 1.00 eq) at rt, followed by addition of $CeCl_3 \cdot 7H_2O$ (5.98 g, 16.1 mmol, 1.00 eq) in one portion. The suspension was stirred at rt for 12 hr. The suspension was diluted with H_2O (500 mL), and then extracted with EtOAc (2 x 500 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered through a pad of

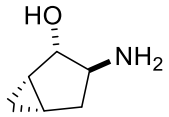
celite, and the filtrate was concentrated to give crude product. The crude product was purified by silica gel column chromatography (EtOAc:PE = 1:5 to 1:2) to give *tert*-butyl *N*-[*rel*-(1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (2.50 g, 66% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.83 (s, br, 1H), 3.98 (m, 1H), 2.71 (dd, *J* = 8.1, 12.7, 1H), 2.12 (m, 1H), 1.91 (m, 2H), 1.46 (s, 9H), 1.30 (m, 2H).

Step 2: *tert*-butyl *N*-[*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxy-3-bicyclo[3.1.0]hexanyl]carbamate



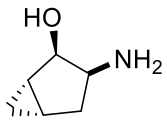
To a solution of *tert*-butyl *N*-[*rel*-(1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (2.00 g, 8.5 mmol, 1.00 eq) in absolute EtOH (120 mL) at -78 °C was added $Li(OtBu)_3AlH$ (4.33 g, 17.0 mmol, 2.00 eq) in small portions. The mixture was allowed to slowly warm to rt and then stirred for 12 hr. The mixture was quenched with H_2O (600 mL) and filtered through a pad of celite. The filtrate was extracted with EtOAc (2 x 500 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give crude product. The crude product was purified by silica gel column chromatography (EtOAc:PE = 1:5 to 1:2) to afford tert-butyl N-[(rel-1S,2S,3S,5S)-2-hydroxy-3-bicyclo[3.1.0]hexanyl]carbamate (1.30 g, 60% yield, 85% purity) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 4.13 (dd, *J* = 4.9, 7.7, 1H), 3.34 (m, 1H), 2.06 (dd, *J* = 7.7, 12.6, 1H), 1.67 (m, 1H), 1.44 (s, 9H), 1.27 (m, 1H), 0.60 (m, 1H), 0.39 (dt, *J* = 5.6, 7.9, 1H).

Step 3: *rel*-(1*S*,2*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol

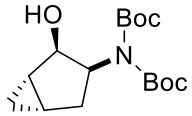


To a round bottom flask charged with HCl in EtOAc (4 M, 42.5 mL, 38.7 eq) was added *tert*-butyl *N*-[*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxy-3-bicyclo[3.1.0]hexanyl]carbamate (1.10 g, 4.38 mmol, 1.0 eq) in portions at 0 °C. The mixture was allowed warm to rt and stirred for 5 hr. A white suspension formed. The reaction was concentrated under vacuum to afford *rel*-(1*S*,2*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (650 mg, 100% yield, HCl salt) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 4.36 (m, 1H), 2.91 (td, *J* = 7.7, 10.0, 1H), 2.22 (dd, *J* = 7.6, 12.7, 1H), 1.88 (m, 1H), 1.57 (m, 1H), 1.44 (m, 1H), 0.63 (m, 1H), 0.51 (m, 1H).

rel-(1*S*,2*R*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol

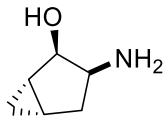


Step 1: *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxy-3-bicyclo [3.1.0]hexanyl]carbamate



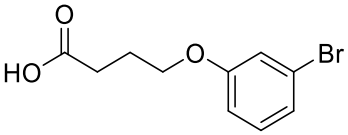
To a stirred solution of *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-(1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]carbamate (400 mg, 1.28 mmol, 1.0 eq) in THF (3 mL) at -78 °C under N_2 was added $LiBH_4$ (55 mg, 2.56 mmol, 2.0 eq). The resulting reaction mixture was stirred at -78 °C for 2 hr, then the reaction mixture was allowed to warm to rt and stirred for 10 hr. The reaction mixture was concentrated under reduced pressure. The crude *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxy-3-bicyclo [3.1.0]hexanyl]carbamate (440 mg) was isolated as a yellow oil and was used in the next step without further purification.

Step 2: *rel*-(1*S*,2*R*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol

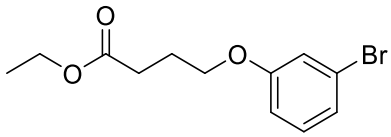


To a solution of *tert*-butyl *N*-*tert*-butoxycarbonyl-*N*-[*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxy-3-bicyclo[3.1.0]hexanyl]carbamate (440 mg, 1.40 mmol, 1.0 eq) in DCM (5 mL) was added TFA (1.60 g, 14.0 mmol, 1.04 mL, 10.0 eq). The resulting mixture was stirred at rt for 5 hr. The mixture was concentrated under reduced pressure to afford the crude product *rel*-(1*S*,2*R*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (400 mg, TFA salt) as a brown solid. The material was used without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.89 (m, 1H), 3.70 (m, 1H), 2.19 (m 1H), 1.87 (m, 1H), 1.57 (m, 1H), 1.44 (m, 1H), 0.51 (m, 1H), 0.29 (m, 1H).

4-(3-bromophenoxy)butanoic acid



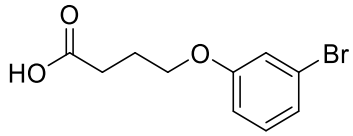
Step 1: ethyl 4-(3-bromophenoxy)butanoate



To a solution of 3-bromophenol (5.00 g, 28.90 mmol, 1.00 eq) and ethyl 4-bromobutanoate (6.20 g, 31.79 mmol, 4.56 mL, 1.10 eq) in DMF (70 mL) was added K_2CO_3 (8.00 g, 57.80 mmol, 2.00 eq). The resulting mixture was stirred at rt for 0.5 hr and then heated to 95 °C for 1 hr. The reaction mixture was then cooled to rt, diluted with H_2O (300 mL) and extracted with DCM (3 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue,

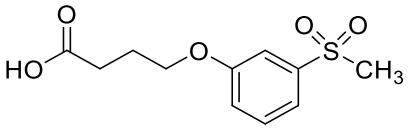
which was purified by silica gel column chromatography (PE:EtOAc = 100:1 to 20:1) to give ethyl 4-(3-bromophenoxy)butanoate (6.40 g, 77% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.09 (m, 3H), 6.83 (d, *J* = 8.4, 1H), 4.16 (q, *J* = 7.1, 2H), 4.00 (t, *J* = 6.2, 2H), 2.51 (t, *J* = 7.3, 2H), 2.11 (m, 2H), 1.26 (t, *J* = 8.0, 3H); LCMS calculated for $C_{12}H_{15}BrO_3$: m/z = 286; found: m/z = 287 (M+H).

Step 2: 4-(3-bromophenoxy)butanoic acid

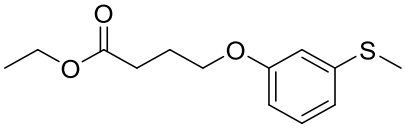


To a solution of ethyl 4-(3-bromophenoxy)butanoate (6.40 g, 22.26 mmol, 1.00 eq) in THF (40 mL) and H_2O (40 mL) was added $LiOH·H_2O$ (1.87 g, 44.52 mmol, 2.00 eq). The resulting mixture was heated to 50 °C and stirred for 3 hr under N_2 atmosphere. The reaction mixture was cooled to rt and concentrated under reduced pressure to give a residue. The residue was diluted with H_2O (200 mL) and extracted with DCM (3 x 200 mL). The organic layers were was discarded. HCl (3M) was added the aqueous phase to adjust to pH = 2 and the mixture was extracted with DCM (3 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford 4-(3-bromophenoxy)butanoic acid (5.22 g) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.16 (dd, *J* = 8.0, 8.0, 1H), 7.07 (m, 2H), 6.88 (dd, *J* = 2.3, 8.0, 1H), 4.00 (t, *J* = 6.3, 2H), 2.48 (t, *J* = 7.4, 2H), 2.05 (m, 2H); LCMS calculated for $C_{10}H_{11}BrO_3$: m/z = 258; found: m/z = 259 (M+H).

4-(3-(methylsulfonyl)phenoxy)butanoic acid



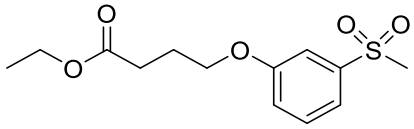
Step 1: ethyl 4-(3-(methylthio)phenoxy)butanoate



To a solution of 3-(methylthio)phenol (5.00 g, 35.66 mmol, 1.00 eq) in DMF (20 mL) was added K_2CO_3 (14.79 g, 106.98 mmol, 3.00 eq) , KI (592 mg, 3.57 mmol, 0.10 eq) and ethyl 4-bromobutanoate (8.35 g, 42.79 mmol, 6.14 mL, 1.20 eq). The resulting reaction mixture was then heated to 100 °C and stirred for 12 hr. The reaction mixture was cooled to rt and quenched by adding H_2O (30 mL) then transferred to a separatory funnel and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 100:1 to 50:1) to

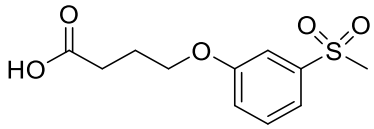
give ethyl 4-(3-(methylthio)phenoxy)butanoate (6.00 g, 62% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 (dt, *J* = 3.0, 8.0, 1H), 6.87 - 6.77 (m, 2H), 6.66 (dd, *J* = 2.1, 8.3, 1H), 4.15 (q, *J* = 7.1, 2H), 4.00 (dt, *J* = 2.8, 6.1, 2H), 2.59 - 2.40 (m, 5H), 2.11 (m, 2H), 1.26 (t, *J* = 7.2, 3H); LCMS calculated for $C_{13}H_{18}O_3S$: m/z = 254; found: m/z = 255 (M+H).

Step 2: ethyl 4-(3-(methylsulfonyl)phenoxy)butanoate



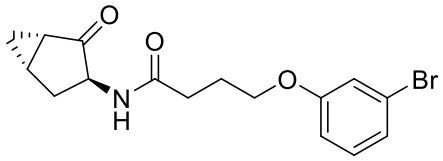
To a solution of ethyl 4-(3-(methylthio)phenoxy)butanoate (500 mg, 1.84 mmol, 1.00 eq) in $CHCl_3$ (10 mL) at 0 °C was added *m*-CPBA (2.27 g, 9.19 mmol, 70% purity, 5.00 eq), and the resulting reaction mixture was warmed to rt and stirred for 5 hr. The reaction was quenched with saturated $Na_2S_2O_3$ solution (50 mL) and the resulting mixture was extracted with EtOAc (2 x 50 mL). The combined organic phases were then washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (PE:EtOAc = 20:1-1:1) to give ethyl 4-(3-methylsulfonylphenoxy) butanoate (490 mg, 86% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.47 (m, 3H), 7.16 (dd, *J* = 1.3, 8.0, 1H), 4.16 (q, *J* = 7.1, 2H), 4.08 (t, *J* = 6.1, 2H), 3.05 (s, 3H), 2.52 (*t*, J = 7.2, 2H), 2.14 (m, 2H), 1.27 (t, *J* = 7.2, 3H); LCMS calculated for $C_{13}H_{18}O_5S$: m/z = 286; found: m/z = 287 (M+H).

Step 3: 4-(3-(methylsulfonyl)phenoxy)butanoic acid



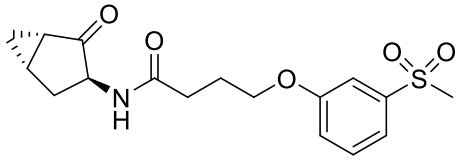
To a solution of ethyl 4-(3-methylsulfonylphenoxy)butanoate (490 mg, 1.58 mmol, 1.00 eq) in THF (5 mL) / H_2O (1 mL) was added $LiOH \cdot H_2O$ (199 mg, 4.74 mmol, 3.00 eq). The resulting reaction mixture was stirred at rt for 12 hr. The reaction mixture was diluted with H_2O (5 mL), then extracted with EtOAc (10 mL) (discarded). The aqueous phase was acidified with citric acid to pH=4 and extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 4-(3-methylsulfonylphenoxy)butanoic acid (360 mg, 88% yield) as a white solid which was used without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.55-7.45 (m, 3H), 7.26 (m, 1H), 4.12 (t, *J* = 6.2, 2H), 3.12 (s, 3H), 2.51 (t, *J* = 7.3, 2H), 2.11 (m, 2H); LCMS calculated for $C_{11}H_{14}O_5S$: m/z = 258; found: m/z = 259 (M+H).

4-(3-bromophenoxy)-*N*-(*rel*-(1*S*,3*S*,5*S*)-2-oxobicyclo[3.1.0]hexan-3-yl)butanamide (BB0020)



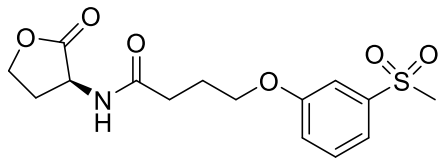
To a solution of 4-(3-bromophenoxy)butanoic acid (300 mg, 1.16 mmol, 1.00 eq) and DMF (58 µmol, 4.46 uL, 0.05 eq) in DCM (7 mL) was added oxalyl chloride (147 mg, 1.16 mmol, 100 µL, 1.00 eq) in DCM (3 mL). The reaction mixture was stirred at rt for 0.5 hr. Then the reaction mixture was added into a suspension of *rel*-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one (129 mg, 1.16 mmol, 1.00 eq ,TFA salt) in DCM (7 mL). Next, DIPEA (525 mg, 4.06 mmol, 710 µL, 3.50 eq) in DCM (3 mL) was added. The resulting reaction mixture was stirred at 25 °C for 2.5 hr. The reaction mixture was concentrated to give a residue which was purified by silica gel column chromatography (DCM: MeOH = 600:1) followed by recrystallization from hot EtOAc:PE 1:20 to give 220 mg of 4-(3-bromophenoxy)-*N*-(*rel*-(1*S*,3*S*,5*S*)-2-oxo-3-bicyclo[3.1.0]hexanyl]butanamide (54% yield) as a white solid (97% pure by HLPC-UV). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.16 (m, 1H), 7.07 (m, 2H), 6.90 (dd, *J* = 1.2, 8.4, 1H), 4.26 (t, *J* = 9.2, 1H), 4.00 (t, *J* = 6.2, 2H), 2.41 (m, 3H), 2.16 - 1.96 (m, 4H), 1.83 (ddd, *J* = 3.6, 5.1, 8.7, 1H), 1.29 (m, 2H); LCMS calculated for $C_{16}H_{18}BrNO_3$: m/z = 351; found: m/z = 352 (M+H).

4-(3-(methylsulfonyl)phenoxy)-*N*-(*rel*-(1*S*,3*S*,5*S*)-2-oxobicyclo[3.1.0]hexan-3-yl)butanamide (BB0126)



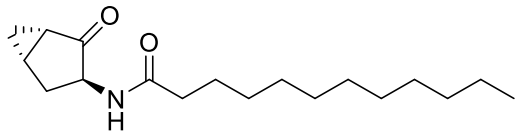
To a solution of 4-(3-(methylsulfonyl)phenoxy)butanoic acid (100 mg, 387 µmol, 1.00 eq) in THF (3 mL) at 0 °C under N_2 atmosphere was added isopropyl chloroformate (47 mg, 387 µmol, 54 µL, 1.00 eq) and Et_3N (78 mg, 774 µmol, 107 µL, 2.00 eq). The resulting reaction mixture was stirred at 0 °C for 1 hr. Then the mixture was added to a solution of *rel*-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one (87 mg, 387 µmol, 1.00 eq, TFA salt) in THF (2 mL). The resulting reaction mixture was stirred at rt for 1 hr. The reaction mixture was diluted with $NaHCO_3$ (5 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were then washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (C18 column, 20-80% MeOH in H_2O) to give 4-(3-(methylsulfonyl)phenoxy)-*N*-(*rel*-(1*S*,3*S*,5*S*)-2-oxobicyclo[3.1.0]hexan-3-yl)butanamide (50 mg, 37% yield) as a white gum (100% pure by HPLC-UV). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.51 (m, 2H), 7.47 (dd, *J* = 1.6, 2.0, 1H), 7.27 (td, *J* = 2.2, 7.5, 1H), 4.27 (m, 1H), 4.12 (t, *J* = 6.3, 2H), 3.12 (s, 3H), 2.44 (m, 3H), 2.11 (m, 3H), 2.00 (m, 1H), 1.83 (m, 1H), 1.32 (m, 2H); LCMS calculated for $C_{17}H_{21}NO_5S$: m/z = 351; found: m/z = 352 (M+H).

(*S*)-4-(3-(methylsulfonyl)phenoxy)-N-(2-oxotetrahydrothiophen-3-yl)butanamide (BB0221)



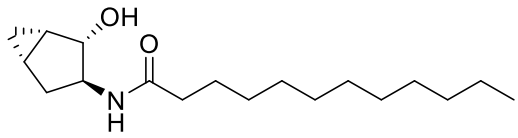
To a solution of 4-(3-methylsulfonylphenoxy)butanoic acid (0.15 g, 580 µmol, 1.0 eq) in THF (1 mL) was added isopropyl carbonochloridate (71mg, 580 µmol, 81 µL, 1.0 eq) and Et_3N (118 mg, 1.16 mmol, 162 µL, 2.0 eq) at 0 °C under N_2 atmosphere. The resulting reaction mixture was stirred at 0 °C for 1 hr, then the mixture was added to the solution of (*S*)-3-aminotetrahydrofuran-2-one (59 mg, 580 µmol, 1.0 eq, HBr salt) in THF (1 mL). The resulting reaction mixture was stirred at rt for 1 hr. The reaction mixture was diluted with $NaHCO_3$ (20 mL), and then extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (10 mL) dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (CH_3CN:EtOAc = 1:20) to give (*S*)-4-(3-(methylsulfonyl)phenoxy)-N-(2-oxotetrahydrothiophen-3-yl)butanamide (160 mg, 81% yield) as a white solid (99% pure by HPLC-UV).). ^1H NMR (400 MHz, DMSO-d_6) δ = 8.44 (d, br, *J* = 7.7, 1H), 7.56 (m, 1H), 7.48 (m, 1H), 7.41 (m, 1H), 7.29 (d, br, *J* = 6.7, 1H), 4.54 (m, 1H), 4.34 (m, 1H), 4.21 (m, 1H), 4.08 (m, 2H), 3.22 (s, 3H), 2.41 - 2.28 (m, 3H), 2.15 (m, 1H), 1.97 (m, 2H); LCMS calculated for $C_{15}H_{19}NO_6S$: m/z = 341; found: m/z = 342 (M+H).

N-(*rel*-(1*S*,3*S*,5*S*)-2-oxobicyclo[3.1.0]hexan-3-yl)dodecanamide (BB0231)



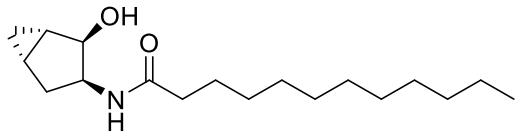
To a solution of *rel*-(1*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-one (206 mg, 914 µmol, 1.0 eq, TFA salt) in DCM (10 mL) at 0 °C under N_2 was added Et_3N (185 mg, 1.83 mmol, 255 µL, 2.0 eq). After stirring for 5 min, dodecanoyl chloride (0.2 g, 914 µmol, 211 µL, 1.0 eq) was added dropwise to the reaction mixture. The resulting reaction mixture was warmed to rt and stirred for 4 hr. The reaction mixture was diluted with H_2O (20 mL) and extracted with DCM (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by preparative TLC (SiO_2, DCM:MeOH = 15:1) to give N-(rel-(1S,3S,5S)-2-oxobicyclo [3.1.0]hexan-3-yl)dodecanamide (123 mg, 46% yield) as a white solid (100% pure by HPLC-ELSD). ^1H NMR (400 MHz, $CDCl_3$) δ = 5.73 (s, br, 1H), 4.15 (m, 1H), 2.80 (dd, *J* = 8.1, 12.9, 1H), 2.21 (t, *J* = 7.5, 2H), 2.13 (m, 1H), 1.92-1.77 (m, 2H), 1.61 (m, 2H), 1.33-1.22 (m, 18H), 0.88 (t, *J* = 6.8, 3H); LCMS calculated for $C_{18}H_{31}NO_2$: m/z = 293; found: m/z = 294 (M+H).

N-(*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)dodecanamide (BB0232)



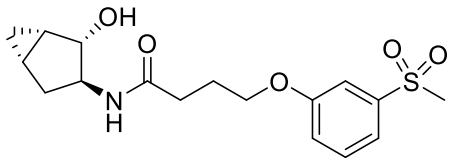
To a solution of *rel*-(1*S*,2*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (120 mg, 802 µmol, 1.0 eq, HCl salt) and Et_3N (162 mg, 1.60 mmol, 223 µL, 2.0 eq) in DCM (2 mL) at 0°C was added dodecanoyl chloride (175 mg, 802 µmol, 185 µL, 1.0 eq). The resulting reaction mixture was warmed to rt and stirred for 3 hr. The reaction mixture was diluted with 2M HCl (20 mL) and extracted with DCM (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by preparative TLC (SiO_2, DCM:MeOH = 15:1) to give *N*-(*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)dodecanamide (47 mg, 20% yield) as a white solid (100% pure by HPLC-ELSD). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.46 (s, br, 1H), 4.78 (s, 1H), 4.24 (t, *J* = 5.7, 1H), 3.57 (m, 1H), 2.25 (dd, *J* = 7.9, 12.0, 1H), 2.18 (t, *J* = 7.6, 3H), 1.65 (m, 1H), 1.59 (m, 4H), 1.33-1.24 (m, 16H), 0.89 (t, *J* = 6.8, 3H), 0.68 (m, 1H), 0.51 (m, 1H); LCMS calculated for $C_{18}H_{33}NO_2$: m/z = 295; found: m/z = 296 (M+H).

N-(*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)dodecanamide (BB0233)



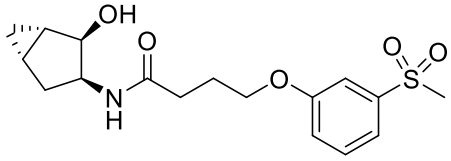
To a solution of *rel*-(1*S*,2*R*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (450 mg, 1.98 mmol, 1.5 eq, TFA salt) and Et_3N (267 mg, 2.64 mmol, 368 µL, 2.0 eq) in DCM (10 mL) at 0°C was added dodecanoyl chloride (289 mg, 1.32 mmol, 305 µL, 1.0 eq). The resulting reaction mixture was warmed to rt and stirred for 3 hr. The reaction mixture was diluted with 2M HCl (20 mL) and extracted with DCM (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 20:1 to 3:1). The resulting solid obtained after evaporation of solvents was washed with n-hexane (50 mL) and filtered. The filter cake was dried under reduced pressure to give *N*-(*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)dodecanamide (220 mg, 56% yield) as a white solid (100% pure by HPLC-ELSD). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.91 (d, br, *J* = 7.5, 1H), 4.07 (d, *J* = 4.4, 1H), 3.92 (m, 1H), 2.16 (m, 3H), 1.94 (s, br, 1H), 1.61 (m, 3H), 1.43 (m, 2H), 1.34-1.21 (m, 16H), 0.88 (t, *J* = 6.7, 3H), 0.52 (m, 1H), 0.37 (m, 1H); LCMS calculated for $C_{18}H_{33}NO_2$: m/z = 295; found: m/z = 296 (M+H).

N-(*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)-4-(3-(methylsulfonyl)phenoxy)butanamide (BB0272)



To a solution of 4-(3-methylsulfonylphenoxy)butanoic acid (20 mg, 77.4 µmol, 1.0 eq) in DMF (0.5 mL) at rt was added *rel*-(1*S*,2*S*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (12 mg, 77.4 µmol, 1.0 eq, HCl salt), HOBt (17 mg, 116.2 µmol, 1.5 eq), DIPEA (30 mg, 232.3 µmol, 46 µL, 3.0 eq) and EDCI (22 mg, 116.2 µmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 5 hr. The reaction mixture was diluted with brine (15 mL) extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (DCM:MeOH = 100:1 to 30:1) to give *N*-(*rel*-(1*S*,2*S*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)-4-(3-(methylsulfonyl)phenoxy) butanamide (19 mg, 66% yield) as a white solid (99% pure by HPLC-UV). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.56-7.42 (m, 3H), 7.17 (dd, *J* = 1.3, 8.0, 1H), 5.57 (s, br, 1H), 4.53 (s, 1H), 4.23 (t, *J* = 5.5, 1H), 4.08 (t, *J* = 6.2, 2H), 3.58 (m, 1H), 3.07 (s, 3H), 2.41 (t, *J* = 7.2, 2H), 2.23 (m, 1H), 2.13 (m, 2H), 1.72 (m, 1H), 1.60 (m, 1H), 1.37 (m, 1H), 0.68 (m, 1H), 0.50 (m, 1H); LCMS calculated for $C_{17}H_{23}NO_5S$: m/z = 353; found: m/z = 354 (M+H).

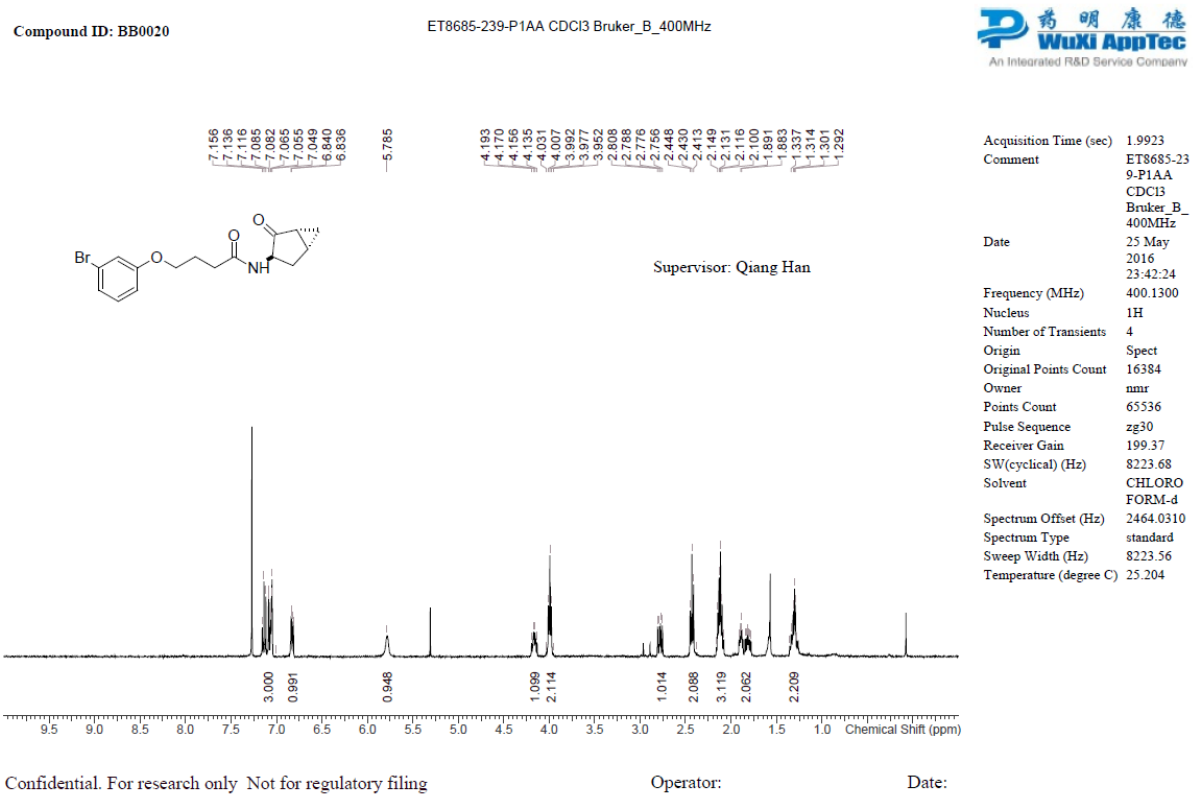
N-(*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)-4-(3-(methylsulfonyl)phenoxy)butanamide (BB0273)



To a solution of 4-(3-methylsulfonylphenoxy)butanoic acid (300 mg, 1.16 mmol, 1.0 eq) in DMF (2 mL) at rt was added *rel*-(1*S*,2*R*,3*S*,5*S*)-3-aminobicyclo[3.1.0]hexan-2-ol (317 mg, 1.39 mmol, 1.2 eq, HCl salt), HOBt (235 mg, 1.74 mmol, 1.5 eq), DIPEA (450 mg, 3.48 mmol, 607 µL, 3.0 eq) and EDCI (334 mg, 1.74 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 hr. The reaction mixture was diluted with brine (15 mL) extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (DCM:MeOH = 100:1 to 30:1) to give a product, which was further purified by recrystallization from hot DCM/PE (5mL / 40mL). The material was isolated by filtration and the filter cake was dried under high vacuum to give *N*-(*rel*-(1*S*,2*R*,3*S*,5*S*)-2-hydroxybicyclo[3.1.0]hexan-3-yl)-4-(3-(methylsulfonyl) phenoxy)butanamide (158 mg, 38% yield) as a white solid (99% pure by HPLC-UV). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.58-7.38 (m, 3H), 7.17 (dd, *J* = 1.3, 8.0, 1H), 5.97 (d, br, *J* = 7.8, 1H), 4.07 (m, 3H), 3.94 (m, 1H), 3.06 (s, 3H), 2.40 (t, *J* = 7.2, 2H), 2.16 (m, 3H), 1.80 (d, *J* = 5.6, 1H), 1.56 (m, 1H), 1.42 (m, 2H), 0.54 (m, 1H), 0.36 (m, 1H); LCMS calculated for $C_{17}H_{23}NO_5S$: m/z = 353; found: m/z = 354 (M+H).

Copies of Analytical Data for New Compounds

BB0020:



Confidential. For research only Not for regulatory filing Operator: Date:

LCMS REPORT

Compound ID : BB0020 ->
Sample ID : ET8685-239-P1A1
Injection Date : 26. May. 2016
Inj. Vol. : 0.5 ul
Location : P1-B-05
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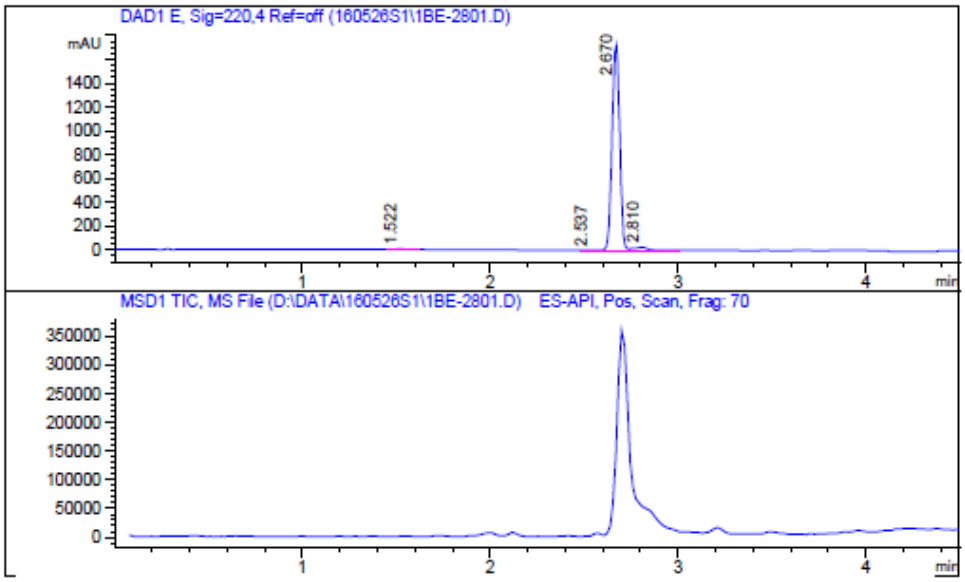
Data Filename : D:\DATA\160526S1\1BE-2801.D
Instrument : S



Integration Result

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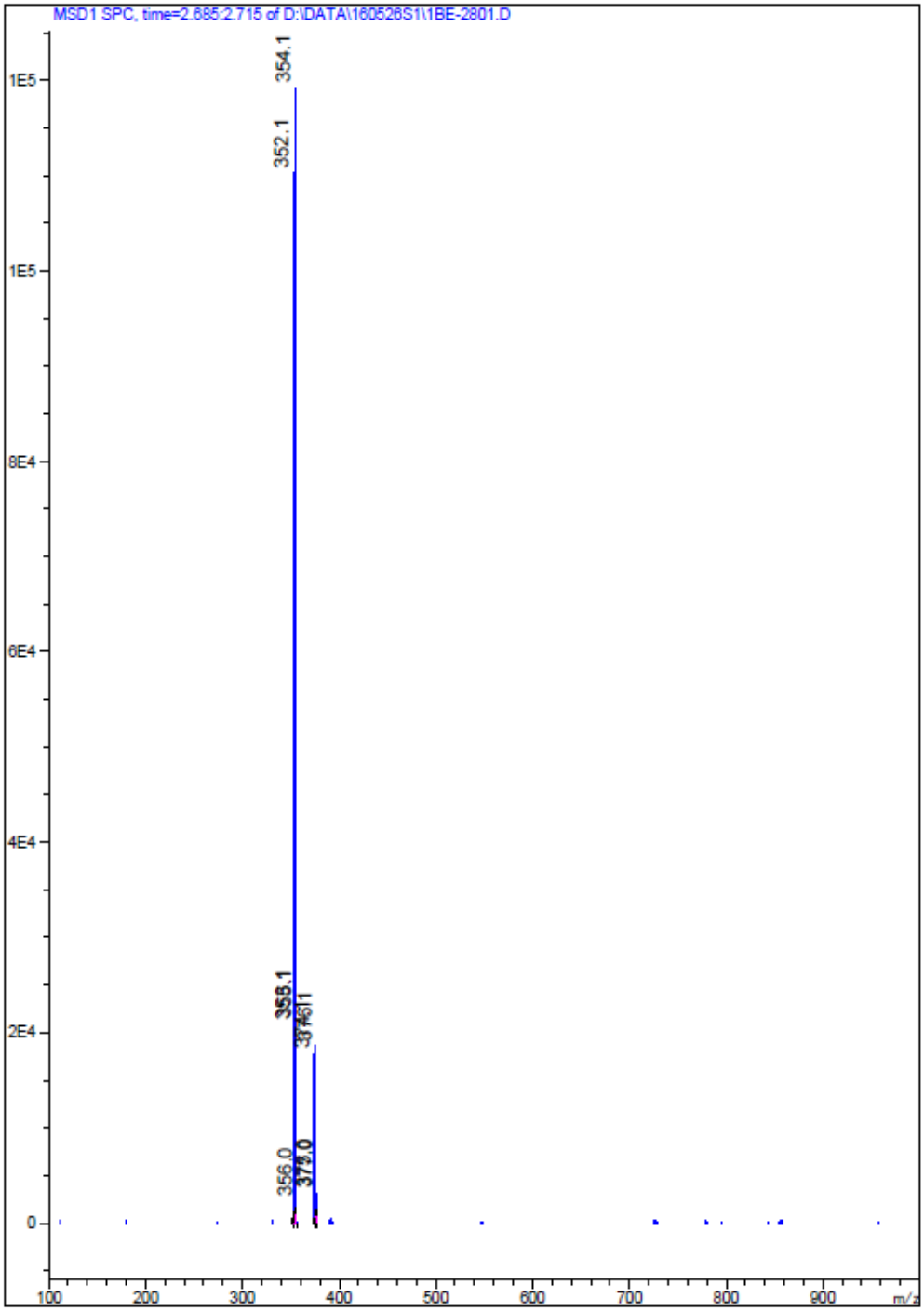
2	2.537	4.746	1.899	0.108	0.043	0.094
3	2.670	4910.489	1731.674	98.091	0.045	96.822
4	2.810	142.151	27.813	1.575	0.072	2.803

Operator:_____

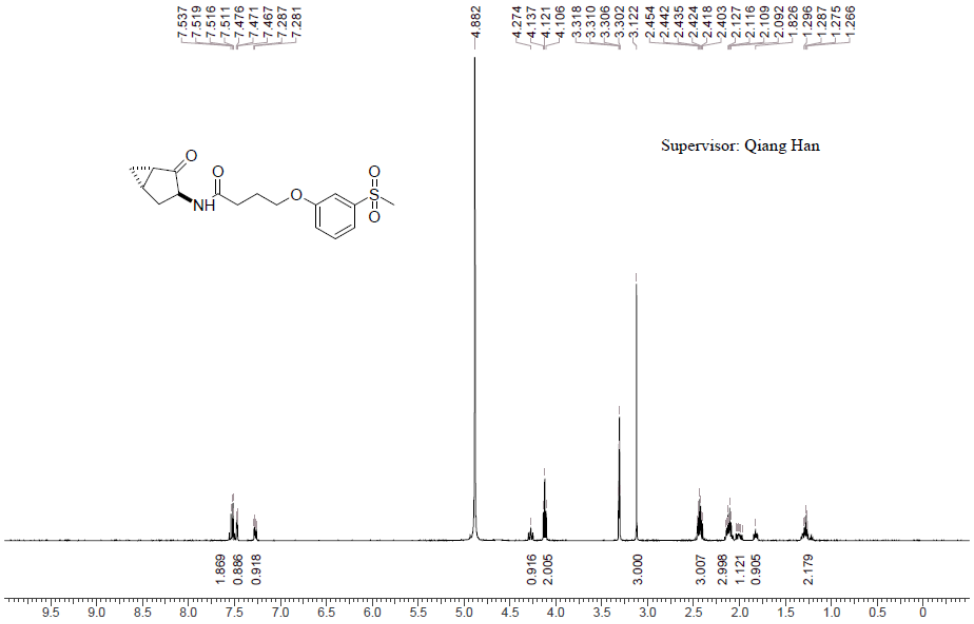
Date:_____



BB0126:

Compound ID: BB0126

ET9659-164-p1m MeOD Varian_D_400MHz



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Receiver Gain	34.00
SW(cyclical) (Hz)	7225.43
Solvent	METHANOL-d4
Spectrum Offset (Hz)	2799.3481
Spectrum Type	standard
Sweep Width (Hz)	7225.21
Temperature (degree C)	AMBIENT TEMPERATURE

Operator:

Date:

Confidential. For research only NOT for regulatory filing

LCMS REPORT

Compound ID : BB0126 ->
Sample ID : ET9659-164-plk
Injection Date : 7. Jun. 2016
Inj. Vol. : 1.00 ul
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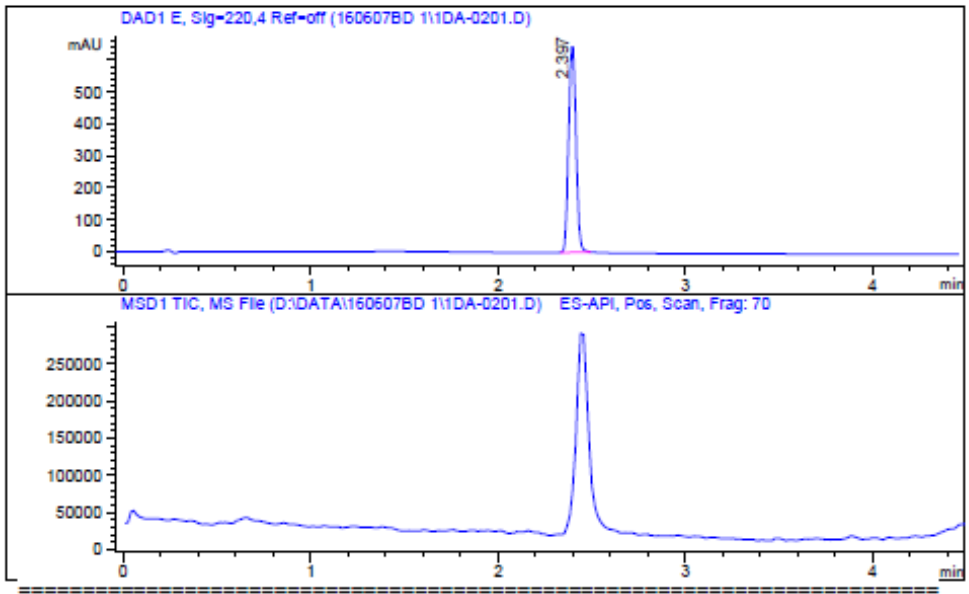
Instrument : B



Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

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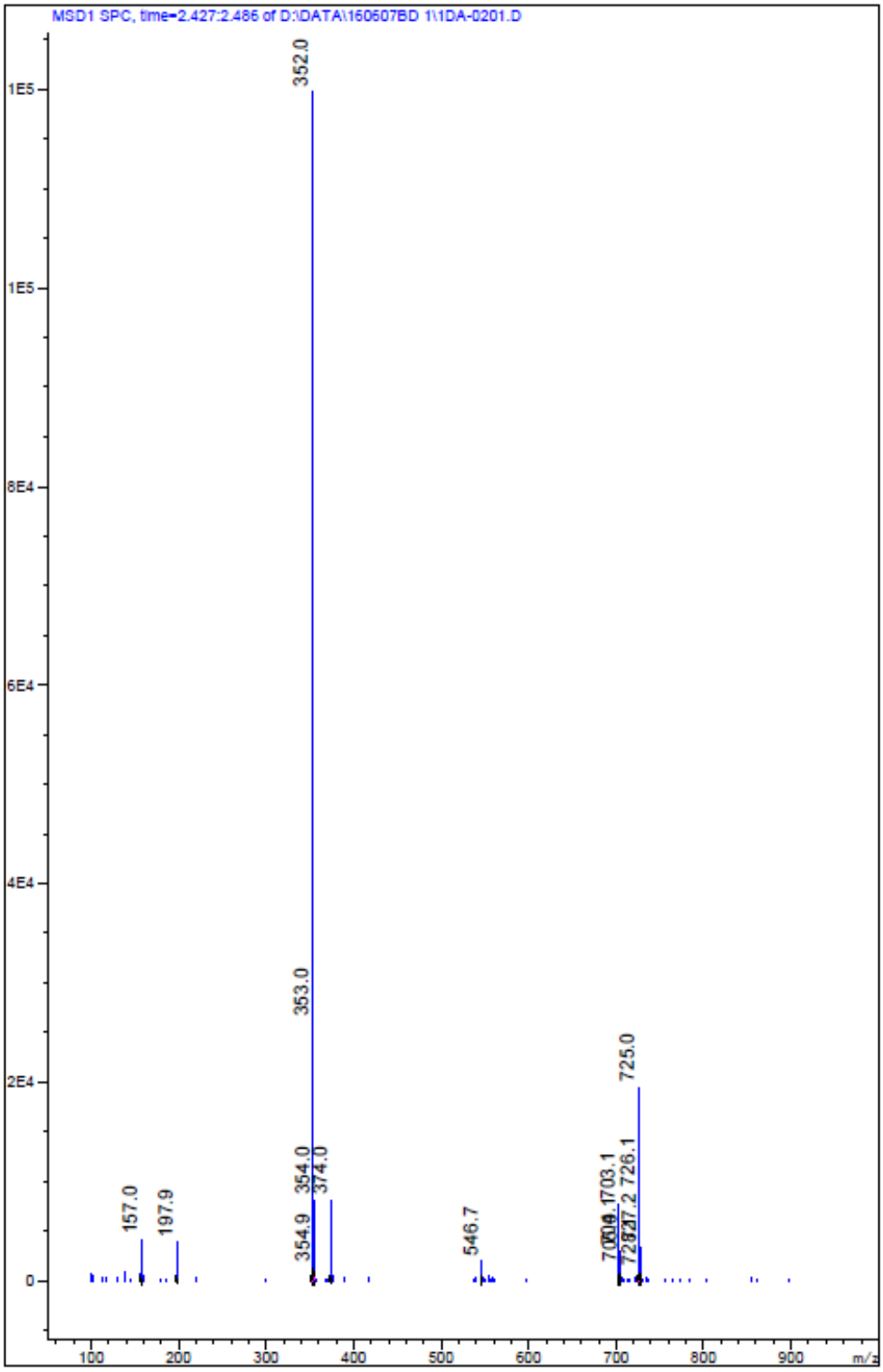
Operator:_____

Date:_____



HPLC REPORT

Compound ID :
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Injection Date : 6/4/2016 3:42:32 PM
Injection Vol : 1ul
Location : tray1 vial13
Acq Method : D:\method\10-80HPLC.lcm
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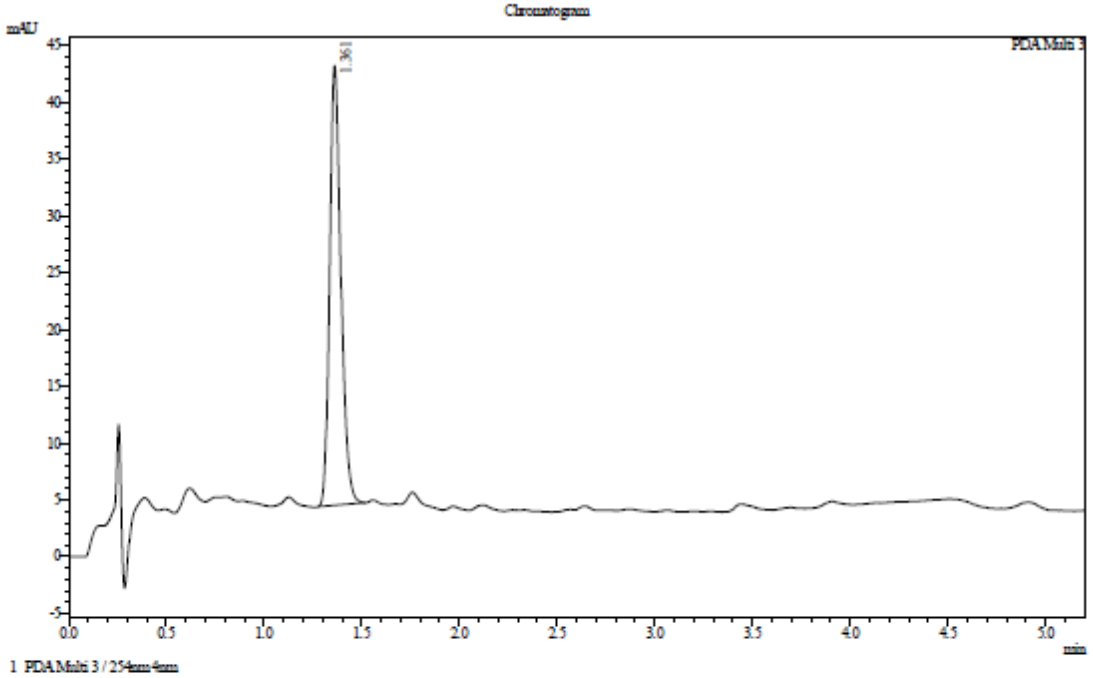
Instrument & Column: HPLC-015(11#-312) Xbridge RP18, 5um



1 PDA Multi 3 / 254nm 4nm

Integration Result

Ch3 254nm 4nm

Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
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Operator:_____

Date: _____

BB0221:

Compound ID: BB0221

ET12347-184-P1BB DMSO Bruker_C_400MHz

WuXi AppTec
An Integrated R&D Service Company

8.446
8.427
7.581
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7.469
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7.295
7.278
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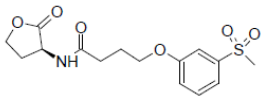
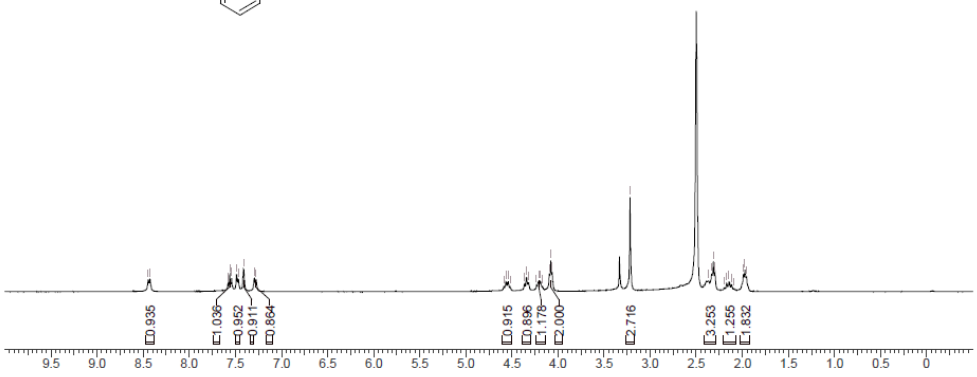
Supervisor: Qiang Han





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Points Count	65536
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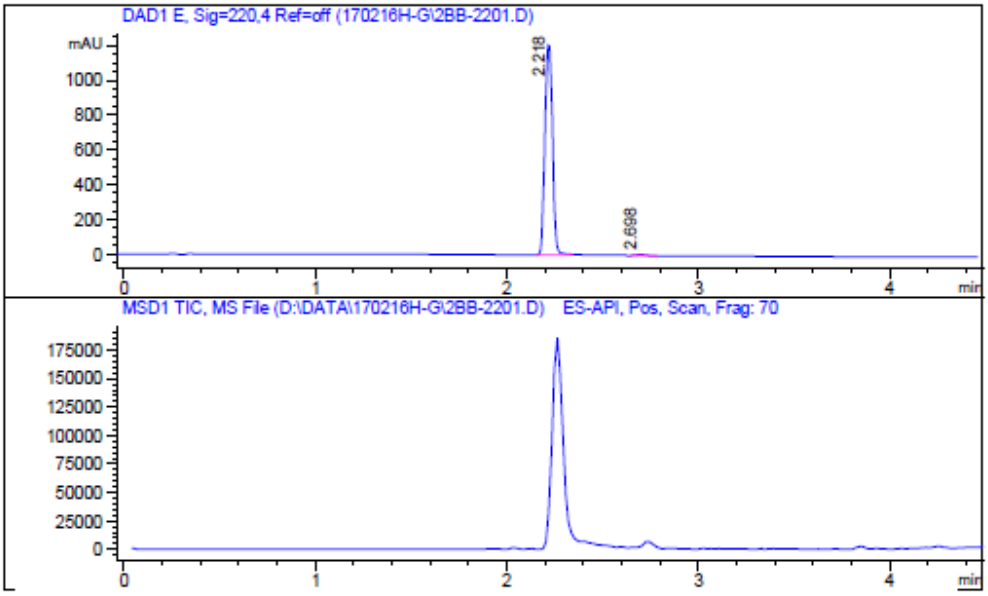
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	-273.000

Operator:

Date:

LCMS REPORT

Compound ID : BB0221 ->
Sample ID : ET12347-184-P1A1
Injection Date : 16. Feb. 2017
Inj. Vol. : 0.50 ul
Location : P2-B-02
Acq Method : D:\DATA\170216H-G\WUXIAB01_W.M
Data Filename : D:\DATA\170216H-G\2BB-2201.D
Instrument : H



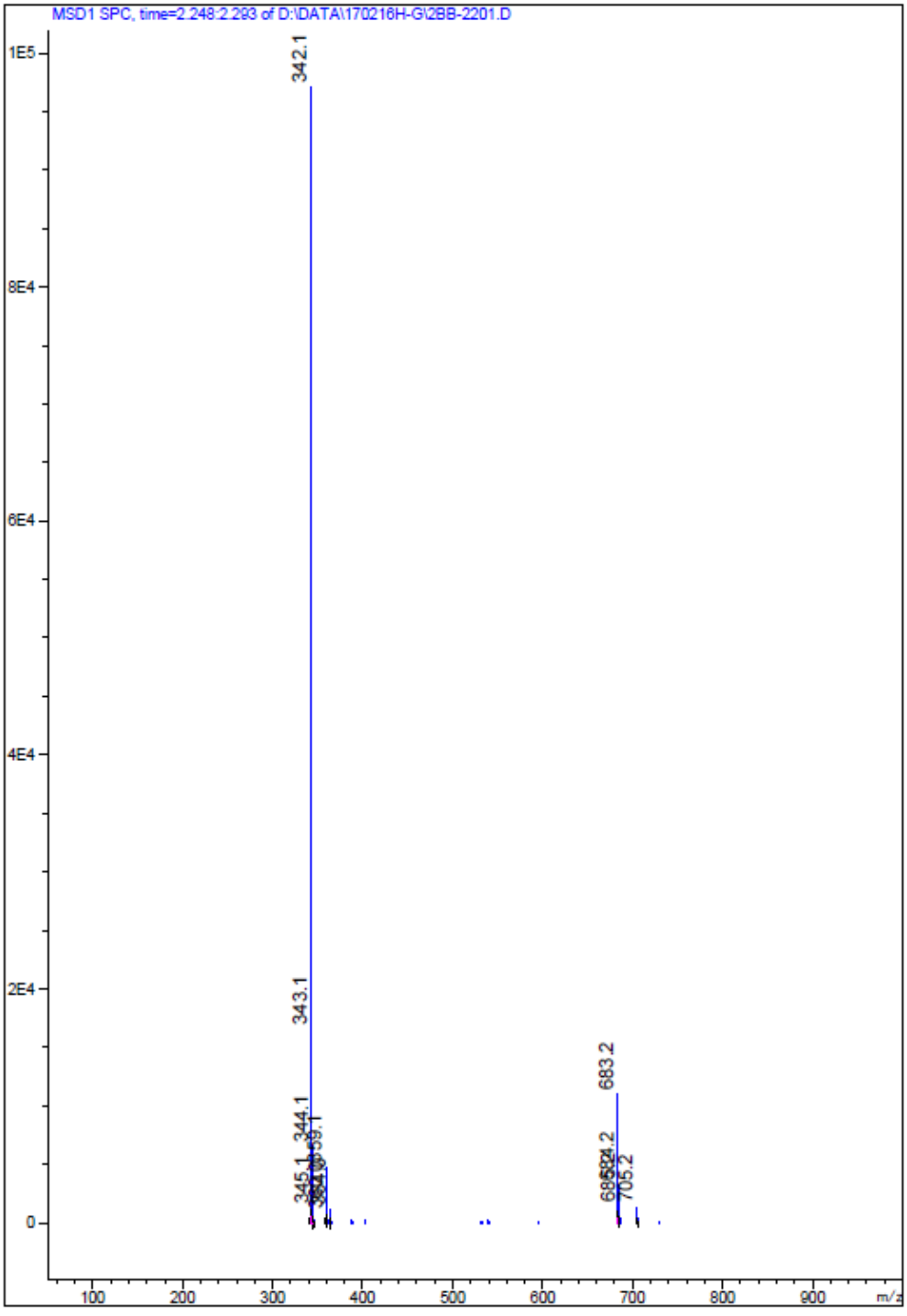
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Height	Height %	Width [min]	Area	Area %
1	2.218	1220.948	99.500	0.044	3312.138	99.476
2	2.698	6.134	0.500	0.045	17.432	0.524

Operator:_____

Date:_____



BB0231:

Compound ID: BB0231

ET9659-608-P1N CDCl3 Varian_T_400MHz

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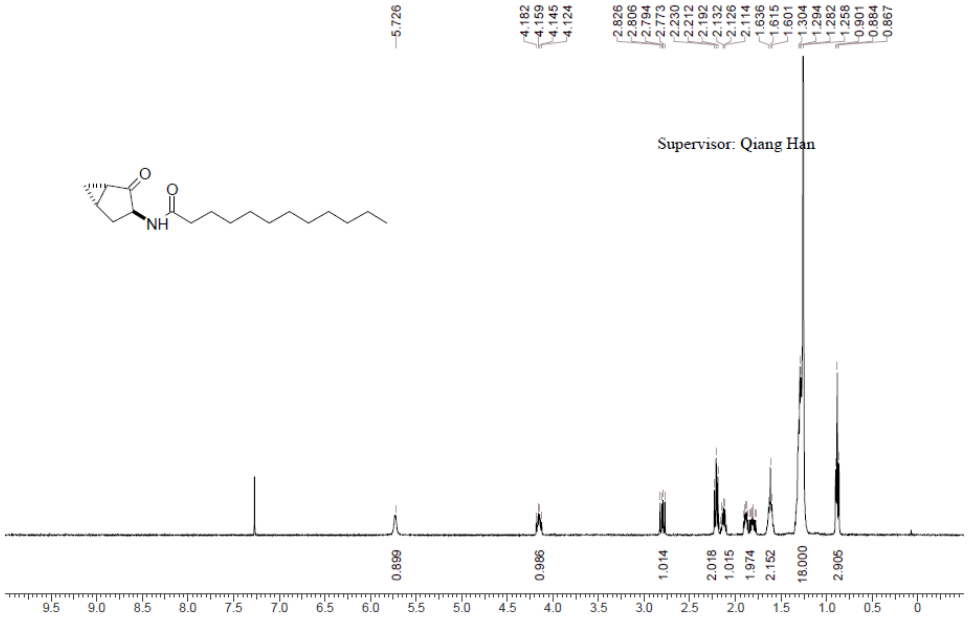
An Integrated R&D Service Company



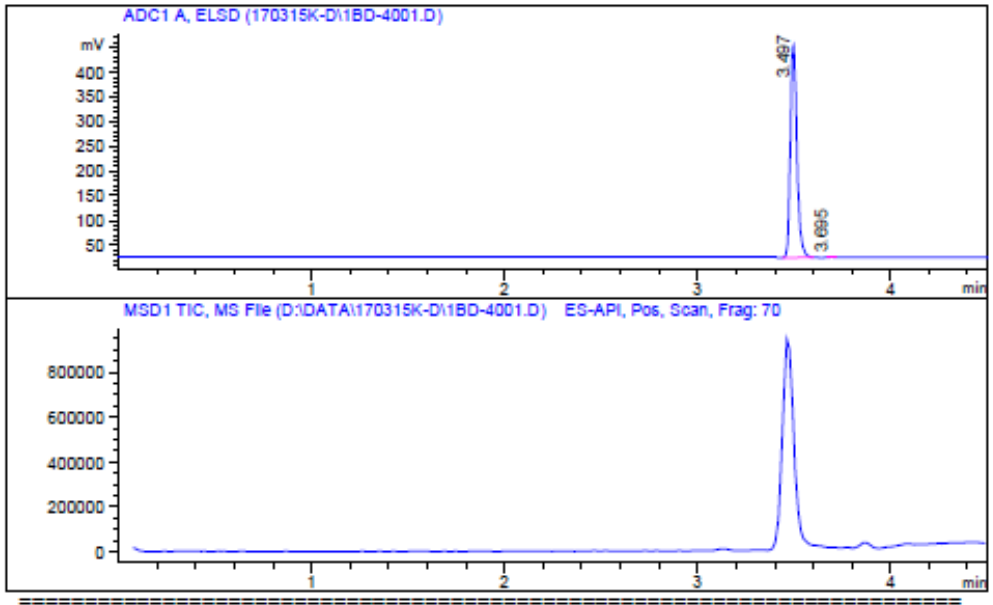
Acquisition Time (sec)	2.2807
Comment	ET9659-608-P1N CDCl3 Varian_T_400MHz
Date	Mar 15 2017
Frequency (MHz)	399.9124
Nucleus	1H
Number of Transients	8
Original Points Count	16384
Points Count	16384
Pulse Sequence	s2pul
Receiver Gain	48.00
SW(cyclical) (Hz)	7183.91
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2803.6741
Spectrum Type	standard
Sweep Width (Hz)	7183.91
Temperature (degree C)	AMBIENT TEMPERATURE

Operator:

Date:

LCMS REPORT

Compound ID : BB0231 ->
Sample ID : ET9659-608-P1K
Injection Date : 15. Mar. 2017
Inj. Vol. : 0.7 ul
Location : P1-B-04
Acq Method : D:\DATA\170315K-D\WUXIAB10.M
Data Filename : D:\DATA\170315K-D\1BD-4001.D
Instrument : K



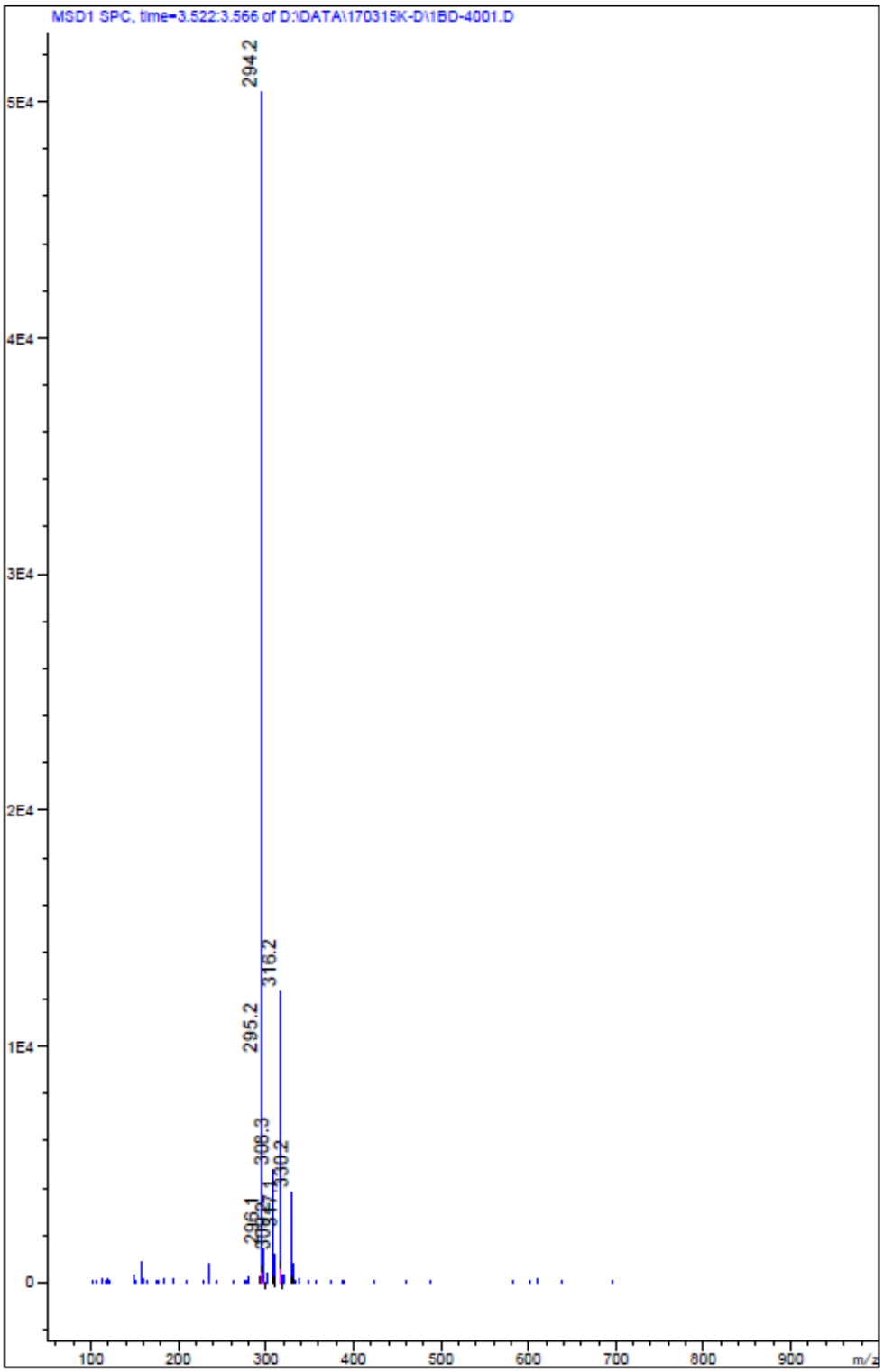
Integration Result

Signal 1 : ADC1 A, ELSD

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	3.497	1016.314	435.194	99.809	0.039	99.863
2	3.695	1.395	0.835	0.191	0.028	0.137

Operator:_____

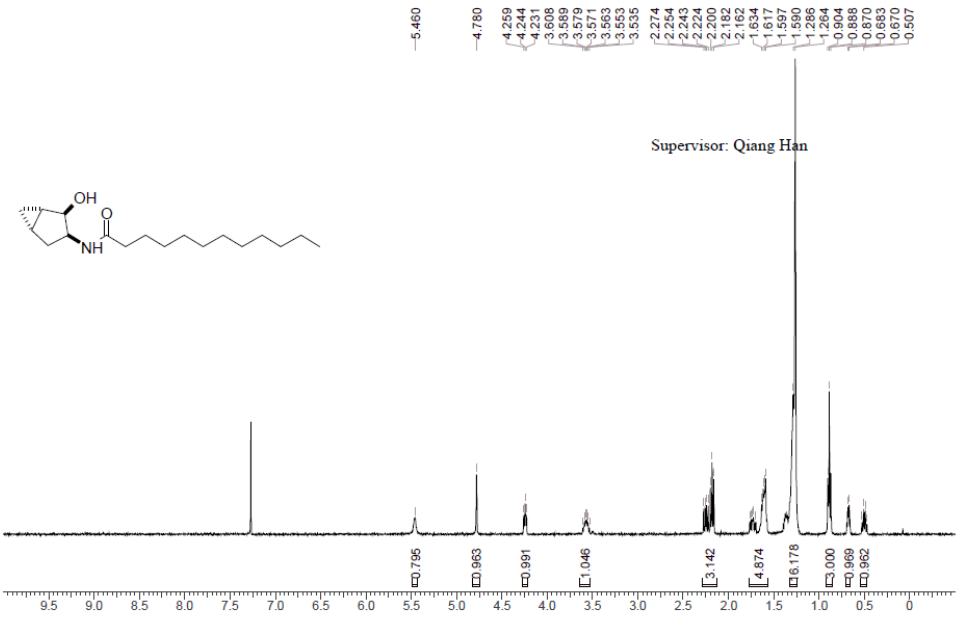
Date:_____



BB0232:

Compound ID: BB0232

ET12347-231-P1AA CDCl3 Varian_Y_400MHz



Acquisition Time (sec)	2.0486
Comment	ET12347-231-P1AA CDCl3 Varian_Y_400MHz
Date	Mar 16 2017
Frequency (MHz)	399.6733
Nucleus	1H
Number of Transients	8
Original Points Count	14802
Points Count	32768
Pulse Sequence	s2pul
Receiver Gain	54.00
SW(cyclical) (Hz)	7225.43
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2802.0835
Spectrum Type	standard
Sweep Width (Hz)	7225.21
Temperature (degree C)	25.000

Operator:

Date:

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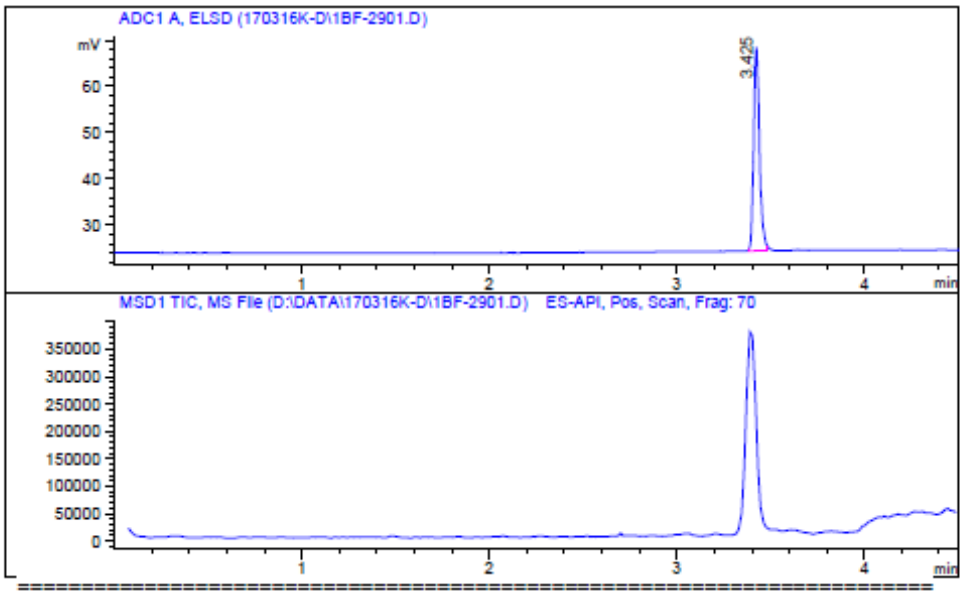
LCMS REPORT

Compound ID : BB0232 ->
Sample ID : ET12347-231-P1A1
Injection Date : 16. Mar. 2017
Inj. Vol. : 0.7 ul
Location : P1-B-06
Acq Method : D:\DATA\170316K-D\WUXIAB10.M
Data Filename : D:\DATA\170316K-D\1BF-2901.D
Instrument : K



Integration Result

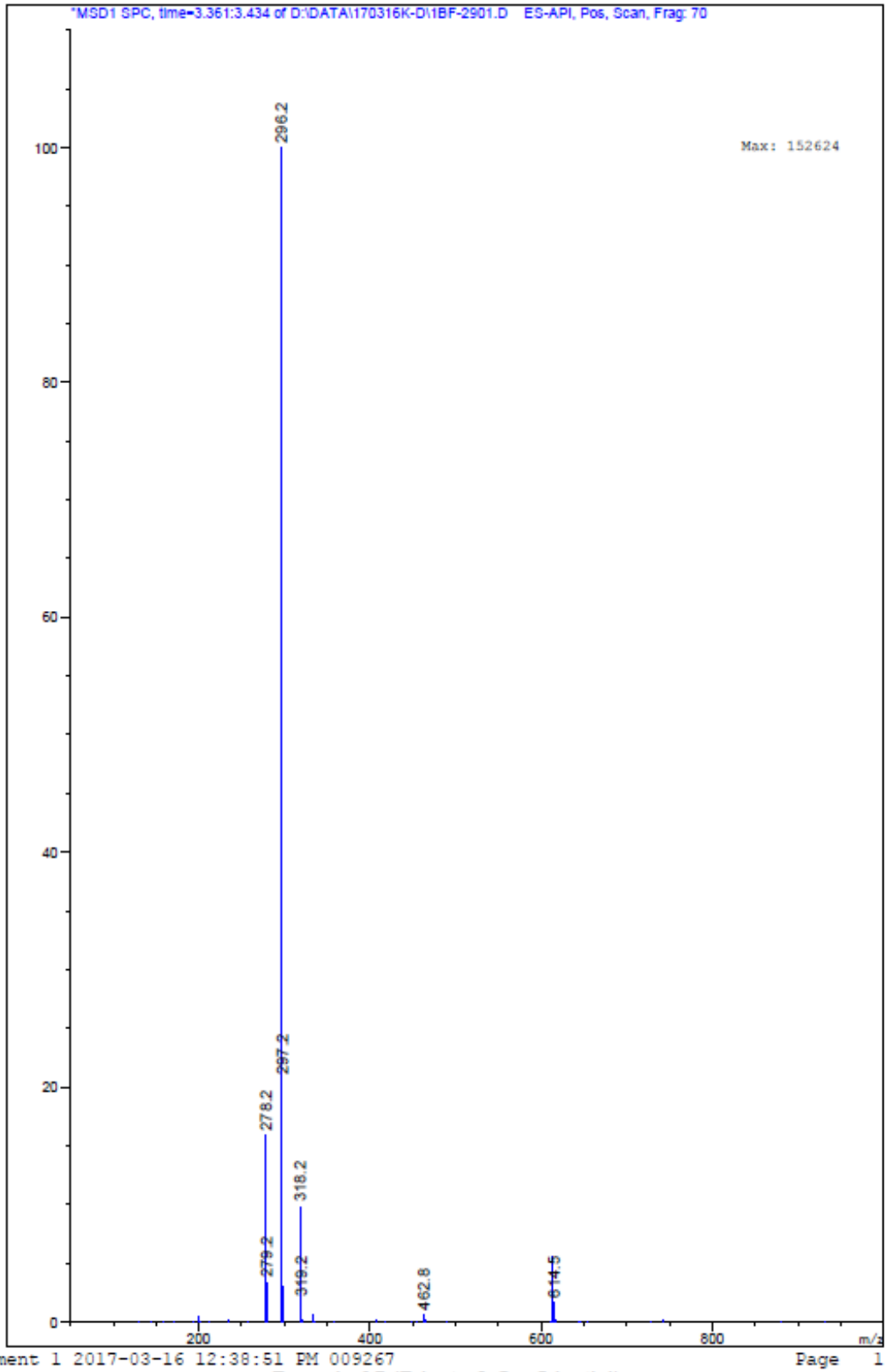
Signal 1 : ADC1 A, ELSD

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	3.425	94.781	43.845	100.000	0.033	100.000

Operator:_____

Date:_____

MS Spectrum

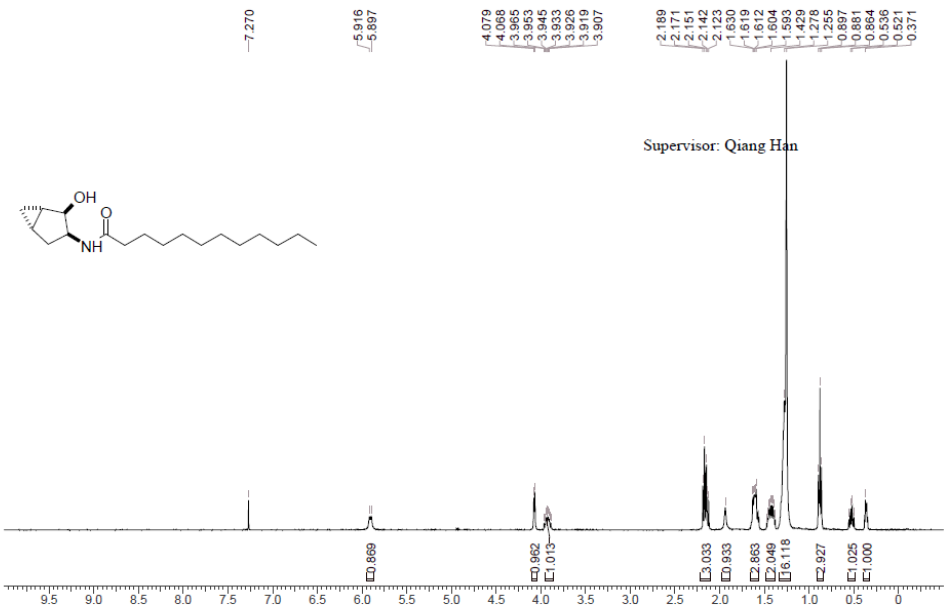


nstrument 1 2017-03-16 12:38:51 PM 009267 Page 1 of 1

BB0233:

Compound ID: BB0233

ET12347-255-P1AA CDCl3 Varian_Y_400MHz



Acquisition Time (sec)	2.0486
Comment	ET12347-255-P1AA CDCl3 Varian_Y_400MHz
Date	Mar 27 2017
Frequency (MHz)	399.6733
Nucleus	1H
Number of Transients	8
Original Points Count	14802
Points Count	32768
Pulse Sequence	s2pul
Receiver Gain	44.00
SW(cyclical) (Hz)	7225.43
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2802.0881
Spectrum Type	standard
Sweep Width (Hz)	7225.21
Temperature (degree C)	25.000

Operator:

Date:

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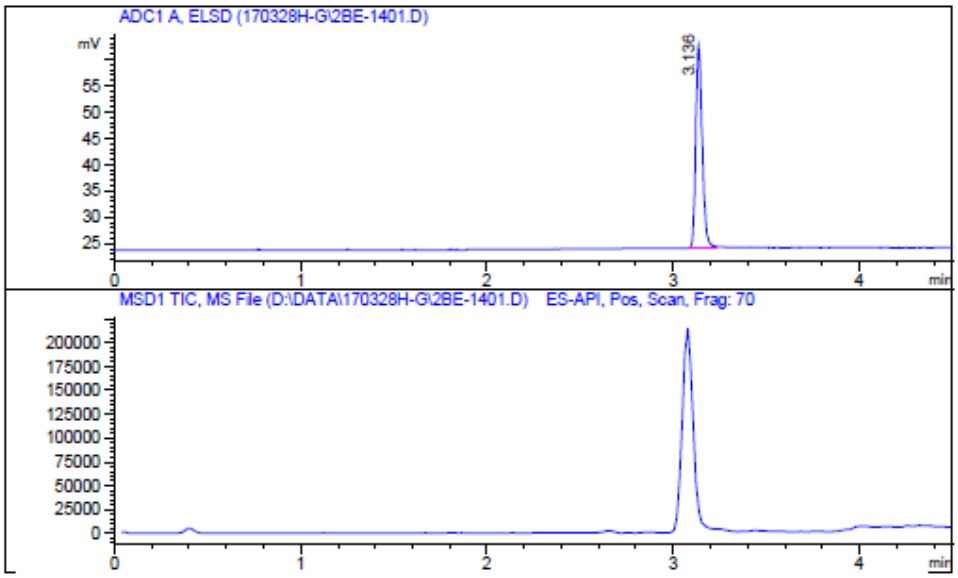
LCMS REPORT

Compound ID : BB0233 ->
Sample ID : ET12347-255-P1A1
Injection Date : 28. Mar. 2017
Inj. Vol. : 0.70ul
Location : P2-B-05
Acq Method : D:\DATA\170328H-G\WUXIAB25.M
Data Filename : D:\DATA\170328H-G\2BE-1401.D
Instrument : H



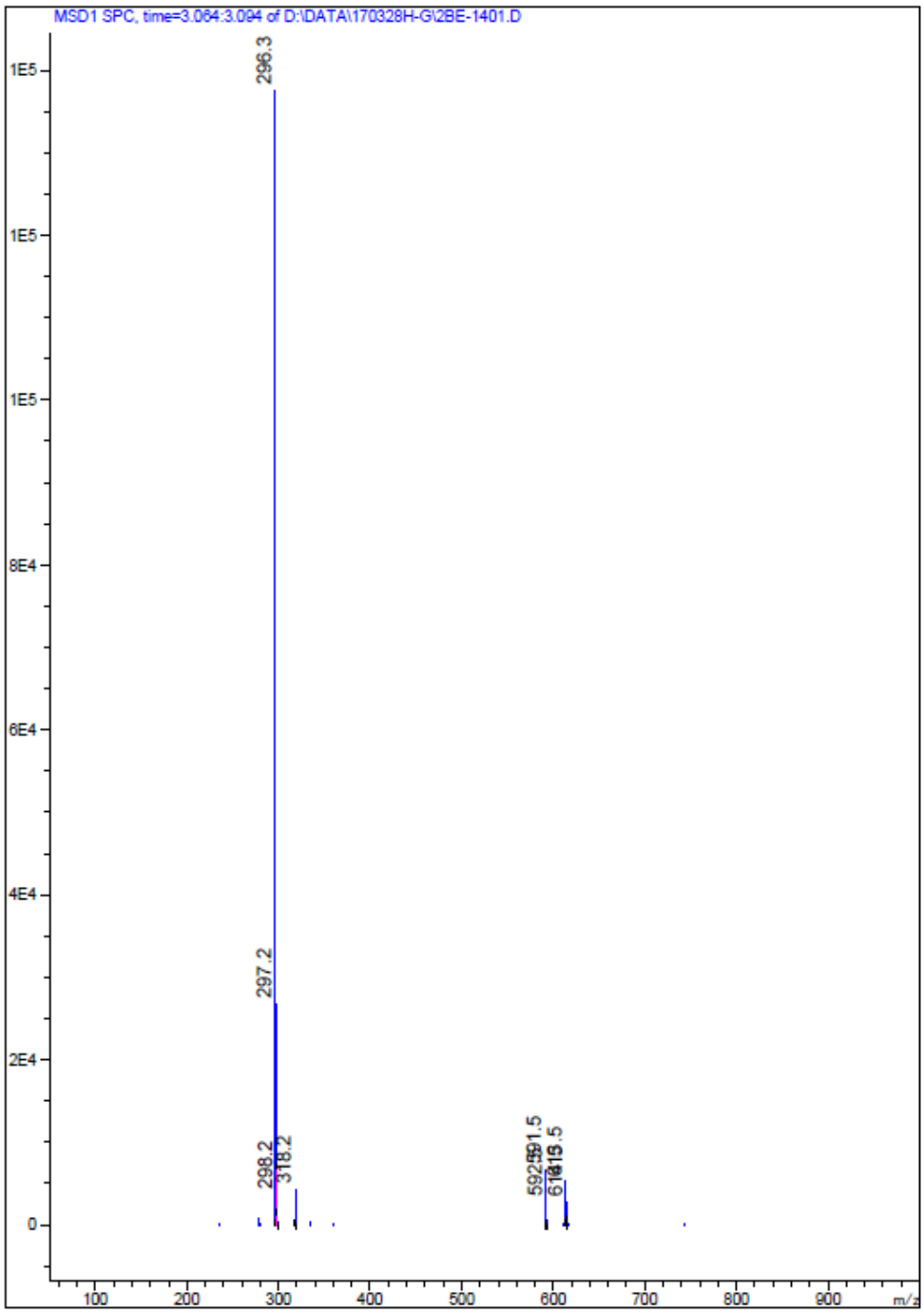
Integration Result

Signal 1 : ADC1 A, ELSD

Peak #	RT [min]	Height	Height %	Width [min]	Area	Area %
1	3.136	38.588	100.000	0.037	93.116	100.000

Operator:_____

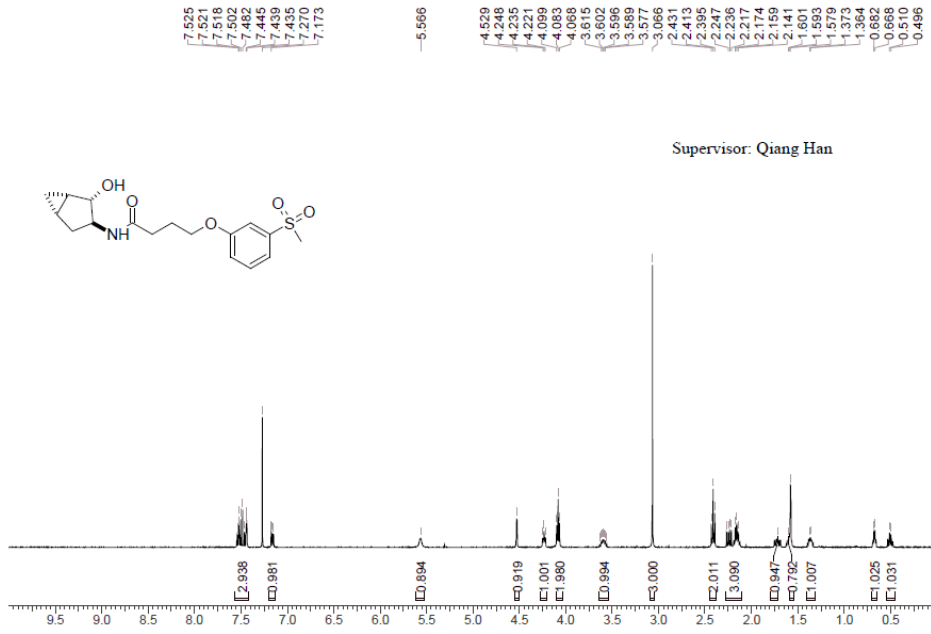
Date:_____



BB0272:

Compound ID: BB0272

ET12347-481-P1AA CDCl3 Bruker_E_400MHz



Acquisition Time (sec)	2.0447
Comment	ET12347-481-P1AA CDCl3 Bruker_E_400MHz
Date	19 Jul 2017 18:21:04
Frequency (MHz)	400.1300
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	174.64
SW(cyclical) (Hz)	8012.82
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2465.2009
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	-273.000

Operator:

Date:

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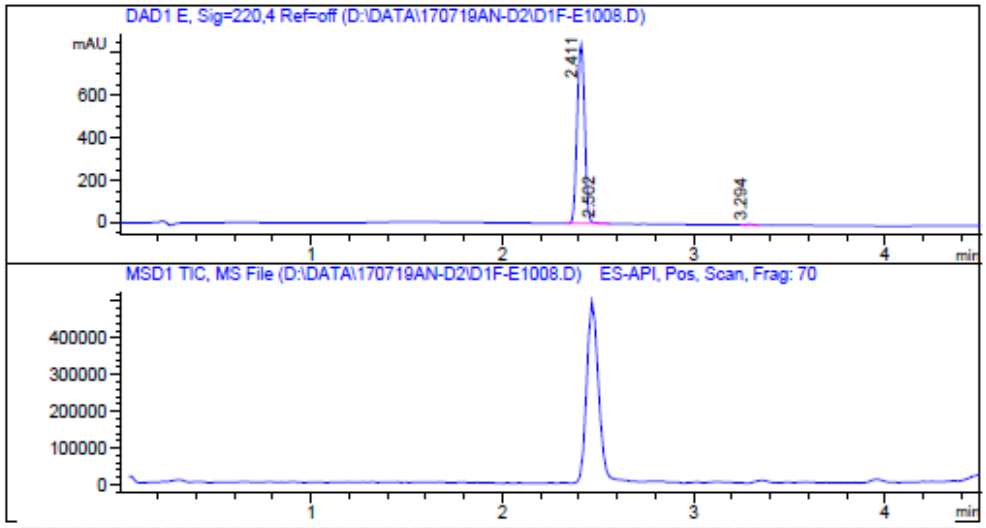
LCMS REPORT

Compound ID : BB0272
Sample ID : ET12347-481-P1A1
Injection Date : 20. Jul. 2017
Inj. Vol. : 2.0 ul
Location : D1F-E1
Acq Method : D:\Method\WUXIAB01.M
Data Filename : D:\DATA\170719AN-D2\D1F-E1008.D
Instrument : AN



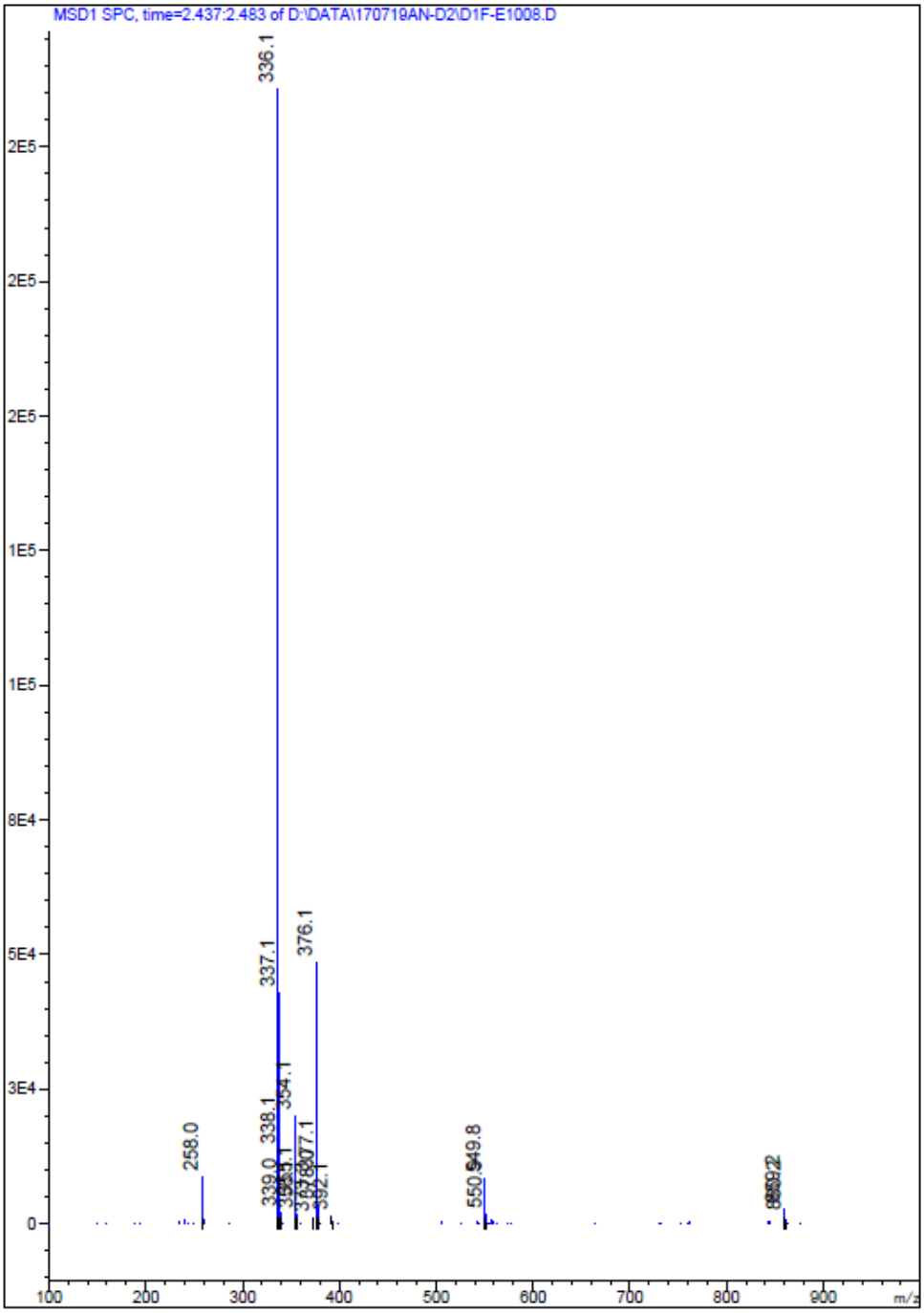
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.411	2288.674	846.584	99.354	0.045	99.427
2	2.502	4.191	2.044	0.240	0.034	0.182
3	3.294	8.993	3.461	0.406	0.043	0.391

Operator:_____

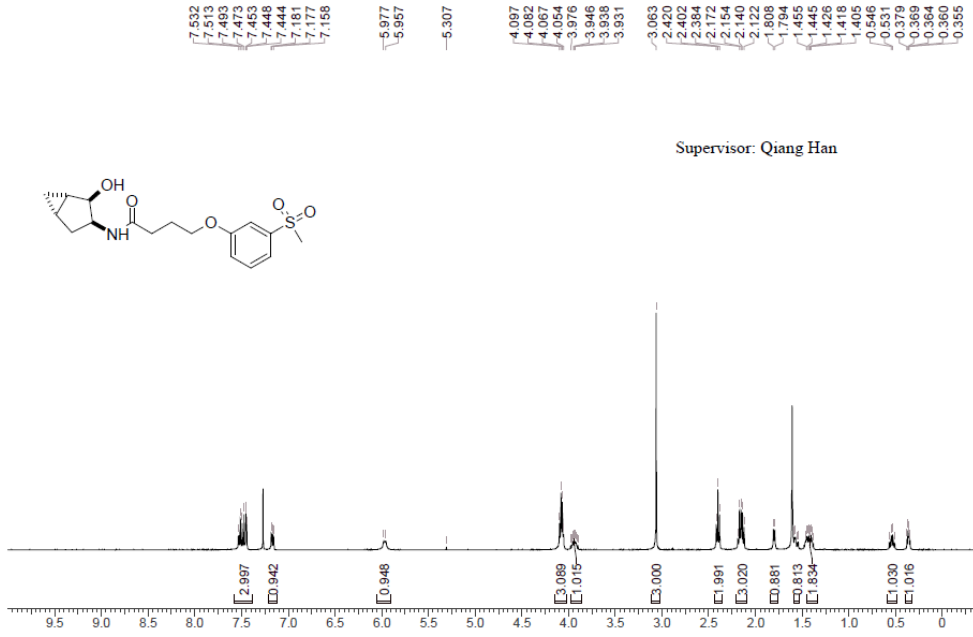
Date:_____



BB0273:

Compound ID: BB0273

ET12347-480-P1BB CDCl3 Bruker_C_400MHz



Acquisition Time (sec)	2.0447
Comment	ET12347-480-P1BB CDCl3 Bruker_C_400MHz
Date	19 Jul 2017 13:42:34
Frequency (MHz)	400.1500
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	115.64
SW(cyclical) (Hz)	8012.82
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2465.0835
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	25.614

Operator:

Date:

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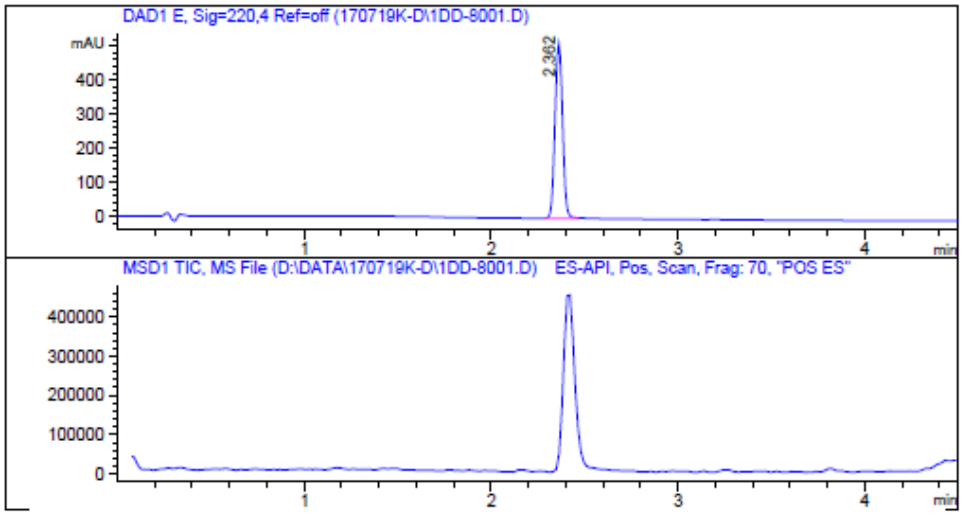
LCMS REPORT

Compound ID : BB0273 ->
Sample ID : ET12347-480-P1A1
Injection Date : 19. Jul. 2017
Inj. Vol. : 2.0 ul
Location : P1-D-04
Acq Method : D:\DATA\170719K-D\WUXIAB01_W.M
Data Filename : D:\DATA\170719K-D\1DD-8001.D
Instrument : K



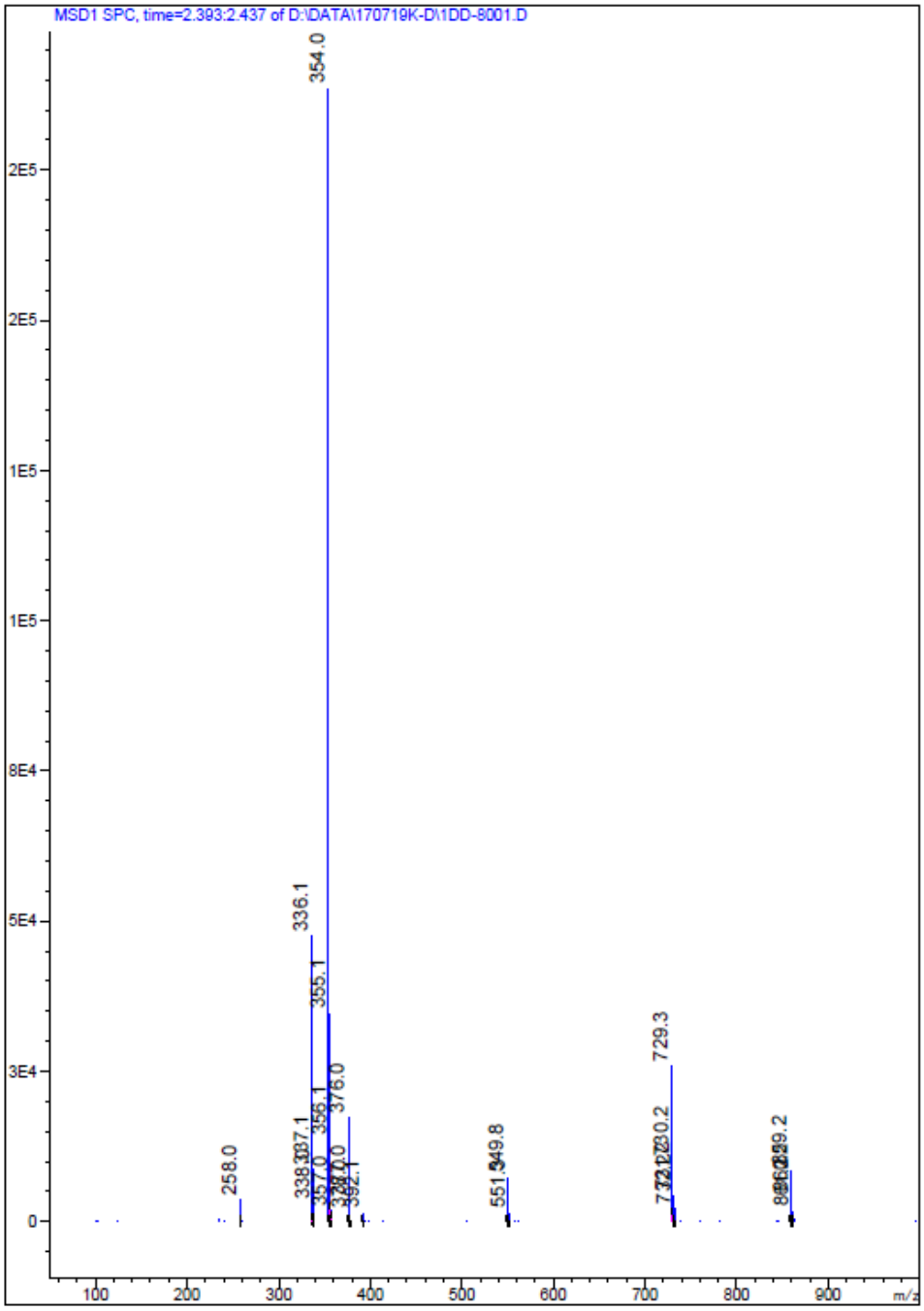
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.362	1456.844	513.269	100.000	0.045	100.000

Operator:_____

Date:_____



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