

## Supplementary Materials for

### Antimalarial pantothenamide metabolites target acetyl–coenzyme A biosynthesis in *Plasmodium falciparum*

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##### Materials and Methods

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Fig. S3. Pantothenate competition assays.

Fig. S4. PANK activity.

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**Other Supplementary Material for this manuscript includes the following:**

(available at [stm.sciencemag.org/cgi/content/full/11/510/eaas9917/DC1](http://stm.sciencemag.org/cgi/content/full/11/510/eaas9917/DC1))

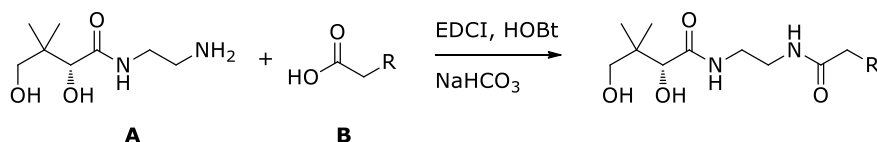
Table S10 (Microsoft Excel format). Source data for Figs. 1C, 2 (A to E), 3C, 4B, and 5 (A to C).

## Materials and Methods

### Synthesis and quality control of compounds described in the main text

#### Synthesis of CXP18.6-006, CXP18.6-017, CXP18.6-026

General procedure A: to a solution of carboxylic acid **B** (0.5 mmol) in MeCN/H<sub>2</sub>O (30:1, 4.3 mL) were added HOBt (0.6 mmol), NaHCO<sub>3</sub> (0.6 mmol), EDCI (0.6 mmol) and a solution of amine **A** (for the synthesis see ref 2, 0.6 mmol) in MeCN/H<sub>2</sub>O (0.7 mL). The progress of the reaction was monitored using LC-MS and upon completion, the reaction was quenched by the addition of saturated aqueous NH<sub>4</sub>Cl solution (15 mL) and the mixture was extracted twice using EtOAc (15 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and filtered before concentration under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH = 98:2 → 80:20) to afford the product.



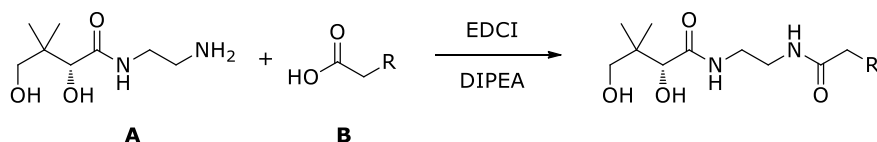
CXP18.6-006: According to general procedure A. Yield: 51%, pale yellow oil.

CXP18.6-017: According to general procedure A. Yield: 35%, colorless oil.

CXP18.6-026: According to general procedure A. Yield: 56%, colorless oil

#### Synthesis of CXP18.6-052:

General procedure B: to a suspension of carboxylic acid **B** (0.72 mmol) in MeCN/H<sub>2</sub>O (19:1, 4 mL) was added EDCI (0.79 mmol). After stirring for 10 min an almost clear solution was obtained. Amine **A** (for the synthesis see ref 2, 0.79 mmol) was added, followed by DIPEA (0.79 mmol). The progress of the reaction was monitored using LC-MS and upon completion, silica gel was added. The mixture was then concentrated under reduced pressure and purified by flash column chromatography (MeCN/MeOH = 4:1 → 2:1) to afford the product.



CXP18.6-052: According to general procedure B. Yield: 30%, colorless oil.

#### Synthesis of MMV689258 (numbers in bold refer to Figure below)

To a stirred solution of ((S)-2-Hydroxy-1-methyl-ethyl)-carbamic acid tert-butyl ester **1** (2.5 g, 14.26 mmol) in dry THF (28 ml) were added phthalimide **2** (2.3g, 15.69 mmol) and PPh<sub>3</sub> (4.11g, 15.69 mmol). DEAD (2.73 g, 15.69 mmol) was then added dropwise to the stirred solution at room temperature and maintained for 16h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography (70:30 -50:50 hexanes-EtOAc) to afford compound **3** (4 g, 92%) as white solid.

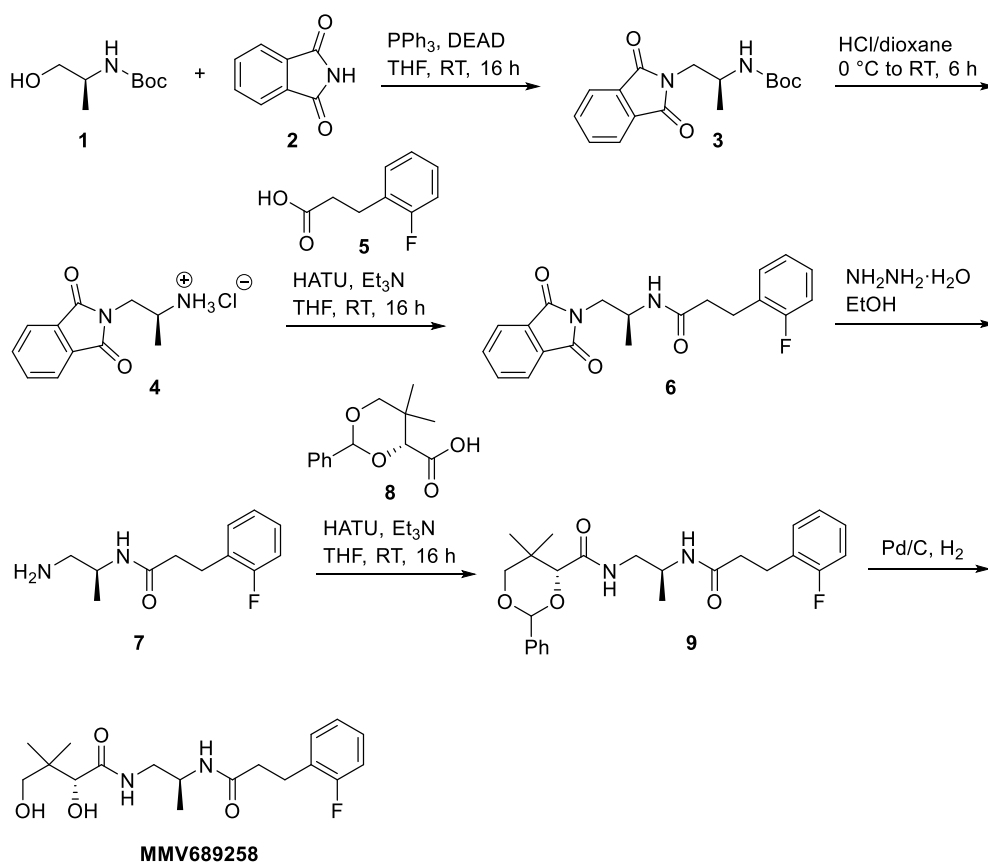
To a stirred solution of compound **3** (2.0 g, 6.57 mmol) in dioxane (20 ml) was added 4M dioxane-HCl (20 ml) at 0°C. It was stirred at RT for 6h. It was concentrated under reduced pressure to afford compound **4** (1.5 g, 95%) as white solid.

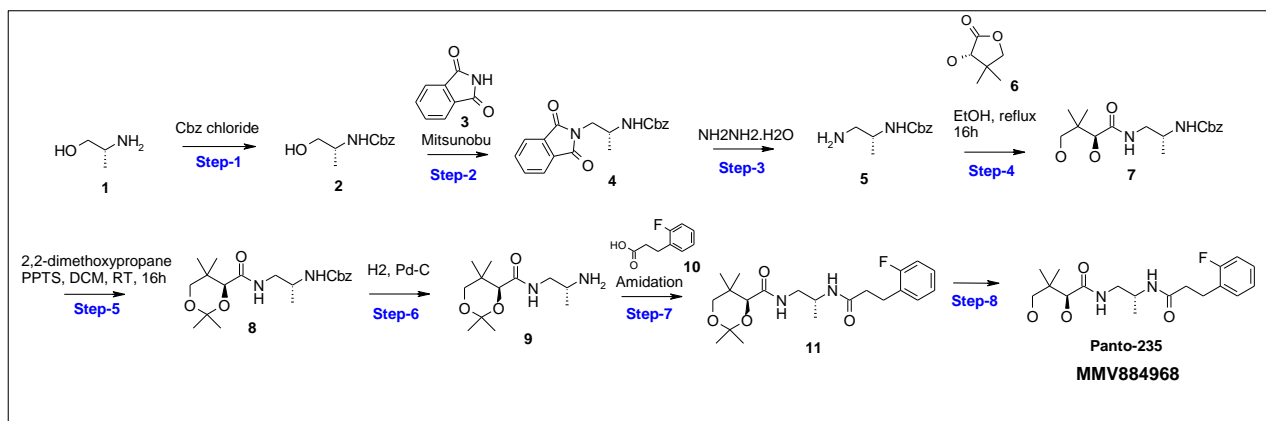
To a stirred solution of compound **5** (180 mg, 1.07 mmol) in THF (10 ml) were added Et<sub>3</sub>N (0.747 ml, 5.36 mmol), HATU (611 mg, 1.61 mmol) and compound **4** (515 mg, 2.14 mmol). It was stirred at RT for 16h. TLC (30% EtOAc-Hexane) showed completion of the reaction. It was diluted with water (20 ml) and extracted with EtOAc (50 ml), washed with brine (10 ml), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. It was purified by combiflash (30% EtOAc-Hexane) to afford compound **6** (330 mg, 87%) as off white solid.

To a stirred solution of compound **6** (330 mg, 0.93 mmol) in EtOH (20 ml) was added hydrazine hydrate (745 mg, 14.90 mmol). It was heated at 50°C for 2h. It was cooled to RT, filtered and concentrated under reduced pressure. The resulting residue was suspended in Et<sub>2</sub>O (20 ml) and filtered, washing thoroughly with Et<sub>2</sub>O (20 ml). The combined filtrates were concentrated under reduced pressure to afford compound **7** (150 mg, 72 %) as gum.

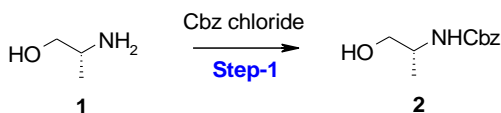
To a stirred solution of compound **8** (75 mg, 0.32 mmol) in THF (20 ml) were added Et<sub>3</sub>N (0.221ml, 1.59 mmol), HATU (181.25 mg, 0.48 mmol) and compound **7** (142.37 mg, 0.64 mmol). It was stirred at room temp for 16h. It was diluted with water (20 ml) and extracted with EtOAc (50 ml), washed with saturated NaHCO<sub>3</sub> (20 ml), brine (10 ml), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. It was purified by column chromatography (3% MeOH-DCM) to afford compound **9** (110 mg, 78%) as gum.

Compound **9** (90 mg, 0.203 mmol) was taken in MeOH (10 ml) and was degassed with argon for 10 minutes. Pd/C (50 mg, 10% moist) was added and the mixture was subjected to hydrogenation in a Parr vessel at 50psi for 16h. It was filtered through celite, concentrated under reduced pressure and purified by preparative TLC (4% MeOH-DCM) to afford MMV689258 (40 mg, 55 %) as colourless oil.



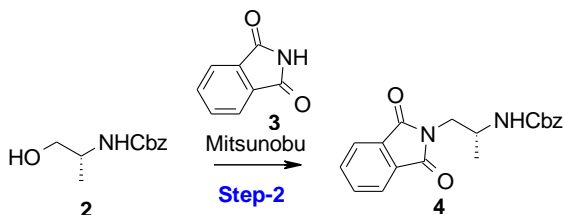


#### Synthesis of compound ((R)-2-Hydroxy-1-methyl-ethyl)-carbamic acid benzyl ester (2):



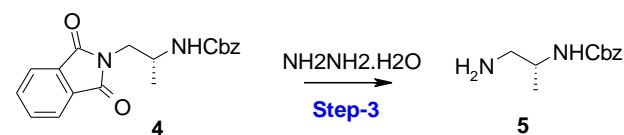
**Procedure:** Same as ((S)-1-Hydroxymethyl-propyl)-carbamic acid benzyl ester (step 1 of MMV692676 synthesis) with (R)-2-Amino-propan-1-ol (**1**) (1 g, 13.314 mmol) to afford ((R)-2-Hydroxy-1-methyl-ethyl)-carbamic acid benzyl ester (**2**) as gum in 54.56% yield, 1.52 g.

#### Synthesis of compound [(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (4):



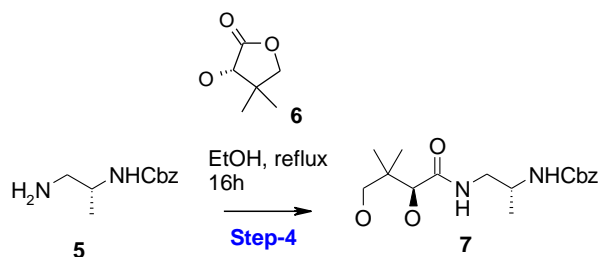
**Procedure:** Same as [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (step 2 of MMV692676 synthesis) with ((R)-2-Hydroxy-1-methyl-ethyl)-carbamic acid benzyl ester (**2**) (1.52 g, 7.264 mmol) to afford [(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (**4**) as sticky solid in 81.37% yield, 2 g.

#### Synthesis of compound ((R)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (5):



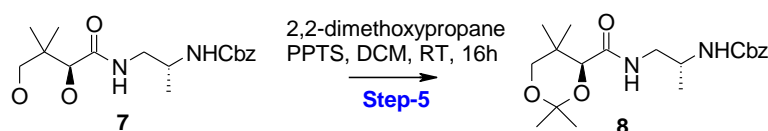
**Procedure:** Same as ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (step 3 of MMV692676 synthesis) with [(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (**4**) (2.5 g, 7.388 mmol) to afford ((R)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (**5**) as gum in 97.49% yield, 1.5 g.

**Synthesis of compound [(R)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (7):**



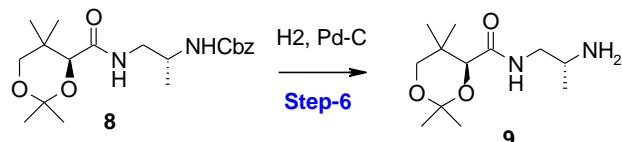
**Procedure:** Same as ((S)-1-[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl)-carbamic acid benzyl ester (step 4 of MMV692676 synthesis) with ((R)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (5) (180 mg, 0.864 mmol) and L-(+)-Pantolactone (5) (337.44 mg, 2.593 mmol) to afford [(R)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (7) as gum in 63.25% yield, 185 mg.

**Synthesis of compound {(R)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (8):**



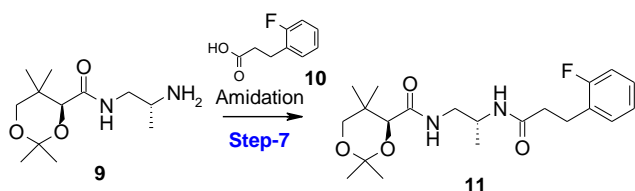
**Procedure:** Same as ((S)-1-[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl)-propyl)-carbamic acid benzyl ester (step 5 of MMV692676 synthesis) with [(R)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (7) (185 mg, 0.547 mmol) to afford {(R)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (8) as gum in 50.75% yield, 105 mg.

**Synthesis of compound (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((R)-2-amino-propyl)-amide (9):**

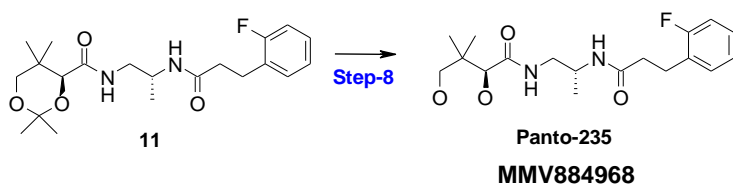


**Procedure:** Same as (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (step 6 of MMV692676 synthesis) with {(R)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (8) (105 mg, 0.277 mmol) to afford (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((R)-2-amino-propyl)-amide (9) as gum in 88.51% yield, 60 mg.

**Synthesis of compound (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (11):**



**Procedure:** Same as general procedure A with **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(R)-2-amino-propyl}-amide (9)** (60 mg, 0.245 mmol) and **3-(2-Fluoro-phenyl)-propionic acid (10)** (49.53 mg, 0.295 mmol) to afford **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (11)** as gum in 92.94% yield, 90 mg.



**Procedure:** Same as general procedure B with **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (11)** (90 mg, 0.228 mmol) to afford **MMV884968** as colorless sticky solid in 37.06% yield, 30 mg.

**Quality control: 1H-NMR, 13C-NMR and HRMS spectra of compounds described in the main text**

p8 Methods

p9 EIC OF CXP18.6-017

p10 MS OF CXP18.6-017

p11 MS/MS OF CXP18.6-017

p12 1H-assignment OF CXP18.6-017

p13 13C-assignment OF CXP18.6-017

p14 EIC OF CXP18.6-052

p15 MS OF CXP18.6-052

p16 MS/MS OF CXP18.6-052

p17 1H-assignment OF CXP18.6-052

p18 13C-assignment OF CXP18.6-052

p19 Heteronuclear single quantum coherence OF CXP18.6-052

p20 EIC OF CXP18.6-026

p21 MS OF CXP18.6-026

p22 MS/MS OF CXP18.6-026

p23 1H-assignment OF CXP18.6-026

p24 13C-assignment OF CXP18.6-026

p25 EIC OF MMV689258

p26 MS OF MMV689258

p27 MS/MS OF MMV689258

p28 1H-assignment OF MMV689258

p29 13C-assignment OF MMV689258

p30 EIC OF MMV884968

p31 MS OF MMV884968

p32 MS/MS OF MMV884968

p33 1H-assignment OF MMV884968

p34 13C-assignment OF MMV884968

p35 EIC OF CXP18.6-006

p36 MS OF CXP18.6-006

p37 MS/MS OF CXP18.6-006

p38 1H-assignment OF CXP18.6-006

p39 13C-assignment OF CXP18.6-006



## Methods

For HRMS - A 5  $\mu$ L aliquot of sample (1  $\mu$ M) was separated by reverse phase HPLC using a Prominence 20 UFLCXR system (Shimadzu, Columbia MD) with a Waters (Milford, MA) BEH C18 column (100mm x 2.1mm 1.7  $\mu$ m particle size) maintained at 55  $^{\circ}$ C and a 20-minute aqueous acetonitrile gradient, at a flow rate of 250  $\mu$ L/min. Solvent A was HPLC grade water with 0.1% formic acid and Solvent B was HPLC grade acetonitrile with 0.1% formic acid. The initial condition were 97% A and 3 % B, increasing to 45% B at 10 min., 75% B at 12 min. where it was held at 75% B until 17.5 min. before returning to the initial conditions. The eluate was delivered into a 5600 (QTOF) TripleTOF using a Duospray<sup>TM</sup> ion source (all AB Sciex, Framingham, MA). The capillary voltage was set at 4.5 kV in negative ion mode, with a declustering potential of 80V. The mass spectrometer was operated in IDA (Information Dependent Acquisition) mode with a 100 ms survey scan from 100 to 1200 m/z, and up to 20 MS/MS product ion scans (100 ms) per duty cycle using a collision energy of 50V with a 20V spread.

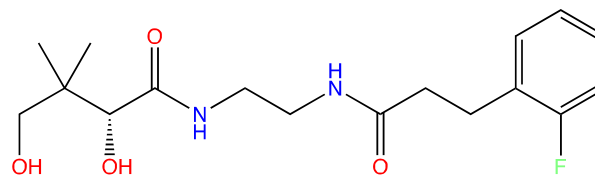
Sample data was loaded into mzMine to visualize the EIC and MS or MS/MS spectra. EICs are displayed for the parent ion  $[M-H]^{-}$  using a 20 ppm mass window. The EIC was used to select the corresponding MS spectra and the two most prominent peaks are displayed ( $[M-H]^{-}$  and  $[M+FA-H]^{-}$ ). High abundance MS/MS spectra were selected using the  $[M-H]^{-}$  precursor information and are annotated for the two most intense product ions. These product ions were annotated and validated using the mass fragmentation tool within ChemDraw.

For NMR – Samples were freeze-dried to remove solvents and were resuspended to 10-20mM in deuterated methanol (MeOD) containing 0.03% TMS reference. Following resuspension 0.5mL of sample was loaded into an NMR tube and spectra were acquired at the Penn State NMR core using a Bruker AVIII-HD-500.

For  $^1H$  NMR spectra (1D), proton assignments are annotated accordingly using letters to assign proton groups. Peak integration values are displayed on the x-axis for relevant peaks using Bruker TopSpin software – the TMS reference is not annotated but is displayed at 0 ppm for all  $^1H$  NMR. Instances where solvent overlap/interference occurs between MeOD and a chemical feature, a zoomed inset is provided for further clarity. The y-axis is scaled to visualize the peaks of interest. For  $^{13}C$  NMR spectra, a JMOD approach was used to determine  $^{13}C$  multiplicities and carbon assignments are annotated accordingly using numbers to assign carbons. CH and  $CH_3$  signals are positive and C and  $CH_2$  signals are negative using this method. All relevant peaks are annotated on the x-axis and their chemical shift is displayed in red using the Bruker TopSpin software. The y-axis is scaled to visualize the peaks of interest. A two-dimensional (2D) heteronuclear single quantum coherence (HSQC) approach was utilized for compound CXP18.6\_052 to accurately assign proton attachment to corresponding  $^{13}C$  heteronuclei ( $^1H$ - $^{13}C$  HSQC). Spectra are displayed with  $^1H$ -NMR on the x-axis and  $^{13}C$ -NMR on the y-axis, using the same assignment schemes previously mentioned. Peaks are identified using the Bruker TopSpin software and are numbered accordingly. Annotations in red are chemical features that can be assigned to the compound of interest and are listed with their corresponding structural feature. ‡ denotes the  $^1H$ - $^{13}C$  correlations for a given feature. Peaks annotated in black are due to solvent background.

XIC (base peak), m/z: 339.1620 - 339.1820

**CXP18.6-017**  
(EIC  $\pm$  20 ppm)



Base Peak Intensity

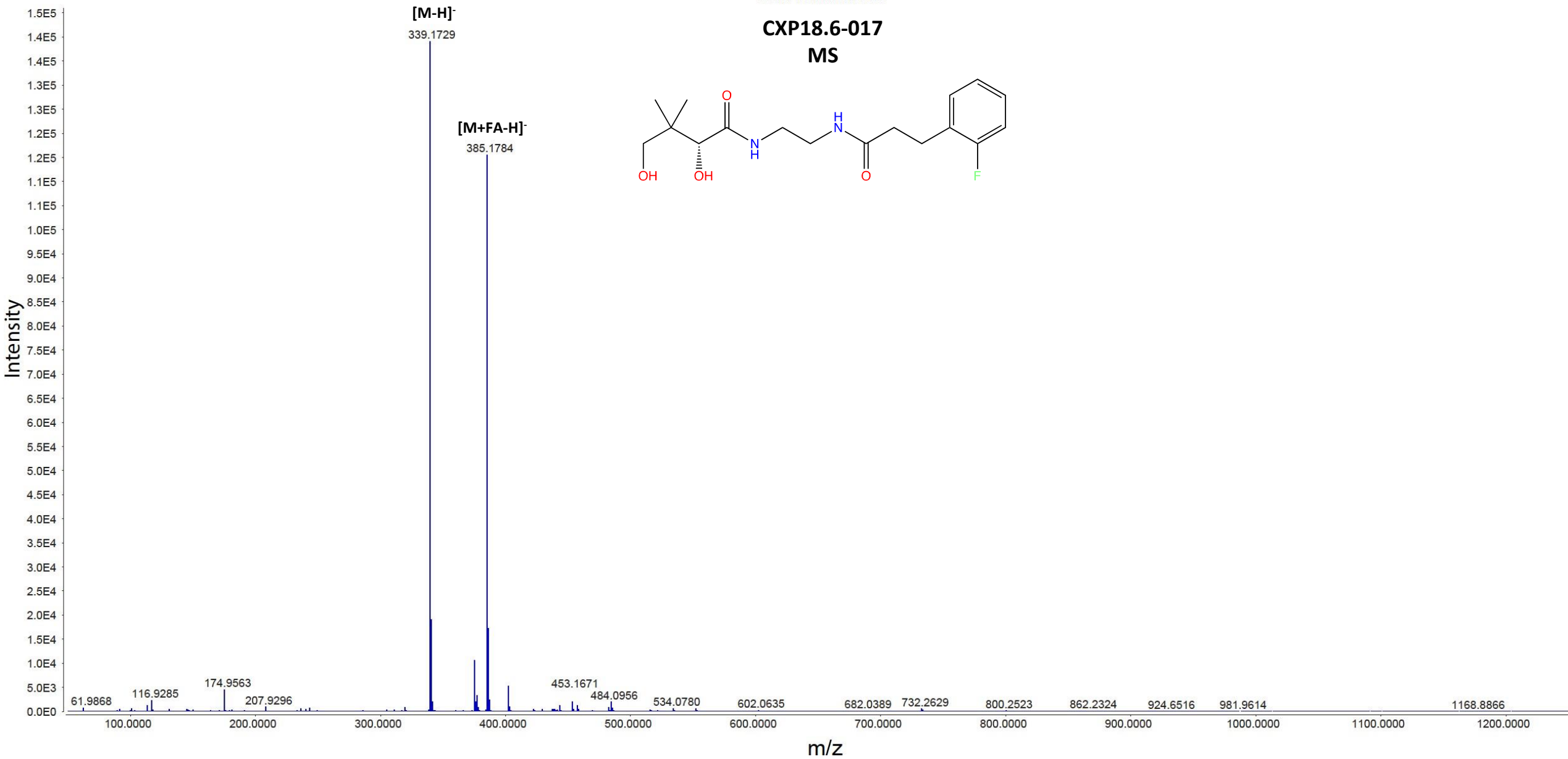
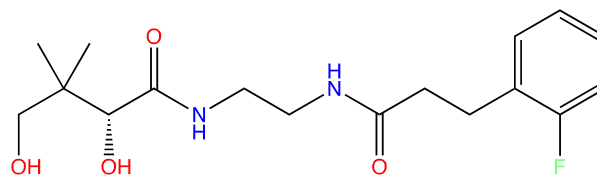
Retention time

339.1703

339.1767

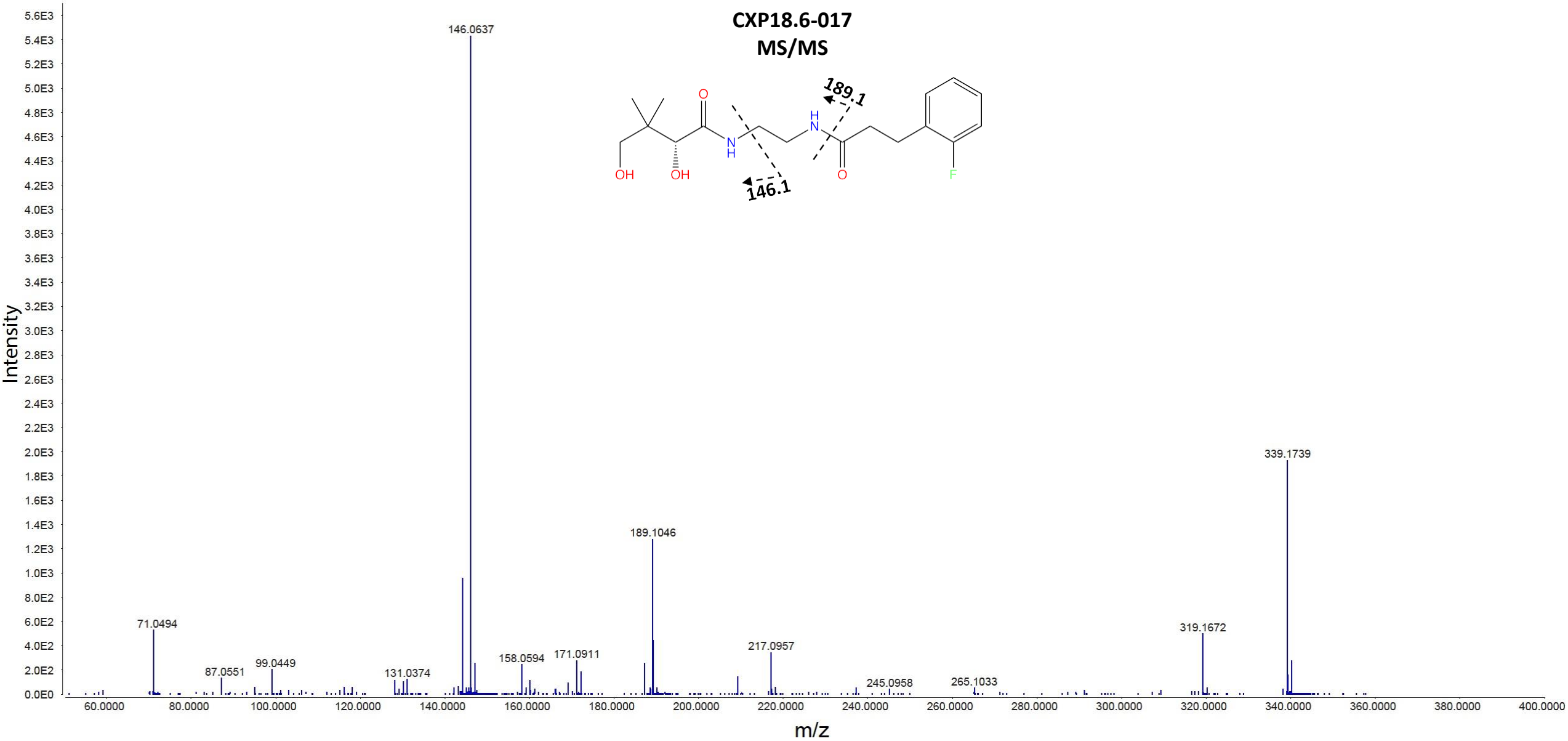
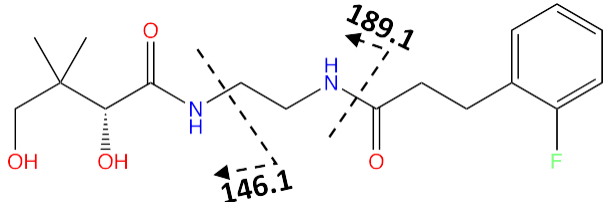
scan#4153 @7.6 MS1 c -, base peak: 339.1729 m/z (1.4E5)  
Scan definition: Full

**CXP18.6-017**  
**MS**

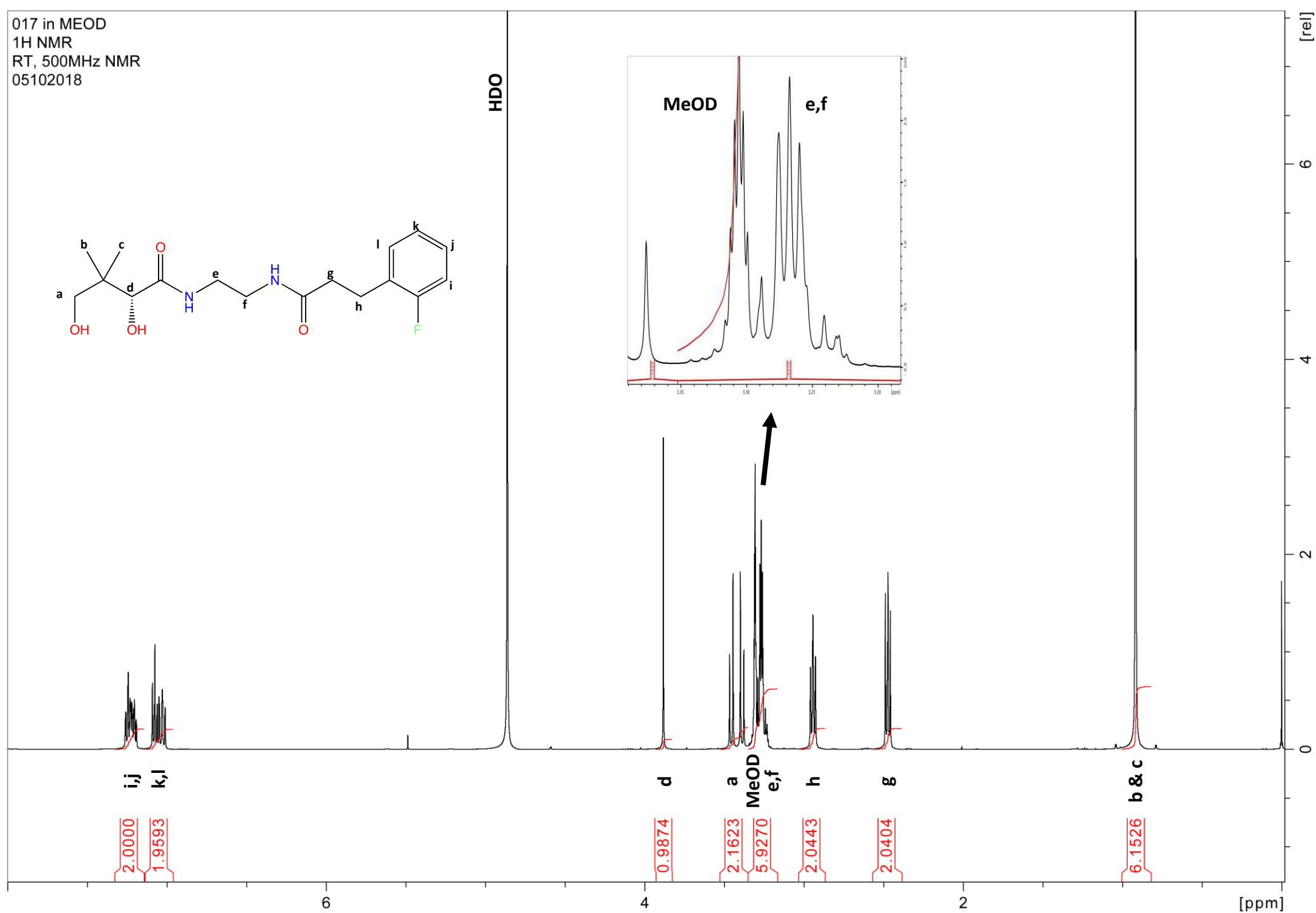
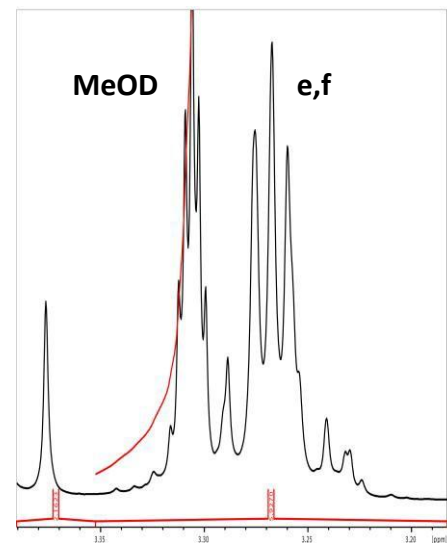
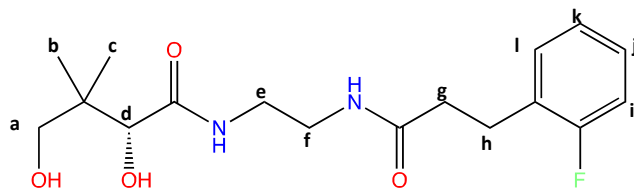


scan#4160 @7.6 MS2 (339.2000) c -, base peak: 146.0637 m/z (5.4E3)  
Scan definition: Full

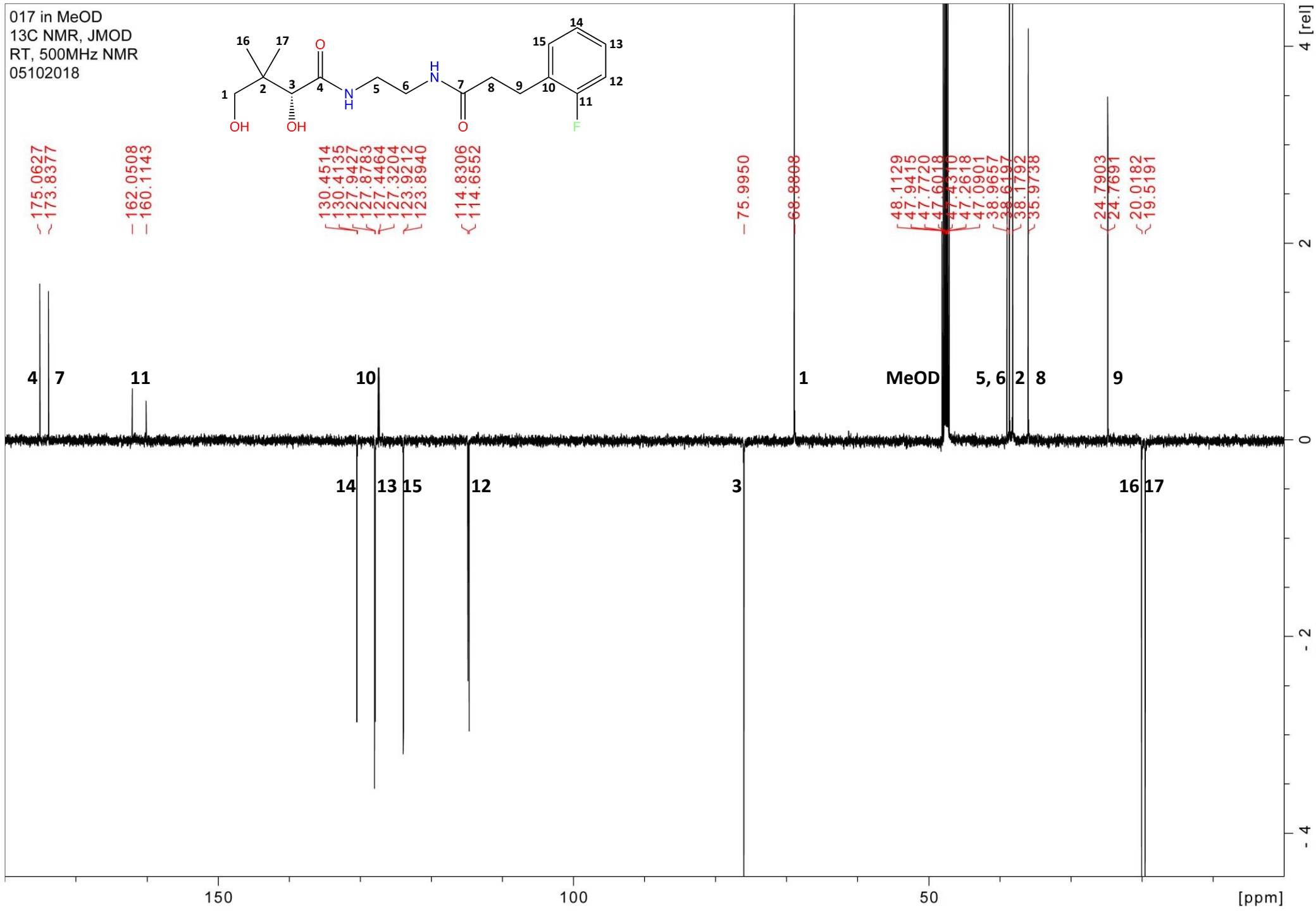
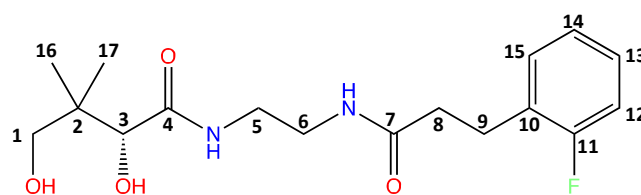
CXP18.6-017  
MS/MS



017 in MEOD  
1H NMR  
RT, 500MHz NMR  
05102018

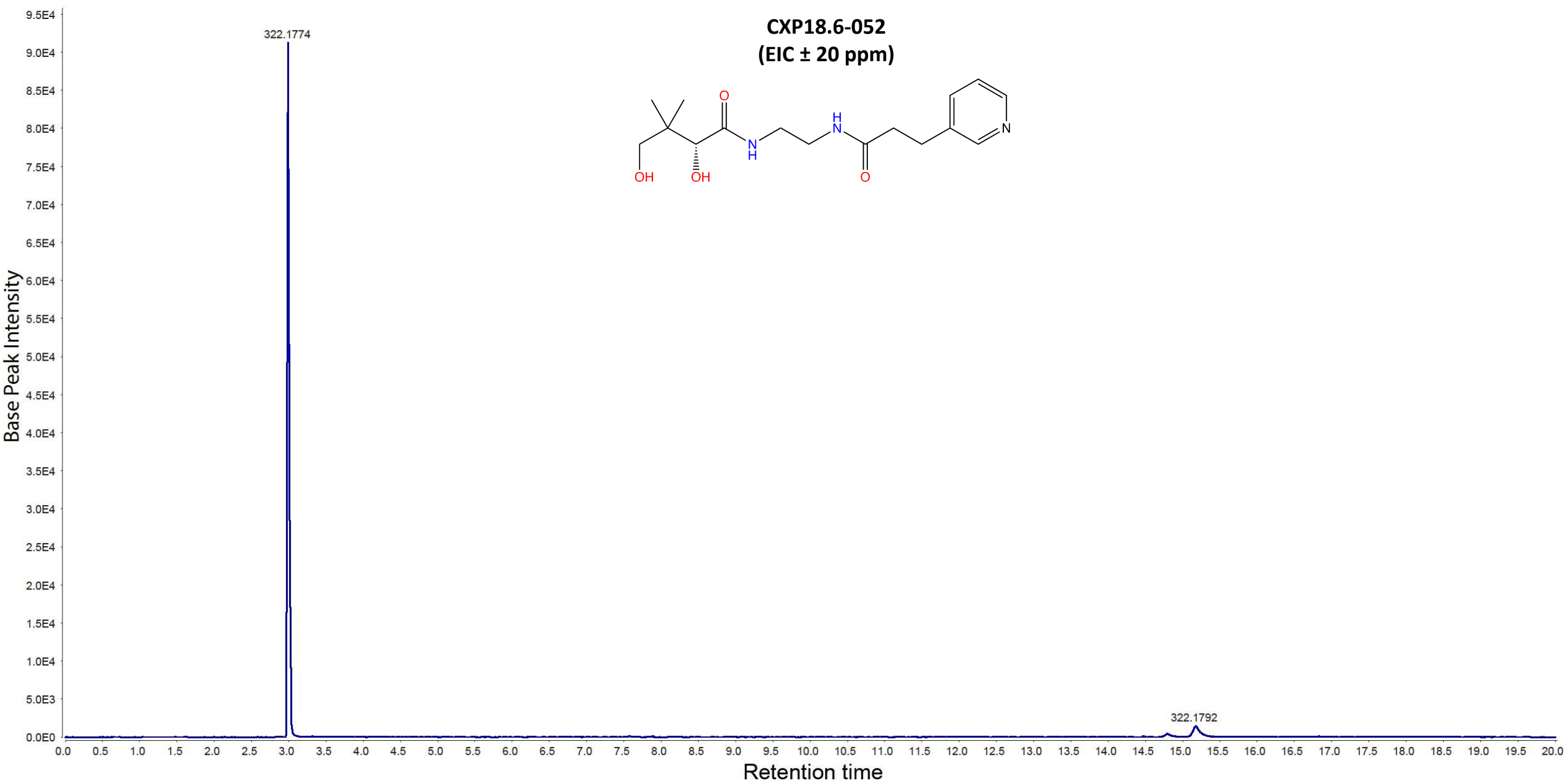
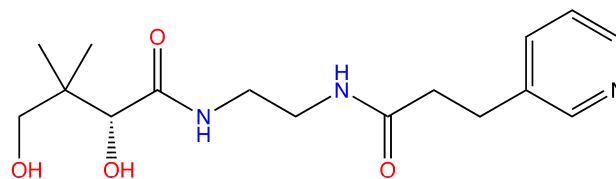


017 in MeOD  
 13C NMR, JMOD  
 RT, 500MHz NMR  
 05102018



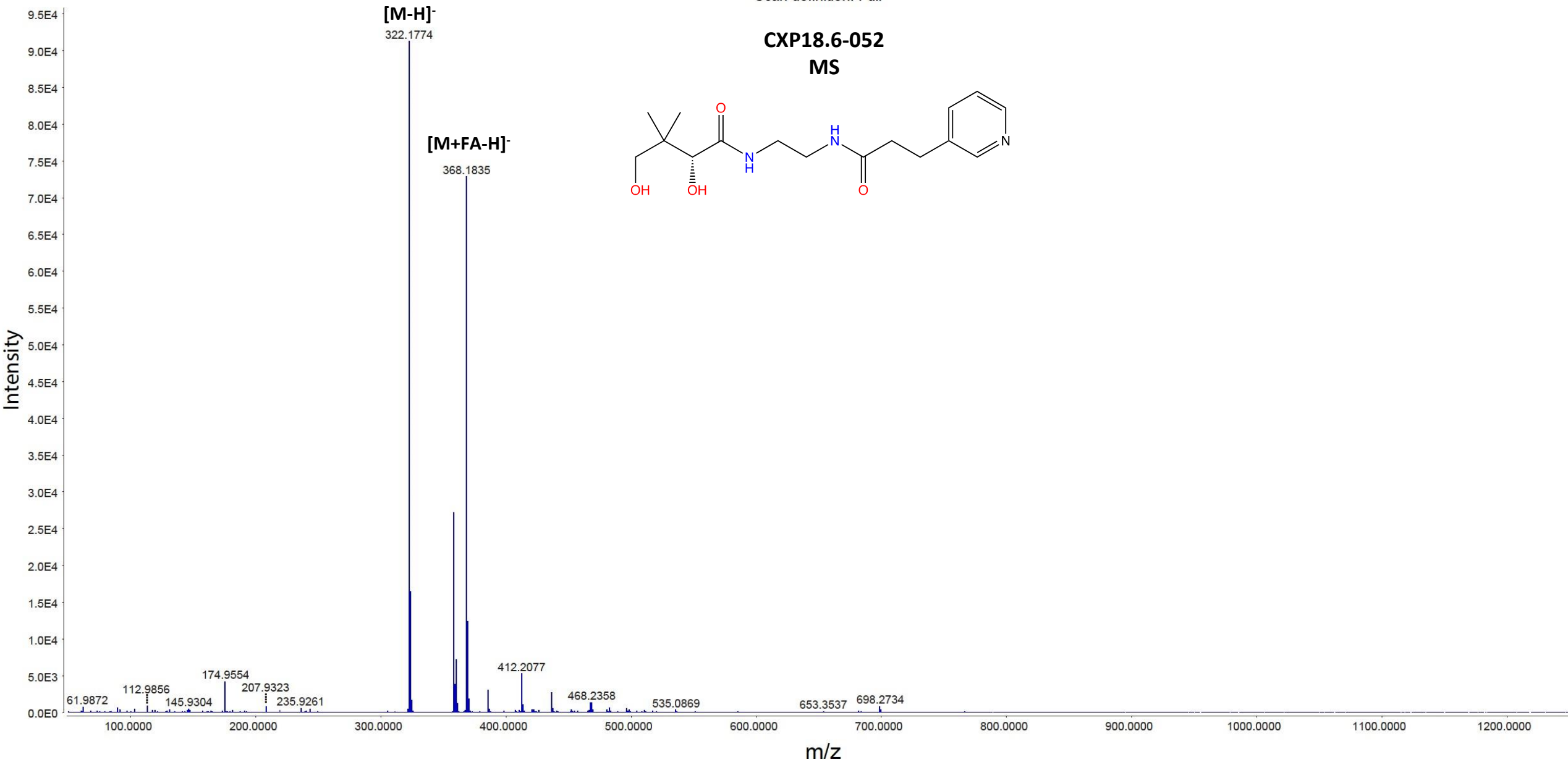
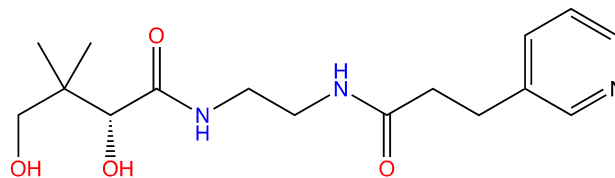
XIC (base peak), m/z: 322.1661 - 322.1862

**CXP18.6-052**  
(EIC  $\pm$  20 ppm)



scan#493 @3.0 MS1 c -, base peak: 322.1774 m/z (9.1E4)  
Scan definition: Full

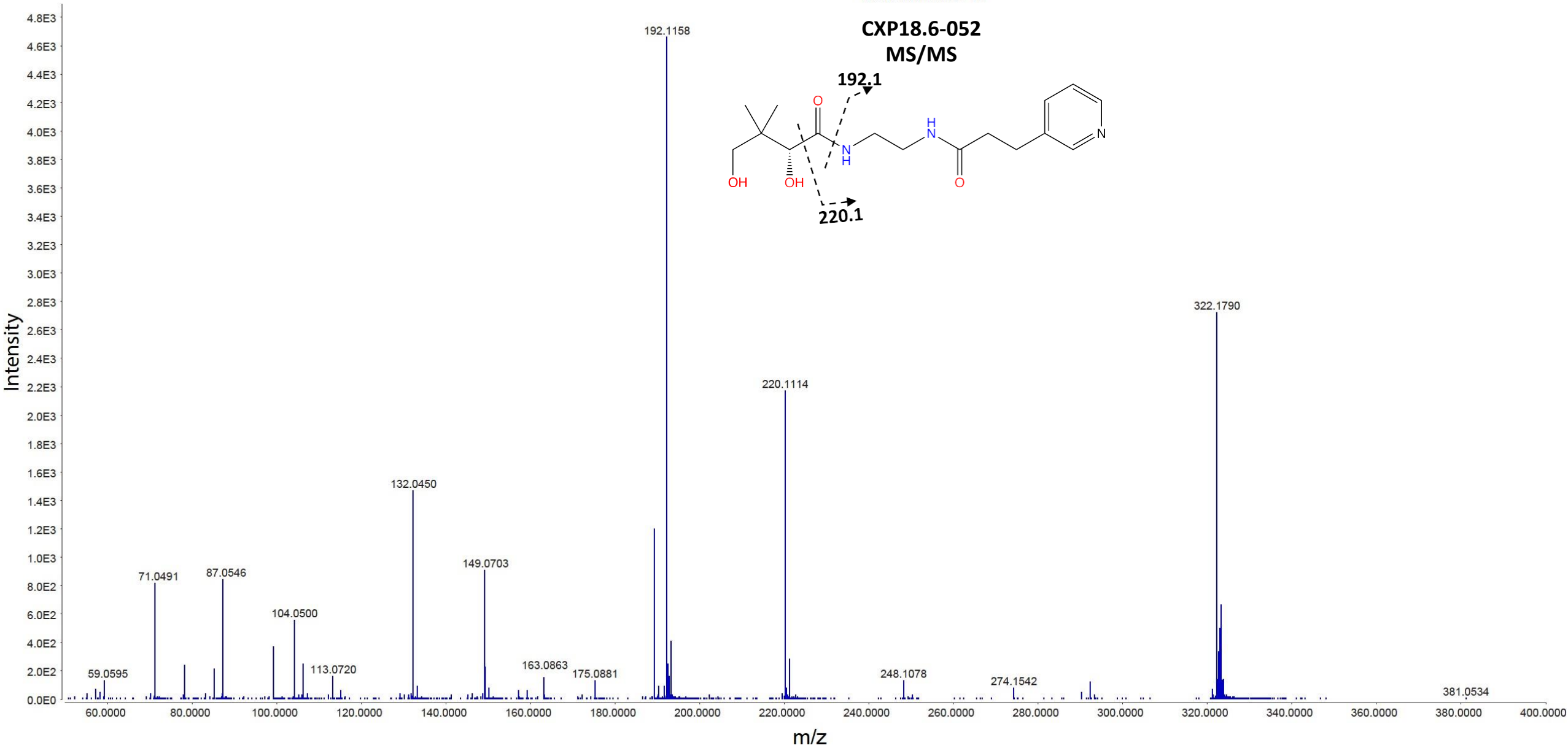
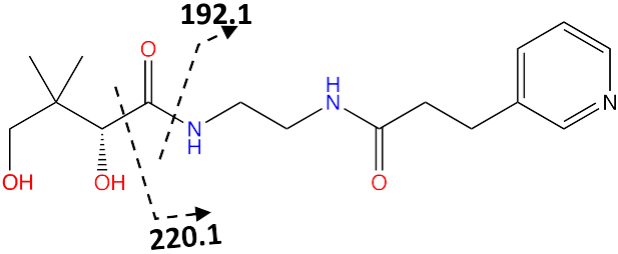
**CXP18.6-052**  
**MS**



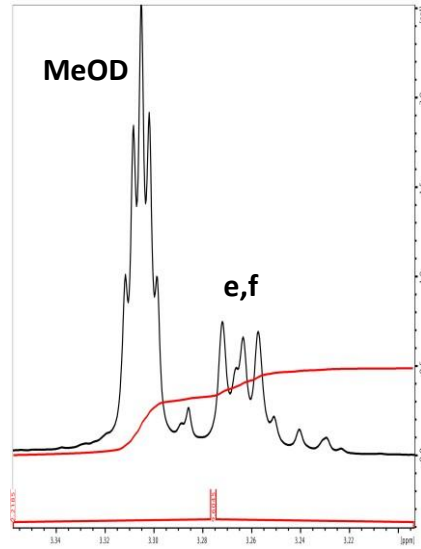
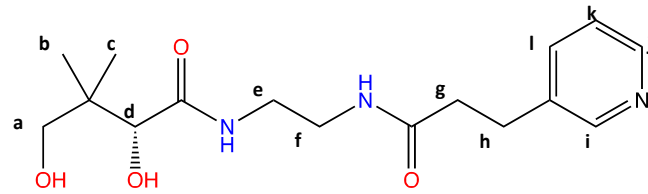


scan#494 @3.0 MS2 (322.1780) c -, base peak: 192.1158 m/z (4.7E3)  
Scan definition: Full

**CXP18.6-052**  
**MS/MS**



052 in MEOD  
1H NMR  
RT, 500MHz NMR  
05102018



H<sub>2</sub>O

DMSO

[rel]

2.0

1.5

1.0

0.5

-0.0

[ppm]

1H-assignment

ij

l

k

d

a

MeOD

e,f

h

g

b,c

1.9008

0.9733

0.9746

0.9676

2.2185

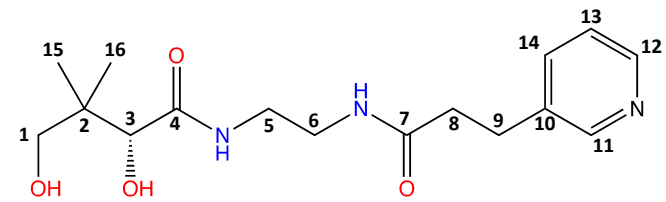
9.6045

1.9629

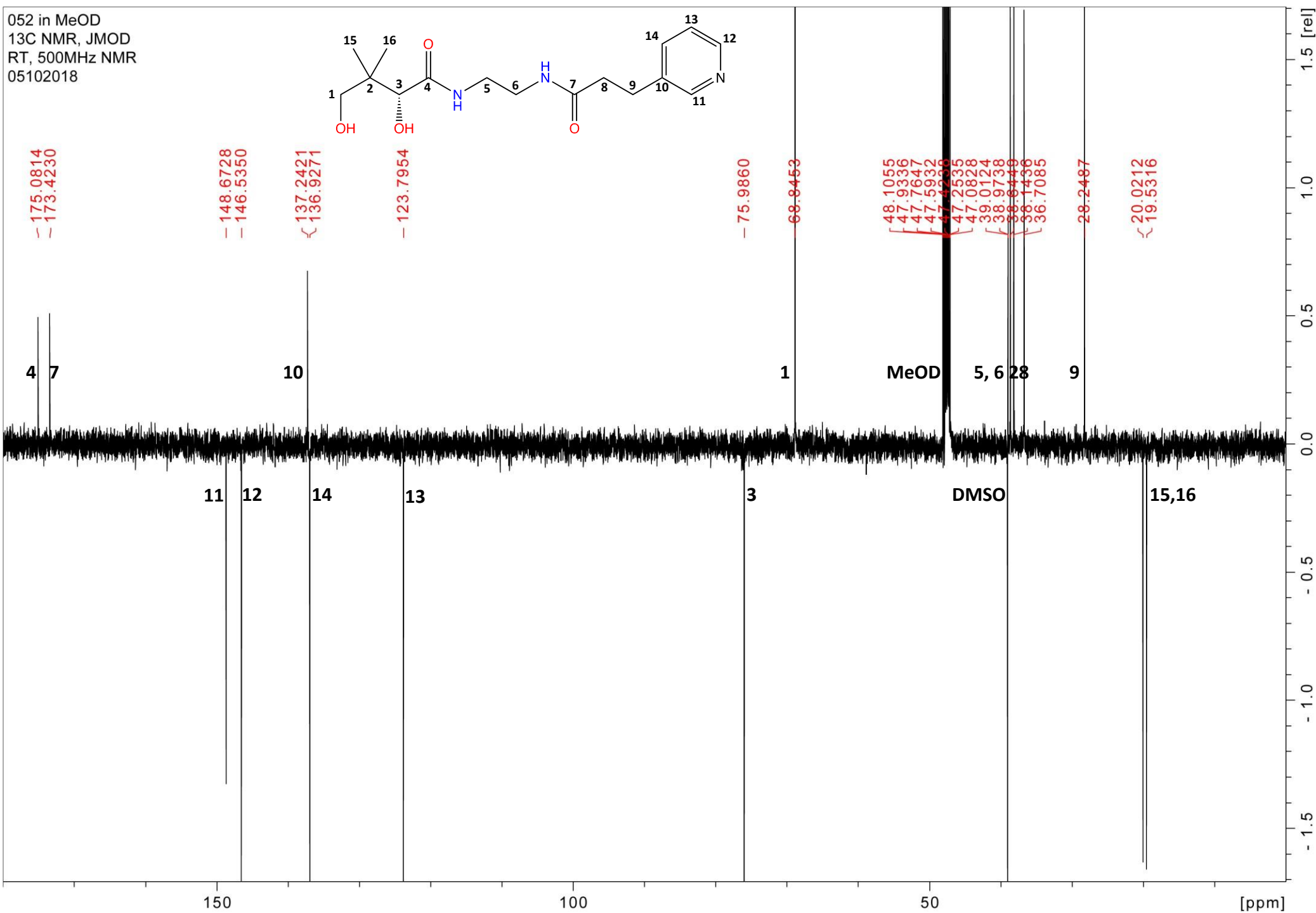
2.0408

6.0000

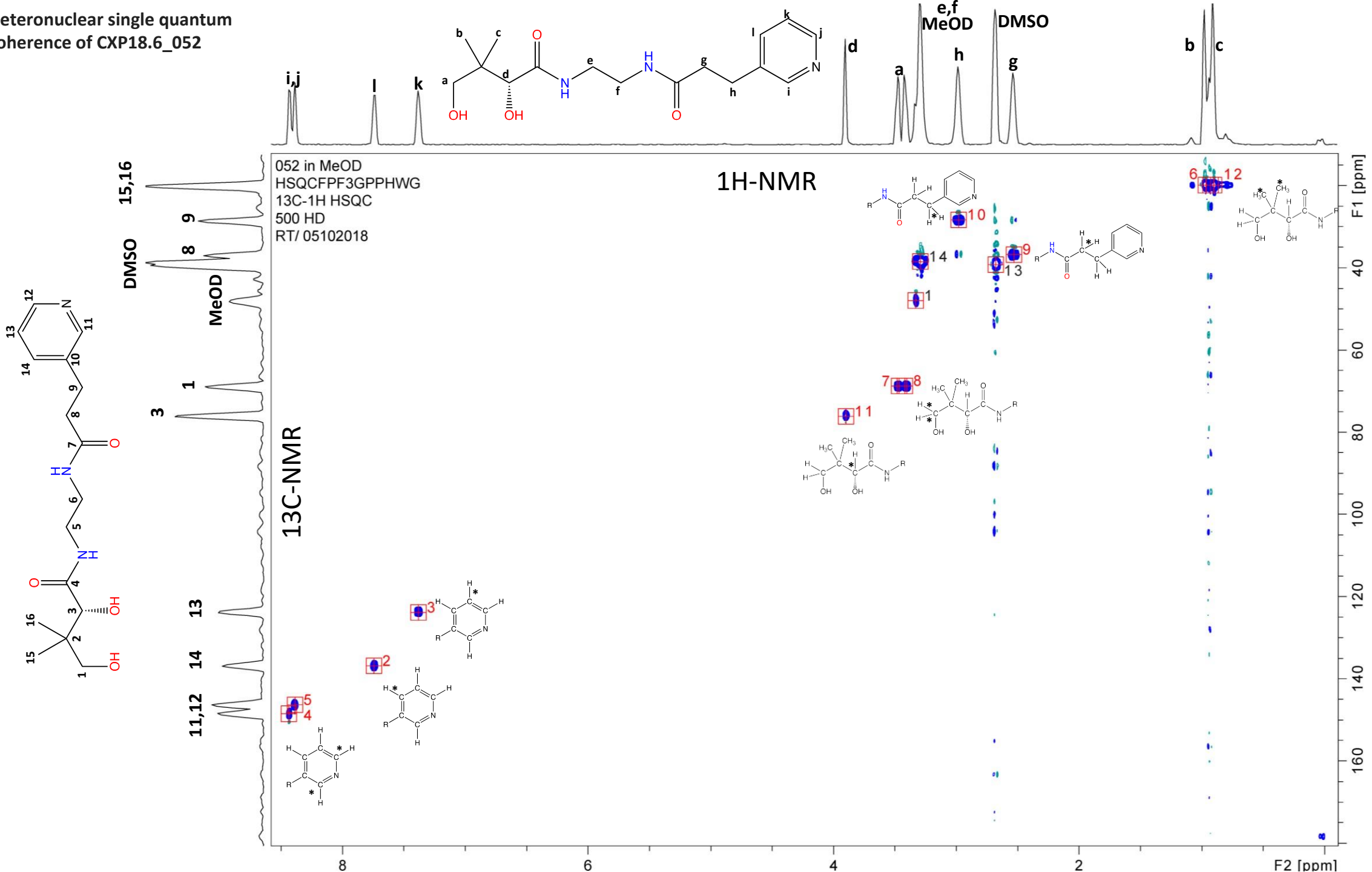
052 in MeOD  
13C NMR, JMOD  
RT, 500MHz NMR  
05102018



13C-assignment

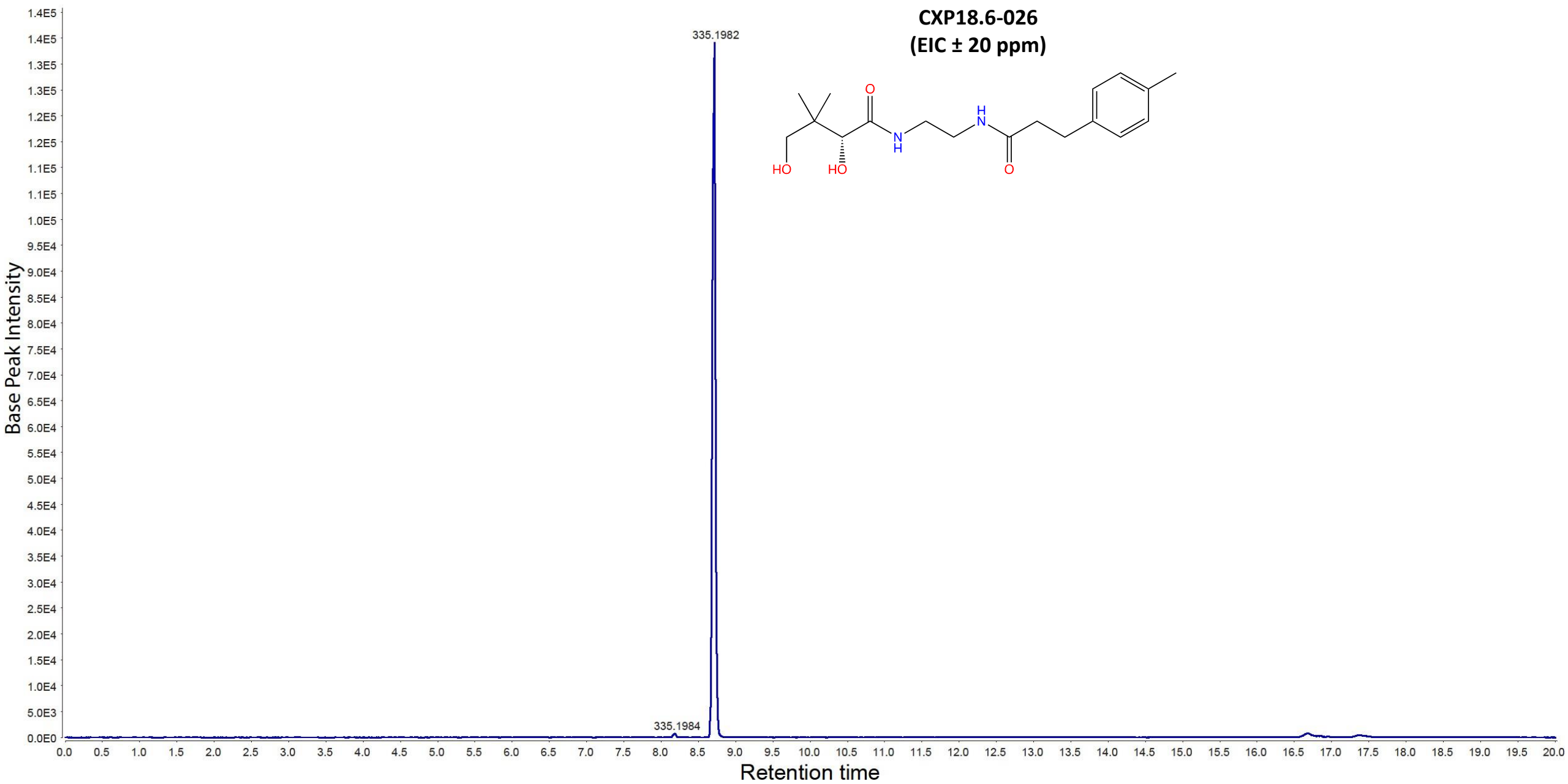
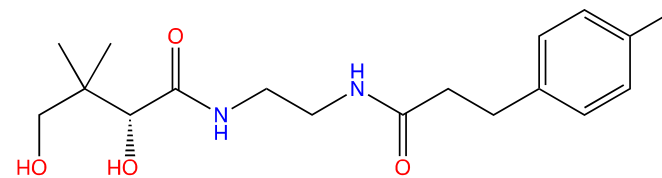


Heteronuclear single quantum  
coherence of CXP18.6\_052

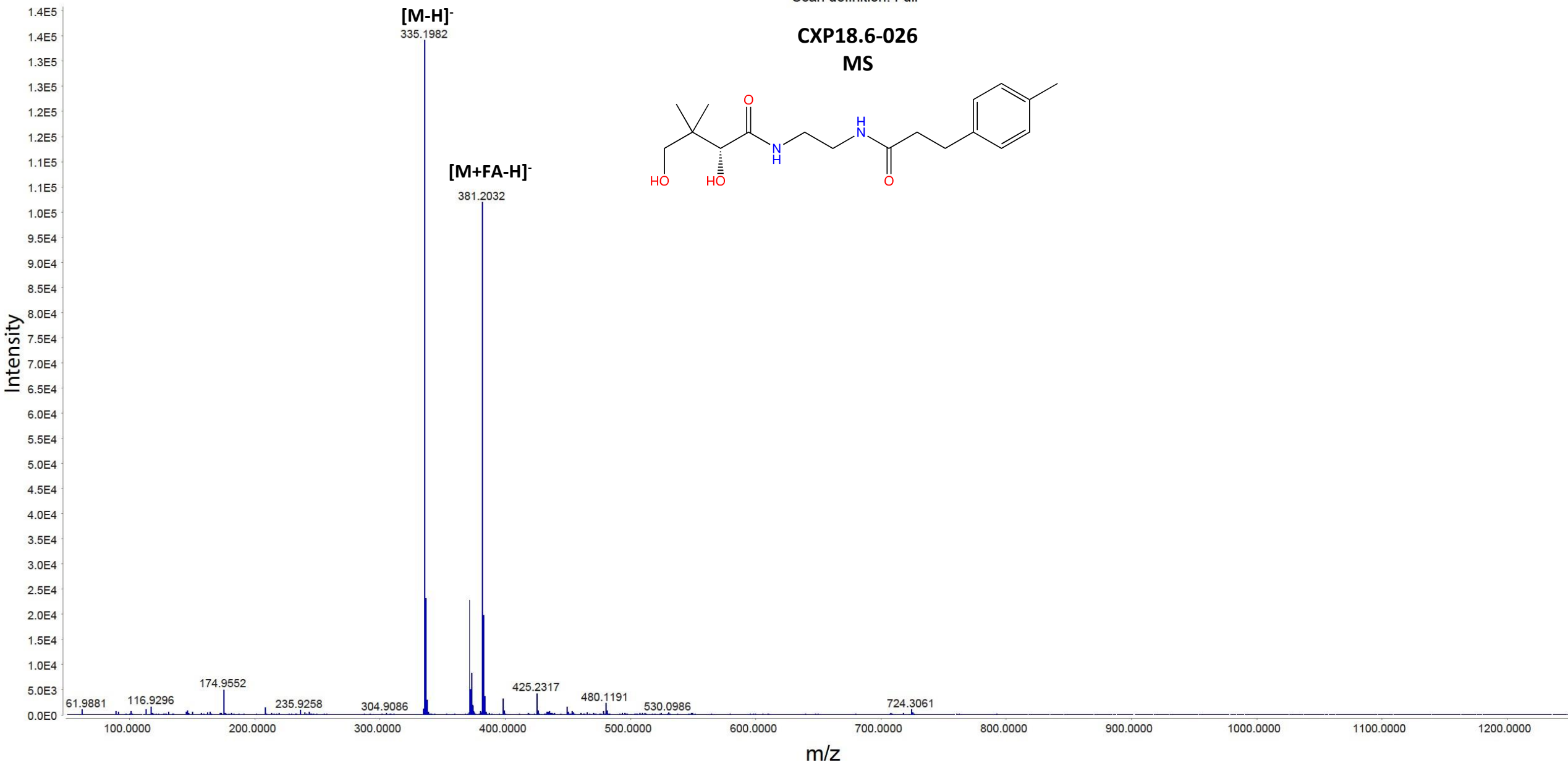


XIC (base peak), m/z: 335.1865 - 335.2066

**CXP18.6-026**  
(EIC  $\pm$  20 ppm)

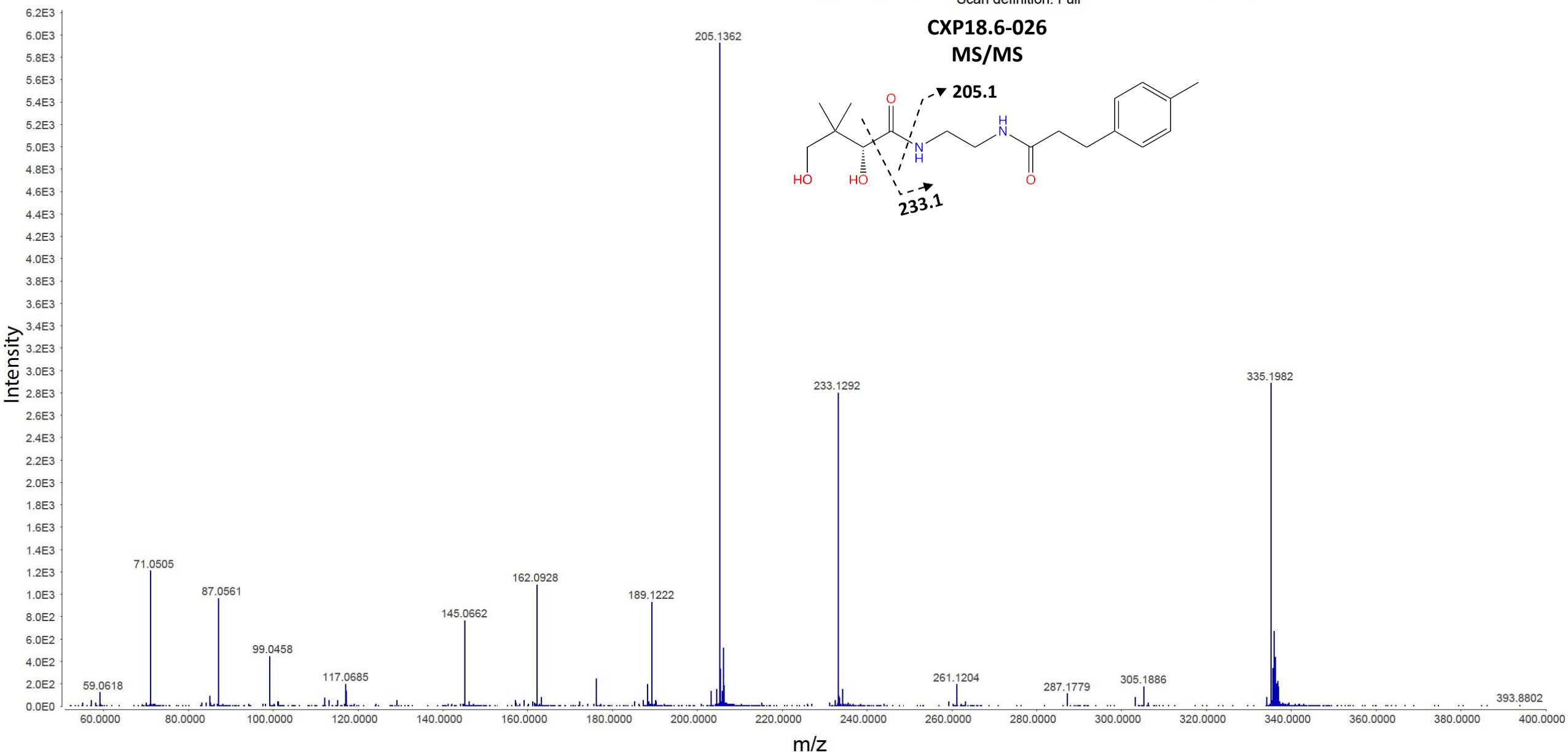


scan#1415 @8.7 MS1 c -, base peak: 335.1982 m/z (1.3E5)  
Scan definition: Full

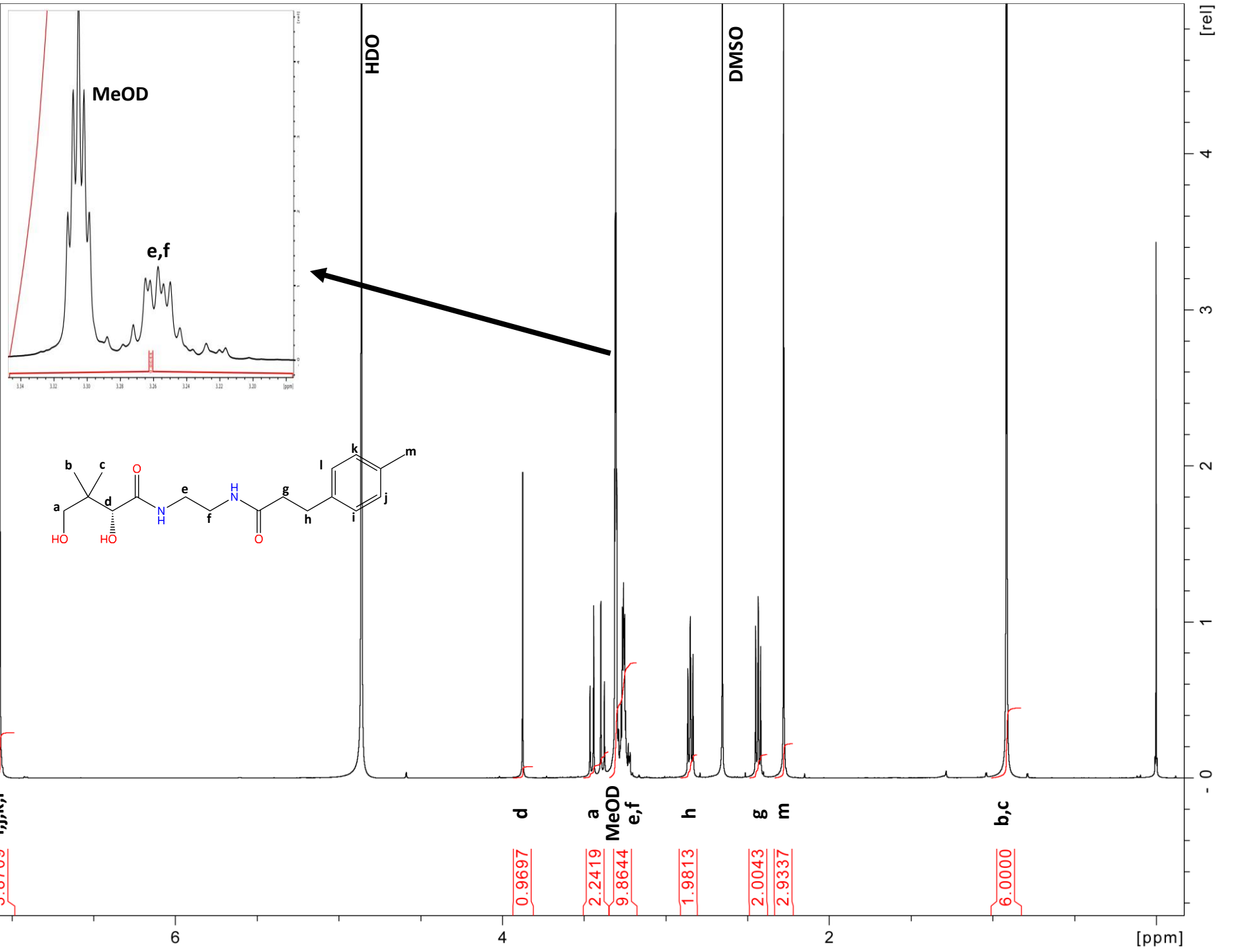


scan#1405 @8.7 MS2 (335.1984) c -, base peak: 205.1362 m/z (5.9E3)  
Scan definition: Full

**CXP18.6-026**  
**MS/MS**



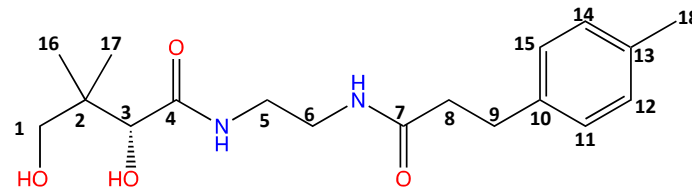
026 in MEOD  
1H NMR  
RT, 500MHz NMR  
05102018



1H-assignment



026 in MeOD  
 13C NMR, JMOD  
 RT, 500MHz NMR  
 05102018



~ 175.0678  
 ~ 174.3191

- 137.6006  
 - 135.3671

~ 128.6730  
 ~ 127.8463

- 75.9905

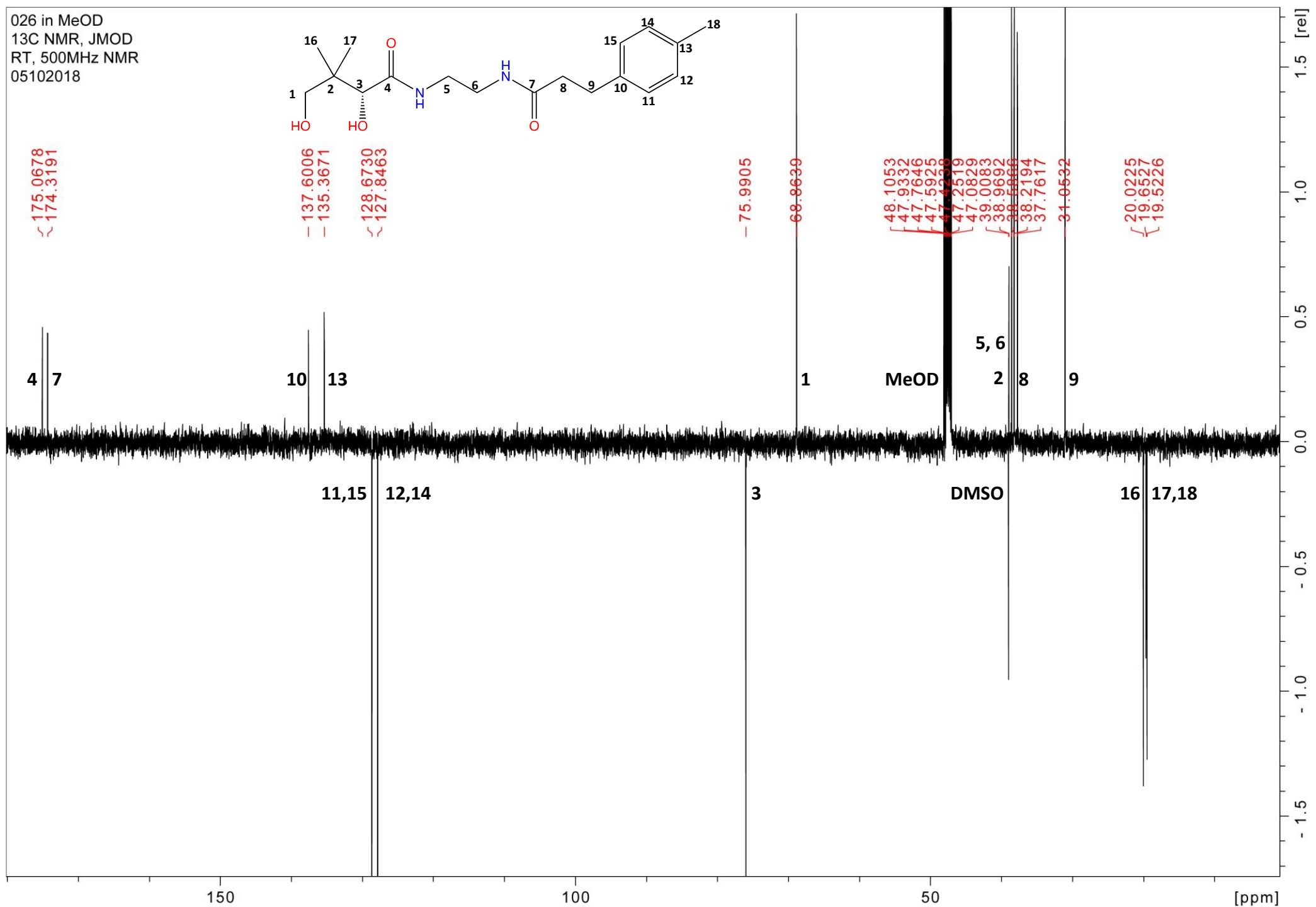
- 68.8639

{ 48.1053  
 { 47.9332  
 { 47.7646  
 { 47.5925  
 { 47.4238  
 { 47.2519  
 { 47.0829  
 { 39.0083  
 { 38.9692  
 { 38.8006  
 { 38.6294  
 { 37.7617

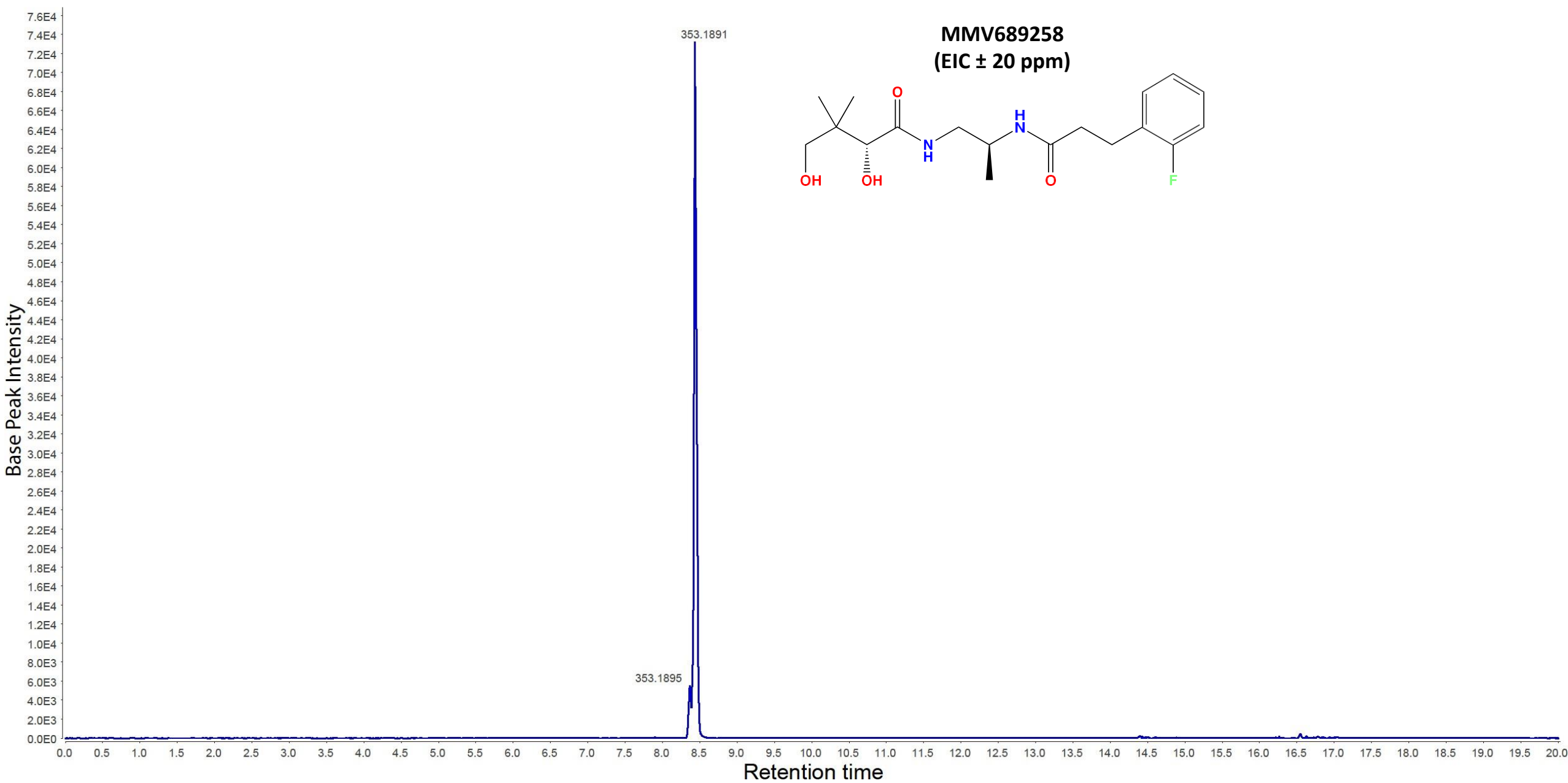
- 31.0532

{ 20.0225  
 { 19.6527  
 { 19.5226

13C-assignment

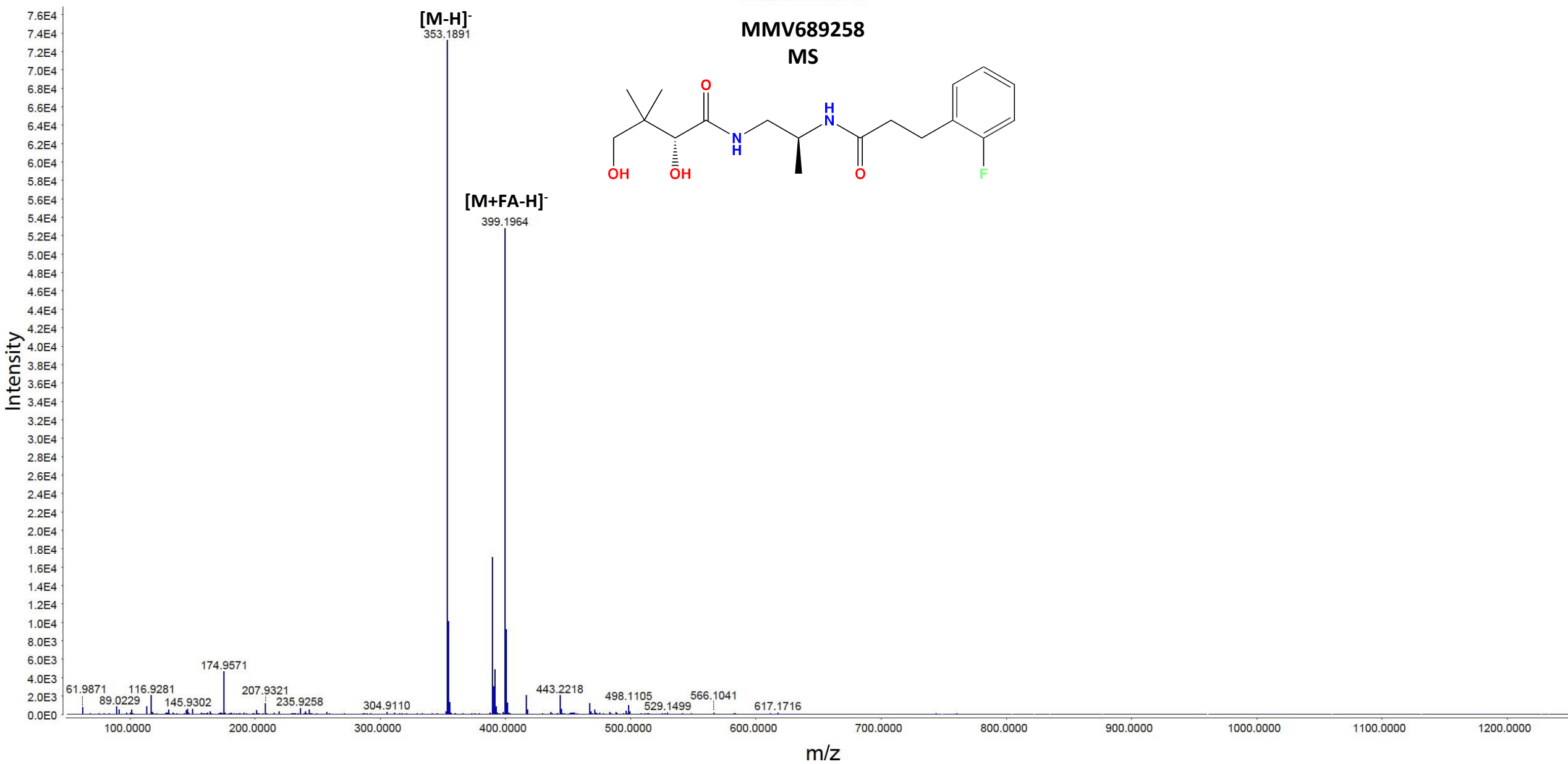
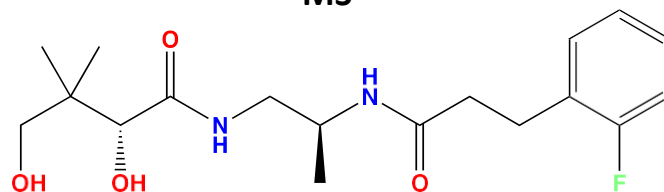


XIC (base peak), m/z: 353.1771 - 353.1972

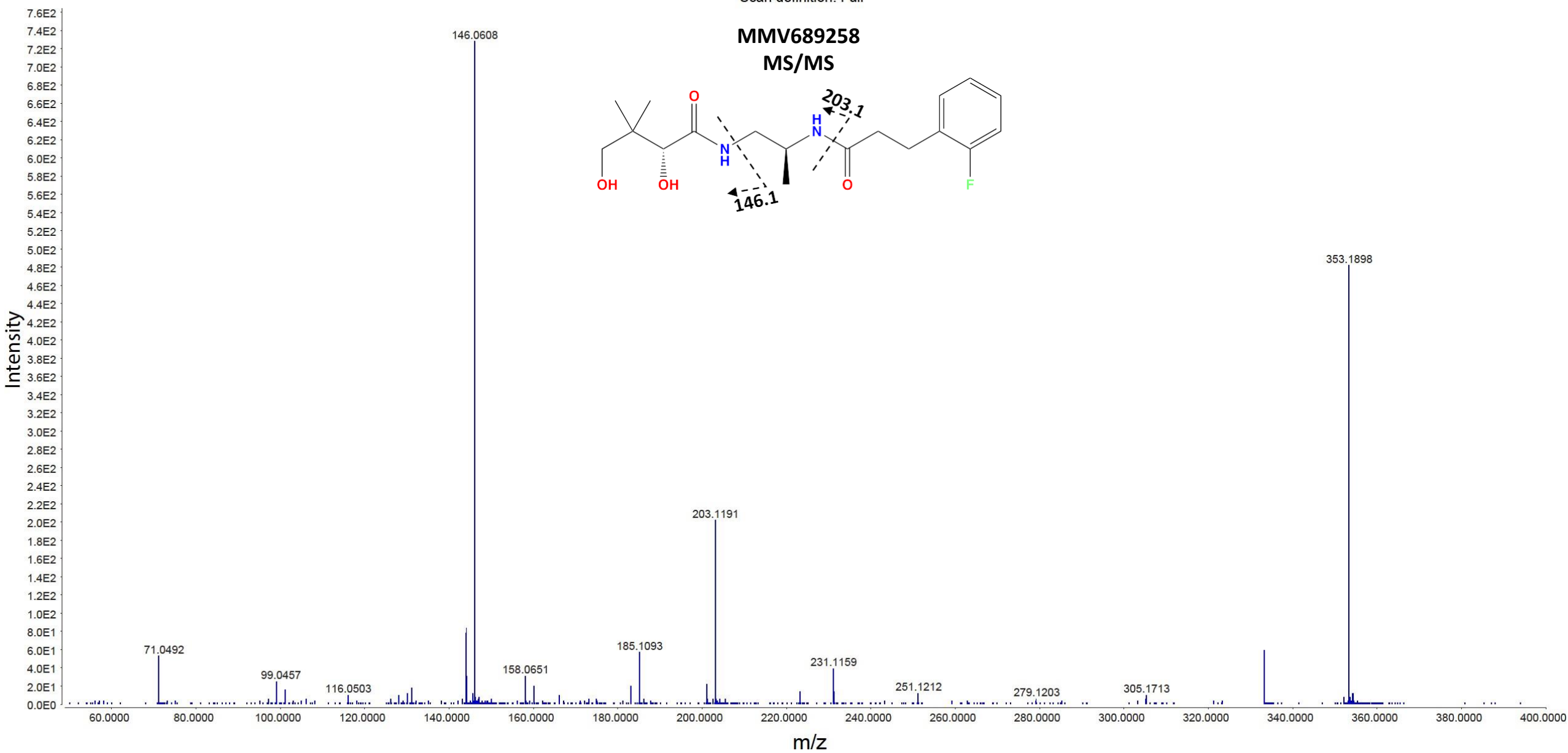


scan#1354 @8.4 MS1 c -, base peak: 353.1891 m/z (7.3E4)  
Scan definition: Full

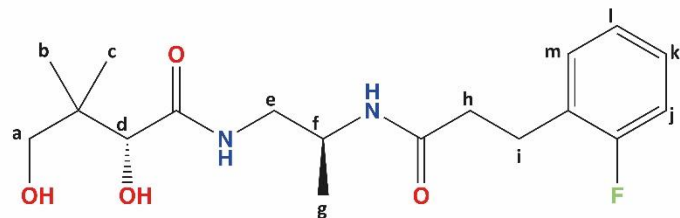
**MMV689258**  
**MS**



scan#1344 @8.4 MS2 (353.1886) c -, base peak: 146.0608 m/z (7.3E2)  
Scan definition: Full



258 in MEOD  
1H NMR  
RT, 500MHz NMR  
05102018



1H-assignment

j,k  
l,m

2.0000  
1.9420

d

0.9766

a

2.2391

f + MeOD

5.0808

e

2.1664

i

1.9665

h

1.9543

g

3.0484

b,c

6.1512

H<sub>2</sub>O

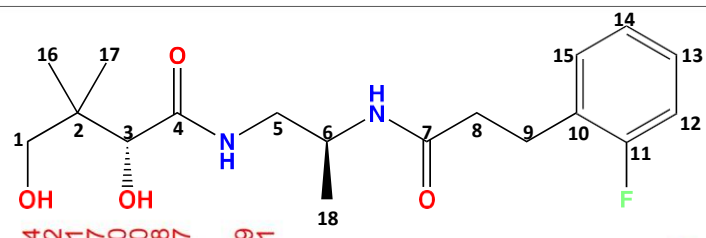
DMSO

TMS

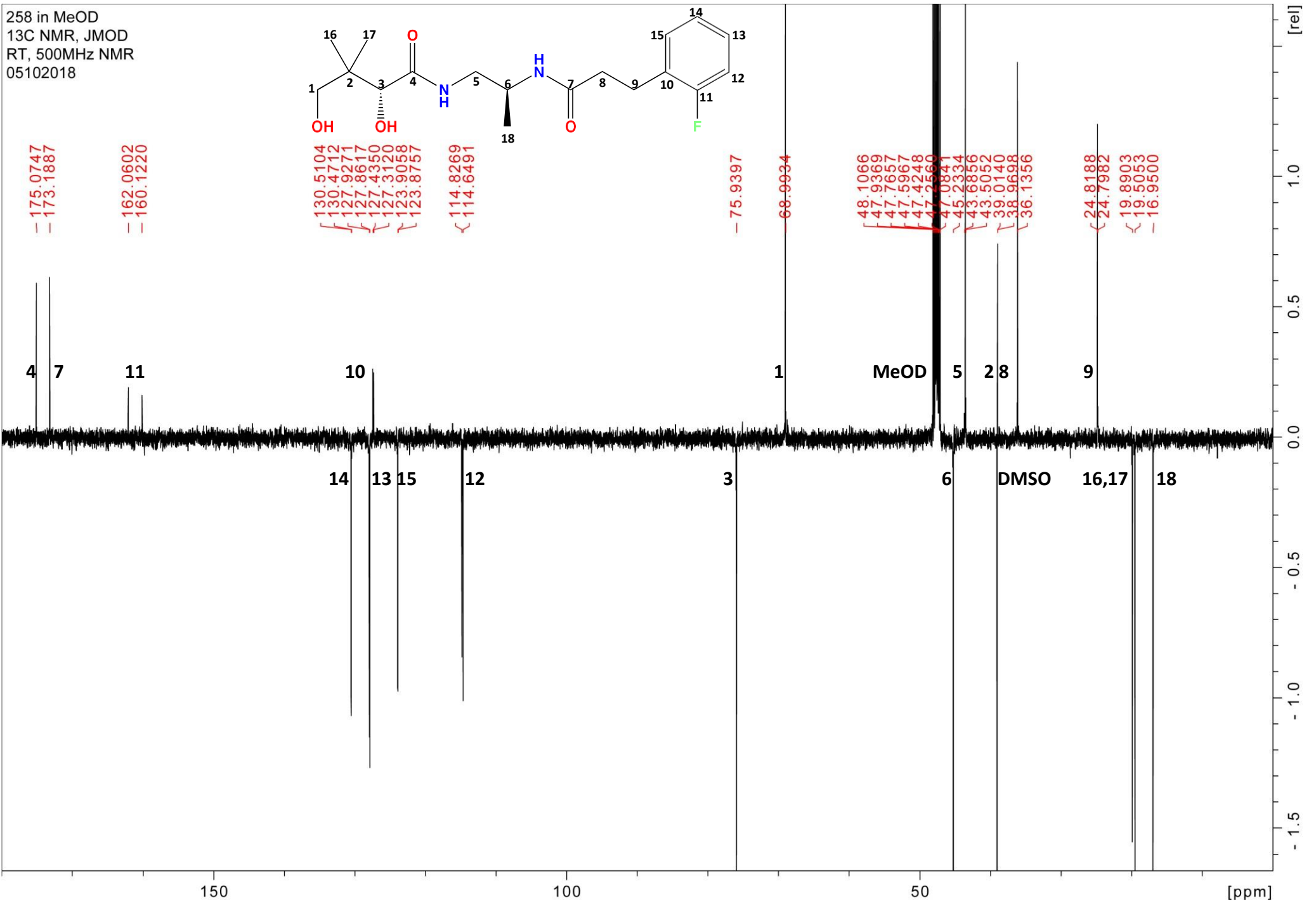
[rel]

[ppm]

258 in MeOD  
13C NMR, JMOD  
RT, 500MHz NMR  
05102018

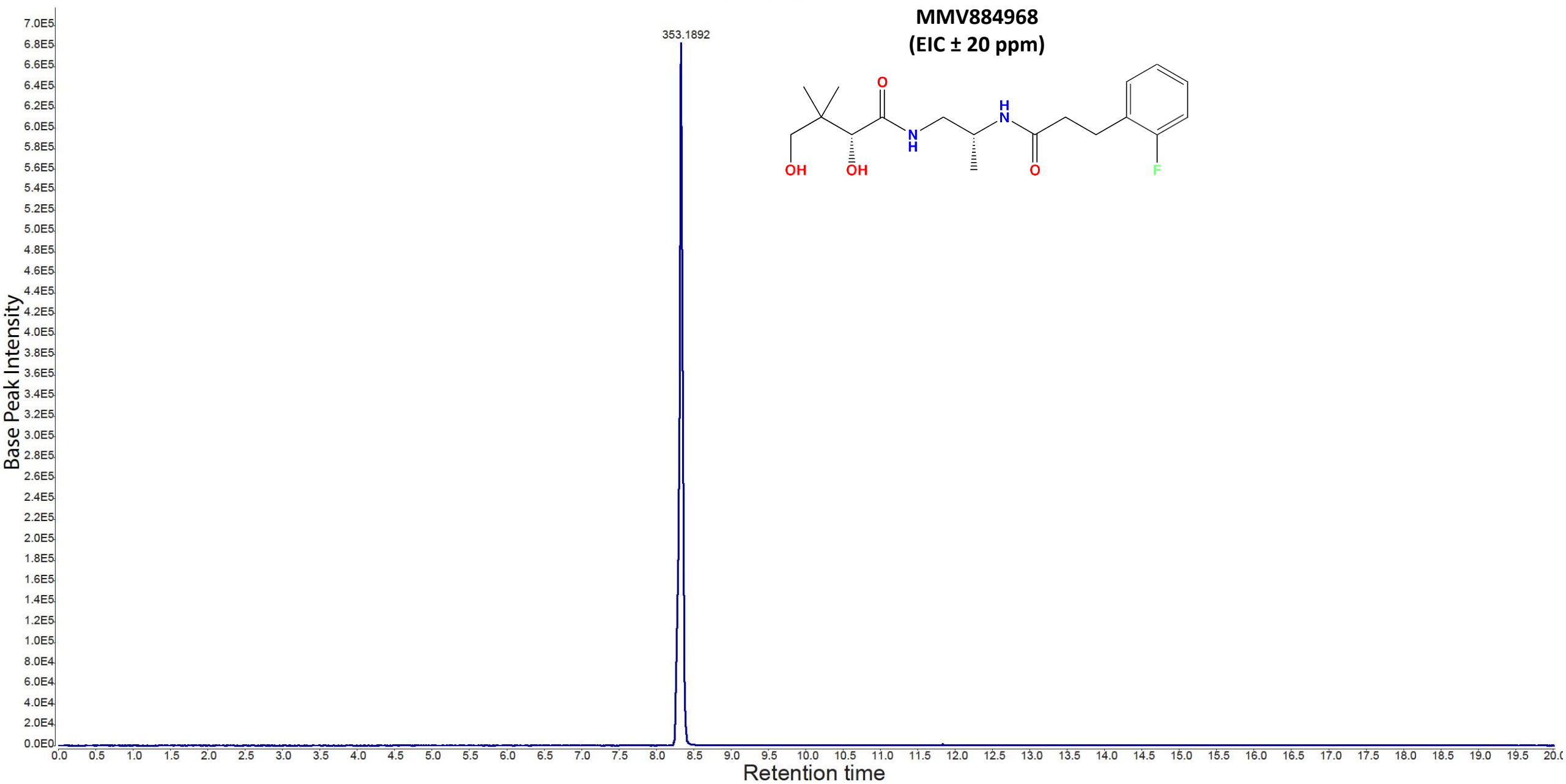
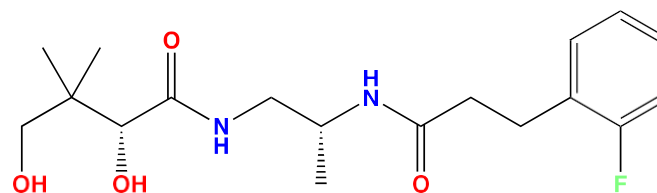


13C-assignment



XIC (base peak), m/z: 353.1771 - 353.1972

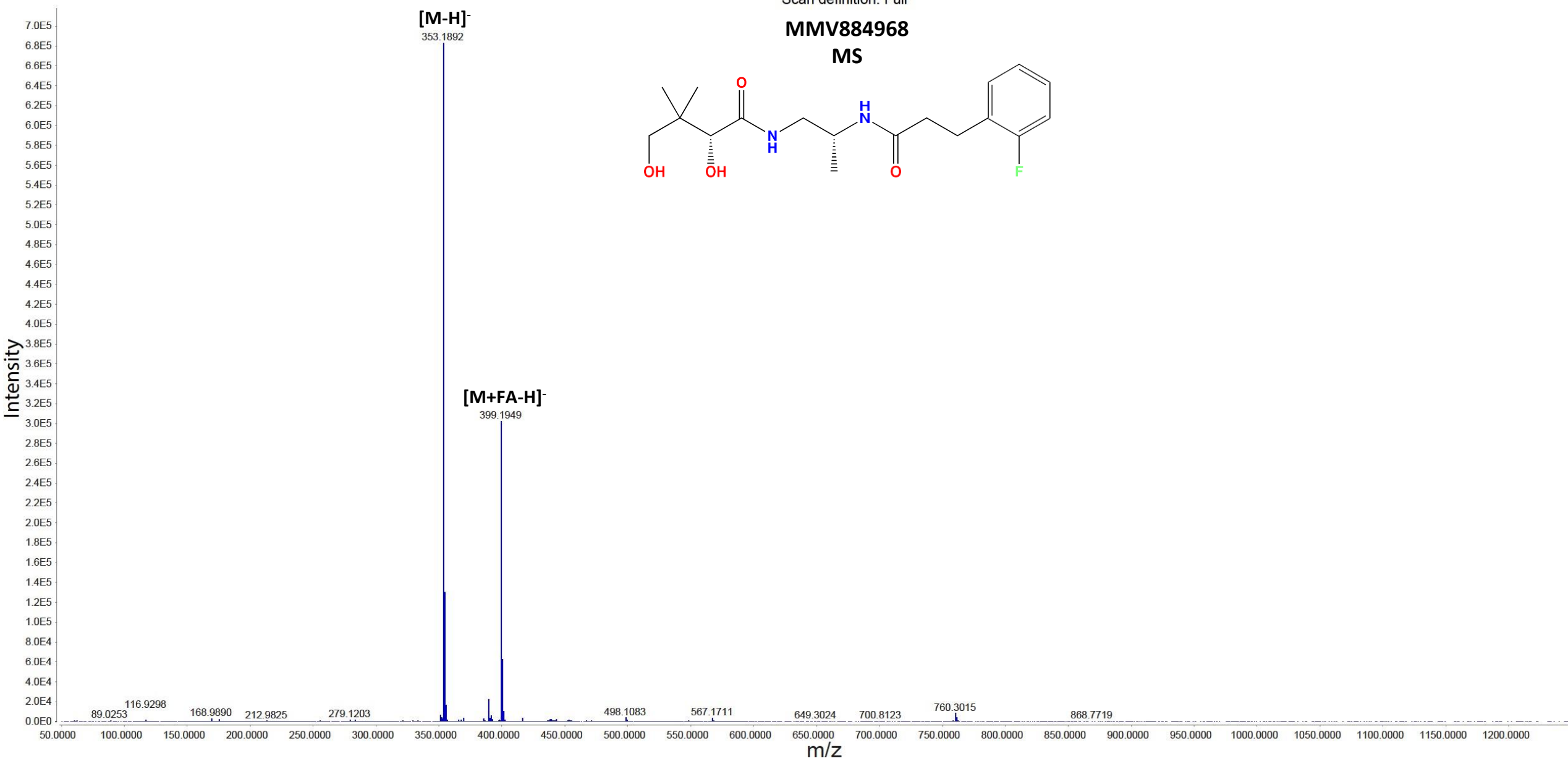
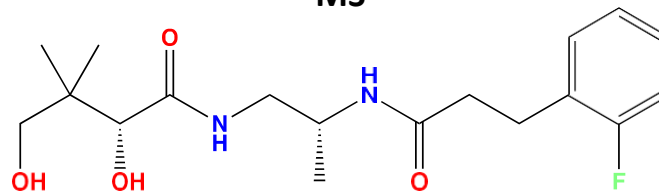
**MMV884968**  
(EIC  $\pm$  20 ppm)



scan#1502 @8.3 MS1 c-, base peak: 353.1892 m/z (6.8E5)  
Scan definition: Full

MMV884968

MS



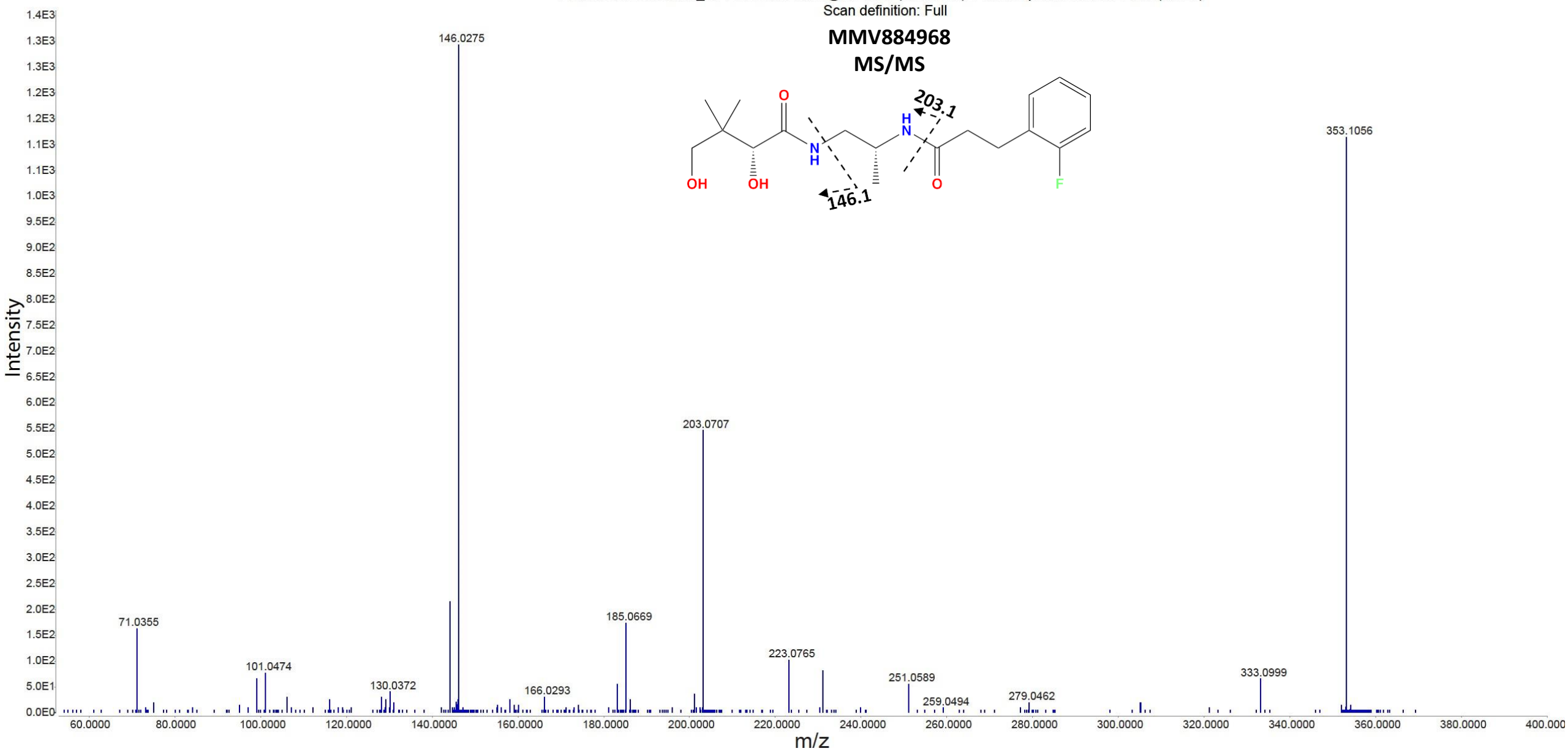
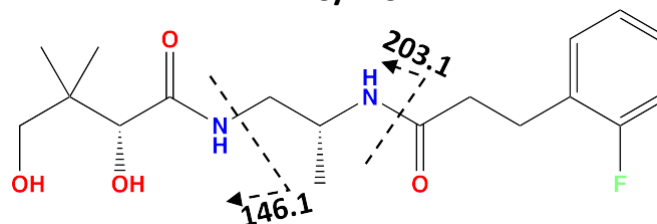


AQNEGSTD518MAY\_05-968 scan#1476 @8.3 MS2 (353.1887) c -, base peak: 146.0275 m/z (1.3E3)

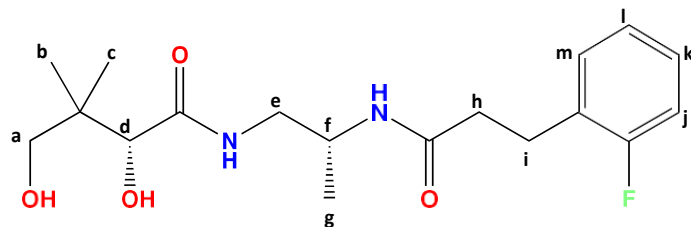
Scan definition: Full

MMV884968

MS/MS



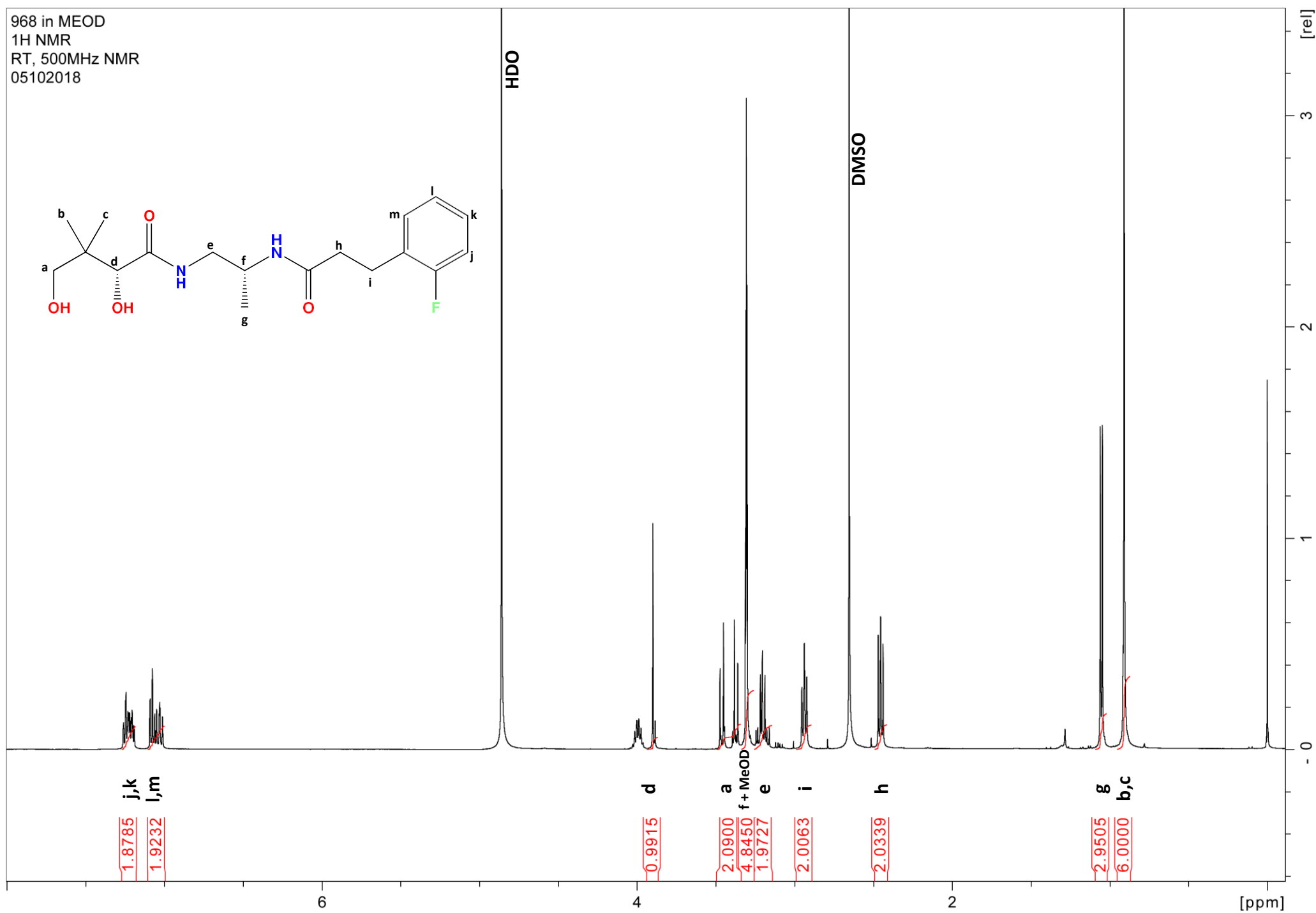
968 in MEOD  
1H NMR  
RT, 500MHz NMR  
05102018



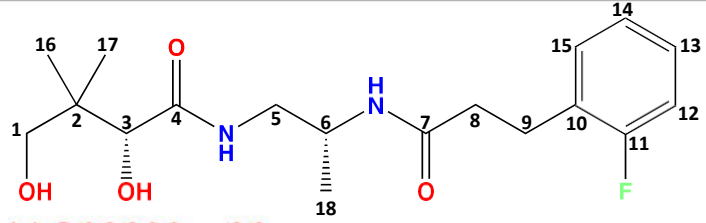
H<sub>2</sub>O

DMSO

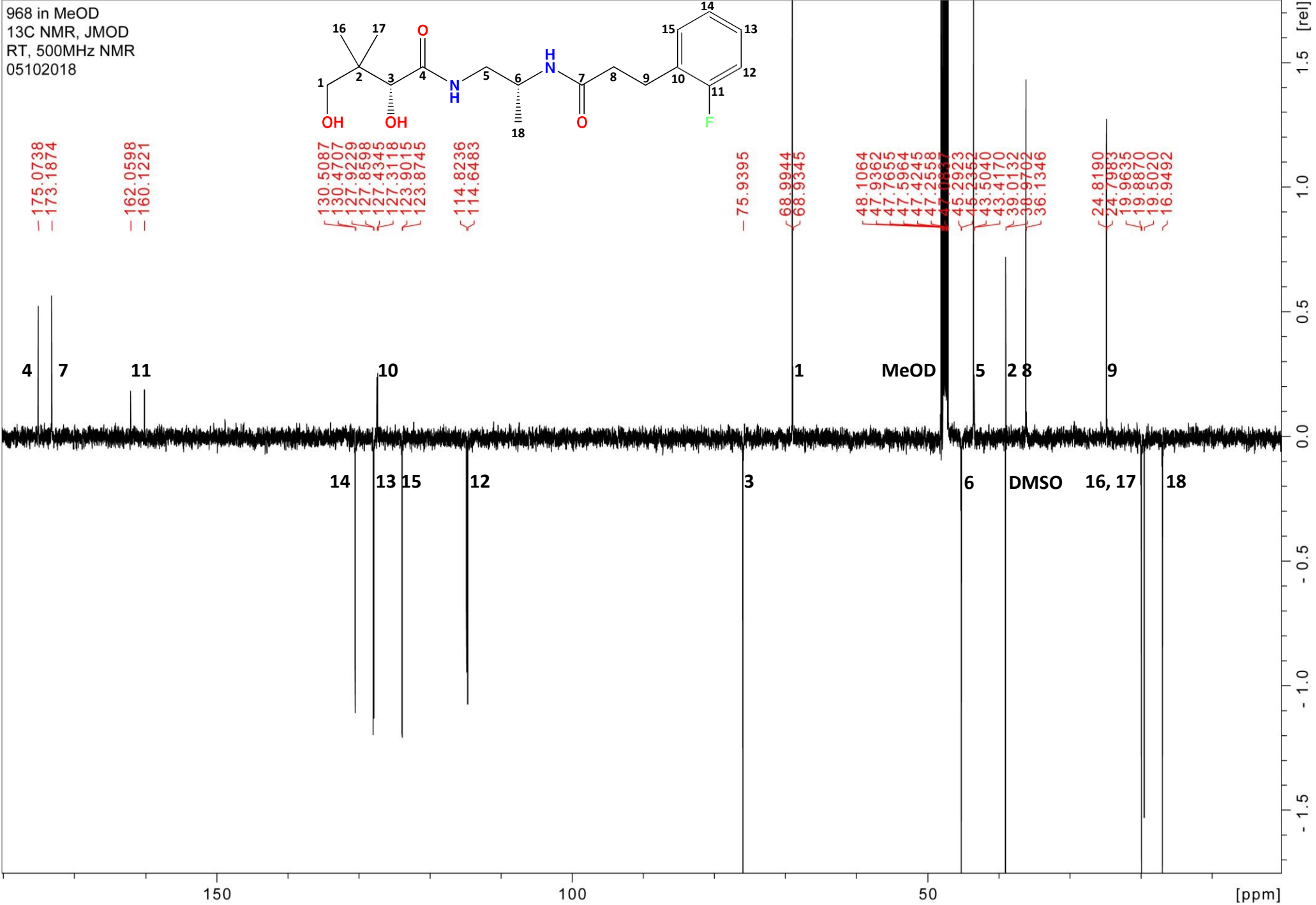
1H-assignment



968 in MeOD  
13C NMR, JMOD  
RT, 500MHz NMR  
05102018



13C-assignment

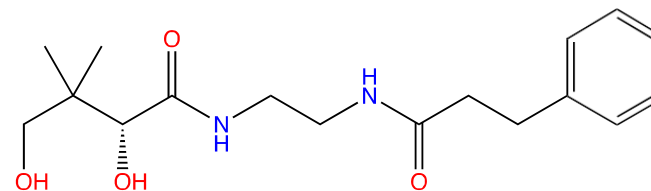


XIC (base peak), m/z: 321.1708 - 321.1909

Base Peak Intensity

321.1872

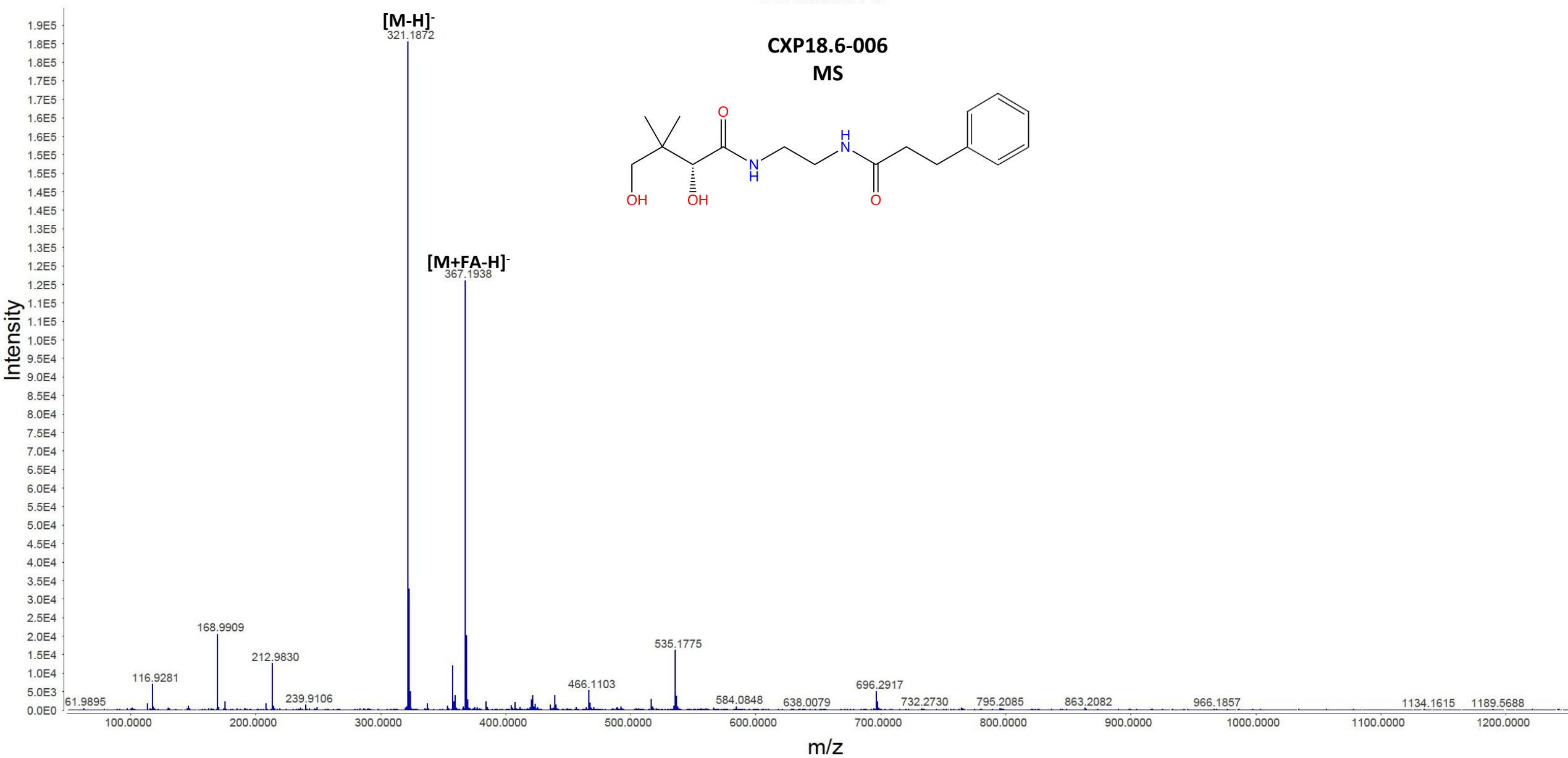
**CXP18.6-006**  
**(EIC ± 20 ppm)**



Retention time

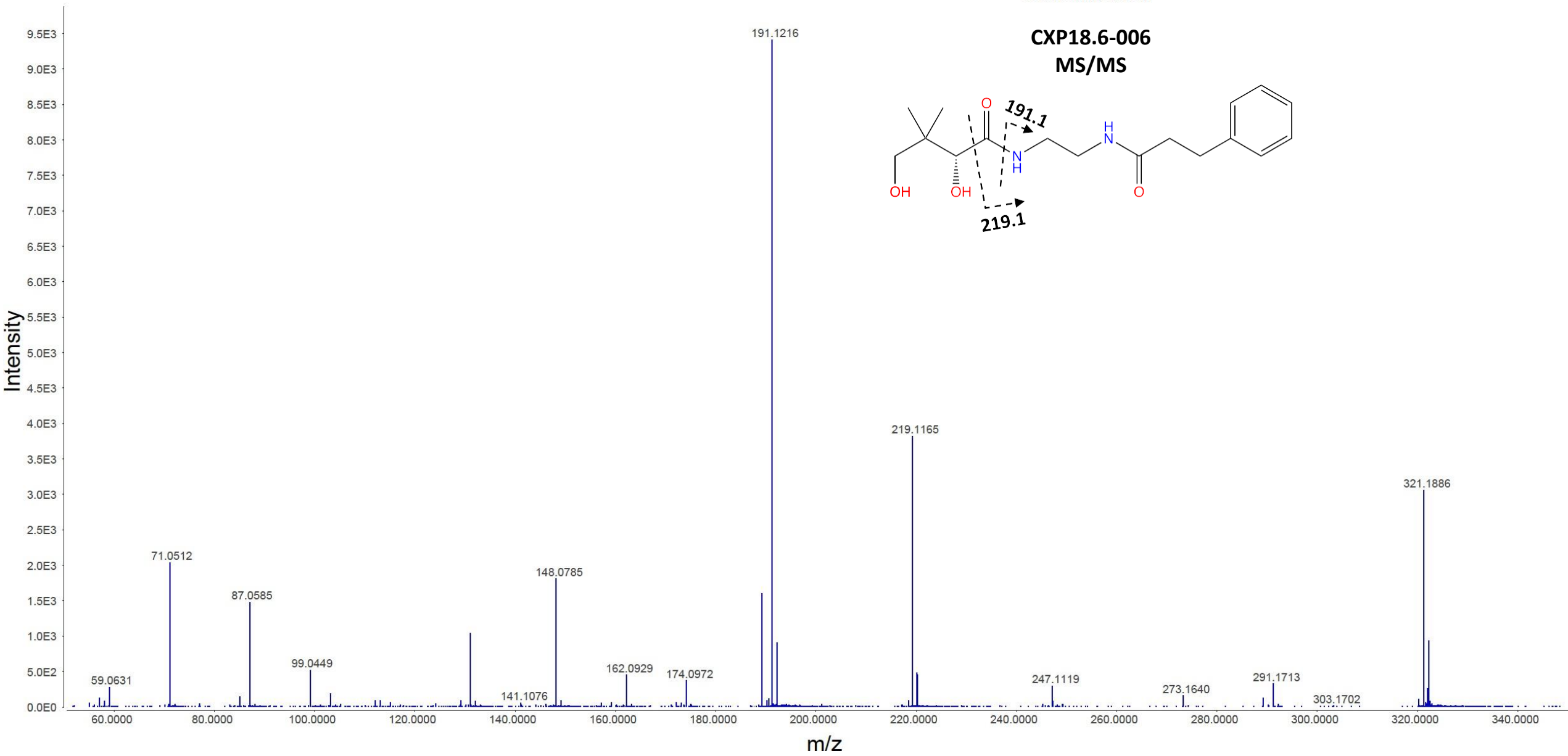
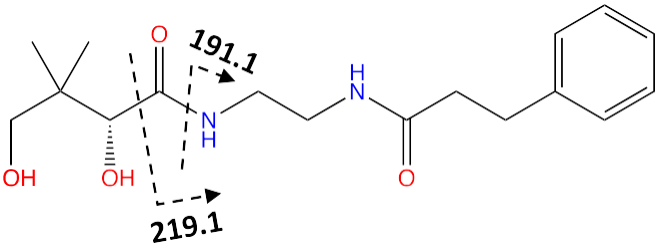
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 12.5 13.0 13.5 14.0 14.5 15.0 15.5 16.0 16.5 17.0 17.5 18.0 18.5 19.0 19.5 20.0

scan#1418 @7.8 MS1 c -, base peak: 321.1872 m/z (1.8E5)  
Scan definition: Full

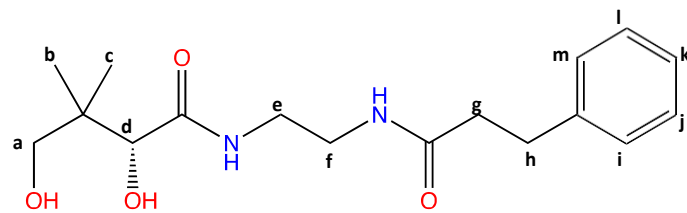


scan#1409 @7.8 MS2 (321.1875) c -, base peak: 191.1216 m/z (9.4E3)  
Scan definition: Full

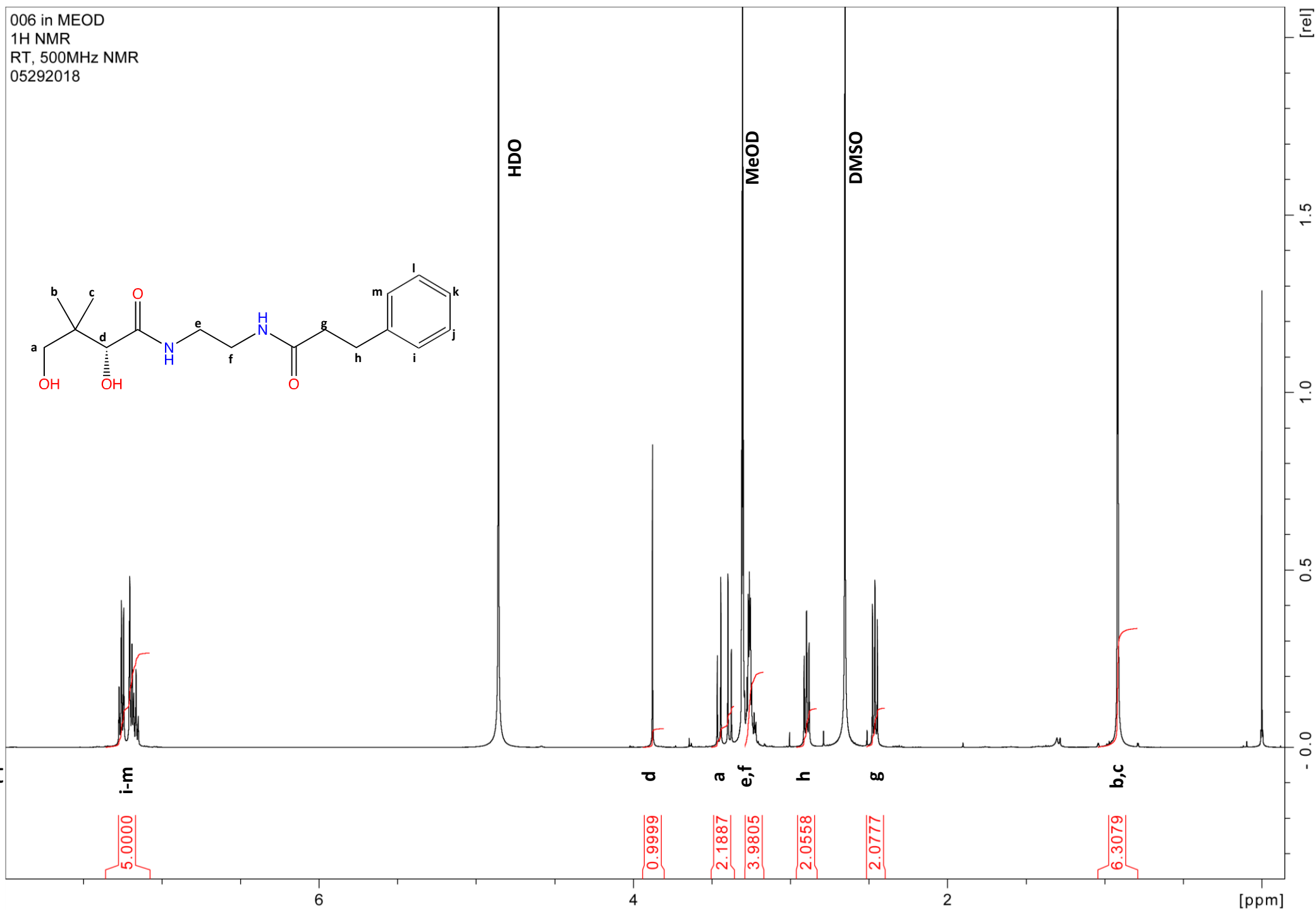
CXP18.6-006  
MS/MS



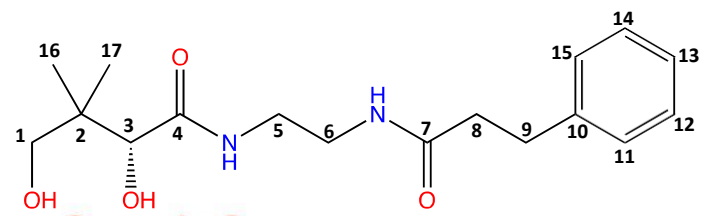
006 in MEOD  
1H NMR  
RT, 500MHz NMR  
05292018



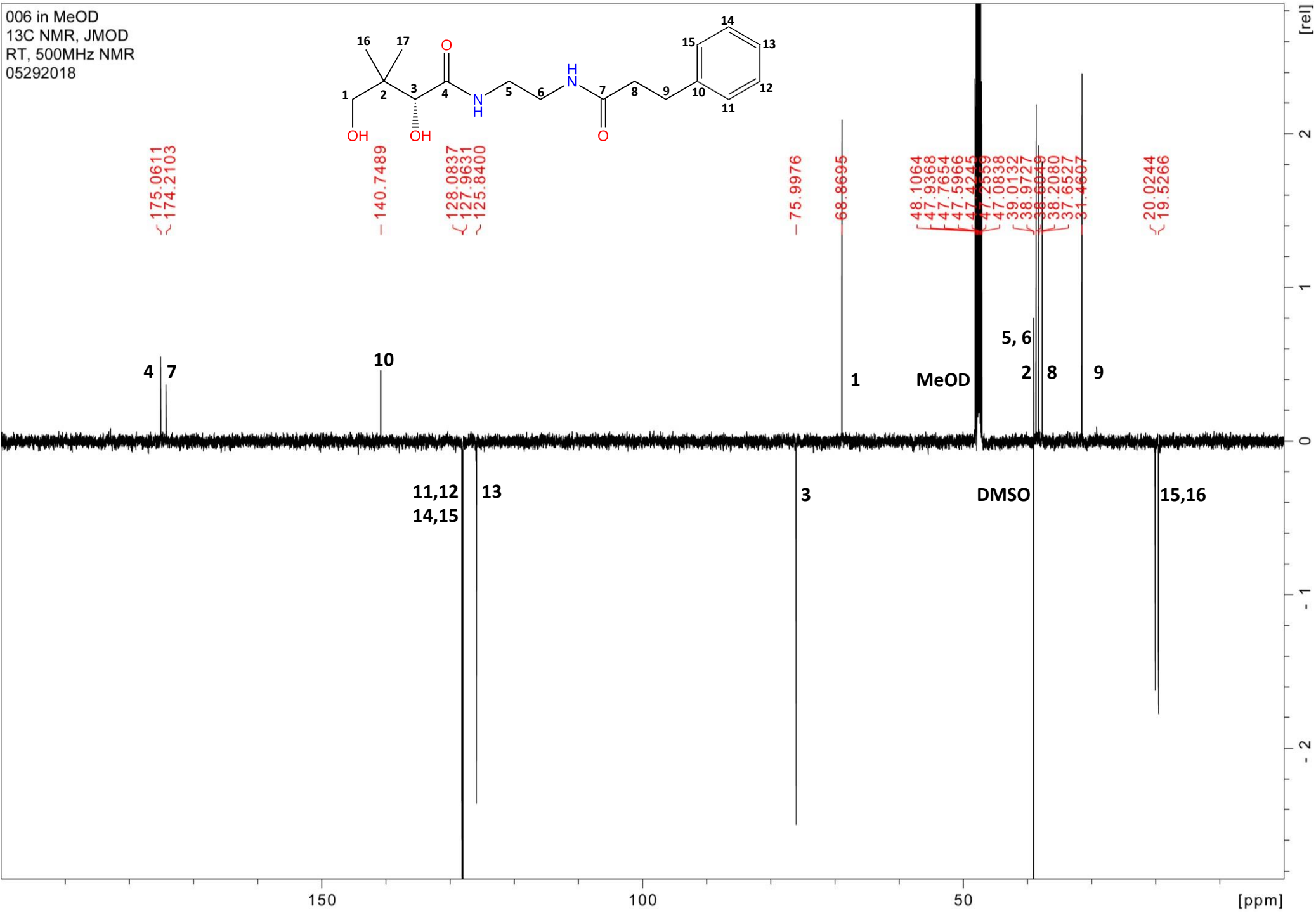
1H-assignment



006 in MeOD  
13C NMR, JMOD  
RT, 500MHz NMR  
05292018



13C-assignment





## Synthesis and quality control of compounds to illustrate SAR (as listed in Supplemental Table S1)

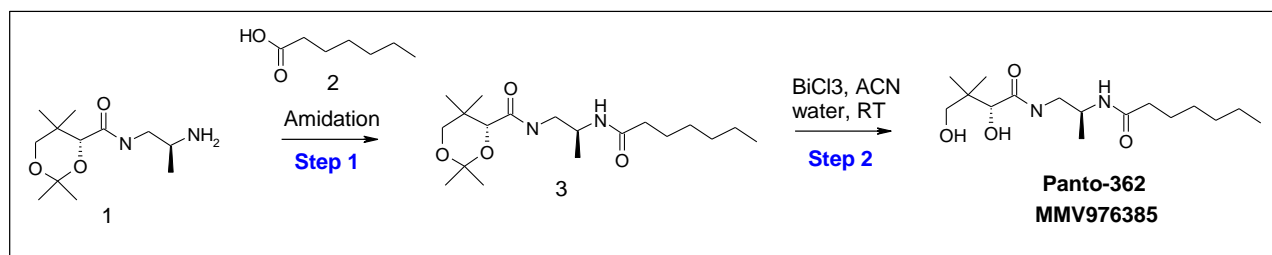
General Procedure for Amidation-

**General Procedure A:** To a stirred solution of **Amine (1)** (1 eqv) and **Acid (2)** (1.2 eqv) in THF (5 ml/mmol) were added HATU (1.5 eqv) and Et<sub>3</sub>N (5 eqv) at 0°C. Reaction mixture was stirred at room temperature for 16h. Reaction mixture was quenched with satd. NaHCO<sub>3</sub> solution and extracted with EtOAc. The organic layer was washed with water, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified over prep TLC plate (MeOH-DCM) to afford desired **amide (3)**.

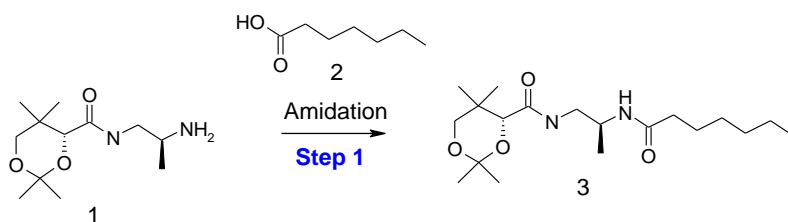
General Procedure for acetonide deprotection-

**General Procedure B:** To a stirred solution of **amide (3)** (1 eqv) in CH<sub>3</sub>CN (5 ml/mmol) was treated with Bi(III)Cl<sub>3</sub> (0.1 eqv) and H<sub>2</sub>O (0.04 ml/mmol). The reaction mixture was stirred at room temperature for 16h. Reaction mass was concentrated under reduced pressure and purified over prep TLC (MeOH in DCM) to afford **Final compound**.

### MMV976385-



### Synthesis of (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-heptanoylamino-propyl)-amide (3):



**Procedure:** Same as general procedure A with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (1)** (100 mg, 0.409 mmol) and **Heptanoic acid (2)** (63.9 mg, 0.491 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-heptanoylamino-propyl)-amide (3)** as gum in 82.28% yield, 120mg.

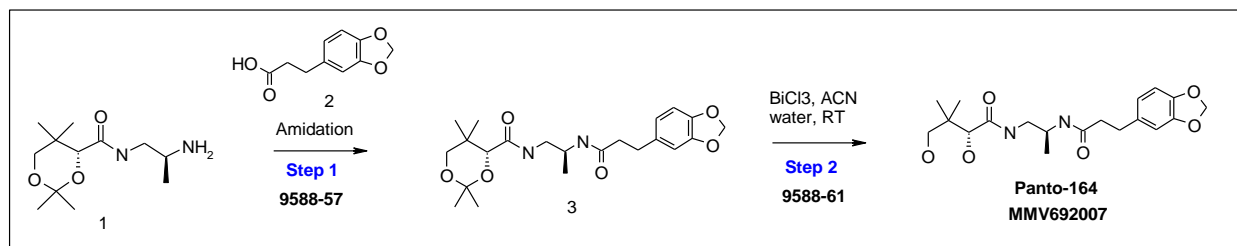
### Synthesis of compound MMV976385:



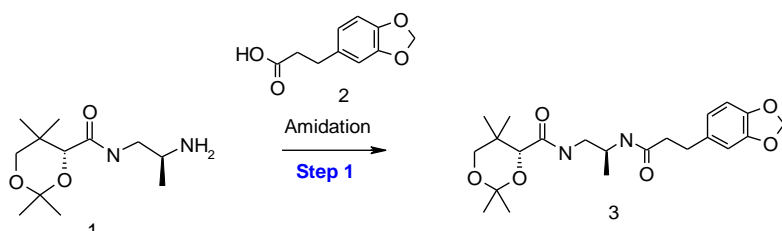
**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-heptanoylamino-propyl)-amide (3)** (100 mg, 0.409 mmol) to afford **Panto-362 (MMV976385)** as gum in 82.28% yield, 120mg.

acid ((S)-2-heptanoylamino-propyl)-amide (3) (186 mg, 0.522 mmol) to afford **MMV976385** as colorless sticky mass in 12.1% yield, 20 mg.

#### **MMV692007-**



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-benzo[1,3]dioxol-5-yl-propionylamino)-propyl]-amide (3):**



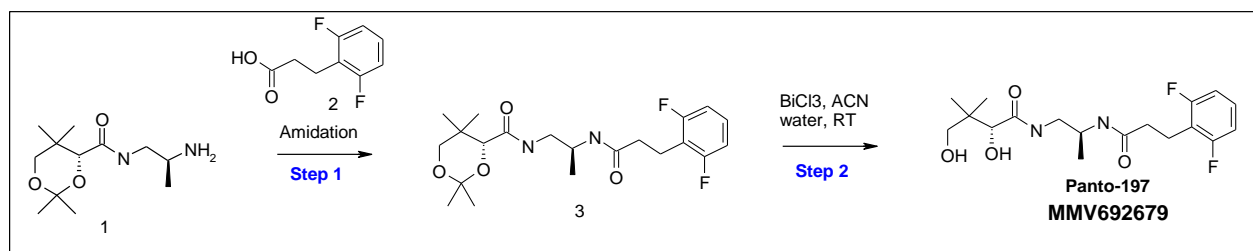
**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (**1**) (125 mg, 0.512 mmol) and 3-Benzo[1,3]dioxol-5-yl-propionic acid (**2**) (118.59 mg, 0.614 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-benzo[1,3]dioxol-5-yl-propionylamino)-propyl]-amide (**3**) as gum in 78.1% yield, 168 mg.

**Synthesis of compound MMV692007:**



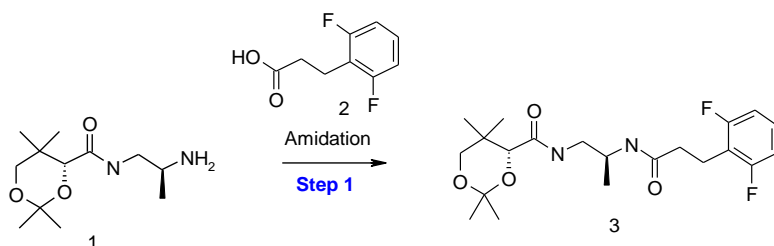
**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-benzo[1,3]dioxol-5-yl-propionylamino)-propyl]-amide (**3**) (168 mg, 0.415 mmol) to afford **MMV692007** as colorless gum in 60.13% yield, 95 mg.

#### **MMV692679-**



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[2,6-**

**difluoro-phenyl)-propionylamino]-propyl}-amide (3):**



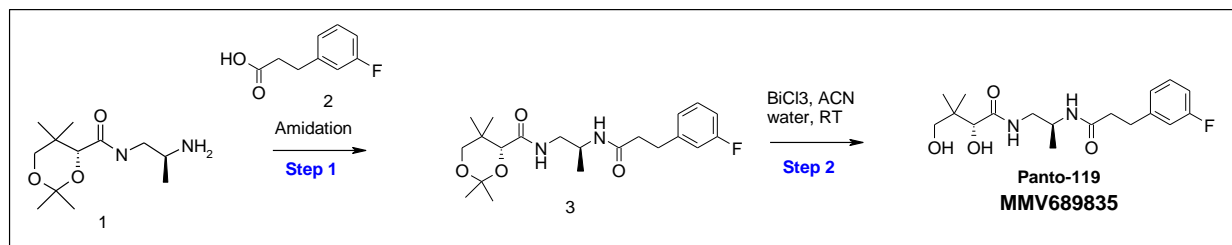
**Procedure:** Same as general procedure A with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (1)** (100 mg, 0.409 mmol) and **3-(2,6-Difluoro-phenyl)-propionic acid (2)** (91.42 mg, 0.491 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-[3-(2,6-difluoro-phenyl)-propionylamino]-propyl)-amide (3)** as gum in 94.78% yield, 160 mg.

**Synthesis of compound MMV692679:**

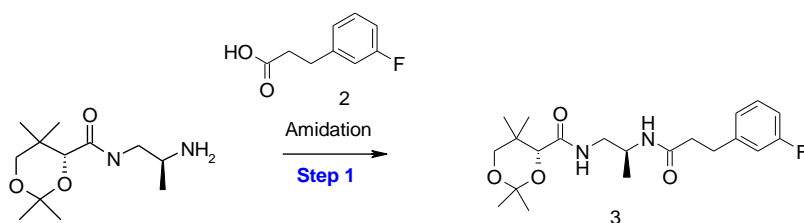


**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-[3-(2,6-difluoro-phenyl)-propionylamino]-propyl)-amide (3)** (213 mg, 0.516 mmol) to afford **MMV692679** as colorless gum in 52.52% yield, 101 mg.

**MMV689835-**



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-[3-(3-fluoro-phenyl)-propionylamino]-propyl)-amide (3):**



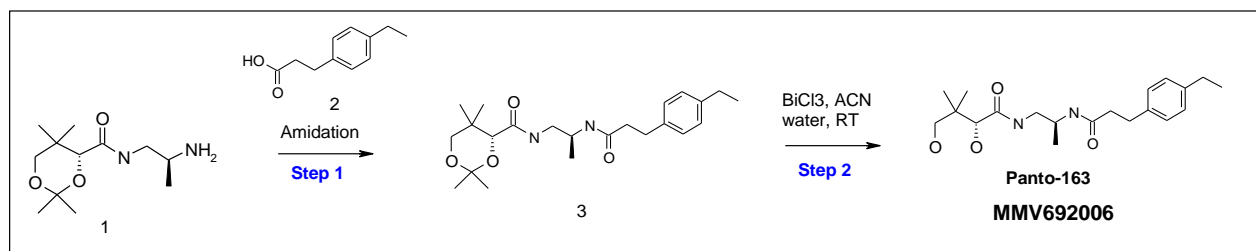
**Procedure:** Same as general procedure A with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (1)** (100 mg, 0.409 mmol) and **3-(3-Fluoro-phenyl)-propionic acid (2)** (82.5 mg, 0.491 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-[3-(3-fluoro-phenyl)-propionylamino]-propyl)-amide (3)** as gum in 86.72 % yield, 140 mg.

### Synthesis of compound MMV689835:

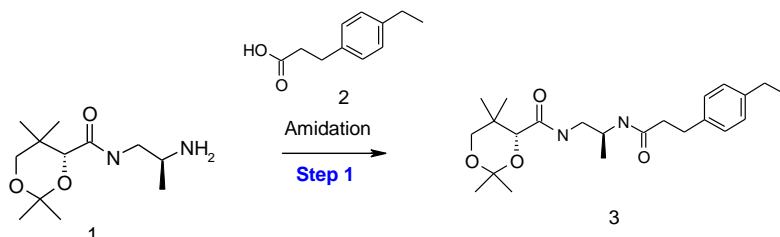


**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(3-fluorophenyl)propionylamino]propyl}-amide (3)** (140 mg, 0.355 mmol) to afford **MMV689835** as colorless gum in 47.7% yield, 60 mg.

### MMV692006-



### Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(4-ethylphenyl)propionylamino]propyl}-amide (3):



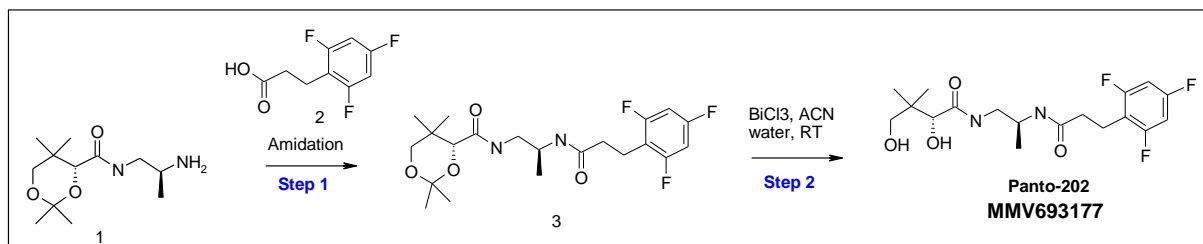
**Procedure:** Same as general procedure A with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-amino-propyl}-amide (1)** (125 mg, 0.512 mmol) and **3-(4-Ethylphenyl)propionic acid (2)** (109.4 mg, 0.614 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(4-ethylphenyl)propionylamino]propyl}-amide (3)** as gum in 68.61% yield, 142 mg.

### Synthesis of compound MMV692006:

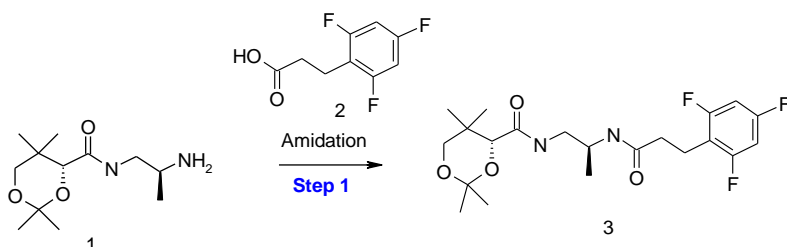


**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(4-ethylphenyl)propionylamino]propyl}-amide (3)** (142 mg, 0.351 mmol) to afford **MMV692006** as colorless gum in 42.99% yield, 55 mg.

### MMV693177-



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2,4,6-trifluoro-phenyl)-propionylamino]-propyl}-amide (3):**



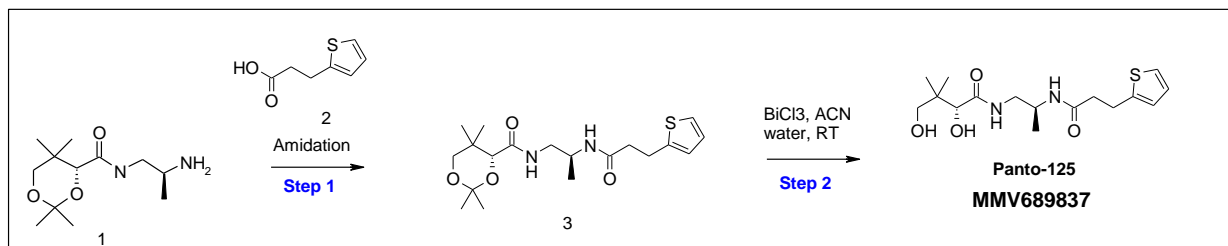
**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (1) (150 mg, 0.614 mmol) and 3-(2,4,6-Trifluoro-phenyl)-propionic acid (2) (150.39 mg, 0.737 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2,4,6-trifluoro-phenyl)-propionylamino]-propyl}-amide (3) as gum in 79.47 % yield, 210 mg.

**Synthesis of compound MMV693177:**

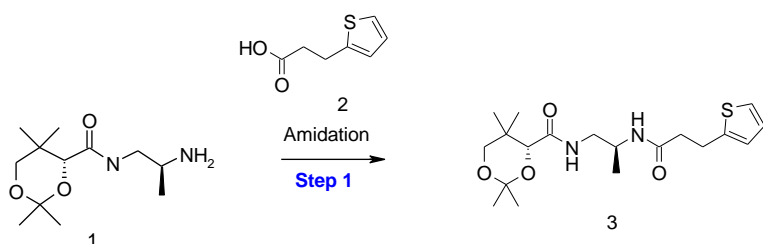


**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2,4,6-trifluoro-phenyl)-propionylamino]-propyl}-amide (3) (100 mg, 0.233 mmol) to afford MMV693177 as colorless sticky gum in 82.61% yield, 75 mg.

### MMV689837-



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-thiophen-2-yl-propionylamino)-propyl]-amide (3):**



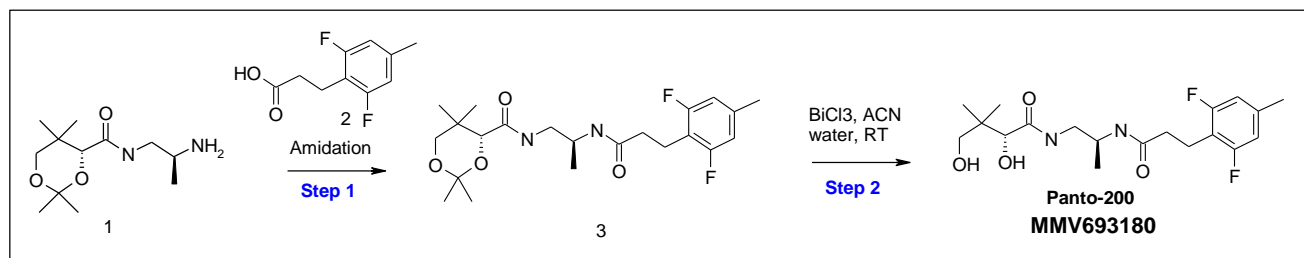
**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (**1**) (100 mg, 0.409 mmol) and 3-Thiophen-2-yl-propionic acid (**2**) (76.7 mg, 0.491 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-thiophen-2-yl-propionylamino)-propyl]-amide (**3**) as gum in 79.85 % yield, 125 mg.

#### Synthesis of compound MMV689837:

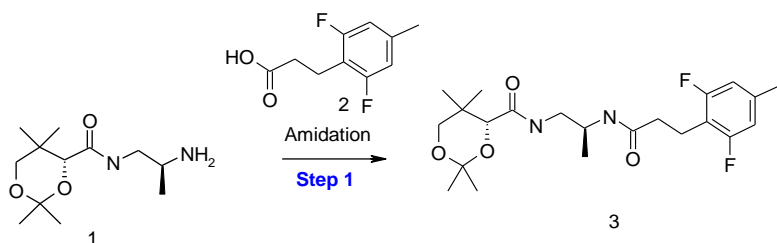


**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-thiophen-2-yl-propionylamino)-propyl]-amide (**3**) (140 mg, 0.366 mmol) to afford MMV693177 as colorless sticky gum in 63.83 % yield, 80 mg.

#### MMV693180-



**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2,6-difluoro-4-methyl-phenyl)-propionylamino]-propyl}-amide (**3**):**



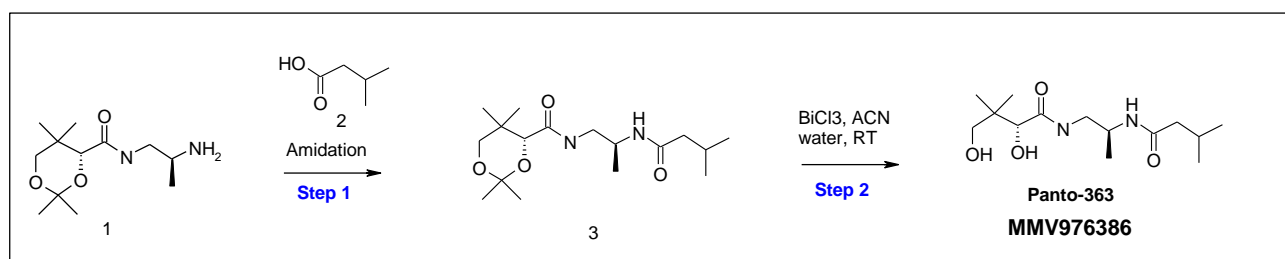
**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (**1**) (175 mg, 0.716 mmol) and 3-(2,6-Difluoro-4-methyl-phenyl)-propionic acid (**2**) (172.05 mg, 0.859 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2,6-difluoro-4-methyl-phenyl)-propionylamino]-propyl}-amide (**3**) as gum in 85.12 % yield, 260 mg.

### Synthesis of compound MMV693180:

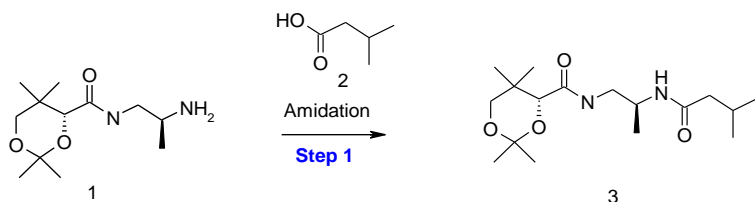


**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-[3-(2,6-difluoro-4-methyl-phenyl)-propionylamino]-propyl]-amide (3) (260 mg, 0.61 mmol) to afford MMV693180 as colorless sticky gum in 46.7% yield, 110 mg.

### MMV976386-



### Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-methyl-butrylamino)-propyl]-amide (3):

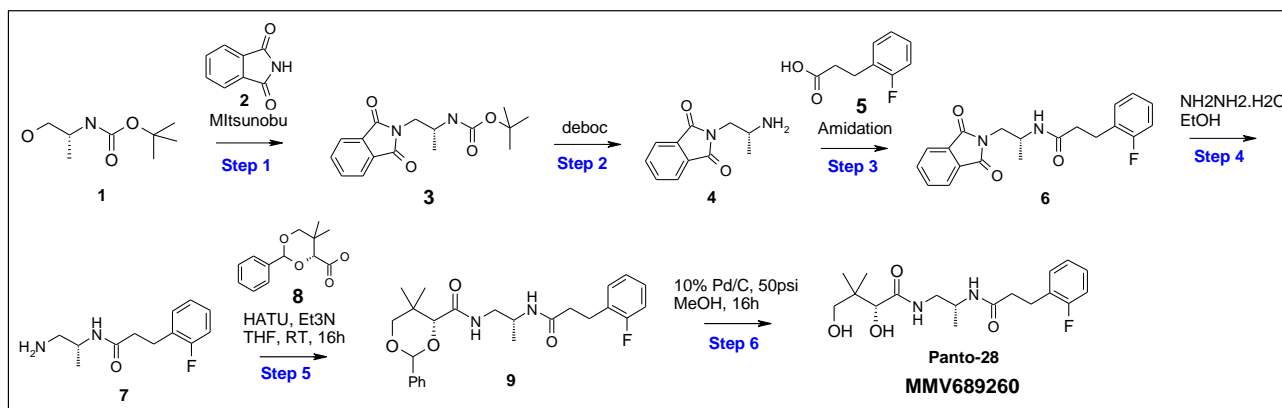


**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (1) (100 mg, 0.409 mmol) and 3-Methyl-butyric acid (2) (50.13 mg, 0.491 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-methyl-butrylamino)-propyl]-amide (3) as gum in 89.3% yield, 120 mg.

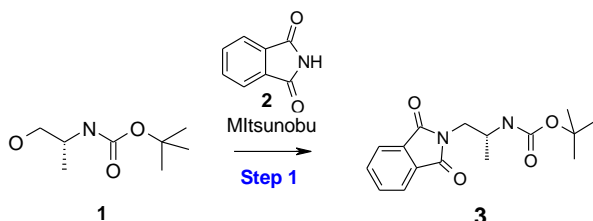
### Synthesis of compound MMV976386:



**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid [(S)-2-(3-methyl-butrylamino)-propyl]-amide (3) (120 mg, 0.366 mmol) to afford MMV976386 as colorless sticky gum in 28.43% yield, 30 mg.

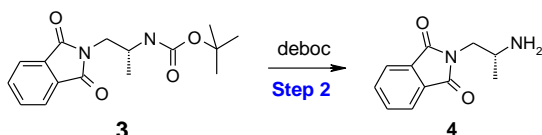
**MMV689260-**

**Synthesis of compound [(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid tert-butyl ester (3):**



**Procedure:** To a stirred solution of **[(R)-2-Hydroxy-1-methyl-ethyl]-carbamic acid tert-butyl ester (1)** (2.5 g, 14.267 mmol) in dry THF (28 ml) were added phthalimide **(2)** (2.3 g, 15.694) and PPh<sub>3</sub> (4.1 g, 15.694). Then DEAD (2.73 g, 15.694 mmol) was added drop wise to the stirred solution at room temperature and stirred for 16h. The reaction mixture was then concentrated and the residue was purified by combiflash (40 g silica column, 35% EtOAc-Hexane) to afford **[(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid tert-butyl ester (3)** as white solid in 96.73% yield, 4.2 g.

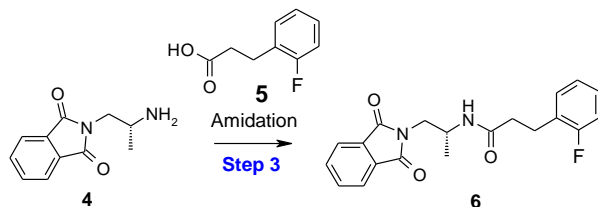
**Synthesis of compound 2-((R)-2-Amino-propyl)-isoindole-1,3-dione (4):**



**Procedure:** To the stirred solution of **[(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid tert-butyl ester (3)** (1.5 g, 4.929 mmol) in Dioxane (15 ml) was added 4M Dioxane-HCl (15 ml) at 0°C. Reaction mixture was stirred at RT for 6h. Then reaction mixture was concentrated under reduced pressure to afford **2-((R)-2-Amino-propyl)-isoindole-1,3-dione (4) (HCl salt)** as white solid in 92.76% yield, 1.1 g.

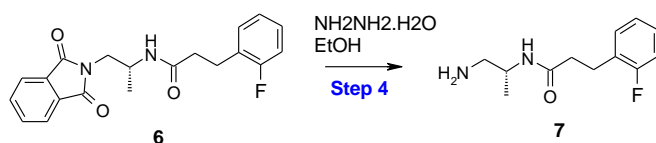


**Synthesis of N-[(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-3-(2-fluoro-phenyl)-propionamide (6):**



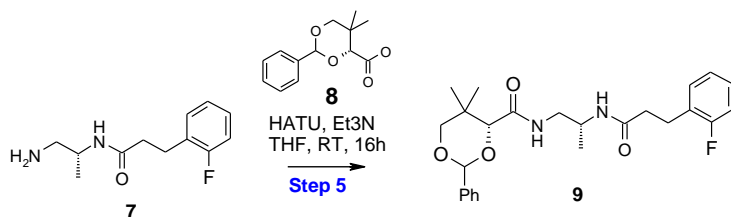
**Procedure:** To a stirred solution of **3-(2-Fluoro-phenyl)-propionic acid (5)** (350 mg, 2.083 mmol) in THF (10 ml) were added Et<sub>3</sub>N (1.45 ml, 10.417 mmol), HATU (1.19 g, 3.125 mmol) and **2-((R)-2-Amino-propyl)-isoindole-1,3-dione (4)** (751.56 mg, 3.125 mmol). Reaction mixture was stirred at RT for 16h. The reaction mixture was quenched with NaHCO<sub>3</sub> solution and extracted with EtOAc. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by combiflash (12 g silica column, 30% EtOAc-Hexane) to afford **N-[(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-3-(2-fluoro-phenyl)-propionamide (6)** as white solid in 98.88% yield, 730 mg.

**Synthesis of N-((R)-2-Amino-1-methyl-ethyl)-3-(2-fluoro-phenyl)-propionamide (7):**



**Procedure:** To the stirred solution of **N-[(R)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-3-(2-fluoro-phenyl)-propionamide (6)** (730 mg, 2.06 mmol) in MeOH (20 ml) was added hydrazine hydrate (1.65 g, 32.959 mmol). Reaction mixture was heated at 50°C for 2h. It was cooled to RT, filtered and concentrated under reduced pressure. The resulting residue was suspended in Et<sub>2</sub>O and filtered. The filtrate was concentrated under reduced pressure to afford **N-((R)-2-Amino-1-methyl-ethyl)-3-(2-fluoro-phenyl)-propionamide (7)** as white solid in 99.57% yield, 460 mg.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (9):**



**Procedure:** To the stirred solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (8)** (130 mg, 0.551 mmol) in THF (20 ml) were added Et<sub>3</sub>N (0.38 ml, 2.754 mmol), HATU (314 mg, 0.826 mmol) and **N-((R)-2-Amino-1-methyl-ethyl)-3-(2-fluoro-phenyl)-propionamide (7)** (246.78 mg,

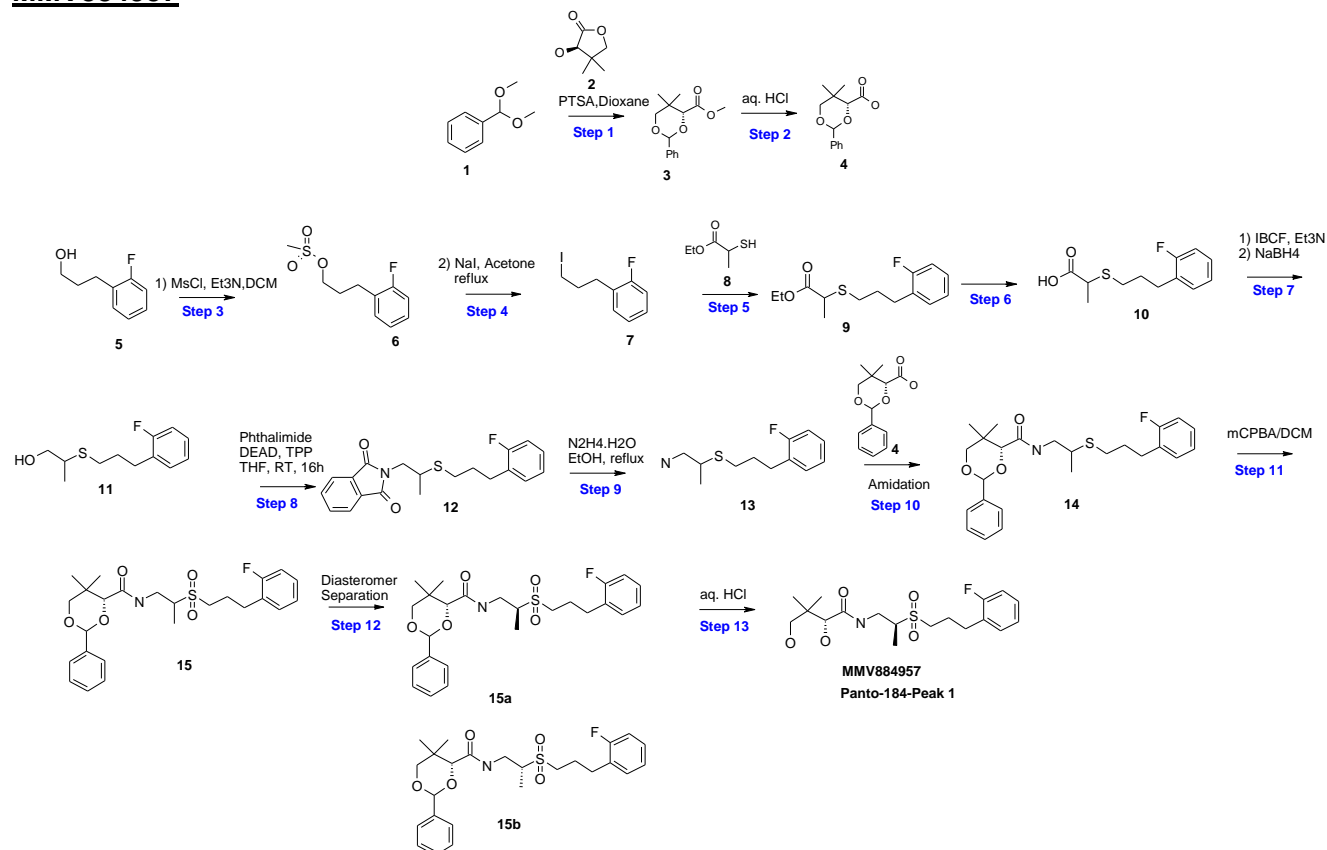
1.102 mmol). Reaction was stirred at RT for 16h. Reaction mixture was quenched with NaHCO<sub>3</sub> solution and extracted with EtOAc. Organic layer washed with water, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by combiflash (12 g, silica column, 25% EtOAc-DCM) to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (9)** as gum in 73.84% yield, 180 mg.

### Synthesis of MMV689260:

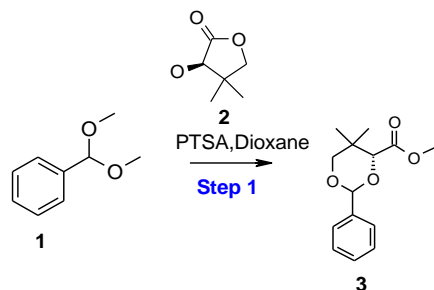


**Procedure:** A solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (9)** (180 mg, 0.407 mmol) in MeOH (20 ml) was degassed with argon for 10 minutes. Then Pd/C (10%, 20 mg) was added and placed in a parr-shaker under hydrogen at 50psi for 16h. The reaction mixture was filtered, the filtrate was concentrated under reduced pressure and the crude was purified by preparative TLC (4% MeOH-DCM) to afford **MMV689260** as colorless gum in 83.24% yield, 120 mg.

### MMV884957-

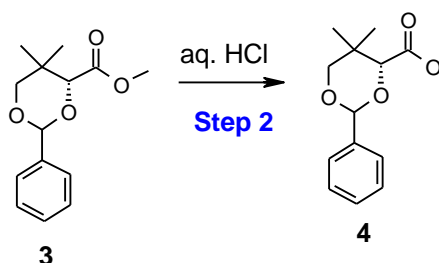


### Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid methyl ester (3):



**Procedure:** To a stirred solution of **Dimethoxymethyl-benzene (1)** (30 g, 230.5 mmol) and **(R)-3-Hydroxy-4,4-dimethyl-dihydro-furan-2-one (2)** (59.6 g, 391.4 mmol) in dioxane (200 mL) was added PTSA (0.79 g, 4.61 mmol) and the reaction mixture was stirred at RT for 2 days. The reaction mixture was quenched with NaHCO<sub>3</sub> solution and stirred at RT for 3h. The mixture was extracted with Ether. Organic layers were washed with water, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to get crude mass which was purified by column chromatography (silica gel, 100-200 mesh, 15% EtOAc in hexane) to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid methyl ester (3)** (20 g, 34.6%) as colorless liquid.

### Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (4):



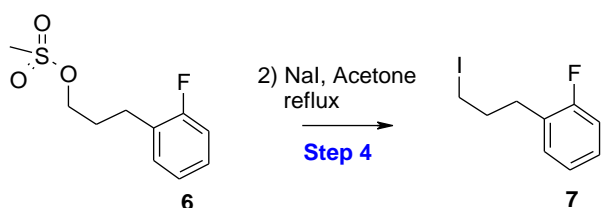
**Procedure:** To a stirred solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid methyl ester (3)** (10 g, 40 mmol) in MeOH (80 mL) was added a solution of LiOH in water (20 mL) and stirred at RT for 2h. The reaction was acidified with 2N HCl and extracted with DCM. Organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (4)** (8 g, 84%) as off white solid.

### Synthesis of Methanesulfonic acid 3-(2-fluoro-phenyl)-propyl ester (6):



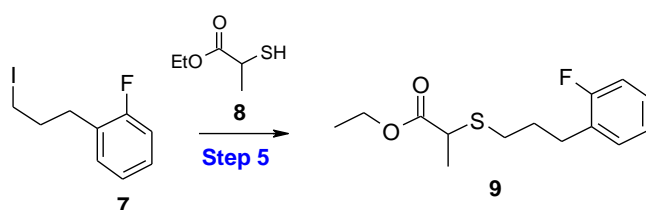
**Procedure:** To a solution of **3-(2-Fluoro-phenyl)-propan-1-ol (5)** ( 3.2 g, 20.77 mmol) in DCM (60 mL) were added Mesylchloride (2.24 mL, 31.16 mmol) and Et<sub>3</sub>N (8.74 mL, 66.33 mmol) at 0°C. Reaction was stirred at RT for 3h. Reaction mass was diluted with sat NaHCO<sub>3</sub> and extracted with EtOAc. Organic layer was washed with water, brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **Methanesulfonic acid 3-(2-fluoro-phenyl)-propyl ester (6)** (4.5 g, 93%) as colorless sticky gum.

#### Synthesis of 1-Fluoro-2-(3-iodo-propyl)-benzene (7):



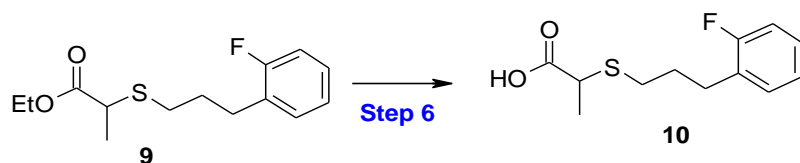
**Procedure:** To stirred solution of **Methanesulfonic acid 3-(2-fluoro-phenyl)-propyl ester (6)** (4.5 g, 19.39 mmol) in acetone (60 mL) was added NaI (6.45 g, 43.06 mmol) in acetone (40 mL) and the resulting mixture was stirred at RT under an atmosphere of argon for 7h. The reaction mixture was then filtered and the solid was washed well with acetone. The combined filtrate and washings were concentrated to get crude mass which was partitioned between ether (200 mL) and water (20 mL). The ethereal layer was washed sequentially with water, 10% aqueous sodium thiosulfate, brine and then dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **1-Fluoro-2-(3-iodo-propyl)-benzene (7)** (4.9 g, 96%) as Colorless sticky gum.

#### Synthesis of 2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid ethyl ester (9):



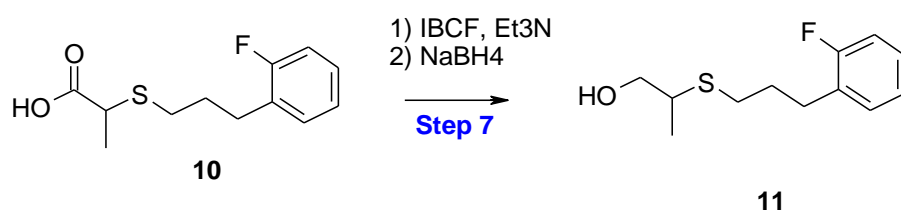
**Procedure:** To a solution of **8** (2.34 g, 18.23 mmol) in EtOH (60 mL), K<sub>2</sub>CO<sub>3</sub> (6.06 g, 43.93 mmol) and **1-Fluoro-2-(3-iodo-propyl)-benzene (7)** (5.8 g, 21.97 mmol) were added at about 0°C. Reaction was stirred at RT for 2h. EtOH was removed under reduced pressure to get crude mass which was diluted with water and extracted with EtOAc. Organic layers were combined and dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatograph using 6% ethyl acetate in hexane to afford **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid ethyl ester (9)** (3.75 g, 63%) as colorless sticky gum.

#### Synthesis of 2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid (10):



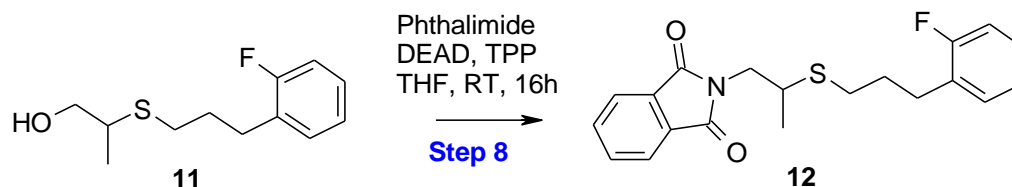
**Procedure:** To a stirred solution of **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid ethyl ester (9)** (3.75 g, 13.88 mmol) in THF (40 mL) was added a solution of LiOH (1.45 g, 34.72 mmol) in water (10 mL) and stirred at RT for 2h. Reaction was acidified with 2N HCl and extracted with DCM. Organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to afford **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid (10)** (2 g, 59%) as colorless sticky gum.

#### Synthesis of 2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propan-1-ol (**11**):



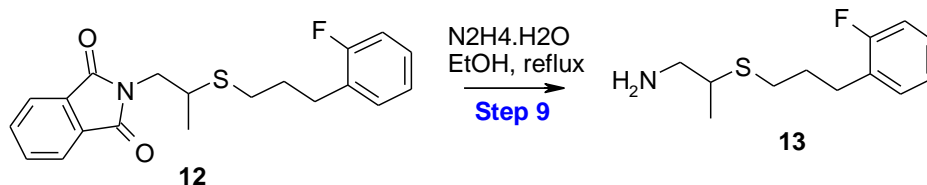
**Procedure:** To a solution of **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propionic acid (10)** (2 g, 8.26 mmol) in THF (25 mL) were added Et<sub>3</sub>N (2.3 mL, 16.52 mmol) and IBCF (1.29 mL, 9.91 mmol) at 0°C. Reaction mass was stirred at RT for 2h. Reaction mass was filtered, NaBH<sub>4</sub> (1.56 g, 41.32 mmol) (in 1 mL water) was added to filtrate at 0°C. Reaction was stirred at RT for 16h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using 10% EtOAc in hexane to afford **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propan-1-ol (11)** (1.5 g, 79%) as colorless sticky gum.

#### Synthesis of 2-{2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propyl}-isoindole-1,3-dione (**12**):



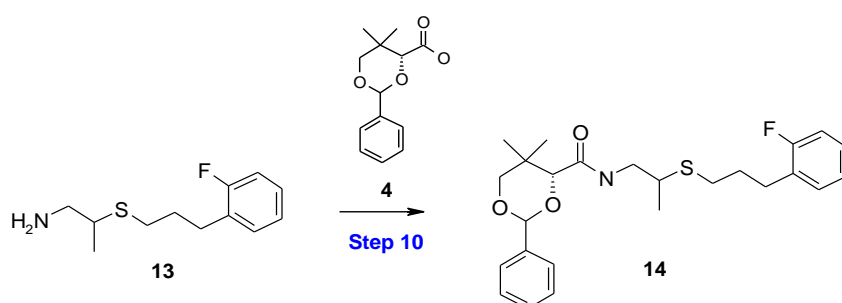
**Procedure:** To a solution of **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propan-1-ol (11)** (1.5 g, 6.57 mmol) in THF (30 mL) were added Phthalimide (1.61 g, 1.61 mmol), PPh<sub>3</sub> (2.06 g, 7.89 mmol) and DEAD (1.31 mL, 7.23 mmol) at 0 deg C. Reaction was stirred at RT for 16h. Reaction mass was concentrated under reduced pressure to get crude mass which was purified by combi flash column chromatography using 15% EtOAc in hexane to afford **2-{2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propyl}-isoindole-1,3-dione (12)** (2.2 g, 94%) as colorless sticky gum.

**Synthesis of 2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propylamine (13):**



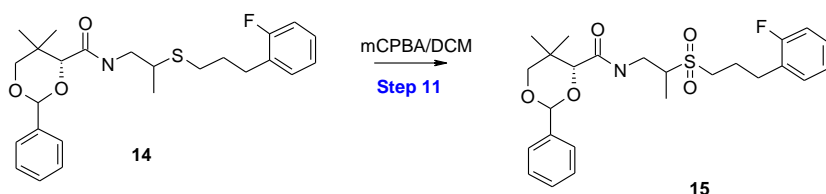
**Procedure:** To a solution of **2-[2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propyl]-isoindole-1,3-dione (12)** (1 g, 2.93 mmol) in EtOH (20 mL) was added  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$  (1.43 mL, 29.32 mmol) and reaction was refluxed for 2 h. Reaction mass filtered and concentrated under reduced pressure to afford **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propylamine (13)** (600 mg, 97%) as colorless sticky mass.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluoro-phenyl)-propylsulfanyl]-propyl}-amide (14):**



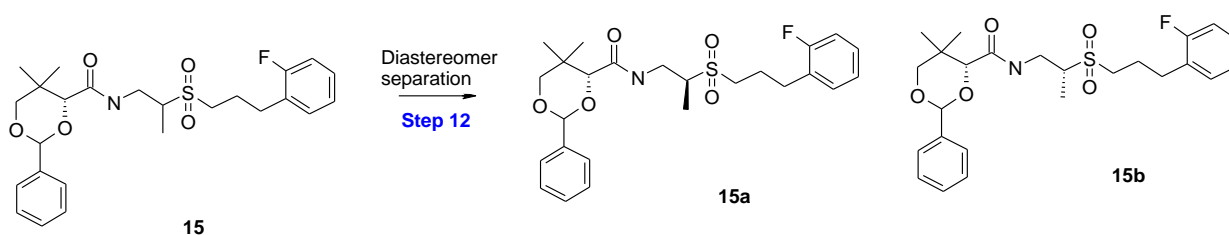
**Procedure:** To a solution of **2-[3-(2-Fluoro-phenyl)-propylsulfanyl]-propylamine (13)** (1 g, 4.39 mmol) in DCM (20 mL) were added  $\text{Et}_3\text{N}$  (1.84 mL, 13.16 mmol), EDC (1.25 g, 6.58 mmol), HOBT (0.89 g, 6.58 mmol) and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (4)** (1.03 g, 4.39 mmol). Reaction was stirred at RT for 16 h. Reaction mass was diluted with sat  $\text{NaHCO}_3$  and extracted with DCM. Organic layer was washed with water, brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using by 2% MeOH in DCM to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluoro-phenyl)-propylsulfanyl]-propyl}-amide (14)** (500 mg, 25%) as colorless sticky gum.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluoro-phenyl)-propane-1-sulfonyl]-propyl}-amide (15):**



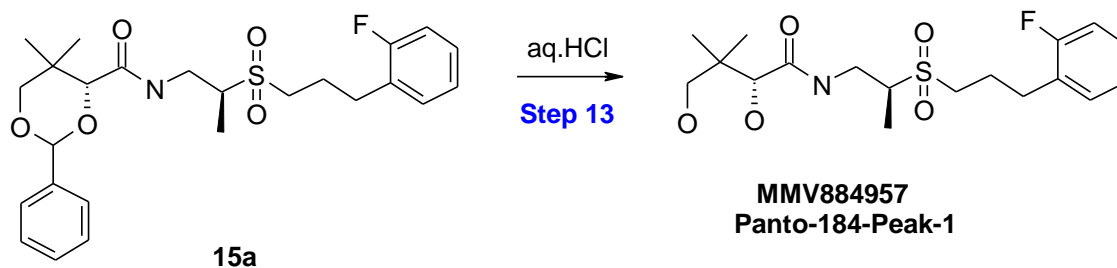
**Procedure:** To a stirred solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluorophenyl)-propylsulfanyl]-propyl}-amide (14)** ( 600 mg, 1.34 mmol) in DCM ( 20 mL), mCPBA (580 mg, 3.36 mmol) was added portion wise at 0°C. Reaction mixture was stirred for 3 hours at 0°C. Reaction mixture was quenched with saturated NaHCO<sub>3</sub> solution and extracted with DCM. Organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to get the crude compound which was purified by column chromatography by silica gel (100-200 mesh) using 20% EtOAc in hexane to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluorophenyl)-propane-1-sulfonyl]-propyl}-amide (15)** (360 mg, 56%) as colorless sticky gum.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluorophenyl)-propane-1-sulfonyl]-propyl}-amide (15a):**



**Procedure:** **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluorophenyl)-propane-1-sulfonyl]-propyl}-amide (15)** (360 mg, 0.75 mmol) racemic compound was subjected to diastereomer separation by SFC. After evaporation of fractions afforded **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluorophenyl)-propane-1-sulfonyl]-propyl}-amide (15a)** 110 mg peak 1 and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(R)-2-[3-(2-fluorophenyl)-propane-1-sulfonyl]-propyl}-amide (15b)** 110 mg peak 2.

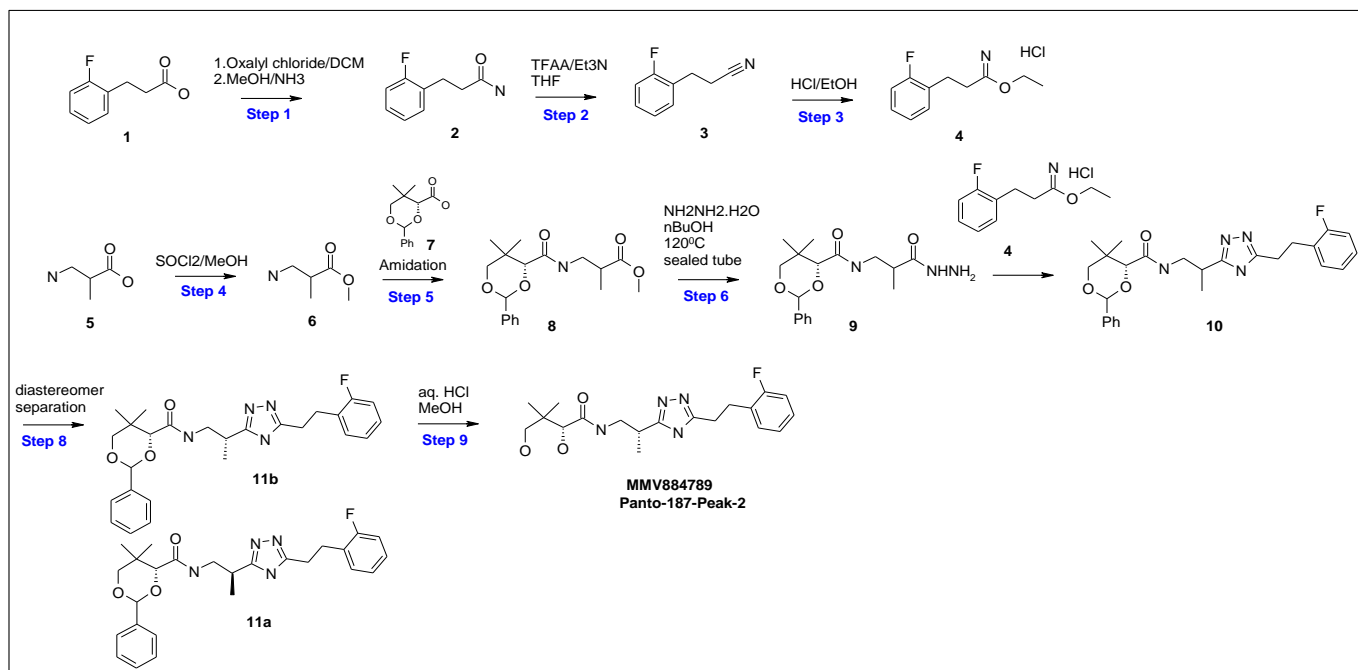
**Synthesis of MMV884957:**



**Procedure:** To a solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-**

**fluoro-phenyl)-propane-1-sulfonyl]-propyl)-amide (15a)** (110 mg, 0.23 mmol) in MeOH (2 mL) was added 1N HCl (1 mL). Reaction was stirred at RT for 2h. MeOH of the reaction mass was concentrated under reduced pressure, diluted with saturated NaHCO<sub>3</sub> solution and extracted with EtOAc. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure which was purified by prep TLC using 2% MeOH in DCM to afford **MMV884957** (20 mg, 22%) as colorless sticky gum.

#### MMV884789-



#### Synthesis of 3-(2-Fluoro-phenyl)-propionamide (2):



**Procedure:** To a solution of **3-(2-Fluoro-phenyl)-propionic acid (1)** (1.5 g, 8.9 mmol) in DCM (20 mL) were added Oxalyl chloride (2.28 mL, 26.7 mmol) at 0°C and catalytic DMF (20 µl). Reaction was stirred at RT for 1h. Reaction mass was evaporated under reduced pressure which was dissolved in THF (5 mL) and added to aq NH<sub>3</sub> (20 mL) dropwise at 0°C. Reaction was stirred at RT for 2h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **3-(2-Fluoro-phenyl)-propionamide (2)** (1.2 g, 80%) as off white solid.

#### Synthesis of 3-(2-Fluoro-phenyl)-propionitrile (3):





**Procedure:** To a solution of **3-(2-Fluoro-phenyl)-propionamide (2)** (1.3 g, 7.7 mmol) 1 in dry THF (20 mL) were added Et<sub>3</sub>N (5.4 mL, 3.8 mmol) and TFAA (2.75 mL, 19.4 mmol) at 0°C. Reaction mixture was stirred for 2h. Reaction mass was evaporated under reduced pressure to get crude mass which was diluted sat aq. NaHCO<sub>3</sub> and extracted with EtOAc washed with water, brine. Organic layer was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **3-(2-Fluoro-phenyl)-propionitrile (3)** (1 g, 86%) as colorless sticky gum.

#### Synthesis of 3-(2-Fluoro-phenyl)-propionimidic acid ethyl ester hydrochloride (4):



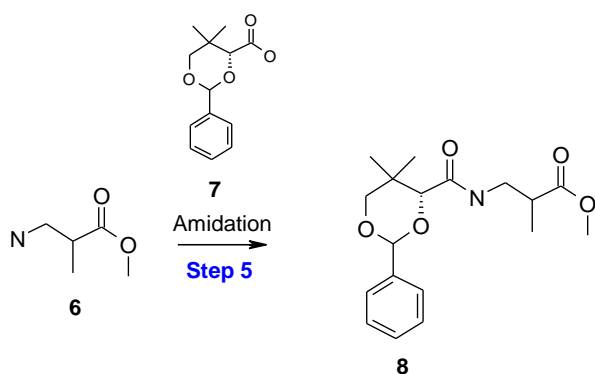
**Procedure:** To a solution of **3-(2-Fluoro-phenyl)-propionitrile (3)** (300 mg, 2.01 mmol) in EtOH (10 mL) was passed hydrogen chloride gas for 30 mins at 0°C. The resulting reaction mixture was stirred at RT for 2h. Reaction mass was concentrated under reduced pressure to afford **3-(2-Fluoro-phenyl)-propionimidic acid ethyl ester hydrochloride (4)** (350 mg, 75%) as colorless sticky gum.

#### Synthesis of 3-Amino-2-methyl-propionic acid methyl ester (6):



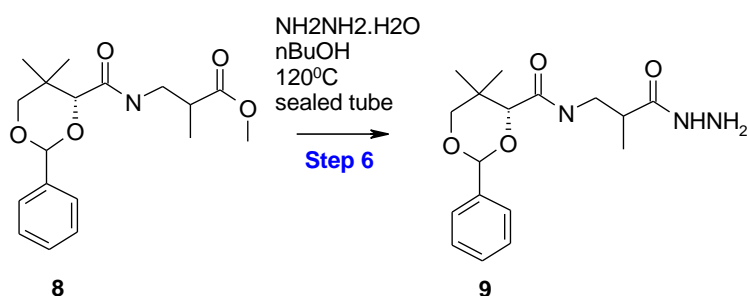
**Procedure:** To a solution of **3-Amino-2-methyl-propionic acid (5)** (500 mg, 4.8 mmol) in MeOH (10 mL) was added SOCl<sub>2</sub> (0.74 mL, 9.6 mmol) at 0°C and reaction mixture was stirred at RT for 16h. Reaction mixture was evaporated under reduced pressure and azeotroped with toluene to afford **3-Amino-2-methyl-propionic acid methyl ester (5)** (550 mg, 97%) as off white solid.

#### Synthesis of 3-[(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carbonyl)-amino]-2-methyl-propionic acid methyl ester (8):



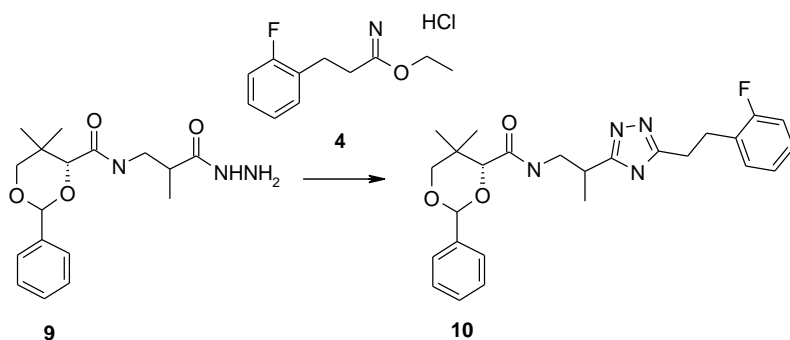
**Procedure:** To a solution of **3-Amino-2-methyl-propionic acid methyl ester (6)** (2.5 g, 21.5 mmol) in DCM (10 mL) were added Et<sub>3</sub>N (8.9 mL, 64.1 mmol), EDCI (6.12 g, 32 mmol), HOBt (4.32 g, 32 mmol) and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid 7** (5.04 g, 21.3 mmol). Reaction was stirred at RT for 16h. Reaction mass was diluted with sat NaHCO<sub>3</sub> and extracted with DCM. Organic layer was washed with water brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using 15% EtOAc in hexane to afford **3-(((R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carbonyl)-amino)-2-methyl-propionic acid methyl ester (8)** (3 g, 42%) as colorless sticky gum.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-hydrazinocarbonyl-propyl)-amide (9):**



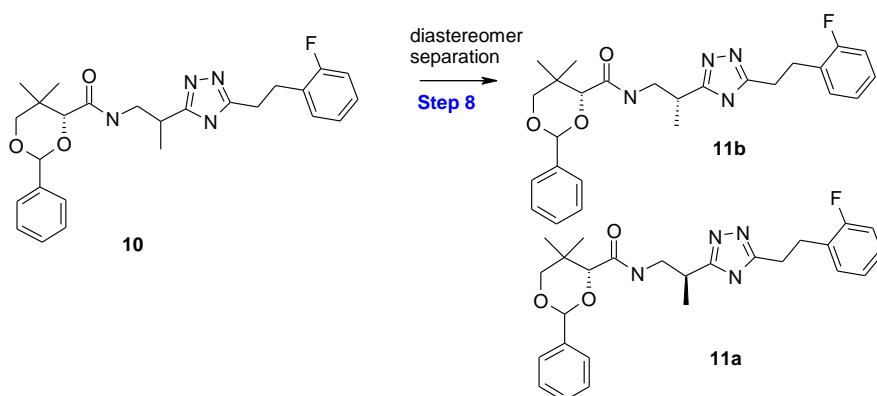
**Procedure:** To a solution of **3-(((R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carbonyl)-amino)-2-methyl-propionic acid methyl ester (8)** (3.7 g, 11 mmol) in n-butanol (5 mL) was taken in a sealed tube was added NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O (8.03 g, 165.4 mmol). Reaction mass was stirred at 120°C for 16h. Reaction mixture was evaporated under reduced pressure to get crude which was purified by silica gel (100-200 mesh) column chromatography (100-200 mesh) by using 4% MeOH in DCM to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-hydrazinocarbonyl-propyl)-amide (9)** (3.2 g, 87%) as colorless sticky gum.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (10):**



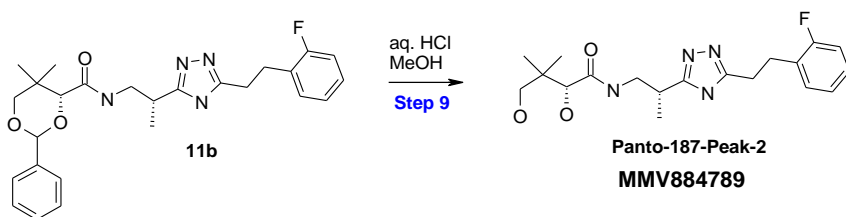
**Procedure:** To a solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-hydrazinocarbonyl-propyl)-amide (9)** (1 g, 2.98 mmol) in EtOH ( 6 mL) were added **3-(2-Fluorophenyl)-propionimidic acid ethyl ester hydrochloride (4)** (694 mg, 2.98 mmol) and Et<sub>3</sub>N (1.25 mL, 8.95 mmol). Reaction mass was refluxed for 16h. Reaction mass was concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using 2% MeOH in DCM to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (10)** (400 mg , 29%) as colorless sticky gum.

**Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid ((R)-2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (11b):**



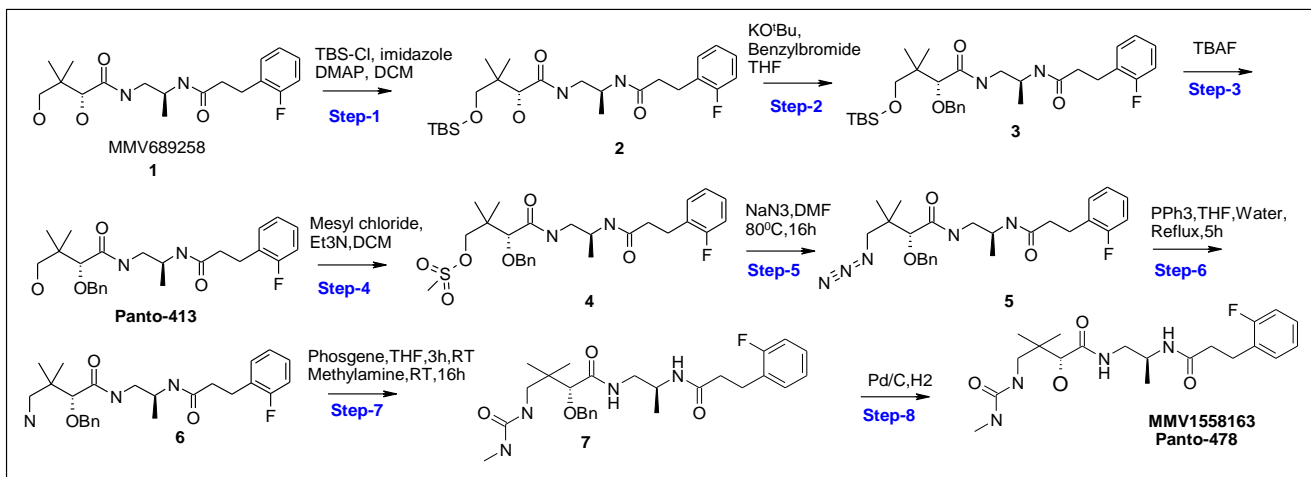
**Procedure:** **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (10)** (400 mg, 0.85 mmol) racemic compound was subjected to diastereomer separation by SFC. After evaporation of fractions afforded **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid ((S)-2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide 11a** 110 mg peak 1 and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid ((R)-2-{5-[2-(2-fluoro-phenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (11b)** 110 mg peak 2.

**Synthesis of MMV884789:**

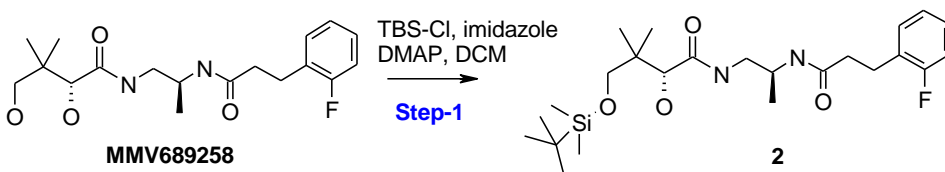


**Procedure:** (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid ((R)-2-{5-[2-(2-fluorophenyl)-ethyl]-4H-[1,2,4]triazol-3-yl}-propyl)-amide (**11b**) (110 mg, 0.23 mmol) in MeOH (2 mL) was added 1N HCl(1 mL). Reaction was stirred at RT for 1h.MeOH of the reaction mass was concentrated under reduced pressure, diluted with sat NaHCO<sub>3</sub> soln and extracted with EtOAc. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure which was purified by prep TLC using 2% MeOH in DCM to afford **MMV884789 (Panto-187-Peak-2)** (14 mg, 16%) as Colorless sticky gum.

### MMV1558163-



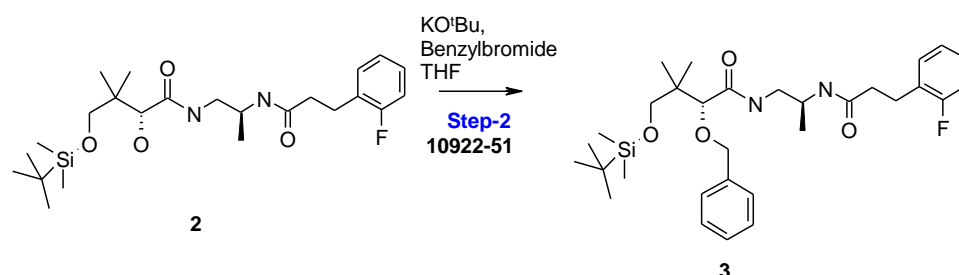
### Synthesis of (R)-4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluorophenyl)-propionylamino]-propyl)-2-hydroxy-3,3-dimethyl-butyramide (**2**):



**Procedure:** (R)-N-((S)-2-[3-(2-Fluoro-phenyl)-propionylamino]-propyl)-2,4-dihydroxy-3,3-dimethyl-butyramide (**MMV689258**) (2.25 g, 6.36 mmol) was dissolved in DCM (50 mL). Imidazole (0.86 g, 12.7

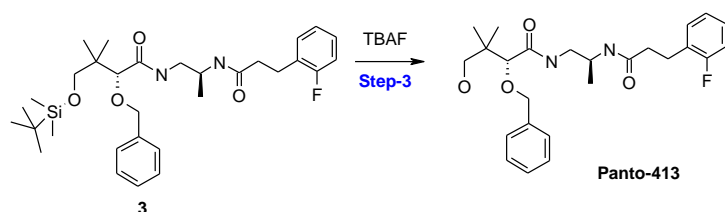
mmol) and DMAP (0.38 g, 3.18 mmol) were added at 0°C. Reaction mass was stirred at RT for 15 mins. TBSCl (1.43 g, 9.54 mmol) was added and reaction mass was stirred at RT for 16h. Reaction mass was diluted with water and extracted with DCM. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get the crude mass which was purified by silica gel (100-200 mesh) column chromatography using 2% MeOH in DCM to afford **(R)-4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-hydroxy-3,3-dimethyl-butyramide (2)** (2.3 g, 77%) as colorless sticky gum.

**Synthesis of (R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (3):**



**Procedure:** To a solution of **(R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (2)** (2.3 g, 4.91 mmol) in THF (15 mL) was added KtBuO (1M soln in THF) (5.4 mL, 5.4 mmol) at 0°C. Benzylbromide (0.62 mL, 5.4 mmol) was added and reaction mass was stirred at RT for 16h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using 2% MeOH in DCM to afford **(R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (3)** (1.8 g, 66%) as colorless sticky gum.

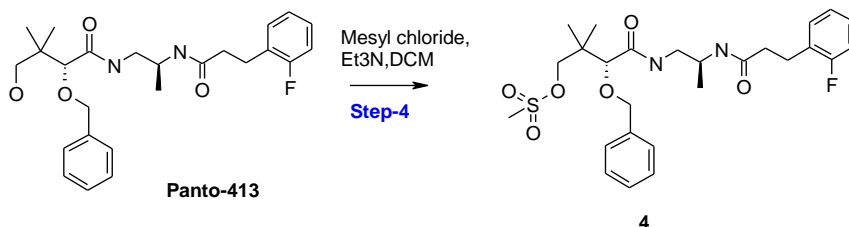
**Synthesis of (R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (Panto-413):**



**Procedure:** To a solution of **(R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (3)** (1.4 g, 2.5 mmol) in THF (8 mL) was added TBAF (1M solution in THF) (3 mL, 3 mmol) at 0°C. Reaction mixture was stirred at RT for 16h. Reaction mixture was quenched with saturated ammonium chloride solution. Organic layer was separated and washed with water, brine. Organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure to afford **(R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (Panto-413)**.

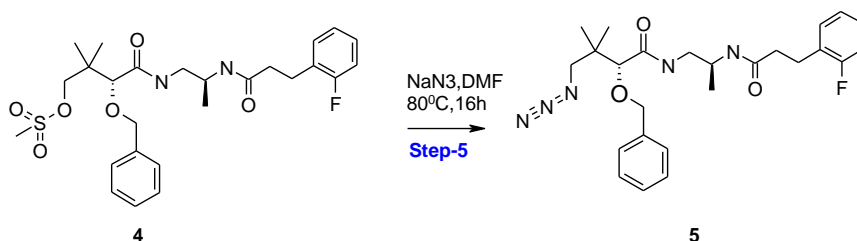
**{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-3,3-dimethyl-butylamide (Panto-413)** (850 mg, 76%) as gum.

**Synthesis of Methanesulfonic acid (R)-3-benzyloxy-3-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl}-2,2-dimethyl-propyl ester (4):**



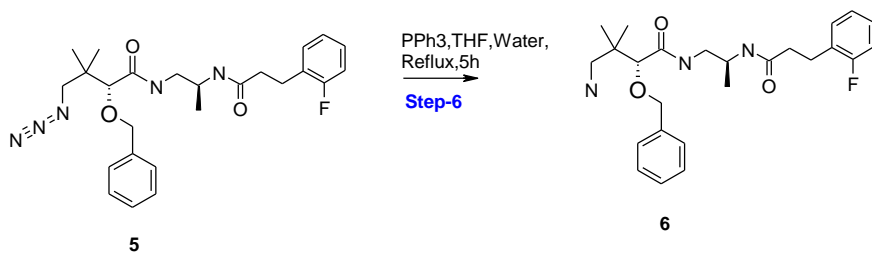
**Procedure:** To a solution of **(R)-2-Benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-N-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-3,3-dimethyl-butylamide (Panto-413)** (300 mg, 0.67 mmol) in DCM (3 mL) were added Mesylchloride (0.078 mL, 1 mmol) and Et<sub>3</sub>N (0.28 mL, 2 mmol) at 0°C. Reaction was stirred at RT for 3h. Reaction mass was diluted with sat NaHCO<sub>3</sub> and extracted with EtOAc. Organic layer was washed with water, brine. Organic layer was dried over anh. sodium sulfate, filtered and concentrated under reduced pressure to afford **Methanesulfonic acid (R)-3-benzyloxy-3-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl}-2,2-dimethyl-propyl ester (4)** (350 mg, 99%) as colorless sticky gum.

**Synthesis of (R)-4-Azido-2-benzyloxy-N-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-3,3-dimethyl-butylamide (5):**



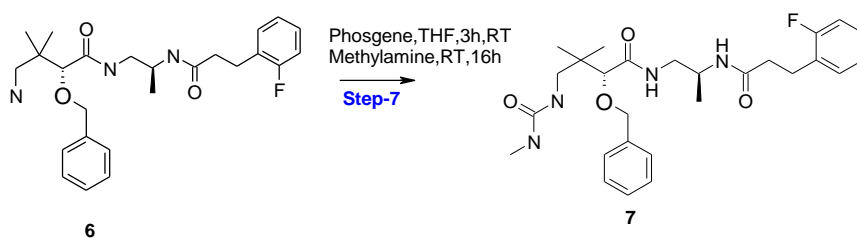
**Procedure:** To a solution of **Methanesulfonic acid (R)-3-benzyloxy-3-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl}-2,2-dimethyl-propyl ester (4)** (350 mg, 0.67 mmol) in DMF (1 mL) was added NaN<sub>3</sub> (87 mg, 1.33 mmol) and reaction was stirred at 80°C for 8h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **(R)-4-Azido-2-benzyloxy-N-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-3,3-dimethyl-butylamide (5)** (150 mg, 48%) as colorless sticky gum.

**Synthesis of (R)-4-Amino-2-benzyloxy-N-{{(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-3,3-dimethyl-butylamide (6):**



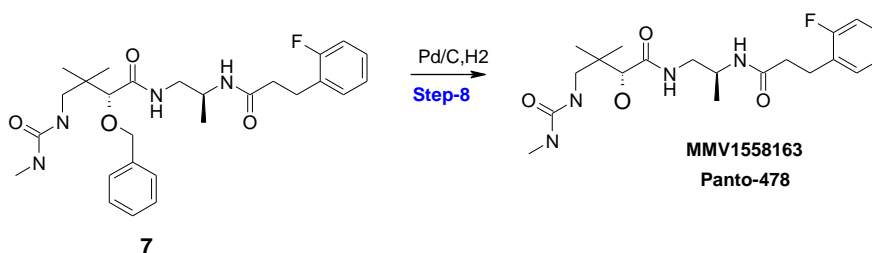
**Procedure:** To a solution of **(R)-4-Azido-2-benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butylamide (5)** (130 mg, 0.27 mmol) in THF (2 mL) was added PPh<sub>3</sub> (145 mg, 0.55 mmol) and water (0.015 mL, 0.83 mmol). Reaction was refluxed for 16h. Reaction mass was diluted 10% MeOH in DCM and dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by prep TLC plate using 2% MeOH in DCM to afford **(R)-4-Amino-2-benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butylamide (6)** (40 mg, 33%) as colorless sticky gum.

**Synthesis of (R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-4-(3-methyl-ureido)-butylamide (7):**



**Procedure:** To a solution of **(R)-4-Amino-2-benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butylamide (6)** (140 mg, 0.31 mmol) in THF (2 mL) was added COCl<sub>2</sub> (20% in Toluene) at 0°C. Reaction was stirred at RT for 2h. Reaction mass was concentrated under reduced pressure to get crude mass which dissolved in THF (2 mL) was added Methylamine (2M soln) (1.5 mL, 3.1 mmol) and reaction was stirred at RT for 16h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to get crude mass which was purified by prep TLC using 2% MeOH in DCM to afford **(R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-4-(3-methyl-ureido)-butylamide (7)** (28 mg, 17%) as colorless sticky mass.

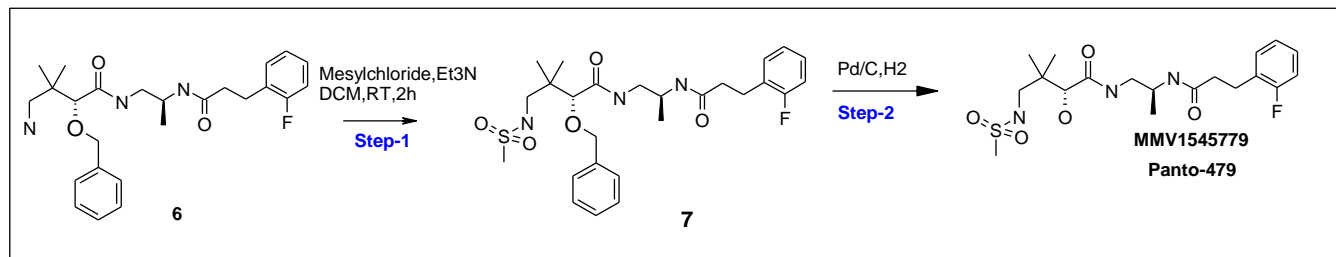
**Synthesis of MMV1558163:**



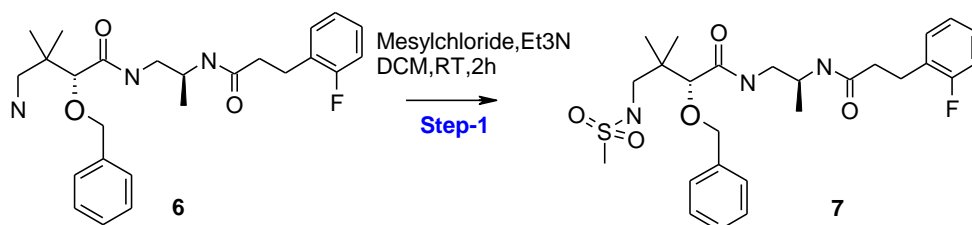
**Procedure:** To a solution of **(R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-4-(3-methyl-ureido)-butylamide (7)** (28 mg, 0.05 mmol) in MeOH (1 mL) was degassed with argon for 10mins. 10% Pd/C (20 mg) was added and reaction was stirred at RT for 16h

under hydrogen atmosphere. Reaction mass was filtered celite bed and filtrate was concentrated under reduced pressure to get crude mass which was purified by prep TLC using 2% MeOH in DCM to afford **MMV1558163 (Panto-478)** (10 mg, 44%) as colorless sticky gum.

### **MMV1545779-**

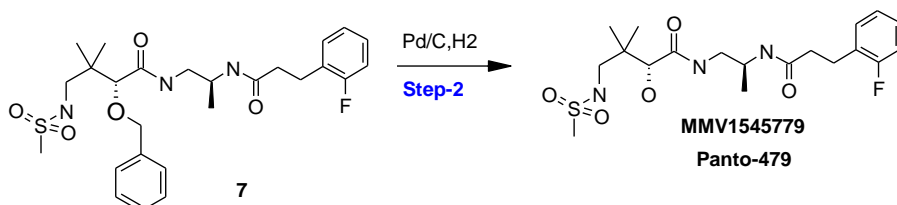


### **Synthesis of (R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-4-methanesulfonylamino-3,3-dimethyl-butyramide (7):**



**Procedure:** To a solution of **(R)-4-Amino-2-benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-butyramide (6)** (30 mg, 0.06 mmol) in DCM (2 mL) were added Mesylchloride (0.06 mL, 0.08 mmol) and Et<sub>3</sub>N (0.4 mL, 0.33 mmol) at 0°C. Reaction was stirred at RT for 2h. Reaction mass was diluted with sat NaHCO<sub>3</sub> and extracted with EtOAc. Organic layer was washed with water, brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **(R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-4-methanesulfonylamino-3,3-dimethyl-butyramide (7)** (28 mg, 79%) as colorless sticky mass.

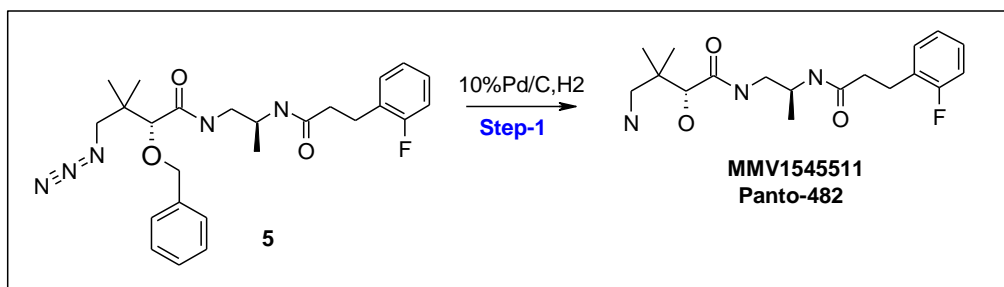
### **Synthesis of MMV1545779:**



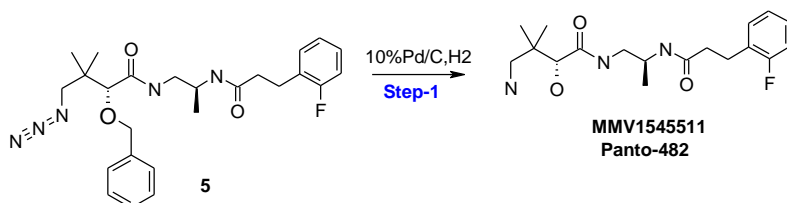
**Procedure:** To a solution of **(R)-2-Benzyloxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-4-methanesulfonylamino-3,3-dimethyl-butyramide (7)** (28 mg, 0.05 mmol) in MeOH (2 mL) was degassed with nitrogen gas. 10% Pd/C (20 mg) was added to the reaction mass and stirred at RT under hydrogen atmosphere for 16h. Reaction mass was filtered through celite bed and concentrated under reduced pressure to get crude mass which was purified by prep TLC using 2% MeOH in DCM to afford **MMV1545779 (Panto-479)** (7 mg, 30%) as colorless sticky mass.



### MMV1545511-

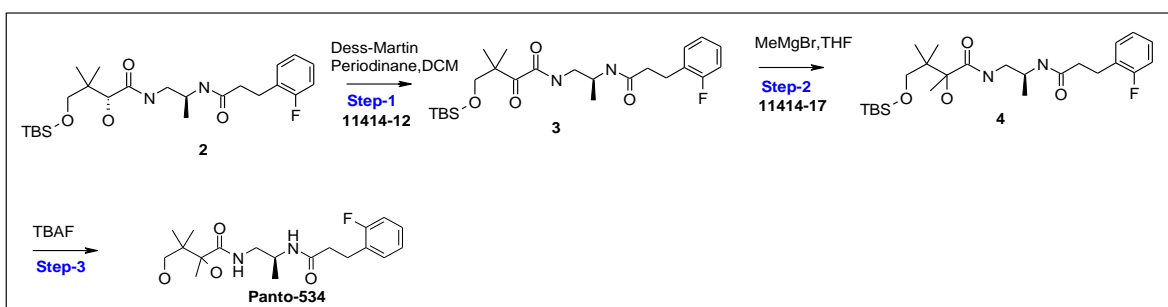


### Synthesis of MMV1545511:

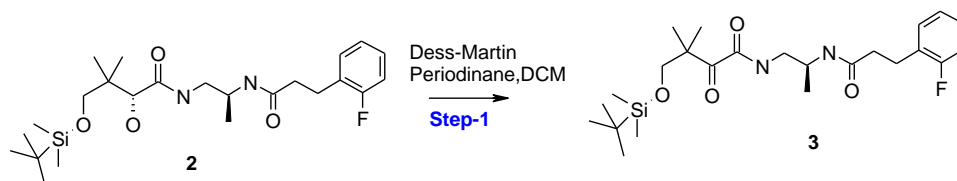


**Procedure:** To a stirred solution of compound **(R)-4-Azido-2-benzyloxy-N-((S)-2-[3-(2-fluorophenyl)propionylamino]-propyl)-3,3-dimethyl-butyrarnide (5)** (50 mg, 0.1 mmol) in MeOH (2 mL) was degassed with nitrogen gas. 10% Pd/C (30 mg) was added to the reaction mass and stirred at RT under hydrogen atmosphere for 4h. Reaction mass was filtered through celite and concentrated under reduced pressure to get crude mass which purified by prep TLC using 6% MeOH in DCM to afford **MMV1545511 (Panto-482)** (4 mg, 11%) as colorless sticky gum.

### MMV1545785-



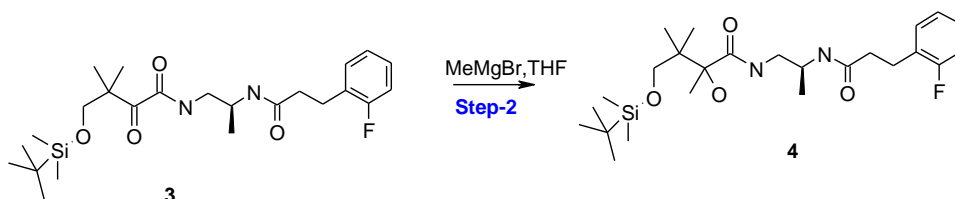
**Synthesis of 4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluorophenyl)propionylamino]-propyl)-3,3-dimethyl-2-oxo-butyrarnide (3):**



**Procedure:** To a solution of **(R)-4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluorophenyl)propionylamino]-**

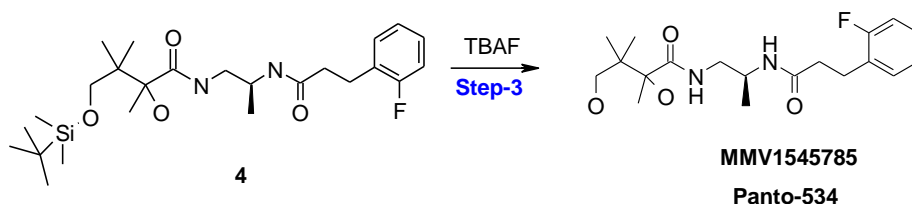
**propionylamino]-propyl]-2-hydroxy-3,3-dimethyl-butamide (2)** (200 mg, 0.43 mmol) in DCM (2 mL) was added Dess-Martin periodinane (279 mg, 0.69mmol) at 0°C. Reaction was stirred at RT for 16h. Reaction mass was quenched with sat NaHCO<sub>3</sub> soln and extracted with DCM. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-2-oxo-butamide (3)** (150 mg, 75%) as colorless sticky mass.

**Synthesis of 4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl]-2-hydroxy-2,3,3-trimethyl-butamide (4):**



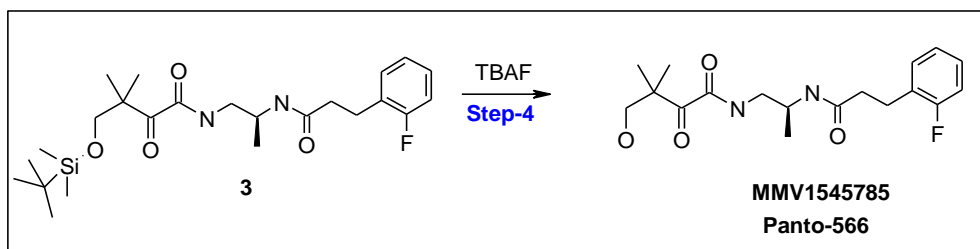
**Procedure:** To a stirred solution of **4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-2-oxo-butamide (3)** (110 mg, 0.23 mmol) in dry THF (2 mL), MeMgBr (3M soln in DEE) (0.32 mL, 0.94 mmol) was added at 0°C. Reaction mass was stirred at RT for 1h. Reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution, extracted with EtOAc, washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to get the crude mass which was purified by prep TLC plate using 30% ethyl acetate in hexane to afford **4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-hydroxy-2,3,3-trimethyl-butamide (4)** (55 mg, 49%) as colorless sticky mass.

**Synthesis of MMV1545785:**

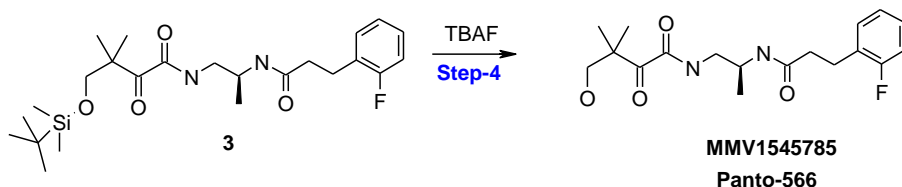


**Procedure:** To a stirred solution of **4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-hydroxy-2,3,3-trimethyl-butamide (4)** (50 mg, 0.1 mmol) in THF (2 mL) was added TBAF (1M solution in THF, 0.12 mL, 0.12 mmol) at 0°C. Reaction mixture was stirred at RT for 16h. Reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution. Organic layer was separated and washed with water, brine. Organic layer was dried over anh. Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduce pressure to get crude compound which was purified by prep TLC plate using 2% MeOH in DCM to afford **MMV1545785 (Panto-534)** (15 mg, 39%) as colorless sticky gum.

**MMV1545786-**

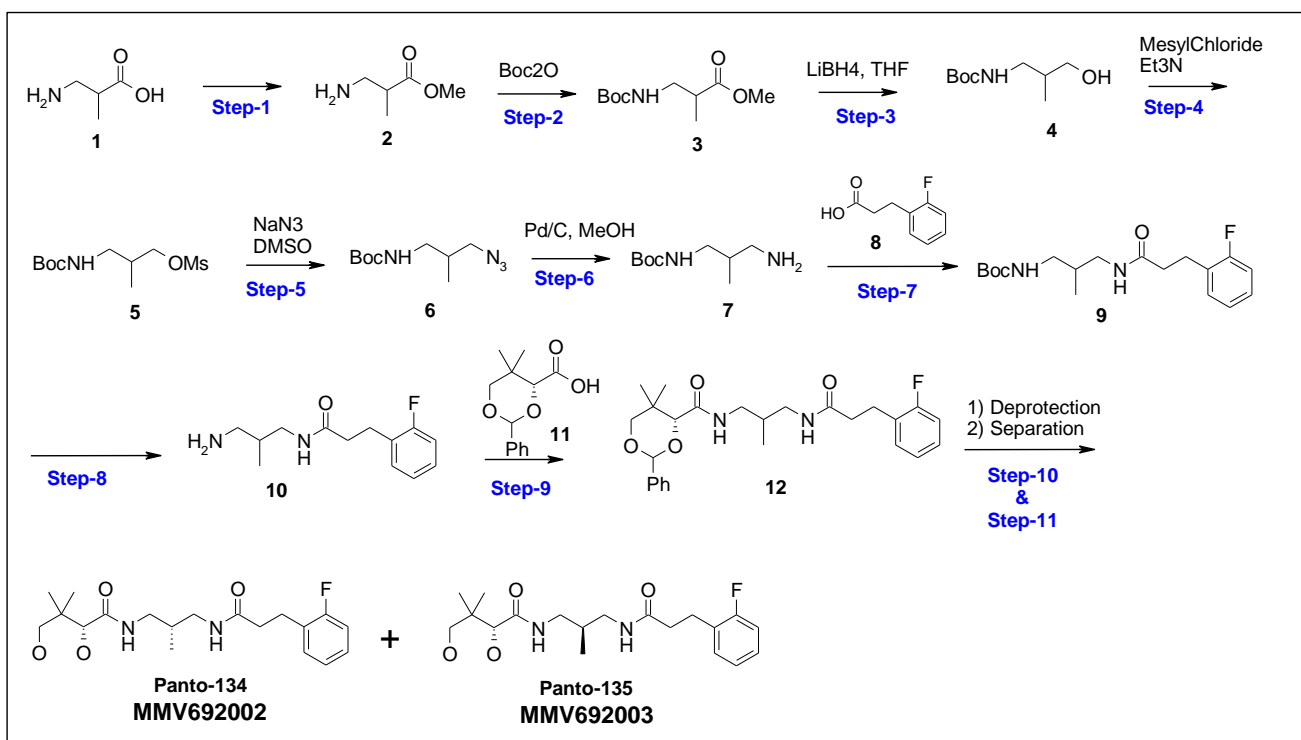


#### Synthesis of MMV1545786:



**Procedure:** To a stirred solution of 4-(tert-Butyl-dimethyl-silanyloxy)-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-3,3-dimethyl-2-oxo-butylamide (**3**) (100 mg, 0.21 mmol) in THF (3 mL) was added TBAF (1M solution in THF, 0.25 mL, 0.25 mmol) at 0°C. Reaction mixture was stirred at RT for 16h. Reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution. Organic layer was separated and washed with water, brine. Organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure to get crude compound which was purified by prep TLC plate using 2% MeOH in DCM to afford **MMV1545786** (**Panto-566**) (16 mg, 21%) as colorless sticky gum.

#### MMV692002 and MMV692003-



#### Synthesis of compound 3-Amino-2-methyl-propionic acid methyl ester (2):



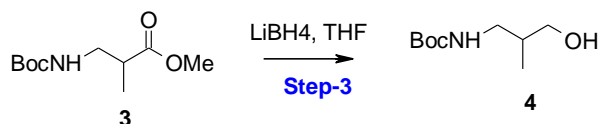
**Procedure:** To a solution of **3-Amino-2-methyl-propionic acid (1)** (500 mg, 4.854 mmol) in MeOH (5 ml) at 0°C was added SOCl<sub>2</sub> (0.704 ml, 9.709 mmol) slowly. The resulting reaction mixture was stirred at room temperature overnight. Solvent was removed in vacuo to afford **3-Amino-2-methyl-propionic acid methyl ester (2) (HCl salt)** as off white solid in 96.72% yield, 550 mg.

#### Synthesis of compound 3-tert-Butoxycarbonylamino-2-methyl-propionic acid methyl ester (3):



**Procedure:** To a suspension of **3-Amino-2-methyl-propionic acid methyl ester (2)** (550 mg, 4.701 mmol) in DCM (10 ml) were added (BOC)<sub>2</sub>O (1 ml, 4.701 mmol), Et<sub>3</sub>N (1.98 ml, 14.103 mmol) and DMAP (5.73 mg, 0.047 mmol). Reaction was stirred at RT for 16h. Reaction mass was diluted with water and extracted with DCM. Organic layer was washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by combiflash (12 g silica column, 2% MeOH-DCM) to afford **3-tert-Butoxycarbonylamino-2-methyl-propionic acid methyl ester (3)** as colorless oil in 63.64% yield, 650 mg.

### Synthesis of compound (3-Hydroxy-2-methyl-propyl)-carbamic acid tert-butyl ester (4):



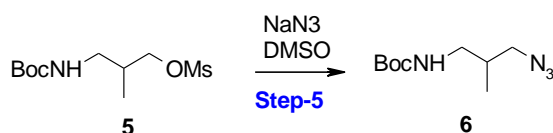
**Procedure:** To a solution of **3-tert-Butoxycarbonylamino-2-methyl-propionic acid methyl ester (3)** (550 mg, 2.535 mmol) in THF (20 ml) was added  $\text{LiBH}_4$  (3M Soln, 1 ml) at 0 deg C. Reaction was stirred at RT for 16h. Reaction mass was quenched with water and extracted with EtOAc. Organic layer was washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **(3-Hydroxy-2-methyl-propyl)-carbamic acid tert-butyl ester (4)** as colorless oil in 93.81% yield, 450 mg.

### Synthesis of compound Methanesulfonic acid 3-tert-butoxycarbonylamino-2-methyl-propyl ester (5):



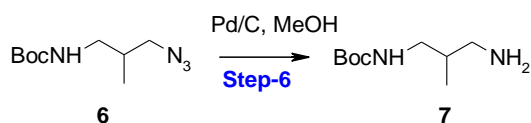
**Procedure:** To a solution of **(3-Hydroxy-2-methyl-propyl)-carbamic acid tert-butyl ester (4)** (530 mg, 2.804 mmol) in DCM (5 ml) were added  $\text{CH}_3\text{SO}_2\text{Cl}$  (0.303 ml, 4.206 mmol) and  $\text{Et}_3\text{N}$  (1.18 ml, 8.413 mmol) at 0 deg C. Reaction was stirred at RT for 3h. Reaction mass was diluted with sat  $\text{NaHCO}_3$  and extracted with EtOAc. Organic layer was washed with water, brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **Methanesulfonic acid 3-tert-butoxycarbonylamino-2-methyl-propyl ester (5)** as colorless sticky mass in 99.91% yield, 749 mg.

### Synthesis of compound (3-Azido-2-methyl-propyl)-carbamic acid tert-butyl ester (6):



**Procedure:** To a solution of **Methanesulfonic acid 3-tert-butoxycarbonylamino-2-methyl-propyl ester (5)** ( ) in DMF ( ) was added  $\text{NaN}_3$  ( ). The reaction was stirred at 80 deg C for 16h. Reaction mass was diluted with water and extracted with EtOAc. Organic layer was washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford **(3-Azido-2-methyl-propyl)-carbamic acid tert-butyl ester (6)** as colorless sticky mass in 99.82% yield, 600 mg.

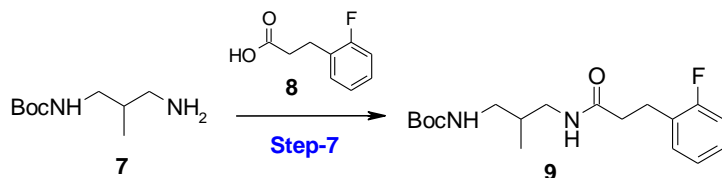
### Synthesis of compound (3-Amino-2-methyl-propyl)-carbamic acid tert-butyl ester (7):



**Procedure:** A solution of **(3-Azido-2-methyl-propyl)-carbamic acid tert-butyl ester (6)** (600 mg,

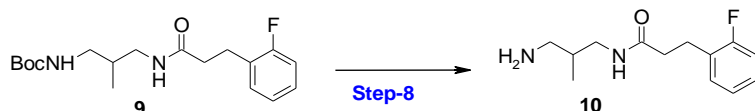
2.804 mmol) in MeOH (8 ml) was degassed with nitrogen. Then 10% Pd/C (100 mg) was added and the reaction was stirred at RT for 2h. Reaction mass was filtered through celite bed. Filtrate was concentrated under reduced pressure to afford **(3-Amino-2-methyl-propyl)-carbamic acid tert-butyl ester (7)** as colorless sticky mass in 56.83% yield, 300 mg.

**Synthesis of compound {3-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid tert-butyl ester (9):**



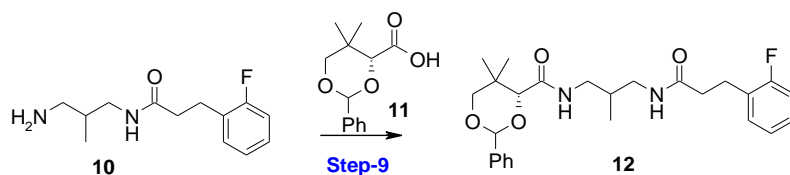
**Procedure:** To the stirred solution of **(3-Amino-2-methyl-propyl)-carbamic acid tert-butyl ester (7)** (2.5 g, 13.279 mmol) in THF (20 ml) were added DIPEA (7.137 ml, 39.836 mmol), HATU (7.57 g, 19.918 mmol) and **3-(2-Fluoro-phenyl)-propionic acid (8)** (2.23 g, 13.279 mmol). Reaction mixture was stirred at RT for 16h. Reaction mass was diluted with EtOAc, washed with satd NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and was concentrated under reduced pressure. Reaction mass was purified by silica gel (100-200 mesh) column chromatography using 2% MeOH in DCM to afford **{3-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid tert-butyl ester (9)** as colorless sticky mass in 10.9% yield, 490 mg.

**Synthesis of compound N-(3-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (10):**



**Procedure:** To stirred a solution of **{3-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid tert-butyl ester (9)** (490 mg, 1.448 mmol) in dioxane (2 ml) was added dioxane-HCl (4M, 4 ml) at 0°C and reaction mixture was stirred at RT for 16h. The reaction mixture was evaporated under reduced pressure to afford **N-(3-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (10)** as HCl salt in 86.31% yield, 389 mg.

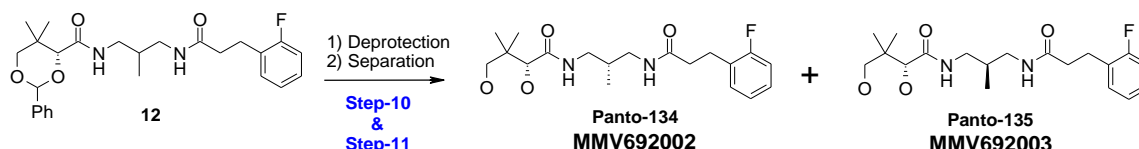
**Synthesis of compound (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {3-[3-(2-fluoro-phenyl)-propionylamino]-2-methyl-propyl}-amide (12):**



**Procedure:** To a solution of **N-(3-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (10)** (210 mg, 0.765 mmol) in DMF (4 ml) were added DIPEA (0.41 ml, 2.295 mmol), HATU (436 mg, 1.148

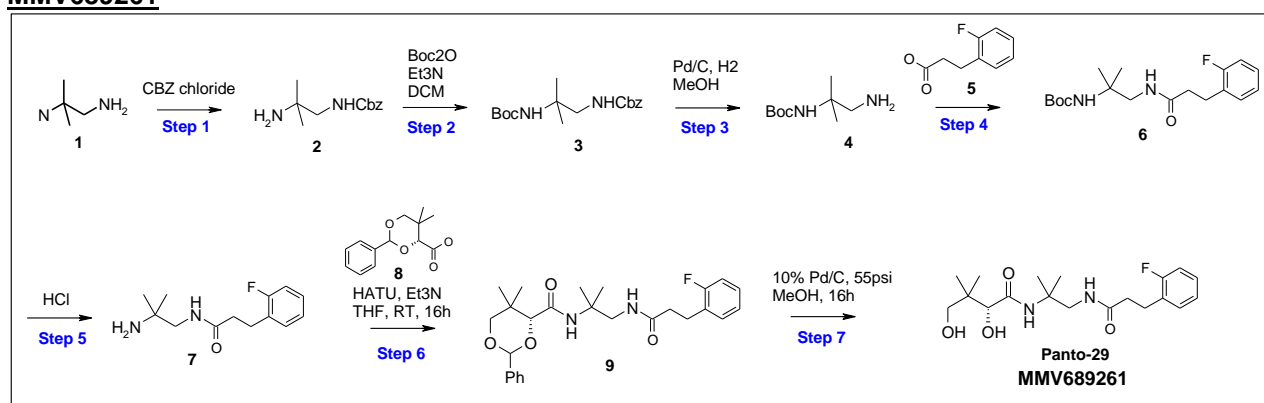
mmol) and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (11)** (180.55 mg, 0.765 mmol). Reaction was stirred at RT for 16h. Reaction mass was diluted with sat NaHCO<sub>3</sub> and extracted with EtOAc. Organic layer was washed with water and brine. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude mass which was purified by silica gel (100-200 mesh) column chromatography using 2% MeOH in DCM to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {3-[3-(2-fluoro-phenyl)-propionylamino]-2-methyl-propyl}-amide (12)** as colorless sticky mass in 42.37% yield, 148 mg.

#### Synthesis of compound MMV692002 and MMV692003:

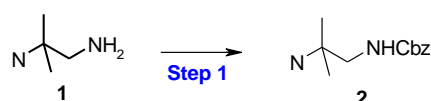


**Procedure:** To a solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {3-[3-(2-fluoro-phenyl)-propionylamino]-2-methyl-propyl}-amide (12)** (435 mg, 0.953 mmol) in MeOH (5 ml) was added 1N HCl (2 ml). Reaction was stirred at RT for 1h. MeOH was concentrated under reduced pressure, diluted with sat NaHCO<sub>3</sub> soln and extracted with EtOAc. Organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure which was purified by prep TLC using 2% MeOH in DCM to afford 180 mg fraction as a mixture of two diastereomers. 180 mg fraction was purified by Chiral prep HPLC (Column Name: Chiralpak ID (250x4.6mm) 5μ, ARD/K/Mobile phase: Hexane/IPA/TFA: 85/15/0.1, Flow Rate: 1.0 ml/min, Solubility: -Mobile phase) to afford 50 mg of peak 1 as off White Gummy Solid (first eluted fraction, registered as **MMV692002** with unknown absolute stereochemistry) and 55 mg of peak 2 off White Gummy Solid (second eluted fraction, registered as **MMV692003** with unknown absolute stereochemistry).

#### MMV689261-

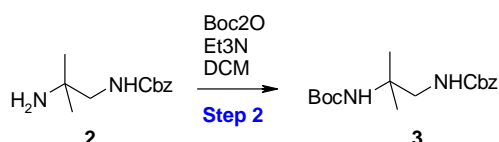


#### Synthesis of compound (2-Amino-2-methyl-propyl)-carbamic acid benzyl ester (2):



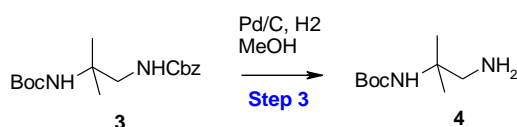
**Procedure:** To a solution of the **2-Methyl-propane-1,2-diamine (1)** (1.1 g, 12.479 mmol) in dichloromethane (10 ml) at 0°C was added benzyl chloroformate (1.79 ml, 12.479 mmol). The reaction mixture was stirred at RT for 16h. Then reaction mixture was basified by satd NaHCO<sub>3</sub> solution and was extracted with EtOAc, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by column chromatography (silica gel, 100-200 mesh, eluted at 9% MeOH-DCM) to afford **(2-Amino-2-methyl-propyl)-carbamic acid benzyl ester (2)** as brown gum in 39.66% yield, 1.1 g.

**Synthesis of compound (2-tert-Butoxycarbonylamino-2-methyl-propyl)-carbamic acid benzyl ester (3):**



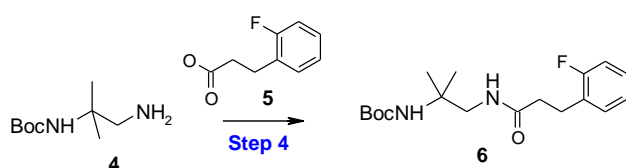
**Procedure:** To the stirred solution of **(2-Amino-2-methyl-propyl)-carbamic acid benzyl ester (2)** (560 mg, 2.523 mmol) in DCM (10 ml) was added Et<sub>3</sub>N (0.255 ml, 2.523 mmol) and followed by BOC anhydride (500 mg, 2.294 mmol). The reaction mixture was stirred at RT for 16h. Reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with 1N HCl, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to afford **(2-tert-Butoxycarbonylamino-2-methyl-propyl)-carbamic acid benzyl ester (3)** as brown gum in 99.53% yield, 736 mg.

**Synthesis of compound (2-Amino-1,1-dimethyl-ethyl)-carbamic acid tert-butyl ester (4):**



**Procedure:** To the stirred solution of **(2-tert-Butoxycarbonylamino-2-methyl-propyl)-carbamic acid benzyl ester (3)** (736 mg, 2.283 mmol) in MeOH (20 ml) was added 10% Pd/C (200 mg). The reaction mixture was stirred at RT under the hydrogen atmosphere (balloon pressure) for 16h. The reaction mixture was filtered and concentrated under reduced pressure to afford **(2-Amino-1,1-dimethyl-ethyl)-carbamic acid tert-butyl ester (4)** as gum in 97.48% yield, 500 mg.

**Synthesis of compound {2-[3-(2-Fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-carbamic acid tert-butyl ester (6):**

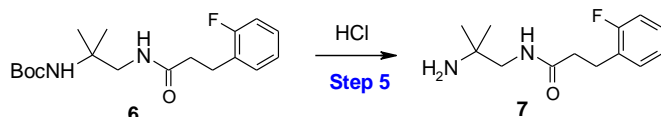


**Procedure:** To the stirred solution of **(2-Amino-1,1-dimethyl-ethyl)-carbamic acid tert-butyl ester**



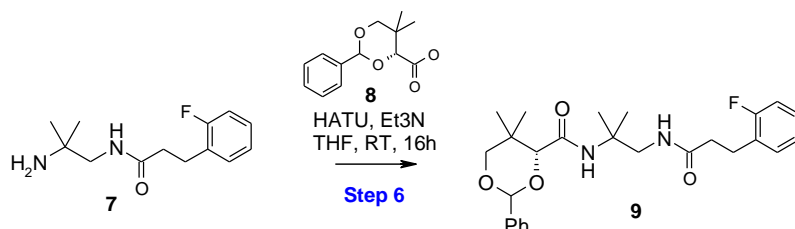
**(4)** (107.58 mg, 0.571 mmol) in THF (10 ml) were added Et<sub>3</sub>N (0.2 ml, 1.429 mmol), HATU (271.59 mg, 0.714 mmol) and **3-(2-Fluoro-phenyl)-propionic acid (5)** (80 mg, 0.476 mmol). Reaction mixture was stirred at RT for 16h. Then the reaction mixture was diluted with water and extracted with EtOAc. Organic layer was washed with satd NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by combiflash (12 g silica column, 20% EtOAc-DCM) to afford **{2-[3-(2-Fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-carbamic acid tert-butyl ester (6)** as off white solid in 68.26% yield, 110 mg.

#### Synthesis of N-(2-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (7):



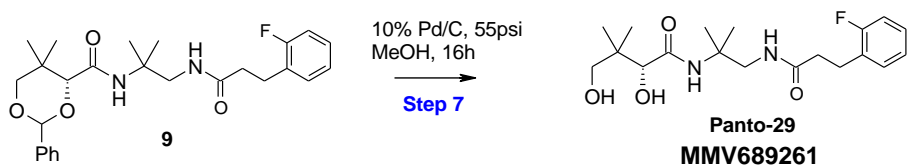
**Procedure:** To stirred a solution of **{2-[3-(2-Fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-carbamic acid tert-butyl ester (6)** (110 mg, 0.325 mmol) in dioxane (2 ml) was added dioxane-HCl (4M, 4 ml) at 0°C and reaction mixture was stirred at RT for 16h. The reaction mixture was evaporated under reduced pressure to afford **N-(2-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (7)** (HCl salt) as white solid in 98.52% yield, 88 mg.

#### Synthesis of (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-amide (9):



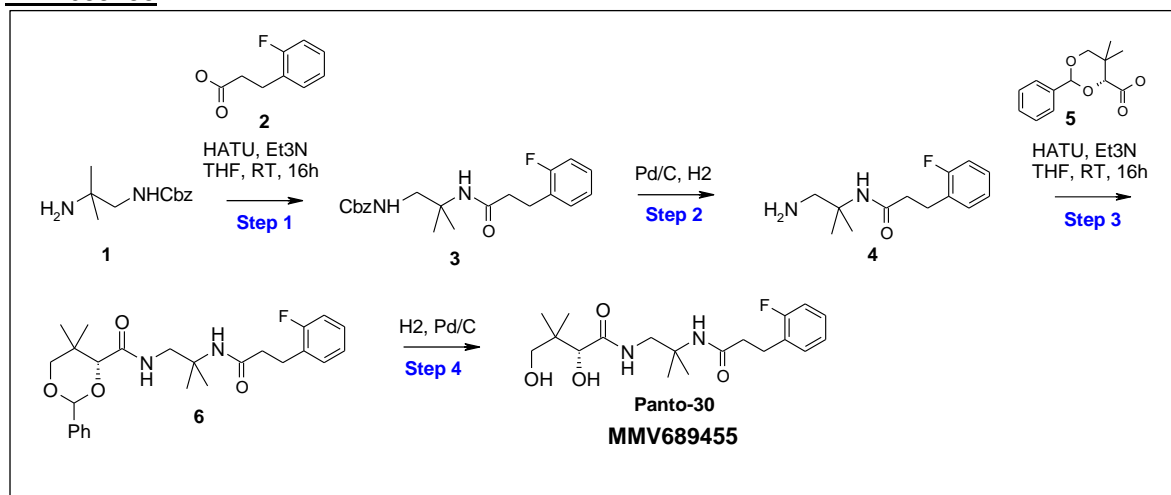
**Procedure:** To the stirred solution of **N-(2-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (7)** (83.83 mg, 0.305 mmol) in THF (20 ml) were added Et<sub>3</sub>N (0.177 ml, 1.271 mmol), HATU (145 mg, 0.381 mmol) and **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (8)** (60 mg, 0.254 mmol). It was stirred at RT for 16h. The reaction mixture was diluted with water and extracted with EtOAc. Organic layer was washed with satd NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by column chromatography (silica gel, 100-200 mesh, 3% MeOH-DCM) to afford **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-amide (9)** as gum in 96.49% yield, 112 mg.

#### Synthesis of MMV689261:

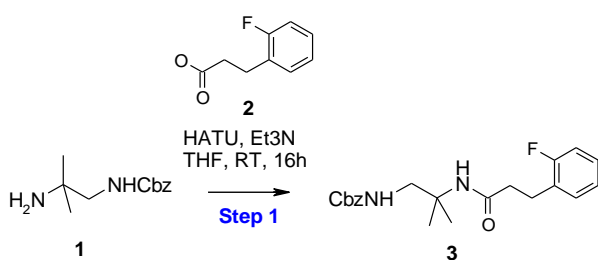


**Procedure:** A solution of **(R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2-fluorophenyl)-propionylamino]-1,1-dimethyl-ethyl}-amide (9)** (120 mg, 0.271 mmol) in MeOH (20 ml) was degassed with argon for 10 minutes. Pd/C (10%, 80 mg) was added and placed under H<sub>2</sub> atmosphere in a parr shaker at 50 psi for 16h. The reaction mixture was filtered, concentrated and purified by column chromatography (silica gel, 100-200 mesh, 4%MeOH-DCM) to afford **MMV689261** as gum in 60.06% yield, 60 mg.

#### **MMV689455-**

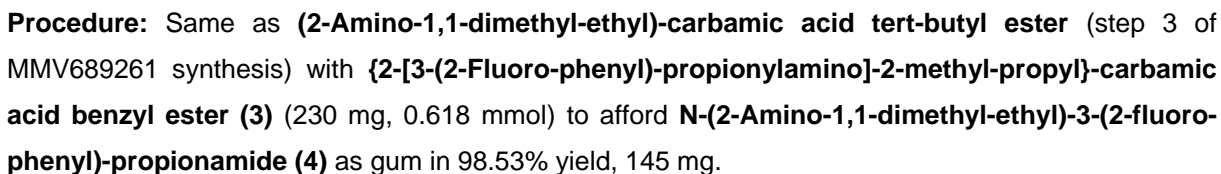


**Synthesis of compound {2-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid benzyl ester (3):**



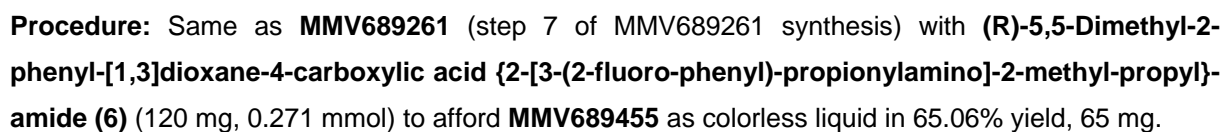
**Procedure:** Same as {2-[3-(2-Fluoro-phenyl)-propionylamino]-1,1-dimethyl-ethyl}-carbamic acid tert-butyl ester (step 4 of MMV689261 synthesis) with **(2-Amino-2-methyl-propyl)-carbamic acid benzyl ester (1)** (254 mg, 1.143 mmol) and **3-(2-Fluoro-phenyl)-propionic acid (2)** (160 mg, 0.952 mmol) to afford **{2-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid benzyl ester (3)** as gum in 81.76% yield, 290 mg.

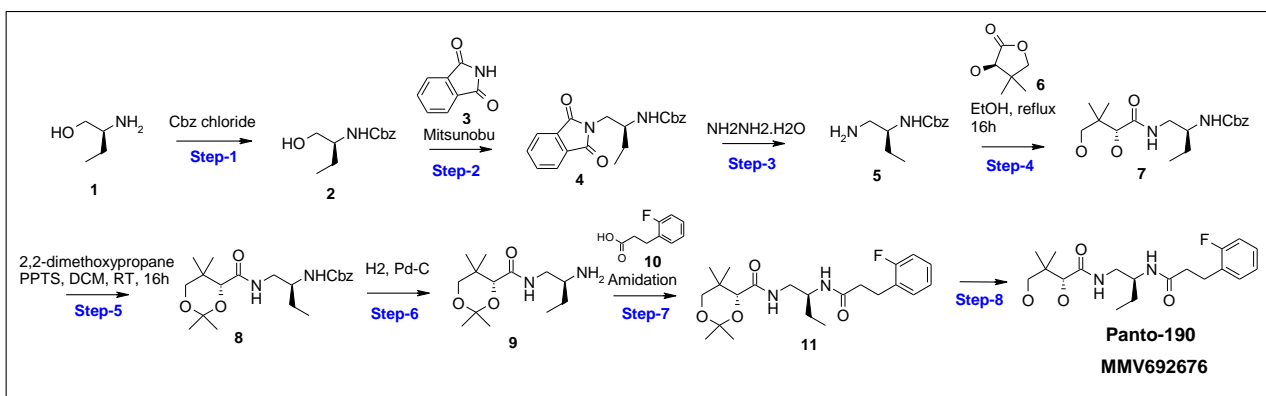
**Synthesis of compound N-(2-Amino-1,1-dimethyl-ethyl)-3-(2-fluoro-phenyl)-propionamide (4):**



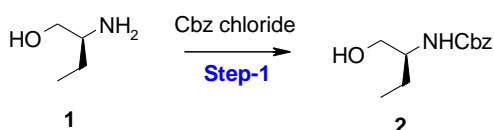
Reaction of compound **4** (N-(2-amino-2-methylpropyl)-2-(4-fluorophenyl)acetamide) with compound **5** (2-(2-((2-oxo-2-phenyl-1,3-dioxolane-5-ylidene)amino)propan-2-yl)acetic acid) in the presence of HATU, Et<sub>3</sub>N, THF, RT, 16h, yielding compound **6** (N-(2-((2-((2-oxo-2-phenyl-1,3-dioxolane-5-ylidene)amino)propan-2-yl)acetyl)amino)-2-methylpropyl)-2-(4-fluorophenyl)acetamide).

### Synthesis of MMV689455:

**MMV692676-**

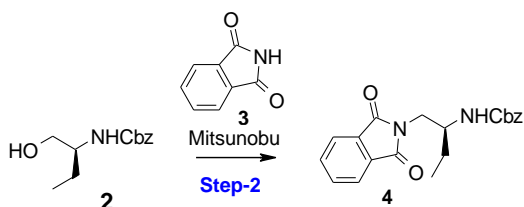


### Synthesis of compound ((S)-1-Hydroxymethyl-propyl)-carbamic acid benzyl ester (2):



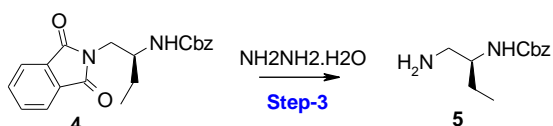
**Procedure:** To a solution of the **((S)-2-Amino-butan-1-ol (1)** (1 g, 11.218 mmol) in dichloromethane (20 ml) at 0°C was added benzylchloroformate (1.6 ml, 11.218 mmol) and triethylamine (1.876 ml, 13.462 mmol). The reaction mixture was stirred at RT for 16h. Reaction mixture was diluted with DCM, washed with satd NaHCO<sub>3</sub> solution, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified by column chromatography (silica gel, 100-200 mesh, 5% MeOH-DCM) to afford **((S)-1-Hydroxymethyl-propyl)-carbamic acid benzyl ester (2)** as gum in 55.94% yield, 1.4 g.

### Synthesis of compound [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (4):



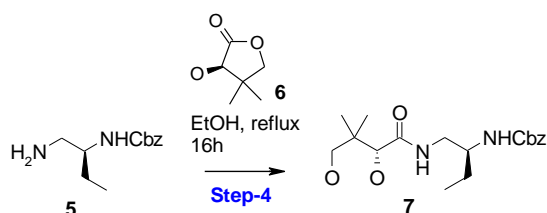
**Procedure:** To a stirred solution of **((S)-1-Hydroxymethyl-propyl)-carbamic acid benzyl ester (2)** (1.4 g, 6.691 mmol) in dry THF (15 ml) were added phthalimide (1.08 g, 7.36 mmol) and PPh<sub>3</sub> (1.93 g, 7.36 mmol). Then DEAD (1.15 ml, 7.36 mmol) was added drop wise to the reaction mixture at room temperature and stirred for 16h. The reaction mixture was then concentrated and the residue was purified by column chromatography (silica gel, 100-200 mesh) using 25%EtOAc-Hexane to afford **[(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (4)** as gum in 63.62% yield, 1.5 g.

### Synthesis of compound ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (5):



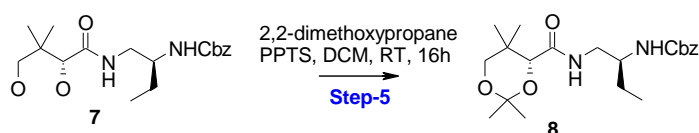
**Procedure:** To the stirred solution of [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (**4**) (1.5 g, 4.257 mmol) in MeOH (10 ml) was added hydrazine hydrate (3.3 ml, 68.106 mmol) and reaction mixture was heated at 50°C for 2h. Then reaction mixture was cooled to RT, filtered and concentrated under reduced pressure. The resulting residue was suspended in Et<sub>2</sub>O and filtered. The combined filtrates were concentrated under reduced pressure to afford ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (**5**) as gum in 99.35% yield, 940 mg.

**Synthesis of compound {(S)-1-[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl}-carbamic acid benzyl ester (**7**):**



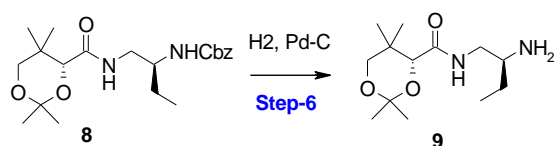
**Procedure:** To the stirred solution of ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (**5**) (940 mg, 4.229 mmol) and D-(-)-Pantolactone (**6**) (1.65 mg, 12.686 mmol) in ethanol (20 ml) was added triethyl amine (2.06 ml, 14.8 mmol). Reaction mass was refluxed for 16h. Reaction mixture was concentrated under reduced pressure. Crude was purified by column chromatography (silica gel, 100-200 mesh) using 4% MeOH-DCM to afford desired compound to afford {(S)-1-[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl}-carbamic acid benzyl ester (**7**) as gum in 49.25% yield, 734 mg.

**Synthesis of compound ((S)-1-[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl)-propyl)-carbamic acid benzyl ester (**8**):**



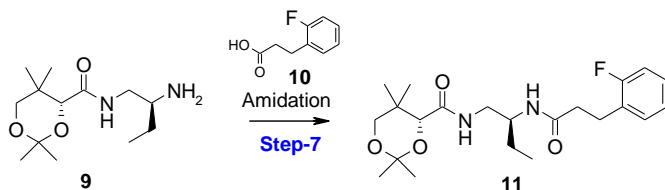
**Procedure:** To a solution of {(S)-1-[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl}-carbamic acid benzyl ester (**7**) (734 mg, 2.083 mmol) and pyridinium-p-toluenesulfonate (209 mg, 0.833 mmol) in dry DCM (20 ml) was added 2,2-dimethoxypropane (2.56 ml, 20.827 mmol) and stirred at RT for 16h. Then solvent was removed under reduced pressure and crude product was purified by silica gel (100-200 mesh) column chromatography by using 1%MeOH in DCM to afford ((S)-1-[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl)-propyl)-carbamic acid benzyl ester (**8**) as gum in 37.92% yield, 310 mg.

**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (**9**):**



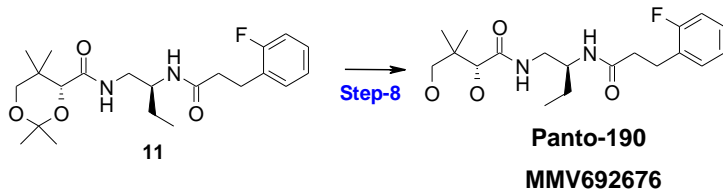
**Procedure:** To the stirred solution of ((S)-1-[[[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl]-amino]-methyl]-propyl)-carbamic acid benzyl ester (**8**) (310 mg, 0.79 mmol) in MeOH (20 ml) was added 10% Pd/C (80 mg). Reaction mixture was stirred at RT under the hydrogen atmosphere (balloon pressure) for 1h. Reaction mass was filtered and the filtrate was concentrated under reduced pressure to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (**9**) as gum in 93.11% yield, 190 mg.

**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-butyl}-amide (**11**):**



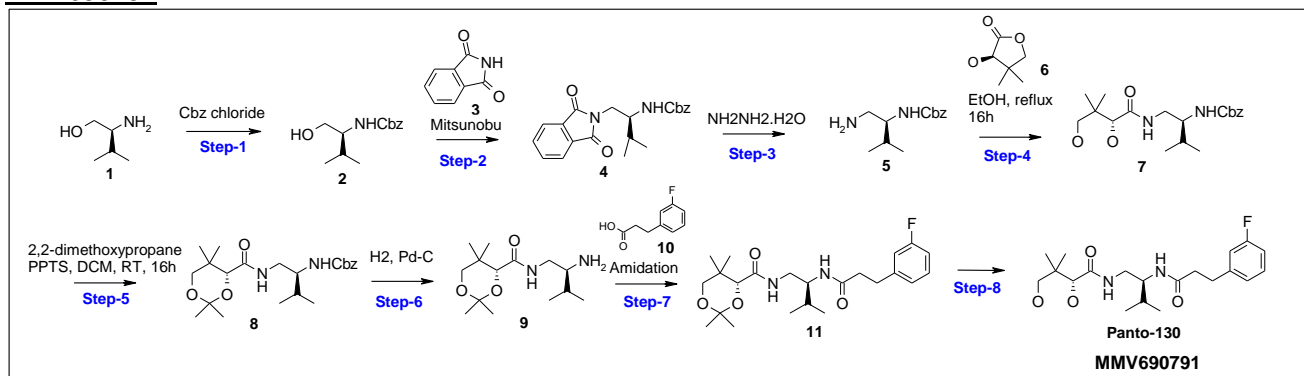
**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (**9**) (150 mg, 0.581 mmol) and 3-(2-Fluoro-phenyl)-propionic acid (**10**) (117.16 mg, 0.697 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-butyl}-amide (**11**) as gum in 96.98% yield, 230 mg.

**Synthesis of MMV692676:**

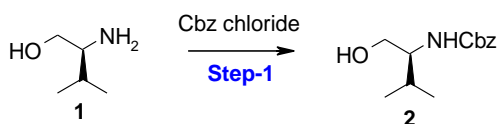


**Procedure:** Same as general procedure B with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-butyl}-amide (**11**) (230 mg, 0.563 mmol) to afford MMV692676 as colorless sticky gum in 60.26% yield, 125 mg.

#### MMV690791-

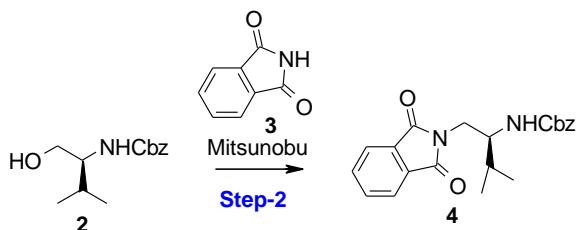


**Synthesis of compound ((S)-1-Hydroxymethyl-2-methyl-propyl)-carbamic acid benzyl ester (**2**):**



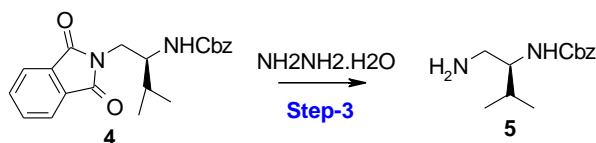
**Procedure:** Same as ((S)-1-Hydroxymethyl-propyl)-carbamic acid benzyl ester (step 1 of MMV692676 synthesis) with (S)-2-Amino-3-methyl-butan-1-ol (1) (3 g, 29.078 mmol) to afford ((S)-1-Hydroxymethyl-2-methyl-propyl)-carbamic acid benzyl ester (2) as gum in 72.17% yield, 4.98 g.

**Synthesis of compound [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-methyl-propyl]-carbamic acid benzyl ester (4):**



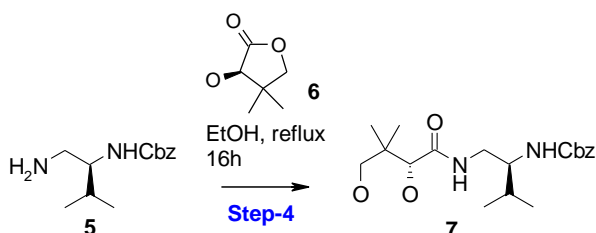
**Procedure:** Same as [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (step 2 of MMV692676 synthesis) with ((S)-1-Hydroxymethyl-2-methyl-propyl)-carbamic acid benzyl ester (2) (2 g, 8.439 mmol) to afford [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-methyl-propyl]-carbamic acid benzyl ester (4) as white solid in 66.62% yield, 2.06 g.

**Synthesis of compound ((S)-1-Aminomethyl-2-methyl-propyl)-carbamic acid benzyl ester (5):**



**Procedure:** Same as ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (step 3 of MMV692676 synthesis) with [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-methyl-propyl]-carbamic acid benzyl ester (4) (2.06 g, 5.622 mmol) to afford ((S)-1-Aminomethyl-2-methyl-propyl)-carbamic acid benzyl ester (5) as gum in 97.85% yield, 1.3 g.

**Synthesis of compound {(S)-1-[[((R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-2-methyl-propyl]-carbamic acid benzyl ester (7):**



**Procedure:** Same as {(S)-1-[[((R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl]-carbamic acid benzyl ester (step 4 of MMV692676 synthesis) with ((S)-1-Aminomethyl-2-methyl-

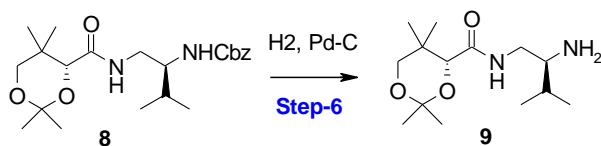
propyl)-carbamic acid benzyl ester (5) (500 mg, 2.116 mmol) to afford {(S)-1-[[[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-2-methyl-propyl]-carbamic acid benzyl ester (7) as gum in 79.97% yield, 620 mg.

**Synthesis of compound ((S)-2-Methyl-1-[[[(R)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl]-propyl)-carbamic acid benzyl ester (8):**



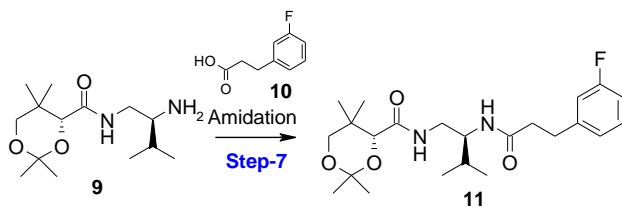
**Procedure:** Same as ((S)-1-[[[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl]-propyl)-carbamic acid benzyl ester (step 5 of MMV692676 synthesis) with {(S)-1-[[[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-2-methyl-propyl]-carbamic acid benzyl ester (7) (620 mg, 1.692 mmol) to afford ((S)-2-Methyl-1-[[[(R)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl]-propyl)-carbamic acid benzyl ester (8) as gum in 46.53 % yield, 320 mg.

**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-3-methyl-butyl)-amide (9):**



**Procedure:** Same as (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (step 6 of MMV692676 synthesis) with ((S)-2-Methyl-1-[[[(R)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl]-propyl)-carbamic acid benzyl ester (8) (520 mg, 1.279 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-3-methyl-butyl)-amide (9) as gum in 76.06% yield, 265 mg.

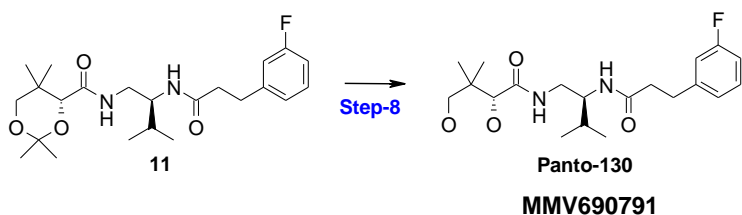
**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(3-fluoro-phenyl)-propionylamino]-3-methyl-butyl}-amide (11):**



**Procedure:** Same as general procedure A with (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-3-methyl-butyl)-amide (9) (265 mg, 0.973 mmol) and 3-(3-Fluoro-phenyl)-propionic acid (10) (196.33 mg, 1.167 mmol) to afford (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(3-fluoro-phenyl)-propionylamino]-3-methyl-butyl}-amide (11) as gum in 77.85% yield, 320 mg.

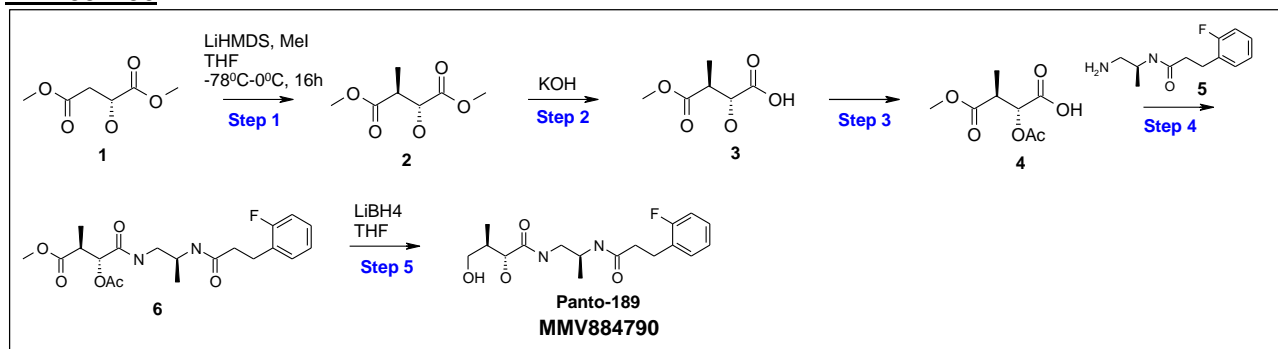
**Synthesis of MMV690791:**



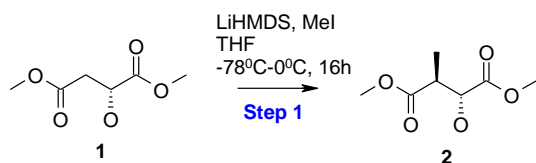


**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(3-fluoro-phenyl)-propionylamino]-3-methyl-butyl}-amide (11)** (320 mg, 0.757 mmol) to afford **MMV690791** as colorless gummy solid in 37.98% yield, 110 mg.

#### **MMV884790-**



#### **Synthesis of compound (2S,3R)-2-Hydroxy-3-methyl-succinic acid dimethyl ester (2):**



**Procedure:** To a stirred solution of **(R)-2-Hydroxy-succinic acid dimethyl ester (1)** (3 g, 18.503 mmol) in THF (30 ml) was added LHMDS (1M in THF, 46 ml) drop wise at -78°C and stirred at same temperature for 1h. Then MeI (1.735 ml, 27.754 mmol) was added and continued for another 3h at same temperature. Reaction was slowly allowed warm to 0°C and stirred for overnight at 0°C. Reaction mass was quenched with saturated ammonium chloride solution and extracted with EtOAc. Organic layer was separated, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford **(2S,3R)-2-Hydroxy-3-methyl-succinic acid dimethyl ester (2)** as gum in 76.7% yield, 2.5 g.

#### **Synthesis of compound (2S,3R)-2-Hydroxy-3-methyl-succinic acid 4-methyl ester (3):**



**Procedure:** To a stirred solution of **(2S,3R)-2-Hydroxy-3-methyl-succinic acid dimethyl ester (2)** (1.2 g, 6.812 mmol) in MeOH-H<sub>2</sub>O(7:1, 5 ml), was added KOH (572 mg, 10.218 mmol) at 0°C and allowed to stir at RT 25 min. Reaction was monitored by TLC. Reaction mass was acidified with 1(N) HCl and extracted with EtOAc. Organic layer was separated, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford **(2S,3R)-2-Hydroxy-3-methyl-succinic acid 4-methyl**

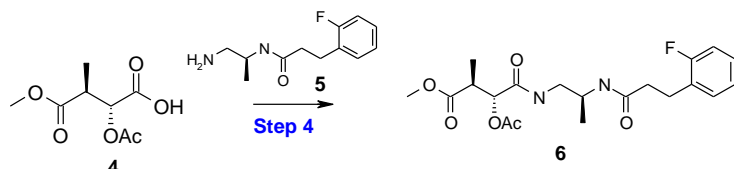
ester (**3**) as sticky solid in 90.54% yield, 1 g.

#### Synthesis of compound (2S,3R)-2-Acetoxy-3-methyl-succinic acid 4-methyl ester (**4**):



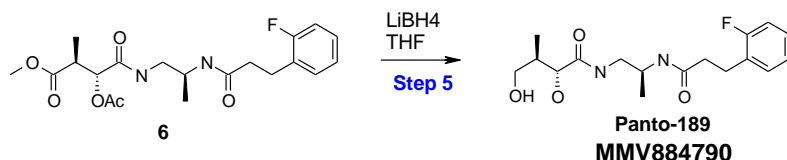
**Procedure:** To a stirred solution of (2S,3R)-2-Hydroxy-3-methyl-succinic acid 4-methyl ester (**3**) (1.2 g, 7.401 mmol) was added acetyl chloride (2.64 ml, 37.005 mmol) at RT and stirred at RT for overnight. Reaction was diluted with EtOAc and water. Organic layer was separated, wash with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford (2S,3R)-2-Acetoxy-3-methyl-succinic acid 4-methyl ester (**4**) as gum in 66.18% yield, 1 g.

#### Synthesis of compound (2S,3R)-3-Acetoxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-methyl-succinamic acid methyl ester (**6**):

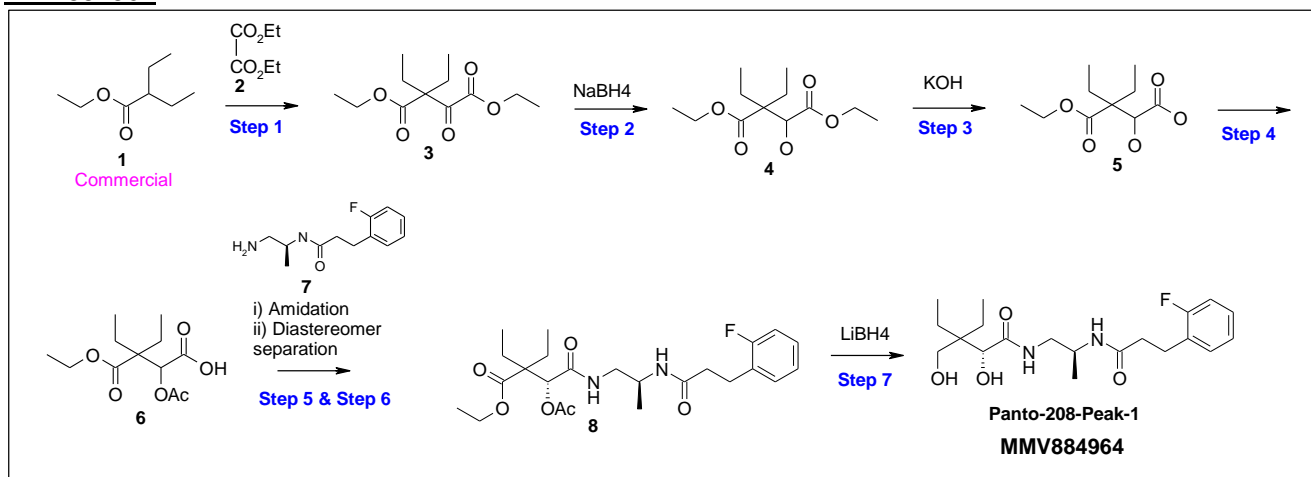
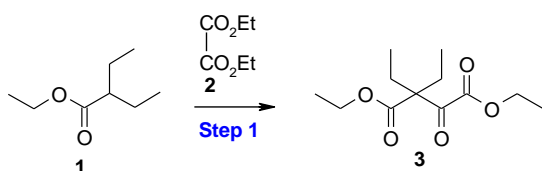


**Procedure:** To a stirred solution of (2S,3R)-2-Acetoxy-3-methyl-succinic acid 4-methyl ester (**4**) (1.2 g, 5.877 mmol) in THF (10 ml) were added Et<sub>3</sub>N (1.47 ml, 11.754 mmol) and CDI (1.43 gm 8.816 mmol) at RT and stirred for 1h. Then N-((S)-2-Amino-1-methyl-ethyl)-3-(2-fluoro-phenyl)-propionamide (**5**) (1.32 g, 5.877 mmol) was added and stirred at RT for overnight. Reaction was quenched with water and extracted with EtOAc. Organic layer was dried over sodium sulphate and concentrated under reduced pressure. Crude was purified by column chromatography (silica gel, 100-200 mesh) using 2% MeOH in DCM to afford (2S,3R)-3-Acetoxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-methyl-succinamic acid methyl ester (**6**) as gum in 10.36% yield, 250 mg.

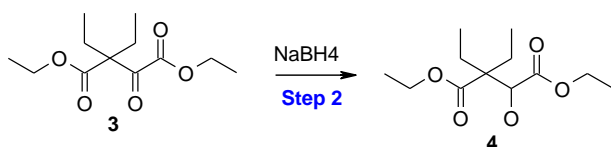
#### Synthesis of MMV884790:



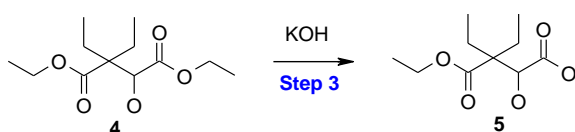
**Procedure:** To a solution of (2S,3R)-3-Acetoxy-N-((S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl)-2-methyl-succinamic acid methyl ester (**6**) (80 mg, 0.195 mmol) in THF (1 ml) at 0°C was added LiBH<sub>4</sub> solution (2M in THF, 0.1 ml). Then the reaction mixture was stirred at RT for 2 hrs. The reaction mixture was quenched with NH<sub>4</sub>Cl solution, filtered through celite bed and washed with ethyl acetate. The filtrate was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Crude was purified over prep TLC (5% MeOH-DCM) to afford MMV884790 as off white solid in 18.09% yield, 12 mg.

**MMV884964-****Synthesis of compound 2,2-Diethyl-3-oxo-succinic acid diethyl ester (3):**

**Procedure:** To a solution of LDA (2M in THF, 17 ml) in dry THF (50 ml) at  $-78^{\circ}\text{C}$  was added slowly **2-ethyl-butyrac acid ethyl ester (1)** (5 g, 34.669 mmol) and stirred for one hour at that temperature. Then **oxalic acid diethyl ester (2)** (5.07 g, 34.669 mmol) was added at  $-78^{\circ}\text{C}$  and stirred at same temperature for 1h. Then reaction mixture was slowly allowed to warm up to  $-20^{\circ}\text{C}$ . Acetic acid was added at  $-20^{\circ}\text{C}$  and warm up to RT. Reaction mixture was quenched with water and extracted with EtOAc. Organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure to afford **2,2-Diethyl-3-oxo-succinic acid diethyl ester (3)** as gum in 59.04% yield, 5 g.

**Synthesis of compound 2,2-Diethyl-3-hydroxy-succinic acid diethyl ester (4):**

**Procedure:** To a stirred solution of **2,2-Diethyl-3-oxo-succinic acid diethyl ester (3)** (3 g, 13.376 mmol) in THF (15 ml) was added  $\text{NaBH}_4$  (1.01 g, 26.751 mmol) at RT and stirred for 8h. Reaction was quenched with water and extracted with EtOAc. Organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford **2,2-Diethyl-3-hydroxy-succinic acid diethyl ester (4)** as gum in 88.03% yield, 2.9 g.

**Synthesis of compound 2,2-Diethyl-3-hydroxy-succinic acid 1-ethyl ester (5):**

**Procedure:** Same as **(2S,3R)-2-Hydroxy-3-methyl-succinic acid 4-methyl ester** (step 2 of MMV884790 synthesis) with **2,2-Diethyl-3-hydroxy-succinic acid diethyl ester (4)** (1.3 g, 5.278

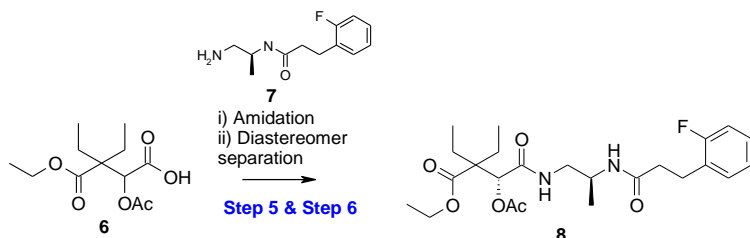
mmol) to afford **2,2-Diethyl-3-hydroxy-succinic acid 1-ethyl ester (5)** as gum in 73.79% yield, 850 mg.

#### Synthesis of compound **3-Acetoxy-2,2-diethyl-succinic acid 1-ethyl ester (6)**:



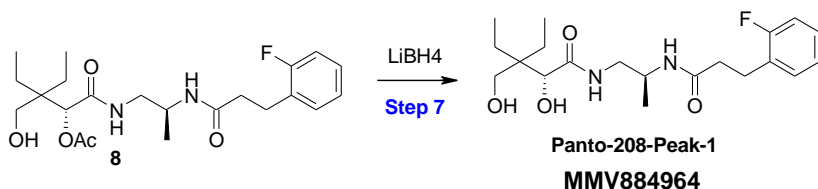
**Procedure:** Same as **(2S,3R)-2-Acetoxy-3-methyl-succinic acid 4-methyl ester** (step 3 of MMV884790 synthesis) with **2,2-Diethyl-3-hydroxy-succinic acid 1-ethyl ester (5)** (1 g, 4.582 mmol) to afford **3-Acetoxy-2,2-diethyl-succinic acid 1-ethyl ester (6)** as gum in 58.7 % yield, 700 mg.

#### Synthesis of compound **Acetic acid (R)-2-ethyl-1-[(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl]-2-hydroxymethyl-butyl ester (8)**:



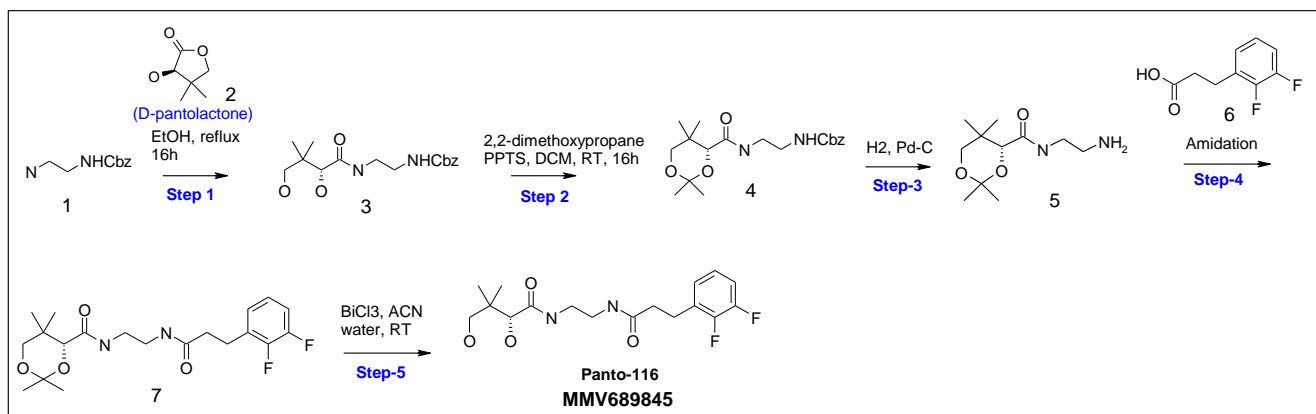
**Procedure:** To a stirred solution of **3-Acetoxy-2,2-diethyl-succinic acid 1-ethyl ester (6)** (1.1 g, 4.226 mmol) in THF (10 ml) were added Et<sub>3</sub>N (1.625 ml, 12.678 mmol), HATU (2.41 g, 6.339 mmol) and **N-[(S)-2-Amino-1-methyl-ethyl]-3-(2-fluoro-phenyl)-propionamide (7)** (0.948 g, 4.226 mmol). The reaction mixture was stirred at room temp for 16h. Then reaction mixture was quenched with water and extracted with EtOAc. Organic layer was dried over sodium sulphate and concentrated under reduced pressure. Crude material was purified by column chromatography (silica gel, 100-200 mesh, 30% EtOAc-Hexane) to afford 900 mg of mixture of diastereomers as a gummy liquid. The fraction was purified by prep HPLC (COLUMN NAME : CHIRALPAK IE (21.0 x 250 mm ,5 μ), FLOW RATE : 21.0 ml/min, MOBILE PHASE : HEX/IPA/FA:75/25/0.1, SOLUBILITY : MeOH/MOBILE PHASE) to afford **Acetic acid (R)-2-ethyl-1-[(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl]-2-hydroxymethyl-butyl ester (8)** as gum in 11.14% yield, 200 mg.

#### Synthesis of MMV884964:

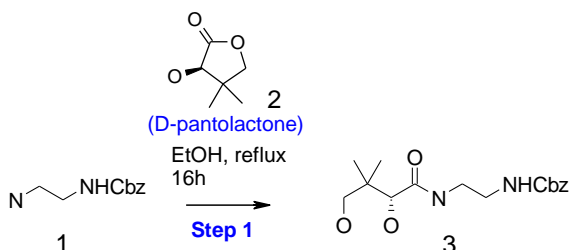


**Procedure:** Same as **MMV884790** (step 5 of MMV884790 synthesis) with **Acetic acid (R)-2-ethyl-1-[(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propylcarbamoyl]-2-hydroxymethyl-butyl ester (8)** (180 mg, 0.386 mmol) to afford **MMV884964** as colorless gum in 7.45% yield, 11 mg.

## MMV689845-

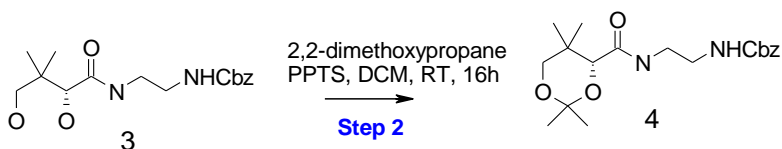


### Synthesis of compound [2-((R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-ethyl]-carbamic acid benzyl ester (3):



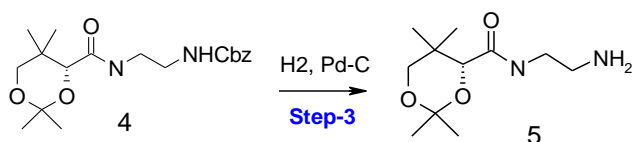
**Procedure:** Same as ((S)-1-[[[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-methyl]-propyl]-carbamic acid benzyl ester (step 4 of MMV692676 synthesis) with (2-Amino-ethyl)-carbamic acid benzyl ester (1) (10 g, 51.483 mmol) to afford [2-((R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-ethyl]-carbamic acid benzyl ester (3) as gum in 77.85% yield, 13 g.

### Synthesis of compound {2-[[[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl]-carbamic acid benzyl ester (4):



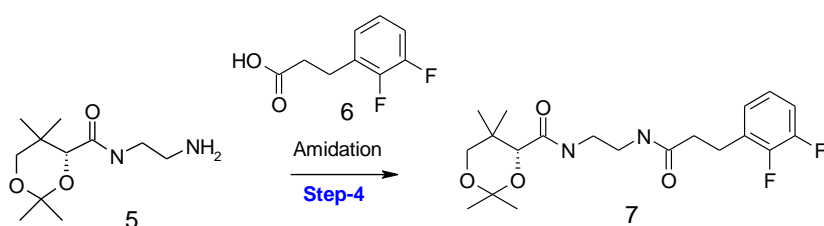
**Procedure:** Same as ((S)-1-[[[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl]-propyl)-carbamic acid benzyl ester (step 5 of MMV692676 synthesis) with [2-((R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-ethyl]-carbamic acid benzyl ester (3) (13.7 g, 42.284 mmol) to afford {2-[[[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl]-carbamic acid benzyl ester (4) as gum in 64.89% yield, 10 g.

### Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid (2-amino-ethyl)-amide (5):



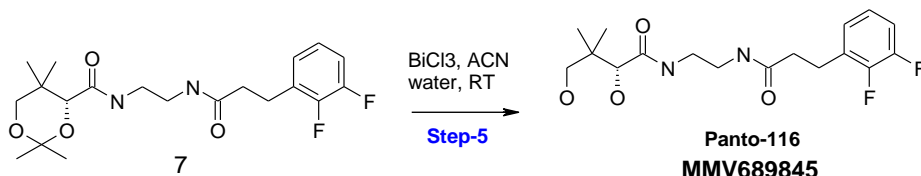
**Procedure:** Same as **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide** (step 6 of MMV692676 synthesis) with **{2-[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (4)** (10 g, 28.409 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid (2-amino-ethyl)-amide (5)** as gum in 99.35% yield, 6.5 g.

**Synthesis of compound (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2,3-difluoro-phenyl)-propionylamino]-ethyl}-amide (7):**



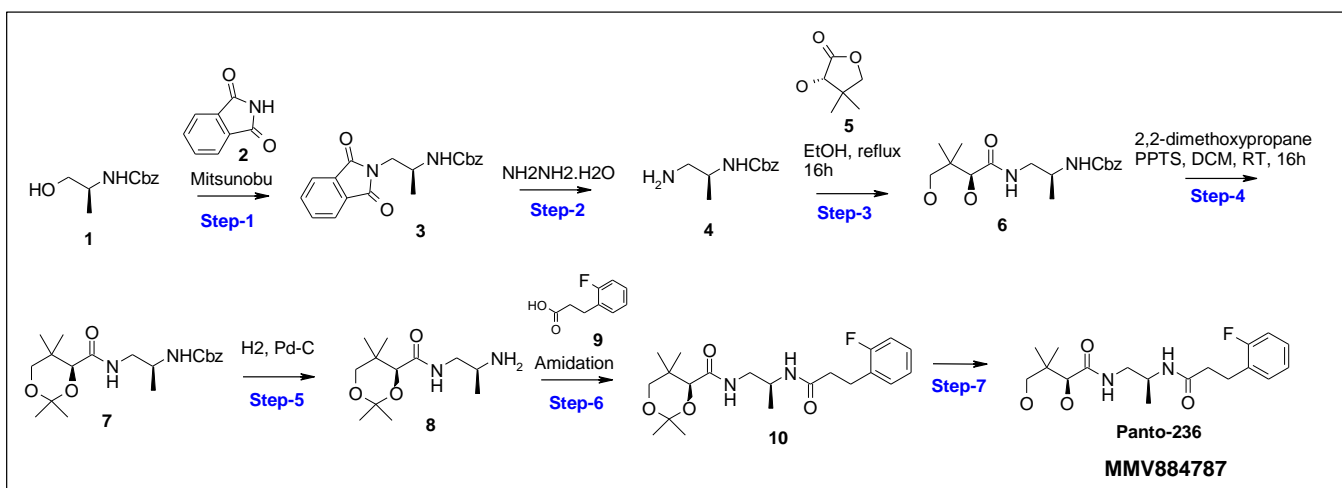
**Procedure:** Same as general procedure A with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid (2-amino-ethyl)-amide (5)** (125 mg, 0.543 mmol) and **3-(2,3-Difluoro-phenyl)-propionic acid (6)** (121.24 mg, 0.651 mmol) to afford **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2,3-difluoro-phenyl)-propionylamino]-ethyl}-amide (7)** as gum in 36.99% yield, 80 mg.

**Synthesis of MMV689845:**

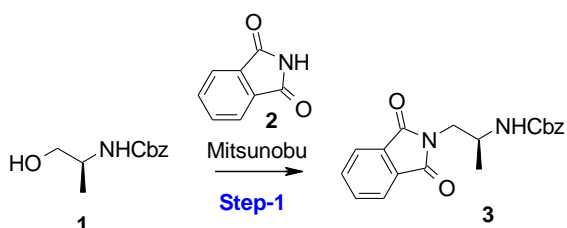


**Procedure:** Same as general procedure B with **(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {2-[3-(2,3-difluoro-phenyl)-propionylamino]-ethyl}-amide (7)** (80 mg, 0.201 mmol) to afford **MMV689845** as colorless gum in 34.74% yield, 25 mg.

**MMV884787-**

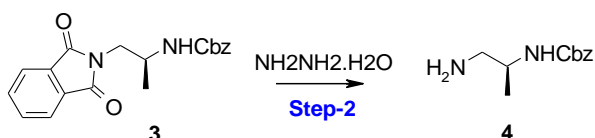


**Synthesis of compound [(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (3):**



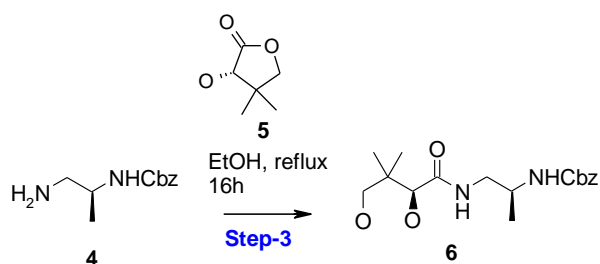
**Procedure:** Same as [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (step 2 of MMV692676 synthesis) with ((S)-2-Hydroxy-1-methyl-ethyl)-carbamic acid benzyl ester (1) (10 g, 47.79 mmol) to afford [(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (3) as off white solid in 87.82% yield, 14.2 g.

**Synthesis of compound ((S)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (4):**



**Procedure:** Same as ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (step 3 of MMV692676 synthesis) with [(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid benzyl ester (3) (14.2 g, 40.296 mmol) to afford ((S)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (4) as gum in 98.9% yield, 8.3 g.

**Synthesis of compound [(S)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (6):**



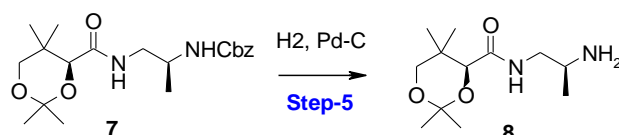
**Procedure:** Same as {(S)-1-[(R)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino]-methyl}-propyl-carbamic acid benzyl ester (step 4 of MMV692676 synthesis) with ((S)-2-Amino-1-methyl-ethyl)-carbamic acid benzyl ester (4) (250 mg, 1.2 mmol) and L-(+)-Pantolactone (5) (468.67 mg, 3.601 mmol) to afford [(S)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (6) as gum in 53% yield, 215 mg.

**Synthesis of compound {(S)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (7):**



**Procedure:** Same as ((S)-1-[(R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-methyl-propyl-carbamic acid benzyl ester (step 5 of MMV692676 synthesis) with [(S)-2-((S)-2,4-Dihydroxy-3,3-dimethyl-butyrylamino)-1-methyl-ethyl]-carbamic acid benzyl ester (6) (215 mg, 0.635 mmol) to afford {(S)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (7) as gum in 91.5% yield, 220 mg.

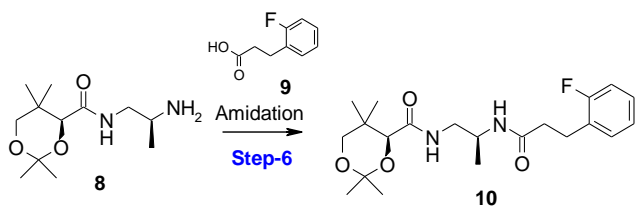
**Synthesis of compound (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (8):**



**Procedure:** Same as (R)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-butyl)-amide (step 6 of MMV692676 synthesis) with {(S)-1-Methyl-2-[(S)-2,2,5,5-tetramethyl-[1,3]dioxane-4-carbonyl)-amino]-ethyl}-carbamic acid benzyl ester (7) (220 mg, 0.561 mmol) to afford (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (8) as gum in 87.62% yield, 120 mg.

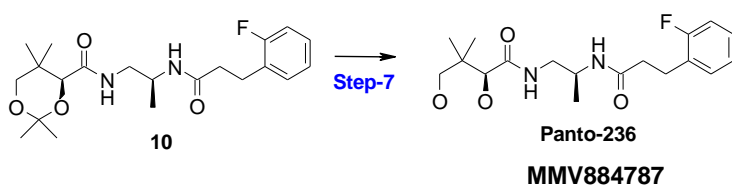
**Synthesis of compound (S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (10):**





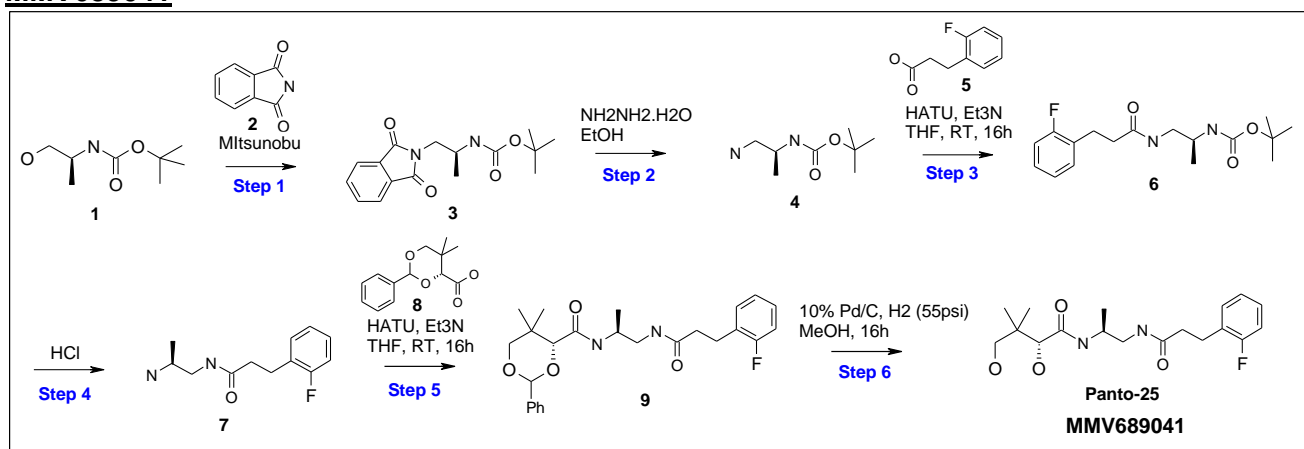
**Procedure:** Same as general procedure A with **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid ((S)-2-amino-propyl)-amide (8)** (130 mg, 0.532 mmol) and **3-(2-Fluoro-phenyl)-propionic acid (9)** (107.33 mg, 0.638 mmol) to afford **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (10)** as colorless sticky solid in 69.59% yield, 146 mg.

#### Synthesis of MMV884787:

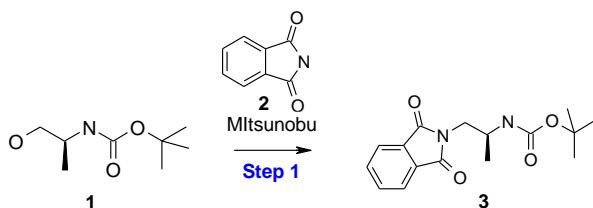


**Procedure:** Same as general procedure B with **(S)-2,2,5,5-Tetramethyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-propyl}-amide (10)** (146 mg, 0.37 mmol) to afford **MMV884787** as colorless sticky solid in 30.5% yield, 40 mg.

#### MMV689041-



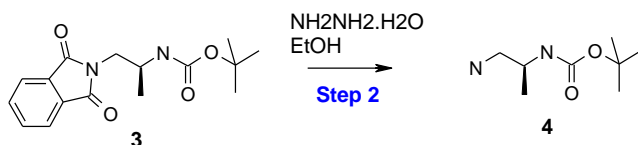
#### Synthesis of compound [(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid tert-butyl ester (3):



**Procedure:** Same as [(S)-1-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-propyl]-carbamic acid benzyl ester (step 2 of MMV692676 synthesis) with **((S)-2-Hydroxy-1-methyl-ethyl)-carbamic acid tert-butyl ester (1)** (500 mg, 2.853 mmol) to afford **[(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-**

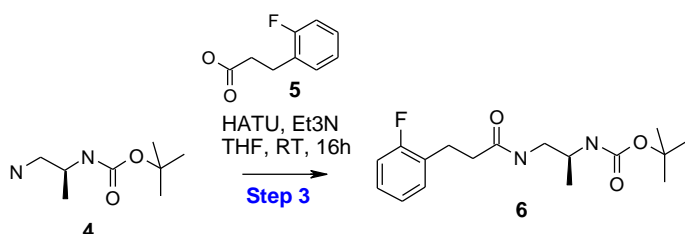
methyl-ethyl]-carbamic acid tert-butyl ester (3) as off white solid in 97.88% yield, 850 mg.

**Synthesis of compound ((S)-2-Amino-1-methyl-ethyl)-carbamic acid tert-butyl ester (4):**



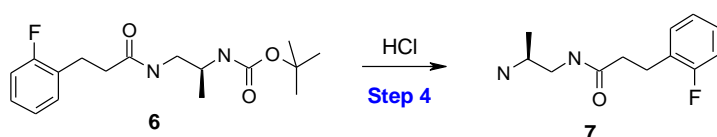
**Procedure:** Same as ((S)-1-Aminomethyl-propyl)-carbamic acid benzyl ester (step 3 of MMV692676 synthesis) with [(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-1-methyl-ethyl]-carbamic acid tert-butyl ester (3) (500 mg, 1.645 mmol) to afford ((S)-2-Amino-1-methyl-ethyl)-carbamic acid tert-butyl ester (4) as gum in 99.45% yield, 285 mg.

**Synthesis of compound {(S)-2-[3-(2-Fluoro-phenyl)-propionylamino]-1-methyl-ethyl}-carbamic acid tert-butyl ester (6):**



**Procedure:** Same as {3-[3-(2-Fluoro-phenyl)-propionylamino]-2-methyl-propyl}-carbamic acid tert-butyl ester (Step 7 of MMV692002 synthesis) with ((S)-2-Amino-1-methyl-ethyl)-carbamic acid tert-butyl ester (4) (279.64 mg, 1.607 mmol) and 3-(2-Fluoro-phenyl)-propionic acid (5) (135 mg, 0.804 mmol) to afford {(S)-2-[3-(2-Fluoro-phenyl)-propionylamino]-1-methyl-ethyl}-carbamic acid tert-butyl ester (6) as off white solid in 95.91% yield, 250 mg.

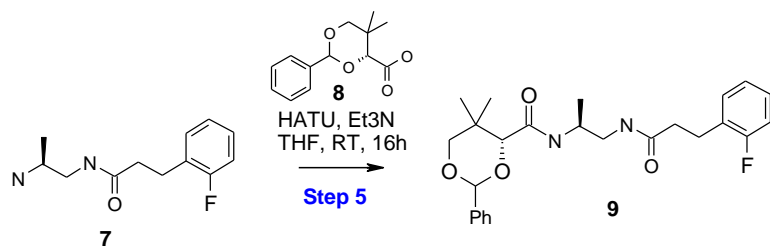
**Synthesis of compound N-((S)-2-Amino-propyl)-3-(2-fluoro-phenyl)-propionamide (7):**



**Procedure:** Same as N-(3-Amino-2-methyl-propyl)-3-(2-fluoro-phenyl)-propionamide (Step 8 of MMV692002 synthesis) with {(S)-2-[3-(2-Fluoro-phenyl)-propionylamino]-1-methyl-ethyl}-carbamic acid tert-butyl ester (6) (250 mg, 0.772 mmol) to afford N-((S)-2-Amino-propyl)-3-(2-fluoro-phenyl)-propionamide (7, HCl salt) as off white solid in 92.46% yield, 160 mg.

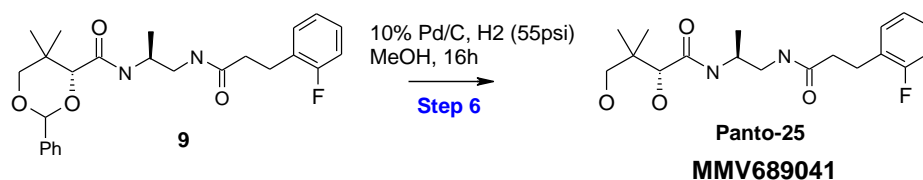
**Synthesis of compound (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-**

**fluoro-phenyl)-propionylamino]-1-methyl-ethyl)-amide (9):**



**Procedure:** Same as (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {3-[3-(2-fluoro-phenyl)-propionylamino]-2-methyl-propyl}-amide (Step 9 of MMV692002 synthesis) with N-((S)-2-Amino-propyl)-3-(2-fluoro-phenyl)-propionamide (**7**) (154.65 mg, 0.593 mmol) and (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid (**8**) (70 mg, 0.297 mmol) to afford (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-1-methyl-ethyl)-amide (**9**) as colorless gum in 76.19% yield, 100 mg.

#### Synthesis of MMV689041:



**Procedure:** Same as MMV689261 (Step 7 of MMV689261 synthesis) with (R)-5,5-Dimethyl-2-phenyl-[1,3]dioxane-4-carboxylic acid {(S)-2-[3-(2-fluoro-phenyl)-propionylamino]-1-methyl-ethyl)-amide (**9**) (120 mg, 0.271 mmol) to afford MMV689041 as colorless gum in 83.24% yield, 80 mg.

## **Quality control: <sup>1</sup>H-NMR and HRMS data of compounds from Table S1**

Pantothenamide\_NMR and LCMS

### **MMV884957:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.92 (brs, 1H), 7.34-7.26 (m, 2H), 7.18-7.13 (m, 2H), 5.52 (d, J = 5.24Hz, 1H), 4.45 (brs, 1H), 3.75 (d, J = 5.24Hz, 1H), 3.62-3.58 (m, 1H), 3.26-3.16 (m, 6H), 2.78 (t, J = 7.48Hz, 2H), 2.01-1.95 (m, 2H), 1.19 (d, J = 6.68Hz, 3H), 0.82 (s, 3H), 0.79 (s, 3H).

LCMS (HCOOH:ACN): M+H=390, R<sub>t</sub>=1.47 min in 3 mins run.

### **MMV884789:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 13.31 (brs, 1H), 7.79-7.69 (m, 1H), 7.28-7.24 (m, 2H), 7.16-7.07 (m, 2H), 5.39 (d, J = 5.48Hz, 1H), 4.47 (t, J = 5.48Hz, 1H), 3.71 (d, J = 5.48Hz, 1H), 3.42-3.35 (m, 1H), 3.32-3.25 (m, 2H), 3.18-3.14 (m, 1H), 2.98-90 (m, 5H), 1.17 (d, J = 6.64Hz, 3H), 0.77 (s, 3H), 0.74 (s, 3H).

LCMS (HCOOH:ACN): M+H=379, R<sub>t</sub>=1.37 min in 3 mins run.

### **MMV692002:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.83 (brs, 1H), 7.74 (brs, 1H), 7.29-7.22 (m, 2H), 7.14-7.07 (m, 2H), 5.38 (d, J = 5.56Hz, 1H), 4.47 (t, J = 5.44Hz, 1H), 3.72 (d, J = 5.56Hz, 1H), 3.31-3.30 (m, 1H), 3.20-3.16 (m, 1H), 2.96-2.90 (m, 3H), 2.85-2.78 (m, 3H), 2.41-2.37 (m, 2H), 1.70-1.62 (m, 1H), 0.81 (s, 3H), 0.80 (s, 3H), 0.72 (d, J = 6.68Hz, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=369, R<sub>t</sub>=2.71 min in 5 mins run.

### **MMV692003:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.83 (brs, 1H), 7.74 (brs, 1H), 7.29-7.22 (m, 2H), 7.14-7.07 (m, 2H), 5.38 (d, J = 5.56Hz, 1H), 4.47 (t, J = 5.44Hz, 1H), 3.72 (d, J = 5.56Hz, 1H), 3.31-3.30 (m, 1H), 3.20-3.16 (m, 1H), 2.96-2.90 (m, 3H), 2.85-2.78 (m, 3H), 2.41-2.37 (m, 2H), 1.70-1.62 (m, 1H), 0.81 (s, 3H), 0.80 (s, 3H), 0.72 (d, J = 6.68Hz, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=369, R<sub>t</sub>=2.67 min in 5 mins run.

### **MMV689041:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.86 (t, J = 5.48Hz, 1H), 7.46 (d, J = 8.28Hz, 1H), 7.28-7.21 (m, 2H), 7.14-7.08 (m, 2H), 5.33 (d, J = 5.52Hz, 1H), 4.47 (t, J = 5.52Hz, 1H), 3.90-3.83 (m, 1H), 3.68 (d, J = 5.52Hz, 1H), 3.28-3.26 (m, 1H), 3.19-3.15 (m, 1H), 3.13-3.08 (m, 1H), 3.05-2.99 (m, 1H), 2.81 (t, J = 7.68Hz, 2H), 2.35 (t, J = 7.68Hz, 2H), 0.96 (d, J = 6.64Hz, 3H), 0.79 (s, 6H).

LCMS (HCOOH:ACN): M+H=355, R<sub>t</sub>=1.38 min in 3 mins run.

### **MMV689261:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.99 (brs, 1H), 7.30-7.24 (m, 3H), 7.15-7.08 (m, 2H), 5.23 (d, J = 5.44Hz, 1H), 4.48 (t, J = 5.32, 1H), 3.59 (d, J = 5.44Hz, 1H), 3.27-3.23 (m, 2H), 3.18-3.12 (m, 2H), 2.84 (t, J = 7.52Hz, 2H), 2.41 (t, J = 7.5Hz, 2H), 1.19 (s, 6H), 0.79 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=369, R<sub>t</sub>=1.44 min in 3 mins run.

### **MMV692676:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.66-7.59 (m, 2H), 7.27-7.20 (m, 2H), 7.14-7.10 (m, 2H), 5.38 (d, J = 5.44Hz, 1H), 4.46 (brs, 1H), 3.77-3.70 (m, 2H), 7.32-7.31 (m, 1H), 3.18-3.15 (m, 2H), 3.04-3.01 (m, 1H), 2.82 (t, J = 7.48Hz, 2H), 2.34 (t, J = 7.48Hz, 2H), 1.44-1.36 (m, 1H), 1.27-1.21 (m, 1H), 0.79-0.71 (m, 9H).

LCMS (HCOOH:ACN): M+H=369, R<sub>t</sub>=1.41 min in 3 mins run.

### **MMV689455:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.77 (t, J = 6.12Hz, 1H), 7.58 (s, 1H), 7.28-7.21 (m, 2H), 7.14-7.085 (m, 2H), 5.48 (d, J = 5.44Hz, 1H), 4.48 (t, J = 5.48, 1H), 3.76 (d, J = 5.44Hz, 1H), 3.34-3.31 (m, 2H), 3.20-3.12 (m, 2H), 2.80 (t, J = 7.64Hz, 2H), 2.30 (t, J = 7.64Hz, 2H), 1.18 (s, 3H), 1.16 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H).

LCMS (HCOOH:ACN): M+H=369, R<sub>t</sub>=1.47 min in 3 mins run.

### **MMV690791:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.55 (d, J = 9.4Hz, 1H), 7.49 (t, J = 4.88Hz, 1H), 7.32-7.26 (m, 1H), 7.05-6.96 (m, 3H), 5.38 (d, J = 5.56Hz, 1H), 4.45 (t, J = 5.56Hz, 1H), 3.75-3.69 (m, 2H), 3.29-3.27 (m, 1H), 3.18-3.06 (m, 3H), 2.86-2.80 (m, 2H), 2.45-2.23 (m, 2H), 1.67-1.62 (m, 1H), 0.83-0.70 (m, 12H).

LCMS (HCOOH:ACN): M+H=383, R<sub>t</sub>=1.47 min in 3 mins run.

**MMV1545511:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.75-7.68 (m, 2H), 7.30-7.20 (m, 2H), 7.18-7.10 (m, 2H), 3.86-3.80 (m, 2H), 3.75 (brs, 1H), 3.20-3.12 (m, 1H), 3.08-2.93 (m, 3H), 2.79 (t, J = 8.32Hz, 2H), 2.32 (t, J = 8.32Hz, 2H), 0.95 (d, J = 6.2Hz, 3H), 0.83-0.79 (m, 6H).

LCMS (HCOOH:ACN): M+H=354, R<sub>t</sub>=1.35 min in 3 mins run.

**MMV1558163:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.75-7.70 (m, 2H), 7.28-7.20 (m, 2H), 7.14-7.10 (m, 2H), 6.04 (brs, 1H), 5.91 (brs, 1H), 5.84 (brs, 1H), 3.84-3.80 (m, 1H), 3.54 (brs, 1H), 3.17-2.90 (m, 3H), 2.81 (t, J = 7.68Hz, 2H), 2.71-2.67 (m, 1H), 2.53 (brs, 3H), 2.31 (t, J = 7.68Hz, 2H), 0.94 (d, J = 6.16Hz, 3H), 0.82 (s, 3H), 0.73 (d, J = 6.56Hz, 3H).

LCMS (HCOOH:ACN): M+H=411, R<sub>t</sub>=1.50 min in 3 mins run.

**MMV1545779:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.85-7.69 (m, 2H), 7.28-7.20 (m, 2H), 7.18-7.10 (m, 2H), 6.68 (brs, 1H), 5.54 (brs, 1H), 3.87-3.80 (m, 1H), 3.64 (brs, 1H), 3.20-2.89 (m, 4H), 2.86 (s, 3H), 2.84-2.70 (m, 2H), 2.38-2.30 (m, 2H), 0.95 (d, J = 6.36Hz, 3H), 0.84 (brs, 6H).

LCMS (HCOOH:ACN): M+H=432, R<sub>t</sub>=1.52 min in 3 mins run.

**MMV884790:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.85 (t, J = 5.56Hz, 1H), 7.50 (d, J = 8.32Hz, 1H), 7.28-7.21 (m, 2H), 7.14-7.08 (m, 2H), 5.39 (d, J = 5.4Hz, 1H), 4.40 (t, J = 5.36Hz, 1H), 3.87-3.84 (m, 1H), 3.75 (t, J = 4.8Hz, 1H), 3.44-3.40 (m, 1H), 3.27-3.23 (m, 1H), 3.10-3.04 (m, 2H), 2.82 (t, J = 7.78Hz, 2H), 2.35 (t, J = 7.78Hz, 2H), 1.90-1.85 (m, 1H), 0.96 (d, J = 6.68Hz, 3H), 0.84 (d, J = 6.92Hz, 3H).

LCMS (HCOOH:ACN): M+H=383, R<sub>t</sub>=1.50 min in 3 mins run.

**MMV884964:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.87 (t, J = 5.78Hz, 1H), 7.76 (d, J = 8.16Hz, 1H), 7.28-7.21 (m, 2H), 7.14-7.08 (m, 2H), 5.33 (d, 5.96Hz, 1H), 4.45 (t, J = 5.38Hz, 1H), 3.84-3.82 (m, 1H), 3.78 (d, J = 5.96Hz, 1H), 3.40-3.31 (m, 2H), 3.08 (t, J = 6.08Hz, 2H), 2.83 (t, J = 7.72Hz, 2H), 2.37 (t, J = 7.72Hz, 2H), 1.34-1.23 (m, 4H), 0.95 (d, J = 6.56Hz, 3H), 0.78-0.74 (m, 6H).

LCMS (HCOOH:ACN): M+H=341, R<sub>t</sub>=1.34 min in 3 mins run.

**MMV1545786:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.36 (brs, 1H), 7.71 (d, J = 7.68Hz, 1H), 7.28-7.22 (m, 2H), 7.14-7.10 (m, 2H), 4.83 (t, J = 5.12Hz, 1H), 3.92-3.85 (m, 1H), 3.67-3.62 (m, 2H), (m, 2H), 2.81 (t, J = 7.52Hz, 2H), 2.33 (t, J = 7.52Hz, 2H), 1.12 (s, 6H), 0.95 (d, J = 6.48Hz, 3H).

LCMS (HCOOH:ACN): M+H=353, R<sub>t</sub>=1.56 min in 3 mins run.

**MMV1545785:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.80-7.69 (m, 2H), 7.31-7.24 (m, 2H), 7.14-7.10 (m, 2H), 5.38 (brs, 1H), 4.90 (brs, 1H), 3.90-3.81 (m, 1H), 3.38-3.26 (m, 2H), 3.20-3.09 (m, 1H), 3.08-2.92 (m, 1H), 2.82 (brs, 2H), 3.32 (brs, 2H), 1.21 (s, 3H), 0.95 (d, J = 5.2Hz, 3H), 0.85 (s, 6H).

LCMS (HCOOH:ACN): M+H=369, R<sub>t</sub>=1.56 min in 3 mins run.

**MMV976385:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.68 (brs, 1H), 7.62 (d, J = 7.6Hz, 1H), 5.39 (d, J = 5.16Hz, 1H), 4.46 (t, J = 4.96Hz, 1H), 3.86-3.81 (m, 1H), 3.72 (d, J = 5.16Hz, 1H), 3.29-3.25 (m, 1H), 3.17-3.13 (m, 2H), 3.04-3.01 (m, 1H), 2.00 (t, J = 7.32Hz, 2H), 1.49-1.42 (m, 2H), 1.30-1.21 (m, 6H), 0.98 (d, J = 6.36Hz, 3H), 0.85-0.78 (m, 9H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=317, R<sub>t</sub>=2.70 min in 5 mins run.

**MMV692007:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.70-7.65 (m, 2H), 6.79 (s, 1H), 6.77 (d, J = 6.92Hz, 1H), 6.63 (d, J = 6.92Hz, 1H), 5.94 (s, 2H), 5.39 (d, J = 5.48Hz, 1H), 4.47 (t, J = 5.6Hz, 1H), 3.88-3.81 (m, 1H), 3.72 (d, J = 5.48Hz, 1H), 3.31-3.27 (m, 1H), 3.19-3.12 (m, 2H), 3.03-2.98 (m, 1H), 2.70 (t, J = 7.12Hz, 2H), 2.27 (t, J = 7.12Hz, 2H), 0.95 (d, J = 6.6Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=381, R<sub>t</sub>=2.28 min in 5 mins run.

**MMV692679:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.75-7.68 (m, 2H), 7.33-7.27 (m, 1H), 7.07-7.02 (m, 2H), 5.39 (d, J = 5.28Hz, 1H), 4.45 (t, J = 5.12Hz, 1H), 3.88-3.82 (m, 1H), 3.72 (d, J = 5.28Hz, 1H), 3.28-3.23 (m, 1H), 3.18-3.13 (m, 2H), 3.02-2.98 (m, 1H), 2.82 (t, J = 7.6Hz, 2H), 2.27 (t, J = 7.6Hz, 2H), 0.95 (d, J = 6.44Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=373, R<sub>t</sub>=1.38 min in 3 mins run.

**MMV689835:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.70-7.66 (m, 2H), 7.33-7.27 (m, 1H), 7.04-6.97 (m, 3H), 5.39 (d, J = 5.48Hz, 1H), 4.46 (t, J = 5.58Hz, 1H), 3.86-3.82 (m, 1H), 3.72 (d, 5.48Hz, 1H), 3.31-3.27 (m, 1H), 3.18-3.10 (m, 2H), 3.03-2.98 (m, 1H), 2.81 (t, J = 7.18Hz, 2H), 2.33 (t, J = 7.18Hz, 2H), 0.95 (d, J = 6.6Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=355, R<sub>t</sub>=1.41 min in 3 mins run.

**MMV692006:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.77-7.66 (m, 2H), 7.12-7.06 (m, 4H), 5.39 (d, J = 5.52Hz, 1H), 4.47 (t, J = 5.4Hz, 1H), 3.88-3.81 (m, 1H), 3.72 (d, J = 5.52Hz, 1H), 3.31-3.26 (m, 1H), 3.18-3.12 (m, 2H), 3.04-2.99 (m, 1H), 2.74 (t, J = 7.5Hz, 2H), 2.57-2.52 (m, 2H), 2.29 (t, J = 7.5Hz, 2H), 1.14 (t, J = 7.52Hz, 3H), 0.95 (d, J = 6.56Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=365, R<sub>t</sub>=2.52 min in 5 mins run.

**MMV693177:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.74-7.67 (m, 2H), 7.13 (t, J = 8.44Hz, 2H), 5.39 (d, J = 5.4Hz, 1H), 4.46 (brs, 1H), 3.86-3.80 (m, 1H), 3.72 (d, J = 5.4Hz, 1H), 3.31-3.25 (m, 1H), 3.18-3.12 (m, 2H), 3.01-2.98 (m, 1H), 2.78 (t, J = 7.42Hz, 2H), 2.26 (t, J = 7.42Hz, 2H), 0.95 (d, J = 6.48Hz, 3H), 0.79 (s, 3H), 0.77 (s, 3H).

LCMS (HCOOH:ACN): M+H=391, R<sub>t</sub>=1.41 min in 3 mins run.

**MMV689837:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.76-7.70 (m, 2H), 7.29 (d, J = 4.44Hz, 1H), 6.91 (d, J = 4.28Hz, 1H), 6.83 (bs, 1H), 5.38 (d, J = 5.36Hz, 1H), 4.47 (bs, 1H), 3.88-3.84 (m, 1H), 3.72 (d, J = 5.36Hz, 1H), 3.31-3.27 (m, 1H), 3.18-3.16 (m, 2H), 3.04-2.98 (m, 3H), 2.39-2.32 (m, 2H), 0.97 (d, J = 6.48Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=343, R<sub>t</sub>=1.36 min in 3 mins run.

**MMV693180:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.72-7.66 (m, 2H), 6.87 (d, J = 8.24Hz, 2H), 5.38 (d, J = 5.48Hz, 1H), 4.45 (t, J = 5.6Hz, 1H), 3.86-3.82 (m, 1H), 3.72 (d, J = 5.48Hz, 1H), 3.31-3.27 (m, 1H), 3.18-3.11 (m, 2H), 3.04-2.99 (m, 1H), 2.77 (t, J = 7.56Hz, 2H), 2.28-2.22 (m, 5H), 0.96 (d, J = 6.86Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=387, R<sub>t</sub>=1.46 min in 3 mins run.

**MMV689845:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.87 (brs, 1H), 7.75 (brs, 1H), 7.26-7.24 (m, 1H), 7.09 (m, 2H), 5.35 (d, J = 5.32Hz, 1H), 4.46 (brs, 1H), 3.70 (d, J = 5.32Hz, 1H), 3.31-3.27 (m, 1H), 3.24 (m, 2H), 3.17-3.07 (m, 3H), 2.86 (t, J = 7.27Hz, 2H), 2.36 (t, J = 7.72Hz, 2H), 0.79 (s, 3H), 0.78 (s, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=359, R<sub>t</sub>=2.87 min in 5 mins run.

**MMV976386:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.69 (brs, 1H), 7.64 (d, J = 7.56Hz, 1H), 5.39 (d, J = 5.28Hz, 1H), 4.46 (brs, 1H), 3.86-3.83 (m, 1H), 3.72 (d, J = 5.28Hz, 1H), 3.31-3.27 (m, 1H), 3.19-3.13 (m, 2H), 3.05-3.02 (m, 1H), 1.96-1.89 (m, 3H), 0.98 (d, J = 6.44Hz, 3H), 0.86 (d, J = 3.96Hz, 6H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=289, R<sub>t</sub>=2.05 min in 5 mins run.

**MMV689260:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.69-7.68 (m, 2H), 7.26-7.24 (m, 2H), 7.14-7.10 (m, 2H), 5.38 (d, J = 5.52Hz, 1H), 4.46 (t, J = 5.56 Hz, 1H), 3.82-3.81 (m, 1H), 3.71 (d, J = 5.52 Hz, 1H), 3.31-3.26 (m, 1H), 3.19-3.14 (m, 2H), 2.96-2.93 (m, 1H), 2.81 (t, J = 7.6Hz, 2H), 2.32 (t, J = 7.92Hz, 2H), 0.95 (d, J = 6.36Hz, 3H), 0.79 (s, 3H), 0.77 (s, 3H).

LCMS (NH<sub>4</sub>OAc:ACN): M+H=355, R<sub>t</sub>=2.41 min in 5 mins run.

**MMV884787:**

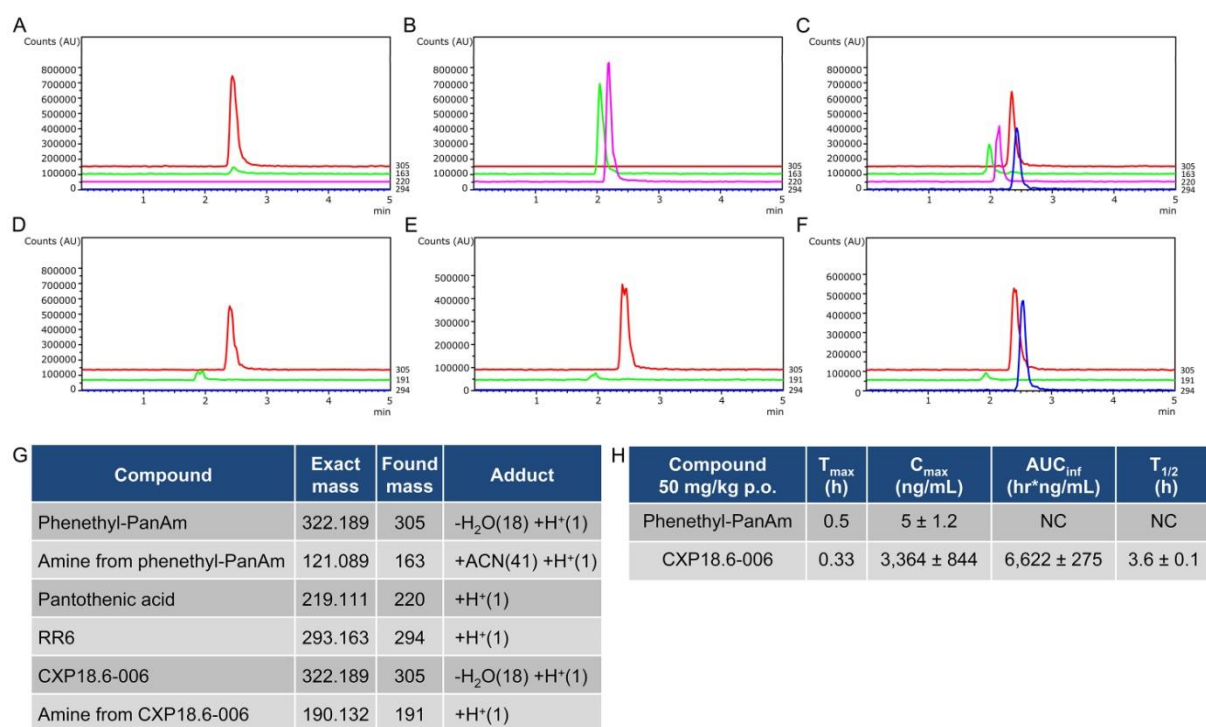
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.69-7.67 (m, 2H), 7.28-7.21 (m, 2H), 7.14-7.08 (m, 2H), 5.39 (d, J = 5.48Hz, 1H), 4.46 (t, J = 5.56Hz, 1H), 3.82-3.79 (m, 1H), 3.71 (d, J = 5.48Hz, 1H), 3.31-3.26 (m, 1H), 3.19-3.14 (m, 2H), 2.98-2.93 (m, 1H), 2.81 (t, J = 7.6Hz, 2H), 2.32 (t, J = 7.92Hz, 2H), 0.95 (d, J = 6.56Hz, 3H), 0.79 (s, 3H), 0.77 (s, 3H).

LCMS (HCOOH: ACN): M+H=355, R<sub>t</sub>=1.39 min in 3 mins run.

**MMV884968:**

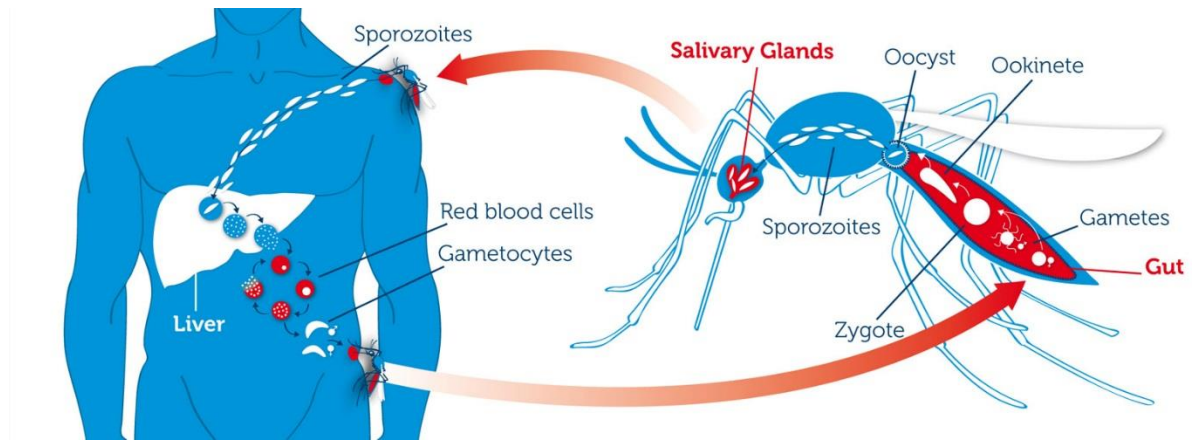
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.72-7.68 (m, 2H), 7.28-7.21 (m, 2H), 7.14-7.08 (m, 2H), 5.39 (d, J = 5.48Hz, 1H), 4.46 (t, J = 5.44Hz, 1H), 3.86-3.83 (m, 1H), 3.72 (d, J = 5.48Hz, 1H), 3.31-3.18 (m, 1H), 3.19-3.11 (m, 2H), 3.04-3.00 (m, 1H), 2.81 (t, J = 7.76Hz, 2H), 2.32 (t, J = 8.23Hz, 2H), 0.95 (d, J = 6.56Hz, 3H), 0.80 (s, 3H), 0.78 (s, 3H).

LCMS (HCOOH:ACN): M+H=355, R<sub>t</sub>=1.40 min in 3 mins run.

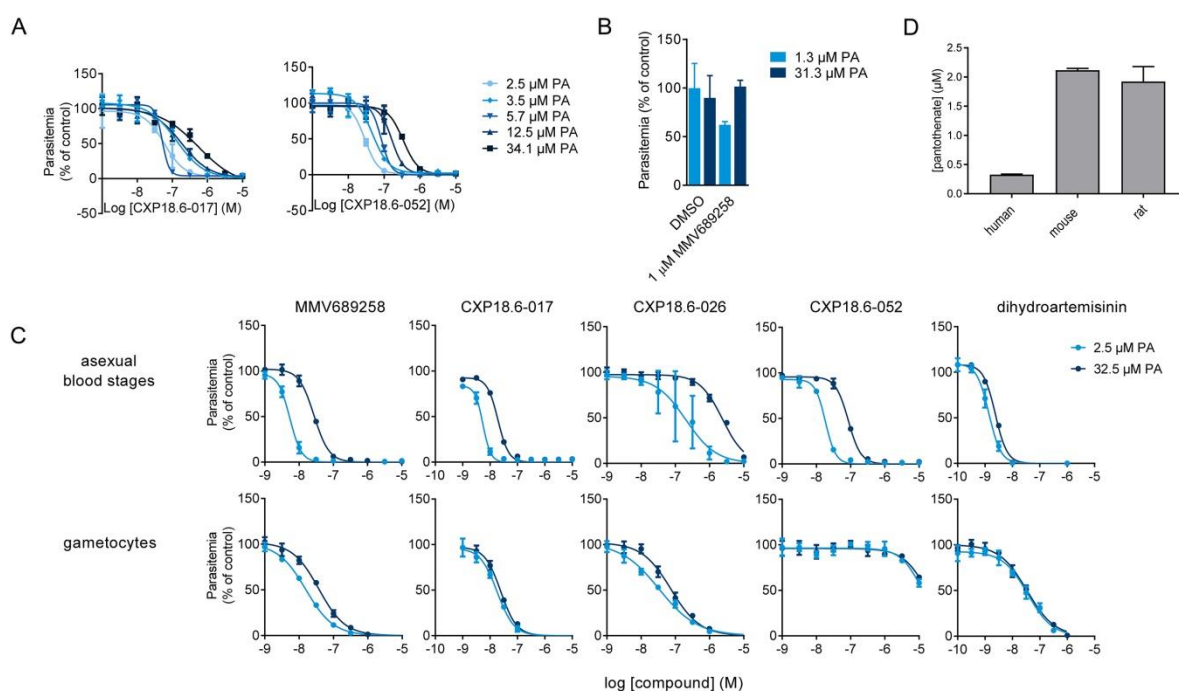


**Fig. S1. Pantothenamide stability.** The stability of phenethyl-PanAm, in **panels A-C**, and its corresponding inverted amide CXP18.6-006 (**panels D-F**) were measured using LC-MS analysis after overnight incubation in buffer (A and D), buffer with 10% fetal bovine serum (B and E) and buffer with 10% FBS supplemented with the vanin inhibitor RR6 (C-F). Fetal bovine serum contains a high level of vanin (pantetheinase) activity. Both compounds are stable after incubation in buffer alone (red lines in figure A and D). The small peaks in the amine channels (green), with molecular masses of 163 (A) and 191 (D), are either the result of disruption of compound during ionization (phenethyl-PanAm sample) or background from the buffer (CXP18.6-006 sample). Overnight incubation in 10% serum resulted in complete degradation of phenethyl-PanAm resulting in pantothenic acid (purple line) and the liberated amine (green line) in figure B. CXP18.6-006 remains intact in serum-containing buffer in figure E. Note that the hypothetical hydrolysis product phenylpropionic acid could not be detected with the used apparatus (E). Addition of vanin inhibitor RR6 (blue line) leads to the protection of phenethyl-PanAm, although some degradation is found leading to peaks corresponding to the amine and pantothenic acid (C). Addition of RR6 to CXP18.6-006 had no effect, since CXP18.6-006 was already stable in serum (F). Compounds (panels A-F) and their corresponding LC-MS information are listed in panel **G**. **H**) Pharmacokinetic analysis of phenethyl-PanAm or CXP18.6-006. Compounds were dosed orally to male Sprague Dawley rats (8-12 weeks old) at 50 mg/kg. Blood was collected at t = 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. Plasma levels of the compounds were measured by LC-MS/MS. The inversion of the susceptible amide bond leads to a dramatic improvement of the half life in vivo.

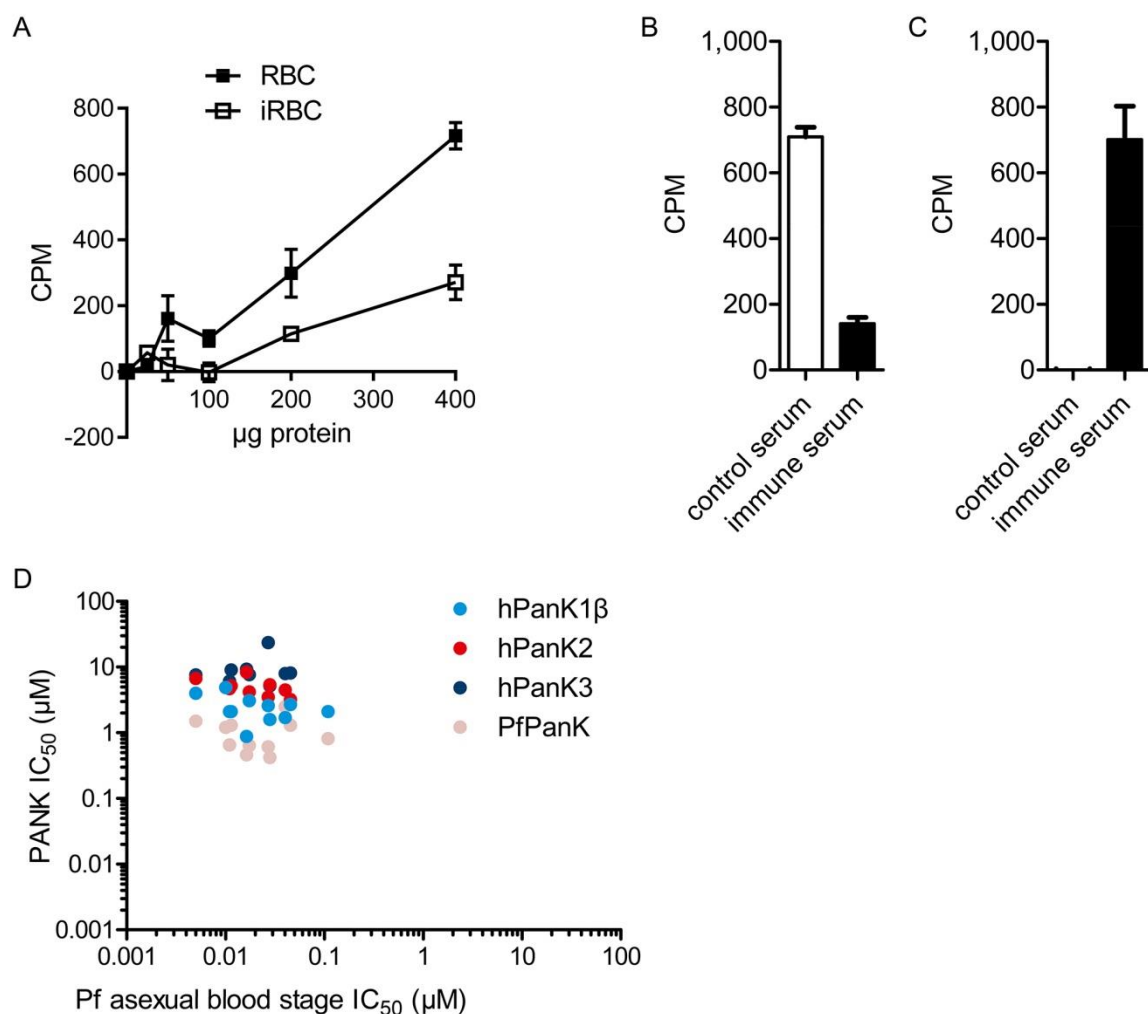




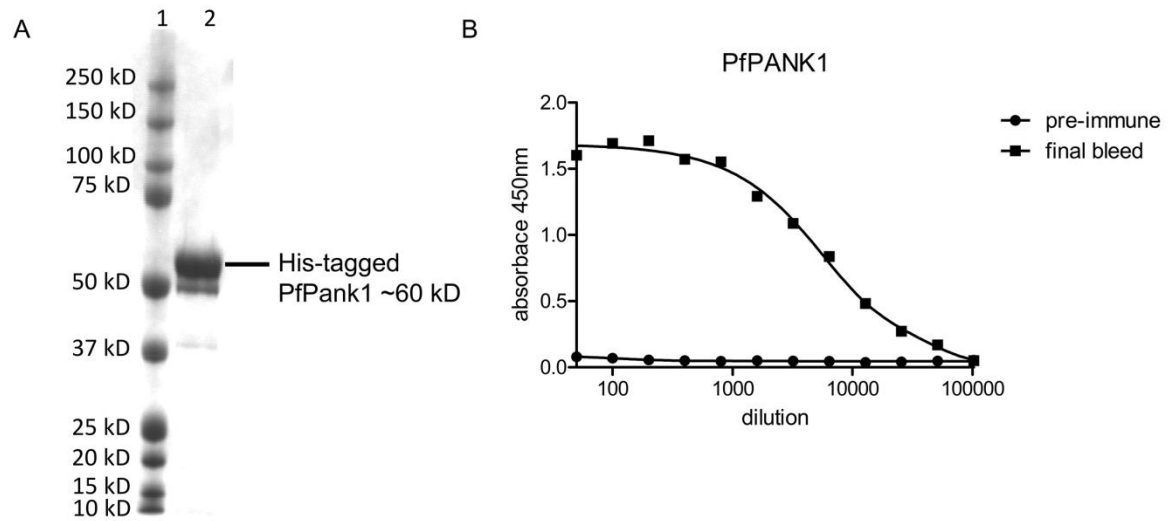
**Fig. S2. Parasite life cycle.** Life cycle of the human malaria parasite indicating the key parasite stages in the human and mosquito host.



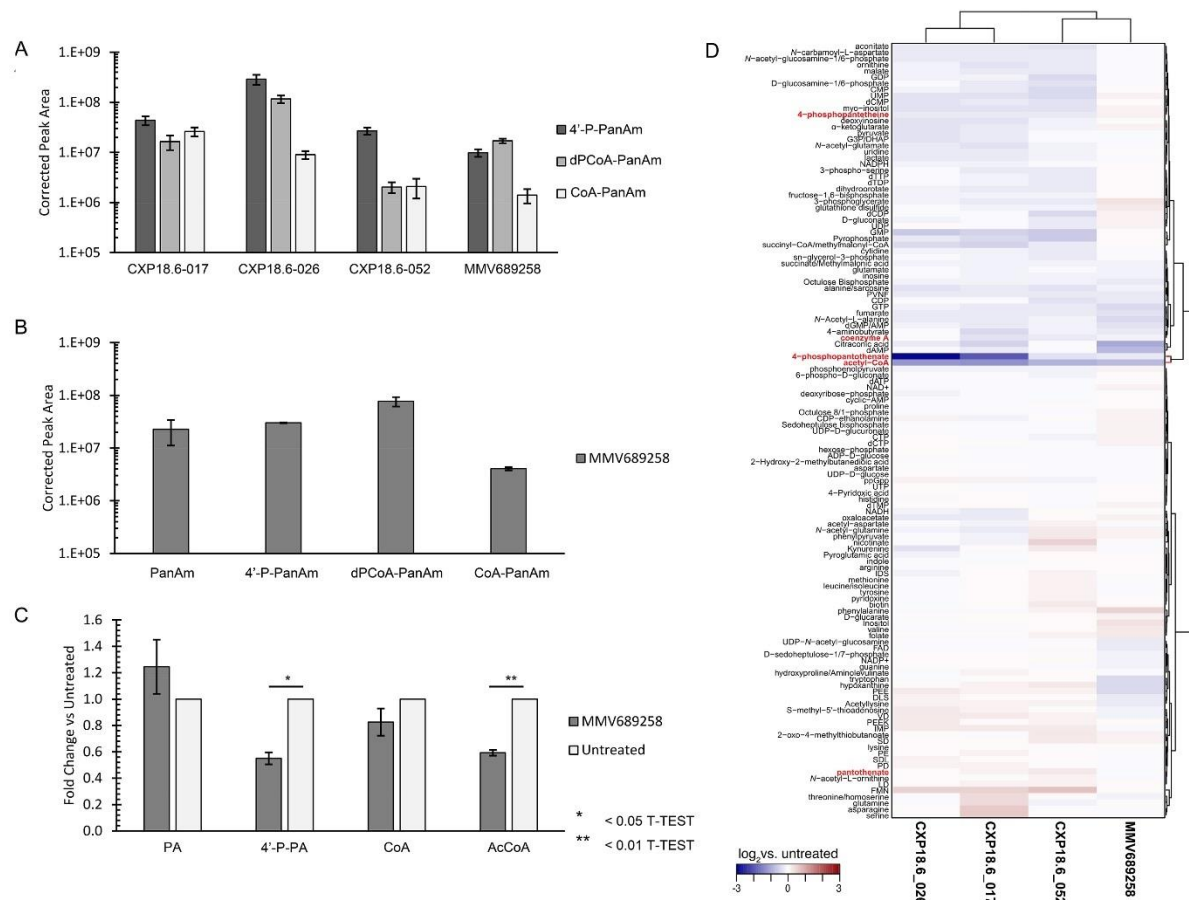
**Fig. S3. Pantothenate competition assays.** **A)** *P. falciparum* NF54 asexual blood stage parasites were exposed to various doses of CXP18.6-017 or CXP18.6-052 in the presence of varying amounts of pantothenate (PA) as indicated in the figure legend. Parasites were allowed to replicate for three days and parasitemia relative to control (0.1% DMSO) was determined through SYBR Green assays. The figure shows average values and standard deviations from two independent measurements. **B)** Sensitivity of the activity of MMV689258 against liver stage development to an increase in the pantothenate concentration. **C)** Pantothenate competition experiments for a larger set of compounds, comparing asexual blood stage parasites and gametocytes. The figure shows average values and standard deviations from four independent measurements. **D)** Pantothenate concentrations in serum from various species as determined by LC-MS.



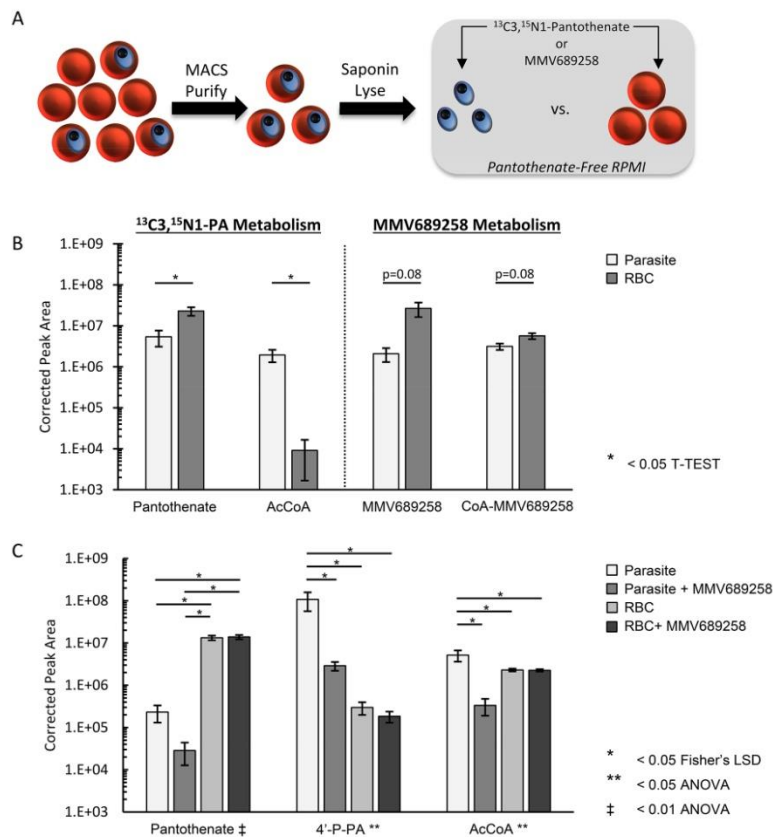
**Fig. S4. PANK activity.** **A)** PANK activity in lysates of infected red blood cells (open squares) and uninfected red blood cells (closed squares) was measured using  $^{14}\text{C}$ -labeled pantothenic acid. Activity is expressed as counts per minute (cpm) on the Y-axis versus microgram of protein on the X-axis. The figure shows the mean and standard deviations of duplicate measurements. **B)** PANK activity in parasite lysates that were treated with protein A/G beads loaded with pre-immune serum (open bar) or anti-*Pf*PANK1 serum (closed bar). About 80% of the PANK activity is removed from the lysate by the specific antiserum. **C)** The protein A/G beads from panel B were washed and assayed for PANK activity. Only the beads loaded with the specific anti *Pf*PANK antibodies showed PANK activity. **D)** Inhibition of *P. falciparum* and human PANK enzymes versus inhibition of parasite replication. The figure shows IC<sub>50</sub> values derived from PANK assays using parasite lysate and human recombinant PANK enzymes and from *P. falciparum* replication assays for 11 pantothenamide compounds, including the compounds listed in table S1. Linear regression analyses showed a lack of correlation between parasite replication and PANK inhibition IC<sub>50</sub> values ( $R^2 < 0.32$  for all PANKs).



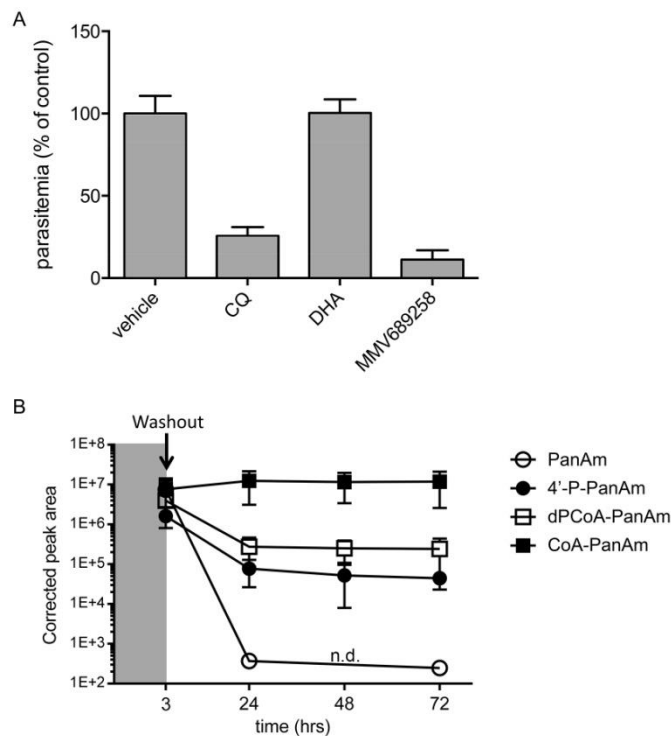
**Fig. S5. Expression of *PfPANK1*.** **A)** SDS-PAGE of baculovirus-expressed recombinant *PfPANK1* (lane 2) next to the marker (lane 1). **B)** Reactivity of rabbit pre-immune and immune sera ('final bleed') against recombinant *PfPANK1*.



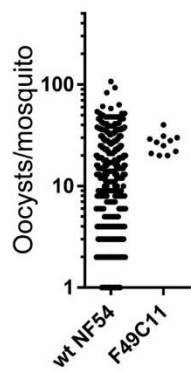
**Fig. S6. Cellular pantothenamide metabolism and targeted metabolomics.** **A)** Pantothenamide analogs detection in MACS-purified trophozoite parasites treated with pantothenamide at  $10 \times \text{IC}_{50}$  for 2.5 hours. The Y-axis denotes corrected peak areas ( $\pm \text{SE}$ ) and the X-axis denotes the specific metabolite. Samples were collected in technical triplicate for  $n = 3$  biological replicates. **B)** Antimetabolite detection in stage III/IV gametocytes (Day +7) treated with  $1 \mu\text{M}$  MMV689258 for 2.5 hours. The Y-axis denotes corrected peak areas ( $\pm \text{SE}$ ) and the X-axis denotes the specific metabolite. Untreated samples displayed little to no signal above background for the respective metabolites and have been removed for clarity. Samples were collected as 2-3 technical replicates for  $n = 3$  biological replicates. **C)** Endogenous pantothenate/CoA biosynthesis in stage III/IV gametocytes (Day +7) treated with  $1 \mu\text{M}$  MMV689258 for 2.5 hours. The Y-axis denotes fold change ( $\pm \text{SE}$ ) relative to untreated control and the X-axis denotes the specific metabolite. Samples were collected as 2-3 technical replicates for  $n = 3$  biological replicates. **D)** Heatmap of targeted metabolomics following pantothenamide treatment. The Y-axis denotes the pantothenamide used and the X-axis denotes cellular metabolites. Values are average log<sub>2</sub> fold change relative to a paired untreated control. Samples were collected in technical triplicate for  $n = 3$  biological replicates and only metabolites detected in all assays are displayed. Pantothenate/CoA biosynthesis metabolites are highlighted in red on the Y-axis. Clustering is euclidean distance and Ward linkage, using the heatmap function in the SupraHex R package.



**Fig. S7. Pantothenate and pantothenamide metabolism in saponin-isolated parasites versus uninfected red blood cells. A)** Schematic of the experimental setup where purified iRBCs were lysed using saponin to generate a pure population of parasites, which were deemed viable using trypan blue. Cells were washed with PBS and all subsequent comparative assays were performed in pantothenate-free media. **B)** <sup>13</sup>C<sub>3</sub>, <sup>15</sup>N<sub>1</sub>-pantothenic acid and MMV689258 metabolism in isolated parasites versus uninfected RBCs. Cells were treated with either 1 μM <sup>13</sup>C<sub>3</sub>, <sup>15</sup>N<sub>1</sub>-pantothenic acid or MMV689258 for 3 hours prior to metabolite extraction. **C)** Endogenous pantothenate/CoA metabolism in saponin isolated parasites and uninfected red blood cells treated with 1 μM MMV689258 for 3 hours. All samples (B & C) were collected in technical triplicate for n = 3 biological replicates. Y-axis denotes corrected peak areas (±SE) and x-axis denotes the specific metabolite for all graphs. Statistics are denoted within their respective figures.

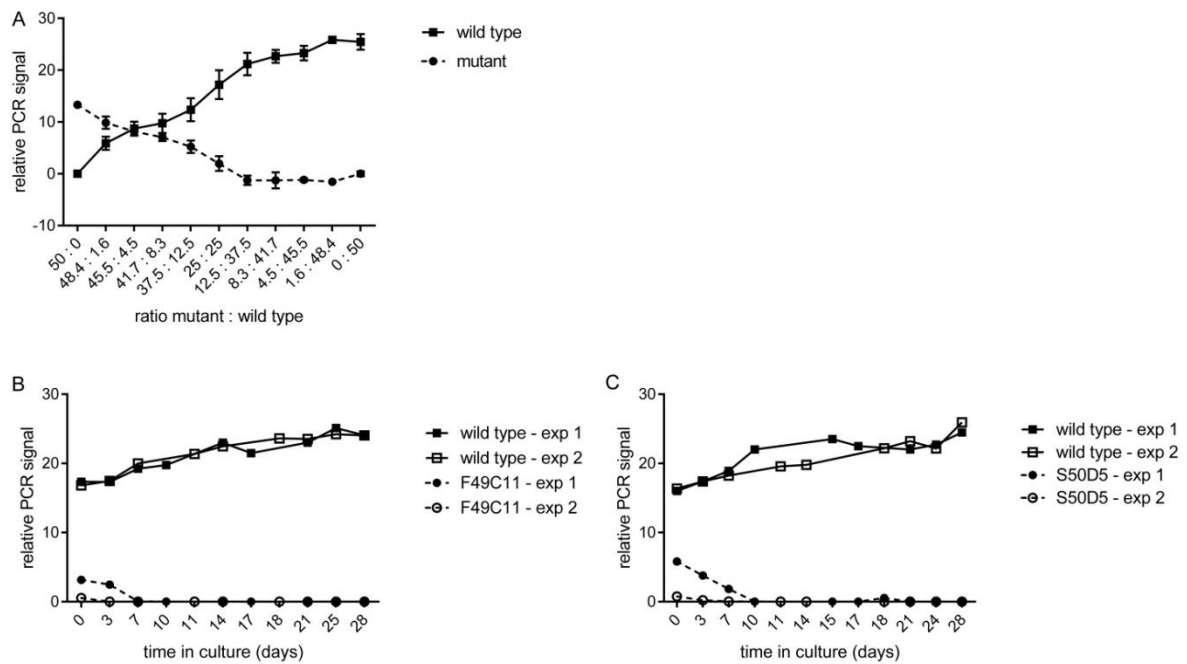


**Fig. S8. Erythrocytes preexposed to MMV689258 are less susceptible to malaria infection. A)** Erythrocytes were exposed to 100 µM of dihydroartemisinin (DHA), chloroquine (CQ) or MMV689258 for three hours followed by a thorough wash. Twenty-four hours later, purified *P. falciparum* trophozoites/schizonts were added to a final parasitemia of 0.83% in a haematocrit of 3%. Seventy-two hours later, parasitemia was measured using a SYBR Green assay. The figure shows values and standard deviations of the mean from 3 independent experiments. **B)** RBC pulse/chase metabolomics using 1 µM MMV689258. Cells were pulsed with 1 µM MMV689258 for 3 hours, washed, and returned to culture for up to 72 additional hours. Samples were taken at 3 hours, preceding washout, and at 24-hour intervals post-washout as displayed on the x-axis. Shading indicates the presence of external drug and washout is marked with a black arrow, subsequent data points are hours post-washout. Samples were collected in technical triplicate with n = 3 biological replicates. Values on the y-axis are corrected peak areas  $\pm$  SE, following removal of blank and untreated sample background. n.d. is not detected.

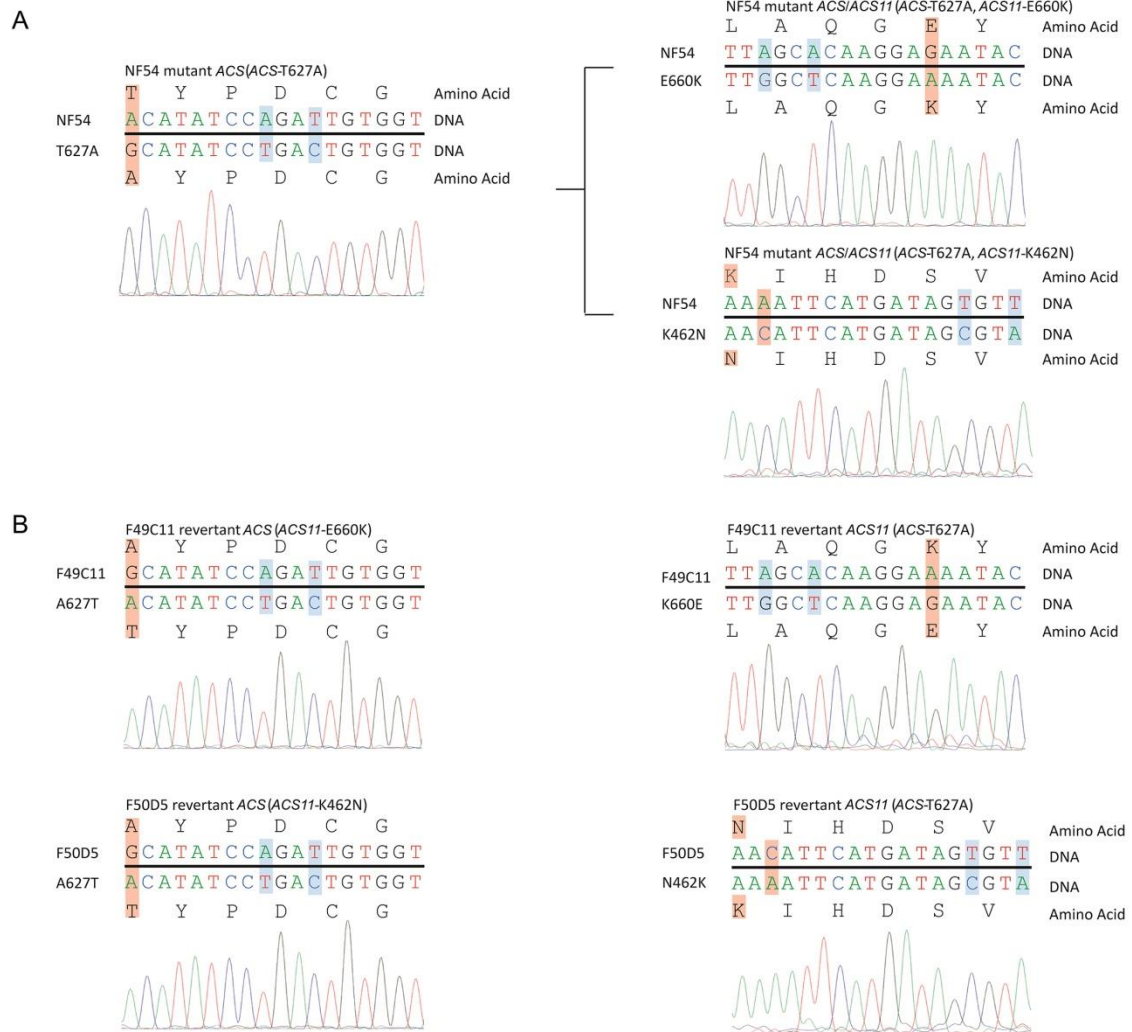


**Fig. S9. Drug-resistant parasite (ACS-T627A and ACS11-E660K) transmission to mosquitoes.** The figure shows oocyst densities at day 7 post infection in midguts of *A. stephensi* mosquitoes infected with gametocytes derived from drug resistant clone F49C11 and, for comparison, with gametocytes derived from the wild type NF54 *P. falciparum* strain.

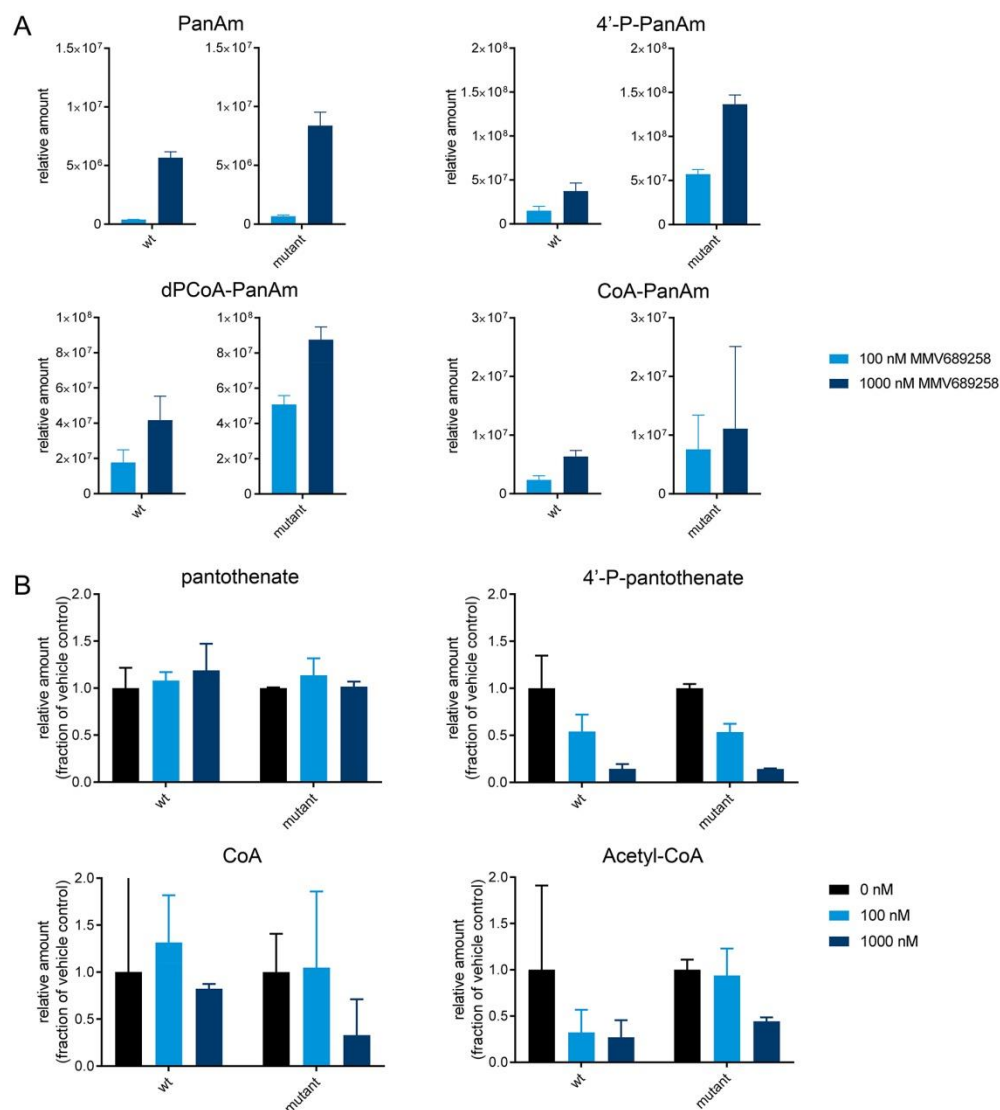




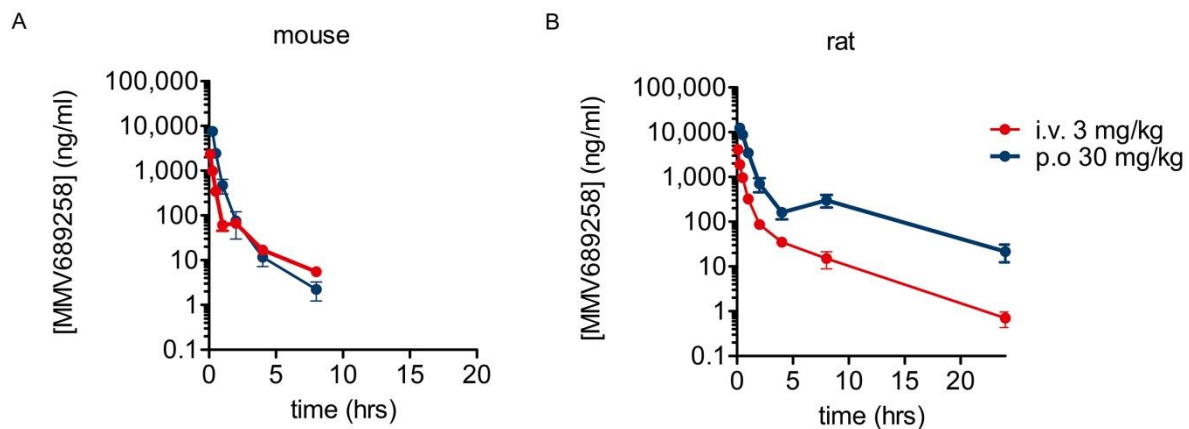
**Fig. S10. Pantothenamide-resistant parasites (ACS-T627A and ACS11-E660K) have reduced fitness.** Resistant parasites were mixed with wild type parasites and cultured for 4 weeks with biweekly passaging. Relative amounts of wild type and resistant parasites were determined by allele-specific PCR. **A)** Control PCR. DNA from drug-selected resistant and wild type parasites was mixed at the ratio's indicated at the X-axis. The two alleles were amplified in a single PCR and quantified by gel densitometry. Intensities were normalized to the total amount of amplified DNA on the gel. **B)** F49C11 mutant parasites (ACS-T627A; ACS11-E660K) were mixed with wild type NF54 parasites at a 1:1 ratio and subcultured biweekly. At the indicated time intervals, samples were analyzed for the presence of wild type and mutant alleles. The experiment was repeated and the figure shows data from both independent replicates. **C)** Competition experiments with F50D5 mutant parasites (ACS-T627A; ACS11-K462N) using the same methodology.



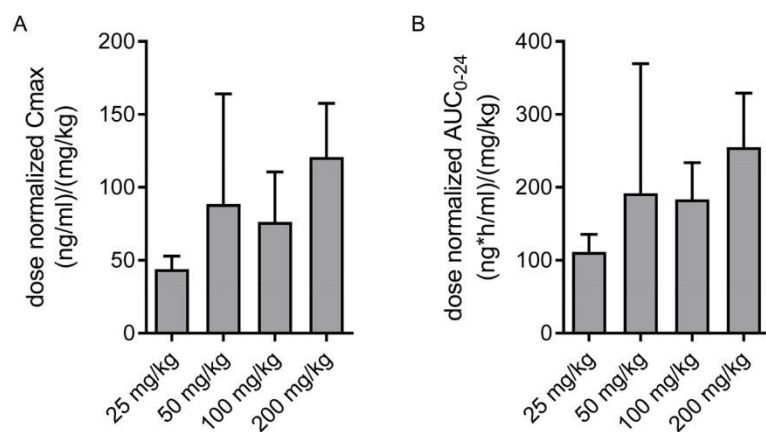
**Fig. S11. Sequence verification of CRISPR-Cas9-engineered mutations. A)** The ACS-T627A mutation was introduced in NF54 wild type parasites by CRISPR/Cas9 mediated genome editing. The resulting mutant strain was then used to introduce either the ACS11-E660K or the ACS11-K462N mutations. **B)** Sequencing results of parasites where mutations acquired through *in vitro* evolution where reverted back to wild type.



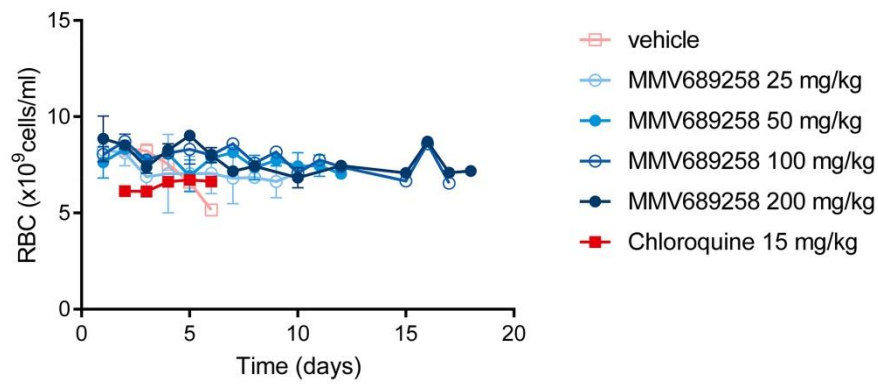
**Fig. S12. Metabolomics of wild-type versus drug-resistant parasites.** NF54 (wt) and drug-resistant (clone F50D5, mutant) parasites, cultured in RPMI1640 containing 10% human serum, were MACS-purified and treated with DMSO or 100nM or 1000nM MMV689258 for 3 hours. Samples were collected for  $n = 3$  biological replicates for NF54 and  $n = 1$  for clone F50D5 measured in duplicate. **A)** Pantothenamide metabolism in wild type and mutant parasites. Y-axis denotes the relative amount ( $\pm$ SD). **B)** Endogenous pantothenate/CoA metabolism in wild type and mutant parasites. Y-axis denotes the relative amount compared to DMSO control ( $\pm$ SD).



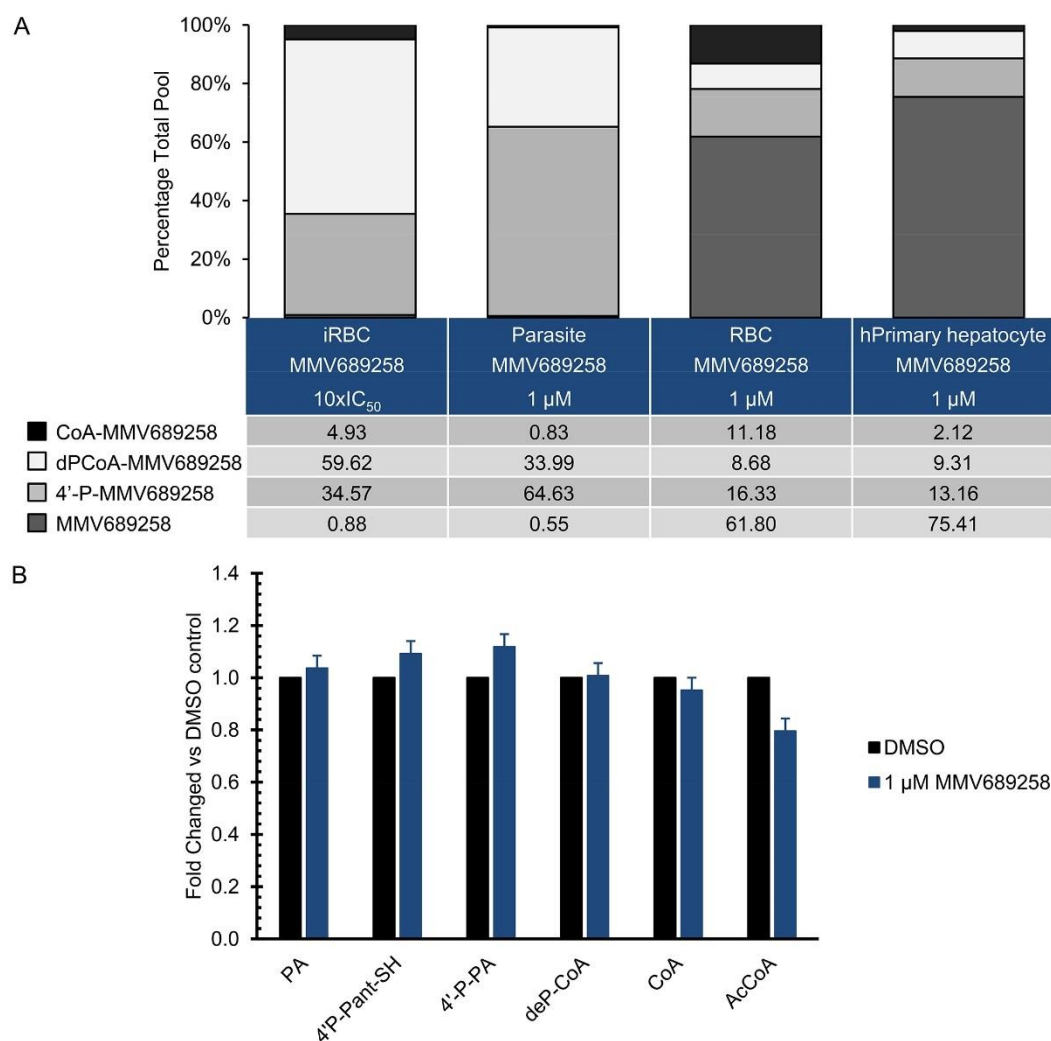
**Fig. S13. Pharmacokinetics of MMV689258 in rodents.** Mice (**A**) and rats (**B**) were dosed intravenously (i.v.) with 3 mg/kg or orally (p.o) with 30 mg/kg. Plasma concentrations of MMV689258 were measured as a function of time for 24 hours post-dosing. In mice, plasma levels were below the detection limit at the 24 hour timepoint. The figure shows average values and standard deviations from three animals.



**Fig. S14. Dose-normalized plasma exposure of MMV689258 in NODscidIL2Ry<sup>null</sup> mice.** The figure shows **(A)** dose-normalized C<sub>max</sub> and **(B)** AUC from mice that received oral dosages indicated at the x-axis. The figure shows average values and standard deviations from two replicate experiments.

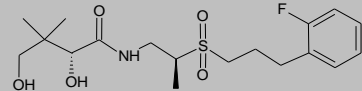
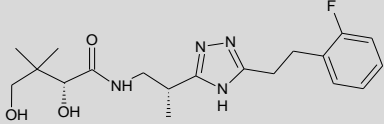
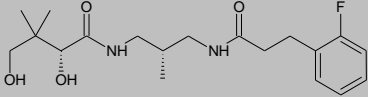
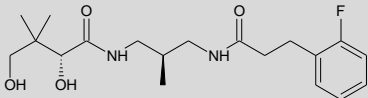
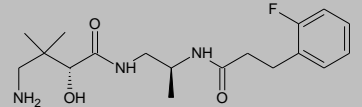
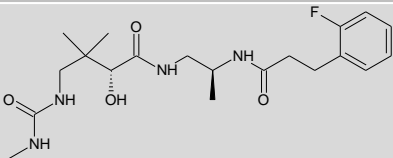
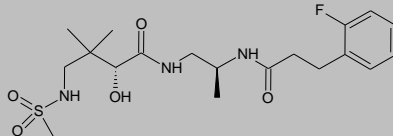
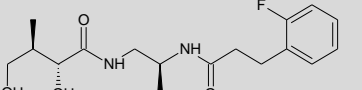
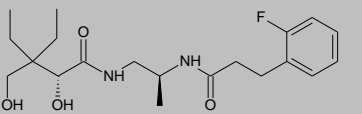
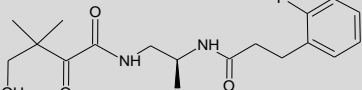


**Fig. S15. Red blood cell counts in *Pf*SCID mice treated with MMV689258.** The figure shows the total number of red blood cells per milliliter as determined by flow cytometry. Values represent averages from two mice. Error bars indicate standard deviations.



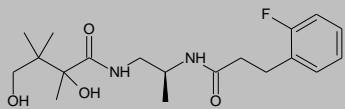
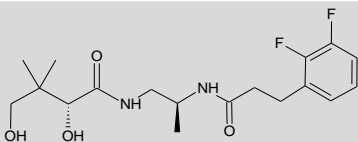
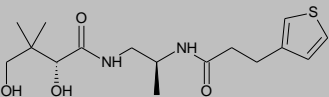
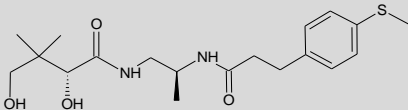
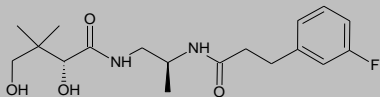
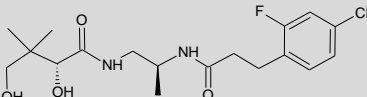
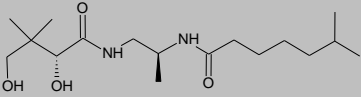
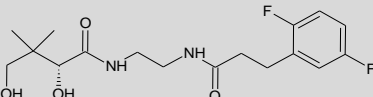
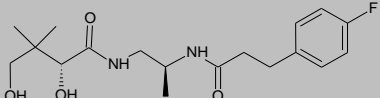
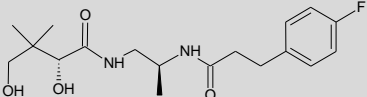
**Fig. S16. Cell-type specificity and primary human hepatocyte metabolomics.** iRBCs were treated for 2.5 hours (see Figure 3), while RBC (Figure 4) and human primary cells were treated for 3.5 hours at the concentrations listed. Biological replicates: iRBC (n = 3), uRBC (n=3), and human primary hepatocytes (n = 2), with all sample collected as 2-3 technical replicates. A) Percentage of analog detected as a percentage of total pantothene-related metabolite pool. B) Pantothenate/CoA biosynthesis pathway in primary human hepatocytes treated with 1  $\mu$ M MMV689258 for 3.5 hours. The X-axis denotes endogenous metabolites and Y-axis fold changes compared to DMSO treated cells.

**Table S1. Selection of compounds to illustrate structure-activity relationship.**

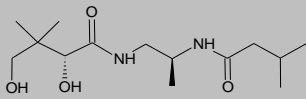
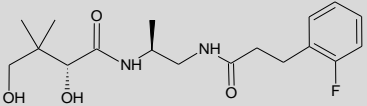
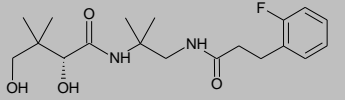
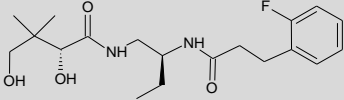
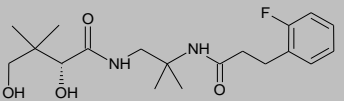
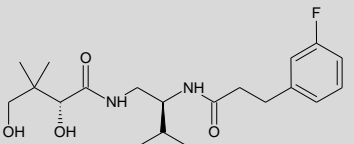
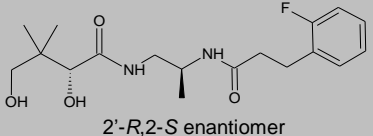
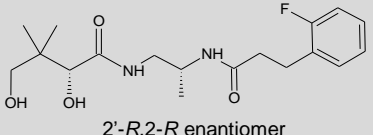
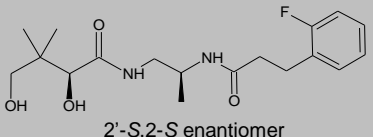
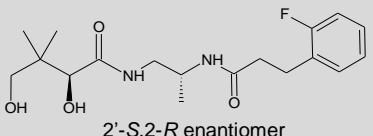
Structural modification/variation	Structure	Compound code	IC <sub>50</sub> against asexuals and gametocytes (μM)
bioisosteres		MMV884957	0.139 / >1
bioisosteres		MMV884789	0.299 / >1
linker length between amides		MMV692002	>1 / >1
linker length between amides		MMV692003	>1 / >1
primary hydroxyl		MMV1545511	>1 / >1
primary hydroxyl		MMV1558163	>1 / >1
primary hydroxyl		MMV1545779	>1 / >1
geminal dimethyl		MMV884790	>1 / >1
geminal dimethyl		MMV884964	>1 / >1
secondary hydroxyl		MMV1545786	>1 / >1



**Table S1. Selection of compounds to illustrate structure-activity relationship; continued**

Structural modification/variation	Structure	Compound code	IC <sub>50</sub> against asexuals and gametocytes (μM)
secondary hydroxyl		MMV1545785	>1 / >1
right-hand side		MMV692005	0.006 / 0.001
right-hand side		MMV689838	0.017 / 0.004
right-hand side		MMV692008	0.021 / 0.001
right-hand side		MMV689835	0.028 / 0.044
right-hand side		MMV692750	0.037 / 0.005
right-hand side		MMV976104	0.040 / >1
right-hand side		MMV976397	0.053 / 0.532
right-hand side		MMV1542106	0.147 / 0.036
right-hand side		MMV689845	0.151 / 0.104

**Table S1. Selection of compounds to illustrate structure-activity relationship; continued**

Structural modification/variation	Structure	Compound code	IC <sub>50</sub> against asexuals and gametocytes (μM)
right-hand side		MMV976386	0.175 / >1
substitutions of the ethyl linker		MMV689041	>1 / >1
substitutions of the ethyl linker		MMV689261	>1 / >1
substitutions of the ethyl linker		MMV692676	0.122 / 0.349
substitutions of the ethyl linker		MMV689455	>1 / >1
substitutions of the ethyl linker		MMV690791	>1 / >1
stereochemistry	 2'-R,2-S enantiomer	MMV689258	0.005 / 0.012
stereochemistry	 2'-R,2-R enantiomer	MMV689260	0.210 / >1
stereochemistry	 2'-S,2-S enantiomer	MMV884787	0.235 / 0.388
stereochemistry	 2'-S,2-R enantiomer	MMV884968	>1 / >1

Compounds used for evaluation of the structure-activity relationship (SAR). Examples are given for modifications at various positions in the pantotheneamide core structure. IC<sub>50</sub> values against asexual and gametocytes are indicated to illustrate the effect of the modifications on the antimalarial potency.

**Table S2. IC<sub>50</sub> values of compounds shown in Fig. 1.** Derived from parasite assays shown in Figure 2; IC<sub>50</sub> values from *in vitro* pantothenate kinase assays against *P. falciparum* and human enzymes; *in vitro* cytotoxicity assays against HepG2, HC04 and primary human hepatocytes, and *in vitro* CYP450 inhibition assays. All IC<sub>50</sub> values are listed in nM. Data are based on 2 to 6 independent replicate measurements. Numbers between brackets indicate 95% confidence intervals. Nd: not determined.

	CXP18.6-006	CXP18.6-017	CXP18.6-026	CXP18.6-052	MMV689258	MMV884968
<b><i>P. falciparum</i></b>						
Asexual blood stages	135 (108-170)	27 (26-29)	109 (79-149)	10 (9-11)	5 (4-7)	>10,000
Gametocytes	200 (150-260)	28 (23-33)	174 (60-508)	>1,000	16 (11-24)	>10,000
Oocysts	nd	20 (14-28)	107 (78-147)	>1,000	17 (7-42)	nd
Liver stages	nd	>4,000	>4,000	>4,000	~200 (nd)	nd
<b>PANK</b>						
PfPANK	nd	640 (526-779)	916 (616-1,061)	1,489 (692-3,205)	1,437 (1,025-2,014)	nd
hPANK1 $\beta$	nd	2,509 (1,474-4,270)	2,188 (1,343-3,565)	5,788 (2,296-14,590)	4,690 (2,585-8,509)	nd
hPANK2	nd	2,914 (2,565-3,310)	4,254 (3,506-5,161)	2,668 (2,092-3,403)	6,148 (4,386-8,618)	nd
hPANK3	nd	>10,000	1,796 (1,070-3,014)	>10,000	~9,123 (nd)	nd
<b>Hepatocytes</b>						
Hep2G	nd	>10,000	>10,000	>10,000	>10,000	nd
HC04	nd	>10,000	>10,000	>10,000	>10,000	nd
primary human hepatocytes	nd	>10,000	>10,000	>10,000	>10,000	nd
<b>CYP450</b>						
(CYP1A2, 2D6, 2C9, 2C19, 3A4)	nd	>20,000	>20,000	>20,000	>20,000	nd

**Table S3. Description of strains used in resistance panel.** The table lists the geographic origin, sensitivity to antimalarial drugs chloroquine (CQ), sulfadoxine (SUL), pyrimethamine (PYR), cyclosporin (CYC), mefloquine (MEF), atovaquone (ATO) and artemisinin (ART) and the genetic alterations underlying the reported resistance phenotypes.

Strain	Geographic origin	CQ	SUL	PYR	CYC	MEF	ATO	ART	resistance gene mutation/copy number variation
NF54	West Africa	S	R	S	S	S	S	S	pfdhps
K1	Thailand	R	R	R	R	S	S	S	pfcr, pfmdr1, pfdhfr, pfdhps
HB3	Honduras	S	S	R	S	S	S	S	pfmdr1, pfdhfr
7G8	Brazil	<b>R</b>	S	R	R	S	S	S	pfcr, pfmdr1, pfdhfr, pfdhps
TM90C2B	Thailand	R		R	R	<b>R</b>	R	S	pfcr, pfmdr1, pfdhfr, pfdhps, pfcytb
D6	Sierra Leone	S	S	S	S	<b>R</b>	S	S	none
V1/S	Vietnam	R	R	R	R	S	S	S	pfcr, pfmdr1, pfdhfr, pfdhps
Dd2	Indochina	R	R	R	R	<b>R</b>	S	S	pfcr, pfmdr1, pfdhfr, pfdhps/3xpfmdr1
FCB	Columbia	R		S	R	S	S	S	pfcr, pfmdr1, pfdhfr, pfdhps/4xpfmdr1
MRA1240	Cambodia							R	pfkelch13

S sensitive  
R resistant (>10x IC<sub>50</sub> shift)  
**R** resistant (<10x IC<sub>50</sub> shift)

**Table S4. Targeted metabolomics values for select compounds of interest.** The data includes the molecular formula which was used to calculate the molecular weight, monoisotopic mass, and expected m/z [M-H]<sup>-</sup> values. The listed 'actual m/z' corresponds to the average of experimental values from the various drug trials, which were used to calculate the Δppm value from expected. Retention time (RT) values are listed as the average of experimental values from all trials. \* denotes compounds for which pure standards were available to validate the expected m/z and retention time values.

Compound	Formula	MW	Monoisotopic mass	Expected m/z [M-H] <sup>-</sup>	Actual m/z [M-H] <sup>-</sup>	Δppm	RT (min.)
Pantothenate*	C <sub>9</sub> H <sub>17</sub> NO <sub>5</sub>	219.2350	219.1107	218.1029	218.1040	5.04	11.31
Pantetheine*	C <sub>11</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub> S	278.3684	278.1300	277.1222	277.1226	1.44	9.17
4-Phosphopantothenate*	C <sub>9</sub> H <sub>18</sub> NO <sub>8</sub> P	299.2149	299.0770	298.0692	298.0700	2.68	13.94
4-Phosphopantetheine*	C <sub>11</sub> H <sub>23</sub> N <sub>2</sub> O <sub>7</sub> PS	358.3483	358.0964	357.0886	357.0898	3.36	13.57
Dephospho-CoA*	C <sub>21</sub> H <sub>35</sub> N <sub>7</sub> O <sub>13</sub> P <sub>2</sub> S	687.5542	687.1489	686.1411	686.1429	2.62	15.08
CoA*	C <sub>21</sub> H <sub>36</sub> N <sub>7</sub> O <sub>16</sub> P <sub>3</sub> S	767.5341	767.1152	766.1074	766.1093	2.48	15.93
Acetyl-CoA*	C <sub>23</sub> H <sub>38</sub> N <sub>7</sub> O <sub>17</sub> P <sub>3</sub> S	809.5708	809.1258	808.1180	808.1201	2.60	16.18
CXP18.6-052*	C <sub>16</sub> H <sub>25</sub> N <sub>3</sub> O <sub>4</sub>	323.3874	323.1845	322.1767	322.1776	2.79	11.39
CXP18.6-017*	C <sub>17</sub> H <sub>25</sub> FN <sub>2</sub> O <sub>4</sub>	340.3898	340.1798	339.1720	339.1705	-4.42	15.70
MMV689258*	C <sub>18</sub> H <sub>27</sub> FN <sub>2</sub> O <sub>4</sub>	354.4164	354.1955	353.1877	353.1895	5.10	16.30
CXP18.6-026*	C <sub>18</sub> H <sub>28</sub> N <sub>2</sub> O <sub>4</sub>	336.4259	336.2049	335.1971	335.1965	-1.79	16.92
4'-P-CXP18.6-052	C <sub>16</sub> H <sub>26</sub> N <sub>3</sub> O <sub>7</sub> P	403.3673	403.1508	402.1430	402.1427	-0.75	13.91
4'-P-CXP18.6-017	C <sub>17</sub> H <sub>26</sub> FN <sub>2</sub> O <sub>7</sub> P	420.3697	420.1462	419.1384	419.1387	0.72	16.53
4'-P-MMV689258	C <sub>18</sub> H <sub>28</sub> FN <sub>2</sub> O <sub>7</sub> P	434.3963	434.1618	433.1540	433.1556	3.60	16.85
4'-P-CXP18.6-026	C <sub>18</sub> H <sub>29</sub> N <sub>2</sub> O <sub>7</sub> P	416.4058	416.1712	415.1634	415.1623	-2.65	17.38
dPCoA-CXP18.6-052	C <sub>26</sub> H <sub>38</sub> N <sub>8</sub> O <sub>13</sub> P <sub>2</sub>	732.5732	732.2034	731.1956	731.1932	-3.28	15.00
dPCoA-CXP18.6-017	C <sub>27</sub> H <sub>38</sub> FN <sub>7</sub> O <sub>13</sub> P <sub>2</sub>	749.5756	749.1987	748.1909	748.1920	1.47	16.80
dPCoA-MMV689258	C <sub>28</sub> H <sub>40</sub> FN <sub>7</sub> O <sub>13</sub> P <sub>2</sub>	763.6022	763.2143	762.2065	762.2042	-3.02	17.00
dPCoA-CXP18.6-026	C <sub>28</sub> H <sub>41</sub> N <sub>7</sub> O <sub>13</sub> P <sub>2</sub>	745.6118	745.2238	744.2160	744.2134	-3.49	17.38
CoA-CXP18.6-052	C <sub>26</sub> H <sub>39</sub> N <sub>8</sub> O <sub>16</sub> P <sub>3</sub>	812.5531	812.1697	811.1619	811.1595	-2.96	15.65
CoA-CXP18.6-017	C <sub>27</sub> H <sub>39</sub> FN <sub>7</sub> O <sub>16</sub> P <sub>3</sub>	829.5555	829.1650	828.1572	828.1583	1.33	17.37
CoA-MMV689258	C <sub>28</sub> H <sub>41</sub> FN <sub>7</sub> O <sub>16</sub> P <sub>3</sub>	843.5821	843.1807	842.1729	842.1746	2.02	17.63
CoA-CXP18.6-026	C <sub>28</sub> H <sub>42</sub> N <sub>7</sub> O <sub>16</sub> P <sub>3</sub>	825.5917	825.1901	824.1823	824.1797	-3.15	17.72

**Table S5. Pharmacokinetic parameters derived from the data shown in fig. S13.**

Species	Route	dose	Parameter	Units	animal 1	animal 2	animal 3	Mean	SD
mouse	i.v.	3 mg/kg	$t_{1/2}$	hr	1.91	1.60	1.80	<b>1.77</b>	0.16
mouse	i.v.	3 mg/kg	AUC <sub>0-t</sub>	hr*ng/mL	927.1	997.1	1025.2	<b>983.1</b>	50.5
mouse	i.v.	3 mg/kg	Clearance	mL/min/kg	3175.2	2973.0	2889.8	<b>50.2</b>	146.8
mouse	i.v.	3 mg/kg	V <sub>dss</sub>	L/kg	2909.3	2448.5	2192.5	<b>2.52</b>	363.2
mouse	p.o.	30 mg/kg	t <sub>max</sub>	hr	0.25	0.25	0.25	<b>0.25</b>	0.00
mouse	p.o.	30 mg/kg	C <sub>max</sub>	ng/mL	6920.0	7530.0	8090.0	<b>7513.3</b>	585.2
mouse	p.o.	30 mg/kg	AUC <sub>0-t</sub>	hr*ng/mL	3227.2	3578.6	3108.1	<b>3304.6</b>	244.6
mouse	p.o.	30 mg/kg	%BA <sub>0-t</sub>		32.8	36.4	31.6	<b>33.6</b>	2.5
rat	i.v.	3 mg/kg	$t_{1/2}$	hr	3.60	3.66	3.38	<b>3.55</b>	0.15
rat	i.v.	3 mg/kg	AUC <sub>0-t</sub>	hr*ng/mL	2252.5	2192.4	2016.0	<b>2153.6</b>	122.9
rat	i.v.	3 mg/kg	Clearance	mL/min/kg	1329.1	1365.7	1486.7	<b>23.2</b>	82.5
rat	i.v.	3 mg/kg	V <sub>dss</sub>	L/kg	1899.0	1834.0	1387.3	<b>1.71</b>	278.6
rat	p.o.	30 mg/kg	t <sub>max</sub>	hr	0.25	0.25	0.25	<b>0.25</b>	0.00
rat	p.o.	30 mg/kg	C <sub>max</sub>	ng/mL	13300.0	12650.0	11100.0	<b>12350.0</b>	1130.3
rat	p.o.	30 mg/kg	AUC <sub>0-t</sub>	hr*ng/mL	14232.7	12254.2	14474.9	<b>13653.9</b>	1218.2
rat	p.o.	30 mg/kg	%BA <sub>0-t</sub>		66.1	56.9	67.2	<b>63.4</b>	5.7

**Table S6. Renal excretion of MMV689258 in rats.** The compound was dosed intravenously to three individual rats at the dosages indicated in the table. Compound levels were determined in urine collected at 0-6, 6-24 and 24-48 hour intervals post dosing.

	Rat 3	Rat 4	Rat 5
Dose concentration (mg/mL)	1.5	1.5	1.5
Dose volume (mL)	0.63	0.73	0.70
Dose administered (µg)	947	1099	1043

MMV689258 (IV)	Time	Concentration detected in urine (µg/g)	Net weight of urine (g)	Amount in urine (µg)	% Dose in urine
Rat 3	0 - 6 hour	31	5.7	178	19
	6 - 24 hour	1.5	18	28	3.0
	24 - 48 hour	0.16	28	4.7	0.49
	Total		53	211	22
Rat 4	0 - 6 hour	111	3.5	387	35
	6 - 24 hour	1.4	15	20	1.8
	24 - 48 hour	0.20	18	3.6	0.33
	Total		36	411	37
Rat 5	0 - 6 hour	33	3.5	116	11
	6 - 24 hour	4.0	16	66	6.4
	24 - 48 hour	0.86	18	16	1.5
	Total		38	198	19
			Mean % dose in urine		26

**Table S7. Biliary excretion of MMV689258 in rats.** The compound was dosed intravenously to three individual bile-duct cannulated rats at the dosages indicated in the table. Post-dosing, compound levels were determined in bile collected at the intervals indicated in the table.

	Rat 1	Rat 2
Dose concentration (mg/mL)	1.5	1.5
Dose volume (mL)	0.52	0.48
Dose administered (µg)	784	720

MMV689258 (IV)	Time	Concentration detected in bile (µg/g)	Net weight of bile (g)	Amount in bile (µg)	% Dose in bile
Rat 1	0 - 1 hour	2.02	1.2	2.5	0.32
	1 - 3 hour	0.12	2.2	0.26	0.03
	3 - 8 hour	0.01	5.3	0.06	0.01
	8 - 24 hour	0.03	17	0.57	0.07
	24 - 48 hour	<LLOQ	16	-	-
	Total		42.3	<b>3.4</b>	<b>0.43</b>
Rat 2	0 - 1 hour	1.7	1.4	2.3	0.32
	1 - 3 hour	0.4	2.0	0.76	0.11
	3 - 8 hour	<LLOQ	5.8	-	-
	8 - 24 hour	<LLOQ	15	-	-
	24 - 48 hour	<LLOQ	16	-	-
	Total		40.8	<b>3.0</b>	<b>0.42</b>
			<b>Mean % dose in bile</b>		<b>0.43</b>



**Table S8. In vitro ADME parameters of MMV689258.** The table shows clearance in human primary hepatocytes, plasma protein binding, inhibition of human CYP450 enzymes and results from Caco-2 permeability assays.

Parameter	Unit	Mouse	Rat	Human
hepatocyte Clint	$\mu\text{l}/\text{min}/10^6 \text{ cells}$	3	2	0.8
plasma protein binding	% bound	32.8	50.2	36.2
CYP inhibition (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) $\text{IC}_{50}$	$\mu\text{M}$	n.d.	n.d.	>20
Caco-2 permeability	cm/s	n.a.	n.a.	$3.5 \times 10^{-6}$ (A->B) $9.6 \times 10^{-6}$ (B->A)

**Table S9. Primers used for genetic studies.**

Primer name	Primer sequence
<b>General primers</b>	
Sequencing-AcCS-F	GGAAGAGATATAGATGGTACTGC
Sequencing-AcCS-R	CGTCCAGATATCCAATAGTATCC
Sequencing-ACS11-F	GATAAGCTAAAGAAGGAACAAGC
Sequencing-ACS11-R	TAACATGTCTTACAATTTTATGAGTTGG
Sequencing guide 1	CGGGAATTCGATTGTGGA
Sequencing guide 2	GCTTGGGGAGATCCGCC
<b>Acetyl-CoA synthetase (PF3D7_0627800)</b>	
Guide-F T627A mutation	ATTGATACCAACATATCCAGATTG
Guide-R T627A mutation	AAACCAATCTGGATATGTTGGTAT
Guide-F A627T reverse mutation	ATTGATACCAGCATATCCAGATTG
Guide-R A627T reverse mutation	AAACCAATCTGGATATGCTGGTAT
Mutation-F T627A	GTTATTTTTTCTTCTATACCAGCATATCCTGACTGTGGTAGATACT
Mutation-R T627A	AGTATCTACCACAGTCAAGGATATGCTGGTATAGAAGAAAAAATAAC
Reverse mutation-F A627T	GTTATTTTTTCTTCTATACCAACATATCCTGACTGTGGTAGATACT
Reverse mutation-R A627T	AGTATCTACCACAGTCAAGGATATGTTGGTATAGAAGAAAAAATAAC
HR-F-EcoRI	TATAAAGAATTTCGGATCAACTGGTAAACCTAAAGG
HR-R-AatII	AATATAGACGCTCTCTGAACGCTCCATCTCC
<b>Acyl-CoA synthetase 11 (PF3D7_1238800)</b>	
Guide-F K462N mutation	ATTGAAAAATTCATGATAGTGTTA
Guide-R K462N mutation	AAACTAACACTATCATGAATTTTT
Guide-F N462K reverse mutation	ATTGAAACATTTCATGATAGTGTTA
Guide-R N462K reverse mutation	AAACTAACACTATCATGAATGTTT
Mutation-F K462N	TATTACAAAAACATTTCATGATAGCGTAATGGAAGGA
Mutation-R K462N	TCCTTCCATTACGCTATCATGAATGTTTTGTAATA
Reverse mutation-F N462K	TATTACAAAAAATTTCATGATAGCGTAATGGAAGGA
Reverse mutation-R N462K	TCCTTCCATTACGCTATCATGAATTTTTGTAATA
HR-F-EcoRI	TATTTTGAATTCTCAGGTTCTTCAGGGACACC
HR-R-AatII	AATATAGACGTCCTACTTCATGTTTTGAACTTTGACC
Guide-F E660K mutation / Guide-F K660E reverse mutation	ATTGAATATATTTAAGTTAGCACA
Guide-R E660K mutation / Guide-R K660E reverse mutation	AAACTGTGCTAACTTAAATATATT
Mutation-F E660K	TTTAAGTTGGCTCAAGGAAATACATAGAACCAGAAAAACT
Mutation-R E660K	AGTTTTTCTGGTTCTATGTATTTTCCTTGAGCCAACTTAAA
Reverse mutation-F K660E	TTTAAGTTGGCTCAAGGAGAATACATAGAACCAGAAAAACT
Reverse mutation-R K660E	AGTTTTTCTGGTTCTATGTATTTCTCCTTGAGCCAACTTAAA
HR-F-EcoRI	TATAAAGAATTCCCTCATTGAAAAATAAACTAGTGC
HR-R-AatII	AATATAGACGTCAGAATGGTGTGGATGTTAAATGG
<b>Multiplex PCR primers</b>	
MWV511	GGAAGAGATATAGATGGTACTGC
MWV512	GATGATTTCCATATACTGTACGTAAC
MWV516	CTACAGTTATTTTTTCTTCTATACCAG
MWV519	TCTACCACAATCTGGATATGT