Supplementary Material for:

Derivatives of the β-crinane Amaryllidaceae Alkaloid Haemanthamine as Multi-Target Directed Ligands for Alzheimer´s Disease

Eliška Kohelová [1], Rozálie Peřinová [1], Negar Maafi[1], Jan Korábečný [2,3], Daniela Hulcová [1,4], Jana Maříková [5], Tomáš Kučera [2], Loreto Martine-Gonzalez [6], Martina Hrabinova [2], Katarina Vorčáková [7], Lucie Nováková [8], Angela De Simone [9], Radim Havelek [10],Lucie Cahlíková [1]*

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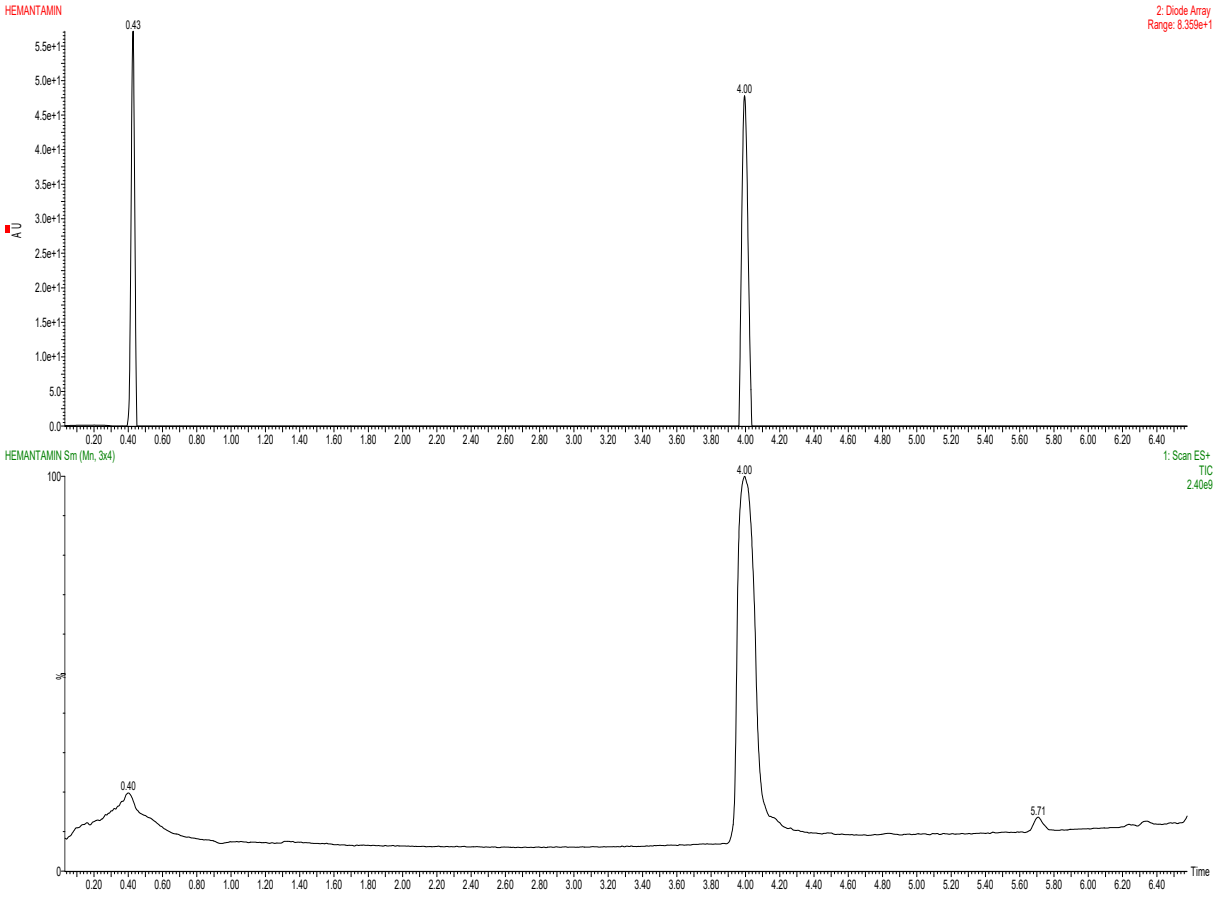
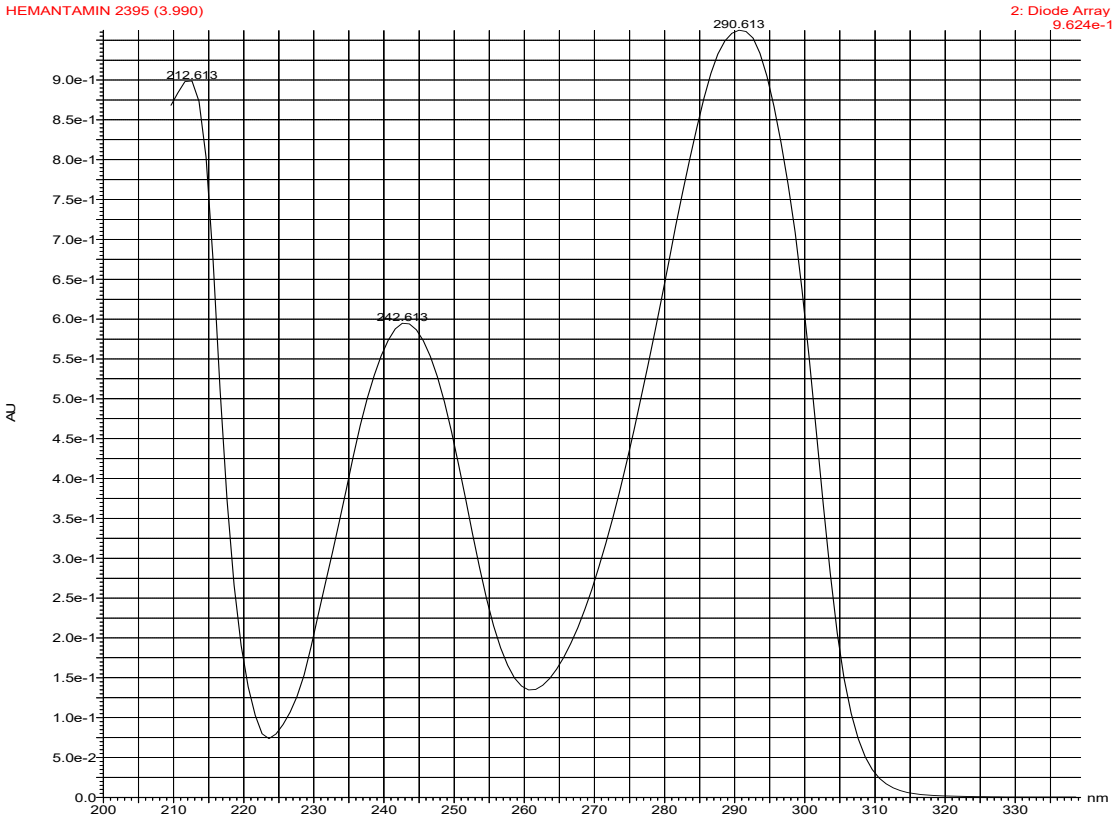
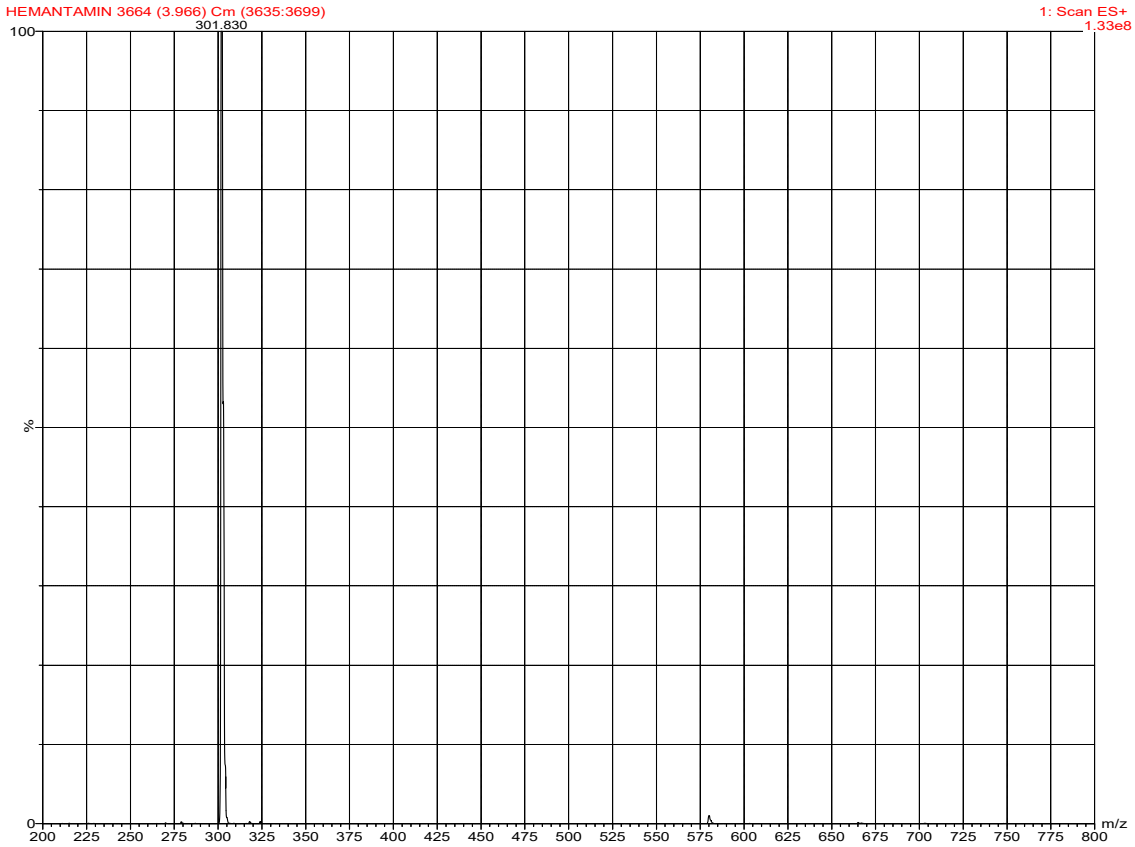
Analytical spectra of haemanthamine:

HPLC (UV/VIS)

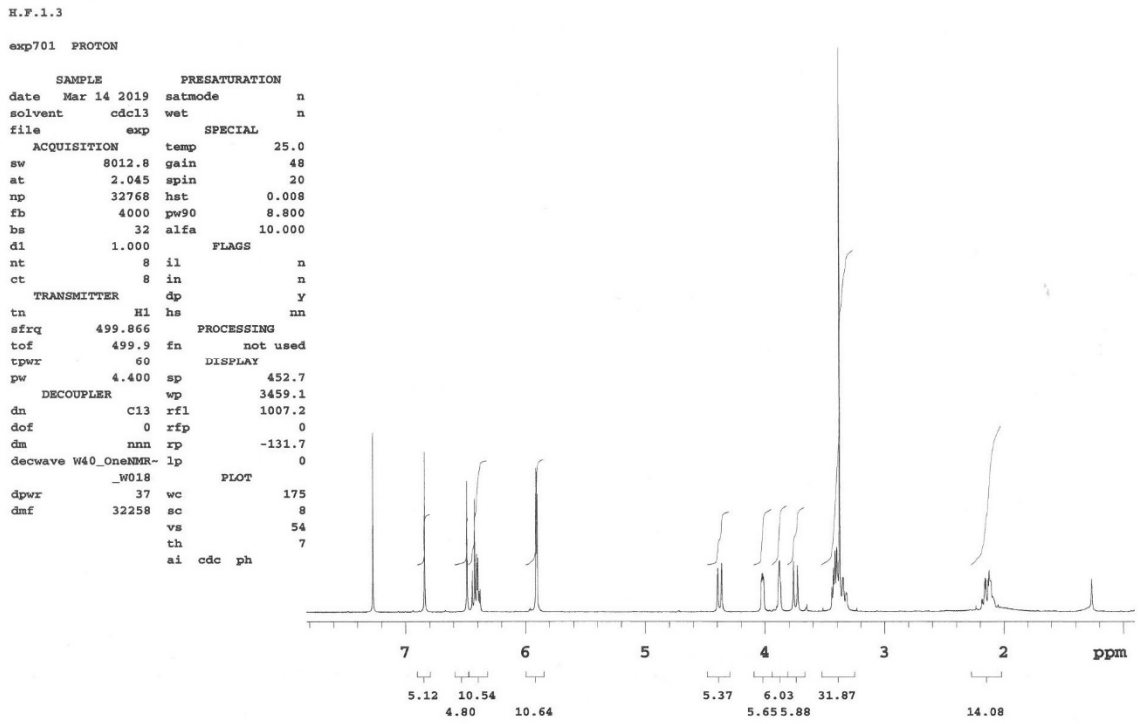



HEMANTAMIN 2395 (3.990)
2: Diode Array
9.624e-1
290.613
212.613
242.613
AU
nm

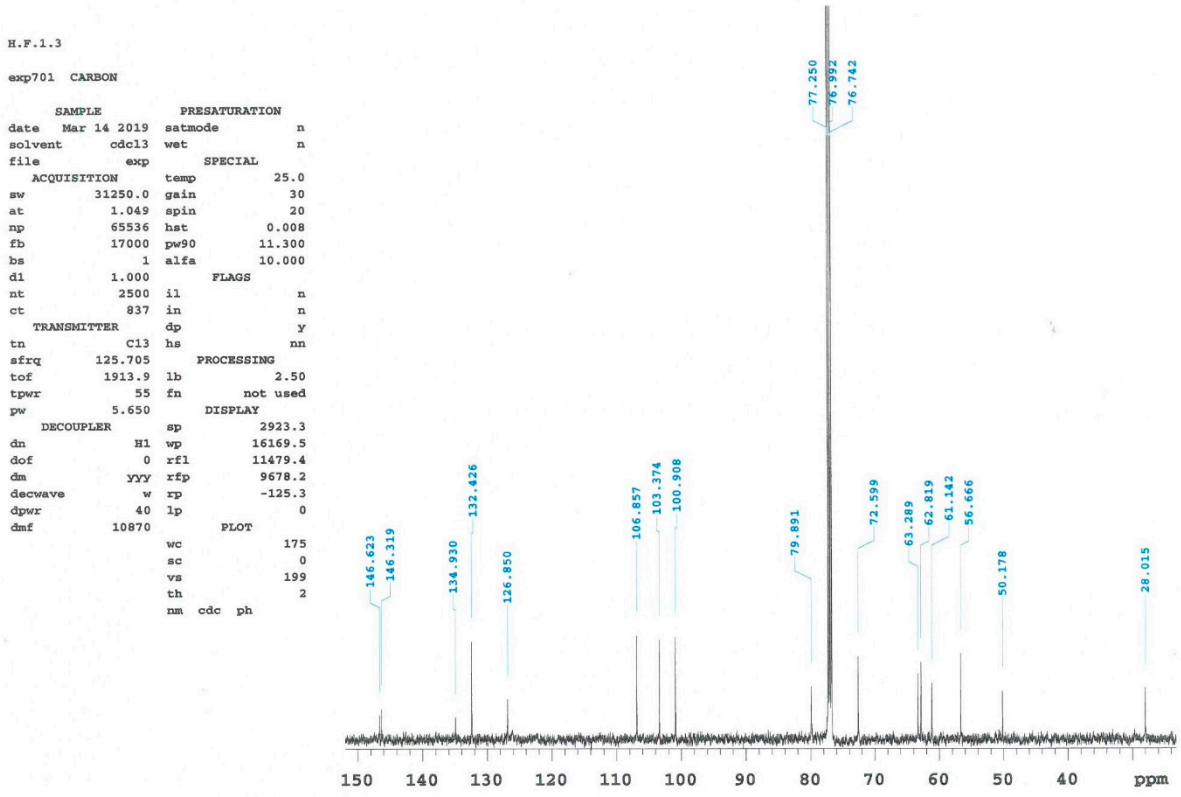
HPLC/MS


HEMANTAMIN 3664 (3.966) Cm (3635:3699)
1: Scan ES+
1.33e8
301.830
%
m/z

^{1}H-NMR



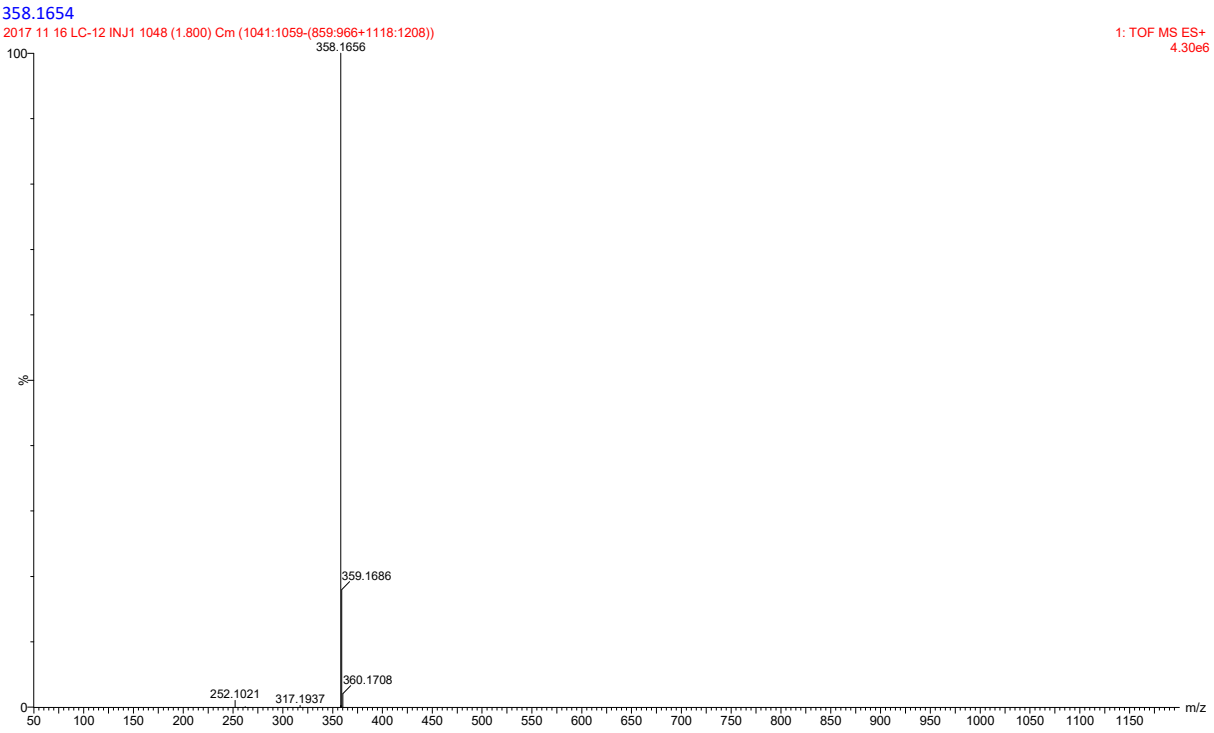
^{13}C-NMR



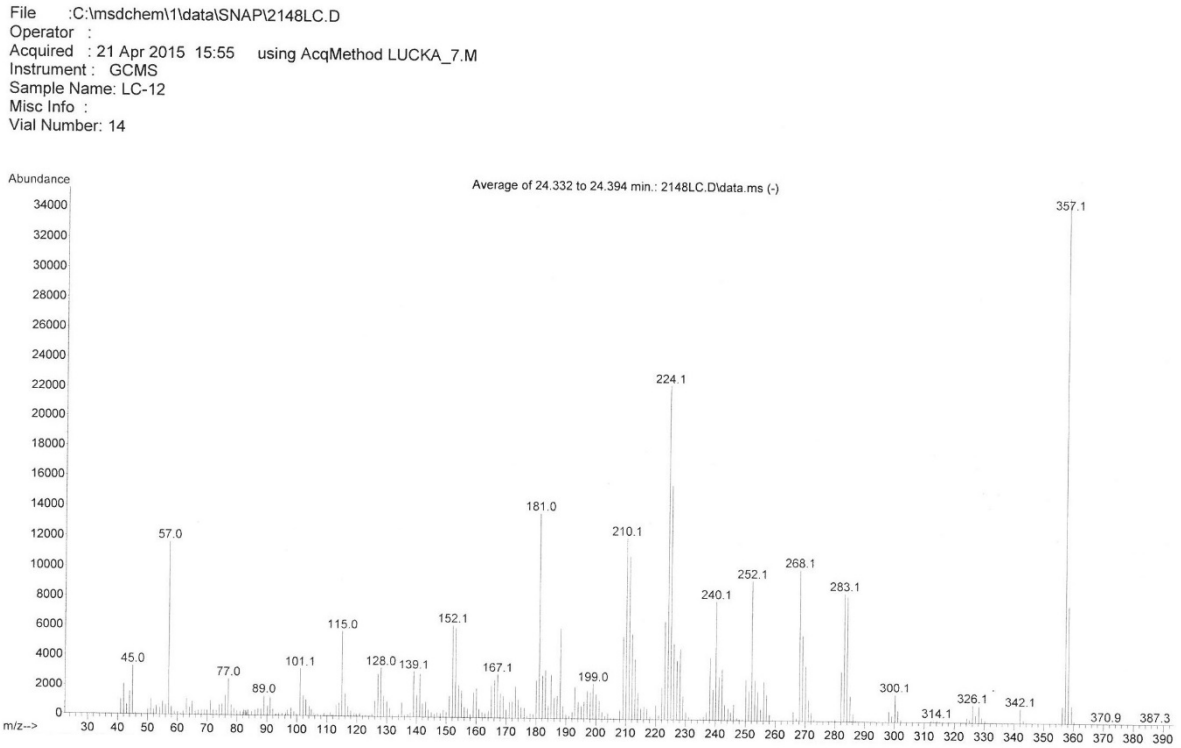
Analytical spectra of prepared derivatives:

11-*O*-Propionylhaemanthamine (1b)

ESI-HRMS



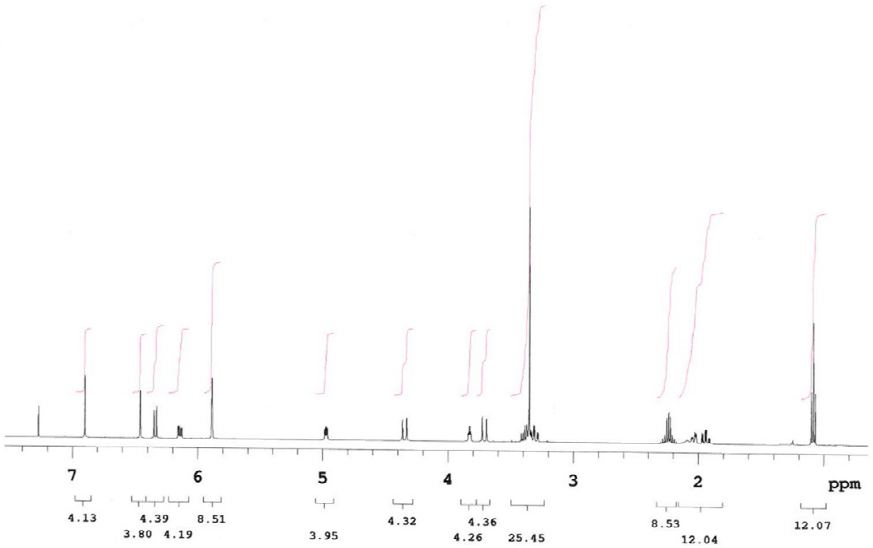
EI-MS



^{1}H-NMR

exp109 PROTON

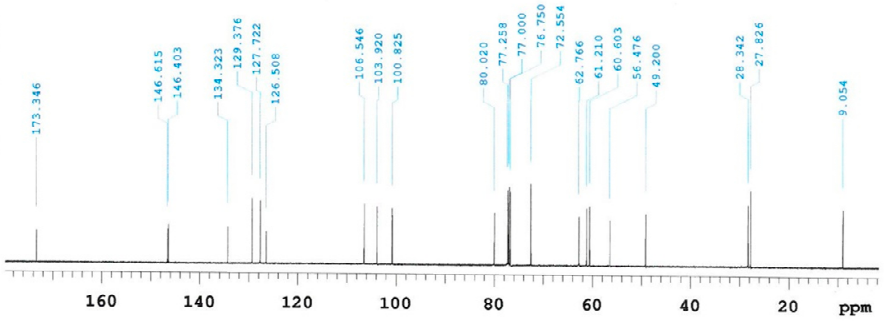
SAMPLE		PRESATURATION	
date	Aug 4 2015	satmode	n
solvent	cdcl3	wet	n
file	/home/vnmrl/v~	SPECIAL	
nmrsys/data/Lucie/~		temp	25.0
Obmeny/LC-12/LC-12~		gain	30
	_H.fid	spin	not used
ACQUISITION		hst	0.008
sw	8012.8	pw90	9.100
at	2.045	alfa	10.000
np	32768	FLAGS	
fb	4000	il	n
bs	32	in	n
d1	1.000	dp	y
nt	8	hs	nn
ct	8	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	499.866	sp	326.5
tof	499.9	wp	3441.5
tpwr	60	rfl	1007.2
pw	4.550	rfp	0
DECOUPLER		rp	-163.2
dn	C13	lp	0
dof	0	PLOT	
dm	nnn	wc	190
decwave	W40_OneNMR~	sc	8
	_W018	vs	14
dpwr	37	th	7
dmf	32258	ai cdc ph	



^{13}C-NMR

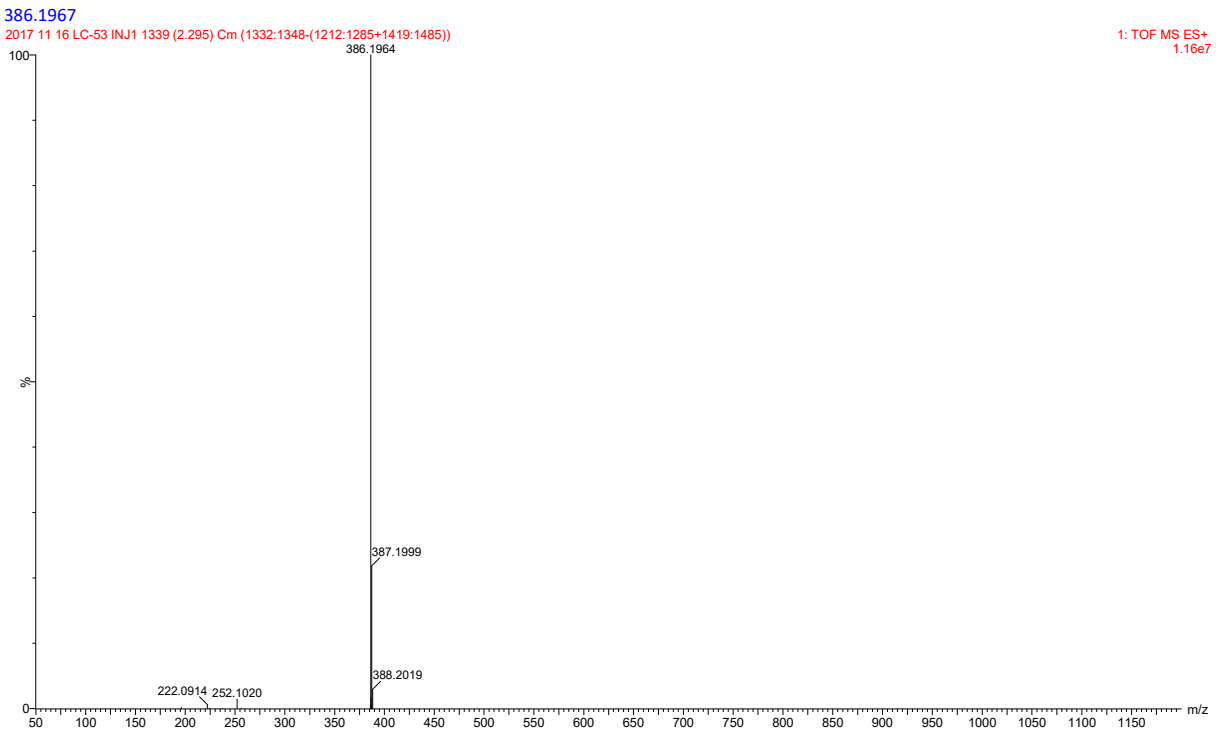
exp109 CARBON

SAMPLE		PRESATURATION	
date	Aug 4 2015	satmode	n
solvent	cdcl3	wet	n
file	/home/vnmrl/v~	SPECIAL	
nmrsys/data/Lucie/~		temp	25.0
Obmeny/LC-12/LC-12~		gain	30
	_C.fid	spin	not used
ACQUISITION		hst	0.008
sw	31250.0	pw90	11.300
at	1.049	alfa	10.000
np	65536	FLAGS	
fb	17000	il	n
bs	1	in	n
d1	1.000	dp	y
nt	1500	hs	nn
ct	790	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.705	DISPLAY	
tof	1913.9	sp	218.7
tpwr	55	wp	22357.9
pw	5.650	rfl	11482.2
DECOUPLER		rfp	9678.2
dn	H1	rp	48.3
dof	0	lp	0
dm	yyy	PLOT	
decwave	w	wc	190
dpwr	41	sc	8
dmf	12346	vs	17
		th	4
		nm cdc ph	



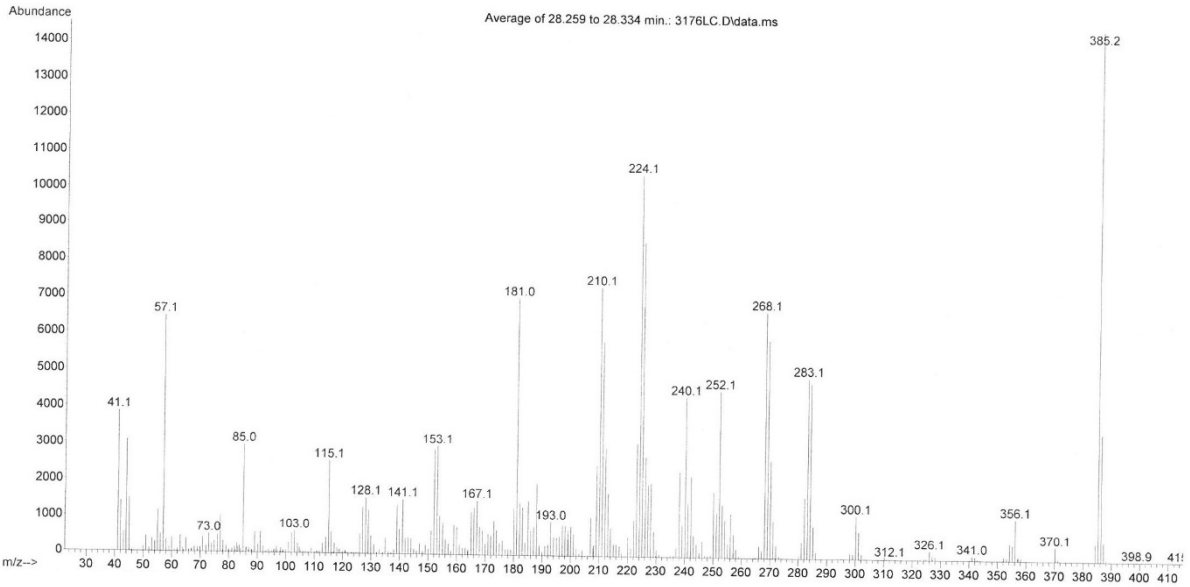
11-*O*-Pentanoylhaemanthamine (1d)

ESI-HRMS



EI-MS

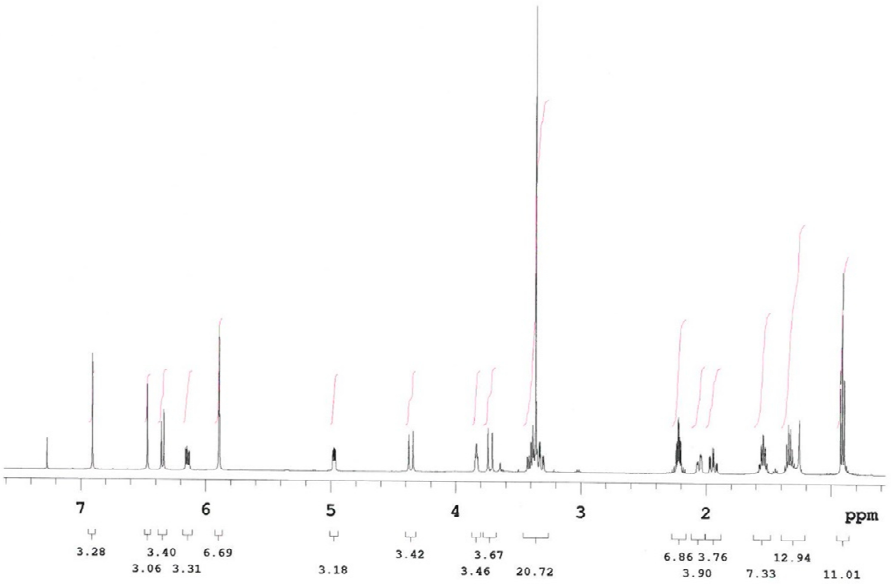
File :C:\msdchem\1\data\SNAP\3176LC.D
Operator :
Acquired : 24 Oct 2016 14:47 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-53
Misc Info :
Vial Number: 8



^{1}H-NMR

exp100 PROTON

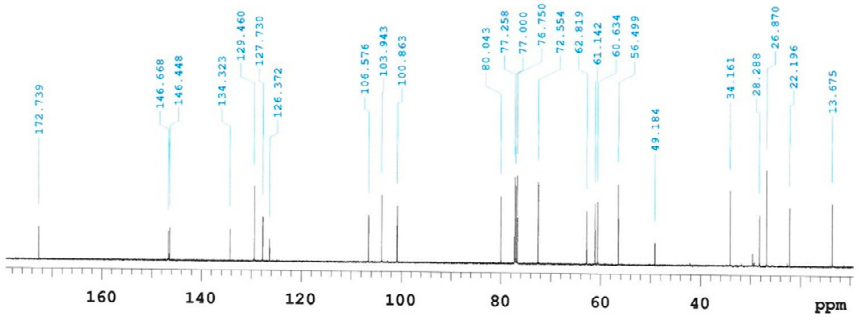
SAMPLE		PRESATURATION	
date	Apr 4 2017	satmode	n
solvent	cdcl3	wet	n
file	exp	SPECIAL	
ACQUISITION		temp	25.0
sw	8012.8	gain	28
at	2.045	spin	20
np	32768	hst	0.008
fb	4000	pw90	9.100
bs	32	alfa	10.000
dl	1.000	FLAGS	
nt	8	il	n
ct	8	in	n
TRANSMITTER		dp	y
tn	H1	hs	nn
sfrq	499.866	PROCESSING	
tof	499.8	fn	not used
tpwr	60	DISPLAY	
pw	4.550	sp	284.3
DECOUPLER		wp	3523.2
dn	C13	rfl	1007.3
dof	0	rfp	0
dm	nnn	rp	-160.2
decwave	W40_OneNMR~_W018	lp	0
		PLOT	
dpwr	37	wc	190
dmf	32258	sc	8
		vs	48
		th	7
		ai cdc ph	



^{13}C-NMR

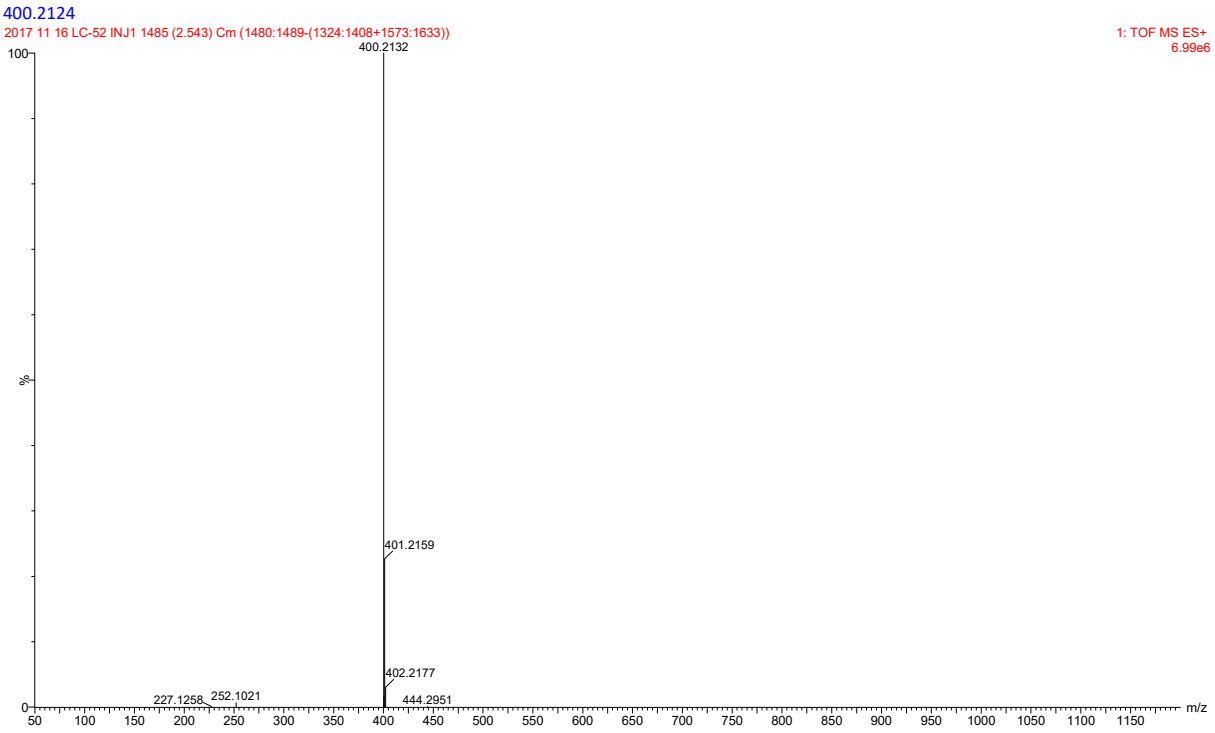
exp100 CARBON

SAMPLE		PRESATURATION	
date	Apr 4 2017	satmode	n
solvent	cdcl3	wet	n
file	exp	SPECIAL	
ACQUISITION		temp	25.0
sw	31250.0	gain	30
at	1.049	spin	20
np	65536	hst	0.008
fb	17000	pw90	11.300
bs	1	alfa	10.000
dl	1.000	FLAGS	
nt	1500	il	n
ct	750	in	n
TRANSMITTER		dp	y
tn	C13	hs	nn
sfrq	125.705	PROCESSING	
tof	1913.9	lb	0.50
tpwr	55	fn	not used
pw	5.650	DISPLAY	
DECOUPLER		sp	1180.9
dn	H1	wp	21351.8
dof	0	rfl	11480.3
dm	yyy	rfp	9678.2
decwave	w	rp	-177.7
dpwr	40	lp	0
dmf	11173	PLOT	
		wc	190
		sc	0
		vs	21
		th	3
		nm cdc ph	



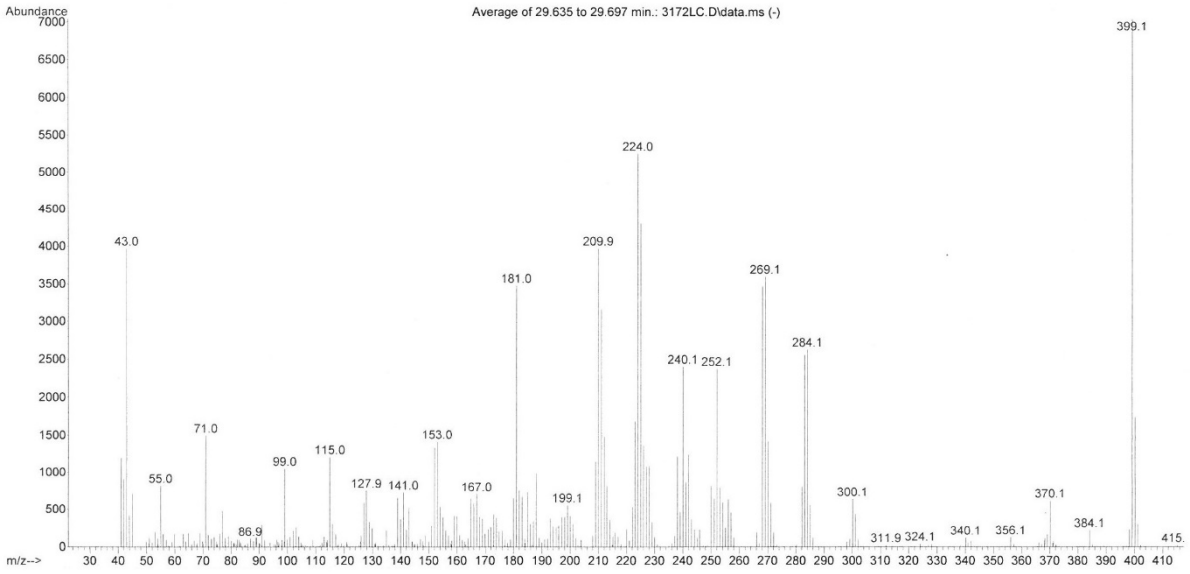
11-O-Hexanoylhaemanthamine (1e)

ESI-HRMS



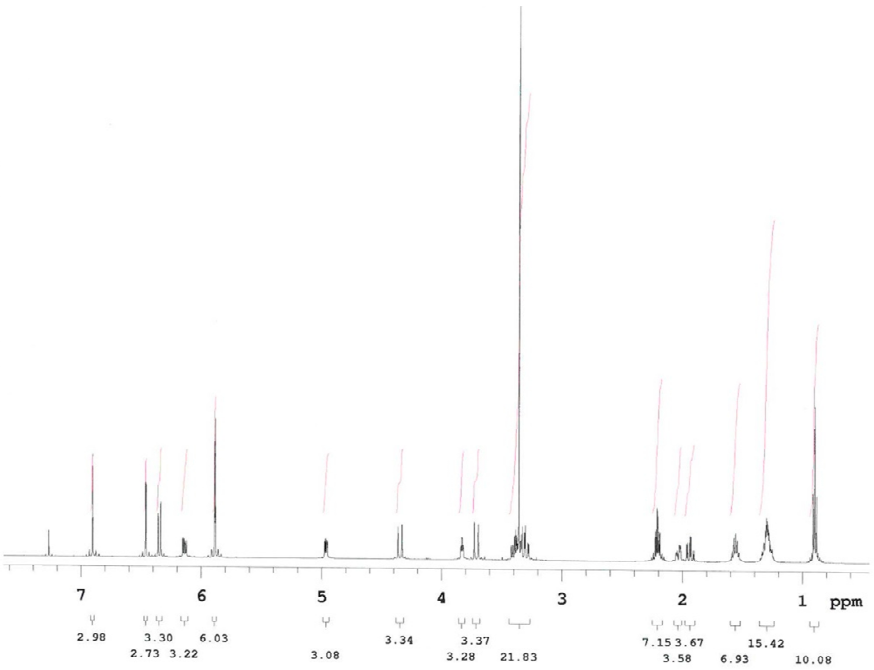
EI-MS

File :C:\msdchem\1\data\SNAP\3172LC.D
Operator :
Acquired : 18 Oct 2016 13:31 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-52
Misc Info :
Vial Number: 5



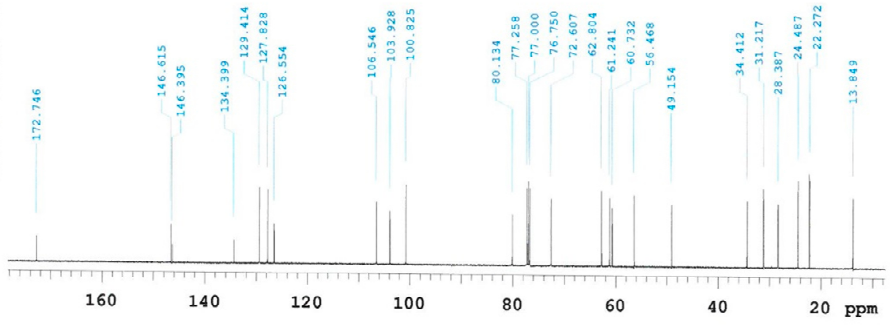
^{1}H-NMR

```
exp8  PROTON

      SAMPLE
date   Mar 21 2017
solvent       cdcl3
file /home/vnmr1/v~
nmrsys/data/Lucie/~
Obmeny/LC-52_H.fid
   ACQUISITION
sw           8012.8
at            2.045
np            32768
fb             4000
bs               32
dl            1.000
nt                8
ct                8
   TRANSMITTER
tn               H1
sfrq        499.866
tof           499.9
tpwr             60
pw            4.550
    DECOUPLER
dn              C13
dof               0
dm              nnn
decwave W40_OneNMR~
              _W018
dpwr             37
dmf           32258

  PRESATURATION
satmode           n
wet               n
     SPECIAL
temp           25.0
gain             28
spin             20
hst           0.008
pw90          9.100
alfa         10.000
      FLAGS
il                n
in                n
dp                y
hs               nn
    PROCESSING
fn         not used
     DISPLAY
sp           223.3
wp          3600.0
rfl         1007.2
rfp              0
rp           161.8
lp               0
       PLOT
wc             190
sc               8
vs              34
th               7
ai  cdc  ph
```



^{13}C-NMR

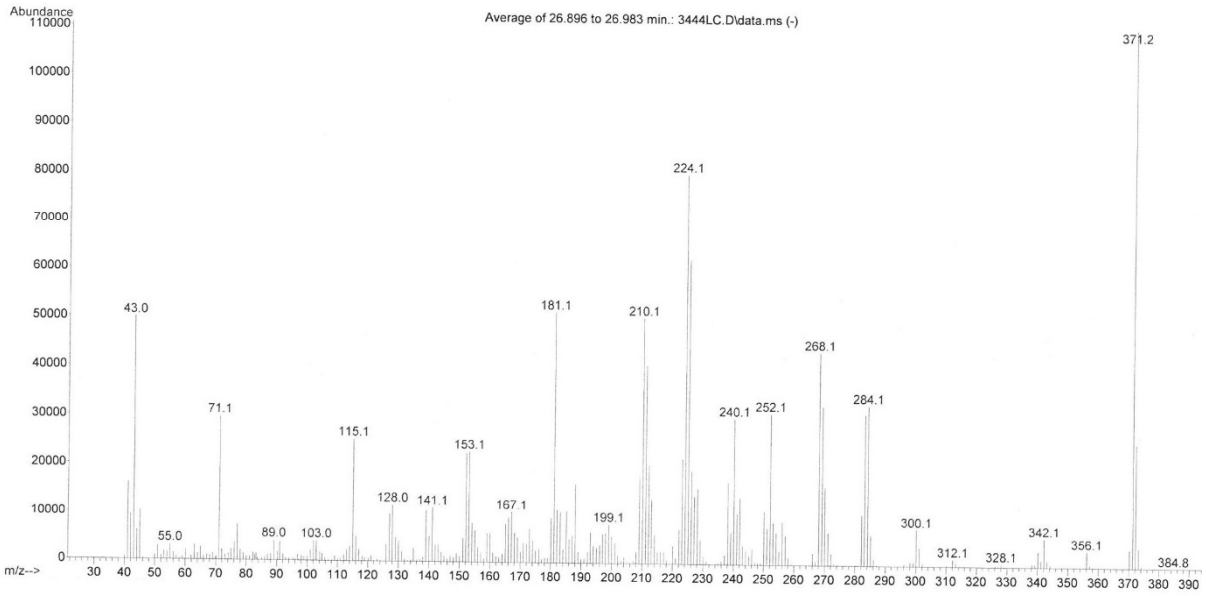
```
exp8  CARBON

      SAMPLE
date   Mar 21 2017
solvent       cdcl3
file /home/vnmr1/v~
nmrsys/data/Lucie/~
Obmeny/LC-52_C.fid
   ACQUISITION
sw          31250.0
at            1.049
np            65536
fb            17000
bs                1
dl            1.000
nt             1000
ct              703
   TRANSMITTER
tn              C13
sfrq        125.705
tof          1913.9
tpwr             55
pw            5.650
    DECOUPLER
dn               H1
dof               0
dm              yyy
decwave           w
dpwr             40
dmf           11173

  PRESATURATION
satmode           n
wet               n
     SPECIAL
temp           25.0
gain             30
spin             20
hst           0.008
pw90         11.300
alfa         10.000
      FLAGS
il                n
in                n
dp                y
hs               nn
    PROCESSING
lb             0.50
fn         not used
     DISPLAY
sp           955.9
wp         21457.7
rfl        11481.3
rfp         9678.2
rp           133.9
lp               0
       PLOT
wc             190
sc               8
vs              20
th               2
nm  cdc  ph
```



11-O-Butanoylhaemanthamine (1f)

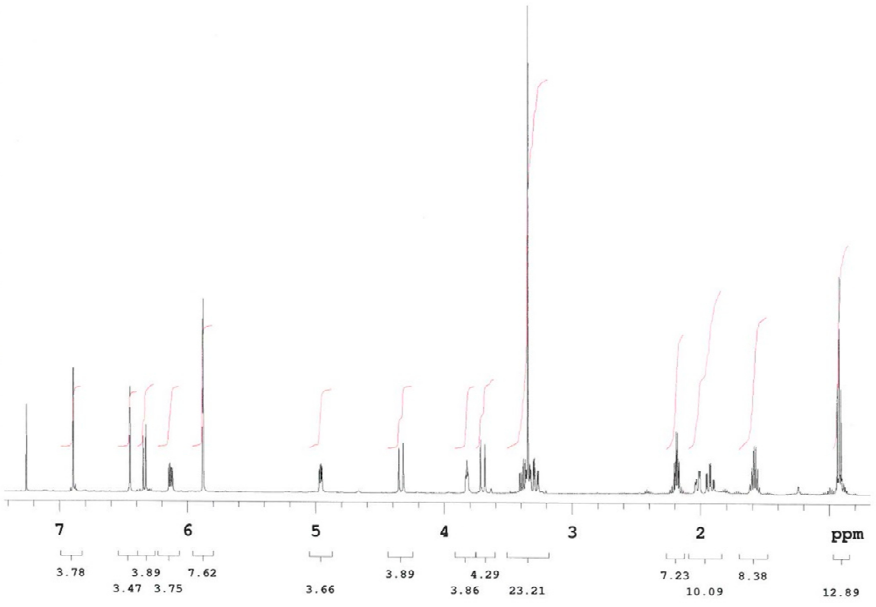
EI-MS

File :C:\msdchem\1\data\SNAP\3444LC.D
Operator : Lucka
Acquired : 24 Mar 2017 13:19 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: JM-1
Misc Info :
Vial Number: 8



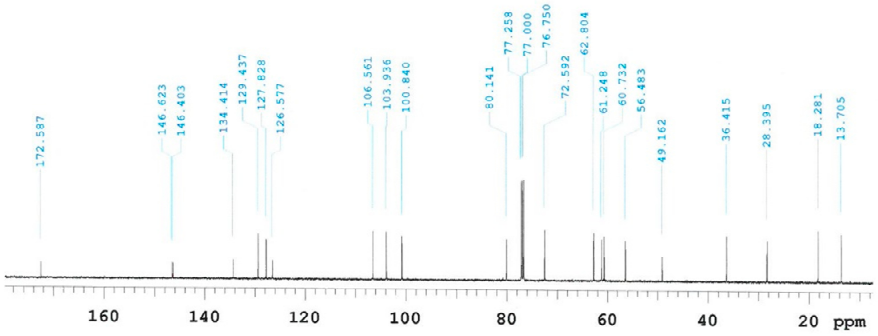
^{1}H-NMR

```
exp9  PROTON

      SAMPLE          PRESATURATION
date   Nov 10 2015  satmode          n
solvent      cdcl3  wet              n
file /home/vnmr1/v~      SPECIAL
nmrsys/data/Zalohy~ temp          25.0
/Hrabalek-160825/N~ gain            30
emecek_Jan/Botanik~ spin       not used
      a/JM-1_H.fid  hst           0.008
   ACQUISITION      pw90          9.100
sw          8012.8  alfa         10.000
at           2.045        FLAGS
np           32768  il               n
fb            4000  in               n
bs              32  dp               y
d1           1.000  hs              nn
nt               8      PROCESSING
ct               8  fn        not used
   TRANSMITTER           DISPLAY
tn              H1  sp           340.5
sfrq       499.866  wp          3372.1
tof          499.9  rfl         4642.7
tpwr            60  rfp         3629.0
pw           4.550  rp             1.8
    DECOUPLER       lp               0
dn             C13         PLOT
dof              0  wc             190
dm             nnn  sc               8
decwave W40_OneNMR~ vs              59
             _W018  th               7
dpwr            37  ai  cdc  ph
dmf          32258
```



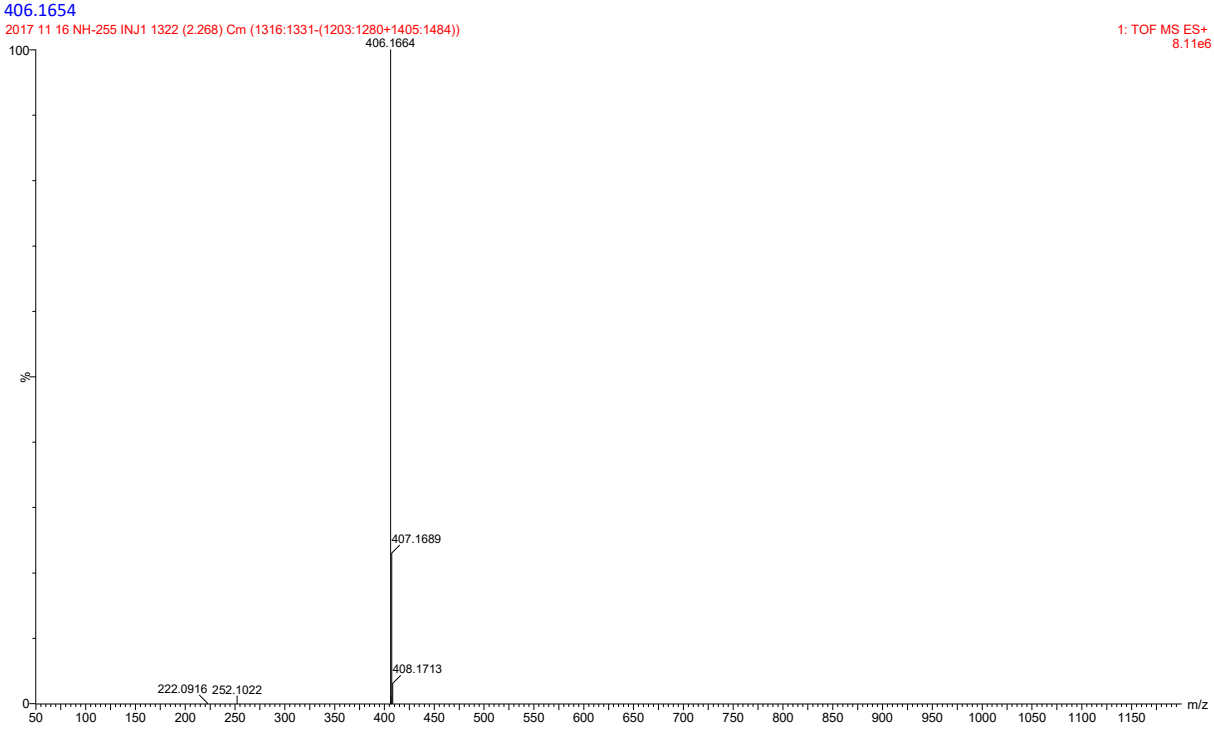
^{13}C-NMR

```
exp9  CARBON

      SAMPLE          PRESATURATION
date   Nov 10 2015  satmode          n
solvent      cdcl3  wet              n
file /home/vnmrl/v~      SPECIAL
nmrsys/data/Zalohy~ temp          25.0
/Hrabalek-160825/N~ gain            30
emecek_Jan/Botanik~ spin       not used
      a/JM-1_C.fid  hst           0.008
   ACQUISITION      pw90         11.300
sw         31250.0  alfa         10.000
at           1.049        FLAGS
np           65536  il               n
fb           17000  in               n
bs               1  dp               y
d1           1.000  hs              nn
nt            1000     PROCESSING
ct             345  lb            1.00
   TRANSMITTER      fn       not used
tn             C13       DISPLAY
sfrq       125.705  sp           934.9
tof         1913.9  wp         21648.4
tpwr            55  rfl        11480.3
pw           5.650  rfp         9678.2
    DECOUPLER       rp          -183.6
dn              H1  lp               0
dof              0        PLOT
dm             yyy  wc             190
decwave          w  sc               8
dpwr            41  vs              22
dmf          12346  th               2
                    nm  cdc  ph
```



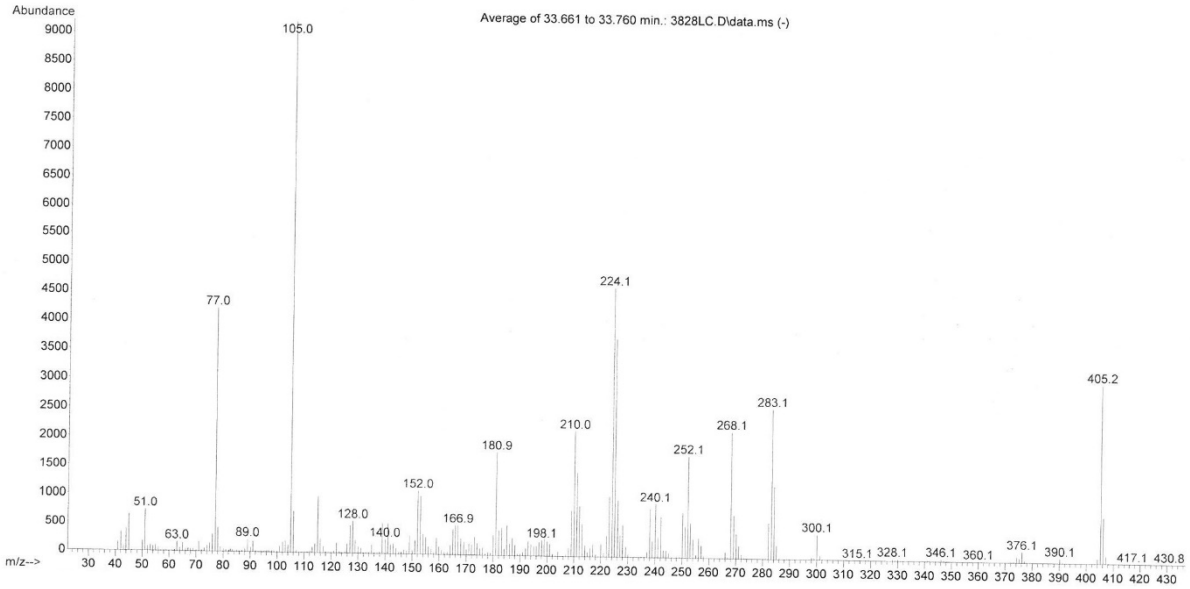
11-O-Benzoylhaemanthamine (1g)

ESI-HRMS



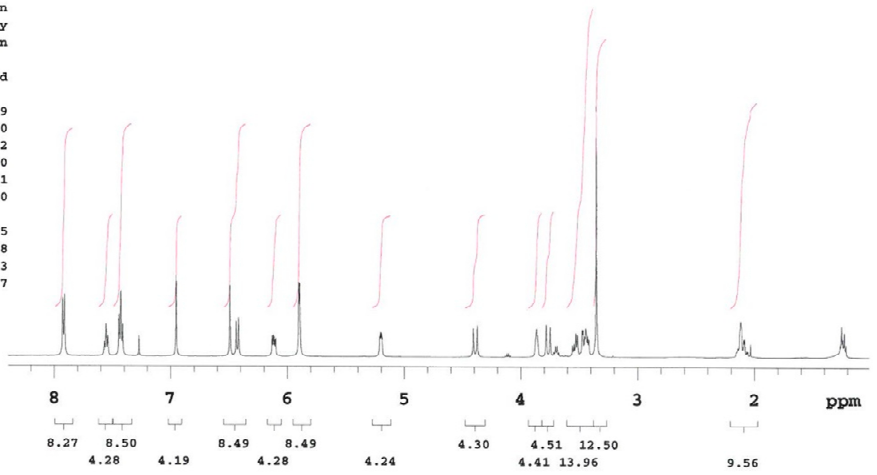
EI-MS

File :C:\msdchem\1\data\SNAP\3828LC.D
Operator : Lucka
Acquired : 17 Jan 2018 10:14 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-118
Misc Info :
Vial Number: 13

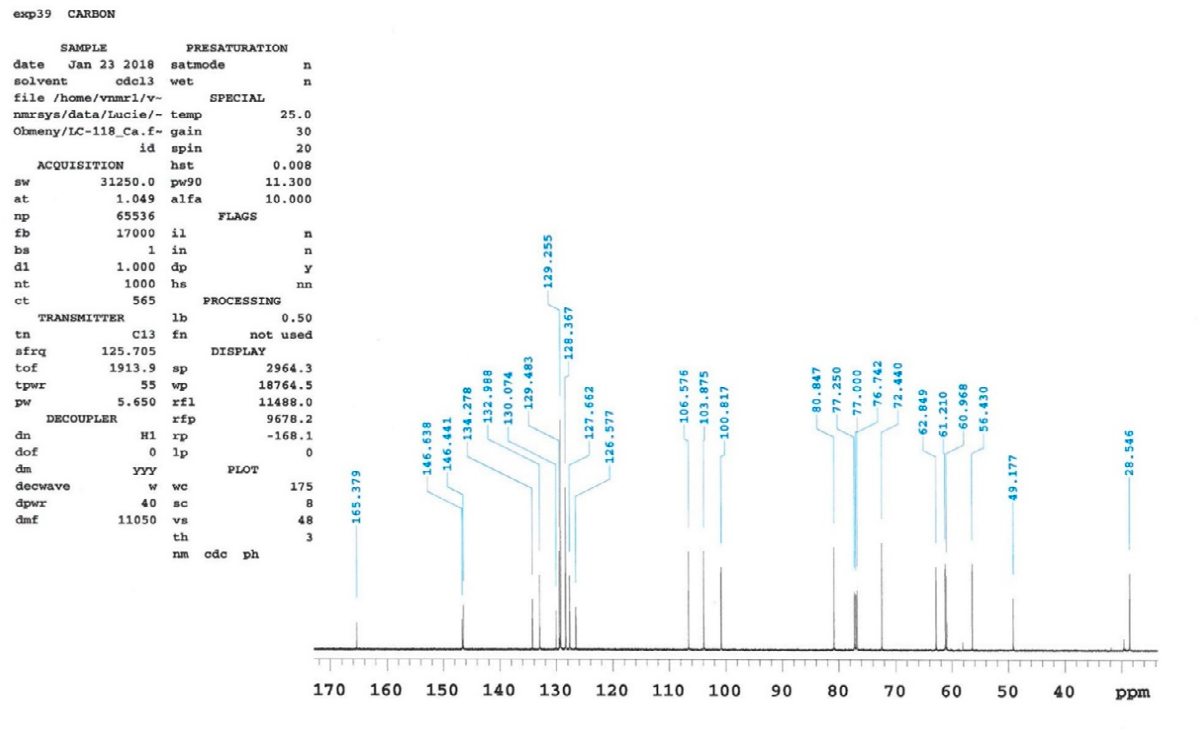


^{1}H-NMR

```
exp39  PROTON

      SAMPLE          PRESATURATION
date   Jan 23 2018  satmode          n
solvent      cdcl3  wet              n
file /home/vnmr1/v~      SPECIAL
nmrsys/data/Lucie/~ temp          25.0
Obmeny/LC-118_Ha.f~ gain            24
                id  spin            20
   ACQUISITION      hst          0.008
sw          8012.8  pw90         8.700
at           2.045  alfa        10.000
np           32768       FLAGS
fb            4000  il               n
bs              32  in               n
d1           1.000  dp               y
nt              8  hs              nn
ct              8      PROCESSING
   TRANSMITTER      fn        not used
tn              H1       DISPLAY
sfrq       499.866  sp           507.9
tof          499.9  wp          3689.0
tpwr            60  rfl         1007.2
pw           4.350  rfp            0
     DECOUPLER      rp          -97.1
dn             C13  lp             0
dof              0        PLOT
dm             nnn  wc           175
decwave W40_OneNMR~ sc             8
              _W018  vs            43
dpwr            37  th             7
dmf          32258  ai  cdc  ph
```

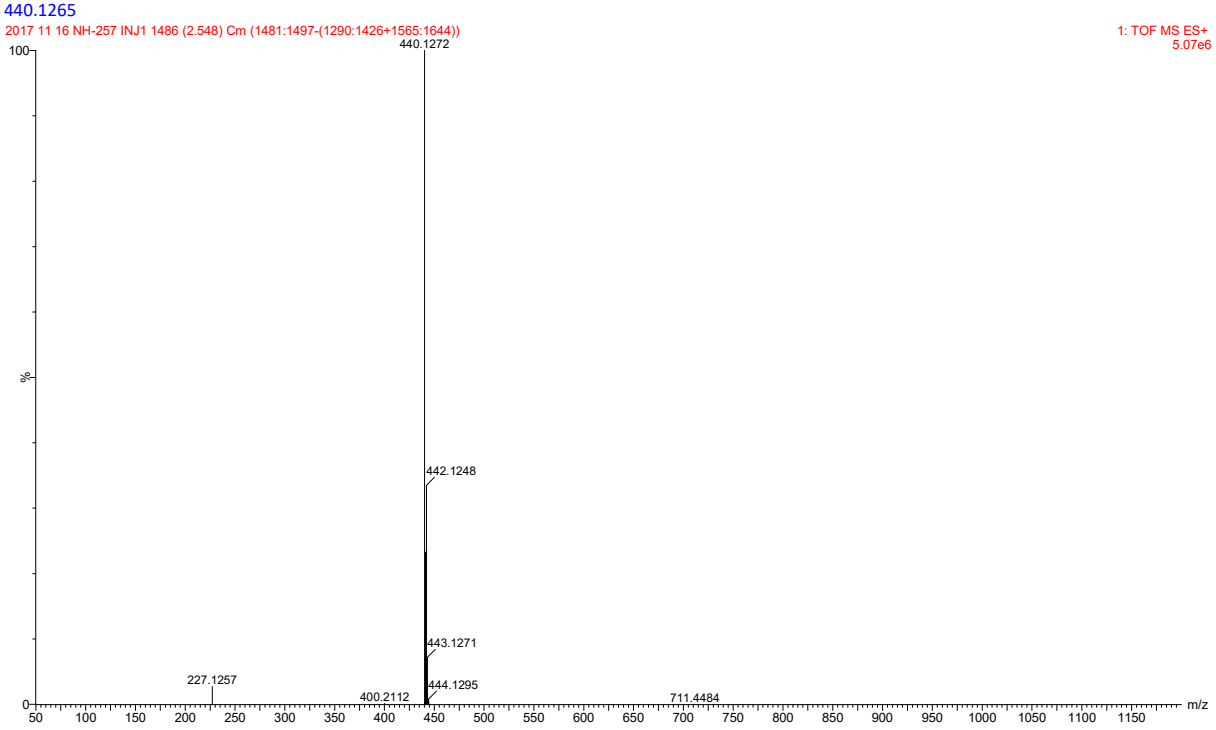


^{13}C-NMR



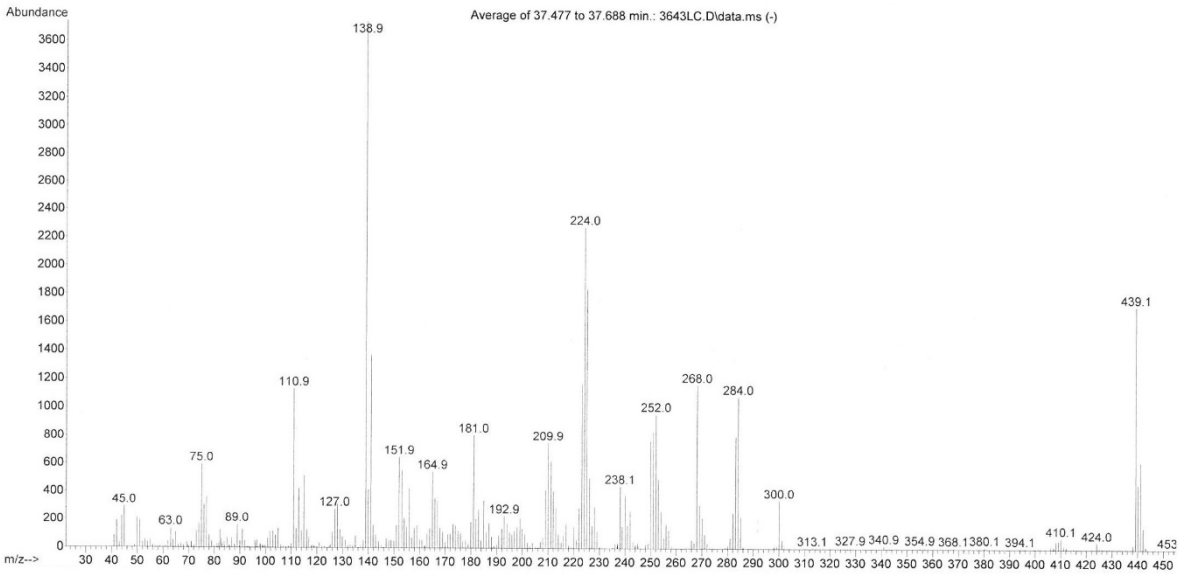
11-O-(3-chlorobenzoyl)haemanthamine (1h)

ESI-HRMS



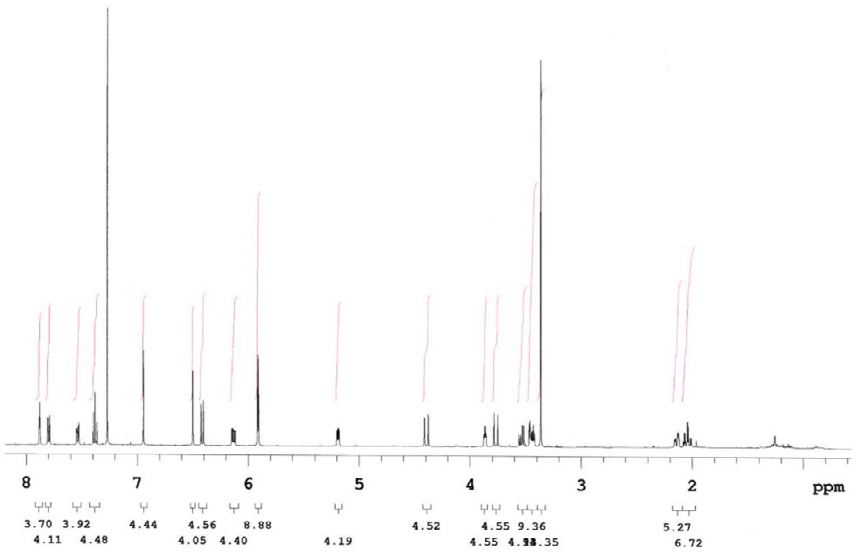
EI-MS

File :C:\msdchem\1\data\SNAP\3643LC.D
Operator :
Acquired : 19 Oct 2017 13:55 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-91
Misc Info :
Vial Number: 12

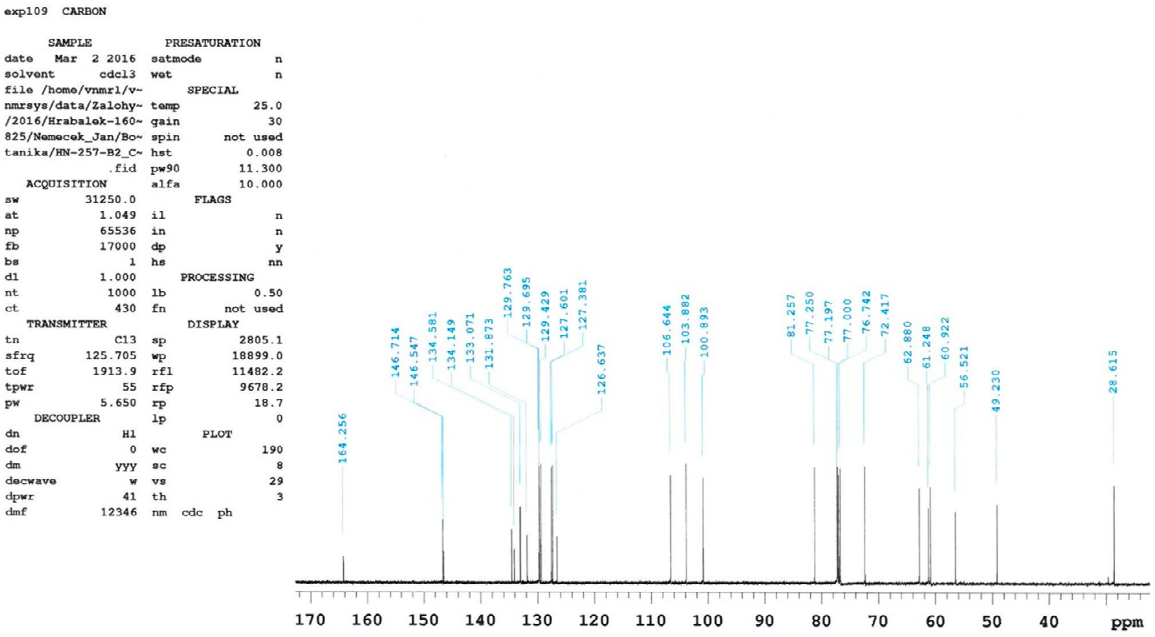


^{1}H-NMR

```
exp109  PROTON

      SAMPLE          PRESATURATION
date   Mar  2 2016  satmode          n
solvent      cdcl3  wet              n
file /home/vnmr1/v~      SPECIAL
nmrsys/data/Zalohy~ temp          25.0
/2016/Hrabalek-160~ gain            30
825/Nemecek_Jan/Bo~ spin       not used
tanika/HN-257-B2_H~ hst           0.008
             .fid   pw90          9.100
   ACQUISITION      alfa         10.000
sw          8012.8        FLAGS
at           2.045  il               n
np          32768  in               n
fb           4000  dp               y
bs             32  hs              nn
d1          1.000      PROCESSING
nt             8  fn        not used
ct             8       DISPLAY
   TRANSMITTER      sp           285.4
tn            H1  wp          3808.8
sfrq       499.866  rfl         1007.2
tof          499.9  rfp            0
tpwr            60  rp          30.5
pw           4.550  lp             0
    DECOUPLER             PLOT
dn            C13  wc           190
dof             0  sc             8
dm            nnn  vs            24
decwave W40_OneNMR~ th           7
             _W018  ai  cdc  ph
dpwr           37
dmf         32258
```

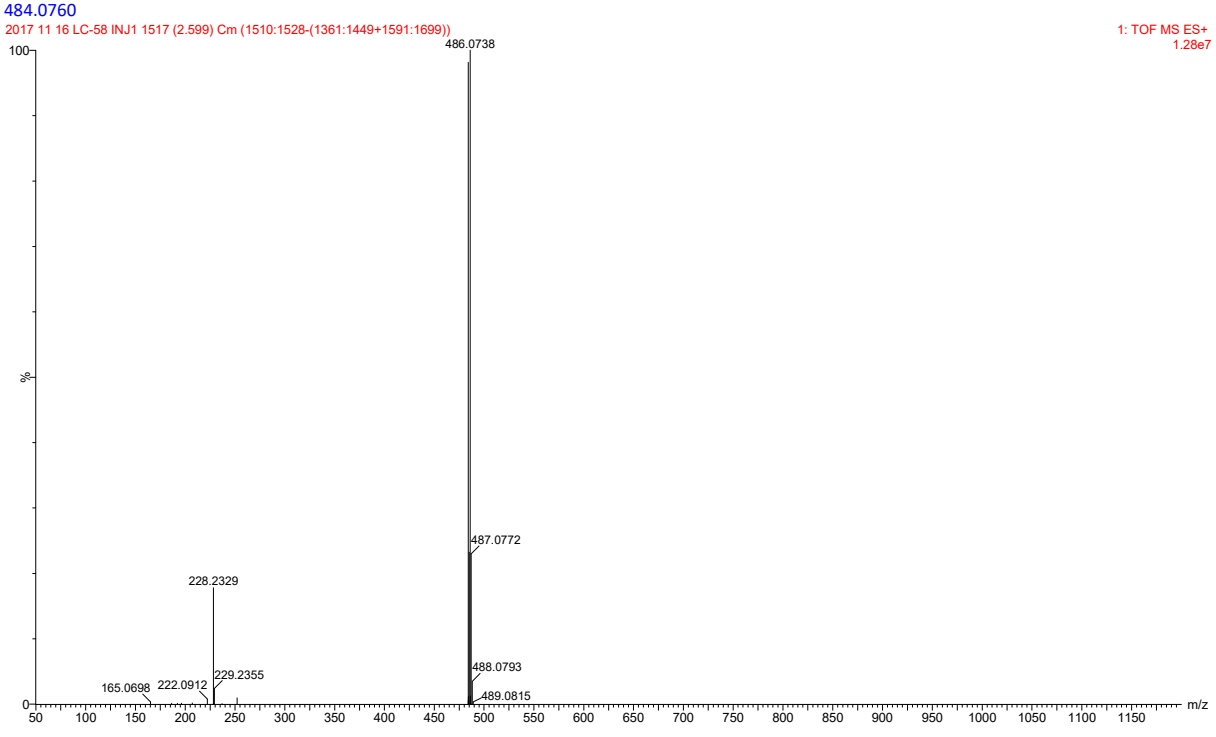


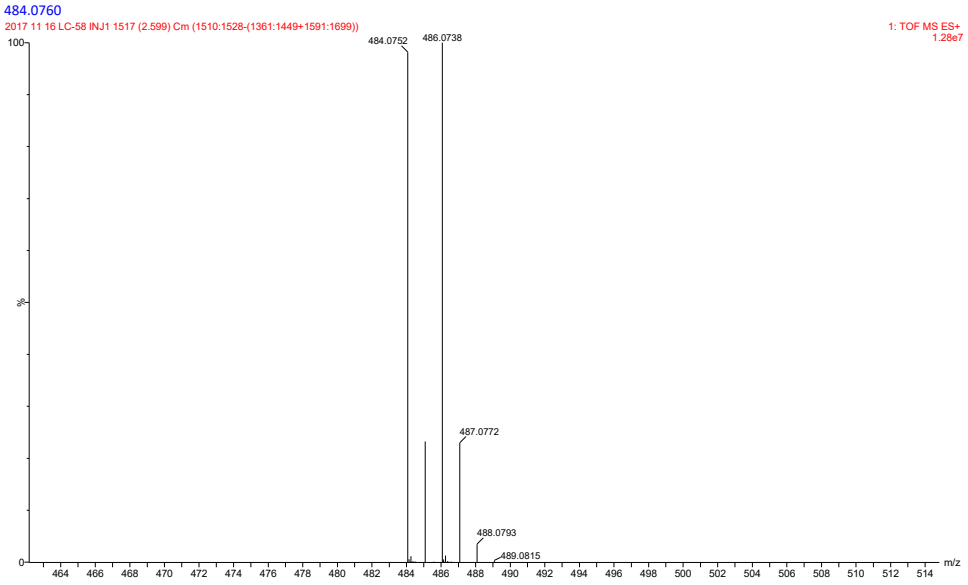
^{13}C-NMR



11-O-(3-bromobenzoyl)haemanthamine (1i)

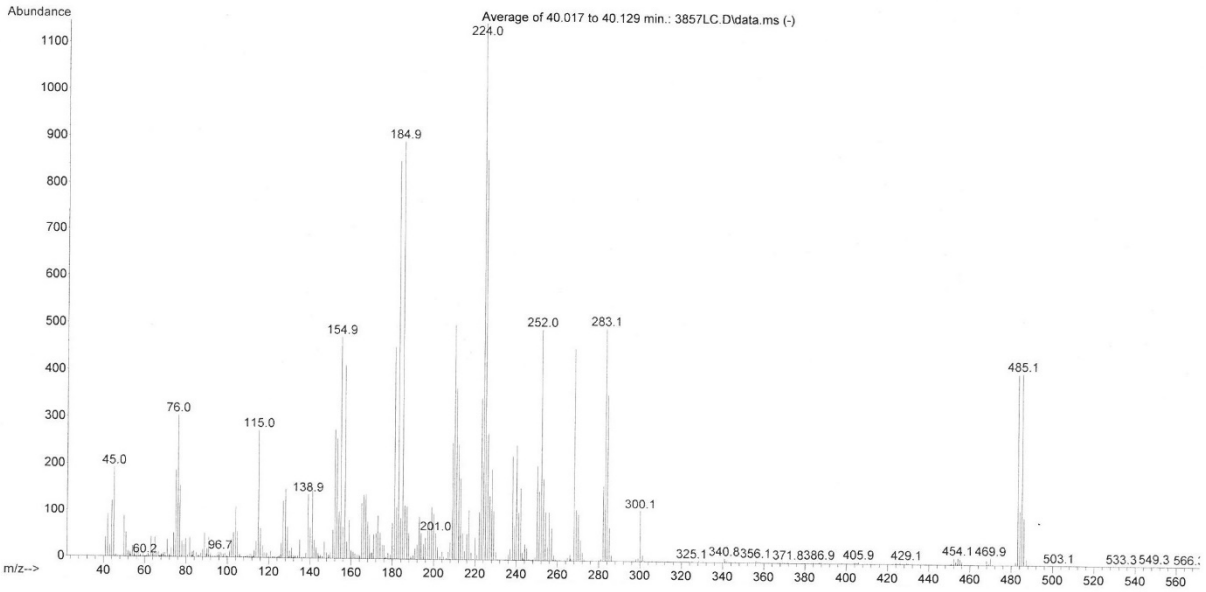
ESI-HRMS





EI-MS

File :C:\msdchem\1\data\SNAP\3857LC.D
Operator : Lucka
Acquired : 25 Jan 2018 15:19 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-58
Misc Info :
Vial Number: 7



^{1}H-NMR

```
exp108  PROTON

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date   Apr 24 2017
solvent       cdcl3
file            exp
   ACQUISITION
sw           8012.8
at            2.045
np            32768
fb             4000
bs               32
d1            1.000
nt                8
ct                8
   TRANSMITTER
tn                H1
sfrq         499.866
tof            499.9
tpwr              60
pw            4.550
    DECOUPLER
dn               C13
dof                0
dm               nnn
decwave W40_OneNMR~
                _W018
dpwr              37
dmf            32258

   PRESATURATION
satmode           n
wet               n
      SPECIAL
temp          25.0
gain            26
spin            20
hst          0.008
pw90         9.100
alfa        10.000
      FLAGS
il                n
in                n
dp                y
hs               nn
    PROCESSING
fn        not used
     DISPLAY
sp           420.9
wp          3753.6
rfl         1007.2
rfp               0
rp             56.5
lp                0
      PLOT
wc              200
sc                8
vs               32
th                7
ai  cdc  ph
```
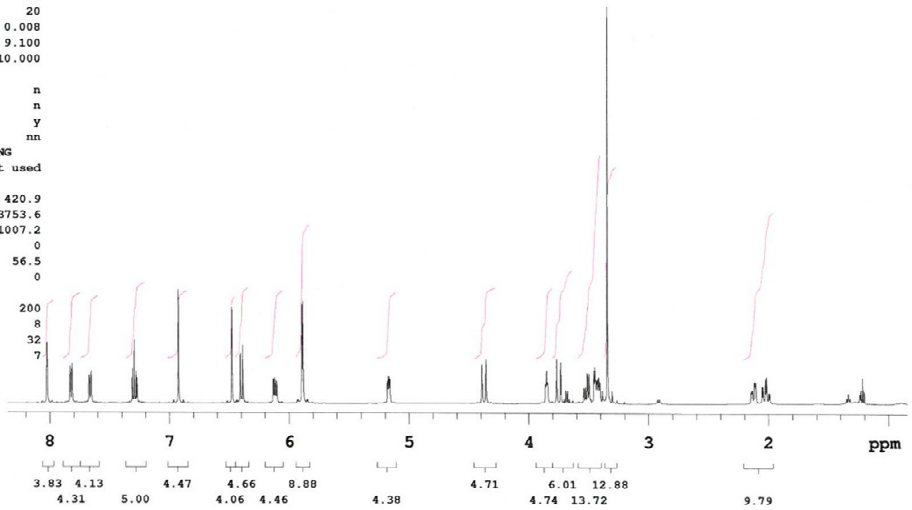
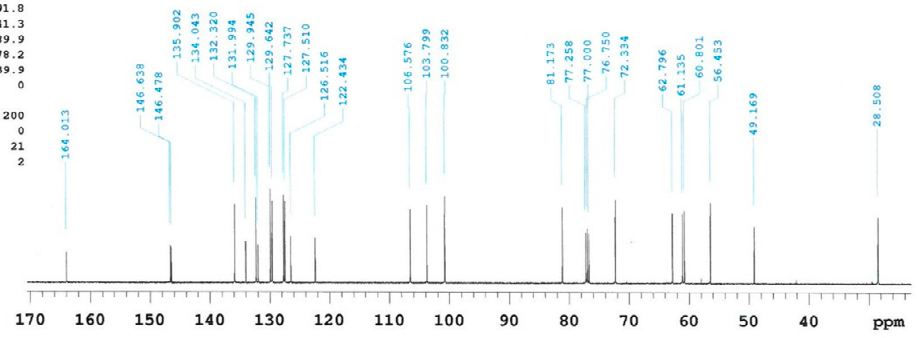



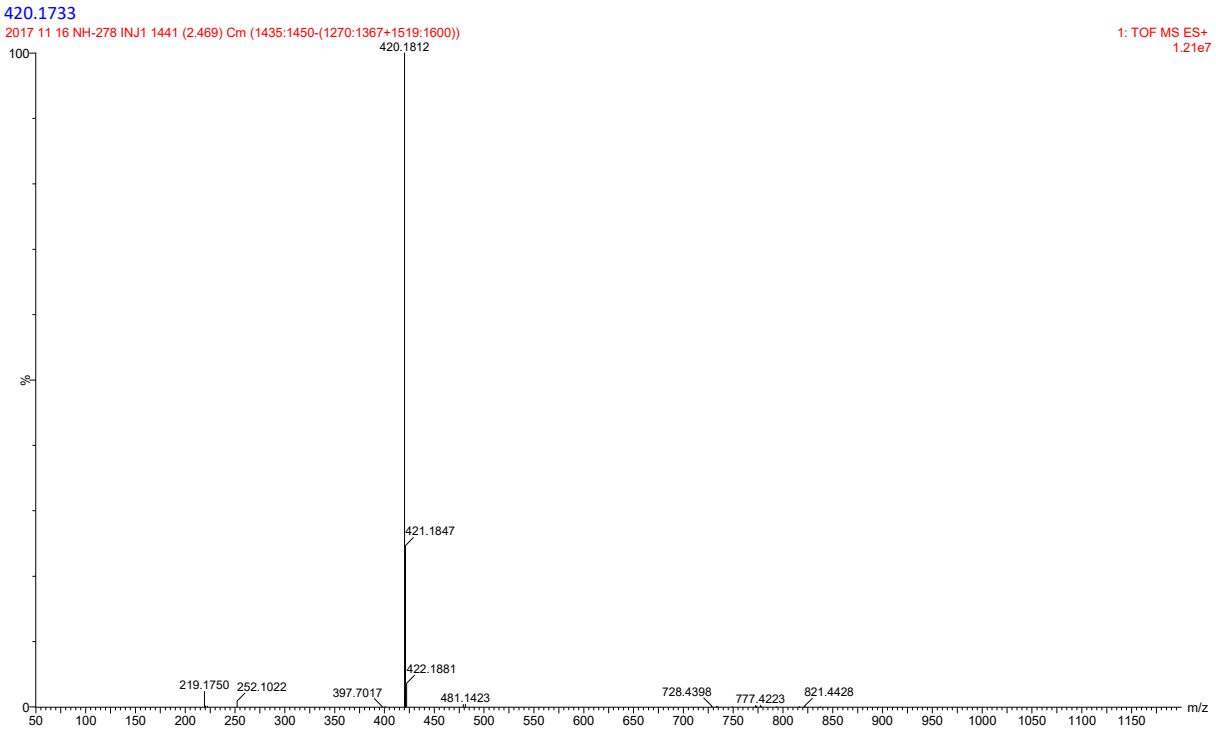
^{13}C-NMR

```
exp109  CARBON

      SAMPLE
date   Apr 24 2017
solvent       cdcl3
file            exp
   ACQUISITION
sw          31250.0
at            1.049
np            65536
fb            17000
bs                1
d1            1.000
nt             1000
ct              275
   TRANSMITTER
tn               C13
sfrq         125.705
tof           1913.9
tpwr              55
pw            5.650
    DECOUPLER
dn                H1
dof                0
dm               yyy
decwave            w
dpwr              40
dmf            11173

   PRESATURATION
satmode           n
wet               n
      SPECIAL
temp          25.0
gain            30
spin            20
hst          0.008
pw90        11.300
alfa        10.000
      FLAGS
il                n
in                n
dp                y
hs               nn
    PROCESSING
lb             1.00
fn        not used
     DISPLAY
sp          2891.8
wp         18541.3
rfl        11489.9
rfp         9678.2
rp           139.9
lp                0
      PLOT
wc              200
sc                0
vs               21
th                2
nm  cdc  ph
```

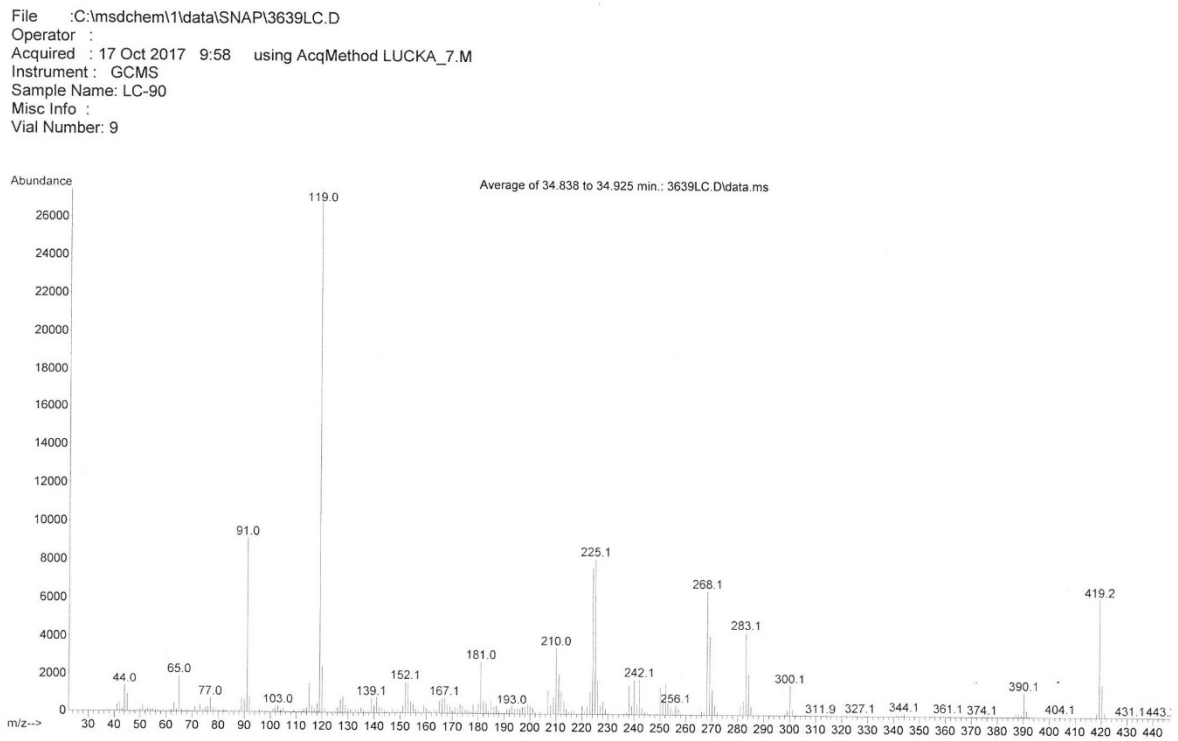


11-O-(2-methylbenzoyl)haemanthamine (1j)

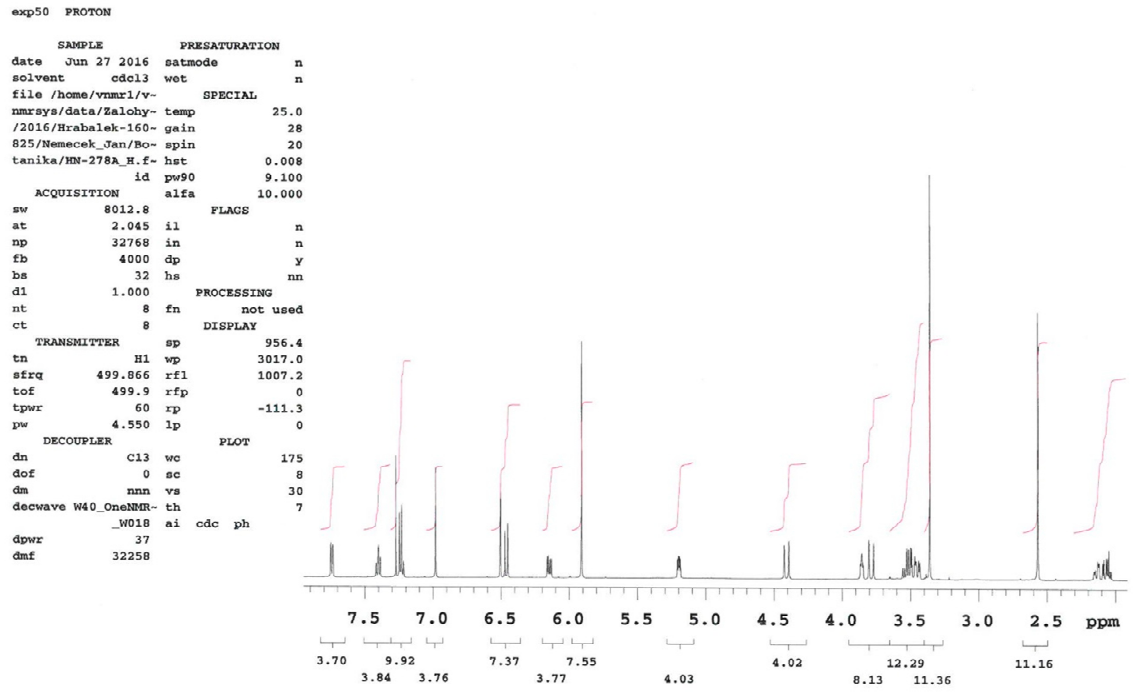
ESI-HRMS



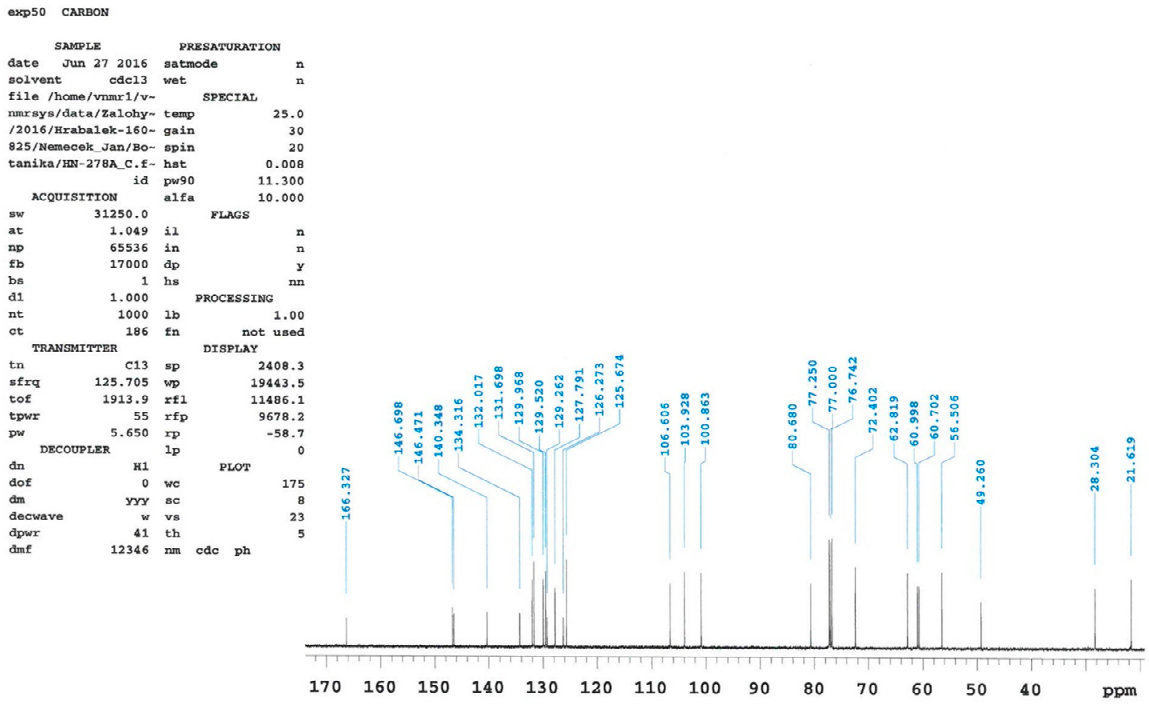
EI-MS



^{1}H-NMR

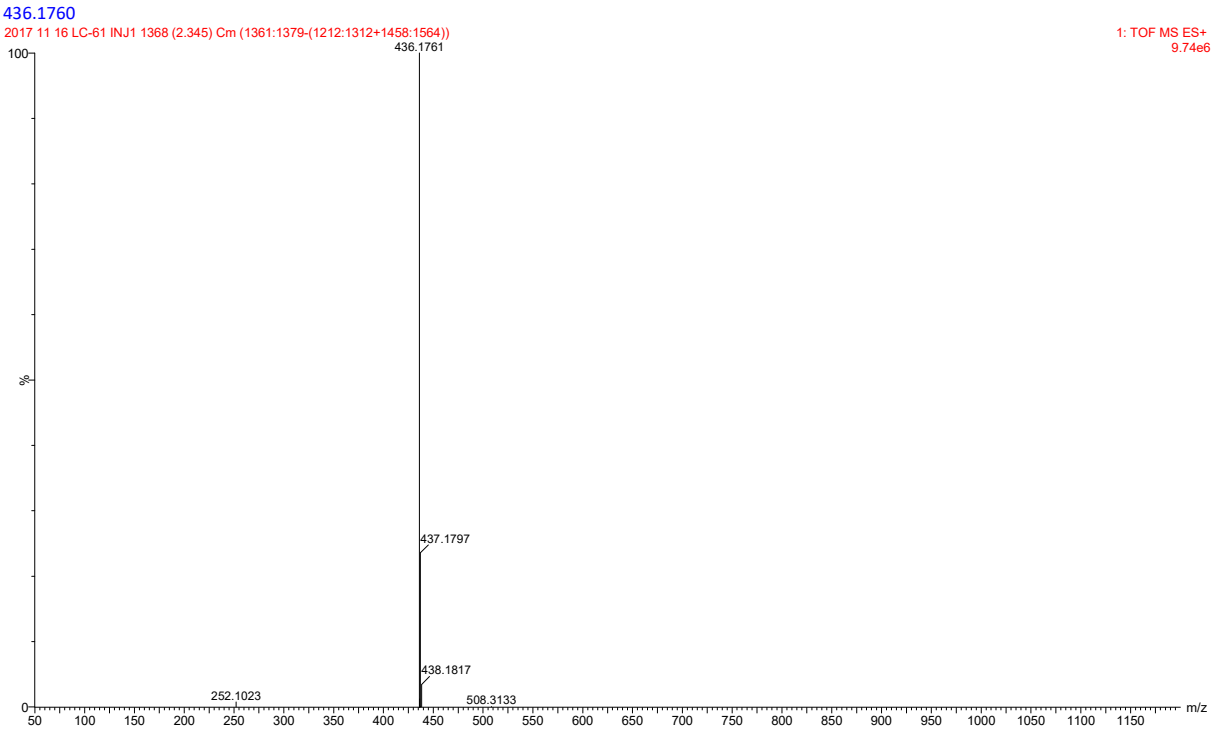


^{13}C-NMR



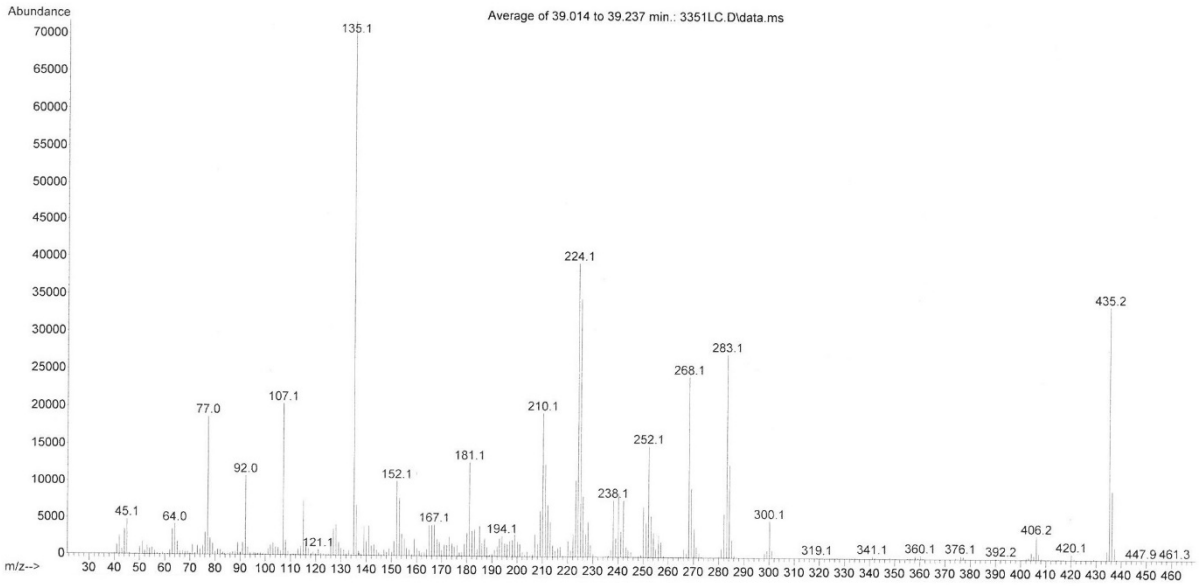
11-O-(3-methoxybenzoyl)haemanthamine (1k)

ESI-HRMS



EI-MS

File :C:\msdchem\1\data\SNAP\3351LC.D
Operator : Lucka
Acquired : 19 Jan 2017 17:22 using AcqMethod LUCKA_7.M
Instrument : GCMS
Sample Name: LC-61
Misc Info :
Vial Number: 6

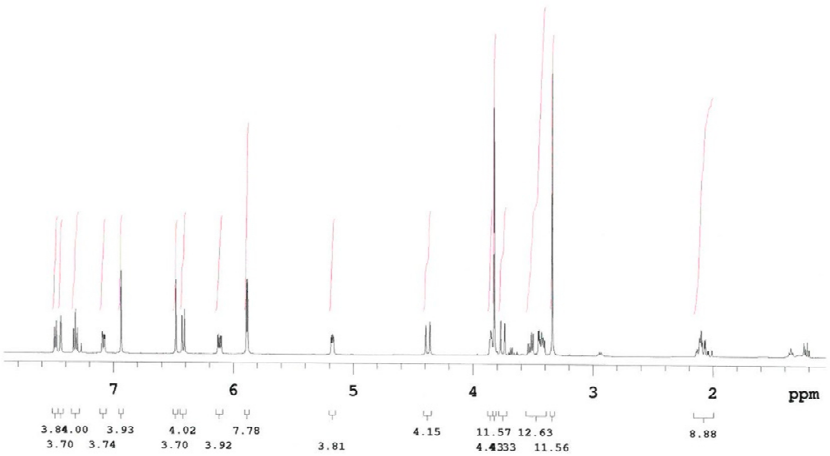


^{1}H-NMR

exp8 PROTON

SAMPLE	
date	Mar 24 2017
solvent	cdcl3
file	exp
ACQUISITION	
sw	8012.8
at	2.045
np	32768
fb	4000
bs	32
d1	1.000
nt	8
ct	8
TRANSMITTER	
tn	H1
sfrq	499.866
tof	499.9
tpwr	60
pw	4.550
DECOUPLER	
dn	C13
dof	0
dm	nnn
decwave	W40_OneNMR~_W018
dpwr	37
dmf	32258

PRESATURATION	
satmode	n
wet	n
SPECIAL	
temp	25.0
gain	22
spin	20
hst	0.008
pw90	9.100
alfa	10.000
FLAGS	
il	n
in	n
dp	y
hs	nn
PROCESSING	
fn	not used
DISPLAY	
sp	529.9
wp	3426.9
rfl	1007.2
rfp	0
rp	163.2
lp	0
PLOT	
wc	190
sc	8
vs	33
th	7
ai	cdc ph

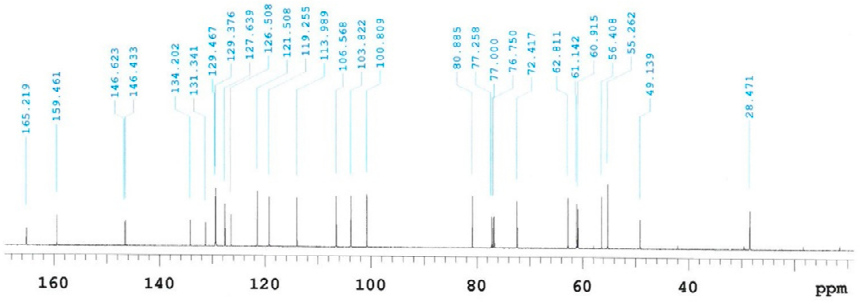


^{13}C-NMR

exp7 CARBON

SAMPLE	
date	Mar 24 2017
solvent	cdcl3
file	exp
ACQUISITION	
sw	31250.0
at	1.049
np	65536
fb	17000
bs	1
d1	1.000
nt	1000
ct	620
TRANSMITTER	
tn	C13
sfrq	125.705
tof	1913.9
tpwr	55
pw	5.650
DECOUPLER	
dn	H1
dof	0
dm	yyy
decwave	w
dpwr	40
dmf	11173

PRESATURATION	
satmode	n
wet	n
SPECIAL	
temp	25.0
gain	30
spin	20
hst	0.008
pw90	11.300
alfa	10.000
FLAGS	
il	n
in	n
dp	y
hs	nn
PROCESSING	
lb	1.00
fn	not used
DISPLAY	
sp	1105.6
wp	20169.3
rfl	11489.9
rfp	9678.2
rp	135.4
lp	0
PLOT	
wc	190
sc	0
vs	14
th	2
nm	cdc ph



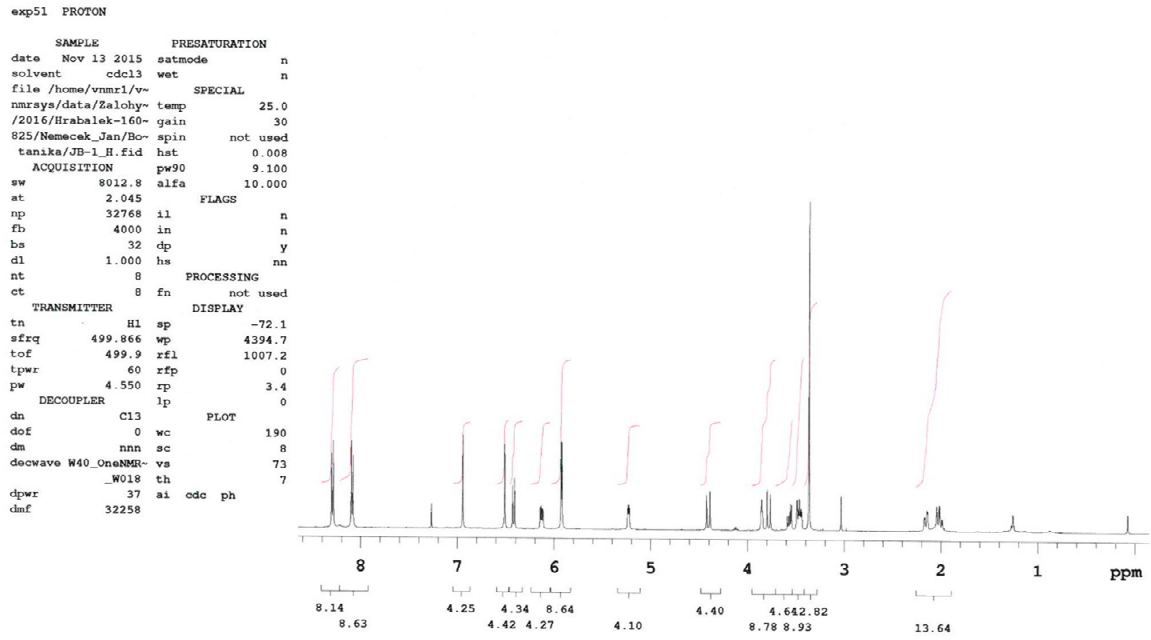
11-O-(4-nitrobenzoyl)haemanthamine (1m)

ESI-HRMS

EI-MS

No ionization under EI-MS conditions

^{1}H-NMR



^{13}C-NMR

exp51 CARBON

SAMPLE	
date	Nov 13 2015
solvent	cdcl3
file	/home/vnmr1/v~
	nmrsys/data/Zalohy~
	/2016/Hrabalek-160~
	825/Nemecek_Jan/Bo~
	tanika/JB-1_C.fid
ACQUISITION	
sw	31250.0
at	1.049
np	65536
fb	17000
bs	1
d1	1.000
nt	1000
ct	380
TRANSMITTER	
tn	C13
sfrq	125.705
tof	1913.9
tpwr	55
pw	5.650
DECOUPLER	
dn	H1
dof	0
dm	yyy
decwave	w
dpwr	41
dmf	12346

PRESATURATION	
satmode	n
wet	n
SPECIAL	
temp	25.0
gain	30
spin	not used
hst	0.008
pw90	11.300
alfa	10.000
FLAGS	
il	n
in	n
dp	y
hs	nn
PROCESSING	
lb	1.00
fn	not used
DISPLAY	
sp	2923.3
wp	18513.7
rfl	11480.3
rfp	9678.2
rp	177.0
lp	0
PLOT	
wc	190
sc	8
vs	30
th	2
nm	cdc ph

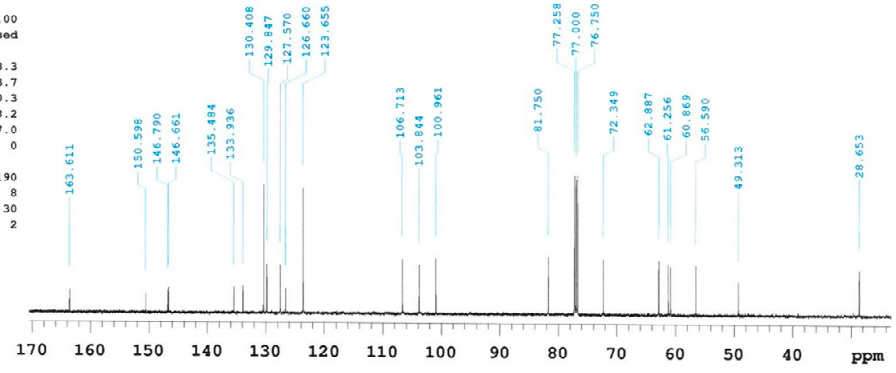


Table S1. Permeability (*Pe* 10^{-6} cm s^{-1}) in the PAMPA-BBB assay for 10 commercial drugs (used in the experiment validation) and compounds **1g**, **1j** and **1m** with their predictive penetration in the CNS.[a]

Compound	Bibl.[b]	*Pe* (10^{-6} cm s^{-1})[c]	Prediction
Atenolol	0,8	0,3 ± 0,3	
Caffeine	1,3	0,1 ± 0,1	
Desipramine	12	7,7 ± 0,4	
Enoxacin	0,9	0,6 ± 0,5	
Hydrocortisone	1,9	0,8 ± 0,2	
Ofloxacin	0,8	0,3 ± 0,1	
Piroxicam	2,5	0,9 ± 0,2	
Promazine	8,8	8,6 ± 0,5	
Testosterone	17	13,6 ± 0,3	
Verapamil	16	15,5 ± 0,8	
18g		8,4 ± 0,2	CNS +
18j		5,8 ± 0,9	CNS +
18m		7,1 ± 0,3	CNS +

[a]PBS:EtOH (70:30) was used as solvent. [b]Reference Di et al. [c]Data are the mean ± SD of 2 independent experiments.

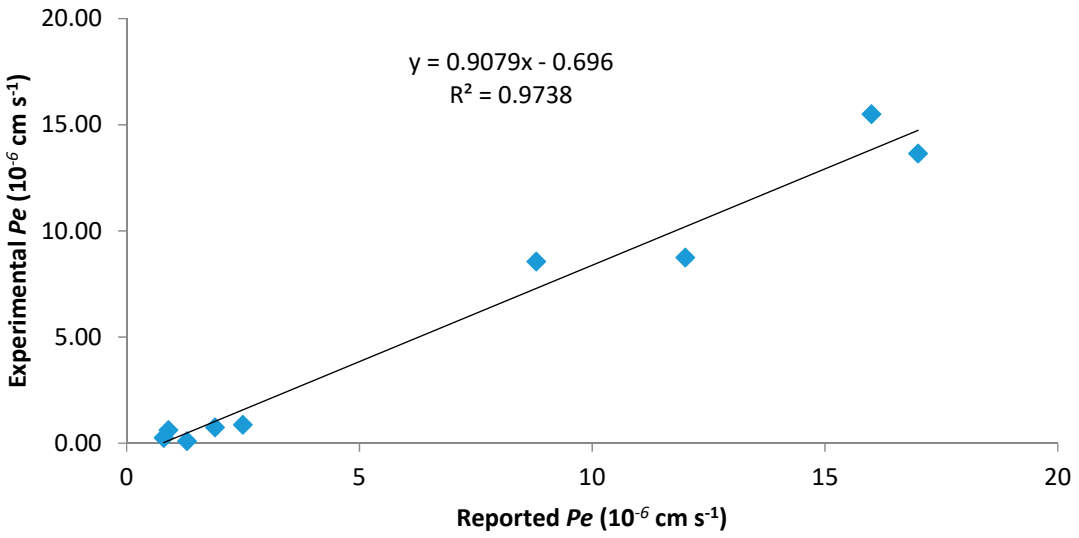


Figure S1. Linear correlation between experimental and reported permeability of commercial drugs using the PAMPA-BBB assay.

Table S2. Cell proliferation of haemanthamine derivatives (**1a – 1m;** c = 10μM) for 10 human cell lines.

	1	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1**	**1i**	**1j**	**1k**	**1m**	**DOX**
Jurkat	13±3	100±13	104±3	110±3	92±1	100±7	90±12	83±11	69±6	133±10	95±11	83±1	69±7	0±3
MOLT-4	1±1	106±9	97±8	99±8	96±1	97±3	101±18	110±19	135±22	115±15	91±3	94±18	76±12	0±1
A549	30±6	95±9	84±3	84±3	99±7	105±9	106±12	98±2	75±25	122±5	96±8	102±5	74±6	66±16
HT-29	37±6	97±10	79±15	82±17	94±4	100±3	101±3	110±17	97±23	120±4	98±6	93±3	73±2	77±12
PANC-1	37±3	114±4	70±13	85±13	88±3	96±4	99±6	100±10	118±9	108±2	95±6	88±5	71±7	59±9
A2780	30±5	107±17	104±3	123±9	99±2	106±5	104±8	120±10	122±24	162±9	129±11	108±7	86±4	5±1
HeLa	16±2	83±6	69±11	74±8	93±6	98±3	88±3	98±2	72±4	93±5	104±4	84±12	71±1	7±10
MCF-7	11±1	97±19	85±7	103±7	93±5	95±4	96±13	108±10	90±11	105±4	112±3	100±2	62±10	41±7
SAOS-2	28±3	88±6	96±16	85±15	88±6	96±8	104±7	104±9	135±6	97±10	97±17	89±15	69±4	73±8
MRC-5	30±2	113±12	102±13	95±7	89±3	96±4	87±14	101±5	100±5	105±3	112±9	92±1	61±8	40±4

Values represent cell proliferation after haemanthamine analogue treatment and are expressed as percent of proliferation of untreated control cells. Each value is a mean of three independent experiments. Doxorubicin was used as a reference drug.