Supporting Information

Figure 1. ^{1}H NMR spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Figure 2. dqfCOSY spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Figure 3. HMQC spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Figure 4. HMBC spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Figure 5. NOESY spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Table 1. List of compounds with alternate naming and Chemical Abstracts Service Registry Number.

Chemistry: Syntheses of compounds **10**, **11**, and **12**.

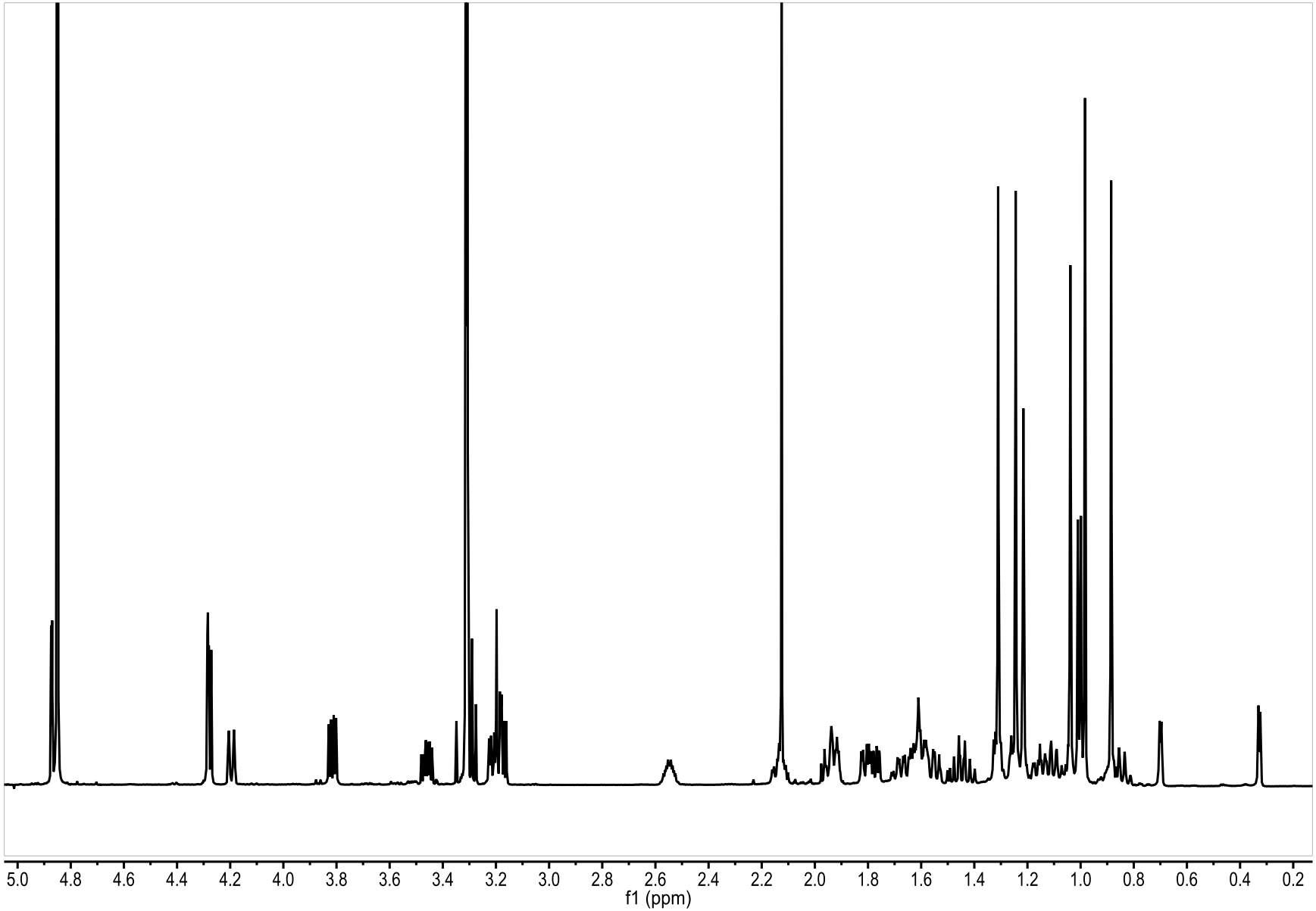
Scheme: Fractionation of *Actaea racemosa* extract.

Pharmacological selectivity screening panel assays.

Analytical Method for PK analyses.

Three-step procedure for the isolation of compounds 1, 5, 6, 7, 8, 9.

IP-MS analysis of Aβ(1-X) of cell-conditioned medium.



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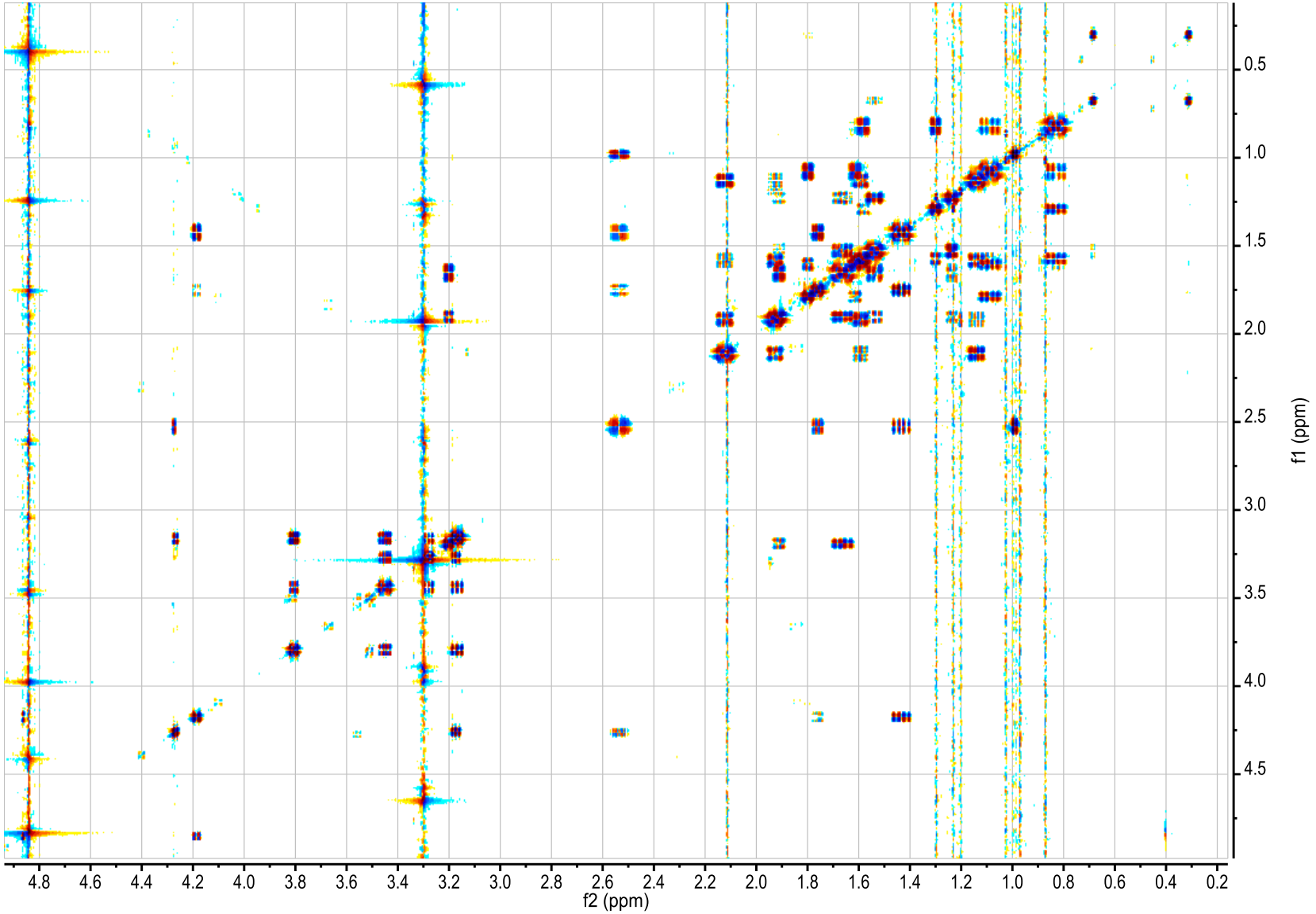


Figure 2. dqfCOSY spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

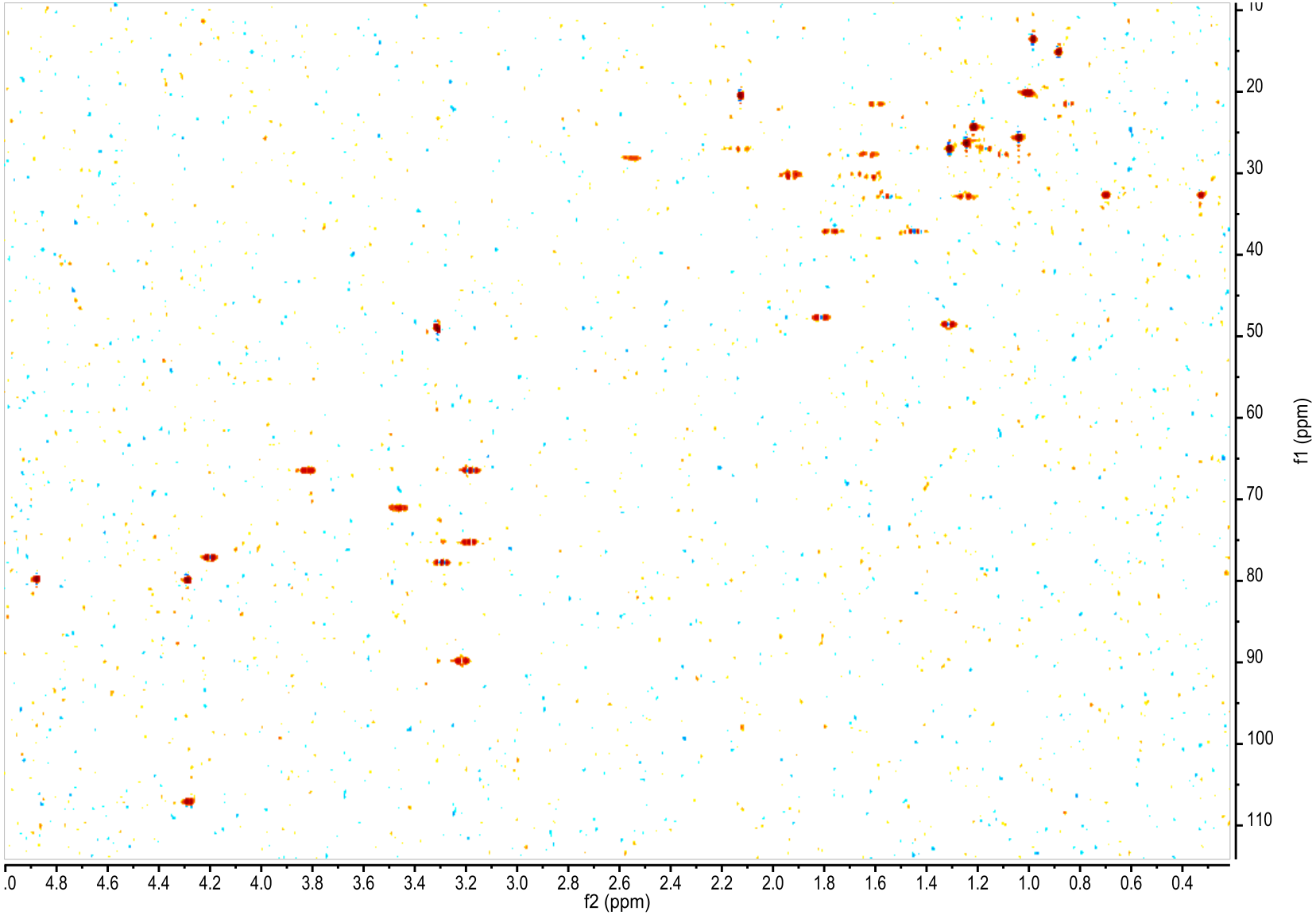


Figure 3. HMQC spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

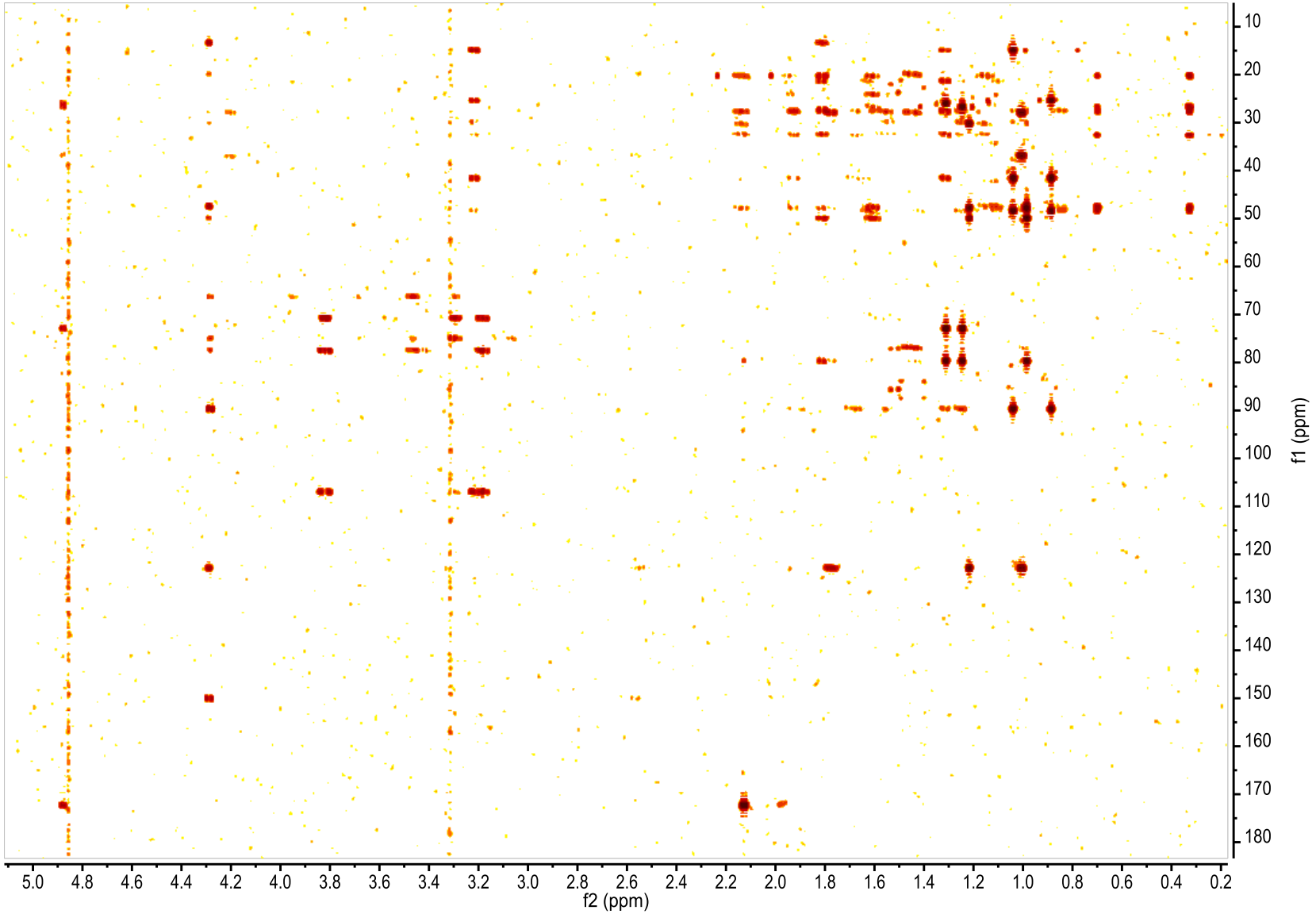


Figure 4. HMBC spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

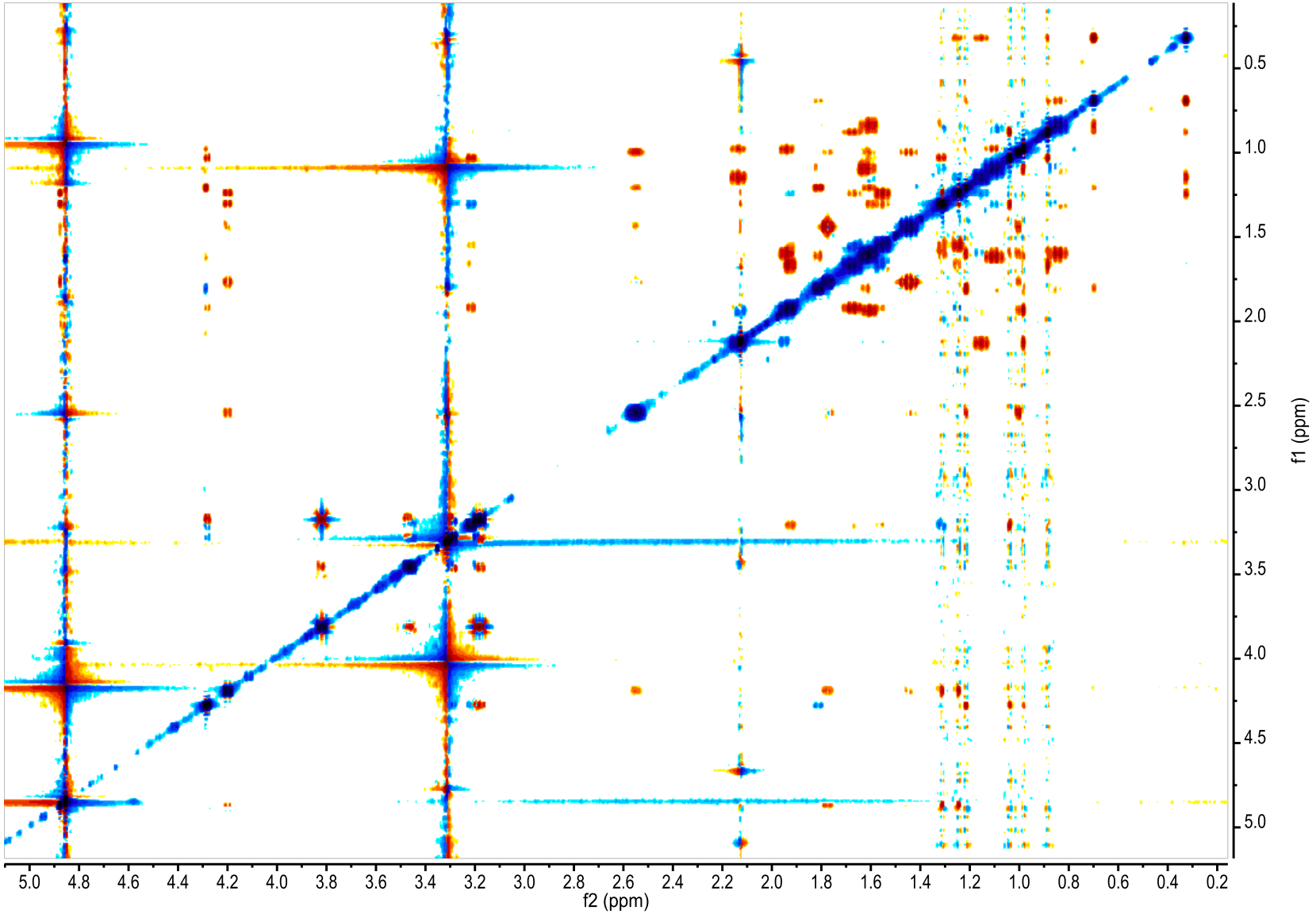


Figure 5. NOESY spectrum (600 MHz, methanol-d_4) of 24-O-acetylhydroshengmanol 3-O-β-D-xylopyranoside-$\Delta^{16,17}$-enol ether (**1**).

Table 1. List of compounds with alternate naming and Chemical Abstracts Service Registry Number.

#	Trivial Chemical Name	CAS Entry Name	CAS Registry Number
1	24-*O* -Acetylhydroshengmanol (delta-16,17)-enol ether-3-*O* -beta-D-xylopyranoside, (24S)	β-D-Xylopyranoside, (3β,15α,23*R* ,24*S*)-24-(acetyloxy)-16,23-epoxy-15,25-dihydroxy-9,19-cyclolanost-16-en-3-yl	915277-86-0
2	Actein	β-D-Xylopyranoside, (3β,12β,16β,23R,24R,25S,26S)-12-(acetyloxy)-16,23:23,26:24,25-triepoxy-26-hydroxy-9,19-cyclolanostan-3-yl	18642-44-9
3	Cimigenol-3-*O* -beta-D-xylopyranoside	β-D-Xylopyranoside, (3β,15α,16α,23*R* ,24*S*)-16,23:16,24-diepoxy-15,25-dihydroxy-9,19-cyclolanostan-3-yl	27994-11-2
4	Cimigenol-3-*O* -alpha-L-arabinopyranoside	α-L-Arabinopyranoside, (3β,15α,16α,23*R* ,24*S*)-16,23:16,24-diepoxy-15,25-dihydroxy-9,19-cyclolanostan-3-yl	256925-92-5
5	24-*O* -Acetylhydroshengmanol-3-*O* -beta-D-xylopyranoside, (24S)	9,19-Cyclolanostane-15,16,24,25-tetrol, 16,23-epoxy-3-(β-D-xylopyranosyloxy)-, 24-acetate, (3β,15α,16β,23R,24S)-	78213-32-8
6	24-*O* -Acetylhydroshengmanol (delta-16,17)-enol ether-3-*O* -beta-D-xylopyranoside, (24R)	ß-D-Xylopyranoside, (3ß,15a,23R,24R)-24-(acetyloxy)-16,23-epoxy-15,25-dihydroxy-9,19-cyclolanost-16-en-3-yl	915277-88-2
7	24-*O* -Acetylhydroshengmanol-3-*O* -alpha-L-arabinopyranoside, (24S)	9,19-Cyclolanostane-15,16,24,25-tetrol, 3-(a-L-arabinopyranosyloxy)-16,23-epoxy-, 24-acetate, (3ß,15a,16ß,23R,24S)-	915277-93-9
8	24-*O* -Acetylhydroshengmanol (delta-16,17)-enol ether-3-*O* -alpha-L-arabinopyranoside, (24S)	α-L-Arabinopyranoside, (3β,15α,23R,24S)-24-(acetyloxy)-16,23-epoxy-15,25-dihydroxy-9,19-cyclolanost-16-en-3-yl	915277-87-1
9	24-*O* -Acetylhydroshengmanol-3-*O* -beta-D-xylopyranoside, (24R)	9,19-Cyclolanostane-15,16,24,25-tetrol, 16,23-epoxy-3-(β-D-xylopyranosyloxy)-, 24-acetate, (3β,15α,16β,23R,24R)-	942578-88-3
10	Hydroshengmanol (delta-16,17)-enol ether-3-*O* -beta-D-xylopyranoside, (24S)	ß-D-Xylopyranoside, (3ß,15a,23R,24S)-16,23-epoxy-15,24,25-trihydroxy-9,19-cyclolanost-16-en-3-yl	915277-92-8
11	24-*O* -Acetylhydroshengmanol (delta-16,17)-enol ether, (24S)	9,19-Cyclolanost-16-ene-3,15,24,25-tetrol, 16,23-epoxy-, 24-acetate, (3ß,15a,23R,24S)-	942578-77-0
12	Hydroshengmanol (delta-16,17)-enol ether, (24S)	9,19-Cyclolanost-16-ene-3,15,24,25-tetrol, 16,23-epoxy-, (3ß,15a,23R,24S)-	942578-79-2

CHEMISTRY

Synthesis of 10. Enol ether **1** (52 mg) was dissolved in MeOH (1 mL) and methanolic KOH (2 mL, 0.5% w/v) was added. The mixture was stirred at room temperature for 3 hours and concentrated under vacuum to dryness. The residue was purified by chromatography on silica gel with MeOH-CH_2Cl_2 (1:9 v/v) to obtain 21 mg of the des-acetyl derivative **12**. MS and NMR data were consistent with removal of the acetyl group. As an alternate procedure, **1** was treated with 1% ammonia in MeOH and heated at 85 °C for 1 hour. Solvent was removed under reduced pressure to afford **10**. MS and NMR data were consistent with removal of the acetyl group.

Synthesis of 11

Compound **1** (10 mg) was dissolved in 5 mL of MeOH. Potassium phosphate buffer (50 mM, pH 6.0, 10 mL) was added to the MeOH solution. Cellulase (Sigma product # C0615, 20 mg) was dissolved in 10 mL of the potassium phosphate buffer solution. The solution of compound **1** was then added to the cellulase solution and the mixture was incubated for 3 days at 37° C.

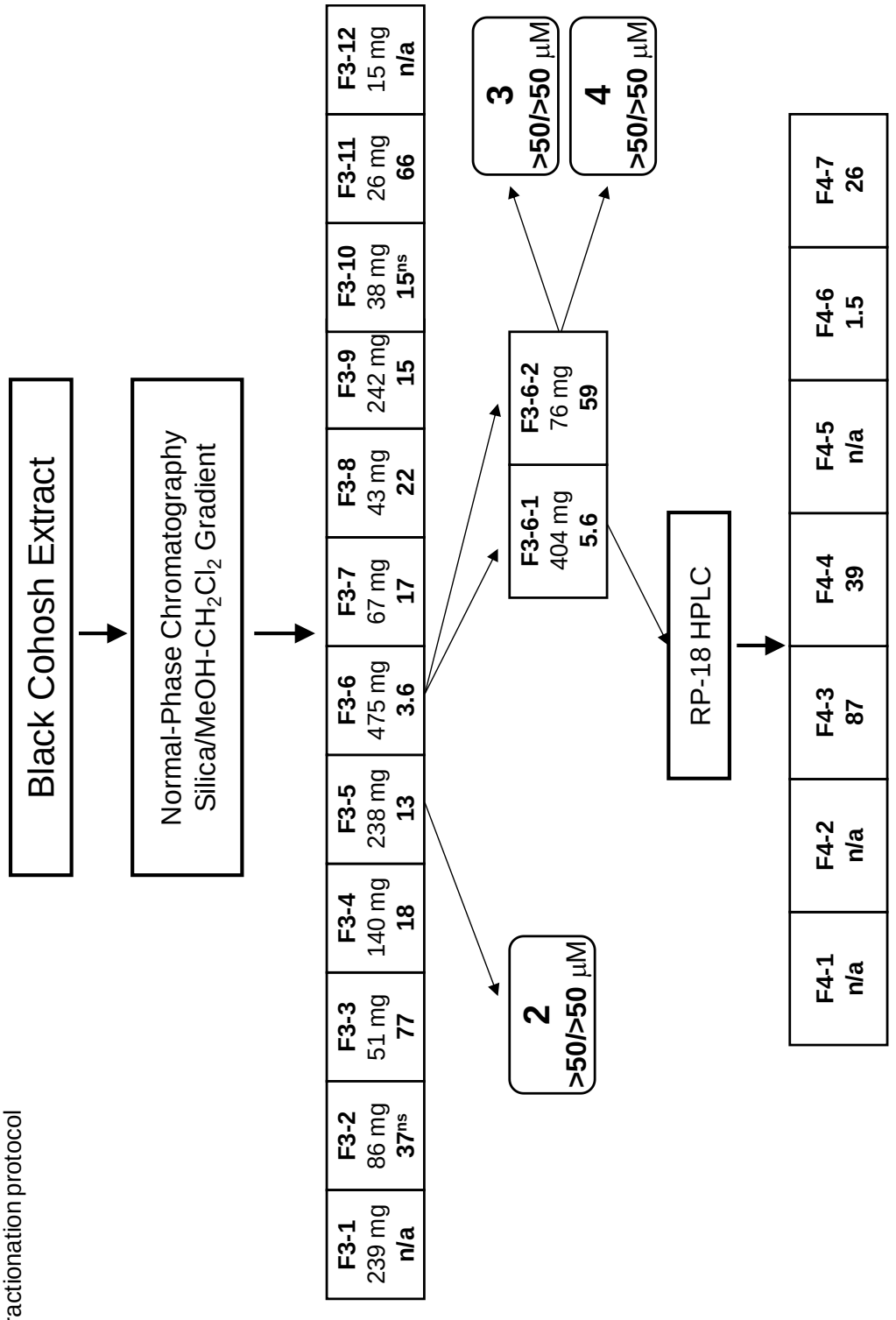
Progress of the reaction was monitored by HPLC as follows: an aliquot of the reaction mixture (20 μL) was added to MeOH (80 μL) containing 0.1% acetic acid (v/v). The sample was then analyzed on a 25 cm x 4.6 mm ID SUPELCOSIL LC-8 reversed phase HPLC column (5 μm particle size) employing a 20 minute MeOH-water gradient.

After the three day incubation, 20 mL of CH_2Cl_2 was added to the reaction mixture which was shaken vigorously followed by centrifugation at 2000 rpm for three minutes. The aqueous layer was removed and extracted two more times with 20 mL of CH_2Cl_2. The organic layers were combined and the solvent was removed to afford 9.6 mg of crude product. The crude product was dissolved in a minimal volume of 20:1 CH_2Cl_2-MeOH and chromatographed over a silica column eluted with the same solvent mixture. Column development was monitored by

TLC using a cerium molybdate stain (1% $CeSO_4$, 5% $(NH_4)Mo_7O_{24}\cdot 4H_2O$ (w/v) and 10% concentrated H_2SO_4 (v/v)) and a 15:1 CH_2Cl_2-MeOH solvent mixture. Fractions containing material with an R_f of 0.26 were pooled to afford compound **11**. MS and NMR data were consistent with removal of the acetyl group.

Synthesis of 12. Acetyl aglycone **11** (12.3 mg) was dissolved in MeOH (0.4 mL) and KOH (0.5 mg) was added. The resulting mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was purified by chromatography on silica gel with MeOH-CH_2Cl_2 (1:9 v/v) to obtain 5.4 mg of tetraol **12**. MS and NMR data were consistent with removal of the acetyl group.

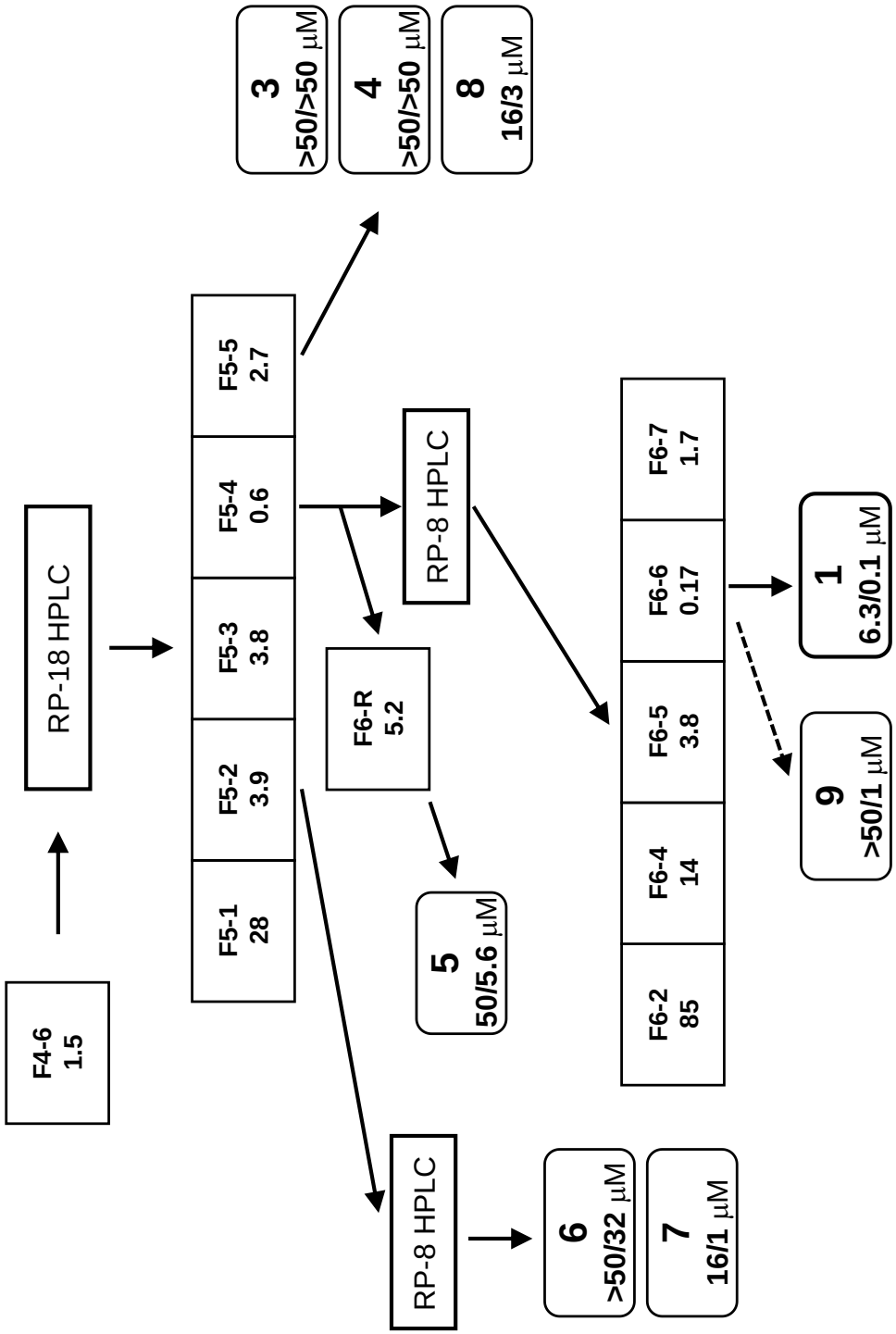
Scheme: Black cohosh extract fractionation protocol.



Scheme. Black cohosh fractionation protocol

Notes: n/a: not active; ns: non-specific. Fraction activities expressed as IC50 in mg/L. Isolated compound activities expressed as IC50 in micromolar to lower Aβ40/Aβ42.

Scheme: Black cohosh extract fractionation protocol (continued)



Notes: n/a: not active; ns: non-specific. Fraction activities expressed as IC50 in mg/L. Isolated compound activities expressed as IC50 in micromolar to lower Aβ40/Aβ42.

Pharmacological selectivity screening panel assays (A). Data for 1 at 1 μM (B), and 10 μM (C). Values within ± 20% are not considered beyond baseline. Values ± 20~49% are considered to be marginal effects. Values ≥ 50% are considered to be "active".

(A) Novascreen General Side Effect Panel, 63 assays plus COX-1 and COX-2

NEUROTRANSMITTER RELATED
Adenosine, Non-selective
Adrenergic, Alpha-1, Non-selective
Adrenergic, Alpha-2, Non-selective
Adrenergic, Beta, Non-selective
Dopamine Transporter, DAT
Dopamine, Non-selective
GABA-A, Agonist Site
GABA-A, BDZ, Alpha-1 Site
GABA-B
Glutamate, AMPA Site
Glutamate, Kainate Site
Glutamate, NMDA, Agonist Site
Glutamate, NMDA, Glycine [Strychnine-insensitive]
Glycine, Strychnine-sensitive Site
Histamine, H1
Histamine, H2
Histamine, H3
Melatonin, Non-selective
Muscarinic, M1 (hr)
Muscarinic, M2 (hr)
Muscarinic, Non-selective, Central
Muscarinic, Non-selective, Peripheral
Nicotinic [a-Bungarotoxin Insensitive, Neuronal]
Norepinephrine Transporter, NET
Opioid, Non-selective
Orphanin-1 (hr)
Serotonin Transporter, SERT
Serotonin, Non-selective
Sigma, Non-selective, Classical

STEROIDS
Estrogen
Testosterone, Cytosolic

ION CHANNELS
Calcium Channel, Type L (Dihydropyridine Site)
Calcium Channel, Type N
Potassium Channel, ATP Sensitive (KATP)
Potassium Channel, Ca2+ Activated, VI
Potassium Channel, I[Kr], hERG (hr)
Sodium channel, Site 2

SECOND MESSENGERS
Nitric Oxide Synthase, NOS (neuronal-binding)

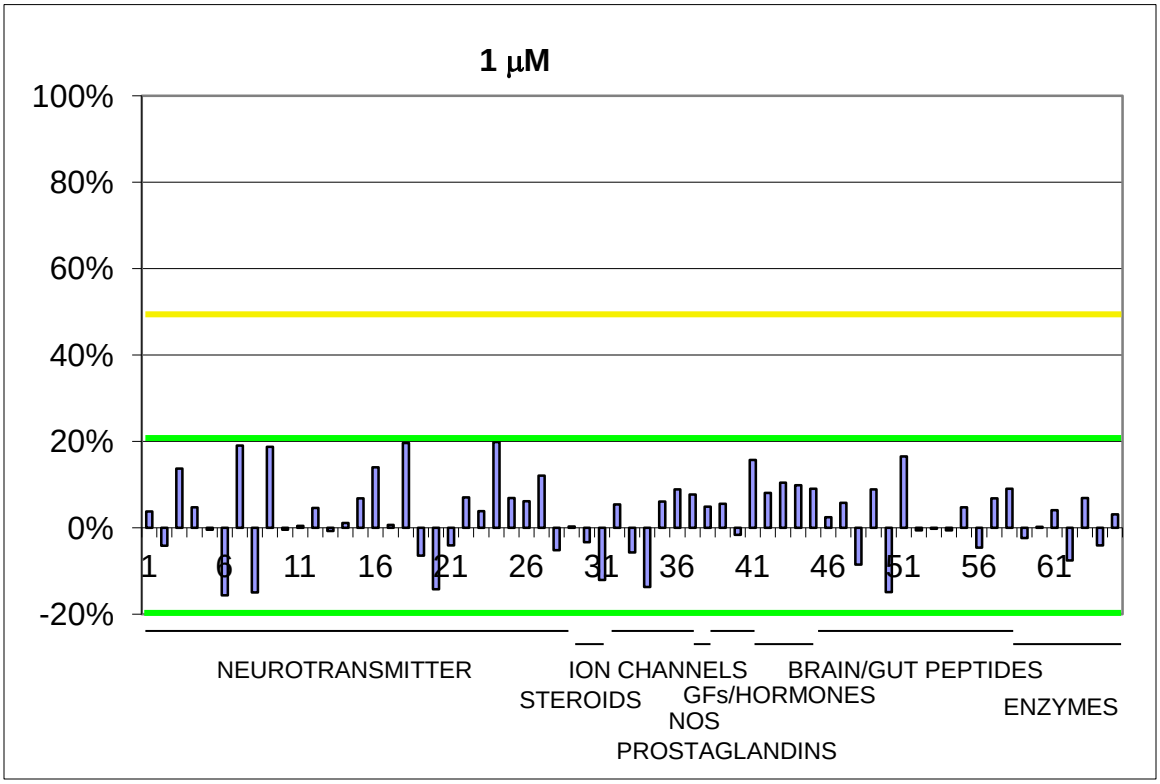
PROSTAGLANDINS
Leukotriene, LTB4 (BLT)
Leukotriene, LTD4 (CysLT1)
Thromboxane, TXA2 (h)

GROWTH FACTORS/HORMONES
Corticotropin Releasing Factor, Non-Selective
Oxytocin
Platelet Activating Factor, PAF
Thyrotropin Releasing Factor, TRH

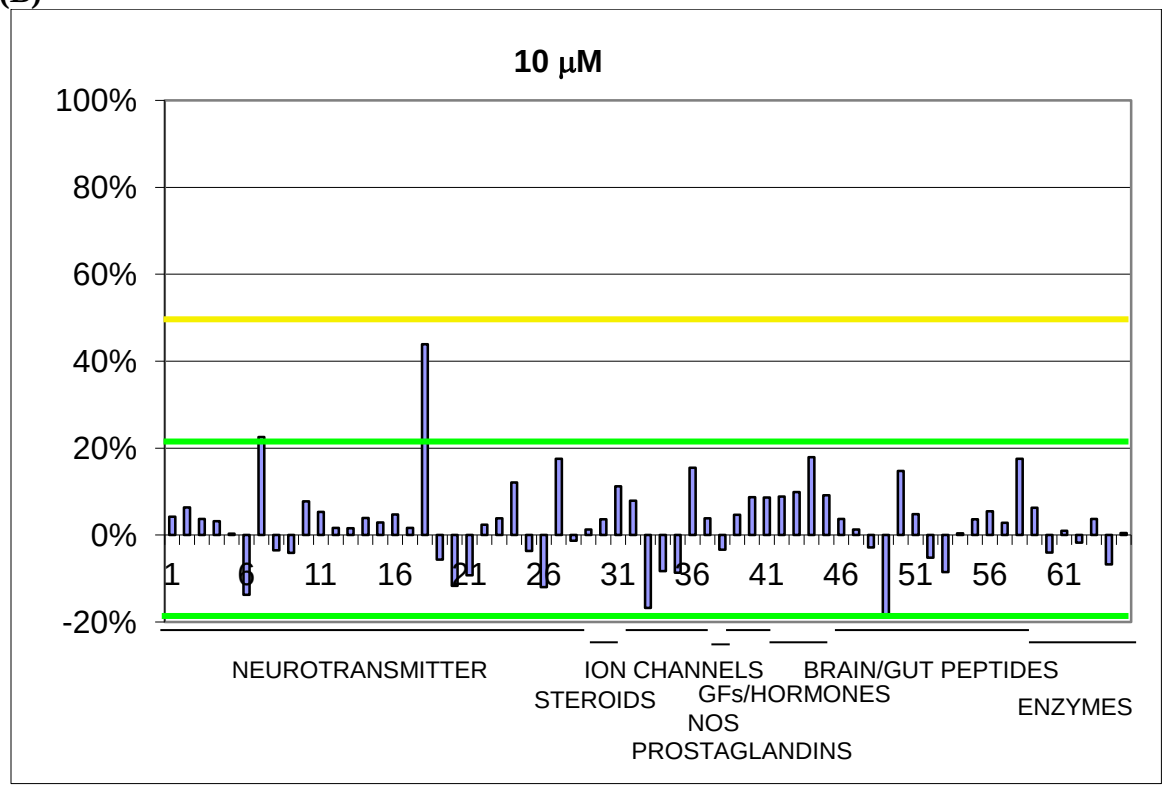
BRAIN/GUT PEPTIDES
Angiotensin II, AT1 (h)
Angiotensin II, AT2, Central
Bradykinin, BK2
Cholecystokinin, CCKA
Cholecystokinin, CCKB
Endothelin, ETA (h)
Endothelin, ETB (h)
Galanin, Non-Selective
Neurokinin, NK1
Neurokinin, NK2, NKA (hr)
Neurokinin, NK3, NKB
Vasoactive Intestinal Peptide, Non-selective
Vasopressin, V1

ENZYMES
Choline Acetyl Transferase, ChAT
Esterase, Acetylcholine
Glutamic Acid Decarboxylase, GAD
Oxidase, MAO-A, peripheral
Oxidase, MAO-B, peripheral
Oxygenase, Cyclooxygenase-1
Oxygenase, Cyclooxygenase-2

(A)


1 μM
100%
80%
60%
40%
20%
0%
-20%
1
6
11
16
21
26
31
36
41
46
51
56
61
NEUROTRANSMITTER
ION CHANNELS
BRAIN/GUT PEPTIDES
STEROIDS
GFs/HORMONES
NOS
ENZYMES
PROSTAGLANDINS

(B)


10 μM
100%
80%
60%
40%
20%
0%
-20%
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36
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NEUROTRANSMITTER
ION CHANNELS
BRAIN/GUT PEPTIDES
STEROIDS
GFs/HORMONES
NOS
ENZYMES
PROSTAGLANDINS

Analytical Method for PK analyses.

Sample Preparation	
Sample Volume	50 µL mouse plasma, 10 µL mouse brain homogensates
Extraction	Protein Precipitation
Sample Analysis	
Number of Standards	9 concentrations (1 - 10,000 ng/m), front and back end
HPLC Pump	LC_01 (Rheos CPS-LC 2000)
HPLC Column	Agilent SB C8 column, 2.0 x 30 mm
Mobile Phase A	100% H_2O, 0.1% formic acid
Mobile Phase B	90/10 ACN/H_2O, 0.1 % formic acid
Flow Rate	0.45 mL/min
Injection Volume	5 µL
MS	MS_01 (API4000)
Interface	TIS positive
Scan	Multiple Reaction Monitoring (MRM)
Analyte: P/D Ion Pair	Cmpd 5: 685.7 $(M+Na)^+$/528.6 Cmpd 10: 553.5 $(M+Na)^+$/395.4 Cmpd 11 511.7 $(M+Na)^+$/395.5 Cmpd 12: 643.6 $(M+Na)^+$/527.6
IS: P/D Ion Pair	Amiodarone: 646.2/100.2
Resolution	Low
Source Temp	500°C
Total Run Time	2 minutes

Time (min)	% Mobile Phase B	Flow Rate (µL/min)
0	10	450
0.1	10	450
0.5	100	450
1.3	100	450
1.4	10	450
2	10	450

Three-step procedure for the isolation of compounds 1, 5, 6, 7, 8, 9.

(**1**) (24*S*)-24-O-acetylhydroshengmanol 3-O-beta-D-xylopyranoside (delta-16,17)-enol ether

(**5**) (24*S*)-24-O-acetylhydroshengmanol 3-O-beta-D-xylopyranoside

(**6**) (24*R*)-24-O-acetylhydroshengmanol 3-O-beta-D-xylopyranoside (delta-16,17)-enol ether

(**7**) (24*S*)-24-O-acetylhydroshengmanol 3-O-beta-D-arabinopyranoside

(**8**) (24*S*)-24-O-acetylhydroshengmanol 3-O-beta-D-arabinopyranoside (delta-16,17)-enol ether

(**9**) (24*R*)-24-O-acetylhydroshengmanol 3-O-beta-D-xylopyranoside

1. Flash column chromatography.

Black cohosh raw extract was obtained from BI Nutriceuticals. Extract (15.6 g) was suspended in 150 ml of a 4-to-1 (v/v) methanol-water mixture at 25 °C. Using a mechanical stirrer, the resulting slurry was vigorously stirred for 30 min at this temperature, which resulted in a brown emulsion. To this emulsion, 51 g of silica gel (ICN silica 32-63 60 Å) was added with continued stirring. Subsequently, the mixture was concentrated at 25 °C in vacuo using a rotary evaporator, until a largely homogenous beige-brown powder was obtained. This material was subjected to column chromatography on silica gel (ICN silica 32-63 60 Å) using a 60 cm long glass column with 50 mm inner diameter.

In preparation for the column chromatography, silica gel was poured into 500 ml of a 20-to-1 dichloromethane-methanol mixture, and the resulting slurry was poured into the glass column. Special care was taken to ensure a dense and even filling of the column. The silica gel was allowed to settle for 30 min, and covered with a 1 cm thick layer of sand. Subsequently, the extract absorbed on silica was poured in a suitable amount of the 20-to-1 dichloromethane-

methanol mixture, and the resulting slurry was carefully poured onto the sand layer on top of the column.

The silica column was eluted with 1.0 l of dichloromethane-methanol 20-to-1, followed by 770 ml of dichloromethane-methanol 10-to 1, followed by 800 ml of dichloromethane-methanol 7-to-1, followed by 550 ml of dichloromethane-methanol 5-to-1. For elution, a pressure of 0.4 bar (argon) was applied.

Fractions were collected using Erlenmeyer flasks. Eight 200 ml-fractions (labeled as F14-0 through F14-7) were collected, followed by eleven 100-ml fractions (labeled as F14-8 through F14-18). All fractions were analyzed by thin-layer chromatography (TLC), using Bakerflex silica plates, and a 5-to-1 dichloromethane-methanol mixture as solvent. After development the silica gel plates were stained with anisaldehyde stain (see Figure 1a). Based on the results of the TLC analyses, fractions F14-9 through F14-12 were evaporated to dryness in vacuo at 25 °C, and 10-mg samples of these fractions were analyzed by ^{1}H-NMR spectroscopy, using CD_3OD as solvent (Figure 2a). Spectra were analyzed with regard to the presence of a broad multiplet at 2.53 ppm, and a 2.2-Hz doublet at 4.86 ppm, because these signals are characteristic for compounds **1** and **7**. Additional dqf-COSY spectra of these four samples confirmed that the signals at 2.53 and 4.86 ppm in fact belong to compounds **1** and **8**. From the NMR spectra of fraction F14-10 it was concluded that this sample contained the highest concentration of **1** and **7**, while slightly smaller amounts of these compounds could be detected in fraction F14-9. Fraction F14-11 appeared to contain traces of **1** and **8**, whereas these compounds could not be detected in fraction F14-12. Based on these results, fraction F14-10 was chosen for further purification via HPLC. Fraction F14-9 could be used in addition, in case larger amounts of compounds **1, 5, 7**, or **8** are needed.

Amounts of fractions F14-9 through F14-12:

F14-9: 190 mg

F14-10: 287 mg

F14-11: 354 mg

F14-12: 215 mg

Major component of fraction F14-10 was actein (JNP 2002, 65, 601-605), which crystallized from a methanolic solution of this fraction. Pure actein was obtained through recrystallization. Major components of fraction F14-11 were cimigenol beta-D-xylopyranoside (**3**) and cimigenol alpha-L-arabinoside (**4**), which crystallized from this fraction as a mixture of roughly 2:1 (JNP 2000, 65, 905-910 and 1391-1397).

2. Reversed-phase HPLC fractionation on C-18 column.

Fraction F14-10 was dissolved in 3.5 ml of methanol. This solution was fractionated by HPLC using a SUPELCO Discovery RP-18 column (25 cm length, 10 mm inner diameter), and a AGILENT 1100 series HPLC system, including autoinjector and a diode array detector used for detection of wavelength from 190-400 nm. A solvent gradient was employed, starting with 30% (v/v) water in methanol for the first two minutes, followed by a linear decrease of water content reaching 100% methanol at 20 min. After 2 min at 100 % methanol, water content was increased to 30% and maintained at that concentration for another 8 min. For separation of the entire sample F14-10, 100 injections of 35 µl each were required. Nine fractions were collected, which were labeled F15-1 through F15-9 (Figure 3a). Compounds **1, 5, 7**, and **8** were eluted in fractions F15-1, 15-2, 15-4 and15-5:

F15-1: 44.3 mg (contains **7** as one of two major compounds**)**

F15-2: 4.0 mg (major component is **7**, second most abundant component is **8**)

F15-4: 20.3 mg (major component: **5**, about 80% pure)

F15-5: 13.6 mg (mixture of **1** and **5** in a ratio of roughly three-to-two, plus small amounts of **6**)

F15-8: 10.5 mg (contains **9** as a major component)

^{1}H NMR spectra of fractions F15-1, 15-2, 15-4 and15-5 are shown in Figure 4a and 4b.

3. Reversed-phase HPLC fractionation on C-8 column for the isolation of 1, 5, and 6.

Fraction F15-5 was dissolved in 1.5 ml of methanol. This solution was fractionated by HPLC using a SUPELCO supelcosil LC-8 column (25 cm length, 10 mm inner diameter), and the AGILENT 1100 series HPLC system described above. A solvent gradient was employed, starting with 40% (v/v) water in methanol for the first two minutes, followed by a linear decrease of water content reaching 100% methanol at 20 min. After 2 min at 100 % methanol, water content was increased to 40% and maintained at that concentration for another 8 min. For separation of the entire sample F15-5, 50 injections of 30 µl each were required. Five fractions were collected, which were labeled F16-1 through F16-5 (Figure 5). Compound **1** was eluted in fraction F16-3, whereas compound **5** eluted in fraction F16-1. A small amount of pure **6** was obtained in fraction F15-5. Figure 6 shows the ^{1}H NMR spectrum of the 9.8 mg of 98% pure **1** obtained.

Amounts obtained:

F16-1: 14.3 mg of pure **5**.

F16-3: 7.1 mg of pure **1**.

F16-5: 0.5 mg of pure **6**.

4. Reversed-phase HPLC fractionation on C-8 column for the isolation of 9.

Fraction F15-8 was dissolved in 0.65 ml of methanol. This solution was fractionated by HPLC using a SUPELCO supelcosil LC-8 column (25 cm length, 10 mm inner diameter), and the

AGILENT 1100 series HPLC system described above. A solvent gradient was employed, starting with 40% (v/v) water in methanol for the first two minutes, followed by a linear decrease of water content reaching 100% methanol at 20 min. After 2 min at 100 % methanol, water content was increased to 40% and maintained at that concentration for another 8 min. For separation of the entire sample F15-8, 22 injections of 30 µl each were required. Seven fractions were collected, which were labeled F18-1 through F18-7. Compound **9** was eluted in fraction F18-6. 2.5 mg of compound **9** were obtained. NMR-spectroscopic analyses including NOESY spectra showed that in methanolic solution compound **9** interconverts with the corresponding ketone. Dilute methanolic solutions contain about 4% ketone and 96% of the hemiacetal form.

5. Reversed-phase HPLC fractionation on C-8 column for the isolation of 7 and 8.

Fraction F15-2 was dissolved in 0.5 ml of methanol. This solution was fractionated by HPLC using a SUPELCO supelcosil LC-8 column (25 cm length, 10 mm inner diameter), and the AGILENT 1100 series HPLC system described above. A solvent gradient was employed, starting with 40% (v/v) water in methanol for the first two minutes, followed by a linear decrease of water content reaching 100% methanol at 20 min. After 2 min at 100 % methanol, water content was increased to 40% and maintained at that concentration for another 8 min. For separation of the entire sample F15-2, 17 injections of 30 µl each were required. Five fractions were collected, which were labeled F19-3 through F19-7. Pure **8** (0.5 mg) was obtained in fraction F19-7, whereas pure **7** (2.3 mg) was obtained in fraction F19-5.

Figure 1a. Silica gel fractionation of black cohosh extract. TLC of fractions F14-3 through F14-18.

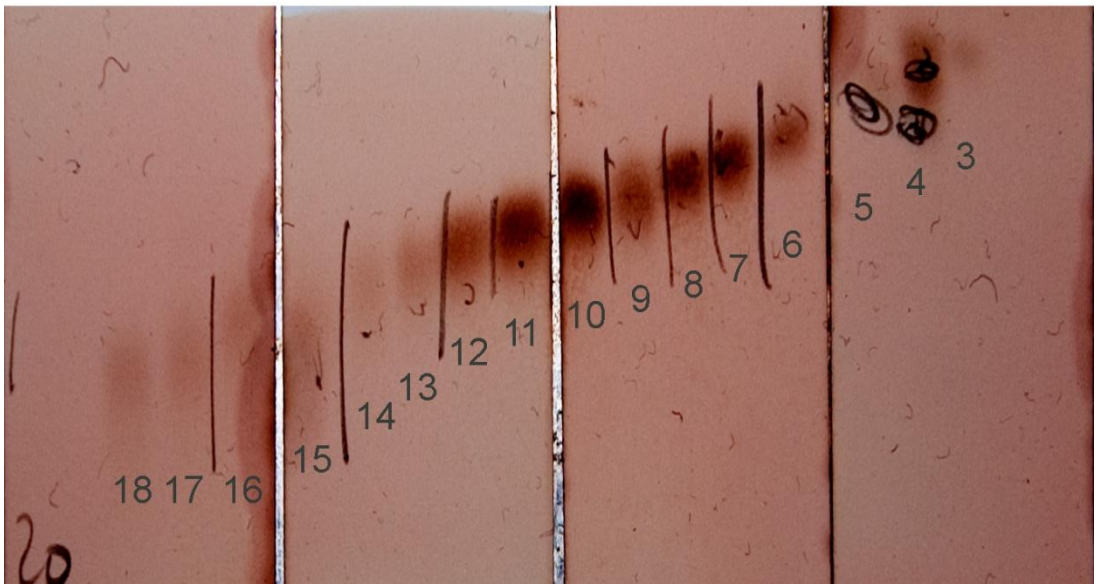


Figure 2a. ^{1}H NMR spectra of fractions F14-9 and F14-10.

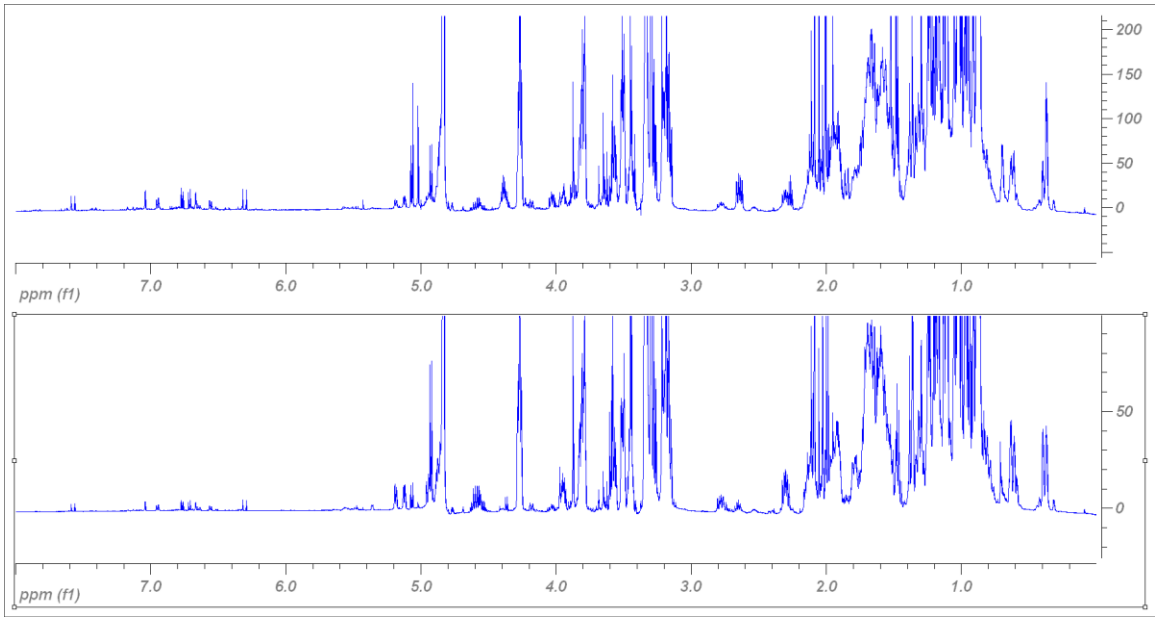


Figure 2b. ^{1}H NMR spectra of fractions F14-11 and F14-12.

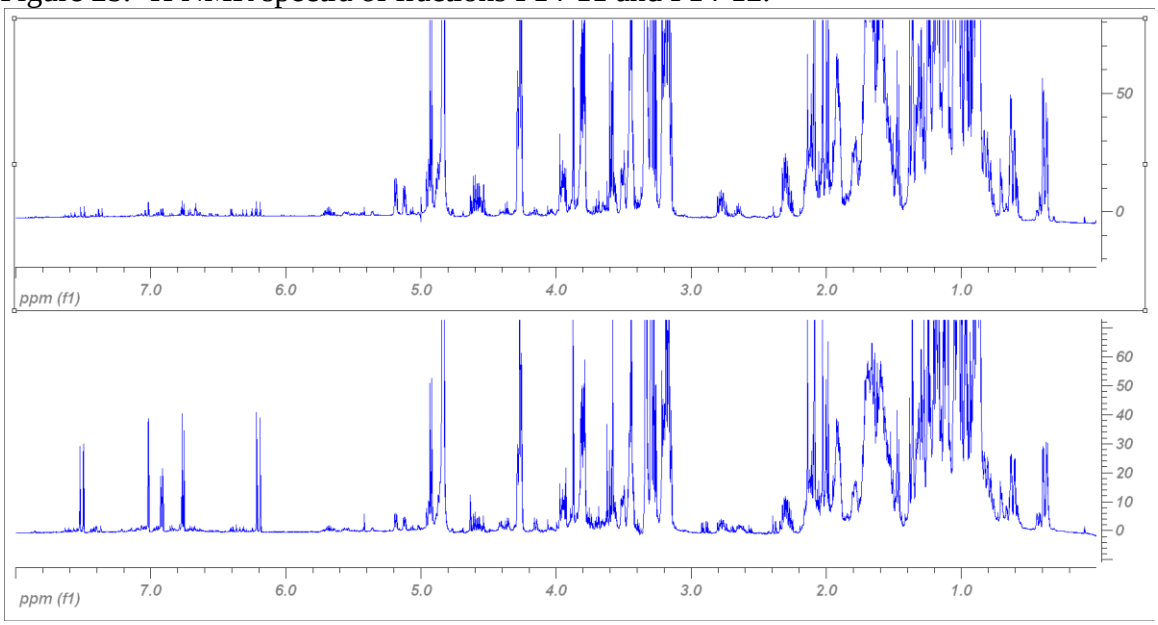


Figure 3a. Chromatogram of RP-18 HPLC separation of F14-10.

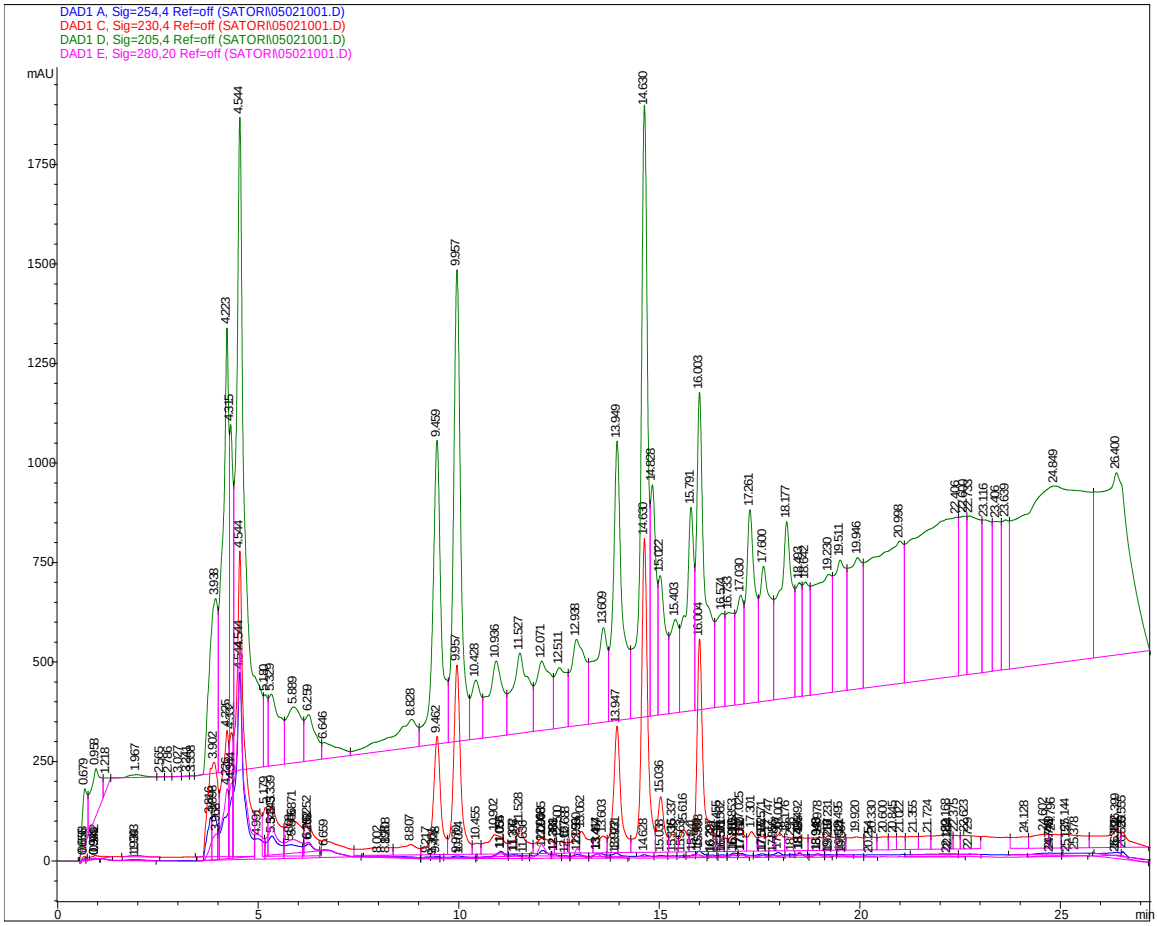


Figure 3b. Enlargement of chromatogram of F14-10 separation. Numbers 1 through 9 correspond to time windows for fractions F15-1 through F15-9.

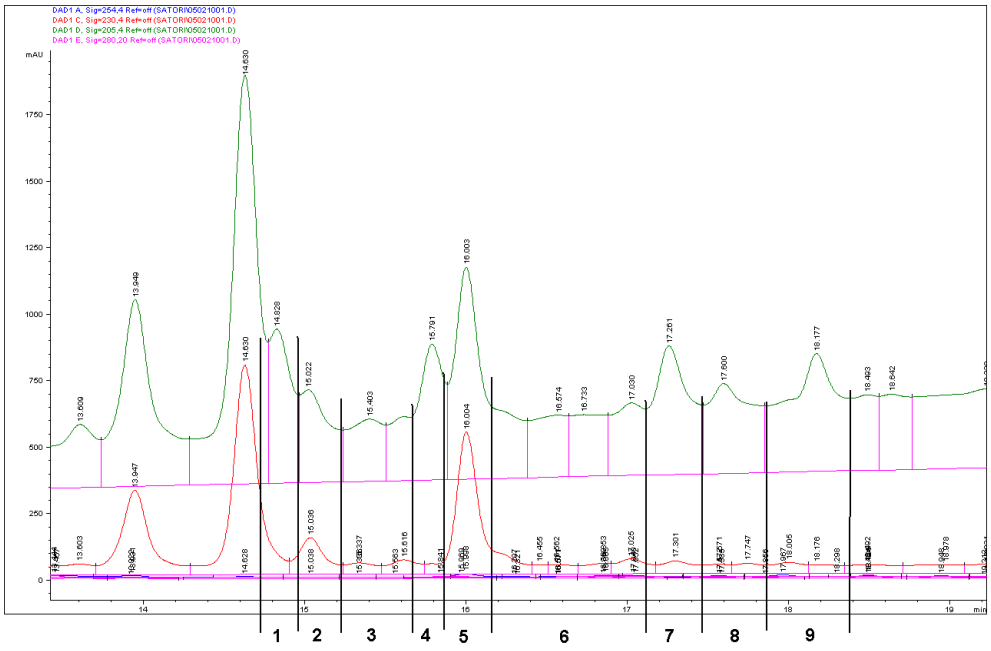


Figure 4a. ^{1}H NMR spectra of fractions F15-1 (top) and F15-2 (bottom).

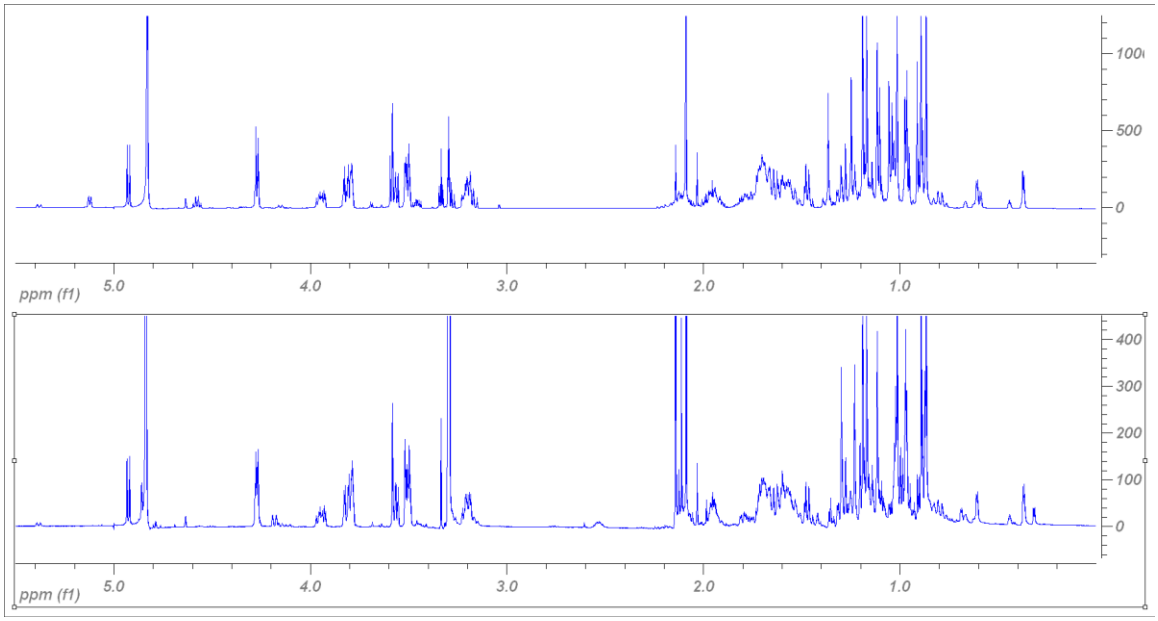


Figure 4b. ^{1}H NMR spectra of fractions F15-4 (top) and F15-5 (bottom).

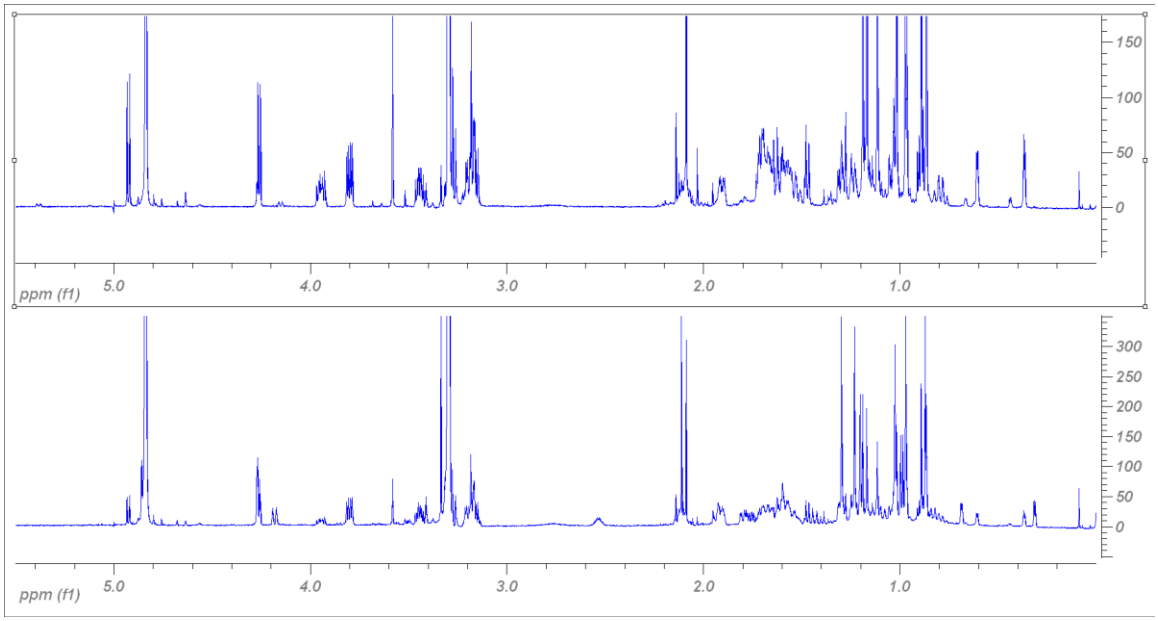


Figure 5a. Chromatogram of RP-18 HPLC separation of F15-5.

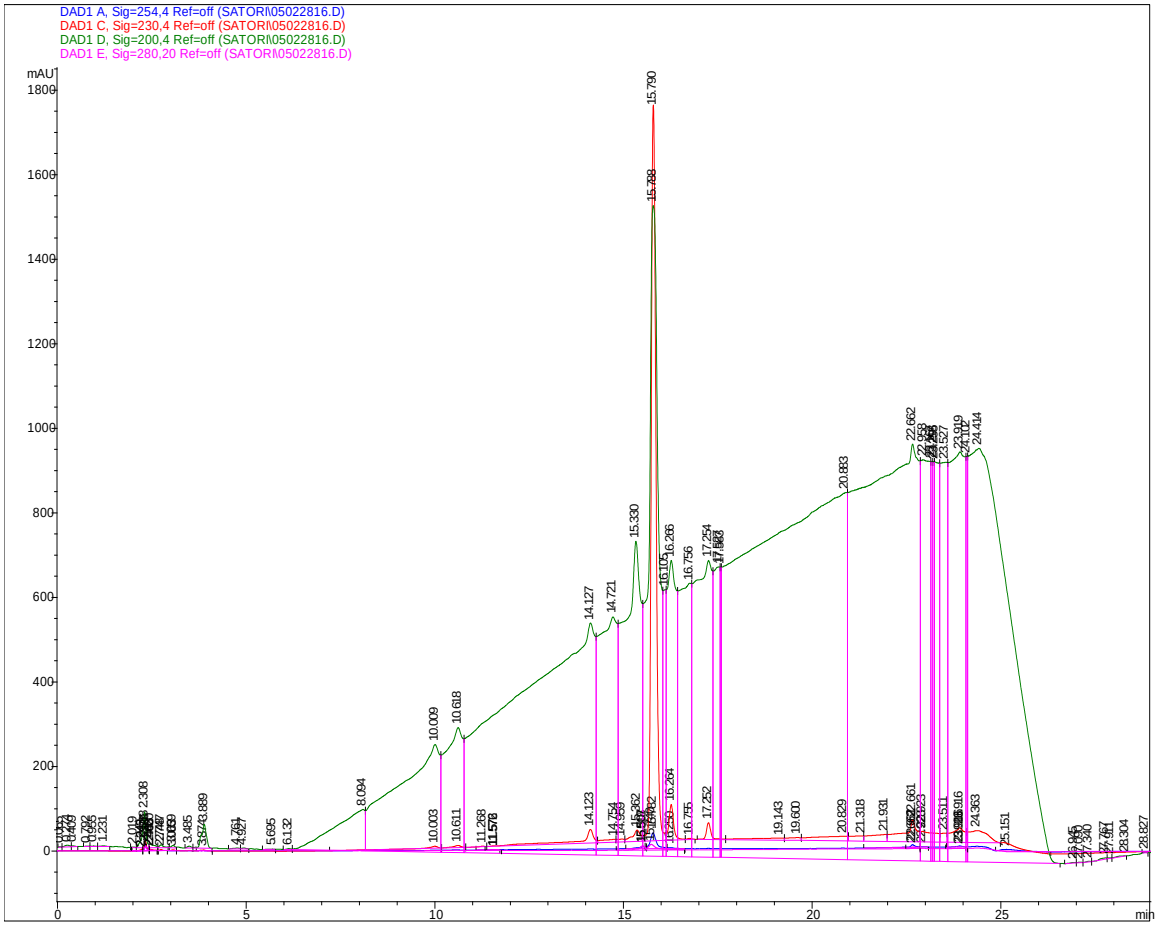


Figure 5b. Enlargement of chromatogram of F15-5 separation. Numbers 1 through 5 correspond to time windows for fractions F16-1 through F16-5.

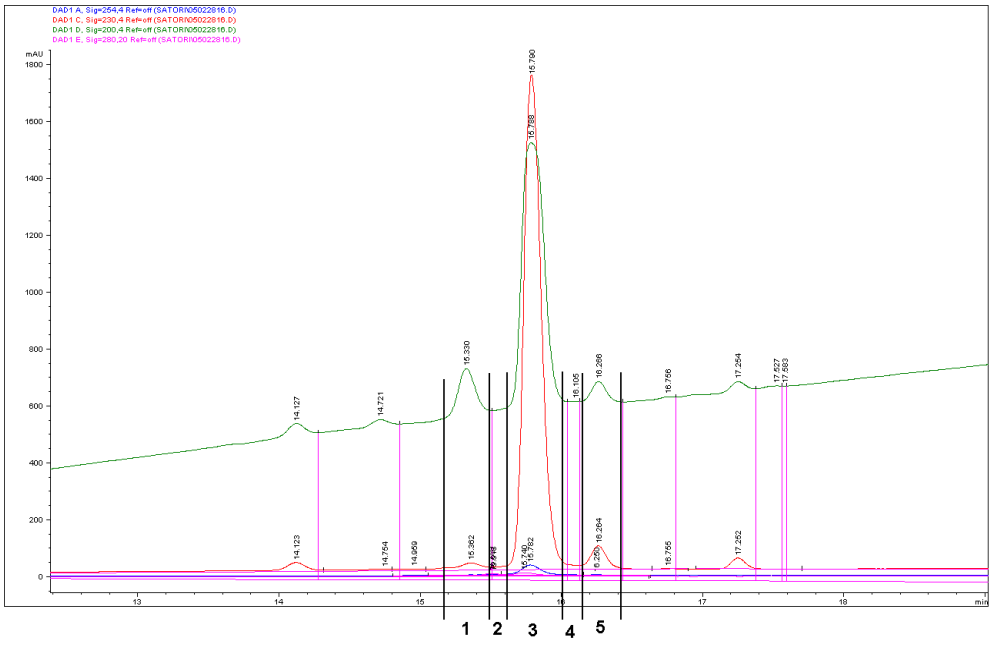
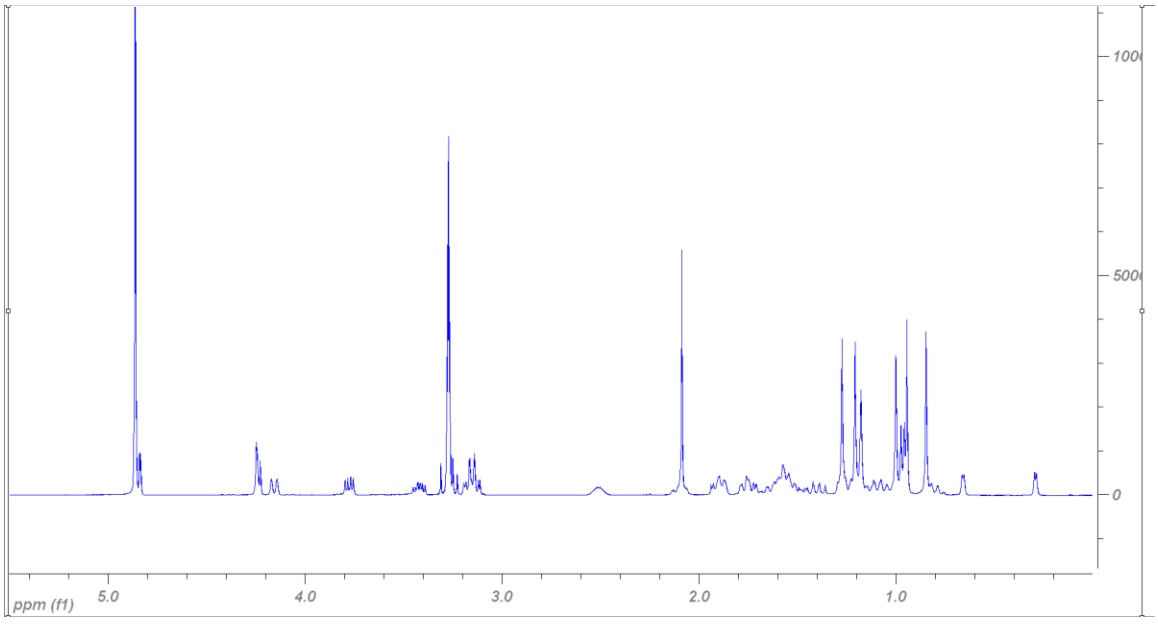


Figure 6. ^{1}H NMR spectrum of fraction F16-3 (98% pure **1**).



IP-MS analysis of Aβ(1-X) of cell-conditioned medium. CHO-7w and CHO-7PA2 cells cultured in DMEM/10% fetal bovine serum were plated in 10-cm dishes and treated during 24 h with DMSO as vehicle control, **1** (0.1, 1 μM), the γ-secretase inhibitor DAPT (20 μM), or the γ-secretase modulator sulindac sulfide (100 μM). The conditioned media were harvested and frozen for subsequent analysis. IP-MS analysis for Aβ(1-X) was performed as previously described.[30,31]

Treatment with compound **1** lowered the level of Aβ42 and increased the proportion of Aβ37 and Aβ39 in both cell lines. DAPT lowered the production of total Aβ as did sulindac sulfide at the concentration tested.

Mass spectral data attached:

For CHO-7W and CHO-7PA2 cells, data are shown for treatment with:

1) DMSO vehicle control

2) Compound **1** (100 nM)

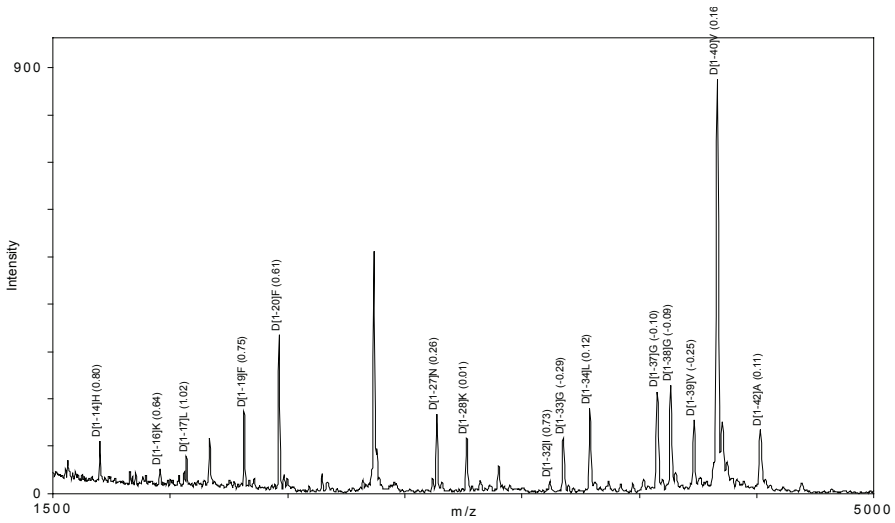
3) Compound **1** (1 μM)

4) DAPT (20 μM)

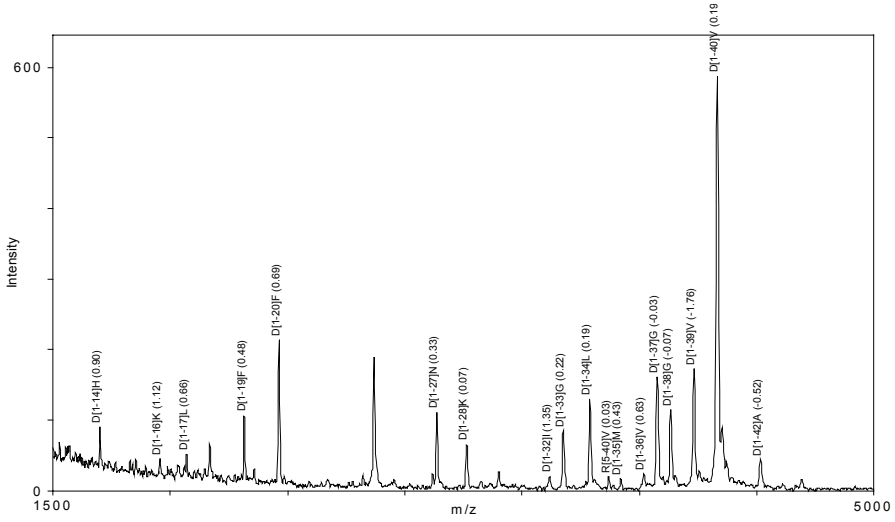
5) Sulindac sulfide (100 μM)

7W samples

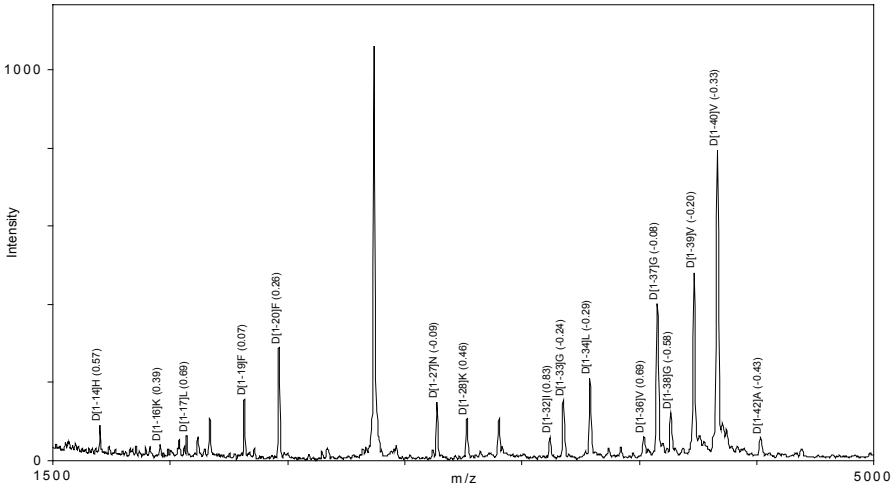
DMSO vehicle control



Compound 1 100 nM

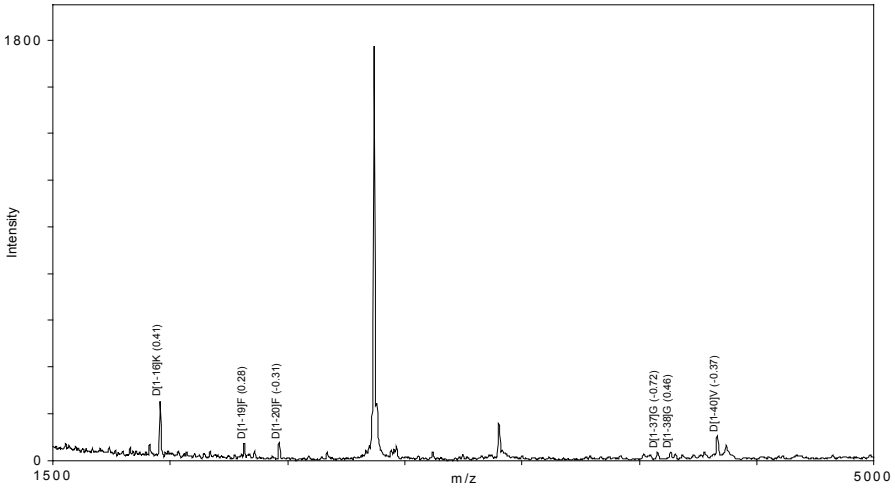


Compound 1 1 μM

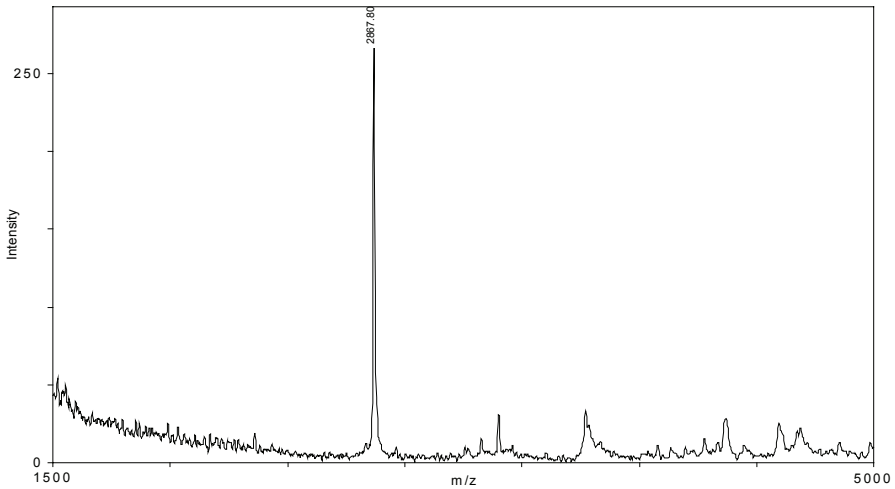


7W samples

DATP
20 μM

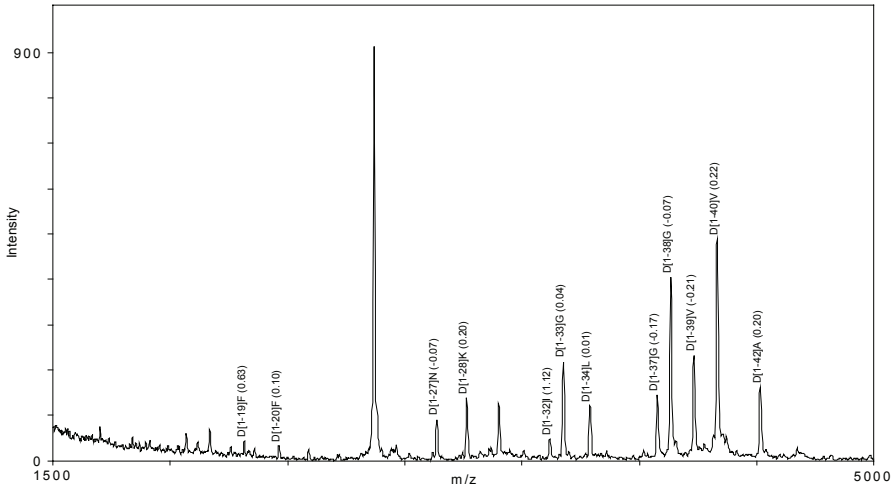


Sulindac sulfide
100 μM

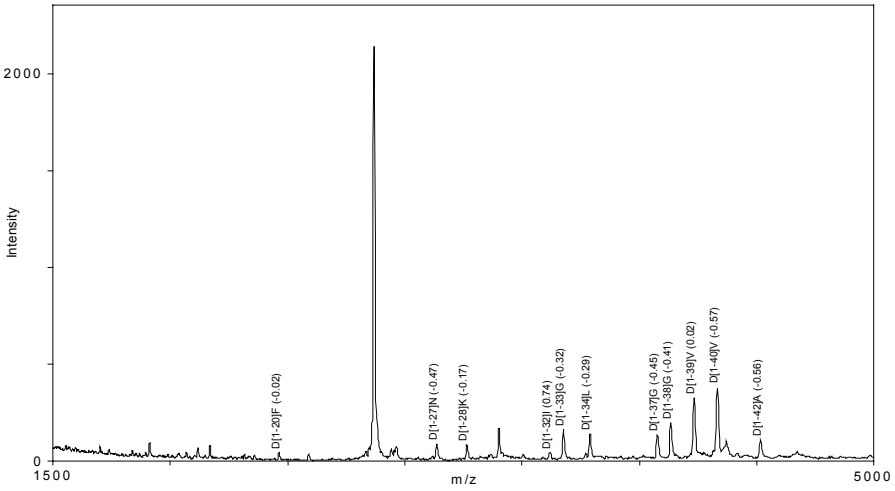


7PA2 samples

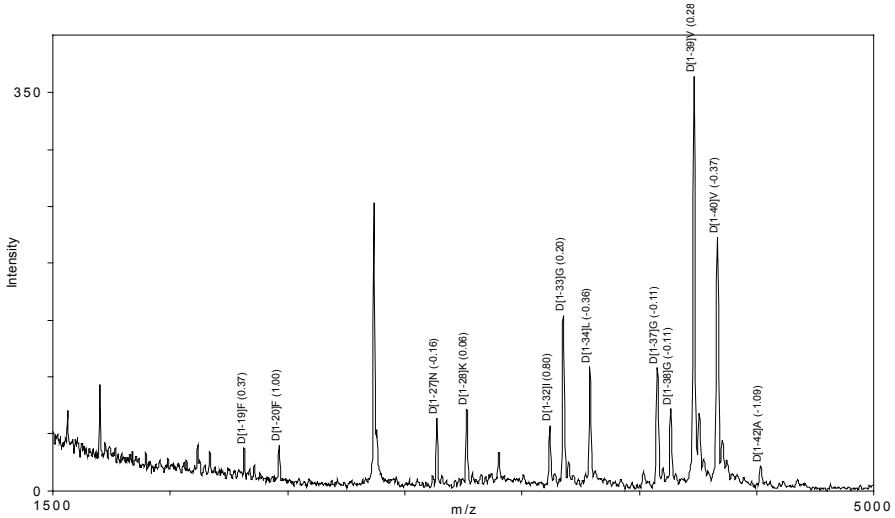
DMSO vehicle control



Compound 1 100 nM

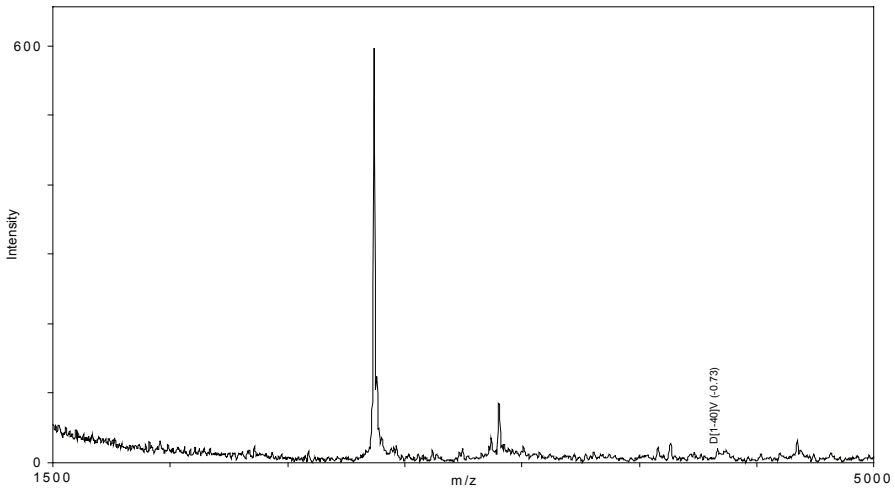


Compound 1 1 μM



7PA2 samples

DATP
20 μM



Sulindac sulfide
100 μM