Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPLEMENTARY DATA FOR

'Isolation and characterisation of amphotericin B analogues and truncated polyketide intermediates produced by genetic engineering of *Streptomyces nodosus*'

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Further information available from corresponding author, Dr Bernard Rawlings.
Microbiological and molecular biology information available from Dr Patrick Caffrey.

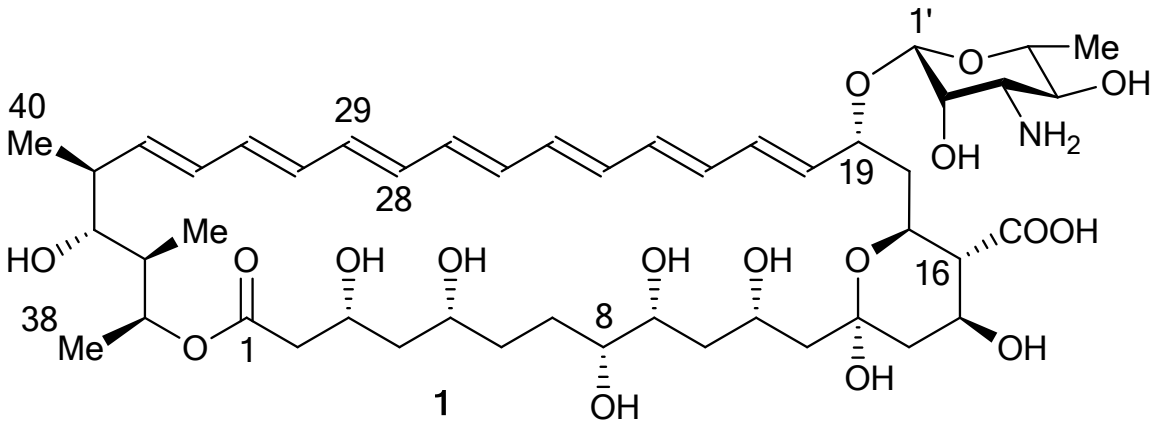
Index for Supplementary Information

Flasks used for GYE medium, and growth after 48 h

250 mL culture with XAD16 resin in trigrooved 2 L flask (4 day old)

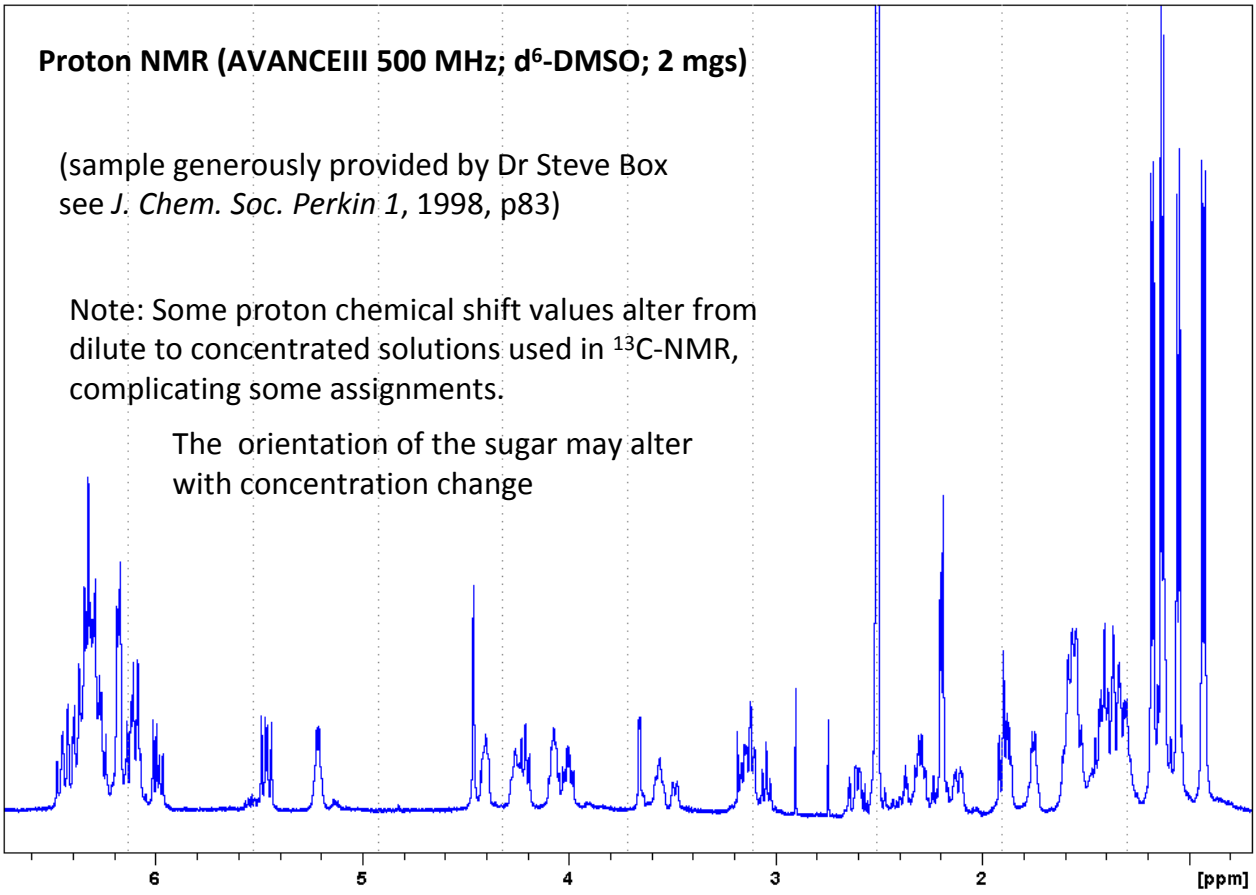
S. nodosus **(amphNM+perDIDII mutant)**

Selected data for amphotericin B (1).

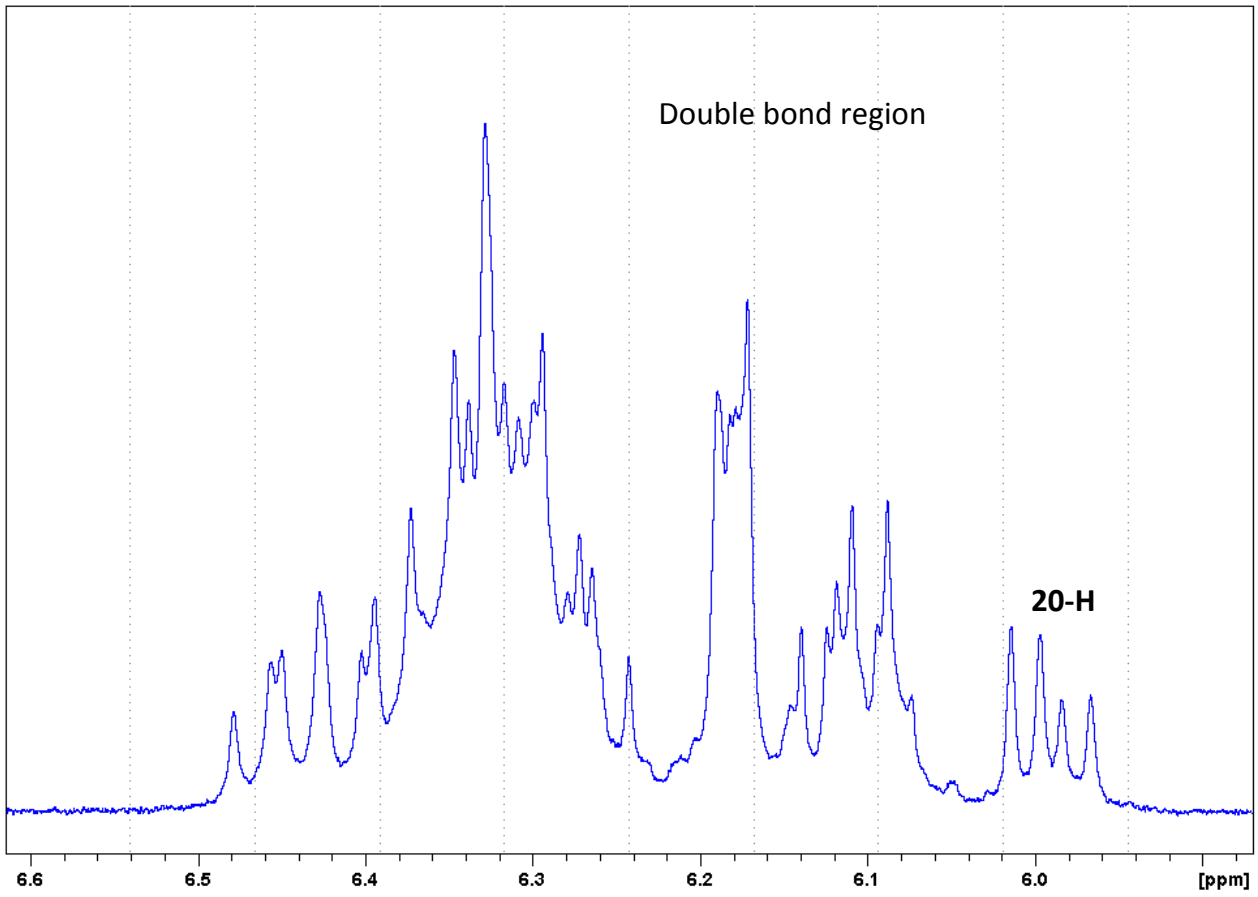


Amphotericin B 1

Amphotericin B 1



Amphotericin B 1



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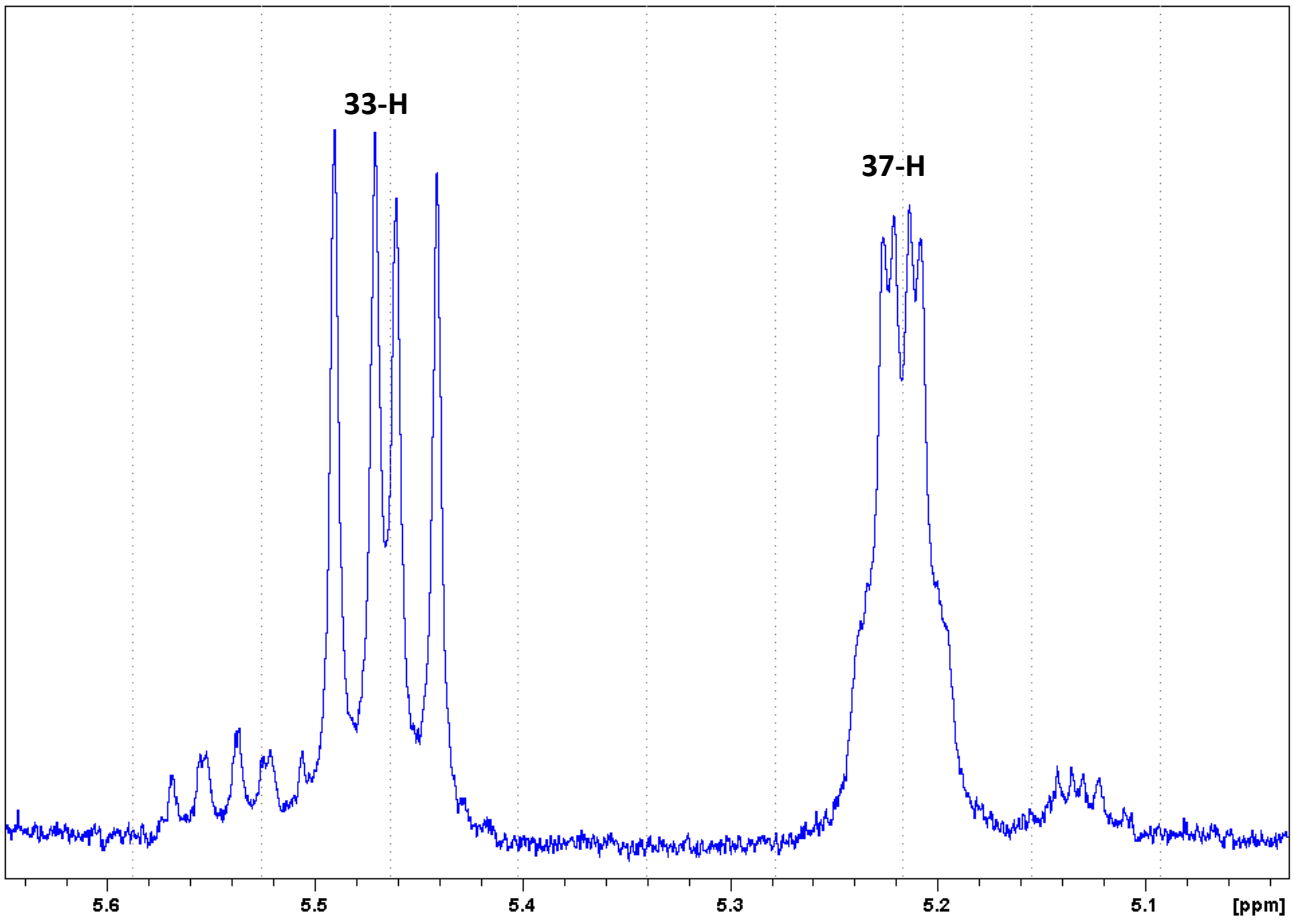

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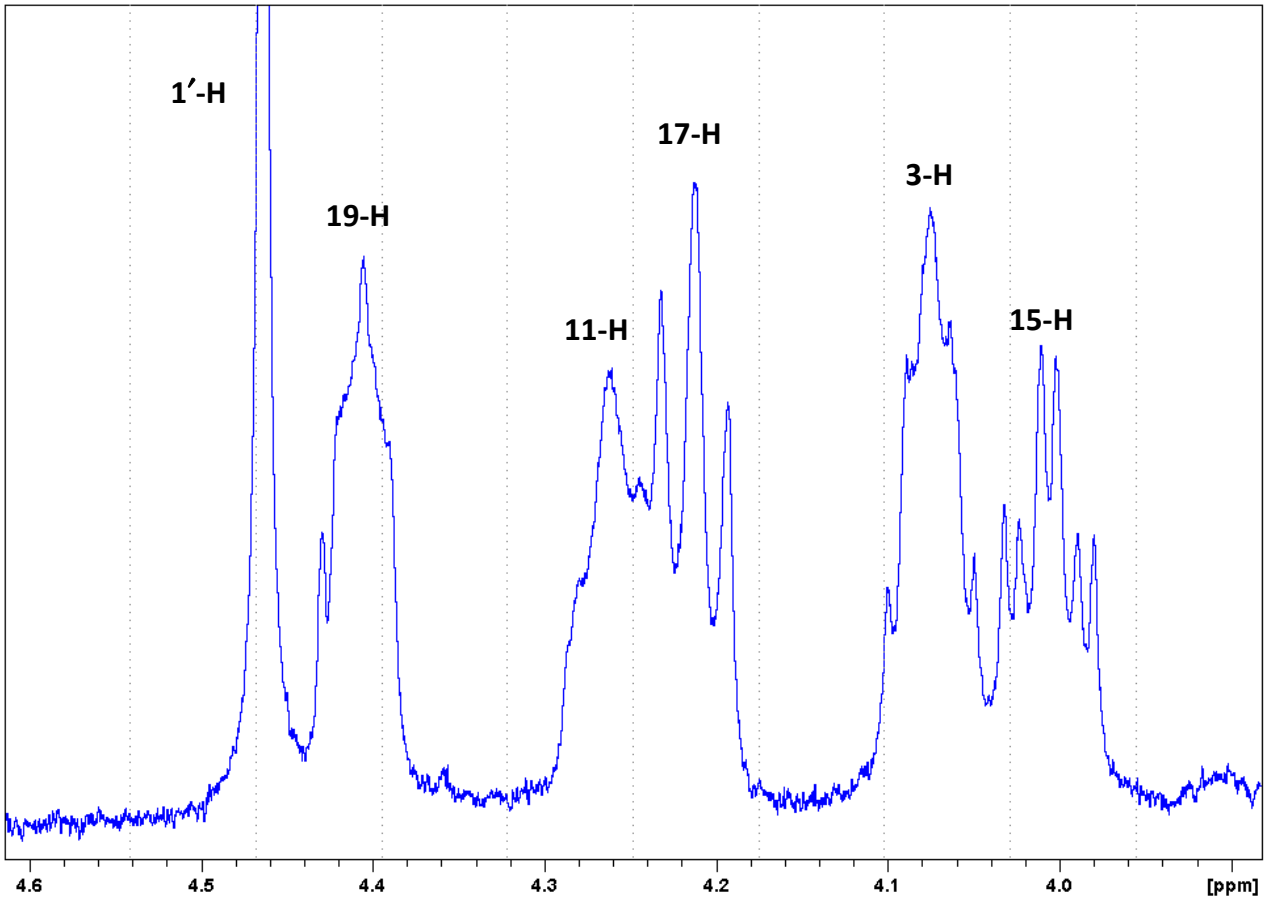

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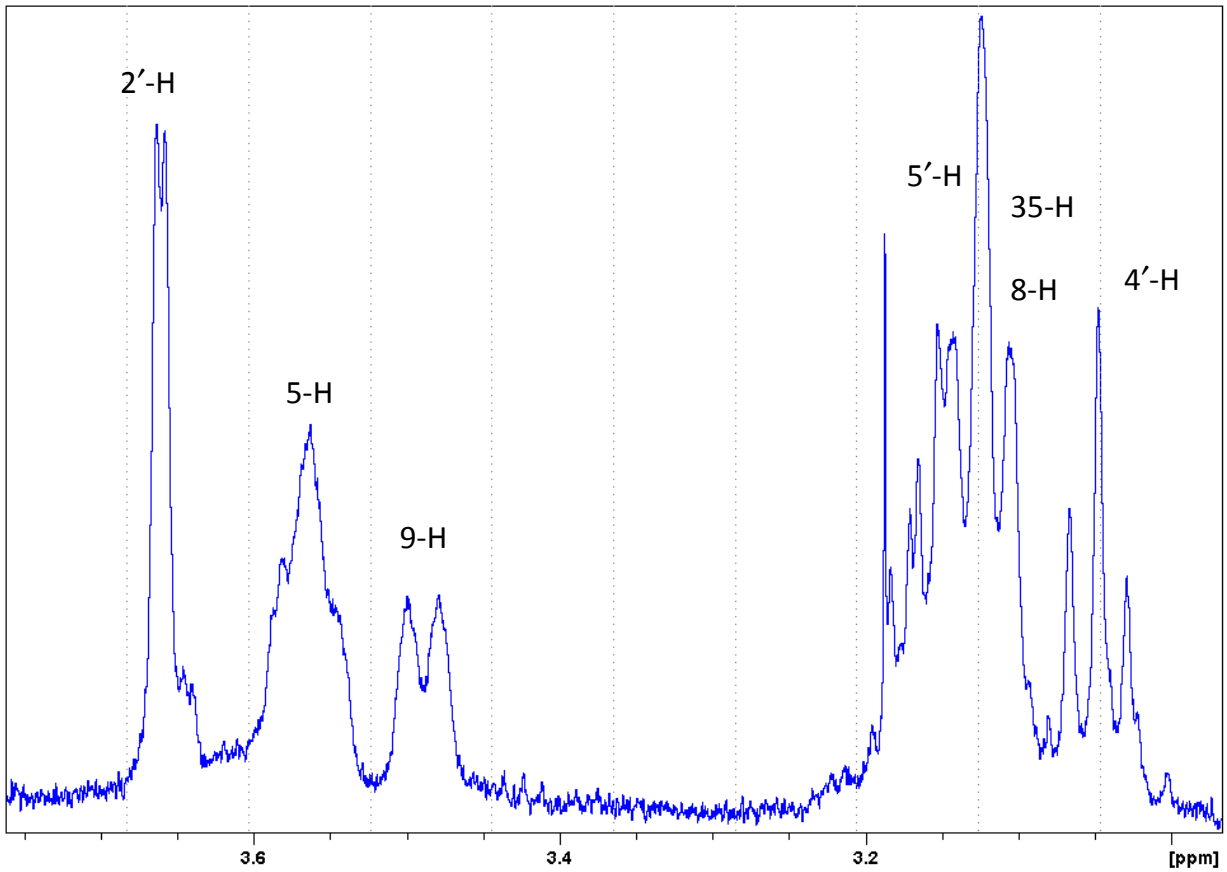

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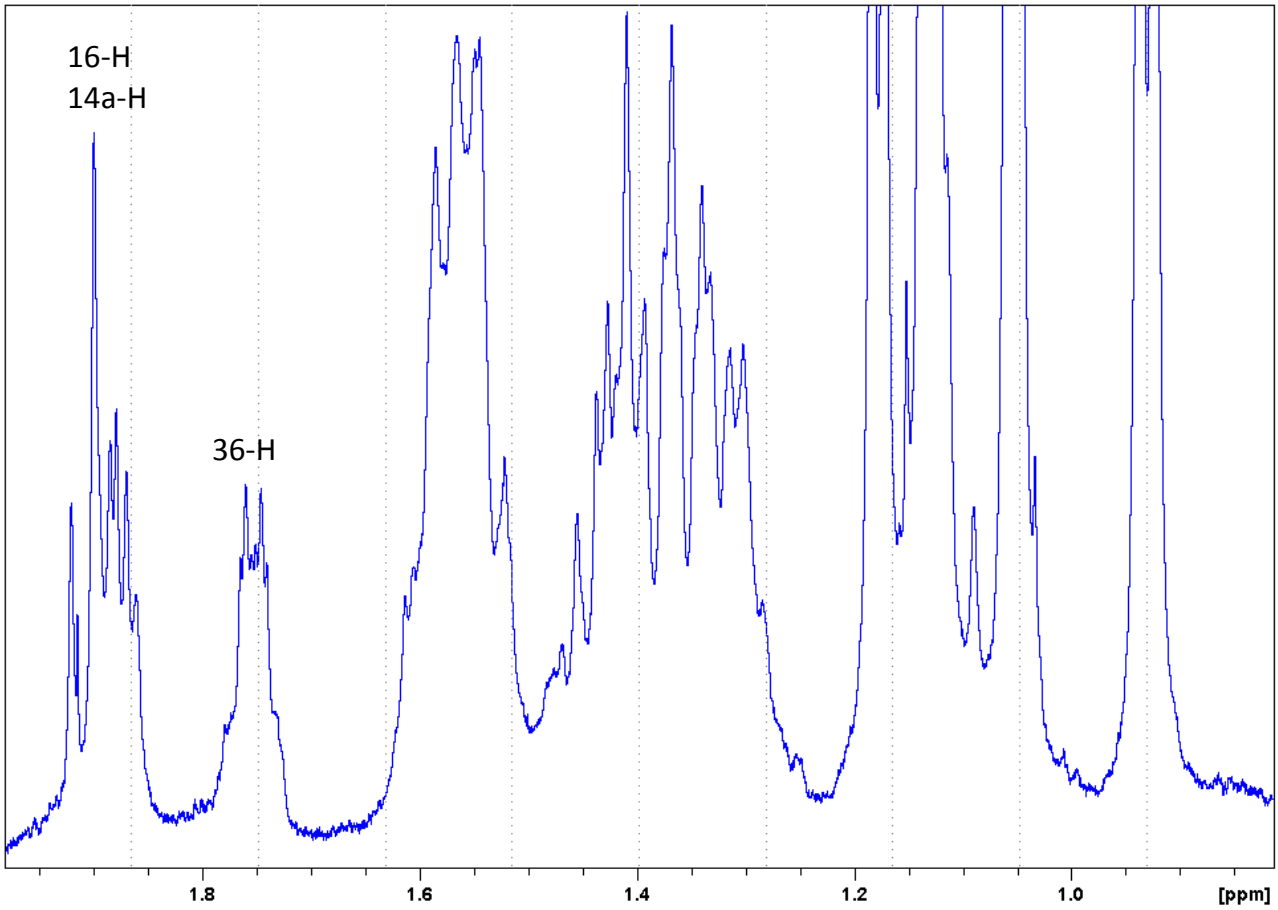
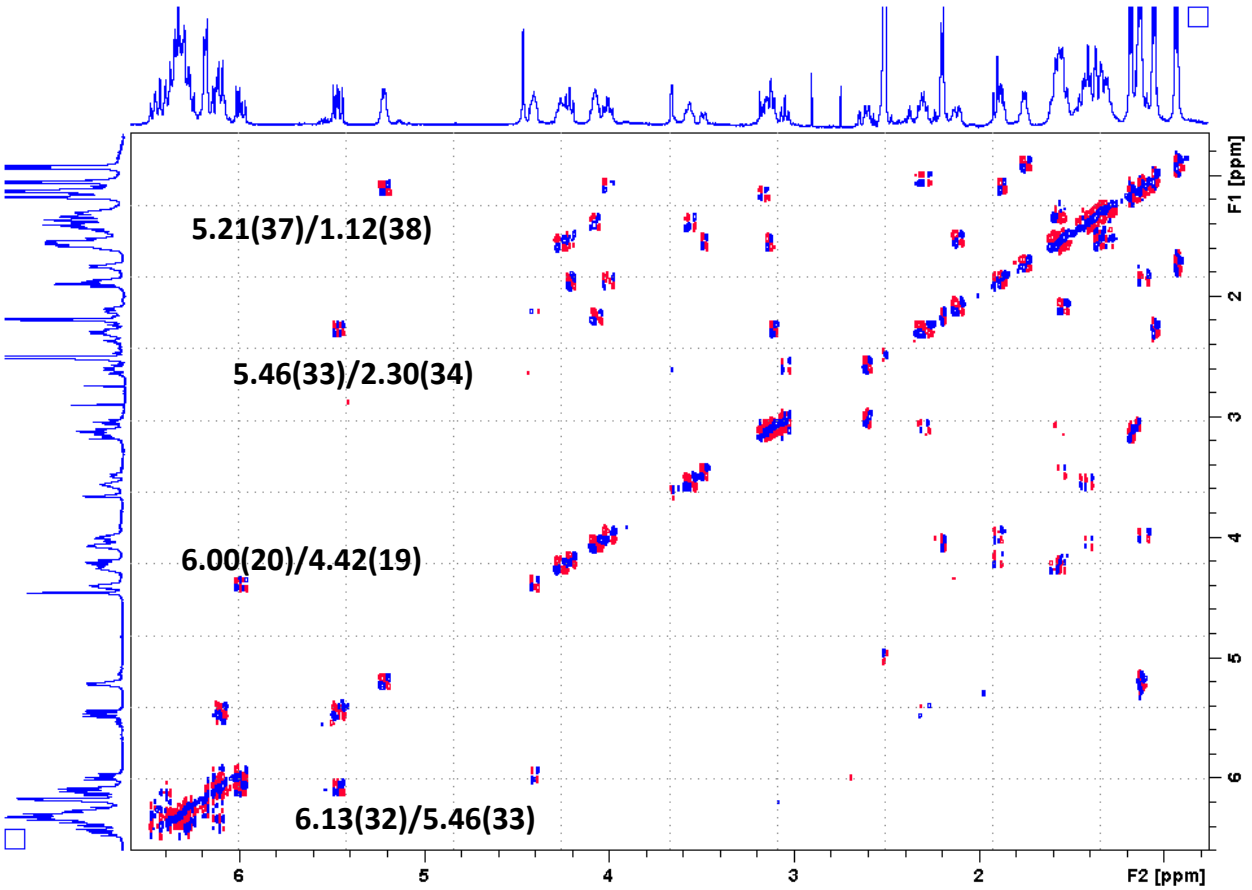


Amphotericin B 1 Proton-proton COSY



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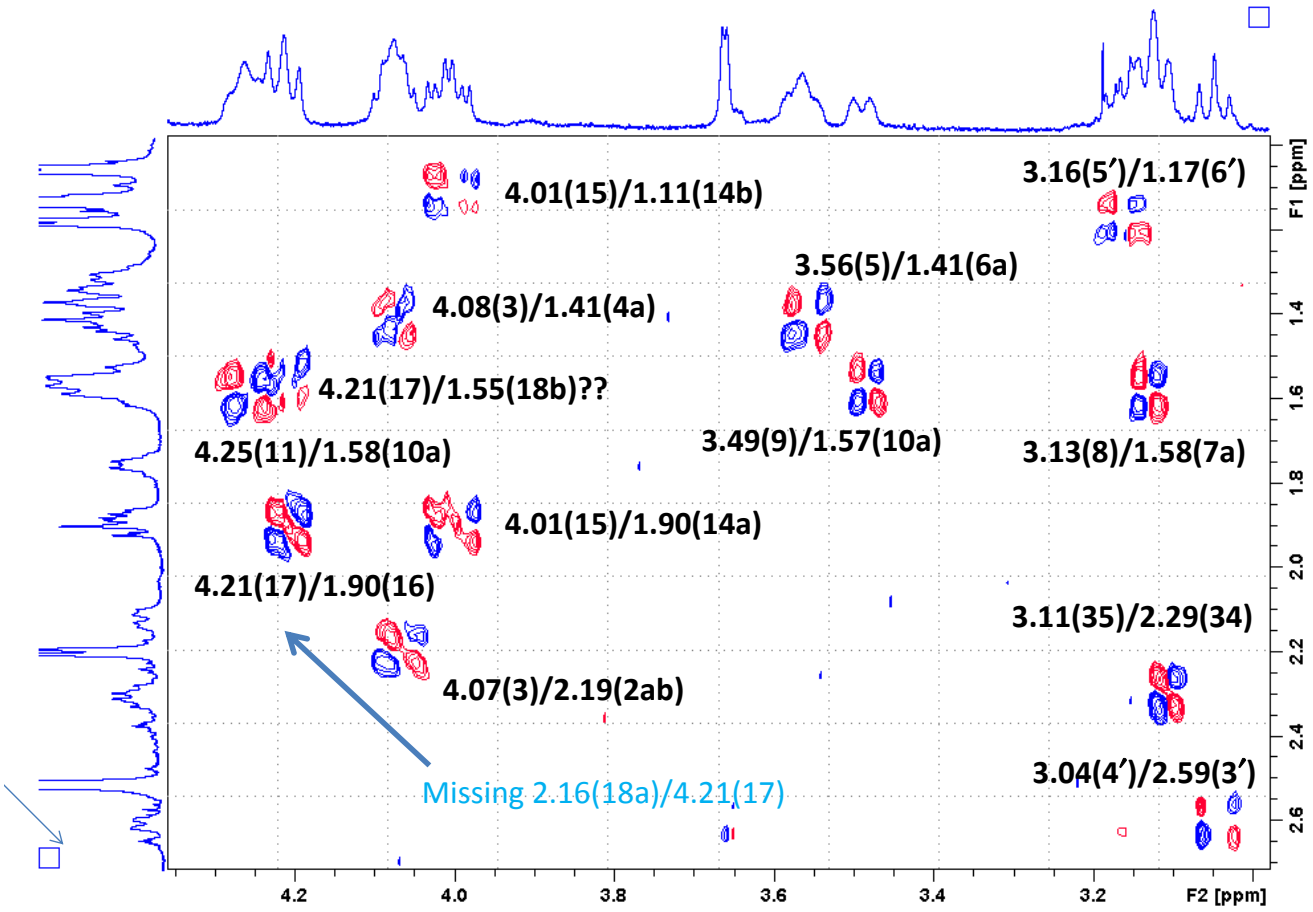

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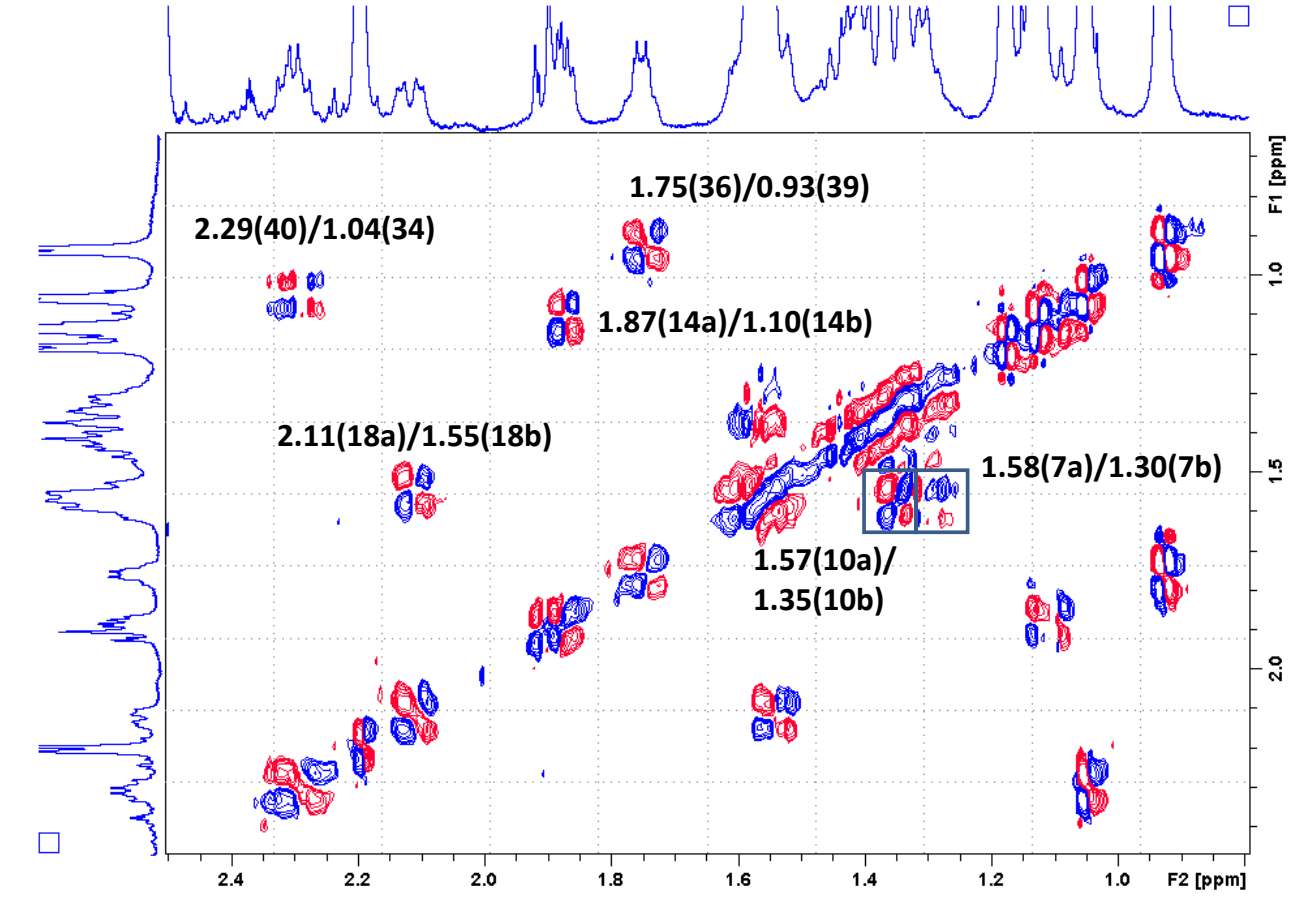

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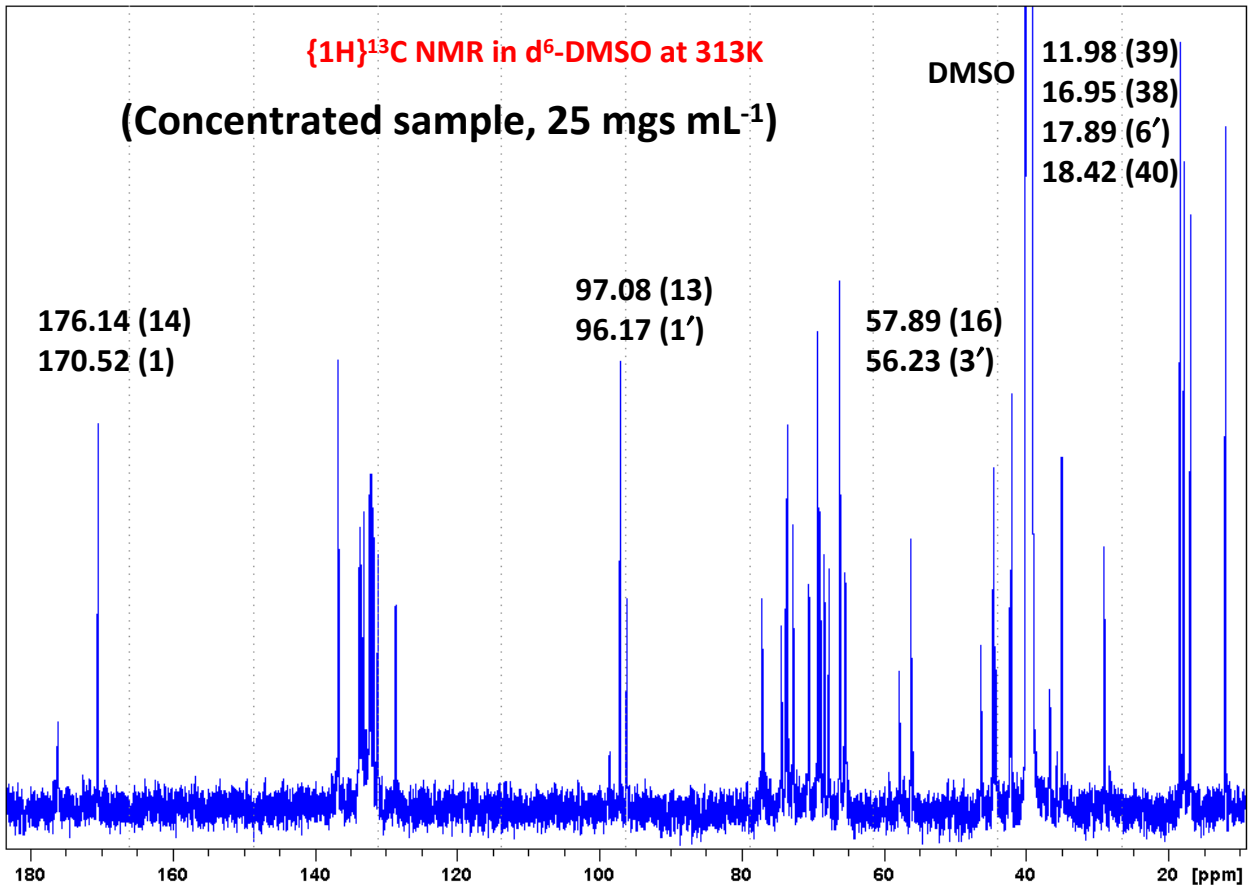

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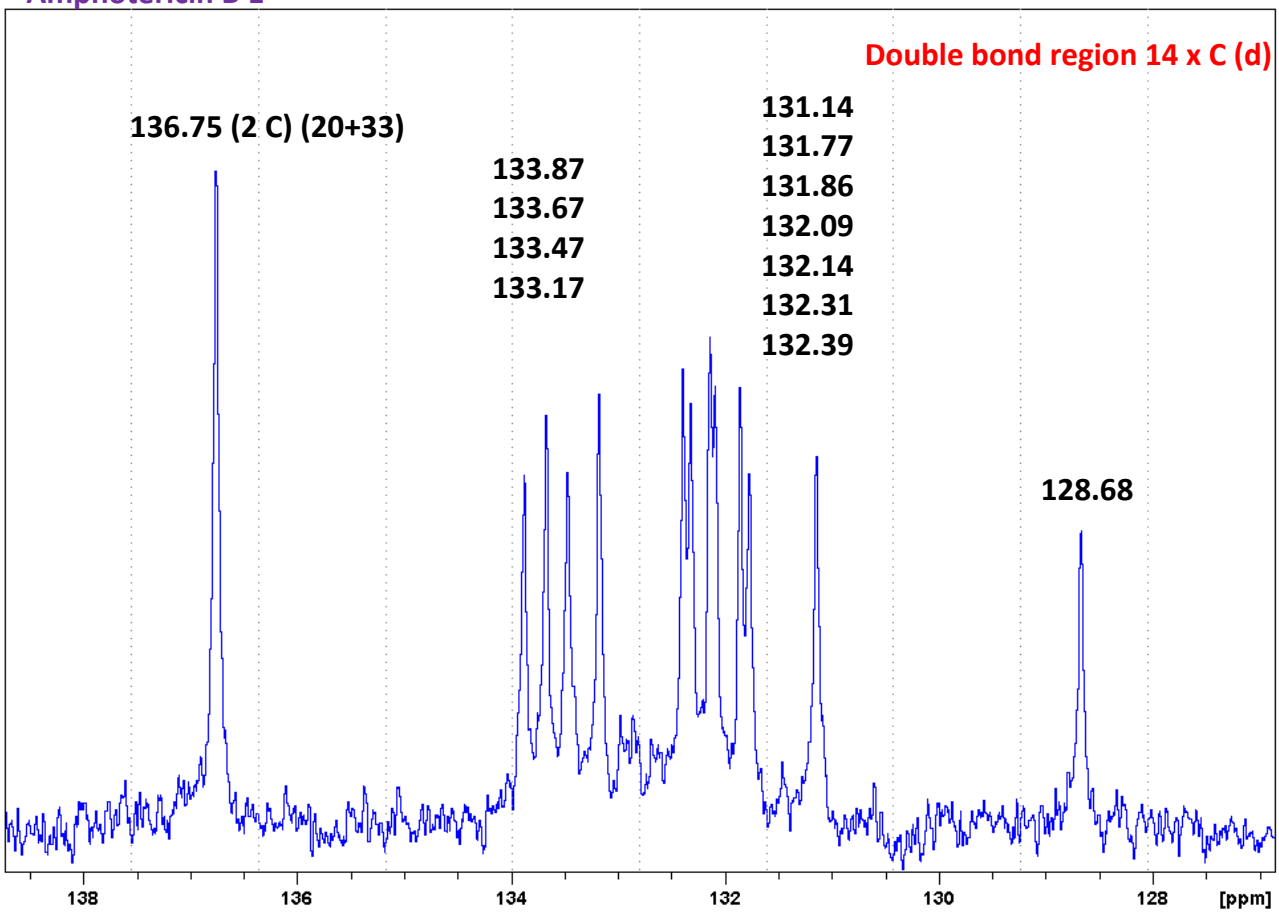

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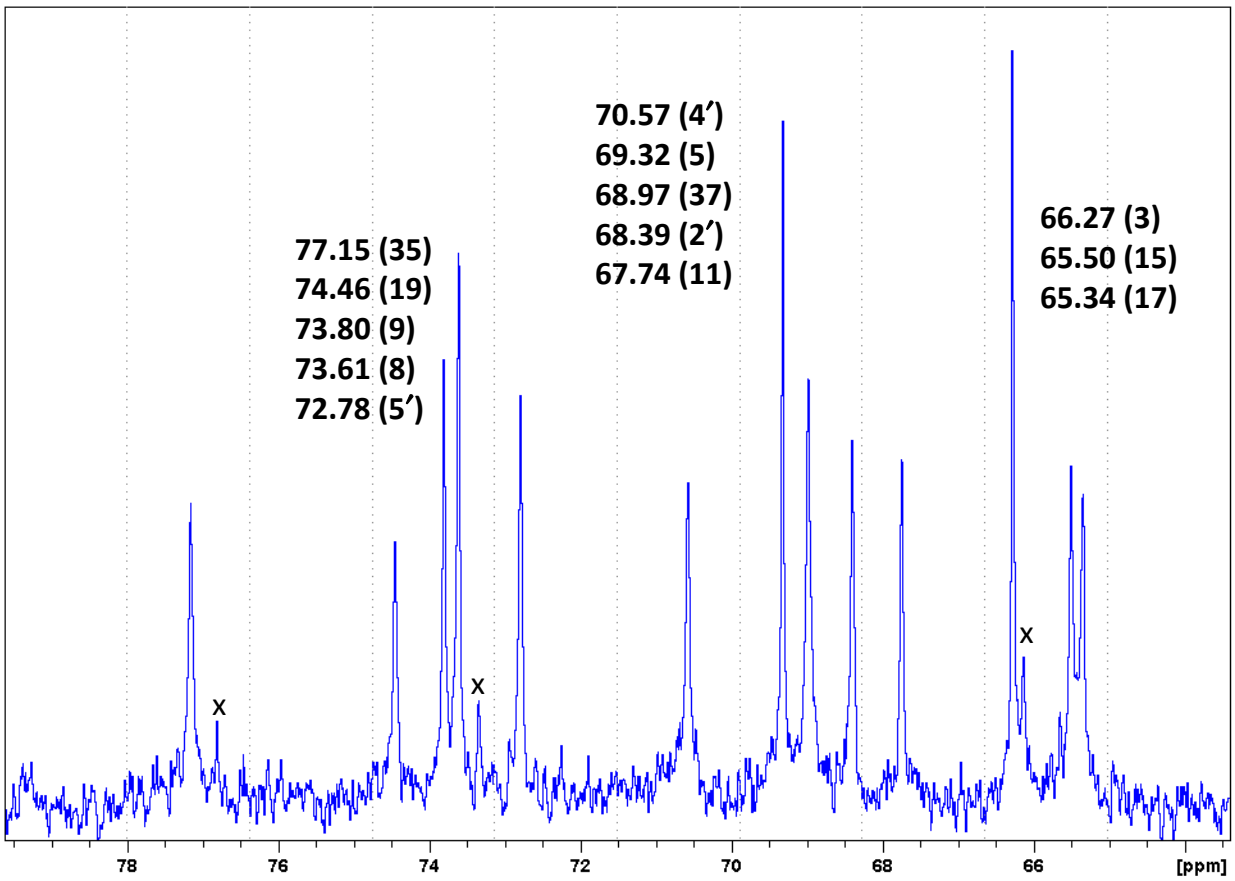
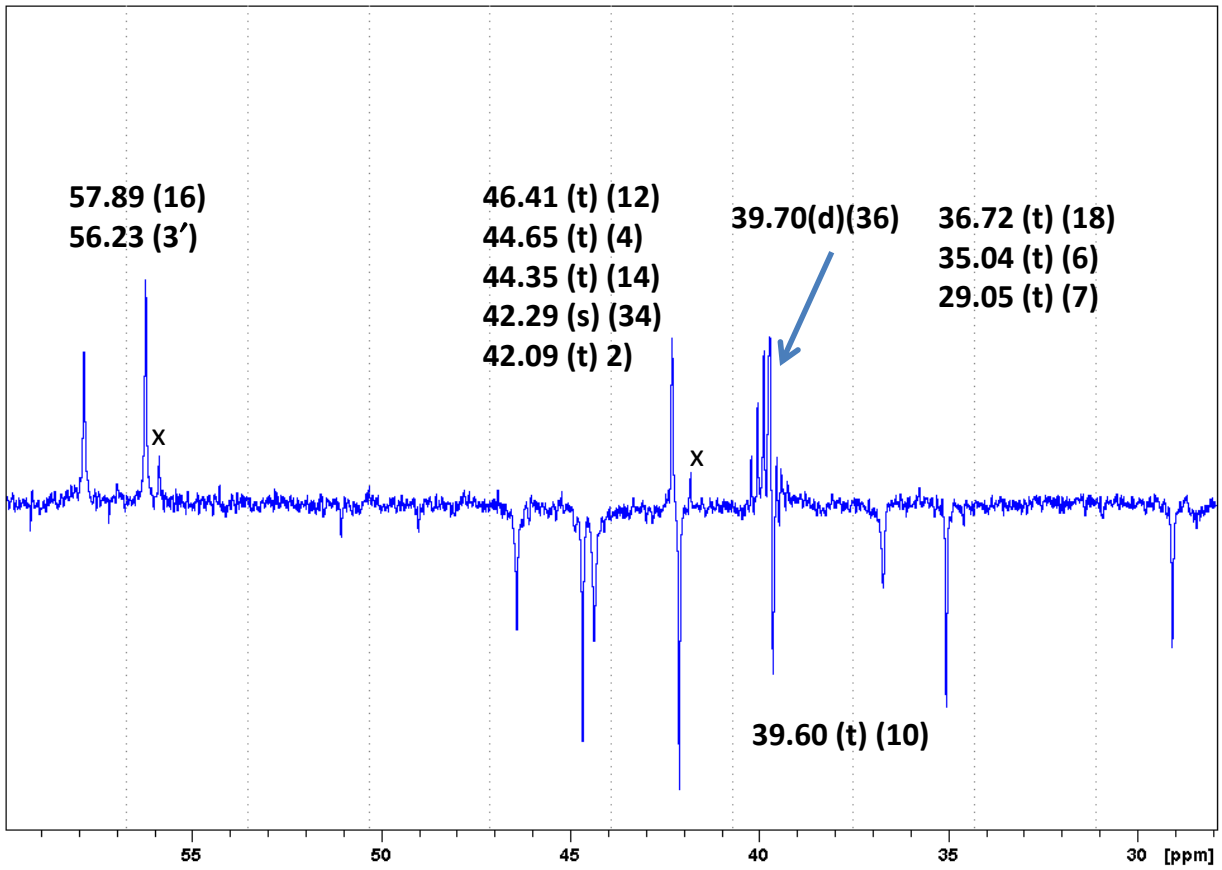
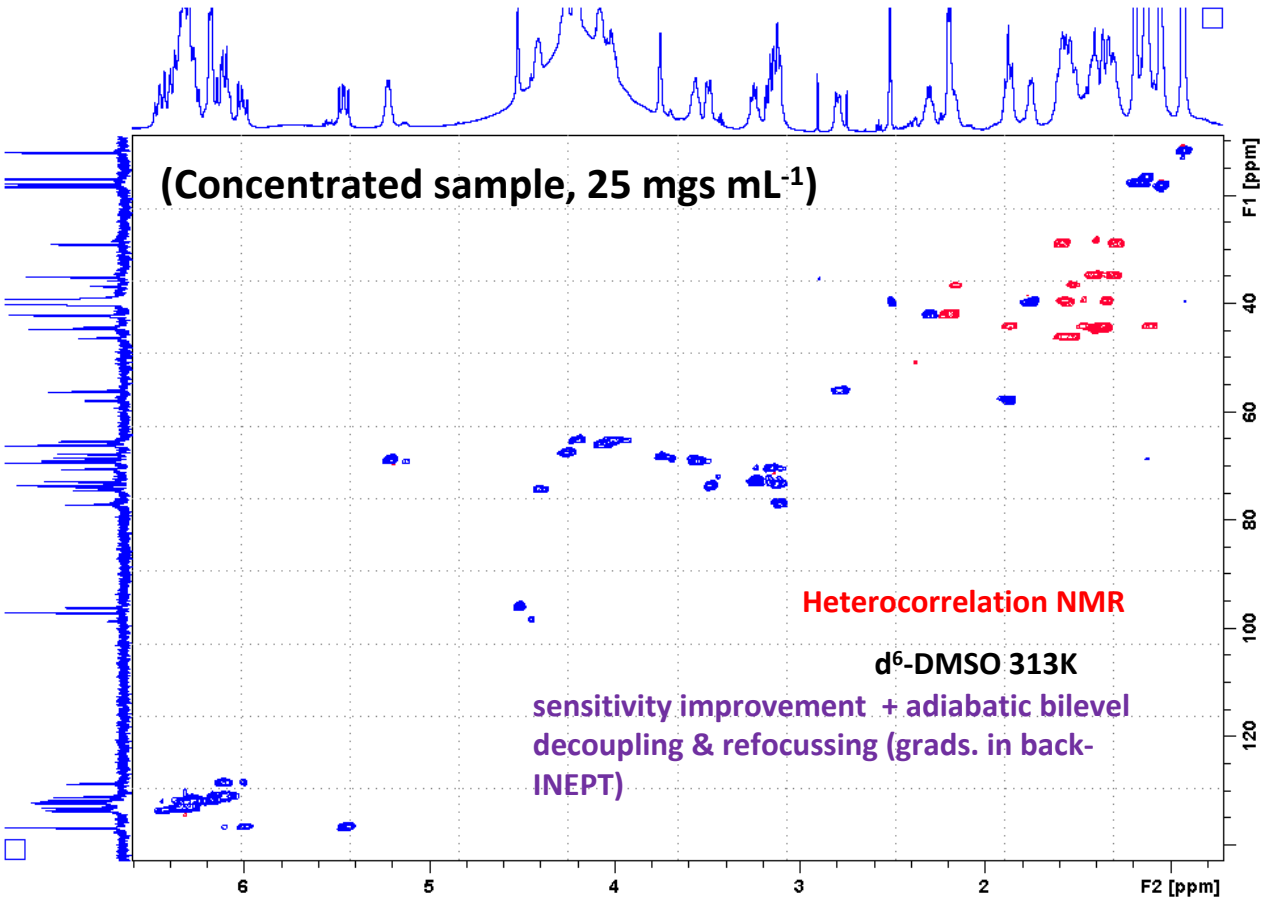
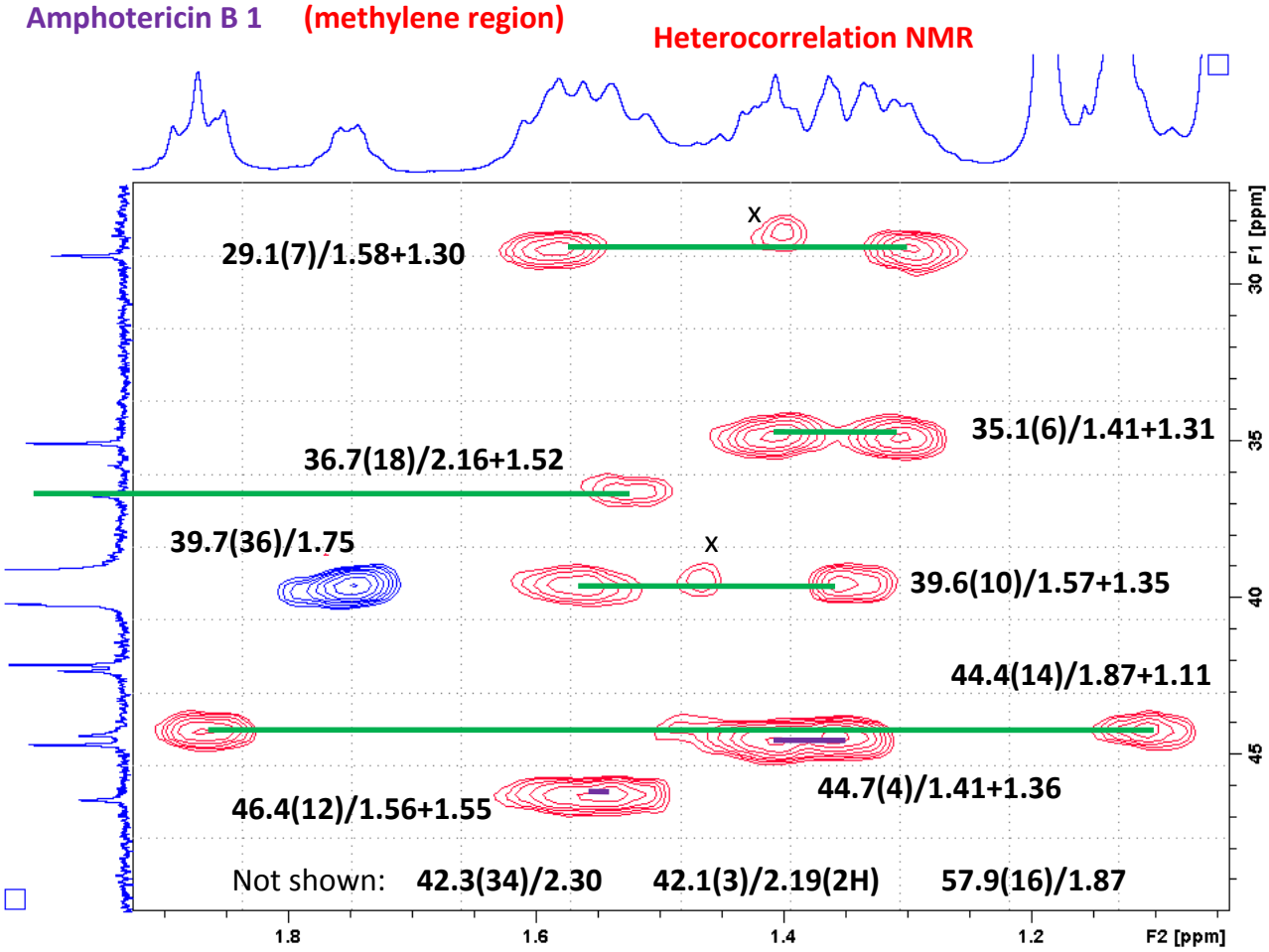


Amphotericin B 1 DEPT, showing resonance under DMSO



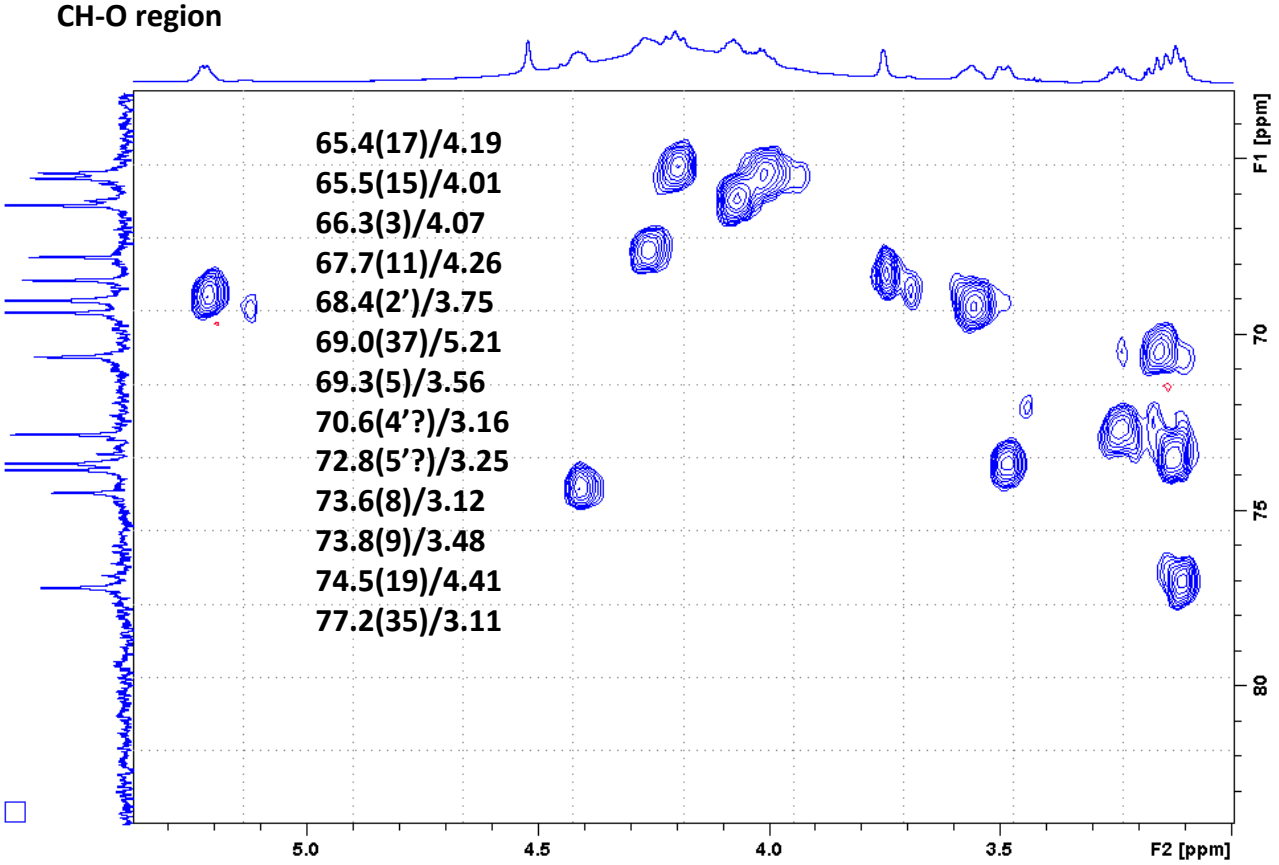

Amphotericin B 1
(Concentrated sample, 25 mgs mL-1)
Heterocorrelation NMR
d6-DMSO 313K
sensitivity improvement + adiabatic bilevel decoupling & refocussing (grads. in back-INEPT)
F1 [ppm]
F2 [ppm]


Amphotericin B 1
(methylene region)
Heterocorrelation NMR
29.1(7)/1.58+1.30
35.1(6)/1.41+1.31
36.7(18)/2.16+1.52
39.7(36)/1.75
39.6(10)/1.57+1.35
44.4(14)/1.87+1.11
44.7(4)/1.41+1.36
46.4(12)/1.56+1.55
Not shown: 42.3(34)/2.30 42.1(3)/2.19(2H) 57.9(16)/1.87
F1 [ppm]
F2 [ppm]

Amphotericin B 1

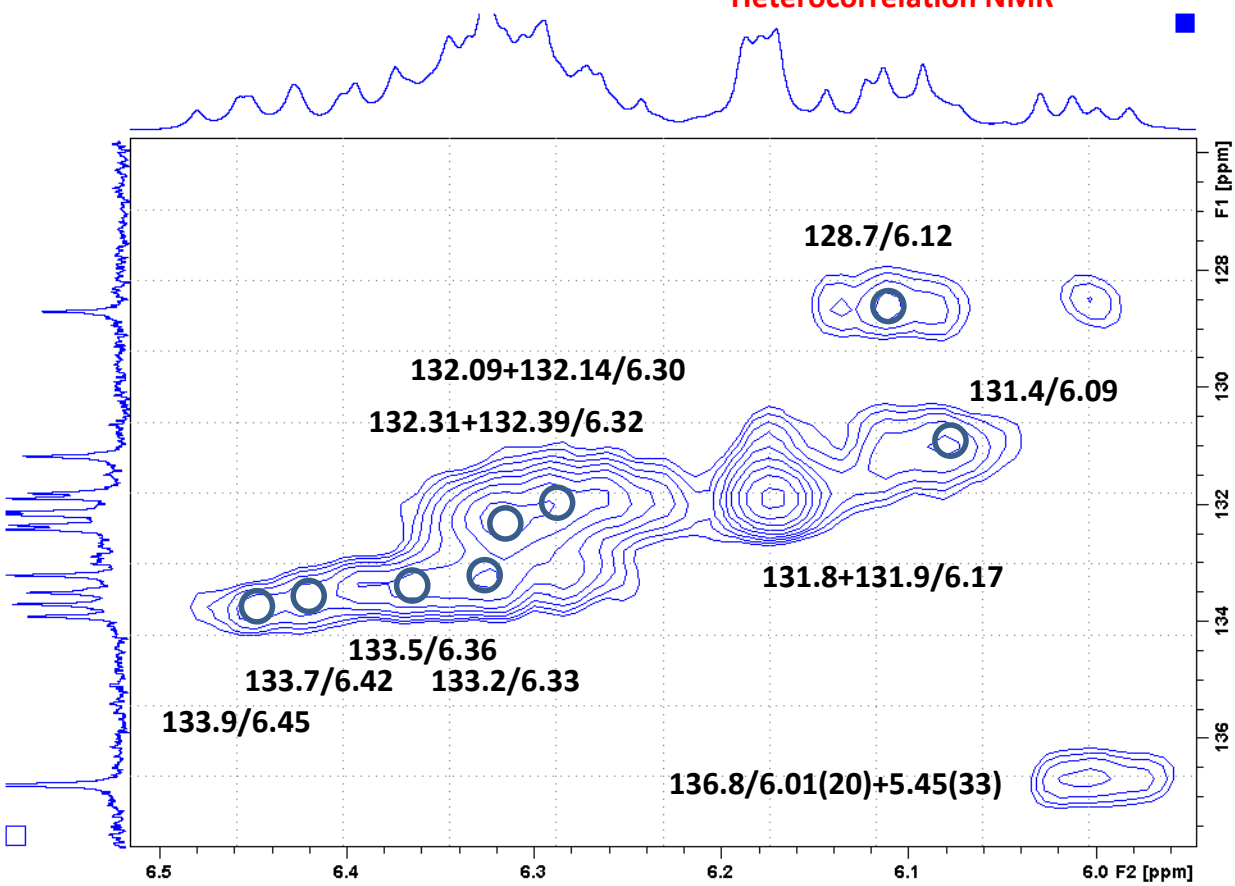
Heterocorrelation NMR

CH-O region



Amphotericin B 1

Heterocorrelation NMR



Amphotericin B 1

NMR(500 MHz) in d^6-DMSO at 313K

(some proton chemical shift values shift from dilute to concentrated solution)

E.g. 3.04 in dilute solution has moved downfield.

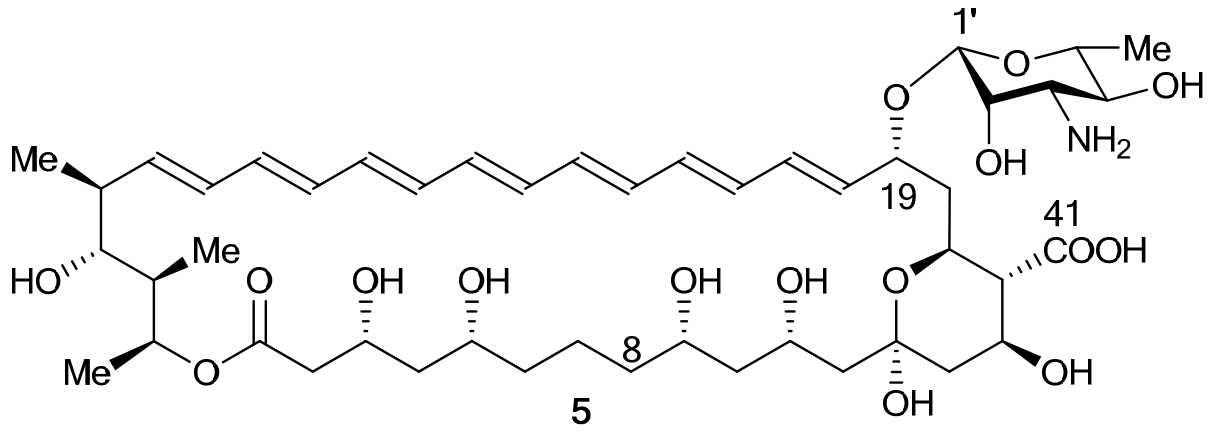
H-3′ is 2.59 in [dilute] and 2.79 in concentrated DMSO

11.98 (C-39)/0.93 (H)
16.95(38)/1.13
17.89(6')/1.18
18.42(40)/1.05
29.1(7)/1.58+1.30
35.1(6)/1.41+1.31
36.7(18)/2.16+1.52
39.6(10) 1.57+1.35
39.7(36) 1.75
42.1(2ab)/2.19
42.3(34)/2.30
44.4(14)/1.87+1.11
44.7(4)/1.41+1.36
46.4(12)/1.56+1.55
56.2(3')/2.79[2.59]
57.9(16)/1.87
65.4(17)/4.19
65.5(15)/4.01
66.3(3)/4.07
67.7(11)/4.26
68.4(2')/3.75
69.0(37)/5.21
69.3(5)/3.56
70.6(4'?)/3.16 [3.04 dil]
72.8(5'?)/3.25 [3.16 dil]
73.6(8)/3.12
73.8(9)/3.48
74.5(19)/4.41
77.2(35)/3.11
96.2(1')/4.51
97.1(13)
128.7(32)/6.12
131.44/6.09
131.77/6.17
131.86/6.17
132.09/*ca* 6.30
132.14/*ca* 6.30
132.31/*ca* 6.32
132.39/*ca* 6.32
133.18/6.33
133.47/6.36
133.67/6.42
133.87/6.45
136.8(33)/5.45
136.8(20)/6.01
170.50(1)
176.14(41)

Discrepancy with Perkin 1998 p83 (NMR at RT):
C-6 is 1.41+1.31.
Typo error in earlier paper: 33-H = 5.51 not 4.51.

AmphL : 8-Deoxyamphotericin B (5)

8-Deoxyamphotericin B 5



Isolated from the 'amphL' mutant

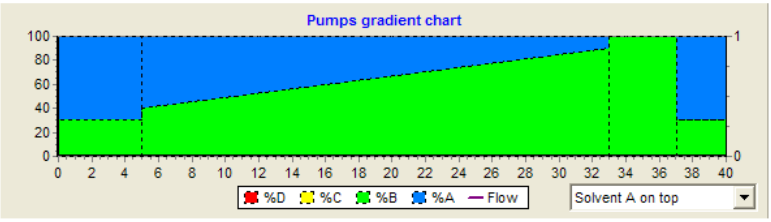
RMM 907.6

AmphL : 8-Deoxyamphotericin B (5)

HPLC analysis of amph L extractions (8-deoxy amphotericin B)

A – Water B – Methanol + 0.1% Formic Acid

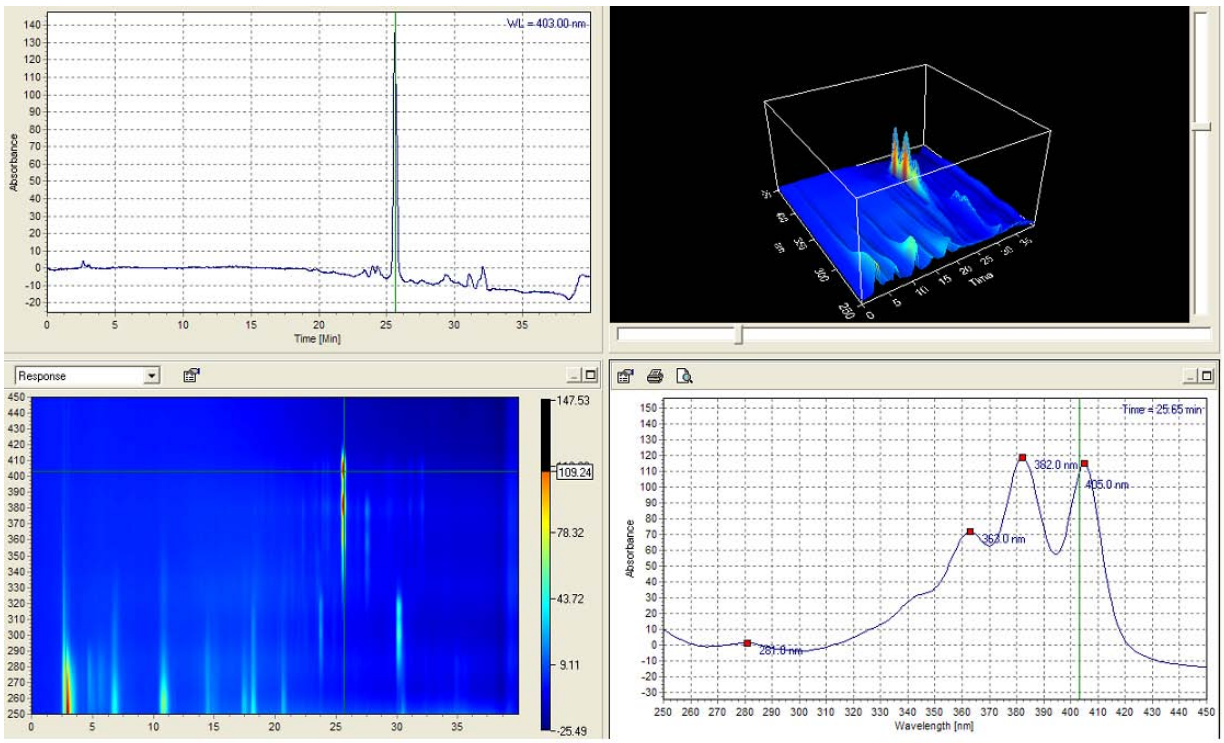
Method name – BM RP C8 Amph L analytical.meth (1 mL/min)
- BM RP C8 Amph L Prep.meth (14.8 mL/min)



Prerun	1.000 ml/min	A=70.00% B=30.00%
5.0 min	1.000 ml/min	A=70.00% B=30.00%
5.0 min	1.000 ml/min	A=60.00% B=40.00%
33.0 min	1.000 ml/min	A=10.00% B=90.00%
33.0 min	1.000 ml/min	A=0.00% B=100.00%
37.0 min	1.000 ml/min	A=0.00% B=100.00%
37.0 min	1.000 ml/min	A=70.00% B=30.00%
40.0 min	1.000 ml/min	A=70.00% B=30.00%

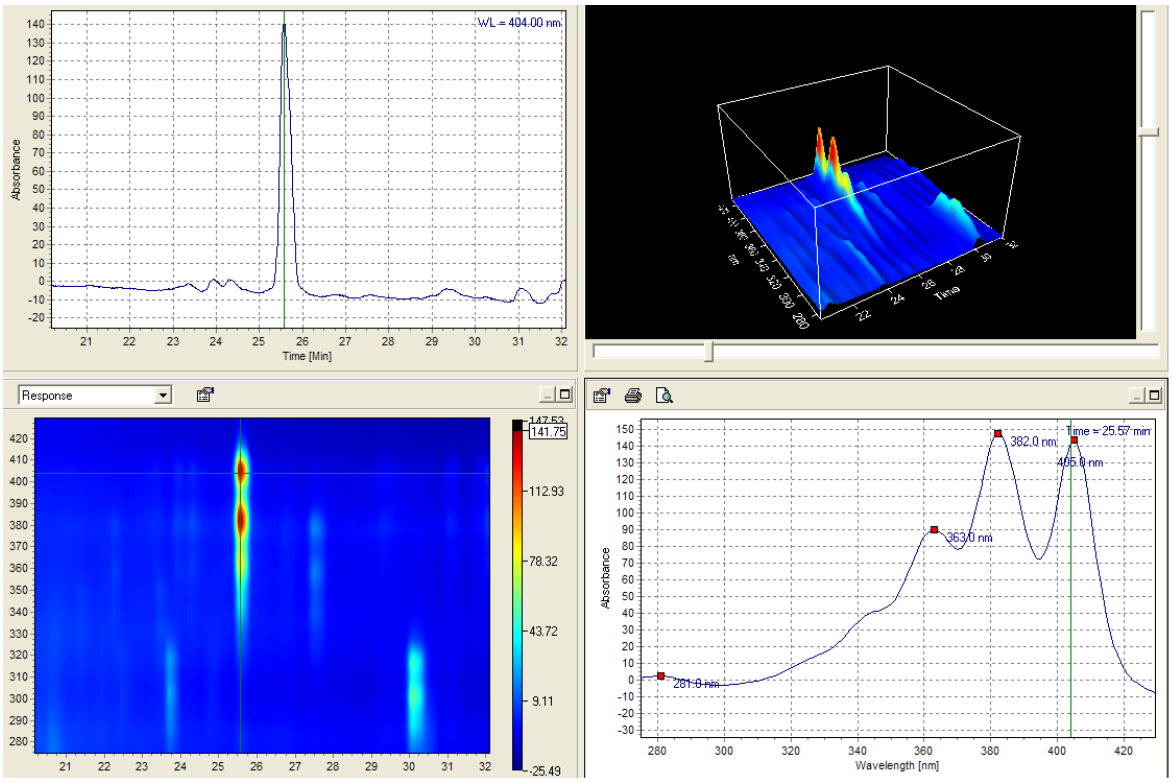
AmphL : 8-Deoxyamphotericin B (5)

amphL: Crude methanolic extract (best growth, least amphB)



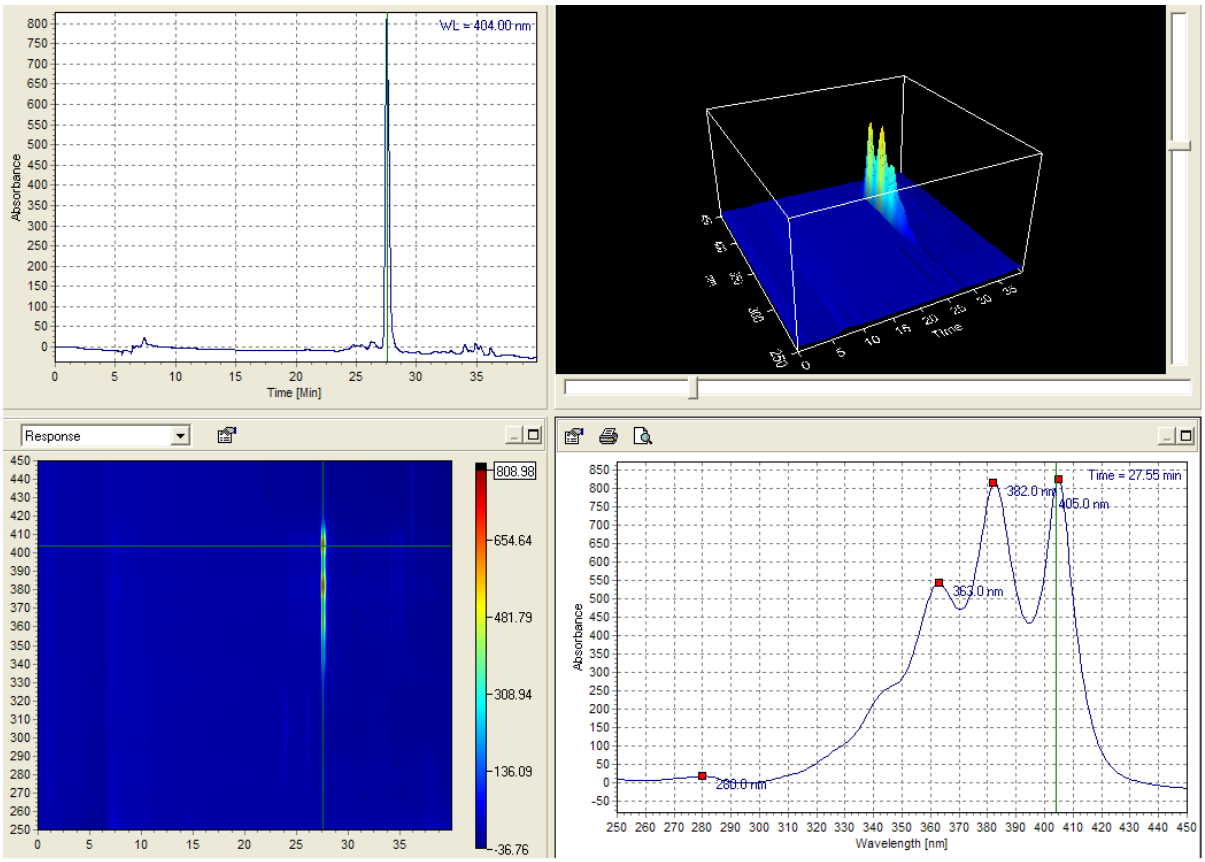
AmphL : 8-Deoxyamphotericin B (5)

amphL: Timescale zoom of crude methanolic extract



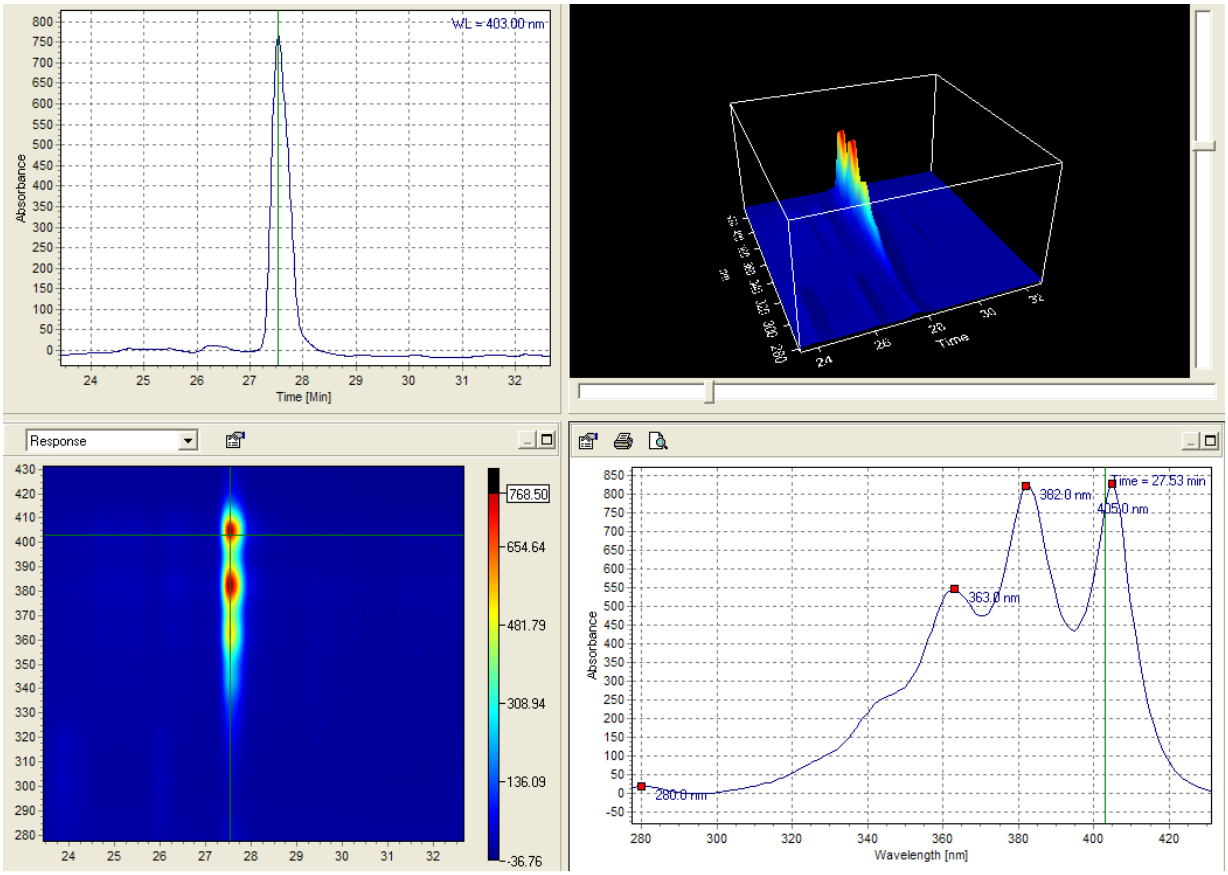
AmphL

amphL: Ppte after water, CHCl3 and MeOH/CHCL3 washes



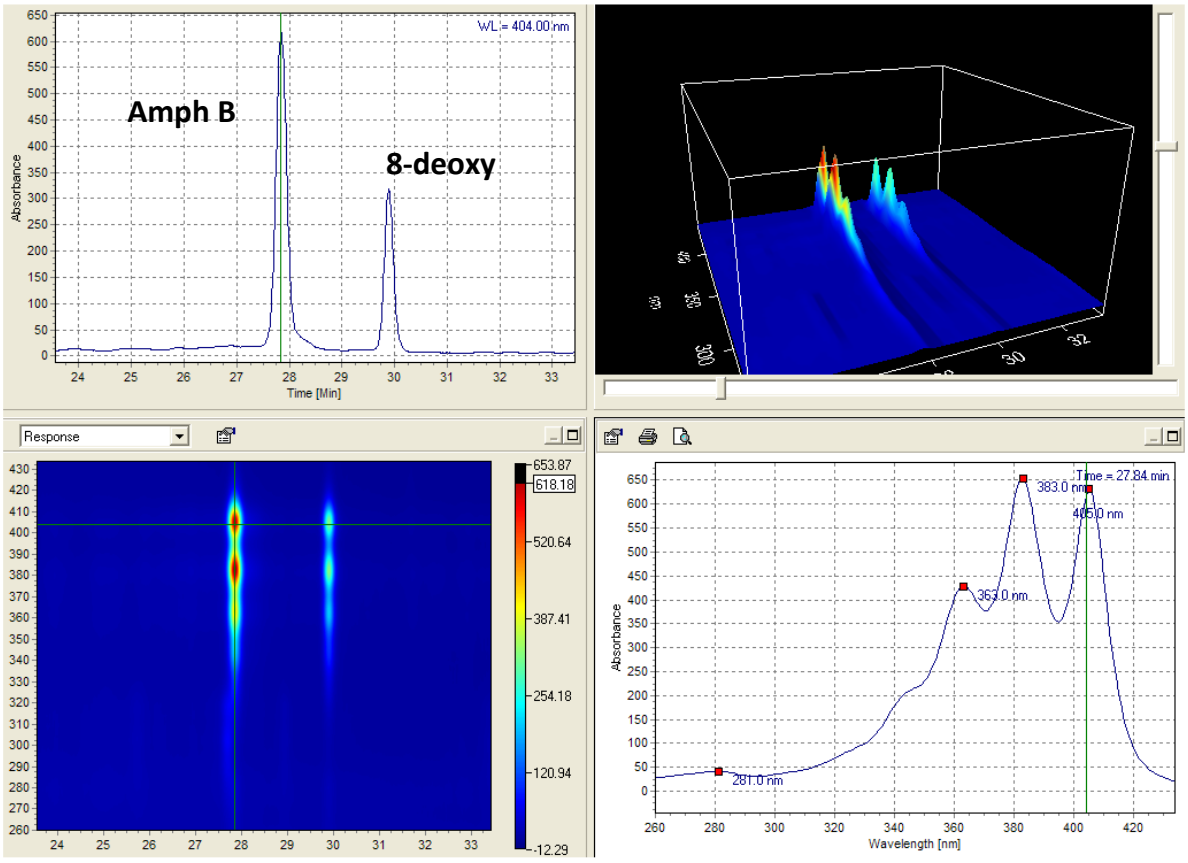
AmphL : 8-Deoxyamphotericin B (5)

Amph L Ppt after washes zoom

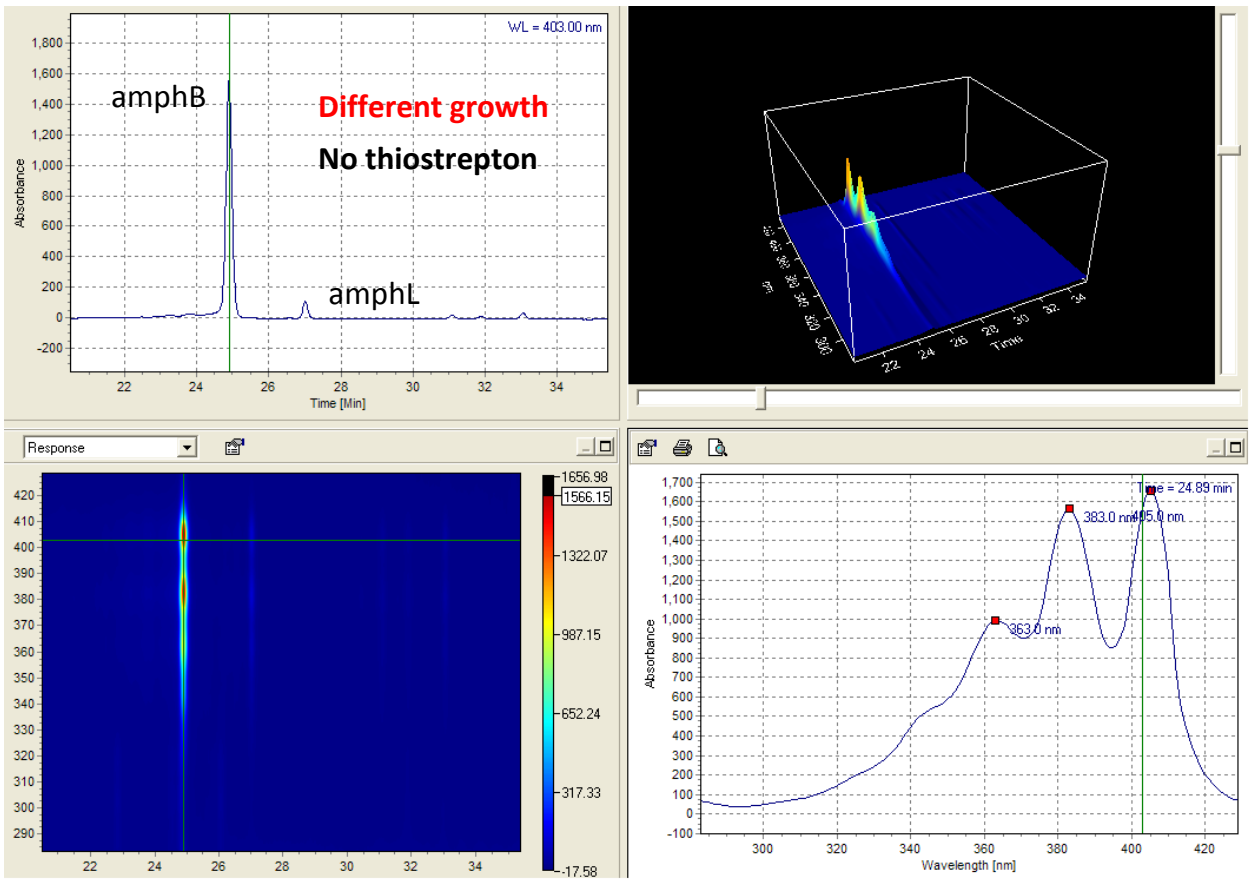


amphL: 8-deoxyamphotericin B sample (29.8 min) spiked with amphotericin B (28 min)

AmphL



amphL: showing full reversion and production of amphotericin B in crude MeOH extract

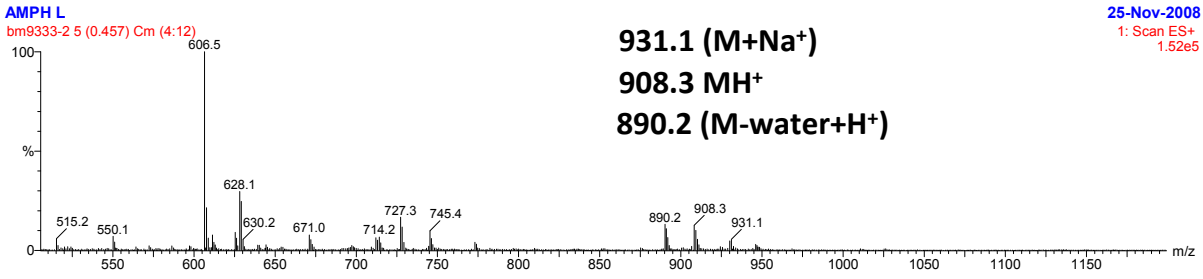


AmphL : 8-Deoxyamphotericin B (5)

8-Deoxyamphotericin B 5

ESMS (+ve) on washed solid

RMM 907.6

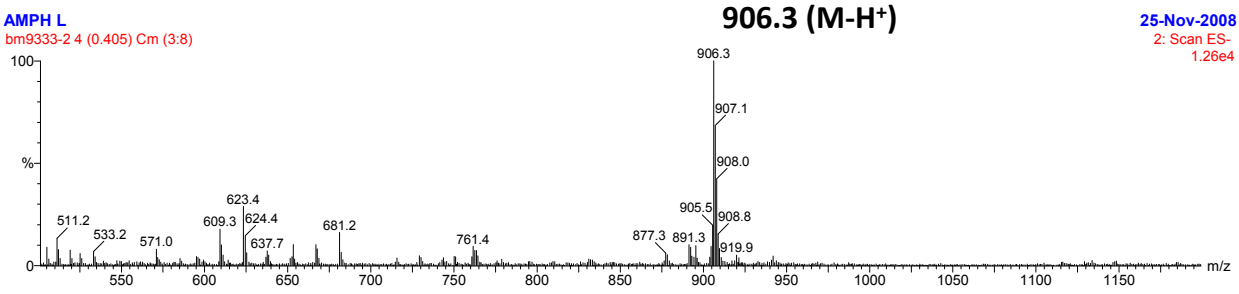


931.1 ($M+Na^+$)

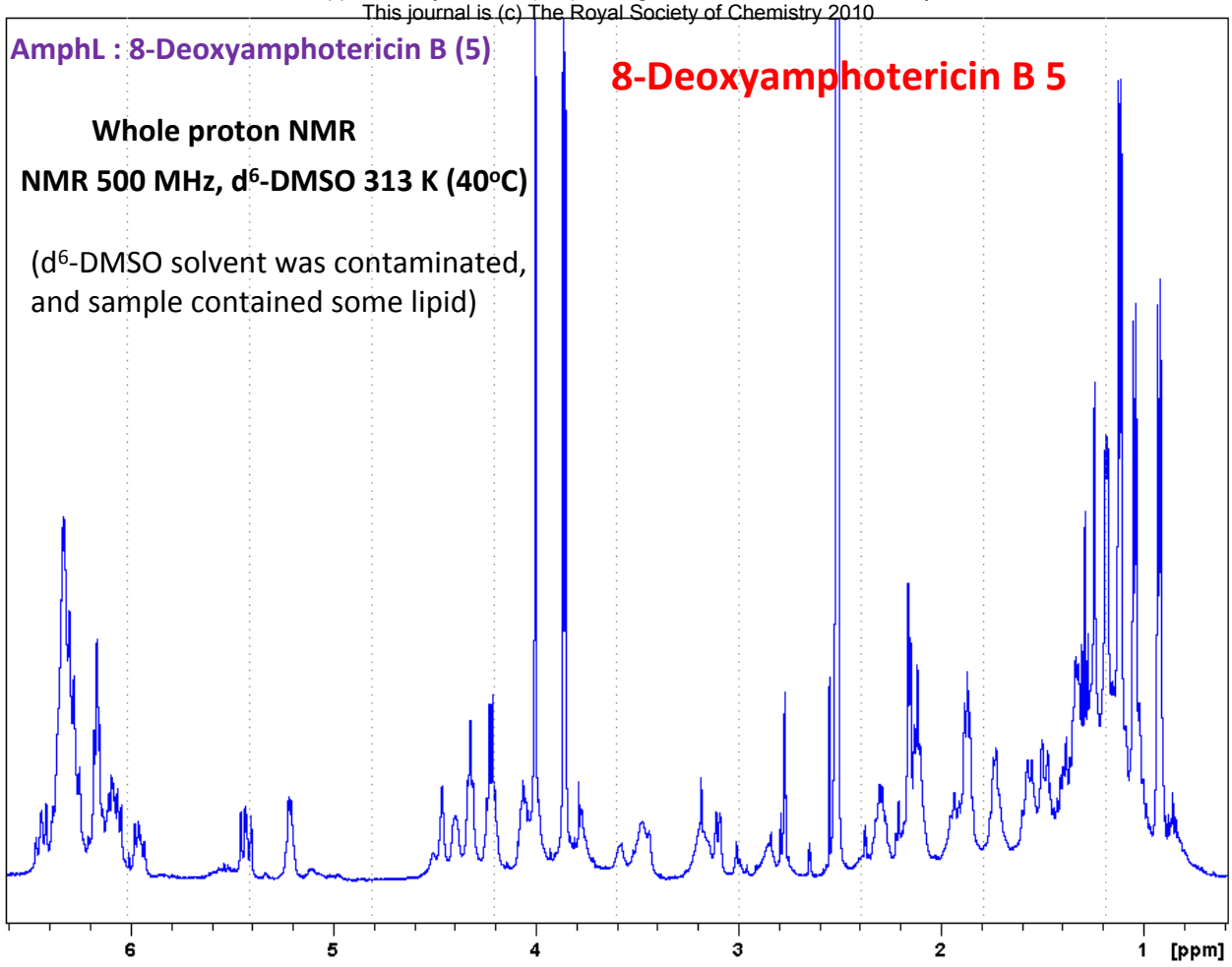
908.3 MH^+

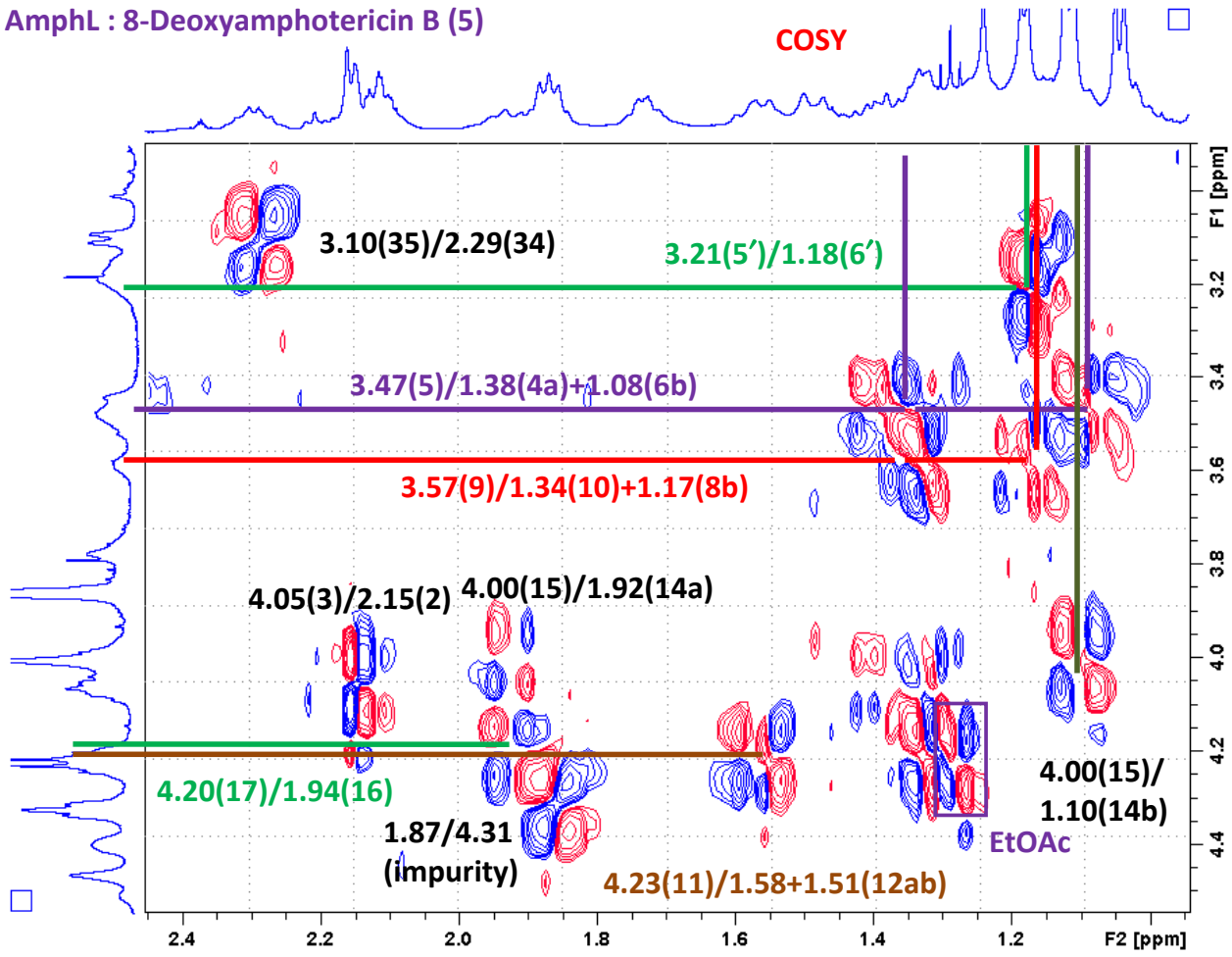
890.2 ($M-water+H^+$)

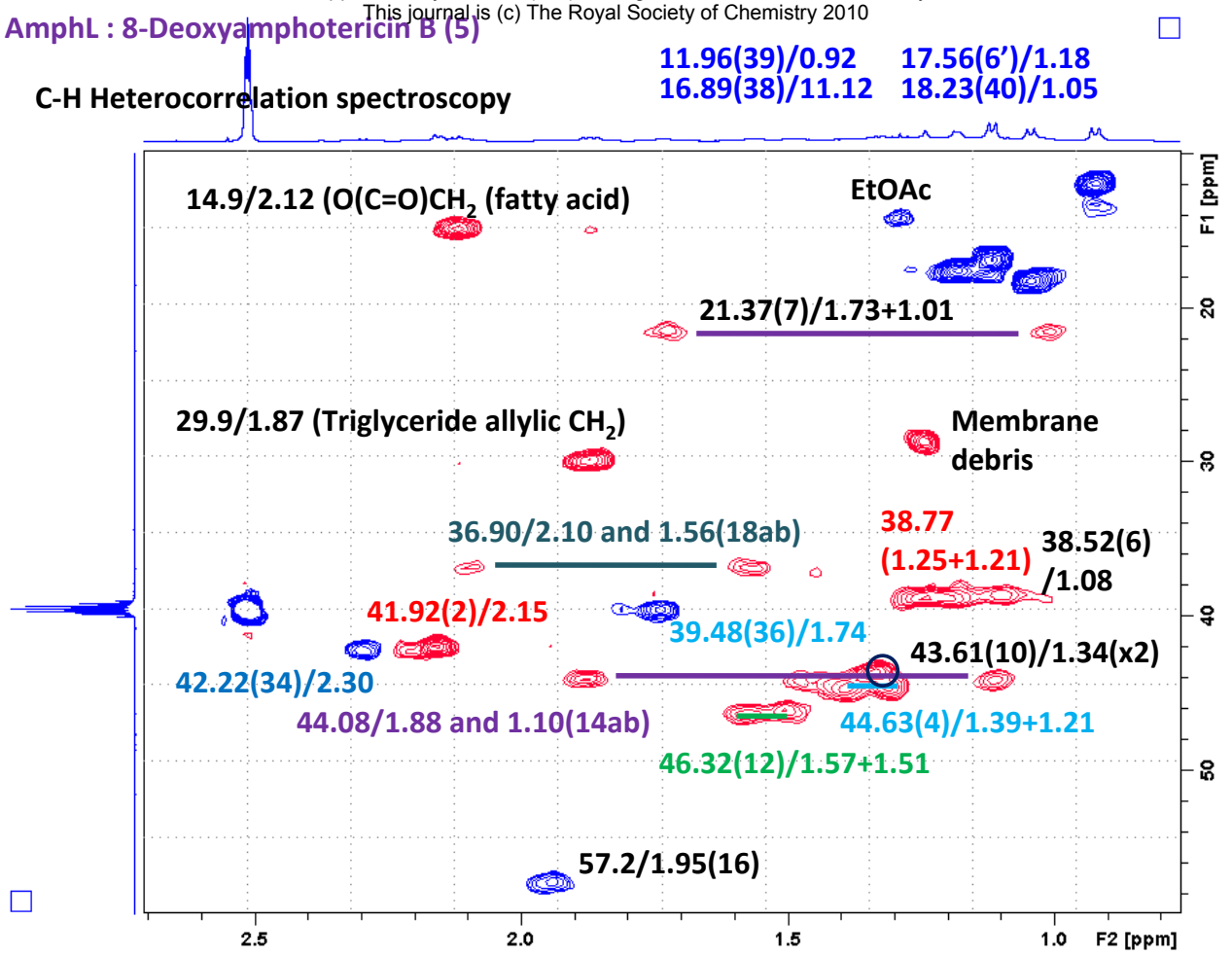
ESMS (-ve) on washed solid

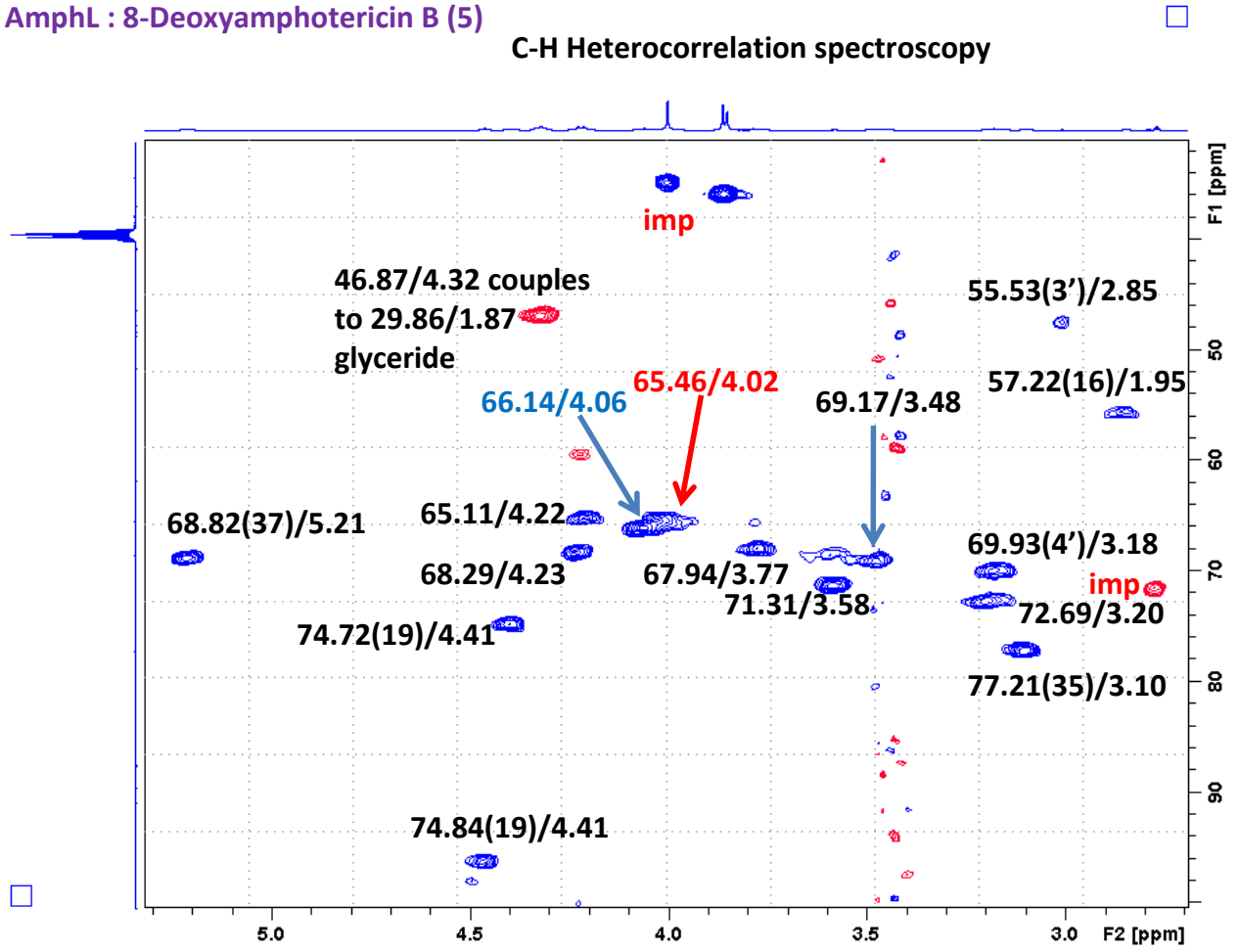


906.3 ($M-H^+$)









AmphL **Heterocorrelation NMR: d^6-DMSO 39.45**

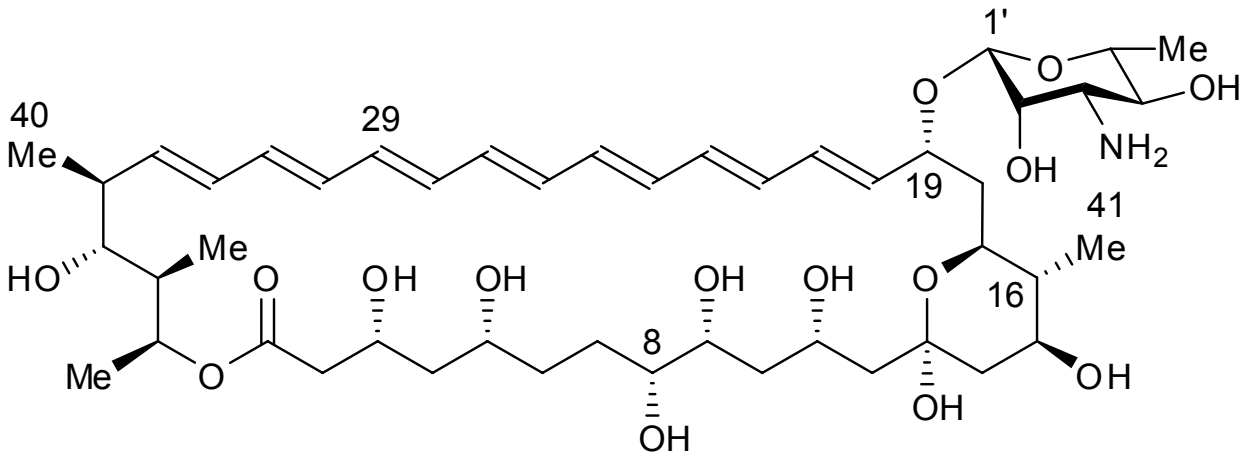
Red are methylenes

From H-C analysis: (*c.f.* amph B)

11.93(39)/0.93	11.9/0.91
14.9/2.12 (imp)	
16.82(38)/1.12	16.8/1.11
17.52(6')/1.18	17.8/1.13
18.21(40)/1.04	18.4/1.04
14.78/2.12 (imp)	
21.37(7)/1.73+1.01	29.0/1.56+1.29
28.64/1.24(imp)	
29.86/1.87 (imp)	
34.82/4.00 (imp)	
35.82/3.86 (imp)	
36.90(18)/2.10+1.56	36.3/1.54+2.05
38.52(6)/1.24+1.08	35.0/1.59+1.26
38.80(8)/1.24+1.17	73.4/3.09
39.48(36)/1.74	39.7/1.73
41.94(2)/2.16(x2)	41.9/2.16
42.21(34)/2.29	42.4/2.28
43.61(10)/1.34(x2)	
44.08(14)/1.91+1.10	44.6/1.86+1.09
44.61(4)/1.38+1.31	44.3/1.32+1.38
46.29(12)/1.58+1.51	46.3/1.53
46.87/4.32(imp)	
55.13(3')/2.85	56.0/2.81
57.17(16)/1.94	58.4/1.93
65.17(17)/4.20	65.2/4.18
65.46(15)/4.02	65.4/3.99
66.12(3)/4.06	66.1/4.06
68.28(11)/4.23	67.7/4.26
68.81(37)/5.21	69.0/5.22
68.88(2')/3.77	68.7/3.74
69.13(5)/3.46	69.7/3.51
69.93(4')/3.18	70.5/3.21
71.26(9)/3.58	73.8/3.48
72.66(5')/3.21	72.5/3.25
74.75(19)/4.40	74.1/4.40
77.20(35)/3.10	77.1/3.09
96.25(1')/4.46	95.5/4.61
128.93/6.11	
130.89(21)/6.07	/6.08
136.18/5.97(20)	/5.97
136.81/5.43(33)	/5.51

AmphNM+perDIDII: 16-Descarboxyl-16-methyl Amph B (8)

16-Descarboxyl-16-methyl-amphotericin B (8)



RMM = 893.5

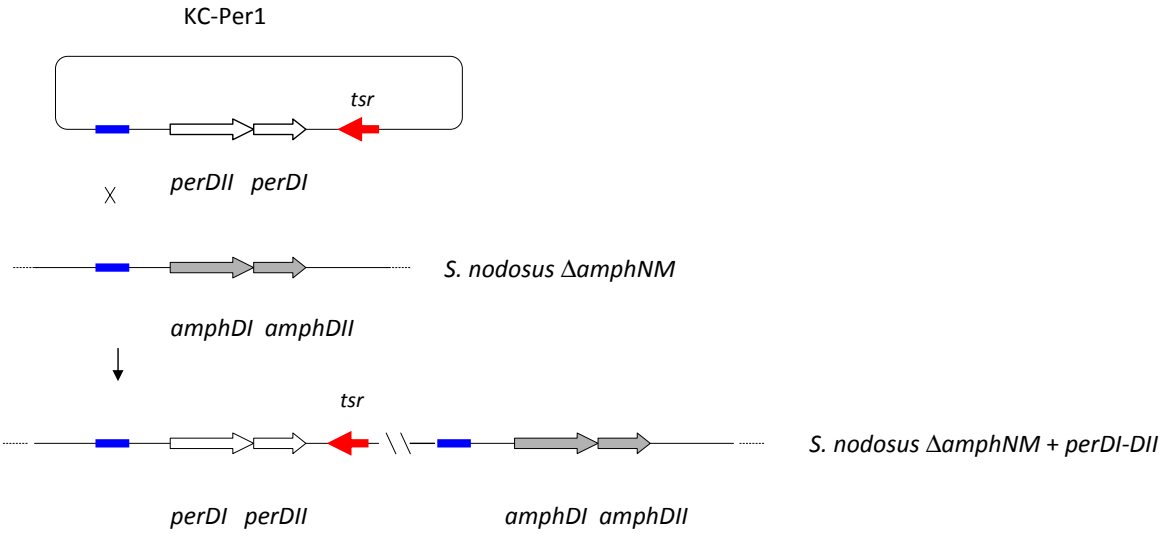
AmphNM+perDIDII: 16-Descarboxyl-16-methyl Amph B (8)

Construction of *S. nodosus* Δ*amphNM* + *perDI-DII*

Phage KC-Per1 contains a StuI-BamHI insert consisting of the *amphDI* upstream region (nucleotides 65861 to 68195 of sequence with GenBank accession number AF357202) fused to the *perDI* and *perDII* genes. Both *amphDI* and *perDI* genes have an NcoI site (5′ CCATGG 3′) surrounding the start codon. The *S. nodosus* DNA was ligated to the *S. aminophilus* DNA through this conserved site. This positioned the *perDI* start codon at the optimum distance from the *amphDI* promoter and ribosome-binding site. The KC-Per1 phage integrated into the *S. nodosus* Δ*amphNM* chromosome by recombination between the homologous *amphDI* upstream sequences. This gave a thiostrepton-resistant lysogen *S. nodosus* Δ*amphNM* + *perDI-DII* which contains the related *perDI-perDII* and *amphDI-DII* regions in direct repeat orientation. The genotype was verified by Southern hybridisation.

AmphNM+perDIDII: 16-Descarboxyl-16-methyl Amph B (8)

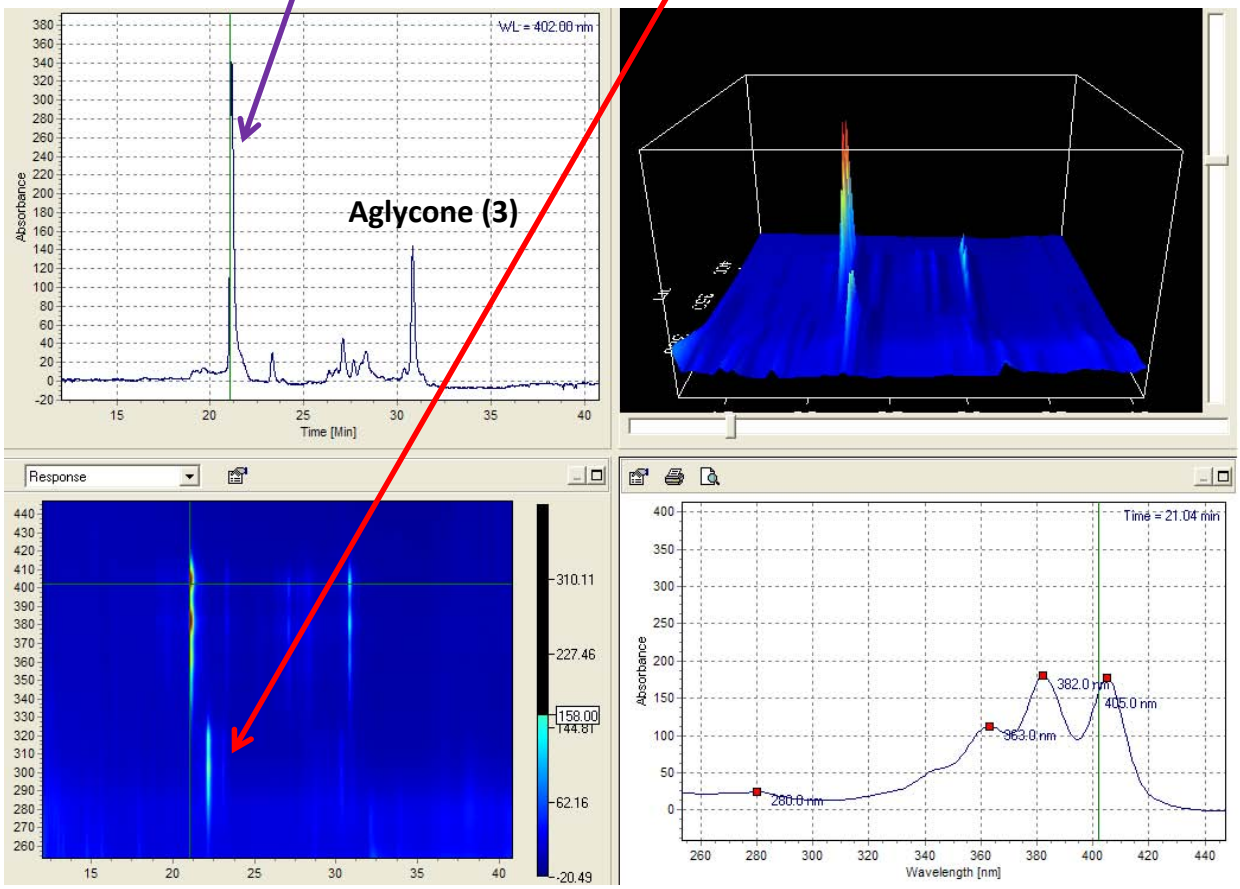
Construction of *S. nodosus* Δ*amphNM* + *perDI-DII*



16-Descarboxyl-16-methyl Amph B (8)

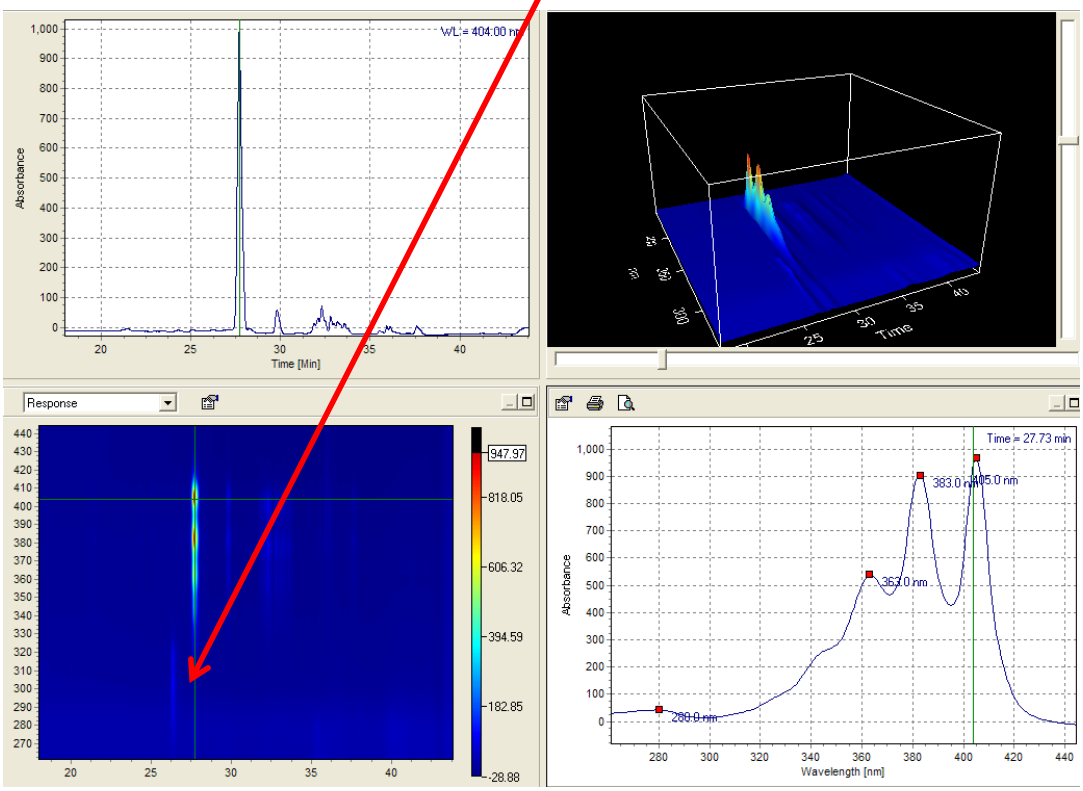
Extraction 1 in MeOH run on C8 RP-HPLC

(tetraene slightly later than heptaene)

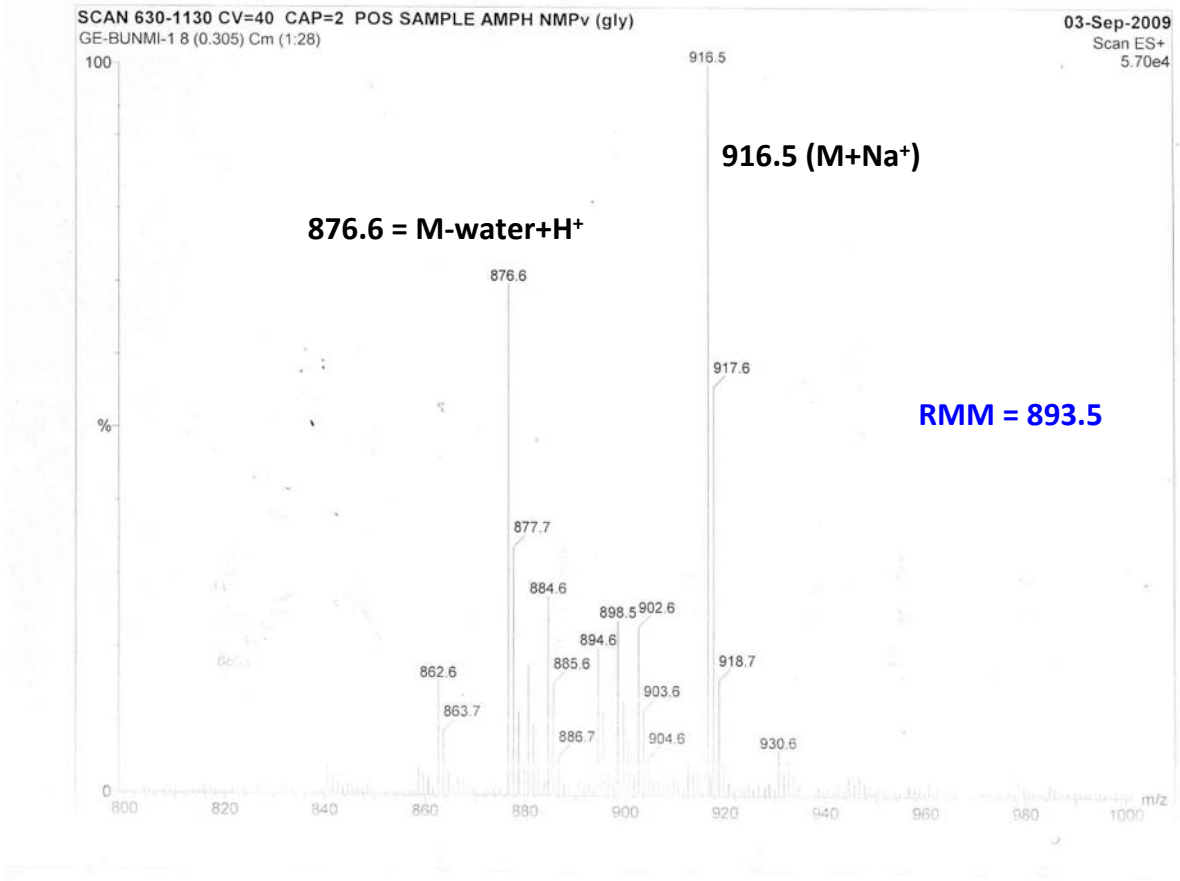


16-Descarboxyl-16-methyl Amph B (8)

Ext 1 ppte water washed in MeOH + 2 vol H_2O, ppte from MeOH to 5% MeOH EtOAc RPHPLC on C18. Note on C18 silica the tetraene elutes before the heptaene.

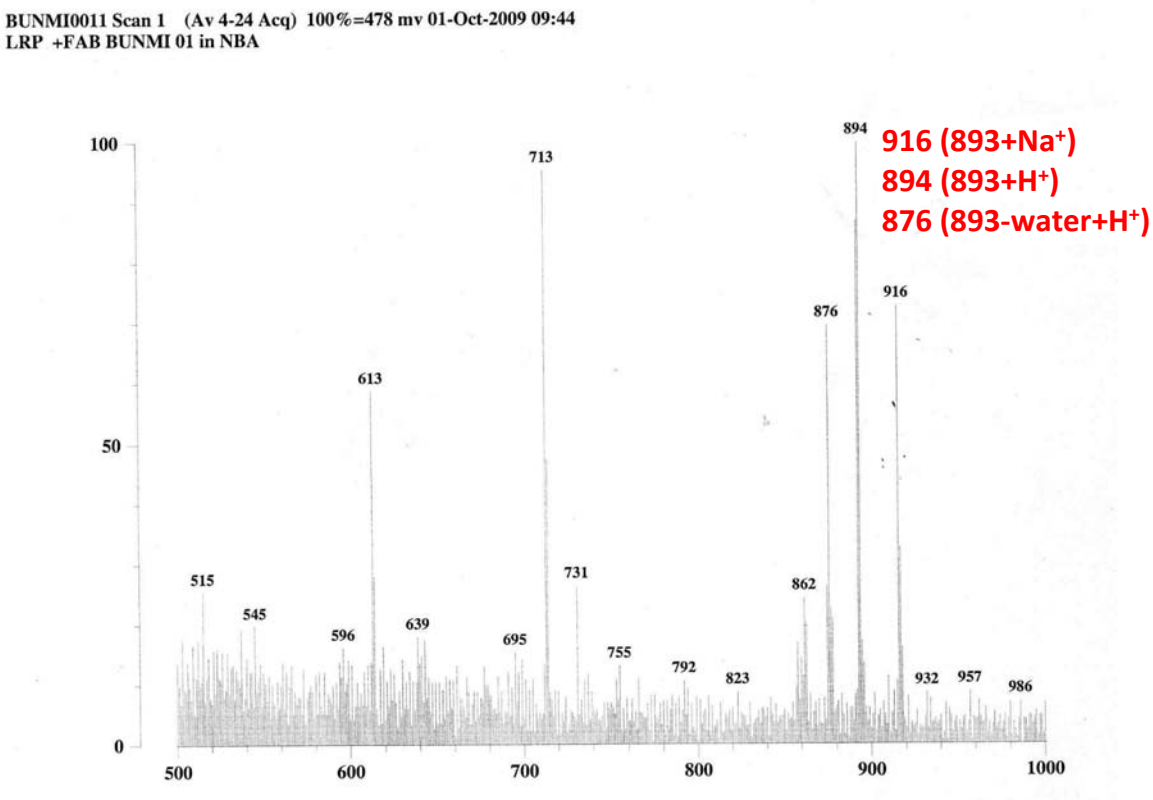


16-Descarboxyl-16-methyl Amph B ESMS(+ve) expanded scale

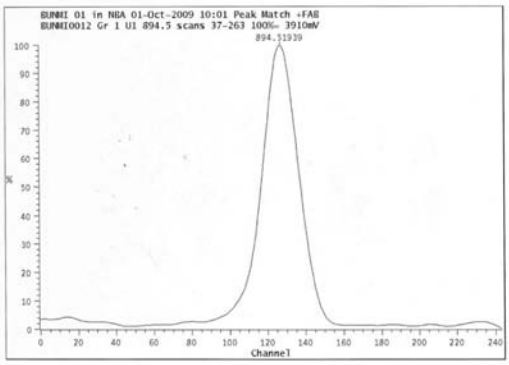


16-Descarboxyl-16-methyl Amph B (8) FAB MS

RMM = 893.5



16-Descarboxyl-16-methyl Amph B (8)



Accurate MS:

Run : BUNMI0012 Scan : 1

Mass	Int	Dev ppm	12 C	1 H	14 N	16 O
894.51939	9006799	-0.2	47	76	1	15

Observed: 894.51939. Calc for $C_{47}H_{76}NO_{15}$ = 894.51959

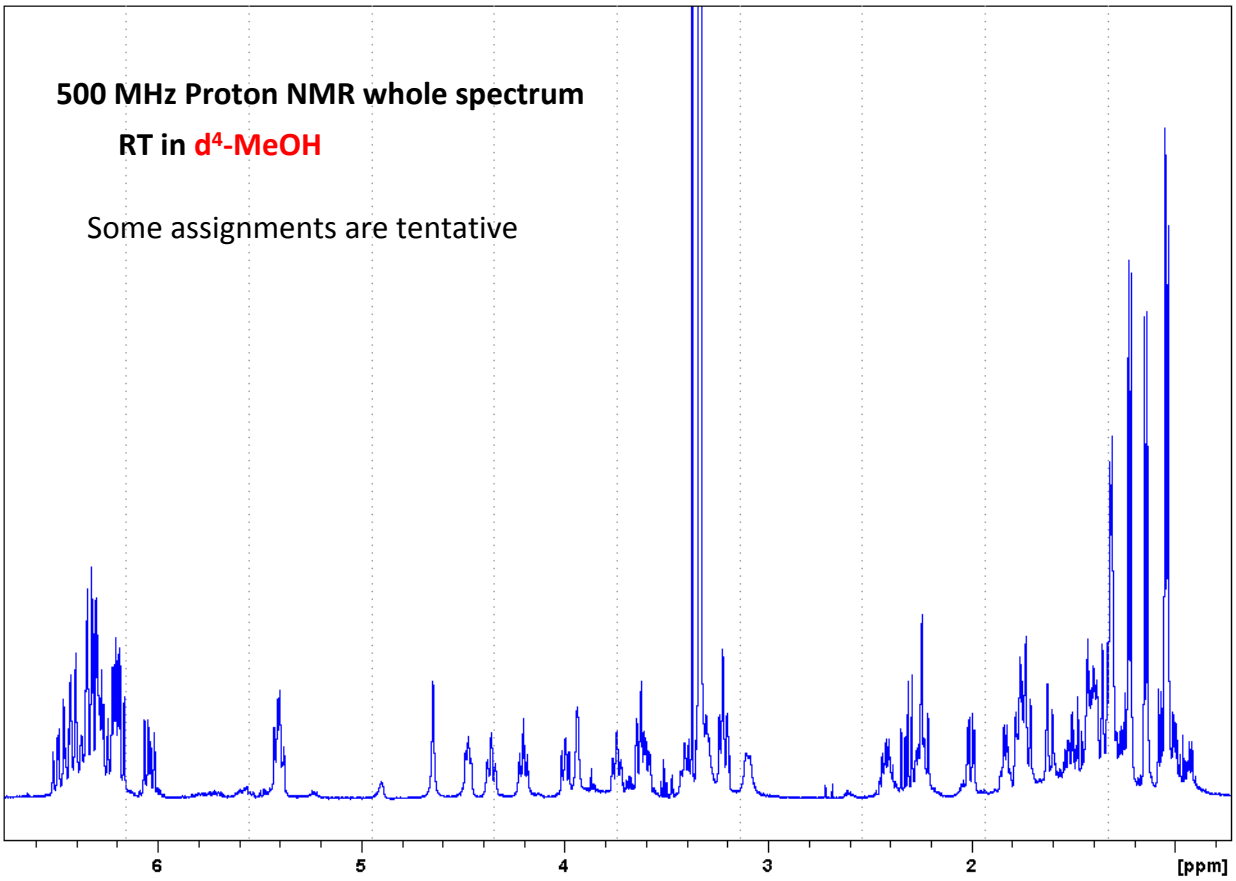
Mass	Abundance	12 C	13 C	1 H	14 N	15 N	16 O
894.51959	59.2879	47	0	76	1	0	15
895.52295	31.2777	46	1	76	1	0	15
896.52630	8.0748	45	2	76	1	0	15
897.52966	1.3595	44	3	76	1	0	15

16-Descarboxyl-16-methyl Amph B (8)

500 MHz Proton NMR whole spectrum

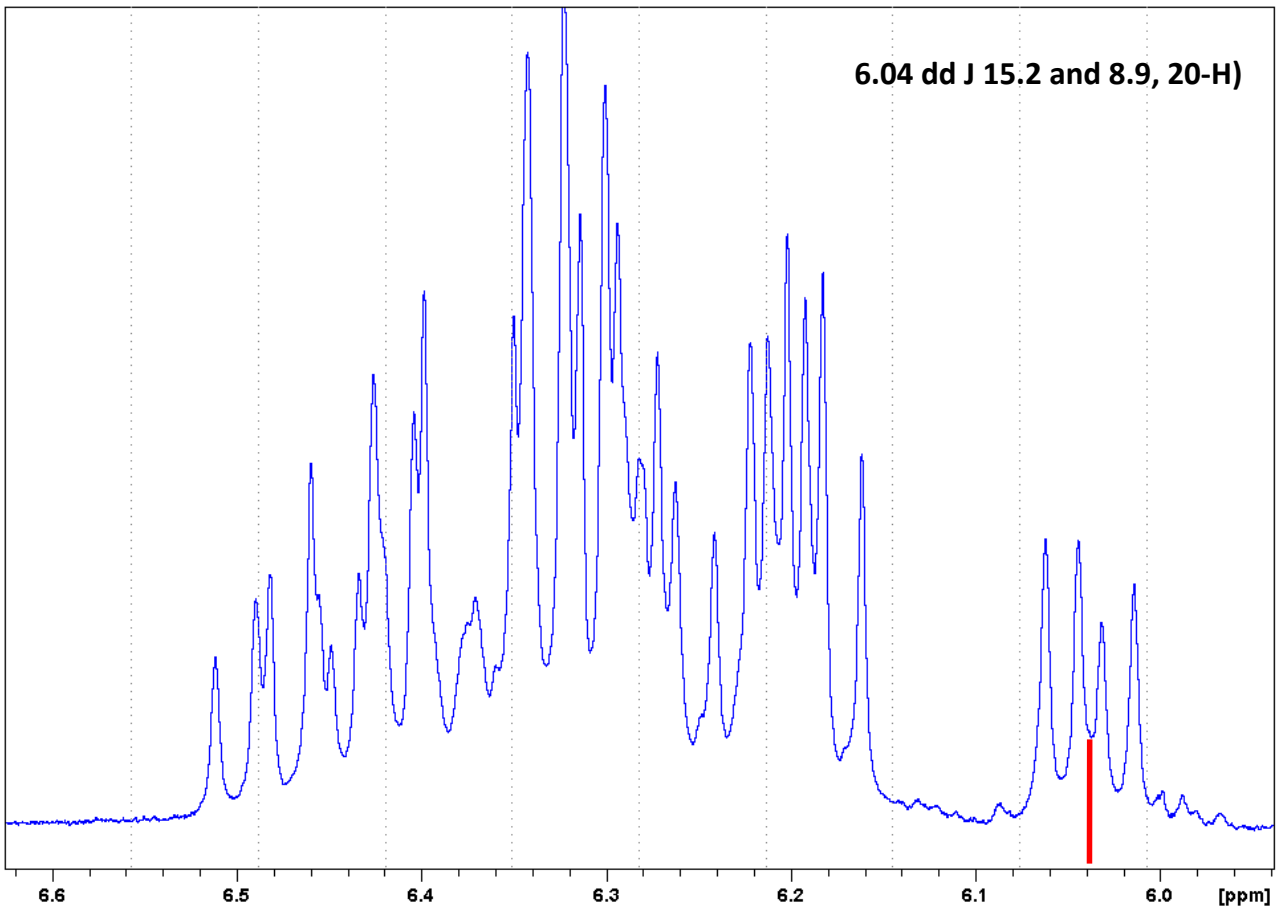
RT in d⁴-MeOH

Some assignments are tentative

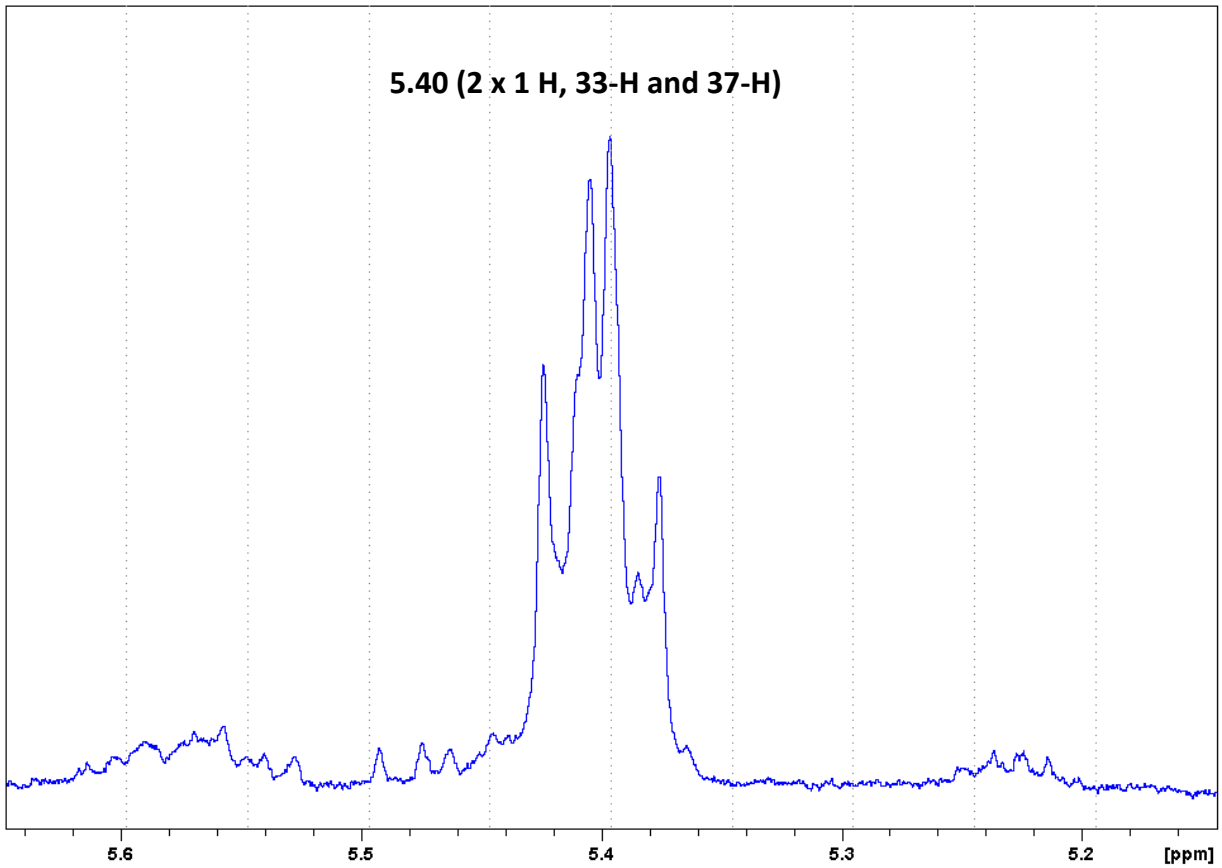


16-Descarboxyl-16-methyl Amph B

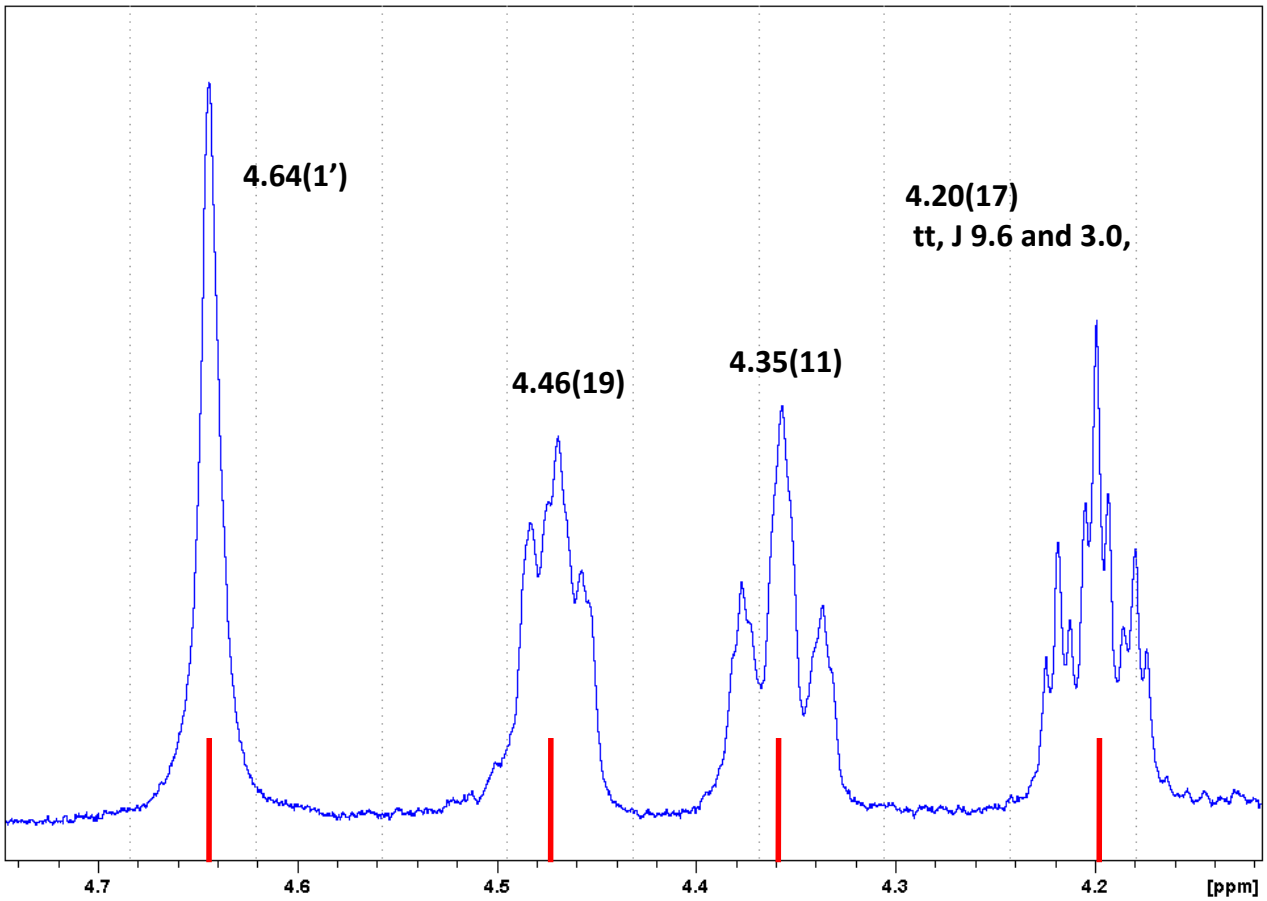
Double bond region



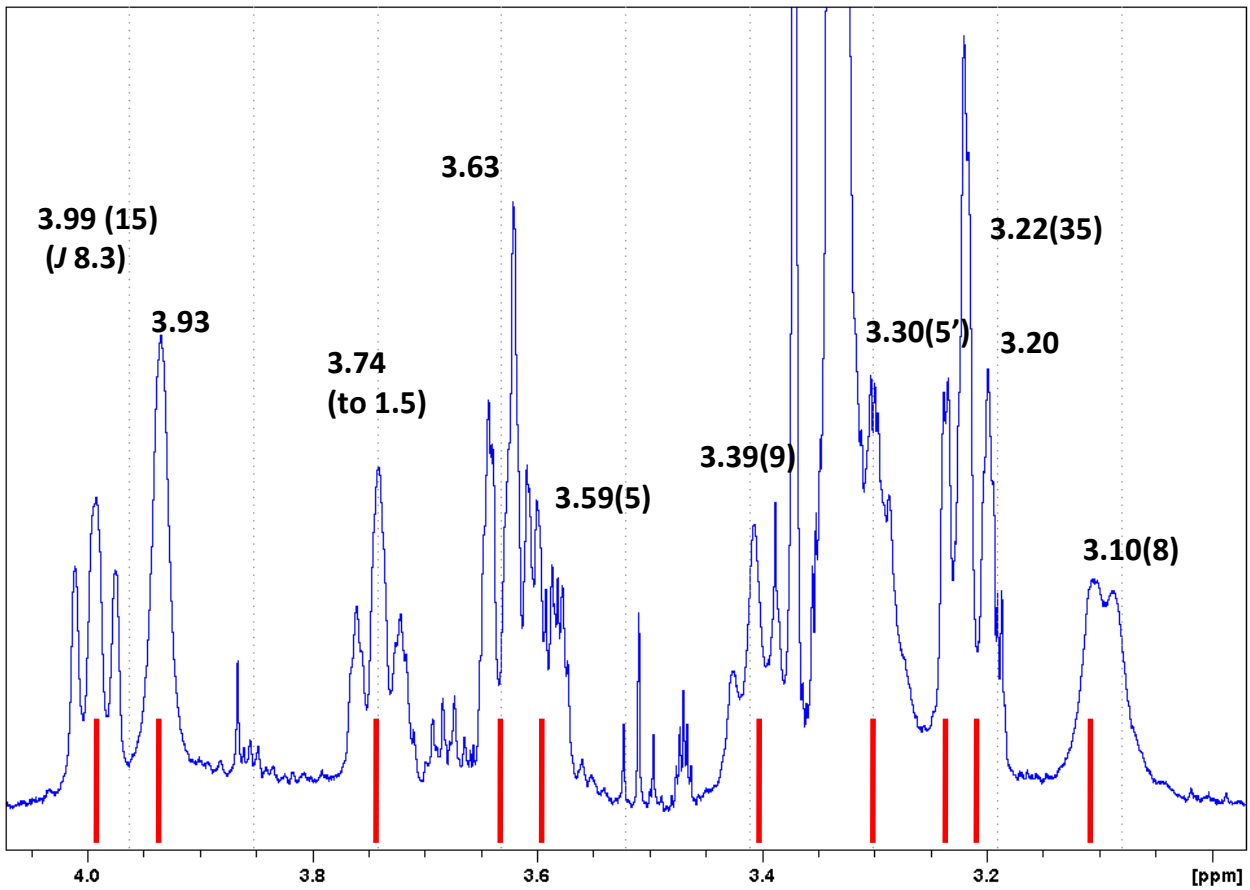
16-Descarboxyl-16-methyl Amph B



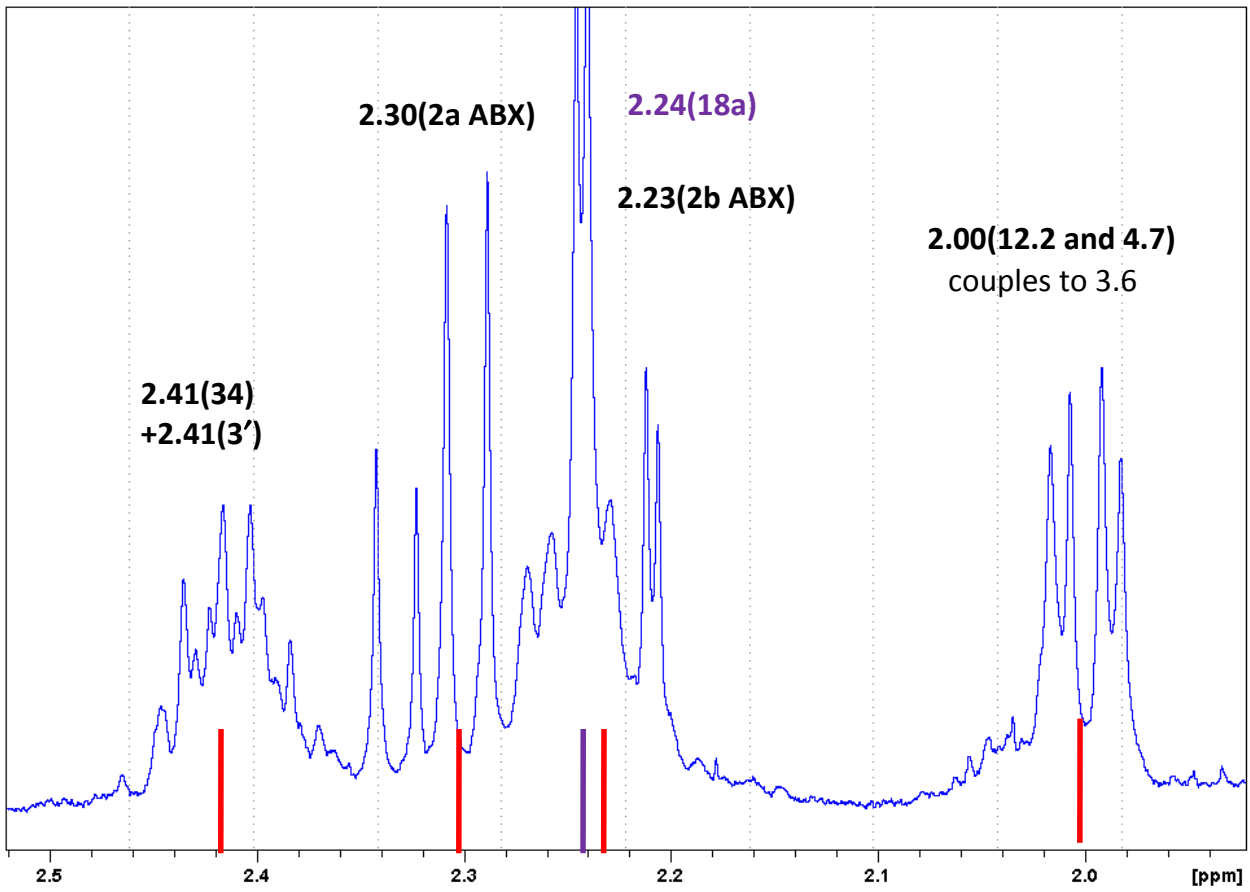
16-Descarboxyl-16-methyl Amph B (8)



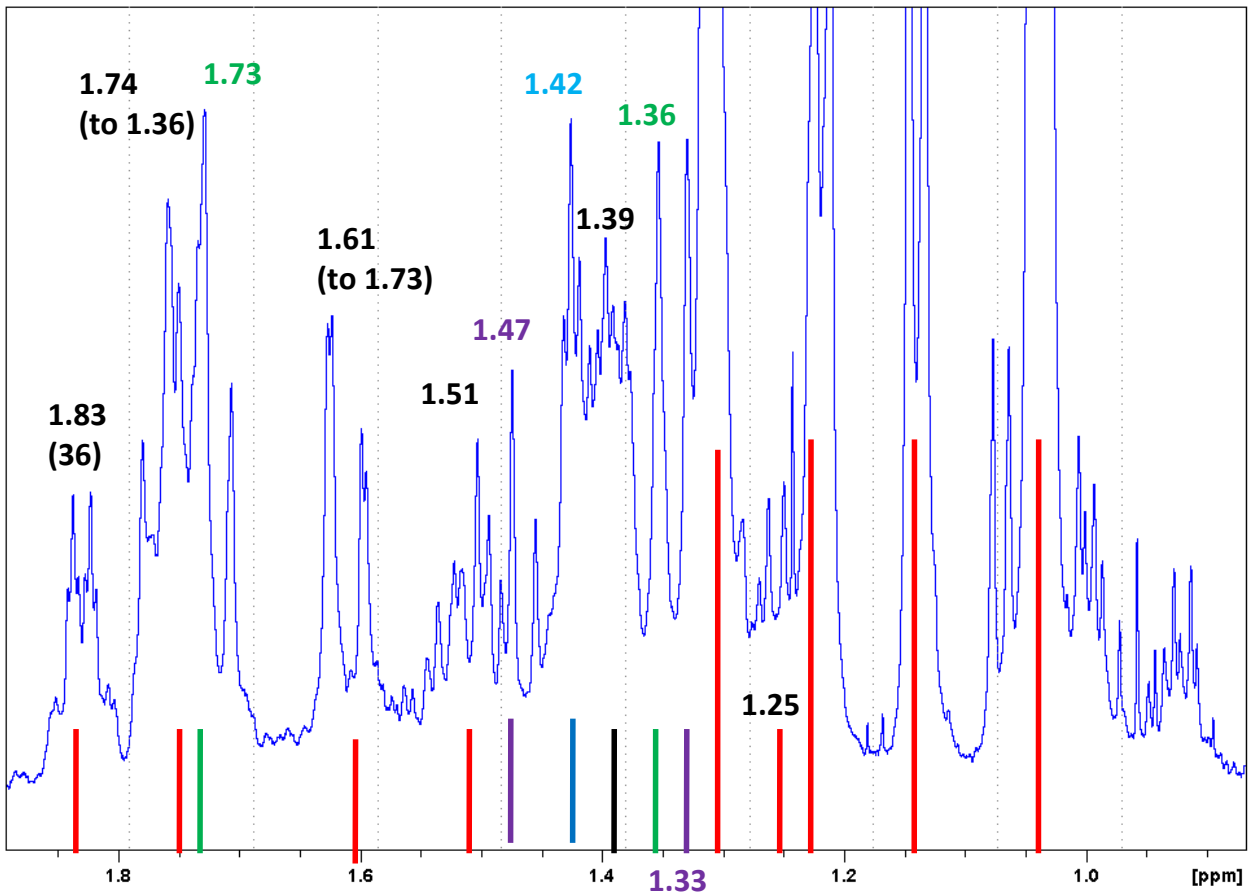
16-Descarboxyl-16-methyl Amph B (8)



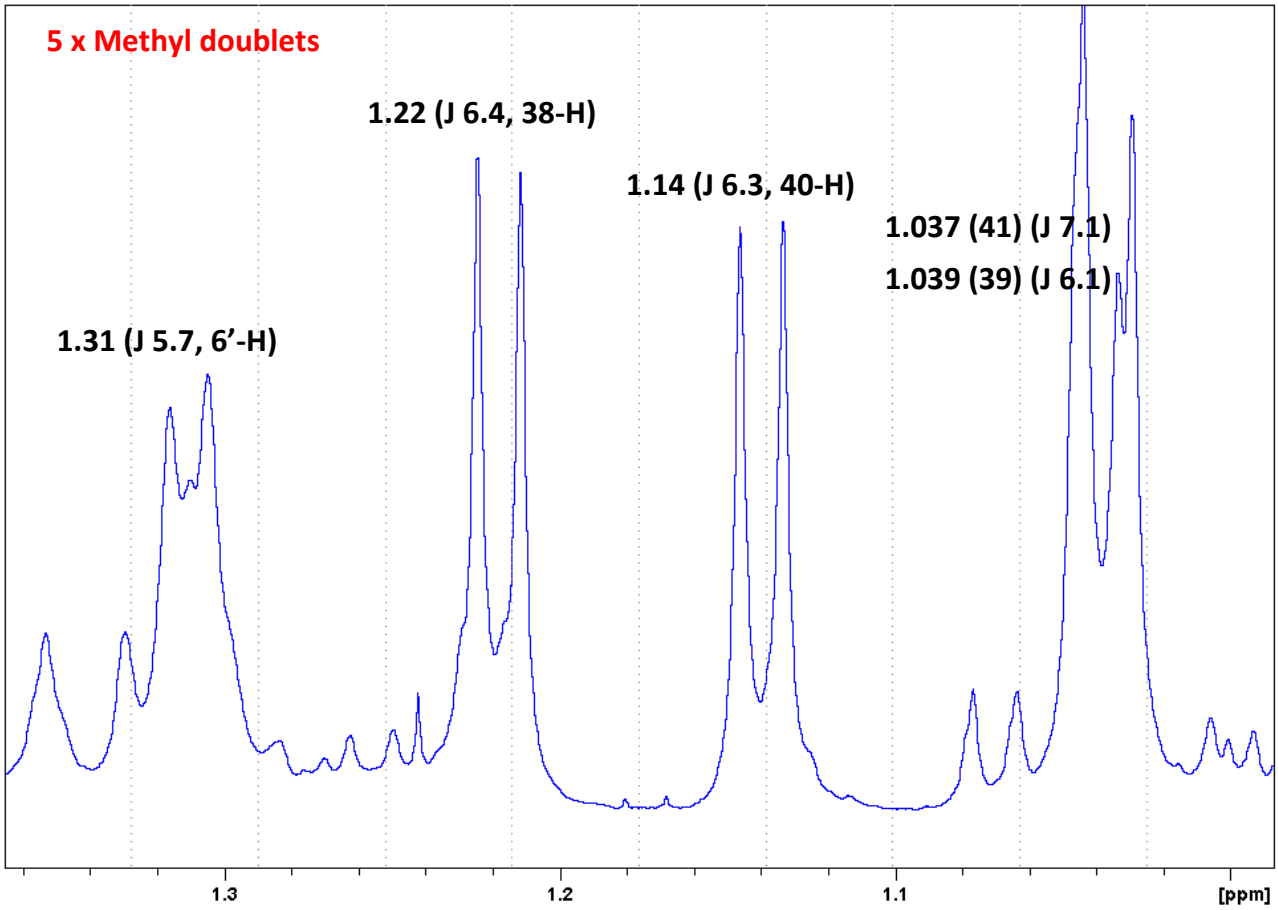
16-Descarboxyl-16-methyl Amph B (8)



16-Descarboxyl-16-methyl Amph B (8)



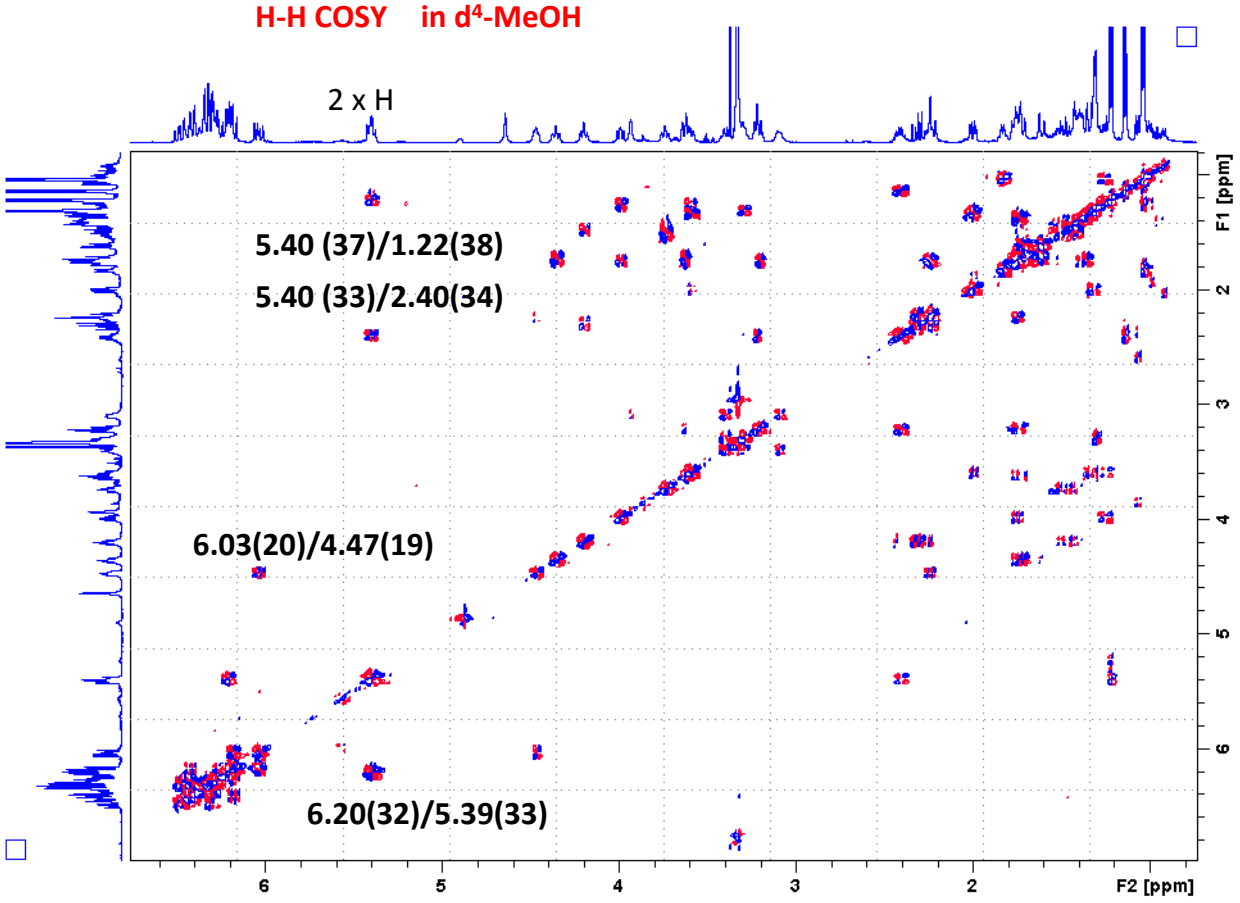
16-Descarboxyl-16-Methyl Amph B



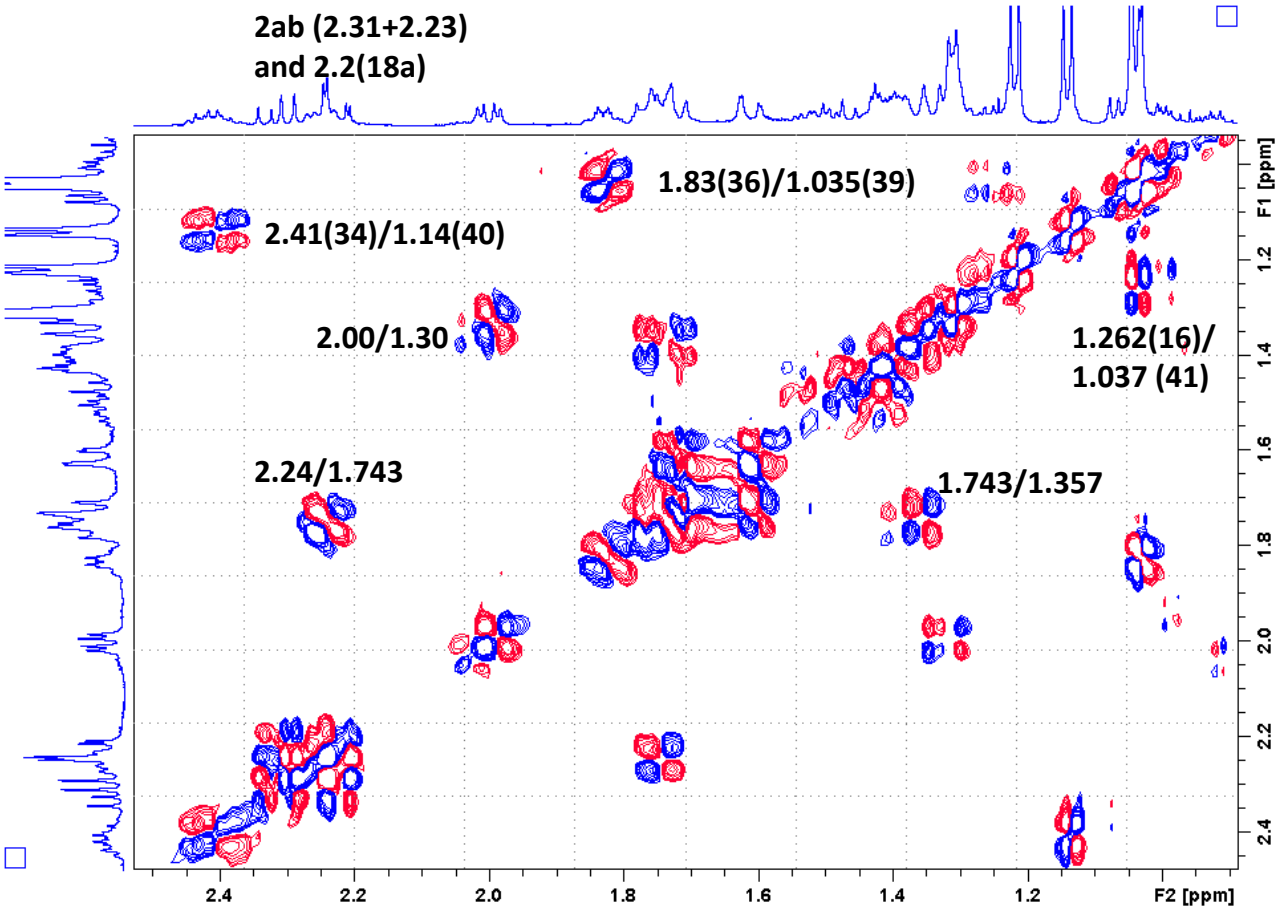
16-Descarboxyl-16-methyl Amph B (8)

Whole spectrum

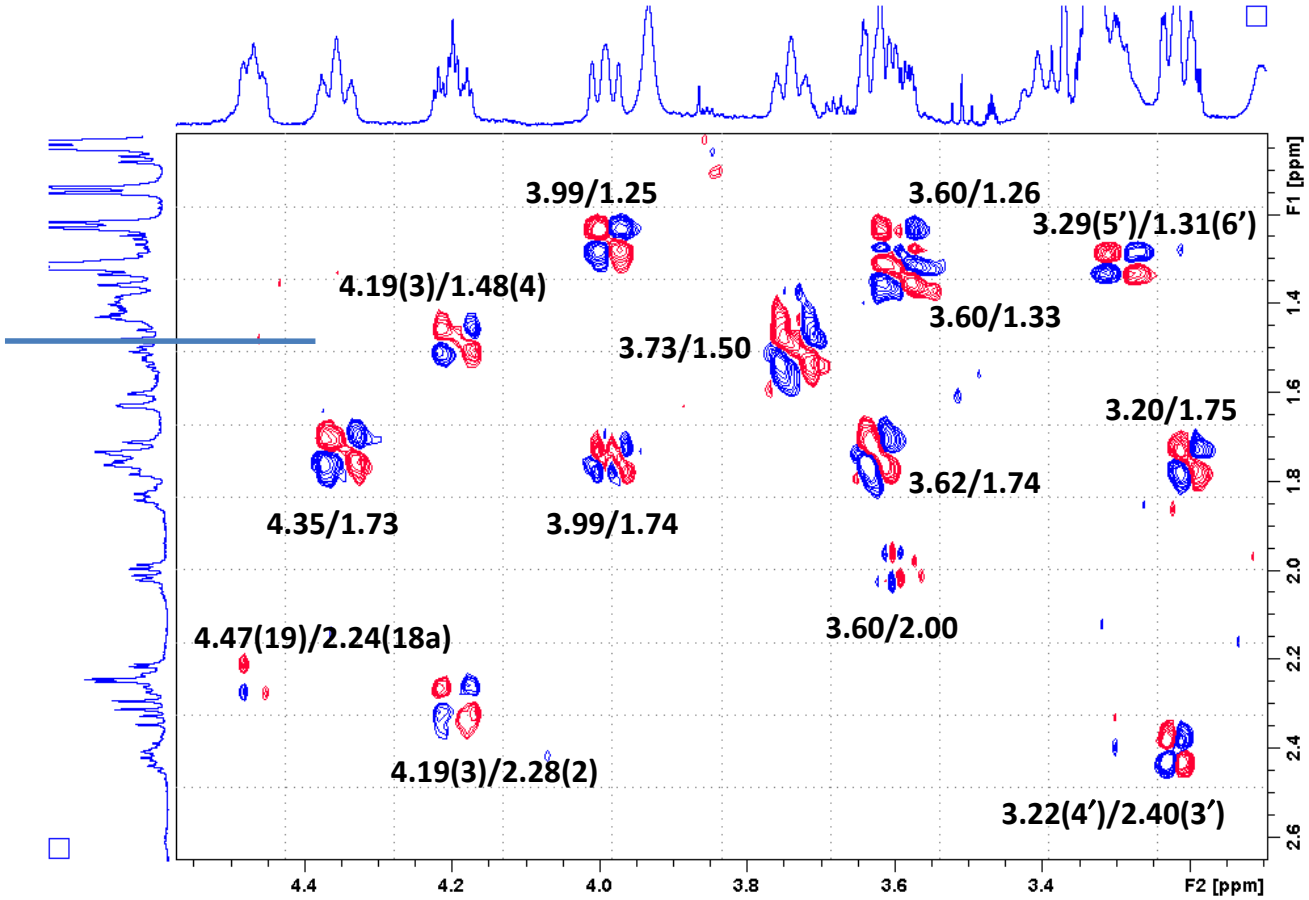
H-H COSY in d⁴-MeOH



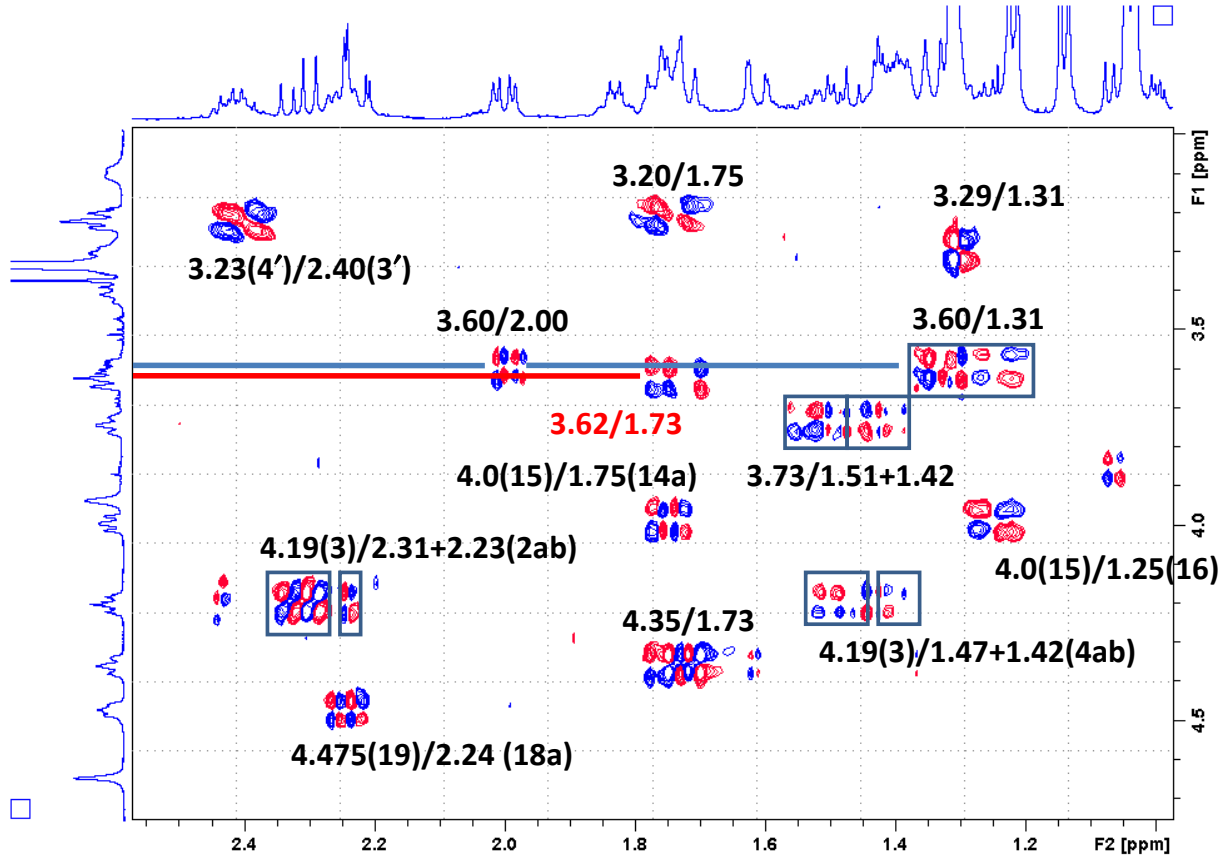
16-Descarboxyl-16-methyl Amph B (8)



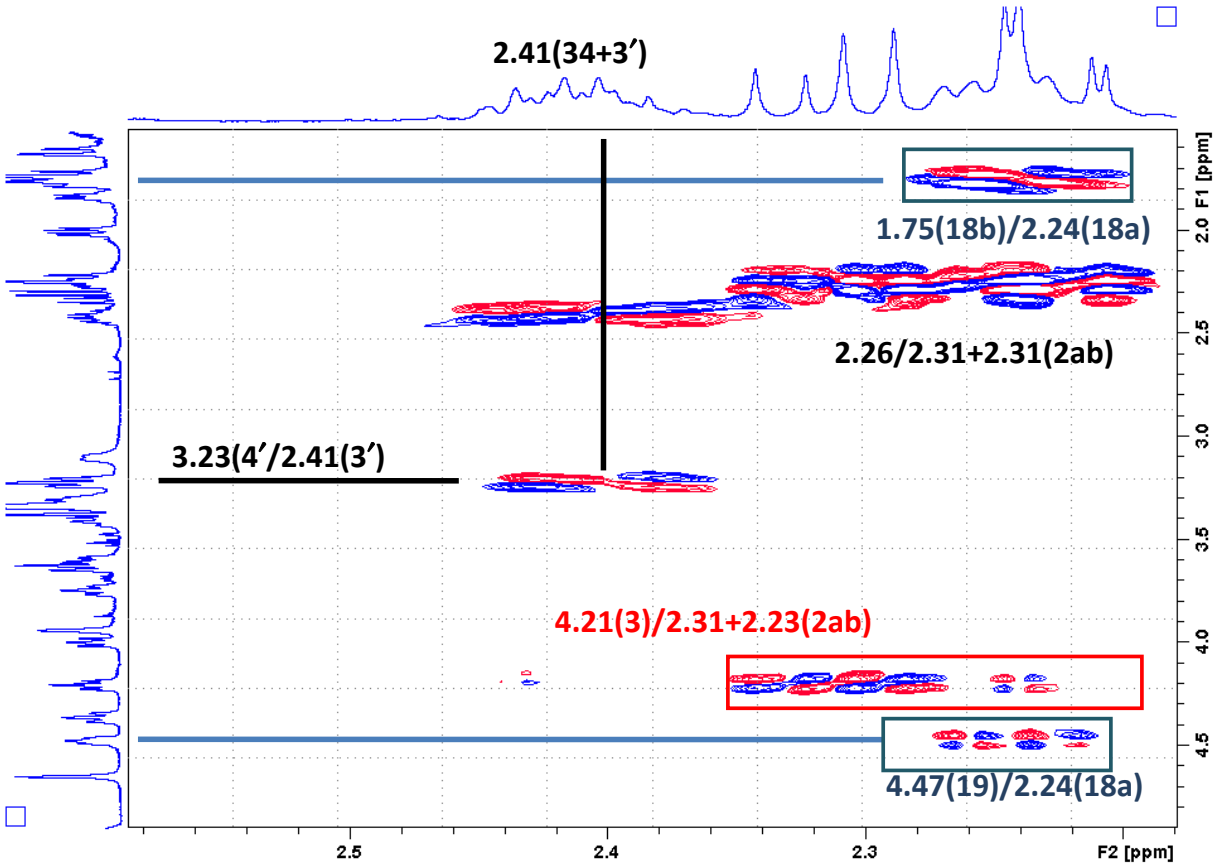
16-Descarboxyl-16-methyl Amph B (8) H-H COSY



16-Descarboxyl-16-methyl Amph B (8) H-H COSY

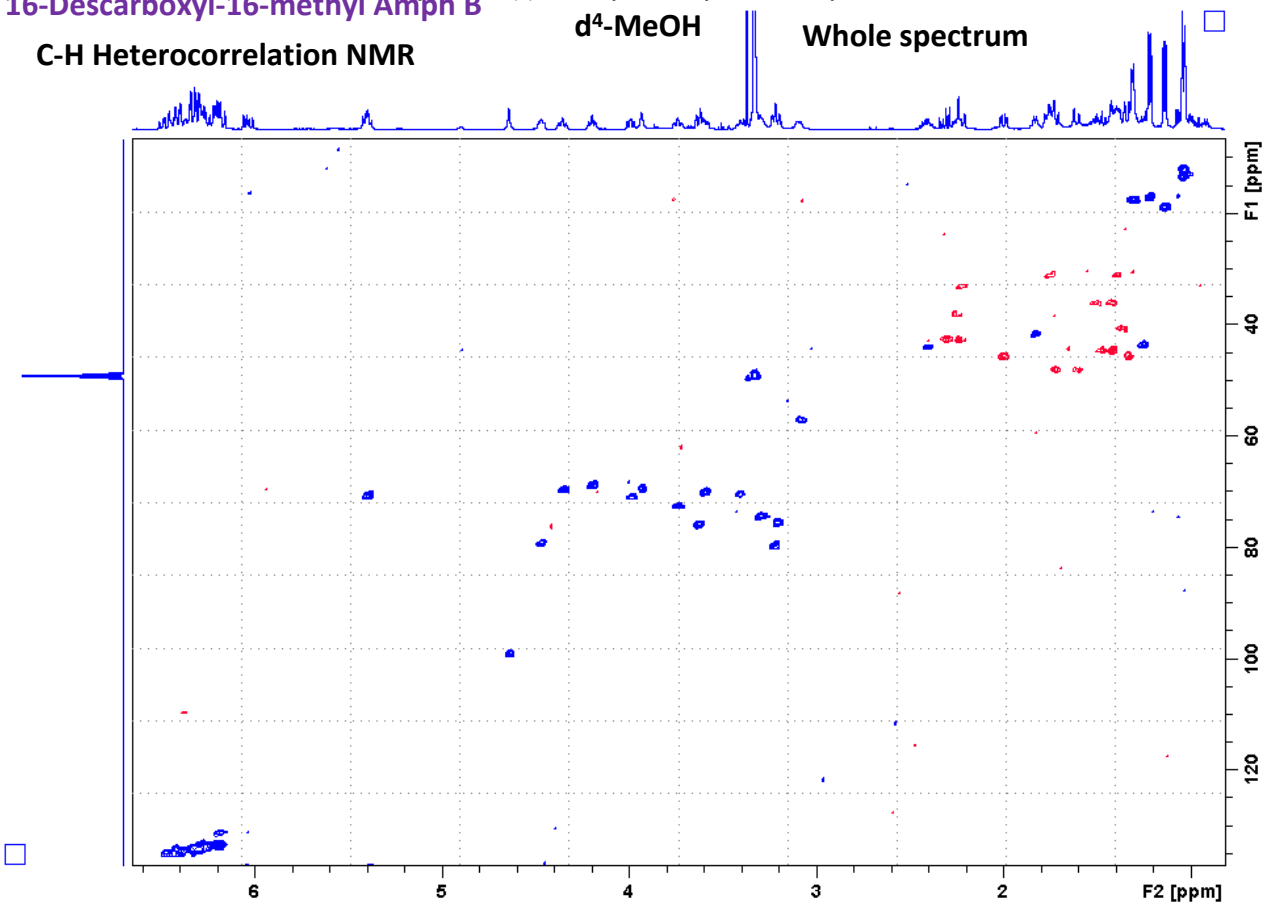


16-Descarboxyl-16-methyl Amph B (8)



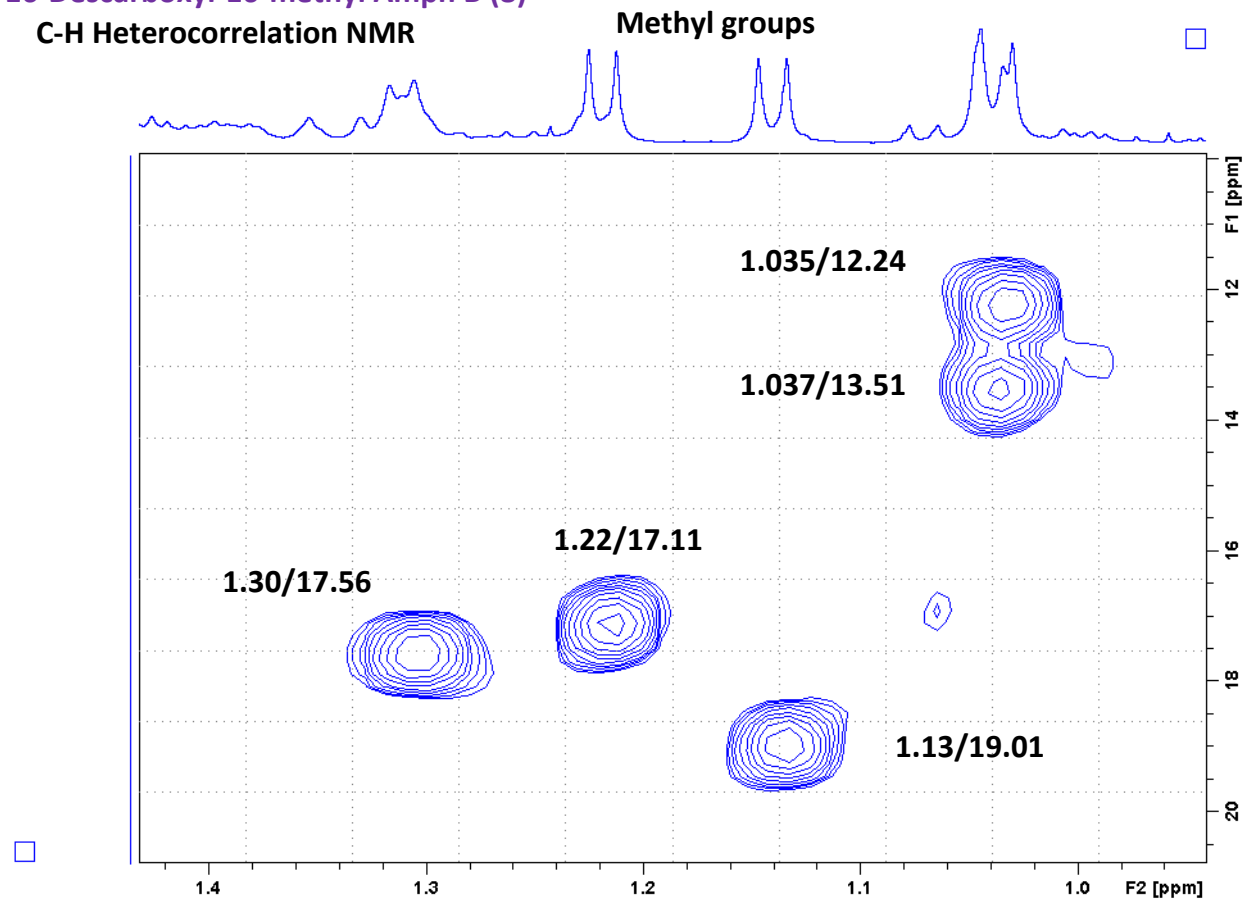
16-Descarboxyl-16-methyl Amph B

C-H Heterocorrelation NMR



16-Descarboxyl-16-methyl Amph B (8)

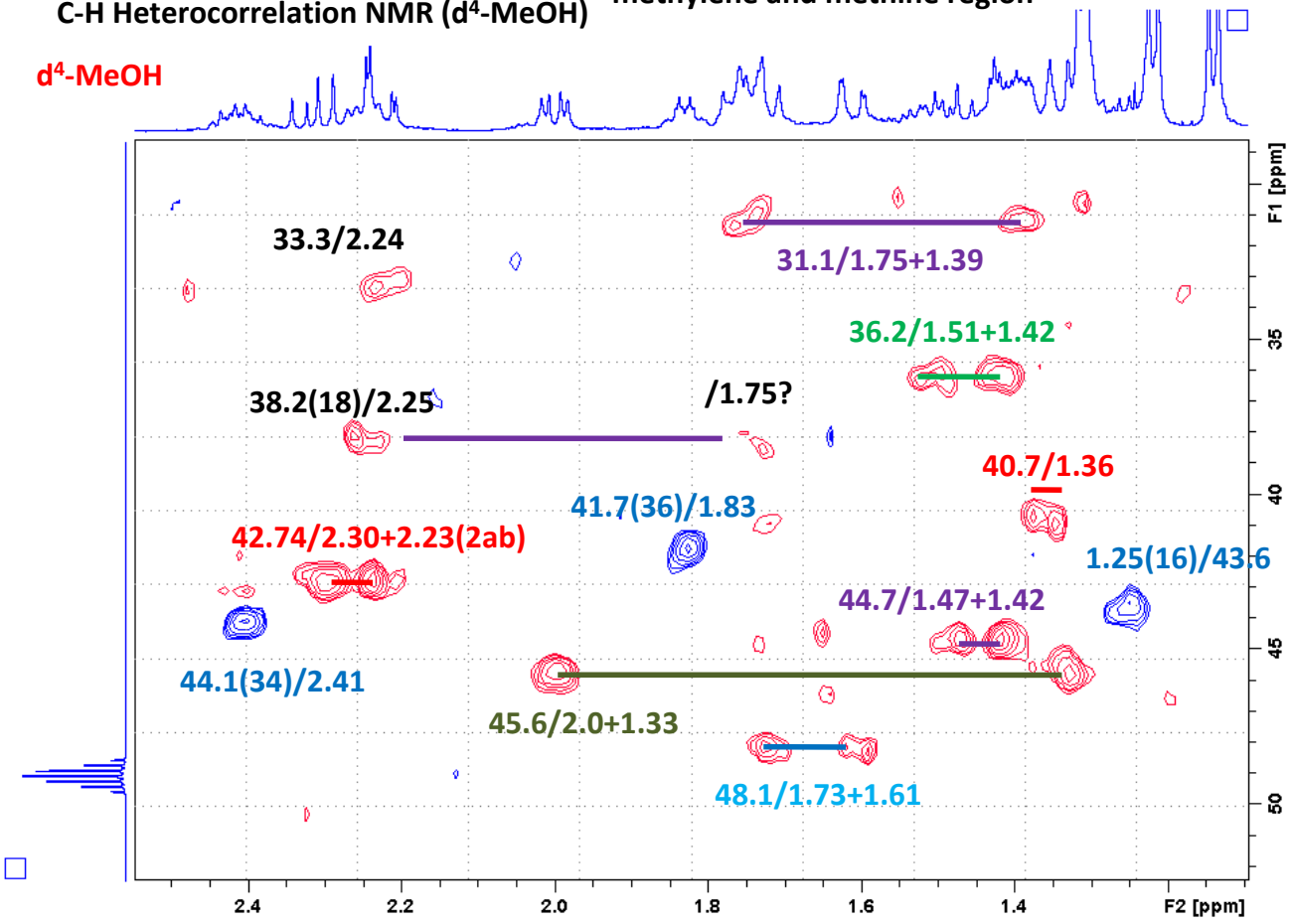
C-H Heterocorrelation NMR



16-Descarboxyl-16-methyl Amph B (8)

C-H Heterocorrelation NMR (d^4-MeOH)

methylene and methine region

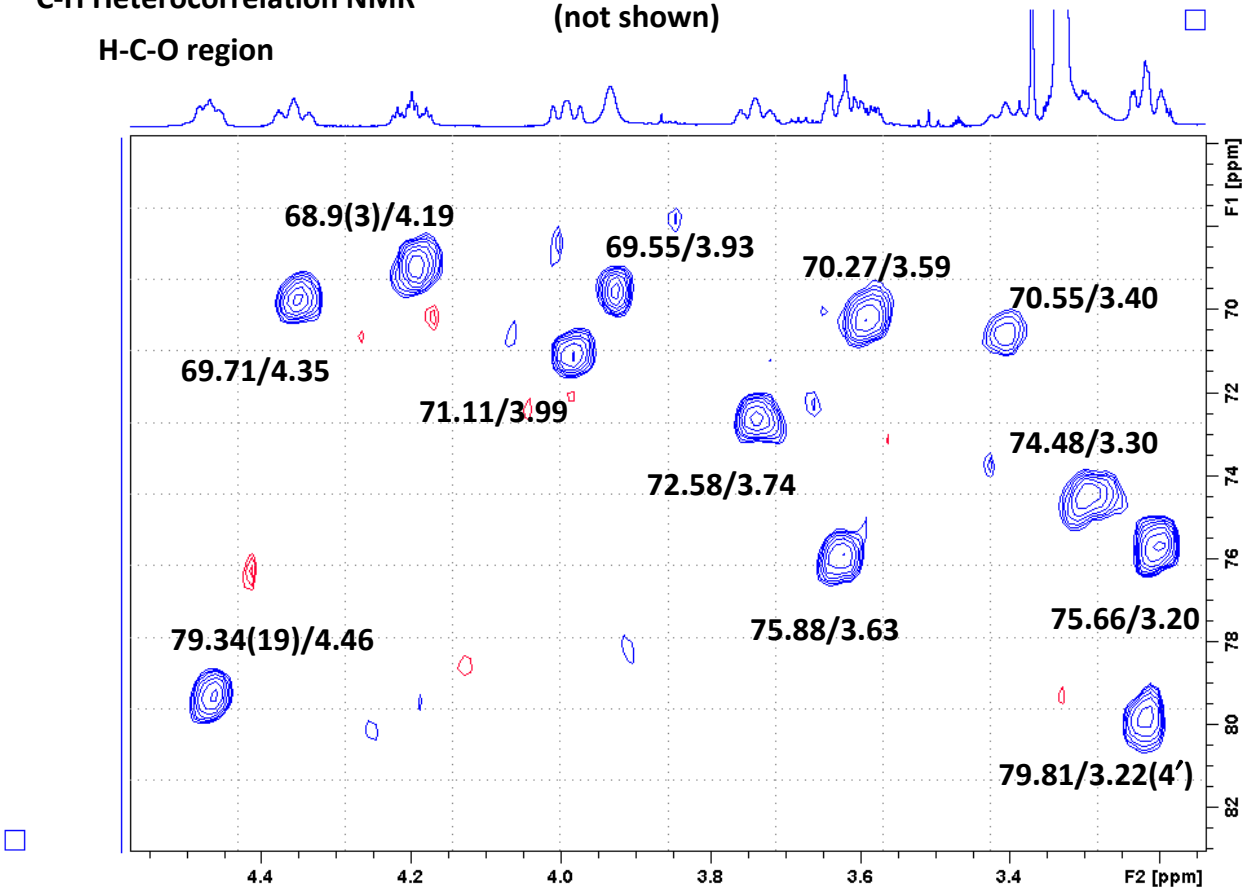


16-Descarboxyl-16-methyl Amph B

C-H Heterocorrelation NMR

H-C-O region

Also 57.19/3.08; 99.1/4.64 and 70.70/5.40 (not shown)



16-Descarboxyl-16-methyl Amph B (8)

No ^{13}C spectra run, only heterocorrelation NMR

12.24 (39)/1.035
13.51(41)/1.037
17.11(38)/1.22
17.56(6')/1.30
19.01(40)/1.13

31.1/1.75+1.39
33.3(18?)/2.24
36.2/1.51+1.42
38.2(18)/2.24+1.75
40.7(36)/1.36
41.7/1.83
42.74(2)/2.30+2.23
43.6(16)/1.25
44.7(4)/1.47+1.42
44.1/2.41(34)
45.6/2.0+1.33
48.1/1.73+1.61

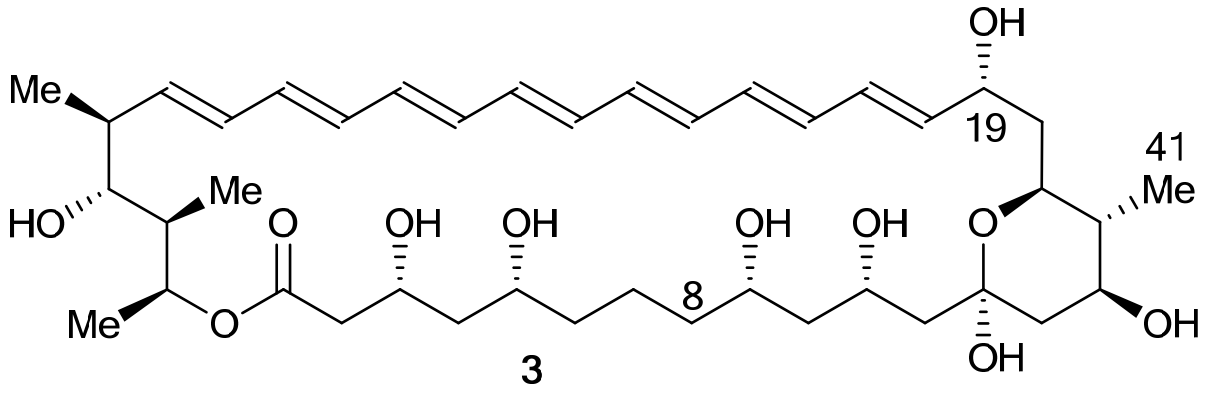
57.19(8)/3.08
68.9/4.19(3)
69.55/3.93
69.71/4.35
70.27/3.59
70.55(9)/3.40
70.70(37)/5.40
71.11/3.99
72.58/3.74
74.48(5')/3.30
75.66/3.20
75.88/3.63
79.34/4.47(19)
79.81(35)/3.22
99.1(1')/4.64

/6.21(33)
/6.036(20)

2.41 = 34+3'

AmphNM+perDIDII : aglycone (3)

8-Deoxy-16-descarboxyl-16-methyl-amphoteronolide B (3)

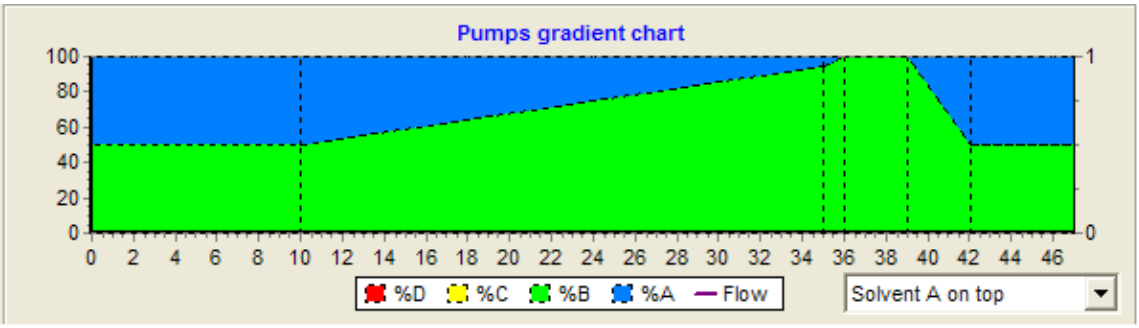


RMM 732.5

AmphNM+perDIDII (aglycone) 3 Example of HPLC analysis

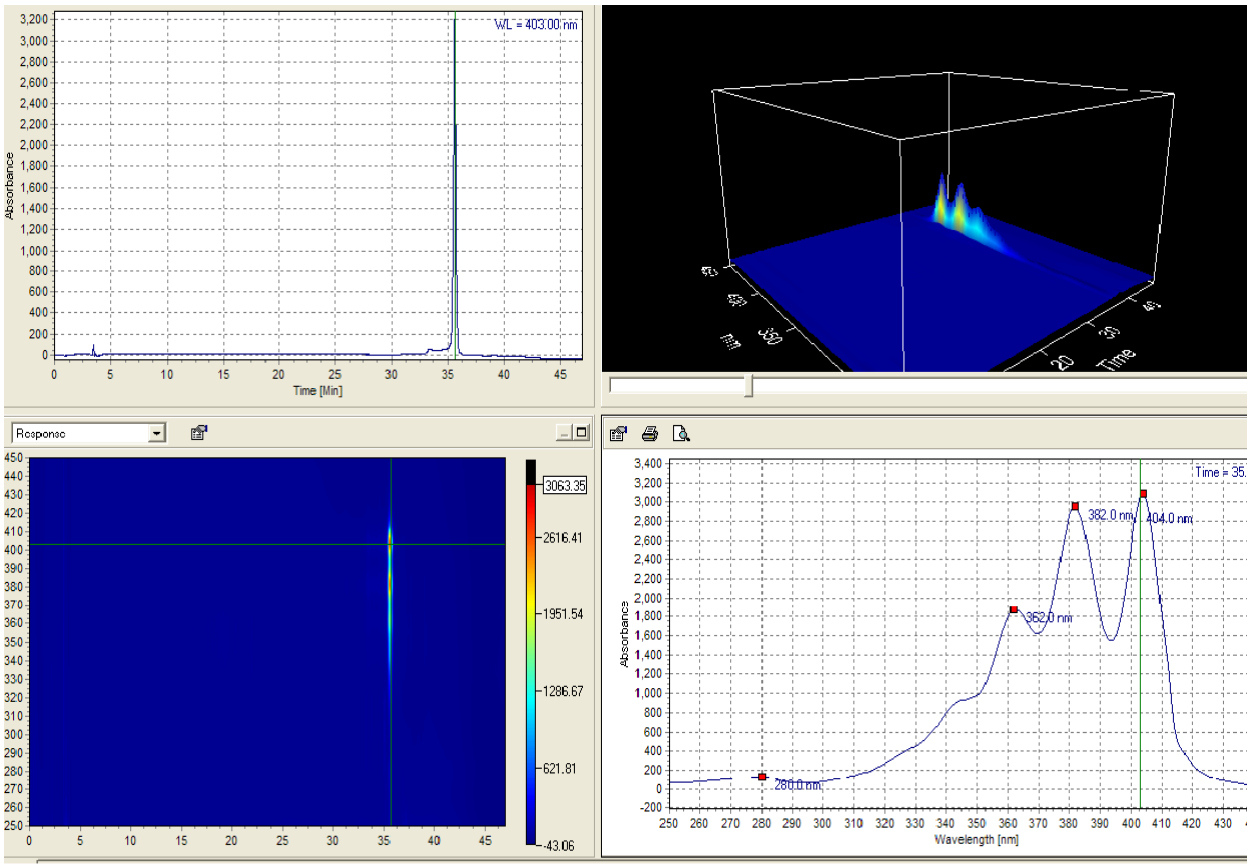
Analytical HPLC purification of amphNM+PerDIDII aglycone (3)
A – Water B – Methanol + 0.1% Formic acid

Gradiant less than 2% per minute



Prerun	1.000 ml/min	A=50.00% B=50.00%
10.0 min	1.000 ml/min	A=50.00% B=50.00%
35.0 min	1.000 ml/min	A=5.00% B=95.00%
36.0 min	1.000 ml/min	A=0.00% B=100.00%
39.0 min	1.000 ml/min	A=0.00% B=100.00%
42.0 min	1.000 ml/min	A=50.00% B=50.00%
47.0 min	1.000 ml/min	A=50.00% B=50.00%

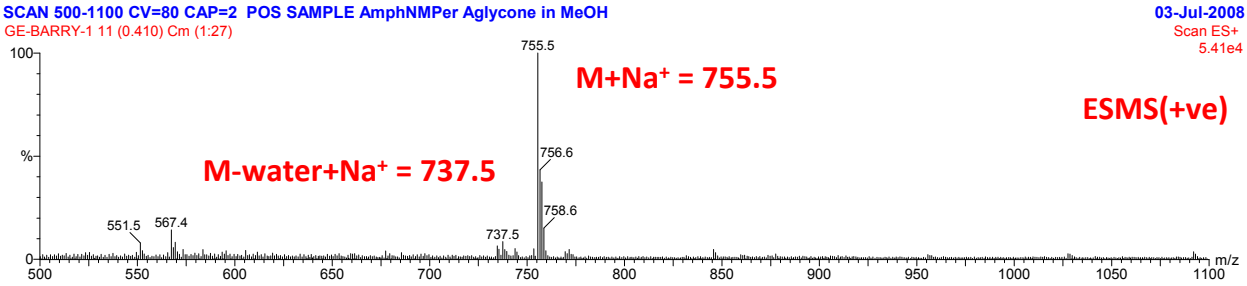
AmphNM+per DIDII : aglycone (3), RP-HPLC of methanol washed ppte

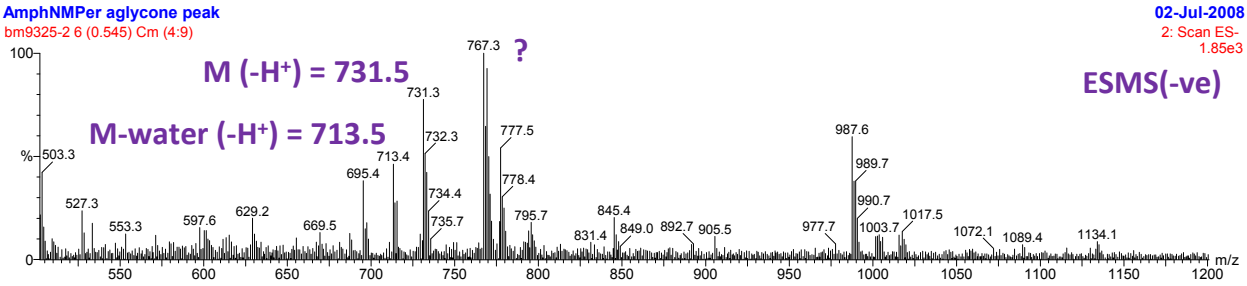


AmphNM+perDIDII aglycone (3)

Electrospray MS (Quattro triple quad)

$C_{41}H_{64}O_{11}$ = 732 **RMM aglycone (3) = 732.5**

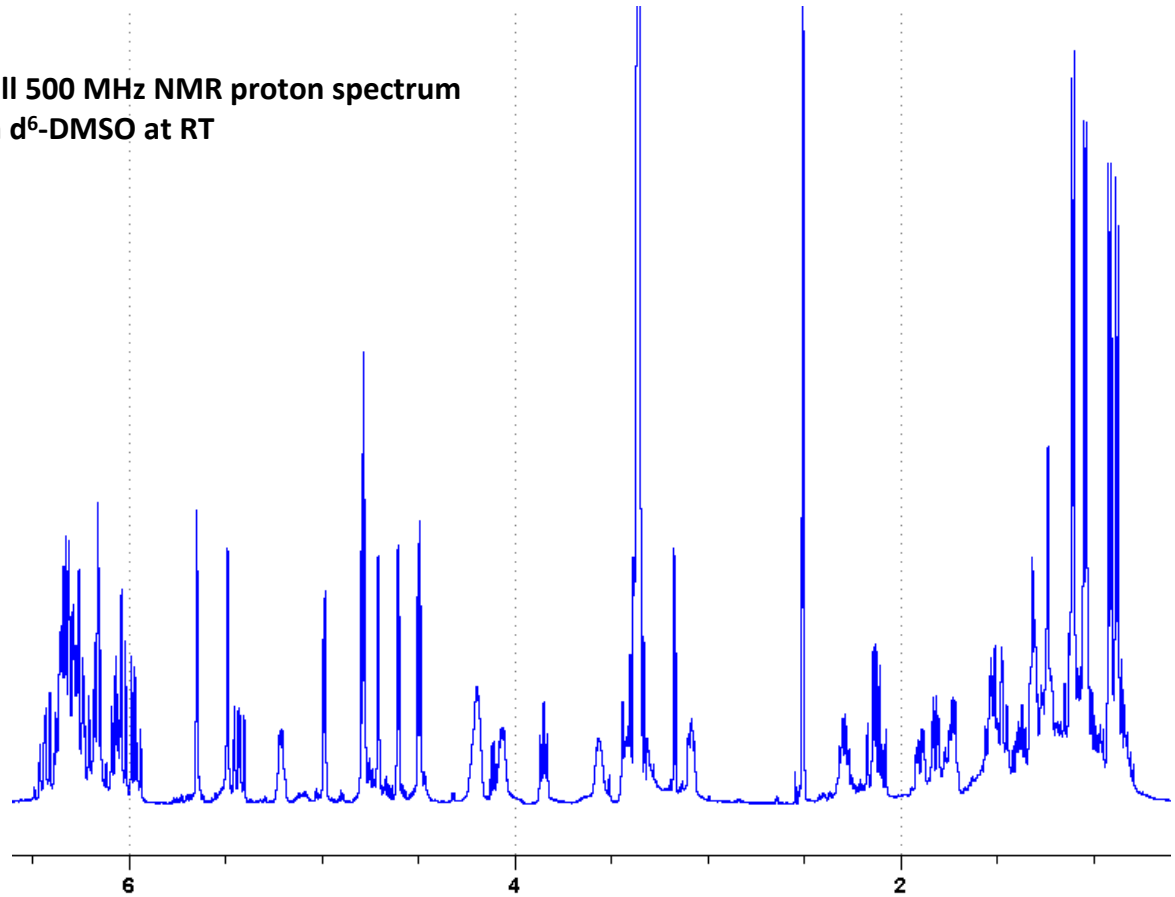


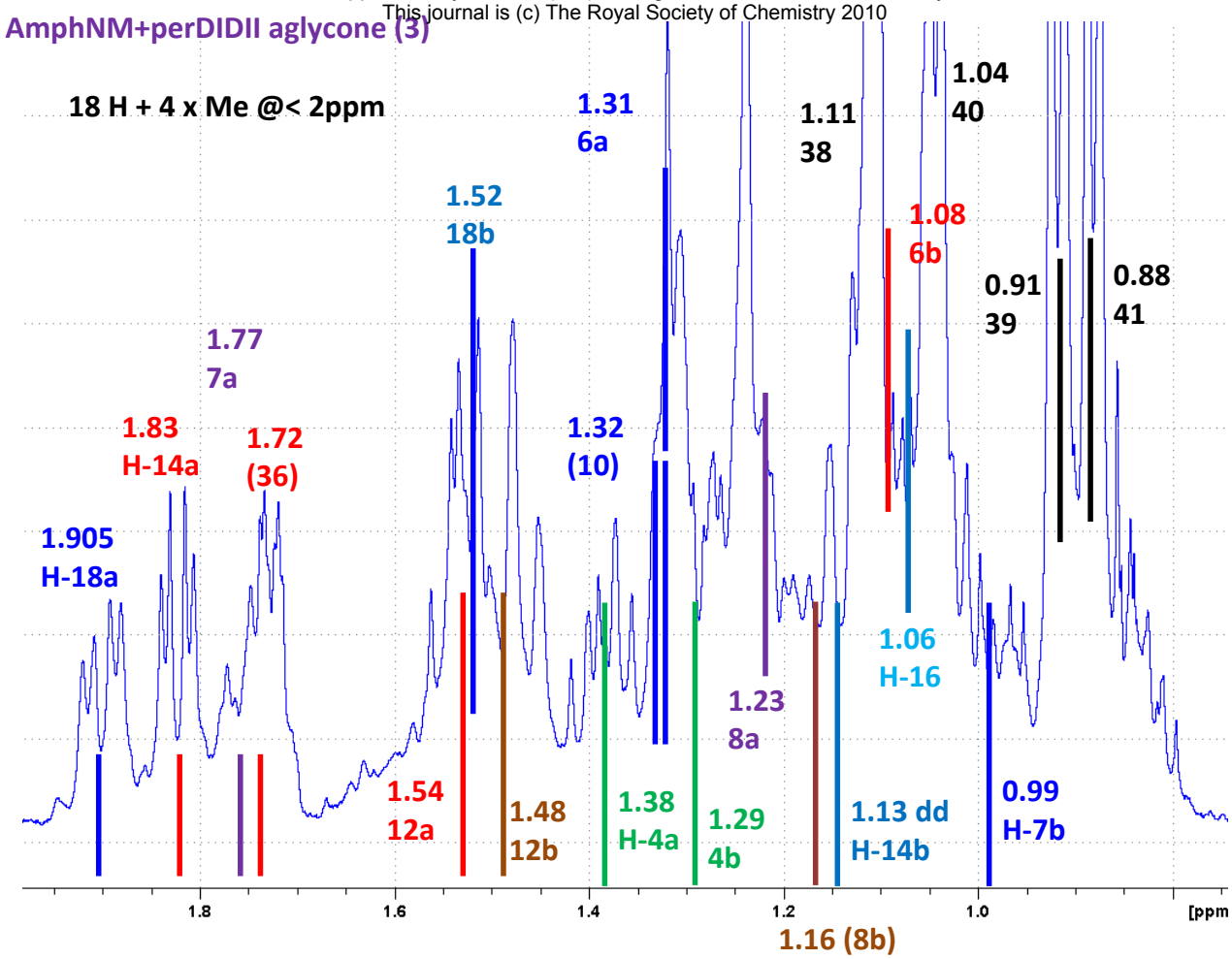


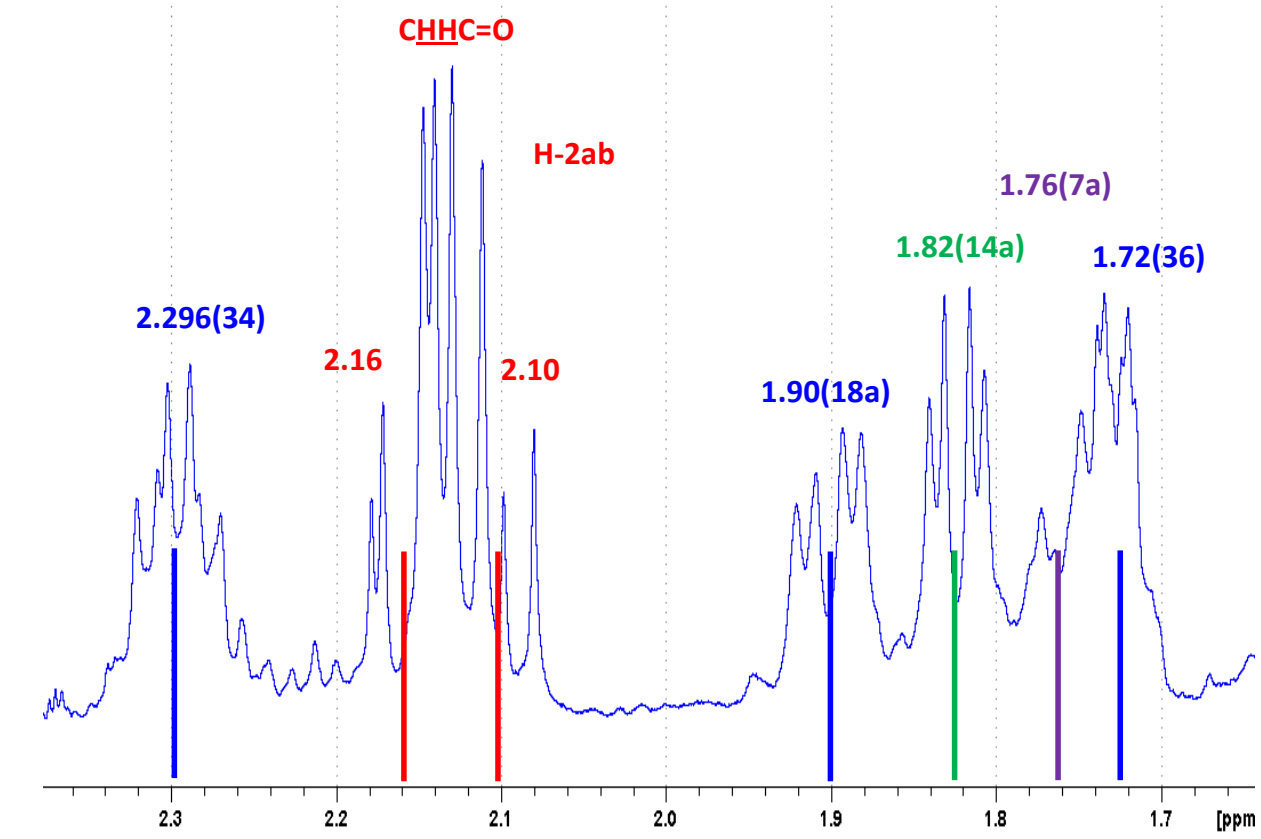
FAB did not work, hence no high resolution MS.

AmphNM+perDIDII aglycone (3)

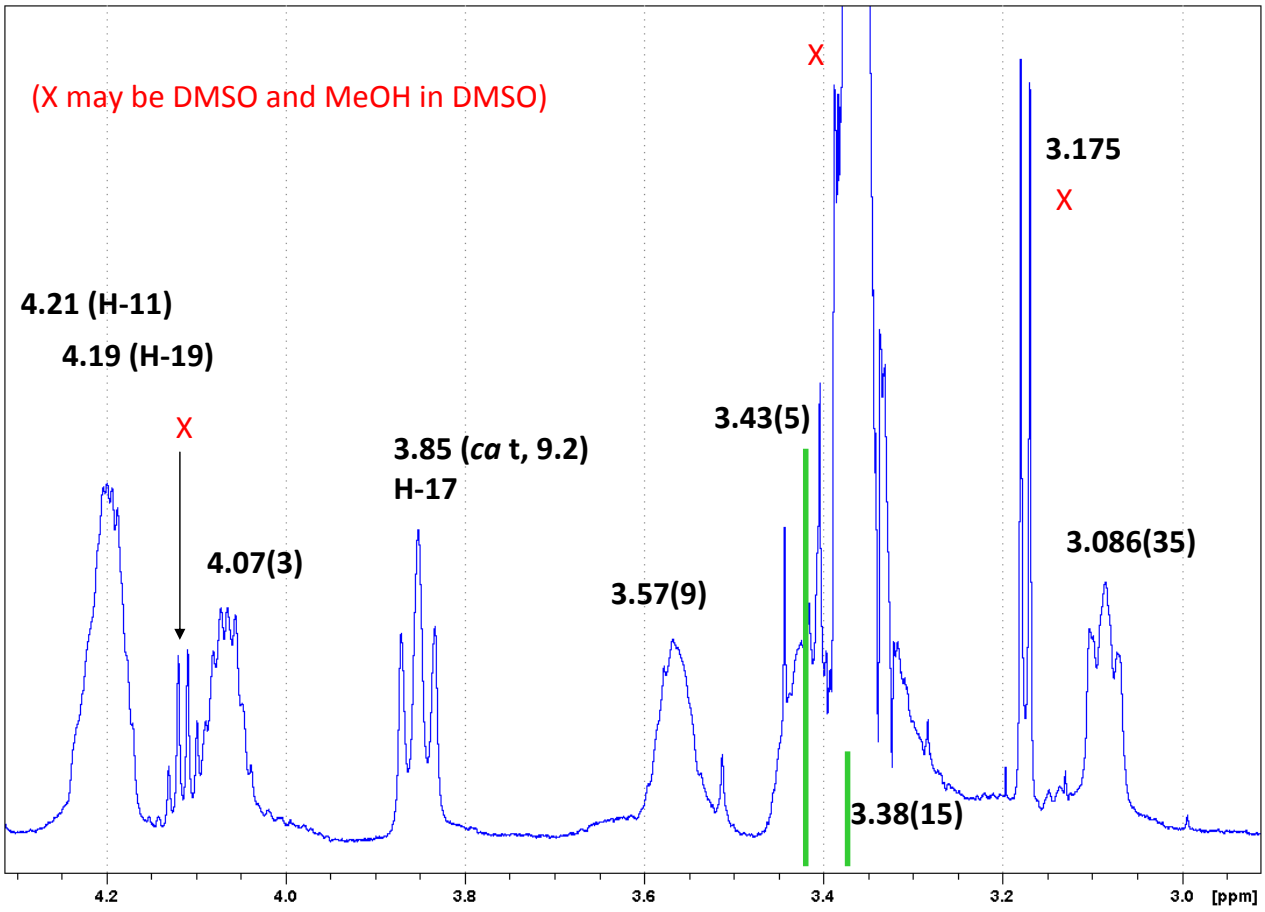
Full 500 MHz NMR proton spectrum in d^6-DMSO at RT



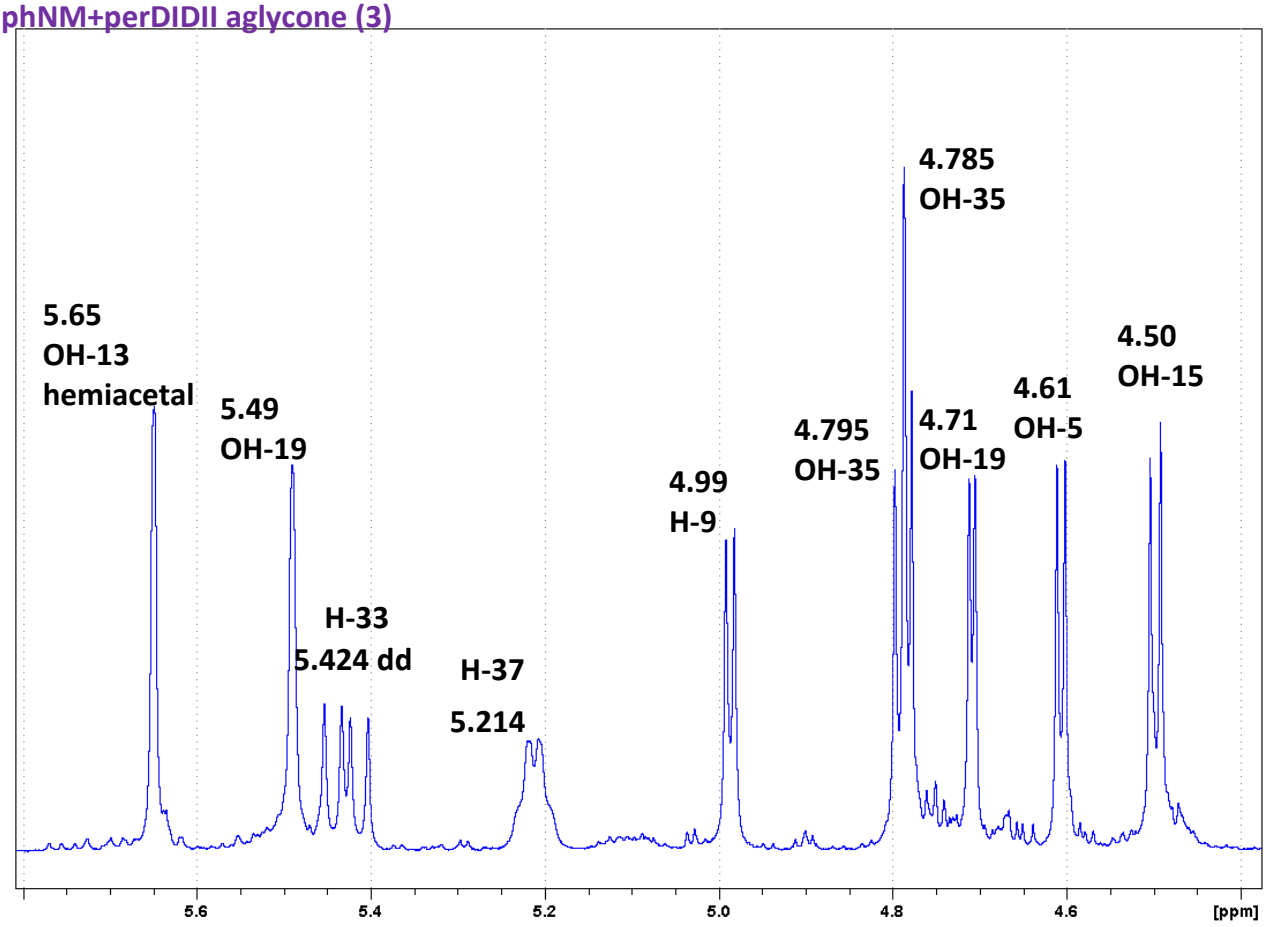

AmphNM+perDIDII aglycone (3)
18 H + 4 x Me @< 2ppm
1.31
6a
1.11
38
1.04
40
1.52
18b
1.08
6b
0.91
39
0.88
41
1.77
7a
1.83
H-14a
1.72
(36)
1.32
(10)
1.905
H-18a
1.06
H-16
1.23
8a
1.54
12a
1.48
12b
1.38
H-4a
1.29
4b
1.13 dd
H-14b
0.99
H-7b
1.16 (8b)
1.8
1.6
1.4
1.2
1.0
[ppm


AmphNM+perDIDII aglycone (3)
CHHC=O
H-2ab
1.76(7a)
1.82(14a)
1.72(36)
2.296(34)
2.16
2.10
1.90(18a)
2.3
2.2
2.1
2.0
1.9
1.8
1.7
[ppm

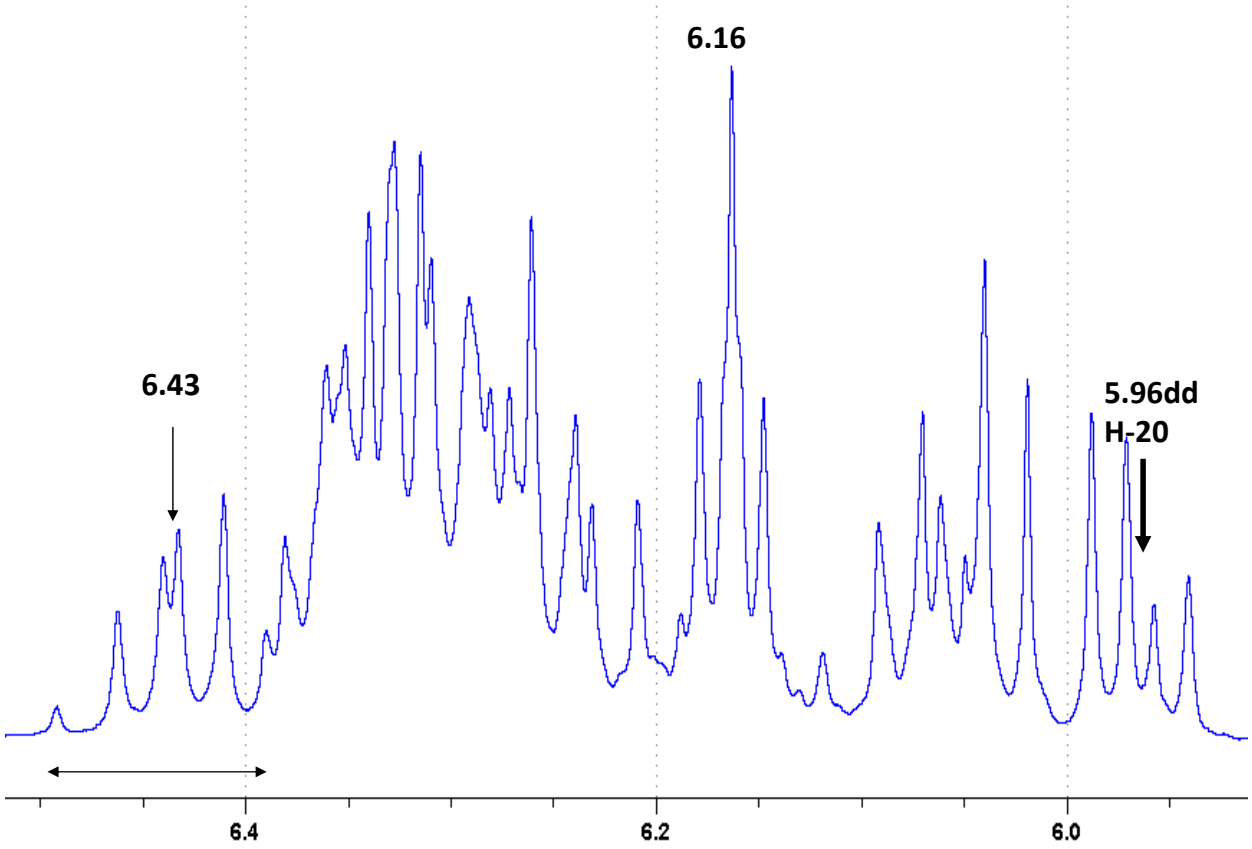
AmphNM+perDIDII aglycone (3)



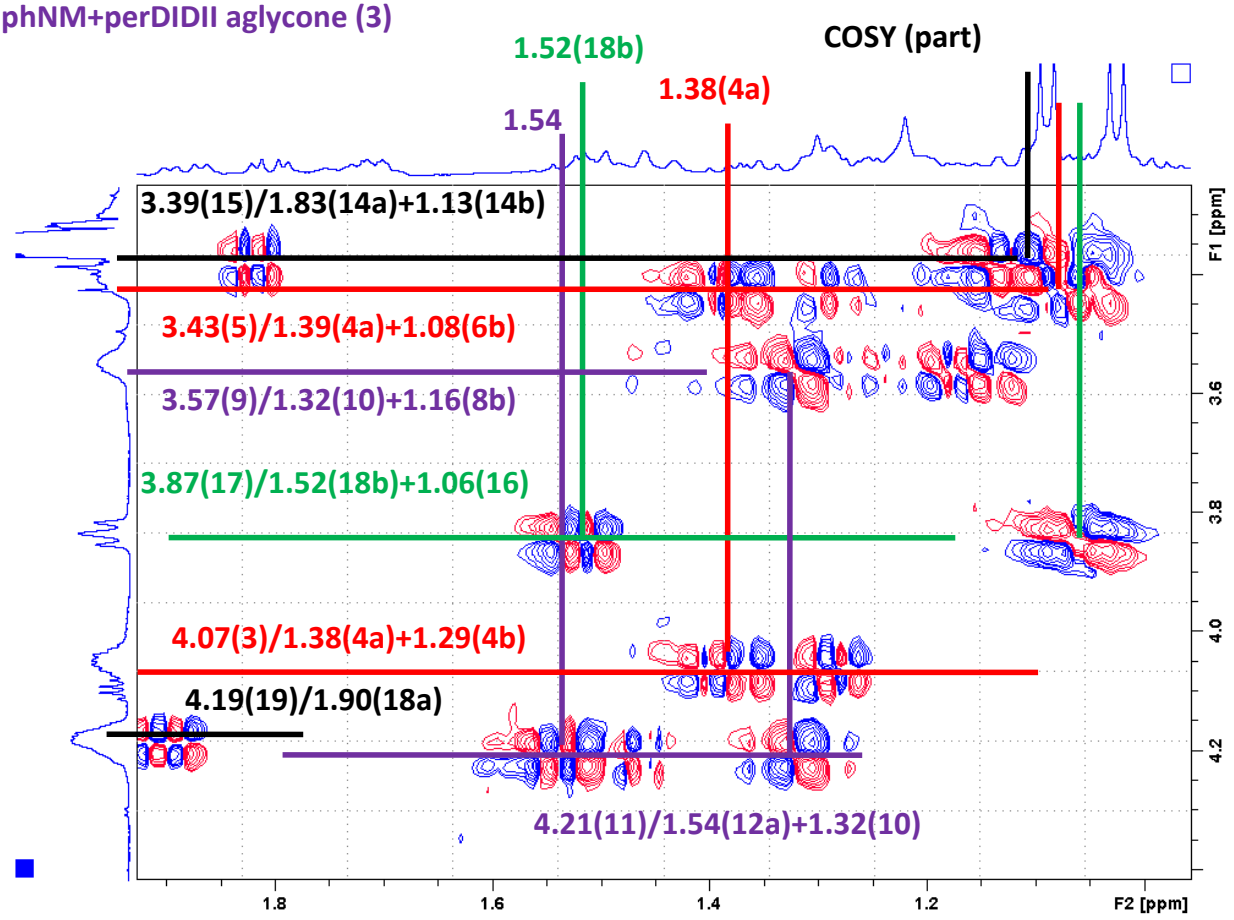
AmphNM+perDIDII aglycone (3)



AmphNM+perDIDII aglycone (3)



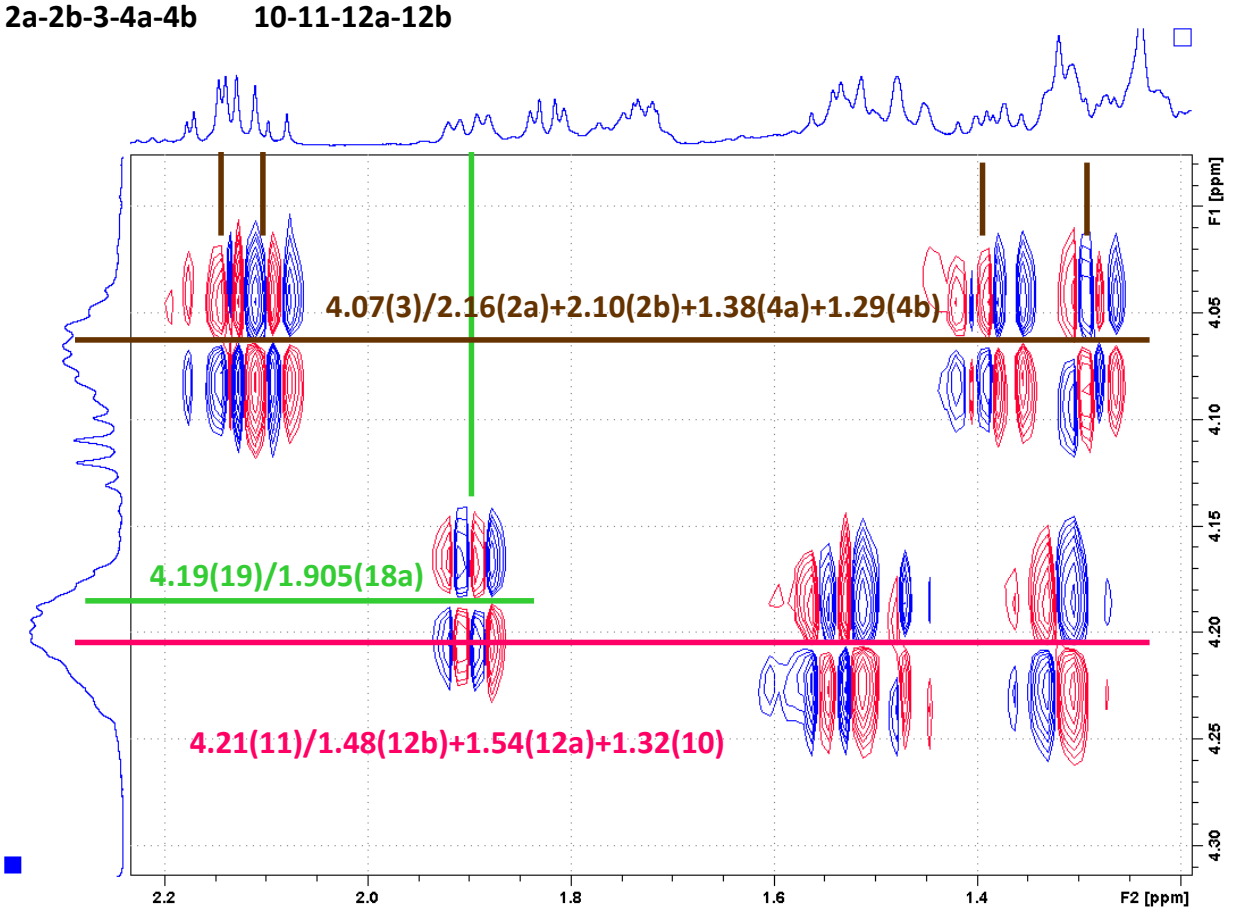
AmphNM+perDIDII aglycone (3)



3-4a-4b-5-6b 8b-9-10ab-11-12a 16-17-18b 18a-19

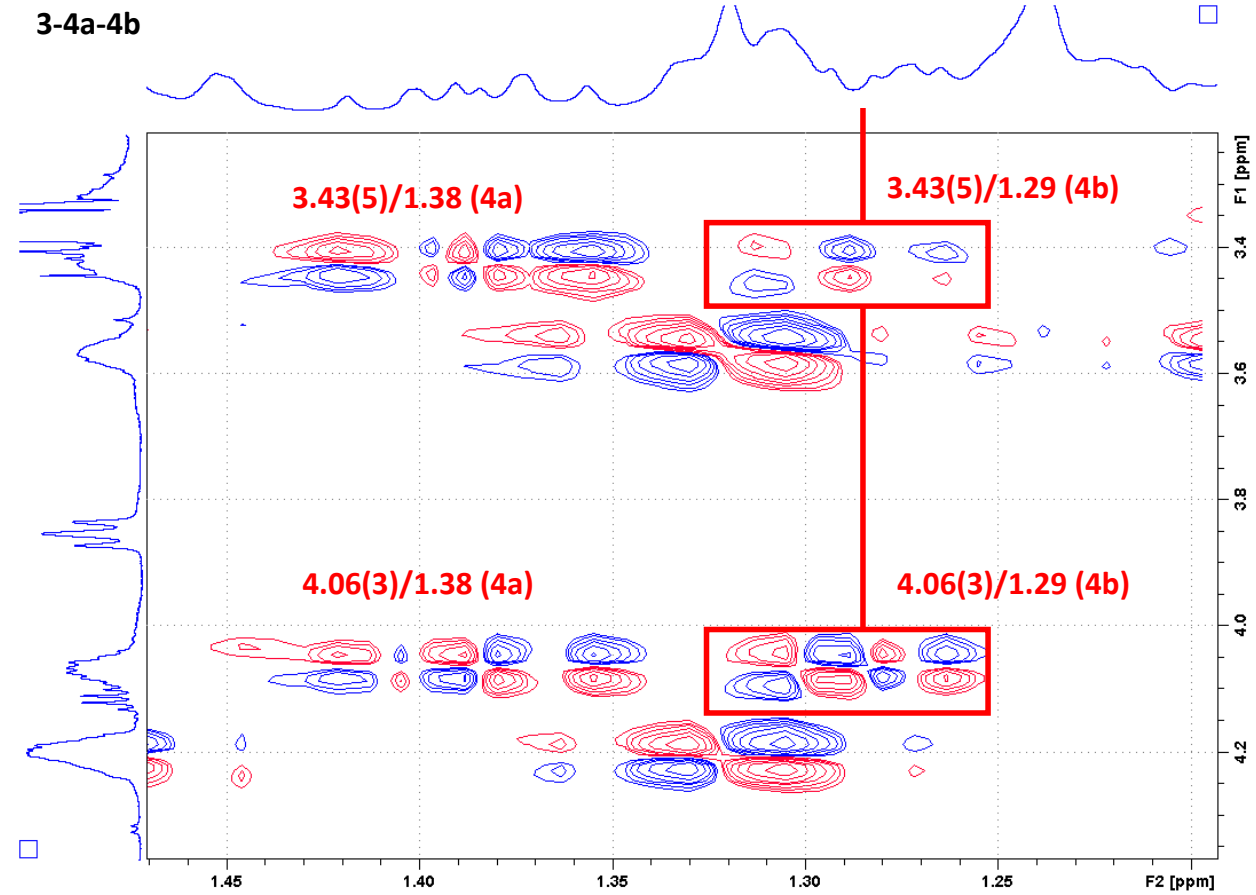
AmphNM+perDIDII aglycone (3)

2a-2b-3-4a-4b **10-11-12a-12b**

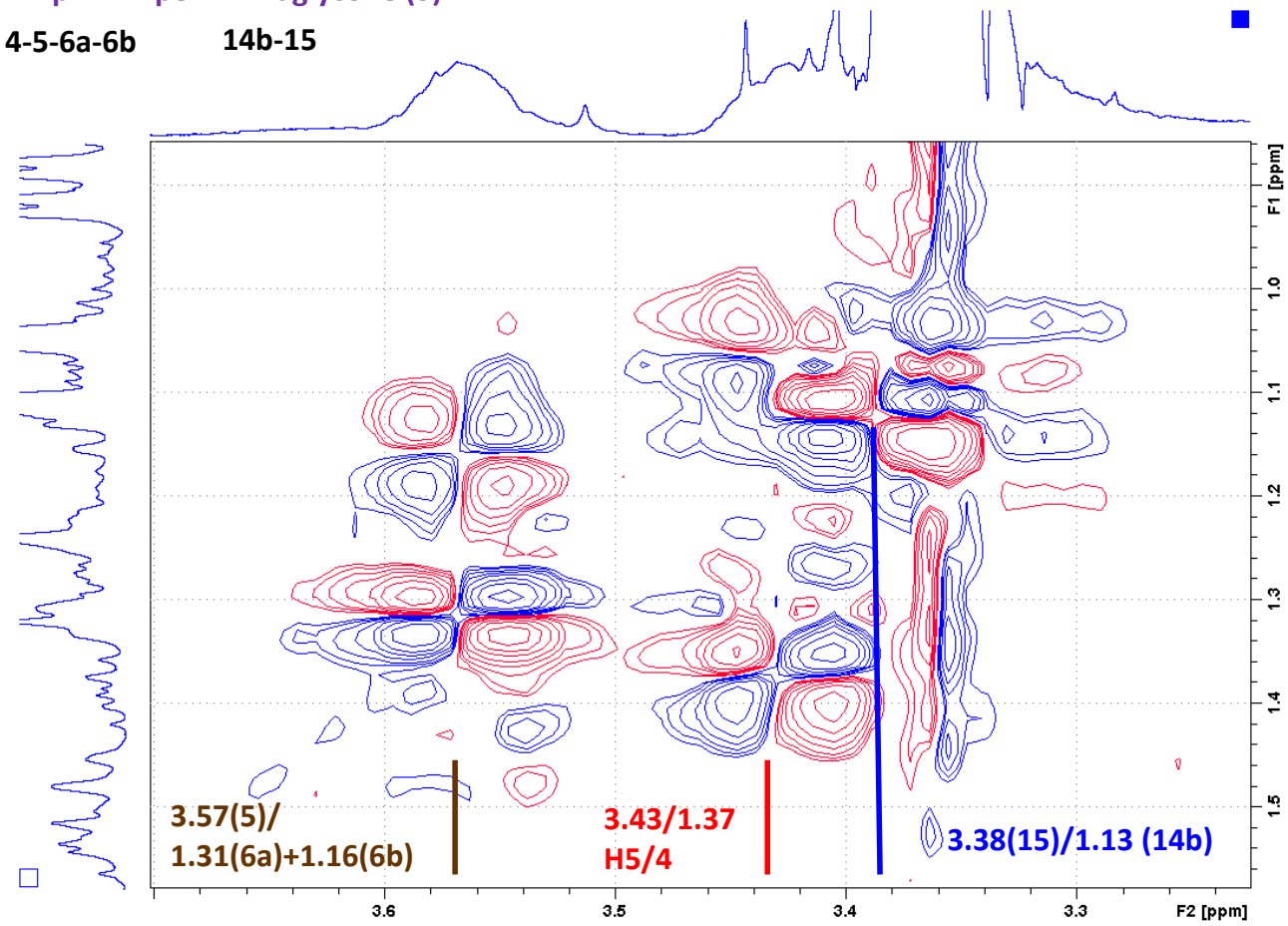


AmphNM+perDIDII aglycone (3)

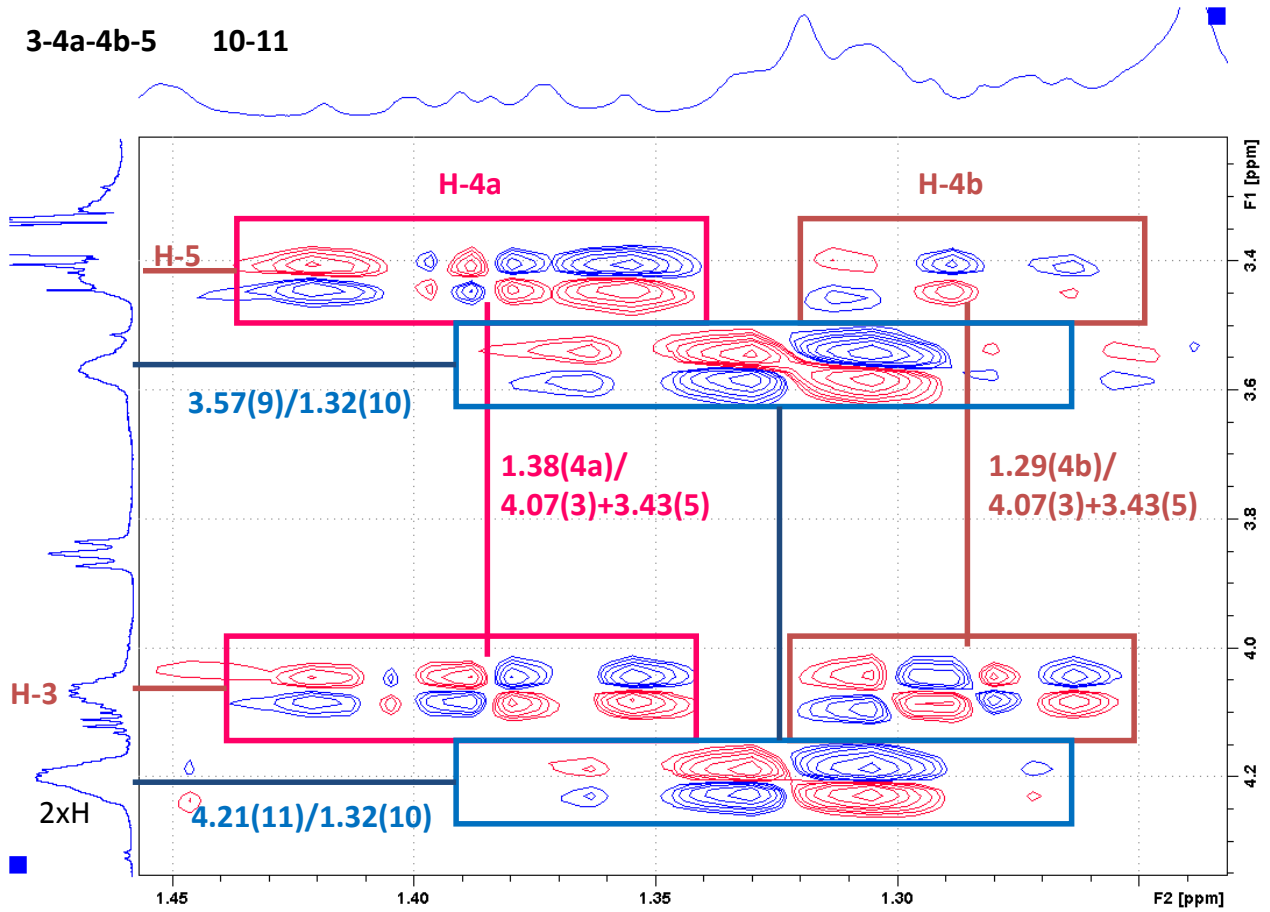
3-4a-4b



AmphNM+perDIDII aglycone (3)



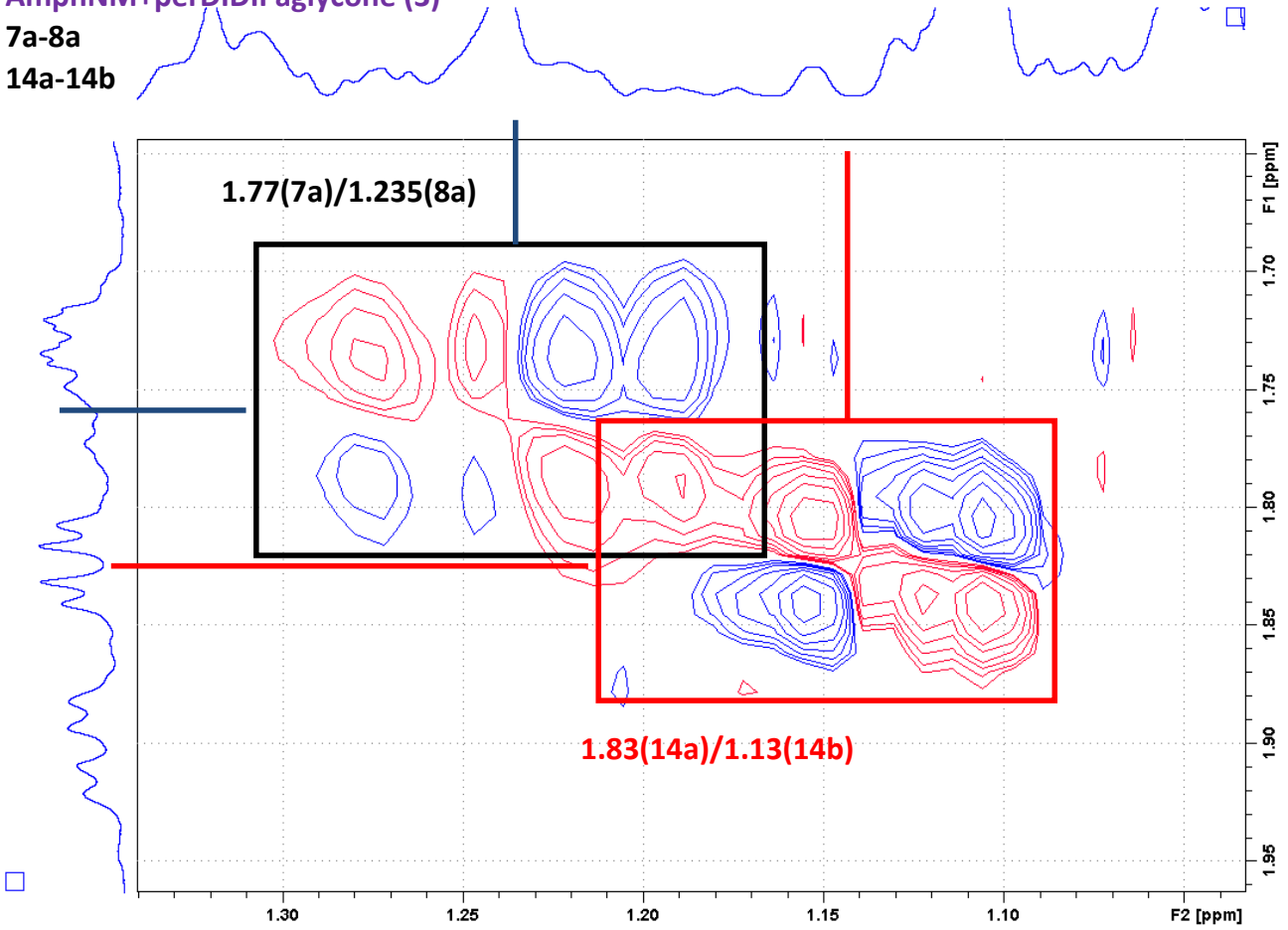
AmphNM+perDIDII aglycone (3)



AmphNM+perDIDII aglycone (3)

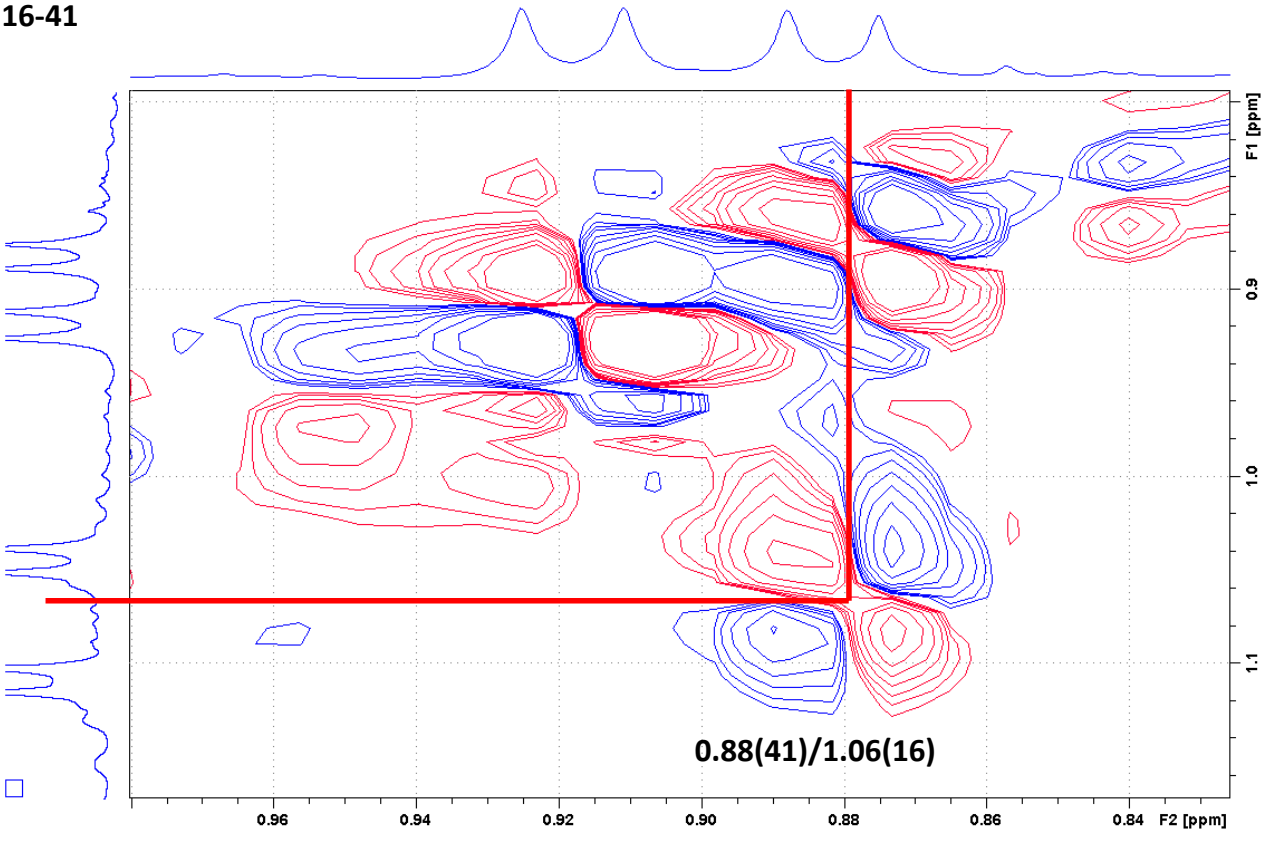
7a-8a

14a-14b



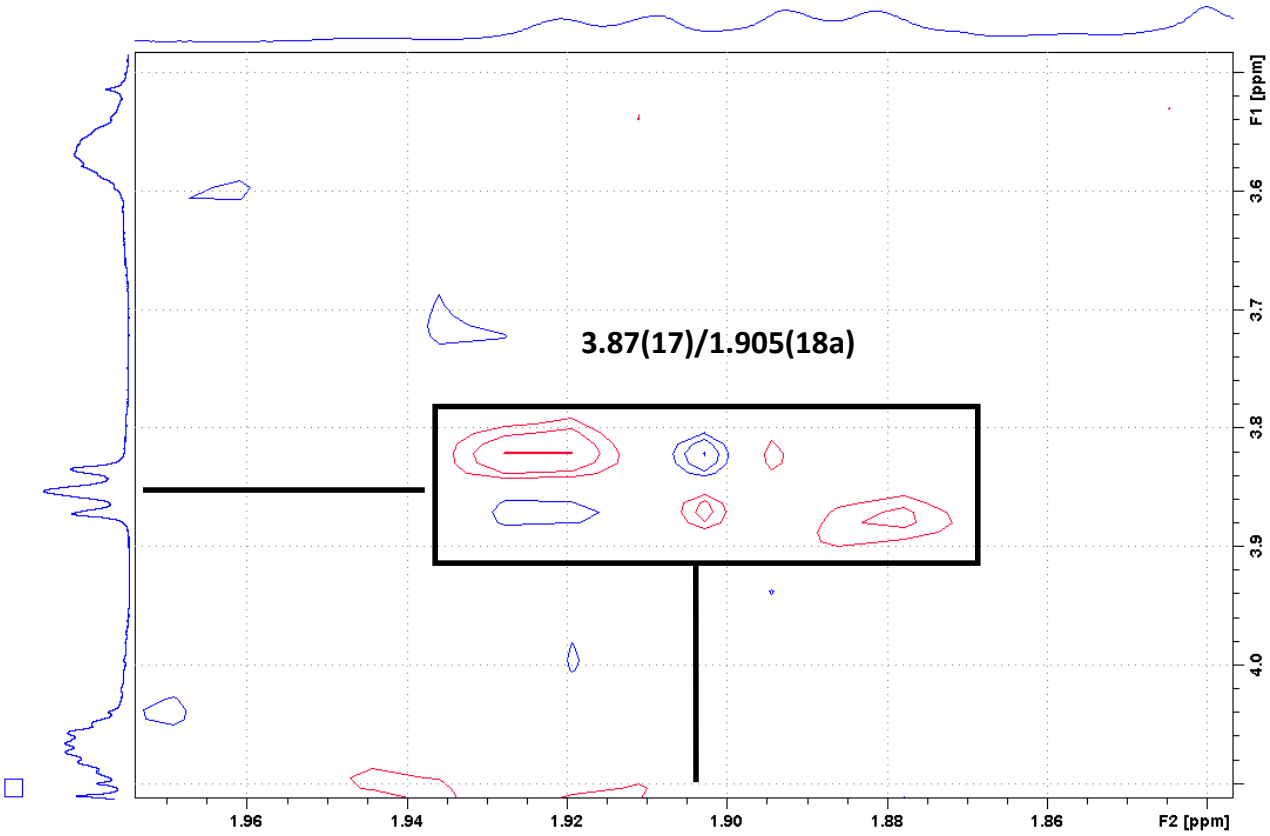
AmphNM+perDIDII aglycone (3)

16-41



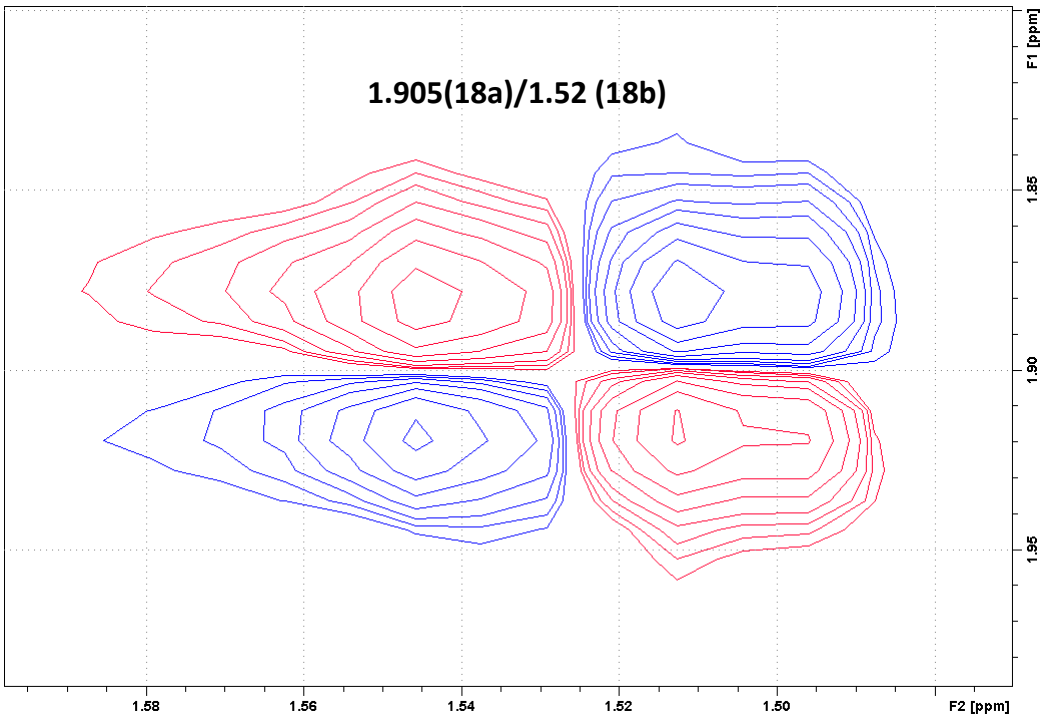
AmphNM+perDIDII aglycone (3)

17-18a



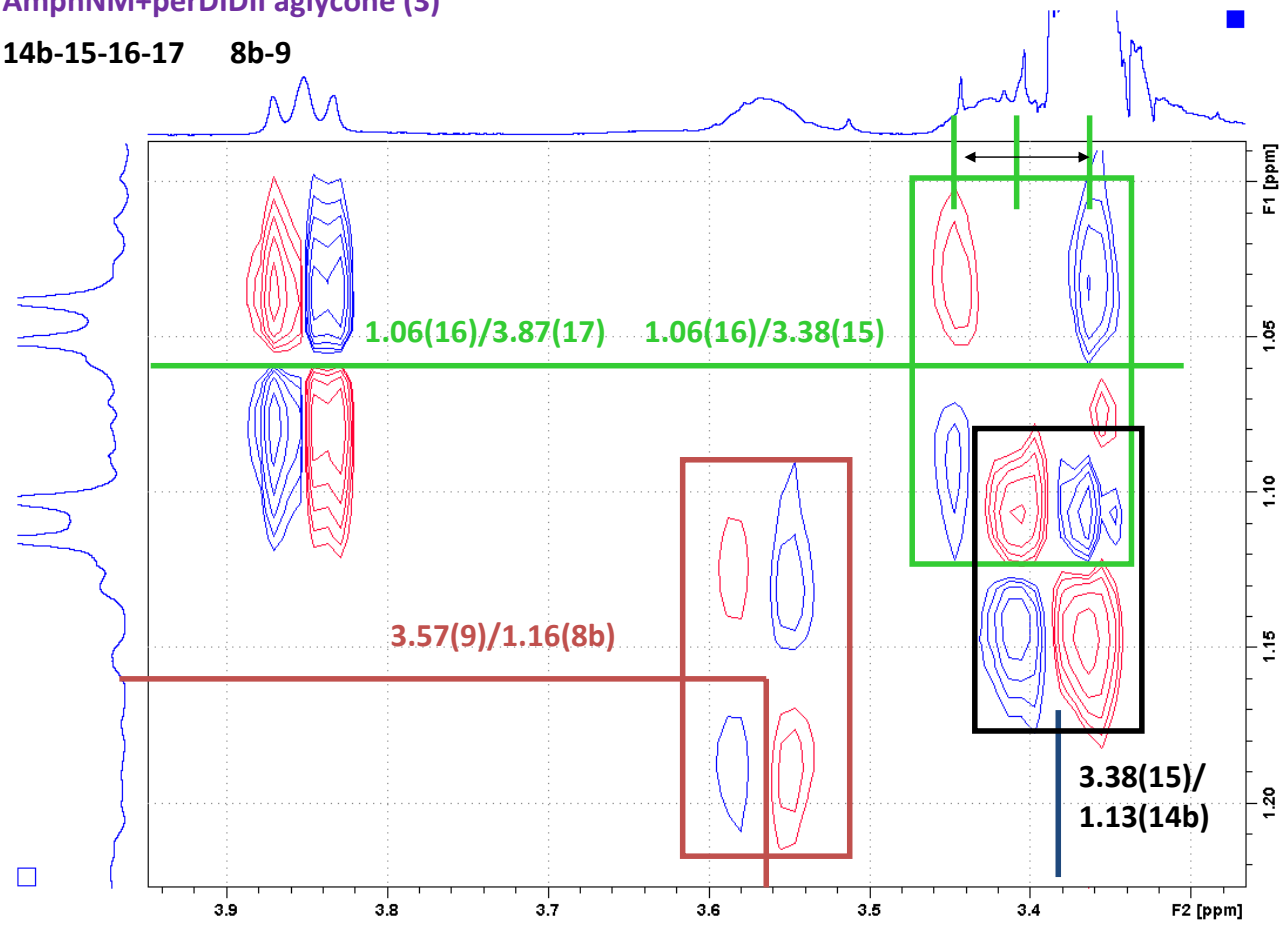
AmphNM+perDIDII aglycone (3)

18a-18b



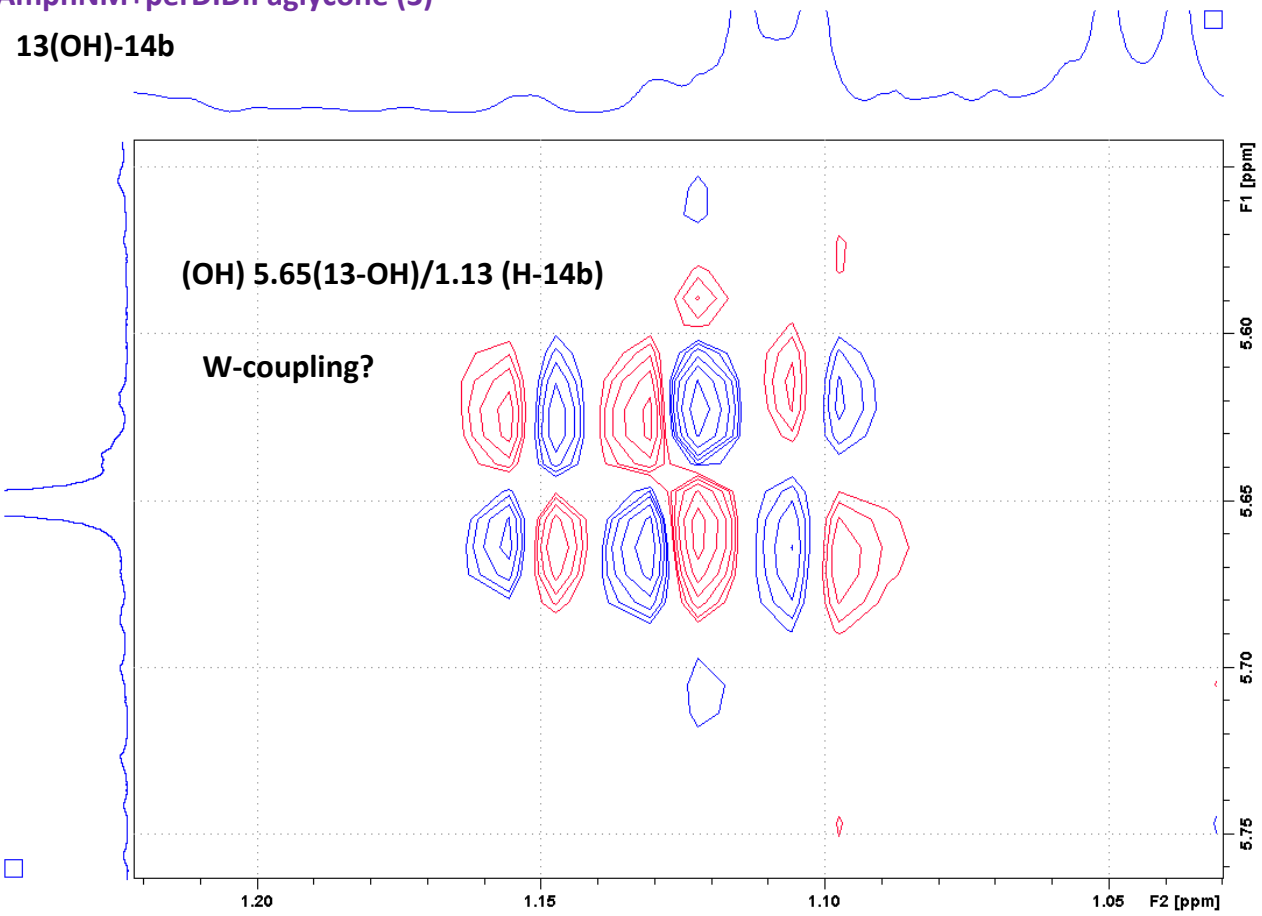
AmphNM+perDIDII aglycone (3)

14b-15-16-17 8b-9

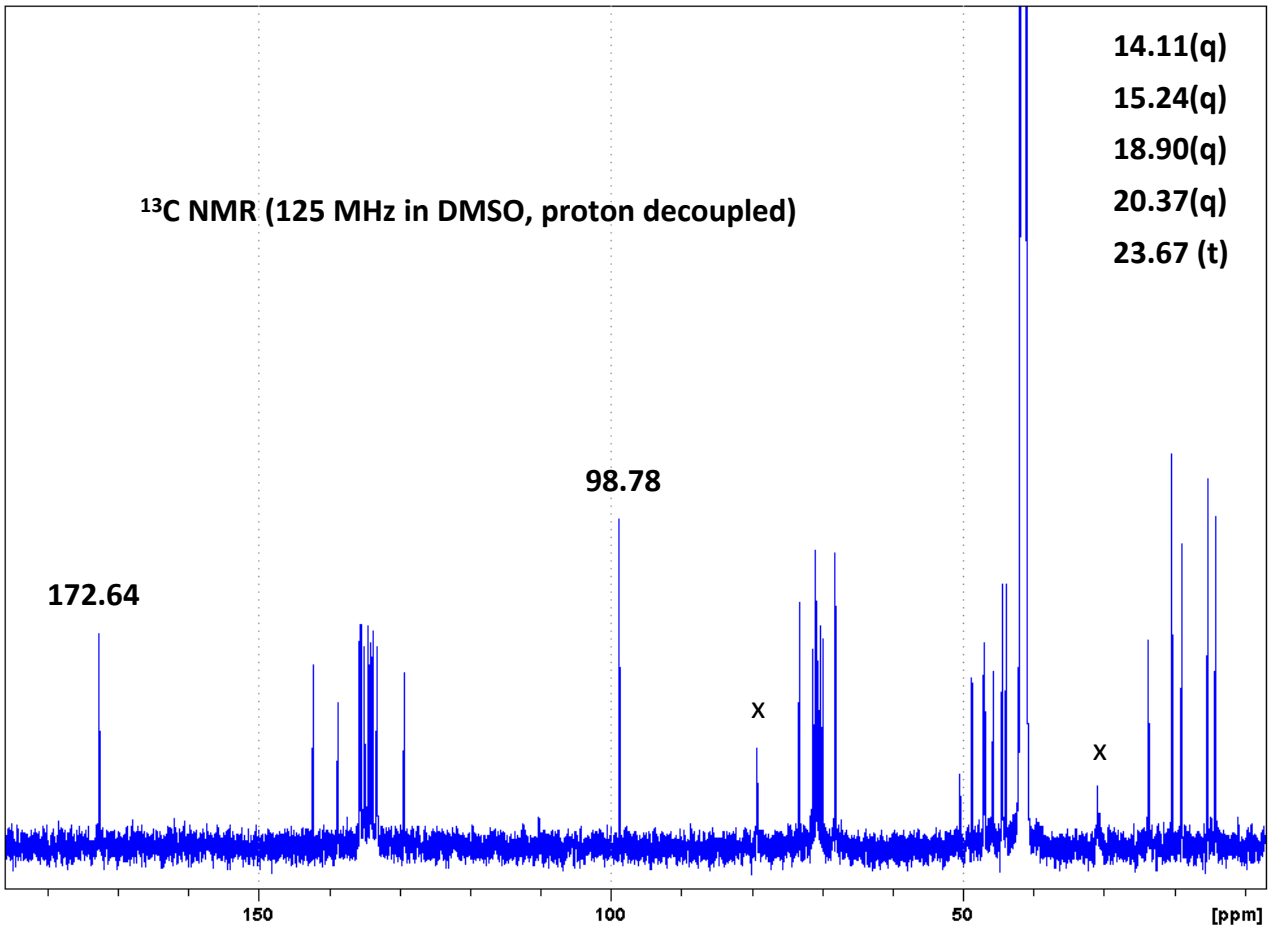


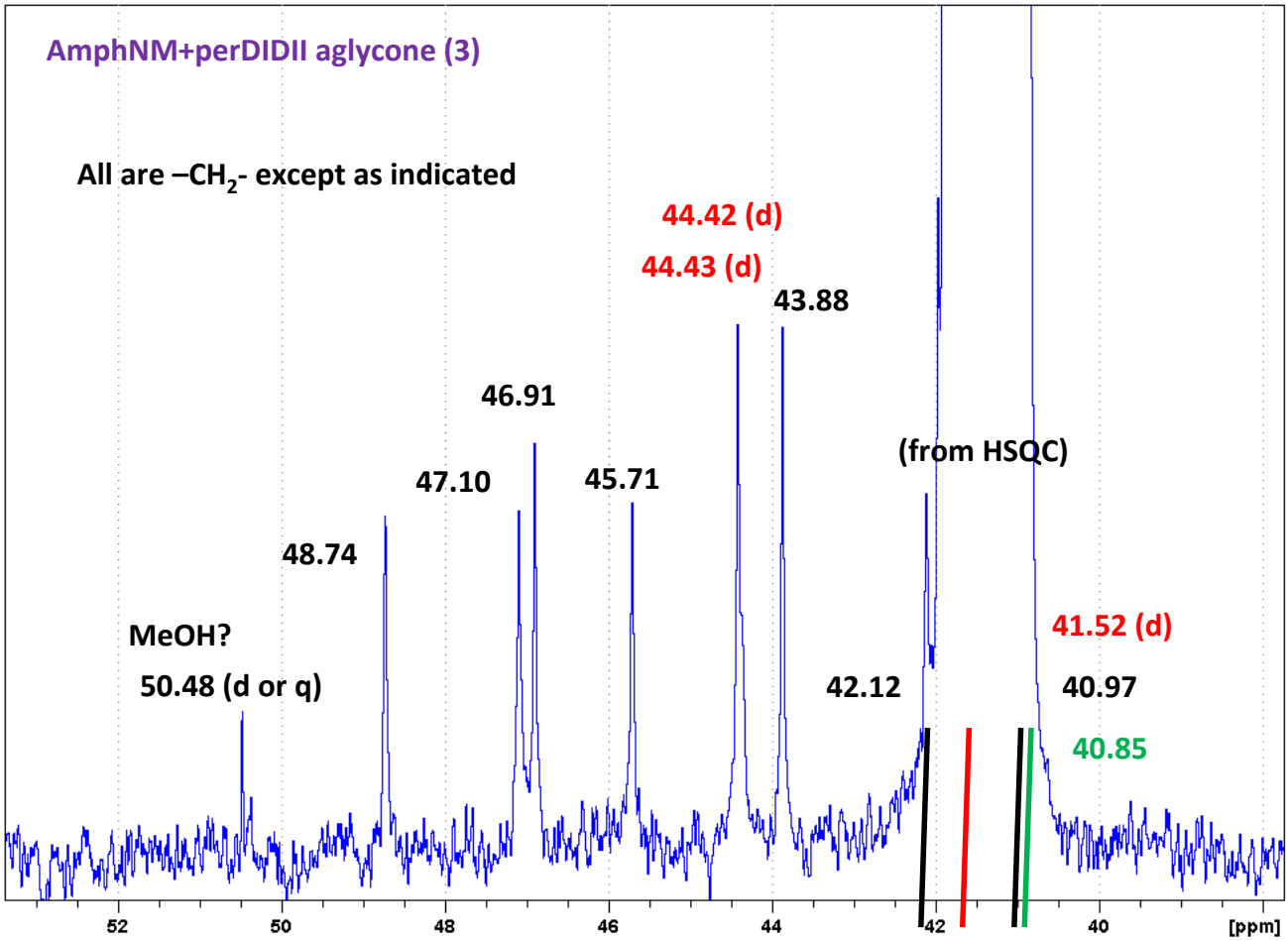
AmphNM+perDIDII aglycone (3)

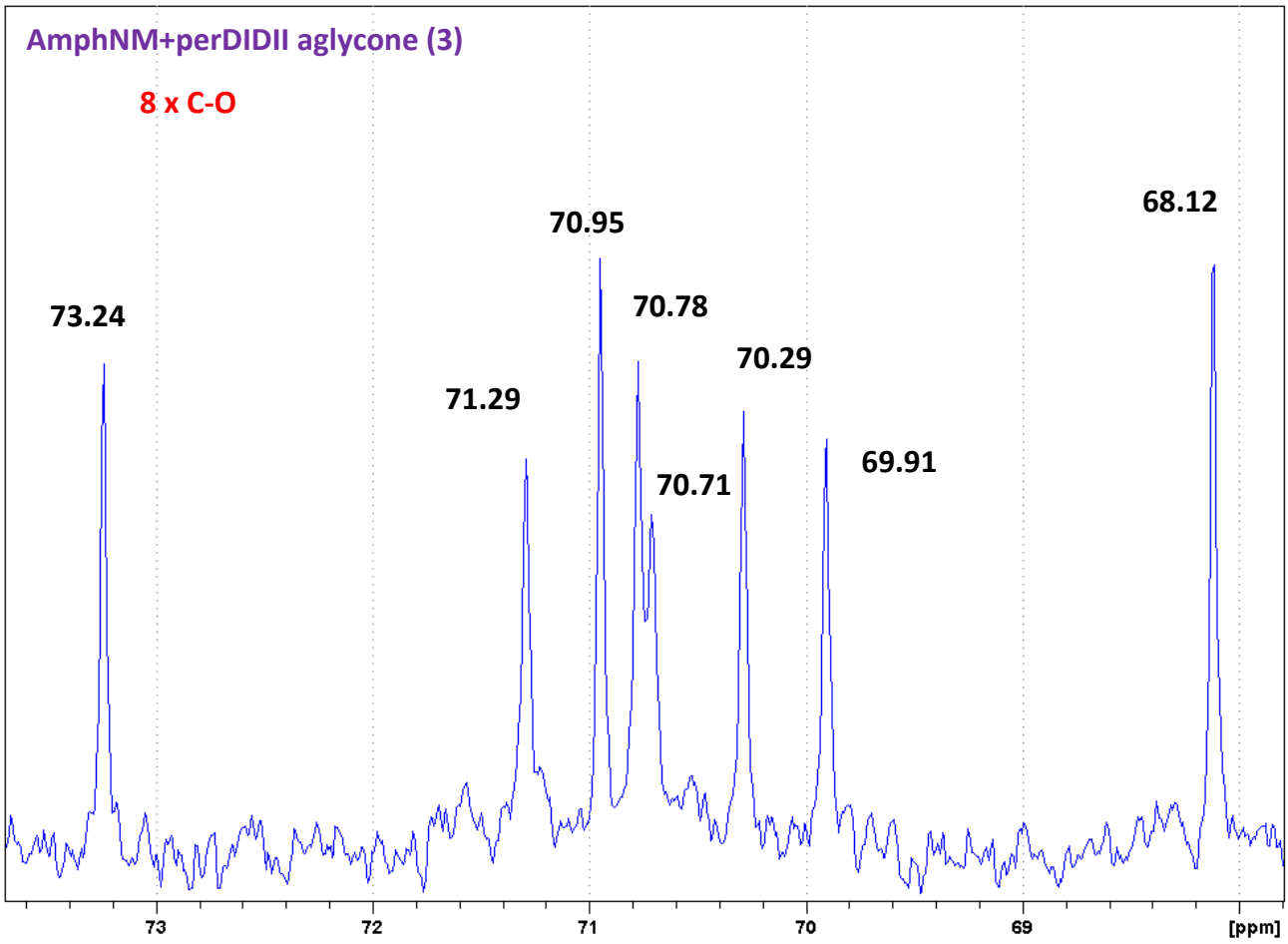
13(OH)-14b

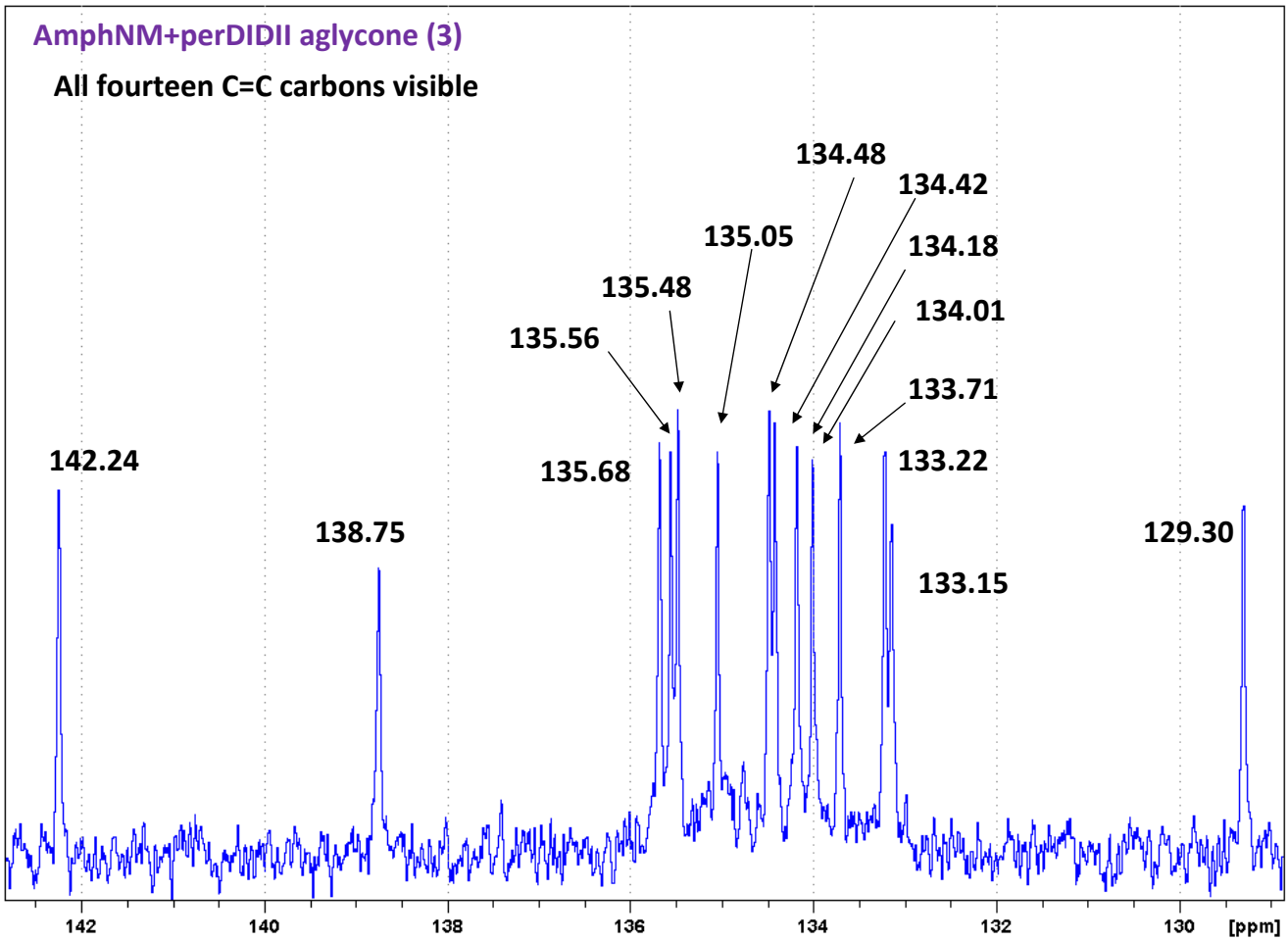


AmphNM+perDIDII aglycone (3)

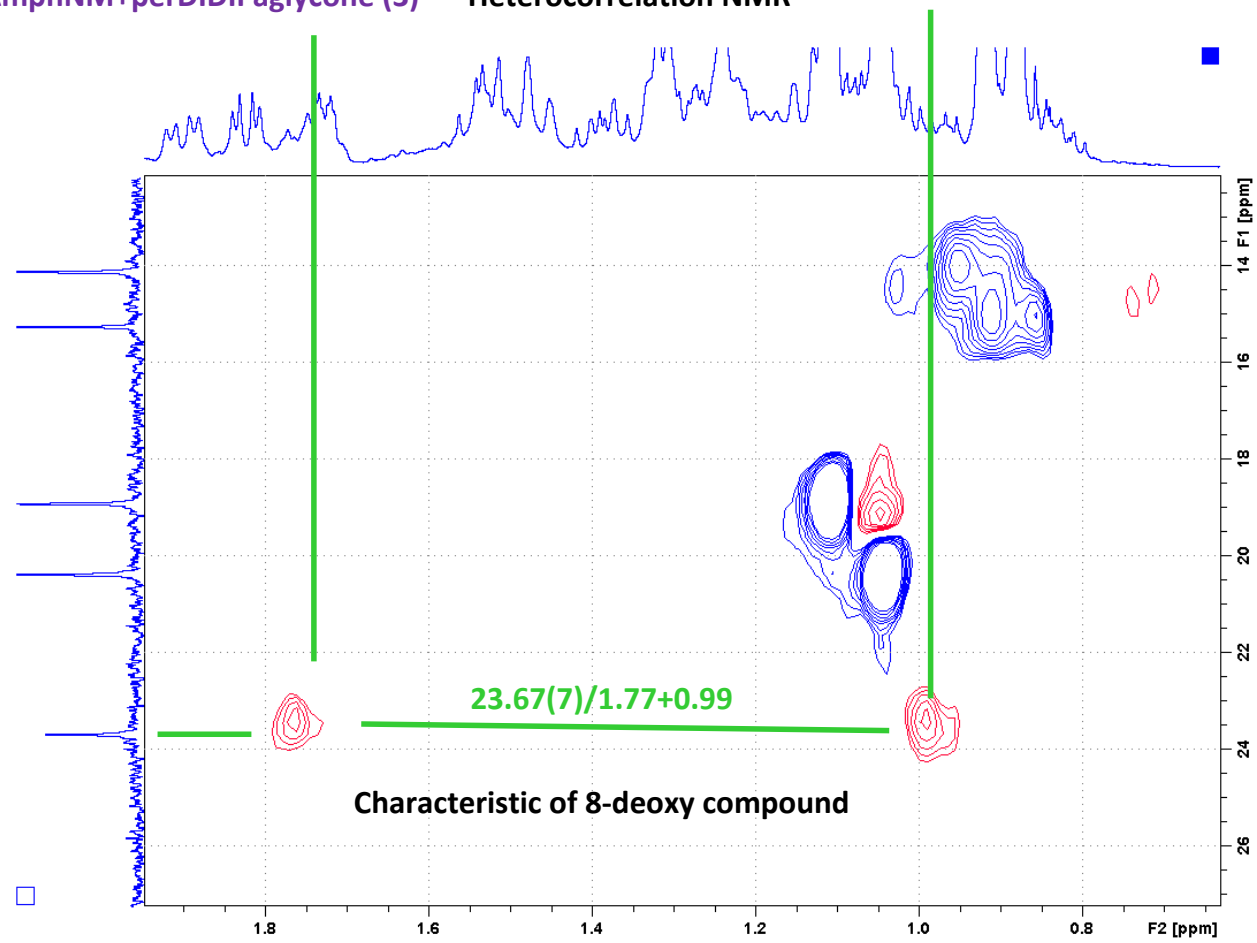




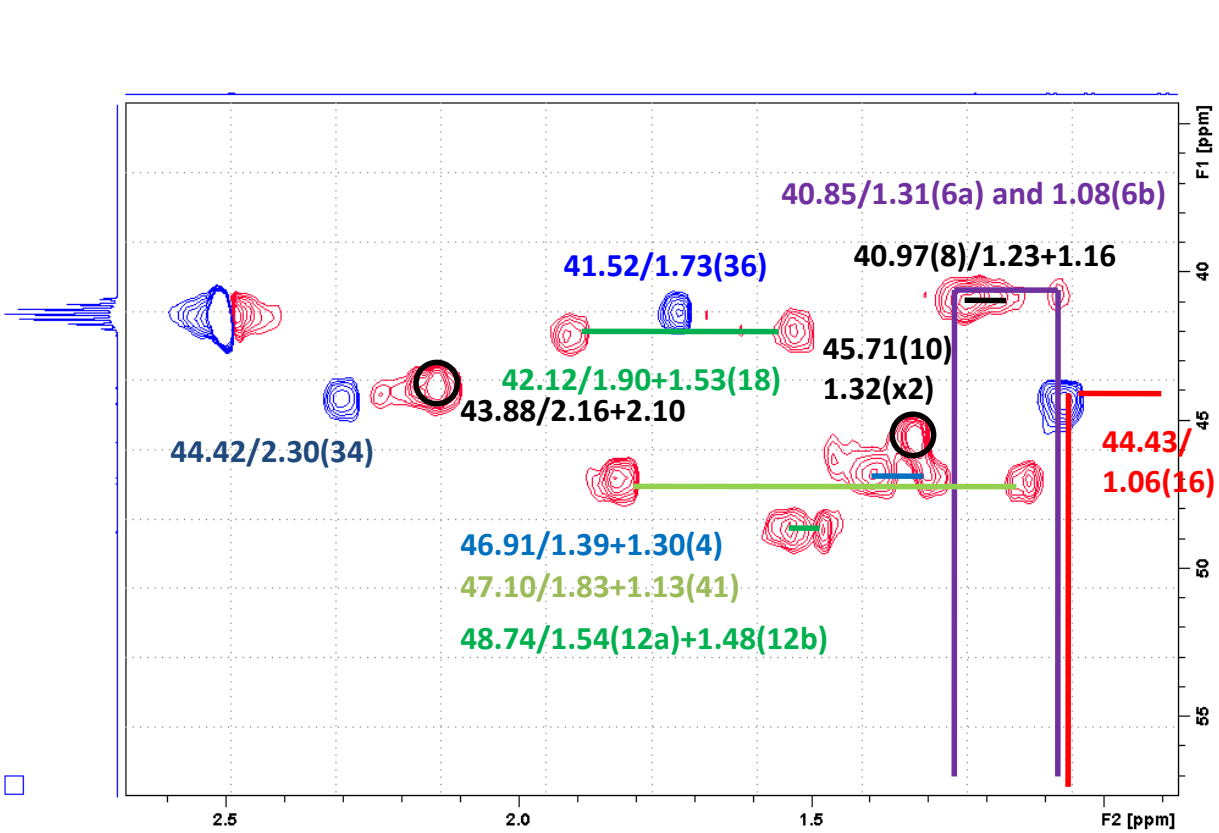

AmphNM+perDIDII aglycone (3)
8 x C-O
73.24
71.29
70.95
70.78
70.71
70.29
69.91
68.12
73
72
71
70
69
[ppm]


AmphNM+perDIDII aglycone (3)
All fourteen C=C carbons visible
142.24
138.75
135.68
135.56
135.48
135.05
134.48
134.42
134.18
134.01
133.71
133.22
133.15
129.30
142
140
138
136
134
132
130
[ppm]

AmphNM+perDIDII aglycone (3) **Heterocorrelation NMR**

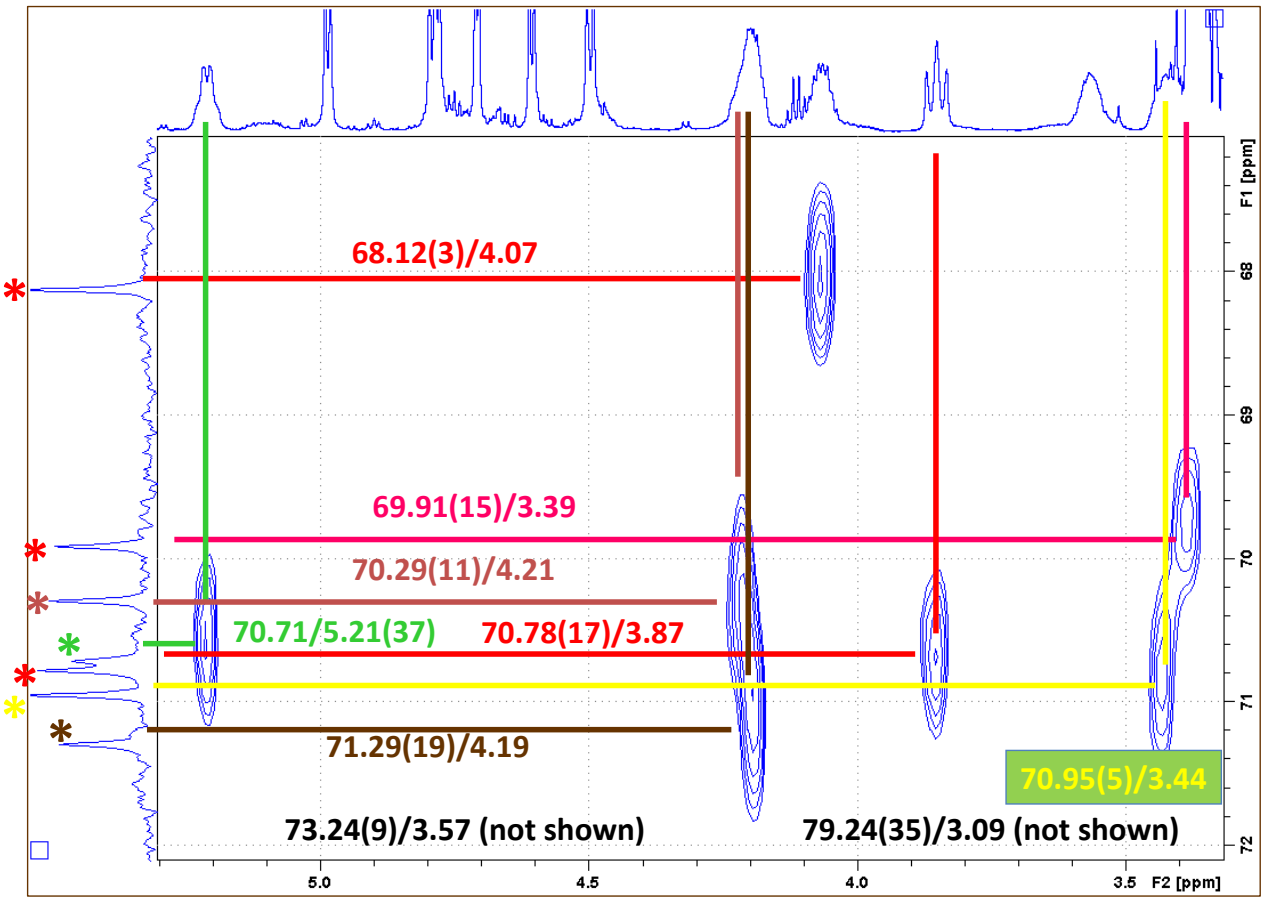


AmphNM+perDIDII aglycone (3) **Heterocorrelation NMR**

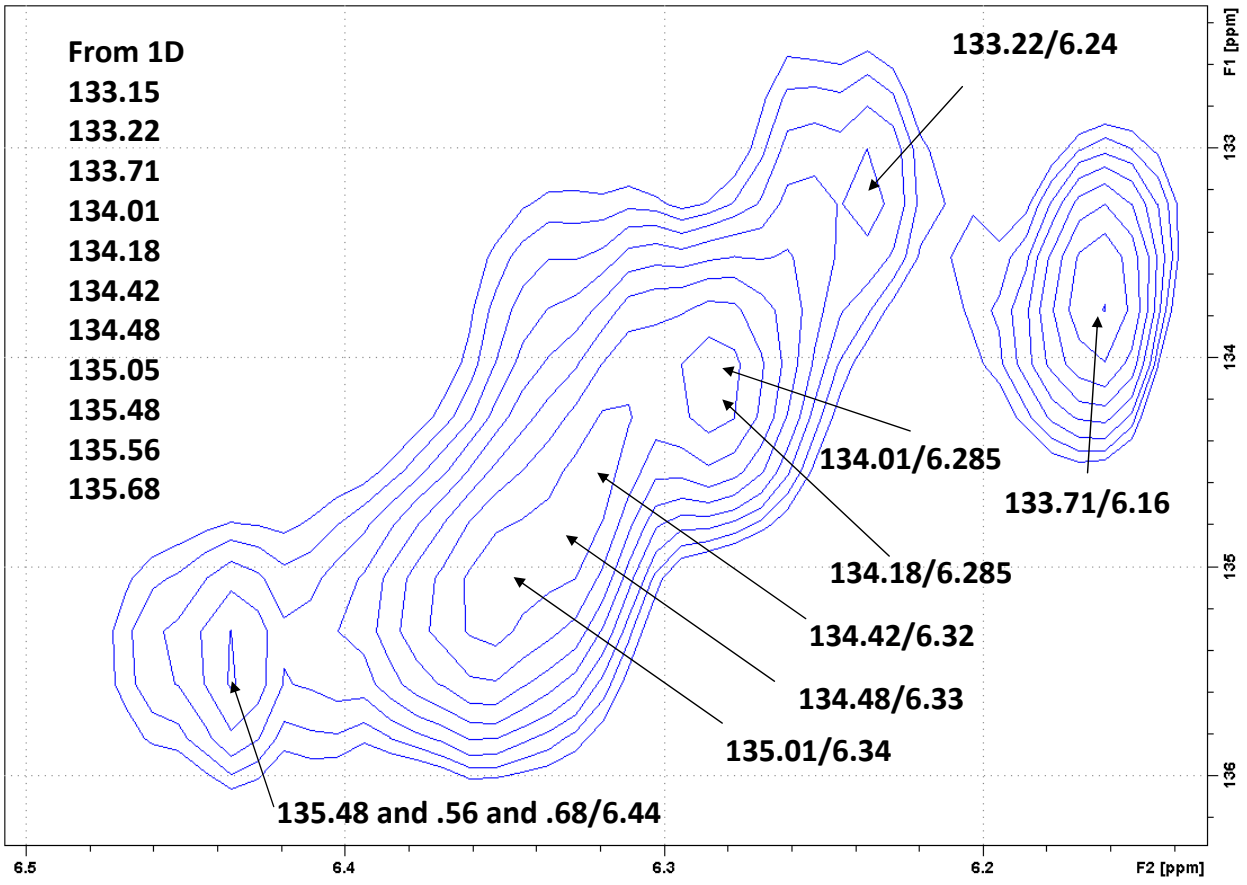


AmphNM+perDIDII aglycone (3)

Heterocorrelation NMR

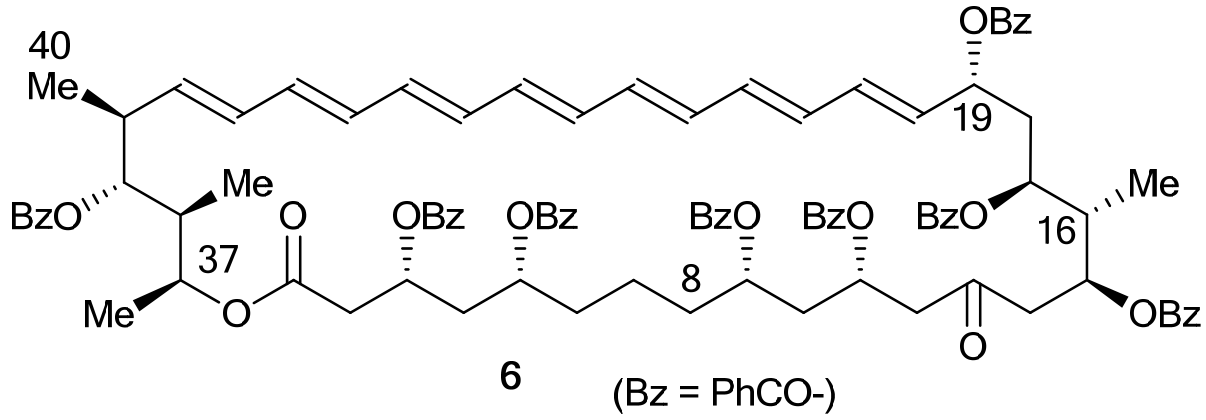


AmphNM+perDIDII aglycone (3)



Octabenzoyl-aglycone (10)

AGLYCONE OCTABENZOYL DERIVATIVE (10)

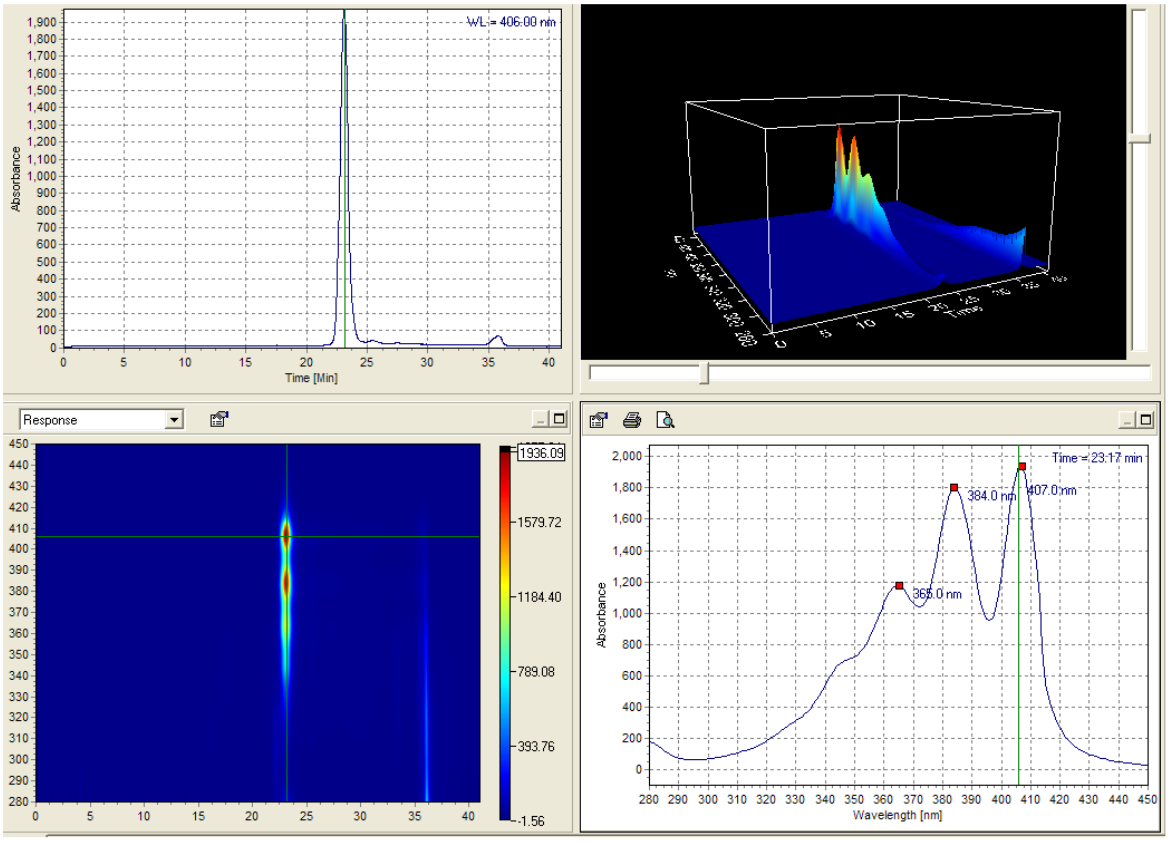


(3*R*,5*R*,9*S*,11*S*,15*R*,17*S*,19*S*,35*S*)-Octabenzoyloxy-13-oxo-(16*S*,34*S*,36*S*)-trimethyl-(20*E*,22*E*,24*E*,26*E*,28*E*,30*E*,32*E*)-octatriacontahepteno-(37*S*)-lactone

Octabenzoylaglycone 10

After purification (silica flash column)

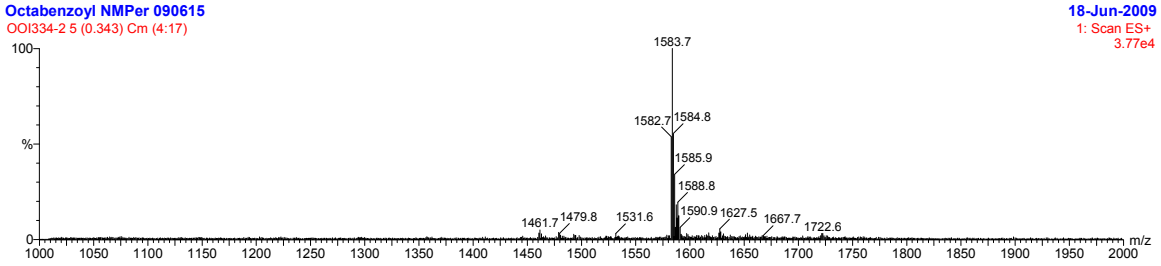
Silica HPLC, EtOAc/Hexane 0-2 min 15% EtOAc, 30 min 25% EtOAc in Hexane, 31 min 80%

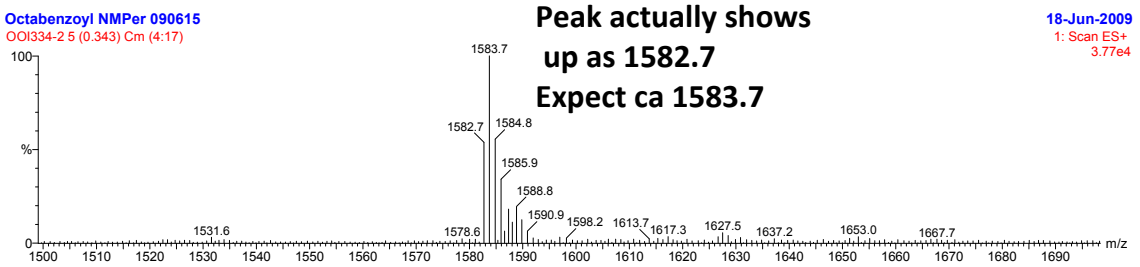


Octabenzoylaglycone (10)

$C_{97}H_{96}O_{19}$=1564; + water = 1582.

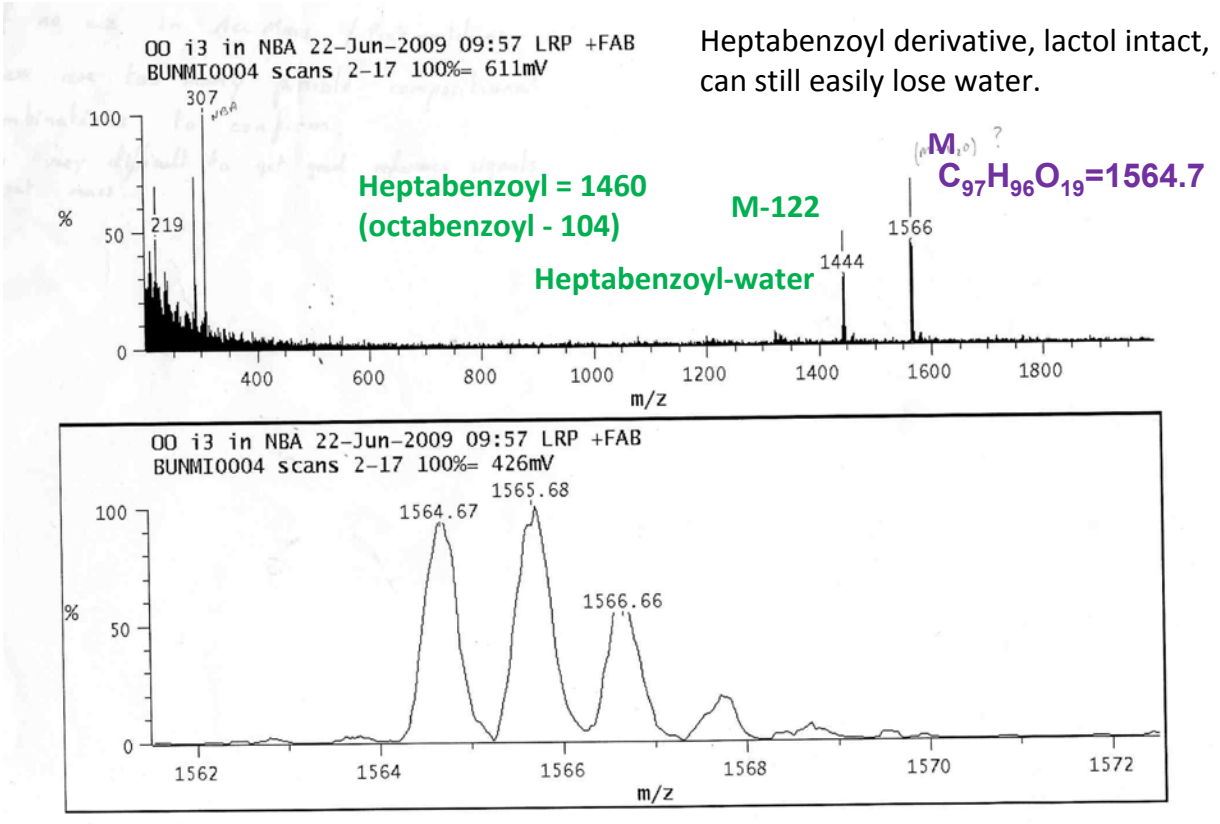
ESMS (+ve) = 1583.7 (M+water+H$^+$)





Peak actually shows up as 1582.7
Expect ca 1583.7

Octabenzoylaglycone (10) FAB-MS (This sample, mixed hepta and octabenzoyl)



Heptabenzoyl derivative, lactol intact, can still easily lose water.

Heptabenzoyl = 1460 (octabenzoyl - 104)

M-122

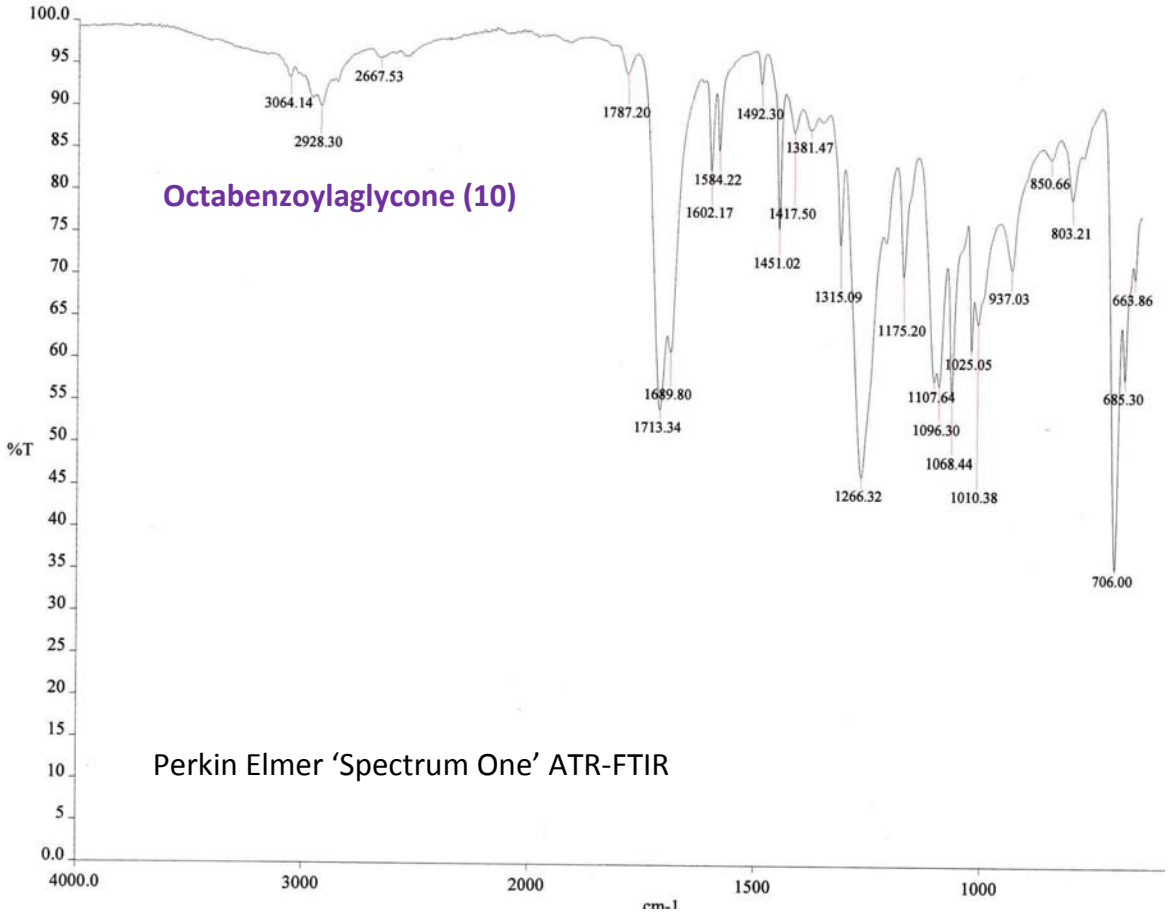
Heptabenzoyl-water

1444

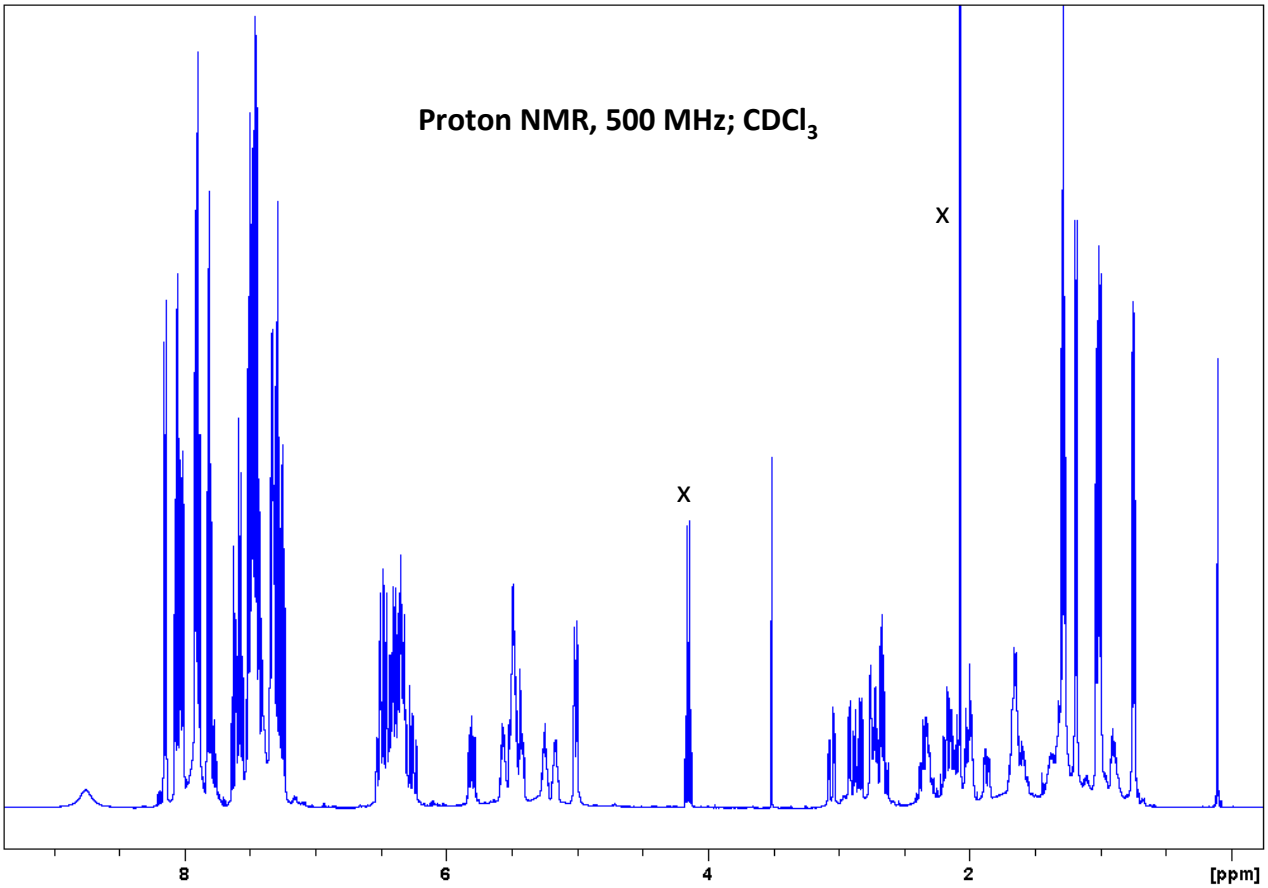
1566

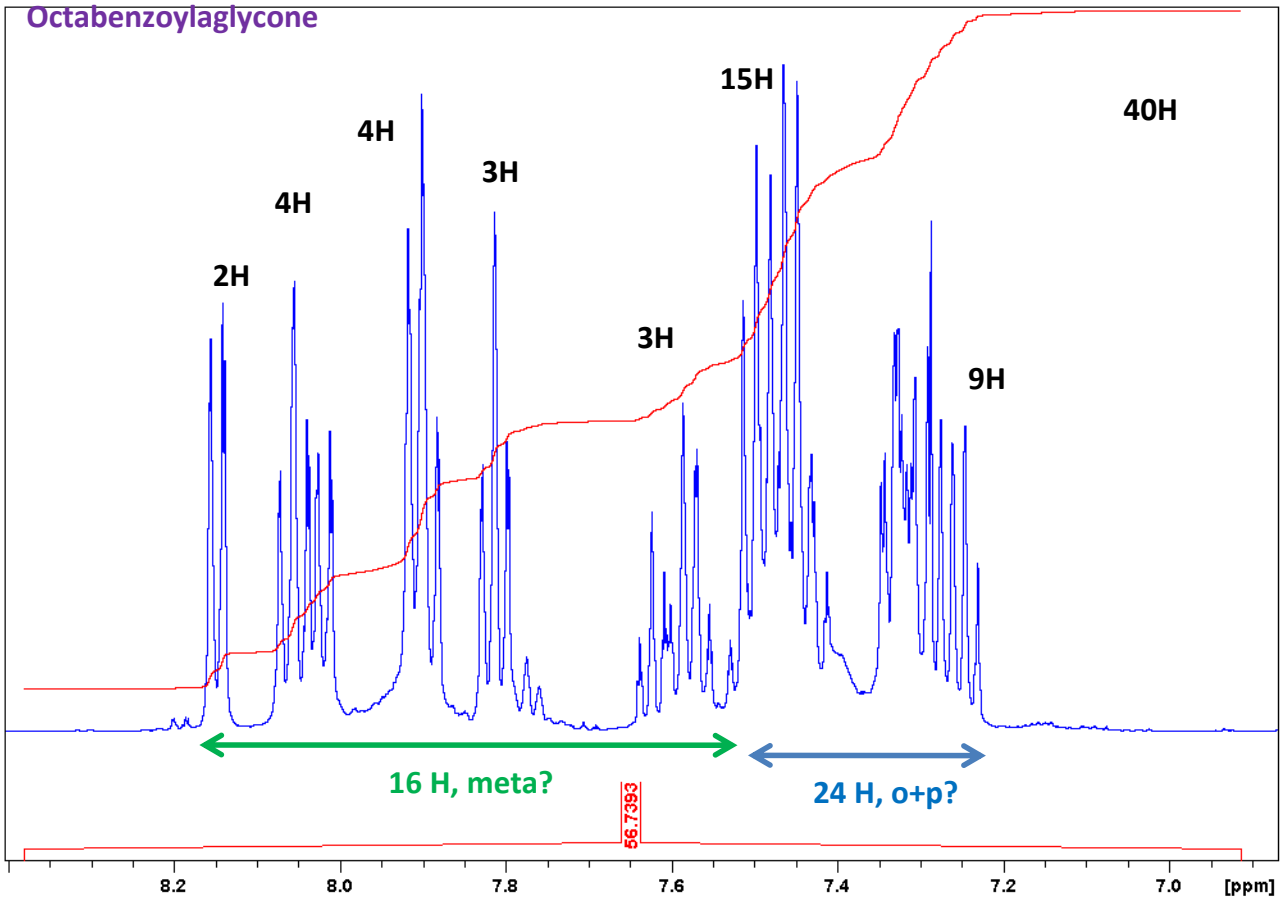
$M_{(+)}$?

$C_{97}H_{96}O_{19}$=1564.7

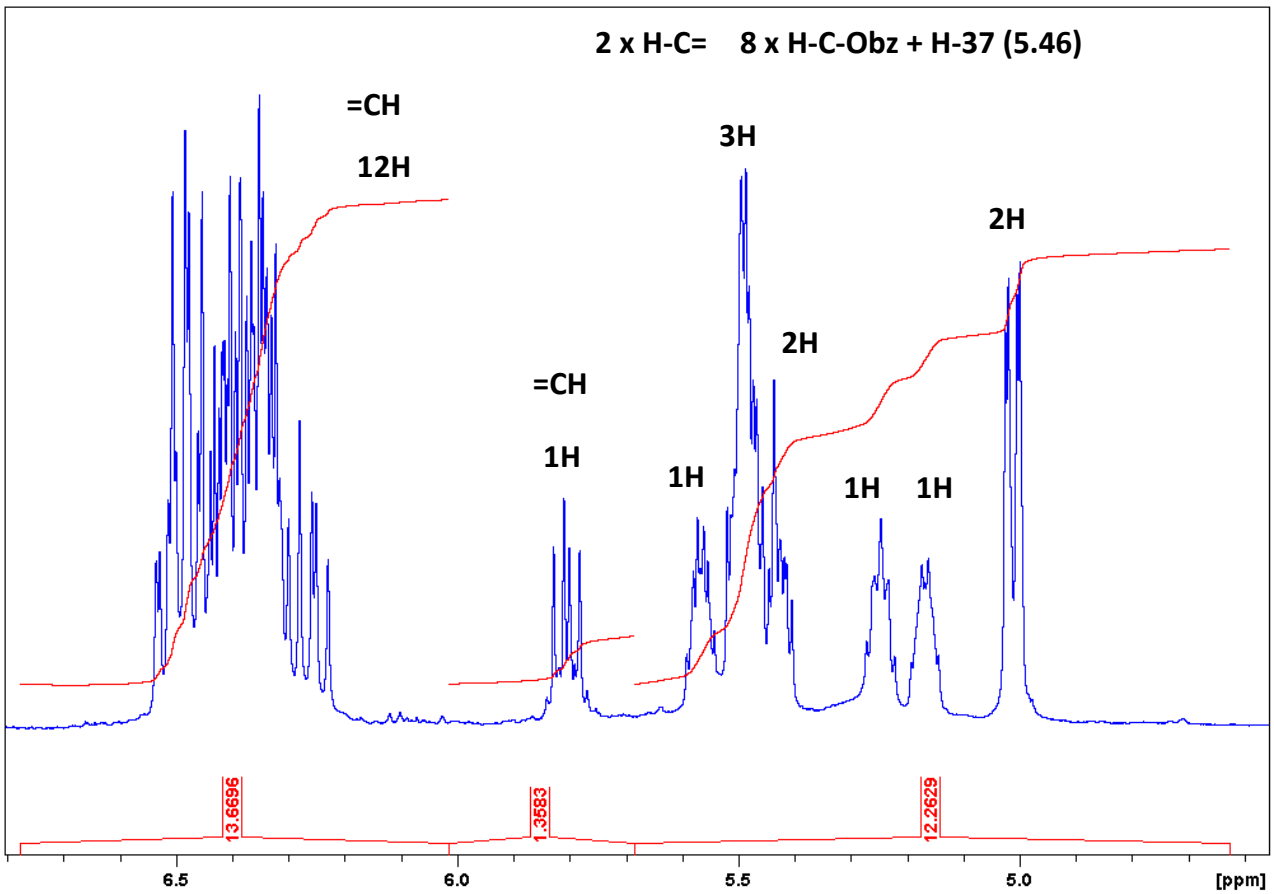


Octabenzoylaglycone (10)

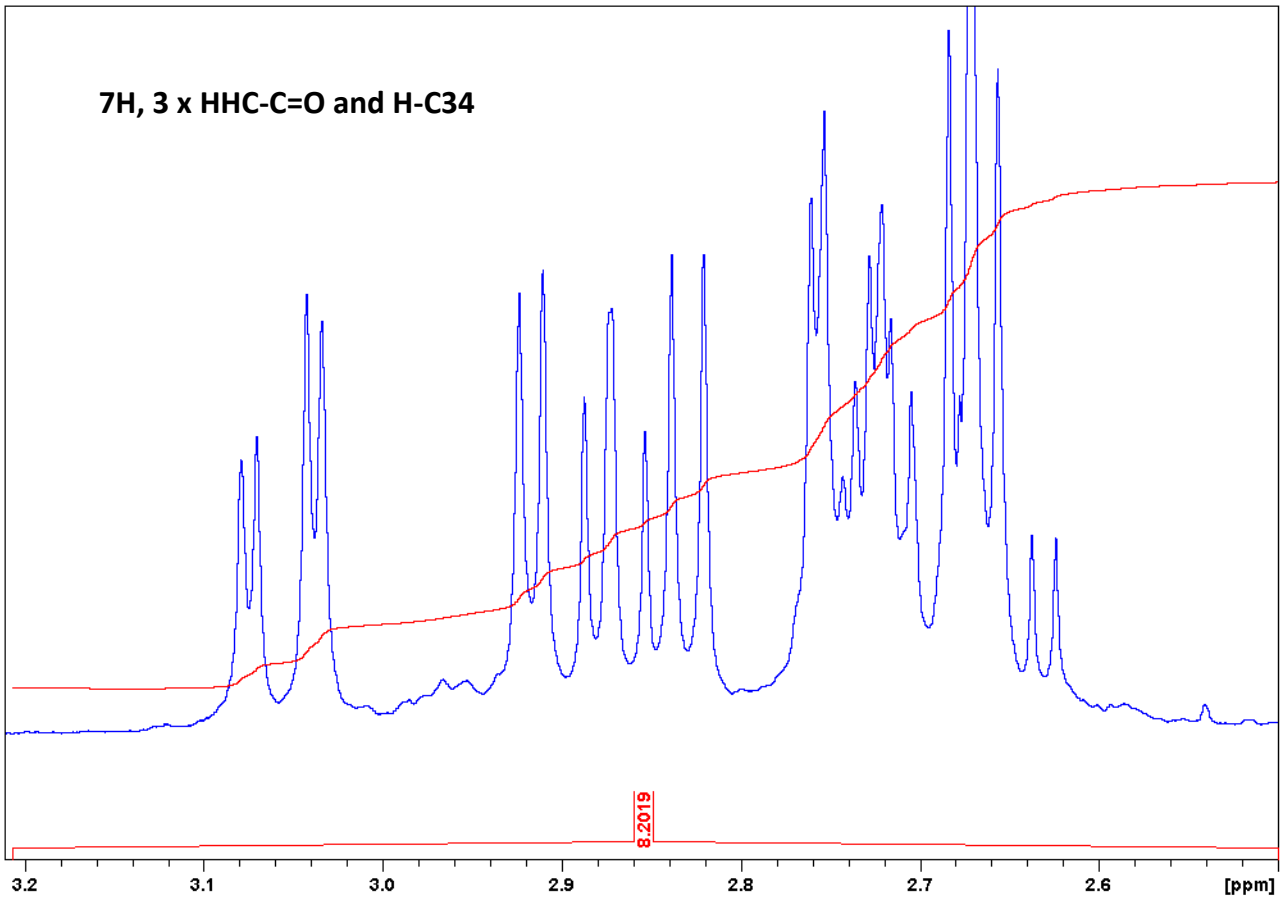




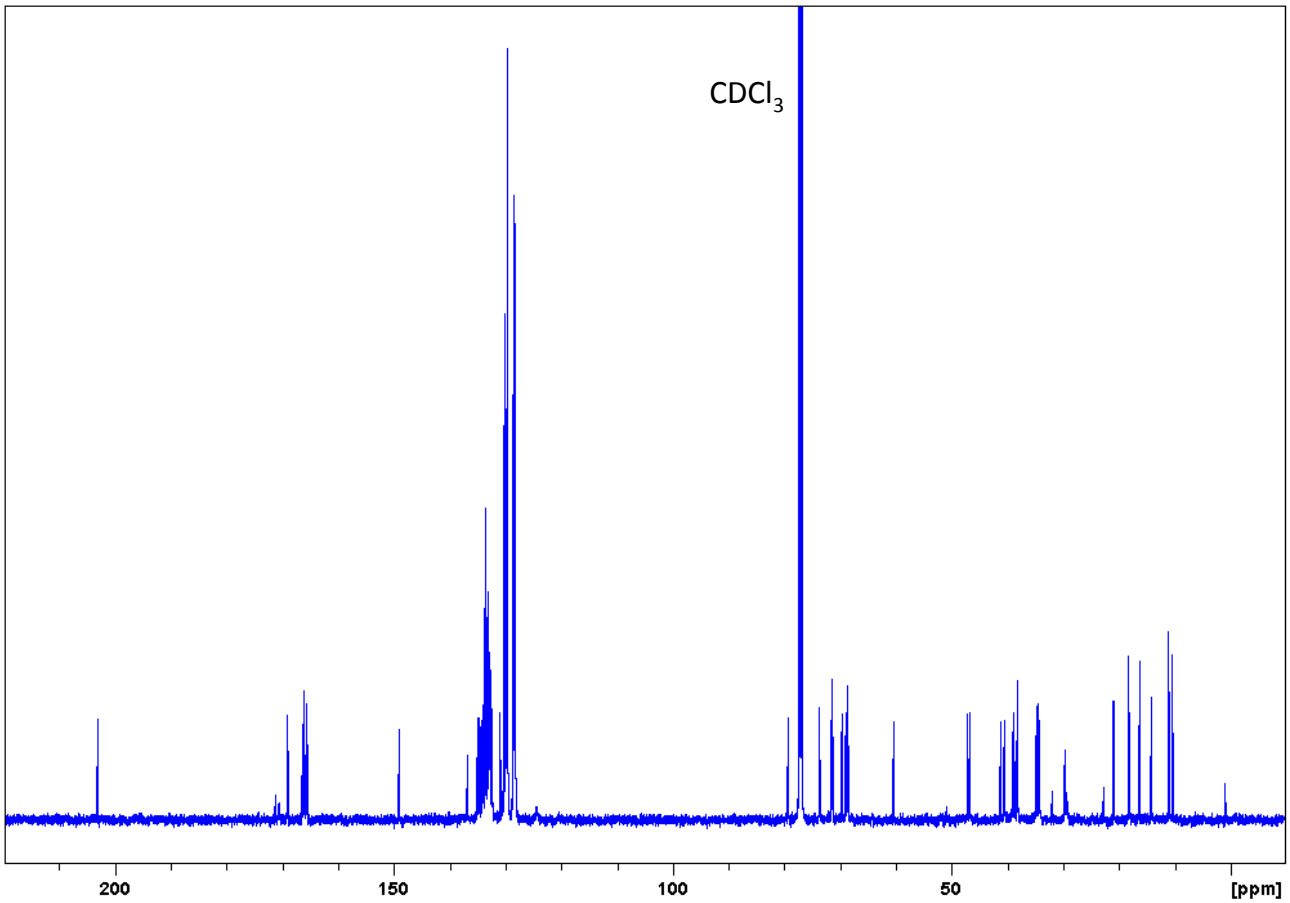
Octabenzoylaglycone (10)



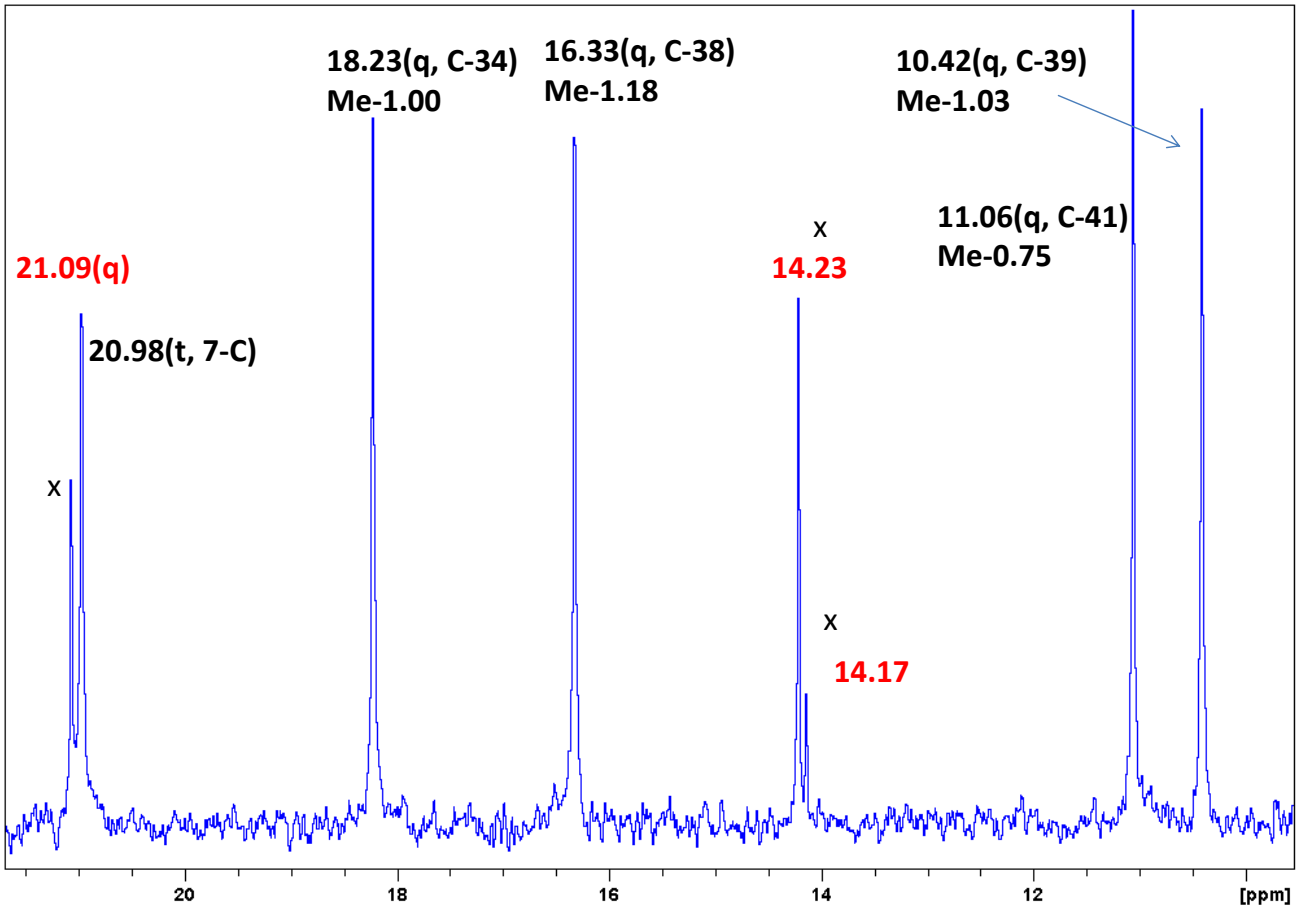
Octabenzoylaglycone



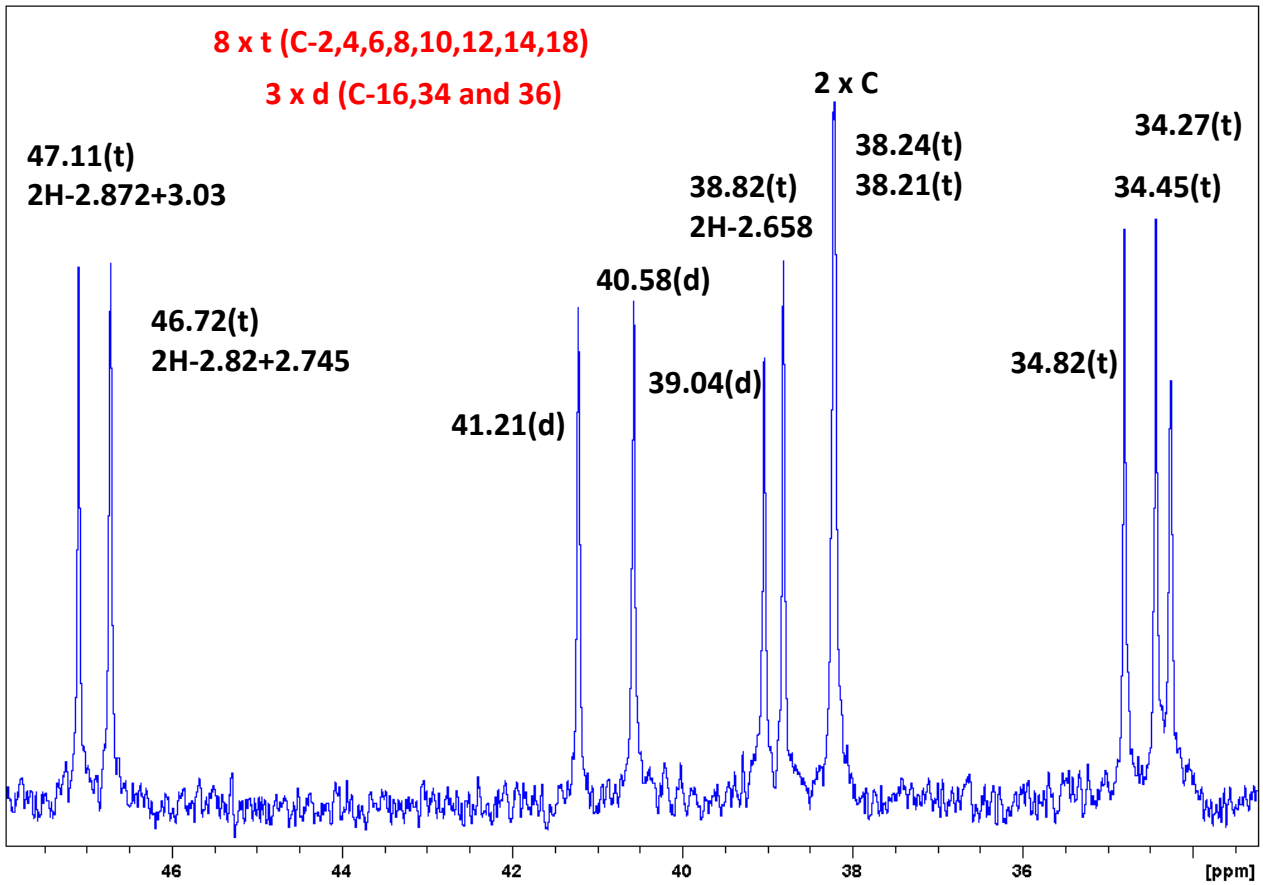
Octabenzoylaglycone (10) ^{13}C proton decoupled NMR (125 MHz)



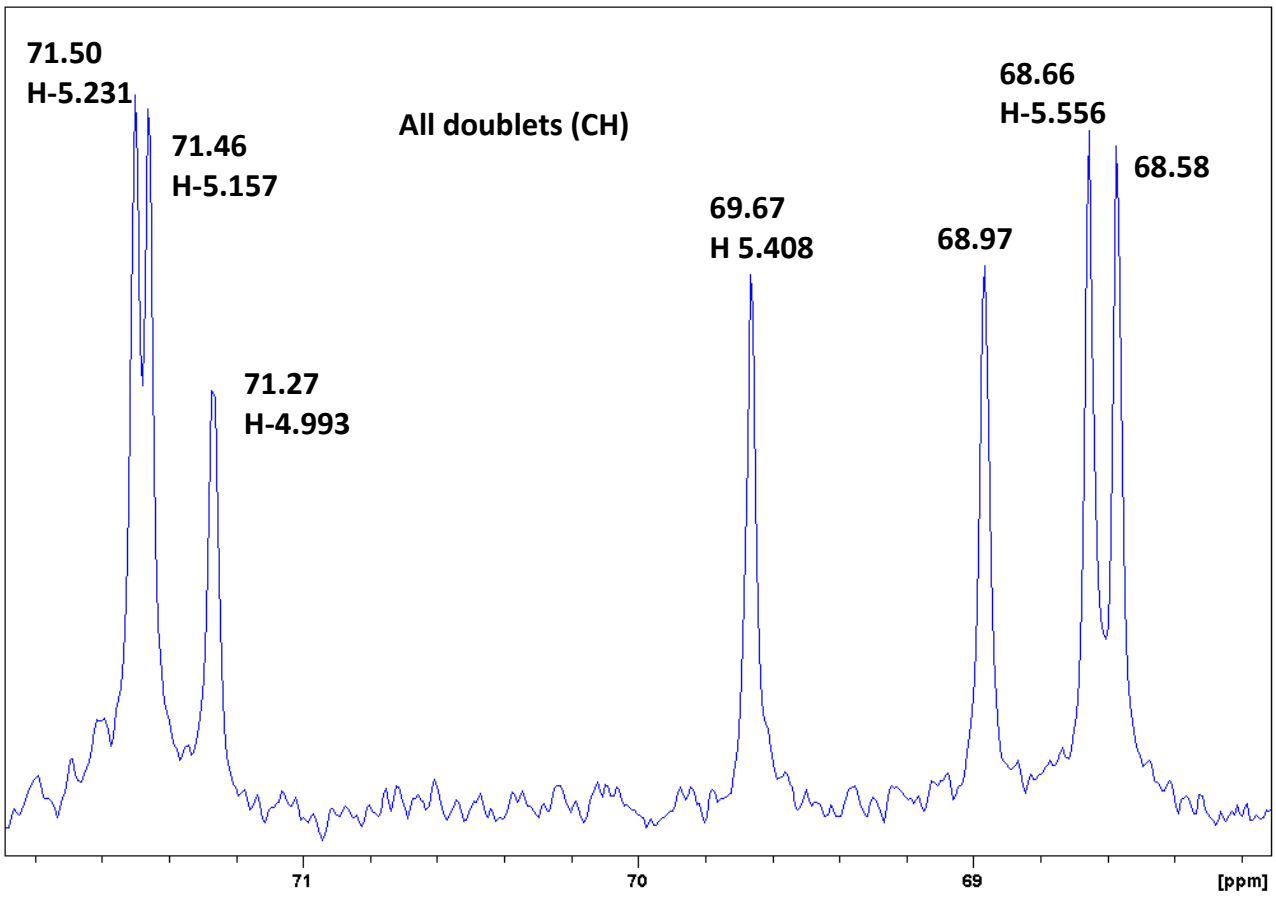
Octabenzoylaglycone (10) 4 x Me and 1 x non-polar $-CH_2-$ at 7-C



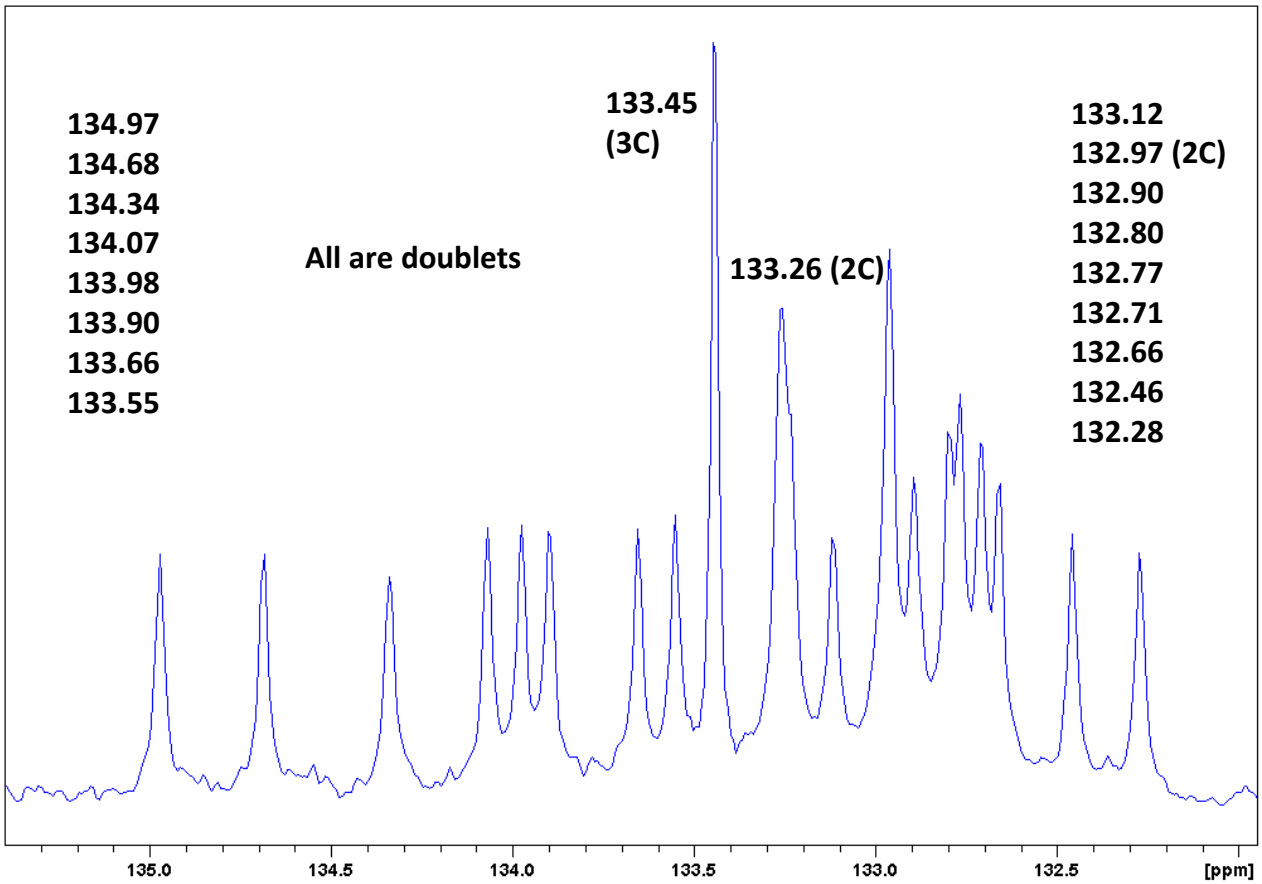
Octabenzoylaglycone (10) ^{13}C NMR



Octabenzoylaglycone **7 x H-C-O + 73.72 + 79.29 ppm: 8 x CH-benzoyl + 37-C.**

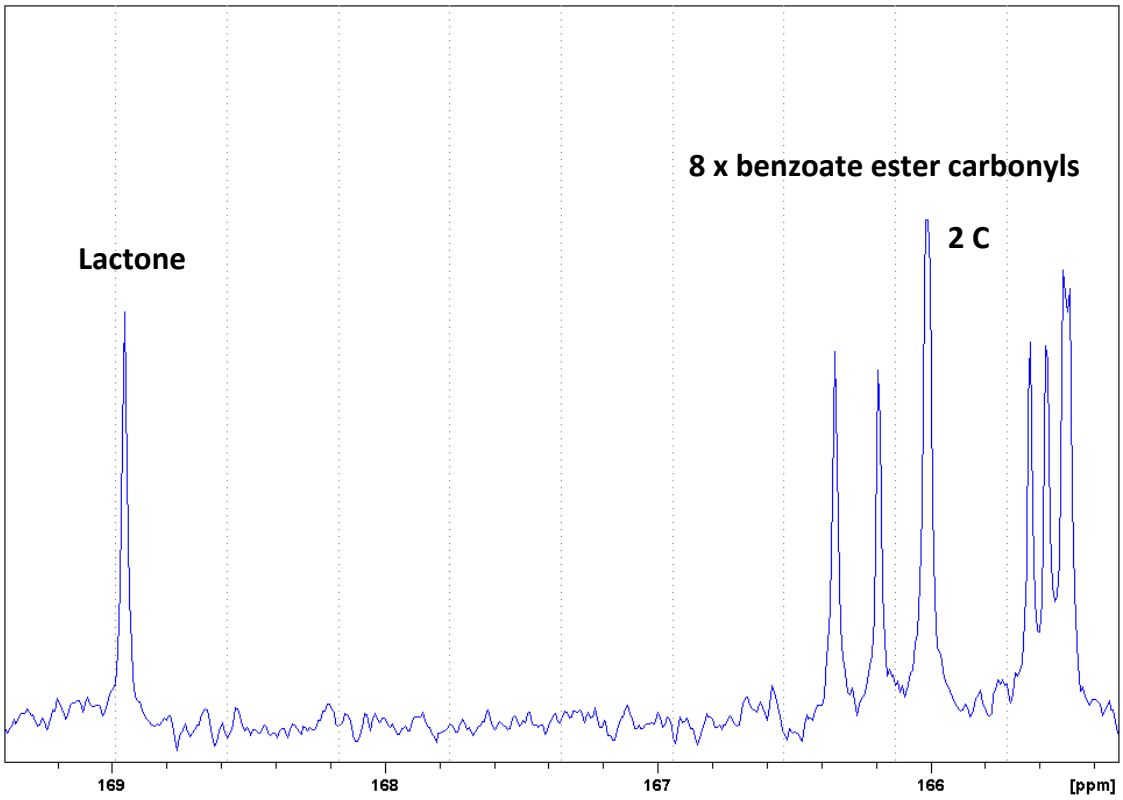


Octabenzoylaglycone (10) **14 x =C-H and 8 x Ar-H** **132-136 ppm**

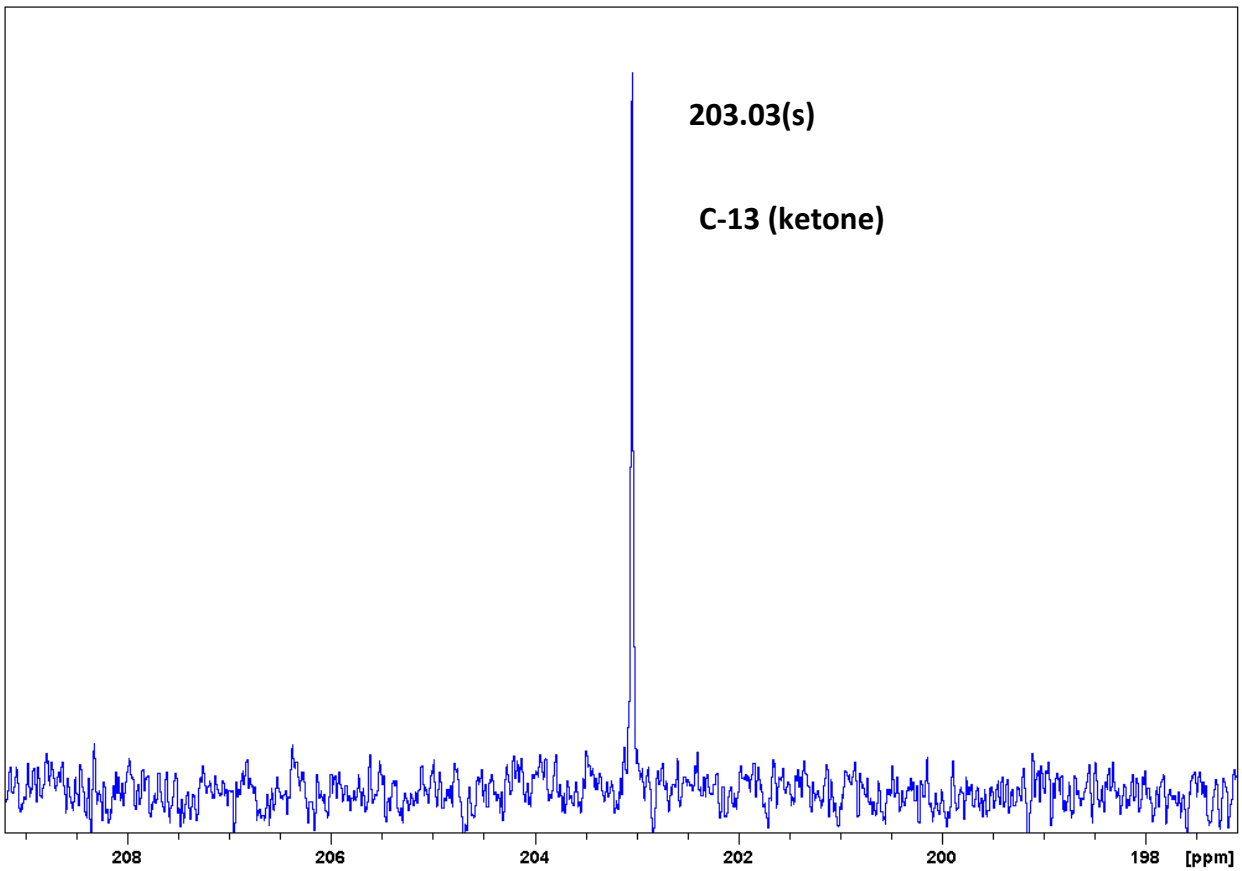


Octabenzoylaglycone (10)

^{13}C NMR



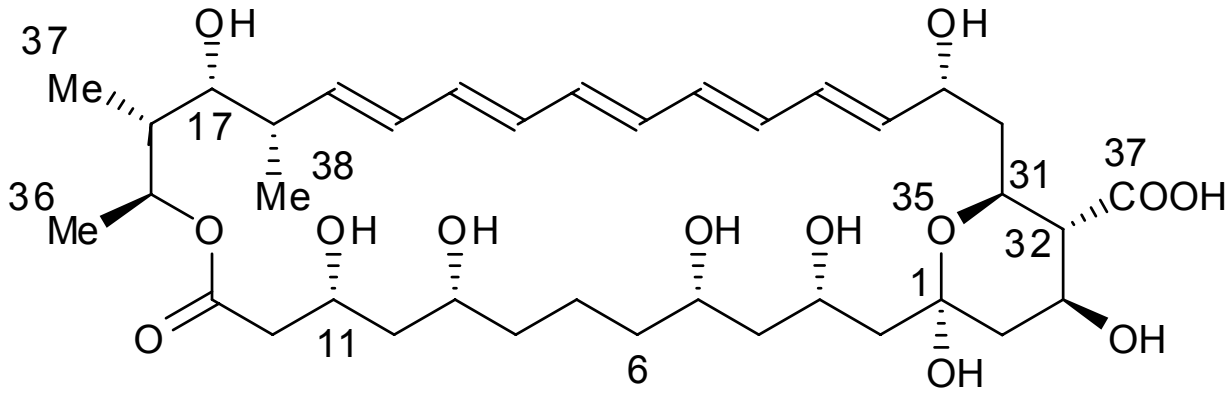
Octabenzoylaglycone



AmphC

PENTAENES 6-Deoxy, aglycone, [O] at 37-C

(Stereochemistry assumed from amphotericin B)



11 (methyl ester = 12)

(1*R*,3*S*,5*S*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,21*E*,23*E*,25*E*,27*E*,29*R*,31*S*,32*R*,33*S*)-1,3,5,9,11,17,29,33-Octahydroxy-15,16,18-trimethyl-13-oxo-14,35-dioxabicyclo[29.3.1]pentatriaconta-19,21,23,25,27-pentaene-32-carboxylic acid

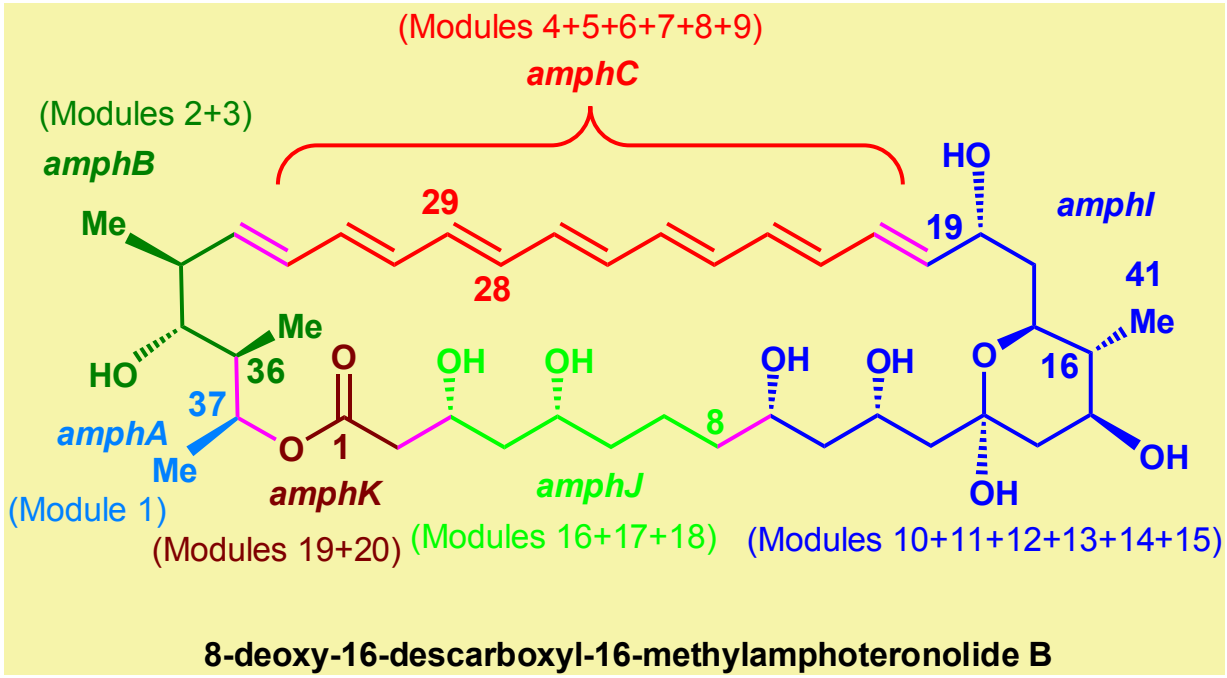
6-Deoxypentaene-aglycone 11 = $C_{37}H_{58}O_{13}$ = 710

AmphC

16 x acetate + 3 x propionate

PKS genes in *S. nodosus*

PKS (*amphABCIJK,* 19 modules

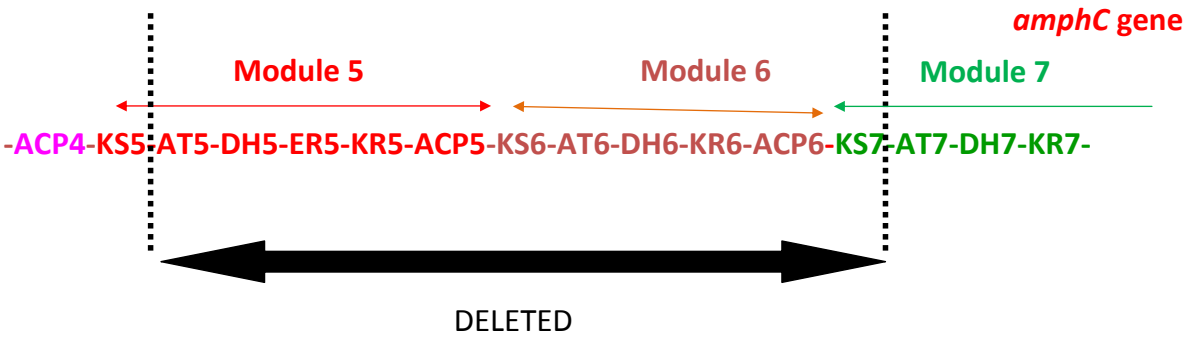


8-deoxy-16-descarboxyl-16-methylamphoteronolide B

AmphC

Region deleted within 'amphC'

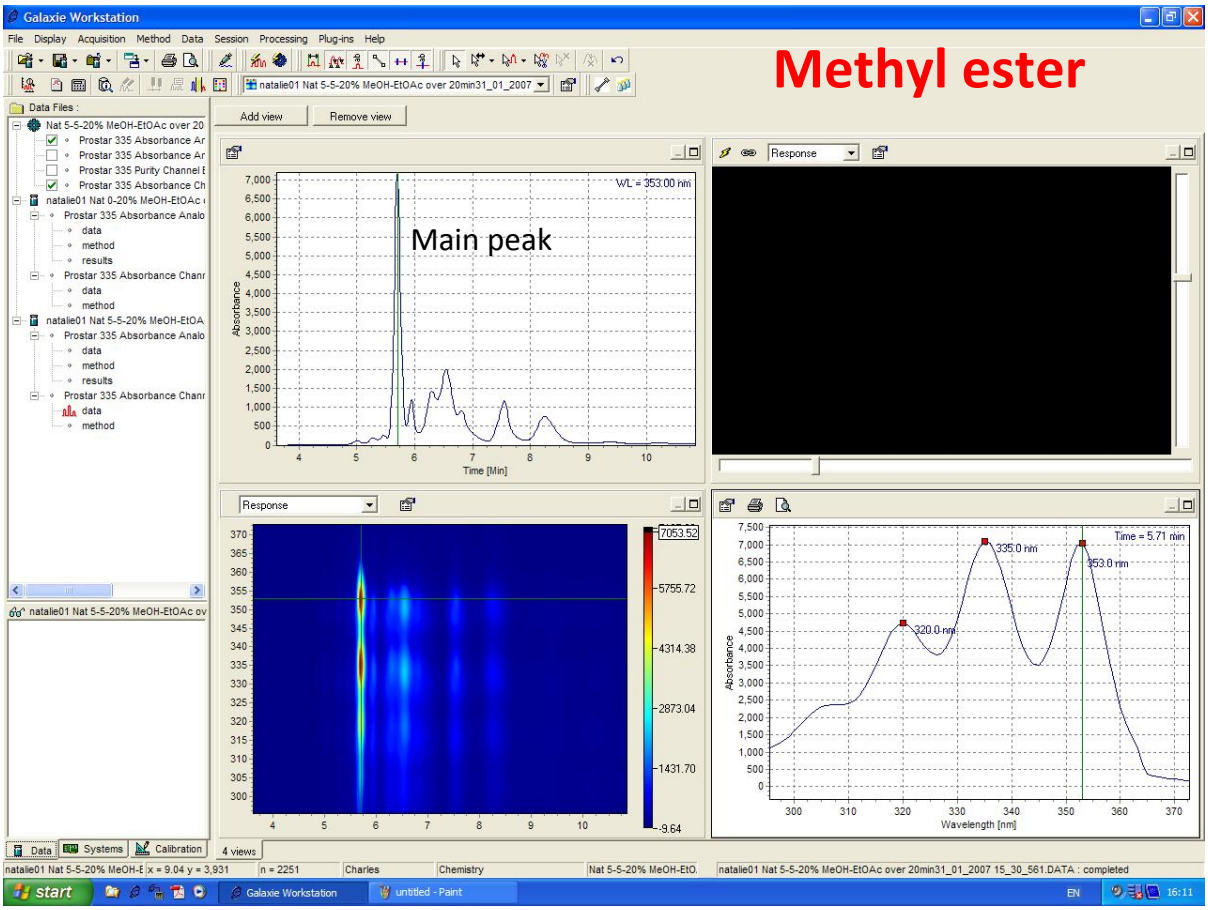
Module 4



-ACP4-KS5-AT7-DH7-KR7-ACP7-

***i.e.* missing domains associated with two whole modules**

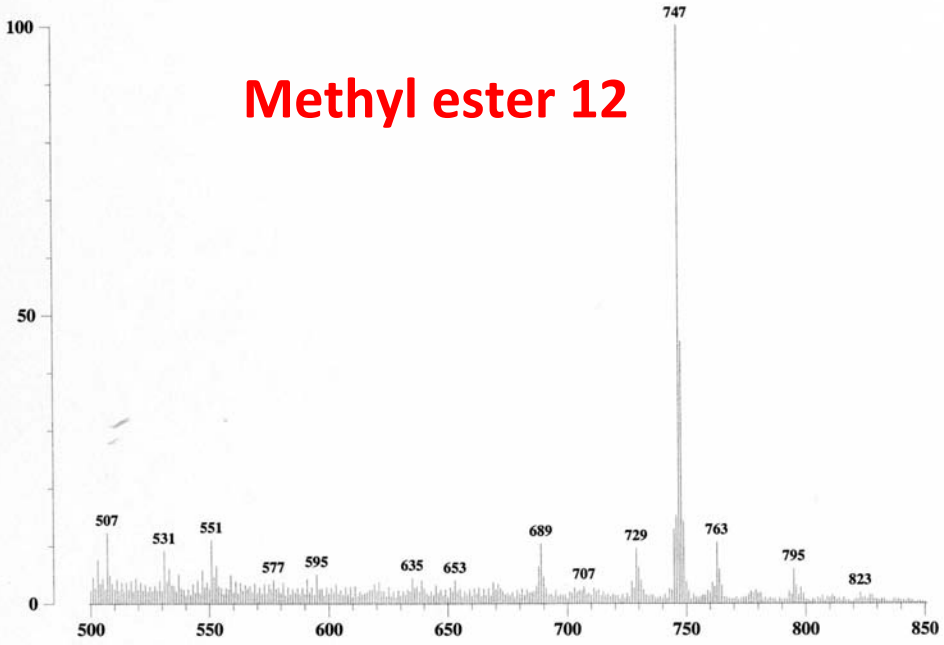
AmphC **Normal phase HPLC on methyl ester extract (MeOH/EtOAc)**



AmphC

FAB MS of methyl ester of 6-deoxyaglycone (12)(pentaene , Me ester) = 747

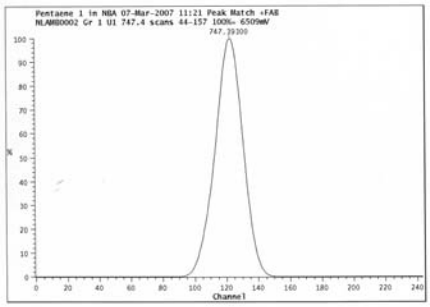
NLAMB0001 Scan 2 (Av 7-20 Acq) 100%=18650 mv 07-Mar-2007 10:44
LRP +FAB Pentaene 1 in NBA



Methyl ester 12

AmphC

High Res FAB MS of methyl ester of 6-deoxyaglycone (pentaene 1, Me ester) $C_{38}H_{60}O_{13}Na$



Run : NLAMB0002 Scan : 1

	Mass	Int	Dev ppm	12 C	1 H	16 O	23 Na
Actual	747.39300	7957179	-0.2	38	60	13	1

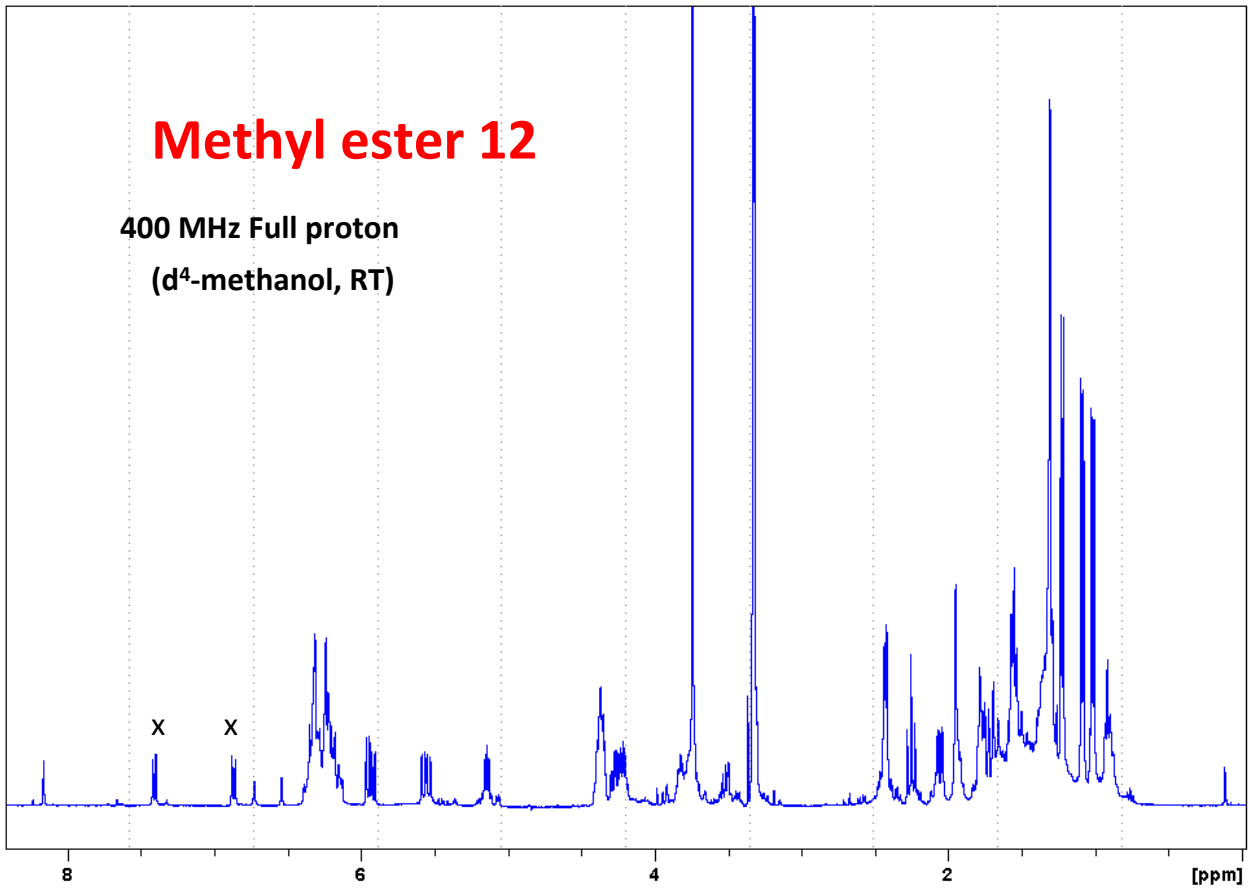
Methyl ester 8

Found: 747.39300 $C_{38}H_{60}O_{13}Na$ requires 747.39319

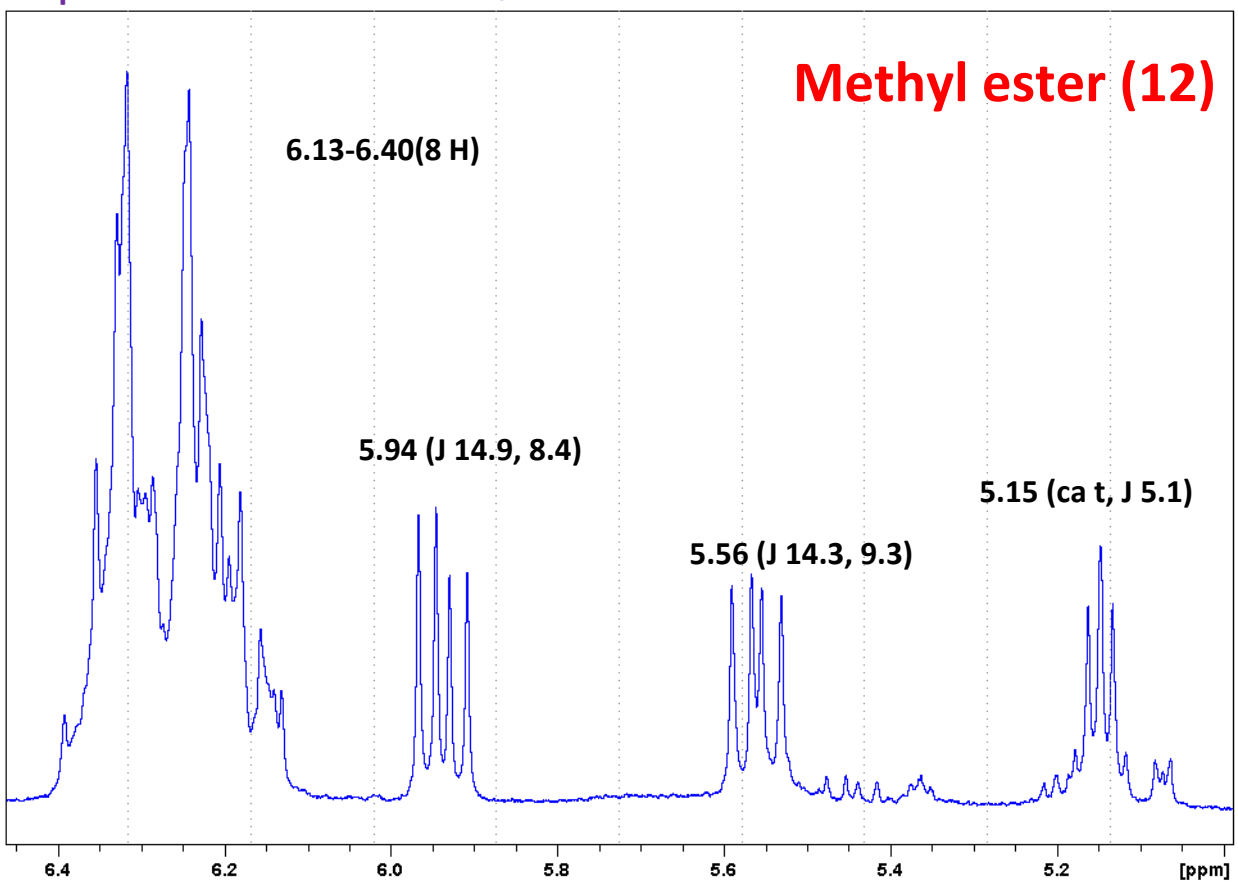
Calculated values for high resolution mass

Mass	Abundance	12 C	13 C	1 H	16 O	23 Na
747.39319	66.0020	38	0	60	13	1
748.39655	28.1521	37	1	60	13	1
749.39990	5.8459	36	2	60	13	1

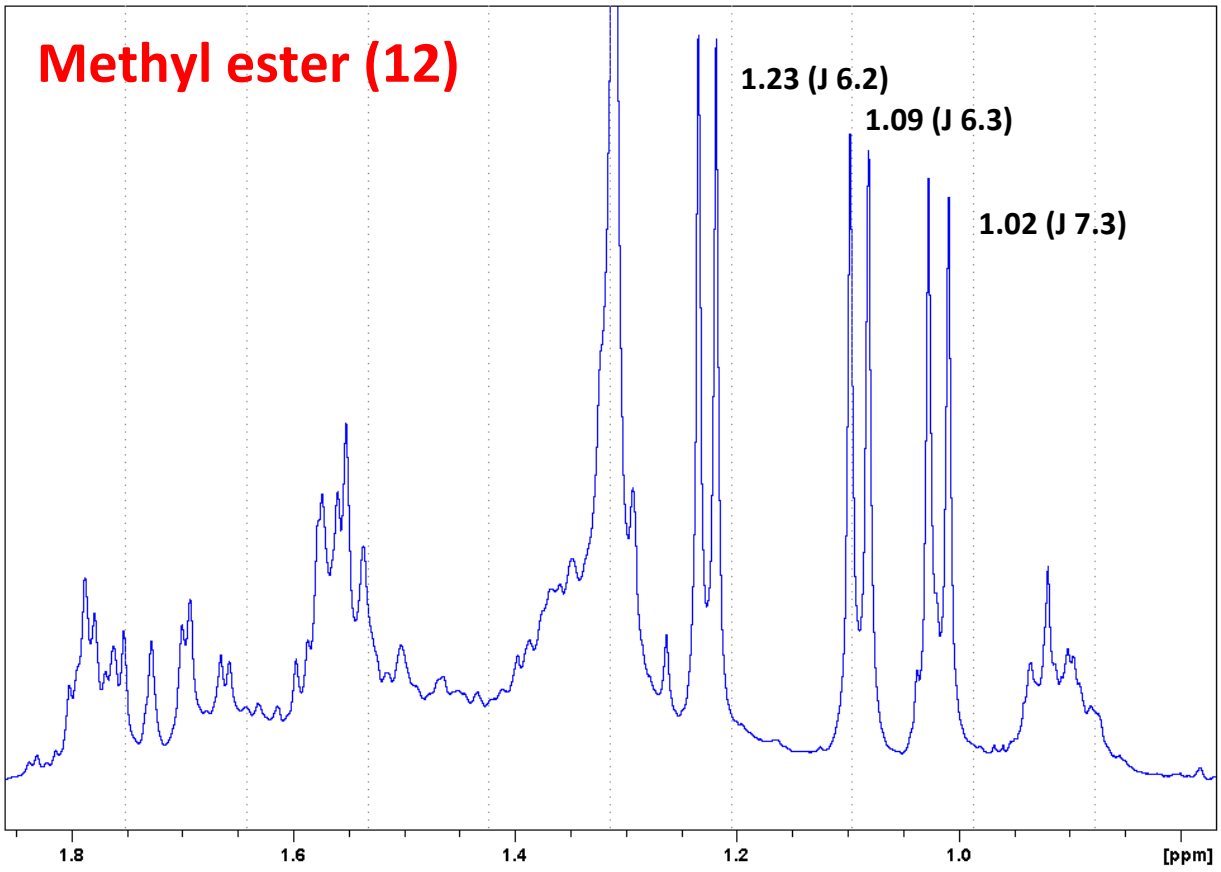
AmphC **Proton NMR of main Pentaene as methyl ester (12)**



AmphC **NMR of Pentaene methyl ester**

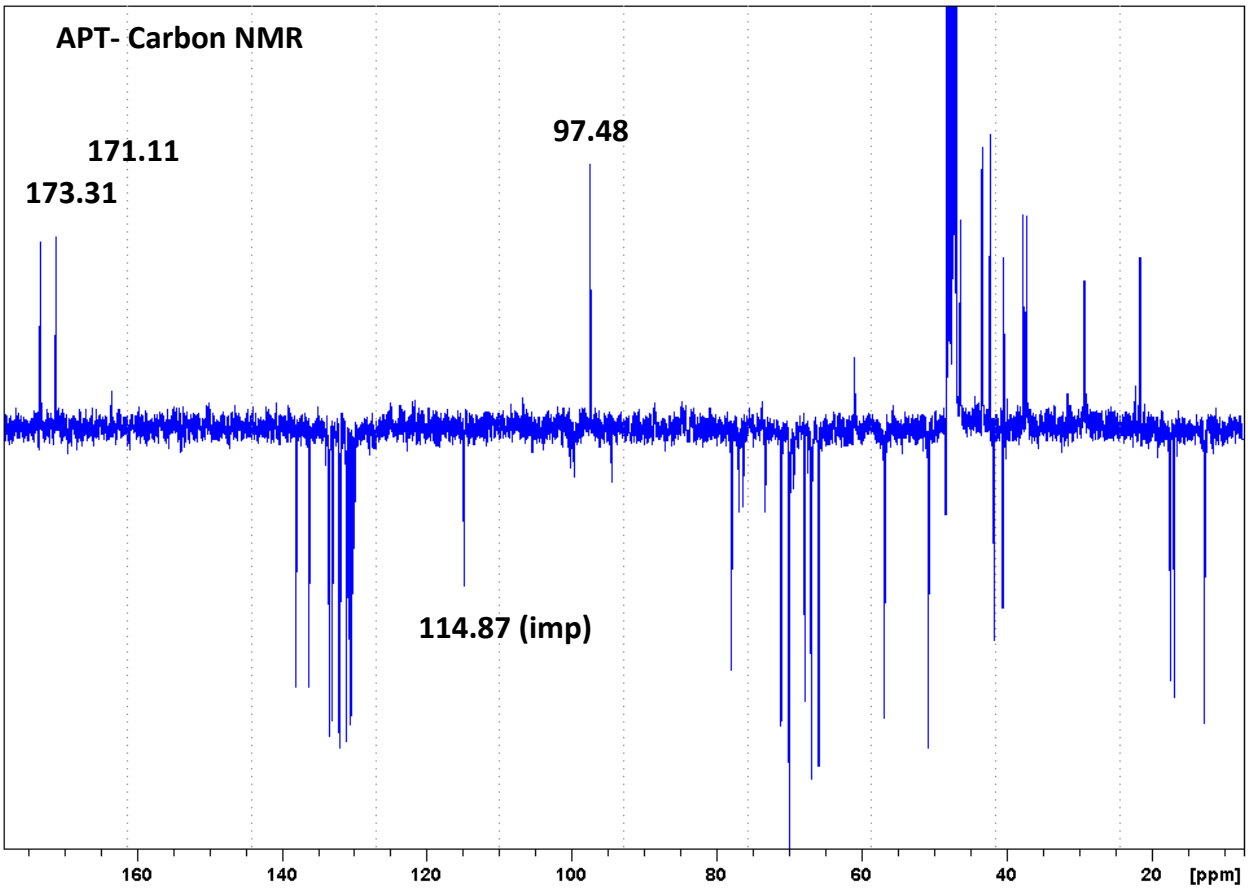


AmphC: NMR of methyl ester of 'purified' pentaene



AmphC

Methyl ester (12)



AmphC

ANALYSIS OF NATIVE PENTAENE EXTRACTS

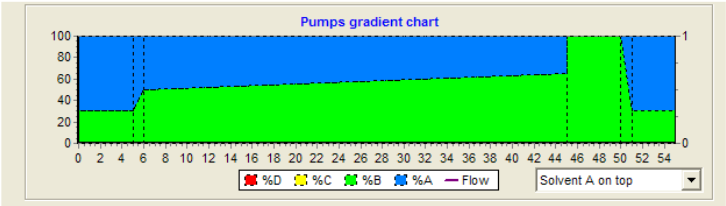
RP-HPLC on native amphC extracts (no methylation)

Solvents: A is water, B is MeOH (+ 0.1% HCOOH)

Analytical HPLC flow rate 1 mL per min ,

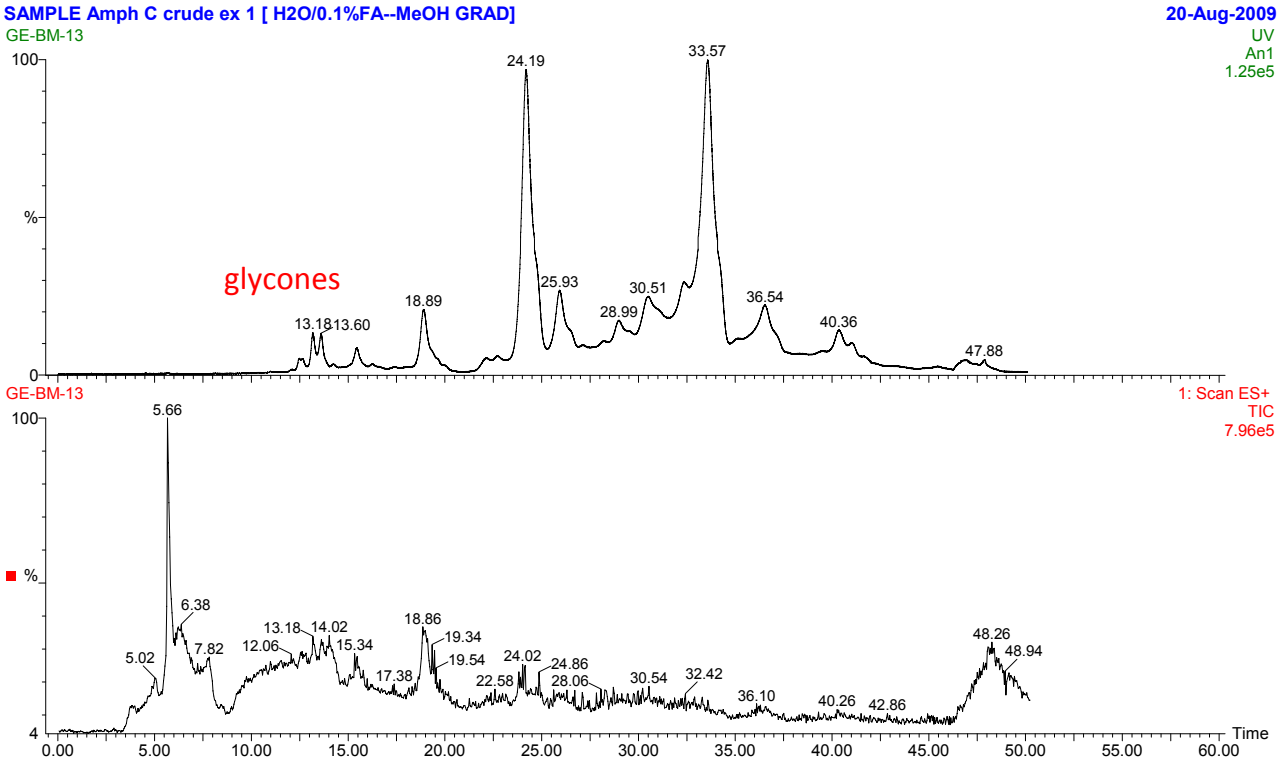
LCMS, same but 0.5 mL per minute, but twice the time scale to compensate.

Prerun	1.000 ml/min	A=70.00% B=30.00%
5.0 min	1.000 ml/min	A=70.00% B=30.00%
6.0 min	1.000 ml/min	A=50.00% B=50.00%
45.0 min	1.000 ml/min	A=30.00% B=70.00%
45.0 min	1.000 ml/min	A=0.00% B=100.00%
50.0 min	1.000 ml/min	A=0.00% B=100.00%
51.0 min	1.000 ml/min	A=70.00% B=30.00%
55.0 min	1.000 ml/min	A=70.00% B=30.00%



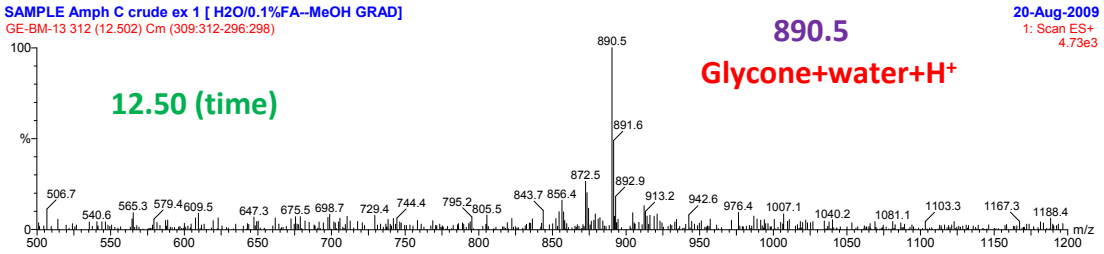
AmphC

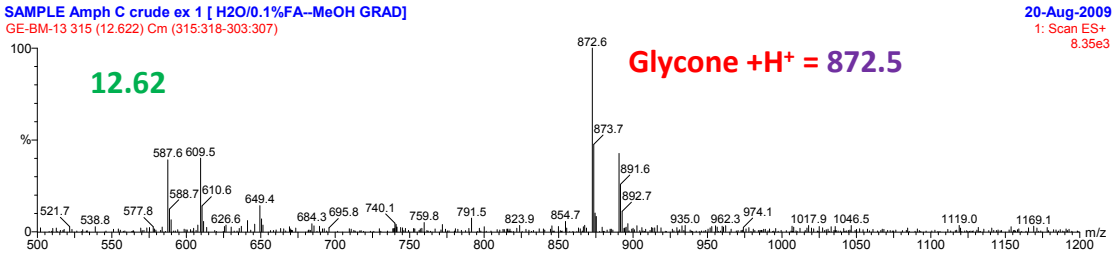
LCMS (+ve) on methanolic extract of mycelia + resin from *amph C*

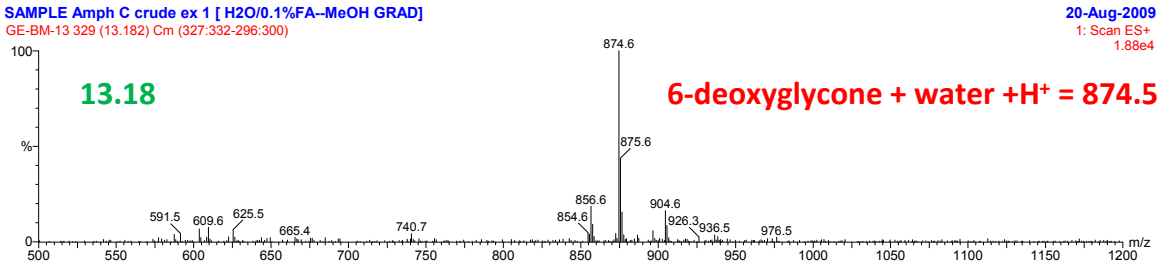


AmphC

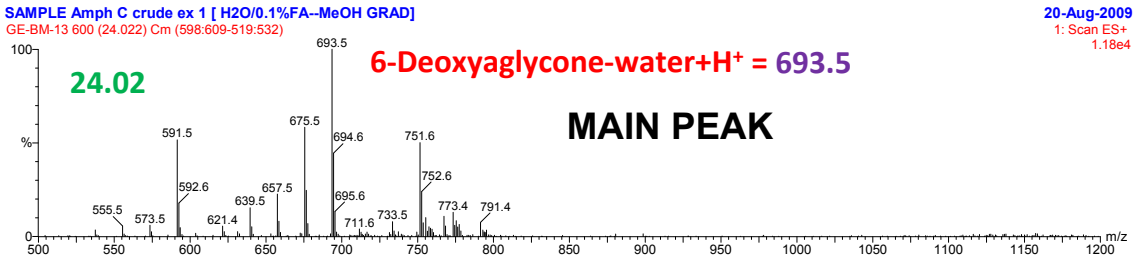
Some glycones (and 6-OH) in early fractions

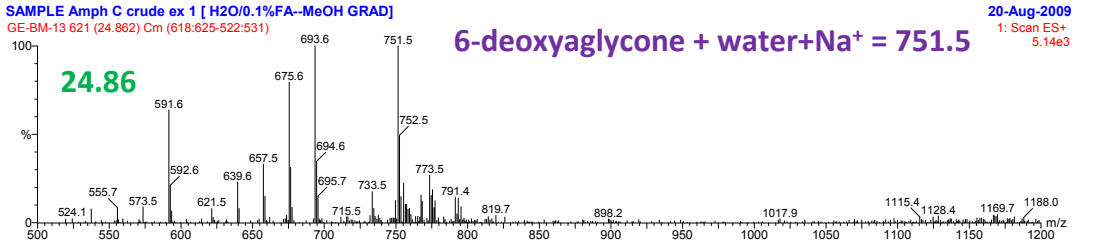


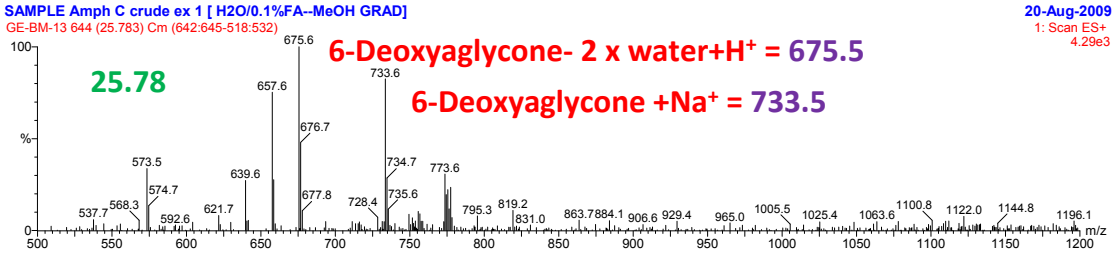


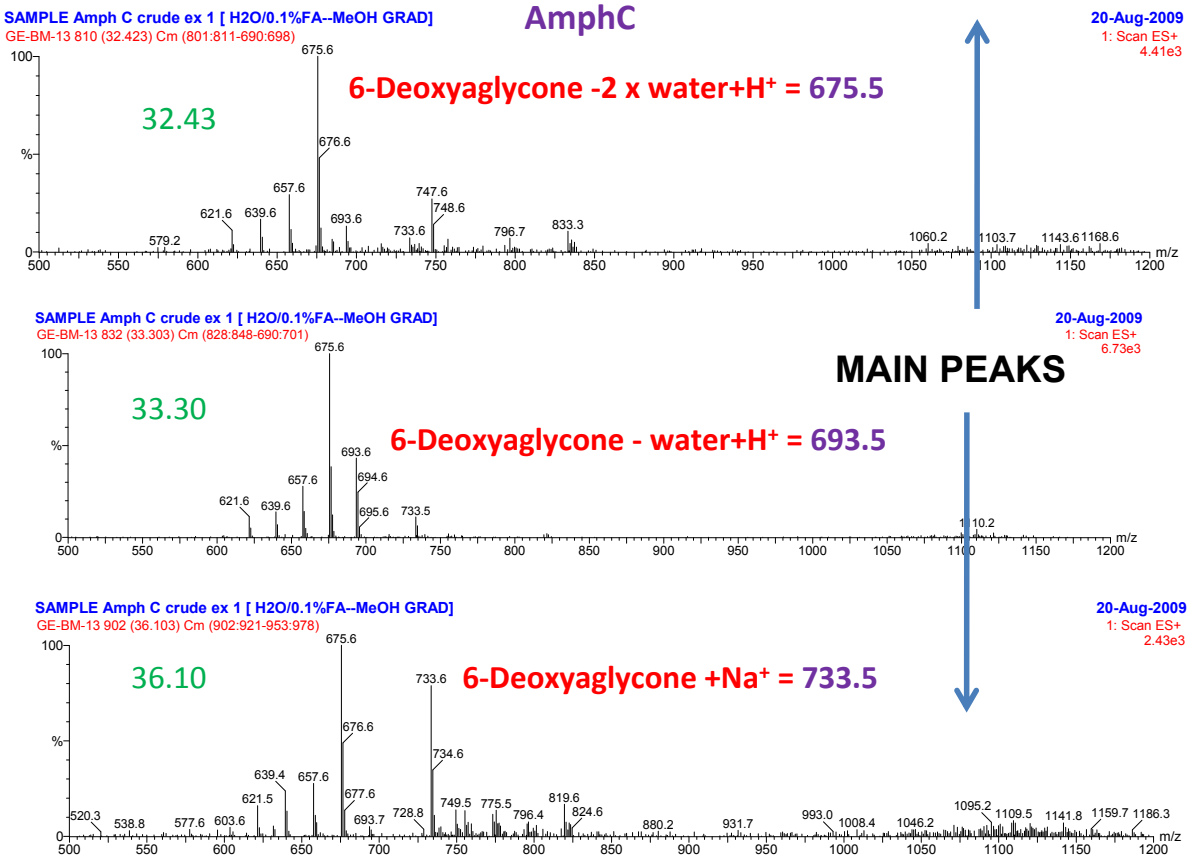


AmphC

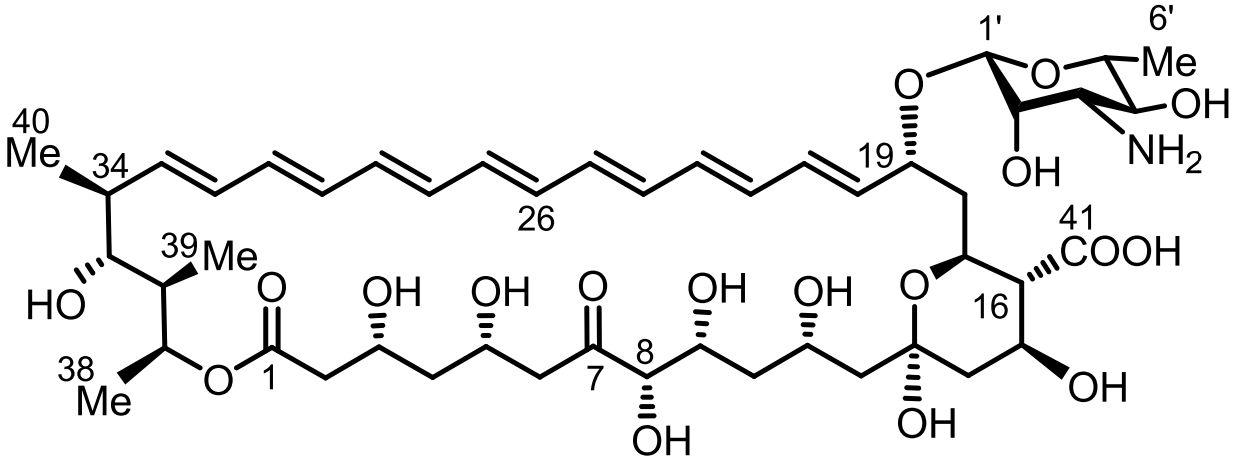








KR16: 7-Oxo-amphotericin B (9)



7-Oxo-amphotericin B (9)

$C_{47}H_{70}NO_{18}$ = 937

RMM 937.5

KR16: 7-Oxo-amphotericin B (9)

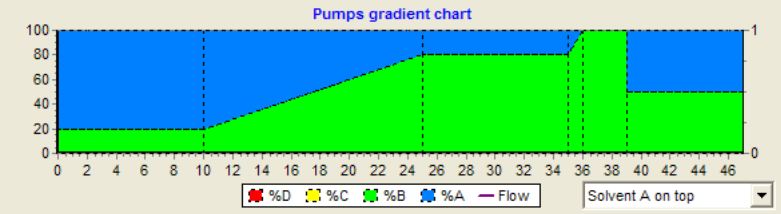
Extraction of 28 x 2 L trigrooved flasks with 250 mL culture (example)

Cells and beads from all uncontaminated flasks are centrifuged in GSA tubes for 10 minutes at 10,000 rpm
In a Sorvall RC5B centrifuge. Spent media is decanted and all solid material is collected in a beaker. Methanolic extractions are carried out on the combined mycelia and bead pellets. All solid material is broken up and dispersed evenly using a large holed ladle or spoon. Approximately 10 L of methanol is added to the dispersed pellet and stored in 5 L conical flasks. Extractions are carried out for a period of 1h to overnight with intermittent swirling to ensure mixing of solid and liquid phases. After the appropriate extraction time the contents of the flasks are allowed to settle. Once settled the methanol fraction can carefully be decanted off into a new vessel. If desired, more methanol can be added to the mycelia and beads and a second extraction can be performed as before. It is advisable to carry out at least two extractions with UV assays being carried out to access if further extractions are necessary. Extract 1 contains approx 400mg Heptaene and 500mg Tetraene, Ext 2 300mg H and 250mg T, Ext 3 150mg H 100mg T.

KR16: 7-Oxo-amphotericin B (9)

HPLC purification of 7-oxo-amphotericin B

C18 Supelco RP columns 5 micron partical,
A – Water B – Methanol + 0.1% v/v formic acid

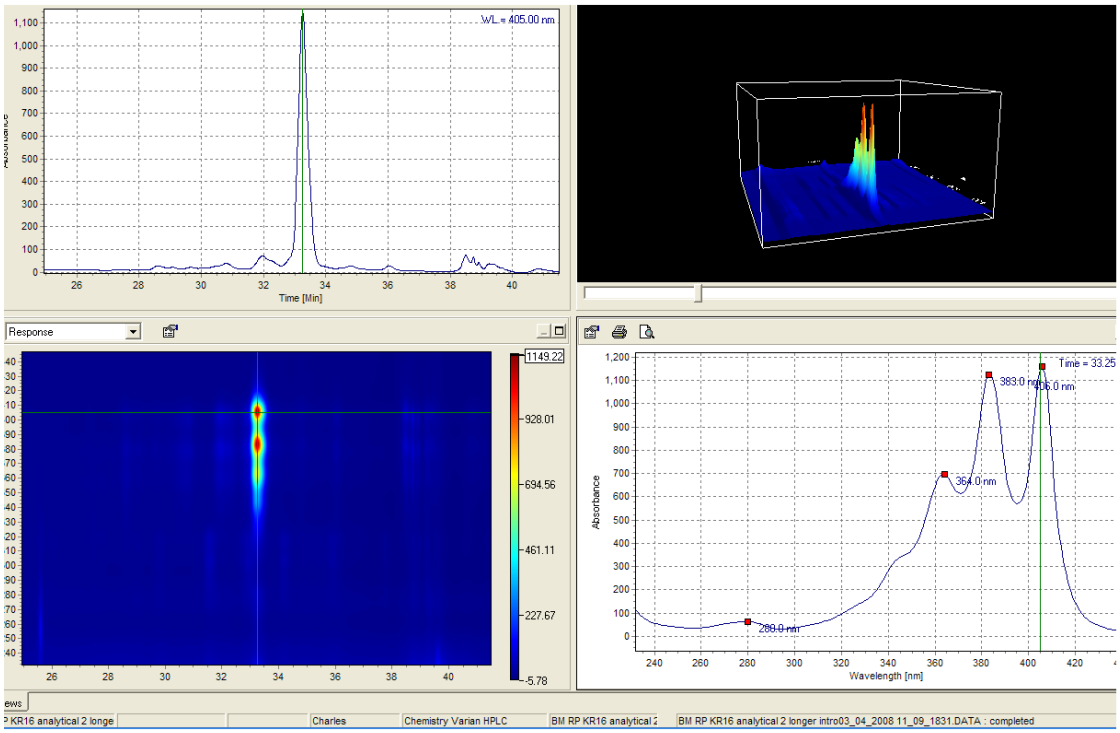


Prerun	1.000 ml/min	A=80.00% B=20.00%
10.0 min	1.000 ml/min	A=80.00% B=20.00%
25.0 min	1.000 ml/min	A=20.00% B=80.00%
35.0 min	1.000 ml/min	A=20.00% B=80.00%
36.0 min	1.000 ml/min	A=0.00% B=100.00%
39.0 min	1.000 ml/min	A=0.00% B=100.00%
39.0 min	1.000 ml/min	A=80.00% B=20.00%
47.0 min	1.000 ml/min	A=80.00% B=20.00%

Analytical 1 mL/min, preparative flow rate 14.8ml/min

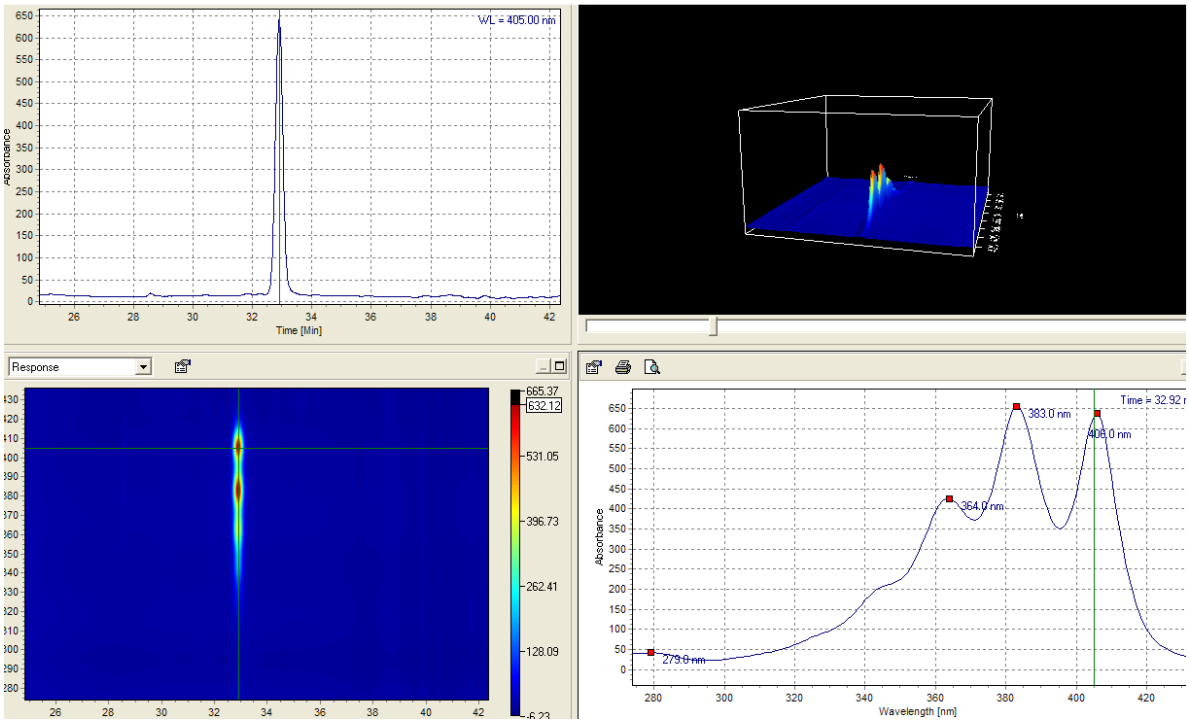
KR16: 7-Oxo-amphotericin B (9)

KR16 ppte from methanolic extractions, before water washes



KR16: 7-Oxo-amphotericin B (9)

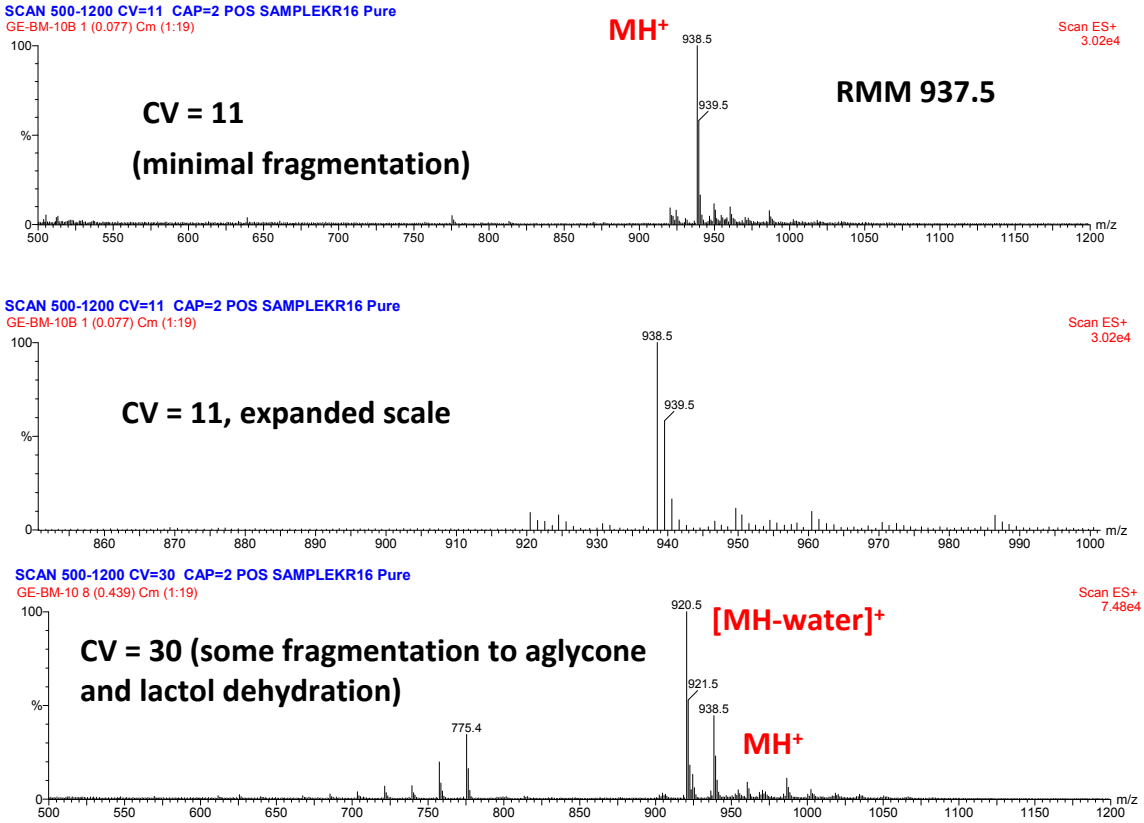
KR16 after 2 x RPHPLC



KR16: 7-Oxo-amphotericin B (9)

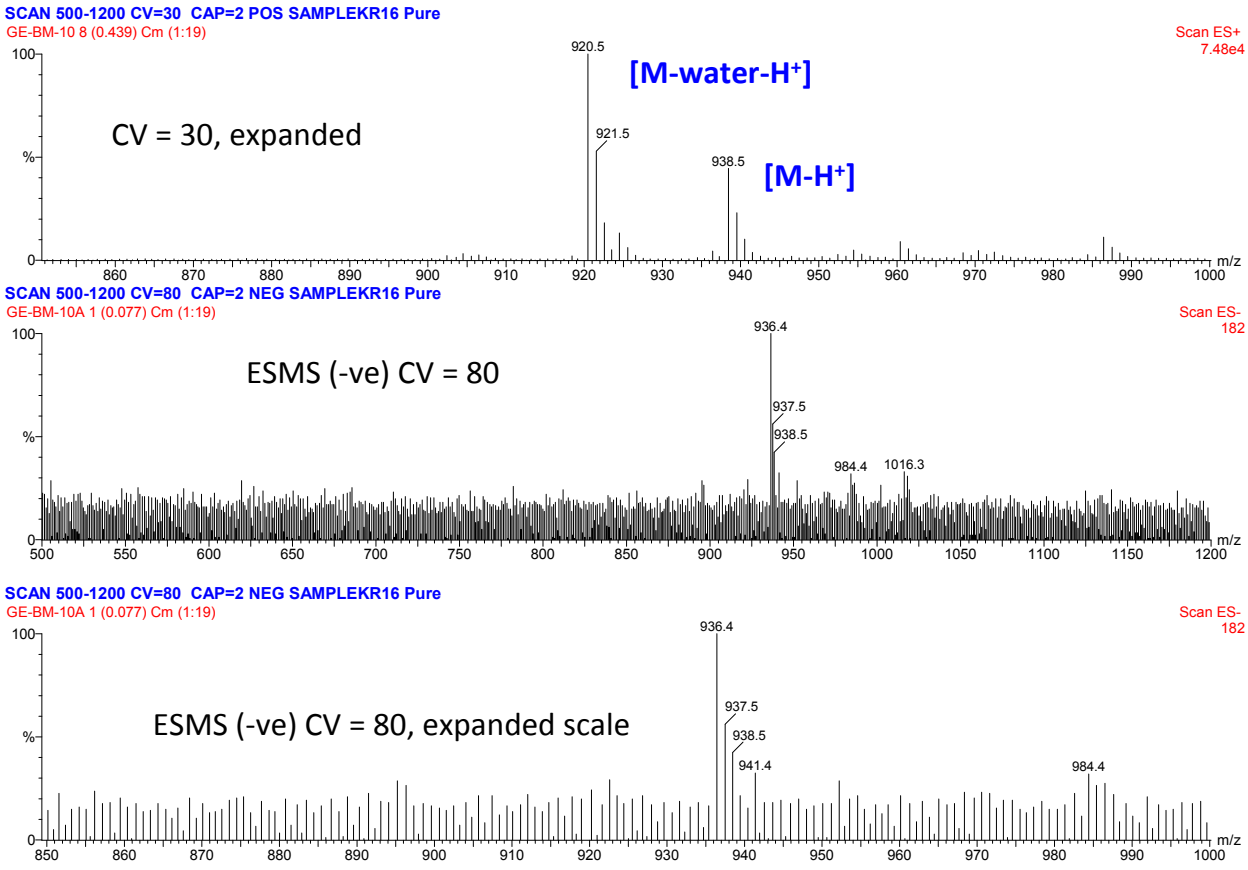
FAB MS was too weak for High resolution

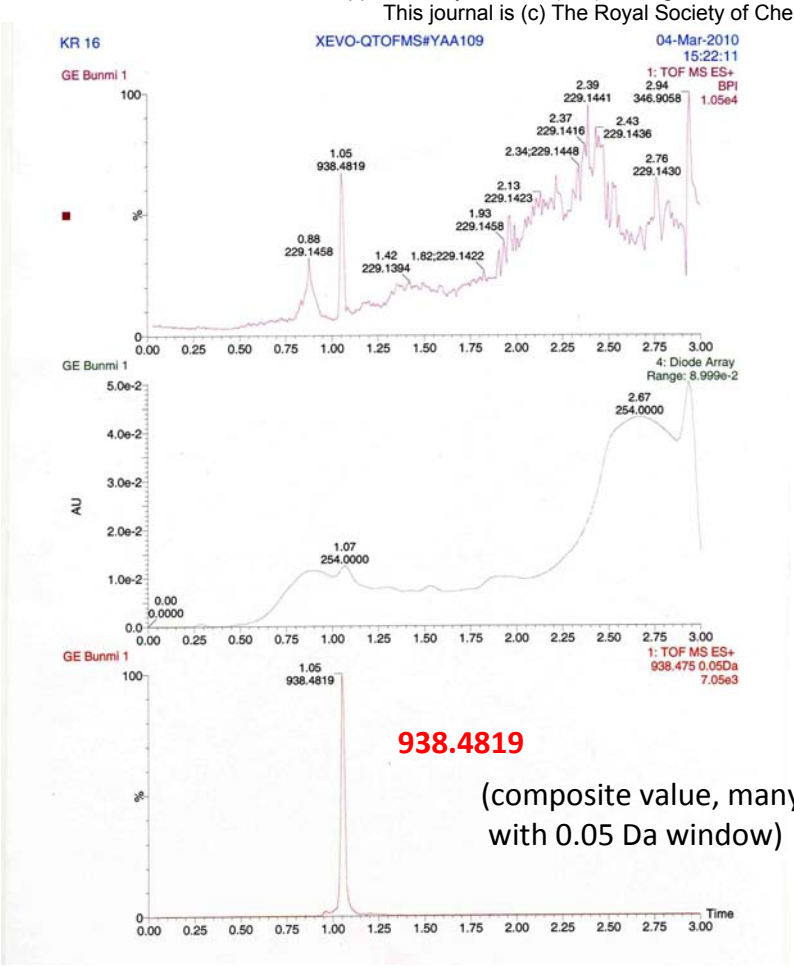
Triple quad electrospray MS **ESMS (+ve) with different cone voltages (CV)**



KR16: 7-Oxo-amphotericin B (9)

ESMS(-ve)





KR16: 7-Oxo-amphotericin B (9)

XEVO QTOFMS

Quadropole electrospray (+ve) with time of flight MS

Waters Ultraperformance Accuity LC

938.4819

(composite value, many scans over peak with 0.05 Da window)

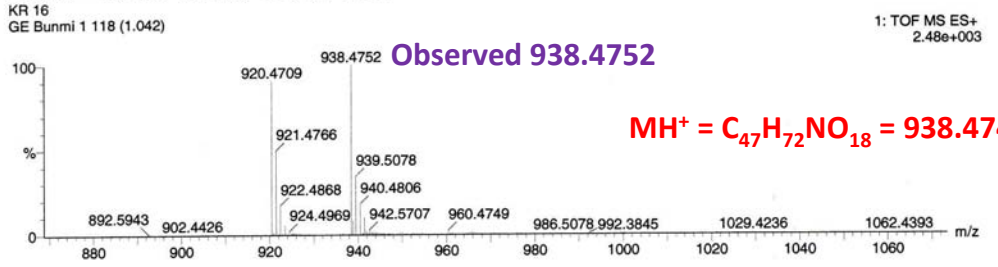
KR16: 7-Oxo-amphotericin B (9)

XEVO QTOFMS

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3929 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-20 S: 0-1
KR 16
GE Bunmi 1 118 (1.042)

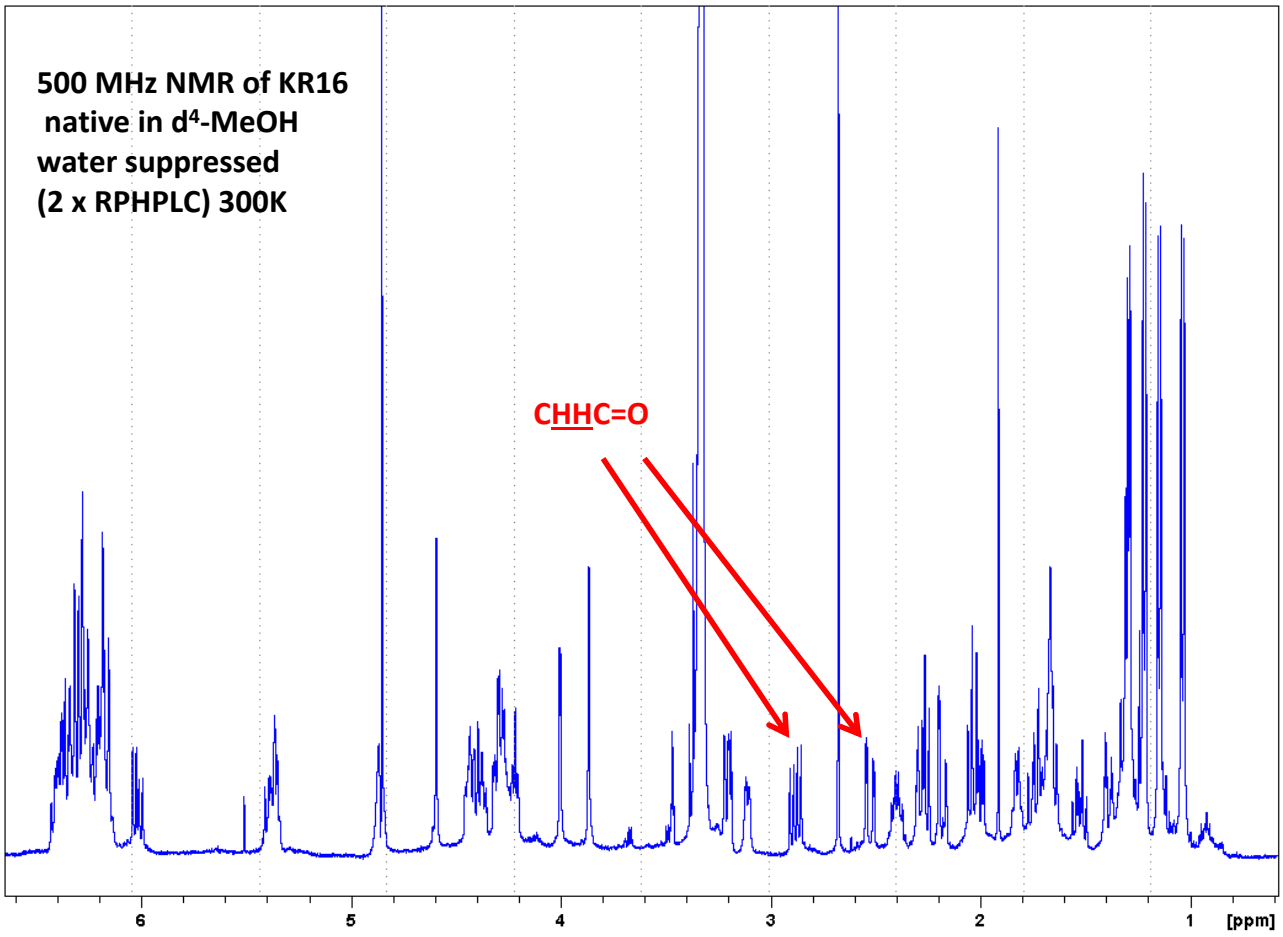


Observed 938.4752

$MH^+ = C_{47}H_{72}NO_{18} = 938.4749$

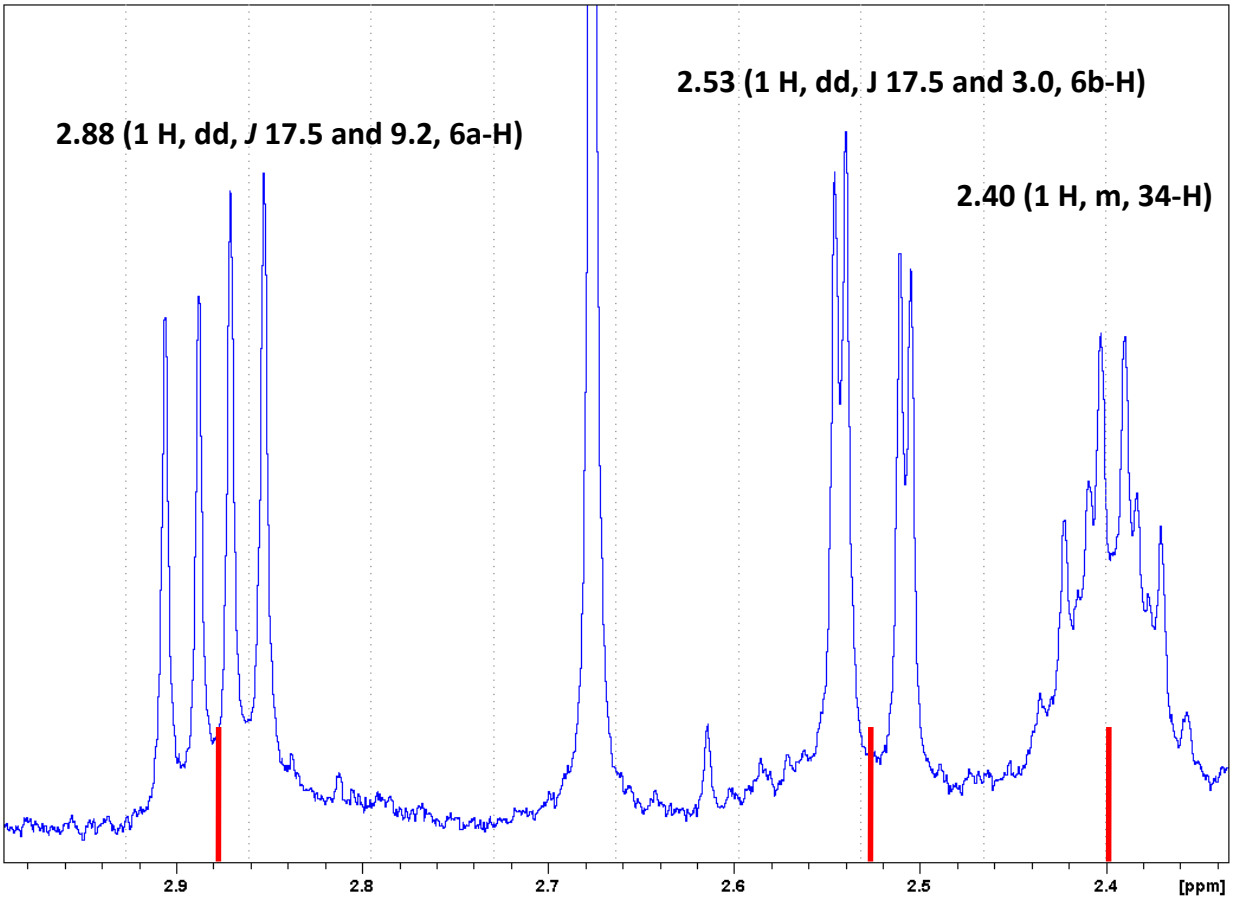
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		3.0	1.0	50.0			
938.4752	938.4743	0.9	1.0	3.5	40.2	0.7	C39 H76 N3 O20 S
	938.4756	-0.4	-0.4	8.5	40.7	1.2	C40 H72 N7 O16 S
	*938.4749	0.3	0.3	12.5	41.8	2.3	C47 H72 N O18
	938.4751	0.1	0.1	26.5	43.0	3.4	C53 H64 N9 O5 S
	938.4744	0.8	0.9	30.5	43.4	3.9	C60 H64 N3 O7
	938.4758	-0.6	-0.6	35.5	43.5	4.0	C61 H60 N7 O3
	938.4759	-0.7	-0.7	38.5	44.1	4.5	C69 H64 N S

KR16: 7-Oxo-amphotericin B (9)



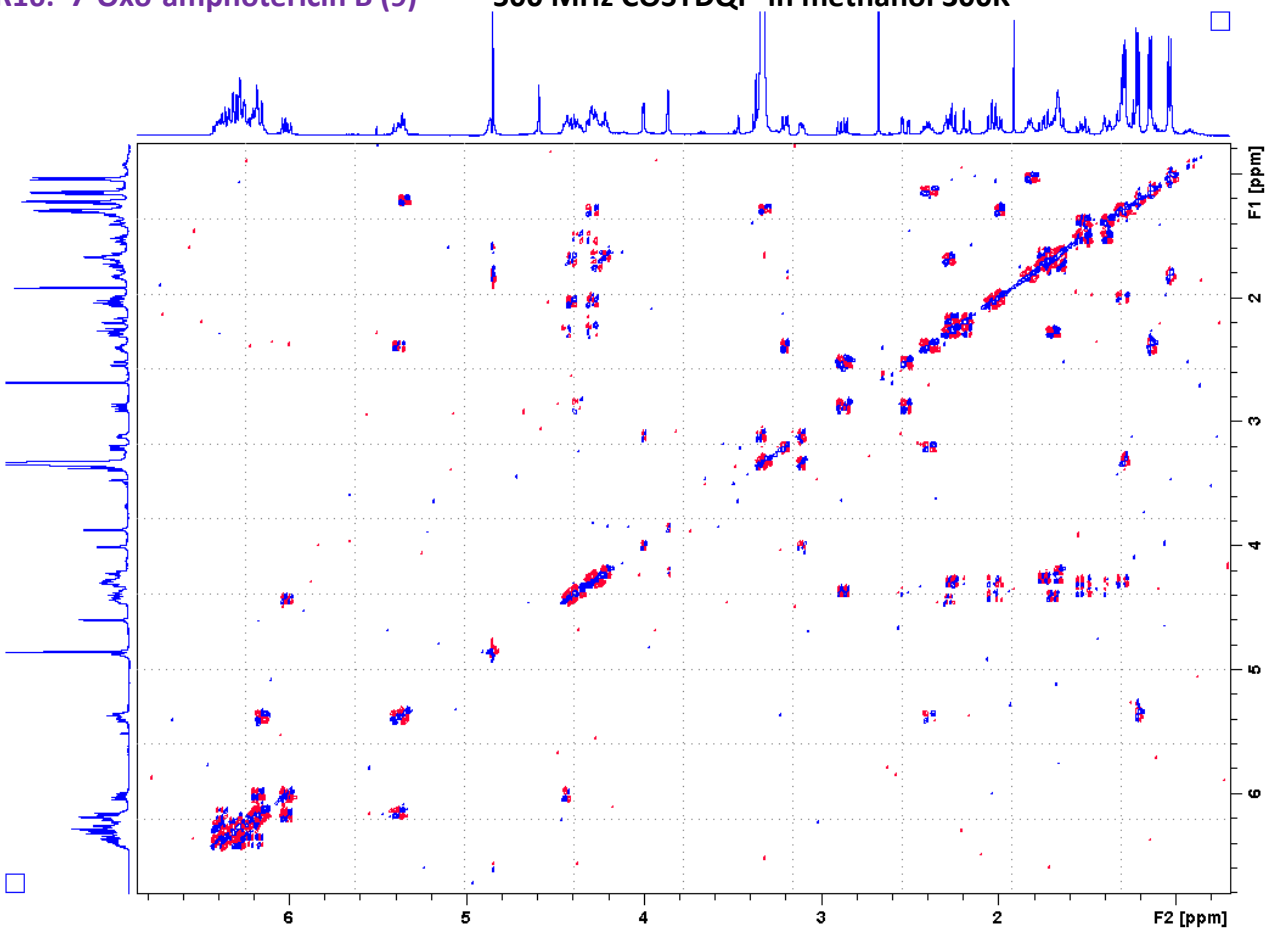
KR16: 7-Oxo-amphotericin B (9)

Selected part of proton NMR showing C-6 protons

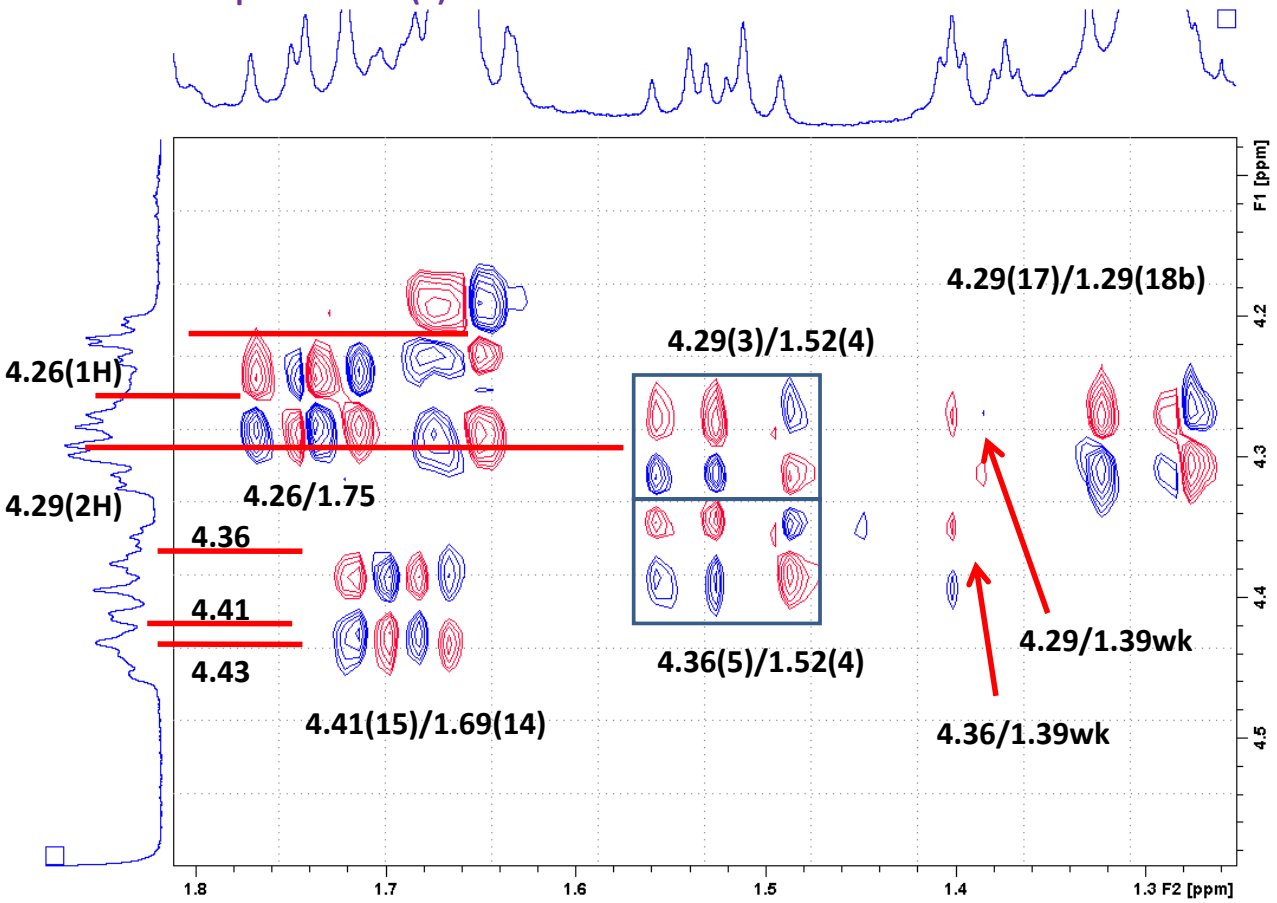


KR16: 7-Oxo-amphotericin B (9)

500 MHz COSYDQF in methanol 300K

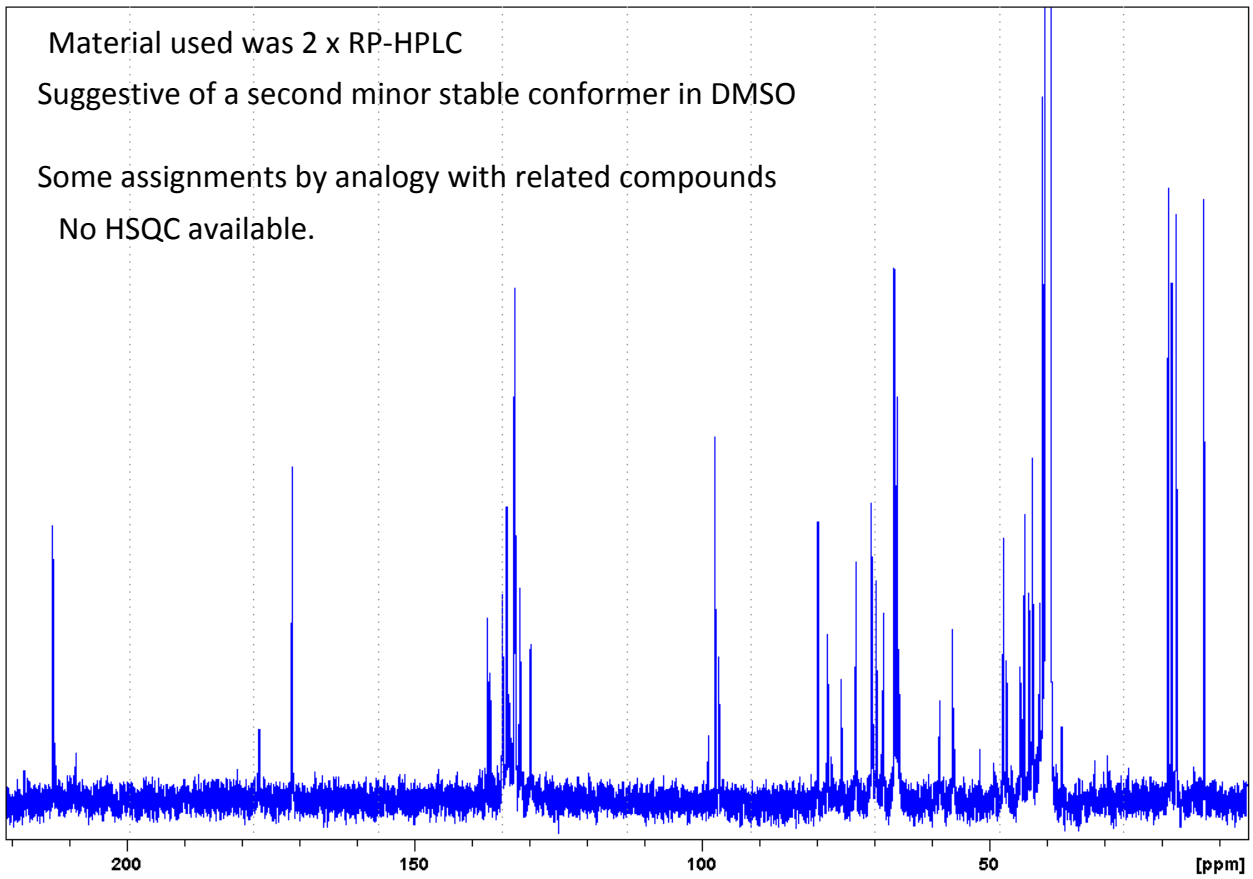


KR16: 7-Oxo-amphotericin B (9)



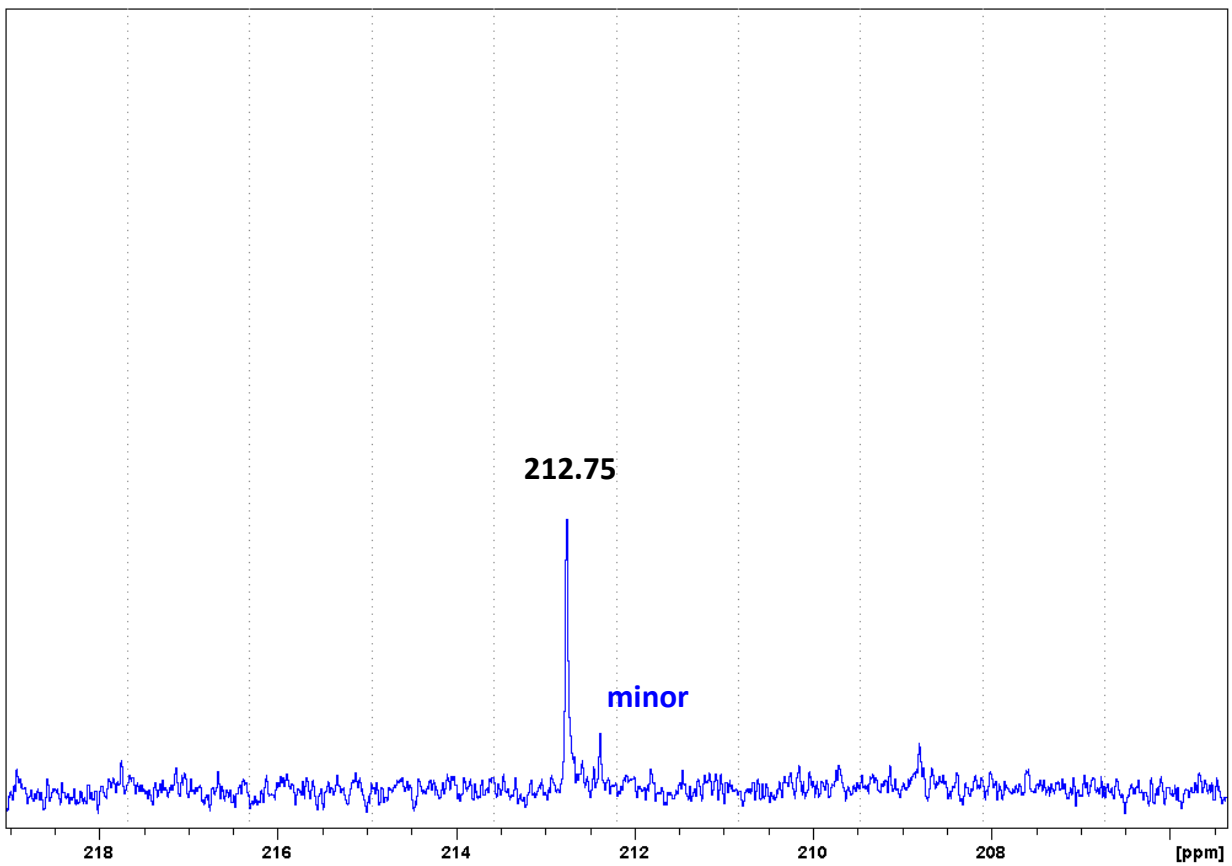
KR16: 7-Oxo-amphotericin B (9)

KR16 NATIVE x2 RPHPLC DMSO 13C{1H} / 308K

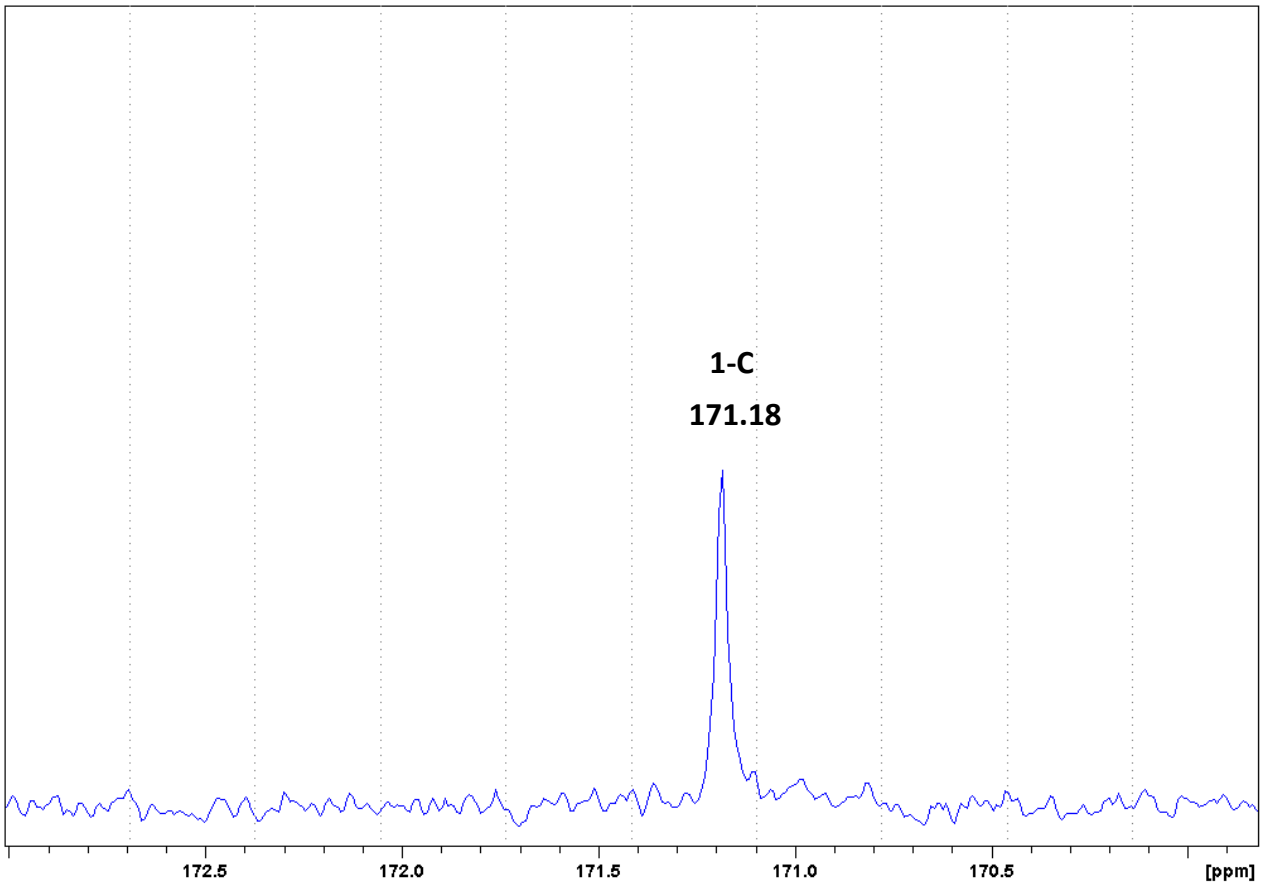


KR16: 7-Oxo-amphotericin B (9)

^{13}C NMR showing ketone (7-C) resonance

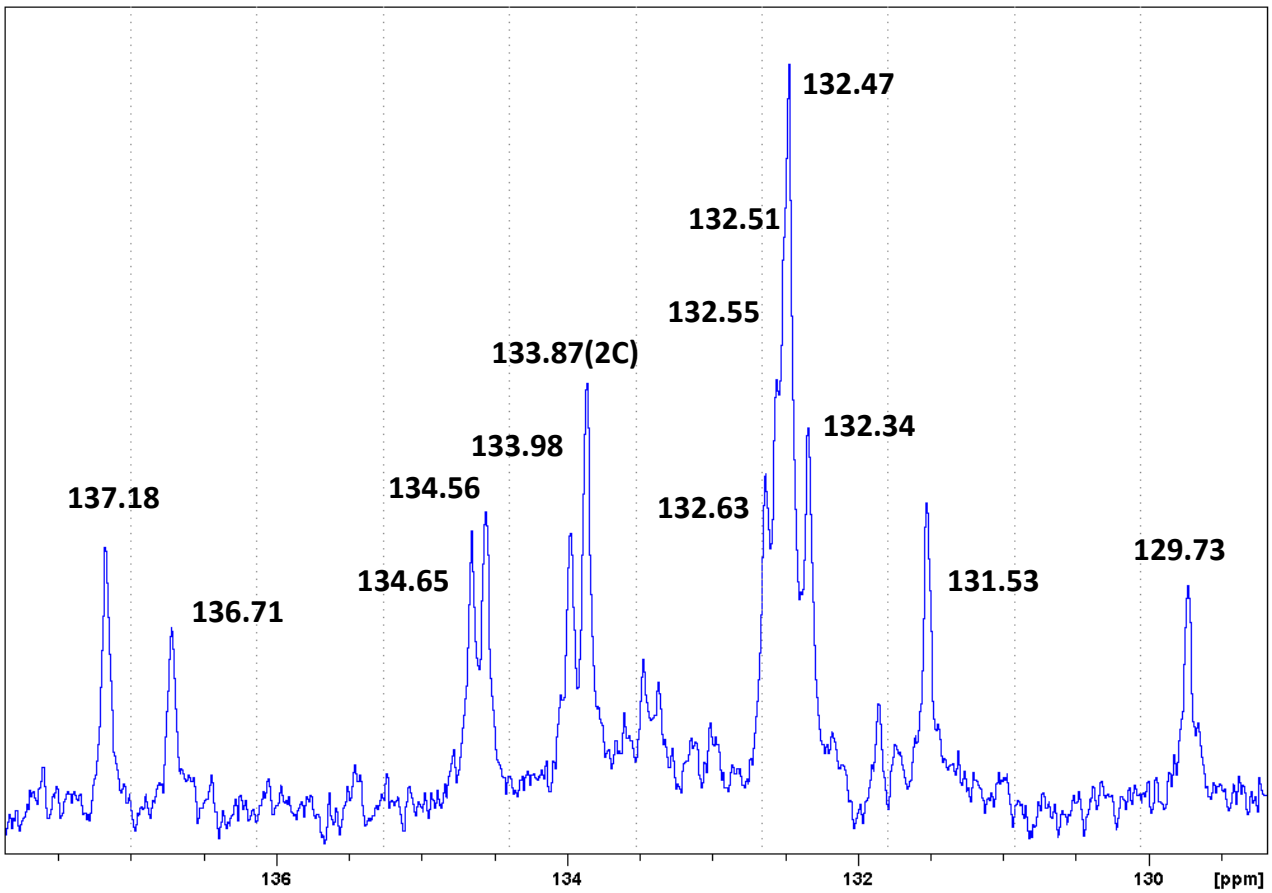


KR16: 7-Oxo-amphotericin B (9)

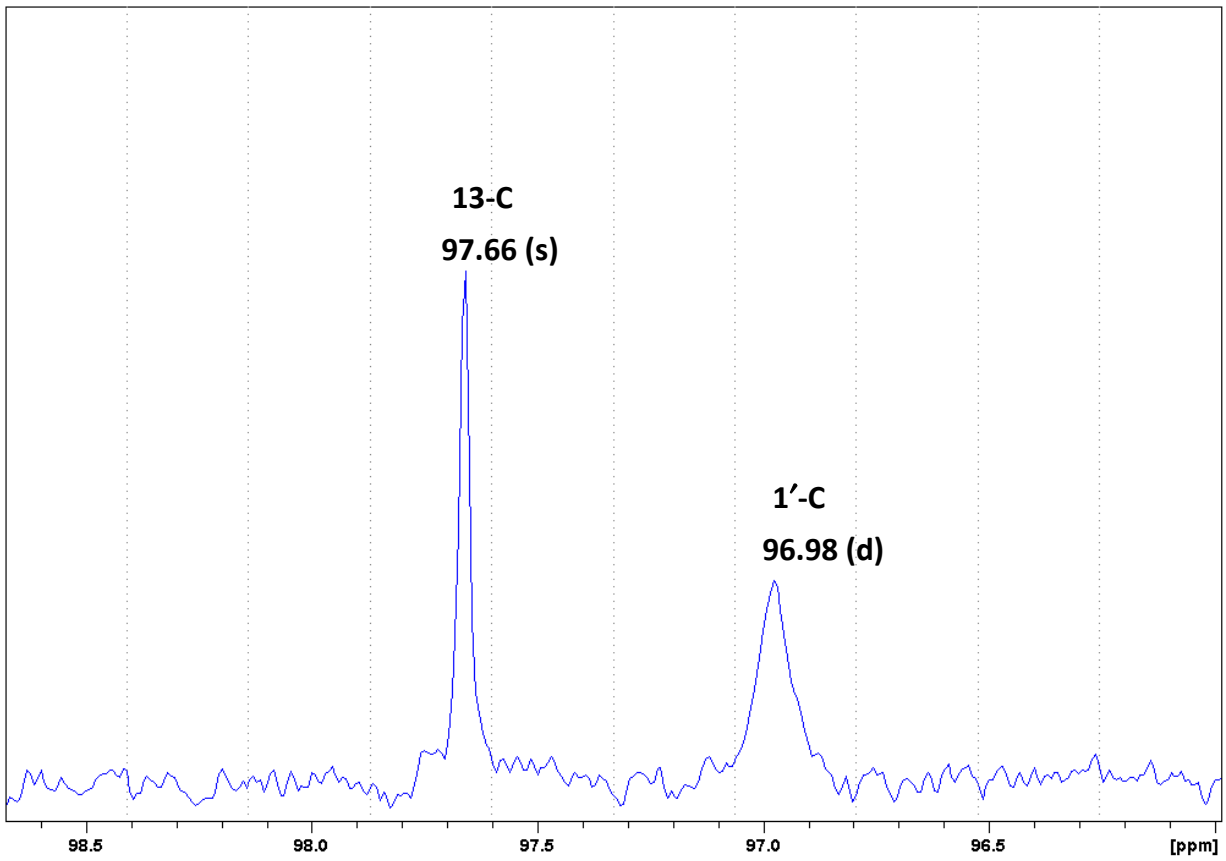


KR16: 7-Oxo-amphotericin B (9)

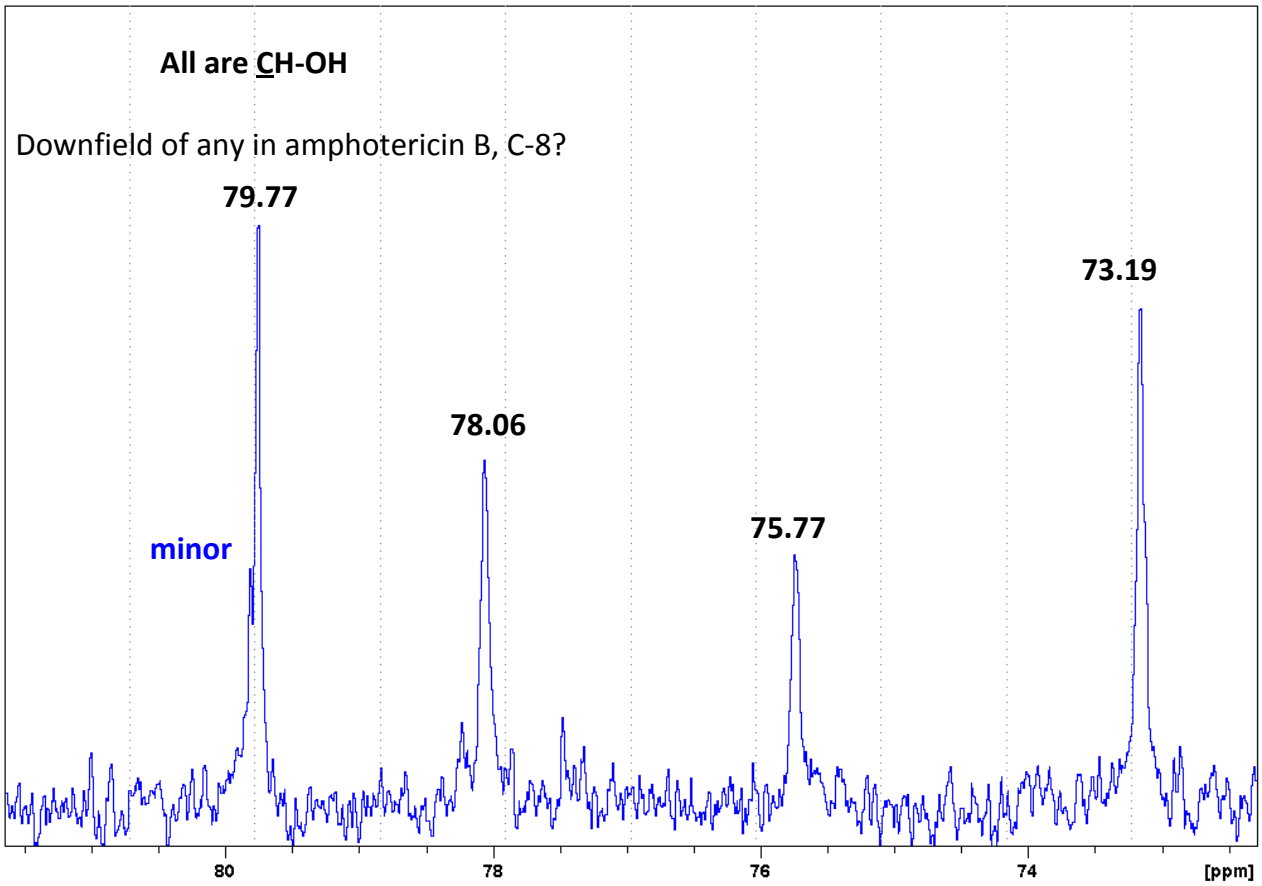
14 x H-C=C



KR16: 7-Oxo-amphotericin B (9)

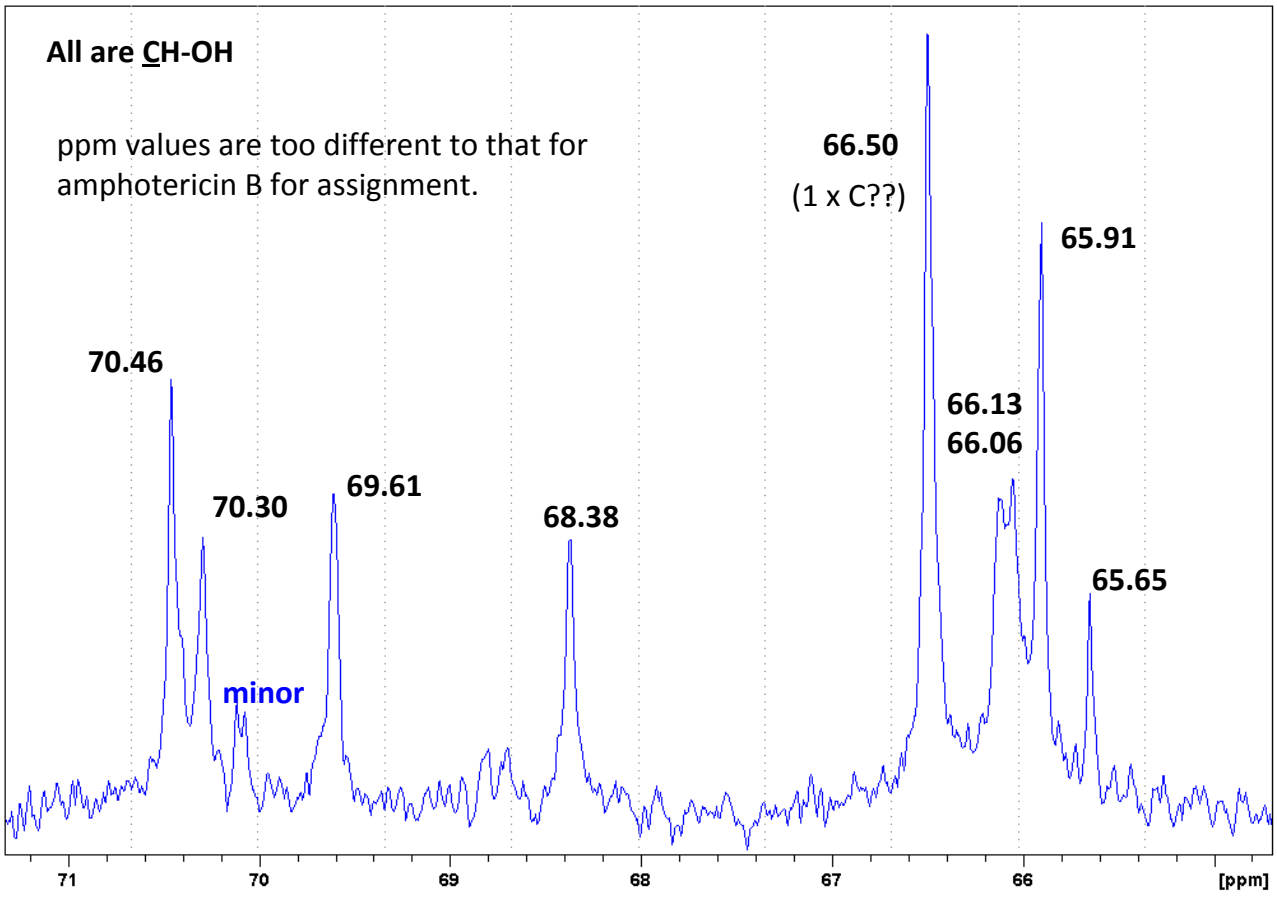


KR16: 7-Oxo-amphotericin B (9)

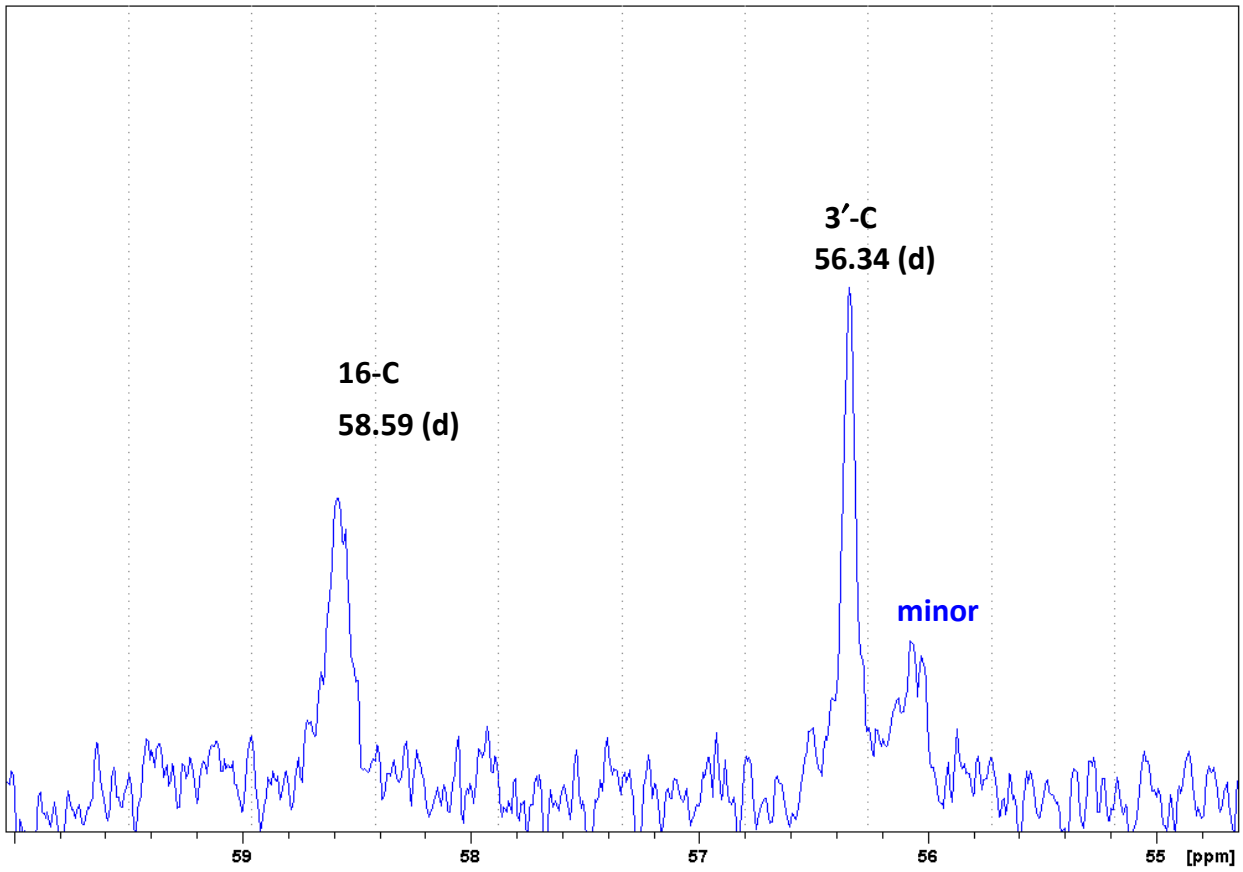


KR16: 7-Oxo-amphotericin B (9)

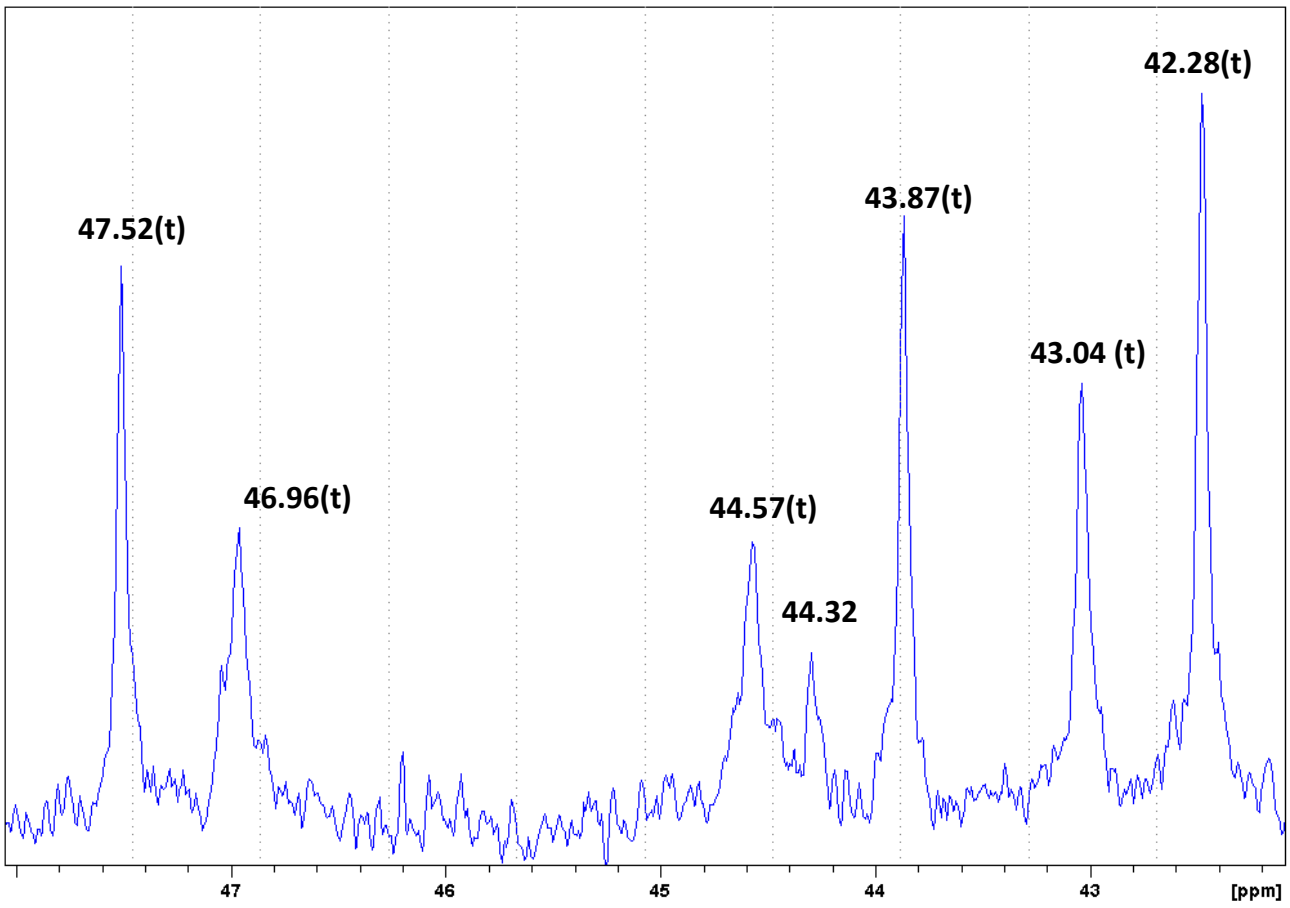
13 resonances between 65 and 80 ppm



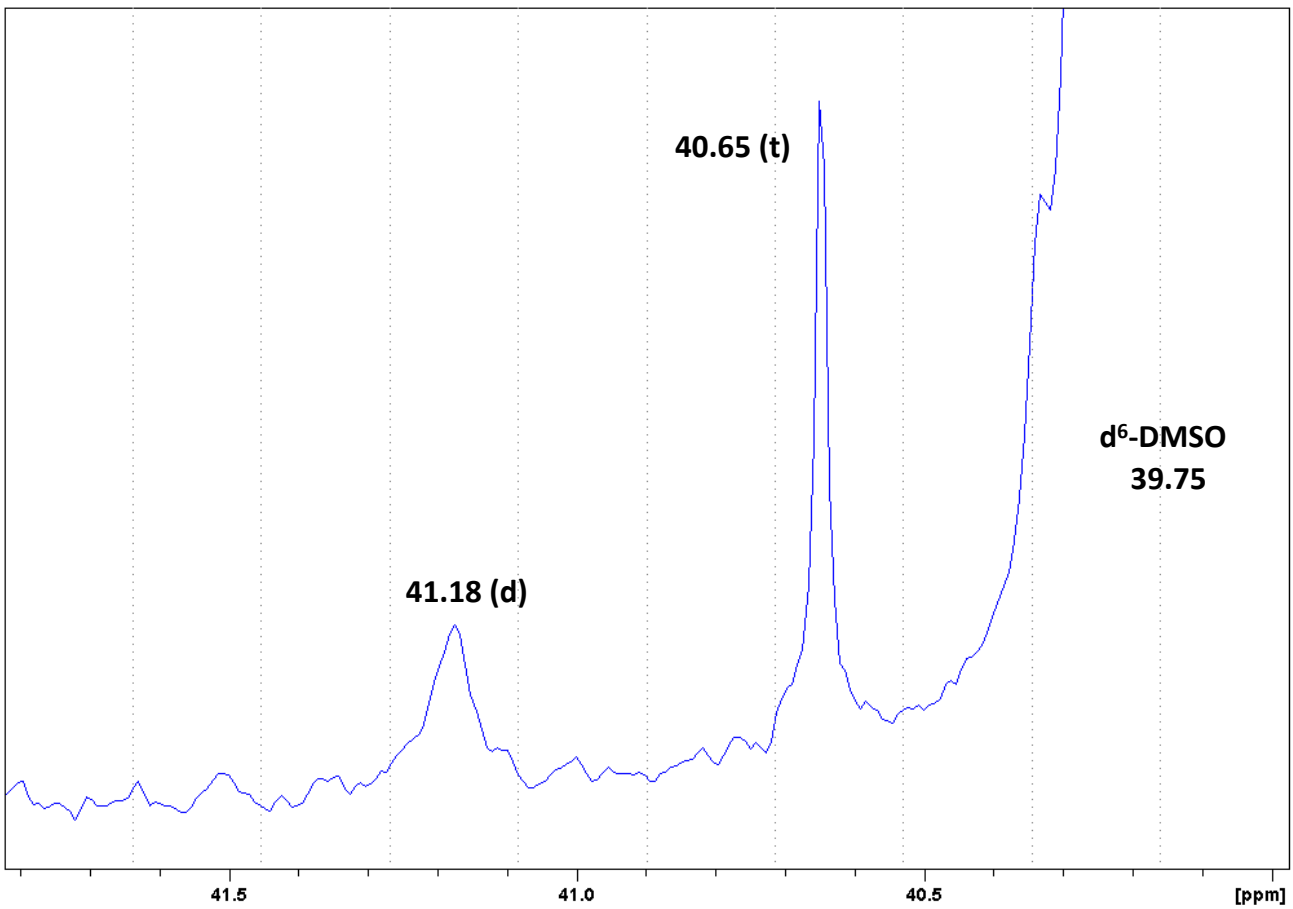
KR16: 7-Oxo-amphotericin B (9)



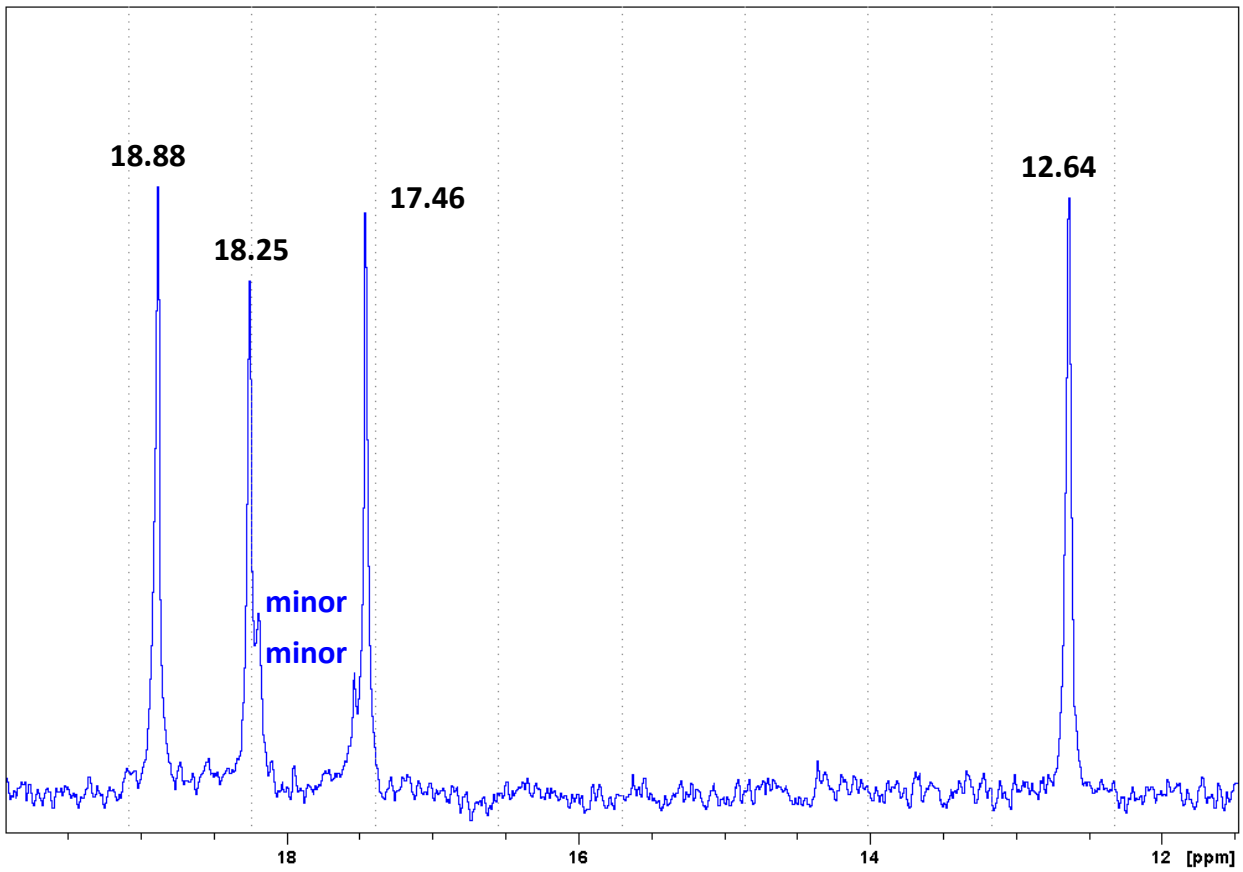
KR16: 7-Oxo-amphotericin B (9)



KR16: 7-Oxo-amphotericin B (9)

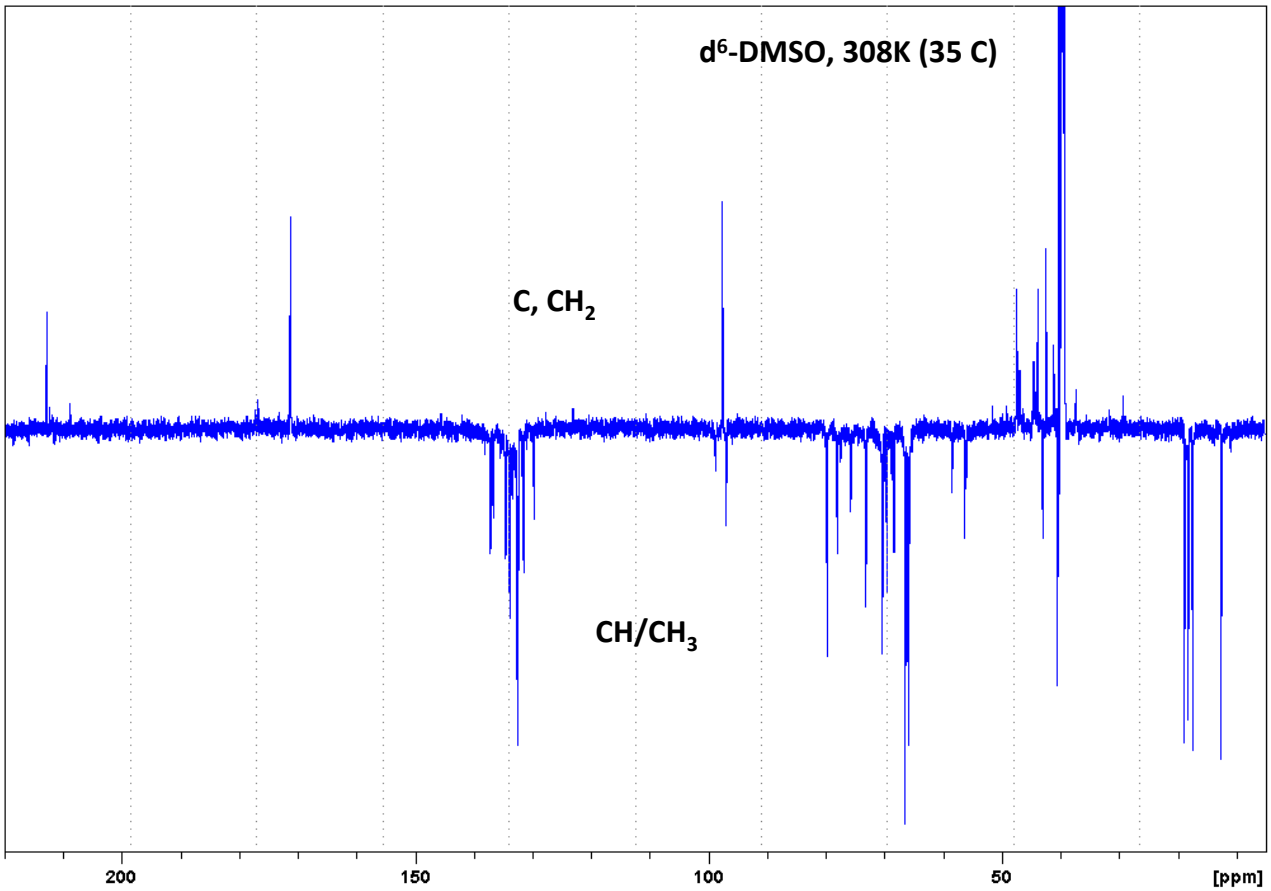


KR16: 7-Oxo-amphotericin B (9)

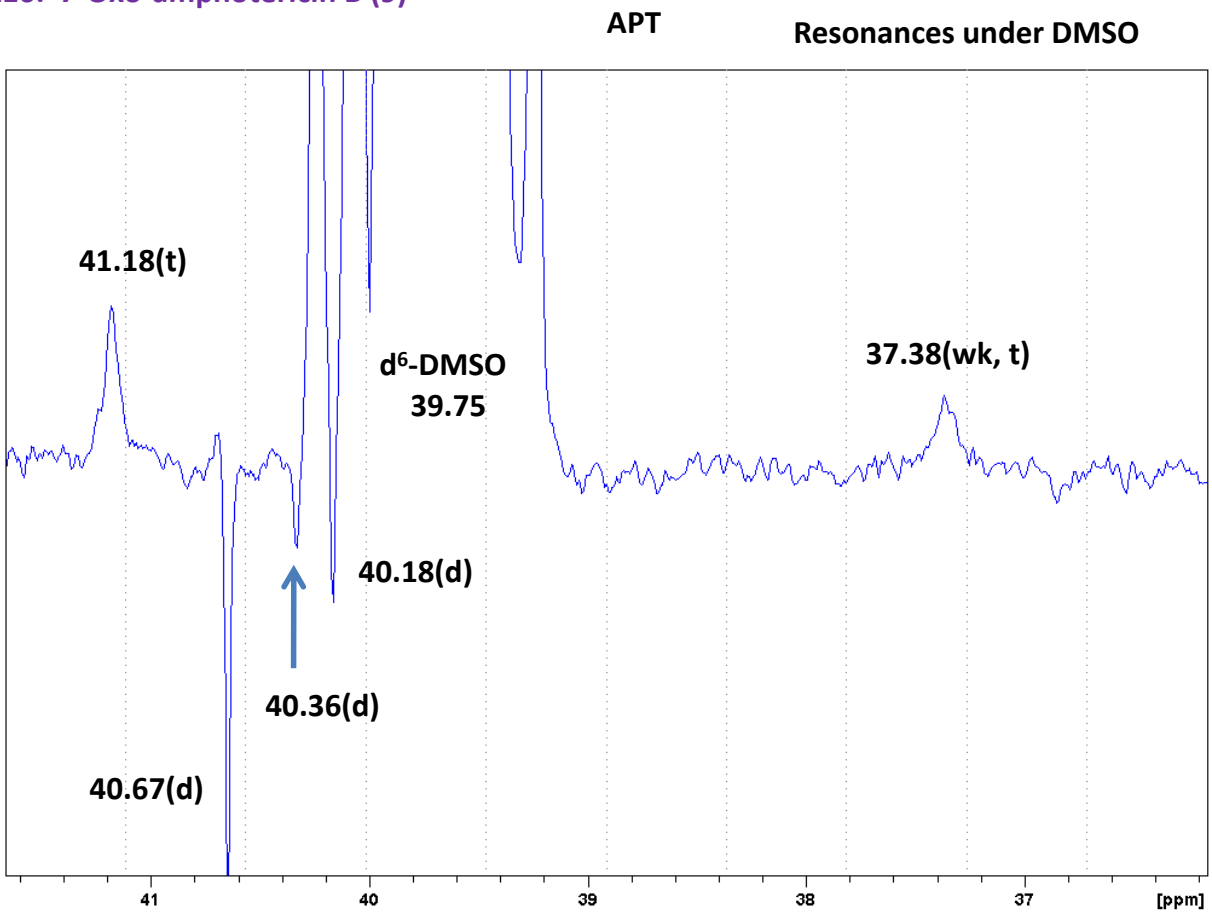


KR16: 7-Oxo-amphotericin B (9)

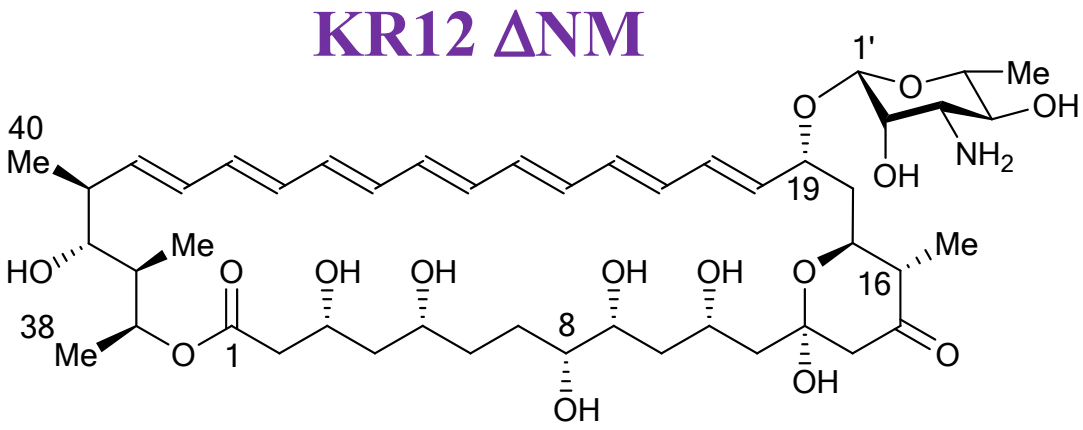
APT –attached proton test.



KR16: 7-Oxo-amphotericin B (9)



KR12 ΔNM



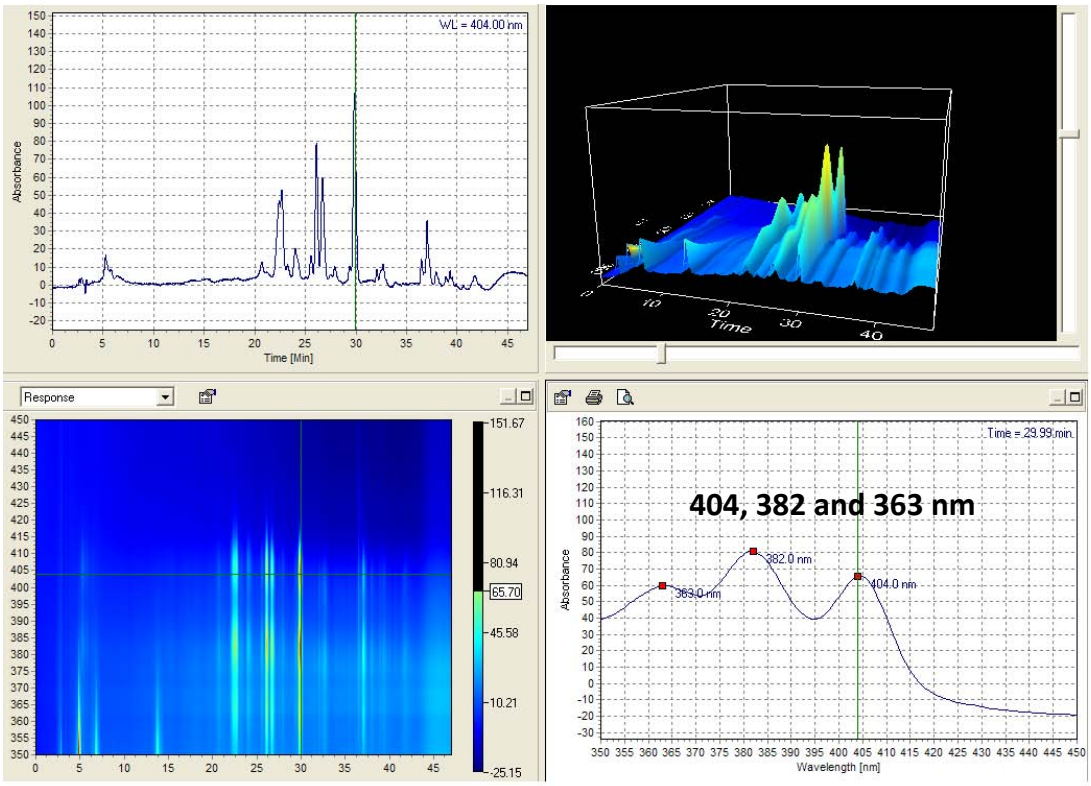
15-Deoxy-16-descarboxyl-16-methyl-15-oxo-amphotericin B (16) RMM= 891.6

and the 8-deoxy analogue:

16-descarboxyl-8,15-Dideoxy-16-methyl-15-oxo-amphotericin B (20) RMM = 875.5

KR12 ΔNM

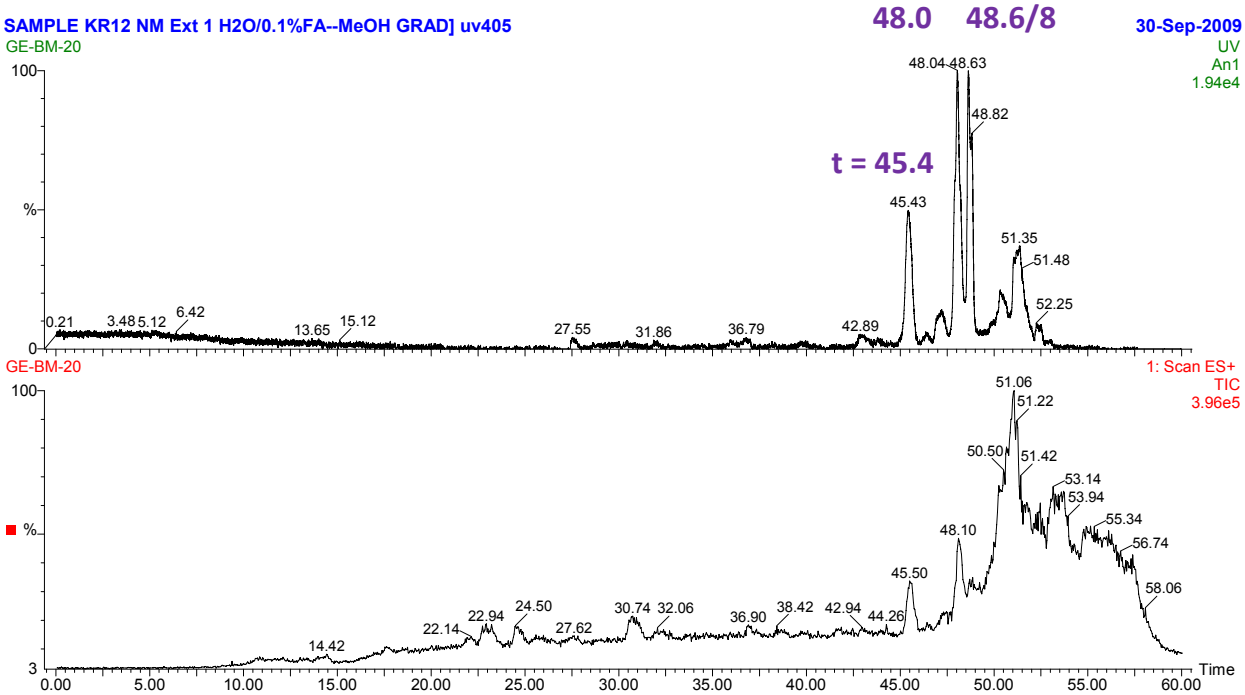
Precipitate (from MeOH extraction) redissolved in MeOH



KR12 ΔNM

ESMS(+ve)

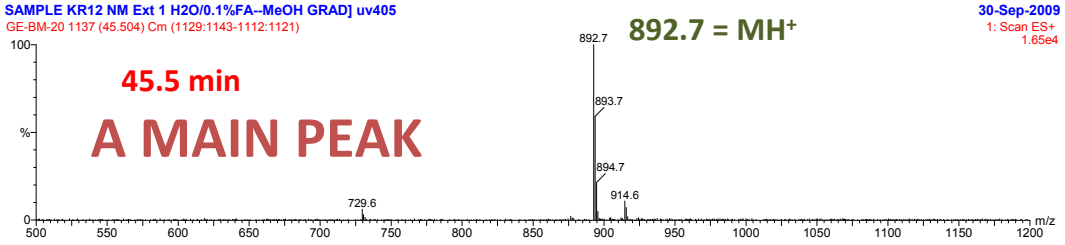
RP-LCMS of methanol extract, concentrated and redissolved in MeOH.

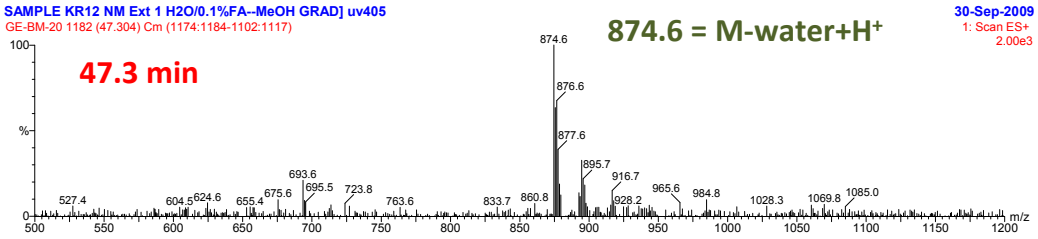


KR12 ΔNM

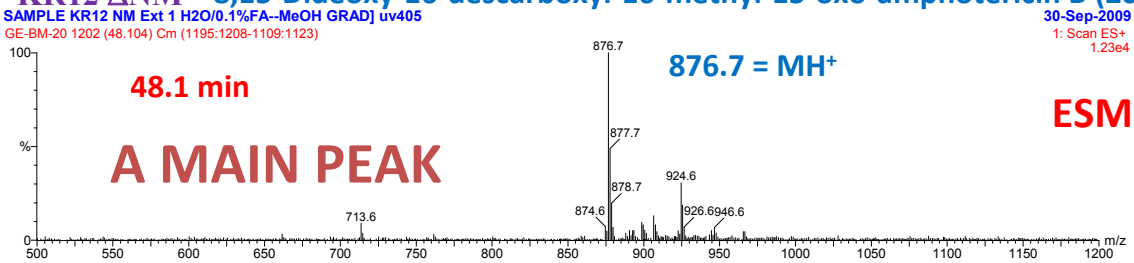
ESMS(+ve)

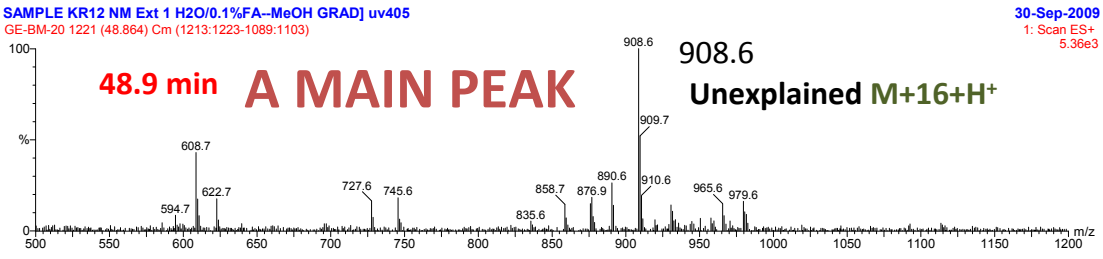
15-Deoxy-16-descarboxyl-16-methyl-15-oxo-amphotericin B (16) = 891.6

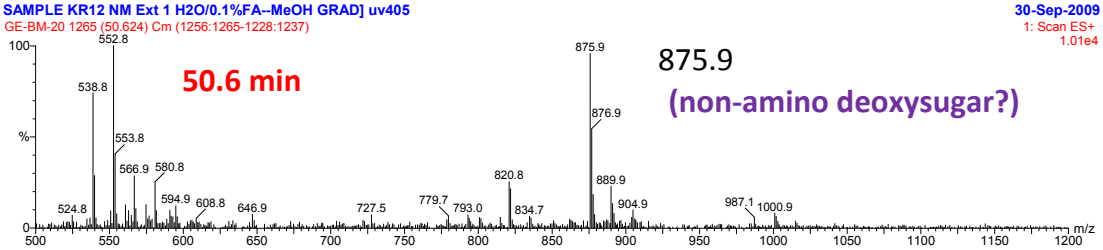




KR12 ΔNM 8,15-Dideoxy-16-descarboxyl-16-methyl-15-oxo-amphotericin B (20) = 875.5

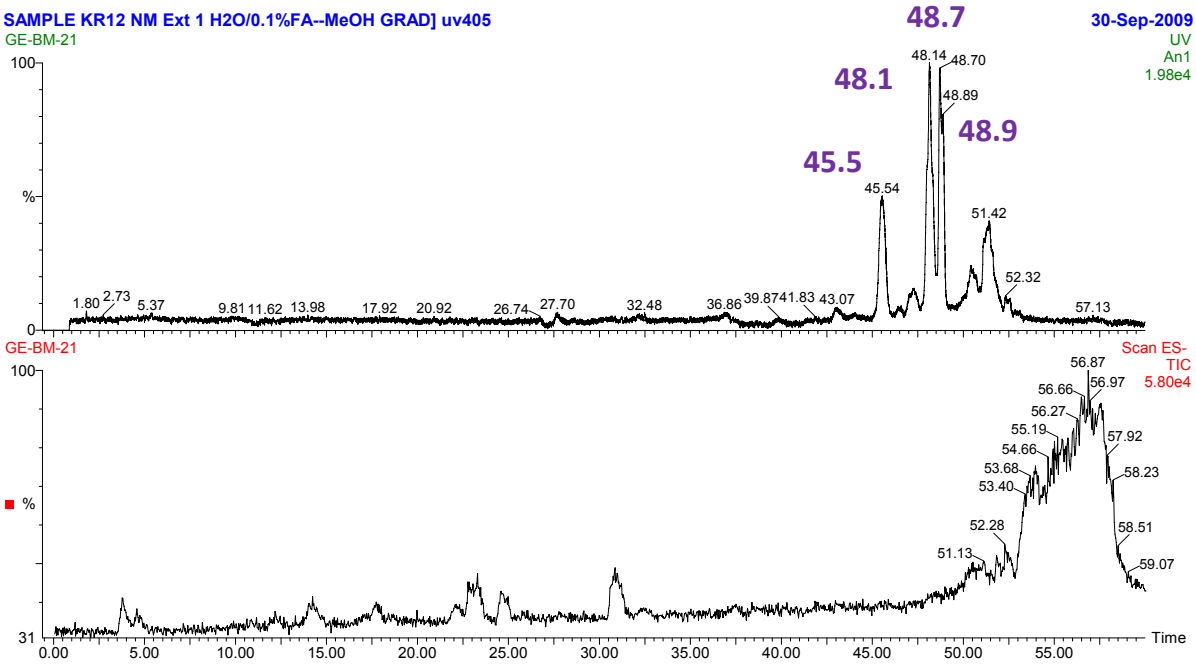




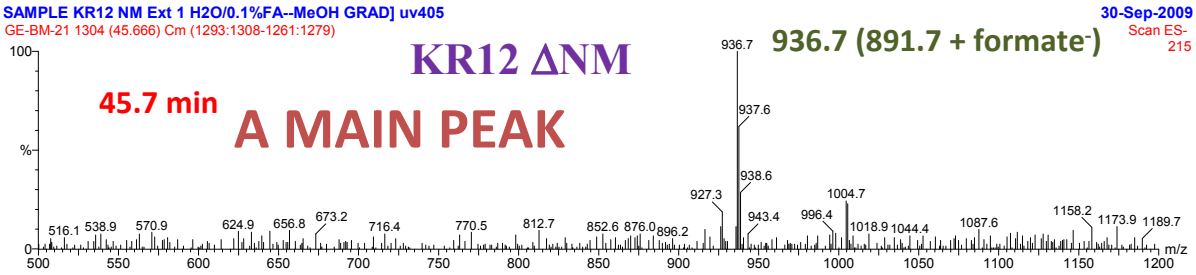


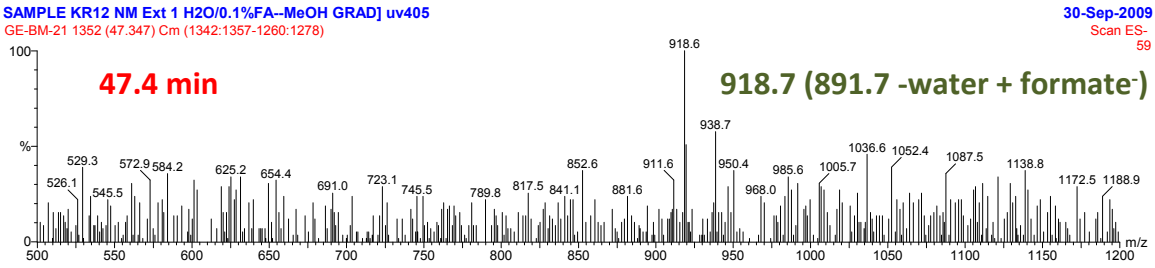
KR12 ΔNM

ESMS(-ve) LCMS

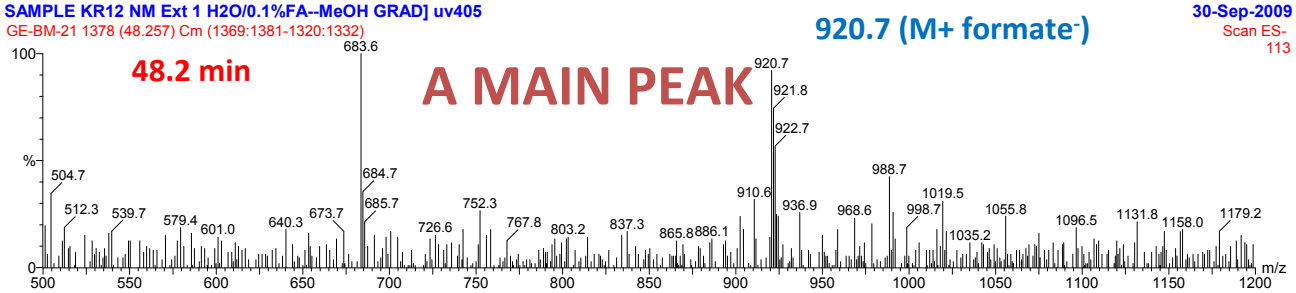


ESMS(-ve) 15-Deoxy-16-descarboxyl-16-methyl-15-oxo-amphotericin B = 891.6

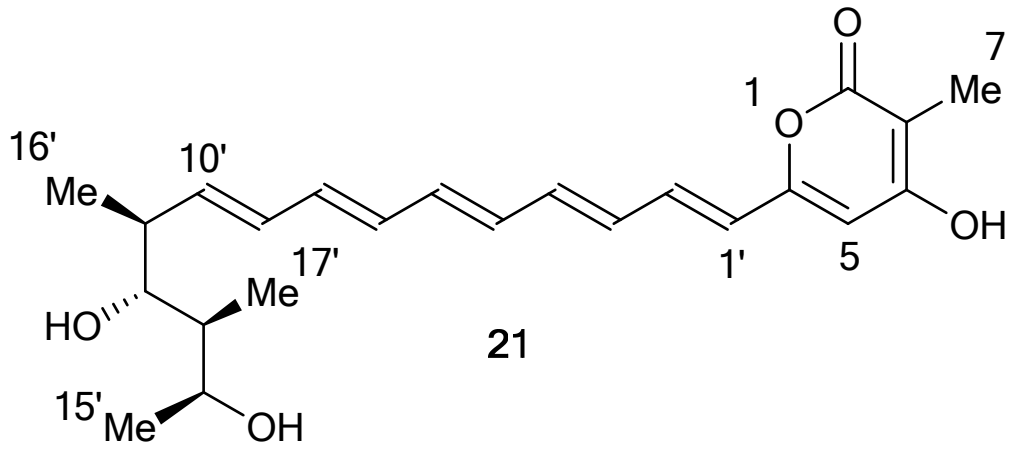




8,15-Dideoxy-16-descarboxyl-16-methyl-15-oxo-amphotericin B = 875.5



KR10-1 Decaketide (21)

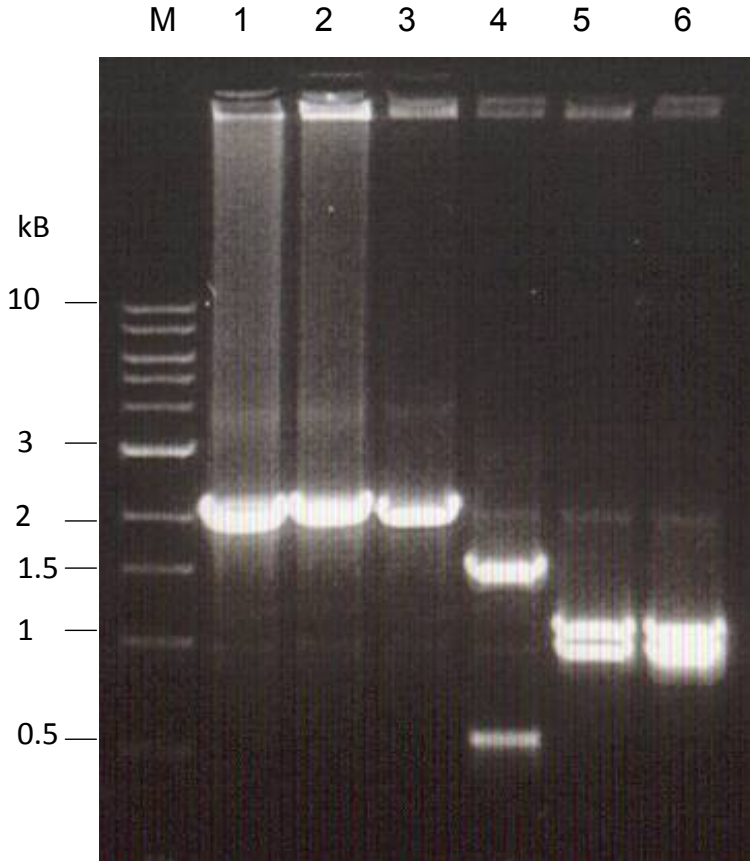


Decaketide product 21 from KR10-1 mutant

6-[(1*E*,3*E*,5*E*,7*E*,9*E*,11*S*,12*S*,13*S*,14*S*)-12,14-Dihydroxy-11,13-dimethyl-1,3,5,7,9-pentadecapenten-1-yl]-4-hydroxy-3-methyl-2-pyrone

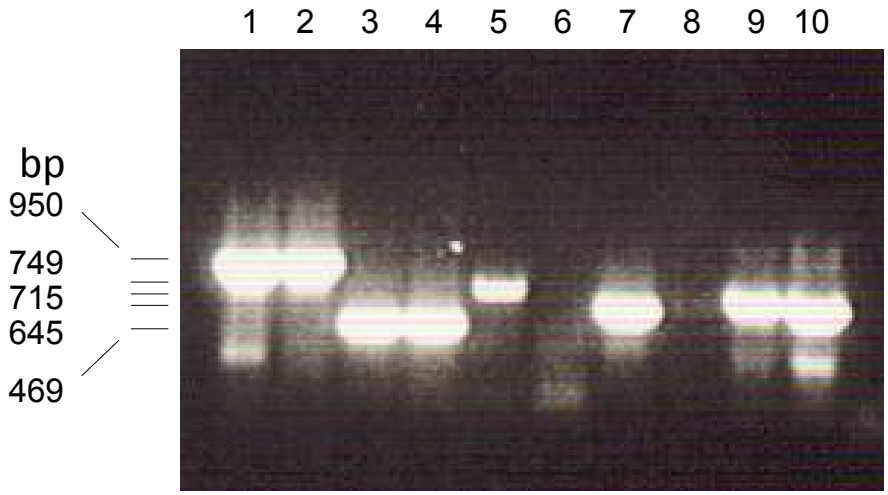
KR10-1 Decaketide (21)

Evidence for inactivation of KR10-1 coding sequence



Analysis of the KR10 coding region in wild-type and mutant strains. PCR primers KR10CF and KR10CR2 were used to amplify the KR10 coding region from *S. nodosus* (lane 1) and the KR10-1 mutant (lane 2). Treatment with HindIII revealed that the DNA amplified from *S. nodosus* was resistant to digestion (lane 3) whereas that from the mutant contained the expected site (lane 4). In control digests, Bcl I cut the PCR products from both strains at an internal site (lanes 5 and 6).

Analysis of AmphC ACP coding sequences in *S. nodosus* and the KR10-1 mutant

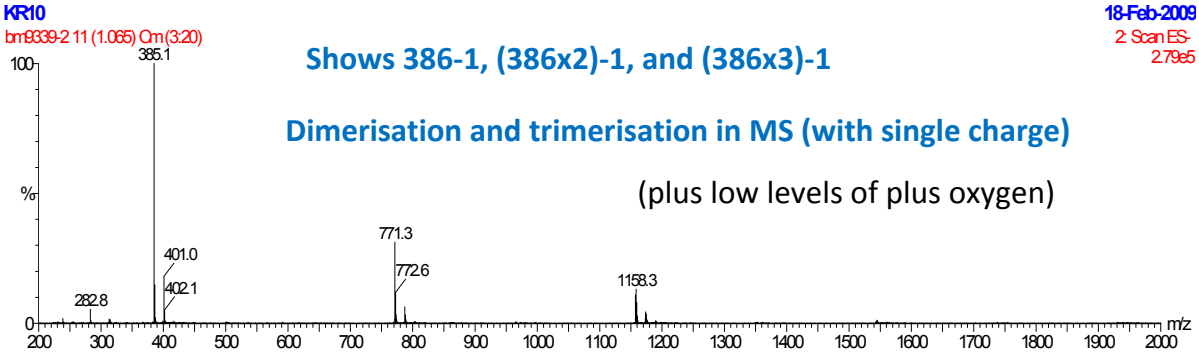


PCR analysis of ACP coding sequences in modules 3 to 8. Lanes 1, 3, 5, 7 and 9, DNA amplified from *S. nodosus* genomic DNA with primers for sequences encoding ACPs 3, 4, 5, 6 and 7, respectively. Lanes 2, 4, 6, 8 and 10, DNA amplified from *S. nodosus* KR10-1 genomic DNA with primers for sequences encoding ACPs 3, 4, 5, 6 and 7, respectively. The ACP5 and ACP6 coding sequences are missing from the KR10-1 mutant (lanes 6 and 8).

The sequences of the oligonucleotide primers were (5′ – 3′):
ACP3F, TTCGTGCTCTTCTCGTCCGTC; ACP3R, CCGAAGAAGTCGGCGTCGAAG;
ACP4F, ACCGATGAGGCCGCTCTCGT; ACP4R, GATCACGATCGGGTCGTCGT;
ACP5F , AACGCCTTCCTGGACGCACT; ACP5R, GAGCAGTCGCCACAGGTCCT;
ACP6F, CAACGCGGGTCAGGCCAACTA; ACP6R, GAAGCTCGTCCAGGATGAAGT;
ACP7F, TCTGGACCTGGACGCGTTCAT; ACP7R, GGCTCCGAAGAGTTCGTCGTG.

KR10-1 Decaketide (21)

KR10 ESMS Negative Ion mode

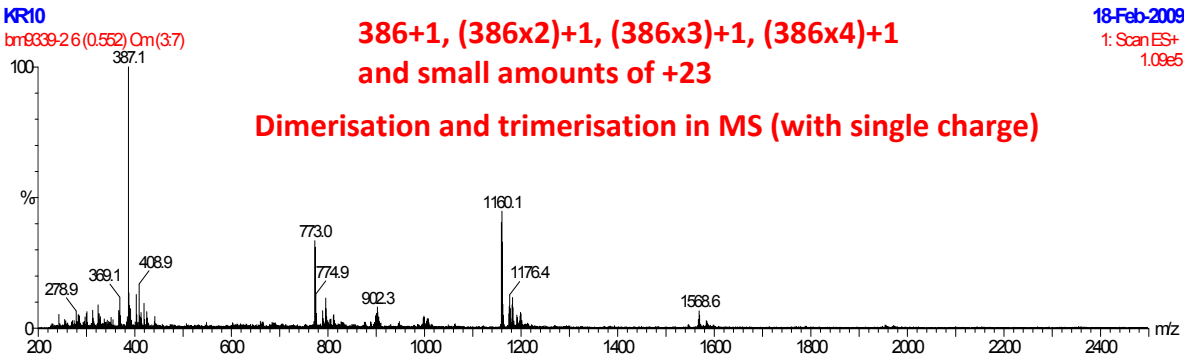


Shows 386-1, (386x2)-1, and (386x3)-1

Dimerisation and trimerisation in MS (with single charge)

(plus low levels of plus oxygen)

KR10 ESMS Positive Ion mode

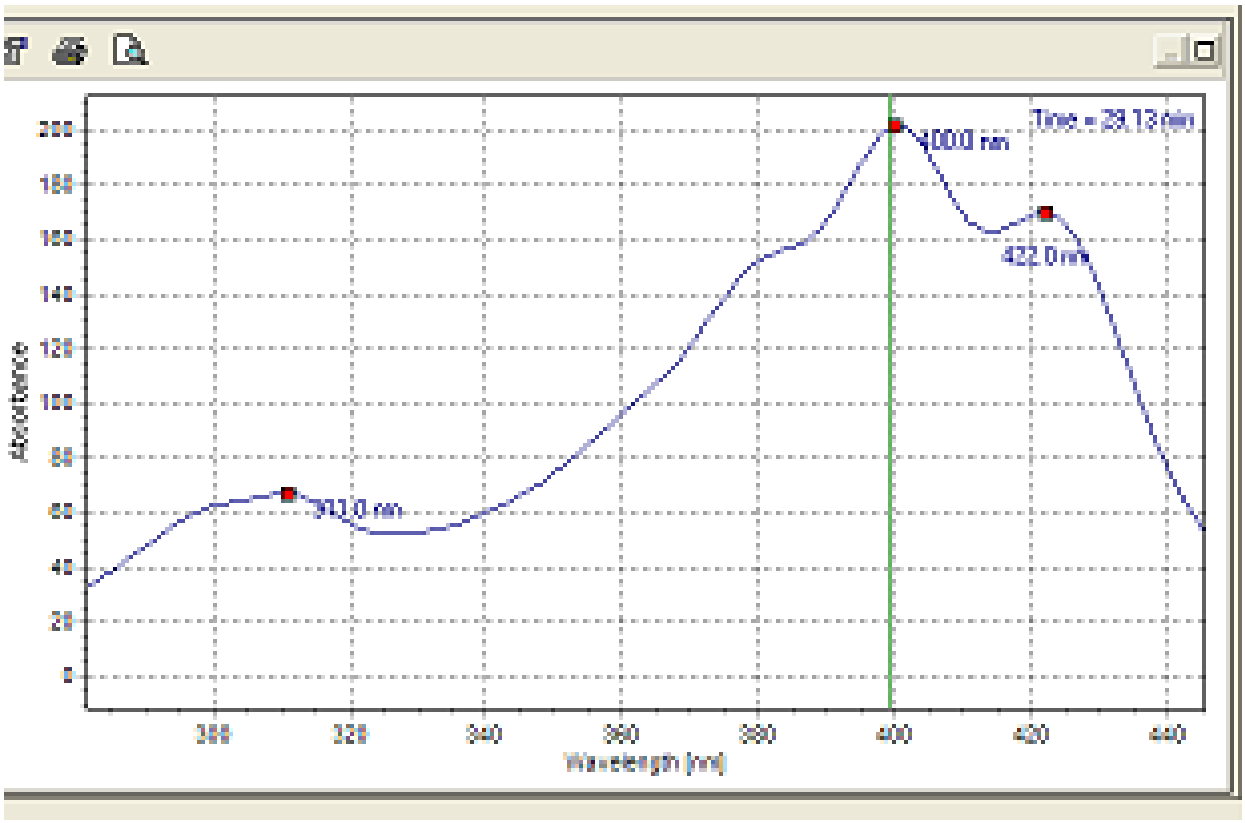


386+1, (386x2)+1, (386x3)+1, (386x4)+1
and small amounts of +23

Dimerisation and trimerisation in MS (with single charge)

KR10-1 Decaketide (21)

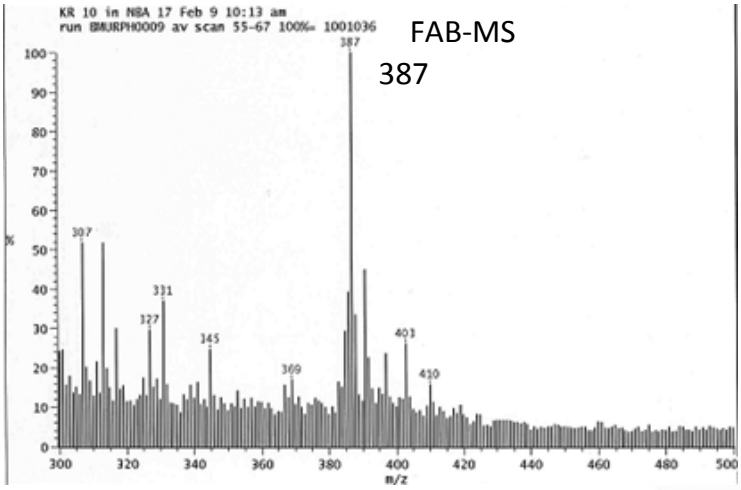
UV spectrum. Max at 422, 400(max), 380(sh) and 311.



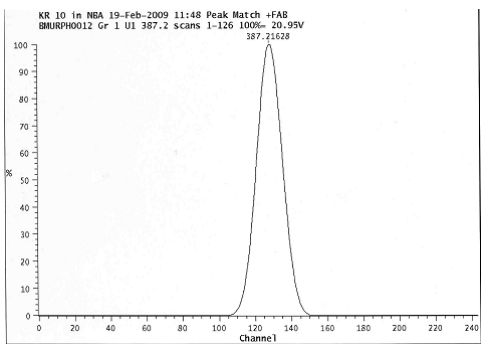
KR10-1 Decaketide (21)

FAB-MS

387



FAB Accurate Mass Data



Observed: 387.21628

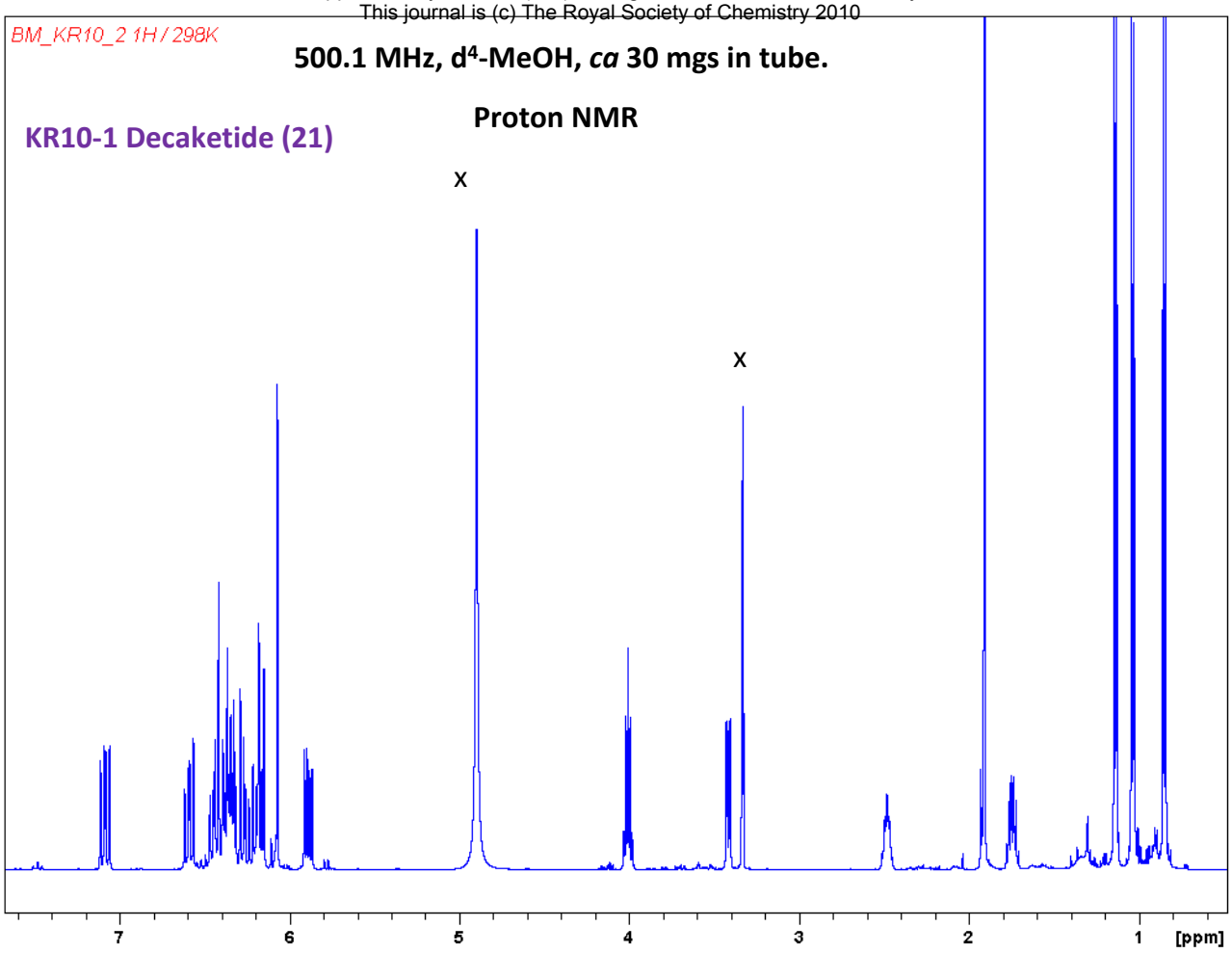
Run : BMURPH0012 Scan : 1

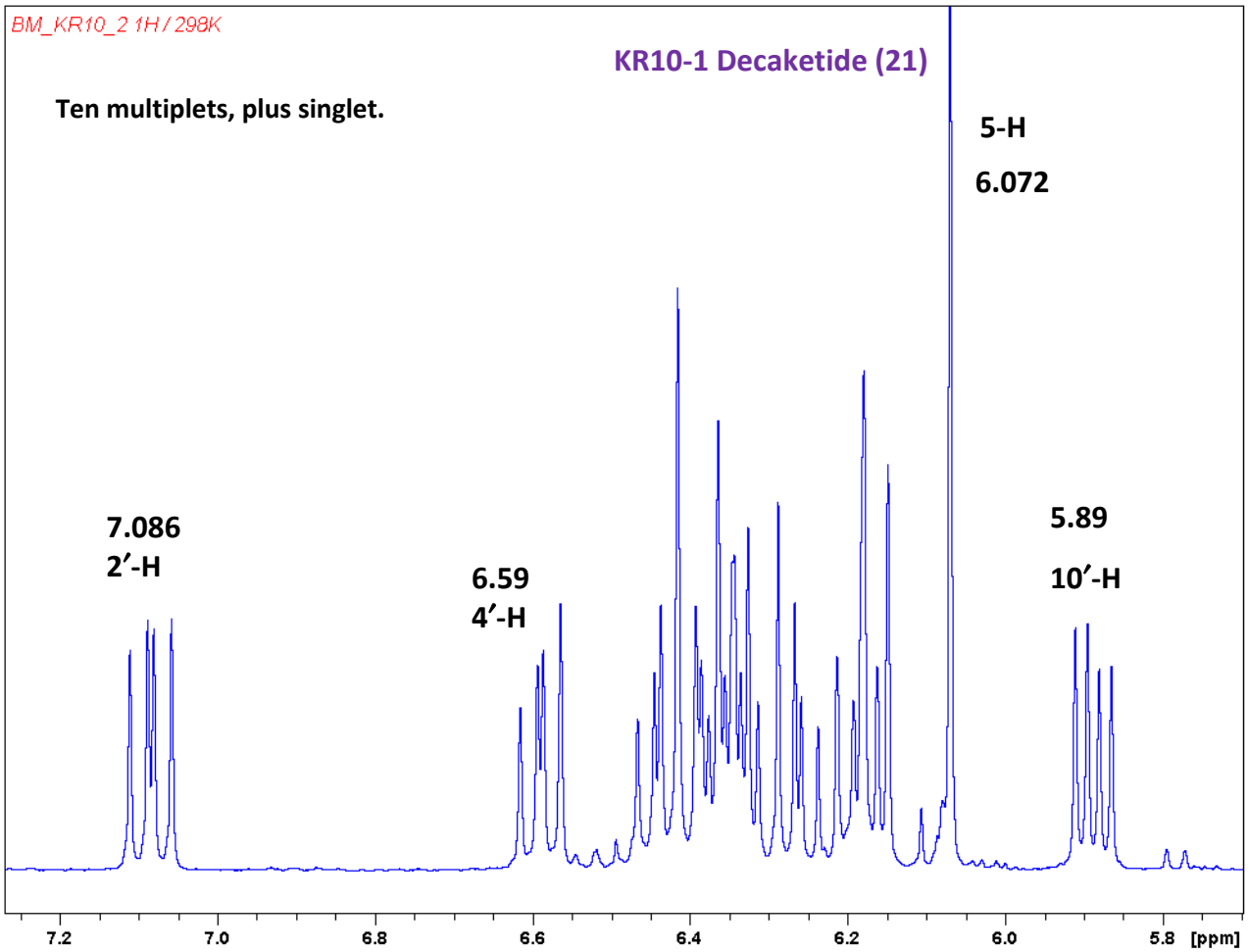
Mass	Int	Dev ppm	12 C	1 H	16 O
387.21628	30602556	-0.2	23	31	5

$C_{23}H_{31}O_5$ requires

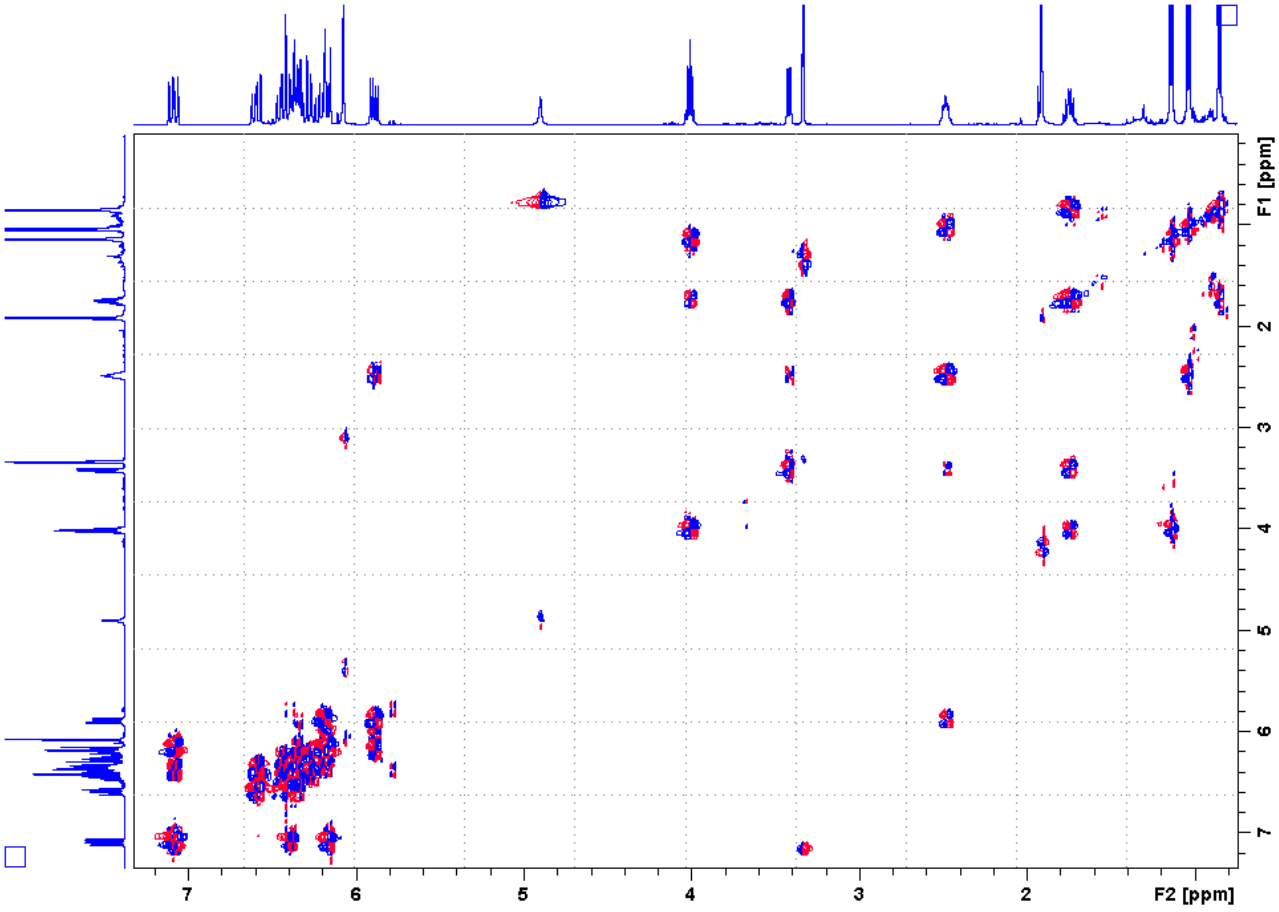
387.21637

Mass	Abundance	12 C	13 C	1 H	16 O
387.21637	77.5169	23	0	31	5
388.21973	20.0122	22	1	31	5
389.22308	2.4709	21	2	31	5

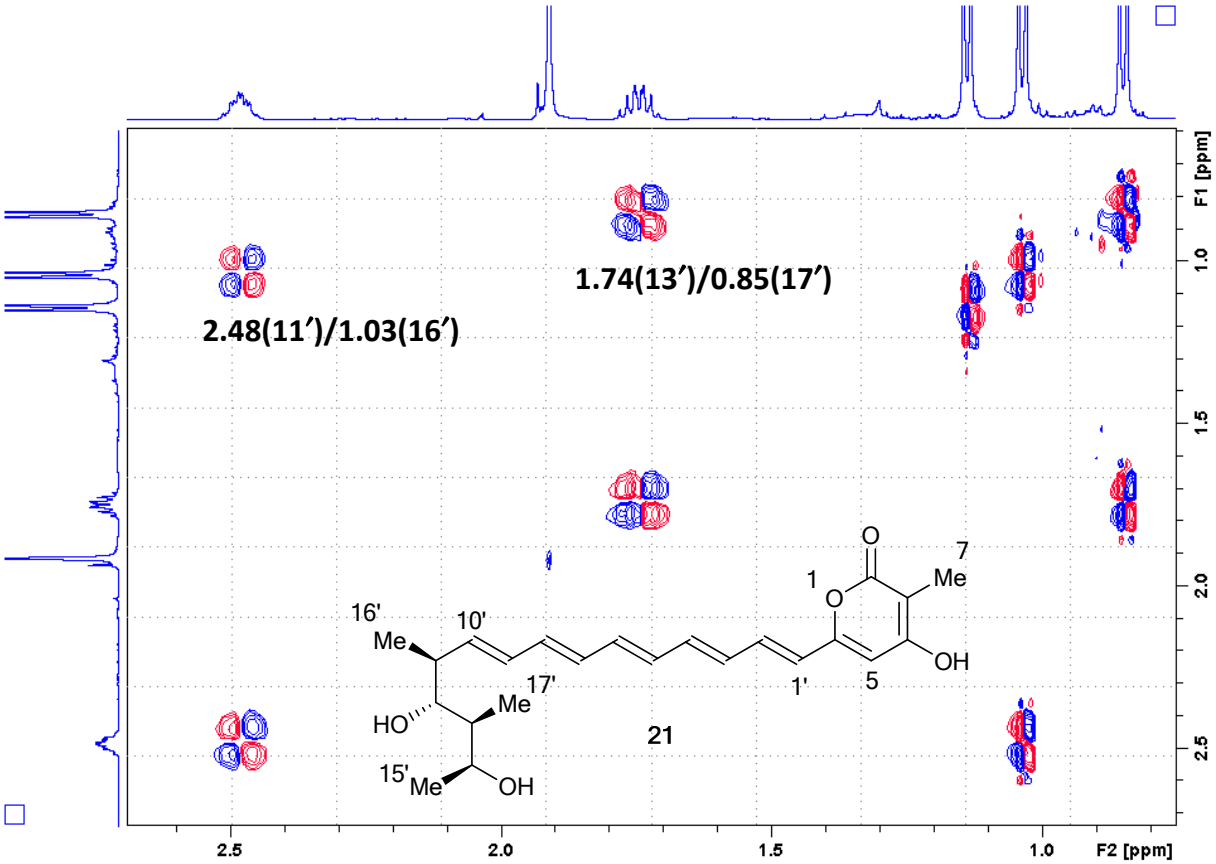




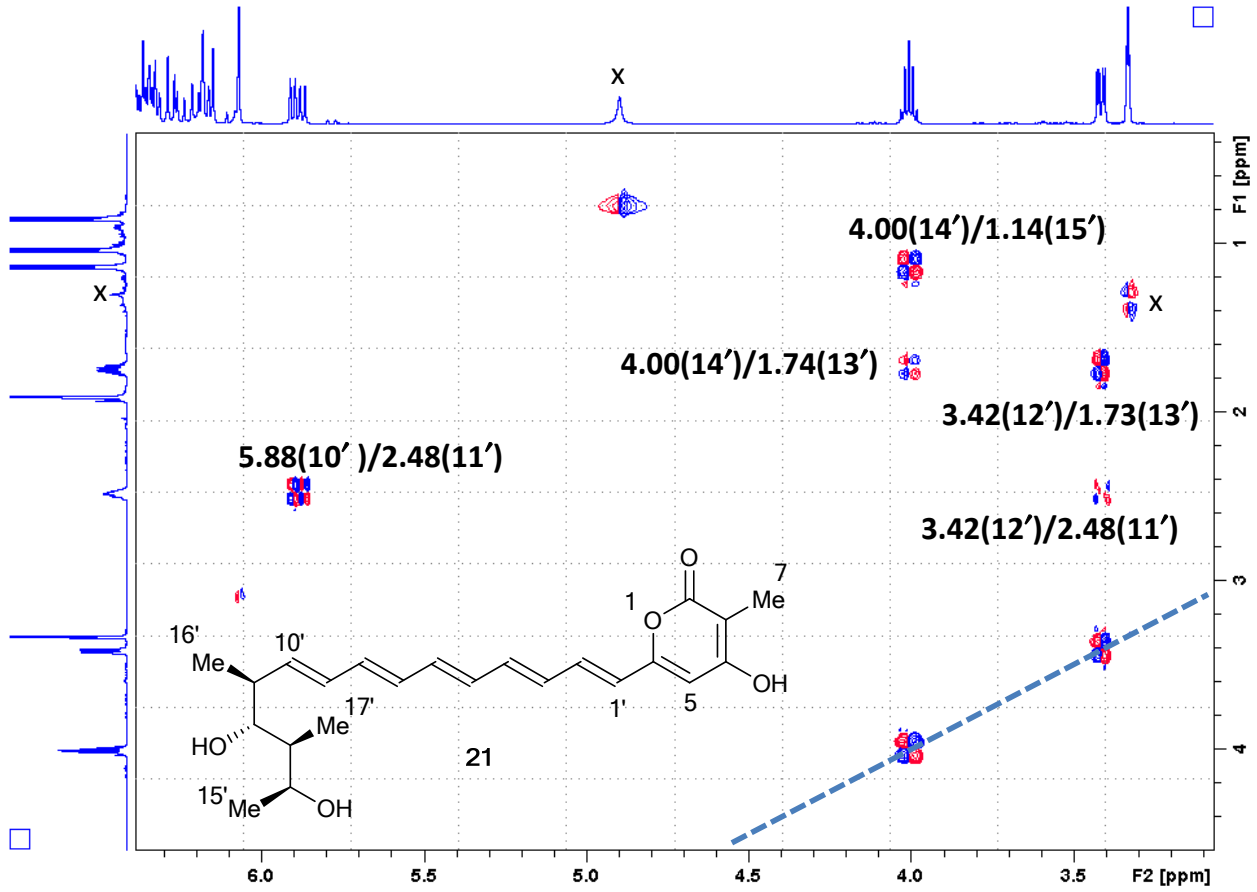
KR10-1 Decaketide (21)



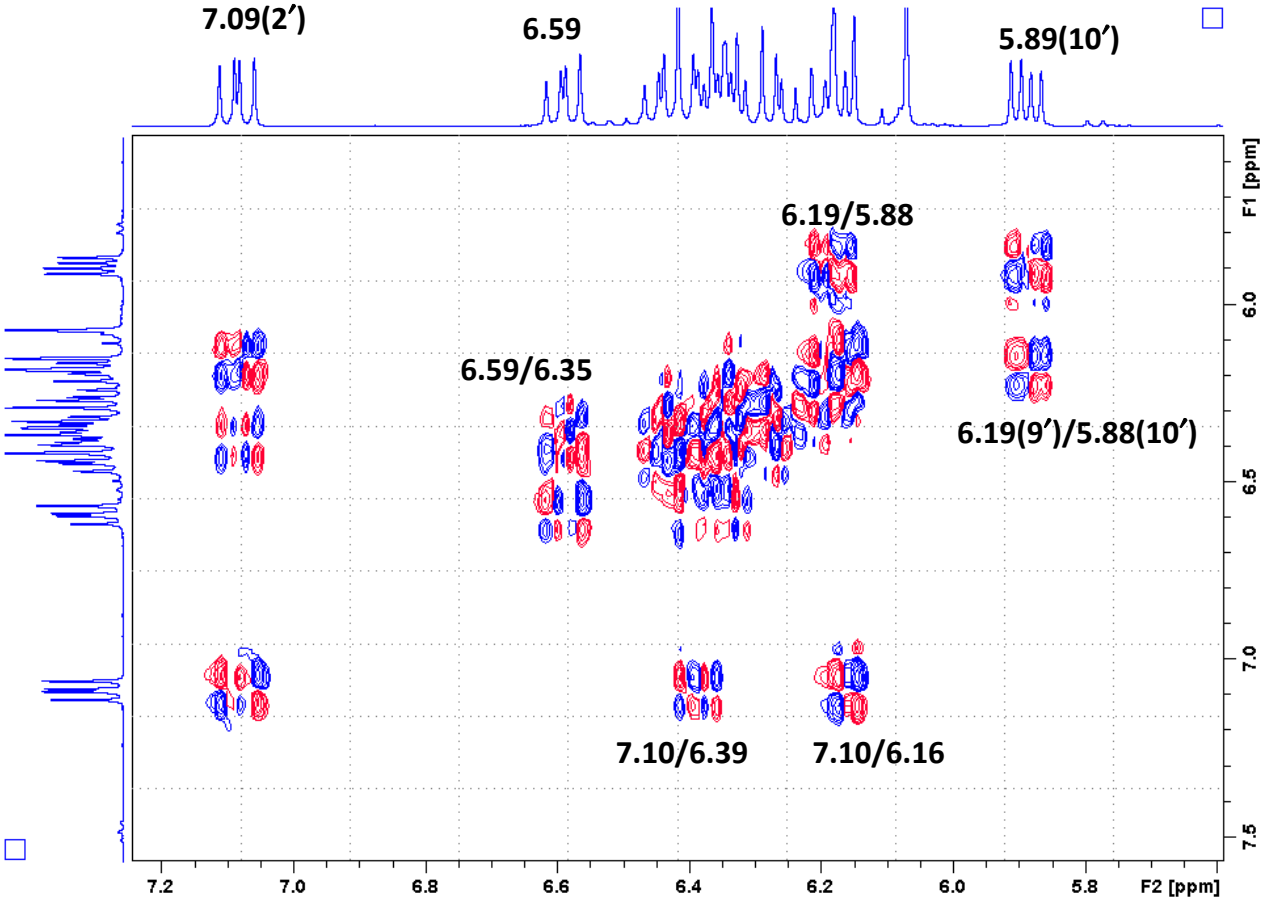
KR10-1 Decaketide (21)

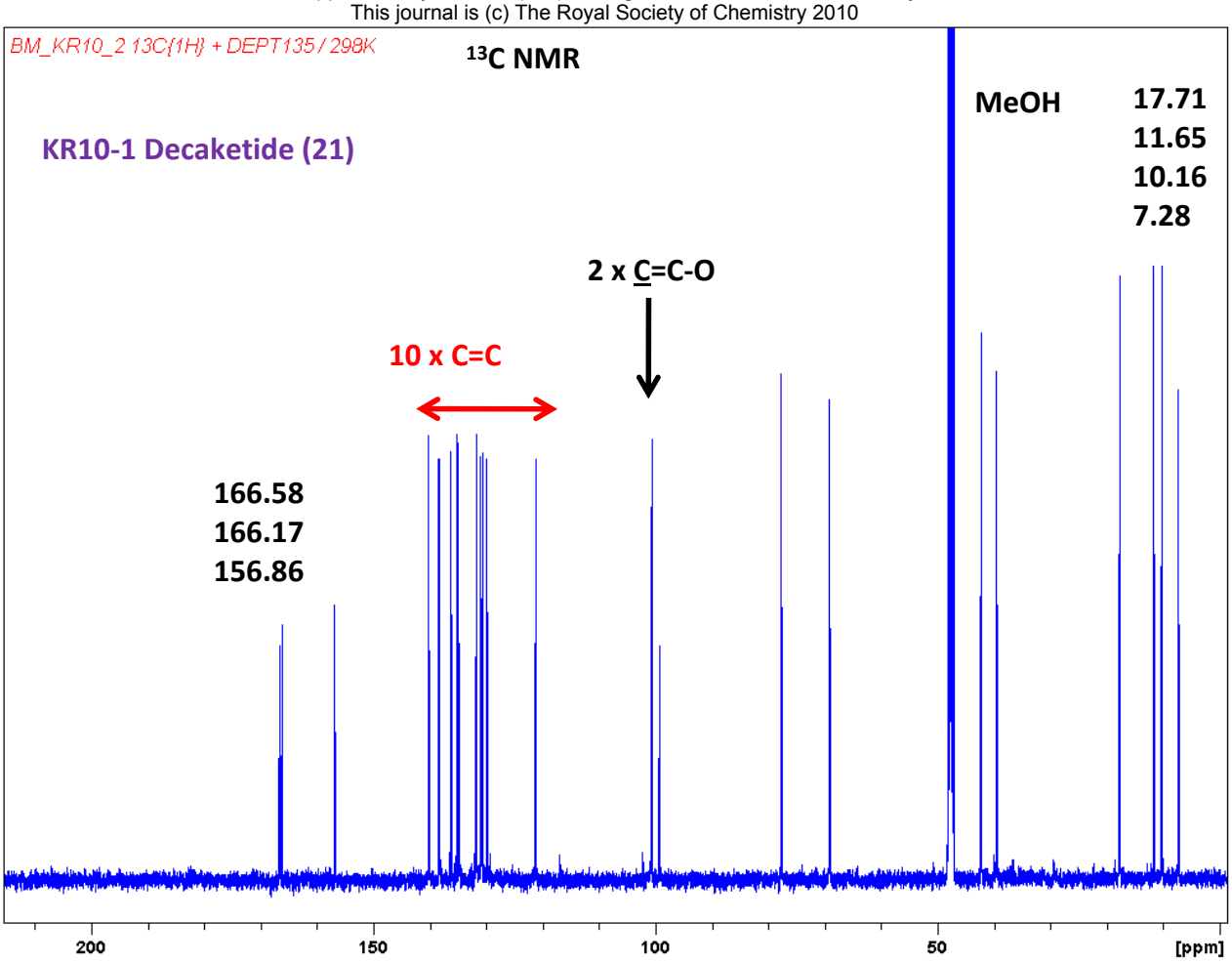


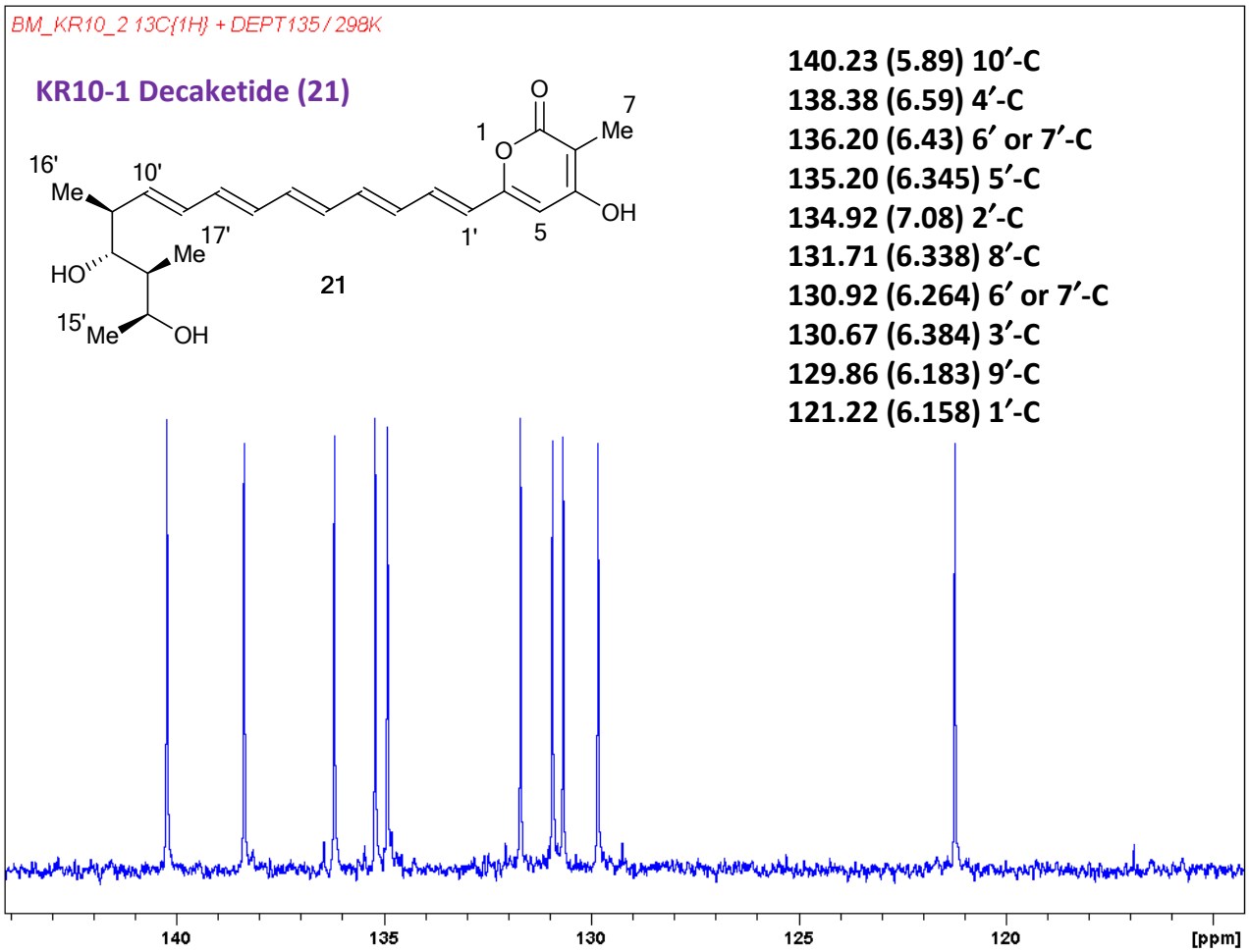
KR10-1 Decaketide (21)

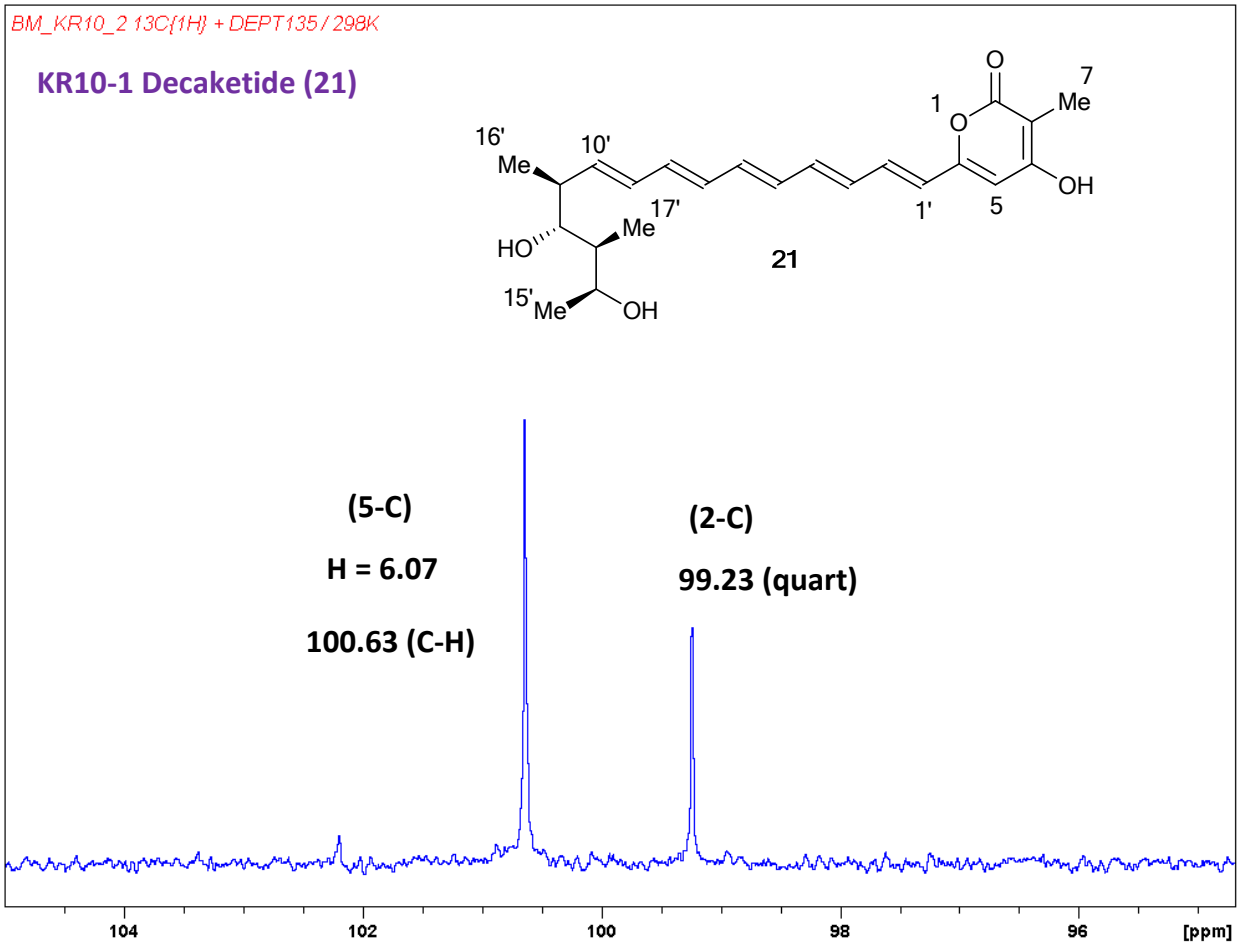


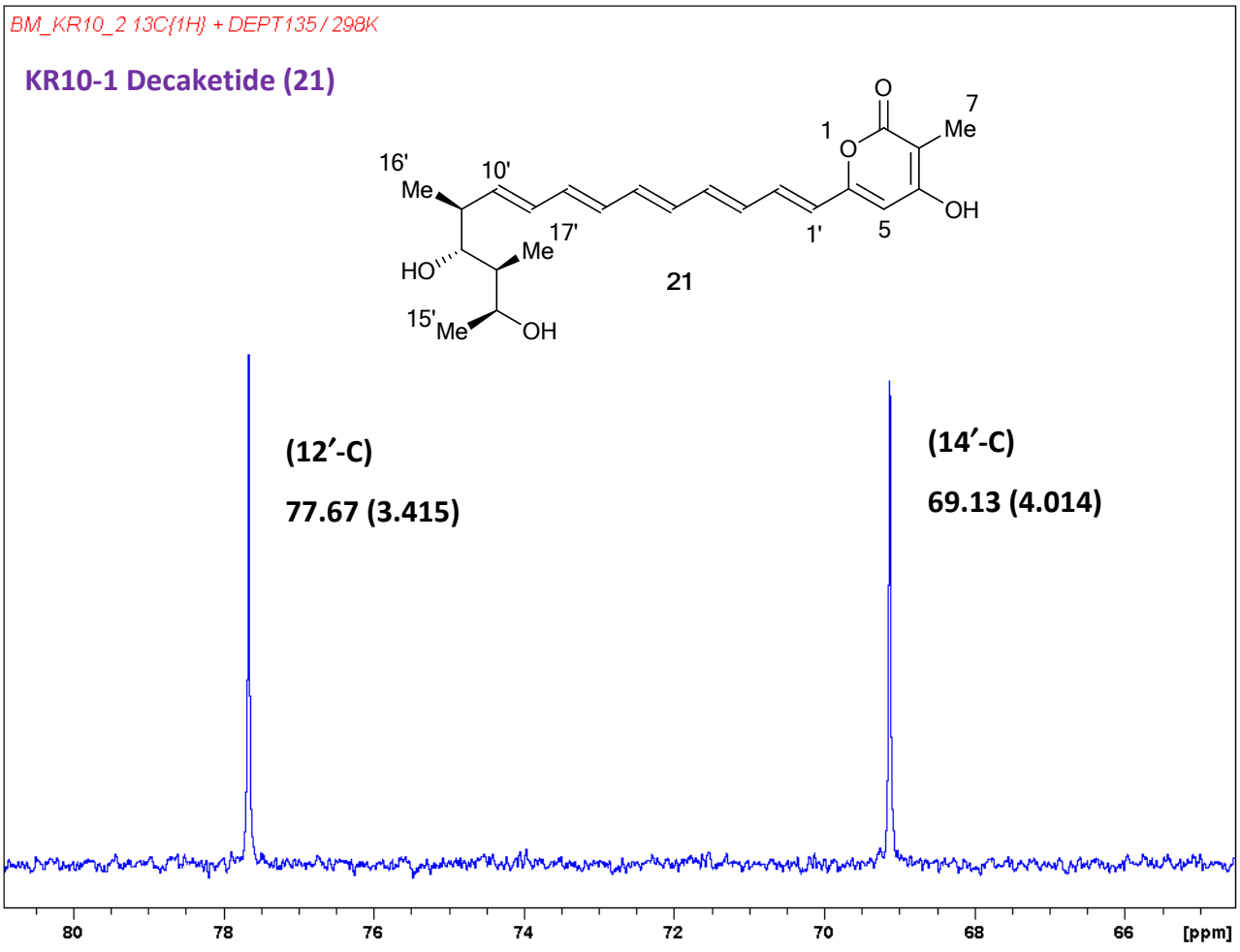
KR10-1 Decaketide (21)

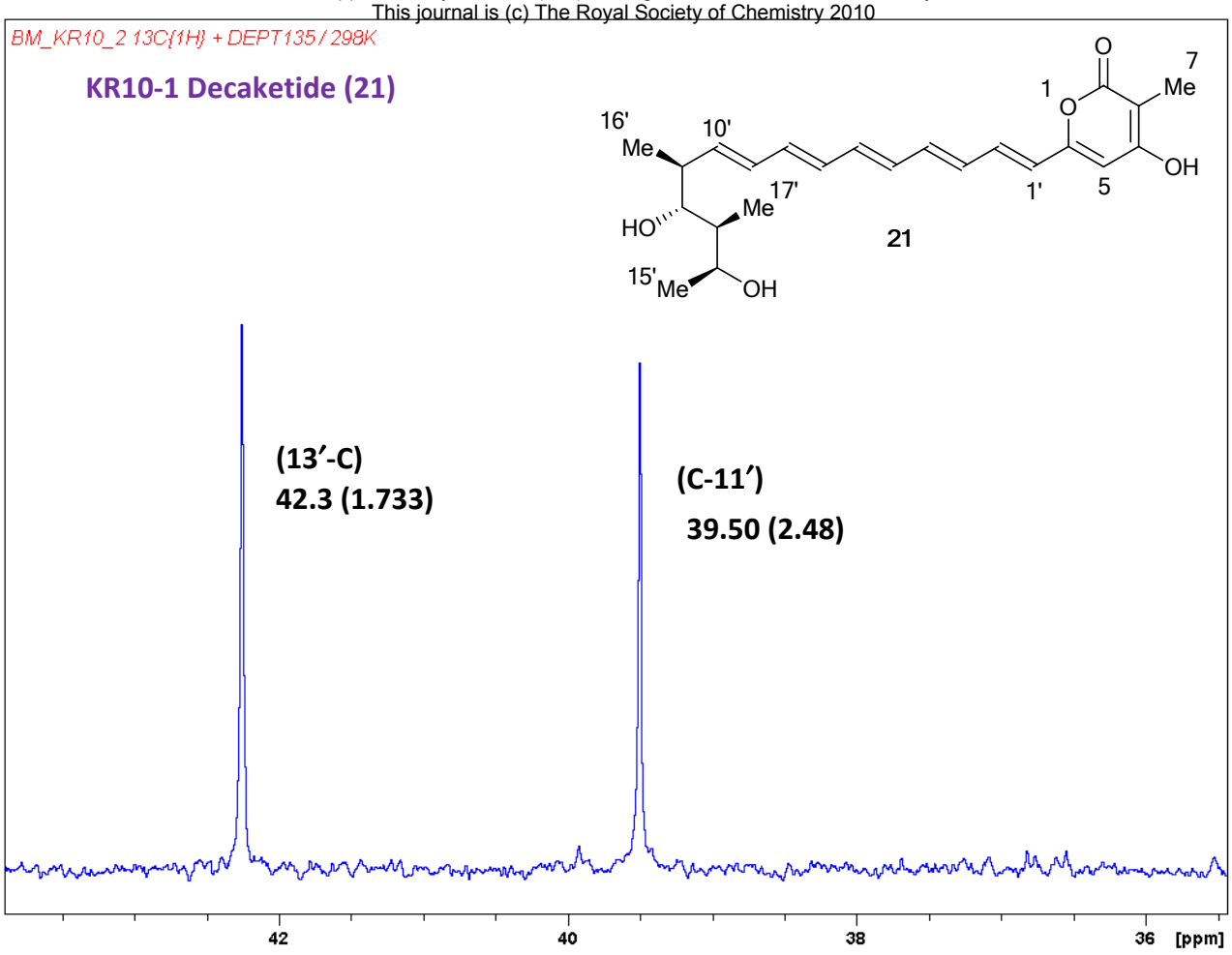


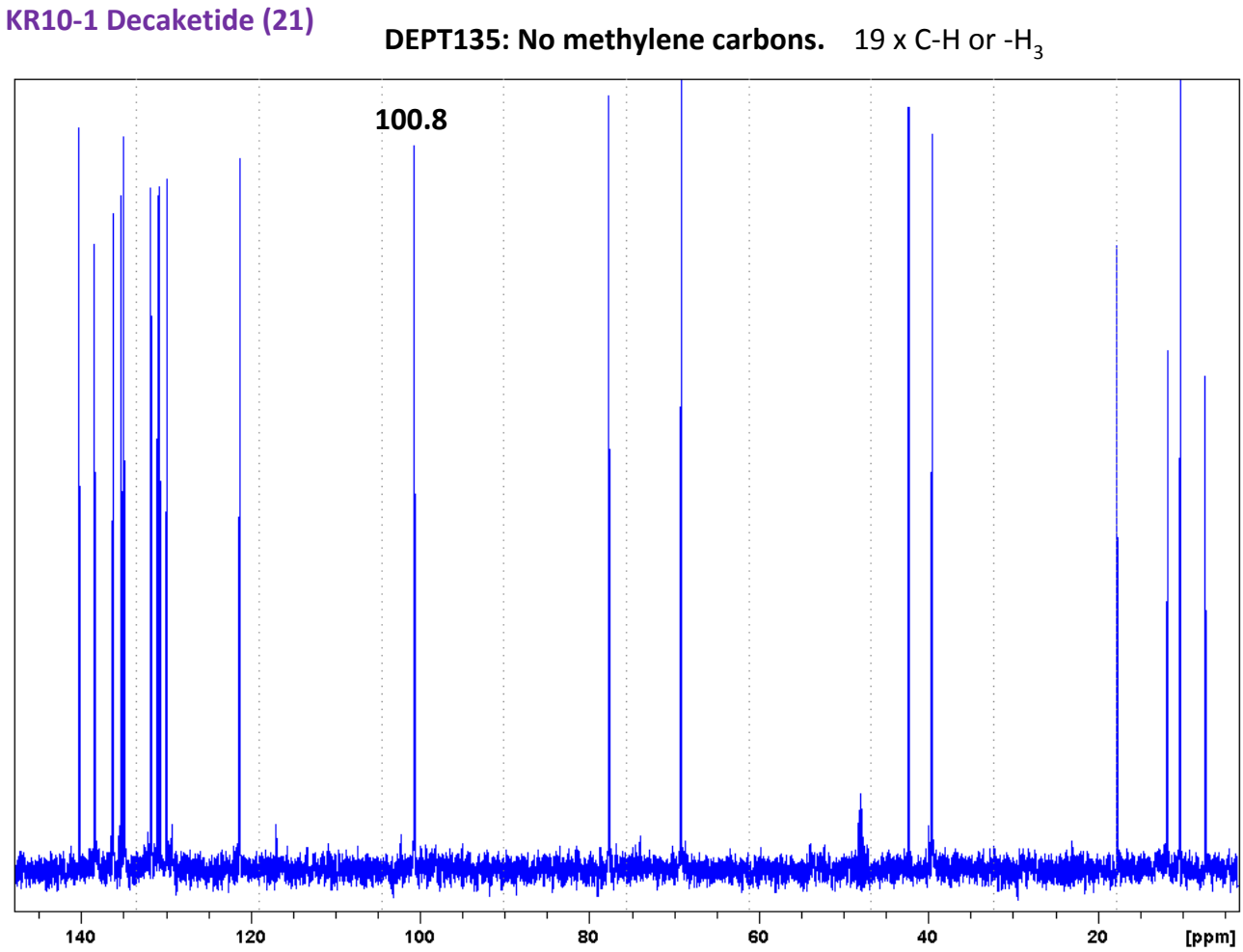

BM_KR10_2 13C{1H} + DEPT135 / 298K
^{13}C NMR
KR10-1 Decaketide (21)
MeOH
17.71
11.65
10.16
7.28
2 x C=C-O
10 x C=C
166.58
166.17
156.86
200
150
100
50
[ppm]


BM_KR10_2 13C{1H} + DEPT135 / 298K
KR10-1 Decaketide (21)
O
7
Me
1
O
OH
16'
Me
10'
17'
Me
HO
15'
Me
OH
1'
5
21
140.23 (5.89) 10′-C
138.38 (6.59) 4′-C
136.20 (6.43) 6′ or 7′-C
135.20 (6.345) 5′-C
134.92 (7.08) 2′-C
131.71 (6.338) 8′-C
130.92 (6.264) 6′ or 7′-C
130.67 (6.384) 3′-C
129.86 (6.183) 9′-C
121.22 (6.158) 1′-C
140
135
130
125
120
[ppm]

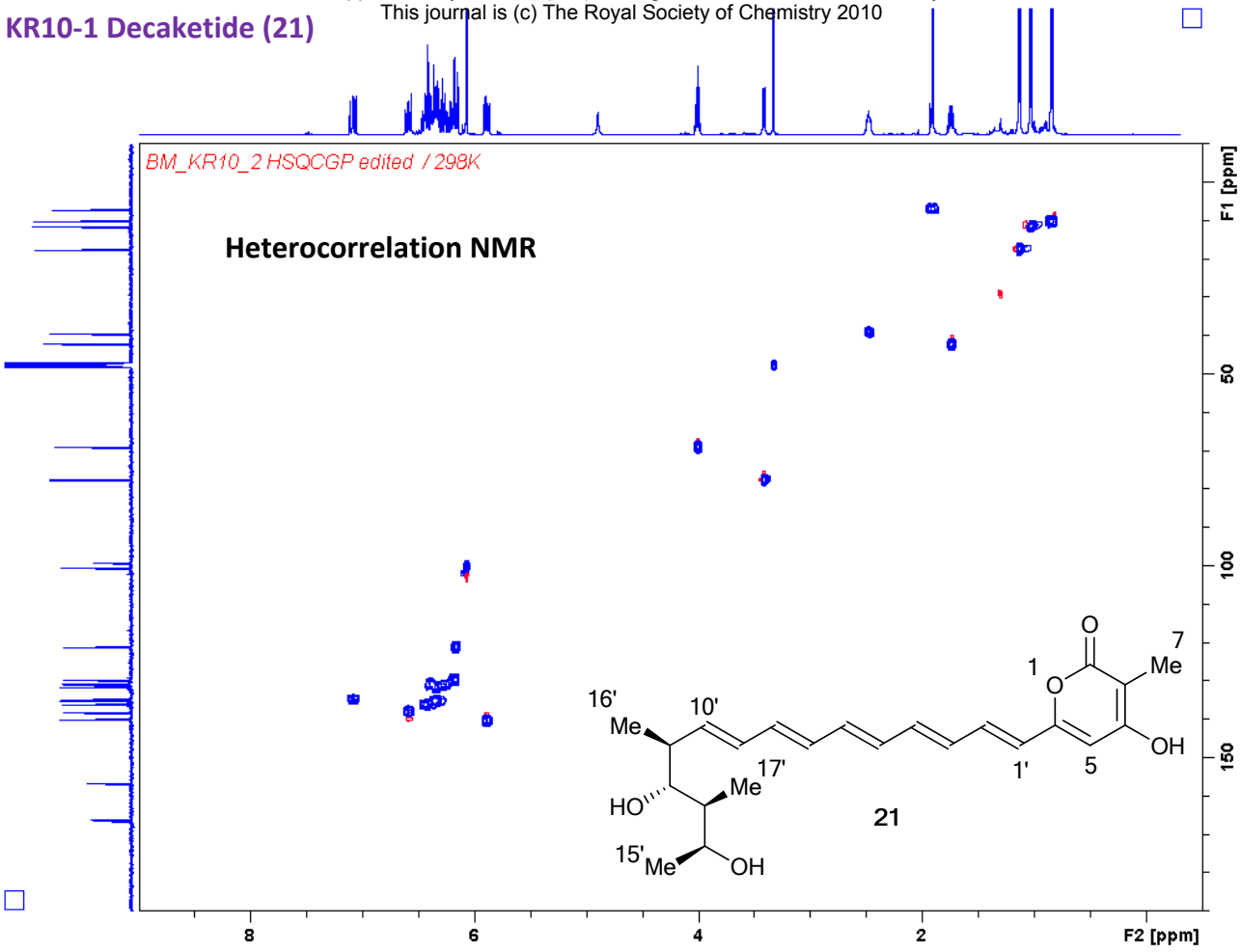

BM_KR10_2 13C{1H} + DEPT135 / 298K
KR10-1 Decaketide (21)
O
7
Me
1
O
16'
Me
10'
OH
17'
1'
5
Me
HO
21
15'
Me
OH
(5-C)
H = 6.07
100.63 (C-H)
(2-C)
99.23 (quart)
104
102
100
98
96
[ppm]


BM_KR10_2 13C{1H} + DEPT135 / 298K
KR10-1 Decaketide (21)
O
7
Me
1
O
16'
Me
10'
OH
17'
1'
5
Me
HO
21
15'
Me
OH
(12′-C)
77.67 (3.415)
(14′-C)
69.13 (4.014)
80
78
76
74
72
70
68
66
[ppm]

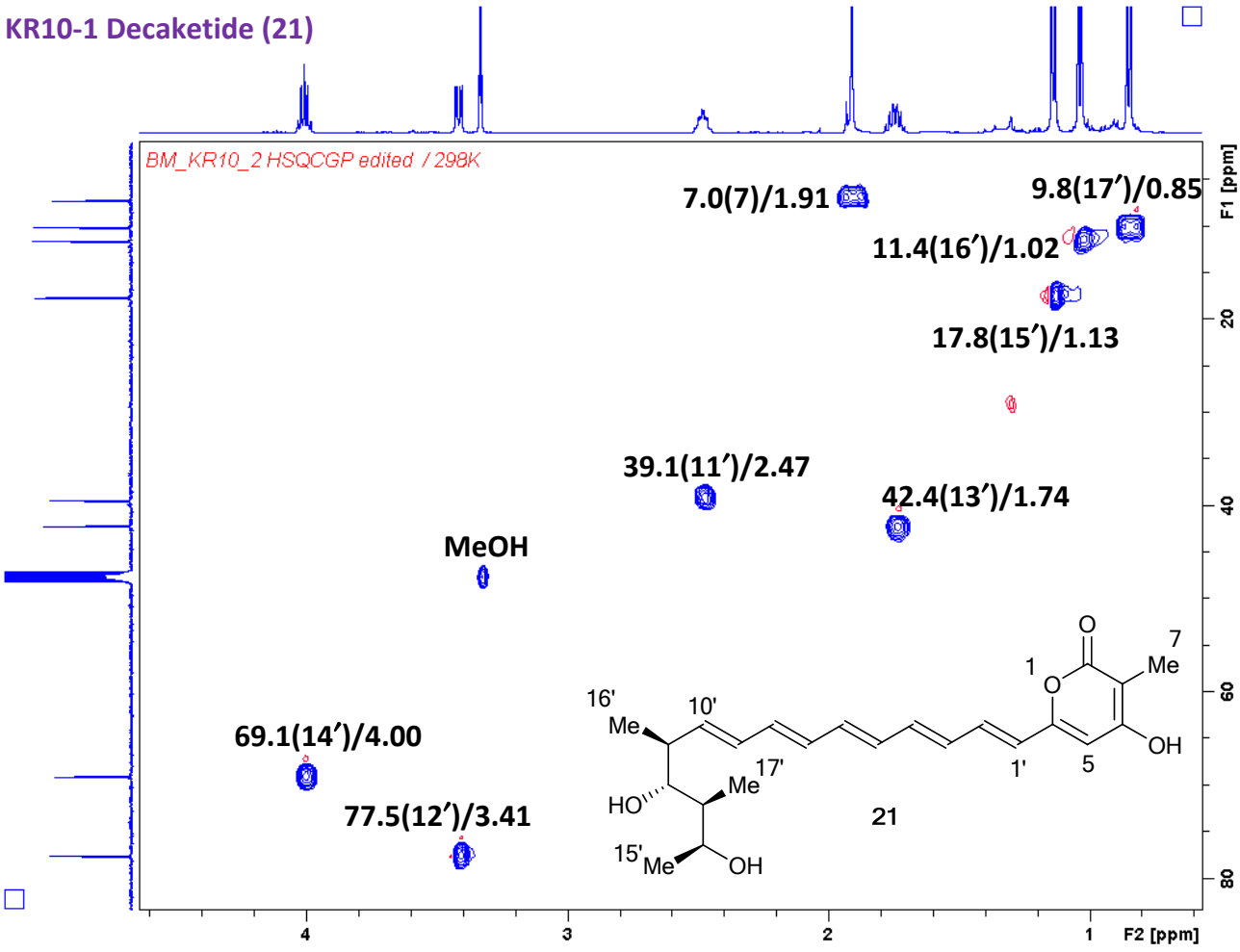

BM_KR10_2 13C{1H} + DEPT135 / 298K
KR10-1 Decaketide (21)
16'
Me
10'
17'
Me
HO
15'
Me
OH
1
O
O
7
Me
OH
5
1'
21
(13′-C)
42.3 (1.733)
(C-11′)
39.50 (2.48)
42
40
38
36
[ppm]


KR10-1 Decaketide (21)
DEPT135: No methylene carbons. 19 x C-H or -H3
100.8
140
120
100
80
60
40
20
[ppm]

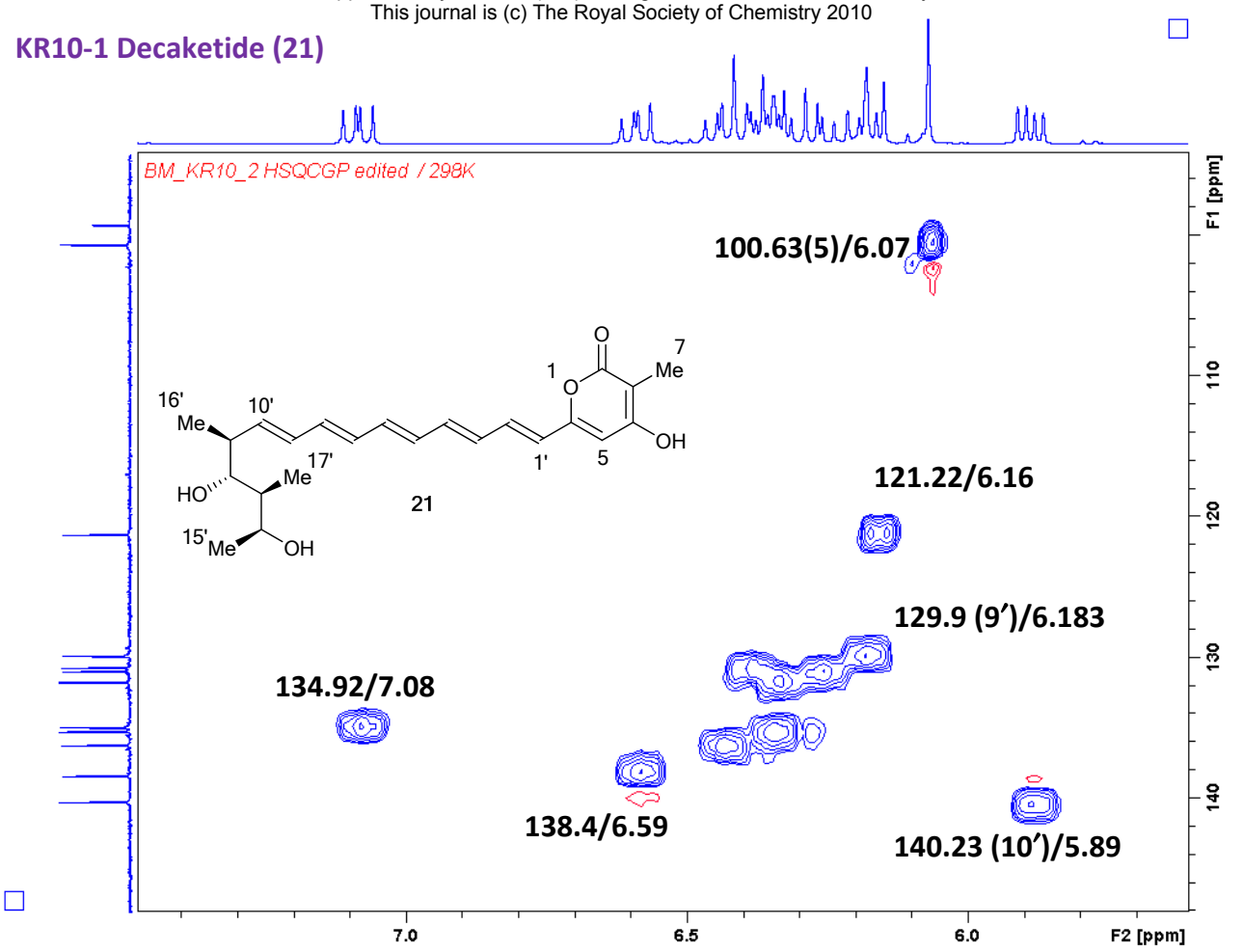
KR10-1 Decaketide (21)



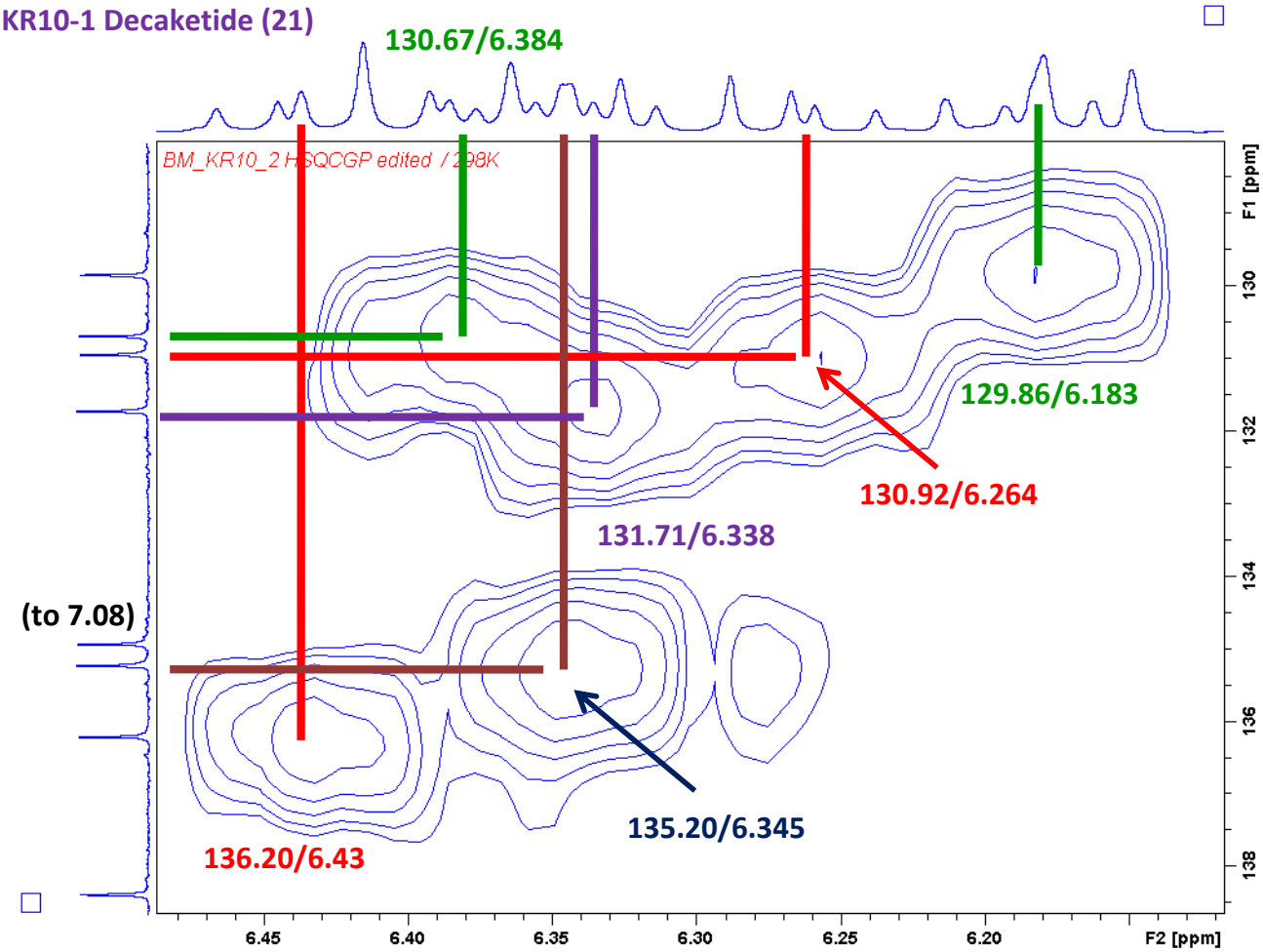
KR10-1 Decaketide (21)



KR10-1 Decaketide (21)



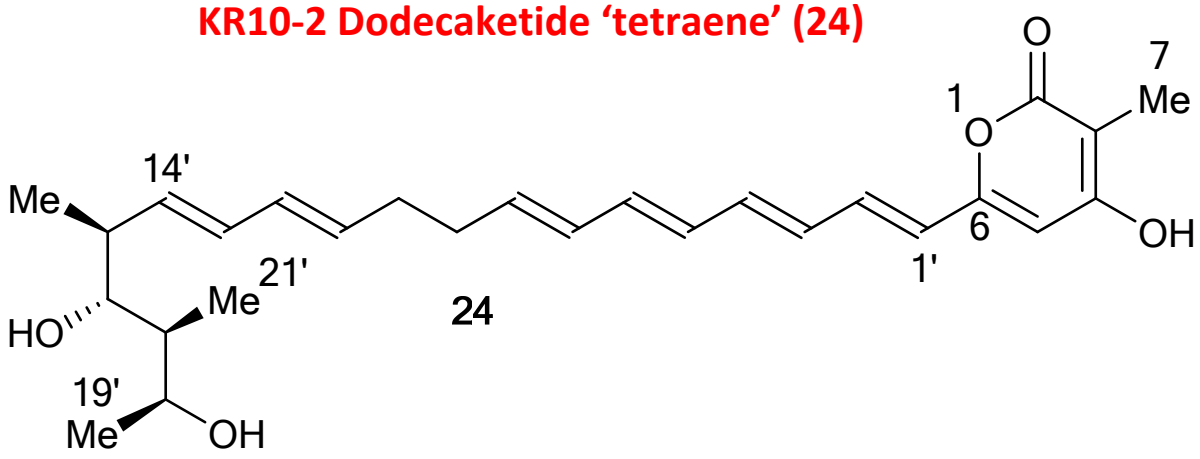
KR10-1 Decaketide (21)



KR10-2 Dodecaketide 'tetraene' (24)

(and trace 'heptaene' analogue)

KR10-2 Dodecaketide 'tetraene' (24)

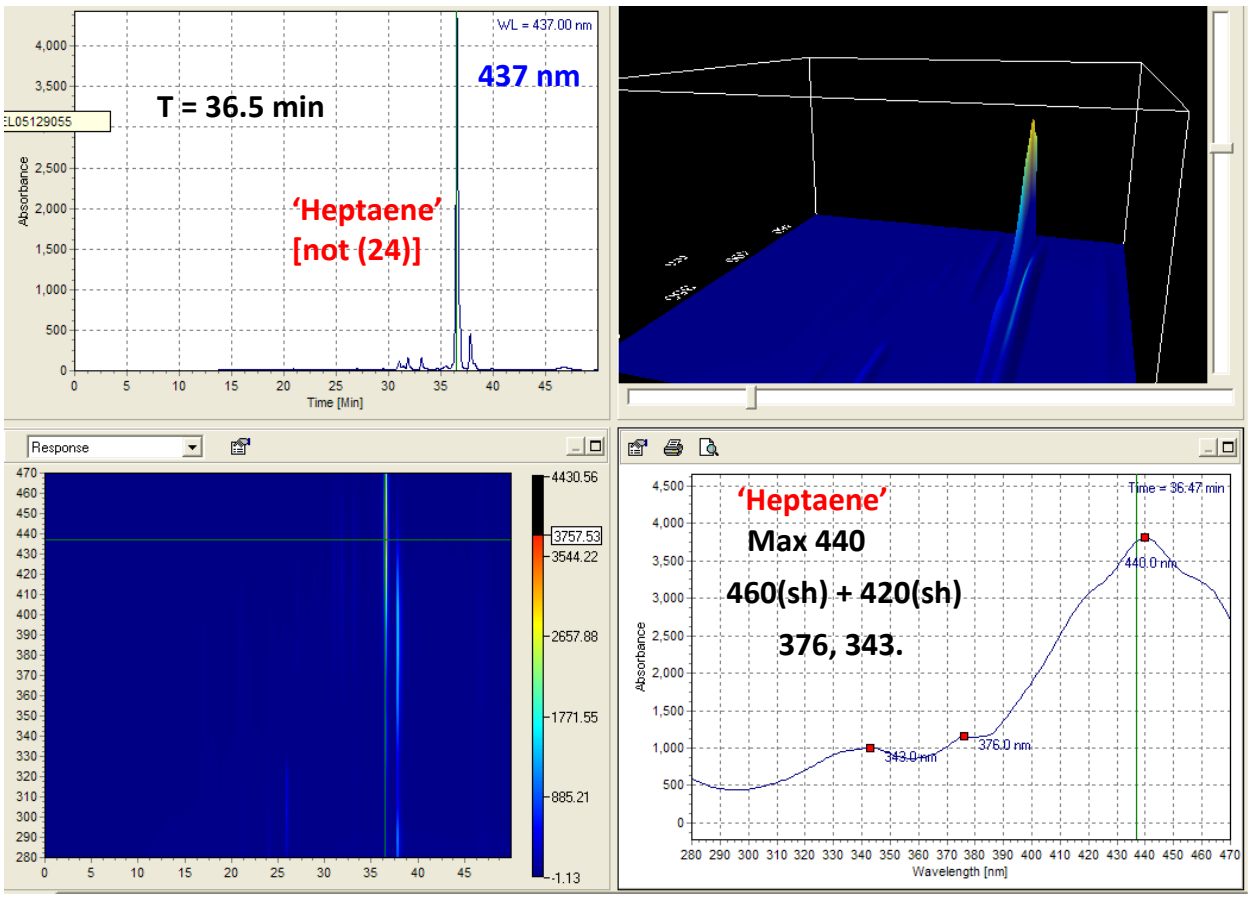


$C_{27}H_{36}O_5$ = 440.4

6-[(1*E*,3*E*,5*E*,7*E*,11*E*,13*E*,15*S*,16*S*,17*S*,18*S*)-16,18-Dihydroxy-15,17-dimethyl-1,3,5,9,11,13-heptadecahexen-1-yl]-4-hydroxy-3-methyl-2-pyrone (24)

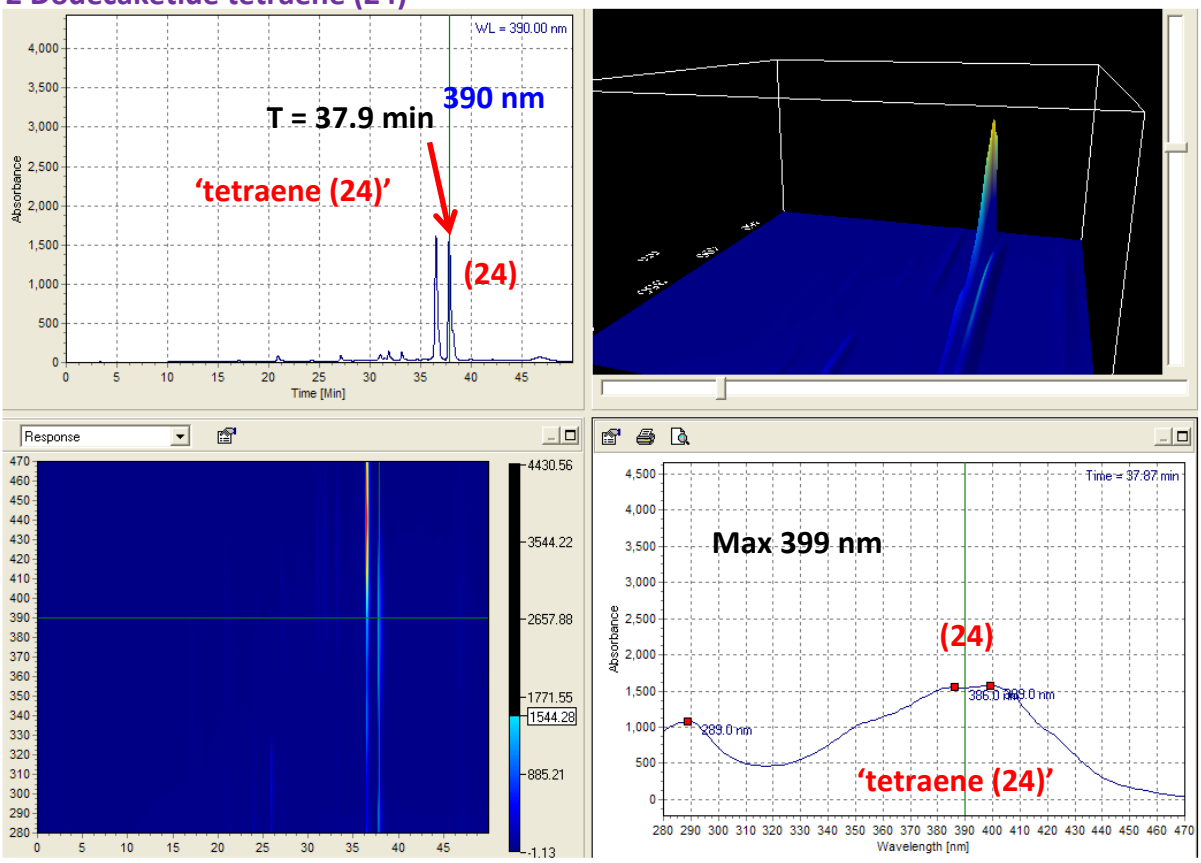
Position of the dihydro, and stereochemistry assumed from amphotericin B

KR10-2 Dodecaketide **HPLC of ppte (prior to washing) RPHPLC Heptaene peak**



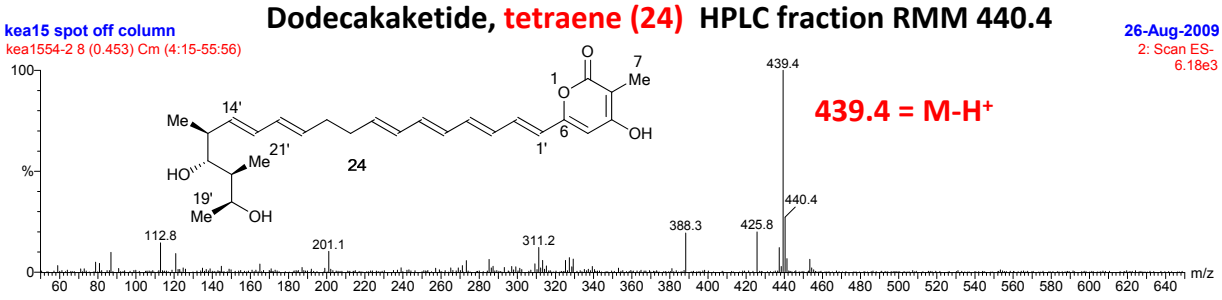
HPLC of ppte (prior to washing) RPHPLC Tetraene peak (24)

KR10-2 Dodecaketide tetraene (24)

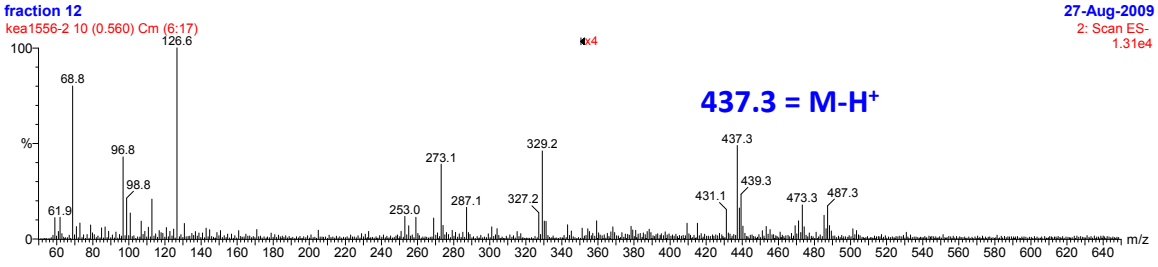


KR10-2 Dodecaketide tetraene (24) ESMS(-ve) analysis

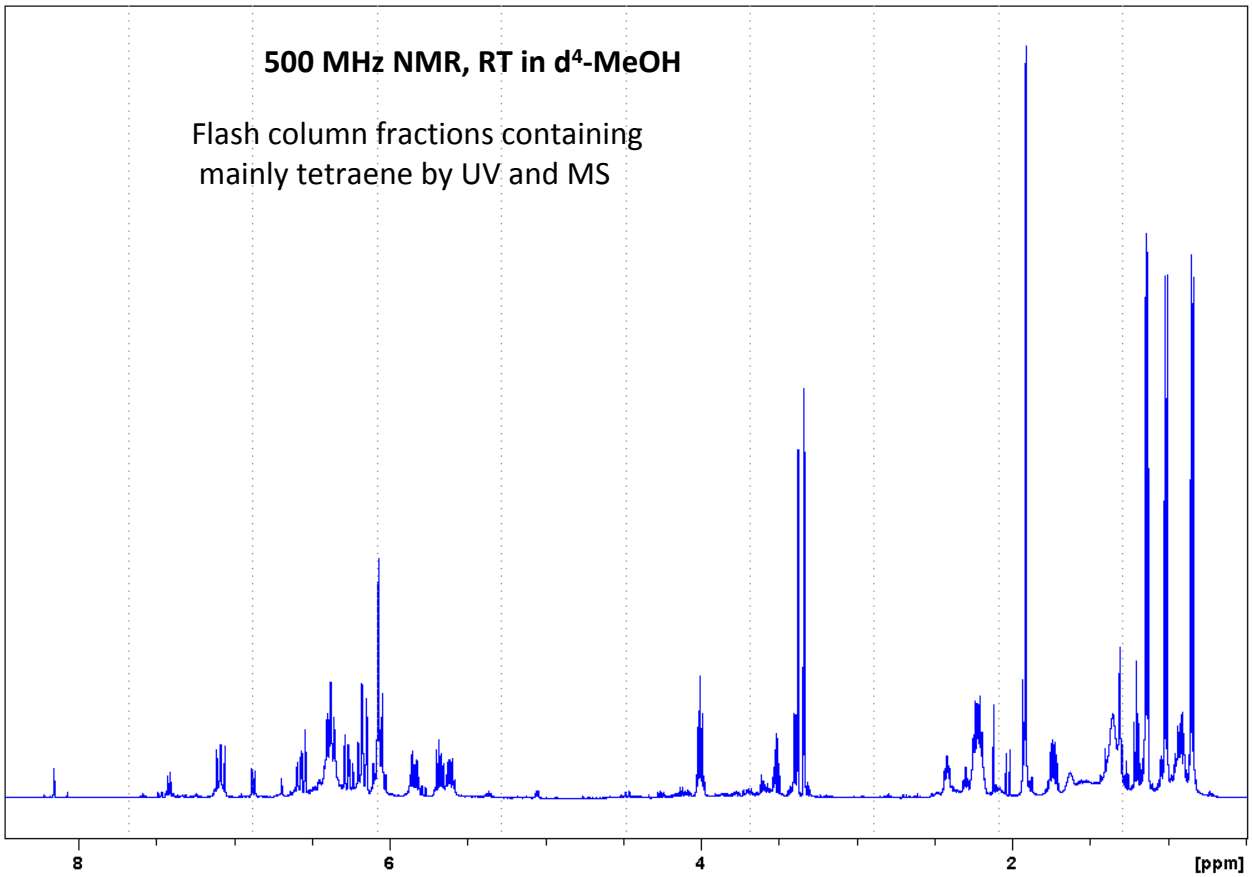
Dodecakaketide, tetraene (24) HPLC fraction RMM 440.4



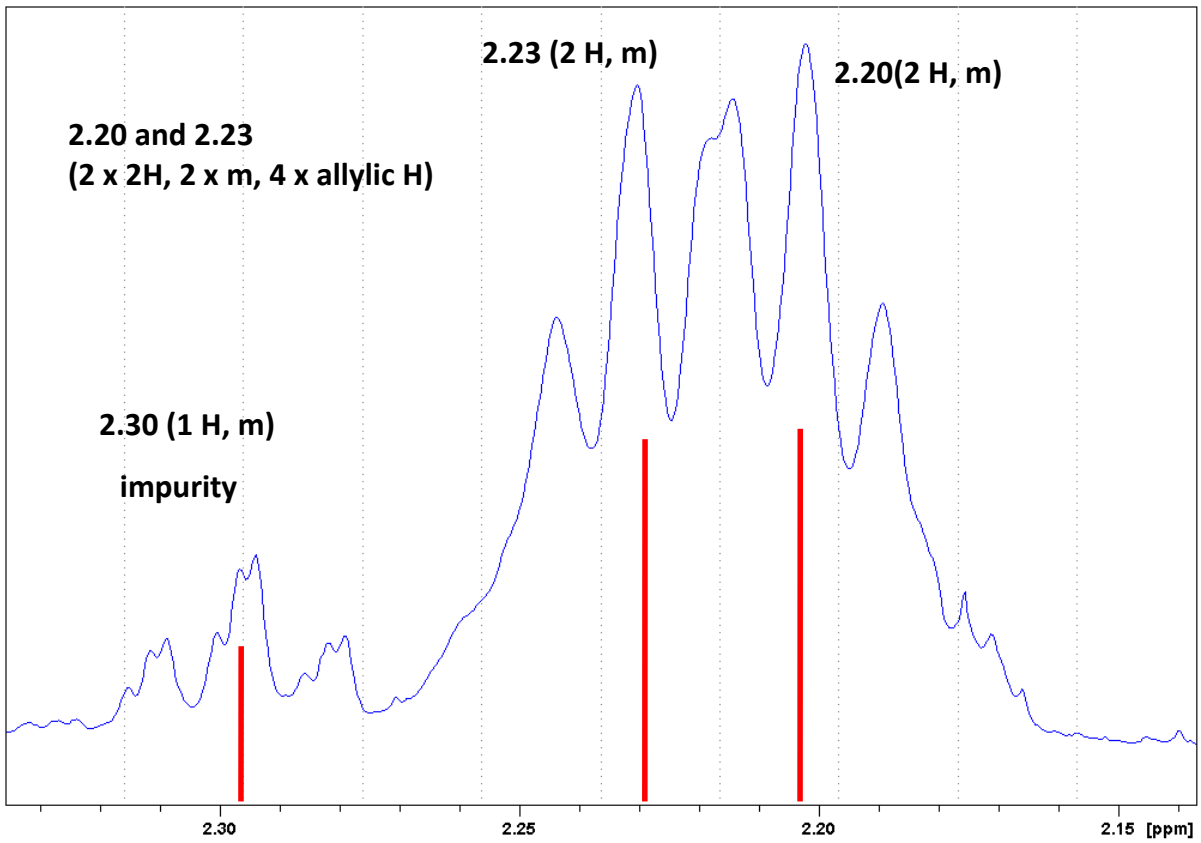
Dodecakaketide, heptaene HPLC fraction, RMM 438.4



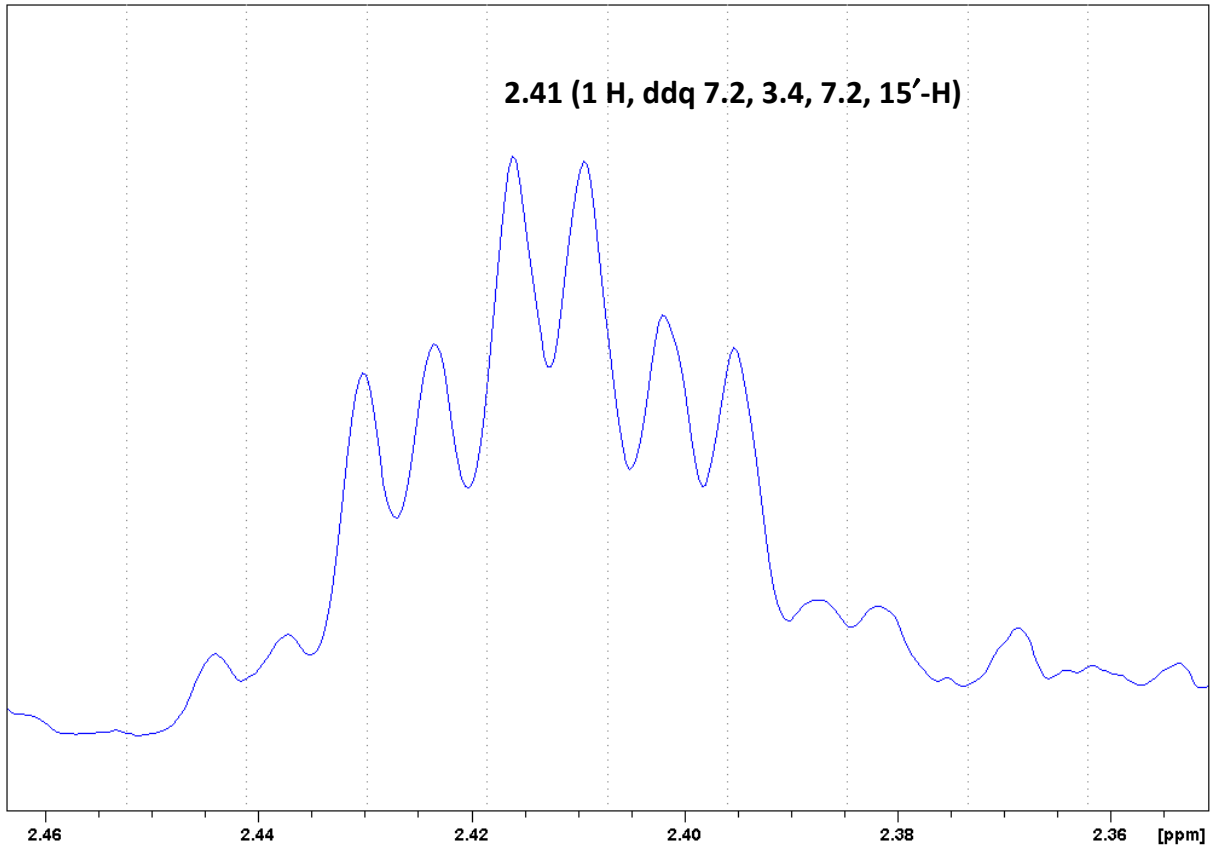
KR10-2 Dodecaketide tetraene (24)



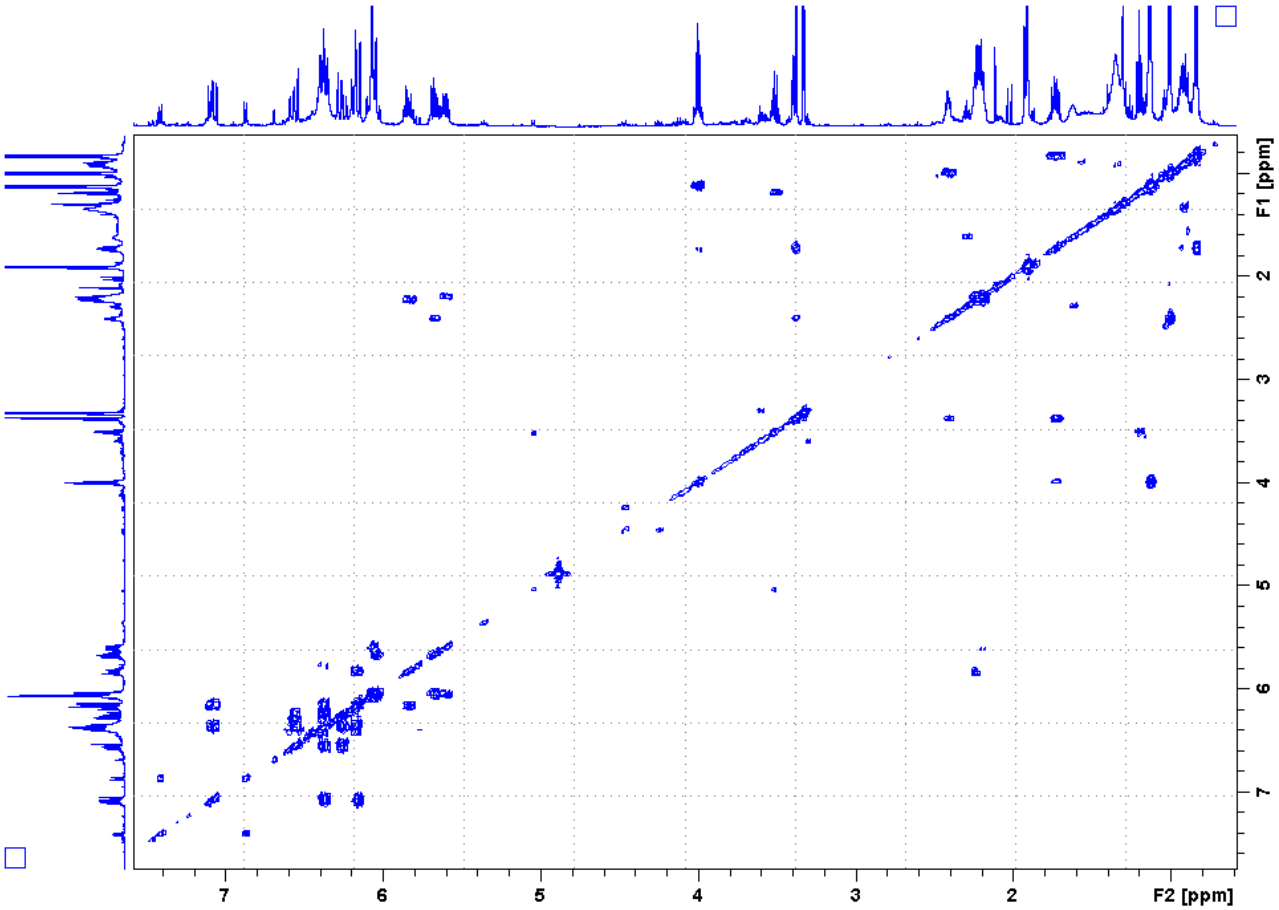
KR10-2 Dodecaketide tetraene (24)



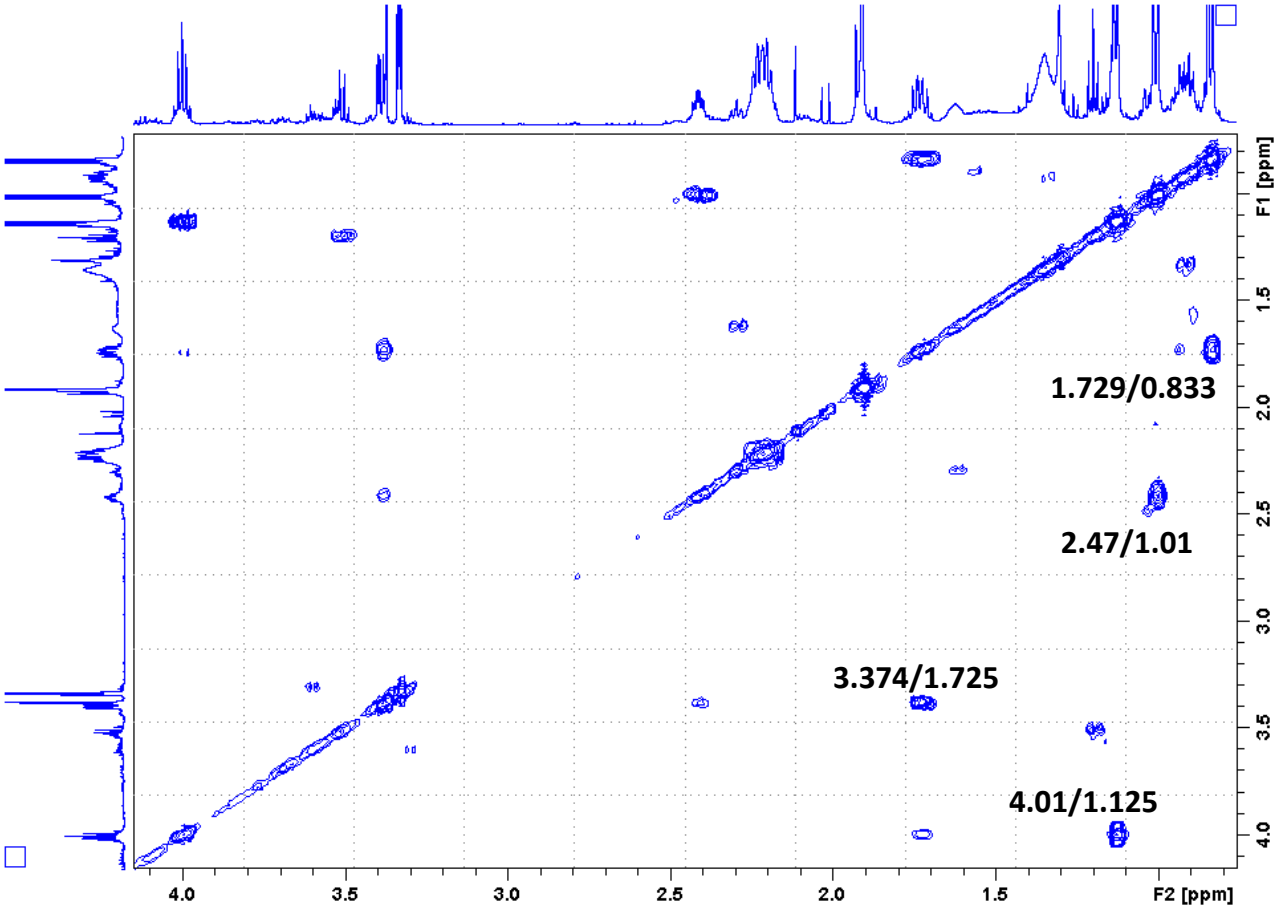
KR10-2 Dodecaketide tetraene (24)



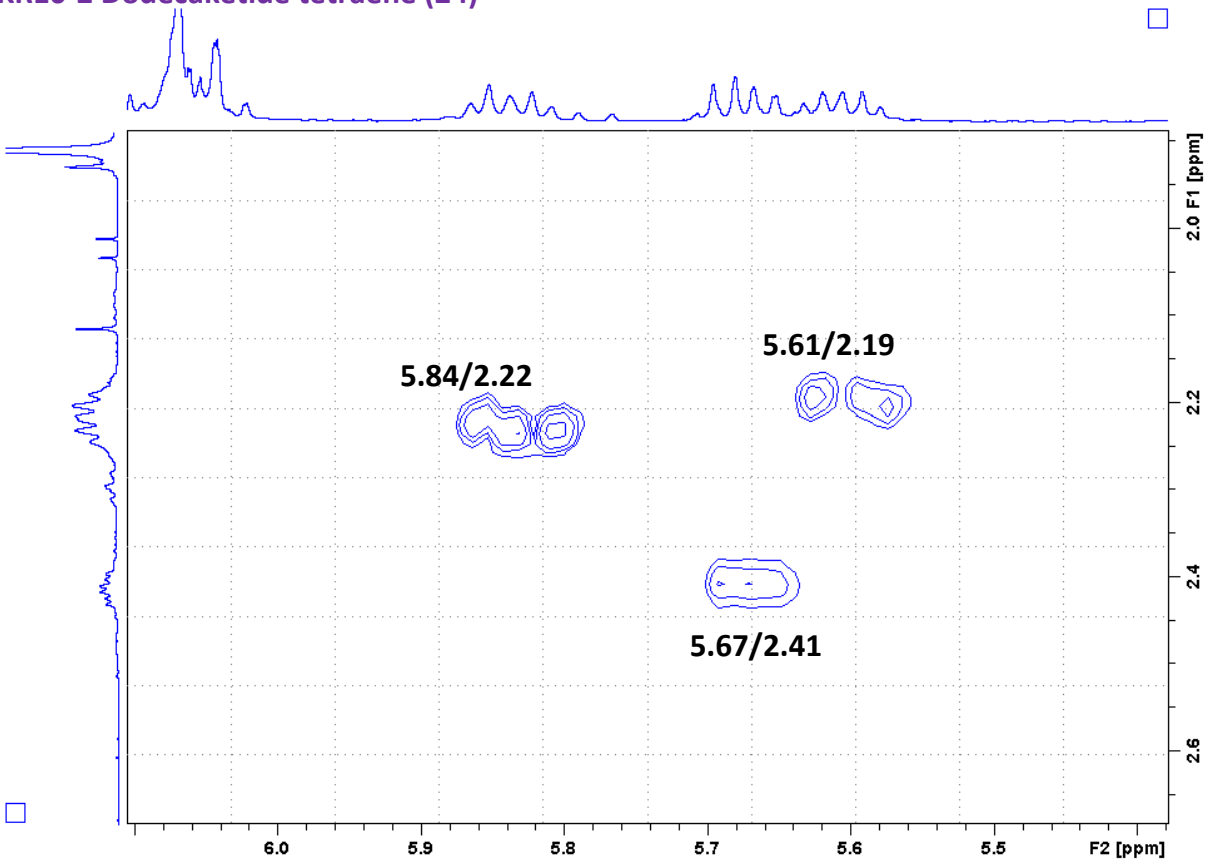
KR10-2 Dodecaketide tetraene (24)



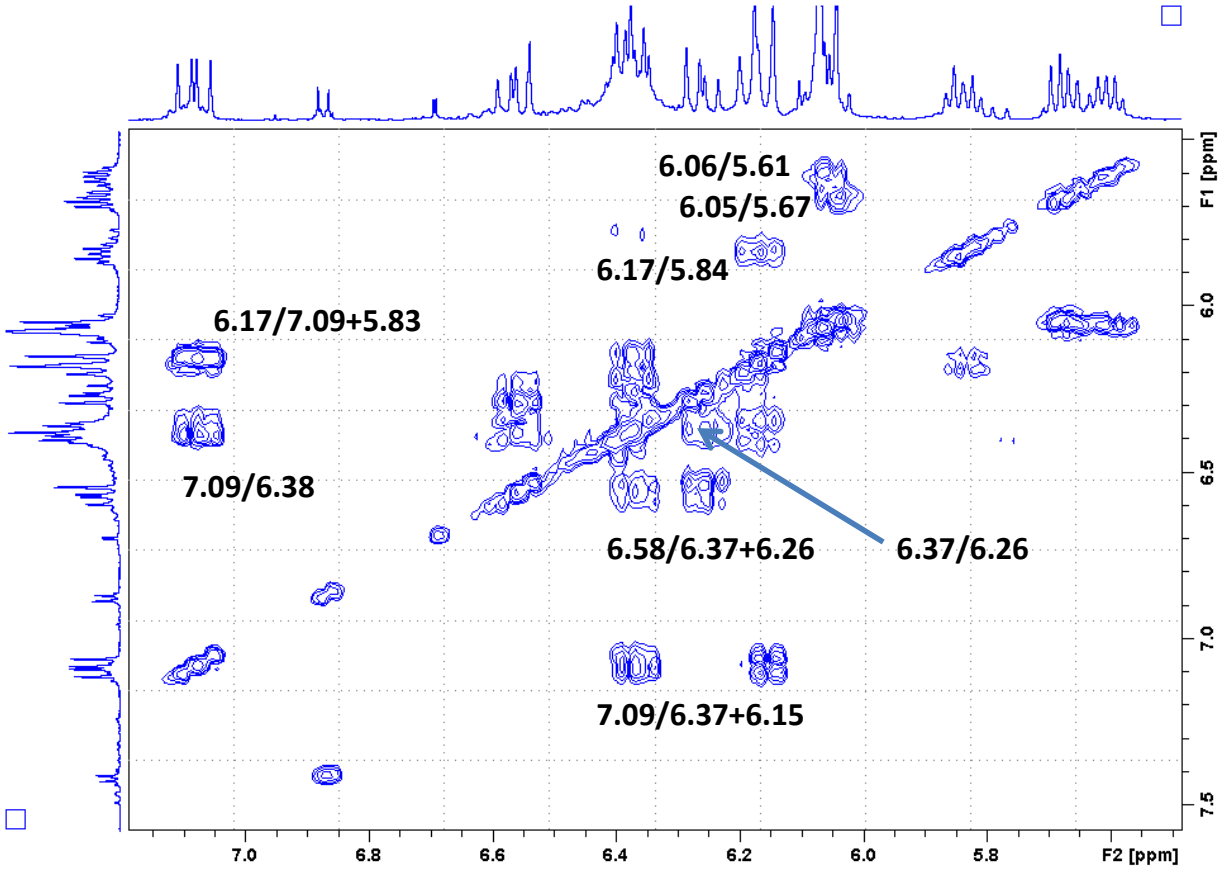
KR10-2 Dodecaketide tetraene (24)



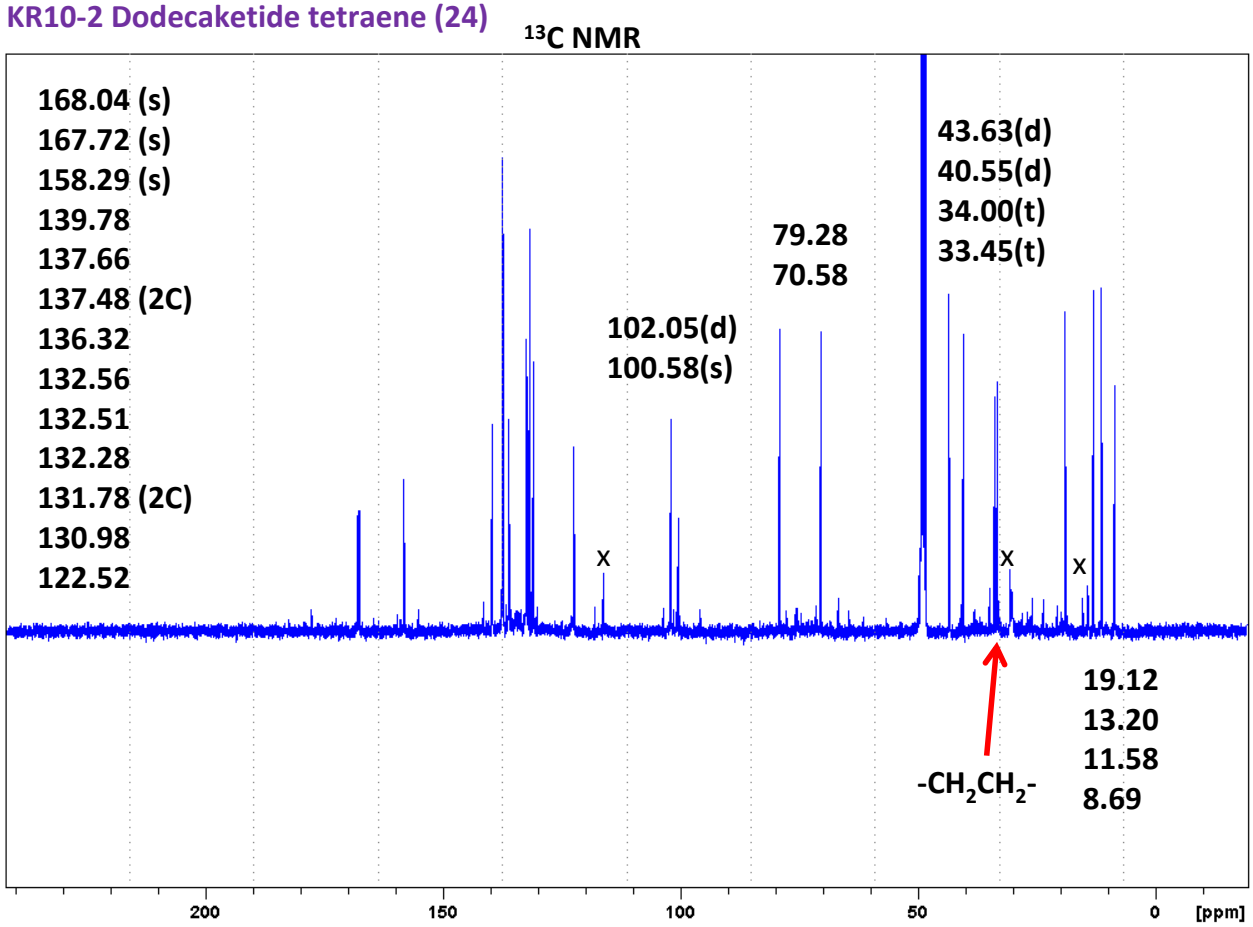
KR10-2 Dodecaketide tetraene (24)

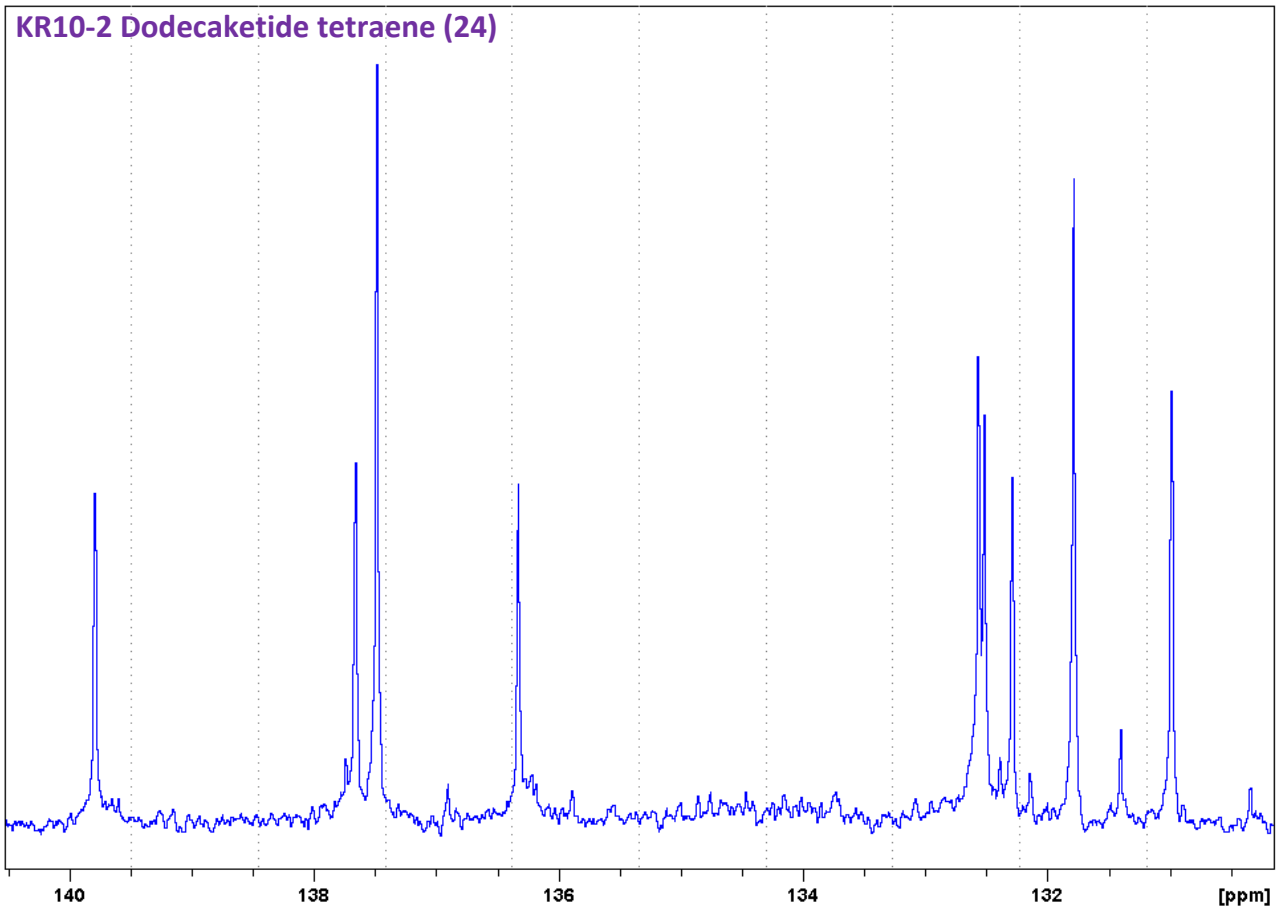


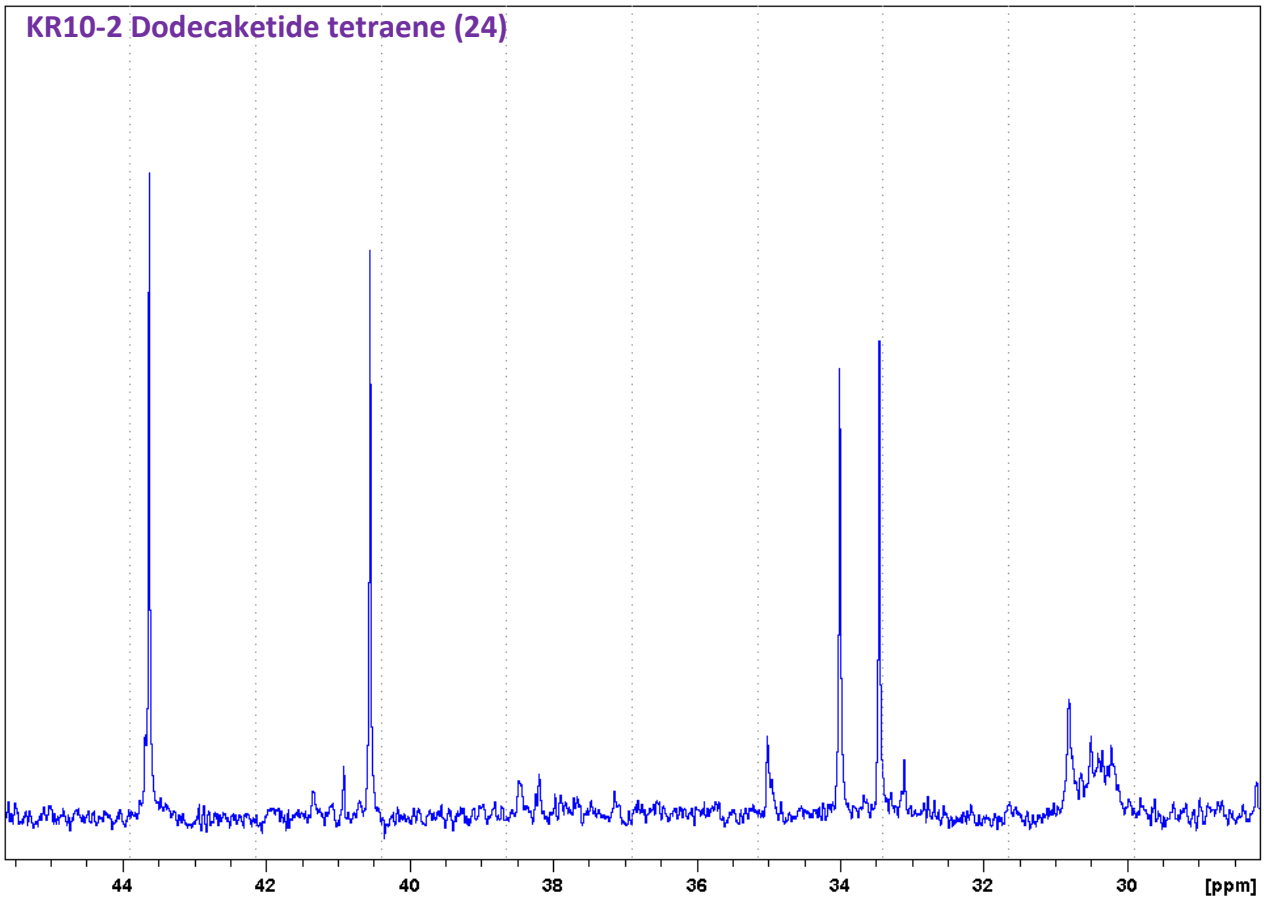
KR10-2 Dodecaketide tetraene (24)



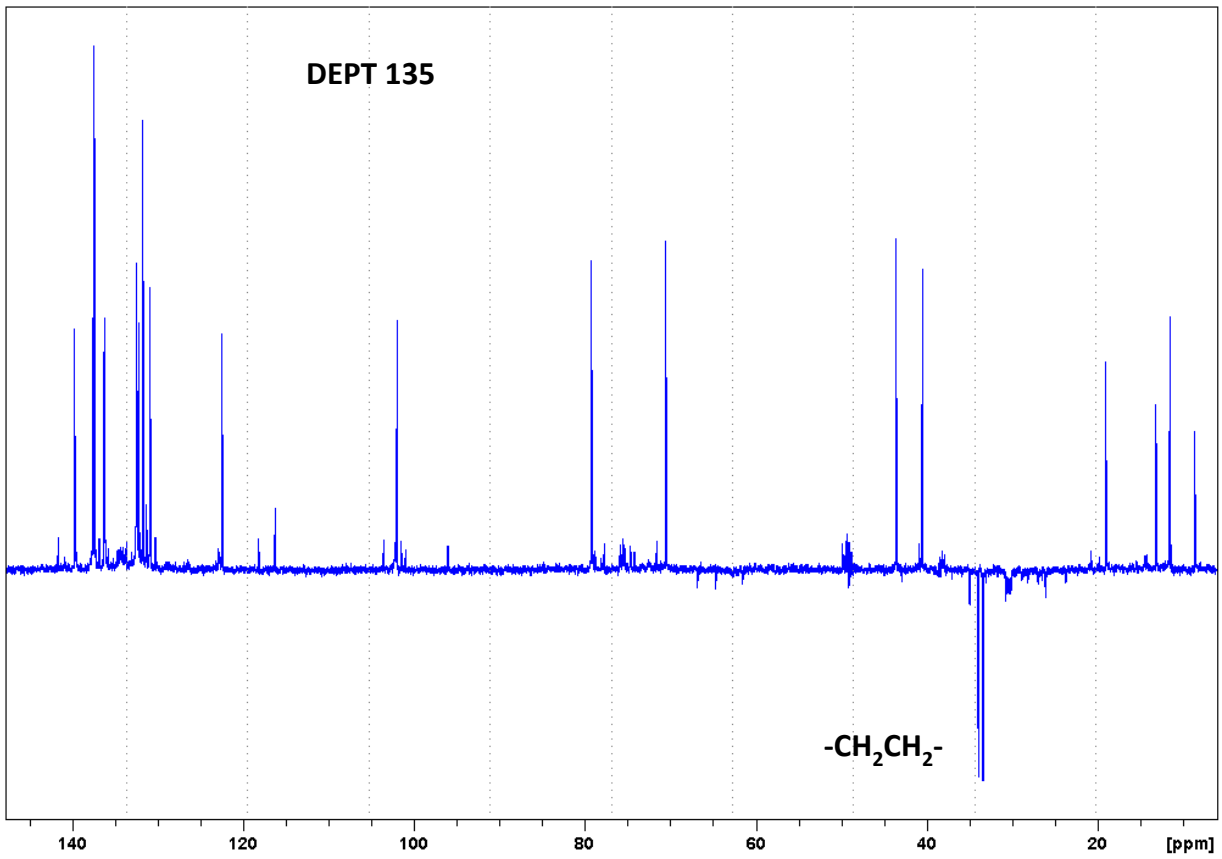
KR10-2 Dodecaketide tetraene (24)



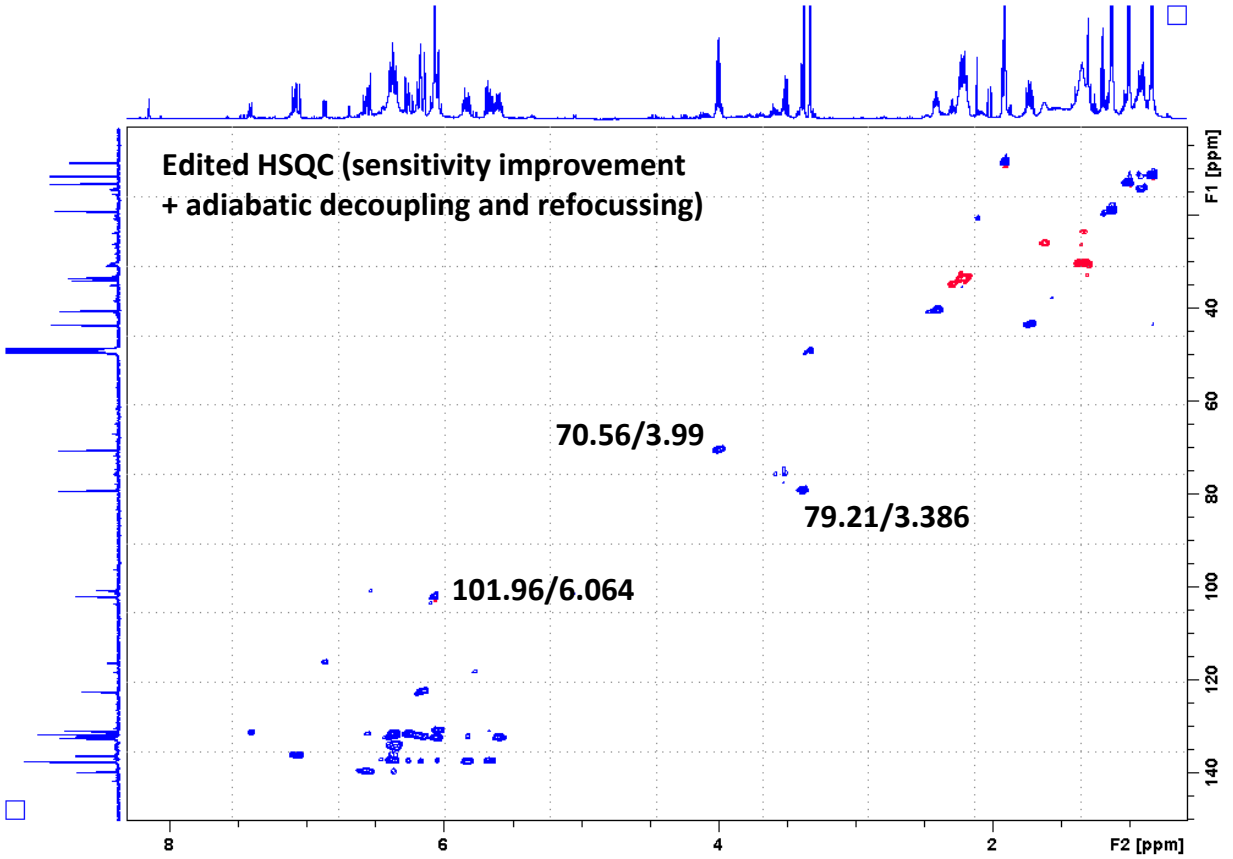

KR10-2 Dodecaketide tetraene (24)
140
138
136
134
132
[ppm]


KR10-2 Dodecaketide tetraene (24)
44
42
40
38
36
34
32
30
[ppm]

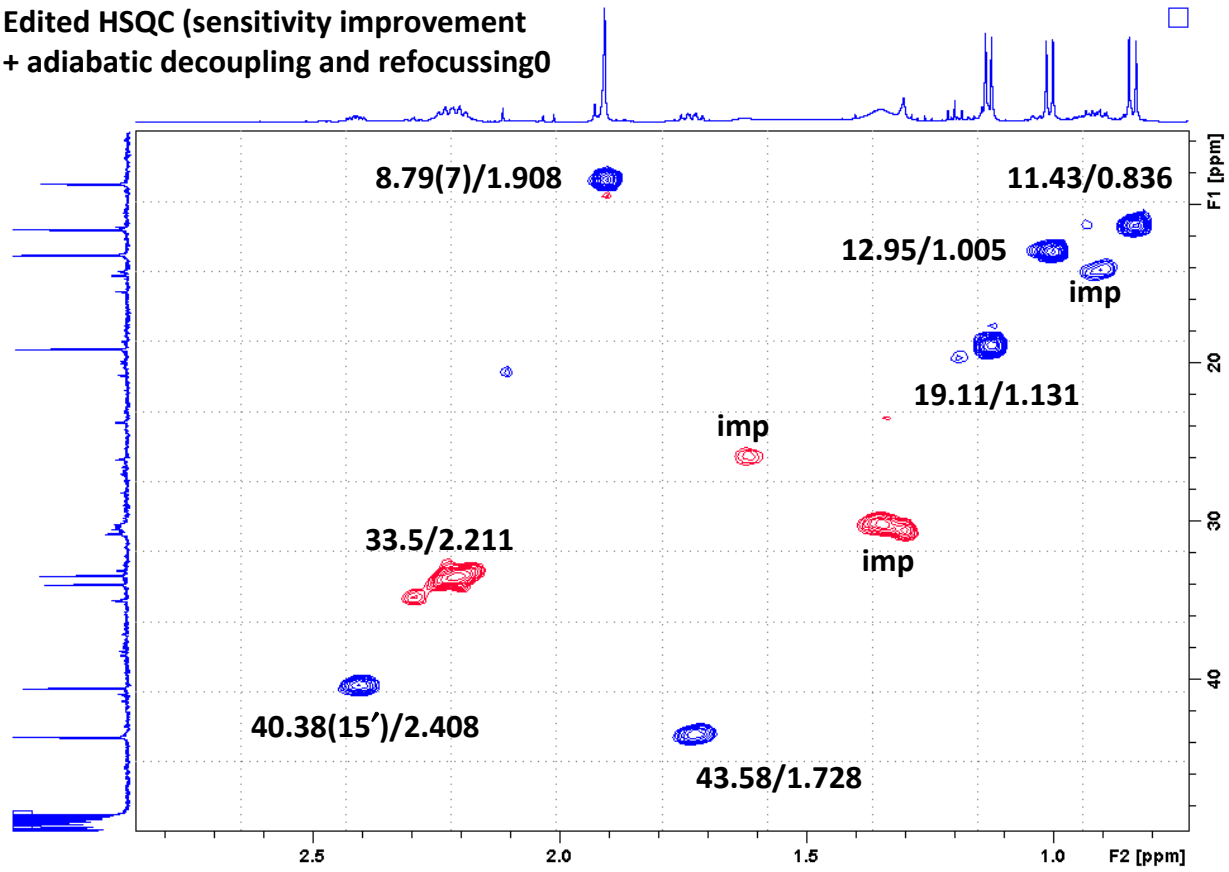
KR10-2 Dodecaketide tetraene (24)



KR10-2 Dodecaketide tetraene (24) HSQC (heterocorrelation NMR)



KR10-2 Dodecaketide tetraene (24)



KR10-2 Dodecaketide tetraene (24)