Supporting Information for

Fully enzymatic esterification/transesterification sequence for the preparation of symmetrical and unsymmetrical trehalose diacyl conjugates

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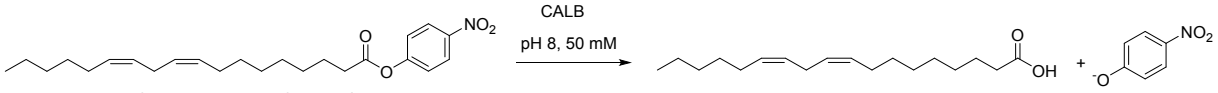
Enzyme activity after thermal or microwave exposure

The enzyme activity was monitored thanks to a colorimetric assay corresponding to the enzymatic hydrolysis of p-nitrophenyl linoleate that produces the UV-vis *p*-nitrophenolate (λ = 405nm).

The enzyme was exposed to a temperature of 46°C and to microwave irradiation and both experiments were compared to enzyme activity of an untouched enzyme.



Chemical Formula: $C_{24}H_{35}NO_4$
Molecular Weight: 401,55

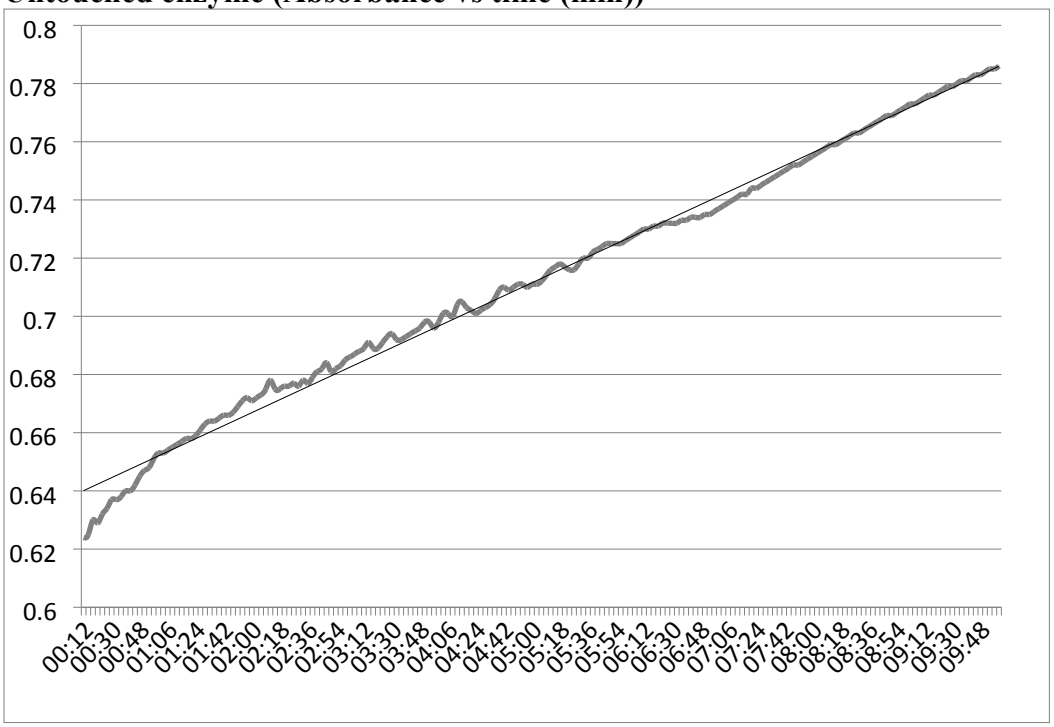
Reactants:

	Reactant	MW	Masse (mg)	n (mmol)	Volume (µL)	C	Volume total
1	pNP linoleate	401.55	7,3	0.032		0.1	2 mL
3	CalB		0.3		300		

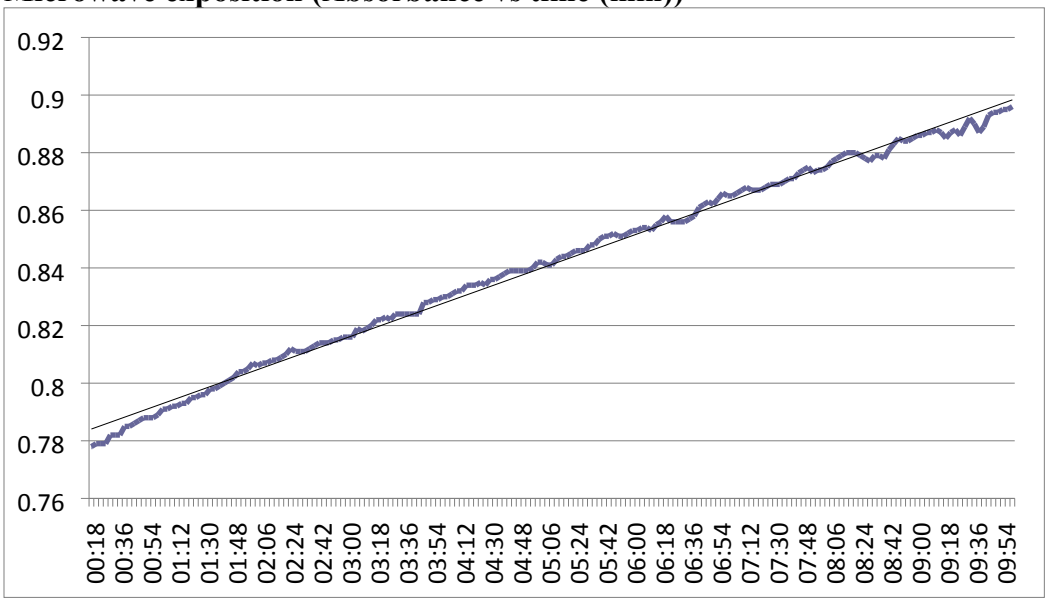
Solvents:

	Name	Volume (µL)
	Phosphate buffer pH 8	300
	DMSO	30

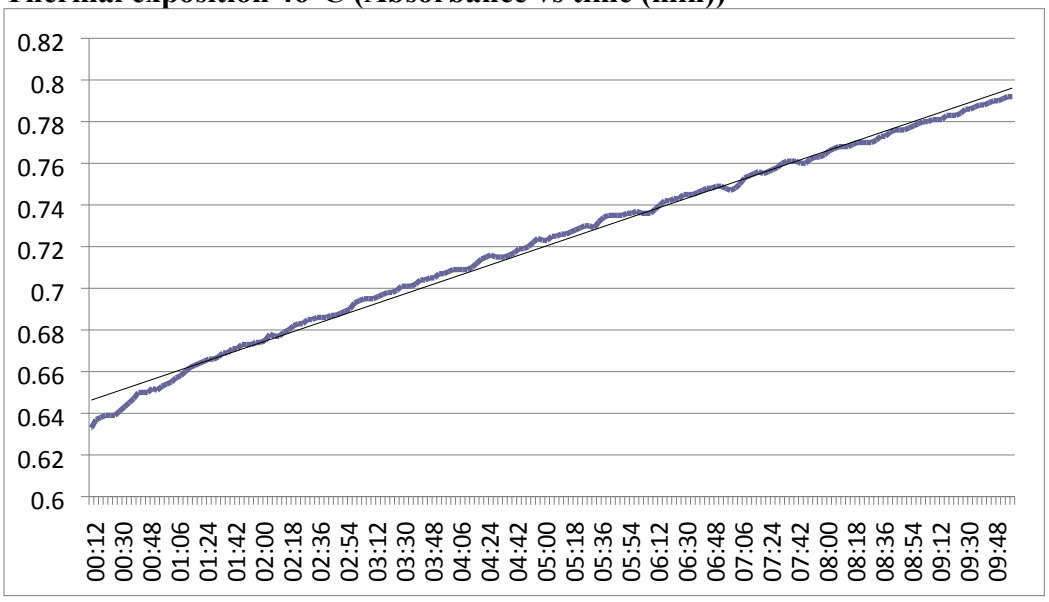
Untouched enzyme (Absorbance vs time (min))



Microwave exposition (Absorbance vs time (min))



Thermal exposition 46°C (Absorbance vs time (min))



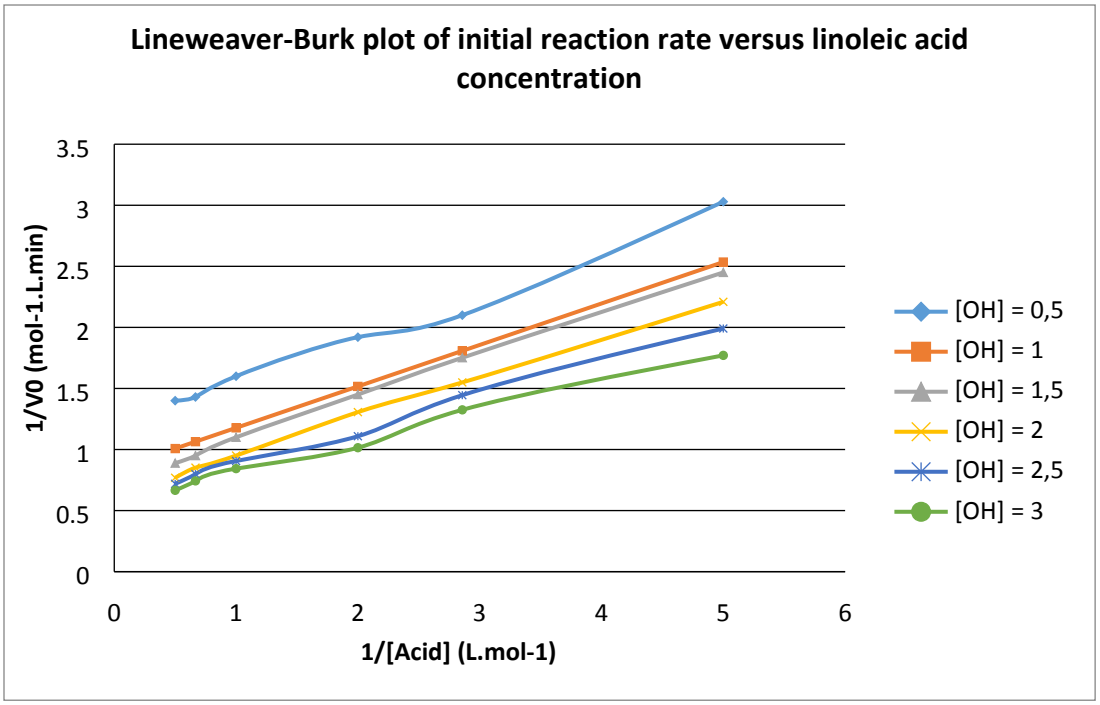
Enzyme kinetics

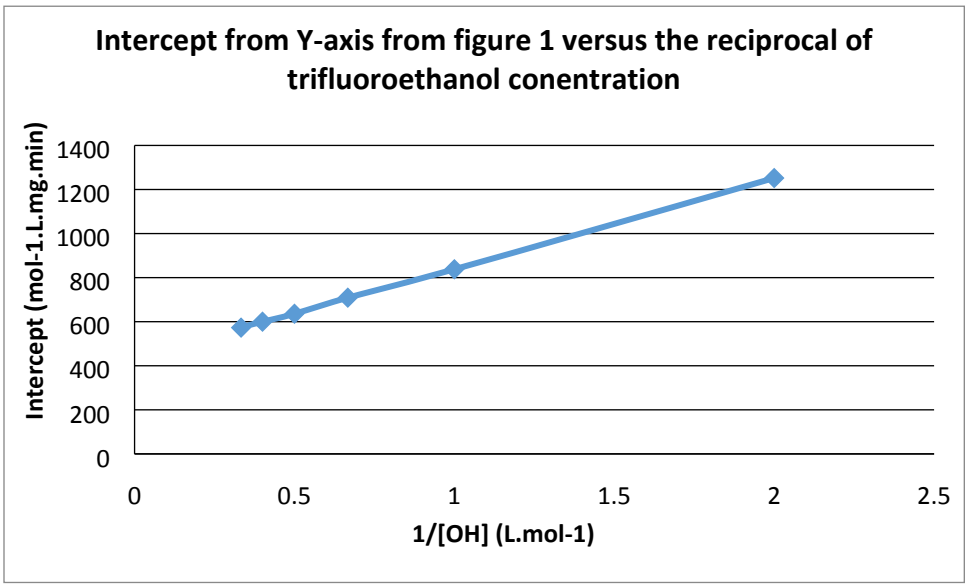
Lipase-catalyzed esterification of linoeic acid with trifluoroethanol was investigated varying acid concentration between 0.2 and 2 mol.L^{-1} and alcohol concentration between 0.5 and 3 mol.L^{-1}.
Briefly, to a suspension of Imb CalB (30 mg) in tBuOH was added M.S. 4A (30 mg) and linoleic acid. After 5 min. stirring at 46 °C trifluoroethanol was added. The different tubes were sealed and shacked at 250 rpm. 100µL of the reaction media was withdrawn at 19, 34, 49 and 64 min. Each sample was dissolved in 900 µL MeOH to which was added 50 µL of a solution of methyl myristate in MeOH (from a 10 mg.mL^{-1} solution). Each sample was analyzed using a GC2014 gas chromatograph (Shimadzu) equipped with a flame ionization detector (FID). The column used was a DB-23 (Agilent J&W Scientific), 30 m x 0.25 mm, 0.25 µm film thickness with helium as carrier gas at constant linear velocity u=36 cm/sec. The oven temperature started at 80°C during 4.5 min, increased by 4°C/min until 280°C and held it during 8 min. Concentration of trifluoroethyl linoleate was determined thanks to a calibration curve with methyl myristate as internal standard. The quantified data obtained for 10% conversion was plotted to calculate initial rates of the reaction.

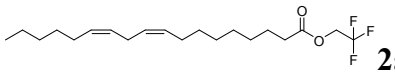
The reaction rate calculated from primary plots of substrate concentration versus time were used to construct the Lineweaver-Burk plot (Figure 1). A set of parallel lines was obtained indicating a ping-pong bi-bi mechanism with no significant inhibition within the studied concentration range. Consequently the kinetic parameters were obtained according to the simplified expression:

$$\frac{1}{V0} = \left[1 + \frac{Km(acid)}{[acid]} + \frac{Km(OH)}{[OH]}\right]\frac{1}{Vmax}$$

Where Km(acid) and Km(OH) are the Michaelis-Menten constant with respect to linoleic acid and trifluoroethanol, Vmax is the maximum esterification rate and [acid] and [OH] represent the initial concentration of linoleic acid and trifluoroethanol respectively. The slopes of the parallel lines of figure 1 are independent of the alcohol concentration yielding an average value of $K_{m(Acid)}/V_{max}$ = 10.2 mg.min. The y-axis intercepts from figure 1 when plotted against the reciprocal of trifluoroethanol concentration. The corresponding plot (Figure 2) gave a slope of 13.5 mg.min ($K_{m(OH)}/V_{max}$) and intercept the y-axis at 14 mol^{-1}.L.mg.min. It allowed to calculate both $K_{m(Acid)}$ = 0.7 mol.L^{-1} and $K_{m(OH)}$ = 0.9 mol.L^{-1}.


Lineweaver-Burk plot of initial reaction rate versus linoleic acid concentration
1/V0 (mol-1.L.min)
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
1/[Acid] (L.mol-1)
[OH] = 0,5
[OH] = 1
[OH] = 1,5
[OH] = 2
[OH] = 2,5
[OH] = 3


Intercept from Y-axis from figure 1 versus the reciprocal of trifluoroethanol conentration
Intercept (mol-1.L.mg.min)
1400
1200
1000
800
600
400
200
0
0
0.5
1
1.5
2
2.5
1/[OH] (L.mol-1)



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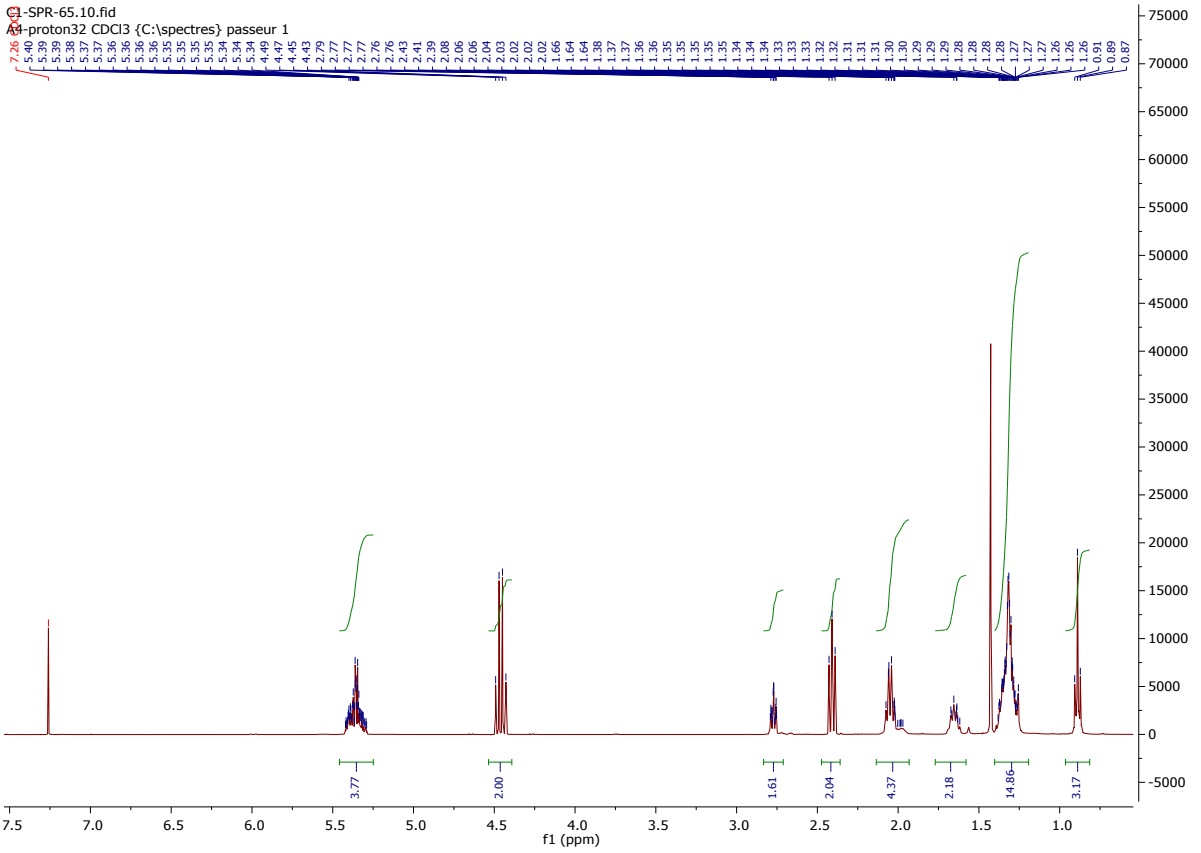
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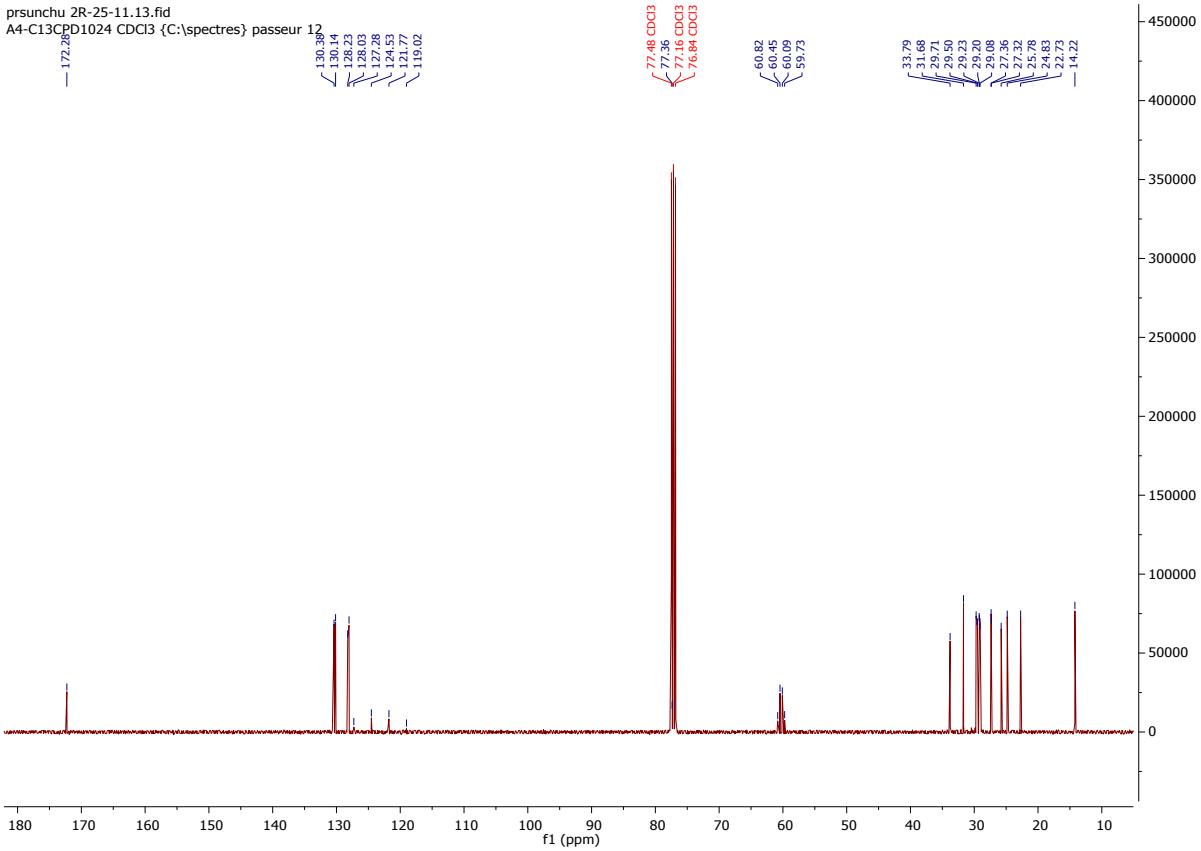
f1 (ppm)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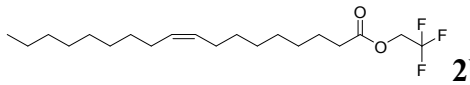
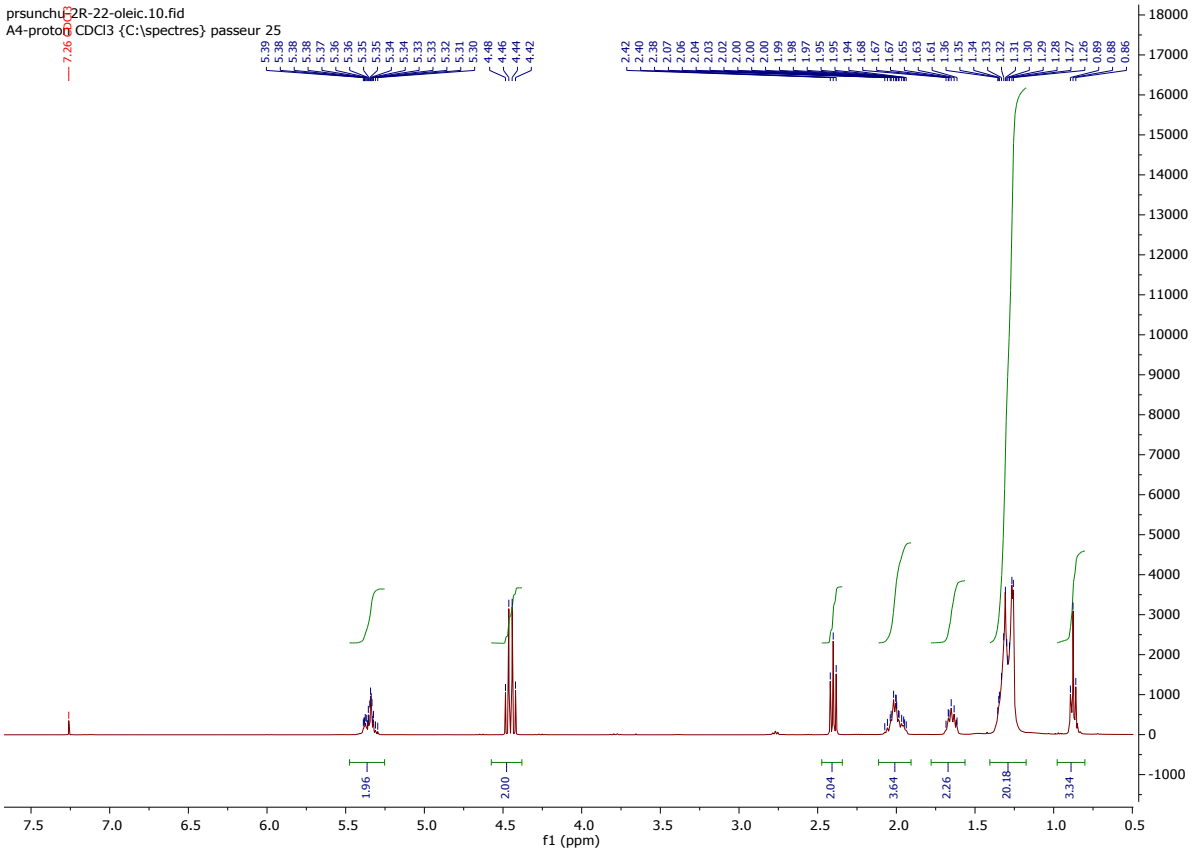
f1 (ppm)


O
O
F
F
F

2b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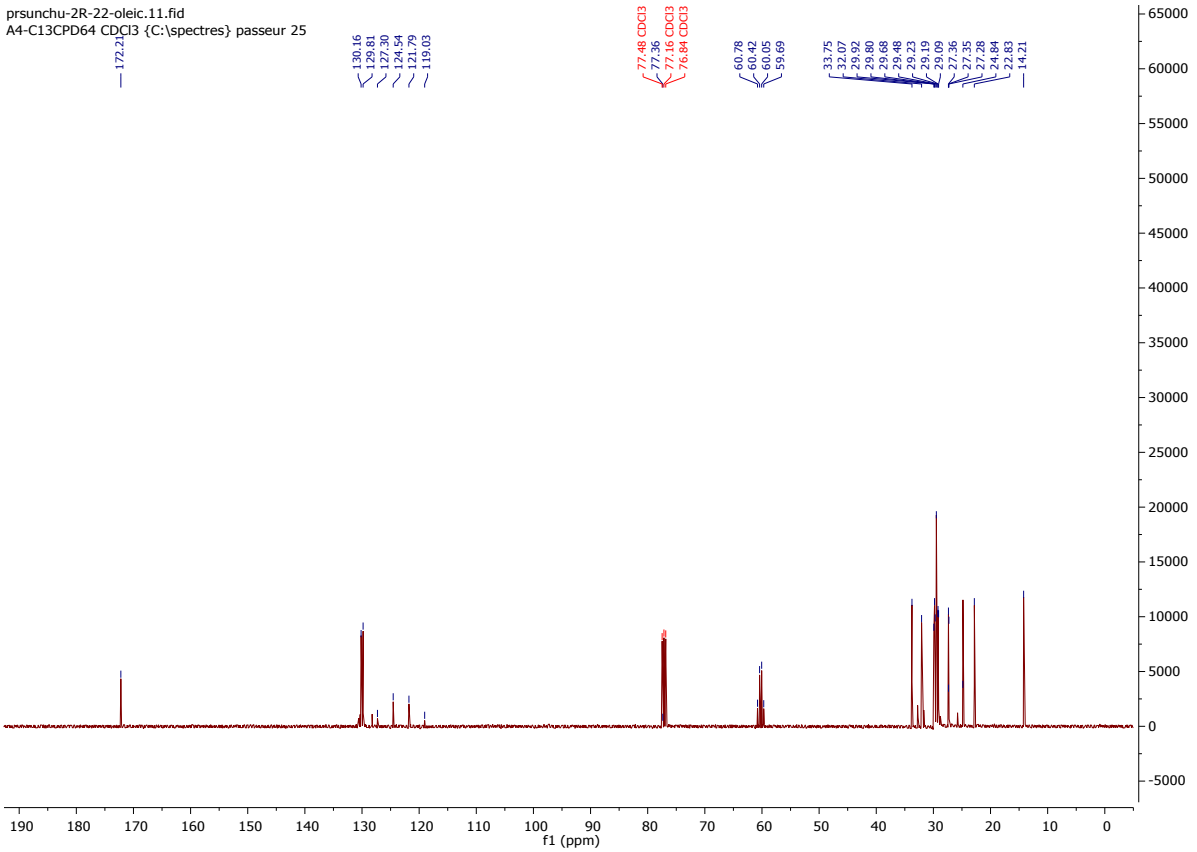
A4-proton CDCl3 {C:\spectres} passeur 25
7.26 CHCl3
5.39 5.38 5.38 5.38 5.37 5.36 5.36 5.35 5.35 5.34 5.34 5.33 5.33 5.32 5.31 5.30
4.48 4.46 4.44 4.42
2.42 2.40 2.38 2.07 2.06 2.04 2.03 2.02 2.00 2.00 2.00 1.99 1.98 1.97 1.95 1.95 1.94 1.68 1.67 1.67 1.65 1.63 1.61 1.36 1.35 1.34 1.33 1.32 1.31 1.30 1.29 1.28 1.27 1.26 0.89 0.88 0.86
1.96
2.00
2.04
3.64
2.26
20.18
3.34
f1 (ppm)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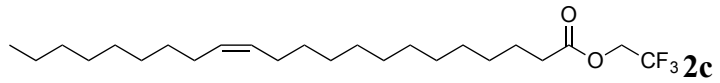
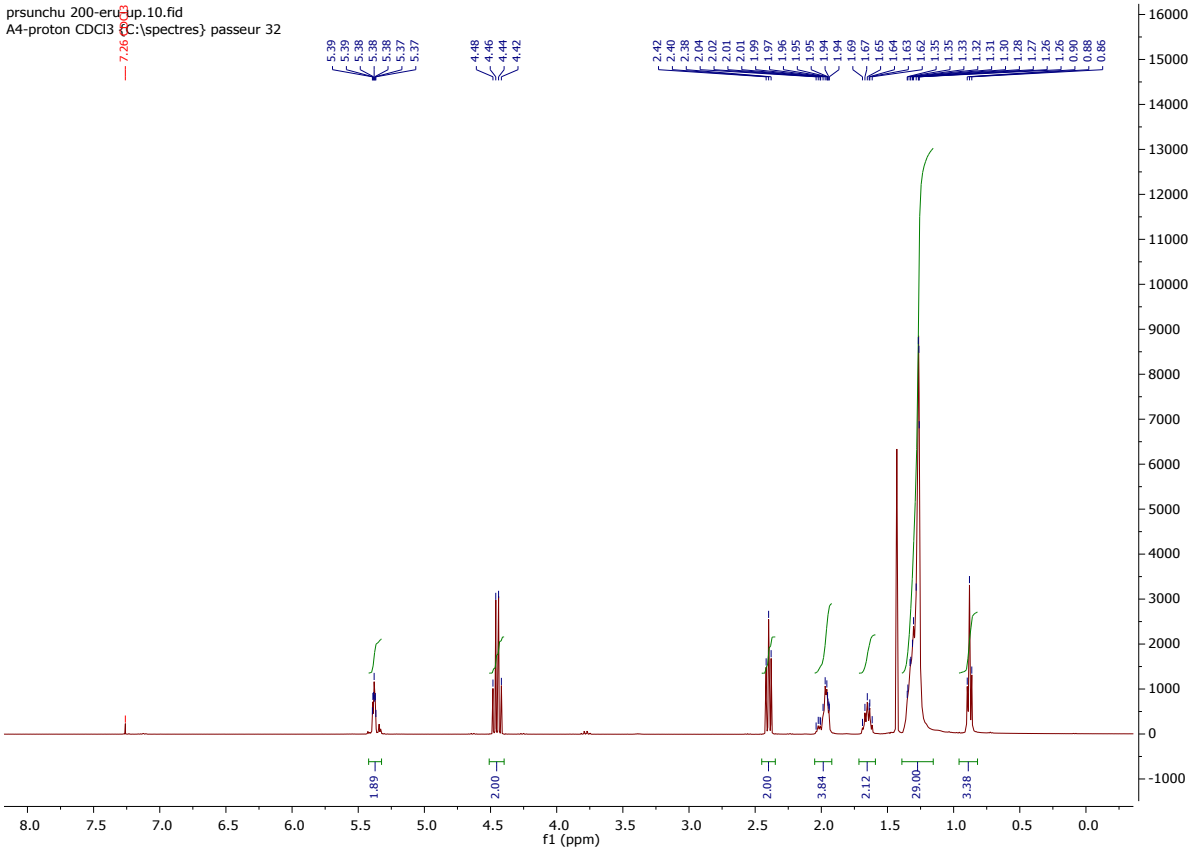

O
O
CF3

2c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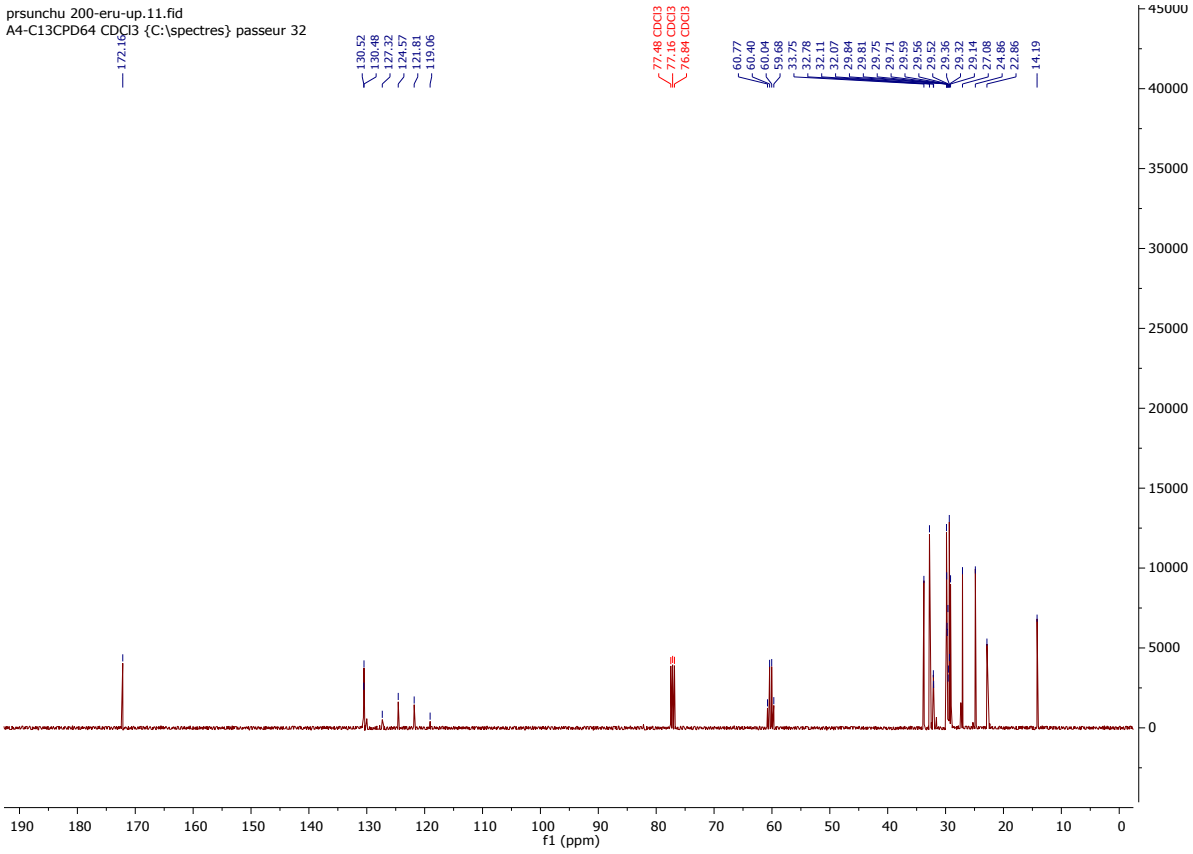
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130.52
130.48
127.32
124.57
121.81
119.06
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
60.77
60.40
60.04
59.68
33.75
32.78
32.11
32.07
29.84
29.81
29.75
29.71
29.59
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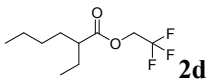
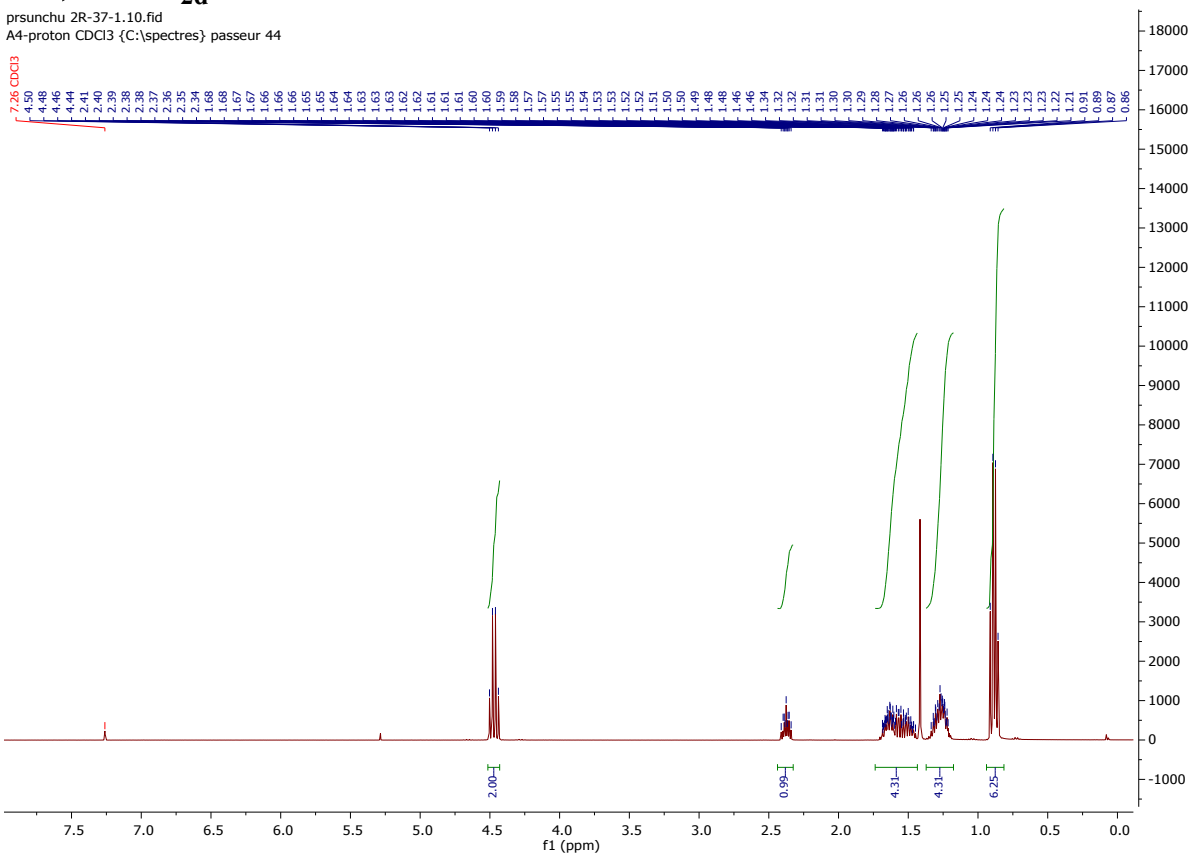
29.52
29.36
29.32
29.14
27.08
24.86
22.86
14.19
f1 (ppm)


O
O
F
F
F

2d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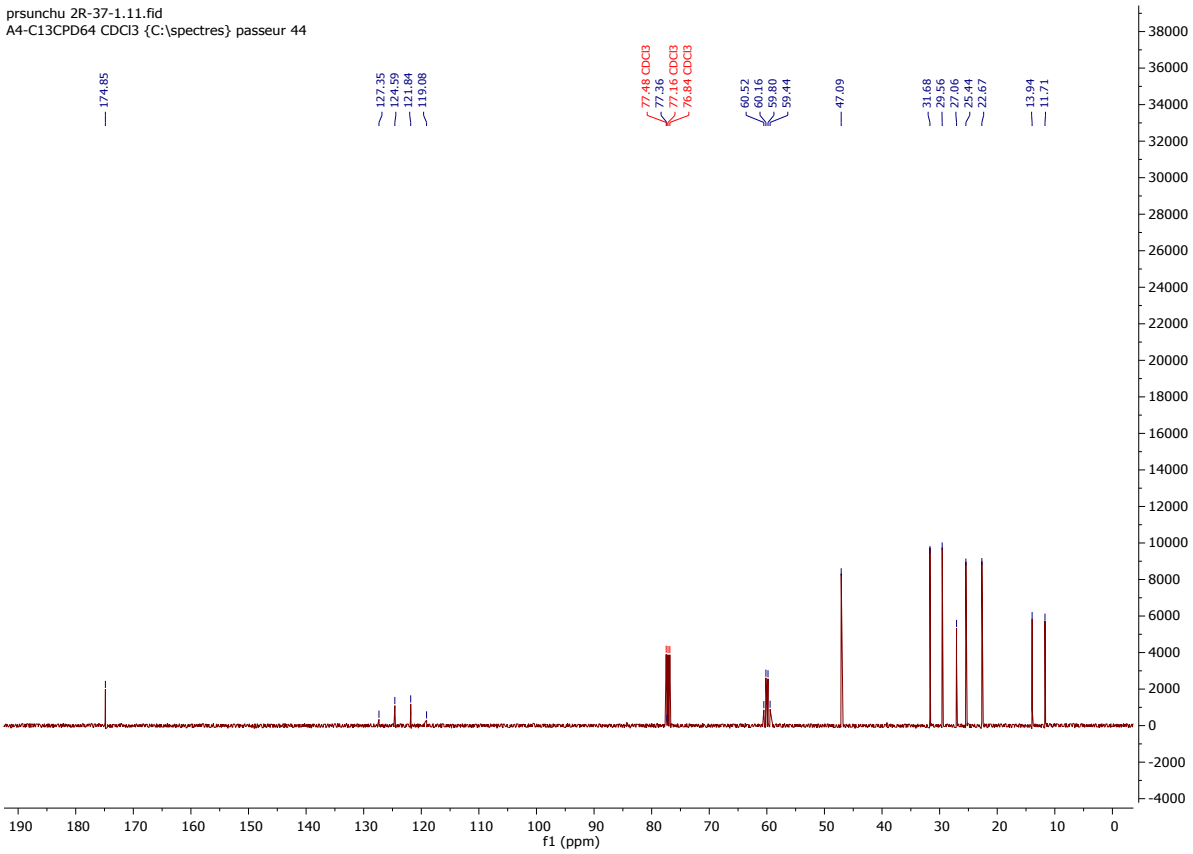
7.26 CDCl3
2.00
0.99
4.31
4.31
6.25
f1 (ppm)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174.85
127.35
124.59
121.84
119.08
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
60.52
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31.68
29.56
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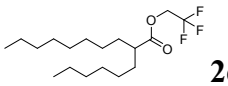
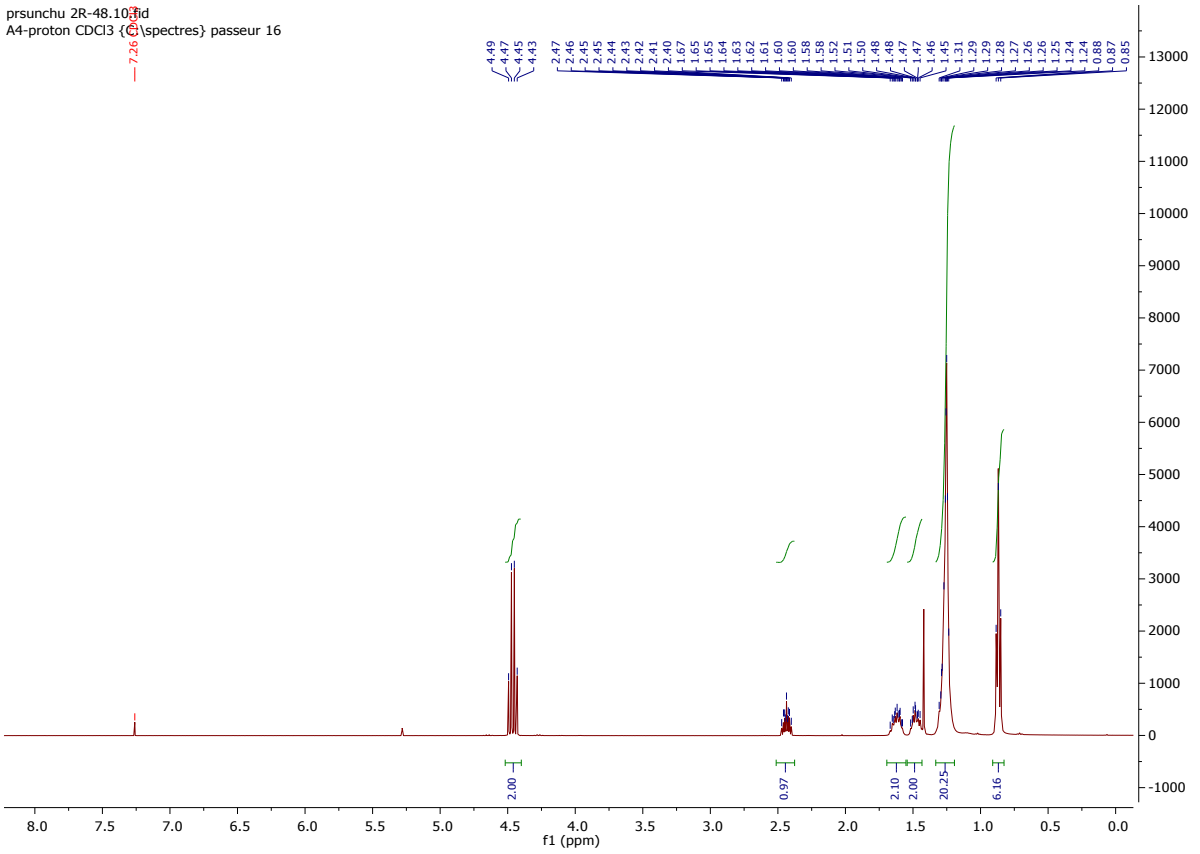
22.67
13.94
11.71
f1 (ppm)


O
F
F
F
O

2e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2.46
2.45
2.45
2.44
2.43
2.42
2.41
2.40
1.67
1.65
1.65
1.64
1.63
1.62
1.61
1.60
1.60
1.58
1.58
1.52
1.51
1.50
1.48
1.48
1.47
1.47
1.46
1.45
1.31
1.29
1.29
1.28
1.27
1.26
1.26
1.25
1.24
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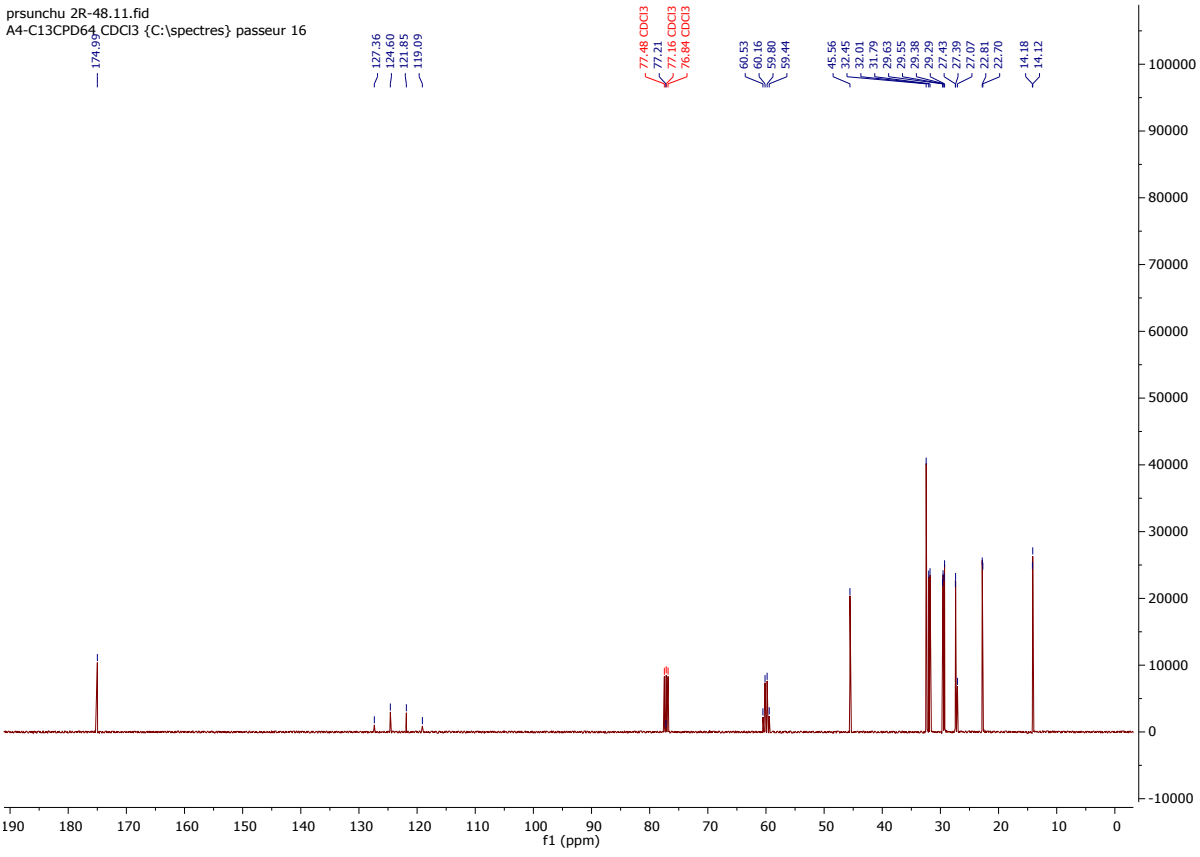
f1 (ppm)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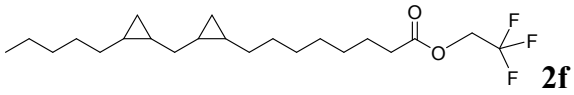
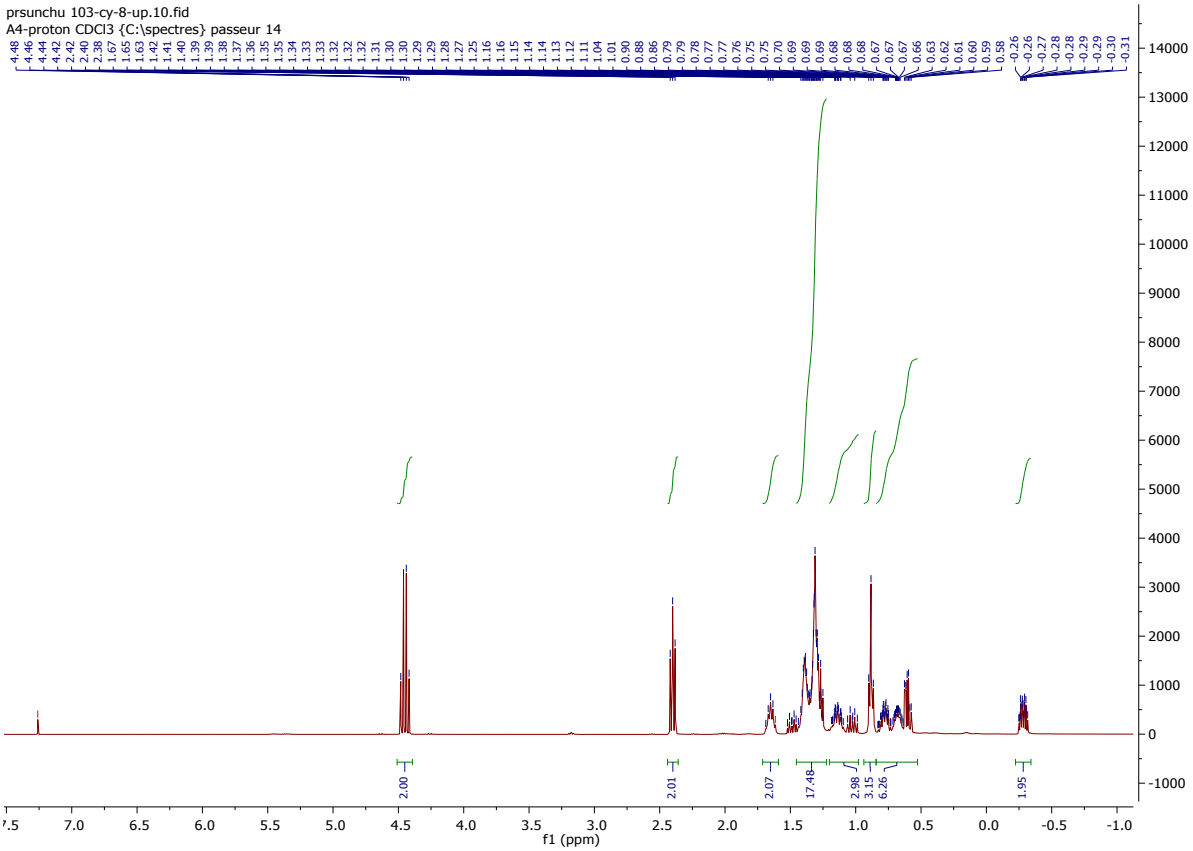

O
O
F
F
F

2f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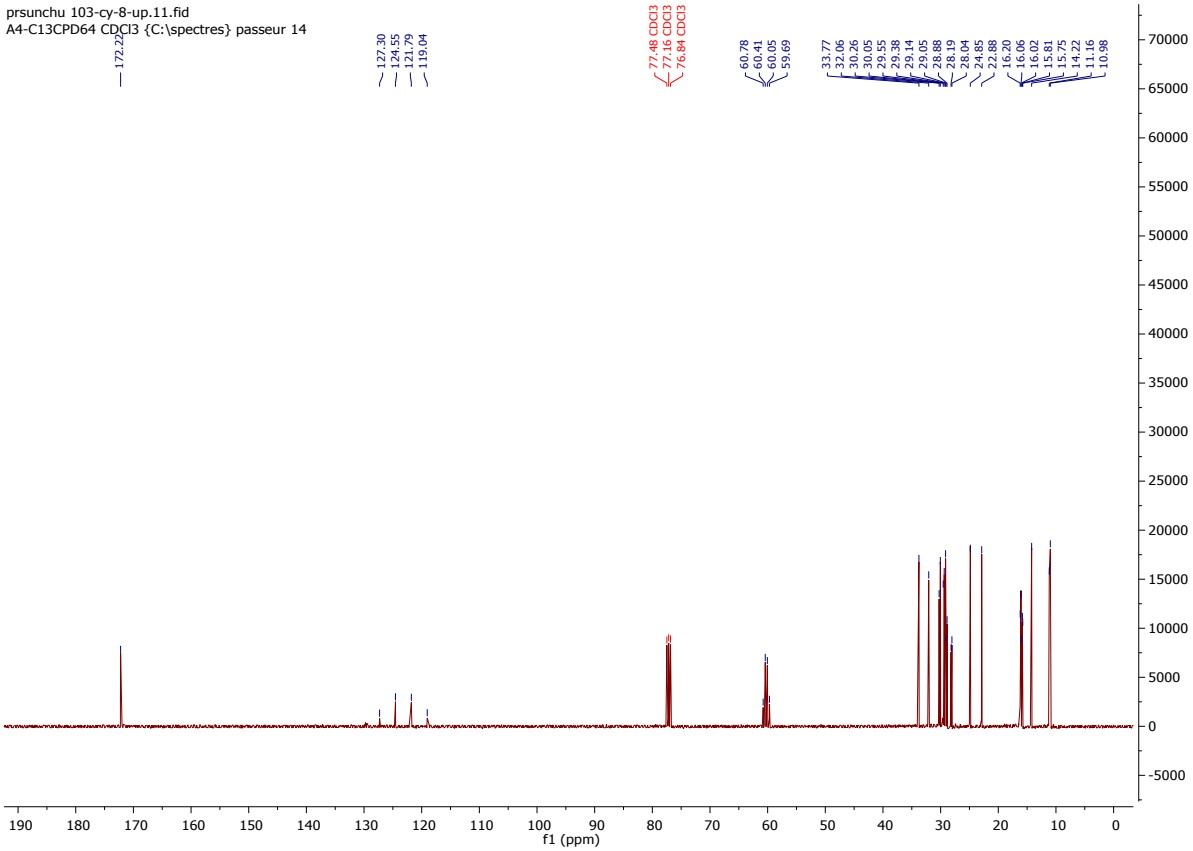
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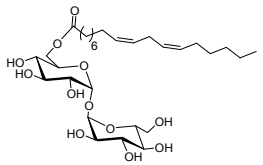
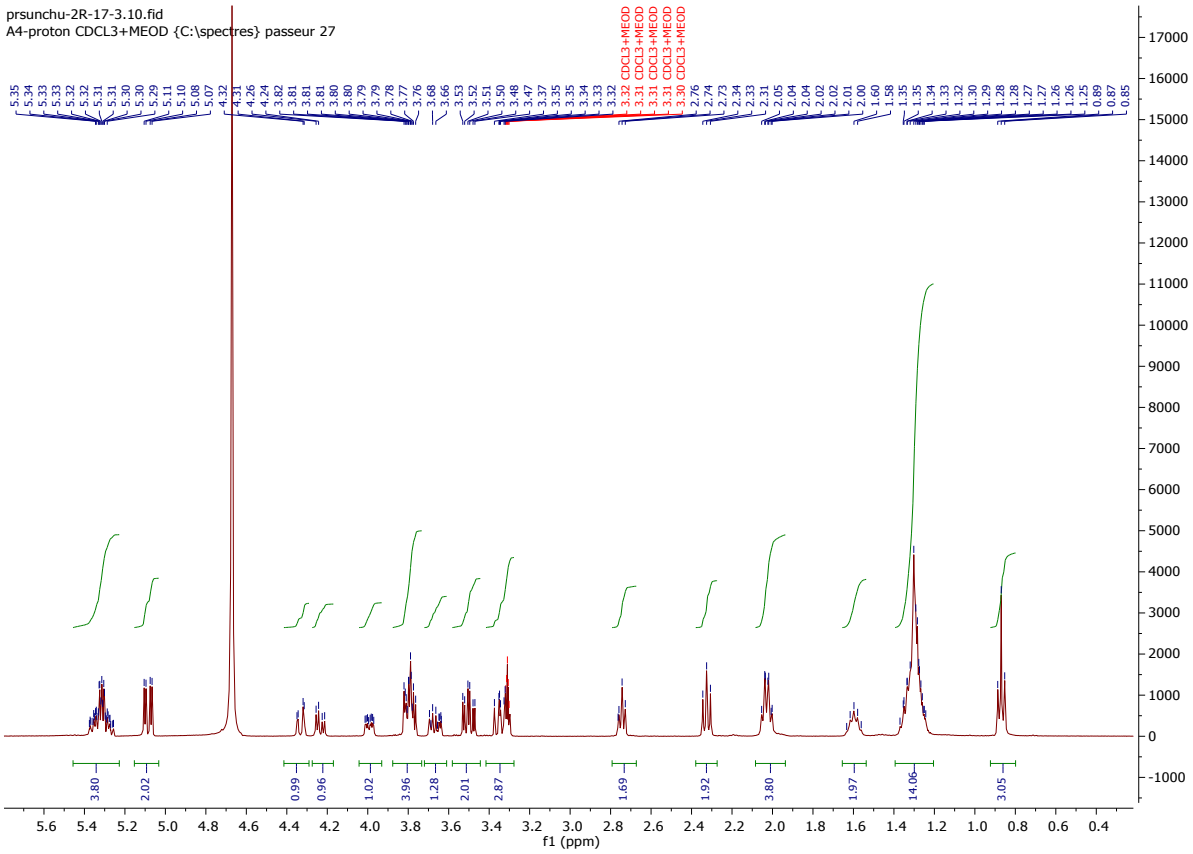


3a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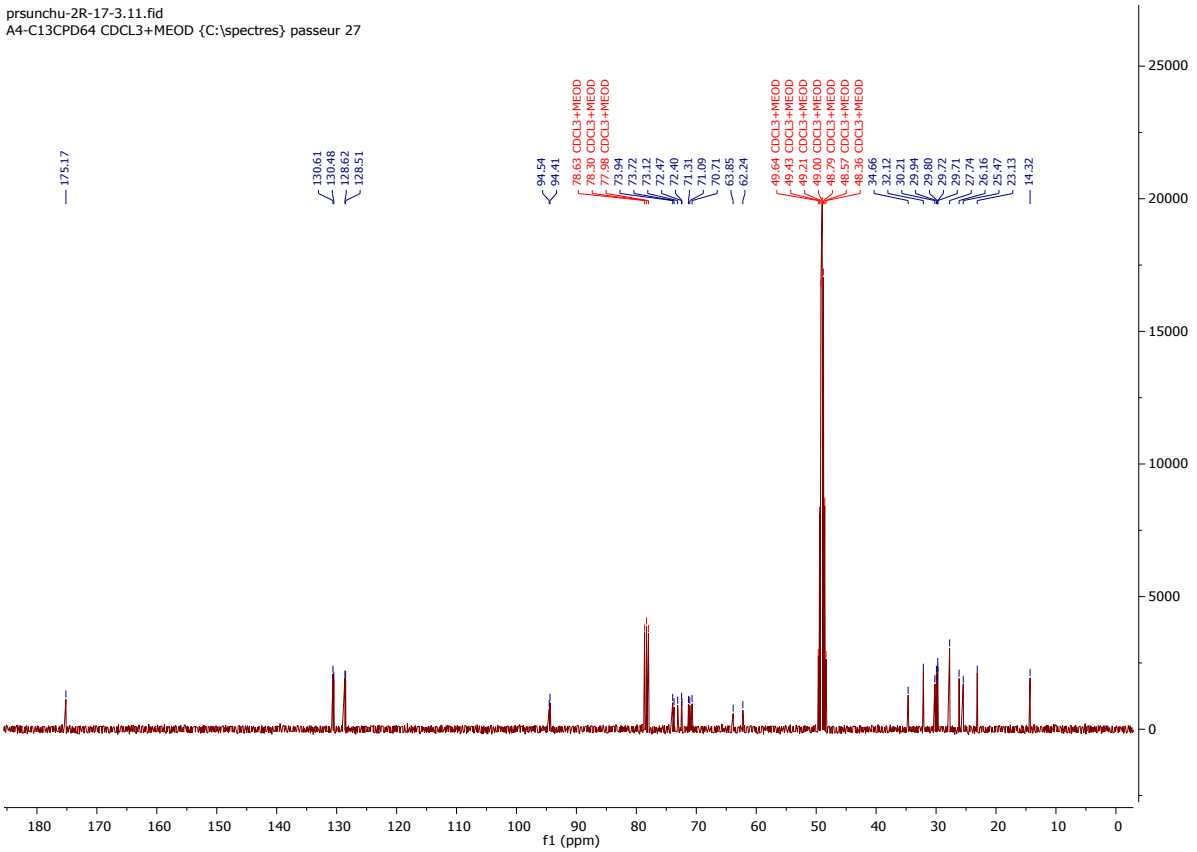
f1 (ppm)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175.17
130.61
130.48
128.62
128.51
94.54
94.41
78.63 CDCL3+MEOD
78.30 CDCL3+MEOD
77.98 CDCL3+MEOD
73.94
73.72
73.12
72.47
72.40
71.31
71.09
70.71
63.85
62.24
49.64 CDCL3+MEOD
49.43 CDCL3+MEOD
49.21 CDCL3+MEOD
49.00 CDCL3+MEOD
48.79 CDCL3+MEOD
48.57 CDCL3+MEOD
48.36 CDCL3+MEOD
34.66
32.12
30.21
29.94
29.80
29.72
29.71
27.74
26.16
25.47
23.13
14.32
25000
20000
15000
10000
5000
0
180
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150
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40
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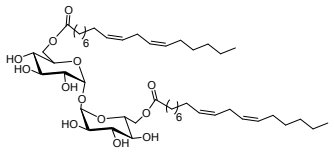
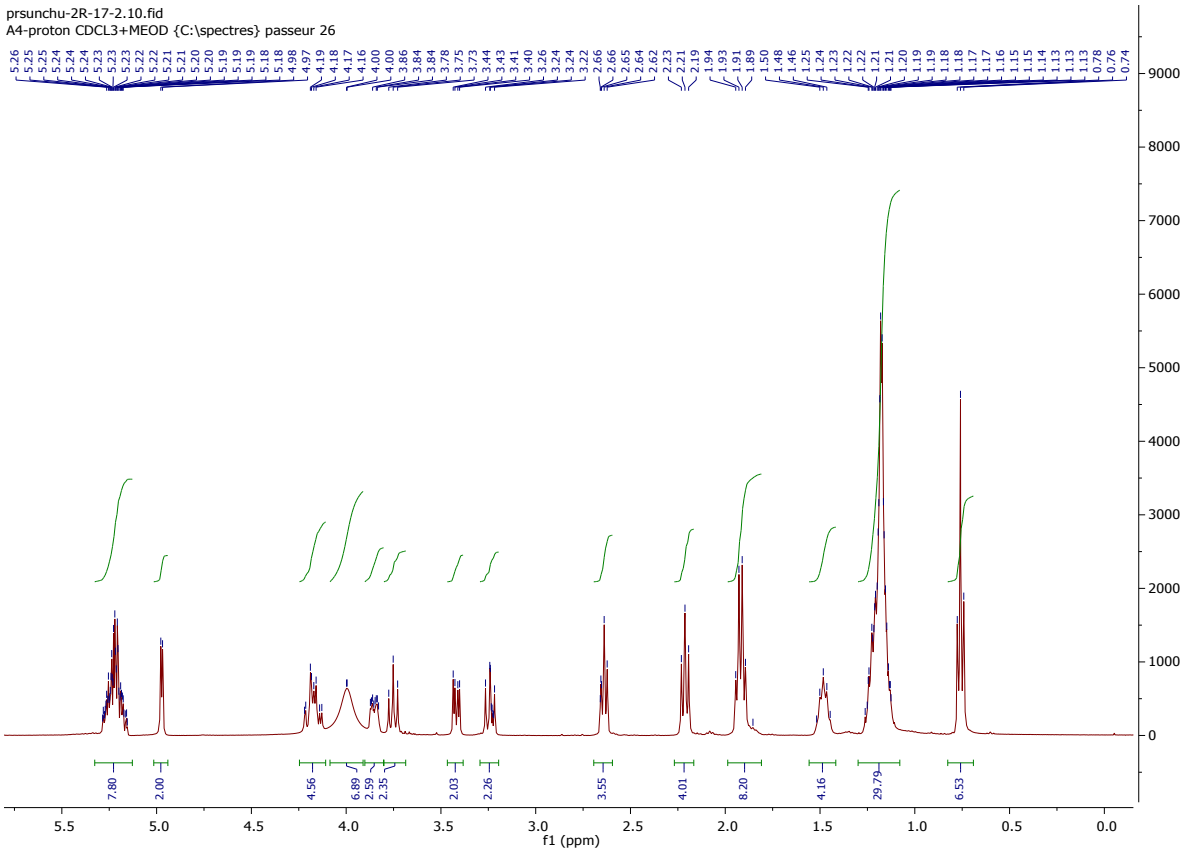
20
10
0
f1 (ppm)


HO
HO
OH
O
O
6
HO
OH
OH

4a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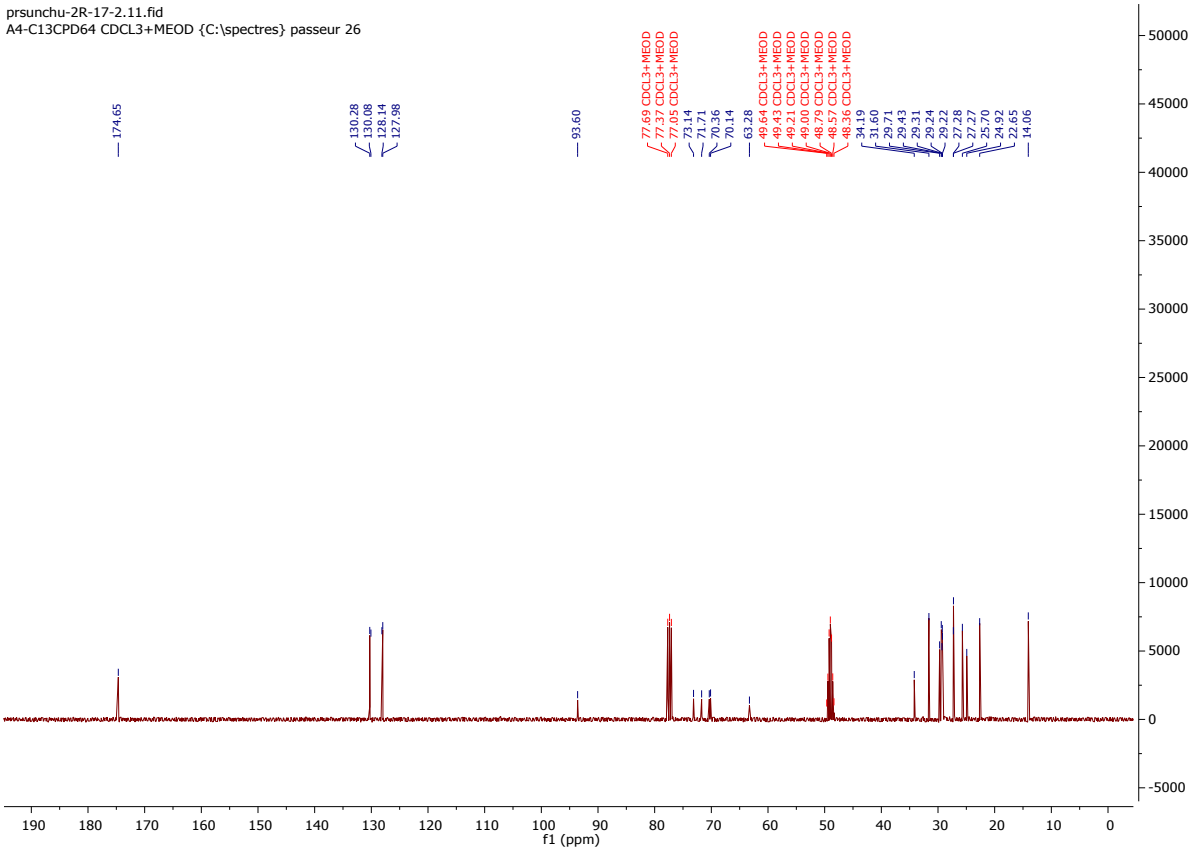
f1 (ppm)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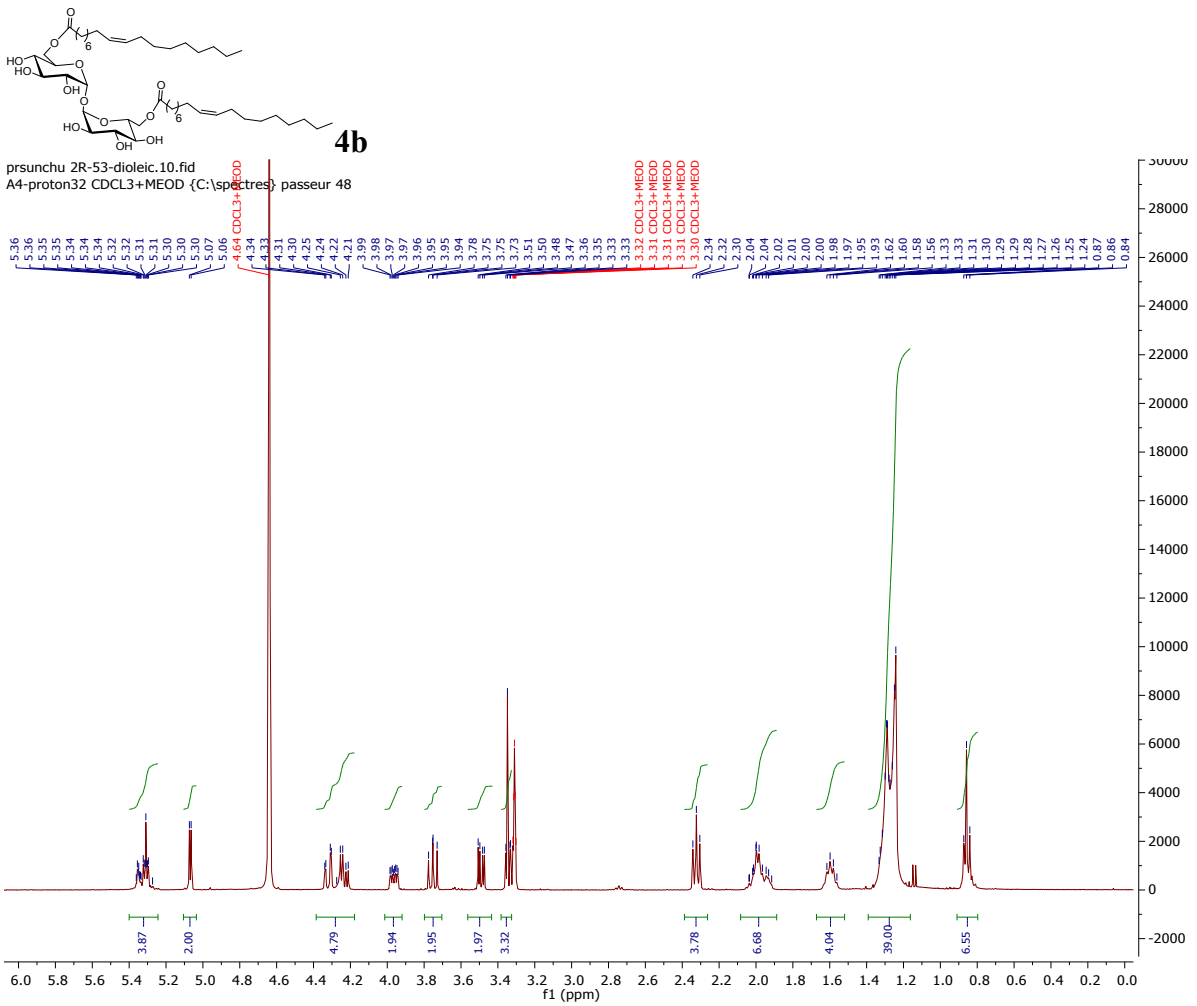
4b
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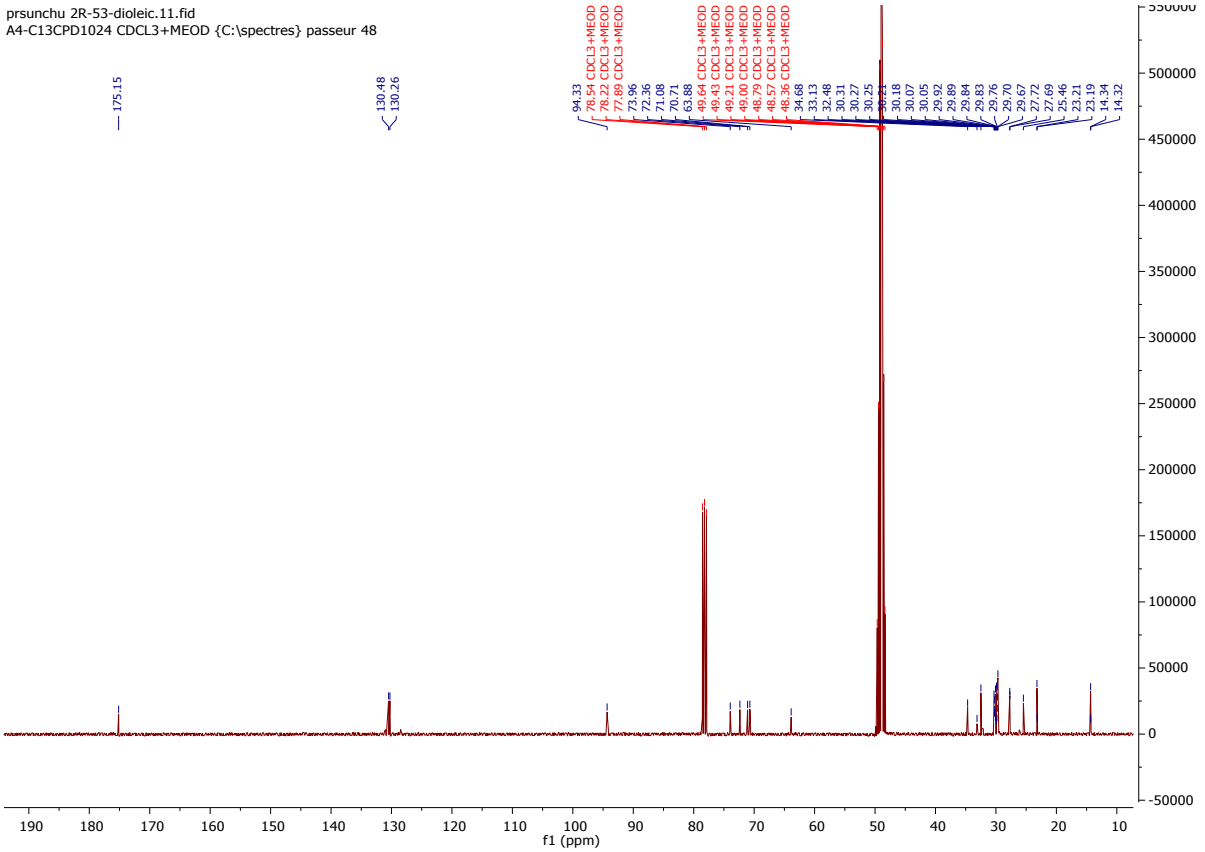
f1 (ppm)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A4-C13CPD1024 CDCL3+MEOD {C:\spectres} passeur 48
175.15
130.48
130.26
94.33
78.54 CDCL3+MEOD
78.22 CDCL3+MEOD
77.89 CDCL3+MEOD
73.96
72.36
71.08
70.71
63.88
49.64 CDCL3+MEOD
49.43 CDCL3+MEOD
49.21 CDCL3+MEOD
49.00 CDCL3+MEOD
48.79 CDCL3+MEOD
48.57 CDCL3+MEOD
48.36 CDCL3+MEOD
34.68
33.13
32.48
30.31
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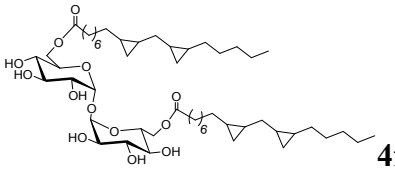
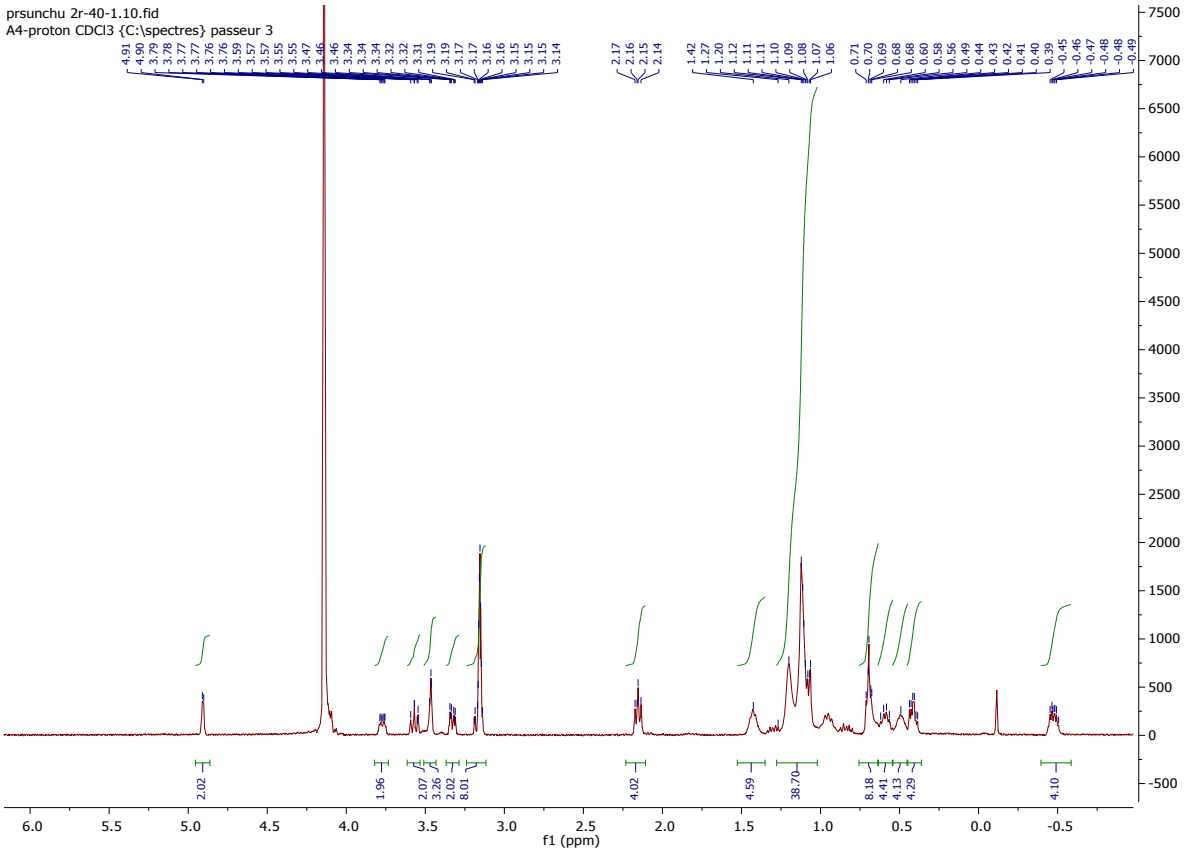
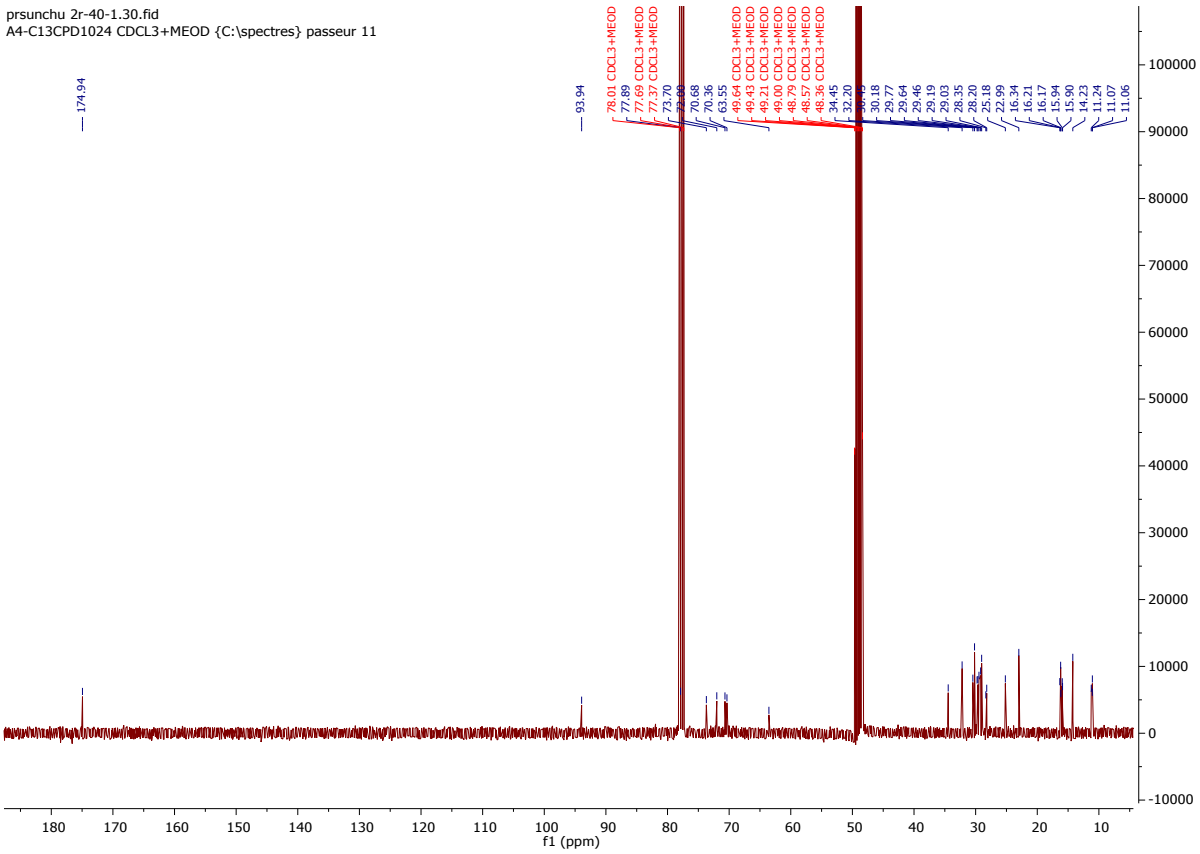
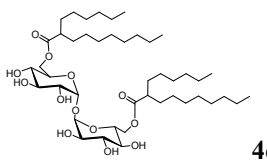
25.46
23.21
23.19
14.34
14.32
f1 (ppm)



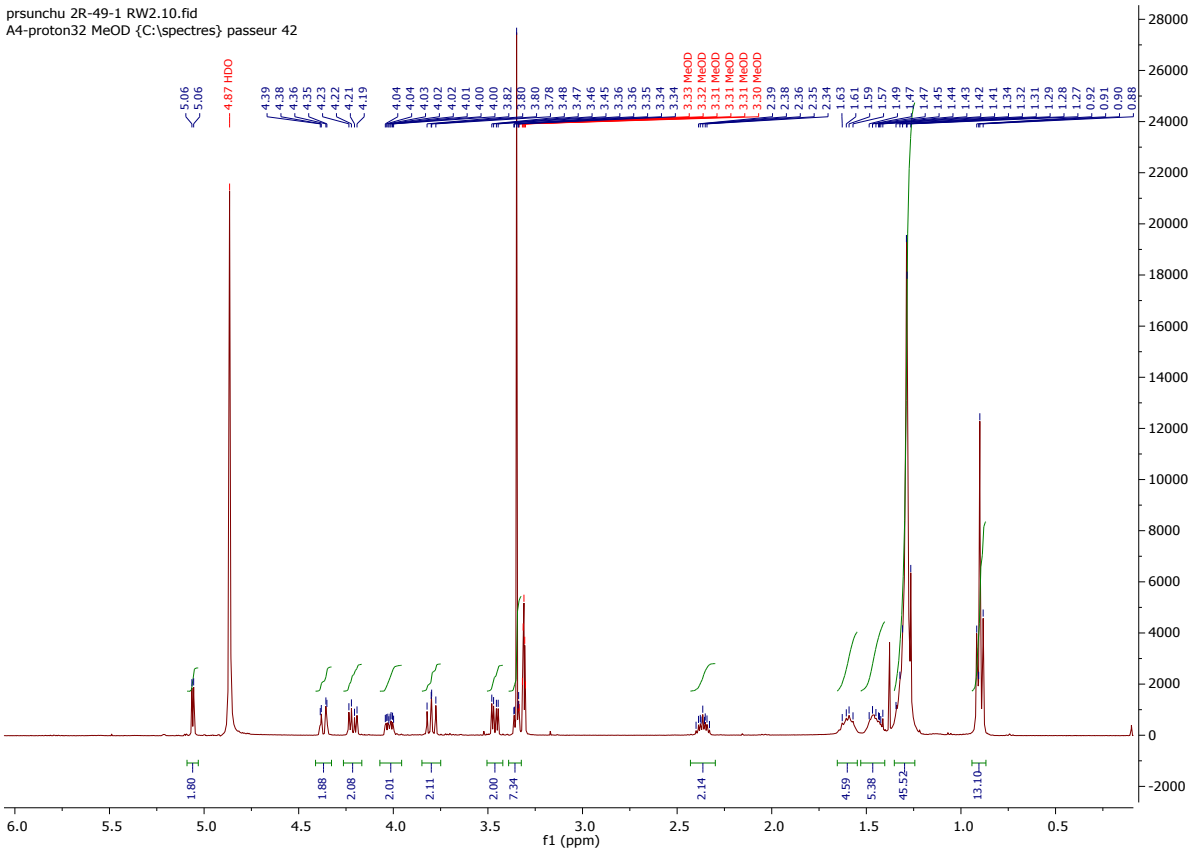
4f

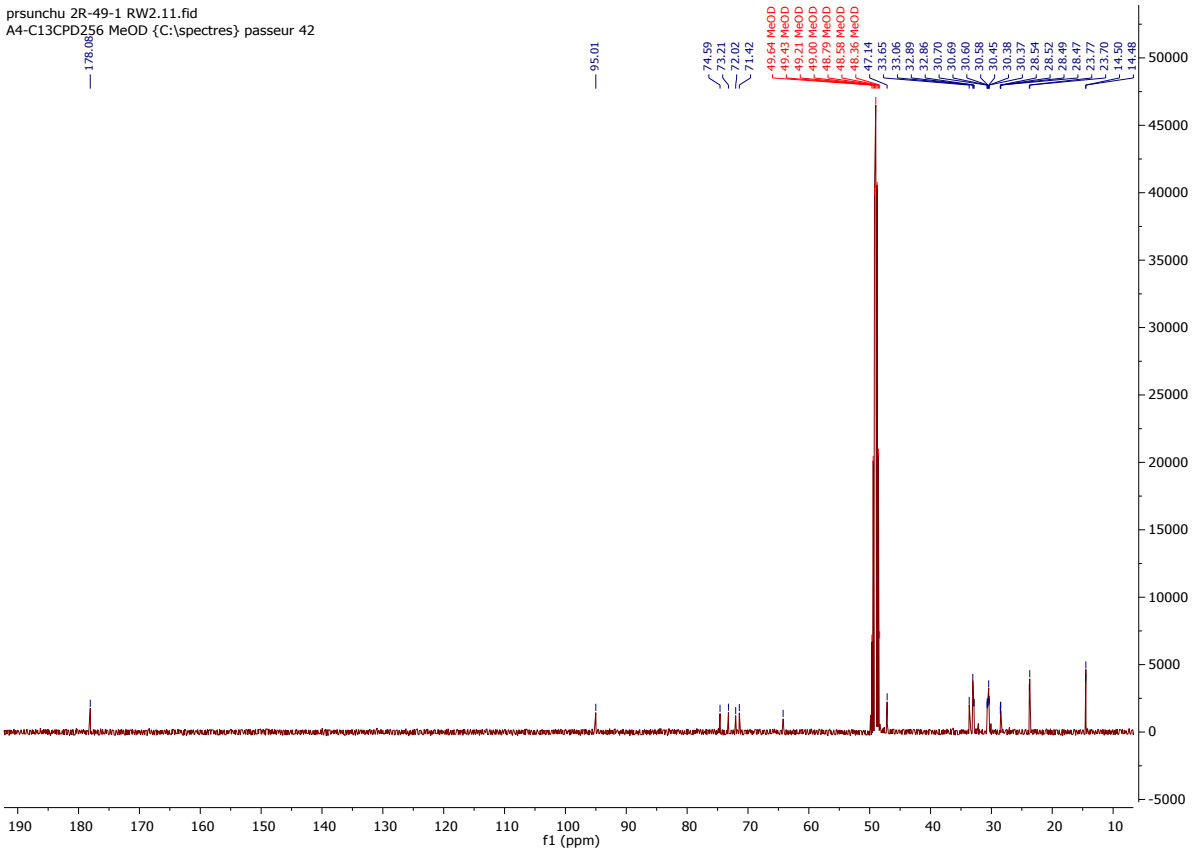

prsunchu 2r-40-1.10.fid
A4-proton CDCl3 {C:\spectres} passeur 3
f1 (ppm)

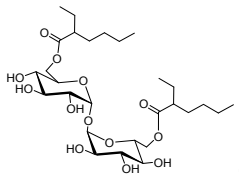

prsunchu 2r-40-1.30.fid
A4-C13CPD1024 CDCL3+MEOD {C:\spectres} passeur 11
f1 (ppm)



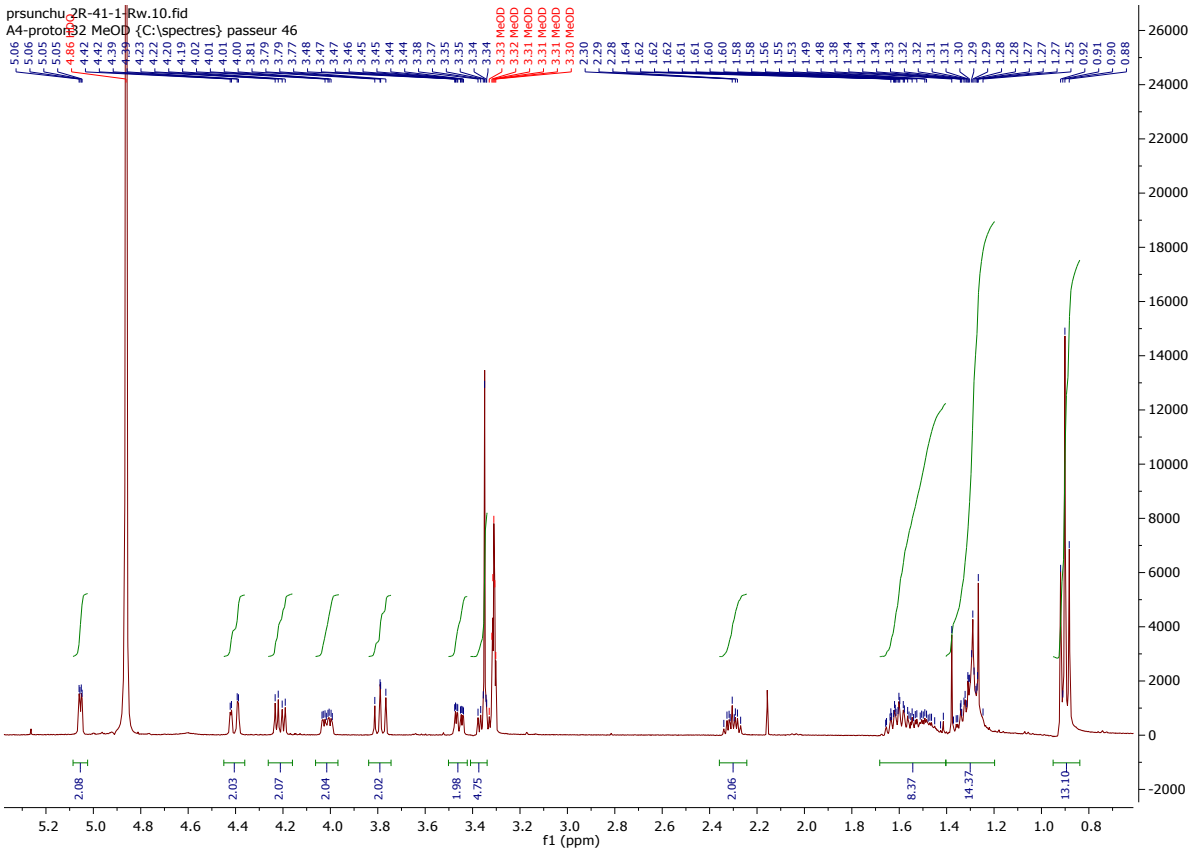
4e

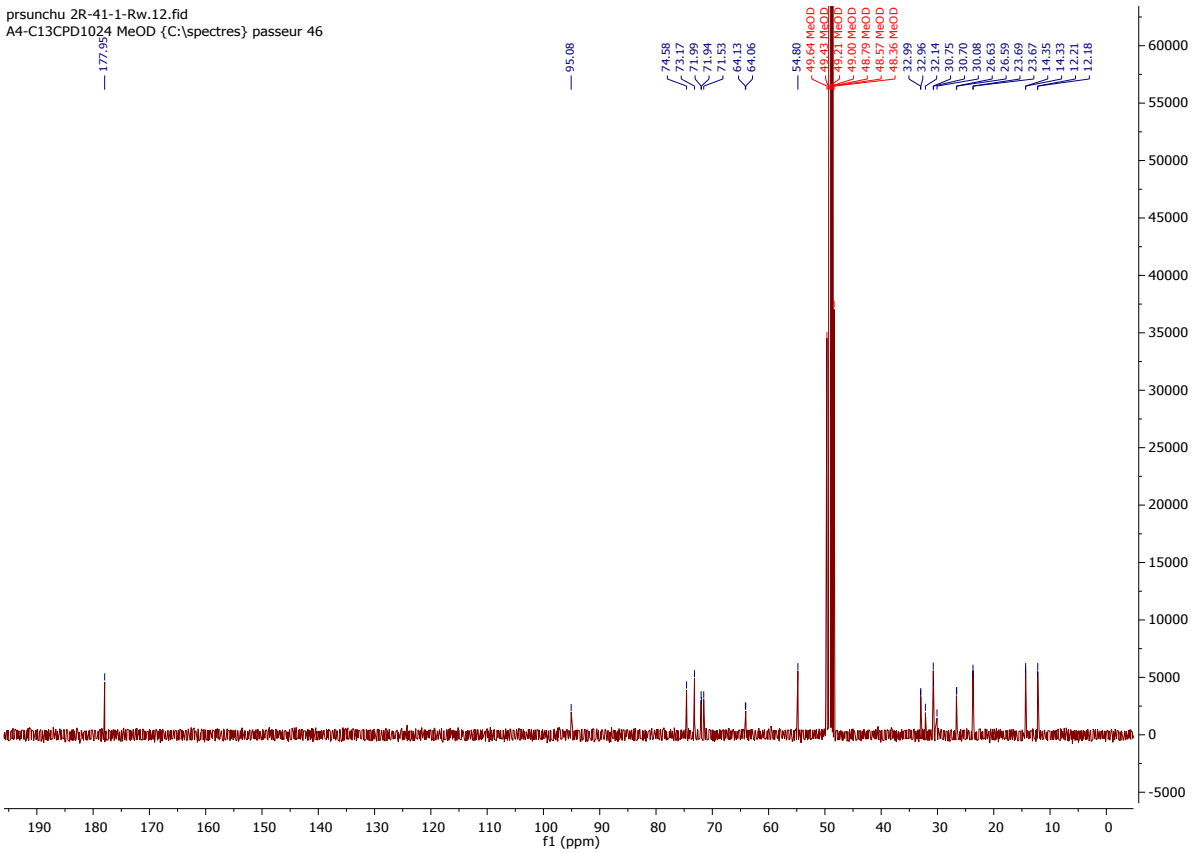

prsunchu 2R-49-1 RW2.10.fid
A4-proton32 MeOD {C:\spectres} passeur 42
4.87 HDO
3.33 MeOD
3.32 MeOD
3.31 MeOD
3.30 MeOD
f1 (ppm)

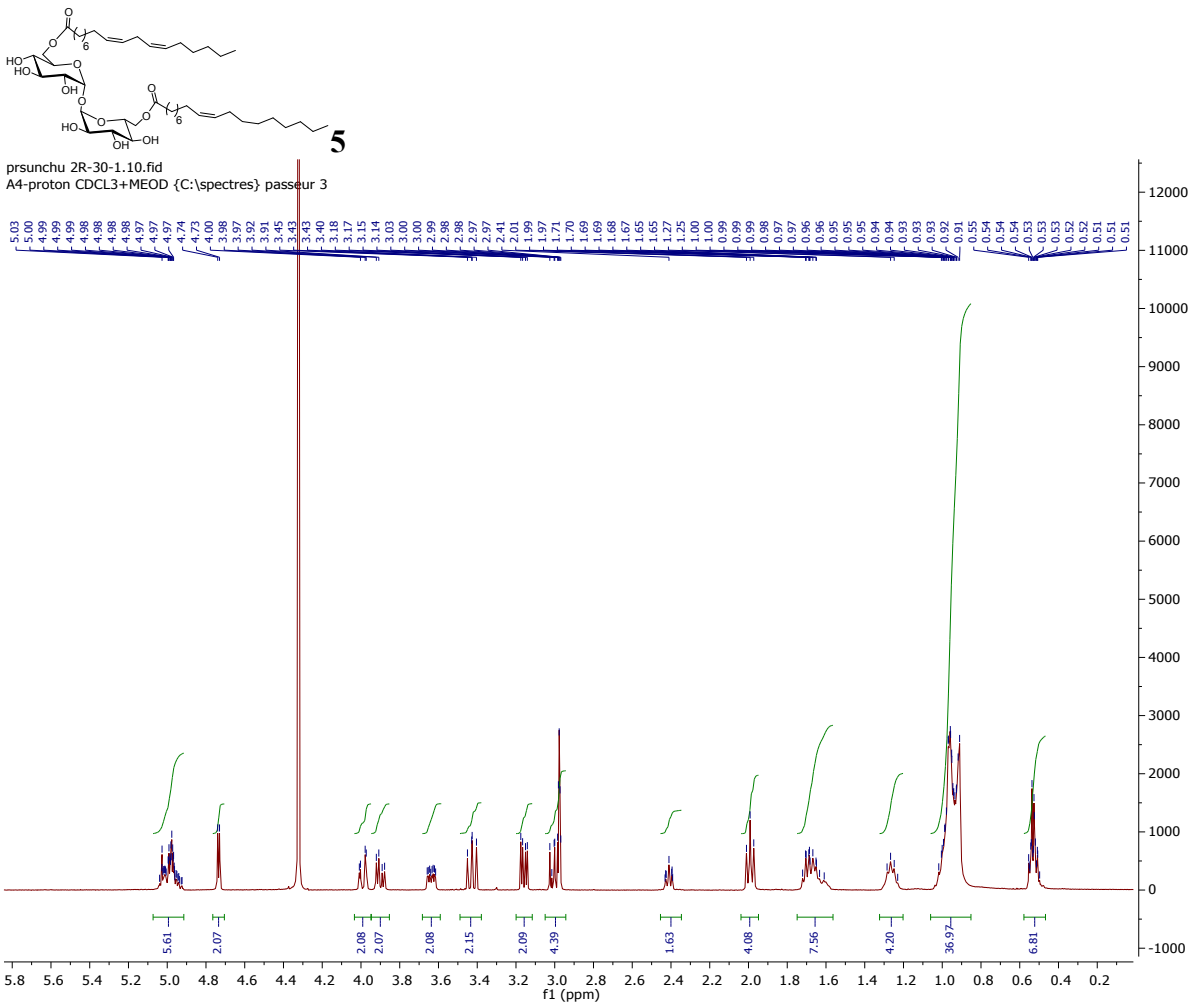

prsunchu 2R-49-1 RW2.11.fid
A4-C13CPD256 MeOD {C:\spectres} passeur 42
178.08
95.01
74.59
73.21
72.02
71.42
49.64 MeOD
49.43 MeOD
49.21 MeOD
49.00 MeOD
48.79 MeOD
48.58 MeOD
48.36 MeOD
47.14
33.65
33.06
32.89
32.86
30.70
30.69
30.60
30.58
30.45
30.38
30.37
28.54
28.52
28.49
28.47
23.77
23.70
14.50
14.48
f1 (ppm)

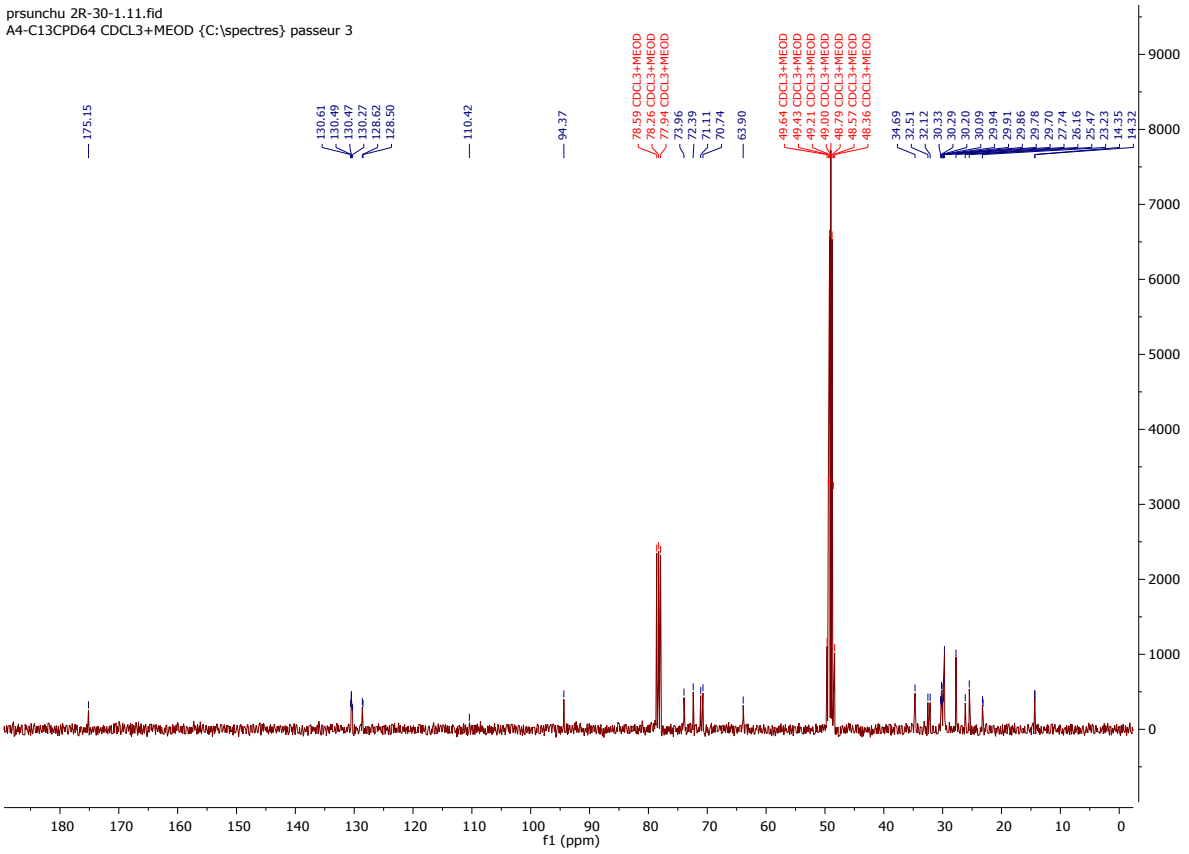


4d


prsunchu 2R-41-1-Rw.10.fid
A4-proton32 MeOD {C:\spectres} passeur 46
f1 (ppm)


prsunchu 2R-41-1-Rw.12.fid
A4-C13CPD1024 MeOD {C:\spectres} passeur 46
f1 (ppm)


5
prsunchu 2R-30-1.10.fid
A4-proton CDCL3+MEOD {C:\spectres} passeur 3
f1 (ppm)


prsunchu 2R-30-1.11.fid
A4-C13CPD64 CDCL3+MEOD {C:\spectres} passeur 3
175.15
130.61
130.49
130.47
130.27
128.62
128.50
110.42
94.37
78.59 CDCL3+MEOD
78.26 CDCL3+MEOD
77.94 CDCL3+MEOD
73.96
72.39
71.11
70.74
63.90
49.64 CDCL3+MEOD
49.43 CDCL3+MEOD
49.21 CDCL3+MEOD
49.00 CDCL3+MEOD
48.79 CDCL3+MEOD
48.57 CDCL3+MEOD
48.36 CDCL3+MEOD
34.69
32.51
32.12
30.33
30.29
30.20
30.09
29.94
29.91
29.86
29.78
29.70
27.74
26.16
25.47
23.23
14.35
14.32
f1 (ppm)