Supplementary Information (SI)

Applied Microbiology and Biotechnology

Updated component analysis method for naturally occurring sophorolipids from *Starmerella bombicola*

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F1 axis: ^{1}H-NMR spectrum ranging from 3.0 to 4.8 ppm; F2 axis: ^{1}H-NMR spectrum ranging from 3.0 to 4.8 ppm

Fig. S9 HSQC spectrum of compound Y, isolated from *S. bombicola*

F1 axis: ^{13}C-NMR spectrum ranging from 10 to 140 ppm; F2 axis: ^{1}H-NMR spectrum ranging from 0.8 to 5.6 ppm

Fig. S10 LC-MS analysis of compound Z, isolated from *S. bombicola*.

Fig. S11 Partial ^{1}H-^{1}H COSY spectrum of compound Z, isolated from *S. bombicola*

F1 axis: ^{1}H-NMR spectrum ranging from 1.0 to 1.8 ppm; F2 axis: ^{1}H-NMR spectrum ranging from 3.0 to 5.5 ppm

Fig. S12 HSQC spectrum of compound Z, isolated from *S. bombicola*

F1 axis: ^{13}C-NMR spectrum ranging from 10 to 140 ppm; F2 axis: ^{1}H-NMR spectrum ranging from 0.8 to 5.6 ppm

Fig. S13 Culture profiles of jar fermentation of *S. bombicola*

(a) Rapeseed oil, (b) rice bran oil; circle: glucose; triangle: oil; square: OD_{600}

Table S1 NMR data for the compound X (methanol-*d4*, 400 MHz)

	^{13}C-NMR		^{1}H-NMR	
	δ (ppm)		δ (ppm)	*J* (Hz)
Saccharides (G1~4)				
C-1	92.6 (G3), 101.2 (G1), 104.2 (G2,4)	H-1	4.48 d (G1), 4.59 d (G2,4), 5.63 d (G3)	7.7
C-2	74.6 (G2,4), 81.1 (G3), 82.4 (G1),	H-2	3.58 t (G3), 3.20-3.65 m	9.0
C-3	76.1–76.8	H-3	3.20–3.65 m	
C-4	69.3–70.0	H-4	3.20–3.65 m	
C-5	73.5–74.2	H-5	3.20–3.65 m	
C-6	60.8–61.3	H-6	3.70, 3.85 m	
C-6 (acetylated)	63.4–63.6	H-6 (acetylated)	4.22, 4.39 m	
Acetyl groups				
–C=O (C-6)	171.6			
–CH_3 (C-6)	19.4, 19.6		2.07, 2.09 s	
Acyl group				
–C=O	173.7			
–CO-$\underline{CH_2}$–	33.5		2.44 m	
–CO-$CH_2\underline{CH_2}$–	24.2		1.64 b	
–$(CH_2)_n$–	24.8–29.6		1.3–1.4 b	
–CH=CH-$\underline{CH_2}$–	26.8		2.0–2.1 b	
–CH=CH–	129.5		5.37 m	
–OCH(CH_3)-$\underline{CH_2}$–	36.5		1.64 b	
–O$\underline{CH}$(CH_3)–	77.3		3.74 m	
–CH_3	20.5		1.22 d	6.2

Table S2 NMR data for the compound Y (methanol-*d4*, 400 MHz)

	^{13}C-NMR		^{1}H-NMR	
	δ (ppm)		δ (ppm)	*J* (Hz)
Saccharides				
C-1'	101.2	H-1'	4.47 d	7.7
C-2'	82.4	H-2'	3.2–3.5 m	
C-3'	76.1 or 76.4	H-3'	3.2–3.5 m	
C-4'	70.0 or 70.1	H-4'	3.2–3.5 m	
C-5'	73.5 or 74.2	H-5'	3.2–3.5 m	
C-6'	63.4 or 63.5	H-6'a	4.21 m	
		H-6'b	4.37 m	
C-1''	104.3	H-1''	4.57 d	7.8
C-2''	74.7	H-2''	3.2–3.5 m	
C-3''	76.1 or 76.4	H-3''	3.2–3.5 m	
C-4''	70.0 or 70.1	H-4''	3.2–3.5 m	
C-5''	73.5 or 74.2	H-5''	3.2–3.5 m	
C-6''	63.4 or 63.5	H-6''a	4.21 m	
		H-6''b	4.37 m	
Acetyl groups				
–C=O (C-6',6'')	171.3			
–CH_3 (C-6',6'')	19.4, 19.5		2.06, 2.08 s	
Glycerol groups				
–CH_2O–	65.1		4.08, 4.15 m	
–CHO–	69.7		3.85 m	
–CH_2OH	62.7		3.57 m	
Acyl group				
–C=O	174.2			
–CO-$\underline{CH_2}$–	33.6		2.37 t	7.4
–CO-$CH_2\underline{CH_2}$–	24.6		1.62 b	
–$(CH_2)_n$–	24.7–29.6		1.2–1.4 b	
–CH=CH-$\underline{CH_2}$–	26.8		2.0–2.1 b	
–CH=CH–	129.5		5.36 m	
–OCH(CH_3)-$\underline{CH_2}$–	36.5		1.62 b	
–O$\underline{CH}$(CH_3)–	77.2		3.76 m	
–CH_3	20.5		1.21 d	6.2

Fig. S1

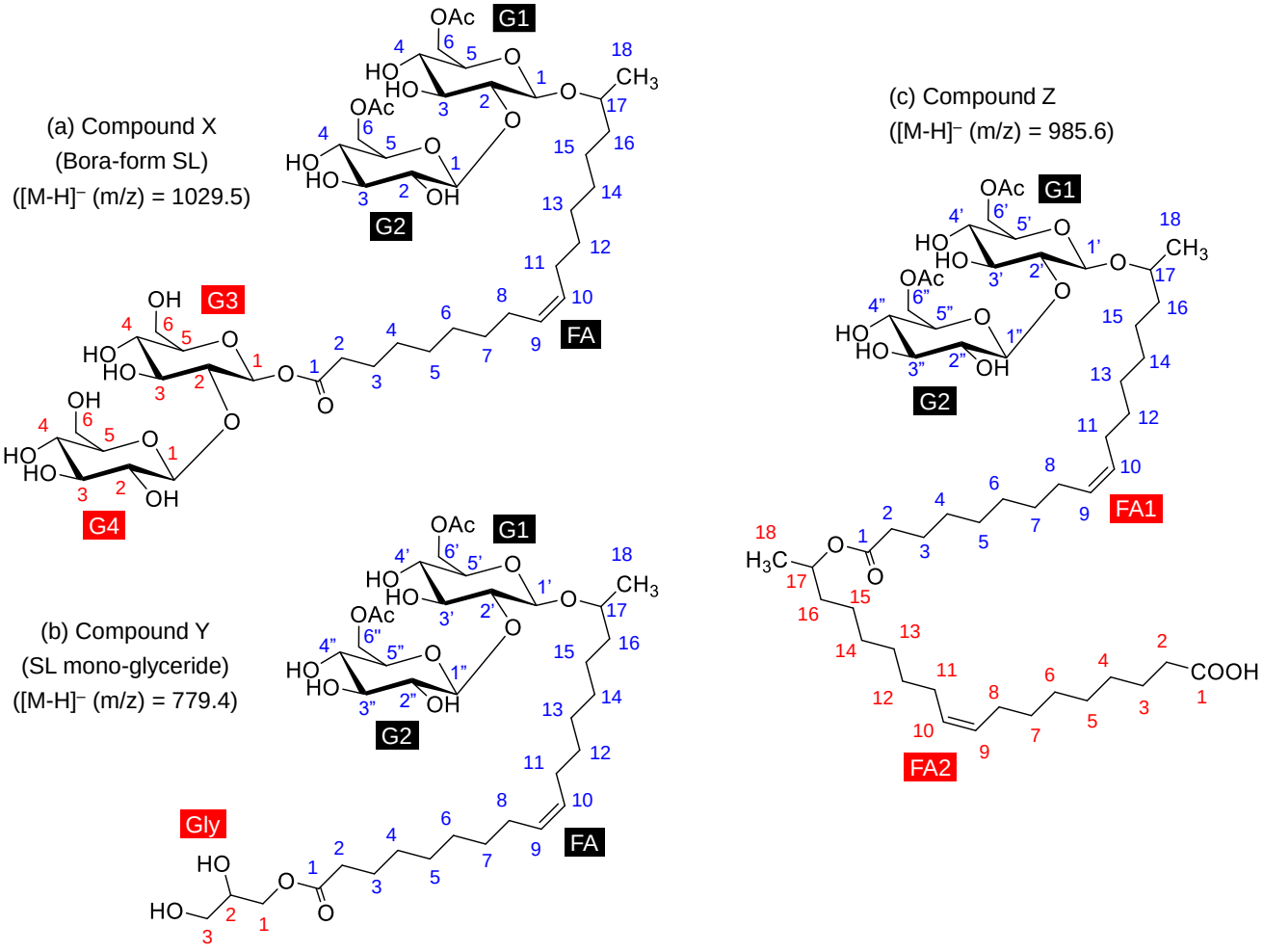

(a) Compound X
(Bora-form SL)
([M-H]⁻ (m/z) = 1029.5)
(b) Compound Y
(SL mono-glyceride)
([M-H]⁻ (m/z) = 779.4)
(c) Compound Z
([M-H]⁻ (m/z) = 985.6)
G1
G2
G3
G4
FA
FA1
FA2
Gly
OAc
OH
HO
CH3
COOH

Fig. S2

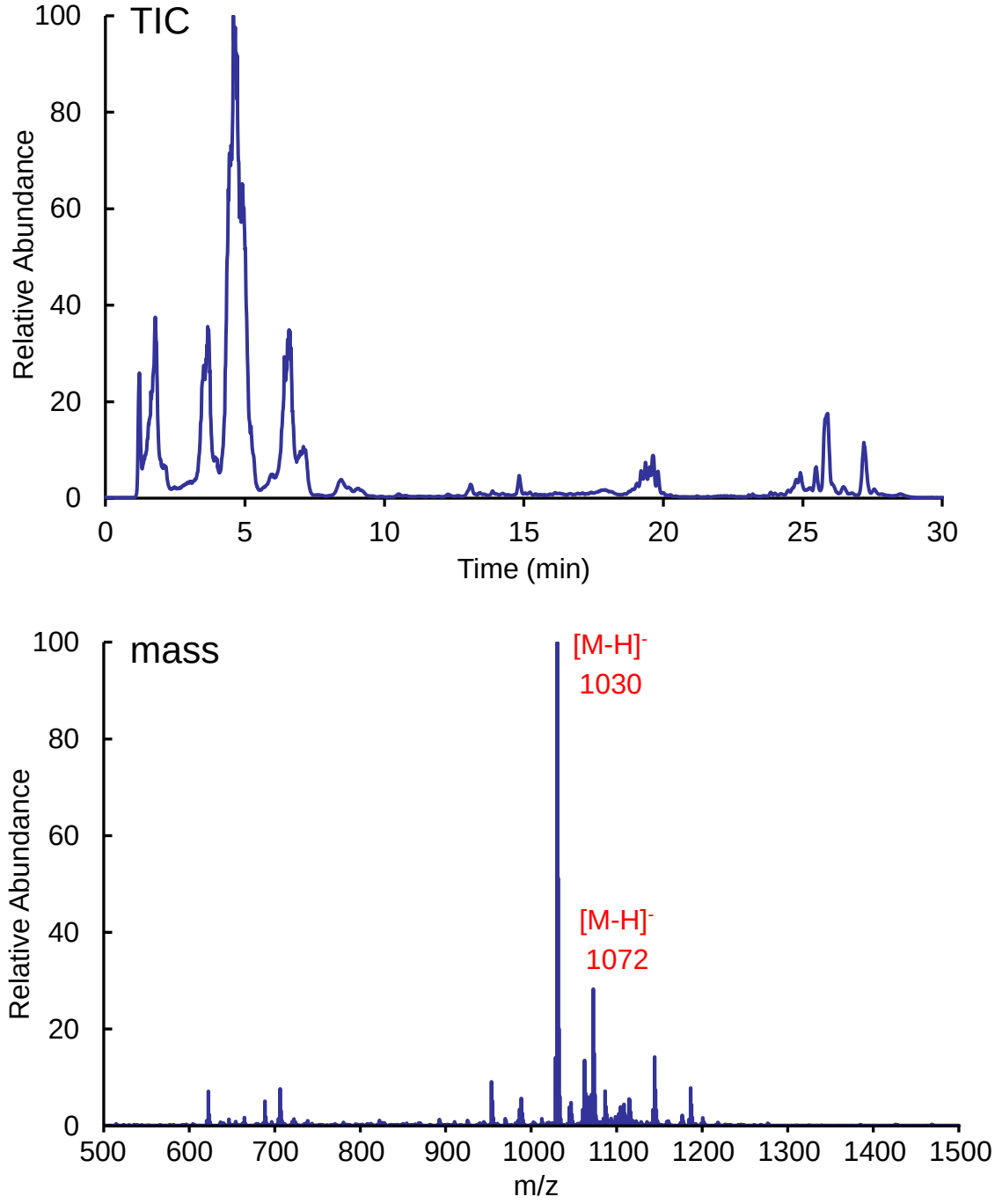

TIC
Relative Abundance
Time (min)
mass
[M-H]-
1030
[M-H]-
1072
Relative Abundance
m/z

Fig. S3

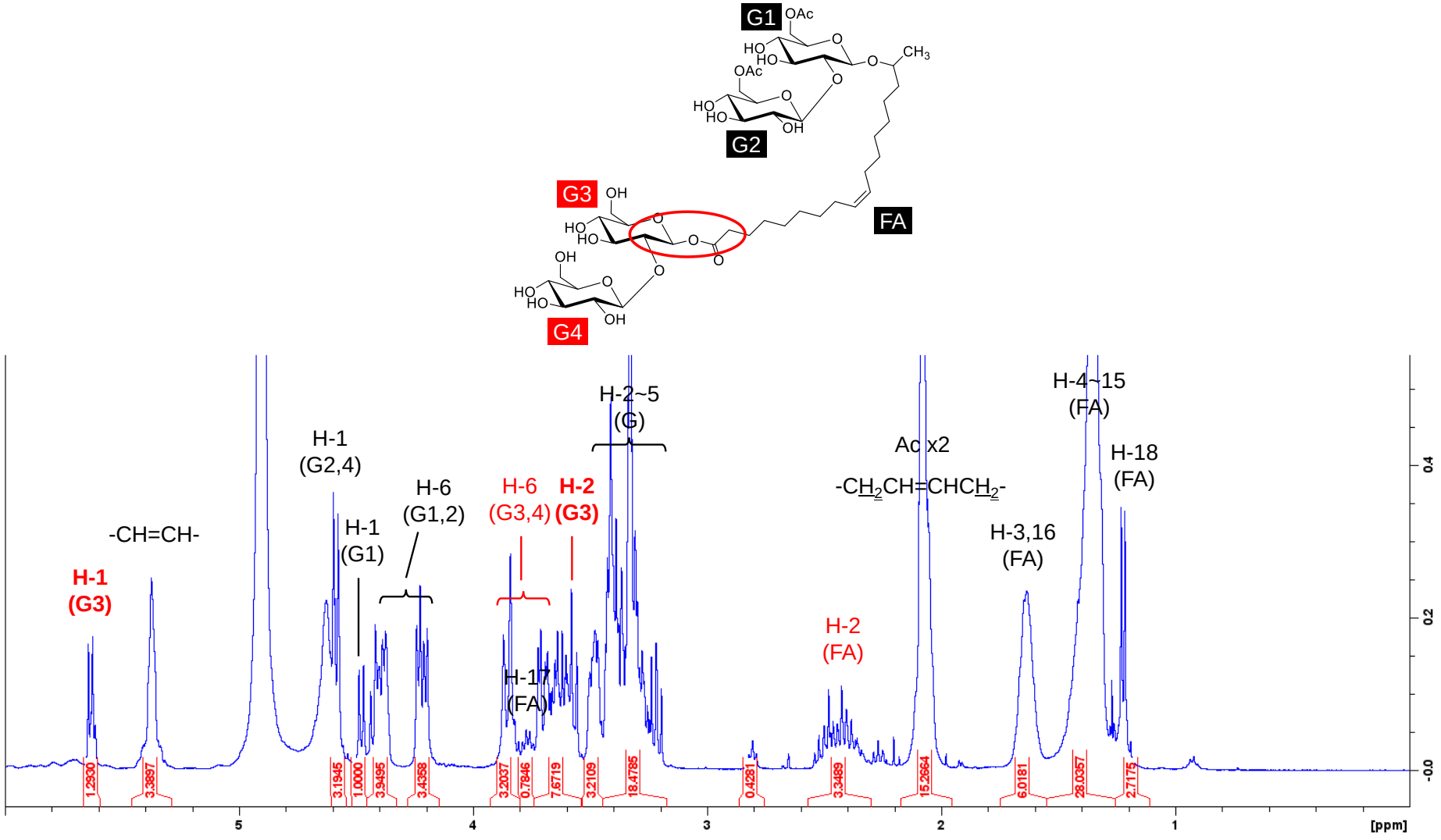

G1
OAc
HO
HO
O
CH3
OAc
HO
HO
OH
G2
G3
OH
HO
HO
FA
OH
HO
HO
G4
H-1
(G3)
-CH=CH-
H-1
(G2,4)
H-1
(G1)
H-6
(G1,2)
H-6
(G3,4)
H-2
(G3)
H-2~5
(G)
H-17
(FA)
H-2
(FA)
Ac x2
-CH2CH=CHCH2-
H-3,16
(FA)
H-4~15
(FA)
H-18
(FA)
1.2930
3.3897
3.1945
1.0000
3.9495
3.4358
3.2037
0.7846
7.6719
3.2109
18.4785
0.4281
3.3489
15.2664
6.0181
28.0357
2.7175
5
4
3
2
1
[ppm]
0.4
0.2
-0.0

Fig. S4

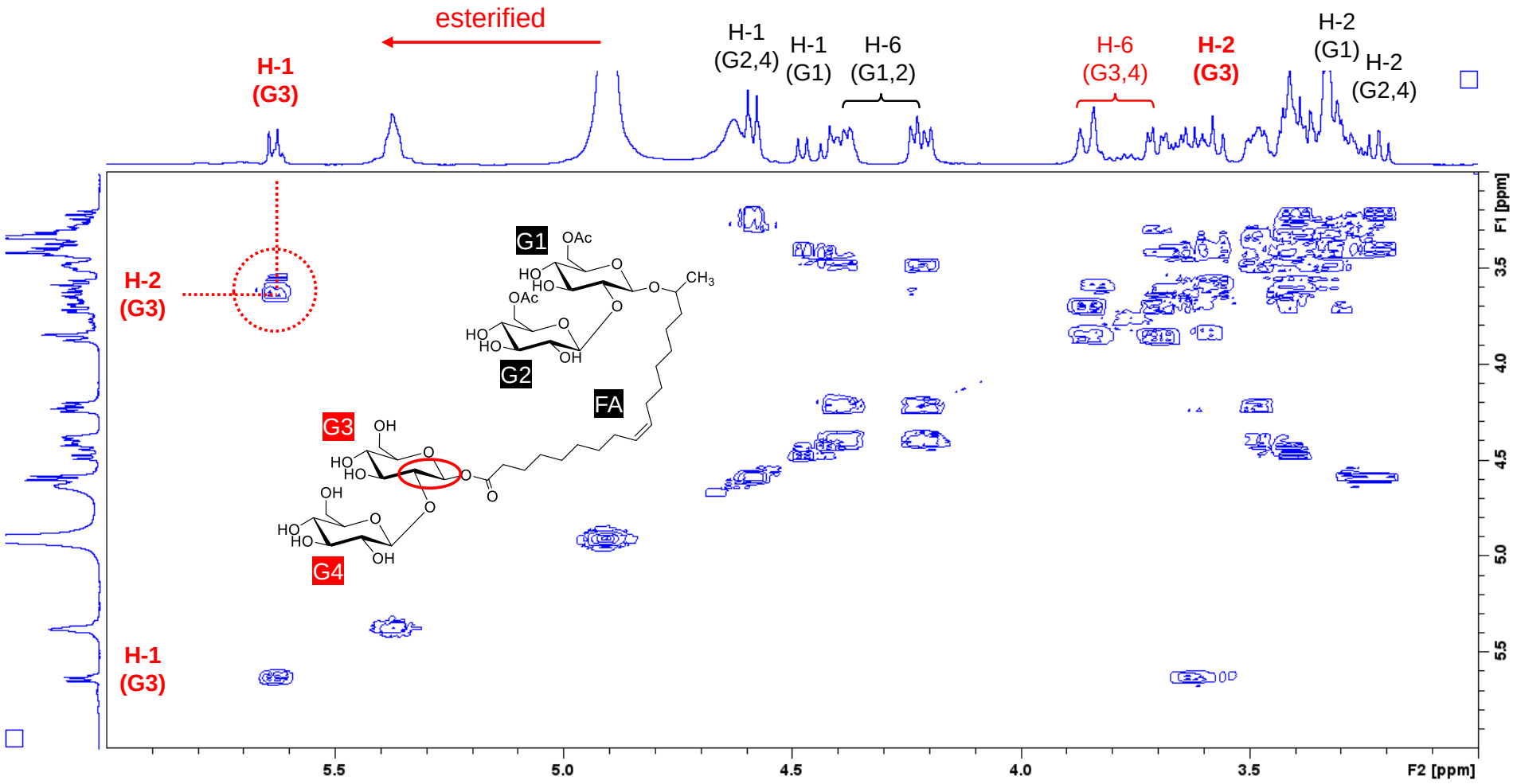

esterified
H-1
(G3)
H-1
(G2,4)
H-1
(G1)
H-6
(G1,2)
H-6
(G3,4)
H-2
(G3)
H-2
(G1)
H-2
(G2,4)
H-2
(G3)
H-1
(G3)
G1
G2
G3
G4
FA
OAc
OH
HO
CH3
F1 [ppm]
F2 [ppm]
5.5
5.0
4.5
4.0
3.5

Fig. S5

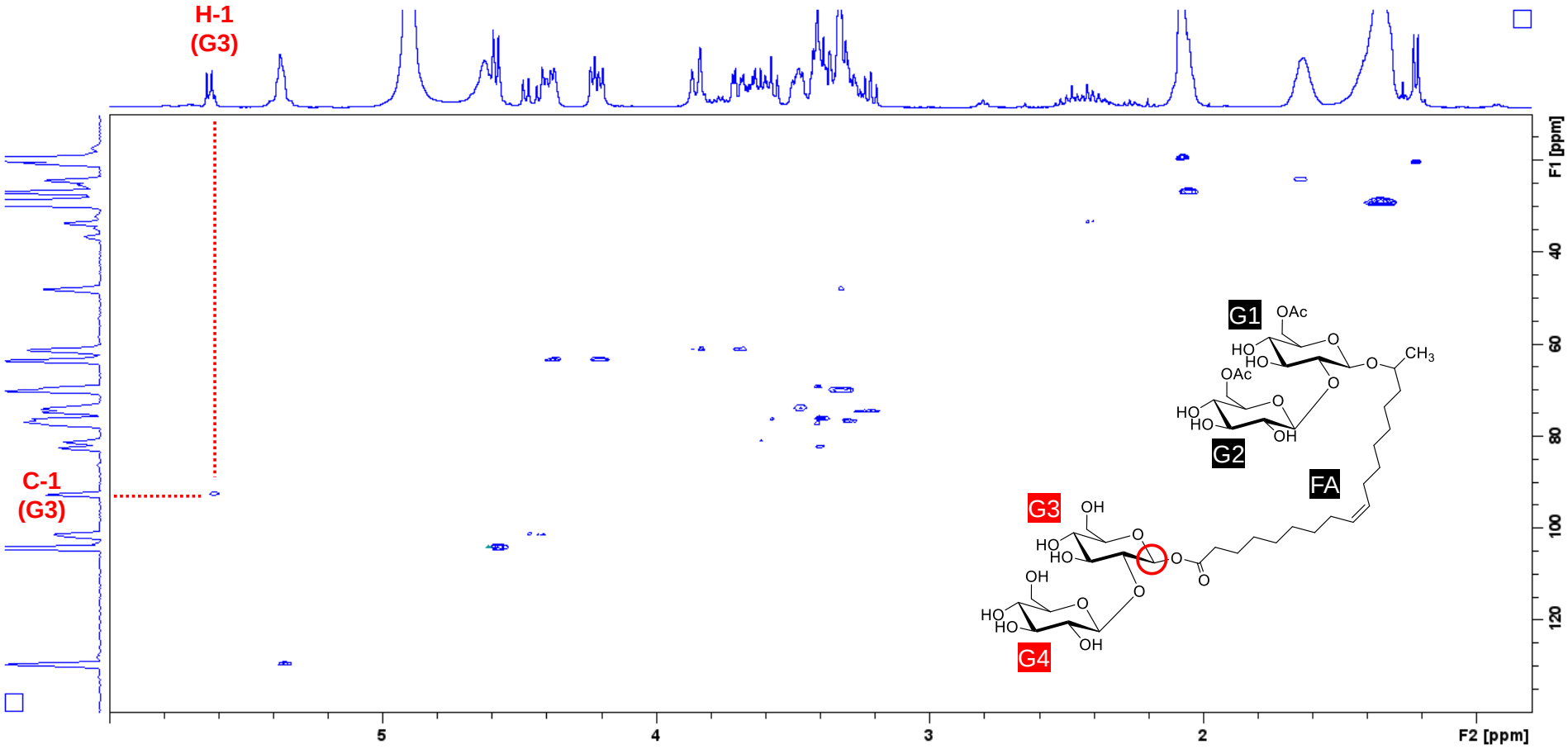

H-1
(G3)
C-1
(G3)
G1
OAc
HO
HO
O
CH3
OAc
HO
HO
O
OH
G2
FA
G3
OH
HO
HO
O
O
O
OH
HO
HO
O
OH
G4
F1 [ppm]
40
60
80
100
120
5
4
3
2
F2 [ppm]

Fig. S6

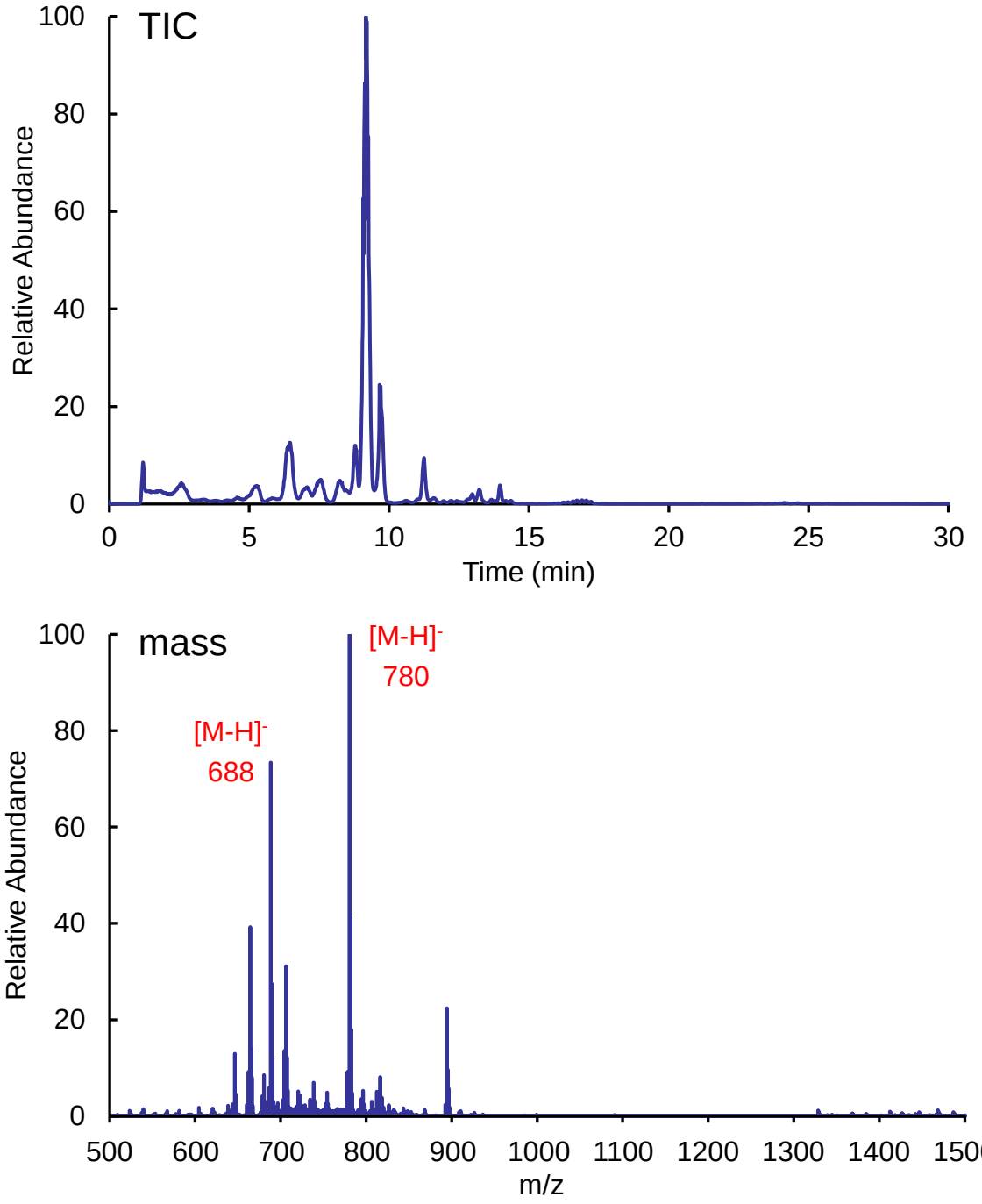

TIC
Relative Abundance
Time (min)
mass
[M-H]-
780
[M-H]-
688
Relative Abundance
m/z

Fig. S7

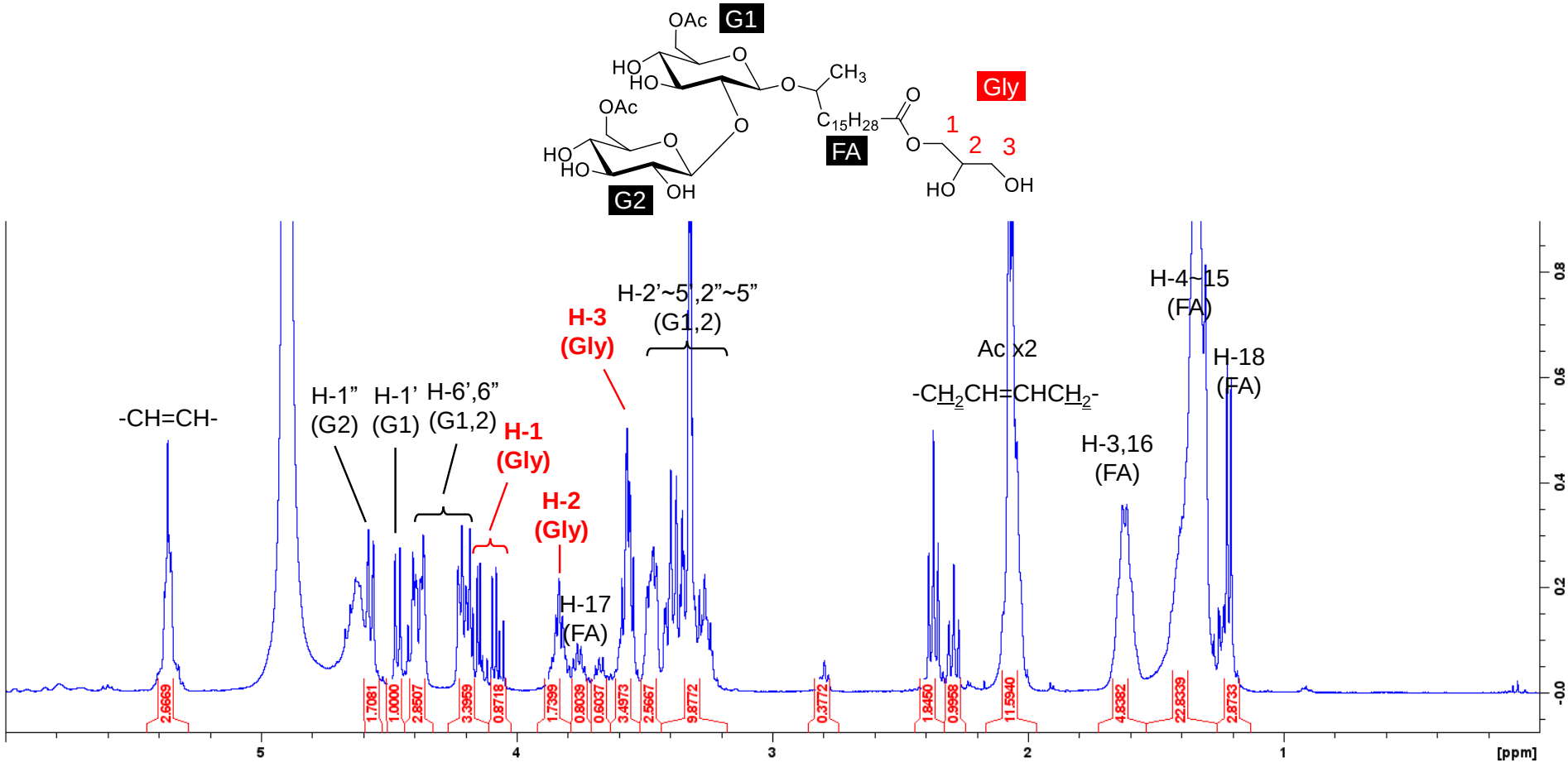

OAc
G1
HO
HO
O
O
CH3
C15H28
Gly
FA
1
2
3
OAc
HO
HO
O
O
HO
OH
G2
H-2'~5',2"~5"
(G1,2)
H-3
(Gly)
Ac x2
-CH2CH=CHCH2-
H-4~15
(FA)
H-18
(FA)
-CH=CH-
H-1"
(G2)
H-1'
(G1)
H-6',6"
(G1,2)
H-1
(Gly)
H-2
(Gly)
H-17
(FA)
H-3,16
(FA)
2.6669
1.7081
1.0000
2.8507
3.3959
0.8718
1.7399
0.8039
0.6037
3.4973
2.5667
9.8772
0.3772
1.8450
0.9958
11.5940
4.8382
22.8339
2.8733
5
4
3
2
1
[ppm]
0.8
0.6
0.4
0.2
-0.0

Fig. S8

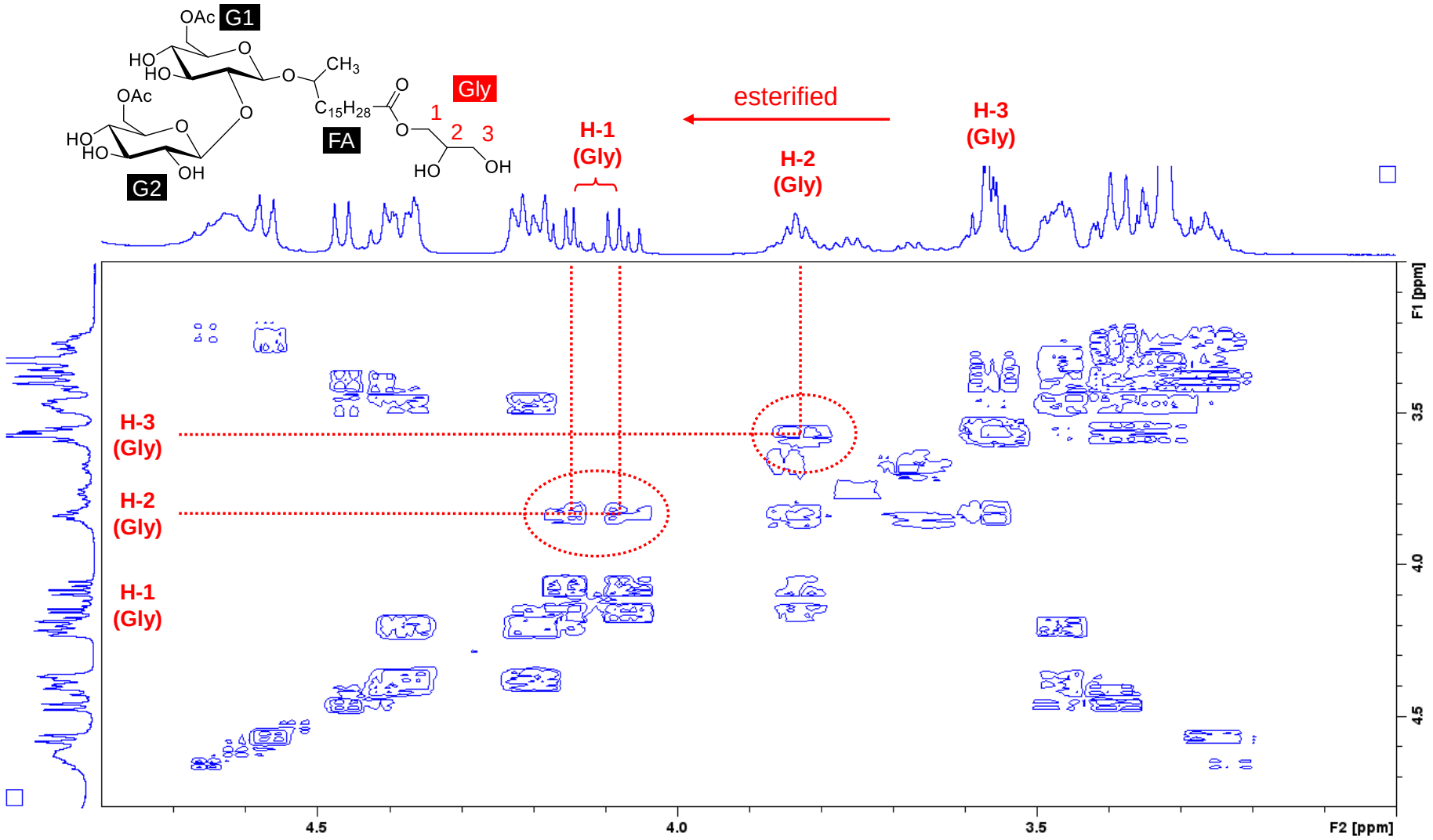

OAc
G1
HO
HO
O
CH3
OAc
C15H28
FA
Gly
1
2
3
HO
OH
HO
HO
O
OH
G2
esterified
H-1
(Gly)
H-2
(Gly)
H-3
(Gly)
H-3
(Gly)
H-2
(Gly)
H-1
(Gly)
F1 [ppm]
3.5
4.0
4.5
4.5
4.0
3.5
F2 [ppm]

Fig. S9

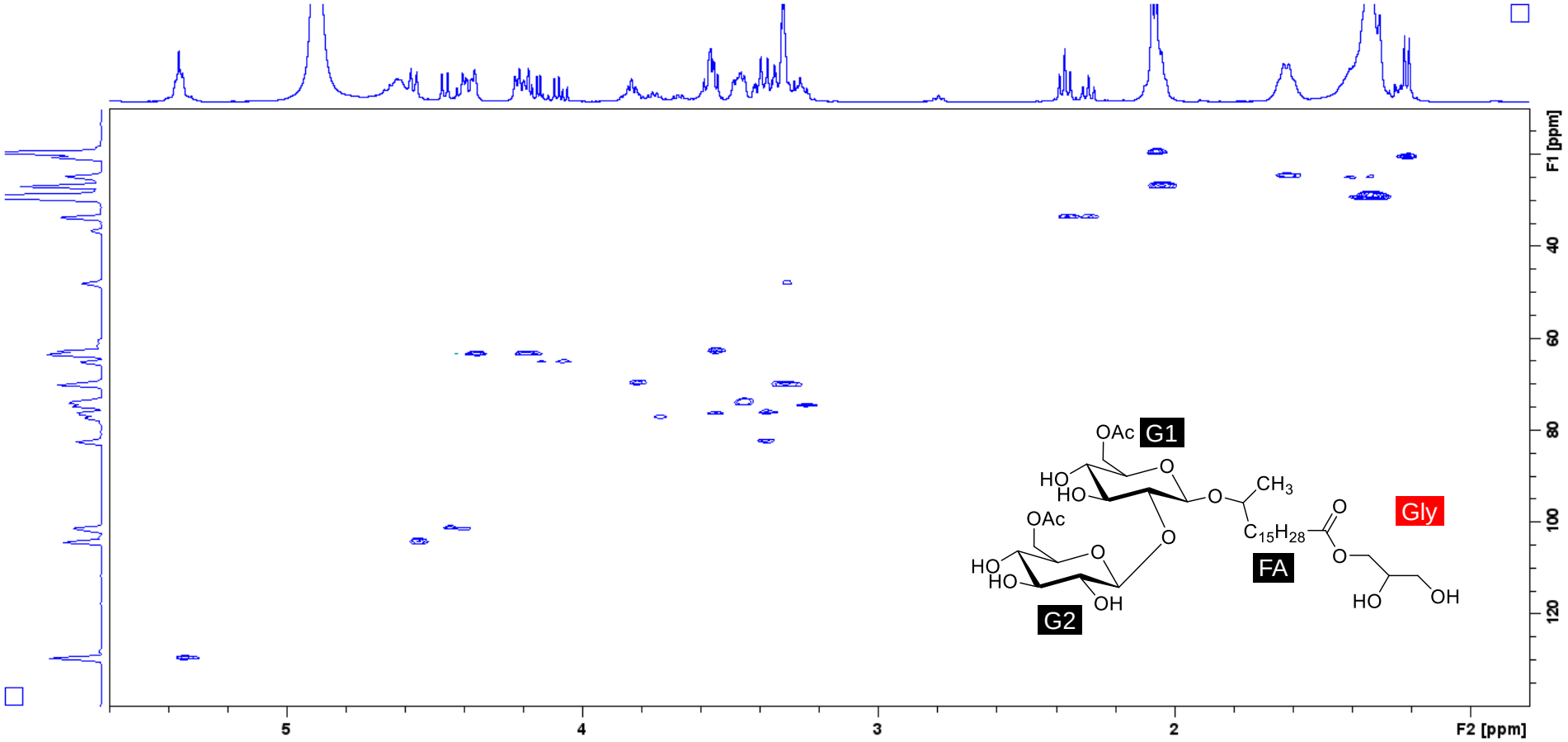

F1 [ppm]
40
60
80
100
120
5
4
3
2
F2 [ppm]
OAc
G1
HO
HO
O
O
CH3
OAc
HO
HO
O
O
C15H28
O
Gly
FA
O
HO
OH
OH
G2

Fig. S10

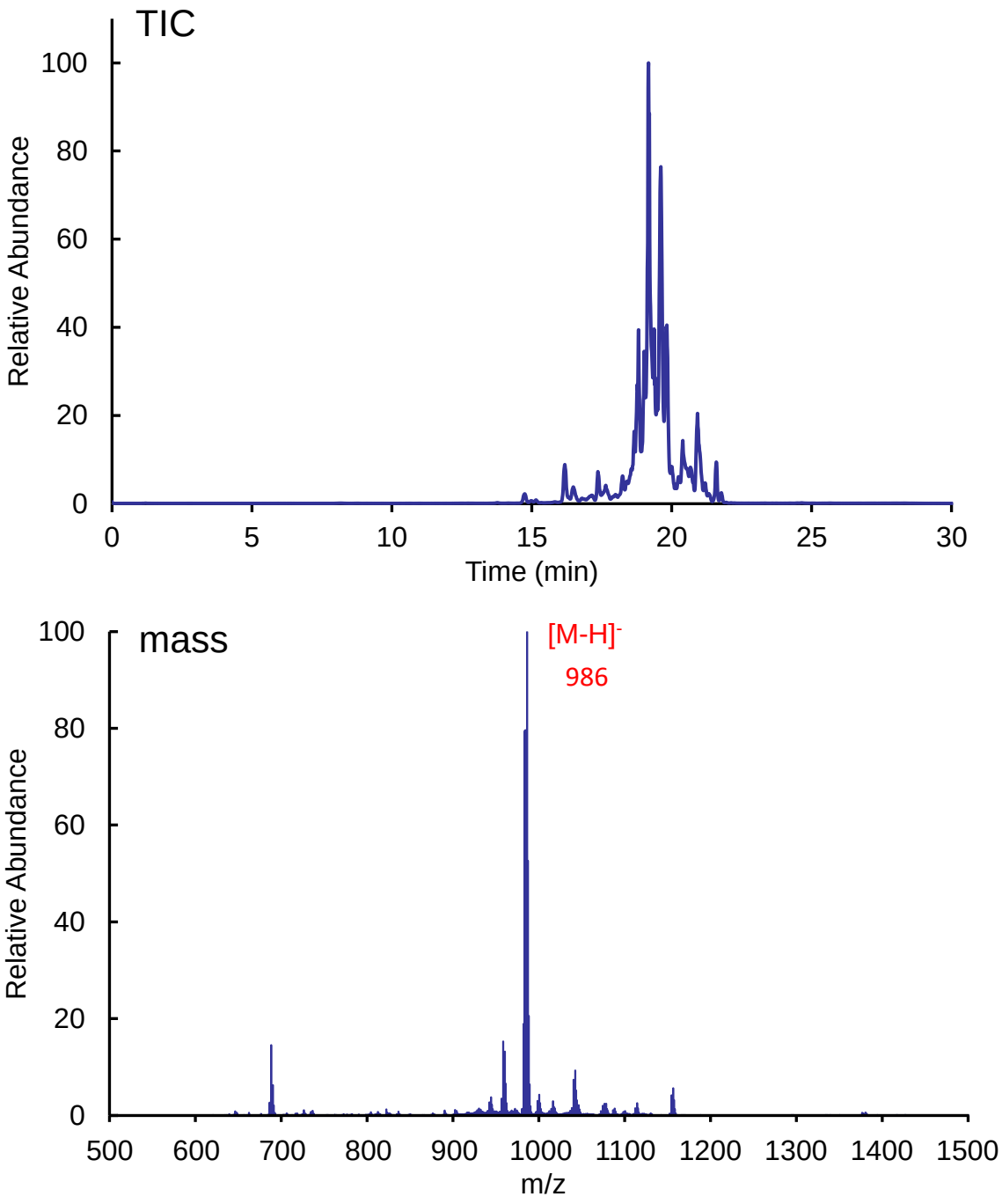

TIC
Relative Abundance
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (min)
mass
[M-H]-
986
Relative Abundance
100
80
60
40
20
0
500
600
700
800
900
1000
1100
1200
1300
1400
1500
m/z

Fig. S11

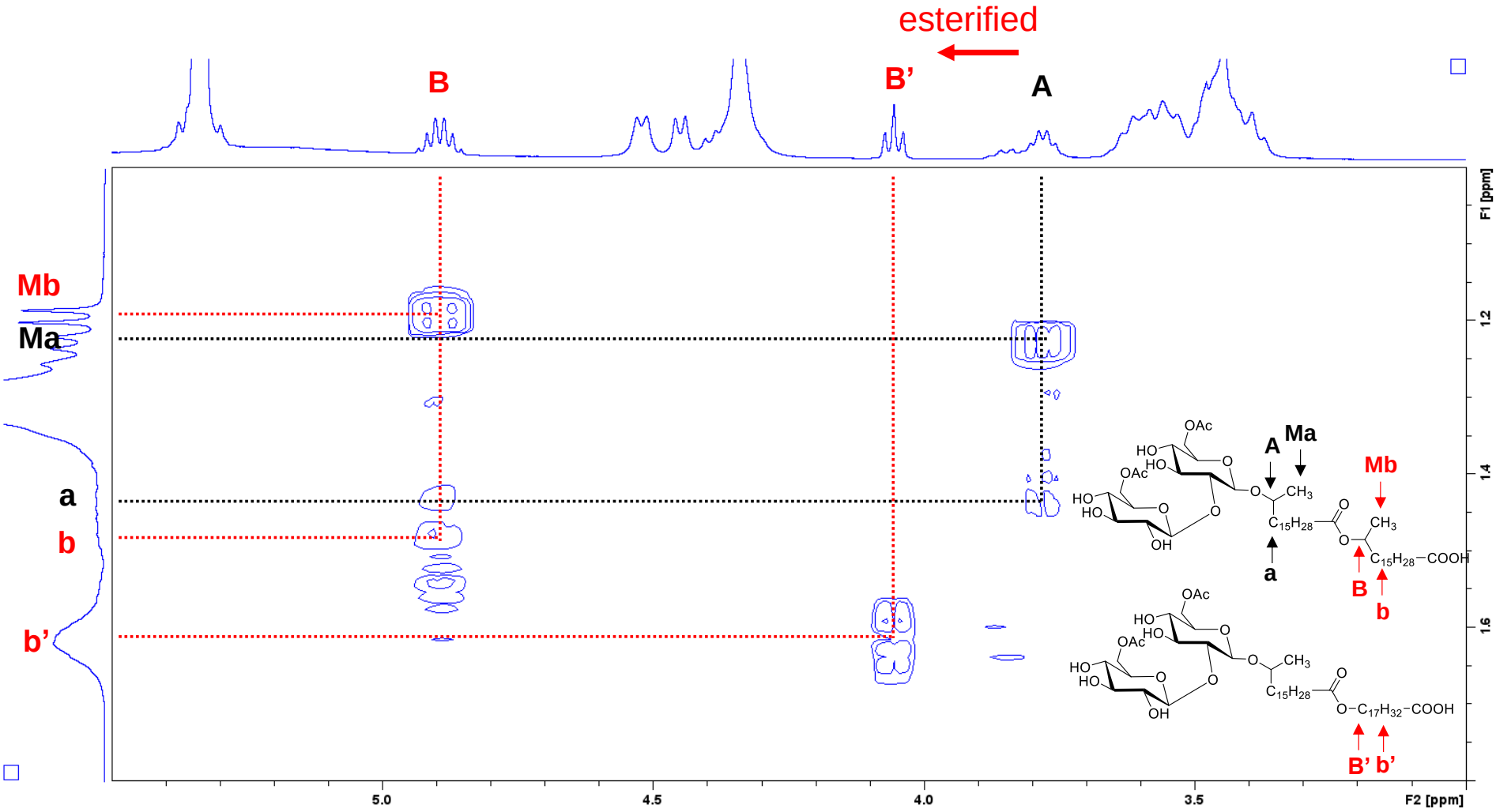

esterified
B
B'
A
Mb
Ma
a
b
b'
F1 [ppm]
1.2
1.4
1.6
5.0
4.5
4.0
3.5
F2 [ppm]
OAc
HO
OAc
HO
HO
HO
O
O
OH
A
Ma
a
CH3
C15H28
O
Mb
CH3
C15H28–COOH
B
b
O–C17H32–COOH
B' b'

Fig. S12

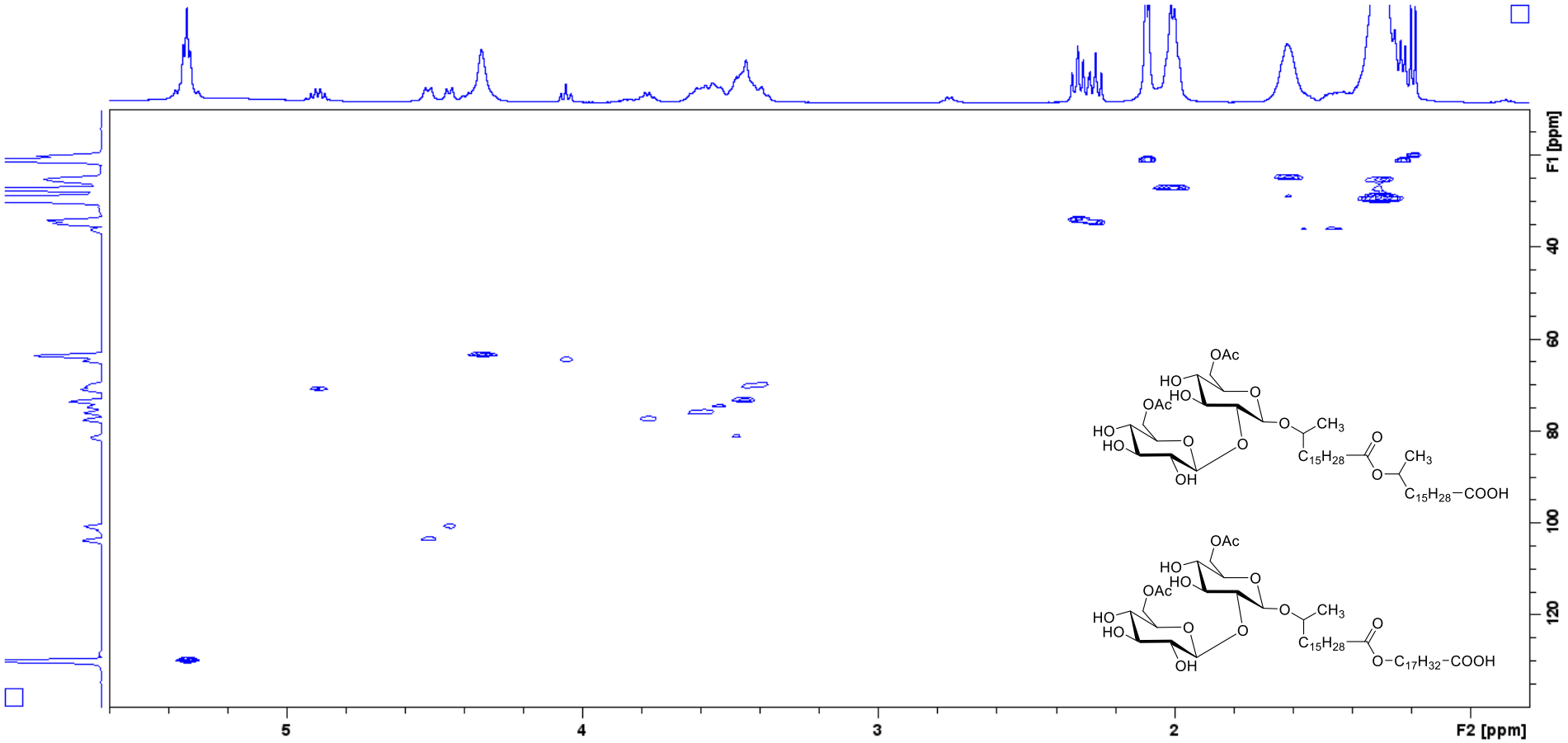
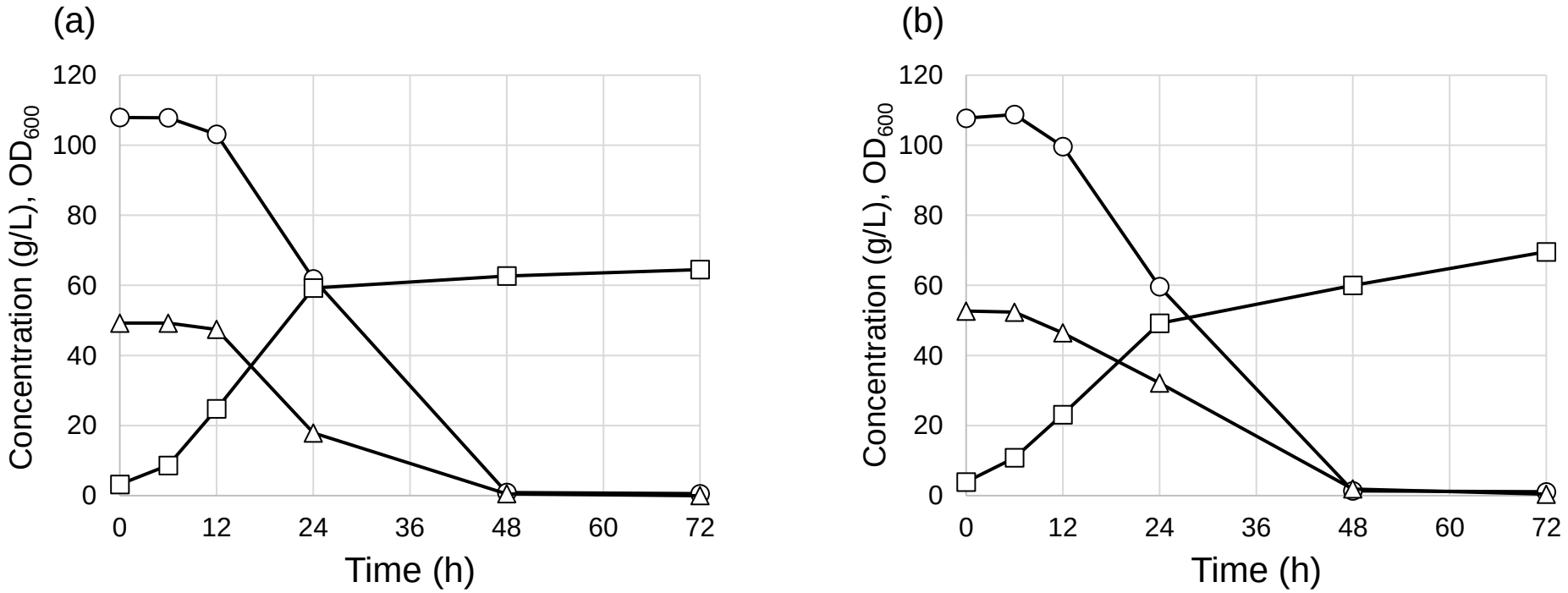

F1 [ppm]
40
60
80
100
120
5
4
3
2
F2 [ppm]
OAc
HO
HO
OAc
HO
HO
OH
O
O
O
CH3
C15H28
O
O
CH3
C15H28−COOH
OAc
HO
HO
OAc
HO
HO
OH
O
O
O
CH3
C15H28
O
O−C17H32−COOH

Fig. S13



(a) rapeseed oil, (b) rice oil, circle: glucose, triangle: oil, square: OD_{600}

Measurement of glucose

The sampled medium was centrifuged to remove oil and precipitate, and the aqueous phase was diluted with 5 mM sulfuric acid for glucose concentration measurements by HPLC. The Aminex HPX-87 H column (Bio-Rad Laboratories, Hercules, CA, USA) with a guard column (Cation H Cartridges 30 × 4.6 mm; Bio-Rad) was used under the following conditions: the column oven temperature of at 65°C, 5 mM H_2SO_4 was used as the mobile phase buffer, and flow rate of 0.6 mL/min.

Measurements of oil and cell growth

Hexane was added to the sampled medium. The mixture was stirred vigorously and then centrifuged to separate the hexane and aqueous phases. The hexane phase was collected and dried under reduced pressure, and the remaining oil was weighed. Next, methanol was added to the aqueous phase, the mixture was stirred vigorously, and the yeast cells were precipitated by centrifugation. After washing with water once, the turbidity (OD_{600}) was measured.