

# Supplementary information for

## **Integration of LC/MS-based molecular networking and classical phytochemical approach allows in-depth annotation of the metabolome of non-model organisms - The case study of the brown seaweed *Taonia atomaria***

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## Detailed MS/MS fragmentation of a selection of nodes

### Cluster 1A (See Figure S6)

- **Example of TG:** node at  $m/z$  794.7233 ( $[\text{C}_{49}\text{H}_{96}\text{NO}_6]^+$ , calc.  $m/z$  794.7232,  $\Delta$  -0.1 ppm;  $[\text{M} + \text{NH}_4]^+$ )

This  $m/z$  feature was identified as TG (46:1) which ionized as a cationic adduct  $[\text{M} + \text{NH}_4]^+$ . The analysis of its MS/MS fragmentation pattern allowed its annotation as **TG (14:0/16:0/16:1)** since three groups of fragment ions were observed in the MS/MS spectrum of this compound: (i) diglyceride ions resulting of the loss of one fatty acid chain at  $m/z$  549.4879 ( $[\text{C}_{35}\text{H}_{65}\text{O}_4]^+$ , calc.  $m/z$  549.4877,  $\Delta$  -0.3 ppm;  $[\text{M} - \text{C}_{14:0} + \text{H}]^+$ ),  $m/z$  523.4720 ( $[\text{C}_{33}\text{H}_{63}\text{O}_4]^+$ , calc.  $m/z$  523.4721,  $\Delta$  0.3 ppm;  $[\text{M} - \text{C}_{16:1} + \text{H}]^+$ ), and  $m/z$  521.4567 ( $[\text{C}_{33}\text{H}_{61}\text{O}_4]^+$ , calc.  $m/z$  521.4564,  $\Delta$  -0.4 ppm;  $[\text{M} - \text{C}_{16:0} + \text{H}]^+$ ), (ii) monoglyceride ions obtained after the loss of two fatty acids at  $m/z$  313.2741 ( $[\text{C}_{19}\text{H}_{37}\text{O}_3]^+$ , calc.  $m/z$  313.2737,  $\Delta$  -1.1 ppm;  $[\text{M} - \text{C}_{14:0} - \text{C}_{16:1} + \text{H}]^+$ ),  $m/z$  311.2581 ( $[\text{C}_{19}\text{H}_{35}\text{O}_3]^+$ , calc.  $m/z$  311.2581,  $\Delta$  -0.1 ppm;  $[\text{M} - \text{C}_{14:0} - \text{C}_{16:0} + \text{H}]^+$ ) and  $m/z$  285.2429 ( $[\text{C}_{17}\text{H}_{33}\text{O}_3]^+$ , calc.  $m/z$  285.2424,  $\Delta$  -1.5 ppm;  $[\text{M} - \text{C}_{16:0} - \text{C}_{16:1} + \text{H}]^+$ ), and (iii) acylium ions at  $m/z$  239.2367 ( $[\text{C}_{16}\text{H}_{31}\text{O}]^+$ , calc.  $m/z$  239.2369,  $\Delta$  1.2 ppm),  $m/z$  237.2213 ( $[\text{C}_{16}\text{H}_{29}\text{O}]^+$ , calc.  $m/z$  237.2213,  $\Delta$  -0.1 ppm) and  $m/z$  211.2053 ( $[\text{C}_{14}\text{H}_{27}\text{O}]^+$ , calc.  $m/z$  211.2056,  $\Delta$  1.5 ppm).

- **Example of SQDG:** node at  $m/z$  834.5396 ( $[\text{C}_{43}\text{H}_{80}\text{NO}_{12}\text{S}]^+$ , calc.  $m/z$  834.5396,  $\Delta$  0.0 ppm;  $[\text{M} + \text{NH}_4]^+$ )

This  $m/z$  feature was annotated as **SQDG (16:0/18:3)**, which ionized as a cationic adduct  $[\text{M} + \text{NH}_4]^+$ , using: (i) fragment ions at  $m/z$  573.4883 ( $[\text{C}_{37}\text{H}_{65}\text{O}_4]^+$ , calc.  $m/z$  573.4877,  $\Delta$  -1.0 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} - \text{H}_2\text{O} + \text{H}]^+$ ) and  $m/z$  591.4988 ( $[\text{C}_{37}\text{H}_{67}\text{O}_5]^+$ , calc.  $m/z$  591.4983,  $\Delta$  -0.2 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} + \text{H}]^+$ ) caused by the detachment of the polar head and generating neutral losses of  $m/z$  261 and 243 Da from the adduct ion, respectively, and (ii) two other fragment ions at  $m/z$  335.2582 ( $[\text{C}_{21}\text{H}_{35}\text{O}_3]^+$ , calc.  $m/z$  335.2581,  $\Delta$  -0.5 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} - \text{H}_2\text{O} - \text{C}_{16:0} + \text{H}]^+$ ) and  $m/z$  313.2739 ( $[\text{C}_{19}\text{H}_{37}\text{O}_3]^+$ , calc.  $m/z$  313.2737,  $\Delta$  -0.4 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} - \text{H}_2\text{O} - \text{C}_{18:3} + \text{H}]^+$ ) which allowed to defined the two acyl chains as C16:0 and C18:3, respectively.

- **Example of MGDG:** node at  $m/z$  746.5770 ( $[\text{C}_{41}\text{H}_{80}\text{NO}_{10}]^+$ , calc.  $m/z$  746.5777,  $\Delta$  0.9 ppm;  $[\text{M} + \text{NH}_4]^+$ )

This  $m/z$  feature (ionized as a cationic adduct  $[\text{M} + \text{NH}_4]^+$ ) was identified as a MGDG (32:1) on the basis of the fragment ion at  $m/z$  549.4874 ( $[\text{C}_{35}\text{H}_{65}\text{O}_4]^+$ , calc.  $m/z$  549.4877,  $\Delta$  0.7 ppm;  $[\text{M} - \text{Gal} + \text{H}]^+$ ) which corresponded to a neutral loss of 197.0900 Da. Its annotation as **MGDG (14:0/18:1)** was fulfilled thanks to fragment ions at  $m/z$  339.2890 ( $[\text{C}_{21}\text{H}_{39}\text{O}_3]^+$ , calc.  $m/z$  339.2894,  $\Delta$  1.2 ppm;  $[\text{M} - \text{Gal} - \text{C}_{14:0} + \text{H}]^+$ ), and  $m/z$  285.2420 ( $[\text{C}_{17}\text{H}_{33}\text{O}_3]^+$ , calc.  $m/z$  285.2424,  $\Delta$  1.5 ppm;  $[\text{M} - \text{Gal} - \text{C}_{18:1} + \text{H}]^+$ ), and the two acylium ions at  $m/z$  265.2524 ( $[\text{C}_{18}\text{H}_{33}\text{O}]^+$ , calc.  $m/z$  265.2526,  $\Delta$  0.7 ppm) and  $m/z$  211.2054 ( $[\text{C}_{14}\text{H}_{27}\text{O}]^+$ , calc.  $m/z$  211.2056,  $\Delta$  1.2 ppm).

### Cluster 1B (See Figure S8)

- **Example of DGDG:** node at  $m/z$  976.5977 ( $[\text{C}_{53}\text{H}_{86}\text{NO}_{15}]^+$ , calc.  $m/z$  976.5992,  $\Delta$  1.6 ppm;  $[\text{M} + \text{NH}_4]^+$ )

This  $m/z$  feature was observed as an adduct ion ( $[\text{M} + \text{NH}_4]^+$ ) and its MS/MS spectrum showed fragment ions at  $m/z$  617.4559 ( $[\text{C}_{41}\text{H}_{61}\text{O}_4]^+$ , calc.  $m/z$  617.4564,  $\Delta$  0.8 ppm;  $[\text{M} - 2 \text{ Gal} - \text{H}_2\text{O} + \text{H}]^+$ ) and  $m/z$  635.4668 ( $[\text{C}_{41}\text{H}_{63}\text{O}_5]^+$ , calc.  $m/z$  635.4670,  $\Delta$  0.4 ppm;  $[\text{M} - 2 \text{ Gal} + \text{H}]^+$ ). These two fragment ions corresponding to neutral losses of 359 and 341 Da from the adduct ion were characteristic of digalactosylacylglycerols (DGDGs). Further fragment ions attributed to monoglyceride ions at  $m/z$  359.2580 ( $[\text{C}_{23}\text{H}_{35}\text{O}_3]^+$ , calc.  $m/z$  359.2581,  $\Delta$  0.2 ppm;  $[\text{M} - 2 \text{ Gal} - \text{H}_2\text{O} - \text{C}_{18:4} + \text{H}]^+$ ) and  $m/z$  333.2423 ( $[\text{C}_{21}\text{H}_{33}\text{O}_3]^+$ , calc.  $m/z$  333.2424,  $\Delta$  0.5 ppm;  $[\text{M} - 2 \text{ Gal} - \text{H}_2\text{O} - \text{C}_{20:5} + \text{H}]^+$ ), and to acylium ions at  $m/z$  285.2213 ( $[\text{C}_{20}\text{H}_{29}\text{O}]^+$ , calc.  $m/z$  285.2213,  $\Delta$  0.0 ppm) and  $m/z$  259.2057 ( $[\text{C}_{18}\text{H}_{27}\text{O}]^+$ , calc.  $m/z$  259.2056,  $\Delta$  -0.2 ppm) allowed the annotation of this metabolite as **DGDG (18:4/20:5)**.

### Cluster 1C (See Figure S9)

- **Example of MGTA:** node at  $m/z$  446.3481 ( $[\text{C}_{24}\text{H}_{48}\text{NO}_6]^+$ , calc.  $m/z$  446.3476,  $\Delta$  2.0 ppm)

This  $m/z$  feature, annotated as **MGTA (14:0)**, showed a characteristic MS fragmentation pathway with: (i) a fragment ion at  $m/z$  428.3379 ( $[\text{C}_{24}\text{H}_{46}\text{NO}_5]^+$ , calc.  $m/z$  428.3370,  $\Delta$  -2.1 ppm;  $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ ) resulting from the loss of water from the molecular ion, (ii) a fragment ion at  $m/z$  285.2429 ( $[\text{C}_{17}\text{H}_{33}\text{O}_3]^+$ , calc.  $m/z$  285.2424,  $\Delta$  -1.8 ppm;  $[\text{M} - \text{C}_7\text{H}_{15}\text{NO}_3]^+$ ) resulting from the loss of the hydroxymethyl-*N,N,N*-trimethyl- $\beta$ -alanine group, and (iii) several ions, including the characteristic fragment ion at  $m/z$  236.1496 ( $[\text{C}_{10}\text{H}_{22}\text{NO}_5]^+$ , calc.  $m/z$  236.1492,  $\Delta$  -1.5 ppm) resulting from the loss of the acyl chain, obtained by successive fragmentations of the polar head group, such as at  $m/z$  218.1387 ( $[\text{C}_{10}\text{H}_{20}\text{NO}_4]^+$ , calc.  $m/z$  218.1387,  $\Delta$  -0.3 ppm; loss of water),  $m/z$  162.1127 ( $[\text{C}_7\text{H}_{16}\text{NO}_3]^+$ , calc.  $m/z$  162.1125,  $\Delta$  -1.4 ppm; loss of glycerol) and  $m/z$  144.1023 ( $[\text{C}_7\text{H}_{14}\text{NO}_2]^+$ , calc.  $m/z$  144.1019,  $\Delta$  -2.9 ppm; loss of glycerol and water).

### Cluster 1D (See Figure S10)

- **Example of SQMG:** node at  $m/z$  600.3411 ( $[\text{C}_{27}\text{H}_{54}\text{NO}_{11}\text{S}]^+$ , calc.  $m/z$  600.3412,  $\Delta$  0.1 ppm;  $[\text{M} + \text{NH}_4]^+$ )

This  $m/z$  feature, observed as an adduct ion ( $[\text{M} + \text{NH}_4]^+$ ), was annotated as **SQMG (18:1)** on the basis of the characteristic fragment ions at  $m/z$  339.2895 ( $[\text{C}_{21}\text{H}_{39}\text{O}_3]^+$ , calc.  $m/z$  339.2894,  $\Delta$  -0.3 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} - \text{H}_2\text{O} + \text{H}]^+$ ) and  $m/z$  357.3002 ( $[\text{C}_{21}\text{H}_{41}\text{O}_4]^+$ , calc.  $m/z$  357.2999,  $\Delta$  -0.7 ppm;  $[\text{M} - \text{C}_6\text{H}_9\text{O}_7\text{S} + \text{H}]^+$ ), which corresponded to the loss of the sulfoquinovosyl group (neutral losses of 261 and 243 Da, respectively, from the ammoniated adduct), and the acylium ion at  $m/z$  265.2525 ( $[\text{C}_{18}\text{H}_{33}\text{O}]^+$ , calc.  $m/z$  265.2526,  $\Delta$  0.2 ppm).

**Cluster 1E** (See Figure S11)

- **Example of PC:** node at  $m/z$  542.3237 ( $[\text{C}_{28}\text{H}_{49}\text{NO}_7\text{P}]^+$ , calc.  $m/z$  542.3241,  $\Delta$  0.8 ppm;  $[\text{M} + \text{H}]^+$ )

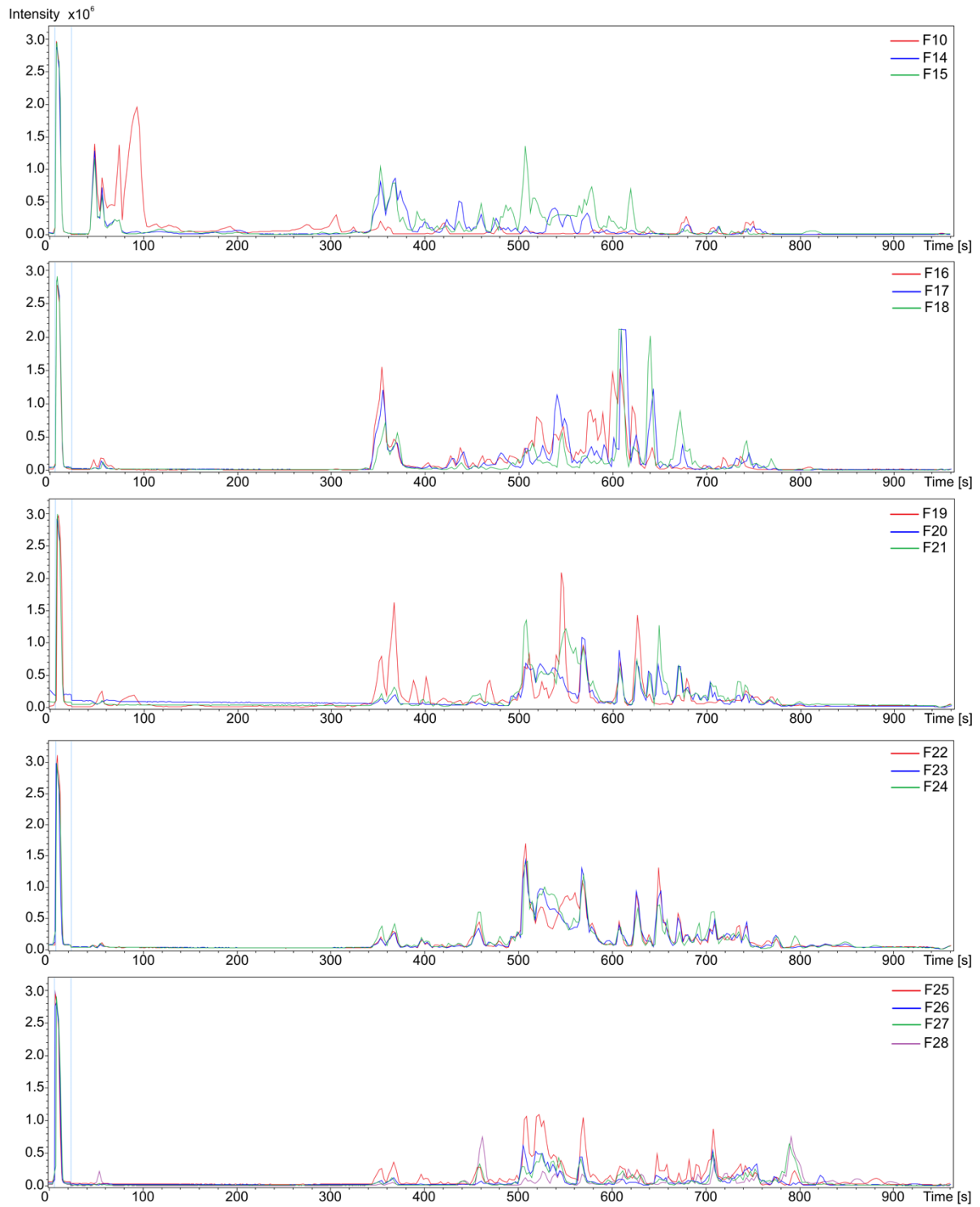
This  $m/z$  feature was annotated as **lyso-PC (20:5)** using the typical fragment ion of the polar head at  $m/z$  184.0729 ( $[\text{C}_5\text{H}_{15}\text{NO}_4\text{P}]^+$ ,  $m/z$  184.0733,  $\Delta$  2.5 ppm), but also those due to its subsequent fragmentation at  $m/z$  104.1069 ( $[\text{C}_5\text{H}_{14}\text{NO}]^+$ , calc.  $m/z$  104.1070,  $\Delta$  0.8 ppm) and  $m/z$  86.0964 ( $[\text{C}_5\text{H}_{12}\text{N}]^+$ , calc.  $m/z$  86.0964,  $\Delta$  0.6 ppm), while the acyl chain was deduced from the molecular formula and the fragment ions at  $m/z$  359.2576 ( $[\text{C}_{23}\text{H}_{35}\text{O}_3]^+$ , calc.  $m/z$  359.2581,  $\Delta$  1.2 ppm;  $[\text{M} - \text{C}_5\text{H}_{15}\text{NO}_4\text{P} + \text{H}]^+$ ), and  $m/z$  258.1099 ( $[\text{C}_8\text{H}_{21}\text{NO}_6\text{P}]^+$ , calc.  $m/z$  258.1101,  $\Delta$  0.9 ppm;  $[\text{M} - \text{C}_{20:5} + \text{H}]^+$ ).

**Table S1.** Summary of the fractions obtained after fractionation of the crude extracts of *Taonia atomaria* by flash-chromatography

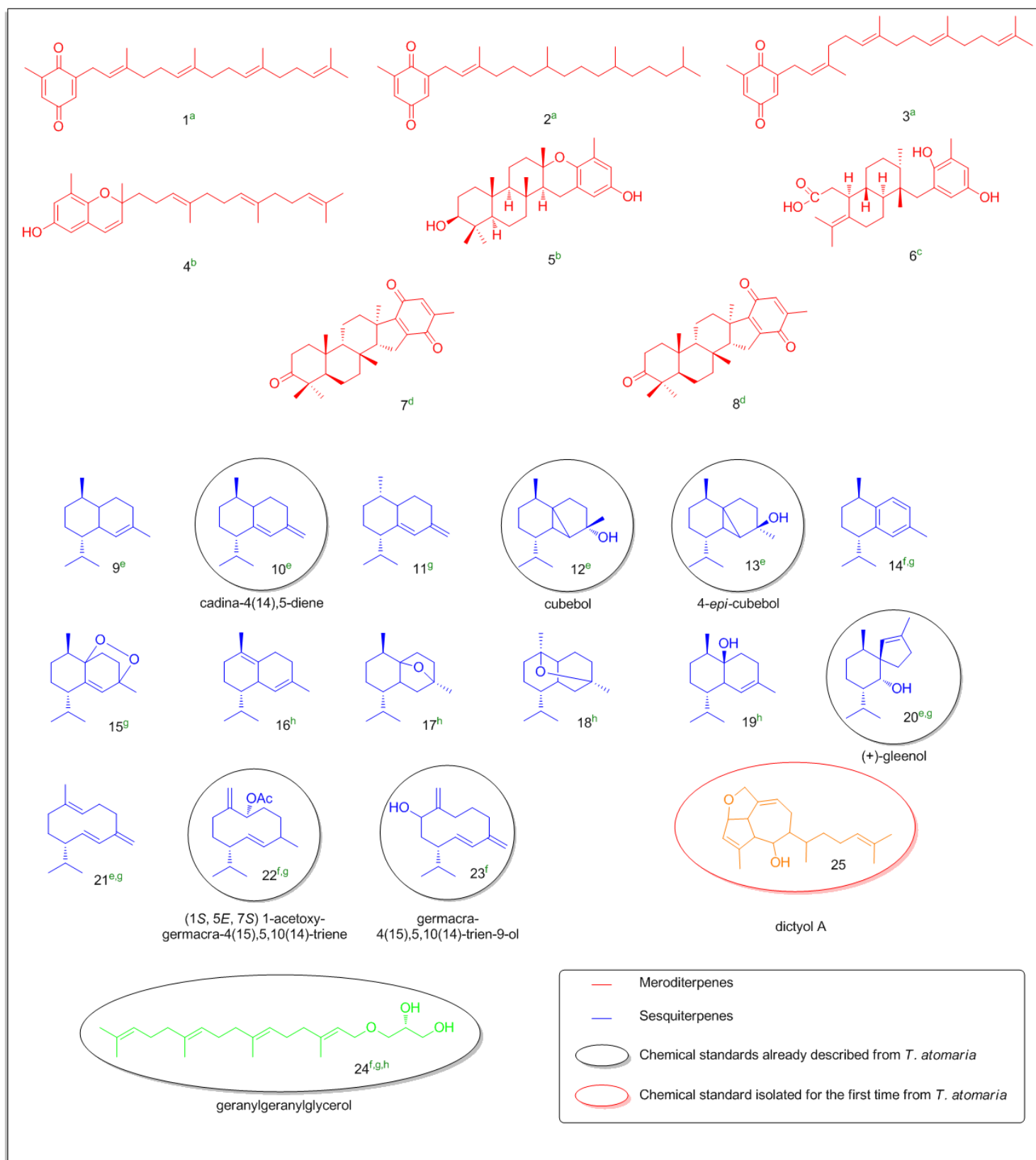
Fraction N°	Elution gradient			Color	Mass (mg)	Semi-preparative HPLC	Purified compounds*	LC-MS/MS analysis
	CH <sub>2</sub> Cl <sub>2</sub>	MeOH	H <sub>2</sub> O					
1	0	10	90	Colorless	< 0.5			
2	0	10	90	Colorless	< 0.5			
3	0	18	82	Light pink	< 0.5			
4	0	27	73	Light purple	< 0.5			
5	0	36	64	Light yellow	< 0.5			
6	0	45	55	Light yellow	< 0.5			
7	0	54	46	Light yellow	< 0.5			
8	0	63	37	Light yellow	< 0.5			
9	0	72	28	Light yellow	< 0.5			
10	0	81	19	Light yellow	< 0.5			X
11	0	90	10	Yellow/Orange	< 0.5			
12	0	99	1	Yellow/Orange	22			
13	0	100	0	Orange	4			
14	0	100	0	Orange	12			X
15	0	100	0	Orange	99			X
16	0	100	0	Orange	241	X		X
17	0	100	0	Dark orange	326	X	20, 22, 24	X
18	0	100	0	Dark green	257	X	20, 22, 25	X
19	9	91	0	Green	216	X	10, 12, 13, 20, 22, 23, 24	X
20	19	81	0	Green	213	X	10, 23	X
21	29	71	0	Light green	152	X	23	X
22	39	61	0	Light green	97			X
23	49	51	0	Green	64			X
24	59	41	0	Dark green	43			X
25	69	31	0	Dark green	53			X
26	79	21	0	Green	78			X
27	89	11	0	Green	132			X
28	99	1	0	Green	116			X
29	100	0	0	Green	32			
30	100	0	0	Green	28			
31	100	0	0	Yellow	20			
32	100	0	0	Yellow	10			
33	100	0	0	Light yellow	16			
34	100	0	0	Light yellow	8			
35	100	0	0	Light yellow	7			
36	100	0	0	Light yellow	11			
37	100	0	0	Light yellow	1			
38	100	0	0	Colorless	< 0.5			
39	100	0	0	Colorless	< 0.5			

\*See Fig. S2 for the corresponding chemical structures. **10**: cadina-4(14),5-diene; **12**: cubebol; **13**: 4-*epi*-cubebol; **20**: (+)-gleenol; **22**: (1*S*, 5*E*, 7*S*) 1-acetoxygermacra-4(15),5,10(14)-triene; **23**: germacra-4(15),5,10(14)-trien-9-ol; **24**: geranylgeranylglycerol; **25**: dictyol A.

**Figure S1.** BPC chromatograms of a selection of fractions obtained from the crude extracts of *Taonia atomaria*



**Figure S2.** Compounds isolated from *Taonia* spp.



<sup>a</sup>Tziveleka *et al.* Chem. Biodivers. 2 (2005) 901; <sup>b</sup>González *et al.* Tetrahedron. 29 (1973) 1605; <sup>c</sup>González *et al.* Tetrahedron Letters. 15 (1974) 3951; <sup>d</sup>Abatis *et al.* Tetrahedron Letters. 46 (2005) 8525; <sup>e</sup>De Rosa *et al.* Phytochemistry. 37 (1994) 1327; <sup>f</sup>Othmani *et al.* Biofouling. 32 (2016) 801; <sup>g</sup>Othmani *et al.* J. Appl. Phycol. 28 (2016) 1975; <sup>h</sup>Tringali *et al.* Phytochemistry. 40 (1995) 827.

**Figure S3.** Chemical structure of dictyol A and main correlations observed on the HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY 2D NMR spectra

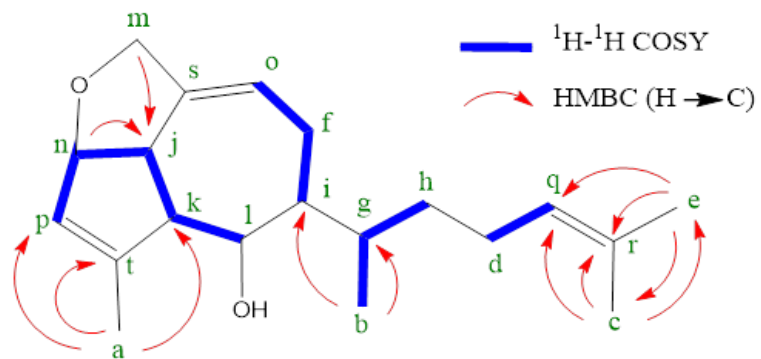




Figure S4-A.  $^1\text{H}$  NMR spectrum of dictyol A ( $\text{CDCl}_3$ , 400 MHz)

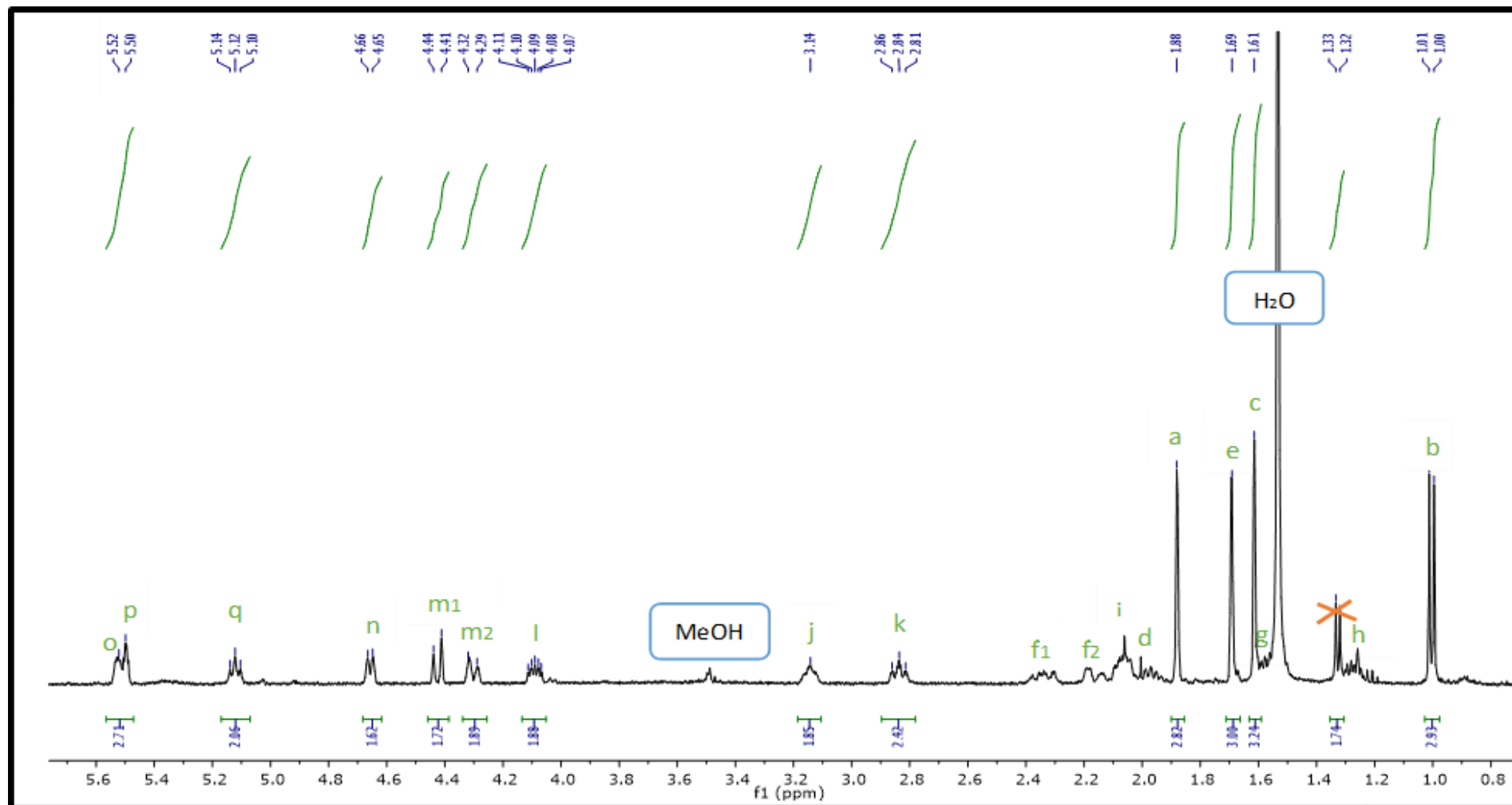
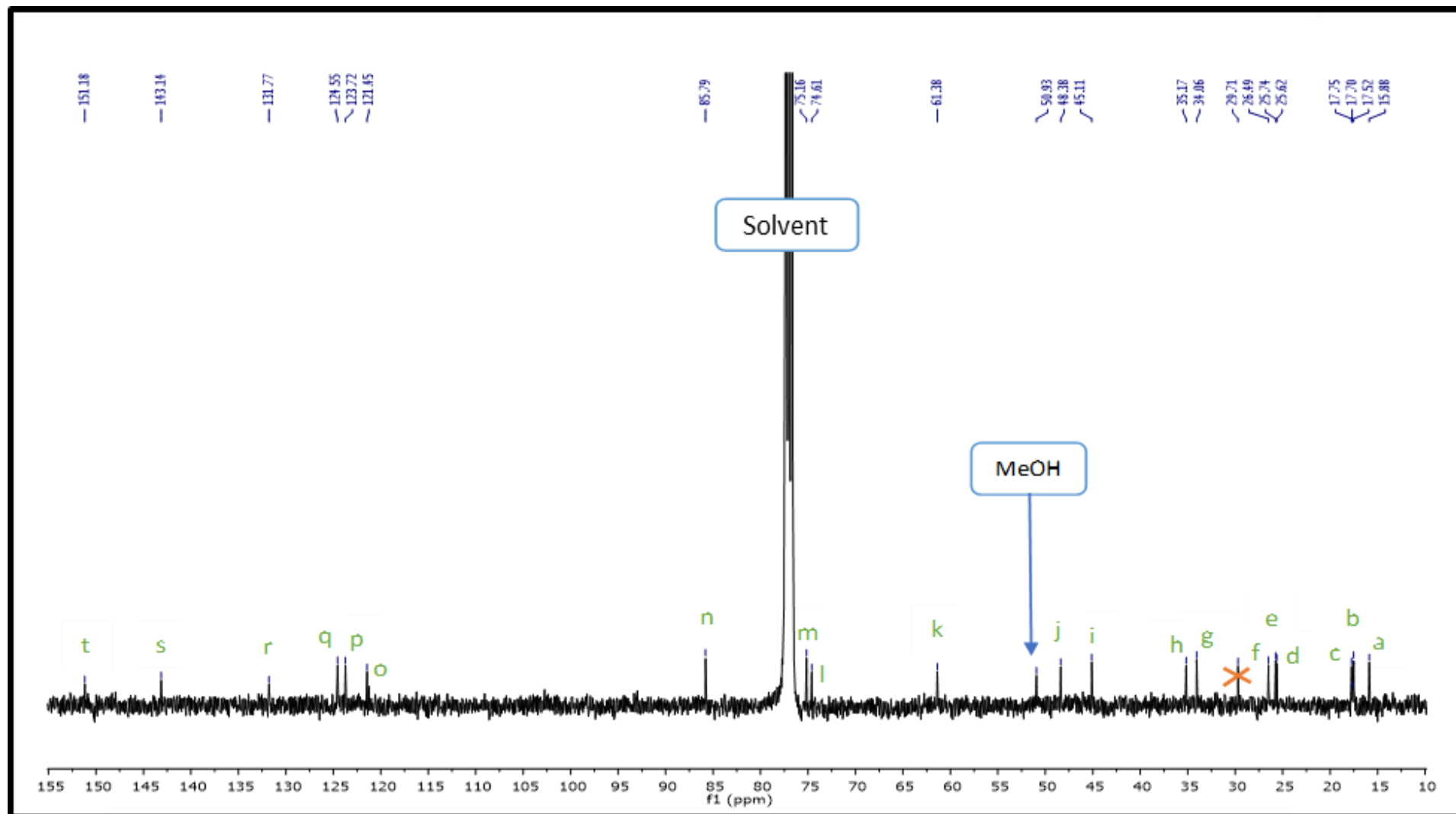
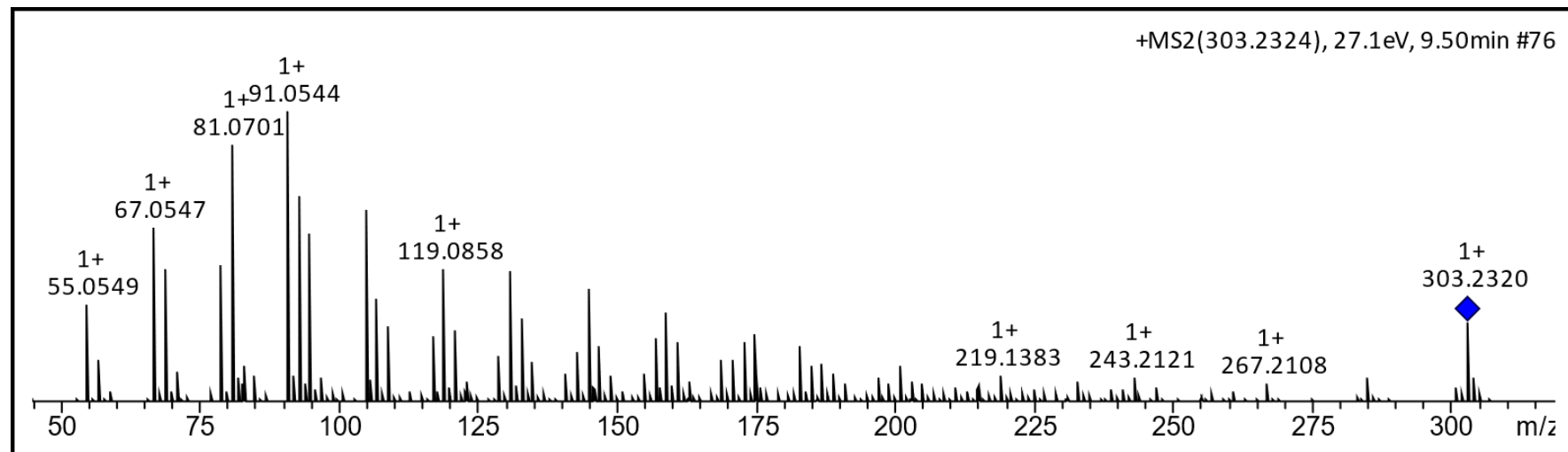


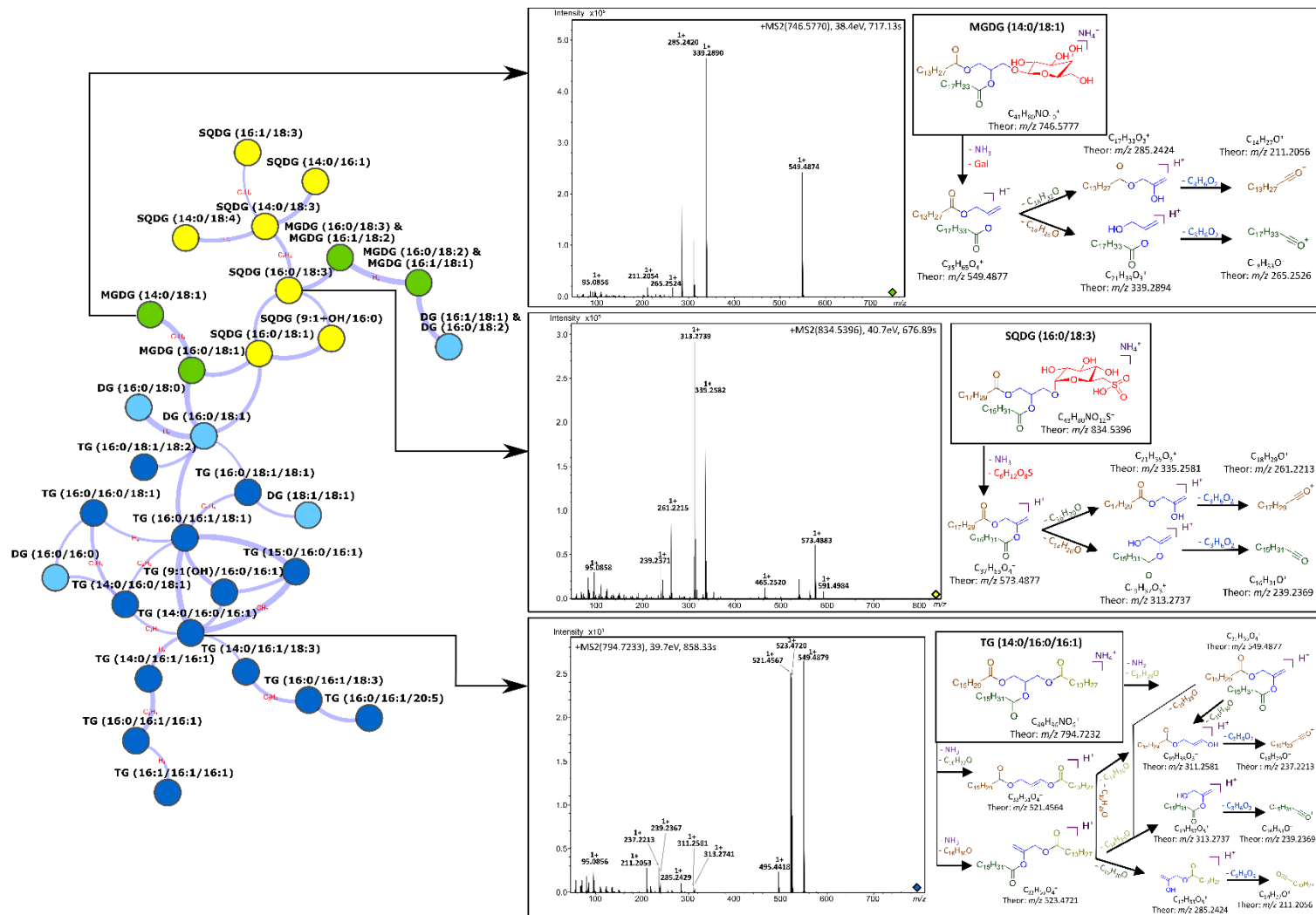
Figure S4-B.  $^{13}\text{C}$  NMR spectrum of dictyol A ( $\text{CDCl}_3$ , 100 MHz)



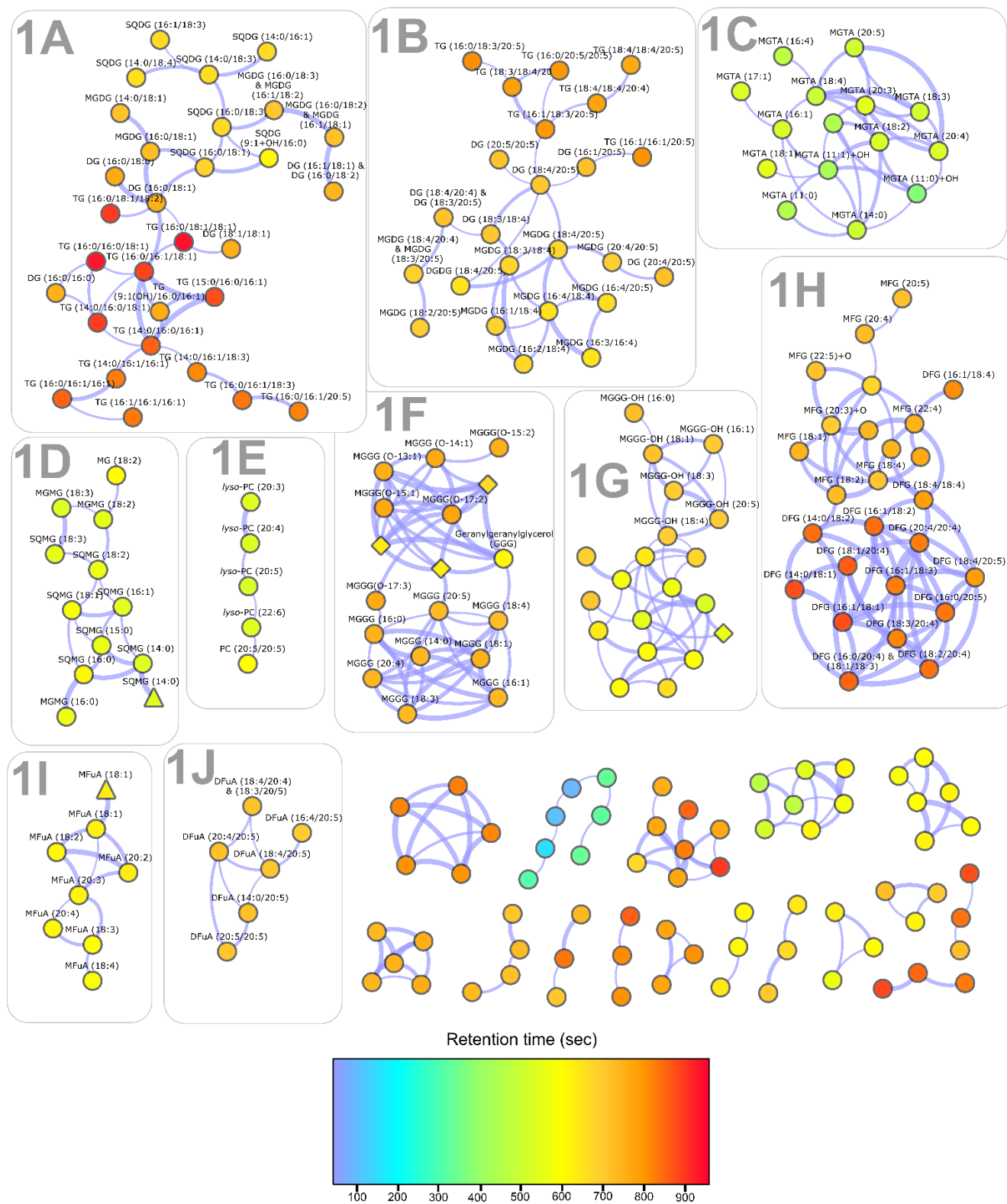
**Figure S5.** (+)-ESI-HRMS spectrum of dictyol A



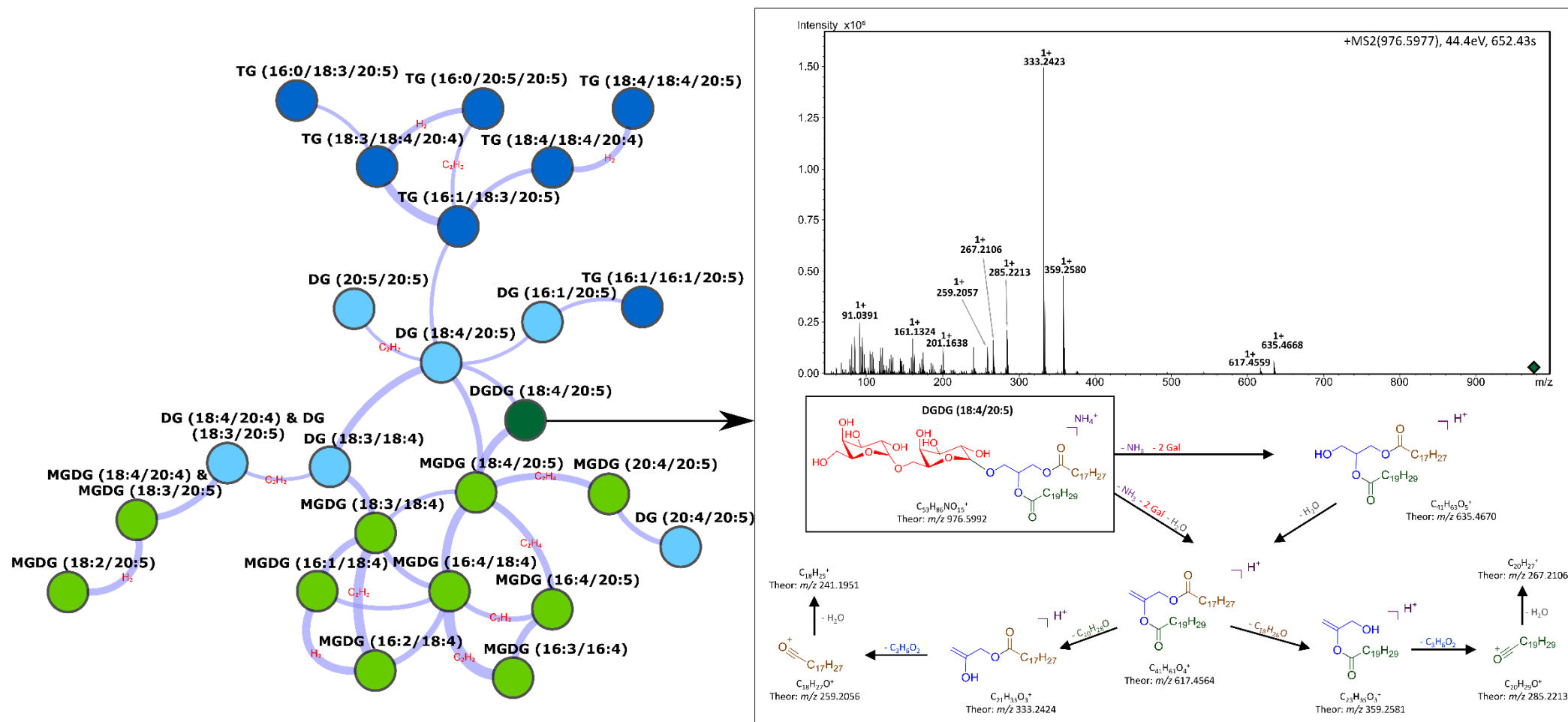
**Figure S6.** Annotation of cluster 1A and proposed MS/MS fragmentation pattern for the compounds MGDG (14:0/18:1), SQDG (16:0/18:3) and TG (14:0/16:0/16:1) (Abbreviations: DG: Diacylglycerol; MGDG: Monogalactosyldiacylglycerol; SQDG: Sulfoquinovosyldiacylglycerol; TG: Triacylglycerol)



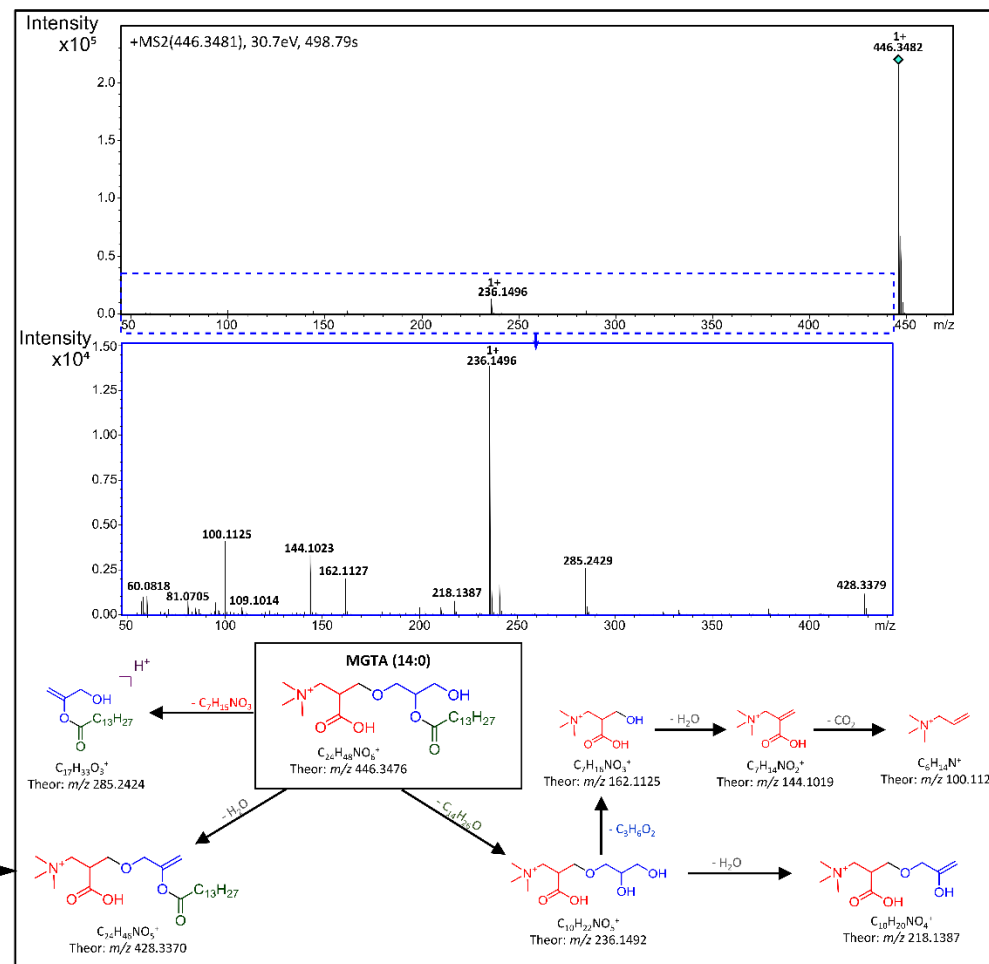
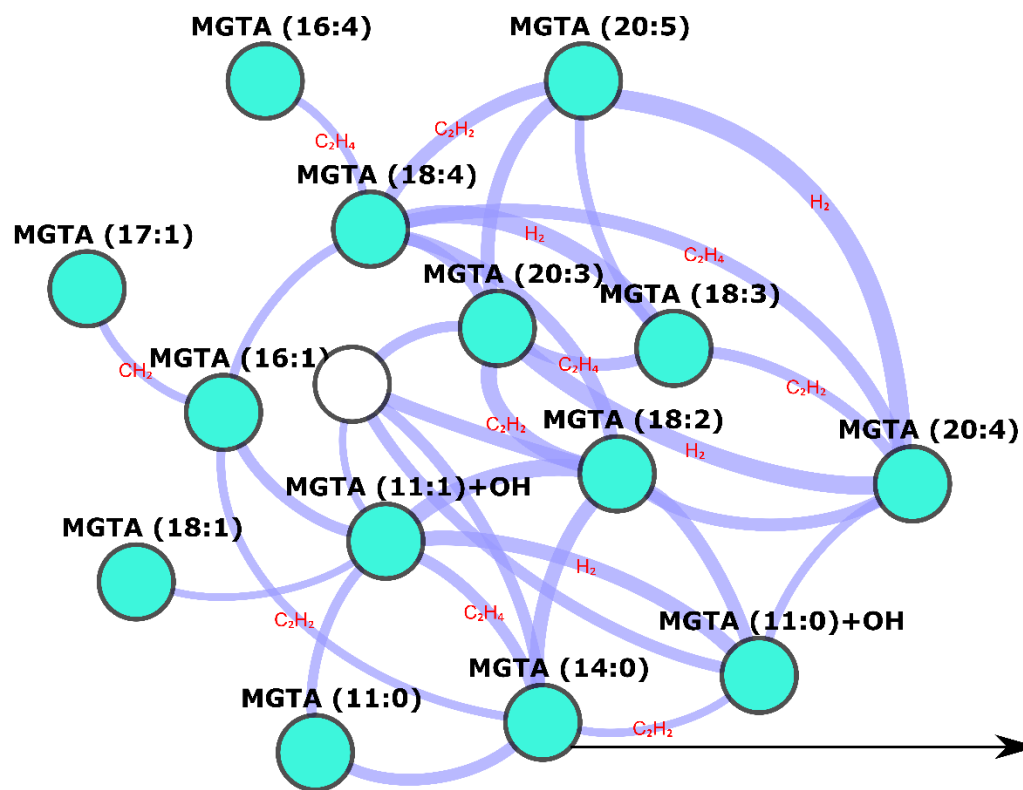
**Figure S7.** Restrictive molecular network (MN-1) representing nodes with a color gradient according to their corresponding retention time (**Abbreviations:** DFG: Diacylfarnesylglycerol; DFuA: Diacylfulvellic acid; DG: Diacylglycerol; DGDG: Digalactosyldiacylglycerol; MFG: Monoacylfarnesylglycerol; MFuA: Monoacylfulvellic acid; MG: Monoacylglycerol; MGDG: Monogalactosyldiacylglycerol; MGGG: Monoradylgeranylgeranylgerlycerol; MGGG-OH: Monoradylhydroxygeranylgeranylgerlycerol; MGMG: Monogalactosylmonoacylglycerol; MGTA: Monoacylglyceryl-*N,N,N*-trimethyl- $\beta$ -alanine; PC: Phosphatidylcholine; SQDG: Sulfoquinovosyldiacylglycerol; SQMG: Sulfoquinovosylmonoacylglycerol; TG: Triacylglycerol) (Circles: metabolites; diamonds: fragments; triangles: adducts)



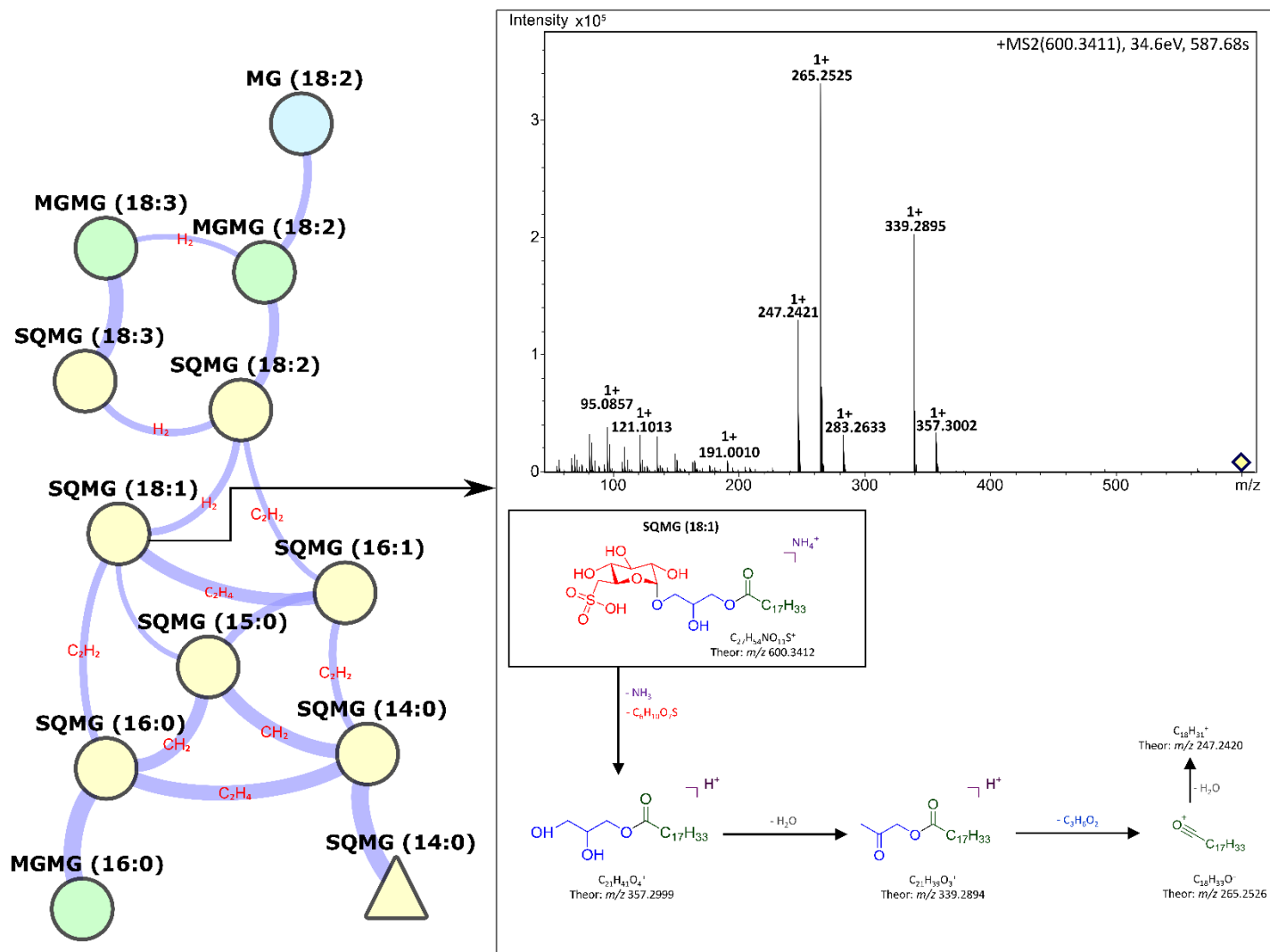
**Figure S8.** Annotation of cluster **1B** and proposed MS/MS fragmentation pattern for the compound DGDG (18:4/20:5) (Abbreviations: DG: Diacylglycerol; DGDG: Digalactosyldiacylglycerol; MGDG: Monogalactosyldiacylglycerol, TG: Triacylglycerol)



**Figure S9.** Annotation of the cluster **1C** and proposed MS/MS fragmentation pattern for the compound MGTA (14:0) (Abbreviation: MGTA: Monoacylglyceryl-*N,N,N*-trimethyl- $\beta$ -alanine)

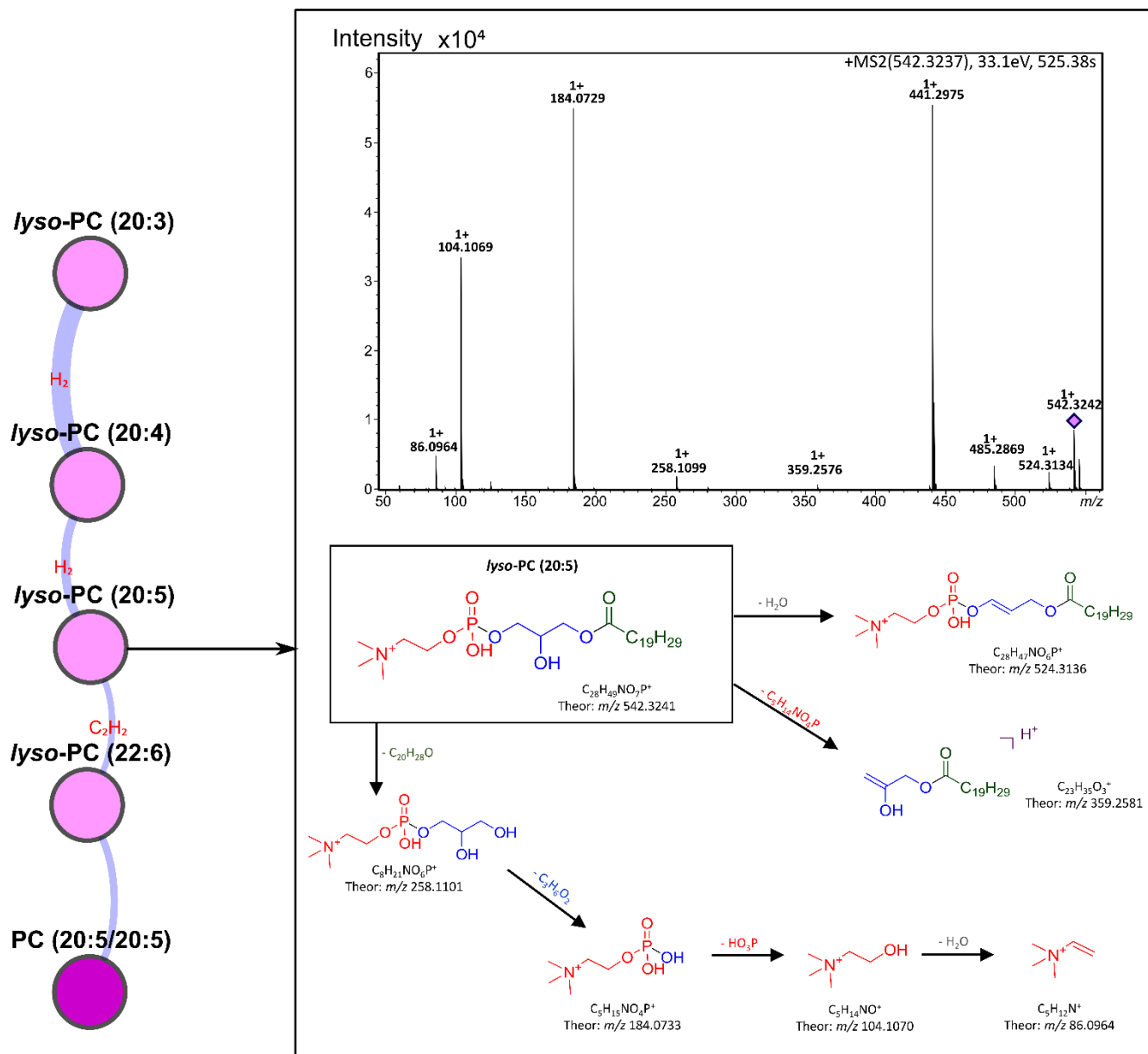


**Figure S10.** Annotation of cluster **1D** and proposed MS/MS fragmentation pattern for the compound SQMG (18:1) (Abbreviations: MG: Monoacylglycerol; MGMG: Monogalactosylmonoacylglycerol; SQMG: Sulfoquinovosylmonoacylglycerol) (circles: metabolites; triangle: adduct)

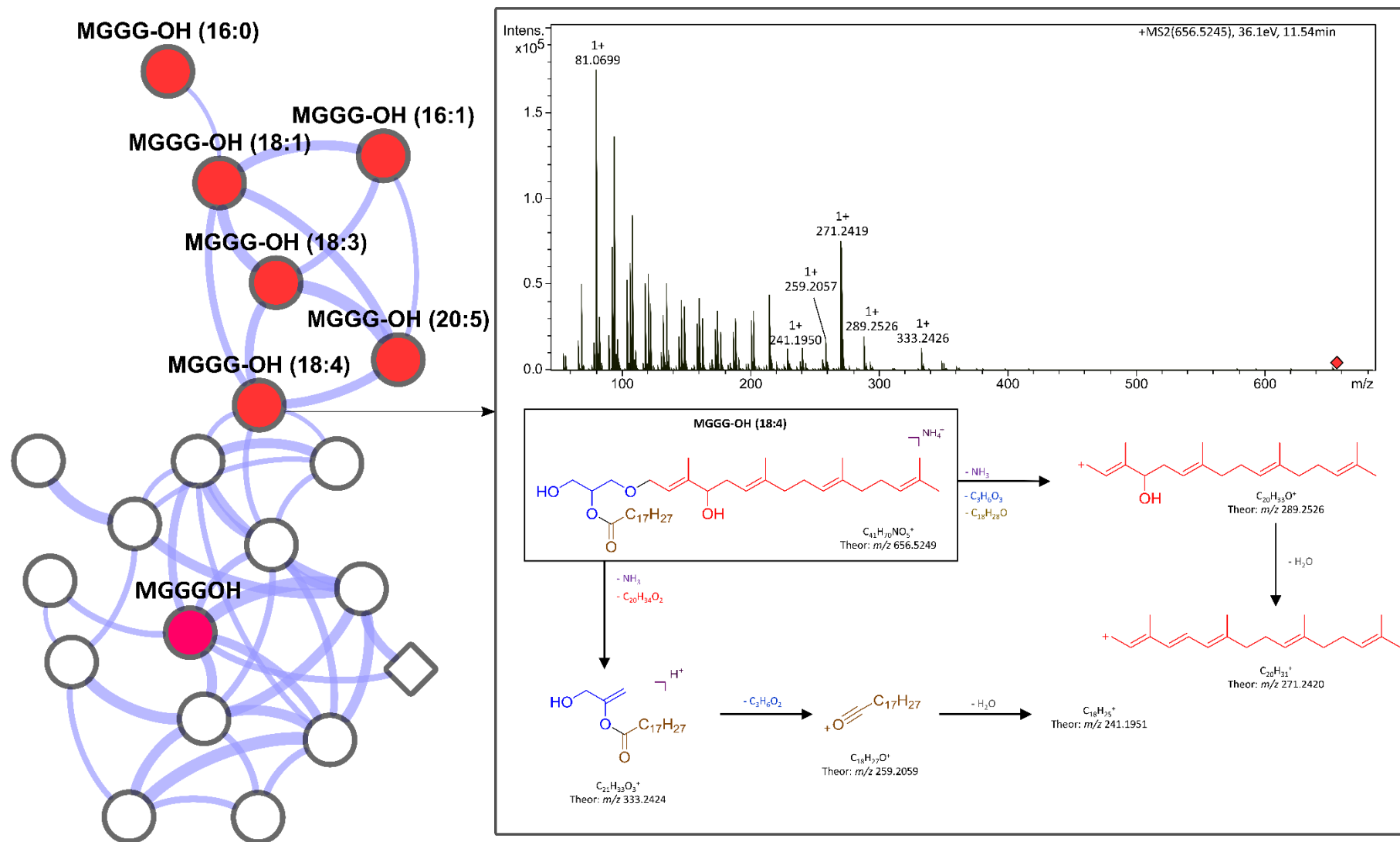




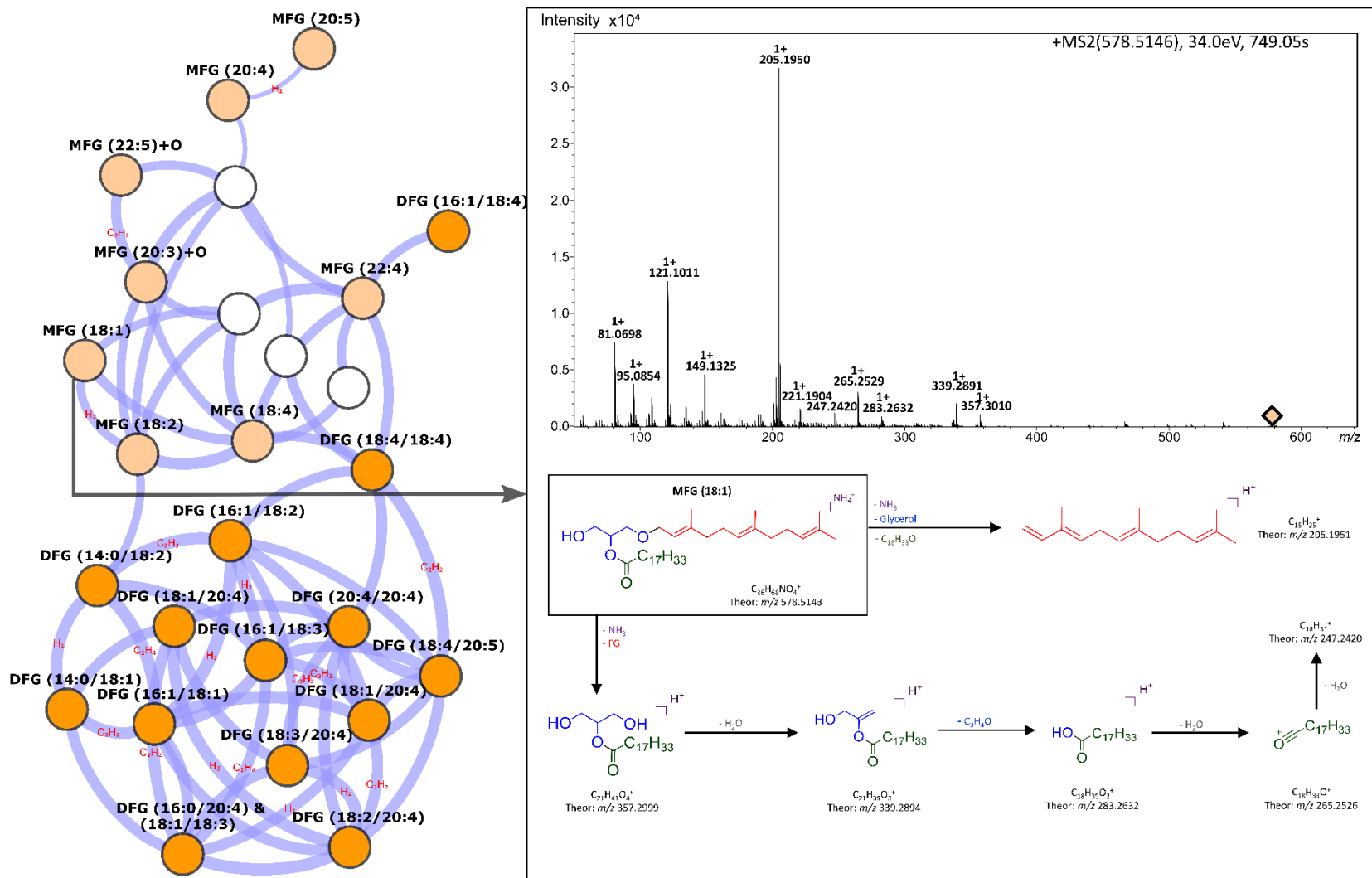
**Figure S11.** Annotation of cluster **1E** and proposed MS/MS fragmentation pattern for the compound *lyso*-PC (20:5) (Abbreviation: PC: Phosphatidylcholine)



**Figure S12.** Annotation of cluster **1G** and proposed MS/MS fragmentation pattern for the compound MGGG-OH (18:4) (**Abbreviation:** MGGG-OH: Monoradylhydroxygeranylgeranylglycerol) (Circles: metabolites; diamond: fragment)



**Figure S13.** Annotation of cluster **1H** and proposed MS/MS fragmentation pattern for the compound MFG (18:1) (Abbreviations: DFG: Diacylfarnesylglycerol; MFG: Monoacylfarnesylglycerol)



**Figure S14.** Annotation of cluster **1J** and proposed MS/MS fragmentation pattern for the compound DFuA (20:4/20:5) (**Abbreviation:** DFuA: Diacylfulvellic acid)

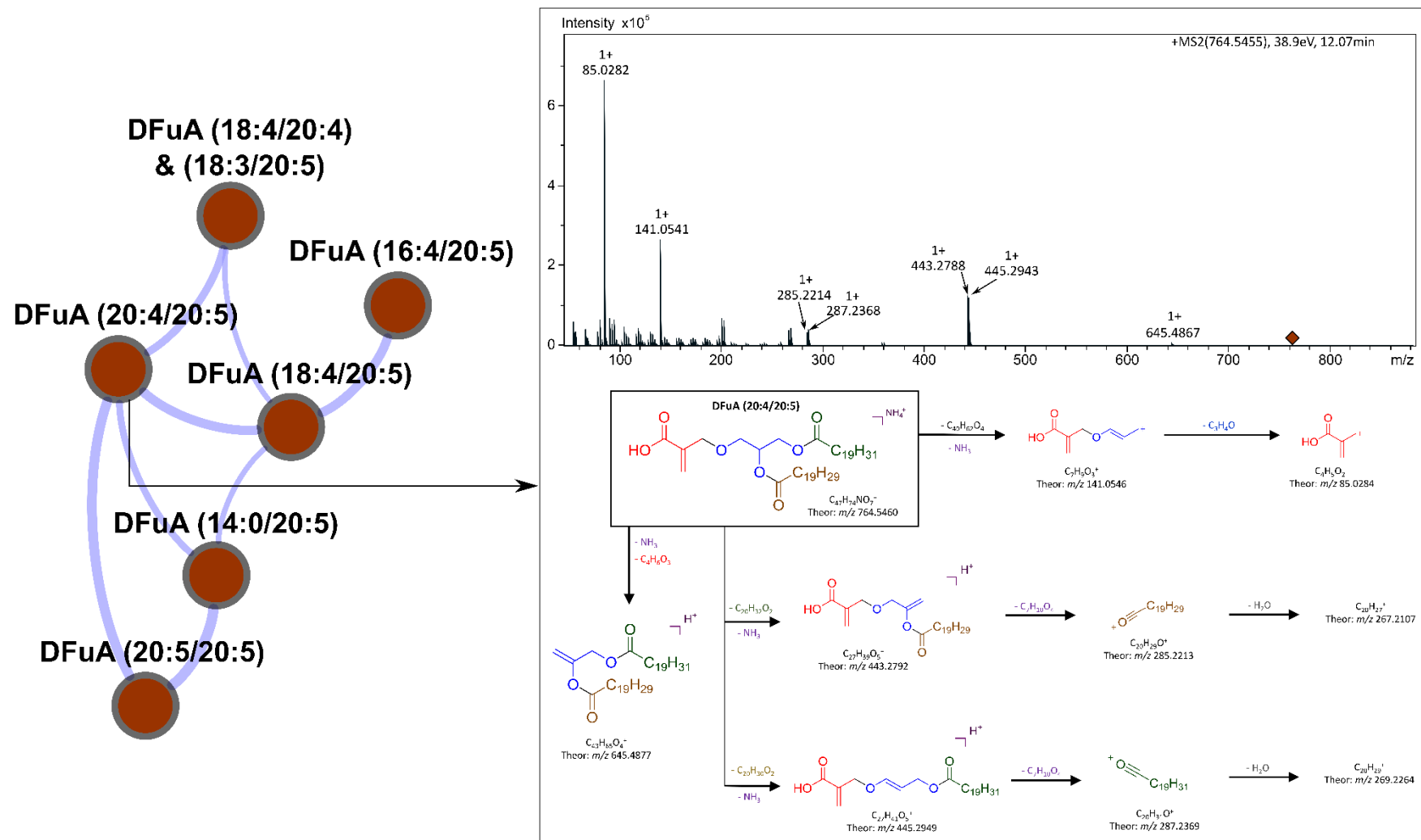


Figure S15. Less restrictive molecular network (MN-2) built with MS/MS data of fractions obtained from the crude extracts of the brown alga *Taonia atomaria* (Black circles: *m/z* features annotated using MN-2) (Circles: metabolites; diamonds: fragments; triangles: adducts)

