

Supplements

The changes of glycosyl inositol phosphoceramides during the cell cycle of the red alga *Galdieria*

Milada Vítová, Mária Čížková, Vít Náhlík, Tomáš Řezanka

Algal cell cultures and growth conditions

The alga was grown in a modified *Galdieria*-nutrient medium (Vítová et al., 2016). The alga was routinely sub-cultured on culture plates containing *Galdieria*-nutrient medium solidified with 3% agar every three weeks.

The cultures were grown at pH 3 in glass flat (2.5 L) photobioreactors. Photobioreactors were placed in a thermostatic water bath and illuminated from one side by a panel of dimmable fluorescent lamps (OSRAM DULUX L55 W/950 Daylight, Milano, Italy). Algal suspensions in the photobioreactors were supplied with a gas mixture of air and CO₂ (2% v/v) at a flow rate of 15 L h⁻¹. The experiments were carried out in a batch culture regime, over at 40 °C, and mean irradiance of 500 μmol photons m⁻² s⁻¹.

Dry matter determination

To follow the growth of cultures dry matter was determined from 5 mL of algal suspension centrifuged at 2100 g for 3 min in dried and pre-weighed 5-mL test tubes. The pellet was dried at 105 °C for 12 h and weighed on a Sartorius TE214S-0CE analytical balance (Sartorius, Germany) (Brányiková et al., 2011).

Confocal microscopy

The algal cells were stained with SYBR Green, according to Vítová et al. (2005). In short, five microliters of freshly defrosted cell pellet were combined with 2.5 μL of, vortexed and kept for 5 - 10 minutes in the dark at room temperature. Next, the cells were observed under a confocal microscope LSM Zeiss 880 (Carl Zeiss, Jena, Germany) using the following excitation wavelengths: chlorophyll - 633 nm, and SYBR Green I - 405 nm.

References

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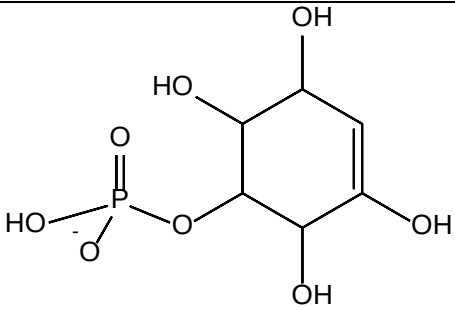
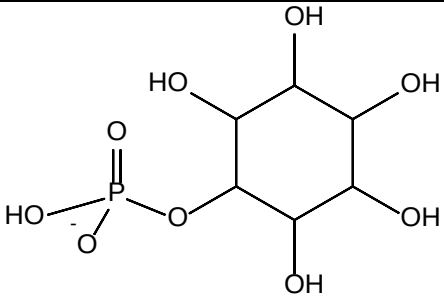
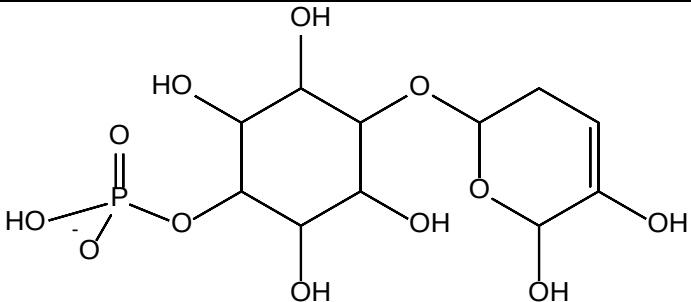
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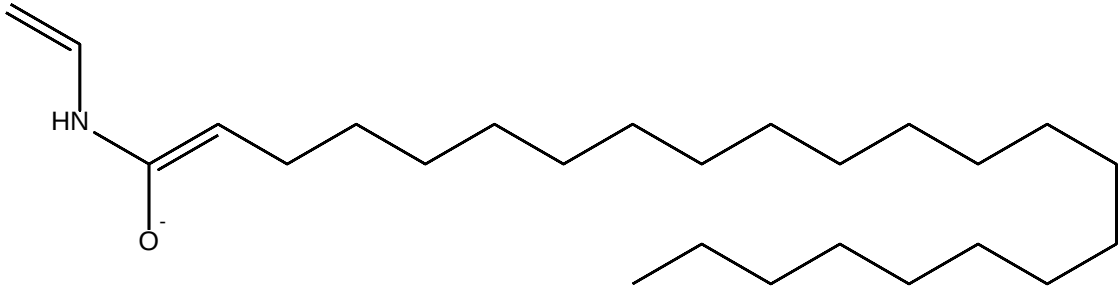
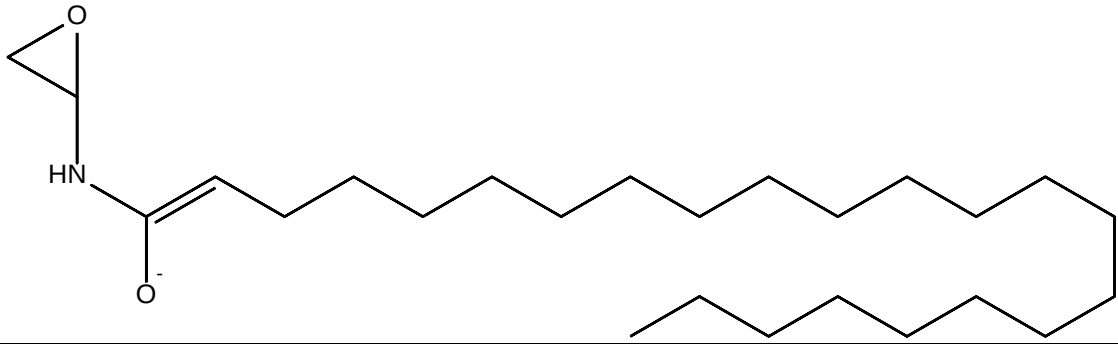
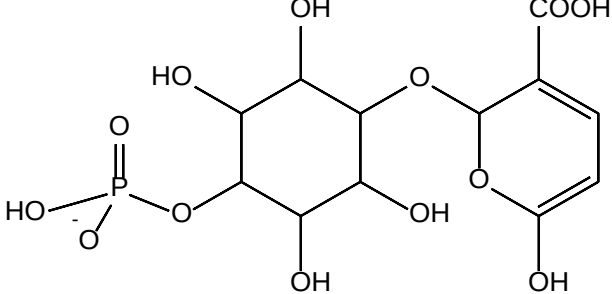
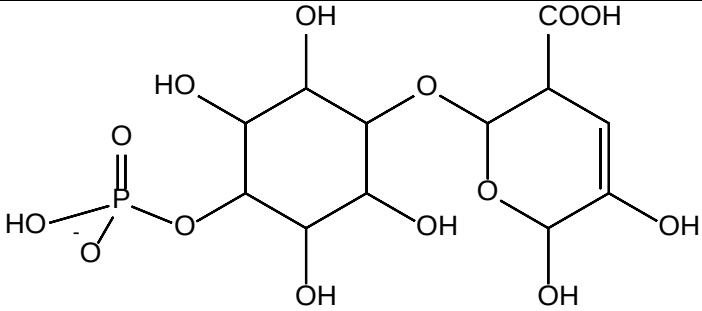
Table 1S. Total shotgun GIPC.

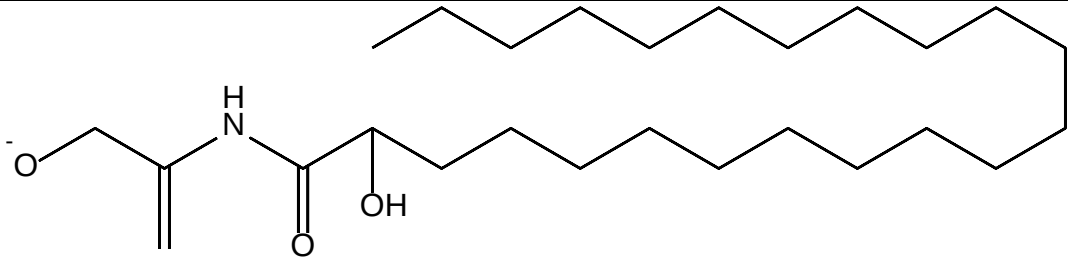
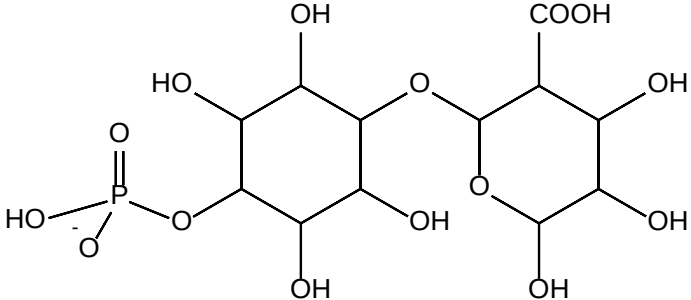
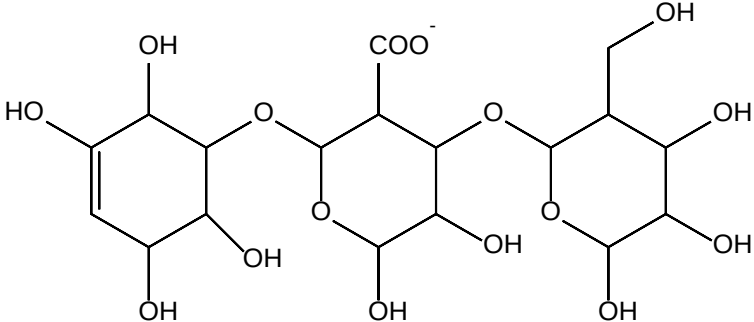
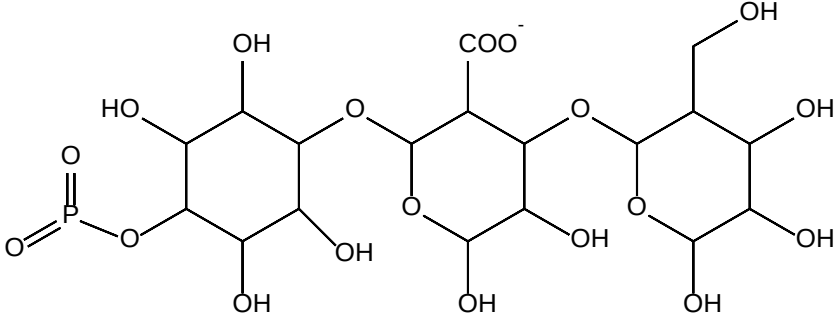
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1058.6760	Hex-IPC-t18:0/OH22:0	
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1086.7075	Hex-IPC-t18:0/OH24:0	Hex-IPC-t20:0/OH22:0
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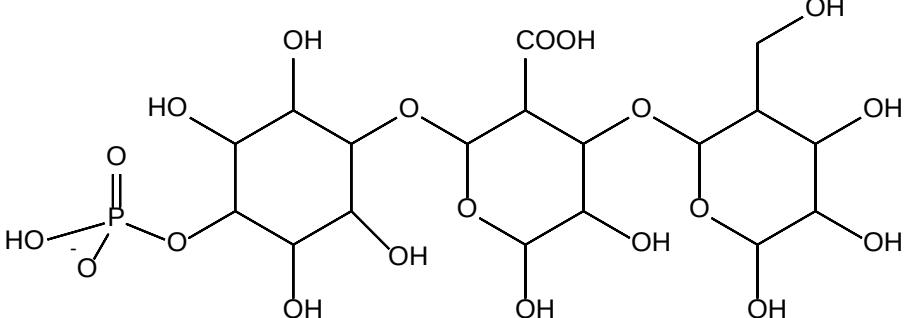
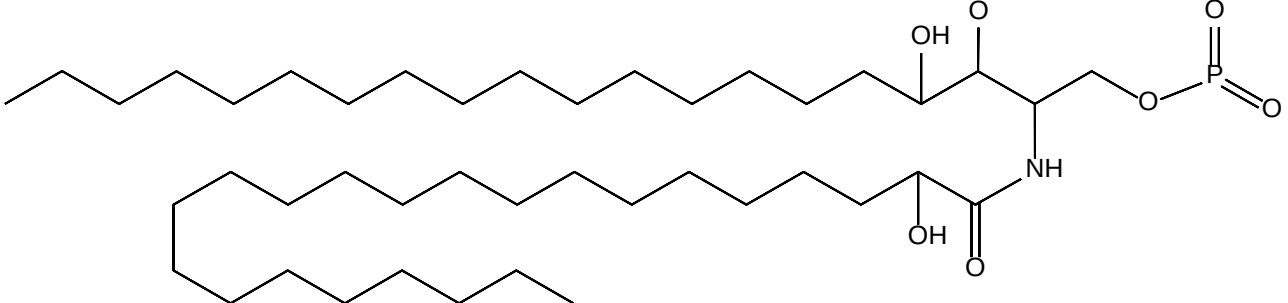
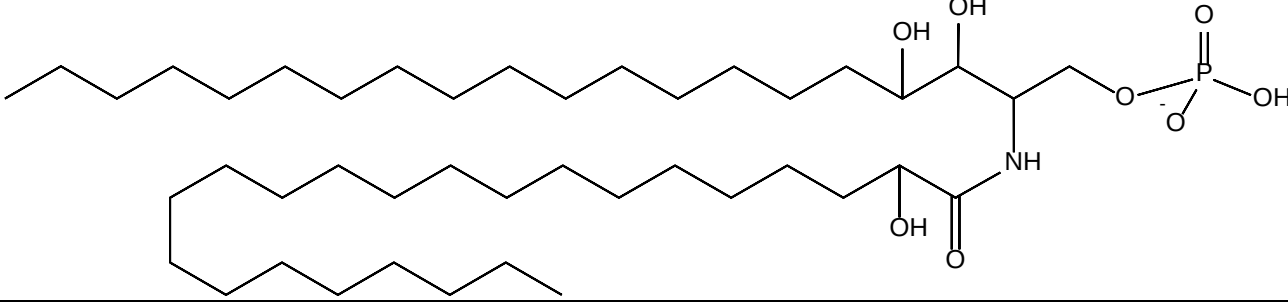
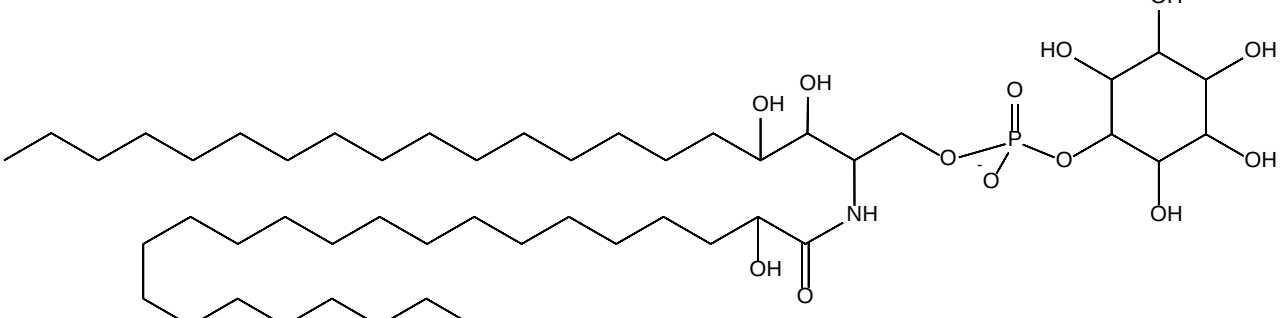
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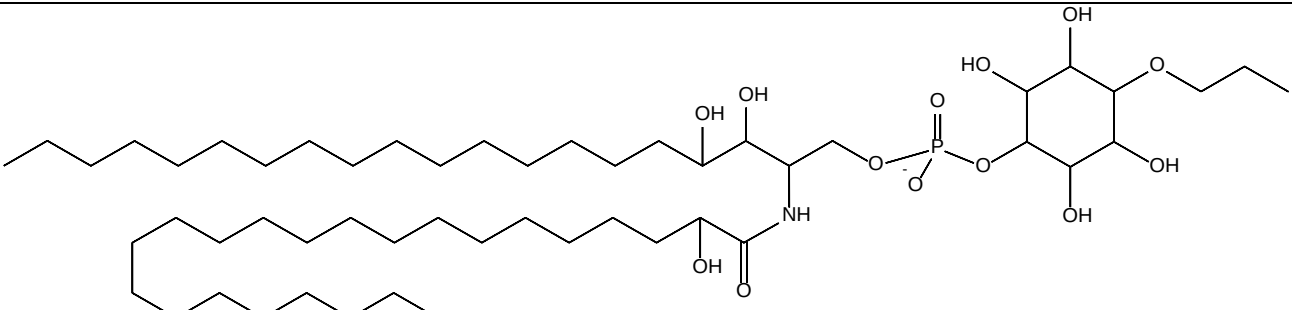
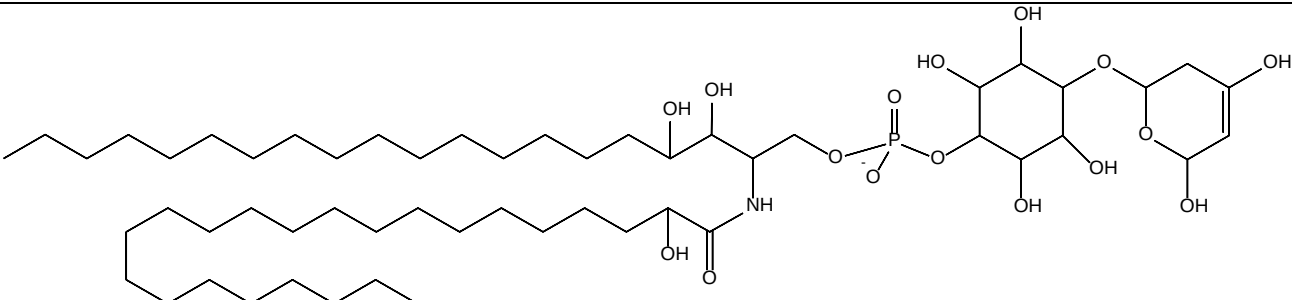
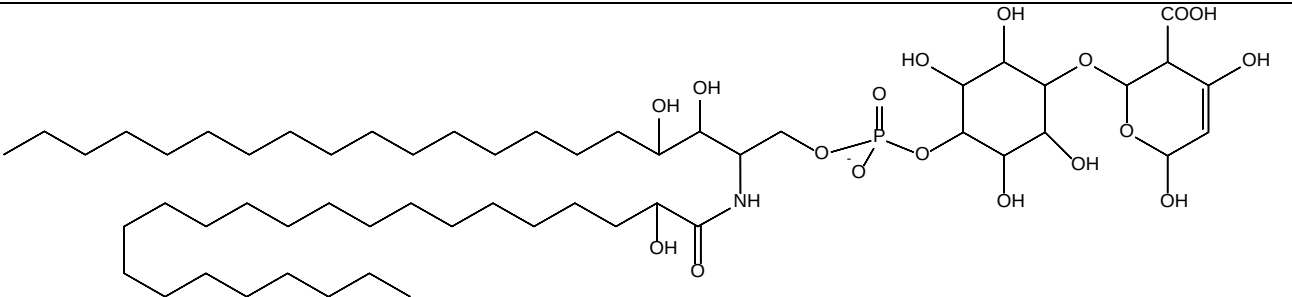
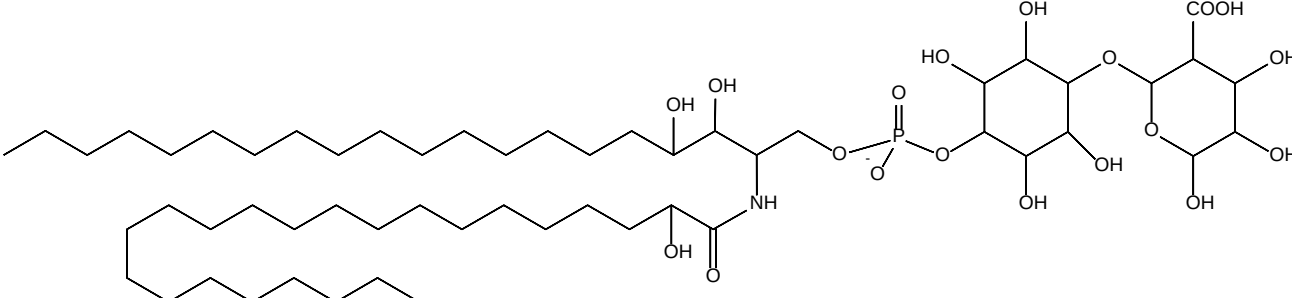
Table 2S. Tandem MS of Hex-HexA-IPC-t20:0/OH-23:0.

[fragment ion] [−]	<i>m/z</i>	Relative abundance (%)	
[IP-H ₂ O] [−]	241.0119	8	
[IP] [−]	259.0223	6	
[C ₃ PO ₃ -C ₁ -CO ₂ -H] [−]	373.0542	4	

S ion	378.3744	6	 The structure shows a long, zigzag hydrocarbon chain. At the left end, there is a vinyl group (CH=CH-) attached to a nitrogen atom (HN-), which is in turn attached to a carbonyl group (C=O) with a negative charge (O⁻). The chain continues as a long alkyl tail.
T ion	394.3693	9	 The structure is similar to the one above, but instead of a vinyl group, it features an epoxide ring (a three-membered ring with one oxygen atom) at the left end, attached to the nitrogen atom (HN-) and the carbonyl group (C=O) with a negative charge (O⁻). The long alkyl chain continues to the right.
$[\text{C}_3\text{PO}_3\text{-C}_1\text{-H}_2\text{O-H}]^-$	399.0331	4	 The structure is a complex molecule. It features a central six-membered ring with multiple hydroxyl (OH) groups. Attached to this ring is a phosphate group (HO-P(=O)(OH)-O-) and a side chain that includes a carboxylic acid group (COOH) and several other hydroxyl groups. The molecule is highly branched and contains multiple oxygen atoms.
$[\text{C}_3\text{PO}_3\text{-C}_1\text{-H}]^-$	417.0441	5	 This structure is very similar to the one above, but the side chain is different. It still has the central six-membered ring with hydroxyl groups and the phosphate group, but the arrangement of the carboxylic acid group (COOH) and other hydroxyl groups is distinct, reflecting a different chemical composition.

[FA] ⁻ ion	424.3799	4	
[C ₃ PO ₃ -B ₁ -H] ⁻	435.0544	5	
[B ₃ -H ₂] ⁻	499.1307	14	
[B ₃ PO ₃ -H] ⁻	579.0971	35	

$[\text{C}_3\text{PO}_3\text{-H}]^-$	597.1077	63	
$[\text{Z}_0\text{PO}_3\text{-H}]^-$	758.6072	31	
$[\text{Y}_0\text{PO}_3\text{-H}]^-$	776.6177	10	
$[\text{Y}_1\text{-H}]^-$	938.6706	100	

$[Y_1+42-H]^-$	980.7177	8	
$[Z_2-CO_2-H]^-$	1052.7022	14	
$[Z_2-H]^-$	1096.6921	12	
$[Y_2-H]^-$	1114.7027	17	

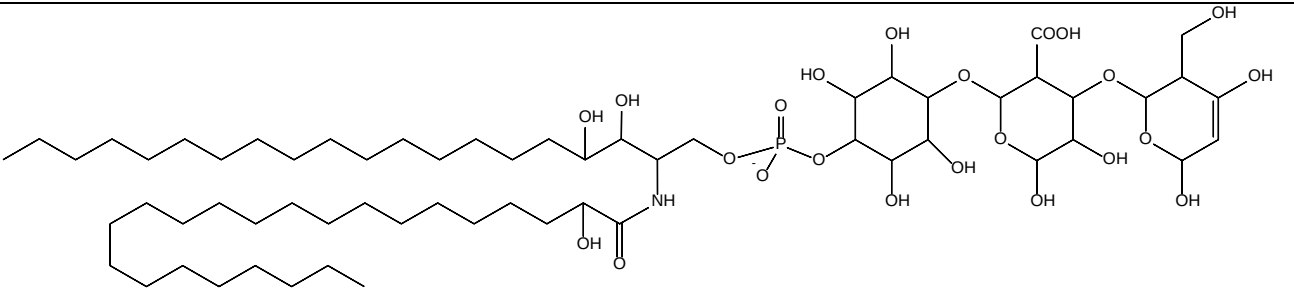
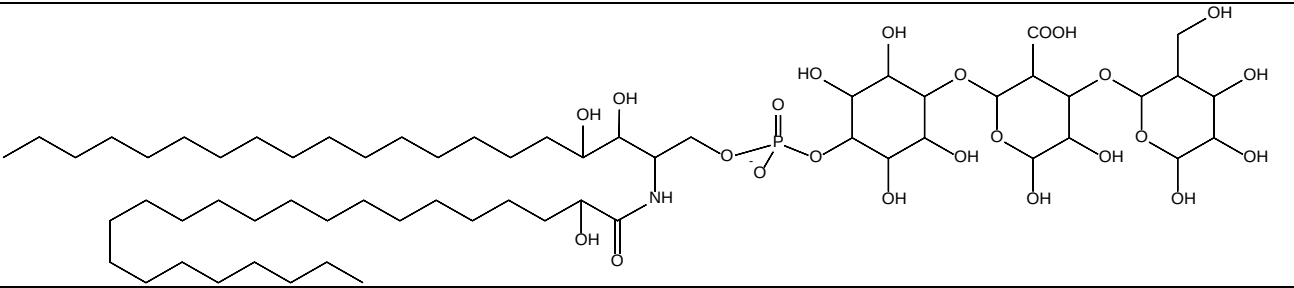
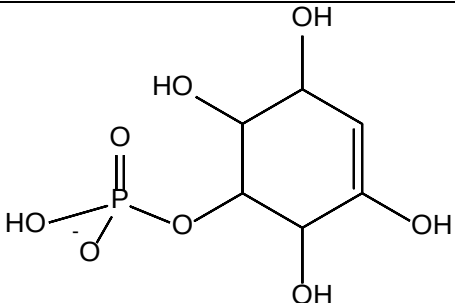
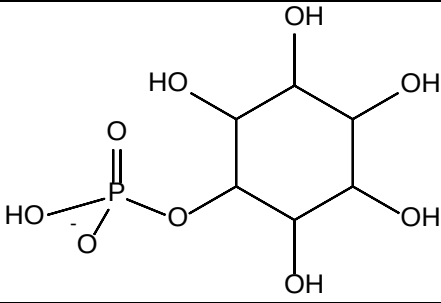
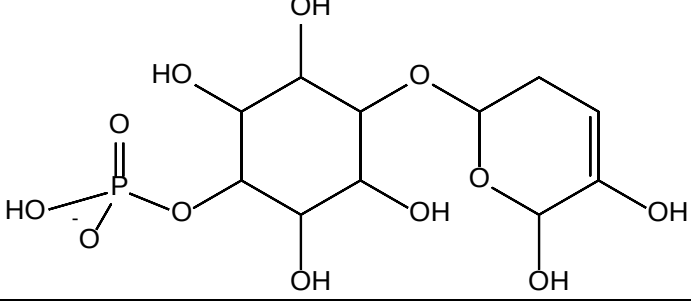
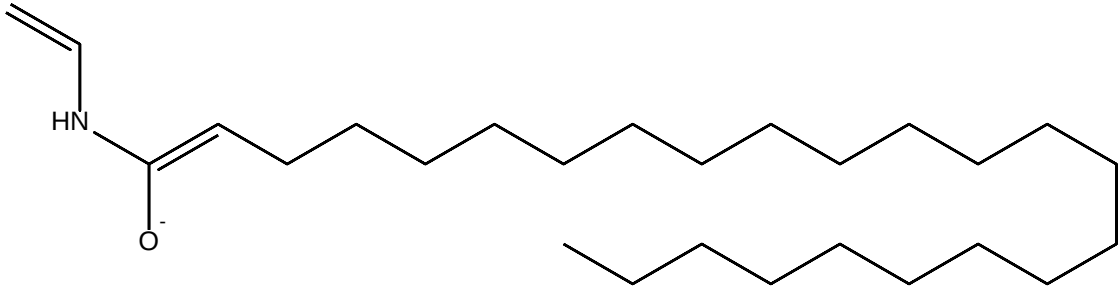
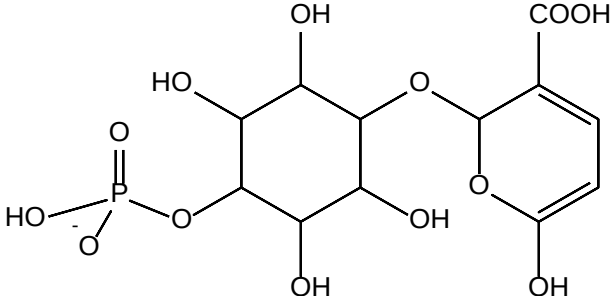
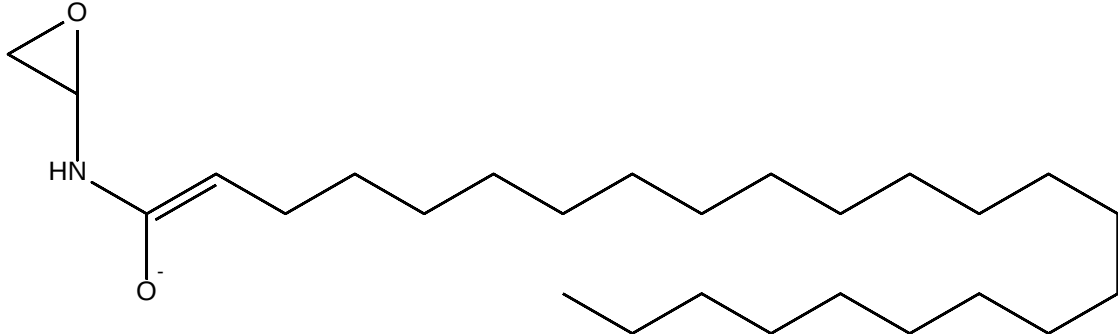
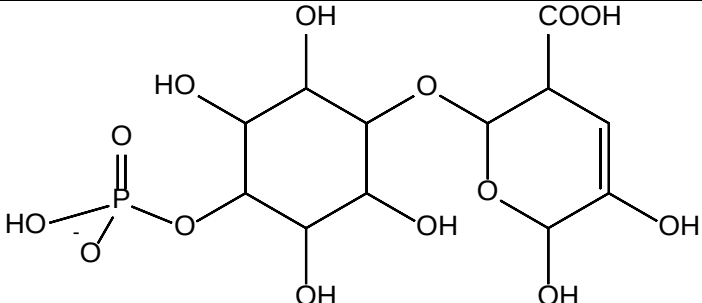
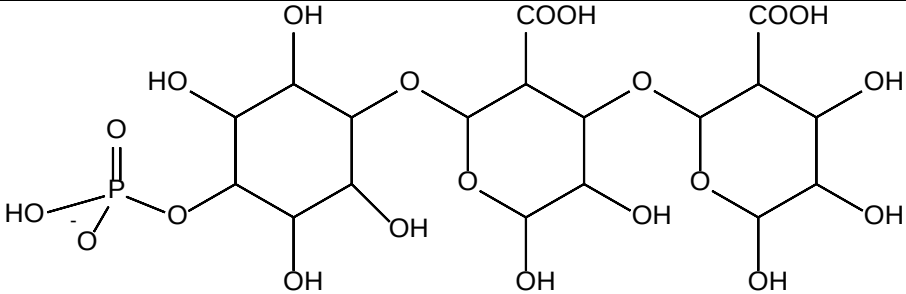
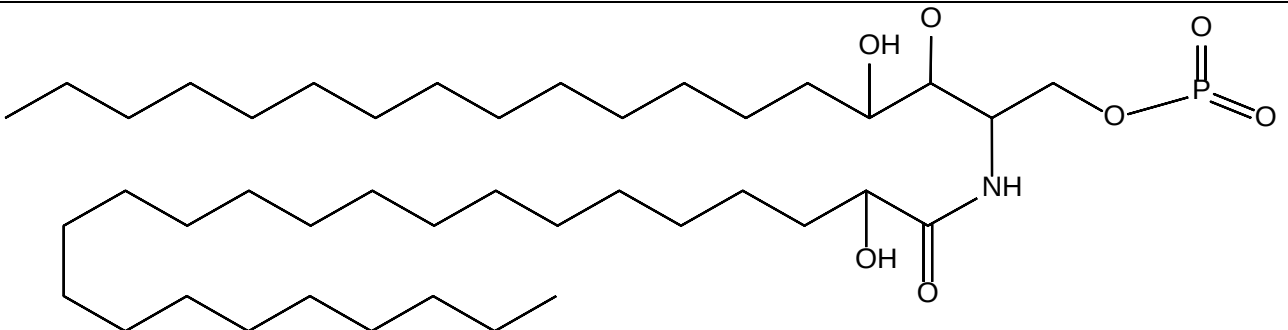
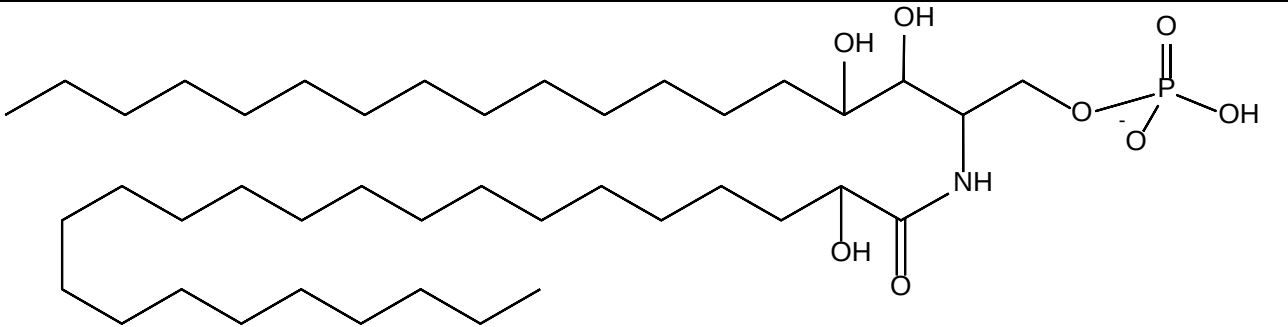
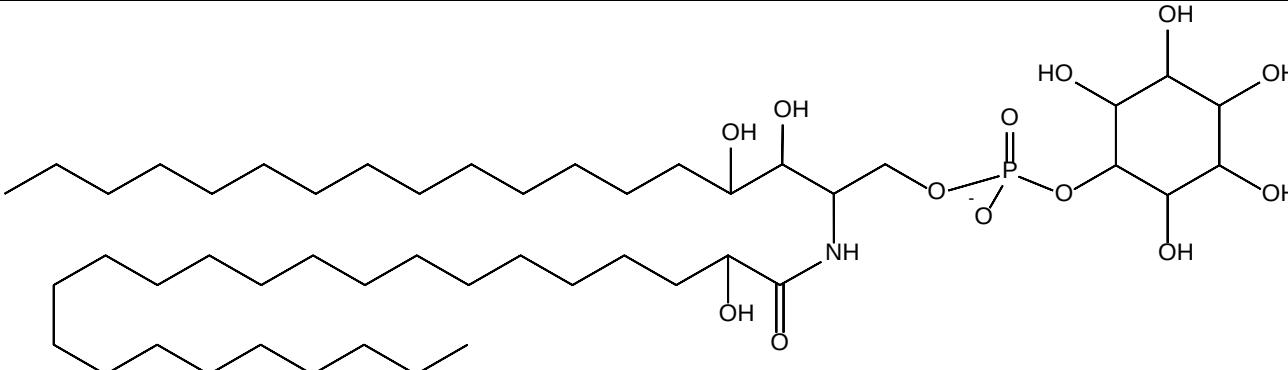
$[M-H_2O-H]^-$	1258.7449	5	 Chemical structure of the $[M-H_2O-H]^-$ ion. It features a long alkyl chain (C18) attached to a glycerol backbone. The glycerol backbone is linked via a phosphate group to a trisaccharide moiety. The trisaccharide consists of three glucose units linked by $\alpha(1\rightarrow6)$ glycosidic bonds. The terminal glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1. The middle glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1. The terminal glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1.
$[M-H]^-$	1276.7554	41	 Chemical structure of the $[M-H]^-$ ion. It features a long alkyl chain (C18) attached to a glycerol backbone. The glycerol backbone is linked via a phosphate group to a trisaccharide moiety. The trisaccharide consists of three glucose units linked by $\alpha(1\rightarrow6)$ glycosidic bonds. The terminal glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1. The middle glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1. The terminal glucose unit has a carboxylic acid group (COOH) at C6 and a hydroxyl group (OH) at C1.

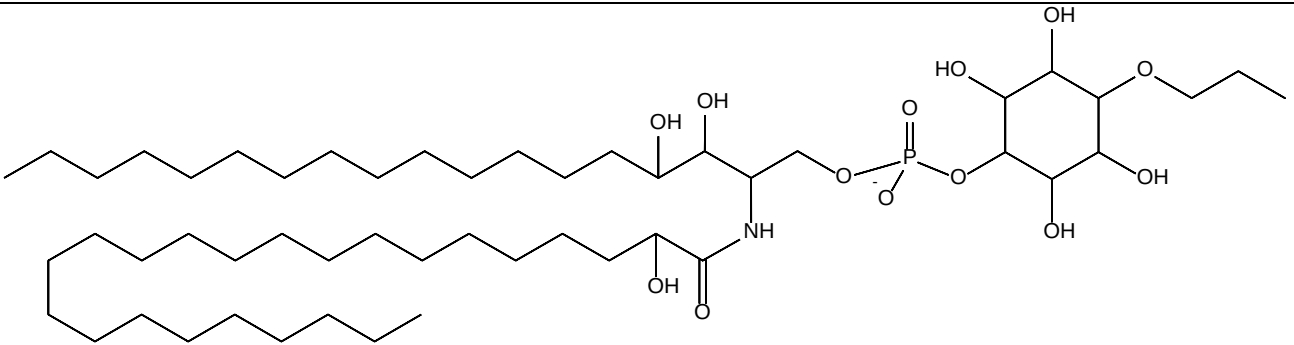
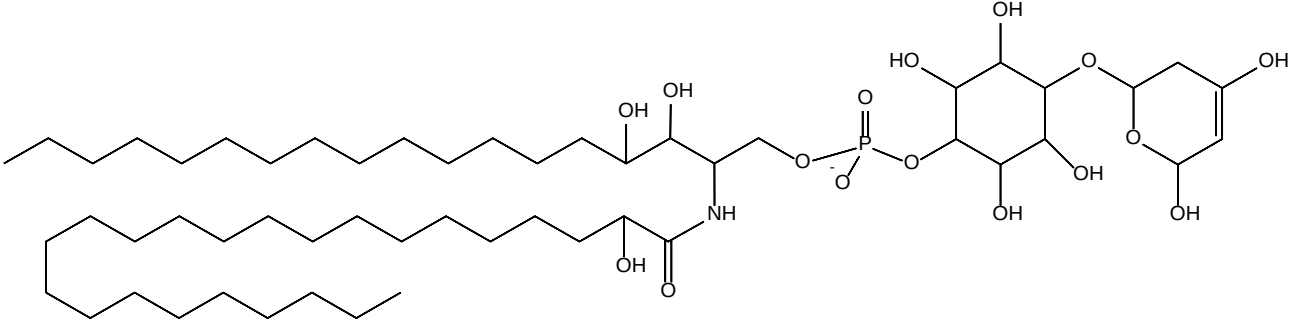
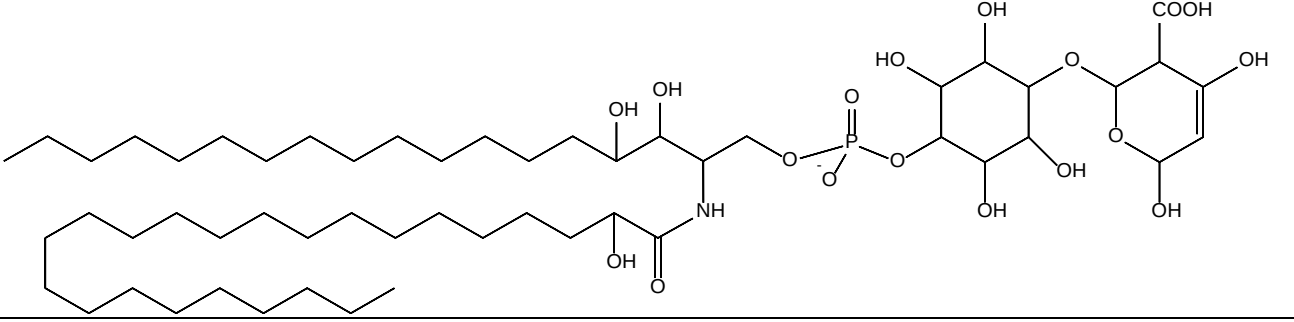
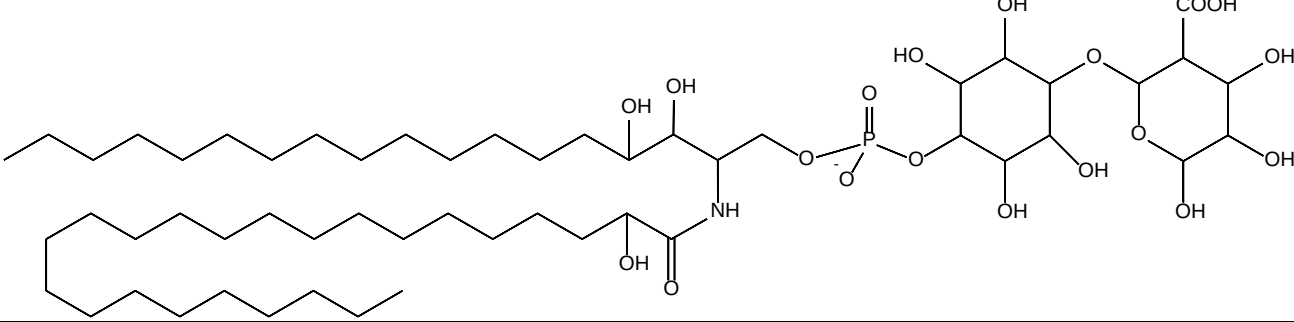
Table 3S. Tandem MS of HexA-HexA-IPC-t18:0/OH-24:0.

[fragment ion] [−]	<i>m/z</i>	Relative abundance (%)	
[IP-H ₂ O] [−]	241.0116	8	
[IP] [−]	259.0221	6	
[C ₃ PO ₃ -C ₁ -CO ₂ -H] [−]	373.0539	4	

S ion	392.3895	6	 Chemical structure of a long-chain fatty acid derivative. It features a vinyl group (CH₂=CH-) attached to a carboxylate group (-COO⁻). The chain continues as a long, zigzag hydrocarbon tail.
[C ₃ PO ₃ -C ₁ -H ₂ O-H] ⁻	399.0331	4	 Chemical structure of a complex molecule. It consists of a central ring system with multiple hydroxyl groups (-OH) and a carboxylic acid group (-COOH). The structure is highly branched and includes a phosphate group (-PO₃⁻).
T ion	408.3844	9	 Chemical structure of a long-chain fatty acid derivative. It features an epoxide group (three-membered ring with an oxygen atom) attached to a carboxylate group (-COO⁻). The chain continues as a long, zigzag hydrocarbon tail.
[C ₃ PO ₃ -C ₁ -H] ⁻	417.0438	5	 Chemical structure of a complex molecule. It consists of a central ring system with multiple hydroxyl groups (-OH) and a carboxylic acid group (-COOH). The structure is highly branched and includes a phosphate group (-PO₃⁻).

$[\text{C}_3\text{PO}_3\text{-B}_1\text{-H}]^-$	435.0542	5	
$[\text{FA}]^-$ ion	438.3950	4	
$[\text{B}_3\text{-H}_2]^-$	513.1094	14	
$[\text{B}_3\text{PO}_3\text{-H}]^-$	593.0758	35	

$[\text{C}_3\text{PO}_3\text{-H}]^-$	611.0862	63	
$[\text{Z}_0\text{PO}_3\text{-H}]^-$	744.5910	31	
$[\text{Y}_0\text{PO}_3\text{-H}]^-$	762.6014	10	
$[\text{Y}_1\text{-H}]^-$	924.6545	100	

$[Y_1+42-H]^-$	966.7013	8	
$[Z_2-CO_2-H]^-$	1038.6860	14	
$[Z_2-H]^-$	1082.6759	12	
$[Y_2-H]^-$	1100.6865	17	

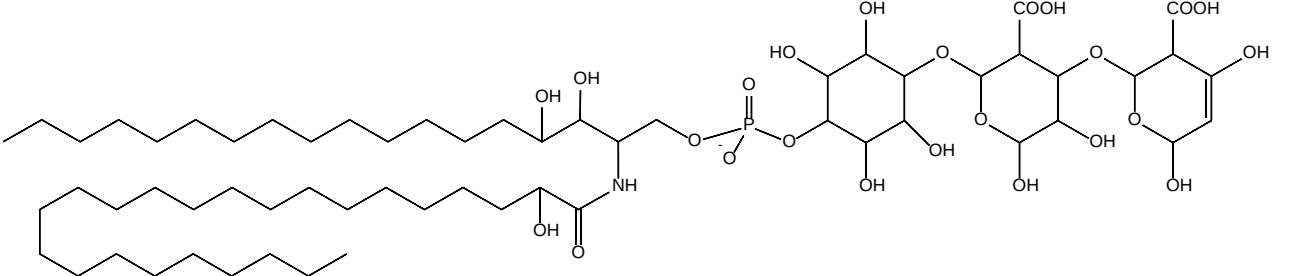
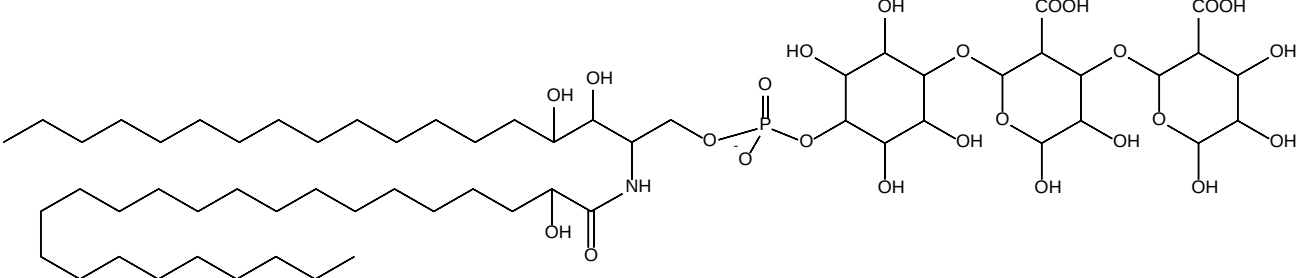
$[M-H_2O-H]^-$	1258.7080	5	 The chemical structure represents the $[M-H_2O-H]^-$ ion. It features a long-chain fatty acid (top) and a branched fatty acid (bottom) linked via an amide bond. The amide nitrogen is connected to a side chain containing two hydroxyl groups and a phosphate group. The phosphate group is linked to a chain of three pyranose rings. The first pyranose ring has hydroxyl groups at C2, C3, and C6. The second pyranose ring has a carboxylic acid group at C1 and hydroxyl groups at C2, C3, and C6. The third pyranose ring has a carboxylic acid group at C1, a hydroxyl group at C2, and a double bond between C3 and C4.
$[M-H]^-$	1276.7184	41	 The chemical structure represents the $[M-H]^-$ ion. It is identical to the structure in the first row, showing a long-chain fatty acid (top) and a branched fatty acid (bottom) linked via an amide bond. The amide nitrogen is connected to a side chain containing two hydroxyl groups and a phosphate group. The phosphate group is linked to a chain of three pyranose rings. The first pyranose ring has hydroxyl groups at C2, C3, and C6. The second pyranose ring has a carboxylic acid group at C1 and hydroxyl groups at C2, C3, and C6. The third pyranose ring has a carboxylic acid group at C1, a hydroxyl group at C2, and a double bond between C3 and C4.

Table 4S. The structures of all known GIPC found in algae

Alga	FA	LCB	[M-H] ⁻	Structure	References
<i>Chondrocanthus acicularis</i>	h24:0; h25:0; h26:0; h24:1; h25:1	t18:1	1084.79; 1098.78; 1112.76; 1082.79; 1096.78	Hex-IPC	Bure et al., 2014 and Cacas et al., 2013
<i>Fucus vesiculosus</i>	h25:0; h26:0; h27:0; h28:0; h25:1; h26:1; h27:1	t18:1	1276.71; 1290.67; 1304.73; 1378.73; 1274.71; 1288.67; 1302.73	Hex-HexA - IPC	Bure et al., 2014 and Cacas et al., 2013
<i>Ulva lactuca</i>	h16:0; h22:0; h16:0; h22:0	t18:1	1502.97; 1587.01; 1516.98; 1601.09	Hex-HexA ₃ - IPC, HexA ₄ - IPC	Bure et al., 2014 and Cacas et al., 2013
<i>Chlorella sorokiniana</i>	h24:0	t18:0	1251	Hex ₂ -IPC	Rose, 2015
<i>Chlorella variabilis</i>	h24:1	t18:0	1249	Hex ₂ -IPC	Rose, 2015
<i>Chlamydomonas reinhardtii</i>	h26:0	t18:0	1802	Hex ₄ -HexA-IPC	Rose, 2015
<i>Coccomyxa subellipsoidea</i>	h24:1	t18:0	1426	Hex ₂ -HexA-IPC	Rose, 2015

Table 5S. Dependence of Sph and FA content on the cell cycle.

molspecFA	0. h	3. h	6. h	9. h	12. h	15. h	18. h
C16:0	5,2	3,5	2,1	1,5	2,2	3,4	5,0
C18:0	3,4	1,9	1,7	1,4	1,8	2,0	3,3
C24:0	3,0	3,1	3,0	3,0	3,0	3,1	3,0
OH22:0	12,5	10,5	9,1	8,6	9,0	10,6	12,8
OH23:0	3,7	3,5	3,0	2,7	2,9	3,6	3,9
OH24:0	64,9	71,6	76,3	78,6	76,1	71,2	64,5
OH25:0	3,0	2,4	2,0	1,7	2,1	2,8	3,3
OH26:0	4,3	3,5	2,8	2,5	2,9	3,3	4,2

molspecSph	0. h	3. h	6. h	9. h	12. h	15. h	18. h
t18:0	70,1	64,7	56,4	47,5	55,5	63,9	69,7
t20:0	29,9	35,3	43,6	52,5	44,5	36,1	30,3

Table 6S. The change of abundance of GIPC molecular species during cell cycle.

mol spec	0. h	3. h	6. h	9. h	12. h	15. h	18. h
Hex-IPC-t18:0/OH22:0	1.9	1.7	1.4	0.8	1.4	1.7	1.9
Hex-IPC-t18:0/OH24:0	14.6	12.7	11.4	10.3	11.8	12.9	15.0
Hex-IPC-t18:0/OH26:0	5.4	4.9	4.7	4.6	4.5	4.7	5.7
Hex-IPC-t20:0/OH24:0	3.3	2.9	2.6	2.7	2.8	3.0	3.4
HexA-IPC-t18:0/C18:0	0.7	0.6	0.5	0.3	0.6	0.7	0.8
HexA-IPC-t20:0/C16:0	1.4	1.3	0.8	0.4	0.9	1.5	0.2
HexA-IPC-t18:0/OH22:0	2.6	2.3	2.4	1.9	2.0	2.5	2.8
HexA-IPC-t18:0/OH24:0	17.0	16.9	15.0	13.7	16.0	17.0	17.6
HexA-IPC-t20:0/OH22:0	1.7	1.4	1.2	1.2	1.1	1.6	2.0
HexA-IPC-t18:0/OH26:0	5.5	5.2	4.9	5.0	5.2	5.2	5.8
HexA-IPC-t20:0/OH24:0	4.2	4.3	4.1	3.3	4.0	4.4	4.8
HexA-IPC-t20:0/OH26:0	2.3	1.7	1.8	1.5	2.0	2.0	2.4
Hex-HexA-IPC-t18:0/OH24:0	10.2	10.7	10.2	10.8	10.2	10.5	11.0
Hex-HexA-IPC-t18:0/OH26:0	2.6	2.7	3.0	2.7	3.1	3.0	2.8
Hex-HexA-IPC-t20:0/OH24:0	2.4	2.5	2.5	2.8	2.7	2.6	2.5
HexA-HexA-IPC-t18:0/OH22:0	1.8	2.1	3.2	4.3	3.0	2.0	1.5
HexA-HexA-IPC-t18:0/OH24:0	7.5	8.6	10.1	11.0	9.5	8.4	6.5
HexA-HexA-IPC-t20:0/OH22:0	0.9	1.0	1.4	1.5	1.3	0.9	0.8
HexA-HexA-IPC-t18:0/OH26:0	2.1	2.5	3.0	3.2	2.9	2.4	1.9
HexA-HexA-IPC-t20:0/OH24:0	1.9	2.7	3.0	3.2	2.9	2.5	1.8
Hex-Hex-HexA-IPC-t18:0/OH24:0	5.4	6.0	6.9	8.1	6.7	5.5	4.7
Hex-Hex-HexA-IPC-t18:0/OH26:0	1.4	1.6	1.7	1.7	1.6	1.4	1.2
Hex-Hex-HexA-IPC-t20:0/OH24:0	1.3	1.4	1.4	1.5	1.3	1.4	1.1
Hex-Hex-Hex-HexA-IPC-t18:0/OH24:0	1.9	2.3	2.8	3.5	2.5	2.2	1.8
Polar head	0. h	3. h	6. h	9. h	12. h	15. h	18. h
Hex-IPC	25.2	22.2	20.1	18.4	20.5	22.3	26.0
HexA-IPC	35.4	33.7	30.7	27.3	31.8	34.9	36.4
Hex-HexA-IPC	15.2	15.9	15.7	16.3	16.0	16.1	16.3
HexA-HexA-IPC	14.2	16.9	20.7	23.2	19.6	16.2	12.5
Hex-Hex-HexA-IPC	8.1	9.0	10.0	11.3	9.6	8.3	7.0
Hex-Hex-Hex-HexA-IPC	1.9	2.3	2.8	3.5	2.5	2.2	1.8

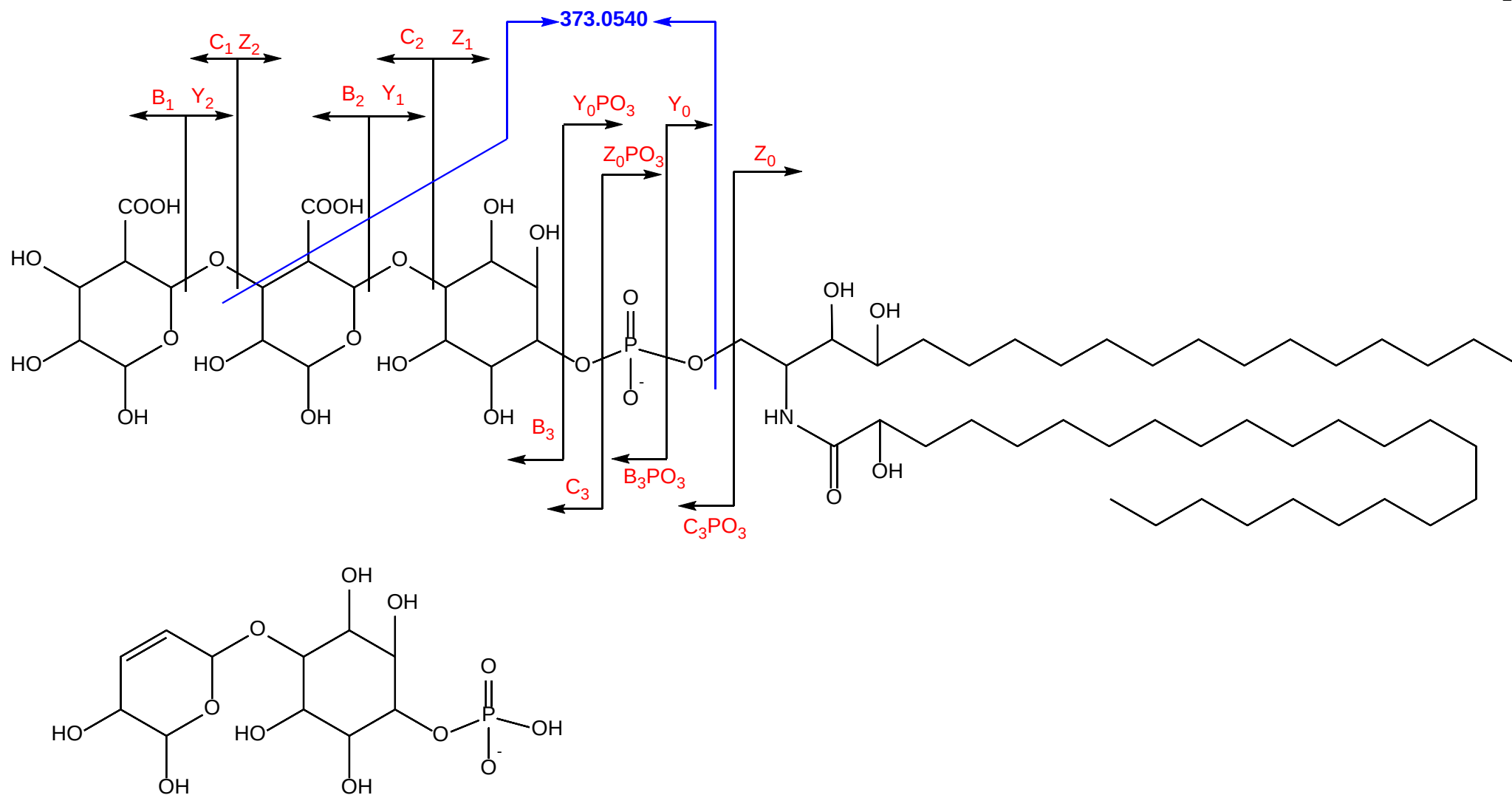


Fig. 1S. The structure of the ion $[C_3PO_3-C_1-CO_2]^-$ at m/z 373.0540 ($C_{11}H_{18}O_{12}P^-$).

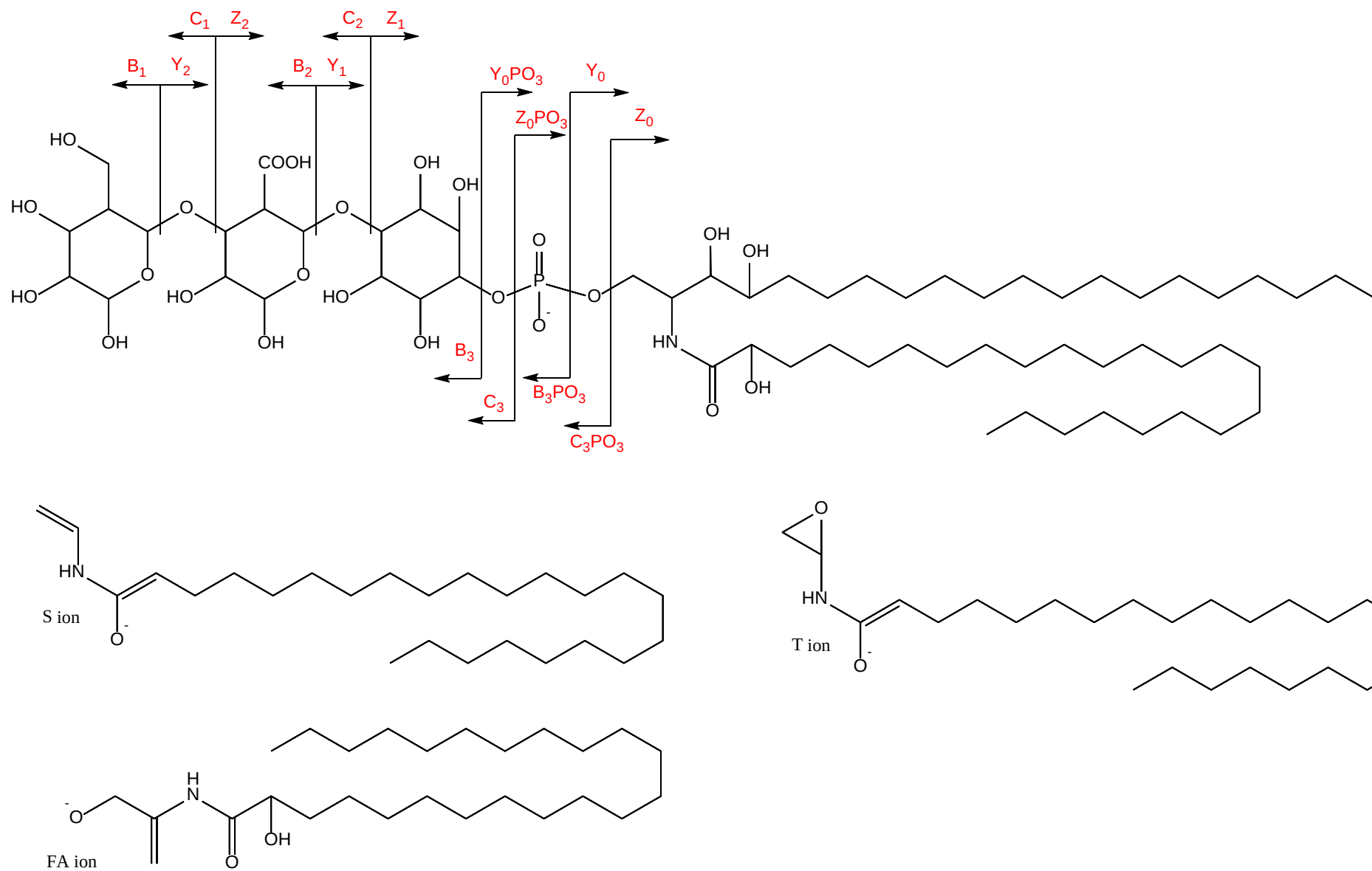


Fig. 2S. The major fragmentation pathways proposed for formation of ions from the Hex-HexA-IPC-t20:0-OH-23:0 (structure of hexose-hexuronic acid inositol phosphate).

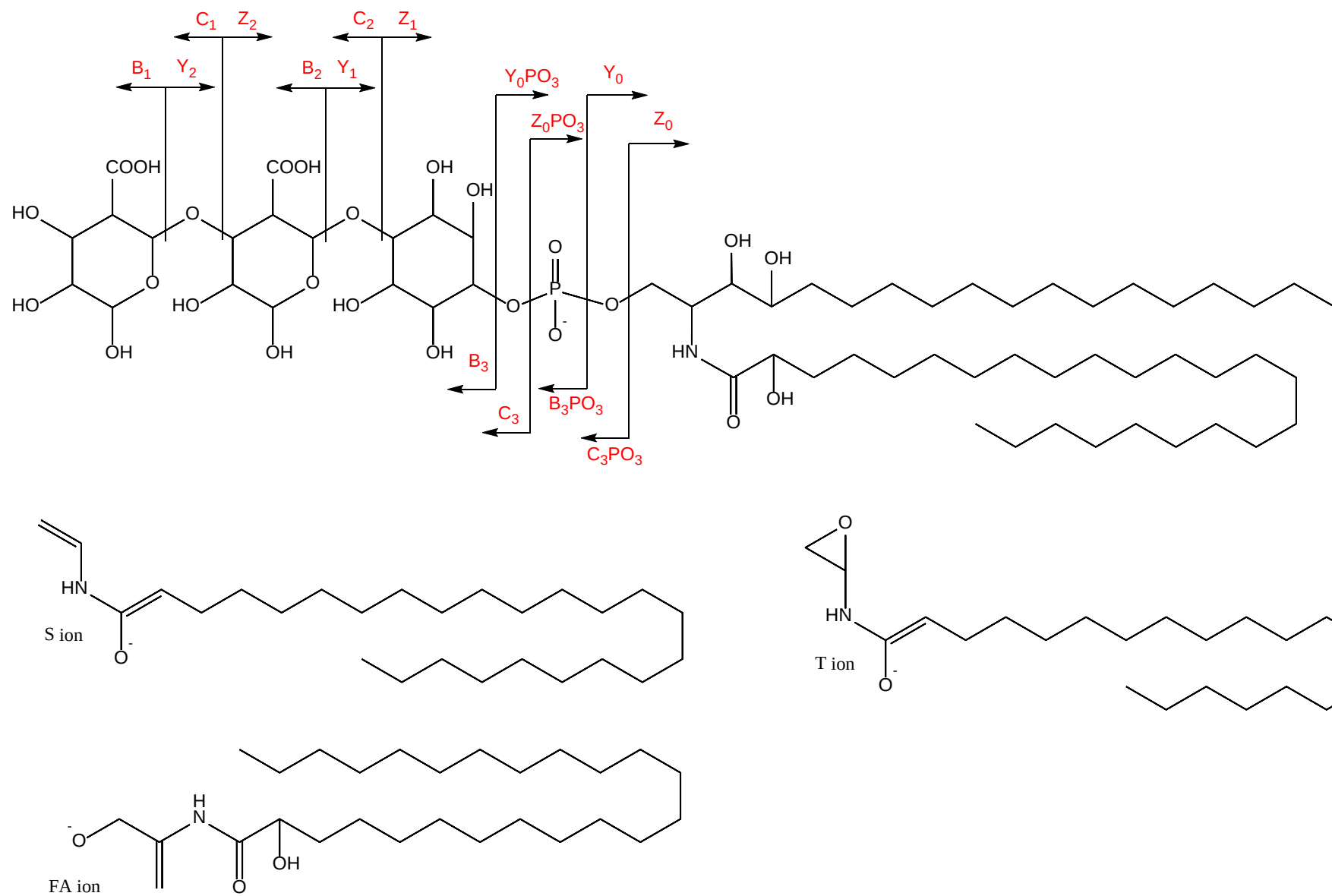


Fig. 3S. The major fragmentation pathways proposed for formation of ions from the HexA-HexA-IPC-t18:0-OH-24:0 (structure of hexuronic acid-hexuronic acid inositol phosphate).

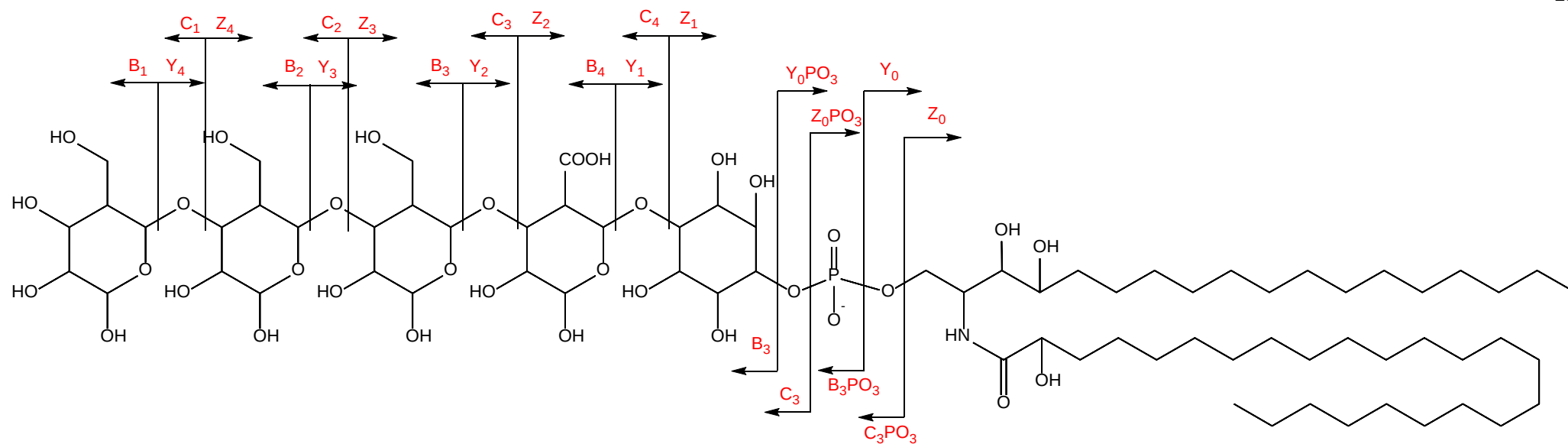


Fig. 4S. The major fragmentation pathways proposed for formation of ions from the $[M-H]^-$ at 1586.8455 Da (Hex-Hex-Hex-HexA-IPC-t18:0-OH24:0).

Galdieria XP_005707108.1	-----	0
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	-----	0
Cyanidioschyzon XP_005536876.1	MDAQEQNDYVVTQLDQSEBAQPFQTAQSAAMIEATGSGFRHIEVAVAAAAAPRHARSAS	60
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	-----PANLVLS-----	7
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	-----	0
Cyanidioschyzon XP_005536876.1	LPSTIVDLSTQMRPPSRNNPSPMAEARQQLVGRTRVPSLGLTSSVQTGILLASGGEPFSE	120
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	-----NSCKKETEIGNG-----DINRLNRRRAASDFVLVEGTNSLDAAPGISGSRFKTRR	57
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	-----	0
Cyanidioschyzon XP_005536876.1	SFSSRTAPEKEQELYTGRRRSQSLN--SERF-----VHGSES-----RIRA	159
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	ASISESTSANNNVCVWGHKKSLPLVSYKFFELPYAPRTSSPKSLFEQ-----	107
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	-----	0
Cyanidioschyzon XP_005536876.1	T--ERGRE-----SSVMNFRLLGYRLARFVFPERSPCSVSYSSTSGGSGDS	208
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	-----VGL-IRRSKVCQYFSKLFQH-TQEKKL-----ESSFV-GP	142
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	-----	5
Cyanidioschyzon XP_005536876.1	EPSPSKRGRSRKRRSGHSGRSFDDOGKALHSKTELQSGTLVLSKADSMSSSFEVGS	268
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	QC-CEQQYRASSEAS-----SQNNRLKLDSSSQSTWISFCAQQNFPSPESFQVGV	193
Galdieria XP_0057073814.1	-----	0
Porphyridium KAA8496974.1	RRSAPNGLNNE-----DRIEPVKNADA-AGGAVL-----GS	36
Cyanidioschyzon XP_005536876.1	ASSTPEAVYDASSSSDAEANEHSSARHSELEHRLPAA--TEGNPOLESUQSNARFCVYDA	327
Gracilariopsis PXF47232.1	-----	0
Galdieria XP_005707108.1	NETKRTPEQRSPVT-----DIAERSESQSQNNVC-----YGLD	227
Galdieria XP_0057073814.1	-----HNNAKT-----LQAKRQSLTSHSPNKDYKCTRQKSNMNEFLR	40
Porphyridium KAA8496974.1	DOSEYEKASKD--TGLNSGAGGSGVAPQSPVSGSHFVSIGAR-----AVRQ	82
Cyanidioschyzon XP_005536876.1	NEERFSPSGETFLYRAWNSCTTAWGRAADHCTRLQLTVFRGVSTQ-----PPLREELNRR	382
Gracilariopsis PXF47232.1	-----NG-----LLPSHSVSSSGS-----PPAPSSPRRP	24
Galdieria XP_005707108.1	RFLRLSGTAGEEITQVVRVGVESVLSHMLPLVWFLVFNYPRL-SLSWRPSTINTLL	286
Galdieria XP_0057073814.1	NFMNLS-----DHTVVRQLFLGVMPFIHLVTVESAA-IPSEYPRITHVRL	87
Porphyridium KAA8496974.1	TVIRITL-----EGISIQEILISFAPVLWILLYAVLAGQIPGSKPRINTVTL	130
Cyanidioschyzon XP_005536876.1	SSLSAK-----AVFLLRTVWVLPVIAWLSVFNFDVIPSANPKYITHVRL	430
Gracilariopsis PXF47232.1	S--AP-----YTPSVKLLALCFVPVILHLLFTCGVALTPDEKRTINTVLL	69
Galdieria XP_005707108.1	PKLEGLLHFPYRNFATRS-----SKAADFVAAPVYTIHAVLPGLFFLYLLTD-GRK	337
Galdieria XP_0057073814.1	SHIEMLGGKRNISYLPHEALSKQTNLAKDIFACIAYSLHAFPLHMFYLCFRS-NT	146
Porphyridium KAA8496974.1	PKIEALLYPHNFNRP-----HTVFDVLAALPYTHLPVPLINLVR-VVARSGAR	181
Cyanidioschyzon XP_005536876.1	PTLESYLLYPHNFNFPFGYD--GRGASFDFLAAPFYTVHAGLPFAFIALLLRTPAR	487
Gracilariopsis PXF47232.1	PTLDKHFVLSSTHFFPSNT--STISSVLDLVAAPVYTHLPFAFLTVSLPSRVRRRA	126
Galdieria XP_005707108.1	EAFCFASLGLVNTSAVLQTILFPFAPPVYFELNGLAKAEHUNSGNPGAARVDKLFGI	397
Galdieria XP_0057073814.1	HAAMFGITFGHNLILAVSHFFLPTAAPVYKEYGQLEAHYHVPGSA-AGLVRIIDKALGF	205
Porphyridium KAA8496974.1	RGLVFARCFGTMCFLGLVTLQVLPPIAPPVYFKEYGFPADYSMKGDA-AVLARVDLAGN	240
Cyanidioschyzon XP_005536876.1	KLPQFGLRLGLVSLGLVTLHVPAPPVYFKEYGFPADYTMKGP-ALLDRIDDAFPI	546
Gracilariopsis PXF47232.1	KLPVFLAFGTMLAAVCTHLTFPTAPPVYFKEYGVPANYSMKGP-ATLARLDQRYNM	185
Galdieria XP_005707108.1	VFYQETVYTT-QNRIPFGSPSLHAAMPVSLVAFCFPTRGSHLFVRLATCVCLVNAAYYL	456
Galdieria XP_0057073814.1	PLFESLYKR--NKVVFGSPSLHVAAPSLIALVEPLPR-ILGGIVPLVTVIMGMAAYL	262
Porphyridium KAA8496974.1	QHYQSYGLSANPIVFGSPSLHGAAMPYLIAAFEP-----SPGPMWAYTFMMAAYL	295
Cyanidioschyzon XP_005536876.1	HFYNTIYAT-AGKVVFGTSPSLHGAAMPYLMAFEP-----QQGRFLWAYTLVMAAYL	600
Gracilariopsis PXF47232.1	QLYHMYAK-GGKVVFGAAPSLHGAAMPYLIAFSPHFP-SLLARVNLVYALLVMAAYL	243
Galdieria XP_005707108.1	QHHYVIDLLGGTYAYLYTYRNFARKLDIPSFFKTQSDNLTEDLT--YSSGKRPPLO	513
Galdieria XP_0057073814.1	EHHYMDVMGGILYAYSVPFCHGQLYRKECVS-----	296
Porphyridium KAA8496974.1	QHIFLTDLLGSAIYAVALFVLGDQGLTLGHIVQKFRASR-NGSHNYRQVRSE-----E	348
Cyanidioschyzon XP_005536876.1	QHYYLLDIGGALYEVVYIAGRLADTESGV-----PDKALYSAPAEAGAYQPLE	652
Gracilariopsis PXF47232.1	RHHYLDITVGAIZFAIALRVAMFVIRSWDDH-----LQEDLCTCKSQQ--RQCFI	293
Galdieria XP_005707108.1	PAYSRIIVSS---TSQ-----DSLTNQRKS--THLGHYS-----	543
Galdieria XP_0057073814.1	-----	296
Porphyridium KAA8496974.1	VPDR-----EANGISRTNSVY-----A--DY-	367
Cyanidioschyzon XP_005536876.1	MPQASTYASRRGHLPSAPMPFTAIRPLTPSRNGPGRSPQRYRSSSQASSIDL	706
Gracilariopsis PXF47232.1	LPY----FRRL-----	300

Fig. 5S. Multiple sequence alignment (MSA) of inositol phosphorylceramide synthase-like protein of different red algae *Galdieria sulphuraria* (strain 074W), *Cyanidioschyzon merolae*, *Porphyridium purpureum*, *Gracilariopsis chorda* with their NCBI Reference Sequence numbers. MSA was performed by using Clustal Omega tool (Sievers et al., 2011). The conserved residues are marked with asterisks (*). A colon (:) indicates conservation between amino acid groups of strongly similar properties. A period (.) indicates conservation between groups of weakly similar properties. Sequences contain conserved protein domain PAP2_Aur1_like. All eight residues that compose the active site on this conserved domain were mapped to the query sequence (Marchler-Bauer et al., 2017).

CLUSTAL O(1.2.4) multiple sequence alignment

```

Galdieria|XP_005708838.1  ---MYGHHGLSELDNKRLLRLLKAAASRVROIKEEKYAMTYRSIETISWPKQYVPFCNW 56
Fistulifera|GAX25383.1  MEDVVVELHEENTLN-E-RFRVLRPAPT-----KGILSLDDO 35
      : * . * : : * : * :
      :
      :
      :

Galdieria|XP_005708838.1  FLLPCNTTCTAKCISLNYTIYISHDOMRRAARRNLSDIILRFGGAGTSQVYNELRRSN 116
Fistulifera|GAX25383.1  I-----LRPCIAYNFAKWTQHPSRIVGFDARSHEIASDGR-WKYAYMSTTEKTN 85
      :          **: : : : : : : * .. * ** * . : . : : *
      :
      :
      :

Galdieria|XP_005708838.1  RVTQNASDACYFIAENKGRHIYKQLQDLPYWNLGINHFWLDFLGKCEGQHDVGFAALLKT 176
Fistulifera|GAX25383.1  KYSLTLTRCCFVHV-----DYLNNWYENP----- 109
      : : . : . * : . . * * :
      :
      :
      :

Galdieria|XP_005708838.1  HLSSDIFRSNFDKSLPLPPRQHFRILEHPKKFLITFKGTSYPLHTAGGIRSILNIFHNGK 236
Fistulifera|GAX25383.1  -----FFAPIRNMVADYNNICE 125
      :          * . : : : : * :
      :
      :
      :

Galdieria|XP_005708838.1  DIIF---VTKPQFSVPTPEYDLMSYEELVEQSKF-CLVPQGRSPSTFRLLVMSAGCIPV 292
Fistulifera|GAX25383.1  DIALSLLVSSQTDGKPLLADLWAVKSMIKLDNNQGISAGKD-----KSLRDNCVNT 179
** : * : . * * : : : : : : : * : : : * :
      :
      :
      :

Galdieria|XP_005708838.1  FLFD-----TTAAKYIMPISDEIANEQISFIAPNYPAEFQAFLEGLRLVMINKKVE 342
Fistulifera|GAX25383.1  FSQQLGLKNKLAVKKLRHGSLFEYGDVQNEAATHMNT-SQRIKRNKEDANLLKSELMD 238
* : . : : * : * : . . : : * . * : : :
      :
      :
      :

Galdieria|XP_005708838.1  NMSNLV-----VKVRFN----- 354
Fistulifera|GAX25383.1  LRAGAAVAAYEAGLVEGSDPWQRRFHGLNSSIHN 272
      : . : : * :
      :
      :
      :

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Fig. 6S. Sequence alignment of glucuronyl/N-acetylglucosaminyl transferase EXT1 protein of red alga *Galdieria sulphuraria* (strain 074W) and diatom *Fistulifera solaris* with their NCBI Reference Sequence numbers. Sequence alignment was performed by using Clustal Omega tool (Sievers F. et al., 2011). The conserved residues are marked with asterisks (*). A colon (:) indicates conservation between amino acid groups of strongly similar properties. A period (.) indicates conservation between groups of weakly similar properties. Sequences contain conserved protein domain of exostosin (EXT) family (Marchler-Bauer et al., 2017). This exostosin family protein is similar to *Arabidopsis thaliana* xylogalacturonan β -1,3-xylosyltransferase that catalyzes the transfer of xylose from UDP-xylose onto oligogalacturonides and endogenous acceptors (Lu et al., 2020).