Supplements

Characterization of Archaea Membrane Lipids in Radioactive Springs Using Shotgun Lipidomics

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Table 1S. Information about six springs, including their locations.

Spring	Water volume produced	Water temperature	Radon content	Latitude	Longitude
Agricola	8 L/min	28 °C	24 kBq/L[a]	50.3725911N	12.9116636E
Behounek	294 L/min	36.8 °C	7.55 kBq/L[b]	50.3725911N	12.9116636E
C1	30 L/min	30 °C	10 kBq/L[b]	50.3725911N	12.9116636E
Curie	30 L/min	28 °C	5 kBq/L[b]	50.3725911N	12.9116636E
Radonka[c]	2.7 L/min	11 °C	13.2 kBq/L[d]	50.2058433N	12.3400597E
Wettinquelle	not detected	10.5 °C	25 kBq/L[e]	50.2218700N	12.2996247E

[a] see photo, Fig. 4S
[b] Krejbichova (1999)
[c] Sometimes also called after the first name of the discoverer, i.e., Břetislav.
[d] Goliáš et al. (2018)
[e] Heinicke et al. (1993)

References

Krejbichova Z (1999) Radioactivity of mineral waters in Bohemia. Czech J Phys. https://doi.org/10.1007/s10582-999-0017-4

Goliáš V, Hrušková L, Fanta M (2018) Radioactive medicinal springs: searching for activating geomaterials. Czech Chem Soc Symp Ser 16:Id725.

Heinicke J, Koch U, Krbetschek M (1993) Zusammenhänge Zwischen Radonanomalien und Schwarm-Erdbeben im Oberen Vogtland. Isot Environ Health Stud. https://doi.org/10.1080/10256019308046123

Table 2S. The content of individual molecular species in ng/L in six springs determined by shotgun lipidomics using an internal standard (β-L-gulopyranosyl-caldarchaetidyl-glycerol).

name	mass (adduct $[M+H]^+$)	Agricola#	Behounek	C1	Curie	Radonka	Wettinquelle
archaeol	653.6812	52.1	28.1	17.7	12.4	34.9	61.4
OH-archaeol	669.6761	13.0	16.4	9.9	9.9	41.2	58.0
2OH-archaeol	685.6711	4.3	4.7	2.0	0.0	12.7	27.3
phosphoglycerol-archaeol	807.6843	6.5	11.7	7.9	2.5	28.5	40.9
Hex-archaeol	815.7341	8.7	7.0	9.9	5.0	38.0	54.6
phosphoglycerol-OH-archaeol	823.6792	0.0	2.3	2.0	0.0	25.4	30.7
2Hex-archaeol	977.7869	0.0	9.4	5.9	5.0	28.5	37.5
2Hex-OH-archaeol	993.7817	0.0	4.7	3.9	0.0	22.2	20.5
GDGT-5*	1292.2444	97.7	138.1	100.5	181.0	386.7	354.6
GDGT-3	1296.2757	17.4	28.1	17.7	42.2	72.9	228.5
GDGT-2	1298.2914	23.9	39.8	33.5	47.1	206.1	364.9
GDGT-1	1300.3070	36.9	42.1	55.2	59.5	355.0	596.8
GDGT-0	1302.3227	78.1	105.3	124.1	141.4	627.7	719.5
OH-GDGT-5*	1308.2393	19.5	11.7	13.8	22.3	69.7	64.8
OH-GDGT-3	1312.2706	6.5	16.4	9.9	17.4	60.2	37.5
OH-GDGT-2	1314.2863	13.0	18.7	7.9	12.4	82.4	98.9
OH-GDGT-1	1316.3019	15.2	14.0	11.8	19.8	123.6	112.5
OH-GDGT-0	1318.3176	26.0	28.1	15.8	24.8	82.4	160.3
2OH-GDGT-5*	1324.2342	17.4	9.4	3.9	7.4	69.7	98.9
2OH-GDGT-0	1334.3125	13.0	4.7	2.0	0.0	72.9	126.2
phosphoglycerol-GDGT-3	1450.2788	17.4	21.1	15.8	17.4	101.4	139.8
phosphoglycerol-GDGT-2	1452.2945	23.9	21.1	21.7	37.2	152.2	194.4
Hex-GDGT-5*	1454.2972	47.7	23.4	35.5	39.7	310.7	381.9
phosphoglycerol-GDGT-1	1454.3101	19.5	25.7	29.6	47.1	114.1	160.3
Hex-GDGT-4	1456.3129	30.4	42.1	45.3	42.2	133.1	177.3
phosphoglycerol-GDGT-0	1456.3258	67.3	58.5	51.2	69.4	215.6	242.1
Hex-GDGT-3	1458.3286	17.4	16.4	17.7	22.3	57.1	78.4
Hex-GDGT-2	1460.3442	19.5	7.0	21.7	19.8	69.7	92.1
Hex-GDGT-1	1462.3599	30.4	42.1	31.5	42.2	98.3	143.2
Hex-GDGT-1	1462.3599	49.9	51.5	53.2	57.0	168.0	262.6

Hex-GDGT-0	1464.3756	62.9	44.5	43.3	71.9	212.4	293.3
Hex-P-GDGT-2	1540.3105	17.4	16.4	13.8	12.4	60.2	95.5
Hex-P-GDGT-1	1542.3262	13.0	14.0	9.9	9.9	76.1	139.8
Hex-P-GDGT-0	1544.3417	19.5	18.7	17.7	7.4	88.8	119.4
2DHex-GDGT-4	1586.3760	0.0	0.0	0.0	0.0	2.8	1.4
2DHex-GDGT-3	1588.3917	0.0	0.0	0.0	0.0	1.2	1.3
2DHex-GDGT-2	1590.4073	0.0	0.0	0.0	0.0	3.6	2.6
2DHex-GDGT-1	1592.4229	0.0	0.0	0.0	0.0	4.6	4.7
2DHex-GDGT-0	1594.4385	0.0	0.0	0.0	0.0	5.2	3.1
DHexHex-GDGT-4	1602.3709	0.0	0.0	0.0	0.0	7.1	5.8
2phosphoglycerol-GDGT-3	1604.2819	17.4	21.1	15.8	19.8	79.3	133.0
DHexHex-GDGT-3	1604.3866	0.0	0.0	0.0	0.0	9.3	7.4
2phosphoglycerol-GDGT-2	1606.2976	23.9	23.4	27.6	29.8	133.1	173.9
DHexHex-GDGT-2	1606.4022	0.0	0.0	0.0	0.0	9.2	8.7
2phosphoglycerol-GDGT-1	1608.3132	36.9	39.8	37.4	37.2	199.7	269.4
DHexHex-GDGT-1	1608.4178	0.0	0.0	0.0	0.0	11.7	14.3
2phosphoglycerol-GDGT-0	1610.3289	41.2	39.8	35.5	49.6	339.2	422.8
DHexHex-GDGT-0	1610.4334	0.0	0.0	0.0	0.0	14.8	16.7
2Hex-GDGT-5*	1616.3507	23.9	21.1	15.8	22.3	88.8	143.2
2Hex-GDGT-4	1618.3658	2.2	0.0	2.0	0.0	28.5	37.5
2Hex-GDGT-3	1620.3815	2.2	0.0	0.0	0.0	44.4	52.4
2Hex-GDGT-2	1622.3971	6.5	7.0	3.9	0.0	41.6	88.7
2Hex-GDGT-1	1624.4127	10.9	14.0	7.9	7.4	49.7	85.7
2Hex-GDGT-0	1626.4283	19.5	18.7	11.8	12.4	86.5	75.5
2Hex-P-GDGT-5*	1696.3166	4.3	7.0	5.9	5.0	25.4	44.3
2Hex-P-GDGT-4	1698.3322	0.0	0.0	2.0	0.0	9.5	27.3
2Hex-P-GDGT-3	1700.3477	0.0	2.3	0.0	0.0	19.0	47.7
2Hex-P-GDGT-2	1702.3633	0.0	0.0	0.0	0.0	34.9	75.0
2Hex-P-GDGT-1	1704.3790	4.3	7.0	3.9	2.5	34.9	98.9
2Hex-P-GDGT-0	1706.3946	6.5	9.4	3.9	2.5	57.1	115.9
3DHex-GDGT-4	1732.4338	0.0	0.0	0.0	0.0	1.7	1.1
3DHex-GDGT-3	1734.4495	0.0	0.0	0.0	0.0	1.7	2.1
3DHex-GDGT-2	1736.4652	0.0	0.0	0.0	0.0	1.8	3.5
3DHex-GDGT-1	1738.4808	0.0	0.0	0.0	0.0	2.4	4.6

3DHex-GDGT-0	1740.4965	0.0	0.0	0.0	0.0	2.9	5.3
2DHexHex-GDGT-4	1748.4287	0.0	0.0	0.0	0.0	3.3	5.7
2DHexHex-GDGT-3	1750.4444	0.0	0.0	0.0	0.0	5.2	6.2
2DHexHex-GDGT-2	1752.4601	0.0	0.0	0.0	0.0	5.5	9.8
2DHexHex-GDGT-1	1754.4757	0.0	0.0	0.0	0.0	5.6	11.2
2DHexHex-GDGT-0	1756.4914	0.0	0.0	0.0	0.0	7.7	14.2
DHex2Hex-GDGT-4	1764.4236	0.0	0.0	0.0	0.0	5.4	8.3
DHex2Hex-GDGT-3	1766.4393	0.0	0.0	0.0	0.0	8.4	17.7
DHex2Hex-GDGT-2	1768.4551	0.0	0.0	0.0	0.0	11.3	18.7
DHex2Hex-GDGT-1	1770.4706	0.0	0.0	0.0	0.0	17.8	23.8
DHex2Hex-GDGT-0	1772.4863	0.0	0.0	0.0	0.0	19.3	27.8
phosphoglycerol2Hex-GDGT-3	1774.3845	0.0	0.0	0.0	0.0	6.3	10.2
phosphoglycerol2Hex-GDGT-2	1776.4001	2.2	2.3	0.0	0.0	9.5	17.1
3Hex-GDGT-5*	1778.4029	0.0	0.0	0.0	0.0	28.5	44.3
phosphoglycerol2Hex-GDGT-1	1778.4158	4.3	11.7	5.9	9.9	34.9	58.0
3Hex-GDGT-4	1780.4185	0.0	2.3	2.0	2.5	37.6	95.5
phosphoglycerol2Hex-GDGT-0	1780.4314	15.2	21.1	15.8	27.3	111.0	150.0
3Hex-GDGT-3	1782.4342	2.2	2.3	0.0	2.5	47.1	103.2
3Hex-GDGT-2	1784.4499	2.2	0.0	2.0	5.0	62.9	250.1
3Hex-GDGT-1	1786.4655	6.5	11.7	5.9	9.9	72.4	328.1
3Hex-GDGT-0	1788.4812	15.2	14.0	5.9	7.4	168.2	434.0
3Hex-P-GDGT-5*	1858.3695	0.0	2.3	3.9	0.0	28.5	51.2
3Hex-P-GDGT-3	1862.4007	0.0	0.0	0.0	2.5	72.9	92.1
3Hex-P-GDGT-2	1864.4163	2.2	0.0	0.0	2.5	104.6	153.5
3Hex-P-GDGT-1	1866.4319	2.2	4.7	3.9	7.4	149.0	248.9
3Hex-P-GDGT-0	1868.4474	6.5	11.7	7.9	7.4	310.7	484.2
4Hex-GDGT-5*	1940.4560	0.0	0.0	0.0	0.0	53.9	92.1
4Hex-GDGT-4	1942.4716	4.3	0.0	3.9	0.0	142.7	177.3
4Hex-GDGT-3	1944.4872	4.3	9.4	7.9	7.4	149.0	235.3
4Hex-GDGT-2	1946.5027	6.5	16.4	9.9	12.4	215.6	266.0
4Hex-GDGT-1	1948.5184	10.9	14.0	15.8	22.3	377.2	422.8
4Hex-GDGT-0	1950.5340	19.5	23.4	23.6	42.2	554.8	743.4
Sum of core lipids		434.0	505.6	425.7	597.6	2298.1	3110.1

Sum of intact polar lipids	755.3	795.5	725.2	865.5	6178.7	9278.8
Sum of total lipids	1189.3	1301.1	1150.9	1463.1	8476.8	12388.9

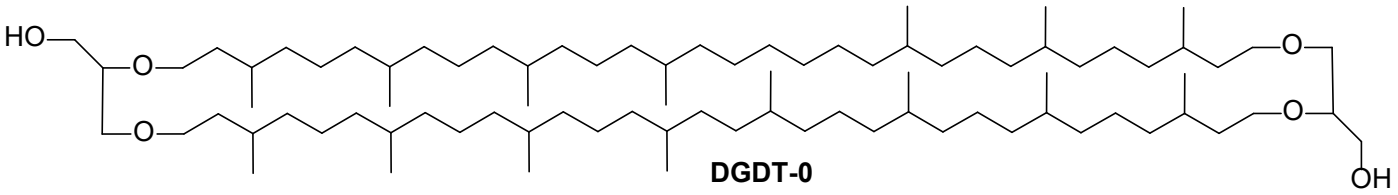
Blue names - core lipids
Black names - intact polar lipids
* GDGT-5 or crenarchaeol or crenarchaeol regio-isomer
# ng/L of water

Table 3S. Tandem MS of Hex-Hex-GDGT-0.

Ion	*m/z*
$[M+H]^+$	1626.4283
$[M+H]^+$-1xHex	1464.3750
$[M+H]^+$-2xHex	1302.3221
$[M+H]^+$-2xHex - 1xH_2O	1284.3121
$[M+H]^+$-2xHex - 2xH_2O	1266.3015
$[M+H]^+$-2xHex - 3xH_2O	1248.2909
$[M+H]^+$-2xHex - 1xC_3H_4O	1246.2965
$[M+H]^+$-2xHex - 1xH_2O-1xC_3H_4O	1228.2859
$[M+H]^+$-2xHex - 2xH_2O-1xC_3H_4O	1210.2753
$[M+H]^+$-2xHex - 1x$C_{40}H_{78}$	743.7123
$[M+H]^+$-2xHex - 1xH_2O-1x$C_{40}H_{78}$	725.7017
$[M+H]^+$-2xHex - 2xH_2O-1x$C_{40}H_{78}$	707.6911
$[M+H]^+$-2xHex - 3xH_2O-1x$C_{40}H_{78}$	689.6805
$[M+H]^+$-2xHex - 1xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	669.6755
$[M+H]^+$-2xHex - 2xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	651.6649
$[M+H]^+$-2xHex - 3xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	633.6543
$[M+H]^+$-2xHex - 4xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	615.6437

Table 4S. Tandem MS of DHex-Hex-GDGT-0

Ion	*m/z*
$[M+H]^+$	1610.4334
$[M+H]^+$-DHex	1464.3750
$[M+H]^+$-DHexHex	1302.3221
$[M+H]^+$-DHexHex-1xH_2O	1284.3121
$[M+H]^+$-DHexHex-2xH_2O	1266.3015
$[M+H]^+$-DHexHex-3xH_2O	1248.2909
$[M+H]^+$-DHexHex-1xC_3H_4O	1246.2965
$[M+H]^+$-DHexHex-1xH_2O-1xC_3H_4O	1228.2859
$[M+H]^+$-DHexHex-2xH_2O-1xC_3H_4O	1210.2753
$[M+H]^+$-DHexHex-1x$C_{40}H_{78}$	743.7123
$[M+H]^+$-DHexHex-1xH_2O-1x$C_{40}H_{78}$	725.7017
$[M+H]^+$-DHexHex-2xH_2O-1x$C_{40}H_{78}$	707.6911
$[M+H]^+$-DHexHex-3xH_2O-1x$C_{40}H_{78}$	689.6805
$[M+H]^+$-DHexHex-1xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	669.6755
$[M+H]^+$-DHexHex-2xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	651.6649
$[M+H]^+$-DHexHex-3xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	633.6543
$[M+H]^+$-DHexHex-4xH_2O-1xC_3H_4O-1x$C_{40}H_{78}$	615.6437



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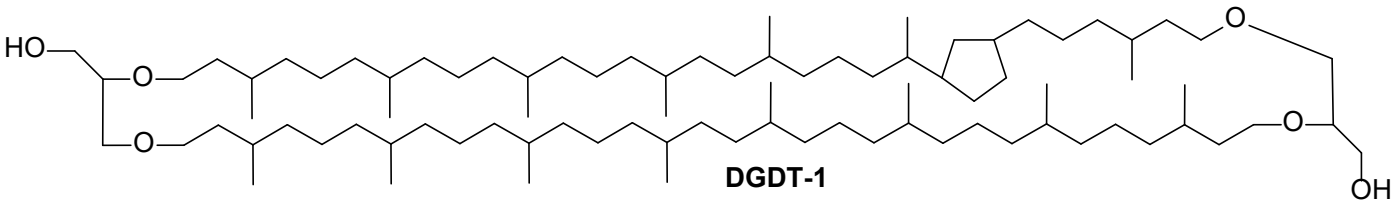

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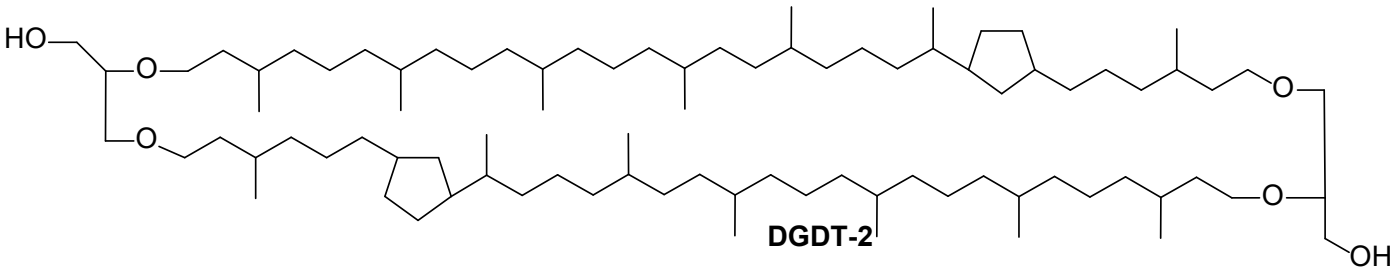

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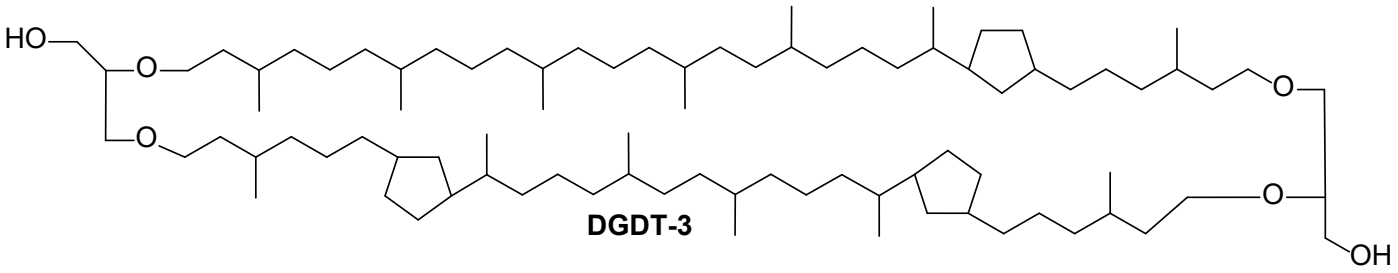

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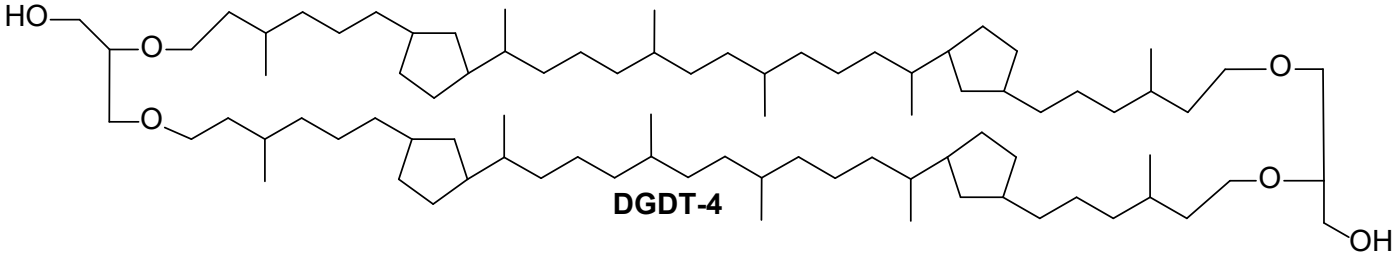

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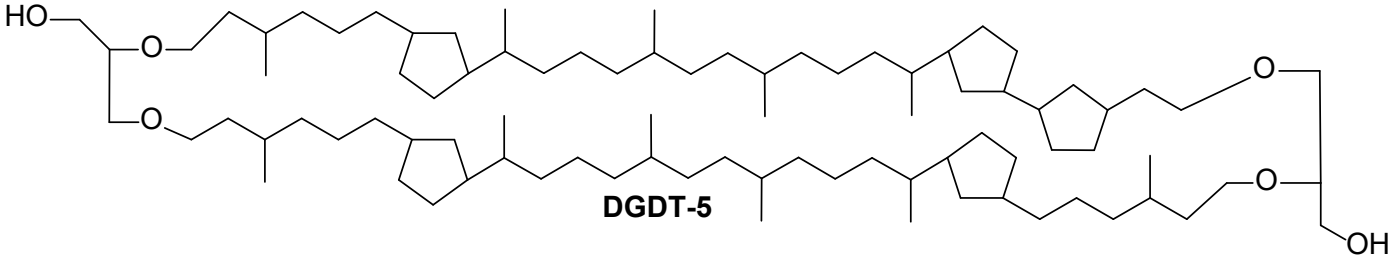

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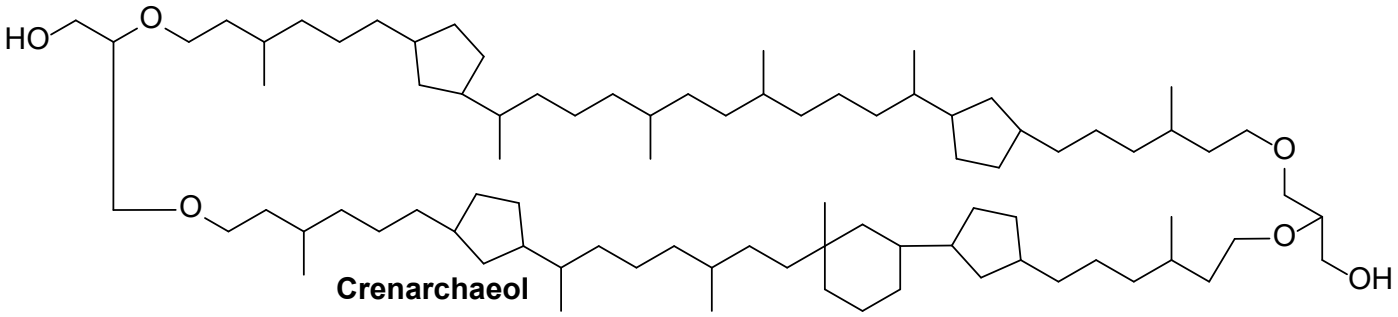
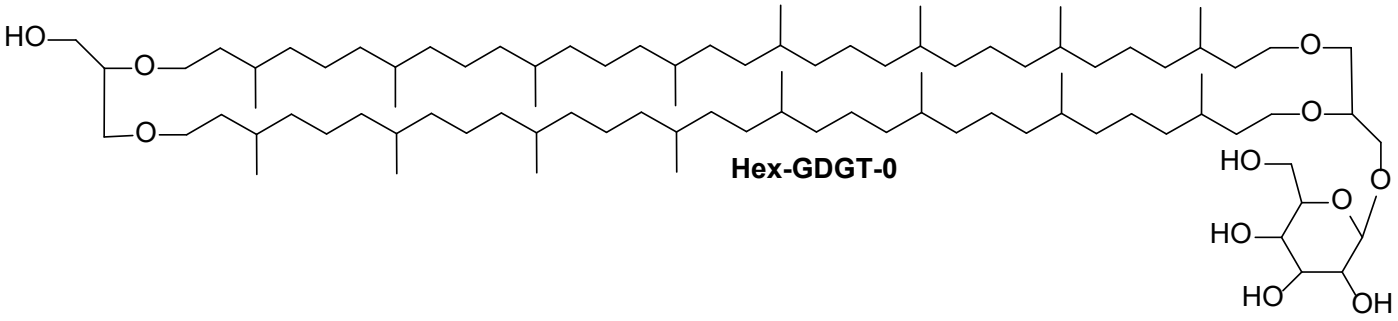
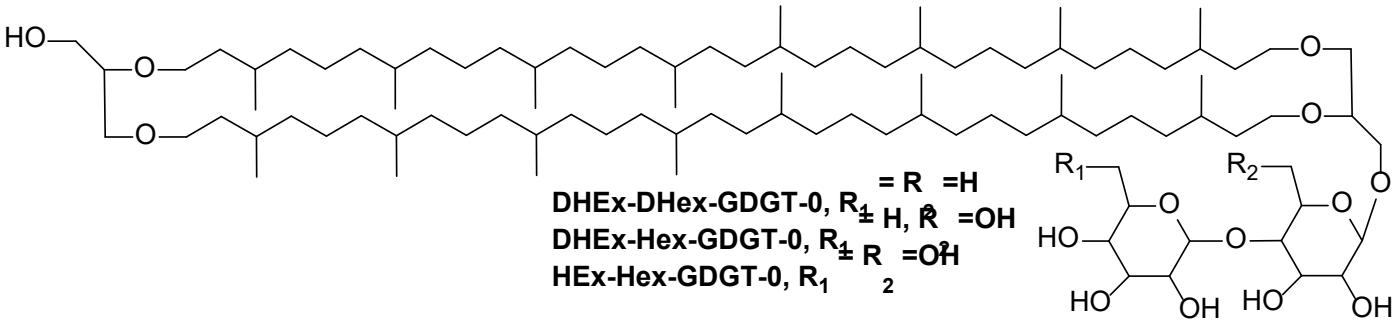


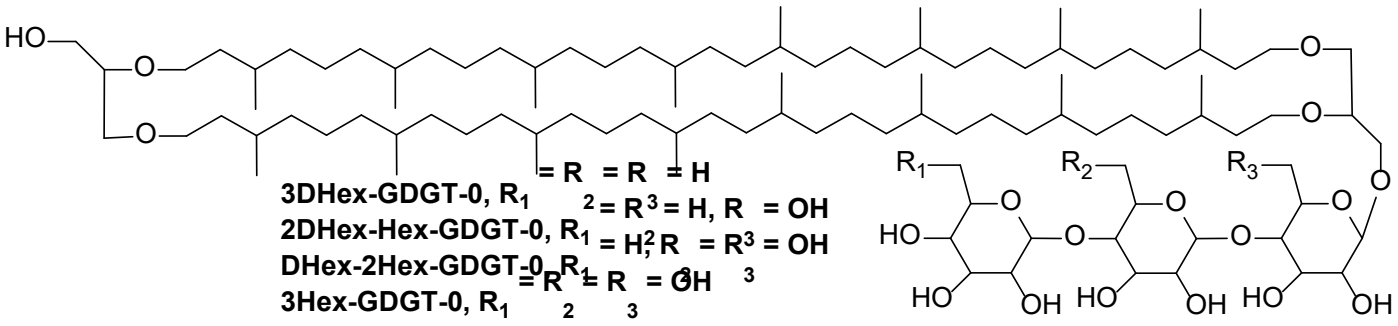
Crenarchaeol



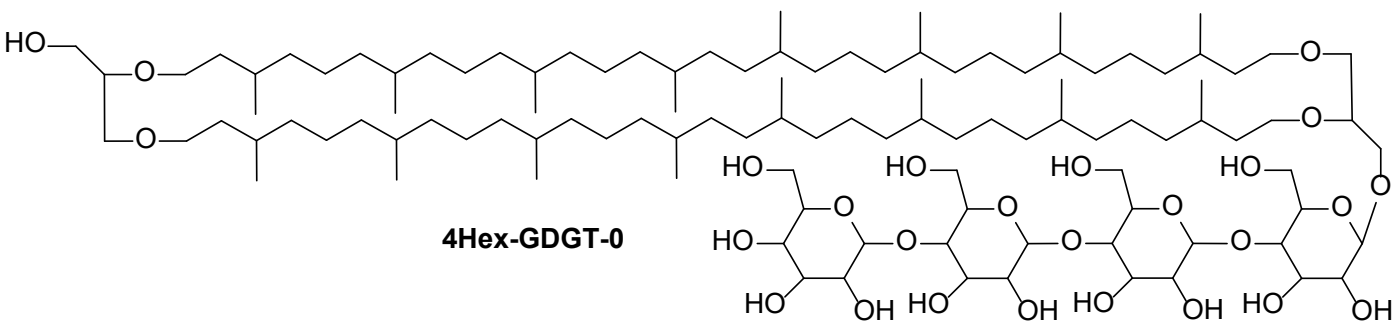
Hex-GDGT-0



DHEx-DHex-GDGT-0, R_1 = R_2 =H
DHEx-Hex-GDGT-0, R_1 = H, R_2 =OH
HEx-Hex-GDGT-0, R_1 = R_2 =OH



3DHex-GDGT-0, R_1 = R_2 = R_3 = H
2DHex-Hex-GDGT-0, R_1 = H, R_2 = R_3 = OH
DHex-2Hex-GDGT-0, R_1 = H, R_2 = R_3 = OH
3Hex-GDGT-0, R_1 = R_2 = R_3 = OH



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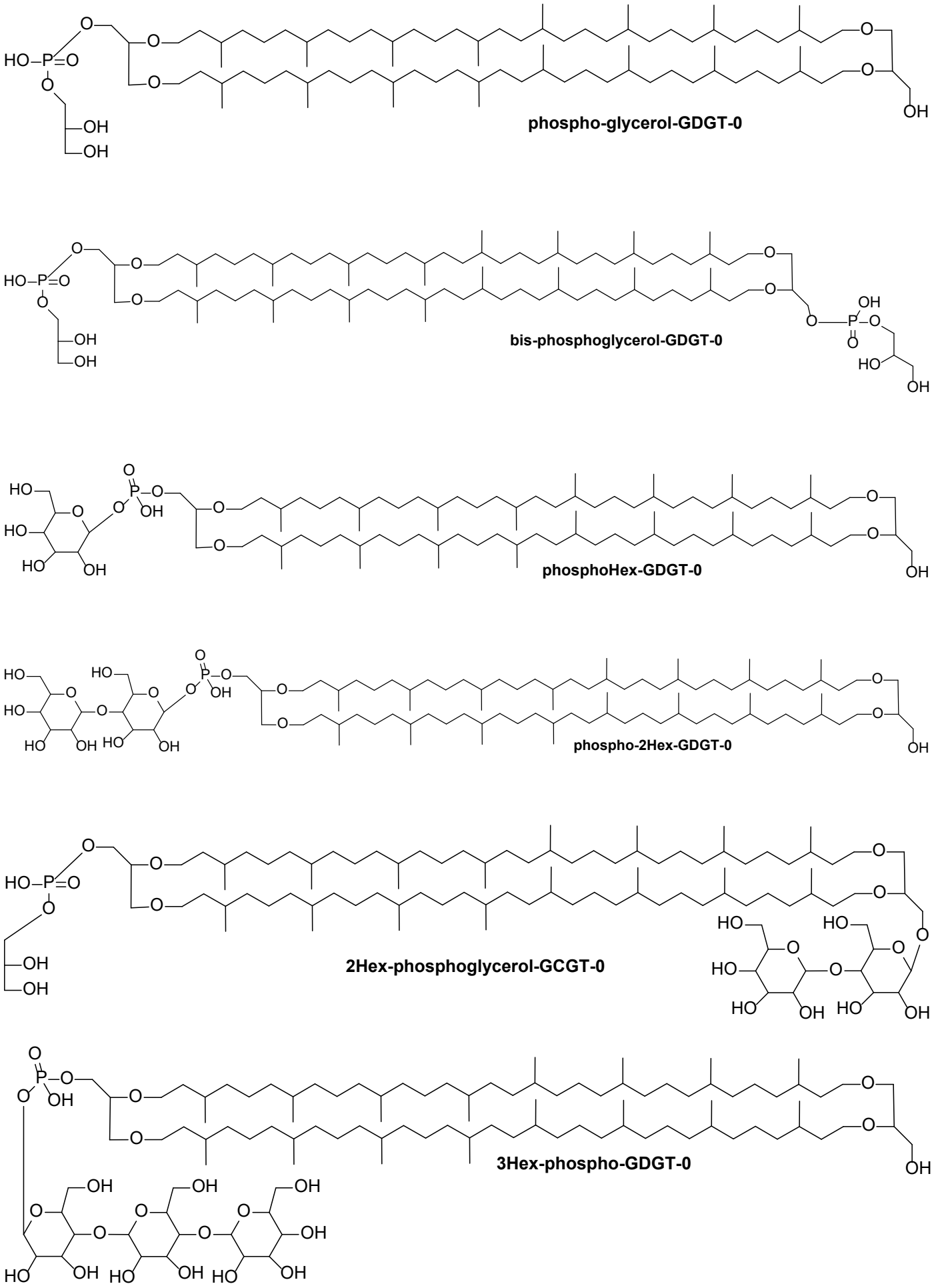

Fig. 1S. Examples of core lipids and intact polar lipids structures that have been identified in Archaea.

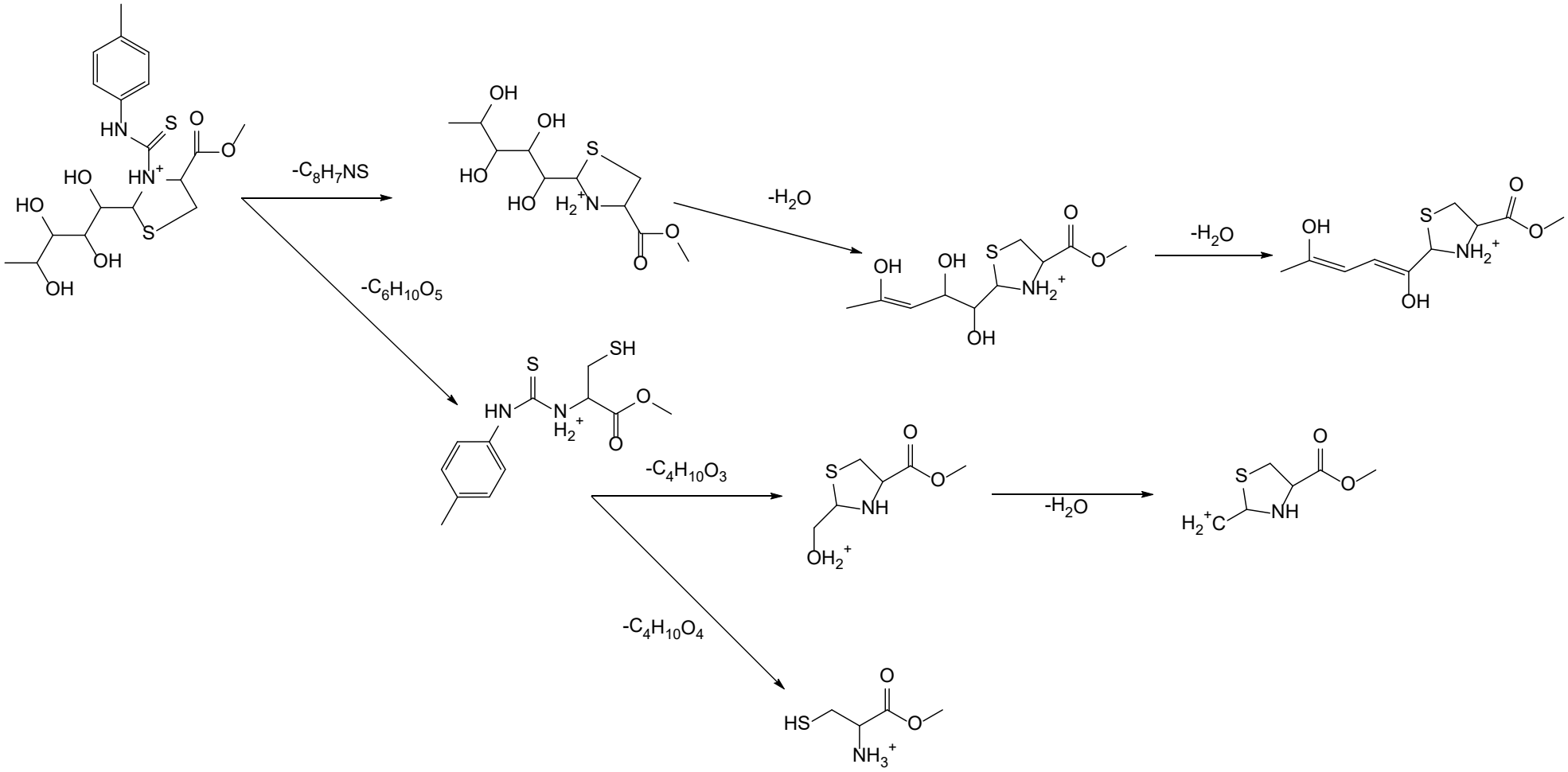

Fig. 2S. The probable fragmentation pathway of deoxyhexose derivative.

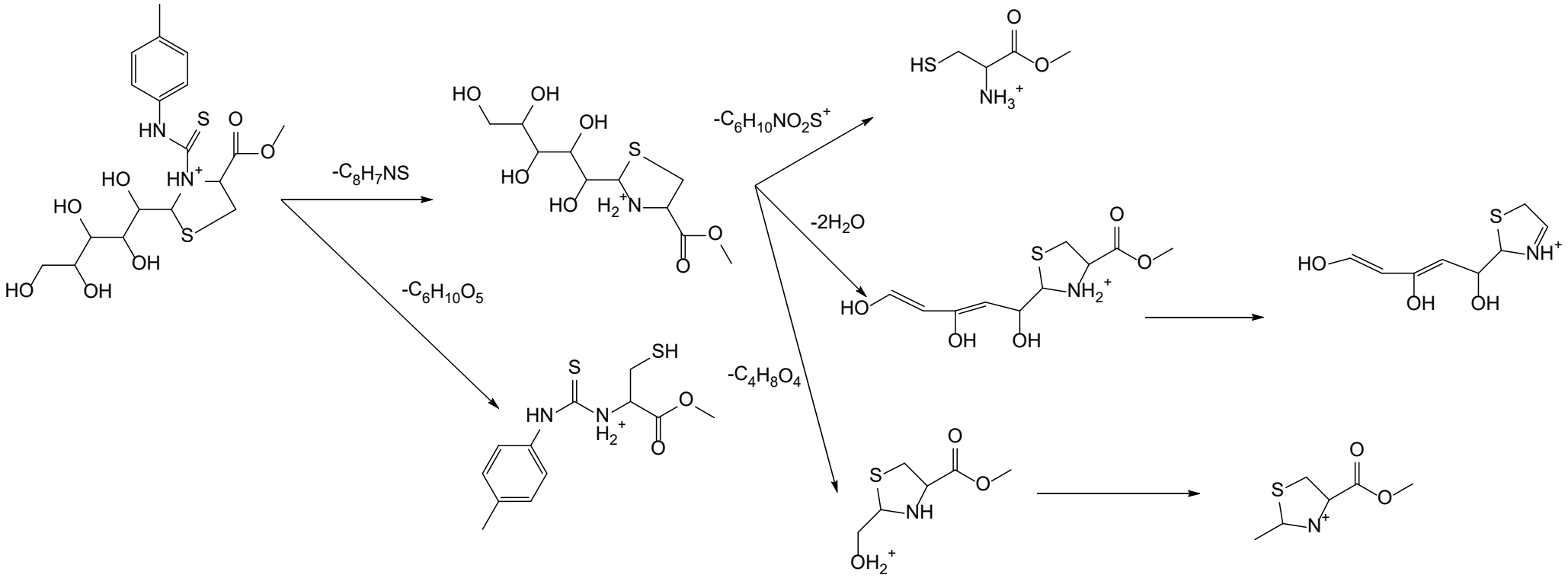

Fig. 3S. The probable fragmentation pathway of hexose derivative.

Fig. 4S. Measuring equipment directly in the mine while taking water from the spring (measured in kBq/L; photographed by the author T.Ř.).

Fig. 5S. An open waste (drain) valve (photographed by the author T.Ř.).

Fig. 6S. Pumps that draw water from depths exceeding 500 meters for use in the spa (photographed by the author T.Ř.).