

Supplements

Analysis of glycosylated cardiolipins from thermophilic bacteria using GC–MS and LC–ESI–MS/MS methods

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Table S1. List of eight strains obtained from the Czech Collection of Microorganisms, Brno, Czech Republic.

Abbreviation	Cultiv. Temp.	Bacterium	Reference
CCM 2062/70	70	<i>Geobacillus stearothermophilus</i> CCM 2062 (no information about source)	[1]
CCM 5965/70	70	<i>Geobacillus stearothermophilus</i> CCM 5965 (Evaporated milk)	[1]
CCM 2062/55	55	<i>Geobacillus stearothermophilus</i> CCM 2062	[2]
CCM 4212/70	70	<i>Meiothermus ruber</i> CCM 4212 (Thermal pools, Hveragherti, Iceland)	[1]
CCM 4212/65	65	<i>Meiothermus ruber</i> CCM 4212	[3]
CCM 4212/55	55	<i>Meiothermus ruber</i> CCM 4212	[2]
CCM 3488/70	70	<i>Thermus aquaticus</i> CCM 3488 (Thermally polluted river near Brussel)	[1]
CCM 3488/65	65	<i>Thermus aquaticus</i> CCM 3488,	[3]

- [1] L. Siristova, K. Melzoch, T. Rezanka, Fatty acids, unusual glycopospholipids and DNA analyses of thermophilic bacteria isolated from hot springs, *Extremophiles*. 13 (2009) 101–109. <https://doi.org/10.1007/s00792-008-0202-6>.
- [2] L. Siristova, R. Luhovy, K. Sigler, T. Rezanka, Biosynthesis of ω -alicyclic fatty acids induced by cyclic precursors and change of membrane fluidity in thermophilic bacteria *Geobacillus stearothermophilus* and *Meiothermus ruber*, *Extremophiles*. 15 (2011) 423–429. <https://doi.org/10.1007/s00792-011-0373-4>.
- [3] T. Rezanka, L. Siristova, K. Sigler, Rhamnolipid-producing thermophilic bacteria of species *Thermus* and *Meiothermus*, *Extremophiles*. 15 (2011) 697–709. <https://doi.org/10.1007/s00792-011-0400-5>.

Table S2. Characteristic mass differences such as 14, 28 or 162 are listed below (color codes - monoglucosides in red, diglucosides in green, triglucosides in blue, tetraglucosides in purple, and pentaglucosides in black.).

<i>m/z</i>	Relative abundance (%)	Sum of carbons in acyls
1401.8926	0.1	56
1415.9083	1.1	57
1429.9240	4.7	58
1443.9397	6.1	59
1457.9554	24.9	60
1471.9711	28.4	61
1485.9868	32.0	62
1500.0025	86.4	63
1514.0182	97.5	64
1528.0339	89.0	65
1542.0496	89.7	66
1556.0653	100.0	67
1563.9454	0.1	56
1570.0810	63.4	68
1577.9611	0.5	57
1584.0967	39.1	69
1591.9768	2.2	58
1598.1124	41.4	70
1605.9925	2.8	59
1612.1281	29.2	71
1620.0082	11.4	60
1626.1438	10.9	72
1634.0239	13.0	61
1640.1595	7.7	73
1648.0396	14.7	62
1654.1752	2.4	74
1662.0553	39.6	63
1668.1909	0.9	75
1676.0710	44.7	64
1682.2066	0.1	76
1690.0867	40.8	65
1704.1024	41.1	66
1718.1181	45.8	67
1725.9982	0.1	56
1732.1338	29.0	68
1740.0139	0.4	57
1746.1495	17.9	69
1754.0296	1.5	58
1760.1652	19.0	70
1768.0453	2.0	59
1774.1809	13.4	71
1782.0610	8.1	60
1788.1966	5.0	72
1796.0767	9.2	61
1802.2123	3.5	73
1810.0924	10.4	62
1816.2280	1.1	74
1824.1081	28.0	63
1830.2437	0.4	75
1838.1238	31.6	64
1844.2594	0.1	76
1852.1395	28.8	65
1866.1552	29.1	66
1880.1709	32.4	67
1888.0510	0.1	56
1894.1866	20.5	68
1902.0667	0.2	57
1908.2023	12.7	69
1916.0824	0.9	58
1922.2180	13.4	70
1930.0981	1.1	59
1936.2337	9.5	71
1944.1138	4.6	60
1950.2494	3.5	72
1958.1295	5.3	61
1964.2651	2.5	73
1972.1452	6.0	62
1978.2808	0.8	74
1986.1609	16.1	63
1992.2965	0.3	75
2000.1766	18.1	64
2006.3122	0.1	76
2014.1923	16.6	65
2028.2080	16.7	66
2042.2237	18.6	67
2050.1039	0.1	56
2056.2394	11.8	68
2064.1196	0.1	57
2070.2551	7.3	69
2078.1353	0.4	58
2084.2708	7.7	70
2092.1510	0.5	59
2098.2865	5.4	71
2106.1667	1.9	60
2112.3022	2.0	72
2120.1824	2.2	61
2126.3179	1.4	73
2134.1981	2.5	62
2140.3336	0.4	74
2148.2138	6.7	63
2154.3493	0.2	75
2162.2295	7.5	64
2168.3650	0.1	76
2176.2452	6.9	65
2190.2609	6.9	66
2204.2766	7.7	67
2218.2923	4.9	68
2232.3080	3.0	69
2246.3237	3.2	70
2260.3394	2.2	71
2274.3551	0.8	72
2288.3708	0.6	73
2302.3865	0.2	74
2316.4022	0.1	75
2330.4179	0.1	76

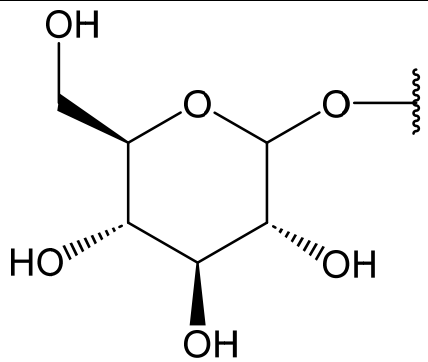
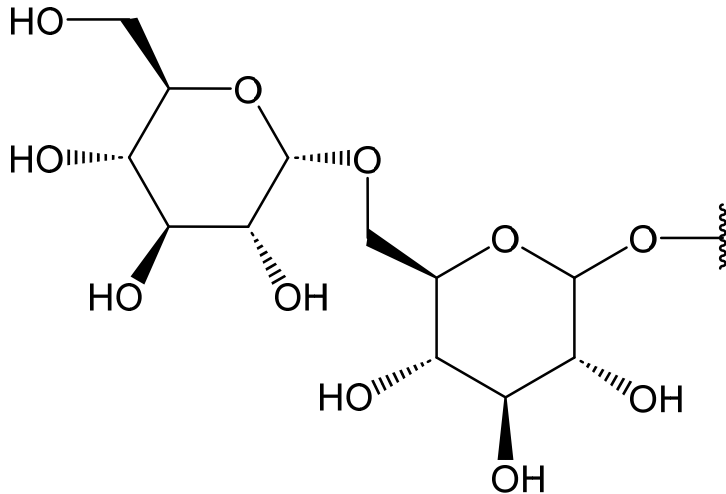
Table S3. The content of molecular species of glucosylated cardiolipins from eight thermophilic bacteria (molar percent).

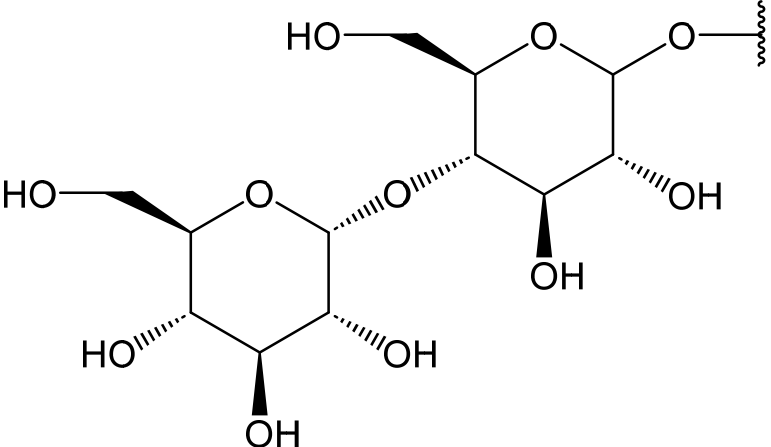
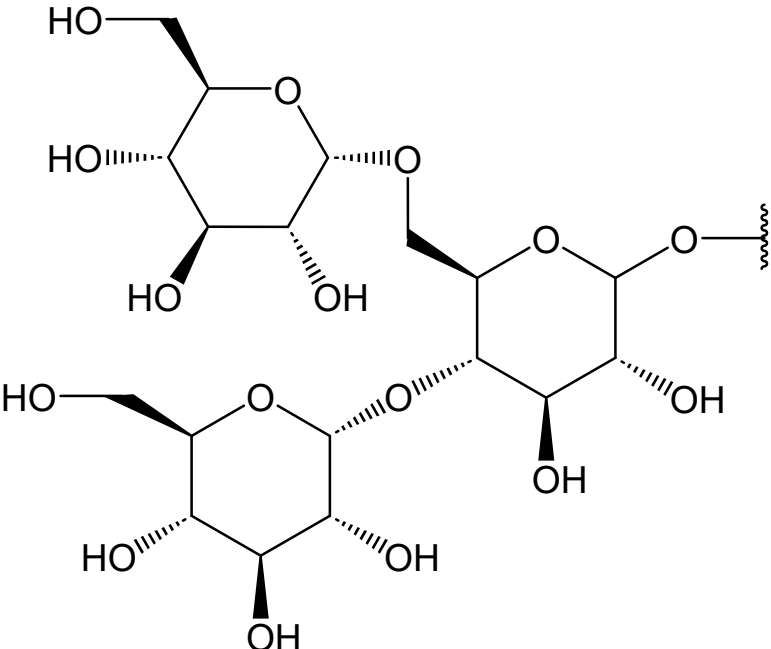
[M-H] ⁻	sum FAs	sum Glc	CCM 4212/55	CCM 4212/65	CCM 4212/70	CCM 3488/65	CCM 3488/70	CCM 2062/55	CCM 2062/70	CCM 5965/70
1401.8926	56	Glc	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01
1415.9083	57	Glc	0.03	0.05	0.07	0.00	0.07	0.07	0.00	0.00
1429.9240	58	Glc	0.19	0.26	0.30	0.01	0.40	0.30	0.00	0.00
1443.9397	59	Glc	0.26	0.32	0.39	0.07	0.46	0.30	0.07	0.07
1457.9554	60	Glc	0.96	1.29	1.61	0.31	1.40	1.81	0.38	0.31
1471.9711	61	Glc	1.28	1.55	1.84	0.40	2.00	1.74	0.32	0.46
1485.9868	62	Glc	1.60	1.68	1.94	1.64	2.18	2.07	1.61	1.44
1500.0025	63	Glc	3.84	4.52	6.40	1.88	5.93	3.70	1.84	2.04
1514.0182	64	Glc	5.50	6.01	7.30	2.11	6.56	5.97	2.07	1.97
1528.0339	65	Glc	6.33	6.07	5.75	5.70	5.85	5.60	5.59	4.75
1542.0496	66	Glc	5.46	5.68	6.91	6.55	5.81	5.67	6.31	6.24
1556.0653	67	Glc	4.74	6.49	5.95	5.88	6.98	6.48	5.76	5.25
1570.0810	68	Glc	6.36	5.04	4.10	5.08	4.27	3.94	6.04	6.44
1584.0967	69	Glc	3.25	2.84	2.16	6.60	2.73	2.53	6.47	6.57
1598.1124	70	Glc	3.45	3.04	2.47	4.19	2.93	2.68	4.10	3.68
1612.1281	71	Glc	2.32	2.13	1.71	2.58	2.07	1.89	2.53	2.96
1626.1438	72	Glc	1.11	0.90	0.59	2.73	0.85	0.71	2.68	3.55
1640.1595	73	Glc	0.70	0.58	0.50	1.93	0.62	0.39	1.89	1.58
1654.1752	74	Glc	0.45	0.32	0.16	0.72	0.23	0.16	0.71	0.87
1668.1909	75	Glc	0.19	0.13	0.06	0.51	0.06	0.06	0.44	0.51
1682.2066	76	Glc	0.13	0.06	0.00	0.16	0.00	0.00	0.16	0.16
1563.9454	56	(Glc) ₂	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
1577.9611	57	(Glc) ₂	0.03	0.03	0.03	0.00	0.03	0.03	0.02	0.00
1591.9768	58	(Glc) ₂	0.04	0.05	0.14	0.00	0.13	0.22	0.03	0.01
1605.9925	59	(Glc) ₂	0.06	0.06	0.18	0.03	0.15	0.26	0.03	0.02
1620.0082	60	(Glc) ₂	0.06	0.14	0.74	0.15	0.54	0.81	0.03	0.03
1634.0239	61	(Glc) ₂	0.08	0.18	0.84	0.18	0.69	0.91	0.14	0.14
1648.0396	62	(Glc) ₂	0.14	0.74	0.95	0.75	0.83	1.00	0.18	0.18
1662.0553	63	(Glc) ₂	0.18	0.84	2.56	0.86	2.40	2.61	0.74	0.75
1676.0710	64	(Glc) ₂	0.73	0.95	2.89	0.97	2.70	3.00	0.84	0.85
1690.0867	65	(Glc) ₂	0.83	2.56	2.64	2.61	2.40	2.75	0.95	0.72
1704.1024	66	(Glc) ₂	0.94	2.89	2.66	2.95	2.48	2.72	2.56	2.89
1718.1181	67	(Glc) ₂	2.53	2.63	2.96	2.69	2.76	3.07	2.89	2.43

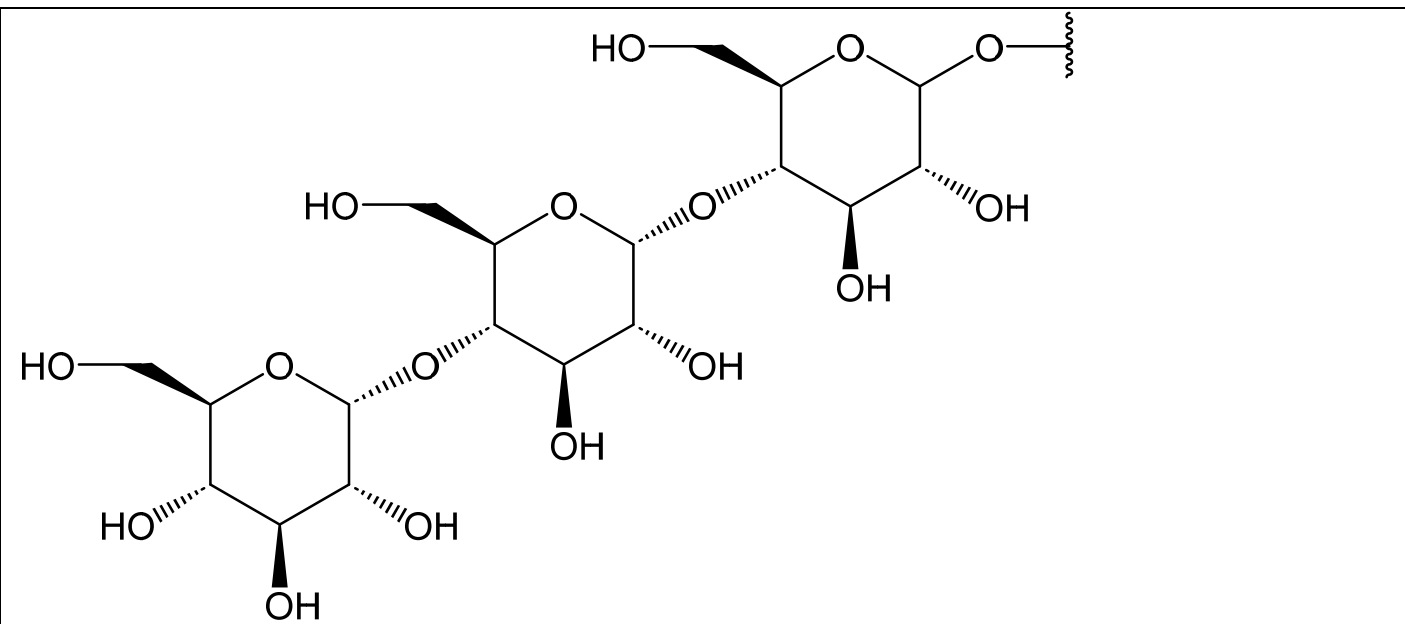
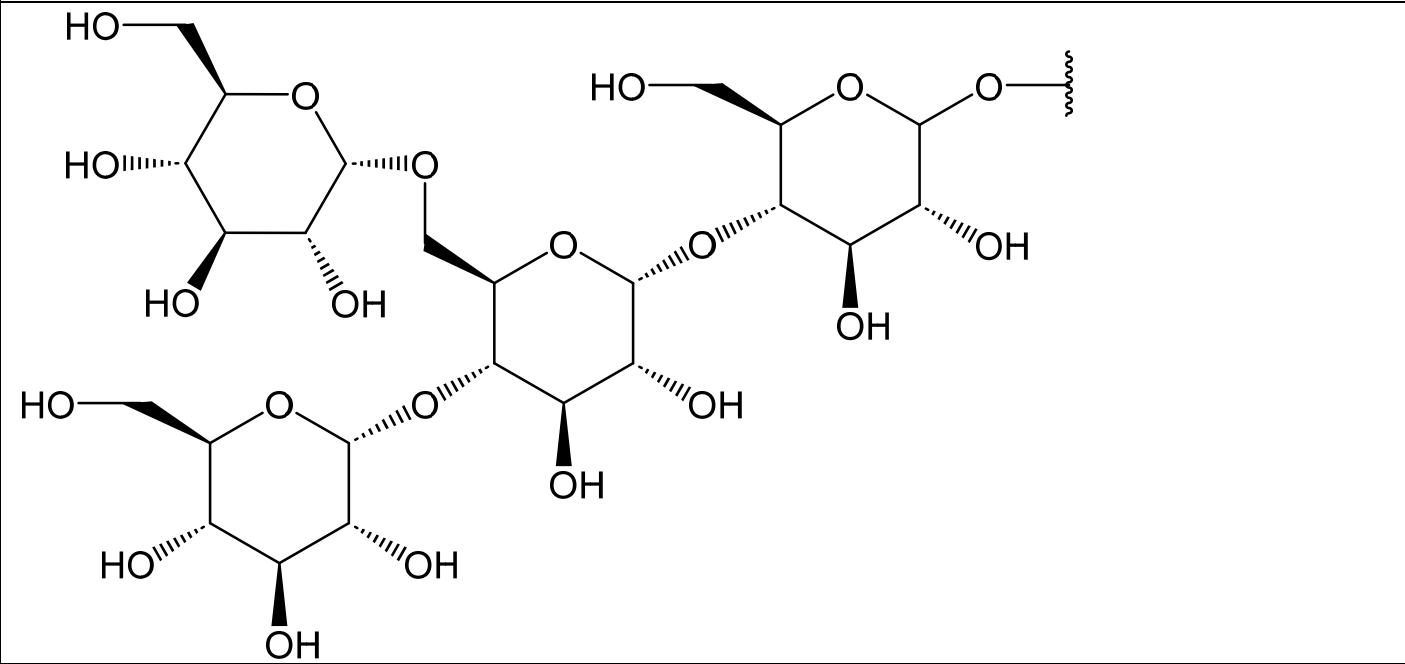
1732.1338	68	(Glc) ₂	2.86	2.65	1.87	2.71	1.70	1.99	2.64	2.91
1746.1495	69	(Glc) ₂	2.61	2.96	1.16	3.02	1.01	1.23	2.66	2.30
1760.1652	70	(Glc) ₂	2.63	1.87	1.23	1.91	1.12	1.32	2.96	3.61
1774.1809	71	(Glc) ₂	2.93	1.16	0.87	1.18	0.77	0.96	1.88	1.44
1788.1966	72	(Glc) ₂	1.86	1.23	0.32	1.25	0.26	0.42	1.16	0.99
1802.2123	73	(Glc) ₂	1.15	0.87	0.23	0.88	0.16	0.29	1.23	1.18
1816.2280	74	(Glc) ₂	1.22	0.32	0.07	0.33	0.07	0.07	0.87	0.72
1830.2437	75	(Glc) ₂	0.86	0.23	0.03	0.23	0.03	0.03	0.32	0.33
1844.2594	76	(Glc) ₂	0.32	0.07	0.00	0.07	0.00	0.00	0.23	0.23
1725.9982	56	(Glc) ₃	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
1740.0139	57	(Glc) ₃	0.03	0.03	0.03	0.00	0.03	0.03	0.03	0.03
1754.0296	58	(Glc) ₃	0.07	0.03	0.10	0.00	0.10	0.12	0.03	0.02
1768.0453	59	(Glc) ₃	0.13	0.03	0.11	0.02	0.13	0.17	0.05	0.03
1782.0610	60	(Glc) ₃	0.14	0.05	0.39	0.03	0.52	0.57	0.06	0.05
1796.0767	61	(Glc) ₃	0.15	0.10	0.53	0.10	0.60	0.65	0.10	0.10
1810.0924	62	(Glc) ₃	0.19	0.13	0.61	0.11	0.67	0.70	0.13	0.18
1824.1081	63	(Glc) ₃	0.26	0.52	1.66	0.43	1.81	1.88	0.52	0.53
1838.1238	64	(Glc) ₃	0.52	0.59	1.89	0.61	2.05	2.11	0.60	0.60
1852.1395	65	(Glc) ₃	0.59	0.67	1.79	0.69	1.87	1.98	0.67	0.59
1866.1552	66	(Glc) ₃	0.67	1.81	1.88	1.85	1.94	1.88	1.70	2.04
1880.1709	67	(Glc) ₃	1.79	2.04	2.09	2.09	2.19	1.93	2.04	2.30
1894.1866	68	(Glc) ₃	2.02	1.86	1.32	1.90	1.37	1.42	1.86	1.64
1908.2023	69	(Glc) ₃	1.84	1.88	0.71	1.92	0.82	0.89	1.88	2.17
1922.2180	70	(Glc) ₃	1.86	2.09	0.71	2.14	0.87	0.95	2.10	1.71
1936.2337	71	(Glc) ₃	2.07	1.32	0.57	1.35	0.62	0.70	1.33	1.25
1950.2494	72	(Glc) ₃	1.31	0.82	0.19	0.84	0.23	0.28	0.82	0.99
1964.2651	73	(Glc) ₃	0.81	0.87	0.10	0.88	0.16	0.20	0.87	1.18
1978.2808	74	(Glc) ₃	0.86	0.61	0.05	0.63	0.05	0.05	0.61	0.62
1992.2965	75	(Glc) ₃	0.61	0.19	0.02	0.23	0.02	0.02	0.19	0.29
2006.3122	76	(Glc) ₃	0.22	0.11	0.00	0.17	0.00	0.00	0.16	0.21
1888.0510	56	(Glc) ₄	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
1902.0667	57	(Glc) ₄	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00
1916.0824	58	(Glc) ₄	0.06	0.02	0.06	0.01	0.04	0.09	0.01	0.01
1930.0981	59	(Glc) ₄	0.10	0.03	0.07	0.02	0.06	0.09	0.01	0.01
1944.1138	60	(Glc) ₄	0.06	0.03	0.30	0.03	0.24	0.34	0.06	0.06
1958.1295	61	(Glc) ₄	0.09	0.04	0.34	0.06	0.29	0.39	0.07	0.07

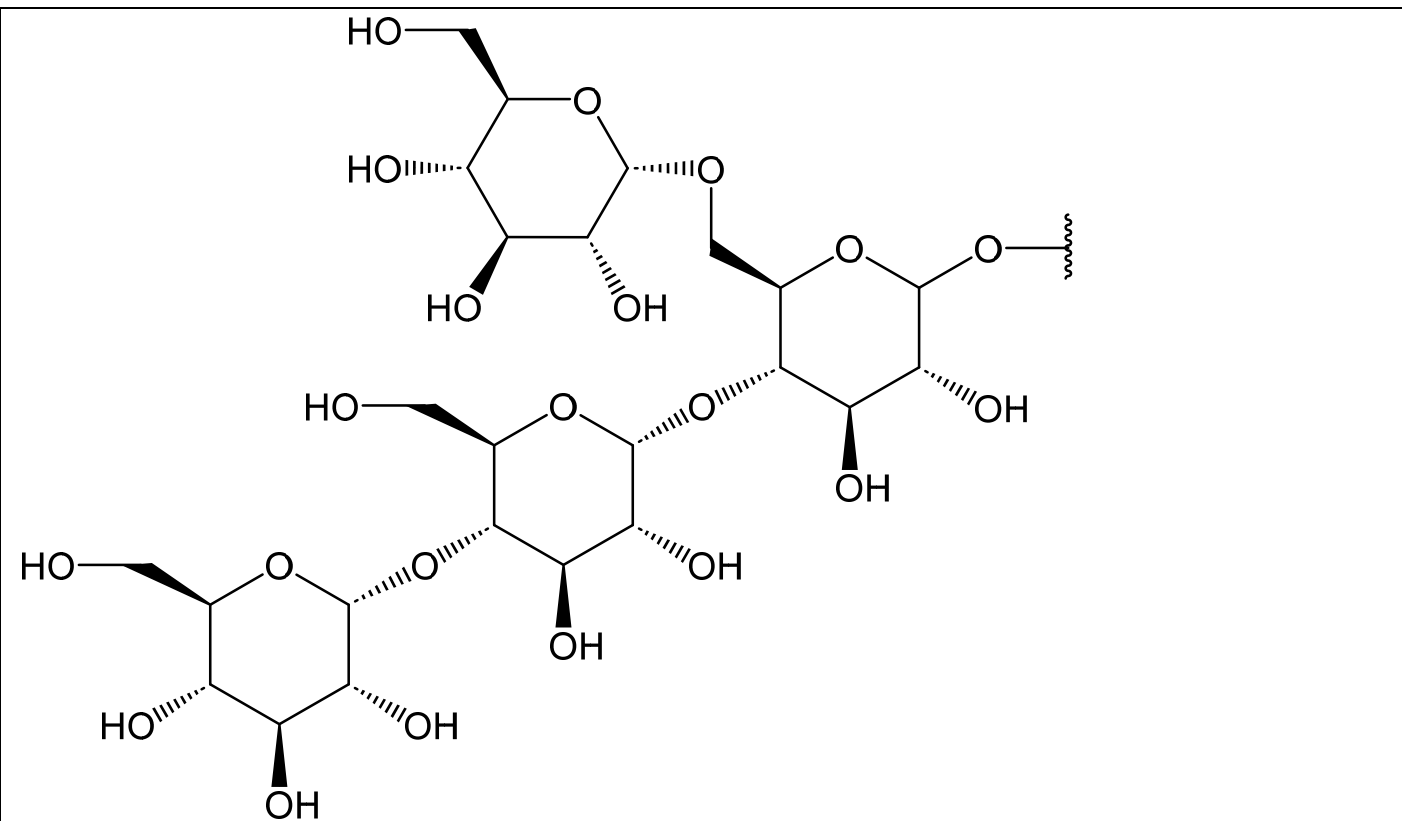
1972.1452	62	(Glc) ₄	0.10	0.06	0.39	0.07	0.32	0.42	0.30	0.32
1986.1609	63	(Glc) ₄	0.12	0.07	1.04	0.30	0.95	1.10	0.34	0.35
2000.1766	64	(Glc) ₄	0.17	0.30	1.17	0.35	1.08	1.22	0.39	0.47
2014.1923	65	(Glc) ₄	0.29	0.34	1.07	0.40	1.00	1.14	1.04	0.92
2028.2080	66	(Glc) ₄	0.34	0.39	1.08	1.06	0.98	1.12	1.17	1.25
2042.2237	67	(Glc) ₄	0.38	1.04	1.20	1.20	1.10	1.30	1.07	0.99
2056.2394	68	(Glc) ₄	1.03	1.17	0.76	1.10	0.66	0.83	1.08	1.12
2070.2551	69	(Glc) ₄	1.16	1.07	0.47	0.86	0.40	0.56	1.20	0.99
2084.2708	70	(Glc) ₄	1.06	1.08	0.50	0.73	0.46	0.56	0.76	0.59
2098.2865	71	(Glc) ₄	1.07	1.20	0.35	0.59	0.28	0.44	0.47	0.48
2112.3022	72	(Glc) ₄	1.19	0.76	0.13	0.48	0.10	0.17	0.50	0.51
2126.3179	73	(Glc) ₄	0.75	0.47	0.09	0.51	0.06	0.09	0.35	0.40
2140.3336	74	(Glc) ₄	0.47	0.50	0.03	0.36	0.03	0.03	0.10	0.13
2154.3493	75	(Glc) ₄	0.49	0.35	0.01	0.13	0.01	0.01	0.09	0.13
2168.3650	76	(Glc) ₄	0.35	0.13	0.00	0.09	0.00	0.00	0.03	0.03
2050.1039	56	(Glc) ₅	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
2064.1196	57	(Glc) ₅	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01
2078.1353	58	(Glc) ₅	0.03	0.02	0.03	0.00	0.04	0.03	0.03	0.03
2092.1510	59	(Glc) ₅	0.03	0.04	0.03	0.00	0.04	0.03	0.02	0.03
2106.1667	60	(Glc) ₅	0.12	0.03	0.10	0.00	0.15	0.12	0.04	0.04
2120.1824	61	(Glc) ₅	0.14	0.03	0.12	0.01	0.17	0.14	0.05	0.05
2134.1981	62	(Glc) ₅	0.14	0.03	0.14	0.03	0.18	0.16	0.05	0.07
2148.2138	63	(Glc) ₅	0.14	0.03	0.40	0.03	0.46	0.43	0.06	0.09
2162.2295	64	(Glc) ₅	0.15	0.03	0.43	0.13	0.49	0.48	0.12	0.13
2176.2452	65	(Glc) ₅	0.15	0.12	0.45	0.15	0.49	0.46	0.09	0.13
2190.2609	66	(Glc) ₅	0.15	0.14	0.45	0.17	0.50	0.47	0.17	0.14
2204.2763	67	(Glc) ₅	0.15	0.16	0.47	0.44	0.53	0.50	0.43	0.46
2218.2923	68	(Glc) ₅	0.16	0.43	0.30	0.50	0.35	0.32	0.49	0.43
2232.3080	69	(Glc) ₅	0.43	0.48	0.17	0.46	0.23	0.19	0.45	0.48
2246.3237	70	(Glc) ₅	0.48	0.45	0.19	0.46	0.25	0.21	0.45	0.41
2260.3394	71	(Glc) ₅	0.45	0.45	0.11	0.51	0.18	0.14	0.50	0.53
2274.3551	72	(Glc) ₅	0.44	0.48	0.04	0.33	0.09	0.05	0.32	0.36
2288.3708	73	(Glc) ₅	0.49	0.32	0.03	0.20	0.07	0.04	0.19	0.22
2302.3865	74	(Glc) ₅	0.31	0.19	0.01	0.23	0.01	0.01	0.21	0.24
2316.4022	75	(Glc) ₅	0.19	0.21	0.01	0.15	0.01	0.01	0.14	0.16
2330.4179	76	(Glc) ₅	0.20	0.14	0.00	0.05	0.00	0.00	0.05	0.05

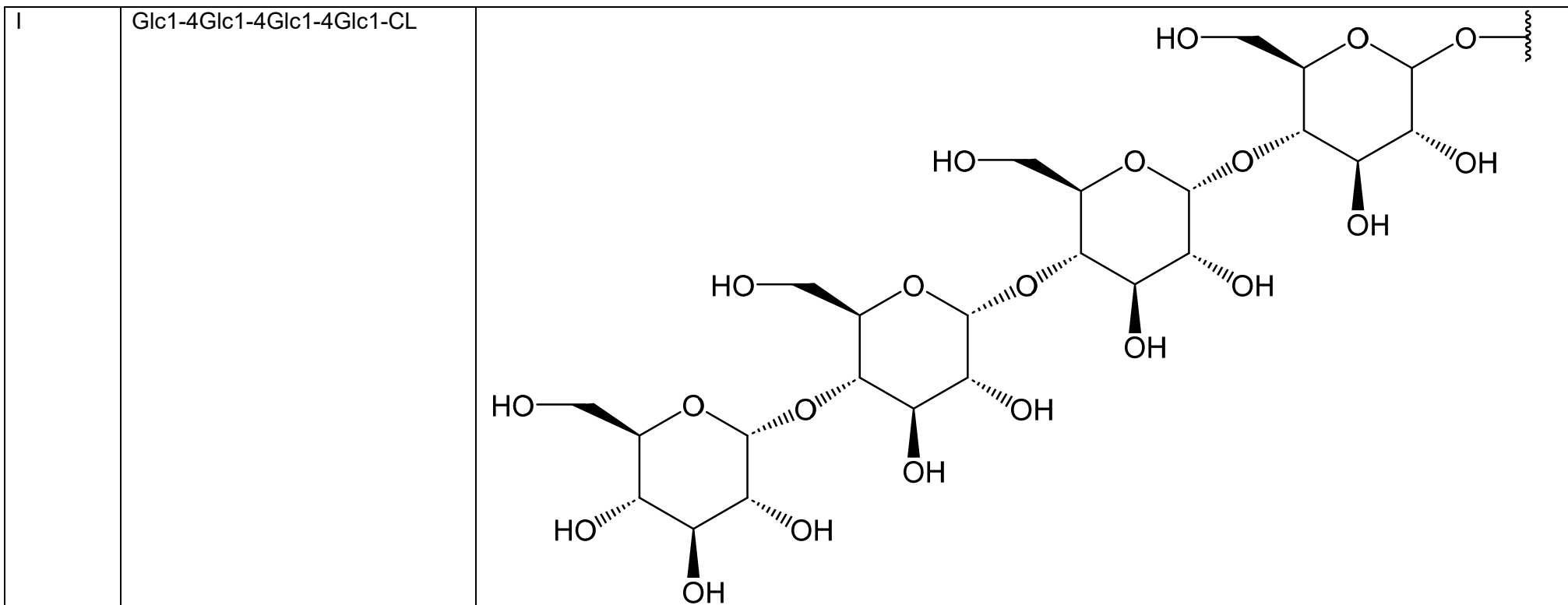
Table S4. Structure o peaks from HILIC.

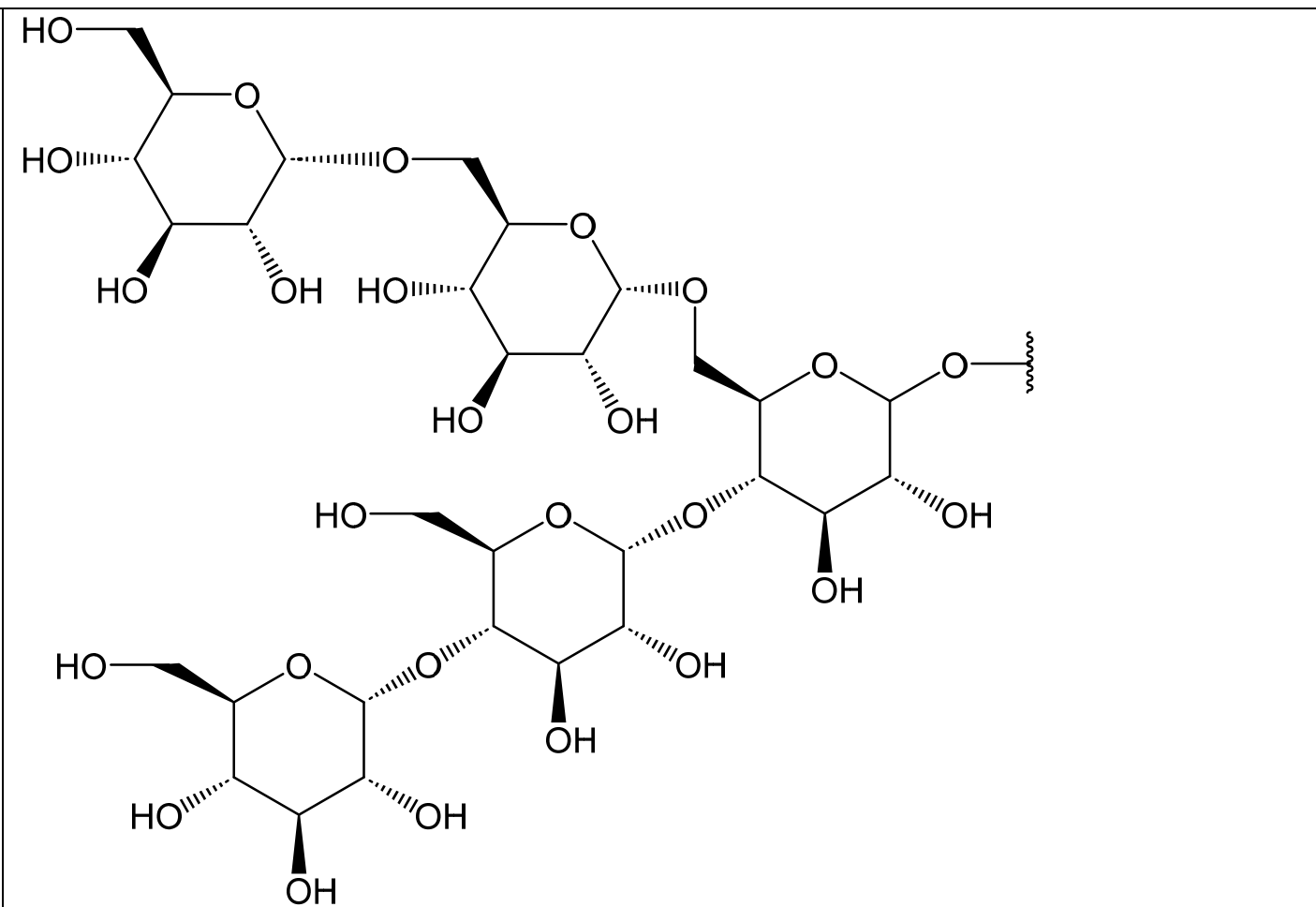
Peak No.	Abbreviation	Structure
B	Glc-CL	
C	Glc-1-6Glc-1-CL	

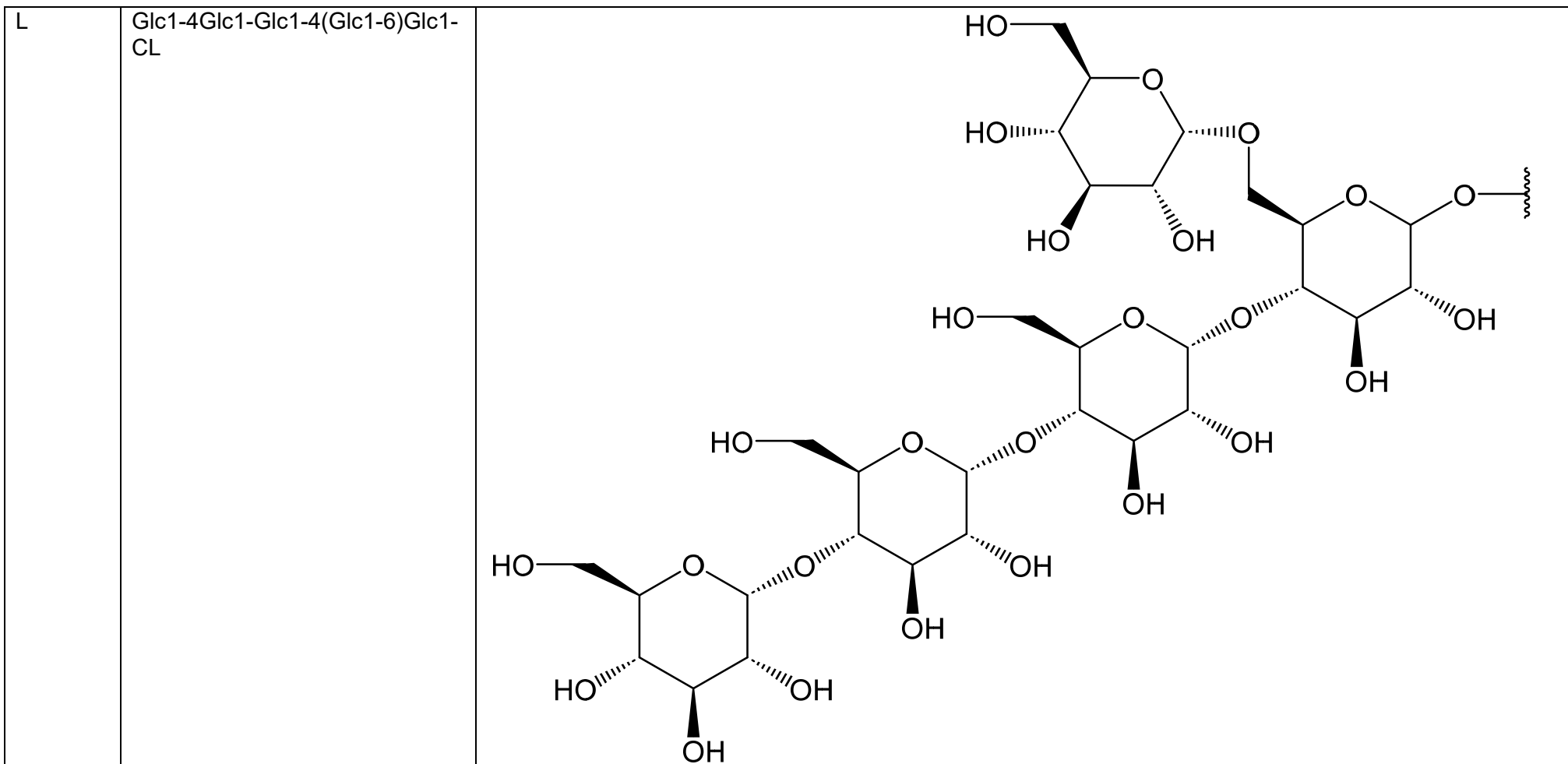
D	Glc-1-4-Glc-1-CL	 The diagram shows two glucose molecules in their cyclic Haworth projection. The left glucose is linked to the right glucose via a 1-4 glycosidic bond. The bond is formed between the C1 of the left glucose (pointing down) and the C4 of the right glucose (pointing up). The right glucose has a wavy line at its C1 position, indicating a linkage to a lipid carrier. Stereochemistry is indicated with wedges and dashes: C2-OH is up, C3-OH is down, and C6-CH₂-OH is up for both glucose units.
E	Glc1-4(Glc1-6)Glc1-CL	 The diagram shows a branched disaccharide structure. A central glucose molecule is linked to two other glucose molecules. The central glucose is linked to the top-right glucose via a 1-4 glycosidic bond (C1 of central to C4 of top-right). It is also linked to the bottom-right glucose via a 1-6 glycosidic bond (C1 of central to C6 of bottom-right). The top-right and bottom-right glucose molecules are linked to lipid carriers via wavy lines at their C1 positions. Stereochemistry is indicated with wedges and dashes: C2-OH is up, C3-OH is down, and C6-CH₂-OH is up for all glucose units.

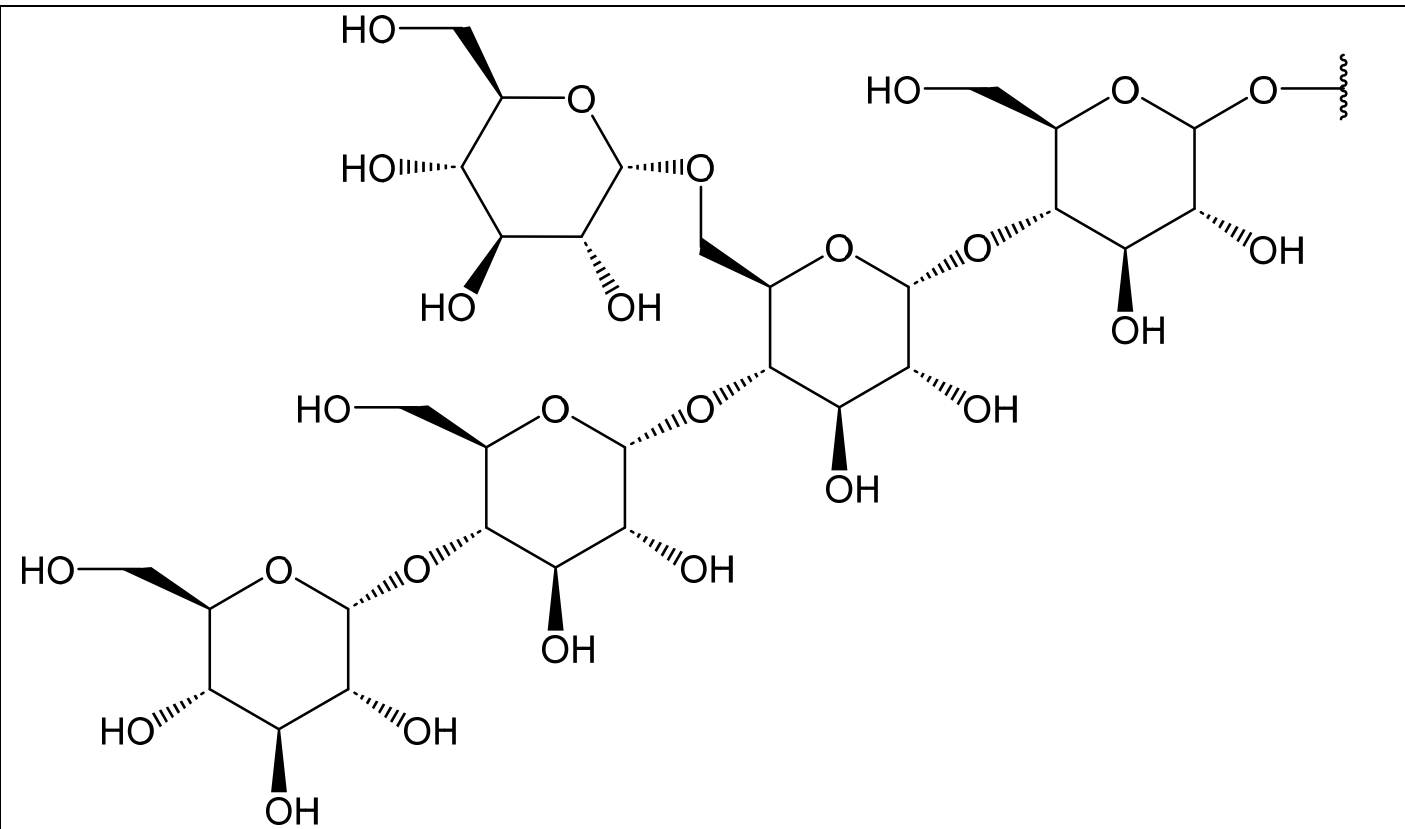
F	Glc1-4Glc1-4Glc1-CL	
G	Glc1-4(Glc1-6)Glc1-4Glc1-CL	

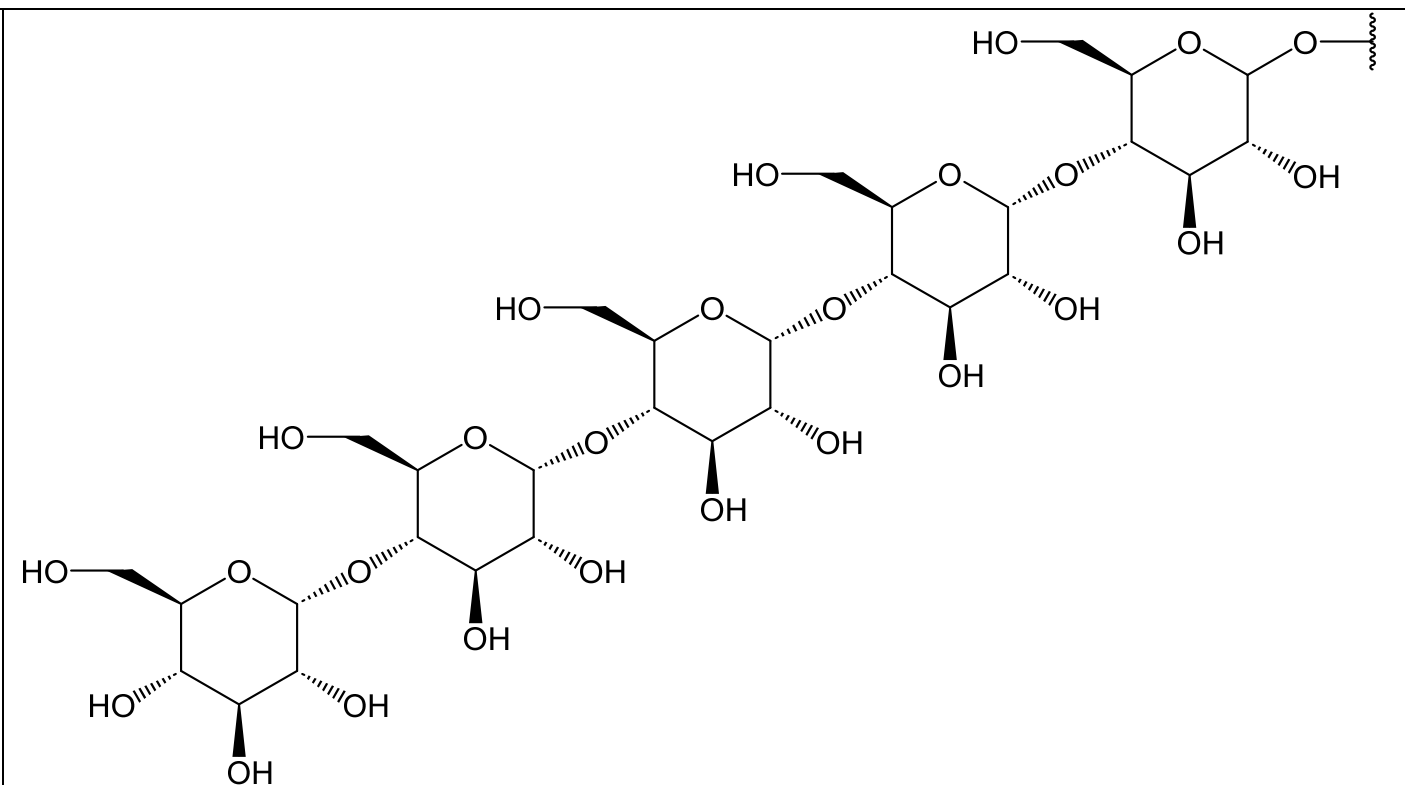
H	Glc1-4Glc1-4(Glc1-6)Glc1-CL	
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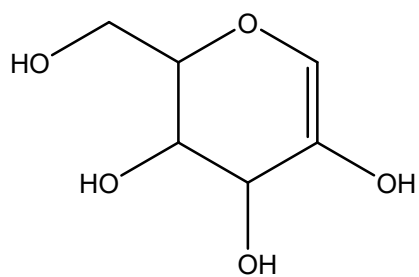


K	Glc1-4Glc1-4(Glc1-6Glc1-6)Glc1-CL	
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M	Glc1-4Glc1-4(Glc1-6)Glc1-4Glc1-CL	 The chemical structure represents a branched oligosaccharide. It consists of a central glucose unit (Glc1) linked to four other glucose units. One branch is a 1-6 linked glucose unit, and the other three are 1-4 linked glucose units. The terminal glucose units are linked to a common point, indicated by a wavy line.
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N	Glc1-4Glc1-4Glc1-4Glc1-CL	 The image shows a chemical structure of a branched oligosaccharide. It consists of four glucose units in their cyclic pyranose form, linked by 1-4 glycosidic bonds. The units are arranged in a chain, with a fourth unit branching off the third unit at its C4 position. Each glucose unit has its hydroxyl groups explicitly shown with stereochemistry: C2-OH is up, C3-OH is down, and C6-OH is up. The terminal unit on the right is linked to a chlorine atom (CL) via an oxygen atom at its C1 position. The entire structure is drawn in a zig-zag fashion from bottom-left to top-right.
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Chemical Formula: C₆H₁₀O₅

Exact Mass: 162.0528

Fig. S1. The assumed structure of the neutral fragment with mass 162.0528 Da.