Electronic Supplementary Material (ESI) for Chemical Science.

Top-Down Strategies for the Structural Elucidation of Intact Gram-negative Bacterial Endotoxins

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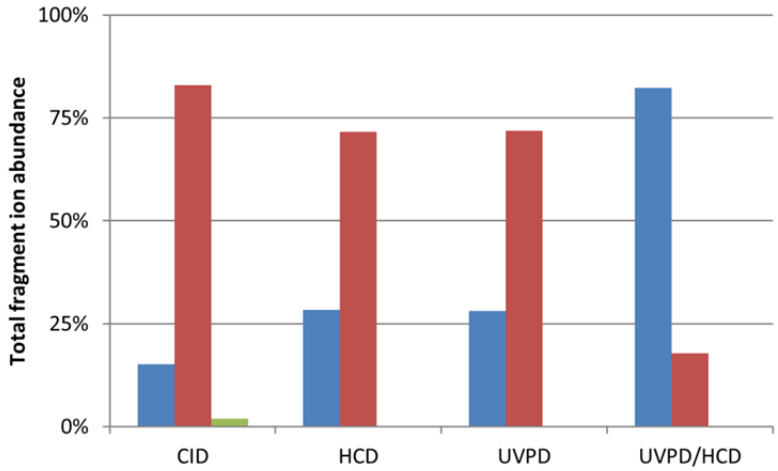

Figure S1. Histograms showing the distribution of multiply charged *E. coli* Kdo_2-Lipid A fragment ions and singly changed fragment ions. Singly charged fragments are shown as blue bars, doubly charged fragments as red bars and triply charged fragments are shown as green bars.

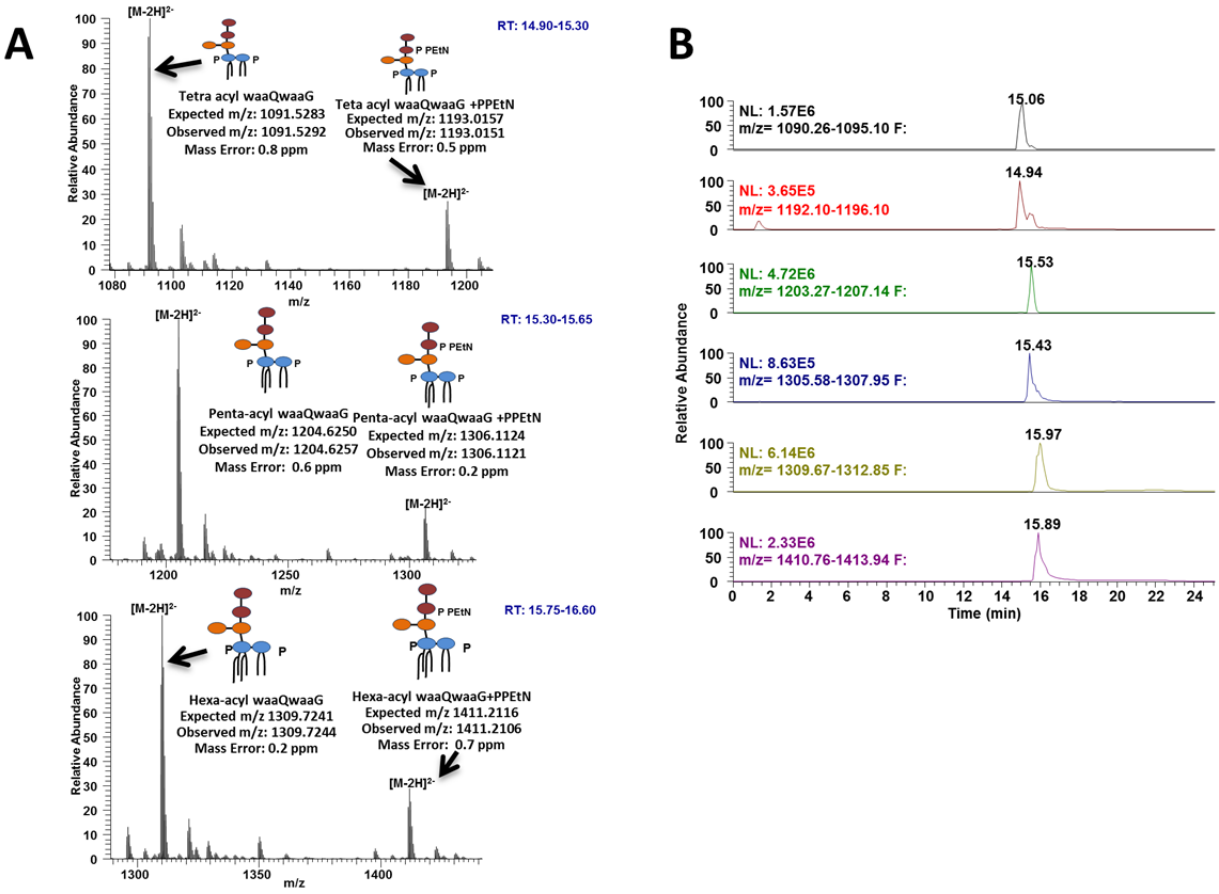

Figure S2. A) ESI-mass spectra of LOS separated within the BN1 ΔwaaQwaaG sample and B) the extracted ion chromatograms of the key LOS identified.

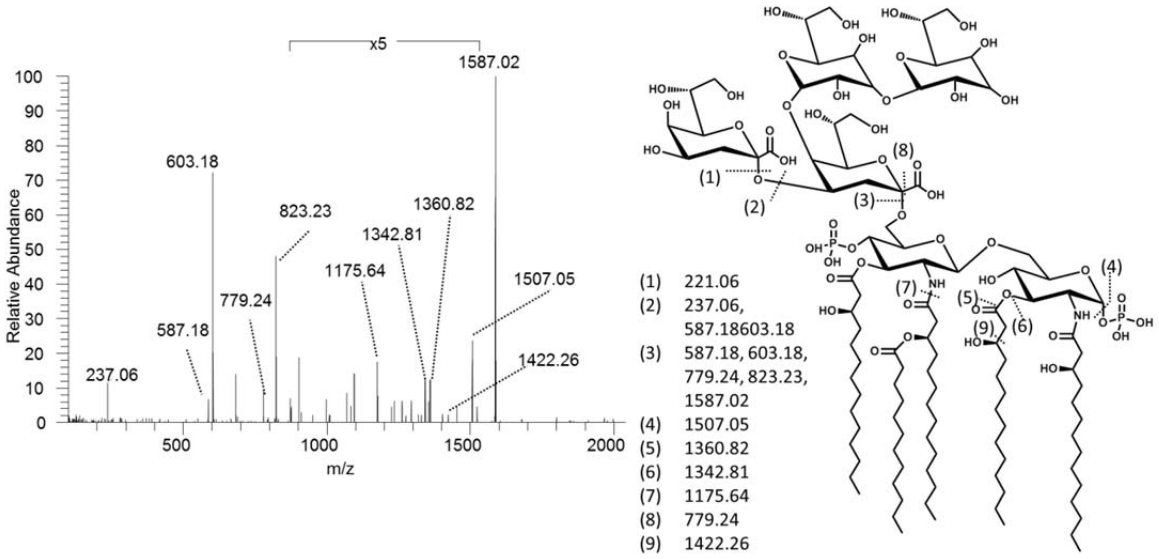

Figure S3. UVPD/HCD mass spectrum of penta-acyl waaQwaaG (z = 3-) [Mr = 2411.27] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

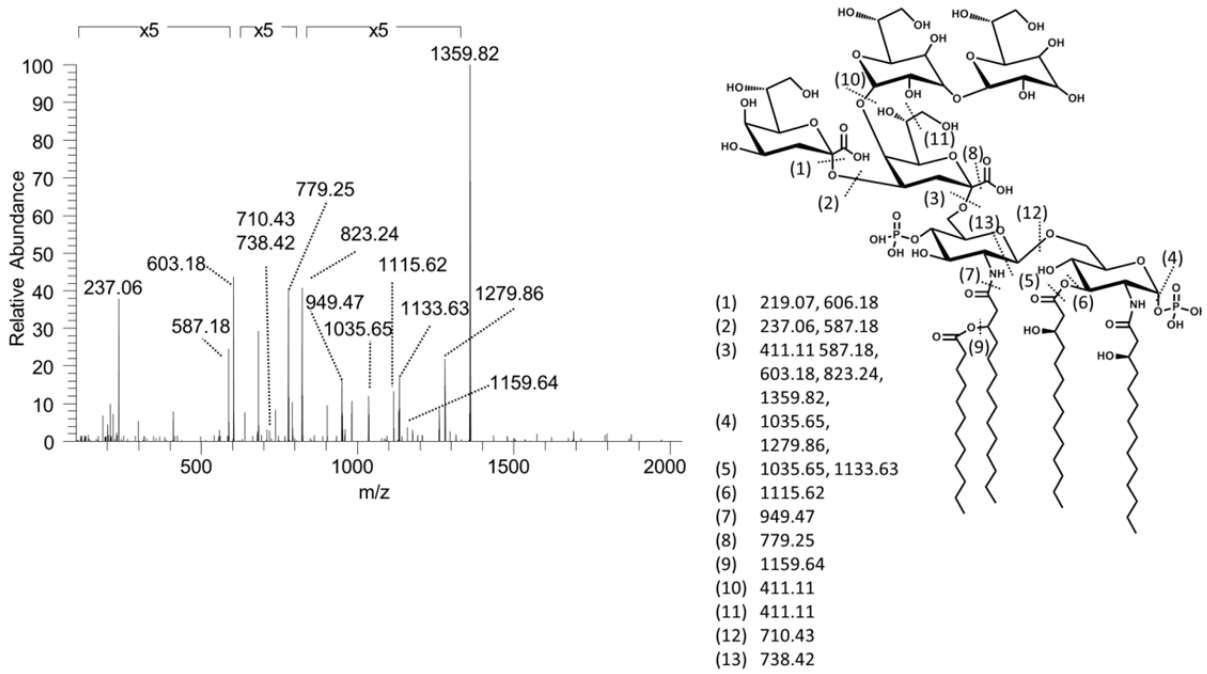

Figure S4. UVPD/HCD mass spectrum of tetra-acyl waaQwaaG (z = 3-) [Mr = 2185.07] BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

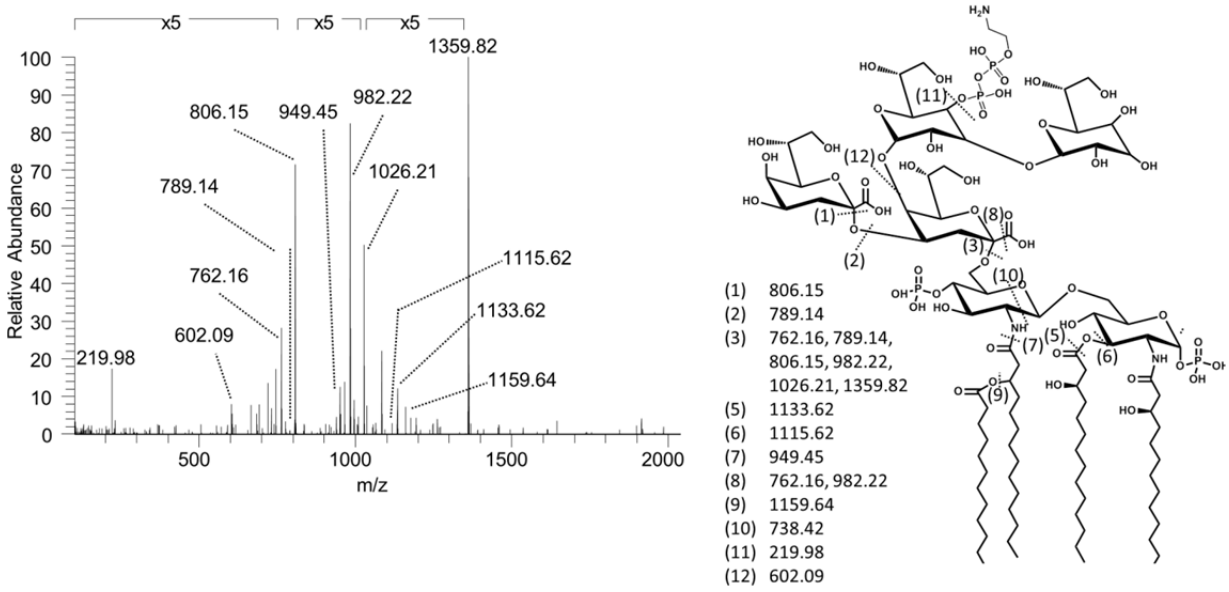

Figure S5. UVPD/HCD mass spectrum of tetra-acyl waaQwaaG + PPEtN (z = 3-) [Mr = 2388.06] BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

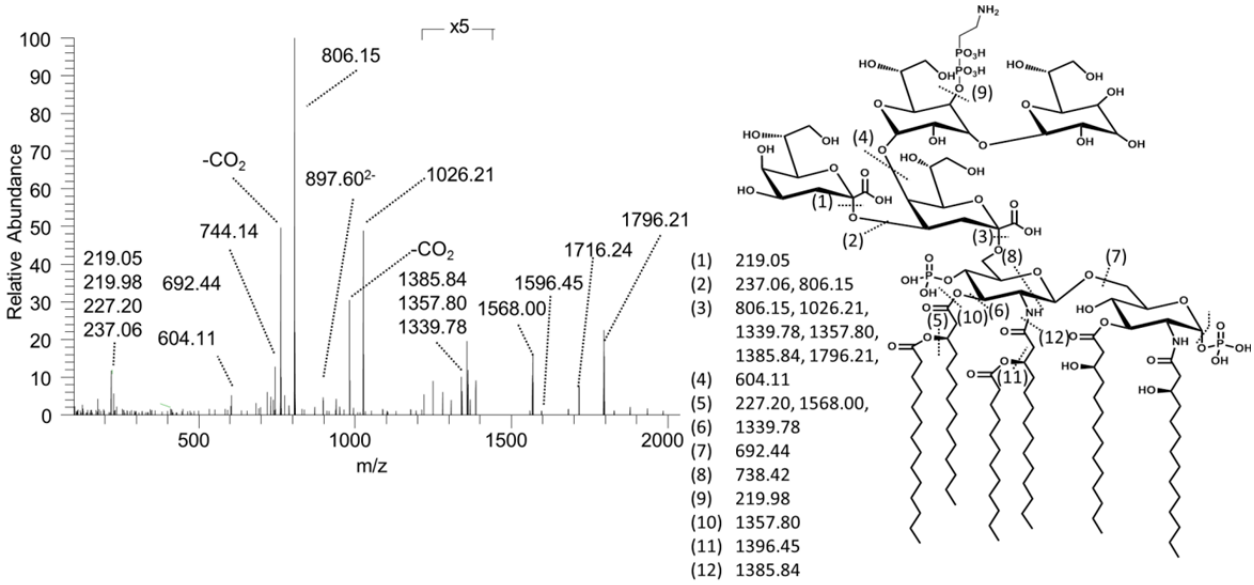

Figure S6. UVPD/HCD mass spectrum of waaQwaaG + PPEtN (z = 3-) [Mr = 2824.44] BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

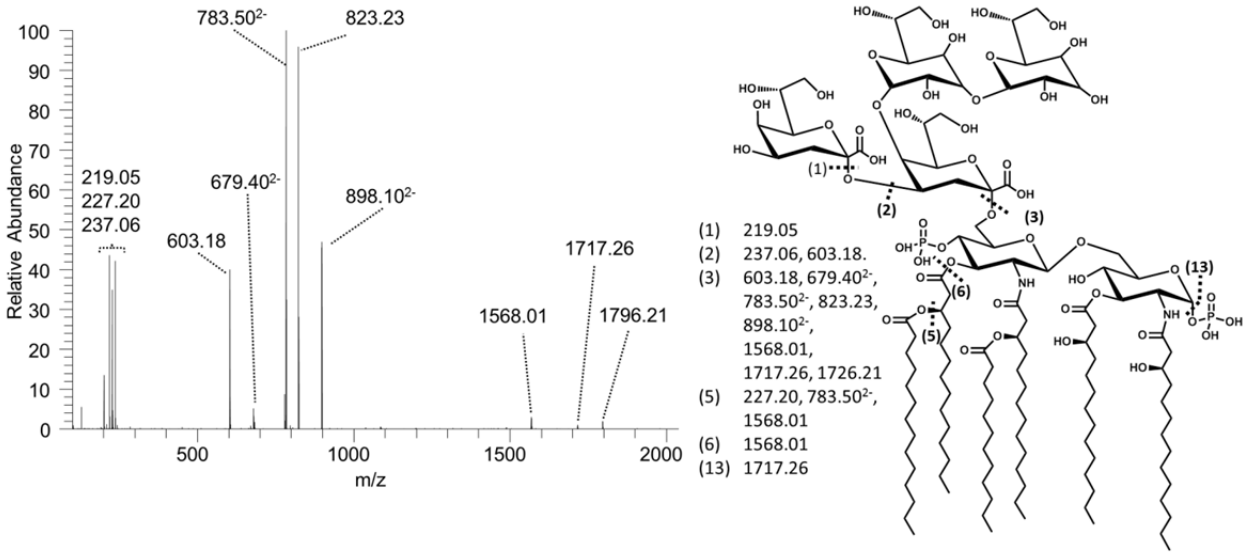

Figure S7. HCD mass spectrum of hexa-acylated waaQwaaG (z = 3-) [Mr = 2621.46] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

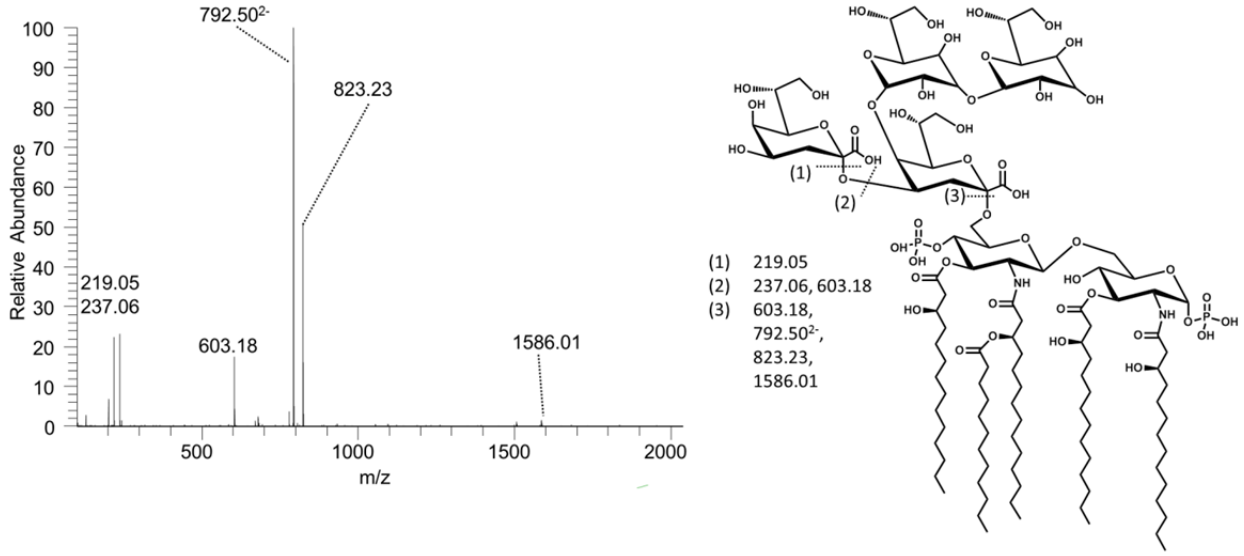

Figure S8. HCD mass spectrum of penta-acyl waaQwaaG (z = 3-) [Mr = 2411.27] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

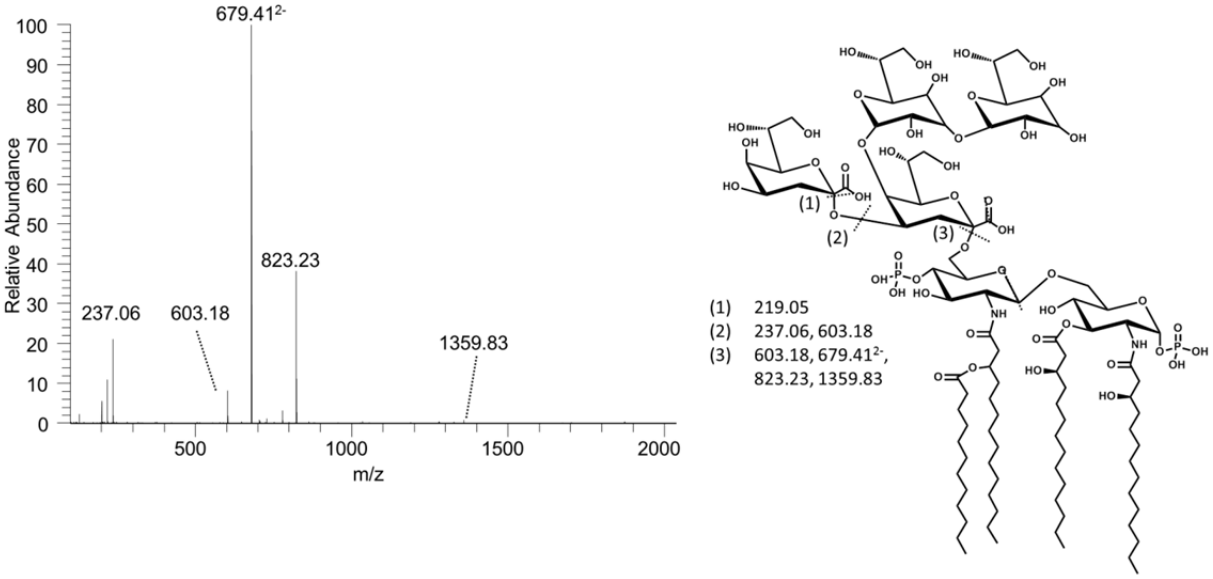

Figure S9. HCD mass spectrum of tetra-acyl waaQwaaG (z = 3-) [Mr = 2185.07] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

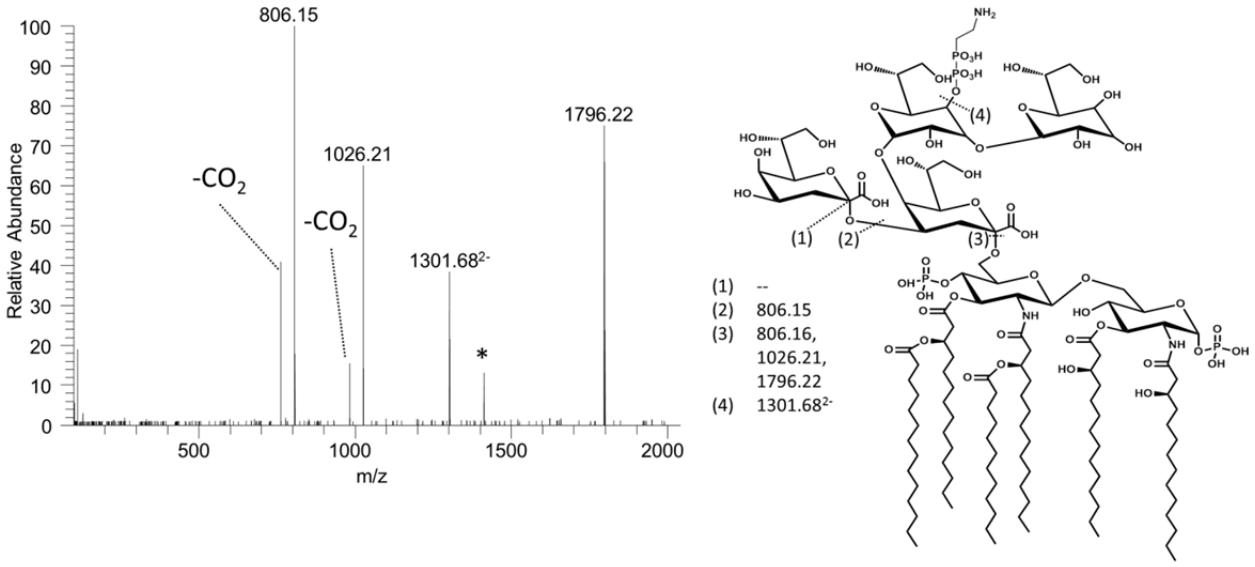

Figure S10. HCD mass spectrum of waaQwaaG + PPEtN (z = 2-) [Mr = 2824.44] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

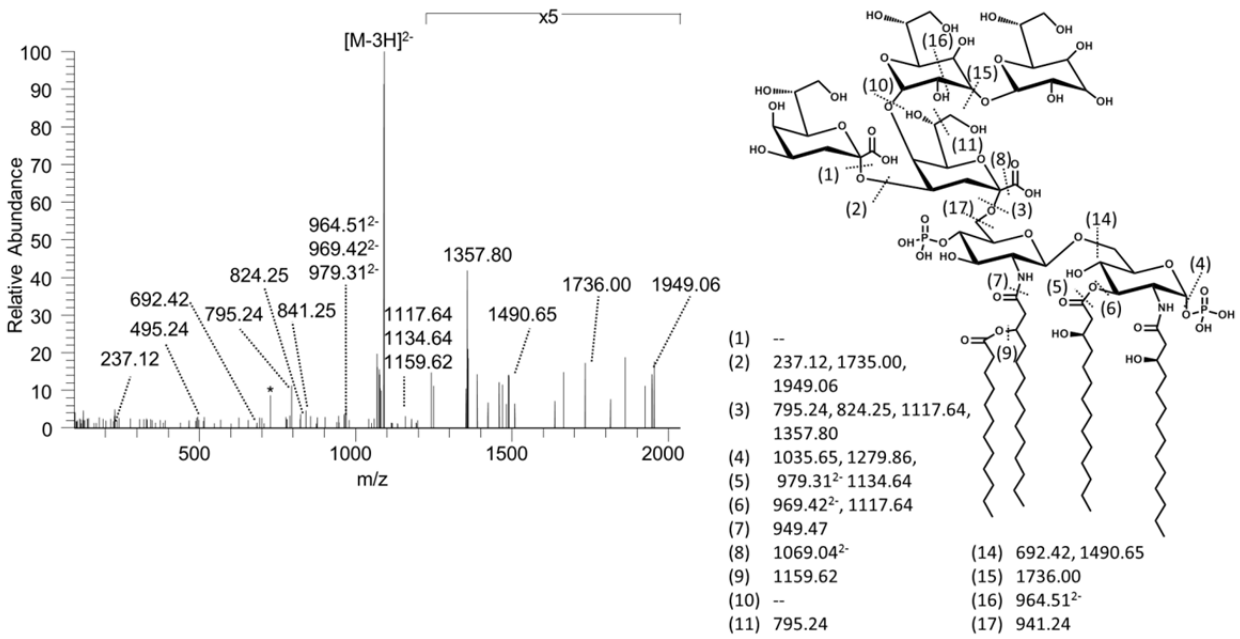

Figure S11. UVPD mass spectrum of tetra-acyl waaQwaaG (z = 3-) [Mr = 2185.07] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

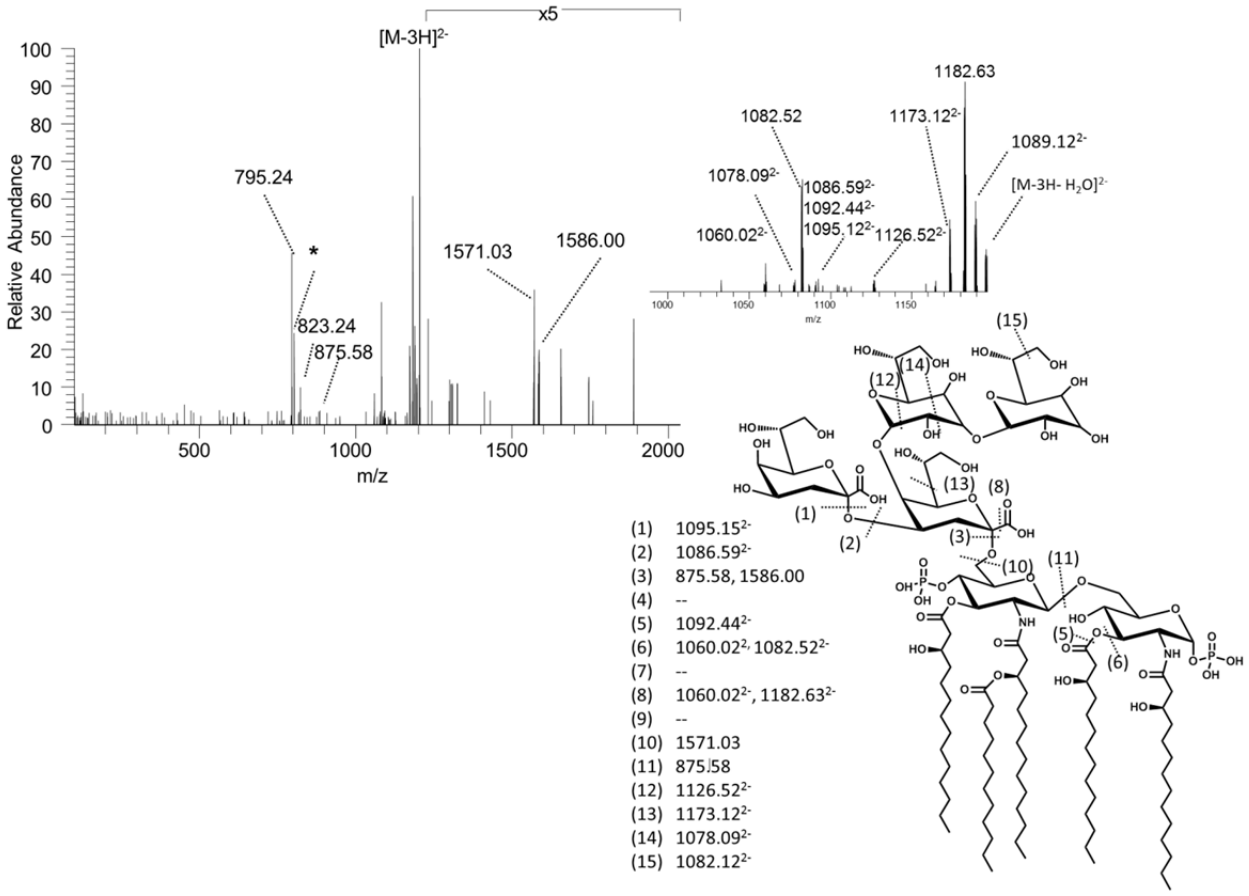

Figure S12. UVPD mass spectrum of penta-acyl waaQwaaG (z =3-) [Mr = 2411.27] from BN1 ΔwaaQwaaG. The corresponding fragmentation map is shown on the right.

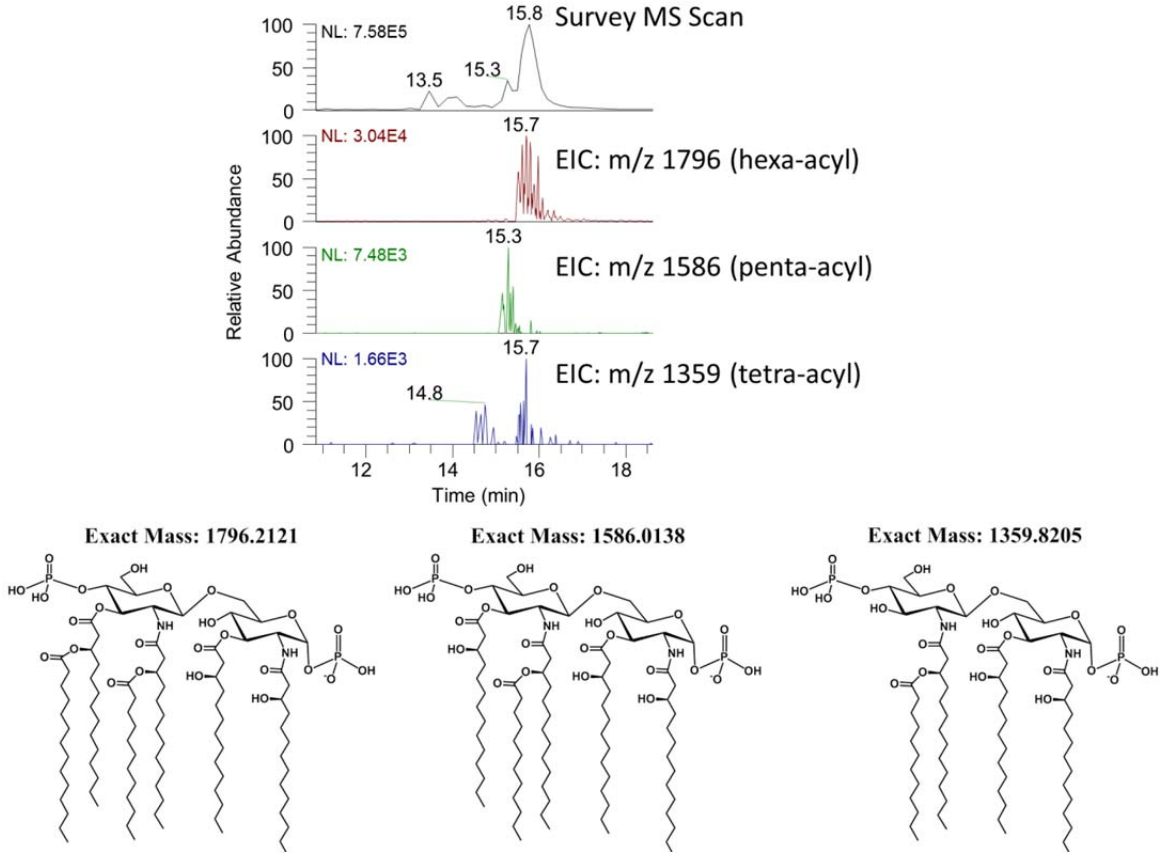

Figure S13: LC-MS trace and extracted ion chromatograms of the UVPD/HCD fragment ions associated with the identified BN1 LOS lipid A anchors . The structures of the different fragment ions are shown below.

Fragment Ion (m/z)	Cleavage Site(s)
219.05 (B_1)	1
227.20	5
237.06 (C_1)	2
395.12	4, 20
411.11	3, 20
439.11 (B_2)	3
692.41 (Z_1),	19
710.42 (Y_1)	18
738.42 ($^{1,5}X_1$)	17
890.46^{2-}	8
897.60^{2-} (Y_2),	3
898.96^{2-}	7
913.48^{2-}	12
926.97^{2-}	11
934.48^{2-}	10
972.55^{2-},	14, 22
995.56^{2-}	14
982.06^{2-}	13, 22
999.63^{2-} (Z_3)	2
1004.56^{2-}	5
1007.63^{2-} (Y_3)	1
1018.07^{2-}	9
1026.07^{2-}	16
1040.07^{2-}	15
1043.14^{2-} ($^{0,2}X_3$)	23
1056.15^{2-} ($^{0,3}X_3$)	24
1066.14^{2-} ($^{4,5}X_3$)	25
1086.66^{2-}	20
1095.66^{2-}	22
1105.15^{2-}	26
1243.82	3, 6, 8
1324.83	4, 8
1341.81	3, 8
1369.84	4, 12
1538.98	13, 21
1541.89 (C_3)	19
1551.99	3, 14
1569.00	3, 5
1585.93 ($^{0,4}A_4$)	27
1596.02	3, 9
1613.93 ($^{0,3}A_4$)	28
1717.24	3, 6
1779.22	4
1780.91	8
1796.22	3
1825.95	12
1853.95	11
1867.19 ($^{1,5}X_2$)	30
1946.11	14, 22
1954.27 ($^{2,4}X_2$)	29
1964.13	13, 22
1991.12	14
1999.27 (Z_3)	2

Table S1. List of fragment ions from UVPD of triply deprotonated Kdo2-lipid A [Mr = 2237.34] and their corresponding cleavage sites. The fragmentation cleavage map is shown in Figure 3.

LOS	Total (percentage)
Tetra Acyl waaQwaaG	11 ± 1%
Tetra Acyl waaQwaaG+PPEtN	4 ± 1%
Penta Acyl waaQwaaG	20 ± 1%
Penta Acyl waaQwaaG+PPETN	6 ± 1%
Hexa Acyl waaQwaaG	42 ± 2%
Hexa Acyl waaQwaaG+PPETN	17 ± 1%
Lipid A Anchor	Total (percentage)
Tetra Acyl waaQwaaG + Tetra Acyl waaQwaaG+PPEtN	15 ± 1%
Penta Acyl waaQwaaG + Penta Acyl waaQwaaG+PPETN	27 ± 1%
Hexa Acyl waaQwaaG + Hexa Acyl waaQwaaG+PPETN	58 ± 2%
LOS Glycoform	Total (percentage)
waaQwaaG without PPEtN	73 ± 2%
waaQwaaG with PPEtN	27 ± 2%
PPEtN Modified:Unmodified	Ratio
Tetra Acyl waaQwaaG+PPETN: Tetra Acyl waaQwaaQ	0.32:1
Penta Acyl waaQwaaG+PPETN:Penta Acyl waaQwaaQ	0.31:1
Hexa Acyl waaQwaaG+PPEtN:Hexa Acyl waaQwaaG	0.40:1

Table S2. Calculated acylation patterns and glycoforms observed for LOS identified within the BN1 ΔwaaQwaaG sample. Percentages were calculated using the extracted ion chromatogram areas listed in Supplemental Table 3, based on the LCMS data in Figure 4A.

	Injection 1		Injection 2		Injection 3		Mean	Coefficient of Variation (%)
	Area	RT	Area	RT	Area	RT		
Tetra Acyl waaQwaaG	64425241	14.91	68980738	14.94	72720858	14.95	68708946	6%
Tetra Acyl waaQwaaG+PPEtN	23758295	14.8	21179094	14.83	21605118	14.85	22180836	6%
	Area	RT	Area	RT	Area	RT		
Penta Acyl waaQwaaG	118269085	15.50	130787353	15.46	121327502	15.44	123461313	5%
Penta Acyl waaQwaaG+PPETN	34726036	15.42	40327010	15.37	40387047	15.35	38480031	8%
		RT	Area	RT	Area	RT		
Hexa Acyl waaQwaaG	239684831	16.01	248163403	15.92	268538772	15.95	252129002	6%
Hexa Acyl waaQwaaG+PPETN	97530633	15.76	104989335	15.83	98301572	15.78	100273847	4%

Table S3. Extracted ion chromatogram areas, retention times, mean peak areas and mean coefficient of variation for observed from BN1 ΔwaaQwaaG LOS based on the LCMS data shown in Figure 4A.

LPS	Observed m/z	Calculated m/z	Mass Error (ppm)	Retention Time (minutes)	Percent
Tetra Acyl waaOwaaQ + P	1293.5631	1293.5638	-5.02	14.8 ± 0.1	2 ± 0%
Tetra Acyl waaOwaaQ + PPEtN	1355.0669	1355.0680	-8.12	14.8 ± 0.1	2 ± 0%
Penta Acyl waaOwaaQ +P	1406.6606	1406.6604	1.42	15.0 ± 0.3	4 ± 0%
Penta Acyl waaOwaaQ + PPEtN	1468.1648	1468.1647	1.02	15.4 ± 0.1	9 ± 1%
Hexa Acyl waaOwaaQ + P	1511.7593	1511.7596	-1.98	15.8 ± 0.1	32 ± 3%
Hexa Acyl waaOwaaQ+PPEtN	1573.2643	1573.2639	2.86	15.8 ± 0.1	35 ± 5%
Hexa Acyl waaOwaaQ+PPEtN+PEtN	1634.7681	1634.7681	0.00	15.7 ± 0.1	10 ± 1%
Hexa Acyl waaO+P	1709.2782	1709.2787	-2.93	15.6 ± 0.1	7 ± 1%

Table S4. List of identified LOS within the BN1 E. coli sample via LC-MS. Percentages were calculated using the extracted ion chromatogram areas listed in Supplemental Table 5 based on the LCMS data in Figure 5A.

LPS	Injection 1	Injection2	Injection3	Mean	Coefficient of Variation (%)
lpxR waaOwaaQ + P	851794	932533	896764	893697	5%
lpxR waaOwaaQ + PPEtN	876229	1202284	1021826	1033446	16%
lpxM waaOwaaQ +P	2145795	2036369	1868549	2016904	7%
lpxM waaOwaaQ + PPEtN	4501591	4889663	4476764	4622673	5%
waaOwaaQ + P	15487393	14771080	17766181	16008218	10%
waaOwaaQ+PPEtN	14746187	18520179	19092010	17452792	14%
waaOwaaQ+PPEtN+PEtN	4157964	5093298	5353392	4868218	13%
waaO+ P	3233134	3764114	3894881	3630710	10%

Table S5. Extracted ion chromatogram areas, mean peak areas and mean coefficient of varaiation observed from BN1 based on the LCMS data shown in Figure 5A.