Supplementary Figures and Tables

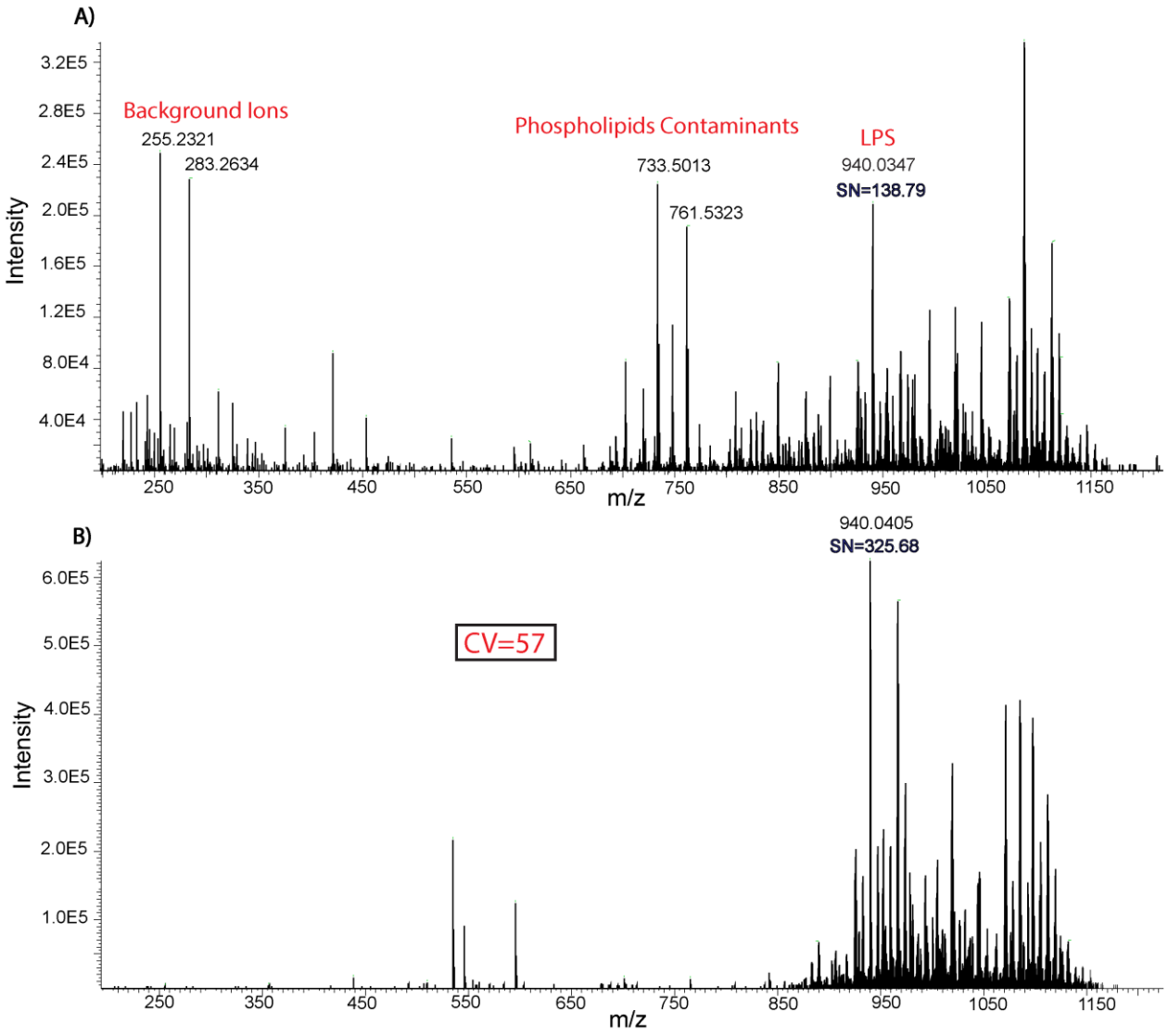


Figure S1: A) ESI-MS of the J5 *E. coli* LPS (FAIMS off). **B)** Corresponding mass spectrum at CV=57 showing the S/N ratio enhancement of *m/z* 940.04 ratio by 2.4 fold compared to when the FAIMS voltages were off

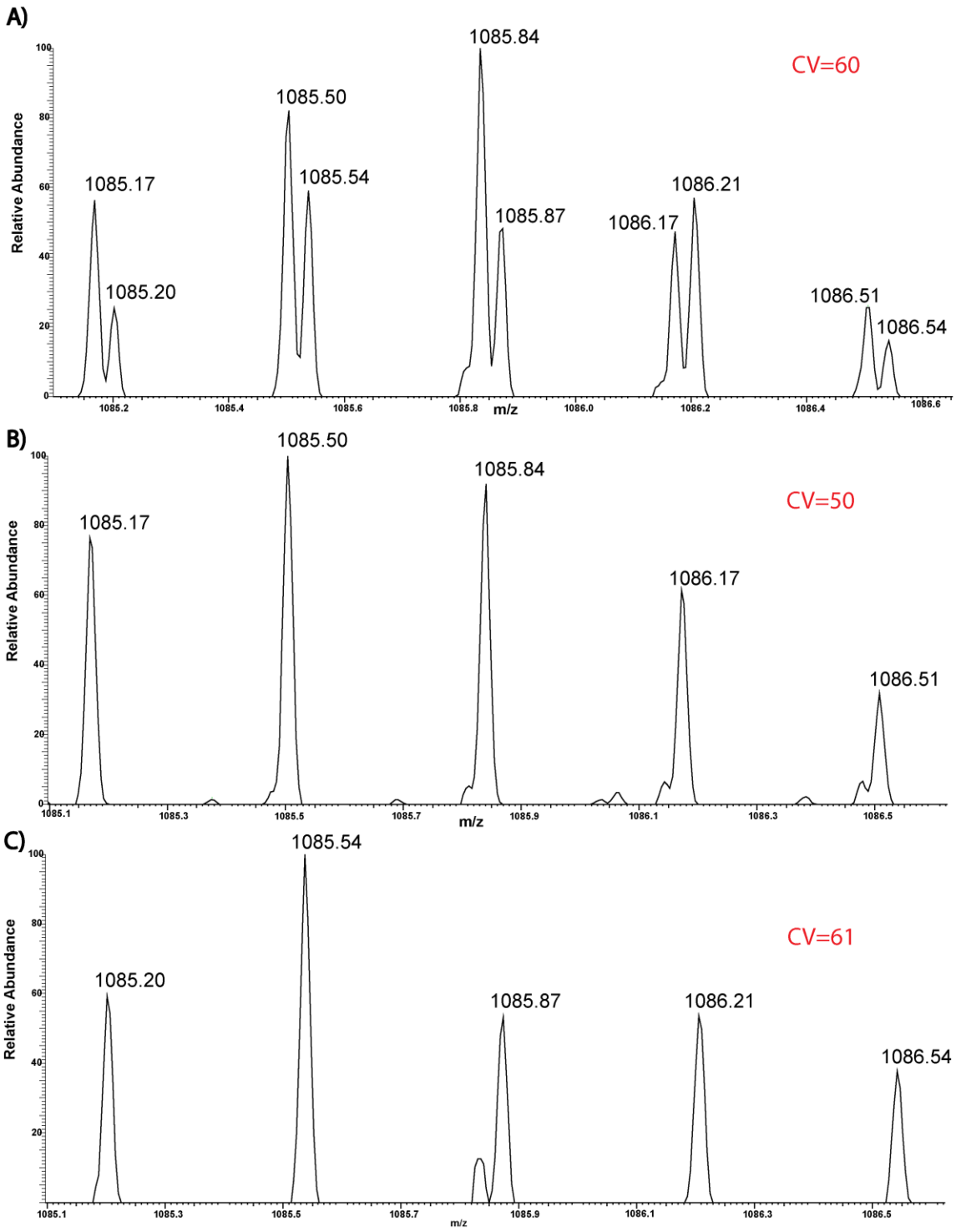


Figure S2: Separation of LPS isobaric ion at *m/z* 1085. **a)** Overlapping isobars at *m/z* 1085.17 and 1085.20 at CV=60 V. **b)** Separated isobar at *m/z* 1085.17 at CV=50 V. **c)** Separated isobar at *m/z* 1085.20 at CV= 61V.

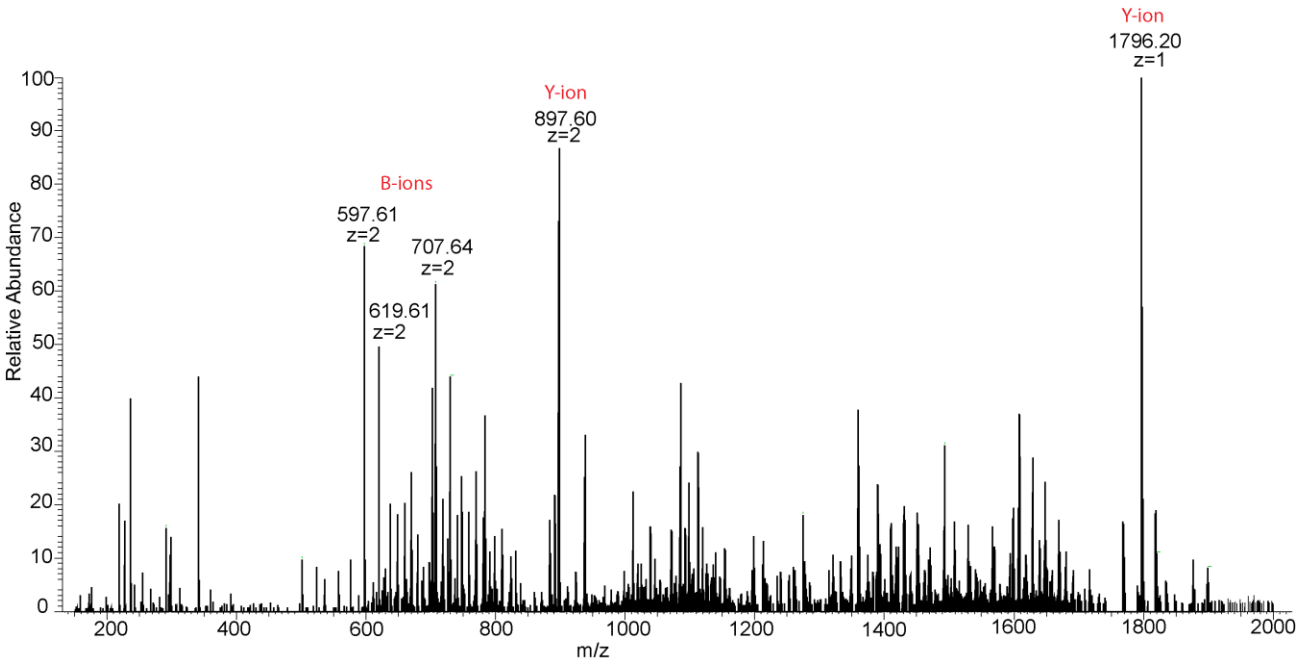


Figure S3: Source induced dissociation (SID) of *E. coli* J5 LPS. Fragmentation of the LOS ions in the source were accomplished by applying an energy of 100 V producing *m/z* 597.61 corresponding to the major oligosaccharide fragment of *m/z* 1085.17.

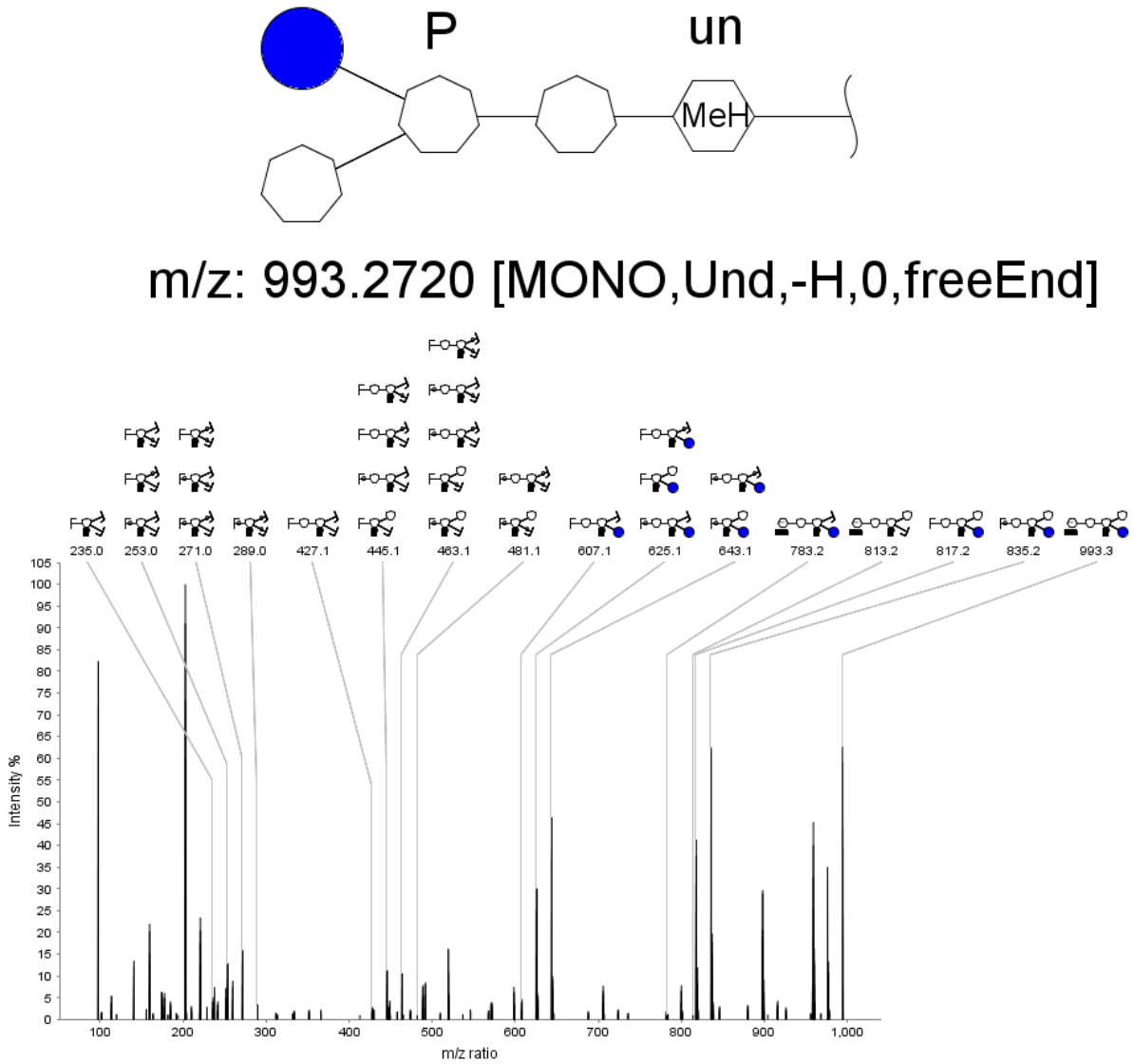


Figure S4: Annotated pseudo MS^3 of *m/z* 597.61 (*Z* = -2) generated using the GlycoWork bench software showing product ions originating from the singly charged key ion at *m/z* 993.26. The [Kdo-H_2O-CO_2] or $C_7H_{10}O_4$ residue in *m/z* 993.26 is represented in the software by an unsaturated methyl hexose residue [$C_7H_{12}O_5$-H_2O = $C_7H_{10}O_4$]

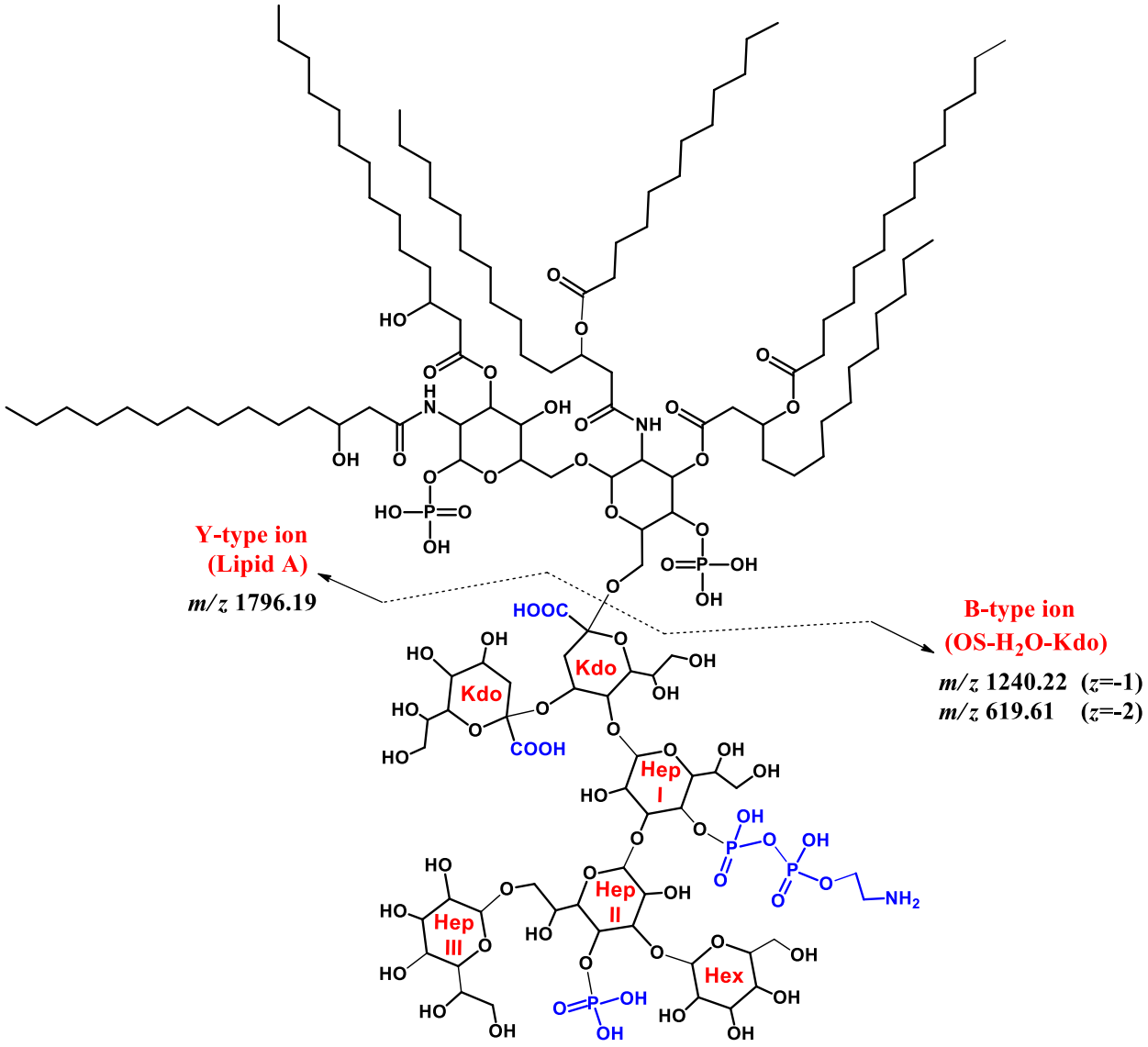
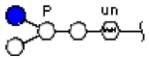
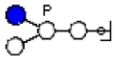
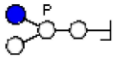
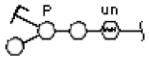
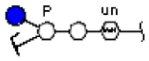
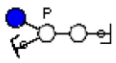
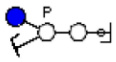
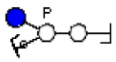
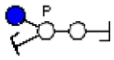
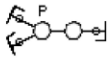
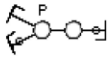
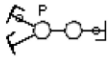
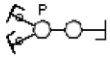
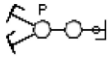
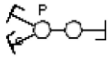
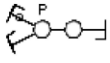
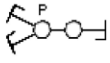


Figure S5: Proposed structure of the isobar at *m/z* 1085.17 showing the lipid A and oligosaccharide compositions. It also shows how the Y-type (Lipid A) and B-type (Oligosaccharides) ions/fragments are formed during the source induced dissociation of 100 V.

Table S1: List of all product ions originating from the singly charged key ion at *m/z* 993.26. Annotation was created using the GlycoWork bench software and all assignments were within 10 ppm

Mass to charge	Intensity	Relative Intensity	Ion	Type	Score	Accuracy	Accuracy PPM	Ion m/z	Charges	Neutral Exchanges
993.2628	53741.3448	62.7753			0.0000	0.0092	9.2443	993.2720	-H	0
835.2069	53516.4789	62.5127		C	0.0000	0.0057	6.7826	835.2126	-H	0
817.1986	35348.8035	41.2910		B	0.0000	0.0034	4.1424	817.2020	-H	0
813.2030	944.6947	1.1035		Z	0.0000	0.0057	7.0290	813.2087	-H	0
783.1923	1047.6561	1.2238		Z	0.0000	0.0058	7.4242	783.1981	-H	0
643.1459	39730.1194	46.4088		C	0.0000	0.0033	5.1001	643.1492	-H	0
643.1459	39730.1194	46.4088		CY	0.0000	0.0033	5.1001	643.1492	-H	0
625.1350	25702.5073	30.0231		B	0.0000	0.0036	5.8216	625.1386	-H	0
625.1350	25702.5073	30.0231		CZ	0.0000	0.0036	5.8216	625.1386	-H	0
625.1350	25702.5073	30.0231		BY	0.0000	0.0036	5.8216	625.1386	-H	0

Mass to charge	Intensity	Relative Intensity	Ion	Type	Score	Accuracy	Accuracy PPM	Ion m/z	Charges	Neutral Exchanges
607.1246	4008.8580	4.6828		BZ	0.0000	0.0035	5.7654	607.1281	-H	0
481.0937	959.7565	1.1211		CY	0.0000	0.0027	5.5632	481.0964	-H	0
481.0937	959.7565	1.1211		CYY	0.0000	0.0027	5.5632	481.0964	-H	0
463.0828	9004.8476	10.5186		CZ	0.0000	0.0031	6.6023	463.0858	-H	0
463.0828	9004.8476	10.5186		BY	0.0000	0.0031	6.6023	463.0858	-H	0
463.0828	9004.8476	10.5186		CYZ	0.0000	0.0031	6.6023	463.0858	-H	0
463.0828	9004.8476	10.5186		CZY	0.0000	0.0031	6.6023	463.0858	-H	0
463.0828	9004.8476	10.5186		BYY	0.0000	0.0031	6.6023	463.0858	-H	0
445.0724	9604.0290	11.2185		BZ	0.0000	0.0029	6.4587	445.0753	-H	0
445.0724	9604.0290	11.2185		CZZ	0.0000	0.0029	6.4587	445.0753	-H	0
445.0724	9604.0290	11.2185		BYZ	0.0000	0.0029	6.4587	445.0753	-H	0
445.0724	9604.0290	11.2185		BZY	0.0000	0.0029	6.4587	445.0753	-H	0
427.0626	2408.4354	2.8133		BZZ	0.0000	0.0021	5.0157	427.0647	-H	0

Mass to charge	Intensity	Relative Intensity	Ion	Type	Score	Accuracy	Accuracy PPM	Ion m/z	Charges	Neutral Exchanges
289.0316	2956.8246	3.4539		CYY	0.0000	0.0014	4.8562	289.0330	-H	0
271.0209	13544.2466	15.8210		CYZ	0.0000	0.0015	5.5391	271.0224	-H	0
271.0209	13544.2466	15.8210		CZY	0.0000	0.0015	5.5391	271.0224	-H	0
271.0209	13544.2466	15.8210		BYY	0.0000	0.0015	5.5391	271.0224	-H	0
253.0106	11022.3107	12.8752		CZZ	0.0000	0.0013	5.0784	253.0119	-H	0
253.0106	11022.3107	12.8752		BYZ	0.0000	0.0013	5.0784	253.0119	-H	0
253.0106	11022.3107	12.8752		BZY	0.0000	0.0013	5.0784	253.0119	-H	0
234.9999	4348.7691	5.0798		BZZ	0.0000	0.0014	5.9868	235.0013	-H	0

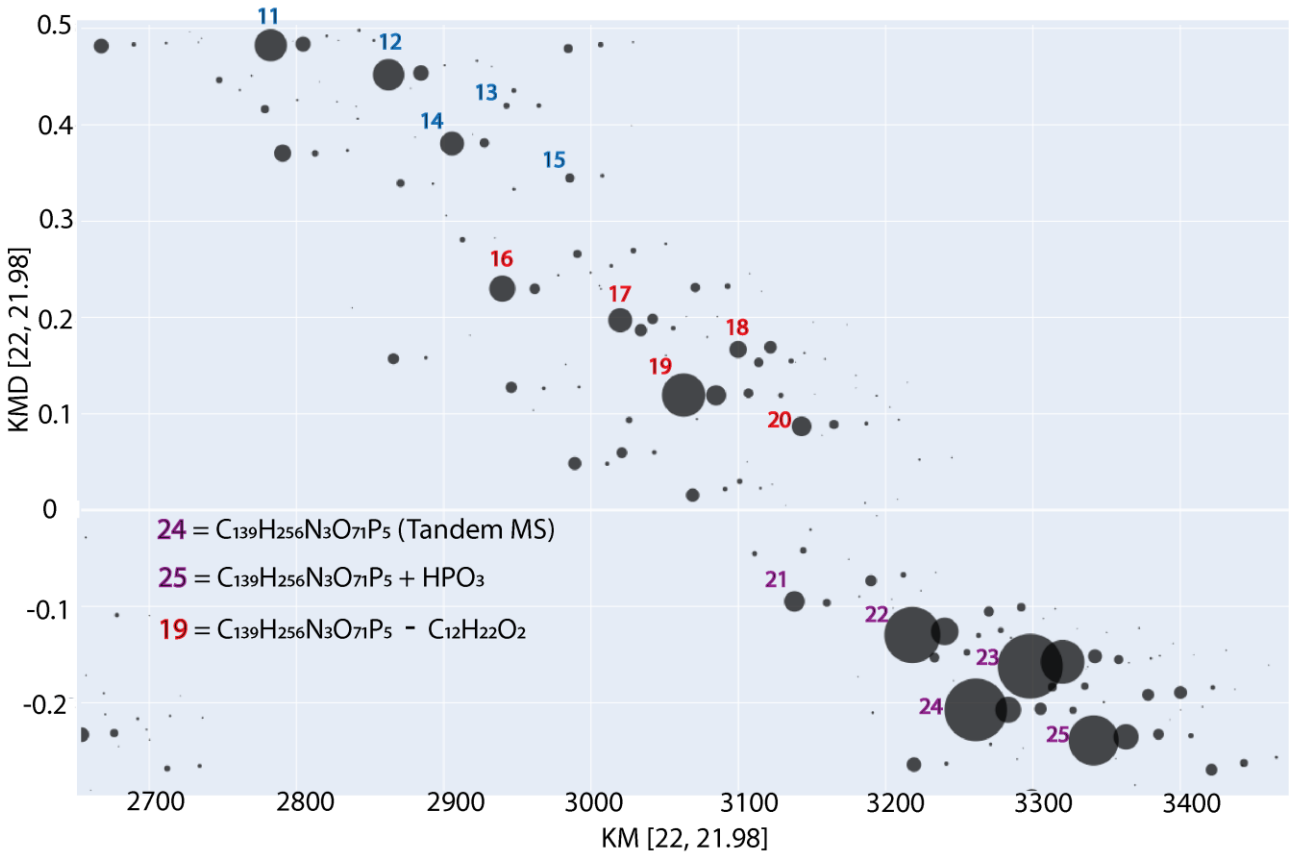


Figure S6: KMD plot region showing a constant structural difference of 157.12 Da between ion clusters **11-15 (blue)** and **16-20** (red) and a constant structural difference of 198.16 Da between ion clusters **16-20** (red) and **21-25** (purple). Chemical compositions of ions **24, 25** and **19** are shown on the plot as a representative example for how all the 74 ions compositions were assigned

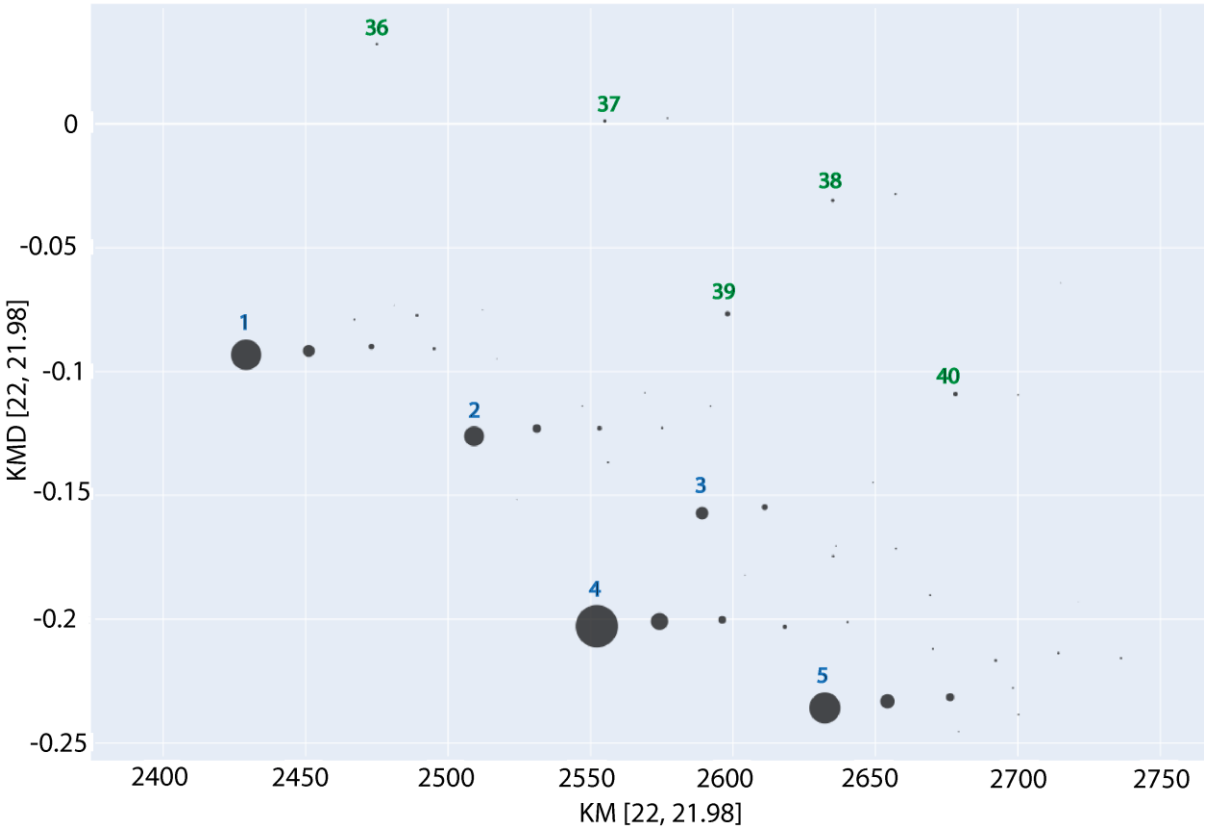


Figure S7: KMD plot region showing a constant structural difference of 45.84 Da between ion clusters **1-5** and **36-40** (annotated in blue and green, respectively).

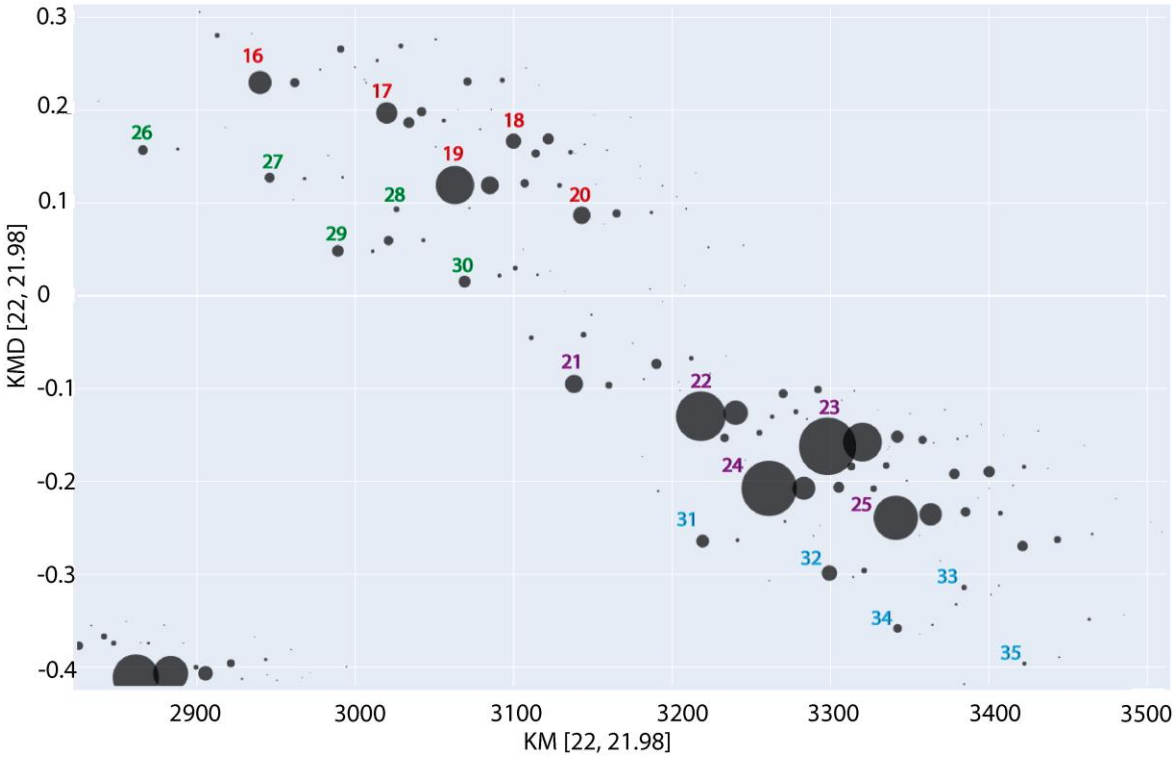


Figure S8: KMD plot region showing a constant structural difference of 73.87 Da between ion clusters **16-20** and **26-30** (annotated in red and green, respectively) and a constant structural difference of 81.10 Da between ion clusters **21-25** and **31-35** (annotated in purple and blue, respectively).

Table S2: Chemical composition of the *E. coli* J5 LPS (Rc mutant) species revealed form the in-depth KMD plot analysis using the custom base unit of [Na-H]. Abbreviations used are LA: *E. coli* lipid A, Kdo: 3-deoxy- d-manno-oct-2-ulosonic acid, Hep: heptose, Hex: hexose, GlcN: glucosamine, P: phosphate, EN: ethanolamine, 3-hydroxy-tetradecanoic acid: C14:0(3-OH), dodecanoic acid: C12:0, tetradecanoic acid: C14:0

a) LOS ion species with a complete hexa-acylated lipid A

	Molecular weight (Da)	Chemical Composition	Chemical Formula	Error (ppm)	Sodiated Forms
1	2863.491	LA+ Kdo2+ Hep2+ Hex+ P	$C_{130}H_{237}N_2O_{59}P_3$	3.2	2885.4718
2	2943.455	LA+ Kdo2+ Hep2+ Hex+ P2	$C_{130}H_{238}N_2O_{62}P_4$	2.3	2965.438
3	2986.4986	LA+ Kdo2+ Hep2+ Hex+ P2+ EN	$C_{132}H_{243}N_3O_{62}P_4$	2.7	3008.4809
4	3023.4233	LA+ Kdo2+ Hep2+ Hex+ P3	$C_{130}H_{239}N_2O_{65}P_5$	2.9	3045.4008
5	3066.4659	LA+ Kdo2+ Hep2+ Hex+ P3+ EN	$C_{132}H_{244}N_3O_{65}P_5$	3.0	3088.4415
6	3108.492	LA+ Kdo2+ Hep2+ Hex+ P3+ EN+ [C_3H_6]	$C_{135}H_{250}N_3O_{65}P_5$	-3.8	
7	3135.5195	LA + Kdo2+ Hep3+ Hex+ P2	$C_{137}H_{250}N_2O_{68}P_4$	2.5	3157.5029, 3179.4783
8	3146.436	LA+ Kdo2+ Hep2+ Hex+ P4+ EN	$C_{132}H_{245}N_3O_{68}P_6$	4.08	
9	3187.4553	LA (-C_2H_4) + Kdo2+ Hep3+Hex+P3	$C_{135}H_{247}N_2O_{71}P_5$	2.7	3209.4313, 3231.4107
10	3215.4886	LA + Kdo2+ Hep3+ Hex+ P3	$C_{137}H_{251}N_2O_{71}P_5$	3.3	3237.4669
11	3216.6223	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P	$C_{143}H_{260}N_3O_{69}P_3$	2.5	3238.6032
12	3258.531	LA + Kdo2+ Hep3+ Hex+ P3+ EN	$C_{139}H_{256}N_3O_{71}P_5$	3.3	3280.5129, 3302.4938, 3324.4772
13	3267.4216	LA (-C_2H_4) + Kdo2+ Hep3+Hex+P4	$C_{135}H_{248}N_2O_{74}P_6$	2.6	3289.399
14	3295.4554	LA + Kdo2+ Hep3+ Hex+ P4	$C_{137}H_{252}N_2O_{74}P_6$	3.4	3317.4328, 3339.4089
15	3296.5911	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P2	$C_{143}H_{261}N_3O_{72}P_4$	3.2	3318.5703
16	3338.4971	LA + Kdo2+ Hep3+ Hex+ P4+ EN	$C_{139}H_{257}N_3O_{74}P_6$	3.2	3360.4753, 3382.4546, 3404.438

17	3339.6153	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN	$C_{145}H_{266}N_4O_{72}P_4$	-2.2	3361.5935
18	3375.4194	LA + Kdo2+ Hep3+ Hex+ P5	$C_{137}H_{253}N_2O_{77}P_7$	2.6	3397.399, 3419.3757
19	3381.5368	LA + Kdo2+ Hep3+ Hex+ P4+ EN2	$C_{141}H_{262}N_4O_{74}P_6$	2.4	3403.5167
20	3381.6409	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN+ Ac	$C_{147}H_{268}N_4O_{73}P_4$	2.3	
21	3418.4618	LA + Kdo2+ Hep3+ Hex+ P5+ EN	$C_{139}H_{258}N_3O_{77}P_7$	2.6	3440.4367, 3462.4128
22	3419.5876	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN	$C_{145}H_{267}N_4O_{75}P_5$	-0.4	3441.5628
23	3461.6094	LA+ Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN+ Ac	$C_{147}H_{269}N_4O_{76}P_5$	2.8	

b) LOS ion species with penta-acylated lipid A

	Molecular weight (Da)	Chemical Composition	Chemical Formula	Error (ppm)	Sodiated forms
1	2665.3291	(LA - C12:0 - O) + Kdo2+ Hep2+ Hex+ P	$C_{118}H_{215}N_2O_{57}P_3$	3.4	2687.3094, 2709.2899
2	2745.2986	(LA - C12:0 - O) + Kdo2+ Hep2+ Hex+ P2	$C_{118}H_{216}N_2O_{60}P_4$	4.5	2767.2762
3	2776.3035	(LA - C14:0) + Kdo2+ Hep2+ Hex+ P2+ EN	$C_{118}H_{217}N_3O_{61}P_4$	4.1	2798.2761
4	2788.3393	(LA - C12:0 - O) + Kdo2+ Hep2+ Hex+ P2+ EN	$C_{120}H_{221}N_3O_{60}P_4$	3.9	2810.3214, 2832.3003
5	2868.3048	(LA - C12:0 - O) + Kdo2+ Hep2+ Hex+ P3+ EN	$C_{120}H_{222}N_3O_{63}P_5$	3.5	2890.2872
6	2910.3289	(LA - C12:0 - O) + Kdo2+ Hep2+ Hex+ P3+ EN+ [C_3H_6]	$C_{123}H_{228}N_3O_{63}P_5$	-4.4	2932.3091
7	2937.3575	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P2	$C_{125}H_{228}N_2O_{66}P_4$	2.6	2959.3396
8	3017.3246	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P3	$C_{125}H_{229}N_2O_{69}P_5$	2.8	3039.3052
9	3018.4612	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ GlcN+ P	$C_{131}H_{238}N_3O_{67}P_3$	3.0	3040.4428
10	3060.367	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P3+ EN	$C_{127}H_{234}N_3O_{69}P_5$	2.9	3082.3491, 3104.329, 3126.3131
11	3097.2893	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P4	$C_{125}H_{230}N_2O_{72}P_6$	2.2	3119.2689
12	3098.4252	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ GlcN+ P2	$C_{131}H_{239}N_3O_{70}P_4$	2.2	3120.4102

13	3140.3338	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P4+ EN	$C_{127}H_{235}N_3O_{72}P_6$	2.9	3162.3138, 3184.2947, 3206.2727
14	3141.4617	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN	$C_{133}H_{244}N_4O_{70}P_4$	0.3	
15	3220.3026	(LA - C12:0 - O) + Kdo2+ Hep3+ Hex+ P5+ EN	$C_{127}H_{236}N_3O_{75}P_7$	3.6	3242.2824

c) LOS ion species with tetra-acylated lipid A

	Molecular weight (Da)	Chemical Composition	Chemical Formula	Error (ppm)	Sodiated forms
1	2427.0997	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep2+ Hex+ P	$C_{102}H_{185}N_2O_{56}P_3$	3.9	2449.0801, 2471.0603, 2493.0431
2	2507.0669	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep2+ Hex+ P2	$C_{102}H_{186}N_2O_{59}P_4$	4.1	2529.0458, 2551.0276, 2573.0094
3	2550.1083	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep2+ Hex+ P2+ EN	$C_{104}H_{191}N_3O_{59}P_4$	3.7	2572.0883, 2594.0696, 2616.0544, 2638.0344
4	2587.0323	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P3	$C_{102}H_{187}N_2O_{62}P_5$	3.6	2609.0118
5	2630.0756	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P3+ EN	$C_{104}H_{192}N_3O_{62}P_5$	4.0	2652.0549, 2674.0352, 2696.0134, 2717.9967
6	2666.9997	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P4	$C_{102}H_{188}N_2O_{65}P_6$	3.9	
7	2673.1167	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P3+ EN2	$C_{106}H_{197}N_4O_{62}P_5$	3.5	2695.0965, 2717.0761
8	2699.1303	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P2	$C_{109}H_{198}N_2O_{65}P_4$	3.8	2721.1101, 2743.0912, 2765.0741, 2787.0531
9	2710.0424	(LA- C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P4+ EN	$C_{104}H_{193}N_3O_{65}P_6$	4.0	2732.0217
10	2753.083	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep2+ Hex+ P4+ EN2	$C_{106}H_{198}N_4O_{65}P_6$	3.3	2775.0616, 2797.0381
11	2779.0984	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P3	$C_{109}H_{199}N_2O_{68}P_5$	4.3	2801.077, 2823.0583, 2845.0376,

					2867.0194
12	2780.2339	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P	$C_{115}H_{208}N_3O_{66}P_3$	4.1	2802.2146
13	2822.1404	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P3+ EN	$C_{111}H_{204}N_3O_{68}P_5$	4.2	2844.1206, 2866.1015, 2888.0858
14	2859.063	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P4	$C_{109}H_{200}N_2O_{71}P_6$	3.6	2881.0406, 2903.0224
15	2860.1988	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ GlcN+ P2	$C_{115}H_{209}N_3O_{69}P_4$	3.5	2882.179
16	2902.1054	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P4+ EN	$C_{111}H_{205}N_3O_{71}P_6$	3.6	2924.084, 2946.0642, 2968.0464, 2990.028, 3012.0138, 3033.9932
17	2903.2349	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN	$C_{117}H_{214}N_4O_{69}P_4$	1.3	2925.2162
18	2939.028	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ P5	$C_{109}H_{201}N_2O_{74}P_7$	3.0	2961.0041
19	2940.1653	(LA - C14:0(3-OH) - C14:0) +Kdo2+ Hep3+ Hex+ GlcN+ P3	$C_{115}H_{210}N_3O_{72}P_5$	3.4	2962.1471
20	2945.2477	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN+ AC	$C_{119}H_{216}N_4O_{70}P_4$	2.1	
21	2982.0715	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ P5+ EN	$C_{111}H_{206}N_3O_{74}P_7$	3.4	3004.0495, 3026.0287
22	2983.205	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN	$C_{117}H_{215}N_4O_{72}P_5$	2.5	3005.1845
23	2988.2797	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN2+ AC	$C_{121}H_{221}N_5O_{70}P_4$	-1.4	
24	3026.245	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN2	$C_{119}H_{220}N_5O_{72}P_5$	1.8	3048.2200
25	3031.3235	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P2+ EN3+ AC	$C_{123}H_{226}N_6O_{70}P_4$	-0.8	3053.3033
26	3068.2489	(LA - C14:0(3-OH) - C14:0) + Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN2+ AC	$C_{121}H_{222}N_5O_{73}P_5$	-0.4	3090.2294
27	3111.2912	(LA - C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ GlcN+ P3+ EN3+ AC	$C_{123}H_{227}N_6O_{73}P_5$	-0.36	3133.2716

d) LOS ion species with tri-acylated lipid A

	Molecular weight (Da)	Chemical Composition	Chemical Formula	Error (ppm)	Sodiated forms
1	2323.9143	(LA - 2 x C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P2+ EN	$C_{90}H_{165}N_3O_{57}P_4$	3.8	2345.8944
2	2367.9416	(LA - C14:0(3-OH) -C14:0 - C12:0) + Kdo2+ Hep2+ Hex+ P2+ EN	$C_{92}H_{169}N_3O_{58}P_4$	4.2	2389.9217
3	2403.8815	(LA - 2 x C14:0(3-OH) - C14:0) + Kdo2+ Hep2+ Hex+ P3+ EN	$C_{90}H_{166}N_3O_{60}P_5$	4.0	2425.8609
4	2472.9366	(LA - 2 x C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P2	$C_{95}H_{172}N_2O_{63}P_4$	4.0	
5	2552.902	(LA - 2 x C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P3	$C_{95}H_{173}N_2O_{66}P_5$	3.5	2574.8828
6	2595.9445	(LA - 2 x C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P3+ EN	$C_{97}H_{178}N_3O_{66}P_5$	3.5	2617.9248
7	2632.8684	(LA - 2 x C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P4	$C_{95}H_{174}N_2O_{69}P_6$	3.4	2654.8478
8	2675.9112	(LA - 2 x C14:0(3-OH) - C14:0)+ Kdo2+ Hep3+ Hex+ P4+ EN	$C_{97}H_{179}N_3O_{69}P_6$	3.6	2697.8935