Typical Preparation of *Francisella tularensis O*-antigen Yields a Mixture of Three Types of Saccharides[†]

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Supporting Information

Supplemental Table 1 Observed fragment ions for [4,2,2][0,0,0] from multi-stage tandem MS in positive ion mode on an LTQ-Orbitrap XL mass spectrometer.

Product ion (m/z)	Charge	Saccharide Composition[1]	Mass Error (ppm)	Fragmentation
MS^2 Precursor ion *m/z* 824.2961 (2+)				
412.1321	1	[1,0,1][0,0,0]º+Na^+	-1.4	B_2
426.1476	1	[1,1,0][0,0,0]º+Na^+	-1.7	Z_2
430.1426	1	[1,0,1][0,0,0] +Na^+	-1.5	C_2
444.1581	1	[1,1,0][0,0,0] +Na^+	-1.8	Y_2
455.1378	1	[2,0,0][0,0,0]º+Na^+	-1.5	Y_7/B_3; Z_3/C_7; Y_3/B_7; Z_7/C_3
473.1482	1	[2,0,0][0,0,0] +Na^+	-1.8	Y_7/C_3; Y_3/C_7
628.2063	1	[2,0,1][0,0,0]º+Na^+	-1.6	B_3
629.7244	2	[3,2,1][0,0,0] +2Na^+	-1.5	Y_6
635.2143	2	[4,1,1][0,0,0]º+2Na^+	-1.3	Y_7/B_7; Z_7/C_7
642.2221	1	[2,1,0][0,0,0]º+Na^+	-1.3	Z_3
644.2194	2	[4,1,1][0,0,0] +2Na^+	-1.6	Y_7/C_7
646.2168	1	[2,0,1][0,0,0] +Na^+	-1.7	C_3
660.2324	1	[2,1,0][0,0,0] +Na^+	-1.7	Y_3
721.7491	2	[4,1,2][0,0,0]º+2Na^+	-0.6	B_7
730.7540	2	[4,1,2][0,0,0] +2Na^+	-1.1	C_7
737.7610	2	[4,2,1][0,0,0] +2Na^+	-2.2	Y_7
815.2906	1	[2,1,1][0,0,0]º+Na^+	-1.4	B_4; Z_4; Y_7/B_5; Z_7/C_5; Y_6/B_6; Z_6/C_6; Y_5/B_7; Z_5/C_7
833.3009	1	[2,1,1][0,0,0] +Na^+	-1.7	C_4; Y_4; Y_7/C_5; Y_6/C_6; Y_5/C_7
988.3593	1	[2,1,2][0,0,0]º+Na^+	-1.3	B_5
1002.3752	1	[2,2,1][0,0,0]º+Na^+	-1.0	Z_5
1020.3858	1	[2,2,1][0,0,0] +Na^+	-1.0	Y_5
1031.3651	1	[3,1,1][0,0,0]º+Na^+	-1.2	Y_7/B_6; Z_7/C_6; Y_6/B_7; Z_6/C_7
1049.3757	1	[3,1,1][0,0,0] +Na^+	-1.2	Y_7/C_6; Y_6/C_7
1204.4340	1	[3,1,2][0,0,0]º+Na^+	-1.0	B_6
1218.4495	1	[3,2,1][0,0,0]º+Na^+	-1.1	Z_6
1222.4445	1	[3,1,2][0,0,0] +Na^+	-1.0	C_6
1236.4599	1	[3,2,1][0,0,0] +Na^+	-1.2	Y_6
1438.5188	1	[4,1,2][0,0,0] +Na^+	-1.1	C_7
1452.5338	1	[4,2,1][0,0,0] +Na^+	-1.5	Y_7
1625.6023	1	[4,2,2][0,0,0] +Na^+	-1.6	$[M+Na]^+$
MS^3 Precursor ion *m/z* 833.3009 (1+), to simplify the nomenclature of the MS^3 fragment ions, Y_4 is assumed to be the precursor ion of MS^3 unless specified.				
239.0634	1	[1,0,0][0,0,0]º+Na^+	-2.0	Y_3/B_6; Z_3/C_6; Y_2/B_7; Z_2/C_7
257.0738	1	[1,0,0][0,0,0] +Na^+	-2.6	Y_3/C_6; Y_2/C_7
412.1316	1	[1,0,1][0,0,0]º+Na^+	-2.7	Y_4/B_6
426.1472	1	[1,1,0][0,0,0]º+Na^+	-2.7	Z_2
444.1576	1	[1,1,0][0,0,0] +Na^+	-3.0	Y_2
455.1372	1	[2,0,0][0,0,0]º+Na^+	-2.9	Y_3/B_7; Z_3/C_7
473.1476	1	[2,0,0][0,0,0] +Na^+	-3.2	Y_3/C_7
545.1685	1		-3.1	$^{0,2}A_3$ from C_4

599.2156	1	[1,1,1][0,0,0]º+Na+	-2.6	Y_6/B_5; Z_6/C_5 from Y_7/C_5; Y_6/C_6 or Y_5/B_6; Z_5/C_6 from Y_6/C_6; Y_5/C_7
628.2053	1	[2,0,1][0,0,0]º+Na^+	-3.3	Y_4/B_7
642.2210	1	[2,1,0][0,0,0]º+Na^+	-3.1	Z_3
646.2158	1	[2,0,1][0,0,0] +Na^+	-3.3	Y_4/C_7
660.2314	1	[2,1,0][0,0,0] +Na^+	-3.3	Y_3
815.2895	1	[2,1,1][0,0,0]º+Na^+	-2.8	Z_4
MS^4 Precursor ion *m/z* 646.2158 (1+)				
239.0632	1	[1,0,0][0,0,0]º+Na^+	-3.0	Y_3/B_6; Z_3/C_6; Y_2/B_7; Z_2/C_7
257.0739	1	[1,0,0][0,0,0] +Na^+	-2.2	Y_3/C_6; Y_2/C_7
412.1316	1	[1,0,1][0,0,0]º+Na^+	-2.7	Y_4/B_6; Z_4/C_6
430.1421	1	[1,0,1][0,0,0] +Na^+	-2.7	Y_4/C_6
455.1372	1	[2,0,0][0,0,0]º+Na^+	-2.9	Y_3/B_7; Z_3/C_7
473.1475	1	[2,0,0][0,0,0] +Na^+	-3.4	Y_3/C_7
545.1684	1		-3.3	$^{0,2}A_3$ from C_4
628.2055	1	[2,0,1][0,0,0]º+Na^+	-2.9	Y_4/B_7; Z_4/C_7
MS^4 Precursor ion *m/z* 660.2314 (1+)				
228.0836	1	[0,1,0][0,0,0] +Na^+	-3.1	Y_1
239.0632	1	[1,0,0][0,0,0]º+Na^+	-3	Y_3/B_6; Z_3/C_6; Y_2/B_7; Z_2/C_7
257.0738	1	[1,0,0][0,0,0] +Na^+	-2.6	Y_3/C_6; Y_2/C_7
426.1471	1	[1,1,0][0,0,0]º+Na^+	-3.0	Z_2
444.1575	1	[1,1,0][0,0,0] +Na^+	-3.2	Y_2
455.1371	1	[2,0,0][0,0,0]º+Na^+	-3.2	Y_3/B_7; Z_3/C_7
473.1474	1	[2,0,0][0,0,0] +Na^+	-3.6	Y_3/C_7
642.2208	1	[2,1,0][0,0,0]º+Na^+	-3.4	Z_3

[1]Saccharide compositions are given as [GalNAcAN, QuiNAc, Qui4NFm] [HexNAc, Hex, Kdo].

º represents loss of water.

Supplemental Table 2 Observed fragment ions for ^{18}O labeled [4,2,2][0,0,0] from multi-stage tandem MS in positive ion mode on an LTQ-Orbitrap XL mass spectrometer.

Product ion (m/z)	Charge	Saccharide Composition[1]	Mass Error (ppm)	Fragmentation
MS^2 Precursor ion *m/z* 825.2987 (2+)				
428.1519	1	[1,1,0][0,0,0]º+^{18}O-^{16}O+Na^+	-1.6	Z_2
646.2168	1	[2,0,1][0,0,0]+Na^+	-1.7	C_3
662.2367	1	[2,1,0][0,0,0]+^{18}O-^{16}O+Na^+	-1.6	Y_3
730.7539	2	[4,1,2][0,0,0]+2Na^+	-1.2	C_7
738.7635	2	[4,2,1][0,0,0]+^{18}O-^{16}O+2Na^+	-1.7	Y_7
815.2906	1	[2,1,1][0,0,0]º+Na^+	-1.4	B_4; Y_7/B_5; Z_7/C_5; Y_6/B_6; Z_6/C_6; Y_5/B_7; Z_5/C_7
835.3053	1	[2,1,1][0,0,0]+^{18}O-^{16}O+Na^+	-1.5	Y_4
988.3594	1	[2,1,2][0,0,0]º+Na^+	-1.2	B_5
1004.3794	1	[2,2,1][0,0,0]º+^{18}O-^{16}O+Na^+	-1.1	Z_5
1022.3901	1	[2,2,1][0,0,0]+^{18}O-^{16}O+Na^+	-0.9	Y_5
1049.3758	1	[3,1,1][0,0,0]+Na^+	-1.1	Y_7/C_6; Y_6/C_7
1204.4342	1	[3,1,2][0,0,0]º+Na^+	-0.8	B_6
1222.4447	1	[3,1,2][0,0,0]+Na^+	-0.9	C_6
1238.4646	1	[3,2,1][0,0,0]+^{18}O-^{16}O+Na^+	-0.8	Y_6
1438.5191	1	[4,1,2][0,0,0]+Na^+	-0.9	C_7
1627.6078	1	[4,2,2][0,0,0]+^{18}O-^{16}O+2Na^+	-0.8	$[M+Na]^+$
MS^3 Precursor ion *m/z* 835.3053 (1+)				
412.1317	1	[1,0,1][0,0,0]º+Na^+	-2.4	Y_4/B_6
428.1516	1	[1,1,0][0,0,0]º+^{18}O-^{16}O+Na^+	-2.3	Z_2
430.1422	1	[1,0,1][0,0,0] +Na^+	-2.5	Y_4/C_6
446.1621	1	[1,1,0][0,0,0]+^{18}O-^{16}O+Na^+	-2.4	Y_2
473.1479	1	[2,0,0][0,0,0] +Na^+	-2.5	Y_3/C_7
628.2057	1	[2,0,1][0,0,0]º+Na^+	-2.6	Y_4/B_7; Z_4/C_7
646.2163	1	[2,0,1][0,0,0]+Na^+	-2.5	Y_4/C_7
662.2362	1	[2,1,0][0,0,0]+^{18}O-^{16}O+Na^+	-2.4	Y_3

[1]Saccharide compositions are given as [GalNAcAN, QuiNAc, Qui4NFm] [HexNAc, Hex, Kdo].

º represents loss of water.

Supplemental Table 3 Observed fragment ions for [4,2,2][0,0,0] and [6,3,3][0,0,0] from tandem MS in negative ion mode on an FT-ICR mass spectrometer.

Observed Peaks (m/z)	Charge	Saccharide Composition[1]	Mass Accuracy (ppm)	Fragmentation
MS^2 of Precursor ion *m/z* 846.3049 (2-)				
215.0674	1	[1,0,0][0,0,0]º-[H^+]	0.3	Y_7/B_2; Z_7/C_2; Y_6/B_3; Z_6/C_3; Y_3/B_6; Z_3/C_6; Y_2/B_7; Z_2/C_7
232.0827	1	[0,1,0][0,0,0]º,** -[H^+]	0.2	Z_1
236.0776	1	[0,0,1][0,0,0]** -[H^+]	0.1	C_1
305.0991	1		0.2	$^{0,2}A_2$
402.1518	1	[1,1,0][0,0,0]º-[H^+]	0.0	Z_2
431.1419	1	[2,0,0][0,0,0]º-[H^+]	-0.2	Y_7/B_3; Z_7/C_3; Y_3/B_7; Z_3/C_7
448.1572	1	[1,1,0][0,0,0]º,** -[H^+]	-0.2	Z_2
575.2204	1	[1,1,1][0,0,0]º-[H^+]	-0.4	Y_6/B_5; Z_6/C_5; Y_5/B_6; Z_5/C_6
618.2261	1	[2,1,0][0,0,0]º-[H^+]	-0.5	Z_3
656.2338	2		-0.9	$^{0,2}A_7$
664.2314	1	[2,1,0][0,0,0]º,** -[H^+]	-0.8	Z_3
706.7577	2	[4,1,2][0,0,0] -2[H^+]	-0.8	C_7
727.7631	2	[4,2,1][0,0,0]º,** -2[H^+]	-0.6	Z_7
729.7605	2	[4,1,2][0,0,0]** -2[H^+]	-0.7	C_7
791.2946	1	[2,1,1][0,0,0]º-[H^+]	-0.8	B_4; Z_4
800.2995	2	[4,2,2][0,0,0] -2[H^+]	-1.3	$[M-2H]^{2-}$
823.3025	2	[4,2,2][0,0,0]** -2[H^+]	-0.9	$[M+FA-2H]^{2-}$
846.3053	2	[4,2,2][0,0,0]2**-2[H^+]	-0.8	$[M+2FA-2H]^{2-}$
982.3734	1	[2,1,2][0,0,0] -[H^+]	-1.2	C_5
1007.3688	1	[3,1,1][0,0,0]º-[H^+]	-1.0	Y_7/B_6; Z_7/C_6; Y_6/B_7; Z_6/C_7
1024.3839	1		-1.2	$^{2,4}A_6$
1028.3788	1	[2,1,2][0,0,0]** -[H^+]	-1.2	C_5
1097.4002	1		-1.2	$^{0,2}A_6$
1180.4371	1	[3,1,2][0,0,0]º-[H^+]	-1.3	B_6
1198.4477	1	[3,1,2][0,0,0] -[H^+]	-1.3	C_6
1223.4428	1	[4,1,1][0,0,0]º-[H^+]	-1.4	Y_7/B_7; Z_7/C_7
1240.4581	1		-1.4	$^{2,4}A_7$
1244.4529	1	[3,1,2][0,0,0]** -[H^+]	-1.5	C_6
1295.4640	1		-1.2	$^{0,2}A_7$-H_2O
1313.4740	1		-1.6	$^{0,2}A_7$
1396.5116	1	[4,1,2][0,0,0]º-[H^+]	-1.2	B_7
1410.5256	1	[4,2,1][0,0,0]º-[H^+]	-2.4	Z_7
1414.5215	1	[4,1,2][0,0,0] -[H^+]	-1.7	C_7
1460.5269	1	[4,1,2][0,0,0]** -[H^+]	-1.7	C_7
MS^2 of Precursor ion *m/z* 1242.4535 (2-)				
402.1516	1	[1,1,0][0,0,0]º-[H^+]	-0.5	Z_2
431.1418	1	[2,0,0][0,0,0]º-[H^+]	-0.4	Y_{11}/B_3; Z_{11}/C_3; Y_7/B_7; Z_7/C_7; Y_3/B_{11}; Z_3/C_{11}
575.2202	1	[1,1,1][0,0,0]º-[H^+]	-0.7	Y_{10}/B_5; Z_{10}/C_5; Y_9/B_6; Z_9/C_6; Y_6/B_9; Z_6/C_9; Y_5/B_{10}; Z_5/C_{10}

618.2258	1	[2,1,0][0,0,0]º-[H+]	-1.0	Z_3
664.2311	1	[2,1,0][0,0,0]º,** -[H+]	-1.2	Z_3
791.2939	1	[2,1,1][0,0,0]º-[H+]	-1.7	B_4; Z_4
837.3001	1	[2,1,1][0,0,0]º,** -[H+]	-0.7	B_4; Z_4
944.3460	2		-2.5	$^{0,2}A_{10}$
982.3728	1	[2,1,2][0,0,0] -[H+]	-1.8	C_5
1007.3685	1	[3,1,1][0,0,0]º-[H+]	-1.3	Y_{11}/B_6; Z_{11}/C_6; Y_{10}/B_7; Z_{10}/C_7; Y_7/B_{10}; Z_7/C_{10}; Y_6/B_{11}; Z_6/C_{11}
1015.8758	2	[5,3,2][0,0,0]º,** -2[H+]	-1.7	Z_{10}
1028.3780	1	[2,1,2][0,0,0]** -[H+]	-2.0	C_5
1052.3834	2		-2.2	$^{0,2}A_{11}$
1093.9021	2	[6,2,3][0,0,0]º-2[H+]	-2.0	B_{11}
1097.3993	1		-2.0	$^{0,2}A_6$
1100.9096	2	[6,3,2][0,0,0]º-2[H+]	-2.3	Z_{11}
1102.9063	2	[6,2,3][0,0,0] -2[H+]	-2.9	C_{11}
1123.9125	2	[6,3,2][0,0,0]º,** -2[H+]	-2.1	Z_{11}
1180.4363	1	[3,1,2][0,0,0]º-[H+]	-2.0	B_6
1194.4516	1	[3,2,1][0,0,0]º-[H+]	-2.3	Z_6
1196.4491	2	[6,3,3][0,0,0] -2[H+]	-2.2	$[M-2H]^{2-}$
1205.4533	2	[6,3,3][0,0,0]º-2[H+]	-3.1	$[M-H_2O-2H]^{2-}$
1219.4512	2	[6,3,3][0,0,0]** -2[H+]	-2.7	$[M+FA-2H]^{2-}$
1223.4419	1	[4,1,1][0,0,0]º-[H+]	-2.1	Y_{11}/B_7; Z_{11}/C_7; Y_7/B_{11}; Z_7/C_{11}
1367.5196	1	[3,2,2][0,0,0]º-[H+]	-2.6	Y_{10}/B_7; Z_{10}/C_7; Y_9/B_{10}; Z_9/C_{10}
1410.5249	1	[4,2,1][0,0,0]º-[H+]	-2.9	Z_7
1583.5931	1	[4,2,2][0,0,0]º-[H+]	-2.9	B_8; Z_8
1774.6715	1	[4,2,3][0,0,0] -[H+]	-3.2	C_9
1889.6987	1		-2.8	$^{0,2}A_{10}$

[1]Saccharide compositions are given as [GalNAcAN, QuiNAc, Qui4NFm] [HexNAc, Hex, Kdo].

º represents loss of water; $^{+}$º represents water adduct; ** stands for formic acid adduct; and * stands for loss of formic acid.

Supplemental Table 4 Observed fragment ions for [6,3,3][0,0,0] from multi-stage tandem MS in positive ion mode on an LTQ-Orbitrap XL mass spectrometer.

Product ion (m/z)	Charge	Saccharide Composition[1]	Mass Error (ppm)	Fragmentation
MS^2 Precursor ion *m/z* 1220.4479 (2+)				
412.1320	1	[1,0,1][0,0,0]º+Na^+	-1.7	B_2
426.1476	1	[1,1,0][0,0,0]º+Na^+	-1.7	Z_2
430.1424	1	[1,0,1][0,0,0] +Na^+	-2.0	C_2
444.1581	1	[1,1,0][0,0,0] +Na^+	-1.8	Y_2
455.1376	1	[2,0,0][0,0,0]º+Na^+	-2.0	Y_{11}/B_3; Z_{11}/C_3; Y_7/B_7; Z_7/C_7; Y_3/B_{11}; Z_3/C_{11}
473.1480	1	[2,0,0][0,0,0] +Na^+	-2.3	Y_{11}/C_3; Y_7/C_7; Y_3/C_{11}
628.2062	1	[2,0,1][0,0,0]º+Na^+	-1.8	B_3
642.2219	1	[2,1,0][0,0,0]º+Na^+	-1.6	Z_3
646.2162	1	[2,0,1][0,0,0] +Na^+	-2.6	C_3
660.2323	1	[2,1,0][0,0,0] +Na^+	-1.9	Y_3
815.2897	1	[2,1,1][0,0,0]º+Na^+	-2.6	B_4; Z_4
833.3003	1	[2,1,1][0,0,0] +Na^+	-2.5	C_4; Y_4
901.8249	2	[4,2,3][0,0,0]º+2Na^+	-1.4	B_9
910.8305	2	[4,2,3][0,0,0]+2Na^+	-1.0	C_9
917.8388	2	[4,3,2][0,0,0]+2Na^+	-0.5	Y_9
923.3280	2	[5,2,2][0,0,0]º+2Na^+	-1.2	Y_{10}/B_{11}; Z_{10}/C_{11}; Y_{11}/B_{10}; Z_{11}/C_{10}
932.3331	2	[5,2,2][0,0,0]º+2Na^+	-1.3	Y_{11}/C_{10}; Y_{10}/C_{11}
968.3439	2		-1.0	$^{0,2}A_{10}$
988.3593	1	[2,1,2][0,0,0]º+Na^+	-1.3	B_5
1002.3752	1	[2,2,1][0,0,0]º+Na^+	-1.0	Z_5
1006.3701	1	[2,1,2][0,0,0]+Na^+	-1.0	C_5
1009.8623	2	[5,2,3][0,0,0]º+2Na^+	-1.2	B_{10}
1016.8703	2	[5,3,2][0,0,0]º+2Na^+	-1.0	Z_{10}
1018.8675	2	[5,2,3][0,0,0]+2Na^+	-1.2	C_{10}
1025.8756	2	[5,3,2][0,0,0]+2Na^+	-0.9	Y_{10}
1031.3652	2	[6,2,2][0,0,0]º+Na^+	-1.1	Y_{11}/B_{11}; Z_{11}/C_{11}
1040.3705	2	[6,2,2][0,0,0]+2Na^+	-1.1	Y_{11}/C_{11}
1049.3756	1	[3,1,1][0,0,0] +Na^+	-1.3	Y_{11}/C_6; Y_{10}/C_7; Y_7/C_{10}; Y_6/C_{11}
1117.8993	2	[6,2,3][0,0,0]º+2Na^+	-1.3	B_{11}
1126.9037	2	[6,2,3][0,0,0]+2Na^+	-2.1	C_{11}
1204.4327	1	[3,1,2][0,0,0]º+Na^+	-2.1	B_6
1236.4599	1	[3,2,1][0,0,0] +Na^+	-1.2	Y_6
1247.4393	1	[4,1,1][0,0,0]º+Na^+	-1.4	Y_{11}/B_7; Z_{11}/C_7; Y_7/B_{11}; Z_7/C_{11}
1265.4499	1	[4,1,1][0,0,0]+Na^+	-1.3	Y_{11}/C_7; Y_7/C_{11}
1337.4713	1		-1.0	$^{0,2}A_7$
1434.5242	1	[4,2,1][0,0,0]º+Na^+	-0.9	Z_7
1438.5187	1	[4,1,2][0,0,0] +Na^+	-1.2	C_7
1452.5350	1	[4,2,1][0,0,0] +Na^+	-0.7	Y_7
1607.5910	1	[4,2,2][0,0,0]º+Na^+	-2.0	B_8; Z_8
1625.6009	1	[4,2,2][0,0,0] +Na^+	-2.4	C_8; Y_8

1780.6608	1	[4,2,3][0,0,0]º+Na^+	-1.3	B_9
MS^3 Precursor ion m/z 833.3003 (1+), to simplify the nomenclature of the MS^3 fragment ions, Y_4 is assumed to be the precursor ion of MS^3 unless specified.				
412.1320	1	[1,0,1][0,0,0]º+Na^+	-1.7	Y_4/B_{10}
426.1469	1	[1,1,0][0,0,0]º+Na^+	-3.5	Z_2
430.1417	1	[1,0,1][0,0,0] +Na^+	-3.7	Y_4/C_{10}
444.1574	1	[1,1,0][0,0,0] +Na^+	-3.5	Y_2
473.1473	1	[2,0,0][0,0,0] +Na^+	-3.8	Y_3/C_{11}
628.2051	1	[2,0,1][0,0,0]º+Na^+	-3.6	Y_4/B_{11}
646.2155	1	[2,0,1][0,0,0] +Na^+	-3.7	Y_4/C_{11}
660.2312	1	[2,1,0][0,0,0] +Na^+	-3.6	Y_3
815.2890	1	[2,1,1][0,0,0]º+Na^+	-3.4	Z_4
833.2993	1	[2,1,1][0,0,0]+Na^+	-3.7	Y_4

[1]Saccharide compositions are given as [GalNAcAN, QuiNAc, Qui4NFm] [HexNAc, Hex, Kdo].

º represents loss of water.

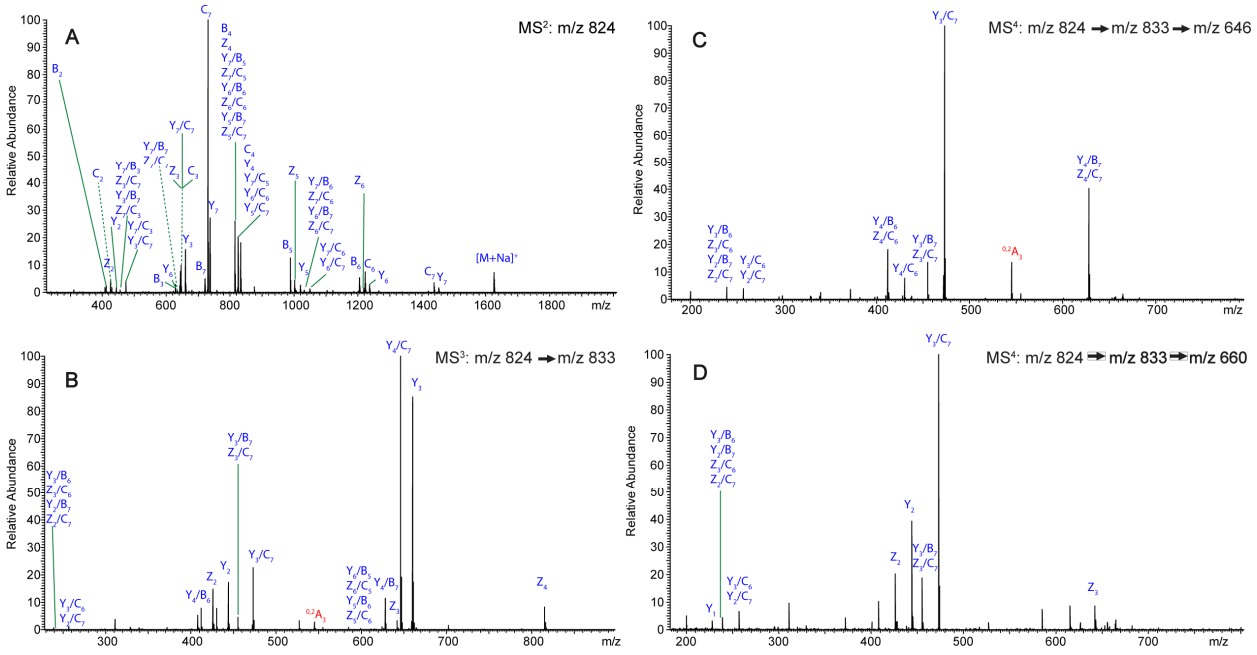
Supplemental Table 5 Observed fragment ions for ^{18}O labeled [6,3,3][0,0,0] from multi-stage tandem MS in positive ion mode on an LTQ-Orbitrap XL mass spectrometer.

Product ion (m/z)	Charge	Saccharide Composition[1]	Mass Error (ppm)	Fragmentation
MS^2 Precursor ion *m/z* 1221.4487 (2+)				
428.1518	1	[1,1,0][0,0,0]º+^{18}O-^{16}O+Na^+	-1.8	Z_2
446.1624	1	[1,1,0][0,0,0]+^{18}O-^{16}O+Na^+	-1.7	Y_2
473.1480	1	[2,0,0][0,0,0]+Na^+	-2.3	Y_{11}/C_3; Y_7/C_7; Y_3/C_{11}
628.2063	1	[2,0,1][0,0,0]º+Na^+	-1.6	B_3
646.2166	1	[2,0,1][0,0,0]+Na^+	-2.0	C_3
662.2365	1	[2,1,0][0,0,0]+^{18}O-^{16}O+Na^+	-1.9	Y_3
815.2903	1	[2,1,1][0,0,0]º+Na^+	-1.8	B_4
835.3052	1	[2,1,1][0,0,0]+^{18}O-^{16}O +Na^+	-1.6	Y_4
988.3592	1	[2,1,2][0,0,0]º+Na^+	-1.4	B_5
1004.3791	1	[2,2,1][0,0,0]º+^{18}O-^{16}O+Na^+	-1.4	Z_5
1018.8668	2	[5,2,3][0,0,0]+2Na^+	-1.9	C_{10}
1022.3896	1	[2,2,1][0,0,0]+^{18}O-^{16}O+Na^+	-1.4	Y_5
1031.3651	2	[6,2,2][0,0,0]º+2Na^+	-1.2	Y_{11}/B_{11}; Z_{11}/C_{11}
1040.3703	2	[6,2,2][0,0,0]+2Na^+	-1.3	Y_{11}/C_{11}
1076.3807	2		-1.4	$^{0,2}A_{11}$
1117.8996	2	[6,2,3][0,0,0]º+2Na^+	-1.1	B_{11}
1126.9044	2	[6,2,3][0,0,0]+2Na^+	-1.5	C_{11}
1204.4337	1	[3,1,2][0,0,0]º+Na^+	-1.2	B_6
1438.5182	1	[4,1,2][0,0,0]+Na^+	-1.5	C_7
1454.5386	1	[4,2,1][0,0,0]+^{18}O-^{16}O+Na^+	-1.1	Y_7
1607.5918	1	[4,2,2][0,0,0]º+Na^+	-1.5	B_8
1627.6068	1	[4,2,2][0,0,0]+^{18}O-^{16}O+Na^+	-1.4	Y_8
1780.6605	1	[4,2,3][0,0,0]º+ Na^+	-1.4	B_9

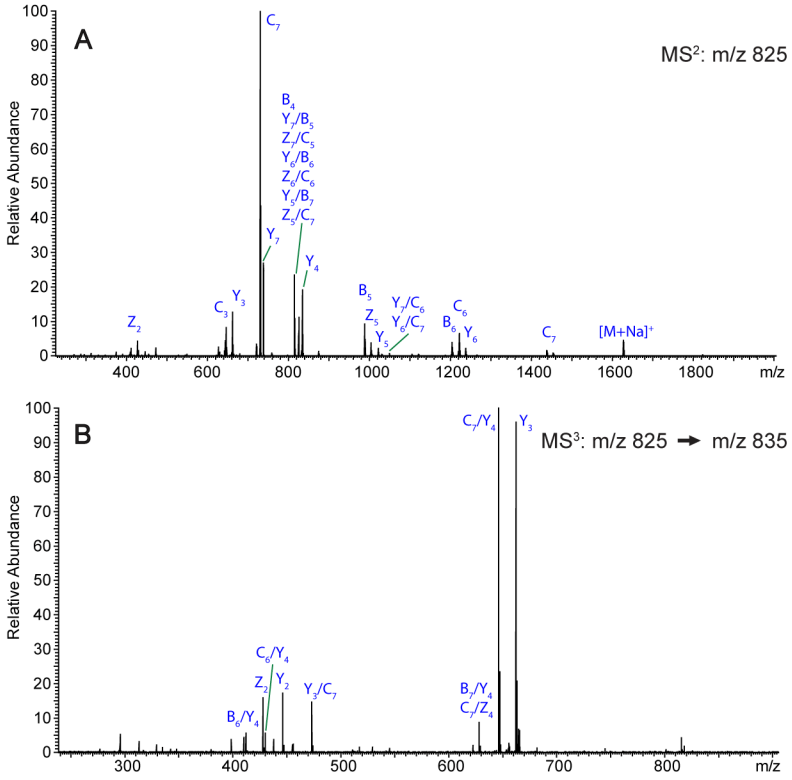
[1]Saccharide compositions are given as [GalNAcAN, QuiNAc, Qui4NFm] [HexNAc, Hex, Kdo].

º represents loss of water.

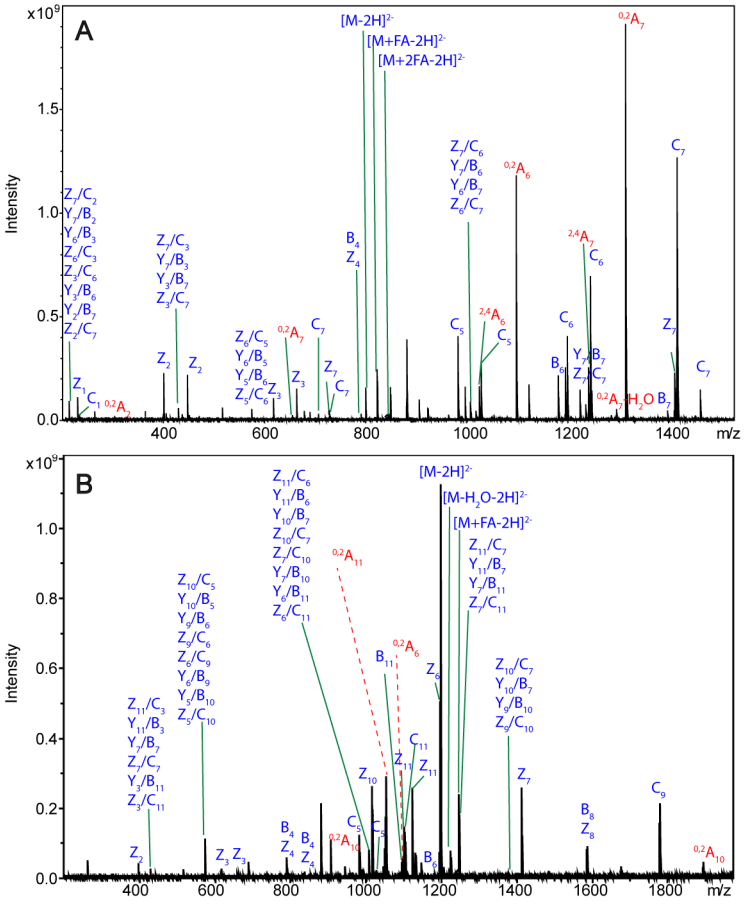
Supplemental Figure 1 Multi-stage tandem MS reveals the structural linkage for [4,2,2][0,0,0]. Tandem mass spectra of the SEC fraction enriched with [4,2,2][0,0,0] was recorded with an Oribtrap mass spectrometer in positive ion mode. CID with 20% normalized collision energy was applied to achieve fragmentation of the $[M + 2Na]^{2+}$ at *m/z* 824.2961 (A). The ion observed at *m/z* 833.3009 in the MS^2 spectrum was isolated and CID was applied to obtain the MS^3 spectrum (B). Two dominant fragment ions at *m/z* 646.2158 and 660.2314 in the MS^3 spectrum were isolated and fragmented to achieve MS^4 spectra (C and D). The fragment ions are designated using the Domon and Costello nomenclature (*27*). "/" is used for internal cleavages, and alternative assignments are shown above one another.



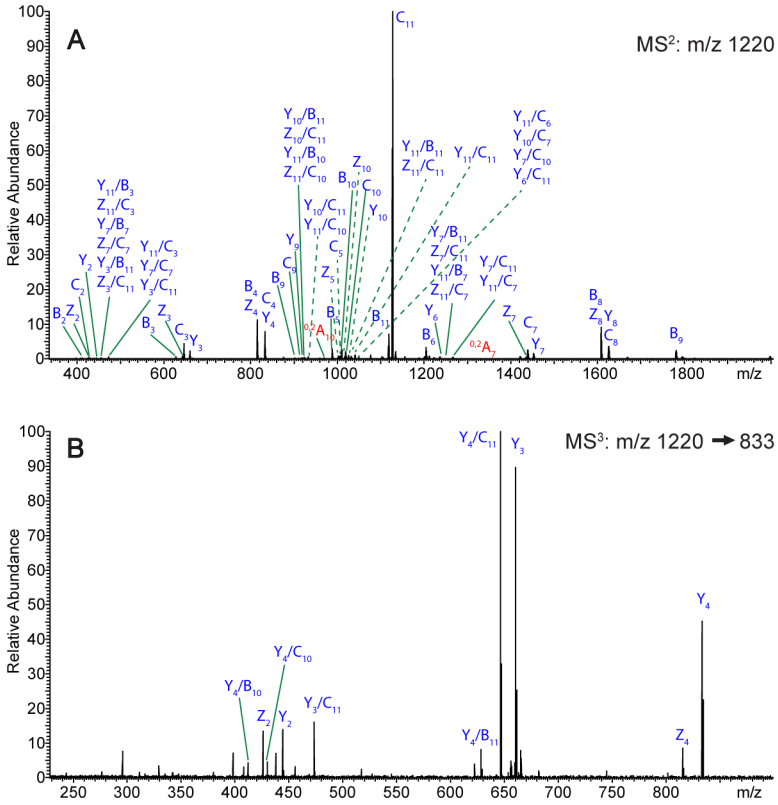
Supplemental Figure 2 Multi-stage tandem mass spectra of the ^{18}O labeled saccharide [4,2,2][0,0,0] reveals the reducing-end residue. After quadrupole ion trap isolation of the ^{18}O labeled saccharide [4,2,2][0,0,0] at *m/z* 825.2987, CID with 20% normalized collision energy was applied to achieve fragmentation (A). The ion at *m/z* 835.3053 further isolated from the MS^2 spectrum and CID was applied to obtain MS^3 spectrum (B). All fragments contain sodium.



Supplemental Figure 3 Similar fragment ions are observed for saccharides in negative ion mode. CID MS^2 spectra of saccharide chains [4,2,2][0,0,0] at *m/z* 846.3049 (A) and [6,3,3][0,0,0] at *m/z* 1242.4535 (B) were obtained in negative ion mode using a Solarix FT-ICR mass spectrometer.



Supplemental Figure 4 Multi-stage tandem MS of the $[M + 2Na]^{2+}$ *m/z* 1220.4479 of the saccharide [6,3,3][0,0,0]. After quadrupole ion trap isolation *m/z* 1220.4479, CID with 20% normalized collision energy was applied to achieve fragment ions (A). The ion at *m/z* 833.3003 was further isolated from the MS^2 spectrum and CID was applied to obtain MS^3 spectrum (B).



Supplemental Figure 5 Tandem MS of the $[M + 2Na]^{2+}$ *m/z* 1221.4487 of the ^{18}O-labeled saccharide [6,3,3][0,0,0] reveals the reducing-end residue. After quadrupole isolation *m/z* 1221.4487, CID with 20% normalized collision energy was applied to achieve fragmentation of the ^{18}O labeled saccharide [6,3,3][0,0,0].