SUPPLEMENTAL INFORMATION

Biosynthesis of the Polymannose Lipopolysaccharide O Antigens from *Escherichia coli* Serotypes O8 and O9a Requires a Unique Combination of Mannosyltransferase Modules in Single- and Multi-Active Site Enzymes

Laura K. Greenfield, Michele R. Richards, Jianjun Li, Warren W. Wakarchuk, Todd L. Lowary, and Chris Whitfield*

*To whom correspondence may be addressed: Chris Whitfield, Department of Molecular and Cellular Biology, Science Complex, University of Guelph, Guelph, Ontario N1G 2W1 Canada. Tel.: 519-824-4120, Ext. 53361; FAX: 519-837-3273; E-mail: cwhitfie@uoguelph.ca.

Table S1: Primer sequences used for the production of recombinant plasmids and chromosomal mutants.

Primer	Sequence[a,b] (5'→3')	Features
WaaLK12a	GCAGTTTTGGAAAAGTTATCATC ATTATAAAGGTAAAACATgtgtagg ctggagctgcttcg	Forward primer used for the amplification of *waaL*$^{K-12}$ in the creation of CWG1006
WaaLK12b	AGTGAGTTTTAACTCACTTCTTA AACTTGTTTATTCTTAAcatatgaatat cctccttag	Reverse primer used for the amplification of *waaL*$^{K-12}$ in the creation of CWG1006
O8WbdBLRFw	AAGAGGGGTTAGATAATGTGTC ATTTATTATGAAAATTATTTTTG CTACTgtgtaggctggagctgcttc	Forward primer used for the amplification of *wbdB*^{O8} in the creation of CWG1007
CB53	ACTGCGGTGGCCCTTGTGATGAG TTCGTTATGAAAATTATTTTTGC TACTgtgtaggctggagctgcttc	Forward primer used for the amplification of *wbdB*^{O9a} in the creation of CWG1009
CB54	TTAGAGTAATTTATAGGCGTTAA TGGTCTGGGTCGTACAGTTCTCC CACGAcatatgaatatcctcctta	Reverse primer used for the amplification of *wbdB*^{O8} and *wbdB*^{O9a} in the creation of CWG1007 and CWG1009
CB55	ACGCCTATAAATTACTCTAAGGG TGTCAGTTGAGAGTTCTACACGT CTATgtgtaggctggagctgcttc	Forward primer used for the amplification of *wbdC*^{O8} and *wbdC*^{O9a} in the creation of CWG1008 and CWG1010
LG75	GGTTTATGAAATTTATCTCAAAC TAACGAAAAATAAATAAGGAGA TTAACgtgtaggctggagctgcttc	Forward primer used for the amplification of *wbdA*^{O9a} in the creation of CWG1105
LG93	TCATAACGAACTCATCACAAGG GCCACCGCAGTAGCCCTGTTGAT AGCGAcatatgaatatcctcctta	Reverse primer used for the amplification of *wbdA*^{O9a} in the creation of CWG1105
LG76	GCGATGAGTATTTATAACGAATT AAAAAATAAAAATACGGAGAAA TAACGgtgtaggctggagctgcttc	Forward primer used for the amplification of *wbdA*^{O8} in the creation of CWG1104
LG77	TTATCTAACCCCTCTTGTATTCA AAGGCAAAAAATACAAGAGAGA TATTTcatatgaatatcctcctta	Reverse primer used for the amplification of *wbdA*^{O8} in the creation of CWG1104
CB56	TCAGGATTTGCTTTCCAGCAATG TAGTGTAGAGATTGACATAGGC GTCAATcatatgaatatcctcctta	Reverse primer used for the amplification of *wbdC*^{O8} and *wbdC*^{O9a} in the creation of CWG1008 and CWG1010
CB57	tgtgat<u>gaattc</u>accATGAAAATTATTTT TGCTACTGAGCCAATTAAATAC	Forward primer used for the amplification of *wbdB*$^{O9a/O8}$ in the creation of pWQ576, pWQ578, pWQ580 and pWQ582; *Eco*RI restriction site
CB59	ctctaa<u>gaattc</u>accATGAGAGTTCTACA CGTCTATAAGACCTA	Forward primer used for the amplification of *wbdC*^{O9a} in the creation of pWQ579; *Eco*RI restriction site
WbdCR1	gatc<u>aagctt</u>TCAGGATTTGCTTTCCA GCAATGTAGTG	Reverse primer used for the amplification of *wbdC*$^{O9a/O8}$ in the creation of pWQ575, pWQ577, pWQ579, pWQ580, pWQ581, and pWQ582; *Hind*III restriction site
WbdCMalE	TTGAGAGTTCTACACGTCTATAA GACC	FForward primer used for the amplification of *wbdC*^{O9a} in the creation of pWQ575
LG8	gatc<u>aagctt</u>TTAGAGTAATTTATAGG	Reverse primer used for the amplification

	CGTTAATGG	of *wbdB*$^{O9a/O8}$ in the creation of pWQ576 and pWQ578; *Hind*III restriction site
LG45	ctctaagaattcaccTTGAGAGTTCTACA CGTCTATAAGACTTAC	Forward primer used for the amplification of *wbdC*^{O8} in the creation of pWQ577; *Eco*RI restriction site
LG68	ctctaagaattcaccATGAGAGTTCTACA CGTCTATAAGACTTACTATCCCG	Forward primer used for the amplification of *wbdC*^{O8} in the creation of pWQ581; *Eco*RI restriction site
LG104	gatcgatctagaTTATTTAAAATATTTA GCTTTAGCTAAGCC	Reverse primer used for the amplification of *wbdA*^{O8} in the creation of pWQ588; *Xba*I restriction site
LG108	gatcgaccatggtacatcatcatcatcatcatcatc atcatCGTATTGTCATAGATTTACA AGGCG	Forward primer used for the amplification of *wbdA*^{O8} in the creation of pWQ588; *Nco*I restriction site
LCWbdAF1	ccgctcgagATGCGTATTGTCATAGA TTTAC	Forward primer used for the amplification of *wbdA*^{O8} in the creation of pWQ587; *Xho*I restriction site
LCWbdAR2	ccgcagctgAACCCCTCTTGTATTCA AAGGC	Reverse primer used for the amplification of *wbdA*^{O8} in the creation of pWQ587; *Pvu*II restriction site

[a]restriction sites are underlined
[b]non-chromosomal sequence is shown in lower-case letters

Table S2: MS/MS confirmation of the products generated by WbdC^{O9a} and WbdCB^{O9a} using the synthetic GlcNAc-PP-C_{13} acceptor.

MS peak (m/z)	MS/MS peaks (m/z)	Identity[a]
724.8[b]	726.5[b]	Man-GlcNAc-PP-C_{13}
	366	Man-GlcNAc
	204	GlcNAc
	186	Dehydration product of GlcNAc
	168	Double dehydration product of GlcNAc
	138	Double dehydration product of GlcNAc with further neutral loss of CH_2OH
1048.8[b]	1050.5[b]	$(Man)_3$-GlcNAc-PP-C_{13}
	690	$(Man)_3$-GlcNAc
	528	$(Man)_2$-GlcNAc
	366	Man-GlcNAc
	204	GlcNAc
	186	Dehydration product of GlcNAc
	168	Double dehydration product of GlcNAc
1210.8[b]	1212.5[b]	$(Man)_4$-GlcNAc-PP-C_{13}
	852	$(Man)_4$-GlcNAc
	690	$(Man)_3$-GlcNAc
	528	$(Man)_2$-GlcNAc
	366	Man-GlcNAc
	204	GlcNAc
	186	Dehydration product of GlcNAc
	168	Double dehydration product of GlcNAc

[a]Man not ionized
[b]A m/z difference of 1.7 is observed because CE-MS were acquired in negative-ion mode and MS/MS in positive-ion mode

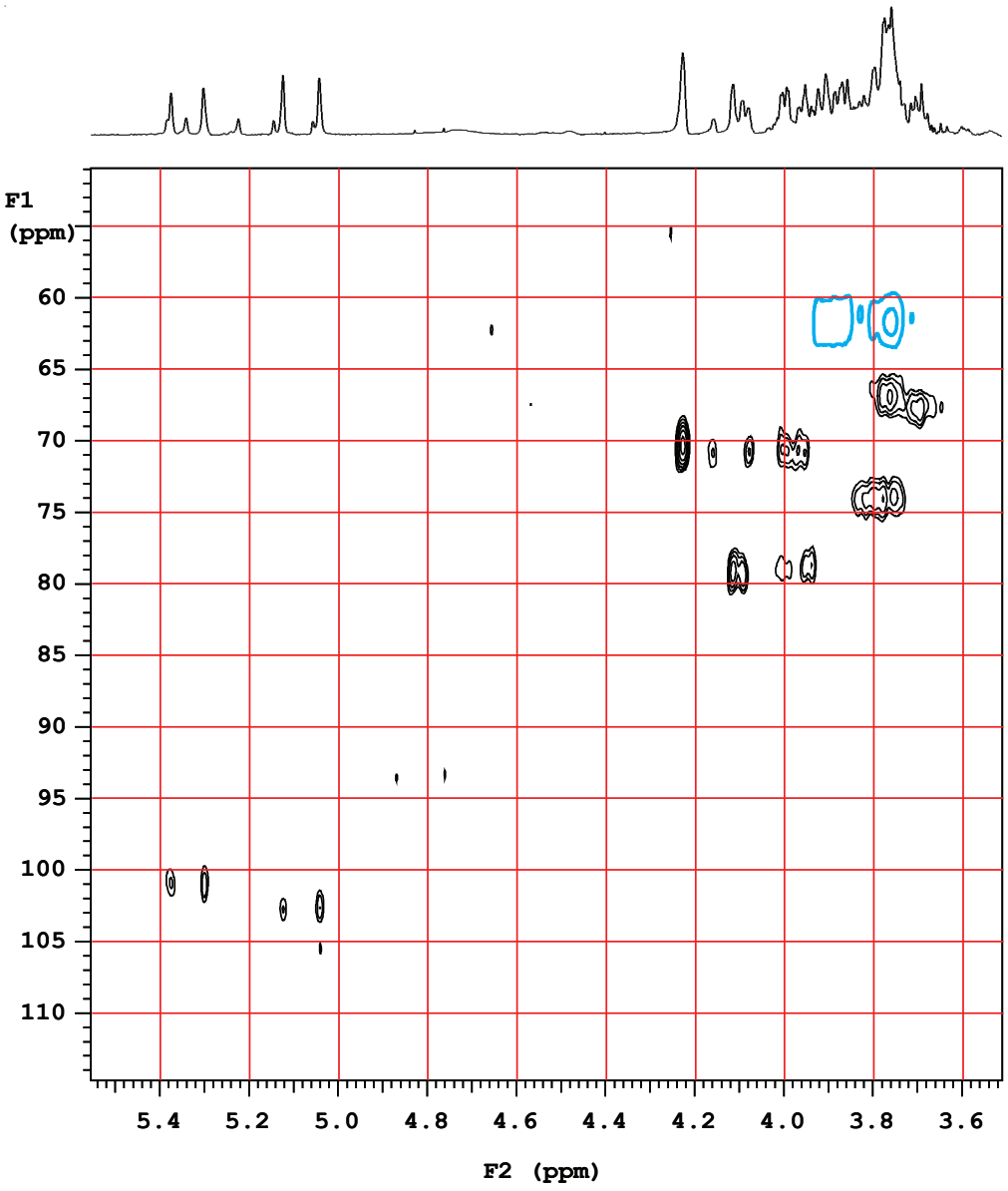

FIGURE S1: ^{1}H-^{13}C gHSQC spectrum for the products synthesized by WbdA^{O9a} using Acceptor B.

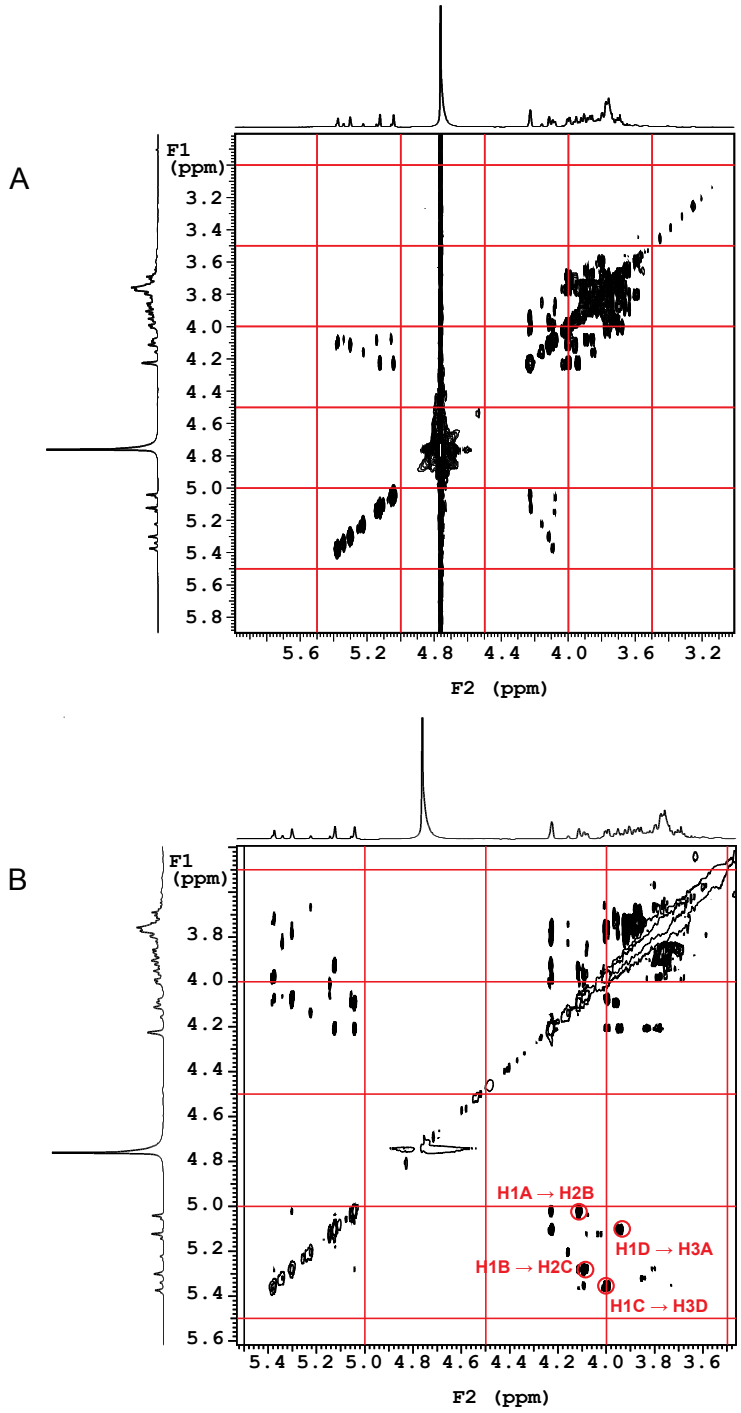

FIGURE S2: gCOSY and tROESY spectra for the products synthesized by WbdA^{O9a} using Acceptor B. *Panel A* shows the gCOSY spectrum and *panel B,* the tROESY spectrum for the reaction products generated by WbdA^{O9a} using Acceptor B.

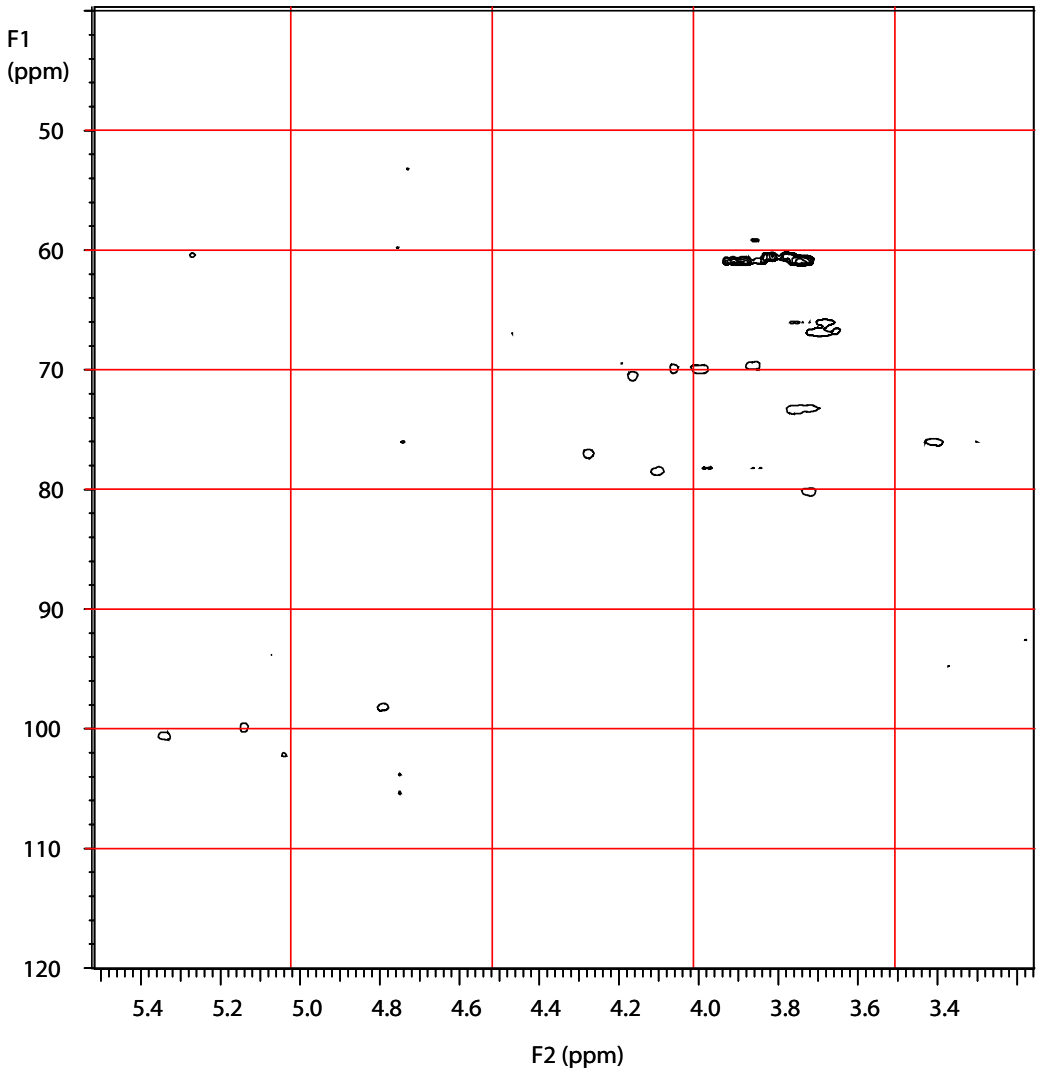

FIGURE S3: ^{1}H-^{13}C gHSQC spectrum for the products synthesized by WbdA^{O8} using Acceptor A.

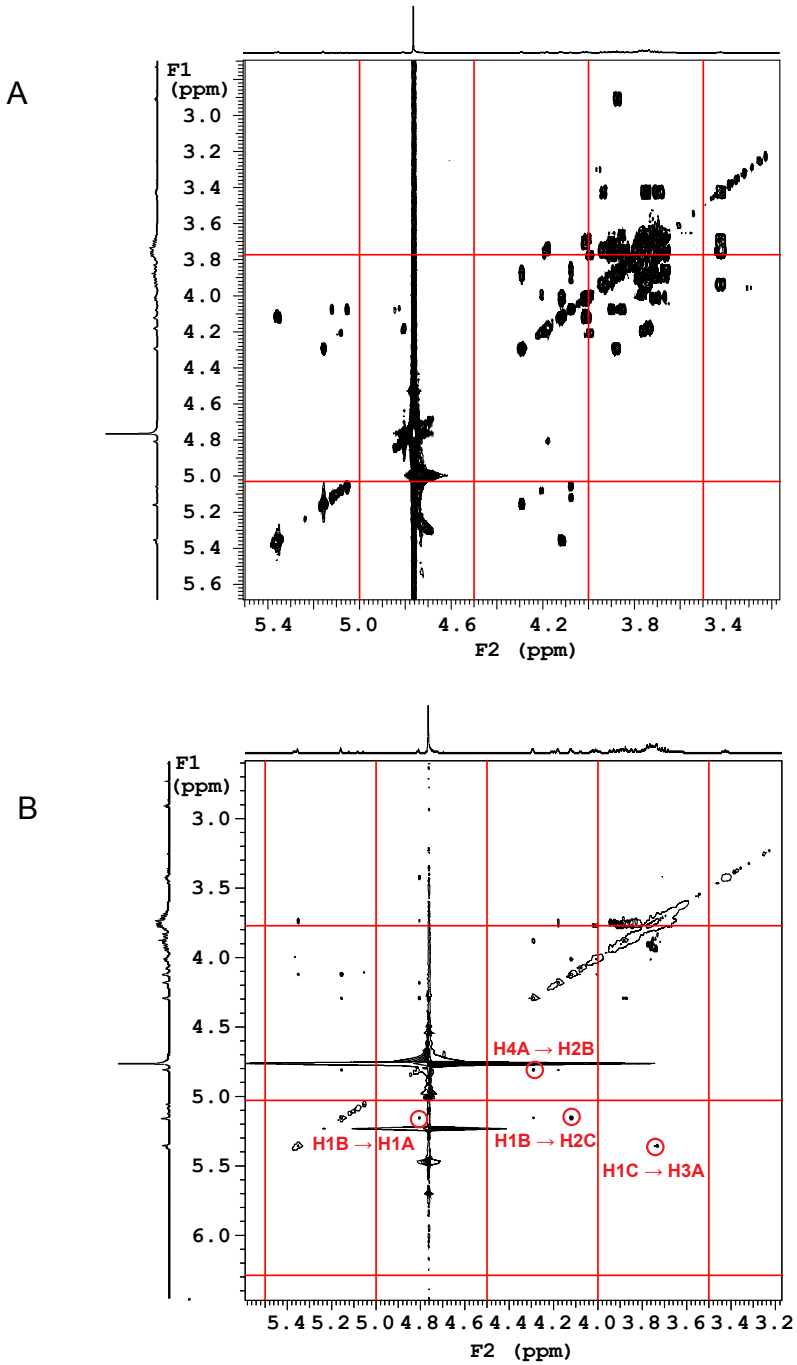

FIGURE S4: gCOSY and tROESY spectra for the products synthesized by WbdA^{O8} using Acceptor A. *Panel A* shows the gCOSY spectrum and *panel B,* the tROESY spectrum for the reaction products generated by WbdA^{O8} using Acceptor A.