Supplementary Data

Identification and biochemical characterization of a novel α2,3-sialyltransferase WbwA from pathogenic *Escherichia coli* serotype O104

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Supplementary Figure 1. Sequence comparisons of WbwA^{O104} and sites of mutations. Alignments of sialyltransferases were done using ClustalW2 (35) and were adjusted slightly so that the HP motifs were aligned. The alignments were edited using GeneDoc (36, http://www.psc.edu/biomed/genedoc). WbwA was from *E. coli* O104, NST from *Neisseria meningitidis*, WaaH from *Salmonella enterica*, PM0188 from *Pasteurella multocida* and ST3Gal-I was human. HP mutation sites for WbwA are shown in light blue, whereas the HP motifs in other sequences are shown in dark blue. The ED mutations for WbwA are shown in coral red, whereas the E(D)E(D)G motifs in other sequences are shown in pink. ST3Gal1 has an HP sequence but does not have an E(D)E(D)G motif.

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           0         *        20         *        40         *        60         *        80         *       100
WbwA     : ----------------------------------------MKMRNNVFVFDSPYCLLIYCILFNHQLH-----------------DTIYIYSDNVSLRDINFPG---- :  47
NST      : MFNLSEWSFRDMGLKKACLTVLCLIVFCFGIFYTFDRVNQGERNAVSLLKDKLFNEEGEPVNLIFCYTILQMKVAERIMAQHPGERFYVVLMSENRNEKYDYYFNQIKD : 109
WaaH     : ------------------------------------------------------------MFLFICMTNLQLLIARSIIEKEQLKSVDILFIGDVHNVKNQYYLKKIQP :  49
PM0188   : MKNRRLNFKLFFLIIFSLFSTLSWSKTITLYLDPASLPALNQLMDFTQNNEDKTHPRIFGLSRFKIPDNIITQYQNIHFVELKDNRPTEALFTILDQYPGNIELDIHLN : 109
ST3GalI  : ----------------------------------------------------MVTLRKRTLKVLTFLVLFIFLTSFFLNYSHTMVATTWFPKQMVLELSENLKRL :  53

            *       120         *       140         *       160         *       180         *       200         *
WbwA     : -KERYIIKKGNNKTSKIFYYFCFLFRL-IINPKLRKLISNRKDYRFLGQDHLFFSKPFLNEFILLEDDGLANYRYPH------------YSRLYKLIIGGHTFGRSTKVN : 142
NST      : KAERAYFFHLPYGLNKSFNFIPTMAELKVKAMLLPKVKRIYLASLEKVSIAAFLSTYPDAEIKTFDDG-----TGN-----------LIQSSSYLGDEFSVNGTIKRNF : 202
WaaH     : LCRHSSIVSQVSKFAAFKTIHRTRYAKKIMESYAKEYHTVFFANFHVPLIHHILSCISFSEINTFDDG-----TNN------------INQKS--IMYENKNISSTSKL : 139
PM0188   : IAHSVQLIRPILAYRFKHLDRVSIQRLNLYDDGSMEYVDLEKEENKDISAEIKQAEKQLSHYLLTGKIKFDNPTIARYVWQSAFPVKYHFLSTDYFEKAEFLQPLKEYL : 218
ST3GalI  : IKHRPCTCTHCIGQRKLSAWFDERFNQTMQPLLTAQNALLEDDTYRWWLRLQREKKPNNLNDTIKELFRVVPGNVDPMLEKRSVGCRRCAVVGNSGNLRESSYGPEIDS : 162

           220         *       240         *       260         *       280         *       300         *       320
WbwA     : KILLSGMIDIIDPTINDKVEFFDLIAAWEKLNSVQKKEINHIFNYCIEDELVADVMILTQPLSEDGFISENEKIRLYDEIIKEYDDKKIVIRQHPRELTDYSLYFKDVK : 251
NST      : ARMMIGDWSIAKTRNASDEHYTIFKGLKNIMDDGRRKMTYLPLFDASELKAGDETGGTVRILLGSPDKEMKEISEKAAKNFNIQYVAP-----HP------RQTYGLSG : 300
WaaH     : IRKLMGRKYHKDEILKLDAKDYTLFPNRTNIIEKTEGIILVHHNGLPDTNNGFKKVLLGTVYTDALKNKEDECVFLQHLQRFIKKKAVDIYIPHPRYDSHQFNGVLNVN : 248
PM0188   : AENYQKMDWTAYQQLTPEQQAFYLTLVGFNDEVKQSLEVQQAKFIFTGTTTWEGNTDVREYYAQQQLNLLNHFTQAGGDLFIGDHYKIYFKG-HPRGGEINDYILNNAK : 326
ST3GalI  : HDFVLRMNKAPTAGFEADVGTKTTHHLVYPESFRELGDNVSMILVPFKTIDLEWVVSAITTGTISHTYIPVPAKIRVKQDKILIY--------HPAFIKYVFDNWLQGH : 263

              *       340         *       360         *       380         *       400         *
WbwA     : VNKVKAPVELVVLNSP--MIKTAVTLFSGGIFNIPCREKIFKGTSFSPLLSKHFGNISNKFEVKGKK---------------------- : 316
NST      : VTTLNSPYVIEDYILREIKKNPHTRYEIYTFFSGAALTMKDFPNVHVYALKPASLPEDYWLKPVYALFTQSGIPILTFDDKD----- : 382
WaaH     : SEMIAEDIILEYLEQG--MSLEIYGFNSTVQYNLNNISTIKNYKITSP-FLKDSFNHGLGFDFNQVSV-------------------- : 313
PM0188   : NITNIPANISFEVLMMTGLLPDKVGGVASSLYFSLPKEKISHIIFTSNKQVKSKEDALNNPYVKVMRRLGIIDESQVIFWDSLKQL- : 412
ST3GalI  : GRYPSTGILSVIFSMHVCDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDADFESNVTATLASINKIRIFKGR---------- : 340
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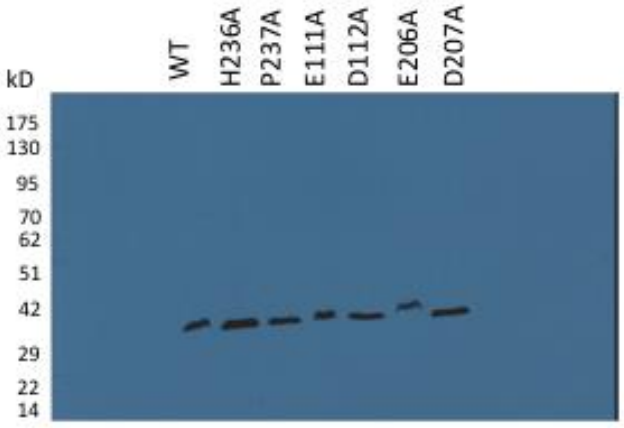
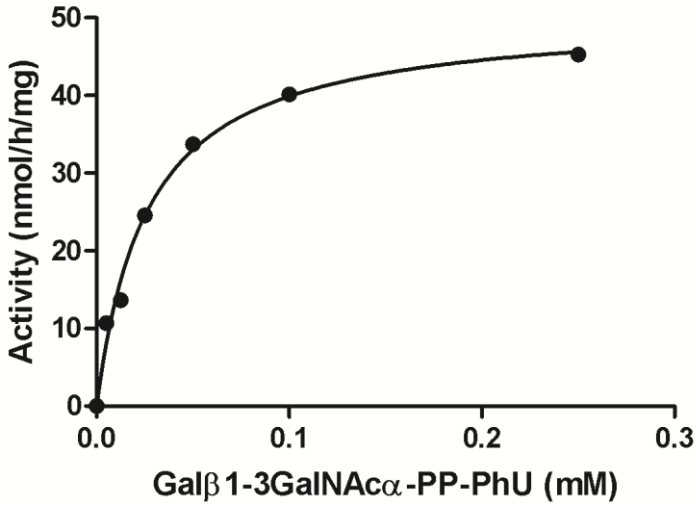
Supplementary Figure 2

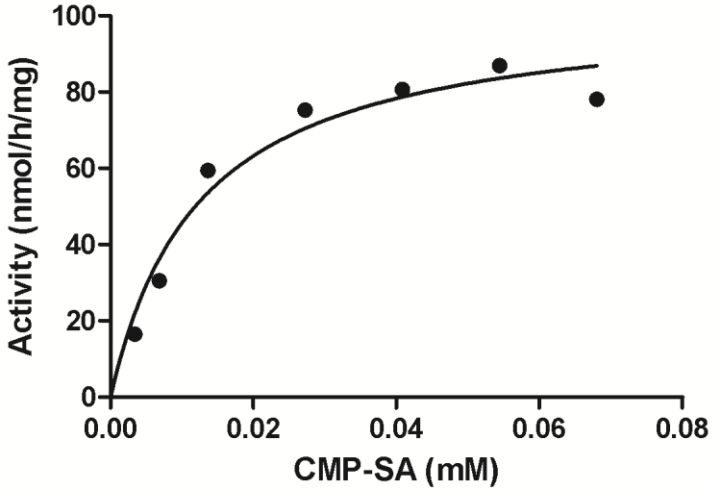
Western blot of wild type and mutant WbwA. Lysates of bacteria containing wild type WbwA (WT) and six WbwA mutants wer run on SDS-PAGE and visualized on a Western blot after treatment with mouse anti-His antibody, goat anti-mouse antibody linked to horseradish peroxidase, and Lightning Plus ECL Chemiluminescence Substrate. All proteins show only one band at about 38 kDa in mass.



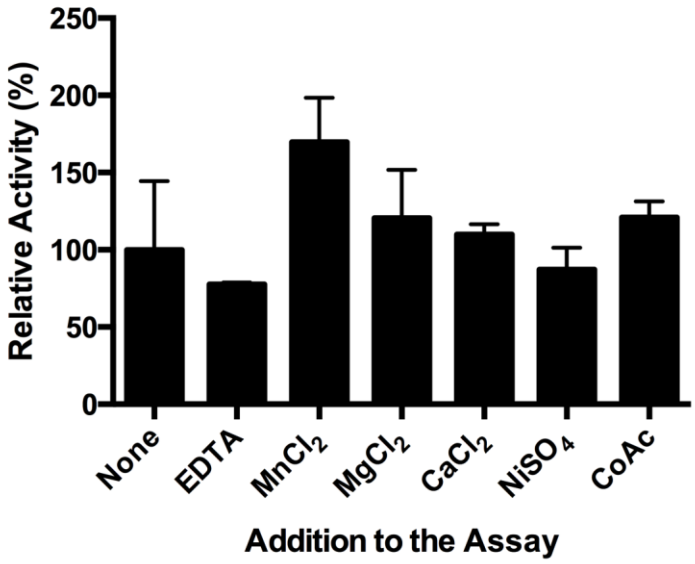
Supplementary Figure 3A. Kinetics of WbwA^{O104} using acceptor Galβ1-3GalNAcα-PP-PhU and bacterial lysate. Assays for SiaT WbwA were carried out as described in the Methods section. The CMP-Sia concentration in the assays was 0.8 mM. The apparent K_M for the acceptor Galβ1-3GalNAcα-PP-PhU was 0.02 mM and the apparent V_{max} was 50 nmol/h/mg.



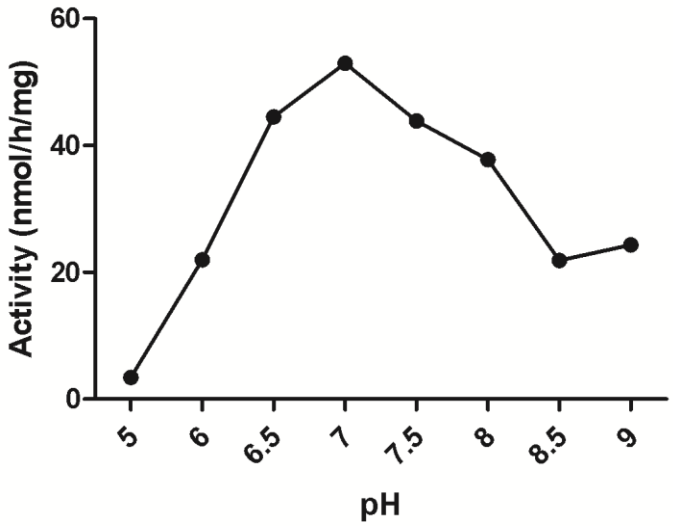
Supplementary Figure 3B. Kinetics for CMP-sialic acid of WbwA^{O104} using acceptor Galβ1-3GalNAcα-PP-PhU and bacterial lysate. Assays for SiaT WbwA were carried out as described in the Methods section. The acceptor concentration in the assays was 0.1 mM. The apparent K_M value for CMP-sialic acid (CMP-SA) was 0.013 mM and the apparent V_{max} was 90 nmol/h/mg.



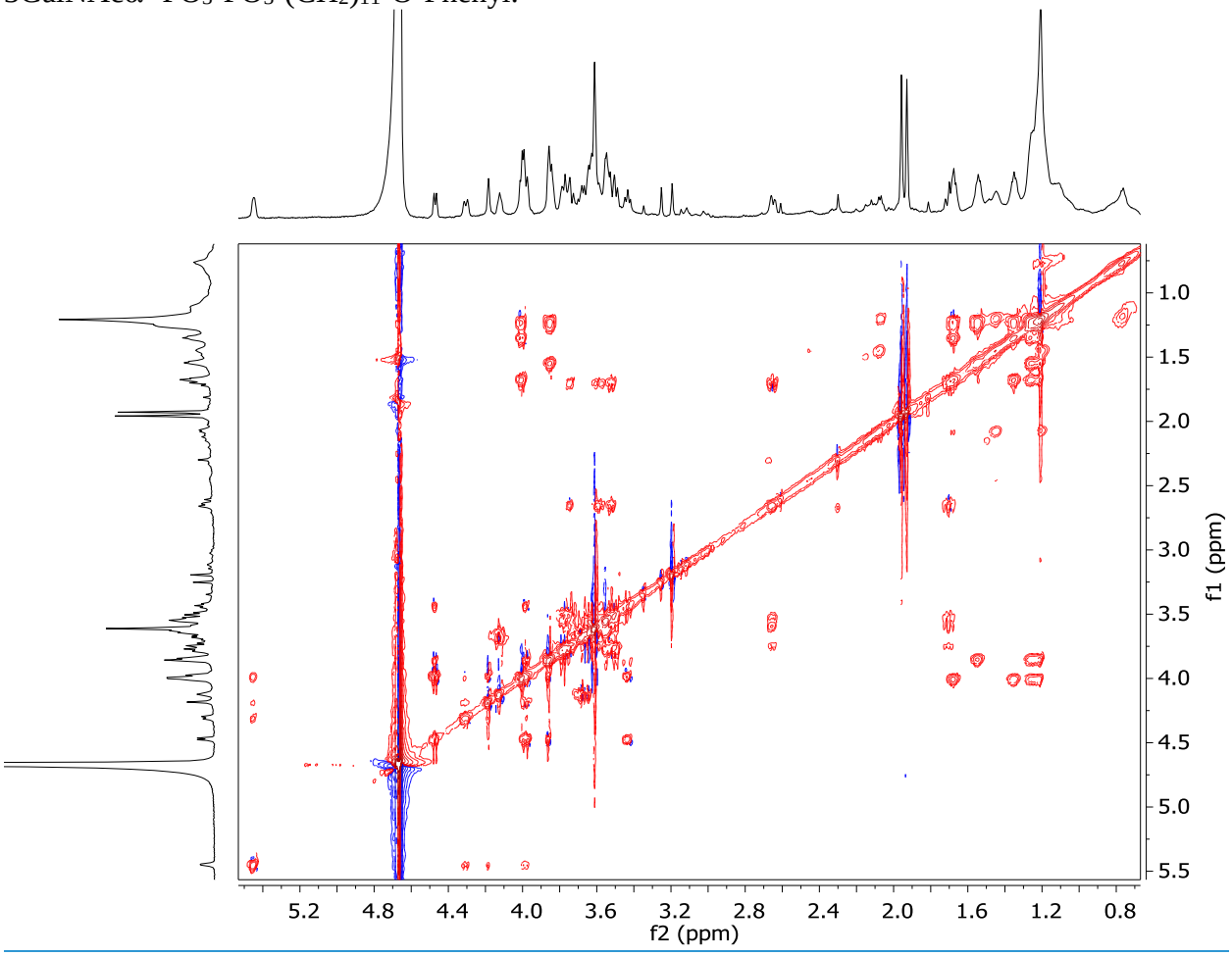
Supplementary Figure 4. Metal ion dependence of $WbwA^{O104}$ activities.
Sialyltransferase assays were carried out using CMP-sialic acid donor and Galβ1-3GalNAc-PP-PhU acceptor as described in the Methods section. Metal ions were added at 5 mM concentration to the assays. Assays with EDTA at 5 mM and no addition (None) served as negative controls. CoAc, Cobalt acetate. No metal ions were required but Mn^{2+} showed a slight stimulation of activity.



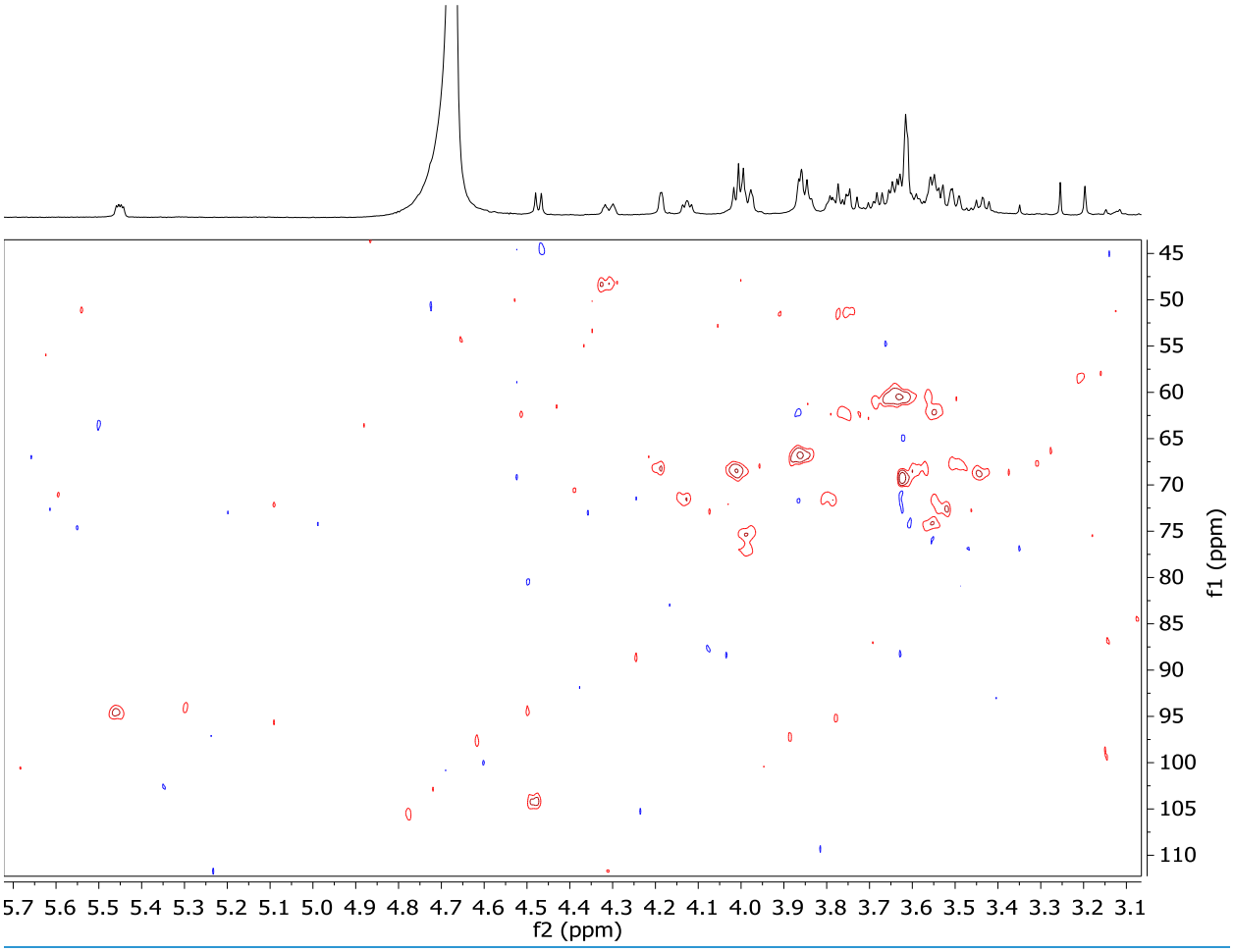
Supplementary Figure 5. $WbwA^{O104}$ activity at different buffer pH values.
Sialyltransferase activity of $WbwA^{O104}$ was assayed as described in the Methods section at different buffer pH values.



Supplementary Figure 6A. 600 MHz TOCSY NMR spectrum of WbwA^{O104} product Sialylα2-3Galβ1-3GalNAcα- PO_3-PO_3-$(CH_2)_{11}$-O-Phenyl.



Supplementary Figure 6B. 600 MHz HSQC NMR spectrum of $WbwA^{O104}$ product Sialylα2-3Galβ1-3GalNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-Phenyl.



Supplementary TABLE I. Glycosyltransferases potentially related to WbwA

Enzyme	GT family	UniProt #	%Identity
β3-Gal-transferase, WbwC (*E. coli* O104)	2	Q93NP5	11.8
α4-Gal-transferase, WbwB, putative (*E. coli* O104)	4	Q93NP7	14.7
α3-SiaT, ST3Gal1, human	29	Q11201	13.6
α3-SiaT, CstI (*Campylobacter jejuni*)	42	D2KQ02	13.1
α3/8-SiaT, bifunctional, CstII (*Campylobacter jejuni*)	42	Q9L9Q5	12.7
α3-SiaT, WbwA (*E. coli* O104)	**52**	**Q93NP9**	**100**
α3/6-SiaT, NST bifunctional (*Neisseria meningitidis*)	52	C6S6J1	12.4
α3-SiaT, (*Neisseria gonorrhea*)	52	P72074	14.2
α3-SiaT (*Neisseria meningitidis)*	52	P72097	13.0
α3-SiaT, Lst, PMST2 *(Pasteurella multocida)*	52	Q9CNC4	14.3
α2-Glc-transferase, WaaH (*Salmonella enterica)*	52	Q7B6E0	14.6
α3-SiaT, PdST, multifunctional (*Pasteurella dagmatis)*	80	C9PR45	12.9
α3-SiaT (*Photobacterium phosphoreum)*	80	A5LHX0	13.2
α6-SiaT, PM0188 (*Pasteurella multocida)*	80	Q9CP67	14.8
α6-SiaT (*Photobacterium sp)*	80	A8QYL1	11.7

Glycosyltransferases are listed that are encoded by the *E. coli* O104 gene cluster (WbwC, WbwA, WbwB), as well as other bacterial mono- and multi-functional sialyltransferases, possibly related to WbwA, from CAZy GT families 42, 52 and 80, as well as a Glc-transferase from the GT52 family. Human sialyl-T synthase (ST3Gal1) is also included. Uniprot alignments were used to determine the percentage of sequence identity with WbwA from *E. coli* O104. The UniProt ID numbers are shown. The identities of these enzymes with WbwA are low. With the exception of putative Gal-transferase WbwB, all of the bacterial enzymes have an HP sequence, and with the exception of Gal-transferase WbwC, all of the bacterial enzymes have at least one (E(D)-E(D)-G motif.

Supplementary TABLE II. Inhibition of ST3Gal1 and WbwA activities by bis-imidazolium salts

Compound	Number of carbons	Inhibition (%)	
		WbwA	ST3Gal1
QT147, 1,4-bis(3-methyl-1*H*-imidazolium-1-yl)butane dichloride	4	10	<1
QT141, 1,8-bis(3-methyl-1*H*-imidazolium-1-yl)octane dichloride	8	12	<1
QT142, 1,14-bis(3-methyl-1*H*-imidazolium-1-yl)tetradecane dichloride	14	19	<1
QT140, 1,15-bis(3-methyl-1*H*-imidazolium-1-yl)pentadecane dichloride	15	48	<1
QT138, 1,16-bis(3-methyl-1*H*-imidazolium-1-yl)hexadecane dichloride	16	66	>90
QT136, 1,18-bis(3-methyl-1*H*-imidazolium-1-yl)octadecane dichloride	18	64	>90
QT135, 1,20-bis(3-methyl-1*H*-imidazolium-1-yl)eicosane dichloride	20	90	94
QT148, 1,20-bis(3-methyl-1*H*-imidazolium-1-yl)eicosane dimesylate	20	96	>99
QT149, 1,22-bis(3-methyl-1*H*-imidazolium-1-yl)docosane dimesylate	22	88	>99

Sialyltransferase assays using purified ST3Gal1 and WbwA in bacterial lysates were performed as described in Material and Methods. Inhibitor concentration in the assays was 0.5 mM. All assays contained 10% MeOH. The concentration of ST3Gal1 acceptor Galβ1-3GalNAcα-Bn was 0.5 mM. For WbwA assays, the concentration of the acceptor Galβ1-3GalNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl was 0.1 mM. The percentage of inhibition compared to control activity without inhibitor is shown. Number of carbons, the length of the aliphatic carbon chain linking the two imidazolium groups in the chloride or mesylate salts.