Supplementary Information (SI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2024

Supporting Information

Enzymatic Synthesis of *N*-Formylated Sialosides *via* a Five-Enzyme Cascade

Zi-Xuan Hu, Shu-Rui Li, Qing-Jun Xia, Ting Wang, Josef Voglmeir, Göran Widmalm, and Li Liu

Contents

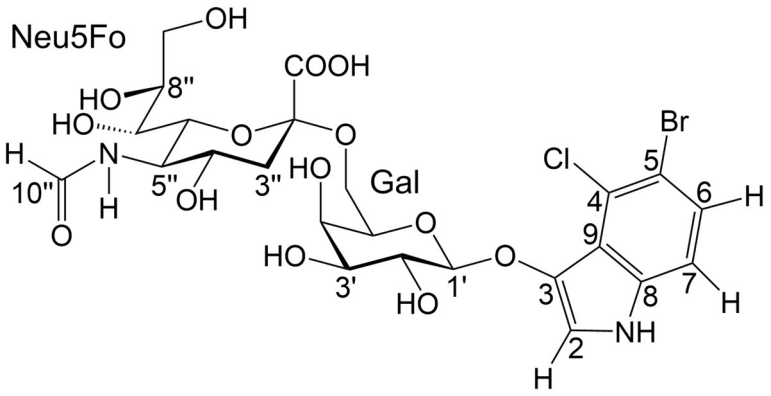

Figure S1. Schematic structure of Neu5FoGalX with selected atom labeling.

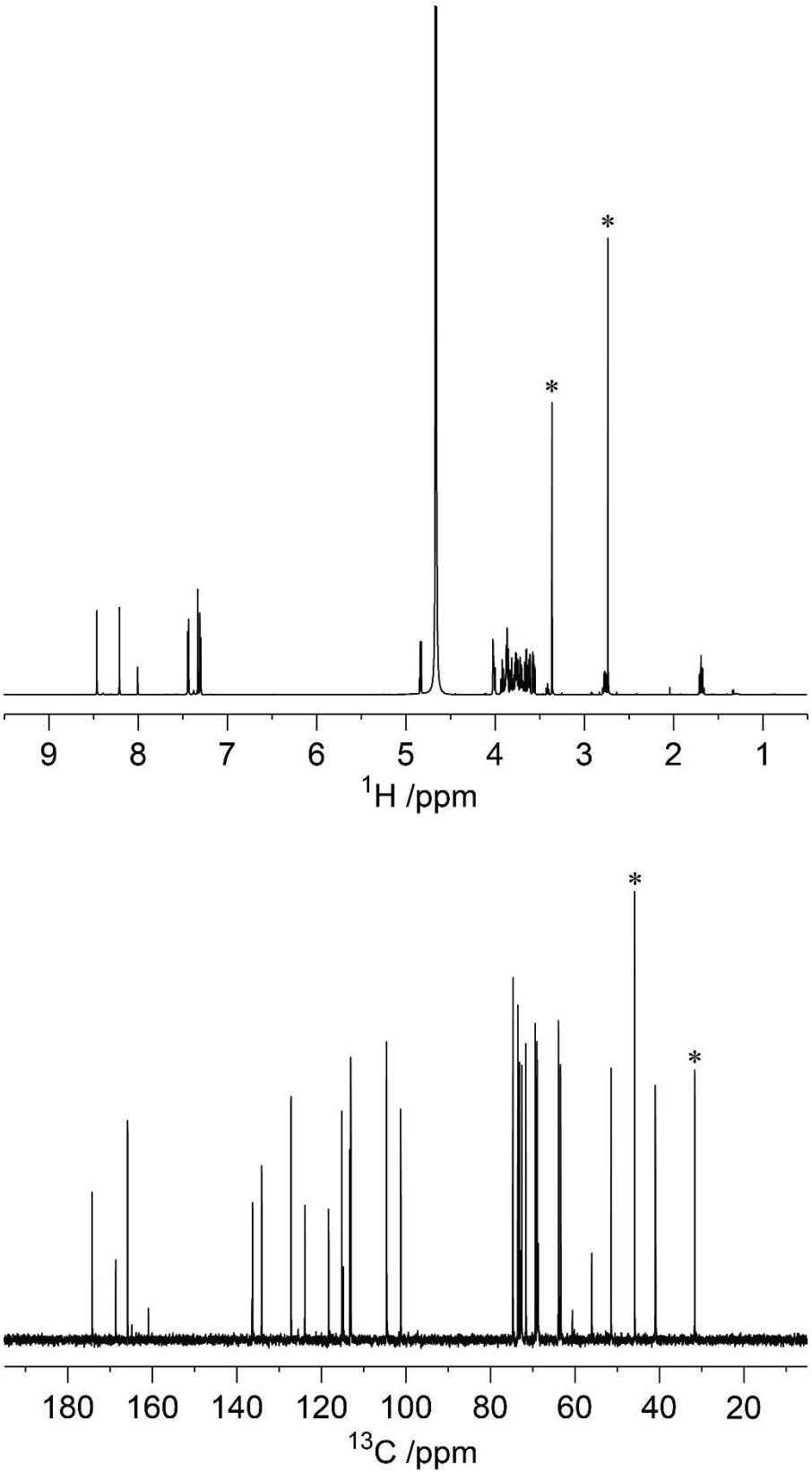

Figure S2. ^{1}H and ^{13}C NMR spectra (top and bottom, respectively) of a Neu5FoGalX-preparation. Resonances from an impurity are indicated by asterisks. The ^{1}H NMR signals originating from the small molecule impurity were possible to suppress by a diffusion-edited ^{1}H NMR experiment.

Table S1. Negative ESI-MS of pseudomolecular ion $[M-H]^-$ from Neu5FoGalX, which as a neutral compound has a molecular formula of $C_{24}H_{30}BrClN_2O_{14}$.

Experiment (*m*/*z*)	Relative intensity	Simulation (*m*/*z*)	Relative intensity
683.0508	74	683.0496	74
684.0578	28	684.0530	20
685.0502	100	685.0476	100
686.0503	30	686.0509	27
687.0485	32	687.0448	29
688.0569	13	688.0480	7

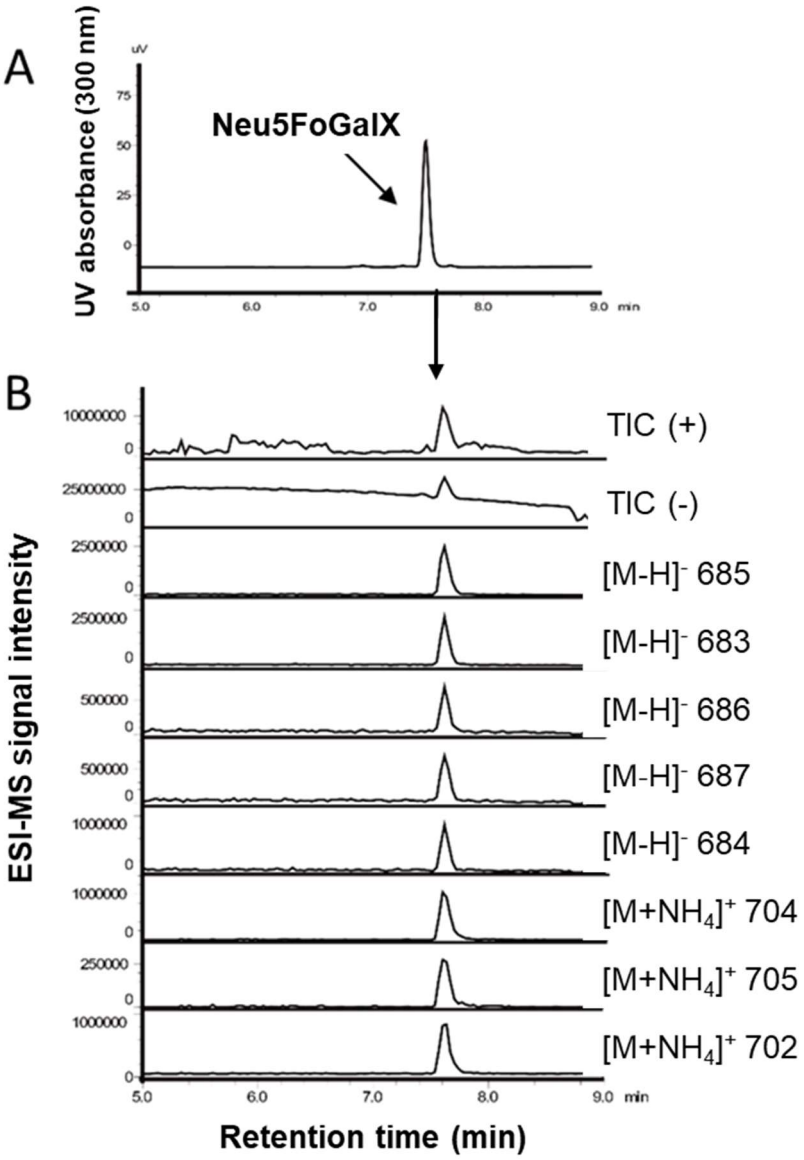

Figure S3. UPLC-ESI-MS identification of Neu5FoGalX after five-enzyme synthesis reaction. (**A**) UV absorbance spectra (300 nm) of Neu5FoGalX. (**B**) Total ion count (TIC) and extracted ion count (EIC) chromatogram profiles of Neu5FoGalX. The conversion was 100 % calculated from the UV-ratios of Neu5FoGalX to GalX.

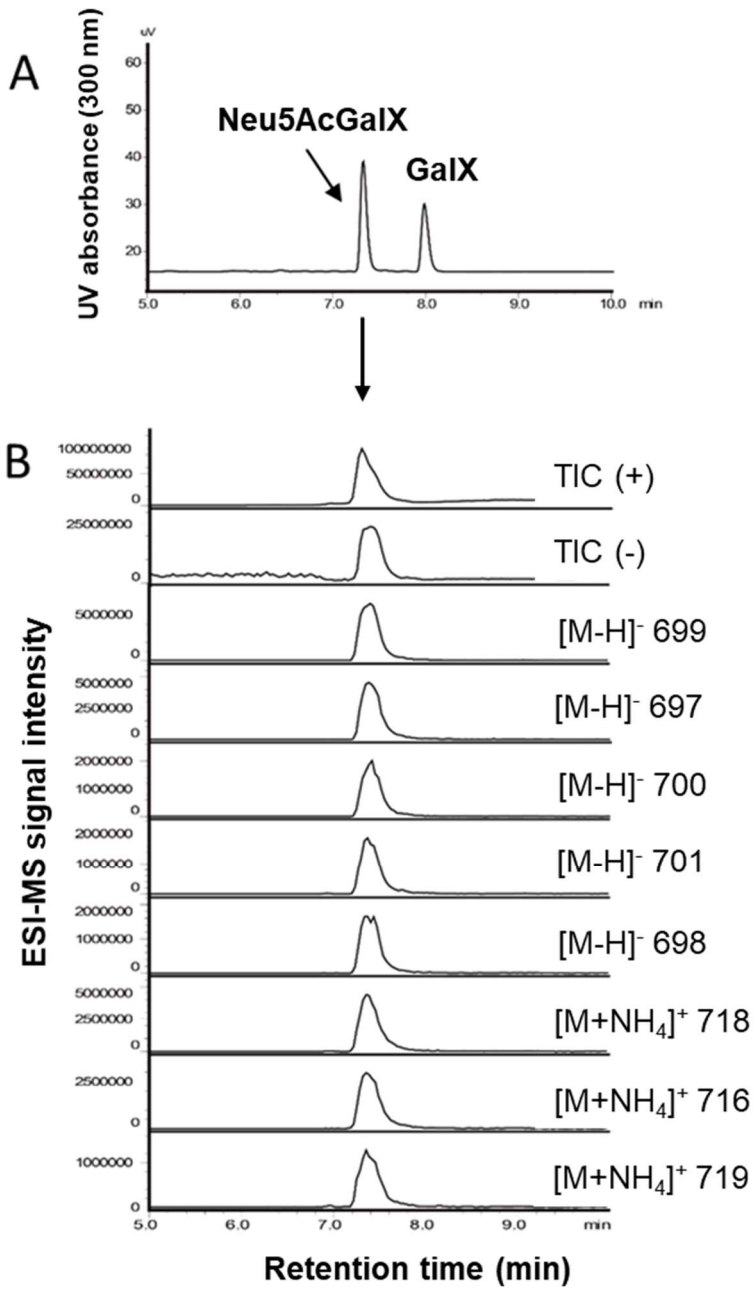

Figure S4. UPLC-ESI-MS identification of Neu5AcGalX after five-enzyme synthesis reaction. (**A**) UV absorbance spectra (300 nm) of Neu5AcGalX. (**B**) Total ion count (TIC) and extracted ion count (EIC) chromatogram profiles of Neu5AcGalX. The conversion was 70% calculated from the UV-ratios of Neu5AcGalX to GalX.

Table S2. UPLC elution procedure of preparative C18 reverse phase separation for Neu5FoGalX purification.

Time (min)	Module	Command	Value
0	Pumps	Solvent B Conc.	10
40	Pumps	Solvent B Conc.	30
41	Pumps	Solvent B Conc.	90
50	Pumps	Solvent B Conc.	90
55	Pumps	Solvent B Conc.	10
75	Pumps	Solvent B Conc.	10
75	Controller	Stop	

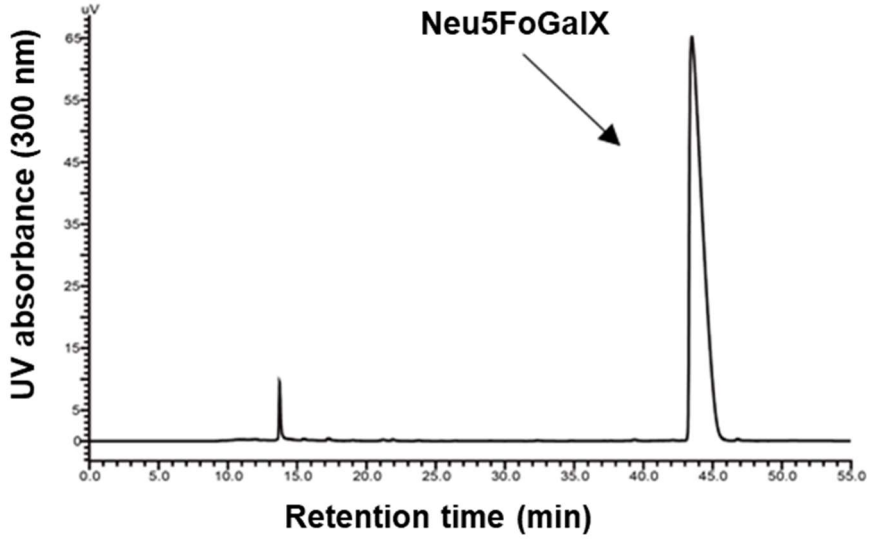

Figure S5. UV spectra for Neu5FoGalX purification by UPLC with C18 preparative reverse phase column.

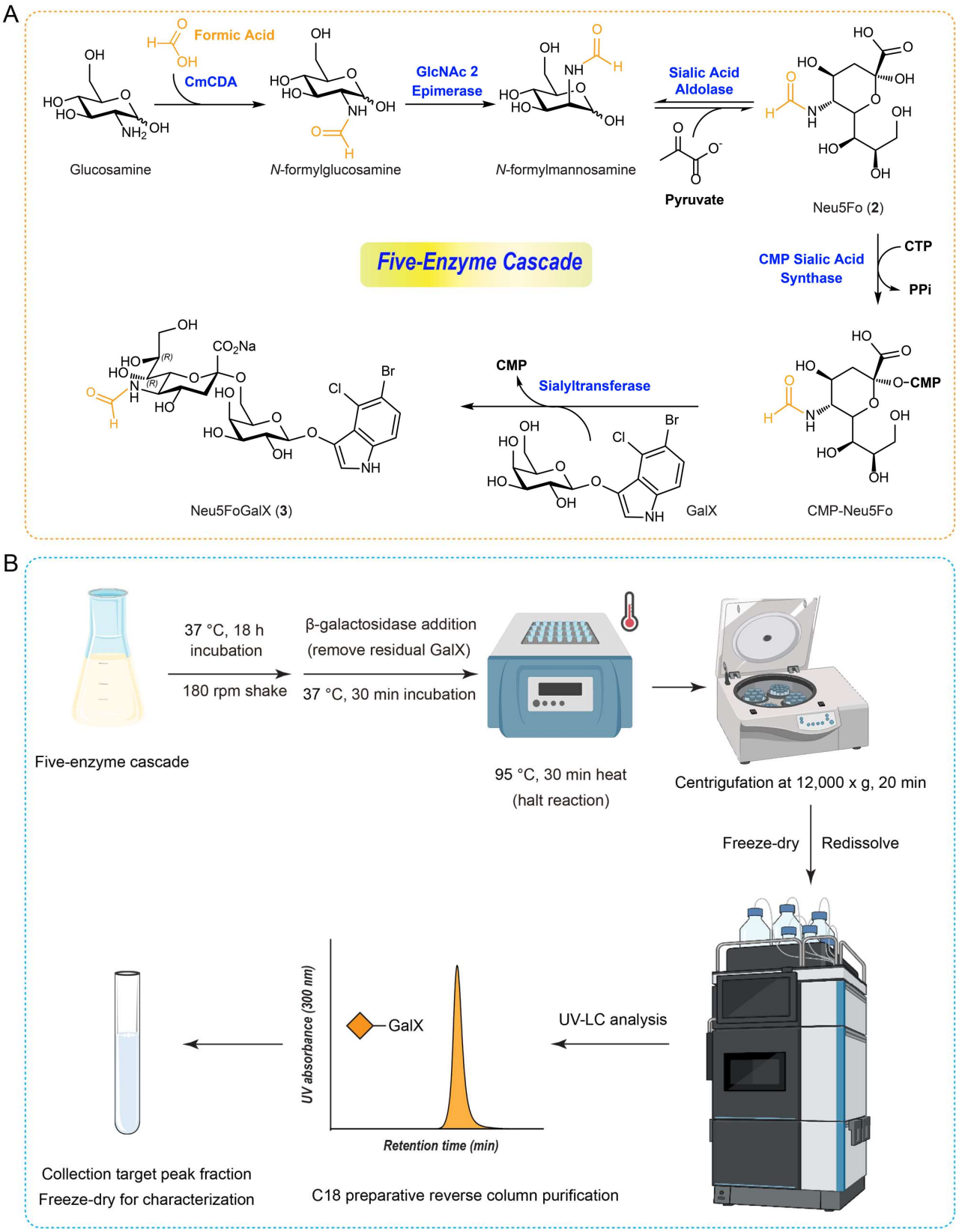

Figure S6. Scheme of five-enzyme cascade (**A**) and procedure of synthesis and purification for Neu5FoGalX (**B**).