



S3 Fig. ¹H NMR spectrum (D₂O, 600 MHz) of SialH trans-sialylation products. To confirm the obtained reaction products, mixed samples were analysed by NMR. As expected, the ¹H NMR spectrum of the reaction products reveals the presence of 3'-sialyllactose (3'SL) and 6'-sialyllactose (6'SL). Of special interest was the signal at δ 4.537 (Gal H-1) ppm, rendered by the unknown reaction product. Additionally, a possible downfield shift of Glc H-3 (δ 3.56 \rightarrow δ 3.73 ppm) suggested sialylation at O-3 of the Glc residue. Since there are no extra Neu5Ac axial or equatorial H-3 signal in the ¹H NMR spectrum, the Neu5Ac signals at δ 1.795 (Neu5Ac H-3a) and δ 2.734 (Neu5Ac H-3e) ppm also suggest an (α 2,3)-linked Neu5Ac residue. Thus, the unknown sialylation product was identified as 3-sialyllactose (3SL).