Supporting figures S1-5

High-throughput profiling of protein N-glycosylation by MALDI-TOF-MS employing linkage-specific sialic acid esterification

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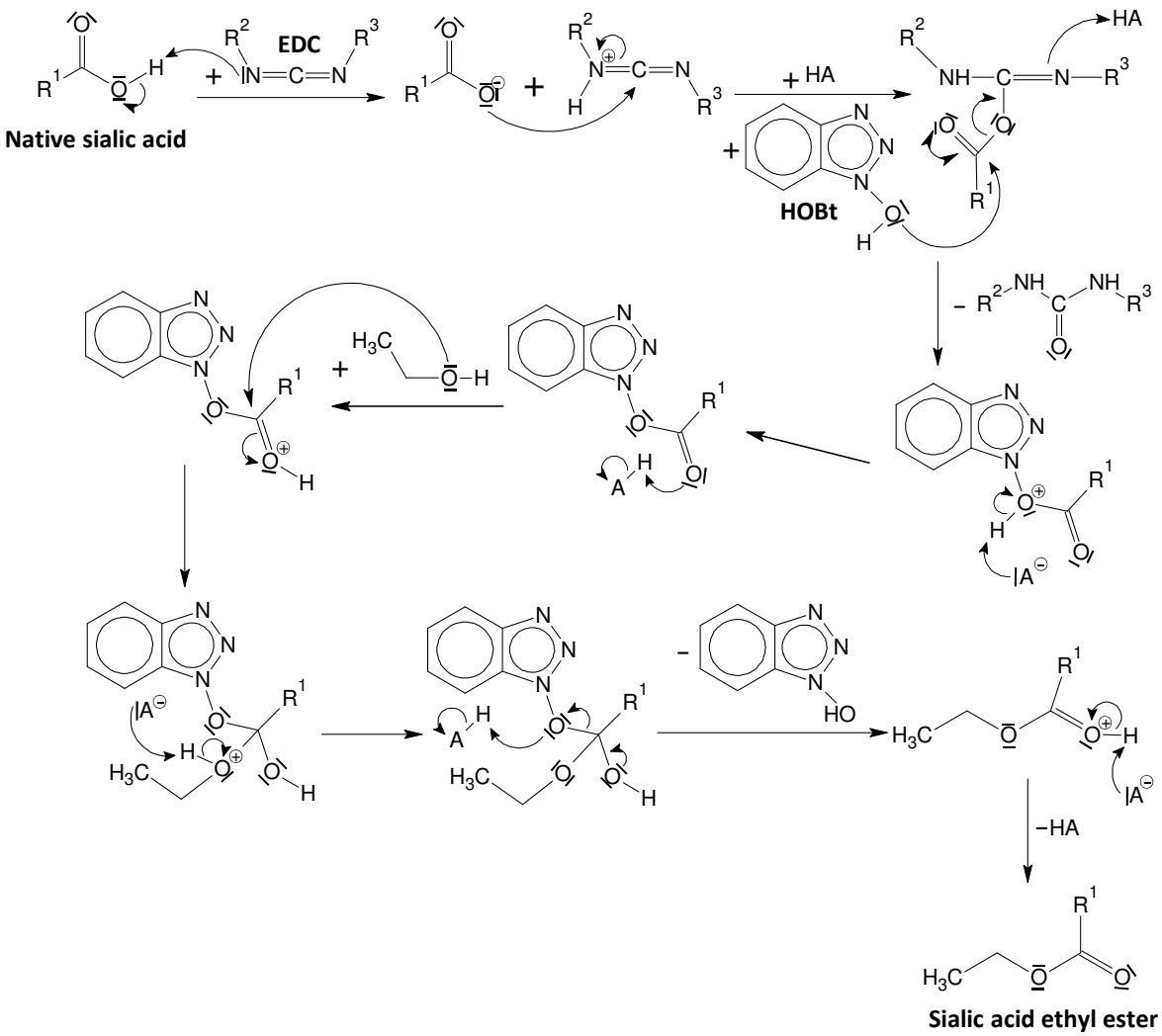

Figure S-1. Reaction mechanism of ethyl esterification, assisted by EDC and HOBt activation, of a carboxyl group as present on an α2,6-linked sialic acid. R1 = glycan, R2 = 1-ethyl, R3 = 3-dimethylaminopropyl, A = base, HA = acid.

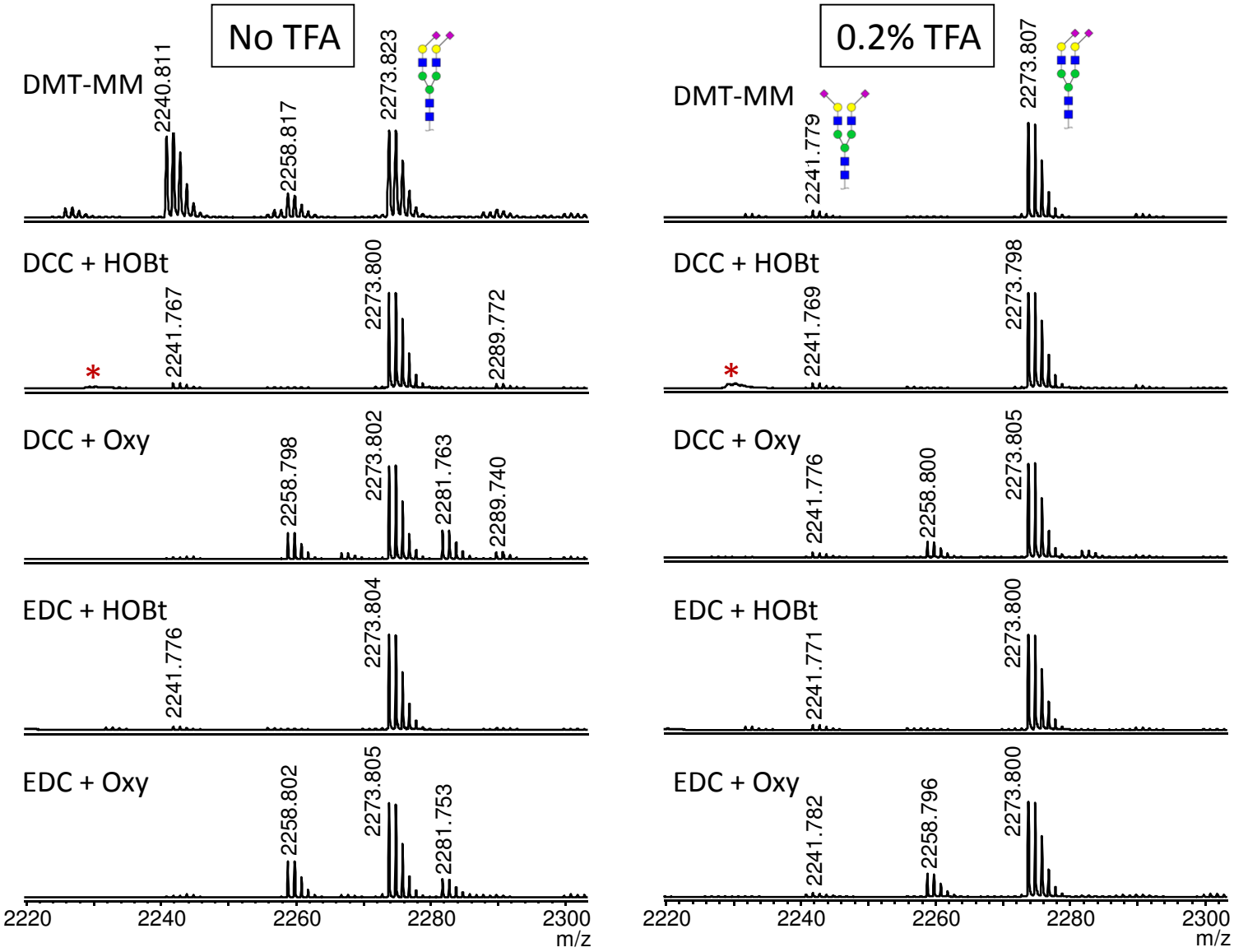

Figure S-2. RP MALDI-TOF-MS analysis of PNGase F-released plasma N-glycome after methyl esterification. The samples were incubated in a variety of activator combinations and acidic conditions in methanol. Shown are the reaction products obtained for the most abundant disialylated N-glycan. The mass of 2273.823 Da corresponds to a fully methyl esterified reaction product $[M+Na]^+$, whereas masses of 2281.763 Da and 2289.740 Da correspond to species lacking one or two methyl groups with resulting sodium salt formation ($[M-H+2Na]^+$ and $[M-2H+3Na]^+$). Reaction products at 2258.817 Da indicate amidation of a carboxylic acid group, whereas 2241.767 Da indicates a lactonized reaction product. EDC + HOBt was selected as most promising on the basis of modification completeness and lack of side reactions, without the need for acid. The asterisk indicates metastable peaks.

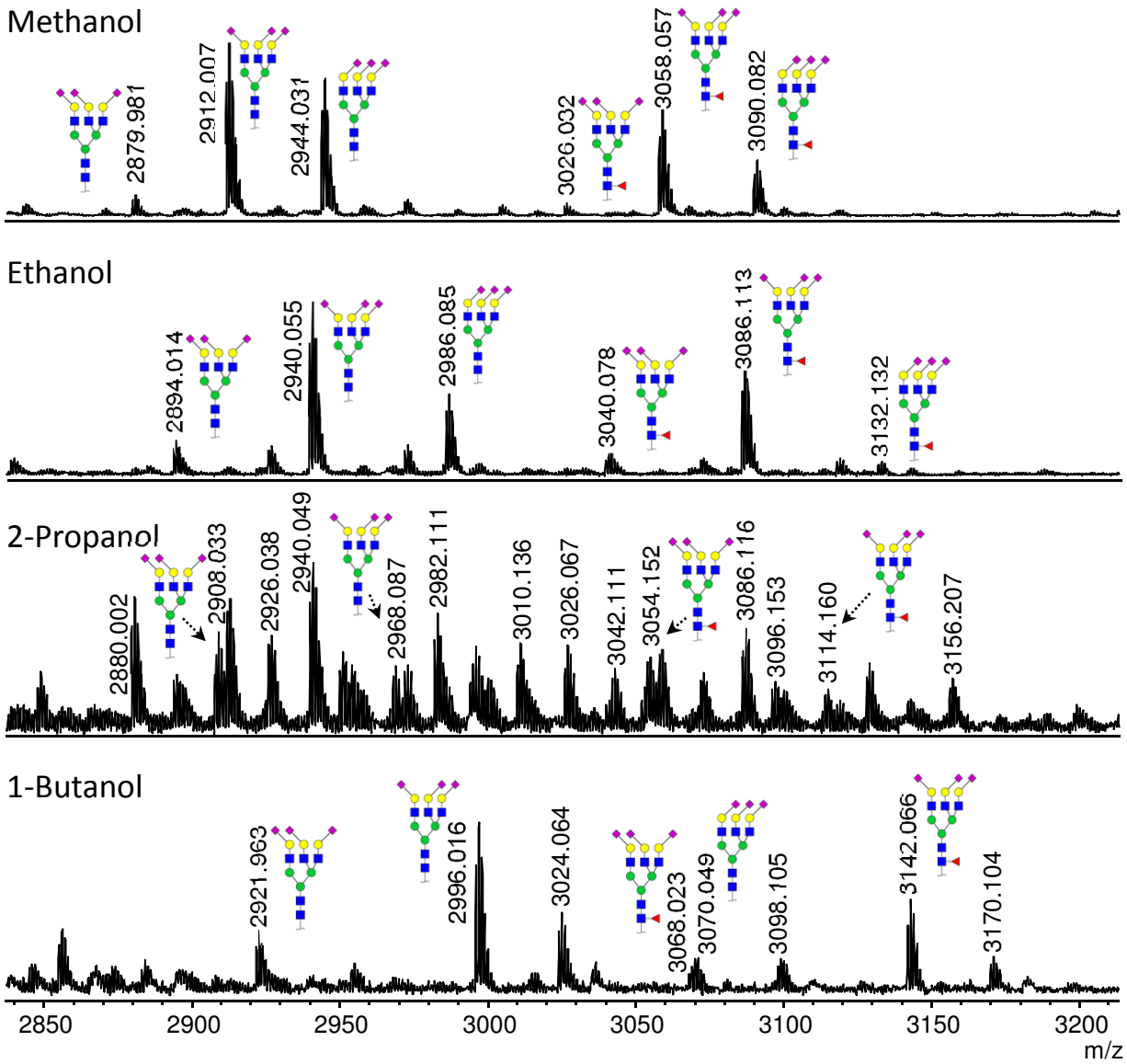

Figure S-3. RP MALDI-TOF-MS of the plasma N-glycome after esterification with methanol, ethanol, 2-propanol and 1-butanol as solvents and alkyl donors using EDC + HOBt activators. Shown is the part of the mass spectrum covering trisialylated compositions with various sialic acid linkage types.[50] Mass differences can be observed between lactonization, and esterification by methanol (32.026 Da), ethanol (46.042 Da), 2-propanol (60.058 Da) and 1-butanol (74.073 Da). All alcohols show to be an alkyl group donor for linkage-specific sialic acid derivatization, while methanol and ethanol showed the highest reaction efficiency with the chosen conditions. Relative ratios of the lactonized and alkyl esterified signals differ per alcohol.

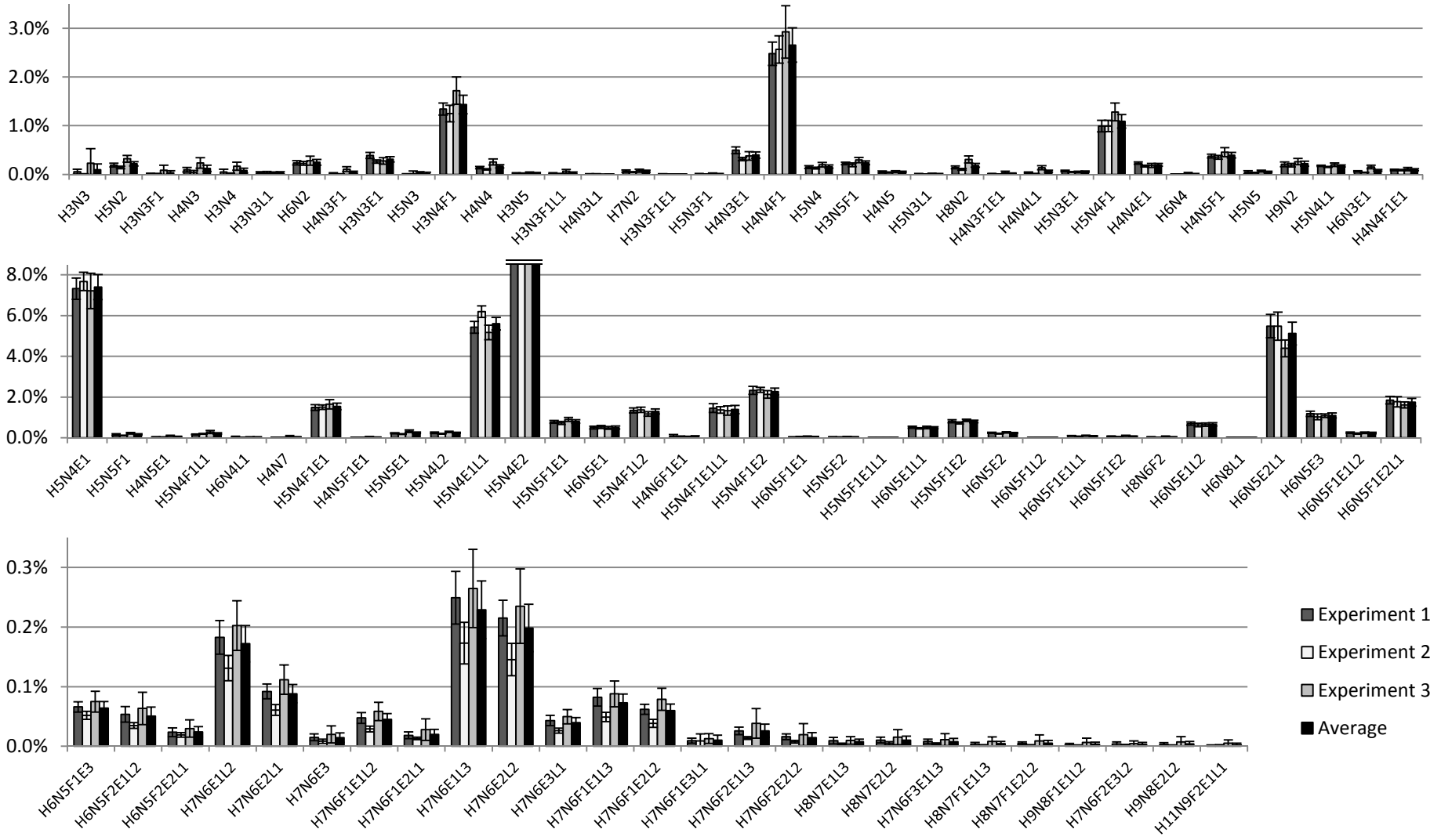

Figure S-4. Repeatability of plasma N-glycan profiling by MALDI-TOF-MS after 1 h 37°C EDC + HOBt ethyl esterification. 24 samples originating from a stock of control plasma were independently released by PNGase F, ethyl esterified, purified by HILIC SPE, crystallized with matrix, recrystallized with ethanol, and analyzed by RP MALDI-TOF-MS. The experiment was performed three times on separate days, indicated as experiment 1-3. The graph shows the average relative intensities for each glycan species (normalized to the overall sum of intensities), with error bars for standard deviation. Abbreviations used are: hexose (H), *N*-acetylhexosamine (N), fucose (F), and *N*-acetylneuraminic acids with either unspecified linkage (S), α2,3-linkage as indicated by lactonization (L), or α2,6-linkage as indicated by esterification (E).

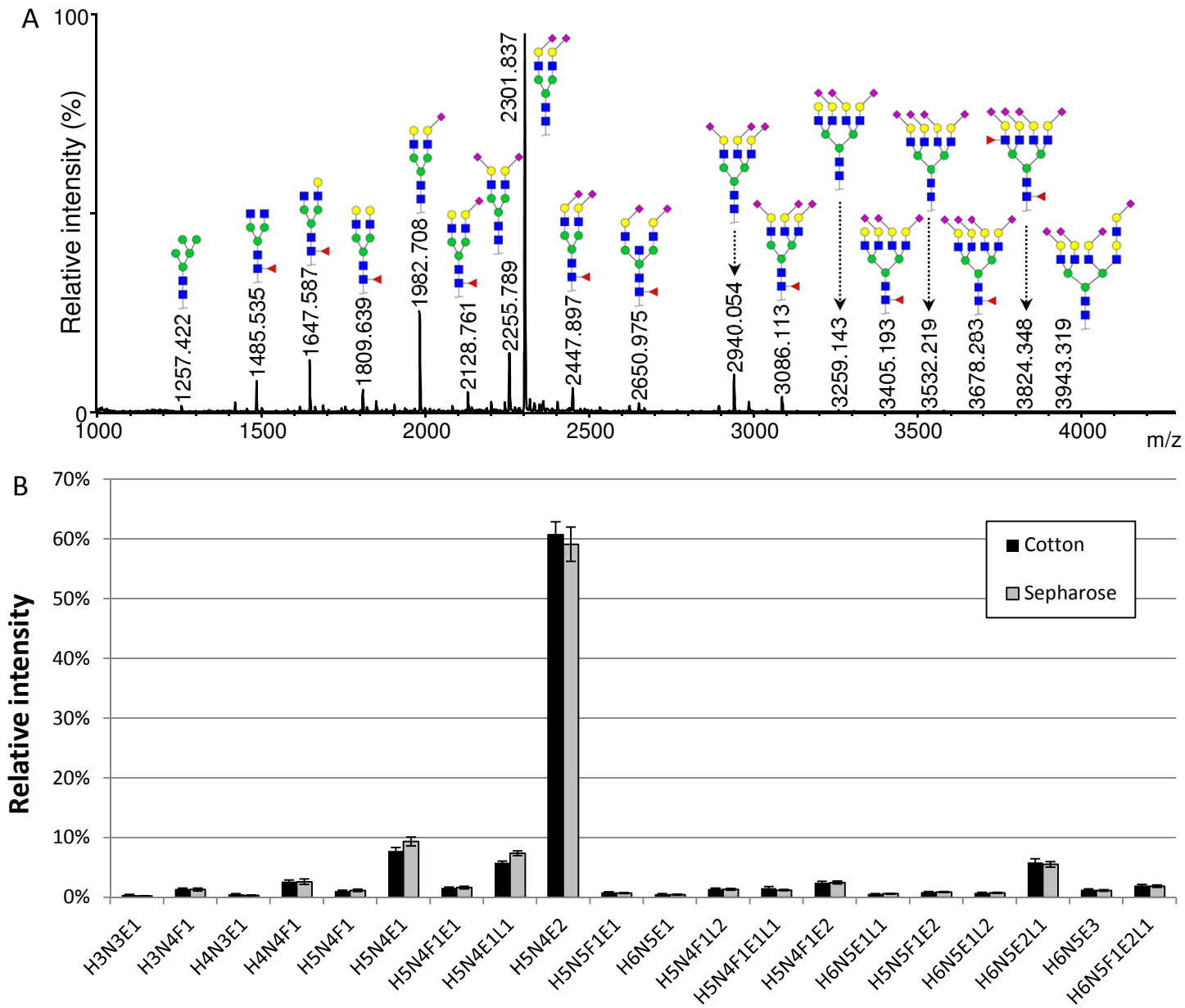

Figure S-5. A) RP MALDI-TOF-MS example spectrum of Sepharose purified plasma N-glycans after ethyl esterification. B) Comparison of relative intensities obtained from cotton HILIC and Sepharose HILIC plate purification procedures. Spectra were acquired with low laser power settings. Error bars show standard deviation of respectively 24 and 96 samples with parallel work-up.