

Supporting Information

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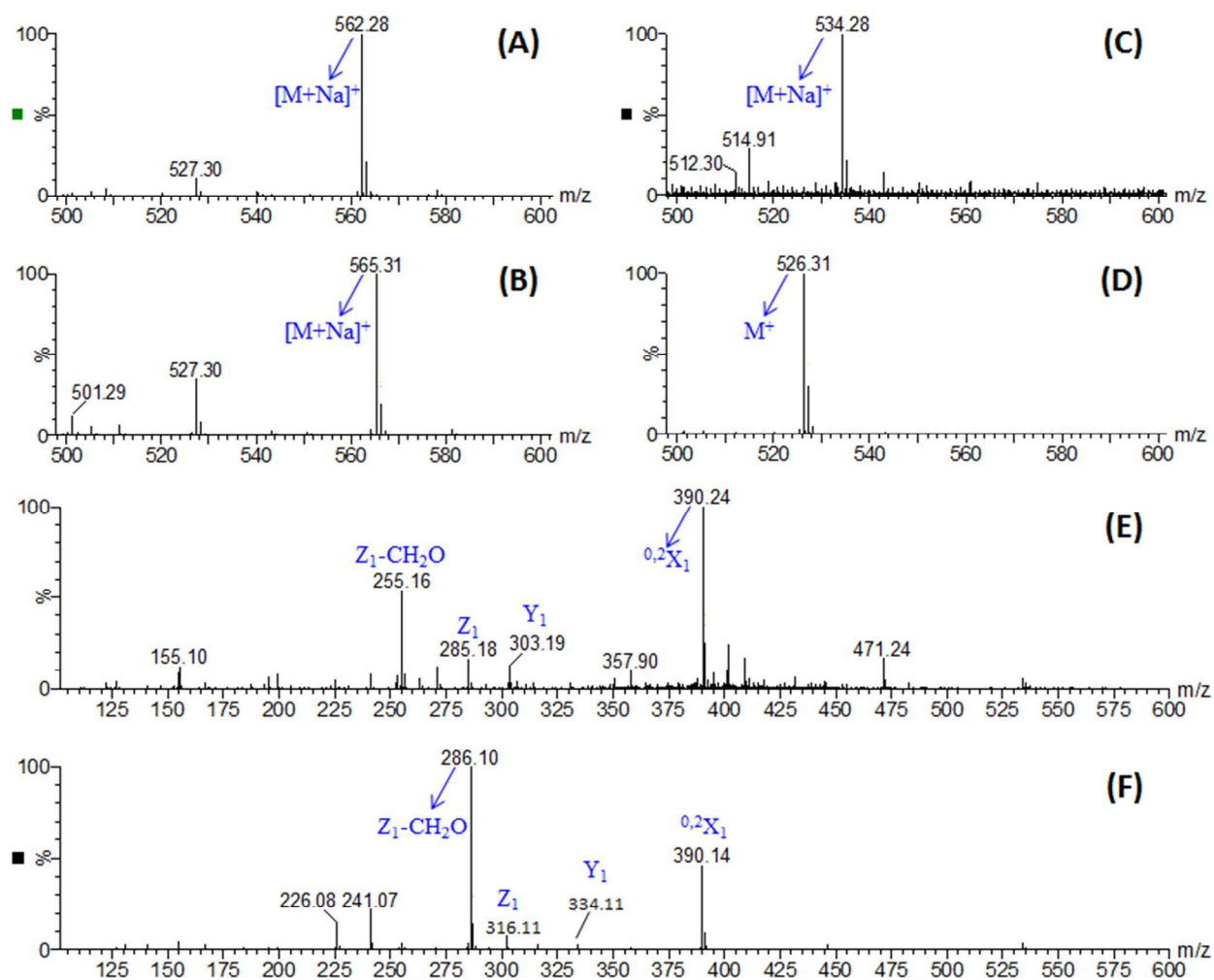


Figure S-1. MS spectra for derivatized Δ UA2S-GlcNAc6S (A), Δ UA2S-GlcNS6S (B), Δ UA2S-GlcNS (C), Δ UA2S-GlcN6S (D) and MS/MS spectra for Δ UA2S-GlcNS (E), Δ UA-GlcNS6S (F). A mass difference of 3Da were observed for derivatized Δ UA2S-GlcNAc6S and Δ UA2S-GlcNS6S, indicating the use trideuteroacetylation enable the differentiation between native N-acetyl group and trideuteroacetyl group from peracetylation step (spectra A and B). For derivatized Δ UA2S-GlcNS and Δ UA2S-GlcN6S, a mass difference of 14Da was observed between derivatized Δ UA2S-GlcN6S (detected as M^+) and Δ UA2S-GlcNS (detected as $[M+H]^+$), or a mass difference of 8Da between derivatized Δ UA2S-GlcN6S (detected as M^+) and Δ UA2S-GlcNS (detected as $[M+Na]^+$) (spectra C and D). For another pair of sulfation positional isomers, the Y_1 and Z_1 ions containing the GlcN residue were 31Da larger for Δ UA-GlcNS6S than Δ UA2S-GlcNS, indicating an original sulfation modification was on GlcN for Δ UA-GlcNS6S and one on Δ UA for Δ UA2S-GlcNS (spectra E and F).

MS spectra of derivatized NS-decamer

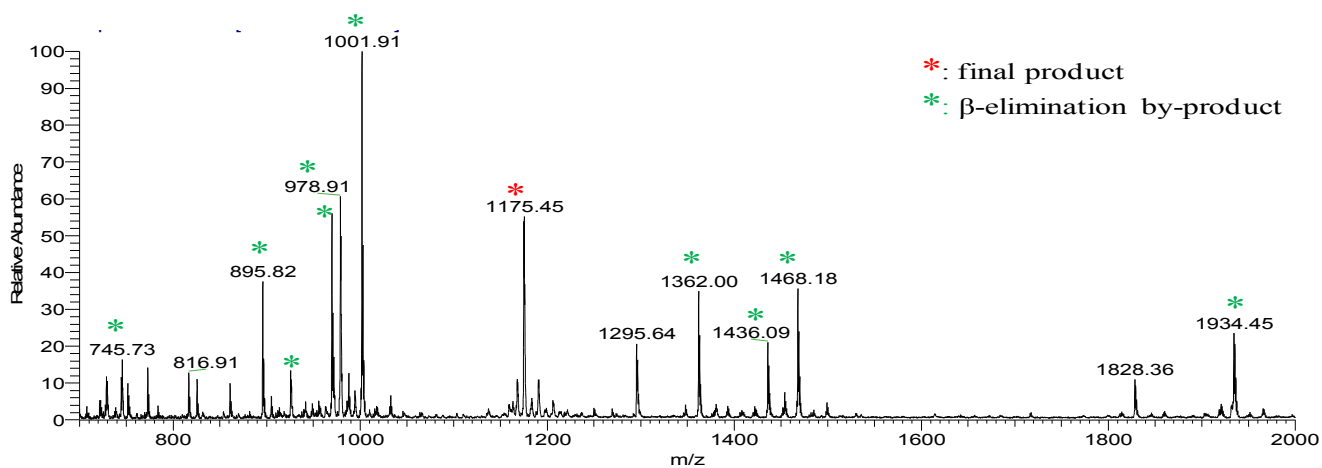


Figure S-2. Direct infusion MS spectrum of the desalted derivatization products of the NS-decamer sequenced in **Figure 2A**. The byproducts are all beta-elimination products that are separated in the reverse phase LC run, and do not result in erroneous sequencing of the product, just in lower sensitivity.

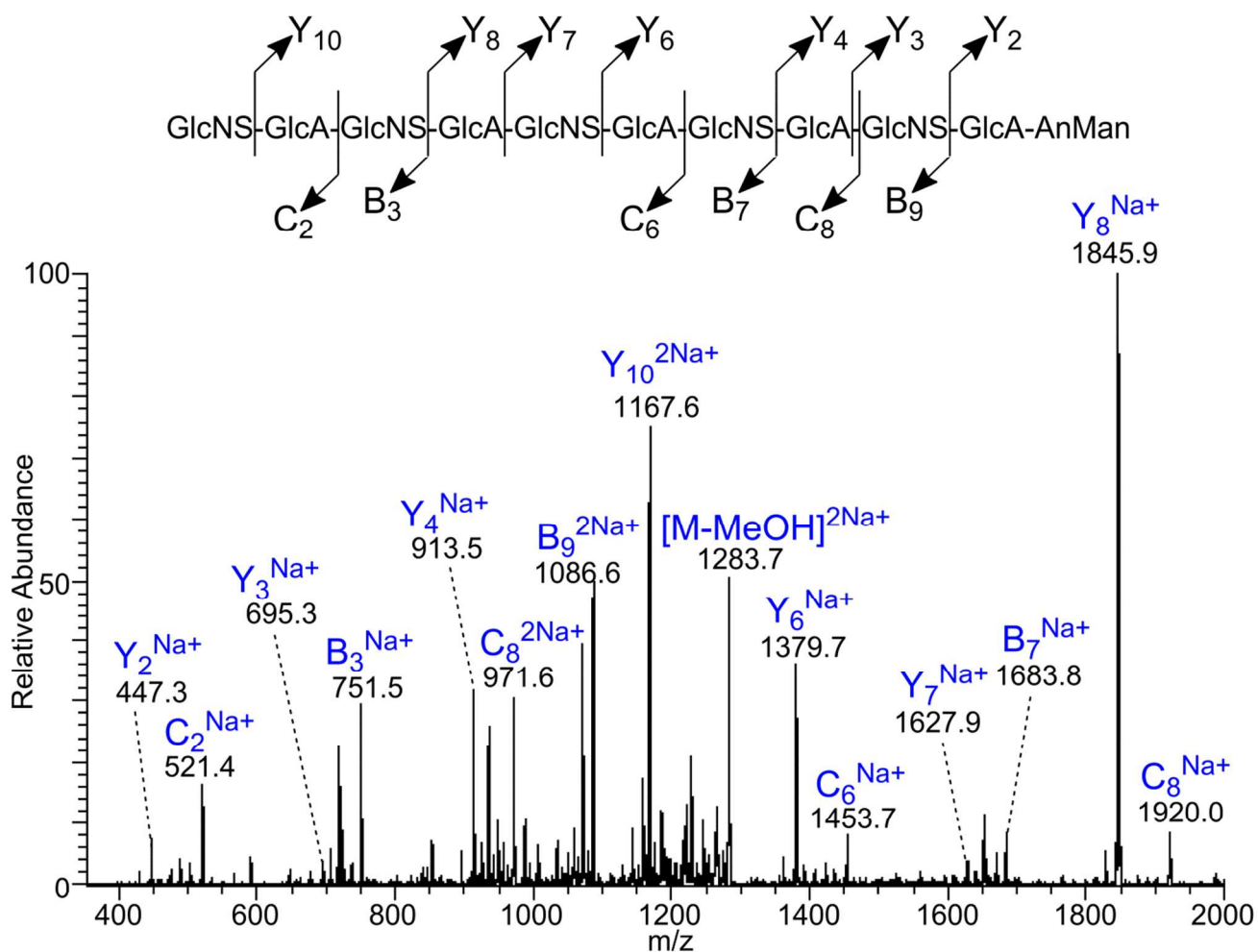


Figure S-3. MS/MS spectra for fully derivatized synthesized NS-undecamer. Sufficient sequential glycosidic bond cleavage fragments were observed, which enable the accurate and complete structural sequencing.

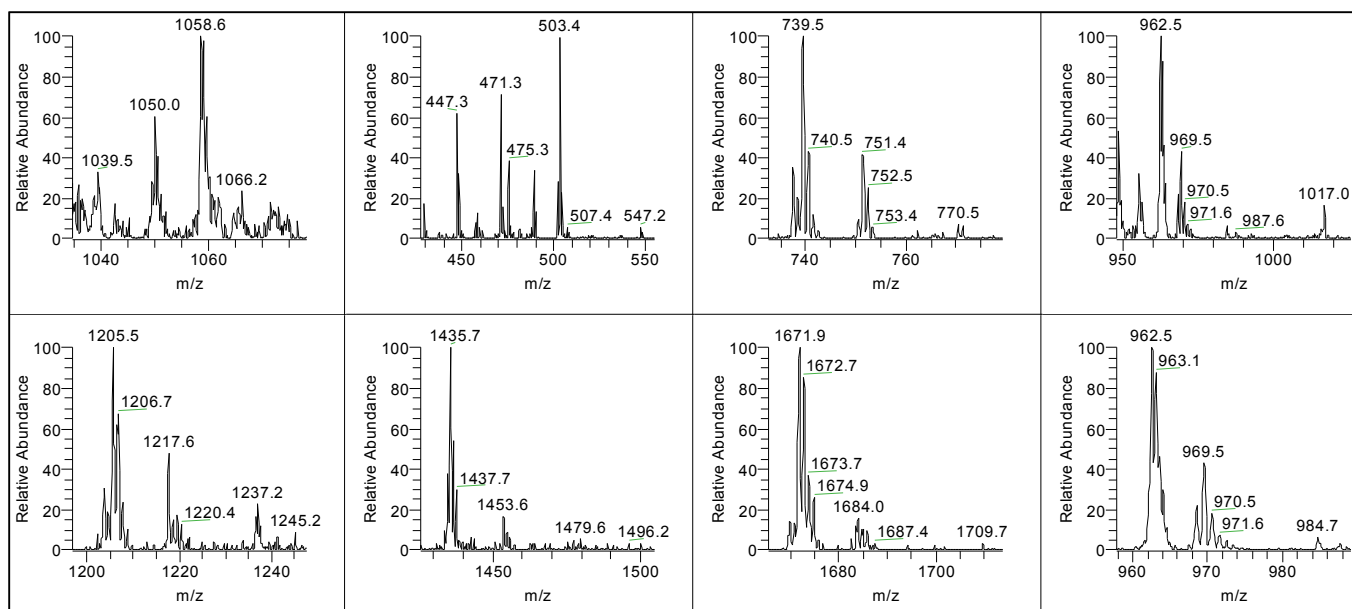


Figure S-4. Zoomed view of MS/MS spectra for the fully derivatized synthesized NS-decamer, shown in **Figure 2A**. These zoomed-in views of the MS/MS spectra clearly show, as described in the manuscript, how an unambiguous interpretation of the MS/MS spectra of fully derivatized HS oligomers can be achieved.

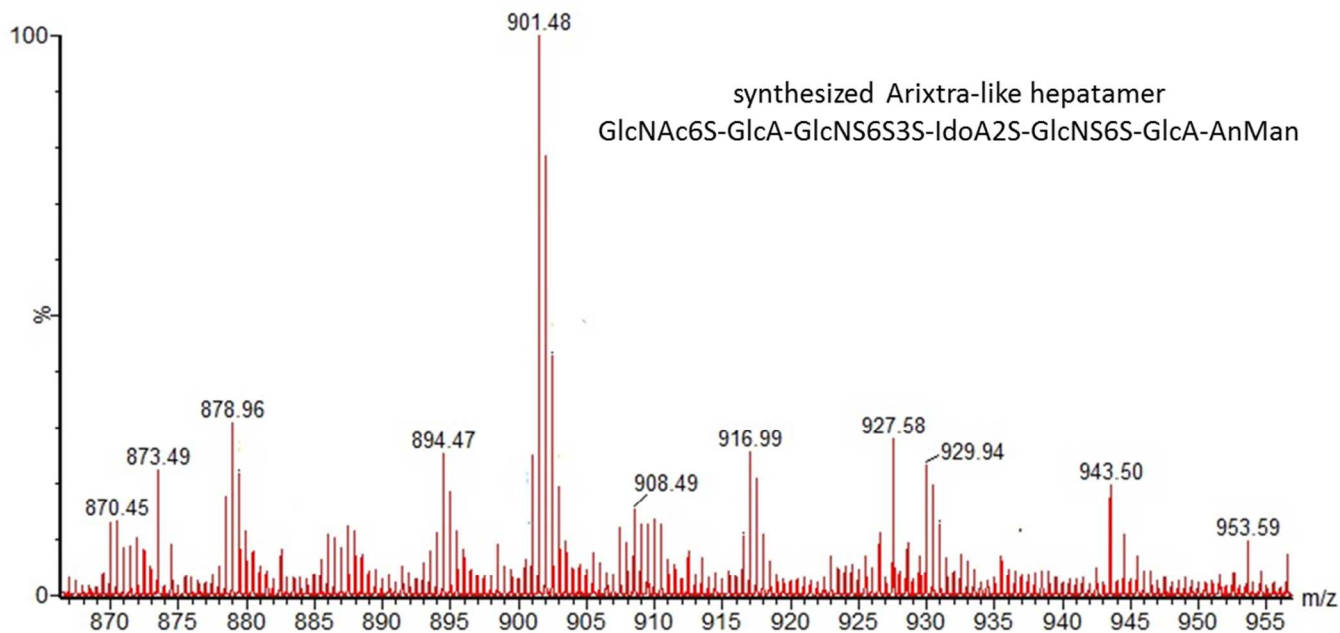


Figure S-5. MS spectra for the fully derivatized synthesized Arixtra-like hepatamer. As we can see from the spectra, after derivatization the most abundant ion we observed is 901.48, while the theoretical value for a fully permethylated, desulfated and acetylated product should give a m/z of 908.49 for $[M+2Na]^{2+}$ ion. The mass difference between hydrogen and methyl group is also 14Da, indicating that there is one under-permethylation site which could not be further acetylated.