Supplementary Materials

Exploration of the Transglycosylation Activity of Barley Limit Dextrinase for Production of Novel Glycoconjugates

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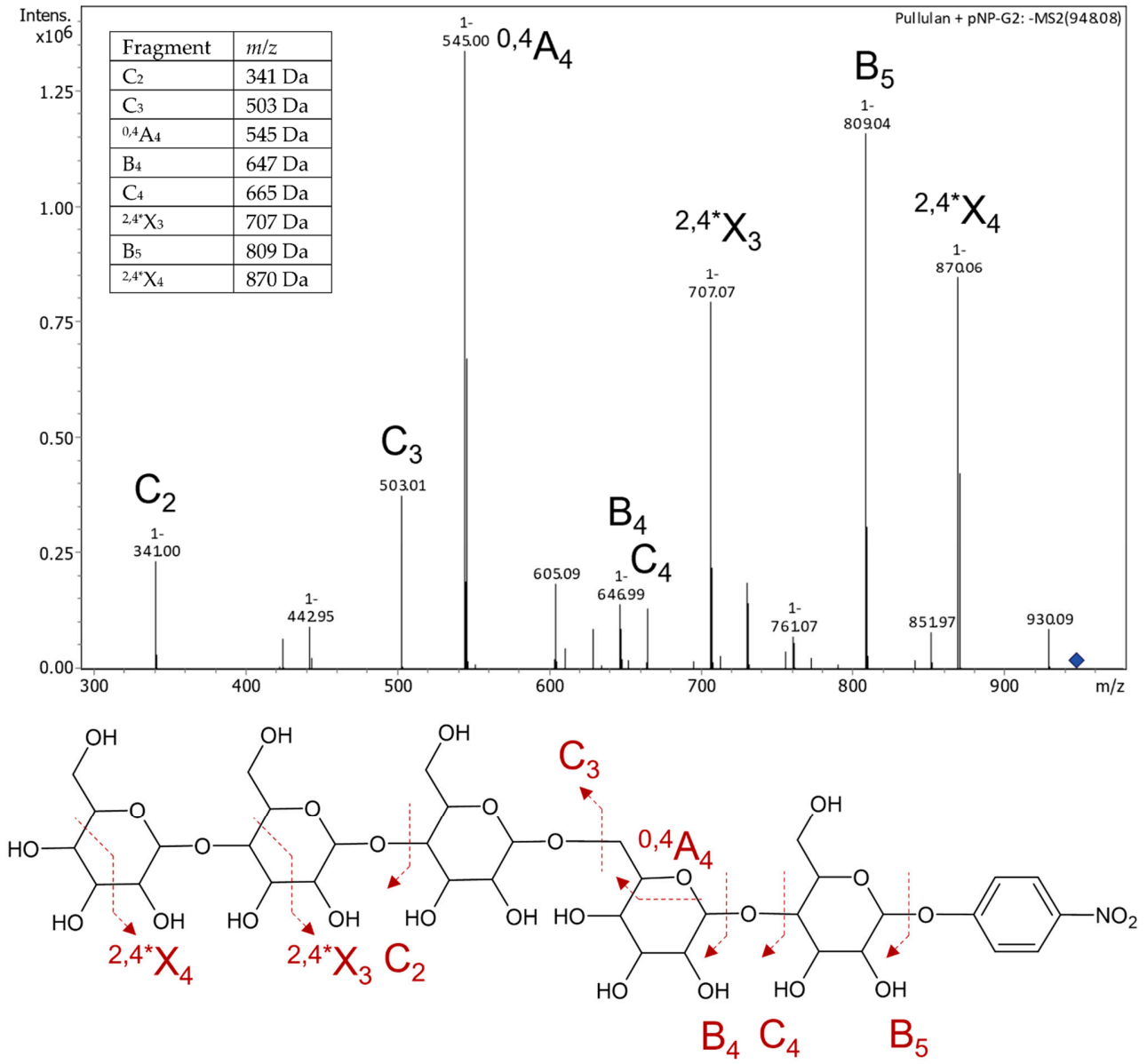


Figure S1. Example of transglycosylation (TG) product identification by liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). Top: MS^2 fragmentation spectrum of *p*-nitrophenyl 6^2-α-maltotriosyl-maltoside (pNP-G5) *m*/*z* 948.1 Da with identified fragments. Bottom: fragmentation pattern of pNP-G5. Nomenclature was suggested by Domon and Castello.

Domon, B.; Costello, C.E. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconj. J.* **1988**, *5*, 397–409, doi:10.1007/BF01049915.

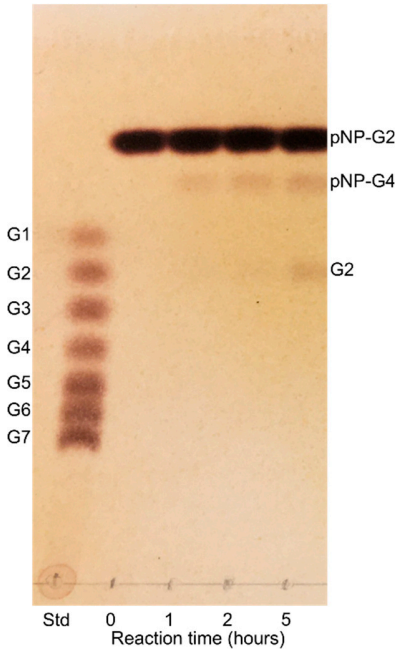


Figure S2. Thin layer chromatography (TLC) analysis of the *Hv*LD TG reaction with pNP maltoside (pNP-G2) functioning both as donor and acceptor. Glucose and maltooligosaccharides (G2 to G7) are included as standards. Released para-nitrophenol (pNP) is not stained by the stainer used.

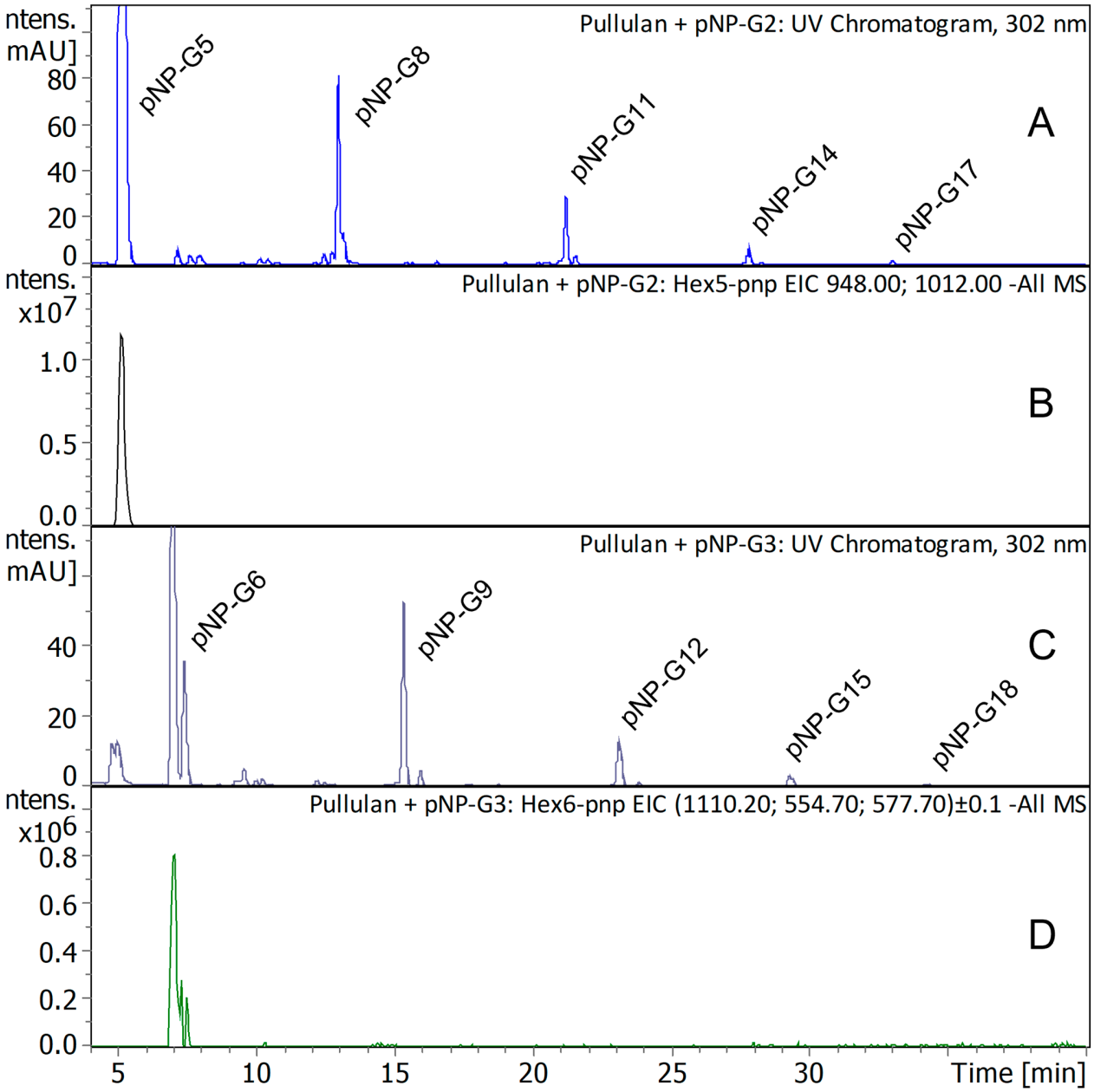


Figure S3. Dual peaks observed in both UV and extracted ion chromatograms indicating the presence of more than one structural isomer. Representative UV chromatograms of reactions with pullulan and pNP-G2 (**A**) or pNP-G3 (**C**). Extracted ion chromatograms of TG products pNP-G5 (**B**) and pNG-G6 (**D**). The TG reaction time was 2 hours.

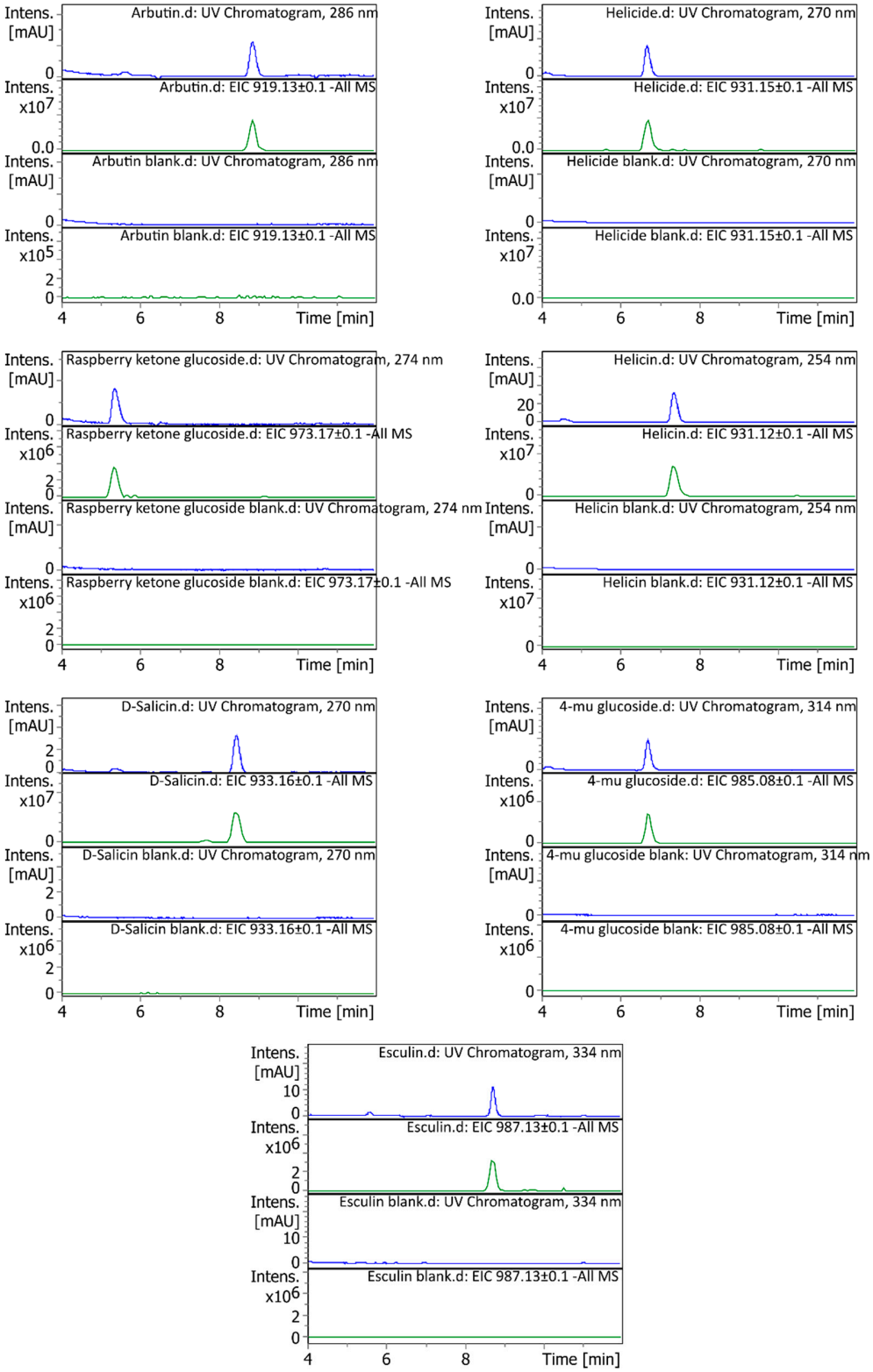


Figure S4. Extracted UV chromatograms and the corresponding extracted ion chromatogram of TG products using alternative acceptors compared with blank reactions. The TG reaction time was 2 hours.

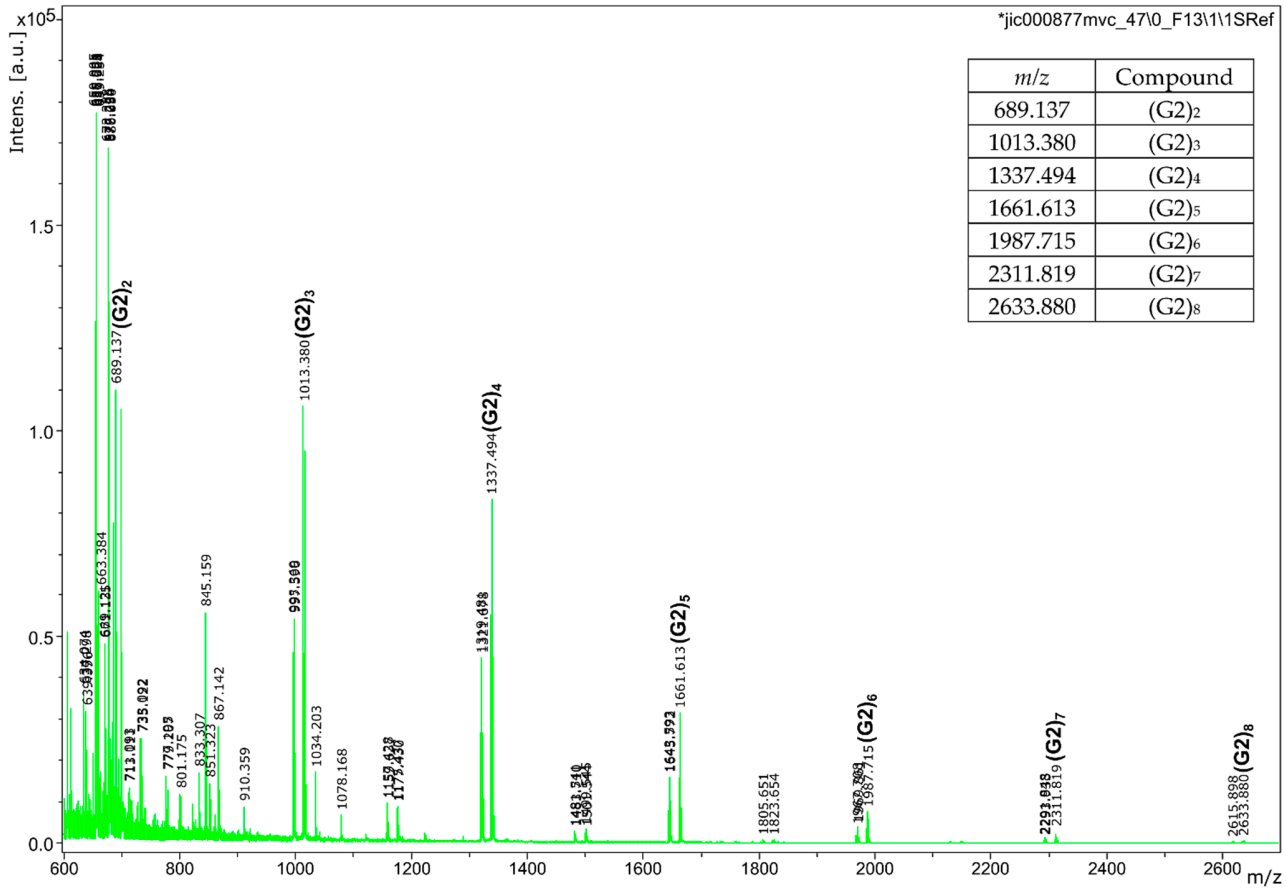
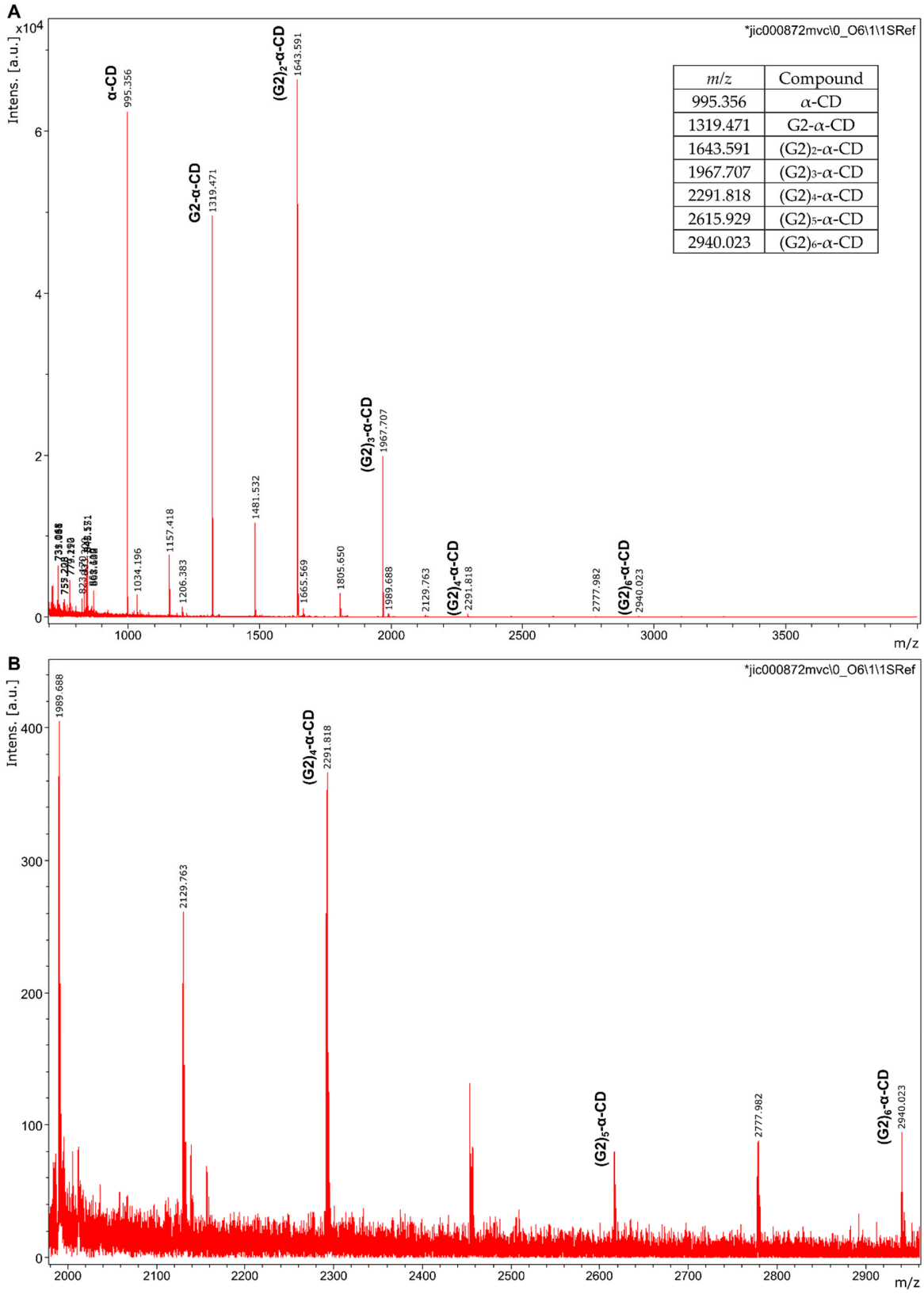


Figure S5. MALDI-TOF spectrum of TG products from 60 min reaction including G2F as donor and maltose (G2) as acceptor.



m/z	Compound
995.356	α-CD
1319.471	G2-α-CD
1643.591	$(G2)_2$-α-CD
1967.707	$(G2)_3$-α-CD
2291.818	$(G2)_4$-α-CD
2615.929	$(G2)_5$-α-CD
2940.023	$(G2)_6$-α-CD

Figure S6. MALDI-TOF spectrum of TG products from 180 min reaction including α-maltosyl fluoride (G2F) as donor and α-CD as acceptor. (**A**) Full range and (**B**) zoomed range of panel **A**.

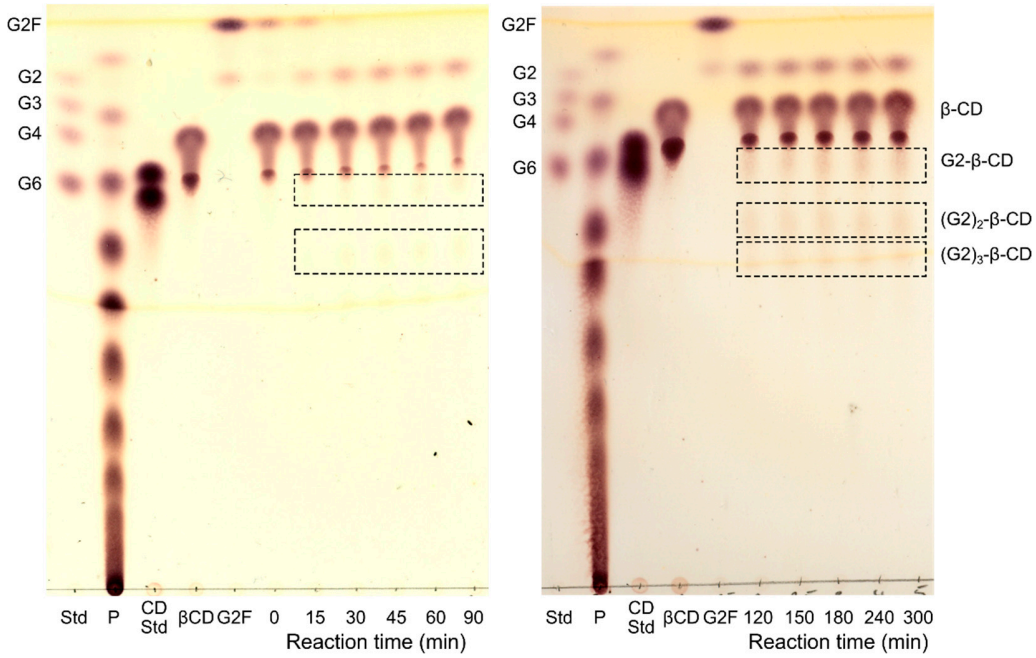


Figure S7. TLC analysis of TG reaction using G2F as donor and β-CD as acceptor. Donor:acceptor ratio of 1:1 (10 mM:10 mM). TG products are framed. Maltooligosaccharides (G2, G3, G4, and G6) are included as standards (Std) together with a pullulan standard (P; see Figure 5 for content) and a mix of G-β-CD and G2-β-CD (CD Std).

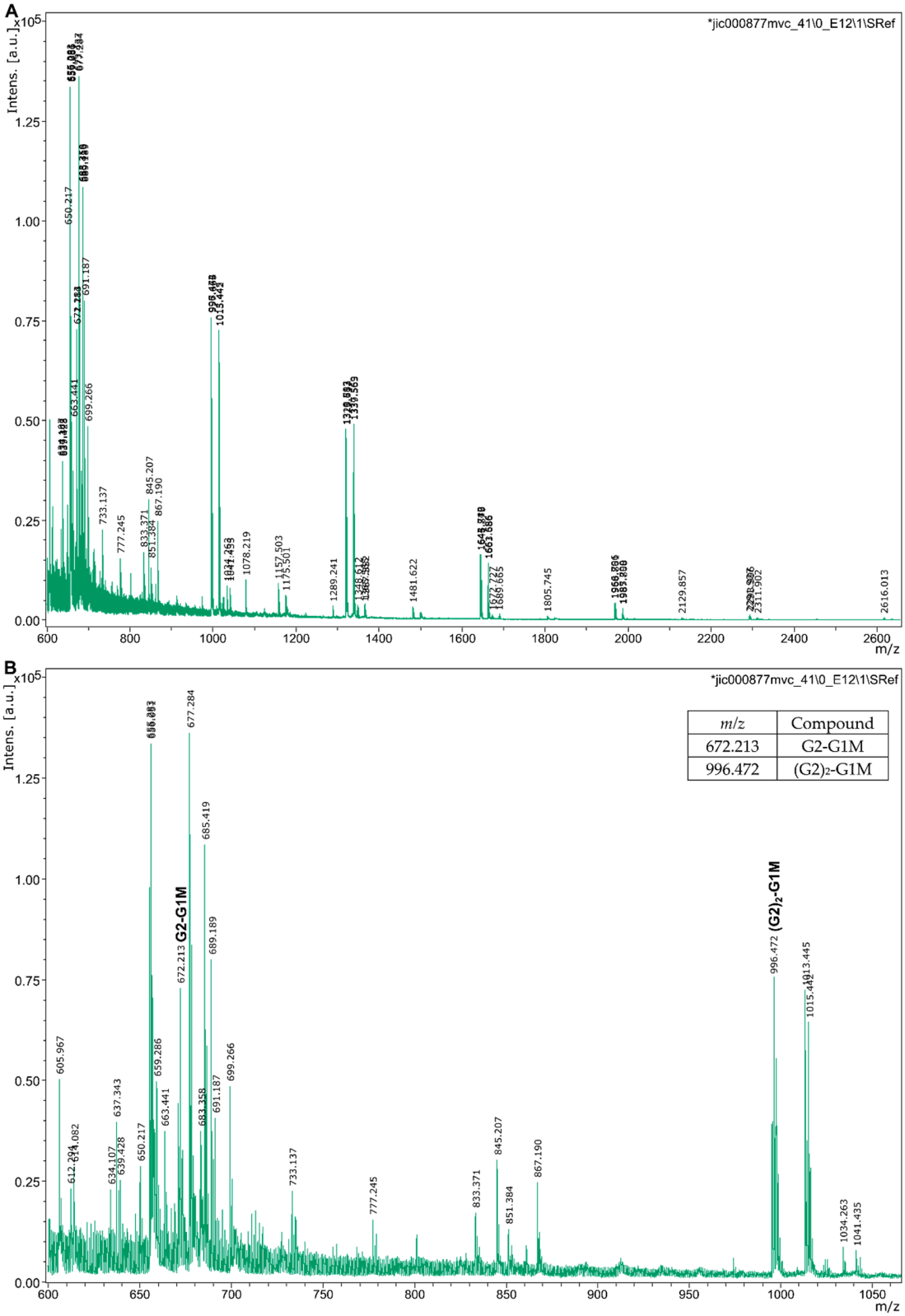


Figure S8. MALDI-TOF spectrum of TG products from 120 min reaction including α-maltosyl fluoride (G2F) as donor and 4-*O*-α-D-glucopyranosyl-moranoline (G1M) as acceptor. (**A**) Full range and (**B**) zoomed range of panel **A** on the lower *m*/*z* range.

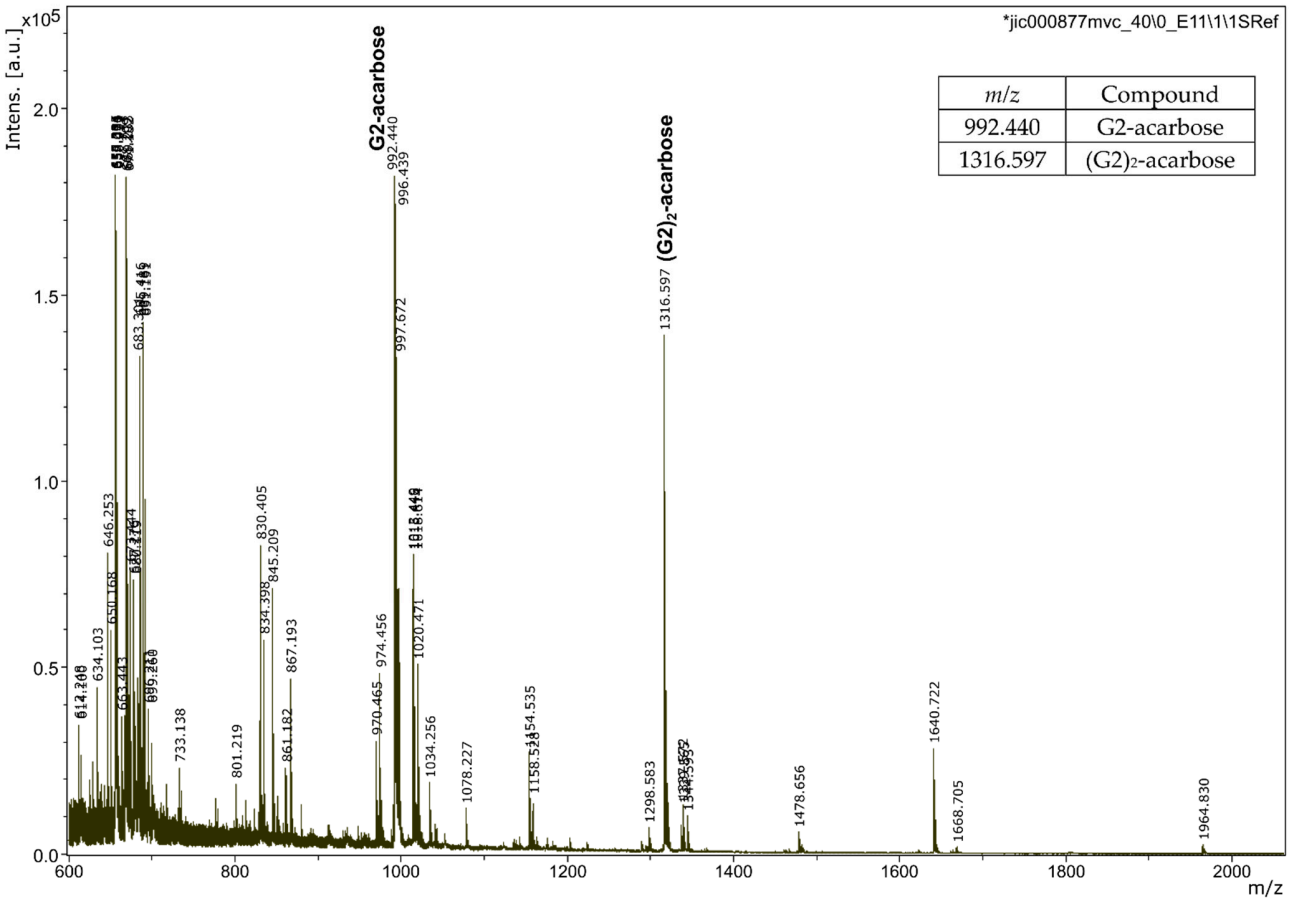


Figure S9. MALDI-TOF spectrum of TG products from 120 min reaction including α-maltosyl fluoride (G2F) as donor and acarbose as acceptor.