applied sciences

MDPI

Supplementary Material

A Comparison of the Transglycosylation Capacity between the Guar GH27 Aga27A and *Bacteroides* GH36 *Bo*Gal36A α-Galactosidases

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* Correspondence: henrik.stalbrand@biochemistry.lu.se

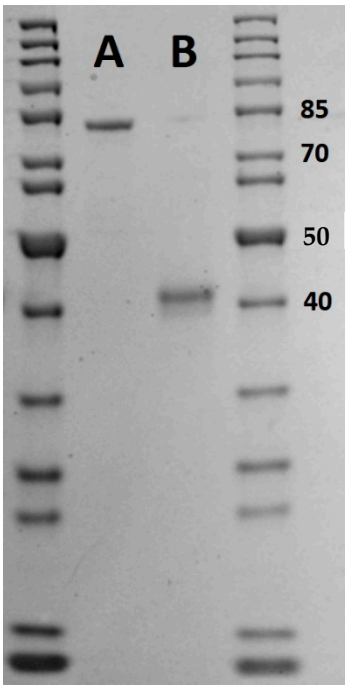
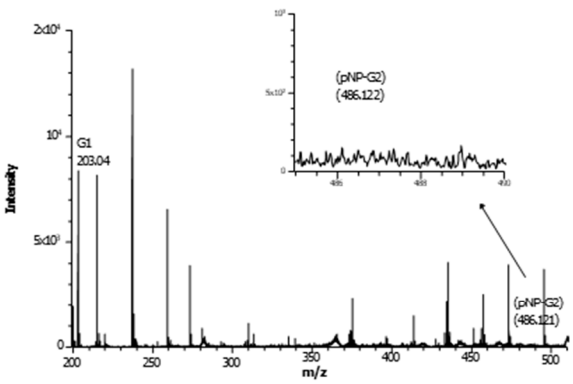


Figure S1. SDS-PAGE gel of the purified *Bo*Gal36A and Aga27A. Lane A was loaded with purified *Bo*Gal36A, with the band having an apparent molecular weight of 83 kDa, and lane B was loaded with Aga27A, with the band having an apparent molecular weight of 42 kDa. The apparent molecular weights correspond well with the theoretical masses of the proteins, with *Bo*Gal36A having a theoretical mass of 81 kDa and Aga27A of 40 kDa.

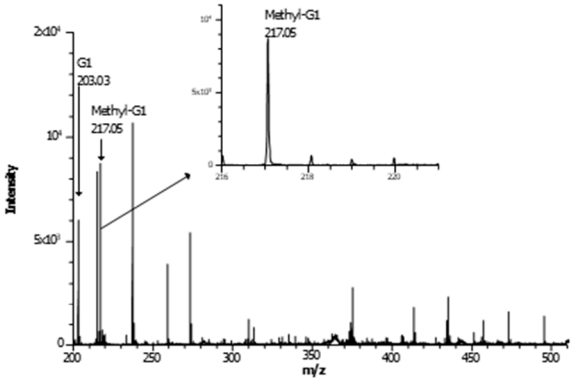
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101 WAELNRDSEG NMVPNAAAFP SGIKALADYV HSKGLKLGVY SDAGNQTCSK
151 RMPGSLGHEE QDAKTFASWG VDYLKYDNCE NLGISVKERY PPMGKALLSS
201 GRPIFFSMCE WGWEDPQIWA KSIGNSWRTT GDIEDNWNSM TSIADSNDKW
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301 DDTTHELISN AEVIAVNQDK LGVQGKKVKS TNDLEVWAGP LSDNKVAVIL
351 WNRSSSRATV TASWSDIGLQ QGTTVDARDL WEHSTQSLVS GEISAEIDSH
401 ACKMYVLTPR S
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Start - End	Observed	Mr(expt)	Mr(calc)	ppm	M	Peptide
165 - 175	1286.6130	1285.6057	1285.6343	-22.2	0	K.TFASWGVDYLK.Y
188 - 195	977.4698	976.4626	976.4800	-17.9	1	K.ERYPPMGK.A
222 - 228	819.3888	818.3815	818.4035	-26.8	0	K.SIGNSWR.T
279 - 288	1159.6084	1158.6011	1158.6186	-15.1	0	R.SHFSIWALAK.A
321 - 326	601.3272	600.3199	600.3595	-65.9	0	K.LGVQGK.K
346 - 353	970.5622	969.5549	969.5760	-21.7	0	K.VAVILWNR.S
404 - 410	879.4629	878.4556	878.4684	-14.5	0	K.MYVLTPR.S
404 - 410	895.4634	894.4561	894.4633	-8.06	0	K.MYVLTPR.S + Oxidation (M)

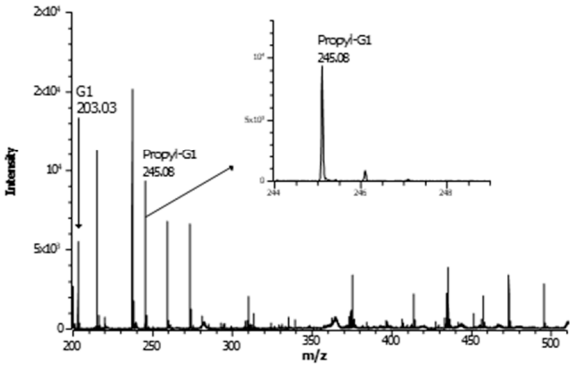
Figure S2. Peptide mass fingerprinting of Aga27A. Procedure: Aga27A was digested with trypsin, and the resulting cleavage products were analyzed with MALDI-TOF MS to confirm protein identity. The matching peptides of the trypsin digested Aga27A are highlighted in red in the displayed amino acid sequence, and the individual fragments are listed with theoretical m/z for [m] and observed m/z [m+H$^+$] below. Eight of the peaks detected in the spectra had m/z corresponding to the m/z for [m+H$^+$] of seven expected peptide products from in-silica trypsin digested Aga27A, with one of the detected peaks having an m/z corresponding to one of the peptides carrying oxidation on a methionine. The seven matched peptides gave a 13% sequence coverage and a significant hit against Cyamopsis tetragonoloba GH27 α-galactosidase with UniProt ID P14749.



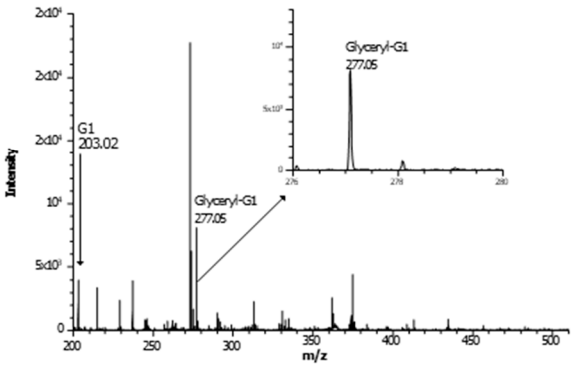
(a) No acceptor (pNP-G2, m/z: 486.122)



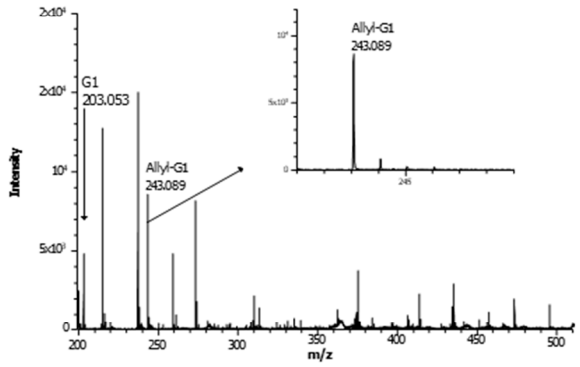
(b) 10% MeOH (Methyl-G1, m/z: 217.068)



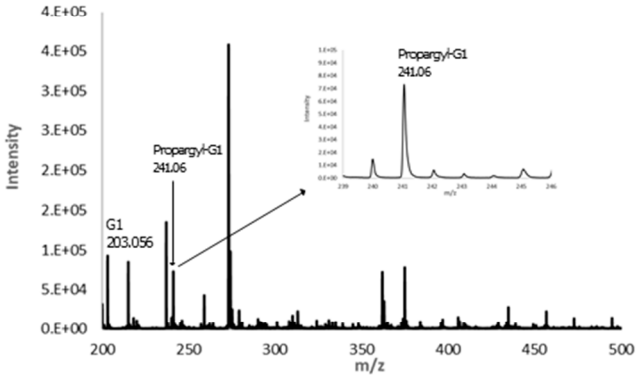
(c) 5% PrOH (Propyl-G1, m/z: 245.100)



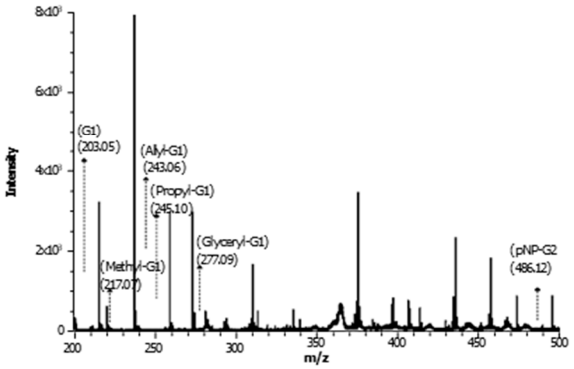
(d) 10% Glycerol (Glyceryl-G1, m/z: 277.089)



(e) 5% Allyl-OH (Allyl-G1, m/z: 243.084)

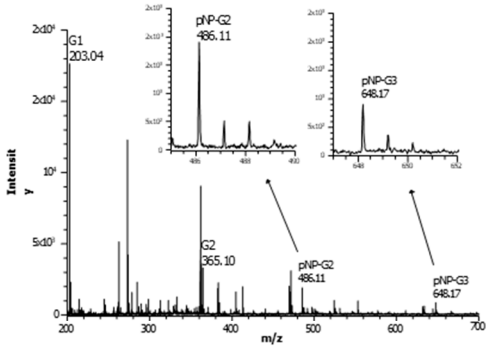


(f) 2.5% Propargyl-OH (Allyl-G1, m/z: 241.068)

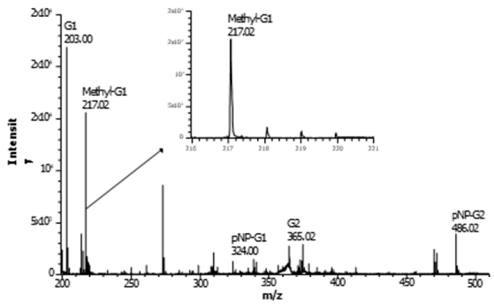


(g) Control: all acceptors and inactivated enzyme

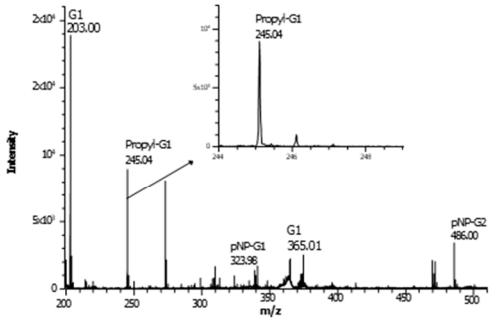
Figure S3. MALDI-TOF spectra of BoGal36A incubated with pNP-Gal and various acceptors. 8 nkat/ml BoGal36A was incubated in 45 mM NaCi pH 6 with 40 mM pNP-Gal alone (**a**) or with 10 v/v% methanol (**b**), 5 v/v% propanol (**c**), 10 v/v% glycerol (**d**), 5 v/v% allyl alcohol (**e**) or 2.5 v/v% propargyl alcohol (**f**) added, at 37 °C for 1h. Detected peaks are marked with labels without parenthesis with the observed m/z listed, while peaks that are not present are marked with labels with parenthesis. In each spectrum, there is a blown-up portion of the spectrum limited to the mass range where potential transglycosylation products are expected to be, with an arrow showing which peak in the full spectrum it corresponds to. Below each spectrum, the acceptor and theoretical m/z for the [m+Na^+] ion of potential transglycosylation products are listed. A control with inactivated enzyme, pNP-Gal and the acceptors are shown in the spectrum (**g**).



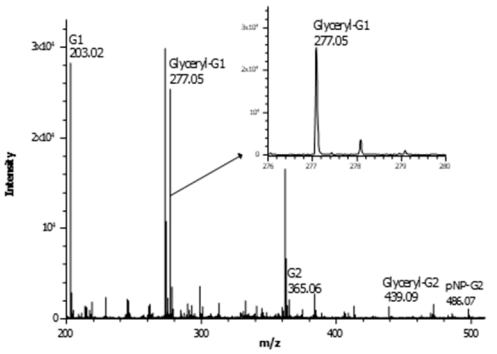
(a) No acceptor (pNP-G2, m/z: 486.122)



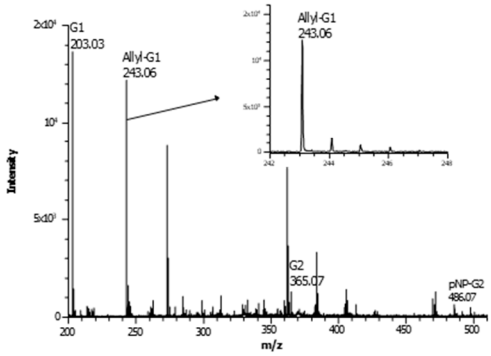
(b) 10% MeOH (Methyl-G1, m/z: 217.068)



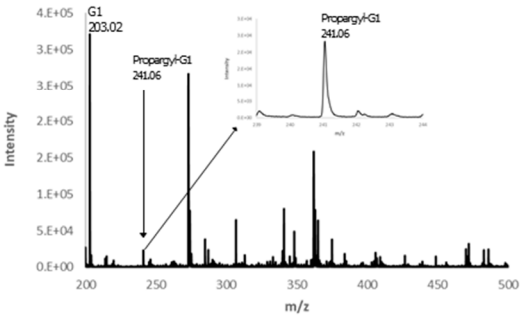
(c) 5% PrOH (Propyl-G1, m/z: 245.100)



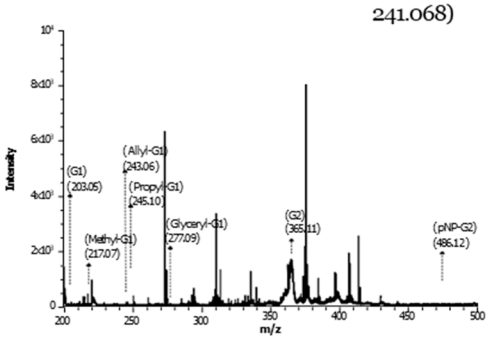
(d) 10% Glycerol (Glyceryl-G1, m/z: 277.089)



(e) 5% Allyl-OH (Allyl-G1, m/z: 243.084)

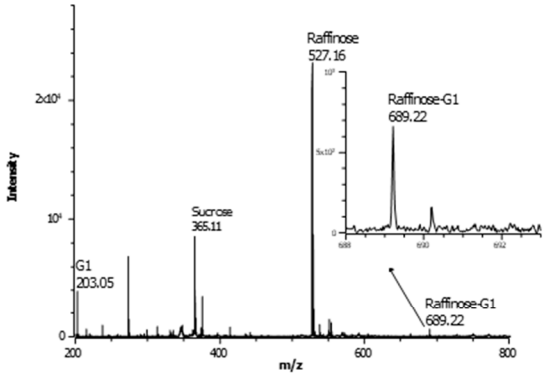


(f) 2.5% Propargyl-OH (Allyl-G1, m/z: 241.068)

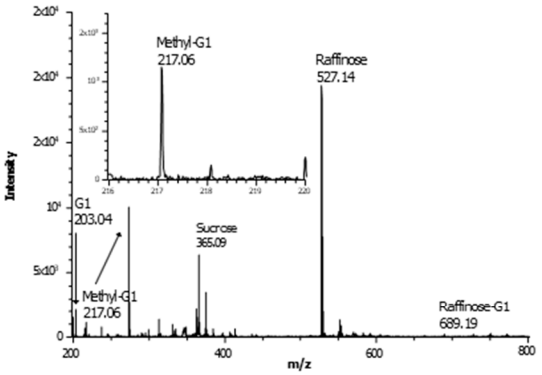


(g) Control: all acceptors and inactivated enzyme

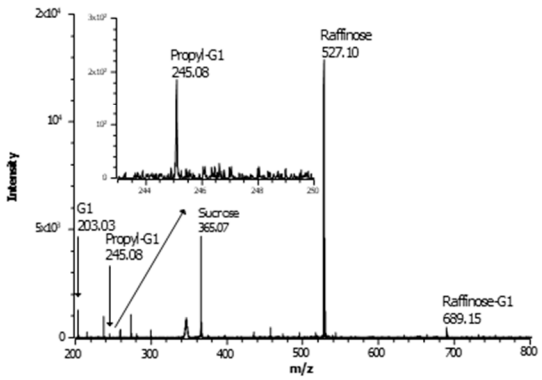
Figure S4. MALDI-TOF spectra of Aga27A incubated with pNP-Gal and various acceptors. 8 nkat/ml Aga27A was incubated in 45 mM NaAc pH 4.5 with 40 mM pNP-Gal alone (**a**) or with 10 v/v% methanol (**b**), 5 v/v% propanol (**c**), 10 v/v% glycerol (**d**), 5 v/v% allyl alcohol (**e**) or 2.5 v/v% propargyl alcohol (**f**) added, at 37 °C for 1h. Detected peaks are marked with labels without parenthesis with the observed m/z listed, while peaks that are not present are marked with labels with parenthesis. In each spectrum, there is a blown-up portion of the spectrum limited to the mass range where potential transglycosylation products are expected to be, with an arrow showing which peak in the full spectrum it corresponds to. Below each spectrum, the acceptor and theoretical m/z for the [m+Na^+] ion of potential transglycosylation products are listed. A control with inactivated enzyme, pNP-Gal and the acceptors are shown in the spectrum (**g**).



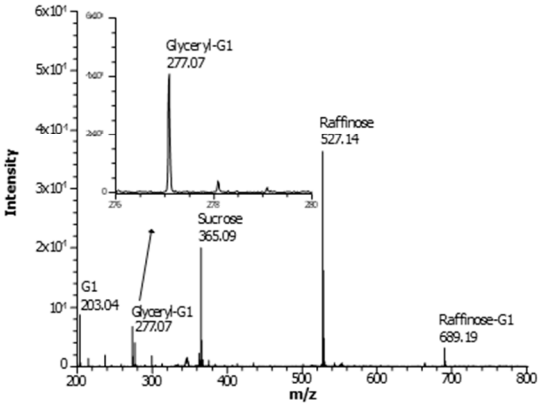
(a) No acceptor (Raf-G1, m/z: 689.212)



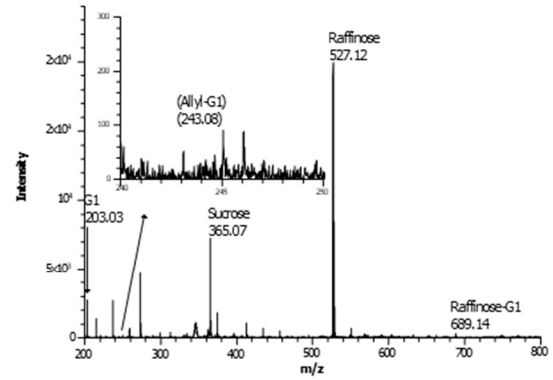
(b) 10% MeOH (Methyl-G1, m/z: 217.068)



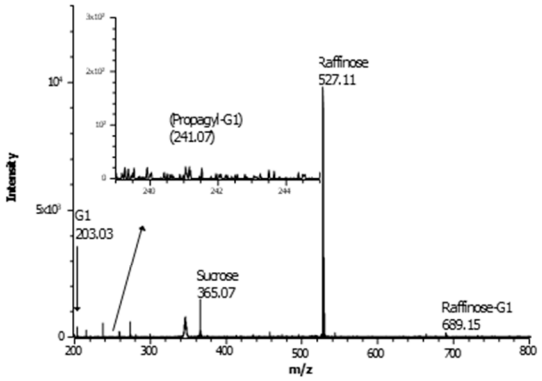
(c) 5% PrOH (Propyl-G1, m/z: 245.100)



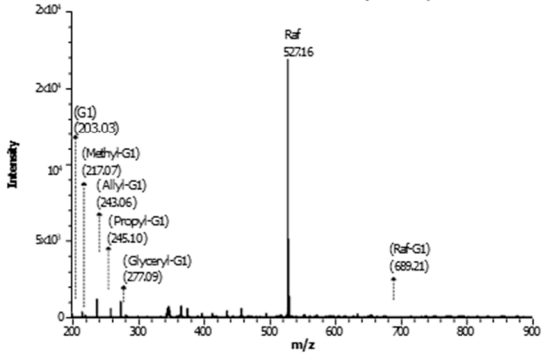
(d) 10% Glycerol (Glyceryl-G1, m/z: 277.089)



(e) 5% Allyl-OH (Allyl-G1, m/z: 243.084)

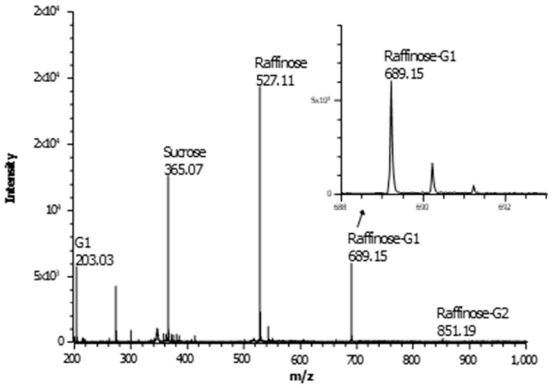


(f) 2.5% propargyl-OH (propargyl-G1, m/z: 241.068)

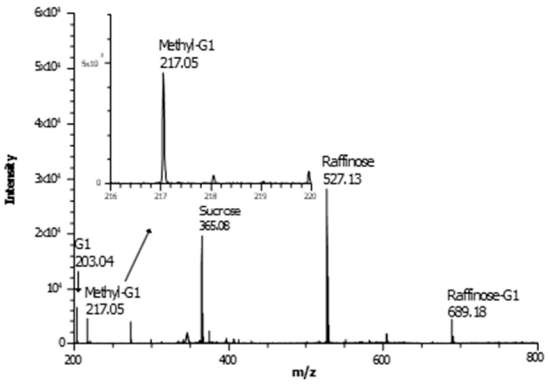


(g) Control: Raffinose, all acceptors and inactivated enzyme

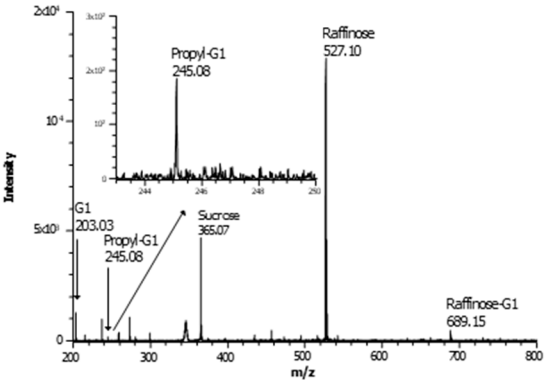
Figure S5. MALDI-TOF spectra of BoGal36A incubated with raffinose and various acceptors. In total, 8 nkat/ml BoGal36A was incubated in 45 mM NaCi pH 6 with 0.4 M raffinose alone (**a**) or with 10 v/v% methanol (**b**), 5 v/v% propanol (**c**), 10 v/v% glycerol (**d**), 5 v/v% allyl alcohol (**e**) or 2.5 v/v% propargyl alcohol (**f**) added, at 37 °C for 24h. Detected peaks are marked with labels without parenthesis, with the observed m/z listed, while peaks that are not present are marked with labels with parenthesis. In each spectrum, there is a blown-up portion of the spectrum limited to the mass range where potential transglycosylation products are expected to be, with an arrow showing which peak in the full spectrum it corresponds to. Below each spectrum, the acceptor and theoretical m/z for the [m+Na^+] ion of potential transglycosylation products are listed. A control with inactivated enzyme, raffinose and the acceptors are shown in the spectrum (**g**).



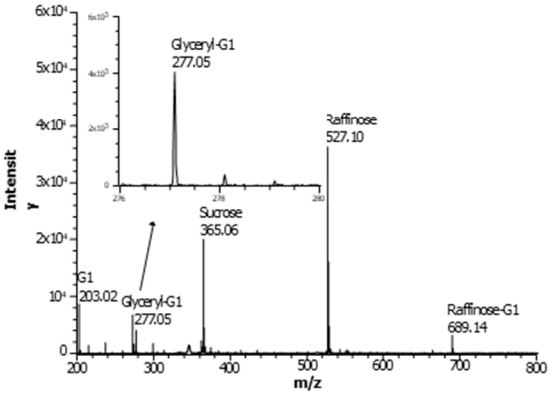
(a) No acceptor (Raf-G1, m/z: 689.212)



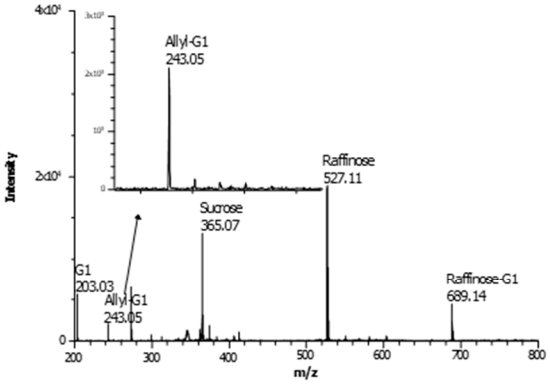
(b) 10% MeOH (Methyl-G1, m/z: 217.068)



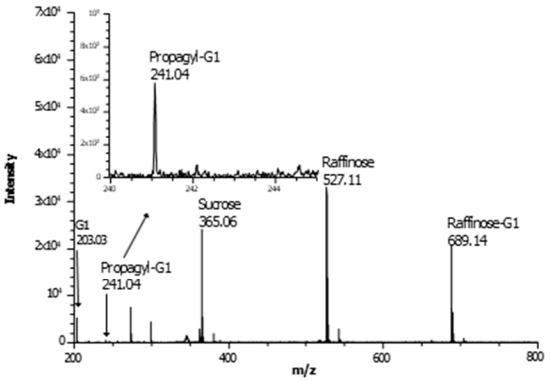
(c) 5% PrOH (Propyl-G1, m/z: 245.100)



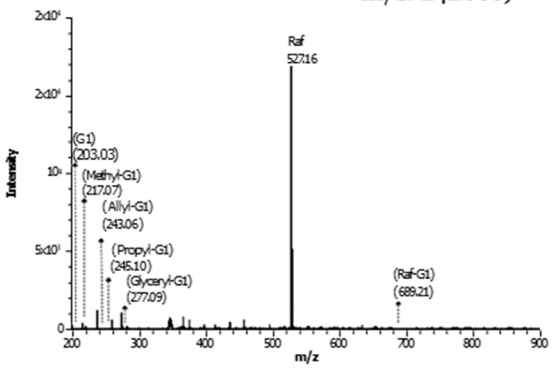
(d) 10% Glycerol (Glyceryl-G1, m/z: 277.089)



(e) 5% Allyl-OH (Allyl-G1, m/z: 243.084)



(f) 2.5% propargyl-OH (propargyl-G1, m/z: 241.068)



(g) Control: Raffinose, all acceptors and inactivated enzyme

Figure S6. MALDI-TOF spectra of Aga27A incubated with raffinose and various acceptors.In total, 8 nkat/ml Aga27A was incubated in 45 mM NaAc pH 4.5with 0.4 M raffinose alone (**a**) or with 10 v/v% methanol (**b**), 5 v/v% propanol (**c**), 10 v/v% glycerol (**d**), 5 v/v% allyl alcohol (**e**) or 2.5 v/v% propargyl alcohol (**f**) added, at 37 °C for 24h. Detected peaks are marked with labels without parenthesis, with the observed m/z listed, while peaks that are not present are marked with labels with parenthesis. In each spectrum, there is a blown-up portion of the spectrum limited to the mass range where potential transglycosylation products are expected to be, with an arrow showing which peak in the full spectrum it corresponds to. Below each spectrum, the acceptor and theoretical m/z for the $[m+Na^+]$ ion of potential transglycosylation products are listed. A control with inactivated enzyme, raffinose and the acceptors are shown in the spectrum (**g**).

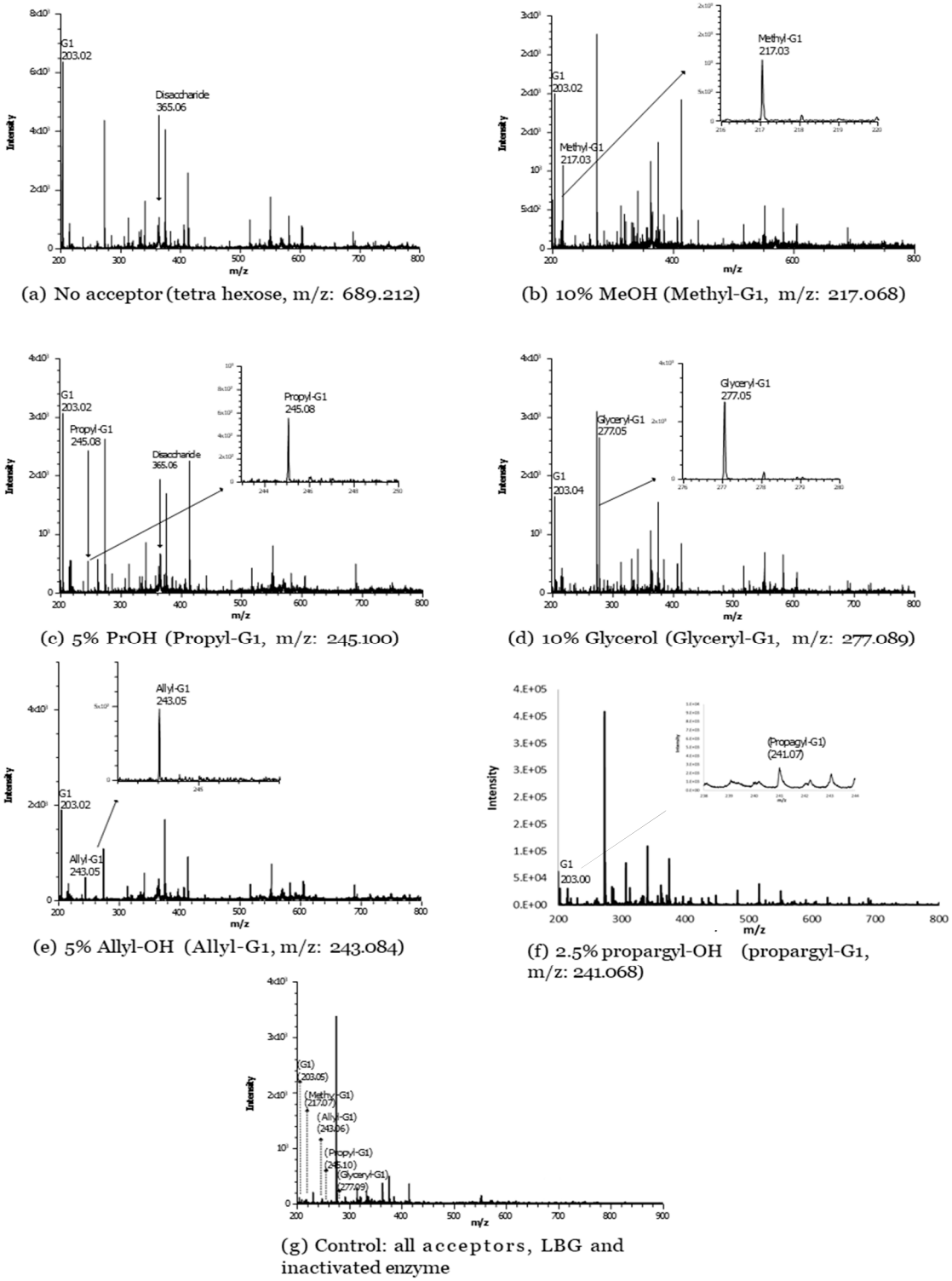


Figure S7. MALDI-TOF spectra of Aga27A incubated with LBG and various acceptors. In total, 8 nkat/ml Aga27A was incubated in 45 mM NaAc pH 4.5 with 0.4 w/v% LBG alone (**a**) or with 10 v/v% methanol (**b**), 5 v/v% propanol (**c**), 10 v/v% glycerol (**d**), 5 v/v% allyl alcohol (**e**) or 2.5 v/v% propargyl alcohol (**f**) added, at 37 °C for 24h. Detected peaks are marked with labels without parenthesis with the observed m/z listed, while peaks that are not present are marked with labels with parenthesis. In each spectrum, there is a blown-up portion of the spectrum limited to the mass range where potential transglycosylation products are expected to be, with an arrow showing which peak in the full spectrum it corresponds to. Below each spectrum, the acceptor and theoretical m/z for the [m+Na^+] ion of potential transglycosylation products are listed. A control with inactivated enzyme, LBG and the acceptors are shown in the spectrum (**g**).