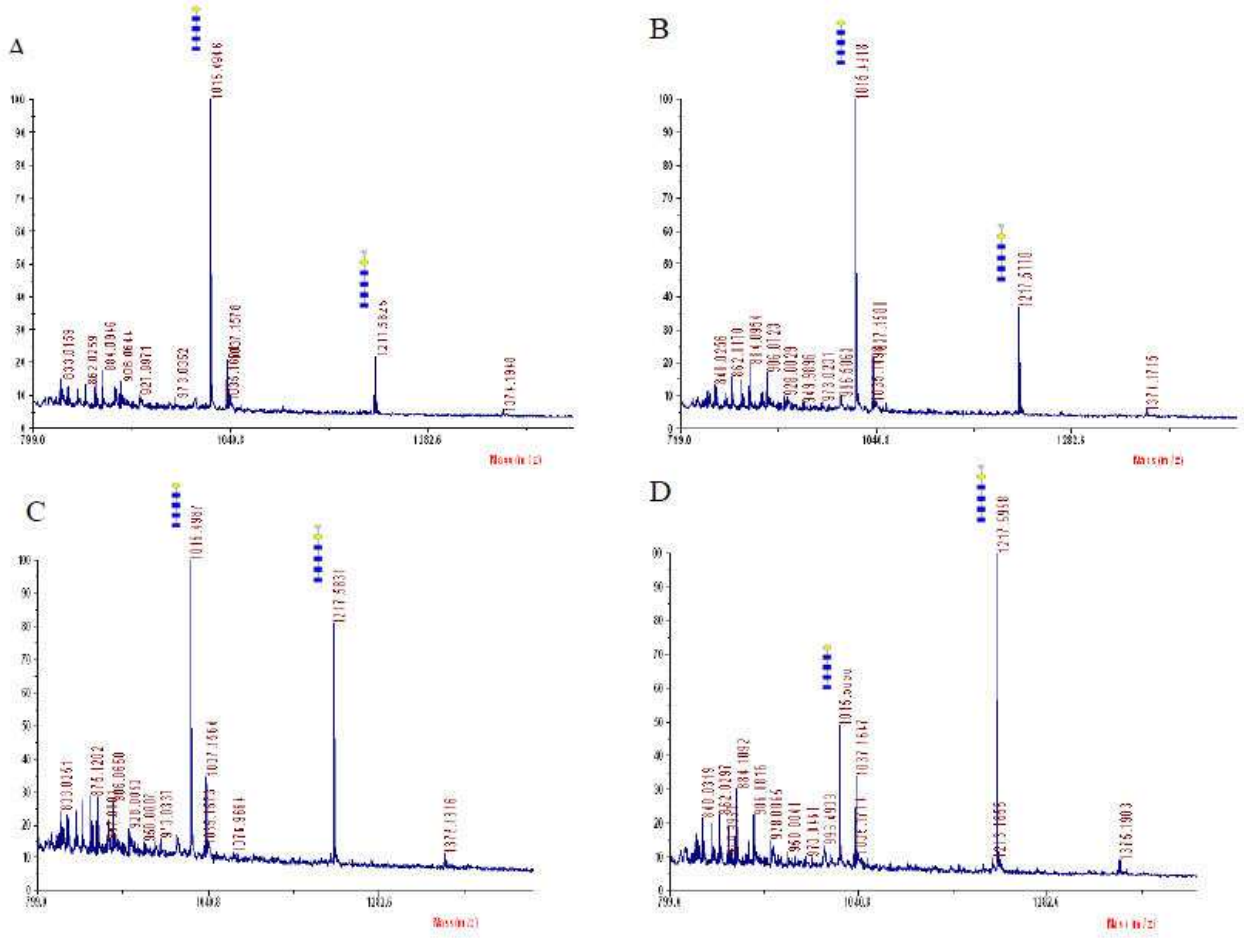
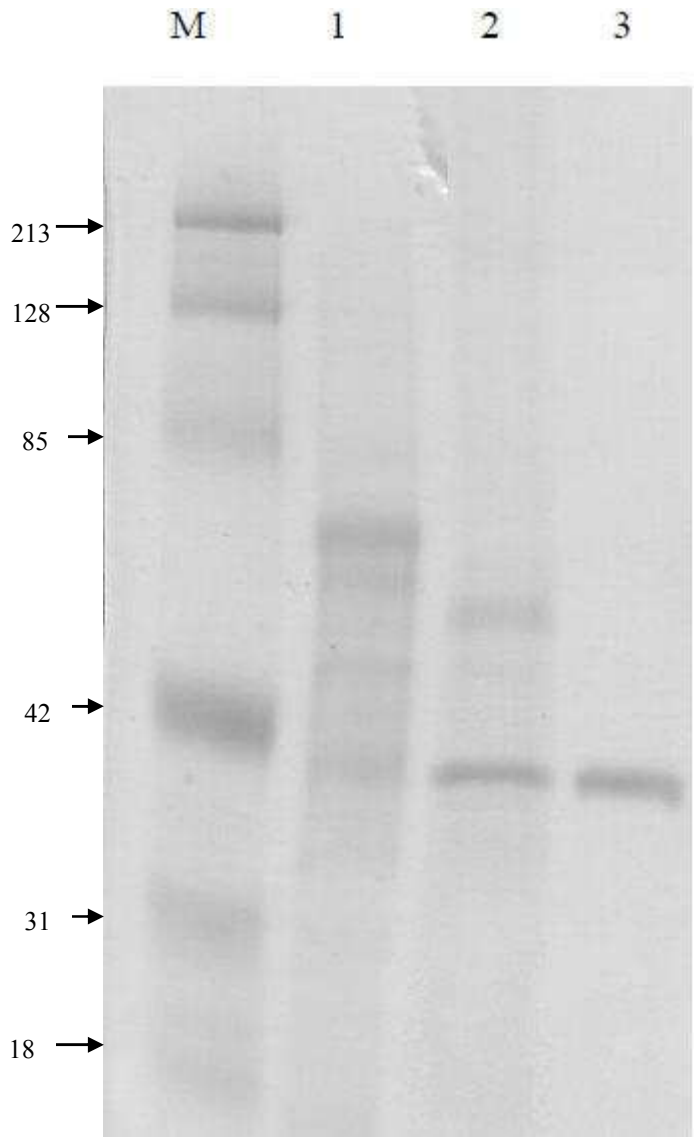
SM-Table 1 Comparison of the kinetic parameters of α3 Gal-T S347 (wt) (a) used in the current studies, with the α3 Gal-T A347 constructed in the lab (b) and that used in the references (26 (c)) and (28 (d)).

Enzyme	K_a (mM)	K_b (mM)	K_{ia} (mM)	K_{ib} (mM)	k_{cat} (s -1)	k_{cat}/K_{ia} (s -1 mM)	k_{cat}/K_{ib} (s -1 mM)
α3 Gal-T 280HAA282..S347[a]	0.790±0.001	4.50±0.01	0.13±0.01	0.90±0.02	3.4	4.3	7.5x10-1
α3 Gal-T 280HAA282..A347[b]	0.400 ±0.001	1.80±0.02	0.20±0.01	0.20±0.01	2.3	5.7	1.3
α3 Gal-T 280HAA282..A347[c]	0.430±0.070	19.9±3.40	0.14±0.03	N/A	6.4	2.3	N/A
α3 Gal-T 280HAA282..A347[d]	0.16	30	N/D	N/D	4.8	N/D	N/D

a α3Gal-T wild type with Ser at 347 used in this paper
b α3Gal-T wild type mutated at position 347 to Ala
c α3Gal-T wild type published (26)
d α3Gal-T wild type published (28)



SM-Figure 1. MALDI mass spectra of glycans after the transfer of 2-keto-Gal by the mutant enzyme SGG to a sugar acceptor galacto-chitotetrose Galβ1,4-GlcNAcβ1,4-(GlcNAcβ1,4-)$_3$. Incubation mixtures of 25 µl, containing 0.5 µg of the enzyme, 0.1 mM acceptor, 500 µM UDP-2-keto-Gal, 5 mM $MnCl_2$ and 25 mM Tris/HCl (pH 7.0), were incubated at 37^0 C for 1 h, 2 h, 6 h and overnight and analyzed by MS (A–D). The major peaks are annotated with the carbohydrate structure shown in the symbols for monosaccharides, according to the nomenclature adopted by the consortium for functional glycomics, http://www.functionalglycomics.org/static/consortium/. GlcNAc (blue squares), and 2-keto-Gal (yellow stars). MS analysis shows a shift in the molecular mass from the starting ion at 1015 *m/z* to a peak of 1217 *m/z* after the addition of 2-keto-Gal moiety (see Material and Methods section), for 1 h (A), 2 h (B), 6 h (C) and overnight incubation (D). A peak after the transfer of 2-keto-Gal moiety (1217 *m/z*) increases with time compared to the starting ion 1015 *m/z,* showing that the enzyme is stable and transfers modified sugar donor during the entire incubation time.



SM-Figure 2 SDS-PAGE analysis of asialofetuin under reduced conditions showing the protein stained bands. (M) Marker, (1) asialofetuin under reduced conditions, (2) asialofetuin under reduced condition after removal of N-glycans by PNGase F treatment, and (3) the PNGase F protein alone under reduced conditions. The chemiluminescence bands in the Figure 7 of the manuscript, with a main band of about 55 kD, correspond to the stained protein bands in the lane 1.