Fig S1. **Superimposition of the structures of raffinose** found in the ScAGal complex (orange) and the free raffinose structure (white) (36). There is a change of 150˚ in the C5-C6 (ω) torsion of the glycosidic linkage. This conformation is stabilized by the multiple interactions that the substrate is making with the protein and also with some high ordered water molecules. This change in the orientation of positions +1 and +2 of the substrate allows the recognition of the sugar by the enzyme, whereas the low energy conformation of the sugar would crash with loop L6 and the neighbour protein molecule.

Fig S2. **Structural alignment of GH27 members.** The structural alignment of ScAGal (3lrkA), the α-galactosidases from *U. vinacea* (3a5vA), rice (1uasA), *T. reesei* (1t0oA), *H. sapiens* (1r47A), the α-N-acetyl-galactosaminidases from *H. sapiens* (3h54A) and *G. gallus* (1ktcA) and the α-galactosidase from *Bacillus halodurans* (3cc1) was generated with the DALI server (45) and ESPript (46). ScAGal secondary structure is shown above the sequence alignment. The black squares indicate sequence similarity. The insertions in ScAGal loops (L6, I1, I2, I3) are highlighted with a blue box. Those insertions involved in dimerization in human and chicken enzymes are highlighted with orange boxes whereas the "2-position recognition loop", at L5, is within the magenta square. The insertion in *T. reesei* L4 is highlighted with a red box. Grey numbers refer to disulphide bonds in the ScAGal structure. Orange marks refer to glycosylated asparagines. Blue arrows highlight the residues involved in substrate recognition.

Figure S1

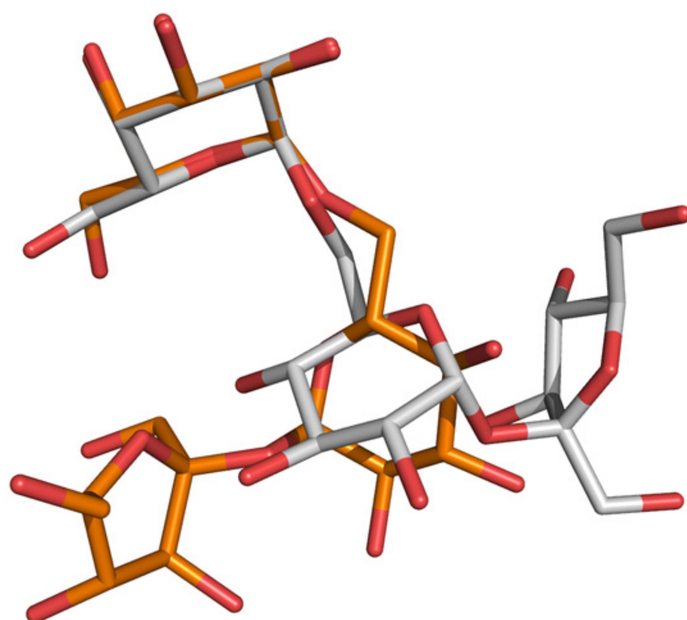

Figure S2