Supporting information

1 - Synthesis procedure of N-MANT labelled maltose 9 and Lactose 10

Solvents were dried by standard methods, molecular sieves were activated by heating for 4 h at 500°C and *N*-methylisatoic anhydride was recrystallized in acetone prior to use. Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–25°C with a Perkin-Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 25°C with a Bruker Advance II 400 instrument, with Me_4Si as internal standard, unless otherwise stated. Assignments were based on homo- and heteronuclear correlations using the supplier's software. In the NMR data for maltose derivatives, Glc^{I} (with roman numeral superscripts) refer to "reducing" D-glucose residue whereas Glc^{II} refer to "non-reducing" D-glucose residue. High-resolution mass spectra (HRESIMS) were performed on a Bruker maXis mass spectrometer by the 'Fédération de Recherche ICOA/CBM' (FR 2708) platform. Flash-silica chromatography was performed on Silica gel 60 (0.040–0.063 mm, Merck, Darmstadt). The reactions were monitored by TLC on coated aluminium sheets (silica gel 60 GF_{254}, Merck), and spots were detected under UV light and by charring with a 95/5 mixture of ethanol and sulfuric acid.

2-benzyloxycarbonylaminoethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl- (1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (5): A mixture of imidate **3**[1] (758 mg, 0.62 mmol), 2-benzyloxycarbonylaminoethanol (195 mg, 1.0 mmol) and 4 Å powdered molecular sieves (0.2 g) in anhydrous CH_2Cl_2 (8 mL) was stirred for 40 min at rt under dry argon then cooled at 0°C. A solution of TMSOTf in toluene (1M, 75 µL) was added and the mixture was stirred for 30 min at 0°C, then was quenched with NEt_3 (100 µL), filtrated and concentrated. Flash silica chromatography (EtOAc/petroleum ether 4:3, containing 0.1 % of NEt_3) afforded the glycoside **5** (690 mg, 89 %) as a white foam. $[\alpha]_D^{20}$ = +61 (*c* = 1 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16-7.10 (m, 40H, Ar-H), 6.07 (dd, $J_{2,3} = J_{3,4}$ = 10.0 Hz, 1H, Glc^{II} H-3), 5.73 (dd, $J_{2,3} = J_{3,4}$ = 8.0 Hz, 1H, Glc^{I} H-3), 5.72 (d, $J_{1,2}$ = 4 Hz, 1H, Glc^{II} H-1), 5.64 (dd, $J_{4,5}$ = 10.0 Hz, 1H, Glc^{II} H-4), 5.25 (m, 2H, Glc^{I} H-2, Glc^{II} H-2), 5.12 (m, 1H, N*H*), 4.97 (ABq, 2H, CH_2-Ar), 4.95 (dd, $J_{5\text{-}6a}$ = 2.5 Hz, $J_{6a\text{-}6b}$ = 12 Hz, 1H, Glc^{I} H-6a), 4.73 (d, $J_{1,2}$ = 7.5 Hz, 1H, Glc^{I} H-1), 4.68 (dd, $J_{5\text{-}6b}$ = 4 Hz, 1H, Glc^{I} H-6b), 4.43 (m, 3H, Glc^{I} H-4, Glc^{II} H-5, H-6a), 4.28 (dd, $J_{5\text{-}6b}$ = 4 Hz, $J_{6a\text{-}6b}$ = 12 Hz, 1H, Glc^{II} H-6b), 4.03 (m, 1H, Glc^{I} H-5), 3.84 (m, 1H, O-C*H*), 3.67 (m, 1H, O-C*H*), 3.30 (m, 2H, N-CH_2); HRMS (ESI): *m/z*: calcd for $C_{71}H_{61}NNaO_{20}$: 1270.36791 [*M*+Na]$^+$; found: 1270.36824.

2-benzyloxycarbonylaminoethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl- (1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (6): A mixture of imidate **4**[1] (820 mg, 0.67 mmol) and 2-benzyloxycarbonylaminoethanol (195 mg, 1.0 mmol) was submitted to the same procedure as described for the preparation of compound **5**. Flash silica chromatography (EtOAc/petroleum ether 4:3, containing 0.1 % of NEt_3) afforded the glycoside **6** (764 mg, 91 %) as a white foam. $[\alpha]_D^{20}$ = +46 (*c* = 1 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10-7.10 (m, 40H, Ar-H), 5,78 (dd, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 1H, Glc H-3), 5.73 (dd, $J_{3,4}$ = 3,5 Hz, $J_{4,5}$ < 1.0 Hz, 1H, Gal H-4), 5.71 (dd, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10 Hz,1H, Gal H-2), 5.40 (dd, $J_{1,2}$ = 8 Hz, 1H, Glc H-2), 5.38 (dd, 1H, Gal H-3), 5.11 (m, 1H, N*H*), 4.92 (ABq, CH_2-Ar), 4.87 (d, 1H, Gal H-1), 4.65 (d, 1H, Glc H-1), 4.59 (dd, $J_{5\text{-}6a}$ = 1.5 Hz, $J_{6a\text{-}6b}$ = 12 Hz, 1H, Glc H-6a), 4.43 (dd, $J_{5\text{-}6b}$ = 4 Hz, 1H, Glc H-6b), 4.21 (dd, $J_{4\text{-}5}$ = 9.5 Hz, 1H, Glc H-4), 3.90 (m, 1H, Gal H-5), 3.78 (m, 2H, Glc H-5, O-C*H*), 3.71 (m, 2H, Gal H-6a, H-6b), 3.62 (m, 1H, O-C*H*), 3.30 (m, 2H, N-CH_2); HRMS (ESI): *m/z*: calcd for $C_{71}H_{65}N_2O_{20}$: 1265.41252 $[M+NH_4]^+$; found: 1265.41349.

2-benzyloxycarbonylaminoethyl α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (7): A solution of compound **5** (674 mg, 0.54 mmol) was treated for 3 h with methanolic sodium methoxide (1M, 0.5 mL), then was deionized with Amberlite IR-120 [H+] resin, filtered and concentrated. The resulting solid was washed with a mixture of petroleum ether/diethyl ether (1/1, 2x10 mL) then was dried to afford the disaccharide **7** (280 mg, 97%) as a amorphous solid. $[\alpha]_D^{20}$ = +56 (*c* = 1 in MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (m, 5H, Ar-H), 5.12 (d, $J_{1,2}$ 3.5 Hz, 1H, Glc$_{II}$ H-1), 5.05 (m, 2H, CH_2-Ph), 4.28 (d, $J_{1,2}$ = 8.0 Hz, 1H, Glc$_I$ H-1), 3.90 (m, 1H, O-C*H*), 3.80 (m, 3H, Glc$_I$ H-6a, Glc$_{II}$ H-6a, Glc$_{I \text{ or } II}$ H-6b), 3.60 (m, 5H, Glc$_I$ H-3, Glc$_{II}$ H-3, H-5, Glc$_{I \text{ or } II}$ H-6b, O-C*H*), 3.50 (dd, $J_{3,4} = J_{4,5}$ = 9.0 Hz, 1H, Glc$_I$ H-4), 3.42 (dd, $J_{2\text{-}3}$ = 9.5 Hz, 1H, Glc$_{II}$ H-2), 3.33 (m, 2H, N-CH_2), 3.25 (m, 3H, Glc$_I$ H-2, H-5, Glc$_{II}$ H-4); HRMS: *m/z*: calcd for $C_{22}H_{33}NaNO_{13}$: 542.18441 $[M+Na]^+$; found: 542.18153.

2-benzyloxycarbonylaminoethyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (8): Compound **6** (730 mg, 0.58 mmol) was submitted to the same procedure as described for the preparation of compound **7**. Disaccharide **8** (300 mg, 96 %) was obtained as an amorphous solid. $[\alpha]_D^{20}$ = +0.5 (*c* = 1 in MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (m, 5H, Ar-H), 5.08 (m, 2H, CH_2-Ph), 4.35 (d, $J_{1,2}$ = 7.5 Hz, 1H, Gal H-1), 4.30 (d, $J_{1,2}$ 8.0 Hz, 1H, Glc H-1), 3.90 (m, 1H, O-C*H*), 3.85 (m, 3H, Glc H-6a, Gal H-6a, Glc or Gal H-6b), 3.80 (dd, $J_{3,4}$ = 3,0 Hz, $J_{4,5}$ < 1.0 Hz, 1H, Gal H-4), 3.70 (m, 1H, Glc or Gal H-6b), 3.65 (m, 1H, O-C*H*), 3.55 (m, 5H, Glc H-3, H-4, Gal H-2, H-3, H-5), 3.40 (m, 2H, N-CH_2), 3.30 (m, 1H, Glc H-5), 3.25 (dd,

$J_{2\text{-}3}$ = 8.5 Hz, 1H, Glc H-2); HRMS (ESI): *m/z*: calcd for $C_{22}H_{33}NaNO_{13}$: 542.18441 [*M*+Na]$^+$; found: 542.183739.

2-(N-methylanthranilyl)aminoethyl α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (9) (Maltose-N-MANT): A solution of **7** (280 mg, 0.54 mmol) in a mixture water/MeOH 4/1 (10 mL) was hydrogenolyzed in the presence of 10% Pd/C catalyst (50 mg) for 20 h at rt. The catalyst was removed by filtration through Celite pad and the filtrate was concentrated to give the intermediate amine. To a solution of the amine and $NaHCO_3$ (1M, 1 mmol) in water (5 mL) was added dropwise a solution of *N*-methylisatoic anhydride (200 mg, 1 mmol) in 1,4-dioxane (2 mL). The mixture was stirred for 2 h at rt then was concentrated. The residue was extracted with a mixture of MeOH/CH_2Cl_2 (1/1, 2x10 mL) then concentrated. Flash silica chromatography (CH_2Cl_2/MeOH 5:2) afforded the maltose N-MANT **9** (205 mg, 79 %) as a white foam. [α] $_D^{20}$ = +57 (*c* = 1 in MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.44 (m, 1H, Ar-H), 7.32 (m, 1H, Ar-H), 6.64 (m, 1H, Ar-H), 6.56 (m, 1H, Ar-H), 5.12 (d, $J_{1,2}$ = 4.0 Hz, 1H, Glc$_{II}$ H-1), 4.31 (d, $J_{1,2}$ = 8.0 Hz, 1H, Glc$_I$ H-1), 3.97 (m, 1H, O-C*H*), 3.80 (m, 3H, Glc$_I$ H-4, Glc$_{II}$ H-6a, H-6b), 3.73 (m, 1H, O-C*H*), 3.62 (m, 3H, Glc$_I$ H-6a, H-6b, N-C*H*), 3.60 (m, 2H, Glc$_I$ H-3, Glc$_{II}$ H-4), 3.50 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, Glc$_{II}$ H-3), 3.48 (m, 1H, N-C*H*), 3.42 (dd, 1H, Glc$_{II}$ H-2), 3.36 (m, 1H, Glc$_{II}$ H-5), 3.28 (m, 1H, Glc$_I$ H-5), 3.23 (dd, $J_{2,3}$ 9.0 Hz, 1H, Glc$_I$ H-2), 2.78 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 172.05 (1 C, NH-CO), 151.08, 133.47, 129.05, 116.96, 115.69, 111.66 (6C, Ar-C), 104.16 (1C, Glc$_I$ C-1) 102.60 (1C, Glc$_{II}$ C-1), 80.90 (1C, Glc$_I$ C-4), 77.34 (1C, Glc$_I$ C-3), 76.28 (1C, Glc$_I$ C-5), 74.71, 74.46, 74.33 (3C, Glc$_{II}$ C-2, 3, 5), 73.78 (1C, Glc$_{II}$ C-4), 71.22 (1C, Glc$_I$ C-2), 69.51 (1C, O*C*H$_2$), 62.39, 61.76 (2C, Glc$_I$ C-6, Glc$_{II}$ C-6), 40.38 (1C, N-*C*H$_2$), 29.68 (1C, N-*C*H$_3$); HRMS (ESI): *m/z*: calcd for $C_{22}H_{35}N_2O_{12}$: 519.71205 [*M*+H]$^+$; found: 519.71228.

2-(N-methylanthranilyl)aminoethyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (10) (Lactose N-MANT): Compound **8** (210 mg, 0.40 mmol) was submitted to the same procedure as described for the preparation of compound **9**. Flash silica chromatography (CH_2Cl_2/MeOH 5:2) afforded the lactose N-MANT **10** (156 mg, 75 %) as a white foam. [α] $_D^{20}$ = +9 (*c* = 1 in MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ =7.45 (m, 1H, Ar-H), 7.36 (m, 1H, Ar-H), 6.66 (m, 1H, Ar-H), 6.54 (m, 1H, Ar-H), 4.35 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal H-1), 4.34 (d, $J_{1,2}$ = 7.5 Hz, 1H, Glc H-1), 3.98 (m, 1H, O-C*H*), 3.82 (m, 3H, Glc H-4, Gal H-6a, H-6b), 3.75 (m, 1H, O-C*H*), 3.62 (m, 2H, Glc H-6a, H-6b), 3.61 (m, 1H, N-C*H*), 3.60 (m, 3H, Glc H-5, Gal H-2, 4), 3.48 (dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.6 Hz, 1H, Gal H-3), 3.45 (m, 1H, N-C*H*), 3.42 (m, 1H, Glc H-5), 3.36 (m, 1H, Gal H-5), 3.26 (dd, 1H, Glc H-2), 2.80 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.94 (1C, NH-CO), 151.0, 133.37, 128.97, 116.91, 115.59, 111.56 (6C,

Ar-C), 104.73 (1C, Glc C-1) 103.96 (1C, Gal C-1), 80.29 (1C, Glc C-4), 76.66 (1C, Glc C-3), 76.11 (1C, Glc C-5), 75.87, 74.41, 74.36, 74.33, 72.14 (5C, Glc C-2, Gal C-2, 3, 4, 5), 69.92 (1C, O*C*H_2), 62.14, 61.49 (2C, Glc C-6, Gal C-6), 40.31 (1C, N-*C*H_2), 29.58 (1C, N-*C*H_3); HRMS (ESI): *m/z*: calcd for $C_{22}H_{35}N_2O_{12}$: 519.71205 [*M*+H]$^+$; found: 519.71232.

2 - Expression and purification of recombinant 6His-tagged Galectin-1 (6His-Gal-1) and 6His-tagged Maltose Binding Protein (6His-MBP)

Both MBP and Gal-1 were expressed as soluble 6His-tagged proteins in *E. coli* Rosetta2 (DE3) bacteria.

To produce 6His-MBP, pETM-41 expression vector containing the 6His-MBP cDNA sequence was used. After transformation, the recombinant bacteria were plated on a selective medium containing 50 µg/mL kanamycin (Sigma-Aldrich) and 35 µg/mL chloramphenicol (Sigma-Aldrich). A single recombinant colony was inoculated in fresh Luria-Bertani (LB) medium (Sigma-Aldrich) and cultured overnight at 37°C in an orbital shaker under mild agitation. The pre-culture was transferred into fresh LB medium containing 50 µg/mL kanamycin and 35 µg/mL chloramphenicol and incubated at 37°C until OD600nm reached 0.6. Induction of protein expression was carried out by adding 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma-Aldrich). The resulting suspension was incubated overnight at 20°C under mild orbital shaking. The bacteria were harvested by centrifugation at 6,000×g for 15 min at 4°C. The pellets were washed with Phosphate Buffered Saline (PBS) (Sigma-Aldrich) and centrifuged at 6,000 ×g for 10 min at 4 °C before being frozen at -80 °C during 30 min. After a thawing step, the pellets were suspended in lysis buffer containing 50 mM sodium phosphate pH 6.9, 300 mM NaCl, 5 mM imidazole. Cell lysis was carried out by 8 cycles of 1 min sonication at 50% power (Branson Sonifier 250). Soluble proteins were collected from the supernatant after 30 min centrifugation at 12,000×g. The supernatant was treated with endonuclease (Benzonase® Nuclease, 250 units/10 ml, Sigma-Aldrich) and 2 mM $MnCl_2$, before being incubated 1h at 4°C. The protein suspension was filtrated through a 0.45 µm membrane (Supor®, PALL-Life Science) and finally loaded onto a 5 mL HiTrap TALON Crude column (GE Healthcare) to purify soluble 6His-MBP. Columns were connected to a Medium-Pressure Chromatography System (Biorad). 50 mL elution buffer (50mM sodium phosphate pH 6.9, 300 mM NaCl and 150 mM Imidazole) were used to eluate 6His-MBP. The proteins were desalted with ultracentrifugal filters Amicon Ultra-15 MWCO 10kDa (Millipore). During the concentration step, imidazole was progressively removed from 6His-MBP solution by washing steps using a 50 mM sodium phosphate pH 6.9, 300 mM NaCl buffer. Expression

and purity of the protein were checked by SDS-PAGE 15% (w/v), after Coomassie Brillant Blue gel staining (Figure S1). 6His-MBP concentration was determined measuring OD_{280nm} on a UV spectrophotometer (UV-1800 Shimadzu) using the molar extinction coefficient ε_{280nm} = 66,000 $M^{-1}.cm^{-1}$.

A similar protocol was used to produce 6His-Gal-1. We first generated the recombinant plasmid pET-28b-hGal-1 by inserting the human cDNA sequence of Gal-1 (GenBank® accession n°NM_002305.3), provided by GeneART® Gene Synthesis (Invitrogen), into the vector pET-28b using NdeI and XhoI restriction sites. Bacteria transformation and protein production steps were all carried out as described above, exception made for the protein extraction step which required a 20 mM sodium phosphate pH 7.4, 500 mM NaCl and 5 mM imidazole lysis buffer. After bacteria lysis and centrifugation of the lysate, the supernatant was treated as above with endonuclease, filtered, and 6His-Gal-1 was purified on a 1 mL HiTrap HP column (GE Healthcare) using 10 mL of a 20 mM sodium phosphate pH 7.4, 500mM NaCl and 500 mM imidazole buffer. Then, we proceeded to protein desalination and concentration using PD-10 Desalting columns (Amersham Biosciences) and centrifugal filters Amicon Ultra-15 MWCO 3 kDa (Millipore), respectively. As above, a 20 mM sodium phosphate pH 7.4, 500 mM NaCl buffer was used for these steps to remove imidazole. Finally, the concentration of the protein was estimated by UV spectroscopy using ε_{280nm} = 8,400 $M^{-1}.cm^{-1}$ and its expression and purity was checked by SDS-PAGE 15% (w/v), after Coomassie Brillant Blue gel staining (Figure S2). pETM-41 and pET-28b vectors were kindly provided by Dr. Behm-Ansmant I. and Dr. Kriznick A. (UMR 7365 CNRS-University of Lorraine, France). Chemicals were all purchased from Sigma-Aldrich and used as received without further purification.

3 - Binding assays

To check whether the glycoconjugates efficiently bind with their respective target, we first proceeded to binding assays monitored by FP or FRET in opaque black half-area 96-well microplates (GREINER BIO-ONE) at room temperature. By FP, 10 µM of Maltose-N-MANT or Lactose-N-MANT were mixed with 0 to 300 µM 6His-MBP or 0 to 1,500 µM 6His-Gal-1, respectively. A 20 mM sodium phosphate pH 7.4, 500 mM NaCl buffer was used for assays performed with 6His-Gal-1 whilst a 50 mM sodium phosphate pH 6.9, 300 mM NaCl buffer was preferred for assays with 6His-MBP. FP measurements were carried out on the Xenius XC spectrofluorometer equipped with a monochromator coupled with a light polarizer and a plate reader (SAFAS MONACO). FP signals were collected at 430 nm upon a polarized excitation

at 320 nm. G-factor was systematically measured then fixed before each assay. In parallel, fluorescence intensity at 430 nm upon an excitation at 320 nm was also systematically collected to ensure that changes in FP signals were not caused by artefactual changes in the fluorescence intensity of our conjugates during the assays (especially upon protein addition). For an optimal detection, the photomultiplier module (PMT) was set at 616 V for assays performed with Maltose-N-MANT/6His-MBP and 650 V for Lactose-N-MANT/6His-Gal-1. For FRET-monitored assays, 50 µM 6His-MBP or 150 µM 6His-Gal-1 were mixed with 0 to 250 µM Maltose-N-MANT or 0 to 2 mM Lactose-N-MANT, respectively. FRET signals at 430 nm were collected upon an excitation at 274 nm on a Xenius XC spectrofluorometer. Slit width was set at 5 nm for both excitation and emission and PMT was set at 650 V. In parallel, fluorescence spectra from 300 to 500 nm, upon an excitation at 274 nm, were systematically recorded. For both approaches, 15 min incubation at room temperature were taken before plate reading. Binding experiments were performed as duplicate, and repeated three times for an accurate statistical analysis.

4 - Competition assays

4.1 - Competition assays using non UV-active competitor candidates

We checked the capacity of the labelled-glycoconjugates to report competition assays by FP and FRET. The buffers were those presented above. The assays were performed in opaque black half-area 96-well microplates and three independent experiments were performed in duplicate. All mixtures were incubated 15 min at room temperature before plate reading. For FP-monitored assays, 10 µM Maltose-N-MANT or Lactose-N-MANT were first mixed with 50 µM 6His-MBP or 150 µM 6His-Gal-1, respectively. 0 to 5 mM maltose or 0 to 100 mM lactose were then added to compete against Maltose-N-MANT or Lactose-N-MANT in binding complex with 6His-MBP or 6His-Gal-1, respectively. As above, FP signals at 430 nm were measured on Xenius XC spectrofluorometer upon a polarized excitation light at 320 nm. PMT was set to 700 V for assays carried out with Maltose-N-MANT/6His-MBP or 650 V for those with Lactose-N-MANT/6His-Gal-1, respectively. G-factor was systematically measured and fixed before each assay. For FRET-monitored assays, 50 µM Maltose-N-MANT were mixed with 50 µM 6His-MBP and 150 µM Lactose-N-MANT were first added onto 150 µM 6His-Gal-1 to form the complexes. 0 to 5 mM maltose or 0 to 100 mM lactose were then loaded as competitors. FRET signals at 430 nm were collected upon an excitation at 274 nm on the Xenius XC spectrofluorometer. Fluorescence spectra from 300 to 500 nm, upon an excitation at 274

nm, were systematically recorded. PMT was set at 650 V to ensure that at least about 15% of the maximum detection at the FRET acceptor wavelength was reached in our conditions.

4.2 - Competition assays using UV-active competitor candidates in HTS conditions

To check whether our glycoprobes can be used in competition experiments with UV-active compounds, we performed additional FRET-based assays with 4-nitrophenyl-β-D-Lactoside (pNP-Lac) (LIBIOS), and 4-methyl-umbelliferyl-β-Lactoside (4MU-Lac) (BIOSYNTH®, Carbosynth) as competitors. To this end, 250 µM of competitor candidate were mixed with 100 µM Lactose-*N*-MANT **10** in the presence of 150 µM 6His-Gal-1, in a 20 mM sodium phosphate pH 7.4 buffer. Samples were excited at 280 nm and the fluorescence spectra were recorded from 290 to 500 nm on a Xenius XC spectrofluorometer. PMT was set at 760 V. The experiments were all performed in triplicate in opaque black half-area 96-well microplates (GREINER BIO-ONE) at room temperature. The fluorescence spectra related to the competition assay with pNP-Lac and 4MU-Lac are shown in figure S3 A and B, respectively. To quantify the probe displacement by the competitors, we collected the fluorescence at 449 nm to ensure that the signal intensity measured corresponds to a FRET effect only. The average and standard deviation of the signals from three independent experiments (n = 3) are reported in figure S3 C. Briefly, in our conditions, FRET signals reached 4.00 ± 0.12 a.u. with 100 µM Lactose-*N*-MANT alone, 6.37 ± 0.10 a.u. with 100 µM Lactose-*N*-MANT mixed with 150 µM 6His-Gal-1, and 4.91 ± 0.08 a.u. in the presence of 250 µM pNP-Lac and 4.18 ± 0.20 a.u. in the presence of 250 µM 4MU-Lac. Z-factor was equal to 0.73.

5 - Data analysis

5.1 - The binding model

For all assays, we assumed that the fluorescent glycoconjugates bind their respective target according to a 1:1 stoichiometry. Such an approach is obviously raw as it does not reflect the capacity of some CBPs to form multimeric complexes in some specific conditions.[2] However, the use of simple but robust binding model is required to generate standardized values of binding parameters such as Kd which are widely used in the context of biomolecules interaction studies. The following model was thus used:

$$L + P \leftrightarrow LP$$

in which, L represents the fluorescent probe, P the protein of interest, and LP the complex formed between the probe and its biological target.

In agreement, with this model:

$$Lt = [L] + [LP] \quad (S1)$$

in which Lt is the total concentration of the fluorescent probe, [L] is the concentration of the fluorescent glycoconjugate in its unbound state, and [LP] represents the concentration of the glycoconjugate in its bound state.

$$Pt = [P] + [LP] \quad (S2)$$

in which Pt is the total concentration of protein, [P] the concentration of the protein in its unbound state and [LP] the concentration of complex at the equilibrium state, respectively.

As Kd is usually defined as:

$$Kd = [L]*[P] / [LP] \quad (S3)$$

solving together equations (S1), (S2) and (S3) leads to:

$$[LP] = (½) \{(Lt+Pt+Kd) – [(Lt+Pt+Kd)_2 – 4LtPt]_{1/2}\} \quad (S4)$$

As a consequence, [L] and [P] could be deduced from equation (S1) and (S2) respectively, knowing the respective values of Lt and Pt.

5.2 - Curve fitting equation used for FP-monitored binding assays

FP signal of a sample generally depends on the rate of the fluorescent reporter in its unbound form to its bound form in solution. For each stage of a binding experiment, the FP value of a sample can be expressed as:

$$r = r_1*[LP]/Lt + r_0*[L]/Lt \quad (S5)$$

in which r is the FP value collected at 430 nm, r0 and r2 correspond to the intrinsic FP values of the fluorescent conjugate in its unbound and bound form respectively, and Lt, [L] and [LP] are as previously defined.

Thanks to equation (S1), equation (S5) can be rewritten as:

$$r= (r_1-r_0)*[LP]/Lt + r_0 \quad (S6)$$

in which [LP] is expressed according to equation (S4).

The mean of FP values at 430 nm determined from the three repeats performed in duplicate for each protein concentration was used to build the binding curve. The binding curve was fitted to equation (S6) using the software Prism7 (GraphPad). r and Pt were set as dependent and independent parameters, respectively. Lt was fixed at 10 µM which was the concentration of Maltose-N-MANT and Lactose-N-MANT. r0 was fixed at the FP value measured in the absence of protein whilst r1 and Kd were let varied to extract Kd value and the corresponding standard

deviation (*sd*). As explained above, we also checked that the emission obtained at 430 nm under excitation at 340 nm remained stable upon the successive addition of proteins.

5.3 - Curve fitting equation used for FRET monitored binding assays

Binding assays involving N-MANT labelled molecules were also monitored by FRET. Hence, fluorescence intensities at 430 nm were measured upon excitation of the sample at 274 nm reflect complex formation. Nevertheless, excitability of the dye at 274 nm was also taken into account in the total fluorescence at 430 nm as its intrinsic absorbance spectra was shown broad in aqueous buffer.[3] As a consequence, the total fluorescence intensities collected at 430 nm was expressed as:

$$F = F_0 + F_1*[L] + F_2*[LP] \quad (S7)$$

in which, F_0 corresponds to the residual fluorescence at 430 nm, F_1 is the fluorescence coefficient describing the excitability of the conjugate at 274 nm and F_2 is the fluorescence coefficient associated to FRET acceptor signal caused by the complex formation. [L] and [LP] are as defined previously.

Thanks to equation (S1), equation (S7) could be rewritten as:

$$F = F_0 + F_1*Lt + (F_2 - F_1)*[LP] \quad (S8)$$

in which [LP] is the concentration of the LP complex expressed according to equation (S4) and Lt is the total concentration of the fluorescent glycoconjugate.

The mean of FRET values at 430 nm determined from the three repeats of the binding experiment performed in duplicate was used to build the binding curve. The binding curve obtained was then fitted to equation (S8). Again, Prism7 (GraphPad) was used as software. For the fitting process, F and Lt were defined as the dependent and the independent parameters, respectively. F_0 were used fixed at the fluorescence measured in the absence of the fluorescent probe. Pt value was set to 50 µM for experiments performed with 6His-MBP and 150 µM for those performed with 6His-Gal-1.

5.4 - The competition model

Assuming a 1:1 stoichiometry between L and P, and that I, a competitor, competes against L for the binding pocket of the protein of interest, we used the following competitive model:

$$LP \leftrightarrow L + P \ (Kd)$$

$$P + I \leftrightarrow IP \ (Ki)$$

in which, L is the fluorescent probe, P the protein of interest and I the competitor whilst Kd and Ki respectively reflect the binding strength of L and I for P.

Competition assays were also monitored by FP and FRET by using the capacity of Maltose-N-MANT or Lactose-N-MANT, to report the potency of maltose and lactose to dissociate either Maltose-N-MANT/6His-MBP and Lactose-N-MANT/6His-Gal-1 complexes, respectively. The mean of FP or FRET signals (at 430 nm) determined from the three independent competition experiments performed in duplicate were reported against the concentration of the competitor to build the competition curve. Thus, the following curve-fitting equation was used:

$$Ft = F_{min} + (F_{max}-F_{min}) / [1+(IC_{50}/It)]_n \quad (S9)$$

in which, Ft indistinctively represents the total FP or FRET signals read at 430 nm in the presence of the total concentration of competitor It, F_{min} and F_{max} are the FP or FRET signals respectively measured at the bottom and the upper plateau of the competition curve, and IC_{50} is the concentration of inhibitor required to dissociate half of the LP complex preformed. “n” is the Hill number reflecting the slope of the decreasing part of the competition curve.
For an accurate estimate of IC_{50}, all parameters were let varied.

Then, Ki values were estimated thanks to an adapted expression of Cheng and Prussof’s model:[4,5]

$$Ki = IC_{50} / [1 + [L]_{50}/Kd)] \quad (S10)$$

in which, Ki and Kd respectively quantify the binding strength of IP and LP complexes, IC_{50} is the value previously determined from the curve fitting with equation (S9) and $[L]_{50}$ is the concentration of the free fluorescent conjugate reached when It equals to IC_{50}.
Assuming that disaggregation of half of the preformed LP complex upon addition of IC_{50} competitor systematically generates a corresponding increase of the free fluorescent glycoconjugate concentration, $[L]_{50}$ was determined according to:

$$[L]_{50} = [L]_0 + [LP]_0/2 \quad (S11)$$

in which, $[LP]_0$ is calculated from equation (S4) knowing Kd, Lt and Pt values and $[LP]_0$ can be deduced from equation (S1), knowing Lt and $[LP]_0$.
Taking into account the uncertainties from Kd and IC_{50} values, the total differential expression of Ki (*d*Ki) allowed to estimate *sd* of the Ki determined according to equation (S12):

$$dKi = [Kd / (Kd + [L]_{50})] * d\,IC_{50} + [(IC_{50} * [L]_{50}) / (Kd + [L]_{50})_2] * dKd \quad (S12)$$

in which, *d*IC_{50} and *d*Kd are respectively the *sd* of IC_{50} and Kd previously determined and $[L]_{50}$ is the concentration of the fluorescent glycoconjugate in its free form at IC_{50}.

Finally, to assess the quality of the competition assays, and since the glycoconjugates have been designed towards fluorescent-based HTS, Z-scores (Z') were systematically determined as:

$$Z' = 1 - 3*(\sigma_p + \sigma_n) / (\mu_p - \mu_n) \quad \text{(S13)}$$

in which, μ_p and μ_n are the mean value of FRET (or FP) signals at 430nm read in the absence and in the presence of the maximum concentration of competitor (lower plateau of the competition curve), respectively, whilst σ_p and σ_n are the standard deviations corresponding to μ_p and μ_n, respectively.

Supplemental figures

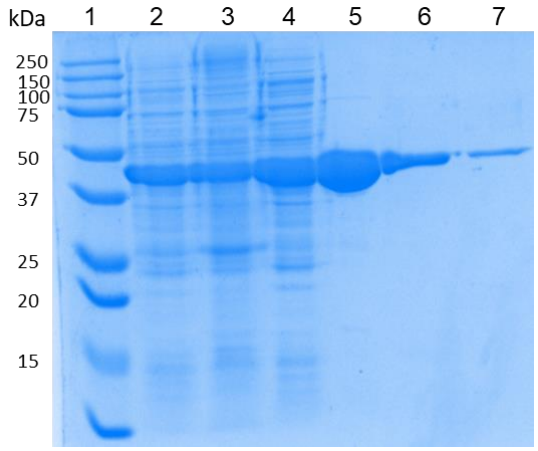

Fig. S1: Expression and purification of 6His-MBP. 6His-MBP was expressed in *E. coli* and purified from supernatant obtained from 12 000 × g centrifugation then eluted by affinity chromatography. Protein expression and purification were analysed by SDS–PAGE 15% stained by Coomassie Brilliant Blue staining. From left to right: lane 1: molecular mass marker, lane 2: total protein expressed in bacteria cultured after overnight induction with IPTG, lane 3: total protein in bacteria pellets from the first 6,000 × g centrifugation step, lane 4: total proteins in the supernatant from the 12,000 xg centrifugation on bacteria lysis, lane 5-7: 6His-MBP elution by affinity chromatography using a HiTrap TALON Crude column (GE Healthcare). 15 µg of total protein were loaded into wells 1 to 5 (elution pic). When not possible, the largest possible volume of sample was loaded (wells 6 and 7).

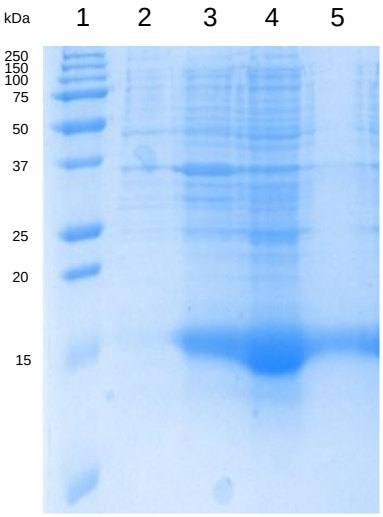

Fig. S2: Expression and purification of 6His-Gal-1. 6His-Gal-1 was expressed in *E. coli* and purified from supernatant obtained from a 12,000 × g centrifugation from bacteria lysis then eluted by affinity chromatography. Protein expression and purification were analysed by SDS–PAGE 15% stained by Coomassie Brilliant Blue staining. From left to right: lane 1: molecular mass, lane 2 and 3: total protein expressed in bacteria culture before and after IPTG induction, respectively, lane 4: total protein in supernatant from 12,000 × g centrifugation on bacteria lysis, lane 5: 6His-Gal-1 elution by affinity chromatography using a HiTrap HP column (GE Healthcare). 15 µg of total protein were loaded into well 4. When not possible, the largest possible volume of sample was loaded (wells 2, 3 and 5).

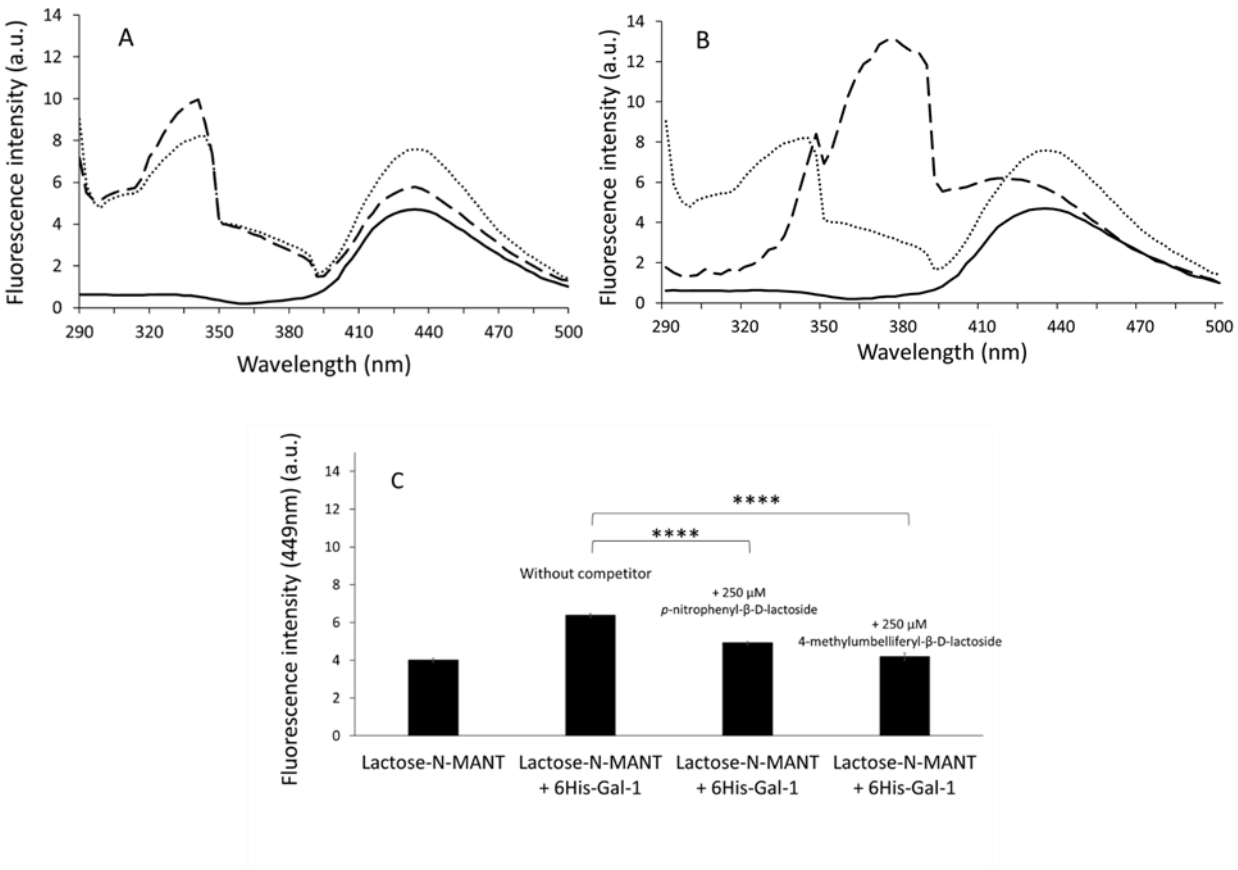

Fig. S3: Competition assays performed with *p*-nitrophenyl-β-D-Lactoside (pNP-Lac) or 4-methyl-umbelliferyl-β-Lactoside (4MU-Lac). (A) Fluorescence spectra of 100 µM Lactose-N-MANT **10** (—), of 100 µM Lactose-N-MANT **10** mixed with 150 µM 6His-Gal-1 in the absence of pNP-Lac (•••) or in the presence of 250 µM pNP-Lac (---), at an excitation wavelength of 280 nm. (B) Fluorescence spectra of 100 µM Lactose-N-MANT **10** (—), of 100 µM Lactose-N-MANT **10** mixed with 150 µM 6His-Gal-1 in the absence of 4MU-Lac (•••) or in the presence of 250 µM 4MU-Lac (---), at an excitation wavelength of 280 nm. (C) Comparison of the florescence intensity at 449 nm of 100 µM Lactose-N-MANT **10** alone, 100 µM Lactose-N-MANT **10** mixed with 150 µM 6His-Gal-1without any competitor, or in the presence of 250 µM pNP-Lac or 250 µM 4MU-Lac. λexc = 280 nm. The average and standard deviation of the FRET signals at 449 nm were from three independent experiments (n = 3). About 62% and 93% displacement of the fluorescent probe were obtained with 250 µM pNP-Lac and 4MU-Lac, respectively. Signals obtained with the competitors significantly differed from the positive control, **** *p-value* < 0.0001 (Student's t-test), Z-factor = 0.73.

Supplemental bibliography

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