Supporting Information

Oxime-based glycocluster microarray

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1. General methods

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. All protected amino acids and Fmoc-Gly-Sasrin® resin was obtained from Advanced ChemTech Europe (Brussels, Belgium), BachemBiochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). Lectins were obtained from Sigma (Saint Quentin Fallavier, France).

For glycopeptides, analytical RP-HPLC was performed on a Waters alliance 2695 separation module, equipped with a Waters 2489 UV/visible detector. Analyses were carried out at 1.23 mL/min (Interchim UPTISPERSE X-SERIE, C_{18}, 5 µm, 125x3.0 mm) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF_{3}CO_{2}H in water; buffer B: 0.09% CF_{3}CO_{2}H in 90% acetonitrile). Preparative HPLC was performed on Waters equipment consisting of a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. Purifications were carried out at 22.0 mL/min (VP 250x21 mm nucleosil 100-7 C_{18}) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient. ESI mass spectra of peptides and glycopeptides were measured on an Esquire 3000 spectrometer from Bruker. Microarray chips have been scanned using a fluorescence scanner Genomic Solutions Genetac LSIV.

2. Synthetic procedures

**Glycosylated compounds 1a-b**

![Chemical Structures](image)

1) BocAoaOSu, DIMEA, DMF, rt, 30 min
2) TFA/CH_{2}Cl_{2} (v/v, 1/1), rt, 15 min

1a R= β-Lac
1b R= α-GalNAc
To a solution of A<ref1> (20.0 mg, 16.1 µmol for 5a; 16.0 mg, 12.9 µmol for 5b) in 0.1 % TFA/water aminooxy glycans (2a:<ref2> 57.0 mg, 161 µmol for 5a; 2b:<ref2> 30.0 mg, 129 µmol for 5b) was added and the reaction mixture stirred at room temperature for 1 h and the crude mixture purified by by semi-preparative RP-HPLC. 5a: yield: 55% (23 mg); analytical RP-HPLC: Rt = 5.8 min (C<sub>18</sub>, λ = 214 nm, 5-60% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>103</sub>H<sub>169</sub>N<sub>19</sub>O<sub>58</sub>K [M+K]<sup>+</sup>: 2639.0, found 2638.8. 5b: yield: 78% (22 mg); analytical RP-HPLC: Rt = 7.6 min (C<sub>18</sub>, λ = 214 nm, 5-40% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>87</sub>H<sub>142</sub>N<sub>23</sub>O<sub>38</sub> [M+H]<sup>+</sup>: 2118.0, found 2117.7. The resulting product 5a (23 mg, 8.8 µmol) and 5b (21 mg, 9.5 µmol) were dissolved in DMF (2 mL) and Boc-Aoa-OSu<ref3> (2.5 mg, 9.7 µmol for 5a; 4.5 mg, 10.5 µmol for 5b) was added and the pH was adjusted at 8 with DIPEA. After 30 min at room temperature, the solution was concentrated under vaccum, the product precipitated in diethyl ether and white solid washed several times with diethetyl ether. The crude mixture was finally dissolved in TFA/CH<sub>2</sub>Cl<sub>2</sub> (v/v, 1/1, 2 mL) and the solution stirred 15 min at room temperature. After evaporation, aminooxy glycoclusters 1 have been obtained by semi-preparative HPLC. 1a: yield: 38% (9 mg); analytical RP-HPLC: Rt = 6.9 min (C<sub>18</sub>, λ = 214 nm, 5-40% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>105</sub>H<sub>173</sub>N<sub>20</sub>O<sub>60</sub> [M+H]<sup>+</sup>: 2674.1, found 2674.5. 1b: yield: 38% (8 mg); analytical RP-HPLC: Rt = 8.0 min (C<sub>18</sub>, λ = 214 nm, 5-40% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>89</sub>H<sub>145</sub>N<sub>24</sub>O<sub>40</sub> [M+H]<sup>+</sup>: 2191.0, found 2191.1.

Unglycosylated compounds 3-4

These two compounds have been prepared according to the procedure described in ref 4. Compound 3: analytical RP-HPLC: Rt = 5.9 min (C<sub>18</sub>, λ = 214 nm, 5-40% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>61</sub>H<sub>109</sub>N<sub>20</sub>O<sub>20</sub> [M+H]<sup>+</sup>: 1441.8, found 1441.5. Compound 4: analytical RP-HPLC: Rt = 5.8 min (C<sub>18</sub>, λ = 214 nm, 5-60% B in 15 min); ESI-MS (positive mode): m/z: calcd for C<sub>43</sub>H<sub>73</sub>N<sub>14</sub>O<sub>14</sub> [M+H]<sup>+</sup>: 1009.5, found
1009.2; m/z: calcd for C_{43}H_{72}N_{14}O_{14}Na [M+Na]^+: 1031.5, found 1031.1; m/z: calcd for C_{43}H_{72}N_{14}O_{14}K [M+K]^+: 1047.5, found 1047.0.

3. HPLC and mass spectra

![Figure S1. ESI-MS (positive mode) of compound 1a.](image)

![Figure S2. RP-HPLC of compound 1a (C_{18}, \lambda = 214 nm, 5-40% B in 15 min).](image)
**Figure S3.** ESI-MS (positive mode) of compound 1b.

**Figure S4.** RP-HPLC of compound 1b (C_{18}, \lambda = 214 \text{ nm}, 5-40\% \text{ B in } 15 \text{ min}).
Figure S5. ESI-MS (positive mode) of compound 3.

Figure S6. RP-HPLC of compound 3 (C₁₈, λ = 214 nm, 5-40% B in 15 min).
Figure S7. ESI-MS (positive mode) of compound 4.

Figure S8. RP-HPLC of compound 4 (C₁₈, λ = 214 nm, 5-60% B in 15 min).

4. Spotting of aminooxy compounds on solid support
Around 0.2 µL volume of each aminooxy glycoclusters at 50 µM in water was manually deposited onto the aldehyde surface of the substrate. After one night under ambient atmosphere, the support was rinsed successively with water, 0.2% SDS and water again. Before the recognition step by the lectins, the substrate was saturated with a BSA solution for 15 minutes (100 mg of BSA in 10 mL of phosphate buffer with 1% tween) and finally rinsed with water and dried.
5. Binding with lectins

A volume of 15 µL of the FITC labelled lectins (2 µL of the initial concentration = 1 mg in 1 mL water, diluted in 198 µL phosphate buffer) was deposited on the compounds areas on the surface and covered with a HybriSlip™ piece. After 1 hour at 37°C incubation, the substrate was rinsed with the phosphate / 1% tween buffer and dried.

6. Serine cluster oxidative cleavage and spotting of aminooxy glycans

6.1. Serine cluster grafting

A volume of 15 µL of RAFT-serine at 50 µM in water was manually deposited onto the aldehyde surface of the substrate and covered with a HybriSlip™ piece. After 2 hours incubation at room temperature, the substrate was rinsed with water, dried and then treated with a methoxyamine (1% v/v in water) solution for 1 hour. The substrate was finally rinsed successively with water, 0.2% SDS and water again and dried.

6.2. Serine cluster oxidative cleavage

The RAFT-serine bearing substrate was treated with a sodium periodate solution at 50 mM in water for 1 hour at room temperature and then rinsed with water and dried.

6.3. Spotting of aminooxy glycans on -serin cluster- support and binding with lectin

See sections 4 and 5 above.
7. Isothermal titration calorimetry (ITC)

Figure S9. Structure of compounds tested by ITC.

HPA was purchased from Sigma Aldrich (Saint Quentin Fallavier, France). ITC experiments were performed with a PEAQ-ITC isothermal titration microcalorimeter (Malvern Instruments, Malvern, UK). The experiments were performed at 25 °C. Lyophilized glycoconjugates and HPA were dissolved in the same buffer composed of Tris-HCl (20 mM) containing NaCl (100 mM) and CaCl$_2$ (100 μM) at pH 7.5. HPA (50 μM) was placed in the 200 μL sample cell operating at 25 °C. Titrations were performed with 20 injections of sugar derivatives (200 μM to 1 mM, 2 μL) one injection every 120 s. The experimental data were fit to a theoretical titration curve using the supplied MicorCal PEAQ-ITC analysis software, with ΔH (enthalpy change), $K_d$ (dissociation constant) and $n$ (number of binding sites per monomer) as adjustable parameters. Free energy change (ΔG) and entropy contributions (TΔS) were derived from the equation ΔG=ΔH-TΔS=-RT ln $K_a$ (where $T$ is the absolute temperature and $R$=8.314 J.mol$^{-1}$K$^{-1}$ and $K_a$=1/$K_d$). Two independent titrations were performed for each tested ligand.
Figure S10. Top: Thermograms obtained by injections of $N$-acetylgalactosamine (1 mM) in a solution of HPA (50 µM). Bottom: corresponding integrated titration curve.
Figure S11. Top: Thermograms obtained by injections of compound 7 (1 mM) in a solution of HPA (50 µM). Bottom: corresponding integrated titration curve.
Figure S12. Top: Thermograms obtained by injections of compound 6 (200 µM and 250 µM) in a solution of HPA (50 µM). Bottom: corresponding integrated titration curve.

8. References