On-bead synthesis and binding assay of chemoselectively template-assembled multivalent neoglycopeptides†

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The investigation of recognition events between carbohydrates and proteins, especially the control of how spatial factors and binding avidity are correlated in, remains a great interest for glycomics. Therefore, the development of efficient methods for the rapid evaluation of new ligands such as multivalent glycoconjugates is essential for diverse diagnostic or therapeutic applications. In this paper we describe the synthesis of chemoselectively-assembled multivalent neoglycopeptides and the subsequent recognition assay on a solid support. Aminooxylated carbohydrates (βLac-ONH_2 **4**, αGalNAc-ONH_2 **9** and αMan-ONH_2 **13**) have been prepared as carbohydrate-based recognition elements and assembled as clusters onto a cyclopeptidic scaffold by an oxime-based strategy in solid phase. Further binding tests between lectins and beads of resin derivatized with neoglycopeptides displaying clustered lactoses, *N*-acetylgalactoses and mannoses (**18–20**) have shown specific recognition and enhanced affinity through multivalent interactions, suggesting that the local density of carbohydrate-based ligands at the bead surface is crucial to improve the interaction of proteins of weak binding affinity. This solid phase strategy involving both molecular assembly and biological screening provides a rapid and efficient tool for various applications in glycomics.

Introduction

A large number of biological phenomena involve recognition processes between oligomeric proteins and clustered carbohydrates expressed on the cell surface.[1] In order to investigate the functional and structural features of these complex multivalent interactions between proteins and oligosaccharides, the design of well-defined compounds displaying oriented glycosylated recognition elements is essential for good affinity. Recently, the fabrication of biosensors and high-throughput carbohydrate microarrays has been reported for glycomic studies.[2] For example, some glyco-based arrays have been used to identify carbohydrate tumor antigens and to simultaneously detect the presence of pathogens or specific antibodies in a given sample.[3] However, while such glyco-arrays show promising potential for diagnostic and therapeutic applications, the development of rapid chemical and screening methods to evaluate the diverse supply of immobilized carbohydrate-based ligands as well as to profile the specificity of carbohydrate-binding proteins remains an exciting challenge. For this purpose, we report herein the fully solid phase synthesis of chemoselectively template-assembled neoglycopeptides exhibiting clusters of carbohydrates as recognition elements and the subsequent binding assays with suitable carbohydrate-binding proteins directly on the beads of resin (Fig. 1).

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR data for **6** and **11**, ^{1}H and ^{13}C spectra for **3**, **4**, **7**, **9**, **12** and **13**, ESI mass spectra for **4**, **9**, **13**, **18–20** and **24–26**. See DOI: 10.1039/b604391g

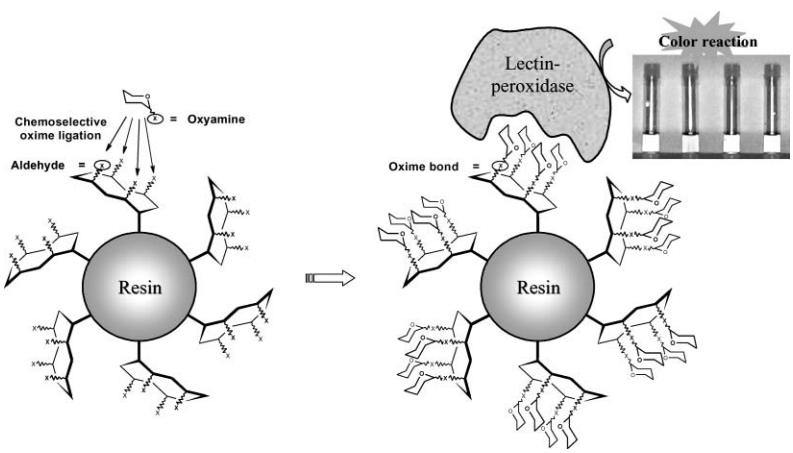


Fig. 1 General strategy for solid phase preparation of multivalent neoglycopeptides and biological evaluation with lectins.

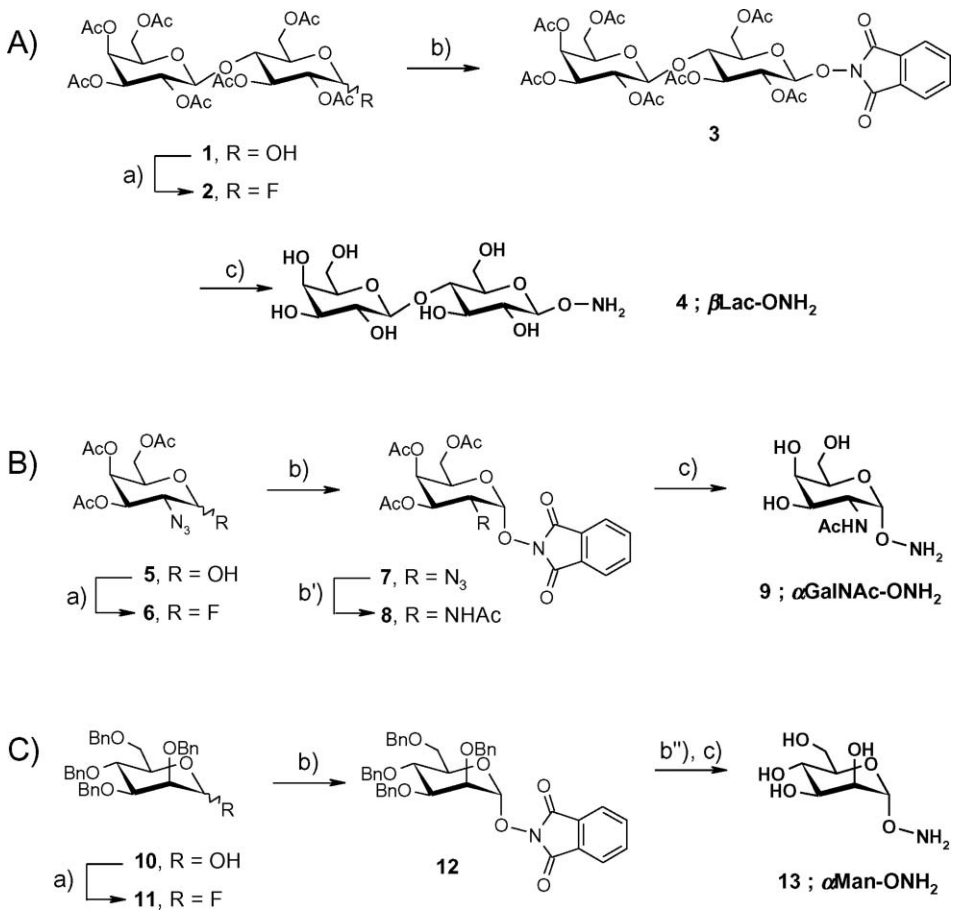
Careful control of the molecular assembly is necessary to warrant the well-defined structure of the target molecules immobilized on the solid support. To date, many synthetic methodologies have been reported to prepare multivalent glycosylated templates[4] for a wide range of biological applications.[5] While recent advancements regarding the solid phase strategies have facilitated the preparation of structurally complex molecules, the formation of stereoselective glycosidic linkages remains critical and still involves time-consuming manipulations of protecting groups and purifications.[6] To overcome these limitations, we and others have demonstrated that chemoselective oxime bond formation represents an attractive approach for the convergent assembly of multivalent biomolecules.[7] Particularly, we have developed recently in our laboratory some applications of the RAFT[8] platforms as vectors for neo-vasculature targeting in tumor therapy,[9] as cell surface mimics[10] or as synthetic vaccine candidates.[11] Such topological cyclodecapeptidic templates were prepared by a combined solid phase and solution convergent strategy and ensured effective recognition with specific receptors. To improve our previous synthetic method and extend the utility of RAFT molecules as convenient tools in glycomics, we have designed the peptidic template for a fully supported synthesis. Thereby, the cyclization of the peptide, the chemoselective incorporation of sugars as well as the biological evaluation of ligands have been entirely realized on solid phase.

Results and discussion

Synthesis of aminooxylated carbohydrates

Chemoselective oxime ligation requires the aminooxylated carbohydrate to be assembled onto a cyclic decapeptide template displaying aldehyde functions. We have reported previously a convenient method for the synthesis of such compounds using fluoride-activated donor sugars, followed by glycosylation reaction with *N*-hydroxyphthalimide (*N*-PhtOH) as a precursor of the oxyamine moiety.[12] In all cases, the syntheses start from the fully protected carbohydrates presenting a free anomer position.

In the lactose series (Scheme 1-A), the compound **1** was prepared from the per-*O*-acetylated lactopyranosyl which was stereoselectively deacetylated at the anomer carbon.[13] The fluorine was then introduced by treating **1** with diethylaminosulfur trifluoride (DAST)[14] in tetrahydrofuran to get quantitatively the activated compound **2** which was used directly for glycosylation without further purification. The *N*-PhtOH was coupled to the donor sugar in dichloromethane in the presence of triethylamine and boron trifluoride-diethyl etherate ($BF_3{\cdot}Et_2O$) as a promoter.[15] The derivative **3** was obtained in the β configuration in 70% yield after silica gel chromatography and precipitation. The removal of *O*-acetyl and phthalimide groups was finally accomplished by treating **3** with methylhydrazine in ethanol overnight to get



Scheme 1 Synthesis of aminooxylated lactose **4** (A), *N*-acetylgalactose **9** (B) and mannose **13** (C). Reagents and conditions: a) DAST, THF; b) *N*-hydroxyphthalimide, $BF_3{\cdot}Et_2O$, Et_3N, CH_2Cl_2; b′) H_2, Pd/C, MeOH–Ac_2O (9 : 1); b″) H_2, Pd/C, MeOH–CH_2Cl_2 (1 : 1); c) $MeHNNH_2$–EtOH (1 : 1).

the pure aminooxylated lactose moiety **4** in 71% yield after evaporation and precipitation from a mixture of methanol and dichloromethane.

The αGalNAc **9** containing an aminooxy group was obtained following the same strategy (Scheme 1-B). The compound **5** was converted to the corresponding fluoride derivative **6** with DAST after the azidonitration[16] of the commercial triacetyl galactal and anomeric deprotection by denitration[17] reaction following the published procedures. Glycosylation with *N*-PhtOH using $BF_3{\cdot}Et_2O$ provided the α and β-anomers which were isolated by column chromatography. After recrystallisation, compound **7** was obtained in 38% yield (88% yield for the glycosylation) as single crystals. X-Ray analysis combined with the determination of the coupling constant between H-1 and H-2 ($J = 3.4$ Hz) confirmed that the compound **7** has the desired α-anomer configuration (Fig. 2).[18] After conversion of the azido moiety into the –NHAc function by catalytic hydrogenation in CH_3OH–Ac_2O and complete deprotection with methylhydrazine, the aminooxylated αGalNAc derivative **9** was obtained in 72% yield.

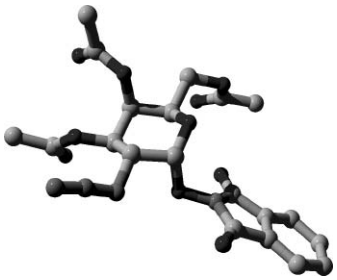


Fig. 2 X-Ray structure of compound **7** ($C_{20}H_{20}N_4O_{10}$, FW = 476.11, triclinic, *P*1, $a = 10.061(2)$, $b = 10.820(2)$, $c = 16.603(5)$ Å, $V = 1801.7(7)$ Å^3, $Z = 3$, $D_{calcd} = 1.511$ g cm^{-3}, $R = 0.0664$, $R_w = 0.0631$).[18]

The last mannose derivative was prepared from the tetra-*O*-benzylated mannopyranoside **10** (Scheme 1-C). The activation of the anomer position with fluoride followed by glycosylation with *N*-PhtOH were achieved using procedures described above. Compound **12** bearing the phthalimido group in an α configuration was purified by silica gel chromatography (75% yield). The final deprotection was more critical. The *O*-benzyl groups were first removed by short catalytic hydrogenation which was carefully controlled by TLC to prevent the cleavage of the N–O bond under these conditions. This reaction led generally to the expected debenzylated derivative which was isolated from partially deprotected compounds by extraction with ethyl acetate. This compound was then treated with methylhydrazine to cleave the remaining phthalimido group to provide the aminooxylated αMan derivative **13** after final silica gel chromatography in a modest deprotection yield (50%).

Solid phase synthesis of mono/tetravalent neoglycopeptides

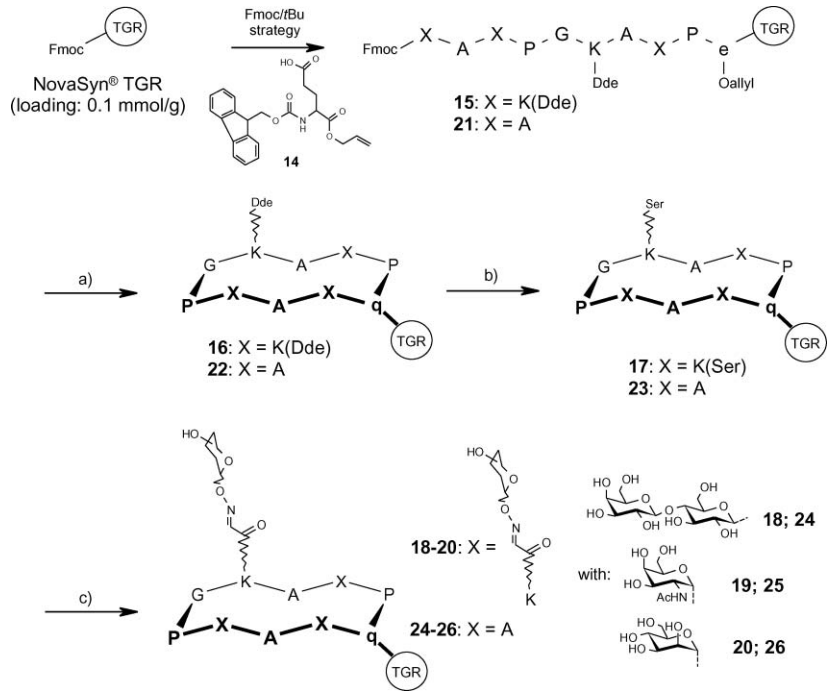
The choice of a suitable resin and orthogonal protecting groups for amino acids was an important consideration for such an approach. In view of further synthetic conditions, we considered that the acid-labile NovaSyn® Tentagel resin (TGR) would provide a convenient support for the whole molecular assembly. Furthermore, it might improve the accessibility of the ligand to the screened lectin and might also prevent unspecific interactions by inclusion at the interior of the beads.[19] Dde[20] was employed as the protecting group for the lysine side-chains providing the anchoring sites for carbohydrates. The type-II β-turn conformation traditionally promoted by a P–G sequence[21] was ensured by the incorporation of the allyl protected D-glutamic acid **14** whose side chain serves as the attachment site on the resin (Scheme 2).[22]

The building block **14** was prepared from the commercial Fmoc-Dglu(O*t*Bu)OH which was protected with an allyl group by treatment of the corresponding caesium salt with allyl bromide and then was deprotected by acidolysis to provide the free carboxylic side chain.[23] Starting from **14**, the orthogonally protected linear peptide **15** was then assembled by solid phase synthesis using standard Fmoc–*t*Bu protocol. Each coupling step was achieved with PyBOP[24] as the coupling reagent and diisopropylethylamine (DIPEA) as the base in DMF. The *O*-allyl protecting group was removed following the standard procedure with $PhSiH_3$–$Pd(PPh_3)_4$ in CH_2Cl_2.[25] The subsequent Fmoc deprotection of *N*-terminal lysine by classical treatment with a solution of piperidine–DMF (1 : 4) gave the linear peptide presenting a free *N*- and *C*-terminal end. The head-to-tail cyclization between the amine function of terminal lysine and the carboxylic acid of glutamic acid was the key step of the synthesis. This reaction occurred quantitatively in DMF after two successive cycles using PyAOP as the coupling reagent[26] and DIPEA as the base to obtain **16**. At this stage, no evidence of side-products was detected by reverse phase HPLC (RP-HPLC) and mass spectrometric analysis of an aliquot of resin. Further functionalization of the cyclic template was realized after removal of Dde protecting groups by treatment of **16** with 4% of hydrazine in DMF[20] to provide four free lysine side chains pointing to the upper side of the template. FmocSer(Trt) was then incorporated using standard conditions on each anchoring site. After removal of *O*-Trt with 1% TFA in dichloromethane, the loading of the resin was calculated by measuring the optical density at 299 nm of the dibenzofulvene–piperidine adduct resulting from the Fmoc deprotection. Finally, the oxidative cleavage[27] of **17** with a large excess of sodium periodate in DMF–H_2O (1 : 1) afforded the desired RAFT molecule presenting four glyoxyaldehyde functions. As for coupling and cyclization steps, the completeness of the oxidation was followed by TNBS[28] and Kaiser[29] colorimetric tests.

The final incorporation of carbohydrate recognition motifs was realized following an oxime-based strategy from aminooxylated modified sugars.[12] Chemoselective conjugation with an excess of aminooxylated sugars (βLac-ONH_2 **4**, αGalNAc-ONH_2 **9** and αMan-ONH_2 **13**) was performed on a solid support in aqueous acetic acid. After 8 hours stirring at room temperature, the excess of sugar was recovered by filtration and the resin was washed carefully. Subsequent analysis of an aliquot of resin after cleavage with pure TFA has shown a single peak by RP-HPLC, which corresponds to the expected final neoglycopeptides **18–20** as confirmed by ES-MS (Fig. 3).

Biological evaluation with lectins

Several studies describe the biological evaluation of carbohydrate-based ligands on a solid support using one-bead-one-compound combinatorial technology.[30] Particularly, new lectin ligands have been discovered from encoded neoglycopeptide libraries by screening with fluorescent or enzyme-labeled proteins and mass spectrometry analysis. Due to the efficiency of our solid phase synthesis



Scheme 2 Solid phase synthesis of mono/tetravalent neoglycopeptides **18–20** and **24–26**. Reagents and conditions: a) 1. DMF–piperidine (4 : 1) 2. $Pd(Ph_3P)_4$, $PhSiH_3$, CH_2Cl_2 3. PyAOP, DIPEA, DMF; b) 1. DMF–hydrazine (24 : 1) 2. FmocSer(Trt), PyBOP, DIPEA, DMF 3. CH_2Cl_2–TFA (99 : 1) 3. DMF–piperidine (4 : 1); c) 1. $NaIO_4$, DMF–H_2O (1 : 1) 2. **4**, **9** or **13**, AcOH–H_2O (1 : 1).

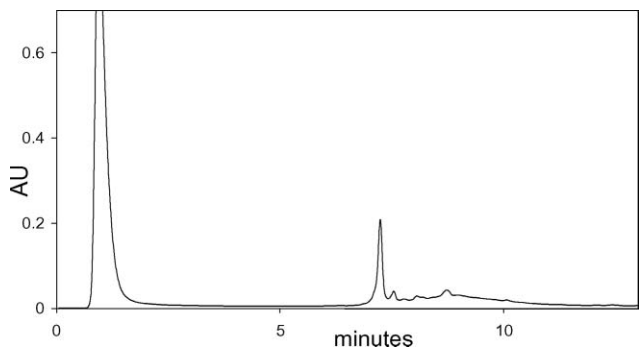


Fig. 3 RP-HPLC profile of crude **20** (Nucleosil 120 Å 3 μm C_{18} particles, 30 × 4.6 mm^2; solv. B: 0.09% TFA in 90% CH_3CN and solv. A: 0.09% TFA, 1.3 mL min^{-1}, detection $\lambda = 214$ and 250 nm, linear gradient 95 : 5 A : B to 60 : 40 A : B in 15 min).

protocol, we investigated the binding of lectins with the beads of resin derivatized with neoglycopeptides **18–20**. To prevent the non specific interaction of lectins with the solid support, the resin was first pre-incubated for one hour at 37 °C in the presence of bovine serum albumin (BSA). The binding assay was then realized by incubating the resin displaying clustered sugars **18–20** for one hour at 37 °C with solutions of horseradish peroxidase (HRP) labeled lectins from *Arachis hypogaea* (PNA, galactoside-binding lectin), concanavalin A from *Canavalia ensiformis* (ConA, mannoside-binding lectin) and lectin from *Helix pomatia agglutinin* (HPA, *N*-acetylgalactoside-binding lectin) in HEPES buffer 0.1 M pH 7.2 containing 0.9 M NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$. After washings, a peroxidase substrate was added and the binding was visualized by measuring the optical density of the resulting solution at 450 nm.

As shown in Fig. 4-A, the peptides displaying clustered sugars bind to the corresponding lectins in a selective manner. RAFT(βLac)$_4$ **18**, RAFT(αGalNAc)$_4$ **19** or RAFT(αMan)$_4$ **20** interacted with PNA, HPA and ConA respectively while no significant recognition was detected with the non-functionalized RAFT(Ser)$_4$ **18**. This suggests that neither the solid support nor the RAFT core interfere with the binding. In addition, an inhibition experiment was realized with a high concentration (10 mM) of D-galactose, methyl *N*-acetyl-α-D-galactopyranoside and methyl α-D-mannopyranoside. We observed that the binding of the resin derivatized by the neoglycoconjugate was inhibited by the corresponding oligosaccharide, confirming unambiguously the specificity of the interactions with lectins.

In addition, Kahne *et al.* have demonstrated that a solid support derivatized with monovalent carbohydrate ligands could bind with lectins in a polyvalent manner due to the spatial organization at the resin surface.[31] Thus, in order to assess the influence of multivalency for such interactions, we prepared the corresponding monovalent neoglycopeptides as a control. Starting from **14**, the linear decapeptide **21** was cyclized after C- and N-terminal deprotection to give **22** using the procedure described above (Scheme 2). The subsequent incorporation of serine followed by oxidation and chemoselective coupling of sugars afforded the monovalent neoglycopeptides **24–26**. To compare the affinity of immobilized mono/tetravalent neoglycopeptides with lectins, binding tests were realized in parallel, using an equal quantity of resin displaying one or four carbohydrate ligands (Fig. 4-B). Interestingly, the interaction of tetravalent ligands exhibiting βLac **18** and αMan **20** with PNA and ConA was enhanced compared

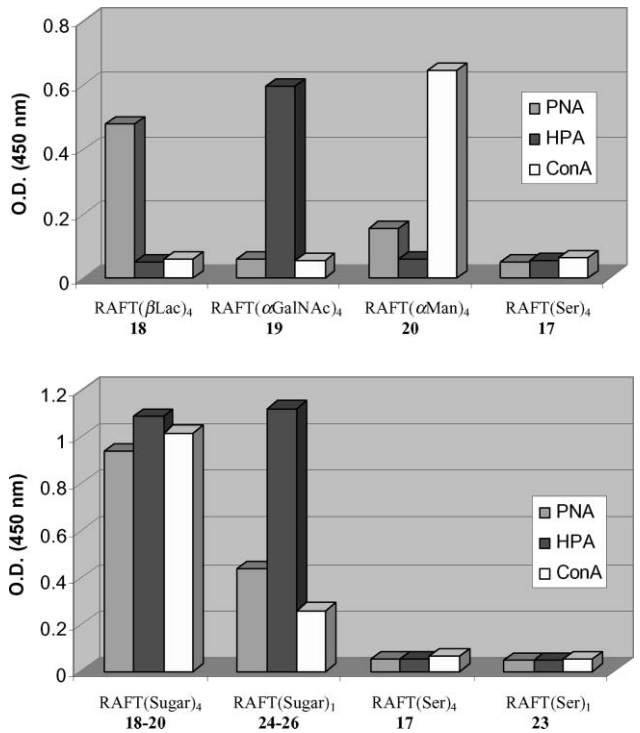


Fig. 4 A) Binding assay of tetravalent neoglycopeptide derivatized beads **18–20** was achieved with 100 μL solutions of horseradish peroxidase (HRP) labeled lectins from *Arachis hypogaea* (peanut, Sigma L 7759), concanavalin A from *Canavalia ensiformis* (Jack bean, Sigma L 6397) and lectin from *Helix pomatia agglutinin* (Sigma L 6387) at 2 μg mL^{-1} (PNA and HPA) or 5 μg mL^{-1} (ConA) in HEPES buffer 0.1 M pH 7.2 containing 0.9 M NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$; B) Comparison of affinity between monovalent **24–26** and tetravalent neoglycopeptides **18–20** with PNA, ConA and HPA (same conditions).

to the corresponding monovalent ligands **24** and **26**, whereas no significant difference was observed for the binding of **19** and **25** with HPA. Such a difference of binding could be attributed to the lower affinity of ConA and PNA for mannose and lactose[32] in comparison with the binding of HPA with GalNAc.[33] Since presentation at the resin surface is multivalent formally for all systems (*e.g.* **18–20** *vs.* **24–26**), the enhancement obtained with our multivalent carbohydrate-based ligands emphasizes that it is not only the multivalent presentation of the sugar ligands that is important to improve the interaction but also that the control of its local density is crucial. These results highlight the potential of such template assembled multivalent ligands for the recognition of proteins with weak binding affinities.

Conclusions

In conclusion, we have prepared resin derivatized with cyclopeptides displaying clustered carbohydrate-based ligands through a combined oxime ligation and solid phase protocol. This new polymer-supported methodology including peptide cyclization and chemoselective assembly occurred cleanly and appears as a significant improvement of our previous solution protocol,[10] suggesting that such compounds might be fully assembled using an automatic parallel peptide synthesizer. Additionally, the immobilized neoglycopeptides were tested on-bead for recognition with lectins. This study has emphasized specific recognition and enhanced affinity through multivalent protein–carbohydrate interactions, suggesting that the local density of sugars at the bead surface is essential to improve the affinity. In view of these results, we anticipate that this supported recognition system might enable the rapid discovery of new selective carbohydrate-based ligands as well as the analysis of protein glycopatterns in glycomics. Thus, this approach comprises a prerequisite for high-throughput screening of diverse multivalent glycoconjugate libraries through combinatorial techniques. Particularly, the optimization of recognition might be further explored rapidly by tuning the size of the spacer between the template and relevant carbohydrate binding elements. This is currently under investigation in our laboratory.

Experimental

General methods

All chemical reagents and solvents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. Thin layer chromatography was performed on 0.2 mm silica 60 coated aluminium foils with F-254 indicator (Merck) and detected under UV light and developed with aqueous sulfuric acid (100 mL, H_2SO_4–H_2O 15%) containing molybdic acid (2 g) and cerium(IV) sulfate hydrate (1 g). Preparative column chromatography was done using silica gel (Merck 60, 200–63 μm). Melting points were measured on an Electrothermal Serie IA9100 apparatus. ^1H and ^{13}C NMR spectra were recorded on Bruker AC300 spectrometers and chemical shifts (δ) were reported in parts per million (ppm). Spectra were referenced to the residual proton solvent peaks. Proton and carbon assignments were obtained from GCOSY and ^1H,^{13}C GHMQC experiments. The α-anomeric configuration of all carbohydrates was established by determination of the coupling constant (J) between H-1 and H-2. Mass spectra were obtained by electron spray ionization (ES-MS) on a VG Platform II in the positive mode. Lectins were obtained from Sigma (Saint Quentin Fallavier, France) for peptides and neoglycopeptides. For the synthesis of peptides, protected amino acids were obtained from Advanced ChemTech Europe (Brussels, Belgium), Bachem Biochimie SARL (Voivins-Le-Bretonneux, France) and France Biochem S. A. (Meudon, France). NovaSyn® tentagel resin and PyBOP were purchased from France Biochem. Reverse phase HPLC analyses were performed on Waters equipment using C_{18} columns. The analytical (Nucleosil 120 Å 3 μm C_{18} particles, 30 × 4.6 mm^2) was operated at 1.3 mL min^{-1} and the preparative column (Delta-Pak 300 Å 15 μm C_{18} particles, 200 × 25 mm^2) at 22 mL min^{-1} with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF_3CO_2H in water; buffer B: 0.09% CF_3CO_2H in 90% acetonitrile).

Synthesis of aminooxylated carbohydrates (4), (9) and (13)

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4′)-(2′,3′,6′-tri-*O*-acetyl-D-glucopyranosyl)-*N*-oxyphthalimide (3).** Compound **1** (5.24 g, 8.24 mmol) was dissolved in dry THF (70 mL) and the solution was cooled at −30 °C. Diethylaminosulfur trifluoride (1.31 mL, 9.89 mmol) was then added and the solution stirred under inert gas at room temperature for 30 minutes.

After addition of methanol (5 mL) at −30 °C, the solution was concentrated and taken up with CH_2Cl_2. The organic layer was washed successively with a saturated solution of $NaHCO_3$ then water, dried over sodium sulfate and evaporated to get **2** as a colorless oil (5.10 g, quantitative yield) which was used directly without further purification. To a stirring solution of compound **2** (1.67 g, 2.63 mmol), *N*-hydroxyphthalimide (0.43 g, 2.63 mmol) and triethylamine (0.47 mL, 2.63 mmol) in CH_2Cl_2 (40 mL) was added $BF_3{\cdot}Et_2O$ (1.66 mL, 13.15 mmol). The reaction was stirred at room temperature for 1 h (monitored by thin layer chromatography) and CH_2Cl_2 was added to the mixture (40 mL). The organic layer was washed two times with 10% aqueous sodium hydrogencarbonate then water, dried under sodium sulfate and evaporated. Compound **3** was finally purified by silica gel chromatography (CH_2Cl_2–EtOAc, 4 : 1) and precipitation from CH_2Cl_2–pentane to give compound **5** (1.43 g, 70%). Mp 109–111 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86–7.74 (m, 4 H, $H_{ar.}$), 5.34 (dd, 1 H, $J_{4',5'}$ = 0.9 Hz, $J_{3',4'}$ = 3.3 Hz, H-4′), 5.28–5.18 (m, 2 H, H-2, H-3), 5.14 (d, 1 H, $J_{1,2}$ = 6.9 Hz, H-1), 5.10 (dd, 1 H, $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 10.4 Hz, H-2′), 4.95 (dd, 1 H, $J_{3',4'}$ = 3.3 Hz, $J_{2',3'}$ = 10.4 Hz, H-3′), 4.53 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.42 (dd, 1 H, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b), 4.11–4.07 (m, 3 H, H-4, H-6′), 3.88 (td, 1 H, $J_{4',5'}$ = 0.9 Hz, $J_{5',6'}$ = 6.8 Hz, H-5′), 3.78–3.73 (m, 1 H, H-5), 2.16, 2.13, 2.08, 2.06, 2.05, 2.03, 1.94 (7s, 21 H, 7OCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8 (C=O_{Ac}), 170.7 (C=O_{Ac}), 170.5 (C=O_{Ac}), 170.4 (C=O_{Ac}), 170.0 (C=O_{Ac}), 169.9 (C=O_{Ac}), 169.5 (C=O_{Ac}), 163.1 (C=O_{Pht}), 135.1 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 104.5 (C-1), 101.5 (C-1′), 76.3 (C-4), 73.3, 73.1 (C-3, C-5), 71.4, 71.1 (C-3′, C-5′), 70.4 (C-2), 69.4 (C-2′), 67.1 (C-4′), 62.5 (C-6), 61.3 (C-6′), 21.2 (OCOCH$_3$), 21.1 (OCOCH$_3$), 21.0 (OCOCH$_3$), 20.9 (OCOCH$_3$); ES-MS (positive mode): calcd for $C_{34}H_{39}NO_{20}K$ 820.17 [M + K]$^+$, found: 820.15.

***O*-(β-D-Galactopyranosyl)-(1 → 4′)-(β-D-glucopyranosyl) oxyamine (4).** The acetylated compound **3** (0.40 g, 0.51 mmol) was dissolved in a solution of ethanol–methylhydrazine (1 : 1, 10 mL) and stirred at room temperature overnight. After evaporation, the fully depotected β-aminooxylated lactose **4** was precipitated in MeOH–CH_2Cl_2 to get a white powder (0.13 g, 71%). ^{1}H NMR (300 MHz, D_2O): δ = 4.62 (d, 1 H, $J_{1',2'}$ = 8.3 Hz, H-1′), 4.47 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1), 4.03 (dd, 1 H, $J_{5',6a'}$ = 1.3 Hz, $J_{6a',6b'}$ = 11.6 Hz, H-6a′), 3.95 (br d, 1 H, $J_{3,4}$ = 3.1 Hz, H-4), 3.84 (dd, 1 H, $J_{5',6b'}$ = 4.6 Hz, $J_{6a',6b'}$ = 11.6 Hz, H-6b′), 3.79–3.63 (m, 7 H, H-3, H-5, H-6, H-3′, H-4′, H-5′), 3.66 (dd, 1 H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.38 (br t, 1 H, $J_{2',3'}$ = 8.4 Hz, H-2′); ^{13}C NMR (75 MHz, D_2O): δ = 105.2 (C-1′), 103.3 (C-1), 78.6, 75.7, 75.1, 74.8, 72.8, 71.7 (C-2′), 71.3 (C-2), 68.9 (C-4), 61.4 (C-6′), 60.4 (C-6). ES-MS (positive mode): calcd for $C_{12}H_{24}NO_{11}$ 358.13 [M + H]$^+$, found: 358.15.

***O*-(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-oxyphthalimide (7).** The oxyphthalimide derivative **7** was prepared from **6** (4.30 g, 13.0 mmol) using the procedure described for **3**. A mixture of α and β anomers was obtained and each anomer was separated by silica gel chromatography (CH_2Cl_2–ethyl acetate, 9 : 1) to give pure compound **7** (2.35 g, 38%) after crystallization from diethyl ether–pentane; mp 105–107 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.85–7.75 (m, 4 H, $H_{ar.}$), 5.57 (br d, 2 H, $J_{1,2}$ = $J_{3,4}$ = 3.6 Hz, H-1, H-4), 5.47 (dd, 1 H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 11.3 Hz, H-3), 5.17 (br t, 1 H, $J_{5,6a}$ = 6.4 Hz, H-5), 4.23 (dd, 1 H, $J_{5,6a}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 3.97–3.92 (m, 2 H, H-2, H-6b), 2.14, 2.06, 2.02 (3s, 9 H, 3OCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.8 (C=O_{Ac}), 170.3 (C=O_{Ac}), 169.9 (C=O_{Ac}), 163.3 (C=O_{Pht}), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 103.5 (C-1), 69.2 (C-5), 68.2 (C-4), 67.7 (C-3), 61.6 (C-6), 57.0 (C-2), 21.1 (OCOCH$_3$); ES-MS (positive mode): calcd for $C_{20}H_{19}N_4O_{10}Na$: 498.09 [M + Na]$^+$, found: 497.96. The corresponding β anomer (3.09 g; 50%) was also obtained. Mp 78–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.92–7.78 (m, 4 H, $H_{ar.}$), 5.37 (dd, 1 H, $J_{4,5}$ = 1.1 Hz, $J_{3,4}$ = 3.3 Hz, H-4), 5.03 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 4.89 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, $J_{2,3}$ = 10.7 Hz, H-3), 4.20–4.13 (m, 2 H, H-6), 4.00 (dd, 1 H, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 10.7 Hz, H-2), 3.91 (td, 1 H, $J_{4,5}$ = 1.1 Hz, $J_{5,6}$ = 6.9 Hz, H-5), 2.18, 2.09, 1.97 (3s, 9 H, 3OCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6 (C=O_{Ac}), 170.4 (C=O_{Ac}), 170.0 (C=O_{Ac}), 163.0 (C=O_{Pht}), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.3 ($CH_{ar.}$), 106.2 (C-1), 71.8 (C-5), 71.4 (C-3), 66.1 (C-4), 61.1 (C-6), 59.5 (C-2), 21.0 (OCOCH$_3$), 20.9 (OCOCH$_3$), 20.9 (OCOCH$_3$).

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-*N*-oxyphthalimide (8).** Compound **7** (1.68 g, 3.5 mmol) was dissolved in a solution of methanol–acetic anhydride (9 : 1, 40 mL) and 10% Pd/C (0.38 g, 0.3 mmol) was added. After stirring the mixture at room temperature under an atmosphere of hydrogen for one hour, the catalyst was removed by filtration under celite and washed with methanol. The solvent was evaporated under reduced pressure and the *N*-acetylated derivative **6** (0.92 g, 53%) was finally obtained as a white powder after silica gel chromatography (CH_2Cl_2–EtOAc, 4 : 1 then 0 : 1) followed by precipitation from CH_2Cl_2–diethyl ether. Mp 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86–7.77 (m, 4 H, $H_{ar.}$), 6.07 (d, 1 H, $J_{2,NH}$ = 9.6 Hz, NH), 5.54 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4), 5.38 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 11.3 Hz, H-3), 5.08 (br t, 1 H, $J_{5,6}$ = 6.4 Hz, H-5), 4.80 (ddd, 1 H, $J_{1,2}$ = 3.4 Hz, $J_{2,NH}$ = 9.6 Hz, $J_{2,3}$ = 11.3 Hz, H-2), 4.30 (dd, 1 H, $J_{5,6}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.00 (dd, 1 H, $J_{5,6}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6b), 2.18, 2.12, 2.09, 2.04 (4s, 12 H, 3OCOCH$_3$, NHCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.2 (C=O), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 105.4 (C-1), 71.5, 69.5, 67.7, 61.9 (C-3, C-4, C-5, C-6), 47.7 (C-2), 21.1 (OCOCH$_3$).

***O*-α-D-Galactopyranosyl oxyamine (9).** The aminooxylated derivative **9** was prepared from **8** (0.86 g, 1.7 mmol) using the procedure described for **4**. Compound **9** (0.37 g, 90%) was purified by flash silica gel chromatography (CH_2Cl_2–ethanol, 7 : 3 then 0 : 1) to get a white powder after lyophilisation. ^{1}H NMR (300 MHz, D_2O): δ = 4.99 (d, 1 H, $J_{1,2}$ = 4.1 Hz, H-1), 4.24 (dd, 1 H, $J_{1,2}$ = 4.1 Hz, $J_{2,3}$ = 11.3 Hz, H-2), 4.03–3.99 (m, 2 H, H-4, H-5), 3.89–3.76 (m, 3 H, H-3, H-6), 2.08 (s, 3 H, HNCOCH$_3$); ^{13}C NMR (75 MHz, D_2O): δ = 175.0 (HNCOCH$_3$), 101.0 (C-1), 71.4, 68.8 (C-4, C-5), 68.0 (C-3), 61.5 (C-6), 49.6 (C-2), 22.3 (HNCOCH$_3$); MS-CI (positive mode): calcd for $C_8H_{17}N_2O_6$ 237.10 [M + H]$^+$, found: 237.00.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-*N*-oxyphthalimide (12).** Compound **12** was prepared from **11** (1.27 g, 2.3 mmol) using the procedure described for **3**. This product (1.20 g, 75%) was obtained as a colorless oil after silica gel chromatography (hexane–EtOAc, 7 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74–7.63 (m, 4 H, $H_{ar.(Pht)}$), 7.37–7.10 (m, 20 H,

$H_{ar.(Bn)}$), 5.48 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.81–4.33 (m, 8 H, $4CH_2$), 4.50 (m, 1 H, H-5), 4.13–4.03 (m, 2 H, H-2, H-4), 3.91 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 8.9 Hz, H-3), 3.81 (dd, 1 H, $J_{5,6a}$ = 3.7 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6a), 3.61 (dd, 1 H, $J_{5,6b}$ = 1.9 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6b); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.6 (C=O), 138.9 ($C_{ar.(Bn)}$), 138.8 ($C_{ar.(Bn)}$), 138.7 ($C_{ar.(Bn)}$), 138.2 ($C_{ar.(Bn)}$), 134.9 ($CH_{ar.(Pht)}$), 128.8 ($C_{ar.(Pht)}$), 128.7 ($CH_{ar.(Bn)}$), 128.7 ($CH_{ar.(Bn)}$), 128.6 ($CH_{ar.(Bn)}$), 128.5 ($CH_{ar.(Bn)}$), 128.3 ($CH_{ar.(Bn)}$), 128.2 ($CH_{ar.(Bn)}$), 128.0 ($CH_{ar.(Bn)}$), 128.0 ($CH_{ar.(Bn)}$), 127.9 ($CH_{ar.(Bn)}$), 127.8 ($CH_{ar.(Bn)}$), 123.9 ($CH_{ar.(Pht)}$), 104.1 (C-1), 79.7 (C-3), 75.3 (CH_2), 74.6, 74.1, 73.6 (CH_2), 73.5, 73.3 (CH_2), 72.7 (CH_2), 69.0 (C-6).

***O*-α-D-Mannopyranosyl oxyamine (13).** A mixture of compound **12** (6.3 g, 9.19 mmol) and 10% Pd/C (1.8 g, 3.3 mmol) in MeOH–CH_2Cl_2 (100 mL, 1 : 1) was stirred at room temperature under a hydrogen atmosphere during 2 hours. After removal of the catalyst by filtration under celite and evaporation, ethyl acetate was added and the fully debenzylated compound was separated from partial deprotected products after washing with water. This compound was immediately treated after lyophilisation following the procedure described for **3**. After silica gel chromatography and lyophilisation, the aminooxylated derivative **13** (0.9 g, 50% for two steps) was obtained as a colorless oil. ^{1}H NMR (300 MHz, D_2O): δ = 5.00 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.03 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 2.8 Hz, H-2), 3.97 (dd, 1 H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 3.88 (dd, 1 H, $J_{5,6b}$ = 3.7 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6b), 3.76–3.72 (m, 3 H, H-3, H-4, H-5); ^{13}C NMR (75 MHz, D_2O): δ = 103.8 (C-1), 73.4, 71.2, 69.4 (C-2), 67.2, 61.4 (C-6); MS-CI (positive mode): calcd for $C_6H_{14}NO_6$ 196.08 $[M + H]^+$, found: 195.94.

Supported assembly of mono/tetravalent neoglycopeptides (18–20) and (24–26)

Fmoc-Dglu(Oallyl)OH (14). Commercial Fmoc-Dglu-(O*t*Bu)OH (2.89 g, 6.8 mmol) was dissolved in a mixture of MeOH–H_2O (20 mL, 20 : 1) and a solution of caesium carbonate (3 M) was added dropwise until pH 8. The solution was evaporated to dryness and the white solid taken up with acetonitrile (100 mL). Allyl bromide (4 mL, 44.2 mmol) was added to the solution and the mixture was kept at room temperature overnight. The solution was concentrated and AcOEt (100 mL) was added then the organic layer was washed with water, an aqueous solution of $NaHCO_3$ and water, dried over sodium sulfate and evaporated. After silica gel chromatography (CH_2Cl_2 then CH_2Cl_2–AcOEt, 9 : 1), the resulting residue was dissolved with a solution of CH_2Cl_2–TFA (20 mL, 1 : 1) and the mixture stirred at room temperature for one hour. The solvent was finally evaporated and product **14** was obtained as a white powder (1.59 g, 57%) after precipitation from diethyl ether–pentane. ^{1}H NMR (300 MHz, DMSO): δ = 12.10 (br s, 1 H, CO_2H), 7.89–7.33 (m, 8 H, $H_{ar.}$), 7.71 (d, 1 H, $J_{NH,CH\alpha}$ = 7.5 Hz, NH), 5.98–5.82 (m, 1 H, CH=), 5.30 (dd, 1 H, $J_{=CH2',CH2''}$ = 1.2 Hz, $J_{=CH2',CH=}$ = 17.1 Hz, $=CH_2'$), 5.20 (dd, 1 H, $J_{=CH2',CH2''}$ = 1.2 Hz, $J_{=CH2'',CH=}$ = 10.5 Hz, $=CH_2''$), 4.58 (d, 2 H, $J_{OCH2,CH=}$ = 5.1 Hz, OCH_2), 4.32–4.21 (m, 3 H, CH_{Fmoc}, CH_{2Fmoc}), 4.16–4.08 (m, 1 H, CHα), 2.32 (t, 2 H, $J_{CH\beta,CH\gamma}$ = 7.2 Hz, $CH_2\gamma$), 2.06–1.76 (m, 2 H, $CH_2\beta$); ^{13}C NMR (75 MHz, DMSO): δ = 174.0 (HOC=O), 172.1 (C=O), 156.5 (C=O), 144.2 ($C_{ar.}$), 144.1 ($C_{ar.}$), 141.1 ($C_{ar.}$), 132.7 (CH=), 128.0 ($CH_{ar.}$), 127.4 ($CH_{ar.}$), 125.6 ($CH_{ar.}$), 120.5 ($CH_{ar.}$), 118.1 (CH_2=), 66.1 (CH_{2Fmoc}), 65.2 (OCH_2), 53.5 (CHα), 47.0 (CH_{Fmoc}), 30.3 ($CH_2\gamma$), 26.3 ($CH_2\beta$).

Cyclic templates RAFT(Dde)$_4$ (16) and RAFT(Dde)$_1$ (22).

*Synthesis of linear peptides **15** and **21**.* The synthesis of linear peptides **15** and **21** was carried out following the Fmoc–*t*Bu strategy in a glass reaction vessel fitted with a sintered glass frit with the NovaSyn® Tentagel resin (1 g, 0.1 mmol). The resin was swollen with CH_2Cl_2 (10 mL, 2 × 15 min) and DMF (10 mL, 1 × 15 min). The coupling reactions were performed with *N*-α-Fmoc-protected amino acid (0.4 mmol), PyBOP (0.21 g, 0.4 mmol) and DIPEA (140 µL, 0.8 mmol) in DMF (10 mL) for 30 min. After washing with DMF (10 mL, 4 × 1 min) and CH_2Cl_2 (10 mL, 2 × 1 min), the completeness of the coupling reaction was controlled by Kaiser and TNBS tests. Fmoc protecting groups were removed by treatment with a piperidine–DMF solution (10 mL, 1 : 4, 3 × 10 min). After washing with DMF (10 mL, 6 × 1 min), the completeness of deprotection was verified by the UV absorption of the piperidine washings at 299 nm. *Allyl deprotection*: the NovaSyn® Tentagel resin bearing the linear peptides **15** or **21** (1 g, 0.1 mmol) was swollen in a glass reaction vessel fitted with a sintered glass frit with CH_2Cl_2 (10 mL, 2 × 15 min) and DMF (10 mL, 1 × 15 min). The resin was treated with $PhSiH_3$ (617 µL, 5 mmol) in dry CH_2Cl_2 (10 mL) for 5 minutes. $Pd(Ph_3P)_4$ (46 mg, 0.04 mmol) was then added and the resin was stirred under argon gas for 20 minutes. The reagents were removed by filtration, the resin washed with CH_2Cl_2 (10 mL, 4 × 1 min) and the cycle was repeated once. The resin was finally washed with CH_2Cl_2 (10 mL, 2 × 1 min), dioxane–water (10 mL, 9 : 1, 2 × 1 min) and DMF (10 mL, 2 × 1 min). *N-terminal Fmoc deprotection*: the resin was treated three times with a solution of piperidine in DMF (10 mL, 1 : 4, 10 min) then washed with DMF (10 mL, 6 × 1 min). The completeness of deprotection was controlled by the UV absorption of the piperidine washings at 299 nm (loading: ≈0.07 mmol g^{-1} of resin). *Cyclisation*: PyAOP (73 mg, 0.14 mmol) and DIPEA (61 µL, 0.35 mmol) were added to the resin in DMF (10 mL) and the mixture was stirred at room temperature. After 30 minutes, the reagents were removed by filtration, the resin washed with DMF (10 mL, 2 × 1 min) then the procedure was repeated. An aliquot of resin was taken and treated with pure TFA for 30 minutes then the filtrate was analyzed by reverse-phase HPLC. RAFT(Dde)$_4$ **16**: R_t = 10.6 min; linear gradient: 95 : 5 to 0 : 100 A : B in 15 min; detection: λ = 214 and 250 nm. RAFT(Dde)$_1$ **22**: R_t = 7.0 min; linear gradient: 95 : 5 to 0 : 100 A : B in 15 min; detection: λ = 214 and 250 nm.

Cyclic templates RAFT(Ser)$_4$ (17) and RAFT(Ser)$_1$ (23).

Dde deprotection. The resin was treated four times with a solution of hydrazine in DMF (10 mL, 1 : 24, 10 min) then washed with DMF (10 mL, 4 × 1 min), CH_2Cl_2 (10 mL, 2 × 1 min) and DMF (10 mL, 2 × 1 min). *Coupling of protected serine*: FmocSer(OTrt) (319 mg, 0.56 mmol for **16**; 80 mg, 0.17 mmol for **22**), PyBOP (291 mg, 0.56 mmol for **16**; 73 mg, 0.17 mmol for **22**) and DIPEA (244 µL, 1.4 mmol for **16**; 71 µL, 0.35 mmol for **22**) in DMF (10 mL) were added to the resin and stirred for 30 minutes at room temperature. After removing the reagents by filtration, the resin was washed with DMF (10 mL, 4 × 1 min) and CH_2Cl_2 (10 mL, 2 × 1 min) and the completeness of the coupling reaction was controlled by TNBS and the Kaiser test on a few beads of resin. *Trt deprotection*: the resin was treated with

a solution of TFA in CH_2Cl_2 (10 mL, 1 : 99) until the mixture remains uncolored (10 mL, 5 × 3 min) and was then washed with CH_2Cl_2 (10 mL, 5 × 1 min) and DMF (10 mL, 2 × 1 min). *Fmoc deprotection*: Fmoc groups were removed following the procedure described for **16** and **22**. The completeness of deprotection was controlled by the UV absorption of the piperidine washings at 299 nm (loading: ≈0.07 mmol g^{-1} of resin). An aliquot of resin was taken and treated with pure TFA for 30 minutes then the filtrate was analyzed by reverse-phase HPLC. RAFT(Ser)$_4$ **17**: R_t = 6.2 min; linear gradient: 95 : 5 to 60 : 40 A : B in 15 min; detection: λ = 214 and 250 nm; ES-MS (positive mode): calcd for $C_{59}H_{104}N_{19}O_{19}$ 1382.77 $[M + H]^+$, found: 1381.71. RAFT(Ser)$_1$ **23**: R_t = 6.3 min; linear gradient: 95 : 5 to 60 : 40 A : B in 15 min; detection: λ = 214 and 250 nm; ES-MS (positive mode): calcd for $C_{41}H_{67}N_{13}O_{13}$ 950.50 $[M + H]^+$, found: 950.41.

Cyclic templates RAFT(Sugar)$_4$ (18–20) and RAFT(Sugar)$_1$ (24–26).

Oxidation of serines. The resin was treated with sodium periodate (1.30 g, 5.6 mmol for **17**; 0.30 g, 1.4 mmol for **23**) in a solution of DMF–water (10 mL, 1 : 1) for one hour. The completeness of the coupling reaction was controlled by TNBS and the Kaiser test. The resin was finally washed with water (10 mL, 3 × 1 min) and DMF (10 mL, 3 × 1 min). *Chemoselective coupling of sugars*: to the resin (40 mg, ≈2.8 μmol) bearing the aminooxy sugar, (for RAFT(CHO)$_4$: 20 mg of **4**, 13.2 mg of **9** and 10.9 mg of **13**, 56 μmol; for RAFT(CHO)$_1$: 5 mg of **4**, 3.3 mg of **9** and 2.7 mg of **13**, 14 μmol) was added in solution in AcOH–H_2O (7 : 3, 1 mL). The resin was stirred at room temperature overnight. The excess of unreacted aminooxy sugar was recovered by filtration, then the resin was washed successively with AcOH–H_2O (1 mL, 1 : 1, 2 × 1 min), DMF (1 mL, 2 × 1 min) and water (1 mL, 2 × 1 min). An aliquot of resin was taken and treated with pure TFA for 30 minutes then the filtrate was analyzed by reverse-phase HPLC and mass spectrometry. RAFT(βLac)$_4$ **18**: R_t = 6.7 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): calcd for $C_{103}H_{168}N_{19}O_{59}$ $[M + H]^+$, 2615.07, found: 2614.93. RAFT(αGalNac)$_4$ **19**: R_t = 7.7 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): $C_{87}H_{140}N_{23}O_{39}$ calcd for 2130.96 $[M + H]^+$, found: 2130.86. RAFT(αMan)$_4$ **20**: R_t = 7.2 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): $C_{79}H_{128}N_{19}O_{39}$ calcd for 1966.86 $[M + H]^+$, found: 1966.81. RAFT(βLac)$_1$ **24**: R_t = 6.6 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): calcd for $C_{52}H_{84}N_{13}O_{23}$ 1258.58 $[M + H]^+$, found: 1258.45. RAFT(αGalNac)$_1$ **25**: R_t = 7.1 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): $C_{48}H_{77}N_{14}O_{18}$ calcd for 1137.55 $[M + H]^+$, found: 1137.50. RAFT(αMan)$_1$ **26**: R_t = 7.0 min (linear gradient: 95 : 5 to 60 : 40 A : B in 15 min, detection: λ = 214 and 250 nm); ES-MS (positive mode): $C_{46}H_{74}N_{13}O_{18}$ calcd for 1096.52 $[M + H]^+$, found: 1096.42.

Recognition tests with lectins and mono/tetravalent neoglycopeptides (18–20) and (24–26)

Binding assay. All experiments were done in duplicate. The resin bearing neoglycopeptides **18–20** and **24–26** (≈2 mg) was pre-incubated in a microtube with a solution of 5% BSA (Bovine Serum Albumin) in PBST buffer (Phosphate Buffer Saline 0.01 M pH 7.4 containing 0.01% v/v Tween 20) at 37 °C for one hour. After filtration and washing with PBST buffer (5 × 1 mL), the binding assay was realized in a microtube with solutions of horseradish peroxidase (HRP) labelled lectins from *Arachis hypogaea* (peanut, Sigma L 7759, PNA), concanavalin A from *Canavalia ensiformis* (Jack bean, Sigma L 6397, ConA) and lectin from *Helix pomatia agglutinin* (Sigma L 6387, HPA) (100 μL, 2 μg mL^{-1} for PNA and HPA) or (100 μL, 5 μg mL^{-1} for ConA) in HEPES buffer 0.1 M pH 7.2 containing 0.9 M NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$ at 37 °C for one hour. After filtration and washing with PBST (5 × 1 mL), the beads of resin were transferred in a microtube and the HRP substrate (200 μL, TMB (3,3′,5,5′-tetramethylbenzidine) Substrate Reagent Set, BD Biosciences Cat. 555214) was added. The enzymatic reaction was finally quenched with 10% aqueous sulfuric acid (100 μL) after 5 minutes and a fraction of the yellow solution was transferred in a multiwell plate (200 μL) to measure the binding at 450 nm with a UV microplates reader. Experiments were done in duplicate.

Inhibition assay. The resin bearing neoglycopeptide **18–20** or **24–26** (≈2 mg) was pre-incubated with a solution of 5% BSA in PBST buffer at 37 °C for one hour. After filtration and washing with PBST buffer (5 × 1 mL), the binding assay was realized in a microtube with solutions of PNA–HRP, ConA–HRP and HPA–HRP (100 μL, 2 μg mL^{-1} for PNA and HPA) or (100 μL, 5 μg mL^{-1} for ConA) in HEPES buffer containing inhibitor (10 mM, respectively D-galactose, methyl *N*-acetyl-α-D-galactopyranoside or methyl α-D-mannopyranoside) at 37 °C for one hour. After filtration and washing with PBST (5 × 1 mL), the beads of resin were transferred in a microtube and the HRP substrate (200 μL) was added. The enzymatic reaction was quenched with 10% aqueous sulfuric acid (100 μL) after 5 minutes and the binding measured at 450 nm with a UV microplates reader.

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