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PAPER

CuAAC synthesis of resorcin[4]arene-based glycoclusters as multivalent ligands of lectins†

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Synthetic multivalent glycoclusters show promise as anti-adhesives for the treatment of bacterial infections. Here we report the synthesis of a family of tetravalent galactose and lactose functionalised macrocycles based on the resorcin[4]arene core. The development of diastereoselective synthetic routes for the formation of lower-rim propargylated resorcin[4]arenes and their functionalistion *via* Cu-catalyzed azide–alkyne click chemistry is described. ELLA binding studies confirm that galactose sugar clusters are effective ligands for the PA-IL bacterial lectin of *Pseudomonas aeruginosa* while poor binding for the lactose-based monovalent probe and no binding could be measured for the multivalent glycoclusters was observed for the human galectin-1.

Introduction

The first step of bacterial infection is the colonization of tissues through adhesion of bacteria to host cells. This process usually relies on the recognition of carbohydrate epitopes present at the surface of cells by receptors (lectins) displayed on the surface of bacteria.[1] *Pseudomonas aeruginosa* is an opportunistic bacterium responsible for more than 20% of hospital acquired infections and is also largely involved in pulmonary infections for cystic fibrosis and intensive care unit patients.[2] This bacterium expresses two lectins which are involved in the binding event responsible for infection: the galactose-specific lectin LecA from *Pseudomonas aeruginosa* (PA-IL) and the fucose-specific lectin LecB from *Pseudomonas aeruginosa* (PA-IIL).[3] Even though the affinity of the lectins for their carbohydrate is relatively weak, the lung bacterial infection could be diminished by using galactose and fucose as lectin competitors.[4] The design of high affinity ligands of PA-IL[5] and/or PA-IIL[6] is therefore a promising anti-adhesive strategy[7] for fighting bacterial infection.

We have previously examined the role of multivalent ligand topology in determining binding affinity at the PA-IL lectin using a series of tetrafunctionalised calixarenes.[5d] We have also studied the influence of other core scaffolds such as porphyrins,[5g,5h] β-peptoids,[5h] calix[6]arenes[5h] or fullerenes.[5e,5f] Resorcin[4]arenes offer the opportunity to investigate topology further through the preparation of macrocycles bearing sugars at the lower-rim in the two readily available *rccc* boat and *rctt* chair diastereoisomers. Resorcin[4]arenes, octafunctionalised with sugar moieties at the upper-rim have previously been prepared through amine induced ring opening of a lactone.[8] Such derivatives have been investigated for a number of biological applications including DNA delivery[9] and protein binding studies. Treatment with maltonolactone resulted in glucose terminated derivatives which could bind effectively to concanavalin A, whereas reaction with lactonolactone gave specificity for binding to peanut lectin, a galactose specific protein.[10] When sialic acid residues were introduced at the upper-rim, binding to Japanese horseshoe crab lectin was observed in addition to inhibition of virus mediated haemagglutination in the presence of influenza A.[11] This ability to interfere with cellular interactions has been further explored by the synthesis of resorcin[4]arenes functionalised with polysaccharide chondroitin sulphate which were able to inhibit adhesion-triggered cell growth in baby hamster kidney cells.[12]

Functionalisation of resorcin[4]arenes at the lower rim with sugars has received considerably less attention, with a single publication outlining the stepwise synthesis of carbohydrate containing macrocycles in which the product adopted the *rctc* diamond configuration and the sugar is in its open chain form.[13]

In this study, we report the synthesis of tetravalent glycoclusters based on the resorcin[4]arene core in two specific topologies, the *rccc* boat and the *rctt* chair stereoisomers (Fig. 1) and their biological evaluation against two lectins, the galactose-specific PA-IL

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† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C NMR and mass spectra for all new compounds. See DOI: 10.1039/c1ob05676j

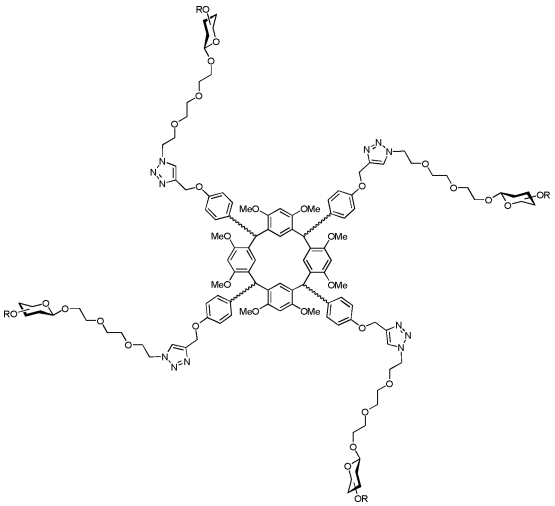


Fig. 1 Sugar functionalised resorcin[4]arenes.

and lactose-specific galectin-1 (Gal-1). The synthetic route allows introduction of lower-rim sugar motifs through the synthesis of resorcin[4]arenes bearing alkynyl groups suitable for reaction with azide functionalised sugar moieties through Cu-catalyzed azide–alkyne click chemistry (CuAAC).[14]

Results and discussion

We have recently developed methods for the direct preparation of octaalkyl resorcin[4]arenes[15] from 1,3-dimethoxybenzene and a number of different aldehydes. Such methods offer the potential of selective synthesis of one of the two major diastereoisomers of resorcin[4]arenes; the *rccc* boat and the *rctt* chair, with additional functionality at the lower rim. These two diastereoisomers (Fig. 2) allow an evaluation of the topological role in binding to lectins as the *rccc* boat enables clustering of the sugar residues on one face of the core and the *rctt* chair provides two discrete binding regions on opposite faces. Interestingly, few alkyne derivatives of resorcin[4]arenes have been described and to date these have exclusively been upper-rim derivatives and have been used for the preparation of organometallic complexes, particularly highly valent cobalt complexes, rather than for cycloadditions.[16]

A two step procedure was devised for the preparation of alkyne bearing resorcin[4]arenes, involving pre-functionalisation of a suitable aldehyde prior to cyclisation. 4-(Propargyloxy)benzaldehyde was synthesised according to literature procedures[17] and treated with 1,3-dimethoxybenzene in the presence of concentrated sulphuric acid in an acetic acid medium (Scheme 1). Similarly to our other reported derivatives, treatment at room temperature and for short time periods at elevated temperature (80 °C) resulted in a mixture of the two diastereoisomers with selectivity towards the *rctt* chair. However, full stereoselectivity could be obtained, with high macrocyclisation yield, over a 7 day reaction time period. This elongated reaction time prompted an investigation of additional routes to the *rctt* diastereoisomer focusing on microwave irradiation. Using the same reaction solvent and catalyst, a 90% yield of the single *rctt* diastereoisomer was obtained in 5 min heating at 70 °C, 100 W power and 20 bar pressure. However, on changing the solvent to dichloromethane, no macrocyclisation was achieved, a result also seen when using dichloromethane and

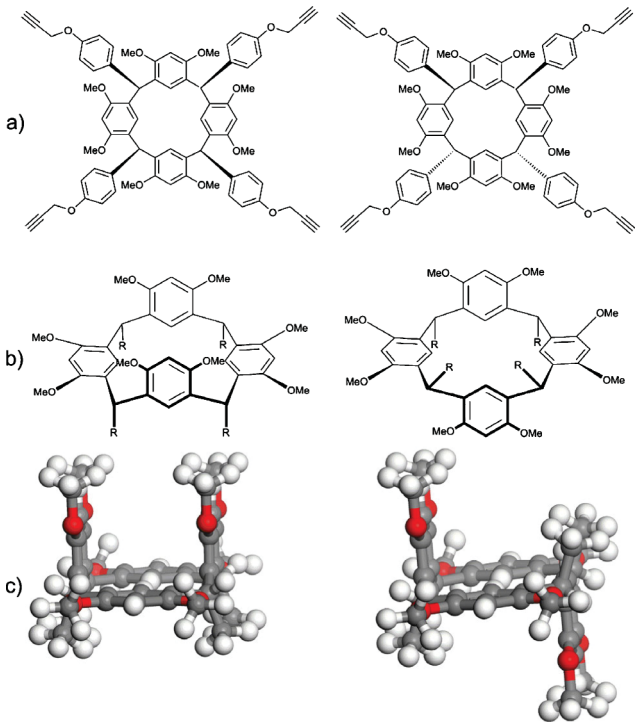
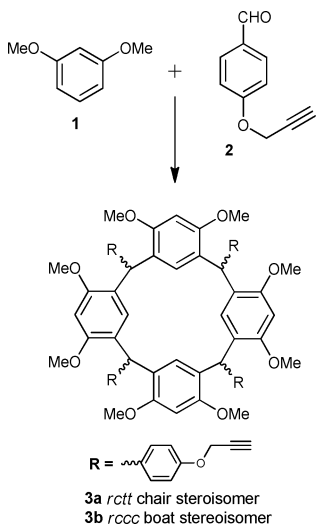


Fig. 2 *rccc* boat and *rctt* chair diastereoisomers of resorcin[4]arenes a) flat representation; b) 3-dimensional representation; c) calculated structures of the diastereoisomers for a simple octa-*O*-alkyl resorcin[4]arene.[15]



Scheme 1 Direct synthesis of tetrapropargylated resorcin[4]arenes using a pre-functionalised aldehyde.

a Lewis acid catalyst ($SnCl_4$), conditions previously developed by Iwanek[18] for the synthesis of *O*-alkyl resorcin[4]arenes under standard conditions.

The *rccc* boat diastereoisomer can be isolated, from the mixture obtained at room temperature, *via* column chromatography using 9 : 1 $CHCl_3$: EtOAc, following precipitation of the majority chair diastereoisomer (45 : 55 boat : chair) from dichloromethane.

The ^{1}H NMR (DMSO-d_6) for the reaction at room temperature clearly shows the presence of the two isomers through the appearance of two non-equivalent singlets in the characteristic peak range (δ 4.9–5.5) for the methylene bridge hydrogens (Fig. 3a and

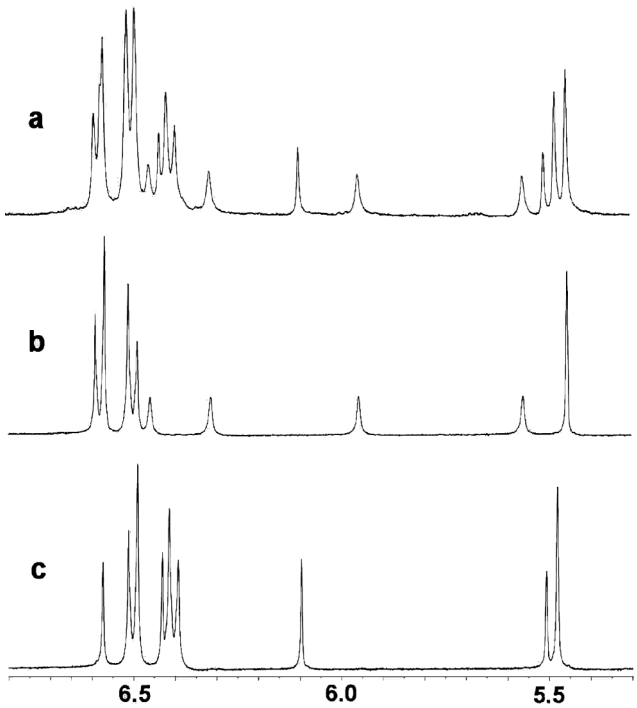


Fig. 3 Expansion of the aromatic region of the ^{1}H NMR spectra (400 MHz, DMSO-d_6) of **3**: a) Products isolated from reaction at 25 °C in acetic acid using H_2SO_4 catalysis, showing presence of **3a** and **3b**; b) purified *rccc* boat diastereoisomer **3b**; c) purified *rctt* chair diastereoisomer **3a**.

Supplementary Information). Additionally, two series of peaks, with integrals corresponding to the two individual bridge signals can be identified, leading to the conclusion that, as expected, the mixture contains both boat and chair isomers. However, for the same reaction performed at 80 °C or under microwave conditions, a single diastereoisomer is isolated (Fig. 3c). This shows the expected C_{2h} symmetry of the *rctt* chair, as indicated by the presence of two signals for the OMe protons (δ 3.58, 3.62, see Supplementary Information), four signals for the protons in the aromatic core of the resorcin[4]arene (δ 5.51, 6.09, 6.43, 6.50), but only a single pair of doublets for the identical pendant aromatic groups (δ 6.40, 6.50) and the expected triplet and doublet for the alkynes (δ 3.48, 4.60, see Supplementary Information). The ^{1}H NMR of the second diastereoisomer, isolated after column chromatography, shows that, as previously observed for large pendant groups,[15b] the *rccc* isomer exists in the C_{2v} flattened boat form, with characteristically broadened signals for the four inequivalent protons of the aromatic core of the resorcin[4]arene (δ 5.56, 5.96, 6.31, 6.46) and two signals for the OMe protons (δ 3.41, 3.56) (Fig. 3b and Supplementary Information).

These two tetravalent propargylated scaffolds were then conjugated with two carbohydrate epitopes (galactose and lactose) in order to study the potential interactions with biologically relevant lectins. The galactose-functionalised glycoclusters were evaluated against PA-IL and the lactose-functionalized derivatives against Gal-1, a human lectin implicated in cancer, inflammation and host-pathogen interactions.[19]

The conjugation of the topoisomeric propargylated resorcin[4]arene **3a–b** was achieved by CuAAC with azido-functionalised galactoside $\mathbf{4_G}$[5f] and lactoside $\mathbf{4_L}$[20] (Scheme 2), using CuI, DIPEA in DMF, to afford the acetylated glycoclusters **5** in high yields (58–85%). The reaction was performed under microwave activation and the cycloaddition was complete within 15 min. The unmasking of the acetate protecting groups was then achieved under smooth solvolysis conditions to obtain the desired resorcin[4]arene-based hydroxylated glycoclusters in near quantitative yields **6**.

The stereochemistry, including the flattened boat of the *rccc* boat, of the propargylated resorcin[4]arene-based scaffolds **3a-b** was conserved in the conjugated macromolecules. This is clearly demonstrated in the ^{1}H NMR spectra, where for the *rctt* chair diastereoisomers $\mathbf{5a_G}$, $\mathbf{5a_L}$, $\mathbf{6a_G}$ and $\mathbf{6a_L}$ (Fig. 4 a,b,e,f respectively), the characteristic high field signal (δ ~5.7) of one of the aromatic residues is observed whereas for the *rccc* boat diastereoisomers (Fig. 4 c,d,g,h) the same proton is downfield (δ ~5.9). Interestingly in the case of the *rctt* chair diastereoisomers the signals for the pendant aromatic groups have fully coalesced in the protected sugar derivatives whereas in the *rccc* boat diastereoisomers the coalescence is less pronounced.

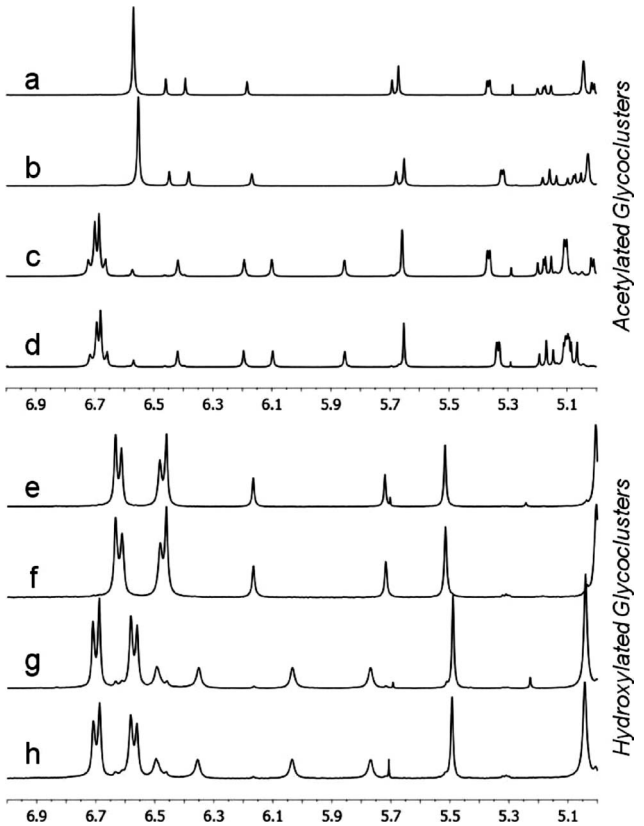
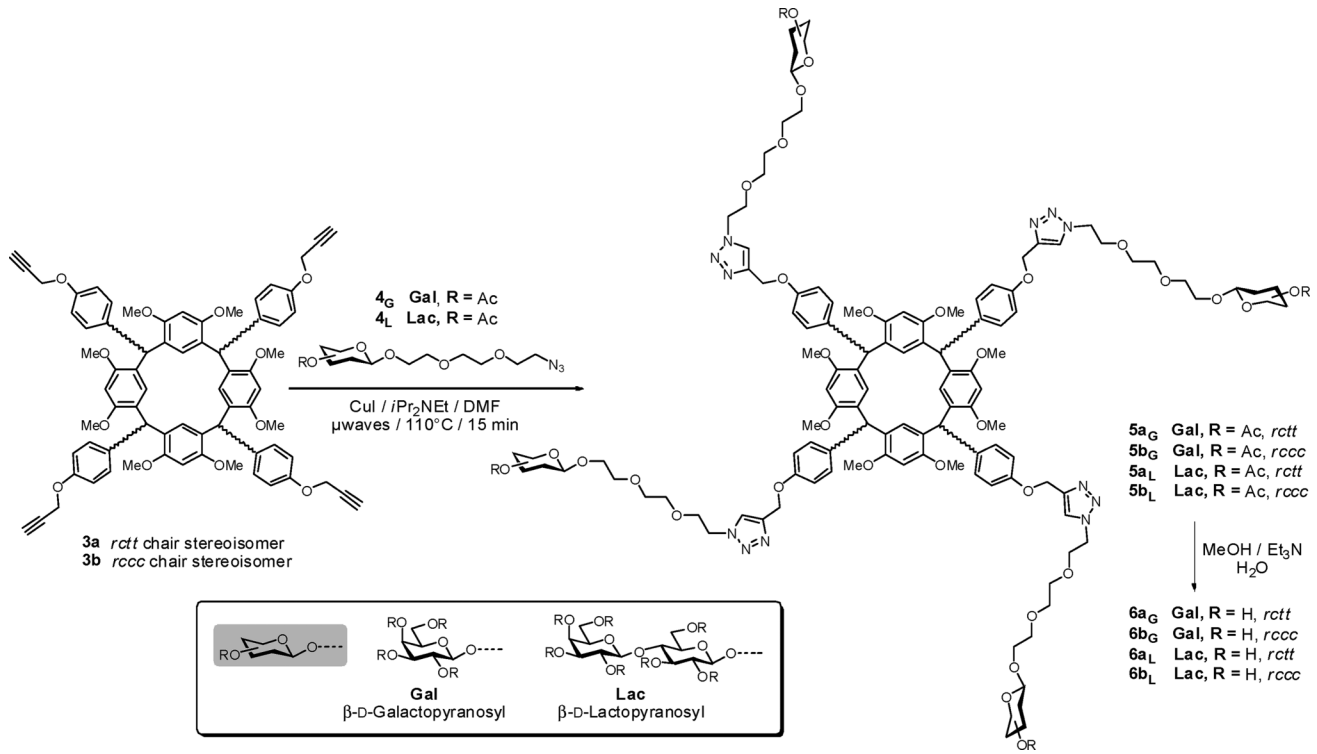


Fig. 4 Partial ^{1}H NMR spectra (aromatic region) of acetylated glycoclusters **5** ($CDCl_3$, 400 MHz, 298 K) **a**: *rctt* chair diastereoisomer $\mathbf{5a_G}$, **b**: *rctt* chair diastereoisomer $\mathbf{5a_L}$, **c**: *rccc* boat diastereoisomer $\mathbf{5b_G}$, **d**: *rccc* boat diastereoisomer $\mathbf{5b_L}$) and hydroxylated glycoclusters **6** (DMSO-d_6 + εD_2O, 400 MHz, 298 K) **e**: *rctt* chair diastereoisomer $\mathbf{6a_G}$**f**: *rctt* chair diastereoisomer $\mathbf{6a_L}$, **g**: *rccc* boat diastereoisomer $\mathbf{6b_G}$, **h**: *rccc* boat diastereoisomer $\mathbf{6b_L}$).

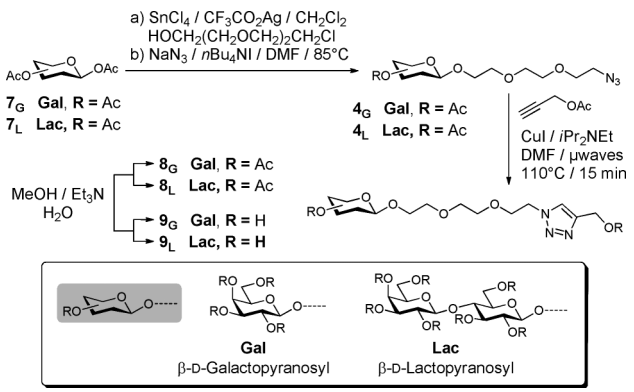
The synthetic route employed was determined based on a number of factors. Conjugation by CuAAC was preferred to amide bond conjugation to avoid the use of excess of either the resorcin[4]arenes or functionalized sugars. The CuAAC reaction



Scheme 2 Synthesis of four topoisomeric resorcin[4]arene-based glycoclusters.

is fast and high yielding (usually >95% for a single reaction) and therefore avoids the potential of incomplete reaction of the alkynyl groups which may lead to mono-, di- or tri-functionalised resorcin[4]arene byproducts. In addition, acetylated carbohydrate derivatives were employed rather than unprotected sugars in order to obtain glycoclusters which are readily soluble in organic solvents and can be easily purified using standard silica gel chromatography. The subsequent deprotection step is facile and gives the desired glycoclusters in high yield and purity.

In order to assess the role of multivalency on lectin binding and to ascertain the influence of the linker on binding, two monovalent carbohydrate probes bearing only the linker arm terminated by a triazole ring were also synthesised (Scheme 3). Glycosylation of peracetyl galactopyranose $\mathbf{7}_G$ or lactose $\mathbf{7}_L$ with 2-[2-(2-chloroethoxy)ethoxy]ethanol followed by azidation of the chloroethyl moiety afforded the acetylated glycosides $\mathbf{4}_G$ and $\mathbf{4}_L$. 1,3-Dipolar cycloaddition under CuAAC conditions with propargyl acetate provided the acetylated cycloadducts $\mathbf{8}_G$[5f] and $\mathbf{8}_L$ and subsequent deprotection of the acetate groups afforded the hydroxylated monovalent carbohydrate probes $\mathbf{9}_G$[5f] and $\mathbf{9}_L$.



Scheme 3 Synthesis of the monovalent carbohydrate probes.

The binding affinity of the glycoclusters was investigated towards PA-IL and Gal-1 using a range of techniques including a haemagglutination inhibition assay (HIA), an enzyme-linked lectin assay (ELLA) and surface plasmon resonance (SPR).

Haemagglutination assays (HIA) were performed for both lectins. The monovalent probes $\mathbf{9}_G$[5h] and $\mathbf{9}_L$ displayed minimum inhibitory concentrations (MIC) in the millimolar range towards PA-IL and Gal-1 respectively (Table 1). The multivalent glycoclusters **6** when evaluated under the same conditions were poorly soluble in the buffer solutions used, however they were of acceptable solubility under the conditions used. Nevertheless they caused the degradation of the erythrocytes and a haemolytic solution of the cells was obtained and thus MIC values could not be determined. Similarly, surface plasmon resonance (SPR) experiments could be readily performed with the monovalent probes $\mathbf{9}_G$[5d] and $\mathbf{9}_L$ (Figure 27 supporting information) towards PA-IL and Gal-1 but the limited solubility of the glycoclusters **6** did not allow the determination of IC_{50} values for these compounds (Table 1). The enzyme-linked lectin assay (ELLA) could be performed only with PA-IL and the galactosylated derivatives $\mathbf{9}_G$,[5d] $\mathbf{6a}_G$ and $\mathbf{6b}_G$. Due to the experimental conditions required for the assay with Gal-1, IC_{50} values could not be determined. The IC_{50} value measured for the monovalent probe $\mathbf{9}_G$ was in the high micromolar range (Table 1). In contrast, the tetravalent glycoclusters $\mathbf{6a}_G$ and $\mathbf{6b}_G$ displayed much stronger inhibition potency with IC_{50} values in the sub-micromolar range corresponding to a 314-fold and 244-fold improvement respectively (Fig. 5). It is

Table 1 Measurement of inhibition for the monovalent probes $\mathbf{9}_G$ and $\mathbf{9}_L$ and for the resorcin[4]arene-based glycoclusters **6**

	PA-IL			Gal-1	
	HIA	ELLA	SPR	HIA	SPR
Ligand (Valency)	MIC[a] (mM)	IC_{50} (μM)	IC_{50} (μM)	MIC[a] (mM)	IC_{50} (μM)
$\mathbf{9}_G$ (1)	10[5h]	220[5f]	63.5[5d]	–/–	–/–
$\mathbf{9}_L$ (1)	–/–	–/–	–/–	1.25	229
$\mathbf{6a}_G$ (4)	Haemol.[b]	0.7	n.s.[c]	–/–	–/–
$\mathbf{6b}_G$ (4)	Haemol.[b]	0.9	n.s.[c]	–/–	–/–
$\mathbf{6a}_L$ (4)	–/–	–/–	–/–	Haemol.[b]	n.s.[c]
$\mathbf{6b}_L$ (4)	–/–	–/–	–/–	Haemol.[b]	n.s.[c]

[a] Minimum inhibitory concentration. [b] Haemolysis of erythrocytes. [c] Not soluble under the experimental conditions required.

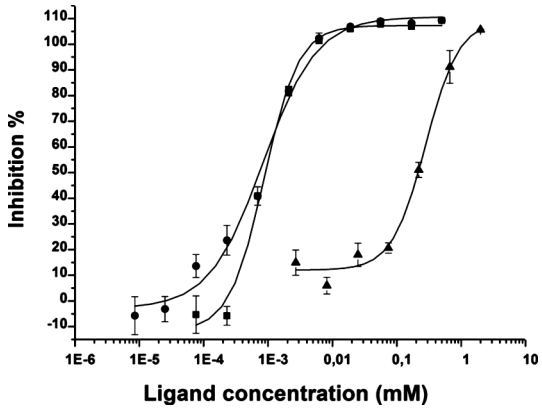


Fig. 5 Inhibition of PA-IL adhesion to a galactosylated surface measured by ELLA for compounds $\mathbf{9}_G$ (▲), $\mathbf{6a}_G$ (●) and $\mathbf{6b}_G$ (■). Error bars represent standard deviation over triplicates, lines are sigmoidal fits.

interesting to note, that in this case, the influence of topology is less clear than previously observed for calix[4]arene scaffolds.[5d] Nevertheless, these improved binding properties towards PA-IL are still significant in the series of multivalent glycoclusters previously studied[5f,5h] by ELLA.

A number of our previously reported calixarene-,[5d,5h] porphyrin-,[5h] β-peptoid-[5h] or fullerene-based[5e,5f] glycoclusters showed better affinities towards PA-IL using SPR analysis (IC_{50} in the sub-micromolar range). The galactosylated glycoclusters $\mathbf{6}_G$ also displayed IC_{50} values (measured by ELLA) in the sub-micromolar range but these are rather higher values in comparison. Thus their binding properties towards PA-IL play a highly important role in further developing this family of multivalent ligands for PA-IL. Rationalisation of this slightly reduced affinity is difficult since isothermal titration microcalorimetry (ITC) measurements could not be performed due to solubility problems and thus the thermodynamic aspects of the interactions and their stoichiometry could not be determined.

However, in the light of the good binding abilities observed, we performed a molecular modelling study of the two new resorcin[4]arene glycoclusters $\mathbf{6a}_G$ and $\mathbf{6b}_G$ in comparison to the most efficient PA-IL ligand identified to-date, a calixarene fixed in the 1,3 alternate conformation that features identical carbohydrate arms.[5d] Comparison of the minimised structures for the three ligands indicates that similarly to the calixarene ligand, in both the *rccc* boat and *rctt* chair diastereoisomers it is possible for the two carbohydrates to be orientated to bind to two neighbouring binding sites on the tetrameric protein (Fig. 6**)** which may account for the highly similar inhibition potencies observed for the two diastereoisomers Modelling of the resorcin[4]arenes in the presence of the protein indicates in both cases that the glycocluster can span two binding sites on a single protein (Fig. 7) and as proposed with the 1,3 alternate calixarene molecule a chelate-like binding may be possible in which the remaining two carbohydrates are available to bind to an additional protein.

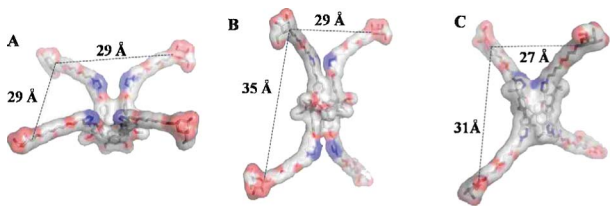


Fig. 6 Low energy conformations of compounds $\mathbf{6b}_g$ (A), $\mathbf{6a}_G$ (B) and 1,3 alternate galactosyalted calix[4]arene (C)[5d] with the linkers built in a extended conformation. Distances have been measured from atom C1 of galactose units.

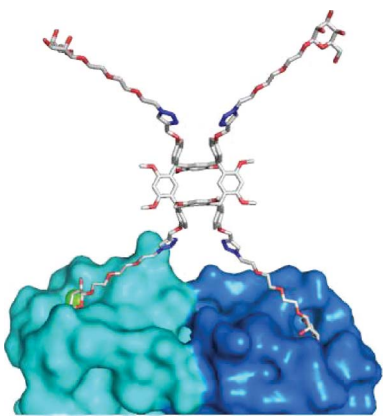


Fig. 7 Molecular modeling of the interaction between two adjacent PA-IL monomers and compound $\mathbf{6b}_G$. The protein is represented with its accessible surface, calcium ions are represented as green spheres.

Conclusion

Two new resorcin[4]arenes suitably functionalised for 'click' chemistry, by the inclusion of alkynes at the lower-rim, were synthesised in a short synthetic sequence. A suitable choice of reaction conditions allowed for the selective formation of the *rctt* chair diastereoisomer through either prolonged treatment at elevated temperature or the use of microwave heating. The conjugation of these propargylated resorcin[4]arene scaffolds with azido-functionalized carbohydrate probes allowed for the efficient preparation of topological isomers of galactosylated and lactosylated glycoclusters. These cyclic architectures offer a general platform for the design of conformationally restricted isomers and the display of binding moieties in a specific spatial arrangement. Although the range of techniques available for measurement of affinity are limited due to solubility problems, the ELLA results obtained for the galactosylated glycoclusters demonstrated a strong affinity for PA-IL. However, no difference in binding properties could be observed due to the topology of the glycoclusters. Our efforts are now focused on the design of more water soluble resorcin[4]arene-based glycoclusters in order to improve their binding properties towards biologically relevant lectins.

Experimental

All reagents for synthesis were commercial (highest purity available for reagent grade compounds) and used without further purification. Solvents were distilled over CaH_2 (CH_2Cl_2), Mg/I_2 (MeOH) or purchased dry. All reactions were performed under an argon atmosphere. Reactions under microwave activation were performed on a Biotage Initiator system. NMR solvents were purchased from Euriso-Top (Saint Aubin, France) or Apollo. NMR spectra were recorded at 293 K, except where stated, using a 300 MHz or a 400 MHz spectrometer. Shifts are referenced relative to deuterated solvent residual peaks. Complete signal assignments from 1D and 2D NMR were based on COSY, HSQC and HMBC correlations. High resolution (HR-ESI-QToF) mass spectra were recorded using a Bruker MicroTOF-Q II XL spectrometer. Optical rotation was measured using a Perkin Elmer polarimeter. Melting points were determined using IA9000 digital melting point apparatus and are uncorrected. Compounds $\mathbf{4}_G$,[5d] $\mathbf{8}_G$,[5d] $\mathbf{9}_G$[5d] and $\mathbf{4}_L$[20] were prepared as previously described.

Recombinant lectins

Recombinant PA-IL was produced in *E. coli* according to our previously reported protocols.[21] Human galectin-1 was generated *via* transformation of pET-3a-gal-1 plasmid, that encodes non-tagged gene LGALS1, into *E. coli* strain BL21 DE3 and expressed and purified following protocols that we have reported previously.[22]

Haemagglutination inhibition assays (HIA)

Haemagglutination inhibition assays (HIA) were performed in U-shaped *96-well* microtitre plates. Rabbit erythrocytes were purchased from Biomérieux and used without further washing. Erythrocytes were diluted to a 4% solution in NaCl (150 mM). Lectin solutions of 2 mg mL^{-1} were prepared in Tris-HCl 20 mM, NaCl 100 mM and $CaCl_2$ 100 μM. The haemagglutination unit (HU) was obtained by the addition of 25 μL of the 4% erythrocyte solution to 25 μL aliquots of sequential (2-fold) lectin dilutions. The mixture was incubated at 25 °C for 60 min. The HU was measured as the minimum lectin concentration required to observe haemagglutination. For the lectin-inhibition assays, lectin concentrations of 4 HU were used. This concentration was found to be 8 μg mL^{-1} for PA-IL and 6 μg mL^{-1} for Gal-1. Subsequent inhibition assays were then carried out by the addition of 12.5 μL lectin solution to 25 μL of sequential dilutions of glycoclusters, monovalent probes and controls. These solutions were then incubated at 25 °C for 2 h then 12.5 μL of 4% erythrocyte solution was added followed by an additional incubation at 25 °C for 30 min. The minimum inhibitory concentration for each molecule was determined for each duplicate.

Enzyme-linked lectin assays (ELLA)

ELLA were conducted by using 96-well microtitre plates (Nunc Maxisorb) coated with PAA-α-D-Gal: 100 μL of a 5 μg mL^{-1} solution in carbonate buffer, pH 9.6 for 1 h at 37 °C then blocking at 37 °C for 1 h with 100 μL per well of 3% (w/v) BSA in PBS. Inhibitor solutions (50 μL) were subjected to serial dilutions (3-fold) with PBS-BSA 0.3% (w/v). Then, biotinylated PA-IL solution (50 μL, 0.5 μg mL^{-1}) was added to each well and the plates were incubated for 1 h at 37 °C. After three washings with T-PBS (50 μL; PBS containing 0,05% Tween 20) for 5 min, horseradish streptavidin-peroxidase conjugate (HRP, 100 μL) (dilution 2 : 5000; Boehringer-Mannheim) was added and left for 1 h at 37 °C. After three more washings, colouration was developed by using 100 μL per well of 0.05 M phosphate/citrate buffer containing *o*-phenylenediamine dihydrochloride (0.4 mg mL^{-1}) and urea hydrogen peroxide (0.4 mg mL^{-1}; OPD kit, Sigma-Aldrich) for 15 min and stopped with 30% sulfuric acid (50 μL). Absorbance was then read at 490 nm by using a microtitre plate reader (BioRad 680) and transformed into inhibition percentages with the help of positive and negative controls. Plots (inhibition percentage *versus* inhibitor concentration) and sigmoidal fitting provided IC_{50} values.

Surface plasmon resonance (SPR)

SPR inhibition experiments were performed on a Biacore 3000 instrument at 25 °C. Measurements were carried out on 3 channels with 3 immobilised sugars: α-L-fucose (channel 1), β-D-galactose (channel 2) and α-D-lactose (channel 3). Immobilization of sugars was performed at 25 °C using running buffer (HBS) at 5 μL min^{-1}. Immobilization on each channel (CM5 Chip) was performed independently. First, the channel was activated by injecting a fresh mixture of EDC/NHS (35 μL, 420 s). Then, a solution of streptavidin (100 μg mL^{-1} in AcONa pH 5 buffer) was injected (50 μL, 600 s). The remaining reactive species were quenched by injecting ethanolamine (1 M, 35 μL, 420 s). Finally a solution of the desired biotinylated PAA–sugar (Lectinity, 200 μg mL^{-1}) was coated onto the surface (50 μL, 600 s) through streptavidin–biotin interaction. This procedure led to 804 RU (resonance unit) (fucoside), 796 RU (galactoside) and 1022 RU (lactoside) of immobilized sugars on channel 1, 2 and 3 respectively. Inhibition experiments were performed with the galactosylated channel 2

and the lactosylated channel 3 and plots represent subtracted data (channel 2–channel 1 or channel 3–channel 1).

Conditions for PA-IL. The running buffer for PA-IL experiments is HEPES 10 mM, NaCl 150 mM, $CaCl_2$ 10 mM, Tween P20 0.005%, pH 7.4. Inhibition experiments with PA-IL are measured with galactosylated channel (2). Inhibition studies consisted of the injection (150 µL, 10 µL min^{-1}, dissociation: 120 s) of incubated (>1 h, r.t.) mixtures of PA-IL (5 µM) and various concentrations of inhibitor (2-fold cascade dilutions). For each inhibition assay, PA-IL (5 µM) without inhibitor was injected to observe the full adhesion of the lectin onto the sugar-coated surface (0% inhibition). The CM5 chip was fully regenerated by successive injections of D-galactose (2 × 30 µL, 100 mM in running buffer).

Conditions for Gal-1. The running buffer for Gal-1 experiments is HEPES 10 mM, NaCl 150 mM, Tween P20 0.005%, pH 7.4. Inhibition experiments with Gal-1 are measured with lactosylated channel (3). Inhibition studies consisted of the injection (80 µL, 10 µL min^{-1}) of incubated (>1 h, r.t.) mixtures of Gal-1 (40 µM) and various concentrations of inhibitor (2-fold cascade dilutions). For each inhibition assay, Gal-1 (40 µM) without inhibitor was injected to observe the full adhesion of the lectin onto the sugar-coated surface (0% inhibition). The CM5 chip was fully regenerated by successive injections of NaCl (10 µL, 1 M in running buffer) and D-galactose (10 µL, 100 mM in running buffer).

For all experimental settings (PA-IL and Gal-1), binding was measured as RU over time after blank subtraction, and data were then evaluated using the BIAevaluation Software, version 4.1. For IC_{50} evaluation, the response (R_{eq} – fitted) was considered as the amount of lectin bound to the carbohydrate-coated surface at equilibrium in the presence of a defined concentration of inhibitor. Inhibition curves were obtained by plotting the percentage of inhibition against the inhibitor concentration (on a logarithmic scale) by using Origin 7.0 software (OriginLab Corp.) and IC_{50} values were extracted from sigmoidal fit of the inhibition curve.

Compound 3a *rctt* diastereoisomer, traditional synthesis

1,3-Dimethoxybenzene (2.0 g, 14 mmol) and 4-(propargyloxy)-benzaldehyde (2.3 g, 14 mmol) were stirred in acetic acid (76 mL) in the presence of concentrated H_2SO_4 (0.85 mL) at 80 °C for 7 d. The reaction mixture was cooled and the resulting precipitate isolated by filtration and washed with Et_2O to yield the desired product (3.30 g, 85%).

Compound 3a *rctt* diastereoisomer, microwave synthesis

Concentrated H_2SO_4 (0.045 mL) was added to 1,3-dimethoxybenzene (0.1 g, 0.072 mmol) and 4-(propargyloxy)benzaldehyde (0.11 g, 0.072 mmol) in acetic acid (5 mL) and irradiated in a CEM-Discover single cavity microwave under stirring for 5 min at 70 °C under 100 W power and 20 Bar pressure. The reaction mixture was cooled and the resulting precipitate isolated by filtration and washed with ether to yield the desired product (3.49 g, 90%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.57 (2H, s, ArH_{resorc}), 6.50 (8H, d, J = 9 Hz, $ArH_{pendant}$), 6.43 (2H, s, ArH_{resorc}), 6.40 (8H d, J = 9 Hz, $ArH_{pendant}$), 6.09 (2H, s, ArH_{resorc}), 5.51 (2H, s, ArH_{resorc}), 5.48 (4H, s, ArC*H*), 4.60 (8H, d, J = 2.5 Hz, OCH_2C), 3.62 (12H, s, OCH_3), 3.58 (12H, s, OCH_3), 3.48 (4H, t, J = 2.5 Hz C≡C*H*).^{13}C NMR (100 MHz, DMSO-d_6 + εDMF 363 K) 156.5, 155.9, 155.8, 136.3, 131.6, 130.1, 128.3, 125.9, 124.7, 114.4, 98.1, 97.4, 80.1, 77.6, 56.6, 56.3, 56.3, 42.2. IR 3265, 3006, 2941, 2830, 1608, 1581, 1504, 1463, 1450, 1438, 1396, 1382, 1291, 1228, 1219, 1200, 1182, 1162, 1097, 1032. HR-ESI-MS (positive mode): m/z calcd for $C_{72}H_{68}NO_{12}$ $[M + NH_4]^+$ 1138.4736, found 1138.4742. Mp 270 °C decomp.

Compound 3b *rccc* diastereoisomer, traditional synthesis

1,3-Dimethoxybenzene (2.0 g, 14 mmol) and 4-(propargyloxy)-benzaldehyde (2.3 g, 14 mmol) were stirred in acetic acid (76 mL) in the presence of concentrated H_2SO_4 (0.85 mL) at 25 °C for 18 h. The reaction mixture was cooled and the resulting precipitate isolated by filtration and washed with ether. Precipitation from dichloromethane yielded pure *rctt* isomer. The filtrate was purified by column chromatography (9 : 1 hexane : ethyl acetate) to yield the *rccc* isomer (0.61 g, 16%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.57 (8H, d, J = 9 Hz, $ArH_{pendant}$), 6.50 (8H, d, J = 9 Hz, $ArH_{pendant}$), 6.46 (2H, s, ArH_{resorc}), 6.31 (2H, s, ArH_{resorc}), 5.96 (2H, s, ArH_{resorc}), 5.56 (2H, s, ArH_{resorc}), 5.46 (4H, s, ArC*H*), 4.65 (8H, d, J = 2.5 Hz, OCH_2C), 3.56 (12H, s, OCH_3), 3.52 (4H, t, J = 2.5 Hz C≡C*H*), 3.41 (12H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.2, 155.9, 155.6, 137.0, 129.9, 124.1, 123.9, 114.2, 80.3, 78.6, 56.3, 56.1, 55.8, 42.3. IR 3276, 3002, 2941, 2833, 1609, 1583, 1506, 1465, 1438, 1360, 1375, 1294, 1217, 1200, 1178, 1159, 1097, 1031. HR-ESI-MS (positive mode): m/z calcd for $C_{72}H_{68}NO_{12}$ $[M + NH_4]^+$ 1138.4733, found 1138.4742. Mp 242–244 °C

General procedure for 1,3-dipolar cycloaddition (Method A)

Unless otherwise stated, the alkyne-functionalized compound, copper iodide, DIPEA and azido-derivative in DMF were introduced into a Biotage Initiator 2–5 mL vial. The vial was flushed with argon and the solution was sonicated for 30 s. The vial was sealed with a septum cap and heated at 110 °C for 15 min under microwave irradiation (solvent absorption level: high). After uncapping the vial, the mixture was diluted with EtOAc (250 mL). The organic layer was washed with 150 mL portions of 1 N HCl, saturated $NaHCO_3$, water, and brine successively. The organic layer was dried (Na_2SO_4), filtered and evaporated. The crude product was purified by flash silica gel column chromatography to afford the desired cycloadducts.

Acetylated *rctt* glycocluster $5a_G$

Obtained as a white foam (189 mg, 85%) following Method A: Compound **3a** (80 mg, 71 µmol, 1 eq.), $\mathbf{4}_G$ (225 mg, 430 µmol, 6 eq.), CuI (6.8 mg, 36 µmol, 0.5 eq.) and DIPEA (63 µL, 0.36 mmol, 5 eq.) in DMF (3 mL). Microwave irradiation: 15 min at 110 °C. After workup, the residue was purified by silica gel flash chromatography (EtOAc then EtOAc/MeOH, 9 : 1). $[\alpha]_D$ = –5.3 (c 0.75, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 4H, H-triaz), 6.57 (s, 16H, *exo*-CH-ar), 6.46, 6.39, 6.18, 5.69 (4 s, 4 × 2H, *endo*-CH-ar), 5.67 (s, 4H, ArC*H*RAr), 5.37 (d, J = 3.3 Hz, 4H, H-4), 5.18 (dd, J = 10.3 Hz, J = 8.2 Hz, 4H, H-2), 5.04 (s, 8H, PhOCH_2C-triaz), 5.00 (dd, J = 10.3 Hz, J = 3.3 Hz, 4H, H-3), 4.54–4.45 (m, 12H, H-1, OCH_2CH_2N), 4.17–4.10 (m, 8H, H-6a, H-6b), 3.96–3.81 (m, 16H, H-5, ½ GalOCH_2,

OCH_2CH$_2$N), 3.72–3.64 (m, 28H, OCH$_3$, $\frac{1}{2}$ GalOCH_2), 3.60–3.52 (m, 24H, GalOCH$_2$CH_2OCH_2CH_2O), 2.12, 2.03, 2.01, 1.96 (4 s, 12H, CH_3CO). ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.5, 170.4, 170.2, 169.5 (4 s, 4×CH$_3$*C*O), 156.2, 156.1, 155.7 (3 s, *C*^{IV}OCH$_3$, *C*^{IV}OCH$_2$Ctriaz), 144.5 (C^{IV}-triaz), 136.06, 136.04 (2 s, *exo*-C^{IV}-ar), 131.8 (*endo*-CH-ar), 130.1 (*exo*-CH-ar), 128.5 (*endo*-CH-ar), 125.3, 124.6 (2 s, *endo*-CH-*C*^{IV}-ar), 123.9 (CH-triaz), 113.8 (*exo*-CH-ar), 101.4 (C-1), 96.6, 95.3 (*endo*-CH-ar), 71.0, 70.8 (C-3, C-5), 70.7, 70.6, 70.3 (GalOCH$_2$*C*H$_2$O*C*H$_2$*C*H$_2$O), 69.5, 69.2 (GalO*C*H$_2$-, O*C*H$_2$CH$_2$N), 68.9 (C-2), 67.2 (C-4), 62.2 (PhOCH_2C-triaz), 61.4 (C-6), 56.40, 56.37, 55.9 (O*C*H$_3$), 50.2 (OCH$_2$*C*H$_2$N), 42.1 (Ar*C*HRAr), 20.9, 20.8, 20.7 (3 s, 4×*C*H$_3$CO). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for C$_{152}$H$_{190}$N$_{12}$O$_{60}$ [M + 2H]$^{++}$ 1571.6087, found 1571.6112.

Acetylated *rccc* glycocluster 5b$_G$

Obtained as a white foam (185 mg, 83%) following Method A: Compound **3b** (80 mg, 71 µmol, 1 eq.), **4**$_G$ (225 mg, 430 µmol, 6 eq.), CuI (6.8 mg, 36 µmol, 0.5 eq.) and DIPEA (63 µL, 0.36 mmol, 5 eq.) in DMF (3 mL). Microwave irradiation: 15 min at 110 °C. After workup, the residue was purified by silica gel flash chromatography (EtOAc then EtOAc/MeOH, 9 : 1). $[\alpha]_D$ = −3.3 (*c* 0.9, CH$_2$Cl$_2$). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 (s, 4H, H-triaz), 6.77–6.62 (m, 16H, *exo*-CH-ar), 6.42, 6.19, 6.10, 5.85 (4 s, 4 × 2H, *endo*-CH-ar), 5.66 (s, 4H, ArC*H*RAr), 5.37 (d, *J* = 3.0 Hz, 4H, H-4), 5.18 (dd, *J* = 10.4 Hz, *J* = 8.0 Hz, 4H, H-2), 5.11, 5.10 (2 s, 8H, PhOCH_2C-triaz), 5.00 (dd, *J* = 10.4 Hz, *J* = 3.0 Hz, 4H, H-3), 4.55–4.45 (m, 12H, H-1, OCH$_2$CH_2N), 4.18–4.06 (m, 8H, H-6a, H-6b), 3.96–3.81 (m, 16H, H-5, $\frac{1}{2}$ GalOCH_2, OCH_2CH$_2$N), 3.73–3.43 (m, 52H, OCH$_3$, $\frac{1}{2}$ GalOCH_2, GalOCH$_2$CH_2OCH_2CH_2O), 2.12, 2.03, 2.01, 1.96 (4 s, 12H, CH_3CO). ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.5, 170.4, 170.2, 169.6 (4 s, 4×CH$_3$*C*O), 156.3, 156.14, 156.07 (3 s, *C*^{IV}OCH$_3$, *C*^{IV}OCH$_2$C-triaz), 144.5 (C^{IV}-triaz), 136.8 (*exo*-C^{IV}-ar), 132.8 (*endo*-CH-ar), 129.9 (*exo*-CH-ar), 128.4 (*endo*-CH-ar), 124.9 (*endo*-CH-*C*^{IV}-ar), 124.0 (CH-triaz), 114.0 (*exo*-CH-ar), 101.5 (C-1), 96.6, 95.6 (*endo*-CH-ar), 71.0, 70.75 (C-3, C-5), 70.72, 70.6, 70.3 (GalOCH$_2$*C*H$_2$O*C*H$_2$*C*H$_2$O), 69.5, 69.3 (GalO*C*H$_2$, O*C*H$_2$CH$_2$N), 68.9 (C-2), 67.2 (C-4), 62.2 (PhOCH_2C-triaz), 61.4 (C-6), 56.3, 56.1 (O*C*H$_3$), 50.2 (OCH$_2$*C*H$_2$N), 42.3 (Ar*C*HRAr), 20.9, 20.8, 20.7 (3 s, 4×*C*H$_3$CO). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for C$_{152}$H$_{190}$N$_{12}$O$_{60}$ [M + 2H]$^{++}$ 1571.6087, found 1571.6142.

Acetylated *rctt* glycocluster 5a$_L$

Obtained as a white foam (94 mg, 58%) following Method A: Compound **3a** (42 mg, 37 µmol, 1 eq.), **4**$_L$ (178 mg, 224 µmol, 6 eq.), CuI (3 mg, 16 µmol, 0.5 eq.) and DIPEA (27 µL, 157 µmol, 5 eq.) in DMF (3 mL). Microwave irradiation: 15 min at 110 °C. After workup, the residue was purified by silica gel flash chromatography (EtOAc/MeOH, 95 : 5 then 9 : 1). $[\alpha]_D$ = −2.3 (*c* 0.43, CH$_2$Cl$_2$). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.67 (s, 4H, H-triaz), 6.55 (s, 16H, *exo*-CH-ar), 6.45, 6.38, 6.17, 5.68 (4 s, 4 × 2H, *endo*-CH-ar), 5.65 (s, 4H, ArC*H*RAr), 5.32 (d, *J* = 3.3 Hz, 4H, H-4′), 5.16 (t, *J* = 9.3 Hz, 4H, H-3), 5.07 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 4H, H-2′), 5.03 (s, 8H, PhOCH_2C-triaz), 4.93 (dd, *J* = 10.4 Hz, *J* = 3.4 Hz, 4H, H-3′), 4.86 (dd, *J* = 9.3 Hz, *J* = 8.0 Hz, 4H, H-2) 4.56–4.40 (m, 24H, H-1, H-1′, H-6a, H-6b, OCH$_2$CH_2N), 4.15–3.99 (m, 8H, H-6′a, H-6′b), 3.90–3.80 (m, 16H, H-5, $\frac{1}{2}$ LacOCH_2, OCH_2CH$_2$N), 3.77 (t, *J* = 9.4 Hz, 4H, H-4), 3.71–3.56 (m, 28H, H-5′, OCH$_3$, $\frac{1}{2}$ LacOCH_2), 3.52 (bs, 24H, GalOCH$_2$CH_2OCH_2CH_2O), 2.12, 2.07, 2.02, 2.01 1.98, 1.94 (6 s, 21H, 7×CH_3CO). ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.40, 170.39, 170.2, 170.1, 169.8, 169.7, 169.1 (7 s, 7×CH$_3$*C*O), 156.14, 156.08, 155.6 (3 s, *C*^{IV}OCH$_3$, *C*^{IV}OCH$_2$C-triaz), 144.4 (C^{IV}-triaz), 136.0 (*exo*-C^{IV}-ar), 131.8 (*endo*-CH-ar), 130.1 (*exo*-CH-ar), 128.5 (*endo*-CH-ar), 125.2, 124.5 (2 s, *endo*-CH-*C*^{IV}-ar), 123.8 (CH-triaz), 113.8 (*exo*-CH-ar), 101.4, 100.7 (C-1, C-1′), 96.6, 95.3 (*endo*-CH-ar), 76.3 (C-4), 72.9, 72.7 (C-3, C-5′), 71.7 (C-2), 71.0 (C-3′), 70.7 (C-5), 70.62, 70.56, 70.2 (GalOCH$_2$*C*H$_2$O*C*H$_2$*C*H$_2$O), 69.4, 69.19 (GalO*C*H$_2$, O*C*H$_2$CH$_2$N), 69.17 (C-2′), 66.7 (C-4′), 62.1, 62.0 (C-6, PhOCH_2C-triaz), 60.8 (C-6′), 56.3, 55.93, 55.90 (O*C*H$_3$), 50.2 (OCH$_2$*C*H$_2$N), 42.0 (Ar*C*HRAr), 20.92, 20.86, 20.75, 20.69, 20.68, 20.6 (6 s, 7×*C*H$_3$CO). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for C$_{200}$H$_{252}$N$_{12}$Na$_2$O$_{92}$ [M + 2Na]$^{++}$ 2169.7597, found 2169.7651.

Acetylated *rccc* glycocluster 5b$_L$

Obtained as a white foam (82 mg, 61%) following Method A: Compound **3b** (35 mg, 31 µmol, 1 eq.), **4**$_L$ (150 mg, 189 µmol, 6 eq.), CuI (3 mg, 16 µmol, 0.5 eq.) and DIPEA (27 µL, 0.16 mmol, 5 eq.) in DMF (3 mL). Microwave irradiation: 15 min at 110 °C. After workup, the residue was purified by silica gel flash chromatography (EtOAc/MeOH, 95 : 5 then 9 : 1). $[\alpha]_D$ = −2.7 (*c* 0.7, CH$_2$Cl$_2$). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.74 (s, 4H, H-triaz), 6.72–6.66 (m, 16H, *exo*-CH-ar), 6.42, 6.20, 6.10, 5.85 (4 s, 4 × 2H, *endo*-CH-ar), 5.65 (s, 4H, ArC*H*RAr), 5.37 (d, *J* = 2.6 Hz, 4H, H-4′), 5.17 (t, *J* = 9.4 Hz, 4H, H-3), 5.13–5.04 (m, 12H, H-2′, PhOCH_2C-triaz), 4.95 (dd, *J* = 10.4 Hz, *J* = 3.4 Hz, 4H, H-3′), 4.87 (dd, *J* = 9.4 Hz, *J* = 8.0 Hz, 4H, H-2′), 4.54–4.42 (m, 24H, H-1, H-1′, H-6a, H-6b, OCH$_2$CH_2N), 4.17–4.02 (m, 8H, H-6′a, H-6′b), 3.91–3.81 (m, 16H, H-5, $\frac{1}{2}$ LacOCH_2, OCH_2CH$_2$N), 3.78 (t, *J* = 9.4 Hz, 4H, H-4), 3.71–3.59 (m, 16H, OCH_3, $\frac{1}{2}$ LacOCH_2), 3.59–3.49 (m, 28H, H-5′, LacOCH$_2$CH_2OCH_2CH_2O), 3.47 (s, 12H, OCH_3), 2.14, 2.08, 2.04, 2.03, 2.02, 1.99, 1.95 (7 s, 21H, CH_3CO). ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.48, 170.46, 170.3, 170.2, 169.9, 169.7, 169.2 (7 s, 7×CH$_3$*C*O), 156.3, 156.14, 156.06 (3 s, *C*^{IV}-OCH$_3$, *C*^{IV}-OCH$_2$C-triaz), 144.5 (C^{IV}-triaz), 136.8 (*exo*-C^{IV}-ar), 132.8 (*endo*-CH-ar), 129.9 (*exo*-CH-ar), 128.4 (*endo*-CH-ar), 124.9 (*endo*-CH-*C*^{IV}-ar), 124.0 (CH-triaz), 114.0 (*exo*-CH-ar), 101.2, 100.8 (C-1, C-1′), 96.6 (1 s, 2C, *endo*-CH-ar), 76.4 (C-4), 72.9, 72.7 (C-3, C-5′), 71.7 (C-2), 71.1 (C-3′), 70.8 (C-5), 70.7, 70.6, 70.3 (LacOCH$_2$*C*H$_2$O*C*H$_2$*C*H$_2$O), 69.5 (O*C*H$_2$CH$_2$N), 69.26 (LacO*C*H$_2$), 69.22 (C-2′), 66.7 (C-4′), 62.2, 62.1 (C-6, PhOCH_2C-triaz), 60.9 (C-6′), 56.3, 56.2, 56.1 (O*C*H$_3$), 50.2 (OCH$_2$*C*H$_2$N), 42.3 (Ar*C*HRAr), 21.0, 20.9, 20.82, 20.77, 20.75, 20.6 (6 s, 7C, 7×*C*H$_3$CO). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for C$_{200}$H$_{254}$N$_{12}$O$_{92}$ [M + 2H]$^{++}$ 2147.7777, found 2147.7853.

General procedure for deacetylation (Method B)

Unless otherwise stated, the acetylated glycoside or glycocluster (1 eq.) was suspended in distilled MeOH, ultra-pure water and ultra-pure triethylamine (5 : 1 : 1, v/v/v). The mixture was stirred under argon at room temperature for 2 to 4 days. Solvents were evaporated, co-evaporated with toluene three times and the

resulting white foam was dissolved in ultra-pure water (5 mL) and freeze-dried to afford pure glycoconjugates.

Hydroxylated *rctt* glycocluster 6a$_G$

Obtained as a freeze-dried white solid (122 mg, 97%) following Method B: Compound **5a$_G$** (160 mg) was suspended in methanol (4 mL), water (1 mL) and triethylamine (1 mL). After stirring at r.t. for 3 days and concentration, the mixture was dissolved in ultra-pure water (5 mL) then freeze-dried to afford the pure deacetylated glycocluster. $[\alpha]_D = -6.7$ (*c* 0.64, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6 + εD$_2$O) δ 8.04 (s, 4H, H-triaz), 6.69–6.57 (m, 10H, *exo*-CH-ar, *endo*-CH-ar), 6.51–6.42 (m, 10H, *exo*-CH-ar, *endo*-CH-ar), 6.17, 5.72 (2 s, 2 × 2H, *endo*-CH-ar), 5.59 (s, 4H, ArC*H*RAr), 5.01 (s, 8H, PhOCH_2C-triaz), 4.48 (t, *J* = 5.0 Hz, 8H, OCH$_2$CH_2N), 4.08 (d, *J* = 7.2 Hz, 4H, H-1), 3.85–3.74 (m, 12H, $\frac{1}{2}$ GalOCH_2, OCH_2CH$_2$N), 3.64 (s, 12H, OCH$_3$), 3.62 (s, 12H, OCH$_3$), 3.58–3.41 (m, 40H, H-3, H-6a, H-6b, $\frac{1}{2}$ GalOCH_2, GalOCH$_2$CH_2OCH_2CH_2O), 3.35–3.28 (m, 4H, H-5), 3.28–3.20 (m, 8H, H-2, H-4). ^{13}C NMR (100 MHz, DMSO-d_6 + ε D$_2$O) δ 156.0, 155.9, 155.2 (3 s, C^{IV}OCH$_3$, C^{IV}OCH$_2$C-triaz), 143.2 (C^{IV}-triaz), 135.2 (*exo*-C^{IV}-ar), 131.4* (*endo*-CH-ar), 130.0 (*exo*-CH-ar), 127.7* (*endo*-CH-ar), 125.0 (CH-triaz), 124.6, 123.6 (*endo*-CH-C^{IV}-ar), 113.8 (*exo*-CH-ar), 103.7 (C-1), 96.0* (*endo*-CH-ar), 75.3, 73.5, 70.6 (C-2, C-4, C-5), 69.9, 69.8, 69.7 (GalOCH$_2$CH$_2$OCH$_2$CH$_2$O), 68.8 (OCH$_2$CH$_2$N), 68.2 (C-3), 67.9 (GalOCH$_2$), 61.2 (PhOCH$_2$C-triaz), 60.6 (C-6), 56.0 (OCH$_3$), 49.6 (OCH$_2$CH$_2$N), 41.7 (ArCHRAr). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for $C_{120}H_{156}N_{12}Na_2O_{44}$ [M + 2Na]$^{++}$ 1257.5061, found 1257.5066.

* Peaks not observed in the ^{13}C NMR spectrum but clearly identified from HSQC experiments.

Hydroxylated *rccc* glycocluster 6b$_G$

Obtained as a freeze-dried white solid (138 mg, 98%) following Method B: Compound **5b$_G$** (180 mg, 5 μmol) was suspended in methanol (4 mL), water (1 mL) and triethylamine (1 mL). After stirring at r.t. for 3 days and concentration, the mixture was dissolved in ultra-pure water (5 mL) then freeze-dried to afford the pure deacetylated glycocluster. $[\alpha]_D = -2.5$ (*c* 1.3, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6 + ε D$_2$O) δ 8.06 (s, 4H, H-triaz), 6.70 (d, *J* = 8.5 Hz, 8H, *exo*-CH-ar), 6.57 (d, *J* = 8.5 Hz, 8H, *exo*-CH-ar), 6.49, 6.35, 6.03, 5.77 (4 s, 4 × 2H, *endo*-CH-ar), 5.49 (s, 4H, ArC*H*RAr), 5.04 (s, 8H, PhOCH_2C-triaz), 4.47 (t, *J* = 5.0 Hz, 8H, OCH$_2$CH_2N), 4.07 (d, *J* = 7.4 Hz, 4H, H-1), 3.87–3.69 (m, 12H, $\frac{1}{2}$ GalOCH_2, OCH_2CH$_2$N), 3.70–3.40 (m, 64H, H-3, H-6a, H-6b, $\frac{1}{2}$ GalOCH_2, GalOCH$_2$CH_2OCH_2CH_2O, OCH$_3$), 3.34-3.28 (m, 4H, H-5), 3.28–3.20 (m, 8H, H-2, H-4). ^{13}C NMR (100 MHz, DMSO-d_6 + ε D$_2$O) δ 156.1, 155.9, 155.7 (3 s, C^{IV}OCH$_3$, C^{IV}OCH$_2$C-triaz), 143.2 (C^{IV}-triaz), 136.2 (*exo*-C^{IV}-ar), 132.1 (*endo*-CH-ar), 129.7 (*exo*-CH-ar), 127.7* (*endo*-CH-ar), 125.0 (CH-triaz), 124.1, 123.6 (*endo*-CH-C^{IV}-ar), 114.0 (*exo*-CH-ar), 103.7 (C-1), 96.8,* 96.1* (*endo*-CH-ar), 75.3, 73.5, 70.7 (C-2, C-4, C-5), 69.95, 69.87, 69.7 (GalOCH$_2$CH$_2$OCH$_2$CH$_2$O), 68.9 (OCH$_2$CH$_2$N), 68.3 (C-3), 68.0 (GalOCH$_2$), 61.2 (PhOCH$_2$C-triaz), 60.6 (C-6), 56.0, 55.0 (OCH$_3$), 49.7 (OCH$_2$CH$_2$N), 42.0 (ArCHRAr). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for $C_{120}H_{156}N_{12}Na_2O_{44}$ [M + 2Na]$^{++}$ 1257.5061, found 1257.5115.

* Peaks not observed in the ^{13}C NMR spectrum but clearly identified from HSQC experiments.

Hydroxylated *rctt* glycocluster 6a$_L$

Obtained as a freeze-dried white solid (65 mg, 97%) following Method B: Compound **5a$_L$** (92 mg) was suspended in methanol (3 mL), water (0.5 mL) and triethylamine (0.5 mL). After stirring at r.t. for 4 days and concentration, the mixture was dissolved in ultra-pure water (5 mL) then freeze-dried to afford the pure deacetylated glycocluster. $[\alpha]_D = -8.6$ (*c* 0.80, DMSO). ^{1}H NMR (400 MHz, DMSO-d$_6$ + ε D$_2$O) δ 8.04 (s, 4H, H-triaz), 6.69–6.55 (m, 10H, *exo*-CH-ar, *endo*-CH-ar), 6.54–6.39 (m, 10H, *exo*-CH-ar, *endo*-CH-ar), 6.17, 5.72 (2 s, 2 × 2H, *endo*-CH-ar), 5.51 (s, 4H, ArC*H*RAr), 5.00 (s, 8H, PhOCH_2C-triaz), 4.47 (bs, 8H, OCH$_2$CH_2N), 4.24–4.09 (m, 8H, H-1, H-1′), 3.88–3.76 (m, 12H, $\frac{1}{2}$ LacOCH_2, OCH_2CH$_2$N), 3.76–3.36 (m, 40H, H-4, H-4′, H-6a, H-6b, H-6′a, H-6′b, $\frac{1}{2}$ LacOCH_2, LacOCH$_2$CH_2OCH_2CH_2O, OCH$_3$), 3.36–3.22 (m, 20H, H-2′, H-3, H-3′, H-5, H-5′), 3.00 (t, *J* = 8.2 Hz, 4H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6 + ε D$_2$O) δ 156.0, 155.9, 155.2 (3 s, C^{IV}OCH$_3$, C^{IV}OCH$_2$C-triaz), 143.2 (C^{IV}-triaz), 135.2 (*exo*-C^{IV}-ar), 131.3 (*endo*-CH-ar), 130.0 (*exo*-CH-ar), 127.8 (*endo*-CH-ar), 124.8 (CH-triaz), 124.6, 123.6 (*endo*-CH-C^{IV}-ar), 113.8 (*exo*-CH-ar), 104.0, 102.8 (C-1, C-1′), 96.4 (*endo*-CH-ar), 80.8, 75.7, 75.04, 75.00, 73.3, 73.2, 70.7 (C-2, C-2′, C-3, C-3′, C-4′, C-5, C-5′), 69.9, 69.8 (LacOCH$_2$CH_2OCH_2CH_2O), 68.8, 68.6 (OCH_2CH$_2$N, LacOCH_2), 61.2 (PhOCH_2C-triaz), 60.6 (C-6, C-6′), 55.9 (OCH$_3$), 49.6 (OCH$_2$CH$_2$N), 41.7 (ArCHRAr). HR-ESI-QToF MS (positive mode): calcd for $C_{144}H_{198}N_{12}O_{64}$ [M + 2H]$^{++}$ 1559.6298, found 1559.6286.

Hydroxylated *rccc* glycocluster 6b$_L$

Obtained as a freeze-dried white solid (54 mg, 99%) following Method B: Compound **5b$_L$** (75 mg) was suspended in methanol (3 mL), water (0.5 mL) and triethylamine (0.5 mL). After stirring at r.t. for 4 days and concentration, the mixture was suspended in ultra-pure water (5 mL) then freeze-dried to afford the pure deacetylated glycocluster. $[\alpha]_D = -6.9$ (*c* 0.75, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6 + ε D$_2$O) δ 8.06 (s, 4H, H-triaz), 6.70 (d, *J* = 8.5 Hz, 8H, *exo*-CH-ar), 6.57 (d, *J* = 8.5 Hz, 8H, *exo*-CH-ar), 6.48, 6.35, 6.03, 5.77 (4 s, 4 × 2H, *endo*-CH-ar), 5.49 (s, 4H, ArC*H*RAr), 5.04 (s, 8H, PhOCH_2C-triaz), 4.47 (t, *J* = 4.7 Hz, 8H, OCH$_2$CH_2N), 4.23-4.14 (m, 8H, H-1, H-1′), 3.88–3.75 (m, 12H, $\frac{1}{2}$ LacOCH_2, OCH_2CH$_2$N), 3.75–3.39 (m, 76H, H-4, H-4′, H-6, H-6′, OCH$_3$, GalOCH$_2$CH_2OCH_2CH_2O, $\frac{1}{2}$ LacOCH_2), 3.33–3.20 (m, 20H, H-2′, H-3, H-3′, H-5, H-5′), 3.00 (t, *J* = 8.2 Hz, 4H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6 + ε D$_2$O) δ 156.0, 155.8, 155.6 (3 s, C^{IV}OCH$_3$, C^{IV}OCH$_2$C-triaz), 143.2 (C^{IV}-triaz), 136.2 (*exo*-C^{IV}-ar), 132.1* (*endo*-CH-ar), 129.6 (*exo*-CH-ar), 127.8* (*endo*-CH-ar), 124.9 (CH-triaz), 124.0, 123.5 (*endo*-CH-C^{IV}-ar), 113.9 (*exo*-CH-ar), 104.0, 102.8 (C-1, C-1′), 96.7* (*endo*-CH-ar), 80.8, 75.7, 75.03, 74.98, 73.3, 73.2, 70.7 (C-2, C-2′, C-3, C-3′, C-4, C-5, C-5′), 69.8, 69.7 (LacOCH$_2$CH_2OCH_2CH_2O), 68.8 (OCH_2CH$_2$N), 68.2 (C-4′, LacOCH_2), 61.2 (PhOCH_2C-triaz), 60.6 (C-6, C-6′), 55.9 55.8 (OCH$_3$), 49.6 (OCH$_2$CH$_2$N), 42.0 (ArCHRAr). HR-ESI-QToF MS (positive mode): calcd for $C_{144}H_{198}N_{12}O_{64}$ [M + 2H]$^{++}$ 1559.6298, found 1559.6288.

* Peaks not observed in the ^{13}C NMR spectrum but clearly identified from HSQC experiments.

1-[1,2,3-Triazol-4-yl-(acetoxy)methyl]-3,6-dioxaoct-8-yl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactoside 8_L

Obtained as a colorless oil (95 mg, 73%) following Method A: propargyl acetate (18.5 mg, 0.19 mmol, 1.3 eq.), $\mathbf{4}_L$ (115 mg, 0,145 mmol, 1 eq.), CuI (2.4 mg, 0.013 mmol, 0.1 eq.) and DIPEA (66 µL, 0.435 mmol, 3 eq.). Purified by silica gel flash chromatography (EtOAc). $[\alpha]_D$ = +0.9 (*c* 1.07, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, H-triaz), 5.33 (dd, *J* = 3.4 Hz, *J* = 0.9 Hz, 1H, H-4′), 5.19 (s, 2H, OCH_2C-triaz), 5.16 (t, *J* = 9.2 Hz, 1H, H-3), 5.09 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 1H, H-2), 4.94 (dd, *J* = 10.4 Hz, *J* = 3.4 Hz, 1H, H-3′), 4.87 (dd, *J* = 9.6 Hz, *J* = 8.0 Hz, 1H, H-2), 4.56–4.45 (m, 6H, H-1, H-1′, H-6a, H-6b, OCH_2CH_2N), 4.15–4.00 (m, 2H, H-6′a, H-6′b), 3.92–3.83 (m, 4H, H-5, OCH_2CH_2N, $\frac{1}{2}$ $LacOCH_2$), 3.78 (t, *J* = 9.4 Hz, 1H, H-4), 3.67 (ddd, *J* = 10.8 Hz, *J* = 6.4 Hz, *J* = 4.0 Hz, 1H, $\frac{1}{2}$ $LacOCH_2$), 3.62–3.54 (m, 7H, $LacOCH_2CH_2OCH_2CH_2$, H-5′), 2.13, 2.09, 2.06, 2.04, 2.03, 2.02, 2.00, 1.95 (8 s, 8 × 3H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.4, 170.3, 170.2, 169.9, 169.7, 169.2 (7 s, 8×CH_3CO), 142.7 (C^{IV}-triaz), 125.0 (CH-triaz), 101.2, 100.7 (C-1, C-1′), 76.3 (C-4), 72.9 (C-3), 72.8 (C-5′), 71.7 (C-2), 71.1 (C-3′), 70.8 (C-5), 70.7, 70.3 (2 s, $LacOCH_2CH_2OCH_2CH_2O$), 69.5, 69.2 ($LacOCH_2$, OCH_2CH_2N), 69.2 (C-2′), 66.7 (C-4′), 62.1 (C-6), 60.9 (C-6′), 57.7 (OCH_2C-triaz), 50.4 (OCH_2CH_2N), 21.00, 20.97, 20.9, 20.8, 20.7, 20.6 (6 s, 8×CH_3CO). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for $C_{37}H_{54}N_3O_{22}$ $[M + H]^+$ 892.3193, found 892.3168.

1-[1,2,3-Triazol-4-yl-(hydroxy)methyl]-3,6-dioxaoct-8-yl β-D-lactoside 9_L

Obtained as a lyophilized white powder (57 mg, 99%) following Method B: $\mathbf{8}_L$ (93 mg, 0.1 mmol, 1 eq.), MeOH (2 mL), water (0.5 mL) and triethylamine (0.5 mL). $[\alpha]_D$ = −2.9 (*c* 0.31, H_2O). ^1H NMR (400 MHz, DMSO-d_6 + ε D_2O) δ 7.92 (s, 1H, H-triaz), 4.53–4.43 (m, 4H, OCH_2CH_2N, OCH_2C-triaz), 4.24–4.15 (m, 2H, H-1, H-1′), 3.87–3.72 (m, 3H, OCH_2CH_2N, $\frac{1}{2}$ $LacOCH_2$), 3.68–3.40 (m, 13H, H-4′, H-3, H-6a, H-6b, H-6′a; H-6′b, $LacOCH_2CH_2OCH_2CH_2$, $\frac{1}{2}$ $LacOCH_2$) 3.34–3.21 (m, 5H, H-2, H-3′, H-4, H-5, H-5′), 3.00 (t, *J* = 8.1 Hz, 1H, H-2′). ^{13}C NMR (100 MHz, DMSO-d_6 + ε D_2O) δ 148.0 (C^{IV}-triaz), 123.4 (CH-triaz), 104.0, 102.8 (C-1, C-1′), 80.8, 75.7, 75.04, 75.00, 73.3, 73.2, 70.7 (C-2, C-2′, C-3, C-3′, C-4, C-5, C-5′), 69.8, 69.7 (2 s, $LacOCH_2CH_2OCH_2CH_2O$), 69.0 (OCH_2CH_2N), 68.3 ($LacOCH_2$), 68.3 (C-4′), 60.6 (C-6, C-6′), 55.1 (OCH_2C-triaz), 49.5 (OCH_2CH_2N). HR-ESI-QToF MS (positive mode): *m*/*z* calcd for $C_{21}H_{37}N_3NaO_{14}$ $[M + Na]^+$ 578.2168, found 578.2143.

Modeling of glycocluster and PA-IL

Three-dimensional structures of compounds **6a** and **6b** were built using Sybyl software (Tripos, St Louis). Core scaffold coordinates were taken from the crystal structures of resorcin[4]arenes in *rccc* boat conformation[23] and *rctt* chair conformation (CCDC-637642).[24] Atomic partial charges were then calculated (MOPAC/MNDO). Galactosylated epitopes with the triethyleneglycol-based linker were taken from previous modeling of calixarene glycoclusters.[5d] Scaffold and carbohydrates epitopes were connected and the charges were derived and symmetrized in order to obtain a neutral global charge. The resulting glycocluster was then energy minimized using conjugate gradient method and TRIPOS Force Field[25] with addition of carbohydrate parameters.[26]

Two adjacent PA-IL monomers were considered for docking compound **6b**, using a procedure previously described.[5d] Briefly one galactose epitope of glycocluster **6b** was fitted into one of the two binding sites of the dimer and a manual conformational search was performed around the linker rotatable bonds for bringing another galactose into the second binding site. Geometry optimization was performed on the ligand, first with appropriate constraints for determining the perfect orientation of the galactose residues in the binding site, then with no constraints.

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