Synthesis and biological evaluation of multivalent carbohydrate ligands obtained by click assembly of pseudo-rotaxanes†

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Multivalent carbohydrate ligands have been prepared by assembling α-cyclodextrin-based pseudo-rotaxanes through "click chemistry". The inclusion complex formed by a lactosyl-α-CD conjugate and a decane axle carrying a lactosyl stopper at one extremity and an azido group at the other end was dimerized by bis-propargyl spacers of different lengths to provide oligorotaxanes having adjustable threading ratios. For the first time, saccharidic ligands have been introduced on rotaxanes both as a biological recognition element and as a capping group. The supramolecular species have been isolated and characterized by mass spectrometry as well as by 1D and DOSY NMR experiments. Their ability to inhibit the binding of *Arachis hypogaea* agglutinin to asialofetuin, assayed by enzyme linked lectin assays (ELLA), was shown to be valency-dependent.

Introduction

Carbohydrate-protein interactions taking place at the cell surface are critical in numerous normal and pathological processes including fertilization, viral and bacterial infection, inflammatory response, cell adhesion, and metastatic spreading.[1] Although the intrinsic binding ability of monovalent carbohydrates to proteins is generally low, multivalent presentation of saccharidic epitopes results usually in highly specific and effective recognition. The so-called glycoside cluster effect accounts for the high affinities observed in natural cellular recognition processes in which multiple glycans bind simultaneously to multiple receptors.[2] Thus an increasing number of polyvalent saccharidic clusters with various scaffolds, including polymers, dendrimers, and nanoparticles, have been synthesized to mimic biological systems, and their binding abilities toward specific lectins or cell surface receptors have been extensively investigated.[3]

In recent years, polyrotaxanes have emerged as novel and promising scaffolds for the preparation of high affinity polyvalent ligands.[4] Polyrotaxanes are supramolecular assemblies in which cyclic molecules (cyclodextrin, calixarene, cucurbituril or crown ether) are threaded onto a linear polymer chain capped with bulky end groups.[5] The cylindrical host molecules are able to spin around the polymer axle as well as to move back and forth along the chain. This polyvalent presentation offers the advantage of polymeric features such as the ability to span nanometer lengths as well as the dynamic presentation of biological ligands. Stoddart *et al.* have developed a self-assembled pseudopolyrotaxane consisting of lactosyl-substituted cyclodextrins threaded onto a linear polyviologen.[6] The macromolecular assembly, which was investigated for its ability to inhibit galectin-1 mediated T-cell agglutination, exhibited an important affinity enhancement over lactose that could not be reached either by trivalent lactosyl clusters or by lactosyl-grafted chitosan polymers. In a different approach, Yui *et al.* prepared maltosyl-functionalized supramolecular species by chemical grafting of the saccharide moiety onto poly(ethylene glycol)/cyclodextrin-based polyrotaxanes.[7] The inhibition of *Concanavalin A*-induced hemagglutination emphasized the remarkable effect of the polyrotaxane structure which displayed a 3000 fold enhancement over maltose. Moreover, comparison of polyrotaxanes having different threading ratios suggested that high mobility of maltose ligands along the PEG chain contributes much to the affinity enhancement.

In the present work we devise an original approach for the preparation of low molecular weight oligorotaxanes with adjustable ligand densities as potential multivalent lectin inhibitors. The construction of these supramolecular systems was based on the assembly by "click chemistry" of host–guest inclusion complexes constituted by a glycosylated-α-cyclodextrin conjugate and a decane guest. Oligorotaxanes carrying up to 5 cyclodextrins were isolated and characterized by MS and NMR experiments (1D and DOSY). Their ability to inhibit the binding of *Arachis hypogaea* agglutinin to asialofetuin was evaluated by enzyme linked lectin assays (ELLA).

Results and discussion

The preparation of glycosyl-appended oligorotaxanes followed the pathway depicted in Scheme 1. The approach relied on the formation and assembly by "click chemistry" of a pseudo-rotaxane composed of a carbohydrate-monofunctionalized α-cyclodextrin and an alkane chain blocked at one end by a saccharidic stopper and carrying an azido group at the other end. A lactosyl moiety was introduced both as biological recognition element on the cyclodextrin and as capping group of the system. The use of a sugar moiety as blocking reagent aimed at solubilizing the guest part in aqueous media, thus allowing complexation to proceed

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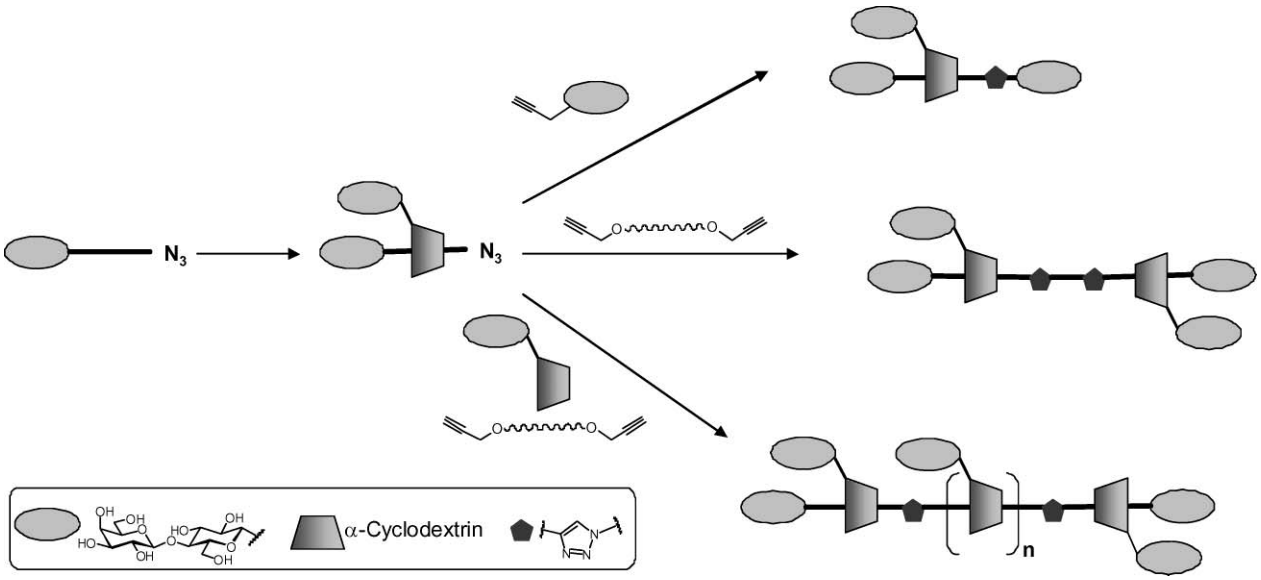
† Electronic supplementary information (ESI) available: MS spectra, TLC profile and graphical comparison of the coefficient of diffusion for the purified polyrotaxanes. See DOI: 10.1039/b822976g



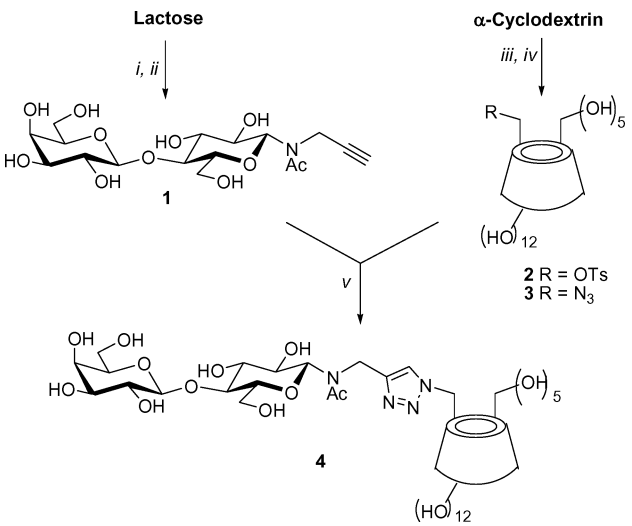
Scheme 1 Synthetic approach towards click-assembled oligorotaxanes.

with α-cyclodextrin. It should also be available for lectin binding, thus participating in the multivalency of the ligand. The size and hydrophilicity of the lactosyl group were both expected to prevent unthreading of the cyclodextrin from the rotaxanes. Its affinity for various galactose binding proteins such as peanut lectin[8] has been deeply investigated in the literature and was also of particular interest for potential biological applications. The guest part consisted of a C-10 alkane chain. Although an abundant literature reported the synthesis and characterization of poly(ethylene glycol)-based polyrotaxanes, an alkane axle was expected to favor the complexation process. Indeed, Weickenmeier *et al.* reported that cyclodextrins threaded onto a polyester of octanedicarboxylic acid and poly(ethylene glycol) occupy preferably the alkyl moiety rather than the PEG part.[9] The decane backbone was selected to form a 1:1 complex in the presence of an equimolar amount of CD-conjugate while a 2:1 complex of CD and decane could be expected with an excess of host cyclodextrin as recently reported by Rodriguez-Llamazares in the first crystallographic structure of an α-CD complex with decanoic acid.[10]

Generation of multivalent species with different cyclodextrin ratios involved either a capping reaction of the inclusion complex with a propargyl lactoside or assembling two complexes together by a bis-propargyl spacer. Taking advantage of the ability of the linker to form inclusion complexes with the α-cyclodextrin allowed the isolation of supramolecular structures with adjustable threading ratios. Both capping and assembling steps occurred through copper catalyzed Huisgen azide-alkyne cycloaddition as reported by Sharpless[11] and Meldal[12] and referred to as "click chemistry". The azide-alkyne "click chemistry" has been efficiently used by Leigh[13] and Stoddart's[14] groups for the synthesis of rotaxanes and one example of a pH-responsive pseudo-polyrotaxane alternating cyclodextrins and cucurbituril macrocycles was prepared by "click polymerization".[15] However, to the best of our knowledge, we report for the first time the use of "click chemistry" for the preparation of glycosylated oligorotaxanes. Besides the obvious efficiency of the copper catalyzed cycloaddition as a ligation method, the α-CD is able to pass across the 1,4-disubstituted 1,2,3-triazole ring[16] thus making this coupling reaction very attractive for the design of new polyrotaxanes.

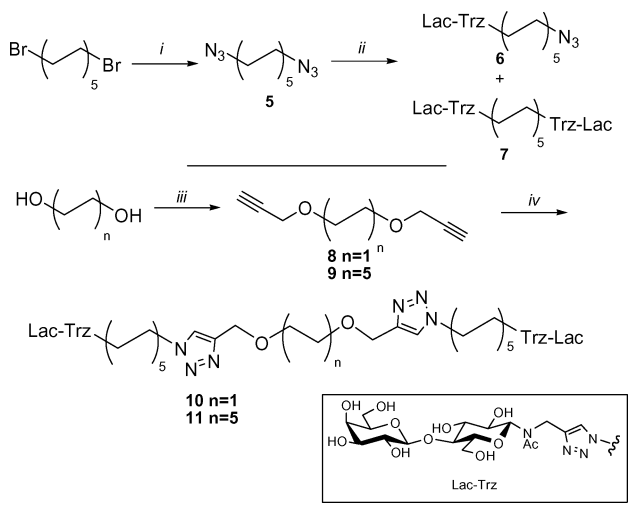
Chemical synthesis of host and guest blocks

Considering that polysubstitution of the cyclodextrin may hamper the inclusion and threading process of the decane part into the cavity of the host molecule[17] we focused on the preparation of a monofunctionalized lactosyl-α-CD (Scheme 2). *N*-Propargyl β-lactosylamide (**1**) was prepared in nearly quantitative yield by reacting lactose in neat propargylamine to afford a transient glycosylamine that was made non-hydrolyzable by selective *N*-acylation in methanol. Initially reported by Spevak *et al.*[18] with allylamine as nucleophile, this procedure proved to be effective with other amines and has been recently applied to the efficient preparation of propargylated xyloglucooligosaccharides.[19] Tertiary amides such



Scheme 2 *i)* Neat propargylamine; *ii)* Ac_2O, MeOH (91% over 2 steps); *iii)* TsCl, pyridine (26%); *iv)* NaN_3, DMF, 140 °C (93%); *v)* $CuSO_4$/sodium ascorbate, H_2O/*i*PrOH (75%).

as **1** are known to undergo a *cis-trans* isomerization in solution which can make their characterization by NMR difficult.[18,20,21] At room temperature, two sets of signals corresponding to *endo* and *exo* rotamers are commonly observed. Peak coalescence is generally achieved by increasing the temperature thus making the NMR spectra simpler. On heating a solution of **1** in D_2O, peak coalescence was accompanied by a substantial signal broadening for the atoms in the proximity of the amide group. At 353K, the ^{13}C signals of the anomeric carbon, the methyl group and the propargyl moiety became so broadened that they were difficult to distinguish from the baseline. This phenomenon has already been reported by Rebek *et al.*[22] in a recent study about tertiary amide rotation in a constrained environment and may reflect some tendency of **1** to form aggregates in water. Nonetheless the structure of **1** was unambiguously confirmed by a complementary NMR experiment in pyridine-d^5 and by mass spectrometry. Selective tosylation of α-CD according to the procedure described by Melton and Slessor[23] with minor modifications afforded pure compound **2** in 26% yield. Tosyl displacement by sodium azide in DMF gave **3**[23] in 93% yield. Coupling of lactosyl compound **1** onto the cyclodextrin in the presence of a catalytic amount of copper sulfate and sodium ascorbate as reducing agent afforded the single 1,4-triazolyl regioisomer **4** in 75% yield. For the synthesis of lactosyl-decane-azide **6** (Scheme 3), commercially available 1,10-dibromodecane was first converted into the diazide **5** by action of sodium azide in DMF in nearly quantitative yield. Mono-functionalization of **5** by lactosyl compound **1** was conducted in DMF with the $CuSO_4$/sodium ascorbate system. Using a 15 fold excess of diazide **6**, we favored a single addition of lactosyl unit, affording compound **6** with a satisfactory yield of 57%, whereas the lactosyl bolaform **7** was isolated in 28% yield. Bis-propagyl spacers **8** and **9** were prepared in respectively 71 and 78% yields from the corresponding alcohols in the presence of sodium hydride and propargyl bromide. While **8** was chosen to prepare a dimerized [2]rotaxane, the longer spacer **9** would form inclusion complexes with α-CD, allowing the preparation of oligorotaxanes with a final number of 3 to 6 cyclodextrins theoretically (1 or 2 CD per decane block). The efficiency of the coupling conditions was first evaluated with **6** and spacers **8** and **9**, in the absence of the host **4**. The lactosyl bolaforms **10** and **11** were isolated in satisfactory yields (70 and 56% respectively).



Scheme 3 *i)* NaN_3, DMF, 60 °C (96%); *ii)* **1**, $CuSO_4$/sodium ascorbate, DMF (**6** 57%, **7** 28%); *iii)* NaH, propargyl bromide (**8** 71%, **9** 78%); *iv)* **6**, $CuSO_4$/sodium ascorbate, H_2O/*i*PrOH (**10** 70%, **11** 56%).

Isolation and characterization by NMR spectroscopy of the oligorotaxanes

NMR spectroscopy was used to provide evidence of the threading processes. Fig. 1 displays the 1D proton spectrum of the guest lactosyl-decane **6** before and after addition of one equivalent of cyclodextrin **4**. Within the 15 min that followed the addition of **4**, all the protons from the triazolyl and decane units were significantly shifted downfield as a result of the inclusion and threading processes. No evolution was further observed after longer incubation times. The unsymmetrical structure of both the cyclodextrin and the axle can lead to a mixture of orientational pseudo-rotaxane isomers in which the orientation of the cyclodextrin along the axle depends on the solvent and molecular structure of the guest.[24] The structural study to determine whether a conformer is favored is under way and will be reported elsewhere in due course. For this reason, it should be emphasized that the schematic representations of **12** and polyrotaxanes are arbitrary and do not prefigure the exact cyclodextrin orientation along the chain. The ^1H NMR spectrum of **12** provided evidence that a lactosyl moiety acts as an efficient capping reagent since the H^a signal of the anomeric proton at 5.7 ppm remained unchanged upon complexation with **4** confirming that no interaction occurs with the cyclodextrin. The impossibility for the axle with lactosyl end groups to thread the cyclodextrin was also highlighted by the fact that the ^1H NMR spectrum of **7** did not display signal modification upon addition of an equimolar amount of **4** (data not shown).

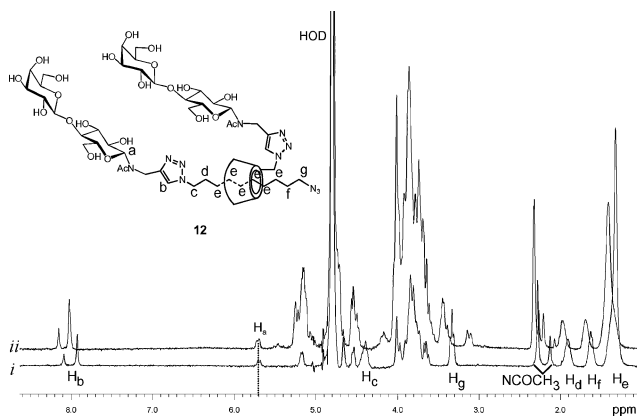
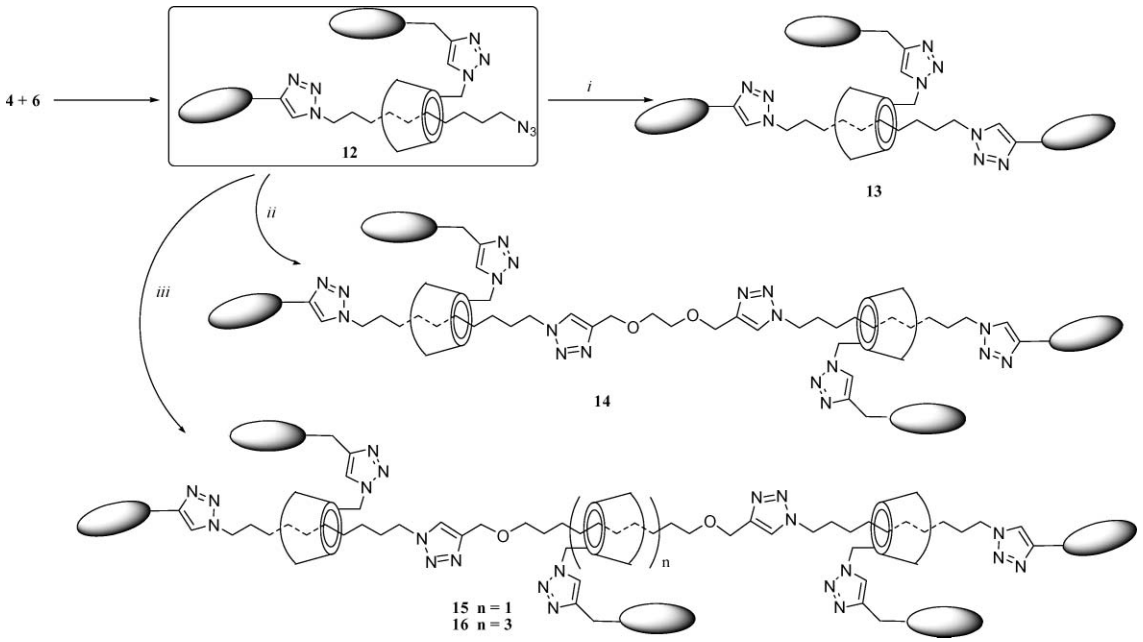


Fig. 1 ^1H NMR spectra at 298 K of guest compound **6**: *i)* alone, *ii)* in the presence of lactosyl-α-CD **4** to form the reversible complex **12**.

Cycloaddition between pseudo[2]rotaxane **12** and lactosyl compound **1** afforded rotaxane **13** which could be isolated in 54% yield and pure form after conventional flash chromatography over silica gel (Scheme 4). The inclusion of one cyclodextrin was confirmed by comparing the integral values of the ^1H NMR signals of the triazolyl moieties, the CH_3 of acetyl groups and the methylene backbone. MALDI-TOF mass spectrometry analysis confirmed the expected structure with a peak at m/z 2508 corresponding to $[M+H]^+$. A "click reaction" between 2 equivalents of the pseudo-rotaxane **12** and 1 equivalent of short spacer **8** was



Scheme 4 *i)* **1** (1.2 equiv.), $CuSO_4$/sodium ascorbate, H_2O (54%); *ii)* **8** (0.5 equiv.), $CuSO_4$/sodium ascorbate, H_2O (24%); *iii)* for **15**: **9** (0.5 equiv.), **4** (0.5 equiv.), $CuSO_4$/sodium ascorbate, H_2O (27%); for **16**: **9** (0.5 equiv.), **4** (15 equiv.), $CuSO_4$/sodium ascorbate, H_2O (67%).

expected to afford an oligorotaxane with 2 cyclodextrins. Size exclusion chromatography of the reaction mixture over a Bio-Gel P6 column allowed the removal of unthreaded cyclodextrin and the high molecular weight fraction **14** was isolated. MALDI-TOF analysis of **14** supported the presence of species with 1, 2 and 3 cyclodextrins with peaks at *m/z* 2871, 4305 and 5723 respectively (see ESI†). Although MS provided evidence for the formation of oligorotaxanes, we observed and confirmed previous findings[25,5b] that the purity and the relative distribution of each species could not be determined unambiguously by this technique. The average number of threaded cyclodextrins in **14** was determined by ^{1}H NMR spectroscopy. The presence of 6 protons from triazolyl groups and 12 protons from acetyl groups supported the presence of the rotaxane with 2 cyclodextrins as the main component and the yield for the preparation of **14** was estimated at around 24%. Oligorotaxane **15** was prepared in a one pot procedure with 1 equivalent of spacer **9**, 2 equivalents of lactosyl-decane-azide **6** and 3 equivalents of lactosyl-α-CD **4** (1 equivalent per guest block). After removal of low molecular weight building blocks by size exclusion chromatography, the high molecular weight fraction **15** was analyzed by MS. The formation of oligorotaxanes with 2, 3 and 4 cyclodextrins was confirmed with peaks at *m/z* 4403, 5864 and 7243. ^{1}H NMR integration indicated an average number of 3 threaded cyclodextrins per oligorotaxane and a yield of 27% was estimated. The same reaction, performed with 15 equivalents of **4** (5 equivalents per guest block), afforded oligorotaxane **16**. Although 6 CDs could theoretically thread, NMR analysis indicated an average number of 5 CDs which was consistent with MS analysis. In this case the excess of **4** favored the formation of the inclusion complex and the yield was increased to 67%.

By comparing the ^{1}H NMR spectra in D_2O of free lactosyl-α-CD **4** and rotaxanes **13**, **14** and **16**, a broadening of the peaks corresponding to the CD and methylene protons can be observed when the number of threaded CD molecules is increased (see Fig. 2), which may reflect the progressive reduction of the mobility of the CD molecules along the aliphatic chain. Indeed, the presence of several CD molecules likely hampers their rotational spinning around the aliphatic chain and their back and forth movement along the chain. The resulting decrease of dynamic conformational averaging thus leads to chemical shift dispersion and residual dipolar coupling and, hence, broadens peaks.

In the next step, diffusion-ordered NMR spectroscopy was used to measure the apparent diffusion coefficients (*D*) of the supramolecular species. Such measurements were expected to provide additional proof of the threaded architectures since the *D* value depends on the molecular size. DOSY data were processed by assuming a monoexponential diffusion decay and thus, a single diffusion coefficient for every proton signal in the ^{1}H NMR spectra. This assumption is not valid in regions of the spectra where the proton signals overlap, since in such regions the calculated diffusion coefficients represent some average of the components whose signals overlap. However, it has been previously reported that in the case of purified rotaxane molecules, assuming a monoexponential diffusion decay is good enough to demonstrate the threading of CD.[26]

Table 1 compares the apparent diffusion coefficient of the rotaxane-type molecules together with free α-CD, lactosyl-α-CD **4** and lactosyl-decane-azide **6**. It can be noted first that the free lactosyl-α-CD **4** exhibits a lower diffusion coefficients than natural α-CD, as might be expected on the basis of the presence of an additional disaccharide unit on the primary face. Concerning oligorotaxanes, the diffusion coefficients calculated from the proton signals of the lactosyl-α-CD **4** and decane chain are similar (data not shown), demonstrating that these components are moving together in solution, as required by a rotaxane-type structure. The measurement of the diffusion coefficients thus allowed us to confirm not only the threaded architecture but also the average number of threaded CD molecules as a decrease of the

Table 1 Diffusion coefficients of α-CD, lactosyl-α-CD **4**, lactosyl-decane-azido **6**, and oligorotaxanes in D_2O at 298 K

Compound	Concentration (M)	$D \times 10^{10}$ (m^2/s)
α-CD	0.01	2.8 (2.9)[a]
4	0.004	2.2
6	0.004	2.2
13	0.004	1.9
14	0.004	1.5
16	0.004	1

[a] The value in brackets was measured at 300 K for comparison with literature data.[27] A value of $D = 2.9 \times 10^{10}$ m^2/s was reported for α-CD in D_2O ([α-CD] = 0.01 M).

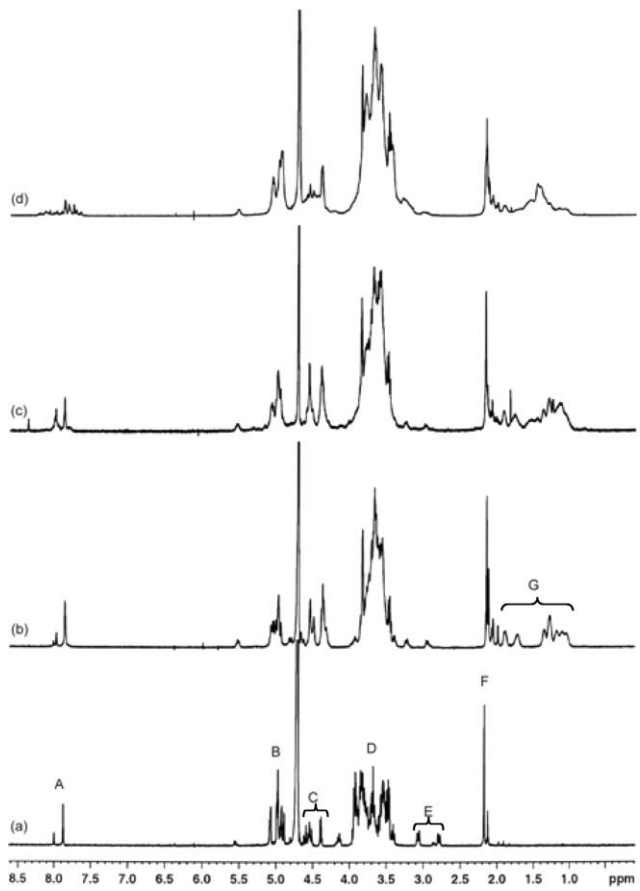


Fig. 2 ^{1}H NMR spectra (500 MHz, 298K, D_2O, 4 mM) of (a) lactosyl-α-CD **4**, (b) rotaxane **13**, (c) polyrotaxane **14** and (d) polyrotaxane **16**. A: H triazolyl moiety; B: H-1 CD; C: H-1 Gal, N-CH_2-triazolyl and H decane; D: H-2,3,4,5 lactosyl moiety and CD and H-6 lactosyl moiety; E: H-6 CD; F: CH_3-CO; G: H decane.

D values could be clearly observed for rotaxanes **13**, **14**, and **16**, respectively.

Competitive inhibition assay of asialofetuin/PNA lectin binding by ELLA

The polyvalent lactosides were next evaluated for their ability to exhibit enhanced binding to *Arachis hypogaea* peanut lectin (PNA) in a solid phase competitive assay. The abundant literature on its specificity and structure makes it appropriate for use as a model in the development of synthetic multivalent ligands. PNA is a homotetrameric protein with a molecular weight of 110 kDa and has one saccharide binding site per subunit. The crystal structure disclosed an unconventional subunit association for a legume lectin into an open quaternary structure. Multivalency of this lectin with clustered or polymeric synthetic ligands has been respectively investigated by X-ray crystallography[28] as well as by isothermal titration calorimetry.[8b]

In the enzyme linked lectin assay, the soluble oligorotaxanes inhibit the association between PNA labelled with horseradish peroxidase and a polymeric ligand fixed on the microtiter plate. The concentration needed to achieve 50% inhibition (IC_{50}) is then assumed to be inversely proportional to the lectin-saccharide binding free energy. In this work, bovin asialofetuin, a glycoprotein possessing nine terminal beta galactoside residues, was used as the immobilized ligand. Examination of literature data reveals, however, a relatively high dispersion of ELLA IC_{50} values for identical systems, which makes a direct comparison of results difficult. The presentation of ligand on microtiter plates either as part of a glycoprotein or as covalently immobilized oligosaccharides has been shown to strongly influence the outcome of the assay. Although the apparent dissociation constants (K_d) are similar for both surfaces, considerable differences have been observed in IC_{50} values.

To have a common reference, propargyl lactosylamide **1** was included as a monovalent model compound in all of the series of measurements. The IC_{50} determined for **1** using the four-parameter logistic equation (GraFit, Erithacus Software) was 0.9 mM which is consistent with data reported in the literature for this lectin.[29]

The affinities of rotaxanes **13**, **14**, **15**, **16** were evaluated and compared to monovalent lactosyl compound **1** as well as the bis-lactosyl compounds **7**, **10**, **11** (Table 2).

The unthreaded bis-lactosylated derivatives **10** and **11** displayed comparable affinities for PNA with a 6-fold increased potency over lactosyl compound **1**. In contrast, the monodecanyl compound **7** was less potent than **1**. The short spacer arm probably induced a folded conformation of the lactosyl bolaform hindering the bioavailability of the sugar epitope. The threading of one lactosyl-α-CD onto the decane chain improved the binding to a lesser extent. The similar affinities of **10**, **11** and **13** for the lectin suggest that only two of the lactosyl groups of **13** are involved in the interaction with the protein. Oligorotaxanes **14**, **15** and **16** displayed respectively 9, 18 and 30-fold increased potency. The density of lactosyl groups along the linear backbone clearly improves the lectin affinity. This multivalent presentation of

Table 2 Inhibition of asialofetuin/HRP-PNA lectin binding by the synthetic multivalent lactosyl ligands

Compound	Lactose unit per molecule	IC_{50} (mM)	Relative potency	Relative potency per lactose unit
1	1	0.9 ± 0.2	1	1
7	2	1.8 ± 0.2	0.5	0.25
10	2	0.15 ± 0.01	6	3
11	2	0.14 ± 0.01	6	3
13	3	0.18 ± 0.01	5	2
14	4	0.1 ± 0.05	9	2
15	5	0.05 ± 0.01	18	4
16	7	0.03 ± 0.01	30	4

the carbohydrate ligand apparently allows the binding of each carbohydrate unit. However the relative potency calculated per lactosyl unit is not as good as expected and the 30-fold increased potency of **16** only translates to a 4-fold improvement per lactosyl. Whereas all the threaded cyclodextrins seem to participate in binding enhancement, only a low cooperativity or multivalent effect is reached. This phenomenon may arise from insufficient mobility of the cyclodextrins. To investigate this hypothesis current work is now focused on the preparation of other polyrotaxanes with more extended polymer backbones.

Conclusion

In conclusion we have developed a new approach to prepare carbohydrate-appended oligorotaxanes with adjustable threading ratios by conjugation of α-CD/decane pseudo-rotaxanes through click chemistry. Multivalent glycoconjugates carrying up to seven lactosyl groups were isolated and characterized by MS and NMR experiments. The carbohydrate ligands inhibited PNA lectin/asialofetuin interactions in a valency-dependent manner but no significant synergic effect was observed. Efforts are in progress to extend the present methodology to polyrotaxanes of higher molecular weight and improved dynamic features.

Experimental

Mass spectrometry and optical rotation measurements

MALDI-TOF measurements were performed on a Bruker Daltonics Autoflex apparatus. ESI experiments were performed on a Waters Micromass ZQ spectrometer. Optical rotation was measured at 20 ± 2 °C with a Perkin-Elmer Model 341 digital polarimeter by using a 10 cm, 1 mL cell.

NMR spectroscopy

^{1}H NMR experiments were performed using Bruker DRX500, Bruker DRX400 and AC300 spectrometers operating at 500, 400 and 300 MHz, respectively. ^{13}C NMR spectra were recorded with Bruker DRX400 and AC300 spectrometers operating at 100 and 75 MHz, respectively. Chemical shifts (δ in ppm) are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide and deuterated chloroform were obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the scheme captions.

The DOSY experiments were carried out at 500 MHz and 298K using the bipolar pulse pair and longitudinal eddy current delay (BPP-LED) sequence.[30] Field gradient calibration was performed using the self-diffusion coefficient of water at 298K. The gradients were applied for 5 ms (δ), and the diffusion time (Δ) was varied from 100 to 200 ms. Gradient settling times were 0 ms, and the eddy current elimination duration was 500 μs. The gradients (g) were increased from 5.7 to 54.1 G/cm in 18 steps, resulting in an attenuation of the molecule resonances to approximately 10% of their original intensities. A total of 16 free induction decays containing 32K data points were collected at each gradient amplitude. The delay between each scan was 5 s, and 0 dummy scans were applied before the first data were collected. All DOSY experiments were constructed by assuming monoexponential diffusion decays for all chemical shifts.

Synthesis

Reactions were monitored by TLC using silica gel 60 F254 precoated plates (E. Merck, Darmstadt) and detection by charring with sulfuric acid solution 3:45:45 H_2SO_4–MeOH–H_2O. For flash chromatography, Merck silica gel 60 was used.

***N*-(4-*O*-β-D-Galactopyranosyl-β-D-glucopyranosyl)-3-acetamido-1-propyne (1).** A solution of lactose (1.2 g, 3,3 mmol) in neat propargylamine (3.7 mL, 67 mmol) was stirred for 48 h at room temperature. After evaporation under reduced pressure, the residue was taken up in MeOH (24 cm^3) and acetic anhydride (3.8 cm^3, 40 mmol) was added. The solution was stirred overnight, concentrated and coevaporated three times with toluene. Purification by column chromatography (CH_3CN/H_2O, 9:1) followed by freeze-drying afforded **1** as a amorphous solid (1.28 g, 91%). $[\alpha]_D^{20}$ +19 (c = 0.5, H_2O); δ_H(D_2O, 353 K, 400 MHz) 2.86 (s, 3 H), 4.17 (dd, J 10 and 8, 1 H), 4.24–4.43 (m, 8 H), 4.53 (m, 2 H), 5.07 (d, J 8, 1 H); δ_C(D_2O, 353 K, 100 MHz) 22.3, 31.1, 61.4, 62.1, 69.8, 71.1, 72.1, 73.8, 76.2, 76.4, 78.0, 79.2, 87.6, 104.0, 171.7; δ_C(CD_3N, 303 K, 75 MHz) 22.4, 30.4, 62.4, 62.5, 70.4, 71.7, 72.2, 72.8, 75.5, 77.7, 77.9, 79.2, 82.1, 82.5, 88.4, 106.2, 171.7; HRMS (ESI) calcd for $C_{17}H_{27}NO_{11}Na$ [M+Na]$^+$: 444.1482; found: 444.1478.

6-*O*-*p*-Toluenesulfonyl-α-cyclodextrin (2)[23,31]. Tosyl chloride (9.8 g, 51 mmol) was added to a stirred solution of α-cyclodextrin (5 g, 5 mmol) in pyridine (160 cm^3). After 15 min, the reaction was quenched by addition of water (30 cm^3). The solution was evaporated under reduced pressure. The crude residue was purified by column chromatography (CH_3CN/H_2O, 9:1) to give **2** (1.5 g, 26%) as an amorphous solid after freeze-drying. δ_H(D_2O, 298 K, 400 MHz) 2.52 (s, 3 H), 3.53 (t, J 9, 1 H), 3.64 (m, 12 H), 3.81–4.07 (m, 22 H), 4.42 (dd, J 11 and 6, 1 H), 4.52 (d, J 11, 1 H), 4.99 (brs, 2 H), 5.08 (brd, J 4, 4 H), 7.58 (d, J 8, 2 H), 7.90 (d, J 8, 2 H); δ_C(D_2O, 298 K, 100 MHz) 21.4, 60.4, 60.5, 60.6, 60.7, 70.0, 70.6, 71.7, 72.0, 72.2, 72.3, 72.4, 73.4, 73.6, 73.7, 81.2, 81.4, 81.5, 81.6, 101.0, 101.7, 101.8, 101.9, 102.0, 128.2, 130.7, 131.6, 147.0; MS-ESI: m/z = 1150 [M+Na]$^+$.

6-Azido-6-deoxy-α-cyclodextrin (3)[23,31]. Sodium azide (0.88 g, 14 mmol) was added to a solution of mono-tosyl-α-CD **2** (1.5 g, 1.3 mmol) in DMF (30 cm^3). The solution was stirred at 140 °C during 2 h and evaporated under reduced pressure. The crude residue was purified by column chromatography (CH_3CN/H_2O, 8:2) and freeze-dried, affording **3** as an amorphous white solid (1.2 g, 93%). MS-ESI: m/z = 1020 [M+Na]$^+$.

6-Lactosyl-α-cyclodextrin conjugate (4). Sodium ascorbate (0.04 g, 0.2 mmol) and copper sulfate (0.013 g, 0.08 mmol) were added to a solution of azido cyclodextrin **3** (0.4 g, 0.4 mmol) and lactosyl **1** (0.17 g, 0.4 mmol) in 2:1 H_2O/iPrOH (15 cm^3). The reaction mixture stirred at room temperature overnight and evaporated under reduced pressure. The crude residue was purified by column chromatography (CH_3CN/H_2O, 85:15 to 75:25) and freeze-dried to give **4** as an amorphous white solid (0.43 g, 75%). $[\alpha]_D^{20}$ +80 (c = 0.5, H_2O); δ_H(D_2O, 353 K, 400 MHz) 2.82 (s, 3 H), 3.62 (d, J 12, 1 H), 3.84 (d, J 12, 1 H), 4.02 (t, J 9, 1 H), 4.11–4.60 (m, 42 H), 4.80 (m, 2 H), 5.05 (d, J 8, 1 H), 5.23 (m, 3 H), 5.53

(m, 1 H), 5.56 (d, *J* 3, 1 H), 5.59 (d, *J* 3, 1 H), 5.64 (m, 3 H), 5.72 (d, *J* 3, 1 H), 8.50 (brs, 1 H); δ_C(D_2O, 353 K, 100 MHz) 22.2, 37.3, 52.2, 60.6, 61.6, 61.7, 62.1, 69.7, 71.2, 71.5, 72.1, 72.7, 72.8, 72.9, 73.1, 73.2, 73.3, 73.8, 74.0, 74.2, 74.4, 74.5, 75.9, 76.4, 78.0, 79.4, 81.9, 82.3, 82.4, 84.0, 88.1, 102.1, 102.4, 102.5, 104.0, 127.2, 145.9, 171.5; MALDI: $m/z = 1441$ $[M+Na]^+$; HRMS calcd for $C_{53}H_{87}N_4O_{40}$ $[M+H]^+$: 1419.4897; found: 1419.4887; calcd for $C_{53}H_{86}N_4O_{40}Na$ $[M+Na]^+$: 1441.4716; found: 1441.4726; calcd for $C_{53}H_{86}N_4O_{40}K$ $[M+K]^+$: 1457.4455; found: 1457.4489.

1,10-Diazidodecane (5)[32]. A solution of 1,10-dibromodecane (5 cm^3, 0.02 mol) and sodium azide (5.8 g, 89 mmol) in DMF (50 cm^3) was stirred overnight at 60 °C. The mixture was evaporated under reduced pressure and the residue was diluted in water (150 cm^3) and extracted with CH_2Cl_2 (2 × 150 cm^3). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc 95:5) to give **5** as a liquid (4.79 g, 96%). δ_H($CDCl_3$, 298 K, 400 MHz) 1.30 (m, 12 H), 1.58 (m, 4H), 3.23 (t, *J* 7, 4 H); δ_C($CDCl_3$, 298 K, 100 MHz) 26.8, 29.0, 29.2, 29.5, 51.6.

Lactosyl-decane-azido conjugate (6) and bis-lactosyl-decane (7). Sodium ascorbate (321 mg, 2 mmol) and copper sulfate (400 mg, 2 mmol) in water (4 cm^3 each) were added to a solution of compound **1** (0.85 g, 2 mmol) and 1,10-diazidodecane **5** (6.8 g, 30 mmol) in DMF (120 cm^3). The reaction mixture was stirred for 3h and concentrated under reduced pressure. The residue was purified by column chromatography (CH_3CN/H_2O 95:5 to 75:25) and freeze-dried, affording **6** (0.75 g, 57%) and **7** (0.6 g, 28%) as amorphous white solids. Data for **6**: $[\alpha]_D^{20}$ +12.2 ($c = 0.5$, H_2O); δ_H(D_2O, 353 K, 400 MHz) 1.89 (m, 12 H), 2.14 (quint, *J* 7, 2 H), 2.42 (brt, *J* 7, 2 H), 2.74 (brs, 3 H), 3.81 (t, *J* 7, 2 H), 4.17 (dd, *J* 10 and 8, 1 H), 4.22–4.40 (m, 10 H), 4.48 (d, *J* 12, 1 H), 4.53 (d, *J* 3, 1 H), 4.88 (brt, *J* 7, 2 H), 5.05 (d, *J* 8, 1 H), 5.19 (m, 2 H), 8.40 (brs, 1 H); δ_C(D_2O, 353 K, 100 MHz) 22.4, 27.1, 27.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.6, 37.3, 51.3, 52.3, 61.7, 62.0, 69.7, 71.2, 72.1, 73.9, 75.9, 76.4, 78.0, 79.7, 88.1, 104.1, 124.9, 146.1, 175.1; MS-MALDI: $m/z = 668$ $[M+Na]^+$, 646 $[M+H]^+$; HRMS calcd for $C_{27}H_{47}N_7O_{11}Na$ $[M+Na]^+$: 668.32313; found: 668.3227. Data for **7**: $[\alpha]_D^{20}$ +8.6 ($c = 0.5$, H_2O); δ_H(D_2O, 353 K, 400 MHz) 1.76 (m, 12 H), 2.41 (brt, *J* 7, 4 H), 2.79 (brs, 6 H), 4.16 (dd, *J* 10 and 8, 2 H) 4.22–4.38 (m, 20 H), 4.50 (dd, *J* 12 and 2, 2 H), 4.53 (d, *J* 3, 2 H), 4.93 (brt, *J* 7, 4 H), 5.04 (d, *J* 8, 2 H), 5.25 (m, 4 H), 8.45 (brs, 2 H); δ_C(D_2O, 353 K, 100 MHz) 22.3, 26.5, 28.8, 29.2, 30.1, 37.4, 51.4, 61.6, 62.0, 69.7, 71.1, 72.1, 73.9, 76.0, 76.4, 78.0, 79.5, 88.0, 104.0, 125.4, 146.3, 175.9; MS-MALDI: $m/z = 1089$ $[M+Na]^+$; HRMS calcd for $C_{44}H_{74}N_8O_{22}Na$ $[M+Na]^+$: 1089.4815; found: 1089.4805; calcd for $C_{44}H_{74}N_8O_{22}K$ $[M+K]^+$: 1105.4555; found: 1105.4591.

1,2-Bis(propargyloxy)ethane (8)[33]. NaH (2.15 g, 89 mmol) was added to a solution of ethylene glycol (1.1 g, 18 mmol) in DMF (20 cm^3) at 0 °C. After 15 min, propargyl bromide (6 cm^3, 0.05 mol) was added. The reaction mixture was stirred overnight at room temperature and evaporated under reduced pressure. The residue was diluted in water (50 cm^3) and extracted with ether (2 × 50 cm^3). The organic layer was dried over anhydrous Na_2SO_4, filtered and then evaporated under reduced pressure. Column chromatography (petroleum ether/EtOAc 80:20 to 75/25) afforded **8** as a liquid (1.76 g, 71%). δ_H($CDCl_3$, 298 K, 400 MHz) 2.41 (t, *J* 3, 2 H), 3.71 (s, 4 H), 4.20 (d, *J* 3, 4 H); δ_C($CDCl_3$, 298 K, 100 MHz) 58.4, 68.9, 74.6, 79.5.

1,10-Bis(propargyloxy)decane (9)[34]. NaH (0.86 g, 34 mmol) was added to a solution of 1,10-decanediol (1 g, 0.06 mol) in DMF (15 cm^3) at 0 °C. After 15 min, propargyl bromide (1.5 cm^3, 17 mmol) was added. The reaction mixture was stirred overnight and evaporated under reduced pressure. The residue was diluted in water (50 cm^3) and extracted with ether (2 × 50 cm^3). The dried organic layers (Na_2SO_4) were then evaporated under reduced pressure and purified by column chromatography (petroleum ether/EtOAc 98:2) to give **9** as a liquid (1.12 g, 78%). δ_H($CDCl_3$, 298 K, 400 MHz) 1.27 (m, 12 H), 1.57 (m, 4 H), 2.38 (m, 2 H), 3.48 (t, *J* 7, 4 H), 4.11 (m, 4 H); δ_C($CDCl_3$, 298 K, 100 MHz) 26.2, 29.5, 29.7, 58.2, 70.5, 74.1, 80.3; MS-ESI: $m/z = 289$ $[M+K]^+$, 268 $[M+NH_4]^+$, 251 $[M+H]^+$.

Bis-lactosyl-didecane (10). Sodium ascorbate (0.014 g, 0.07 mmol) and copper sulfate (0.012 g, 0.07 mmol) were added to a solution of **8** (0.010 g, 0.07 mmol) and **6** (0.094 g, 0.14 mmol) in 1:1 H_2O/*i*PrOH (2 cm^3). The solution was stirred overnight and the solvents evaporated under reduced pressure. The residue was purified by column chromatography (CH_3CN/H_2O 85:15 to 8:2) over silica gel followed by filtration through reverse phase C_8 cartridge to give **10** as an amorphous white solid after lyophilization (0.07 g, 70%). $[\alpha]_D^{20}$ +14.6 ($c = 0.5$, H_2O); δ_H(D_2O, 353 K, 400 MHz) 1.70 (m, 24 H), 2.36 (brs, 4 H), 2.76 (m, 6 H), 4.17 (dd, *J* 10 and 8, 2 H), 4.22–4.38 (m, 24 H), 4.49 (m, 2 H), 4.53 (d, *J* 3, 2 H), 4.87 (m, 8 H), 5.05 (d, *J* 8, 2 H), 5.16 (m, 8 H), 8.52 (m, 4 H); δ_C(D_2O, 353 K, 100 MHz) 22.3, 26.8, 29.2, 29.5, 30.3, 30.4, 37.3, 51.3, 51.4, 61.6, 62.0, 64.5, 69.7, 70.1, 72.1, 73.9, 75.9, 76.4, 78.0, 79.7, 88.1, 104.1, 125.3, 146.1, 175.5; MS-MALDI: $m/z = 1451$ $[M+Na]^+$; HRMS calcd for $C_{62}H_{104}N_{14}O_{24}Na$ $[M+Na]^+$: 1451.7246, found: 1451.7255; calcd for $C_{62}H_{103}N_{14}O_{24}Na_2$ $[M-H+2Na]^+$: 1473.7065, found: 1473.7079.

Bis-lactosyl-tridecane (11). Sodium ascorbate (0.024 g, 0.12 mmol) and copper sulfate (0.019 g, 0.12 mmol) were added to a solution of **9** (0.015 g, 0.06 mmol) and **6** (0.077 g, 0.12 mmol) in 1:1 H_2O/*i*PrOH (3 cm^3). The solution was stirred for 2 h and the solvents evaporated under reduced pressure. The residue was purified by column chromatography (CH_3CN/H_2O 9:1 to 85:15) over silica gel followed by filtration through a reverse phase C_8 cartridge (H_2O/CH_3OH 10:0 to 4:6) to give **11** as an amorphous white solid after lyophilization (0.052 g, 56%). $[\alpha]_D^{20}$ +7 ($c = 0.2$, H_2O); δ_H(D_2O, 353 K, 400 MHz) 1.75 (m, 36 H), 2.03 (m, 4 H), 2.36 (m, 8 H), 2.74 (m, 6 H), 3.98 (t, *J* 7, 4 H), 4.18 (dd, *J* 10 and 8, 2 H), 4.22–4.39 (m, 20 H), 4.48 (d, *J* 12, 2 H), 4.53 (d, *J* 3, 2 H), 4.86 (t, *J* 7, 8 H), 5.05 (d, *J* 8, 2 H), 5.09 (brs, 4 H), 5.18 (m, 4 H), 8.40 (brs, 4 H); δ_C(D_2O, 353 K, 100 MHz) 22.4, 26.8, 27.0, 29.5, 29.8, 30.1, 30.2, 30.3, 30.5, 30.8, 37.3, 51.1, 51.2, 61.6, 62.0, 64.6, 69.7, 71.1, 72.1, 73.9, 75.9, 76.4, 78.0, 79.7, 88.1, 104.1, 124.4, 124.9, 145.9, 146.0, 175.0; MS-MALDI: $m/z = 1563$ $[M+Na]^+$; HRMS calcd for $C_{70}H_{120}N_{14}O_{24}Na$ $[M+Na]^+$: 1563.8497, found: 1563.8502.

Rotaxane 13. A mixture of lactose-decane-azide **6** (0.023 g, 0.036 mmol) and cyclodextrin **4** (0.051 g, 0.036 mmol) in water (2 cm^3) was allowed to stand at room temperature for 15 min before **1** (0.018 g, 0.043 mmol), sodium ascorbate (2.8 mg, 14.4 μmol) and

copper sulfate (1.2 mg, 7.2 μmol) were added. The reaction mixture was stirred overnight and concentrated under reduced pressure. Purification by column chromatography (CH_3CN/H_2O 80:20 to 65:35) followed by freeze-drying afforded **13** as an amorphous white solid (0.048 g, 54%). $[\alpha]_D^{20}$ +33.8 (c = 0.4, H_2O); δ_H(D_2O, 353 K, 400 MHz) 1.77–2.08 (m, 12 H), 2.40 (m, 2 H), 2.59 (m, 2 H), 2.80 (s, 9 H), 3.82 (d, J 12, 1 H), 3.98 (m, 2 H), 4.14–4.55 (m, 67 H), 4.64 (m, 1 H), 4.96 (m, 2 H), 5.05 (m, 5 H), 5.23 (m, 7 H), 5.60 (d, J 3, 1 H), 5.65 (m, 5 H), 5.67 (d, J 3, 1 H), 5.73 (d, J 3, 1 H), 8.50 (brs, 3 H); δ_C(D_2O, 353K, 100 MHz) 22.2, 27.8, 28.3, 30.9, 31.0, 31.3, 31.6, 31.7, 32.0, 37.4, 51.4, 51.5, 51.7, 60.5, 60.9, 61.0, 62.0, 69.7, 71.2, 71.7, 72.1, 72.7, 72.8, 72.9, 73.1, 73.4, 73.8, 74.1, 74.7, 74.8, 75.9, 76.4, 78.0, 79.4, 79.5, 82.4, 82.5, 82.6, 84.0, 88.1, 102.4, 102.5, 103.0, 103.1, 103.2, 104.0, 125.3, 127.2, 146.0, 176.1; MS-MALDI: m/z = 2508 $[M+H]^+$; HRMS calcd for $C_{97}H_{160}N_{12}O_{62}Na_2$ $[M+2Na]^{2+}$: 1265.47657, found: 1265.4771.

Rotaxane 14. Lactose-decane-azide **6** (0.052 g, 0.08 mmol) and cyclodextrin **4** (0.113 g, 0.08 mmol) were dissolved in water (1 cm^3) and vigorously stirred for 5 min. The solution was allowed to stand for 1 h at room temperature and **8** (0.005 g, 0.04 mmol), sodium ascorbate (0.016 g, 0.08 mmol) and copper sulfate (0.013 g, 0.08 mmol) were added. The reaction was stirred overnight and the solvent was evaporated under reduced pressure. The mixture was purified on a Bio-Gel P-6 column and then desalted by gel permeation on a TKHW40F/50F (Interchrom) column (500/21.2mm) coupled to a refractive index detector and eluted with water at a flow rate of 4 cm^3/min, affording **14** as an amorphous solid after freeze-drying (38 mg, 24%). An average number of 2 threaded cyclodextrins was determined by ^{1}H NMR spectroscopy.

Oligorotaxane 15. Lactose-decane-azide **6** (0.062 g, 0.096 mmol), spacer **9** (0.01 g, 0.04 mmol) and cyclodextrin **4** (0.193 g, 0.14 mmol) were dissolved in water (1 cm^3) and vigorously stirred for 5 min. The solution was allowed to stand for 1 h at room temperature and sodium ascorbate (0.019 g, 0.096 mmol) and copper sulfate (0.015 g, 0.096 mmol) were added. The reaction was stirred overnight and the solvent was evaporated under reduced pressure. The mixture was purified on a Bio-Gel P-6 column and then desalted by gel permeation on a TKHW40F/50F (Interchrom) column (500/21.2mm) coupled to a refractive index detector and eluted with water at a flow rate of 4 mL/min affording **15** as an amorphous solid after freeze-drying (63 mg, 27%). An average number of 3 threaded cyclodextrins was determined by ^{1}H NMR spectroscopy.

Oligorotaxane 16. Lactose-decane-azide **6** (0.062 g, 0.096 mmol), spacer **9** (0.01 g, 0.04 mmol) and cyclodextrin **4** (0.850 g, 0.6 mmol) were dissolved in water (2 cm^3) and vigorously stirred for 5 min. The solution was allowed to stand for 1 h at room temperature and sodium ascorbate (0.019 g, 0.096 mmol) and copper sulfate (0.015 g, 0.096 mmol) were added. The reaction was stirred overnight and the solvent was evaporated under reduced pressure. The mixture was purified on a Bio-Gel P-6 column and then desalted by gel permeation on a TKHW40F/50F (Interchrom) column (500/21.2mm) coupled to a refractive index detector and eluted with water at a flow rate of 4 mL/min affording **16** as an amorphous solid after freeze-drying (0.23 g, 67%). An average number of 5 threaded cyclodextrins was determined by ^{1}H NMR spectroscopy.

Enzyme linked lectin assays (ELLA)

BD Falcon™ (Microtest™) microtitration plates were coated with 50 μL/well of asialofetuin (ASF) (0.02 mg/mL in PBS, pH = 7.3) and incubated for 2 h at 37 °C. The wells were then washed 4 times with 200 μL of PBS-Tween 20 0.05%. BSA solution (1% in PBS, 150 μL/well) was added to each well and incubated for 1 h at 37 °C. After washing 4 times with 200 μL of PBS-Tween 20 0.05%, the wells were filled with 50 μL of lactosylated competitors at different concentrations (in PBS 0.1 mM $MnCl_2$, 0.1 mM $CaCl_2$, 0.1% BSA) and with 50 μL of peanut lectin (PNA) (peroxidase labeled, 1.5 μg/mL in PBS 0.1 mM $MnCl_2$, 0.1 mM $CaCl_2$, 0.1% BSA) and incubated at 37 °C for 2 h. The plates were washed 4 times with 200 μL of PBS-Tween 20 0.05% and 100 μL of *o*-phenylenediamine dihydrochloride (SIGMA*FAST*™ OPD) was added. The reaction was stopped after 5 min with H_2SO_4 (3 M, 50 μL) and the optical density was measured at 490 nm.

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