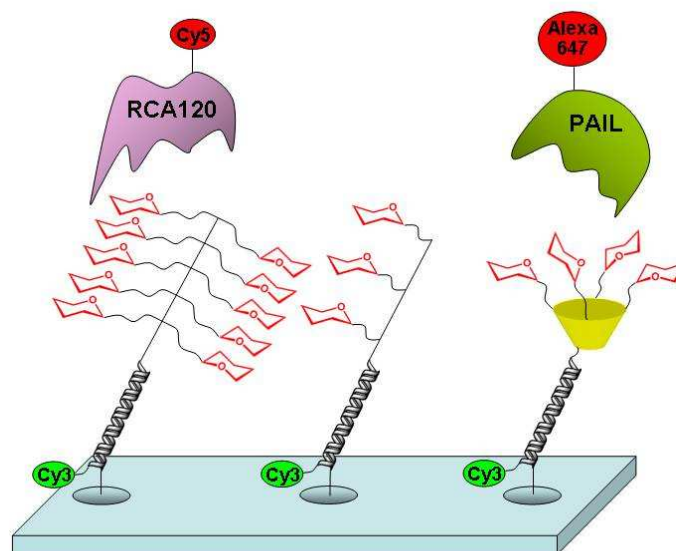CAPITOLO 4: *GLYCOCLUSTERS* **PER** *ARRAY* **DI DNA**

4.1 Introduction

Many key physiological and pathological events occur through interactions between carbohydrate and sugar-binding proteins called lectins. The host-pathogen recognitions offer many examples of such interactions. For example, the first step of the infection of influenza virus involves the adhesion of the virion to sialic acid residues present onto the surface of endothelial cells mediated by the viral hemagglutinin. A similar approach is exploited by bacteria and parasites.

Therefore, understanding the structure-function relationships of this molecular recognition process may provide access to innovative therapeutic strategies. Nevertheless, since natural carbohydrate-lectin interactions are usually weak, glycochemists have devoted many efforts in designing high affinity artificial ligands, including glycosides bearing hydrophobic aglycons[1] and, more recently, glycoclusters,[2] to take advantage of the so-called cluster effect.[3-5] It is worth noting that, in the case of glycoclusters, the affinity is not only related to the multivalency.[6] In fact,

different authors have demonstrated the importance of the spatial distribution of the glycoside residues for obtaining high affinities.[7]

Calix[4]arenes can be synthesized in various blocked conformations, thus providing a series of well-defined geometries for the display of sugar ligands. A recent study demonstrated that *N*-glycosylated calix[*n*]arenes of variable valencies and geometries are capable of distinguishing among lectins of a family.[8]

We report herein the synthesis of *C*-glycosylated calix[4]arenes in which four galactose residues are linked through an alkyl triazole tether to the upper rim of the macrocycle cavity while an azido group is present on the opposite side. Next, the calixsugars were grafted through copper-mediated azide-alkyne cycloaddition (CuAAC) on mono or dipropargyloxymethyl-propanediol moieties allowing the synthesis of oligonucleotides bearing one or two calixarene glycoclusters, respectively. Finally, their affinity for lectins PA-IL[9] and RCA 120, galactose specific lectins from *Pseudomonas aeruginosa* and *Ricinus communis*, respectively, were compared to that displayed by linear and antenna-type glycoclusters.[10, 11] The evaluation of the affinity of each glycomimic was performed on a DNA-based glycoarray platform as previously described[12] by direct fluorescence scanning and by determination of their IC_{50} values.[13]

4.2 Synthesis of *C*-galactosyl calix[4]arene clusters

We designed calix[4]arene based glycoclusters **1** and **2** (Figure 1) functionalized with a single azido group with the aim of introducing these multi-glycosylated molecules into oligonucleotides via the copper catalyzed azide-alkyne cycloaddition (click chemistry).[14] The calix[4]arenes were chosen as platforms because they can be easily derivatized at both the upper (wide) and lower (narrow) rims and they are endowed with a well organized three-dimensional architecture.[15] Among the four possible conformations (cone, partial cone, 1,2-alternate, 1,3-alternate) adopted by calix[4]arenes, the cone structure allows a spatially close arrangement of the four sugar ligands at one side of the macrocycle.
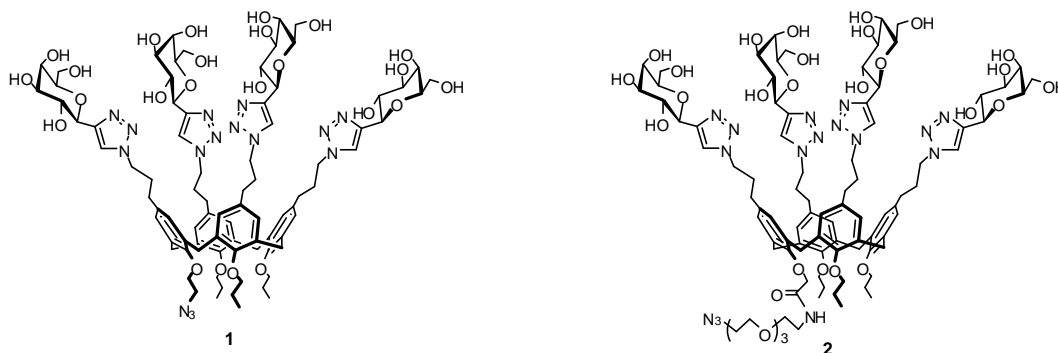

Figure 1.

The synthesis of densely substituted calix[4]arenes **1** and **2** is succinctly described. The key intermediate **7** was first targeted from the known[16] tetra-allyl-calix[4]arene **3** via calixarene chemistry (Scheme 1). Compound **7** featured four azidopropyl groups at the upper rim and only one free hydroxy group at the lower rim, while the other three hydroxy groups were protected as *O*-propyl ethers to insure a blocked cone conformation of the macrocycle. Unfortunately, the regioselective, direct protection of three phenolic hydroxy groups of calix[4]arene derivatives is a not trivial task. In fact, the synthesis of lower rim di-alkylated[17] and tetra-alkylated[18] calix[4]arenes in cone conformation is a straightforward transformation, whereas the preparation of the corresponding tri-alkyl ethers is troublesome and often requires multistep methods.[19, 20] A well known procedure[18, 21] for the synthesis of tri-*O*-alkylated calix[4]arenes involves the treatment of the tetra-hydroxy derivative with an excess of alkyl halides in the presence of BaO and $Ba(OH)_2$. However, when the tetra-allyl-calix[4]arene **3** was submitted to these reaction conditions, both the di- and tri-propyl ethers were formed in low yield (ca. 25% each). Thus, the target tetra-azidated calixarene **7** was prepared starting from **3** employing an alternative strategy to differentiate one of the four hydroxy groups at the lower rim. Taking advantage of our previous studies on the synthesis of calix[4]arene based glycoclusters,[22-25] **3** was mono-glycosylated by reaction with commercially available α-D-mannofuranose diacetonide **4** (1.1 equiv.) under Mitsunobu conditions[22] and then the three residual hydroxy groups were protected as *O*-propyl ethers to give compound **5** in 80% isolated yield (two steps). The first step of this new procedure afforded a mono-protected calix[4]arene in similar or even better yield than those registered exploiting previous methods based on the use of NaOMe[20] (70-80%), LiOH[26] (73-85%), K_2CO_3[27] (37-88%), CsF[27] (60-85%), or $(Bu_3Sn)_2O$[28] (47-80%) and very simple alkyl halides.[29]
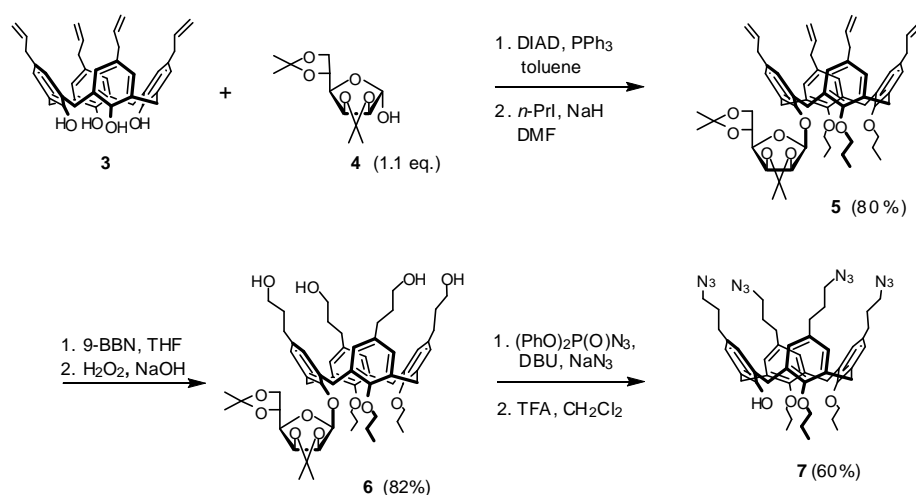
Scheme 1

With compound **5** in hand having all hydroxy groups suitably protected, the multiple hydroboration-oxidation of the four allyl groups at the upper rim was safely carried out to give the

tetrol **6**. Subsequent transformation by azidation with diphenyl phosphoryl azide and sodium azide followed by removal of the mannofuranose fragment by acidic hydrolysis afforded the tetra-azide **7**. In order to obtain the glycocluster **1** featuring the azido group in close proximity of the macrocycle cavity, the free hydroxy group of **7** was alkylated with the short *N*-Boc ethylamino chain to give **8** (Scheme 2). The fixed cone conformation of the calix[4]arene macrocycle adopted by **8** was supported by the presence in its ^{1}H NMR spectrum of signals for the equatorial and axial protons of the methylene bridges as doublets at ca. 3.1 and 4.3 ppm, respectively. Then, click chemistry was performed on the tetra-azide **8** as described in earlier reports from our laboratory.[24, 25] Accordingly, **8** was allowed to react with a stoichiometric amount (4.0 equiv.) of known[30] ethynyl 2,3,4,6-tetra-*O*-acetyl-β-D-*C*-galactopyranoside **9** in the presence of CuI and Hünig's base to give the triazole-tethered tetravalent glycosylated calix[4]arene **10**. Quite remarkably, the four click reactions on the same substrate **8** must have occurred quite efficiently as compound **10** was obtained in 75% yield, corresponding to a 93% average yield for each cycloaddition reaction. This good result is very likely due to the postulated[25] complexation of a copper(I)-triazolide intermediate with the sugar alkyne, which is therefore placed in proximity of unreacted azido groups and can readily undergo an intramolecular cycloaddition. The regioisomeric assignment of the 1,4-disubstituted 1,2,3-triazole rings was established by ^{13}C NMR spectroscopy as described in our earlier works.[24] Removal of the *N*-Boc group from **10** under acidic conditions followed by a diazo-transfer reaction[31] to convert the amino function into the azido group afforded **11**. Finally, the latter compound was converted into the target calix[4]arene **1**, bearing carbohydrate residues at the upper rim and a single azido group at the lower rim, by treatment with ammonia in methanol.
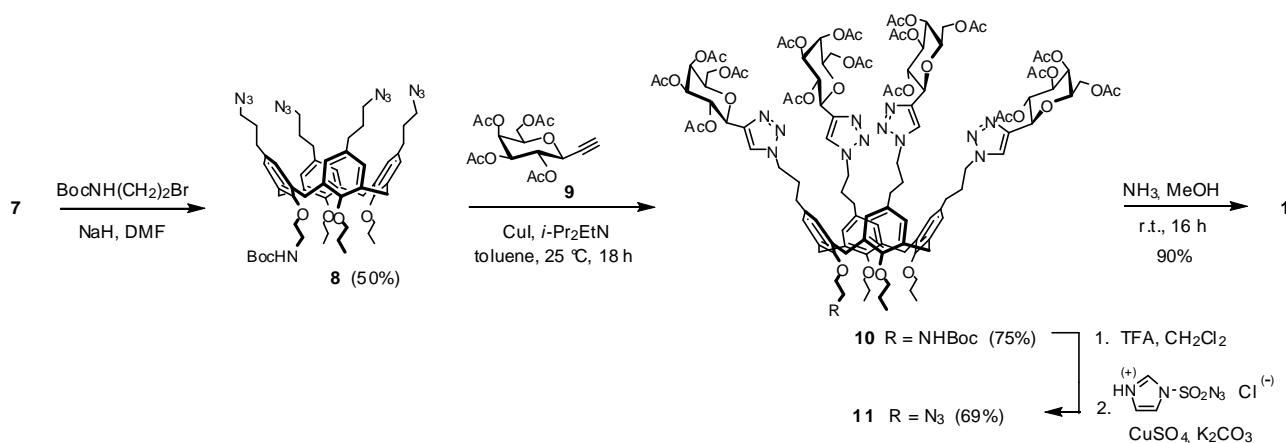
Scheme 2

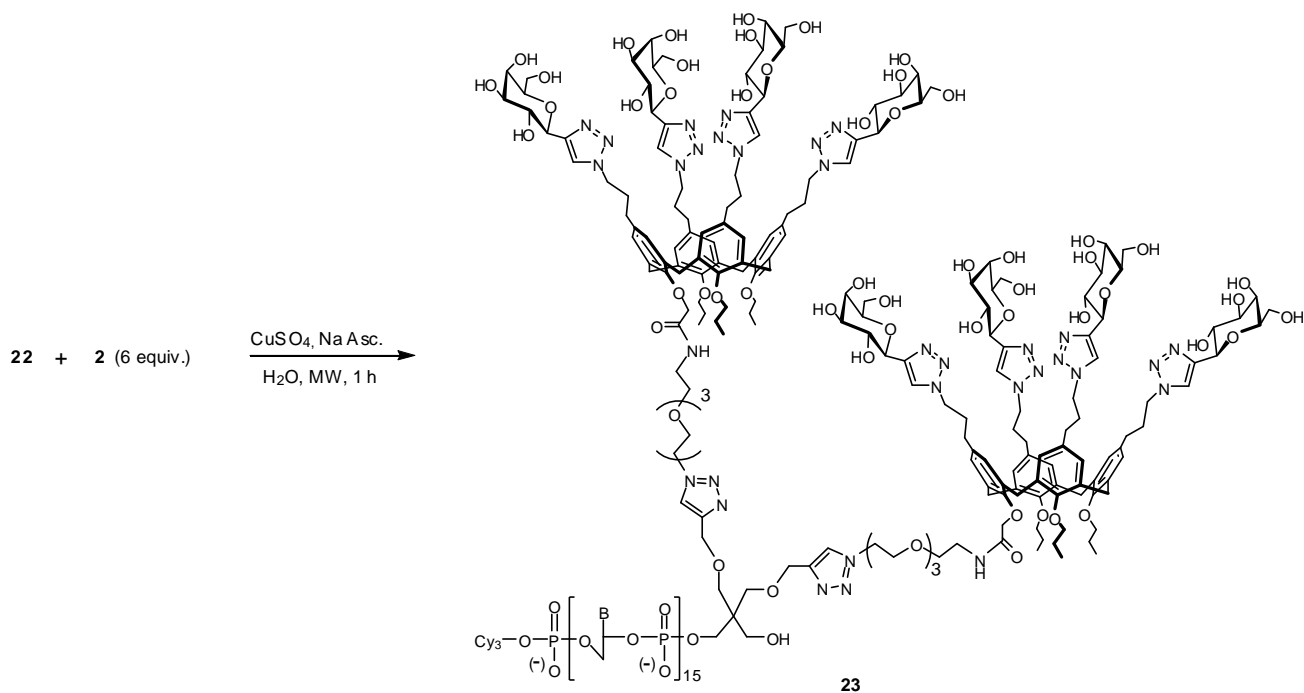To obtain the glycocluster **2**, displaying a long tether holding the azido group at the lower rim, the calix[4]arene derivative **7** was derivatized as the ethyl ester **12** (Scheme 3), which was submitted to the standard click reaction with the sugar alkyne **9** to give the glycocluster **13** in high yield (80%). Also in this case, the fixed cone conformation of the calix[4]arene scaffold and the

1,4-disubstitution pattern of the triazole rings were readily confirmed by ^{1}H and ^{13}C NMR analyses, respectively. Transesterification of **13** followed by basic hydrolysis, afforded the compound **14** wherein not only the ester group at the lower rim of the macrocycle but also those of the sugar residues had been removed. Finally the azido group was introduced in **14** by *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide (EDC) activated amidic coupling[32] of the free carboxylic group with the commercially available 11-azido-3,6,9-trioxaundecan-1-amine **15** to give the target product **2**. Compounds **1** and **2** were purified by reversed-phase column chromatography and characterized by NMR and MS analyses.

Scheme 3
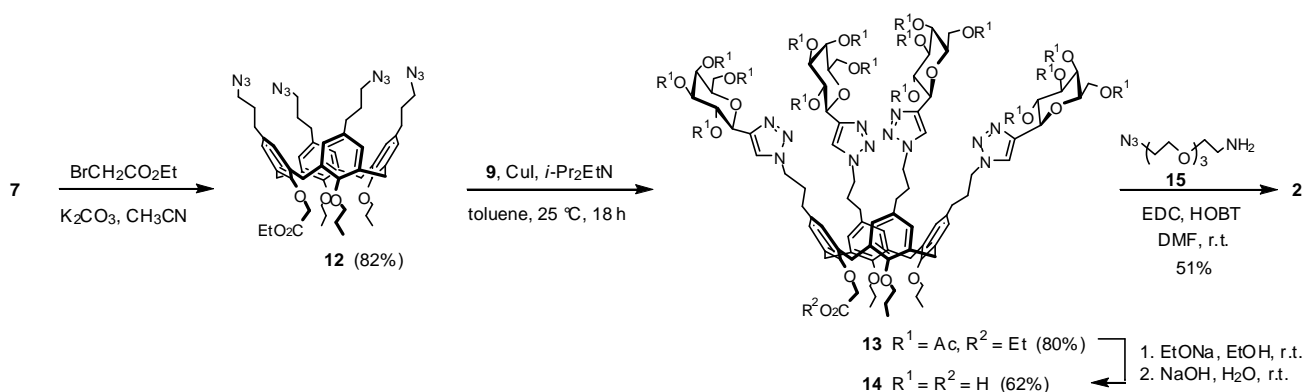
4.3 Synthesis and characterization of glycocluster-oligonucleotide hybrids.

The assembly of glycoclusters **1** and **2** with oligonucleotide chains was carried out in order to immobilize the resulting glycoconjugates on a microarray surface. To this aim, oligonucleotides were prepared by a DNA synthesizer using the standard phosphoramidite chemistry[33] on CPG (Controlled Pore Glass) solid support, a porous borosilicate material frequently used for DNA synthesis. Two different solid supported materials **16**[34] and **17**[35] (Scheme 4), displaying one and two alkyne residues respectively, were used for the synthesis of oligonucleotides **18** and **19** displaying the same sequence (CTG CCT CTG GGT TCA)[12] and labeled on the 5'-end with the fluorescent dye Cy3 (Scheme 4). Treatment of **18** and **19** with concentrated aqueous ammonia released the oligonucleotides from the solid support and removed the protecting groups (*i.e.* β-cyanoethyl, benzoyl, and isobutyryl) (Scheme 4). The oligonucleotides **20** and **21** were isolated, their purity was established by analytical HPLC, and they were characterized by MALDI- TOF mass spectrometry.

Scheme 4. Solid-phase oligonucleotide synthesis (SPOS) of 5'-fluorescently labeled DNA 3'-mono- or di-alkynes: (1) 2.5% Cl_2CHCO_2H, CH_2Cl_2; (2) phosphoramidite derivative + benzylthiotetrazole; (3) Ac_2O, N-methyl-imidazole, 2,6-lutidine; (4) 0.1 M I_2, $THF-H_2O$-pyridine. The grey ball represents long chain alkyl CPG. $B^* = A^{bz}$, C^{bz}, G^{ibu} or T and B = A, C, G or T.

Since the alkyne-functionalized oligonucleotides **20** and **21** were water soluble compounds, we planned to carry out their coupling with azide functionalized glycoclusters **1** (Scheme 5) and **2** (Scheme 6), respectively, in water using $CuSO_4$ and sodium ascorbate as the source of copper(I). Both reactions were performed under microwave irradiation[10] in order to achieve high reaction rates and therefore avoid some phosphodiester hydrolysis due to the presence of copper(I) ion.[36] The crude products from the click reactions were purified by preparative HPLC and characterized by MALDI-TOF mass spectrometry to give the final glycoconjugates **22** and **23**.

Scheme 5

Scheme 6

4.4 Preparation of DNA-based glycoarrays to probe lectin-carbohydrate interactions

Our methodology[12] for the surface immobilization of glycoconjugates on the microarrays was used as follows: a) construction of DNA chips, b) hybridization of the prepared glycocluster-oligonucleotide conjugates bearing the complementary DNA sequence and the fluorescent dye Cy3, and c) addition of the fluorescently labeled lectins Cy5 or Alexa 647 (Figure 2).
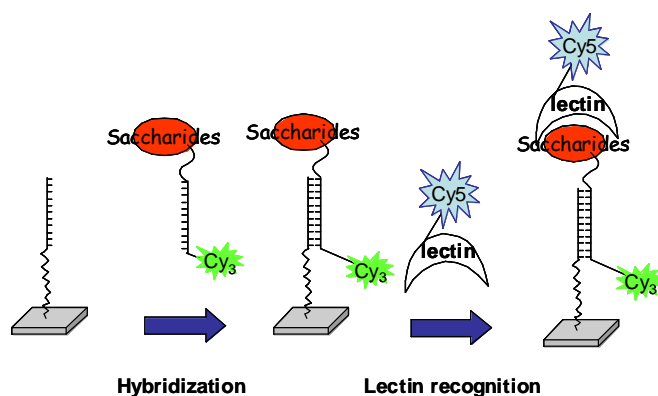

Figure 2. Schematic representation of the method used to study lectin-carbohydrate interactions.

Accordingly, first the 3'-amino-oligonucleotides (whose sequence was complementary to that of the glycoconjugates prepared) were covalently immobilized on functionalized[37] 52-well glass slides.[38] Then, the glycocluster oligonucleotide derivatives **22** and **23,** the previously tested[12] galactosyl conjugates **24a-d** (Figure 3)**,** and an additional galactosyl conjugate **25** bearing ten

galactose residues (Figure 4) were hybridized onto the chip in order to compare their lectin binding properties. Moreover, the tri-mannosyl conjugate **24e** was immobilized as a negative control.

Figure 3. Structures of previously reported[12] linear glycoconjugates **24a-e**.

Glycoconjugate **25** was synthesized as described for the synthesis of **24a-e**, but in this case the alkyne functions were introduced using a dialkyne phosphoramidite derivative.[11, 37] Thus, starting from universal solid support 1,3-propanediol, five dialkyne phosphoramidite derivatives were incorporated by phosphoramidite chemistry. The introduction of the ten galactose residues was performed by click chemistry assisted by microwaves[10] and then the oligonucleotide was synthesized and labeled with a Cy3 phosphoramidite. The desired glycoconjugate **25** was obtained after ammonia treatment and HPLC purification.

Figure 4. Structure of the deca-galactose antenna glycoconjugate **25** prepared.

Scanning of the Cy3 fluorescence signal with the Microarray scanner GenePix 4100A displayed a homogeneous hybridization of all conjugates, except for **25**, which included an oligonucleotide sequence different from that of the linear glycoconjugates **24a-e** (Figure 5). Two different Cy3 signals were observed for compound **24e** (each value was the mean of four spots on the same row): for one row, the signal intensity was similar to that observed for the compounds **24a-d**, whereas it was approximately one third for the other row. Further experiments confirmed that compound **24e** hybridizes similarly to compounds **24a-d**.
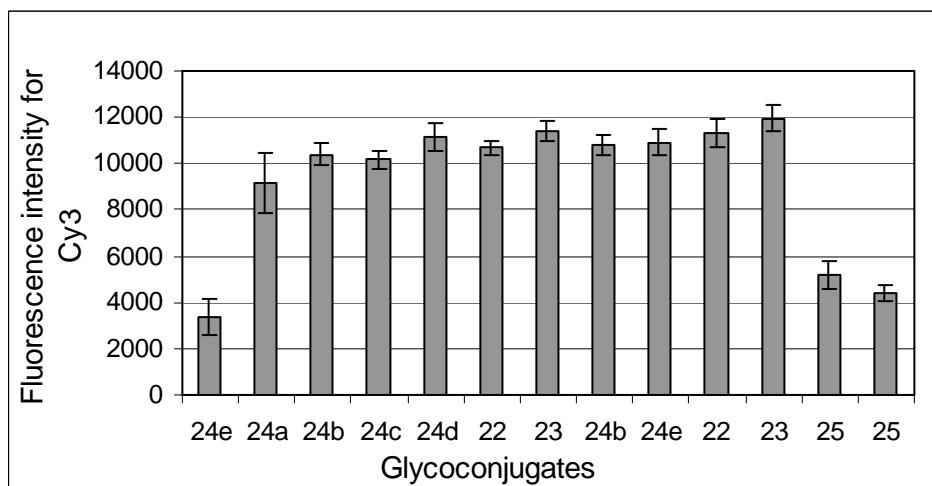
Figure 5. Fluorescence signals (a. u.) at 532 nm after hybridization monitoring the immobilization of glycoconjugates **22-25** on the DNA chip.

Next, we studied the interaction of **22** and **23** with two different galactose-binding lectins: PA-IL[9] (*Pseudomonas aeruginosa* lectin) and RCA 120 (*Ricinus communis* agglutinin). PA-IL, the first Gram-negative bacterium *Pseudomonas aeruginosa* lectin isolated,[39] features a narrow specificity spectrum for D-galactose containing molecules. This protein is constituted by four identical monomers in which four calcium-dependent binding sites are located. After hybridization, the Alexa 647-labeled PA-IL was deposited in each well at 2.8 µM concentration and, after incubation and washing of the glass slide with 0.02% Tween 20 in PBS solution, the chip was scanned at 532 and 635 nm. The fluorescence signal of each conjugate was determined as the average of the mean fluorescence signal of four spots (Figure 6). The fluorescence image of Cy3 (Figure 6, left) proved that the glycoconjugates were still present after lectin incubation, while fluorescence image of Alexa 647 (Figure 6, right) was observed as a result of the binding of PA-IL. The selectivity of PA-IL for the corresponding galactose derivatives **24a-d** and **25** was demonstrated by the Alexa 647 fluorescence signal at background level for mannose bearing glycoconjugate **24e** (Figure 7). The relative affinity of PA-IL to the glycoconjugates can be directly monitored through the intensity of the lectin's fluorescence signal.

As previously observed with RCA120,[12] glycoconjugates exhibiting three galactose residues (**24b** and **24d**) presented a better affinity for PA-IL than those with only one residue (**24a** and **24c**) due to the expected cluster effect.[4, 5] The nature of the linker (L1 and L2) between the galactose residues has little effect (**24b** *vs*. **24d**). Surprisingly, lower affinities were observed when the number of galactose moieties was increased. Thus, the deca-galactosyl conjugate (**25**) displayed a two-fold decreased fluorescence signal while they were at background level for both glycoconjugates **22** and **23** as well as for the negative control **24e**, thus indicating that **22** and **23** did not have affinity for PA-IL lectin (Figure 6-7). Actually, it was expected that PA-IL would strongly recognize **22** and **23** because they featured a triazole ring β-D-linked to the galactose moiety, a molecular motif closely related to phenyl β-D-galactoside, the most potent ligand for PA-IL. Two possible explanations for this finding can be advanced by considering that 1) steric hindrance can arise or 2) glycosylated calixarenes can sequester calcium ions, thus removing them from the binding site of the lectin. Hence, ^{1}H NMR experiments were carried out to evaluate the complexation ability of glycoclusters **1** and **2** toward calcium(II) ions. The addition of anhydrous $Ca(ClO_4)_2$ to a solution of **1** in CD_3OD led to an upfield shift (ca. 0.1 ppm) and broadening of the signals due to the four aromatic protons resonating at δ = 6.47-6.40 and to two (δ = 2.1) of the four upper rim benzyl-type methylene groups. Downfield shifts of other signals in the central part of the spectrum (δ = 4.5-3.7) were also observed. In a similar way, after treatment of glycocluster **2** with $Ca(ClO_4)_2$, its ^{1}H NMR spectrum (in CD_3OD) showed some changes. In particular, the signals of two (δ = 7.92) of the four H-5 triazole atoms were slightly shifted downfield whereas the signals of four aromatic protons (δ = 6.3) and four upper rim aliphatic protons (δ = 2.0) underwent a ca. 0.1 ppm upfield shift. To confirm the cation selectivity of the recognition process, the same experiments were repeated replacing $Ca(ClO_4)_2$ with anhydrous $NaClO_4$ in the CD_3OD solutions. In this case, the spectra of **1** and **2** did not show any significant modification, suggesting that both glycoclusters can complex Ca^{2+} ions when installed onto the oligonucleotide chains and exposed to the lectin. This conclusion contrasted with the finding that no molecular recognition was detected even by increasing the amounts of calcium ions in the tris/HCl buffer used instead of phosphate buffer. Therefore, we conclude that the calcium sequestration cannot be taken as the causing effect for the lack of binding of **22** and **23** to PA-IL lectin. At this stage a convincing explanation for that observation is open to conjectures and may rather be related to steric hindrance.

The present data showed that PA-IL recognizes more efficiently glycoclusters with linear spatial structure than those with antenna (**25**) and calixarene (**22** and **23**) structure. This result suggests that a too close proximity between the galactose moieties has a negative effect on the recognition by PA-IL.
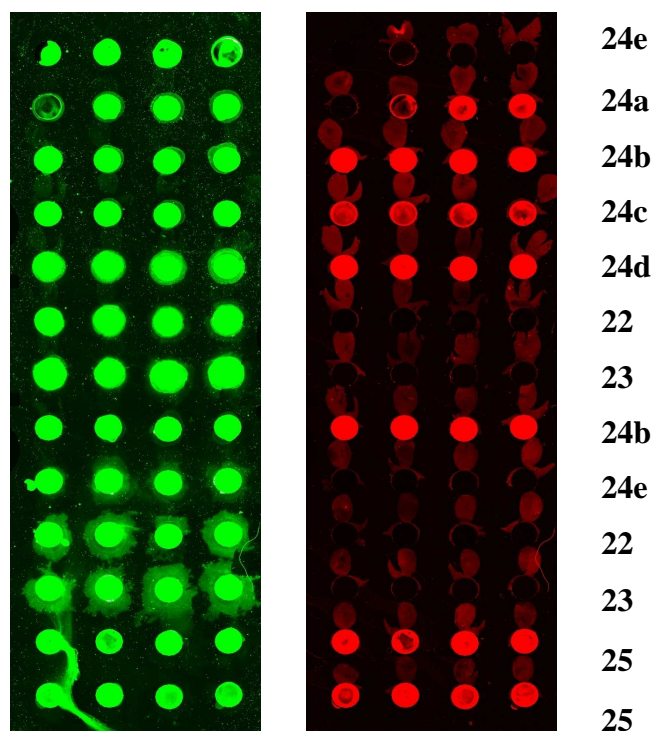
Figure 6. Fluorescence images (a. u.) recorded after incubation of immobilized glycoconjugates **22**-**25** with Alexa 647-labeled PA-IL at 532 nm (left) and at 635 nm (right).
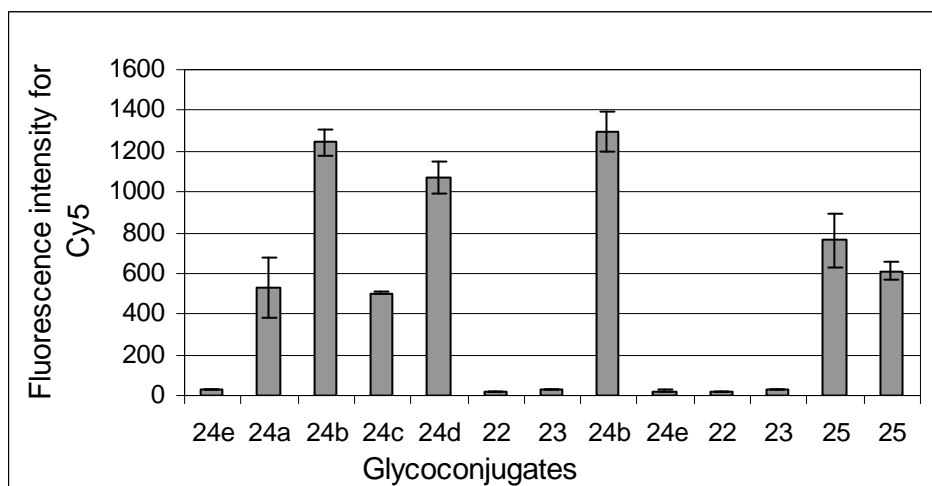

Figure 7. Fluorescence signals (a. u.) recorded at 635 nm after incubation of immobilized glycoconjugates **22**-**25** with Alexa 647-labeled PA-IL.

A similar affinity study was then performed employing RCA 120, another lectin recognizing galactose residues. RCA 120[40] is a heterodimeric protein with a MW of 60 kDa, made up of two S-S linked chains A and B, the latter containing two carbohydrate binding sites specific for galactose. Cy5-labeled RCA 120 was deposited in each well at 2 µM concentration and, after incubation and washing of the glass slide with 0.02% Tween 20 in PBS solution, it was scanned at 532 and 635 nm. The fluorescence image of Cy3 (Figure 8, left) proved that the glycoconjugates were still

present after lectin incubation, while the fluorescence image of Cy5 (Figure 8, right) was observed as a result of the binding of RCA 120 with galactose residues.
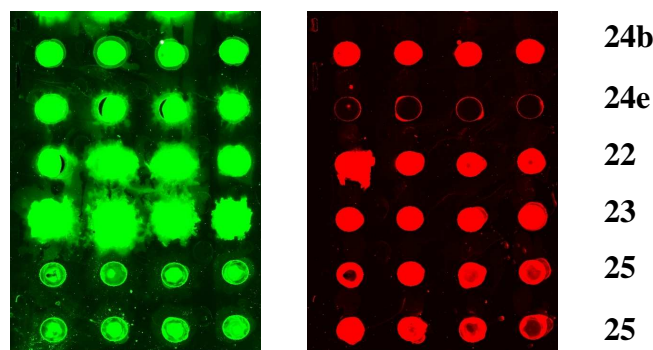

Figure 8. Fluorescence images (a. u.) recorded at 532 nm (left) and 635 nm (right) after incubation of immobilized glycoconjugates **22**, **23**, **24b**, **24e**, and **25** with Cy5-labeled RCA 120.

The fluorescence signal of each conjugate was determined as the average of the fluorescence signal of four spots. As expected, the Cy5 signal was at background level for the mannose bearing glycoconjugate **24e**, highlighting the selectivity of RCA 120 for galactose (Figure 9).
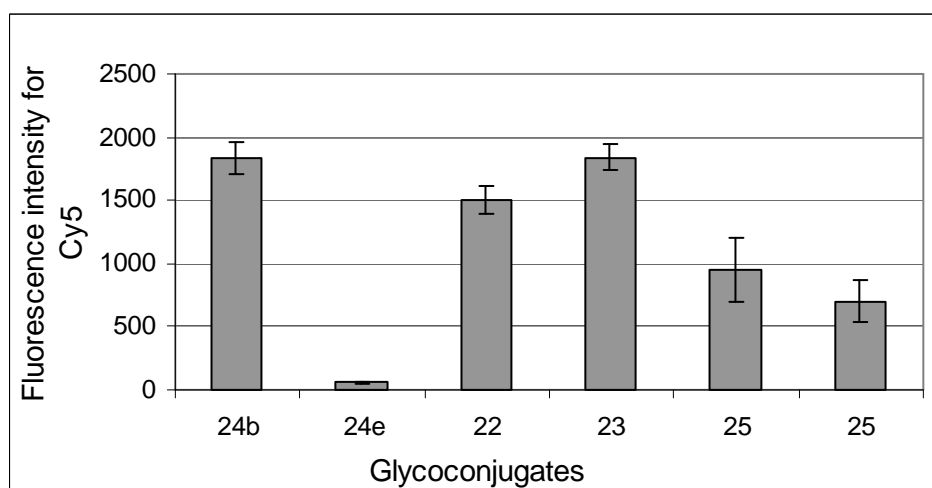

Figure 9. Fluorescence signals (a. u.) at 635 nm after incubation with RCA 120.

Interestingly and in contrast to the data obtained with PA-IL, we found that all galactosylated-glycoconjugates were able to bind RCA 120. Therefore, in this case, the use of the non-natural calixarene scaffold and triazole linker did not prevent the molecular recognition of the sugar ligand by the lectin. The glycoconjugate **23**, bearing eight galactose residues, displayed an affinity similar to that observed for **24b**, the most active compound, featuring only three galactose moieties. The ratio of the intensities of the Cy5 signals for **23** and **22** was in the 1.2-2 range (from independent experiments) while the ratio of the galactose residues linked to these glycoconjugates was of 2. Surprisingly, RCA 120 bound with a lower affinity to compound **25** bearing ten residues in an

antenna spatial arrangement. These results indicate that the three-dimensional orientation of the sugar units is more important than their number. In fact **24b**, bearing three galactose residues in a linear arrangement, was the most potent ligand among the seven glycoconjugates tested in the present study.

In order to have a quantitative analysis of the binding affinities between lectin RCA 120 and glycoconjugates **22** and **23**, the corresponding IC_{50} values were measured as previously reported[41] and compared with the IC_{50} values determined for **24a** and **24b**.[13] We determined IC_{50} values as the concentration of lactose required for the removal of 50% of RCA 120 bound to the immobilized glycoconjugates. The glycoconjugates **22** or **23** were immobilized by hybridization at the bottom of the microwells and further incubated with mixtures of Cy5-labeled RCA 120 (2 µM) containing increasing concentrations of lactose (0.05 μM to 30 mM). Fluorescence images at 532 and 635 nm were then obtained after washing of the glass slides to remove the unbound lectins (Figure 10). Each experimental point is an average value of four spots.
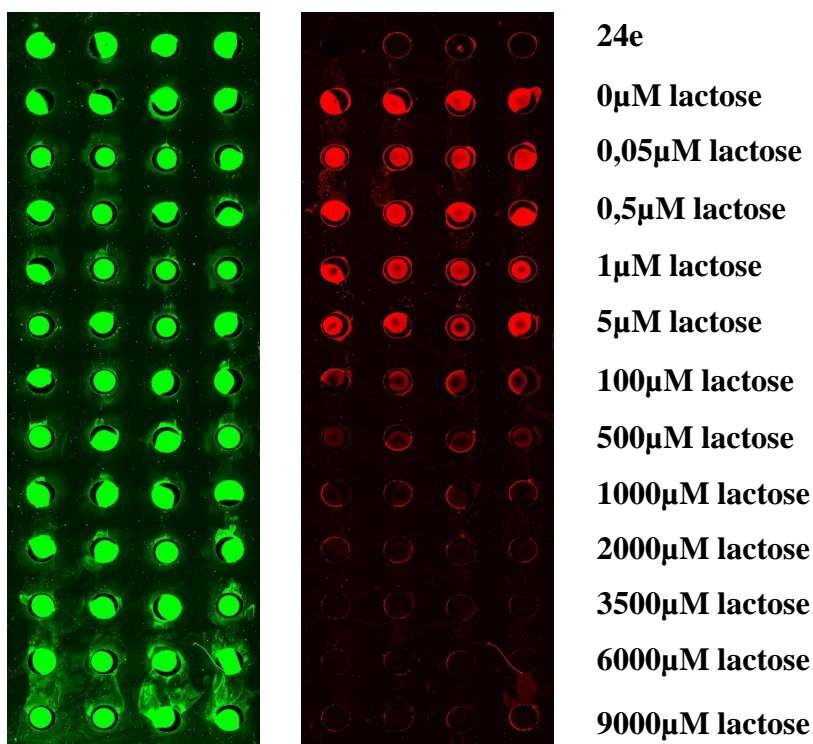

24e

0µM lactose

0,05µM lactose

0,5µM lactose

1µM lactose

5µM lactose

100µM lactose

500µM lactose

1000µM lactose

2000µM lactose

3500µM lactose

6000µM lactose

9000µM lactose

Figure 10. Typical fluorescence images recorded after incubation with Cy5-labeled RCA 120 and increasing concentrations of lactose.

The Cy5 fluorescence intensities were tabulated versus logarithmic lactose concentrations (Figure 11).
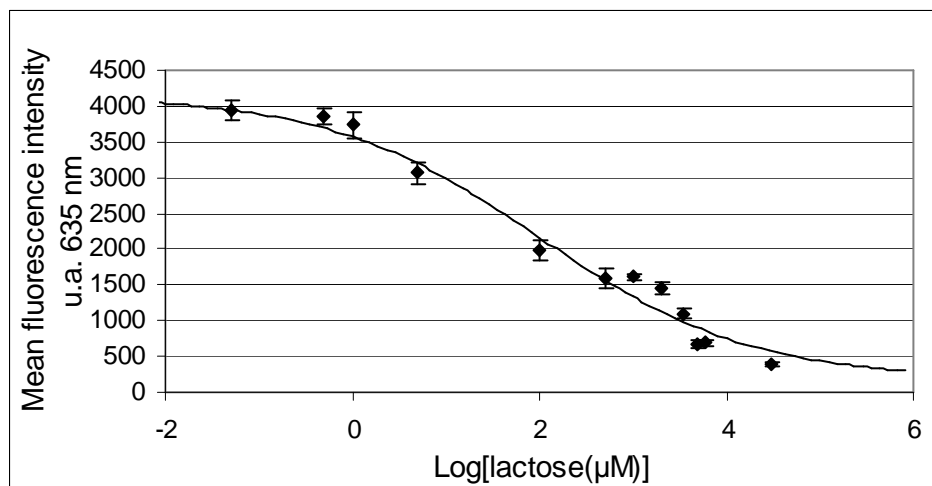
Figure 11. Competitive curves of Cy5-RCA120 with **22** immobilized on DNA chip in presence of an increasing concentration of lactose. Fluorescence intensity (a.u.) at 635 nm.
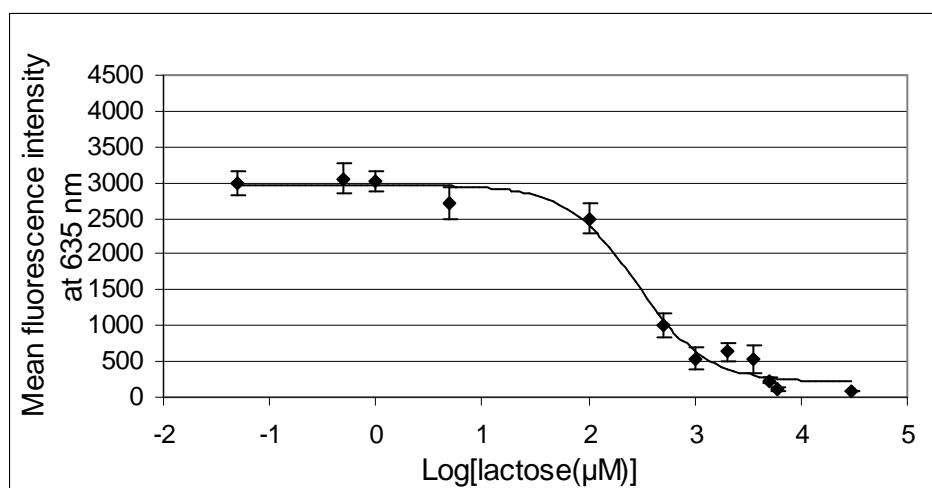

Figure 11. Competitive curves of Cy5-RCA120 with **23** immobilized on DNA chip in presence of an increasing concentration of lactose. Fluorescence intensity (a.u.) at 635 nm.

The IC_{50} values for the mono-galactose **24a**,[13] the tri-galactose **24b,**[13] the tetra-galactose **22** and the octa-galactose **23** glycoconjugates are displayed in Table 1.

Table 1. IC_{50} of glycoconjugates-RCA 120 binding.				
Glycoconjugate	Valency	IC_{50} µM	Relative potency[a]	Potency per galactose residue[b]
24a	1	5.6 ± 2.8[13]	1	1.0
24b	3	385 ± 45[13]	69	23
22	4	114 ± 14	20	5
23	8	305 ± 22	54	7
[a] Calculated as the ratio of monomer glycoconjugate **24a** to other glycoconjugates IC_{50} values. [b] Calculated as the ratio of relative potency to the number of galactose residues.				

As expected, we found that the affinity of RCA 120 increased along with the number of galactose residues due to a higher local galactose concentration. IC_{50} measurements confirmed that RCA 120 had affinity for compounds **24b** and **23** in the same range. Moreover, the IC_{50} for **23** was 2.7 times higher than that for **22** leading to an affinity per residues increased by a factor of 1.3, whereas values in the 1.2-2 range were obtained when analyzed by fluorescence of Cy5 (Figure 9). Therefore, as already reported,[5] the measured cluster effect depends on the assay conditions.

4.5 Conclusion

The present study showed that each class of galactose cluster (linear, calixarene and antenna) is recognized with different affinity by PA-IL and RCA 120 lectins. Our result showed that the spatial arrangement is more important than the number of galactose residues since the linear trivalent clusters (**24b** and **24d**) were more able to bind lectins than antenna (**25**) and calixarene (**22** and **23**) ones exhibiting 10, 4 and 8 galactose moieties, respectively. Furthermore, we showed that PA-IL is more selective than RCA 120 since galactosyl-calixarene derivatives **22** and **23** were not recognized by PA-IL. The importance of spatial arrangement of the glycoside residues in the lectin recognition process has been assessed for the asialoglycoprotein receptor.[12, 42, 43] It was showed that the trigalactose cluster with the higher distance between the sugar residues presents the optimal recognition.[39, 42]

The recognition study was performed by direct fluorescence scanning and by the determination of the IC_{50} values, both techniques leading to similar results.

The carbohydrate microarray used in this study required only minute amounts of material. The synthesis of the glycoconjugates could therefore be performed on a rather small scale but the miniaturization through the microarray technology provided the biological data for a complete study.

4.6 Experimental Section

All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Anhydrous solvents were dried over standard drying agents[44] and freshly distilled prior to use. Reactions were monitored by TLC on silica gel 60 F_{254} with detection by charring with sulfuric acid. Flash column chromatography[45] was performed on silica gel 60 (40-63 μm). Melting points were determined with a capillary apparatus. Optical rotations were measured at 20 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in deg·mL·g^{-1}·dm^{-1}. ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR spectra (75 and 100 MHz) were recorded from CDCl$_3$ solutions at room temperature unless otherwise specified. Peak assignments were aided by ^{1}H-^{1}H COSY and gradient-HMQC

experiments. In the ^{1}H NMR spectra reported below, the *n* and *m* values quoted in geminal or vicinal proton-proton coupling constants $J_{n,m}$ refer to the number of the corresponding sugar protons.

High performance liquid chromatography (HPLC) analyses and purifications were performed on a Waters-Millipore instrument equipped with a Rheodyne injector, a 600S controller and a model 996 photodiode array detector. For analyses, a reverse phase C18 Nucleosil (5 μm) column (150 x 4.6 mm; Macherey-Nagel, Germany) was used at a flow rate of 1 mL·min^{-1} using a linear gradient of acetonitrile 5% to 60% in 0.05 M aqueous triethylammonium acetate (pH 7) for 25 min. For purifications, a reverse phase C18 Delta-Pak (15 μm) column (7.8 x 300 mm; Waters, Japan) was used at a flow rate of 2 mL·min^{-1} using a linear gradient of acetonitrile 24% to 48% in 0.05 M aqueous triethylammonium acetate (pH 7) for 25 min.

MALDI-TOF mass spectra were recorded on a Voyager mass spectrometer (Perspective Biosystems, Framingham, MA) equipped with a nitrogen laser. MALDI conditions: accelerating voltage 24 kV; guide wire 0.05% of the accelerating voltage; grid voltage 94% of the accelerating voltage; delay extraction time 500 ns. 1 μL of sample was mixed with 5 μL of a saturated solution of hydroxypicolinic acid (HPA) in acetonitrile-water (1:1, v/v) containing 10% of ammonium citrate, then a few beads of DOWEX 50W-X8 ammonium sulfonic acid resin were added. 1 μL of the above mixture was placed on a plate and dried at room temperature and pressure.

5,11,17,23-Tetraallyl-25-(2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranosyl)-26,27,28-tripropoxy-calix[4]arene (5).
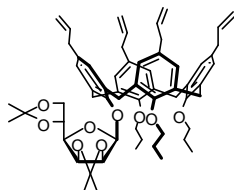
To a stirred solution of tetrol **3** (500 mg, 0.86 mmol) and triphenylphosphine (335 mg, 1.28 mmol) in anhydrous toluene (10 mL) was added diisopropyl azodicarboxylate (250 μL, 1.28 mmol) and, after 15 min, hemiacetal **4** (243 mg, 0.94 mmol). Stirring was continued for an additional 1.5 h, then the mixture was concentrated. To a stirred solution of the residue in DMF (20 mL) was added NaH (0.30 g, 7.7 mmol, of a 60% dispersion in oil) and, after 10 min, 1-iodopropane (0.75 mL, 7.7 mmol). The mixture was stirred at room temperature for 1.5 h, then diluted with CH₃OH (0.5 mL) and, after 30 min, diluted with 1 M phosphate buffer at pH 7 (80 mL) and extracted with Et₂O (2 x 100 mL). The combined organic phases were dried (Na₂SO₄) and concentrated. The residue was eluted from a column of silica gel with CH₂Cl₂-cyclohexane (from 1:1 to 3:1) to give **5** (652 mg, 80%) as a syrup; $[\alpha]_D$ = -19.2 (*c* 0.6, CHCl₃). ¹H NMR (400 MHz): δ 6.98-6.92 (m, 4H, Ar), 6.08 (ddt, 2H, *J* = 6.0, 12.0, 16.5 Hz, 2 CH₂=C*H*CH₂), 6.05-5.96 (m, 4H, Ar), 5.59-5.48 (m, 2H, 2 CH₂=C*H*CH₂), 5.12-5.01 (m, 4H, 2 C*H*₂=CHCH₂), 4.87-4.75 (m, 4H, 2 C*H*₂=CHCH₂), 4.78 (dd, 1H, $J_{1,2}$ = 3.0, $J_{2,3}$ = 6.3 Hz, H-2), 4.71 (dd, 1H, $J_{3,4}$ = 4.0 Hz, H-3), 4.69 (d, 1H, H-1), 4.63 and 3.01 (2d, 2H, *J* = 13.8 Hz, ArC*H*₂Ar), 4.48-4.43 (m, 1H, H-5), 4.42 (d, 1H, *J* = 13.0 Hz, H$_{ax}$ of ArC*H*₂Ar), 4.40 (d, 1H, *J* = 13.0 Hz, H$_{ax}$ of ArC*H*₂Ar), 4.35 (d, 1H, *J* = 13.0 Hz, H$_{ax}$ of ArC*H*₂Ar), 4.23 and 3.88 (2dt, 2H, *J* = 5.5, 11.3 Hz, CH₃CH₂C*H*₂O), 4.14-3.97 (m, 4H, 2 H-6, CH₃CH₂C*H*₂O), 3.65-3.57 (m, 2H, CH₃CH₂C*H*₂O), 3.42-3.36 (m, 5H, H-4, 2 CH₂=CHC*H*₂), 3.08 (d, 1H, *J* = 13.0 Hz, H$_{eq}$ of ArC*H*₂Ar), 3.07 (d, 1H, *J* = 13.0 Hz, H$_{eq}$ of ArC*H*₂Ar), 3.05 (d, 1H, *J* = 13.0 Hz, H$_{eq}$ of ArC*H*₂Ar), 2.77-2.72 (m, 4H, 2 CH₂=CHC*H*₂), 2.12-1.98 (m, 4H, 2 CH₃C*H*₂CH₂O), 1.94-1.82 (m, 2H, CH₃C*H*₂CH₂O), 1.60, 1.44, 1.39 (3s, 12H, 4 Me), 1.10 (t, 3H, *J* = 7.0 Hz, C*H*₃CH₂CH₂O), 0.87 (t, 6H, *J* = 7.0 Hz, 2 C*H*₃CH₂CH₂O). ¹³C NMR (75 MHz): δ 156.2 (C), 156.1 (C), 153.6 (C), 152.4 (C), 138.75 (CH), 138.66 (CH), 138.0 (CH), 137.8 (CH), 137.1 (C), 136.9 (C), 136.8 (C), 136.3 (C), 134.0 (C), 133.8 (C), 132.9 (C), 132.7 (C), 131.8 (C), 129.4 (CH), 129.0 (CH), 127.3 (CH), 127.1 (CH), 115.0 (CH₂), 114.9 (CH₂), 114.8 (CH₂), 114.6 (CH₂), 112.9 (C), 109.0 (C), 106.6 (CH), 79.1 (CH), 78.9 (CH), 76.2 (CH), 73.3 (CH), 77.1 (CH₂), 76.5 (CH₂), 76.4 (CH₂), 66.6 (CH₂), 39.5 (CH₂), 39.35 (CH₂), 39.30 (CH₂), 31.7 (CH₂), 31.0 (CH₂), 30.9 (CH₂), 26.9 (CH₃), 26.1 (CH₃), 25.5

(CH$_3$), 24.7 (CH$_3$), 23.5 (CH$_2$), 22.7 (CH$_2$), 10.8 (CH$_3$), 10.1 (CH$_3$), 9.7 (CH$_3$). ESI MS (953.25): 971.7 (M + NH$_4^+$).

5,11,17,23-Tetrakis(3-hydroxypropyl)-25-(2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranosyl)-26,27,28-tripropoxy-calix[4]arene (6).
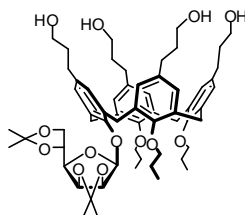

To a cooled (0 °C), stirred solution of **5** (950 mg, 1.00 mmol) in anhydrous THF (10 mL) was added dropwise 9-boracyclo[3.3.1]nonane (32 mL, 16.0 mmol, of a 0.5 M solution in hexane). The solution was allowed to reach room temperature in 3 h, then cooled to 0 °C and slowly diluted with 10 M NaOH (1 mL) and 35% H_2O_2 (3 mL). The mixture was stirred at room temperature for 15 min and then warmed to 60 °C. Stirring was continued for an additional 2 h, then the mixture was cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (80 mL), concentrated to remove the organic solvents, and extracted with AcOEt (2 x 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of Sephadex LH 20 with 1:1 CH_2Cl_2-MeOH to give **6** (840 mg, 82%) as a white solid. An analytical sample was obtained by preparative TLC (silica gel 60, 0.5 mm, 10:1 $CHCl_3$-CH_3OH); $[\alpha]_D$ = -15.6 (*c* 0.3, $CHCl_3$). ^{1}H NMR (300 MHz): δ 7.01-6.95 (m, 4H, Ar), 6.18-6.07 (m, 4H, Ar), 4.82 (dd, 1H, $J_{1,2}$ = 3.0, $J_{2,3}$ = 5.6 Hz, H-2), 4.73 (dd, 1H, $J_{3,4}$ = 3.9 Hz, H-3), 4.71 (d, 1H, H-1), 4.66 and 3.03 (2d, 2H, *J* = 13.5 Hz, ArC*H*₂Ar), 4.50-4.45 (m, 1H, H-5), 4.46 (d, 1H, *J* = 13.0 Hz, H_{ax} of ArC*H*₂Ar), 4.43 (d, 1H, *J* = 13.0 Hz, H_{ax} of ArC*H*₂Ar), 4.38 (d, 1H, *J* = 13.0 Hz, H_{ax} of ArC*H*₂Ar), 4.26 and 3.89 (2dt, 2H, *J* = 5.9, 11.0 Hz, CH₃CH₂C*H*₂O), 4.16-3.98 (m, 4H, 2 H-6, CH₃CH₂C*H*₂O), 3.64 (t, 6H, *J* = 7.0 Hz, CH₃CH₂C*H*₂O, 2 CH₂CH₂C*H*₂OH), 3.49 (t, 4H, *J* = 7.0 Hz, 2 CH₂CH₂C*H*₂OH), 3.42 (dd, 1H, $J_{4,5}$ = 6.8 Hz, H-4), 3.10 (d, 1H, *J* = 13.0 Hz, H_{eq} of ArC*H*₂Ar), 3.09 (d, 1H, *J* = 13.0 Hz, H_{eq} of ArC*H*₂Ar), 3.07 (d, 1H, *J* = 13.0 Hz, H_{eq} of ArC*H*₂Ar), 2.72 (t, 4H, *J* = 6.5 Hz, 2 C*H*₂CH₂CH₂OH), 2.19-1.98 (m, 16H, 4 CH₂C*H*₂CH₂OH, 2 C*H*₂CH₂CH₂OH, 2 CH₃C*H*₂CH₂O), 1.90 (tq, 2H, *J* = 7.0, 7.0 Hz, CH₃C*H*₂CH₂O), 1.60, 1.42, 1.40 (3s, 12H, 4 Me), 1.09 (t, 3H, *J* = 7.0 Hz, C*H*₃CH₂CH₂O), 0.89 (t, 6H, *J* = 7.0 Hz, 2 C*H*₃CH₂CH₂O). ^{13}C NMR (75 MHz): δ 155.9 (C), 155.8 (C), 153.2 (C), 152.0 (C), 137.1 (C), 137.0 (C), 136.8 (CH), 136.3 (C), 135.8 (C), 134.8 (C), 134.6 (C), 133.8 (C), 132.7 (C), 132.5 (C), 131.6 (C), 129.0 (CH), 128.7 (CH), 127.0 (CH), 126.9 (CH), 126.8 (CH), 112.8 (C), 109.0 (C), 106.7 (CH), 79.1 (CH), 78.9 (CH), 77.4 (CH₂), 76.9 (CH₂), 76.5 (CH₂), 76.1 (CH), 73.3 (CH), 66.6 (CH₂), 62.45 (CH₂), 62.39 (CH₂), 62.2 (CH₂), 62.1 (CH₂), 34.2 (CH₂), 33.64 (CH₂), 33.57 (CH₂), 31.6 (CH₂), 31.1 (CH₂), 30.8 (CH₂), 26.9 (CH₃), 26.1 (CH₃), 25.5 (CH₃), 24.6

(CH$_3$), 23.5 (CH$_2$), 22.7 (CH$_2$), 10.8 (CH$_3$), 10.2 (CH$_3$), 9.8 (CH$_3$). ESI MS (1025.33): 1048.2 (M + Na$^+$).

**5,11,17,23-Tetrakis(3-azidopropyl)-25-hydroxy-26,27,28-tripropoxy-calix[4]arene (7).**
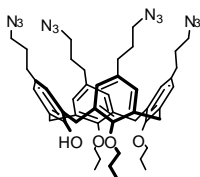
A mixture of calixarene tetrol **6** (774 mg, 0.76 mmol), sodium azide (390 mg, 6.00 mmol), diphenyl phosphoryl azide (980 μL, 4.53 mmol), 1,8-diazabicyclo[5.4.0.]undec-7-ene (450 μL, 3.02 mmol), and anhydrous DMF (4 mL) was stirred at 110 °C for 16 h then cooled to room temperature, diluted with Et_2O (100 mL), washed with H_2O (20 mL), dried (Na_2SO_4), and concentrated. A solution of the residue in 2:1 CH_2Cl_2-CF_3CO_2H (6 mL) was kept at room temperature for 3 h, then concentrated. The residue was eluted from a column of silica gel with 2:1 CH_2Cl_2-cyclohexane to give **7** (397 mg, 60%) as a syrup. ^{1}H NMR (300 MHz): δ 6.99 (s, 2H, Ar), 6.90 (s, 2H, Ar), 6.25-6.20 (m, 4H, Ar), 4.84 (s, 1H, OH), 4.38 and 3.36 (2d, 4H, J = 13.0 Hz, 2 ArC*H*$_2$Ar), 4.35 and 3.24 (2d, 4H, J = 13.6 Hz, 2 ArC*H*$_2$Ar), 3.86-3.80 (m, 2H, CH_3CH_2C*H*$_2$O), 3.73 (t, 4H, J = 7.0 Hz, 2 CH_3CH_2C*H*$_2$O), 3.33 (t, 2H, J = 7.0 Hz, CH_2CH_2C*H*$_2$N$_3$), 3.30 (t, 2H, J = 7.0 Hz, CH_2CH_2C*H*$_2$N$_3$), 2.96 (t, 4H, J = 6.8 Hz, 2 CH_2CH_2C*H*$_2$N$_3$), 2.75 (t, 2H, J = 7.0 Hz, C*H*$_2$CH$_2$CH$_2$N$_3$), 2.68 (t, 2H, J = 7.0 Hz, C*H*$_2$CH$_2$CH$_2$N$_3$), 2.37-2.24 (m, 2H, CH_3C*H*$_2$CH$_2$O), 2.14 (t, 4H, J = 7.5 Hz, 2 C*H*$_2$CH$_2$CH$_2$N$_3$), 2.04-1.82 (m, 12H, 2 CH$_2$C*H*$_2$CH$_2$N$_3$, 2 CH_3C*H*$_2$CH$_2$O), 1.45 (tt, 4H, J = 7.0, 7.0 Hz, 2 CH$_2$C*H*$_2$CH$_2$N$_3$), 1.13 (t, 6H, J = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.94 (t, 3H, J = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 154.9 (C), 152.8 (C), 151.4 (C), 136.8 (C), 135.1 (C), 134.8 (C), 133.2 (C), 132.6 (C), 131.2 (C), 130.7(C), 129.0 (CH), 128.3 (CH), 127.7 (CH), 127.6 (CH), 77.6 (CH$_2$), 76.6 (CH$_2$), 50.6 (CH$_2$), 50.3 (CH$_2$), 32.1 (CH$_2$), 31.9 (CH$_2$), 31.8 (CH$_2$), 30.8 (CH$_2$), 30.7 (CH$_2$), 30.5 (CH$_2$), 30.4 (CH$_2$), 23.4 (CH$_2$), 22.2 (CH$_2$), 10.8 (CH$_3$), 9.5 (CH$_3$). ESI MS (883.10): 901.4 (M + NH$_4^+$).

5,11,17,23-Tetrakis(3-azidopropyl)-25-(2-*t*-butoxycarbonyl-aminoethoxy)-26,27,28-tripropoxy-calix[4]arene (8).
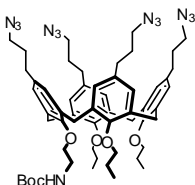

To a stirred solution of alcohol **7** (110 mg, 0.12 mmol) in anhydrous DMF (2 mL) was added NaH (15 mg, 0.37 mmol, of a 60% dispersion in oil) and, after 15 min, 1-bromo-2-*t*-butoxycarbonylaminoethane (84 mg, 0.38 mmol). The mixture was stirred at 50 °C for 18 h, then cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (15 mL) and extracted with Et_2O (2 x 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 1:1 CH_2Cl_2-cyclohexane (containing 0.3% of Et_3N) to give **8** (64 mg, 50%) as a syrup; ^{1}H NMR (400 MHz): δ 6.98 (bt, 1H, *J* = 6.0 Hz, NH), 6.85 (s, 4H, Ar), 6.08 (s, 2H, Ar), 6.05 (s, 2H, Ar), 4.40 and 3.06 (2d, 4H, *J* = 12.8 Hz, 2 ArC*H*$_2$Ar), 4.25 and 3.09 (2d, 4H, *J* = 13.2 Hz, 2 ArC*H*$_2$Ar), 3.99 (t, 2H, *J* = 4.5 Hz, OC*H*$_2$CH$_2$N), 3.98-3.92 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.86-3.79 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.77-3.71 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.64 (dt, 2H, *J* = 4.5, 6.0 Hz, OCH$_2$C*H*$_2$N), 3.28 (t, 4H, *J* = 6.8 Hz, 2 CH$_2$CH$_2$C*H*$_2$N$_3$), 3.04 (t, 4H, *J* = 7.2 Hz, 2 CH$_2$CH$_2$C*H*$_2$N$_3$), 2.67 (t, 4H, *J* = 6.8 Hz, 2 C*H*$_2$CH$_2$CH$_2$N$_3$), 2.07 (t, 4H, *J* = 7.2 Hz, 2 C*H*$_2$CH$_2$CH$_2$N$_3$), 1.93 (tt, 4H, *J* = 6.8, 6.8 Hz, 2 CH$_2$C*H*$_2$CH$_2$N$_3$), 1.87-1.76 (m, 6H, 3 CH$_3$C*H*$_2$CH$_2$O), 1.52 (s, 9H, *t*-Bu), 1.47 (tt, 4H, *J* = 7.2, 7.2 Hz, 2 CH$_2$C*H*$_2$CH$_2$N$_3$), 0.94 (t, 6H, *J* = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.86 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 156.5 (C), 155.3 (C), 154.9 (C), 153.1 (C), 136.3 (C), 136.2 (C), 134.8 (C), 134.1 (C), 133.9 (C), 133.2 (C), 133.0 (C), 129.0 (CH), 128.7 (CH), 127.6 (CH), 127.4 (CH), 78.9 (C), 77.2 (CH$_2$), 76.5 (CH$_2$), 75.2 (CH$_2$), 50.6 (CH$_2$), 50.5 (CH$_2$), 41.0 (CH$_2$), 32.0 (CH$_2$), 31.0 (CH$_2$), 30.9 (CH$_2$), 30.6 (CH$_2$), 30.4 (CH$_2$), 28.7 (CH$_3$), 22.7 (CH$_2$), 22.5 (CH$_2$), 10.2 (CH$_3$). ESI MS (1026.28): 1044.7 (M + NH$_4^+$).

5,11,17,23-Tetrakis{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-(2-*t*-butoxycarbonyl-aminoethoxy)-26,27,28-tripropoxy-calix[4]arene (10).
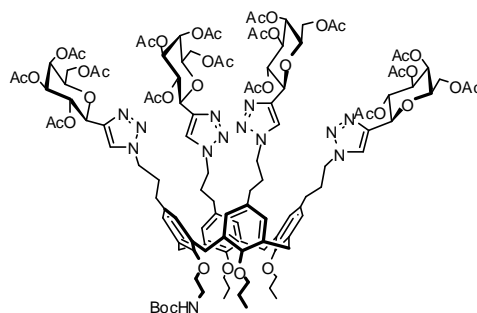

A mixture of calix[4]arene tetra-azide **8** (34 mg, 0.03 mmol), ethynyl *C*-galactoside **9** (47 mg, 0.12 mmol), freshly distilled *N,N*-diisopropylethylamine (105 μL, 0.60 mmol), CuI (6 mg, 0.03 mmol), and anhydrous toluene (1 mL) was stirred in the dark at room temperature for 18 h, then concentrated. The residue was eluted from a column of silica gel with AcOEt (containing 0.3% of Et$_3$N) to give **10** (55 mg, 75%), a white foam; $[\alpha]_D$ = -8.8 (*c* 0.7, CHCl$_3$). ^{1}H NMR (400 MHz): δ 7.72 (s, 1H, H-5 Tr.), 7.71 (s, 1H, H-5 Tr.), 7.58 (s, 2H, 2 H-5 Tr.), 6.90 (bt, 1H, *J* = 5.5 Hz, NH), 6.84 (s, 4H, Ar), 6.07 (s, 2H, Ar), 6.04 (s, 2H, Ar), 5.53-5.51 (4dd, 4H, 4 H-4), 5.47-5.38 (4dd, 4H, 4 H-2), 5.21-5.17 (4dd, 4H, 4 H-3), 4.76 (d, 2H, $J_{1,2}$ = 10.0 Hz, 2 H-1), 4.72 (d, 2H, $J_{1,2}$ = 10.0 Hz, 2 H-1), 4.40 and 3.07 (2d, 4H, *J* = 13.2 Hz, 2 ArC*H*$_2$Ar), 4.36-4.30 (4ddd, 4H, 4 H-5), 4.27 and 3.09 (2d, 4H, *J* = 13.2 Hz, 2 ArC*H*$_2$Ar), 4.14-4.07 (m, 16H, 8 H-6, 4 ArCH$_2$CH$_2$C*H*$_2$), 4.01-4.00 (m, 2H, OC*H*$_2$CH$_2$N), 3.97-3.93 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.85-3.81 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.76-3.72 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.63-3.60 (m, 2H, OCH$_2$C*H*$_2$N), 2.63-2.58 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.24-2.19 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.17, 2.16, 2.03, 2.02, 1.99, 1.91, and 1.88 (7s, 48H, 16 Ac), 2.04-1.85 (m, 8H, 4 ArCH$_2$C*H*$_2$CH$_2$), 1.85-1.74 (m, 6H, 3 CH$_3$C*H*$_2$CH$_2$O), 1.52 (s, 9H, *t*-Bu), 0.95 (t, 6H, *J* = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.86 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 170.4 (C), 170.1 (C), 170.0 (C), 169.7 (C), 169.6 (C), 156.4 (C), 155.3 (C), 155.1 (C), 153.3 (C), 144.4 (C), 144.1 (C), 136.3 (C), 136.2 (C), 134.0 (C), 133.4 (C), 133.1 (C), 129.0 (CH), 128.9 (CH), 128.7 (CH), 128.6 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.7 (CH), 127.6 (CH), 127.3 (CH), 122.1 (CH), 78.9 (C), 77.5 (CH$_2$), 76.7 (CH$_2$), 75.2 (CH$_2$), 74.7 (CH), 73.7 (CH), 71.8 (CH), 68.7 (CH), 67.6 (CH), 61.5 (CH$_2$), 49.7 (CH$_2$), 49.5 (CH$_2$), 40.9 (CH$_2$), 31.8 (CH$_2$), 31.0 (CH$_2$), 28.6 (CH$_3$), 22.7 (CH$_2$), 20.65 (CH$_3$), 20.57 (CH$_3$), 10.2 (CH$_3$). ESI MS (2451.58): 1227.4 (M + 2H$^+$)/2.

5,11,17,23-Tetrakis{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-(2-azidoethoxy)-26,27,28-tripropoxy-calix[4]arene (11).
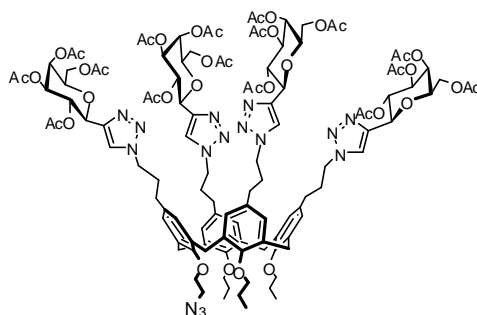

A solution of **10** (196 mg, 0.08 mmol) in CH_2Cl_2 (8 mL) and CF_3CO_2H (4 mL) was kept at room temperature for 1 h, then concentrated. To a mixture of the crude ammonium salt, K_2CO_3 (60 mg, 0.44 mmol), $CuSO_4 \cdot 5H_2O$ (2 mg, 8 μmol), CH_3OH (4 mL), and CH_3CN (1 mL), was added imidazole-1-sulfonyl azide hydrochloride[29] (40 mg, 0.20 mmol). The mixture was stirred at room temperature for 6 h, then concentrated, diluted with acetic anhydride (2 mL) and pyridine (2 mL), stirred for an additional 3 h, and concentrated. The residue was diluted with H_2O (10 mL) and extracted with AcOEt (2 x 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with AcOEt to give **11** (132 mg, 69%) as a white foam; $[\alpha]_D = -10.2$ (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz): δ 7.69 (s, 1H, H-5 Tr.), 7.67 (s, 1H, H-5 Tr.), 7.59 (s, 2H, 2 H-5 Tr.), 6.71 (s, 4H, Ar), 6.22 (s, 2H, Ar), 6.17 (s, 2H, Ar), 5.54-5.51 (4dd, 4H, 4 H-4), 5.44 (dd, 2H, $J_{1,2} = J_{2,3} = 10.0$ Hz, 2 H-2), 5.42 (dd, 2H, $J_{1,2} = J_{2,3} = 10.0$ Hz, 2 H-2), 5.22-5.16 (4dd, 4H, 4 H-3), 4.76 (d, 2H, 2 H-1), 4.73 (d, 2H, 2 H-1), 4.40 and 3.07 (2d, 4H, $J = 13.1$ Hz, 2 ArC*H*$_2$Ar), 4.34 and 3.10 (2d, 4H, $J = 13.1$ Hz, 2 ArC*H*$_2$Ar), 4.32-4.25 (m, 4H, 2 ArCH$_2$CH$_2$C*H*$_2$), 4.18-4.07 (m, 18H, 4 H-5, 8 H-6, 2 ArCH$_2$CH$_2$C*H*$_2$, OCH$_2$C*H*$_2$N$_3$), 3.92-3.86 (m, 4H, OC*H*$_2$CH$_2$N$_3$, CH$_3$CH$_2$C*H*$_2$O), 3.78-3.69 (m, 4H, 2 CH$_3$CH$_2$C*H*$_2$O), 2.53-2.47 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.17, 2.16, 2.03, 2.02, 1.99, 1.90, and 1.88 (7s, 48H, 16 Ac), 2.18-2.08 (m, 8H, 2 ArC*H*$_2$CH$_2$CH$_2$, 2 ArCH$_2$C*H*$_2$CH$_2$), 1.94-1.84 (m, 10H, 2 ArCH$_2$C*H*$_2$CH$_2$, 3 CH$_3$C*H*$_2$CH$_2$O), 1.04 (t, 6H, $J = 7.4$ Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.94 (t, 3H, $J = 7.4$ Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 170.4 (C), 170.1 (C), 170.0 (C), 169.7 (C), 155.6 (C), 155.0 (C), 154.1 (C), 144.4 (C), 144.2 (C), 135.8 (C), 135.6 (C), 134.0 (C), 133.8 (C), 133.6 (C), 133.4 (C), 128.5 (CH), 127.8 (CH), 127.5 (CH), 122.1 (CH), 77.2 (CH$_2$), 76.5 (CH$_2$), 74.7 (CH), 73.8 (CH), 71.9 (CH), 71.3 (CH$_2$), 68.6 (CH), 67.6 (CH), 61.5 (CH$_2$), 50.7 (CH$_2$), 49.7 (CH$_2$), 49.6 (CH$_2$), 31.9 (CH$_2$), 30.9 (CH$_2$), 30.8 (CH$_2$), 29.6 (CH$_2$), 23.3 (CH$_2$), 23.0 (CH$_2$), 20.7 (CH$_3$), 20.6 (CH$_3$), 10.5 (CH$_3$), 10.0 (CH$_3$). ESI MS (2377.46): 1189.7 $(M + 2H^+)/2$; 1200.7 $(M + Na^+ + H^+)/2$.

5,11,17,23-Tetrakis{3-[4-(β-D-galactopyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-(2-azidoethoxy)-26,27,28-tripropoxy-calix[4]arene (1).
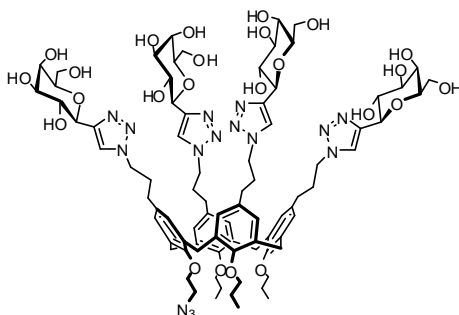

A solution of **11** (15 mg, 0.006 mmol) in a 2 M solution of NH_3 in CH_3OH (1 mL) was kept at room temperature for 16 h, then concentrated. The residue was eluted from a C18 silica gel cartridge with H_2O-CH_3OH (from 1:1 to 1:5), then CH_3OH, to give **1** (9.7 mg, 90%) as an amorphous solid; $[\alpha]_D$ = +14.2 (*c* 0.4, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 8.06, 8.03, 7.92, and 7.91 (4s, 4H, 4 H-5 Tr.), 6.79 (s, 4H, Ar), 6.47-6.45 (m, 2H, Ar), 6.40 (bs, 2H, Ar), 4.44 and 3.12 (2d, 4H, *J* = 12.7 Hz, 2 ArC*H*$_2$Ar), 4.39-4.34 (3d, 4H, 4 H-1), 4.37 and 3.14 (2d, 4H, *J* = 12.8 Hz, 2 ArC*H*$_2$Ar), 4.19 (t, 4H, *J* = 7.3 Hz, 2 ArCH$_2$CH$_2$C*H*$_2$), 4.10-4.05 (m, 6H, 2 ArCH$_2$CH$_2$C*H*$_2$, OC*H*$_2$CH$_2$N$_3$), 3.99-3.87 (m, 12H, 4 H-4, 4 H-2, CH$_3$CH$_2$C*H*$_2$O, OCH$_2$C*H*$_2$N$_3$), 3.79-3.64 (m, 16H, 4 H-5, 8 H-6, 2 CH$_3$CH$_2$C*H*$_2$O), 3.64-3.59 (3d, 4H, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.2 Hz, 4 H-3), 2.42-2.36 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.11-2.04 (m, 8H, 2 ArC*H*$_2$CH$_2$CH$_2$, 2 ArCH$_2$C*H*$_2$CH$_2$), 2.02-1.90 (m, 6H, 3 CH$_3$C*H*$_2$CH$_2$O), 1.84-1.76 (m, 4H, 2 ArCH$_2$C*H*$_2$CH$_2$), 1.08 (t, 6H, *J* = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 1.00 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz, CD_3OD): δ 155.3 (C), 147.5 (C), 136.7 (C), 135.3 (C), 130.0 (CH), 129.3 (CH), 124.9 (CH), 80.9 (CH), 78.3 (CH$_2$), 76.3 (CH), 72.1 (CH), 71.0 (CH), 62.9 (CH$_2$), 52.2 (CH$_2$), 50.3 (CH$_2$), 33.0 (CH$_2$), 31.7 (CH$_2$), 24.6 (CH$_2$), 11.1 (CH$_3$), 10.6 (CH$_3$). ESI MS (1704.87): 1706.3 (M + H$^+$).

**5,11,17,23-Tetrakis(3-azidopropyl)-25-(ethoxycarbonylmethoxy)-26,27,28-tripropoxy-calix[4]arene (12).**
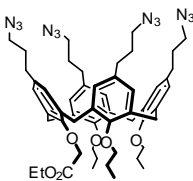
To a stirred solution of alcohol **7** (159 mg, 0.18 mmol) in anhydrous DMF (3 mL) was added NaH (15 mg, 0.37 mmol, of a 60% dispersion in oil) and, after 15 min, ethyl bromoacetate (60 μL, 0.54 mmol). The mixture was stirred at 55 °C for 18 h, then cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (15 mL) and extracted with Et_2O (2 x 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 8:1 cyclohexane-AcOEt to give **12** (144 mg, 82%) as a syrup; ^{1}H NMR (400 MHz): δ 6.67 (s, 2H, Ar), 6.65 (s, 2H, Ar), 6.23 (s, 4H, Ar), 4.77 (s, 2H, OCH_2CO_2), 4.60 and 3.12 (2d, 4H, J = 13.5 Hz, 2 ArCH_2Ar), 4.39 and 3.07 (2d, 4H, J = 13.0 Hz, 2 ArCH_2Ar), 4.19 (q, 2H, J = 7.2 Hz, CO_2CH_2CH$_3$), 3.88-3.83 (m, 2H, CH_3CH_2CH_2O), 3.80-3.70 (m, 4H, 2 CH_3CH_2CH_2O), 3.23 (t, 2H, J = 6.8 Hz, ArCH$_2$CH$_2$CH_2), 3.22 (t, 2H, J = 6.8 Hz, ArCH$_2$CH$_2$CH_2), 3.10 (t, 4H, J = 6.8 Hz, 2 ArCH$_2$CH$_2$CH_2), 2.55-2.50 (m, 4H, 2 ArCH_2CH$_2$CH$_2$), 2.22 (t, 4H, J = 7.5 Hz, 2 ArCH_2CH$_2$CH$_2$), 2.01-1.78 (m, 10H, 3 CH_3CH_2CH$_2$O, 2 ArCH$_2$CH_2CH$_2$), 1.66-1.56 (m, 4H, 2 ArCH$_2$CH_2CH$_2$), 1.30 (t, 3H, J = 7.2 Hz, CO_2CH$_2$CH_3), 1.04 (t, 6H, J = 7.5 Hz, 2 CH_3CH$_2$CH$_2$O), 0.98 (t, 3H, J = 7.5 Hz, CH_3CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 170.4 (C), 155.3 (C), 154.3 (C), 135.5 (C), 135.2 (C), 134.4 (C), 134.0 (C), 133.8 (C), 128.6 (CH), 128.3 (CH), 127.7 (CH), 127.6 (CH), 77.0 (CH_2), 70.3 (CH_2), 60.2 (CH_2), 50.6 (CH_2), 32.0 (CH_2), 31.3 (CH_2), 30.8 (CH_2), 30.5 (CH_2), 23.2 (CH_2), 14.2 (CH_3), 10.5 (CH_3), 10.1 (CH_3). ESI MS (969.18): 987.4 (M + NH_4^+).

5,11,17,23-Tetrakis{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-(ethoxycarbonyl-methoxy)-26,27,28-tripropoxy-calix[4]arene (13).
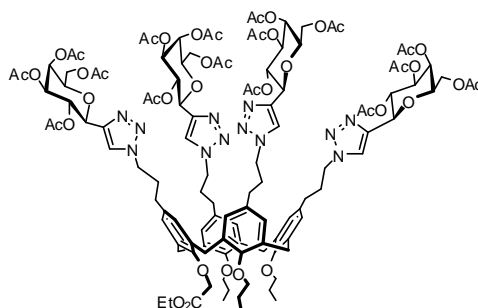

The cycloaddition between the tetra-azide **12** (140 mg, 0.14 mmol) and the ethynyl *C*-galactoside **9** (226 mg, 0.63 mmol) was carried out as described for the preparation of **10** to give, after column chromatography of silica gel (AcOEt), **13** (277 mg, 80%) as a white foam; $[\alpha]_D$ = -8.6 (*c* 0.9, CHCl$_3$). ^{1}H NMR (400 MHz): δ 7.68 (s, 1H, H-5 Tr.), 7.66 (s, 1H, H-5 Tr.), 7.59 (s, 2H, 2 H-5 Tr.), 6.66 (s, 2H, Ar), 6.65 (s, 2H, Ar), 6.23 (s, 2H, Ar), 6.22 (s, 2H, Ar), 5.53-5.51 (4dd, 4H, 4 H-4), 5.44 (dd, 2H, $J_{1,2} = J_{2,3}$ = 10.0 Hz, 2 H-2), 5.42 (dd, 2H, $J_{1,2} = J_{2,3}$ = 10.0 Hz, 2 H-2), 5.19 (dd, 2H, $J_{3,4}$ = 3.2 Hz, 2 H-3), 5.18 (dd, 2H, $J_{3,4}$ = 3.2 Hz, 2 H-3), 4.76 (s, 2H, OC*H*$_2$CO$_2$), 4.75 (d, 2H, 2 H-1), 4.73 (d, 2H, 2 H-1), 4.61 and 3.11 (2d, 4H, *J* = 13.5 Hz, 2 ArC*H*$_2$Ar), 4.39 and 3.06 (2d, 4H, *J* = 13.0 Hz, 2 ArC*H*$_2$Ar), 4.30-4.25 (m, 4H, 2 ArCH$_2$CH$_2$C*H*$_2$), 4.18 (q, 2H, *J* = 7.0 Hz, CO$_2$C*H*$_2$CH$_3$), 4.16-4.08 (m, 16H, 4 H-5, 8 H-6, 2 ArCH$_2$CH$_2$C*H*$_2$), 3.87-3.86 (m, 2H, CH$_3$CH$_2$C*H*$_2$O), 3.79-3.69 (m, 4H, 2 CH$_3$CH$_2$C*H*$_2$O), 2.50-2.43 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.17-2.09 (m, 8H, 2 ArC*H*$_2$CH$_2$CH$_2$, 2 ArCH$_2$C*H*$_2$CH$_2$), 2.16, 2.02, 2.01, 1.99, 1.90, and 1.87 (6s, 48H, 16 Ac), 1.96-1.84 (m, 10H, 2 ArCH$_2$C*H*$_2$CH$_2$, 3 CH$_3$C*H*$_2$CH$_2$O), 1.27 (t, 3H, *J* = 7.0 Hz, CO$_2$CH$_2$C*H*$_3$), 1.01 (t, 6H, *J* = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.94 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz): δ 170.3 (C), 170.1 (C), 170.0 (C), 169.6 (C), 155.5 (C), 154.5 (C), 144.3 (C), 144.1 (C), 135.6 (C), 135.3 (C), 133.9 (C), 133.7 (C), 133.2 (C), 128.3 (CH), 127.6 (CH), 122.1 (CH), 76.9 (CH$_2$), 74.6 (CH), 73.7 (CH), 71.8 (CH), 70.2 (CH$_2$), 68.6 (CH), 67.5 (CH), 61.4 (CH$_2$), 60.2 (CH$_2$), 49.6 (CH$_2$), 31.8 (CH$_2$), 31.3 (CH$_2$), 30.8 (CH$_2$), 23.1 (CH$_2$), 20.6 (CH$_3$), 20.5 (CH$_3$), 14.1 (CH$_3$), 10.4 (CH$_3$), 10.0 (CH$_3$). ESI MS (2394.48): 1198.7 (M + 2H[+])/2.

5,11,17,23-Tetrakis{3-[4-(β-D-galactopyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-carboxylmethoxy-26,27,28-tripropoxy-calix[4]arene (14).
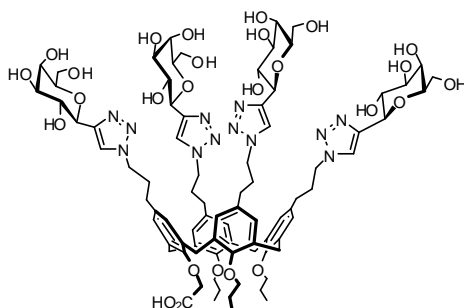
A solution of **13** (100 mg, 0.04 mmol) in a 0.2 M solution of NaOEt in EtOH (2 mL, prepared from Na and EtOH immediately before the use) was kept at room temperature for 3 h in a nitrogen atmosphere, then neutralized with Dowex 50 X2-400 resin (H$^+$ form, activated and washed with H_2O and EtOH immediately before the use), and filtered through a sintered glass filter. The resin was washed with H_2O and DMF, and the solution was concentrated. A solution of the residue in 0.2 M aqueous NaOH (2 mL) was kept at room temperature for 24 h in a nitrogen atmosphere, then neutralized with Dowex 50 X2-400 resin (H$^+$ form, activated and washed with H_2O and EtOH immediately before the use), and filtered through a sintered glass filter. The resin was washed with H_2O and DMF, and the solution was concentrated. The residue was eluted from a C18 silica gel cartridge with 1:1 H_2O-CH_3OH, then CH_3OH, to give **14** (44 mg, 62%) as an amorphous solid; $[\alpha]_D$ = +14.2 (*c* 0.5, DMF). ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) selected data: δ 8.04 (s, 2H, 2 H-5 Tr.), 7.96 (s, 2H, 2 H-5 Tr.), 6.76 (s, 4H, Ar), 6.50 (s, 2H, Ar), 6.47 (s, 2H, Ar), 4.60 (s, 2H, OC*H*$_2$CO$_2$), 4.45 and 3.14 (2d, 4H, *J* = 13.0 Hz, 2 ArC*H*$_2$Ar), 4.31 and 3.14 (2d, 4H, *J* = 13.0 Hz, 2 ArC*H*$_2$Ar), 2.37-2.26 (m, 4H, 2 ArC*H*$_2$CH$_2$CH$_2$), 2.18-2.08 (m, 4H), 2.04-1.75 (m, 14H), 0.96 (t, 6H, *J* = 7.5 Hz, 2 C*H*$_3$CH$_2$CH$_2$O), 0.93 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O). ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) selected data: δ 146.9 (C), 135.5 (C), 134.7 (C), 128.5 (CH), 124.0 (CH), 80.0 (CH), 79.9 (CH), 75.5 (CH), 70.8 (CH), 69.5 (CH), 61.4 (CH$_2$), 49.3 (CH$_2$), 31.9 (CH$_2$), 23.3 (CH$_2$), 11.0 (CH$_3$). ESI MS (1693.84): 1694.7 (M + H$^+$).

5,11,17,23-Tetrakis{3-[4-(β-D-galactopyranosyl)-1*H*-1,2,3-triazol-1-yl]propyl}-25-[(11-azido-3,6,9-trioxaundecan-1-amino)carbonyl-methoxy]-26,27,28-tripropoxy-calix[4]arene (2).
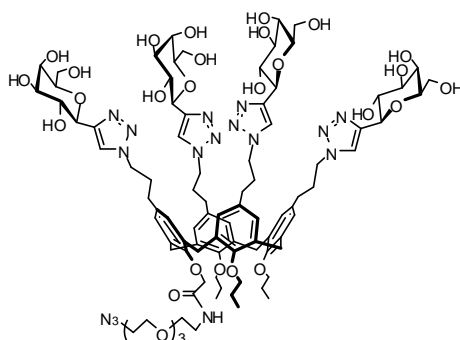
A mixture of **14** (21 mg, 0.012 mmol), 1-hydroxybenzotriazole hydrate (HOBT, 3 mg, 0.024 mmol), and *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide (EDC, 14 mg, 0.072 mmol) in anhydrous DMF (2 mL) was stirred at room temperature for 30 min, then commercially available 11-azido-3,6,9-trioxaundecan-1-amine **15** (10 µL, 0.036 mmol) was added. Stirring was continued for an additional 48 h, then the solvent was removed under vacuum. The residue was eluted from a C18 silica gel cartridge with H_2O-CH_3OH (from 1:1 to 1:5), then CH_3OH, to give **2** (12 mg, 51%) as an amorphous solid; $[\alpha]_D$ = +14.5 (*c* 0.4, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.11, 8.10, 7.90, and 7.89 (4s, 4H, 4 H-5 Tr.), 6.94 (s, 2H, Ar), 6.91 (s, 2H, Ar), 6.30-6.26 (m, 4H, Ar), 4.82 (s, 2H, OCH_2CO_2), 4.43 and 3.27 (2d, 4H, *J* = 13.5 Hz, 2 ArCH_2Ar), 4.40 and 3.14 (2d, 4H, *J* = 13.0 Hz, 2 ArCH_2Ar), 4.39 (d, 2H, $J_{1,2}$ = 9.8 Hz, 2 H-1), 4.34 (d, 2H, $J_{1,2}$ = 9.8 Hz, 2 H-1), 4.28-4.22 (m, 4H, 2 ArCH$_2$CH$_2$CH_2), 4.11-4.06 (m, 4H, 2 ArCH$_2$CH$_2$CH_2), 3.99-3.91 (8 dd, 8H, 4 H-2, 4 H-4), 3.91-3.78 (m, 6H, 3 CH$_3$CH$_2$CH_2O), 3.76-3.56 (m, 30H), 3.31-3.29 (m, 2H), 2.58-2.51 (m, 4H, 2 ArCH_2CH$_2$CH$_2$), 2.27-2.16 (m, 4H, 2 ArCH$_2$CH_2CH$_2$), 2.05-1.97 (m, 4H, 2 ArCH_2CH$_2$CH$_2$), 1.95-1.72 (m, 10H, 2 ArCH$_2$CH_2CH$_2$, 3 CH$_3$CH_2CH$_2$O), 1.02 (t, 6H, *J* = 7.5 Hz, 2 CH_3CH$_2$CH$_2$O), 0.92 (t, 3H, *J* = 7.5 Hz, CH_3CH$_2$CH$_2$O). ^{13}C NMR (75 MHz, CD_3OD): δ 173.3 (C), 157.2 (C), 156.3 (C), 154.4 (C), 147.7 (C), 147.2 (C), 137.5 (C), 135.9 (C), 135.7 (C), 135.4 (C), 134.9 (C), 134.0 (C), 131.1 (CH), 130.3 (CH), 129.3 (CH), 128.9 (CH), 125.1 (CH), 124.9 (CH), 80.9 (CH), 78.9 (CH$_2$), 77.8 (CH$_2$), 76.3 (CH), 76.2 CH), 75.0 (CH$_2$), 72.2 (CH), 72.0 (CH), 71.8 (CH$_2$), 71.7 (CH$_2$), 71.6 (CH$_2$), 71.2 (CH$_2$), 71.0 (CH), 70.9 (CH$_2$), 62.9 (CH$_2$), 51.7 (CH$_2$), 50.8 (CH$_2$), 50.4 (CH$_2$), 33.0 (CH$_2$), 32.5 (CH$_2$), 32.1 (CH$_2$), 24.2 (CH$_2$), 24.0 (CH$_2$), 10.8 (CH$_3$), 10.4 (CH$_3$). ESI MS (1894.08): 1895.3 (M + H$^+$).

Mono- (16)[36] and di-alkyne (17)[36] solid supports were synthesized as previously described.

Synthesis of 5'-Cy3-oligonucleotides with 3'-mono- or 3'-di-alkyne functions.

The oligonucleotides were synthesized on a DNA synthesizer (ABI 394) using standard phosphoramidite chemistry starting from the monoalkyne **16** or the dialkyne **17** solid supports. Detritylation was performed with 3% DCA in CH_2Cl_2 for 35 s for the dimethoxytrityl groups and for 60 s for the monomethoxytrityl group (Cy3). For the coupling step, benzylmercaptotetrazole was used as activator (0.3 M in anhydrous CH_3CN), commercially available phosphoramidites (A, T, C, G, Cy3) (0.075 M in anhydrous CH_3CN) were introduced with a 20 s coupling time. The capping step was performed with acetic anhydride using commercial solution (Cap A: Ac_2O, pyridine, THF 10/10/80 and Cap B: 10% *N*-methylimidazole in THF) for 15 s. Oxidation was performed using a commercially available solution of iodine (0.1 M I_2, THF-pyridine-water 90:5:5) for 13 s.

Synthesis of 5'-Cy3-CCG CGT TGG ATT AGC (PePO galactosyl$_2$)$_5$ propanol (25).

Starting from 1,3-propanediol solid support five 3-(4,4'-dimethoxytrityloxy)- 2,2-bis(propargyloxymethyl)-propyl (2-cyanoethyl *N,N*-diisopropyl)-phosphoramidite[11] were coupled according to the phosphoramidite elongation cycle (see above) and the three first nucleotides. Solid supported DMTr-A^{bz}G^{ibu}C^{bz} decaalkyne derivative (0.6 µmol) was transferred into a microwave vial and 1-azido-3,6-dioxaoct-8-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[12, 45] (30 µmol, 15.2 mg, in 100 µL of methanol), $CuSO_4$ (1.2 µmol, 6 µL of 200 mM water solution) and sodium ascorbate (6 µmol, 24 µL of 250 mM water solution) were added. The sealed vial was irradiated with microwaves for 20 min at 60 °C under magnetic stirring. After filtration, washing with methanol-water and dry acetonitrile, and drying under dessicator, the beads were transferred into a DNA column and the sequence was completed on the DNA synthesizer with a Cy3 at the 5'-end. After deprotection and HPLC purification the pure **25** was characterized by MALDI-TOF MS; *m/z* calculated for $C_{353}H_{531}N_{87}O_{208}P_{21}$ (M-H)$^-$ 9972.02, found 9971.03.

General procedure for deprotection.

The beads were treated with concentrated aqueous ammonia (~1mL) at 55 °C for 6 h, then the supernatant was withdrawn and evaporated to dryness. The residue was dissolved in water for subsequent analyses.

Cu(I)-catalyzed cycloaddition for mono- and di-calix[4]arene oligonucleotide conjugates (22-23).

To monoalkyne oligonucleotide **20** (0.5 μmol) were added **1** (4 equiv., 100 μL of a 0.02 M solution in water), $CuSO_4$ (5 equiv., 2.5 μmol, 13 μL of a 0.2 M solution in H_2O), freshly prepared sodium ascorbate (25 equiv., 12.5 μmol, 64 μL of a 0.2 M solution in H_2O), and water (300 μL). The mixture was heated at 60 °C for 1 h in a sealed tube using a microwave synthesizer Initiator from Biotage (temperature monitored with an internal infrared probe). 500 μL of saturated aqueous EDTA solution was then added to the mixture and the resulting solution was desalted on NAP10. The crude product was purified on reversed-phase preparative HPLC to yield 111 nmol of pure compound **22**, determined by spectrophotometry at 550 nm ($\varepsilon_{(Cy3)} = 150000$). RT (HPLC) = 15.42 min; MALDI-TOF MS: *m/z* calculated for $C_{264}H_{345}N_{68}O_{124}P_{16}$ (M-H)$^-$ 6950.62 found 6949.63.

The synthesis of conjugate **23** (70 nmol) was performed as described above using 6 equiv. of **2**. RT (HPLC) = 17.18 min; MALDI-TOF MS: *m/z* calculated for $C_{366}H_{490}N_{85}O_{157}P_{16}$ (M-H)$^-$ 9087.98 found 9090.22.

Fabrication of Microarrays[12]

Silanisation of the glass slides. Borosilicate glass slides (Schott) bearing 52 microreactors[39] (2 mm diameter, 65-100 µm deep) were functionalised according to the protocol developed by Dugas *et al.*[37] In brief, after piranha treatment, the slides were heated under dry nitrogen for 2 h at 150 °C. Next, dry pentane (250 ml) and *tert*-butyl-11-(dimethylamino)silylundecanoate (300 µl) were added at room temperature. After 20 min of incubation, pentane was evaporated and the slides were heated at 150 °C overnight. Functionalised slides were obtained after washing in THF and rinsing in water. Deprotection of the ester function was performed using formic acid (7 h at room temperature). *N-*

Hydroxysuccinimide (0.1 M) and di(isopropyl)-carbodiimide (0.1 M) in dry THF were used for the activation of the acid functions.

Immobilization of DNA strands. 3'-Amino modified oligonucleotides were purchased from Eurogentec. Sequence 1 S1: 5'-GTG AGC CCA GAG GCA GGG-$(CH_2)_7$-NH_2, Sequence 2 S2: 5'-GCT AAT CCA ACG CGG GCC AAT CCT T-$(CH_2)_7$-NH_2 and Sequence 3 (negative control) S3: 5'-ATG CCC TCT TTG ATA TT-$(CH_2)_7$-NH_2 were used. At the bottom of the corresponding well, 1 µl of the desired sequence (25 µM in PBS 10x (pH 8.5) were deposited. The coupling reaction was performed overnight at room temperature. Water was then allowed to slowly evaporate. The slides were washed with SDS 0.1% at 70 °C for 30 min and rinsed with deionized water.

Blocking. After immobilization all slides were blocked with Bovine Serum Albumin (BSA). Blocking was performed with a 4% BSA solution in PBS 1x (pH 7.4), at room temperature for 2 h. The glass slides were then washed in PBS 1x (pH 7.4)-Tween20 0.05% 3 x 3 minutes followed by PBS 1x (pH 7.4) 2-3 times, rinsed with deionized water, and dried by centrifugation.

Hybridization of the glycoconjugates on DNA array. Glycoconjugates bearing a DNA tag were hybridized with 1 µL of a solution at the desired concentrations (SSC 5x, 0.1% SDS) placed at the bottom of the corresponding well and allowed to hybridize overnight at room temperature in a H_2O vapor saturated chamber. The slides were washed in SSC 2x, 0.1% SDS at 51 °C for 1 min followed by SSC 2x at room temperature for an additional 5 min, and then rinsed with deionized water and dried by centrifugation.

Probing Microarrays

Cy5 Labelling of RCA 120 lectin. RCA 120 (Sigma) was labelled with Amersham Biosciences Cy5 Ab Labelling Kit according to the manufacturer's protocol. Protein concentration and the dye to protein ratio were estimated by optical density (nanodrop) reading the absorbance at 280 and 650 nm. Lectin concentration was estimated to be 4 µM bearing an average of 4 dyes per protein.

Labelling of PA-IL lectin (gift from A. Imberty, CERMAV) with Alexa 647 was performed with the microscale labelling kit from Invitrogen. Protein concentration was estimated according to the manufacturer protocol by reading the absorbance at 280 and 650 nm. The final lectin concentration was estimated at 28 µM with a degree of labelling of 0.4.

The labeled lectin was diluted in PBS 1x (pH 7.4), $CaCl_2$ (final concentration 5 µM) and 20% BSA (final concentration 2%) to the desired concentration. For IC_{50} experiments, lactose was added into the solution at different final concentrations. 1 µL of each solution was placed at the bottom of each well and incubated at 37 °C in a H_2O vapor saturated chamber for 2 h. Then the slides were washed in PBS1x (pH 7.4)-Tween20 0.02% for 10 min, dried by centrifugation, and scanned.

Fluorescence scanning. Fluorescent scanning was performed using a Microarray scanner, GenePix 4100A software package (Axon Instruments) (excitation 532/635 nm and emission 575/670 nm). The fluorescence signal of each conjugate was determined as the average of the mean fluorescence signal of four spots.

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