Achieving High Affinity towards a Bacterial Lectin through Multivalent Topological Isomers of Calix[4]arene Glycoconjugates

ARTICLE *in* CHEMISTRY - A EUROPEAN JOURNAL · OCTOBER 2009

Impact Factor: 5.73 · DOI: 10.1002/chem.200901799 · Source: PubMed

CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200901799

Achieving High Affinity towards a Bacterial Lectin through Multivalent Topological Isomers of Calix[4]arene Glycoconjugates

Samy Cecioni,[a, b] Ruth Lalor,[c] Bertrand Blanchard,[b] Jean-Pierre Praly,[a] Anne Imberty,*[b] Susan E. Matthews,*[c] and Sébastien Vidal*[a]

Abstract: A family of seven topologically isomeric calix[4]arene glycoconjugates was prepared through the synthesis of a series of alkyne-derivatised calix[4]arene precursors that are suitable for the attachment of sugar moieties by microwave-assisted copper(I)-catalysed azide–alkyne cycloaddition (CuAAC). The glycoconjugates thus synthesised comprised one mono-functionalised derivative, two 1,2- or 1,3-divalent regioisomers, one trivalent and three tetravalent topoisomers in the cone, partial cone or 1,3-alternate conformations. The designed glycoconjugates were evaluated as ligands for the galactose-binding lectin PA-IL from the opportunistic bacterium *Pseudomonas aeruginosa*, a major causative agent of lung infections in cystic fibrosis patients. Binding affinities were determined by isothermal titration calorimetry (ITC), and the interaction with the lectin was shown to be strongly dependant on both the valence and the topology. Whereas the trivalent conjugate displayed enhanced affinity when compared to a monosaccharide model, the tetravalent conjugates are to-date the highest-affinity ligands measured by ITC. The topologies presenting carbohydrates on both faces of calixarene are the most potent ones with dissociation constants of approximately 200 nM. Molecular modelling suggests that such a multivalent molecule can efficiently chelate two of the binding sites of the tetrameric lectin; this explains the 800-fold increase of affinity achieved by the tetravalent molecule. Surface plasmon resonance (SPR) experiments confirmed that this glycoconjugate is the strongest inhibitor for binding of PA-IL to galactosylated surfaces for potential applications as an anti-adhesive agent.

Keywords: calixarenes · click chemistry · isothermal titration calorimetry · lectin · surface plasmon resonance

Introduction

Lectin–carbohydrate interactions[1] play a prominent role in biological recognition events involving cells and proteins.[2] Although these multi-modal interactions are generally highly specific, the affinity for monovalent interactions is usually weak. However, strong binding can be achieved in natural systems due to the 'glycoside cluster effect'[3] in which several saccharide ligands are presented to interact with several binding sites on one (or more) receptors at the same time. This concept of a multivalent effect[4] has been particularly studied through the development of synthetic glycoclusters,[5] glycodendrimers[6] and glycopolymers.[7]

The use of 1,3-dipolar cycloaddition reactions between alkynes and azides[8] for the introduction of sugars to the calix[4]arene scaffold[9] was first proposed by the group of Santoyo-González,[10] and they were able to prepare glycoconjugates as a mixture of 1,4- and 1,5-regioisomers about the triazole ring. Since the observation by Sharpless[11] and Meldal[12] that the Huisgen[13] cycloaddition reaction can be

[a] S. Cecioni, Dr. J.-P. Praly, Dr. S. Vidal
Institut de Chimie et Biochimie Moléculaires et Supramoléculaires
Laboratoire de Chimie Organique 2 - Glycochimie, UMR 5246
Université Lyon 1 and CNRS
43 Boulevard du 11 Novembre 1918, 69622,Villeurbanne (France)
Fax: (+33) 478-898-914
E-mail: sebastien.vidal@univ-lyon1.fr

[b] S. Cecioni, B. Blanchard, Dr. A. Imberty
CERMAV - CNRS, Université Joseph Fourier and ICMG
BP 53, 38041, Grenoble(France)
Fax: (+33) 476-547-203
E-mail: anne.imberty@cermav.cnrs.fr

[c] Dr. R. Lalor, Dr. S. E. Matthews
School of Chemical Sciences and Pharmacy
University of East Anglia
Norwich, NR4 7TJ (UK)
Fax: (+44) 160-345-8553
E-mail: susan.matthews@uea.ac.uk

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200901799.

catalysed by Cu^{I} to give exclusively the 1,4-disubstituted regioisomer, the use of 'click' chemistry as a straightforward linking strategy has been used in a wide variety of areas by us[14] and others.[15]

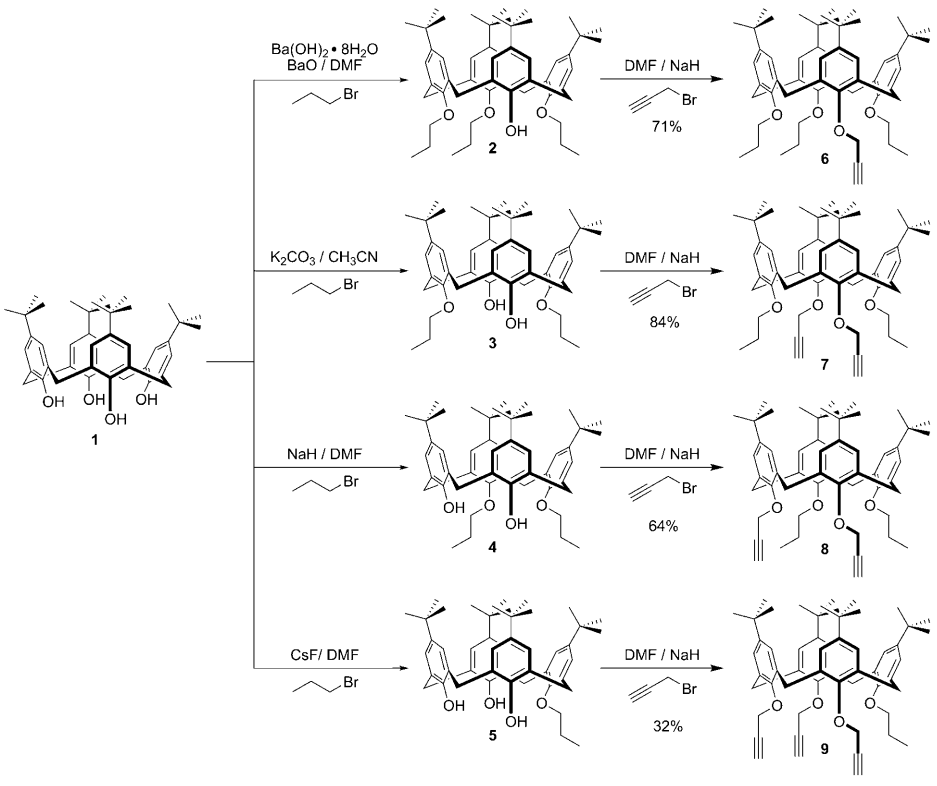
Recently the groups of Dondoni[16] and Bew[17] have described the synthesis of glycoclusters with azido-functionalised calix[4]arenes. This process involves multiple synthetic steps and, in the case of the upper-rim derivatives, conformational locking through the introduction of lower-rim propyl groups. In contrast, functionalisation of the lower-rim, with propargyl groups allowing the preparation of scaffolds with a range of valences and conformations is straightforward and requires a maximum of two synthetic steps from commercial starting materials.

The biological applications of calixarene glycoconjugates have been examined against a range of lectins including wheat germ agglutinin,[18] cholera toxin,[19] NKR-P1,[20] galectins[21] and the influenza A viruses.[22] The opportunistic pathogen *Pseudomonas aeruginosa* is the causative agent of lung infections and an important morbidity and mortality factor for immuno-compromised and cystic fibrosis patients. The bacterium contains several carbohydrate-binding proteins, including two soluble lectins, PA-IL (LecA) and PA-IIL (LecB), which are specific for D-galactose and L-fucose, respectively.[23] Treatment with fucose and galactose derivatives has been shown to be efficient against acute pneumonia in mice models.[24] Inhalation of fucose and galactose also decreased significantly *P. aeruginosa* counts in cystic fibrosis patients.[25] Higher-affinity ligands can be expected to compete even more efficiently with receptor binding and thus are promising compounds for antimicrobial therapy.[9b] Several glycomimetics and glycodendrimers have been recently tested[14d,26] against the fucose-binding lectin PA-IIL, and some dendrimers demonstrated biofilm dispersion properties.[27] Until now, only one class of multivalent galactosylated glycoconjugates[28] has been directed against PA-IL, a tetrameric lectin for which the crystal structure is available,[29] and the binding properties were analysed through turbidimetric assays.

As part of an ongoing programme[14] to prepare a library of multivalent glycoclusters and evaluate the effect of three-dimensional shape on selectivity between lectins, we report here the use of readily prepared alkyne functionalised calix[4]arenes as tuneable platforms for the preparation of glycoclusters. The affinity of PA-IL for each of the different glycoclusters was evaluated by titration microcalorimetry and surface plasmon resonance studies, and the high-affinity results obtained with tetrameric compounds have been rationalised by molecular modelling.

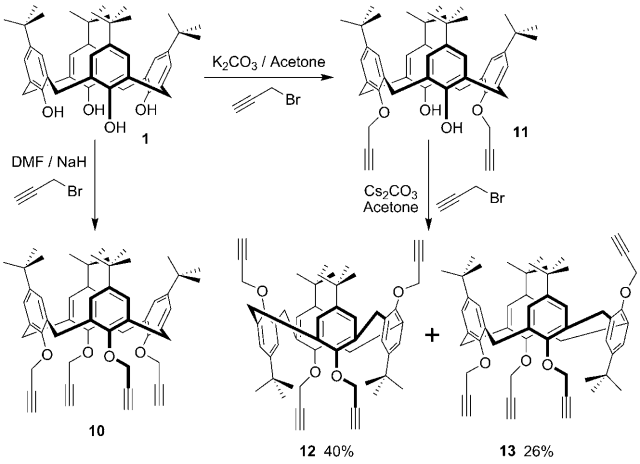
Results and Discussion

Synthesis of calix[4]arene multivalent glycoconjugates: The synthesis of calix[4]arenes that are locked in the cone conformation, featuring one, two or three alkyne moieties was achieved by using established procedures for the preparation of selectively functionalised derivatives.[30] By using the known partially propylated derivatives **2**,[31] **3**,[31] **4**[30] and **5**,[32] treatment with an excess of sodium hydride and propargyl bromide resulted in the fully alkylated derivatives **6**, **7**, **8** and **9**, in good yield (Scheme 1).



Scheme 1. Synthesis of partially propargylated calix[4]arenes.

The fully propargylated derivative **10**, in the cone conformation, was prepared by following the method of Ryu and Zhao[33] (Scheme 2). Although single-step preparative methods[32,34] are available for the synthesis of calix[4]arene derivatives fixed in the 1,3-alternate conformation, in our hands these gave complex mixtures of partially and fully alkylated derivatives in various conformations. However, a two-step



Scheme 2. Synthesis of the three conformational isomers of tetrapropargylated calix[4]arenes.

procedure, involving first the synthesis and isolation of the 1,3-dipropargyl derivative **11**[35] followed by reaction with excess propargyl bromide in the presence of caesium carbonate gave a mixture that could be resolved by column chromatography to afford the desired 1,3-alternate conformer **12** in 40% yield and, interestingly, the partial cone conformational isomer **13** in 26% yield (Scheme 2). This isolation of two conformers is in contrast to a recent report from Chetcuti et al.[36] in which similar conditions resulted in the exclusive formation of **12**.

Microwave-assisted copper(I)-catalysed azide–alkyne cycloaddition (CuAAC) was initially applied to the simple monovalent derivative **6** (Table 1, Scheme 3) by using 1.5 equivalents of the galactose azide **14**.[14a] Thus by using

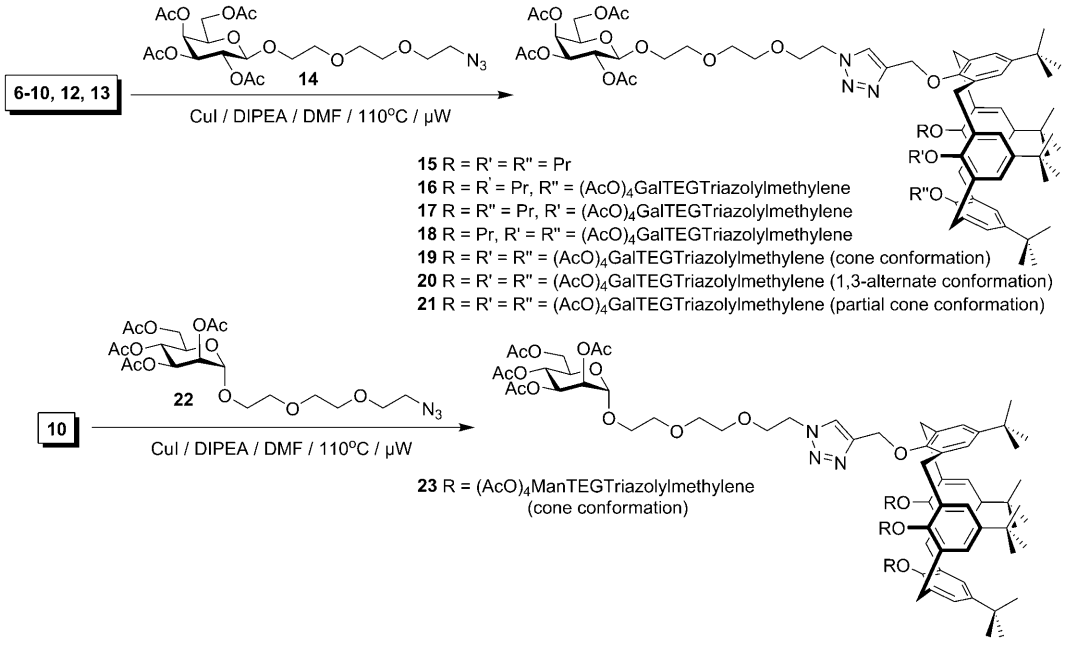
Table 1. Determination of optimum conditions for the cycloaddition of **6** and **14** (1.5 equiv).

Catalytic system	Solvent	Temperature [°C]	Duration[a]
CuI/*i*Pr$_2$NEt[b]	toluene	20	11 days
CuI/*i*Pr$_2$NEt[[b]]	toluene	70	7 h
CuI/*i*Pr$_2$NEt[[b]]	toluene	70[d]	5×15 min
CuI/*i*Pr$_2$NEt[[b]]	toluene	110[d]	3×15 min
CuI/*i*Pr$_2$NEt[[b]]	DMF	20	6 days
CuI/*i*Pr$_2$NEt[[b]]	DMF	110	40 min
CuI/*i*Pr$_2$NEt[b]	DMF	110[d]	15 min
$CuSO_4$/Na Ascorbate[c]	DMF	110[d]	15 min

[a] Total conversion (monitored by TLC). [b] CuI (0.5 equiv)/*i*Pr$_2$NEt (5 equiv). [c] $CuSO_4$ (0.5 equiv)/sodium ascorbate (2 equiv). [d] Microwave irradiation.

the standard catalytic conditions applied by Meldal and coworkers,[12] namely CuI, and *i*Pr$_2$NEt in toluene, a significant rate increase for the reaction was observed both upon thermal and microwave heating. These rates could be further enhanced by the use of DMF, a solvent in which both reagents are freely soluble. Interestingly, similar results were also obtained by using Sharpless' conditions ($CuSO_4$, sodium ascorbate).[11]

With the optimised reaction conditions in hand, the synthesis of calix[4]arene glycoconjugates incorporating galactose and mannose moieties (Scheme 3) was extended to the derivatives **6**–**10**, **12** and **13**. All compounds were fully characterised by NMR spectroscopy (Figure 1) and mass spectrometry. Enhanced yields were observed for less sterically crowded systems, with the mono- and 1,3-disubstituted derivatives **15** and **16**, respectively, being prepared in the highest yield. In the case of the cone conformer **10**, in which all the sugars reside on the same side of the molecule the yield is the lowest (**19**: 52%), and with the 1,3-alternate confor-



Scheme 3. Synthesis of calix[4]arene glycoconjugates by using microwave-assisted CuAAC.

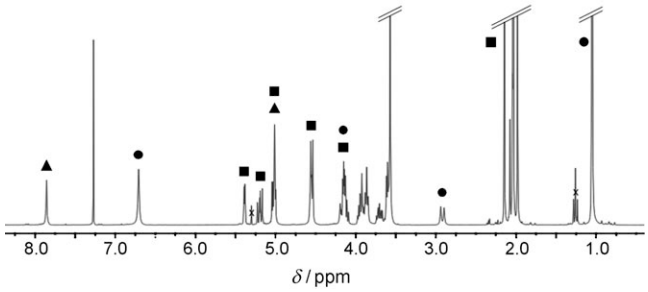


Figure 1. Typical ^{1}H NMR (298 K, $CDCl_3$, 300 MHz) for glycoconjugate **19** and signal assignment. (x) residual solvents, (●) calix[4]arene, (▲) triazolyl, (■) galactosyl moieties.

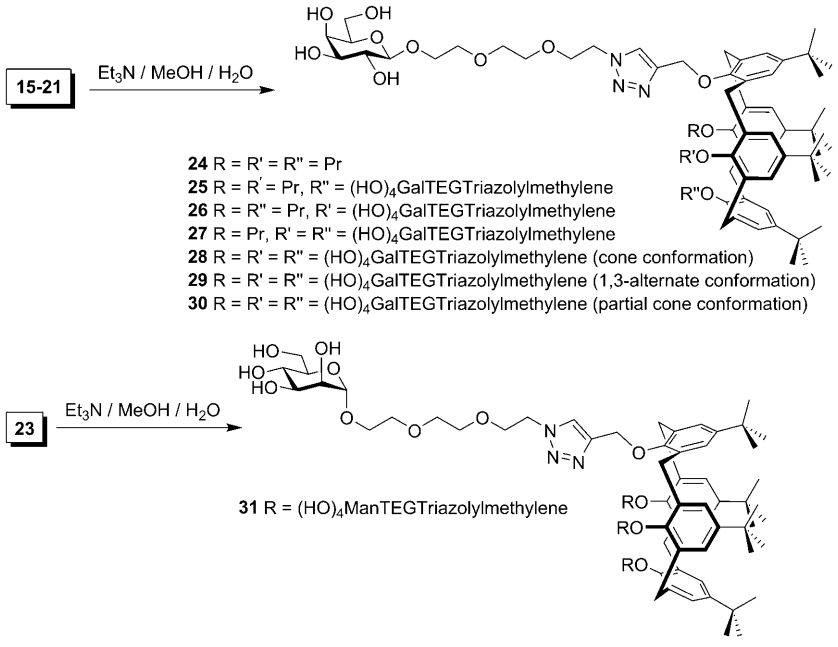
mer **12**, in which the propargyl groups are optimally spaced out relative to each other, the highest (**20**: 79%).

In all cases, the conjugation of carbohydrate moieties to the calix[4]arene scaffolds preserved the symmetry of the parent molecule under the click reaction and hydrolysis synthetic conditions. This was demonstrated by NMR spectroscopic analyses by which the conformation of the glycoconjugates could be clearly identified; this indicated that no ring inversion occurred. Thus, for the tetra-derivatised series **10**, **19** and **23** the ^{1}H NMR signals for the methylene bridge protons appear as a pair of doublets between $\delta = 3$ and 4.5 ppm in each molecule while the singlet that is observed for these protons between $\delta = 3$ and 4 ppm in the 1,3-alternate conformation is conserved on conversion of **12** to **20** and **29**.

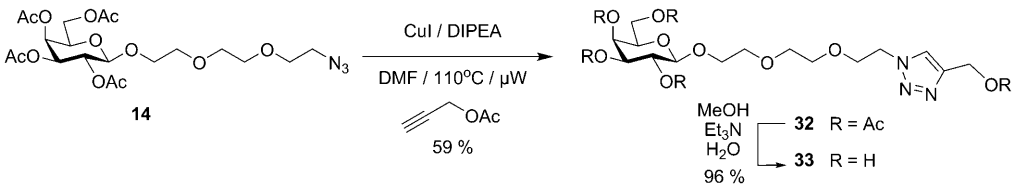
De-acetylation of the glycoconjugates was achieved by using a triethylamine/water/methanol mixture to yield the derivatives **24**–**31**, which were considered for biological studies (Scheme 4). Because the monovalent calix[4]arene glycocluster **24** was not soluble enough in water or a water/DMSO mixture (turbid samples) to enable biological studies, an additional monovalent derivative **33** was synthesised to provide a water-soluble monovalent probe. Cycloaddition of **14** with propargyl acetate afforded the peracetylated cycloadduct **32**, which upon hydrolysis of the ester groups yielded **33** (Scheme 5). The regioisomeric divalent glycoconjugates **25** and **26** also showed poor water solubility but were soluble in a mixture of water and 3% DMSO, whereas the trivalent and tetravalent glycoclusters **27**–**31** were fully soluble in water (>25 mM). Measurement of the critical micelle concentration (CMC) by the pendant-drop method for the water-soluble derivatives **27**–**30** showed that self-assembly occurred only when reaching high-micromolar concentrations.

A cartoon representation of the seven glycoconjugates synthesised (Figure 2) provides a three-dimensional view of the discrete topologies adopted by each glycocluster. We assigned a topology-related designation to each conjugate in which **24**, for example, has a 1:0 topology; that is, each number depicts the number of carbohydrate epitopes present at the lower and upper rim of the calix[4]arene, respectively. The designation for **25** and **26** is 2:0, and 3:0 is the designation for **27**, 4:0 for **28**, 2:2 for **29**, and finally 3:1 for **30**.

The affinities of the designed glycoconjugates were determined by isothermal titration calorimetry (ITC) with addition of ligands to the solution of lectin (see the Supporting Information). Dissociation constants (K_d) and thermody-



Scheme 4. Hydrolysis of the ester protecting groups.



Scheme 5. Synthesis of a water-soluble monovalent probe **33**.

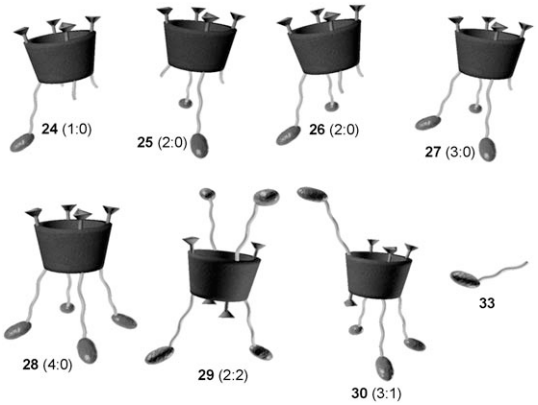


Figure 2. Schematic representation of the seven calix[4]arene glycoconjugates synthesised **24**–**30**, **33** and their topological designation in parentheses. Pyramids = *t*Bu groups, waves = triethyleneglycol-based linkers, ellipsoids = carbohydrate epitopes.

Table 2. Microcalorimetry data of the synthesised glycoconjugates **25**–**31** and monovalent probe **33** binding to PA-IL.[a]

Ligand	Topology	n	$-\Delta H°$ [kJ mol^{-1}][b]	$-T\Delta S°$ [kJ mol^{-1}][b]	$-\Delta G°$ [kJ mol^{-1}][b]	K_d [nM][b]	β[c]
33	n.a.[d]	1[e]	36 ± 1	14 ± 1	22 ± 1	150 000 ± 33000	1
25	2:0	n.a.[d]			no binding observed[f]		
26	2:0	n.a.[d]			no binding observed[f]		
27	3:0	0.79 ± 0.03	28.1 ± 0.5	−4.4 ± 1	32.5 ± 0.5	2050 ± 389	73
28	4:0	0.33 ± 0.01	71 ± 6	34 ± 7	37 ± 1	420 ± 160	357
30	3:1	0.26 ± 0.01	98 ± 9	60 ± 9	38 ± 1	200 ± 5	750
29	2:2	0.24 ± 0.01	104 ± 1	65 ± 1	39 ± 1	176 ± 6	852
31	4:0	n.a.[d]			no binding observed		

[a] The glycoconjugates were titrated into a solution of PA-IL (150, 100 and 50 μM lectin monomer concentrations for experiments with monovalent, trivalent and tetravalent glycoclusters) at 298 K to obtain a final ratio of ligand/lectin of 3:1, 1.5:1 and 0.5:1 for monovalent, trivalent and tetravalent structures respectively. [b] Errors are the standard deviations of 2 or 3 experiments. [c] β is the potency of binding by using the monovalent galactoside **33** as a reference. [d] n.a. = not applicable. [e] Fixed parameter for the fitting procedure. [f] The divalent glycoconjugates **25** and **26** were tested by using 3% DMSO in water and did not display any binding below 0.12 mM representing the solubility limit.

namics parameters are listed in Table 2, together with the binding stoichiometry (n), which is defined as the number of glycocluster per monomer of PA-IL. ITC experiments involving multivalent lectins and multivalent ligands (both are tetravalent in the present study) would typically be prone to pick up aggregation between the binding partners. Even though the ITC peaks for each injection displayed a small portion at their base, which could suggest the formation of aggregates, this did not influence the measurements significantly and provided reproducible and reliable data were obtained (Figure 3).

The best natural ligands of PA-IL are α-linked di-galactosides with dissociation constants (K_d) of about 50 μM as determined by ITC.[29b] The K_d value for the monovalent probe **33** was determined by ITC to be 150 μM and this value was used as the monovalent reference value. Because the binding affinity of **33** is comparable to that observed for galactose and galactoside derivatives, it can be concluded that the triethyleneglycol–triazolyl–methylene linker used in the design of the calix[4]arene glycoconjugates does not influence the binding to the lectin. In addition, the absence of binding observed with the mannosylated derivative **31** can rule out non-specific binding events due to the calixarene core.

The divalent glycoclusters **25** and **26** are not soluble in water but are apparently soluble in a solution containing 3% DMSO. However, no binding to PA-IL could be observed for these compounds at a concentration of 0.12 mM. Experiments involving higher concentrations of divalent ligands could not be performed due to their poor solubility. It was confirmed that this absence of binding is not due to the presence of 3% DMSO because the same concentration did not affect the binding of tetravalent glycocluster **28**.

The trivalent glycoconjugate **27** displays a K_d value of 2 μM. The enthalpic contribution is slightly less favourable than for the monovalent reference ligand **33**, but the entropic parameter highlights a relatively favourable binding event that results in an improvement in the binding to PA-IL by 73-fold (24-fold per galactose residue). The number of PA-IL monomers per molecule of ligand **27** is 0.79; this indicates that a mixture of one ($n = 1$) and two ($n = 0.5$) lectin monomer(s) bound per trivalent ligand is observed in solution.

The tetravalent glycoclusters **28**–**30** are considerably more efficient in terms of dissociation constants with K_d values in the sub-micromolar range. Nevertheless, strong differences are observed in the thermodynamic behaviour of the glycoclusters, depending on their topology. The introduction of a fourth galactose residue in the cone tetravalent conformer **28** in comparison to the trivalent glycocluster **27** creates a 5-fold improvement in affinity and a 360-fold enhancement relative to the monovalent probe **33**. The stoichiometry indicates that three carbohydrate residues are bound to PA-IL ($n = 0.33$), but the enthalpic contribution is doubled when compared to the monovalent probe **33**. This highly positive enthalpic contribution is partly counter-balanced by a slight entropic cost in comparison to the reference compound **33**.

The tetra-galactosylated partial cone compound **30** or 1,3-alternate **29** display a 3:1 or 2:2 topology. Both compounds have very similar binding behaviour. The simple topological change between the cone **28** and the partial cone conformer **30** generates a 2-fold improvement in binding to PA-IL. The observed stoichiometry of $n = 0.26$ suggests that the four carbohydrate epitopes bind to a monomer of PA-IL. The enthalpic contributions of glycocluster **29** and **30** are three times higher than for the monovalent probe **33**, and the entropic costs are more unfavourable as multivalency occurs.[37]

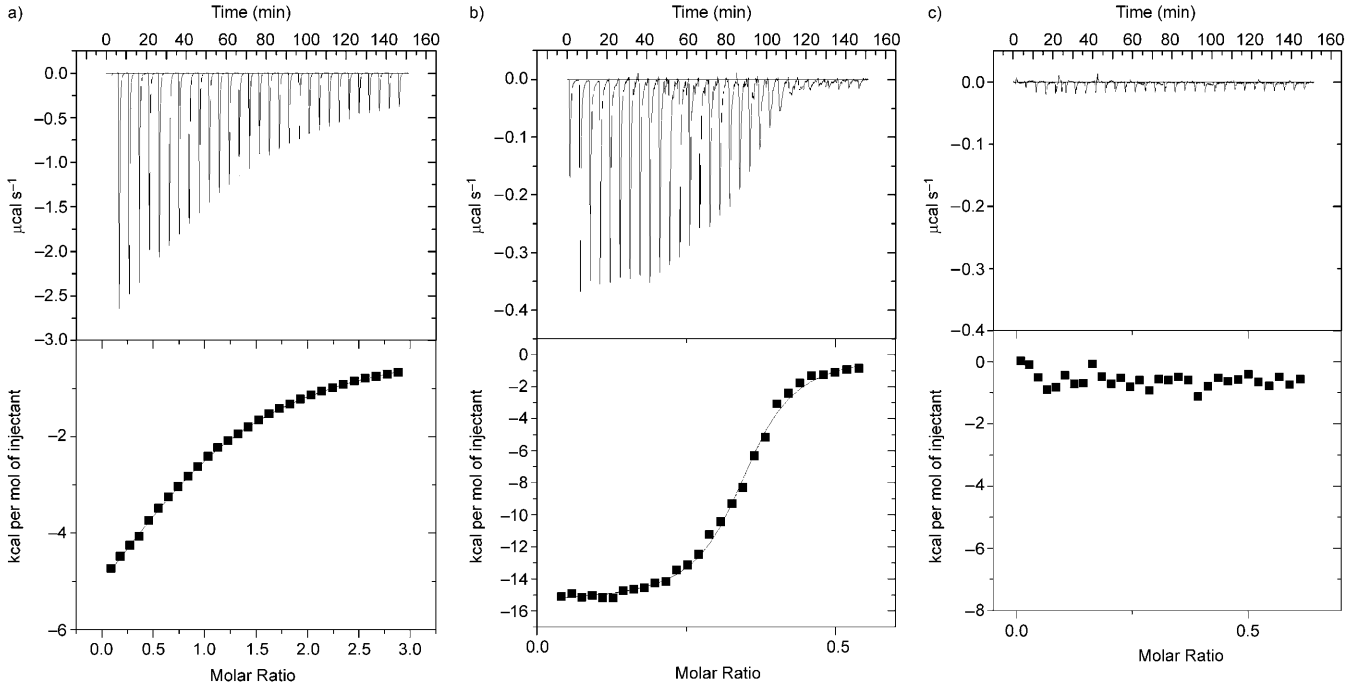


Figure 3. Typical ITC measurements representing the raw ITC data (top) and integrated titration curves (bottom) for the binding of a) monovalent galactoside **33**, b) the galactosylated **28** and c) mannosylated **31** tetravalent cone conformers to PA-IL.

Such tetravalent clusters could interact either with four different PA-IL tetramers, or chelate the binding sites of two PA-IL tetramers. Chelation of the four binding sites of the same PA-IL molecules cannot be envisaged due to the geometry of the protein (Figure 5b). The intermolecular interaction of a single tetravalent ligand with four different tetramers of PA-IL would lead to large aggregates. An intramolecular interaction of two galactose residues with two binding sites of PA-IL on the same face of the rectangular arrangement of the lectin tetramer would result in a so-called "chelate" complex characterised by a highly enthalpic-favourable contribution that is not fully opposed by the entropic cost.[37] The multivalent ligands **29** and **30** display a large enhancement in binding, with a 200-fold increase of efficiency per galactose, which is more in agreement with a chelation mechanism. Limited amount of precipitation was observed in the ITC cell, indicating that aggregation is also occurring during the titration. Additional experiments with dynamic light scattering (see the Supporting Information) also indicated the co-existence of tetrameric and larger oligomeric forms upon addition of small amounts of compound **30**.

To evaluate the anti-adhesive potential of such glycoconjugates, adhesion inhibition experiments were designed. Surface plasmon resonance by using biotinylated polymeric saccharides 'trapped' on a streptavidin-coated chip is an appropriate technique because it mimics the binding of the lectin to glycosylated cell surfaces. The interaction between the lectin and the galactosylated surface was monitored in the absence or presence of high-affinity glycoconjugates that can inhibit the adhesion of the protein (Figure 4). Again,

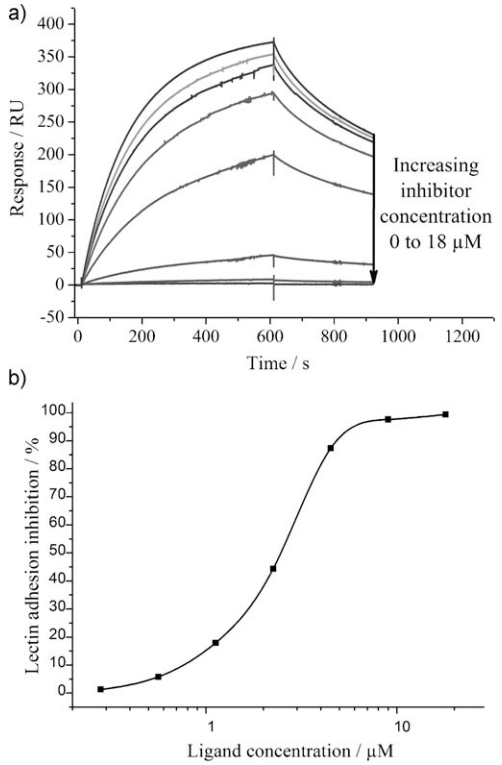


Figure 4. Typical SPR sensorgram measured for tetravalent compound **28** incubated with PA-IL (10 µM) and injected on a CM5 chip coated with Streptavidin/Biotin–PAA–α-D-Galactose. a) sensorgram, b) corresponding inhibition curve. PAA = Polyacrylamide.

the best inhibitors are the tetravalent compounds **29** and **30**, which display galactose residues on both faces (Table 3). In

Table 3. Inhibition of the adhesion of PA-IL to a surface of galactosides determined by SPR.

Ligand	Valency	Topology	K_d[a] [nM]	IC_{50}[b] [nM]	β[c]
33	1	–	150000	71900	1
27	3	3:0	2050	6400	11
28	4	4:0	420	2500	29
30	4	3:1	200	1700	42
29	4	2:2	176	500	144

[a] Dissociation constants obtained from ITC experiments. [b] Minimum concentration of the inhibitor required to prevent 50% of the adhesion of PA-IL onto the galactosylated surface. [c] Relative potency of the multivalent inhibitors compared to the monovalent compound.

addition to confirming results from ITC experiments, the SPR data demonstrate that this family of multivalent glycoconjugates are highly efficient for inhibiting the binding of the bacterial lectin to a glycosylated surface.

Molecular modelling studies were undertaken to assess the ability of the most efficient ligand, **29** to bind to two adjacent binding sites of the tetrameric lectin PA-IL as postulated from ITC experiments. A three-dimensional model of the tetravalent glycocluster **29** was built from the X-ray crystal data of the tetrapropargylated calixarene **12**.[36] From conformational analysis and taking into account the flexibility of the linkers, the maximum distances between galactose residues (O-4) were estimated to be 28 Å for branches on the same side of the calixarene scaffold and 39 Å for branches on opposite faces. Such distances are compatible with the topology of the PA-IL tetramer that has a rectangular shape with binding sites (Ca^{2+}) separated by 32 Å on the small face and 71 Å on the long one (Figure 5a).

Docking calculations were therefore performed on model **29** with two of its galactose residues in both binding sites on the small face of one PA-IL tetramer (Figure 5b). The best results in terms of energy and geometry were obtained with two galactose residues from opposite faces of compound **29**. The two other monosaccharides are therefore available for binding to another PA-IL tetramer as displayed in Figure 5c. Each linker can adopt such an extended conformation without energy penalty. The two lectin dimers face each other without steric conflict. The calixarene scaffold presenting two galactose residues on each of the opposite faces appears therefore to have the optimal topology to provide both intra-tetramer and inter-tetramer bridging.

Conclusions

In conclusion, we have demonstrated that a range of calix[4]arene ligand platforms, with a variety of alkyne loadings and conformational arrangements, are readily prepared in two steps from commercial starting material. Microwave-assisted CuAAC coupling afforded of a series of multivalent calix[4]sugars suitable for investigation of multivalent effects upon binding to lectins. Isothermal titration calorimetry measurements demonstrate that multivalency plays a part in binding to the PA-IL lectin with tetravalent galactose ligands binding most strongly. The enhanced binding of ligands locked in the partial cone and 1,3-alternate conformations indicate the important role that is played by three-dimensional interactions and point towards a chelate-based binding interaction at the protein surface. Surface plasmon resonance studies highlighted a similar behaviour in terms of affinity for PA-IL but also demonstrated the ability of such glycoclusters to act as anti-adhesive molecules for potential applications as antimicrobials. Docking calculations were also performed to confirm the proposed chelate mode of binding of the 1,3-alternate glycocluster, which is the best

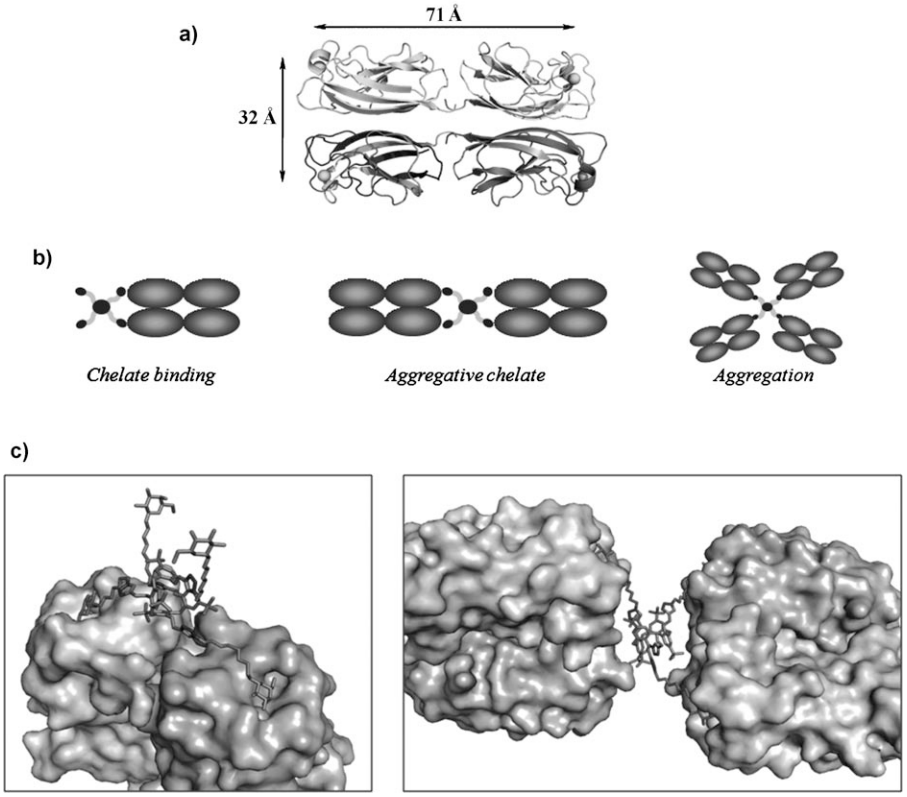


Figure 5. a) Three-dimensional structure of PA-IL tetramer. Calcium ions in binding sites are represented as grey spheres. Distances are measured between calcium ions in binding sites. b) Representation of three possible binding modes. c) Molecular modeling pictures of the chelate (left) and aggregative chelate (right) complexes.

inhibitor known to date for PA-IL with a 800-fold increase in affinity for the lectin in comparison to a monovalent probe.

Experimental Section

Isothermal titration microcalorimetry (ITC): Recombinant PA-IL was produced in *E. coli* and ITC measurements were performed according to our previously reported protocols.[29b] Purified and lyophilised PA-IL was dissolved in buffer (0.1 M Tris–HCl buffer containing 6 μM $CaCl_2$, pH 7.5) at a concentration of 0.05 mM and degassed. Protein concentration was checked by measurement of optical density by using a theoretical molarity extinction coefficient of 28000 (1 cm). Carbohydrate ligands were dissolved directly into the same buffer at a concentration of 0.12 mM, degassed and placed in the injection syringe. Isothermal titration calorimetry was performed with a VP-ITC MicroCalorimeter from MicroCal Incorporated. PA-IL was placed into the 1.4478 mL sample cell, at 25 °C, by using 10 μL injections of carbohydrate every 300 s. Carbohydrate ligands were also titrated alone against the buffer. Data were fitted with MicroCal Origin 7 software, according to standard procedures. Fitted data yielded the stoichiometry (n), the association constant (K_a) and the enthalpy of binding (ΔH). Other thermodynamic parameters (i.e., changes in free energy, ΔG, and entropy, ΔS) were calculated from the equation $\Delta G = \Delta H - T\Delta S = -RT\ln K_a$ in which T is the absolute temperature and $R = 8.314\ \mathrm{J\,mol^{-1}\,K^{-1}}$. Two or three independent titrations were performed for each ligand tested.

Surface plasmon resonance studies (SPR): SPR experiments were performed by using a Biacore T100 instrument at 25 °C by using HBS (Hepes-buffered saline: 10 mM Hepes and 150 mM NaCl, pH 7.4) containing 0.005 % (v/v) Tween 20 and a flow rate of 10 $\mu L\,min^{-1}$. Measurements were carried out on two channels by using two immobilised sugars: α-L-fucose (channel 1) and α-D-galactose (channel 2). Biot–PAA (biotinylated polyacrylamide) saccharidic probes (Lectinity Corp., Moscow, Russia) were trapped on a CM5 sensor chip that was coated with streptavidin by using the following procedure. The chip was activated with EDC (*N*-ethyl-*N*-[3-dimethylaminopropyl]carbodiimide)/NHS (*N*-hydroxysuccinimide) solution (400 s, 5 $\mu L\,min^{-1}$) and streptavidin (600 s, 5 $\mu L\,min^{-1}$) in 10 mM acetate buffer (pH 4.5) was injected into the flow channel. Finally, the sensor surface was blocked with 1 M ethanolamine (400 s, 5 $\mu L\,min^{-1}$); this led to final responses of 1637 and 1594 RU (resonance units) on channel 1 and 2 respectively. Then, each Biot–PAA–monosaccharide (50 μL at concentration 200 $\mu g\,mL^{-1}$, 600 s) was injected into the selected channel; this led to immobilization of 309 RU on channel 1 (fucose) and 283 RU on channel 2 (galactose). Inhibition studies consisted in the injection (600 s, 10 $\mu L\,min^{-1}$) of incubated (1 h, RT) mixtures of PA-IL (10 μM) and various concentrations of inhibitor (2-fold cascade dilutions from 360 to 1.41 μM for monovalent compounds and from 18 to 0.14 μM for multivalent compounds). For each inhibition assay, PA-IL (10 μM) without inhibitor was injected to observe the full adhesion of the lectin onto the sugar-coated surface (0 % inhibition). The CM5 chip was fully regenerated by using two successive injections of D-galactose (30 s, 100 mM in running buffer). Binding was measured as RU over time after blank subtraction (channel 1), and data were evaluated by using the BIAcore T100 evaluation Software, version 1.1. For IC_{50} evaluation, Req (RU corresponding to steady state equilibrium) was taken as the amount of PA-IL bound to the sugar surface in the presence of competing inhibitor. Inhibition curves were obtained by plotting the percentage of inhibition against the inhibitor concentration (on a logarithmic scale) by using Origin 7.0 software (OriginLab Corp.).

Modelling of glycocluster and PA-IL: Construction of the core scaffold of glycocluster **29** was built by using the X-ray structure of **12**.[36] Atomic partial charges were then calculated (MOPAC/MNDO). Carbohydrates epitopes with the triethyleneglycol-based linker were built, and their atomic charges were set by using carbohydrate specific charges.[38] Scaffold and carbohydrates epitopes were connected and the charges were derived and symmetrised to obtain a neutral global charge. The resulting glycocluster was then energy minimised by using the conjugate gradient method and the TRIPOS force field[39] with the addition of carbohydrate parameters.[38]

Only half of the PA-IL tetramer was considered for docking because this was the minimum unit presenting two neighbouring binding sites in close vicinity and accessible for glycocluster **29**. This dimeric lectin was prepared from the X-ray structure (PDB code: 1OKO) after removal of water molecules and carbohydrate ligands. Hydrogen atoms were added, and the Pullman charges were calculated, except for the calcium ions, which were treated with a charge of 2. The positions of hydrogen atoms were optimised with Tripos force field.

Modelling of the interaction between partners: A procedure was developed to model compound **29** with two galactoses in neighbouring of PA-IL binding sites. One galactose epitope of glycocluster **29** was fitted into one of the two binding sites of the dimeric PAIL by overlaying its atoms with the galactose molecule from the PDB structure. A systematic conformational search was performed around 12 rotatable bonds with distance constraint between atom O4 of the second galactose and the calcium ion in the second binding site. The ligands with appropriate conformations were optimised with inclusion of constraint for minimising the distance between the docked galactose (second site) and the "ghost" of the bound galactose in the crystal structure of the complex. After removal of these dummy atoms, several steps of energy minimisation were performed. The last cycle included full optimisation of the whole ligand, with no constraints, to check the stability of the proposed interaction.

Acknowledgements

The authors thank the Association for International Cancer Research (03–256, 08–0062), the University of East Anglia, the University Claude Bernard Lyon 1 and the CNRS for financial support. S.C. thanks the Région Rhône-Alpes (Cluster de Recherche Chimie) and the CNRS (Programme Interdisciplinaire: Chimie pour le Développement Durable) for additional funding. Financial help is acknowledged from Vaincre la Mucoviscidose, the GDR Pseudomonas and the ANR Glycoasterix. The authors thank the EPSRC Mass Spectrometry Service, Swansea for providing analyses. Catherine Gautier's help with lectin production and handling has been much appreciated. The SPR experiments were run thanks to the access to Biacore T100 at Plateforme Nanobio–Grenoble. Dr Yves Chevalier is gratefully acknowledged for CMC measurements and helpful discussions. Professor Jacques Vicens, Dr Rachid Souane and Professor Pierre Thuéry are thanked for their useful discussions and provision of the X-ray crystal data for **12**. Dr D. Bouchu, C. Duchamp and N. Henriques are gratefully acknowledged for mass spectrometry analyses.

[1] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674.
[2] a) A. Varki, *Glycobiology* **1993**, *3*, 97–130; b) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720; c) C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357–2364.
[3] a) Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321–327; b) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555–578.
[4] a) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794; b) R. J. Pieters, *Org. Biomol. Chem.* **2009**, *7*, 2013–2025.
[5] a) R. Roy in *The Chemistry of Neoglycoconjugates* (Ed.: G.-J. Boons), Blackie, London, **1998**, pp. 243–321; b) A. Imberty, Y. M. Chabre, R. Roy, *Chem. Eur. J.* **2008**, *14*, 7490–7499.
[6] M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742–748.
[7] S. L. Flitsch, *Curr. Opin. Chem. Biol.* **2000**, *4*, 619–625.
[8] M. Meldal, C. W. Tornøe, *Chem. Rev.* **2008**, *108*, 2952–3015.
[9] A. Dondoni, *Chem. Asian J.* **2007**, *2*, 700–708.
[10] F. G. Calvo-Flores, J. Isac-Garcia, F. Hernandez-Mateo, F. Perez-Balderas, J. A. Calvo-Asin, E. Sanchez-Vaquero, F. Santoyo-Gonzalez, *Org. Lett.* **2000**, *2*, 2499–2502.

[11] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
[12] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[13] R. Huisgen, G. Szeimies, L. Möbius, *Chem. Ber.* **1967**, *100*, 2494–2507.
[14] a) C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J. P. Cloarec, J. P. Praly, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700–4702; b) A. Meyer, C. Bouillon, S. Vidal, J. J. Vasseur, F. Morvan, *Tetrahedron Lett.* **2006**, *47*, 8867–8871; c) Y. Chevolot, C. Bouillon, S. Vidal, F. Morvan, A. Meyer, J.-P. Cloarec, A. Jochum, J.-P. Praly, J.-J. Vasseur, E. Souteyrand, *Angew. Chem.* **2007**, *119*, 2450–2454; *Angew. Chem. Int. Ed.* **2007**, *46*, 2398–2402; d) F. Morvan, A. Meyer, A. Jochum, C. Sabin, Y. Chevolot, A. Imberty, J.-P. Praly, J.-J. Vasseur, E. Souteyrand, S. Vidal, *Bioconjugate Chem.* **2007**, *18*, 1637–1643; e) L. Moni, G. Pourceau, J. Zhang, A. Meyer, S. Vidal, E. Souteyrand, A. Dondoni, F. Morvan, Y. Chevolot, J.-J. Vasseur, A. Marra, *ChemBioChem* **2009**, *10*, 1369–1378; f) J. Zhang, G. Pourceau, A. Meyer, S. Vidal, J.-P. Praly, E. Souteyrand, J.-J. Vasseur, F. Morvan, Y. Chevolot, *Biosens. Bioelectron.* **2009**, *24*, 2515–2521.
[15] a) M. V. Gil, M. J. Arévalo, O. López, *Synthesis* **2007**, 1589–1620; b) G. Pourceau, A. Meyer, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2008**, *73*, 6014–6017; c) G. Pourceau, A. Meyer, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2009**, *74*, 1218–1222.
[16] a) A. Dondoni, A. Marra, *J. Org. Chem.* **2006**, *71*, 7546–7557; b) A. Vecchi, B. Melai, A. Marra, C. Chiappe, A. Dondoni, *J. Org. Chem.* **2008**, *73*, 6437–6440.
[17] S. P. Bew, R. A. Brimage, N. L'Hermite, S. V. Sharma, *Org. Lett.* **2007**, *9*, 3713–3716.
[18] G. M. L. Consoli, F. Cunsolo, C. Geraci, V. Sgarlata, *Org. Lett.* **2004**, *6*, 4163–4166.
[19] D. Arosio, M. Fontanella, L. Baldini, L. Mauri, A. Bernardi, A. Casnati, F. Sansone, R. Ungaro, *J. Am. Chem. Soc.* **2005**, *127*, 3660–3661.
[20] K. Krenek, M. Kuldova, K. Hulikova, I. Stibor, P. Lhotak, M. Dudic, J. Budka, H. Pelantova, K. Bezouska, A. Fiserova, V. Kren, *Carbohydr. Res.* **2007**, *342*, 1781–1792.
[21] S. André, F. Sansone, H. Kaltner, A. Casnati, J. Kopitz, H. J. Gabius, R. Ungaro, *ChemBioChem* **2008**, *9*, 1649–1661.
[22] A. Marra, L. Moni, D. Pazzi, A. Corallini, D. Bridi, A. Dondoni, *Org. Biomol. Chem.* **2008**, *6*, 1396–1409.
[23] a) N. Gilboa-Garber, *Methods Enzymol.* **1982**, *83*, 378–385; b) A. Imberty, M. Wimmerová, E. P. Mitchell, N. Gilboa-Garber, *Microbes Infect.* **2004**, *6*, 221–228.
[24] C. Chemani, A. Imberty, S. de Bentzmann, M. Pierre, M. Wimmerova, B. P. Guery, K. Faure, *Infect. Immun.* **2009**, *77*, 2065–2075.
[25] H. P. Hauber, M. Schulz, A. Pforte, D. Mack, P. Zabel, U. Schumacher, *Int. J. Med. Sci.* **2008**, *5*, 371–376.
[26] a) K. Marotte, C. Préville, C. Sabin, M. Moumé-Pymbock, A. Imberty, R. Roy, *Org. Biomol. Chem.* **2007**, *5*, 2953–2961; b) K. Marotte, C. Sabin, C. Préville, M. Moumé-Pymbock, M. Wimmerova, E. P. Mitchell, A. Imberty, R. Roy, *ChemMedChem* **2007**, *2*, 1328–1338; c) E. M. V. Johansson, S. A. Crusz, E. Kolomiets, L. Buts, R. U. Kadam, M. Cacciarini, K.-M. Bartels, S. P. Diggle, M. Cámara, P. Williams, R. Loris, C. Nativi, F. Rosenau, K.-E. Jaeger, T. Darbre, J.-L. Reymond, *Chem. Biol.* **2008**, *15*, 1249–1257.
[27] E. Kolomiets, M. A. Swiderska, R. U. Kadam, E. M. Johansson, K. E. Jaeger, T. Darbre, J. L. Reymond, *ChemMedChem* **2009**, *4*, 562–569.
[28] I. Deguise, D. Lagnoux, R. Roy, *New J. Chem.* **2007**, *31*, 1321–1331.
[29] a) G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerova, D. Sudakevitz, S. Pérez, N. Gilboa-Garber, A. Imberty, *FEBS Lett.* **2003**, *555*, 297–301; b) B. Blanchard, A. Nurisso, E. Hollville, C. Tétaud, J. Wiels, M. Pokorná, M. Wimmerová, A. Varrot, A. Imberty, *J. Mol. Biol.* **2008**, *383*, 837–853.
[30] S. E. Matthews, M. Saadioui, V. Bohmer, S. Barboso, F. Arnaud-Neu, M. J. Schwing-Weill, A. G. Carrera, J. F. Dozol, *J. Prakt. Chem.* **1999**, *341*, 264–273.
[31] K. Iwamoto, K. Araki, S. Shinkai, *Tetrahedron* **1991**, *47*, 4325–4342.
[32] K. Iwamoto, K. Araki, S. Shinkai, *J. Org. Chem.* **1991**, *56*, 4955–4962.
[33] E. H. Ryu, Y. Zhao, *Org. Lett.* **2005**, *7*, 1035–1037.
[34] W. Verboom, S. Datta, Z. Asfari, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1992**, *57*, 5394–5398.
[35] Z. Asfari, A. Bilyk, C. Bond, J. M. Harrowfield, G. A. Koutsantonis, N. Lengkeek, M. Mocerino, B. W. Skelton, A. N. Sobolev, S. Strano, J. Vicens, A. H. White, *Org. Biomol. Chem.* **2004**, *2*, 387–396.
[36] M. J. Chetcuti, A. M. J. Devoille, A. B. Othman, R. Souane, P. Thuery, J. Vicens, *Dalton Trans.* **2009**, 2999–3008.
[37] T. K. Dam, C. F. Brewer, *Chem. Rev.* **2002**, *102*, 387–430.
[38] A. Imberty, E. Bettler, M. Karababa, K. Mazeau, P. Petrova, S. Pérez in *Building Sugars: The Sweet Part of Structural Biology* (Eds.: M. Vijayan, N. Yathindra, A. S. Kolaskar), Indian Academy of Sciences and Universities Press, Hyderabad, **1999**, pp. 392–409.
[39] M. Clark, R. D. I. Cramer, N. van den Opdenbosch, *J. Comput. Chem.* **1989**, *10*, 982–1012.

Received: June 30, 2009
Published online: October 26, 2009

