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Thiourea-linked upper rim calix[4]arene neoglycoconjugates: synthesis, conformations and binding properties †

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The thiourea group has been exploited to link two or four carbohydrate units at the upper rim of tetrapropoxycalix[4]-arene derivatives in the cone conformation. Two synthetic methodologies were used, the first one consisting of the condensation of di- and tetraminocalix[4]arenes with the isothiocyanate of monosaccharides in dry CH_2Cl_2 at room temperature and the second one exploiting the condensation of an aminolactoside with a calixarene isothiocyanate. The first method allows the glycoconjugates to be obtained in 75–80% overall yields. The difunctionalised derivatives exist in a closed flattened cone conformation in $CDCl_3$ and CD_3OD due to the formation of intramolecular hydrogen bonds involving the thiourea groups which are broken in DMSO-d_6 to give an open flattened cone conformation. The thiourea groups act not only as linkers but also as binding units for anionic substrates as evidenced by solution ^{1}H NMR and ESI-MS experiments. Turbidimetric analysis indicates that the tetraglucoside and tetragalactoside clusters give specific interactions with Concanavalin A (Con A) and peanut lectin (PNA), respectively. Both features show that the neoglycoconjugates could also be used as site specific molecular delivery systems.

Introduction

In the last few years, we have been engaged in the synthesis of hybrid macrocyclic receptors characterised by the presence of polar binding groups in close proximity to a hydrophobic cavity, which are attractive for studying the selective recognition of bio-relevant species.[1]

In this context our attention was primarily devoted to calixarene conjugates with amino acids[1b,2] and carbohydrates.[3] The main motivation for the synthesis of macrocycle-based glycoconjugates[4] is the possibility they offer of studying the so-called cluster glycoside effect,[5] which is responsible for the tight binding of sugars to proteins *via* multivalency.[6] Moreover, calixarene-based glycoconjugates are also attractive as a novel type of amphiphilic carbohydrates able to self-assemble and give interesting supramolecular aggregates.[7]

Several synthetic procedures to link sugar moieties to phenolic oxygen atoms of calix[4]arenes (lower rim) are known,[3a,8] but only a few are reported for the upper rim (aromatic nuclei) which use the formation of ether[3b,9] or carbon–carbon bonds.[9c,10] We envisaged the possibility of synthesising new upper rim glycocalix[4]arenes exploiting the formation of thioureas, a method which has been successfully used to obtain cyclodextrin-scaffolded glycoclusters.[11] In addition, the thiourea groups could be used as binding units for anionic species thus conferring host–guest properties to the glycoclusters and making them attractive as novel site specific molecular delivery systems.

Results and discussion

Synthesis

The synthesis of calix[4]arene glycoconjugates **9**–**12** is outlined in Scheme 1.

We decided, for our purposes, to use as glycosyl donors the isothiocyanates **3** and **4** in the β-form which were synthesised through the method proposed by Lindhorst and co-workers,[12] melting the corresponding glycosyl bromides[13] with KSCN. In the case of the galactosyl derivative we found that a more reproducible procedure, which gives the β-galactosyl isothiocyanate **4** in 50% yield (β/α = 8/1), is to heat the bromide dissolved in acetone with KSCN at 110 °C for 3 h in a sealed tube.

As glycosyl acceptors we selected the conformationally locked cone di- and tetraminocalix[4]arenes **1** and **2**. They are both known compounds,[14] but we modified the procedures for their synthesis in order to improve the yield and the isolation of the products in pure form. To this end, we treated the corresponding nitro derivatives[15] with hydrazine in presence of Pd/C (10%) at 70 °C or, alternatively, with Pd/C in hydrogen atmosphere (2 bar), achieving in both cases the desired amino derivatives in 95% yield, after very easy work-up procedures.[16]

The condensation reactions between the di- and tetraminocalix[4]arenes **1** and **2** and the glycosyl isothiocyanates **3** and **4** were performed in dry CH_2Cl_2 at room temperature for 24 h. The compounds **5**–**8** were isolated after flash chromatography in 75–80% yield and quantitatively deprotected to the corresponding derivatives **9**–**12** with the Zemplen method (CH_3ONa, CH_3OH).

We tried to extend this procedure to the synthesis of lactose containing derivatives, but we were not able to prepare the corresponding lactosyl isothiocyanate, either by melting or by solution methods. For this reason, we investigated an alternative possibility *via* the β-lactosyl azide[17] which was reduced to the amino derivative **13**. This was then reacted with the diisothiocyanate calix[4]arene **14**[14b] to give the bislactosyl compound **15**, subsequently deprotected to **16** (Scheme 2). However, the yield of this reversed condensation procedure was significantly lower than the previous ones and therefore we decided not to apply it to the known tetraisothiocyanate calix[4]arene derivative.[14b] All new compounds were fully characterised by NMR, MS and elemental analysis.

The difunctionalised glycocalixarenes **9** and **10** are soluble in acetone, methanol and DMSO, while the tetraglucoside derivative **11** is soluble in pure water up to 5×10^{-5} M concentration which increases to 1×10^{-4} M in 80/20 H_2O/DMSO. The tetragalactoside receptor **12** is less soluble in water compared with **11** (1.2×10^{-5} M).

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra in different solvents of compounds **5**–**12**, **15** and **16**, dynamic ^{1}H NMR experiments on compounds **5** and **9** and NOESY 2D spectra of compounds **5** and **12**. See http://www.rsc.org/suppdata/ob/b3/b301595e/

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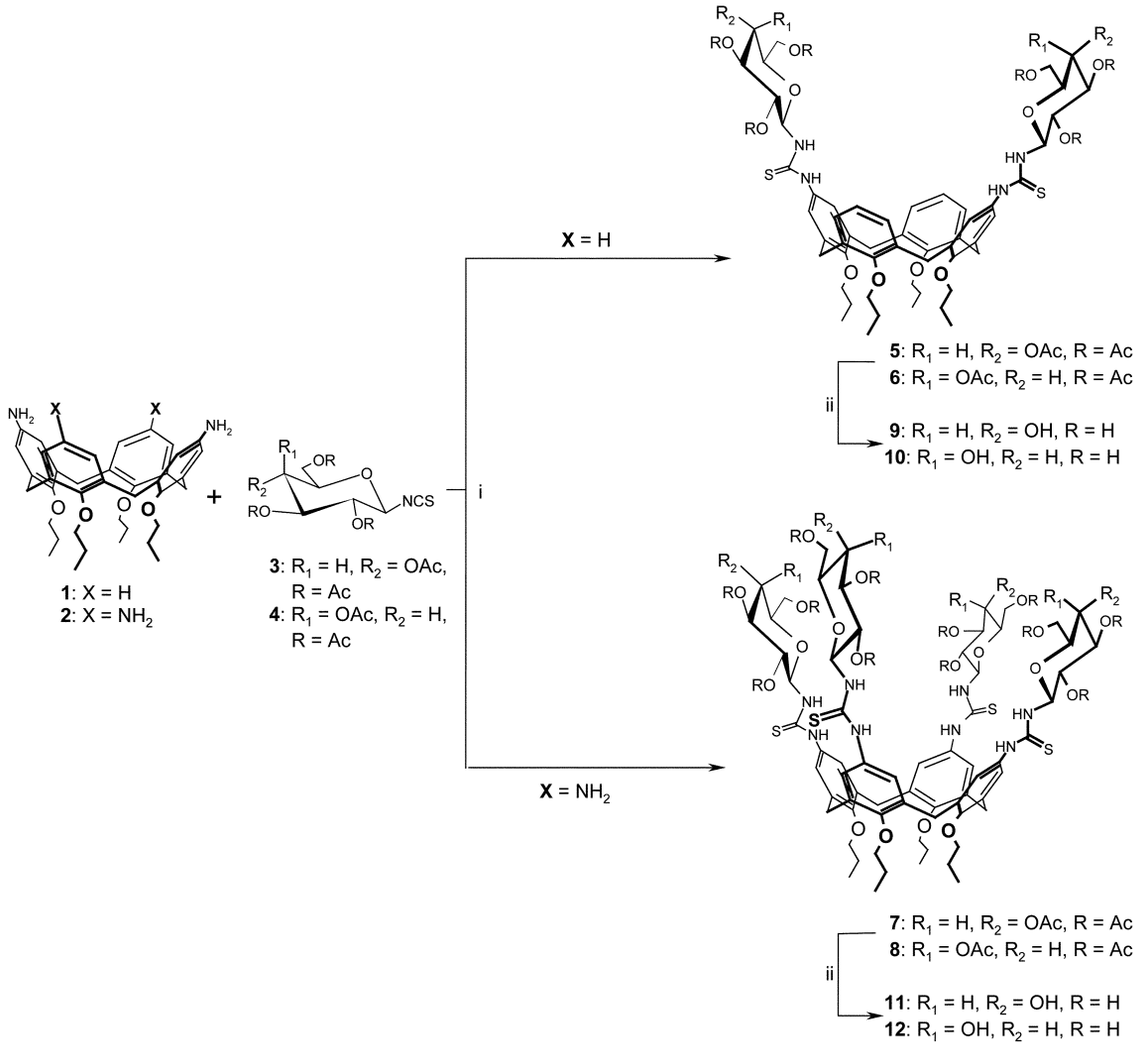
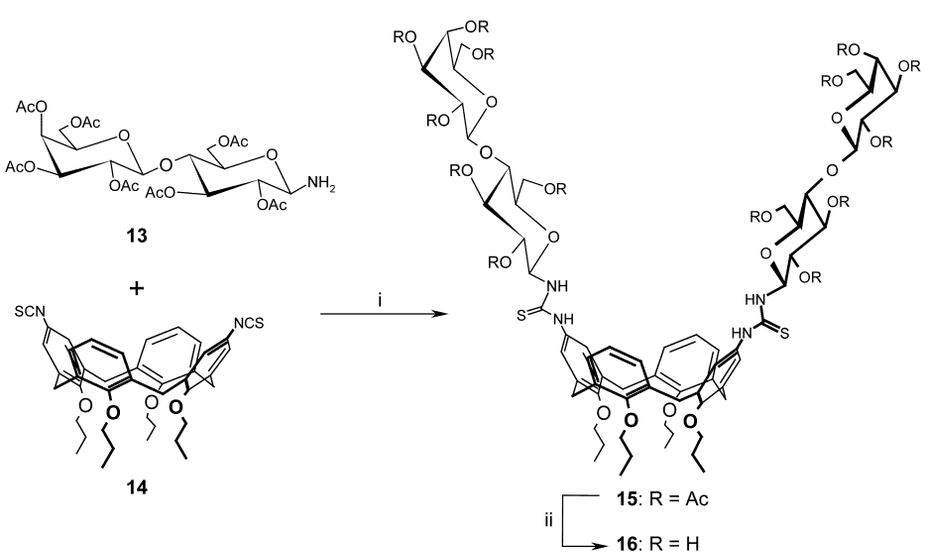


Scheme 1 *Reagents and conditions*: i, CH_2Cl_2, r.t., 24 h; ii, $NaOCH_3$, CH_3OH, r.t., 30 min.



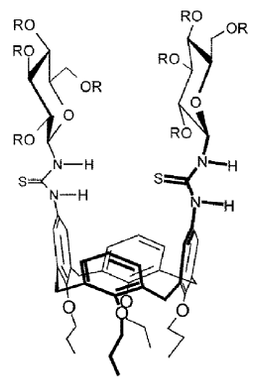
Scheme 2 *Reagents and conditions*: i, DMF, 100 °C, 24 h; ii, $NaOCH_3$, CH_3OH, r.t., 30 min.

Conformational properties

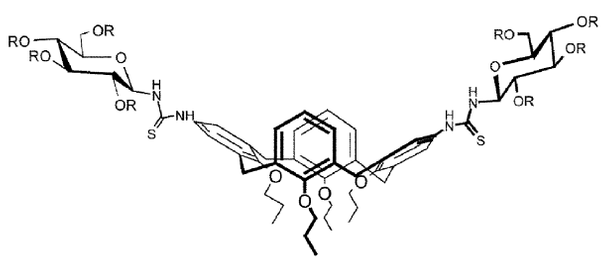
It is well known that cone tetraalkylated calix[4]arenes are not rigid molecules but they experience a residual mobility between two flattened cone (also called pinched cone) conformations, which are usually fast exchanging on the NMR time scale at room temperature.[18] The populations of the two conformers and the rate of interconversion between them depend on solvent, steric bulkiness and hydrogen bonding ability of the substituents at the upper rim.[2e,19] The ^{1}H NMR spectrum of compound **5** in $CDCl_3$ (see ESI †) shows, at room temperature, two doublets at $\delta = 7.10$ and 7.07 and a triplet at $\delta = 6.91$ which account for the six protons of the unsubstituted aromatic rings. The two signals (one broad) belonging to the functionalised

aromatic rings appear at a quite high field (δ = 6.08 and 6.03). In DMSO-d_6(see ESI †) the relative positions of the two sets of aromatic protons are inverted, the signals belonging to the substituted rings moving downfield (δ = 7.03 and 7.01) and those relative to the unsubstituted rings moving upfield (6.48–6.32 ppm). Similar data were also obtained with compound **6**.

This behaviour is indicative[2e,19] of a conformational change (Fig. 1) from a closed flattened cone conformation, stable in $CDCl_3$ because of intramolecular hydrogen bonding between the two upper rim substituents, to the more classical open flattened cone conformation, evidenced in DMSO-d_6.



closed flattened cone



open flattened cone

Fig. 1 The two flattened cone conformations of the difunctionalised glycosylthioureido calix[4]arenes.

Surprisingly, the ^{1}H NMR spectra in CD_3OD of compounds **5** (see ESI †) and **6** are similar to those observed in $CDCl_3$, thus indicating the closed conformation is more stable even in a polar, protic solvent such as methanol. Since a very similar solvent dependent conformational behaviour is also observed with the *O*-deprotected derivatives **9** and **10**, which have several additional OH groups potentially able to form hydrogen bonds, we conclude that the H-bonding interactions which stabilise the closed conformation are those involving the thiourea groups and, possibly, only the glycoside endocyclic oxygen atoms, which are know to interact with hydrogen bonding donor groups.[20a,d]

The ^{1}H and ^{13}C NMR spectra of the tetrafunctionalised receptors **7**, **8**, **11** and **12** show the expected C_4 symmetry and no particular conformational feature connected with the calix[4]-arene ring mobility in all solvents tested ($CDCl_3$, CD_3OD and DMSO-d_6).

As observed with most thioureido sugars,[20] the ^{1}H NMR spectra of all glycocalix[4]arenes synthesised in this work show some broad signals, in particular the NHs, the aromatic and the anomeric protons, even in solvents of high donicity such as DMSO-d_6. This broadening must be mainly attributed to the restricted rotation of the pseudo-amide NH–C=S bonds of the thiourea units and the consequent equilibrium among different rotamers (Fig. 2), excluding the *E*,*E* rotamer because of the two bulky substituents. The relatively low-field chemical shifts of the H1 anomeric proton (δ = 5.90–5.20) observed for these compounds suggest the parallel disposition of the C–H1 bond with respect to the C=S bond[20] and, as a consequence, the *anti* disposition of the sulfur atom and H_B. NOESY experiments performed in DMSO-d_6 at 300 K with the tetragalactosyl-thioureido derivative **12** (see ESI †) evidenced the presence of an intense cross peak between H-2 of the glycoside unit and the closest NH proton (H_B), which, thus, is in *anti* position with respect to H1. The much more intense cross peak between the two different NH protons (H_A and H_B) indicates, together with the other spectral data, *Z*,*Z* (Fig. 2) as the predominant conformation of the thiourea units at room temperature. On the other hand, a weaker but significant cross peak between H2 and $H_A$can be explained by the presence in solution of a lower percentage of a *Z*,*E* conformation (*Z*,E_1 in Fig. 2). Moreover, a very weak correlation between H_A and H-1 is consistent with a second *Z*,*E* rotamer (*Z*,E_2 in Fig. 2), while the correlation between H_B and the aromatic protons in *ortho* positions to the thiourea suggests the presence of the *E*,*Z* rotamer (Fig. 2). In the case of the disubstituted, protected compound **5** similar results are observed, even if the *Z*,*Z* rotamer seems to be largely the most abundant in solution on the basis of the cross peak intensities. Similar data were also collected for all other glycocalixarenes **6**–**11**.

Dynamic ^{1}H NMR studies in $CDCl_3$, CD_3OD and DMSO-d_6 with both the protected and the deprotected glycosyl derivatives show a progressive sharpening of the signals by heating. Upon cooling (273–213 K) in $CDCl_3$ and CD_3OD, the signals split into more complicated patterns because of the progressive freezing of the different rotamers of the thiourea units. The observed coalescence temperatures in $CDCl_3$ for the thioureido NH protons of the glycocalix[4]arenes **5**–**8** and **15**(290–280 K) are in agreement with literature data on sugar thiourea derivatives.[21] As expected, in the explored temperature range (350–213 K), no interconversion is evidenced from a flattened cone conformer to the other, showing that the observed dynamic phenomenon is only related to the thiourea isomerism.

No evidence for the formation of self-assembled dimeric capsules of compounds **7** and **8**, which are known to be easily formed with upper rim tetraurea calix[4]arene derivatives,[22] was obtained by MALDI-TOF mass spectra, ^{1}H NMR dilution

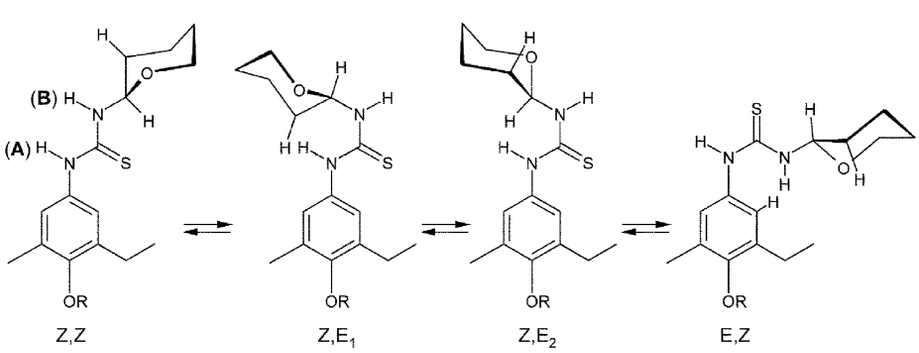


Fig. 2 Possible rotamers of the glycosylthioureido groups in the calixarene derivatives.

experiments and CD spectra[15e] in apolar solvents. With the same type of experiments, the presence of dimeric species of the bisglycosylated compounds **5**, **6** and **9**, **10**, which could be also in agreement with the observed ^{1}H NMR data, was also ruled out in both $CDCl_3$ and CD_3OD.

Binding properties

Multivalent glycosylated ligands can simultaneously interact with several equivalent binding sites of macromolecular receptors, such as lectins and antibodies.[23] Tailored ligands having a number of exposed carbohydrate units corresponding to that of the receptor recognition sites can sometime form 1 : 1 complexes with it,[24] although, more generally, the synthetic glycoclusters give cross-linking interactions among different receptor molecules, causing their agglutination.[25] An easy method to detect this ligand-mediated aggregation of proteins is turbidimetric analysis,[25] which allows microprecipitation in a solution of the protein after addition of the cluster to be checked, following the absorbance increase. In this way, we evaluated the ability of the tetraglycosylated derivatives **11** and **12** to interact with Concanavalin A (Con A), a selective α-D-glucoside and α-D-mannoside binding lectin, and with peanut lectin (PNA, *Arachis hypogaea*), that specifically binds β-D-galactosides and *N*-acetyl-D-galactosamine.[26] The addition of glycocalixarene **11** to a solution of Con A caused a rapid increase of the absorbance values which were registered over a period of 4.5 h (Fig. 3), until they were stable. The observation for 4.5 h of a reference suspension of the calixarene derivative showed that no absorbance changes occur in the absence of Con A, as for a Con A solution in the absence of **11**. At that point, a 400-fold molar amount of D-glucose was added and a decrease of turbidity took place, although it was not sufficient to reduce the absorbance to the starting value. The same amount of D-galactose did not cause any change in the absorbance values of the Con A/**11** mixture evidencing the specific involvement of the glucose units of **11** in the interaction with this lectin. Moreover, a solution of PNA did not become turbid with compound **11** and no turbidity change was observed by adding the galactose containing calixarene **12** to the Con A solution. On the other hand, the galactocalix[4]arene **12** was able to agglutinate PNA lectin and this process was also partially inhibited by adding an excess of D-galactose, as expected on the basis of the known carbohydrate selectivity properties of this lectin.

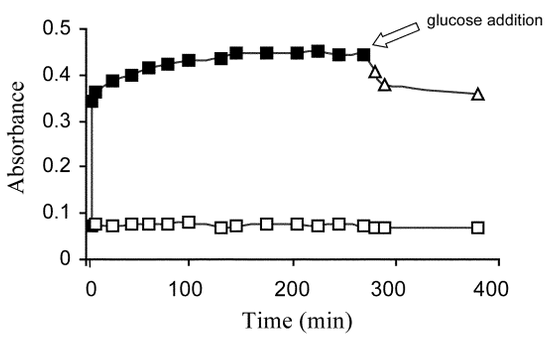


Fig. 3 Turbidimetric analysis (25 °C, $\lambda = 350$ nm): absorbance increase for a mixture of **11** and Concanavalin A (-■-); absorbance decrease after addition of D-glucose (-Δ-); absorbance of **11** (-□-) used as reference.

A second attractive feature of the glycoconjugates synthesised is the presence of a hydrophobic cavity in close proximity to the thiourea groups, a circumstance which could confer host properties to our compounds. This would be interesting since selected guests could be transported by the glycocluster toward specific organs which recognise the exposed sugar units. Since the thiourea groups are known to interact with anionic guests[27] we investigated the anion recognition properties of the glycocalixarenes **9**–**12**. For the tetrafunctionalised receptors **11** and **12** the addition of the tetrabutylammonium salts of several anions in DMSO-d_6 causes a large broadening of the signals relative to the thiourea N–H protons, which did not allow the evaluation of the association constants. However, in the case of the bisglucosyl derivative **9** consistent downfield shifts of the N–H protons were observed upon titration with several anionic species in the same solvent. Moreover, a downfield shift of the signals of the unsubstituted aromatic rings and a more evident upfield shift of those relative to the substituted ones suggest a conformational rearrangement of the receptor to bring both the thiourea units close to the anionic substrates. Job plots[28] and non-linear least squares analysis of the titration data[29] gave a 1 : 1 stoichiometry for the complexes and allowed the determination of the association constants reported in Table 1. The values of K_{ass} are low because of the competition of a solvent with high donicity such as DMSO. Considering that going from DMSO to an apolar solvent such as $CDCl_3$ one has to expect an increase of the association constants of at least one order of magnitude,[30] the data reported in Table 1 reveal a trend which deserves some comments. The evidenced selectivity order benzyl phosphonate > benzoate > dihydrogen phosphate > *N*-acetyl-L-alaninate > β-D-glucose-6-phosphate > chloride > acetate indicates that, beside the primary interaction of the anionic centre with the thiourea N–H groups, which seems to be stronger with the phosphate containing guests, other factors affect binding. The presence of the aromatic ring in the guest improves the binding, probably because of favourable interactions with the sugar backbone (Fig. 4).[31]

Table 1 Association constants (K_{ass}/M^{-1}) for receptor **9** determined in DMSO-d_6 by ^{1}H NMR titration experiments (300 MHz, 300 K)

Substrate[a]	K_{ass}[b]
$H_2PO_4^-$	90
Cl^-	31
CH_3COO^-	17
N-Acetyl-L-alaninate	58
β-D-Glucose-6-phosphate	36
Benzoate	103
Benzylphosphonate	170

[a] As tetrabutylammonium salts. [b] Errors within 10%.

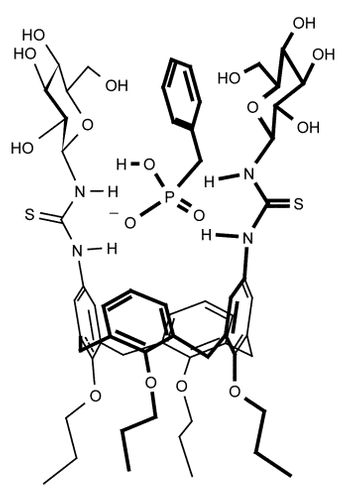


Fig. 4 Proposed mode of binding of benzylphosphonate to **9**.

The preference of the glycoconjugates **9** and **11** for the binding of phosphate containing guests was also confirmed by competitive ESI-MS experiments with mixtures of different anions in methanol. An equivalent of each anionic species (as tetrabutylammonium salts) was allowed to equilibrate with one equivalent of host in methanol solution before registering the mass spectra (Fig. 5). In both cases the molecular peak of

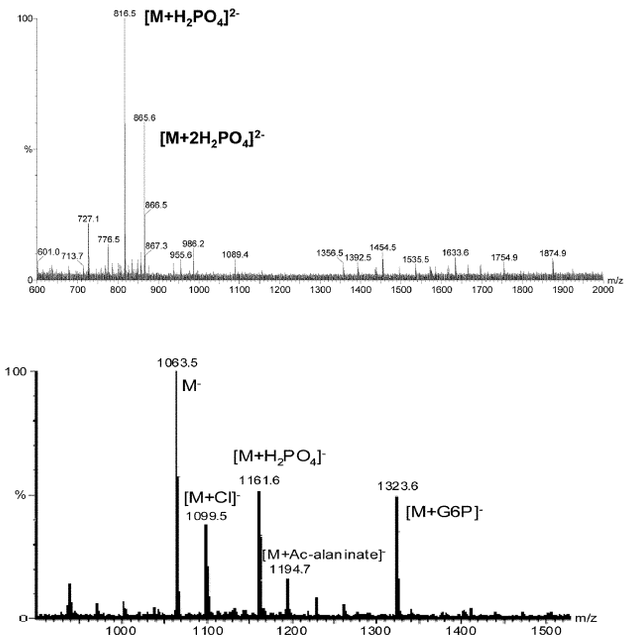


Fig. 5 Top: ESI-MS spectrum of a mixture containing receptor **11** and Cl^-, Br^-, I^-, HSO_4^- and $H_2PO_4^-$. Bottom: ESI-MS spectrum of a mixture containing receptor **9** and Cl^-, Br^-, $H_2PO_4^-$, glucose-6-phosphate (G6P), acetate, acetyl-L-alaninate.

the 1 : 1 host : guest complex with $H_2PO_4^-$ is the most intense in the spectra. Furthermore, since the molecular peak of the free ligand is almost undetectable in the case of the tetrafunctionalised receptor **11**, while it is the base peak in the experiment with the difunctionalised derivative **9**, we conclude that the anion binding ability of the former is significantly higher.

Conclusions

Thiourea linked upper rim calix[4]arene glycoconjugates with exposed two or four glucose, galactose and lactose units have been synthesised for the first time. Dynamic ^{1}H NMR experiments in solvents of different polarity and NOESY two-dimensional spectra allowed the disclosure of the conformational features of the neoglycoconjugates at the level of both the thiourea bonds and the calix[4]arene skeleton. Turbidimetric experiments show that the tetraglucosyl and tetragalactosyl derivatives specifically bind to Concanavalin A (Con A) and peanut lectin (PNA, *Arachis hypogaea*), respectively, through multivalent interactions which are, for the first time, evidenced for upper rim calix[4]arene glycoclusters. The binding studies performed by ^{1}H NMR and electrospray mass spectrometry (ESI-MS) show both di- and tetrafunctionalised macrocyclic hosts **9**–**12** are able to preferentially complex anionic guests having an aromatic tail. These feature make the new glycoclusters attractive as possible site specific molecular delivery systems.

Experimental

All moisture sensitive reactions were carried out under nitrogen atmosphere. All dry solvents were prepared according to standard procedures and stored over molecular sieves. Melting points were determined in capillaries sealed under nitrogen on an Electrothermal apparatus and are uncorrected. ^{1}H (300 and 400 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on Bruker AC300 and AMX400 spectrophotometers (partially deuterated solvents were used as internal standards). Coupling constant (J) values are given in Hz. Mass spectra were recorded in ESI mode on a Micromass ZMD. Optical rotations were measured at 20 °C on a Perkin Elmer 241 Polarimeter, using the wavelength at 589 nm; $[a]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed using a Termoquest 1112 CHNSO instrument and are reported as percentages. TLC was performed on silica gel Merck 60 F_{254}, and flash chromatography using 32–63, 60 Å ICN silica gel. 5,17-Dinitro-25,26,27,28-tetra-*n*-propoxycalix[4]arene,[15a,b] 5,11,17,23-tetranitro-25,26,27,28-tetra-*n*-propoxycalix[4]arene[15] and 5,17-diisothiocyanate-25,26,27,28-tetra-*n*-propoxycalix[4]arene[14b] were prepared according to literature procedures.

NMR Titration experiments

Titration experiments were performed in DMSO-d_6 by titrating a 5×10^{-3} M solution of the receptor with a 4.4×10^{-2} M solution of the guest, varying the host/guest ratio from 2 : 1 to 1 : 9.

Tetrabutylammonium salts

All tetrabutylammonium salts, excluding $H_2PO_4^-$ and Cl^-, were prepared by adding 1 equiv. of a freshly titrated solution of tetrabutylammonium hydroxide in methanol to a solution of the corresponding acid in methanol. The mixture was stirred at room temperature for 2 h and evaporated to dryness under reduced pressure. The resulting deliquescent solid or syrup was dried at high vacuum for 24 h, checked by NMR and stored in a desiccator.

ESI-MS complexation studies

10 µl of 5×10^{-3} M solutions of the receptor and guests were mixed and diluted to 7×10^{-5} M. The mixtures were analysed in negative ionisation mode by direct perfusion in ESI-MS interface; injection flow-rate = 20 µl min^{-1}; desolvation temperature = 150 °C; source block temperature = 80 °C; cone and desolvation gas flow-rates = 1.6 and 8 l min^{-1}, respectively; capillary = 3.0 KV; cone = 30 V; extractor = 3 V. Spectra were registered with a scan time of 6 s.

Turbidimetric analysis

500 µl of a glycocalix[4]arene dispersion (0.3 mg ml^{-1}) were quickly mixed with 500 µl of a lectin solution (0.5 mg ml^{-1}). The turbidity of the solution was monitored by reading the absorbance at 350 nm at regular time intervals until no noticeable changes could be observed, using a Perkin Elmer UV-Vis Lambda BIO 20 spectrophotometer. The sample cell was thermostated by a Peltier device at 25 °C. All experiments were performed in triplicate.

General procedures for the synthesis of the amino calixarene derivatives 1 and 2

Procedure A: to a solution in ethanol of the appropriate nitro derivative, hydrazine hydrate (20 equiv. for each nitro group) and a catalytic amount of Pd/C (10%) were added. The reaction mixture was refluxed for 5 h and quenched by catalyst filtration. The filtrate was evaporated at reduced pressure, the residue dissolved with ethyl acetate and washed with distilled water. The organic layer was separated, dried over $MgSO_4$ and evaporated to dryness at reduced pressure to obtain the pure amino calix[4]arene. **Procedure B**: a solution in ethyl acetate of the appropriate nitro derivative containing Pd/C (10%) was stirred under H_2 (2 bar) at room temperature for 24 h. The catalyst was filtered off and the filtrate was evaporated to dryness to give the amino calixarene without further purification.

5,17-Diamino-25,26,27,28-tetrapropoxycalix[4]arene 1

The compound was obtained in 95% yield through both procedures A and B and showed the same spectroscopic properties as previously reported.[14a]

5,11,17,23-Tetraamino-25,26,27,28-tetrapropoxycalix[4]arene 2

The compound was obtained in 95% yield through both procedures A and B and showed the same spectroscopic properties as previously reported.[14b,15c]

2,3,4,6-Tetraacetyl-β-galactosyl isothiocyanate 4

To a solution of 2,3,4,6-tetraacetyl-β-galactosyl bromide[13] (1.00 g, 2.43 mmol) in acetone (50 ml), KSCN (2.35 g, 24.3 mmol) was added. The mixture was heated at 110 °C in a sealed tube for 3 h, then the reaction was quenched by evaporation of the solvent at reduced pressure. The residue was solved in CH_2Cl_2 (30 ml), washed with distilled water (2 × 20 ml) and evaporated to dryness at reduced pressure. The crude residue was purified by flash column chromatography to obtain **4** as a white solid (475 mg, 50%). The product showed the same spectroscopic properties as previously reported.[12]

General procedure for the preparation of the protected glycosylthioureido calix[4]arenes 5–8

A solution of the aminocalix[4]arene and the appropriate isothiocyanate **3**[12] or **4** (2 equiv. for each NH_2 group) in dry CH_2Cl_2 (2.5 ml per 0.1 mmol of calixarene) was stirred for 24 h at room temperature. The reaction was diluted with CH_2Cl_2 and quenched by addition of distilled water. The organic layer was separated, washed with distilled water and evaporated to dryness at reduced pressure. The pure products were isolated by flash column chromatography.

5,17-Bis[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 5

Eluent for flash column chromatography: AcOEt/hexane 1/1; white solid; yield 75%; mp 164–167 °C; (Found: C 59.85, H 6.21, N 4.02. Calc for $C_{70}H_{88}N_4O_{22}S_2$: C 59.99, H 6.33, N 4.00%); $[\alpha]_D$ −31.5 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 3369, 1755, 1222; δ_H (300 MHz, DMSO-d_6) 9.70 (2 H, br s, NH), 7.80 (2 H, br d, NH), 7.03 (2 H, d, *J* 2.0, Ar), 7.01 (2 H, d, *J* 2.0, Ar), 6.48–6.32 (6 H, m, Ar), 5.89 (2 H, br t, H-1), 5.36 (2 H, t, *J* 9.5, H-3), 5.01 (2 H, t, *J* 9.5, H-2), 4.96 (2 H, t, *J* 9.5, H-4), 4.34 (4 H, d, *J* 12.9, H_{ax} of ArCH_2Ar), 4.19 (2 H, dd, *J* 11.2 and 4.6, H-6a), 4.05–3.91 (4 H, m, H-5 and H-6b), 3.88 (4 H, t, *J* 7.9, OCH_2), 3.67 (4 H, t, *J* 6.8, OCH_2), 3.16 (4 H, d, *J* 12.9, H_{eq} of ArCH_2Ar), 2.09, 2.02, 1.99, 1.95, (3 H each, 4 s, CH_3CO), 2.08–1.81 (8 H, m, OCH_2CH_2), 1.04 (6 H, t, *J* 7.3, CH_3), 0.93 (6 H, t, *J* 7.4, CH_3); δ_H (400 MHz, $CDCl_3$) 7.45 (2 H, br s, NH), 7.10 (2 H, d, *J* 6.8, Ar), 7.07 (2 H, d, *J* 6.2, Ar), 6.91 (2 H, t, *J* 6.8, Ar), 6.59 (2 H, br s, NH), 6.08, 6.03 (2 H each, br s, Ar), 5.85 (2 H, t, *J* 9.6, H-1), 5.32 (2 H, t, *J* 9.6, H-3), 5.08 (2 H, t, *J* 9.6, H-2), 4.99 (2 H, t, *J* 9.6, H-4), 4.45 (4 H, d, *J* 13.2, H_{ax} of ArCH_2Ar), 4.41–4.36 (2 H, m, H-6a), 4.12 (2 H, d, *J* 12.5, H-6b), 3.99 (4 H, t, *J* 7.9, OCH_2), 3.88–3.80 (2 H, m, H-5), 3.74 (4 H, t, *J* 7.9, OCH_2), 3.19 (4 H, d, *J* 13.2, H_{eq} of ArCH_2Ar), 2.11, 2.10, 2.02, 2.01, (3 H each, 4 s, CH_3CO), 1.98–1.85 (8 H, m, OCH_2CH_2), 1.10 (6 H, t, *J* 7.4, CH_3), 0.93 (6 H, t, *J* 7.5, CH_3); δ_C (75 MHz, DMSO-d_6) 181.2 (CS), 169.8, 169.3, 169.2 (CO), 155.0, 154.0, 135.4, 133.0, 131.9, 127.5, 123.8, 121.8 (Ar), 81.3 (C-1), 76.4, 76.1 (OCH_2), 72.6 (C-5), 71.9 (C-3), 70.4 (C-2), 67.8 (C-4), 61.6 (C-6), 30.5, 30.0 (ArCH_2Ar), 22.8, 22.5 (OCH_2CH_2), 20.4, 20.3, 20.2, 20.1 (CH_3CO), 10.3, 9.7 (CH_3); *m*/*z* (ESI) 1423.3 (100, $[M + Na]^+$).

5,17-Bis[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 6

Eluent for flash column chromatography: AcOEt/hexane 1/1; white solid; yield 80%; mp 154–156 °C; (Found: C 59.87, H 6.23, N 4.05. Calc. for $C_{70}H_{88}N_4O_{22}S_2$: C 59.99, H 6.33, N 4.00%); $[\alpha]_D$ −3.83 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 3415, 1754, 1217; δ_H (300 MHz, DMSO-d_6) 9.68 (2 H, br s, NH), 7.91 (2 H, br d, NH), 7.08 (4 H, m, Ar), 6.34 (6 H, m, Ar), 5.87 (2 H, br t, H-1), 5.36 (2 H, dd, *J* 9.6 and 3.3, H-3), 5.35–5.25 (2 H, m, H-4), 5.07 (2 H, t, *J* 9.6, H-2), 4.33 (4 H, d, *J* 12.9, H_{ax} of ArCH_2Ar), 4.31–4.21 (2 H, m, H-5), 4.02 (4 H, d, *J* 5.9, H-6a and H-6b), 3.92 (4 H, t, *J* 7.9, OCH_2), 3.67 (4 H, t, *J* 6.8, OCH_2), 3.16 (4 H, d, *J* 12.9, H_{eq} of ArCH_2Ar), 2.11, 2.05, 1.99, 1.93, (3 H each, 4 s, CH_3CO), 1.89–1.80 (8 H, m, OCH_2CH_2), 1.05 (6 H, t, *J* 7.4, CH_3), 0.92 (6 H, t, *J* 7.3, CH_3); δ_C (75 MHz, DMSO-d_6) 181.5 (CS), 169.9, 169.3 (CO), 155.1, 154.4, 135.8, 133.0, 127.5, 124.2, 121.9, 116.8 (Ar), 81.7 (C-1), 76.6, 76.2 (OCH_2), 71.3 (C-5), 70.7 (C-3), 68.3 (C-2), 67.6 (C-4), 61. 2 (C-6), 30.6, 30.2 (ArCH_2Ar), 22.9, 22.6 (OCH_2CH_2), 20.6, 20.4 (CH_3CO), 10.5, 9.9 (CH_3); *m*/*z* (ESI) 1423.3 (100, $[M + Na]^+$).

5,11,17,23-Tetrakis[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 7

Eluent for flash column chromatography: Et_2O/MeOH 25/1; white solid; yield 80%; mp 175–178 °C; (Found: C 54.27, H 5.66, N 5.11. Calc. for $C_{100}H_{128}N_8O_{40}S_4$: C 54.34, H 5.84, N 5.07%); $[\alpha]_D$ −6.1 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 3373, 1754, 1222; δ_H (400 MHz, $CDCl_3$) 7.98 (4 H, br s, NH), 6.82 (4 H, br s, NH), 6.52 (8 H, br s, Ar), 5.82 (4 H, t, *J* 9.2, H-1), 5.34 (4 H, dd, *J* 9.5 and 9.3, H-3), 5.04 (4 H, dd, *J* 9.3 and 9.2, H-2), 4.93 (4 H, dd, *J* 9.7 and 9.5, H-4), 4.46 (4 H, d, *J* 13.7, H_{ax} of ArCH_2Ar), 4.38 (4 H, dd, *J* 12.5 and 4.2, H-6a), 4.17–4.01 (8 H, m, H-6b), 3.90–3.80 (12 H, m, OCH_2 and H-5), 3.22 (4 H, d, *J* 13.7, H_{eq} of ArCH_2Ar), 2.15–1.91 (56 H, m, CH_3CO and OCH_2CH_2), 1.03 (12 H, t, *J* 7.3, CH_3); δ_C (75 MHz, $CDCl_3$) 181.8 (CS), 171.5, 170.6, 169.7, 169.5 (CO), 155.6, 136.4, 129.8, 124.9, 124.3 (Ar), 83.0 (C-1), 76.5 (OCH_2), 73.7 (C-5), 72.7 (C-3), 70.8 (C-2), 68.2 (C-4), 61.6 (C-6), 30.8 (ArCH_2Ar), 23.1 (OCH_2CH_2), 20.9, 20.7, 20.5, 20.4 (CH_3CO), 10.1 (CH_3); *m*/*z* (ESI) 1105.7 (100, $[M + 2H]^+$).

5,11,17,23-Tetrakis[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 8

Eluent for flash column chromatography: CH_2Cl_2/MeOH 50/1; white solid; yield 75%; mp 180–182 °C; (Found: C 54.19, H 5.71, N 5.13. Calc. for $C_{100}H_{128}N_8O_{40}S_4$: C 54.34, H 5.84, N 5.07%); $[\alpha]_D$ +35.8 (*c* 0.5, DMSO); ν_{max}/cm^{-1} (KBr) 3371, 1752, 1224; δ_H (300 MHz, $CDCl_3$) 7.99 (4 H, br s, NH), 6.80 (4 H, br s, NH), 6.60 (4 H, br s, Ar), 6.52 (4 H, br s, Ar), 5.83 (4 H, br t, H-1), 5.45 (4 H, d, *J* 3.0, H-4), 5.20 (4 H, dd, *J* 9.6 and 3.0, H-3), 5.14 (4 H, t, *J* 9.6, H-2), 4.48 (4 H, d, *J* 13.6, H_{ax} of ArCH_2Ar), 4.20–4.01 (12 H, m, H-5, H-6a and H-6b), 3.96–3.83 (8 H, m, OCH_2), 3.22 (4 H, d, *J* 13.6, H_{eq} of ArCH_2Ar), 2.17–1.83 (56 H, m, CH_3CO and OCH_2CH_2), 0.98 (12 H, t, *J* 7.7, CH_3); δ_C (75 MHz, $CDCl_3$) 181.8 (CS), 171.3, 171.5, 170.0, 169.6 (CO), 156.0 (Ar *ipso*), 83.4 (C-1), 76.5 (OCH_2), 72.7 (C-5), 70.9 (C-3), 68.4 (C-2), 67.3 (C-4), 61.0 (C-6), 30.8 (ArCH_2Ar), 23.2 (OCH_2CH_2), 20.9, 20.7, 20.6, 20.4 (CH_3CO), 10.1 (CH_3); *m*/*z* (ESI) 1127.7 (100, $[M + 2Na]^{2+}$), 2231.4 (30, $[M + Na]^+$).

General procedure for the deprotection from acetyl groups

The [(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)thioureido]-25,26,27,28-tetrapropoxycalix[4]arenes were dissolved in MeOH and the solution was adjusted to pH 8–9 by addition of freshly prepared methanolic NaOMe. The solution was stirred at room temperature for 1 h and then neutralised with Amberlite IR-120 (H^+) ion exchange resin. The resin was removed by filtration and after evaporation of the solvent the unprotected compounds **9**–**12** were obtained as white solids.

5,17-Bis(β-D-glucopyranosylthioureido)-25,26,27,28-tetrapropoxycalix[4]arene 9

Yield 82%; decomposition without melting at 168 °C; (Found: C 60.76, H 6.65, N 5.20. Calc. for $C_{54}H_{72}N_4O_{14}S_2$: C 60.88, H 6.81, N 5.26%); $[\alpha]_D$ −4.2 (*c* 0.5, EtOH); ν_{max}/cm^{-1} (KBr)

3415, 1218; δ_H (300 MHz, CD_3OD) 6.99 (4 H, d, *J* 7.1, Ar), 6.81 (2 H, t, *J* 7.1, Ar), 6.42 (2 H, s, Ar), 6.34 (2 H, s, Ar), 5.43 (2 H, d, *J* 8.8, H-1), 4.48 (4 H, d, *J* 13.3, H_{ax} of ArCH_2Ar), 4.01 (4 H, t, *J* 7.7, OCH_2), 3.84 (2 H, dd, *J* 11.8 and 2.4, H-6a), 3.76 (4 H, t, *J* 7.2, OCH_2), 3.66 (2 H, dd, *J* 11.8 and 4.9, H-6b), 3.42 (2 H, t, *J* 8.8, H-3), 3.38–3.25 (6 H, m, H-2, H-4 and H-5), 3.17 (4 H, d, *J* 13.3, H_{eq} of ArCH_2Ar), 2.02–1.88 (4 H, m, OCH_2CH_2), 1.11 (6 H, t, *J* 7.4, CH_3), 0.94 (6 H, t, *J* 7.4, CH_3); δ_C (75 MHz, CD_3OD) δ 183.2 (CS), 158.6, 155.1, 137.4, 137.2, 136.1, 130.1, 130.0, 124.5, 123.7 (Ar), 85.6 (C-1), 79.5, 79.0 (OCH_2), 78.2 (C-5), 77.9 (C-3), 74.0 (C-2), 71.6 (C-4), 62.8 (C-6), 32.0, 31.9 (ArCH_2Ar), 24.6, 24.2 (OCH_2CH_2), 11.2, 10.4 (CH_3); *m*/*z* (ESI) 1087.3 (100, $[M + Na]^+$).

5,17-Bis(β-D-galactopyranosylthioureido)-25,26,27,28-tetrapropoxycalix[4]arene 10

Yield 85%; mp 182–183 °C; (Found: C 60.74, H 6.73, N 5.18. Calc. for $C_{54}H_{72}N_4O_{14}S_2$: C 60.88, H 6.81, N 5.26%); $[\alpha]_D$ +2.45 (*c* 0.5, EtOH); ν_{max}/cm^{-1} (KBr) 3414, 1217; δ_H (300 MHz, CD_3OD) 6.97 (4 H, d, *J* 7.4, Ar), 6.80 (2 H, t, *J* 7.4, Ar), 6.50 (2 H, s, Ar), 6.32 (2 H, s, Ar), 5.39 (2 H, br s, H-1), 4.47 (4 H, d, *J* 13.3, H_{ax} of ArCH_2Ar), 4.00 (4 H, t, *J* 7.7, OCH_2), 3.88 (2 H, d, *J* 2.6, H-4), 3.76 (4 H, t, *J* 6.9, OCH_2), 3.71–3.49 (10 H, m, H-2, H-3, H-5, H-6a and H-6b), 3.18, 3.16 (2 H each, 2 d, *J* 13.3, H_{eq} of ArCH_2Ar), 2.03–1.91 (4 H, m, OCH_2CH_2), 1.12 (6 H, t, *J* 7.4, CH_3), 0.94 (6 H, t, *J* 7.5, CH_3); δ_C (75 MHz, CD_3OD) 183.1 (CS), 158.5, 155.1, 137.2, 137.0, 136.2, 133.1, 130.0, 124.5, 123.7 (Ar), 86.1 (C-1), 78.3, 78.2 (OCH_2), 77.9 (C-5), 75.8 (C-3), 71.4 (C-2), 70.5 (C-4), 62.5 (C-6), 32.0, 31.9 (ArCH_2Ar), 24.6, 24.2 (OCH_2CH_2), 11.2, 10.4 (CH_3); *m*/*z* (ESI) 1087.3 (100, $[M + Na]^+$).

5,11,17,23-Tetrakis(β-D-glucopyranosylthioureido)-25,26,27,28-tetrapropoxycalix[4]arene 11

Yield 89%; decomposition without melting at 225 °C; (Found: C 52.96, H 6.13, N 7.30. Calc. for $C_{68}H_{96}N_8O_{24}S_4$: C 53.11, H 6.13, N 7.27%); $[\alpha]_D$ +28.0 (*c* 0.5, DMSO); ν_{max}/cm^{-1} (KBr) 3419, 1221; δ_H (300 MHz, CD_3OD) 6.86, 6.77 (4 H each, 2 bs, Ar), 5.47 (4 H, br s, H-1), 4.50 (4 H, d, *J* 13.2, H_{ax} of ArCH_2Ar), 3.92–3.83 (10 H, m, H-6b and OCH_2), 3.69 (4 H, dd, *J* 11.8 and 5.0, H-6a), 3.49–3.29 (16 H, m, H-2, H-3, H-4 and H-5), 3.22 (4 H, d, *J* 13.2, H_{eq} of ArCH_2Ar), 2.04–1.94 (8 H, m, OCH_2CH_2), 1.06 (12 H, t, *J* 7.3, CH_3); δ_C (75 MHz, $CDCl_3$) 183.4 (CS), 171.5, 156.2, 137.1, 133.7, 126.0 (Ar), 85.9 (C-1), 79.7 (OCH_2), 79.3 (C-5), 78.5 (C-3), 74.5 (C-2), 71.7 (C-4), 63.0 (C-6), 32.3 (ArCH_2Ar), 24.7 (OCH_2CH_2), 11.1 (CH_3); *m*/*z* (ESI) 767.5 (100, $[M - 2H]^{2-}$), 1535.7 (22, $[M - H]^-$).

5,11,17,23-Tetrakis(β-D-galactopyranosylthioureido)-25,26,27,28-tetrapropoxycalix[4]arene 12

Yield 90%; decomposition without melting at 184 °C; (Found: C 52.92, H 6.20, N 7.29. Calc. for $C_{68}H_{96}N_8O_{24}S_4$: C 53.11, H 6.13, N 7.27%); $[\alpha]_D$ +35.8 (*c* 0.5, DMSO); ν_{max}/cm^{-1} (KBr) 3425, 1545; δ_H (300 MHz, CD_3OD) 6.85, 6.75 (4 H each, 2 bs, Ar), 5.51 (4 H, br s, H-1), 4.51 (4 H, d, *J* 13.2, H_{ax} of ArCH_2Ar), 3.98–3.59 (34 H, m, H-2, H-3, H-4, H-5, H-6a, H-6b and OCH_2), 3.21 (4 H, d, *J* 13.2, H_{eq} of ArCH_2Ar), 2.04–1.91 (8 H, m, OCH_2CH_2), 1.06 (12 H, t, *J* 7.4, CH_3); δ_C (75 MHz, CD_3OD) 183.2 (CS), 156.0, 137.5, 136.8, 133.8, 133.2, 125.6 (Ar), 86.4 (C-1), 78.6 (OCH_2, C-5), 76.1 (C-3), 72.1 (C-2), 70.6 (C-4), 62.9 (C-6), 32.3 (ArCH_2Ar), 24.7 (OCH_2CH_2), 11.0 (CH_3); *m*/*z* (ESI) 1535.4 (100, $[M]^-$).

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-β-D-glucopyranosylamine 13

A suspension of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-β-D-glucopyranosylazide[17] (450 mg, 0.69 mmol) and Pd/C (10%) in CH_3CN (30 ml) was stirred under H_2 (2 bar) for 2 h. The reaction mixture was filtered and evaporated to give **13** as a white solid (330 mg, 75%); mp 95–97 °C; (found: C 49.02, H 5.95, N 2.37. Calc. for $C_{26}H_{37}NO_{17}$: C 49.13, H 5.87, N 2.20%); $[\alpha]_D$ +4.4 (*c* 0.5, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 5.30 (1 H, dd, *J* 3.4 and 0.6, H-4′), 5.18 (1 H, dd, *J* 9.4 and 9.3, H-3), 5.05 (1 H, dd, *J* 10.4 and 7.6, H-2′), 4.91 (1 H, dd, *J* 10.4 and 3.4, H-3′), 4.68 (1 H, dd, *J* 9.4 and 8.6, H-2), 4.44 (1 H, d, *J* 8.6, H-1), 4.41 (1 H, dd, *J* 12.0 and 1.8, H-6a), 4.13–3.98 (3 H, m, H-6b, H-6′a and H-6′b), 4.05 (1 H, d, *J* 7.6, H-1′) 3.83 (1 H, td, *J* 6.3 and 1.0, H-5′), 3.69 (1 H, dd, *J* 9.9 and 9.3, H-4), 3.55 (1 H, ddd, *J* 9.9, 5.1 and 1.8, H-5), 2.15, 2.12, 2.06, 2.05, 2.04, 1.96 (3 H each, 6 s, CH_3CO); δ_C (75 MHz, $CDCl_3$) 170.4, 170.1, 170.0, 169.6, 169.0 (CO), 101.2 (C-1′), 84.6 (C-1), 73.7, 73.0,72.5, 71.0 (C-4, C-5, C-3, C-3′, C-2), 70.7 (C-5′), 69.1 (C-2′), 66.6 (C-4′), 62.3 (C-6), 60.8 (C-6′), 20.8, 20.6, 20.4 (*C*H_3CO).

5,17-Bis[(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-β-D-glucopyranosyl)thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 15

To a solution of 5,17-bisisothiocyanate-25,26,27,28-tetrapropoxycalix[4]arene **14**[14b] (140 mg, 0.20 mmol) in dry DMF (10 ml) 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-β-D-glucopyranosylamine **13** (277 mg, 0.44 mmol) was added. The reaction mixture was stirred for 24 h at 100 °C. After cooling to room temperature the reaction was quenched with 1 M HCl (20 ml). After extraction with CH_2Cl_2 (3 × 25 ml), the combined organic layers were washed with distilled water and evaporated to dryness at reduced pressure. The residue was purified by flash column chromatography (AcOEt/toluene 3/2) to afford **14** as a white solid (180 mg, 46%); mp 165–167 °C; (found: C 56.89, H 5.94, N 2.91. Calc. for $C_{94}H_{120}N_4O_{38}S_2$: C 57.03, H 6.12, N 2.83%); $[\alpha]_D$ −31.5 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 3385, 1753, 1220; δ_H (400 MHz, DMSO-d_6) 9.66 (2 H, br s, NH), 7.78 (2 H, br d, NH), 7.05–7.02 (4 H, m, Ar), 6.40–6.29 (6 H, m, Ar), 5.79 (2 H, br t, H-1), 5.28–5.10 (6 H, m, H-2′,H-3 and H-4′), 4.94–4.81 (4 H, m, H-2 and H-3′), 4.87 (2 H, d, *J* 8.1, H-1′), 4.40–4.20 (6 H, m, H-4, H-6a and H-6b), 4.28 (4H, d, *J* 13.4, H_{ax} of ArCH_2Ar), 4.07–3.98 (8 H, m, H-5, H-5′, H-6′a and H-6′b), 3.91 (4 H, t, *J* 7.6, OCH_2), 3.68 (4 H, t, *J* 6.6, OCH_2), 3.15 (4 H, d, *J* 13.4, H_{eq} of ArCH_2Ar), 2.11–1.85 (50 H, m, OCH_2CH_2 and CH_3CO), 1.04 (6 H, t, *J* 7.3, CH_3), 0.92 (6 H, t, *J* 7.4, CH_3); δ_C (75 MHz, DMSO-d_6) 170.7–169.2 (CO), 157.1, 154.4, 128.6, 124.1, 123.7, 122.0 (Ar), 100.3 (C-1′), 82.2 (C-1), 77.0, 76.6 (OCH_2), 76.3 (C-4), 75.6 (C-5), 74.0 (C-3), 72.5 (C-3′), 70.8 (C-2), 70.4 (C-5′), 68.8 (C-2′), 66.6 (C-4′), 61.9 (C-6), 60.8 (C-6′), 30.6 (ArCH_2-Ar), 23.1, 22.6 (OCH_2CH_2), 20.5, 20.4, 20.2, 20.0 (*C*H_3CO), 10.3, 9.5 (CH_3); *m*/*z* (ESI) 659.1 (100, $[M + H]^{3+}$), 1011.3 (90, $[M + 2Na]^{2+}$), 1999.6 (80, $[M + Na]^+$).

5,17-Bis[(β-D-galactopyranosyl)-1-β-D-glucopyranosyl)-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 16

The deprotection of **15** (180 mg, 0.09 mmol) to **16** was carried out using the general procedure previously described for the other glycocalixarenes; white solid (107 mg, 85%); mp 196–198 °C; (found: C 56.90, H 6.73, N 3.91. Calc. for $C_{66}H_{92}N_4O_{24}S_2$: C 57.04, H 6.67, N 4.03%); $[\alpha]_D$ +12.25 (*c* 0.4, DMSO); ν_{max}/cm^{-1}(KBr) 3420, 122; δ_H (300 MHz, CD_3OD) 7.01 (4 H, d, *J* 7.4, Ar), 6.82 (2 H, t, *J* 7.4, Ar), 6.35 (4 H, br s, Ar), 5.45 (2 H, d, *J* 8.98, H-1), 4.47 (4 H, d, *J* 13.2, H_{ax} of ArCH_2Ar), 4.38 (2 H, d, *J* 7.4, H-1′), 4.01 (4 H, t, *J* 7.3, OCH_2), 3.87–3.70 (20 H, m, H-2, H-3, H-4, H-2′, H-3′ and H-4′), 3.62–3.44 (12 H, m, H-5, H-5′, H-6a, H-6b, H-6′a and H-6′b), 3.18 (4 H, d, *J* 13.1, H_{eq} of ArCH_2Ar), 2.02–1.87 (8 H, m, OCH_2CH_2), 1.11 (6 H, t, *J* 7.3, CH_3), 0.94 (6 H, t, *J* 7.1, CH_3); δ_C (75 MHz, CD_3OD) 182.5 (CS), 155.6, 155.0, 137.4, 137.2, 136.1, 130.2, 130.1, 124.5, 123.8 (Ar), 105.2 (C-1′), 85.4 (C-1), 80.7 (C-4), 78.3, 78.0, 77.9, 77.4, 77.1 (OCH_2, C-5, C-3, C-3′), 74.8 (C-2), 73.6

(C-5′), 72.5 (C-2′), 70.3 (C-4′), 62.5 (C-6), 62.0 (C-6′), 32.0 (Ar*C*H_2Ar), 24.6, 24.2 (OCH$_2$*C*H$_2$), 11.2, 10.4 (CH_3); *m/z* (ESI) 1411.4 (100, $[M + Na]^+$), 717.1 (65, $[M + 2Na]^{2+}$).

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References

1 (*a*) F. Sansone, M. Segura and R. Ungaro, in *Calixarenes 2001*, ed. Z. Asfari, V. Böhmer, J. Harrowfield and J. Vicens, Kluwer Academic Publishers, Dordrecht, 2001, p. 496; (*b*) F. Sansone, L. Baldini, A. Casnati, M. Lazzarotto, F. Ugozzoli and R. Ungaro, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 4842–4847; (*c*) A. Casnati, F. Sansone and R. Ungaro, *Acc. Chem. Res.*, 2003, **36**(4), in press.

2 (*a*) A. Casnati, M. Fabbi, N. Pelizzi, A. Pochini, F. Sansone, R. Ungaro, E. Di Modugno and G. Tarzia, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2699–2704; (*b*) F. Sansone, S. Barboso, A. Casnati, M. Fabbi, A. Pochini, F. Ugozzoli and R. Ungaro, *Eur. J. Org. Chem.*, 1998, 897–905; (*c*) F. Sansone, S. Barboso, A. Casnati, D. Sciotto and R. Ungaro, *Tetrahedron Lett.*, 1999, **40**, 4741–4744; (*d*) L. Frish, F. Sansone, A. Casnati, R. Ungaro and Y. Cohen, *J. Org. Chem.*, 2000, **65**, 5026–5030; (*e*) M. Lazzarotto, F. Sansone, L. Baldini, A. Casnati, P. Cozzini and R. Ungaro, *Eur. J. Org. Chem.*, 2001, 595–602.

3 (*a*) A. Marra, M.-C. Scherrmann, A. Dondoni, A. Casnati, P. Minari and R. Ungaro, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2479–2481; (*b*) A. Dondoni, A. Marra, M.-C. Scherrmann, A. Casnati, F. Sansone and R. Ungaro, *Chem. Eur. J.*, 1997, **3**, 1774–1782.

4 D. A. Fulton and J. F. Stoddart, *Bioconjugate Chem.*, 2001, **12**, 655–672.

5 (*a*) Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327; (*b*) J. J. Lundquist and E. Toone, *Chem. Rev.*, 2002, **102**, 555–578.

6 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794.

7 L. Guangtao, M.-F. Gouzy and J.-H. Fuhrhop, *Top. Curr. Chem.*, 2002, **218**, 133–158.

8 (*a*) S. J. Meunier and R. Roy, *Tetrahedron Lett.*, 1996, **37**, 5469–5472; (*b*) R. Roy and J. M. Kim, *Angew. Chem., Int. Ed.*, 1999, **38**, 369–372; (*c*) C. Saitz-Barria, A. Torres-Pinedo and F. Santoyo-González, *Synlett*, 1999, **12**, 1891–1894; (*d*) J. Budka, M. Tkadlecová, P. Lhoták and I. Stibor, *Tetrahedron*, 2000, **56**, 1883–1887; (*e*) A. Dondoni, X. Hu, A. Marra and H. D. Banks, *Tetrahedron Lett.*, 2001, **42**, 3295–3298.

9 (*a*) A. Marra, A. Dondoni and F. Sansone, *J. Org. Chem*, 1996, **61**, 5155–5158; (*b*) C. Félix, H. Parrot-Lopez, V. Kalchenko and A. W. Coleman, *Tetrahedron Lett.*, 1998, **39**, 9171–9174; (*c*) A. Dondoni, M. Kleban, X. Hu, A. Marra and H. D. Banks, *J. Org. Chem.*, 2002, **67**, 4722–4733.

10 A. Dondoni, M. Kleban and A. Marra, *Tetrahedron Lett.*, 1997, **38**, 7801–7804.

11 (*a*) C. Ortiz Mellet, J. M. Benito, J. M. García Fernández, H. Law, K. Chmurski, J. Defaye, M. L. O'Sullivan and H. Caro, *Chem. Eur. J.*, 1998, **4**, 2523–2531; (*b*) J. J. García-Lopez, F. Santoyo-González, A. Vargas-Berenguel and J. J. Giménez-Martínez, *Chem. Eur. J.*, 1999, **5**, 1775–1784; (*c*) R. Roy, F. Hernández-Mateo and F. Santoyo-González, *J. Org. Chem.*, 2000, **65**, 8743–8746.

12 T. K. Lindhorst and C. Kieburg, *Synthesis*, 1995, 1228–1230.

13 K. P. Ravindranathan and H. J. Jennings, *J. Carbohydr. Chem.*, 1990, **9**, 778–781.

14 (*a*) P. Timmerman, H. Boerrigter, W. Verboom and D. N. Reinhoudt, *Recl. Trav. Chim. Pays-Bas*, 1995, **114**, 103–111; (*b*) A. M. van Wageningen, E. Snip, W. Verboom, D. N. Reinhoudt and H. Boerrigter, *Liebigs Ann./Recueil*, 1997, 2235–2245.

15 (*a*) E. Kelderman, L. Derhaeg, G. J. T. Heesink, W. Verboom, J. F. J. Engbersen, N. F. Van Hulst, A. Persoons and D. N. Reinhoudt, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 1075–1077; (*b*) W. Verboom, A. Durie, R. J. M. Egberink, Z. Asfari and D. N. Reinhoudt, *J. Org. Chem.*, 1992, **57**, 1313–1316; (*c*) A. M. Rincón, P. Prados and J. de Mendoza, *J. Am. Chem. Soc.*, 2001, **123**, 3493–3498.

16 When we had established these procedures, another similar synthetic protocol to obtain amino derivatives of calix[4]arene was reported.[15c]

17 F. D. Tropper, F. O. Andersson, S. Braun and R. Roy, *Synthesis*, 1992, 618–620.

18 (*a*) A. Yamada, T. Murase, K. Kikukawa, T. Matsuda and S. Shinkai, *Chem. Lett.*, 1990, 455–458; (*b*) T. Harada, J. M. Rudzinski, E. Osawa and S. Shinkai, *Tetrahedron*, 1993, **49**, 5941–5954; (*c*) A. Yamada, T. Murase, K. Kikukawa, T. Arimura and S. Shinkai, *J. Chem. Soc., Perkin Trans. 2*, 1991, 793–797; (*d*) A. Arduini, M. Fabbi, L. Mirone, A. Pochini, A. Secchi and R. Ungaro, *J. Org. Chem.*, 1995, **69**, 1454–1457.

19 (*a*) J. Scheerder, R. H. Vreekamp, J. F. J. Engbersen, W. Verboom, J. P. M. van Duynhoven and D. N. Reinhoudt, *J. Org. Chem.*, 1996, **61**, 3476–3481; (*b*) N. Pelizzi, A. Casnati and R. Ungaro, *Chem. Commun.*, 1998, 2607–2608.

20 (*a*) J. M. García Fernández, J. L. Jiménez Blanco, C. Ortiz Mellet and J. Fuentes, *J. Chem. Soc., Chem. Commun.*, 1995, 57–58; (*b*) J. L. Jiménez Blanco, J. M. Benito, C. Ortiz Mellet and J. M. García Fernández, *Org. Lett.*, 1999, **1**, 1217–1220; (*c*) V. M. Díaz Pérez, C. Ortiz Mellet, J. Fuentes and J. M. García Fernández, *Carbohydr. Res.*, 2000, **326**, 161–175; (*d*) J. M. García Fernández and C. Ortiz Mellet, *Adv. Carbohydr. Chem. Biochem.*, 2000, **55**, 35–135.

21 J. M. Benito, M. Gómez-García, J. L. Jiménez Blanco, C. Ortiz Mellet and J. M. García Fernández, *J. Org. Chem.*, 2001, **66**, 1366–1372.

22 D. M. Rudkevich, in *Calixarenes 2001*, ed. Z. Asfari, V. Böhmer, J. Harrowfield and J. Vicens, Kluwer Academic Publishers, Dordrecht, 2001, p. 155.

23 (*a*) T. K. Lindhorst, *Top. Curr. Chem.*, 2002, **218**, 201–235; (*b*) B. T. Houseman and M. Mrksich, *Chem. Biol.*, 2002, **9**, 443–454.

24 (*a*) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read and D. R. Bundle, *Nature*, 2000, **403**, 669–6672; (*b*) Z. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol and E. Fan, *J. Am. Chem. Soc.*, 2002, **124**, 12991–12998.

25 (*a*) K. Aoi, K. Itoh and M. Okada, *Macromolecules*, 1995, **28**, 5391–5393; (*b*) D. Zanini and R. Roy, *J. Am. Chem. Soc.*, 1997, **119**, 2088–2095; (*c*) T. Fujimoto, C. Shimizu, O. Hayashida and Y. Aoyama, *J. Am. Chem. Soc.*, 1998, **120**, 601–602; (*d*) R. Roy, D. Pagé, F. Figueroa Perez and V. Verez Bencomo, *Glycoconjugate J.*, 1998, **15**, 251–263; (*e*) H. Kitano, Y. Sumi and K. Tagawa, *Bioconjugate Chem.*, 2001, **12**, 56–61.

26 (*a*) R. Lotan, E. Skutelsky, D. Danon and N. Sharon, *J. Biol. Chem.*, 1975, **250**, 8518–8523; (*b*) T. J. Williams, N. R. Plessas, I. J. Goldstein and J. Lönngren, *Arch. Biochem. Biophys.*, 1979, **195**, 145–151.

27 (*a*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 487–516; (*b*) A. Casnati, F. Sansone and R. Ungaro, *Adv. Supramol. Chem.*, 2003, **9**, 165–218.

28 H.-J. Schneider and A. Yatsimirsky, in *Principles and Methods in Supramolecular Chemistry*, John Wiley & Sons, Chichester, 2000, p. 151.

29 C. S. Wilcox, in *Frontiers in Supramolecular Chemistry and Photochemistry*, ed. H.-J. Schneider and H. Dürr, VCH, Weinheim, 1991, p. 123.

30 T. R. Kelly and M. H. Kim, *J. Am. Chem. Soc.*, 1994, **116**, 7072–7080.

31 (*a*) J. Jiménez-Barbero, E. Junquera, M. Martín-Pastor, S. Sharma, C. Vicent and S. Penadés, *J. Am. Chem. Soc.*, 1995, **117**, 11198–11204; (*b*) J. C. Morales and S. Penadés, *Angew. Chem., Int. Ed.*, 1998, **37**, 654–657.