



Exploring calixarene-based clusters for efficient functional presentation of *Streptococcus pneumoniae* saccharides

Marta Giuliani^a, Federica Faroldi^a, Laura Morelli^b, Enza Torre^c, Grazia Lombardi^c,
Silvia Fallarini^{c,*}, Francesco Sansone^{a,*}, Federica Compostella^{b,*}

^a Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

^b Department of Medical Biotechnology and Translational Medicine, University of Milan, Via Saldini 50, 20133 Milano, Italy

^c Department of Pharmaceutical Sciences, University of "Piemonte Orientale", Largo Donegani 2, 28100 Novara, Italy

ARTICLE INFO

Keywords:

Saccharide antigens
Serotype 19F
Streptococcus pneumoniae
Calixarenes
Multivalent ligands

ABSTRACT

Calixarenes are promising scaffolds for an efficient clustered exposition of multiple saccharide antigenic units. Herein we report the synthesis and biological evaluation of a calix[6]arene functionalized with six copies of the trisaccharide repeating unit of *Streptococcus pneumoniae* (SP) serotype 19F. This system has demonstrated its ability to efficiently inhibit the binding between the native 19F capsular polysaccharide and anti-19F antibodies, despite a low number of exposed saccharide antigens, well mimicking the epitope presentations in the polysaccharide. The calix[6]arene mobile scaffold has been selected for functionalization with SP 19F repeating unit after a preliminary screening of four model glycolixarenes, functionalized with *N*-acetyl mannosamine, and differing in the valency and/or conformational properties. This work is a step forward towards the development of new fully synthetic calixarenes comprising small carbohydrate antigens as potential carbohydrate-based vaccine scaffolds.

1. Introduction

Encapsulated bacterial infections are still one of the most prevalent causes of serious disease in humans, especially in young children. Immunization is the most appropriate way to prevent bacterial infections [1]. In the early stages of microbiology, capsular polysaccharides (CPSs) were recognized as relevant virulence factors, and found to be able to stimulate protective immunity against infections, laying the bases for the development of current antibacterial vaccines [2]. Nevertheless, the study of the chemical determinants of immunogenicity to CPSs still requires detailed molecular insights. CPSs are cell-surface polymers consisting of oligosaccharide repeating units, characterized by a huge number of possible structural modifications and linkage combinations and associated to a relative high degree of flexibility. Typically, anti-carbohydrate antibodies show affinities in their recognition that are several factors lower than those observed for antibodies specific for proteins or peptide antigens. This peculiar weakness is partially compensated by the multivalent nature of such antibodies, that face in a clustered form a multiple number of densely displayed antigen molecules to increase the effect of the response [3]. This can explain the existing relationship between molecular weight and antigenicity, established for polysaccharide antigens. In fact, high

molecular mass repetitive polysaccharides are able to simultaneously display a greater number of epitopes capable to effectively interact with specific antibodies. This kind of model where the binding of a glycan-binding protein to a clustered saccharide patch enhances the overall affinity of the interaction has been widely described [4], for instance between tumor associated saccharide antigens and lectins. Lectin-glycan interactions are stabilized by weak hydrogen bonding and van der Waals intermolecular forces, and the multivalent presentation is the basis for a biologically relevant binding. There are several examples demonstrating that lectins bind their targets only when glycans are clustered at high density in a multivalent glycoconjugate backbone [5]. This concept has been exploited for instance in the field of cancer vaccine development. For example, the Tn tumor associated antigen is overexpressed on the surface of tumor cells. It is exposed on the Mucine surface in clusters of 2–5 units. Data show that at least two contiguous antigen molecules are crucial for the binding of the anti-Tn monoclonal antibodies, with an even greater affinity in the presence of three residues [6]. Different studies have been oriented to the selection of the proper scaffold for the presentation of multiple copies of the Tn antigenic unit to generate an anticancer vaccine [7–9]. The choice of the scaffold for multivalent presentation of glycans to target proteins is crucial in determining the biological activity [10–13]. In this context,

* Corresponding authors.

E-mail addresses: silvia.fallarini@uniupo.it (S. Fallarini), francesco.sansone@unipr.it (F. Sansone), federica.compostella@unimi.it (F. Compostella).

<https://doi.org/10.1016/j.bioorg.2019.103305>

Received 18 July 2019; Received in revised form 17 September 2019; Accepted 19 September 2019

Available online 19 September 2019

0045-2068/© 2019 Elsevier Inc. All rights reserved.

the use of calixarenes represents a viable approach explored in the literature for the display of multiple glycan units [14]. This type of macrocycle in fact, due to peculiar structural properties and selective synthetic procedures [15], allows the achievement of small libraries of potential multivalent ligands bearing the same epitopes but with a controlled modulation of their number (valency) and orientation in the space. The conformational behavior of the different possible types of calixarenes strongly impacts on the presentation mode of the conjugated saccharide moieties [14,16], which can be exploited to increase the avidity of the biological recognition. This versatility can make possible the comparison of subtly modified parameters and their effects on selectivity and efficiency in biological activities like interaction with carbohydrate recognition proteins [14]. A calix[4]arene in the so called cone geometry conjugated to LacNAc fragments, for example, was found extremely selective in the inhibition of galectin-3 while totally unable to bind to galectin-1 [17]. Calixarenes functionalized with lactose units showed inhibition activity towards human galectins characterized, in some cases, by an impressive selectivity strictly dependent on their conformational properties and related arrangement of the epitopes [18]. Analogously, different sized and conformationally featured calixarenes exposing galactose units resulted in different efficiency in the binding to *Pseudomonas aeruginosa* lectin A (PA-IL) [19]. Ten years ago, a calix[4]arene decorated with four Tn units has indeed been described as a promising synthetic multivalent vaccine candidate [20]. In this framework, we have decided to explore the potential of calixarenes as scaffolds for the multipresentation of bacterial CPS fragments. Our hypothesis was to verify if such macrocyclic scaffold, presenting multiple, but however limited copies of short CPS fragments, is able to gain affinity and potency towards the natural antibodies approaching those observed for the natural polysaccharide. In principle, a positive assessment would open the possibility to work with shorter saccharide fragments, which can be obtained by chemical synthesis, instead of the longer oligosaccharides, used in vaccine formulations, obtained by size-reduced purified natural polysaccharides [21]. Moreover, the use of calixarenes and short saccharide fragments would have the advantage of giving structurally well-defined and easily reproducible systems.

We focused on the gram positive bacterium *Streptococcus pneumoniae* (SP) which is among the major responsible of severe forms of bacterial infectious diseases [1,22–24]. In particular, serotype 19F (SP19F) is one of the most common causes of invasive pneumococcal disease in children, and included in the current commercial pneumococcal conjugate vaccines. Its CPS is a linear polymer made up of β -D-ManpNAc-(1 $\rightarrow$ 4)- α -D-Glcp-(1 $\rightarrow$ 2)- α -L-Rha trisaccharide repeating units, linked through phosphodiester bridges (Fig. 1). Herein, we report on the preparation and biological evaluation of a family of calixarenes functionalized with saccharide fragments related to the trisaccharide repeating unit of SP19F with the aim to explore the effect of the multipresentation of the ligand mediated by calixarenes on the binding to the antibodies. We found that a calix[6]arene, functionalized with six copies of the trisaccharide repeating unit of SP19F, is capable to

effectively inhibit the binding between the native 19F CPS and the anti-19F antibodies.

2. Results and discussion

2.1. Synthesis

We report the synthesis of a small library of glycolcalixarenes (compounds **1a-d** and **2a-b**, Fig. 2) functionalized at the upper rim with saccharide units present in the CPS structure of SP19F. To this aim, both calix[4]- and calix[6]arene were selected as scaffolds in order to have a modulation of the valency. Moreover, the calix[4]arene based derivatives were designed with three different conformational features, one conformationally mobile, one blocked in the cone geometry, one blocked in the 1,3-alternate one, in order to compare a different spatial arrangement of the epitope units. Also the calix[6]arene based glycoclusters are conformationally mobile. In addition, a monovalent acyclic analogue was planned to have a reference for verifying the role of the multivalency in the interaction with the antibodies.

In a first step of the project, all the selected scaffolds were functionalized with the *N*-acetyl- β -D-mannosamine residue (compounds **1a-d** and **1-MON**, Fig. 2), while subsequently, based on a preliminary set of inhibition studies, only the calixarene scaffold of the best inhibitor of the binding between the 19F polysaccharide and the corresponding anti-19F antibodies was functionalized with the SP19F trisaccharide repeating unit. Thus, two additional glycolcalixarenes (**2a** and **2b**, Fig. 2), were obtained, with increased similarity with respect to the natural structure. This two-steps approach was pursued because of the demanding synthesis of the trisaccharide and the consequent difficulty in producing a sufficient amount for the functionalization of all the available structures.

In order to have an efficient and clean reaction for the coupling between the calixarene scaffolds and the sugar units, we decided to exploit the amine-isothiocyanate condensation. To this aim, the calixarene isothiocyanates **4a-d** and the sugar unit **6**, functionalized at the reducing end with an aminopropyl linker, were synthesized (Scheme 1). Compounds **4a-d** were prepared by treatment of the corresponding, already known aminocalixarenes **3a-d** [25–27] with thiophosgene (caution) in the presence of a base, the cone isomer following a previously reported procedure [28] and the others through equivalent strategies. It may be interesting to underline that for the conformationally mobile tetraisothiocyanate calixarene **4a** the ^1H NMR spectrum in CDCl_3 highlighted the presence of two conformers, identified as the *cone* and the *partial cone*, in equilibrium and in slow exchange on the NMR timescale, in a 1:4 ratio.

On the basis of procedures reported in the literature [29], we then prepared compound **6**, a protected derivative of 3-aminopropyl *N*-acetyl- β -D-mannosamine, benzylated at the sugar hydroxyls. Monosaccharide **6** was deprotected from the Cbz group by hydrogenolysis producing the amino derivative **7** that was subsequently reacted with cone and 1,3-alternate tetraisothiocyanate calix[4]arenes **4b** and **4c**, obtaining in good yields the corresponding tetraglycosylated macrocycles (see SI). Nevertheless, unexpectedly, their deprotection from benzyl groups to give the two planned glycoclusters failed, despite the use of different methods, discouraging the use of this pathway for the preparation of the target calixarenes **1a-d**.

Therefore, we went back to monosaccharide **6** to replace benzyl protecting groups with acetyls being proved that the latter one can efficiently be removed from saccharide units attached to a calixarene scaffold [13,14,30,31]. Therefore, a sequence of protection and deprotection reactions starting from **6** was carried out to finally obtain compound **11** with an overall yield of 50% (Scheme 2). More in detail, after the selective removal of *N*-Cbz from **6** by hydrogenolysis, compound **7** was protected with a Boc group to give **8**. The subsequent catalytic hydrogen transfer under MW irradiation yielded **9**, fully deprotected on the OH groups, without the need of purification. The

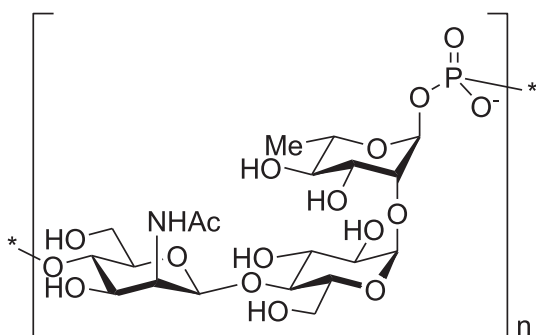


Fig. 1. *Streptococcus pneumoniae* 19F capsular polysaccharide repeating unit.

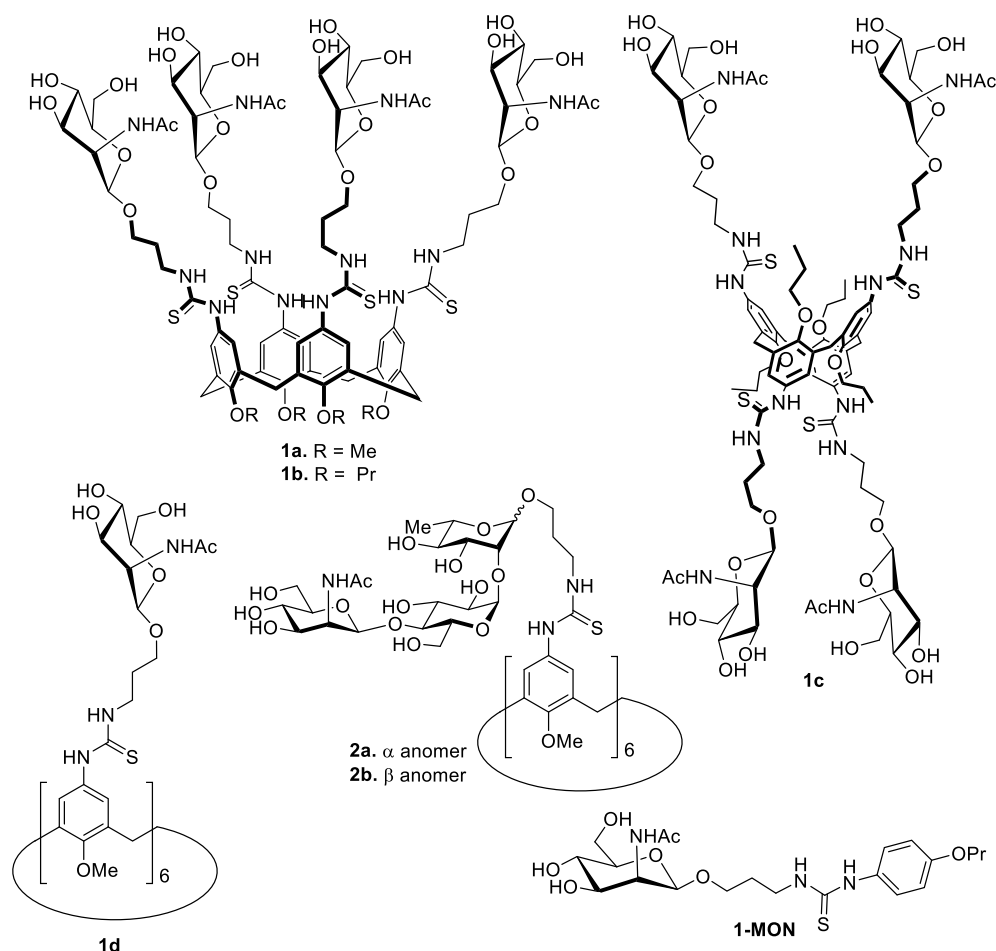


Fig. 2. Library of multivalent glycolixarenes decorated with saccharide units of SP 19F CPS.

acetylation step, performed with acetic anhydride and pyridine, afforded in high yield sugar **10**, which was treated with trifluoroacetic anhydride to remove the Boc group providing **11** in quantitative yield.

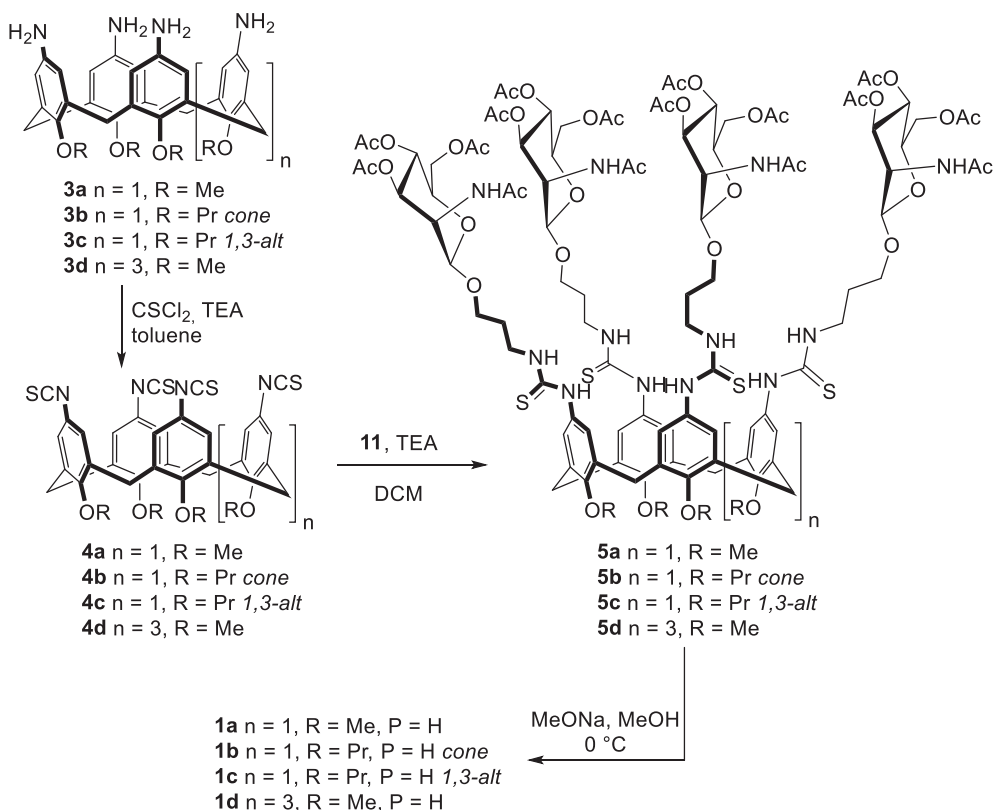
The mannosamine derivative **11** was then reacted with the isothiocyanate calixarenes **4a-d**. The click chemistry reaction between isothiocyanate and amine, used for the conjugation reaction, is one of the most commonly employed methodology for the design and synthesis of multivalent glycoconjugates [32,33]. This methodology does not involve the anomeric position of the glycoside and the newly formed thioureido unit presents an enhanced potential for hydrogen bonding that can increase both the solubility in water of the ligand and the number of interactions with the receptor. Glycolixarenes **5a** [34], **5b**, **5c** [34] and **5d** were obtained in moderate yields (from 25% to 71%) after column chromatography performed to remove the sugar excess and traces of partially functionalized derivatives. Finally, compounds **5a-d** were deacetylated under Zemplén conditions at 0 °C to give the target calixarenes **1a-d**. For the two conformationally mobile derivatives **1a** and **1d**, we proceeded in the investigation by ^1H NMR spectroscopy of their behavior in solution. The ^1H NMR spectrum in MeOD of the former one did not give any clear information about the conformation adopted by the calixarene in solution. A spectrum in D_2O was then recorded, knowing that conformationally mobile calix[4]arene amphiphiles usually tend to adopt in water a 1,3-*alternate* geometry which minimizes the lipophilic surfaces exposed to the solvent [35]. At room temperature the spectrum still presented broad signals, difficult to assign, while by raising the temperature up to 80 °C a rather well-defined spectrum was obtained (see SI at page SI22). It was possible to identify the signals of the H_1 and H_2 protons of the sugar moiety, at 4.69 and 4.43 ppm respectively, and, at the same time, the single signal at

around 7 ppm for all the aromatic protons together with the absence of the typical doublets for the methylene bridge of cone and partial cone conformers demonstrated the adoption of a 1,3-*alternate* geometry. This should plausibly be the geometry adopted by glycolixarene **1a** also in the aqueous environment of the biological tests.

As observed for **1a**, the ^1H NMR spectrum in D_2O at room temperature of calix[6]arene **1d** was characterized by broad signals that became sharp by increasing the temperature at 80 °C (see SI at page SI24). This behaviour could be explained with the low mobility of the aromatic units through the macrocyclic annulus, slowed down by the steric hindrance of the bulky substituents at the upper rim despite the bigger size of the macrocycle. On the other hand, interestingly, in the past it was observed, rather unexpectedly, that methoxyglucocalix[6]- and [8]-arenes tend to self-assemble in aqueous solution [36], despite the absence of a well-defined amphiphilicity, as indeed for **1a** and **1d**. For glycolixarenes **1a** and **1d** self-aggregation cannot then be excluded, determining or at least contributing to the broadening of the signals in the ^1H NMR spectrum. In both cases, the sharpening of the signals by increasing the temperature can be read as a consequence of the assembly disaggregation and/or of the increased mobility of these derivatives in solution.

Following a similar synthetic strategy, monomeric derivative **1-MON** was prepared (Scheme 3) starting from 4-propoxyaniline **12** that was transformed into the corresponding isothiocyanate **13**. Compound **13** was then reacted with sugar unit **11** to give compound **14** which was subsequently deprotected to the final **1-MON** in 78% yield.

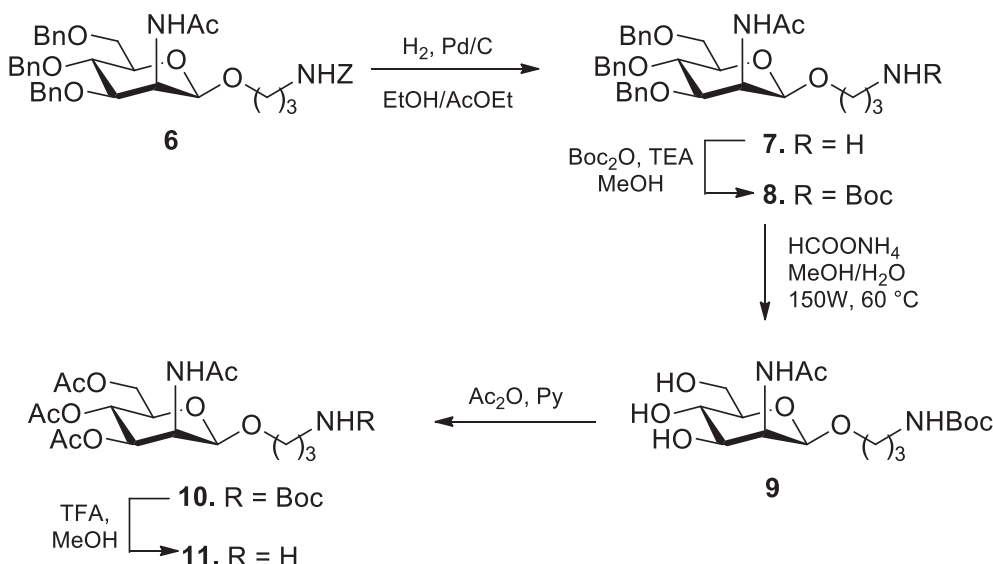
On the basis of the results obtained from biological tests on compounds **1a-d** (see below), the conformationally mobile calix[6]arene was selected as the best scaffold for obtaining the glycolixarenes

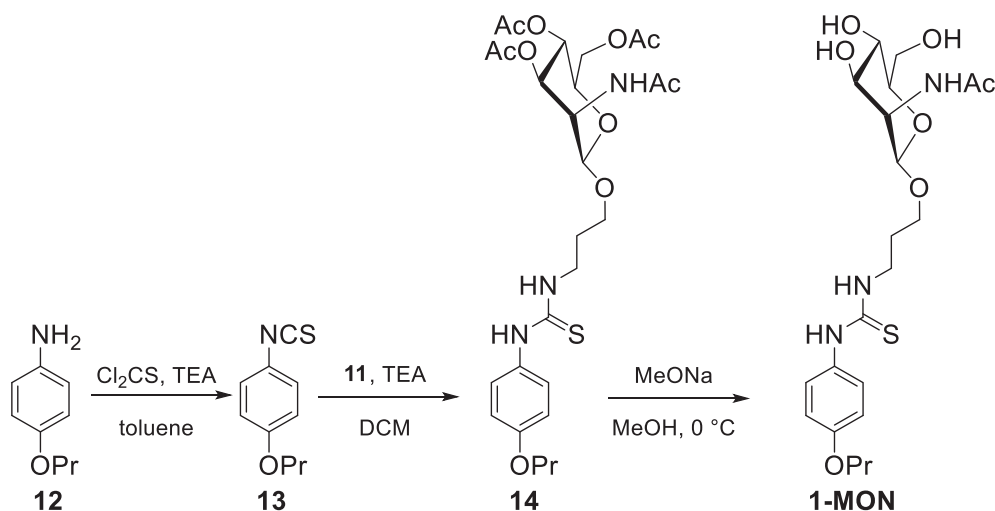
Scheme 1. Synthesis of glycolalix[n]arenes **1a-d**.

displaying the trisaccharide repeating unit of SP19F. To this aim, exploiting our expertise in the synthesis of synthetic fragments of *Streptococcus pneumoniae* [37–39], we planned the preparation of compound **15**, the peracetylated derivative of the SP19F trisaccharide repeating unit, functionalized at the downstream residue with an aminopropyl linker. Compound **15** was synthesized starting from the trisaccharide trichloroacetimidate donor **16** [37], which was glycosylated with Z-aminopropanol (Scheme 4) [38]. The reaction was promoted with trimethylsilyl triflate and trisaccharide **17** was obtained in high yields (96%) as an α/β mixture of the two anomers **17a** and **17b**, which were separated by flash chromatography. Each anomer was

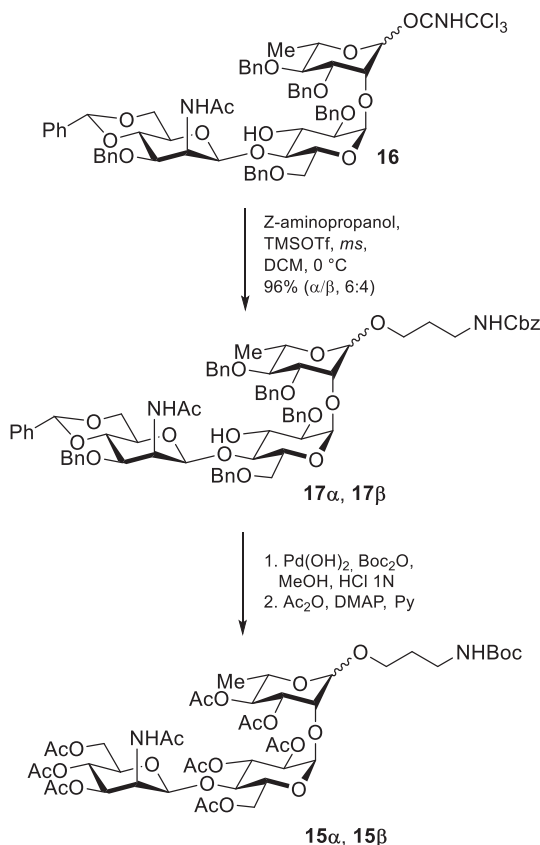
separately subjected to initial exchange of the CBZ amino protecting group of the linker for the BOC one, followed by hydrogenolysis of the benzyl ethers, and final acetylation of the hydroxyl groups with acetic anhydride. This protecting group manipulation sequence was very efficient and allowed to recover in both cases the final trisaccharides **15a** and **15b** in 88% yield.

Hexaisothiocyanate **4d** was separately reacted in good yields with both the α and the β anomer of compound **15**, to evaluate if a different stereochemistry of the anomeric position on the rhamnose residue may have an influence on the biological activity of the glycolalixarene. If not, in perspective, the calixarene could be reacted with the mixture of

Scheme 2. Sequence of protection and deprotection steps to obtain compound **11**.



Scheme 3. Synthesis of monomer 1-MON.



Scheme 4. Synthesis of trisaccharides 15α and 15β.

the two anomers that is produced in the final step of the synthesis of the trisaccharide when the aminopropyl chain is introduced at the anomeric position of rhamnose. The two glycolixarenes **18a** and **18b** obtained by this condensation were deprotected under Zemplen conditions at 0 °C to give the final glycolusters **2a** and **2b** in 81% and 82% yield respectively (Scheme 5).

2.2. Binding affinity measurements

The ability of increasing concentrations (from 10^{-7} mg/mL to 1 mg/mL) of each new compound to inhibit the binding between the native 19F CPS, coated onto plates, and the mouse anti-19F polyclonal

antibodies was evaluated in a classical competitive ELISA. Fig. 3 shows the inhibition curves obtained with the compounds under evaluation.

The relative efficacy of each compound was assessed by determining its maximum effect of inhibition at 1 mg/mL, while the concentration that produces the 50% of the possible maximum effect (IC_{50}) was calculated when the curve reaches a plateau and taken as indirect index of the relative potency (Table 1). The activities of the new compounds were compared with those of the natural 19F polysaccharide and the 19F trisaccharide repeating unit (SP19F-RU, see structure in SI) [38,40] as the reference compound. The maximum inhibition observed for 19F CPS was fixed as the 100%.

Our results demonstrate that the spatial preorganization of the mannosamide units on the calixarene effectively increases their efficacy in a way that depends on the valency and on the tridimensional architecture of the macrocyclic scaffold. In fact, comparing the maximum % of inhibition (Table 1) found for the monomeric model **1-MON** (16%) with that of calixarene **1a** (34%), also considering that the concentration of the ManAc epitope is substantially the same for the two compounds (2.1 and 2.2 mM for **1-MON** and **1a**, respectively), the single mannosamide unit evidences an efficacy almost 2 fold higher when it is displaced on the calix[4]arene scaffold, which raises to 3 fold with the glycolix[6]arene **1d**. The efficacy of the tetravalent calixarenes **1a-c** appears to be related to their conformational properties and the consequent geometry of presentation of the mannosamine units. In fact, the percentage of inhibition increases from the cone isomer to the 1,3-alternate and to the conformationally mobile (**1b** $10 \pm 2\%$, **1c** $21 \pm 3\%$, **1a** $34 \pm 7\%$). This suggests that, despite the same number of saccharide units in these three ligands, the upper rim of ligand **1b** is too crowded for an effective interaction with the antibodies resulting even worse than the monovalent model **1-MON**. The better behavior of **1c** supports this hypothesis since it exposes only two sugar units per part of the space with a reduced steric hindrance and perhaps an overall more convenient arrangement with respect to **1b**. However, also this blocked display of **1c** seems not to be the optimal one, as reflected in its lower level of efficacy with respect to the mobile isomer **1a** that evidently can adapt itself for a better interaction with the antibodies as allows the epitope units to better adapt to the binding sites. Flexibility appears then to play an important role in determining the efficacy. The inability of compound **calix[4]-Gal**, a tetra thioureidoglycolixarene based on the same calix[4]arene scaffold as **1a** but bearing a non-related sugar (galactose), in inhibiting the binding between 19F CPS and anti-19F antibodies confirms the specificity of the sugar recognition and excludes an unspecific cross-reaction with the calixarene scaffold. On the whole, the conformational properties, the related geometry of exposition of the mannosamine units, the flexibility together with the higher

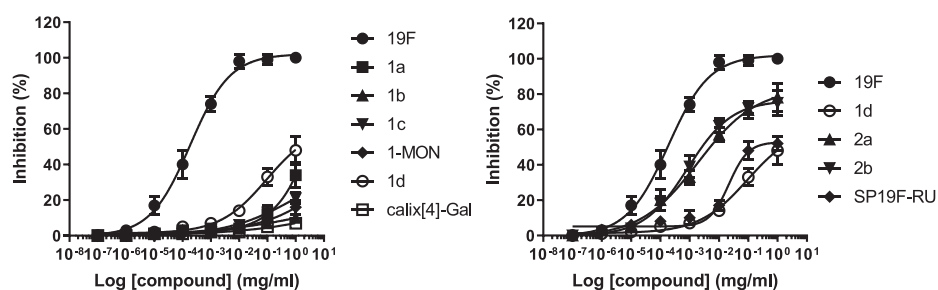
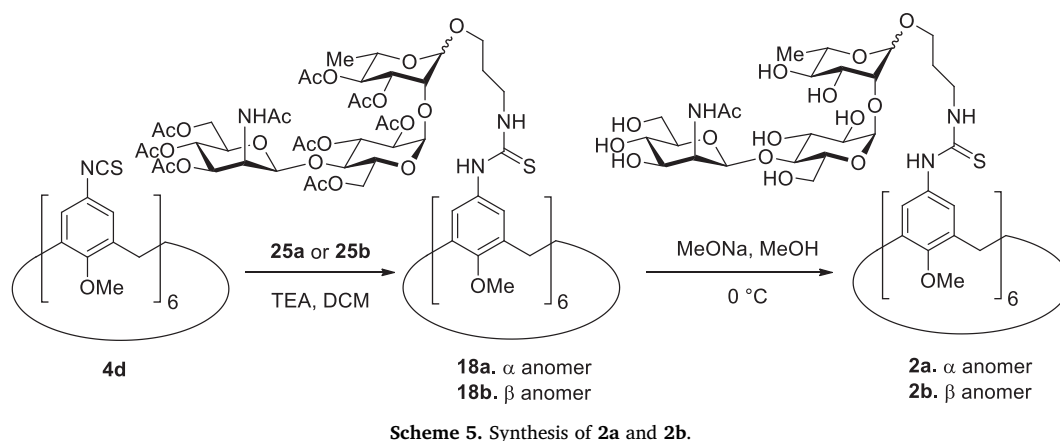


Fig. 3. Results of the Elisa experiments. Concentration/response curves of tested compounds on the inhibition of the binding between SP19F native polysaccharide, coated onto the plates, and the anti-19F antibodies, evaluated by a competitive ELISA method. Values are means of at least four experiments run in triplicate.

Table 1
Results of the competitive Elisa assay.

Compound	IC ₅₀ (mg/mL)	Max inhibition (%) ^a
19F	1.8×10^{-4}	100 ± 2
1a		34 ± 7
1b		10 ± 2
1c	1.2×10^{-1}	21 ± 3
1d	9.5×10^{-2}	48 ± 8
1-MON		16 ± 7
calix[4]-Gal		7 ± 2
SP19F-RU	2.1×10^{-2}	52 ± 4
2a	1.9×10^{-3}	78 ± 8
2b	8.6×10^{-4}	75 ± 7

^a The maximum inhibition elicited by each compound at 1 mg/mL.

valency could explain the highest efficacy (maximal inhibition $48 \pm 8\%$) and potency ($IC_{50} = 9.5 \times 10^{-2}$ mg/mL) showed by glyco-calix[6]arene **1d**. Calix[6]arene was then selected as the more promising scaffold for the functionalization with the SP19F trisaccharide repeating unit analogs **2a** and **2b**.

As shown in Fig. 3 and reported in Table 1, glyco-calix[6]arenes **2a** and **2b**, decorated with six trisaccharide SP19F repeating units, significantly improve efficacy (75–78% of inhibition) and affinity ($IC_{50} = 1.9 \times 10^{-3}$ and 8.6×10^{-4} mg/mL, respectively) towards the antibodies with respect to the simpler cluster **1d** (48% of inhibition, $IC_{50} = 9.5 \times 10^{-2}$ mg/mL). This result can be explained considering the nature of the saccharide ligands on the two clusters: the trisaccharide repeating unit is a more specific epitope for the anti-19F antibodies, whereas the lower, but still non-negligible, activity of compound **1d** could be ascribed to a predominant role of the mannosamine unit into the trisaccharide. Moreover, and more importantly, both glyco-calixarenes **2a** and **2b** are more active than the single trisaccharide unit (52% of inhibition and $IC_{50} = 2.1 \times 10^{-2}$ mg/mL) showing once more and more significantly that the presentation of the epitope units on this type of scaffold increases the strength and the

efficacy of antibody binding. In this framework, at the maximum of inhibition, the trisaccharide attached to the calix[6]arene platform is at a very similar concentration (1.31 mM) as when used alone (1.47 mM), but induces an efficacy 1.5 fold higher than SP19F-RU, determining an inhibition of the binding over 70% significantly higher than the 52% of the SP19F-RU. These data strongly suggest that the glyco-calix[6]arene thanks to its peculiar conformational mobility enables a proper presentation of the trisaccharide repeating units that improves the strength of the ligand-receptor interactions well mimicking the conformational organization of the epitopes generated by the natural 19F polysaccharide and recognized by the specific antibodies. This result is even more significant if we consider that the chemical structure of calixarenes **2a** and **2b** is much simpler than the one of the natural polysaccharide and yet able to provide a significant inhibitory effect. As a consequence, the maximum of inhibition compared with that of 19F CPS is relevant, 75% of inhibition versus the 100%. Furthermore, the structural difference due to the α and β anomeric connection of the trisaccharide to the spacer has no significant influence on the efficacy of the two ligands **2a** and **2b**, although resulted in slightly different potencies. This means that, in perspective, the separation of the two anomers **15a** and **15b** could even be avoided, proceeding in the coupling as a mixture of both with the calixarene isothiocyanate. The statistic products containing randomly both anomers should substantially show the same biological activity as **2a** and **2b** with a not negligible save in the procedure of preparation.

3. Conclusions

Fully synthetic carbohydrate-based vaccines offer the advantage to allow site-selective conjugation of saccharide epitopes [37,41,42] and to incorporate into the nanosystems active mediators to increase vaccine efficacy. The possibility to accurately control the number and type of vaccine active species ensures homogeneous composition, which is important to induce highly reproducible biological properties with a better safety profile. In this work, the calix[6]arene scaffold represents

a valuable platform for the simultaneous presentation of multiple copies of minimized portions of the 19F CPS. In fact, this system, bearing six trisaccharide repeating units linked through a thiourea group to the macrocycle via an aminopropyl spacer, has been able to efficiently bind to anti-19F antibodies with a significant improvement of the inhibition activity compared to the one of the single repeating unit. The overall efficiency is very high, especially considering the low number of exposed saccharide antigens compared to the large number of repeating units that are present in the natural polymer. Evidently, the structural properties of the calixarene can properly present the exposed saccharide units in a tridimensional arrangement that is significantly similar to that of the natural epitopes in CPS. Our data clearly suggest that, in perspective, glycolixarenes **2a** and **2b**, or even closely related systems with slightly longer saccharide fragments, could be functionalized with immunogenic peptides in order to elicit an antibacterial specific immune response. A structurally well-defined and easily reproducible multivalent glycolixarene should avoid the use of complex oligosaccharide species, which are generally required for immunogenicity. Calixarenes seem then to have great potential as carriers for the development of fully synthetic carbohydrate-based vaccines, and this work contributes towards this direction.

4. Experimental section

4.1. Chemical procedures

General information. All moisture sensitive reactions were carried out under a nitrogen or argon atmosphere, using previously oven-dried glassware. All dry solvents were prepared according to standard procedures, distilled before use and stored over 3 or 4 Å molecular sieves. All other reagents were commercial samples and used without further purification. Analytical TLC were performed using prepared plates of silica gel (Merck 60F-254 on aluminum) and then, according to the functional groups present on the molecules, revealed with UV light or using staining reagents: FeCl₃ (1% in H₂O/MeOH 1:1), H₂SO₄ (5% in EtOH), ninhydrin (5% in EtOH), basic solution of KMnO₄ (0.75% in H₂O), molybdic acid solution (molybdatophosphorus acid and Ce(IV) sulphate in 4% sulphuric acid). Reverse phase TLC were performed using silica gel 60 RP-18F-254 on aluminium sheets. Merck silica gel 60 was used for flash chromatography (40–63 µm) and for preparative TLC plates (10–12 µm). Sigma Aldrich C18 reverse phase silica gel was used for flash chromatography. ¹H NMR and ¹³C NMR spectra were recorded on Bruker AV300 and Bruker AV400 spectrometers (observation of ¹H nucleus at 300 MHz and 400 MHz, respectively, and of ¹³C nucleus at 75 MHz and 100 MHz, respectively) and partially deuterated solvents were used as internal standards to calculate the chemical shifts (δ values in ppm). All ¹³C NMR spectra were performed with proton decoupling. For ¹H NMR spectra recorded in D₂O at temperatures higher than 25 °C the correction of chemical shifts was performed using the expression $\delta = 5.060 - 0.0122 \times T(^{\circ}\text{C}) + (2.11 \times 10^{-5}) \times T^2(^{\circ}\text{C})$ [43] to determine the resonance frequency of water protons. Electrospray ionization (ESI) mass analyses were performed with a Waters single-quadrupole spectrometer or with a LTQ Orbitrap XL spectrometer. Melting points were determined on an Electrothermal apparatus in capillaries sealed under N₂ atmosphere. Microwave reactions were performed using a CEM Discovery System reactor.

5,11,17,23-Tetraamino-25,26,27,28-tetramethoxycalix[4]arene [25], 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene cone [27], 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene 1,3-alternate [25], 5,11,17,23,29,35-hexaamino-37,38,39,40,41,42-hexamethoxycalix[6]arene [26], 4-propoxyaniline [44], 5,11,17,23-tetraisothiocyanate-25,26,27,28-tetrapropoxycalix[4]arene cone [28], N-(benzyloxycarbonyl)aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-mannopyranoside [29] were prepared according to literature procedures.

4.1.1. 5,11,17,23-Tetraisothiocyanate-25,26,27,28-tetramethoxycalix[4]arene (4a)

In a two-neck round-bottom flask 5,11,17,23-tetraamino-25,26,27,28-tetramethoxycalix[4]arene **3a** (0.82 g, 1.22 mmol) was dissolved in 65 mL of dry toluene under N₂ atmosphere. Then thiophosgene (1.11 mL, 14.64 mmol, *caution*) and Et₃N (4.07 mL, 29.28 mmol) were added and the mixture was allowed to react at room temperature for 48 h. The solvent was removed under reduced pressure and the crude was redissolved in dichloromethane. The organic phase was washed with water (2 × 70 mL), 1 N HCl (70 mL) and then with a saturated solution of NaCl (2 × 80 mL). The solvent was removed under reduced pressure and the residue purified by column chromatography (hexane/DCM 7/3, v/v) to afford a yellowish solid (0.19 g, 0.28 mmol, 23% yield). Mp: dec > 180 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.14 (s, 1.6H, ArH, partial cone), 7.02 (s, 1.6H, ArH, partial cone), 6.81 (s, 1.6H, ArH, partial cone), 6.65 (s, 1.6H, ArH, cone), 6.29 (s, 1.6H, ArH, partial cone), 4.26 (d, *J* = 13.5 Hz, 0.8H, ArCHH_{ax}Ar, cone), 3.97 (d, *J* = 14.0 Hz, 1.6H, ArCHH_{ax}Ar, partial cone), 3.78 (s, 2.4H, OCH₃, cone), 3.74 (s, 2.4H, OCH₃, partial cone), 3.68 (s, 4.8H, OCH₃, partial cone), 3.56 (s, 3.2H, ArCHH_{eq}Ar, partial cone), 3.13 (d, *J* = 13.5 Hz, 0.8H, ArCHH_{eq}Ar, cone), 3.06 (d, *J* = 14.0 Hz, 1.6H, ArCHH_{eq}Ar, partial cone), 3.04 (s, 2.4H, OCH₃, partial cone). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 156.9, 156.4 (C_{Ar} ipso), 135.6, 134.3, 132.5, 127.7, 126.5, 126.2, 125.7, 125.6 (C_{Ar}, NCS), 61.9, 61.4, 60.2, 59.8 (OCH₃), 35.0 (ArCH₂Ar, partial cone), 30.3, 30.1 (ArCH₂Ar, cone and partial cone). HRMS (ESI-TOF) *m/z*: calcd for C₃₆H₂₈N₄O₄S₄Na [M + Na]⁺ 731.0886, found 731.0861.

4.1.2. 5,11,17,23-Tetraisothiocyanate-25,26,27,28-tetrapropoxycalix[4]arene 1,3-alternate (4c)

In a two-neck round-bottom flask 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene 1,3-alternate **3c** (1.23 g, 1.96 mmol) was dissolved in 25 mL of dry toluene under N₂ atmosphere. Then thiophosgene (1.8 mL, 23.47 mmol, *caution*) and Et₃N (6.53 mL, 46.94 mmol) were added and the mixture was allowed to react at room temperature for 48 h. The solvent was removed under reduced pressure and the crude was redissolved in dichloromethane. The organic phase was washed twice with distilled H₂O (30 mL), once with 1 N HCl (30 mL) and then twice with a saturated solution of NaCl (40 mL). The solvent was removed under reduced pressure and the desired compound was obtained in 33% yield as an off-white solid after purification by column chromatography (cyclohexane/DCM 4/1 v/v) (0.53 g, 0.65 mmol). Mp: 220–221 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 6.90 (s, 8H, ArH), 3.65 (t, *J* = 7.2 Hz, 8H, OCH₂), 3.47 (s, 8H, ArCH₂Ar), 1.87–1.78 (m, 8H, OCH₂CH₂CH₃), 1.09 (t, *J* = 7.2 Hz, 12H, CH₂CH₃). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 155.3 (C_{Ar} ipso), 134.0 (NCS), 127.1 (C_{Ar} ortho), 125.0 (C_{Ar} para), 74.7 (OCH₂), 35.1 (ArCH₂Ar), 23.9 (OCH₂CH₂CH₃), 10.7 (CH₂CH₃). HRMS (ESI-TOF) *m/z*: calcd for C₄₄H₄₄N₄O₄S₄Na [M + Na]⁺ 843.2138, found 843.2156.

4.1.3. 5,11,17,23,29,35-Hexaisothiocyanate-37,38,39,40,41,42-hexamethoxycalix[6]arene (4d)

In a round-bottom flask **3d** (0.15 g, 0.185 mmol) was dissolved in DCM (5 mL) under N₂ atmosphere. Then thiophosgene (254 µL, 3.33 mmol, *caution*), BaCO₃ (0.657 g, 3.33 mmol) and H₂O (3 mL) were added with the remaining amount of DCM (5 mL). The mixture was allowed to react at rt for 48 h and then diluted with DCM/H₂O. The organic phase was separated from the aqueous layer and evaporated under reduced pressure. The crude thus obtained was purified by flash chromatography (hexane/EtOAc 8/2, v/v) and crystallized with CH₃CN to yield the pure product as a white solid (39 mg, 0.037 mmol, 20%). Mp: dec > 200 °C. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 6.74 (s, 12H, ArH), 3.89 (s, 12H, ArCH₂Ar), 3.54 (s, 18H, OCH₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 155.3 (C_{Ar} ipso), 135.2 (C_{Ar} ortho), 133.9 (NCS), 126.6 (C_{Ar} para), 126.2 (C_{Ar} meta), 61.0 (OCH₃), 30.2 (ArCH₂Ar). HRMS (ESI-TOF) *m/z*: calcd for C₅₄H₄₂N₆O₆S₆Na [(4d + Na)⁺] 1085.1388, found

1085.1414 (80%); calcd for $C_{54}H_{42}N_6O_6S_6K$ $[M + K]^+$ 1101.1127, found 1101.1151 (100%).

4.1.4. Aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy- β -D-mannopyranoside (7)

To a solution of N-(benzyloxycarbonyl)aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy- β -D-mannopyranoside (6) (0.54 g, 0.79 mmol) in a 4:1 mixture of AcOEt/EtOH (10 mL), Pd/C (10%) was added and the suspension was shaken in a Parr hydrogenator for 90 min under 1.5 bar of H_2 at room temperature, after which the catalyst was filtered off and the filtrate evaporated under vacuum, to give the desired product as an orange oil (0.373 g, 0.68 mmol, 86%). 1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.38–7.13 (m, 15H, ArH), 6.20 (d, $J = 9.9$ Hz, 1H, NHAc), 4.90–4.79 (m, 3H, H_2 , $2 \times CHHPh$), 4.57 (d, $J = 11.9$ Hz, 1H, $CHHPh$), 4.52–4.42 (m, 4H, H_1 , $3 \times CHHPh$), 3.93–3.82 (m, 1H, $OCHHCH_2$), 3.73 (br, 2H, $H_{6a,b}$), 3.68–3.62 (m, 2H, H_4 , H_5), 3.61–3.51 (m, 1H, $OCHHCH_2$), 3.46–3.38 (m, 1H, H_3), 2.83–2.74 (m, 2H, $CH_2CH_2NH_2$), 2.03 (s, 3H, CH_3CO), 1.77–1.69 (m, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.9 ($COCH_3$), 138.2, 137.9, 137.7, 128.4, 128.33, 128.31, 127.9, 127.85, 127.8, 127.7 (C_{Ar}), 99.5 (C_1), 79.8 (C_5), 75.0 (C_3), 74.8 (CH_2Ph), 74.1 (C_4), 73.3 (CH_2Ph), 71.1 (CH_2Ph), 68.9 (C_6), 67.6 (OCH_2), 49.2 (C_2), 38.5 (CH_2NH_2), 27.9 ($CH_2CH_2CH_2$), 23.6 ($COCH_3$). ESI-MS m/z : calcd for $C_{32}H_{41}N_2O_6$ $[M + H]^+$ 549.3, found 549.0.

4.1.5. N-(Boc)aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy- β -D-mannopyranoside (8)

Aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy- β -D-mannopyranoside (7) (0.34 g, 0.63 mmol) was dissolved in MeOH (8 mL), then Et_3N (348 μ L, 2.5 mmol) and Boc_2O (0.68 g, 3.13 mmol) were subsequently added. The reaction was allowed to react for 2 h, then the solvent was removed under vacuum. The pure product was obtained as a pale oil in quantitative yield (0.40 g, 0.61 mmol). 1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.26–7.03 (m, 15H, ArH), 6.18 (d, $J = 9.4$ Hz, 1H, NHAc), 5.26 (br, 1H, NHBoc), 4.78–4.68 (m, 3H, H_2 , $2 \times CHHPh$), 4.44 (d, $J = 12.0$ Hz, 1H, $CHHPh$), 4.40–4.31 (m, 4H, $3 \times CHHPh$, H_1), 3.75–3.66 (m, 1H, $OCHHCH_2$), 3.64–3.58 (m, 2H, $H_{6a,b}$), 3.56–3.51 (m, 2H, H_4 , H_5), 3.50–3.39 (m, 1H, $OCHHCH_2$), 3.37–3.29 (m, 1H, H_3), 3.11–3.05 (m, 2H, CH_2CH_2NHBoc), 1.89 (s, 3H, $COCH_3$), 1.66–1.54 (m, 2H, $CH_2CH_2CH_2$), 1.31 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.8 ($COCH_3$), 156.1 ($CO(CH_3)_3$), 146.7, 138.2, 137.9, 137.8, 128.4, 128.3, 128.2, 127.8, 127.78, 127.73, 127.6 (C_{Ar}), 99.5 (C_1), 85.0 ($C(CH_3)_3$), 80.3 (C_5), 74.9 (C_3), 74.7 (CH_2Ph), 74.0 (C_4), 73.3 (CH_2Ph), 71.0 (CH_2Ph), 68.8 (C_6), 67.1 (OCH_2), 49.2 (C_2), 37.6 (CH_2NH_2), 29.7 ($CH_2CH_2CH_2$), 27.2 ($C(CH_3)_3$), 23.3 ($COCH_3$). ESI-MS m/z : calcd for $C_{37}H_{48}N_2O_8Na$ $[M + Na]^+$ 671.3, found 671.1.

4.1.6. N-(Boc)aminopropyl 2-acetamido-2-deoxy- β -D-mannopyranoside (9)

In a MW sealed vessel N-(Boc)aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy- β -D-mannopyranoside (8) (0.60 g, 0.93 mmol) was dissolved in a MeOH/ H_2O mixture (5 mL, 1/1 v/v) and Pd/C (10%) in catalytic amount and then NH_4COOH (0.23 g, 3.7 mmol) were added. The mixture was heated at 60 °C under microwave irradiation (150 W) for 1.5 h. The catalyst was filtered off and the solvent removed under reduced pressure, to give the desired product as white foam (0.30 g, 0.79 mmol, 85%) 1H NMR (400 MHz, MeOD) δ (ppm): 4.65 (s, 1H, H_1), 4.47 (d, $J = 3.2$ Hz, 1H, H_2), 3.87 (br, 3H, $H_{6a,b}$, $OCHHCH_2$), 3.68 (dd, $J_{3,2} = 4.0$, $J_{3,4} = 9.5$ Hz, 1H, H_3), 3.63–3.57 (m, 1H, $OCHHCH_2$), 3.53 (t, $J = 9.5$ Hz, 1H, H_4), 3.31–3.25 (m, 1H, H_5), 3.13 (t, $J = 6.4$ Hz, 2H, CH_2CH_2NHBoc), 2.05 (s, 3H, $COCH_3$), 1.73 (t, $J = 6.0$ Hz, 2H, $CH_2CH_2CH_2$), 1.45 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, MeOD) δ (ppm): 173.4 ($COCH_3$), 157.1 ($CO(CH_3)_3$), 99.4 (C_1), 78.5 ($C(CH_3)_3$), 76.9 (C_5), 73.0 (C_3), 66.9 (C_4), 66.3 (OCH_2), 60.5 (C_6), 53.5 (C_2), 36.9 (CH_2NH_2), 29.5 ($CH_2CH_2CH_2$), 27.4 ($C(CH_3)_3$), 21.4 ($COCH_3$). ESI-MS m/z : calcd for $C_{16}H_{32}N_2O_8Na$ $[M + Na]^+$ 401.2, found 401.3.

4.1.7. N-(Boc)aminopropyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-mannopyranoside (10)

N-(Boc)aminopropyl 2-acetamido-2-deoxy- β -D-mannopyranoside (9) (0.18 g, 0.47 mmol) was dissolved in pyridine (6 mL) and then acetic anhydride was added (740 μ L, 7.91 mmol). The reaction mixture was stirred at room temperature for 1 h, then the solvent removed under reduced pressure. The pure compound was obtained after purification via flash column chromatography (EtOAc/hexane 4/1, v/v) as a white foam in quantitative yield (0.23 g, 0.46 mmol). 1H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.94 (br, 1H, NHAc), 5.06 (t, $J = 9.2$ Hz, 1H, H_4), 4.97 (dd, $J_{3,2} = 4.0$, $J_{3,4} = 9.2$ Hz, 1H, H_3), 4.67–4.60 (m, 3H, H_1 , H_2 , NHBoc), 4.27 (dd, $J_{6a,5} = 6.0$, $J_{6a,6b} = 15.0$ Hz, 1H, H_{6a}), 4.12 (dd, $J_{6b,5} = 6.0$, $J_{6b,6a} = 15.0$ Hz, 1H, H_{6b}), 3.90–3.83 (m, 1H, $OCHHCH_2$), 3.68–3.58 (m, 1H, H_5), 3.55 (br, 1H, $OCHHCH_2$), 3.22–3.15 (m, 2H, CH_2CH_2NHBoc), 2.09 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.00 (s, 3H, $NHCOCH_3$), 1.77–1.68 (m, 2H, $CH_2CH_2CH_2$), 1.42 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.4, 170.5, 170.2, 169.7 ($COCH_3$), 156.1 ($CO(CH_3)_3$), 98.7 (C_1), 78.9 ($C(CH_3)_3$), 72.2 (C_5), 71.4 (C_3), 67.0 (OCH_2), 66.2 (C_4), 62.6 (C_6), 49.9 (C_2), 37.3 (CH_2NH_2), 29.5 ($CH_2CH_2CH_2$), 28.3 ($C(CH_3)_3$), 22.9, 20.6, 20.5 ($COCH_3$). ESI-MS m/z : calcd for $C_{22}H_{36}N_2O_{11}Na$ $[M + Na]^+$ 527.2, found 527.4.

4.1.8. Aminopropyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-mannopyranoside (11)

N-(Boc)aminopropyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-mannopyranoside (10) (0.24 g, 0.47 mmol) was dissolved in dry DCM (15 mL), then trifluoroacetic acid (1.27 mL, 16.45 mmol) was added dropwise. The reaction was allowed to stir at room temperature for 1 h, then it was quenched by the addition of Et_3N . The solvent was evaporated under reduced pressure and the desired compound was obtained as a yellow-orange oil in quantitative yield (0.19 g, 0.46 mmol). 1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.85 (d, $J = 9.0$ Hz, 1H, NHAc), 5.08 (t, $J = 9.6$ Hz, 1H, H_4), 4.99 (dd, $J_{3,2} = 4.6$, $J_{3,4} = 9.6$ Hz, 1H, H_3), 4.78–4.70 (m, 2H, H_1 , H_2), 4.24 (dd, $J_{6a,5} = 6.0$, $J_{6a,6b} = 12.4$ Hz, 1H, H_{6a}), 4.13 (dd, $J_{6b,5} = 2.4$, $J_{6b,6a} = 12.0$ Hz, 1H, H_{6b}), 4.01–3.92 (m, 1H, $OCHHCH_2$), 3.81–3.74 (m, 2H, H_5 , $OCHHCH_2$), 3.71–3.64 (m, 2H, $CH_2CH_2NH_2$), 2.07 (s, 3H, CH_3CO), 2.02 (s, 6H, CH_3CO), 1.99 (s, 3H, $NHCOCH_3$), 2.00–1.96 (m, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.5, 161.2, 160.4 ($COCH_3$), 98.2 (C_1), 72.3 (C_5), 71.3 (C_3), 66.6 (OCH_2), 66.0 (C_4), 62.4 (C_6), 49.5 (C_2), 37.4 (CH_2NH_2), 26.3 ($CH_2CH_2CH_2$), 22.4, 20.4 ($COCH_3$). HRMS (ESI-TOF) m/z : calcd for $C_{17}H_{28}N_2O_9Na$ $[M + Na]^+$ 427.1687, found 427.1658.

4.1.9. N-(tert-butyloxycarbonyl)-3-amminopropyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-acetyl-L-rhamnopyranoside (15)

Compound 16 [37] (0.19 g, 0.147 mmol) and N-(benzyloxycarbonyl)-3-amminopropyl (0.12 g, 0.586 mmol), as previously described [38], were dissolved in dry CH_2Cl_2 (3 mL) and activated powder molecular sieves 4 Å (0.10 g) were added. The suspension was stirred under Ar atmosphere at room temperature for 15 min, then it was cooled to 0 °C and TMSOTf 0.1 M in dry CH_2Cl_2 (0.29 mL, 0.029 mmol) was added. After 15 min, the reaction was quenched by the addition of TEA, filtered over a Celite pad and the solvent evaporated under reduced pressure. Purification of the crude through flash chromatography (Hexane/Ethyl Acetate 6:4) afforded 0.48 g of the less polar α -anomer, 0.80 g of a mixture of the two anomers, and 0.63 g of the β -anomer (17 overall yield: 96%). 17 α and 17 β were reacted separately in the next step. To a solution under Argon of compound SP3 in MeOH (0.01 M), Boc_2O (3.5 eq.) and then Pd(OH) $_2$ /C (1/1, w/w_{substrate}) were added. The mixture was stirred under hydrogen atmosphere for 3 h, and checked by TLC (hexane/AcOEt, 1/1) to confirm that the Z-amino protecting group has been exchanged with BOC. Then, one drop of HCl 1 N was added, and the reaction was stirred again under hydrogen atmosphere overnight. TLC (DCM/MeOH, 75/25) control showed that the

reaction was completed, then few drops of dry Py were added, and the reaction was filtered over filter paper. After evaporation of the solvent, the crude was dissolved in dry Py (0.03 M), and acetic anhydride was added (Ac₂O/Py, 1/2) together with a catalytic amount of DMAP. The reaction was stirred at room temperature for 19 h, diluted with MeOH, and then the solvent evaporated. Purification of the crude by flash chromatography (hexane/EtOAc, 1:9) gave compound **15** as an amorphous white solid.

15a: 0.32 g of **15a** were obtained starting from 0.48 g of **17a** (88% yield). $[\alpha]_D^{20} = +22.3$ ($c = 1$ in chloroform). ¹H NMR (CDCl₃): $\delta = 5.91$ (d, 1H, $J_{2',NH} = 7.2$ Hz, NH), 5.39 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.4$ Hz, H_{3'}), 5.29–5.20 (m, 2H, H_{1',H3'}), 5.15–5.03 (m, 2H, H_{4',H4''}), 4.92 (dd, 1H, $J_{2',3'} = 3.8$ Hz, $J_{3',4'} = 10.0$ Hz, H_{3''}), 4.76–4.56 (m, 5H, H₁, H_{1''}, H₂, H_{2''} and NH), 4.36 (dd, 1H, $J_{5'',6a''} = 5.3$ Hz, $J_{6a'',6b''} = 12.5$ Hz, H_{6a''}), 4.30–4.22 (m, 2H, 2H_{6'}), 4.15–4.03 (m, 2H, H_{5'}, H_{6a'}), 4.02–3.98 (m, 1H, H₂), 3.84–3.69 (m, 3H, H_a, H_{4'}, H₅), 3.67–3.59 (m, 1H, H_{5''}), 3.49–3.40 (m, 1H, H_{a'}), 3.28–3.17 (m, 2H, 2H_{6'}), 2.21–1.98 (9 s, 27H, 9 CH₃), 1.85–1.75 (m, 2H, 2H_b), 1.46 (s, 9H, (CH₃)₃CO), 1.20 (d, 3H, $J_{5,6} = 6.0$ Hz, 3H₆). ¹³C NMR (CDCl₃): $\delta = 171.9$ –169.6 (9C, C=O), 155.9 (C=O), 98.2 (C_{1'}), 96.4 (C₁), 93.3 (C_{1'}), 75.5 (C_{4'}), 73.7 (C₂), 72.7 (C_{5'}), 71.9 (C_{3'}), 71.3 (C₄), 71.0 (C_{2'}), 70.7 (C_{3'}), 69.9 (C₃), 68.2 (C_{5'}), 66.6 (C₅), 65.9 (C_a), 65.7 (C_{4''}), 62.2 (C_{6''}), 62.0 (C_{6'}), 50.8 (C_{2''}), 37.83 (C_c), 31.6 (Me₃C), 29.8 (C_b), 28.4 (Me₃C), 21.2–20.6 (9C, CH₃CO), 17.7 (C₆). MS (ESI) m/z (%): 1045.3 (1 0 0) [M + Na]⁺.

15b: 0.42 g of **15b** were obtained starting from 0.63 g of **17b** (85% yield). $[\alpha]_D^{20} = +55.4$ ($c = 1$ in chloroform). ¹H NMR (CDCl₃): $\delta = 5.94$ (d, 1H, $J_{2',NH} = 7.2$ Hz, NH), 5.62 (br d, 1H, $J_{1',2'} = 3.9$ Hz, H_{1'}), 5.41 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H_{3'}), 5.13–5.04 (m, 2H, H₄, H_{4''}), 4.98 (dd, 1H, $J_{2,3} = 3.0$ Hz, $J_{3,4} = 10.0$ Hz, H₃), 4.92 (dd, 1H, $J_{2',3'} = 4.0$ Hz, $J_{3',4'} = 10.0$ Hz, H_{3''}), 4.75 (dd, 1H, $J_{1',2'} = 3.9$ Hz, $J_{2',3'} = 10.2$ Hz, H_{2'}), 4.69 (br s, 1H, H_{1''}), 4.63–4.58 (m, 1H, H_{2''}), 4.47 (s, 1H, H₁), 4.36 (dd, 1H, $J_{5'',6a''} = 5.4$ Hz, $J_{6a'',6b''} = 12.4$ Hz, H_{6''}), 4.25 (dd, 1H, $J_{5',6a'} = 5.0$ Hz, $J_{6a',6b'} = 11.8$ Hz, H_{6a'}), 4.21–4.12 (m, 3H, H₂, H_{5'}, H_{6b'}), 4.06 (dd, 1H, $J_{5'',6a''} = 2.2$ Hz, $J_{6a'',6b''} = 12.4$ Hz, H_{6a''}), 3.87–3.79 (m, 1H, H_a), 3.69 (t, 1H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H_{4'}), 3.65–3.61 (m, 1H, H_{5''}), 3.50–3.41 (m, 2H, H_a, H₅), 3.29–3.19 (m, 1H, H_c), 3.11–3.02 (m, 1H, H_c), 2.17–2.00 (9 s, 27H, 9 CH₃), 1.82–1.66 (m, 2H, 2H_b), 1.45 (s, 9H, (CH₃)₃CO), 1.26 (d, 3H, $J_{5,6} = 6.0$ Hz, 3H₆). ¹³C NMR (CDCl₃): $\delta = 172.1$ –169.5 (9C, C=O), 156.1 (C=O), 100.9 (C₁), 98.2 (C_{1'}), 94.3 (C_{1'}), 75.7 (C_{4'}), 72.7 (C_{5'}), 72.3 (C₂), 71.9 (2C, C₃, C_{3'}), 71.1 (C₄), 70.7 (C₅), 70.5 (C_{3'}), 70.3 (C_{2'}), 67.8 (C_{5'}), 67.6 (C_a), 65.8 (C_{4''}), 62.2 (2C, C₆, C_{6''}), 50.8 (C_{2''}), 37.3 (C_c), 31.6 (Me₃C), 29.4 (C_b), 28.4 (Me₃C), 23.1–20.6 (9C, CH₃CO), 17.7 (C₆). MS (ESI) m/z (%): 1045.3 (1 0 0) [M + Na]⁺.

4.1.10. 5,11,17,23-Tetrakis-N-[3-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-mannopiranosyloxy)-propyl-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene cone (**5b**)

In a two-neck round-bottom flask calixarene **4b** (0.05 g, 0.0609 mmol) was dissolved in dry DCM (6 mL) under N₂ atmosphere, then sugar **11** (0.123 g, 0.305 mmol) and Et₃N (255 μL, 1.83 mmol) were added. The mixture was stirred at rt for 24 h, after which half equivalents of sugar and Et₃N were added and the mixture stirred for additional 16 h. The solvent was removed under reduced pressure and the crude was purified via flash column chromatography (DCM/MeOH 20/1, v/v) yielding cone derivative **5b** in 37% yield as a white solid (0.0547 g, 0.0225 mmol). Mp: dec > 130 °C. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 8.36 (br, 4H, NHCS), 6.65 (br, 8H, ArH), 6.38 (br, 8H, CSNHCH₂, NHAc), 5.15–5.09 (m, 8H, H₃, H₄), 4.78–4.74 (m, 8H, H₂, H₁), 4.41 (d, $J = 13.2$ Hz, 4H, ArCHH_{ax}Ar), 4.27 (dd, $J_{6a,5} = 5.6$, $J_{6a,6b} = 12.0$ Hz, 4H, H_{6a}), 4.13 (dd, $J_{6b,5} = 2.4$, $J_{6b,6a} = 12.0$ Hz, 4H, H_{6b}), 3.97–3.96 (m, 4H, OCHHCH₂CH₂), 3.84 (br, 12H, OCH₂CH₂CH₃, CHHNHCS), 3.70–3.69 (m, 4H, H₅), 3.57 (br s, 4H, OCHHCH₂CH₂), 3.49–3.48 (m, 4H, CHHNHCS), 3.14 (d, $J = 13.2$ Hz, 4H, ArCHH_{eq}Ar), 2.07 (s, 12H, CH₃CO), 2.05 (s, 12H, CH₃CO), 2.03 (s, 12H, CH₃CO),

2.01 (s, 12H, CH₃CO), 1.96–1.90 (m, 16H, OCH₂CH₂CH₃, CH₂CH₂CH₂), 0.94 (t, $J = 7.5$ Hz, 12H, CH₂CH₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 180.4 (CS), 172.1 (NHCOCH₃), 170.7 (COCH₃), 170.5 (COCH₃), 169.8 (COCH₃), 154.5 (C_{Ar} ipso), 136.2 (C_{Ar} ortho), 132.1 (C_{Ar} para), 124.8 (C_{Ar} meta), 99.1 (C₁), 77.3 (OCH₂CH₂CH₃), 72.4 (C₅), 71.4 (C₄), 68.7 (OCH₂CH₂CH₂), 66.0 (C₃), 62.5 (C₆), 50.3 (C₂), 43.0 (CH₂NHCS), 30.9 (ArCH₂Ar), 29.9 (CH₂CH₂CH₂), 28.9 (OCH₂CH₂CH₃), 23.4 (COCH₃), 23.2 (COCH₃), 20.8 (COCH₃), 20.7 (COCH₃), 10.2 (CH₂CH₂CH₃). HRMS (ESI-TOF) m/z : calcd for C₁₁₂H₁₅₆N₁₂O₄₀S₄Na₂ [M + 2Na]²⁺ 1241.4590, found 1241.4586.

4.1.11. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-mannopiranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxycalix[6]arene (**5d**)

To a solution of compound **4d** (0.0416 g, 0.0392 mmol) in DCM (8 mL) sugar **11** (0.111 g, 0.274 mmol) and NEt₃ (273 μL, 1.96 mmol) were added and the mixture stirred at rt for 72 h. The solvent was removed under reduced pressure and the crude purified via flash column chromatography and preparative TLC (DCM/MeOH 94/6, v/v) to afford **5d** as a yellowish solid (0.0317 mg, 0.0091 mmol, 23%). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.66 (br, 6H, NHCS), 6.92 (br, 12H, ArH), 6.57 (s, 12H, CSNHCH₂, NHAc), 5.18 (br, 6H, H₄), 5.03 (br, 6H, H₃), 4.77 (br, 6H, H₂), 4.71 (br, 6H, H₁), 4.29 (br, 6H, H_{6a}), 4.14 (br, 6H, H_{6b}), 4.03–3.17 (overlapped, 60H, OCHHCH₂CH₂, CHHNHCS, H₅, ArCH₂Ar, OCH₃), 2.10 (s, 18H, CH₃CO), 2.07 (s, 36H, CH₃CO), 2.03 (s, 18H, CH₃CO), 1.81 (br, 12H, CH₂CH₂CH₂). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 180.6 (CS), 172.1 (COCH₃), 170.7 (COCH₃), 170.3 (COCH₃), 169.7 (COCH₃), 154.2 (C_{Ar} ipso), 134.8 (br, C_{Ar} ortho, C_{Ar} para), 125.3 (C_{Ar} meta), 99.9 (C₁), 72.5 (C₅), 71.3 (C₃), 68.6 (OCH₂CH₂CH₂), 66.1 (C₄), 62.6 (C₆), 61.0 (OCH₃), 50.4 (C₂), 42.9 (CH₂NHCS), 29.7 (ArCH₂Ar), 29.0 (CH₂CH₂CH₂), 23.4 (NHCOCH₃), 20.9 (COCH₃), 20.8 (COCH₃), 20.7 (COCH₃). HRMS (ESI-TOF) m/z : calcd for C₁₅₆H₂₁₀N₁₈O₆₀S₆Na₂ [M + 2Na]²⁺ 1766.6028, found 1766.6047.

4.1.12. N-[3-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-mannopiranosyloxy)-propyl-thioureido]-4-propoxybenzene (**14**)

To a solution of **13** (0.03 g, 0.155 mmol) in dry DCM (4 mL) under N₂ atmosphere, sugar **11** (0.0943 g, 0.233 mmol) and NEt₃ (324 μL, 2.33 mmol) were added and the mixture stirred for 12 h at rt. The solvent was removed under reduced pressure and the crude purified by flash column chromatography (DCM/MeOH 20/1, v/v) yielding **14** as an off-white solid (0.0490 g, 0.0821 mmol, 53%). Mp: 90–91 °C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.25 (br, 1H, NHCS), 7.18 (d, $J = 8.0$ Hz, 2H, ArH), 6.86 (d, $J = 8.0$ Hz, 2H, ArH), 6.23 (br, 1H, CH₂NHCS), 6.05 (d, $J = 7.6$ Hz, 1H, NHAc), 5.08 (t, $J = 9.6$ Hz, 1H, H₄), 4.99 (dd, $J_{3,2} = 4.0$ Hz, $J_{3,4} = 10.0$ Hz, 1H, H₃), 4.72 (d, $J = 4.0$ Hz, 1H, H₂), 4.63 (s, 1H, H₁), 4.26 (dd, $J_{6a,5} = 5.2$ Hz, $J_{6a,6b} = 12.4$ Hz, 1H, H_{6a}), 4.08 (d, $J = 12.0$ Hz, 1H, H_{6b}), 3.89–3.86 (m, 3H, OCHHCH₂CH₂, OCH₂CH₂CH₃), 3.76 (br, 1H, CHHNHCS), 3.67–3.56 (m, 2H, H₅, OCHHCH₂CH₂), 3.53 (br, 1H, CHHNHCS), 2.06 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 2.02 (s, 3H, COCH₃), 1.97 (s, 3H, COCH₃), 1.75 (m, 4H, OCH₂CH₂CH₃, CH₂CH₂CH₂), 0.98 (t, $J = 7.2$ Hz, 3H, CH₂CH₃). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 181.7 (CS), 171.7 (NHCOCH₃), 170.6 (COCH₃), 170.1 (COCH₃), 169.7 (COCH₃), 157.9 (C_{Ar} ipso), 129.8 (C_{Ar} ortho), 127.3 (C_{Ar} para), 127.3, 125.6 (C_{Ar} meta), 99.1 (C₁), 72.5 (C₅), 71.0 (C₃), 69.8 (OCH₂CH₂CH₃), 68.5 (OCH₂CH₂CH₂), 66.0 (C₄), 62.4 (C₆), 50.3 (C₂), 43.1 (CH₂NHCS), 29.7 (CH₂CH₂CH₂), 23.4 (COCH₃), 20.8 (OCH₂CH₂CH₃), 20.8, 20.7 (COCH₃), 10.5 (OCH₂CH₂CH₃). HRMS (ESI-TOF) m/z : calcd for C₂₇H₃₉N₃O₁₀SNa [M + Na]⁺ 620.2254, found 620.2261.

4.1.13. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-mannopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl-(1 → 2)-3,4-di-O-acetyl-α-L-rhamnopyranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxycalix[6]arene (**18a**)

15a (22 mg, 0.0215 mmol) was dissolved in dry DCM (4 mL) under

Ar atmosphere. The temperature was decreased to 0 °C and 0.5 mL of trifluoroacetic were added. The reaction proceeded at room temperature for 1 h, after which the TLC showed the complete consumption of the reagent, thus the solvent was evaporated at reduced pressure. The obtained deprotected product was dissolved in dry DCM (2 mL) and 5,11,17,23,29,35-hexaisothiocyanate-37,38,39,40,41,42-hexamethoxycalix[6]arene (**4d**) (2.5 mg, 0.00239 mmol) and NEt_3 (17 μL , 0.120 mmol) were added under Ar atmosphere. The mixture was stirred for 48 h at room temperature, after which the reaction was quenched by removal of the solvent at reduced pressure. The desired product was obtained after column chromatography (DCM/MeOH 96:4 v/v) as a white solid in 86% yield (13.5 mg, 0.00205 mmol). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.01 (br, 12H, ArH), 5.91 (br, 6H, NHAc), 5.38 (t, $J_{3',2'} = J_{3',2''} = 9.5$ Hz, 6H, $\text{H}_{3'}$), 5.24 (br, 12H, H_3 , $\text{H}_{1'}$), 5.14–5.03 (m, 12H, H_4 , $\text{H}_{4'}$), 4.93 (br, 6H, $\text{H}_{3''}$), 4.77–4.67 (m, 12H, $\text{H}_{2'}$, $\text{H}_{1''}$), 4.62 (br, 12H, $\text{H}_{2''}$, H_1), 4.37 (dd, $J_{6a,5} = 5.1$, $J_{6a,6b} = 12.5$ Hz, 6H, $\text{H}_{6a''}$), 4.25 (br, 12H, H_6), 4.12–4.03 (m, 12H, H_5 , $\text{H}_{6''}$), 4.00 (br, 6H, H_2), 3.84–3.70 (m, 18H, H_5 , H_4 , $\text{OCHHCH}_2\text{CH}_2$), 3.67 (br, 12H, $\text{H}_{5''}$, $\text{OCHHCH}_2\text{CH}_2$), 3.45 (br, 12H, CH_2NHCS), 2.16 (s, 18H, CH_3CO), 2.13 (s, 18H, CH_3CO), 2.12 (s, 18H, CH_3CO), 2.11 (s, 18H, CH_3CO), 2.09 (s, 18H, CH_3CO), 2.06 (s, 36H, CH_3CO), 2.04 (s, 18H, CH_3CO), 2.02 (s, 18H, CH_3CO), 1.88 (br, 12H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.19 (d, $J = 5.8$ Hz, 18H, H_6). HRMS (ESI-TOF) m/z : calcd for $\text{C}_{288}\text{H}_{390}\text{N}_{18}\text{O}_{144}\text{S}_6\text{Na}_4$ [$\text{M} + 4\text{Na}$] $^{4+}$ 1672.0388, found 1672.0396.

4.1.14. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-acetyl- α -D-glucopyranosyl-(1 $\rightarrow$ 2)-3,4-di-O-acetyl- β -L-rhamnopyranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxycalix[6]arene (6b**)**

15b (29 mg, 0.0283 mmol) was dissolved in dry DCM (4 mL) under Ar atmosphere. The temperature was decreased to 0 °C and 0.5 mL of trifluoroacetic were added dropwise. The reaction proceeded at room temperature for 1 h, after which the TLC showed the complete consumption of the reagent, thus the solvent was evaporated at reduced pressure. The crude was then dissolved in dry DCM (3 mL) and 5,11,17,23,29,35-hexaisothiocyanate-37,38,39,40,41,42-hexamethoxycalix[6]arene (**4d**) (3.3 mg, 0.00314 mmol) and NEt_3 (21 μL , 0.157 mmol) were added under Ar atmosphere. The mixture was stirred for 48 h at room temperature, after which the reaction was quenched by removal of the solvent at reduced pressure. The desired product was obtained after column chromatography (DCM/MeOH 95/5, v/v) as a white solid in 82% yield (17 mg, 0.00258 mmol). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.11 (br, 12H, ArH), 5.93 (d, $J = 7.3$ Hz, 6H, NHAc), 5.60 (br, 6H, $\text{H}_{1'}$), 5.41 (t, $J = 9.1$ Hz, 6H, $\text{H}_{3'}$), 5.17–5.02 (m, 12H, $\text{H}_{4'}$, H_4), 4.96 (dd, $J_{3,2} = 3.0$, $J_{3,4} = 10.1$ Hz, 6H, H_3), 4.93 (dd, $J_{3'',2''} = 5.8$, $J_{3'',4''} = 9.9$ Hz, 6H, $\text{H}_{3''}$), 4.74 (br, 6H, $\text{H}_{1''}$), 4.69 (br, 6H, $\text{H}_{2'}$), 4.62 (br, 6H, $\text{H}_{2''}$), 4.50 (br, 6H, H_1), 4.39 (dd, 6H, $\text{H}_{6a''}$), 4.28 (br, 6H, $\text{H}_{6a'}$), 4.13 (br, 18H, H_2 , H_5 , $\text{H}_{6b'}$), 4.05 (d, $J = 12.0$ Hz, 6H, $\text{H}_{6b''}$), 3.86 (br, 12H, CH_2NHCS), 3.75 (br, 6H, $\text{H}_{4'}$), 3.66 (br, 6H, $\text{H}_{5''}$), 3.45 (br, 18H, H_5 , OCH_2), 2.16 (s, 18H, CH_3CO), 2.12 (s, 36H, CH_3CO), 2.11 (s, 18H, CH_3CO), 2.061 (s, 18H, CH_3CO), 2.056 (s, 18H, CH_3CO), 2.05 (s, 18H, CH_3CO), 2.03 (s, 18H, CH_3CO), 2.01 (s, 18H, CH_3CO), 1.75 (br, 12H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.23 (d, $J = 5.8$ Hz, 18H, H_6). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 172.1, 170.6, 170.5, 170.4, 170.1, 169.7, 169.6, 135.2, 125.0, 100.8, 98.3, 94.0, 75.8, 72.6, 72.3, 71.9, 71.8, 71.0, 70.7, 70.4, 67.8, 65.6, 62.3, 62.1, 60.8, 50.8, 41.8, 32.2, 29.7, 26.4, 23.4, 23.2, 21.3, 20.9, 20.8, 20.76, 20.68, 17.7, 14.1. HRMS (ESI-TOF) m/z : calcd for $\text{C}_{288}\text{H}_{390}\text{N}_{18}\text{O}_{144}\text{S}_6\text{Na}_4$ [$\text{M} + 4\text{Na}$] $^{4+}$ 1672.0388, found 1672.0399.

4.1.15. General procedure for the deacetylation reaction of glycolcalix[n]arenes 5a-d and monomer 14

To a solution of the peracetylated compound in MeOH at 0 °C, freshly prepared MeONa was added till pH 9. The solution was stirred for 3 h, after which Amberlite IR-120 (H^+), and 1 mL of H_2O in the case of the hexacalixarenes, were added and the mixture stirred at rt till neutral pH. The resin was filtered off and the solvent removed under reduced pressure.

4.1.15.1. 5,11,17,23-Tetrakis-N-[3-(2-acetamido-2-deoxy- β -D-mannopiranosyloxy)-propyl-thioureido]-25,26,27,28-tetramethoxycalix[4]arene (1a**). The crude was first purified by C18 reverse phase column chromatography (MeOH/ H_2O 5.5/4.5, v/v), and then by size exclusion chromatography on Sephadex G-25 as stationary phase (MeOH/ H_2O 1.5/8.5, v/v). The final purification step was performed via standard flash chromatography (i-PrOH/ H_2O / Et_3N 8/2/0.5, v/v/v) to afford the mobile calix[4]arene **1a** in 40% yield (0.037 g, 0.0191 mmol). Mp: dec > 140 °C. ^1H NMR (400 MHz, D_2O , 80 °C) δ (ppm): 6.98 (br, 8H, ArH), 4.69 (br, 4H, $\text{H}_{1'}$), 4.43 (br, 4H, H_2), 3.88–3.22 (m, 48H, H_3 , H_4 , H_5 , H_{6a} , H_{6b} , OCH_2CH_2 , CH_2NHCS , OCH_3), 1.97 (s, 12H, NHCOCH_3), 1.80–1.74 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2$). ^{13}C NMR (100 MHz, MeOD, 55 °C) δ (ppm): 180.6 (CS), 173.6 (NHCOCH_3), 155.7 (C_{Ar} ipso), 135.32 (C_{Ar} ortho), 132.4 (C_{Ar} para), 124.4 (C_{Ar}), 99.5 (C_1), 77.0 (C_5), 73.0 (C_3), 67.2 (C_4 , $\text{OCH}_2\text{CH}_2\text{CH}_2$), 61.0 (C_6 , OCH_3), 53.6 (C_2), 42.1 (CH_2NHCS , ArCH_2Ar), 29.2 ($\text{CH}_2\text{CH}_2\text{CH}_2$), 22.2 (NHCOCH_3). HRMS (ESI-TOF) m/z : calcd for $\text{C}_{80}\text{H}_{116}\text{N}_{12}\text{O}_{28}\text{S}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 1843.6803, found 1843.6812.**

4.1.15.2. 5,11,17,23-Tetrakis-N-[3-(2-acetamido-2-deoxy- β -D-mannopiranosyloxy)-propyl-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene cone (1b**). The pure product **1b** was obtained after purification by C18 reverse phase column chromatography (MeOH/ H_2O 4/1, v/v) in 78% yield as a white solid (0.034 g, 0.0176 mmol). Mp: dec > 178 °C. ^1H NMR (400 MHz, MeOD) δ (ppm): 6.71 (br, 8H ArH), 4.71 (s, 4H, $\text{H}_{1'}$), 4.53 (s, 4H, H_2), 4.47 (d, $J = 13.2$ Hz, 4H, $\text{ArCHH}_{ax}\text{Ar}$), 3.98–3.86 (m, 24H, $\text{H}_{6a,b}$, $\text{OCHHCH}_2\text{CH}_2$, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.71–3.63 (m, 16H, H_3 , $\text{OCHHCH}_2\text{CH}_2$, CHHNHCS), 3.58 (t, $J = 9.6$ Hz, 4H, H_4), 3.29–3.27 (m, 4H, H_5), 3.18 (d, $J = 13.2$ Hz, 4H, $\text{ArCHH}_{eq}\text{Ar}$), 2.08 (s, 12H, NHCOCH_3), 2.02–1.96 (m, 8H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 1.86 (br, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.06 (t, $J = 6.8$ Hz, 12H, $\text{OCH}_2\text{CH}_2\text{CH}_3$). ^{13}C NMR (100 MHz, MeOD) δ (ppm): 179 (CS), 173.6 (NHCOCH_3), 154.0 (C_{Ar} ipso), 135.32 (C_{Ar} ortho), 132.2 (C_{Ar} para), 123.9 (C_{Ar}), 99.5 (C_1), 76.9 (C_5), 76.8 (C_6), 72.8 (C_3), 67.3 ($\text{OCH}_2\text{CH}_2\text{CH}_2$), 66.9 (C_4), 60.7 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 53.6 (C_2), 47.7 (CH_2NHCS), 42.2 (C_2), 30.5 (ArCH_2Ar), 28.7 ($\text{CH}_2\text{CH}_2\text{CH}_2$), 23.1 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 21.7 (NHCOCH_3), 9.5 ($\text{OCH}_2\text{CH}_2\text{CH}_3$). HRMS (ESI-TOF) m/z : calcd for $\text{C}_{88}\text{H}_{132}\text{N}_{12}\text{O}_{28}\text{S}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 1955.8055, found 1955.8069.**

4.1.15.3. 5,11,17,23-Tetrakis-N-[3-(2-acetamido-2-deoxy- β -D-mannopiranosyloxy)-propyl-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 1,3-Alternate (1c**). Compound **1c** was obtained after purification via C18 reverse phase column chromatography (MeOH/ H_2O 3/2, v/v) as a white solid (0.023 g, 0.0119 mmol, 83%). Mp: dec > 176 °C. ^1H NMR (400 MHz, MeOD) δ (ppm): 7.09 (s, 8H, ArH), 4.71 (s, 4H, $\text{H}_{1'}$), 4.56 (d, $J = 3.6$ Hz, 4H, H_2), 4.06–3.94 (m, 4H, CHHNHCS), 3.87 (d, $J = 3.2$ Hz, 8H, $\text{H}_{6a,b}$), 3.83–3.64 (m, 24H, H_3 , $\text{OCH}_2\text{CH}_2\text{CH}_2$, CHHNHCS , $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.63–3.50 (m, 12H, ArCH_2Ar , H_4), 3.31–3.25 (m, 4H, H_5), 2.10 (s, 12H, NHCOCH_3), 2.01–1.81 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.96–1.80 (m, 8H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 1.04 (t, $J = 7.6$ Hz, 12H, $\text{OCH}_2\text{CH}_2\text{CH}_3$). ^{13}C NMR (100 MHz, MeOD) δ (ppm): 180.7 (CS), 173.5 (NHCOCH_3), 153.9 (C_{Ar} ipso), 133.6 (C_{Ar} ortho), 131.6 (C_{Ar} para), 125.4 (C_{Ar}), 99.52 (C_1), 76.90 (C_5), 74.9 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 72.8 (C_3), 67.2 (CH_2NCS), 66.9 (C_4), 60.7 (C_6), 53.6 (C_2), 42.2 ($\text{OCH}_2\text{CH}_2\text{CH}_2$), 35.0 (ArCH_2Ar), 28.8 ($\text{CH}_2\text{CH}_2\text{CH}_2$), 23.6 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 21.6 (NHCOCH_3), 9.7 ($\text{OCH}_2\text{CH}_2\text{CH}_3$). HRMS (ESI-TOF) m/z : calcd for $\text{C}_{88}\text{H}_{132}\text{N}_{12}\text{O}_{28}\text{S}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 1955.8055, found 1955.8070.**

4.1.15.4. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-2-deoxy- β -D-mannopiranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxycalix[6]arene (1d**). Purification by C18 reverse phase column chromatography (MeOH/ H_2O 64/36, v/v) afforded **1d** in 54% yield as a off-white solid (0.015 g, 0.00549 mmol). Mp: 186–187 °C. ^1H NMR (400 MHz, D_2O , 80 °C) δ (ppm): 7.45 (s, 12H, ArH), 5.20 (s, 6H, $\text{H}_{1'}$), 4.97 (d, $J = 4.4$ Hz, 6H, H_2), 4.44 (br, 12H,**

ArCH₂Ar), 4.40–4.23 (m, 24H, H₃, H_{6a,b}, OCHHCH₂CH₂), 4.18–4.11 (m, 6H, OCHHCH₂CH₂), 4.07–4.00 (m, 18H, CH₂NHCS, H₄), 3.84 (br, 6H, H₅), 3.77 (br, 18H, CH₃), 2.52 (s, 18H, NHCOCH₃), 2.31 (br, 12H, CH₂CH₂CH₂). ¹³C NMR (100 MHz, D₂O, 80 °C) δ (ppm): 180.7 (CS), 175.4 (NHCOCH₃), 154.8 (C_{Ar} ipso), 135.6 (C_{Ar} ortho), 134.1 (C_{Ar} para), 126.8 (C_{Ar}), 99.9 (C₁), 77.2 (C₅), 75.0 (C₃), 67.8 (C₄), 67.7 (OCH₂CH₂CH₂), 61.2 (OCH₃), 59.9 (C₆), 53.8 (C₂), 42.5 (CH₂NHCS), 30.7 (CH₂CH₂CH₂), 29.2 (ArCH₂Ar), 22.8 (NHCOCH₃). HRMS (ESI-TOF) *m/z*: calcd for C₁₂₀H₁₇₄N₁₈O₄₂S₆K₂ [M + 2 K]²⁺ 1404.6177, found 1404.6204.

4.1.15.5. N-[3-(2-acetamido-2-deoxy-β-D-mannopyranosyloxy)-propyl-thioureido]-4-propoxybenzene (1-MON). The pure product was obtained after purification by C18 reverse phase column chromatography (MeOH/H₂O 1/1, v/v) as a off-white solid (0.0272 g, 0.0577 mmol, 66%). Mp: dec > 76 °C. ¹H NMR (400 MHz, MeOD) δ (ppm): 7.24 (d, *J* = 8.9 Hz, 2H, ArH), 6.93 (d, *J* = 8.9 Hz, 2H, ArH), 4.65 (d, *J* = 1.5 Hz, 1H, H₁), 4.48 (dd, *J*_{2,1} = 1.5, *J*_{2,3} = 4.5 Hz, 1H, H₂), 3.95 (t, *J* = 6.5 Hz, 2H, OCH₂CH₂CH₃), 3.93–3.89 (m, 1H, OCHHCH₂CH₂), 3.88–3.84 (m, 1H, H_{6a,b}), 3.69 (br, 1H, CHHNHCS), 3.67 (dd, *J*_{3,2} = 4.4, *J*_{3,4} = 9.6 Hz, 1H, H₃), 3.61 (m, 2H, OCHHCH₂CH₂, CHHNHCS), 3.53 (t, *J* = 9.6 Hz, 1H, H₄), 3.29–3.23 (m, 1H, H₅), 2.04 (s, 3H, NHCOCH₃), 1.89–1.76 (m, 4H, OCH₂CH₂CH₃, CH₂CH₂CH₂), 1.06 (t, *J* = 7.2 Hz, 3H, OCH₂CH₂CH₃). ¹³C NMR (100 MHz, MeOD) δ (ppm): 181.1 (CS), 173.5 (NHCOCH₃), 157.7 (C_{Ar} ipso), 127.3 (C_{Ar} para), 126.7, 114.5 (C_{Ar} meta), 99.4 (C₁), 76.9 (C₅), 76.8 (OCH₂CH₂CH₃), 69.4 (C₄, OCH₂CH₂CH₂), 60.6 (C₆), 53.5 (C₂), 41.9 (CH₂NHCS), 28.6 (CH₂CH₂CH₂), 22.3 (OCH₂CH₂CH₃), 21.4 (NHCOCH₃), 9.4 (OCH₂CH₂CH₃). HRMS (ESI-TOF) *m/z*: calcd for C₂₁H₃₃N₃O₇Sn [M + Na]⁺ 494.1937, found 494.1955.

4.1.16. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-2-deoxy-β-D-mannopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxycalix[6]arene (2a)

The pure product was obtained after trituration in diethyl ether (7.5 mg, 0.00164 mmol, 80%). ¹H NMR (400 MHz, MeOD/D₂O 75/25) δ (ppm): 8.49 (s, NH), 7.01 (br, 12H, ArH), 5.04 (br, OH), 4.94 (br, 6H, H₁), 4.87 (br, 6H, H₁), 4.85 (br, 6H, H₁), 4.54 (br, 6H, H₂), 4.14–3.22 (overlapped, 144H, H₃, H₄, H₅, H_{6a,b}, H₂, H₃, H₄, H₅, H_{6a,b}, H₂, H₃, H₄, H₅, OCH₂CH₂CH₂, CH₂NHCS, OCH₃, ArCH₂Ar), 2.08 (s, 18H, NHCOCH₃), 1.86 (br, 12H, CH₂CH₂CH₂), 1.29 (br, 18H, H₆). ¹³C NMR (100 MHz, MeOD/D₂O 1:1) δ (ppm): 174.5, 99.4, 98.0, 97.7, 78.8, 76.9, 76.8, 72.5, 72.3, 71.6, 71.5, 70.5, 70.1, 68.9, 66.7, 65.2, 60.4, 59.9, 53.5, 39.9, 28.7, 21.8, 16.8. HRMS (ESI-TOF) *m/z*: calcd for C₁₉₂H₂₉₄N₁₈O₉₆S₆Na₃ [M + 3Na]³⁺ 1550.2248, found 1550.2276.

4.1.17. 5,11,17,23,29,35-Hexakis-N-[3-(2-acetamido-2-deoxy-β-D-mannopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 2)-β-L-rhamnopyranosyloxy)-propyl-thioureido]-37,38,39,40,41,42-hexamethoxy calix[6]arene (2b)

The pure product was obtained after trituration in diethyl ether (9.6 mg, 0.00209 mmol, 81%). ¹H NMR (400 MHz, MeOD/D₂O 9/1) δ (ppm): 7.04 (br, 12H, ArH), 5.11 (br, 6H, H₁), 4.83 (s, 6H, H₁), 4.67 (br, 6H, H₁), 4.63 (br, OH), 4.53 (br, 6H, H₂), 4.24–3.27 (overlapped, 144H, H₃, H₄, H₅, H_{6a,b}, H₂, H₃, H₄, H₅, H_{6a,b}, H₂, H₃, H₄, H₅, OCH₂CH₂CH₂, CH₂NHCS, OCH₃, ArCH₂Ar), 2.07 (s, 18H, NHCOCH₃), 1.90 (br, 12H, CH₂CH₂CH₂), 1.34 (br, 18H, H₆). ¹³C NMR (100 MHz, MeOD/D₂O 9/1) δ (ppm): 173.4, 100.7, 100.4, 99.5, 78.9, 78.4, 77.0, 72.9, 72.6, 72.4, 72.1, 70.4, 66.7, 60.5, 60.0, 53.5, 29.5, 21.6, 16.8. HRMS (ESI-TOF) *m/z*: calcd for C₁₉₂H₂₉₄N₁₈O₉₆S₆Na₃ [M + 3Na]³⁺ 1550.2248, found 1550.2273.

4.2. Competitive ELISA

96-well flat-bottomed plates were incubated overnight at 4–8 °C

with a mixture of *S. pneumoniae* CPS 19F (1 mg/mL, Sanofi-Aventis, France) and methylated human serum albumin (1 mg/mL). A solution of foetal calf serum (5%) in phosphate-buffered saline supplemented with Brij-35 (0.1%) and sodium azide (0.05%) was applied to the plates for blocking of nonspecific binding sites. The plates were incubated overnight at 4–8 °C with a solution (1:200) of rabbit polyclonal anti-19F, used as reference serum (Statens Serum Institut, Artillerivej, Denmark). When compounds were tested, they were added to each well immediately before the addition of the reference serum. The plates were then incubated with alkaline phosphatase conjugate goat anti-rabbit IgG (Sigma-Aldrich, Milan, Italy), stained with *p*-nitrophenylphosphate, and the absorbance was measured at 405 nm with an Ultramark microplate reader (Bio-Rad Laboratories S.r.l., Milan, Italy).

Declaration of Competing interest

The authors declared that there is no conflict of interest.

Acknowledgements

The authors are grateful to Prof. Luigi Lay for kindly providing SP19F-RU used as reference compound in ELISA tests. This work was supported by the Italian Ministry of University and Research (PRIN 2015 grant, prot. 2015RNWJAM, Nanoplatforms for enhanced immune response). FS acknowledges the Centro Interdipartimentale Misura “G. Casnati” of Parma University for the use of NMR and Mass Spectrometry facilities. This work has benefited from the equipment and framework of the COMP-HUB Initiative, funded by the ‘Departments of Excellence’ program of the Italian Ministry for Education, University and Research (MIUR, 2018–2022).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.103305>.

References

- [1] K.A. Geno, G.L. Gilbert, J.Y. Song, I.C. Skovsted, K.P. Klugman, C. Jones, H.B. Konradson, M.H. Nahm, Pneumococcal capsules and their types: past, present, and future, *Clin. Microbiol. Rev.* 28 (2015) 871–899.
- [2] B.H.A. Rehm, Bacterial polymers: biosynthesis, modifications and applications, *Nat. Rev. Microbiol.* 8 (2010) 578–592.
- [3] O. Haji-Ghassemi, R.J. Blackler, N.M. Young, S.V. Evans, Antibody recognition of carbohydrate epitopes, *Glycobiology* 25 (2015) 920–952.
- [4] M. Cohen, A. Varki, Modulation of glycan recognition by clustered saccharide patches, *Int. Rev. Cell Mol. Biol.* 308 (2014) 75–125.
- [5] T.K. Dam, C.F. Brewer, Effects of clustered epitopes in multivalent ligand-receptor interactions, *Biochemistry* 47 (2008) 8470–8476.
- [6] E. Osinaga, S. Bay, D. Tello, A. Babino, O. Pritsch, K. Assemet, D. Cantacuzene, H. Nakada, P. Alzari, Analysis of the fine specificity of Tn-binding proteins using synthetic glycopeptide epitopes and a biosensor based on surface plasmon resonance spectroscopy, *FEBS Lett.* 469 (2000) 24–28.
- [7] R. Lo-Man, S. Vichier-Guerre, S. Bay, E. Deriaud, D. Cantacuzene, C. Leclerc, Antitumor immunity provided by a synthetic multiple antigenic glycopeptide displaying a tri-Tn glycotopope, *J. Immunol.* 166 (2001) 2849–2854.
- [8] S. Grigalevicius, S. Chierici, O. Renaudet, R. Lo-Man, E. Deriaud, C. Leclerc, P. Dumy, Chemoselective assembly and immunological evaluation of multipitopic glycoconjugates bearing clustered Tn antigen as synthetic anticancer vaccines, *Bioconjug. Chem.* 16 (2005) 1149–1159.
- [9] R. Lo-Man, S. Vichier-Guerre, R. Perraut, E. Deriaud, V. Huteau, L. BenMohamed, O.M. Diop, P.O. Livingston, S. Bay, C. Leclerc, A fully synthetic therapeutic vaccine candidate targeting carcinoma-associated Tn carbohydrate antigen induces tumor-specific antibodies in nonhuman primates, *Cancer Res.* 64 (2004) 4987–4994.
- [10] S. Behren, U. Westerlind, Glycopeptides and -mimetics to detect, monitor and inhibit bacterial and viral infections: recent advances and perspectives, *Molecules* 24 (2019).
- [11] F. Compostella, O. Pitirollo, A. Silvestri, L. Polito, Glyco-gold nanoparticles: synthesis and applications, *Beilstein J. Org. Chem.* 13 (2017) 1008–1021.
- [12] B. Lepenies, J. Lee, S. Sonkaria, Targeting C-type lectin receptors with multivalent carbohydrate ligands, *Adv. Drug. Deliver. Rev.* 65 (2013) 1271–1281.
- [13] S.C. Purcell, K. Godula, Synthetic glycoscapes: addressing the structural and functional complexity of the glycocalyx, *Interf. Focus* 9 (2019).

- [14] F. Sansone, A. Casnati, Multivalent glycolixarenes for recognition of biological macromolecules: glycolixarenes capable of multitasking, *Chem. Soc. Rev.* 42 (2013) 4623–4639.
- [15] C.D. Gutsche, Calixarenes: an introduction, 2nd edition, *Monogr. Supramol. Chem* (2008) 1–276.
- [16] L. Legnani, F. Compostella, F. Sansone, L. Toma, Cone calix[4]arenes with orientable glycosylthioureido groups at the upper rim: an in-depth analysis of their symmetry properties, *J. Org. Chem.* 80 (2015) 7412–7418.
- [17] S. Andre, C. Grandjean, F.M. Gautier, S. Bernardi, F. Sansone, H.J. Gabius, R. Ungaro, Combining carbohydrate substitutions at bioinspired positions with multivalent presentation towards optimising lectin inhibitors: case study with calixarenes, *Chem. Commun.* 47 (2011) 6126–6128.
- [18] S. Andre, F. Sansone, H. Kaltner, A. Casnati, J. Kopitz, H.J. Gabius, R. Ungaro, Calix[n]arene-based glycolixarenes: bioactivity of thiourea-linked galactose/lactose moieties as inhibitors of binding of medically relevant lectins to a glycoprotein and cell-surface glycoconjugates and selectivity among human adhesion/growth-regulatory galectins, *ChemBioChem* 9 (2008) 1649–1661.
- [19] S. Cecioni, R. Lalor, B. Blanchard, J.P. Praly, A. Imbert, S.E. Matthews, S. Vidal, Achieving high affinity towards a bacterial lectin through multivalent topological isomers of calix[4]arene glycoconjugates, *Chem.-Eur. J.* 15 (2009) 13232–13240.
- [20] C. Geraci, G.M.L. Consoli, E. Galante, E. Bousquet, M. Pappalardo, A. Spadaro, Calix[4]arene decorated with four antigen glycomimetic units and P3CS immunoadjuvant: Synthesis, characterization, and anticancer immunological evaluation, *Bioconjug. Chem.* 19 (2008) 751–758.
- [21] F. Berti, R. Adamo, Antimicrobial glycoconjugate vaccines: an overview of classic and modern approaches for protein modification, *Chem. Soc. Rev.* 47 (2018) 9015–9025.
- [22] J.N. Weiser, D.M. Ferreira, J.C. Paton, *Streptococcus pneumoniae*: transmission, colonization and invasion, *Nat. Rev. Microbiol.* 16 (2018) 355–367.
- [23] T. Welte, A. Torres, D. Nathwani, Clinical and economic burden of community-acquired pneumonia among adults in Europe, *Thorax* 67 (2012) 71–79.
- [24] A. Arbo, D. Lovera, C. Martinez-Cuellar, Mortality predictive scores for community-acquired pneumonia in children, *Curr. Infect. Disease Rep.* 21 (2019) 10.
- [25] F. Sansone, M. Dudic, G. Donofrio, C. Rivetti, L. Baldini, A. Casnati, S. Cellai, R. Ungaro, DNA condensation and cell transfection properties of guanidinium calixarenes: dependence on macrocycle lipophilicity, size, and conformation, *J. Am. Chem. Soc.* 128 (2006) 14528–14536.
- [26] M. Dudic, A. Colombo, F. Sansone, A. Casnati, G. Donofrio, R. Ungaro, A general synthesis of water soluble upper rim calix[n]arene guanidinium derivatives which bind to plasmid DNA, *Tetrahedron* 60 (2004) 11613–11618.
- [27] F. Sansone, E. Chierici, A. Casnati, R. Ungaro, Thiourea-linked upper rim calix[4]arene neoglycoconjugates: synthesis, conformations and binding properties, *Org. Biomol. Chem.* 1 (2003) 1802–1809.
- [28] A.M.A. vanWageningen, E. Snip, W. Verboom, D.N. Reinhoudt, H. Boerrigter, Synthesis and application of iso(thio)cyanate-functionalized calix[4]arenes, *Liebigs Ann.-Recl* (1997) 2235–2245.
- [29] M.I. Torres-Sanchez, C. Zaccaria, B. Buzzi, G. Miglio, G. Lombardi, L. Polito, G. Russo, L. Lay, Synthesis and biological evaluation of phosphono analogues of capsular polysaccharide fragments from *Neisseria meningitidis* A, *Chem.-Eur. J.* 13 (2007) 6623–6635.
- [30] M. Torvinen, R. Neitola, F. Sansone, L. Baldini, R. Ungaro, A. Casnati, P. Vainiotalo, E. Kalenius, Glucosylthioureidocalix[4]arenes: synthesis, conformations and gas phase recognition of amino acids, *Org. Biomol. Chem.* 8 (2010) 906–915.
- [31] G.M.L. Consoli, F. Cunsolo, C. Geraci, T. Mecca, P. Neri, Calix[8]arene-based glycoconjugates as multivalent carbohydrate-presenting systems, *Tetrahedron Lett.* 44 (2003) 7467–7470.
- [32] J.M.G. Fernandez, C.O. Mellet, Chemistry and developments of N-thiocarbonyl carbohydrate derivatives: Sugar isothiocyanates, thioamides, thioureas, thiocarbamates, and their conjugates, *Adv. Carbohydr. Chem. Bi* 55 (2000) 35–135.
- [33] C. Fessele, S. Wachtler, V. Chandrasekaran, C. Stiller, T.K. Lindhorst, A. Krueger, Thiourea-bridged nanodiamond glycoconjugates as inhibitors of bacterial adhesion, *Eur. J. Org. Chem.* (2015) 5519–5525.
- [34] L. Toma, L. Legnani, F. Compostella, M. Giuliani, F. Faroldi, A. Casnati, F. Sansone, Molecular architecture and symmetry properties of 1,3-alternate calix[4]arenes with orientable groups at the para position of the phenolic rings, *J. Org. Chem.* 81 (2016) 9718–9727.
- [35] M. Giuliani, I. Morbioli, F. Sansone, A. Casnati, Moulding calixarenes for biomacromolecule targeting, *Chem. Commun.* 51 (2015) 14140–14159.
- [36] F. Sansone, L. Baldini, A. Casnati, R. Ungaro, Conformationally mobile glucosylthioureidocalix[6]- and calix[8]arenes: synthesis, aggregation and lectin binding, *Supramol. Chem.* 20 (2008) 161–168.
- [37] L. Legnani, S. Ronchi, S. Fallarini, G. Lombardi, F. Campo, L. Panza, L. Lay, L. Poletti, L. Toma, F. Ronchetti, F. Compostella, Synthesis, molecular dynamics simulations, and biology of a carba-analogue of the trisaccharide repeating unit of *Streptococcus pneumoniae* 19F capsular polysaccharide, *Org. Biomol. Chem.* 7 (2009) 4428–4436.
- [38] M. Vetro, D. Safari, S. Fallarini, K. Salsabila, M. Lahmann, S. Penades, L. Lay, M. Marradi, F. Compostella, Preparation and immunogenicity of gold glyco-nanoparticles as antipneumococcal vaccine model, *Nanomedicine-UK* 12 (2017) 13–23.
- [39] L. Morelli, S. Fallarini, G. Lombardi, C. Colombo, L. Lay, F. Compostella, Synthesis and biological evaluation of a trisaccharide repeating unit derivative of *Streptococcus pneumoniae* 19A capsular polysaccharide, *Bioorg. Med. Chem.* 26 (2018) 5682–5690.
- [40] G. Soliveri, A. Bertolotti, L. Panza, L. Poletti, C. Jones, L. Lay, Synthesis of phosphorylated fragments of *Streptococcus pneumoniae* type 19F capsular polysaccharide, *J. Chem. Soc. Perk Trans.* 1 (2002) 2174–2181.
- [41] C. Colombo, O. Pitirollo, L. Lay, Recent advances in the synthesis of glycoconjugates for vaccine development, *Molecules* 23 (2018).
- [42] I. Calloni, L. Unione, G. Jimenez-Oses, F. Corzana, L. Del Bino, A. Corrado, O. Pitirollo, C. Colombo, L. Lay, R. Adamo, J. Jimenez-Barbero, The Conformation of the mannopyranosyl phosphate repeating unit of the capsular polysaccharide of *neisseria meningitidis* serogroup a and its carba-mimetic, *Eur. J. Org. Chem.* (2018) 4548–4555.
- [43] H.E. Gottlieb, V. Kotlyar, A. Nudelman, NMR chemical shifts of common laboratory solvents as trace impurities, *J. Org. Chem.* 62 (1997) 7512–7515.
- [44] I.T. Ho, J.H. Chu, W.S. Chung, Calix[4]arene with lower-rim beta-amino alpha, beta-unsaturated ketones containing bis-chelating sites as a highly selective fluorescence turn-on chemosensor for two copper(II) ions, *Eur. J. Org. Chem.* (2011) 1472–1481.