Probing Carbohydrate-Lectin Recognition in Heterogeneous Environments with Monodisperse Cyclodextrin-Based Glycoclusters

Marta Gómez-García,[†] Juan M. Benito,*[‡] Anna P. Butera,[†] Carmen Ortiz Mellet,[†] José M. García Fernández,[‡] and José L. Jiménez Blanco*[†]

[†]Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Profesor García González 1, E-41012 Sevilla, Spain

[‡]Instituto de Investigaciones Químicas, CSIC - Universidad de Sevilla, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain

Ⓢ Supporting Information

ABSTRACT: A series of β-cyclodextrin (βCD)-scaffolded glycoclusters exposing heterogeneous yet perfectly controlled displays of α-mannosyl (α-Man) and β-lactosyl (β-Lact) antennas were synthesized to probe the mutual influence of varying densities of the saccharide motifs in the binding properties toward different plant lectins. Enzyme-linked lectin assay (ELLA) data indicated that the presence of β-Lact residues reinforced binding of α-Man to the mannose-specific lectin concanavalin A (Con A) even though homogeneous β-Lact clusters are not recognized at all by this lectin, supporting the existence of synergic recognition mechanisms (*heterocluster effect*). Conversely, the presence of α-Man motifs in the heteroglycoclusters also resulted in a binding-enhancing effect of β-Lact toward peanut agglutinin (PNA), a lectin strongly binding multivalent lactosides but having no detectable affinity for α-mannopyranosides, for certain architectural arrangements. Two-site, sandwich-type ELLA data corroborated the higher lectin clustering efficiency of heterogeneous glycoclusters compared with homogeneous displays of the putative sugar ligand with identical valency. A turbidity assay was also consistent with the previous observations. Most revealingly, the lectin cross-linking ability of heterogeneous glycoclusters was sensitive to the presence of high concentrations of the non-ligand sugar, strongly suggesting that "mismatching" saccharide motifs may modulate carbohydrate-lectin specific recognition in a lectin-dependent manner when present in highly dense displays together with the "matching" ligand, a situation frequently encountered in biological systems.
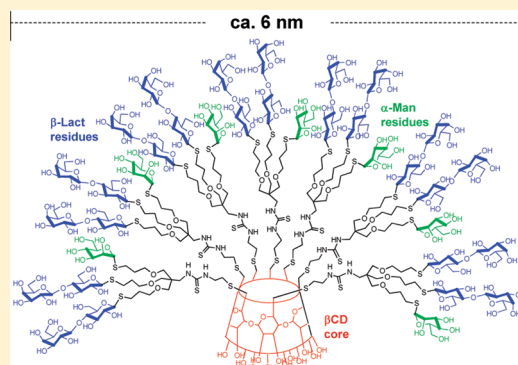
INTRODUCTION

Specific interactions between carbohydrate ligands and carbohydrate-binding proteins (lectins) are known to play key roles in a plethora of fundamental processes in cell daily life.[1] Despite being characteristically weak, Nature has managed to efficiently exploit these interactions through presentation of both the binding motifs and the recognition sites in multiple copies. Affinity enhancements exceeding those expected from the simple addition of individual interactions can be achieved in this way, a phenomenon known as the *cluster* or *multivalent glycoside effect*.[2] First noted by Lee and co-workers,[3] the cluster effect has been extensively investigated. Synthetic polyconjugates with well-defined structures have contributed to unravel the mechanisms at work,[4] leading eventually to useful tools for biotechnological[5] or therapeutic purposes.[6] The great majority of these artificial systems incorporate a single sugar motif, which strongly contrasts with the heterogeneity generally encountered in biological systems. Actually, differences in the surface density of a particular sugar ligand would be expected to be affected by the relative proportion and location of the neighbor saccharides, which might affect the affinity and selectivity toward a complementary receptor.[7]

Multivalent glyco-constructs taking heterogeneity into consideration are rather scarce. Statistical grafting of different saccharidic wedges onto polymeric,[8] dendritic,[9] or self-assembled scaffolds[10] affords polydisperse materials in which the relative position between the sugar components is not defined. On the other hand, orthogonal functionalization of dendritic cores is concomitantly associated, in most cases, to low-throughput synthetic strategies,[11] whereas combinatorial approaches[12] are so far limited to low valency derivatives. Only a few reports have investigated the role that "mismatching" carbohydrates might exert during the recognition of "matching" glycoligands by a specific lectin.[8c,9b,10a,11d,12a] In a previous communication we have reported a synthetic methodology for the preparation of β-cyclodextrin (βCD)-centered heteroglycoclusters[13] that allows sampling monodisperse conjugates with perfectly defined densities and spatial orientation of different saccharidic antennas, e.g., α-D-mannopyranoside (α-Man) and β-D-glucopyranoside (β-Glc) residues.[14] Notably, the affinity of these clusters toward the α-Man-specific lectin concanavalin

A (Con A) was found to be amplified by the presence of the β-Glc motifs, which are supposed to be irrelevant for this particular lectin, in highly dense glycocluster presentations. This unprecedented binding-enhancing role of "mismatching" carbohydrates, termed the *heterocluster effect*,[15] was further confirmed by several monitoring techniques, including competitive enzyme-linked lectin assay (ELLA) isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR).[16]

Thermodynamic analysis indicated that the heterocluster effect relies on a favorable entropic contribution to the free energy of binding. It was speculated that the "mismatching" sugar residue could facilitate sliding of the "matching" epitopes over the lectin binding site by transient exchange.[14,16] Whether or not this phenomenon is a peculiarity of α-Man−Con A binding in the presence of β-Glc or might this synergistic binding mechanism be also operating in other carbohydrate-lectin pairs remains intriguing. In order to address this question, a series of monodisperse homo- and heteromultivalent βCD-centered glycoclusters featuring α-Man and β-lactoside (β-Lact) units have been now synthesized. Their binding affinities toward two different plant lectins, namely, the mannose-specific lectin Con A and the lactose-binding lectin peanut agglutinin (PNA), have been evaluated by ELLA, two-site ELLA, and turbidimetry. Both lectins have been extensively used for the purification and characterization of glycoconjugates in a variety of research areas, including the separation and structural analysis of oligosaccharides or glycopeptides cleaved from glycoproteins, the pattern analysis for tissue comparison, or the detection of different glycoforms in glycoproteins.[17] Results are discussed as a function of the total carbohydrate density, the relative proportion of the α-Man and β-Lact units, and the nature of the lectin.

■ RESULTS AND DISCUSSION

General Synthetic Strategy and Building Blocks. The synthesis of the new series of α-Man and β-Lact homo- and heteroglycoclusters has been carried out by implementing a modular convergent strategy that takes advantage of (i) the stepwise radical addition of thiols to double bonds (ene-thiol addition)[18] for the construction of glycodendrons and (ii) the amine-isothiocyanate coupling reaction (thiourea-forming reaction) to generate the hyperbranched structure.[19] The ene-thiol addition proceeds with anti-Markovnikok regioselectivity and permits the sequential incorporation of saccharidic wedges onto a polyene branching element that can be further armed with an isothiocyanate group (Figure 1A), while the thiourea-forming reaction is very well suited for multiconjugation purposes (Figure 1B).[20] Triallylated pentaerythritol **1**[21] and per-(C-6)-cysteaminyl-βCD **2**[22] (Figure 1C) were chosen as the central building blocks. In this manner, a series of C_7-symmetric homo- and heterofunctional glycoclusters with a total (α-Man + β-Lact) 21-valency became accessible.

The azo-bis(isobutironitrile) (AIBN)-initiated radical addition of either the β-Lact (**3**)[23] or α-Man (**4**)[24] thiosugars to **1** led to the homotrivalent dendrons **5** and **6**[16] in 71% and 83% yield, respectively (Scheme 1). Triflyl activation of the primary hydroxyl group in **5** and **6** followed by azide anion displacement (→ **7** and **8**)[16] and isothiocyanation using the triphenylphosphine−carbon disulfide (TPP−CS$_2$) system[25] led to the corresponding isothiocyanate-armed dendrons **9** and **10**.[16] The fully unprotected trivalent compounds **tri*Lact*-OH** and **tri*Man*-OH**[16] were also prepared as control compounds (Scheme 1).
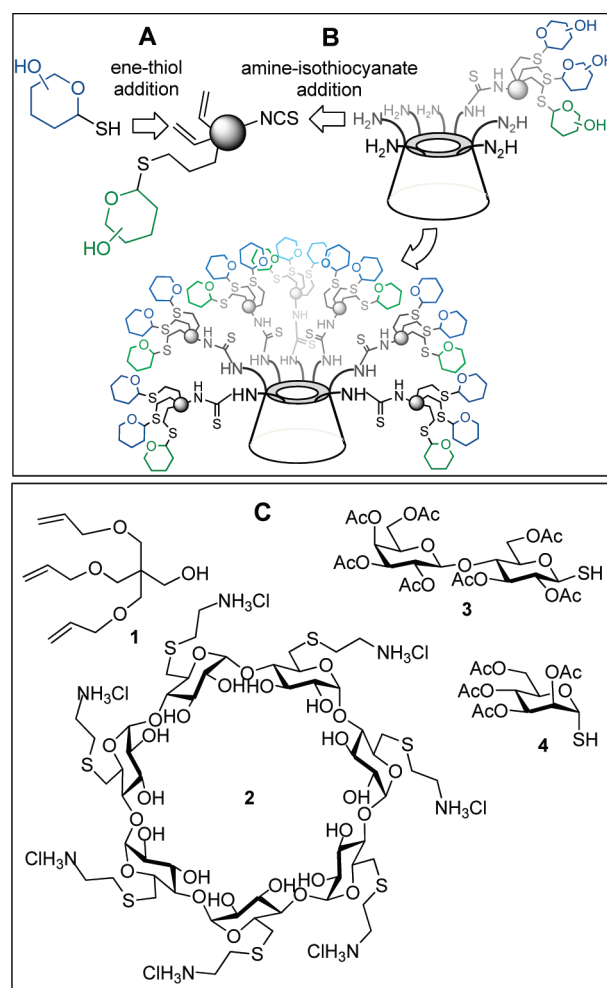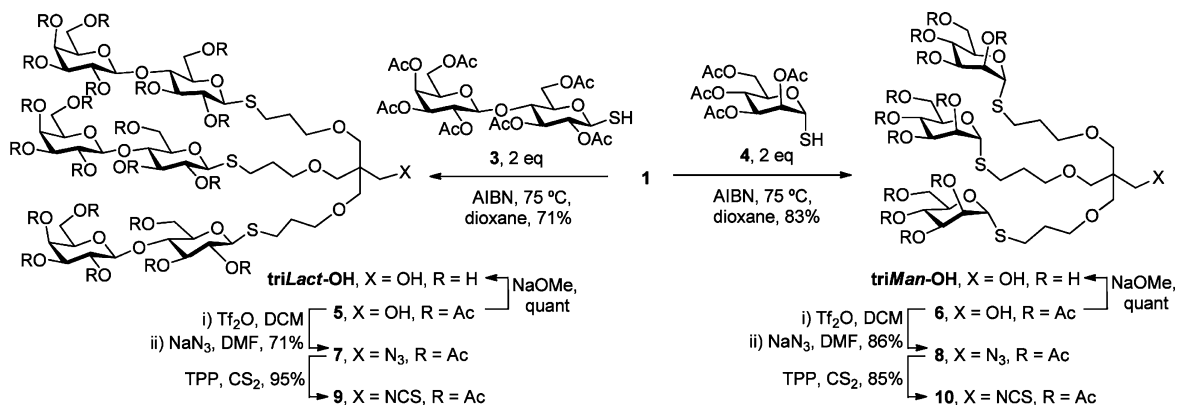

Figure 1. Schematic representation of the modular convergent strategy implemented to build monodisperse CD-scaffolded heteroglycoclusters highlighting the key steps (A, ene-thiol addition; B, amine-isothiocyanate addition) and the corresponding building blocks (C).
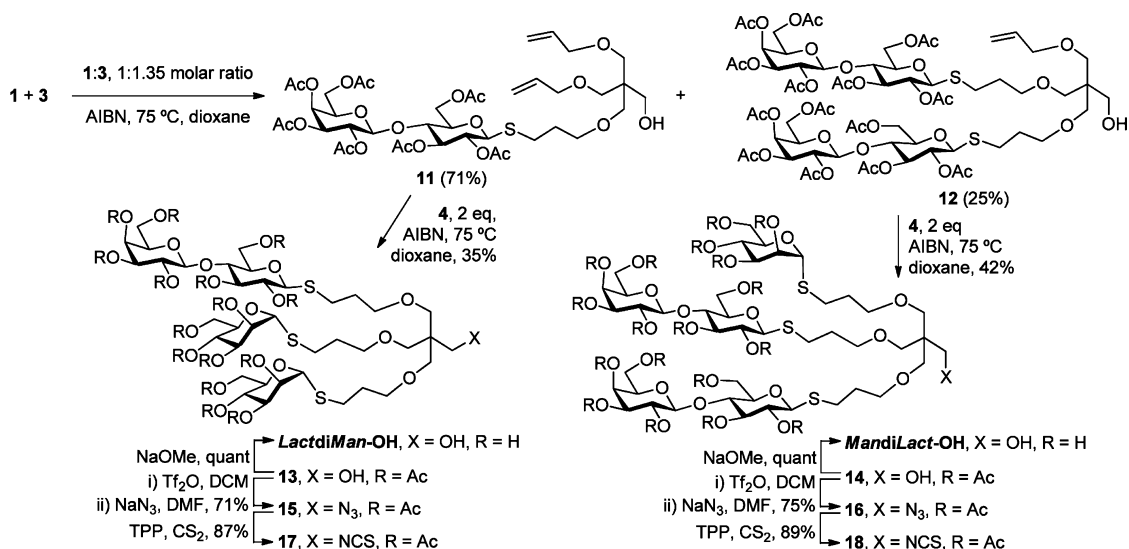
Radical addition of thiosugars **3** and **4** to **1** can be experimentally controlled to preferentially obtain partially glycosylated products by simply adjusting the amount of reactive thiol. Thus, by reacting 0.45 equiv of thiol **3** with the triallylated scaffold **1** a binary mixture of the easily separable mono- (**11**, 71%) and dilactosylated (**12**, 25%) derivatives could be obtained. Further AIBN-mediated reaction of these conjugates with the mannosyl thiol **4** furnished the heterotrivalent adducts **13** and **14**, respectively, which were transformed into the isothiocyanate-armed derivatives **17** and **18** via the corresponding azide intermediates **15** and **16** following a reaction sequence parallel to that above commented for the homogeneous counterparts **9** and **10**. Conventional deacetylation of **13** and **14** was also effected to obtain the corresponding heterotrivalent models **Man*di*Lact-OH** and **Lact*di*Man-OH** (Scheme 2).

In order to compare the multivalent effect in high-density versus low-density conjugates, monovalent building blocks were required. For that purpose, the strategy depicted in Scheme 3 was implemented. Starting from monoallylated propyleneglycol **19**,[26] the lactosylated and mannosylated derivatives **20** and **21** were obtained. Conventional deacetylation furnished ***Lact*-OH**
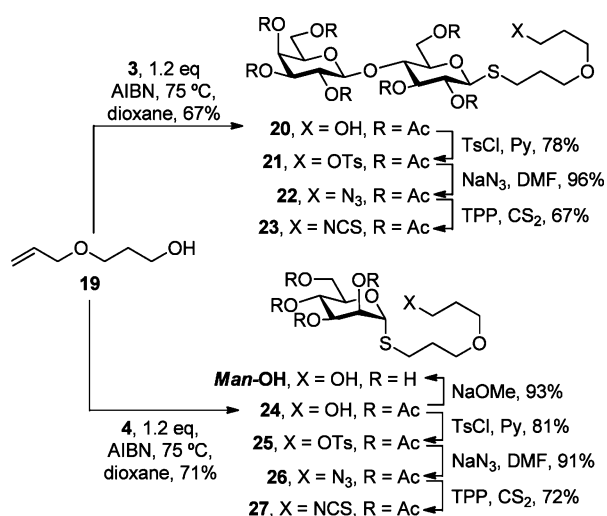
Scheme 1. Synthesis of Trivalent Homoglycodendrons and Building Blocks

triLact-OH, X = OH, R = H
5, X = OH, R = Ac
7, X = N₃, R = Ac
9, X = NCS, R = Ac

NaOMe, quant
i) Tf₂O, DCM
ii) NaN₃, DMF, 71%
TPP, CS₂, 95%

triMan-OH, X = OH, R = H
6, X = OH, R = Ac
8, X = N₃, R = Ac
10, X = NCS, R = Ac

NaOMe, quant
i) Tf₂O, DCM
ii) NaN₃, DMF, 86%
TPP, CS₂, 85%

Scheme 2. Synthesis of Trivalent Heteroglycodendrons and Building Blocks

1 + 3   1:3, 1:1.35 molar ratio
AIBN, 75 °C, dioxane

11 (71%)   **12** (25%)

4, 2 eq, AIBN, 75 °C dioxane, 35%   **4**, 2 eq AIBN, 75 °C dioxane, 42%

LactdiMan-OH, X = OH, R = H
13, X = OH, R = Ac
15, X = N₃, R = Ac
17, X = NCS, R = Ac

NaOMe, quant
i) Tf₂O, DCM
ii) NaN₃, DMF, 71%
TPP, CS₂, 87%

MandiLact-OH, X = OH, R = H
14, X = OH, R = Ac
16, X = N₃, R = Ac
18, X = NCS, R = Ac

NaOMe, quant
i) Tf₂O, DCM
ii) NaN₃, DMF, 75%
TPP, CS₂, 89%

Scheme 3. Synthesis of Monovalent Ligands and Building Blocks

3, 1.2 eq AIBN, 75 °C, dioxane, 67%

20, X = OH, R = Ac
21, X = OTs, R = Ac
22, X = N₃, R = Ac
23, X = NCS, R = Ac

TsCl, Py, 78%
NaN₃, DMF, 96%
TPP, CS₂, 67%

19

4, 1.2 eq, AIBN, 75 °C, dioxane, 71%

Man-OH, X = OH, R = H
24, X = OH, R = Ac
25, X = OTs, R = Ac
26, X = N₃, R = Ac
27, X = NCS, R = Ac

NaOMe, 93%
TsCl, Py, 81%
NaN₃, DMF, 91%
TPP, CS₂, 72%

Synthesis of βCD-Scaffolded Homo- and Heteroglycoconjugates. The thiourea-forming reaction was purposely chosen for the key multiconjugation step because it is totally chemoselective in the presence of free hydroxyl groups, is high yielding, and has no solvent restrictions or byproduct formation, and furthermore the thiourea linkages are biocompatible and physiologically stable. The series of isothiocyanate-armed derivatives 9, 10, 17, and 18 feature all possible combinations of α-mannosyl and β-lactosyl antennas on a trivalent dendron. Amplification of these patterns by coupling with the heptacysteaminyl βCD derivative 2 furnished a diverse set of displays of per-O-acetylated α-Man and β-Lact epitopes (28−31) with a global 21-valency (Scheme 4). Similarly, the monovalent building blocks 23 and 27 afforded the corresponding homogeneous heptavalent glycoclusters 32 and 33. TLC and NMR monitoring of the reaction mixtures evidenced outstanding chemical yields in all cases. Nevertheless, chromatographic purification of the high molecular weight hemiacetates handicapped the final isolation yields in certain cases (see Experimental Section). Final acetyl cleavage by mixed Zemplén transesterification-saponification reaction quantitatively furnished the target fully unprotected multivalent glycoconjugates **triLact-CD triMan-CD, MandiLact-CD, LactdiMan-CD, Lact-CD,** and **Man-CD.**

and **Man-OH,**[16] respectively, while the sequence hydroxyl triflation → azide displacement (to give **22** and **26**)[16] followed by reaction with the CS₂-TPP system furnished the corresponding isothiocyanate-armed building blocks **23** and **27.**[16]

Scheme 4. Synthesis of CD-Scaffolded Homo- and Heteroglycoclusters
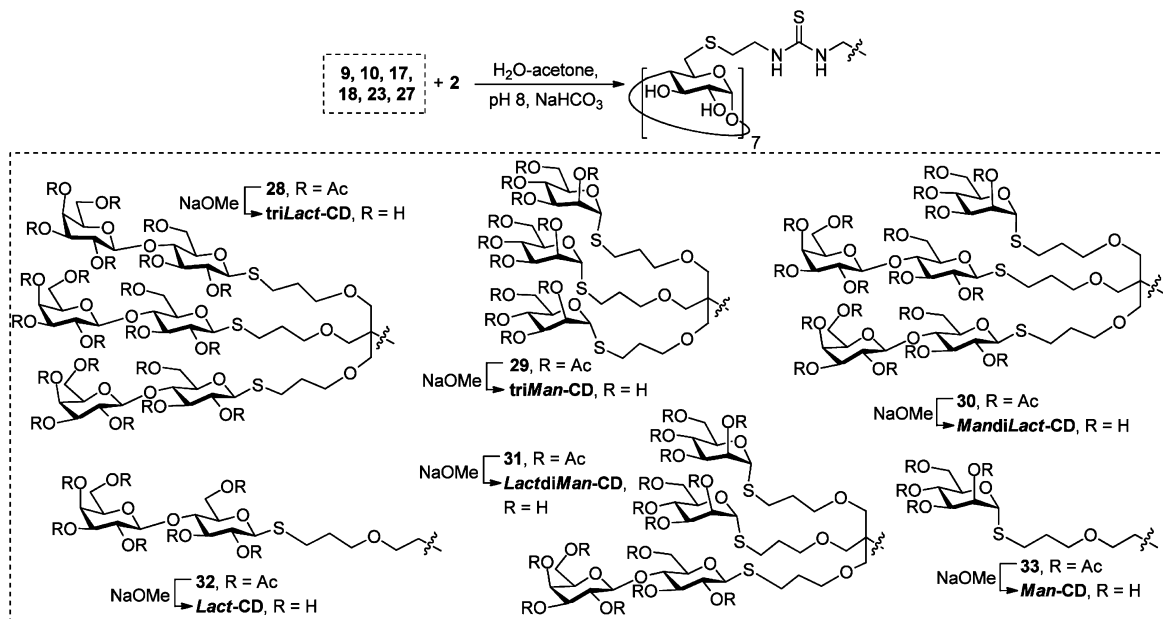
The homogeneity and purity of all structures were confirmed by mass spectrometry, NMR spectroscopy, and combustion analysis. ^{1}H and ^{13}C NMR spectra of the final conjugates in D$_2$O showed the typical line broadening associated with restricted rotation at the pseudoamide NH−C(=S) bonds,[27] which remained evident also at elevated temperatures (333−353 K). The drastic decrease of motion at the central core region of the macromolecular conjugates provokes an increase in the relaxation time for the corresponding carbon atoms that translates into much lower intensities in the ^{13}C NMR spectra in comparison with the carbons of the peripheral glycoligands. Nevertheless, both the ^{13}C and ^{1}H NMR spectra were consistent with the expected C_7 symmetry for homogeneously substituted βCD-centered clusters. In the case of the homoglycoconjugates **triMan-CD** and **triLact-CD**, proton spectra showed two and three spin systems, respectively, corresponding to the cyclodextrin core and the mannosyl (for **triMan-CD**) or glucosyl and galactosyl units (for **triLact-CD**) in 1:3 ratio. For heteroglycoclusters **LactdiMan-CD** and **MandiLact-CD**, four different spin systems were distinguished, their relative intensities perfectly matching the expected mannose/lactose/CD relative proportions (Figure 2).

Evaluation of Lectin Binding Affinity by ELLA. First, the Con A binding avidity of the α-Man/β-Lact glycoclusters was evaluated by enzyme-linked lectin assay (ELLA).[28] This test measures the ability of a soluble saccharide to inhibit the association between a labeled lectin (here Con A or PNA lectin labeled with horseradish peroxidase, Con A-HRP or PNA-HRP) and a ligand immobilized on the microtiter well (here yeast mannan or a lactose-functionalized polyacrylamide polymer). The location of the glycodendrons at the primary face of the CD platform provides a well-defined topology in which the saccharide epitopes share the same space region, featuring a highly dense glycosylated surface (Figure 3, top). Considering that the HRP label is a rather big protein, (40 kDa) this architecture likely prevents two lectin moieties from approaching, as previously demonstrated in analogous systems,[14] resulting in 1:1 binding stoichiometries. On the other hand, molecular simulations[14,29] indicated that the largest
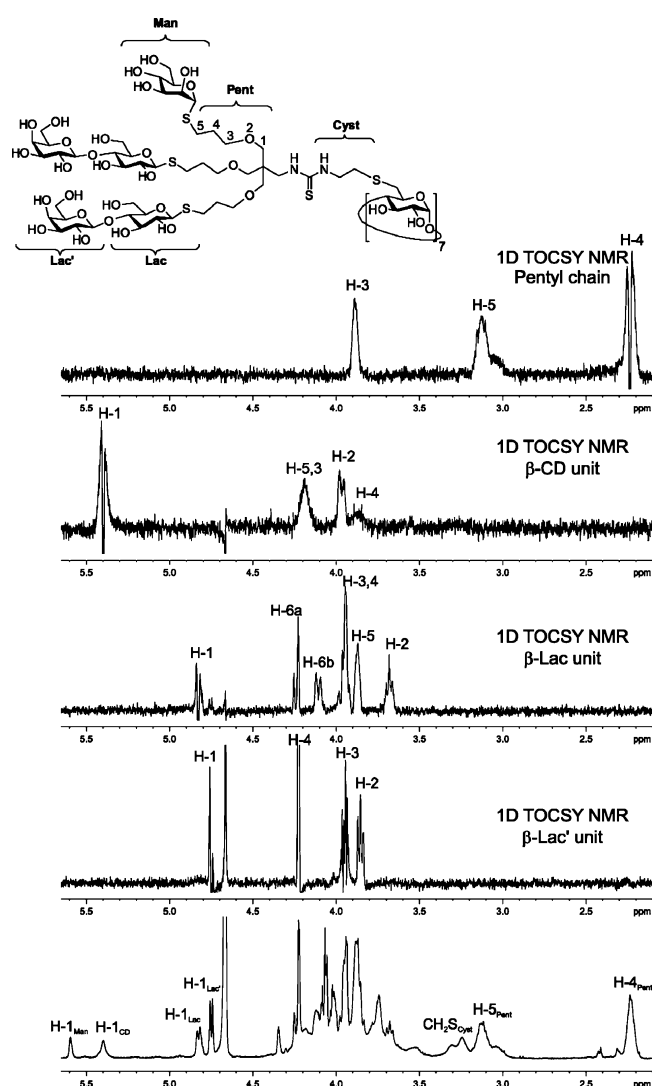

Figure 2. Stacked ^{1}H (below) and 1D TOCSY NMR (500 MHz, D$_2$O, 333 K) spectra of compound **MandiLact-CD**.
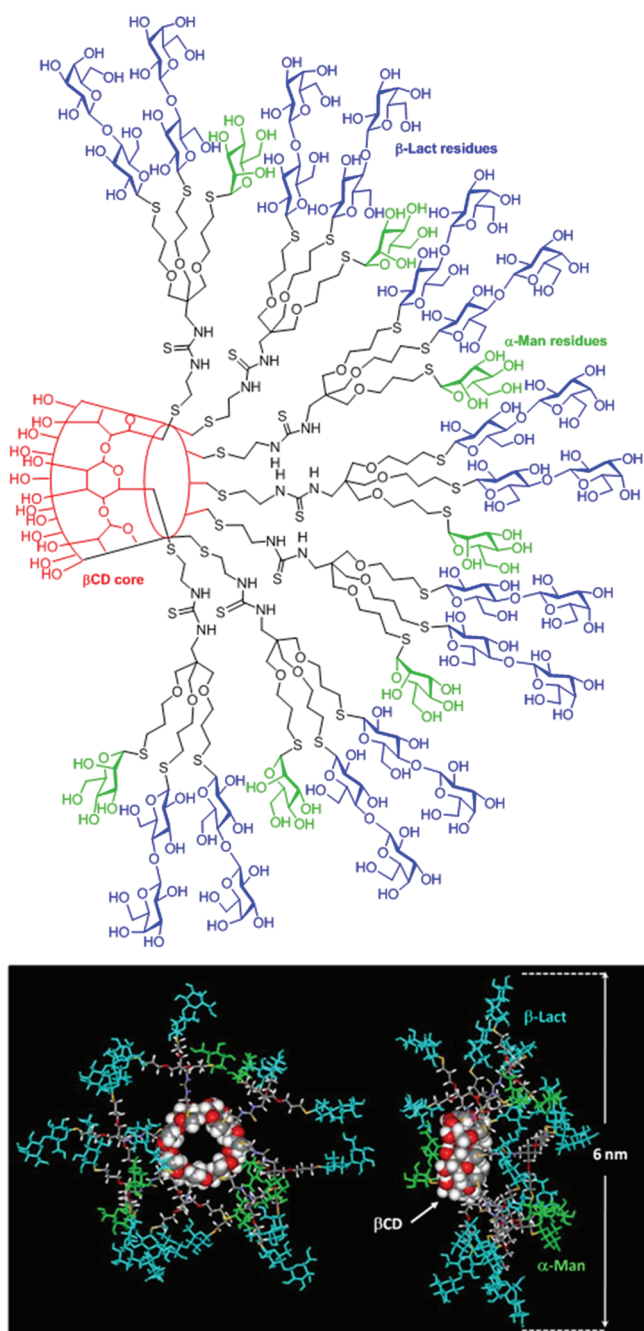
Figure 3. Schematic representation of the *MandiLact-CD* heterogeneous glycocluster (top) and 3D representation obtained by molecular modeling (bottom; an axial view from the primary face of the *β*CD core and a lateral view are shown at the right and left corners, respectively). The CD platform is depicted as a space-filled CPK model, whereas sticks have been used for the rest of the molecule. *β*-Lactosyl and *α*-mannosyl residues are colored blue and green, respectively.

distance between the external sugar units (ca. 6 nm) is shorter than the distance between two adjacent recognition sites in Con A[30] or PNA[31] lectins (Figure 3, bottom). This situation should prevent contributions to the binding energy associated to the chelate effect (e.g., simultaneous interaction of glycotopes in the same glycocluster molecule with more than one lectin binding site).[9] In such circumstances, differences in binding affinities can be ascribed, essentially, to the effect of differences in the relative densities of the sugar motifs in the

interaction of the multivalent conjugate with a single carbohydrate binding site in the lectin.

In addition to the compounds depicted in Scheme 4, the dimannosylated conjugate **diMan-OH**, still bearing an allyl group instead of the lactosyl unit, and the corresponding CD-scaffolded 14-valent homologue **diMan-CD**[16] were included in the binding assay against Con A-HRP as additional controls (Chart 1). The concentration of glycoclusters required to

Chart 1. Structures of the Homogeneous Divalent and Tetradecavalent Glycoclusters diMan-OH and diMan-CD Used As Control Compounds in ELLA Experiments
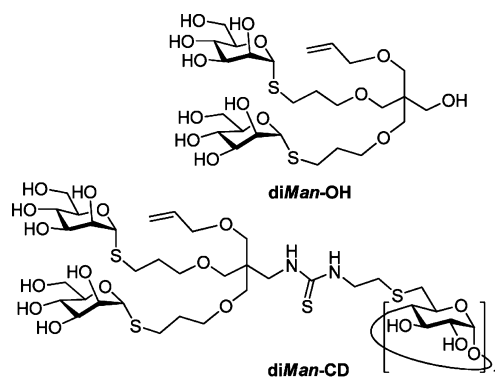
achieve 50% inhibition, the IC$_{50}$ values (Table 1) are assumed to be inversely proportional to the lectin–saccharide free energy

Table 1. Inhibition of Yeast Mannan—Con A Binding by Homo- and Heteromultivalent Glycoclusters Determined by ELLA

compound	Lact units	Man units	IC$_{50}^{j}$ (μM)	rel potency[a]	Man molar rel potency
Me-α-Man	0	1	865 ± 30[b]	1	1
Man-OH	0	1	800 ± 35[b]	1.1	1.1
MandiLact-OH	2	1	370 ± 25[c]	2.3	2.3
diMan-OH	0	2	319 ± 25[d]	2.5	1.3
LactdiMan-OH	1	2	340 ± 30[c]	2.5	1.3
triMan-OH	0	3	46 ± 5[e]	18.8	6.3
lactose	1	0	ni[j]		
triLact-OH	1	0	nij		
Man-CD	0	7	67 ± 5[f]	12.9	1.8
MandiLact-CD	14	7	50 ± 5[g]	17.3	2.5
diMan-CD	0	14	76 ± 8[f]	11.4	0.8
LactdiMan-CD	7	14	14 ± 2[h]	61	4.4
triMan-CD	0	21	5.5 ± 0.5[i]	157	7.5
Lact-CD	7	0	ni[k]		
triLact-CD	21	0	ni[k]		

[a]Relative values are compared to methyl-α-D-mannopyranoside (Me-α-Man, IC$_{50}$ 865 ± 30 μM).[32] The IC$_{50}$ values are expressed as mean values ± SD obtained from at least five independent determinations. [b−i]Differences between data with different superscript letters are statistically significant (P < 0.001). [j]No inhibition at ca. 4 mM. [k]No inhibition at ca. 1 mM.

of binding. For comparative purposes, they were normalized to the affinity shown by methyl α-D-mannopyranoside in a parallel experiment (IC$_{50}$ 865 ± 30 μM).[32] In agreement with previous

results,[12b,33] a substantial amplification of Con A-HRP avidity was observed for conjugates where the mannosyl units were presented in triads, and little, if any, differences were noticed among mono- or dimannosylated dendrons, regardless of the presence or absence of other saccharidic or nonsaccharidic elements. The mannose-devoid ligands **Lact-OH** or **triLact-OH** did not bind at all Con A-HRP, in agreement with the known lectin specificity. Lactose, therefore, has no apparent influence in the recognition of mannose by Con A when the total sugar density is low.

The scenario was substantially different in the hyperbranched CD-scaffolded glycocluster series. Despite lactose not being itself recognized by Con A (no binding observed for the 7- or 21-valent homogeneous conjugates **Lact-CD** or **triLact-CD**), the presence of lactosyl residues significantly enhanced the relative mannose binding potency (i.e., from 1.8 to 2.5 in mannose molar basis when going from **Man-CD** to **MandiLact-CD**; from 0.8 to 4.4 from **diMan-CD** to **LactdiMan-CD**). Actually, the IC_{50} of **LactdiMan-CD** (14 μM) was 5.4-fold lower than that of **diMan-CD**, both bearing 14 α-D-mannopyranoside units, and was fairly close to the value measured for **triMan-CD** (5.5 μM), with 21 α-D-mannopyranoside residues. The lactose-induced affinity increases are slightly less pronounced than those previously measured for β-D-glucopyranose,[16] indicating that the heterocluster effect is dependent on the nature of the secondary "mismatching" sugar.

The results for the competitive binding inhibition test using PNA-HRP are summarized in Table 2. In agreement with the

Table 2. Inhibition of Polylactoside−PNA Binding by Homo- and Heteroglycoclusters Determined by ELLA

compound	Lact units	Man units	IC_{50}[b−g] (μM)	rel potency[a]	Lact molar rel potency
lactose	1	0	1150 ± 65[b]	1	1
LactdiMan-OH	1	2	24% inhibition at 1.7 mM		
MandiLact-OH	2	1	500 ± 35[c]	2.3	1.2
triLact-OH	3	0	320 ± 25[d]	3.6	1.2
Man-OH	0	1	ni[h]		
triMan-OH	0	3	ni[h]		
Lact-CD	7	0	77 ± 8[e]	14.9	2.1
LactDiMan-CD	7	14	50 ± 6[f]	23	3.3
MandiLact-CD	14	7	50 ± 7[f]	23	1.6
triLact-CD	21	0	21 ± 2[g]	54.8	2.6
Man-CD	0	7	ni[i]		
triMan-CD	0	21	ni[i]		

[a]Relative values are compared to lactose (IC_{50} 1150 ± 65 μM). The IC_{50} values are expressed as mean values ± SD obtained from at least five independent determinations. [b−g]Differences between data with different superscript letters are statistically significant ($P < 0.001$). [h]No inhibition at ca. 2 mM. [i]No inhibition at ca. 1 mM.

expected lectin specificity,[34] mannosylated conjugates were not recognized at all by PNA. The presence of mannose residues in combination with lactose in the trivalent derivatives was found to be irrelevant. In the CD-scaffolded series, participation of mannosyl residues in lectin recognition became evident. Thus, the IC_{50} value decreases from 77 to 50 μM when going from **Lact-CD** to **LactdiMan-CD**. In a lactose molar basis, the recognition of the lactose binding motif is more efficient when

surrounded by mannose as in **LactdiMan-CD** (3.3-fold relative to lactose) than in the homogeneous 21-valent conjugate **triLact-CD** (only 2.6-fold). This result is most striking considering that 1:2 lactose/mannose relative proportion was the least efficient in the trivalent series. The heptalactoside **LactdiMan-CD** is actually as potent as the tetradeca lactoside **MandiLact-CD** as a PNA ligand, meaning that the individual lactose motifs are recognized twice more efficiently in the first case. The particular structural features of PNA, with binding sites that are more exposed to the bulk solvent and significantly smaller than those of Con A,[35] surely determine their different behavior.

Evaluation of Lectin Clustering Capability by Two-Site ELLA. To evaluate the lectin clustering abilities of the new glycoclusters, a two-site "sandwich" ELLA experiment was performed.[28b] Unlabeled (therefore cross-linkable) lectin (Con A or PNA) was first laid down onto a microtiter well. Preformed complexes of the glycoclusters at different concentrations with the corresponding HRP-labeled lectin (Con A-HRP or PNA-HRP) were then added. The relative amounts of bound HRP-labeled lectin obtained are represented in Figures 4 and 5, with the
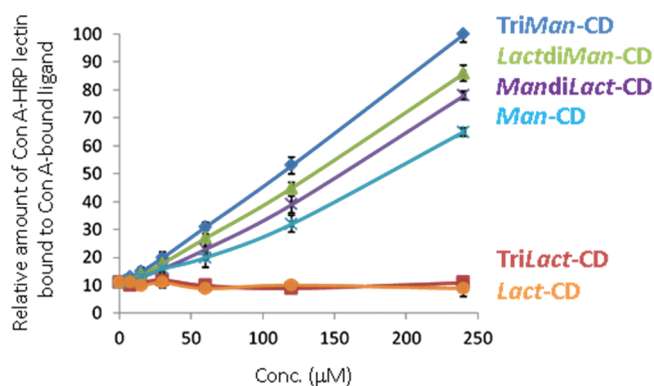

Figure 4. Relative cross-linking efficiencies of CD-glycoclusters against Con A at different concentrations.
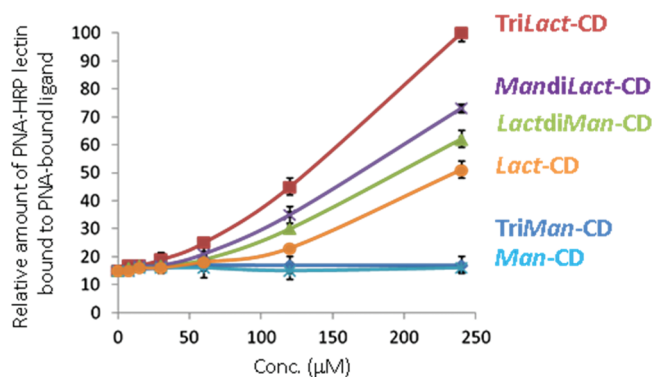

Figure 5. Relative cross-linking efficiencies of CD-glycoclusters against PNA at different concentrations.

maximum for the corresponding homogeneous 21-valent glycocluster **triMan-CD** (for Con A, Figure 4) or **triLact-CD** (for PNA, Figure 5) at the highest concentration (250 μM) set at 100%. The residual value (11−15%) observed without previous addition of ligands can be explained by HRP-protein exchanges with unlabeled lectin at the surface of the well. Relative cross-linking values in the range 10−18%, which were reached in the experiments using methyl α-D-mannopyranoside, lactose, or the

trivalent glycodendrons (data not shown), must be considered within the background.

As expected, homogeneous CD-centered glycoclusters mismatching the known lectin selectivity, that is, **triMan-CD** and **Man-CD** when assayed against PNA and **triLact-CD** and **Lact-CD** when assayed against Con A, showed no clustering capabilities. For matching pairs, the linking potential of the 21-valent cluster was much higher as compared with the 7-valent counterparts (1.5- to 2-fold at 250 μM). The clustering abilities of the **Man/Lact-CD** heteroglycoclusters were, in all cases, between these limits. Most importantly, the pairs **MandiLact-CD**/Con A and **LactdiMan-CD**/PNA consistently exhibited a higher tendency to cross-link as compared with the corresponding **Man-CD**/Con A and **Lact-CD**/PNA pairs, even though they have identical valency in a mannose molar basis, in agreement with the enhanced lectin-binding capabilities in heterogeneous environments observed by classical ELLA.

Evaluation of Lectin Aggregation Capability by Turbidity Assay. To test whether the differences in binding simultaneously two lectin molecules by the glycoclusters correlated with their relative capacity to promote the formation of three-dimensional aggregates, a kinetic turbidity assay was carried out. Turbidity measurements can be used to monitor the formation of cross-linked complexes in real time.[36] For that purpose, the ligands were added to a solution of Con A or PNA in PBS (pH 7.3), and the turbidity of the mixture was screened. The initial rate of precipitation (V_i) was determined by linear fits of the initial portion of the data (Figures 6 and 7).
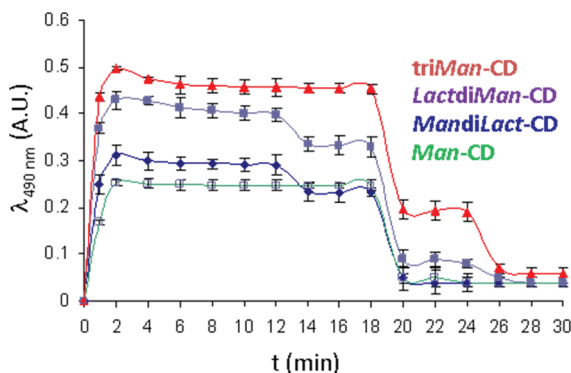

Figure 6. Absorption changes of Con A (1 mg/mL, 50 μL) at 490 nm upon addition of mannose-containing CD-glycoclusters (250 μM, 50 μL) as a function of time. Lactose (100 mM, 50 μL) and D-mannose (100 mM, 2 × 50 μL) were sequentially added to the mixtures after 12, 18, and 24 min. Addition of D-mannose (100 mM, 100 μL) after 12 min led to basal absorption values in all cases. Values are expressed as mean values ± SD obtained from at least three independent determinations.

The corresponding data (Table 3) were consistent with the previous observations by two-site ELLA. Thus, no precipitation occurred for trivalent dendrons or for mismatched CD-centered glycocluster-lectin pairs. The 21-valent homogeneous glycoclusters were very efficient at promoting fast aggregation of the matching lectin. For heterogeneous glycoclusters, the aggregation capacity increased with the putative ligand valency (α-D-mannopyranosyl for Con A and β-lactosyl for PNA). The turbidimetry data also indicated that the heteroglycoclusters with seven copies of the matching ligand, **MandiLact-CD** for Con A and **LactdiMan-CD** for PNA, were more efficient at
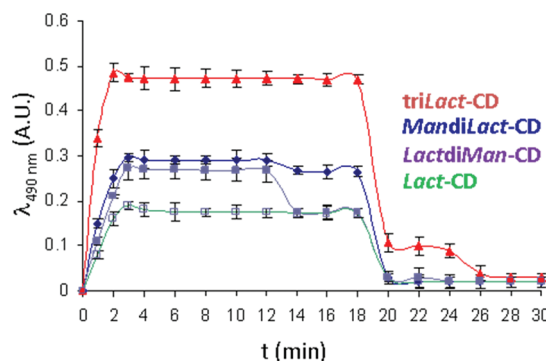

Figure 7. Absorption changes of PNA in PBS (pH 7.3, 1 mg/mL, 50 μL) at 490 nm upon addition of lactose-containing CD-glycoclusters (250 μM, 50 μL) as a function of time. D-Mannose (100 mM, 50 μL) and lactose (100 mM, 2 × 50 μL) were sequentially added to the mixtures after 12, 18, and 24 min. Addition of lactose (100 mM, 100 μL) after 12 min led to basal absorption values in all cases. Values are expressed as mean values ± SD obtained from at least three independent determinations.

Table 3. Initial Aggregation Rates (V_i) of Con A and PNA Lectins in PBS (pH 7.3, 1 mg/mL, 50 μL) by CD-Centered Homo- and Heteroglycoclusters (250 μM, 50 μL) at 25 °C[a]

compound	Lact units	Man units	$V_{i, \text{Con A}}$ (AU min^{-1})	$V_{i, \text{PNA}}$ (AU min^{-1})
triMan-CD	0	21	0.437	0
triLact-CD	21	0	0	0.340
MandiLact-CD	14	7	0.249	0.151
LactdiMan-CD	7	14	0.369	0.111
Man-CD	0	7	0.168	0
Lact-CD	7	0	0	0.080

[a]No aggregation was observed for Con A or PNA in combination with any of the trivalent homo and heteroglycocendrons depicted in Schemes 1 and 2, respectively.

cross-linking the corresponding lectin than the heptavalent homogeneous derivatives **Man-CD** and **Lact-CD**, even though the accessibility of the recognition motifs must be higher in the later. Altogether, those data strongly support an active contribution of the mismatching sugar on the binding of highly dense heteroglycoclusters to both Con A and PNA.

In order to assess whether the observed enhanced capacity of heterogeneous mannose/lactose CD-glycoclusters to aggregate the mannose specific lectin Con A was lactose-specific, 50 μL of a 100 mM solution of lactose in PBS was added to the well containing the suspensions of **MandiLact-CD**/Con A and **LactdiMan-CD**/Con A after 12 min. In both cases a significant decrease of the optical density, corresponding to a decrease in turbidity of about 20%, was observed. In contrast, addition of lactose had no effect on the aggregates of Con A with the homogeneous mannosyl clusters **triMan-CD** and **Man-CD**. Further addition of 50 μL of a 100 mM solution of mannose in PBS (18 min) fully reverted aggregation in the case of the heptamannosyl conjugates **MandiLact-CD** and **Man-CD** and strongly decreased turbidity for the 14- and 21-valent (in mannose molar basis) clusters **LactdiMan-CD** and **triMan-CD**; a second addition of the mannose solution (24 min) was necessary to completely disrupt the aggregates in these cases (Figure 6). Parallel experiments in which the addition of 100 μL of 100 mM solution of mannose in PBS to the suspensions was

effected directly after 12 min resulted in instantaneous reversion for all homogeneous and heterogeneous glycoclusters (data not shown).

The turbidimetry data and initial aggregation rates in the case of PNA (Figure 7 and Table 3) closely reproduced the relative affinities obtained by ELLA (Table 2), with the heterogeneous glycoclusters having similar capacity to bind to the lectin despite *Man*di*Lact*-CD having double lactose-base valency compared with *Lact*di*Man*-CD. A similar experiment was carried out to assess whether the mismatching α-D-mannopyranosyl units were contributing to PNA aggregation in a mannose-specific manner. Notably, addition of 50 μL of a 100 mM solution of mannose in PBS to the suspensions (12 min) had a much more pronounced effect in the *Lact*di*Man*-CD/PNA than in the *Man*di*Lact*-CD/PNA aggregates. This result is consistent with the more pronounced mannose-induced PNA binding efficiency previously determined by ELLA (relative affinities in lactose molar basis 3.3 vs 1.6 for *Lact*di*Man*-CDas compared to *Man*di*Lact*-CD, Table 2). Addition of excess lactose to the precipitates resulted in clear solutions in all cases, demonstrating the lactose-specific character of the aggregation.

Investigation of multivalent recognition is a complicated task because it usually reflects an intricate overlapping of a number of microscopic effects, the individual contributions to the overall process being difficult to dissect.[37] In our case, the molecular design and experimental setup was conceived to exclude contributions from simultaneous binding to several lectin binding sites (chelate effect). The β-cyclodextrin scaffold allows building highly dense carbohydrate surfaces of nanometric dimensions where the relative proportions of the two glycotope constituents, D-mannose and lactose, can be strictly controlled while keeping constant overall topologies. Nevertheless, binding efficiency might be influenced by a number of other effects, such as subsite binding or steric congestion between glycoligands.[36,38] Actually, the ensemble of data clearly indicates that the binding affinity of each of this sugars by a specific lectin (Con A or PNA) is not independent of the presence of the second sugar when both are closely packed at high valency presentation. The effect is observable when considering the interaction of the multivalent display with a single lectin binding site (ELLA), the capacity to clusterize the lectin (two-site ELLA), and the ability to promote three-dimensional aggregates (turbidity assay).

Most relevant is that in the case of pairs of compounds having identical valency of the matching ligand, e.g., the heptamannosylated conjugates *Man*di*Lact*-CD and *Man*-CD when assayed against Con A, the derivative bearing in addition the mismatching epitope showed enhanced lectin binding affinity, even though steric considerations would be unfavorable. This strongly suggests an active participation of the mismatching sugar in the recognition of the glycocluster. The fact that the aggregation potential is decreased in the presence of high concentrations of this mismatching sugar in the solution further supports that this effect is specific in nature.

The current body of evidence does not allow unequivocal deciphering of the mechanism at work for the heterocluster effect. In the case of the α-D-mannopyranose–Con A association, quantitatively significant differences have been encountered dependent on the nature of the mismatching sugar (β-D-glucopyranose or lactose). For identical binary proportions of carbohydrates, the effect is also lectin-dependent, being more pronounced in the case of Con A as compared with PNA. In each case, the optimal increase on binding efficiency is a function of the relative proportion of the matching-mismatching elements. Previous thermodynamic data pointed to an entropic origin for the heterocluster effect, probably through facilitating sliding of the matching sugar over the binding site by transiently binding to the lectin.[14] The fact that this process is someway disrupted in the presence of the mismatching sugar in the solution fits with this hypothesis. It should be emphasized, however, that the mismatching sugar was not recognized by the lectin either in multivalent presentation or at the high concentration used in the competitive turbidimetric experiment. This might indicate that transient binding occurs at the vicinity of the primary binding site and is only relevant when the matching epitope is already bound.

■ CONCLUSION

In this study, we implemented a synthetic methodology to elaborate a library of molecularly well-defined multivalent glycoclusters with a precise display of α-Man and β-Lact antennas by using β-cyclodextrin as the central platform. The resulting monodisperse architectures are very compact, with all dendron branches oriented toward the same face of the βCD cone and the sugar residues sharing an area of ca. 6 nm diameter. These highly dense sugar patches have proven very useful to assess the influence that different distributions of "matching" and "mismatching" sugar ligands play in the specific recognition by the α-D-mannopyranose- and lactose-binding lectins Con A and PNA, respectively. The ensemble of results supports that the vision that gives all the credit to the main actors (the receptor and its putative binding epitope) is probably too simplistic. Saccharidic ligands that are not specifically recognized by a certain lectin might influence the recognition process of the "matching" sugar motif, by mechanisms that cannot be explained only in terms of steric effects, when forced to share the same space region. Altogether, these results support that the proposed *heterocluster effect* is not just a curiosity restricted to Con A but can also influence the binding mechanisms of other lectins. The differences observed in the present study surely arise from structural differences in the binding sites of the selected lectins. Thus, Con A is known to possess an extended binding site, and although it cannot accommodate galactose in the primary site, this monosaccharide residue can bind to a secondary site when attached to mannose.[39] This might also happen in highly dense conjugates and, simultaneously, facilitate sliding of the mannosyl ligands.[36,40] Actually, combining primary and secondary carbohydrate and noncarbohydrate ligands in multivalent displays has already proven to be a very powerful strategy to increase affinity toward biologically relevant receptors.[41] Given the fact that heterogeneity is probably the main feature of the cellular milieu, it is very likely that the heterocluster effect is also operative in biological systems. Further research in this direction is currently sought in our laboratories.

■ EXPERIMENTAL SECTION

General Methods. Native and horseradish peroxidase-labeled concanavalin A (Con A and HRP-Con A) and peanut agglutinin from *Arachis hypogaea* (PNA and HRP-PNA), mannan from *Saccharomyces cerevisiae*, and all other common reagents and materials were purchased from commercial sources. Optical rotations were measured at room temperature in 1-cm or 1-dm tubes. Infrared (IR) spectra were recorded on a FTIR spectrophotometer. ^{1}H (and ^{13}C NMR) spectra were recorded at 300 (75.5 for ^{13}C) and 500 (125.7 for ^{13}C) MHz instruments. 1D ^{1}H TOCSY, 2D COSY, and ^{1}H−^{13}C HMQC

experiments were used to assist NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with Kieselgel 60 F254, with visualization by UV light and by charring with 10% H_2SO_4 or 0.2% ninhydrin. Column chromatography was carried out on silica gel 60 (230−400 mesh). Gel permeation chromatography (GPC) of the fully unprotected CD adducts was carried out on a Sephadex G-25 (eluent H_2O) column attached to a fraction collector system using a UV detector set at 248 nm. The operating conditions of FAB mass spectra were the following: the primary beam consisted of Xe atoms with a maximum energy of 8 keV; the samples were dissolved in thioglycerol, and the positive ions were separated and accelerated over a potential of 7 keV; NaI was added as cationising agent. MALDI-TOF mass spectra were acquired on a spectrometer operating in the positive-ion mode with an accelerating voltage of 28 keV. Samples were dissolved in H_2O at mM concentration and mixed with a standard solution of 2,5-dihydroxy-benzoic acid (DHB; 10 mg/mL in 10% aq EtOH, 2 mL) in 1:1 v/v relative proportions; 1 μL of the mixture was loaded onto the target plate and then allowed to air-dry at room temperature. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain).

Azo-bis(isobutyronitrile), dichloromethane, trifluoromethanesulfonic anhydride, triphenylphosphine, *p*-toluensulfonic chloride, and *N,N*-dimethylformamide are indicated by the acronyms AIBN, DCM, Tf$_2$O, TPP, TsCl, and DMF, respectively. 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-1-thio-β-lactose (**3**) and 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose (**4**) were prepared from the corresponding per-*O*-acetates in three steps by transformation into the corresponding glycosyl halides, treatment with thiourea, and subsequent hydrolysis of the resulting isothiouronium salt with potassium metabisulfite (K$_2$S$_2$O$_5$).[23,24] Heptaamine **2**[22] and allylated derivatives **1**[21] and **19**[26] were prepared following the reported procedures. Mannosylated building dendrons **6**, **8**, **10**, **24**−**27**, **Man-OH**, and **triMan-OH** and CD conjugates **29**, **33**, **Man-CD**, and **triMan-CD** were obtained as previously described.[16] The synthesis of the lactosylated acrylamide polymer used as reference in ELLA experiments with PNA-HRP is depicted in Scheme S1 in the Supporting Information.

2,2,2-Tris[5-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosylthio)-2-oxapentyl]ethanol (5). A solution of **1** (124 mg, 0.48 mmol), **3** (1.9 g, 2.9 mmol, 2 equiv) and AIBN (48 mg, 0.3 mmol) in dry dioxane (20 mL) was stirred under Ar at 75 °C for 3 h. Cyclohexene (1.0 mL) was then added, the solvents were removed under reduced pressure, and the residue was purified by column chromatography using 1:2 → 1:3 petroleum ether/EtOAc and then EtOAc as eluent. Yield: 0.76 g (71%); R_f = 0.20 (1:3 petroleum ether/EtOAc); $[\alpha]_D$ = −6.4 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.30 (d, 3 H, $J_{3',4'}$ = 3.4 Hz, H-4′$_{Lact}$), 5.16 (t, 3 H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3$_{Lact}$), 5.05 (dd, 3 H, $J_{2',3'}$ = 10.4 Hz, $J_{1',2'}$ = 8.0 Hz, H-2′$_{Lact}$), 4.91 (dd, 3 H, H-3′$_{Lact}$), 4.87 (t, 3 H, $J_{1,2}$ = 9.7 Hz, H-2$_{Lact}$), 4.46 (d, 3 H, H-1′$_{Lact}$), 4.44 (d, 3 H, H-1$_{Lact}$), 4.43 (dd, 3 H, $J_{6a,6b}$ = 10.0 Hz, $J_{5,6a}$ = 2.3 Hz, H-6a$_{Lact}$), 4.09 (dd, 3 H, $J_{6a',6b'}$ = 12.5 Hz, $J_{5',6a'}$ = 7.0 Hz, H-6a′$_{Lact}$), 4.05 (dd, 3 H, $J_{5,6b}$ = 5.2 Hz, H-6b$_{Lact}$), 4.04 (dd, 3 H, $J_{5',6b'}$ = 7.0 Hz, H-6b′$_{Lact}$), 3.84 (t, 3 H, H-5′$_{Lact}$), 3.74 (t, 3 H, $J_{4,5}$ = 9.7 Hz, H-4$_{Lact}$), 3.58 (ddd, 3 H, H-5$_{Lact}$), 3.56 (s, 2 H, CH$_2$OH), 3.40 (t, 6 H, $^3J_{H,H}$ = 5.8 Hz, H-3$_{Pent}$), 3.34 (s, 6 H, H-1$_{Pent}$), 2.70 (bs, 1 H, OH), 2.67, 2.64 (2 dt, 6 H, $^3J_{H,H}$ = 6.0 Hz, $^2J_{H,H}$ = 13.0 Hz, H-5$_{Pent}$), 2.11−1.92 (7 s, 63 H, MeCO), 1.79 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3−169.1 (CO), 101.1 (C-1′$_{Lact}$), 83.7 (C-1$_{Lact}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.2 (C-1$_{Pent}$), 71.0 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7 (C-3$_{Pent}$), 69.1 (C-2′$_{Lact}$), 66.6 (C-4′$_{Lact}$), 65.4 (C-1$_{Pent}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 45.0 (C$_q$), 29.9 (C-4$_{Pent}$), 27.3 (C-5$_{Pent}$), 21.0−20.5 (*Me*CO); FABMS *m/z* 2237 [M + Na]$^+$. Anal. Calcd for C$_{92}$H$_{132}$O$_{55}$S$_3$: C 49.90, H 6.01. Found: C 49.92, H 6.04.

2,2,2-Tris[5-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosylthio)-2-oxapentyl]ethyl Azide (7). To a solution of alcohol **5** (0.28 g, 0.13 mmol) in dry DCM (1.5 mL) were added pyridine (60 μL) and Tf$_2$O (26 μL, 0.16 mmol) under Ar at −25 °C. The solution was stirred for 40 min at −25 °C, diluted with DCM (1.0 mL), washed with cold saturated aqueous NaHCO$_3$, dried (MgSO$_4$), and concentrated. The residue was dissolved in DMF (3 mL), and NaN$_3$ (25 mg, 0.38 mmol)

was added. The mixture was stirred at room temperature for 3 h and then concentrated. The resulting residue was dissolved in DCM, washed with water, dried (MgSO$_4$), concentrated, and purified by column chromatography (1:2 → 1:3 petroleum ether/EtOAc). Yield: 0.20 g (71%); R_f = 0.54 (1:3 petroleum ether/EtOAc); $[\alpha]_D$ = −9.1 (*c* 1.0 in DCM); IR (KBr) ν_{max} 2105 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.30 (d, 3 H, $J_{3',4'}$ = 3.5 Hz, H-4′$_{Lact}$), 5.05 (dd, 3 H, $J_{2',3'}$ = 10.5 Hz, $J_{1',2'}$ = 7.9 Hz, H-2′$_{Lact}$), 4.91 (dd, 3 H, H-3′$_{Lact}$), 4.87 (t, 3 H, $J_{1,2}$ = $J_{2,3}$ = 9.9 Hz, H-2$_{Lact}$), 5.16 (t, 3 H, $J_{3,4}$ = 9.6 Hz, H-3$_{Lact}$), 4.45 (d, 3 H, H-1′$_{Lact}$), 4.43 (d, 3 H, H-1$_{Lact}$), 4.42 (dd, 3 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.0 Hz, H-6a$_{Lact}$), 4.09 (dd, 3 H, $J_{6a',6b'}$ = 11.8 Hz, $J_{5',6a'}$ = 7.2 Hz, H-6a′$_{Lact}$), 4.04 (dd, 3 H, $J_{5,6b}$ = 5.3 Hz, H-6b$_{Lact}$), 4.03 (dd, 3 H, $J_{5',6b'}$ = 7.2 Hz, H-6b′$_{Lact}$), 3.84 (t, 3 H, H-5′$_{Lact}$), 3.74 (t, 3 H, $J_{4,5}$ = 9.9 Hz, H-4$_{Lact}$), 3.57 (ddd, 3 H, H-5$_{Lact}$), 3.39 (t, 6 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.25 (s, 2 H, CH$_2$N$_3$), 3.24 (s, 6 H, H-1$_{Pent}$), 2.67, 2.64 (2 dt, 6 H, $^3J_{H,H}$ = 7.0 Hz, $^2J_{H,H}$ = 14.0 Hz, H-5$_{Pent}$), 1.92−2.12 (7 s, 63 H, MeCO), 1.80 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3−169.1 (CO), 101.1 (C-1′$_{Lact}$), 83.8 (C-1$_{Lact}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.0 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.4 (C-2$_{Lact}$), 69.6 (C-1$_{Pent}$), 69.5 (C-3$_{Pent}$), 69.1 (C-2′$_{Lact}$), 66.6 (C-4′$_{Lact}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 52.0 (CH$_2$N$_3$), 45.4 (C$_q$), 30.0 (C-4$_{Pent}$), 27.4 (C-5$_{Pent}$), 21.0−20.5 (*Me*CO); FABMS *m/z* 2262 [M + Na]$^+$. Anal. Calcd for C$_{92}$H$_{131}$N$_3$O$_{54}$S$_3$: C 49.35, H 5.90, N 1.88. Found: C 49.29, H 5.84, N 1.76.

2,2,2-Tris[5-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosylthio)-2-oxapentyl]ethyl Isothiocyanate (9). To a solution of azide **7** (0.44 g, 0.2 mmol) in dry dioxane (10 mL) were added TPP (57 mg, 0.22 mmol) and CS$_2$ (0.12 mL, 1.98 mmol) under Ar. The solution was stirred at room temperature for 24 h. Then the solvents were evaporated, and the residue was purified by column chromatography using 1:2 petroleum ether/EtOAc as eluent. Yield: 0.44 g (95%); R_f = 0.38 (1:3 petroleum ether/EtOAc); $[\alpha]_D$ = −10.5 (*c* 1.0 in DCM); IR (KBr) ν_{max} 2191, 2108 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.32 (d, 3 H, $J_{3',4'}$ = 3.0 Hz, H-4′$_{Lact}$), 5.20 (t, 3 H, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, H-3$_{Lact}$), 5.09 (dd, 3 H, $J_{2',3'}$ = 10.5 Hz, $J_{1',2'}$ = 7.7 Hz, H-2′$_{Lact}$), 4.95 (dd, 3 H, H-3′$_{Lact}$), 4.92 (t, 3 H, $J_{1,2}$ = 9.9 Hz, H-2$_{Lact}$), 4.58 (3 H, dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.5 Hz, H-6a$_{Lact}$), 4.48 (d, 3 H, H-1′$_{Lact}$), 4.47 (d, 3 H, H-1$_{Lact}$), 4.11 (3 H, dd, $J_{6a',6b'}$ = 11.5 Hz, $J_{5',6a'}$ = 7.5 Hz, H-6a′$_{Lact}$), 4.06 (3 H, dd, $J_{5,6b}$ = 5.0 Hz, H-6b$_{Lact}$), 4.05 (3 H, dd, $J_{5',6b'}$ = 8.5 Hz, H-6b′$_{Lact}$), 3.87 (dd, 3 H, H-5′$_{Lact}$), 3.79 (t, 3 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.61 (ddd, 3 H, H-5$_{Lact}$), 3.56 (s, 2 H, CH$_2$NCS), 3.44 (t, 6 H, $^3J_{H,H}$ = 5.7 Hz, H-3$_{Pent}$), 3.32 (s, 6 H, H-1$_{Pent}$), 2.71, 2.68 (2 dt, 6 H, $^2J_{H,H}$ = 13.1 Hz, $^3J_{H,H}$ = 7.3 Hz, H-5$_{Pent}$), 2.14−1.95 (6 s, 63 H, MeCO), 1.83 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.1−168.9 (CO), 130.3 (NCS), 101.0 (C-1′$_{Lact}$), 83.6 (C-1$_{Lact}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.0 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.4 (C-2$_{Lact}$), 69.6 (C-1$_{Pent}$), 69.5 (C-3$_{Pent}$), 69.1 (C-2′$_{Lact}$), 66.6 (C-4′$_{Lact}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 45.8 (CH$_2$NCS), 45.7 (C$_q$), 29.8 (C-4$_{Pent}$), 27.2 (C-5$_{Pent}$), 20.8−20.3 (*Me*CO); FABMS *m/z* 2278 [M + Na]$^+$. Anal. Calcd for C$_{93}$H$_{131}$NO$_{54}$S$_4$: C 49.53, H 5.85, N 0.62. Found: C 49.40, H 5.66, N 0.60.

2,2,2-Tris(5-β-lactosylthio-2-oxapentyl)ethanol (tri*Lact*-OH). Conventional Zemplén deacetylation of **5** (0.20 g, 90 μmol) gave **tri*Lact*-OH**. Yield: 107 mg (90%); R_f = 0.13 (6:3:1 MeCN/H$_2$O/NH$_4$OH); $[\alpha]_D$ = −11.0 (*c* 1.0 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 4.48 (d, 3 H, $J_{1,2}$ = 10.0 Hz, H-1$_{Lact}$), 4.38 (d, 3 H, $J_{1',2'}$ = 8.0 Hz, H-1′$_{Lact}$), 3.89 (dd, 3 H, $J_{6a,6b}$ = 12.05 Hz, H-6a$_{Lact}$), 3.85 (d, 3 H, H-4′$_{Lact}$), 3.73 (dd, 3 H, H-6b$_{Lact}$), 3.71 (dd, 3 H, $J_{6a',6b'}$ = 11.5 Hz, $J_{5',6a'}$ = 3.0 Hz, H-6a′$_{Lact}$), 3.67 (dd, 3 H, $J_{5',6ab'}$ = 3.5 Hz, H-6b′$_{Lact}$), 3.64 (m, 3 H, H-5′$_{Lact}$), 3.60 (dd, 3 H, $J_{3',4'}$ = 3.5 Hz, H-3′$_{Lact}$), 3.59 (t, 3 H, $J_{4,5}$ = 9.0 Hz, H-4$_{Lact}$), 3.58 (t, 3 H, $J_{3,4}$ = 9.0 Hz, H-3$_{Lact}$), 3.53 (m, 6 H, H-3$_{Pent}$), 3.52 (ddd, 3 H, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 4.5 Hz, H-5$_{Lact}$), 3.50 (s, 2 H, CH$_2$OH), 3.47 (dd, 3 H, $J_{2',3'}$ = 10.0 Hz, H-2′$_{Lact}$), 3.38 (m, 6 H, H-1$_{Pent}$), 3.30 (dd, 3 H, $J_{2,3}$ = 9.0 Hz, H-2$_{Lact}$), 2.77, 2.73 (2 dt, 6 H, $J_{4',5'}$ = 7.0 Hz, $J_{5a',5b'}$ = 14.0 Hz, H-5$_{Pent}$), 1.86 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (75.5 MHz, D$_2$O) δ 102.8 (C-1′$_{Lact}$), 85.3 (C-1$_{Lact}$), 78.6 (C-5$_{Lact}$), 78.0 (C-4$_{Lact}$), 75.7 (C-3$_{Lact}$), 75.3 (C-5′$_{Lact}$), 72.4 (C-3′$_{Lact}$), 71.9 (C-2$_{Lact}$), 70.8 (C-2′$_{Lact}$), 69.9 (C-3$_{Pent}$), 69.2 (C-1$_{Pent}$), 68.5 (C-4′$_{Lact}$), 61.3 (CH$_2$OH), 60.9 (C-6′$_{Lac}$), 60.1 (C-6$_{Lac}$), 44.9 (C$_q$), 29.1

(C-4$_{Pent}$), 26.7 (C-5$_{Pent}$); FABMS m/z 1353 [M + Na]$^+$. Anal. Calcd for C$_{50}$H$_{90}$O$_{34}$S$_3$: C, 45.10, H, 6.81. Found: C, 44.83, H, 6.55.

2-[5-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-*β*-lactosylthio)-2-oxapentyl]-2,2-bis(2-oxapent-4-enyl)ethanol (11) and 2,2-Bis[5-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-*β*-lactosylthio)-2-oxapentyl]-2-(2-oxapent-4-enyl)ethanol (12). A solution of **3** (1.78 g, 2.73 mmol), **1** (0.5 g, 1.95 mmol) and AIBN (128 mg, 0.78 mmol) in dry dioxane (25 mL) was stirred under Ar at 75 °C for 1 h. Cyclohexene (2.6 mL) was then added, the solvents were removed under reduced pressure, and the products were separated by column chromatography using 1:2 → 1:3 petroleum ether/EtOAc and then EtOAc as eluent.

Data for **11**. Yield: 0.88 g (71%); R_f = 0.49 (1:2 petroleum ether/EtOAc); [$α$]$_D$ = −9.4 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) $δ$ 5.81 (ddt, 2 H, OCH$_2$C*H*=), 5.30 (d, 1 H, $J_{3′,4′}$ = 3.5 Hz, H-4′$_{Lact}$), 5.24 (dq, 2 H, $^3J_{H,H}$ = 17.5 Hz, =CH$_a$), 5.22 (dq, 2 H, $^3J_{H,H}$ = 10.5 Hz, $^2J_{H,H}$ = 1.5 Hz, =CH$_b$), 5.15 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3$_{Lact}$), 5.04 (dd, 1 H, $J_{1′,2′}$ = 8.0 Hz, H-2′$_{Lact}$), 4.90 (dd, 1 H, $J_{2′,3′}$ = 10.5 Hz, H-3′$_{Lact}$), 4.87 (t, 1 H, $J_{1,2}$ = 9.5 Hz, H-2$_{Lact}$), 4.43 (d, 1 H, H-1′$_{Lact}$), 4.41 (d, 1 H, H-1$_{Lact}$), 4.40 (dd, 1 H, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a$_{Lact}$), 4.07 (dd, 1 H, $J_{6a′,6b′}$ = 11.5 Hz, $J_{5′,6a′}$ = 7.5 Hz, H-6a′$_{Lact}$), 4.04 (dd, 1 H, $J_{5,6b}$ = 5.0 Hz, H-6b$_{Lact}$), 4.03 (dd, 1 H, $J_{5′,6b′}$ = 7.5 Hz, H-6b′$_{Lact}$), 3.89 (dt, 4 H, $^3J_{H,H}$ = 5.5 Hz, $^4J_{H,H}$ = 1.2 Hz, C*H*$_2$CH=), 3.82 (t, 1 H, H-5′$_{Lact}$), 3.73 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.64 (m, 2 H, C*H*$_2$OH), 3.57 (ddd, 1 H, H-5$_{Lact}$), 3.42 (t, 2 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.41 (m, 6 H, H-1$_{Pent}$, C*H*$_2$OAll), 2.82 (bt, 1 H, OH), 2.68, 2.62 (2 dt, 2 H, $^3J_{H,H}$ = 6.5 Hz, $^2J_{H,H}$ = 13.0 Hz, H-5$_{Pent}$), 1.91−2.10 (7 s, 21 H, MeCO), 1.79 (m, 2 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) $δ$ 170.8−168.6 (CO), 134.7 (CH=), 116.9 (CH$_2$=), 101.2 (C-1′$_{Lact}$), 83.8 (C-1$_{Lact}$), 76.6 (C-5$_{Lact}$), 76.3 (C-4$_{Lact}$), 73.7 (C-3$_{Lact}$), 72.4 (OCH$_2$CH=), 71.3 (CH$_2$OAll, C-1$_{Pent}$), 70.9 (C-3′$_{Lact}$), 70.6 (C-5′$_{Lact}$), 70.2 (C-2$_{Lact}$), 69.6 (C-3$_{Pent}$), 69.0 (C-2′$_{Lact}$), 66.5 (C-4′$_{Lact}$), 65.8 (CH$_2$OH), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 44.8 (C$_q$), 29.8 (C-4$_{Pent}$), 27.4 (C-5$_{Pent}$), 21.0−20.6 (*Me*CO); FABMS m/z 931 [M + Na]$^+$. Anal. Calcd for C$_{40}$H$_{60}$O$_{21}$S: C, 52.85, H, 6.65. Found: C, 52.73, H, 6.69.

Data for **12**: Yield: 0.76 g (25%); R_f = 0.25 (1:2 petroleum ether/EtOAc); [$α$]$_D$ = −26.2 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) $δ$ 5.82 (ddt, 2 H, OCH$_2$C*H*=), 5.31 (d, 2 H, $J_{3′,4′}$ = 3.5 Hz, H-4′$_{Lact}$), 5.22 (dq, 1 H, $^3J_{H,H}$ = 10.4 Hz, $^2J_{H,H}$ = 1.5 Hz, =CH$_b$), 5.18 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$_{Lact}$), 5.14 (dq, 1 H, $^3J_{H,H}$ = 17.2 Hz, =CH$_a$), 5.07 (dd, 2 H, $J_{1′,2′}$ = 8.0 Hz, H-2′$_{Lact}$), 4.91 (dd, 2 H, $J_{2′,3′}$ = 10.5 Hz, H-3′$_{Lact}$), 4.90 (t, 2 H, $J_{1,2}$ = 9.5 Hz, H-2$_{Lact}$), 4.45 (dd, 2 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.5 Hz, H-6a$_{Lact}$), 4.44 (d, 2 H, H-1′$_{Lact}$), 4.43 (d, 2 H, H-1$_{Lact}$), 4.10 (dd, 2 H, $J_{6a′,6b′}$ = 12.0 Hz, $J_{5′,6a′}$ = 7.5 Hz, H-6a′$_{Lact}$), 4.05 (dd, 2 H, $J_{5,6b}$ = 5.0 Hz, H-6b$_{Lact}$), 4.04 (dd, 2 H, $J_{5′,6b′}$ = 7.5 Hz, H-6b′$_{Lact}$), 3.92 (dt, 2 H, $^3J_{H,H}$ = 5.5 Hz, $^4J_{H,H}$ = 1.3 Hz, C*H*$_2$CH=), 3.84 (t, 2 H, H-5′$_{Lact}$), 3.75 (t, 2 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.63 (s, 2 H, C*H*$_2$OH), 3.59 (ddd, 2 H, H-5$_{Lact}$), 3.43 (t, 4 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.40 (m, 6 H, H-1$_{Pent}$, C*H*$_2$OAll), 2.88 (bs, 1 H, OH), 2.69, 2.66 (2 dt, 4 H, $^3J_{H,H}$ = 7.0 Hz, $^2J_{H,H}$ = 13.5 Hz, H-5$_{Pent}$), 2.13−1.94 (7 s, 42 H, MeCO), 1.81 (m, 4 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) $δ$ 171.3−170.9 (CO), 134.7 (CH=), 116.6 (CH$_2$=), 101.1 (C-1′$_{Lact}$), 83.8 (C-1$_{Lact}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 72.4 (OCH$_2$CH=), 71.2, 71.0 (CH$_2$OAll, C-1$_{Pent}$), 70.8 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7 (C-3$_{Pent}$), 69.1 (C-2′$_{Lact}$), 66.6 (C-4′$_{Lact}$), 65.8 (CH$_2$OH), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 44.9 (C$_q$), 29.9 (C-4$_{Pent}$), 27.3 (C-5$_{Pent}$), 20.8−20.5 (*Me*CO); FABMS m/z 1583 [M + Na]$^+$. Anal. Calcd for C$_{66}$H$_{96}$O$_{38}$S$_2$: C, 50.76, H, 6.20. Found: C, 50.66, H, 6.01.

2-[5-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-*β*-lactosylthio)-2-oxapentyl]-2,2-bis[5-(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosylthio)-2-oxapentyl]ethanol (13). A solution of **11** (1.25 g, 1.38 mmol), **4** (2.0 g, 5.53 mmol) and AIBN (90 mg, 0.55 mmol) in dry dioxane (30 mL) was stirred under Ar at 75 °C for 30 min. Cyclohexene (1.8 mL) was then added, the solvents were removed under reduced pressure, and the residue was purified by column chromatography using 1:2 → 1:3 petroleum ether/EtOAc as eluent. Yield: 0.77 g (35%); R_f = 0.19 (1:3 petroleum ether/EtOAc); [$α$]$_D$ = +20.9 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) $δ$ 5.29 (d, 1 H, $J_{3′,4′}$ = 3.0 Hz, H-4′$_{Lact}$), 5.28 (dd, 2 H, $J_{2,3}$ = 3.5 Hz, $J_{1,2}$ = 1.5 Hz,

H-2$_{Man}$), 5.26 (t, 2 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4$_{Man}$), 5.20 (d, 2 H, H-1$_{Man}$), 5.19 (dd, 2 H, H-3$_{Man}$), 5.16 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$_{Lact}$), 5.05 (dd, 1 H, $J_{2′,3′}$ = 9.5 Hz, $J_{1′,2′}$ = 7.5 Hz, H-2′$_{Lact}$), 4.90 (dd, 1 H, H-3′$_{Lact}$), 4.87 (t, 1 H, $J_{1,2}$ = H-2$_{Lact}$), 4.45 (m, 2 H, H-1$_{Lact}$, H-6a$_{Lact}$), 4.44 (d, 1 H, H-1′$_{Lact}$), 4.33 (ddd, 2 H, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-5$_{Man}$), 4.26 (dd, 2 H, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Man}$), 4.08 (dd, 1 H, $J_{6a′,6b′}$ = 11.0 Hz, $J_{5′,6a′}$ = 7.0 Hz, H-6a′$_{Lact}$), 4.05 (dd, 2 H, H-6b$_{Man}$), 4.04 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, H-6b$_{Lact}$), 4.03 (dd, 1 H, $J_{5′,6b′}$ = 7.0 Hz, H-6b′$_{Lact}$), 3.83 (t, 1 H, H-5′$_{Lact}$), 3.75 (t, 1 H, $J_{3,4}$ = 9.5 Hz, H-4$_{Lact}$), 3.74 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.59 (m, 2 H, C*H*$_2$OH), 3.58 (ddd, 1 H, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.5 Hz, H-5$_{Lact}$), 3.42 (2 t, 6 H, $^3J_{H,H}$ = 6.5 Hz, H-3$_{Pent}$), 3.35 (s, 6 H, H-1$_{Pent}$), 2.66, 2.62 (2 dt, 6 H, $^2J_{H,H}$ = 12.5 Hz, $^3J_{H,H}$ = 6.5 Hz, H-5$_{Pent}$), 1.91−2.12 (8s, 45 H, MeCO), 1.80 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) $δ$ 170.0−169.1 (CO), 101.1 (C-1′$_{Lact}$), 83.7 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.4, 71.3 (C-1$_{Pent}$), 71.1 (C-2$_{Man}$), 71.0 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7, 69.6 (C-3$_{Pent}$), 69.4 (C-3$_{Man}$), 69.1 (C-2′$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4′$_{Lact}$), 66.3 (C-4$_{Man}$), 65.4 (CH$_2$OH), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 45.1 (C$_q$), 29.9, 29.7 (C-4$_{Pent}$), 28.2, 27.3 (C-5$_{Pent}$), 20.9−20.8 (*Me*CO); FABMS m/z 1661 [M + Na]$^+$. Anal. Calcd for C$_{68}$H$_{100}$O$_{39}$S$_3$: C 49.87, H 6.15. Found: C 49.84, H 5.94.

2,2-Bis[5-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-*β*-lactosylthio)-2-oxapentyl]-2-[5-(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosylthio)-2-oxapentyl]ethanol (14). A solution of **12** (0.60 g, 0.39 mmol), **4** (0.28 g, 0.78 mmol) and AIBN (12 mg, 77 $μ$mol) in dry dioxane (8.4 mL) was stirred under Ar at 75 °C for 30 min. Cyclohexene (0.26 mL) was added, the solvents were removed under reduced pressure, and the residue was purified by column chromatography using 1:2 → 1:3 petroleum ether/EtOAc as eluent. Yield: 0.31 g (42%); R_f = 0.19 (1:3 petroleum ether/EtOAc); [$α$]$_D$ = +9.7 (*c* 1.2 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) $δ$ 5.30 (d, 2 H, $J_{3′,4′}$ = 3.5 Hz, H-4′$_{Lact}$), 5.29 (d, 1 H, $J_{2,3}$ = 3.5 Hz, $J_{1,2}$ = 1.5 Hz, H-2$_{Man}$), 5.27 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4$_{Man}$), 5.20 (bs, 1 H, H-1$_{Man}$), 5.20 (dd, 1 H, H-3$_{Man}$), 5.17 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$_{Lact}$), 5.06 (dd, 2 H, $J_{2′,3′}$ = 10.5 Hz, $J_{1′,2′}$ = 8.0 Hz, H-2′$_{Lact}$), 4.91 (dd, 2 H, H-3′$_{Lact}$), 4.88 (t, 2 H, $J_{1,2}$ = 9.5 Hz, H-2$_{Lact}$), 4.45 (d, 2 H, H-1′$_{Lact}$), 4.43 (m, 4 H, H-1$_{Lact}$, H-6a$_{Lact}$), 4.33 (ddd, 1 H, $J_{5,6a}$ = 5.5 Hz, $J_{5,6b}$ = 2.0 Hz, H-5$_{Man}$), 4.28 (dd, 1 H, $J_{6a,6b}$ = 12.5 Hz, H-6a$_{Man}$), 4.09 (dd, 2 H, $J_{6a′,6b′}$ = 11.5 Hz, $J_{5′,6a′}$ = 7.0 Hz, H-6a′$_{Lact}$), 4.06 (dd, 1 H, $J_{5,6b}$ = 5.0 Hz, H-6b$_{Man}$), 4.05 (dd, 2 H, $J_{6a,6b}$ = 12.0 Hz, H-6b$_{Lact}$), 4.04 (dd, 2 H, $J_{5′,6b′}$ = 7.0 Hz, H-6b′$_{Lact}$), 3.84 (t, 2 H, H-5′$_{Lact}$), 3.75 (t, 2 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.59 (m, 2 H, C*H*$_2$OH), 3.58 (ddd, 2 H, $J_{5,6a}$ = 2.0 Hz, H-5$_{Lact}$), 3.41 (2 t, 6 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.35 (s, 6 H, H-1$_{Pent}$), 2.69, 2.63 (2 dt, 6 H, $^2J_{H,H}$ = 13.0 Hz, $^3J_{H,H}$ = 6.5 Hz, H-5$_{Pent}$), 1.92−2.13 (10 s, 54 H, MeCO), 1.81 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) $δ$ 170.0−169.1 (CO), 101.1 (C-1′$_{Lact}$), 83.7 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.3, 71.1 (C-1$_{Pent}$), 71.2 (C-2$_{Man}$), 71.0 (C-3′$_{Lact}$), 70.7 (C-5′$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7, 69.6 (C-3$_{Pent}$), 69.5 (C-3$_{Man}$), 69.1 (C-2′$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4′$_{Lact}$), 66.3 (C-4$_{Man}$), 65.4 (CH$_2$OH), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6′$_{Lact}$), 45.0 (C$_q$), 29.9, 29.5 (C-4$_{Pent}$), 28.2, 27.3 (C-5$_{Pent}$), 21.0−20.8 (*Me*CO); FABMS m/z 1947 [M + Na]$^+$. Anal. Calcd for C$_{80}$H$_{116}$O$_{47}$S$_3$: C 49.89, H 6.07. Found: C 49.86, H 5.96.

2-[5-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-*β*-lactosylthio)-2-oxapentyl]-2,2-bis[5-(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosylthio)-2-oxapentyl]ethyl Azide (15). Compound **15** was prepared from **13** (0.34 g, 0.21 mmol) by triflation with Tf$_2$O (43 $μ$L, 0.26 mmol) in dry DCM (2.5 mL) in the presence of pyridine (100 $μ$L) and displacement of the resulting triflate with NaN$_3$ (42 mg, 0.64 mmol) in DMF (4.0 mL), following the procedure above-described for the preparation of **7**. Yield: 0.28 g (71%); IR (KBr) $ν_{max}$ 2103 cm^{-1}; R_f = 0.46 (1:3 petroleum ether/EtOAc); [$α$]$_D$ = +37.0 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) $δ$ 5.29 (d, 1 H, $J_{3′,4′}$ = 3.5 Hz, H-4′$_{Lact}$), 5.28 (d, 2 H, $J_{2,3}$ = 4.0 Hz, H-2$_{Man}$), 5.25 (t, 2 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4$_{Man}$), 5.20 (s, 2 H, H-1$_{Man}$), 5.19 (dd, 2 H, H-3$_{Man}$), 5.15 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$_{Lact}$), 5.03 (dd, 1 H, $J_{2′,3′}$ = 10.5 Hz, $J_{1′,2′}$ = 7.5 Hz, H-2′$_{Lact}$), 4.90 (dd, 1 H, H-3′$_{Lact}$), 4.86 (t, 1 H, $J_{1,2}$ = 9.5 Hz, H-2$_{Lact}$), 4.43 (d, 1 H, H-1′$_{Lact}$), 4.42 (d, 1 H, H-1$_{Lact}$), 4.41 (dd, 1 H, $J_{6a,6b}$ = 12.5 Hz, H-6a$_{Lact}$), 4.32 (ddd, 2 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-5$_{Man}$), 4.26 (dd, 2 H, $J_{6a,6b}$ = 12.5 Hz, H-6a$_{Man}$), 4.07

(1 H, dd, $J_{6a',6b'}$ = 11.5 Hz, $J_{5',6a'}$ = 7.0 Hz, 6a'$_{Lact}$), 4.02 (1 H, dd, $J_{5',6b'}$ = 7.0 Hz, H-6b'$_{Lact}$), 4.03 (2 H, dd, H-6b$_{Man}$), 4.02 (1 H, dd, H-6b$_{Lact}$), 3.82 (t, 1 H, H-5'$_{Lact}$), 3.72 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.56 (ddd, 1 H, $J_{5,6b}$ = 5.5 Hz, $J_{5,6a}$ = 2.0 Hz, H-5$_{Lact}$), 3.40 (2 t, 6 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.26 (s, 6 H, H-1$_{Pent}$), 3.25 (m, 2 H, CH$_2$N$_3$), 2.65, 2.61 (2 dt, 6 H, $^2J_{H,H}$ = 12.5 Hz, $^3J_{H,H}$ = 5.5 Hz, H-5$_{Pent}$), 1.93–2.11 (8 s, 45 H, MeCO), 1.80 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3–169.5 (CO), 101.1 (C-1$_{Lact}$), 83.7 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.6 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.1 (C-2$_{Man}$), 70.9 (C-3'$_{Lact}$), 70.7 (C-5'$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.6 (C-1$_{Pent}$), 69.3 (C-3$_{Pent}$, C-3$_{Man}$), 69.1 (C-2'$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4'$_{Lact}$), 66.3 (C-4$_{Man}$), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6'$_{Lact}$), 51.9 (CH$_2$N$_3$), 45.4 (C$_q$), 29.9, 29.5 (C-4$_{Pent}$), 28.3, 27.4 (C-5$_{Pent}$), 21.0–20.6 (MeCO); FABMS m/z 1684 [M + Na]$^+$. Anal. Calcd for C$_{68}$H$_{99}$N$_3$O$_{38}$S$_3$: C 49.12, H 6.00; N 2.53. Found: C 48.84, H 5.62, N 2.47.

2,2-Bis[5-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]-2-[5-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-2-oxapentyl]ethyl Azide (16). Compound 16 was prepared from 14 (0.26 g, 0.13 mmol) by triflation with Tf$_2$O (26 µL, 0.16 mmol) in dry DCM (3.0 mL) in the presence of pyridine (64 µL) and displacement of the resulting triflate with NaN$_3$ (26 mg, 0.41 mmol) in DMF (2.5 mL), following the procedure above-described for the preparation of 7. Yield: 194 mg (75%); IR (KBr) ν$_{max}$ 2103 cm^{-1}; R_f = 0.40 (1:3 petroleum ether/EtOAc); [α]$_D$ = +10.0 (c 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.32 (d, 2 H, $J_{3',4'}$ = 3.5 Hz, H-4'$_{Lact}$), 5.31 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, $J_{1,2}$ = 1.7 Hz, H-2$_{Man}$), 5.29 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4$_{Man}$), 5.23 (d, 1 H, H-1$_{Man}$), 5.22 (dd, 1 H, H-3$_{Man}$), 5.18 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3$_{Lact}$), 5.07 (dd, 2 H, $J_{2',3'}$ = 10.4 Hz, $J_{1',2'}$ = 7.9 Hz, H-2'$_{Lact}$), 4.93 (dd, 2 H, H-3'$_{Lact}$), 4.89 (t, 2 H, $J_{1,2}$ = 9.4 Hz, H-2$_{Lact}$), 4.46 (d, 2 H, H-1'$_{Lact}$), 4.45 (d, 2 H, H-1$_{Lact}$), 4.35(ddd, 1 H, $J_{5,6a}$ = 5.2 Hz, $J_{5,6b}$ = 1.9 Hz, H-5$_{Man}$), 4.45 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a$_{Lact}$), 4.29 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, H-6a$_{Man}$), 4.10 (dd, 2 H, $J_{6a',6b'}$ = 11.1 Hz, $J_{5',6a'}$ = 7.3 Hz, H-6a'$_{Lact}$), 4.07 (dd, 1 H, H-6b$_{Man}$), 4.06 (dd, 2 H, $J_{5,6b}$ = 5.3 Hz, H-6b$_{Lact}$), 4.05 (dd, 2 H, $J_{5',6b'}$ = 7.3 Hz, H-6b'$_{Lact}$), 3.85 (t, 2 H, H-5'$_{Lact}$), 3.76 (t, 1 H, $J_{4,5}$ = 9.4 Hz, H-4$_{Lact}$), 3.59 (ddd, 2 H, H-5$_{Lact}$), 3.42 (2 t, 6 H, $^3J_{H,H}$ = 5.8 Hz, H-3$_{Pent}$), 3.28 (s, 2 H, CH$_2$N$_3$), 3.27, 3.26 (2 s, 6 H, H-1$_{Pent}$), 2.69, 2.66 (2 dt, 6 H, $^2J_{H,H}$ = 13.7 Hz, $^3J_{H,H}$ = 7.5 Hz, H-5$_{Pent}$), 1.93–2.14 (6 s, 54 H, MeCO), 1.85 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3–169.1 (CO), 101.1 (C-1'$_{Lact}$), 83.8 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.2 (C-2$_{Man}$), 71.0 (C-3'$_{Lact}$), 70.7 (C-5'$_{Lact}$), 70.4 (C-2$_{Lact}$), 69.6 (C-1$_{Pent}$), 69.5 (C-3$_{Pent}$, C-3$_{Man}$), 69.1 (C-2'$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4'$_{Lact}$), 66.3 (C-4$_{Man}$), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6'$_{Lact}$), 52.0 (CH$_2$N$_3$), 45.4 (C$_q$), 29.9, 29.6 (C-4$_{Pent}$), 28.3, 27.4 (C-5$_{Pent}$), 20.9–20.6 (MeCO); FABMS m/z 1973 [M + Na]$^+$. Anal. Calcd for C$_{80}$H$_{115}$N$_3$O$_{46}$S$_3$: C, 49.25, H, 5.94; N, 2.15. Found: C, 49.09, H, 6.05; N, 2.17.

2-[5-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]-2,2-bis[5-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-2-oxapentyl]ethyl Isothiocyanate (17). Compound 17 was obtained by isothiocyanation of azide 15 (0.61 g, 0.37 mmol) with TPP (106 mg, 0.40 mmol) and CS$_2$ (0.22 mL, 3.7 mmol) following the procedure above-described for the preparation of 9. Yield: 0.54 g (87%); R_f = 0.61 (1:3 petroleum ether/EtOAc); [α]$_D$ = +33.4 (c 1.0 in DCM); IR (KBr) ν$_{max}$ 2191, 2106 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.32 (d, 2 H, $J_{3',4'}$ = 3.5 Hz, H-4'$_{Lact}$), 5.31 (bd, 2 H, $J_{2,3}$ = 3.5 Hz, H-2$_{Man}$), 5.29 (t, 2 H, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4$_{Man}$), 5.23 (bs, 2 H, H-1$_{Man}$), 5.22 (dd, 2 H, H-3$_{Man}$), 5.18 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$_{Lact}$), 5.07 (dd, 1 H, $J_{2',3'}$ = 10.4 Hz, $J_{1',2'}$ = 8.2 Hz, H-2'$_{Lact}$), 4.93 (dd, 1 H, H-3'$_{Lact}$), 4.90 (t, 1 H, $J_{1,2}$ = 9.5 Hz, H-2$_{Lact}$), 4.46 (d, 1 H, H-1'$_{Lact}$), 4.45 (d, 1 H, H-1$_{Lact}$), 4.44 (m, 1 H, H-6a$_{Lact}$), 4.35 (ddd, 2 H, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-5$_{Man}$), 4.29 (dd, 2 H, $J_{6a,6b}$ = 12.1 Hz, H-6a$_{Man}$), 4.10 (dd, 1 H, $J_{6a',6b}$ = 11.2 Hz, $J_{5',6a}$ = 6.8 Hz, H-6a'$_{Lact}$), 4.07 (dd, 2 H, H-6b$_{Man}$), 4.06 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 5.6 Hz, H-6b$_{Lact}$), 4.05 (dd, 1 H, $J_{5',6'b}$ = 6.8 Hz, H-6'b$_{Lact}$), 3.85 (t, 1 H, H-5'$_{Lact}$), 3.76 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.60 (ddd, 1 H, $J_{5,6a}$ = 1.9 Hz, H-5$_{Lact}$), 3.54 (m, 2 H, CH$_2$NCS), 3.45, 3.44 (2 t, 6 H, $^3J_{H,H}$ = 5.9 Hz, H-3$_{Pent}$), 3.32 (s, 4 H, H-1$_{Pent}$), 3.31 (s, 2 H, H-1$_{Pent}$), 2.69, 2.65 (2 dt, 6 H, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.0 Hz, H-5$_{Pent}$), 1.93–2.14 (6 s, 45 H, MeCO), 1.85 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3–169.1

(CO), 128.5 (NCS), 101.1 (C-1'$_{Lact}$), 83.7 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.8 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.2 (C-2$_{Man}$), 71.0 (C-3'$_{Lact}$), 70.7 (C-5'$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7 (C-1$_{Pent}$), 69.6 (C-3$_{Pent}$), 69.5 (C-3$_{Man}$), 69.1 (C-2'$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4'$_{Lact}$), 66.3 (C-4$_{Man}$), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6'$_{Lact}$), 45.9 (CH$_2$NCS), 45.8 (C$_q$), 29.9, 29.5 (C-4$_{Pent}$), 28.3, 27.3 (C-5$_{Pent}$), 20.9–20.8 (MeCO); FABMS m/z 1702 [M + Na]$^+$. Anal. Calcd for C$_{69}$H$_{99}$NO$_{38}$S$_4$: C 49.37, H 5.94, N 0.83. Found: C 49.30, H 5.72, N 0.80.

2,2-Bis[5-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]-2-[5-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-2-oxapentyl]ethyl Isothiocyanate (18). Compound 18 was obtained by isothiocyanation of azide 16 (194 mg, 99 µmol) with TPP (29 mg, 0.11 mmol) and CS$_2$ (55 µL, 0.91 mmol) following the procedure above-described for the preparation of 9. Yield: 174 mg (89%); R_f = 0.53 (1:3 petroleum ether/EtOAc); [α]$_D$ = +8.7 (c 1.0 in DCM); IR (KBr) ν$_{max}$ 2191, 2108 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.31 (d, 2 H, $J_{3',4'}$ = 3.5 Hz, H-4'$_{Lact}$), 5.30 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, $J_{1,2}$ = 1.5 Hz, H-2$_{Man}$), 5.28 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4$_{Man}$), 5.23 (d, 1 H, H-1$_{Man}$), 5.21 (dd, 1 H, H-3$_{Man}$), 5.17 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3$_{Lact}$), 5.07 (dd, 2 H, $J_{2',3'}$ = 10.5 Hz, $J_{1',2'}$ = 8.0 Hz, H-2'$_{Lact}$), 4.92 (dd, 2 H, H-3'$_{Lact}$), 4.89 (t, 2 H, $J_{1,2}$ = 10.0 Hz, H-2$_{Lact}$), 4.46 (d, 2 H, H-1'$_{Lact}$), 4.45 (dd, 2 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a$_{Lact}$), 4.44 (d, 2 H, H-1$_{Lact}$), 4.34 (ddd, 1 H, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-5$_{Man}$), 4.28 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Man}$), 4.10 (2 H, dd, $J_{6a',6b}$ = 11.0 Hz, $J_{5',6a}$ = 7.0 Hz, H-6'a$_{Lact}$), 4.07 (dd, 1 H, H-6b$_{Man}$), 4.06 (dd, 2 H, H-6b$_{Lact}$), 4.05 (dd, 2 H, $J_{5',6'b}$ = 7.0 Hz, H-6'b$_{Lact}$), 3.85 (t, 2 H, H-5'$_{Lact}$), 3.76 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4$_{Lact}$), 3.59 (ddd, 2 H, H-5$_{Lact}$), 3.52 (s, 2 H, CH$_2$NCS), 3.43 (2 t, 6 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Pent}$), 3.31 (s, 7 H, H-1$_{PentMan}$), 3.30 (s, 4 H, H-1$_{PentLact}$), 2.69, 2.65 (2 dt, 6 H, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.0 Hz, H-5$_{Pent}$), 2.14–1.93 (6 s, 54 H, MeCO), 1.83 (m, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.6–169.1 (CO), 130.3 (NCS), 101.1(C-1'$_{Lact}$), 83.7 (C-1$_{Lact}$), 82.7 (C-1$_{Man}$), 76.7 (C-5$_{Lact}$), 76.2 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.2 (C-2$_{Man}$), 71.0 (C-3'$_{Lact}$), 70.7 (C-5'$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.7 (C-1$_{Pent}$), 69.5 (C-3$_{Pent}$), 69.4 (C-3$_{Man}$), 69.1 (C-2'$_{Lact}$), 69.0 (C-5$_{Man}$), 66.6 (C-4'$_{Lact}$), 66.3 (C-4$_{Man}$), 62.4 (C-6$_{Man}$), 62.2 (C-6$_{Lact}$), 60.8 (C-6'$_{Lact}$), 45.9 (CH$_2$NCS), 45.7 (C$_q$), 29.8, 29.5 (C-4$_{Pent}$), 28.3, 27.3 (C-5$_{Pent}$), 20.9–20.7 (MeCO); FABMS m/z 1990 [M + Na]$^+$. Anal. Calcd for C$_{81}$H$_{115}$NO$_{46}$S$_4$: C 49.46, H 5.89, N 0.71. Found: C 49.23, H 5.75, N 0.70.

2,2-(5-β-Lactosylthio-2-oxapentyl)-2-bis(5-α-D-mannopyranosylthio-2-oxapentyl)ethanol (LactdiMan-OH). Conventional Zemplén deacetylation of 13 (118 mg, 72 µmol) gave **LactdiMan-OH**. Yield: 62 mg (95%); R_f = 0.17 (6:3:1 MeCN/H$_2$O/NH$_4$OH); [α]$_D$ = +32.0 (c 1.0 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 5.23 (bs, 2 H, H-1$_{Man}$), 4.47 (d, 1 H, $J_{1,2}$ = 9.9 Hz, H-1$_{Lact}$), 4.37 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1'$_{Lact}$), 3.98 (dd, 2 H, $J_{2,3}$ = 3.1 Hz, $J_{1,2}$ = 1.3 Hz, H-2$_{Man}$), 3.93 (dd, 2 H, $J_{3,4}$ = 9.7 Hz, H-3$_{Man}$), 3.89 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a$_{Lact}$), 3.86 (d, 1 H, $J_{3,4}$ = 4.4 Hz, H-4'$_{Lact}$), 3.80 (dd, 2 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 2.1 Hz, H-6a$_{Man}$), 3.73 (dd, 1 H, $J_{5,6b}$ = 2.3 Hz, H-6b'$_{Lact}$), 3.70 (M, 6 H, H-5$_{Man}$, H-6'a$_{Lact}$, H-6'b$_{Lact}$, H-6b$_{Man}$), 3.63 (M, 5 H, H-5'$_{Lact}$, H-4$_{Man}$, CH$_2$OH), 3.61 (dd, 2 H, $J_{2,3}$ = 9.8 Hz, H-5$_{Lact}$), 3.59 (2 t, 2 H, $J_{3,4}$ = 9.8 Hz, $J_{4,5}$ = 9.7 Hz, H-3$_{Lact}$, H-4$_{Lact}$), 3.58 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, H-3'$_{Lact}$), 3.54 (t, 6 H, $^3J_{H,H}$ = 5.7 Hz, H-3$_{Pent}$), 3.52 (ddd, 1 H, H-5$_{Lact}$), 3.47 (t, 1 H, H-2'$_{Lact}$), 3.37 (M, 6 H, H-1$_{Pent}$), 3.31 (t, 1 H, H-2$_{Lact}$), 2.70, 2.68 (2 dt, 6 H, $^2J_{H,H}$ = 13.3 Hz, $^3J_{H,H}$ = 6.4 Hz, H-5$_{Pent}$), 1.85 (M, 6 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, D$_2$O) δ 103.0 (C-1'$_{Lact}$), 85.5 (C-1$_{Lact}$), 85.0 (C-1$_{Man}$), 78.7 (C-4$_{Lact}$), 78.3 (C-5$_{Lact}$), 75.9 (C-3$_{Lact}$), 75.4 (C-5'$_{Lact}$), 73.3 (C-3$_{Man}$), 72.6 (C-3'$_{Lact}$), 72.1 (C-2$_{Lact}$), 71.9 (C-2$_{Man}$), 71.2 (C-5$_{Man}$), 71.0 (C-2'$_{Lact}$), 70.0 (C-3$_{Pent}$), 69.6, 69.4 (C-1$_{Pent}$), 68.6 (C-4'$_{Lact}$), 67.1 (C-4$_{Man}$), 61.6 (CH$_2$OH), 61.1 (C-6'$_{Lact}$), 60.9 (C-6$_{Man}$), 60.3 (C-6$_{Lact}$), 45.1 (Cq), 29.3 (C-4$_{PentLact}$), 28.6 (C-4$_{PentMan}$), 27.7 (C-5$_{PentMan}$), 26.8 (C-5$_{PentLact}$); FABMS m/z 1191 [M + Na]$^+$. Anal. Calcd for C$_{44}$H$_{80}$O$_{29}$S$_3$: C, 45.20, H, 6.90. Found: C, 45.21, H, 6.81.

2,2-Bis(5-β-lactosylthio-2-oxapentyl)-2-(5-α-D-mannopyranosylthio-2-oxapentyl)ethanol (MandiLact-OH). Conventional Zemplén deacetylation of 14 (107 mg, 56 µmol) gave **MandiLact-OH**. Yield: 62 mg (95%); R_f = 0.17 (6:3:1 MeCN/H$_2$O/NH$_4$OH); [α]$_D$ = +32.0 (c 1.0 in H$_2$O); ^{1}H NMR (300 MHz, D$_2$O) δ 5.25 (s, 1 H, H-1$_{Man}$), 4.50 (d, 2 H, $J_{1,2}$ = 9.9 Hz, H-1$_{Lact}$), 4.40 (d, 2 H,

$J_{1',2'}$ = 7.57 Hz, H-1'$_{Lact}$), 4.01 (d, 1 H, $J_{2,3}$ = 2.3 Hz, H-2$_{Man}$), 3.93 (dd, 1 H, $J_{3,4}$ = 9.5 Hz, H-3$_{Man}$), 3.91 (bdd, 2 H, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a$_{Lact}$), 3.87 (d, 2 H, $J_{3',4'}$ = 3.3 Hz, H-4'$_{Lact}$), 3.83 (dd, 1 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 2.3 Hz, H-6a$_{Man}$), 3.76 (bd, 2 H, H-6b$_{Lact}$), 3.75 (m, 1 H, H-5$_{Man}$), 3.74 (d, 1 H, H-6b$_{Man}$), 3.73 (d, 2 H, H-6'a$_{Lact}$), 3.70 (d, 2 H, H-6'b$_{Lact}$), 3.67 (dd, 2 H, H-5'$_{Lact}$), 3.62 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4$_{Man}$), 3.61 (dd, 2 H, $J_{3',4'}$ = 3.3 Hz, H-3'$_{Lact}$), 3.60 (t, 2 H, $J_{4,5}$ = 9.6 Hz, H-4$_{Lact}$), 3.59 (t, 2 H, $J_{2,3}$ = 9.6 Hz, H-3$_{Lact}$), 3.56 (s, 2 H, H-3$_{PentMan}$), 3.55 (m, 2 H, H-5$_{Lact}$), 3.55 (m, 4 H, H-3$_{PentLact}$), 3.52 (s, 2 H, CH$_2$OH), 3.49 (dd, 2 H, $J_{2',3'}$ = 10.0 Hz, H-2'$_{Lact}$), 3.41 (s, 2 H, H-1$_{PentMan}$), 3.40 (m, 4 H, H-1$_{PentLac}$), 3.33 (t, 2 H, $J_{2,3}$ = 9.6 Hz, H-2$_{Lact}$), 2.77 (m, 4 H, H-5$_{PentLact}$), 2.70 (m, 2 H, H-5$_{PentMan}$), 1.89 (s, 2 H, H-4$_{PentMan}$), 1.88 (m, 4 H, H-4$_{PentLact}$); ^{13}C NMR (75.5 MHz, D$_2$O) δ 102.9 (C-1'$_{Lact}$), 85.4 (C-1$_{Lact}$), 85.0 (C-1$_{Man}$), 78.7 (C-5$_{Lact}$), 78.2 (C-4$_{Lact}$), 75.8 (C-3$_{Lact}$), 75.4 (C-5'$_{Lact}$), 73.2 (C-3$_{Man}$), 72.6 (C-3'$_{Lact}$), 72.1 (C-2$_{Lact}$), 71.9 (C-2$_{Man}$), 71.2 (C-5$_{Man}$), 71.0 (C-2'$_{Lact}$), 70.0 (C-3$_{Pent}$), 69.4 (C-1$_{Pent}$), 68.6 (C-4'$_{Lact}$), 67.0 (C-4$_{Man}$), 61.5 (CH$_2$OH), 61.0 (C-6'$_{Lact}$), 60.9 (C-6$_{Man}$), 60.3 (C-6$_{Lact}$), 45.1 (C$_q$), 29.2 (C-4$_{PentLact}$), 28.5 (C-4$_{PentMan}$), 26.8 (C-5$_{PentLact}$), 27.7 (C-5$_{PentMan}$); FABMS m/z 1191 [M + Na]$^+$. Anal. Calcd for C$_{44}$H$_{80}$O$_{29}$S$_3$: C, 45.20, H, 6.90. Found: C, 45.21, H, 6.81.

7-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosylthio)-4-oxaheptanol (20). A solution of **19** (0.18 g, 1.53 mmol), **3** (1.2 g, 1.83 mmol), and AIBN (69 mg, 0.42 mmol) in dry dioxane (6 mL), under Ar, was stirred at 75 °C for 3 h. Cyclohexene (1.4 mL) was then added, the solvents were removed under reduced pressure and the residue was purified by column chromatography using 100:1 → 40:1 DCM/MeOH as eluent. Yield: 0.79 g (67%); R_f = 0.15 (1:2 petroleum ether/EtOAc); $[\alpha]_D$ = −123.8 (c 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (d, 1 H, $J_{3',4'}$ = 3.5 Hz, H-4′), 5.19 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3), 5.09 (dd, 1 H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10. Five Hz, H-2′), 4.94 (dd, 1 H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.92 (dd, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.7 Hz, H-2), 4.46 (m, 3 H, H-1, H-1′, H-6a), 4.09 (m, 3 H, H-6b, H-6′a, H-6′b), 3.86 (bt, 1 H, H-5′), 3.77 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 3.73 (t, 2 H, $^3J_{H,H}$ = 5.9 Hz, CH$_2$OH), 3.61 (m, 1 H, H-5), 3.58 (t, 2 H, $^3J_{H,H}$ = 5.9 Hz, H-3$_{Hept}$), 3.50 (m, 2 H, H-5$_{Hept}$), 2.75, 2.69 (2 dt, 2 H, $^2J_{H,H}$ = 13.0 Hz, $^3J_{H,H}$ = 7.2 Hz, H-7$_{Hept}$), 2.14, 2.10, 2.05, 2.04, 2.03, 1.95 (6 s, 21 H, MeCO), 1.85 (m, 2 H, H-6$_{Hept}$), 1.81 (m, 2 H, H-2$_{Hept}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3, 170.1, 170.0, 169.7, 169.1 (CO), 101.0 (C-1′), 83.6 (C-1), 76.7 (C-5), 76.2 (C-4), 73.7 (C-3), 70.9 (C-3′), 70.7 (C-5′), 70.2 (C-2), 69.8 (C-3$_{Hept}$), 69.2 (C-5$_{Hept}$), 69.1 (C-2′), 66.6 (C-4′), 62.2 (C-6), 61.6 (C-1$_{Hept}$), 60.8 (C-6′), 32.0 (C-2$_{Hept}$), 29.8 (C-6$_{Hept}$), 27.1 (C-7$_{Hept}$), 20.8, 20.7, 20.6, 20.5 (MeCO); FABMS m/z 791 [M + Na]$^+$. Anal. Calcd for C$_{32}$H$_{48}$O$_{19}$S: C 49.99, H 6.29, S, 4.17. Found: C 49.83, H 6.18, S, 3.89.

7-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosylthio)-4-oxaheptyl p-Toluenesulfonate (21). To a solution of **20** (0.69 g, 0.9 mmol) in dry DCM (14 mL), tosyl chloride (0.26 g, 1.35 mmol) and DMAP (165 mg, 1.35 mmol) were added. The reaction mixture was stirred at room temperature for 24 h, diluted with DCM (35 mL) and washed with H$_2$O (50 mL). The organic layer was separated, dried (MgSO$_4$) and evaporated, and the residue was purified by column chromatography (1:1 petroleum ether/EtOAc). Yield: 0.65 g (78%); R_f = 0.52 (2:1 petroleum ether/EtOAc); $[\alpha]_D$ = −121.4 (c 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.78, 7.34 (2d, 4 H, Ph), 5.33 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ = 1.0 Hz, H-4′), 5.19 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 5.09 (dd, 1 H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10. Five Hz, H-2′), 4.94 (dd, 1 H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.92 (dd, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2), 4.46 (m, 3 H, H-1, H-1′, H-6a), 4.09 (m, 5 H, H-6b, H-6′a, H-6′b, CH$_2$OTs), 3.86 (bt, 1 H, H-5′), 3.78 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.60 (m, 1 H, H-5), 3.42 (t, 2 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Hept}$), 3.38 (m, 2 H, H-5$_{Hept}$), 2.70, 2.63 (2 dt, 2 H, $^2J_{H,H}$ = 13.0 Hz, $^3J_{H,H}$ = 7.0 Hz, H-7$_{Hept}$), 2.44 (s, 3 H, MePh), 2.14, 2.13, 2.09, 2.08, 2.06, 2.05, 2.04, 2.03, 2.02 1.95 (7 s, 21 H, MeCO), 1.88 (m, 2 H, H-6$_{Hept}$), 1.78 (m, 2 H, H-2$_{Hept}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.6, 169.0 (CO), 144.7, 133.2, 129.84, 127,9 (C−Ar), 101.1 (C-1′), 83.7 (C-1), 76.7 (C-5), 76.2 (C-4), 73.8 (C-3), 71.0 (C-3′), 70.7 (C-5′), 70.4 (C-2), 69.1 (C-2′, C-5$_{Hept}$), 67.6 (C-1$_{Hept}$), 66.6 (C-4′), 66.1 (C-3$_{Hept}$), 62.2 (C-6), 60.8 (C-6′), 29.9 (C-6$_{Hept}$), 29.3 (C-2$_{Hept}$), 27.2 (C-7$_{Hept}$), 21.6 (MePh),

20.8, 20.7, 20.6, 20.5 (MeCO); FABMS m/z 945 [M + Na]$^+$. Anal. Calcd for C$_{39}$H$_{54}$O$_{21}$S$_2$: C 50.75, H 5.90, S, 6.95. Found: C 50.81, H 5.84, S, 6.68.

7-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosylthio)-4-oxaheptyl Azide (22). A mixture of **21** (0.61 g, 0.66 mmol) and NaN$_3$ (130 mg, 2.0 mmol) in dry DMF (8 mL) was vigorously stirred at 80 °C for 2 h. The reaction mixture was concentrated, and the resulting residue was dissolved in DCM (15 mL), washed with H$_2$O (15 mL), dried (MgSO$_4$) and purified by column chromatography (3:2 petroleum ether/EtOAc). Yield: 0.51 g (96%); R_f = 0.24 (1:1 petroleum ether/EtOAc); $[\alpha]_D$ = −10.2 (c 1.0 in DCM); IR (NaCl) ν_{max} 2099 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (bd, 1 H, $J_{3',4'}$ = 3.5 Hz, H-4′), 5.19 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 5.09 (dd, 1 H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10. Five Hz, H-2′), 4.94 (dd, 1 H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.92 (dd, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2), 4.46 (m, 3 H, H-1, H-1′, H-6a), 4.09 (m, 3 H, H-6b, H-6′a, H-6′b), 3.86 (bt, 1 H, H-5′), 3.77 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.60 (m, 1 H, H-5), 3.47 (t, 4 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Hept}$, H-5$_{Hept}$), 3.37 (m, 2 H, CH$_2$N$_3$), 2.71 (m, 2 H, H-7$_{Hept}$), 2.14, 2.10, 2.05, 2.04, 2.03, 1.95 (6s, 21 H, MeCO), 1.83 (m, 4 H, H-2$_{Hept}$, H-6$_{Hept}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.6, 169.0 (CO), 101.0 (C-1′), 83.7 (C-1), 76.7 (C-5), 76.2 (C-4), 73.8 (C-3), 71.0 (C-3′), 70.7 (C-5′), 70.3 (C-2), 69.0 (C-2′, C-5$_{Hept}$), 67.4 (C-3$_{Hept}$), 66.6 (C-4′), 62.2 (C-6), 60.8 (C-6′), 48.44 (C-1$_{Hept}$), 29.9 (C-6$_{Hept}$), 29.1 (C-2$_{Hept}$), 27.2 (C-7$_{Hept}$), 20.8, 20.7, 20.6, 20.5 (MeCO); FABMS m/z 816 [M + Na]$^+$. Anal. Calcd for C$_{32}$H$_{47}$N$_3$O$_{18}$S: C 48.42, H 5.97, N 5.29, S, 4.04. Found: C 48.19, H 5.68, N 5.02, S, 3.76.

7-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosylthio)-4-oxaheptyl Isothiocyanate (23). To a solution of azide **22** (0.46 g, 0.58 mmol) in dioxane (14 mL) were added TPP (166 mg, 0.63 mmol) and CS$_2$ (0.35 mL, 5.76 mmol). The reaction mixture was stirred at room temperature under Ar for 22 h and then concentrated, and the residue was purified by column chromatography using 1:1 petroleum ether/EtOAc as eluent. Yield: 0.34 mg (72%); R_f = 0.20 (1:1 EtOAc/petroleum ether); $[\alpha]_D$ = −12.6 (c 1.0 in DCM); IR (NaCl) ν_{max} 2119 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, H-4′), 5.20 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 5.10 (dd, 1 H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz, H-2′), 4.95 (dd, 1 H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.93 (dd, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2), 4.47 (m, 3 H, H-1, H-1′, H-6a), 4.10 (m, 3 H, H-6b, H-6′a, H-6′b), 3.86 (bt, 1 H, H-5′), 3.78 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.63 (t, 2 H, $^3J_{H,H}$ = 6.0 Hz, CH$_2$NCS), 3.60 (m, 1 H, H-5), 3.51 (t, 2 H, $^3J_{H,H}$ = 6.0 Hz, H-3$_{Hept}$), 3.49 (t, 2 H, $^3J_{H,H}$ = 6.0 Hz, H-5$_{Hept}$), 2.72 (m, 2 H, H-7$_{Hept}$), 2.14, 2.13, 2.09, 2.08, 2.06, 2.05, 2.04, 2.03, 2.02 1.95 (7 s, 21 H, MeCO), 1.93 (m, 2 H, H-2$_{Hept}$), 1.85 (m, 2 H, H-6$_{Hept}$); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.6, 169.0 (CO), 101.1 (C-1′), 83.7 (C-1), 76.7 (C-5), 76.2 (C-4), 73.8 (C-3), 71.0 (C-3′), 70.7 (C-5′), 70.3 (C-2), 69.2, 69.1 (C-2′, C-5$_{Hept}$), 66.8 (C-3$_{Hept}$), 66.6 (C-4′), 62.2 (C-6), 60.8 (C-6′), 42.14 (C-1$_{Hept}$), 30.1 (C-2$_{Hept}$), 29.8 (C-6$_{Hept}$), 27.2 (C-7$_{Hept}$), 20.8, 20.7, 20.6, 20.5 (MeCO); FABMS m/z 832 [M + Na]$^+$. Anal. Calcd for C$_{33}$H$_{47}$NO$_{18}$S$_2$: C 48.94, H 5.85, N 1.73, S 7.92. Found: C 48.63, H 5.65, N 1.45, S, 7.61.

General Procedure for the Preparation of Lactose-Containing CD-Scaffolded Glycoclusters. A solution of **2** (20 mg, 11 μmol) in H$_2$O (1 mL) was adjusted to pH 8−9 with solid NaHCO$_3$ and stirred for 16 h at room temperature. A solution of the corresponding isothiocyanate (115 μmol, 1.5 equiv) in acetone (1 mL) was then added, and the reaction mixture was stirred at room temperature for 24−48 h. Acetone was then evaporated under reduced pressure, the remaining aqueous suspension was freeze-dried, and the solid residue was purified by column chromatography, using MeCN → 10:1 MeCN/H$_2$O as eluent, to give the corresponding hemiacetylated C_7-symmetric adducts. For the notation of atoms in NMR assignments, see Figure S1 in Supporting Information.

Heptakis{6-deoxy-6-{2-{3-{2,2,2-tris[5-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]ethyl}thioureido}-ethyl}thio}cyclomaltoheptaose (28). Reaction time: 48 h. Yield: 86 mg (45%), R_f = 0.53 (10:1:1 MeCN/H$_2$O/NH$_4$OH); $[\alpha]_D$ = +0.1 (c 1.0 in MeOH); ^{1}H NMR (500 MHz, MeOD, 323 K) δ 5.35 (d, 21 H, $J_{3',4'}$ = 3.0 Hz, H-4′$_{Lact}$), 5.20 (t, 21 H, $J_{2,3}$ = $J_{3,4}$ = 8.5 Hz, H-3$_{Lact}$),

5.10 (bd, 21 H, $J_{2',3'}$ = 10.0 Hz, H-3'$_{Lact}$), 5.00 (m, 21 H, H-2'$_{Lact}$), 4.99 (m, 7 H, d, H-1), 4.87 (t, 21 H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2$_{Lact}$), 4.73 (m, 21 H, H-1'$_{Lact}$), 4.72 (bs, 21 H, H-1$_{Lact}$), 4.50 (bd, 21 H, dd, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Lact}$), 4.16 (m, 21 H, H-6b$_{Lact}$), 4.13 (m, 21 H, H-6'a$_{Lact}$), 4.11 (m, 21 H, dd, H-6'b$_{Lact}$), 3.97 (m, 7 H, H-5), 3.89 (m, 21 H, H-4$_{Lact}$), 3.84 (m, 21 H, H-5'$_{Lact}$), 3.82 (m, 7 H, H-3), 3.79 (m, 21 H, H-5$_{Lact}$), 3.71 (m, 14 H, CH$_2$N$_{Cyst}$), 3.52 (m, 42 H, H-3$_{Pent}$), 3.47 (m, 14 H, H-2, H-4), 3.40 (m, 44 H, H-1$_{Pent}$, CH$_2$NH$_{Cyst}$), 3.22 (m, 7 H, H-6a), 2.99 (m, 7 H, H-6b), 2.90 (m, 42 H, CH$_2$S$_{Cyst}$), 2.77 (m, 42 H, H-5$_{Pent}$), 2.11–1.94 (m, 441 H, MeCO), 1.90 (m, 42 H, m, H-4$_{Pent}$); 1D TOCSY (500 MHz, MeOD, 323 K, irradiation at H-2'$_{Lact}$) δ 5.35 (d, 21 H, $J_{3',4'}$ = 3.0 Hz, H-4'$_{Lact}$), 5.10 (bd, 21 H, $J_{2',3'}$ = 10.0 Hz, H-3'$_{Lact}$), 5.00 (m, 21 H, H-2'$_{Lact}$), 4.73 (m, 21 H, H-1'$_{Lact}$), 4.13 (m, 21 H, H-6'a$_{Lact}$), 4.11 (m, 21 H, H-6'b$_{Lact}$), 3.84 (m, 21 H, H-5'$_{Lact}$); 1D-TOCSY (500 MHz, MeOD, 323 K, irradiation at H-3$_{Lact}$) δ = 4.87 (t, 21 H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2$_{Lact}$), 4.72 (bs, 21 H, H-1$_{Lact}$), 4.50 (bd, 21 H, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Lact}$), 4.16 (m, 21 H, H-6b$_{Lact}$), 3.89 (m, 21 H, H-4$_{Lact}$), 3.79 (m, 21 H, H-5$_{Lact}$); ^{13}C NMR (125.7 MHz, MeOD, 323 K) δ 184.0 (CS), 172.0–171.0 (CO), 103.6 (C-1), 101.8 (C-1'$_{Lact}$), 86.0 (C-4), 84.7 (C-1$_{Lact}$), 77.9 (C-5$_{Lact}$), 77.4 (C-4$_{Lact}$), 75.4 (C-3$_{Lact}$), 74.4 (C-3), 74.2 (C-2, C-5), 72.4 (C-3'$_{Lact}$), 72.0 (C-2$_{Lact}$), 71.7 (C-5'$_{Lact}$), 71.0 (C-1$_{Pent}$), 70.8 (C-2'$_{Lact}$, C-3$_{Pent}$), 68.5 (C-4'$_{Lact}$), 63.8 (C-6$_{Lact}$), 62.2 (C-6'$_{Lact}$), 45.7 (C$_q$, CH$_2$N$_{Cyst}$), 40.6 (CH$_2$N$_{Cyst}$), 34.7 (C-6), 33.9 (CH$_2$S$_{Cyst}$), 31.2 (C-4$_{Pent}$), 28.5 (C-5$_{Pent}$), 21.2–19.6 (MeCO). Anal. Calcd for C$_{708}$H$_{1025}$N$_{14}$O$_{406}$S$_{35}$: C, 49.01, H, 5.95, N, 1.13. Found: C, 48.70, H, 5.56, N, 1.00.

Heptakis{6-deoxy-6-{2-{3-{2,2-bis{[5-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]-2-[5-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-2-oxapentyl]}ethyl}thioureido}ethylthio}}cyclomaltoheptaose (30). Reaction time: 16 h. Yield: 119 mg (70%); R_f = 0.57 (10:1:1 MeCN/H$_2$O/NH$_4$OH); [α]$_D$ = +22.5 (c 1.3 in MeOH); ^{1}H NMR (500 MHz, MeOD, 313 K) δ 5.45 (m, 7 H, H-1$_{Man}$), 5.34 (m, 21 H, H-4'$_{Lact}$, H-2$_{Man}$), 5.27 (t, 7 H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4$_{Man}$), 5.10 (m, 21 H, H-3$_{Man}$, H-3$_{Lact}$), 5.09 (bd, 14 H, $J_{2,3}$ = 9.7 Hz, H-3'$_{Lact}$), 4.99 (t, 14 H, $J_{1,2}$ = 8.1 Hz, H-2'$_{Lact}$), 4.85 (t, 14 H, $J_{1,2}$ = 9.8 Hz, H-2$_{Lact}$), 4.82 (m, 7 H, H-1), 4.72 (m, 28 H, H-1'$_{Lact}$, H-1$_{Lact}$), 4.50 (bd, 14 H, dd, $J_{6a,6b}$ = 10.6 Hz, H-6a$_{Lact}$), 4.31 (m, 14 H, H-5$_{Man}$, H-6a$_{Man}$), 4.13 (m, 63 H, H-6b$_{Man}$, H-6'a$_{Lact}$, H-6'b$_{Lact}$, H-6b$_{Lact}$, H-5'$_{Lact}$), 3.98 (m, 7 H, H-5), 3.95 (m, 14 H, H-4$_{Lact}$), 3.80 (m, 21 H, H-3, H-5$_{Lact}$), 3.65 (m, 14 H, CH$_2$N$_{Cyst}$), 3.54 (m, 56 H, H-2, H-4, H-3$_{Pent}$), 3.40 (m, 56 H, H-1$_{Pent}$, CH$_2$NH$_{Cyst}$), 3.22 (m, 7 H, H-6a), 3.01 (m, 7 H, H-6b), 2.89 (m, 14 H, CH$_2$S$_{Cyst}$), 2.78 (m, 42 H, H-5$_{Pent}$), 2.15–2.05 (m, 378 H, MeCO), 1.95 (m, 42 H, m, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, MeOD) δ 183.5 (CS), 172.3–171.2 (CO), 103.8 (C-1), 102.0 (C-1'$_{Lact}$), 84.8 (C-4), 84.2 (C-1$_{Lact}$), 83.6 (C-1$_{Man}$), 77.9 (C-5$_{Lact}$), 77.7 (C-4$_{Lact}$), 75.5 (C-3$_{Lact}$), 74.6 (C-3), 74.5 (C-2, C-5), 72.8 (C-3'$_{Lact}$), 72.5 (C-2$_{Man}$), 71.8 (C-2$_{Lact}$, C-1$_{Pent}$, C-5'$_{Lact}$), 71.2 (C-3$_{Man}$, C-3$_{Pent}$), 70.8 (C-2'$_{Lact}$), 70.1 (C-5$_{Man}$), 68.7 (C-4'$_{Lact}$), 67.7 (C-4$_{Man}$), 63.8 (C-6$_{Lact}$, C-6$_{Man}$), 62.3 (C-6'$_{Lact}$), 45.7 (C$_q$, CH$_2$N$_{Cyst}$), 40.8 (CH$_2$N$_{Cyst}$), 35.0 (C-6), 34.0 (CH$_2$S$_{Cyst}$), 31.2 (C-4$_{PentLact}$), 30.8 (C-4$_{PentMan}$), 29.5 (C-5$_{PentMan}$), 28.7 (C-5$_{PentLact}$), 21.3–20.4 (MeCO). Anal. Calcd for C$_{623}$H$_{910}$N$_{14}$O$_{350}$S$_{35}$: C, 48.85, H, 5.99, N, 1.28. Found: C, 48.81, H, 5.83, N, 1.11.

Heptakis{6-deoxy-6-{2-{3-{2-{[5-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-2-oxapentyl]-2,2-bis[5-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-2-oxapentyl]}ethyl}thioureido}ethylthio}}cyclomaltoheptaose (31). Reaction time: 16 h. Yield: 32 mg (22%); R_f = 0.55 (10:1:1 MeCN/H$_2$O/NH$_4$OH); [α]$_D$ = +39.9 (c 1.0 in MeOH); ^{1}H NMR (500 MHz, MeOD, 323 K) δ 5.37 (m, 14 H, H-1$_{Man}$), 5.34 (d, 7 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ = 0.0 Hz, H-4'$_{Lact}$), 5.32 (bd, 14 H, $J_{2,3}$ = 3.5 Hz, H-2$_{Man}$), 5.25 (t, 14 H, $J_{3,4}$ = $J_{4,5}$ = 10.5 Hz, H-4$_{Man}$), 5.20 (dd, 14 H, H-3$_{Man}$), 5.19 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3$_{Lact}$), 5.09 (dd, 7 H, $J_{2',3'}$ = 10.0 Hz, H-3'$_{Lact}$), 4.99 (dd, 7 H, $J_{1',2'}$ = 7.5 Hz, H-2'$_{Lact}$), 4.87 (t, 7 H, $J_{1,2}$ = 9.0 Hz, H-2$_{Lact}$), 4.69 (d, 7 H, H-1'$_{Lact}$), 4.68 (d, 7 H, H-1$_{Lact}$), 4.48 (bd, 7 H, dd, $J_{6a,6b}$ = 10.9 Hz, H-6a$_{Lact}$), 4.39 (bdd, 14 H, $J_{5,6a}$ = 5.0 Hz, H-5$_{Man}$), 4.28 (bd, 14 H, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Man}$), 4.14 (bd, 7 H, H-6b$_{Man}$), 4.13 (m, 21 H, H-6'a$_{Lact}$, H-6'b$_{Lact}$, H-6b$_{Lact}$), 3.95 (m, 7 H, H-5'$_{Lact}$), 3.85 (m, 7 H, H-4$_{Lact}$), 3.81 (m, 7 H, t, H-3), 3.75 (m, 7 H, H-5$_{Lact}$), 3.72 (m, 14 H, CH$_2$N$_{Cyst}$), 3.54 (m, 56 H, H-2, H-4, H-3$_{Pent}$), 3.41 (m, 56 H, H-1$_{Pent}$, CH$_2$NH$_{Cyst}$), 3.21

(m, 7 H, H-6a), 3.01 (m, 7 H, H-6b), 2.91 (m, 14 H, CH$_2$S$_{Cyst}$), 2.79, 2.76 (2 dt, 42 H, $^2J_{H,H}$ = 13.5 Hz, $^3J_{H,H}$ = 7.0 Hz, H-5$_{Pent}$), 2.15–2.03 (s, 315 H, MeCO), 1.93 (m, 42 H, m, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, MeOD, 325 K) δ 182.2 (CS), 172.3–171.2 (CO), 103.7 (C-1), 102.0 (C-1'$_{Lact}$), 85.8(C-4), 84.8 (C-1$_{Lact}$), 84.0 (C-1$_{Man}$), 78.1 (C-5$_{Lact}$), 77.6 (C-4$_{Lact}$), 75.6 (C-3$_{Lact}$), 74.3 (C-3), 73.7 (C-2, C-5), 72.8 (C-3'$_{Lact}$), 72.6 (C-2$_{Man}$), 72.1 (C-2$_{Lact}$, C-1$_{Pent}$), 71.9 (C-5'$_{Lact}$), 71.2 (C-3$_{Man}$), 71.0 (C-3$_{Pent}$), 70.9 (C-2'$_{Lact}$), 70.6 (C-5$_{Man}$), 68.8 (C-4'$_{Lact}$), 67.8 (C-4$_{Man}$), 63.9 (C-6$_{Lact}$), 63.8 (C-6$_{Man}$), 62.4 (C-6'$_{Lact}$), 47.1 (C$_q$, CH$_2$N$_{Cyst}$), 41.8 (CH$_2$N$_{Cyst}$), 34.7 (C-6), 33.9 (CH$_2$S$_{Cyst}$), 31.4 (C-4$_{PentLact}$), 30.8 (C-4$_{PentMan}$), 29.6 (C-5$_{PentMan}$), 28.2 (C-5$_{PentLact}$), 21.3–20.4 (MeCO). Anal. Calcd for C$_{539}$H$_{798}$N$_{14}$O$_{294}$S$_{35}$: C, 48.67, H, 6.05, N, 1.47. Found: C, 48.40, H, 5.86, N, 1.31.

Heptakis{6-deoxy-6-{2-{N′-[7-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosylthio)-4-oxaheptyl]thioureido}ethylthio}}-cyclomaltoheptaose (32). Reaction time: 48 h. Yield: 26 mg (37%); R_f = 0.45 (10:1:1 MeCN/H$_2$O/NH$_4$OH). The compound was purified by column chromatography using 10:1 MeCN/H$_2$O as eluent. Compound 32 was deprotected without any further characterization.

General Procedure for the Deprotection of Hemiacetylated CD-Scaffolded Glycoclusters. Deacetylation was effected by treatment with 1 N NaOMe in MeOH (0.1 equiv per mol of acetates) at room temperature. After 5 min a white precipitate appeared, which was redissolved by addition of H$_2$O. The solution was stirred for 15 min, neutralized using Amberlite IR-120 (H$^+$) ion-exchange resin, demineralized with Duolite MB-6113 (H$^+$, OH$^-$) ion-exchange resin and freeze-dried to give the fully unprotected conjugates. Analytically pure samples for lectin-binding studies were obtained by gel permeation chromatography (Sephadex G-25, H$_2$O).

Heptakis{6-deoxy-6-{2-{N′-{2,2,2-tris[5-(β-lactosylthio)-2-oxapentyl]ethyl}thioureido}ethylthio}}cyclomaltoheptaose (triLact-CD). Yield: 56 mg (99%); [α]$_D$ = −1.0 (c 1.0 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O, 343 K) δ 5.47 (bs, 7 H, H-1), 4.89 (d, 21 H, $J_{1,2}$ = 9.0 Hz, H-1$_{Lact}$), 4.83 (d, 21 H, $J_{1',2'}$ = 8.5 Hz, H-1'$_{Lact}$), 4.32 (bd, 21 H, $J_{6a,6b}$ = 11.5 Hz, H-6a$_{Lact}$), 4.30 (d, 21 H, $J_{3',4'}$ = 4.5 Hz, H-4'$_{Lact}$), 4.29 (m, 7 H, H-5), 4.18 (bd, 21 H, H-6b$_{Lact}$), 4.15 (m, 42 H, H-6'a$_{Lact}$, H-6'b$_{Lact}$), 4.12 (m, 21 H, H-5'$_{Lact}$), 4.09 (bs, 14 H, CH$_2$N$_{Cyst}$), 4.02 (dd, 21 H, $J_{2',3'}$ = 8.5 Hz, H-3'$_{Lact}$), 4.02 (m, 42 H, H-3$_{Lact}$, H-4$_{Lact}$), 4.05 (m, 7 H, H-3), 3.97 (m, 42 H, H-3$_{Pent}$), 3.95 (m, 21 H, H-5$_{Lact}$), 3.97 (m, 7 H, H-2), 3.95 (m, 56 H, H-1$_{Pent}$, CH$_2$N$_{Cyst}$), 3.94 (t, 21 H, H-2'$_{Lact}$), 3.76 (t, 21 H, $J_{2,3}$ = 9.0 Hz, H-2$_{Lact}$), 3.54 (t, 7 H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 3.51 (m, 7 H, H-6a), 3.32 (m, 7 H, H-6b), 3.25 (m, 14 H, CH$_2$S$_{Cyst}$), 3.19 (m, 42 H, H-5$_{Pent}$), 2.29 (m, 42 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, D$_2$O, 343 K) δ 181.8 (CS), 103.5 (C-1'$_{Lact}$), 102.6 (C-1), 85.8 (C-1$_{Lact}$), 84.8 (C-4), 79.2 (C-4$_{Lact}$), 76.4 (C-5$_{Lact}$), 75.8 (C-5'$_{Lact}$, C-3$_{Lact}$), 73.2 (C-3'$_{Lact}$), 72.9 (C-3), 72.7 (C-2$_{Lact}$), 71.5 (C-2'$_{Lact}$, C-3$_{Pent}$), 70.6 (C-1$_{Pent}$, C-2, C-5), 69.1 (C-4'$_{Lact}$), 61.4 (C-6'$_{Lact}$), 61.1 (C-6$_{Lact}$), 44.9 (C$_q$, CH$_2$N$_{Cyst}$, CH$_2$N$_{Cyst}$), 32.5 (C-6), 31.2 (CH$_2$S$_{Cyst}$), 29.9 (C-4$_{Pent}$), 27.4 (C-5$_{Pent}$); MALDI-TOFMS m/z 11197 [M + K]$^+$. Anal. Calcd for C$_{419}$H$_{731}$N$_{14}$O$_{259}$S$_{35}$: C, 44.51, H, 6.60, N, 1.76. Found: C, 44.63, H, 6.35, N, 1.53.

Heptakis{6-deoxy-6-{2-N′-{2,2-bis[5-(β-lactosylthio)-2-oxapentyl]-2-[5-(α-D-mannosylthio)-2-oxapentyl]ethyl}-thioureido}ethylthio}}cyclomaltoheptaose (MandiLact-CD). Yield: 77 mg (99%); [α]$_D$ = +35.0 (c 0.7 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O, 333 K) δ 5.60 (s, 7 H, H-1$_{Man}$), 5.40 (bs, 7 H, H-1), 4.82 (d, 14 H, $J_{1,2}$ = 8.0 Hz, H-1$_{Lact}$), 4.75 (d, 14 H, $J_{1',2'}$ = 7.5 Hz, H-1'$_{Lact}$), 4.36 (m, 7 H, H-2$_{Man}$), 4.25 (bd, 14 H, $J_{6a,6b}$ = 12.0 Hz, H-6a$_{Lact}$), 4.24 (m, 7 H, H-5$_{Man}$), 4.23 (d, 14 H, $J_{3',4'}$ = 5.0 Hz, H-4'$_{Lact}$), 4.19 (m, 14 H, H-5, H-3), 4.13 (m, 21 H, H-3$_{Man}$. H-6a$_{Man}$, H-6b$_{Man}$), 4.10 (bd, 14 H, H-6b$_{Lact}$), 4.06 (m, 35 H, H-6'a$_{Lact}$, H-6'b$_{Lact}$, H-4$_{Man}$), 4.01 (m, 28 H, H-5'$_{Lact}$, CH$_2$N$_{Cyst}$), 3.97 (m, 7 H, H-2), 3.95 (m, 28 H, H-3$_{Lact}$, H-4$_{Lact}$), 3.94 (dd, 7 H, $J_{2',3'}$ = 10.5 Hz, H-3'$_{Lact}$), 3.89 (m, 42 H, H-3$_{Pent}$), 3.88 (m, 63 H, H-1$_{Pent}$, H-4, CH$_2$N$_{Cyst}$), 3.87 (m, 14 H, H-5$_{Lact}$), 3.85 (t, 7 H, H-2'$_{Lact}$), 3.68 (t, 14 H, $J_{2,3}$ = 9.5 Hz, H-2$_{Lact}$), 3.53 (m, 7 H, H-6a), 3.31 (m, 7 H, H-6b), 3.24 (m, 42 H, CH$_2$S$_{Cyst}$), 3.12 (m, 42 H, H-5$_{Pent}$), 2.24 (m, 14 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, D$_2$O, 333 K) δ 180.1 (CS), 103.5 (C-1'$_{Lact}$), 102.6 (C-1), 85.9 (C-1$_{Lact}$), 85.6 (C-1$_{Man}$), 85.0 (C-4), 79.2 (C-4$_{Lact}$), 76.4 (C-5$_{Lact}$), 75.8 (C-3$_{Lact}$, C-5'$_{Lact}$), 73.8 (C-5$_{Man}$), 73.2 (C-3'$_{Lact}$), 72.8 (C-3, C-2$_{Lact}$), 72.5 (C-2$_{Man}$), 71.9 (C-3$_{Man}$), 71.5 (C-2'$_{Lact}$, C-3$_{Pent}$), 70.7 (C-1$_{Pent}$, C-2, C-5),

69.1 (C-4'$_{Lact}$), 67.6 (C-4$_{Man}$), 61.5 (C-6'$_{Lact}$, C-6$_{Man}$), 61.1 (C-6$_{Lact}$), 45.0 (C$_q$, CH$_2$N$_{Cyst}$, CH$_2$N$_{Cyst}$), 34.0 (C-6), 32.8 (CH$_2$S$_{Cyst}$), 30.1 (C-4$_{PentLact}$), 29.6 (C-4$_{PentMan}$), 28.4 (C-5$_{PentMan}$), 27.5 (C-5$_{PentLact}$); MALDI-TOFMS m/z 10114 [M + H]$^+$. Anal. Calcd for C$_{371}$H$_{660}$N$_{14}$O$_{226}$S$_{33}$: C 44.60, H 6.66, N 1.96. Found: C, 44.11, H, 6.29, N, 1.71.

Heptakis{6-deoxy-6-{2-{N'-{2-[5-(β-lactosylthio)-2-oxapentyl]-2,2-bis[5-(α-D-mannopyranosylthio)-2-oxapentyl]ethyl}-thioureido}ethylthio}}cyclomaltoheptaose (*LactdiMan*-CD).
Yield: 22 mg (99%); [α]$_D$ = +48.3 (*c* 0.6 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O, 343 K) δ 5.66 (s, 14 H, H-1$_{Man}$), 5.46 (bs, 7 H, H-1), 4.88 (d, 7 H, J$_{1,2}$ = 10.0 Hz, H-1$_{Lact}$), 4.82 (d, 7 H, J$_{1',2'}$ = 8.0 Hz, H-1'$_{Lact}$), 4.41 (s, 14 H, H-2$_{Man}$), 4.30 (m, 14 H, H-5$_{Man}$), 4.29 (bd, 7 H, J$_{6a,6b}$ = 11.0 Hz, H-6a$_{Lact}$), 4.28 (d, 7 H, J$_{3',4'}$ = 4.5 Hz, H-4'$_{Lact}$), 4.27 (m, 7 H, H-5), 4.19 (m, 14 H, H-3$_{Man}$), 4.18 (bd, 7 H, H-6b$_{Lact}$), 4.13 (m, 14 H, H-6'a$_{Lact}$, H-6'b$_{Lact}$), 4.12 (s, 28 H, H-6a$_{Man}$, H-6b$_{Man}$), 4.10 (m, 21 H, H-5'$_{Lact}$, CH$_2$N$_{Cyst}$), 4.05 (m, 7 H, H-3), 4.03 (m, 14 H, H-4$_{Man}$), 4.02 (dd, 7 H, J$_{2',3'}$ = 10.5 Hz, H-3'$_{Lact}$), 4.01 (m, 14 H, H-3$_{Lact}$, H-4$_{Lact}$), 3.94 (m, 42 H, H-3$_{Pent}$), 3.93 (m, 7 H, H-5$_{Pent}$), 3.95 (m, 63 H, H-1$_{Pent}$, H-2, CH$_2$N$_{Cyst}$), 3.92 (t, 7 H, H-2'$_{Lact}$), 3.75 (t, 7 H, J$_{3,4}$ = J$_{4,5}$ = 9.5 Hz, H-4), 3.73 (t, 7 H, J$_{2,3}$ = 9.5 Hz, H-2$_{Lact}$), 3.66 (m, 7 H, H-6a), 3.21, 3.18 (2 dt, 42 H, ^{2}J$_{H,H}$ = 13.0 Hz, ^{3}J$_{H,H}$ = 6.0 Hz, H-5$_{Pent}$), 3.38 (m, 7 H, H-6b), 3.32 (m, 42 H, CH$_2$S$_{Cyst}$), 2.30 (m, 42 H, H-4$_{Pent}$); ^{13}C NMR (125.7 MHz, D$_2$O, 343 K) δ 182.1 (CS), 103.6 (C-1'$_{Lact}$), 102.7 (C-1), 86.0 (C-1$_{Lact}$), 85.7 (C-1$_{Man}$), 85.2 (C-4), 79.4 (C-4$_{Lact}$), 76.5 (C-5$_{Lact}$), 75.9 (C-5'$_{Lact}$, C-3$_{Lact}$), 73.8 (C-5$_{Man}$), 73.4 (C-3'$_{Lact}$), 72.6 (C-3, C-2$_{Lact}$), 72.5 (C-2$_{Man}$), 71.9 (C-3$_{Man}$), 71.6 (C-2'$_{Lact}$, C-3$_{Pent}$), 70.8 (C-1$_{Pent}$, C-2, C-5), 69.1 (C-4'$_{Lact}$), 67.6 (C-4$_{Man}$), 61.5 (C-6'$_{Lact}$, C-6$_{Man}$), 61.1 (C-6$_{Lact}$), 44.8 (C$_q$, CH$_2$N$_{Cyst}$), 40.5 (CH$_2$N$_{Cyst}$), 34.0 (C-6), 33.1 (CH$_2$S$_{Cyst}$), 30.2 (C-4$_{PentLact}$), 29.8 (C-4$_{PentMan}$), 28.5 (C-5$_{PentMan}$), 27.6 (C-5$_{PentLact}$); MALDI-TOFMS m/z 8902 [M + Na]$^+$. Anal. Calcd for C$_{329}$H$_{588}$N$_{14}$O$_{189}$S$_{35}$: C 44.47, H 6.67, N 2.20. Found: C 44.55, H 6.43, N 2.02.

Heptakis{6-deoxy-6-{2-{N'-[7-(β-lactosylthio)-4-oxaheptyl]-thioureido}ethylthio}}cyclomaltoheptaose (*Lact*-CD). Reaction time: 48 h. Deacetylation was achieved following the general procedure above-described. Yield: 21 mg (99%); [α]$_D$ = −51.0 (*c* 0.5 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O, 343 K) δ 5.52 (bs, 7 H, H-1), 4.96 (d, 7 H, J$_{1,2}$ = 10.0 Hz, H-1$_{Lact}$), 4.88 (d, 7 H, J$_{1',2'}$ = 8.0 Hz, H-1'$_{Lact}$), 4.37 (bd, 7 H, J$_{6a,6b}$ = 12 Hz, H-6a$_{Lact}$), 4.36 (bs, 7 H, H-4'$_{Lact}$), 4.36−3.90 (m, 2H, H-3, H-4); 4.32 (bs, 7 H, H-5), 4.23 (bd, 7 H, H-6b$_{Lact}$), 4.20 (m, 14 H, H-6'a$_{Lact}$, H-6'b$_{Lact}$), 4.14 (m, 7 H, H-5'$_{Lact}$), 4.12 (bs, 14 H, CH$_2$N$_{Cyst}$), 4.08 (dd, 7 H, J$_{2',3'}$ = 8.5 Hz, J$_{3',4'}$ = 4.5 Hz, H-3'$_{Lact}$), 4.08 (m, 14 H, H-3$_{Lact}$, H-4$_{Lact}$), 4.10 (m, 7 H, H-2), 4.01 (m, 7 H, H-5$_{Lact}$), 3.96 (m, 7 H, H-2'$_{Lact}$), 3.81 (t, 7 H, J$_{2,3}$ = 9.0 Hz, H-2$_{Lact}$), 3.60−3.30 (m, 14 H, H-6a, H-6b), 3.37 (bs, 14 H, CH$_2$S$_{Cyst}$), 3.92 (bs, 14 H, H-1$_{Hept}$), 2.30 (m, 14 H, H-2$_{Hept}$), 4.01 (m, 14 H, H-3$_{Hept}$), 4.02 (m, 14 H, H-5$_{Hept}$), 2.35 (m, 14 H, H-6$_{Hept}$), 3.23 (m, 14 H, H-7$_{Hept}$); ^{13}C NMR (125.7 MHz, D$_2$O, 343 K) δ 181,0 (CS), 103.5 (C-1'$_{Lact}$), 102.6 (C-1), 85.9 (C-1$_{Lact}$), 85.0 (C-4), 79.2 (C-4$_{Lact}$), 79.2 (C-3$_{Lact}$), 76.4 (C-5$_{Lact}$), 75.8 (C-5'$_{Lact}$), 73.2 (C-3'$_{Lact}$), 72.8 (C-2$_{Lact}$), 72.7 (C-3), 71.5 (C-2'$_{Lact}$), 69.8 (C-5$_{Hept}$), 69.6 (C-2, C-5), 69.2 (C-4'$_{Lact}$), 68.7 (C-3$_{Hept}$), 61.5 (C-6'$_{Lact}$), 61.1 (C-6$_{Lact}$), 44.3 (CH$_2$N$_{Cyst}$), 41.8 (C-1$_{Hept}$), 33.9 (C-6), 32.9 (CH$_2$S$_{Cyst}$), 30.1 (C-6$_{Hept}$), 29.0 (C-2$_{Hept}$), 27.3 (C-7$_{Hept}$); MALDI-TOFMS m/z 5219 [M - 2H + 3Na]$^+$. Anal. Calcd for C$_{189}$H$_{336}$N$_{14}$O$_{105}$S$_{21}$: C 44.01, H 6.57, N 3.80, S, 13.05. Found: C 43.68, H 6.20, N 3.46, S, 12.70.

Synthesis of the Reference Lactosylated Polyacrylamide Polymer for ELLA against PNA. The lactosylated polyacrylamide polymer (**S4**) used as reference ligand in the competitive ELLA experiments using PNA was prepared from 2,3,6,2',3',4',6'-hepta-*O*-acetyl-1-(2-aminooethyl)thio-β-lactose (**S1**) by formation of the corresponding acrylamide derivative (**S2**), de-*O*-acetylation (→ **S3**), and final copolymerization with acrylamide. The corresponding synthetic scheme is depicted in the Supporting Information (Scheme S1).

2,3,6,2',3',4',6'-Hepta-*O*-acetyl-1-(2-aminooethyl)thio-β-lactose (S1). A suspension of cysteamine hydrochloride (0.20 g, 1.8 mmol) in dry toluene (3 mL) was sonicated until complete solution under Ar. Lactose octaacetate (0.5 g, 0.74 mmol) and BF$_3$·Et$_2$O (0.46 mL, 4.39 mmol) were then added and the reaction mixture was vigorously stirred at 50 °C for 16 h. Et$_3$N (1.5 mL) was then added, the solvents

were evaporated and the resulting residue was purified by column chromatography using 9:1 DCM/MeOH. Yield: 0.49 g (50%); R_f = 0.55 (9:1 DCM/MeOH); [α]$_D$ = +12.0 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (d, 1 H, J$_{3',4'}$ = 3.3 Hz, H-4'$_{Lact}$), 5.19 (t, 1 H, J$_{2,3}$ = J$_{3,4}$ = 9.2 Hz, H-3$_{Lact}$), 5.09 (dd, 1 H, J$_{2',3'}$ = 10.4 Hz, J$_{1',2'}$ = 7.9 Hz, H-2'$_{Lact}$), 4.95 (dd, 1 H, H-3'$_{Lact}$), 4.92 (t, 1 H, J$_{1,2}$ = 9.2 Hz, H-2$_{Lact}$), 4.57 (dd, 1 H, J$_{6a,6b}$ = 11.0 Hz, J$_{5,6a}$ = 1.8 Hz, H-6a$_{Lact}$), 4.51 (d, 1 H, H-1'$_{Lact}$), 4.49 (d, 1 H, H-1$_{Lact}$), 4.12 (dd, 1 H, J$_{6'a,6'b}$ = 11.1 Hz, J$_{5',6'a}$ = 6.1 Hz, H-6'a$_{Lact}$), 4.08 (dd, 1 H, J$_{5,6b}$ = 4.0 Hz, H-6b$_{Lact}$), 4.07 (dd, 1 H, J$_{5',6'b}$ = 7.4 Hz, H-6'b$_{Lact}$), 3.88 (dd, 1 H, H-5'$_{Lact}$), 3.79 (t, 1 H, J$_{4,5}$ = 9.2 Hz, H-4$_{Lact}$), 3.66 (ddd, 1 H, H-5$_{Lact}$), 2.98 (m, 1 H, NCHa), 2.91 (m, 2 H, NCHb, SCHa), 2.76 (m, 1 H, SCHb), 2.14−1.95 (7 s, 21 H, MeCO); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 172.0−169.0 (CO ester), 101.2 (C-1'$_{Lact}$), 84.0 (C-1$_{Lact}$), 76.9 (C-5$_{Lact}$), 76.1 (C-4$_{Lact}$), 73.8 (C-3$_{Lact}$), 71.0 (C-3'$_{Lact}$), 70.9 (C-5'$_{Lact}$), 70.3 (C-2$_{Lact}$), 69.3 (C-2'$_{Lact}$), 66.68 (C-4'$_{Lact}$), 62.1 (C-6$_{Lact}$), 61.0 (C-6'$_{Lact}$), 41.6 (NCH$_2$), 33.9 (SCH$_2$), 21.1−20.7 (*Me*CO); FABMS m/z 718 (100%, [M + Na]$^+$). Anal. Calcd for C$_{28}$H$_{41}$NO$_{17}$S: C 48.34, H 5.94, N 2.01. Found: C 49.19, H 5.81, N 1.95.

2,3,6,2',3',4',6'-Hepta-*O*-acetyl-1-(2-acrylamidoethyl)thio-β-lactose (S2). A solution of compound **S1** (0.38 g, 0.55 mmol) and triethylmine (0.32 mL, 3.2 mmol, 6 equiv) in DCM (35 mL) was cooled to 0 °C. Acryloyl chloride (57 μL, 1.25 equiv) in DCM (15 mL) was added dropwise while the temperature was allowed to reach room temperature. The reaction mixture was stirred for 30 min. Methanol (2 mL) was then added, and the reaction mixture was stirred at room temperature for a further 1 h. Water (25 mL) was then added, and the organic phase was then washed successively with 0.5 M HCl (2 × 25 mL), saturated aqueous NaHCO$_3$ and water. The dried (MgSO$_4$) organic phase was filtered and evaporated to dryness. The residue was purified by column chromatography using 1:1 EtOAc/DCM. Yield: 0.24 g (60%); R_f = 0.26 (1:1 EtOAc/DCM); [α]$_D$ = +16.0 (*c* 1.0 in DCM); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.29 (t, 1 H, ^{3}J$_{H,H}$ = 5.1 Hz, NH), 6.27 (dd, 1 H, ^{3}J$_{H,H}$ = 10.5 Hz, ^{2}J$_{H,H}$ = 1.5 Hz, =CHa), 6.12 (dd, 1 H, ^{3}J$_{H,H}$ = 17.0 Hz, =CH), 5.62 (dd, 1 H, =CHb), 5.32 (d, 1 H, J$_{3',4'}$ = 3.3 Hz, H-4'$_{Lact}$), 5.18 (t, 1 H, J$_{2,3}$ = J$_{3,4}$ = 9.2 Hz, H-3$_{Lact}$), 5.08 (dd, 1 H, J$_{2',3'}$ = 10.4 Hz, J$_{1',2'}$ = 5.7 Hz, H-2'$_{Lact}$), 4.94 (dd, 1 H, H-3'$_{Lact}$), 4.88 (t, 1 H, J$_{1,2}$ = 9.8 Hz, H-2$_{Lact}$), 4.57 (dd, 1 H, J$_{6a,6b}$ = 12.1 Hz, J$_{5,6a}$ = 1.9 Hz, H-6a$_{Lact}$), 4.48 (d, 1 H, H-1'$_{Lact}$), 4.45 (d, 1 H, H-1$_{Lact}$), 4.11 (dd, 1 H, J$_{6'a,6'b}$ = 11.1 Hz, J$_{5',6'a}$ = 6.3 Hz, H-6'a$_{Lact}$), 4.06 (dd, 1 H, J$_{5',6'b}$ = 7.7 Hz, H-6'b$_{Lact}$), 4.04 (dd, 2 H, J$_{5,6b}$ = 5.1 Hz, H-6b$_{Lact}$), 3.84 (dd, 1 H, H-5'$_{Lact}$), 3.74 (t, 1 H, J$_{3,4}$ = J$_{4,5}$ = 9.5 Hz, H-4$_{Lact}$), 3.60 (m, 2 H, H-5$_{Lact}$, NCHa), 3.43 (m, 1 H, NCHb), 2.89 (m, 1 H, SCHa), 2.77 (m, 1 H, SCHb), 2.13−1.94 (7 s, 21 H, MeCO); ^{13}C NMR (125.7 MHz, CDCl$_3$) δ 170.5−169.0 (CO ester), 165.6 (CO amide), 130.6 (=CH), 126.7 (=CH$_2$), 101.1 (C-1'$_{Lact}$), 83.8 (C-1$_{Lact}$), 76.8 (C-5$_{Lact}$), 75.9 (C-4$_{Lact}$), 73.6 (C-3$_{Lact}$), 71.0 (C-3'$_{Lact}$), 70.9 (C-5'$_{Lact}$), 70.0 (C-2$_{Lact}$), 69.1 (C-2'$_{Lact}$), 66.6 (C-4'$_{Lact}$), 61.7 (C-6$_{Lact}$), 60.7 (C-6'$_{Lact}$), 39.1 (NCH$_2$), 30.8 (SCH$_2$), 20.8−20.5 (*Me*CO); FABMS m/z 772 [M + Na]$^+$. Anal. Calcd for C$_{31}$H$_{43}$NO$_{18}$S: C, 49.66, H, 5.78; N 1.87. Found: C 49.50, H, 5.60, N, 1.76.

1-(2-Acrylamidoethyl)thio-β-lactose (S3). Conventional Zemplén deacetylation of **S2** (122 mg, 0.16 mmol) gave **S3** (84 mg, 99%). R_f = 0.49 (6:3:1 MeCN/H$_2$O/NH$_4$OH); [α]$_D$ = −3.0 (*c* 1.0 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 6.15 (dd, 1 H, ^{3}J$_{H,H}$ = 10.5 Hz, ^{3}J$_{H,H}$ = 17.1 Hz, =CH), 6.06 (dd, 1 H, ^{2}J$_{H,H}$ = 0.9 Hz, ^{3}J$_{H,H}$ = 17.1 Hz, =CHa), 5.64 (dd, 1 H, ^{2}J$_{H,H}$ = 0.9 Hz, ^{3}J$_{H,H}$ = 10.1 Hz, =CHb), 4.45 (d, 1 H, J$_{1',2'}$ = 9.9 Hz, H-1'$_{Lact}$), 4.32 (d, 1 H, J$_{1,2}$ = 7.8 Hz, H-1$_{Lact}$), 3.83 (dd, 1 H, J$_{6a,6b}$ = 12.3 Hz, J$_{5,6a}$ = 1.5 Hz, H-6a$_{Lact}$), 3.79 (d, 1 H, J$_{3',4'}$ = 3.1 Hz, H-4'$_{Lact}$), 3.66 (dd, 1 H, J$_{5,6b}$ = 5.4 Hz, H-6b$_{Lact}$), 3.63 (m, 2 H, H-6'$_{Lact}$), 3.59 (m, 1 H, H-5'$_{Lact}$) 3.54 (m, 3 H, H-3$_{Lact}$, H-3'$_{Lact}$, H-4$_{Lact}$), 3.48 (m, 1 H, H-5$_{Lact}$), 3.41 (m, 3 H, H-2$_{Lact}$, NCH$_2$), 3.23 (t, 1 H, J$_{2,3}$ = J$_{4,5}$ = 9.9 Hz, H-2'$_{Lact}$), 2.85 (dt, 1 H, ^{2}J$_{H,H}$ = 13.3 Hz, ^{3}J$_{H,H}$ = 7.0 Hz, SCHa), 2.75 (dt, 1 H, SCHb); ^{13}C NMR (125.7 MHz, D$_2$O) δ 165.6 (CO), 129.9 (=CH), 127.5 (=CH$_2$), 102.9 (C-1'$_{Lact}$), 85.3 (C-1$_{Lact}$), 78.7 (C-5$_{Lact}$), 78.1 (C-4$_{Lact}$), 75.7 (C-3$_{Lact}$), 75.4 (C-5'$_{Lact}$), 72.5 (C-3'$_{Lact}$), 72.0 (C-2'$_{Lact}$), 71.0 (C-2$_{Lact}$), 68.6 (C-4'$_{Lact}$), 61.0 (C-6'$_{Lact}$), 60.2 (C-6$_{Lact}$), 39.6 (NCH$_2$), 29.2 (SCH$_2$); FABMS m/z 478 [M + Na]$^+$. Anal. Calcd for C$_{17}$H$_{29}$NO$_{11}$S: C, 44.83, H 6.42, N 3.08, S 7.04 . Found: C 44.82, H 6.19, N 2.97, S 6.98.

Poly[acrylamide-*co*-[*N*-[2-(β-lactosylthio)ethyl]acrylamide] (S4). Compound **S3** (15 mg, 33 μmol) and acrylamide (22 mg, 0.31 mmol) were dissolved in deoxygenated H_2O (0.7 mL), and to the solution were added ammonium persulfate (12 μL, 50 mg/mL solution in H_2O) and *N,N,N′N′*-tetramethylethylenediamine (TMEDA, 4 μL, 0.027 mmol, 0.08 equiv). The reaction mixture was stirred at 90 °C for 12 min under nitrogen. The procedure was repeated until complete disappearance of the starting materials (TLC). Then, the reaction mixture was cooled to room temperature, diluted with H_2O (10 mL), and lyophilized. The residue was dissolved in H_2O (20 mL), extensively dialyzed (2 kD cutoff) against pure H_2O (5 × 2 L), and lyophilized to furnish lactose polymer **S4** (19 mg, 52%). ^1H NMR (300 MHz, D_2O) δ 4.45 (bs, 1 H, $J_{1,2}$ = 7.8 Hz, H-1′$_{Lact}$), 4.30 (bd, 1 H, $J_{1,2}$ = 7.8 Hz, H-1$_{Lact}$), 3.84−3.75 (m, 2 H, H-6a$_{Lact}$, H-4′$_{Lact}$), 3.70−3.20 (m, 1 H, H-2$_{Lact}$, H-3$_{Lact}$, H-4$_{Lact}$, H-5$_{Lact}$, H6b$_{Lact}$, H-2′$_{Lact}$, H-3′$_{Lact}$, H-5′$_{Lact}$, H-6′$_{Lact}$, NCH$_2$), 2.75 (m, 2 H, SCH$_2$), 2.20−2.00 (m, ~10 H, CHCO), 1.65−1.35 (m, ~20 H, *CH$_2$*CHCO); ^{13}C NMR (75.5 MHz, D_2O) δ 179.0 (CO), 103.0 (C-1′$_{Lact}$), 85.5 (C-1$_{Lact}$), 78.9 (C-5$_{Lact}$), 78.3 (C-4$_{Lact}$), 76.0 (C-3$_{Lact}$), 75.6 (C-5′$_{Lact}$), 72.7 (C-3′$_{Lact}$), 72.1 (C-2′$_{Lact}$), 71.2 (C-2$_{Lact}$), 68.8 (C-4′$_{Lact}$), 61.3 (C-6′$_{Lact}$), 60.4 (C-6$_{Lact}$), 42.3−41.8 (CHCO, NCH$_2$), 36.1−34.2 (CH$_2$CHCO), 29.5 (SCH$_2$).

Enzyme-Linked Lectin Assays (ELLA). Nunc-Inmuno plates (MaxiSorp) were coated overnight with yeast mannan or poly-[acrylamide-*co*-2-(acrylamido)ethylthio-β-lactoside)] (**S4**) at 100 μL/well diluted from a stock solution of 10 μg/mL in 0.01 M phosphate buffer saline (PBS, pH 7.3 containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}) at room temperature. The wells were then washed three times with 300 μL of washing buffer (containing 0.05% (v/v) Tween 20) (PBST). The washing procedure was repeated after each of the incubations throughout the assay. The wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h at 37 °C. After washing, the wells were filled with 100 μL of serial dilutions of peroxidase labeled lectins (Con A and Peanut agglutinin) from 10^{-1} to 10^{-5} mg/mL in PBS and incubated at 37 °C for 1 h. The plates were washed and 50 μL/well of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (0.25 mg/mL) in citrate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 μL/well of 1 M H_2SO_4 and the absorbances were measured at 415 nm. Blank wells contained citrate-phosphate buffer. The concentration of lectin displaying absorbances between 0.8 and 1.0 was used for inhibition experiments. In order to carry out the inhibition experiments, each inhibitor was added in a serial of 2-fold dilutions (60 μL/well) in PBS with 60 μL of the desired lectin-peroxidase conjugate concentration on Nunclon (Delta) microtiter plates and incubated for 1 h at 37 °C. The above solutions (100 μL) were then transferred to the mannan-coated microplates, which were incubated for 1 h at 37 °C. The plates were washed, and the ABTS substrate was added (50 μL/well). Color development was stopped after 20 min, and the absorbances were measured. The percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \left(A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}\right)$$
$$/A_{(\text{no inhibitor})} \times 100.$$

Results in triplicate were used for plotting the inhibition curves for each individual ELLA experiment. Typically, the IC_{50} values (concentration required for 50% inhibition of the lectin coating polysaccharide association) obtained from several independently performed tests were in the range of ±15%. Nevertheless, the relative inhibition values calculated from independent series of data were highly reproducible.

Two-Site ELLA (Sandwich Assay). For Con A, Nunc-Inmuno plates (MaxiSorp) microtitration plates were coated with yeast mannan and blocked with BSA as above-described. Con A lectin was then added at 100 μL/well from a stock solution of 5 μg/mL in 0.01 M phosphate buffer (PBS, pH 7.3) for 2 h at 37 °C. In the case of PNA, the plates were coated directed with the lectin and then blocked with BSA. The synthesized multivalent glycoclusters were used as stock solutions of

0.25 mM of PBS. The ligands were added in serial 2-fold dilutions (50 μL/well) in PBS and incubated at 37 °C. After 1 h, horseradish peroxidase-labeled Con A or PNA lectin (Con A-HRP or PNA-HRP, 50 μL/well of 200-fold dilution of a 1 mg/mL stock solution in PBS) was added to the microtiter plates, which were incubated for an additional 1 h at 37 °C. The plates were washed with PBS, and 50 μL/well of ABTS (0.25 mg/mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reactions were stopped after 30 min by adding 50 μL/well of 1 M H_2SO_4, and the optical density was measured at 405 nm relative to 570 nm.

Turbidity Assay. To 50 μL of a solution of Con A or PNA lectin in PBS (pH 7.3, containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}) was added the ligand of interest (50 μL of a 250 μM solution in PBS). The time-dependent turbidity kinetics were recorded by measuring the absorption coefficient at 490 nm at intervals of 1 min for 30 min. After 12 min, addition of mannose in the experiments run with Con A or lactose in experiments run with PNA (100 mM, 100 μL) restored the clear solution state. In parallel experiments, lactose (100 mM, 50 μL) and mannose (100 mM, 2 × 50 μL), for experiments run with Con A, or mannose (100 mM, 50 μL) and lactose (100 mM, 2 × 50 μL), for experiments run with PNA, were sequentially added to the mixtures after 12, 18, and 24 min. In all cases the last addition fully restored the clear solution state.

Data Analysis. All experiments were done in quintuplicate (ELLA) or triplicate (two-site ELLA and turbidity assay). Significance testing using Student's *t* test and regression analysis was done using Anova software. Significance was defined with $P < 0.001$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Scheme S1 depicting the reaction sequence for the synthesis of the lactosylated acrylamide polymer used in ELLA, Figure S1 with the notation used for NMR in the case of compound **32**, and Figures S2−S30 reproducing the ^1H and ^{13}C NMR spectra of newly synthesized compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Email: jljb@us.es; juanmab@iiq.csic.es.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) (a) Gabius, H. *Biochim. Biophys. Acta* **2002**, *1572*, 163−164. (b) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357−2364. (c) Murrey, H. E.; Hsieh-Wilson, L. C. *Chem. Rev.* **2008**, *108*, 1708−1731. (d) Collins, B. E.; Paulson, J. C. *Curr. Opin. Chem. Biol.* **2004**, *8*, 617−625.

(2) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578.

(3) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.

(4) For recent comprehensive reviews of several aspects of the *glycoside cluster effect*, see: (a) Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165−393. (b) Lahmann, M. *Top. Curr. Chem.* **2009**, *288*, 17−65. (c) Jayaraman, N. *Chem. Soc. Rev.* **2009**, *38*, 3463−3483. (d) Deniaud, D.; Julienne, K.; Gouin, S. G. *Org. Biomol. Chem.* **2011**, *9*, 966−979.

(5) (a) Gupta, G.; Surolia, A.; Sampathkumar, S. *Omics* **2010**, *14*, 419−436. (b) Gemeiner, P.; Mislovicova, D.; Tkac, J.; Svitel, J.; Paetoprsty, V.; Hrabarova, E.; Kogan, G.; Kozar, T. *Biotechnol. Adv.* **2009**, *27*, 1−15. (c) McNicholas, S.; Rencurosi, A.; Lay, L.; Mazzaglia, A.; Sturiale, L.; Perez, M.; Darcy, R. *Biomacromolecules* **2007**, *8*, 1851−1857.

(d) Kizhakkedathu, J. N.; Creagh, A. L.; Shenoi, R. A.; Rossi, N. A. A.; Brooks, D. E.; Chan, T.; Lam, J.; Dandepally, S. R.; Haynes, C. A. *Biomacromolecules* **2010**, *11*, 2567−2575. (e) Hoai, N.; Sasaki, A.; Sasaki, M.; Kaga, H.; Kakuchi, T.; Satoh, T. *Biomacromolecules* **2011**, *12*, 1891−1899.

(6) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669−672.

(7) Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *96*, 11782−11786.

(8) (a) Thoma, G.; Patton, J. T.; Magnani, J. L.; Ernst, B.; Oehrlein, R.; Duthaler, R. O. *J. Am. Chem. Soc.* **1999**, *121*, 5919−5929. (b) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823−4830. (c) Miyachi, A.; Dohi, H.; Neri, P.; Mori, H.; Uzawa, H.; Seto, Y.; Nishida, Y. *Biomacromolecules* **2009**, *10*, 1846−1853.

(9) (a) Wolfenden, M. L.; Cloninger, M. J. *J. Am. Chem. Soc.* **2005**, *127*, 12168−12169. (b) Wolfenden, M. L.; Cloninger, M. J. *Bioconjugate Chem.* **2006**, *17*, 958−966.

(10) (a) Vico, R. V.; Voskuhl, J.; Ravoo, B. J. *Langmuir* **2011**, *27*, 1391−1397. (b) Voskuhl, J.; Stuart, M. C. A.; Ravoo, B. J. *Chem.—Eur. J.* **2010**, *16*, 2790−2796.

(11) (a) Pourceau, G.; Meyer, A.; Vasseur, J.; Morvan, F. *J. Org. Chem.* **2009**, *74*, 1218−1222. (b) Deguise, I.; Lagnoux, D.; Roy, R. *New J. Chem.* **2007**, *31*, 1321−1331. (c) Katajisto, J.; Karskela, T.; Heinonen, P.; Loennberg, H. *J. Org. Chem.* **2002**, *67*, 7995−8001. (d) Ortega-Muñoz, M.; Perez-Balderas, F.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Eur. J. Org. Chem.* **2009**, 2454−2473.

(12) (a) Dulery, V.; Renaudet, O.; Wilczewski, M.; Van der Heyden, A.; Labbe, P.; Dumy, P. *J. Comb. Chem.* **2008**, *10*, 368−371. (b) Ramström, O.; Lohmann, S.; Bunyapaiboonsri, T.; Lehn, J.-M. *Chem.—Eur. J.* **2004**, *10*, 1711−1715.

(13) Cyclodextrins have been widely used for the construction of homogeneous glycoclusters. For reviews and selected recent examples, see: (a) Ortiz Mellet, C.; Defaye, J.; Garcia Fernandez, J. M. *Chem.—Eur. J.* **2002**, *8*, 1982−1990. (b) Vargas-Berenguel, A.; Ortega-Caballero, F.; Casas-Solvas, J. M. *Mini-Rev. Org. Chem.* **2007**, *4*, 1−14. (c) Bernardes, G. J. L.; Kikkeri, R.; Maglinao, M.; Laurino, P.; Collot, M.; Hong, S. Y.; Lepenies, B.; Seeberger, P. H. *Org. Biomol. Chem.* **2010**, *8*, 4987−4996. (d) Díaz-Moscoso, A.; Guilloteau, N.; Bienvenu, C.; Méndez-Ardoy, A.; Jiménez Blanco, J. L.; Benito, J. M.; Le Gourriérec, L.; Di Giorgio, C.; Vierling, P.; Defaye, J.; Ortiz Mellet, C.; García Fernández, J. M. *Biomaterials* **2011**, *32*, 7263−7273. (e) Grünstein, D.; Maglinao, M.; Kikkeri, R.; Collot, M.; Barylyuk, K.; Lepenies, B.; Kamena, F.; Zenobi, R.; Seeberger, P. H. *J. Am. Chem. Soc.* **2011**, *133*, 13957−13966.

(14) Gomez-Garcia, M.; Benito, J. M.; Rodriguez-Lucena, D.; Yu, J.-X.; Chmurski, K.; Ortiz Mellet, C.; Gutierrez Gallego, R.; Maestre, A.; Defaye, J.; Garcia Fernandez, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 7970−7971.

(15) The term *herocluster effect* refers to the increase in the intrinsic binding affinity of a carbohydrate ligand towards a lectin in the presence of a second sugar that itself is not a ligand for this lectin (intrinsic affinity means in a per-ligand basis, keeping the same overall topology and preventing contributions to binding from aggregation phenomena).

(16) Gomez-Garcia, M.; Benito, J. M.; Gutierrez-Gallego, R.; Maestre, A.; Ortiz Mellet, C.; Garcia Fernandez, J. M.; Jimenez Blanco, J. L. *Org. Biomol. Chem.* **2010**, *8*, 1849−1860.

(17) (a) *Glyscosciences—Status and Perspectives*; Gabius, H.-J., Gabius, S., Eds.; Chapman & Hall: Weinheim, 1997. (b) Goldstein, I. J.; Poretz, R. D. In *The Lectins—Properties, Functions and Applications in Biology and Medicine*; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic Press: Orlando, 1986; pp 33−243.

(18) (a) Dondoni, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 8995−8997. (b) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540−1573.

(19) Jimenez Blanco, J. L.; Ortega-Caballero, F.; Ortiz Mellet, C.; Garcia Fernandez, J. M. *Beilstein J. Org. Chem.* **2010**, *6*, 20.

(20) For recent examples on the use of the thiourea-forming reaction for multiconjugation of cyclodextrin platforms to different functional elements, see : (a) Méndez-Ardoy, A.; Guilloteau, N.; Di Giorgio, C.; Vierling, P.; Santoyo-González, F.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2011**, *76*, 5882−5894. (b) Ortiz Mellet, C.; Benito, J. M.; García Fernández, J. M. *Chem. Soc. Rev.* **2011**, *40*, 1586−1680. (c) Ortiz Mellet, C.; Benito, J. M.; García Fernández, J. M. *Chem.—Eur. J.* **2010**, *16*, 6728−6742. (d) Díaz-Moscoso, A.; Vercauteren, D.; Rejman, J.; Benito, J. M.; Ortiz Mellet, C.; De Smedt, S. C.; García Fernández, J. M. *J. Controlled Release* **2010**, *143*, 318−325.

(21) Lubineau, A.; Malleron, A.; Le Narvor, C. *Tetrahedron Lett.* **2000**, *41*, 8887−8891.

(22) Defaye, J.; Ortiz Mellet, C.; Garcia Fernandez, J. M.; Gómez-García, M.; Chmurski, K.; Yu, J. X. WO2004/087768, 2004.

(23) Fulton, D. A.; Stoddart, J. F. *J. Org. Chem.* **2001**, *66*, 8309−8319.

(24) Matta, K. L.; Girotra, R. N.; Barlow, J. J. *Carbohydr. Res.* **1975**, *43*, 101−109.

(25) Garcia-Moreno, M. I.; Diaz-Perez, P.; Benito, J. M.; Ortiz Mellet, C.; Defaye, J.; Garcia Fernandez, J. M. *Carbohydr. Res.* **2002**, *337*, 2329−2334.

(26) Dufour, M.; Gramain, J. C.; Husson, H. P.; Sinibaldi, M. E.; Troin, Y. *Synth. Commun.* **1992**, *22*, 189−200.

(27) Ortiz-Mellet, C.; Benito, J. M.; Garcia Fernandez, J. M.; Law, H.; Chmurski, K.; Defaye, J.; O'Sullivan, M. L.; Caro, H. N. *Chem.—Eur. J.* **1998**, *4*, 2523−2531.

(28) (a) Corbell, J. B.; Lundquist, J. J.; Toone, E. J. *Tetrahedron: Asymmetry* **2000**, *11*, 95−111. (b) Gouin, S. G.; Vanquelef, E.; Garcia Fernandez, J. M.; Ortiz Mellet, C.; Dupradeau, F.; Kovensky, J. *J. Org. Chem.* **2007**, *72*, 9032−9045.

(29) Gouin, S. G.; Garcia Fernandez, J. M.; Vanquelef, E.; Dupradeau, F.; Salomonsson, E.; Leffler, H.; Ortega-Muñoz, M.; Nilsson, U. J.; Kovensky, J. *ChemBioChem* **2010**, *11*, 1430−1442.

(30) Naismith, J. H.; Emmerich, C.; Habash, J.; Harrop, S. J.; Helliwell, J. R.; Hunter, W. N.; Raftery, J.; Kalb, A. J.; Yariv, J. *Acta Crystallogr., Sect. D* **1994**, *50*, 847−858.

(31) (a) Banerjee, R.; Das, K.; Raviashankar, R.; Suguna, K.; Surolia, A.; Vijayan, M. *J. Mol. Biol.* **1996**, *259*, 281−296. (b) Ravishankar, R.; Suguna, K.; Surolia, A.; Vijayan, M. *Acta Crystallogr., Sect. D* **1999**, *55*, 1375−1382.

(32) Baussanne, I.; Benito, J. M.; Ortiz Mellet, C.; Garcia Fernandez, J. M.; Defaye, J. *ChemBioChem* **2001**, *2*, 777−783.

(33) (a) Benito, J. M.; Gomez-Garcia, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; Garcia Fernandez, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355−10363. (b) Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen, T. M.; Spencer, N.; Stoddart, J. F.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429−3437.

(34) Lotan, R.; Skutelsky, E.; Danon, D.; Sharon, N. *J. Biol. Chem.* **1975**, *250*, 8518−8523.

(35) Ravishankar, R.; Thomas, C. J.; Suguna, K.; Surolia, A.; Vijayan, M. *Proteins* **2001**, *43*, 260−270.

(36) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922−14933.

(37) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794.

(38) Lindhorst, T. K.; Bruegge, K.; Fuchs, A.; Sperling, O. *Beilstein J. Org. Chem.* **2010**, *6*, 801−809.

(39) Williams, T. J.; Homer, L. D.; Shafer, J. A.; Goldstein, I. J.; Garegg, P. J.; Hultberg, H.; Iversen, T.; Johansson, R. *Archiv. Biochem. Biophys.* **1981**, *209*, 555−564.

(40) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615−1619.

(41) Guiard, J.; Fiege, B.; Kitov, P. I.; Peters, T.; Bundle, D. R. *Chem.—Eur. J.* **2011**, *17*, 7438−7441.