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EXPEDITIOUS SYNTHESIS OF C-GLYCOSYL BARBITURATE LIGANDS OF BACTERIAL LECTINS: FROM MONOMER DESIGN TO GLYCOCLUSTERS AND GLYCOPOLYMERS

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ABSTRACT

The approach developed here offers a straightforward and efficient access to β -C-glycosyl barbiturates ligands, spanning from glycomimetics to multivalent C-neoglycoconjugates, with the aim of deciphering structural parameters impacting the binding to pathogenic lectins. We reinvestigated the Knoevenagel condensation of barbituric acid on protecting-group free carbohydrates and successfully designed sodium and 5,5-disubstituted *N,N*-dimethyl barbiturate forms of D-galactose, L-fucose, melibiose, 2'-fucosyllactose, maltose and evaluated their binding affinity by isothermal titration calorimetry with LecA (galactose-binding lectin) and LecB (fucose-binding lectin) from *Pseudomonas aeruginosa* and RSL (fucose-binding lectin) from *Ralstonia solanacearum*. The barbiturate ring was shown detrimental for binding to LecA (K_D in mM range) and even more to LecB (non-interaction) while RSL is much more tolerant especially in presence of an aromatic group (K_D in μ M range). However, distancing the barbiturate ring from the recognition carbohydrate residue by using oligosaccharides increased affinity up to low micromolar range. Extension of our convenient synthetic approach led in two-steps to melibiose-based C-glycosyl barbiturates clusters and C-glycosyl barbiturate glycopolymers exhibiting a dramatic enhancement of binding avidity for LecA.

INTRODUCTION

It is now well established that carbohydrates and glycoconjugates mediate important biological processes, such as cell–cell communication and pathogen infection, through recognition events with a wide range of carbohydrate-binding biomolecules (proteins, carbohydrates and nucleic acids).¹ As a result, considerable progress has been made in method development of carbohydrate-based diagnostic and/or therapeutic agents. For instance, the design of glycomimetics of higher affinity was developed as synthetic ligands to compete with carbohydrate-binding receptors present on pathogenic micro-organisms (bacteria, viruses).^{2,3} The shortest access to glycomimetics or (neo)glycoconjugates requires chemoselective ligations to the anomeric position of carbohydrates which display an aldehyde function under its opening form.^{4,5} In carbohydrate chemistry, the aldehyde-condensation reaction is traditionally performed by amination with primary amines or with more reactive α -nucleophiles such as oxyamine or hydrazine derivatives. These last condensation reactions were preferred because of the efficiency, chemo- and stereo-selectivity and tolerance to various solvent including water. Accordingly, we have exploited the modified Kochetkov amination⁶ or reductive amination⁷ to prepare clickable neoglycoconjugates useful for the preparation of self-assembled glyco-nanostructures.⁸ Interestingly, the Knoevenagel condensation using β -diketones is another very convenient method that leads to β -C-glycosides in one step directly from unprotected sugar.⁹ However, it has been less developed in spite of its chemical and enzymatic stability and ring integrity of the terminal reducing sugar.^{10,11} The major breakthrough in Knoevenagel condensation with unprotected carbohydrates has been achieved first when Galbis-Perez et al.¹² reported, in the mid-1980s, the direct reaction in neutral aqueous media with barbituric acids, a cyclic β -diketone, resulting in β -C-glycosyl barbiturates. Later, Lubineau et al.¹³ demonstrated the efficient use of acetylacetone, a non-cyclic β -diketone, for obtention of mostly β -C-glycosidic ketones.¹⁴ More specifically, the condensation of commercially available barbituric acids with unprotected sugars have

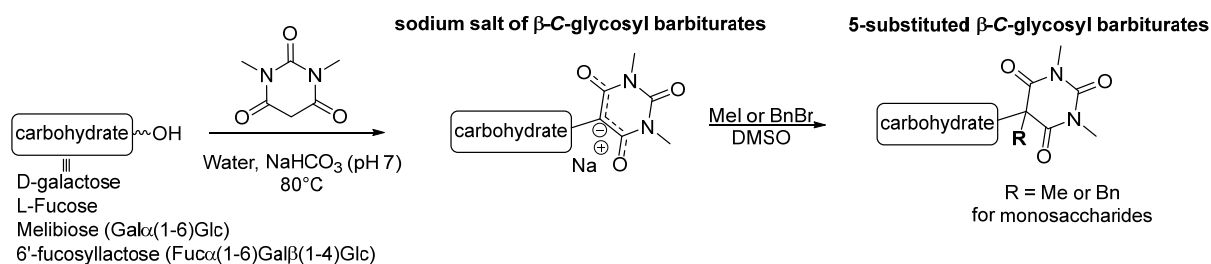
been successfully applied on monosaccharides (hexoses and pentoses)¹⁵ and, more recently on disaccharides¹⁶ to give anionic β -C-glycosyl barbiturates where the negative charge is delocalized among the C-4, C-5 and C-6 carbons of the barbituric ring. Finally, Clarkson et al. benefited from the negative charge to react with various halogenated compounds in order to obtain polymerizable monomers^{17,18} or C-glycosyl lipids.¹⁶

In the light of the convenient and straightforward access to neoglycoconjugates mediated by Knoevenagel condensation with barbituric acids, there is a significant need to gain insight into the determinant structural parameters of β -C-glycosyl barbiturates interacting with lectins from opportunistic pathogens such as LecA (galactose-binding lectin) and LecB (fucose-binding lectin) from *Pseudomonas aeruginosa*¹⁹ and RSL (fucose-binding lectin) from *Ralstonia solanacearum*.²⁰ These lectins are involved in bacterial adhesion to host cells. Specifically, *P. aeruginosa* is an opportunistic pathogen implicated in a large number of opportunistic infections in immunocompromised patients and lethal lung infections in cystic fibrosis patients. *R. solanacearum* is a widely distributed plant pathogen of major economic importance since it leads to lethal wilt in many agricultural crops. Therefore, inhibition of bacterial adhesion to host cells by synthesizing glycomimetics with high and selective affinities for targeted lectins is a promising approach for the development of preventive or therapeutic anti-infectives.^{21,22} For such purpose, we designed a series of specific β -C-glycosyl barbiturates ligands with excellent yields. Next, we established a straightforward and convenient synthetic route to prepare and study the multivalent interactions of a C-glycosyl barbiturates cluster and C-glycosyl barbiturate glycopolymers with LecA.

RESULTS AND DISCUSSION

Synthesis of C-glycosyl barbiturates

The main objective of this work was to evaluate the impact of the structural characteristics of the barbiturate ring linked to carbohydrates on the binding affinities with corresponding lectins. For such purpose, we decided to analyze multiple parameters such as the influence of the negative charge localized along the barbiturate ring, the removal of the charge by reacting it with methyl iodide or benzyl bromide and finally the distance between the charged barbiturate and the recognition carbohydrate module by using oligosaccharides. Thus, a variety of β -C-glycosyl barbiturates were synthesized by Knoevenagel condensation using commercially available *N,N*-dimethylbarbituric acid and protecting-group free carbohydrates in hot water (80 °C) at neutral pH (Scheme 1). Since LecA is a galactose-binding lectin and LecB and RSL are fucose-binding lectins, we selected galactose-based ligands such as D-galactose (entries 1-3; Table 1) and melibiose (entry 7; Table 1) and fucose-based ligands such as L-fucose (entries 4-6; Table 1) and 2'-fucosyllactose (entry 8; Table 1) as starting material, respectively. A glucose-based ligand, *i.e.* maltose (entry 9; Table 1), was synthesized as negative control for probing nonspecific interactions with targeted lectins.

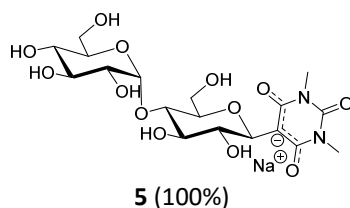


Scheme 1 Synthesis of sodium salt and 5-substituted β -C-glycosyl barbiturates

The Knoevenagel condensation was remarkably efficient (Table 1) and led exclusively to β -C-glycosyl barbiturates (**1-5**) where the β -configuration and the pyran ring were confirmed by ^1H NMR with a strong coupling constant of the anomeric proton ($J_{1,2} = 10$ Hz) (Figures S1, S15, S19, S35 and S40; SI). In most situations, quantitative transformations were observed by T.L.C. and purification were performed with an automatic flash chromatography system affording the product in high purity and excellent isolated yields (70 -100 %).

Table 1 Structures of β -C-glycosyl barbiturate derivatives

Entry	Carbohydrate	β -C-glycosyl barbiturate (Yield %)	5-substituted derivatives (Yield %)
1	D-Galactose	 1 (95%)	
2			 1a (70%)
3			 1b (90%)
4	L-Fucose	 2 (83%)	
5			 2a (90%)
6			 2b (88%)
7	Melibiose	 3 (89%)	
8	2'-Fucosyllactose	 4 (88%)	



The sodium salts of 1,3-dimethyl-(β -C-glycopyranosyl)barbituric acid **1** and **2** were subsequently alkylated with methyl iodide and benzyl bromide in DMSO to afford **1a** and **1b**, respectively, for D-galactose and **2a** and **2b**, respectively, for L-fucose. The methyl group was selected because of its limited steric hindrance and the benzyl group because of the potential favorable π -stacking interactions with the lectin active site. The ^{13}C NMR chemical shift for C-5 of the barbiturate ring (ca. 55-64 ppm) confirmed the formation of 5,5-disubstituted barbiturates (Figure S5, S11, S25, S30 and S47; SI), as opposed to a value of ca. 87 ppm for 5-monosubstituted barbiturates (Figure S2, S16, S20, S36 and S41; SI) where it would form part of an enolic system.¹⁵

Carbohydrate-Lectin recognition studies

In order to assess the structural requirements for efficient binding of monovalent C-glycosylbarbiturates to specific lectins (**1**, **1a**, **1b** and **3** for LecA and **2**, **2a**, **2b** and **4** for LecB and RSL), determination of affinity constant and thermodynamic contributions were performed using titration microcalorimetry (Table 2). (Figures S53-S69; SI)

Table 2 Isothermal titration microcalorimetry data for monovalent β -C-glycosylbarbiturate derivatives binding to bacterial lectins LecA, LecB and RSL. Standard deviation is calculated over 2 or 3 experiments for the three fitted parameters.

Lectin	Ligand	n^a	$-\Delta H$ [kJ mol ⁻¹]	$T\Delta S$ [kJ mol ⁻¹]	$-\Delta G$ [kJ mol ⁻¹]	K_D [μM]
LecA	β -D-GalMe ^c	1 ^b	19.0	5.3	24.3	55.7
	Melibiose ^d	1 ^b	35.9	-10.9	25.0	38.8
	1 β -D-GalBarb	0.8 ^b	5.50 \pm 0.36	7.2	12.7	5900 \pm 800
	1a β -D-GalBarbMe	0.8 ^b	17.2 \pm 0.1	-5.4	11.8	8623 \pm 149
	1b β -D-GalBarbBn	0.8 ^b	22.8 \pm 0.1	-7.9	14.9	2482 \pm 31
	3 MeliBarb	0.92 \pm 0.04	29.5 \pm 0.4	-5.7	23.8	66.3 \pm 2.6
LecB	6 MeliBarb2	0.41 \pm 0.03	55 \pm 2.0	-18.9 \pm 2.1	36.1	0.48 \pm 0.001
	α -L-FucMe ^e	0.77	41.3	-4.9	36.4	0.43
	2'-Fucosyllactose ^f	0.73 \pm 0.02	41.1 \pm 0.1	-6.0 \pm 0.2	35.1	0.70 \pm 0.03
	2 β -L-FucBarb					- ^f
	2a β -L-FucBarbMe					-
	2b β -L-FucBarbBn					-
RSL	4 2'-FucLacBarb	0.94 \pm 0.01	32.5 \pm 0.1	1.4	33.9	1.13 \pm 0.12
	α -L-FucMe ^g	1.98	42.1 \pm 1.3	-7.1	35.0	0.73 \pm 0.03
	2'-Fucosyllactose	1.66 \pm 0.02	51.4 \pm 0.4	-17.5	33.9	1.17 \pm 0.08
	2 β -L-FucBarb	1.04 \pm 0.03	25.6 \pm 0.4	-4.5	21.1	197 \pm 13
	2a β -L-FucBarbMe	0.77 \pm 0.02	37.0 \pm 1.5	-16.4	20.6	252 \pm 31
	2b β -L-FucBarbBn	0.88 \pm 0.02	24.9 \pm 1.5	4.3	29.2	7.6 \pm 1.4
	4 2'-FucLacBarb	1.66 \pm 0.02	31.1 \pm 1.1	-1.9	29.2	11.8 \pm 0.5

^a Stoichiometry, n = number of occupied lectin binding site per ligand. ^b Value fixed during the fitting procedure ^c Data from the literature.²³ ^d Data from the literature.²⁴ ^e Data from the literature.²⁵ ^f The β -C-fucosylbarbiturate derivatives did not show any measurable affinity in ITC (Figures S59-S61; SI). ^g Data from the literature.²⁰

From the ITC data presented in the Table 2, comparison can be made between barbiturate derivatives and methyl derivatives on monosaccharides. The barbiturate ring whether under its anionic or neutral

(methylated or benzylated) form is detrimental for the binding to *Pseudomonas* lectins LecA and LecB, (K_D in millimolar range for LecA, no interaction with LecB). RSL is less affected with a K_D around 200 μM , still losing two order of magnitude when compared to methylated fucose.

In order to rationalize the observed effect of barbiturate substitution on monosaccharide affinity, compounds **1** and **2** have been manually docked in the binding sites of corresponding lectins, in agreement with known location of glycan ligand (Figure 1). Compound **2** conformations was taken from crystal structure of 5- β -D-galactopyranosyl-1,3-dimethylbarbituric acid.²⁶ The structure of compound **2** was built from compound **1** by substituting the D-galactose ring by a L-fucose one.

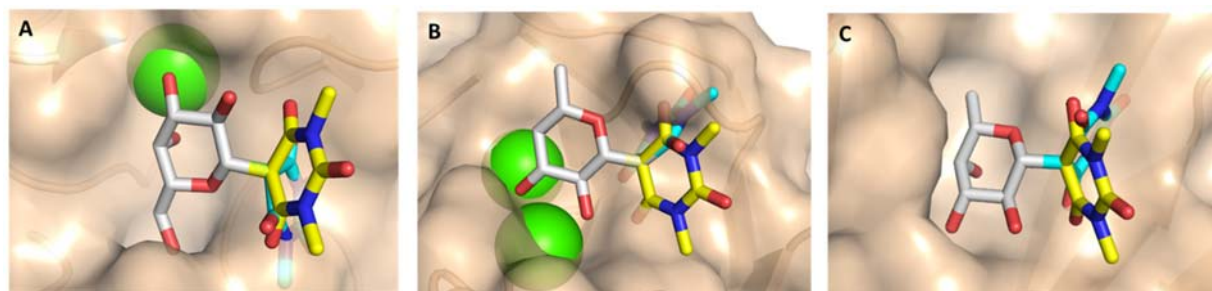


Figure 1. Manual docking of compound **1** and **2** in lectins binding sites, with two conformations for the barbiturate ring, indicated in cyan and yellow. The protein surface is colored in beige and calcium ions are represented as green spheres. A: LecA (Pdb code 1OKO) complex with compound **1**. B and C: LecB (code 1GZT) and RSL (code 1BT9) complexes with compound **2**.

Two different conformations were generated for exploring the flexibility of the barbiturate around the carbon-carbon aglycone bound. In all cases, the conformation observed in the crystal structure²⁶ generates steric conflict with the protein surface, although in a limited way for the RSL complex that presents a flat area close to the fucose binding site. Testing an alternate orientation of the barbiturate could relieve the steric conflicts for LecA and RSL, but not for LecB. This structural analysis is in agreement with the binding data, confirming that LecB cannot accommodate a barbiturate ring on the fucose, while LecA, and in a larger extension RSL can bind to it, albeit with some conformational rearrangement.

When a benzyl group is attached to the barbiturate (compound **1b**) the affinity was significantly enhanced for LecA. Indeed, it was already reported that phenyl aglycon in galactoside have stronger affinity²³ and structural data demonstrated the role of an intermolecular CH- π T-shape interaction between C(ϵ 1)-H of residue His50 in LecA and the aromatic ring of the galactoside aglycone.²⁷ Similarly, a strong binding is obtained for **2b** (K_D of 7.3 μM) with RSL, representing a 28-fold increase compared to **2** and **2a**. Interestingly, **2b** appeared to be an excellent discriminating ligand, strongly preferring the fucophilic lectin RSL rather than LecB. Such fine specificity with a bulky aglycon derivative was previously observed with a conformationally constrained fucoside.²⁸

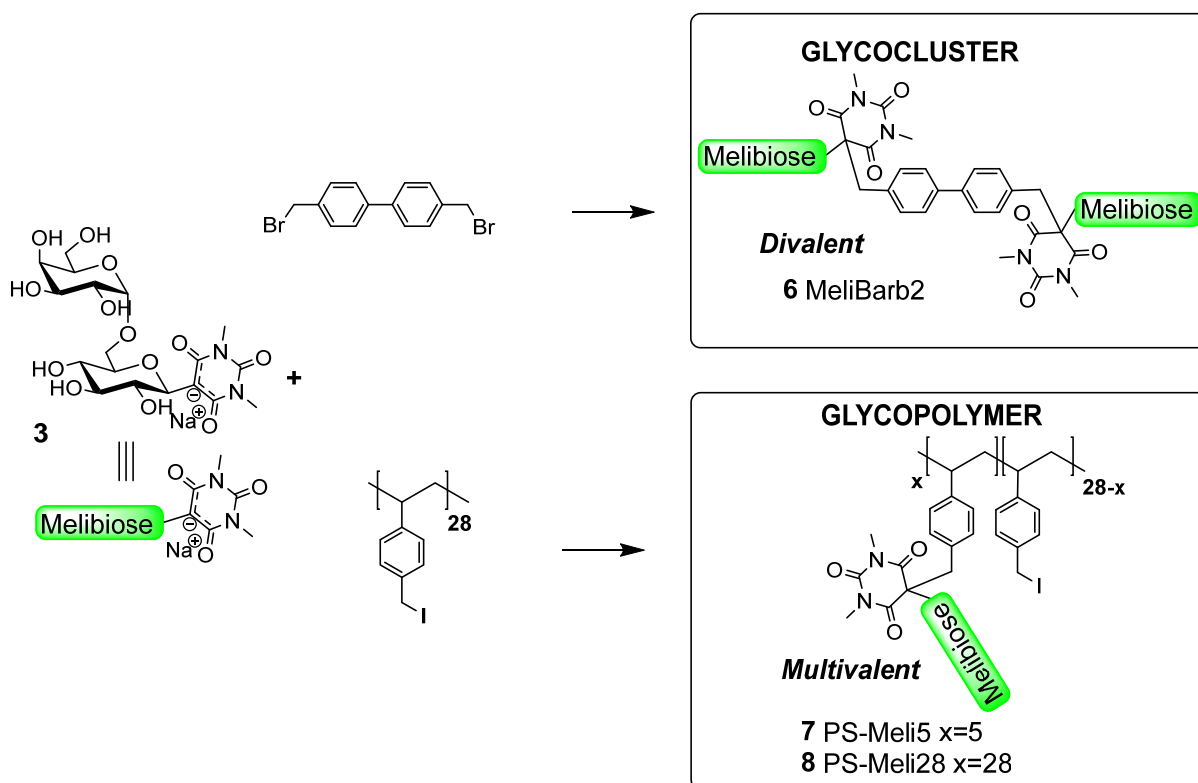
In order to avoid the steric hindrance, the barbiturate ring was distanced from the terminal residue α -D-galactosyl or α -L-fucosyl by using oligosaccharide spacers. Such strategy drastically increased the binding affinity for all studied lectins (K_D **3**/LecA = 66.2 μM , K_D **4**/LecB = 1.3 μM , K_D **4**/RSL = 11.8 μM); iv) with affinity in the same range as the previously published data for corresponding free oligosaccharides (Table 2).

The analysis of the thermodynamic contributions demonstrated some unusual effect of the barbiturate ring. All lectin-ligand interactions in Table 2 are enthalpy-driven as usually observed for carbohydrate-lectin interactions²⁹ with a strong negative enthalpy due to occurrence of many hydrogen bonds.

However, in presence of the barbiturate, the entropy term is not always unfavorable, demonstrating that limitation of the conformational flexibility is a strategy of interest for enhancing affinity. Finally, no interactions were observed between the three lectins and β -C-maltosyl barbiturate derivative **5**, thereby confirming the absence of nonspecific interactions.

Synthesis of glycoclusters and glycopolymers with binding assays

Capitalizing on structural parameters of β -C-glycosyl barbiturates promoting the interactions with studied lectins, we envisioned increasing the potency of our glycomimetics through a rapid and original access to multivalent glycoclusters and glycopolymers according to a two-step procedure. We developed the strategy with β -C-melibiosyl barbiturate derivative which was demonstrated in our study as a potent inhibitor of LecA. As previously, the first step consisted in synthesizing the 1,3-dimethyl-(β -C-melibiosyl)barbituric acid sodium salt **3** and the second step involved the grafting onto various halogenated benzyl scaffolds *via* an alkylation of the barbiturate ring (Scheme 2). We were delighted to find that our two-step process proceeded well with commercially available 4,4'-bis(bromomethyl)-1,1'-biphenyl and poly(4-iodomethylstyrene).



Scheme 2 Synthesis of melibiose-based glycocluster **6** and glycopolymers **7-8**.

Divalent C-galactosylbarbiturate **6** (MeliBarb2) was prepared by coupling 1,3-dimethyl-(β -C-melibiosyl)barbituric acid sodium salt with 4,4'-bis(bromomethyl)-1,1'-biphenyl in DMSO This glycocluster was once again fully characterized by 1D and 2D NMR, IR spectroscopy and further confirmed by mass spectrometry.

The thermodynamic parameters determining the binding mode of the divalent ligand were investigated by ITC and compared to the monovalent reference MeliBarb **3** (Table 2). While the monovalent ligand **3** displayed a K_D value of 66.2 μ M, the divalent one **6** showed a K_D value in the sub-micromolar range ($K_D = 0.41 \mu$ M), resulting in a significant relative potency of 69 and highlighting the remarkable multivalent effect (Figure S57; SI). The stoichiometry (n) of 0.41 (Table 2) for the complex

generated between the lectin LecA and the glycocluster **6** indicates that all the carbohydrate epitopes are engaged. This result demonstrates that even if the molecular design of the linker has not been optimized, we were able to reach, in a rapid and convenient way, a potent lead glycocluster from a protecting- group free carbohydrate.

Similarly, the multivalent C-glycosylbarbiturate were generated from a polymer backbone bearing 4-iodomethylstyrene, which have been substituted to append the C-melibiosylbarbiturate epitopes. The sodium salt C-melibiosylbarbiturate **3** was conjugated along the polystyrene chain to afford a partial or full substitution that was controlled by the stoichiometry ratio. With 1 or 2 equivalents of **3** per 4-iodomethylstyrene monomer, the ¹H NMR spectrometry revealed 18% or 100% of substitution, respectively, which corresponded to 5 or 28 C-melibiosylbarbiturates per polymer chain (Figure S51; SI).

The size of amphiphilic glycopolymers in water were first assessed by dynamic light scattering (DLS) and revealed hydrodynamic diameters of 290 and 16 nm for the partially substituted **7** that corresponds to micellar aggregates and 24 nm for the fully substituted **8** that fits with monodisperse micelles consisting of hydrophobic polystyrene as the micelle core and hydrophilic melibiose as the shell (Figure S52; SI).

Because of poor solubility of **7** in buffer and aggregation phenomena the binding affinity of the glycopolymers **7** and **8** to LecA was evaluated in a hemagglutination assay that measured the inhibition of LecA-induced agglutination of rabbit erythrocytes in comparison to melibiose used as the reference to calculate the relative potency (Table 3). (Figure S70; SI)

Table 3 Hemagglutination assay data for *P. aeruginosa* LecA binding with multivalent β-C-melibiosyl barbiturate derivatives.

Ligand	<i>n</i> ^a	MIC [μM] ^b	r.p. ^c / <i>n</i>
Melibiose	1	3125	1
7 PS-Meli5	5	44	14.2
8 PS-Meli28	28	0.4	279
9 PS-Mal9	9	-	-

^a *n* = Number of melibiose residues per ligand. ^b MIC=minimal inhibitory concentration for the hemagglutination assay. The MIC corresponds to the highest dilution causing a complete inhibition of hemagglutination. ^c r.p./*n* = relative potency per melibiose residue = (MIC_(melibiose)/MIC_(ligand))/*n*.

The glycopolymer **7**, with lower degree of substitution, exhibited a MIC of 44 μM and was 14-fold more potent than the monovalent melibiose reference highlighting the cluster effect. More interestingly, the fully substituted glycopolymer **8** led to strong inhibition with a MIC of 0.4 μM resulting in a significant increase on valency-corrected relative potency (279). In combination with DLS data, this result indicates that increasing the saccharide density along the polystyrene backbone improves the binding towards LecA due to a better accessibility of ligand displayed at the surface of individual spherical micelles and led to a significant greater cluster effect. This result tendency is generally observed with amphiphilic glycopolymer nanoparticles.^{30,31}

CONCLUSION

In summary, we have demonstrated that Knoevenagel condensation using commercially available 1,3-dimethyl barbituric acids and unprotected carbohydrates constitutes a rapid and versatile method for the scalable preparation of monovalent 1,3-dimethyl-(β-C-glycopyranosyl)barbituric acid sodium salt useful for targeting diverse lectins (LecA, LecB and RSL) coming from opportunistic pathogens. An additional alkylation step allowed a versatile molecular design of monovalent carbohydrates but also

the ease access to multivalent scaffolds from glycoclusters to glycopolymers. The β -C-glycosylbarbiturates–lectin recognition studies unraveled the impact of barbiturate ring which is unfavorable for binding *P. aeruginosa* lectins but to a lesser extent for RSL lectin allowing accommodation of hindered aglycon groups. The interactions were restored when oligosaccharides were used enabling distance of the recognition carbohydrate from the barbiturate ring. Thus, the synthetic strategy we have outlined for obtaining glycomimetics and their multivalent counterparts is one of the most efficient and rapid starting from protecting-group free carbohydrates. Obviously, various kind of non-hydrolyzable β -C-glycosyl barbiturate systems could be obtained facilitating the development of glycosciences. In that sense, application of this methodology to attach carbohydrates to other materials are ongoing.

EXPERIMENTAL PROCEDURES

General information

All chemicals were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) or Carbosynth (Berkshire, United Kingdom), and were used as received. The poly(4-iodomethylstyrene) (M_w 6830 g mol⁻¹, $M_w/M_n = 2.2$) was obtained from Specific Polymers (Castries, France). α -L-fucopyranosyl-(1 \rightarrow 2)-lactose was kindly offered by Dr. Eric Samain (CERMAV, Grenoble, France) who developed a biotechnological process to produce oligosaccharides.³² For carbohydrates, the progress of the reactions was monitored by thin layer chromatography using silica gel 60 F254 precoated plates (Merck). Spots were visualized using UV light and by charring with 3% H₂SO₄ in MeOH–water (1:1, v/v). Silica gel 60 (Geduran® Si 60, 40–63 μ m, Merck) was used for column chromatography. IR spectra were recorded using a PerkinElmer spectrometer. The samples were analyzed by transmission from 400 to 4000 cm⁻¹ (4 scans resolution 2). ¹H and ¹³C NMR spectra were recorded at 298 K in appropriate deuterated solvents using Bruker Avance DRX400 (400 MHz) spectrometer and chemical shifts (δ) were reported in parts per million (ppm). Spectra were referenced to the residual proton solvent peaks relative to the signal of D₂O (4.79 ppm for ¹H) and MeOH-d₄ (δ 3.31 and 49 ppm for ¹H and ¹³C, respectively). Assignments were done by using 1D and 2D NMR correlations COSY and HSQC experiments. Mass spectra (ESI-MS) were recorded using on an Esquire 3000 spectrometer from Bruker. High-resolution mass spectra (HRMS-ESI) were performed on a Waters Xevo® G2-S QToF. Dynamic light scattering experiments were carried out at 25°C using an ALV laser goniometer, which consists of a 22 mW HeNe linearly polarized laser operating at a wavelength of 632.8 nm and an ALV-5000/EPP multiple τ digital correlator with 125 ns initial sampling time. Isothermal titration calorimetry (ITC) was performed with a ITC200 Microcalorimeter from Malvern and the data was analyzed using the Microcal Origin software. At least two independent titrations were run. Molecular modeling ????. Hemagglutination inhibition assays were performed on rabbit erythrocytes (details in SI) with glycopolymers.

Synthesis of sodium 5-(β -D-galactopyranosyl)-1,3-dimethylbarbiturate (1). Sodium 5-(β -D-galactopyranosyl)-1,3-dimethylbarbiturate synthesis is adapted from a procedure described by Gonzalez et al.¹⁵ D-galactose (5.54 mmol) and 1,3-dimethylbarbituric acid (6.40 mmol) were dissolved in deionized water (12.5 mL) followed by the slow addition of NaHCO₃ (6.30 mmol) in order to neutralize the pH at 6.5. The mixture was then heated at 80°C and monitored by TLC (EtOAc/MeOH/H₂O, 40/15/3, v/v). After 5h, D-galactose had been totally consumed and the reaction was stopped. Water was evaporated under reduced pressure and the obtained orange solid was redissolved into MeOH (25 mL) using sonication. Thus, **1** was precipitated by adding 25 mL of EtOAc and the white solid was removed by filtration and washed two times with EtOAc and once with a EtOAc/MeOH (1/1) mixture. Finally, **1** was dried under reduced pressure at 60°C and obtained as a white powder (94% yield). ¹H NMR (400 MHz, D₂O, 298K) δ 3.24 (6H, s, N-CH₃), 3.65 (1H, dd, *J* 3.5 Hz,

9.5 Hz, 3-H Gal), 3.70-3.78 (3H, m, 5-H Gal, 6-HH' Gal), 3.98 (1H, d, *J* 3.4 Hz, 4-H Gal), 4.48 (1H, d, *J* 10.0 Hz, 1-H Gal), 4.57 (1H, t, *J* 9.7 Hz, 2-H Gal); ¹³C NMR (100 MHz, D₂O, 298K) 28.3 (N-CH₃), 61.7 (6-C Gal), 68.1 (2-C Gal), 70.4 (4-C Gal), 75.8 (3-C Gal), 77.2 (1-C Gal), 79.4 (5-C Gal), 86.9 (5-C barbiturate), 155.0 (2-C barbiturate); HRMS (ESI) *m/z* 341.0960 ([MNa + H]⁺, C₁₂H₁₈N₂O₈Na calc. 341.0955).

Synthesis of 5-(β-D-galactopyranosyl)-1,3,5-trimethylbarbiturate (1a). **1** (1.47 mmol) was partially dissolved in DMSO (2.0 mL) and iodomethane (2.94 mmol) was added. The reaction mixture was then stirred at room temperature for 24h and monitored by TLC (DCM/MeOH, 8/2, v/v) till complete conversion was achieved. DMSO was removed from the mixture using a Reveleris C18 40g pre-packed column eluted with gradient elution of methanol in water (0-100%). Fractions that contained glycosylated products were collected and adsorbed on silica by evaporation. The silica powder was then placed on a column of silica packed with DCM and was eluted with DCM containing an increasing concentration of methanol (0-20%). Evaporation under reduced pressure of the fractions that contained **1a** gave a yellowish solid (70% yield). ¹H NMR (400 MHz, MeOD, 298K) δ 1.63 (3H, s, 5-CBarb-CH₃), 3.25 (6H, d, *J* 9.6 Hz, N-CH₃), 3.37-3.42 (2H, m, 3-H Gal, 5-H Gal), 3.60-3.69 (3H, m, 1-H Gal, 2-H Gal, 6-HH' Gal), 3.81 (1H, d, *J* 3.1 Hz, 4-H Gal); ¹³C NMR (100 MHz, MeOD, 298K) δ 20.6 (5-CBarb-CH₃), 28.8 (N-CH₃), 29.0 (N-CH₃), 62.5 (6-C Gal), 64.3 (5-C barbiturate), 70.3 (2-C Gal), 70.5 (4-C Gal), 76.8 (3-C Gal), 80.9 (5-C Gal), 85.4 (1-C Gal), 153.0 (2-C barbiturate), 172.2-173.2 (4-C barbiturate, 6-C barbiturate); HRMS (ESI) *m/z* 333.1296 ([M + H]⁺, C₁₃H₂₁N₂O₈ calc. 333.1298).

Synthesis of 5-(β-D-galactopyranosyl)-5-benzyl-1,3-dimethylbarbiturate (1b). 5-(β-D-galactopyranosyl)-5-benzyl-1,3-dimethylbarbiturate synthesis is adapted from a procedure described by Wulff *et al.*¹⁷ **1** (1.47 mmol) was partially dissolved in DMSO (15 mL) and benzylbromide (2.94 mmol) was added. The mixture was then stirred at room temperature for 24h and monitored by TLC (DCM/MeOH, 8/2, v/v) till complete conversion was achieved. The reaction mixture was then precipitated into 50 mL of toluene and the obtained glue was sonicated in order to release entrapped DMSO. The solution was then filtered to retain the solid part. This process was repeated twice on the remaining glue stuck to the round flask and finally, all the solid parts were collected and dissolved in DCM. TLC showed that majority of DMSO was removed during the washing process. The solution was then placed on a column of silica packed with DCM and was eluted with DCM containing an increasing concentration of MeOH (0-20%). Evaporation under reduced pressure of the collected fractions gave **1b** a white powder (90% yield). ¹H NMR (400 MHz, MeOD, 298K) δ 3.03 (3H, s, N-CH₃), 3.09 (3H, s, N-CH₃), 3.42-3.49 (2H, m, 3-H Gal, CH-φ), 3.51 (1H, t, *J* 5.7 Hz, 5-H Gal), 3.57 (1H, d, *J* 13.1 Hz, CH'-φ), 3.71-3.80 (3H, m, 2-H Gal, 6-HH' Gal), 3.88 (1H, d, *J* 3.2 Hz, 4-H Gal), 3.99 (1H, d, *J* 9.8 Hz, 1-H Gal), 6.96-7.03 (2H, m, CH aro.), 7.18-7.24 (3H, m, CH aro.); ¹³C NMR (100 MHz, MeOD, 298K) δ 28.5 (N-CH₃), 28.6 (N-CH₃), 42.8 (CH₂- φ), 61.7 (5-C barbiturate), 62.7 (6-C Gal), 70.4 (2-C Gal), 71.0 (4-C Gal), 76.8 (3-C Gal), 81.5 (5-C Gal), 84.9 (1-C Gal), 128.6 (CH aro.), 129.5 (CH aro.), 130.5 (CH aro.), 136.4 (C aro. quat.), 151.9 (2-C barbiturate), 171.3-172.2 (4-C barbiturate, 6-C barbiturate); HRMS (ESI) *m/z* 409.1612 ([M + H]⁺, C₁₉H₂₅N₂O₈ calc. 409.1605).

Synthesis of sodium 5-(β-D-melibiosyl)-1,3-dimethylbarbiturate (3). Sodium 5-(β-D-melibiosyl)-1,3-dimethylbarbiturate dimethylbarbiturate synthesis is adapted from a procedure described by Critchley *et al.*¹⁶ Melibiose monohydrate (2.78 mmol) and 1,3-dimethylbarbituric acid (3.19 mmol) were dissolved in deionized water (6.5 mL) followed by the slow addition of NaHCO₃ (3.15 mmol) up to reach a pH of approximately 6.5. The mixture was then heated at 80°C and monitored by TLC (Acetonitrile/H₂O, 7.5/2.5, v/v). After 6h, melibiose had been totally consumed and the reaction was stopped. Water was partially evaporated under reduced pressure, mixed with silica and dried. The silica powder was then placed on a column of silica packed with acetonitrile and was eluted with acetonitrile containing an increasing concentration of water (5-30%). All the fractions that contains the

product were then lyophilized to obtain an orange powder. To improve the purity, the solid was solubilized into 30 mL of MeOH heated under reflux and, after cooling at room temperature, **3** was precipitated by the addition of 60 mL of EtOAc. The white precipitate was removed by filtration and washed two times with EtOAc. Finally, **3** was dried under reduced pressure at 60°C and obtained as a white powder (89% yield). ¹H NMR (400 MHz, D₂O, 298K) δ 3.23 (6H, s, N-CH₃), 3.49 (1H, m, 3-H Glu), 3.61 (1H, s, 5'-H Gal), 3.61 (1H, d, *J* 5.6 Hz, 4-H Glu), 3.71-3.74 (3H, m, 6-H Glu, 6'-HH' Gal), 3.80 (1H, dd, *J* 3.8 Hz, 10.3 Hz, 2'-H Gal), 3.90 (1H, dd, *J* 3.3 Hz, 10.3 Hz, 3'-H Gal), 3.97-4.01 (3H, m, 5-H Glu, 6-H' Glu, 4'-H Gal), 4.43 (1H, t, *J* 9.5 Hz, 2-H Glu), 4.53 (1H, d, *J* 10.0 Hz, 1-H Glu); ¹³C NMR (100 MHz, D₂O, 298K) δ 28.3 (N-CH₃), 61.8 (6'-C Gal), 67.0 (6-C Glu), 69.1 (2'-C Gal), 69.9 (4'-C Gal), 70.1 (2-C Glu), 70.2 (3'-C Gal), 70.5 (4-C Glu), 71.5 (5-C Glu), 77.1 (1-C Glu), 78.9 (5'-C Gal), 79.2 (3-C Glu), 86.7 (5-C barbiturate), 98.9 (1'-C Gal), 155.1 (2-C barbiturate); HRMS (ESI) *m/z* 503.1503 ([MNa + H]⁺, C₁₈H₂₈N₂O₁₃Na calc. 503.1489).

Synthesis of sodium 5-(β-L-fucopyranosyl)-1,3-dimethylbarbiturate (2). L-Fucose (12.2 mmol) and 1,3-dimethylbarbituric acid (14.1 mmol) were dissolved in deionized water (27 mL) followed by the slow addition of NaHCO₃ (14.6 mmol) up to reach a pH of 6.5. The mixture was then heated at 80°C and monitored by TLC (ACN/H₂O, 9/1, v/v). After 5h, L-fucose had been totally consumed and the reaction was stopped. Water was then totally evaporated under reduced pressure and the obtained orange solid was dissolved into MeOH (15 mL) using sonication. Thus, **2** was precipitated by adding 45 mL of EtOAc, followed by a dispersion of the precipitate using sonication. The white solid was removed by filtration and washed two times with EtOAc. Finally, **2** was dried under reduced pressure at 60°C and obtained as a white powder (83% yield). ¹H NMR (400 MHz, D₂O, 298K) δ 1.22 (3H, d, *J* 6.5 Hz, 6-CH₃ Fuc), 3.23 (6H, s, N-CH₃), 3.64 (1H, ddd, *J* 8.0 Hz, *J* 3.7 Hz, *J* 1.3 Hz, 3-H Fuc), 3.78 (1H, dd, *J* 3.5 Hz, *J* 0.8 Hz, 4-H Fuc), 3.83 (1H, qd, *J* 6.5 Hz, *J* 0.9 Hz, 5-H Gal), 4.46 (1H, d, *J* 9.8 Hz, 1-H Fuc), 4.48 (1H, q, *J* 9.2 Hz, 2-H Fuc); ¹³C NMR (100 MHz, D₂O, 298K) δ 16.5 (6-CH₃ Fuc), 28.3 (N-CH₃), 67.8 (2-C Fuc), 73.3 (4-C Fuc), 75.2 (5-C Fuc), 75.9 (3-C Fuc), 76.9 (1-C Gal), 87.0 (5-C barbiturate), 155.0 (2-C barbiturate); HRMS (ESI) *m/z* 325.1009 ([MNa + H]⁺, C₁₂H₁₈N₂O₇Na calc. 325.1006).

Synthesis of 5-(β-L-fucopyranosyl)-1,3,5-trimethylbarbiturate (2a). **2** (0.77 mmol) was partially dissolved in DMSO (1.0 mL) and iodomethane (1.54 mmol) was added. The reaction mixture was then stirred at room temperature for 24h and monitored by TLC (DCM/MeOH, 9.5/0.5, v/v) till complete conversion was achieved. **2a** was purified using a Reveleris C18 (40g) pre-packed column eluted with gradient elution of methanol in water (0-100%). Fractions that contained **2a** were collected and lyophilized to give a white solid (90% yield). **2a** is a very hygroscopic compound. ¹H NMR (400 MHz, D₂O, 298K) 1.14 (3H, d, *J* 6.5 Hz, 6-CH₃ Fuc), 1.61 (3H, s, 5-CBarb-CH₃), 3.24 (6H, d, *J* 2.0 Hz, N-CH₃), 3.38 (1H, dd, *J* 8.9 Hz, *J* 3.4 Hz, 3-H Fuc), 3.50 (1H, qd, *J* 6.5 Hz, *J* 1.0 Hz, 5-H Fuc), 3.56 (1H, dd, *J* 3.4 Hz, *J* 1.0 Hz, 4-H Fuc), 3.58 (1H, d, *J* 9.5 Hz, 1-H Fuc), (1H, q, *J* 9.0 Hz, 2-H Fuc); ¹³C NMR (100 MHz, D₂O, 298K) δ 16.8 (6-CH₃ Fuc), 20.4 (5-CBarb-CH₃), 28.7-28.9 (N-CH₃), 55.7 (5-C barbiturate), 70.0 (2-C Fuc), 73.0 (4-C Fuc), 76.4 (5-C Fuc), 77.0 (3-C Fuc), 85.4 (1-C Fuc), 153.1 (2-C barbiturate), 172.2-173.4 (4-C barbiturate, 6-C barbiturate); HRMS (ESI) *m/z* 317.1345 ([M + H]⁺, C₁₃H₂₁N₂O₇ calc. 317.1349).

Synthesis of 5-(β-L-fucopyranosyl)-5-benzyl-1,3-dimethylbarbiturate (2b). **2** (1.47 mmol) was partially dissolved in DMF (1.5 mL) and benzylbromide (2.94 mmol) was added. The reaction mixture was then stirred at room temperature for 24h and monitored by TLC (ACN/H₂O, 9/1, v/v) till complete conversion was achieved. The mixture was then dried under N₂ flow. The resulting orange film was solubilized in MeOH and then adsorbed on silica. **2b** was purified using a Reveleris silica (40μm, 40g) pre-packed column eluted with gradient elution of methanol in DCM (0-100%). Fractions that contained **2b** were collected and lyophilized to give a white foam (88% yield). ¹H NMR (400 MHz, D₂O, 298K) δ 1.21 (3H, d, *J* 6.5 Hz, 6-CH₃ Fuc), 3.05 (6H, d, *J* 9.7 Hz, N-CH₃), 3.43 (1H, dd, *J* 9.2 Hz, *J* 3.3 Hz, 3-

H Fuc), 3.48 (2H, d, J 6.9 Hz, CH- ϕ), 3.59 (1H, d, J 6.8 Hz, 5-H Fuc), 3.61 (1H, d, J 3.0 Hz, 4-H Fuc), 3.78 (1H, d, J 9.5 Hz, 2-H Fuc), 3.93 (1H, d, J 9.6 Hz, 1-H Fuc), 6.99 (2H, m, CH aro.), 7.20 (2H, m, CH aro.); ^{13}C NMR (100 MHz, D_2O , 298K) δ 16.9 (6- CH_3 Fuc), 28.4-28.6 (N- CH_3), 42.5 (CH_2 - ϕ), 62.1 (5-C barbiturate), 70.1 (2-C Fuc), 73.3 (4-C Fuc), 77.0 (5-C Fuc), 77.1 (3-C Fuc), 84.1 (1-C Fuc), 128.6 (CH aro. para), 129.5 (CH aro.), 130.5 (CH aro.), 136.6 (C aro. quat.), 152.0 (2-C barbiturate), 171.4-172.1 (4-C barbiturate, 6-C barbiturate); HRMS (ESI) m/z 415.1473 ($[\text{M} + \text{Na}]^+$, $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_7\text{Na}$ calc. 415.1476).

Synthesis of sodium 5- $[\alpha$ -L-fucopyranosyl-(1 \rightarrow 2)- β -D-lactosyl]-1,3-dimethylbarbiturate (4). α -L-fucopyranosyl-(1 \rightarrow 2)-D-lactose (0.61 mmol) and 1,3-dimethylbarbituric acid (0.74 mmol) were dissolved in deionized water (2 mL) followed by the slow addition of NaHCO_3 (68 mg) up to reach pH 6.5. The mixture was then heated at 80°C and monitored by TLC (Acetonitrile/ H_2O , 7/3, v/v). After 6h, α -L-fucopyranosyl-(1 \rightarrow 2)-D-lactose had been totally consumed and the reaction was stopped. Water was partially evaporated under reduced pressure and the concentrated mixture was then added, drop by drop, to 50 mL of *i*-PrOH in order to induce precipitation of **4**. The white precipitate was removed by filtration and washed three times with 5 mL of acetone. Finally, the precipitate was solubilized in water and freeze dried to give **4** as a white foam (67% yield). ^1H NMR (400 MHz, D_2O , 298K) δ 1.31 (3H, d, J 6.6 Hz, 6''- CH_3 Fuc), 3.26 (6H, N- CH_3), 3.54 (1H, d, J 8.4 Hz, 3''-H Fuc), 3.64 (1H, t, J 9.1 Hz, 3-H Glu), 3.70-4.0 (10H, m, 4-H Glu, 5-H Glu, 6-HH' Glu, 3'-H Gal, 5'-H Gal, 6'-HH' Gal, 2''-H Fuc, 4''-Fuc), 3.78 (1H, s, 2'-H Gal), 3.95 (1H, s, 4'-H Gal), 4.32 (1H, q, J 6.5 Hz, 5''-H Fuc), 4.47 (1H, t, J 9.5 Hz, 2-H Glu), 4.58 (1H, d, J 9.9 Hz, 1-H Glu), 4.64 (1H, d, J 7.8 Hz, 1'-H Gal), 5.37 (1H, d, J 5.1 Hz, 1''-H Fuc); ^{13}C NMR (100 MHz, D_2O , 298K) δ 15.3 (6''- CH_3 Fuc), 27.6 (N- CH_3), 60.1 (6-C Glu), 61.1 (6'-C Gal), 66.9 (5''-C Fuc), 68.2 (2''-C Fuc), 69.2 (4'-C Gal), 69.3 (2-C Glu), 69.6 (5-C Glu), 71.7 (5'-C Gal), 73.6 (4-C Glu), 75.2 (2'-C Gal), 75.5 (4''-C Fuc), 76.0 (1-C Glu), 76.2 (3'-C Gal), 76.7 (3-C Glu), 78.9 (3''-C Fuc), 85.9 (5-C barbiturate), 98.8 (1''-C Fuc), 100.2 (1'-C Gal), 154.3 (2-C barbiturate); HRMS (ESI) m/z 649.2062 ($[\text{M} + \text{H}]^+$, $\text{C}_{24}\text{H}_{38}\text{N}_2\text{O}_{17}$ calc. 649.2068).

Synthesis of sodium 5-(β -D-maltosyl)-1,3-dimethylbarbiturate (5). Sodium 5-(β -D-maltosyl)-1,3-dimethylbarbiturate synthesis is adapted from a procedure described by Critchley *et al.*¹⁶ D-Maltose monohydrate (2.8 mmol) and 1,3-dimethylbarbituric acid (3,32 mmol) were dissolved in deionized water (6 mL) followed by the slow addition of NaHCO_3 (280 mg) up to pH 6.5. The mixture was then heated at 80°C and monitored by TLC (Acetonitrile/ H_2O , 8/2, v/v) till complete conversion was achieved. **5** was purified using a Reveleris C18 40g pre-packed column eluted with gradient elution of methanol in water (0-20%). Fractions that contained **5** were collected and freeze-dried to give a yellowish solid (99% yield). **5** could be also purified by precipitation. After complete conversion of D-maltose, water was partially evaporated under reduce pressure and the concentrated mixture was then added, drop by drop, to 100 mL of *i*-PrOH in order to induce precipitation of **5**. The white precipitate was removed by filtration and washed three times with 10 mL of acetone. Finally, **5** was dried under reduced pressure at 60°C and obtained as a white powder (93% yield). ^1H NMR (400 MHz, D_2O , 298K) δ 3.23 (6H, s, N- CH_3), 3.44 (1H, t, J 9.2 Hz, 4'-H Glu), 3.57-3.62 (2H, m, 2'-H Glu, 4-H Glu), 3.73 (1H, t, J 9.5 Hz, 3'-H Glu), 3.77-3.88 (7H, m, 3-H Glu, 5-H Glu, 6-HH' Glu, 5'-H Glu, 6'-HH' Glu), 4.40 (1H, t, J 9.4 Hz, 2-H Glu), 4.55 (1H, d, J 9.9 Hz, 1-H Glu), 5.45 (1H, d, J 3.9 Hz, 1'-H Glu); ^{13}C NMR (100 MHz, D_2O , 298K) δ 28.4 (N- CH_3), 61.2 (6'-C Glu), 61.5 (6-C Glu), 70.1 (2-C Glu, 4'-C Glu), 72.6 (2'-C Glu), 73.4 (3-C Glu), 73.7 (3'-C Glu), 76.7 (1-C Glu), 77.8 (5-C Glu), 79.0 (4-C Glu), 79.4 (5'-C Glu), 86.8 (5-C barbiturate), 100.6 (1'-C Glu), 155.1 (2-C barbiturate); HRMS (ESI) m/z 503.1498 ($[\text{MNa} + \text{H}]^+$, $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_{13}\text{Na}$ calc. 503.1489).

Synthesis of α,α' -Di[3'',5''-dimethyl-1''-(β -D-melibiosyl)pyrimidine-2'',4'',6''-trione]-*p,p'*-bitolyl (6). **3** (0.4 mmol) was partially dissolved in dry DMSO (1.5 mL) and 4,4'-(bisbromomethyl)biphenyl (0.07 mmol) was added. The mixture was then stirred at 70°C for 5h and monitored by TLC (ACN/ H_2O , 8/2,

v/v). **3** was not totally converted. **6** was purified from the mixture using a Reveleris C18 40g pre-packed column eluted with gradient elution of methanol in water (0-100%). Evaporation under reduced pressure of the fractions that contained **6** gave a syrup which was diluted in water and freeze dried to obtain a white foam (72% yield). ¹H NMR (400 MHz, MeOD, 298K) δ 3.09 (12H, d, *J* 5.7 Hz, N-CH₃), 3.24 (2H, t, *J* 9.3 Hz, 4-H Glu), 3.34 (2H, t, *J* 8.8 Hz, 3-H Glu), 3.47 (2H, m, 5-H Glu), 3.51 (2H, s, 6'-H Gal), 3.54 (2H, s, 6'-H' Gal), 3.56-3.88 (18H, m, CH₂-φ, 2-H Glu, 6-HH' Glu, 2'-H Gal, 3'-H Gal, 4'-H Gal, 5'-H Gal), 4.05 (2H, d, *J* 9.8 Hz, 1-H Glu), 4.9 (2H, 1'-H Gal), 7.05 (4H, d, *J* 8.2 Hz, CH aro.), 7.44 (4H, d, *J* 8.3 Hz, CH aro.); ¹³C NMR (100 MHz, MeOD, 298K) δ 28.7 (N-CH₃), 42.1 (6'-C Gal), 61.9 (5-C barbiturate), 62.9 (6-C Glu), 68.0 (CH₂-φ), 70.4 (2'-C Gal), 71.2 (5'-C Gal), 71.5 (3'-C Gal), 71.7 (4-C Glu), 72.4 (4'-C Gal), 72.9 (2-C Glu), 80.1 (3-C Glu), 81.1 (5-C Glu), 84.2 (1-C Glu), 100.2 (1'-C Gal), 127.8 (CH aro.), 131.1 (C aro. quat.), 135.6 (C aro. quat.), 152.0 (2-C barbiturate), 171.3-172.1 (4-C barbiturate, 6-C barbiturate); HRMS (ESI) *m/z* 1161.3844 ([M + Na]⁺, C₁₃H₂₀N₂O₇Na calc. 1161.3857).

General procedure for the synthesis of meliobiose or maltose bearing glycopolymers (7), (8) & (9).

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ASSOCIATED CONTENT

Supporting Information

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NMR & FT-IR spectra of β-C-glycosylbarbiturates. Raw ITC data and pictures of hemagglutination assays.

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Notes

The authors declare no competing financial interest.

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