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Aïcha Abdallah, Emilie Gillon, Patrice Rannou, Anne Imberty, Sami Halila. Microwave-Assisted Synthesis of β - N -Aryl Glycoamphiphiles with Diverse Supramolecular Assemblies and Lectin Accessibility. *Bioconjugate Chemistry*, 2024, 35 (8), pp.1200-1206. 10.1021/acs.bioconjchem.4c00224 . hal-04727492

HAL Id: hal-04727492

<https://hal.science/hal-04727492v1>

Submitted on 9 Oct 2024

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MICROWAVE-ASSISTED SYNTHESIS OF β -N-ARYL GLYCOAMPHIPHILES WITH DIVERSE SUPRAMOLECULAR ASSEMBLIES AND LECTINS ACCESSIBILITIES

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KEYWORDS. Carbohydrate Chemistry, Microwave, Glycoamphiphiles, Micelles, Lectins.

ABSTRACT: Glycoamphiphiles have attracted considerable interests in a broad range of application fields owing to their solution and bulk-state self-assembly abilities. Despite their importance, the straightforward synthesis of glycoamphiphiles consisting of a hydrophilic carbohydrate linked to a hydrophobic aglycone remains one of the major challenges in glycosciences. Here, a rapid, simple, and efficient synthetic access to chemically stable glycoamphiphiles at physiological pH, namely *N*-(β -D-glycosyl)-2-alkylbenzamide, is reported. It leverages the non-reductive amination of unprotected carbohydrates with *ortho*-substituted aniline derivatives which could be readily obtained by reacting commercially available primary alkylamines with isatoic anhydride. This strategy avoids protection and deprotection of sugar hydroxyl groups and the use of reductive agents, which makes it advantageous in terms of atom and step economy. Moreover, in order to circumvent to cons of classical *N*-aryl glycosylation, we investigate the use of microwave as a heat source that provides fast, clean, and high-yield β -*N*-arylation of unprotected carbohydrates. Their self-assembly into water led to multiple morphologies of dynamic supramolecular glycoamphiphiles that were characterized to assess their ability to bind to lectins from pathogenic bacteria. Biophysical interactions probed by isothermal titration microcalorimetry revealed micromolar affinities for most of the synthesized glycoamphiphiles.

1. INTRODUCTION

Glycoconjugates, defined as carbohydrates or glycans attached to a lipid or a protein, are ubiquitous in Nature, covering the outermost layer of living cells through a thick coating named Glycocalyx. They play critical roles in varied biological and pathological processes, acting as signaling, recognition, and bacterial adhesion, etc. Consequently, major scientific interests in accessing glycoconjugates or glycomimetics stem from the promise to use them as probes for biological research, as well as lead compounds for developing drugs, vaccines, and diagnostic tools. These endeavors are however made difficult by a lack of general methods for the straightforward preparation of these key-enabling carbohydrate derivatives. Standard approaches for the chemical modification of the reducing-end position of carbohydrates rely on tedious and time-consuming multiple protecting group and activation strategies. Although much progress has been made in anomeric functionalization of protecting-group-free carbohydrates,¹ this still represents an intense field of research in carbohydrate chemistry and beyond.

In our continuous effort to develop chemoselective anomeric modification of unprotected carbohydrates,^{2,3} we discovered that *para*-substituted aniline were convenient molecular scaffolds for *N*-arylation, through reductive amination of weakly reactive reducing-end position of chitosan oligosaccharides.⁴ Indeed, the interest of using substituted anilines lies in the exceptionally low pK_a of their primary amino function ($pK_a = 4.6$)⁵ providing a good nucleophilicity at a slightly acidic pH. And since the intermediate electrophilic protonated aniline Schiff base displays a pK_a roughly 2 units below, it is abundant for a subsequent intramolecular *O*-nucleophilic attack to give mainly β -*N*-aryl glycosides.⁶ However, the *N*-glycosylation of aromatic compounds starting from aniline derivatives and carbohydrates requires an extended reaction time, typically ca. 3-6 days.⁷ Therefore There exists therefore a ground to shorten the reaction time for *N*-arylation of unprotected carbohydrates.

Microwaves were described in the mid-1980s⁸ as a relevant heating source for dramatically enhancing reaction rates, decreasing side-products and resulting in improved yields. Microwaves have been elegantly exploited for chemical or enzymatic transformations of carbohydrates,⁹ but, to the best of our knowledge, is unprecedented for the direct *N*-arylation of unprotected carbohydrates.

Over the last two decades, our research team has actively been engaged in the synthesis of carbohydrate-based amphiphiles (or glycoamphiphiles) exhibiting fascinating controlled self-assembly properties in the solution (micelles, supramolecular gels)^{10,11} or in bulk (nano-organized thin-films)¹² states for various applications ranging from nanomedicine to nanoelectronics. Moreover, self-

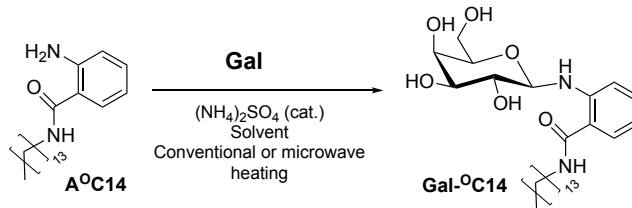
assembled glycoamphiphiles are in important class of drug-delivery system since they improve the biological as well as the pharmacokinetics profile of therapeutic agents.¹³ In this context, the present study describes a straightforward and versatile microwave-assisted *N*-arylation of protecting-group-free carbohydrates, enabling the preparation of a series of glycoamphiphiles that self-assemble into various morphologies in solution and interact with carbohydrate-binding proteins, so called lectins, from pathogens. In the context of antiadhesion therapy that relies on inhibition of bacterial adhesion with competitive lectin-inhibitory carbohydrates, we assessed interactions between these newly synthesized amphiphilic β -*N*-glycosides with lectins from aggressive worldwide-distributed bacteria such as *Pseudomonas aeruginosa*, an opportunistic bacterium responsible for hospital-acquired diseases and pulmonary infections, and the phytopathogenic *Ralstonia solanacearum* that causes a lethal bacterial wilt disease in hundreds plant species. *P. aeruginosa* produces galactose-specific LecA and fucose-specific LecB lectins¹⁴ while *R. solanacearum* produces fucose-specific RSL lectin with structural similarity to AFL from human fungal pathogen *Aspergillus fumigatus*.¹⁵

2. RESULTS AND DISCUSSION

2.1. Synthesis of microwave-assisted amphiphilic β -*N*-aryl glycosides.

N-arylation of carbohydrates is an important chemical transformation to a wealth of biologically active carbohydrates. *N*-aryl glycosides have been reported to possess multiple biological activities including anticancer, antiviral, and antibacterial activities.¹⁶⁻¹⁸ Direct preparation of β -*N*-aryl glycosides was first investigated according to a methodology using ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ as a mildly acid catalyst¹⁹ and heating in protic solvents such as water or methanol. $(\text{NH}_4)_2\text{SO}_4$ proved to be useful for the synthesis of *N*-phenantroline glycosylamines²⁰ or for the preparation of maltose-based hydrogelators.²¹ Since we aimed at synthesizing a series of amphiphilic β -*N*-aryl glycosides with various hydrophobic alkyl chain length and different hydrophilic carbohydrates, we identified that isatoic anhydride could be used as a starting material for producing a range of useful substituted *ortho*-aniline derivatives. Conversion of isatoic anhydride to 2-amino-*N*-alkyl benzamides was readily accomplished by nucleophilic ring opening with primary aliphatic amines in THF for 2h at 60°C (82-92 % yields; See SI page S3-S4). Next, for the synthesis of amphiphilic β -*N*-aryl glycosides, we optimized the reaction conditions by studying the *N*-glycosylation between D-galactose (**Gal**) and 2-amino-*N*-tetradecyl benzamide **A^oC14** (Table 1), as model substrates.

Table 1. *N*-arylation of D-galactose under conventional heating versus microwave (MW).



Entry	Reaction conditions	Yield of Gal-°C14 (%) ^a
1	$(\text{NH}_4)_2\text{SO}_4$, MeOH, reflux, 3 days	24
2	$(\text{NH}_4)_2\text{SO}_4$, H ₂ O, 65°C, 3 days	0
3	$(\text{NH}_4)_2\text{SO}_4$, H ₂ O, 65°C, 1 equiv. β -CD, 3 days	0
4	MeOH, reflux, 3 days	traces
5	3 equiv. of Gal , $(\text{NH}_4)_2\text{SO}_4$, MeOH, reflux for 24 h	50
6	3 equiv. of A^oC14 , $(\text{NH}_4)_2\text{SO}_4$, MeOH, reflux for 24 h	50
7	3 equiv. of A^oC14 , $(\text{NH}_4)_2\text{SO}_4$, MeOH, MW, 90°C for 2 h	73
8	3 equiv. of A^oC14 , $(\text{NH}_4)_2\text{SO}_4$, MeOH, MW, 110°C for 2 h	75
9	3 equiv. of A^oC14 , $(\text{NH}_4)_2\text{SO}_4$, MeOH, oil bath, 110°C for 2 h	20

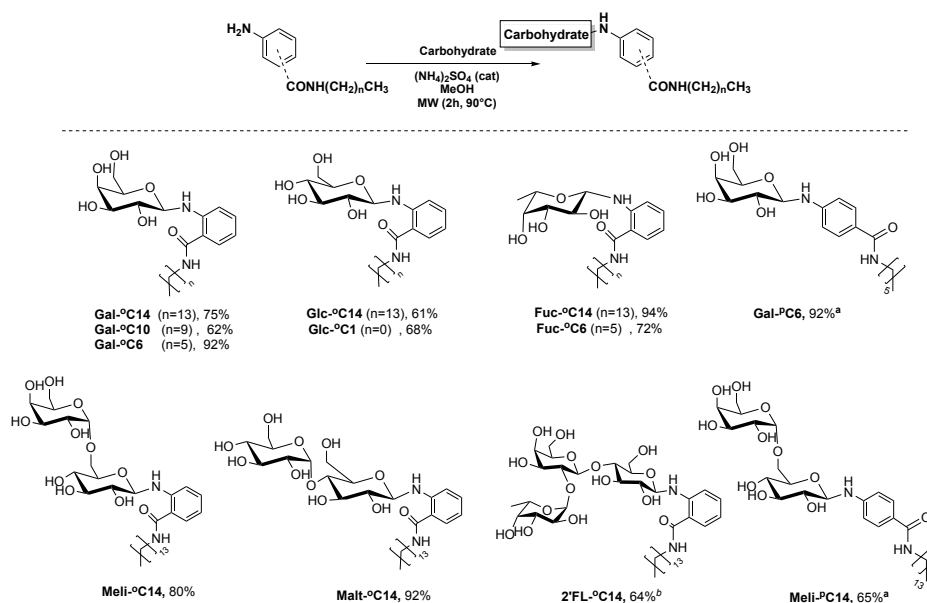
^a isolated yield.

(Entry 1) **Gal** was treated with an equimolar amount of **A^oC14**, in the presence of a catalytic amount of $(\text{NH}_4)_2\text{SO}_4$ in MeOH at reflux following M. Yamanaka's conditions²² to afford, after 3 days of reaction, only 24% of isolated β -*N*-tetradecyl galactosides **Gal-°C14**. Water has been tested as solvent in presence or absence of β -cyclodextrin which should aid in solubilization of **Gal-°C14** through host-guest inclusion²³ (entries 2-3). However, no reactivity was observed. This can be rationalized though invoking hydrolysis or the poor water-solubility of **A^oC14**. The need of $(\text{NH}_4)_2\text{SO}_4$ as an acid catalyst was proved since without the latter almost no reaction occurred (entry 4). The reaction was well-improved by using an excess of **Gal** or **A^oC14** with a satisfactory yield of 50% (entries 5-6). We then investigated the effect of microwave heating on the synthesis of **Gal-°C14**, again using $(\text{NH}_4)_2\text{SO}_4$ as a catalyst and 3 equiv. excess of **A^oC14** (Entries 7-8). Our procedure used microwave irradiation at both 90°C (entry 7) and 110°C (entry 8) for only 2 h, compared with 3 days at reflux with conventional heating, to give ca. 70% yield. To ensure that the improved yield was not promoted thanks to a simple elevated temperature, we reproduced the reaction at 110°C with a conventional heating (oil bath) in

a closed tube (entry 9). After 2 hours, we isolated only 20% of **Gal-°C14** clearly demonstrating the beneficial microwave effect. The impressive acceleration of reaction time (few hours compared to days) with excellent β -stereoselectivity of *N*-arylation prompted us to study this new and optimized protocol with other carbohydrates, that could interact with fucophilic LecB or RSL lectin or galactophilic LecA lectin, and with a series of 2-amino-*N*-alkyl benzamides.

For such purpose, we selected galactose-based ligands such as **Gal** or the disaccharide melibiose (**Mel**) and fucose-based ligands such as L-fucose (**Fuc**) or the trisaccharide 2'-fucosyllactose (**2'FL**) for interaction studies with LecA and LecB or RSL, respectively. Glucose-based ligands, *i.e.*, D-glucose (**Glc**) and maltose (**Mal**), were synthesized as negative control for probing nonspecific interactions. Other structural parameters such as the influence of the chain length and position, *i.e.* *ortho* vs. *para*, of the *n*-alkyl chain on the aniline, on the self-assembling properties in aqueous media and on affinities for targeted lectins were addressed (Scheme 1).

Scheme 1. Scope of the microwave-assisted non-reductive *N*-aryl-amination.

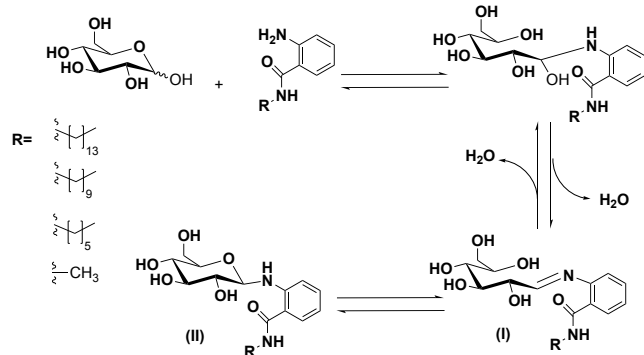


To demonstrate the effectiveness of our innovative approach, the optimized protocol was applied to all experiments of β -*N*-arylation with unprotected carbohydrates (MW, 2h, 90°C, 3 equiv. arylamine, 0.2 equiv. (NH₄)₂SO₄), except for **2'FL** where 4 h and the addition of 4% vol. H₂O were needed to drive the reaction to completion). For the sake of clarity and reading, we have decided to codify the glycoamphiphiles as follows: The carbohydrates are named according to the nomenclature previously cited, then the position of the substitution on the aniline is represented by superscript with an O for *ortho*- or P for *para*-substitution and finally C_n where n designates the carbon number of the *n*-alkyl chain (Scheme 1). To our delight, the β -*N*-arylation, with various previously synthesized aniline derivatives, proceeded efficiently and gave the desired β -*N*-aryl glycosides with a satisfactory yield ranging from 61 to 94%. **Gal** was *N*-arylated with derivatives in C14, C10 and C6 located in *ortho*-position and *para*-position for C6. All amphiphilic β -*N*-aryl glycosides were fully characterized by (1D & 2D) NMR spectroscopy and high-resolution mass spectrometry (HRMS) (Supporting information). The β -stereoselectivity, rigorously confirmed by ¹H NMR signal of H-1 anomer at the reducing-end (³J_{1,2} = 5.1–8.8 Hz), was conserved whatever the nature of carbohydrates or substituted aniline derivatives. It is noteworthy that amination with a primary amine, such as tetradecyl amine displayed a very low reactivity in the same reaction conditions emphasizing the importance of aniline derivatives for such coupling reactions (data not shown here).

2.2. Stability studies in aqueous solution.

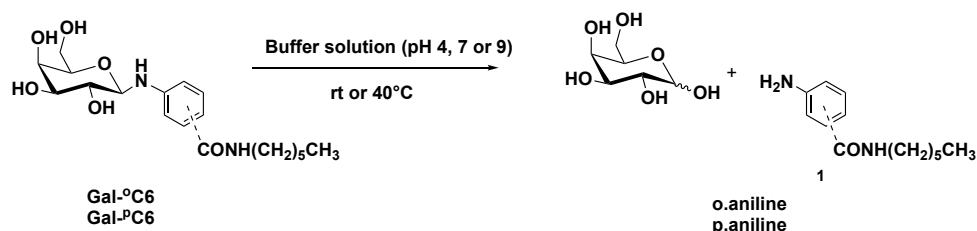
With our glycoamphiphiles in hand, we undertook a stability study and rates of hydrolysis under aqueous conditions, since they have to be stable at least within the timescale (few seconds) of the detection of lectin/carbohydrate interactions. Even though the *N*-glycosidic bond is known to be unstable to chemical hydrolysis, Thierry Maugard and his coworkers⁶ have shown that β -*N*-aryl glycosides were stable at physiological pH, *ca.* 7.4, with a half-time life ranging from 13h to 3 days depending on the nature of the aryl substituent. It is noteworthy that *N*-glycosidic bond was found more stable than the *O*-glycosidic bond to enzymatic hydrolysis. Detailed in Scheme 2, the mechanism of the aryl *N*-glycosylation highlights the possible reversibility of the reaction in water.

Scheme 2. Mechanism of aryl *N*-glycosylation and reverse reaction of hydrolysis



To determine the hydrolysis rates and the kinetic according to the position of the substitution on the aniline derivative, the glycoamphiphiles **Gal-^oC6** and the isomer **Gal-^pC6**, that are macroscopically water-soluble, were incubated at varying representative pH (4, 7 and 9) and temperature (RT or 40°C), since lectins preferentially work at specific conditions of buffer and temperature. The hydrolysis reaction was monitored by high-performance liquid chromatography (HPLC) and the observed rate constants for hydrolysis were determined by directly fitting the integrated areas relative to glycoamphiphiles remaining. The half-lives for hydrolysis of **Gal-^oC6** and **Gal-^pC6** are presented in Table 2.

Table 2. Half-lives in buffer solution of Gal-^oC6 (2c) and Gal-^pC6 under different pH values and temperatures



Product	pH	t _{1/2} at rt	t _{1/2} at 40°C
Gal- ^o C6, Gal- ^p C6	4	≈ 1h	≈ 20min
	7	≈ 24h	≈ 8h
	9	> 1day	>1 day

First, the HPLC results clearly demonstrated the same hydrolysis profile for **Gal-^oC6** and **Gal-^pC6** indicating that the position of the bulky alkyl chain does not interfere with the kinetic of hydrolysis reaction. Second, the best stability was observed in alkaline conditions at RT ($t_{1/2} > 1$ day) and the worst one for acidic conditions and at a warmer temperature, *ca.* 40°C ($t_{1/2} = 1$ h). The effect of higher temperature reflects an increase of the kinetic of hydrolysis reaction while the pH-dependance stems mainly from the position of the equilibrium between the ring closed species II and the open-chain iminium (Schiff base) species I, where I is favored in acidic conditions so prompt to hydrolysis (Scheme 2). At neutral conditions (pH 7, RT), the samples showed a limited rate of hydrolysis with $t_{1/2}$ of one day. In contrast, under acidic conditions, the hydrolysis was found to be faster with a half-life time of *ca.* 1h at RT and 20 min at 40°C. Compared to existing data of the literature,⁶ our compound **Gal-^oC6** or **Gal-^pC6** is by far more stable than non-substituted aromatic derivatives ($t_{1/2} = 24$ min at 40°C for pH 6.5) suggesting that the *n*-alkyl chain, either in *ortho* or *para* position, could create an hydrophobic pocket around the anomeric position, thus limiting access of water molecules and therefore hydrolysis. More importantly, this study, demonstrating the good stability of amphiphilic β-*N*-aryl glycosides, allows us to propose a new class of glycoamphiphiles as potential ligands for lectins.

2.3. Self-assembly properties in aqueous solution and morphological characterizations:

Carbohydrate-based amphiphiles exhibit fascinating solution self-assembly properties due to their ability to generate a diverse range of discrete morphologies (*e.g.* spherical micelles, vesicles, tubes, fibers, lamellae and so on) applicable in fundamental and practical areas. The shapes are typically determined by the chemical composition and physical assembly method. In the frame of carbohydrate-lectin interactions, self-assembly is an elegant bottom-up approach that uses supramolecular interactions (reversible) to create dynamic multivalent systems advantageous to enhance binding affinity.²⁴ Our family of glycoamphiphiles, that could be considered as minimalistic mimics of native glycolipids that are key components of cell membranes, is featured by a hydrophilic carbohydrate responsible of hydrogen bonding, a hydrophobic *n*-alkyl chain promoting van der Waals interactions and an additional aromatic ring that contributes to stabilize self-assemblies by π-π or/and C-H⋯π stacking.²⁵ The expected self-assembled micelles in water should consist in alkyl-aryl cores surrounded by sterically-stabilized carbohydrate shells. The aggregation behavior of some

water-soluble glycoamphiphiles, **Meli-^oC14**, **Meli-^pC14**, **Gal-^oC6** and **Gal-^pC6**, directly dissolved into water above their critical micellar concentration (CMC) values, have been imaged by means of transmission electron microscopy (TEM) (Figure 1). Estimated from the change of the surface tension with increasing concentration (See Supporting Information), the CMC values for **Gal-^oC6**, **Meli-^oC14** and **2'FL-^oC14** were found to be 2.4 mM, 12 μ M and 12.2 μ M, respectively.

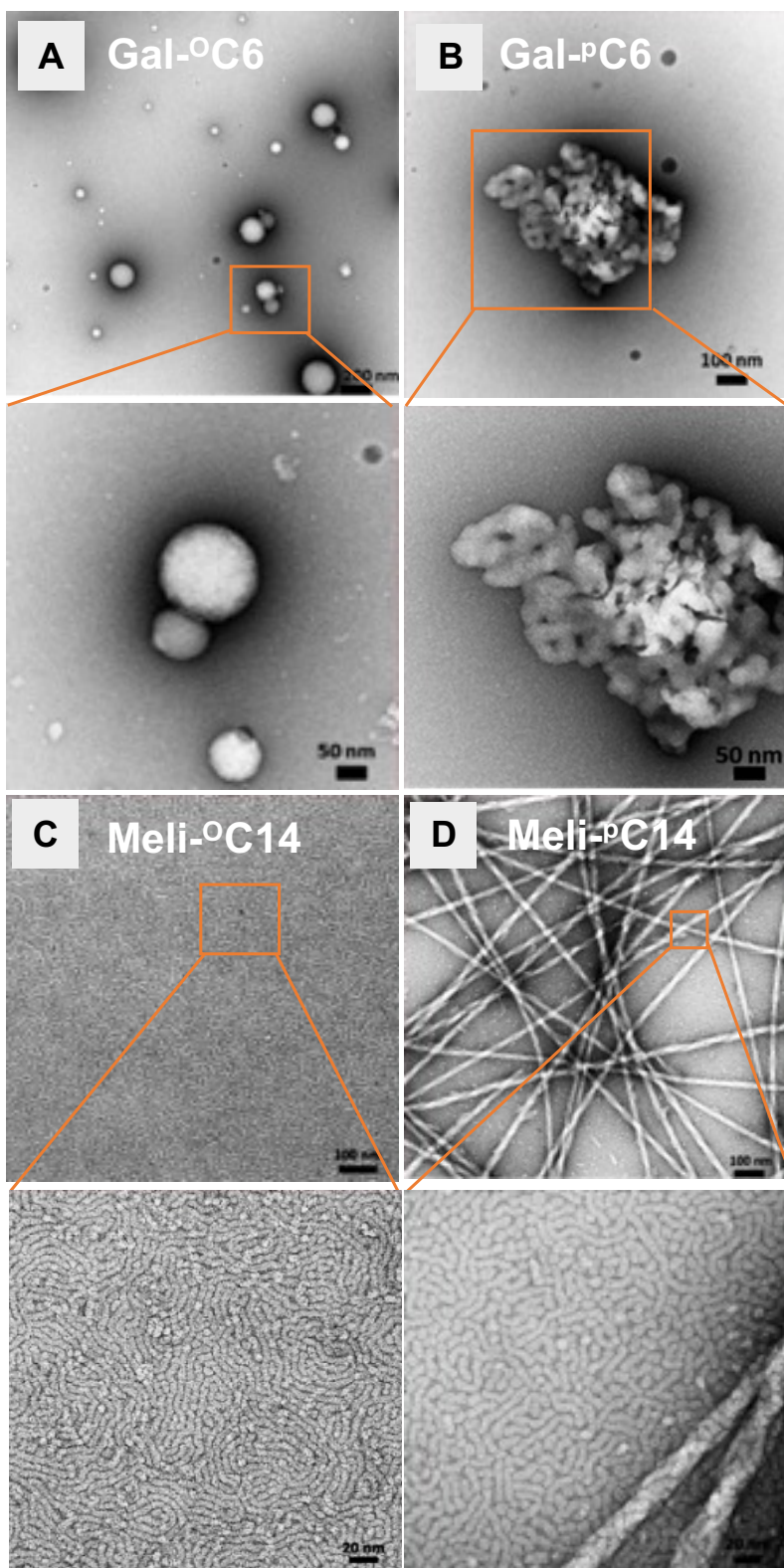


Figure 1. TEM images with enlarged zones of A) Gal-^oC6 (2.6 mM), B) Gal-^pC6 (2.6 mM), C) Meli-^oC14 (0.15 mM) and D) Meli-^pC14 (0.15 mM) aggregates in water.

Polydisperse spherical micelles with a cross-sectional diameter within a wide range of 5 to 300 nm were observed for **Gal-^oC6** (Figure 1A), while for the position isomer **Gal-^pC6** (Figure 1B), elongated aggregates of ring-shaped micelles (nanodonuts) have been obtained with an outer diameter of ~100 nm, and an inner diameter of ~10 nm. The average thickness of the nanodonuts is ca. 20 nm on the sides and ca. 75 nm on the ends. The nanodonut structure is supposed to be the elongated micelle and is curved to be a closed ring since some spherical micelles coexist (Figure 1B and S48). Remarkably, well-homogeneous tortuous worm-like micelles were formed for **Meli-^oC14** (Figure 1C), which could be clearly observed in the enlarged picture, and characterized by a uniform thickness of ~4nm and few micrometers length. For **Meli-^pC14**, worm-like micelles were still observed but in mixture with micrometer-long helicoidal rigid nanofibers with a mean core diameter of ~20 nm (Figure 1D). In addition, the product **2'FL-^oC14** also forms well-organized worm-like aggregates in water (Figure 2A).

The different morphologies are undoubtedly attributed to the molecular geometric considerations and subsequent molecular packing. Indeed, it is well-established that the self-assembly process is roughly governed by geometrical constraints impacting the surface curvature of individual uncharged amphiphile molecules (packing parameter),²⁶ in addition to the attractive hydrophobic interactions between the lipophilic segments and repulsive steric interactions between the hydrophilic headgroups.^{27,28} This packing parameter (p) is based on the formula $p=V/al$, where V , a , and l denote the volume of hydrophobic chains, the cross-sectional area of the hydrophilic head and the length of the hydrophobic chain respectively. Spherical micelles can be formed if $p \leq 1/3$, cylindrical or rod-like micelles if $1/3 \leq p \leq 1/2$, bilayer or lamellar sheets forms if $1/2 \leq p \leq 1$. Attempting to rationalize the results, we performed p calculations and found that for **Meli-^oC14** or **Meli-^pC14**, the p -value was ca. $1/3$ confirming the rod-like aggregates. Concerning **Gal-^oC6** or **Gal-^pC6**, a p -value of 0.29 was consistent with the spherical micelles showed by TEM (details of calculation in supporting information, page S39). However, the most striking result is the positional isomeric effect on morphological transitions. By comparing morphologies of **Gal-^oC6** (spheres) vs. **Gal-^pC6** (donuts) and **Meli-^oC14** (worms) vs. **Meli-^pC14** (worms/fibers), it appears that the orientation of the alkyl chain around the aryl ring profoundly impacts the self-assembly properties. In first approximation, if one considers that these glycoamphiphiles adopt “head-to-head, tail-to-tail” packing models with partial interdigitation of the hydrophobic alkyl chain, the *para*-isomer would have the largest average interfacial area per molecule compared to the *ortho*-isomer, and subsequently exert subtle difference in molecular packing. The shape transition in solution with respect to the position of the hydrophobic *n*-alkyl chain on the aromatic ring for amphiphilic β -*N*-aryl glycosides had not yet been studied in the literature.²⁹

The morphologies of the self-assembled glyconanostructures have been obtained in pure water that are quite far from buffered aqueous solutions needed for optimizing the interactions with lectins. When deionized water was replaced by Tris buffer, TEM images (Figure 2) of **2'FL-^oC14** showed that the worm-like micelles were still existing but in shorter lengths and thicknesses. Noteworthy, when 10% of DMSO was added to ensure better solubilization of glycoamphiphiles since it is a good solvent as well for the carbohydrate as for the alkyl chain, a solvent-driven evolution of glycoamphiphiles morphology was identified, *i.e.*, morphology transition from worm-like (0% DMSO) to spherical micelles (10% DMSO) (Figure 2).

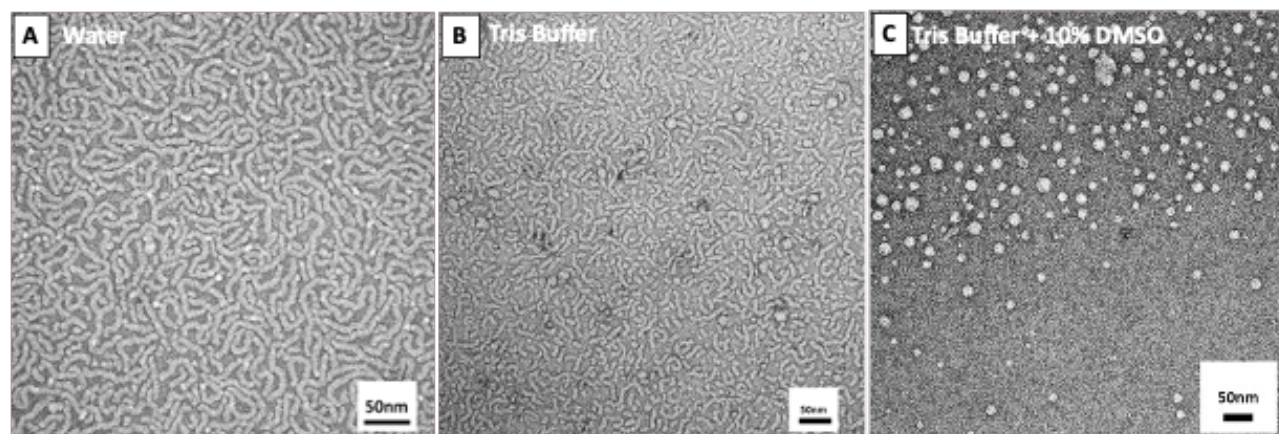


Figure 2. TEM images of morphological transition of 2'FL-^oC14 with respect of solvent composition.

The same shape evolution has been observed for **Meli-^oC14** (Figure S46). **Gal-^oC6** and **Gal-^pC6** were fully dissolved avoiding the presence of self-assembled glyconanostructures while **Meli-^pC14** moved to a mixture of spherical and remarkable helical stack bilayer micelles (Figure S47). Even if **Mal-^oC14** was synthesized as negative control for lectin binding tests, we checked its morphology in same aqueous conditions and observed tortuous worm-like micelles (Figure S47). The nature of the solvent as well as the salt concentration/ionic strength in solution affect the balance between the supramolecular interactions and the degree of swelling that result in different shapes of self-assembled nanostructures.³⁰

2.4. Studies of the interaction of glycoamphiphile micelles with lectins

Self-assembly of amphiphilic β -*N*-aryl glycosides into supramolecular nanostructures coated by carbohydrates suggests that they may be used as supramolecular multivalent ligands.³¹

Table 3. ITC Data for *N*-(β -D-glycosyl)2-alkylbenzamide derivatives binding to bacterial lectins LecA, LecB, and RSL. Standard deviation is calculated over 2 when possible

Lectin	Ligand	n	$-\Delta H$ [kJ mol ⁻¹]	$T\Delta S$ [kJ mol ⁻¹]	$-\Delta G$ [kJ mol ⁻¹]	K_D [μ M]	Morphologies observed by TEM	
LecA	PNPGal ^a	0.95	40.9	-18.4	26.3	26.1	-	
	Gal- ^o C6	0.97±0.05	39.6±1.8	-14.6	25.0	41.6±4.7	-	
	Gal- ^p C6 ^b	0.98	38.5	-13.0	25.5	34.7	-	
	Melibiose ^b	1 ^c	33.6±2.8	-8.20	25.4	36.0±3.0	-	
	Meli- ^o C14	1 ^c	26.7±2.7	-5.8	20.9	197±47	Spheres	
	Meli- ^p C14	Weak titration – no quantification						Spheres + helical stack bilayer
	Malt- ^o C14	No binding						Worms
LecB	2'FL ^b	1.06	31.4	4.6	36	0.50		
	2'FL- ^o C14	Weak titration – no quantification						Spheres
	Malt- ^o C14	No binding						Worms
RSL	2'FL ^d	2.0	42.1	-7.1	35.0	1.4	-	
	2'FL- ^o C14	1.58±0.13	23.9±0.3	12.8	36.8	0.365±0.016	Spheres	

^aReference data for LecA/PNPGal from ref 34. ^bOne experiment only for Gal-^pC6 and for reference oligosaccharides. ^cStoichiometry fixed to 1 during fitting procedure due to low affinity. ^dReference data for RSL/2'FL from ref 15.

An assessment of the interaction of **Gal-^oC6**, **Gal-^pC6** and **Meli-^oC14** with LecA and **2'FL-^oC14** with LecB and RSL was conducted by means of isothermal titration calorimetry (ITC). The solubility of **2'FL-^pC14** and **Fuc-^oC6** were not sufficient to conduct experiments. Corresponding non-lipidated glycans, *i.e.* *p*-nitrophenyl β -galactoside (PNPGal), melibiose and 2'-fucosyllactose (2'FL) were also assayed for comparison and for checking the binding activity of the lectins. For these soluble glycans, data were in agreement with those reported in literature, when available (Table 3).

Different types of thermograms were obtained for the amphiphilic compounds, as displayed in Figures S2-S4. Strong heat of interaction, with titration at expected values of one ligand per monomer (LecA) or two ligands (RSL) were obtained for most assays, except with LecB that gave non interpretable thermogram with 2'FL-based glycoamphiphiles. This indicates the sugar head is in general accessible for binding to lectins. When possible, dissociation constant (K_D) and thermodynamic contributions were obtained from fitting procedure and are reported in Table 3.

The galactose-based monosaccharides ligands interacted with LecA in the same micromolar range than the PNPGal, and with comparable enthalpy and entropy contribution, indicating that both **Gal-^oC6** and **Gal-^pC6** compounds are fully soluble in the tested experimental conditions and that the galactose moiety is accessible for binding. Melibiose-derived compounds gave mixed results, with **Meli-^oC14** acting as a slightly weaker binder than the soluble disaccharide **Meli** while **Meli-^pC14** did not interact clearly with the lectin. The longer *n*-alkyl chain seems to induce some aggregation, that is also dependent on the linkage between the disaccharide and the aliphatic substituent. In the conditions of ITC measurements, **Meli-^oC14** exists as spherical micelles while **Meli-^pC14** self-assembles into coexisting spherical and helical stack bilayer micelles. The difference of binding with LecA could stem from this difference of morphologies where spherical micelles are more prone and accessible for interaction with LecA lectin.

The effect of long *n*-alkyl chain is very impacting for the binding of **2'FL-^oC14** to the tetrameric lectin LecB. While the soluble trisaccharide **2'FL** has a strong affinity of 500 nM for the lectin, the heat release by **2'FL-^oC14** is very weak (Figure S3(E)) and even if some titration occurs, it is not possible to interpret it in a quantitative manner. The aggregative state of the amphiphilic compound does not allow for an effective binding of the lectin. Interestingly the same compound binds very strongly to RSL with K_D of 356 nM, *ca.* 4 times better than the soluble trisaccharide 2'FL. All binding sites are not occupied ($n = 1.58$ instead of expected $n = 2$) resulting in a loss of enthalpy contribution compared to the free oligosaccharide ($\Delta H = -23.9$ kJ/mol compared to $\Delta H = -42.1$ kJ/mol), but this

is more than compensated by the entropic contribution that is strongly favorable for the amphiphilic compound ($-\Delta S = -12.8$ kJ/mol) instead of classical unfavorable entropy barrier of ($-\Delta S = 7.1$ kJ/mol) observed for 2'FL. The micellar aggregation of 2'FL-**C14** therefore prevents the fucose-dependent binding by LecB but enhances the one by RSL. This could be correlated to the multivalent architecture of the two lectins: LecA has a ball shape with four binding sites pointing in different directions, while RSL has a donut shape with six binding sites on the same face. It has been previously demonstrated that multivalent presentation of fucose result in several order of magnitude enhancement of binding constant for RSL and related lectins BambL and AFL.^{32,33}

3. CONCLUSION

In summary, we have developed an expeditious synthetic access to amphiphilic β -*N*-aryl glycosides *via* a microwave-assisted β -*N*-arylation of protecting group-free carbohydrates. This method allowed the introduction *ortho*-substituted aniline derivatives, obtained from isatoic anhydride, on the reducing end of carbohydrates in a short, efficient, and concise way. The solution self-assembly of a series of glycoamphiphiles was systematically investigated showing that morphologies of the glyconanoparticles were related to the molecular geometric considerations as evidenced by the positional isomeric effect on self-assembly properties. The *n*-alkyl chain position provides an excellent approach for controlling shapes of the self-assembled objects. Finally, glyconanoparticles were successfully applied in lectin recognition from pathogenic bacteria and demonstrated the potential of amphiphilic β -*N*-aryl glycosides micelles as possible anti-adhesives for the prevention of pathogen infection or as ligands for the detection of lectins from pathogens in a biosensor device.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Materials and methods, synthesis of glycoamphiphiles, characterization data (NMR, Mass spectrometry, HPLC) and additional experimental data and images on self-assembly of glycoamphiphiles and interaction studies with ITC (PDF)

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The manuscript was written through contributions of all authors and all authors have given approval to the final version of the manuscript.

Funding Sources

French Research National Agency (ANR) via the PRC project “Sweet-Display” (ANR-21-CE07-0050).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors acknowledge the financial support from the French Research National Agency (ANR) via the PRC project “Sweet-Display” (ANR-21-CE07-0050). We thank the Institut Carnot PolyNat (ANR-21-CARN-0025-0), CBH-EUR-GS (ANR-17-EURE-0003) and Glyco@Alps (ANR-15-IDEX-02) for additional supports. We acknowledge the NanoBio ICMG (UAR 2607) for providing facilities for mass spectrometry, NMR analyses and electronic microscopy. We thank the members of the chromatography platform of CERMAV for their help in performing HPLC analyses.

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