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***N,N'*-Disubstituted Barbituric Acid: A Versatile and Modular Multifunctional Platform for Obtaining β -C-Glycoconjugates from Unprotected Carbohydrates in Water**

François Portier, Justine Solier and Sami Halila*

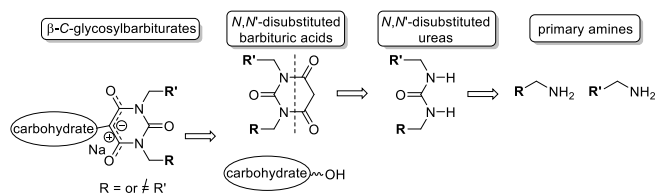
Abstract: Direct access to non-hydrolyzable glycoconjugates is highly mandatory for stabilizing their intrinsic biological or physical-chemistry properties in the surrounding environment. Here, a simple and versatile approach for the preparation of multifunctional clickable β -C-glycoconjugates has been developed. Readily accessible *N,N'*-disubstituted barbituric acids have been synthesized and ligated in water to the reducing end of unprotected carbohydrates via Knoevenagel condensation. Additionally, divalent glycoclusters have been built from bis-urea backbones and showed ability to interact with a model lectin, the *peanut agglutinin*. This concept can be readily adapted for the preparation of other C-glycoconjugates applicable in diverse area of glycosciences.

Carbohydrate-binding proteins are ubiquitous in Nature and include lectins, glycosyl-enzymes, growth-factors, cancer-related antibodies, carbohydrate receptors in plants and many more.^[1] Therefore, neoglycoconjugates, characterized by having a non-native and non-hydrolyzable sugar-aglycon linkage, has received considerable recent attention due to the increasing need to develop stable molecular tools in glycobiology and carbohydrate-based therapeutic and diagnostic substances. Neoglycoconjugate formation that enable direct functionalization of the reducing end of mono or oligosaccharides while maintaining the cyclic structure is particularly appealing for keeping the biological activity.^[2,3] In this regard, the chemoselective conjugations so far developed on free carbohydrates can be split roughly into two main streams: The first one consists in selective activation of the anomeric hydroxyl group such as the use of 2-chloro-1,3-dimethylimidazolium chloride^[4] and the second one involves the nucleophilic attack on the terminal or reducing end aldehyde, typically with an amine, oxyamine or hydrazine, and more occasionally, with an activated β -diketone.^[3,4] The latter is known as Knoevenagel condensation to afford β -C-glycosides that are an important class of hydrolytically stable glycomimetic finding development as type II anti-diabetes (dapagliflozin, canagliflozin, and empagliflozin) or as anti-aging cosmetic agent (Pro-Xylane).^[5] Moreover, compare to oxyamine or hydrazine that are highly reactive and thus quite hardly handled, β -diketone is a latent C-nucleophile activatable upon deprotonation. Surprisingly, since the first report of Knoevenagel condensation on unprotected sugars using barbituric acid (a cyclic β -diketone) or *N,N'*-dimethylbarbituric acid in 1983 by Galbis Perez et al.,^[6] only few published works have benefited from this chemo- and β -stereo-selective reaction.^[7-9] Advantageously, the Knoevenagel condensation was performed in water at neutral pH and led to sodium salt of β -C-glycosylbarbiturates in excellent yields. In continuation of our long-term program of chemo-selective synthesis of (neo)glycoconjugates with the ultimate goal of facilitating the development of glycosciences,^[10-12] we were interested in exploring barbituric acid-mediated Knoevenagel condensation and determined structural parameters of β -C-glycosylbarbiturates required for the interaction with pathogenic lectins.^[13]

Here we propose a new generation of barbituric acid derivatives incorporating clickable functions on each of the nitrogens of the barbituric ring and perform the Knoevenagel condensation on unprotected carbohydrates (Scheme 1) in water. Thanks to the ease and versatility of the barbituric ring formation, we were able to produce a wide range of derivatives. The *N,N'*-disubstituted barbituric acids were produced in two steps consisting in synthesizing first the *N,N'*-disubstituted urea which was next cyclized with malonyl chloride. Using this methodology, three set of barbiturate derivatives were synthesized affording homo-bis, hetero-bis or mono-clickable derivatives. Clickable functions were targeted because of the multitude of relevant glycoconjugate architectures accessible through click reactions.^[14] Additionally, we present an elegant way to prepare a divalent ligand of *peanut agglutinin* (PNA) lectin starting from readily accessible diamine derivatives.

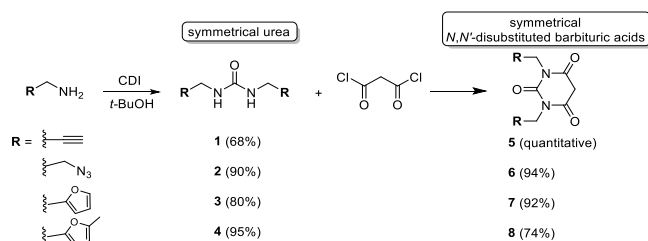
[*] Dr. F. Portier, J. Solier, Dr. S. Halila
Department of Physical-Chemistry and Self-Assembly of
Glycopolymers
Univ. Grenoble Alpes, CNRS, CERMAV
BP53, 38041 Grenoble Cedex 9, France.
Correspondence: sami.halila@cermav.cnrs.fr

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Scheme 1. Retrosynthetic pathway for the synthesis of clickable β -C-glycosylbarbiturate derivatives.

The symmetrical clickable barbituric acid derivatives were easily prepared in two steps and good overall yields (~75%) from the corresponding clickable primary amine. First, the propargylamine or 2-azidoethylamine or furfurylamine or 5-methylfurfurylamine was reacted with carbonyldiimidazole (CDI) in *t*-BuOH affording the homo-bis-clickable *N,N'*-disubstituted urea **1**, **2**, **3** and **4**, respectively (Scheme 2).^[15] Interestingly, the urea precipitated from the reaction mixture and was purified by simple filtration. The preparation of urea also worked with water but with led to lower yield certainly because of the poor stability of CDI in water. Next, cyclization of the urea with malonyl chloride afforded the corresponding cyclic barbituric acids **5**, **6**, **7** and **8**.^[16] Attempts to form the *N,N'*-disubstituted barbituric acids by microwave-assisted cyclization of *N,N'*-disubstituted ureas with malonic acid in a mixture of acetic anhydride and acetic acid were performed but the desired products were always accompanied with a minor side-product consisting in 5-acetyl form.^[17] The propargyl or azide function and furan derivatives were selected due to the possibility to perform a Huisgen 1, 3-dipolar cycloaddition^[18] and Diels-Alder^[19] click reaction, respectively.



Scheme 2. Synthesis of symmetrical clickable barbituric acid derivatives. (For details, see SI)

With clickable barbituric acid derivatives in hand, we then set out to determine the efficiency of Knoevenagel condensation with galactose (Gal) in water at neutral pH and at 80°C. The condensation of **5** and **6** proceeded smoothly and with complete β -stereoselectivity (¹H NMR: $J_{1,2} \sim 10$ Hz) to give sodium salts of β -C-galactosylbarbiturates **9** and **10** in 63% and 70% yield, respectively (Entries 1-2; Table 1). The condensation of difurfuryl barbituric acid **7** led to a complex mixture where a dimeric glycosylbarbiturate adduct, resulting probably from an homocoupling of furan cycle, was detected by mass spectrometry (data not shown). We guessed that the Knoevenagel condensation of 5-methylfuran derivative **8** could prevent the formation of side-products. And in fact, we obtained the expected di(5-methylfurfuryl) β -C-D-galactosylbarbiturate **11** with a satisfactory isolated yield (63%) (Entry 3; Table 1). Next, the reaction scope was investigated with respect to a selection of mono- (*N*-acetylglucosamine (GlcNAc)), di- (lactose (Lac) and maltose (Mal)) and oligo-saccharides (maltoheptaose (MH)) (Entries 4-7; Table 1). The desired β -C-glycosylbarbiturates were obtained in a completely stereoselective manner with yields ranging from 50% to 83%. These results clearly show that the conjugation successfully proceeded with numerous carbohydrates and could be certainly optimized for the oligosaccharide, MH.

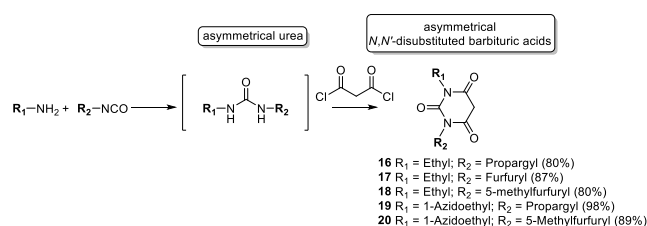
Table 1. Knoevenagel condensation of unprotected reducing carbohydrates in water with homo-bis-clickable barbituric acids.

Entry ^[a]	Carbohydrate	Product ^[b]	Yield ^[c]
<i>Homo-bis-clickable</i>			
1	Gal		63%

2	Gal		70%
		10	
3	Gal		64%
		11	
4	GlcNAc		83%
		12	
5	Lac		60%
		13	
6	Mal		78%
		14	
7	MH		50%
		15	

[a] The reactions were carried out in water at pH 7 for 5 h (entries 1–5) and 24 h (entry 6) at 80°C by using 1 equivalent of barbituric acid derivative. [b] In all cases, only pure β products were formed. [c] Isolated yields.

Aiming to demonstrate the versatility of the barbituric acid platform for the construction of various C-glycoconjugates in water, we extended the repertoire by preparing asymmetrical clickable barbituric acids containing either mono-clickable or hetero-bis-clickable functions (Scheme 3).




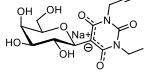
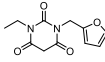
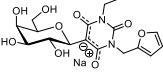

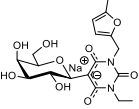
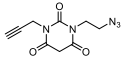
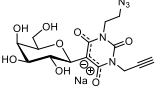
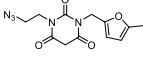
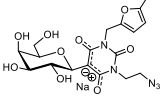
Scheme 3. Synthesis of asymmetrical clickable barbituric acid derivatives. (For details, see SI)

The asymmetrical barbituric acids were obtained from a simple and rapid two successive steps procedure. First, commercially available reagents such as furfurylamine, 5-methylfurfurylamine or propargylamine were reacted with ethyl isocyanate in dry dichloromethane generating mono-clickable asymmetrical ureas as intermediates (Scheme 3). For the hetero-bis-clickable urea, 2-chloroethylisocyanate was reacted either with propargylamine or 5-methylfurfurylamine and the chloro atom was readily substituted with sodium azide in DMF to afford *N*-azidoethyl-*N'*-propargylurea or *N*-azidoethyl-*N'*-(5-methylfurfuryl)urea with 98% and 89% overall yield, respectively. As previously, the formation of *N,N'*-disubstituted barbituric acids was performed by urea cyclization with malonyl chloride affording **16–20** with excellent overall yields (80–98%) (Scheme 3).

We next turned our attention to the Knoevenagel condensation of barbituric acids **16–20** with galactose according to our previous optimal conditions. Gratifyingly, the efficacy of the coupling was still high (~60–70%) and once again in a β -stereoselective manner affording the corresponding mono-clickable β -C-galactosylbarbiturates **21** (alkyne) and **23** (methylfuran), or the hetero-bis-clickable one **25** (methylfuran/azide) (Table 2). The Knoevenagel condensation with the furan barbituric derivative **17** led again to a complex mixture, confirming the instability of the furan in the coupling

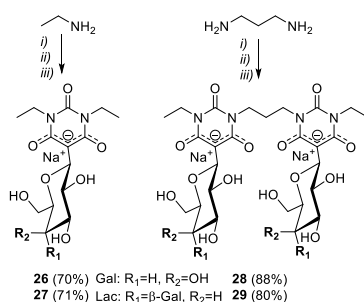
conditions. A similar behavior was observed for the azide/alkyne barbituric derivative **19** where the ^1H NMR indicated the presence of peaks around 7-8 ppm that could be attributed to *H*-triazole protons resulting from the cycloaddition of the azide and alkyne function (data not shown). Nevertheless, thanks to simple and efficient chemistries whose versatility core is based on the readily accessible symmetrical or asymmetrical ureas, we were able to design multifunctional “clickable” C-glycoconjugates.

Table 2. Knoevenagel condensation of D-galactose in water with mono- and hetero-bis-clickable barbituric acids.

Entry	Barbituric acid derivatives ^[a]	Product ^[b]	Yield ^[c]
<i>Mono-clickable</i>			
1	 16	 21	72%
2	 17	 22	impure
3	 18	 23	73%
<i>Hetero-bis-clickable</i>			
4	 19	 24	impure
5	 20	 25	58%

[a] The reactions were carried out in water at pH 7 for 5 h at 80°C by using 1 equivalent of barbituric acid derivative. [b] In all cases, only pure β products were formed. [c] Isolated yields.

Following the perspective to expand our synthetic method, we next investigated the possibility to perform the Knoevenagel condensation of carbohydrates with polybarbituric acids that could be obtained from the corresponding polyureas and earlier from polyamines. This extension could lead to hydrolytically stable multivalent carbohydrate ligands useful for greatly improving carbohydrate/lectin interactions (glycoside cluster effect)^[20] and *in fine* for the development of carbohydrate-based anti-adhesion drugs tackling microbial diseases.^[21] As proof of concept, we prepared galactose-based divalent clusters toward the well-studied *peanut agglutinin* lectin (PNA), an homotetrameric protein which preferentially binds galactose (Gal)-containing carbohydrates such as tumour-associated Thomsen-Friedenreich antigen (T-antigen; Gal β 1-3GalNAc) or lactose (Lac; Gal β 1-4Glc).



i) EtNCO; ii) Malonyl chloride; iii) Knoevenagel condensation on Gal and Lac

Scheme 4. Monovalent and divalent galactose-based ligands obtained from monoamine and diamine starting materials, respectively. (For details, see SI)

The divalent galactosylated ligands integrating a short aliphatic linker was based upon the coupling of commercially available 1,3-diaminopropane with ethylisocyanate followed by cyclization with malonyl chloride to afford the bis-barbituric acid derivative (Scheme 4). The latter was condensed with galactose or lactose in water at 80°C to give the divalent ligands **28** and **29**, respectively, with good yields. The monovalent C-galactoside and lactoside ligand **26** and **27** were synthesized as reference standards for comparative binding studies studied by Isothermal Titration Calorimetry (ITC).

The experimental ITC data (see SI) evidenced that galactosyl monosaccharides whether the monovalent **26** or the divalent **28** ones, do not interact with PNA. These results confirmed our recent observation^[13] revealed that the presence of the barbituring ring directly linked to the recognition carbohydrate is detrimental for the related lectin binding relying on steric hindrances. However, the interaction could be restored by using oligosaccharides enabling distance of the recognition carbohydrate from the barbituric ring. Indeed, a dissociation constant (K_D) of the monovalent C-lactoside **27** in interaction with PNA was found around 260 μM , which is a typical value range for monovalent carbohydrate/lectin interactions. On a valency-corrected basis, the divalent C-lactoside **29** (K_D of 10,3 μM) exhibited a 13-fold affinity gain compared to the corresponding monovalent ligand **27**, which highlighted a moderate multivalent effect. Nevertheless, most important we showed that barbituric-based scaffold is a new convenient candidate for preparing glycoclusters. Finally, the work could be easily extended to prepare multivalent clusters with varying interligand spacing^[22] in order to exhibit a chelate cooperativity where significant enhancements of several orders of magnitude can be achieved when the tethered ligands of a glycocluster fit the distance between two carbohydrate binding domains of a single lectin.^[23] Works are ongoing in this direction.

In conclusion, we developed a new entry to C-glycoconjugates bearing clickable functions via the use of readily accessible *N,N'*-disubstituted barbituric acids. Experimentally simple and environmentally friendly, the Knoevenagel condensation was performed in water onto reducing sugars without the need for any protecting groups. Mono, homo-bis, and hetero-bis clickable carbohydrates have been designed and prepared according to an unprecedented modular and tunable ligation method. We also demonstrated that this process can be extended to the preparation of galactose-based glycoclusters interacting with PNA lectin. We believe this work is a significant contribution to the field of glycosciences, providing a versatile ligation platform that are synthetically accessible to a wide range of researchers. By the way, application of this methodology brings a new opportunity for the modification of aldehyde-containing materials.

Acknowledgements

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Keywords: carbohydrates • Knoevenagel condensation • barbituric acids • click chemistry • glycoclusters

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