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## Stable glycosylamines at the reducing ends of cellulose nanocrystals†

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**The reaction of reducing end groups in cellulose nanocrystals with dodecylamine was examined. Using a direct-dissolution solution-state NMR protocol, the regioselective formation of glucosylamines was shown. This provides an elegant approach to sustainably functionalize these bio-based nanomaterials, that may not require further reduction to more stable secondary amines.**

Regio-selective modifications of cellulose nanocrystals (CNCs) are experiencing increasing attention as an avenue toward designing functional nanomaterials; from liquid crystals<sup>1</sup> to complex 2D nanostructures.<sup>2,3</sup> Functionalization of the reducing end groups (REGs) of the cellulose chains, by means of aldehyde-specific chemistry, is probably the most prominent avenue, yielding anisotropically modified nanoparticles.<sup>4,5</sup> Two synthetic approaches have been mainly explored, by targeting the reducing end ‘aldehydes’ directly with hydrazine derivatives (ligation reactions),<sup>6–9</sup> or primary amines through reductive amination.<sup>10,11</sup> A new approach using dicarbonyls<sup>12</sup> (Knoevenagel condensation), may also show promise in the future. All these reactions are carried out under heterogeneous conditions, *i.e.*, modifying the CNCs while dispersed in water, typically at a low consistency. To compensate for the high dilutions and to drive the reaction to completion, reagents are often used in excessive stoichiometries, at the cost of scalability and atom economy. Moreover, the chemistry applied to the reducing end of CNCs is often not well understood, due to the lack of suitable methods for structural characterisation. Recently, a solution-state NMR technique using the electrolyte [P<sub>4444</sub>][OAc]:DMSO-*d*<sub>6</sub> to dissolve crystalline celluloses has emerged,<sup>13–15</sup> enabling profound analysis at the molecular level. Combined with model reactions on water-soluble

saccharides, this method allowed to confirm the selectivity of Knoevenagel condensation<sup>12</sup> and reductive amination<sup>16</sup> toward CNC REGs.

Given the commercial availability of different amines, reductive amination chemistry seems promising for CNC derivatization. However, there are drawbacks when upscaling. Most reports involve the use of significant excesses of reagents (*e.g.*, 300 eq. reducing agent in a recent report<sup>16</sup>). Additionally, the use of typical borohydride reductants, *e.g.*, sodium cyanoborohydride, causes contamination with residual boron-containing by-products. Common dialysis protocols needed to successfully tackle such contaminations of modified CNCs are not economical at scale. The same applies for azeotropic distillation, which was proposed for the removal of 2-picoline-borane (PICB)<sup>17</sup> or 5-ethyl-2-picoline borane (PEMB).<sup>18</sup> Another challenge emerges in identifying if the reduction step has even occurred. Even though several recent articles use reductive amination to modify the reducing ends of CNCs and plenty of indirect evidence shows the success of such reactions,<sup>10,11,19,20</sup> only one article gives direct NMR spectroscopic evidence of secondary amine formation.<sup>1</sup>

In general, when targeting REGs for the selective modification of CNCs, the reactivity of the REG is presumed to be as the ‘aldehyde’ form, as in organic chemistry textbooks. However, the peculiar and complex chemistry of sugar aldehydes is well known in other research areas. The presence of several hydroxy functionalities in the structure influences the overall reactivity of the aldehyde over the presence of ring-chain equilibria or the potential occurrence of further rearrangements. Especially in investigations towards reactions of saccharides with amines these phenomena prove to be consequential. This is well documented in the Maillard reaction, where initial steps involve imine formation and subsequent rearrangements, or further cascade reactions, leading to a wide variety of products, generally associated with food aromas.<sup>21</sup> These initial reactions are also important for the synthetic stabilization and bioavailability of proteins,<sup>22,23</sup> with the first naturally occurring protein glycosylamine linkages identified in 1961.<sup>24</sup> Aside from the synthesis of complex organic molecules, such chemistry has

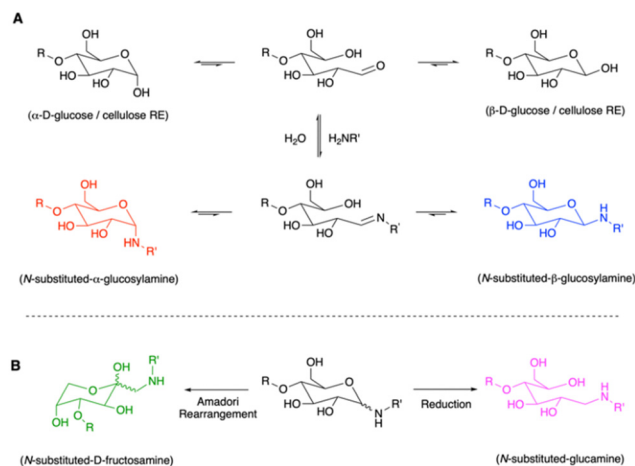
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**Scheme 1** Known pathways involved in the reaction of glucose with primary amines – probed for the modification of the REGs of CNCs in this work: (A) In contact with solvent the more stable  $\alpha$  and  $\beta$  anomers of the reducing sugars are in equilibrium with their open chain form. In the presence of an amine the aldehyde can react under formation of an imine (Schiff base), which is in turn in an equilibrium with the  $\alpha$  and  $\beta$  glucosylamines; (B) Potential side reactions during glucosylamine preparation are Amadori rearrangements, resulting in fructosamines. These represent intermediates in the Maillard reaction cascade. The reductive amination of CNCs to glucamines can circumvent this and potential hydrolytic stability issues but at cost. ( $R = H$  for glucose;  $R =$  anhydroglucose units (AGUs) for cellulose;  $R' =$  alkyl).

also been proven to be commercially relevant, *e.g.*, in the production of surfactants *via*: (A) the reductive amination of sugars with fatty amines,<sup>25</sup> or (B) the lipase-catalysed reaction of fatty acid carbonyls with *N*-methylglucamines, to form acylglucamides.<sup>26</sup>

Irrespective of the intended products the first stage in the reaction of sugar REGs with an amine is believed to involve the formation of an imine at the intermediate open-chain aldehyde of the terminal saccharide (Scheme 1). The carbonyl and imine species are in equilibrium with their respective anomeric cyclic structures. In the case of glucose, as an intensively studied model compound, these include its  $\alpha$  and  $\beta$  anomers and the derivative  $\alpha$  and  $\beta$  glucosylamines (Scheme 1A). In water and in the absence of reducing conditions, the bond between amine and sugar is known to be rather hydrolytically labile. However, the equilibria can be influenced by the amine substituents, *e.g.*, over their electron donating or withdrawing nature, their steric demand and hydrophobicity. Also, solvent effects and pH of the media are important factors.<sup>27</sup>

While rapid hydrolysis can be expected in many cases, certain *N*-substituted glucosylamines and other glycosylamines (derived from other monosaccharides than glucose) are stable, isolable, and well-studied, especially concerning glycoconjugation, further Amadori rearrangements<sup>28</sup> and Maillard cascade reactions.<sup>21</sup> Many synthetic procedures exist for isolation of glycosylamines. They are reported to become more hydrolytically stable at higher pH ranges,<sup>27</sup> with the Amadori rearrangement (Scheme 1B) promoted under addition of catalytic amounts of weak acids.<sup>29–31</sup>

In the case of nanocellulose modification, as mentioned before, excess reducing agent and amine is typically applied to drive the reaction to completion, to yield a hydrolytically stable *N*-substituted glucamine terminus (*i.e.*, secondary amine, Scheme 1B). Considering the discussed drawbacks, we were interested if REG modifications with primary amines can also be performed in a more sustainable and scalable manner. While this represents a rather trivial challenge from an organic chemist's preparative perspective – apart from the heterogenous reaction conditions needed for CNC modification – the unambiguous identification of the planned chemistry remains complex. However, understanding of the formed structures is important as they strongly influence the stability of the derivatization and, thus, set the scope for potential applications. If we wish to move toward sustainably modified cellulose and more specifically nanocellulose REG modifications, we need to begin to chart the chemistry at the REG – by providing direct high-resolution spectroscopic proof, as is standard in organic chemistry.<sup>4</sup>

Moreover, scalable, economically, and ecologically viable alternatives for CNC modifications must be sought, where the mechanistic understanding is the critical starting point, to avoid the existing problems. In this sense we showcase, herein, how formation of glycosylamines, at the REGs of CNCs, can be elucidated by applying a developing solution state NMR protocol utilising direct dissolution of the CNCs into an ionic liquid-DMSO- $d_6$  electrolyte.<sup>13–15</sup>

After a preliminary screening study (ESI,† S2), reaction of glucose with the long-chain aliphatic primary amine dodecylamine (DDA) gave a stable product for further analysis. Consequently, a model glucosylamine (anomeric mixture of  $\alpha$  and  $\beta$ ) was prepared by reaction of glucose with DDA (1.2 eq. to glucose). Recrystallization of the  $\alpha$  and  $\beta$  mixture resulted in the isolation of a sample of pure  $\beta$  glucosylamine, for  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR spectral assignment. The assignments for the  $\beta$  anomer and the  $\alpha$  and  $\beta$  mixture are shown in ESI,† Fig. S2. For comparison, the reductive amination of glucose was performed by addition of PEMB to yield the dodecylglucamine for HSQC NMR signal assignment (ESI,† Fig. S3). Clearly there is a difference between the 1- and 2-position saccharide resonances in the  $^1\text{H}$  and HSQC spectra for the dodecylglucamine – the main diagnostic peaks in the HSQC spectrum (the anomeric CH-1 correlations) are missing in the dodecylglucamine sample. The *gem*- $\alpha$  correlations are also now shifted downfield in both spectral ranges.

Given the successful preparation of the model compound we applied the reaction conditions (0.24 eq. of DDA vs AGU, heating at 70 °C for 3 d) to commercial CNCs. To examine solvent effects the reactions were performed both as dispersions in water and anhydrous DMSO. It was assumed that the reaction in DMSO would be a lot more amenable to shifting the equilibrium towards the product and allow for easier access to the reducing ends, due to lower aggregation of the CNC dispersion in dipolar aprotic solvents. The products were analysed by diffusion-edited  $^1\text{H}$  NMR (ESI,† Fig. S4), in the  $[\text{P}_{4444}][\text{OAc}]:\text{DMSO}-d_6$  electrolyte (direct dissolution NMR solvent),<sup>13,14</sup> which clearly indicated that the reaction had occurred in both solvents. Despite strong



peak superposition with the used electrolyte in other NMR experiments, the diffusion-editing allows to straightforwardly examine the derivatization. The editing removes the fast-diffusing species, *e.g.* H<sub>2</sub>O, [P<sub>4444</sub>][OAc], DMSO-d<sub>6</sub> and DDA, leaving only the slow-diffusing polymeric species (including DDA covalently attached to the CNCs) visible in the spectra. Expectedly, the reaction in DMSO showed a higher conversion to the aminated product, evident by the increase in intensity of the aliphatic signals (<3 ppm), relative to the water reaction. At this point it is unclear if this is due to decreased aggregation giving access to REGs (in DMSO) *vs.* equilibrium shift in the presence of H<sub>2</sub>O, or some additional kinetic effect. However, it is clear that the aminated products, in the absence of reducing agent, are relatively stable in both DMSO and H<sub>2</sub>O.

The DDA-CNC glycosylamine material prepared in DMSO was further analysed using multiplicity-edited HSQC, in [P<sub>4444</sub>][OAc]:DMSO-d<sub>6</sub>, using a higher field 850 MHz <sup>1</sup>H frequency spectrometer equipped with a N<sub>2</sub>-cooled cryoprobe. As can be seen from the HSQC spectrum (Fig. 1), both glucosylamine end-group anomers as identified in the glucose model compounds (ESI,† Fig. S2) are present. Moreover, they are in the correct proportions (allowing for HSQC quantification error), relative to the  $\alpha$  and  $\beta$  glucosylamine model mixture and are in excess over the residual REGs of cellulose. The reaction has not gone to completion, with a healthy  $\sim$ 80% conversion based on the relative intensities in Fig. 1. Considering the heterogenous reaction conditions this represents an acceptable yield. One can speculate that aggregation or sterics play a role in the non-quantitative transformation, in addition to the potential for slow hydrolysis during workup. Noteworthy, there were also no apparent signs for further follow-up reactions, conceivably following Amadori rearrangements or even the Maillard reaction cascade. This proves that the formed glucosylamine structures in the DDA-CNC derivatives are stable towards work-up and dissolution in the

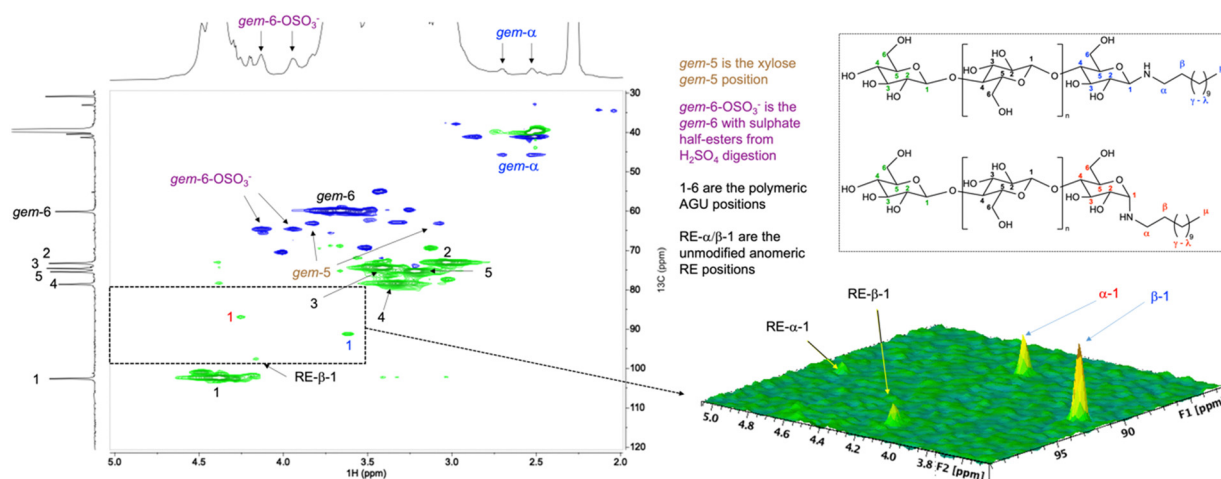
NMR electrolyte over prolonged times, at 65 °C. Another aspect that can affect redispersibility and reactivity of the CNCs is the presence of sulphate half esters on the CNC surface. These do not seem to be removed or converted to dodecylsulphonamide, due to the visible retention of the 6-OH sulphate half ester functionalities (*gem*-6-OSO<sub>3</sub><sup>−</sup>) in the HSQC spectrum (Fig. 1).

For comparison, the reductive amination of the commercial CNCs was also tested using DDA and PEMB as reducing agent. An HSQC spectrum was also obtained using the more sensitive equipment (ESI,† Fig. S5). By comparison with our glucamine and glucosylamine models, it was clear that the reductive amination had not occurred, by the absence of corresponding glucamine correlations. Moreover, glycosylamine resonances were also missing, as were the resonances corresponding to the REGs. This tentatively indicates that reduction of the REGs seems to occur much faster than reduction of the Schiff base.

While the above analyses all demonstrate the stability of dodecyl-*N*-glycosylamine conjugates, with glucose and CNCs, their presence in the solid-state is still under question; all previous and literature spectra demonstrate stability only in the solution-state. As such, the <sup>13</sup>C solution-state NMR spectrum of the  $\alpha$  and  $\beta$  mixture of the dodecylglucamine was compared with the solid-state <sup>13</sup>C spectrum, utilizing cross-polarization (CP) and magic-angle spinning (MAS) (ESI,† Fig. S6). This demonstrates the stability of the glucosylamine moiety in both the solid-state, and in the solution-state, including under ambient neutral aqueous conditions.

As such, the modification of the REGs of CNCs is clearly possible, in the absence of reducing reagents, yielding a water-stable glycosylamine product, dependent on the starting amine. This brings the application of REG-functionalized cellulotics a step closer to reality, particularly in the areas of materials and biomedical sciences.

One should also now consider that indirect proof of REG reductive aminations, using current protocols, cannot exclude



**Fig. 1** Selective modification of the REGs with DDA applied to a commercial sulphated CNC. This is a more complex cellululosic substrate with relevance in materials science. (a) The multiplicity-edited <sup>1</sup>H-<sup>13</sup>C HSQC spectrum ([P<sub>4444</sub>][OAc]:DMSO-d<sub>6</sub> (1 : 4 wt%), 850 MHz, 65 °C, 5 wt% CNC) showed signals characteristic for the  $\alpha$  and  $\beta$  glucosylamine structures (see Scheme 1). (b) 3D expansion of the C1-H spectral area demonstrating a conversion of around 80%, based on the relative intensities of the REG and glucosylamine anomeric signals –CH and CH<sub>3</sub> groups are shown in green, CH<sub>2</sub> groups are shown in blue. Diffusion-edited <sup>1</sup>H trace shown on top and <sup>13</sup>C trace on the left.



the presence of hydrolytically unstable non-reduced glycosylamine structures. For the current approach, hydrolytic stability of different REG substituents now needs to be charted, as a function of the differing aqueous conditions, the electron-withdrawing nature, and the hydrophobic nature of the substituents. Reaction optimization and the effect of aggregation of CNCs under 'green' processing conditions clearly also should be probed. It is important to consider that the presented derivatization approach might not be applicable to a wider variety of primary amines, owing to rearrangement side reactions. This was suggested by the brown coloration of mixtures when employing short chain aliphatic amines, in our pre-screening experiments, hinting towards the occurrence of Maillard reactions (also known as 'browning' reactions). However, this may also provide another angle on derivatization strategies over the isolation of the intermediately formed and more stable Amadori rearrangement product, which may avoid potential hydrolysis over wider pH conditions. There are a plethora of reports exploiting the peculiar properties of sugar REGs for conjugation reactions. Arguably, adapting these protocols for cellulose modification, after fine tuning the reaction conditions using suitable analytical tools, represents a more elegant derivatization approach than employing super-stoichiometric amounts of potentially toxic co-reagents. Overall, we hope that this work demonstrates that it is possible to elevate cellulose chemical optimization studies to a more fundamental bottom-up approach, by applying similar analytical methodology to that which has been applied in organic chemistry since the 1960's, *i.e.*, solution-state NMR.

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## Conflicts of interest

There are no conflicts to declare.

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