Unlocking the Potential of Bio-Based Nitrogen-Rich Furanic Platforms as Biomass Synthons

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Abstract: The demand for new biomass-derived fine and commodity chemicals propels the discovery of new methodologies and synthons. Whereas furfural and 5-hydroxymethylfurfural are cornerstones of sustainable chemistry, 3-acetamido-5-acetyl furan (3A5AF), an N-rich furan obtained from chitin biomass, remains unexplored, due to the poor reactivity of the acetyl group relative to previous furanic aldehydes. Here we developed a reactive 3-acetamido-5-furfuryl aldehyde (3A5F) and demonstrated the utility of this synthon as a source of bio-derived nitrogen-rich heteroaromatics, carbon cycles, and as a bioconjugation reagent.

Introduction

The ever-growing demand for chemical products aligned with societal concerns has propelled the development of green and sustainable chemistry. In compliance with one of the 12 principles of green chemistry, the use of renewable raw materials is of the utmost importance. Carbohydrate-containing biomass is the focus of intense research since it can be used as an alternative source of carbon-based platform chemicals, thus alleviating our dependence on fossil-based feedstocks. Amongst the several examples, furanic platforms obtained from lignocellulosic biomass have emerged as a cornerstone for the sustainable development of new valuable chemicals, as a replacement for oil-based products, and as a starting material for the preparation of “drop-in” chemicals. In fact, furfural is currently being produced in over 250 kTonne/year with over 80 synths being prepared from it.[1] Despite being highly versatile platform chemicals, a major feature of furfural and 5-hydroxymethylfurfural (HMF) is the high content in oxygen and carbon. This translates to derivatives seldom bearing amines or amides. Often introducing external nitrogen requires non-sustainable sources, the most common being ammonia. Knowing that circa 1.5 % of the total world energy consumption is used to produce ammonia, which is then introduced in fine and commodity chemicals, several academia and industry-based groups have turned their attention to nitrogen-rich biomass sources.[2,3]

Thankfully, nature provides us with a solution for this shortcoming. Besides lignocellulosic biomass, chitin is one of the most abundant waste byproduct. This bio-polymer is comprised of N-acetyl-glucosamine (NAG) units, which can be used as a source of bio-renewable nitrogen.

Recently, a variety of methodologies have been described for the transformation of both NAG and chitin to an interesting furan, 3-acetamido-5-acetylfuran (3A5AF).[4]

Synthetic access to this furan often requires ionic liquids as solvent, high temperatures (commonly using microwave irradiation) and mixtures of boric acid and strong acids such as HCl. Yields range from 0.5 to 60 %, with the best yields employing NAG directly (Figure 1).[5–8] Additionally, Minnaard and co-workers have recently described an interrupted dehydration of NAG yielding the corresponding dihydroxethyl acetamidofuran (DiHAF) (Figure 1A).[9] These novel furanic blocks have been transformed into a variety of useful molecules, however the poor reactivity of the 5-acetyl group impairs the possible synthetic operations in comparison with the highly reactive aldehyde encountered in furfural and HMF (Figure 1B). We envision that obtaining an aldehyde derivative from NAG will grant access to a new range of chemical reactivity thus increasing the utility of the 3-amino-furfuryl platform (Figure 1C).
Results and Discussion

Initial studies on the preparation of the desired aldehyde focused on the oxidative C–C cleavage of DiHAF. Using 1.5 equivalents of NaIO 4, the 3-acetamido-5-furfuryl aldehyde (3ASF) was isolated in 76 % yield as a yellow solid. In an attempt to reduce operational steps, and supported by previous reports,[10] we tested the possibility of the direct oxidative cleavage of the phenylboronic ester intermediate obtained from the interrupted dehydration of NAG.[9] Indeed, treating the reaction mixture with an aqueous solution of NaIO 4 yielded 3ASF in 85 % yield (Table 1, Entry 1). The reaction was further optimized by screening various acid promoters and additives. Surprisingly, replacing phenylboronic acid with significantly cheaper boric acid did not lead to a decrease of the yield of the aldehyde, originating 3ASF in 92 % (Table 1, Entry 2). Employing other Brønsted acids yielded the desired product although in lower yield (Table 1, Entries 3–6).

The optimal catalyst load was found to be 0.5 equivalents of triflic acid affording 3ASF in a slightly better yield of 94 % (Table 1, Entry 7).

At this point, replacing the pyridine with triethylamine would significantly facilitate the purification of the product, however, no product was obtained from the reaction. The reaction was scaled to 2 grams, yielding 76 % of pure product (Table 1, Entry 2).

With the aldehyde product 3ASF in hands, we explored the chemical reactivity of this new synthon. As depicted in Figure 2, 3ASF was able to undergo several functional group transformations typical of furyl aldehydes, thus proving its utility as a sustainable synthon for the synthesis of fine chemicals.

Furfuryl alcohol and bis-hydroxymethylfurfural are two of the most important derivatives of furfural and HMF and are extensively used in polymer and pharmaceutical industries.[11–13] Additionally, furylamines can be used as intermediaries for the synthesis of biologically relevant products, polymers, and commodity chemicals.[14–17] Nevertheless, nitrogen-rich furans are less common relative to furfuryl alcohol,[18] highlighting the need to obtain N-rich furans directly from biomass. To address this need, 3ASF was submitted to common reduction conditions. Sodium borohydride afforded the desired alcohol 1 in high yield.

Table 1: Selected examples of one-pot transformation of N-acetylgluco- samine (NAG) to 3-acetamido-furfural (3ASF).[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>Additive</th>
<th>3ASF Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFOH</td>
<td>PhB(OH) 3</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>TFOH</td>
<td>B(OH) 3</td>
<td>92 (76)[9]</td>
</tr>
<tr>
<td>3</td>
<td>Acetic acid</td>
<td>B(OH) 3</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>pTSA</td>
<td>B(OH) 3</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>H 2 SO 4</td>
<td>B(OH) 3</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>Amberlyst</td>
<td>B(OH) 3</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>TFOH[b]</td>
<td>B(OH) 3</td>
<td>94</td>
</tr>
<tr>
<td>8</td>
<td>TFOH[c]</td>
<td>B(OH) 3</td>
<td>0</td>
</tr>
</tbody>
</table>

[a] N-acetylgulcosamine (100 mg, 0.45 mmol, 1 equiv), pyridine (2.25 mL, 0.2 M), boric acid (145.4 mg, 0.68 mmol, 1.5 equiv), molecular sieves 4 Å (500 mg, 5 mass equiv), NaIO 4 (145.4 mg, 0.68 mmol, 1.5 equiv), r.t., 1 h 30 min. [b] 0.5 equiv TFOH was used. [c] Triethylamine was used instead of pyridine. [d] 2 gram scale.
Silylation of the alcohol with TBSCI afforded the desired product 2 as a white solid in high yield.

Reductive amination with propargylamine and NaCNBH₃ afforded the corresponding furyllamine 3. Importantly, the propargyl handle may be of use for further orthogonal modifications. In this sense, we demonstrate the reduction of 3A5F to the corresponding alcohol and amines, allowing their use as building blocks for posterior applications. Importantly, the novel reduced furans provide either primary amines in contrast with the previous furanic 3A5AF. Thus, the increased reactivity and decreased sterical hindrance of the alcohol may be further exploited for the development of new derivatives.

Renewable long-chain alkanes are known as relevant fuel additives. They can be obtained from a tandem aldol condensation/hydrogenation of ketones and furanic aldehydes. An example of this aldol condensation is the NaOH promoted condensation of furfural and cyclohexanone.[19] Extending the methodology to our aldehyde yielded the desired condensation C20 product 4 in high yields containing 2 terminal biomass derived acetamides. Noteworthy, base-promoted hydrolysis of the acetamide was not observed. Additional carbon-extension reactions such as Wittig reaction or Horner–Emmons were previously demonstrated on furfural and 5-HMF.[20] Using a robust procedure described by Maulide and co-workers for the conversion of aldehydes to Z-olefines we were able to perform the olefination of 3A5F.[21] By employing the reported thiouronium ylide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) the Z-olefin 5 was isolated as a single isomer in medium yield. The olefination experiments we describe allow easy access to furanic olefins, useful for the development of new biofuels and as synthons for polymer chemistry (i.e. polymerization by metathesis or free-radical polymerization), amongst other potential applications.

Cyclopentenones (CP) are powerful building blocks in organic synthesis, due to the wide-ranging chemical reactivity of the enone structural motif. CPs are often used for the synthesis of a variety of bioactive molecules,[22,23] including natural products.[24,25] A broad variety of CP are prepared from furanic platforms such as furfuryl alcohol,[26] furfural[27–31] and hydroxymethylfurural.[32,33] These have been obtained either via the Piancatelli rearrangement or the furan ring opening to Stenhouse-salts followed by Nazarov electrocyclization. A recent report discloses the Piancatelli rearrangement of a 3A5AF derivative, yielding CP containing bio-renewable nitrogen.[34]

Prompted by these results, 3A5F was used to obtain triamino-CPs 6. To this end, morpholine was selected as a model amine and the aldehyde was reacted in the presence of catalytic amounts of Lewis and Brønsted acid in acetonitrile. In the presence of 10% ZnCl₂, compound 6a was isolated in 81% yield as the trans-4,5-diastereomer bearing an acetamide substituent at position 3 (for the complete optimization data, please see Supporting Information, Table S1). Selecting ZnCl₂ as the optimal catalyst, the scope of CPs was expanded to other alkyl and aliphatic amines as depicted in Scheme 1. Cyclic secondary amines reacted with 3A5F to give the corresponding diamine derivatives in low (6c, 24%) to high yields (6a-b, 81 and 66%). The low yield obtained for 6c can be explained by the formation of side products, a common occurrence when using furanic aldehydes in the presence of pyridoline.

Linear secondary amines, such as the unsaturated diallyl amine and the saturated diethy1l and dioctyl amines were also well tolerated giving origin to derivatives 6d-f in 56%, 56% and 64% yields, respectively. Surprisingly, reaction with diethylamine afforded the desired product 6e whereas in the case of furfural the amine is unreactive.[27–31] When bis(2-methoxyethyl) amine was used, the corresponding diamine derivative 6g was obtained in good yield (85%). The reaction of 3-acetamide furfural with the unsymmetric secondary amine N-allylmethylamine also proceeded smoothly to generate derivative 6h in 75% yield. Finally, we also tested the reactivity of primary amines, which generally represent more challenging substrates. While benzylamine showed no reactivity with 3A5F, aniline succeeded in originating the desired product 6i in 57% yield.

The utility of the cyclopentenone scaffold was showcased by performing relevant modification of the core structure by using the CP6d as model substrate (Scheme 2).

On one hand, Luche reduction of the CP yielded the desired allylic alcohol 8 in 53% isolated yield. However, when attempting the complete reduction using sodium borohydride, the free enaminone 9 was isolated as the sole product in quantitative yield. It is noteworthy that the reaction occurs with the retention of the initial nitrogen,
thus contributing to the discovery of complex structures harnessing sustainable nitrogen.

Additionally, we were interested to explore the potential of the aldehyde as a biorthogonal handle in chemical biology. Protein and antibody site-selective modification is a powerful tool in drug development, with 11 Food and Drug Administration (FDA) approved antibody drug conjugates (ADCs) in 2022 and 80 ADCs in development. The importance of obtaining well defined homogeneous bioconjugates propelled the investigation of site-selective modification. The high reactivity of the thiol group is commonly exploited for the modification of cysteine residues, either by exploring the thiol nucleophilicity in the presence of electrophiles (i.e. Michael acceptors) or by elimination of the thiol group thus generating electrophilic dehydroalanines. Emerging technologies aim at increasing orthogonality, namely targeting the modification of N-terminal cysteines using NHS-activated acrylates, condensation of free cysteine with 2-cyanobenzothiazol (CBT), cyclopropenones (CPO) and activated aldehydes to form thiazolidines. Concerning thiazolidines, most conditions employed are incompatible with the current gold standards of bioconjugation (i.e. acidic pH, long reaction times). Recent advances take advantage of a proximity-based approach generating thiazolidines from 2-formylbenzeneboronic acids (2-FBBA) that lead to more stable constructs.

We envisioned that the 3-acetamido group would promote the stabilization of a furanyl-thiazolidine by electron-donating effects, thus 3A5F could be employed as a sustainable bioconjugation handle for N-cystein modification. To this end, the new aldehyde was smoothly reacted with cysteamine hydrochloride in acetonitrile affording the corresponding thiazolidine in good yield.

The reaction was extended to a model peptide containing an N-terminal cysteine to evaluate its reactivity. Cysteine was incubated with 5 equiv of aldehyde in aqueous medium at pH 6.0. As shown in Figure 3, full conversion to the conjugate was observed in 30 min and no hydrolysis occurred in solution for up to 72 h.

Other N-terminal Cys containing peptides were reacted with 3A5F and we observed compatibility of the reaction with other nucleophilic amino acids, including serine and lysine. It is noteworthy to mention the chemoselective nature of the reaction in C-ovalbumin, where only one
modification was observed, with the lysine remaining unreacted (Figure 3B).

The site-selectivity was evaluated using vasopressin, which contains both an N-terminal and an in-chain cysteine. The peptide was incubated with 5 equiv of aldehyde in aqueous medium at pH 6.0. As shown in Figure 3C, full conversion to the conjugate was observed in 30 min. Additionally, the stability of the construct allowed for a site-selective dual-orthogonal modification by reaction of the internal cysteine with a maleimide.

When N-acetyl calcitonin was used, a peptide containing 2 Lys, an N-terminal acetylated Cys and an in-chain Cys, no reaction occurred which further corroborates the site-selectivity towards N-terminal cysteine of the 3A5F reagent.

The selective modification of the in-chain cysteine in the presence of the thiazolidine further supports the stable nature of the constructs since no thiol scrambling was observed up to 24 h incubation with 20 equiv of maleimide.

In addition, the reactivity towards more complex biomacromolecules such as proteins was accessed. To this end, a recombinant enhanced green fluorescent protein containing an N-terminal cysteine residue (cys-GFP) was produced as described in a previous report by Bernardes and co-workers.[44]

Incubation of cys-GFP with 3A5F (100 equiv) yielded the desired adduct (Figure 4) with high efficiency as confirmed by LC–MS spectrometry.

The possibility of introducing a broad scope of amides and carbamates in the furyl moiety allows the decoration of the bioconjugates with orthogonal handles, thus 3A5F may emerge as a viable sustainable synthon for chemical biology studies.

Conclusion

In summary, the tandem dehydration/C–C cleavage of N-acetylglucosamine (NAG) promoted by triflic acid followed by treatment with sodium periodate yields 3-acetamido-5-furyl aldehyde (3A5F) in high yield.[47] The novel furan 3A5F overcomes one of the main drawbacks of lignocellulosic-based biomass, the absence of nitrogen. Additionally, 3A5F can undergo various reactions such as reduction, reductive amination, aldol condensation, olefination, cyclopentenone and thiazolidine formation. Importantly, the site-selective modification of N-terminal cysteine residues is one of the few examples of renewable handles for chemical biology.

When comparing with the most broadly used biomass furanics (furfural and 5-hydroxymethylfurfural) the acetamide in position 3 not only introduces bio-based nitrogen to the pipeline of biomass valorization, but also increase its reactivity by rendering the furan more electron-rich. When comparing with the previous acetamido furan, 3A5AF, the replacement of the parent ketone with an aldehyde drastically altered its reactivity, allowing for the use of the furan in the aforementioned applications. The high reactivity displayed by 3A5F aligned with its facile preparation will propel the discovery of novel chemical entities and technologies based on the new furanic.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

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