Ultrasound-Assisted Synthesis of 1-N-β-D-Glucopyranosyl-1H-1,2,3-triazole Benzoheterocycles and their Anti-Inflammatory Activities

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Neste trabalho, a preparação de vários glicosídeos triazólicos a partir da reação entre a azida de 2,3,4,6-tetra-O-acetil-β-D-glicopiranosila e alcinos terminais foi desenvolvida em moderados a excelentes rendimentos (63-99%). Em todas as etapas de reação foi aplicada a energia de ultrasom para aumentar a reatividade química. Adicionalmente, os compostos conjugados com benzoetcrbiclos revelaram potente atividade anti-inflamatória.

In this work, the preparation of various glucosyl triazoles from a reaction between 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide and terminal alkynes was developed in moderate to excellent yields (63-99%). Ultrasound energy was applied at each step of the reaction to increase chemical reactivity. In addition, the compounds conjugated with benzoheterocycles moieties revealed potent anti-inflammatory activity.

Keywords: triazole, click chemistry, ultrasound, anti-inflammatory activity, carbohydrate, benzoheterocycle

Introduction

Much modern research in organic synthesis has promoted the development of new methodologies and their optimization. Over the past decade, one fast-growing area in organic chemistry has been the synthesis of compounds employing ultrasound irradiation.¹ In recent years, sonochemistry has been applied to accelerate a large number of organic reactions and enhance their chemical yields.² The use of this technology in organic synthesis has been reported in a variety of areas, for instance heterocycles³ and carbohydrates,⁴,⁵ and the latter represent a vast field for the exploration of chemical reactivity.

A research area in carbohydrate chemistry has been centered on the need to induce the formation of glycoside linkages towards the glycoconjugate mimics.⁶ A diversity of carbohydrate structures is conjugated with heterocyclic moieties, e.g., via the aglycon part which promotes the formation of small molecules that show some biological activity. Hybrid compounds are often a prerequisite for biological activity and can influence drug design and discovery of new chemical entities (NCEs) based on sugar scaffolds.⁷ Glucopyranosyl triazoles have shown biological activities such as enzyme inhibition,⁸ and as antitumor,⁹ antiviral¹⁰ and anti-tuberculosis agents.¹¹ For anti-inflammatory activity, the literature describes a few examples based on carbohydrates.¹² In this context, glycocoumpounds with a benzoheterocyclic aglycon to evaluate their anti-inflammatory activities were selected.

The 1,2,3-triazoles linked to carbohydrate scaffolds have been synthesized employing a copper-based catalyst.¹³ The effect of ultrasound on carbohydrate chemistry⁴,⁵,¹⁴ and specifically on the click chemistry in the synthesis of 1,2,3-triazoles has been reported very recently.¹⁵,¹⁶

Motivated by our recent projects involving the synthesis¹⁷ and biological activities¹²,¹⁸ of a series of new 1,2,3-triazole derivatives, our group became interested in N-glycosyl-1,2,3-triazoles formed from 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoysl azide and terminal alkynes via 1,3-dipolar cycloaddition reaction using the application of

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ultrasound energy. Our strategy to obtain these compounds containing a 1,2,3-triazole moiety was developed using only ultrasound irradiation in four steps, as shown in Scheme 1.

Results and Discussion

Firstly, our attention was focused on the preparation of the starting materials, namely 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide 1 and terminal alkynes 2c-g. In order to simplify the synthesis of 1 a three-step procedure employing ultrasound irradiation was performed. Compound 1 was prepared from D-glucose under modified conditions using ultrasonic energy in the acetylation (Ac₂O, 3.5 mol% I₂, ultrasound, 20 min), bromination (HBr/AcOH, ultrasound, 50 min) and azidation (acetone/H₂O/NaN₃, ultrasound, 40 min). After these three steps, compound 1 was obtained with overall yields of 61%. Comparatively, the results without ultrasound energy are overall lower yields and longer-time processes to obtain the azide-tagged sugar 1 (Table 1).

Our research group has been interested in the synthesis of benzoheterocyclic derivatives, and recently, developed a stereoselective functionalization of unsaturated carbohydrates using palladium reagents that resulted in an efficient strategy for constructing allylic N- and S-benzoheterocycles linked to carbohydrate moieties.

To continue along this line, compounds 2c-g were prepared through a reaction between propargyl bromide and benzoheterocycles in the presence of K₂CO₃ under sonication conditions where the desired compounds 2c-g were synthesized, as shown in Table 2. This protocol furnished the desired compounds in 45-77%. In comparison, the reactions using the silent conditions afforded the same compounds 2c-g with similar yields, albeit after 20-24 h.

To begin our study towards producing glucopyranosyl 1,2,3-triazoles (Scheme 2), the Sharpless protocol was

![Scheme 1. Strategy to obtain 1,2,3-triazole sugars in four-steps from D-glucose and benzoheterocycles using ultrasound irradiation](image)

**Table 1. Synthesis of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide (1)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Method A - Ultrasound</th>
<th>Method B - Silent condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time / min</td>
<td>Yield / %</td>
</tr>
<tr>
<td>Acetylation</td>
<td>20</td>
<td>95⁵</td>
</tr>
<tr>
<td>Bromination</td>
<td>50</td>
<td>70⁴</td>
</tr>
<tr>
<td>Azidation</td>
<td>40</td>
<td>92⁵</td>
</tr>
<tr>
<td>Total (three steps)</td>
<td>110</td>
<td>61</td>
</tr>
</tbody>
</table>

*Yields of crude materials: no secondary spots were observed by TLC. *⁶Isolated yield after column chromatography.
applied to promote the reaction between 1 equiv. of 1 and 1.2 equiv. of 2a using 20 mol% CuSO$_4$·H$_2$O, 40 mol% sodium ascorbate in 50% tert-BuOH:H$_2$O at ambient temperature, but a very low level of conversion was observed (thin layer chromatography (TLC) analysis), even after 12 h stirring. Field and co-workers$^{13}$ also described similar results when employing this protocol in the synthesis of α- and β-D-glucopyranosyl triazoles via CuAAC click chemistry. Based on our recent results,$^{17}$ however, the protocol was changed to 10 mol% CuI in dichloromethane, and a partial conversion (examined by TLC) was observed after 24 h. Fortunately, after adding 10 mol% of triethylamine, the reaction was completed in 20 h. The total conversion obtained by using a base (Et$_3$N) can be explained via deprotonation of the transient π-complex (RC≡CH[CuL$_n$]) to form the copper-acetylide, as reported in the literature.$^{29}$ In order to obtain a shorter reaction time, the ultrasound energy at room temperature was applied, and 1,2,3-triazole-sugars 3a were obtained in 20 min in 79% yield (Table 3, entry 1). This shorter time, under these conditions, can be accounted for by the sonocatalysis in 1,3-dipolar cycloaddition reaction.$^{3,15,30}$ Driowya et al.$^{16}$ recently described the synthesis of 3a in 77% yield, for 20 min under ultrasound irradiation using 2 equiv. of CuI and 2 equiv. of DIEA (N,N-diisopropylethylamine). Hence, comparatively, our procedure appeared more efficient because employing catalytic amounts of copper(I) iodide and triethylamine.

After optimization of the conditions, our group decided to apply our protocol to obtain N-glycosyl-1,2,3-triazoles 3a-g using various terminal alkynes (Scheme 2). Ultrasound-assisted reaction of 1 with various functionalized alkynes 2a-g afforded the corresponding β-glucopyranosyl triazole derivatives 3a-g in moderate to excellent yields (63-96%) within short reaction times (Scheme 2 and Table 3).

Compounds 3a,$^{31}$ 3c,$^{32}$ and 3d,$^{33}$ were recently reported in the literature. Compounds 3a and 3c were prepared via the click chemistry procedure under conventional conditions.$^{31,32}$

Table 2. Synthesis of propargyl N- and S-benzoheterocycles (2c-g)

<table>
<thead>
<tr>
<th>Product</th>
<th>Method A - Ultrasound</th>
<th>Method B - Silent condition</th>
<th>Melting point / °C (from Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time / min</td>
<td>Yield$^\dagger$ / %</td>
<td>time / min</td>
</tr>
<tr>
<td>2c$^\text{b}$</td>
<td>10</td>
<td>55</td>
<td>1440 (24 h)</td>
</tr>
<tr>
<td>2d</td>
<td>20</td>
<td>76</td>
<td>1200 (20 h)</td>
</tr>
<tr>
<td>2e</td>
<td>60</td>
<td>77</td>
<td>1380 (23 h)</td>
</tr>
<tr>
<td>2f</td>
<td>60</td>
<td>45</td>
<td>1440 (24 h)</td>
</tr>
<tr>
<td>2g</td>
<td>60</td>
<td>60</td>
<td>1320 (22 h)</td>
</tr>
</tbody>
</table>

$^\dagger$Isolated yield after column chromatography. $^x$In this case, the reaction was performed without addition of K$_2$CO$_3$.

Scheme 2. Synthesis of N-glucosyl-1,2,3-triazoles under ultrasound activation.
The compound 3d was obtained via a thermal cycloaddition reaction along with its 1,5-isomer and separated by column chromatography. When applying our click protocol, compound 3d was synthesized in 88% isolated yield (Table 3, entry 4). To our knowledge, compounds 3b, 3e, 3f and 3g have not been previously described.

The structures of the compounds 3a-g were analyzed using 1H and 13C nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) or elemental analysis. The appearance of a singlet in 1H NMR spectrum data in the region between 7.5 and 7.9 ppm was assigned to H5' (methine proton) of the triazolyl ring. In order to ascertain the preferential conformations in the 1,2,3-triazole-sugars 3a-g, our study was decided on the base of the proton coupling constants (J) in the glucopyranose ring. The 1H and 13C NMR spectra were in concordance with the proposed structure. The methylene protons (−CH−Het) in 3c, 3e-g do not appear as a singlet, but as a double doublet, the non-equivalence between hydrogen atoms is a case of diastereotopic geminal protons. The 1H NMR spectra of compounds 3a-g showed the anomeric proton (H1) as a doublet in the range δ 5.81-5.93 ppm and JH1,H2 8.8-9.2 Hz. This vicinal coupling indicates a 1,2-trans relationship and a β-anomeric configuration. Large vicinal coupling constants were observed for protons H2, H3 and H4 (9.2-10.0 Hz) that appeared in the δ 5.17-5.55 ppm region. These results are in agreement with a trans diaxial relationship between H1/H2, H2/H3, H3/H4 and H4/H5, suggesting that all hydrogens are in axial position in a C4′ conformation.

### Table 3. Ultrasound-assisted synthesis of N-glucosyl-1,2,3-triazoles (3a-g)

<table>
<thead>
<tr>
<th>entry</th>
<th>Product</th>
<th>Reaction time / min</th>
<th>Melting point / °C (from Reference)</th>
<th>[α]D, †</th>
<th>Yield / % (from Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="3a.png" alt="Structure" /></td>
<td>20</td>
<td>189-193 (195-198)†</td>
<td>−19.0</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td><img src="3b.png" alt="Structure" /></td>
<td>30</td>
<td>153-155</td>
<td>−32.8</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td><img src="3c.png" alt="Structure" /></td>
<td>20</td>
<td>204-205 (203-204)†</td>
<td>−22.0</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td><img src="3d.png" alt="Structure" /></td>
<td>30</td>
<td>182-184</td>
<td>−21.5</td>
<td>88 (78)†</td>
</tr>
<tr>
<td>5</td>
<td><img src="3e.png" alt="Structure" /></td>
<td>25</td>
<td>152-153</td>
<td>−17.7</td>
<td>69</td>
</tr>
<tr>
<td>6</td>
<td><img src="3f.png" alt="Structure" /></td>
<td>20</td>
<td>156-158</td>
<td>−20.8</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td><img src="3g.png" alt="Structure" /></td>
<td>20</td>
<td>161-162</td>
<td>−25.7</td>
<td>88</td>
</tr>
</tbody>
</table>

† = 0.01 g mL⁻¹ in CH₂Cl₂, † = after chromatography column. †Two isomers: 1,4 and 1,5.††
Ultrasound-Assisted Synthesis of 1-N-β-D-Glucopyranosyl-1H-1,2,3-triazole Benzoheterocycles


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for the β-D-glucopyranosyl ring. The proton H₅ is located upfield (ca 4 ppm) and the multiplicity appears as ddd due to H₅ coupling with protons H₁ (J₅,4 8.4-10.0 Hz), H₆ (J₅,6 3.6-5.0 Hz) and H₆' (J₅,6' 1.6-2.8 Hz), as expected for D-series sugar compounds 3a-g. The relations between H₁, H₂, H₃ and H₄ were shown using a H,H-correlation spectrum (COSY). Nuclear Overhauser effect (NOE) contact between H₁Νa and H₅Νa was observed employing H,H-nuclear Overhauser effect spectroscopy (NOESY) experiment.

Furthermore, a spatial NOE contact between H₅ and H₁ or H₂ was detected, these results confirmed the exclusive formation of 1,4-regioisomer. For the experiment of nuclear Overhauser effect difference spectroscopy (NOE DIFF), the proton H₅ (H₅triazole) for irradiation in compounds 3b and 3e, such as representative sampling, was chosen. These experiments can be monitored by increasing the H₁ or H₂ signals. When performing the experiments in CDCl₃ or DMSO-d₆, a more accurate analysis of the NOE DIFF spectrum revealed solvent effects on the population distribution of rotamers of the N-glucosyl-1,2,3-triazole series, as shown in Figure 1.

Srivastava and co-workers described ab initio (HF/6-31G* method) molecular orbital calculations in vacuum of a glucosyl-triazole linked to 1,2,4-oxadiazole moiety, indicating a more stable B-type conformer. Through H₁ irradiations, the authors also observed a NOE contact of 6% between H₁ and H₅ in DMSO-d₆. In our case, the NOE-DIFF experiment indicates that the conformer A-type is preferred in CDCl₃ for compound 3b (ca. 5% to H₂ and < 1.0% to H₁, Figure 1). On the other hand, in DMSO-d₆, the experiments revealed a substantial increase of conformer B-type, displaying a rotational equilibrium of N-glucosyl-1,2,3-triazoles between the conformer A- and B-types. These results are consistent with polarity parameters involving hydrogen-bond donating solvents. Probably, this tendency can be explained in terms of H₅ acidity allowing an intramolecular CH-O hydrogen bond formation between H₅ and the endocyclic oxygen of glucopyranose, as shown in Figure 1 (structure A-type).

In this case, as expected, the less polar CDCl₃ favored the conformer A-type, whereas the more polar DMSO-d₆ destabilized the intramolecular hydrogen-bond, thus increasing the conformer B-type. These are interesting results that can play important roles in biological activities through conformational stabilization. Further investigation of the correlation of conformational behavior and biological activity of 1,2,3-triazole-carbohydrates is currently under way in our laboratory.

Having synthesized and characterized 1,2,3-triazole-sugars 3a-g, and considering the results of Shafi et al. related to the anti-inflammatory activity of benzothiazole-2-thio-linked 1,2,3-triazoles, the benzoheterocyclic series 3d-g was selected to have studies their acute anti-inflammatory activity profiles, the results are summarized in Table 4 and Figure 2. The above compounds exhibited moderate to good anti-inflammatory activity with the percentage inhibition of edema formation ranging from 49.2 to 64.7%, while the reference drug ASA and ibuprofen both showed 77% inhibition (Table 4).

Compound 3d showed moderate activity (49.2%). It was observed that when the thiomethyl group (Y=SCH₂-) was introduced in the structure, the activity increased from 55 to 65% (Figure 2). Our results are in agreement with the

Figure 1. Solvent effect observed on the conformational equilibrium of 3b and 3e.
Table 4. Acute anti-inflammatory activity for the benzoheterocyclic glucosyl 1,2,3-triazoles 3d-g.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose / (mg kg⁻¹)</th>
<th>Mean ± standard deviation / g</th>
<th>Edema inhibition / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline 0.9%)</td>
<td>–</td>
<td>0.1512</td>
<td>–</td>
</tr>
<tr>
<td>ASA</td>
<td>250</td>
<td>0.0347 ± 0.001</td>
<td>77.0</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>250</td>
<td>0.0347 ± 0.007</td>
<td>77.0</td>
</tr>
<tr>
<td>CMC</td>
<td>–</td>
<td>0.1412 ± 0.040</td>
<td>6.7</td>
</tr>
<tr>
<td>3d</td>
<td>250</td>
<td>0.0767 ± 0.001</td>
<td>49.2</td>
</tr>
<tr>
<td>3e</td>
<td>250</td>
<td>0.0667 ± 0.001</td>
<td>55.8</td>
</tr>
<tr>
<td>3f</td>
<td>250</td>
<td>0.0633 ± 0.002</td>
<td>58.0</td>
</tr>
<tr>
<td>3g</td>
<td>250</td>
<td>0.0533 ± 0.002</td>
<td>64.7</td>
</tr>
</tbody>
</table>

Significant differences: 'p < 0.001; 'p < 0.05.

Figure 2. Structure and anti-inflammatory activity of 1-N-β-D-glucopyranosyl-1H-1,2,3-triazole benzoheterocycles 3d-g.

In particular, the substitution at the third position of benzoheterocyclic, when X = S, NH or O, shows growth in anti-inflammatory activity for 3e (55.8%), 3f (58.0%) and 3g (64.7%), respectively (Figure 2). The potency for acute anti-inflammatory activity was optimized in compound 3g, which exhibited a higher diversity of atoms (N, S and O) at the benzoheterocyclic site and showed a relatively similar profile when compared with the positive controls, ibuprofen and acetylsalicylic acid.

Conclusion

In summary, regioselectively 1,4-disubstituted N-β-D-glucopyranosyl-1,2,3-triazoles 3a-g were synthesized under ultrasound irradiation in moderate to excellent yields of 63 to 99% at short reaction time of 20-30 min using catalytic amounts of CuI and Et₃N at room temperature. The compounds containing the benzoheterocyclic moieties showed moderate to good acute anti-inflammatory activity. The current results demonstrate that these glycoconjugates represent a promising starting point for further design of potential anti-inflammatory drugs.

Experimental

All organic solvents were analytical grade (Vetec, Brazil). All reactions were monitored by TLC analysis on GF-254 (Merck-Darmstadt, Germany). Reactions were carried out in a USC-1400A Ultraceaner ultrasound cleaning bath with an operating frequency of 40 kHz. Column chromatography was performed on Merck silica gel 60 (Darmstadt, Germany). Melting points were determined in a FPM II BioSan apparatus and are uncorrected. Optical rotations were measured in a Krüss Model P1000 polarimeter. ¹H (300 or 400 MHz), ¹³C NMR (75 or 100 MHz), COSY, NOESY and NOE-DIFF spectra were obtained with Varian Unity Plus spectrometers in CDCl₃ or DMSO-d₆. Elemental analysis were carried out in a CA EA1110 CHNS-O analyzer, and HRMS analysis were recorded with a Shimadzu Liquid Chrom MS LCMS-IT-TOF using acetonitrile or methanol as the solvent. IR spectra were recorded on a IFS66 Bruker spectrophotometer using KBr discs.

Acute anti-inflammatory activity

Bio-activity tests were performed by the following procedure of Winter et al., on groups of 10 Swiss white mice. The acute anti-inflammatory activity used 250 mg kg⁻¹ of the compounds which had been evaluated by the carrageenan-induced paw edema method. The control group received 1% carboxymethylcellulose. Two positive and negative anti-inflammatory tests were performed on three animal groups by oral administration of aspirin, ibuprofen and aqueous saline solution, respectively. The results for the compounds are expressed as mean ± standard deviation using the paired student-t test. In all cases, p < 0.001 was used as the criterion for statistical significance.

Supplementary Information

Supplementary information (spectral data and figures containing IR, ¹H and ¹³C NMR) are available free of charge at http://jbcs.sbq.org.br as a PDF file.
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