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Chitosan-Oxanorbornadiene: A Convenient Chitosan Derivative for Click Chemistry without Metal Catalyst Problem

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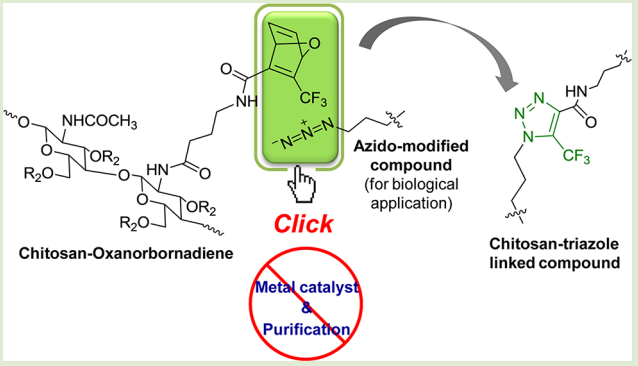
Supporting Information

ABSTRACT: Click chemistry is considered to be a good pathway to conjugate chitosan with functional molecules due to the ease of the reaction at room temperature. However, as chitosan forms a complex with metal ions, there is a problem with the existence of metal ions in the derivative. The present work demonstrates that chitosan-oxanorbornadiene can provide metal-free Click by showing the optimal condition to introduce oxanorbornadiene, with 80% substitution, and clarifies model reactions of chitosan with azido-modified substrates for the ligation of bioactive molecules.



Chitosan, known for its specific properties, for example, biocompatibility,[1] nontoxicity,[2] and biodegradability[3]—has had favorable reports in regard to its high value-added applications in the biological and biomedical fields.[4] For chitosan derivative production, most of the approaches are based on the ligation of biological active, organic molecules (BSA,[5] antibody[4c]), or inorganic molecules (magnetite,[6] silica[7]). Thereby, the functionalizations of chitosan in aqueous and organic solvents by using conjugating/coupling reagents[8] need to be considered. It is accepted that an ideal condition in modifying chitosan with bioactive compounds should be done in aqueous at room temperature and, if possible, without the use of toxic coupling or conjugating agents.

Recently, Click chemistry has received much attention in macromolecular chemistry due to its high chemoselectivity in mild reaction conditions with a variety of functionalizations. The copper-catalyzed Click chemistry based on the reaction between an azido- and an alkyno- group was first reported by Medal[9] and Sharpless.[10] The bioorthogonality of the components avoids interaction with the environment of biological or biomedical systems.[11] For this reason, Click chemistry is a good reaction pathway to design biostructured molecules, for example, on the basis of chitosan.[12]

It is important to note that one of the specific properties of aminopolysaccharide chitosan is the complexation with metal ions[13] or Cu(I)-catalyzed Click chemistry. Therefore, this might lead to Cu contamination after the reaction. In that case, the risks of metal toxicity may prevent its use in biomedical application.

The present work focuses on a new pathway to obtain metal-free Click chemistry for chitosan. In the past, Agard et al. reported a good example of the metal-free Click reaction called "the Strain-Promoted Azide–Alkyne Cycloaddition (SPPC)", which can be further conjugated with peptides.[14] In this work, oxanorbornadiene moieties, which were first reported by Rutjes,[15] are considered to be effective functional molecules to bring in a reaction with azido-modified substrates, resulting in a triazole linkage between chitosan and the substrates. An attractive point of chitosan-oxanorbornadiene is the reaction progress at room temperature without any additives or catalysts. Recently, Krause et al. have successfully linked the modified polysaccharide alginate with cyclic RGD-pentapeptides via this oxanorbornadiene based metal-free Click technique. The regio- and chemoselectivity of the cycloaddition, resulting in different products, were investigated by using ^{19}F NMR.[16] To the best of our knowledge, an oxanorbornadiene-based Click reaction with aminopolysaccharide chitosan has not yet been reported.

Scheme 1 shows the introduction of trifluoromethylated oxanorbornadienes (**1**–**3**) to chitosan (**CS**). All reaction conditions were performed by dissolving **CS** in water containing 1.2 equiv HOBt.[17] To study the reaction efficiency of the oxanorbornadiene derivatives, three types of oxanorbornadienes (**1**–**3**) having different spacer lengths were considered. Typically, the trifluoromethyl group shows a ^{19}F NMR chemical shift at ∼−61 ppm, as shown in the case of **1** (Figure 1a). For **1** and **2**, the conditions were similar to each other based on the use of a conjugating agent (EDC) and DMAP to

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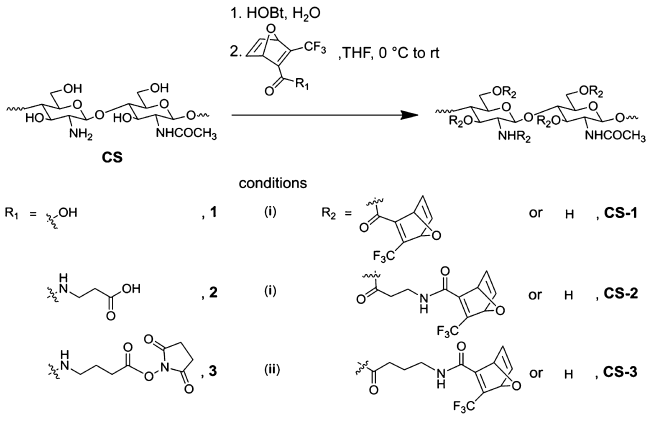
Scheme 1. Synthesis of Chitosan-Oxanorbornadiene with (i) EDC, DMAP, 1 d, and (ii) DiPEA, 1 d; Purification by Intense Dialysis against an Aqueous NaCl Solution and DI Water



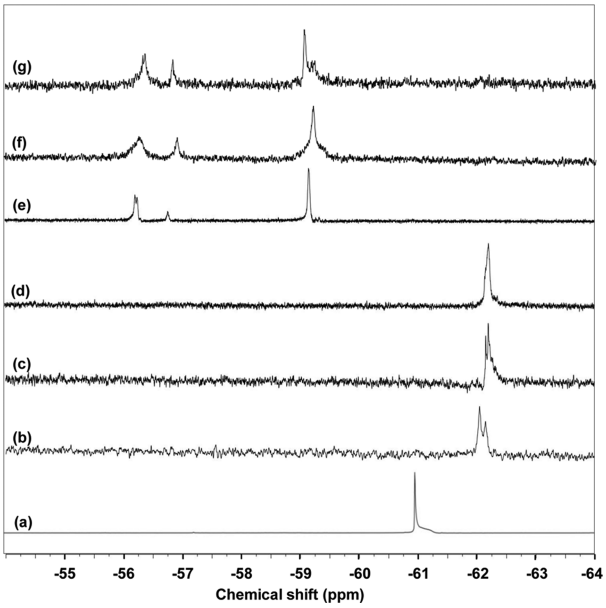


Figure 1. ^{19}F NMR spectra of (a) **1**, (b) **CS-1**, (c) **CS-2**, (d) **CS-3**, (e) **CS-4**, (f) **CS-5**, and (g) **CS-6** in 2% CD_3COOD/D_2O.

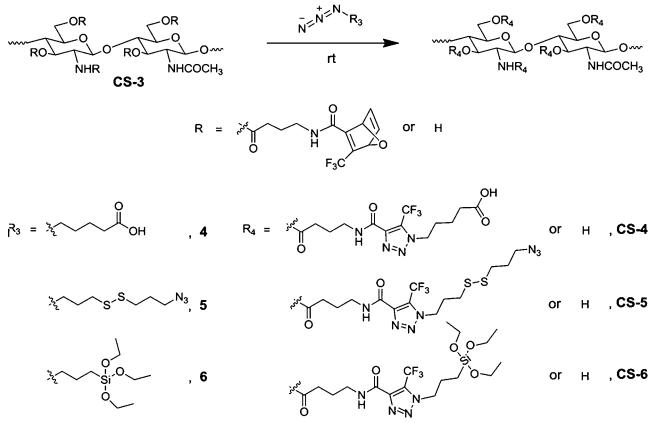
obtain **CS-1** and **CS-2**, respectively. Figure 1b,c confirms the success of the reaction.

The FTIR spectra of **CS-1** and **CS-2** show a new peak at 1740 cm^{-1} and an increase of the peak at 1550 cm^{-1}. The changes in peak intensity were clarified by the curve fitting technique to confirm that the substitution of oxanorbonadiene resulted in ester and amide bonds (Figure S1b,c, Table S1). The percentage of substitution, which could be easily quantified by ^{1}H NMR (Figure S3A,B), were found to be only 15% for both cases.

To increase the percentage of oxanorbornadiene substitution, a NHS-active ester oxanorbornadiene derivative, **3**, was prepared prior to obtaining **CS-3** in alkaline condition (DiPEA) without EDC (Figure 1d, Figure S1d, Table S1). ^{1}H NMR confirms the percentage of substitution for **CS-3** to be the highest at ~80 (Figure S3C). This suggests how reactive NHS-ester species play an important role in the introduction of oxanorbonadiene to **CS**. In other words, in the cases of **CS-1** and **CS-2**, the low yield might come from the side reaction of *O*-acylurea resulting in *N*-acylurea (Figure S4). The spacer chain length in R_1 is of interest whether it shows any effect to the reaction or not. Here, the short chain length of the oxanorbornadiene-NHS ester, **3a** (Supporting Information), was also applied by using condition (ii). It was found that derivatization was not successful. The results indicated that oxanorbonadiene with a certain chain length is necessary as it might help minimize the steric hindrance in the reaction. In the condition (ii), the substitution of oxanorbornadiene on chitosan could be controlled at ~20, ~70, and ~80%, by varying the content of oxanorbornadiene **3** for 0.5, 1.5, and 3 equiv to chitosan, respectively. It should be noted that a higher degree of oxanorbonadiene leads to a decrease in solubility of the derivative in water.

To apply Click chemistry for chitosan functionalizations, a series of azido-modified substrates, that is, **4**, **5**, and **6**, were used as model compounds. The success of the metal-free cycloaddition between **CS-3** and **4**, **5**, and **6** will then be a guideline for coupling chitosan with amino acids, peptides, and antibodies, as well as being used for surface modification with inorganic particles and nanoparticles. The ligation of **CS-3** with **4**, **5**, and **6** was carried out by using metal-free cycloaddition (Scheme 2) to obtain **CS-4**, **CS-5**, and **CS-6**, respectively. After

Scheme 2. Cu-Free Cycloaddition of Azido-Modified Substrates; 5-Azidopentanoic Acid, 4; 1,2-*Bis*(3-azido-propyl)disulfane, 5; and (3-Azidopropyl)triethoxysilane, 6; to CS-Oxanorbornadiene, CS-3, Yielding Chitosan Conjugation Products CS-4, CS-5, and CS-6 in 2% CD_3COOH/H_2O



mixing both compounds in 2% (v/v) acetic acid aqueous solution at room temperature, the cycloaddition was monitored by using ^{1}H NMR. The success of the reaction could be traced by the disappearance of the chemical shift at δ ~−62 ppm belonging to oxanorbornadiene in ^{19}F NMR (Figure 1e−g). After the reaction, a new set of ^{19}F signals could be identified. This is related to the regioisomeric form (1,4-cycloaddition (*trans*) and 1,5-cycloaddition (*cis*)) of triazole, which shows a signal at δ ~ −59 for *trans* and a signal at δ ~ −56 for *cis*. This result is relevant to the report by Krause et al. that showed *cis* and *trans* triazoles of monomeric model substrates (azido valeric acid and Boc-protonated ε-azido-lysine) and alginate. Another possible way to trace the reaction is the appearance of methine protons of the furan byproduct as well as the disappearance of methine protons of oxanorbonadiene in ^{1}H NMR. For example, in the case of **CS-4**, the appearance of chemical signals at 6.4 and 7.4 ppm, as well as disappearance of

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signals at 5.6, 5.7, and 7.3 ppm confirms the successful click addition of **4** to **CS-3** (Figure 2B).

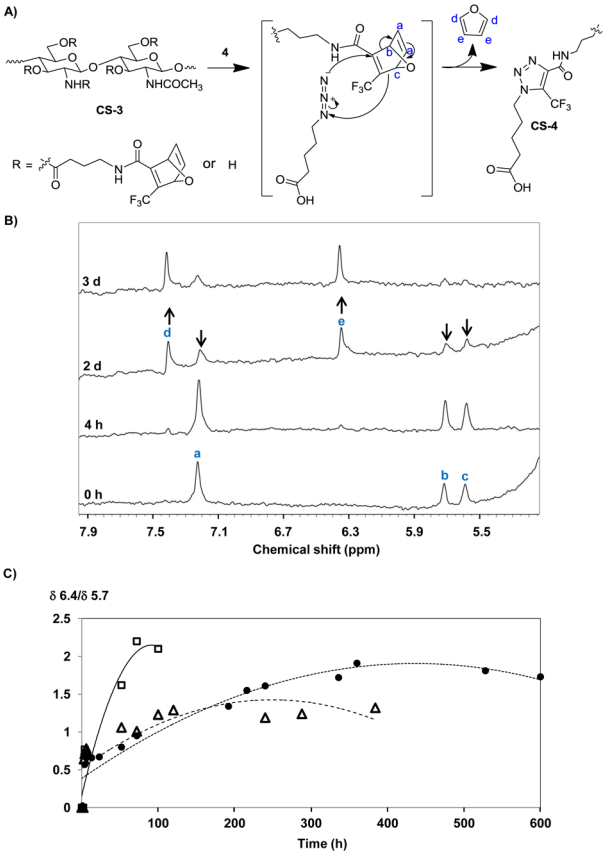

Figure 2. (A) Mechanism of cycloaddition between **CS-3** and **4**, (B) ^{1}H NMR spectra of ligation progress in 2% CD_3COOD/D_2O over time between **CS-3** and **4**, and (C) Ratio of furan and oxanorbornadiene (integral ratio of δ 6.4/δ 5.7) over the reaction time detected by ^{1}H NMR based on the integration; (□) from **CS-3** and **4**, (Δ) from **CS-3** and **5**, and (●) from **CS-3** and **6**.

Figure 2C shows the plots between the ratios of furan and oxanorbonadiene and the reaction time. It is clear that azido-carboxylic acid, **4**, performs the best to accomplish the reaction within 3 days, whereas azido-disulfide substrate, **5**, and azido-silane substrate, **6**, show the completion of the reaction after more than 10 days. The reason why **4** shows higher reactivity might be related to good solubility in water. In addition, the bulkiness of **5** and **6** might hinder the ability to Click the oxanorbornadiene. Especially in the case of **5**, a cross-link via two units of triazoles might be possible as can be traced from the disappearance of the N_3 group (2090 cm^{-1}) in FT-IR spectrum (see Supporting Information, Figure 2d). The disappearance of the azido peak at 2090 cm^{-1} during the reaction could confirm the conversion of this metal-free Click reaction, while the original fingerprint of the chitosan-oxanorbornadiene derivative remained unchanged (Figure S2).

In summary, novel chitosan derivatives, chitosan-oxanorbornadienes, were successfully synthesized enebling metal free Click chemisty. The hydroxyl and the amine groups at the polymeric chain acted as nucleophiles to perform the reaction with the NHS-oxanorbornadiene. The high degree of oxanorbornadiene substitution on chitosan (~80%) could be accomplished when oxanorbonadiene was used with a certain spacer chain length in the form of a NHS-active ester, **3**. A series of water-based model reactions at room temperature between chitosan-oxanorbonadiene (**CS-3**) and azido-carboxylic acid derivative, disulfide derivative, and silane derivative proved a successful triazole linkage. Consequently, the chitosan oxanorbornadiene derivative, combined with metal-free Click chemistry, is a convenient derivative to provide simple ligation to other functional molecules like polymers or inorganic particles (e.g., magnetite, gold, silica particles) and makes chitosan useful in advanced applications, especially in the biomedical field.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, mechanism of **CS-2** and *N*-acylurea, curve fitting, and ^{1}H NMR spectra used for the present study are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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