Journal of Applied Polymer Science

Preparation of a C_6 Quaternary Ammonium Chitosan Derivative through a Chitosan Schiff Base with Click Chemistry

Yu Chen,[1] Fengju Wang,[1] Dongran Yun,[1] Yanwen Guo,[2] Yanchun Ye,[2] Yanxi Wang,[2] Huimin Tan[1]

[1]School of Material Science and Engineering, Beijing Institute of Technology, Beijing 100081, People's Republic of China

[2]School of Science, Beijing Institute of Technology, Beijing 100081, People's Republic of China

Correspondence to: Y. Chen (E-mail: cylsy@163.com)

ABSTRACT: In this study, we explored a new method for preparing C_6 quaternary ammonium chitosan (CTS) derivatives. The C_2—NH_2 of CTS was first protected by benzaldehyde. The C_6—OH of CTS was then transformed into a sulfonyl ester, which was then reacted with NaN_3 through nucleophilic substitution to introduce the —N_3 group at the CTS C_6 position. This intermediate was reacted in a click chemistry reaction with a terminal alkynyl quaternary ammonium salt; this was followed by the deprotection of C_2—NH_2 with acid to furnish the C_6 quaternary ammonium CTS derivative. The structures and properties of synthesized products in the reactions were characterized by Fourier transform infrared spectroscopy, NMR, X-ray diffraction, and thermogravimetric analysis, respectively. The effects of the reaction conditions on the degree of Schiff-base CTS quaternization were evaluated by elemental analysis. The largest inhibition zone test and the minimum inhibitory concentration test showed that compared with CTS, the prepared CTS derivative had significantly improved antibacterial activity toward *Staphylococcus aureus* and *Escherichia coli*. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

KEYWORDS: functionalization of polymers; modification; polysaccharides

Received 21 January 2012; accepted 19 July 2012; published online
DOI: 10.1002/app.38431

INTRODUCTION

Chitosan (CTS) is the deacetylation product of chitin and the only readily available basic amino polysaccharide in natural polysaccharides. Because of its unique C_2—NH_2 moiety, CTS has excellent material properties and wide application as a wastewater treatment agent, filter aid, enhancer, surface sizing agent, and bactericidal/antibacterial agent in the paper industry.[1–5] However, the application of CTS is restricted to only acidic conditions where the —NH_2 group becomes protonated and, thus, has good solubility and enables antibacterial effects.[6,7] The further enhancement of the antibacterial activity of CTS over a broader pH range will promote the better application of CTS in the paper industry. The quaternization of CTS is an important means for improving its antibacterial properties. A number of researchers have reported the preparation and applications of quaternized CTS derivatives.[8,9] The preparation of the quaternary ammonium salt of CTS includes reactions on C_2—NH_2 and C_6—OH. There are several methods for modifying C_2—NH_2 to prepare CTS quaternary ammonium salt derivatives, such as the direct quaternization of C_2—NH_2,[10,11] coupling with the small-molecule quaternary ammonium salt bearing reactive functional groups,[12,13] and graft copolymerization of vinyl quaternary ammonium salt on the side chain of CTS.[14,15] The attachment of quaternary ammonium groups on the C_6—OH of CTS can form a dual antibacterial functionality together with C_2 polycationic amino groups to greatly enhance the antibacterial properties of the CTS derivatives. C_6 quaternized CTS also has higher cationic properties and interacts more strongly with fibers and fillings that have negative surface charges; this provides it with enhanced filter aid performance. C_6 quaternized CTS has wider potential application in the paper industry. Furthermore, C_6 quaternized CTS can also be widely used in the fields of antibacterial wound dressings, cosmetics, textile printing and dyeing, food preservation materials, and so on. The preparation of C_6 quaternized CTS derivatives has been reported. Usually, the C_2 amino group in CTS is first protected by a Schiff base; after the reaction between the C_6—OH of the CTS Schiff base and 2,3-epoxypropyltrialkyl ammonium chloride, the Schiff base is removed to obtain an O-substituted CTS quarternary ammonium salt.[16–18] Nevertheless, there are relatively few reports on new routes for performing the C_6 quaternization of CTS.

Recently, click chemistry has become a powerful tool for materials modification by material chemists.[19] The most representative click reaction is the Cu(I)-catalyzed [3 + 2] cycloaddition of azide with an alkyne to form a triazole heterocycle; this method

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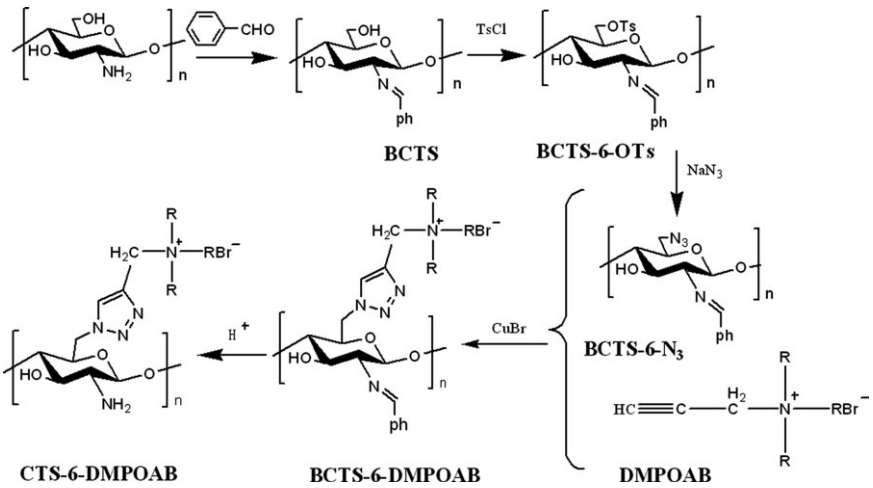

Scheme 1. Reaction routes for the preparation of a C_6 quaternary ammonium CTS derivative through the CTS Schiff base with click chemistry.

was developed by Sharpless,[20] who is a Nobel laureate in chemistry (2001). The click reaction has a number of advantages, including a high reaction rate, readily available starting material, high reliability, high oxygen and moisture stability, high yield, mild reaction conditions, good stereoselectivity, simple workup, and easy purification.[21,22] Click chemistry not only has broad applications in synthetic organic chemistry[23] but also has been employed in the immobilization of small molecules, dendrimers, linear polymers, and biological macromolecules into the pyran ring of carbohydrate polymers.[24,25] The modification of carbohydrate polymers by click chemistry will help to overcome their disadvantages, such as their low selectivity, complicated reaction conditions, various side reactions, and low yields, and will remarkably improve their substitution efficiency. The preparation of CTS derivatives via click chemistry has also been reported recently. Gruskiene et al.[26] prepared new CTS–poly (ethylene glycol) monomethyl ether derivatives containing intermediate triazolyl moieties at the C_6 position of glucosamine units by coupling via a 1,3-dipolar cycloaddition between azide and propargyl groups of CTS and poly(ethylene glycol) monomethyl ether. Ifuku et al.[27] recently presented the preparation of highly regioselective CTS derivatives via click chemistry with a 6-azido-6-deoxy CTS derivative with two different kinds of terminal alkynes compounds. Zampano et al.[28] prepared *N*-phthaloyl chitosan to protect the 2-NH_2 of CTS; then, selective C-6 azidation was achieved via a two-step synthesis via tosylation and a one-pot procedure, respectively. Finally, partially *O*-phthaloylated *N*-phthaloyl chitosan was prepared via a click reaction. Zhang et al.[29] selectively modified CTS with either azide or alkyne groups and then demonstrated a new way to fabricate CTS microcapsules, using click chemistry as the driving force of the layer-by-layer assembly.

In this study, after the protection of CTS C_2—NH_2 into the Schiff base, we activated CTS C_6—OH and prepared a C_6—OH modified quaternary ammonium salt CTS derivative through click chemistry. The reaction routes are shown in Scheme 1. We thus explored a new route for preparing quaternary ammonium CTS derivatives.

EXPERIMENTAL

Materials

CTS (biological grade), with a deacetylation degree of 91.05% or greater and a molecular weight of 1.0×10^6, was supplied by Zhejiang Yuhuan Biochemical Co., Ltd. (China). *N,N*-Dimethyl-2-propynylamine (analytical reagent) was supplied by Acros Organic. Sodium ascorbate was purchased from Aladdin Reagent Co., Ltd. (China). Benzaldehyde, *p*-toluene sulfonyl chloride, sodium azide, *n*-octyl bromine, CuBr, triethylamine, glacial acetic acid, NaOH, acetone, methanol, aether, chloroform ($CHCl_3$), *N,N*-dimethylformamide, ethanol, tetrahydrofuran (THF), cyclohexane, and dimethyl sulfoxide were all analytical grade and were used as received.

Synthesis of *N,N*-Dimethyl-*N*-propynyl-*N*-octyl Ammonium Bromine (DMPOAB)

n-Octyl bromine (5.38 g) was dissolved in THF (20 mL), and *N,N*-dimethyl-2-propynylamine (3.6 g) was added. The mixture was heated at 50°C for 48 h and then concentrated at room temperature under reduced pressure. The residue was dissolved in THF, and the DMPOAB product was precipitated by the addition of cyclohexane. DMPOAB was obtained at a 71% yield.

Fourier transform infrared (FTIR) spectroscopy (KBr, cm^{-1}): 3311 ($\nu_{\equiv CH}$), 2962 (ν_{CH2}), 2862 (ν_{CH3}), 2120 ($\nu_{C\equiv C}$), 1633 (ν_{C-N}), 1464 (δ_{CH2}), 1377 (δ_{CH3}), 1067 (ν_{C-N}), 721 (γ_{CH2}), 679 ($\delta_{\equiv CH}$). ^{13}C-NMR ($CDCH_3$, ppm): 81 (—C≡), 71 (≡C), 64 (alkynyl—CH_2—N^+), 54 (N^+—CH_2), 51 (CH_3—N^+), 32 (CH_2), 29 (CH_2), 26 (CH_2), 23 (CH_2), 22 (CH_2), 14 (CH_3). ANAL.: Calcd: C, 54.96%; H, 9.16%; N, 5.34%. Found: C, 55.11%; H, 9.07%; N, 5.16%.

Synthesis of 2-Benzaldehyde Schiff-Base Chitosan (BCTS)

To a three-necked, round-bottom flask equipped with a condenser and nitrogen protection, 1 g of CTS was added into a glacial acetic acid/methanol solution (2 wt %) and dissolved for 2 h; then, benzaldehyde ($n_{benzaldehyde}/n_{CTS} = 1.5$; where n is amount of a substance) dissolved in methanol was added. After stirring at 60°C for 4 h, the pH value of the reaction mixture was adjusted to neutrality by a NaOH/methanol solution (5 wt

%) and continued to react at 60°C for 4 h. The reaction mixture was filtered and successively washed with methanol, ethanol, and ether and was then dried *in vacuo* to afford BCTS.

FTIR (KBr, cm^{-1}): 3449 ($\nu_{O-H} + \nu_{N-H}$), 2931 (ν_{-CH2-}), 2878 (ν_{-CH3}), 1644 (ν_{C-N}), 1594 ($\delta_{N-H} + \nu_{C-N}$), 1575 (ν_{C-C}, benzene ring), 1447 (δ_{-CH2-}), 1365 (δ_{-CH3}), 1315 ($\nu_{C-N} + \delta_{N-H}$), 1230 (ν_{C-O-C}), 1152 (ν_{C-O-C}), 1033 (ν_{C-N}), 758 (δ_{C-H}, benzene ring), 693 (δ_{C-H}, benzene ring). ANAL. Found: C, 61.28%; N, 5.20% (for CTS, C 45.22%, N 8.57%).

The degree of substitution with Schiff groups (0.91) was estimated by the increase in the molar C/N ratio between CTS (C/N = 5.28) and BCTS (C/N = 11.78).

Synthesis of 2-Benzaldehyde Schiff-Base Chitosan–6-*p*-Sulfonyl Ester (BCTS–6-OTs)

To a 9 wt % aqueous solution of NaOH (40 mL) was added 0.5 g of BCTS. The mixture was stirred at 30°C for 2 h to allow BCTS to fully swell; then, a chloroform solution of *p*-toluene sulfonyl chloride (n_{BCTS}/n_{TsCl} = 1:20) was added dropwise to the flask. After it was stirred at 30°C for 2 h, the reaction mixture was filtered and washed with ethanol two times and then dried *in vacuo* to give BCTS–6-OTs.

FTIR (KBr, cm^{-1}): 3439 ($\nu_{O-H} + \nu_{N-H}$), 2924 (ν_{-CH2-}), 2872 (ν_{-CH3}), 1642 (ν_{C-N}), 1594 ($\delta_{N-H} + \nu_{C-N} + \nu_{C-C}$), 1497 ($\nu_{C-C}$, *p*-tosyl), 1451 ($\delta_{-CH2-}$), 1364 ($\delta_{-CH3} + \nu_{asS-O}$), 1171 ($\nu_{S-O}$), 1157 ($\nu_{C-O-C}$), 1031 ($\nu_{C-N}$), 816 ($\delta_{C-H}$, *p*-substituted benzene ring), 750 (δ_{C-H}, benzene ring), 693 (δ_{C-H}, benzene ring). ANAL. C, 56.50%; N, 3.15%; S, 7.36%.

The substitution degree of *p*-sulfonyl ester was 91.62%, as determined by elemental analysis.

Synthesis of 2-Benzaldehyde Schiff-Base Chitosan–6-Azide (BCTS–6-N_3)

To a three-necked, round-bottom flask equipped with a magnetic bar and a condenser, BCTS–6-OTs (0.5 g) and *N,N*-dimethylformamide (10 mL) were added and stirred at room temperature. Sodium azide ($n_{NaN3}/n_{BCTS-OTs}$ = 1:1) was added to the solution, and then, the mixture was stirred at 80°C for 4 h. Afterward, the reaction was quenched with excess acetone, and the formed precipitate was filtered, successively washed with water, ethanol, and ether, and vacuum-dried to afford BCTS–6-N_3.

ANAL. Found: C, 68.96%; N, 13.72%; S, 3.19%.

The substitution degree of —N_3 was 77.9%, as determined by elemental analysis.

FTIR (KBr, cm^{-1}): 3435 ($\nu_{O-H} + \nu_{N-H}$), 2926 (ν_{-CH2-}), 2879 (ν_{-CH3}), 2106 ($\nu_{-N-N\equiv N}$), 1651 (ν_{C-N}), 1594 ($\delta_{N-H} + \nu_{C-N}$, which became weaker than the corresponding peaks in the spectrum of BCTS–6-OTs), 1458 (δ_{-CH2-}), 1377 (δ_{-CH3}), 1157 (ν_{C-O-C}), 1063 (ν_{C-N}), 756 (δ_{C-H}, benzene ring), 693 (δ_{C-H}, benzene ring).

Synthesis of the C_6—OH-Substituted Quaternary Ammonium Salt of 2-Benzaldehyde Schiff-Base Chitosan (BCTS–6-DMPOAB) through Click Chemistry

To a solution of BCTS–6-N_3 in dimethyl sulfoxide (2.5% w/v) was added CuBr (0.5 mmol), triethylamine (0.5 mmol), sodium ascorbate (0.25 mmol), and DMPOAB. The reaction was allowed to proceed for and at a certain time and temperature, precipitated sequentially in 1:1 H_2O/EtOH and H_2O, filtered, washed sequentially with H_2O, EtOH, and acetone, and then dried *in vacuo*.

FTIR (KBr, cm^{-1}): 3439 ($\nu_{O-H} + \nu_{N-H}$), 3127 (ν_{-CH-}), 2920 (ν_{-CH2-}), 2869 (ν_{-CH3}), 1622 ($\delta_{N-H} + \nu_{C-N} + \nu_{C-N}$), 1464 ($\delta_{-CH2-}$), 1352 ($\delta_{-CH3}$), 1157 ($\nu_{C-O-C}$), 1054 ($\nu_{C-N}$), 1020 ($\nu_{N-N}$), 756 ($\delta_{C-H}$, benzene ring), 722 ($\gamma_{CH2}$), 693 ($\delta_{C-H}$, benzene ring).

Deprotection of BCTS–6-DMPOAB

BCTS–6-DMPOAB was soaked in a 5 wt % acetic acid aqueous solution and stirred for 24 h at 30°C. Then, ethanol was added to the reaction mixture, and the formed precipitate was filtered, washed, and dried to give the amino-deprotected substitution product CTS-*g*-DMPOAB.

FTIR (KBr, cm^{-1}): 3430 ($\nu_{O-H} + \nu_{N-H}$), 3120 (ν_{-CH-}), 2923 (ν_{-CH2-}), 2864 (ν_{-CH3}), 1640 ($\delta_{N-H} + \nu_{C-N} + \nu_{C-N}$), 1464 ($\delta_{-CH2-}$), 1364 ($\delta_{-CH3}$), 1150 ($\nu_{C-O-C}$), 1059 ($\nu_{C-N}$), 1020 ($\nu_{N-N}$), 718 ($\gamma_{CH2}$).

Characterization

The FTIR spectra were obtained on a NEXUS-470 series FTIR spectrometer (Nicolet Co.) with a scanning range of 500–4000 cm^{-1}. KBr pellets of the samples were used.

^{13}C-NMR spectra were obtained on a 300-MHz NMR AL-300 spectrometer (JEOL, Japan) with tetramethylsilane as an internal standard.

Wide-angle X-ray diffraction (XRD) of the samples was performed on an X'Pert Pro MPD type X-ray diffractometer (PaNalytical Co., Holland) with Cu Kα characteristic radiation (λ = 0.154 nm) at a voltage of 40 kV and a current of 40 mA. The scanning rate was 5°/min and the scanning scope of 2θ was from 5 to 60° at room temperature.

Thermogravimetric analysis (TGA) was carried out on a Q5000 IR thermogravimetric analyzer (TA Co.) from 25 to 800°C at a heating rate of 20°C/min under nitrogen.

Ultraviolet–visible spectra were recorded with a Pgeneral TU-1810 ultraviolet–visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

Elemental analysis was carried out with a Vario EL elemental analyzer (Germany). The C, H, and N contents of BCTS–6-N_3 were determined, and the substitution degree of —N_3 was calculated. Ultimately, the C, H, and N contents of BCTS–6-DMPOAB were determined, and the substitution degree of quaternization (DQ) was calculated.

Antibacterial Properties Test

According to a method from the literature,[30] the inhibitory effects of CTS and C_6—OH-substituted quaternary ammonium salt of chitosan (CTS–6-DMPOAB) against *Staphylococcus aureus* and *Escherichia coli* were compared with a largest inhibition zone test and the minimum inhibitory concentrations (MICs).

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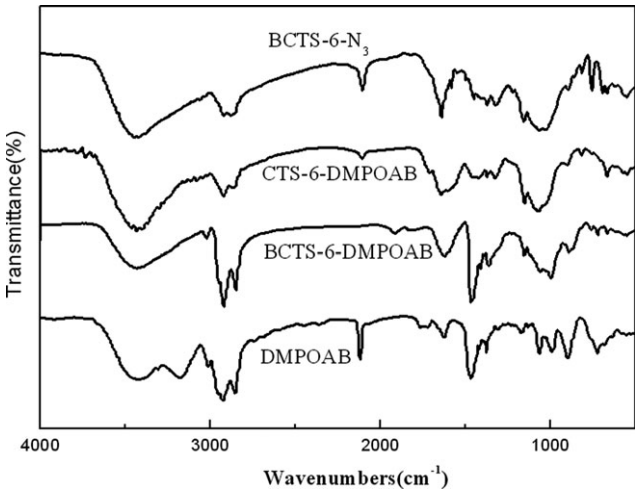


Figure 1. FTIR spectroscopy of the synthesized products in the reactions.

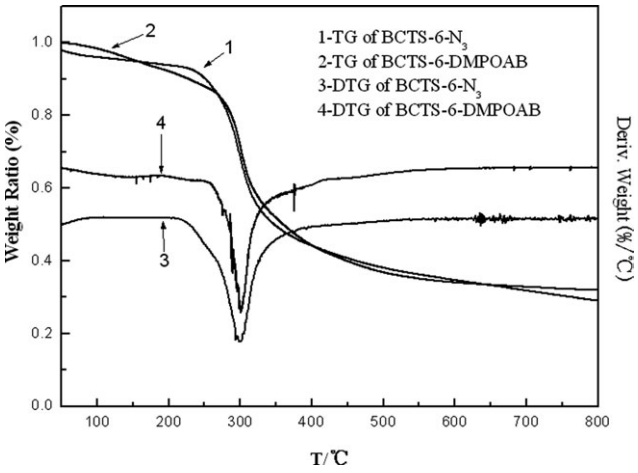


Figure 3. TGA and differential thermal analysis curves of BCTS–6-N_3 and BCTS–6-DMPOAB.

RESULTS AND DISCUSSION

FTIR Characterization

The structures of BCTS–6-N_3, DMPOAB, and BCTS–6-DMPOAB were identified and compared by FTIR spectroscopy (Figure 1). After the click chemistry reaction, the absorption peaks of the terminal alkyne in DMPOAB at 2120 cm^{-1} ($\nu_{C\equiv C}$), 3311 cm^{-1} ($\nu_{\equiv C-H}$), and 679 cm^{-1} ($\delta_{\equiv C-H}$) all disappeared. Also, the characteristic absorption of the azido group in BCTS–6-N_3 at 2106 cm^{-1} also disappeared. There was a large broad peak at over 3000 cm^{-1}, which was the hydrogen bonding of —OH and —NH in CTS, which also masked the stretching absorption of unsaturated C—H in the triazole at around 3127 cm^{-1}. Compared with BCTS–6-N_3 and DMPOAB, a broad and strong peak formed in the range 1657–1565 cm^{-1} because of the overlap of C=N in the triazole and the C=O stretching of amide I of the CTS framework. The overlap of the N—N stretching of triazole and the C—N stretching in CTS and DMPOAB formed a strong broad peak near 1020 cm^{-1}. Also, the bending absorptions of methyl and methylene groups appeared at 1352 and 1464 cm^{-1}, and the characteristic absorption peaks of the —$(CH_2)_n$— chain ($n \geq 4$) appeared at 722 cm^{-1}. These observations further indicated that the click chemistry reaction between DMPOAB and BCTS–6-N_3 occurred and introduced the long-chain quaternary ammonium group to the BCTS molecule.

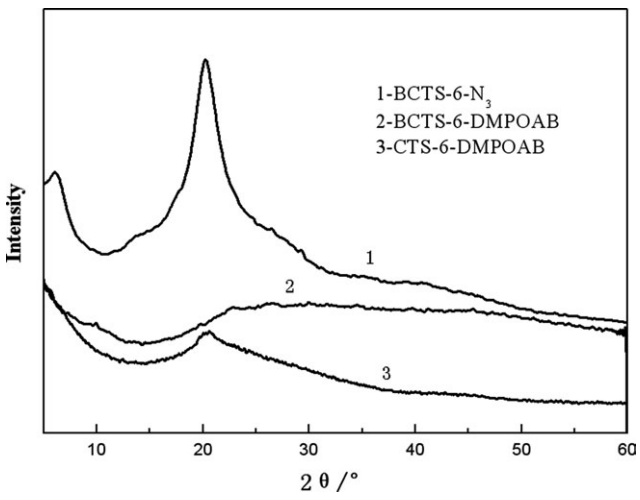


Figure 2. XRD curves of the synthesized products in the reactions (T = temperature).

After the Schiff base was deprotected from BCTS–6-DMPOAB, the —NH_2 stretching absorption at 3424 cm^{-1} and the —NH_2 bending absorption at 1600 cm^{-1} all strengthened more than the bending absorption of methyl and methylene groups at 1352 and 1464 cm^{-1}, and the bending vibrations of benzene at 756 and 693 cm^{-1} disappeared for CTS–6-DMPOAB. The results show that the C_2 of CTS was deprotected from the Schiff base to form —NH_2 again, and the final product CTS–6-DMPOAB was obtained.

XRD Characterization

The XRD patterns of the products are shown in Figure 2. We observed that the BCTS–6-N_3 still had strong diffraction at $2\theta = 7°$ and 20°. However, after the click chemistry reaction, the long alkyl chain quaternary ammonium salt was introduced to the C_6—OH of CTS, which greatly increased the distance between the CTS molecular chains and weakened the intermolecular hydrogen bonds. As a result, there was no sharp diffraction peak but only a broad peak in the range $2\theta = 5–60°$ in the XRD spectra of BCTS–6-DMPOAB. After the hydrolysis of the Schiff base, the amino groups and the residual —OH groups in CTS formed hydrogen bonding; this resulted in the appearance of a new diffraction peak around $2\theta = 20°$. Combined with the FTIR data, the XRD data further confirmed the occurrence of the click chemistry reaction.

TGA Characterization

The TGA and differential thermogravimetry curves of BCTS–6-N_3 and BCTS–6-DMPOAB are compared in Figure 3. The weight loss of BCTS–6-N_3 occurred mainly in the range 207–413°C, and the maximum decomposition rate was observed at 299°C. The total weight loss in this temperature range was 50.47%. This weight loss mainly corresponded to the thermal

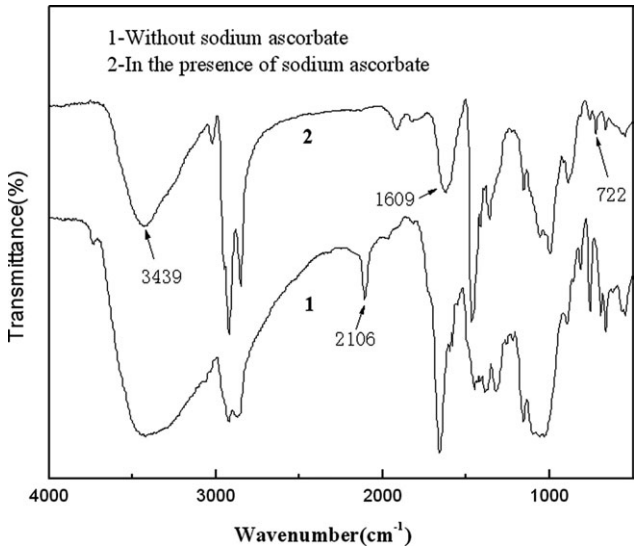


Figure 4. FTIR spectroscopy of the click chemistry products without and in the presence of sodium ascorbate.

decomposition of the CTS Schiff base bearing the azido group. Due to the unstable nature of the azido functional group and Schiff base, the temperature of maximum thermal decomposition rate for BCTS–6-N_3 was lower than that of CTS (315°C) and BCTS (311°C).

The weight loss of the click chemistry reaction product BCTS–6-DMPOAB mainly occurred in the range 257–348°C, the maximum decomposition rate was observed at 304°C, and the total weight loss in this temperature range was 35.13%. Compared with BCTS–6-N_3, BCTS–6-DMPOAB had a lower total weight loss but a higher initial decomposition temperature and higher maximum decomposition rate temperature. This indicated that the thermal stability of BCTS–6-DMPOAB was improved compared to that of BCTS–6-N_3 after the connection of DMPOAB and BCTS through the triazole structure.

Impact Factors of the Click Chemistry Reaction

We compared the structures of the BCTS–6-DMPOAB prepared via the click chemistry reaction without and in the presence of 0.25 mmol of sodium ascorbate. As shown in Figure 4, the stretching absorption of N—H at 3439 cm^{-1}, and the bending vibration of N—H at 1609 cm^{-1} decreased obviously for the product prepared via sodium ascorbate addition. The characteristic absorption of the azido group at 2106 cm^{-1} disappeared, and the characteristic absorption peaks of the —$(CH_2)_n$— chain ($n \geq 4$) appeared at 722 cm^{-1} for the previous product. In contrast, for the product prepared without sodium ascorbate, there was still a strong stretching vibration peak of N—H at 3439 cm^{-1} and a bending vibration peak of N—H at 1609 cm^{-1}. The characteristic absorption of the azido group at 2106 cm^{-1} did not disappear, and the characteristic absorption peaks of —$(CH_2)_n$— chain ($n \geq 4$) did not appear at 722 cm^{-1}. The previous results confirm that the addition of sodium ascorbate facilitated the click chemistry reaction.

Cu^+ is an effective catalyst in the click chemistry reaction, but it is very unstable in aqueous solutions. The d^{10} electronic subshell in Cu^+ tends to lose one electron to form the Cu^{2+} ion. The hydrated ion $[Cu(H_2O)_6]^{2+}$ in aqueous solution is also more stable than the $[Cu(H_2O)_6]^+$ ion.[31] CTS and its derivatives can also easy chelate with the Cu^{2+} ion to deactivate the catalyst.[32] Therefore, in the Cu^+-catalyzed click chemistry reaction of the CTS derivative, the addition of sodium ascorbate stabilized the Cu^+ and facilitated the click chemistry reaction.

As previously mentioned, with regard to the determination of the degree of quaternization by elemental analysis, we investigated the impact of the click reaction conditions, such as the molar ratio of DMPOAB to BCTS–6-N_3 and the reaction time and temperature, on the degree of quaternization of BCTS–6-DMPOAB. The results are listed in Table I.

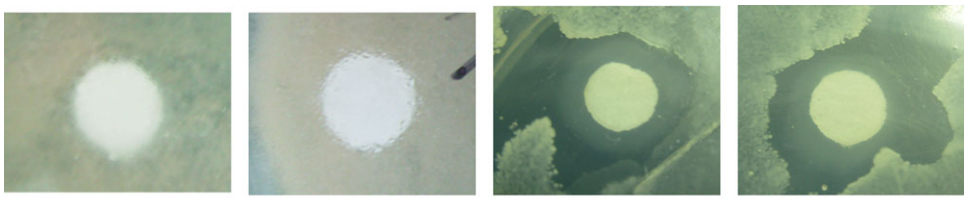
As shown in Table I, with increasing addition of DMPOAB, the degree of quaternization of the prepared BCTS-*g*-DMPOAB also gradually increased. However, because DMPOAB had a large molecular volume, its introduction to the C_6—OH position of CTS resulted in significant steric hindrance. Therefore, when $n_{DMPOAB}/n_{BCTS-6-N3}$ exceeded 1.25, a further increase in the addition of DMPOAB did not significantly increase the degree of quaternization of the product but only complicated the reaction workup.

As also shown in Table I, the degree of quaternization kept increasing when the reaction time was prolonged. However, the rate increase became slow after the reaction proceeded for 3 h, which might have been the most appropriate reaction time for high efficiency.

The degree of quaternization was low in the product BCTS–6-DMPOAB when the click reaction was carried out at a lower temperature. With the reaction temperature was increased, the degree of quaternization significantly increased and hit a peak at 35°C. However, the degree of quaternization dropped when

Table I. Impact of the Click Reaction Conditions on the Degree of Quaternization of BCTS–6-DMPOAB

$n_{DMPOAB}/n_{BCTS-6-N3}$	Reaction time (h)	Reaction temperature (°C)	DQ (%)
0.5:1			21.35
0.75:1			37.63
1:1	3	35	53.59
1.25:1			66.98
1.5:1			68.26
	1		40.69
	2		57.33
1.25:1	3	40	61.51
	4		64.23
	5		66.53
		20	19.53
		30	40.69
1.25:1	3	35	66.98
		40	61.51
		50	53.75



A, Inhibitory effect of 1% CTS solution on *Staphylococcus aureus*;
B, Inhibitory effect of 1% CTS solution on *Escherichia*;
C, Inhibitory effect of 1% CTS-6-DMPOAB solution on *Staphylococcus aureus*;
D, Inhibitory effect of 1% CTS-6-DMPOAB solution on *Escherichia*.

Figure 5. Inhibitory effects of CTS and CTS–6-DMPOAB against *S. aureus* and *E. coli*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the reaction temperature was increased further. Thus, 35°C was considered to be the optimum reaction temperature.

Antibacterial Properties of CTS–6-DMPOAB

Using the largest inhibition zone test, we compared the inhibitory effects of CTS and CTS–6-DMPOAB against *S. aureus* and *E. coli*. It can be seen from Figure 5 that the 1% CTS solution had a very weak inhibitory effect on *S. aureus* and *E. coli*, and the bacteria multiplied greatly in the vicinity of the sample. In contrast, the 1% CTS–6-DMPOAB solution effectively inhibited the two bacteria, and no bacteria survived in the vicinity of the sample. The MICs of CTS against *S. aureus* and *E. coli* were examined; the values were 0.0125 and 0.050%, respectively. The MICs of CTS–6-DMPOAB with DQ = 40.10% were 0.00625 and 0.00313%, respectively. When DQ of CTS–6-DMPOAB was increased to 58.75%, the MICs were 0.00313 and 0.00156%, respectively.

As stated previously, the long-chain quaternary ammonium group in CTS–6-DMPOAB had an antibacterial effect. In addition, the DMPOAB group at the C_6 position of CTS synergized with the C_2—NH_2 of CTS to further improve the antibacterial properties of CTS–6-DMPOAB. The structure–activity relationship of CTS–6-DMPOAB will be examined in-depth in a future study.

CONCLUSIONS

In this study, a new route for preparing C_6 quaternary ammonium CTS derivatives was explored. The DMPOAB group at the C_6 position of CTS synergized with the C_2—NH_2 of CTS to further improve the antibacterial properties of CTS–6-DMPOAB. The structures and properties of the synthesized products in the reactions were characterized. The effects of the sodium ascorbate addition, molar ratio of DMPOAB to BCTS–6-N_3, and reaction time and temperature on the degree of quaternization of BCTS–6-DMPOAB were evaluated by elemental analysis. By using the largest inhibition zone test and the MIC test, we compared the inhibitory effects of CTS and CTS–6-DMPOAB against *S. aureus* and *E. coli*. We found that the prepared CTS–6-DMPOAB had significantly improved antibacterial activity toward *S. aureus* and *E. coli* compared to CTS.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Natural Science Foundation of China (contract grant number 50903008) and the Basic Study Program of Beijing Institute of Technology (contract grant numbers 20080942002 and 20090942004).

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