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Research Article

The Synthesis of the Locating Substitution Derivatives of Chitosan by Click Reaction at the 6-Position of Chitin

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A novel method to prepare the macrocyclic compound locating substitution derivatives of chitosan was investigated, by using cyclodextrin as the model of macrocyclic compound. The method combines the advantages of activated 6-OH of chitin and high efficiency of click reaction. Chitin C_6-OH *p*-toluenesulfonate (CTN-6-OTs) was generated and subsequently transferred to chitin C_6-N_3 via nucleophilic substitution. Afterwards, β-cyclodextrin was immobilized at 6-OH of chitin via click reaction to afford CTN-6-CD. Ultimately, CTS-6-CD was obtained by removing the acetyl group of chitin unit. The structures and properties of these products were characterized by FTIR, TG, and XRD, respectively. It was found that CTN-6-CD synthesized at the optimum conditions has an immobilized loading of 1.6126×10^{-4} mol/g and that of the corresponding CTS-6-CD, generated by removal of the acetyl group, was 1.6891×10^{-4} mol/g.

1. Introduction

Macrocyclic compounds, such as cyclodextrin, calixarene, crown ether, and cucurbituril, and so forth, have attracted more and more attention in recent years. With the aid of their specific cavity structures, they could be widely used in the fields of functional carrier materials or medicine [1, 2].

Grafting macrocyclic molecules into chitosan-reactive sites may lead to a molecular carrier that possesses the cumulative effects of inclusion, size specificity, and transport properties of macrocyclic compounds as well as the properties of easily modifying, good biodegradability, film-forming property, biocompatibility, antibacterial property, and low toxicity of the polymeric matrix [3–6]. The products obtained by macrocyclic compound grafting to chitosan using different methods and their properties have been studied extensively [7, 8].

However, in most cases the immobilizations were made on the 2-NH_2 of chitosan [9–12]. As the amino group of chitosan gives it great physiological activities and at the same time brings a good modification handle, the above derivatives are not conducive to perform further amino group modification and manipulate its unique properties.

If macrocyclic compound could be immobilized at the 6-OH position of chitosan, and at the same time reserve the 2-NH_2 group of chitosan, the supramolecular structure with particular properties could be formed and the applications of chitosan derivatives could be greatly expanded.

In the various kinds of macrocyclic compounds, β-cyclodextrin is a very important species due to their unique hydrophobic internal cavity and hydrophilic external surface. Recently, the host-guest interactions of β-cyclodextrin or its derivatives with size-compatible hydrophobic molecules have been extensively studied and applied in the fields of chiral recognition, controlled drug release, chemical analysis, analogue enzyme, molecular switch, and so forth [13, 14]. The preparation of 6-OH immobilized β-cyclodextrin derivatives of chitosan has been reported [15–19]. Since the 6-OH group of chitosan has a lower reactivity than the 2-NH_2, preparation of the above derivatives was more difficult and yielded substitution degrees were relatively low ($<7 \times 10^{-5}$ mol/g). In comparison, derivatives of chitosan immobilizing with β-cyclodextrin at the 2-NH_2 position reported in the literature could attain 2.4×10^{-4} mol/g [20]. Practical application of 6-OH β-cyclodextrin immobilized derivatives of chitosan was restricted for the complicated preparation route and

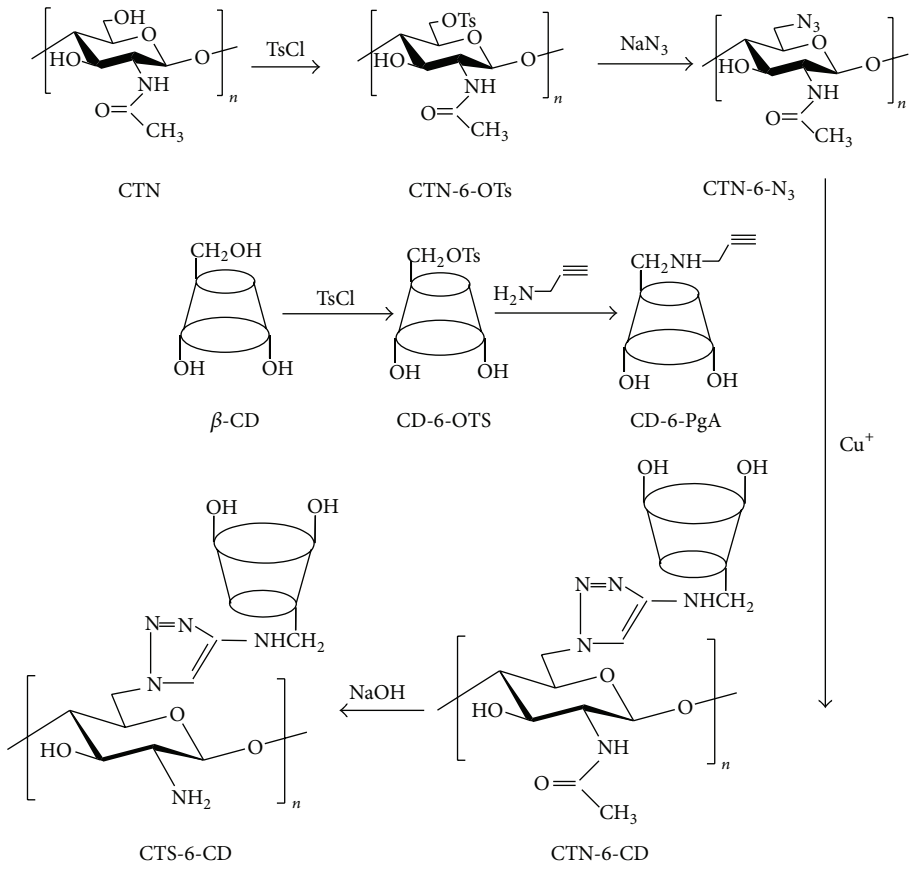

Figure 1: The main reaction route for preparation of C_6-immobilized β-cyclodextrin of chitosan via click reaction of chitin derivative.

lower substitution degree. Then simplification of the reaction route and promoting the substitution degree of 6-OH β-cyclodextrin immobilized derivatives of chitosan are very necessary and useful.

Recently, efficient and high yielding "click reaction" as a useful and versatile tool in organic synthesis has attracted much attention. In particular, the Cu(I)-catalyzed 1,3-dipolar cycloaddition of terminal alkynes with azides to give 1,4-regioselectively substituted triazole heterocycle has been widely used in the synthesis and postpolymerization modification of polymers [21, 22]. Click chemistry has also been employed in immobilizing small molecules, linear polymers, dendrimers, and biological macromolecules on the pyran ring of carbohydrate polymers [23, 24]. Modification of carbohydrate polymers by click chemistry will help to overcome their shortages such as complicated reaction conditions, low selectivity, various side reactions, and low yields and remarkably improve their immobilizing efficiency. Preparation of the chitosan derivatives via click chemistry has also been reported recently [25–27]. However, to the best of our knowledge, there is no report about immobilizing β-cyclodextrin derivatives in chitosan at a specific position via click chemistry.

On the basis of substitution reactions of chitin, removing the acetyl of the derivatives is an important way to synthesize the orienting substitutive derivatives at 6-OH of chitosan. In order to prepare diethylaminoethyl- (DEAE-) chitosan, where the DEAE groups were specifically introduced onto 6-OH groups of chitosan with minimal by-product, Kim and Chun prepared DEAE-chitin by reacting chitin with *N*, *N*-diethylaminoethyl chloride firstly and then carried out direct *N*-deacetylation to prepare the aim structure [28]. In previous study of our group [29], tosylated chitin at 6-OH positions was prepared and then displaced by the monoamino-β-cyclodextrin derivative via nucleophilic substitution of the tosyl group to afford the 6-OH substituted cyclodextrin derivatives. Further removal of acetyl groups on the chitin main chain yielded the C_6 substituted cyclodextrin derivatives. However, as the reaction process of nucleophilic substitution was easy to be influenced by the volume of the substituting group, especially for that of the β-cyclodextrin, the substitution capacity of the product was a little low (1.2868×10^{-4} mol/g). By the way, the reaction process of nucleophilic substitution was complicated.

In this study, after 6-OH groups on chitin were activated, β-cyclodextrins, as a model of macrocyclic compound, were immobilized via click reactions. Further removal of the acetyl groups on chitin skeleton afforded supramolecular substituted β-cyclodextrins on 6-OH positions of chitosan. The main reaction route is shown in Figure 1. The above reaction route would be a high efficiency and simple route for preparation of 6-OH located substitution derivatives of

chitosan with free 2-NH_2. By substitution of β-cyclodextrin on 6-OH of chitin and then removing the acetyl groups of chitin, the route for preparation of the derivatives of chitosan with free 2-NH_2 could be simplified. In addition, for the high efficiency of click reaction, the substitution degree of β-cyclodextrin on 6-OH of chitosan is hopeful to be improved.

Depend on the special structure of 6-OH β-cyclodextrin immobilized derivatives of chitosan, the 2-NH_2 of it is easy to be further chemical modified or coupled, and the β-cyclodextrin immobilized bears the hydrophobic internal cavity and hydrophilic external surface property. Then the above derivatives could be used as a good skeleton for drug release carrier, sensitivity film for the electrochemical sensor or biochemical sensor, functional fiber, chromatographic support, and so forth.

2. Experimental

2.1. Materials. Chitin was chemical grade and was supplied by Zhejiang Golder-Shell Biochemical Co., Ltd. (China). *p*-Toluenesulfonyl chloride was of analytical grade and was supplied by Tianjin Guangfu Fine Chemical Research Institute (China). LiCl was of analytical grade and was purchased from Shanghai Shengke Bio-tech Co., Ltd. (China). Sodium azide (NaN_3, A.R.) was supplied by Tianjin Fucheng Chemical Reagent Co., Ltd. (China). β-Cyclodextrin (β-CD) was of analytical grade and was purchased from Sigma-Aldrich Chemical Reagent Co., Ltd. (America). Propargylamine (chemical pure grade, purity > 98%) was supplied by Nanjing Honest New Materials Co., Ltd. (China). Sodium ascorbate, with purity > 99%, was purchased from Beijing Chemical Reagents Company (China). Other reagents were of analytical grade all and were used as received. Mono-6-tosylated-β-cyclodextrin (CD-6-OTs) was synthesized according to the method in the literature [30].

2.2. Preparation of Mono-6-Propargyl Amino-β-Cyclodextrin (CD-6-PgA). CD-6-OTs and propargylamine (PgA) (molar ratio = 1 : 1.5) was added into a 3-neck round bottom flask equipped with a magnetic bar and condenser. Then the reaction was stirred at 60°C for 6 hours with nitrogen protection. Afterwards, the reaction was quenched with acetonitrile, and the formed precipitate was filtered and successively washed with acetonitrile. CD-6-PgA in 63% yield was obtained after being dried under vacuum. FTIR (KBr, cm^{-1}): 3432 (ν_{O-H} + ν_{N-H}), 2930 (ν_{asCH_2}), 2129 ($\nu_{C\equiv C}$), 1603 (δ_{N-H}), 1446 (δ_{CH_2}), 1407 (δ_{O-H}), 1339 (δ_{CH}), 1182 (ν_{C-O-C}), 1035 (ν_{C-N} + ν_{C-O}, the strength was stronger than 6-OTS-β-CD). ^{13}C-NMR (DMSO-d_6, δ): 104.7 (C_1, β-CD), 81.6 (C_4, β-CD), 79.6 (–C≡), 73.3 (C_3, β-CD), 72.1 (C_5, β-CD), 71.9 (C_2, β-CD), 60.1 (C_6, β-CD), 58.8 (≡C), 31.5 (propargylamine, NH–C–). Elemental analysis is as follows: measured values: C 45.50%, H 6.22%, and O 48.28%, and theoretical values: C 46.08%, H 6.23%, and O 47.69%.

2.3. Synthesis of 6-Tosylated-Chitin (CTN-6-OTs). Chitin (0.5 g) and LiCl (1.4 g) were added to a three-neck flask. Then the *reaction apparatus* was sealed and protected by nitrogen. 26 mL of dimethylacetamide (DMAc) was injected by needle tube. The mixture was stirred at room temperature to let chitin fully swell and then triethylamine (7.8 mL) and 9.0 g of *p*-toluenesulfonyl chloride dissolved in DMAc (26 mL) were added. After stirring at 8°C for 24 h, the reaction mixture was quenched by pouring into a large excess of acetone with stirring to precipitate the brown crude product. The solid was filtered and successively washed thoroughly with methanol, water, and ethanol and then dried under vacuum to give CTN-6-OTs. The yield of CTN-6-OTs was 73%. Elemental analysis is as follows: C 54.93%, H 5.34%, N 4.18%, and S 7.77%. The substitution degree of *p*-toluenesulfonyl was 81.27%, as determined by elemental analysis.

2.4. Preparation of 6-Azide-Chitin (CTN-6-N_3). To a 3-neck round bottom flask equipped with a magnetic bar and condenser, CTN-6-OTs (0.5 g) and DMF (10 mL) were added and stirred at room temperature. 0.096 g of sodium azide was added to the solution and then the mixture was stirred at 80°C for 8 hours. Afterwards, the reaction was quenched with excess acetone, and the formed precipitate was filtered and successively washed with water, ethanol, and ether and vacuum dried at 60°C to afford CTN-6-N_3. CTN-6-N_3 was obtained in 63% yield. Elemental analysis is as follows: C 44.28%, H 5.45%, N 19.68%, and S 0.84%. The substitution degree of -N_3 was 75.19%, as determined by elemental analysis.

2.5. Synthesis of Chitin C_6 Immobilized β-Cyclodextrin Derivatives (CTN-6-CD) via Click Chemistry. To a 3-neck round bottom flask equipped with a magnetic bar and condenser, 0.5 g of CTN-6-N_3 was added and dissolved in certain organic solvent (Such as dimethyl sulfoxide, N-methylpyrrolidone, 5% LiCl-DMAc solution, and triethylamine, resp.). A solution of 0.025 g $CuSO_4 \cdot 5H_2O$ in 2 mL of *dimethyl sulfoxide* (DMSO), 0.060 g sodium ascorbate, and some deionized water were added into the flask, followed by adding CD-6-PgA according to certain molar ratio ($n_{CD\text{-}6\text{-}PgA} : n_{CTN\text{-}6\text{-}N_3}$, the molar ratio of CD-6-PgA to CTN-6-N_3, range from 0.5 : 1 to 5 : 1). Then the mixture was stirred for a while at a certain temperature (range from 30°C to 90°C). Afterwards, the reaction was quenched with excess deionized water, and the precipitate was filtered and dried to furnish the CTN-6-CD with 57% yield.

2.6. Deacetylation and Preparation of Chitosan C_6 Immobilized β-Cyclodextrin Derivatives (CTS-6-CD). CTN-6-CD was soaked in 45% of NaOH solution. The mixture was added in a round bottom flask equipped with condenser, stirred, gradually warmed up to 80°C, and reacted for 12 h. Upon completion of the reaction, the reaction mixture was diluted by pouring into water, standing overnight. The supernatant was removed after the precipitate settled and the precipitate was repeatedly washed with plenty of water to a neutral pH and then dried under vacuum to give the aim immobilized product CTS-6-CD. The yield of CTS-6-CD of this step was 78%.

2.7. Characterization. FTIR spectrum was obtained on a NEXUS-470 series FTIR spectrometer (Nicolet Co., USA). KBr pellets of the samples were used.

Wide angle X-ray diffraction (XRD) of samples was recorded by X'Pert Pro MPD type X-ray diffractometer (PANalystical Co., Holland), with 2θ angle from 5° to 50°.

Thermogravimetric analysis (TGA) of the samples was produced on a TG-Pyris 1 thermogravimetric analyzer (Shimadzu Co., Japan) at a heating rate of 20°C/min with temperature range from 50°C to 600°C and nitrogen was used as the purge gas.

UV-Vis spectra were recorded by using Pgeneral TU-1810 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

^{13}C-NMR spectra of CD-6-OTs (with DMSO-d_6 as solvent) was obtained on a 300 MHz NMR AL-300 (JEOL, Japan) spectrometer with tetramethylsilane as an internal standard.

Elemental analysis was carried out with a vario EL elemental analyzer (Germany). C, H, N contents of the samples were determined.

2.8. Determination of the Immobilized Loading. According to [31], concentrated sulfuric acid (5 mL) was slowly dropped in the β-cyclodextrin standard solutions, with which the concentrations were 0.025, 0.035, 0.040, 0.050, 0.080, and 0.100 g/L, respectively, and then reacted at room temperature for 30 min. The UV-Vis absorbance around 490 nm of the products from β-cyclodextrin hydrolysis was tested and the standard curve between the UV absorbance and the concentration could be deduced:

$$Y'_{CD} = 17.20854X'_{CD} - 0.26257 \quad (R = 0.99809), \tag{1}$$

where Y'_{CD} is the UV absorbance of the product from CD hydrolysis and X'_{CD} is the concentration of CD ($g{\cdot}L^{-1}$).

In the presence of concentrated sulfuric acid, β-cyclodextrin or its immobilized products will be hydrolyzed into monosaccharides and subsequently the carbohydrates will form uronic derivatives by rapid dehydration, which could condense with phenol to afford orange compounds with stable color. The maximum absorption peak could be measured around 490 nm. Within certain concentration range, the absorbance and the content of polysaccharide present linear relationship. Then the concentration of β-cyclodextrin in the sample can be determined by using the standard curve, and the substitution degree of β-cyclodextrin could be calculated.

However, it was found that under concentrated sulfuric acid hydrolysis conditions in the presence of phenol in an aqueous solution, the hydrolyzed pyranose ring from chitin or its deacetylation product also has certain degree of UV absorption around 490 nm. The standard working curves based on the hydrolysis of chitin and chitosan, prepared through the same deacetylation reaction conditions of Section 2.6, in concentrated sulfuric acid, were determined via experiment. They could be expressed as follows:

$$\begin{aligned} Y'_{CTN} &= 0.09142X'_{CTN} + 0.04117 \quad (R = 0.98997), \\ Y'_{CTS} &= 0.15011X'_{CTS} - 0.04002 \quad (R = 0.98878), \end{aligned} \tag{2}$$

where Y'_{CTN} is the absorbance of the products from chitin hydrolysis under the test conditions and X'_{CTN} is the concentration of chitin in the solution (g/L). Y'_{CTS} is the absorbance of the products from chitosan (deacetylation product of chitin) hydrolysis under the test conditions and X'_{CTS} is the concentration of chitosan in the solution (g/L).

If X_i represents the concentration of CTN-6-CD in the test solution, X_{CD} is the concentration of immobilized CD, and X_{CTN} is the concentration of chitin, then the following equation can be derived:

$$X_i = X_{CTN} + X_{CD}. \tag{3}$$

Assuming Y_i is the total absorbance of the hydrolyzed CTN-6-CD around 490 nm, Y_{CD} is the absorbance around 490 nm from the CD hydrolysis product, and Y_{CTN} is the absorbance around 490 nm from pyranose absorbance of chitin hydrolysis, the following equations could be obtained:

$$Y_i = Y_{CTN} + Y_{CD}, \tag{4}$$

$$Y_{CD} = 17.20854X_{CD} - 0.26257, \tag{5}$$

$$Y_{CTN} = 0.09142X_{CTN} + 0.04117. \tag{6}$$

Equations (3), (5), and (6) can be combined into (4) to give the concentration of immobilized CD in the CTN-6-CD test solution as

$$X_{CD} = \frac{Y_i - 0.09142X_{CTN} + 0.2214}{17.20854}. \tag{7}$$

In a similar way, the concentration of immobilized CD in the CTS-6-CD test solution could be calculated as

$$X_{CD} = \frac{Y_i - 0.15011X_{CTS} + 0.30259}{17.20854}. \tag{8}$$

The substitution degree of CD in CTN-6-CD or CTS-6-CD could be calculated through the following formula:

$$Q = \frac{X_{CD} \times 1000}{X_i \times 1135}, \tag{9}$$

where Q is the substitution capacity (mol/g) of CD and 1135 is the molar mass of CD.

3. Results and Discussion

3.1. FTIR Characterization. Structures of synthesized products in the reactions were identified and confirmed by FTIR spectrums (Figure 2).

In the FTIR spectrum of CTN-6-OTs, the peak at 1367 cm^{-1} from the asymmetrical stretching vibration and

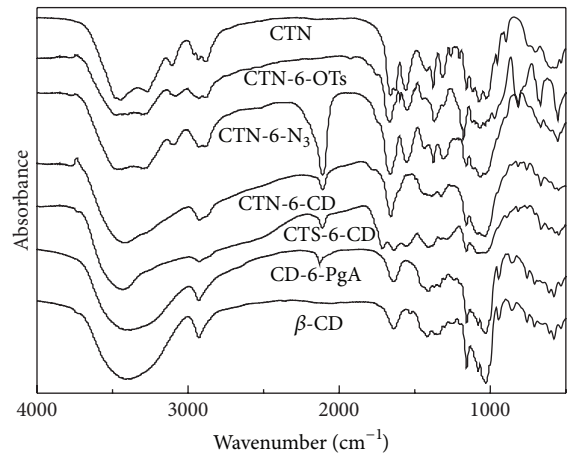

Figure 2: FTIR spectrums of the synthesized products in the reactions.

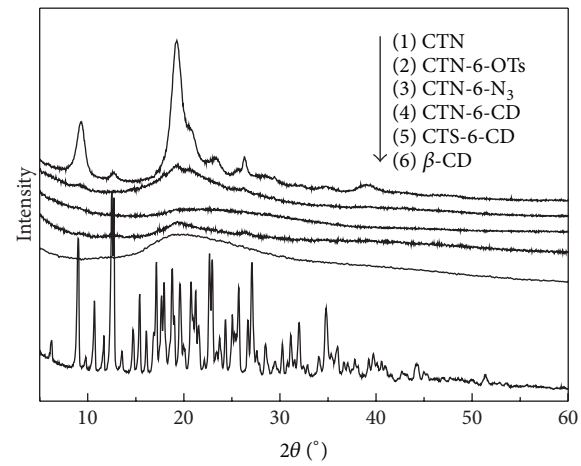

Figure 3: XRD patterns of the synthesized products in the reactions.

1176 cm^{-1} from the asymmetrical stretching vibration of S=O of the tosyl group could be found. The absorption peak at 1600 cm^{-1} attributed to the stretching vibration of the benzene ring appeared and the absorption peak from C–H bending vibration of the benzene ring at 811 cm^{-1} was also able to be found. From all these IR peak changes, we conclude that the tosyl group has been immobilized on the C_6 position of the pyranose ring of chitin.

Comparing the characteristic peaks in FTIR spectrum between CTN-6-OTs and CTN-6-N_3, a stretching vibration absorption peak of the azide group at 2108 cm^{-1} was found in the spectrum of CTN-6-N_3. On the other hand, the absorption peak at 1367 cm^{-1} and 1176 cm^{-1} of the tosyl group in the spectrum of CTN-6-OTs became weak for the corresponding peaks in the spectrum of CTN-6-N_3. In addition, the absorption peak at 1600 cm^{-1} and 815 cm^{-1} assigned to benzene ring in the spectrum of CTN-6-OTs also became weak for the corresponding peaks in the spectrum of CTN-6-N_3. These results indicated that the tosyl group on the C_6 position of the pyranose ring of chitin was successfully replaced by azide group.

In the FTIR spectrum of the product CTN-6-CD generated from the click reaction of CTN-6-N_3 and CD-6-PgA, the stretching vibration absorption peak of the azide group at 2108 cm^{-1} and the stretching vibration absorption peak of the triple bond at 2129 cm^{-1} were decreased sharply, and only a very weak overlapped peak existed. On the other hand, at 1487 cm^{-1} an absorption peak appeared which could be assigned to the C=N bond in the triazole ring. Nevertheless, the stretching vibration absorption peak of the unsaturated C–H bond in the triazole ring, assumed at near 3130 cm^{-1}, was overlapped by the hydrogen bond of the hydroxyl group in chitin and β-cyclodextrin above 3000 cm^{-1} and a broad peak was formed. Meanwhile, the stretching vibration absorption peak of the N–N bond in the triazole ring, assumed at near 1020 cm^{-1}, was overlapped by the broad stretching vibration absorption peak of C–N and C–O bonds in chitin and β-cyclodextrin. For introducing β-cyclodextrin, the peak around 1405 cm^{-1} assigned to the bending vibration of O–H was increased observably. The above results indicated that CTN-6-CD was gained.

After the acetyl group of CTN-6-CD was removed, the absorption peak at 1551 cm^{-1} attributed to the bending vibration of N–H bond of amide II appeared. The absorption peak at 1663 cm^{-1} assigned to C=O of chitin was decreased and shifted to the lower wavenumber. The broad peak above 3000 cm^{-1} and the peak around 1405 cm^{-1} assigned to the bending vibration of O–H deduced from introducing of β-cyclodextrin were still reserved. It was therefore clear from the results of FTIR spectrum that the chitosan structure was obtained by the deacetylated reaction and the immobilized β-cyclodextrin still existed. Thus CTS-6-CD, the aim product, was produced.

3.2. XRD Characterization. XRD characterization was carried out for the structure analysis of the products in the reactions.

As can be seen in the XRD patterns (Figure 3), CTN has strong absorption peaks near 9°, 21°, and 26°. The diffraction peak of CTN-6-OTs around 21° became weak and diffused, and the strength of the peaks near 9° and 26° were sharply decreased and became very weak. As a result of the introduction of the large size tosyl group, the distance between the molecular chains of CTN was increased, the *degree of crystallinity* of CTN was decreased, and the *amorphous state* of the product was increased. For the azide product CTN-6-N_3, only a wide peak appeared and not any diffraction peak. After β-cyclodextrin was introduced on the 6-OH position of chitin via the click reaction, as the hydrogen bonds could be formed between the –OH of β-cyclodextrin and the amide structure of chitin, the diffuse diffraction peak around $2\theta = 20°$ was increased. Strong and sharp peaks were found in the XRD curve of β-CD, showing its high crystalline structure. However, the diffraction peaks in the figure of CTN-6-CD disappeared. Combined with the characterization results of FTIR, a conclusion could be drawn that the β-cyclodextrin which was not immobilized

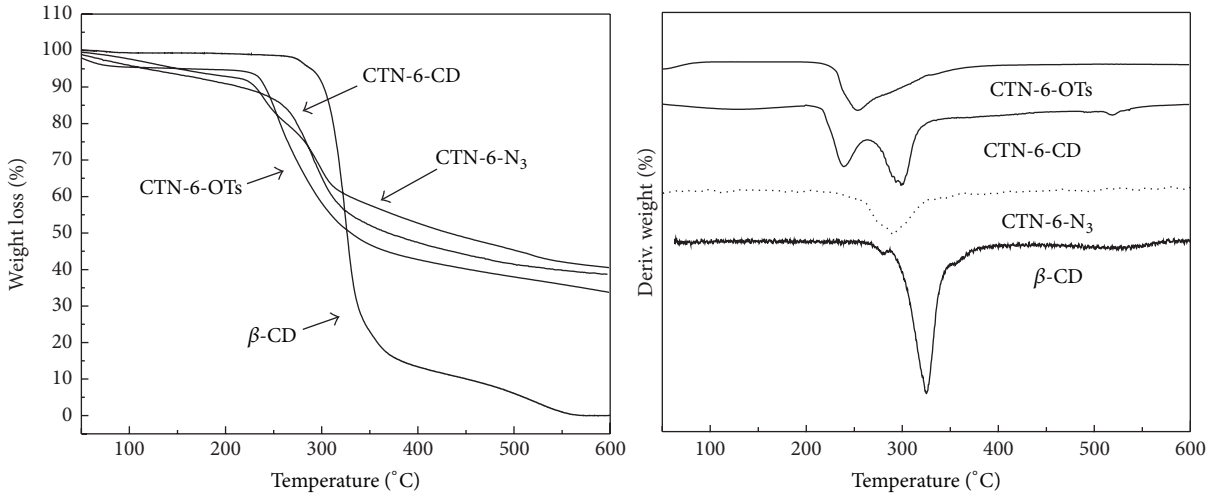

Figure 4: The TG and DTG curves of the synthesized products in the reactions.

with chitin has been fully washed off. After the removal of acetyl group from the skeleton of chitin, the diffraction peak in CTS-6-CD around $2\theta = 20^{\circ}$ was increased further as the intramolecular and intermolecular hydrogen bonds of the products were enhanced due to the arising of the $-NH_2$ structure. XRD characterization combined infrared data provided further proof of the abovementioned occurrence of the step-by-step reaction.

3.3. TG Analysis. The TG and DTG curves of the synthesized products in the reactions were compared in Figure 4.

The weight loss of CTN-6-OTs happened in the temperature range of 210–393°C, within which the weight loss percentage was 51.40% and the highest decomposition rate was at 253°C. The thermolysis within this range mostly came from the thermal decomposition of the tosyl group of C_6 position of chitin and the skeleton of chitin.

After tosyl on C_6 position of chitin was substituted by azide group, the weight loss occurred in two stages, 209–261°C and 269–335°C, respectively. The corresponding weight loss percentage was 12.10% and 19.36%, and the highest decomposition rate was at 238°C and 300°C, respectively.

The weight loss of BCTS-6-N_3 happening within the temperature range of 209–261°C came from the thermal decomposition of the azide group. As the lower thermostability of the azide group, the thermal decomposition temperature of it was lower than that of the sulfonate group of CTN-6-OTs. The thermolysis within the range of 269–235°C mostly came from the thermal decomposition of the skeleton of chitin. As the molar mass of the azide group was lower than that of the tosyl group, the total weight loss percentage of CTN-6-N_3 in the above two stages was lower than that of CTN-6-OTs within the temperature range of 210–393°C.

After CTN-6-CD was gained via the click reaction, the weight loss of it happened in the temperature range of 236–360°C, within which the weight loss percentage was 37.44% and the highest decomposition rate was at 291°C. Compared with CTN-6-OTs and CTN-6-N_3, the temperature beginning to decompose and the temperature at the highest decomposition rate were all significantly delayed. The above results show that the stability of the CTN-6-CD is higher than the first two intermediates. However, as hydrophilicity of β-cyclodextrin, CTN-6-CD showed more apparent slow weightlessness around the lower temperature than the above two intermediates.

3.4. Effects of Reaction Conditions on the Loading Capacities. As the abovementioned determining method of the immobilized loading, we investigated the impact of the click reaction conditions on the immobilized loading of the β-cyclodextrin in CTN-6-CD. The results were listed in Table 1.

It could be seen from the table that the immobilized loading of the β-cyclodextrin could be improved as increasing the molar ratio of CD-6-PgA to CTN-6-N_3. However, when $n_{\text{CD-6-PgA}} : n_{\text{CTN-6-N}_3}$ was higher than 1.5 : 1, the increasing ratio of the immobilized loading became slowly and the reaction efficiency of CD-6-PgA was decreased if the raw ratio of CD-6-PgA continued to increase. In view of the above phenomenon, $n_{\text{CD-6-PgA}} : n_{\text{CTN-6-N}_3} = 1.5 : 1$ is the suitable molar ratio.

It could be seen from Table 1 that the immobilized loading kept increasing as prolonging the reaction time. However, the increasing rate became slow after the reaction proceeded for 8 hours. To keep the high reaction efficiency, 8 hours were ordered as the most appropriate reaction time.

The immobilized loading of β-cyclodextrin was low in the product when the click reaction was carried out at lower temperature. With the reaction temperature increased, the immobilized loading of β-cyclodextrin significantly went up and hit the peak at 60°C. However, the immobilized loading dropped if the reaction temperature kept increasing. Thus, 60°C was considered as the optimum reaction temperature.

Comparing the immobilized loading of the products prepared by CTN-6-N_3 swelled in different organic solvents, it was found that the highest immobilized loading was obtained when triethylamine was employed as the solvent.

Table 1: The impact of the click reaction conditions on the immobilized amount of the β-cyclodextrin in the product.

$n_{\text{CD-6-PgA}} : n_{\text{CTN-6-N}_3}$	Reaction time/h	Reaction temperature/°C	Solvents	Immobilized/10^{-4} mol/g amount/μmol·g^{-1}
0.5 : 1	8	40	Dimethyl sulfoxide	0.8802
1 : 1				0.9198
1.5 : 1				1.1762
2 : 1				1.1805
2.5 : 1				1.2017
5 : 1				1.1901
1 : 1	2	60	Dimethyl sulfoxide	0.6759
	4			0.7365
	6			0.7959
	8			0.9759
	10			1.0107
1 : 1	8	30	Triethylamine	0.4375
		40		0.8659
		55		1.2365
		60		1.5110
		65		1.3079
		75		1.1358
		90		0.9159
1 : 1	8	60	Dimethyl sulfoxide	0.9759
			N-methylpyrrolidone	0.7856
			5% LiCl-DMAc	1.2723
			Triethylamine	1.5110

For the Cu(I)-catalyzed click reaction, the empty orbital of the electron-deficient Cu(I) ion can form a coordination bond with electron-rich alkyne and promote the click reaction [32]. Therefore, the removal of the alkynylic proton under basic condition of triethylamine promotes the coordination between the copper catalyst and the alkyne substrate and thus improves the reactivity of the alkyne. Besides, 5% LiCl-DMAc solvent system could improve the swelling property of the chitin derivatives and is helpful for increasing the immobilized loading of β-cyclodextrin.

By comparing the loading capacity of CTN-6-CD synthesized at different conditions, we found that the optimal conditions to synthesize CTN-6-CD with the highest loading capacity were as follows: $n_{\text{CD-6-PgA}} : n_{\text{CTN-6-N}_3} = 1.5 : 1$, triethylamine as the solvent of CTN-6-N_3 in click reaction, and 8 hr reaction time at 60°C. CTN-6-CD synthesized at these optimal conditions has an immobilized loading of 1.6126×10^{-4} mol/g.

After removing of acetyl group to afford CTS-6-CD, the immobilized loading of the final product was 1.6891×10^{-4} mol/g. The improvement of the immobilized loading came from the fact that the molecular weight of the product CTS-6-CD became smaller when the acetyl group was removed. However, the immobilized loading would be reduced if the triazole ring was not stable and could be destroyed when removing acetyl group. Therefore, the improvement of the immobilized loading also indicated that the triazole ring was relatively stable and the immobilization was not destroyed during the process of deprotection. These results demonstrated that the CTS-6-CD product prepared from this reaction route had an advantage of high stability. After CTN-6-CD was deacetylated, the deacetylation degree of chitosan of the corresponding CTS-6-CD was 86.1%, as determined by the alkalimetry method.

4. Conclusion

The Cu(I)-catalyzed click reaction was carried out between CTN-6-N_3 with CD-6-PgA to furnish the chitin 6-OH immobilized β-cyclodextrin derivatives CTN-6-CD which was further transformed to CTS-6-CD by removal of the acetyl group of chitin structure unit. The immobilized loading of the CTS-6-CD prepared at the optimal conditions was 1.6891×10^{-4} mol/g, which was obviously higher than the substitution degree of the chitosan C_6-substituted cyclodextrin derivative reported in the literature and close to the loading capacity of the derivatives of chitosan immobilizing with β-cyclodextrin at the 2-NH_2 position. This method has been demonstrated to be highly efficient and selective synthetic methodology for preparation of 6-OH substituted β-cyclodextrin derivatives of chitosan with free 2-NH_2. The exploration of this study built a new route for preparation of the 6-OH located macrocyclic compound derivatives of chitosan with a high degree of substitution and reliable structures and also established a basis for the investigations on other applications of the above derivatives. The prepared 6-OH substituted macrocyclic compound derivatives of chitosan with high loading capacity have good application

prospect in the field of chemical biosensor, slow release drug carrier, chromatographic support, and so forth.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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