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Exclusive and complete introduction of amino groups and their *N*-sulfo and *N*-carboxymethyl groups into the 6-position of cellulose without the use of protecting groups☆

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Abstract

A new regioselective synthesis of 6-amino-6-deoxycellulose with a DS 1.0 (degree of substitution) at C-6, and its 6-*N*-sulfonated and its 6-*N*-carboxymethylated derivatives, without using protecting groups is described in this paper. The reaction conditions were optimized for preparing cellulose tosylate with full tosylation at C-6 and partial tosylation at C-2 and C-3. The nucleophilic substitution (S_N) reaction of the tosyl group by NaN_3 at low temperature of 50 °C in Me_2SO was achieved completely at C-6, whereas the tosyl groups at C-2 and C-3 were not displaced. In contrast to this, at 100 °C the tosyl groups at C-6, and also those at C-2 and C-3, were replaced by azido groups. This regioselective reaction that depends on temperature makes it possible to reach a selective and quantitative S_N reaction at C-6 at low temperatures. In the subsequent reduction step with $LiAlH_4$, the azido group at C-6 was reduced to the amino group, and the tosyl groups at C-2 and C-3 were simultaneously completely removed. Also reported is a temperature-dependent, regioselective and complete iodination by nucleophilic substitution of the tosyl group at C-6 at 60 °C. At higher temperatures from 75 to 130 °C, substitution is also observed to occur at C-2. The selective iodination at 60 °C was employed to confirm the complete tosylation at C-6 of cellulose. The reaction products were identified by four different independent quantitative methods, namely ^{13}C NMR, elemental analysis, ESCA, and fluorescence spectroscopy. 6-*N*-Sulfonated and 6-*N*-carboxymethylated cellulose derivatives were also synthesized. The new derivatives are potent candidates for structure–function studies, e.g., studies in relation to regioselectively 2-*N*-sulfonated and 2-*N*-carboxymethylated chitosan derivatives.

Keywords: 6-Amino-6-deoxycellulose; 6-Azido-6-deoxy-2,3-di-*O*-tosylcellulose; Tosylation; Regioselective introduction of the azido group; Regioselective removal of C-2 and C-3 tosyl groups; Regioselective iodination; Chitosan chemistry; N-Sulfonation; N-Carboxymethylation; ^{13}C NMR spectroscopy; FITC analysis; Elemental analysis; ESCA analysis

1. Introduction

Numerous attempts have been made to synthesize aminodeoxycellulose by replacing hydroxyl groups by amino groups in order to change the nature of cellulose. In 1926 Karrer[1] prepared the first aminodeoxycellulose by the reaction of cellulose tosylate (DS 0.1) with ammonia. Later Sakurada[2] and Scherer and Field[3] synthesized aminodeoxycellulose with DS of 0.5 and 1.0, respectively.

Attempts have also been made to synthesize 6-amino-6-deoxycellulose selectively by using protecting groups. A common pathway is to prepare 6-*O*-tritylcellulose and protect C-2 and C-3, e.g., by acetyl or phenylcarbamoyl groups. After removing the trityl group, the tosyl group is introduced into C-6. The tosyl group is used as a leaving group to perform an S_N reaction. Haskins and Weinstein[4] were the first who introduced the phthalimido group into C-6 with a DS of 0.25 by previously protecting the C-2 and C-3 positions with acetyl groups. However, they failed to remove the phthalic acid residues. In 1973, Usov et al.[5] prepared

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6-amino-6-deoxy-2,3-di-*O*-phenylcarbamoylcellulose with a DS of 0.89 at the 6-position. Using the same procedure, Teshirogi et al.[6] obtained a 6-amino-6-deoxycellulose derivative with a DS of 0.90.

Cramer[7] claimed a synthesis of 6-amino-6-deoxy-D-glucose from 6-*O*-sulfonic acid esters of D-glucose via an intermediate of 6-azido-6-deoxy-α-D-glucopyranoside. In this synthesis, no protecting groups at C-2 and C-3 were used to prepare specific 6-*O*-sulfonic acid esters of D-glucose. It is however known that tosylation is a nonselective reaction at C-6.[8,9]

To prepare 6-amino-6-deoxycellulose derivatives, S_N reactions were used by treating tosyl or tresyl cellulose derivatives with amines. Kern et al.[10] synthesized 6-deoxy-6-pyridinium-2,3-di-*O*-methylcellulose with a DS of 0.8 by the reaction of 6-*O*-tresyl-2,3-di-*O*-methylcellulose with pyridine in five steps. Heinze et al.[11] reported on the synthesis of 6-amino-6-deoxycellulose derivatives with DS of 0.4–0.6 without using protecting groups. These derivatives were obtained by the reaction of cellulose tosylates with (+)-*R*-, (−)-*S*- or racemic amines, e.g., 1-phenylethylamine in Me_2NCHO–water. Koschella and Heinze[12] claimed a preparation of 6-deoxy-6-trialkylammonium cellulose derivatives with a DS in the range of 0.2–0.5 by the reaction of tosylcellulose with triethylamine, *N*,*N*-dimethyl-1,3-diaminopropane, or 2,4,6-tris(*N*,*N*-dimethylaminomethyl)phenol in Me_2NCHO–water. However, C-2 and C-3 were also partially tosylated.

6-Azido-6-deoxycellulose and 6-deoxy-6-halocellulose, which are excellent intermediates for the preparation of 6-amino-6-deoxycellulose, have also been synthesized. Clode and Horton[13] reported on a method for the introduction of azide into C-6 of cellulose. Two levels of DS (0.25 and 0.68) were achieved after having used the above-mentioned activating and protecting group strategy. Saad and Furuhata[14] obtained 6-chloro-6-deoxycellulose (DS 0.87) and 6-bromo-6-deoxycellulose (DS 0.92). 6-Deoxy-6-iodocellulose derivatives with a DS in the range of 0.46–0.94 were prepared by nucleophilic exchange of cellulose tosylate with NaI in acetylacetone.[9] However, these 6-deoxycellulose intermediates were not used to introduce the primary amino group into C-6.

In fact, all the methods described so far in the literature are incomplete substitution reactions at C-6 of cellulose. In most cases the C-2 and C-3 positions of cellulose were not deprotected after the reaction at C-6 having been carried out. Additionally there are side-reaction products at C-2 and C-3. In this paper we report on a new synthetic strategy for introducing the primary amino group into C-6. For drug design, the complete and regioselective introduction of the primary amino group into C-6 is needed. 6-Amino-6-deoxycellulose also was employed as an intermediate for synthesizing defined 6-*N*-sulfonated and 6-*N*-carboxymethylated derivatives without using a protecting group strategy. The starting material is a cellulose fully tosylated at C-6, which is as well partially tosylated at C-2 and C-3. Then a quantitative and specific nucleophilic substitution of the leaving group at C-6 takes place with sodium azide at low temperatures without affecting the tosyl groups at C-2 and C-3. The following reduction step results in 100% reduction of the azido group to the amino group at C-6, and in addition, in complete removal of tosyl groups at C-2 and C-3. We also studied iodination reactions as an analytical method to determine the degree of substitution of tosylation at C-6 of cellulose. It was shown that complete and specific iodination at low temperature only occurred at C-6. Moreover, we investigated the nucleophilic exchange reaction of 6-*O*-tosylcellulose with ammonia in an autoclave. The reaction conditions were chosen in such a way that all reactions could be carried out in homogeneous solutions.

2. Experimental

Starting materials.—Cellulose was purchased from Fluka Chemical Co. (Avicel PH-101) with DP 280. (+)-D-Glucosamine·HCl was purchased from Serva. Other chemicals and solvents were purchased either from Fluka or Aldrich Chemical Co. All chemicals and reagents, unless otherwise specified, were not further purified, dried or pretreated. Solvents (*N*,*N*-dimethyl sulfoxide, dimethylacetamide, dioxane) were dried over calcium hydride and subsequently distilled in vacuum. Lithium chloride was dried overnight at 130 °C in vacuum before use.

Analytical methods.—Dialysis tubes (Visking) with the pore size 15–20 Å from Serva were used to purify the cellulose derivatives. ^{13}C NMR spectra of cellulose derivatives were measured with a Bruker AC 300 spectrometer operating at 75.47 MHz or a Unity-500 spectrometer operating at 125.63 MHz in Me_2SO-d_6 or in D_2O. The degree of substitution was calculated by the "triangle method" according to Casu et al.[15] The infrared spectra were measured in KBr pellets with an Impact 400 FTIR spectrometer from Nicolet. An A-5082 (Tecan) was used to determine the fluorescence properties of FITC-labeled 6-amino-6-deoxycellulose.

Dissolution of cellulose in N,N-*dimethylacetamide-(DMAc)–LiCl.*—Cellulose (5.0 g, 30.8 mmol) was suspended in DMAc (120 mL) and stirred at 160 °C for 1 h. In order to replace the water bound to cellulose, about 10 mL of DMAc was removed by distillation under an N_2 atmosphere. The mixture was cooled to 100 °C, and 10.0 g of anhyd LiCl were added. By cooling down to rt and with continuous stirring, the cellulose dissolved completely within several hours, resulting in a bright-yellow, viscous solution.

p-*Toluenesulfonylation of cellulose*.—In a typical preparation, a mixture of Et_3N (14.9 mL, 106.8 mmol) in DMAc (10.2 mL) was added to the solution of cellulose (5.0 g, 30.8 mmol) in DMAc–LiCl under stirring. After cooling to 8 °C, a solution of *p*-toluenesulfonyl chloride (26 g, 136.9 mmol) in DMAc (25 mL) was added dropwise within 30–60 min. The homogeneous reaction mixture was stirred for 24 h at 8 °C under an N_2 atmosphere and then slowly poured into ice-cold water (1.2 L). The resulting precipitate was filtered off, washed carefully with about 5 L of distilled water, and 600 mL EtOH, suspended in 250 mL acetone, and reprecipitated in 800 mL of distilled water. After filtration and washing with EtOH, the product was dried at 50 °C in vacuum. Yield: 82%. IR (KBr): *v* 3515 (OH), 3075 (C–H_{arom}), 2924 (CH), 1600 and 1458 (C–C_{arom}), 1370 and 1180 cm^{-1} (SO_2). ^{13}C NMR (Me_2SO-d_6): δ 144.9–127.7 (C–H_{arom}), 101.5 (C-1), 97.9 (C-1′), 83.3–71.6 (C-2, C-2–OTs, C-3, C-3–OTs, C-4, C-5), 68.2 (C-6–OTs), 59.4 (C-6), 21.1 (CH_3) ppm.

Iodination of cellulose tosylate.—In a typical preparation anhyd NaI (2.5 g, 16.7 mmol) was added to a solution of cellulose tosylate **1a** (1.5 g, 4 mmol) in acetylacetone (50 mL). The reaction mixture was kept at 130 °C for 2 h. After cooling the mixture to rt, the product was isolated by precipitation in EtOH (300 mL), filtered off, and thoroughly washed with distilled water. The product was soaked in EtOH (250 mL) overnight and then in 0.1 M $Na_2S_2O_3$ solution (250 mL) for 1 h. The residue was washed with distilled water and EtOH, and the product was dried at 50 °C in vacuum overnight. Yield: 75%. IR (KBr): *v* 3505 (OH), 3070 (C–H_{arom}), 2921 (CH), 1598 and 1452 (C–C_{arom}), 1371 and 1176 cm^{-1} (SO_2). ^{13}C NMR (Me_2SO-d_6): δ 144.9–127.7 (C–H_{arom}), 104.3 (C-1″), 101.6 (C-1), 97.2 (C-1′), 82.1–70.4 (C-2, C-2–OTs, C-3, C-3–OTs, C-4, C-5), 67.9 (C-6–OTs), 59.6 (C-6), 21.1 (CH_3), 7.3 (C-6–I) ppm.

Nucleophilic displacement of cellulose tosylate with sodium azide.—In a typical preparation cellulose tosylate **1b** (1 g, 2.1 mmol) was dissolved in dry Me_2SO (100 mL). After addition of NaN_3 (682.5 mg, 10.5 mmol), the mixture was stirred at 50 °C for 24 h under an N_2 atmosphere. The product was separated by precipitation in ice-cold water (100 mL). The product was filtered off, washed with distilled water (1 L) and EtOH (400 mL) and dried at 50 °C in vacuum overnight. Yield: 94%. IR (KBr): *v* 3500 (OH), 3070 (C–H_{arom}), 2924 (CH), 2112 (N_3), 1600 and 1458 (C–C_{arom}), 1370 and 1180 cm^{-1} (SO_2). ^{13}C NMR (Me_2SO-d_6): δ 144.9–127.7 (C–H_{arom}), 101.5 (C-1), 97.9 (C-1′), 81.8–71.2 (C-2, C-2–OTs, C-3, C-3–OTs, C-4, C-5), 59.4 (C-6), 50.1 (C-6–N_3), 21.1 (CH_3) ppm.

Reduction of the azido group at C-6 of cellulose to the amino group with simultaneous removal of the tosyl groups at C-2 and C-3 of cellulose with $LiAlH_4$.—In a typical preparation, 6-azido-6-deoxycellulose tosylate **2q** (1 g, 3 mmol) was dissolved in dry dioxane (100 mL). After addition of $LiAlH_4$ (570 mg, 15 mmol), the mixture was stirred at 54 °C for 24 h. Thereafter a solution of distilled water (2 mL) in dioxane (8 mL) and at last distilled water (8 mL) were added dropwise at rt. After stirring for 24 h, the resulting precipitate was filtered off, washed with EtOH, and dissolved in 2 N HCl (30 mL). The solution was dialyzed against deionized water for 3 days and then freeze-dried. Yield: 44%. IR (KBr): *v* 3432 (O–H and N–H), 2924 (C–H), 1064 cm^{-1} (O–C–O). ^{13}C NMR (1% DCl in D_2O): δ 140–130 (C–H_{arom}), 105.5 (C-1), 81.9–69.6 (C-2, C-3, C-4, C-5), 43.2 (C-6) ppm.

Complete regioselective N-sulfonation of 6-amino-6-deoxycellulose.—6-Amino-6-deoxycellulose (280 mg, 1.74 mmol) was dispersed in water (50 mL). To this mixture sodium carbonate (600 mg, 3.3 equiv) and $SO_3{\cdot}NMe_3$ complex (1.2 g, 5 equiv) were added. After stirring for 20 h at 65 °C under an N_2 atmosphere, the solution was dialyzed against deionized water for 30 h, against 25 mM NaOH for 6 h, against deionized water for 3 days, and then it was freeze dried.[16] Yield: 85%. IR (KBr): *v* 1192 cm^{-1} (O=S=O). ^{13}C NMR (D_2O): δ 105.4 (C-1), 82.8–70.0 (C-2, C-3, C-4, C-5), 47.0 (C-6) ppm.

Complete regioselective N-carboxymethylation of 6-amino-6-deoxycellulose.—6-Amino-6-deoxycellulose (400 mg, 2.5 mmol) was suspended in water (30 mL). After addition of glyoxylic acid monohydrate (0.4 g, 4.3 mmol), the aminocellulose dissolved within the next 90 min. The solution was adjusted to pH 12 by addition of 2 N NaOH. A solution of 0.29 g sodium cyanoborohydride in water was added and stirred for 60 min. The product was precipitated in EtOH (200 mL), filtered off, washed with EtOH, and dried in vacuum at rt overnight (a variation of 17). Yield: 80%. IR (KBr): *v* 1638 cm^{-1} (C=O) carboxylic acid. ^{13}C NMR (D_2O): δ 181.5 (C=O), 105.6 (C-1), 83.2–69.8 (C-2, C-3, C-4, C-5), 54.8 (CH_2), 51.8 (C-6) ppm.

Determination of the amount of amino groups in 6-amino-6-deoxycellulose by a fluorescence labeling method using fluorescein-5-isothiocyanate (FITC) as a marker.—Fluorescein-5-isothiocyanate (FITC) was dissolved in EtOH, a sample of 6-amino-6-deoxycellulose was dissolved in distilled water, and (+)-D-glucosamine·HCl was used as a standard. The concentrations of the samples were in the range of 9.7–388 pmol/L. The measurements were made with an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

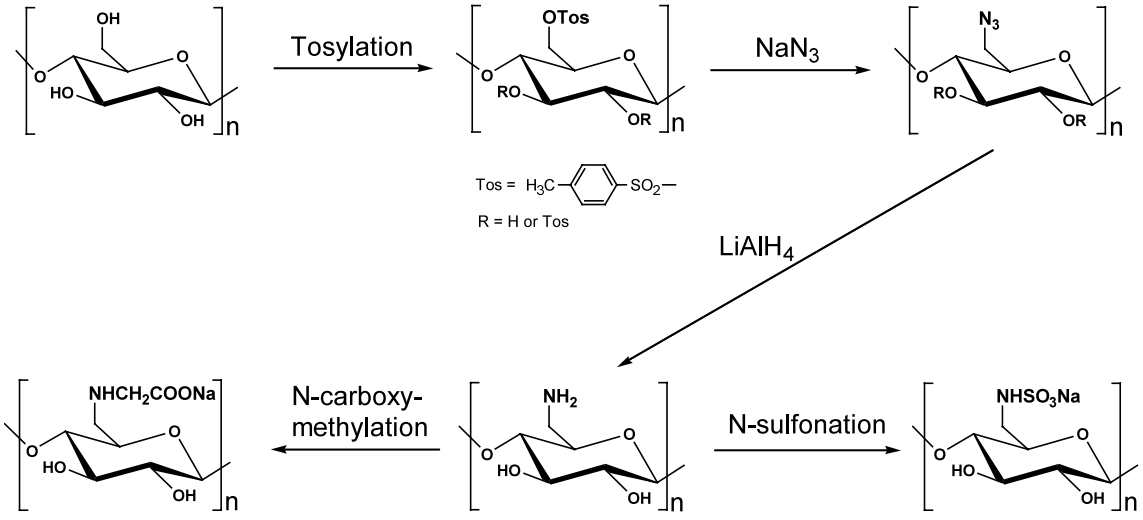
Attempts for the synthesis of 6-amino-6-deoxycellulose by nucleophilic substitution of cellulose tosylate with ammonia.—In a typical preparation cellulose tosylate with a DS (C-6–OTs) 0.90 and a DS (C-2–OTs) 0.42 (1 g, 2.1 mmol) was dissolved in DMAc (20 mL) in a 65

mL autoclave, and 10 mL liquid ammonia was added. This solution was stirred at 125 °C for 18 h. After cooling down to rt, the product was precipitated by pouring the reaction mixture into 50 mL of ice-cold water. The product was filtered off, washed with distilled water and EtOH and dried in vacuum at rt overnight. Yield: 56%. IR (KBr): ν 3458 (O–H and N–H), 2924 (C–H), 1044 cm^{-1} (O–C–O).

3. Results

Tosylation of cellulose.—Scheme 1 outlines the synthetic procedure for the regioselective and quantitative introduction of amino groups and their *N*-sulfonyl and *N*-carboxymethyl groups into C-6 of cellulose without using protecting groups. Firstly, we tried to use methods known in the literature for the preparation of cellulose tosylate with full tosylation at C-6, which is a prerequisite for the subsequent nucleophilic substitution with azido groups, the subsequent reduction step to amino groups, and for the reactions to the related N-substituted derivatives, respectively. We selected two different reaction conditions described by Rahn et al.[9] with DS values of 1.36 and 2.30, which had been reported to result in fully tosylated C-6 along with a small amount of chlorine substituted at C-6. The results in Table 1 show that only 90 mol% of the C-6–OH groups of cellulose (**1a**, DS of 1.39) were tosylated, whereas cellulose with the higher total DS of 2.02 (**1b**) resulted in 100 mol% tosylation at C-6. This was confirmed by ^{13}C NMR spectroscopy. There was no shift at 59.4 ppm, which means that all C-6–OH groups were quantitatively tosylated. The peak at 59 ppm described by Rahn et al.[9] for C-6–Cl was not observed. There are only two splitting peaks at 101.5 ppm (C-1) and 97.9 ppm (C-1′, affected by C-2–OTos), which means that no chlorination at C-2 (see Fig. 1) occurred. Pyridine–water extraction is known to remove the ionic-bound chlorine. After pyridine extraction no chlorine was observed by ESCA measurement.

Improving the regioselectivity and completeness of the nucleophilic substitution of cellulose tosylates by sodium azide at C-6.—Additionally to prove that complete



Scheme 1. Scheme for the regioselective synthesis of 6-amino-6-deoxycellulose and its *N*-sulfonated and *N*-carboxymethylated derivatives.

Table 1
Conditions and results of the homogeneous reaction of cellulose with *p*-toluenesulfonyl chloride in DMAc/LiCl

Entry	Tos-Cl/AGU (mol ratio)	Temperature/°C	Time/h	Cellulose tosylates (partial DS [a])		Total DS [b]	Yield (%)
				$C_{2\text{-Tos}}$	$C_{6\text{-Tos}}$		
1a	1.8	8	24	0.42	0.90	1.39	84
1b	4.5	8	24	0.85	1.0	2.02	82

[a] Degree of substitution calculated by ^{13}C NMR spectroscopy (Me_2SO-d_6).
[b] Calculated by sulfur elemental analysis.

Table 2
Conditions and results of the regioselective nucleophilic reaction of *O*-tosylcellulose with sodium azide

Entry	Tos-cell	Solvent	NaN_3/Tos-AGU (mol)	Temperature/°C	Time/h	Product (partial DS [a])			Yield (%)
						$C_{6\text{-OH}}$	$C_{6\text{-Tos}}$	$C_{6\text{-azido}}$	
2a	1a	acetone–H_2O	5.0	100	24	0.11	0.55	0.34	78
2b	1a	acetone–H_2O	5.0	100	48	0.10	0.44	0.46	75
2c	1a	acetone–H_2O	5.0	100	72	0.11	0.38	0.51	79
2d	1a	acetone–H_2O	5.0	100	96	0.12	0.32	0.56	78
2e	1a	acetone–H_2O	5.0	100	120	0.10	0.28	0.62	80
2f	1a	acetone–H_2O	5.0	100	144	0.10	0.28	0.62	78
2g	1a	Me_2SO	5.0	100	24	0.11	0.28	0.61	82
2h	1a	Me_2SO	5.0	25	24	0.11	0.71	0.18	80
2i	1a	Me_2SO	5.0	25	72	0.12	0.22	0.66	75
2j	1a	Me_2SO	5.0	50	6	0.12	0.33	0.55	88
2k	1a	Me_2SO	5.0	50	12	0.11	0.19	0.70	82
2l	1a	Me_2SO	5.0	50	18	0.12	0.14	0.74	90
2m	1a	Me_2SO	5.0	50	24	0.10	0.10	0.80	85
2n	1a	Me_2SO	2.5	50	24	0.12	0.21	0.67	87
2o	1a	Me_2SO	7.5	50	24	0.12	0.13	0.75	85
2p	1b	Me_2SO	5.0	50	24	0.00	0.10	0.90	90
2q	1b	Me_2SO	5.0	50	38	0.00	0.00	1.00	92

[a] Degree of substitution calculated by ^{13}C NMR spectroscopy (Me_2SO-d_6).

tosylation has occurred at C-6, we investigated the S_N reaction of the tosyl group with sodium azide, resulting in an optimized regioselective and quantitative substitution at C-6. Both cellulose tosylates **1a** and **1b** were used to study the effects of solvents, reaction temperature, reaction time, and molar ratio of sodium azide/anhydroglucose unit (AGU). The reaction conditions and results are shown in Table 2.

Firstly, we studied a mixed water–acetone solvent system according to Cramer[7] to perform this reaction under conditions of the molar ratio of 5.0 of NaN_3/AGU, a constant temperature of 100 °C and variable reaction time (**2a**–**2f**). The results showed that azido groups displace tosyl groups at C-6 as well at C-2. In the ^{13}C NMR spectra, three new signals at 50.1, 63.2, and 104.1 ppm were observed. The signal at 50.1 ppm could be assigned to the C-6–N_3, which is close to the shift of C-6–N_3 in 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-D-glucopyranose.[18] The signal at 63.2 ppm is assigned to C-2–N_3, which is close to the shift of C-2–N_3 in a 2-azido-2-deoxy analogue of a galactobiose-containing saccharide.[19] The third signal is assigned to C-1′ affected by C-2–N_3. Additionally, the FTIR spectra support the introduction of azido groups and show the typical absorption of azido groups at 2100 cm^{-1}.

The percentage of tosyl groups replaced by azido groups at C-6 increased from 37.8% to 68.9% while the reaction time was prolonged from 24 to 120 h. But a higher percentage of azido groups at C-6 could not be reached even by prolonging the reaction time up to 144 h. We conclude that the mixed solvent system is unsuitable to obtain a regioselective displacement of the tosyl group by an azido group at C-6 under these conditions.

Also dimethyl sulfoxide was systematically studied with **1a** and **1b** to perform this S_N reaction. The effects of temperature, reaction time, and the molar ratio of tosylcellulose to azide are shown in Table 2 (**2g**–**2q**). The reaction temperature has a great influence on the regioselective S_N reaction. At 100 °C displacement of tosyl groups by azido groups at C-6 and also at C-2 and C-3 takes place (**2g**). The ^{13}C NMR spectrum shows four new peaks: one at 50.1 ppm for C-6–N_3, the second at 63.2 ppm for C-2–N_3, the third at 65.6 ppm can be assigned to C-3–N_3 according to Hattori et al.,[20] and the last one at 104.1 ppm for C-1′ as affected by C-2–N_3. In contrast to this, in the same system at lower temperatures of 25 and 50 °C, we observed only one new peak at 50.1 ppm in the ^{13}C NMR spectra. This means that there is a selective nucleophilic substitution of the tosyl group at C-6 by an azido group (**2h**–**2q**). By prolonging the reaction time, the completeness of the reaction increased at 50 °C. At 38 h, a complete and exclusive introduction of azido groups at C-6 was obtained with derivative **1b** (**2q**).

Preparation of 6-amino-6-deoxycellulose by completely removing residual tosyl groups at C-2 and C-3 and with complete reduction of the azido groups at C-6 to amino groups.—There are a lot of reduction procedures described in literature[22–24] that may be used to reduce azido groups to amino groups. It is well known that lithium aluminum hydride effectively reduces azido species to the corresponding amines.[21] In the case of **2q**, the azido group at C-6 must be reduced to an amino group, while the residual tosyl groups at C-2 and C-3 must simultaneously be removed completely (Scheme 1). The reaction conditions and ^{13}C NMR results of the reduction of 6-azido-6-deoxy-2,3-*O*-tosylcellulose (**2q**) by $LiAlH_4$ in dioxane are shown in Table 3. The azido groups were reduced completely in all cases (**3a**–**3d**). No peak at 50.1 ppm for C-6–N_3 was observed in the ^{13}C NMR spectra. The typical absorptions of the azido group at 2100 cm^{-1} disappeared in the FTIR spectra. However, the tosyl groups were not so easily removed. In all cases there was a small number of tosyl groups left at C-2 and C-3 of cellulose derivatives with DS values ranging from 0.02 to 0.08. The tosyl groups could not be removed completely by one reduction step even if the reduction time is increased to 96 h (**3d**). The residual tosyl groups in the cellulose derivatives could, however, be completely removed by a second reduction step (**3e**). The FTIR spectrum of **3e** showed that all typical absorption bands of the tosyl group and of the azido group disappeared. In the ^{13}C NMR spectrum of **3e** (see Fig. 1) the signals at 97.9 ppm (C-1′, affected by C-2–OTos), 50.1 ppm (C-6–N_3) and 21.1 ppm (CH_3) completely disappeared, and a new signal at 43.2 ppm appeared, which was assigned to C-6–NH_2. After having derivatized the amino groups with FITC and measured the fluorescence, the DS of amino groups in 6-amino-6-deoxycellulose was determined to be 0.99.

Exclusive and complete 6-N-sulfonation of 6-amino-6-deoxycellulose.—N-Sulfonation in homogeneous aqueous solution by using the trimethylamine–SO_3 complex according to Holme and Perlin[16] and Baumann and Faust,[25] respectively, resulted in regioselectively and completely 2-*N*-sulfonated chitosan. The N-sulfonation method of chitosan was applied successfully to 6-amino-6-deoxycellulose and resulted in selective and complete 6-N-sulfonation. It is interesting that in the ^{13}C NMR spectrum the C-6–*N*-sulfate signal was shifted downfield by 3.8 ppm compared to the C-6–NH_2 signal at 43.2 ppm. The infrared spectrum of the *N*-sulfonated derivative showed the typical vibration of the O=S=O group at 1192 cm^{-1}.

Exclusive and complete 6-N-carboxymethylation of 6-amino-6-deoxycellulose.—Introduction of *N*-carboxymethyl groups into 6-amino-6-deoxycellulose according to the method of Muzzarelli et al.[17] and Baumann and Faust[25] with chitosan having variable NAc content shows highest regioselectivity at C-6. The reaction can be carried out with 6-amino-6-deoxycellulose quantitatively up to an overall substituted amino DS of 1. The ^{13}C NMR spectrum showed a new signal at 51.8 ppm for the C-6–NCM, and no C-6–NH_2 signal was observed (see Fig. 1). The IR spectrum showed a new signal at 1600 cm^{-1}, which could be assigned to a typical vibration of a carbonyl group.

Iodination of cellulose tosylates.—When heated with an excess of sodium iodide, the tosyl group at C-6 of glucose and of cellulose acetate were exclusively and quantitatively replaced by iodine.[26,27] This method was applied by Rahn et al.[9] to determine the DS of tosylation at C-6 of cellulose tosylates (total DS in a range of 0.46–1.79). They also found by ^{13}C NMR measurements that some iodinated derivatives showed an exchange of the tosyl groups at C-2 by iodo groups. Additionally a small amount of tosyl groups were left at C-6, even in the case of the derivative with the highest DS 0.94 of iodo groups. In this paper we firstly try to prove the selectivity and completeness of the iodination reaction at C-6 before we apply this method to derivative **1b** showing complete 6-O-tosylation (Scheme 2). The reaction conditions and results are shown in Table 4.

Table 3
Removal of tosyl groups and reduction of azido groups of 6-azido-6-deoxycellulose (**2q**) by $LiAlH_4$ in dioxane

Entry	$LiAlH_4$/azido-AGU (mol)	Temperature/°C	Time/h	Partial DS (^{13}C NMR)		
				$C_{6\text{-azido}}$	$C_{6\text{-amino}}$	$C_{2,3\text{-}O\text{-Tos}}$
3a	5.0	54	24	0	1.0	0.08
3b	5.0	54	48	0	1.0	0.06
3c	5.0	54	72	0	1.0	0.03
3d	5.0	54	96	0	1.0	0.02
3e [a]	5.0	54	24	0	1.0	0

[a] After twofold reduction.

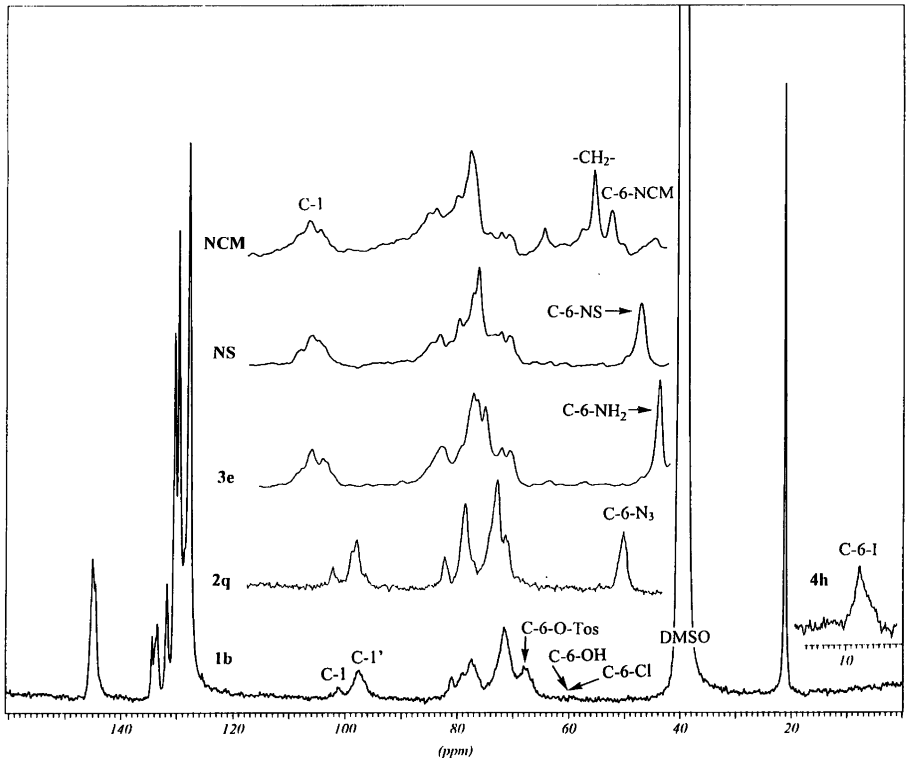
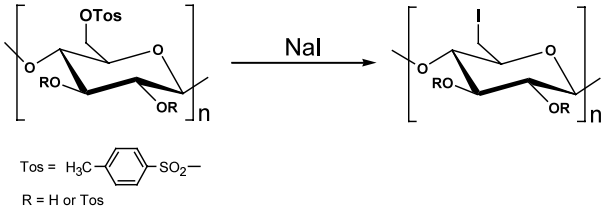


Fig. 1. ^{13}C NMR spectra of cellulose derivatives. **1b**, Tosylcellulose with a total DS of 2.02, respectively, of DS 1.0 at C-6 (tosyl group); **2q**, 6-azido-6-deoxy-2,3-di-*O*-tosylcellulose with a DS of 1.0 (azido group); **3e**, 6-amino-6-deoxycellulose; **4h**, 6-deoxy-6-iodo-2,3-di-*O*-tosylcellulose with a DS of 1.0 (iodo group); **NS**, 6-*N*-sulfonated cellulose; **NCM**, 6-*N*-carboxymethylated cellulose.

Firstly we repeated the reaction conditions of Rahn et al.,[9] and then we investigated new reaction conditions. Our results of **4b** showed that the iodination of **1a** was incomplete and unselective at C-6 under the conditions of 130 °C, and 2 h, with the molar ratio of NaI/AGU 4.0 in acetylacetone.[9] Only 92% of the 6-*O*-tosyl groups in **1a** were substituted by iodo groups, and 18% of the 2-*O*-tosyl groups were displaced. In the case of **4a** that was obtained from fully 6-*O*-tosylated cellulose **1b**, 91% of the 6-*O*-tosyl groups and 14% of the 2-*O*-tosyl groups were substituted by iodo groups. In order to improve the selectivity of the iodination at C-6, we investigated the effect of reaction temperature to show that selective iodination at C-6 of cellulose tosylate is a temperature-dependent reaction. At different temperatures of 130, 100, 75, 60, and 45 °C iodated derivatives **4b**–**4f** were obtained. At 130, 100, and 75 °C, the S_N reaction occurred at C-6 as well as at C-2, which can be seen in the ^{13}C NMR spectra. Compared with the ^{13}C NMR spectrum of cellulose tosylate, two new chemical shifts at 104.3 and 7.3 ppm were observed. The signal at 104.3 ppm was assigned to C-1′, an affected by C-2–I. The signal at 7.3 ppm was assigned to C-6–I. It is not yet clear whether the tosyl groups at C-3 are substituted by iodo groups, because we could not find a new separate peak for C-3–I by ^{13}C NMR spectroscopy. Probably the signal for C-3–I is overlapped by another peak. In contrast to these results, at low temperatures of 60 and 45 °C, only one new signal at 7.3 ppm appears in the ^{13}C NMR spectrum, which shows an exclusive iodination at C-6. At low temperatures the completeness of selective iodination at C-6 is dependent on the reaction time. At 60 °C for 25 h, 72% of the 6-*O*-tosyl groups of **1b** were substituted by iodo groups (**4g**). At 60 °C a complete and exclusive introduction of the iodo group at C-6 of **1b** was obtained when the reaction time was prolonged to 42 h (**4h**).

Analytical proof of the derivatives.—It is a prerequisite for quantitative estimation of all functional groups of derivatives as well the completeness of the reactions to use several analytical methods, e.g., elemental analyses, and quantitative NMR measurements. In Fig. 1, all significant NMR signals are shown for each derivative.



Scheme 2. Iodination of tosylcellulose with NaI in acetylacetone.

Table 4
Iodination of cellulose tosylate at the molar ratio of NaI/AGU 4.0 in acetylacetone

Entry	Tos-cell [a]	Temperature/°C	Time/h	Partial DS of Products calculated from ^{13}C NMR						DS_I	DS_{Tos}
				$C_{2\text{-OH}}$	$C_{2\text{-Tos}}$	$C_{2\text{-iodo}}$	$C_{6\text{-OH}}$	$C_{6\text{-Tos}}$	$C_{6\text{-iodo}}$		
4a	1b	130	2	0.14	0.72	0.14	0.00	0.09	0.91	1.26	0.56
4b	1a	130	2	0.56	0.36	0.08	0.13	0.07	0.80	0.92	0.51
4c	1a	100	2	0.58	0.35	0.07	0.13	0.13	0.74	0.83	0.56
4d	1a	75	2	0.55	0.42	0.03	0.12	0.21	0.66	0.64	0.81
4e	1a	60	2	0.58	0.42	0.00	0.13	0.67	0.20		
4f	1a	45	2	0.58	0.42	0.00	0.12	0.84	0.04		
4g	1b	60	25	0.14	0.86	0.00	0.00	0.28	0.72		
4h	1b	60	42	0.14	0.86	0.00	0.00	0.00	1.00	1.03	1.05

[a] In all cases the amount of cellulose tosylates was 1.0 g.

Table 5
Quantitative analysis results by different methods

Derivatives	C-6 (mol%)			C-2+C-3 (mol%)	Total DS
Tosylcellulose (**1b**)	100%	tosyl (^{13}C NMR, 68.2 ppm)	51%	tosyl (elemental)	2.07 tosyl
	0%	OH (^{13}C NMR, 59.4 ppm)			
	0%	chlorine (^{13}C NMR, 59.0 ppm)	2.6%	chlorine (elemental)	
6-Azido-6-deoxycellulose (**2q**)	100%	N_3 (^{13}C NMR, 50.1 ppm)			1.0 azido
	98%	N_3 (elemental)			
	0%	tosyl (^{13}C NMR)	50.4%	tosyl (elemental)	1.07 tosyl
	0%	OH (^{13}C NMR)			
	0%	chlorine (^{13}C NMR)	1.5%	chlorine (elemental)	
6-Amino-6-deoxycellulose (**3f**)	100%	NH_2 (^{13}C NMR)			1.0 amino
	99%	NH_2 (FITC)			
	98%	NH_2 (elemental)			
	0%	OH (^{13}C NMR)			
	0%	chlorine (^{13}C NMR)			
	0%	tosyl (^{13}C NMR)	0% [a]	tosyl (elemental)	
	0%	N_3 (^{13}C NMR)			

[a] After twofold reduction.

Additionally, in Table 5, the percentage of quantitative estimation of functional groups by different analytical methods is shown. We did not find any hint of a chloro group at C-6 in tosylcellulose **1b** by ^{13}C NMR spectroscopy as had been reported by Rahn et al.[9] after tosylation of cellulose. The reason for this may be that there is complete tosylation at C-6 and the subsequent possible susceptibility of the tosylester group by nucleophilic attack of released chloride is too low under the conditions used.

Further results support the completeness of tosylation at C-6 (100%). No signal of C-6–OH at 59.4 ppm was observed in the ^{13}C NMR spectrum. The iodine analysis of subsequently modified sample **4h** (S_N reaction with sodium iodide takes place selectively at C-6 at 60 °C) additionally supports the above conclusion that C-6 is completely tosylated (see Table 4).

The exchange of tosyl groups by azido groups was complete and regioselective at C-6 (**2q**) at low-temperature conditions of 50 °C. This was shown by estimating the peak intensity at 50.1 ppm (100%) for the azido group at C-6 as well as by elemental analysis (98 mol%). No C-6–OTs signal at 68.2 ppm and no C-6–OH signal at 59.4 ppm were observed in the ^{13}C NMR spectrum. This is the proof for complete reaction at C-6. The subsequent reduction step results in completely transferring the azido group at C-6 into an amino group (^{13}C NMR) and additionally 98% amino groups (elemental analysis) and 99% primary amino groups (fluorescence measurement with FITC reagent). The small deviation to 100% is attributed to the analytical error of the methods used. In the case of a usually accepted standard deviation of 2% of the analytical methods used for direct estimation, only a few groups

lead to precise results with negligible deviation, whereas the estimation of a bulk of groups (98–99%) has to take fully into account the 2% deviation in the results. This is the reason why we could not reach 100% estimated functional groups in the later cases, although the reaction was complete.

S_N reaction of cellulose tosylates with ammonia.—Another known concept was tested to prepare pure 6-amino-6-deoxycellulose by optimizing S_N reaction conditions of cellulose tosylate (DS 1 at C-6) with partial tosylation at C-2 and C-3 in the presence of ammonia and Me_2NAc as solvent in an autoclave. At 125 °C a complete displacement of tosyl groups by amino groups was observed by FTIR spectroscopy (see Table 6). In this case the infrared spectrum (**5d**) showed neither asymmetric nor the symmetric valence vibrations of the O=S=O group at 1180 cm^{-1} and at 1370 cm^{-1}, which means that this group had completely disappeared. The product was colored brown and was insoluble in water or common organic solvents. Therefore, we regret not being able to show the exact regioselective displacement reaction of tosyl groups by ammonia in a ^{13}C NMR spectrum. The reason for the brown color and insolubility might be the side reaction of end groups of cellulose with ammonia and a possible crosslinking reaction. We are studying this reaction further to get an alternative amination at C-6 of cellulose.

4. Discussion

Cellulose, the most abundant biopolymer with one anomeric center and four asymmetric carbon atoms and three different OH groups in the repeating unit, is a potential candidate not only for preparing a variety of new materials, but it is also important for drug design.

New concepts are needed to introduce, for example, heparin-like or other functional groups into the cellulose backbone with the highest possible regioselectivity and completeness. Such derivatives synthesized with optimized methods are ideally characterized by a DS of 1 for each functional group introduced in each individual position of the cellulose repeating unit. This means there must be a uniform distribution of each functional group along the polysaccharide chain. The synthesis of cellulose derivatives via enzymatic polymerization has until now not been suitable to prepare regioselectively modified cellulose derivatives.[28] The ring-opening reaction of glucose orthoester derivatives via cationic polymerization[29] is limited to ether derivatives and cannot be used for introducing sulfate half ester or carboxylic groups into cellulose. The synthesis of regioselectively modified cellulose derivatives with heparin-like functional groups via solid-phase stepwise synthesis to solve this problem quickly has not been so far developed. The attempts to introduce only one heparin-like functional group into cellulose with such high specificity failed, e.g., sulfonation reactions.[30] The reactivity differences between OH groups at C-6, C-2 and C-3 of cellulose in sulfonation reactions were not sufficient to reach a medium standard of regioselectivity and completeness. However, additionally used protecting groups such as trimethylsilyl groups or acetyl groups improved the sulfation of cellulose.[30,31] It has to be taken into account that after the reaction all protecting groups must be removed completely without affecting functional groups. If the removal of the protecting group is not complete, cell toxicity might be induced, e.g., with trimethylsilyl groups.[30,31] In a case where two or three different functional heparin-like groups such as sulfate half ester and acetyl groups have to be introduced, the preparation becomes much more difficult.

However, to promote an easy and quick reaction step, the regioselective introduction of amino groups can be used to solve the problem because the reactivity differences of OH groups to amino groups are nearly 1:100. In the case of chitosan, the 2-amino-2-deoxycellulose, reactivity differences between OH and amino groups are mostly sufficient to reach highest possible regioselectivity, steroselectivity and completeness with heparin-like functional groups.[25] In the case of heparin with repeating units containing glucosamine, most of the regioselective displacement reactions of functional groups showed highest possible regioselectivity and completeness.[32]

Table 6
Conditions and results of the nucleophilic exchange reaction of cellulose tosylate **1b** with ammonia in an autoclave in DMAc

Entry	Ammonia/g	Pressure/bar	Temperature/°C	Time/h	FTIR results	
					ν_{as} (SO_2)	ν_s (SO_2)
5a	5.0	15	50	18	strong	strong
5b	5.0	22	75	18	weak	strong
5c	5.0	33	100	18	weak	weak
5d	5.0	40	125	18	no	no

In all cases the amount of cellulose tosylates was 1.0 g.

We developed a new strategy to introduce amino groups and related functional groups exclusively into C-6 of cellulose without using protecting groups. The known literature methods for tosylation[9] of cellulose were optimized to reach a DS of 1 at C-6. Because the tosyl group is not bulky enough to give only 6-tosylation,[8,9,33] we have to also accept the partial tosylation at C-2 and C-3. An S_N reaction of the 6-*O*-tosyl groups by phthalimido groups is possible.[4] The exchange is specific at C-6 because of the bulky phthalimido group. However, the S_N reaction is incomplete because the phthalimido group is quite bulky. Therefore we decided to use an azido group for the S_N reaction. Azides are known to be usual intermediates in oligosaccharide synthesis,[34–36] and at the end of the synthetic step, the azido groups are reduced into amino groups.

In our concept the key reaction is the S_N reaction of 6-*O*-tosylcellulose by sodium azide. This near-specificity of the reaction at C-6 at low temperature may be explained by double steric effects of both the tosyl groups at C-2 and C-3 and the glucose ring against nucleophilic attack of the azido group. The tosyl groups at C-2 and C-3 can subsequently be completely removed by reduction with $LiAlH_4$ while the azido group at C-6 is simultaneously and completely reduced to the amino group.

The 6-amino-6-deoxycellulose so obtained was used as an intermediate to introduce additional carboxylic groups or sulfate half ester groups into C-6. These new derivatives were prepared by known sulfonation and carboxymethylation reactions with highest possible regioselectivity and completeness recently tested with chitosan.[31] Structure–function studies are also possible in relation to chitosan and heparin derivatives with comparable functional groups but different backbone structures.

Some derivatives may be immobilized covalently by their amino groups to carboxylic group-containing polymer surfaces. They can be immobilized by bifunctional or heterobifunctional crosslinking agents, e.g., vitamin C, to functionalized surfaces. The derivatives can also be used as surface coating for biomaterials or for sensors etc.[37] Additionally the 6-azido-6-deoxycellulose may be used to introduce aldehyde or carboxylic acid groups[13] into C-6, or it may be used for UV activation and anchoring on non-functionalized polymers such as PE, PP etc.[38] A lot of other regioselective reactions have to be tested to use the high potential of such newly defined regioselectively modified pure cellulose derivatives for drug design.

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References

1. Karrer von, P.; Wehrli, W. *Helv. Chim. Acta* **1926**, *9*, 591–597.
2. Sakurada, I. *J. Soc. Chem. Ind. Jpn.* **1929**, *32*, 11B.
3. Scherer, P. C.; Field, J. M. *Rayon Text. Month.* **1941**, *22*, 51–53.
4. Haskins, J. F.; Weinstein, A. H. *J. Org. Chem.* **1954**, *19*, 67–69.
5. Usov, A. I.; Nosova, N. I.; Firgang, S. I.; Golosa, O. P. *Vysokomol. Soedin., Ser. A* **1973**, *15*, 1150–1153.
6. Teshirogi, T.; Yamamoto, H.; Sakamoto, M.; Tonami, H. *Sen'i Gakkaishi* **1979**, *35*, T525–T529.
7. Cramer, F. D. *Methods Carbohydr. Chem.* **1962**, *1*, 242–246.
8. McCormick, C. L.; Dawsey, T. R.; Newman, J. K. *Carbohydr. Res.* **1990**, *208*, 183–191.
9. Rahn, K.; Diamantoglou, M.; Klemm, D.; Berghmans, H.; Heinze, T. *Angew. Makromol. Chem.* **1996**, *238*, 143–163.
10. Kern, H.; Choi, S.; Wenz, G. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1998**, *39*, 80–81.
11. Heinze, T.; Koschella, A.; Magdaleno-Maiza, L.; Ulrich, A. S. *Polym. Bull.* **2001**, *46*, 7–13.
12. Koschella, T.; Heinze, T. *Macromol. Biosci.* **2001**, *1*, 178–184.
13. Clode, D. M.; Horton, D. *Carbohydr. Res.* **1971**, *19*, 329–337.
14. Saad, G. R.; Furuhata, K.-i. *Egypt. Polym. Int.* **1997**, *42*, 356–362.
15. Casu, B.; Johnson, E. A.; Mantovani, M.; Mulloy, B.; Oreste, P.; Pescador, R.; Prino, G.; Torri, G.; Zopetti, G. *Arzneim. Forsch.* **1983**, *33*, 135–141.
16. Holme, K. R.; Perlin, A. S. *Carbohydr. Res.* **1997**, *302*, 7–12.
17. Muzzarelli, R. A. A.; Tanfani, F.; Emanuelli, M.; Mariotti, S. *Carbohydr. Res.* **1982**, *107*, 199–214.
18. Maunier, V.; Boullanger, P.; Lafont, D.; Chevalier, Y. *Carbohydr. Res.* **1997**, *299*, 49–57.
19. Hansen, H. C.; Magnusson, G. *Carbohydr. Res.* **1999**, *322*, 166–180.
20. Hattori, K; Yoshida, T.; Nakashima, H.; Premanathan, M.; Aragaki, R.; Mimura, T.; Kaneko, Y.; Yamamoto, N.; Uryu, T. *Carbohydr. Res.* **1998**, *312*, 1–8.
21. (a) Rosser, R. M.; Faulkner, D. J. *J. Org. Chem.* **1984**, *49*, 5157–5160;
    (b) Bessodes, M.; Abushanab, E.; Antonakis, K. *Tetrahedron Lett.* **1984**, *25*, 5899–5902;
    (c) Boyer, J. H. *J. Am. Chem. Soc.* **1951**, *73*, 5865–5866.
22. Rolla, F. *J. Org. Chem.* **1982**, *47*, 4327–4329.
23. (a) Lin, T.-S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 544–548;
    (b) Ohrui, H.; Misawa, T.; Meguro, H. *J. Org. Chem.* **1985**, *50*, 3007–3009;
    (c) Willer, R. L. *J. Org. Chem.* **1984**, *49*, 5150–5154;
    (d) Hansen, H. C.; Magnusson, G. *Carbohydr. Res.* **1999**, *322*, 166–180.

24. Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635–646.
25. Baumann, H.; Faust, V. *Carbohydr Res.* **2001**, *331*, 43–57.
26. Oldham, J. W. H.; Rutherford, J. K. *J. Am. Chem. Soc.* **1932**, *54*, 366–378.
27. Cramer, F. B.; Purves, C. B. *J. Am. Chem. Soc.* **1939**, *61*, 3458–3465.
28. Okamoto, E.; Klyosada, T.; Shoda, S.; Kobayashi, S. *Cellulose* **1997**, *4*, 161–172.
29. Nakatsubo, F.; Kamitakahara, H.; Hori, M. *J. Am. Chem. Soc.* **1996**, *118*, 1677–1681.
30. Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. Functionalization of Cellulose. In *Comprehensive Cellulose Chemistry*; Wiley-VCH Verlag: Weinheim, 1998; Vol. 2, pp 115–132.
31. Baumann, H.; Richter, A.; Klemm, D.; Faust, V. *Macromol. Chem. Phys.* **2000**, *201*, 1950–1962.
32. Baumann, H.; Scheen, H.; Huppertz, B.; Keller, R. *Carbohydr. Res.* **1998**, *308*, 381–388.
33. Heuser, E.; Heath, M.; Shockley, W. H. *J. Am. Chem. Soc.* **1950**, *72*, 670–674.
34. Dessinges, A.; Castillon, S.; Olesker, A.; Thang, T. T.; Lukacs, G. *J. Am. Chem. Soc.* **1984**, *106*, 450–451.
35. Croft, A. P.; Bartsch, R. A. *Tetrahedron* **1983**, *39*, 1417–1474.
36. Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495–4537.
37. Tiller, J.; Berlin, P.; Klemm, D. *Biotechnol. Appl. Biochem.* **1999**, *30*, 155–162.
38. Erdtmann, M.; Keller, R.; Baumann, H. *Biomaterials* **1994**, *15*, 1043–1048.