ELSEVIER

Carbohydrate Polymers 45 (2001) 43–51

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Starch derivatives of high degree of functionalization. Part 2. Determination of the functionalization pattern of *p*-toluenesulfonyl starch by peracylation and NMR spectroscopy ☆

R. Dicke, K. Rahn, V. Haack, Th. Heinze*

Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University of Jena, Humboldtstraße 10, D-07743 Jena, Germany

Accepted 18 April 2000

Abstract

In the solvent system *N,N*-dimethyl acetamide/LiCl homogeneously synthesized *p*-toluenesulfonyl starch samples were subsequently peracetylated and perpropionylated in order to evaluate their molecular structure by means of NMR spectroscopy. The total degree of substitution of tosyl groups (DS_{Tos}) was determined using the signals of the methyl protons of the tosyl, acetyl or propionyl moieties. The distribution of the functional groups were accessible as well. By means of two-dimensional methods an unambiguous assignment of the signals was possible. Tosyl starch of DS_{Tos} values of up to one shows a predominate functionalization at position 2. At higher DS_{Tos} the primary position is increasingly functionalized, i.e. the tosyl starch samples with a new functionalization pattern were accessible.

Keywords: *p*-Toluenesulfonyl starch; Degree of substitution; Functionalization pattern; Regioselective derivatization; NMR spectroscopy

1. Introduction

The properties of polysaccharide products obtained by polymeranalogous reactions may be strongly influenced by the distribution of the functional groups within the repeating units and along the polymer chains. Therefore, a recent goal in polysaccharide chemistry is the development of synthesis paths which allow the preparation of polysaccharide-based products with a pre-set functionalization pattern. Especially regioselective etherifications and esterifications of cellulose and starch are increasingly studied, with the substituent located exclusively or at least predominantly at one (or two) deliberately pre-set sites of the anhydroglucose units (Heinze, 1998; Heinze & Glasser, 1998; Klemm, Stein, Heinze, Philipp & Wagenknecht, 1996).

In the field of starch functionalization, products of high degree of substitution (DS) are presently in the center of our interest including the determination of the distribution of the functional groups. For instance, carboxymethyl starch samples synthesized by different methods: (i) by conventional slurry reaction; (ii) homogeneously in dimethyl sulfoxide; and iii) via 6-*O*-triphenylmethyl starch, were very effectively analyzed by means of HPLC and ^{1}H-NMR spectroscopy after chain degradation (Heinze, Liebert, Pfeiffer & Heinze, 1999). Due to the very stable bonds of the carboxymethyl moieties the acid-catalyzed depolymerization can be realized without a splitting of the ether bonds which is an inalienable prerequisite for these analytical methods.

Quite recently, we studied the homogeneous preparation of *p*-toluenesulfonyl (tosyl) ester of starch using *N,N*-dimethyl acetamide (DMA)/LiCl as a solvent (Heinze, Talaba & Heinze, 2000). The standard ^{13}C-NMR spectra of the products indicate that the tosylation reaction is more effective at 2 position than at the 6 and 3 positions. Moreover, samples with a total DS_{Tos} of up to 1.4 can practically not transferred to 6-deoxy-6-iodo starch by a treatment with iodide which is assumed to occur selectively at C atom of tosylated primary hydroxyl groups. To get quantitative information about the partial DS at the different position the above mentioned methods, which includes necessarily a chain degradation, are not suitable since tosylate groups can undergo a broad variety of subsequent reactions such as nucleophilic displacement or elimination reactions. Consequently, different analytical tools have to be applied.

A very powerful method for the structural characterization of polysaccharide derivatives is the NMR spectroscopy,

☆ Part 1, Heinze et al. (2000). *Carbohydrate Polymer* 42, 411–420.

* Corresponding author.
E-mail address: coh@uni-jena.de (Th. Heinze).

0144-8617/01/$ - see front matter
PII: S0144-8617(00)00219-8

Table 1
Description of the *p*-toluenesulfonyl starch samples investigated

Reaction conditions			*p*-Toluenesulfonyl starch				
Molar ratio Tos-Cl/AGU[a]	Temperature (°C)	Time (h)	No.	S (%)	DS_{Tos}[b]	Cl (%)	Yield (%)
0.5	8	24	**2a**	5.27	0.36	0.00	88
1.0	8	24	**2b**	7.61 (9.42)[c]	0.61 (0.87)	0.19	97
1.0	Room temperature	24	**2c**	7.63 (9.43)[c]	0.61 (0.88)	0.11	96
1.0	8	24	**2d**	8.30	0.70	0.00	98
1.5	8	5	**2e**	10.17	1.02	0.27	76
3.0	8	1	**2f**	10.77	1.14	0.00	85
6.0	8	24	**2g**	13.61	2.02	0.32	62

[a] Two mole triethylamine/mol Tos-Cl, AGU: anhydroglucose unit.
[b] DS_{Tos}: degree of substitution of tosyl groups, calculated on the basis of sulfur analysis determined with a LECO CHNS/932 ultimate analyzer.
[c] Sulfur determined with a LECO CS/225 analyzer.

especially the ^{1}H-NMR spectroscopy of polysaccharide acetates or of derivatives which are subsequently modified by complete acetylation reaching a total DS of 3 (Deus, Friebolin & Siefert, 1991; Klemm & Stein, 1995; Matulova, Toffanin, Navarini, Gilli, Paoletti & Cesaro, 1994). In the present paper we report on the determination of the DS values and the functionalization pattern of tosyl starch by means of ^{1}H-NMR spectroscopy including two-dimensional methods using the subsequently acetylated and propionylated tosyl starch samples. Moreover, results of ^{13}C-NMR spectroscopy are discussed.

2. Experimental

2.1. Materials

The *p*-toluenesulfonyl (tosyl) starch samples (**2a**–**g**, see Table 1) were prepared homogeneously in DMA/LiCl (4.3% starch content) using commercial starch "HYLON VII" (**1**, 70% amylose, *National Starch & Chemical GmbH*, Neustadt, Germany), tosyl chloride, and triethylamine as described in detail by Heinze et al. (2000). The solvents were dried and distilled prior to use according to the conventional methods. Other reagent grade chemicals were used without further purification.

Table 2
Degree of substitution of *p*-toluenesulfonyl (tosyl) groups (DS_{Tos}) of peracylated tosyl starch determined from the ^{1}H-NMR spectra

Tosyl starch		Tosyl starch acylate			
No.	DS_{Tos}	No.	DS_{Tos} calculated from ^{1}H-NMR spectra using		
			$(CH_3)_{Tos}$	$(CH_{arom.})_{Tos}$	$(CH_3)_{Acyl}$
2a	0.36	**4a**	–[a]	0.41	0.35
2b	0.61 (0.87)[b]	**3b**	0.94	0.80	0.78
2c	0.61 (0.88)[b]	**3c**	0.94	0.83	0.78
2c	0.61 (0.88)[b]	**4c**	–	0.82	0.80
2d	0.70	**4d**	–	0.86	0.76
2e	1.02	**3e**	1.00	0.97	1.20
2e	1.02	**4e**	–	0.95	1.00
2f	1.14	**3f**	1.21	1.31	1.16
2g	2.02	**3g**	1.99	1.85	–

[a] Separate integration of the signals not possible.
[b] See Table 1.

2.2. Measurements

Elemental analysis was carried out by means of a LECO CHNS/932 ultimate analyzer with a burning temperature at 1000°C (Ag-crucible). For selected tosyl starch samples a LECO CS/225 analyzer with a burning temperature of about 1300°C was additionally used (ceramic crucible). The FTIR spectra were recorded on a Nicolet Impact 400 spectrometer using KBr pellets dried at 100°C for 1 h. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker AMX 400 spectrometer in DMSO-d_6 or $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively, with a 5 mm diameter C–H dual probe. All spectra were recorded at 50°C ($CDCl_3$) and at 60°C (DMSO-d_6) with a concentration of 5% (w/w) and referenced from the residual proton of $CDCl_3$ at 7.30 ppm for ^{1}H and the middle carbon signal at 77.0 ppm for ^{13}C, or of DMSO-d_6 at 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C. Multiple bond C–H correlated 2D spectroscopy measurements were performed by a field-gradient mode heteronuclear multibond coherence (HMBC) technique. The spectrum was subjected to FT-processing after zero-filling to a 1024×512 matrix. The total measurement time was 48 h.

2.3. Methods

2.3.1. Acetylation of tosyl starch (*3e*), typical example

To a stirred solution of tosyl starch **2e** (0.3 g, 0.9 mmol, $DS_{Tos} = 1.02$) in 5 ml anhydrous pyridine, 5 ml (40 mmol) acetic acid anhydride and 0.1 g (0.8 mmol) *N,N*-dimethylaminopyridine were added. The mixture was kept at room temperature for 20 h and additionally for 5 h at 80°C. After

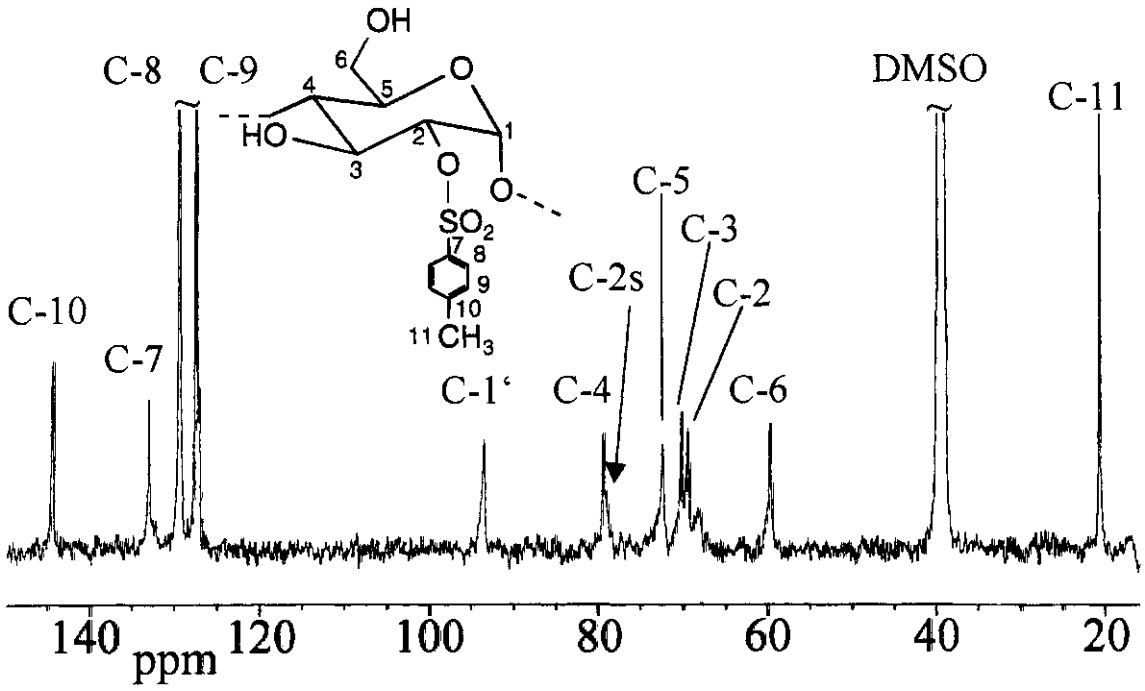


Fig. 1. ^{13}C-NMR spectrum of tosyl starch sample **2e** (DS_{Tos} = 1.02) recorded in DMSO-d_6 at 60°C.

cooling down to ambient temperature, the product was isolated by precipitation in 100 ml methanol, filtered off, thoroughly washed with methanol and dried at 50°C for 5 h under vacuum.

Yield: 0.4 g (90%).

Degree of substitution: DS_{Tos} = 1.00, 0.97 or 1.20, respectively, calculated from the ^{1}H-NMR spectrum using the corresponding signals of CH_3 of tosyl or acetyl moieties or of aromatic proton of tosyl moiety (see Fig. 4 and Table 2). Both tosyl and acetyl groups give a total DS of 3.0.

The tosyl starch acetate **3e** is soluble in acetone, dimethyl sulfoxide (DMSO), DMA, *N,N*-dimethyl formamide (DMF), pyridine and $CHCl_3$.

FTIR (KBr): 2962 (ν CH); 1752 (ν C═O); 1597, 1495, 1447 (ν C–C_{arom}); 1370 (ν_{as} SO_2); 1178 (ν_s SO_2); 835 cm^{-1} (δ C–H_{arom}).

^{13}C-NMR ($CDCl_3$): 20.6–21.5 (*C*H_3, tosylate and acetate); 62.2–96.8 (starch backbone); 127.9–145.6 (*C*H_{arom}); 169.4–169.9 ppm (*C*═O, acetate).

^{1}H-NMR ($CDCl_3$): 1.77–2.08 (C*H*$_3$C═O); 2.45 (C*H*$_3$, tosylate); 3.67–5.17 (starch backbone); 7.24–7.73 ppm (C–*H*$_{arom}$).

*2.3.2. Propionylation of tosyl starch (**4e**), typical example*

Tosyl starch sample **2e** (0.3 g) was allowed to react with 5 ml (39 mmol) propionic acid anhydride/0.1 g *N,N*-dimethylaminopyridine in 5 ml anhydrous pyridine. The mixture was kept at 80°C for 20 h. After cooling down to room temperature, the product was isolated by precipitation in 100 ml of ethanol, filtered off, thoroughly washed with ethanol and dried at 50°C for 5 h under vacuum. *Yield*: 0.4 g (90%).

Degree of substitution: DS_{Tos} = 0.95 or 1.00, calculated from the ^{1}H-NMR spectrum using the signals of the aromatic protons of the tosyl moiety or CH_3 of the propionyl ester (see Fig. 5 and Table 2).

4e is soluble in acetone, DMSO, DMA, DMF, pyridine and $CHCl_3$.

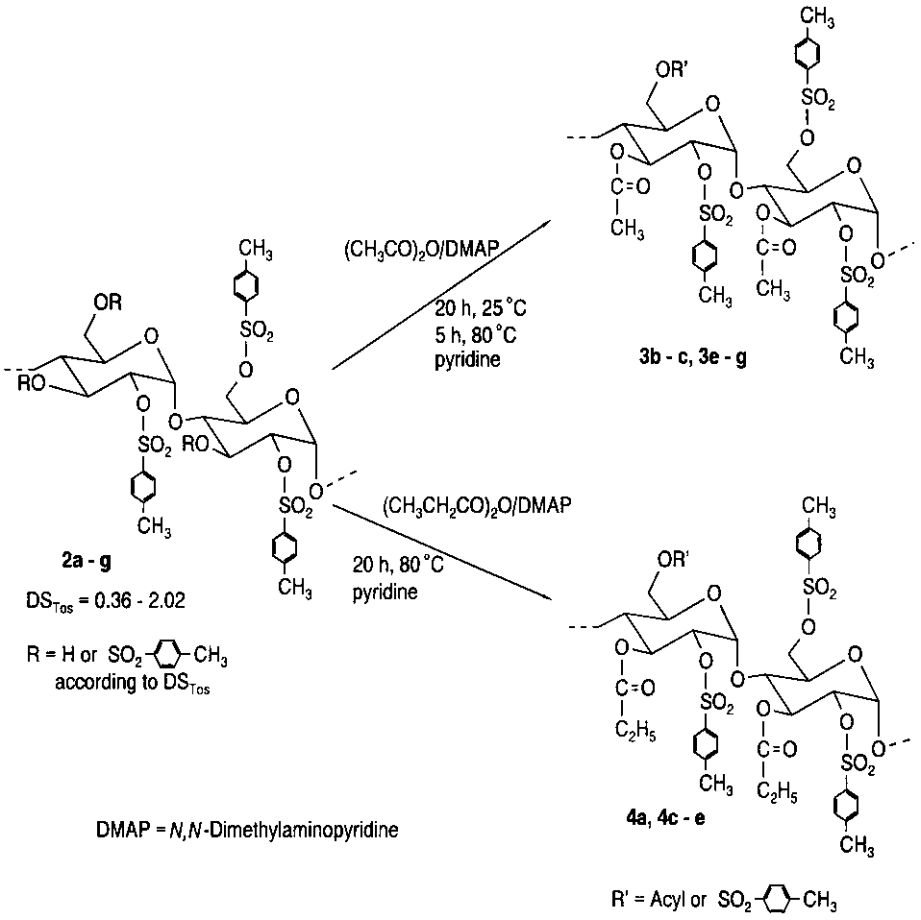
FTIR (KBr): 2982, 2946, 2929 (ν CH); 1749 (ν C═O); 1597, 1463 (ν C–C_{arom}); 1351 (ν_{as} SO_2); 1179 (ν_s SO_2); 836 cm^{-1} (δ C–H_{arom}).

^{13}C-NMR ($CDCl_3$): 8.4–8.9 (CH_3CH_2); 21.5 (*C*H_3, tosylate); 26.8–27.2 (*C*H_3 propionate); 61.8–96.2 (starch backbone); 128.1–145.6 (*C*H_{arom}); 172.7–173.3 ppm (*C*═O, propionate).

^{1}H-NMR ($CDCl_3$): 0.96–1.24 (CH_3CH_2); 1.94–2.32 (CH_3CH_2C═O); 2.57 (C*H*$_3$, tosylate); 3.71–5.20 (starch backbone); 7.11–7.73 ppm (C–*H*$_{arom}$).

3. Results and discussion

The reaction of starch dissolved in *N,N*-dimethyl acetamide/LiCl with *p*-toluenesulfonyl (tosyl) chloride in the presence of triethylamine represents an effective method for the preparation of pure and organo-soluble tosyl starch samples with an insignificant incorporation of chlorodeoxy groups (Heinze et al., 2000). The DS_{Tos} can simply controlled by adjusting the molar ratio of tosyl chloride to anhydroglucose unit (AGU). In order to gain consistent structure–property relations of the polymers an exact



Scheme 1.

knowledge of both the total DS and the distribution of the functional groups dependent on the total DS_{Tos} is absolutely necessary. For this reason we investigated tosyl starch and subsequently acylated samples by means of ^{1}H-NMR spectroscopy.

3.1. Description of tosyl starch samples

The tosyl starch samples (**2a**–**g**) investigated in this work were synthesized under totally homogeneous reaction conditions using maize starch **1** (Hylon VII) as starting material (Heinze et al., 2000). In contrast, the already described tosylation of starch starting from a slurry in pyridine as well as the homogeneous reaction in DMSO leads to products with a preferred reaction at the primary hydroxyl group at position 6 (Clode & Horton, 1971; Whistler & Hirase, 1961; Yalpani, 1985).

As summarized in Table 1, samples with DS_{Tos} values in the range from 0.4 to 2.0 were prepared. The DS_{Tos} was calculated from the sulfur content determined by a LECO CHNS/932 ultimate analyzer at a burning temperature of 1000°C. However, in some cases the estimation of DS_{Tos} is not reproducible. The sulfur content fluctuates extremely and an exact estimation is not possible. Therefore, samples were investigated additionally with a LECO CS/225 analyzer with a burning temperature of about 1300°C leading to an about 2% higher sulfur content as shown by tosyl starch samples **2b** and **2c** with less than 8% sulfur and consequently with a high amount of unmodified OH-groups (Table 1). Obviously, the burning temperature is rather important to determine the DS_{Tos} values by elemental analysis.

Standard ^{13}C-NMR spectra of the tosyl starch derivatives were measured in DMSO-d_6. Fig. 1 shows the ^{13}C-NMR spectrum of sample **2e** ($DS_{Tos} = 1.02$) and the assignment of the signals. The peak for the C-6 atom of the AGU influenced by O-6 tosylation appears at $\delta = 68.4$ ppm (C-6_S), i.e. it exhibits a down field-shift at about 8.7 ppm compared with the corresponding carbon of unmodified starch (C-6, $\delta = 59.8$ ppm). From the signal intensities it may be concluded that the 6 position is not or only slightly tosylated. On the other hand, the signal at $\delta = 93.5$ ppm which is assigned to C-$1'$ (C-1 atom influenced by O-2 tosylation) indicates an extensive reaction at the position 2. Moreover, a signal for C-1 (unmodified C-2 position) is not visible which would appear at about with a high field shift of 3–5 ppm. The tosylated secondary hydroxyl groups itself give a new signal at $\delta = 79.4$ ppm. From the spectra it may be

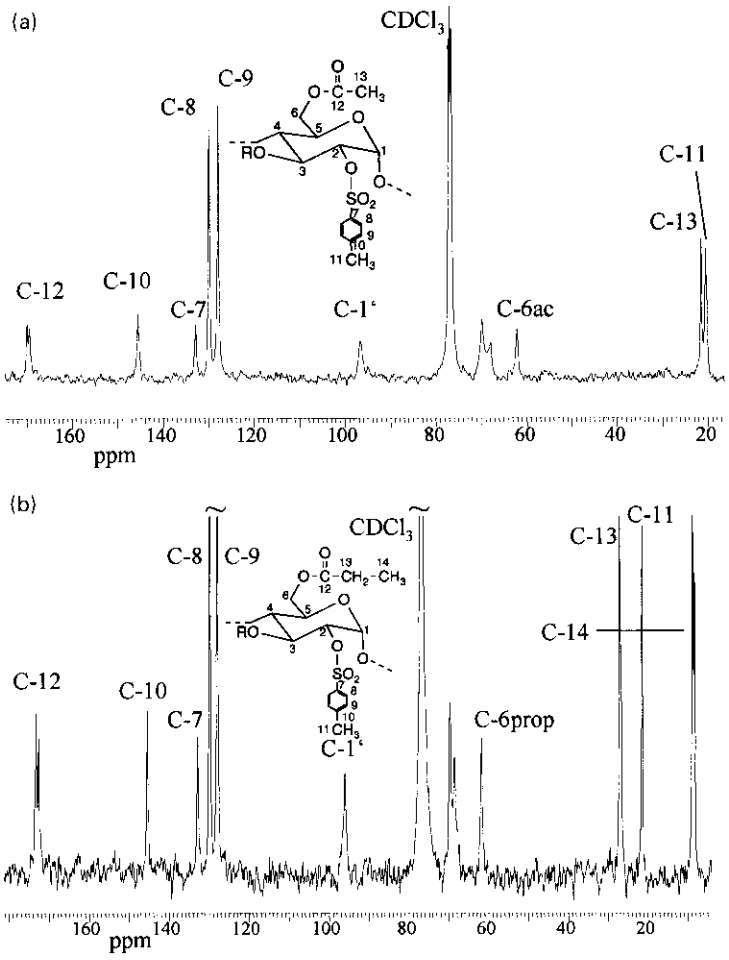


Fig. 2. ^{13}C-NMR spectrum of: (a) tosyl starch acetate **3e**; and (b) tosyl starch propionate **4e** recorded in $CDCl_3$ at 50°C.

concluded that a substituent distribution within the AGU in the order O-2 ⩾ O-6 and O-3 occurred.

The determination of the partial DS_{Tos} of tosyl starch samples at the 6 position by reaction with NaI (in acetyl acetone) under conditions for a selective substitution of the 6 tosylate groups shows that a maximum DS_I of 0.7 can be obtained starting from tosyl starch sample **2g** with a high DS_{Tos} of 2.02. The result indicates that even at this high DS_{Tos} no complete tosylation of the CH_2OH functional groups occurred. At a comparatively low DS_{Tos} of 1.08, the DS_I reached a value of 0.20 only. That means that a remarkable tosylation of secondary OH groups appeared. The results of substituent distribution obtained by analysis of the corresponding iododeoxy derivatives of tosyl starch are in agreement with those of ^{13}C-NMR investigations. From both the methods it may be concluded that the tosylation occurs faster at the secondary hydroxyl groups.

However, it should be noted that in contrast to the generally accepted idea the determination of the extent of tosylation of primary OH groups of polysaccharides by the analysis of the corresponding iododeoxy derivatives is just a semiquantitative method. Deviations from a perfect analysis were already published by various authors (Hall & Horne, 1973; Rahn, Diamantoglou, Klemm, Berghmans & Heinze, 1996; Takahashi, Fujimoto, Bama, Miyamoto & Inogaki, 1986).

3.2. Acylated tosyl starch derivatives

3.2.1. Synthesis of tosyl starch acetates and propionates

In comparison to starch, the acetylation of cellulose derivatives has found considerable interest in connection with the determination of the substituent distribution by using ^{13}C- and ^{1}H-NMR spectroscopy (Deus et al., 1991; Goodlett, Dougherty & Patton, 1971; Iwata, Azuma, Okamura, Muramoto & Chun, 1992; Tezuka & Tsuchiya, 1995; Tezuka, Imai, Oshima & Chiba, 1987). For this purpose, it is necessary that the free OH groups of the polymer are completely acetylated or propionylated in order to obtain well-resolved spectra which can be used for quantitative assessments.

A complete acetylation of the free OH groups of tosyl starch samples (Scheme 1) could be achieved by reacting the polymer with a 40 molar excess of acetic acid anhydride for 20 h at room temperature and additionally 5 h at 80°C

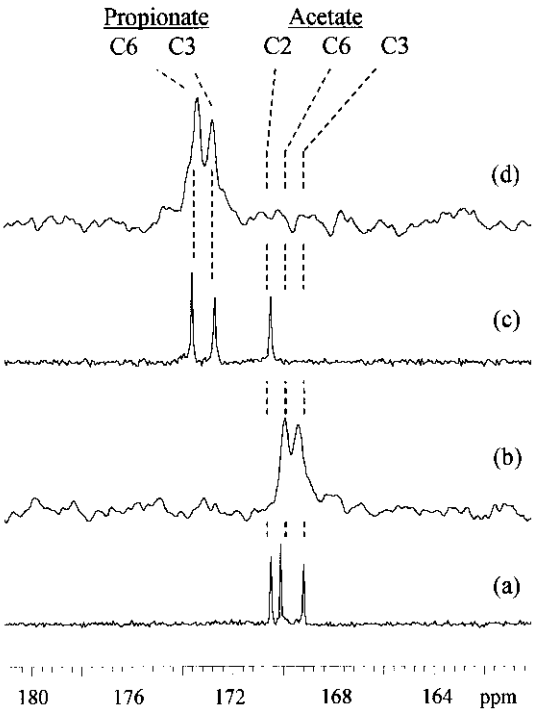


Fig. 3. Carbonyl region of the ^{13}C-NMR spectra of: (a) starch triacetate; (b) tosyl starch acetate **3e** ($DS_{Tos} = 1.02$); (c) 2-mono-*O*-acetyl-3,6-di-*O*-propionyl starch; and (d) tosyl starch propionate **4e** ($DS_{Tos} = 1.02$) measured in $CDCl_3$ at 50°C.

using *N,N*-dimethylaminopyridine (DMAP) as a catalyst (**3b**, **c** and **3e**–**g**, Table 2). In the case of propionylation, a complete esterification could be realized by reacting the polymer with an approximately 40 molar excess of propionic acid anhydride for 20 h at 80°C in the presence of DMAP (samples **4a** and **4c**–**e**, Table 2).

A quantitative acylation is evident in the FTIR spectra from the disappearance of the hydroxyl absorption at 3523 cm^{-1} (ν OH) along with the appearance of the ester carbonyl absorption ν C═O at 1752 cm^{-1} (acetyl) and 1749 cm^{-1} (propionyl). The signals of the tosyl groups ν_{as} SO_2 at 1370 (acetyl) or 1351 cm^{-1} (propionyl), and ν_s SO_2 at 1178 cm^{-1} are still visible which is an indication for the stability of the tosyl ester under the reaction conditions used.

The synthesized tosyl starch acetates and propionates show the same solubility in common organic solvents as the starting tosyl starch samples, however, an additional solubility in $CHCl_3$ appears.

3.2.2. ^{13}C-NMR spectroscopic characterization

Standard ^{13}C-NMR spectra of the tosyl starch acetates and propionates were measured in $CDCl_3$. The ^{13}C-NMR spectrum of the tosyl starch acetate **3e** ($DS_{Tos} = 1.02$) shows the resonances of the polymer backbone between $\delta =$ 62.2–96.8 ppm and signals of the tosyl moieties between $\delta =$ 127.9–145.6 ppm (CH_{arom}). Moreover, at $\delta =$ 20.6–21.5 ppm the signals of the CH_3 (tosylate and acetate) and at $\delta =$ 169.4–169.9 ppm the signals of the acetyl C═O

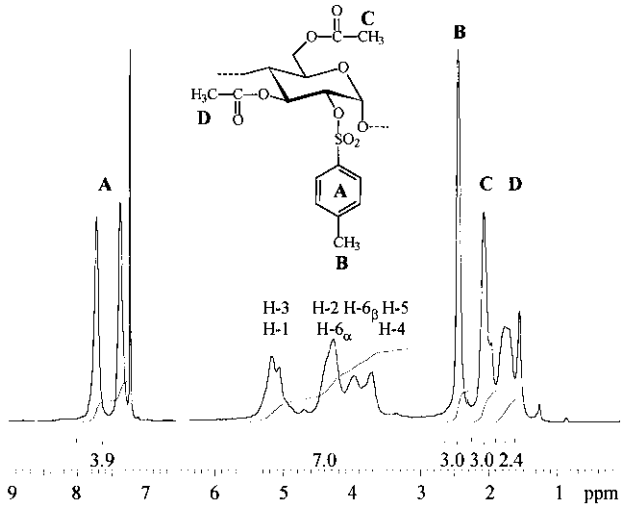


Fig. 4. ^{1}H-NMR spectrum of tosyl starch acetate **3e** ($DS_{Tos} = 1.02$) measured in $CDCl_3$ at 50°C.

groups are found (Fig. 2a). For the tosyl starch propionates the spectra (see e.g. spectrum of sample **4e**, Fig. 2b) show the resonances of the starch backbone between $\delta =$ 61.8–96.2 ppm, signals of the tosyl moieties between $\delta =$ 128.0–145.6 ppm (CH_{arom}). At $\delta = 21.5$ ppm the signals of the CH_3 and at $\delta =$ 172.7–173.3 ppm signals for the ester groups (C═O) appear. Additionally, the peaks of the propionyl moieties occur at $\delta =$ 8.4–8.9 ppm (CH_3CH_2) and at $\delta =$ 26.8–27.2 ppm (CH_3).

Usually in polysaccharide acetates, the signals of the carbonyl groups appear at different chemical shifts depending on the position functionalized within the anhydrosugar units. Therefore, it seems appropriate to gain information about the distribution of the functional groups by using the carbonyl signals of the ^{13}C-NMR spectra. As shown in Fig. 3 (spectrum a), in case of a starch triacetate three separate signals can be detected at 170.5, 170.1, and 169.2 ppm, which can be assigned to a C═O moiety at positions 2, 6 and 3, respectively. In contrast, the ^{13}C-NMR spectrum of the tosyl starch acetate **3e** (Fig. 3b) shows only two carbonyl peaks at 169.9 and 169.4 ppm indicating that the positions 6 and 3 are acetylated. From this results it may be concluded that the tosylation occurred regioselectively at the O-2 atoms. Moreover, spectrum c of Fig. 3 was obtained from a 2-mono-*O*-acetyl-3,6-di-*O*-propionyl starch sample which shows just one C═O signal for the acetyl moiety at C-2 and two signals for the propionyl moieties at C-3 and C-6 as expected. The synthesis of this new starch derivative is described in detail by Dicke (1999). The tosyl starch propionate **4e** (Fig. 3d) gave a ^{13}C-NMR spectrum with two carbonyl peaks at 172.7 and 173.3 ppm, i.e. the propionyl moieties are located at positions 3 and 6. Consequently, the tosyl groups of the starting starch tosylate **2e** with a DS_{Tos} of 1.02 are preferably located at position 2.

From these results, it becomes obvious that the tosylation occurred faster at O-2 compared to O-3 and O-6. Moreover,

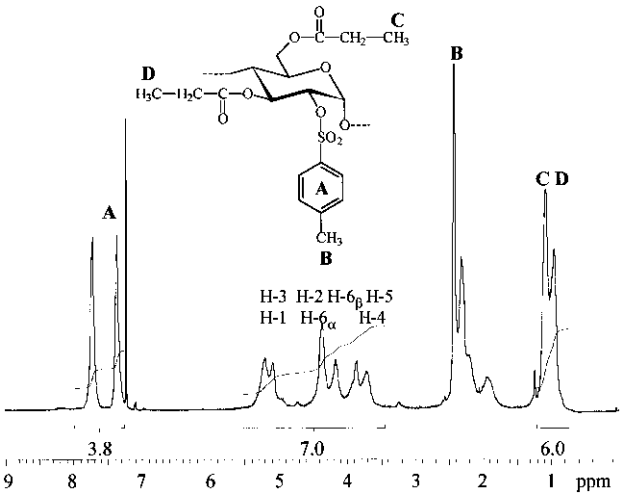


Fig. 5. ^{1}H-NMR spectrum of tosyl starch propionate **4e** ($DS_{Tos} = 1.02$) measured in $CDCl_3$ at 50°C.

^{13}C-NMR spectroscopy is important to assign the signals using 2D NMR spectroscopical methods (see below).

3.2.3. *^{1}H-NMR spectroscopy*

Fig. 4 shows the ^{1}H-NMR spectrum of tosyl starch acetate **3e** ($DS_{Tos} = 1.02$). The signals of the tosyl moiety appear at 2.45 (CH_3) and between 7.24–7.73 ppm (C–H_{arom}) and the peaks of the starch backbone are found in the region of 3.67–5.17 ppm. The signals of the acetyl groups occur between 1.77–2.08 ppm ($CH_3C{=}O$). The ^{1}H-NMR spectra of the perpropionylated tosyl starch samples are also very well resolved. A typical spectrum including the assignment of the signals of sample **4e** is shown in Fig. 5.

From the spectrum it is possible to determine the DS_{Tos} value using Eq. (1). The time between every scan was 4.3 s. In previous experiments it was found that for starch acetate a relaxation time of 4.3 s is sufficient to determine the DS-values by means of ^{1}H-NMR spectroscopy (Günther, 2000). Moreover, the determination of the DS of cellulose esters via NMR spectroscopy was also reported by Deus et al. (1991) and Goodlett et al. (1971).

The DS_{Tos} values may be calculated from the protons of both the methyl groups or the aromatic rings of the tosylate moieties as well as from the methyl groups of the acetyl or propionyl ester function. The DS_{Tos} values obtained are summarized in Table 2 and they are in good agreement with those of elemental analysis.

In some cases even the determination of the distribution of the functional groups within the repeating units is possible concerning the primary versus secondary positions. The calculation of sample **3e** gives partial DS_{Acetyl} values of 0.80 at positions 2 and 3. For sample **3f** a partial $DS_{Propionyl}$ value of 0.82 for O-2 and -3 was determined.

$$DS = \frac{I_{Signal}/n}{\sum I_{AGU}/7} \quad (1)$$

$n =$ H atoms of the signal of aromatic ($n = 4$) or methyl protons ($n = 3$) of tosyl moieties or methyl protons ($n = 3$) of acetyl and propionyl ester moieties, AGU = anhydroglucose unit

From the ^{1}H-NMR measurement it becomes obvious that with increasing DS_{Tos} the resolution of the spectra decrease. This might be resulted from the increasing shielding of the rather bulky aromatic groups. As a consequence, samples of high DS_{Tos} should preferably investigated after perpropionylation products since the distance of the corresponding methyl groups of the propionate esters and the polymer backbone is longer compared to acetate.

The assignment of the signals of the methyl protons concerning their position within the anhydroglucose repeating unit is sure due to the primary versus secondary positions.

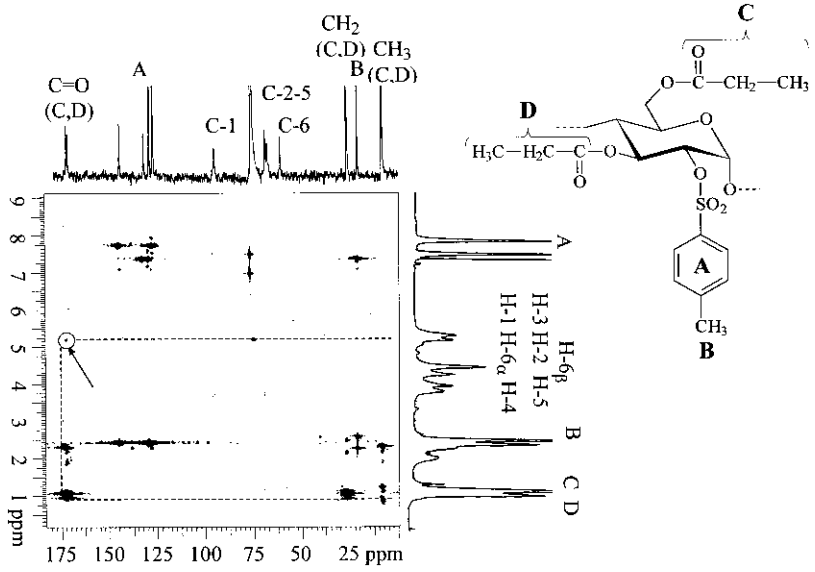


Fig. 6. 400 MHz HMBC NMR-spectrum of tosyl starch propionate **4e** ($DS_{Tos} = 1.02$) measured in $CDCl_3$ at 50°C.

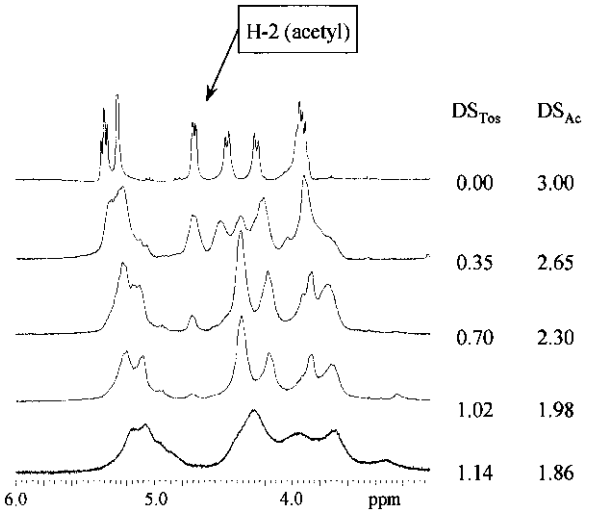


Fig. 7. ^{1}H-NMR spectra (spectral range 3–6 ppm) of tosyl starch acetates of different values of the degree of substitution of tosyl (DS_{Tos}) and acetyl groups (DS_{Ac}).

However, an unambiguous assignment of the methyl protons between the two secondary groups was not published. Therefore, we studied our samples using 2D NMR techniques. By means of the HMBC technique it was possible to gain a clear insight. Fig. 6 shows a HMBC spectrum of the tosyl starch propionate **4e** ($DS_{Tos} = 1.02$). A long-range correlation peak with rather high sensitivity of the CH_3 group of the propionate moiety D with the C═O groups appears. Moreover, a correlation of the latter one with a H-atom was evaluated. From ^{1}H^{1}H-COSY-NMR experiments it was possible to assign the signal of this H-atom to position 3 of the AGU. This correlation peak was marked in Fig. 6. Therefore, it may be concluded that the propionate ester function is located at position 3. Moreover, a propionylation of position 6 can be concluded from the fact that the starch tosylates of DS_{Tos} up to 1.4 cannot be modified by subsequent displacement reactions which are proceed at the primary tosylate groups only.

The high selectivity of the tosylation of starch dissolved in DMA/LiCl can also be concluded from ^{1}H-NMR spectra of peracetylated tosyl starch (Fig. 7). The typical signal for the proton at the acetylated 2 position decreases with increasing DS_{Tos}. Already at a DS_{Tos} of 1.14 the signal disappear completely, i.e. any position 2 is substituted by a tosyl moiety.

4. Conclusions

In the present paper the determination of the distribution of functional groups of tosyl starch, synthesized in the solvent *N,N*-dimethyl acetamide (DMA)/LiCl for the first time, by means of NMR spectroscopical investigations of subsequently acetylated or propionylated samples was studied. Since polysaccharide tosylate cannot be investigated after hydrolytic chain degradation due to a variety of possible side reactions (displacement, elimination and cross-linking) it appears especially important to make a method available for the characterization of the intact polymers. The signals of the NMR spectra were unambiguously assigned using two-dimensional NMR methods especially HMBC technique. From the results it becomes obvious that the new synthesis path applied, i.e. a homogeneous reaction in the solvent system DMA/LiCl (see also Heinze et al., 2000), leads to products with a new functionalization pattern. In any case a preferred tosylation at position 2 occurs. It is worth to mention that a highly regioselective reaction can be carried out without using protective groups. At DS_{Tos} values below 1 the tosylation proceeds predominated at O-2 and this position is completely functionalized already at a total DS_{Tos} of 1.14. At higher DS_{Tos} values the primary position is increasingly functionalized.

Very recently, a preferred acylation of the *O*-2 position of starch in DMSO using vinyl carbonic acid esters and an inorganic salt was found (Dicke, 1999). In these studies the regioselcetivity was explained on the basis of structure of the polymer in solution (DMSO) which forms a helical structure as described by Everett and Foster (1959), Fujii, Honda and Fujita (1973) and Nakanishi, Norisuye, Teramoto and Kitamura (1993). This might be true of the state of dissolution of starch in DMA/LiCl as well, however, up to now no experimental data are available.

The results indicate that starch dissolved in the solvent system DMA/LiCl is reactive and that starch derivatives of high DS can be very effectively obtained even with a regioselective distribution of the functional groups. The remaining OH groups are reactive as well and products of a complete modification can be synthesized subsequently. The reason of the regioselectivity on the molecular level cannot be explained satisfactorily with the present results. Obviously, the reaction system starch/DMA/LiCl/Tos-Cl possesses special "supramolecular" interactions. At present we study the reaction system using NMR spectroscopy including ^{7}Li-NMR.

Acknowledgements

The general financial support of the Bundesministerium für Ernährung, Landwirtschaft und Forsten and the Fachagentur für Nachwachsende Rohstoffe (Agency of Renewable resources) is gratefully acknowledged.

References

Clode, D. M., & Horton, D. (1971). Preparation and characterization of the 6-aldehydo derivatives of amylose and whole starch. *Carbohydrate Research*, *17*, 365–373.

Deus, C., Friebolin, H., & Siefert, E. (1991). Partiell acetylierte Cellulose-Synthese und Bestimmung der Substituentenverteilung mit Hilfe der ^{1}H-NMR-Spektroskopie. *Makromoleculare Chemie*, *192*, 75–83.

Dicke, R. (1999). PhD thesis, Friedrich Schiller University of Jena.

Everett, W. W., & Foster, J. F. (1959). The conformation of amylose in solution. *Journal of American Chemical Society*, *81*, 3464–3469.

Fujii, M., Honda, K., & Fujita, H. (1973). Dilute solution of amylose in dimethyl sulfoxide. *Biopolymers*, *12*, 1177–1195.

Goodlett, V. W., Dougherty, J. T., & Patton, H. W. (1971). Characterization of cellulose acetates by nuclear magnetic resonance. *Journal of Polymer Science: Part A-1*, *9*, 155–161.

Günther, W. (2000). Personal communication.

Hall, D. M., & Horne, J. R. (1973). Model compounds of cellulose: trityl ethers substituted exclusively at C-6 primary hydroxyls. *Journal of Applied Polymer Science*, *17*, 2891–2896.

Heinze, Th. (1998). New ionic polymers by cellulose functionalization. *Macromolecular Chemistry & Physics*, *199*, 2341–2364.

Heinze, Th., & Glasser, W. G. (1998). The role of novel solvents and solution complexes for the preparation of highly engineered cellulose derivatives. In Th. Heinze & W. G. Glasser, *Cellulose derivatives: modification, characterization, and nanostructures, ACS Symposium Series* (pp. 12–18). Series no. 688. ACS.

Heinze, Th., Liebert, T., Pfeiffer, K., & Heinze, U. (1999). Effective approaches for estimating the functionalization pattern of carboxymethyl starch of different origin. *Starch/Stärke*, *51*, 11–16.

Heinze, Th., Talaba, P., & Heinze, U. (2000). Starch derivatives of high degree of functionalization. 1. Effective, homogeneous synthesis of p-toluenesulfonyl (tosyl) starch with a new functionalization pattern. *Carbohydrate Polymers*, *42*, 411–420.

Iwata, T., Azuma, J.-I., Okamura, K., Muramoto, M., & Chun, B. (1992). Preparation and n.m.r. assignment of cellulose mixed esters regioselectively substituted by acetyl and propanoyl groups. *Carbohydrate Research*, *224*, 277–283.

Klemm, D., & Stein, A. (1995). Silylated cellulose materials in design of supramolecular structures of ultrathin cellulose films. *Journal of Macromolecular Science—Pure & Applied Chemistry*, *A32*, 899–904.

Klemm, D., Stein, A., Heinze, Th., Philipp, B., & Wagenknecht, W. (1996). Regioselectively substituted esters and ethers of cellulose. In J. C. Salamone, *Polymeric materials encyclopedia: synthesis, properties and applications* (pp. 1043–1054). Boca Raton, FL: CRC Press.

Matulova, M., Toffanin, R., Navarini, L., Gilli, R., Paoletti, S., & Cesaro, A. (1994). NMR analysis of succinoglycans from different microbial sources: partial assignment of their ^{1}H and ^{13}C NMR spectra and location of the succinate and the acetate groups. *Carbohydrate Research*, *265*, 167–179.

Nakanishi, Y., Norisuye, T., Teramoto, A., & Kitamura, S. (1993). Conformation of amylose in dimethyl sulfoxide. *Macromolecules*, *26*, 4220–4225.

Rahn, K., Diamantoglou, M., Klemm, D., Berghmans, H., & Heinze, Th. (1996). Homogeneous synthesis of cellulose *p*-toluenesulfonates in *N*,*N*-dimethyl acetamide/LiCl solvent system. *Angewandte Makromoleculare Chemie*, *238*, 143–163.

Takahashi, S.-I., Fujimoto, T., Bama, B. M., Miyamoto, T., & Inogaki, H. (1986). ^{13}C-NMR spectral studies on the distribution of substituents in some cellulose derivative. *Journal Polymer Science, Part A: Polymer Chemistry*, *24*, 2981–2993.

Tezuka, Y., & Tsuchiya, Y. (1995). Determination of substituent distribution in cellulose acetate by means of a ^{13}C NMR study on its propanoated derivatives. *Carbohydrate Research*, *273*, 83–91.

Tezuka, Y., Imai, K., Oshima, M., & Chiba, T. (1987). Determination of substituent distribution in cellulose ethers by means of a ^{13}C-NMR study on their acetylated derivatives. 1. Methylcellulose. *Macromolecules*, *20*, 2413–2418.

Whistler, R. L., & Hirase, S. (1961). Introduction of 3,6-anhydro rings into amylose and characterization of the products. *Journal of American Chemical Society*, *26*, 4600–4605.

Yalpani, M. (1985). A survey of recent advances in selective chemical and enzymic polysaccharide modifications. *Tetrahedron*, *41*, 2957–3020.