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Synthesis and characterization of the novel cellulose derivative dansyl cellulose

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Summary

Novel, soluble 5-dimethylamino-1-naphthalenesulfonyl esters of cellulose (**2**) with a degree of substitution from 0.67 to 1.59 were prepared by esterification of cellulose dissolved in *N,N*-dimethylacetamide/LiCl solvent system and characterized by means of FTIR- as well as ^{1}H and ^{13}C NMR-spectroscopy. The remaining OH groups of **2** can be fully acetylated in pyridine using *N,N*-dimethylaminopyridine as a catalyst. The polymers show typical fluorescence and absorption spectra with absorptions at 217, 250 and 317 nm which are in good agreement with quantum chemical calculations.

Introduction

5-Dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride) is widely used in biochemical analysis of peptides, e.g. [1,2]. Due to its appropriate high reactivity and photochemical activity it found interest in carbohydrate analysis, too. Dansyl-β-cyclodextrins were studied as fluorescent sensors responsive to organic compounds [3]. Soluble hydroxyethyl- and methyl cellulose as well as dextran were labeled with dansyl chloride and the fluorescence depolarization was investigated [4].

Unfortunately, until now the synthesis of soluble dansyl celluloses of considerable high degree of substitution (DS) was not studied although various sulfonic acid esters like *p*-toluenesulfonates (tosylates) of polysaccharides have been extensively reported in the literature [5,6]. Especially tosylates of cellulose have found considerable interest as organo-soluble cellulose intermediates. The sulfonyl ester functions may serve as a protecting group in further modifications of the free remaining OH groups or as a leaving group in nucleophilic displacement reactions. In the course of our studies on cellulose tosylates [7-9] our interest was focused on dansylation reactions of cellulose because of both the sterical hindrance compared to tosyl moiety and the fluorescence activity of the dansyl group. The introduction of dansyl groups in cellulose should result in new cellulosics with promising properties. Thus, we wish to communicate about the homogeneous synthesis of dansyl cellulose in the solvent system *N,N*-dimethylacetamide (DMA)/LiCl. The organo-soluble products as well as additionally acetylated ones are characterized by means of FTIR, ^{1}H- and ^{13}C NMR spectroscopy. UV absorption and

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fluorescence of the new cellulosics are discussed in terms of some results of quantum chemical calculations.

Experimental

Dansyl cellulose 2

In a typical procedure, 2g (12.3 mmole) cellulose (AVICEL®, degree of polymerization 280) were slurried in 30 ml *N,N*-dimethylacetamide (DMA, dried with CaH_2) and kept at 130 °C for 1 h. The slurry was allowed to cool to 100 °C where 3 g of anhydrous LiCl (dried at 150 °C for 2 h) was added. By cooling down to room temperature under stirring the sample dissolves completely. After standing overnight, 5.16 ml (37.0 mmole) triethylamine dissolved in 10 ml DMA was added dropwisely. The mixture was cooled to 10 °C and then 5 g (18.5 mmole) dansyl chloride (Fluka) in 15 ml DMA was added within 30 min under stirring. The reaction mixture was stirred for 24 h at 10 °C. The polymer was precipitated in 500 ml methanol, filtered off, carefully washed with distilled water and methanol. The polymer was redissolved in *N,N*-dimethylformamide (DMF) and precipitated in methanol. The collected product **2a** was dried at room temperature under vacuum.

Yield 3.74 g, Degree of substitution (DS_S) calculated on the basis of sulfur analysis 0.67 (6.75 % S). The product is soluble in DMF, DMA, dimethyl sulfoxide, and pyridine.

FTIR (KBr): 3465 (ν OH), 3085 (ν CH_{arom}), 2949, 2889 (ν CH_3), 2836 (ν CH_2), 2791 (ν N-CH_3), 1358 (ν_{as} SO_2), 1177 (ν_s SO_2).

^{13}C NMR (DMSO-d_6); numbering of C atoms see Figs 1 and 2, index s means substituted, δ = 103.2 (C1), 98.5 (C1'), 72.6 (C2), 80.4 ($C2_s$), 75.6 (C3), 83.0 ($C3_s$), 79.0 (C4), 77.1 (C4'), 75.6 (C5), 67.9 ($C6_s$), 61.1 (C6), 116.3 - 151.0 (C7 - C16), 45.6, 46.8 (C17) ppm.

^1H NMR (DMSO-d_6) δ = 2.88 - 2.89 (CH_3), 7.29 - 8.58 ($H_{arom.}$) ppm.

Dansyl cellulose acetate 3

To a solution of 0.5 g dansyl cellulose **2a** (DS_S = 0.67) in 10 ml pyridine and 0.5 g *N,N*-dimethylaminopyridine was slowly added 3.0 ml acetic anhydride in 5 ml pyridine under stirring. The reaction mixture was kept at 45 °C for 90 min. After standing overnight, the product was precipitated in 200 ml methanol, filtered off, dissolved in 10 ml tetrahydrofurane (THF) and reprecipitated in 200 ml methanol. The polymer dried at room temperature is soluble in THF, $CHCl_3$, and dioxane.

FTIR (KBr): 1760 cm^{-1} (ν C=O) and the above mentioned peaks of dansyl cellulose except ν OH.

^{13} C NMR (DMSO-d_6); numbering of C atoms see Figs 1 and 2, index s means substituted, δ = 62.1 ($C6_{s,acetyl}$), 67.1 ($C6_{s,\ dansyl}$), 98.7 ($C1'_{dansyl}$), 99.5 ($C1'_{acetyl}$), 132.6 (C7), 128.8 (C8), 130.6 (C9), 119.8 (C10), 152.4, 152.9 (C11), 116.5 (C12), 130.6 (C13), 129.6 (C14), 131.5 (C15), 120.1 (C16), 45.5 (C17), 20.3 (CH_3, acetyl), 169.3 - 170.5 (C=O, acetyl) ppm.

^1H NMR (DMSO-d_6) δ = 1.95 (CH_3, acetyl), 2.84 - 3.11 (CH_3, dansyl), 7.28 - 8.59 (H_{arom}) ppm.

Measurements

The degree of substitution of the dansyl cellulose esters was determined by C,H,N,S ultimate analysis.

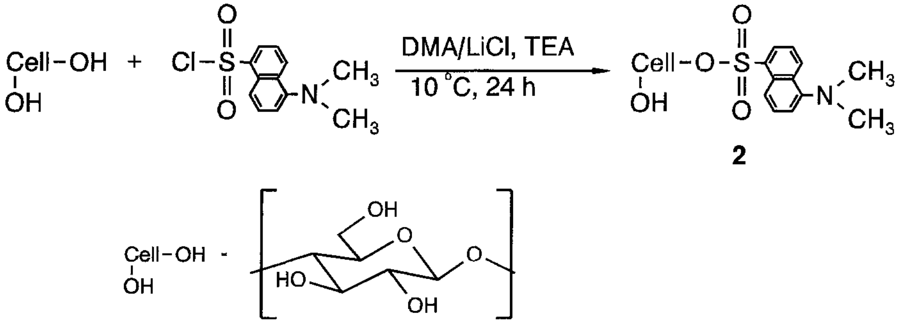
FTIR spectra were recorded on a Impact Nicolet 400 spectrometer using thermally treated (1 h at 100 °C) KBr pellets.

^{13}C NMR spectra (8 % solution in DMSO-d_6 and dioxane-d_8, respectively) were acquired on a Bruker AMX 400 spectrometer operating at 100.63 MHz, the accumulation number was between 25000 and 30000 scans.

UV-absorption, fluorescence and fluorescence excitation spectra were obtained by using Perkin Elmer spectrometers (Lambda 16 and LS 50, respectively).

Results and discussion

The solvent system *N,N*-dimethylacetamide (DMA)/LiCl is now a widely used reaction medium for homogeneous modification of cellulose [10]. Basing on our latest results on tosylation of cellulose in DMA/LiCl [7 - 10] we examined the synthesis of *O*-(5-dimethylamino-1-naphthalenesulfonyl) cellulose (dansyl cellulose).



DMA: N,N-dimethylacetamide; TEA: triethylamine

Table 1: Results of homogeneous dansylation of cellulose in *N,N*-dimethylacetamide/LiCl (triethylamine as a base) at 10 °C for 24 h

Molar ratio[a] (mole/mole)	Sample	Dansyl cellulose DS_S[b]	S (%)	Cl (%)
1.5	**2a**	0.67	6.75	0.99
3.5	**2b**	1.59	9.57	0.82
6.0	**2c**	1.46	9.31	1.55

[a] Mole dansyl chloride per mole anhydroglucose unit

[b] Degree of substitution calculated on the basis of sulfur analysis

The preparation was performed by reacting the cellulose in DMA/LiCl with dansyl chloride at 10 °C within 24 h using triethylamine as a base. Depending on the molar ratio of the dansyl chloride per anhydroglucose unit (AGU) soluble products of a degree of substitution (DS_S) = 0.67 (**2a**, 1.5 mole per mole AGU; soluble in *N,N*-

dimethylformamide (DMF), and dimethyl sulfoxide, (DMSO) and of DS_S = 1.59 (**2b**, 3.5 mole per mole AGU; soluble in DMF, DMSO, tetrahydrofurane (THF), and chloroforme) were obtained (Table 1). An increase of the molar ratio of up to 6.0 mole per mole AGU does not lead to a further increase in DS_S. Compared to tosylation of cellulose [7], in dansylation reaction a higher molar ratio of esterification agent to AGU is necessary to get a comparable DS. Furthermore, the maximum DS reached is lower (DS_{dansyl} 1.6 - DS_{tosyl} 2.5). This behavior may result from the sterical stress of the dansyl substituents. The chlorine analysis of the samples indicates that even at a reaction temperature of 10 °C a minimum amount of chlorine is incorporated into the polymer. This results from the well-known nucleophilic substitution of sulfonyl ester moieties by chloride ions, which are generated during the reaction and are an essential component of the solvent system.

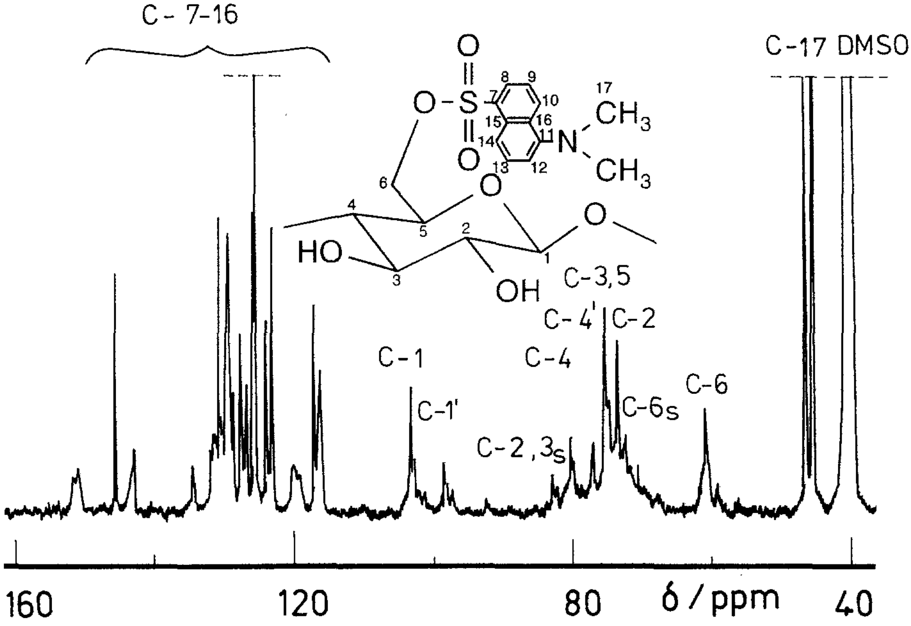


Fig 1. ^{13}C NMR spectrum of dansyl cellulose **2a** in dimethylsulfoxide-d_6, 20000 scans

Fig. 1 shows the standard ring carbon ^{13}C NMR spectrum with proton-decoupling of the dansyl cellulose **2a**. The evaluation was carried out based on the assignment for tosyl cellulose [6,7]. The signal appearing at δ = 67.9 ppm ($C6_S$) is caused by a substituted O6 atom, i.e., it exhibits a down field-shift of about 7 ppm compared with the corresponding carbon of pure cellulose. The small signal at δ = 59.4 ppm might be assigned to a chlorodeoxymethylene group in agreement with the chlorine content determined by ultimate analysis. The unsubstituted O6 atom gives a signal for the C6 at δ = 61.1 ppm as expected [11]. In comparison with pure cellulose a new peak at δ = 80.4 ppm ($C2_S$) indicates an C2 functionalization. Furthermore, the splitting of C1 carbon atom signal (C1') demonstrates the substitution at the O2, too. A small signal at δ = 83.0 ppm indicates a little substitution at the O3 as well. Resonances assigned to the dansyl methyl and aromatic carbons are visible at δ = 45.6 - 46.8 and at δ = 116.3 - 151.0 ppm, respectively. However, this part of the spectrum is too complicated to allow precise assignement. This indicates a strong interaction, probably the formation of H-bonds between the residual free OH groups and the nitrogen atoms of dimethylamino moieties.

In order both to check the reactivity of free OH groups and to simplify the spectrum of dansyl cellulose, the polymer was acetylated in pyridine using acetic anhydride and *N,N*-dimethylaminopyridine as an effective acylation catalyst already employed in cellulose chemistry [12]. Using the described conditions, fully substituted products (soluble in $CHCl_3$, THF, and dioxane) are obtained as confirmed by FTIR analysis. No residual OH groups could be detected. The standard ^{13}C NMR spectrum with proton-decoupling recorded in dioxane solution allows an exact assignment of the aromatic peaks (Fig. 2). Furthermore, typical signals of the carbonyl groups of the acetyl substituent were estimated at $\delta = 169.3$ and 170.5. The signals for C17 and C19 (compare Fig. 2) appear at 45.5 and 20.3 ppm, respectively. The solvent dioxane-d_8 itself shows a broad signal at about 66 ppm. Therefore, the assignments of C atoms of the AGUs is not possible. However, a spectrum recorded in DMSO-d_6 (not shown) proofed the structure of the fully substituted AGU as discussed above.

The cellulose esters were also characterized by means of FTIR spectroscopy. The results are in agreement with the structures described (see Experimental).

In any case, determination of the degree of polymerization by means of GPC measurements in THF solution was unsuccessful. This result migth be explained by strong inter- and intramolecular interactions in solution.

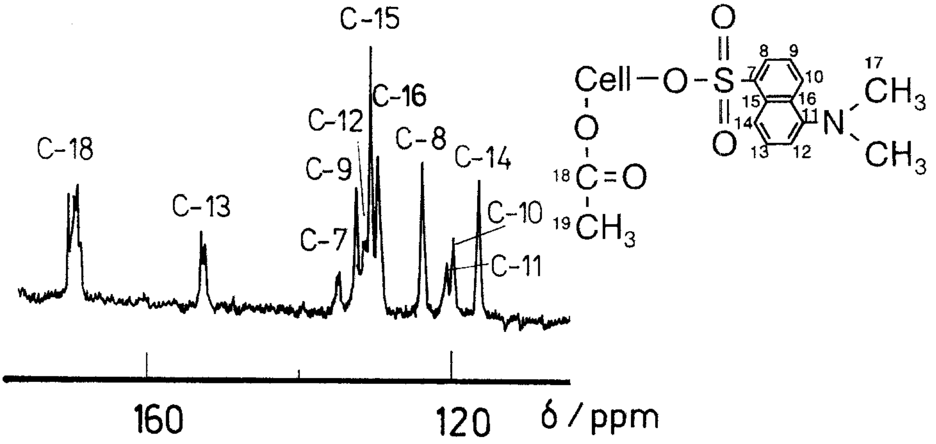


Fig 2. ^{13}C NMR spectrum of danysl cellulose acetate **3a** in the chemical shift range from 110 to 170 ppm (measured in dioxane-d_8, 19500 scans)

The absorption spectrum of acetylated dansyl cellulose in ACN is shown in Fig.3. It is characterized by three bands at 331, 250 and 217 nm. A quantum chemical MNDO-AM1 [13] calculation on a model compound (dansylethylester) yields: 340(0.14), 251(0.24) and 218(0.33) nm (in brackets: oscillator strength). The calculated values are in good agreement with the experimental results. It should be mentioned that according to this calculation the longest-wavelength transition cannot be considered as a pure HOMO-LUMO transition. It contains approximately 30% of the HOMO-1 → LUMO-2 configuration. The fluorescence due to the dansyl fluorophore depends very sensitively on the bonding situation of the sulfonyl group [14]. Thus, dansylchloride itself does not fluoresce at all, the fluorescence maximum of the free acid in ACN occurs at 454 nm and that of the acetylated cellulose derivative at 550 nm. Furthermore, the fluorescence depends strongly on solvent polarity (fluorescence maximum in CH_2Cl_2: 519 nm) and is characterized by a large Stokes shift (in acetonitrile: 201 nm, Fig.4).

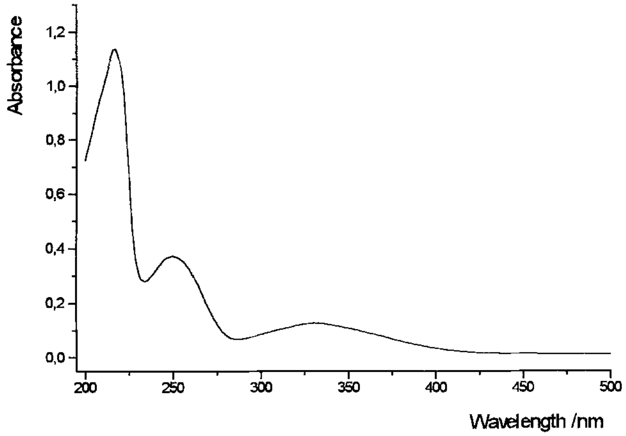


Fig 3. Absorption spectrum of acetylated dansyl cellulose **3a** in acetonitrile

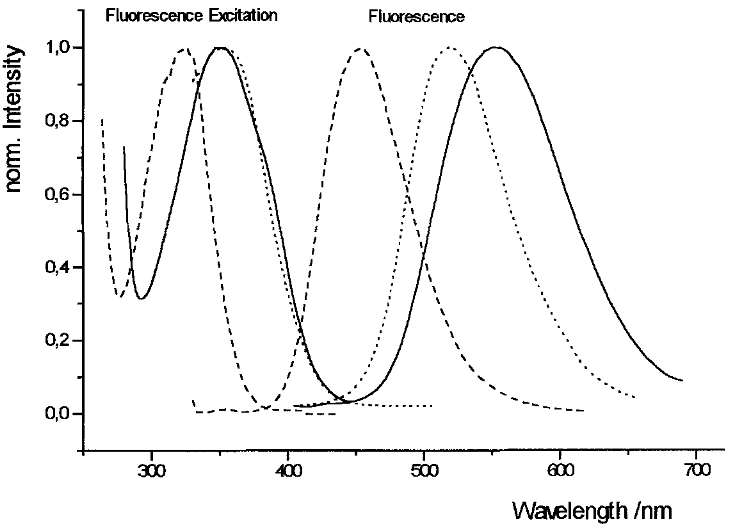


Fig. 4. Fluorescence and fluorescence excitation spectra of acetylated dansyl cellulose in acetonitrile (solid line), in methylene chloride (dotted line) and of the free acid in acetonitrile (dashed line)

Quantum chemical calculations also yield a large Stokes shift (85 nm, without taking into account solvent effects). The respective fluorescence transition (transition from the relaxed S1 state to the Franck-Condon S0 state) is associated with a change of the dipole moment of 5.44 D, making the strong solvent polarity dependence of the fluorescence understandable.

The UV absorption and fluorescence studies of the dansyl cellulose including further oligo- and polysaccharide derivatives will be continued.

Acknowledgments

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