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Structural Characterization of 6-Halo-6-Deoxycelluloses by Direct-Dissolution Solution-State NMR Spectroscopy

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Regioselective modifications of cellulose using activated cellulose derivatives such as 6-halo-6-deoxycelluloses provide a convenient approach for developing sustainable products with properties tailored to specific applications. However, maintaining precise regiochemical control of substituent distribution in 6-halo-6-deoxycelluloses is challenging due to their insolubility in most common solvents and the resulting difficulties in precise structure elucidation by modern instrumental analytical techniques. Herein, an accessible NMR-based approach toward detailed characterization of 6-halo-6-deoxycelluloses, including the determination of the degrees of substitution at carbon 6 (DS_6), is presented. It is shown that the direct-dissolution cellulose solvent, tetrabutylphosphonium acetate:DMSO-d_6, converts 6-halo-6-deoxycelluloses to 6-monoacetylcellulose, enabling in situ solution-state NMR measurements. A range of 1D and 2D NMR experiments is used to demonstrate the quantitivity of the conversion and provide optimum dissolution conditions. In comparison with other NMR-based derivatization protocols for elucidating the structure of 6-halo-6-deoxycelluloses, the presented approach offers major advantages in terms of accuracy, speed, and simplicity of analysis, and minimal requirements for reagents or NMR instrumentation.

1. Introduction

6-Halo-6-deoxycelluloses are modified forms of cellulose in which the primary hydroxy groups of the anhydroglucose unit (AGU) are substituted with a halide. Synthetic pathways toward 6-halo-6-deoxycelluloses are straightforward: 6-chloro- and 6-bromo-6-deoxycelluloses can be prepared directly from cellulose,[1–4] whereas the shortest route to 6-iodo-6-deoxycellulose involves a two-step synthesis via 6-chloro-6-deoxycellulose.[5] These regioselectively functionalized cellulose derivatives are unique in that, under appropriate conditions, the halide can be introduced exclusively and completely onto carbon 6 (C6) of the AGU in a one-step reaction, i.e., without protection/deprotection strategies.[1,6–8] The established preparation methods and high regioselectivity make 6-halo-6-deoxycelluloses promising as activated cellulose derivatives for designing new bio-based materials with fine-tuned properties.

Thanks to the presence of a good leaving group, these compounds are well-suited for nucleophilic substitution (S_N) reactions and, alongside cellulose tosylates[9–13] and cellulose phenyl carbonates,[14,15] offer a convenient pathway to functional cellulose products. 6-Bromo-6-deoxycellulose is perhaps the most interesting in this regard because it can be synthesized with high regioselectivity, which makes it a good starting point for further regioselective transformations, involving a wide array of sulfur- and nitrogen-based nucleophiles.[1,11,16–22] The direct bromination of cellulose involves S_N2 substitution of an oxyphosphonium intermediate—a transformation which is energetically disfavored at secondary hydroxy groups in positions C2 and C3. Following from that, it has been argued that only primary hydroxy groups can be brominated, resulting in bromination occurring at C6 position.[4,23] The degree of substitution at position C6 (DS_6) is adjustable, with a maximum value of 0.98, as reported by Matsui et al.[1] The reported regioselectivity means that 6-bromo-6-deoxycellulose can offer a more uniform molecular skeleton compared to, for instance, tosylated cellulose—although tosylation occurs predominantly at C6, in products with total DS exceeding 0.8, C2 and C3 are also esterified, albeit to a minor extent.[8,24,25]

Still, the potential of 6-halo-6-deoxycelluloses has been somewhat clouded due to their predominant insolubility in common

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molecular organic solvents or water.[6,7,20] Naturally, the inherent poor solubility of 6-halo-6-deoxycelluloses means that further transformations proceed heterogeneously, thus limiting the reaction efficiency to surface modifications and leading to products with unpredictable distribution of substituents along the polymer chain as well as within the AGU. These challenges have hitherto prevented in-depth studies into the structure–property relationship of 6-halo-6-deoxycelluloses.[6,24,26] Clearly, the analytical toolkit of a cellulose chemist should include a method that enables accurate determination of the total DS as well as partial (site-specific) DS for positions C2, C3, and C6 of AGU, all of which are, in principle, available reaction sites.

Commonly utilized approaches toward the determination of DS in 6-halo-6-deoxycelluloses involve either direct or indirect methods. The direct strategies rely on the analysis of solid samples—thus bypassing the issue of insolubility—and include elemental analysis, FTIR spectroscopy, and solid-state NMR spectroscopy. Elemental analysis using the oxygen flask combustion method, also known as the Schöniger flask method, enables a rapid estimation of the total DS based on the halogen content.[1,2,4] However, by definition, this approach fails to discriminate between partial (site-specific) DS values for positions C2, C3, and C6, with any residual impurities leading to overestimated DS. This is a problem for 6-halo-6-deoxycelluloses, as homogeneous syntheses of these compounds typically involve lithium chloride or lithium bromide added in large quantities for complete dissolution of cellulose.[1–4] Furthermore, cellulose derivatives can be prone to moisture uptake, therefore calculations based on elemental composition are not a reliable method for establishing DS. On the other hand, FTIR spectroscopy may in theory be utilized as a direct method for quantifying partial DS from distinct absorption bands corresponding to substituents attached to C2, C3, or C6. For instance, Furuhara et al.[2] identified absorption bands of C3-Cl and C6-Cl, but those two signals were poorly resolved and allowed only for qualitative analysis. Granted, modern instrumental analytical techniques such as attenuated total reflection (ATR)-FTIR may enable fast estimation of the relative DS at C6, C3, and C2, but for rigorous quantitative analyses, one also needs calibration standards with known partial DS values, which are not readily available. Moreover, solid-state NMR has been utilized to further delineate structural characteristics; Roshan et al.[21] specifically used ^{13}C cross-polarization magic-angle spinning (CP-MAS) NMR for the tentative assignments of C1–C6 resonances in unmodified cellulose, 6-bromo-6-deoxycellulose, and two other derivatives. Nonetheless, ^{13}C CP-MAS NMR remains largely restricted to semi-quantitative analysis of substitution patterns in cellulose derivatives due to, for example, poor resolution, very long collection times required for obtaining quantitative data, and the need for dedicated instrumentation.[27,28]

The indirect methods can be subdivided into destructive procedures (e.g., hydrolysis followed by GC-MS)[2–4] and derivatization protocols leading to more soluble 6-deoxycelluloses, which can be analyzed by solution-state NMR. Improved solubility is typically achieved by introducing various functionalities onto positions C2 and C3 whilst preserving the halogen moiety in position C6. For instance, Fox and Edgar[6] prepared soluble 2,3-di-*O*-acylated derivatives of 6-bromo-6-deoxycellulose, and subsequently utilized solution-state ^{13}C NMR for structure elucidation and determination of the degree of substitution in position C6 (DS_6). However, the acylation approach led to problems with insufficient spectral resolution, resulting in unreliable DS_6 values obtained directly from ^{13}C NMR. Consequently, the authors reported DS_6 values based on elemental analysis.[6] It is also important to note that samples with very low DS_6 may require complementary analytical techniques, as ^{13}C NMR is notorious for low signal-to-noise ratios and may require up to 20 000 scans in the quantitative mode.[24] ^{31}P-labeling followed by ^{31}P NMR analysis seems to offer a better alternative for quantification purposes, but the method remains limited to cellulose derivatives soluble in chloroform or chloroform/pyridine mixtures.[29,30] Despite its utility for certain applications that prioritize fast analyses and relative comparisons, the ^{31}P-labeling approach requires costly derivatization agents and may suffer from poor reproducibility.[31]

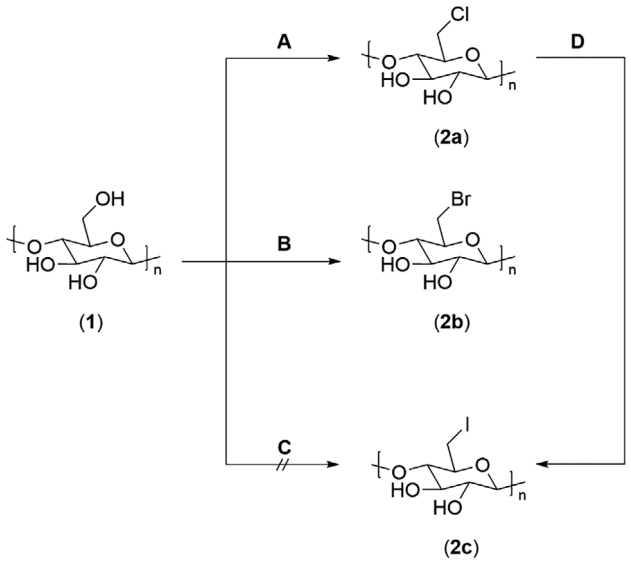
Given these limitations, it becomes imperative to develop a straightforward and accessible method to rapidly elucidate the structures of 6-halo-6-deoxycelluloses, ensuring the accurate determination of DS_6. We previously demonstrated the utility of an ionic liquid (IL) electrolyte, tetrabutylphosphonium acetate ($[P_{4444}][OAc]$):DMSO-d_6, for direct-dissolution solution-state NMR analysis of cellulose and its derivatives, including microcrystalline cellulose, oxidized cellulose, and cellulose acetates.[28,32–34] Herein, we expand the scope of the protocol and describe a simple procedure for detailed structural characterization and accurate DS_6 determination of 6-halo-6-deoxycelluloses by in situ derivatization followed by solution-state NMR spectroscopy.

2. Results and Discussion

2.1. Synthesis of Regioselectively Substituted 6-Halo-6-Deoxycelluloses: Consideration of the Total DS and Solubility

Since the aim of the present work was to demonstrate the utility of the IL electrolyte in solution-state NMR analysis of 6-halo-6-deoxycelluloses, suitable materials were required. To achieve this, we initially selected the most straightforward reaction pathway, i.e., direct halogenation via the Appel reaction under homogeneous conditions[1,2] (**Scheme 1**). This approach allowed us to obtain 6-chloro-6-deoxycellulose (**2a**) and 6-bromo-6-deoxycellulose (**2b**). However, our attempts at direct iodination of cellulose, based on previously published syntheses from simple carbohydrates,[35,36] did not result in the expected 6-iodo-6-deoxycellulose (**2c**). This was likely caused by the presence of excess chloride anion used for facilitating cellulose dissolution, as it had been previously noted that in organic solvent-LiCl systems, the chloride ion participates in reactions.[4] Consequently, we opted for a two-step synthesis, wherein (**2a**) was partially converted to (**2c**) under heterogeneous conditions via the Finkelstein reaction.[5]

The total DS, as determined from the halogen content, for the chlorinated and brominated products (**2a**) and (**2b**) were 0.85 and 0.99, respectively. However, due to methodological limitations, it was not possible to determine the DS for the mixed chloro-*co*-iodo compound (**2c**). Given the high total DS values of the obtained chlorinated and brominated materials, and considering the reportedly high regioselectivity of halogenation under the Appel



Scheme 1. Reaction pathways for the synthesis of 6-halo-6-deoxycelluloses. Summary of reagents and reaction conditions: A) $Ph_3P/BrCCl_3$ in DMA/LiCl, room temperature, 36 h; B) Ph_3P/NBS in DMA/LiBr, 70 °C, 2 h; C) Ph_3P/imidazole/I_2 in DMA/LiCl, 70 °C, 3 h; D) partial conversion from (**2a**) using NaI in acetonylacetone, 120 °C, 5 h.

reaction conditions,[1,2] we wanted to elucidate the detailed structure and confirm the regioselectivity at C6 using solution-state NMR.

The synthesized products (**2a–c**) were therefore tested for solubility in several molecular solvents. Consistent with previous reports,[6,7,20] they remained insoluble, even at prolonged heating and low sample concentrations (**Table 1**). Only the mixed 6-chloro-*co*-6-iodo-6-deoxycellulose (**2c**) was soluble in DMSO. This enabled the acquisition of NMR data, using the perdeuterated DMSO-d_6; however, the ^{1}H and ^{13}C NMR spectra did not provide any meaningful information due to poor resolution and low signal-to-noise ratio.

Thus, in order to fully solubilize compounds (**2a–c**) at a sufficient concentration that would enable high-resolution NMR analyses, we utilized $[P_{4444}][OAc]$:DMSO-d_6 (1:4 wt%).[28] This NMR solvent system is an example of an ionic liquid (IL) electrolyte. The dissolution of an ionic liquid in a co-solvent results in ionic conductivity, hence the term "IL electrolyte" used in the present work. The conditions applied for the direct dissolution of 6-halo-6-deoxycelluloses synthesized herein were based on our prior work regarding the solution-state NMR analysis of cellulose-based materials in the IL electrolyte.[28,32–34] Similarly, the products (**2a–c**) dissolved readily when stirred at 65 °C, typically within ≈15 min, thereby enabling acquisitions of ^{1}H and ^{13}C NMR data with good spectral quality and signal-to-noise ratio (Figures S1–S6, Supporting Information).

Table 1. Solubility of 6-halo-6-deoxycelluloses in molecular solvents, determined at a sample concentration of 10 mg mL^{-1}.

Entry	DMSO	DMF	THF	$CHCl_3$	Toluene	Ethanol	Acetone
2a	–	–	–	–	–	–	–
2b	–	–	–	–	–	–	–
2c	+[a)]	–	–	–	–	–	–

Note: (+), soluble; (-), insoluble; [a)] soluble after heating for 48 h at 80 °C.

2.2. Characterization of 6-Halo-6-Deoxycelluloses by 1D NMR

2.2.1. ^{1}H and ^{13}C NMR of 6-Bromo-6-Deoxycellulose Dissolved in the IL Electrolyte

The following discussion of the 1D ^{1}H and ^{13}C NMR spectra will be focused on 6-bromo-6-deoxycellulose (**2b**), selected as a representative sample. **Figure 1** presents ^{1}H, diffusion-edited ^{1}H and ^{13}C NMR spectra of (**2b**), along with signal assignments for all major peaks of the cellulose backbone, which are also given in **Table 2**.

The ^{1}H NMR spectrum (Figure 1a) is dominated by high-intensity signals attributable to $[P_{4444}][OAc]$, which was used for solubilization, whereas the cellulose-bound proton resonances are characterized by low intensity. The IL resonances appear upfield of the cellulose-backbone region, the latter typically being between 3 and 5 ppm. Nonetheless, based on the standard proton NMR experiment alone, it is not possible to unambiguously determine if the IL peaks are not overlapping any of the polymer-bound signals. Here, the diffusion-edited ^{1}H NMR data (Figure 1b) can provide conclusive evidence, as the effect of diffusion-editing is that one can "filter out" peaks corresponding to the low molecular weight (fast diffusing) species from the ^{1}H spectrum. By comparing the ^{1}H and diffusion-edited ^{1}H spectra, it is possible to determine which signals correspond to species that are covalently bound to cellulose.[28] Importantly, the signals of the cellulose-bound acetyl group at 2.0 ppm are clearly discernible from the acetate ion of the IL at 1.6 ppm, which is present in the standard ^{1}H NMR data but absent from the diffusion-edited ^{1}H spectrum. This is relevant for 6-halo-6-deoxycelluloses, as we initially attempted a nonderivatizing direct dissolution. However, due to the fact that we see the acetate retained in the spectrum after applying diffusion-editing, the logical conclusion is that the halide has been substituted for the acetate (**Scheme 2**, S_N2 displacement). Moreover, it is evident that there is only the 6-OAc forming, as the formation of 2-OAc or 3-OAc would lead to additional acetyl resonances retained in the diffusion-edited ^{1}H spectrum.[32,33] This is consistent with the basicity of acetate anions in aprotic environments, where a rough correlation between basicity and nucleophilicity exists—similar to the "naked anion" concept observed in the reaction of potassium/sodium acetate in aprotic media with crown ethers.[37]

The conversion of 6-bromo-6-deoxycellulose (**2b**) to 6-monoacetylcellulose (**3**) was further confirmed by ^{13}C NMR (Figure 1c), where the C6-OAc resonance is clearly discernable at 63.10 ppm. Conversely, the C6-Br signal expected at 32 ppm[6] was absent from the ^{13}C NMR spectrum; the absence of the C6-OH resonance at 60.03 ppm[34] also indicated that the product was fully substituted at position C6. These observations imply that: a) complete displacement of the bromine moiety by the acetyl functionality had occurred, and b) there was no hydrolytic cleavage of the C6-Br bond caused by traces of water in the IL electrolyte. The remaining two positions bearing the hydroxy group, C2 and C3, which are in principle also available

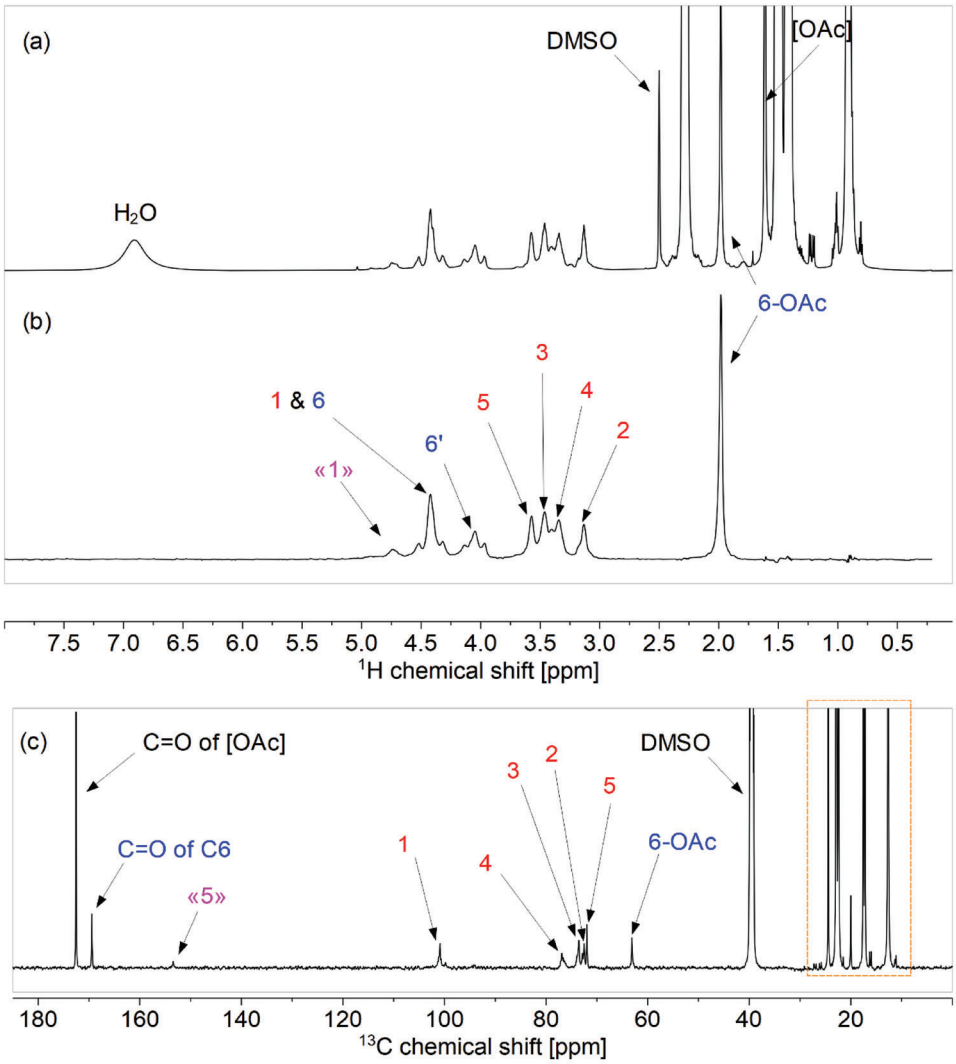


Figure 1. 1D NMR spectra ([P_{4444}][OAc]:DMSO-d_6 1:4 wt%, 65 °C, 5 wt%) for a representative sample of 6-bromo-6-deoxycellulose (**2b**): a) quantitative ^{1}H NMR spectrum; b) diffusion-edited ^{1}H NMR spectrum; c) ^{13}C{^{1}H} NMR spectrum (non-carbonyl IL signals appear within the region marked with a dashed box). Signals attributable to 5,6-cellulosene (**4**) are indicated with angle brackets «».

for bromination, retained their functionality. This was evident from the fact that the C2 and C3 signals, detected at 72.53 and 73.42 ppm, conform to the assignments made by Koso et al.[34] for unmodified cellulose, dissolved under the same conditions (72.97 and 74.39 ppm, respectively). The previously reported[3] shift for C3-Br of 3,6-dibromo-3,6-dideoxycellulose at 62.34 ppm is not observed in the ^{13}C NMR spectrum. Finally, we detected a signal of small intensity at 153.42 ppm, consistent with the ^{13}C chemical shift for C5 of 5,6-cellulosene (**4**) (Scheme 2, elimination), as reported by Ishii.[5] This demonstrates unambiguously that the competing elimination reaction occurs,[38] albeit it seems minor compared to the main substitution reaction.

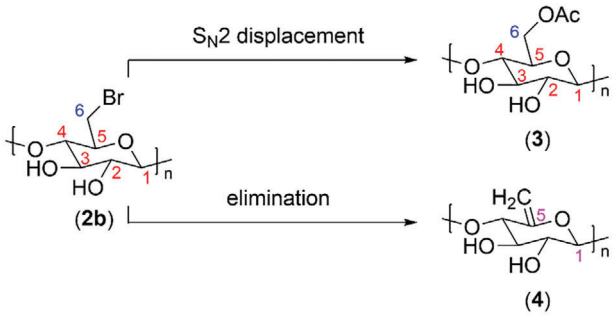
Table 2. ^{1}H and ^{13}C NMR chemical shifts for the cellulose backbone of 6-monoacetylated cellulose (**3**).

Position	1	2	3	4	5	6	6′
^{1}H chemical shift [ppm]	4.43	3.13	3.46	3.34	3.57	4.41	4.05
^{13}C chemical shift [ppm]	100.53	72.37	73.31	76.83	71.67	62.75	–

Note: Spectra were measured at 65 °C, at 5 wt% sample concentration in [P_{4444}][OAc]:DMSO-d_6 (1:4 wt%). All shifts are referenced to the residual DMSO-d_6 signal at 2.50 and 39.52 ppm for ^{1}H and ^{13}C spectral dimensions, respectively. The assignments were made by tracing C—H correlations in 2D NMR spectra, provided in Figure S7 (Supporting Information).

2.2.2. Purity of the IL Electrolyte: Possible Side Reactions

The IL electrolyte used for the reactive dissolution of halodeoxycelluloses should be of sufficient purity and low water content. The presence of water, manifested in the ^{1}H NMR data as a broad peak, may complicate accurate integration, as the residual water peak occasionally overlaps with the cellulose backbone region.



Scheme 2. Dissolution-reaction of 6-bromo-6-deoxycellulose (**2b**) in [P_{4444}][OAc]:DMSO-d_6 (1:4 wt%), leading to the formation of 6-monoacetylated cellulose (**3**) as the main product, and trace amounts of 5,6-cellulosene (**4**).

This problem is illustrated in **Figure 2**, comparing ^{1}H NMR spectra acquired after dissolving the same representative sample of (**2b**) in "wet" and "dry" IL electrolyte. The spectrum recorded in the "wet" IL electrolyte shows a sharp peak at 3.15 ppm, assignable to residual methanol from the preparation of the IL. Traces of triphenylphosphine oxide, produced as a byproduct of the halogenation reaction under the Appel conditions, were also present at ≈7.5 ppm, but these downfield resonances can be disregarded since they do not affect the peak integral area of the cellulose backbone region. The methanol signal, on the other hand, contributes to the error in the cellulose peak integral. Still, the polymer-bound species seem to remain unaffected by potential side reactions with water or methanol, which would lead to the loss of the halide functionality at C6. This was apparent from the diffusion-edited ^{1}H NMR data that showed no differences for the "wet" and "dry" IL electrolyte. Nonetheless, for the sake of accurate processing of the quantitative ^{1}H NMR spectra, moisture uptake from ambient air should be prevented, which can be easily achieved by storing the IL electrolyte under 4 Å molecular sieves.

2.3. Kinetic Studies and Quantitivity of the Dissolution-Reaction in the IL Electrolyte

In the ^{1}H NMR spectral data, the resonances of the acetyl functionality bound to the cellulose backbone are well separated from the peaks associated with the acetate anion of the IL electrolyte. This creates an added benefit, as it is possible that DS_6 could be quickly calculated directly from the ^{1}H NMR data—by integrating the intensities of the cellulose backbone and the polymer-bound acetate peaks—provided that the conversion from (**2**) to (**3**) is quantitative. In order to establish the rate and quantitivity of the dissolution-reaction in [P_{4444}][OAc]:DMSO-d_6, we monitored the conversion by diffusion-edited ^{1}H NMR experiments acquired for samples kept for up to 72 h at 65 °C. These tests also enabled us to determine the minimum time required for complete conversion, which is expected to depend on the halogen. For example, Furuhata et al.[39] studied the reactivity of halide ions in nucleophilic substitution reactions involving halodeoxycelluloses in aprotic solvents. They concluded that the ease of cleavage of the C—X bond in substitution reactions decreases in the following order: C—I > C—Br > C—Cl. Therefore, with 6-chloro-6-deoxycellulose (**2a**) being the least reactive in the series, we estimated its conversion to 6-monoacetylcellulose at different time increments over 72 h.

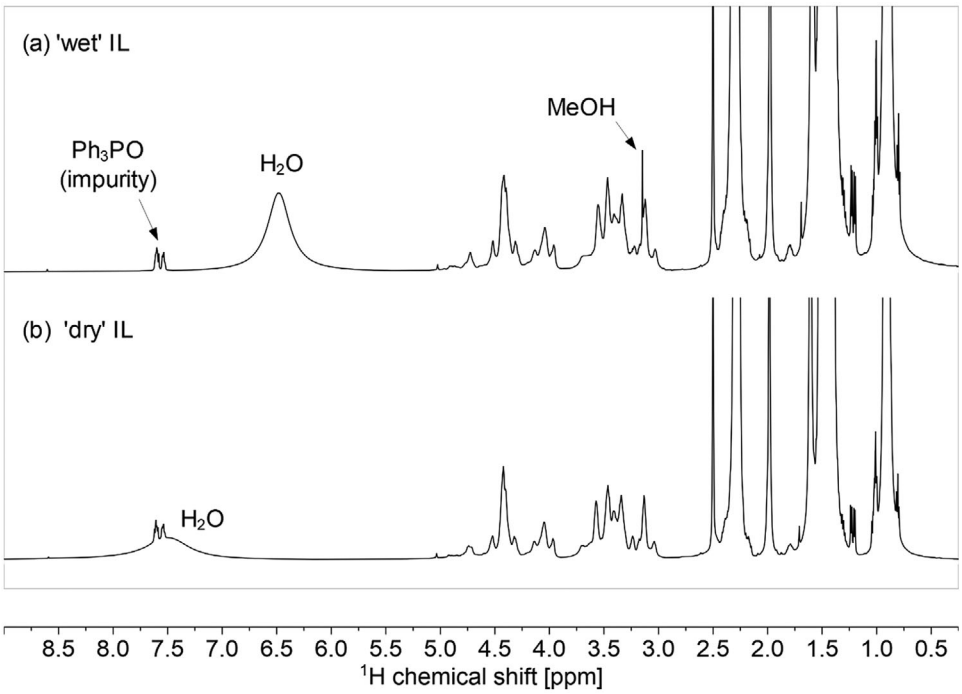


Figure 2. Comparison of ^{1}H NMR spectra of 6-bromo-6-deoxycellulose (**2b**) dissolved in "wet" and "dry" IL electrolyte ([P_{4444}][OAc]:DMSO-d_6 1:4 wt%, 65 °C, 5 wt%).

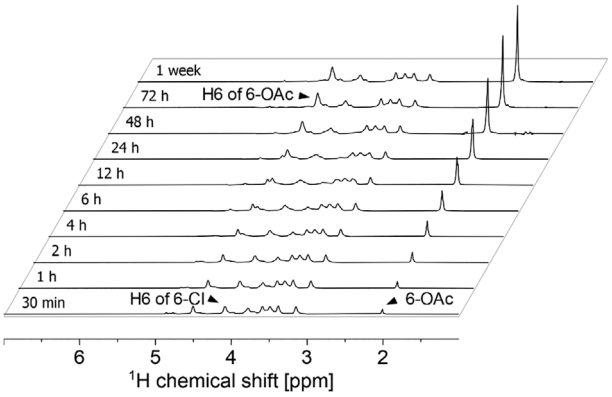


Figure 3. Stacked diffusion-edited ^{1}H NMR spectra of (**2a**) dissolved in the IL electrolyte at 65 °C. The gradual formation of (**3**) due to the reactive dissolution of (**2a**) is apparent from the emerging 6-OAc signal at 2.01 ppm. Peak intensities were normalized to the H2 signal at 3.15 ppm.

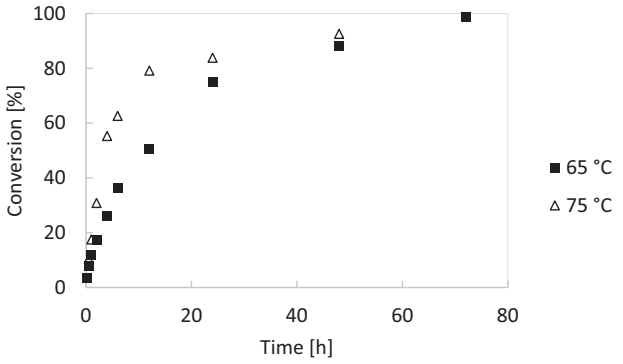


Figure 4. Conversion of (**2a**) to (**3**) as a function of reaction time at 65 °C (squares) and 75 °C (triangles). The percentage of (**3**) formed at a particular time point was estimated from the diffusion-edited ^{1}H NMR data.

Figure 3 presents stacked diffusion-edited ^{1}H NMR spectra of (**2a**) measured after dissolving the sample at 65 °C and maintaining at this temperature for a period up to 72 h. The formation of (**3**) is apparent from the emerging 6-*O*-acetyl resonance at 2.01 ppm. Concurrently, we observed a downfield shift of one of the geminal proton signals (H6) of the cellulose backbone, from 3.78 to 4.42 ppm, consistent with the formation of 6-monoacetylated cellulose.[40] The chlorine moiety was displaced completely within 72 h, and we saw no significant changes in the diffusion-edited ^{1}H spectrum measured again after the sample was kept at 65 °C for one week. Thus, we concluded that the conversion of (**2a**) to (**3**) was complete, and no significant degradation of the polymer chain had occurred as a result of the release of the chloride anion into the reaction mixture.

The fact that the obtained product (**3**) remains stable in the IL electrolyte is important with respect to the accuracy and robustness of DS_6 calculations based on the acetyl signal. Naturally, sufficiently long dissolution time and elevated temperature are required for the substitution to proceed to completion. Conversely, partial degradation of the cellulose polymer chain may occur under prolonged heating, resulting in slightly lower integral values for the cellulose backbone region, which would ultimately lead to erroneous DS_6. However, our observations did not indicate this, as the integral ratio of the acetyl region to the cellulose backbone region, used to calculate DS_6, remained constant and no additional peaks indicative of polymer chain degradation were detected. Furthermore, in a series of parallel measurements conducted at a higher temperature of 75 °C, acceleration in halide displacement was observed initially, but complete conversion to (**3**) still required heating the sample for up to 72 h (**Figure 4**).

Concurrently, the conversion from (**2b**) to (**3**) was investigated. The displacement of the bromine moiety was expected to occur faster, as bromide is a better leaving group in substitution reactions compared to chloride. The results indicated that, under the applied dissolution conditions, the halide displacement was complete within 2 h at 65 °C, as concluded from the diffusion-edited ^{1}H experiments (Figures S8 and S9, Supporting Information). Regarding the possibility of polymeric chain degradation, we did not observe any differences between spectra acquired after heating the brominated compound (**2b**) at 65 °C for 2 h compared to 12 h. This led us to conclude that the cleavage of the C6—Br bond and concurrent release of the bromide ion into the reaction mixture, did not affect the stability of the dissolution-reaction product (**3**).

Importantly, while the direct-dissolution solvent is required to solubilize the cellulosic material, the dissolution occurs independently of the halide displacement reaction. This can be concluded from the fact that complete dissolution was typically achieved within 15 min at 65 °C, whereas the conversion from the halide to acetate required up to 72 h for the chlorinated material.

The results of the kinetic studies, coupled with the ^{13}C NMR data presented in Figure 1c, support our hypothesis regarding the quantitivity of the halide displacement by the acetate—thus, the DS_6 by the acetyl group in (**3**) is equal to the DS_6 by chlorine (**2a**) or bromine (**2b**). Furthermore, as an added benefit, the significantly lower reactivity of (**2a**) in S_N2-type transformations also enabled rapid estimation of DS_6 by the iodine in compound (**2c**), which was obtained from (**2a**), following a heterogeneous synthesis described by Ishii.[5] In that work, it is reported that the transformation of (**2a**) to (**2c**) results in mixed 6-chloro-*co*-6-iodo-6-deoxycelluloses, with DS_6 by iodine being influenced by both solvent selection and reaction time. In the referenced work, the DS_6 calculations were based on relative peak ratios of glucose, 6-chloro-6-deoxyglucose, and 6-iodo-6-deoxyglucose in the GC-MS analysis, a procedure that is labor-intensive and requires hydrolysis, further derivatization, and the preparation of standards. In this context, our approach using the IL-based electrolyte combined with solution-state NMR measurements appears to be more efficient—we were able to calculate the DS_6 by iodine by limiting the time of dissolution-reaction and performing ^{1}H NMR analysis immediately after full solubilization of (**2c**). The subsequent NMR analysis showed a rapid displacement of the iodine group occurring in 15 min at 65 °C (the total approximate duration of the experiment), resulting in a DS_6 value of 0.39. Granted, minor displacement of the chlorine moiety within that time is likely, but not to a significant degree, based on the results of the kinetics of chloride displacement (Figure 4). DS_6 by residual C6—Cl in (**2c**) was calculated by factoring in DS_6 of the starting material (**2a**, DS_6 0.85). This approach allowed us to estimate the distribution pattern in the mixed 6-chloro-*co*-6-iodo-6-deoxycellulose (DS_{Cl} 0.46, DS_I 0.39) directly from ^{1}H NMR data.

We need to acknowledge, however, that the values for DS_{Cl} and DS_1 are only estimates. This is because in the ^{13}C NMR spectrum of (**2c**) (Figure S6, Supporting Information), a prominent C5 peak of 5,6-cellulosene (**4**) was detected. It is unclear at this point if this is caused by direct elimination of the hydrogen halide from (**2c**) or whether the formation of (**4**) occurs as a result of a different reaction mechanism.

2.4. Detailed Structural Characterization of 6-Halo-6-Deoxycelluloses by 2D NMR

In order to further substantiate our claims regarding the quantitivity of the conversion of 6-halo-6-deoxycelluloses (**2a–c**) to (**3**), we utilized multiplicity-edited ^{1}H-$^{13}C\{^{1}H\}$ heteronuclear single quantum coherence (HSQC) NMR spectroscopy. The 2D NMR experiments allowed us to follow geminal resonances in the ^{13}C spectral regions typical for C6—Cl, C6—Br, C6—OAc, and C6—OH (44, 32, 63, and 60 ppm, respectively) (**Figure 5**), with improved sensitivity compared to ^{13}C NMR.

For the least reactive 6-chloro-6-deoxycellulose (**2a**, DS 0.85), the residual geminal signal attributable to C6—Cl at 44 ppm was still prominent after heating the sample at 65 °C for 24 h (Figure 5a), whilst the spectrum acquired after 48 h showed traces of the unreacted material only upon significant magnification (Figure 5b). On the other hand, in the case of fully substituted 6-bromo-6-deoxycellulose (**2b**, DS 0.99), only the geminal pair in the region characteristic for (**3**) was detected, without any residual C6-Br resonances at 32 ppm (Figure 5c). Finally, for the partially substituted 6-bromo-6-deoxycellulose (**2b'**, DS 0.15), two pairs of geminal C6 signals were present at 63 and 60 ppm (Figure 5d). In all the datasets, there was no evidence of 2,3-acetylated cellulose backbone positions, based on the assignments made by Kono et al.[40] Specifically, the two resonances attributable to positions 2 and 3 and were consistent with the chemical shifts reported for unsubstituted cellulose in $[P_{4444}][OAc]$:DMSO-d_6 (1:4 wt%).[34] Thus, our results demonstrate that the displacement of the halide attached to C6 by the acetyl moiety coming from the IL electrolyte is indeed quantitative under standardized dissolution conditions.

The 2D NMR experiments performed for the 6-chloro-*co*-6-iodo-6-deoxycellulose (**2c**) allowed us to identify the residual 6-chloro species that did not undergo exchange within the minimal time required for complete sample solubilization and acquisition of NMR data (Figure S10a, Supporting Information). Conversely, NMR analysis of the product dissolved in DMSO-d_6 revealed a distinctive geminal signal of the 6-iodo species present at 7.6 ppm in the ^{13}C spectral dimension (Figure S10b, Supporting Information). These resonances were absent from the spectra acquired in the IL electrolyte, thus providing further evidence for the rapid displacement of the iodine moiety. The mixed copolymeric sample dissolved in DMSO-d_6 also showed prominent geminal signals for unsubstituted cellulose, consistent with Kono et al.,[40] indicating that the 6-halo moiety (most likely the iodide, as a better leaving group) is labile under the applied dissolution conditions (80 °C, 48 h).

By using 2D NMR methods, we also attempted to explain the presence of several minor peaks downfield of the cellulose backbone region, clearly discernible in the diffusion-edited ^{1}H NMR data. These signals proved difficult to assign due to low signal intensity in the two-dimensional NMR data, but we tentatively attributed these resonances to unknown acetals formed during the dissolution-reaction. Speculative mechanisms for their formation could be: a) halide-catalyzed aerial oxidation of 6-OH to 6-aldehyde, b) Kornblum oxidation with DMSO-d_6 as oxidant,[41] or c) formation of 5,6-cellulosene (**4**) by elimination reaction under basic conditions,[5,38] as presented earlier in Scheme 2. The first mechanism was tested by the inclusion of $Na_2S_2O_3$ or pyridine, as bromide/bromine scavenging agents, during the dissolution-reaction. This yielded no qualitative changes in the NMR spectra downfield of 4.65 ppm. The possibility of the Kornblum oxidation was ruled out by using DMF-d_7 as an alternative to DMSO-d_6. The acetal resonances downfield of the main cellulose backbone region were still present after dissolution in $[P_{4444}][OAc]$:DMF-d_7 (Figure S11, Supporting Information). The final option of a side reaction leading to the formation of 5,6-cellulosene (**4**), postulated earlier in the discussion on the ^{13}C NMR data, does seem to account for the presence of additional peaks in the acetal region. Ishii[5] characterized the acetylated 5,6-cellulosene by ^{13}C NMR, which identifies the C5 alkene position at 151 ppm in DMSO-d_6. This assignment was consistent with theoretical DFT calculations (for full theoretical ^{1}H and ^{13}C assignments for 5,6-cellulosene (**4**), see Table S1, Supporting Information). Likewise, from heteronuclear multiple bond correlation (HMBC) NMR acquisition performed on (**2b**), we observed correlations of C5 atoms that correspond to protons of CH_2-6 and CH-4 in the expected spectral regions (Figure S12, Supporting Information). The formation of (**4**) would automatically add an additional H1 acetal peak in the acetal region, as the elimination to 5,6-cellulosene would preserve the glycosidic linkage. This additional H1 resonance was tentatively assigned at 4.77 and 100.6 ppm for ^{1}H and ^{13}C spectral dimensions, respectively.

2.5. Comparison of DS_6 Obtained from ^{1}H and ^{13}C NMR-Based Calculations

The DS_6 values based on ^{1}H NMR spectral data can be cross-validated with ^{13}C NMR spectra, provided that all data are acquired under quantitative conditions. This is possible, as the ^{13}C NMR-based calculations utilize two well-separated C6 signals at 60 and 63 ppm, which correspond to the unsubstituted and 6-monoacetylated AGU, respectively. The most suitable materials for this type of cross-validation are samples with DS_6 between ≈0.1 and 0.9, as this ensures that both signals are clearly detectable in the ^{13}C NMR spectra. Therefore, for the purpose of cross-validation, we prepared partially substituted 6-bromo-6-deoxycelluloses, dissolved the material in the IL electrolyte under standardized conditions, and performed quantitative ^{1}H and ^{13}C NMR experiments. In order to ensure the quantitivity of the ^{13}C NMR data, we had also determined the ^{13}C spin–lattice relaxation times (T_1) for the cellulose backbone C1–C6 (Table S2, Supporting Information).

The results of the DS_6 obtained for the two partially C6-substituted brominated celluloses are presented in **Table 3**. The obtained integrals for the C6-OAc peak at 63.2 ppm were 0.12 and 0.77 (Figure S14, Supporting Information), which were in agreement with the values obtained from quantitative ^{1}H NMR (DS_6

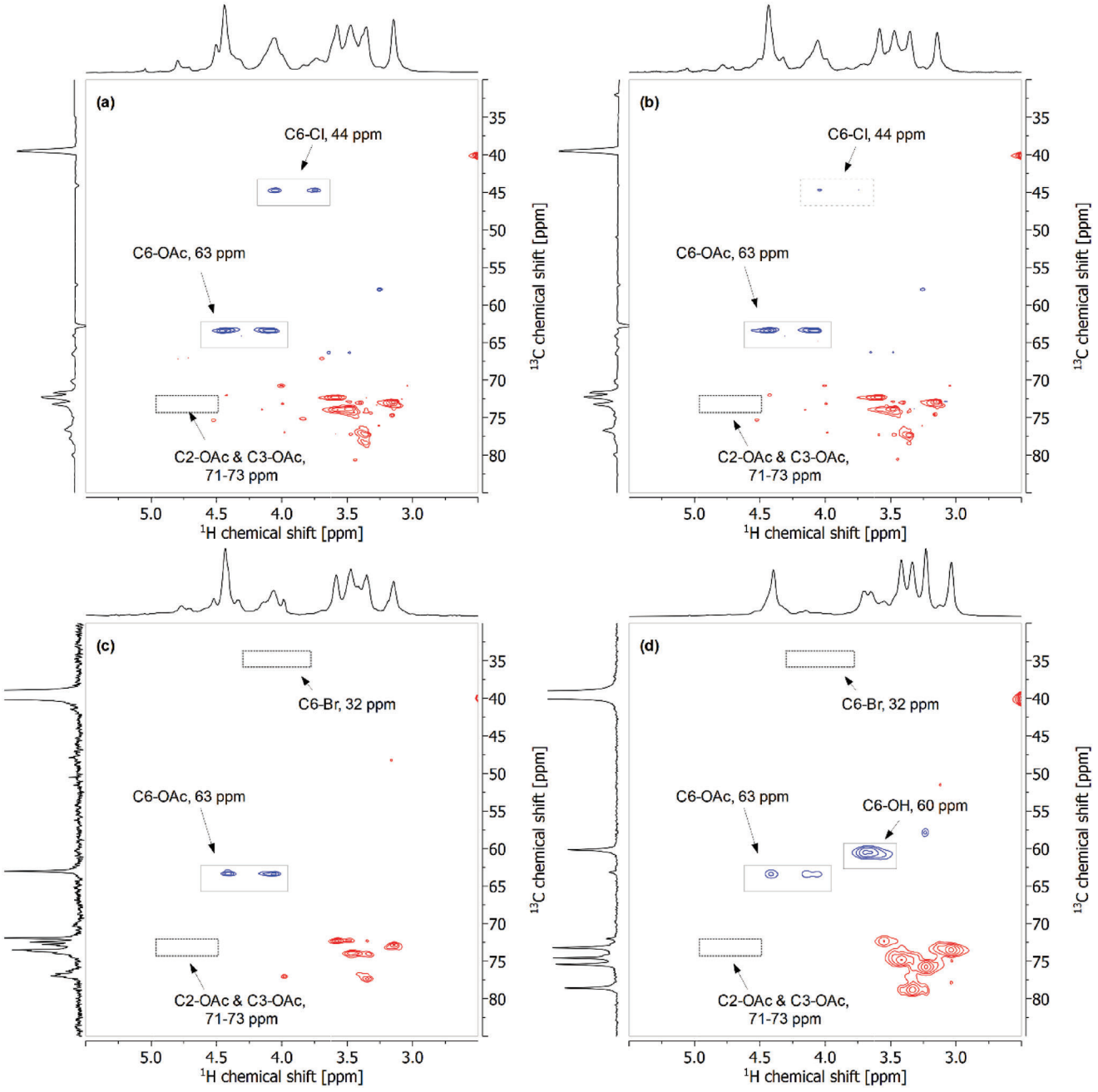


Figure 5. Multiplicity-edited ^{1}H-^{13}C{^{1}H} HSQC spectra of 6-halo-6-deoxycelluloses dissolved at 65 °C in [P_{4444}][OAc]:DMSO-d_6: a) 6-chloro-6-deoxycellulose, DS 0.85, 24 h; b) 6-chloro-6-deoxycellulose, DS 0.85, 48 h; c) 6-bromo-6-deoxycellulose, DS 0.99, 2 h; and d) 6-bromo-6-deoxycellulose, DS 0.15, 2 h.

0.15 and 0.72). The total DS values determined from the halogen content were also consistent with the NMR-based calculations.

By utilizing the ^{13}C NMR measurements, we were also able to quantitatively assess the formation of 5,6-cellulosene (**4**) (Scheme 2, elimination). This was not possible from ^{1}H NMR spectral data due to the fact that the proton signals of (**4**), as calculated for the DFT model (Table S1 and Figure S13, Supporting Information), are overlapped by the resonances of the cellulose backbone. Conversely, in the ^{13}C NMR spectrum, the C5 signal of (**4**) was clearly discernible at 153.4 ppm, but only for the brominated material with the high DS_6 (Figure S14, Supporting Information). The peak integral at 153.4 ppm was relatively small, although it may contribute to the overall dissolution-reaction yield by ≈9% for the highly substituted bromocelluloses. Nonetheless, we concluded that, from the point of view of quantitative analysis, ^{1}H NMR-based calculations give accurate DS_6, at the same time offering a significant advantage in terms of acquisition time and spectral quality over quantitative-mode ^{13}C NMR.

Table 3. DS_6 of two partially brominated cellulose samples (**2b′**) and (**2b″**), based on quantitative ^1H and ^{13}C NMR data.

Entry	DS_6 based on ^1H NMR	DS_6 based on ^{13}C NMR	DS based on Br%[a)]
2b′	0.15	0.12	0.15
2b″	0.72	0.77	0.80

[a)] Total DS values calculated from the bromine content.

This is important especially when high-field instruments or more suitable probes, e.g., cryogenically cooled or broad-band channel optimized probes, are not accessible.[28]

2.6. Comparison of DS_6 Determined by Two Alternative NMR-Based Protocols

2.6.1. Evaluation of ^{31}P-Labeling as an Alternative Method for Determining DS_6

The phosphitylation method, or ^{31}P-labeling, is a derivatization protocol based on integral analysis of ^{31}P NMR data in spectral regions corresponding to an internal standard and a ^{31}P-labeled cellulose derivative, obtained by reacting the polymeric analyte with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (2-Cl-TMDP).[29,42] The ^{31}P-based method allows in situ derivatization and analysis. However, it remains limited to chloroform- or chloroform/pyridine-soluble samples. The long-term stability of the phosphitylated *N*-hydroxy-5-norbornene-2,3-dicarboximide (*e*-HNDI), used as the internal standard, can also be problematic and lead to inconsistent DS values.[31]

The aforementioned limitations make the phosphitylation method unsuitable for the determination of DS_6 of the halogenated cellulose samples. In particular, the representative sample of 6-bromo-6-deoxycellulose (**2b**) remained insoluble in hot pyridine, and the subsequent ^{31}P-labeling proceeded heterogeneously. Consequently, although significant incorporation of TMDP moieties could be assumed under heterogeneous conditions,[42] the actual DS was not determinable by solution-state ^{31}P NMR—the value obtained by this method (0.28) was higher than the total DS determined from the bromine content (0.15). Significant variation of the integral peak areas over time was also apparent (Table S3, Supporting Information), occurring either due to the instability of the internal standard or the analyte itself during the NMR data acquisition. Consequently, we were unable to obtain consistent DS_{31P} values from consecutive measurements.

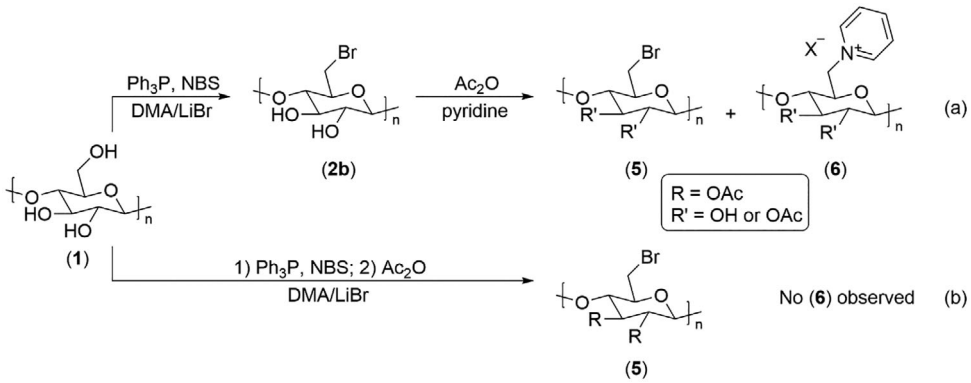
2.6.2. Evaluation of the Peracetylation Protocol for Determining DS_6

The peracetylation approach has been previously proposed as a method for calculating DS of several polysaccharide derivatives directly from ^1H NMR.[43–45] The derivatization protocol involves the functionalization of all unreacted hydroxy groups along the cellulose backbone with acetic anhydride, in the presence of pyridine, to form acetate esters, thus enhancing the solubility of the product in common perdeuterated solvents, such as DMSO-d_6 or $CDCl_3$. Provided that the conversion of the unreacted hydroxy groups is quantitative, it is possible to obtain partial DS values of peracetylated cellulose derivatives at positions C2, C3 and C6 from the respective carbonyl signals present in ^{13}C NMR spectra, typically at 170–168 ppm.[46] Quantitative-mode ^{13}C NMR acquisitions for peracetylated samples are nonetheless time-consuming, requiring up to 20 000 scans for adequate signal-to-noise ratio that could guarantee quantitative results.[24] Still, the peracetylation method seems particularly suitable for structural elucidation of cellulose derivatives with aromatic substituents. In these instances, quantitative conversion of the hydroxyl moieties is not a prerequisite, as the signals of the aromatic ring, which appear downfield from the cellulose backbone region, can be readily utilized for integration and subsequent calculation of the total DS directly from ^1H NMR. This can significantly shorten the NMR data collection time from several hours required by quantitative-mode ^{13}C NMR to several minutes, which is sufficient for acquiring quantitative ^1H NMR spectra. Furthermore, on the assumption that the peracetylation is quantitative, ^1H NMR spectra of the derivatized samples can also be utilized to obtain partial DS values by deconvolution of the well resolved methyl proton signals associated with 2-OAc, 3-OAc, and 6-OAc, as demonstrated recently by Elschner et al.[43]

Naturally, in the case of 6-halo-6-deoxycelluloses, it is necessary to ensure complete functionalization of the remaining hydroxy functionalities in positions 2, 3, and 6. This is because the DS by the acetyl functionality (DS_{Ac}), obtained from the ^1H NMR data, is used to back-calculate the DS by the halogen (DS_X) by subtracting DS_{Ac} from the maximum total DS of 3. One additional assumption must be that the C6—X bond is not (partially) cleaved—an assumption which is problematic, considering that certain cellulose derivatives are reported to be unstable under the peracetylation conditions.[44]

Despite the abovementioned limitations of the method, we attempted to apply peracetylation by suspending (**2b**) in pyridine and adding acetic anhydride, following the heterogeneous route described by Elschner et al.[43] Subsequent isolation and purification of the (per)acetylated 6-bromo-6-deoxycellulose (**5**) allowed us to obtain material only partially soluble in DMSO-d_6 and $CDCl_3$.The DS_6 obtained from ^1H NMR of the DMSO-soluble fraction of (**5**) (Figure S16, Supporting Information) was significantly higher than the DS_6 of the sample before (per)acetylation, as calculated from the ^1H NMR of (**3**), with the exact DS_6 values of 0.62 and 0.15, respectively.

In order to understand why the (per)acetylation results in apparently overestimated DS_6, we considered the possibility of a side reaction that had occurred between the brominated compound (**2b**) and pyridine, leading to the partial formation of the pyridinium salt (**6**), consistent with previous studies reporting on the displacement of bromide by pyridine.[47,48] This hypothesis was confirmed by the diffusion-edited ^1H NMR spectrum, where we observed the retention of pyridinium resonances (Figure S17, Supporting Information). The C6 resonance of the pyridinium moiety was also observed in the ^{13}C NMR data at 126.7 ppm, whereas the expected C6-OAc signal was not present (Figure S18, Supporting Information). The assignment for the 6-pyridinium was confirmed by the fact that a new geminal CH_2 signal appeared in the multiplicity-edited HSQC spectrum (Figure S19, Supporting Information). If not all hydroxy groups of (**2b**) had been converted to the acetyl due to the side reaction with pyridine, the DS_6 value would be artificially overestimated



Scheme 3. a) Stepwise and b) in situ bromination and peracetylation of cellulose (**1**).

upon back-calculation. This scenario, confirmed by the NMR data, indicates that the heterogeneous (per)acetylation in pyridine cannot accurately determine DS_6 for this class of halogenated celluloses.

The quantitivity of the heterogeneous peracetylation could be further questioned on the grounds that, at least in our experience, only homogeneous reactions enable complete functionalization and uniform distribution of substituents along the polymer chain. Conversely, the homogeneous route in DMA/LiBr, leading to 6-bromo-6-deoxy-2,3-di-*O*-acetylcellulose (**5**) by a one-pot regioselective bromination and in situ acetylation[6] (**Scheme 3b**), presented an opportunity to reevaluate the quantitivity of the peracetylation reaction. For this purpose, we carried out two parallel reactions under the same bromination conditions, one involving the isolation of (**2b**), and the other involving in situ peracetylation followed by isolation and purification of (**5**). The two compounds (**2b**) and (**5**), obtained after isolative treatment, were dissolved in the IL electrolyte and DMSO-d_6, respectively, and their ^{1}H and ^{13}C NMR spectra were compared.

The subsequent calculations of DS_6, performed separately for (**2b**) using the dissolution-reaction in the IL electrolyte, and for (**5**) obtained by in situ bromination-acetylation (Figures S20 and S21, Supporting Information), gave consistent values [**Table 4**, entries 1–2 for (**2b**) and 6–7 for (**5**)]. Thus, the peracetylation reaction should be performed in an inert direct-dissolution cellulose solvent (e.g., DMA/LiBr).

3. Conclusion

We have developed a new approach toward detailed structural characterization of 6-halo-6-deoxycelluloses by solution-state NMR, utilizing the IL electrolyte [P_{4444}][OAc]:DMSO-d_6. High-resolution NMR measurements are possible in situ as a result of a direct-dissolution process, in which the insoluble 6-halo-6-deoxycelluloses are quantitively converted into soluble 6-monoacetylcellulose by the nucleophilic substitution reaction mechanism.

Table 4. DS_6 of a representative sample of 6-bromo-6-deoxycellulose (2b), as determined by different NMR-based methods.

Entry	Method	Integrated signals	Integrated regions [ppm]	Calculation	DS_6
1	^{1}H NMR after dissolution-reaction in [P_{4444}][OAc]:DMSO-d_6	Acetyl vs cellulose backbone	2.1–1.9 vs 5.0–2.8	$DS_{Ac} = (I_{Ac}/3)/(I_{Cell}/7) = DS_6$	0.15
2	^{13}C NMR after dissolution-reaction in [P_{4444}][OAc]:DMSO-d_6	C6 of 6-monoacetylcellulose vs C6 of unmodified cellulose	63.5–62.5 vs 61–59	$DS_{Ac} = (I_{C6\text{-}Ac})/(I_{C6\text{-}Ac} + I_{C6\text{-}OH}) = DS_6$	0.12
3	^{31}P NMR after phosphitylation	Internal standard vs alkoxy-TMDP	152.3–151.7 vs 151.3–142.0	Equations S1 and S2, Supporting Information	0.28
4	^{1}H NMR after heterogeneous acetylation	Acetyl vs cellulose backbone	2.3–1.7 vs 5.6–3.3	$DS_{Ac} = (I_{Ac}/3)/(I_{Cell}/7)$ $DS_6 = 3 - DS_{Ac}$	0.62
5	^{13}C NMR after heterogeneous acetylation	C6-Br vs C6-OH	32 vs 61–59	$DS_{Ac} = (I_{Ac}/3)/(I_{Cell}/7)$ $DS_6 = 3 - DS_{Ac}$	n.d.
6	^{1}H NMR after one-pot bromination and acetylation	Acetyl vs cellulose backbone	2.3–1.7 vs 5.6–3.3	$DS_{Ac} = (I_{Ac}/3)/(I_{Cell}/7)$ $DS_6 = 3 - DS_{Ac}$	0.19
7	^{13}C NMR after one-pot bromination and acetylation	C6-Br vs C6-OH	32 vs 61–59	$DS_{Ac} = (I_{Ac}/3)/(I_{Cell}/7)$ $DS_6 = 3 - DS_{Ac}$	0.15
8	Schöniger method[a)]	–	–	$DS_{Br} = (Br\%/100\% * M_{AGU})/(M_{Br} - Br\%/100\% * A_{Br})$	0.15

[a)] The total DS value, determined from the bromine content, is provided for cross-validation with a non-NMR method; n.d., not determined due to the absence of the reference C6-OH signal.

The quantitivity of the dissolution-reaction was demonstrated by kinetic studies using diffusion-edited ^{1}H NMR, and the results were further corroborated by 2D NMR analyses. The quantitative ^{1}H NMR experiments enabled direct calculations of site-specific degrees of substitution at carbon C6 (DS_6). The values obtained from ^{1}H NMR acquisitions were cross-validated against ^{13}C NMR data, which also allowed to quantify the formation of 5,6-cellulosene as a side-product of the competing elimination reaction. The NMR-based values were consistent with the calculations based on halogen content, chosen as an independent method for determining the total DS. Concurrently, the regioselectivity of the halogenation reaction was successfully demonstrated.

The dissolution-reaction approach utilizing the IL electrolyte was compared with the existing alternative derivatization methods, i.e., ^{31}P-labeling and two (per)acetylation protocols. In comparison, the strategy presented herein is experimentally faster and easier for quantitative analysis of structural features of 6-halo-6-deoxycelluloses, including accurate determination of DS_6 from ^{1}H NMR experiments performed in situ, without isolation and purification of the derivatized products. The dissolution-reaction in the IL electrolyte also allows to quickly estimate distribution patterns in mixed 6-halo-6-deoxycelluloses by leveraging the intrinsic differences in the reactivities of halogens.

The presented strategy adds to the currently available analytical toolbox of cellulose chemistry. The approach is particularly suitable for rapid NMR-guided analysis of halogenated cellulose derivatives, which are otherwise insoluble in common perdeuterated solvents.

4. Experimental Section

Materials: Microcrystalline cellulose (MCC, Avicel PH-101), anhydrous lithium chloride (LiCl, ≥99%), anhydrous lithium bromide (LiBr, ≥99%), triphenylphosphine (Ph_3P, 99%), *N*-bromosuccinimide (NBS, ≥99%), bromotrichloromethane ($BrCCl_3$, 99%), imidazole (99%), iodine (I_2, ≥99%), and anhydrous *N*,*N*-dimethylacetamide (DMA, 99.8%) were purchased from Sigma-Aldrich (Finland); anhydrous sodium thiosulfate (98.5%) was acquired from Thermo Scientific; acetonylacetone (>97%) was obtained from TCI; sodium iodide (NaI, ≥99%), pyridine (≥99.7%), and acetic anhydride (99.7%) were purchased from VWR (Finland).

MCC was dried under vacuum at 40 °C for 24 h. LiBr and LiCl were dried under vacuum at 130 °C. DMA and pyridine were stored over 4 Å molecular sieves until use. All other reagents and solvents were used as received.

Measurements: NMR spectra were typically recorded at 65 °C on a Bruker Avance NEO 600 spectrometer (600 MHz ^{1}H-frequency) equipped with 5 mm double resonance (X,^{1}H) broadband probehead (SmartProbe) equipped with Z-gradient coil capable of delivering gradient amplitudes up to 50.1 G cm^{-1}. ^{31}P{^{1}H} NMR spectra were recorded at 25 °C using a Bruker Avance NEO 500 spectrometer (500 MHz ^{1}H-frequency) equipped with 5 mm triple resonance (^{1}H, ^{13}C, X) inverse-detection broadband probehead equipped with triple axis gradient coils capable of delivering X-, Y-, and Z-gradient amplitudes up to 50, 50, and 67 G cm^{-1}, respectively. Details on NMR sample preparation and pulse programs used are given in the Supporting Information.

Determination of the halogen content was performed using the Schöniger oxygen-flask combustion method for the decomposition of the samples and subsequent titration on a Mettler Toledo T50 Titrator.

Chlorination of Cellulose (Scheme 1A): MCC (1.0 g, 6.2 mmol AGU) was suspended in DMA (30 mL) at 120 °C for 2 h under a flow of argon. The temperature was lowered to 90 °C and LiCl (2.8 g) was added. Stirring was continued until all LiCl had dissolved, and the reaction mixture was cooled to room temperature, yielding a clear, viscous, slightly yellow solution within ≈16 h. Subsequently, Ph_3P (3.8 g, 2.0 eq. AGU) dissolved in DMA (10 mL) was added, and the reaction mixture was cooled in an ice bath, followed by dropwise addition of $BrCCl_3$ (1.4 mL, 2.0 eq. AGU). The ice bath was then removed and stirring was continued at room temperature for 36 h. The solution remained clear and homogeneous, and no significant change in color was observed throughout the course of the reaction. The product was precipitated from acetonitrile (500 mL), filtered, stirred in distilled water for 24 h, filtered, stirred in methanol for 72 h, filtered, and finally dried in vacuo to yield 6-chloro-6-deoxycellulose (**2a**) as a white powder. Yield: 0.713 g.

Bromination of Cellulose (Scheme 1B): 6-Bromo-6-deoxycellulose was prepared according to a published procedure.[1] In brief, MCC (1.0 g, 6.2 mmol AGU) was suspended in DMA (60 mL) at 130 °C under a flow of argon. After 2 h, LiBr (14 g) was added at 100 °C, and stirring was continued at that temperature for another 30 min. The heating was switched off, and MCC dissolved completely within ≈16 h. Subsequently, Ph_3P (4.1 g, 2.5 eq. AGU) dissolved in DMA (20 mL) was added, and the reaction mixture was placed in an ice bath. NBS (2.8 g, 2.5 eq. AGU) dissolved in DMA (15 mL) was added dropwise, and additional DMA was added to the total volume of 120 mL. The reaction mixture was kept at 70 °C for 2 h and was then poured into acetonitrile (500 mL), filtered, stirred in acetone for 16 h, filtered, washed with distilled water, stirred in aqueous $NaHCO_3$ (70 mM) for 48 h, filtered, and finally washed with copious amounts of water and dried in vacuo to yield 6-bromo-6-deoxycellulose (**2b**) as a slightly brown powder. Yield: 1.131 g.

Iodination of Cellulose under Homogeneous Conditions (Scheme 1C): MCC (0.10 g, 0.617 mmol AGU) was dissolved in DMA/LiCl, as described for the chlorination of cellulose. The MCC solution was cooled in an ice bath and Ph_3P (0.32 g, 2.0 eq. AGU), imidazole (0.19 g, 4.5 eq. AGU), and I_2 (0.32 g, 2.0 eq. AGU) were added as solutions in DMA (the total volume was 50 mL). The reaction mixture remained clear but turned slightly green when I_2 was added; the color disappeared within minutes. The reaction mixture was kept at 70 °C for 3 h, whereafter the mixture was poured into acetone (400 mL). The work-up was the same as for the chlorination of cellulose. Yield: 80 mg.

Iodination of Cellulose under Heterogeneous Conditions (Scheme 1D): 6-Iodo-6-deoxycellulose (**2c**) was synthesized as previously reported by Ishii.[5] A 100 mL round bottom flask was charged with NaI (747 mg, 4.98 mmol) and acetonylacetone (15 mL). 6-Chloro-6-deoxycellulose (**2a**, 300 mg, 1.66 mmol) was added to the suspension and the reaction mixture was kept at 120 °C for 5 h. After that, the crude heterogeneous reaction mixture was poured over ice-cold water. The cellulosic material was filtered off, washed with water, resuspended in water, and further treated with sodium thiosulfate solution (50 mL, 0.10 M) for 1 h. The cellulosic solids were filtered off, washed with water and suspended again in water overnight before final filtration and washing. The obtained product was dried in vacuo until constant weight to yield 366 mg of (**2c**) as a white powder.

Kinetic Studies: 6-Halo-6-deoxycellulose (50 mg) was placed in a 4 mL glass vial, to which a stock solution of $[P_{4444}][OAc]$ in DMSO-d_6 (1:4 wt%) was added (950 mg). The polymeric material was solubilized completely by stirring for ≈15 min at 65 °C in an oil bath. The mixture was subsequently transferred to an NMR tube and placed inside an NMR probe preheated to 65 °C or 75 °C. The diffusion-edited ^{1}H NMR spectra were acquired typically with 32 scans at 0.5, 1, 2, 4, 6, and 12 h for (**2a**) and (**2b**), and additionally at 24, 48, and 72 h for (**2a**).

Conversion of 6-Halo-6-Deoxycelluloses to 6-Monoacetylcellulose: In a typical procedure, dry 6-halo-6-deoxycellulose (50 mg) was added to a sample vial and made up to 1.00 g with a stock solution of $[P_{4444}][OAc]$ in DMSO-d_6 (1:4 wt%). The vial was placed in an oil bath at 65 °C, stirred for 48 h, and the solution was transferred to an NMR tube for analysis.

^{31}P-Labeling: The phosphitylation method was adapted from a previously published procedure.[29] In brief, 6-bromo-6-deoxycellulose (25 mg) was placed in 8 mL screw-top vial and pyridine (500 µL) was added. The mixture was vortexed until the sample was well dispersed. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (2-Cl-TMDP, 200 µL,

1.27 mmol) was added and the sample was stirred for ≈30 min at 80 °C. The internal standard solution (*endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (*e*-HNDI), 121.5 × 10^{-3} M in pyridine–$CDCl_3$ (3:2 v/v, 125 µL, 152 µmol)) and $Cr(acac)_3$ (1.0 mL, 80 × 10^{-3} M in $CDCl_3$) were subsequently added and the mixture was vortexed for ≈10 s. The ^{31}P NMR data were acquired immediately to minimize any error due to the instability of the internal standard.

Heterogeneous Conversion of 6-Bromo-6-Deoxycellulose to 6-Bromo-6-Deoxy-2,3-Di-O-Acetylcellulose: The peracetylation method was adapted from Elschner et al.[43] Acetic anhydride (5.0 mL) was added to a suspension of 6-bromo-6-deoxycellulose (**2b**, 300 mg) in pyridine (10 mL). The heterogeneous reaction mixture was stirred at 60 °C for 24 h. The reaction was allowed to cool to room temperature and the undissolved fraction was centrifuged off. The clear supernatant solution was poured into aqueous $NaHCO_3$ (150 mL, 40 × 10^{-3} M), and the precipitated product was collected by filtration and washed three times with distilled water (3 × 50 mL). The purified product was dried under vacuum for 16 h to yield 101 mg as a brown powder.

Homogeneous Conversion of 6-Bromo-6-Deoxycellulose to 6-Bromo-6-Deoxy-2,3-Di-O-Acetylcellulose by In Situ Peracetylation: The one-pot bromination-peracetylation reaction was performed as described by Fox and Edgar.[6] MCC (1.0 g, 6.2 mmol AGU) was suspended in DMA (60 mL) under magnetic stirring, flushed with argon, and heated to 160 °C under continuous argon flow. The suspension was stirred at 160 °C for 1 h. The temperature was lowered to 90 °C, and LiBr (9.0 g) was added to the reaction flask. The heating was switched off, and a clear solution was obtained within 16 h. Ph_3P (6.4 g, 4.0 eq. AGU) dissolved in DMA (20 mL) was added, and the reaction mixture was cooled to 0 °C. NBS (4.4 g, 4.0 eq. AGU) dissolved in DMA (20 mL) was added dropwise through an addition funnel. The reaction mixture was heated to 70 °C and stirred at that temperature for 1 h. The in situ peracetylation of the unreacted hydroxy groups was performed by dropwise addition of acetic anhydride (2.9 mL, 5.0 eq. AGU), followed by continued stirring at 70 °C for 16 h. The reaction was quenched by allowing it to cool to room temperature and pouring into a mixture of methanol and deionized water (1:1 v/v, 800 mL). The precipitated product was recovered by filtration, dissolved in acetone, concentrated under reduced pressure, and reprecipitated from ethanol. The purified product was dried under vacuum for 16 h to yield 1.59 g of a white powder.

Solubility Tests: Solubility was tested by stirring 10 mg of sample material in 1.0 mL of solvent, initially at room temperature for 24 h, then at elevated temperature (80 °C or close to bp for low-boiling point solvents) for up to 48 h.

Determination of DS_6 from Quantitative ^1H NMR Data: The degree of substitution at position 6 (DS_6) was calculated from the following formula

$$DS_6 = (I_{Ac}/3) / (I_{Cell}/7) \quad (1)$$

where I_{Ac} is the peak area of the acetate signal (≈1.9–2.1 ppm); I_{Cell} is the peak area of the cellulose backbone signal (≈2.8–5.0 ppm); the integers 3 and 7 correspond to the number of protons present in the acetate moiety and the cellulose backbone, respectively.

The DS_6 can be readily calculated from the ^1H NMR spectrum, after phase correction and baseline correction; however, for accurate results, it is recommended to apply peak fitting and deconvolution in order to exclude possible contribution of signals from residual water or other impurities. The deconvolution of two brominated cellulose samples (**2b′**) and (**2b″**) using the general-purpose peak fitting program Fityk[49] is presented as examples in Figures S22 and S23, Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords

6-halo-6-deoxycelluloses, cellulose, degree of substitution, nuclear magnetic resonance, regioselective modification of cellulose

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