Bottom-up chemoenzymatic synthesis towards novel fluorinated cellulose-like materials

Peterson de Andrade¹‡, Juan C. Muñoz-García², Giulia Pergolizzi¹, Valeria Gabrielli², Sergey A. Nepogodiev¹, Dinu Iuga³, László Fábián,², Rinat Nigmatullin⁴, Marcus A. Johns⁴, Robert Harniman⁵, Stephen J. Eichhorn⁴, Jesús Angulo², Yaroslav Z. Khimyak*², Robert A. Field*¹,⁶

¹Department of Biological Chemistry, John Innes Centre, Norwich NR4 7UH, UK
²School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK
³Department of Physics, University of Warwick, Coventry CV4 7AL, UK
⁴Bristol Composites Institute, CAME School of Engineering, University of Bristol, Bristol BS8 1TR, UK
⁵School of Chemistry, University of Bristol, Bristol BS8 1TS, UK
⁶Present address: Department of Chemistry and Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN, UK

‡ Equal contribution

*Corresponding authors e-mail address: rob.field@jic.ac.uk; robert.field@manchester.ac.uk; y.khimyak@uea.ac.uk

Abstract

Understanding the fine details of self-assembly of building blocks into complex hierarchical structures represents a major challenge en route to the design and preparation of soft matter materials with specific properties. Enzymatically-synthesised cellodextrins are known to have limited water solubility beyond DP9, a point at which they self-assemble into particles resembling the anti-parallel cellulose II crystalline packing. We have prepared and characterized a series of site-selectively fluorinated cellodextrins of different degrees of fluorination and substitution patterns by chemoenzymatic synthesis. The structural characterization of these materials at different length scales, combining advanced NMR and microscopy methods, showed that multiply 6-fluorinated cellodextrin chains assembled into particles presenting morphological and crystallinity features that are unprecedented for cellulose-like materials. In contrast, the introduction of a single fluorine atom per cellodextrin chain had a minor impact on materials structure. Our work emphasizes the strength of combining chemoenzymatic synthesis, fluorinated building blocks and advanced NMR and microscopy methods for the thorough characterization of hierarchical structures, leading to the controlled design of new biomaterials with specific properties.
Introduction

Cellulose is an abundant natural biopolymer used extensively in industry as raw material for the production of paper, textile, food thickeners, explosives, etc.\textsuperscript{1,2} The contemporary use of cellulose is based on nanosized cellulose particles (nanocellulose), which is a promising class of renewable material due to its intrinsic characteristics and potential for broad advanced industrial applications.\textsuperscript{3–5} Hence, the design of nanocellulose-based soft-matter materials relies on the formation of assembly-driven structures, and may prompt to changes in mechanical properties or bring additional functionality.\textsuperscript{6–10}

Production of cellulose nanocrystals and nanofibrillated cellulose, the main classes of nanocellulose, are both top-down methodologies, based on the isolation of nanocellulose from cellulose biomass, which requires high energy consumption.\textsuperscript{5} Moreover, functionalization of nanocellulose to meet requirements for specific applications often requires harsh chemical conditions (i.e. strong acids and bases). As an alternative, significant progress has been made in the synthesis of nanocellulosic materials based on a bottom-up approach via enzymatic polymerization.\textsuperscript{11–13} This approach is highly flexible and is projected to enable the generation of tailor-made functionalized nanocellulosic materials, by opening up the possibility to direct the nanoarchitecture and properties of cellulose oligomers.

Developing strategies to functionalize biocompatible materials giving rise to tuneable properties is becoming increasingly important to their integration into biomedical products.\textsuperscript{14,15,16} In this scenario, the site-selective introduction of probes into cellulose to report on local structure and to modulate material properties is of great importance. Fluorine is well-known for its unique physicochemical properties,\textsuperscript{17} which find widespread use in drugs\textsuperscript{18} and agrochemicals\textsuperscript{19} due to its remarkable impact on conformation, permeability, potency, metabolism, etc.\textsuperscript{20,21} In addition, the absence of fluorine in biological systems makes the introduction of $^{19}$F nuclei a powerful reporter of local structure and environment in complex systems.\textsuperscript{22–29} Nonetheless, its use remains underexplored with respect to biomaterials.

Synthetic strategies for the regio- and stereoselective (deoxy)fluorination of monosaccharides have been successful.\textsuperscript{30–33} In contrast, the corresponding top-down derivatization of cellulose\textsuperscript{8,34} is complicated by solubility challenges, resulting in incomplete control of the sites and extent of fluorination.\textsuperscript{35} The bottom-up chemical synthesis of structurally defined cellodextrins, including 3-fluorinated compounds,\textsuperscript{32} has been achieved recently by automated glycan assembly.\textsuperscript{36} Enzymatic synthesis presents an alternative option to achieve the bottom-up preparation of functionalized oligo- and poly-saccharides\textsuperscript{37} in a regio- and stereo-controlled manner.\textsuperscript{38,39} For instance, glycoside phosphorylases (GPs)\textsuperscript{12,40–43} have recently received attention for the synthesis of amylose and cellulose-like materials. In particular, cellodextrin phosphorylase (CDP, EC 2.4.1.49) has emerged as a powerful tool for the synthesis of differently functionalized cellulose oligomers giving rise to a variety of nanostructures (sheets,\textsuperscript{11,44,45} rods,\textsuperscript{46} or ribbons\textsuperscript{47}) depending on the nature of the substrate.\textsuperscript{11,48–52}

Herein, we have prepared enzymatically produced cellodextrins (EpCs) with different fluorination patterns. Monofluorinated EpCs (2F-, 3F- and 6F-EpC) were obtained by CDP-mediated oligomerisation of α-D-glucose1-phosphate (Glc-1P) as donor and deoxy-fluoro-celluloses as acceptor substrates. Multiply 6-fluorinated EpC (multi-6F-EpC) was prepared from 6-deoxy-6-fluoro-α-D-glucose1-phosphate (6F-Glc-1P) and cellobiose as donor and acceptor substrates, respectively.
The thorough structural characterization of these biomaterials has been performed by a combination electron microscopy, X-ray diffraction, Raman spectroscopy and advanced Nuclear Magnetic Resonance experiments. The presence of a single fluorine atom per cellodextrin chain did not exert a substantial impact on the morphology and crystalline structure of the material. In contrast, the presence of multiple 6-deoxy-6-fluoro-glucose units yielded an unprecedented crystalline allomorph never reported before for a cellulose-like material, thus constituting a new long-range ordering motif for it. Our findings demonstrate how enzymatic catalysis from functionalized monosaccharide building blocks enables the generation of site-specific fluorinated cellulose oligomers with unique structural features, which may open up way for new a class of cellulose-like materials, and highlight the value of advanced NMR methods in detailing the molecular level characterization.

Results and discussion

Enzymatic synthesis of fluorinated cellodextrins

Synthesis of 2-, 3- and 6-monofluorinated cellodextrins (2F-EpC, 4; 3F-EpC, 5; and 6F-EpC, 6)

CDP uses glucose as an acceptor substrate only poorly compared to cellobiose and longer cello-oligosaccharides,\textsuperscript{11,52} to produce cellodextrins containing deoxy-fluoro-glucose at the reducing terminus (compounds 4-6). We therefore initially used cellobiose phosphorylase (CBP) (PRO-GH94-004) to synthesize monofluorinated cellobiose analogues (1-3) (Figure 1A) for use as acceptors for CDP (Figure 1B). CBP was incubated at 37 °C with 1:1 ratio of Glc-1P and deoxy-fluoro-glucose (2F-, 3F- or 6F-Glc) for 16 h, at which point point TLC showed ca. 80% conversion into the disaccharides 1 and 3, and ca. 60% into 2. The different conversion efficiencies may be rationalized based on a study of Cellulomoinas uda cellobiose phosphorylase,\textsuperscript{53} in which $k_{\text{cat}}/K_m$ values for 2F-Glc (2.4%), 3F-Glc (0.013%) and 6F-Glc (31%) acceptors are substantially lower than that of the parent Glc substrate, but all three compounds are indeed productive substrates. CBP was removed from reaction mixture by affinity chromatography (His6-tag nickel column purification) and the desired products were purified by gel filtration chromatography. The purification successfully removed residual deoxy-fluoro-glucose acceptors, but small amounts of cellobiose required removal by HPLC to obtain compounds 1-3 in high purity for characterization (†ESI Figures S1-S3). However, the monofluorinated cellobextrins 4-6 could be obtained in one-pot reactions from the respective sugar-1P and fluorinated gluoses.

Once Glc-1P consumption was almost complete in the CBP reactions, more Glc-1P (4 eq.) was added together with CDP and the reactions were incubated at 37 °C shaking for 16 h. A white precipitate was formed and isolated by centrifugation, followed by resuspension and washing with MQ water to remove enzyme, salts and any soluble sugar. Further 4 eq. of Glc-1P were added to the supernatant, and CDP reaction was further incubated to produce more fluorinated EpCs. In this manner, monofluorinated cellodextrins were obtained with reasonable overall yield based on consumed fluoro-glucose [47% (Glc-β-1,4)\textsubscript{3}F-Glc (4), 30% (Glc-β-1,4)\textsubscript{3}F-Glc (5), 32% (Glc-β-1,4)\textsubscript{6}F-Glc (6)]. MALDI-TOF mass spectrometry analysis showed these materials to have an average DP ca. 9, while the unsubstituted cellobextrin (EpC, 8) produced under the same reaction conditions averaged ca. DP 8 (†ESI Figure S7). Traces of longer monofluorinated cellobextrins were also observed. Solution-state $^{19}$F NMR analysis in 1 M NaOD (†ESI Figure S4) showed two singlets for each material,
reflecting reducing terminal anomers, with peaks at -195.21 and -195.26 ppm (2F-EpC, 4), -190.86 and -197.19 ppm (3F-EpC, 5) and -232.55 and -234.05 ppm (6F-EpC, 6).

**Figure 1. Enzymatic synthesis of fluorinated cellodextrins.** (A) Cellobiose phosphorylase (CBP) catalysed reaction of 6-D-glucose 1-phosphate (Glc-1P) and deoxy-fluoro-D-glucose (2F-Glc, 3F-Glc or 6F-Glc), followed by (B) cellodextrin phosphorylase (CDP) catalysed oligomerization with Glc-1P and monofluorinated cellobiose, to afford enzymatically-produced fluorinated cellodextrins (2F-EpC, 4; 3F-EpC, 5; and 6F-EpC, 6). (C) CDP-catalysed reaction of 6-deoxy-6-fluoro-α-D-glucose 1-phosphate (6F-Glc-1P) or Glc-1P and cellobiose as acceptor, to produce multiply 6-fluorinated cellodextrin (multi-6F-EpC, 7) or the parent Enzymatically produced Cellodextrin (EpC, 8), respectively.

**Synthesis of multiply 6-fluorinated cellodextrin (multi-6F-EpC, 7)**

We also investigated CDP-mediated oligomerization using the chemically modified glucosyl donor 6F-Glc-1P (Figures S5-S6) and cellobiose as acceptor (Figure 1C) to achieve higher structural impact by placing multiple fluorine atoms along the cellodextrin (multiply 6-fluorinated cellodextrin, multi-6F-EpC, 7). The initial tests using 6F-Glc as an acceptor to obtain a fully 6F-substituted cellodextrin proved very slow and inefficient. Alternatively, CBP was tested with 6F-Glc as an acceptor to generate a difluorinated cellobiose, which could be a better substrate for CDP. However, only trace amounts of the product were detected; prompting us to choose the natural acceptor cellobiose. CDP was incubated at 37 °C with a 6:1 ratio of 6F-Glc-1P and cellobiose for 72 h. The resulting precipitate was isolated by centrifugation, followed by re-suspension and washing with MilliQ water to give 7 with 64% yield. $^{19}$F solution-state NMR analysis of 7 dissolved in 1 M NaOD (†ESI Figure S4) showed one major singlet at -233.25 ppm, which may correspond to fluorine from the 6F-Glc internal repeating units, and three smaller singlets at -233.29, -233.31 and -233.35 ppm from 6F-Glc close to the reducing terminal and the non-reducing terminal unit. Analysis by MALDI-TOF mass spectrometry revealed that multi-6F-EpC 7 had a higher average DP (ca. 10) than the parent EpC (ca. DP 8) and that longer chains, up to DP 15, could also be observed in the multiple 6-fluorinated material (†ESI Figure S7).
Morphological characterization

Electron Microscopy (EM) and Atomic Force Microscopy (AFM)

Transmission electron microscopy (TEM) was initially used to observe the morphological differences between EpC and fluorinated EpCs, which were prepared for analysis only by dilution of concentrated suspensions obtained after purification of precipitates formed during enzymatic synthesis. As expected, the TEM images of the monofluorinated 2F-EpC (4), 3F-EpC (5) and 6F-EpC (6) (†ESI Figure S8) show a very similar morphology to EpC (8, Figure 2A, a). This crystalline sheet-like morphology is well-known for enzymatically synthesised cello-oligosaccharides, including derivatised cellulose, such as acrylated cellulose and cellulose conjugated with oligo(ethylene glycol). On the other hand, multi-6F-EpC (7) particles formed predominantly into significantly shorter platelets (< 100 nm length) (Figure 2B, a). These differences were further confirmed by AFM imaging using samples prepared by depositing diluted sample suspensions on freshly cleaved mica (Figure 2A, b and c). Although a few long platelets are present in multi-6F-EpC (7), their fraction is smaller than in EpC (8). As reported in the literature, the thickness of EpC (8) platelets was found to be ~5 nm. Similar thickness was observed for long platelets of multi-6F-EpC (7, Figure 2B, c).

![Figure 2](image.png)

Figure 2. TEM (a) and AFM (b -2 x 2 μm and c - 0.5 x 0.5 μm) images of enzymatically produced celloextrin EpC (8, A) and multi-6F-EpC (7, B). Scale bars correspond to 100 nm. Scale bars on the right of b and c are height measurements.

Long-range structural characterization by powder X-ray diffraction (PXRD)

The PXRD patterns of the monofluorinated 2F-EpC (4), 3F-EpC (5) and 6F-EpC (6) are virtually indistinguishable from the diffraction pattern of EpC (8) (Figure 3). This result indicates that the monofunctionalized celloextrin-like molecules arrange as cellulose type II allomorph, with three intense and sharp peaks at 2θ = 12°, 20° and 23° (d-spacing of 0.74, 0.44 and 0.39 nm, respectively) representing (110), (110) and (020) planes. On the other hand, the experimental PXRD pattern reported for multi-6F-EpC (7) does not correspond to any allomorph previously described for cellulose (Figure 3). The pattern shows two well defined peaks at 2θ = 15° and 23° (d-spacing of 0.59 and 0.39 nm, respectively), as well as four different broad components at 2θ = 21°, 25°, 30° and 36° (d-spacing of 0.42, 0.36, 0.30 and 0.25 nm, respectively).
In order to verify possible similarities with previously reported cellulose structural organisations, we predicted and compared the PXRD spectra of multi-6F-EpC (7) to each known allomorph (†ESI Figure S9 and Table S1). Remarkably, the observed peak positions of multi-6F-EpC (7) are unique when compared to the diffraction patterns of the known allomorphs (Figure 3, †ESI Figure S9 and Table S1), hence demonstrating the formation of a new crystalline structure for this new cellulose-like material.

Figure 3. Powder X-ray diffraction patterns of EpC (8, black), 2F-EpC (4, red), 3F-EpC (5, green), 6F-EpC (6, orange) and multi-6F-EpC (7, blue).

Molecular level characterization

Raman spectroscopy

Figure 4 shows typical Raman spectra of 2F-EpC (4), 3F-EpC (5), 6F-EpC (6), multi-6F-EpC (7) and EpC (8). The bands located at ca. 1462 (HOC and HCH stretching), 1265 (HCC and HCO stretching) and 576 cm\(^{-1}\) (heavy atom stretching) and the dominance of the band located at ca. 354 cm\(^{-1}\) over the band located at ca. 379 cm\(^{-1}\) (both heavy atom stretching) confirmed that EpC (8) arrange into a cellulose type II structure.\(^56\) 4, 5 and 6 are very similar to 8, as expected for a single fluorine atom (at the reducing end) per oligosaccharide chain. The weak band located at 487 cm\(^{-1}\) for the monofluorinated EpCs is probably an amalgamation of the 480 and 496 cm\(^{-1}\) bands as a result of the single fluorine present in each chain.

In contrast, the multi-6F-EpC (7) spectrum is significantly different owing to the presence of multiple fluorine atoms. Multi-6-fluorination results in new Raman bands located at 480, 496 and 924 cm\(^{-1}\). The first two bands correspond to the presence of CHxF, whilst the third relates to CHxF, confirming modification at the C6 position.\(^57\) Multi-6-fluorination also results in the shift of multiple bands, including 1462 to 1451 cm\(^{-1}\) and 1265 to 1268 cm\(^{-1}\), as well as the loss of others, such as the band located at 576 cm\(^{-1}\) (Table S2). Most significantly, the band associated with the glycosidic linkage located at 1097 cm\(^{-1}\) is shifted to 1088 cm\(^{-1}\). This provides some evidence that the crystal structure of the multi-6F-EpC material is neither cellulose type I nor type II.
Figure 4. Normalised, deconvoluted Raman spectra for EpC (black), 2F-EpC (red), 3F-EpC (green), 6F-EpC (orange) and multi-6F-EpC (blue). Dashed lines correspond to boundaries of bands associated with C-O-C stretching (C-O-C) and presence of fluorinated carbon groups (CH$_2$F and CH$_3$F).

Solid-state Nuclear Magnetic Resonance (SSNMR)

Direct polarization $^{19}$F NMR experiments (without $^1$H decoupling) were carried out at 60 kHz MAS rate for the 2F-EpC (4), 3F-EpC (5), 6F-EpC (6) and multi-6F-EpC (7) (Figure 5a). A single very broad and asymmetric peak was observed for the mono-fluorinated materials, centred at -190 (2F-EpC, 4), -197 (3F-EpC, 5) and -232 ppm (6F-EpC, 6), respectively, in good agreement with the solution $^{19}$F NMR data (†ESI Figure S4). 3F-EpC (5) and 6F-EpC (6) showed broad peaks, with line widths at half height of 11.9 and 9.4 kHz, respectively (Figure 5c), while multi-6F-EpC (7) showed a sharper (3.8 kHz width at half height) Lorentzian-shaped peak (Figure 5c). $^1$H-decoupled $^{19}$F ($^{19}$F{$^1$H}) NMR spectra of 3F-EpC recorded at slower MAS rate showed an even broader $^{19}$F peak (†ESI Figure S10), indicating that (i) fast MAS is more efficient at decoupling that radiofrequency decoupling (fast MAS decouples both $^{19}$F-$^{19}$F homonuclear dipolar coupling as well as heteronuclear $^1$H-$^{19}$F coupling), and (ii) the large $^{19}$F line widths of 2F-EpC (4), 3F-EpC (5), 6F-EpC (6) and multi-6F-EpC (7) in the fast MAS spectra (Figure 5a) are mostly due to the large heterogeneity of $^{19}$F chemical environments. This can be easily understood considering that these materials assemble into particles with a specific crystalline packing (cellulose type II$^{14}$ for 2F-EpC (4), 3F-EpC (5) and 6F-EpC (6), and a new organisation for muti-6F-EpC (7); Figures 5d and 6a), and the $^{19}$F nucleus is extremely sensitivity to chemical environment. Upon assembly of nanocellulose the $^{19}$F atoms of each cellulose chain can occupy any position within the nanofibril (surface, core, far from or nearby other fluorinated residues, etc), hence presenting non-equivalent environments within the packing of EpC (Figure 5c). Assuming that $^{19}$F-$^1$H dipolar interactions are practically eliminated at fast MAS, the peak broadening reflects a multitude of orientations sampled by the C-F bonds.
Figure 5. (a) Chemical structures of non-modified EpC (8), monofluorinated 2- (4), 3- (5) and 6F-EpC (6), and multi-6F-EpC (7). (b) 3D model of the crystalline packing of cellulose II allomorph based on the origin-center-origin (o-c-o) chains. The O3-O6 intra-chain (blue dashes) and O2-O2 and O6-O6 inter-sheet (yellow dashes) hydrogen bonds are shown. It should be noted that the substitution of all -OH groups at C6 with fluorine atoms precludes the formation of O6-O6 inter-sheet hydrogen bonds during self-assembly. Note: the intra-chain hydrogen bonds are only shown for the center chain for simplicity. (c) Direct detection $^{19}$F MAS NMR spectra of multi-6F-EpC (7, blue) and 2F- (4, red), 3F- (5, green) and 6F-EpC (6, orange) powders, acquired at 60 kHz MAS rate and 800 MHz $^{19}$F frequency (20 T magnetic field). (d) $^1$H-13C CP/MAS NMR spectra of EpC powder acquired at 10 kHz MAS rate (black), and 2F- (4, red), 3F- (5, green) and 6F-EpC (6, orange) 10 wt% dispersions acquired at 6 kHz MAS, and 100 MHz $^{13}$C frequency.

To characterize the structural organization of 2F-EpC (4), 3F-EpC (5), 6F-EpC (6) and multi-6F-EpC (7) materials at the molecular level, $^1$H-13C CP/MAS experiments were carried out. Each type of cellulose allomorph presents a characteristic $^{13}$C NMR fingerprint. Monofluorinated EpCs (4, 5 and 6) 10 wt% dispersions showed the characteristic cellulose II $^1$H-$^{13}$C CP fingerprint, typical of non-modified EpC (8) (Figure 5d). The only noticeable difference was the presence of a broad peak at ca. 61 ppm, which is characteristic of a surface/disordered population of C6 (sC6, Figure 5d). Hence, the peak at 63 ppm represents the interior/ordered domains of C6 (iC6, Figure 5d). The sC6 broad peak is typically observed in bacterial cellulose, which consists of cellulose particles containing both I$_a$ and I$_b$ crystalline domains and disordered regions. Indeed, surface/disordered and interior/ordered domains are typically found in nanocrystalline cellulose (CNC), bacterial cellulose (BC) and plant cell
walls (PCWs).\(^{61}\) We note that the presence of a fluorine atom substituting the 3-hydroxyl group of glucose might affect the formation of the characteristic O3-O6 intra-chain hydrogen bond between adjacent glucose residues in cellulose II allomorph (Figure 5b). On the other hand, the formation of the O2-O2 and O6-O6 inter-sheet hydrogen bonds of cellulose II would be affected in 2F- and 6F-EpC, respectively (Figure 5b). Spectral deconvolution of the \(^{1}H\)-\(^{13}C\) CP/MAS NMR spectra enabled us to estimate the relative surface area (RSA) of the EpC particles constituting each material (†ESI Figure S11, Table S3). RSAs of about 16-23% were obtained for the three monofluorinated EpCs, with 3F-EpC showing the highest value (23%); †ESI Table S3). Similar values of surface area have been determined before for bacterial cellulose.\(^{60}\)

The \(^{1}H\)-\(^{13}C\) CP/MAS NMR spectrum of multi-6F-EpC (7) showed a pattern of peaks that does not correspond to either cellulose I, II or III allomorphs (†ESI Table S4, S5). This is evidenced by the appearance of C1 (105.9 ppm) as a singlet peak in 7, which is a singlet in cellulose type Ia, and III, and a doublet in cellulose type Ib, II and III (†ESI Table S4).\(^{62,63}\) Notably, multi-6F-EpC (7) presents some spectral features in common with cellulose III, i.e. C1 and non-fluorinated C6 peaks are singlets and appear at very similar chemical shifts (104.8 and 62.1 ppm, respectively; †ESI, Table S5). However, the PXRD pattern of multi-6F-EpC (7, Figure 3) does not correspond to either cellulose III\(^{64}\) or III\(^{65}\) allomorphs, or any other cellulose allomorphs reported so far (†ESI Table S1). Hence, 7 assembles into a crystalline organization which is unprecedented for a cellulose-type material. The formation of this novel structural motif is also supported by the new features observed in the Raman spectra, which do not correspond to either cellulose I or II (Figure 3, †ESI Table S4).

The combination of fast MAS \(^{1}H\)-\(^{13}C\) CP, low MAS \(^{1}H,^{19}F\)-decoupled \(^{19}F\)-\(^{13}C\) CP, water-polarization transfer (WPT) solid-state NMR and \(^{13}C\), COSY and HSQC solution NMR experiments enabled the assignment of the \(^{13}C\) spectrum of multi-6F-EpC (7, Figure 6) to be made. \(^{1}H,^{19}F\)-decoupled \(^{19}F\)-\(^{13}C\) CP enabled the assignment of C6, C4 and C5 peaks of the fluorinated residues (Figure 6a). The highest intensity peak was assigned to C6 (83.8 ppm), as it is the carbon atom closest to 6F (1.3 Å). The peak at 73.1 ppm corresponds to C4 and C5 sites, based on their proximity to fluoride (Figure 6a, †ESI Figure S12), while C2 and C3 are too far away to cross-polarize from fluorne effectively. \(^{13}C\) DEPT135, COSY and HSQC solution NMR experiments confirmed this assignment (†ESI Figure S13), with the methylene carbons of the fluorinated (C6) and non-fluorinated (C6*) glucose units appearing in antiphase with respect to the CH carbons (Figure 6a). Importantly, the \(^{13}C\) peaks at 81.9 and 73.1 ppm observed in the CP spectrum did not appear on the \(^{13}C\) DEPT135 nor \(^{1}H\)-\(^{13}C\) HSQC solution NMR experiments carried out for a diluted dispersion of multi-6F-EpC (7). Hence, these peaks most likely correspond to the immobile interior carbons (iC6 and iC2,3,4,5, respectively) that are too broad to be detectable by solution NMR. The solution-NMR-observed C6 and C2,3,4,5 peaks were therefore assigned to surface/disordered domains (sC6 and sC2,3,4,5, respectively). The assignment of sC6 and iC6 was further validated by water polarization transfer CP (WPT-CP) NMR experiments (Figure 6b).\(^{65}\) The peak intensity in WPT-CP experiments depends on the distance and relative mobility of bound water at the particle surface and the number of interacting water molecules at a particular site. Hence, peaks corresponding to surface domains will show faster WPT growth at short mixing times than interior domains, as we have recently observed for BC.\(^{60}\) At sufficiently long mixing times, WPT become homogeneous for both surface and interior domains due to the efficient spin diffusion. Figure 6b shows the WPT factors for a 25 wt% dispersion of multi-6F-EpC (7) at 16 ms mixing time (under our experimental conditions, homogenization of surface-interior water polarization transfer is achieved around 200 ms). A much higher WPT factor was observed for the sC6
controlled morphology and considerable enabled the detailed characteri-
ization of the nanocellulosic materials (monofluorinated cellobioses and 6F-
ated materials). CBP and CDP proved to be robust enzymes for accepting fluorinated substrates (monofluorinated cellobioses and 6F-Glc-1P, respectively) in order to produce novel nanocellulosic materials. Comparison between the enzymatically produced cellodextrin (EpC) and its monofluorinated analogues (2F-, 3F- and 6F-EpC) clearly shows that the presence of a single fluorine atom in the reducing end glucose unit does not affect to a large extent the crystalline structure and morphology of these materials. In contrast, the presence of multiple 6-fluorinated glucose units along the oligosaccharide chain (multi-6F-EpC) yielded a novel cellodextrin material of different morphology and crystallinity. Importantly, multi-6F-EpC shows a unique long-range ordering motif unprecedented for cellulose-based biomaterials, indicated by diffraction and spectroscopic methods. Advanced solid-state NMR methods enabled the detailed characterization of these novel materials, deciphering the water-exposed and interior chemical environments for different carbon sites. Our findings highlight considerable potential of chemoenzymatic synthesis for generating novel glycomaterials of controlled morphology and molecular structure via easy-to-prepare building blocks of specific

Conclusions

We have developed a bottom-up approach for the chemoenzymatic synthesis and characterization of novel mono and multiply-fluorinated cellodextrins from functionalized monosaccharide building blocks. Enzymatic catalysis has been employed for the rapid production of tailor-made fluorinated materials based on substrates with specific fluorination pattern. CBP and CDP proved to be robust enzymes for accepting fluorinated substrates (monofluorinated cellobioses and 6F-Glc-1P, respectively) in order to produce novel nanocellulosic materials. Comparison between the enzymatically produced cellodextrin (EpC) and its monofluorinated analogues (2F-, 3F- and 6F-EpC) clearly shows that the presence of a single fluorine atom in the reducing end glucose unit does not affect to a large extent the crystalline structure and morphology of these materials. In contrast, the presence of multiple 6-fluorinated glucose units along the oligosaccharide chain (multi-6F-EpC) yielded a novel cellodextrin material of different morphology and crystallinity. Importantly, multi-6F-EpC shows a unique long-range ordering motif unprecedented for cellulose-based biomaterials, indicated by diffraction and spectroscopic methods. Advanced solid-state NMR methods enabled the detailed characterization of these novel materials, deciphering the water-exposed and interior chemical environments for different carbon sites. Our findings highlight considerable potential of chemoenzymatic synthesis for generating novel glycomaterials of controlled morphology and molecular structure via easy-to-prepare building blocks of specific
substitution pattern. Our work opens promising avenues for the substitution of chemical methods for material preparation by environmentally friendly procedures.

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Graphical abstract

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