Carbohydrate Polymers 76 (2009) 60–67

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and structural characterization of 3-*O*-ethylene glycol functionalized cellulose derivatives

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ARTICLE INFO

Article history:
Received 3 September 2008
Received in revised form 15 September 2008
Accepted 18 September 2008
Available online 30 September 2008

Keywords:
Regioselectivity
Synthesis
3-*O*-ethylene glycol 2,6-di-*O*-acetyl cellulose
2,6-Di-*O*-thexyldimethylsilyl cellulose
T1 NMR experiments

ABSTRACT

The synthesis of 3-*O*-ethylene glycol cellulosics via 2,6-di-*O*-thexyldimethylsilyl cellulose was studied. Reaction yield and degree of substitution were dependent on reaction conditions and size of the ethylene glycol group. The presence of tetra-*n*-butylammonium iodide in catalytic amounts and prolonged reaction times significantly increased the degree of substitution of the ethylene glycol substituents. However, the longer reaction times lead to significant degradation of the cellulosic polymer chain. The structure of the 3-*O*-ethylene glycol 2,6-di-*O*-thexyldimethylsilyl cellulose intermediates and the 3-*O*-ethylene glycol 2,6-di-*O*-acetyl celluloses were confirmed by means of one- and two-dimensional NMR spectroscopy. The degree of 3-*O*-ethylene glycol substitution was confirmed by quantitative ^{13}C NMR ratified by T1 experiments.

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1. Introduction

The development of functionalized materials from cellulose and cellulose derivatives is receiving increasing attention. Of particular interest are microporous polymeric films. Such materials have potential applications in medical and non-medical uses (Stenzel, 2002), including photonic crystals (Wijnhoven & Vos, 1998), microreactors, microarray chips (Ramsay, 1998), and scaffolds for tissue growth (Sato et al., 2002). Cellulosics have been reported to form regular structures in films. Stenzel and coworkers (Stenzel, Davis, & Fane, 2003; Hernandez-Guerrero, Davis, Barner-Kowollik, & Stenzel, 2005) recently reported the production of porous films from polystyrene comb polymers built on hydroxyisopropyl cellulose. The regularity of the porous films increased with the density of branches and with the increasing length of each branch; however, poor quality low regularity films were obtained. Similarly, Kondo et al. (Kasai & Kondo, 2004) and Uraki et al. (Nemoto et al., 2005) produced honeycomb-shaped films based on cellulose acetate. Through the direct solvent evaporation process, extremely irregular acetate films were obtained, having macroporous sizes ranging from 1 to 100 μm. However, when prepared by the transcription method, using a Poly(dimethyl siloxane) (PDMS) template, uniform patterned cellulose acetate and regenerated cellulose films were produced (Nemoto et al., 2005).

The nature of the polymer used, specifically, the shape of the polymer in solution is reportedly a critical criteria for the formation of highly regular structures in films (Stenzel, 2002). This is generally accomplished using star polymers, amphiphilic block copolymers or polymers that form micelles in the solvent of interest. Regioselective functionalization of cellulose offers the potential to produce novel cellulosic materials with promising properties (Klemm, Heinze, Philipp, & Wagenknecht, 1997). The regioselective modification of cellulose derivatives with amphiphilic moieties may alter the molecular shape and thereby affect the regularity and size of the micropores in the resulting honeycomb structured films. In fact, ethylene glycol modification of 2,6-thexyldimethylsilyl (TDMS) cellulose resulted in the formation of regular microporous honeycomb structured films (Kadla, Asfour, & Bar-Nir, 2007). Honeycomb films produced from 3-EG_{16}-2,6-TDMS cellulose were extremely uniform with a pore size diameter of approximately 2 μm (Kadla et al., 2007). Increasing ethylene glycol molecular weight led to increased pore uniformity, but did not dramatically affect pore size. This may have been a result of the limited degree of substitution (DS ⩽ 0.2) of the ethylene oxide moieties. In this paper we report the synthesis and detailed characterization of a series of ethylene oxide substituted 2,6-di-*O*-thexyldimethylsilyl (TDMS) cellulose and 2,6-di-*O*-acetyl cellulosics.

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doi:10.1016/j.carbpol.2008.09.026

2. Experimental section

2.1. Materials

Tris(ethylene glycol) monomethyl ether (EG_3), polyethylene glycol 350 monomethyl ether (EG_7), polyethylene glycol 750 monomethyl ether (EG_{16}), dimethylthexylsilyl chloride (TDMSCl), imidazole, lithium chloride, dimethyl acetamide (DMA)/anhydrous DMA, acetic anhydride, tetra-*n*-butylammonium iodide (TBAI), tetrabutylamonium fluoride trihydrate (TBAF) and Chromium(III) acetylacetonate (Cr(III)acac) were purchased from Aldrich and used as received. Tetrahydrofuran (THF) was dried on sodium/benzophenone before use. Cellulose was obtained from the deacetylation of cellulose acetate 30K (Aldrich) using sodium hydroxide in methanol and thoroughly dried prior to use.

2.2. Characterization

One- and two-dimensional NMR spectra were measured using a Bruker AVANCE-300 spectrometer at 40 °C (C_6D_6) or 70 °C (DMSO-d_6) for 50 mg of sample. T1 experiments were performed in 40 °C C_6D_6 or 0.1 M of Chromium(III) acetylacetonate (Cr(III)acac) in C_6D_6. Chemical shifts were referenced to tetramethyl silane (TMS; 0.0 ppm). Infrared spectra were obtained with a Perkin–Elmer Spectrum One FT-IR spectrometer as potassium bromide pellets in a 3:1 sample to salt ratio (insoluble samples) or in $CHCl_3$ (soluble samples). Differential scanning calorimetry (DSC) measurements were run using 10 mg of sample under nitrogen at a heating rate of 20 °C min^{-1} using a TA instruments Q1000 DSC calibrated with indium. Thermogravimetric analysis (TGA) measurements were run under nitrogen using a TA instruments Q500 TGA. Sample mass was ~2 mg and an initial heating rate of 20 °C min^{-1} was used. Elemental analysis was measured using a Perkin-Elmer Series II CHNS/O analyzer. The relative average molecular mass of the cellulosic compounds were determined by gel permeation chromatography (GPC – Agilent Technologies) using Styragel columns (HR-4 and HR-1) at 40 °C and tetrahydrofuran (THF) as the eluting solvent. The GPC system was calibrated using standard polystyrene samples with molecular weights ranging between 580 and 1800K. The injection volume was 100 μL, and the cellulosic concentration was 1 mg mL^{-1} THF (0.1 mol).

2.3. Synthesis

Methoxypoly(ethylene glycol) tosylates were synthesized from tris(ethylene glycol) monomethyl ether (EG_3), polyethylene glycol 350 monomethyl ether (EG_7), and polyethylene glycol 750 monomethyl ether (EG_{16}). Monoethylene glycol (0.1 mol) was dissolved in 50 ml THF and added to a solution of potassium hydroxide (0.22 mol, 2.2 equiv.) dissolved in 50 ml of water and cooled to 0 °C. *p*-tosyl chloride (0.12 mol, 1.2 equiv.) in 100 ml of THF was added dropwise over 30 min. The reaction mixture was held at 5 °C with stirring for 2 h, and then warmed to room temperature and stirred for an additional 18 h. Ethyl acetate (100 mL) was then added to the reaction and the organic phase collected. The aqueous layer was extracted with two 75-mL portions of ethyl acetate, which were combined and extracted with three 75-mL portions of 6 N HCl. The organic layer was dried over magnesium sulfate and the solvent was removed by evaporation in vacuo. Yield: 95%.

^{1}H NMR ($CDCl_3$, 300 MHz) (Kadla et al., 2007; Yue & Cowie, 2002): δ (ppm) = 2.35 (s, 3H), 3.25 (s, 3H), 3.4–3.6 (multiplet, 8/24/60H), 3.68 (t, 2H), 4.08 (t, 2H), 7.25 (d, 2H), 7.67 (d, 2H).

Methoxypoly(ethylene glycol) Iodide (Yue & Cowie, 2002) was synthesized from the corresponding EG tosylate. Ethylene glycol-tosylate (0.05 mol) was dissolved in 50 ml of acetone and carefully added to a refluxing potassium iodide (0.18 mol, 3.6 equiv.) in 50 ml of acetone solution. The mixture was refluxed for 18 h and the acetone was removed under reduced pressure. The reaction mixture was dissolved in 100 mL of chloroform and extracted with 100 mL of water. The organic layer was removed and the aqueous layer was extracted with two 75-mL portions of chloroform. The combined organic layers were washed with brine and dried over magnesium sulfate. The solvent was removed by evaporation in vacuo. Yield: 92%.

Tris(ethylene glycol) iodide monomethyl ether (EG_3-I): ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ (ppm) = 71.9, 70.6, 70.2, 59.0, 2.99.

Polyethylene glycol 350 iodide monomethyl ether (EG_7-I): ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ (ppm) = 71.95, 71.92, 70.64, 71.57, 70.20, 59.02, 2.97.

Polyethylene glycol 750 iodide monomethyl ether (EG_{16}-I): ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ (ppm) = 71.7, 71.4, 70.0, 69.9, 69.7, 58.5, 5.9.

2,6-di-O-Thexyldimethylsilyl cellulose (1) (2,6-TDMS cellulose) (Kadla et al., 2007; Koschella, Heinze, & Klemm, 2001) was prepared according to the procedure of Koschella et al. Cellulose (30 K g/mol, 5.0 g, 0.03 mol) was suspended in 50 ml of DMA and stirred at 120 °C for 2 h. The slurry was then cooled to 100 °C and lithium chloride (20.0 g, 0.45 mol, 15 equiv.) was added and allowed to stir for 15 min. The mixture was then cooled to room temperature and stirred overnight to afford a colorless viscous solution. Imidazole (10 g, 0.15 mol, 5 equiv.) was added to the solution and stirred for 30 min at which time dimethylthexyl silyl chloride (1.7 ml, 0.09 mol, 3 equiv.) was added dropwise. The solution was stirred for 15 min and then heated to 100 °C and allowed to stir for 24 h under nitrogen. The reaction mixture was allowed to cool to room temperature and the polymer was isolated by poring slowly into 500 mL of pH 7 aqueous buffer solution and filtered. Further purification was done by redissolving the crude polymer in chloroform and precipitating in methanol.

Yield: 12 g (89%), DS 1.96; Elemental analysis: calc: 59.14 C%, 10.38 H%; measured: 59.03 C%, 10.58 H%.

FT-IR ($CHCl_3$): 3502 (OH), 2960, 2860 (CH), 1468 (CH_2, CH_3), 1379 (CH_3), 1253 (Si–C), 1120 (Si–O–C), 1080, 1038 (C–O–C_{AGU}), 834, 778 (Si–C/Si–O–C).

^{13}C NMR (10% in C_6D_6, 313 K, 75.4 MHz): δ = 102.5 (C1), 76.9, 75.6, 72.1, 71.9, 60.6 (C6), 35.0 to −3.4 (TDMS group).

3-O-EG-2,6-di-O-Thexyldimethylsilyl cellulose (2a-c) (3-EG_n 2,6-TDMS cellulose). General procedure: Sodium hydride (760 mg, 0.02 mol, 10 equiv.) was washed three times in freshly distilled anhydrous THF under nitrogen. 2,6-di-TDMS cellulose (1 g) was added and stirred in 20 mL anhydrous THF, at room temperature for 1 h. tetra-*n*-Butylammonium iodide (TBAI) (75 mg, 0.02 mmol, 0.01 equiv.) and EG-iodide (3/4.5/8.4 g, 0.01 mol, 5 equiv.) were added. The mixture was stirred for 4 days at room temperature, and then slowly precipitated into methanol while destroying the excess of sodium hydride. Further purification was done by redissolving the crude polymer in chloroform and precipitating in methanol.

3-O-(3,6,9-trioxydecyl)-2,6-di-O-(thexyldimethylsilyl) Cellulose (2a) (3-EG_3 2,6-TDMS cellulose) was prepared using the iodide of tris(ethylene glycol) monomethyl ether. DS (EG_3) 0.42-0.82. Yield: 79%.

FT-IR ($CHCl_3$): 3493 (OH), 2958, 2870.4 (CH), 1465 (CH_2, CH_3), 1378 (CH_3), 1252 (Si-C), 1118(Si-*O*-C), 1076, 1036 (C-*O*-C_{AGU}), 834, 778 (Si–C/Si–O–C).

^{13}C NMR (C_6D_6, 313 K, 75.4 MHz): δ = 102.5 (C1), 76.9, 75.5, 75.3, 72.2 (PEG), 70.8 (EG), 70.7 (EG), 60.73 (C6), 58.5 (OMe), 34.5 to −3.4 (methyl of TDMS).

3-O-(3,6,9,12,15,18,21-heptaoxyicosyl)-2,6-di-O-(thexyldimethylsilyl) Cellulose (2b) (3-EG_7 2,6-TDMS cellulose) was prepared using the iodide of polyethylene glycol monomethyl ether (MW 350 g/mol). DS (EG_7) 0.32–0.62. Yield: 73%.

^{13}C NMR (C_6D_6, 313 K, 75.4 MHz): δ = 102.7 (C1), 75.7, 72.2 (EG), 70.8 (EG), 60.8 (C6), 58.4 (*O*Me), 34.4 to −3.3 (methyl of TDMS).

3-*O*-(3,6,9,12,15,18,21,24,27,30,33,36,−39,42,45,48-hexadecaoxynonatetracontyl)-2,6-di-*O*-(thexyldimethylsilyl) Cellulose (2c) (3-EG_{16} 2,6-TDMS cellulose) was prepared using the iodide of polyethylene glycol monomethyl ether (MW 750 g/mol). DS (EG_{16}) 0.55-0.65. Yield: 31%.

^{13}C NMR (C_6D_6, 313 K, 75.4 MHz): δ = 102.5 (C1), 75.6, 72.2 (EG), 70.8 (EG), 60.89 (C6), 58.4 (*O*Me), 34.4 to −3.3 (methyl of TDMS).

3-O-EG-cellulose (3a-c) (3-EG_n cellulose). General procedure: 3-EG-2,6-TDMS cellulose (**2a-c**) (1 g, 3.4 mmol TDMS/2.6 mmol TDMS/1.7 mmol TDMS) was suspended in 50 mL of freshly distilled THF and tetrabutylamonium fluoride trihydrate (TBAF) (1 g/820 mg/540 mg, 3.4/2.6/1.7 mmol, 1 equiv./TDMS) was added and stirred for 18 h at room temperature. The product was precipitated in methanol, filtered and washed with methanol, then dried in vacuo.

3-O-(3,6,9-trioxydecyl) Cellulose (3a) (3-EG_3 cellulose): FT-IR (KBr): 3431 (OH), 2879 (CH), 1461, 1376, 1320, 1122, 1070, 1025 (C–O–$C_{cellulose}$), 841, 803 (traces: Si–C /Si0–O–C).

3-O-EG_n-2,6-di-O-acetyl cellulose (4a-c). General procedure: 3-*O*-EG-cellulose (**3a-c**) (1 g) and imidazole (2 g, 0.03 mol, 5 equiv.) were stirred in 10 mL of *N,N*-dimethylacetamide (DMA) at room temperature for 1 h. 10 mL of acetic anhydride were added and the mixture was stirred at 80 °C for 48 h. The product was precipitated in water, filtered and washed with water or methanol, then dried in vacuo. Further purification was done by redissolving the crude polymer in chloroform and precipitating in methanol.

3-*O*-(3,6,9-trioxydecyl)-2,6-di-*O*-Acetyl cellulose (4a) (3-EG_3 2,6-di-Ac cellulose): Yield: 84%.

FT-IR ($CHCl_3$): 2932, 2883 (CH), 1748 (C=O), 1455, 1433 (CH_2, CH_3), 1370 (CH_3), 1230, 1113, 1048 (C–O–C_{AGU}).

^{13}C NMR (DMSO-d_6, 343 K, 75.4 MHz): δ = 170.5, 169.6, 169.3 (C=O), 99.8 (C1), 76.4, 72.9, 72.5, 72.2, 71.9, 70.4, 70.2 (EG), 62.8 (C6), 58.5 (OMe), 20.8, 20.5 (Me–Ac).

3-*O*-(3,6,9,12,15,18,21-heptaoxyicosyl)-2,6-di-*O*-acetyl cellulose (4b) (3-EG_6 2,6-di-Ac cellulose): Yield: 72%.

FT-IR ($CHCl_3$): 2932, 2875 (CH), 1752 (C=O), 1455, 1439 (CH_2, CH_3), 1369 (CH_3), 1231, 1113, 1051 (C–O–$C_{cellulose}$).

^{13}C NMR (DMSO-d_6, 343 K, 75.4 MHz): δ = 170.5, 169.6, 169.3 (C=O), 99.8 (C1), 76.4, 72.9, 72.5, 72.2, 71.9, 70.4, 70.2 (EG), 62.8 (C6), 58.5 (*O*Me), 20.8, 20.5 (Me–Ac).

3-*O*-(3,6,9,12,15,18,21,24,27,30,33,36,−39,42,45,48-hexadecaoxynonatetracontyl)-2,6-di-*O*-acetyl cellulose (4c) (3-EG_{16} 2,6-di-Ac cellulose): Yield: 32%.

FT-IR ($CHCl_3$): 2953, 2869 (CH), 1756 (C=O), 1464, 1438 (CH_2, CH_3), 1369 (CH_3), 1231, 1113, 1050 (C–O–$C_{cellulose}$).

^{13}C NMR (DMSO-d_6, 343 K, 75.4 MHz): δ = 170.5, 169.6, 169.3 (C=O), 99.8 (C1), 76.4, 72.9, 72.5, 72.2, 71.9, 70.4, 70.2 (EG), 62.8 (C6), 58.5 (OMe), 20.8, 20.5 (Me–Ac).

3. Results and discussion

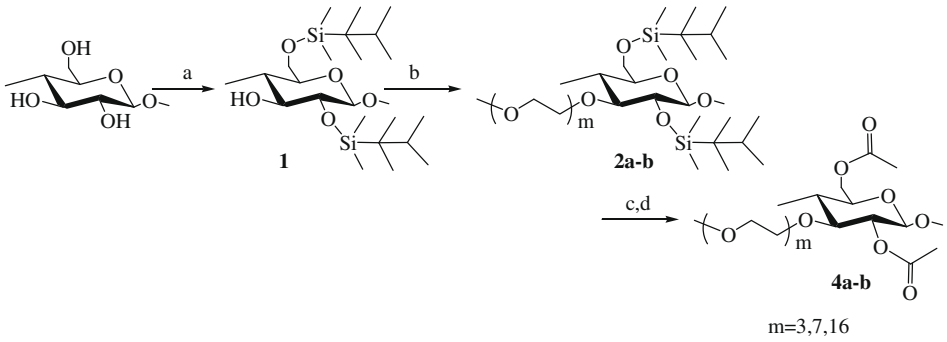
3.1. Synthesis and characterization of 3-EG_n-2,6-TDMS cellulose derivatives 2a–c

Scheme 1 illustrates the reaction scheme for the preparation of 3-*O*-ethylene glycol monomethyl ether (EG_3, EG_7, EG_{16})-2,6-di-*O*-diacetyl celluloses **4a**–**c**. Regioselective substitution was accomplished using bulky thexyldimethylsilyl protecting groups (Koschella et al. 2001). Specifically, cellulose, dissolved in DMA/LiCl was reacted with thexyldimethylsilyl chloride (TDMSCl) and imidazole to form 2,6-di-*O*-thexyldimethylsilyl cellulose (**1**). The degree of substitution (DS) was determined to be 1.96 by elemental analysis (Kadla et al., 2007). Regioselectivity was confirmed by two-dimensional NMR analysis of the 3-*O*-allyl-substituted 2,6-di-*O*-thexyldimethylsilyl cellulose derivative (data not shown)(Koschella et al., 2001) and the final product (**4a**–**c**; see below).

Although characterization of the intermediate products **2a**–**4c** was readily performed using FT-IR and ^{13}C NMR techniques, the degree of substitution (DS) of the ethylene glycol monomethyl ether (EG) component was difficult, and available only by elemental analysis (EA). NMR spectroscopy has been widely used to quantify functional groups in various cellulosics (Buchanan, Edgar, Hyatt, & Wilson, 1991; Buchanan, Edgar, & Wilson,, 1991; Liu & Baumann, 2002). Fig. 1a shows the ^{13}C NMR spectrum for 3-EG_3-2,6-TDMS-cellulose. The signals for the carbon atoms of the cellulose, thexyldimethylsilyl (TDMS) protecting group and the EG group are clearly resolved: δ = 102.5 (C1), 77.4–75.8 (C2–5), 72.2–70.7 (EG–CH_2), 60.7 (C6), 58.8 (EG–CH_3), 34.5 to −3.1 (TDMS–CH_3). However, the ^{13}C NMR spectrum obtained using a standard one Pulse experiment is not quantitative because of the nuclear Overhauser enhancement of the ^{13}C nuclei due to their attached ^{1}H nuclei. Therefore, to obtain quantitative ^{13}C NMR spectra the spin-lattice (T1) relaxation time of the various ^{13}C nuclei of interest needed to be determined. Using an inversion recovery pulse sequence, the length of the relaxation delay (D1) required for quantitative analysis (D1 $\geqslant$ 5T1) of our cellulose derivatives was determined. Specifically, we focused on the T1 relaxation times of the well-resolved signals associated with the TDMS and EG groups (labeled 1–5 in Fig. 1b). In this sequence, cellulose signals were not resolved due to the low number of scans applied. Table 1 lists the spin-lattice (T1) relaxation times for the TDMS and EG group carbons. The methyl group of the ethylene glycol unit (peak 3) had the longest T1 at ~9.5 s; therefore, a D1 of 60 s was used for the quantitative ^{13}C NMR experiments. Much shorter relaxation times were observed repeating the T1 experiment using 0.1 M Cr(III)acac (relaxation reagent) (Table 1). Using the relaxation agent, the T1 for the same peak (peak 3) was measured to be 270.2 ms, and the longest T1 was measured for peak five, at 2.2 s.

From elemental analysis the DS of the TDMS protecting group was determined to be ~2, therefore the integral of the methyl signals of the TDMS groups at 34.5 and 24.5 ppm can be set equal to 2 (two carbons per anhydroglucopyranose unit). As the EG groups contain one CH_3O group per EG group, and at most one per anhydroglucopyranose unit, integration of the CH_3O group signal at 58.5 ppm indicates the DS of the EG groups is 0.50 for the 3-EG_3-2,6-TDMS-cellulose (**2a**) (Fig. 1b and c) and 0.49 for the 3-EG_{16}-2,6-TDMS-cellulose (**2c**) (Fig. 1d). These values are in excellent agreement with those obtained from elemental analysis (Table 2) and confirm the accuracy and precision of the NMR experiment.

Previously, we obtained relatively low EG DS values ($\leqslant$0.2) for our 3-EG_m-2,6-TDMS-cellulosics (Kadla et al. 2007), using tosylated-EGs, imidazole as the base and THF as the solvent. Therefore, in an attempt to increase the DS, we performed a series of experiments comparing the efficiency of using (i) tosylated-EG vs. iodated-EG; (ii) pyridine, sodium hydride, imidazole and triethyl amine as bases and (iii) DMA, THF and DMSO as solvents. Depending on the conditions, varying results were obtained. For example, using sodium hydroxide in anhydrous DMSO, as per the conditions reported for making poly(ethylene oxide) modified cellulose (Yue & Cowie, 2002), led to partial deprotection of the TDMS groups and low EG group substitution. By contrast, Klemm's procedure for the allylation of 2,6-TDMS-cellulose (Koschella et al., 2001) using EG-I and sodium hydride as a base in THF, was very efficient, and therefore was further optimized. The presence of tetra-*n*-butylammonium iodide (TBAI) in catalytic amounts (Petzold, Klemm, Heublein, Burchard, & Savin, 2004) significantly increased the DS of the EG, from 0.11 without the catalyst to 0.39 in the presence of catalyst. Further optimization of the reaction conditions, e.g.



Scheme 1. Synthetic scheme to regioselective cellulose acetate polymer brushes: (a) TDMSCl, imidazole, DMA-LiCl, 100°C; (b) NaH, EG_mI, TBAI, THF; (c) TBAF, THF; (d) imidazole, DMA, AcO_2.

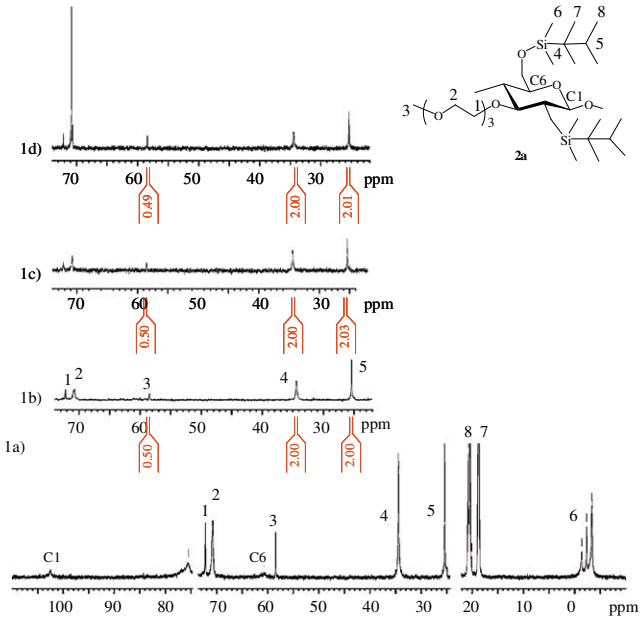


Fig. 1. (a) ^{13}C NMR spectrum of 3-EG_3-2,6-TDMS-cellulose (**2a**); (b) Quantitative ^{13}C NMR spectrum of 3-EG_3-2,6-TDMS-cellulose (**2a**); (c) Quantitative ^{13}C NMR spectrum of 3-EG_3-2,6-TDMS-cellulose (**2a**) using relaxation reagent; (d) Quantitative ^{13}C NMR of 3-EG_{16}-2,6-TDMS-cellulose (**2c**). All experiments were run in C_6D_6 at 40 °C.

Table 1
Spin-lattice (T1) relaxation times for selected peaks associates with the EG and TDMS groups without/with relaxation regent

Peak #[a]	δ (ppm)	T1 no relaxation reagent	T1 with relaxation reagent
1	72.2	3.6 s	777.9 ms
2	70.8	1.5 s	1.3 s
3	58.5	9.5 s	270.2 ms
4	34.5	688.6 ms	585.5 ms
5	25.4	6.4 s	2.2 s

[a] The peak number corresponds to the peaks shown in Fig. 1b.

Table 2
DS values obtained using EA and quantitative ^{13}C NMR

	Compound name	DS by EA	DS by NMR	DS by NMR (+Cr(III)acac)
	2,6-TDMS cellulose	1.96		
Fig. 1b	3-EG_3-TDMScellulose	0.50	0.50	0.50
Fig. 1c	3-EG_{16}-TDMScellulose	0.49	0.49	

temperature and time, were performed and the effect on DS (NMR) and number-average molecular weight (Mn – GPC) were monitored.

Increasing the reaction temperature from room temperature to 60 °C or 80 °C not only lead to a decrease in the molecular weight (Mn) of the polymer, indicating polymer degradation, but also a decrease in DS (Table 3: entry 1 vs. 4 vs. 6). Extending the reaction to longer periods of times lead to an increase in DS, but substantially decreased the Mn (Table 3: entry 5 vs. 6 vs. 8).

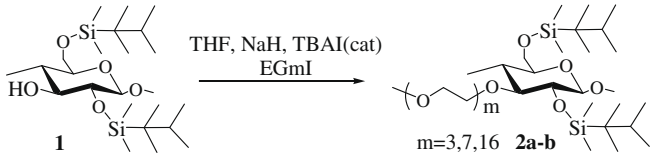
Based on these results, the optimal reaction conditions for C_3–O substitution of **2** were found using EG-iodides, sodium hydride as the base, in the presence of a catalyst (TBAI), using dry THF at room temperature for 4 days (Scheme 2).

3.2. Synthesis and characterization of 3-O-EG-2,6-di-O-acetyl cellulose derivatives 4a–c

Removal of the silicon-containing protecting groups from the C2 and C6 positions of the anhydroglucopyranose unit, **2a–c**, was accomplished using tetrabutylammonium fluoride trihydrate (TBAF) in THF. Again, it was found that the conditions under which the deprotection step was performed had a significant impact on the Mn of the polymer. Therefore, optimization of the reaction conditions, e.g. temperature, time and concentration of TBAF, was required and measured by FT-IR (removal of the Si–C stretch) and GPC analysis (Mn). The deprotected product was not soluble in most common solvents (e.g.: chloroform, THF, DMAc, acetone, DMSO...) and could not be thoroughly characterized. Therefore,

Table 3
Temperature and time comparison of C_3–O substitution of **2a**

Entry	Solvent	Temperature (°C)	Time (days)	DS	Mn (E04)
1	Dioxane	80	4	0.42	2.87
2	THF	60	1	0.36	3.19
3	THF	60	2	0.39	2.5
4	THF	60	4	0.45	3.32
5	THF	rt	1	0.37	4.21
6	THF	rt	4	0.68	3.81
7	THF	rt	7	0.77	2.03
8	THF	rt	14	0.82	2.0



Scheme 2. Optimized conditions for C_3–O substitution of 1.

Table 4
Deprotection of 3-EG3-2,6-TDMS-cellulose in THF

Entry	Fig. #	TBAF (equiv.)	Temp. (°C)	Time (h)	Mn of the Ac product ($\times 10^4$)	TDMS presence (FT-IR)
1[a]		4	50	24	0.57	–
2		4	rt	18	2.56	–
3		2	rt	18	4.01	Traces
4	2c	1	rt	18	5.2	Traces
5	2d	2	50	18	3.6	–
6		1	50	18	3.82	Traces
9	2b	1	rt	8	4.22	+

[a] As per conditions of (Koschella et al., 2001).

the deprotected product was acetylated to the corresponding 3-*O*-EG-2,6-di-*O*-acetyl cellulose, purified and analyzed.

Using an excess of TBAF (4:1 TBAF:TDMS group) and 50 °C for 1 day led to complete deprotection, but also to extensive degradation of the cellulosic backbone and a low Mn (Table 4, entry 1). Therefore, more moderate reaction conditions were examined. It was found that a 1:1 stoichiometry of TBAF:TDMS groups in cellulose was sufficient for deprotection (Table 4: entry 2–4). Likewise, heating the reaction was unnecessary (Table 4: entry 4 vs. 6). Reactions carried out at room temperature had significantly higher Mn values as compared to those run at 50 °C. It was also found that the reactions needed to be run overnight (18 h) as shorter (8 h) reaction times did not sufficiently remove the TDMS groups. Fig. 2b and c show the presence of the TDMS C–Si stretching bands at 834 and 778 cm^{-1}.

The deprotected 3-*O*-EG cellulose (**3a–c**) was treated with acetic anhydride in DMAc and imidazole. The acetylated product was obtained after 48 h in 80 °C producing **4a–c** which were soluble in chloroform, and therefore purification was available. Full acetylation was determined by FT-IR (Fig. 2d) were neither TDMS stretching (834 and 778 cm^{-1}) nor OH stretching (between 3000 and 3600 cm^{-1})(Kadla et al., 2007) bands are present, and there is a strong carbonyl stretching band at 1748 cm^{-1}.

Well-resolved ^1H and ^{13}C NMR spectra were obtained for compounds **4a–c**. Fig. 3 shows the ^{13}C NMR spectrum of 3-*O*-EG_3-2,6-di-*O*-acetyl cellulose (**4a**). The carbon spectrum reveals the carbonyl peak of the acetyl groups at δ = 170.5–169.3 ppm, the cellulose peaks: 99.8 ppm (C1), 76.4–72.2 ppm (C2–5) and 62.8 ppm (C6), EG peaks at 71.9–70.1 ppm, MeO of EG at 58.5 ppm, methyl of the acetyl groups at 20.9–20.5, and traces of the TDMS group at 18.8 ppm. The $DS_{(EG)}$ of the starting material **2a** was determined to be 0.50 by quantitative ^{13}C NMR, and as expected, there was no change in the $DS_{(EG)}$ of 3-*O*-EG_3-2,6-di-*O*-acetyl cellulose (**4a**). The relative integral of the MeO of EG at 58.5 ppm was 0.5 as compared to that of 1.0 for the C1 of cellulose at 99.8 ppm (Fig. 3b). As there was not complete EG group substitution of the cellulose C3 position, integration of the acetyl carbonyl carbons at 170.5–169.3 ppm was greater than 2 but lower than 2.5. This lower than expected value is the result of the trace amount of TDMS groups still present in the 3-*O*-EG_3-2,6-di-*O*-acetyl cellulose (TDMS peak at 18.8 ppm – Fig. 3a).

Regioselectivity can clearly be determined by carbon NMR. The C1 field-shift is influenced by the O2 substituent (Ac), (Heinze, Talaba, & Heinze, 2000) wherein one signal for C1 implies complete substitution of C2 by a single moiety. Likewise, a single signal for the C6 implies a single substituent on the corresponding O6. The regioselectivity of **4a** was confirmed by means of two-dimensional NMR techniques, which are presented in Figs. 4 and 5. (Note: based on the DS of the EG substituent, $DS_{(EG)}$ = 0.5, the NMR spectrum represent both segments **4a** (3-EG_3-2,6-Ac-cellulose) and **4a′** (2,3,6-Ac-cellulose) repeat units).

Signals assignments were determined by crosspeak correlations using both HSQC and COSY spectra. Accordingly, H1 and H6a, b were easily assigned by correlations in the HSQC spectrum (δ_H/

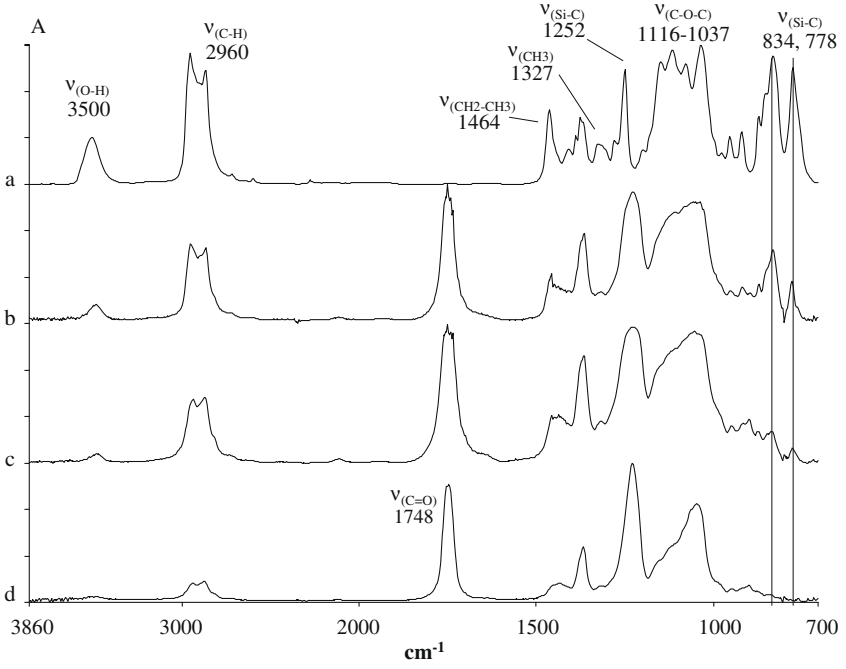


Fig. 2. FT-IR spectrum of 3-EG_3-TDMS-cellulose (a) and 3-EG_3-Ac-cellulose (b–d) corresponding to Table 4.

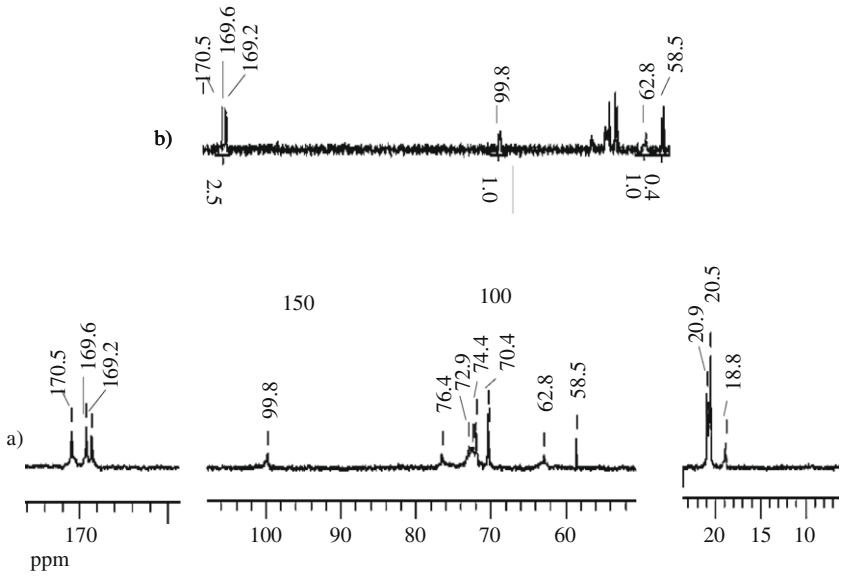


Fig. 3. (a) ^{13}C NMR spectrum of 3-EG$_3$-2,6-Ac-cellulose (**4a**) and (b) quantitative ^{13}C NMR spectrum of 3-EG$_3$-2,6-Ac-cellulose (**4a**). All experiments were run in DMSO-d_6 at 70 °C.

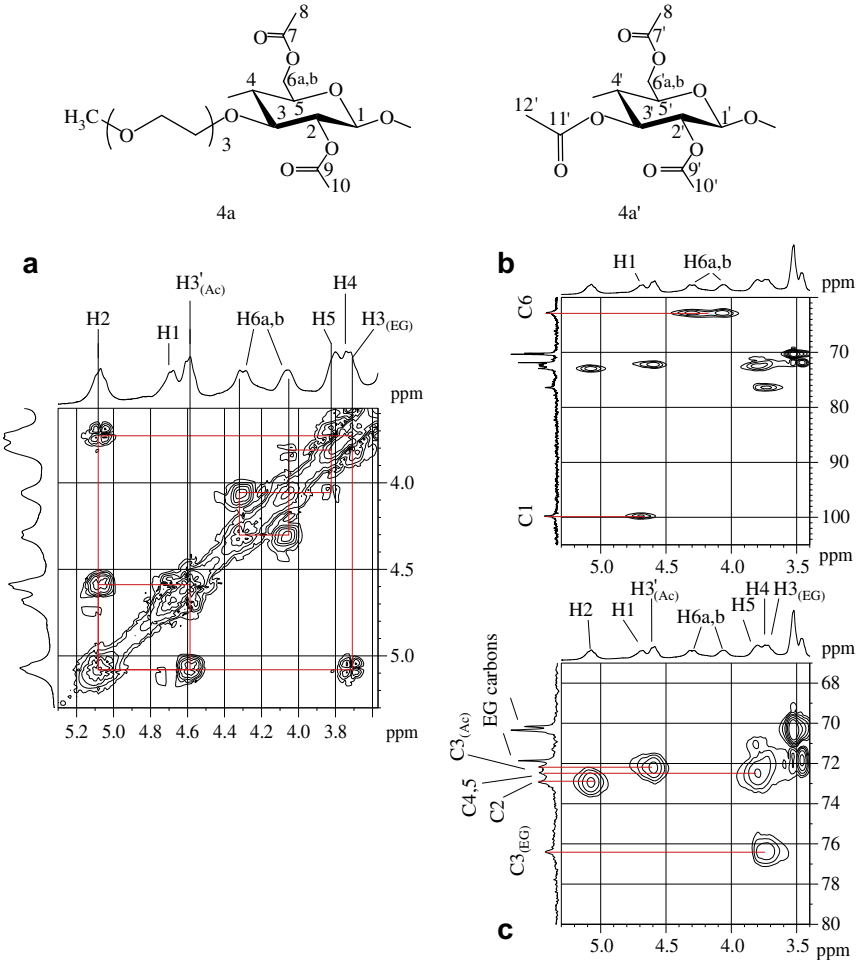


Fig. 4. NMR analysis of 3-EG$_3$-2,6-Ac-cellulose (**4a**): (a) ^{1}H/^{1}H correlated spectrum (COSY); (b) and (c) ^{1}H/^{13}C heteronuclear single quantum correlation (HSQC). All experiments were run in DMSO-d_6 at 70 °C.

δ_C; H1/C1 ~ 4.67/99.8; H6/C6 ~ 4.32–4.06/62.8). COSY correlations assigned H2 (~5.07 pm) by correlation with its neighbors H3$_{(EG)}$ and H3′$_{(Ac)}$. H5 (~3.80 ppm) was assigns by crosspeak correlation with one of the H6 protons (~4.06 pm), and H4 (~3.75 ppm) was assigns by crosspeak correlation with H5. As the DS of the EG groups was 0.5, the acetylated product produced two signals for

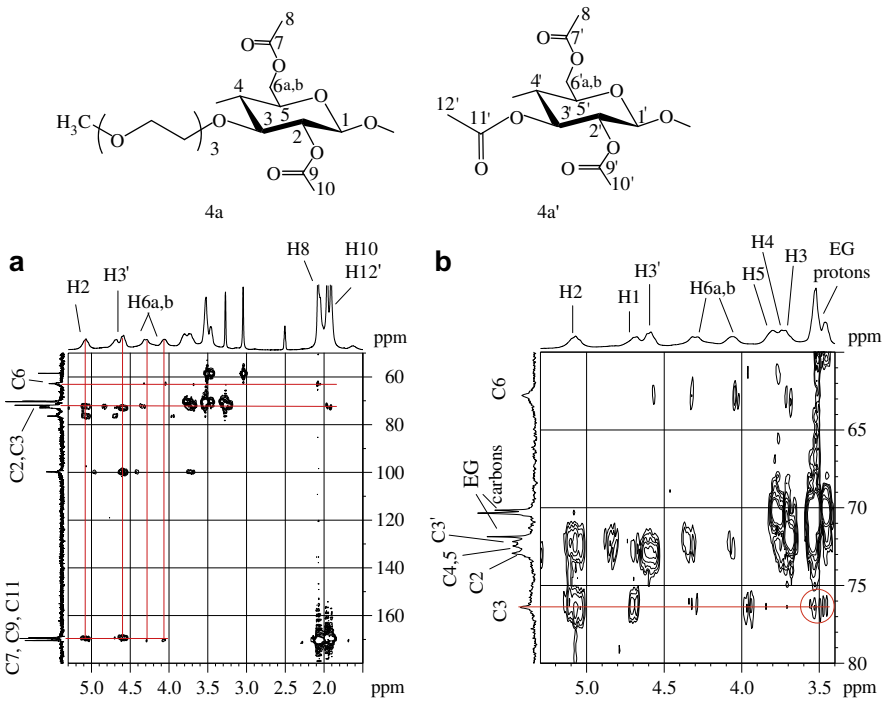


Fig. 5. $^{1}H/^{13}C$ heteronuclear multiple bond correlation experiment (HMBC).

H3, a high field H3 at ~3.72 ppm due to the regioselective EG functionalization, and a lower field H3′ at ~4.59 ppm arising from acetylation of the non-EG derivatized hydroxyl groups. The remaining downfield methine protons correspond to the acetylated OH groups at H6a, H6b (~4.32–4.06 ppm) and H2 (~5.07 ppm). No additional highfield correlations for these protons are present, confirming the regioselectivity and complete acetylation of these positions. Finally, complete carbon peak assignment was made from the HSQC spectrum (Fig. 4b and c); C1 at 99.8 ppm, C5 at 76.4 ppm, C-2,3,4 at 72.9–72.2 ppm, EG carbons at 71.9–70.2 ppm and C6 at 62.8 ppm.

Further structure conformation was obtained from the $^{1}H/^{13}C$ heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 5). Long-range correlations are observed between the C6 and H8, C2 and H10, H6 and C7, and H2 and C9. Similarly, the same correlations exist for the fully acetylated repeat unit **4a**′, i.e. C3′ and H12′, and H3′ and C11′. Further examination of the anhydroglucopyranose region (Fig. 5b), long-range correlations between $C3_{(EG)}$ and EG protons are clearly shown, however no similar correlations are present for C2 or C6.

4. Conclusions

3-*O*-ethylene glycol 2,6-di-*O*-thexyldimethylsilyl cellulose and 3-*O*-ethylene glycol 2,6-di-*O*-acetyl cellulose with ethylene glycol oligomers ($m = 3$, 7, 16) were synthesized and characterized by one- and two-dimensional NMR spectroscopy. COSY, HSQC and HMBC correlations confirmed selective 3-*O*-ethylene glycol substitution. T1 experiments enabled the development of a quantitative ^{13}C NMR technique to determine accurately the degree of ethylene glycol substitution. Through the use of a relaxation agent (Cr(III)-acac), a dramatic reduction in the time to obtain a quantitative ^{13}C NMR spectrum was achieved. Optimization of reaction conditions lead to degrees of C_3–O substitution as high as 0.8 and yields of >70%. Increasing the ethylene glycol oligomer size decreased product yield and DS. Reaction time, temperature and reagents all affected the DS and Mn values of the synthesized polymers.

Acknowledgements

The authors greatly acknowledge NSERC for financial support of this research.

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