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Synthesis of highly functionalized dextran alkyl carbonates showing nanosphere formation

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ABSTRACT

Highly substituted dextran alkyl carbonates were synthesized applying different acylating agents. The influence of the reaction media and the alkyl chain length on the reaction efficiency was systematically studied. Moreover, fluoroformates were successfully adapted as highly efficient and easy to handle reagents for the synthesis of dextran alkyl carbonates. The products obtained were clearly described by means of NMR- and IR spectroscopy. The dextran esters form spherical nanoparticles of a size below 300 nm that might be applied for delivery of therapeutic- or imaging agents.

1. Introduction

Dextran is the generic term for a family of neutral, water soluble polysaccharides consisting of a α-(1→6) linked D-glucose main chain with varying branches. The content and type of branches depend on the origin and ranges from 3 to 50% (DeBelder, 1993). In general branches may arise from α-(1→2), α-(1→3), and α-(1→4) glycosidic bonds (Taylor, Cheetham, & Walker, 1985). Due to the common solubility in water and dipolar aprotic solvents, the biocompatibility, and the ability of degrading in certain physical environments, dextran is already successfully applied in the medical and biomedical field for more than 4 decades (DeBelder, 1996).

In the context of our work to design smart polymers by chemical modification of polysaccharides, including polysaccharide-based nanoparticles our interest is focused on biopolymer derivatives with a balanced hydrophobic/hydrophilic character (Heinze, 2009). Moreover, pH-sensitive polysaccharide materials are of considerable interest for targeted and sustained drug delivery (Bachelder, Beaudette, Broaders, Dashe, & Frechet, 2008; Daus & Heinze, 2010). In particular, carbonic esters of dextran were applied as intermediates in syntheses and as support for delivery of therapeutic or imaging agents (Larsen, 1989; Mehvar, 2000; Vandoorne, Bruneel, Vercauteren, & Schacht, 1991; Vandoorne, Vercauteren, Permentier, & Schacht, 1985). The carbonate linkage hydrolyze under physiological conditions and thus lead to a release of grafted molecules (van Dijk-Wolthuis, van Steenbergen, Underberg, & Hennink, 1997).

First attempts to utilize the carbonate linkage for the covalent binding of different functional moieties were carried out via the activation of dextran with phosgene (Barker, Disney, & Somers, 1972). This approach is limited due to the difficulties in the handling of phosgene and due the fact that it is combined with a number of side reactions. More useful is the conversion of dextran with chloroformates to give the the corresponding carbonates. Chloroformates are known to lead to the formation of cross-linked polysaccharides together with cyclic and linear carbonates (Chaves & Arranz, 1985; Doane, Shasha, Stout, Russell, & Rist, 1968; Rudel, Gabert, & Möbius, 1978). The application of chloroformates for the functionalisation of dextran and other polysaccharides is still challenging due to a number of side reactions arising from their high reactivity (Fig. 1). Beside the formation of the carbonate linkage (Fig. 1a), one of the main side reactions is the hydrolysis of the acylating agent (Fig. 1b). Moreover, the application of common dipolar aprotic solvents, typically applied in polysaccharide chemistry, may result in the formation of salts as shown in Fig. 1c and d (Dang & Olofson, 1990). Finally, the decomposition of chloroformates catalyzed by chloride ions may appear (Fig. 1e) that complicate the application of typical polysaccharide solvents like *N*,*N*-dimethylacetamide/LiCl.

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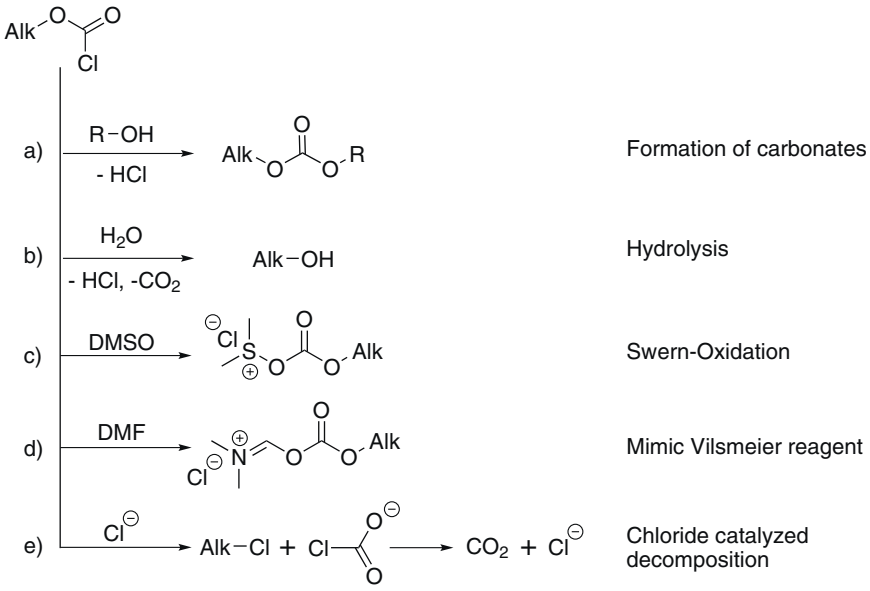

Fig. 1. Reactions of chloroformates.

In the present work we describe the synthesis of highly substituted dextran alkyl carbonates. In order to overcome the drawbacks mentioned the influence of the reaction media, the alkyl chain length, and the type of acylating agent on the reaction efficiency were investigated. Moreover, preliminary results about the formation of nano-scaled particles are described as well.

2. Experimental

2.1. Materials

Dextran (Fluka) produced by *Leuconostoc mesenteroides* strain no. NRRL B-512(F) possesses a $\bar{M}_w$ of 54,400 g/mol and a $\bar{M}_n$ of 34,960 g/mol. The α-(1→6) linked glucose main chain contains about 5% of randomly distributed α-(1→3) branches. A detailed structure analysis of the dextran sample **1** was published recently (Heinze, Liebert, Heublein, & Hornig, 2006). Butyl fluoroformate **3d** was obtained according to the procedure described by (Cuomo & Olofson, 1979). Yield: 80% ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 4.30 (t, J = 6.6 Hz, OCH_2), 1.70 (m, CH_2), 1.40 (m, CH_2), 0.96 (t, 7.3 Hz, CH_3) ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 147.9, 143.4 (d, $J^{C\text{-}F}$ = 284 Hz, C=O), 71.4 (OCH_2), 30.1 (CH_2), 18.6 (CH_2), 13.5 (CH_3). Other chemicals were purchased from Aldrich and were used without further treatment.

2.2. Measurements

NMR spectra were acquired on a Bruker Avance 250 MHz and a Bruker Avance 400 MHz with 16 scans and 25 mg sample per mL solvent for ^{1}H NMR spectroscopy (room temperature) and up to 200,000 scans for ^{13}C NMR spectroscopy (70 °C) applying up to 100 mg sample per mL solvent. FTIR spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer with the KBr technique. Elemental analysis were performed by CHNS 932 Analyzer (Leco). The diameter and polydispersity of the nanoparticles were determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instrument, Malvern, UK). The mean particle size was approximated as the effective (z-average) diameter and the width of the distribution as the polydispersity index (PDI) obtained by the cumulants method assuming spherical shape. For scanning electron microscopy (SEM) studies, one droplet nanoparticle suspension on a mica surface was lyophilized and covered with platinum. The images were obtained with the SEM equipment LEO-1 450 VP (LEO, Oberkochen, Germany) operating at 15 kV.

2.3. Synthesis

2.3.1. Synthesis of dextran ethyl carbonate in dimethyl sulfoxide (DMSO)

Dextran **1** (1 g, 6.17 mmol) was dissolved in 10 mL dry DMSO at 40 °C using a doublewall reactor. Pyridine (2.4 mL, 28 mmol) was added and the solution was cooled to 6 °C. Ethyl chloroformate **2b** (2.7 mL, 28 mmol) was added drop-wise during 1 h. The mixture was allowed to react for 4 h at 6 °C under stirring. The crude product was isolated by precipitation in 150 mL ethanol, washed 4 times with 100 mL ethanol and dried in vacuum at 40 °C. The sample was reprecipitated from 5 mL DMSO in 100 mL ethanol. Yield: 56%, DS = 0.71 (obtained by ^{1}H NMR spectroscopy after peracetylation), ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 154.8 (C=O), 98.7 (C-1), 95.8 (C-1S), 77.0 (C-2S), 73.7 (C-3), 72.3 (C-2), 70.7 (C-4 + C-5), 69.8 (C-3), 66.5-64.7 (C-6), 64.2 (OCH_2), 14.4 (CH_3).

2.3.2. Synthesis of dextran alkyl carbonates in N,N-dimethylformamide (DMF)/LiCl or N,N-dimethylacetamide (DMAc)/LiCl applying chloroformates, general procedure

Dextran **1** (1 g, 6.17 mmol) was stirred in 30 mL dry DMF or DMAc for 2 h at 120 °C. After cooling the suspension to 80 °C, LiCl (0.3 g) was added and the mixture was stirred until a clear solution was obtained. The dextran solution was filled into a double-wall reactor with septum under argon and cooled to 5 °C. After the addition of pyridine, alkyl chloroformate **2** was added slowly, to avoid strong evolution of gas and precipitation. After stirring for 24 h at 5 °C, the reaction mixture was precipitated in 300 mL water or 2-propanol. The precipitate was filtered off and washed 4 times with 150 mL water or 2-propanol. The product was dried in vacuum at 40 °C and purified by reprecipitation from 5 mL DMSO in 100 mL water.

2.3.3. Synthesis of dextran butyl carbonates applying butylfluoroformate, general procedure

To a solution of dextran **1** (1 g, 6.17 mmol) in 20 mL DMF/LiCl or in 10 mL DMSO butylfluoroformate and triethylamine both

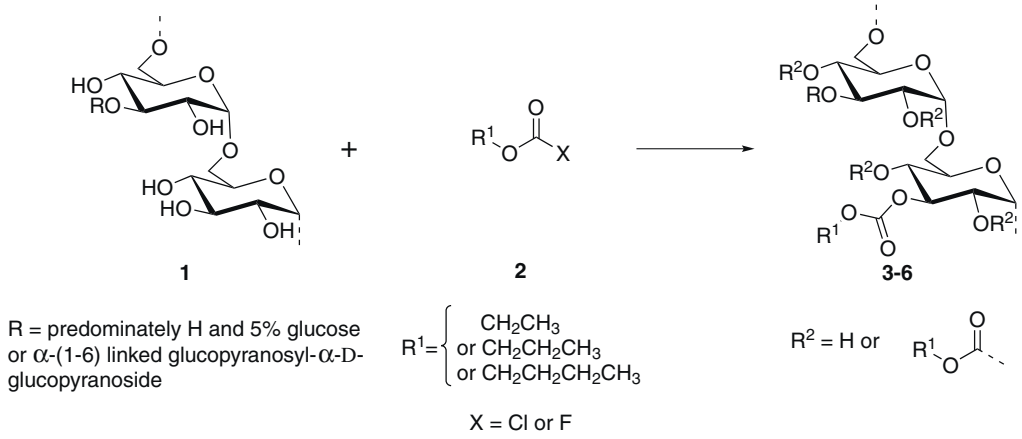

Fig. 2. Reaction scheme of the synthesis of dextran alkyl carbonates (**3–6**) applying alkyl chloroformates and -fluoroformates.

dissolved in 5 mL of DMF or DMSO were added. After stirring for 24 h at 60 °C, the product was isolated by precipitation in 300 mL water. The precipitate was filtered off and washed 4 times with 150 mL water. The crude product was dried in vacuum at 40 °C and purified by reprecipitation from 5 mL DMSO in 100 mL water.

2.3.4. Peracetylation, general procedure

Dextran alkyl carbonate **5** (300 mg) was dissolved in 5 mL DMF. Subsequently 5 mL pyridine, 5 mL acetic anhydride, and 50 mg 4-dimethylaminopyridine were added and the solution was allowed to react for 24 h at 60 °C under stirring. After precipitation into 150 mL of a saturated aqueous solution of $NaHCO_3$, the product was isolated by centrifugation, washed 4 times with 100 mL water and dried in vacuum at 40 °C. IR (KBr): no ν_{OH}.

2.3.5. Titration

Dextran alkyl carbonate (100 mg) was treated with 20 mL NaOH solution (0.1 M) under argon at 60 °C. The mixture was stirred for additional 5 h until the solution becomes clear. After cooling to room temperature, the sample was titrated back with hydrochloric acid (0.1 M) measuring the pH-value.

2.3.6. Synthesis of cyclic dextran carbonate

Dextran **1** (0.5 g, 3.1 mmol) was dissolved in dry DMSO at 40 °C and dioxane (0.75 mL) and triethylamine (2.5 mL, 18 mmol) was added. After cooling to 0 °C with ice, ethyl chloroformate (1.0 mL, 10.5 mmol) was added in 3 min and the reaction mixture was stirred for additional 2 min. Adding 5 M hydrochloric acid (0.8 mL), pH 6 was adjusted. After precipitation in 100 mL ethanol, the product was filtered off, washed 4 times with 100 mL ethanol and dried in vacuum at 40 °C. Yield: 528 mg, IR: 1810 cm^{-1} ($\nu_{C=O}$).

Table 1
Conditions for and results of the synthesis of dextran alkyl carbonates.

Reagent[a]	Solvent[b]	Temperature (°C)	Molar ratio[c]	No.	DS[d]	Yield (%)
EC	DMF/LiCl	4	2.0	**3a**	1.13	52
EC	DMF/LiCl	4	3.0	**3b**	1.41	52
EC	DMSO	6	4.5	**3c**	0.71	56
EC	DMAc/LiCl	4	4.5	**3d**	1.50	69
EC	DMF/LiCl	4	4.5	**3e**	1.71	71
EC	DMF/LiCl	4	10.0	**3f**	2.19	84
EC	DMAc/LiCl	4	15.0	**3g**	2.30	68
EC	DMF/LiCl	4	15.0	**3h**	2.46	69
EC	DMF/LiCl	4	30.0	**3i**	2.80	81
PC	DMF/LiCl	4	2.0	**4a**	1.07	32
PC	DMF/LiCl	4	3.0	**4b**	1.24	71
PC	DMF/LiCl	4	4.5	**4c**	1.54	81
PC	DMF/LiCl	4	10.0	**4d**	2.13	80
PC	DMF/LiCl	4	15.0	**4e**	2.37	78
PC	DMF/LiCl	4	30.0	**4f**	2.77	85
BC	DMF/LiCl	4	1.0	**5a**	0.45	90
BC	DMF/LiCl	4	2.0	**5b**	1.13	86
BC	DMF/LiCl	4	3.0	**5c**	1.38	84
BC	DMF/LiCl	4	4.5	**5d**	1.63	81
BC	DMF/LiCl	4	10.0	**5e**	2.12	84
BC	DMF/LiCl	4	15.0	**5f**	2.34	84
BC	DMF/LiCl	4	30.0	**5g**	2.68	83
BF	DMSO	60	1.0	**6a**	0.91	65
BF	DMF/LiCl	60	1.0	**6b**	0.35	85
BF	DMSO	60	4.5	**6c**	3.00	78
BF	DMF/LiCl	60	4.5	**6d**	3.00	76

[a] EC: ethyl chloroformate, PC: propyl chloroformate, BC: butyl chloroformate, and BF: butylfluoroformate.
[b] DMSO: dimethyl sulfoxide, DMF: *N*,*N*-dimethylformamide, and DMAc: *N*,*N*-dimethylacetamide.
[c] Mole reagent per mole anhydroglucose unit.
[d] Degree of substitution (DS) determined by means of ^{1}H NMR spectroscopy after peracetylation.

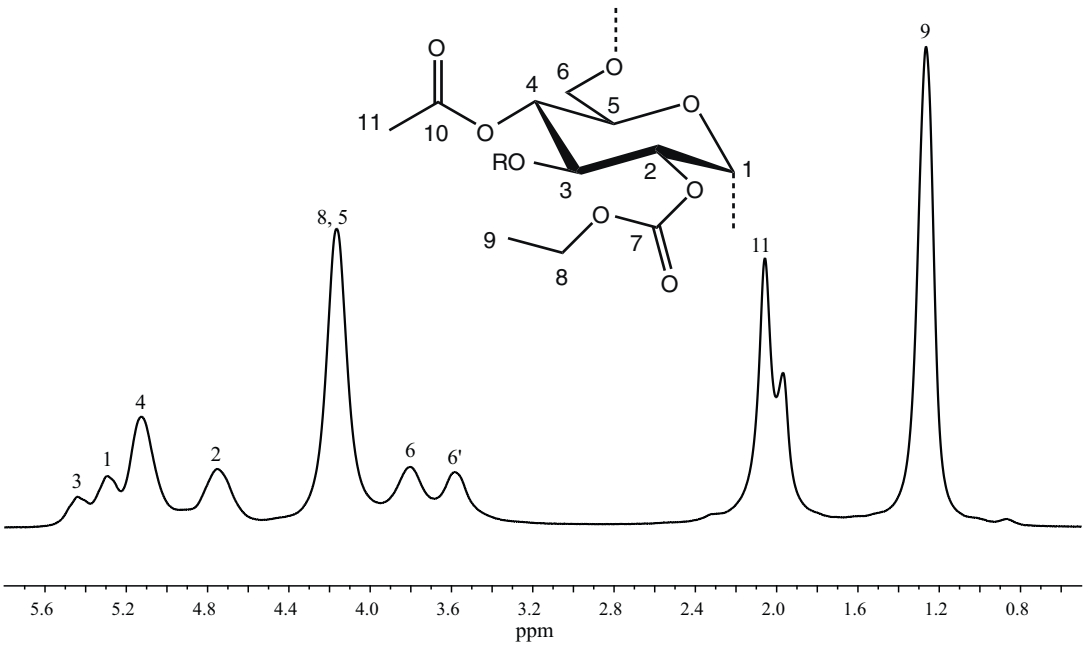

Fig. 3. ^{1}H NMR spectrum of peracetylated dextran ethyl carbonate **3e** (degree of substitution 1.71) recorded in $CDCl_3$.

2.3.7. Nanoparticle preparation by dialysis

The dextran ester (20 mg) was dissolved in 5 mL DMAc and dialyzed against 500 mL distilled water in a regenerated cellulose dialysis membrane with a molecular weight cut off of 3500 g/mol (Spectra/Por®: 3). The water was renewed 5 times after at least 3 h.

3. Results and discussion

3.1. Formation of dextran alkyl carbonates

As schematically shown in Fig. 2, dextran (**1**) was allowed to react with different alkyl chloroformates and -fluoroformates (**2**) in order to obtain highly substituted dextran alkyl carbonates (**3–6**). Table 1 summarizes the conditions for and results of the synthesis of dextran alkyl carbonates.

In a first set of experiments, the dependence of the reaction of dextran and ethyl chloroformate on the solvent used for esterification was studied. The synthesis was carried out in dimethyl sulfoxide (DMSO), *N*,*N*-dimethylacetamide/lithium chloride (DMAc/LiCl) or *N*,*N*-dimethylformamide/lithium chloride (DMF/LiCl), respectively, under comparable conditions (Table 1: **3c**, **3d**, and **3e**). The degrees of substitution (DS) of the products obtained are strongly dependent on the solvent. The results clearly reveal lower DS values and thus a lower reaction efficiency in DMSO compared to the solvents DMAc/LiCl and DMF/LiCl. Moreover, the yield is about 15% lower applying DMSO. This results are in good agreement with literature data (Nichifor, Coessens, & Schacht, 1995; Ramirez, Sanchezchaves, & Arranz, 1995). They may be explained by a nucleophilic attack of the solvent to the reagent (Fig. 1) and a partial oxidative degradation of the polymer backbone (Dang & Olofson, 1990; Heinze, Liebert, & Koschella, 2006). The reactions in DMAc/LiCl or DMF/LiCl lead to products with comparable DS values and yields. The slightly higher DS values obtained in DMF/LiCl may result from a better solubility of the chloroformate in DMF. Moreover, the reaction of chloroformates with tertiary amides (Fig. 1d) may be less pronounced in DMF. Based on these experiments all further reactions of dextran with alkyl chloroformates were carried out in DMF/LiCl.

Dextran (**1**) was allowed to react with ethyl-, propyl-, and butyl chloroformate in order to study the dependence of the DS of carbonate moieties on the alkyl chain length of the chloroformate used (Table 1: **3a–i**, **4a–f**, and **5a–g**). In general, the application of ethyl chloroformate led to the products with highest DS values. For low molar ratios, the reaction with butyl chloroformate showed the same efficiency as the ethyl analogue, while propyl chloroformate led to lower DS values (e.g. samples **3a**, **4a** and **5b**). At high molar

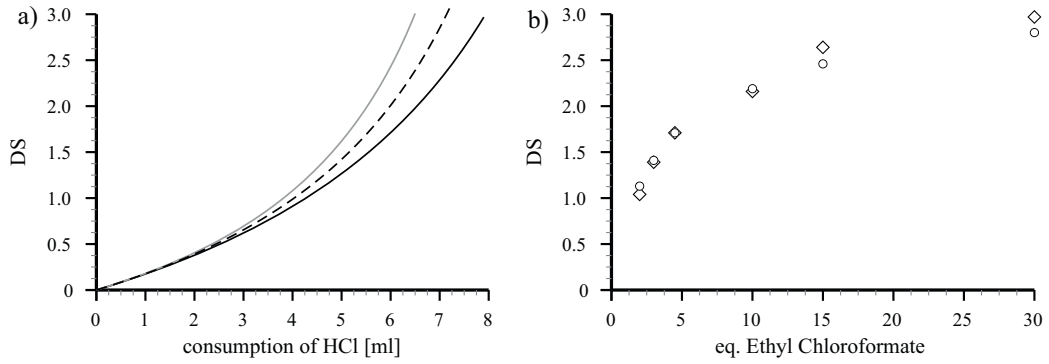

Fig. 4. (a) Graphical plot of the correlation of the degree of substitution (DS) and the consumption of hydrochloric acid during back-titration with HCl after the complete hydrolysis with NaOH: dextran ethyl- (black), propyl- (dashed), and butylcarbonates (gray); (b) (DS) values of dextran ethyl carbonates determined by means of titration (◊) and by means of ^{1}H NMR spectroscopy after peracetylation (○).

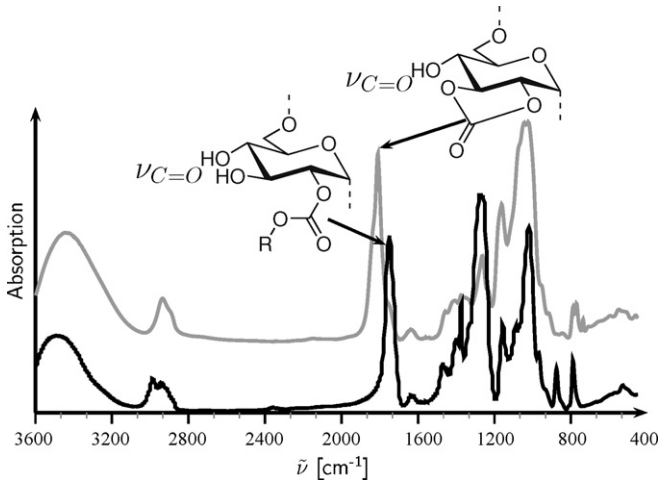

Fig. 5. IR spectra of dextran carbonates bearing cyclic carbonate moieties (gray) and acyclic carbonate moieties **3e** (black).

ratios, propyl- and butyl chloroformate display the same reactivity. The small differences of the DS values make it difficult to give a definitive interpretation. However, different facts can be taken into consideration. First, steric factors may cause a decrease of reaction efficiency with increasing alkyl chain length. Moreover, the reactivity of the carbonyl group of the chloroformate is changed due to the difference of the inductive effects by the different alkyl chains, on one hand. On the other, the decrease in carbonyl activity is known to decrease the decomposition rate of the n-alkyl chloroformates, induced by chloride ions shown in Fig. 1e (Kevill & Weitl, 1968).

The use of chloroformates is not the method of choice for the synthesis of highly substituted dextran carbonates. Due to their hydrolytic instability (Fig. 1b) and due to the other possible side reactions (Fig. 1c–e), a very high molar excess of reagent is needed to obtain high DS values (Table 1: **3i**, **4f**, and **5g**). Therefore, alkyl fluoroformates were studied as alternative reagents. Unlike chloroformates, fluoroformates should be effective acylating agents due to the fact that the stability of fluoroformates in tertiary amide solvents contrasts sharply with the behavior of chloroformates Dang & Olofson, 1990). For example, the reaction could be carried out at the relatively high temperature of 60 °C. Applying chloroformates temperatures of 4–6 °C must be utilized to avoid the complete decomposition of the reagent.

Butyl fluoroformate was allowed to react with dextran in DMF/LiCl and DMSO (Table 1: **6a–d**). Surprisingly, the products obtained in DMF/LiCl do not display higher DS values at a molar ratio of 1:1 (Table 1: **6b**). DS values of 0.45 and 0.35 were obtained applying the chloroformate and fluoroformate, respectively, in DMF/LiCl. In contrast, the reaction in DMSO led to an increase of the DS to 0.91 at this molar ratio. At a molar ratio of 1:4.5 an improved reaction efficiency could be noticed in both solvents DMSO and DMF/LiCl. Thus, a DS of 3 is obtained applying the fluoroformate (independent of the solvent) while in case of the chloroformate only a DS of 1.63 can be achieved. For the reaction in DMF/LiCl, these results seem to be contradictory to each other. However, the presence of Cl^- ions (7.1 mmol per 6.17 mmol dextran, see Section 2) in the solvent DMF/LiCl may cause a halide exchange and thus convert the butyl fluoroformate to its corresponding chloro compound. Once formed, a decomposition of the chloroformate takes place due to the high temperature of 60 °C (Fig. 1). These processes are the reason for the low DS value at a molar ratio of 1:1 applying butyl fluoroformate at 60 °C in DMF/LiCl (Table 1: **6b**). As mentioned above, the reaction in DMSO (no LiCl) led to a significant higher DS value at this molar ratio (Table 1: **6a**). However, we want to emphasize that applying butyl fluoroformate with molar ratio of 1:4.5, completely functionalized products (DS 3) were obtained in both solvents DMSO and DMF/LiCl. Applying butyl chloroformate, it is impossible to obtain a completely functionalized product even at molar ratio of 1:30. Moreover, the reaction of butyl fluoroformate in DMSO shows a very high rate of conversion even at low molar ratio.

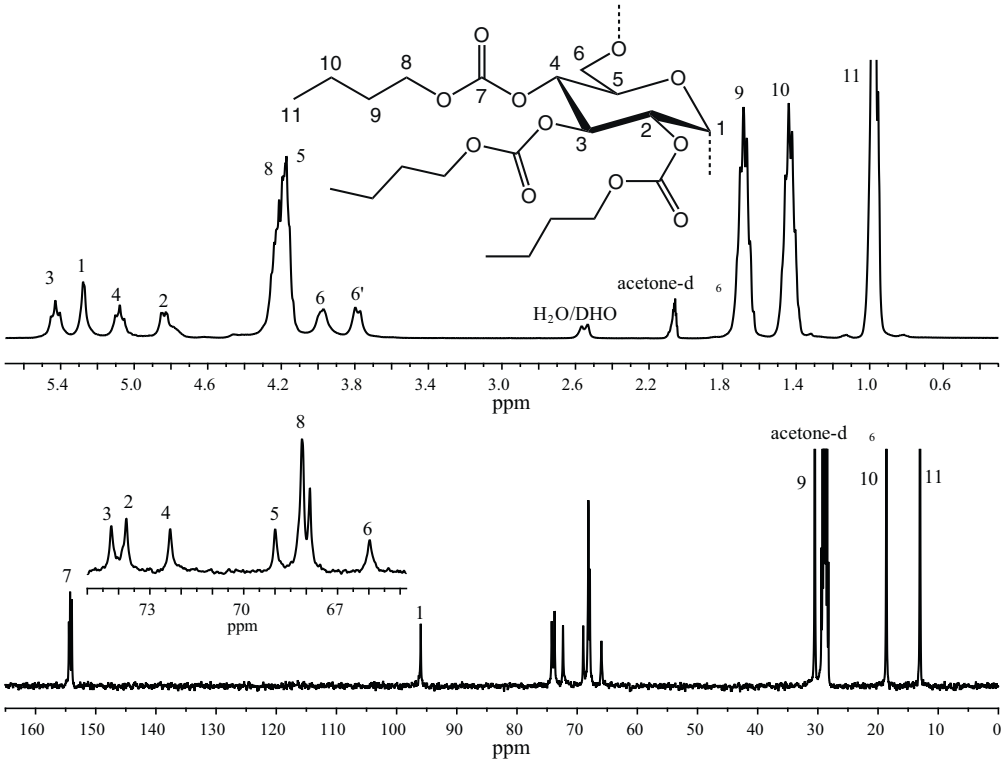

Fig. 6. ^{1}H (top) and ^{13}C NMR (bottom) spectra of dextran butyl carbonate **6d** (degree of substitution 3.0) recorded in acetone-d_6.

3.2. Structure characterization of dextran alkyl carbonates

The DS values were determined by titration applying the procedure first described by Koltsova et al. (1977). Therefore, the samples were hydrolyzed with NaOH solution (0.1 M) under argon until a clear solution was obtained. Subsequently, the sample was titrated back with hydrochloric acid (0.1 M) measuring the pH-value. The correlation of consumption of hydrochloric acid and the DS is shown in Fig. 4a with respect to the alkyl carbonate moiety. Moreover, ^{1}H NMR spectroscopy of the polysaccharide derivatives was applied for the determination of the DS. Therefore, the remaining OH groups were peracetylated to eliminate intra- and intermolecular interactions of the polymer chains by hydrogen bonds and any overlap of the signals of the protons of the AGU and the hydroxyl groups. Fig. 3 shows the ^{1}H NMR spectrum of the peracetylated dextran ethyl carbonate with a DS of 1.71 recorded in $CDCl_3$. The proton resonances of the ethyl carbonate ester moiety appear at 1.265 ppm (CH_3) and 4.168 ppm (CH_2). The ratio of the spectral integrals of the CH_3 protons of the ethyl carbonate versus the CH_3 protons of the acetate moiety (at about 2 ppm) were used to calculate the DS according to:

$$\mathrm{DS} = \frac{3I_{\mathrm{H9}}}{(I_{\mathrm{H9}} + I_{\mathrm{H11}})}$$

In Fig. 4b the DS values obtained by titration and ^{1}H NMR spectroscopy are compared, indicating that they agree very well. However, as shown in Fig. 4a the difference in consumption of hydrochloric acid decreases with increasing DS. Therefore, for highly functionalized carbonates the titration method has a remarkable higher operational error, which might explain the slightly higher DS values. Thus, we apply ^{1}H NMR spectroscopy after peracetylation as standard method for the determination of the DS. In addition to DS measurements, the molecular structure of the dextran esters was investigated applying FTIR-, ^{1}H, and ^{13}C NMR spectroscopy.

FTIR measurements were applied to prove the presence of the carbonate linkage and the absence of cyclic carbonate moieties. Fig. 5 shows the FTIR spectra of dextran carbonates bearing cyclic and acyclic carbonate moieties. Both spectra show an intense absorption arising from the $\nu_{C=O}$ stretching. In case of cyclic carbonate moieties the signal occurs at 1805 cm^{-1} while the acyclic carbonate moieties give rise to a signal at 1745 cm^{-1} (Rannard & Davis, 1999).

As shown for dextran butyl carbonate (**6d**), the dextran derivatives can be clearly described by means of NMR spectroscopy. Fig. 6 shows the ^{1}H and ^{13}C NMR spectra recorded in acetone-d6. Applying total correlation NMR spectroscopy (TOCSY), it was possible to assign all proton resonances. The TOCSY spectrum shown in Fig. 7 (top) was acquired with a spin-lock time of 15 ms. Therefore, only coupled pairs of protons separated by two or three bonds were detected. The assignment of the proton resonances results from the cross-peaks connecting the hydrogen nuclei H-3 and H-4 (solid), H-3 and H-2 (dashed), H-1 and H-2 (dotted), and H-4 and H-5 (gray). The cross-peak connecting H-5 and H-6 is not visible at this spin-lock time because of its weak coupling and the overlap of H-5 and H-8. In the ^{13}C NMR spectrum (Fig. 6, bottom) the resonances were assigned by combining the results of the TOCSY measurement and heteronuclear single quantum coherence (HSQC) NMR spectroscopy. The HSQC spectrum shown in Fig. 7 (bottom) allows a clear assignment of all ^{13}C-signals. It became obvious that the previous assignment of both proton- and ^{13}C-resonances by (Arranz, Roman, & SanchezChaves, 1987) was not completely right. The proton resonances of the butyl moiety appear at 0.97 (H-11), 1.44 (H-10), and 1.68 ppm. The signal of methylene group attached to oxygen (H8) is overlapped by H-5 and appears at about 4.1 ppm. The

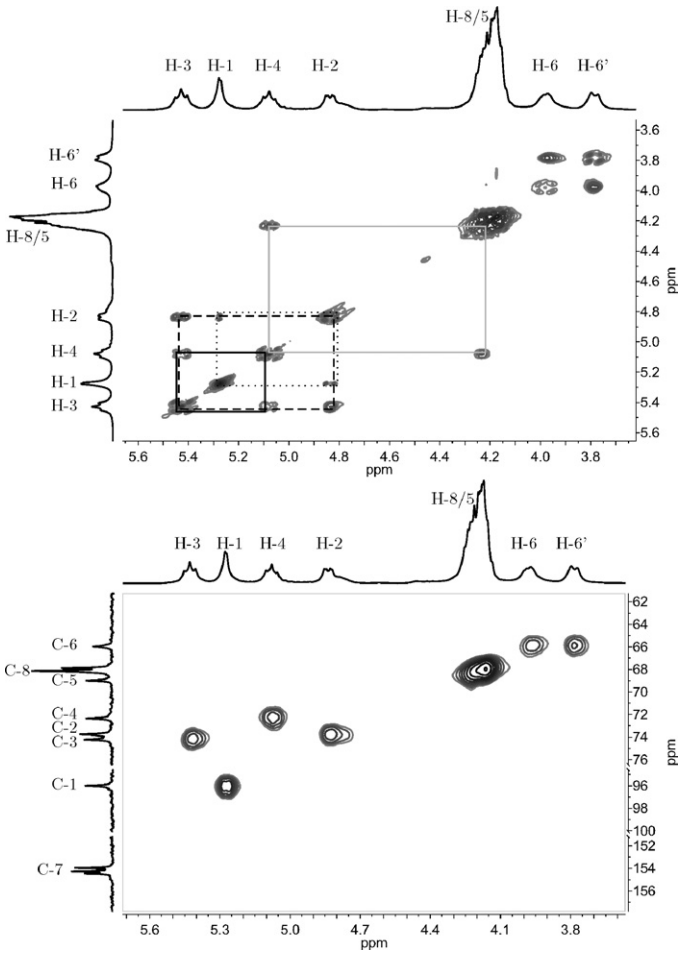

Fig. 7. Detail of the total correlation (TOCSY, top) and heteronuclear single quantum coherence (HSQC, bottom) NMR spectra of dextran butyl carbonate **6d** (degree of substitution 3.0) recorded in acetone-d$_6$.

signals resulting from the hydrogen atoms of the polymer backbone are visible at 5.27 (H-1), 4.83 (H-2), 5.42 (H-3), and 3.98/3.78 ppm (H-6/H-6′). The signals in the ^{13}C NMR spectrum arising from the butyl moiety are visible at 68.1, 67.9 (C8), 30.5 (C9), 18.6 (C10), and 13.1 ppm (C11). The signals resulting from the carbon atoms of the AGU appear at 96.0 (C-1), 73.8 (C-2), 74.2 (C-3), 72.4 (C-4), 69.0 (C-5), and 66.0 ppm (C-6). The esterification of the polymer backbone with butyl carbonate is proved by the appearance of the resonances of the carbonyl group (C7) at about 154 ppm. In further NMR experiments the regiochemistry will be studied with respect to the DS values and the acylating agent used. The results will be published elsewhere.

3.3. Nanosphere formation

The dextran carbonates are of interest for the preparation of nanoscaled particles that might be applied for delivery of therapeutic- or imaging agents. Recently, we found that tailored dextran esters form nanospheres by applying a simple solvent exchange (Heinze & Hornig, 2010; Liebert, Hornig, Hesse, & Heinze, 2005; Wondraczek & Heinze, 2008). Thus, preliminary nanosphere formation experiments were carried out with the dextran carbonates. The dextran carbonates were dissolved in *N*,*N*-dimethylacetamide and an exchange of the organic solvent against the non-solvent water was carried out via dialysis. It was found that nanospheres of different size in the range from 150 to 600 nm are formed depending on the dextran ester used. Moreover, a narrow

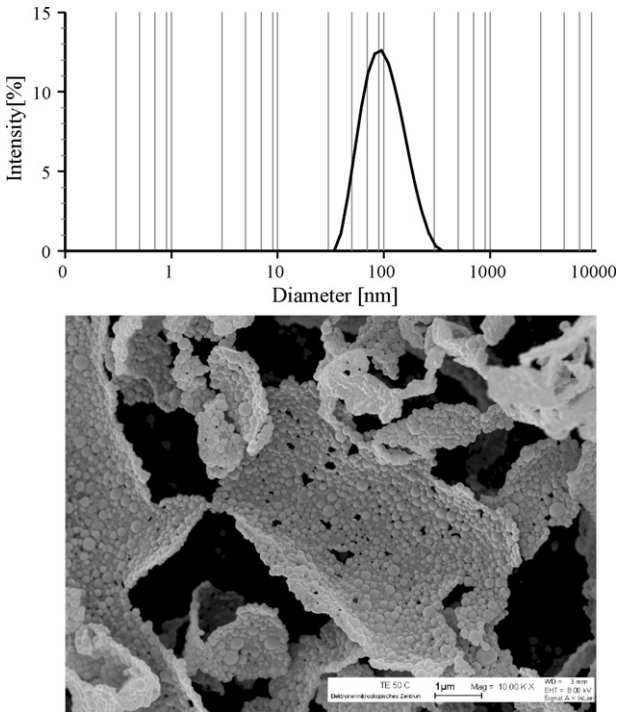

Fig. 8. Nanoparticles obtained by dialysis of dextran propyl carbonate (**4c**): particle size distribution evaluated by means of dynamic light scattering (top) and typical scanning electron microscopy (SEM) image (bottom).

particle size distribution was obtained, as evaluated by dynamic light scattering measurements. Fig. 8 shows the characteristics of nanoparticles obtained by dialysis of dextran propyl carbonate (**4c**). A detailed study about the characteristics and properties of the dextran carbonate nanoparticles is under investigation.

4. Conclusion

The preparation of dextran alkyl carbonates of varying DS levels was carried out. The influences of the reaction media and the alkyl chain length on the reaction efficiency were systematically studied in case of the chloroformate compounds. Moreover, we succeeded in the adaptation of fluoroformates, known from low molecular weight chemistry, as acylating agents for dextran. In comparison to the corresponding chloroformates, fluoroformates are easily manageable and display a much higher reaction efficiency. Thus, completely functionalized dextran carbonates (DS 3) could be obtained applying a relatively low molar excess of the fluoroformate. The structure of the biopolymer derivatives is clearly described based on the NMR and IR spectra. Highly substituted dextran alkyl carbonates form nanospheres with a size in the range from 150 to 600 nm, depending on the dextran derivative used. A detailed characterization of the particles is under investigation and will be reported elsewhere.

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