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Syntheses and detailed structure characterization of dextran carbonates

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ABSTRACT

Dextran alkyl carbonates were synthesized applying ethyl chloroformate, butyl chloroformate, butyl fluoroformate and 1H-imidazole-1-carboxylates. The influence of the reaction conditions on the reaction efficiency and the substitution pattern was studied in detail. The structure of the products obtained was clearly described by means of NMR- and IR-spectroscopy.

1. Introduction

Dextran, a family of neutral polysaccharides possessing a α-(1 → 6) linked D-glucose main chain, is a promising biopolymer for the design of new functional materials. In particular, chemical modification of dextran is of increasing interest for the design of the structure and hence products with novel properties of this important class of polysaccharides (Heinze, Liebert, Heublein, & Hornig, 2006). For example, carbonic acid esters of dextran were applied as intermediates in syntheses and for targeted and sustained delivery of therapeutic or imaging agents (Larsen, 1989; Mehvar, 2000; Vandoorne, Bruneel, Vercauteren, & Schacht, 1991; Vandoorne, Vercauteren, Permentier, & Schacht, 1985; Vansteenkiste, Demarre, & Schacht, 1992). Moreover, it was shown that dextran carbonic acid esters with high degree of substitution (DS) form nanoparticles by applying a simple solvent exchange (Wondraczek, Elschner, & Heinze, 2011).

First approaches to use the carbonate moiety for the covalent functionalization of dextran were carried out with different alcohols after the activation of the polymer with phosgene (Barker, Disney, & Somers, 1972). This method is limited due to the difficulties in the handling of phosgene and the fact that it is combined with a number of side reactions. The conversion of dextran with simple and inexpensive chloroformates is more suitable. However, it must be stated that these acylating agents may 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). Therefore, fluoroformates were successfully adapted as highly efficient and easy to handle reagents for the synthesis of dextran alkyl carbonates (Wondraczek et al., 2011). Fluoroformates are commercially barely available and accessible only by toxic reagents and extensive multistep reactions (Dang, Olofson, Wolf, Piteau, & Senet, 1990; Olah et al., 1979; Svec et al., 2008). Last but not least 1H-imidazole-1-carboxylates are supposed to be efficient reagents for grafting complex molecules, like steroids, to dextran (Staab & Mannschreck, 1962). Therefore, two synthetic routes may be employed. The first is the activation of the polysaccharide with *N*,*N*′-carbonyldiimidazole (CDI) followed by the addition of the desired alcohol. The second is initial preparation of the reactive species of the alcohol. Since the first strategy leads to cross-linking of dextran (Bamford, Middleton, & Allamee, 1986) we choose the synthesis of the 1H-imidazole-1-carboxylates of the alcohols. They are accessible via chloroformates and imidazole in THF (Staab, 1957) or by the conversion of CDI and an alcohol in toluene containing a catalytic amount of KOH (Rannard & Davis, 1999). The synthesis of dextran carbonates applying 1H-imidazole-1-carboxylates was carried out to introduce reactive groups in the polymer backbone that could be finally

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cross-linked (De Geest et al., 2008a, 2008b; van Dijk-Wolthuls, Tsang, Kettenes-van de Bosch, & Hennink, 1997). In our previous work we focus on the preparation of dextran carbonic acid esters with high DS applying alkyl chloro- and fluoroformates and their application for the formation of nanoscaled particles (Wondraczek et al., 2011). From the results the question appeared if the type of acylating agents and synthesis conditions have an influence on the substitution pattern of the resulting dextran alkyl carbonates. Therefore, in the present work we focus on the detailed characterization of dextran alkyl carbonates synthesized via different acylating agents and conditions. The reactivity and the influence of the method applied on the substitution pattern are described. Moreover, the application of 1H-imidazole-1-carboxylates is studied, since it turned out that chloro- and fluoroformates are not suitable to graft complex alcohols to polysaccharides.

2. Experimental

2.1. Materials

Dextran (Fluka) produced by *Leuconostoc mesenteroides* strain no. NRRL B-512(F) possesses a $\overline{M}_w$ of 54,400 g/mol and a $\overline{M}_n$ of 34,960 g/mol. The α-(1 → 6) linked glucose main chain contains about 5% of randomly distributed α-(1 → 3) branches. Dextran ethyl carbonate (DS 0.47) was synthesized from a dextran produced by *Leuconostoc* spp. strain number 10817 ($\overline{M}_w$ 5400 gmol^{-1}) containing α-(1 → 2) and α-(1 → 3) branches. A detailed structure analysis of the dextran sample **1** was published recently (Heinze et al., 2006). Dextran and LiCl (Merck) were dried for 6 h at 105 °C in vacuum over potassium hydroxide prior use. Butyl fluoroformate **2c** was obtained according to the procedure described by Cuomo and 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-F} = 284 Hz, C═O), 71.4 (OCH_2), 30.1 (CH_2), 18.6 (CH_2), 13.5 (CH_3). 1H-imidazole-1-carboxylates **2d–e** were synthesized according to a procedure described by Rannard and Davis (1999). Butyl 1H-imidazole-1-carboxylate **2d** Yield: 90%, ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 8.10 (s, ImH), 7.39 (s, ImH), 7.03 (s, ImH), 4.39 (t, J = 6.6 Hz, OCH_2), 1.74 (m, CH_2), 1.45 (m, CH_2), 0.95 (m, CH_3) ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 148.7 (C═O), 137.0 (C—N), 130.6 (C—N), 117.0 (C—N), 68.2 (OCH_2), 30.4 (CH_2), 18.9 (CH_2), 13.5 (CH_3). *t*-Butyl 1H-imidazole-1-carboxylate **2e** Yield: 95%, ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 8.06 (s, ImH), 7.36 (s, ImH), 7.02 (s, ImH), 1.61 (s, CH_3) ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 147.1 (C═O), 137.0 (C—N), 130.2 (C—N), 117.1 (C—N), 85.5 (C—O), 27.9 (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 was performed by CHNS 932 Analyzer (Leco).

2.3. Synthesis

2.3.1. Synthesis of ergosterol 1H-imidazole-1-carboxylate

Dry toluene (10 mL) was added to a 100 mL Schlenk tube under nitrogen. CDI (1.070 g, 6.6 mmol) was added followed by KOH (2 mg). The mixture was heated at 60 °C and ergosterol (2.380 g, 6.0 mmol) was added during 1 h. After stirring for additional 3 h toluene (50 mL) was added and the mixture was left to cool. The precipitate was filtered off and the clear solution was concentrated in vacuum, dissolved in CH_2Cl_2 (30 mL), and washed three times with water (3 × 15 mL). The solution was dried with anhydrous Na_2SO_4 and concentrated in vacuum to give a pale yellow solid. Yield: 1.653 g (56%), ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 8.17 (s, ImH), 7.44 (s, ImH), 7.08 (s, ImH), 5.62 (m, H-6), 5.42 (m, H-7) 5.21 (m, H-22, H-23), 4.94 (m, H-3), 2.7–0.7 (m), 0.64 (H-18), ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 148.0 (C═O), 142.0 (C-8), 137.1, 137.0 (C-5, C—N), 135.5 (C-23), 132.0 (C-22), 130.3 (C—N), 121.0 (C-6), 117.2 (C—N), 116.2 (C-7), 77.7 (C-3), 55.7 (C-17), 54.5 (C-14), 46.0 (C-9), 42.8 (C-13, C-24), 40.4 (C-20), 39.0 (C-12), 37.7 (C-1), 37.0 (C-10), 36.4 (C-4), 33.1 (C-25), 28.2, 28.0 (C-16, C-2), 23.0 (C-15), 21.1, 21.0 (C-21, C-11), 19.9 (C-26), 19.6 (C-27), 17.6 (C-24[1]), 16.1 (C-19), 12.0 (C-18).

2.3.2. Synthesis of dextran n-alkyl carbonates applying ethyl- or butyl chloroformate, general procedure

Dextran **1** (1 g, 6.17 mmol) was stirred in 30 mL dry DMF 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. In case of DMSO as solvent, dextran was dissolved at 40 °C. The dextran solution was filled into a double-wall reactor with septum under argon and cooled to 4 or 6 °C. After the addition of pyridine, alkyl chloroformate **2a** or **2b** was added slowly, to avoid strong evolution of gas and precipitation. After stirring for 4 or 24 h at 4 or 6 °C, the reaction mixture was precipitated in 300 mL water, ethanol or 2-propanol. The precipitate was filtered off and washed four times with 150 mL water, ethanol or 2-propanol. The product was dried in vacuum at 40 °C and purified by reprecipitation from 5 mL DMSO or acetone in 100 mL water.

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

To a solution of dextran **1** (1 g, 6.17 mmol) in 15 mL DMSO butyl fluoroformate **2c** and triethylamine both dissolved in 5 mL 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 four times with 150 mL water. The crude product was dried in vacuum at 40 °C and purified by reprecipitation from 5 mL DMSO or acetone in 100 mL water.

2.3.4. Synthesis of dextran butyl carbonates applying butyl 1H-imidazole-1-carboxylate, general procedure

To a solution of dextran **1** (1 g, 6.17 mmol) in 20 mL DMSO butyl 1H-imidazole-1-carboxylate **2d** and triethylamine were added. After stirring for 24 h at 60 °C, the product was isolated by precipitation in 300 mL water and worked up as described above.

2.3.5. Synthesis of dextran t-butyl carbonates applying t-butyl 1H-imidazole-1-carboxylate and sodium hydride, general procedure

Sodium hydride (0.4 equivalents to *t*-butyl 1H-imidazole-1-carboxylate **2e**) was dissolved in 5 mL dry DMSO at 60 °C until the evolution of gas stopped. After cooling to room temperature a solution of dextran **1** (1 g, 6.17 mmol) in 30 mL DMSO was added. *t*-Butyl 1H-imidazole-1-carboxylate **2e** in 5 mL DMSO was added and the reaction solution was stirred for 24 h at room temperature. The product was isolated by precipitation in 300 mL $NaHCO_3$ solution. The precipitate was filtered off and washed four times with 150 mL water. The crude product was dried in vacuum at 40 °C and purified by reprecipitation from 5 mL DMF or acetone in 100 mL water.

5e Yield: 92%, DS 2.85 (determined by means of ^{1}H NMR spectroscopy after peracetylation), IR (KBr): 1760 cm^{-1} ($\nu_{C=O}$) ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 5.24 (H-3), 5.06 (H-1), 4.93 (H-4),

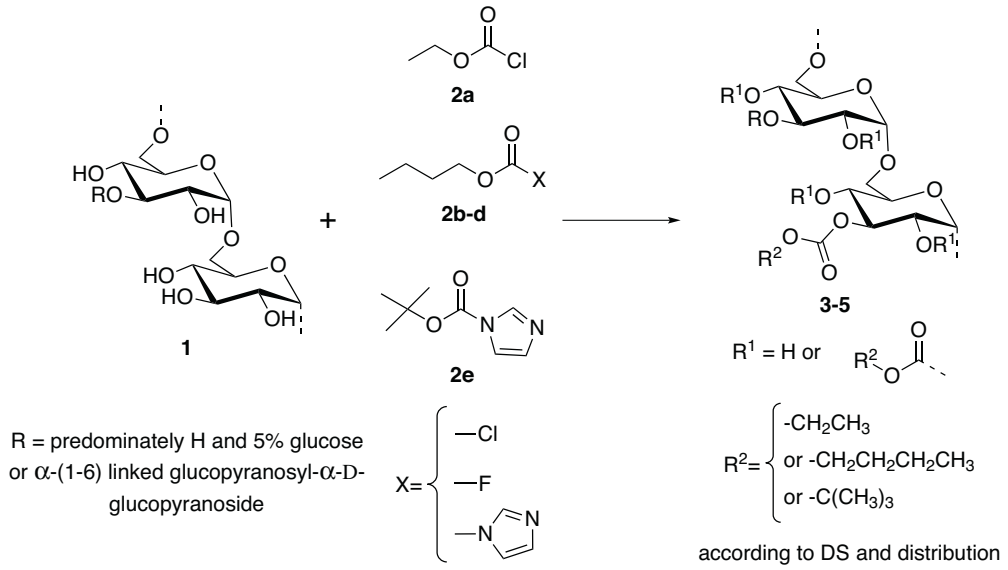

Fig. 1. Reaction scheme of the synthesis of dextran alkyl carbonates.

4.65 (H-2), 3.96 (H-5), 3.76 (H-6), 3.56 (H-6), 1.44 (CH_3) ^{13}C NMR (63 MHz, $CDCl_3$): δ [ppm] = 152.6, 152.1, 151.8 (C=O), 96.7 (C-1′), 82.5, 82.0 ($OC(CH_3)_3$), 73.4, (C-3, C-2) 71.3 (C-4′), 68.8 (C-5), 66.0 (C-6), 27.7 (CH_3).

2.3.6. Synthesis of dextran ergosterol carbonate

To a solution of dextran **1** (200 mg, 1.23 mmol) in 10 mL DMF/LiCl ergosterol 1H-imidazole-1-carboxylate (0.303 mg, 0.62 mmol) and triethylamine (200 μL) were added. After stirring for 24 h at 80 °C, the product was precipitated in 100 mL ethanol, isolated by centrifugation, and washed four times with 50 mL ethanol. The product was dried in vacuum at 40 °C.

Elemental analysis: C, 57.04; H, 7.46 (DS 0.19), further analytic methods were carried out after peracetylation of the sample: IR (KBr): no ν_{OH}, ^{13}C NMR (100 MHz, acetone-d_6): δ [ppm] = 169.6, 169.4 (C=O, acetate), 153.6 (C=O, carbonate), 135.6 (C-23), 132.1 (C-22), 95.6 (C-1), 80-60 (C-2, C-3, C-4, C-5, C-6), 56.0 (C-17), 54.6 (C-14), 42.9 (C-13, C-24), 40.1 (C-20), 39.0 (C-12), 33.0 (C-25), 22.6 (C-15), 20.6, 19.8, 19.4, 19.1 (C-21, C-11, C-26, C-27, CH_3-acetate), 17.1 (C-24[1]), 11.8 (C-18).

2.3.7. Peracetylation, general procedure

Dextran alkyl carbonate **3**–**5** (300 mg) was dissolved in 5 mL DMF. Subsequently 5 mL pyridine, 5 mL acetic anhydride, and 50 mg 4-(dimethylamino) pyridine 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 four times with 100 mL water and dried in vacuum at 40 °C. IR (KBr): no ν_{OH}

3. Results and discussion

The syntheses and characterization of dextran alkyl carbonates was carried out. In particular ethyl-, butyl-, *t*-butyl- and ergosterol carbonate moieties were grafted to the polymer backbone applying different acylating agents.

3.1. Synthesis of dextran n-alkyl carbonates

In order to synthesize dextran *n*-alkyl carbonates, dextran (**1**) was allowed to react with ethyl chloroformate (**2a**), butyl chloroformate (**2b**), butyl fluoroformate (**2c**), and butyl 1H-imidazole-1-carboxylate (**2d**) (Fig. 1). The conditions and the results of the synthesis of dextran alkyl carbonates are summarized in Table 1.

The influence of the solvent on the reaction of dextran with alkyl chloroformates was demonstrated recently (Wondraczek et al., 2011). It was shown that dimethyl sulfoxide (DMSO) leads to dextran ethyl carbonates with low DS values and thus a low reaction efficiency compared to the use of *N*,*N*-dimethylacetamide/lithium chloride (DMAc/LiCl) or *N*,*N*-dimethylformamide/lithium chloride (DMF/LiCl) as solvent (Table 1, **3a**–**c**). Due to the relatively less pronounced side reactions, DMF/LiCl is the most suitable solvent

Table 1
Conditions for and results of the synthesis of dextran ethyl-, butyl- and *t*-butyl carbonates.

Reagent[a]	Solvent[b]	Temp. (°C)	Base[c]	Molar ratio[d]	No.	DS[e]
EtCF	DMSO	6	Py	4.5	**3a**	0.71
EtCF	DMAc/LiCl	4	Py	4.5	**3b**	1.50
EtCF	DMF/LiCl	4	Py	4.5	**3c**	1.71
BuCF	DMF/LiCl	4	Py	1.0	**4a**	0.45
BuCF	DMF/LiCl	4	Py	2.0	**4b**	1.13
BuCF	DMF/LiCl	4	Py	4.5	**4c**	1.63
BuCF	DMF/LiCl	4	Py	30.0	**4d**	2.68
BuFF	DMSO	60	TEA	1.0	**4e**	0.91
BuFF	DMSO	60	TEA	4.5	**4f**	3.00
BuIm	DMSO	60	TEA	1.0	**4g**	0.98
BuIm	DMSO	60	TEA	4.5	**4h**	2.46
BuIm	DMSO	60	–	4.5	**4i**	0.44
BuIm	DMSO	60	KOH	4.5	**4j**	*[f]
BuIm	DMSO	60	NaH	4.5	**4k**	*[f]
t-BuIm	DMSO	20	NaH	0.5	**5a**	0.17
t-BuIm	DMSO	20	NaH	1.0	**5b**	0.56
t-BuIm	DMSO	20	NaH	2.0	**5c**	1.74
t-BuIm	DMSO	20	NaH	3.0	**5d**	2.57
t-BuIm	DMSO	20	NaH	4.5	**5e**	2.85
t-BuIm	DMSO	60	KOH/18-Crown-6	4.5	**5f**	2.43
t-BuIm	DMSO	60	TEA	4.5	**5g**	0.20

[a] EtCF, ethyl chloroformate; BuCF, butyl chloroformate; BuFF, butyl fluoroformate; BuIm, butyl 1H-imidazole-1-carboxylate; *t*-BuIm, *t*-butyl 1H-imidazole-1-carboxylate.
[b] DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide.
[c] Py, pyridine; TEA, triethylamine.
[d] Mole reagent per mole anhydroglucose unit.
[e] Degree of substitution (DS) determined by means of ^{1}H NMR spectroscopy after peracetylation.
[f] Product cross-linked.

for this conversion. Furthermore, the high yields of 80–90% make the procedure quite interesting. However, even at low molar ratios (Table 1, **4a**–**c**) nearly the half amount of reagent decomposes during the reaction. To reach high values of degree of substitution (DS), an even higher molar excess of reagent is needed. For example dextran butyl carbonate (**4d**) with a DS of 2.68 requires a molar ratio of 1:30.

Therefore, alkyl fluoroformates were studied as effective acylating reagents due to their stability in DMSO and tertiary amide solvents (Dang & Olofson, 1990). The reactions 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. In case of fluoroformates DMSO is the most suitable solvent because chloride ions in the solvent DMF/LiCl may cause a halide exchange and thus convert the butyl fluoroformate to the corresponding chloro compound. A DS of 3 (**4f**) is obtained applying 4.5 equivalents butyl fluoroformate while in case of the chloroformate only a DS of 1.63 (**4c**) can be achieved. In spite of the high efficiency of fluoroformates their use is limited due to the poor accessibility. Except for a few examples, fluoroformates are commercially not available. Moreover, the synthesis of fluoroformates based on more complex alcohols requires a multi step reaction with hazardous chemicals like hydrogen fluoride (Olah et al., 1979).

Due to the mild and efficient esterification using *N*,*N*′-carbonyldiimidazole (CDI), the synthesis of carbonate starting from dextran and the alkyl alcohol was adapted. According to a one step procedure, butyl 1H-imidazole-1-carboxylate was prepared from CDI and butanol, described by Rannard and Davis (1999). The reaction of butyl 1H-imidazole-1-carboxylate and dextran in DMSO, using triethylamine as catalyst, shows very high efficiency of conversion even at low molar ratio. For example, a DS of 0.98 (**4g**) was obtained applying one equivalent of reagent only. Highly substituted dextran butyl carbonates of a DS 2.46 (**4f**) can be obtained as well at comparable low molar ratios of 1:4.5. However, the reaction strongly depends on the basicity of the catalyst. A molar ratio of 1:4.5 yields a low substituted product (**4i**, DS 0.44) if no base is added. Catalysis by strong bases like KOH or NaH leads to cross-linking (**4j**–**k**). Bases being significantly less stronger than triethylamine allow a slow conversion only, due to the weak nucleophilicity of the hydroxyl groups of dextran. On the other hand, KOH is known to induce the formation of cyclic carbonates from 1,2-diols (Rannard & Davis, 1999). In case of polysaccharides, a subsequent cross-linking via ring opening reaction induced by a nucleophilic attack of activated OH-groups seems to be possible (Chaves & Arranz, 1985). Obviously the formation of an acyclic carbonate or a cyclic carbonate followed by cross-linking depends on donor–acceptor interactions of reagent, base, substrate, or solvent.

3.2. Synthesis of unconventional dextran alkyl carbonates

Due to the fact that alkyl chloroformates are simple and inexpensive acylating agents to access dextran carbonates based on primary alcohols we apply butyl chloroformate to yield products with low and moderate DS values. In case of fluoroformates completely functionalized polymers (DS 3) are accessible. The mild and efficient esterification using CDI covers almost the whole DS range. Moreover, the use of CDI opens the door to unconventional dextran carbonates from alcohols that could not be converted into chloro- or fluoroformates. For example, *t*-butyl chloroformate is not suitable as acylating reagent due to its hydrolytic and thermal sensitivity (Chopin & Rogers, 1948). Therefore, dextran (**1**) was allowed to react with *t*-butyl 1H-imidazole-1-carboxylate (**2e**) in order to obtain dextran *t*-butyl carbonates (Fig. 1, **5a**–**g**).

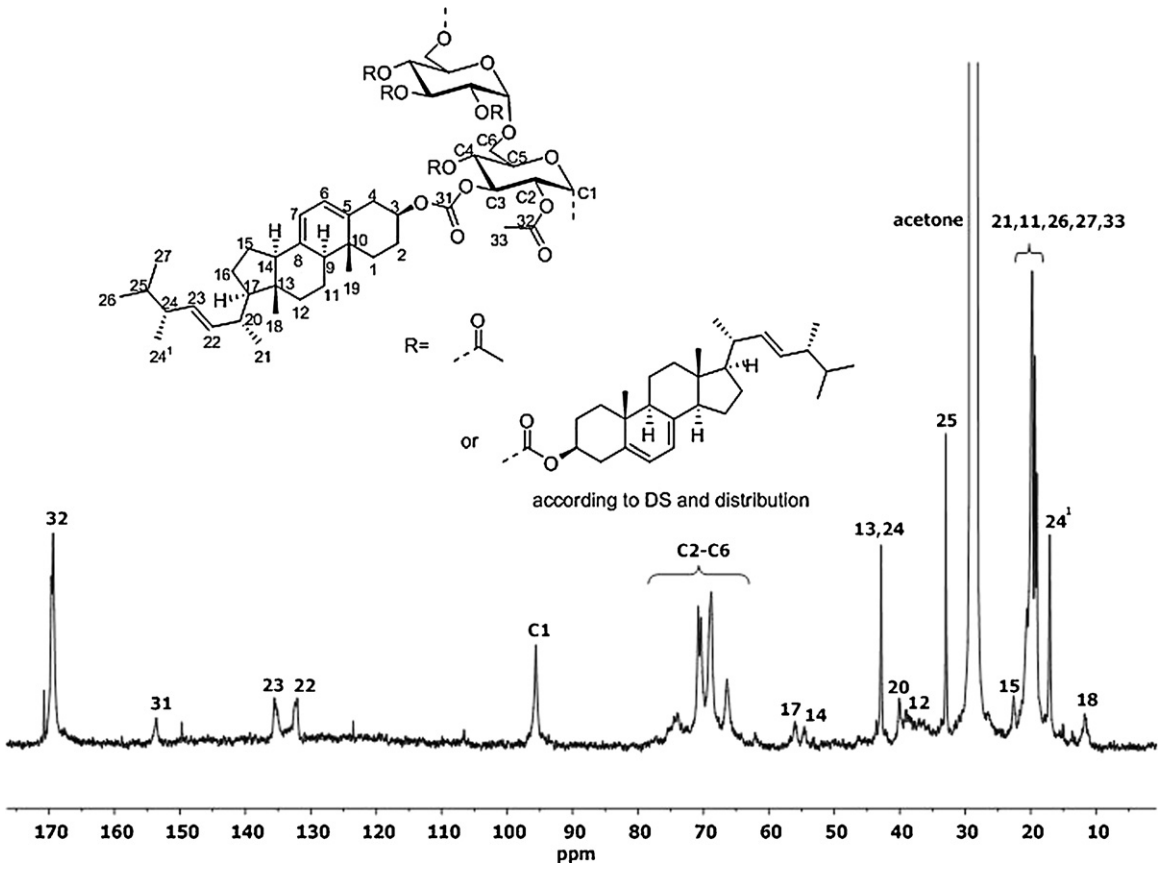

Fig. 2. ^{13}C NMR spectrum of dextran ergosterol carbonate (peracetylated) in acetone-d_6.

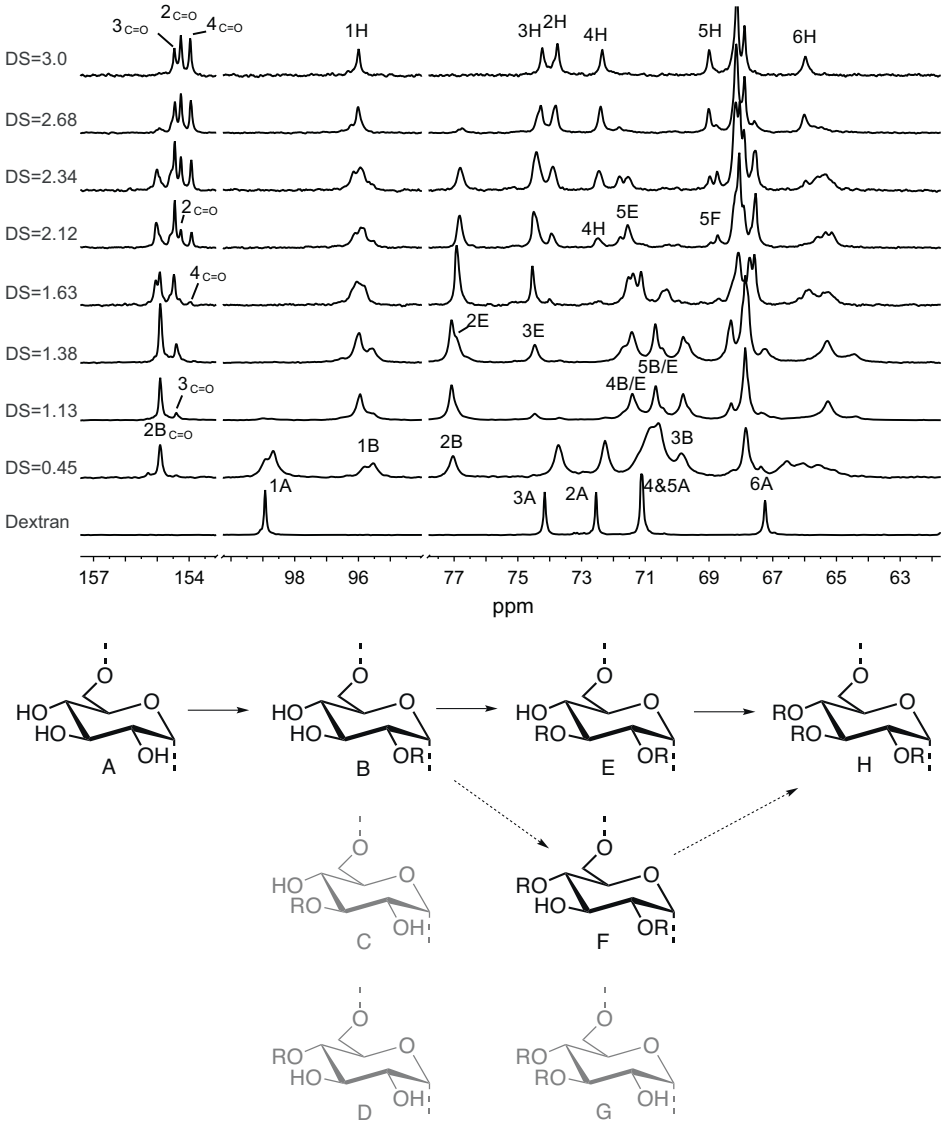

Fig. 3. ^{13}C NMR spectra of dextran and dextran butyl carbonates (top); repeating units that may occur in dextran derivatives (bottom).

As summarized in Table 1 as well, in a set of experiments, the dependence of the reaction of dextran and *t*-butyl 1H-imidazole-1-carboxylate (**2e**) on the catalytic base used was studied. The application of triethylamine leads to a product of low DS value (0.20 at a molar ratio of 1:4.5, **5g**) compared to the *n*-butyl derivative **4h** (DS 2.46). Upon increasing the strength of the base, the DS of the products increases. Using KOH/18-Crown-6 as catalyst, a significantly higher conversion appeared (**5f**, DS 2.43). The application of sodium hydride leads to the product with the highest DS value (**5e**, DS 2.85). Therefore, the dependence of the DS value on the molar ratio was systematically studied using sodium hydride as base. At low molar ratios (Table 1, **5a**–**b**) only about the half amount of reagent reacts with dextran. The efficiency becomes higher applying intermediate molar ratios. For example, two equivalents of reagent led to a product with DS 1.74 (**5c**). About 0.3 equivalents of **2e** are consumed in side reactions under these conditions.

As a result of the strong +I-effect of the *t*-butyl moiety, in contrast to the *n*-butyl group, a low activity of the carbonyl group is apparent. Thus, strong bases are necessary to allow a reaction with secondary or tertiary alcohols that is in agreement with published results about the reactivity of *t*-butyl 1H-imidazole-1-carboxylate (Staab & Mannschreck, 1962). Surprisingly no cross-linking occurs during the grafting of *t*-butyl carbonate moiety to the polymer backbone using strong bases. Probably the carbonyl group of the dextran carbonate is shielded by the *t*-butyl group and no attack of hydroxyl groups is possible.

To demonstrate the potential of the activation using CDI for grafting complex alcohols to dextran, the corresponding ergosterol 1H-imidazole-1-carboxylate was applied (^{13}C NMR spectrum in Fig. 2). The synthesis was carried out in DMF/LiCl due to the poor solubility of ergosterol 1H-imidazole-1-carboxylate in DMSO. A molar ratio of 1:0.5 yields a product with a DS of 0.19 although the alkyl moiety is very bulky. This result shows the high efficiency of 1H-imidazole-1-carboxylates. In contrast to previous studies (Larsen, 1989), hazardous chemicals including phosgene are no longer necessary for grafting pharmacologically active alcohols to dextran. Moreover, the byproducts of the procedure applying CDI are non-toxic and easy to remove.

3.3. Structure characterization of dextran alkyl carbonates

The molecular structure of dextran alkyl carbonates can be studied in detail by means of NMR spectroscopy. ^{1}H NMR spectroscopy of the peracetylated products yields the DS values. Moreover, the

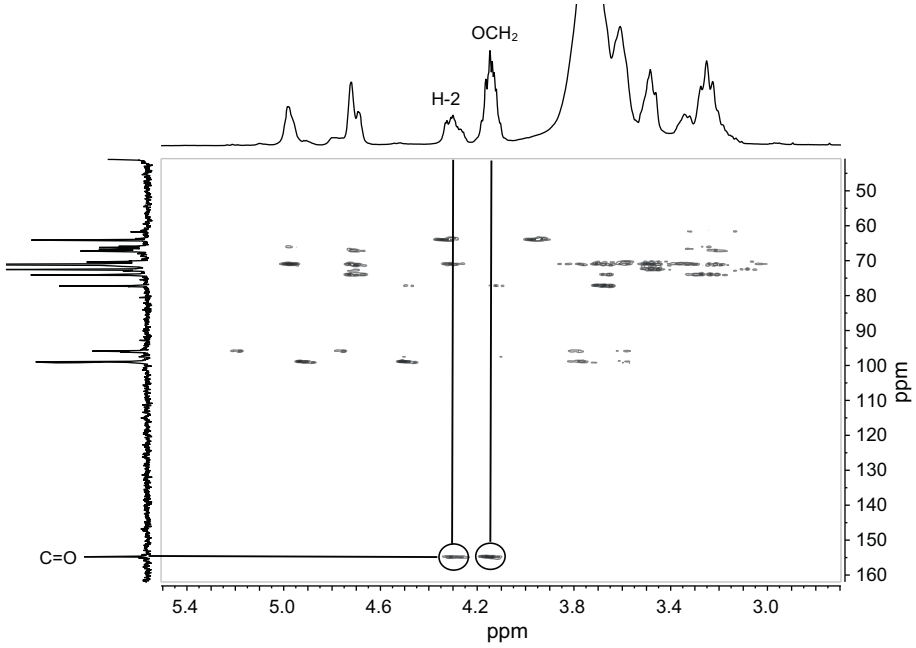

Fig. 4. HMBC NMR-spectrum of dextran ethyl carbonate (DS 0.47) in DMSO-d_6.

signals in the ^{13}C NMR spectra can be assigned to give a detailed view on the structure of the single repeating units. As shown for dextran butyl carbonate, synthesized with butyl chloroformate, we investigated the ^{13}C NMR signals depending on the DS to get more information about the selectivity in the formation of dextran carbonates. Fig. 3 shows ^{13}C NMR spectra of dextran and of dextran butyl carbonates with DS values in the range from 0.45 to 3. In comparison to the totally substituted derivative (DS 3), the spectra of samples with lower DS become more complicated because a unsubstituted (A), three monosubstituted (B–D), three disubstituted (E–G), and a fully substituted repeating unit (H) may occur within the polymer chain (formulas in Fig. 3). The shifts of ^{13}C NMR peaks of dextran (AGU A) were assigned according to literature (Heinze et al., 2006). At DS 0.45, the C-2 signal is shifted about 4.5 ppm to lower field because reaction takes place at position 2 first (B, 2-mono-O substituted). On the other hand, the resonances of the neighbor positions 1 and 3 are high field shifted. Positions 4, 5, and 6 exhibit a small shift to higher field as well. This phenomenon was already observed by NMR studies of acetylated and benzylated dextrans (Gagnaire & Vignon, 1977). Increasing the DS above one results in a further small high field shift of the C-2B signal. The C-3B signal (69.8 ppm, Arranz, Roman, and Sanchez-Chaves (1987)) itself is shifted about 4.5 ppm to lower field. Above DS 2 the C-2H resonance of trisubstituted dextran butyl carbonate occurs at 73.8 ppm. In addition, there are three different ^{13}C NMR signals arising from position 5. On one hand, the signal C-5H belonging to the trisubstituted dextran carbonate is found. On the other, the signal arising from C-5E (E, 2,3-di-O substituted) and furthermore, the resonance C-5F (F, 2,4-di-O substituted) indicating substitution at position 4. The number of C=O and OCH_2 resonances also increases with increasing DS. At low DS values, there is only one C=O signal at 154.9 ppm assigned to the monosubstituted repeating unit B. At DS 1.13 a further C=O resonance appears, which was assigned to a carbonate at position 3 by means of HMBC NMR spectroscopy. The C=O signals related to positions 4 and 2 are visible at 153.9 and 154.3 ppm. From these results the conclusion may be drawn that the reactivity of individual secondary OH groups decreases in the order C-2 > C-3 > C-4. Thus, the repeating units A → B → E → H are observed with increasing DS (lined path, Fig. 3 bottom). Moreover, a minor amount of repeating unit F occurs as indicated by the resonance C-5F (dotted path).

To verify the regioselective reaction of dextran with alkyl chloroformates at low molar ratios, a dextran ethyl carbonate (DS 0.47) was prepared, based on a low molecular weight dextran. The reduced degree of polymerization results in an increased lifetime of longitudinal and transversal magnetization and thus in higher resolution of the recorded NMR spectrum. Fig. 4 shows the HMBC NMR spectrum of the dextran ethyl carbonate (DS 0.47). The correlation of the OCH_2 (alkyl moiety) and the H-2 resonance (repeating unit) by their cross peaks to the C=O signal proves the linkage of the alkyl carbonate to the polymer backbone. The fact that no other cross peaks to the C=O signal are visible provides clear evidence of the regioselective reaction of dextran and alkyl chloroformates under these conditions.

In further studies the dependence of the substitution pattern on the reaction conditions applied was investigated. Fig. 5 shows ^{13}C NMR spectra of dextran butyl carbonates **4b** (DS 1.13), **4e** (DS 0.91), and **4g** (DS 0.98) synthesized applying butyl chloroformate, butyl fluoroformate, and butyl 1H-imidazole-1-carboxylate, respectively. Considering the same DS value of each sample, the C-1′ resonance (C-1 signal influenced by substitution at position 2) is very strong in comparison to the C-1 signal in case of **4b**. This fact indicates the selectivity of chloroformates at position 2 as mentioned above. The ratio of intensities of the C-1′ and the C-1 signal is about 1:2 using butyl fluoroformate or butyl 1H-imidazole-1-carboxylate. Moreover, the multiple C=O signals in case of the butyl carbonates **4e** and **4g** indicate the random substitution pattern. Although the selectivity of alkyl chloroformates seems to be contradictory due to their high reactivity at the first look, the reaction temperature may be the leading parameter. Probably fluoroformates and 1H-imidazole-1-carboxylates might show the same or even better selectivity if they would have a proper reactivity at low temperatures. On the other hand, to study the substitution pattern applying chloroformates is not possible due to their instability at high temperatures.

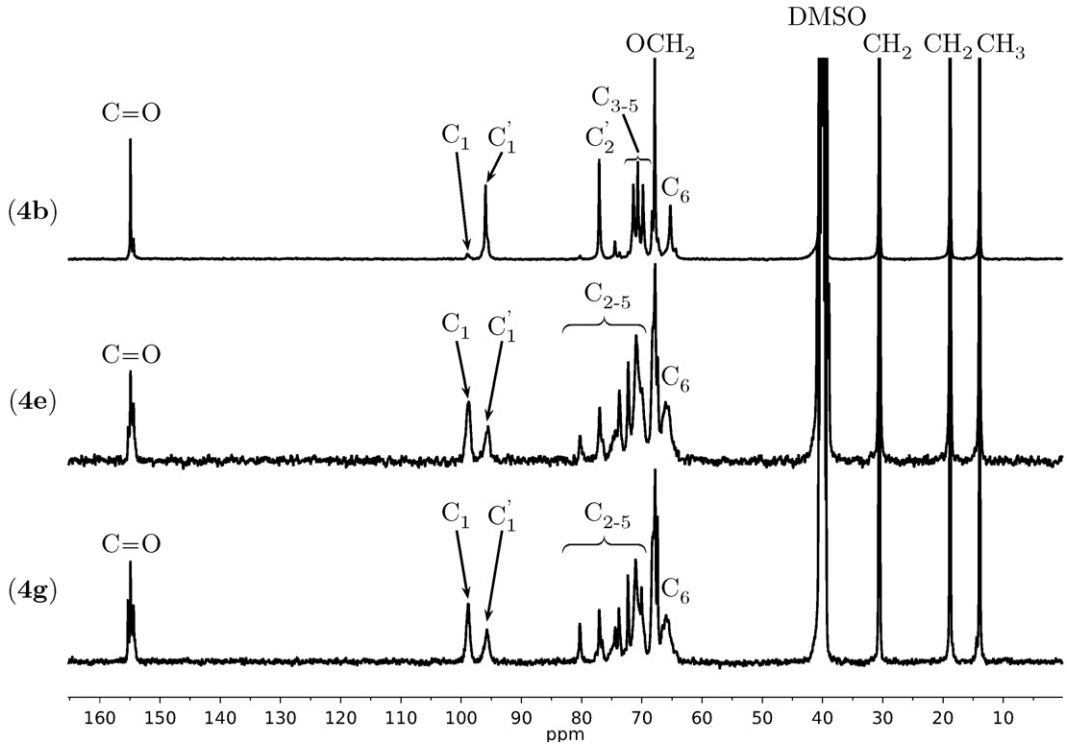

Fig. 5. ^{13}C NMR spectra of dextran butyl carbonates (DS 1.13), (DS 0.91), (DS 0.98) synthesized applying butyl chloroformate, butyl fluoroformate and butyl 1H-imidazole-1-carboxylate.

4. Conclusion

The synthesis of dextran alkyl carbonates applying different acylating agents was carried out. The influence of the reaction conditions on the reaction efficiency and the substitution pattern was studied in detail. It became obvious that alkyl chloroformates are simple and inexpensive acylating agents to access dextran carbonates based on primary alcohols with low and moderate DS values. In case of butyl fluoroformate a completely functionalized polymer (DS 3) can be prepared. The mild and efficient esterification using *N*,*N*′-carbonyldiimidazole opens the door to unconventional dextran carbonates based on tertiary alcohols or complex molecules of pharmacological interest. The structure of the biopolymer derivatives can be clearly described by means of NMR spectroscopy. Applying butyl chloroformate at low temperatures, the substitution takes place in the order C-2 > C-3 > C-4 mainly. However, butyl fluoroformate and butyl 1H-imidazole-1-carboxylate lead to a random substitution pattern due to the required higher reaction temperature. Nevertheless, 1H-imidazole-1-carboxylates are versatile intermediates to overcame specific problems in the syntheses of dextran-based drug conjugates.

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