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Functional Polymers Based on Dextran

Thomas Heinze (✉) · Tim Liebert · Brigitte Heublein · Stephanie Hornig

Kompetenzzentrum Polysaccharidforschung, Friedrich-Schiller-Universität Jena, Humboldtstraße 10, 07743 Jena, Germany
thomas.heinze@uni-jena.de

Abstract Dextran, comprising a family of neutral polysaccharides consisting of an α-(1→6) linked D-glucose main chain with varying proportions of linkages and branches, depending on the bacteria used, is an interesting starting material for chemical modification reactions for the design of new functional polymers with promising properties. The review summarises recent results on structure characterisation of dextran including some comments on biosynthesis of this important class of biopolymers. Applications of dextran are discussed as well. Chemical modification reactions of dextran are increasingly studied for the structure and hence property design. The review highlights recent progress in esterification of dextran, both inorganic and organic polysaccharide esters, etherification reactions towards ionic and non-ionic ethers, and the huge variety of different conversions mainly developed for the binding of drugs. It summarises recent developments in the application of dextran derivatives with a focus on the chemical structures behind these materials such as prodrugs, bioactivity of inorganic dextran esters, heparin sulfate mimics, hydrogels, nanoparticles and self assembly structures for surface modification.

Keywords Functionalised dextran · Structural analysis · Prodrugs · Nanostructures · Bioactivity

Abbreviations

AFM	Atomic force microscopy
AGU	Anhydroglucose units
AT	Antithrombin
AZT	Azidothymidine
BSH	$Na_2B_{12}H_{11}SH$
BSA	Bovine serum albumin
CAC	Critical association concentration
CDI	*N*,*N*′-Carbonyldiimidazole
CMC	Critical micelle concentration
CMD	Carboxymethyl dextran
CMDB	Carboxymethyl dextran benzylamide
CMDBSSu	Carboxymethyl dextran benzylamidesulfonate sulfate
CMDBSu	Carboxymethyl dextran benzylamide sulfate
CMDEE	Carboxymethyl dextran ethyl ester
CMDSu	Carboxymethyl dextran sulfate
COSY	Two-dimensional correlated spectroscopy
CsA	Cyclosporin A
2D	Two-dimensional
Dach-Pt	*cis*-Dihydroxo(cyclohexane-*trans*-L-1,2-diamine)platinum II
DCC	*N*,*N*′-Dicyclohexylcarbodiimide

DCMD	Dicarboxyethyl dextran
DEAE	2-(Diethylamino)ethyl
$DexC_n$	2-Hydroxyoctyl- and 2-hydroxydodecyl
$DexPEG_{10}C_n$	Poly(ethylene glycol)-alkyl dextran ether
DexP	2-Hydroxy-3-phenoxy propyl dextran ethers
DLS	Dynamic light scattering
DMA	*N*,*N*-Dimethylacetamide
DMAP	4-*N*,*N*-Dimethylaminopyridine
DPA	Dextran propionate acetate
DS	Degree of substitution (DS is the average number of functional groups per anhydroglucose repeating unit arriving at a level of three after complete functionalisation of all hydroxyl groups)
EC	Endothelial cells
EDC	1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide
EEDQ	*N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
ELISA	Enzyme-linked immunosorbent assay
EPR	Electron paramagnetic resonance
FITC	Fluorescein isothiocyanate
GI	Gastrointestinal
GLC-MS	Gas–liquid chromatography/mass spectrometry
Glc*p*	Glucopyranose
HCII	Heparin cofactor II
HEMA	2-Hydroxyethyl methacrylate
HIV	Human immunodeficiency virus
HMDS	1,1,1,3,3,3,-Hexamethyldisilazane
HMPC	Hydrophobically modified polycationic dextran
HMQC	Heteronuclear multiple quantum correlation
HPLC	High performance liquid chromatography
HSV	Herpes simplex virus
HTLV-III	Human T-cell lymphotropic virus type III
LbL	Layer-by-layer
LD_{50}	Lethal dose
β-LG	β-Lactoglobulin
Lm	*Leuconostoc mesenteroides*
M_n	Number average molecular weight
M_w	Weight average molecular weight
2M2B	2-Methyl-2-butene
MCA	Monochloroacetic acid
MesCl	Methanesulfonic acid chloride
MRI	Magnetic resonance imaging
MWD	Molecular weight distribution
NHS	*N*-Hydroxysuccinimide
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
PCL	Poly(ε-caprolactone)
PEC	Polyelectrolyte complex
PEG	Poly(ethylene glycol)
PLA	Poly(lactide)
PrPres	Protease-resistant isoform of a host-encoded protein
PSDA	Particle size distribution analyser

PS-DVB	Polystyrene-divinylbenzene
Py	Pyridine
QCM	Quartz crystal microbalance
R_G	Radius of gyration
ROP	Ring-opening polymerisation
RT	Room temperature
S	*Streptococcus*
S_N	Nucleophilic substitution
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SMC	Smooth muscle cells
SPR	Surface plasmon resonance
SRA	Scavenger receptor class A
T-2513	7-Ethyl-10-(3-aminopropyloxy)-camptothecin
TBAH	Tetrabutylammonium hydroxide
TEA	Triethylamine
TMSCl	Chlorotrimethylsilane
TosCl	*p*-Toluenesulfonyl chloride
TOCSY	Total correlated spectroscopy
Trityl	Triphenylmethyl
TTd	Tetanus toxoid
UV/Vis	Ultraviolet/visible light

1 Introduction

Polysaccharides are fascinating macromolecules possessing structural diversity and functional versatility. Focusing on polyglucans (i.e. polysaccharides containing glucose repeating units only) a broad variety of structures appear, resulting from the stereochemistry of the anomeric C-atom, from the regiochemistry of the glycosidic linkage and from the pattern of branching. Cellulose, the β-(1 $\rightarrow$ 4) linked polyglucan of a very uniform molecular structure, is by far the most important polysaccharide. Cellulose is not only the most abundant organic compound but also most intensively used as raw material for the commercial production of fibres, beads and sponges as well as for the commercial synthesis of various esters and ethers [1]. Next to cellulose, the polyglucan starch has to be mentioned. As very well known, starch consists of two primary polymers containing D-glucose, namely the linear α-(1 $\rightarrow$ 4) linked amylose and the amylopectin composing additional α-(1 $\rightarrow$ 6) linked branches. Amylose and amylopectin occur in varying ratios depending on the plant species [2]. Starch is also used to a high extent as raw material for chemical modification reactions, though the commercially produced derivatives possess a low degree of substitution (DS). In addition to these polysaccharides mainly isolated from different plant sources, various fungi and bacteria synthesise polyglucans, e.g. curdlan (β-(1 $\rightarrow$ 3) linked),

scleroglucan and schizophyllan (β-(1 → 3) linked main chain and β-(1 → 6) linked branches) and pullulan (α-(1 → 4) and α-(1 → 6) linked).

However, the most important polysaccharide for medical and industrial applications produced by bacterial strains is dextran, a family of neutral polysaccharides consisting of an α-(1 → 6) linked D-glucose main chain with varying proportions of linkages and branches depending on the bacteria used (Fig. 1). The α-(1 → 6) linkages in dextran may vary from 97 to 50% of total glycosidic bonds. The balance represents α-(1 → 2), α-(1 → 3) and α-(1 → 4) linkages usually bound as branches [3]. Different bacterial strains are able to synthesise dextran mainly from sucrose. In 1861, Pasteur found slime-producing bacteria, which were later named *Leuconostoc mesenteroides* by van Tieghem in 1878 [4, 5]. Scheibler named the segregated carbohydrate

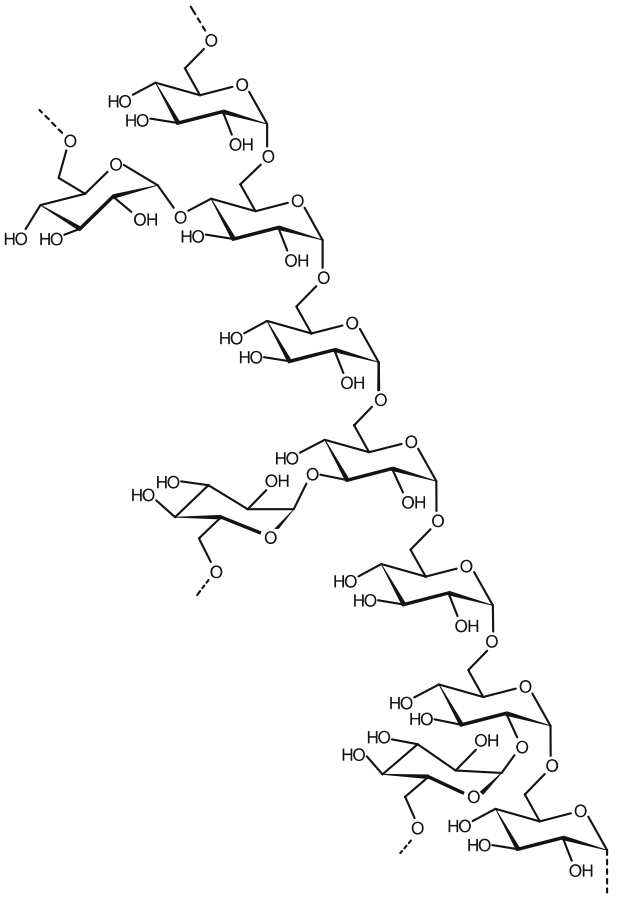


Fig. 1 Part of the α-(1 → 6)-linked glucose main chain of dextran with branching points in 2-, 3- and 4-positions

"dextran". Subsequent investigations have shown that dextran can be formed by several bacterial strains mostly gram-positive, facultatively anaerobe cocci, e.g. *Leuconostoc* and *Streptococcus* strains [6].

The commercial production carried out by various companies is estimated to be ca. 2000 t $year^{-1}$ worldwide [7]. Due to the common solubility in water and various other solvents (e.g. DMSO, formamide), the biocompatibility, and the ability of degrading in certain physical environments, dextran is already successfully applied in the medical and biomedical field [8]. The physiological activity of dextran and its derivatives, indicated also by a very large number of publications in this area of research, is in contrast to inadequate structural analysis of both dextran and their semi-synthetic products. Only a few publications, in contrast to extensive studies in cellulose and starch chemistry [9, 10], deal with the defined functionalisation and characterisation of dextran for adjusting desired features.

From the chemist's point of view, dextran is really an interesting polysaccharide as starting biopolymer for the design of structures and hence of properties by polymeranalogous reactions. The homopolymer structure of dextran composed of D-glucose units without any relevant imperfection and the availability of a number of dextran samples with a narrow molecular weight distribution are advantageous for the chemical modification. In addition to the products obtained from cellulose and starch, dextran derivatives may show different properties due to the differences in the structures of the polysaccharide chain and the three secondary hydroxyl groups, even if the same functional groups are introduced. Multifunctional dextran derivatives can be prepared with tunable properties depending on the introduced substituent. The main goal of this article is to review some aspects of the chemistry of dextran. Emphasis is placed on ethers and esters, which have been a recent focus of interest.

2
Sources, Structure and Properties of Dextran

2.1
Occurrence of Dextran

Leuconostoc mesenteroides and *Leuconostoc* ssp. are found in fermented foods of plant origin [11]. The occurrence of these bacteria in sugar refineries is responsible for problems in filtration processes because of increased viscosity by the presence of soluble dextran [12, 13]. Furthermore, dextran retards the rate of crystallisation of sucrose and adversely affects the crystal shape. The occurrence of dextran in the matrix of dental plaque results from certain *Streptococcus* strains [14]. The principle organism, *Streptococcus mutans*, is able to produce water-soluble glucan (named dextran) and water-insoluble

glucan (mutan) from sucrose [15, 16]. These polysaccharides provide a protective matrix for the colonisation of cariogenic bacteria on the tooth surface and attend as reserve carbohydrates [12]. Consequently, dental plaque promotes caries and can thus be reduced by application of dextranases, enzymes which hydrolyse specific glycosidic bonds [17]. One of the major industrial applications of dextranases is the reduction of sliming in sugar production processes [18].

For industrial, medical and scientific interests, a variety of dextran samples from different origins are commercially available. Depending on the fermentation conditions, dextran with particular features can be produced as described in Sect. 3.2.

2.2 Structure Characterisation

Dextran is a homopolymer of glucose with predominantly α-(1 $\rightarrow$ 6) linkages (50–97%) [12]. Figure 1 shows a part of the dextran main chain with branching points in the 2, 3 and 4 positions. The degree and nature of branching units is dependent on the dextran-producing bacterial strain [6].

Preliminary examinations of dextran structures were conducted by optical rotation, infrared spectroscopy and periodate–oxidation reactions. More detailed results can be achieved by methylation analysis [19]. The hydroxyl groups are methylated with methyl iodide after activation with sodium methylsulfinyl carbanion (Fig. 2). The methyl dextran is hydrolysed to the corresponding different methylated monosaccharides, which are furthermore reduced and peracetylated. The resulting alditol acetates of methylated sugars are separated by gas chromatography and identified by their retention times. In particular, a combined capillary gas–liquid chromatography/mass

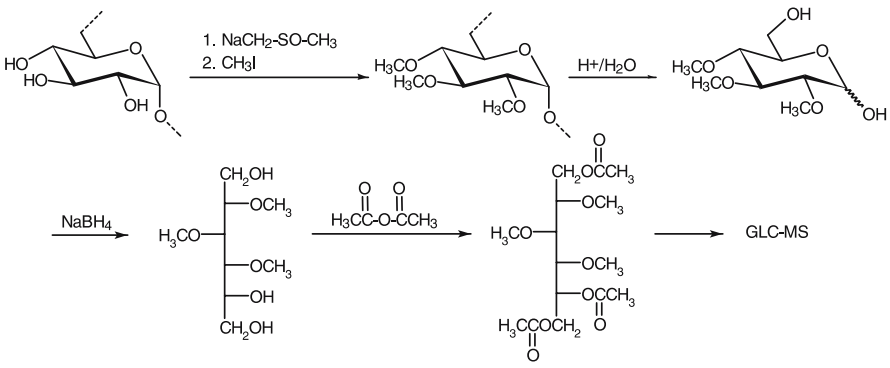


Fig. 2 Methylation analysis with subsequent GLC-MS for structure determination of dextran demonstrated for an α-(1 $\rightarrow$ 6) linked glucose unit

Table 1 Percentage of different glycosidic linkages in dextran of different bacterial strains obtained by methylation analysis

Strain number[a]	Solubility in water	α-Linkages (%) $1 \rightarrow 6$	$1 \rightarrow 2$	$1 \rightarrow 3$	$1 \rightarrow 4$	Refs.
Lm NRRL B-512F	+	95		5		[21, 23]
Lm NRRL B-1355 fraction 1	+	54		46		[20]
Lm NRRL B-1355 fraction 2	–	95		5		[24]
Lm NRRL B-1299 fraction 1	+	68	29	3		[25]
Lm NRRL B-1299 fraction 2	–	63	27	8		[25]
Lm NRRL B-742 fraction 1	+	50		50		[24]
Lm NRRL B-742 fraction 2	–	87			13	[24]
S mutans 6715 fraction 1	+	64		36		[16]
S mutans 6715 fraction 2	–	4		96		[26]
S mutans GS5	+	70		30		[27]
S downei	+	90		10		[28]

[a] *Lm Leuconostoc mesenteroides*, *S Streptococcus*

spectrometry (GLC-MS) is an effective method for structure determination of dextran [20, 21].

Furthermore, structural investigations were carried out by the use of degradative enzymes of known specificity followed by means of thin-layer chromatography, HPLC and ^{13}C NMR spectroscopy [12, 16]. Table 1 shows examples of the linkage analysis of several dextran fractions produced by different bacterial strains.

The length of the side chains in dextran produced by *Lm* NRRL B-512F has been studied by sequential alkaline degradation [22]. The procedure is based on the substitution of the terminal non-reducing glucopyranose (Glc*p*) at position 6 with *p*-toluenesulfonylmethyl groups. Analysis by GLC-MS reveals that about 40% of the side chains contain one glucose residue, 45% are two glucose units long and the remaining 15% are longer than two. These results confirm HPLC studies of enzymatically hydrolysed dextran from *Lm* NRRL B-512F [3].

2.2.1 NMR Spectroscopic Characterisation of Dextran

Beside chemical modification and degradation of the polymer backbone, NMR spectroscopy is a capable method for structure determination of dextran [29–32]. Moreover, from the experiences of the authors in the field of structure characterisation of polysaccharides and their derivatives, NMR spectroscopy is one of the most important methods for obtaining detailed

structure information [33]. The chemical shifts of the hydrogen and carbon atoms (NMR spectra measured in DMSO-d_6) of the glucose unit in the α-(1 → 6) linked main chain are summarised in Table 2. In addition to the ring protons, the protons of the hydroxyl moieties are located at 4.10–4.12 ppm (OH2), 4.51–4.52 ppm (OH3) and 4.63–4.64 ppm (OH4). Anomeric proton resonances of reducing end groups are down-field shifted for α- and high-field for β-anomers [31]. In the ^{13}C NMR spectrum, six signals for the corresponding C-atoms of the dextran main chain are found (Table 2).

In addition to the resonances of the main chain, signals corresponding to certain branches can be observed. Strain *Lm* NRRL-B 512F is known to produce dextran with about 5% α-(1 → 3) linkages, determined by methylation analysis. Beside the resonances of the α-(1 → 6) linked glucose unit, the signal at 100.5 ppm indicates C1 corresponding to an α-(1 → 3) linkage (Fig. 3). The signal at 84.4 ppm indicates C3 of the α-(1 → 3, 6) linked branching unit. The C6 atom of the non-reducing end group (indicated as $C6_s$, where s is substructure) is assigned to the signal at 61.7 ppm. The α-(1 → 3) linkage content can be estimated as 5% by integration of the C1 signals in α-(1 → 6) and α-(1 → 3) linkages.

Table 2 Chemical shifts (ppm) for α-(1 → 6) linked dextran in DMSO-d_6

	Position						
	1	2	3	4	5	6	6′
^{1}H	4.70–4.69	3.19–3.28	3.43–3.47	3.19–3.28	3.63–3.65	3.73–3.77	3.55–3.59
^{13}C	98.9	72.5	74.1	71.0	71.1	67.1	

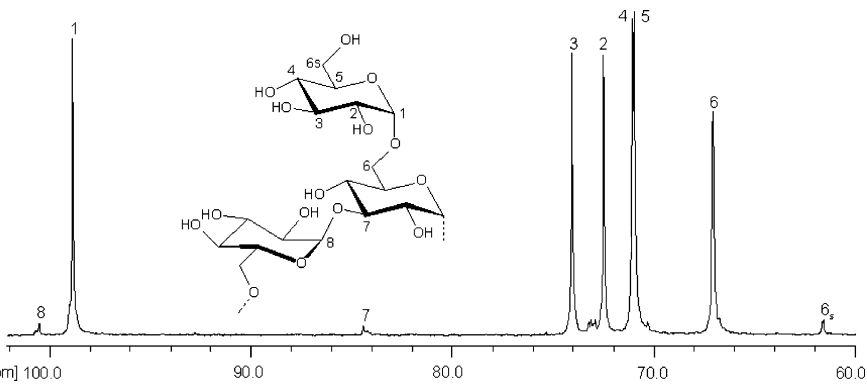


Fig. 3 ^{13}C NMR analysis (in DMSO-d_6) of dextran (M_w 60 000 g mol^{-1}) produced by *Lm* NRRL B-512F in DMSO-d_6

More complex structures need highly resolved NMR spectra applying two-dimensional (2D) spectroscopy for peak assignment. Extensive NMR studies have been carried out for dextran produced by *Leuconostoc ssp.* strain number 10817 (M_w 5400 g mol^{-1}) [34]. A variety of signals beside the resonances for the main chain indicates the structural diversity. A slight signal corresponding to the α-(1 → 3) linkage at 100.5 ppm can be observed. Branching resulting from α-(1 → 2) linkages is predominant. At 97.1 ppm, the anomeric carbon atom appears to participate in the α-(1 → 2) branch [25]. The C1 involved in an α-(1 → 6) linkage and substituted on C2 is situated at 96.3 ppm whereas the C2 is situated at 77.4 ppm. Furthermore, no cross-peak in the HMQC-TOCSY spectrum was observed that corresponded to H1 of the α-(1 → 2) branch at 4.28–4.30 ppm and C6 of the glucopyranosyl residue in the side chain, but one with the C6 of the non-reducing end group. These results indicate branching lengths of one Glc*p* unit. In accord with the low M_w, the amount of reducing end groups is comparatively high and can be detected in the HMQC-TOCSY spectrum. The signal of the α-anomeric (β-anomeric) proton of the reducing end group at 4.91–4.92 ppm (4.28–4.30 ppm) gives a cross-peak with the corresponding carbon at 92.8 ppm (97.5 ppm). A part of the HMQC-TOCSY spectrum is shown in Fig. 4.

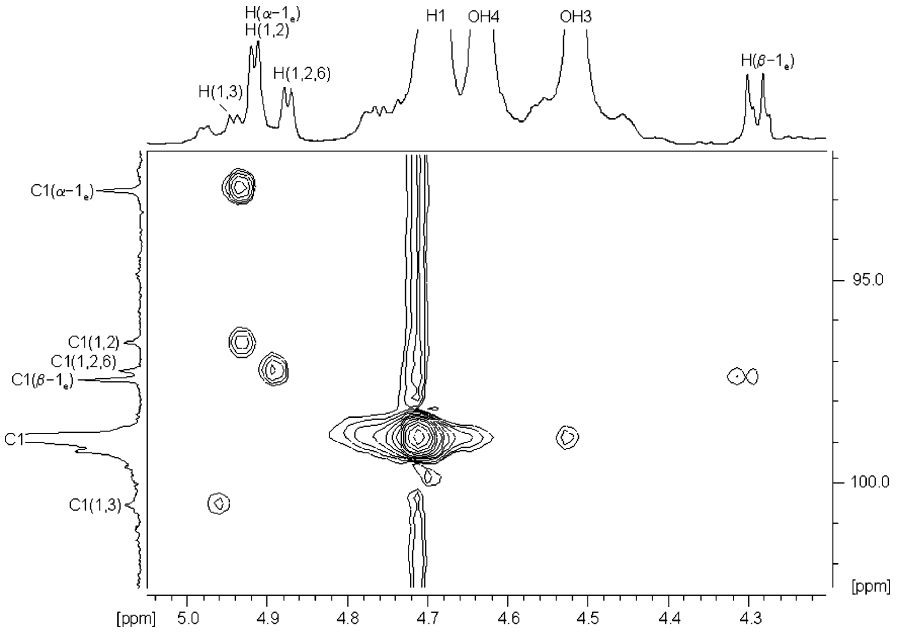


Fig. 4 HMQC-TOCSY spectrum in the region of anomeric atoms of dextran produced by *Leuconostoc ssp.* strain no. 10817 (M_w 5400 g mol^{-1}) in DMSO-d_6 (type of linkage of corresponding AGU and anomeric form of reducing end group, respectively, in *brackets*)

Even with the use of modern NMR instruments, the exact assignment of all resonances beside the α-(1 $\rightarrow$ 6) glycosidic linked main chain is not feasible because of the weak mobility with increasing molecular weight of the polymer. Additionally, a calculation of the degree of branching by integration of relevant signals can not satisfactorily be carried out because of overlay and noise.

2.2.2 Molecular Weight

The weight average molecular weight (M_w) can be determined by light scattering, ultracentrifugation, small-angle neutron scattering and viscometry [35]. Membrane osmometry and end group analysis give information about the number average molecular weight (M_n). Native dextran is generally of a high average molecular weight ranging from 9×10^6 to 5×10^8 g mol^{-1} with a high polydispersity [36–38]. The polydispersity of dextran increases with the molecular weight as a result of increasing branch density [39]. However, defined molecular weight fractions are of interest for many current applications. Beside the fractional precipitation with subsequent molecular weight determination, size exclusion chromatography (SEC) is a useful tool for analysis of the molecular weight distribution (MWD). Figure 5 shows MWD curves obtained by SEC from four dextran samples (a–d), which are used as standards for aqueous SEC, and five further commercially available dextrans (e–i). Even the lower molecular weight dextran samples (e–h)

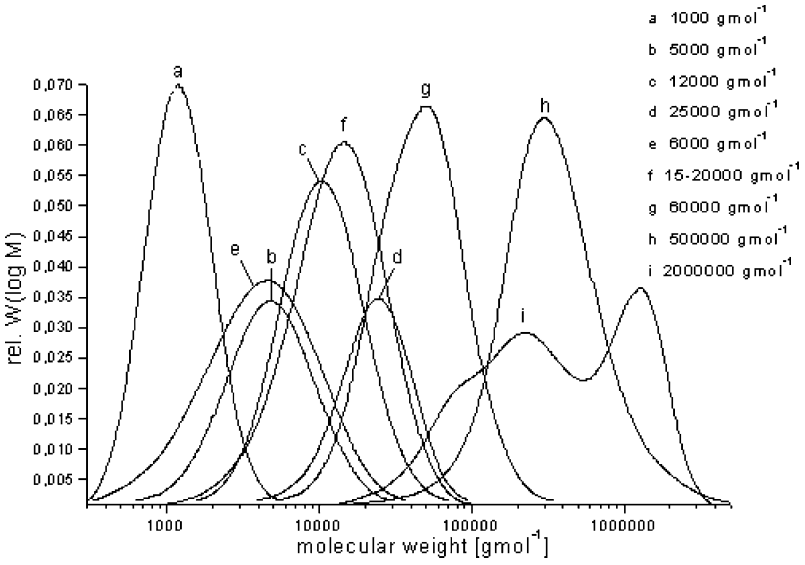


Fig. 5 Molecular weight distribution of dextran fractions (*a–g*) determined by SEC

for technical and scientific use have a low polydispersity and thus a narrow MWD.

2.2.3 Physico-Chemical Characteristics

Being more rod-like below M_w 2000 g mol^{-1}, dextran molecules behave as a random coil in aqueous, low concentration solution, as examined by small angle X-ray scattering (SAXS) measurements [40–42]. If a certain overlap concentration is reached, the individual chains interpenetrate each other and form a transient network of entangled chains resulting in more compact coil geometry and thus a non-Newtonian behaviour [43, 44]. The radius of gyration, R_G, is a useful parameter for estimating the size of dextran molecules in solution. With increasing M_w the R_G increases, whereas higher concentrations or poor solvents lead to decreased R_G values (Table 3) [41, 45].

Naturally occurring dextran is basically amorphous. However, single crystals with lath-like shape can be grown in a mixture of water/polyethylene glycol at temperatures ranging from 120 to 200 °C [47]. Combined electron and X-ray diffraction studies indicate that the unit cell contains two antiparallel dextran chains of two glucopyranosyl residues each [48].

Table 3 Molecular dimensions of dextran from *Lm* NRRL B-512F as radius of gyration (R_G) in nm depending on M_w, concentration and solvent

Solvent/ concentration (mg mL^{-1})	R_G(nm) $M_w \times 10^{-3}$ (g mol^{-1}) 40	70	100	500	1000	2000	Refs.
Water/1.25	8.5	9	–	–	–	–	[41]
Water/5	6	8	9.5	20	27.5	38	[46]
Water/50	30	–	–	–	–	–	[42]
Water/100	–	10	–	–	–	–	[41]
10% Ethanol/50	25	–	–	–	–	–	[42]
1M Urea/50	30	–	–	–	–	–	[42]

2.3 Properties

Depending on the dextran-producing bacterial strain and thus the structural diversities, the properties of dextran may be different for different samples. In general, the occurrence of the α-(1 → 6) glycosidic bond provides an increase of chain mobility and is responsible for the solubility in a variety

of solvents including water, DMSO, DMA/LiCl, formamide, ethylene glycole, glycerol, 6 M aqueous urea, 2 M aqueous glycine and 4-methylmorpholine-4-oxide [13, 45, 49]. The molecular weight of the dextran fraction can influence the time of the dissolution. A 10% aqueous dextran solution (M_w 40 000 g mol^{-1}) will develop precipitates during storage, indicating that dextran solutions are not stable [42, 50]. The adsorption of dextran molecules on the air–liquid interface is the first step of insolubilisation. The precipitates can be resolved in boiling water or DMSO. Even hydrogel formation occurs from concentrated solutions (50–60%) of low molecular weight dextran (M_w 6000 g mol^{-1}) caused by sol–gel conversion, which leads to crystallisation [51].

The colloid osmotic pressure of aqueous dextran solutions can be regulated by molecular weight and concentration of the solute [52]. Dissolved dextran in low concentrations possesses Newtonian flow characteristics [45]. The relationship between viscosity and concentration is shown in Fig. 6 for different dextran fractions [52]. The molecular weight dependence of the intrinsic viscosity can be estimated by several equations [37, 46, 53].

The specific rotation of dextran differs with the solvent and the structural features. Rotations at 25 °C in water and formamide range from + 195 to + 201° and from + 208 to + 233°, respectively [6, 52].

Dextran is a physiologically harmless biopolymer because of its biocompatible, biodegradable, non-immunogenic and non-antigenic properties [54, 55]. It can be depolymerised by different α-1-glycosidases (dextranases) occurring in liver, spleen, kidney and lower part of the gastrointestinal tract.

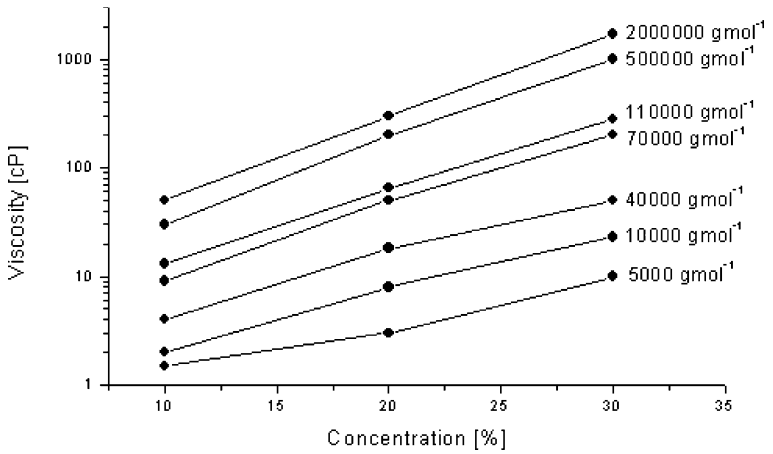


Fig. 6 Dependence of viscosity of dextran fractions on concentration at 25 °C [52]

3
Production and Application of Dextran

3.1
Biosynthesis of Dextran

The majority of dextrans in nature are produced extracellularly via dextransucrase from sucrose by several lactic acid bacteria, principally *Leuconostoc* and *Streptococcus* species [13]. Dextran is also synthesised by dextrinase of different *Gluconobacter* species [56]. Referring to this enzyme, fermentation of maltodextrins leads to α-(1 $\rightarrow$ 4) branched dextrans with comparatively lower M_w. However, dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F has attracted most interest because of commercial use.

Dextransucrase is the active enzyme, which catalyses the transfer of D-glucopyranosyl residues from sucrose to dextran, circumstantially named 1,6-α-D-glucan-6-α-glycosyl tranferase [45, 57]. The relatively high binding energy of the glycosidic bond in sucrose is utilised by the enzyme to synthesise the α-(1 $\rightarrow$ 6) linkages of the polymer backbone [13]. Therefore, no adenosine triphosphate or cofactors are required. Beside sucrose, a number of natural, as well as synthetic, donors yield dextran in the presence of dextransucrase. Lactulosucrose [58], α-D-glucopyranosyl fluoride [59], *p*-nitrophenyl-α-D-glucopyranoside [60] and even dextran [61, 62] can act as donor substrates. For the investigation of the mechanism, pulse-chase studies with ^{14}C-labelled sucrose were conducted [63]. The proposed insertion mechanism involves two nucleophiles at the active site, which attack sucrose and yield two β-glycosyl intermediates by displacing fructose [64]. The primary hydroxyl group of one glycosyl residue attacks nucleophilically the C1 of the second glycosyl intermediate, resulting in the formation of an α-(1 $\rightarrow$ 6) linkage and a free enzyme nucleophile. The unoccupied nucleophile site attacks another sucrose molecule forming a new β-glycosyl intermediate. The primary hydroxyl group of the latter attacks the C1 hydroxyl group of the isomaltosyl unit and is thus conveyed to the glycosyl residue. The glycosyl and dextranosyl groups are alternately transferred between the nucleophiles while a growth of the dextran chain proceeds (Fig. 7). If an acceptor displaces the dextran from the active site, termination of chain extension occurs. Acceptors are polyhydroxyl compounds, for instance maltose, nigerose, methyl-α-D-glycoside and dextran itself [65]. In the latter case, branching results from an attack of the C3 hydroxyl group of a dextran acceptor chain at the glucosyl or dextranosyl unit of the enzyme (Fig. 7, [66]).

Imidazolium groups of histidine are essential for the transfer of a hydrogen ion to the displaced fructosyl moiety [12]. The reprotonation of the imidazole takes place by abstracting a proton from the C6 hydroxyl moiety and facilitating the formation of the glycosidic linkage.

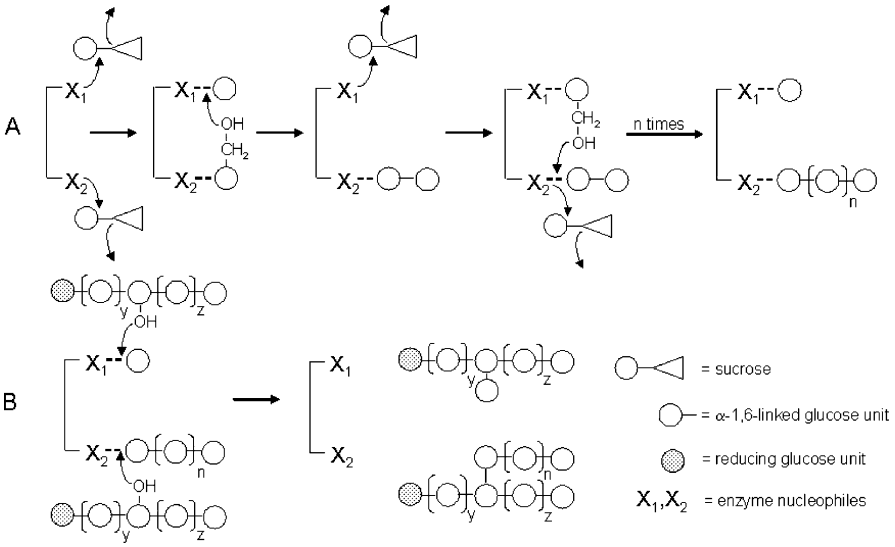


Fig. 7 Mechanism proposed for the synthesis of the α-(1 $\rightarrow$ 6) glycosidic linked dextran backbone (*A*) and α-(1 $\rightarrow$ 3) branches (*B*) by B-512F dextransucrase (adapted from [12])

3.2 Industrial Production

Recently, most major producers of dextran apply a method based on the batch-wise culture of *Leuconostoc mesenteroides* NRRL B512(F) or B512 strains in the presence of sucrose [67]. Aside from serving as energy source for the microorganism, sucrose induces the dextransucrase production [68]. Special nutritional requirements are satisfied by a combination of complex medium components, for instance yeast extracts, acid hydrolysed casein, corn steep liquors or malt extracts with the addition of peptone or tryptone broth [67, 69]. Low levels of calcium and phosphate are necessary for optimal enzyme and dextran yields. Initial pH values for the fermentation media generally lie between 6.7 and 7.2 where maximum enzyme production takes place [70]. Due to the liberation of organic acids, e.g. lactic acid, the pH decreases to approximate 5, the value for maximum enzyme activity [71]. The stability of the enzyme can be improved by the addition of low concentrations of high M_w dextran, methyl cellulose, poly(ethylene glycol) (M_w 20 000 g mol^{-1}) or non-ionic detergents. For practical purpose, the fermentation process for reaching high M_w dextran takes place at a temperature of 25 °C [69, 72]. At lower temperatures, the amount of low M_w dextran increases, while over 25 °C higher branching occurs [73, 74]. Another influence on branching and weight average molecular weight is the concentration of sucrose. With increasing sucrose content, both the degree of branching and

the yield of high M_w dextran decreases; thus optimal conditions are concentrations of 2–10% and continuous feeding with sucrose [74, 75]. After 24–48 h of fermentation, the viscous culture fluid is precipitated in ethanol or methanol [76]. The obtained native dextran has a M_w up to 5×10^8 g mol^{-1} together with a broad distribution [36–38].

Dextran can also be produced enzymatically using cell-free culture supernatants that contain dextransucrase [68]. Low levels of high molecular weight dextran, poly(ethylene glycol) (PEG) or non-ionic detergents stabilise the enzyme [77]. The highest activity of dextransucrase after purification is obtained by the phase partitioning method using aqueous solutions of dextran and PEG 6000 [78]. This procedure is simple, inexpensive and less time-consuming than other purification methods involving ultrafiltration and chromatography.

For clinical and technical dextran, the partial hydrolysis and further fractionation of native dextran gives products with the desired molecular sizes [79, 80]. Variation of the concentration of the hydrochloric or sulfuric acid, the time and temperature of hydrolysis, and the nature and ratio of the precipitant (alcohol/water) for phase separation permits the control of the resultant average molecular weights. Recently, a new fractionation method, called continuous spin fractionation, was developed to improve the efficiency of the separation process [81]. The use of spinning nozzles facilitates the transfer of the soluble, low molecular weight polymer species into the extracting agent to such an extent that successful fractionation becomes possible even with concentrated polymer solutions. The molecular weight distribution and therefore the polydispersity can be significantly narrowed. Attractive alternative methods for producing defined dextran fractions include the use of chain degrading enzymes, dextranases and chain-terminating acceptor reactions [18, 82].

3.3 Chemical Synthesis of Dextran

Beside the natural fermentation process, dextran can be synthesised chemically via a cationic ring-opening polymerisation (ROP) of levoglucosan (1,6-anhydro-β-D-glucose), a pyrolysis product of polyglucans (Fig. 8, [83]).

The polymerisation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose with phosphorous pentafluoride as catalyst under high vacuum and subsequent cleavage of the benzyl ether linkage by Birch reduction leads to dextrans with M_n 41 800–75 750 g mol^{-1} [84, 86]. Furthermore, tri-*O*-methyl- glucopyranose and tri-*O*-ethyl-glucopyranose can serve as monomer but yield a low molecular weight or rather polydisperse polymer (Table 4). The synthetic dextran has features similar to the natural product concerning solubility, optical rotation and spectroscopic characteristics. X-ray powder patterns show higher crystallinity compared to native dextran [87]. A more convenient

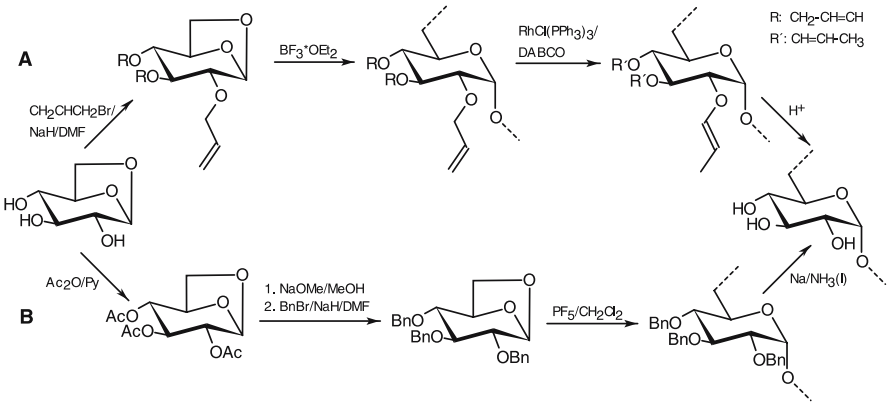


Fig. 8 Synthesis of dextran via a cationic ring-opening polymerisation of 1,6-anhydro-2,3,4-tri-*O*-allyl-β-D-glucopyranose (*path A*) and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (*path B*) [84, 85]

synthesis strategy is the protection of the relevant hydroxyl groups with allyl ether moieties [85]. After the cationic ROP to the stereoregular polymer of 2,3,4-tri-*O*-allyl-α-(1,6)-D-glucopyranan, an isomerisation with $RhCl(PPh_3)_3$ as catalyst results in propenyl ether groups, which can be cleaved under acidic conditions. Polymers with a M_n 21 700 g mol^{-1} and a narrow molecular weight distribution (M_w/M_n 2.1) were obtained (Table 4).

Table 4 Ring-opening polymerisation of 1,6-anhydro-2,3,4-tri-*O*-substituted β-D-glucopyranose in CH_2Cl_2

Monomer [a]	Initiator	[I]/[M] [b]	Temp (°C)	Time (h)	Yield (%)	M_n [c] (g mol^{-1})	M_w/M_n	Refs.
Me	$BF_3 \cdot OEt_2$	0.030	+ 25	157	6	1400	–	[84]
Me	PF_5	0.037	– 28	144	85	19 950	–	[84]
Et	PF_5	0.118	– 78	167	70	14 800	–	[84]
Et	PF_5	0.190	– 78	12	51	47 200	62.5	[84]
Bz	PF_5	0.202	– 78	87	86	56 100	–	[84]
Bz	PF_5	0.106	– 78	91	95	75 750	–	[84]
Allyl	$BF_3 \cdot OEt_2$	0.480	0	144	84	21 700 [d]	2.1	[85]
Allyl	$CF_3SO_3CH_3$	0.480	0	80	25	11 000 [d]	1.2	[85]

[a] Substituent of 1,6-anhydro-β-D-glucopyranose
[b] Molar ratio of monomer (M) to initiator (I)
[c] Determined by membrane osmometry in toluene
[d] Determined by SEC in $CHCl_3$

3.4 Application of Dextran

Clinical grade dextran with molecular weights of 40 000, 60 000 and 70 000 g mol^{-1} (designated dextran 40, 60 and 70) in 6 or 10% aqueous solutions are at present available for replacing moderate blood losses [13, 45]. The polymer essentially substitutes blood proteins, e.g. albumins, in providing colloid osmotic pressure to pull fluid from the interstitial space into the plasma. Because of the low antigenicity and high water solubility, dextran produced by *Lm* NRRL B-512(F) is the material of choice for clinical dextran. Furthermore, the high percentage of α-(1 $\rightarrow$ 6) glycosidic linkages is responsible for the biological stability in the human bloodstream. The antithrombotic effect of clinical dextran fractions provides a prophylactic treatment for deep venous thrombosis and postoperative pulmonary emboli. Dextran 40 has the special feature of improving the blood flow, presumably caused by reduction of blood viscosity and inhibition of erythrocyte aggregation. Hypersensitivity reactions are limited by a preinjection of low molecular weight dextran (M_w 1000 g mol^{-1}) as a monovalent hapten.

Technical dextran fractions are common in various industries [67, 70]. Because of the narrow molecular weight distribution, specific fractions are used as SEC standard for molecular weight determination. Dextran in X-ray and other photographic emulsions improves the efficiency of silver without loss of fineness of grain [12]. The polysaccharide is used as an ingredient for cosmetics and in bakery products due to superior moisturising properties. Advanced heat shock stability and an increase of the melting temperature can be observed after addition of native dextran to frozen dairy products [43]. The presence of dextran in aqueous solutions helps to confer a favourable physiological environment due to the colloid osmotic pressure. Thus, the use of dextran is a benefit in the preservation of viable organs and as an ingredient for ophthalmic formulations such as artificial tears and eye drops. Dextran is able to prevent protein opsonisation and hence is used for surface modification, e.g. coating of ferrite particles for hyperthermia in cancer therapy [88–90]. Dextran and PEG are known to separate in water [91]. This aqueous two-phase system has proved successful for separating mixtures of biomolecules and subcellular particles, for instance in enzyme-catalysed peptide synthesis [92, 93].

3.4.1 Cross-linked Dextran

Cross-linking can be achieved by physical interactions and chemical reactions. Concentrated solutions of low molecular weight dextran (M_w 6000 g mol^{-1}) lead to the formation of hydrogels by crystallisation [51]. Cross-linking with epichlorhydrin gives commercial products known as Sephadex

for gel filtration and Debrisan, a wound-cleansing agent that shortens the healing time [94]. Another matrix for the separation of macromolecules is Sephacryl, an allyl dextran cross-linked by *N*,*N*′-methylene bisacrylamide [95, 96]. Furthermore, hydrogels can be obtained by reaction of the polymer with various bifunctional reagents like diisocyanates and phosphorus oxychloride, or by acylation (Sect. 4.2.1), e.g. with methacrylate groups and subsequent UV irradiation [97–99]. Cross-linking of carboxymethyl dextran (CMD) leads to stimuli-responsive hydrogel membranes (Sect. 5.2.2.6). In general, dextran hydrogels have received increased attention due to their biodegradable and biocompatible properties as an efficient matrix system for industrial polymer separation (gel filtration) [54, 55].

4
Esterification of the Polysaccharide

4.1
Inorganic Esters of Dextran

Among the inorganic esters of dextran, only the sulfuric acid half esters (sulfates) and the phosphoric acid esters (phosphates) have gained significant interest. The introduction of sulfate or phosphate groups leads to polyelectrolytes with an improved water solubility, giving aqueous solutions with defined rheological properties, which are valuable as viscosity-regulating agents. Nevertheless, the pronounced biological activity is the most important feature of such dextran derivatives. The anticoagulating properties of the heparin analogue inorganic dextran esters were revealed as early as the 1940s [100].

4.1.1
Dextran Phosphates

The preparation of dextran phosphate (Fig. 9) is simply achieved by treatment of the polysaccharide with polyphosphoric acid in formamide yielding products with up to 1.7% phosphorus.

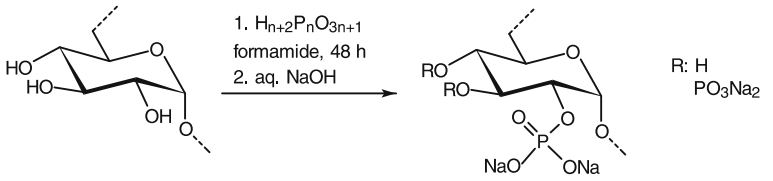


Fig. 9 Preparation of dextran phosphoric acid esters (phosphates) with polyphosphoric acid

These pure dextran phosphates exhibit immunostimulatory effects independent of the molecular weight. It was shown that the mitogenic response of murine splenocytes can be enhanced [101]. Moreover, dextran phosphate (M_w 40 000 g mol^{-1}) increases the survival rate of mice infected with influenza A2 virus (H2N2). Intraperitoneal administration of dextran phosphate, an interferon inducer, shows a 1-day delay in the virus growth in lung, and production of HAI antibody, when compared to the non-treated or dextran-treated mice as controls. More significant was the 2-day delay in the development of lung consolidation, which led to 40% survival of the treated mice [102].

4.1.1.1 Palmitoyldextran Phosphates

Dextran phosphates with additional long chain aliphatic ester functions such as palmitoyldextran phosphates exhibit antitumour activity (Fig. 10).

A growth regression of Sarcoma 183 ascites tumour up to 82% is described [103]. Comparable results were obtained for dextran modified by palmitoylation and/or phosphorylation, which yields three derivatives, i.e. palmitoyldextran phosphate, dextran phosphate and palmitoyldextran. Of these compounds, only palmitoyldextran phosphate showed a growth-inhibitory effect on Ehrlich solid tumour in mice. In combination therapy with mitomycin C, bleomycin, cyclophosphamide and 5-fluorouracil, palmitoyldextran phosphate manifested strong synergistic effects in case of Sarcoma 180 ascites and L1210 leukemic tumours. The compound is not directly cytocidal to Sarcoma 180 ascites tumour, but it appears to act via activation of peritoneal macrophage. The antitumour activity of palmitoyldextran phosphate apparently is mainly due to immunological host-mediated mechanisms [104].

In addition, esters of dextran-bearing palmitoyl groups and phosphate functions can be used for the preparation of specifically modified liposomes, which are exploited for the entrapment of peptides such as hirudin, the most potent inhibitor of thrombin [105].

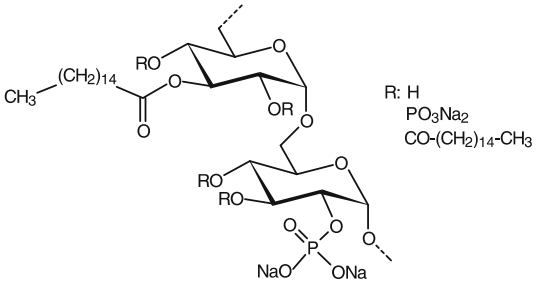


Fig. 10 Typical structural element of palmitoyldextran phosphates

4.1.2
Dextran Sulfuric Acid Half Esters (Dextran Sulfates)

In contrast to dextran phosphate, which is only of scientific interest up to now, the dextran sulfuric acid half ester and its sodium salt, usually simple referred to as dextran sulfate, is a commercially available product today. Its high purity, water solubility and reproducible quality commend it for many applications in molecular biology and the health care sector.

Different approaches for the synthesis of dextran sulfates are known [106]. First attempts exploited treatment of the polysaccharide with concentrated or slightly diluted H_2SO_4 in order to achieve sulfation. A remarkable depolymerisation occurs under these conditions. H_2SO_4 can also be applied in combination with alkyl alcohols yielding alkyl sulfates as reactive species. Here the polymer degradation is comparably low. Powerful sulfating agents are chlorosulfonic acid and sulfur trioxide [107, 108]. A major drawback of these reagents is the sensitivity against moisture. Both compounds strongly react with water. A convenient method to reduce the risk during synthesis is the application of the complexes of $ClSO_3H$ and SO_3 with organic bases, e.g. triethylamine (TEA) and pyridine (Py) or aprotic dipolar solvents, e.g. *N*,*N*-dimethylformamide (DMF, Fig. 11). The commercially available SO_3-DMF- and SO_3-Py complexes are white solids that are easy to use. Homogeneous reactions of dextran with these complexes are established methods [109].

A remarkable new method for the conversion of dextran and dextran derivatives, mainly towards mimetics of heparan sulfate uses comparable sulfation but applies 2-methyl-2-butene (2M2B) as an acid scavenger of neutral character [110]. This procedure shows a more efficient reaction combined with diminished chain degradation, as can be seen in Table 5. The method was used for the sulfation of carboxymethylated dextran.

Although sulfation of dextran is an easy synthetic step, most of the studies directed towards biological activity of dextran sulfate are carried out with commercially available products. The DS of these derivatives is usually rather high. The DS of the most widely applied dextran sulfate from Sigma is 2.3. A typical ^{13}C NMR spectrum of a commercial dextran sulfate is shown in Fig. 12. The spectrum confirms the high degree of sulfation and reveals com-

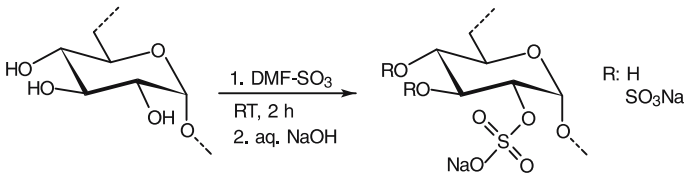


Fig. 11 Preparation of dextran sulfuric acid halfesters with *N*,*N*′-dimethylformamide (DMF)-SO_3 complex

Table 5 Results for the sulfation of carboxymethyl dextran with different sulfating agents applying 2-methyl-2-butene (2M2B) as an acid scavenger (reaction temperature 30 °C, [110])

Sulfating agent	Molar ratio reagent/AGU	DS_{CM}	DS_S	Reducing sugars (nmol Glu mg^{-1})
$ClSO_3H$	2 : 1 [a]	0.37	0.35	12.71
SO_3-Me_3N	2 : 1	0.49	0.05	1.98
SO_3-Et_3N	2 : 1	0.29	0.40	2.61
SO_3-pyridine	2 : 1	0.44	0.17	1.84
SO_3-DMF	2 : 1	0.37	0.23	1.57
SO_3-DMF/2M2B	2 : 1	0.56	0.42	0.82
SO_3-DMF/2M2B	3 : 1	0.57	1.00	0.71
SO_3-DMF/2M2B	5 : 1	0.55	1.20	0.50

[a] At 22 °C

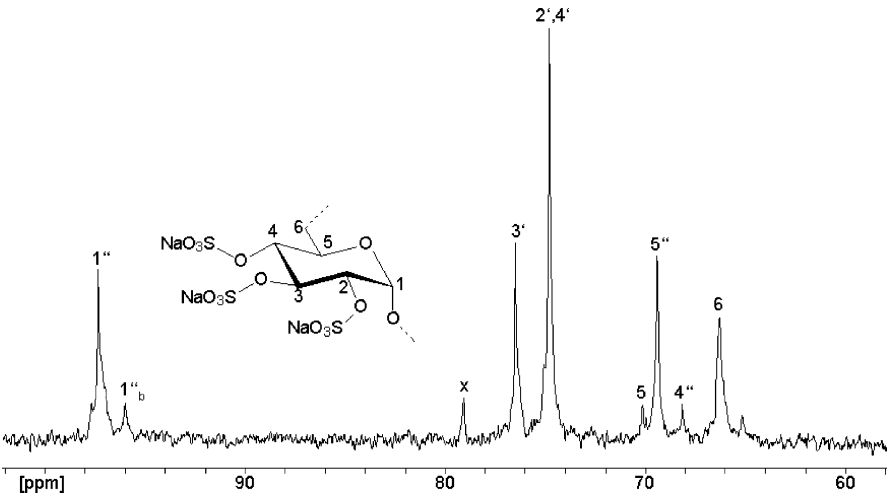


Fig. 12 Typical ^{13}C NMR spectrum of a commercial dextran sulphate; ′ indicates functionalisation at the corresponding position, ″ indicates sulfation at the neighbouring position, *b* indicates a C-atom of a branching structure, *X* corresponds to a signal caused by a 1,3 branching and substitution at C2 and C4 or a 1,2 branching and substitution at C3

plete functionalisation of position 2. The molecular weight of the commercial dextran sulfates is in the range 5000 to 500 000 g mol^{-1}. Besides the use of dextran sulfates with different M_W, the DS and the structure of the derivatives have not usually been considered in most of the studies discussed below.

4.1.2.1 Bioactivity

Both pure dextran sulfates and mixed derivatives of dextran containing sulfate groups exhibit a broad spectrum of biological activities. The structural features of so-called mimetics of heparan sulfate are discussed in Sect. 5.2.2.1. The pure dextran sulfates used are almost exclusively commercial products with DS values above 2.0.

Among the first biological effects recognised for dextran sulfate was the anticoagulating activity [100, 111]. Thus, dextran sulfates with different molecular weight (7000 up to 458 000 g mol^{-1}) have been tested as potential substitutes for heparin in anticoagulant therapy, showing that products with the lowest molecular weight display the highest anticoagulating properties [112–116]. Investigations on sulfates of cellulose and dextran suggest that the anticoagulant activities of these compounds are at least partially mediated through antithrombin III [117]. However, at best this is only 15% of heparin's activity. Nevertheless, the dextran derivative can be applied as mimic or substitute for heparan sulfate for specific interactions. For instance, it was shown that it protects the kidney against endothelial damage in a model of thrombotic microangiopathy [118]. The beneficial effect of dextran sulfate could be attributed to its ability to protect endothelial cells from coagulation and complement activation, i.e. it acts as a "repair coat" of injured glomerular endothelium.

It is also a well established fact that dextran sulfate modulates the immune response with effects on macrophages [119], proliferation of B-lymphocytes [120], and helper T-lymphocytes [121]. It has been implicated as an activator of C3 via the alternative pathway of complement [122]. Numerous reports claim that dextran sulfate is a potent inhibitor of human immunodeficiency virus (HIV), Herpes simplex virus (HSV) and other pathogens [123–125].

Comparison of the activity of sulfated homopolysaccharides such as dextran and cellulose esters with that of neutral homopolysaccharides and sulfated heteropolysaccharides such as heparin and heparan sulfuric acid half esters shows potent virucidal activity against human T-cell lymphotropic virus type III (HTLV-III) for the sulfated homopolysaccharides. In contrast, neutral homopolysaccharides have no effect and sulfated heteropolysaccharides exhibit only a little effect on HTLV-III activities. This suggests that the sulfate moiety and the type of polysaccharide are most important in inhibiting growth of HTLV-III [126].

In vitro studies have shown that dextran sulfate inhibits HIV binding, replication and syncytium formation, probably because they interfere with the ionic interaction between cell surface components such as CD4 or sulfated polysaccharides and positively charged amino acids concentrated in the V3 region of HIV gp120 [127–129]. Again, low molecular weight derivatives with

$M_w < 10\,000$ g mol^{-1} are the most efficient [130]. Dextran sulfate was shown to be a potent inhibitor of, e.g. HSV infectivity in vitro (Table 6, [131]).

In contrast, it has not been possible to provide any protection in murine models of HSV infection. In addition, it was found in preclinical trials that dextran sulfate administered orally [132] and intravenously [133] did not lead to a decrease in circulating HIV antigen. Reasons may be the poor absorption of the polysaccharide derivative from the intestine and desulfation processes [134, 135]. Nevertheless, the treatment with dextran sulfate and derivatives of dextran sulfate of optimised DS for short intervals using high dosages may overcome these problems. In this case, the sulfated polysaccharide is not substantially endocytosed or degraded by cell receptor binding in the mammal and thereby retains antiviral activity in vivo [136]. Additionally, the combination of azidothymidine (AZT), which has shown promise in prolonging life in patients with AIDS but exhibits significant blood toxicities, and dextran sulfate leads to a higher effectivity than either alone [137].

Recent research and development are also concentrated on the cancerostatic effects of dextran sulfate. A number of mechanisms for this specific biological interaction were established. Previous studies centre on the ability of dextran sulfate to reduce peritoneal- [138] and pulmonary metastasis [139]. These studies used derivatives of both low (7000 g mol^{-1}) and high molecular weight (500 000 g mol^{-1}) to reduce adherence of melanoma or lung tumour cells to endothelium of lung and peritoneum. The dextran sulfate was not toxic to cancer cells, therefore the anti-metastatic capacities were thought to be due to the polyanionic nature of the polymer. It was suggested that the dextran sulfate binding to both tumour and endothelial cells rendered them more negatively charged. This increasing electrostatic repulsion altered the adhesiveness between the tumour cells and the vascular endothelium. The altered adhesion properties were thought to reduce or inhibit the settlement of the tumour cells in the capillaries of organs, resulting in a subsequent reduction

Table 6 Infectivity of HSV-1 (Herpes simplex virus) and HSV-2 strains pretreated with different concentrations of dextran sulfate for 1 h at 37 °C

Concentration (nM)	Plaque forming unit (% of control) for HSV-1 (F)[a]	HSV-2 (22)[a]
0.10	88.4 ± 23.7[b]	69.2 ± 1.4
0.25	72.6 ± 30.2	63.1 ± 6.2
0.50	58.8 ± 21.2	55.2 ± 3.1
1.00	49.3 ± 22.7	43.5 ± 3.1
10.00	15.6 ± 9.1	3.2 ± 1.5
50.00	0	0

[a] Wild-type strain
[b] Results are the mean (± the SD) of four independent experiments

in metastasis. Recent findings show that the ability of dextran sulfate to completely inhibit hyaluronidase needs to be invoked [140, 141]. Hyaluronidases have been previously implicated in tumour progression and metastasis. The ability of the dextran derivative to inhibit enzymatic degradation of hyaluronan into biologically active fragments was documented. Consequently, the finding of reduced metastasis after intravenous or intraperitoneal administration of dextran sulfate could be easily attributed to the hyaluronidase inhibitory action as well as an anti-adhesive mechanism of action caused by the polyelectrolyte.

Interestingly, the polysaccharide sulfate is also considered as an antiscapie drug [142]. Thus, it was included in an experimental setting studying the mode and the site of action of the major antiscrapie drugs, investigating their effects on the abnormal protease-resistant isoform of a host-encoded protein (PrPres) and on its accumulation in mouse spleen. Dextran sulfate delayed the beginning of the clearance phase but then blocked PrPres synthesis for a long period of time, probably because of its immunological effects on the spleen.

Although dextran sulfate exhibits such promising biological properties its application is limited because of various side effects. In early studies, clinical trials were discouraging with reports of, inter alia, stiff and painful joints and loss of hair [143]. Likewise chronic toxicity studies revealed retardation in weight gain and osteoporosis [133, 144]. More serious is the fact that dextran sulfate may cause colitis. Today, treatment of mice with the polysaccharide ester has even become a standard method to experimentally induce colitis. It is used both to study the mechanism of inflammatory reactions and to elucidate the reasons for colonic cancer. Thus, in recent investigations the "dextran sulfate-induced colitis" was induced by oral administration for 3 and 7 days to clarify the microvascular changes and the effector sites of lansoprazole during the formation of colitis [145]. Moreover, changes in expression or a redistribution of intercellular tight junction proteins were investigated using acute and chronic dextran sulfate-induced colitis in mice [146]. In acute inflammation, the changes are more pronounced than in chronic inflammation. In addition, studies applying dextran sulfate-induced colitis in mice have suggested that the enhanced release of reactive oxygen species plays an important role in the pathogenesis of inflammatory bowel disease, such as ulcerative colitis and Crohn's disease. It was shown that free radical scavengers (edaravone and tempol) suppressed the colonic shortening and the damage score. A clinical effect for edaravone and tempol in inflammatory bowel disease patients is strongly expected [147].

4.1.2.2
Dextran Sulfates in Supramolecular Assemblies

Besides the bioactivity, dextran sulfate was studied for a broad variety of applications using its polyelectrolyte nature and its ability to form polyelec-

trolyte complexes (PEC) with polycations such as chitosan. The process is based on the sequential deposition of interactive polymers from their solutions by electrostatic, van der Waals, hydrogen bonding and charge transfer interactions [148]. These interactions can be applied to create layer-by-layer (LbL, Fig. 13) assemblies of functional material surfaces with defined biodegradability or bioactivity.

Alternate anti-vs procoagulant activity of human whole blood on a LbL assembly between chitosan and dextran sulfate has been achieved [149, 150]. Furthermore, the technique permits the formation of biodegradable nanostructures with nanometer-order thickness on surfaces, which is an important requirement for biomedical applications. The alternating enzymatic hydrolysis of a LbL assembly formed from chitosan and dextran sulfate by chitosanase was demonstrated via measurements with a quartz crystal microbalance (QCM) [151]. The hydrolysis of the assembly was clearly dependent on the surface component. The hydrolysis of the assembly with the dextran sulfate surface was saturated within 10 min and was much faster than the hydrolysis of the assembly with the chitosan surface, although chitosanase can hydrolyse chitosan (Fig. 14).

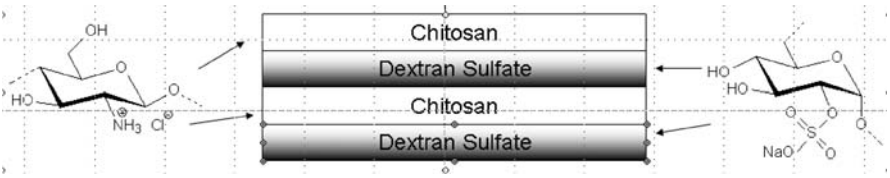


Fig. 13 Polyelectrolyte complex formed by layer-by-layer assembly of dextran sulfate and chitosan

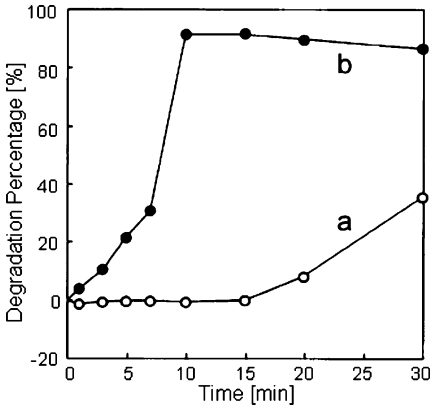


Fig. 14 Enzymatic hydrolysis with chitosanase of (**a**) the layer-by-layer (LbL) assembly of dextran sulfate/chitosan (five steps, total thickness 22 nm) with a chitosan surface and (**b**) a comparable LbL assembly (dextran sulfate/chitosan, six steps, total thickness 55 nm) with a dextran sulfate surface

This unique hydrolysis may appear from the electrostatic condensation of the enzyme with its cationic surface on the outermost surface of dextran sulfate, leading to subsequent hydrolysis of chitosan in the underlayer of the assembly.

In addition, the complexes based on the system dextran sulfate/chitosan show remarkable pH selectivity. Complex gels with approximately equivalent concentrations of amino and sulfate groups are highly sensitive to the change in external pH in a narrow range. The maximum volume of the complex gel was observed at pH 10.5 and was approximately 300 times as large as the initial one. Compared with the swelling behaviour of a complex gel prepared from carrageenan, it seems to be the high density of the ionisable functional groups, as well as the flexibility of acidic polymer chains, which contribute to the high pH sensitivity [152].

The strong interaction of dextran sulfates with cationic functions in porous support materials is exploited to create new highly charged surfaces for adsorption of proteins. It was revealed that new and strong ionic exchange resins are accessible by simple and rapid deposition of dextran sulfates on commercial DEAE- or MANAE-agarose. The material is characterised by an increased charge density on the porous surface of the support, which can perfectly bind protein material, as demonstrated in Fig. 15 [153].

PEC can adsorb the majority (80%) of the proteins contained in crude extracts from *Escherichia coli* and *Acetobacter turbidans* at pH 7 and can be used to immobilise industrially relevant enzymes, e.g. *Candida antarctica* A and B with very high activity recoveries and immobilisation rates. In contrast to covalent binding, the proteins can be recovered and do not undergo irreversible structural alteration.

A comparable strategy is applied to obtain compounds with a pronounced biological selectivity. By treatment of a porous NH_3^+-containing polypropylene membrane with the sulfate of dextran, a material for convenient removal

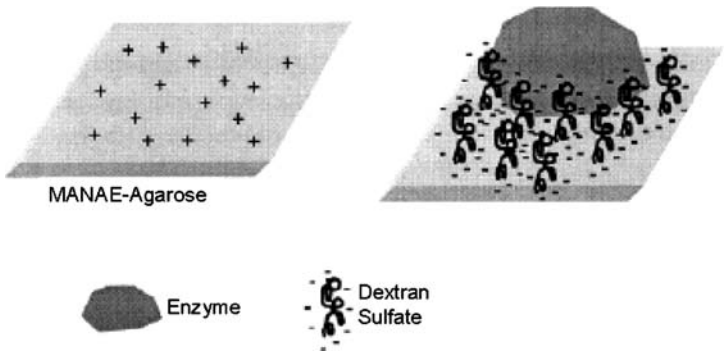


Fig. 15 Strong and non-distorting reversible binding of enzymes on polyelectrolyte complexes [153]

of HIV and related substances from blood, plasma or other body fluids is obtained. Filtration of HIV-containing human plasma results in 99.2% removal of HIV [154].

The "chitosan-dextran sulphate PEC" is widely used for the preparation of colloidal systems as well. Slow drop-wise addition of the components is generally used for the formation of PECs, which allows elaborating both cationic and anionic particles with an excess of chitosan or dextran sulfate, respectively. The PEC particles show a core/shell structure. The hydrophobic core results from the segregation of complexed segments, whereas excess component in the outer shell ensured the colloidal stabilisation against further coagulation. Considering the host/guest concept for the formation of PECs, the influence of the molecular weight of components on particle sizes could be well explained by the chain length ratios of the two polymers and is schematically demonstrated in Fig. 16.

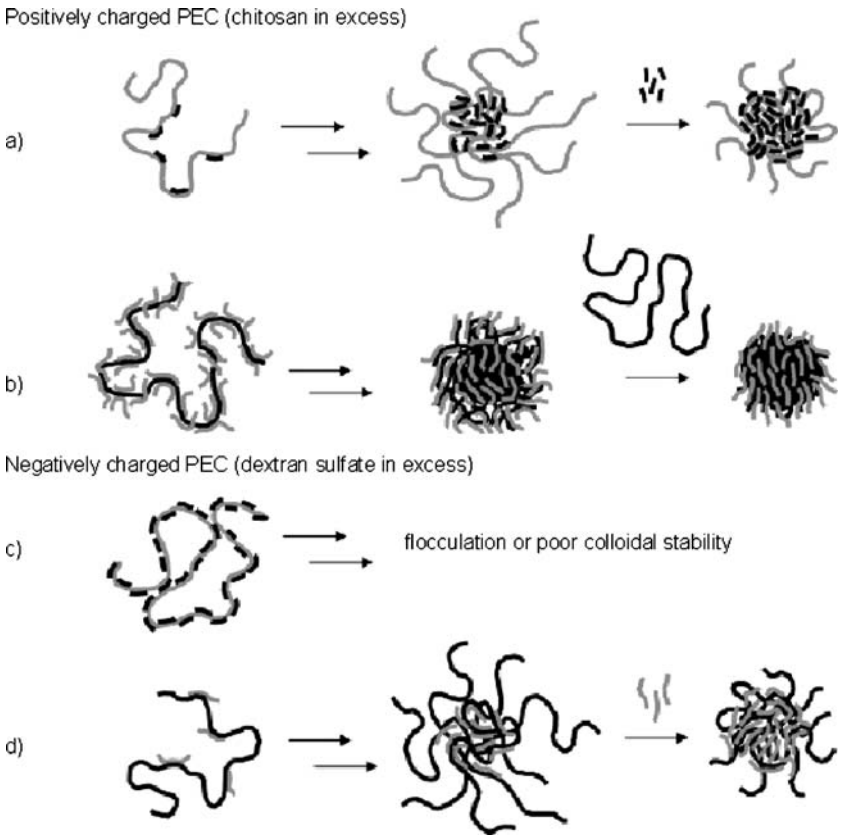


Fig. 16 Preparation of differently charged microparticles, dependent on the chain length of the polymers applied [155]

As an irreversible flocculation occurred with a drop-wise approach for both cationic and anionic PEC particles when the mixing ratio was close to unity, a more versatile and simpler method was found to be the one-shot addition of one solution to the other. Characterisation of particles obtained via this path revealed very similar properties to those obtained by a slow drop-wise approach [155].

Polyelectrolyte multilayer microspheres, prepared by alternating adsorption of dextran sulfate and protamine on melamine formaldehyde cores followed by the partial decomposition of the core, were used to immobilise the peroxidase and glucose oxidase. Retention of enzymic activity of the peroxidase/glucose oxidase system incorporated into the microspheres was demonstrated. These bienzyme system immobilised in the microspheres can be applied for kinetic glucose assays [156].

Highly engineered nanoparticles containing dextran sulfate are prepared for applications in the field of medical diagnostics, e.g. beginning atherosclerosis, which is an inflammatory disease of the arterial walls. A targeted magnetic resonance imaging (MRI) contrast agent for in vivo imaging of early stage atherosclerosis was designed. Early plaque development is characterised by the influx of macrophages, which expresses a class of surface receptors known collectively as the scavenger receptors. The macrophage scavenger receptor class A (SRA) is highly expressed during early atherosclerosis. The macrophage SRA therefore presents itself as an ideal target for labelling of lesion formation. By coupling a known ligand for the scavenger receptor (dextran sulfate) to a MRI contrast agent, early plaque formation can be detected in vivo [157].

It should be mentioned that the defined interaction of dextran sulfate with amino functions is not only applied for the design of structures on the supermolecular level but also on the molecular level. Thus, a preferred handed helical structure was induced into the polyaniline main chains by chemical polymerisation of achiral aniline in the presence of dextran sulfate as a molecular template. This affords a novel chemical route for the synthesis of chiral conducting polymers [158].

4.2 Organic Esters of Dextran

In contrast to the broad variety of applications of $1 \rightarrow 4$ and $1 \rightarrow 3$ linked glucans after reaction with (C_2 to C_4) carboxylic acid anhydrides and chlorides [159], the use of dextran esters of short chain aliphatic acids such as acetates or propionates is rather limited. According to the present knowledge, the commonly applied acetylation of polysaccharides with the acetic acid anhydrides or acetyl chlorides in the presence of triethylamine or pyridine as base does not lead to pure and soluble dextran acetate with significant DS values. In contrast, dextran propionates [160] and butyrates [161] can be

easily prepared in heterogeneous reactions of the polymer suspended in pyridine using the carboxylic acid anhydride. The acylation of dextran yields derivatives with defined hydrophobic character. Thus, the solubility of dextran esters in water varies with the DS and the chain length of the substituent. It was shown that the maximum DS values while maintaining water solubility are 0.26 for C_6 carboxylic acid esters and 0.50 for C_4 carboxylic acid esters of dextran [162]. These values are comparable with data for solubility of ethyl (DS 0.81) and butyl (DS 0.69) carbonate substituted dextran [163]. Such hydrophobically modified dextran derivatives form aqueous biphase systems in combination with dextran [162] or with PEG [164, 165], which are exploited for the separation of biological material. Using dextran esters with C_3, C_4 and C_6 acid groups, a family of biphase systems is accessible by varying the properties of the hydrophobically modified dextran. This approach allows control of the phase boundary defining the onset of biphase formation as well as the solvency properties of the more hydrophobic phase. Dextran esters of aliphatic carboxylic acids are also exploited for pharmaceutical coatings [166].

Acylation reactions of dextran in combination with NMR spectroscopy are tools for the elucidation of the reactivity of dextran [30, 161, 167] and for the analysis of structural features of the polymers. The acetyl group distribution in the Glc*p* units in acetylated amylose, dextran and cellulose was studied by ^{1}H and ^{13}C NMR spectroscopy. It was concluded that the reactivity of OH groups decreased in the order C2 > C3 > C4 for dextran.

Subsequent conversion of dextran derivatives with acetic acid or propionic acid anhydride is an effective method for revealing structural features on the molecular level. This is illustrated on a dextran propionate (M_w 5430 g mol^{-1}) which can be completely acetylated with acetic anhydride/pyridine in a separate step yielding a peracetylated sample (dextran propionate acetate, DPA). The assignment of the chemical shifts of DPA is carried out via 2D NMR (Fig. 17).

DS_{Prop} can be determined precisely from highly resolved polymer-^{1}H NMR spectra using the spectral integrals of the anhydroglucose unit's (AGU) proton region at 3.1–5.3 ppm and the integrals of the acetyl proton signals at 1.8–2.1 ppm. The appearance of three signals for the acetyl protons and the complexity of the COSY signals for H2, H3 and H4 suggest a statistic pattern of propionylation.

The introduction of longer aliphatic acids can also be achieved by acylation with the so-called impeller method. The carboxylic acids or their anhydrides are converted in situ to reactive mixtures of symmetric and mixed anhydrides (Fig. 18).

Chloroacetyl, methoxyacetyl and most importantly trifluoroacetyl moieties are used as impellers. Carboxylic acid esters of polysaccharides with almost complete functionalisation can be obtained. Thus, chloroform-soluble dextran stearates and dextran myristates with DS 2.9 are prepared by

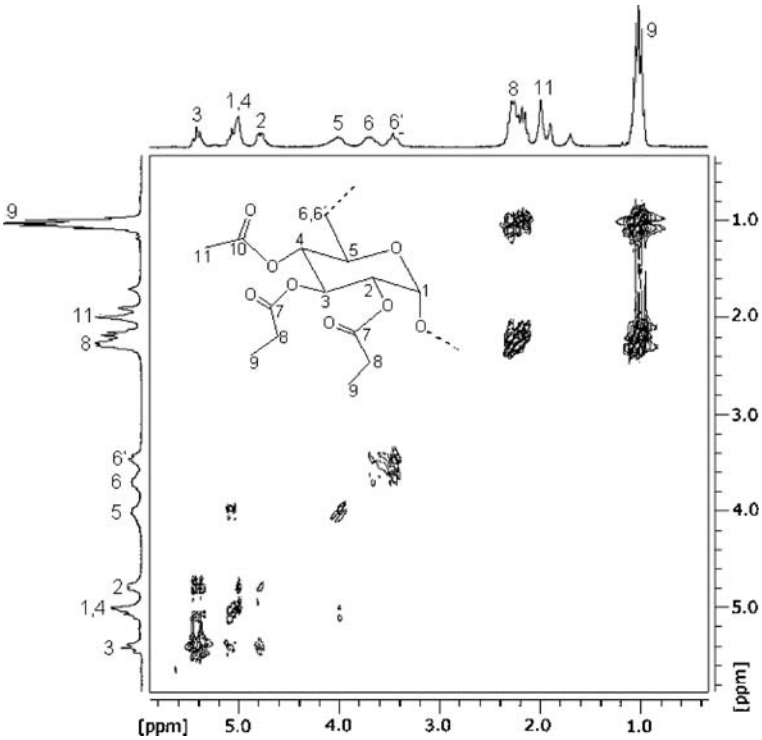


Fig. 17 ^{1}H, ^{1}H-COSY NMR spectrum (in $CDCl_3$) of a dextran acetate propionate (DS_{Prop} 1.70) [160]

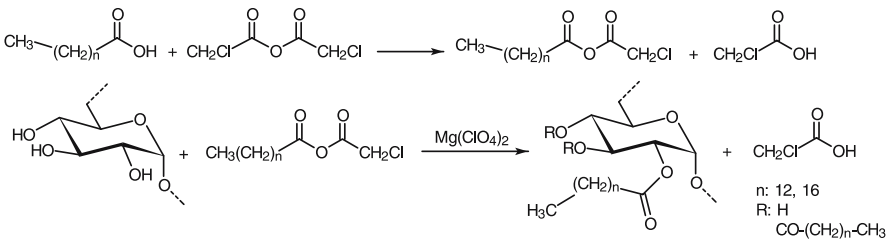


Fig. 18 Formation of mixed anhydrides during esterification of dextran using the impeller method

treating dextran in chloroacetic anhydride with the corresponding acids at 70 °C for 1 h. The presence of magnesium perchlorate as catalyst is necessary [168].

4.2.1
Homogeneous Esterification Reactions

Dextran usually dissolves in water, DMSO, DMA/LiCl, DMF/LiCl, formamid, aqueous urea and glycerin. The solubility may be restricted by a high crystallinity of the polymer (Sect. 2.3). The most versatile solvents for homogeneous acylation reactions are DMSO or mixtures of a polar aprotic solvent and a salt. The broadest application found the combination of substituted amide with LiCl. Thus, dextran dissolves easily in the mixture DMF/LiCl upon heating to 90–100 °C. This solvent can be exploited for the esterification of dextran as displayed in Fig. 19.

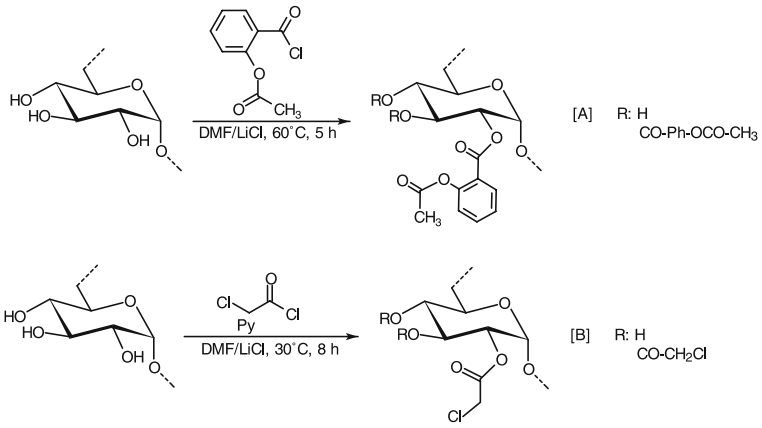


Fig. 19 Dextran esters synthesised homogeneously in DMF/LiCl [169, 170]

Conversion of dextran in DMF/LiCl with succinic anhydride is a popular method for the introduction of spacer-bound large molecules [171, 172]. Moreover, homogeneous esterification is exploited for the binding of bioactive compounds yielding prodrugs and for the introduction of unsaturated moieties giving precursors for hydrogels as discussed in the next sections.

4.2.1.1
Hydrogels Based on Unsaturated Dextran Esters

Hydrogels prepared via homogeneous esterification of dextran with unsaturated carboxylic acids are advanced polysaccharide-based products useful for drug delivery systems and protective encapsulants, e.g. of viruses used in gene therapy [173]. Very promising in this regard is the dextran maleic acid monoester [174], which can be obtained by conversion of dextran in DMF/LiCl with the maleic anhydride in the presence of TEA. The DS of the products can be easily controlled with the amount of anhydride applied but

is also influenced by temperature, amount of catalyst and reaction time, as displayed in Table 7.

The dextran maleates are easily soluble in various common organic solvents such as DMSO, DMF, *N*-methyl-2-pyrrolidone (NMP) and DMA. The hydrogels are manufactured by irradiation of dextran maleate with long-wave UV light (365 nm). The minimum DS required for proper UV cross-linking of the derivatives is 0.60. The hydrogels show a high swelling capacity (swelling ratio up to 1489% at pH 7) depending on DS and the pH of the medium, i.e. highest swelling ratio in neutral pH, followed by acidic (pH 3) and alkaline conditions (pH 10). The swelling ratio increases with increasing DS. The surface and interior structure of a dextran methacrylate hydrogel (DS values up to 0.75), prepared in a comparable manner (Fig. 20), is investigated

Table 7 Influence of the temperature, reaction time and amount of maleic anhydride on the degree of substitution for the esterification of dextran in DMF/LiCl with maleic anhydride

Temp. (°C)	Time (h)	Molar ratio [a]	DS
20	20	1 : 1.0	0.30
40	20	1 : 1.0	0.51
60	20	1 : 1.0	0.99
60	1	1 : 1.0	0.36
60	5	1 : 1.0	0.57
60	10	1 : 1.0	0.84
60	20	1 : 1.0	0.99
60	8	1 : 0.5	0.60
60	8	1 : 1.0	0.90
60	8	1 : 2.0	1.47
60	8	1 : 3.0	1.53

[a] Mol OH group of dextran/mol maleic anhydride

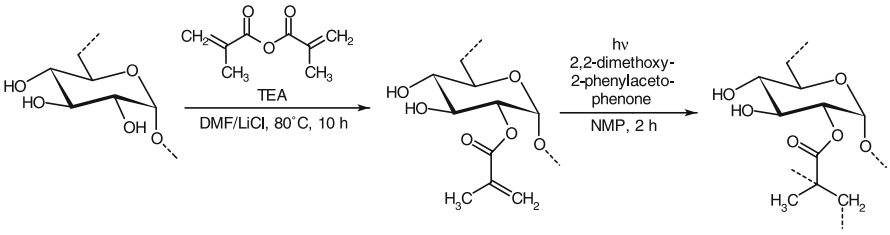


Fig. 20 Synthesis of dextran methacrylate usable for the formation of hydrogels after photo-initiated cross-linking [175]

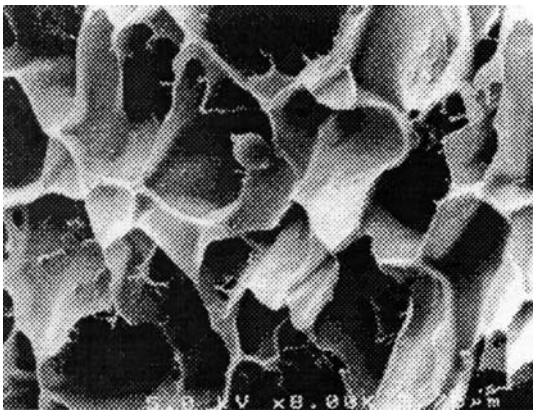


Fig. 21 Three-dimensional porous structure of a dextran methacrylate hydrogel observed by means of SEM

by means of SEM after application of special cryofixation and cryofracturing techniques.

A unique 3D porous structure is observed in the swollen hydrogel (Fig. 21), which is not determined in the unswollen state. Different pore sizes and morphologies between the surface and the interior of swollen hydrogels are visible [175].

Transparent hydrogels useful for adhesion inhibitors, tissue adhesives, wound dressings, hemostatics and embolisation materials are obtained from dextran methacrylates via polymerisation with *N*-isopropylacrylamide in DMSO in the presence of azobisisobutyronitrile [176]. A broad variety of new hydrogels with different sensitivities and tunable degradation behaviour is accessible by grafting L-lactide onto 2-hydroxyethyl methacrylate (HEMA) and binding this polymerisable group on dextran via activation with *N*,*N*′-carbonyldiimidazole (CDI, Sect. 4.2.2) [177].

4.2.2 Synthesis of Dextran Esters with Bioactive Moieties (Prodrugs) via In Situ Activation Reactions

The majority of acylation reactions on dextran are exploited to prepare complex esters of the polymer such as fluorescent-labelled dextran or polymers forming defined superstructures. By far the most important class of esters in this regard are still conjugates, i.e. dextran coupled to drugs, proteins, or hormones [178, 179]. Conjugation to dextran may overcome many problems in drug design including limited solubility of the drugs, short plasma half life, toxicity of the drugs and unspecific interaction with tissue [8]. In addition, binding of pharmaceutical products to the polymer can lead to controlled-release compounds. Polysaccharide esters are well suited for this concept

because of the ease of deesterification by simple hydrolysis or enzymatic attack. A broad variety of drugs can be bound yielding so-called prodrugs, which represents a rapidly growing field of pharmaceutical research. The polysaccharide of choice is almost exclusively dextran to give water-soluble prodrugs.

For the introduction of such complex and sensitive functions, the heterogeneous conversion applying carboxylic acid anhydrides or chlorides is not appropriate. A number of new synthesis paths for the defined, homogeneous conversion of dextran to its carboxylic acid esters were therefore developed. Reaction of dextran with carboxylic acids after in situ activation is the most important path among the new synthesis tools for the defined esterification of dextran with complex and sensitive acids. The most popular in situ activating reagents exploited are sulfonic acid chlorides, reagents of the dialkylcarbodiimide type and *N*,*N*′-carbonyldiimidazole. Besides basic results for these synthesis paths, a selection of results for the preparation of prodrugs is included in this section to show its usefulness.

4.2.2.1 Activation of Carboxylic Acids with Sulfonic Acid Chlorides

Via the in situ activation, applying sulfonic acid chlorides, covalent binding of bioactive molecules onto dextran was achieved by esterification of the polymer with α-naphthylacetic acid (**1**), nicotinic acid (**2**) and naproxen (**3**) homogeneously in DMF/LiCl using *p*-toluenesulfonyl chloride (tosyl chloride, TosCl) or methanesulfonic acid chloride (mesyl chloride, MesCl) and pyridine within 22 h at 30–70 °C (Fig. 22).

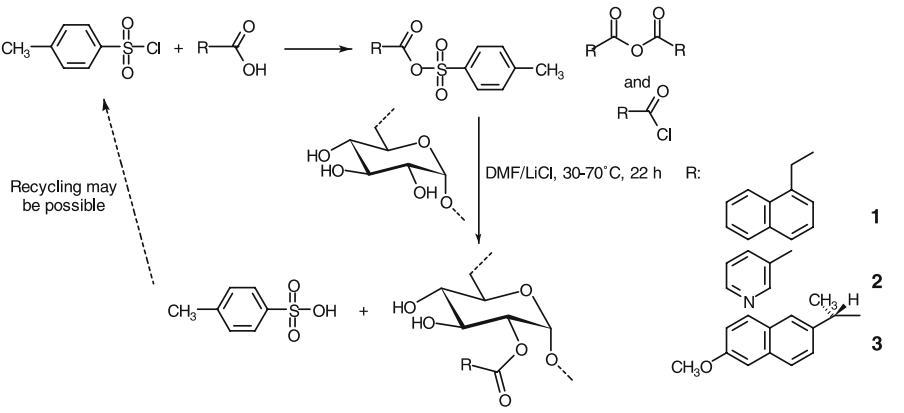


Fig. 22 Schematic plot for the esterification of dextran with α-naphthylacetic acid (*1*), nicotinic acid (*2*), and naproxen (*3*) via in situ activation of carboxylic acids with *p*-toluenesulfonyl chloride (TosCl)

Both the temperature and the concentration of pyridine as well as the type of sulfonic acid chloride show an influence on the reaction, as displayed in Table 8 [180]. The esterification is even possible without the base.

^{13}C NMR spectra of partially modified dextran with α-naphthylacetate moieties show that the reactivity of the individual hydroxyl groups decreases in the order C2 > C4 > C3. A mechanism for the reaction is suggested, which includes formation of an acylium complex as observed for the reaction with acid chlorides (Fig. 23).

These findings support the NMR results for the acylation with acyl chlorides, i.e. the in situ activation with sulfonic acid chloride succeeds mainly via intermediately formed acyl chlorides of the carboxylic acids [180]. The binding of naproxen is a nice example for the preparation of prodrugs as a valuable approach for the transportation of lipophilic agents in a biological environment [45, 178]. The water solubility of naproxen bound to dextran exceeds that of the acid form of the drug by 500 times. Participation of specific acid–base catalysis in the hydrolysis of the ester in aqueous buffer solutions at 60 °C is observed. An almost identical degradation rate is obtained for the ester in 80% human plasma, excluding catalysis of hydrolysis by plasma

Table 8 Influence of the concentration of pyridine [Py], the reaction temperature, and type of sulfonic acid chloride applied for the esterification of dextran (0.12 mol L^{-1}) in DMF/LiCl with α-naphthylacetic acid (0.37 mol L^{-1}) for 22 h

Conditions [Py] (mol L^{-1})	Temp. (°C)	Sulfonic acid chloride	Product DS
–	50	TosCl	0.13
0.37	50	TosCl	0.15
0.74	50	TosCl	0.19
1.48	50	TosCl	0.23
0.74	30	TosCl	0.13
0.74	60	TosCl	0.22
0.74	70	TosCl	0.23
0.74	50	MesCl	0.18

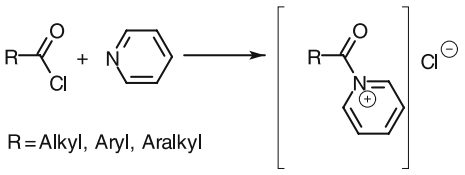


Fig. 23 Formation of an acylium complex

enzymes [181, 182]. In the same manner, dextran esters of ketoprofen, diclofenac, ibuprofen and fenoprofen can be studied showing that the dextran ester prodrug approach provides selective colon delivery systems of drugs possessing a carboxylic acid functional group [183, 184]. The introduction of *N*-acylamino acid into the dextran backbone is achievable in the same manner [185, 186].

4.2.2.2 Dialkylcarbodiimide-Type Coupling Reagents

Coupling reagents of the dialkylcarbodiimide type are most frequently utilised for the esterification of polysaccharides with complex carboxylic acids [179]. The best known condensation agent of this class, particularly in peptide and protein chemistry, is *N*,*N*′-dicyclohexylcarbodiimide (DCC, Fig. 24) [187].

These reagents have a number of drawbacks. First of all, they are toxic especially via contact with skin. The LD_{50} (dermal, rat) of DCC is 71 mg kg^{-1}. This should always be considered if the reaction is used for the preparation of materials for biological applications. Moreover, the *N*,*N*′-dialkylurea formed during the reaction is hard to remove from the polymer except for preparation in DMF and DMSO, where it can be filtered off. In case of esterification of polysaccharides in DMSO in the presence of these reagents, oxidation of hydroxyl functions may occur due to a Moffatt type reaction (Fig. 25, [188]). The oxidation products formed can be detected with the aid of 2,4-dinitrophenylhydrazine, e.g. in case of the conversion of dextran with DCC in DMSO [189].

Moreover during the reaction, decomposition of DMSO to dimethylsulfide occurs resulting in a pungent odour. The treatment with DCC may also lead to the formation of isourea ethers according to reaction shown in Fig. 26.

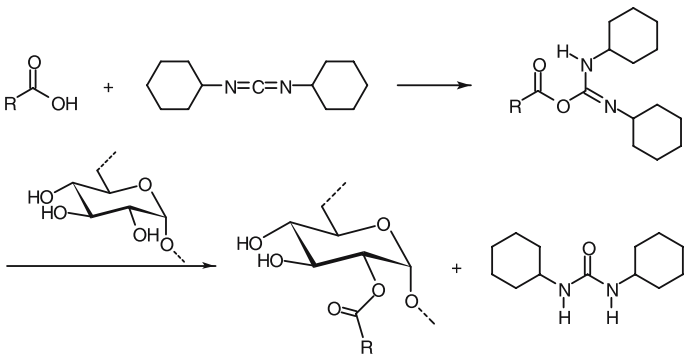


Fig. 24 Esterification of a polysaccharide with carboxylic acid in situ activated with *N*,*N*′-dicyclohexylcarbodiimide (DCC)

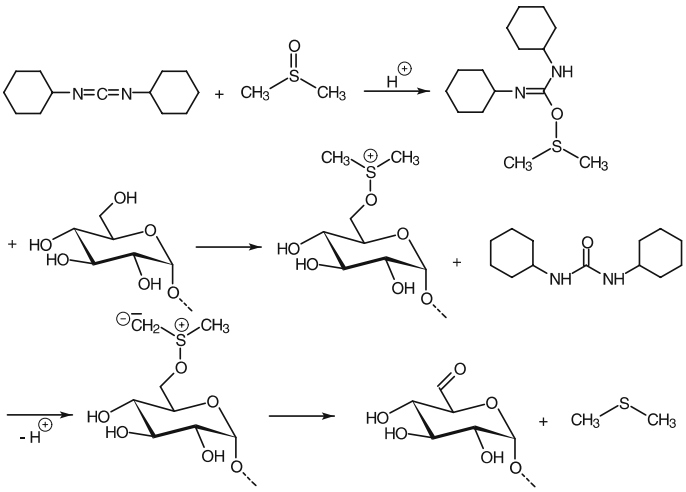


Fig. 25 Moffatt reaction at a non-reducing terminal glucose unit in dextran

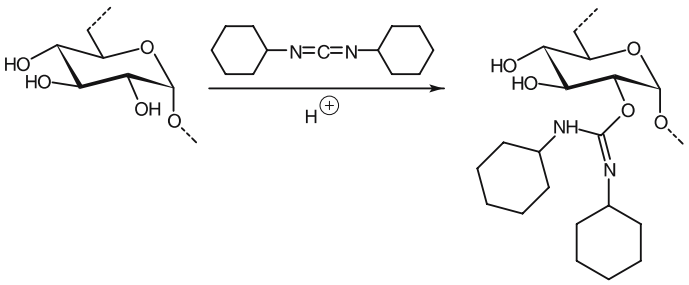


Fig. 26 Formation of isourea ethers as a side reaction during conversion of dextran using DCC as coupling reagent

Nevertheless, the reagent is still used for a large number of esterification reactions of dextran. The synthesis of dextran amino acid esters is achieved by conversion of the polysaccharide in DMSO with the *N*-benzyloxycarbonyl protected acids for 48 h at 20 °C using DCC and pyridine. *O*-(*N*-Benzyloxycarbonylglycyl)dextran with DS 1.1, *O*-(*N*-benzyloxycarbonyl-aminoenanthyl) dextran with DS 2.2, and *O*-(*N*-acetyl-L-histidinyl)dextran with DS 1.1 are accessible. Deprotection was achieved with oxalic acid and Pd/C [190, 191].

Functionalisation with bulky hydrophobic carboxylic acids/DCC was studied for the synthesis of amphiphilic polymers based on dextran. Bile acid is covalently bound to dextran (Fig. 27) through ester linkage in the presence of DCC/DMAP (added in dichloromethane) as coupling reagent.

A homogeneous reaction occurs if the mixture DMF/formamide is used as solvent. The amount of bound acid (determined by UV/Vis spectroscopy)

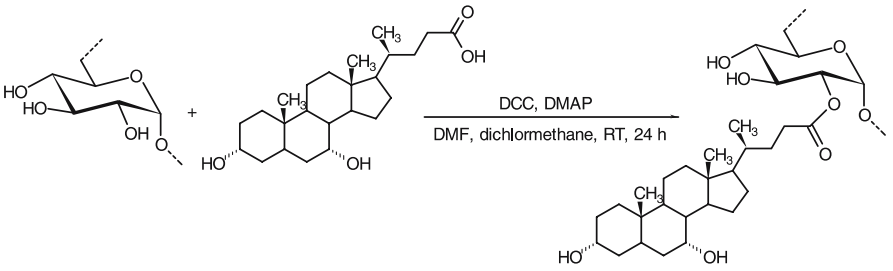


Fig. 27 Synthesis of bile acid esters of dextran using DCC

is in the range from 10.8 to 11.4 mol % [192, 193]. A prodrug accessible via in situ activation of the carboxylic acids with DCC is the metronidazole monosuccinate ester of dextran synthesised in DMSO or DMF using TEA as base (Fig. 28, [194]).

The hydrolysis of the dextran metronidazole succinate over the pH range 7.4–9.2 at 37 °C can be determined with high-performance size exclusion chromatography showing slower release compared to other prodrugs such as the dextran cromoglycate (see below). Interestingly, an intramolecularly catalysed hydrolysis by the neighbouring dextran hydroxyl groups is observed [195]. Metronidazole can also be covalently bound with maleic and glutaric acid. For the dextran metronidazole esters, in which succinic and glutaric acids are incorporated as spacers, the decomposition proceeds through parallel formation of metronidazole and the monoester derivative, as can be demonstrated by reversed-phase HPLC and SEC. Almost identical stability of the individual esters is obtained after incubation in 0.05 M phosphate buffer pH 7.40 and in 80% human plasma, revealing that the hydrolysis in plasma proceeds without enzymic catalysis. The half-lives of the modified dextran derived from maleic, succinic and glutaric acids are 1.5, 32.1 and 50.6 h, respectively [196].

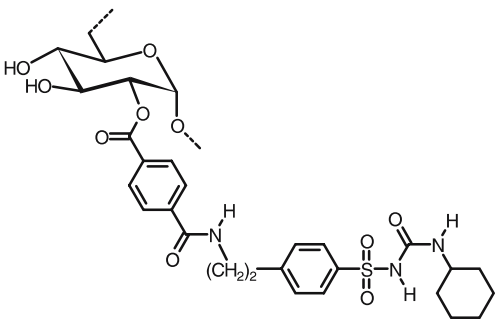


Fig. 28 Structure of dextran monosuccinyl metronidazole

4.2.2.3 *N,N′*-Carbonyldiimidazole (CDI) as Esterification Reagent

A method with an enormous potential for dextran modification is the homogeneous one-pot synthesis after in situ activation of the carboxylic acids with CDI, which is a rather well known technique in general organic chemistry and was published in 1962 [197]. It is especially suitable for the functionalisation of the biopolymers, because during conversion the reactive imidazolide of the acid is generated and only CO_2 and imidazole are formed as by-products (Fig. 29).

The reagent and by-products are non-toxic. The imidazole is freely soluble in a broad variety of solvents including water, alcohol, ether, chloroform and pyridine and can be easily removed. In addition, the pH is not drastically changed during the conversion, resulting in negligible chain degradation.

In comparison to DCC, the application of CDI is much more efficient, avoids most of the side reactions and allows the use of DMSO, which represents a good solvent for most of the complex carboxylic acids. In case of CDI, no oxidation is observed and no decomposition of the DMSO (no odour of dimethylsulfide).

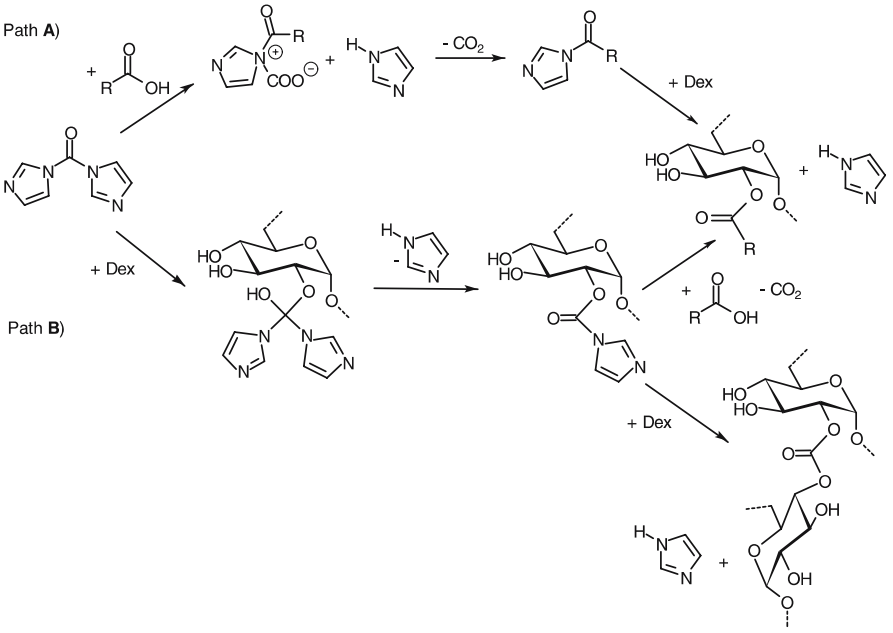


Fig. 29 Reaction paths leading exclusively to esterification (*path A*) or additionally to cross-linking reactions (*path B*) under the action of CDI as reagent for dextran esterification

The conversion is generally carried out as a one-pot reaction in two stages. First, the acid is transformed with the CDI to give the imidazolide. The conversion of the alcohol in the first step is also possible for the esterification but yields undesired cross-linking via carbonate formation in case of a polyol (Fig. 29). The imidazolide of the carboxylic acid should always be firstly synthesised. Model reactions and NMR spectroscopy (Fig. 30) with acetic acid confirm that during a treatment at room temperature CDI is consumed completely within 6 h. Thereby, the tendency of cross-linking initiated by unreacted CDI, which would lead to insoluble products, is avoided.

Basic investigations on conditions for coupling by use of butyric acid and dextran confirm that the imidazolide is formed within 2 h. The reaction at room temperature for 17 h results in butyrate content of 92% of the acid applied. Only 0.25% N is found in the product. The solvent has a pronounced influence; for dextran the solvent of choice is the mixture formamide/DMF/CH_2Cl_2 [189]. 4-Pyrrolidinopyridine is used as catalyst in this process.

Although CDI was applied as early as 1972 as reagent for the esterification of starch and dextran, it has only scarcely been used up to now. Its renaissance during the last few years may be due to the fact that it became an affordable commercially available product. Among the first attempts for the esterification of polysaccharides via CDI is the binding of amino acids onto dextran. Besides CDI, *N*,*N*′-(thiocarbonyl)diimidazole can be utilised to obtain the corresponding imidazolide [198]. The amino acids bound via this path are glycine, L-leucine, L-phenylalanine, L-histidine and L-alanyl-L-histidine. They are protected with *N*-trifluoroacetyl, *N*-benzyloxycarbonyl

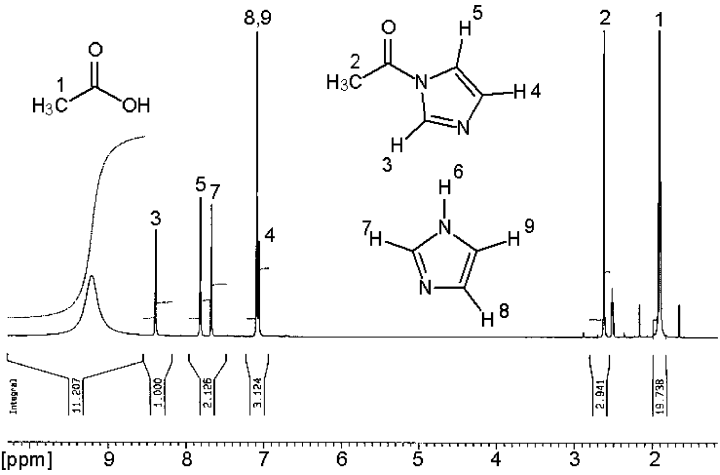


Fig. 30 ^{1}H NMR spectroscopic investigation of the in situ activation of acetic acid with CDI confirming complete consumption of the CDI to the acetyl imidazolide

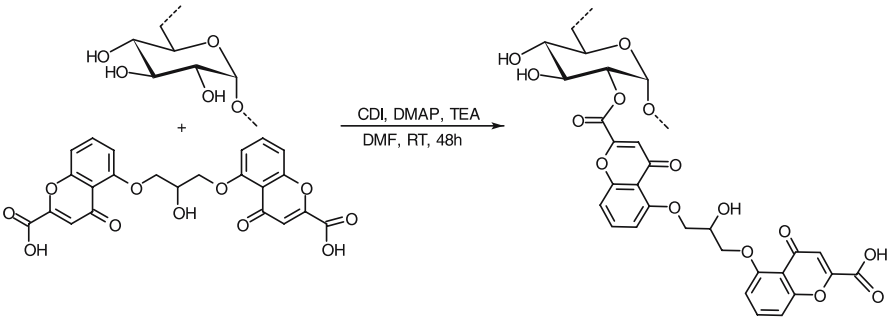


Fig. 31 Esterification of dextran with cromoglycic acid using in situ activation with CDI

and 2,4-dinitrophenyl moieties. The protecting groups can be removed after the esterification of the polysaccharide by hydrolysis or hydrogenation over Pd catalyst [191].

Cromoglycic acid can be covalently bound to dextran (Fig. 31). The acid was transferred into the imidazolide with CDI in DMF in the presence of TEA and 4-*N*,*N*-dimethylaminopyridine (DMAP) within 5 h at room temperature. The conversion with dextran dissolved in DMF is achieved within 48 h at room temperature. The procedure gives high yields (up to 50%) with derivatives containing between 0.8 and 40% (w/w) of the acid (DS can not be calculated because there is no structural information excluding the intermolecular esterification of the acid). Comparison with a route involving chlorination of the free acid in a first step, followed by reaction with dextran in formamide, results in low yields (1.5%) of an ester containing only 2.5% (w/w) cromoglycic acid [199].

Studies on the ester of the antiasthmatic drug with dextran indicate that the cromoglycate is released from the ester with a half-life of 10 h, if the acylation is carried out with the chloride of the drug yielding a loading of 2.5% (w/w). The product obtained via the imidazolide releases the cromoglycate (0.8% w/w) with a half-life of 39 min, while another batch containing 40% (w/w) cromoglycate has a release half-life of 290 min in buffer of pH 7.4 at 37 °C [199].

4.2.2.4
Micro- and Nanoparticles Manufactured by Dextran Esterification with CDI

Interestingly, CDI can also be utilised for the introduction of substituents by inter- or intramolecular coupling of OH moieties of the polysaccharide via a carbonate function. This synthesis was used to obtain dextran with 2-hydroxyethyl methacrylate moieties (dex-HEMA) and dex-HEMA with lactate spacer functions (Fig. 32). A new class of dextran derivatives (DS < 0.2) that can be polymerised containing hydrolysable groups is accessible [177].

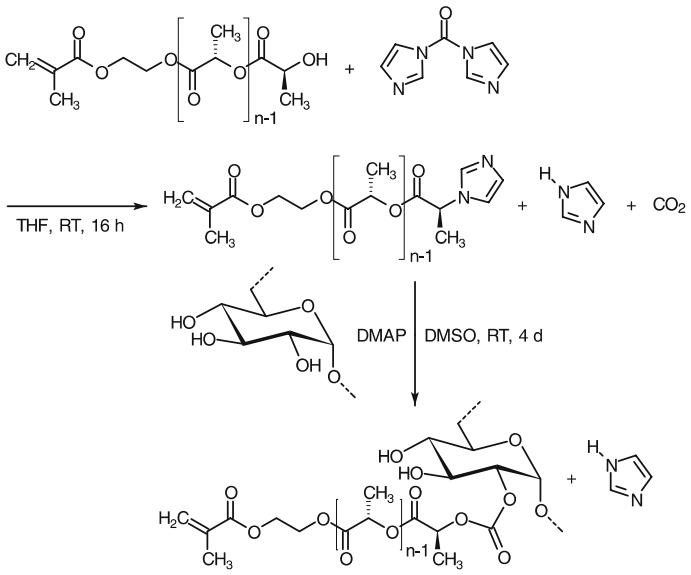


Fig. 32 Conversion of dextran with hydroxyethyl methacrylate lactate using CDI yielding a carbonate-bound ester moiety

These polymerisable dextran derivatives are widely exploited for the preparation of microspheres by water-in-water emulsion polymerisation [200] or for the production of nanogel usable as carriers for intracellular drug delivery with tunable degradation properties [201]. Comparable microgels with a monodisperse size distribution can be obtained using a microfluidic device [202]. Moreover, thermoresponsive hydrogels [203] or self-exploding lipid-coated microgels [204] have been prepared.

The CDI method can be exploited for the adjustment of the hydrophilic–hydrophobic balance, necessary for the formation of polymeric nanoparticles, in a dialysis process. This balance is achieved by esterification of dextran with biocompatible propionate and pyroglutamate moieties, leading to highly functionalised derivatives [34]. The products with DS > 2 can be realised by setting the molar ratio of the two acids to repeating unit in a one-step process or by subsequent propionylation of the remaining hydroxyl groups of the dextran ester. They form spherical nanoparticles by a simple dialysis process [160, 205]. The solvent DMA is slowly exchanged against water, leading to an arrangement of the hydrophobic moieties inside the core. The adjustment of the hydrophilic–hydrophobic balance within the dextran molecule is necessary for the formation of polymeric nanoparticles. The size of the particles can be determined by a particle size distribution analyser (PSDA), dynamic light scattering (DLS), scanning electron microscopy (SEM) and atomic force microscopy. A narrow size distribution of the particles is evidenced by PSDA (Fig. 33).

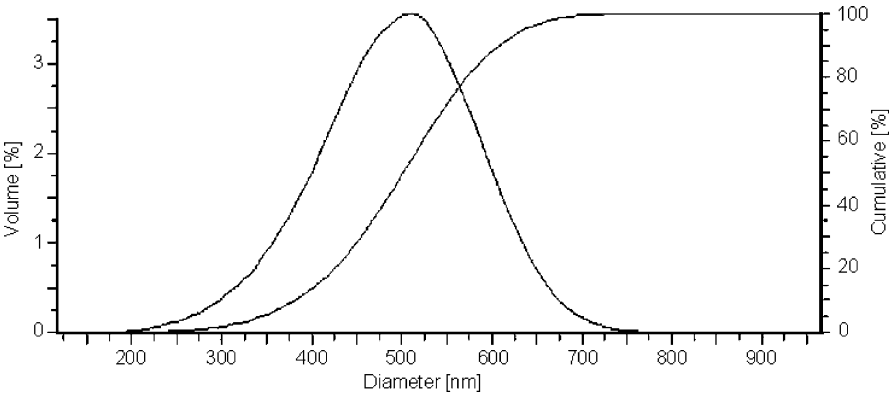


Fig. 33 Particle size distribution of perpropionylated dextran furoate pyroglutamate (DS_{Fur} 0.79; DS_{Pyr} 1.27) nanoparticles

By varying the character and amount of substituents and the M_w of the starting dextran, the design of biopolymer nanoparticles of appropriate size is possible (Table 9). Thus, nanoparticles in the range 90–520 nm are available.

The high DS avoids the collapse of nanoparticles due to the prevention of hydrogen bond formation. The nanospheres in the aqueous suspension do not undergo any morphological changes even after 3 weeks storage. SEM images in Fig. 34 show the uniformity in size and shape and the stability of se-

Table 9 M_w of dextran, DS of dextran furoate pyroglutamate propionate, and mean diameters of nanoparticles determined by DLS or PSDA

Dextran M_w ($g\,mol^{-1}$)	Dextran ester [a] No.	DS_{Fur}	DS_{Pyr}	DS_{Prop}	Per-acylation [b]	Mean diameter DLS/PSDA (nm)
5400	1	0.79	1.27	–	–	520
5400	2	0.79	1.27	–	+	500
5400	3	0.12	1.13	–	+	460
5400	4	–	0.26	1.70	–	384
18 100	5	–	0.33	2.20	–	413
54 800	6	–	0.26	2.16	–	446
54 800	7	0.22	1.34	–	+	260
54 800	8	0.99	0.33	–	–	214
54 800	9	0.99	0.33	–	+	184
54 800	10	–	1.96	–	+	87

[a] *Fur* furoate, *Pyr* pyroglutamate, *Prop* propionate
[b] Propionylation of all free hydroxyl and amine moieties after CDI esterification

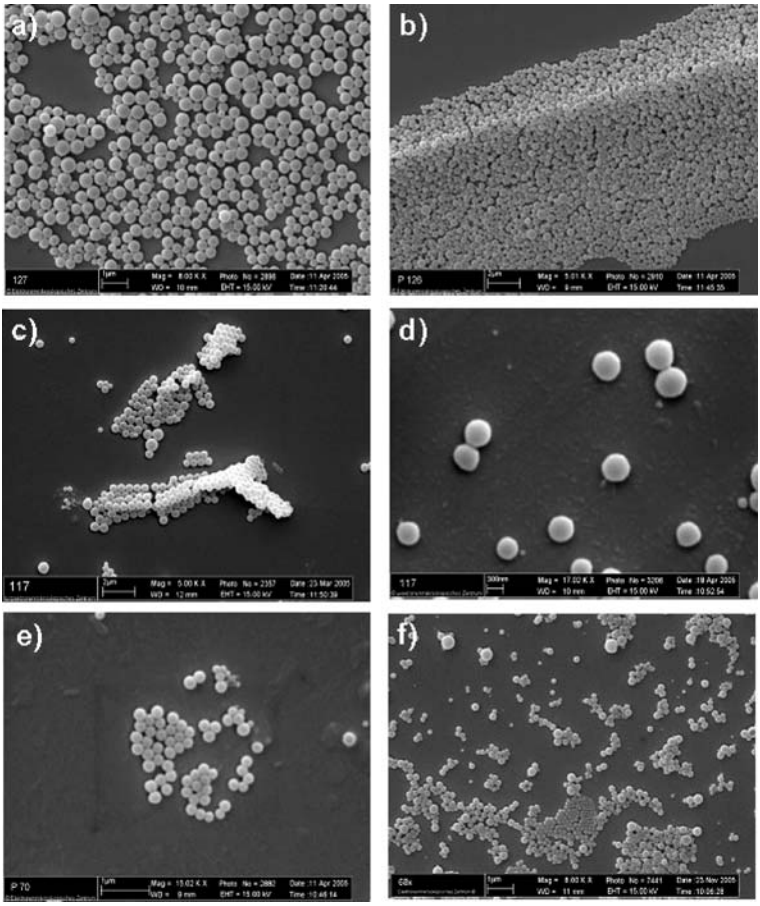


Fig. 34 SEM images of dextran ester nanoparticles (Table 9) from: **a** sample 1, **b** sample 3, **c** sample 4, **d** sample 4 after 3 weeks storage in water, **e** sample 7, and **f** sample 8 (Table 9) on a mica surface

lected dextran nanoparticles. Defined size adjustment, biocompatibility and biodegradability are advantageous for immobilisation of hydrophobic drugs in aqueous systems with subsequent targeting [206].

4.2.2.5 Transesterification

In addition to the conversion of dextran with organic acids after in situ activation, transesterification is an interesting synthesis tool for the introduction of sensitive carboxylic acid moieties, which is illustrated for the acrylation of dextran. Thus, dextran is acylated with vinyl acrylate in the presence of Proleather FG-F and lipase AY, a protease and lipase from *Bacillus sp*. and

Table 10 Ratio of theoretical degree of substitution (DS) versus obtained DS for the conversion of dextran with vinyl acrylate in the presence of the enzyme Proleather FG-F

DS Theoretical	Obtained	Efficiency (%)
0.10	0.072	71.4
0.20	0.151	75.7
0.30	0.224	74.6
0.40	0.315	78.9
0.50	0.370	74.1

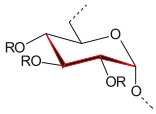
Candida rugosa in anhydrous DMSO. Structure analysis by means of NMR spectroscopy indicates functionalisation of positions 2 and 3 of the AGU in equal amounts [207]. The efficiency of the reaction and the DS accessible in the presence of Proleather FG-F is shown in Table 10.

5 Ethers of Dextran

Basically, modification of the dextran backbone by introduction of ether-type moieties leads to comparatively stable dextran derivatives with altered physicochemical properties. A summary of etherification reactions used most frequently for dextran functionalisation is given in Table 11.

The solubility, the hydrophilic–lipophilic balance, the ionic strength and the resistance against hydrolytic or enzymatic degradation can be tailored by etherification. The hydrophilic–lipophilic balance is adjustable by covalent attachment of hydrophobic groups, e.g. long alkyl chains or phenoxy groups and hydrophilic moieties, like hydroxyalkyl, ethyleneglycol or ionic groups. The amphiphilic ethers have emulsifiying properties and can form micelles in water usable as surfactans or for the encapsulation of hydrophobic material such as drugs. Furthermore, the introduction of ether moieties onto the polymer background influences the degradation behaviour. The degradation of ionic dextran derivatives like diethylaminoethyl dextran [253] or carboxymethyl dextran [254] by α-1-glucosidases proceeds slower than for the parent dextran. Nevertheless, cross-linked dextran samples are still degradable by α-1-glucosidases [255, 256]. This approach is applied to the design of biocompatible and biodegradable hydrogels via etherification. Formation of ether bonds is widely exploited for the insertion of spacers usable for subsequent drug fixation. An appropriate length and chemical structure of the spacer can control the drug release [178]. Selective multistep functionalisation including etherification is investigated for the synthesis of heparin mimetica, which represents a rapidly developing field of research.

Table 11 Summary of etherification reactions used most frequently for dextran modification

Type of dextran ether 	Functional group R	Refs.
Methyl	$-CH_3$	[22, 208–211]
Ethyl	$-C_2H_5$	[22]
Benzyl	$-CH_2C_6H_5$	[30]
Trityl	$-C(C_6H_5)_3$	[22, 212–214]
Trimethylsilyl	$-Si(CH_3)_3$	[215–218]
Carboxymethyl	$-CH_2COOH$	[108, 219–222]
2-Hydroxyethyl	$-C_2H_4OH$	[223, 224]
2-Mercaptoethyl	$-C_2H_4SH$	[225]
2-Cyanoethyl	$-C_2H_4CN$	[226, 227]
2-Hydroxypropyl	$-CH_2CHOHCH_3$	[224, 228]
2-Hydroxyalkyl	$-CH_2CHOH(CH_2)_nCH_3$	[229–231]
2-Hydroxypropyl-3-phenoxy	$-CH_2CHOHCH_2OC_6H_5$	[229, 232–235]
3-Chloro-2-hydroxypropyl	$-CH_2CHOHCH_2Cl$	[236–238]
2-Diethylaminoethyl	$-C_2H_4N(C_2H_5)_2$	[239–244]
3-Amino-2-hydroxypropyl	$-CH_2CHOHCH_2NH_2$	[245]
3-Dimethylalkylammonium-2-hydroxypropyl	$-CH_2CHOHCH_2N^+(CH_3)_2R$	[246–250]
Polyethyleneglycol cetyl	$-(CH_2CH_2O)_{10}C_{16}H_{33}$	[251, 252]
Polyethyleneglycol stearyl	$-(CH_2CH_2O)_{10}C_{18}H_{37}$	[251, 252]

5.1 Non-ionic Dextran Ethers

5.1.1 Alkyl Dextran

Methylation- or combined methylation–ethylation reactions were used for structure analysis of polysaccharides. The alkylation of dextran can be applied to the investigation of the branching pattern, i.e. the number and length of side chains (Sect. 2.2) [22, 23]. The methylation is carried out in liquid ammonia with sodium iodide and methyl iodide, yielding products that are soluble in chloroform and tetrachloroethane [257].

Partially methylated dextran was synthesised with dimethyl sulfate in 19% sodium hydroxide solution (w/v) [208, 209]. Characterisation of the methyl dextran obtained was carried out by complete hydrolysis and separation of the mixture of glucose, mono-, di- and tri-*O*-methylated glucoses by paper chromatography. The mono- and difunctionalised glucose can in turn

be analysed precisely by GLC. From the portions of 2-*O*-, 3-*O*- and 4-*O*-methyl-glucopyranose in the hydrolysate with a low degree of substitution (DS 0.14, only monomethylation was achieved), the initial rate constants were obtained and the ratios of $k_2 : k_3 : k_4$ were found to be 8 : 1 : 3.5. This pronounced reactivity of the C2-hydroxyl group in etherification procedures is also observed for other polysaccharides [258–260]. As an example for the preparation of an arylether of dextran, the triphenylmethylation (tritylation) should be mentioned. The regioselectivity of the tritylation reaction is used for the characterisation of the non-reducing end groups in the polymers. Tritylation was performed by dissolving dry dextran in formamide, addition of the triphenylmethyl chloride in pyridine and keeping the temperature at 120 °C for 2 h. The degree of tritylation increases as the molecular weight of dextran decreases [212].

5.1.2 Hydroxyalkyl and Hydroxyalkyl Aryl Ethers of Dextran as Emulsifying Agents

The conversion of dextran with 1,2-epoxy-3-phenoxypropane, epoxyoctane or epoxydodecane may be exploited for the preparation of amphiphilic dextran derivatives. Polymeric surfactants prepared by hydrophobic modification of polysaccharides have been widely studied, starting with the pioneering work of Landoll [261]. Neutral water-soluble polymeric surfactants can be obtained by reaction of dextran with 1,2-epoxy-3-phenoxypropane in 1 M aqueous NaOH at ambient temperature (Fig. 35, [229, 233]). The number n of hydrophobic groups per 100 Glc*p* units varies between 7 and 22 depending on the reaction conditions. 2-Hydroxy-3-phenoxy propyl dextran ethers (DexP) behave like classical associative polymers in aqueous solution. In dilute solution, the intrinsic viscosity decreases significantly whereas

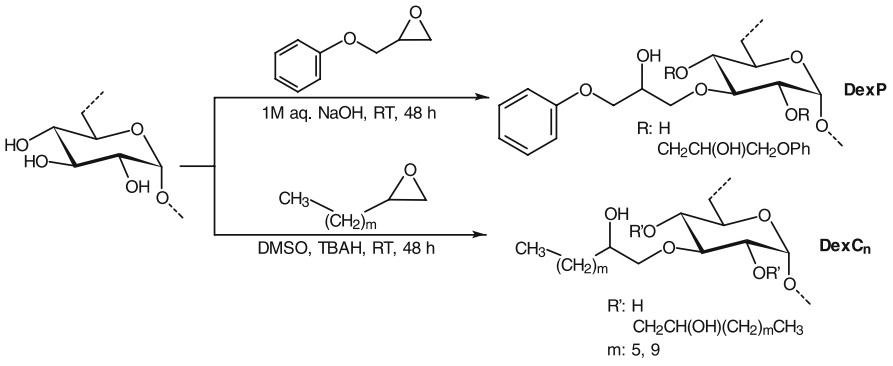


Fig. 35 Synthesis of amphiphilic 2-hydroxy-3-phenoxy propyl (DexP), 2-hydroxyoctyl and 2-hydroxydodecyl dextran ($DexC_n$)

the Huggins coefficient increases with increasing n, interpreted in terms of hydrophobic interaction between the phenoxy groups. Low solvated coils and shrunken conformations are formed. At concentrations above $40\,g\,L^{-1}$ ($DexP_{15}$) and $35\,g\,L^{-1}$ ($DexP_{22}$), the reduced viscosity deviates from linear variation because of intermolecular hydrophobic interactions initiating the formation of aggregates. The surface-active properties were evidenced by surface (air/water) and interfacial (dodecane/water) tension measurements. Both the surface and the interfacial tension decreases with DS and polymer concentration. However, above a critical polymer concentration no further decrease in surface tension is observed, which is assigned to the formation of a dense polymer layer at the liquid–air or liquid–liquid interface. Oil-in-water emulsions can be prepared by sonification in the presence of the amphiphilic copolymer (dissolved in the aqueous phase). The thickness of the adsorbed polymer layer in dodecane/water emulsions was estimated by zeta potential measurements coupled with size measurements. Polymeric surfactants usually show no or only low hemolytic effects.

Amphiphilic 2-hydroxyoctyl and 2-hydroxydodecyl dextran ethers were prepared homogeneously in DMSO at 40 °C in the presence of an aqueous solution of tetrabutylammonium hydroxide (TBAH) with aliphatic epoxide (epoxyoctane or epoxydodecane, Fig. 35). The DS, determined by ^{1}H NMR in DMSO-d_6, can be varied by changing the reaction time and the concentration of the epoxide [262]. Viscometric investigations carried out in dilute and semidilute solutions give information about their solution behaviour [230]. The relation between monodisperse dissolved macromolecules and aggregates and their ability to interact with each other depends on the DS, the length of the hydrocarbon chains and the concentration of the polymer. Static and dynamic light scattering measurements allow precise knowledge of the state formed in dilute solutions with varying DS and increasing hydrocarbon chain length [231].

Amphiphilic 2-hydroxy-3-phenoxy propyl dextran ethers (DexP) were also applied as stabilisers of nanoparticles formed by emulsion polymerisation of styrene, leading to a stable hydrophilic surface and showing reduced non-specific protein adsorption [229, 232, 233]. The styrene-in-water emulsions were prepared by sonification of styrene in the aqueous phase in the presence of DexP, with potassium peroxodisulfate as initiator at 95 °C. The size of the polysaccharide-coated polystyrene nanoparticles prepared was directly correlated to the styrene concentration and the DS of the DexP. For a concentration of styrene of 10% (v/v), the droplet size is around 160 nm at maximal surface coverage and a coagulate amount below 5% (w/w). With increasing concentration of styrene, the particle size and the amount of coagulate increase, indicating that coalescence processes take place during polymerisation. Furthermore, for dextran ethers of low DS, the coalescence becomes predominant and no latex is observed. Polystyrene particles coated with DexP present markedly higher protein repulsion in contrast to unmodified

particles or to dextran-coated particles. The dextran layer formed on the particles is thicker than the layer with DexP, but due to the higher polymer chain flexibility of dextran, the layer is loosely packed and bovine serum albumin (BSA) can penetrate through. In the presence of phenoxy moieties, the stiffness of the adsorbed layer increases due to hydrophobic interactions between the polymer chains and, therefore, the density of the layer increases. The probability of BSA making direct contact to the polystyrene surface is reduced [263].

Comparable experiments were performed with DexP-coated macroporous polystyrene-divinylbenzene (PS-DVB) particles [264] and with DexP, labelled with 4-amino-TEMPO, using EPR spectroscopy to study the conformation of the polymer chains [265]. Low substituted DexP gave thicker layers with lower density than highly substituted derivatives due to the presence of more loops and tails. With increasing DS of DexP, the stiffness of the adsorbed layers and, therefore, the density increases and the non-specific interaction of BSA with the DexP-coated PS-DVB surfaces seems to be restricted to the top of the adsorbed layer.

Moreover, DexP and $DexC_n$ were exploited as stabilisers in an oil-in-water emulsion/evaporation technique for the preparation of biocompatible poly(lactide) (PLA) nanospheres [262]. The influence of the dextran ethers on the particle size, surface density and stability were investigated. DSC studies suggested that the mechanism of interfacial adhesion depends on the nature of the hydrophobic moieties. The dextran ethers produce stable nanoparticles with hydrophilic surfaces and reduced BSA adsorption [266]. Such particles of PLA have been widely applied as carriers for drug delivery [267]. The encapsulation of lidocaine in PLA nanoparticles, adopting DexP and $DexC_n$ as emulsion stabiliser in the preparation of the nanoparticles, was investigated. Using DexP, the encapsulation of lidocaine in PLA nanoparticles is not possible probably due to specific interactions between the drug and DexP. However, lidocaine-loaded nanospheres from about 7 to 18% (w/w) could be obtained in the presence of the 2-hydroxyalkyl dextran ethers. The release of lidocaine of uncoated PLA and of $DexC_n$-coated PLA nanoparticles did not significantly change.

5.1.3 Poly(Ethylene Glycol)-Alkyl Dextran Ether ($DexPEG_{10}C_n$) for Drug Delivery

Amphiphilic poly(ethylene glycol)-alkyl dextran ethers are emerging as vehicles in the oral delivery of poorly water soluble drugs [251, 268, 269]. They form polymer micelles of low critical association concentrations (CAC) and small micelle sizes in aqueous solution. Particulate delivery systems lead to an enhancement of the absorption efficiency and bioavailability of highly lipophilic drugs orally applied, and provide the drug with some level of pro-

tection against degradation within the GI tract, prolonged drug transition time, and improved drug absorption [270].

Low molecular weight surfactant micelles are widely used as drug carrier systems due to their good pharmacological characteristics [271, 272]. They are formed above a critical micelle concentration (CMC) and rapidly break apart upon dilution. In contrast to the low molecular weight surfactant micelles, the association of amphiphilic polymers like $DexPEO_{10}C_n$ in water takes place at concentrations (CAC), which are lower by several orders of magnitude than typical surfactant CMC values. The polymeric micelles consist of a hydrophobic core (cetyl or stearyl groups) and the hydrophilic shell (dextran backbone) exposed to the aqueous environment [273, 274]. The hydrophobic cetyl and stearyl groups are attached via short PEG linker to dextran (Fig. 36).

The poly(ethylene glycol)-cetyl and stearyl dextrans are synthesised as follows: the terminal hydroxyl groups of cetyl or stearyl poly(ethylene glycol) are tosylated using *p*-toluenesulfonyl chloride and either pyridine or a mixture of $Et_3NH^+Cl^-$ and $Me_3NH^+Cl^-$. The tosylated poly(ethylene glycols) are converted with dextran to give the corresponding $DexPEG_{10}C_n$ [251, 252]. The degree of $PEG_{10}C_n$ substitution can be calculated using ^1H NMR spectra. The size of the polymeric micelles ranges from 10 to 100 nm.

CAC values of the copolymers are estimated by fluorescence spectroscopy using pyrene as probe. The excitation spectra of the hydrophobic fluorescence probe, preferentially arranged into the hydrophobic core of the micelle, undergoes a small shift to longer wavelengths (from $\lambda = 333$ nm in a hydrophilic environment to $\lambda = 336$ nm in a hydrophobic environment) [275–277]. An increase in the length of the hydrophobic residue at a given length of the hydrophilic polymer chain causes a decrease in the CAC value and an increase in micelle stability [252, 278]. Cyclosporin A (CsA), a highly effective immunosuppressive agent, was incorporated into $DexPEG_{10}C_n$ micelles by a dialysis method. An aqueous $DexPEG_{10}C_n$ solution was treated with a solution of CsA in ethanol, followed by extensive dialysis against water. The solubility of the lipophilic drug CsA in aqueous solutions of $DexPEG_{10}C_n$ through encapsulation in the hydrophobic core of the micelles can be increased with increasing DS and decreasing molecular weight of the dextran [252]. The cytotoxicity of $DexPEG_{10}C_n$ micelles towards a Caco-2 cell line, deriving from human colon denocarcinoma, is significantly lower than that of unlinked $PEG_{10}C_n$. $DexPEG_{10}C_n$ micelles exhibit high stability in gastric and intestinal

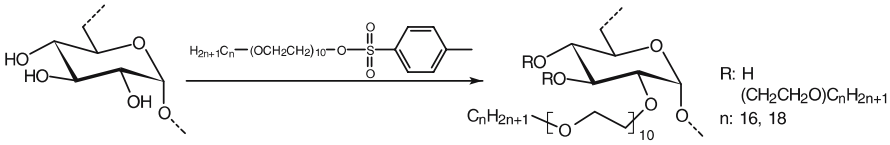


Fig. 36 Synthesis of poly(ethylene glycol)-cetyl and -stearyl dextran ether

fluids and their size is optimal for effective drug delivery. The permeability of CsA encapsulated in $DexPEG_{10}C_n$ micelles across Caco-2 cells is significantly increased compared to free CsA. The application of CsA encapsulated in vitamin B_{12}-modified $DexPEO_{10}C_n$ micelles also enhances the permeability through Caco-2 cell monolayers [279]. The characteristic of the biopolymeric micelle systems indicates that this approach can provide practical opportunities in the oral delivery of hydrophobic drugs.

5.2 Ionic Dextran Ethers

5.2.1 Sulfopropylation

One approach to introduce an anionic moiety onto dextran is sulfopropylation. In combination with a hydrophobic modification, sulfopropylation was utilised for manufacture of anionic amphiphilic dextran ethers. The derivatives were synthesised in two steps. Dextran was reacted with 1,2-epoxy-3-phenoxy propane in aqueous NaOH solution (Fig. 35) or in DMSO using TBAH instead of NaOH. The subsequent step is the conversion with 1,3-propane sultone in DMSO (Fig. 37).

The emulsifying properties of these polymeric surfactants demonstrate that the chemical structure influences the kinetic behaviour of interfacial tension reduction. An increase of sulfopropyl moieties reduces the interfacial tension slower while an increase in 2-hydroxy-3-phenoxy propyl moieties reduces the interfacial tension faster. The ionic strength of the emulsion appears to increase the rate of tension reduction. The average droplet size of oil-in-water emulsions in presence of previously dissolved 2-hydroxy-3-phenoxy propyl sulfopropyl dextran is around 180 nm immediately after preparation and increases with time. The presence of ionic moieties appeared to facilitate emulsification at low polymer concentrations due to electrostatic repulsions between the oil droplets [229].

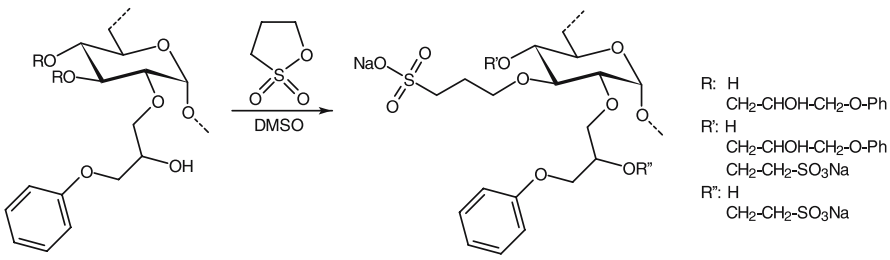


Fig. 37 Sulfopropylation of 2-hydroxy-3-phenoxy propyl applied to the manufacture of anionic amphiphilic 2-hydroxy-3-phenoxy propyl sulfopropyl dextran

5.2.2
Carboxymethyl Dextran (CMD)

Carboxymethylation of dextran in water/organic solvent mixtures using monochloroacetic acid (MCA) under strong alkaline conditions leads to CMD with good reproducibility (Fig. 38).

A DS of up to 1.0 is obtained in one step by applying optimised reaction conditions; 3.8 M aqueous NaOH, reaction temperature of 60 °C for 90 min in tert-butanol/water or isopropanol/water 85 : 15 (v/v) mixtures (Table 12) [219]. The DS of CMD can be increased by repeated carboxymethylations. CMD with DS 1.5 was realised by two-step carboxymethylation. Under optimal conditions, the applied NaOH solution was 3.8 M. The DS value is decreased with lower NaOH concentrations because of incomplete activation of the hydroxyl groups, and also with higher NaOH concentrations due to increasing side reactions of MCA with NaOH forming glycolic acid [280].

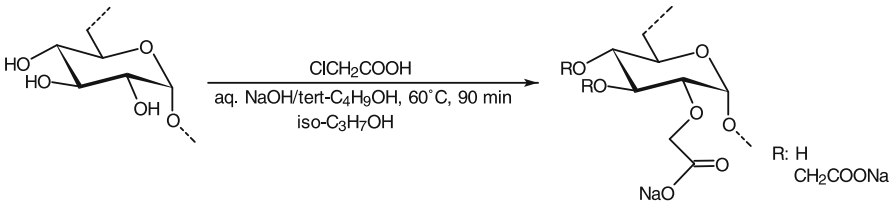


Fig. 38 Synthesis of carboxymethyl dextran (CMD)

Table 12 Influence of solvent mixture and temperature on carboxymethylation of dextran

Solvent mixture (v/v) tert-Butanol	Water	Temp. (°C)	DS_{CM}
100	0	60	0.35
85	15	50	0.82
85	15	60	0.96
85	15	70	0.88
80	20	60	0.74
70	30	60	0.50
60	40	60	0.39
50	50	60	0.31
0	100	60	0.63
85 (isopropanol)	15	60	0.99
85 (M_4U [a])	15	60	0.77

[dextran] = 2 g, [NaOH] = 3.8 M, [MCA] = 3 g, 90 min, total volume 50 mL
[a] 1,1,3,3-Tetramethylurea

Table 13 Molecular weight and polydispersity of carboxymethyl dextran with different degrees of substitution (DS)

Sample	DS_{CM}	M_n	M_w	M_w/M_n
Dextran T40	–	25 500	38 000	1.5
CMD	0.76	49 000	70 400	1.4
CMD	0.83	56 700	77 500	1.4
CMD	1.01	54 700	79 400	1.5
CMD	1.47 [a]	53 500	80 600	1.5

[a] After second carboxymethylation

The isopropanol/water or tert-butanol/water mixtures (85 : 15, v/v) are favourable regarding the miscibility and solubility of the reactants. Carboxymethylation proceeds very fast. After 15 min DS 0.8 and 90 min DS 1.0 is obtained. Further prolongation of the reaction time does not yield higher values. The conversion of dextran T10 (10 000 g mol^{-1}) and T40 (40 000 g mol^{-1}) leads to comparable DS values by using optimal reaction conditions. By carboxymethylation, no degradation of dextran was observed although the molecular weight of the CMD samples compared to the starting dextran (T40) is rather high, obviously due to the problems of SEC measurements of polyelectrolytes (Table 13).

The NMR data of ^{1}H-COSY and HMQC spectra reveal that at the beginning of the reaction (below a DS of about 0.3) only three possible monosubstituted glucoses prevail as building blocks of the polymer chain. The values for the rate coefficients are $k_2 = 1.2$, $k_3 = 0.5$ and $k_4 = 0.8$. Beyond this DS (up to a value of about 0.8), disubstituted units are also observed but no trisubstitution occurs [220, 221].

5.2.2.1 Bioactivity of CMD-Based Derivatives (Heparan Sulfate Mimetics)

CMD exhibits no anticoagulant and anticomplementary activities (Table 14). Nevertheless, carboxymethylation of dextran is a crucial step in the preparation of heparin-like polymers. Heparin is a natural polyanionic polysaccharide with repeating uronic acid-*N*-acetyl-D-glucosamine units, which is able to interfere with blood coagulation. It accelerates the inactivation of coagulation enzymes by natural inhibitors like antithrombin (AT) and heparin cofactor II (HCII). Commercial heparin contains essentially sulfate, sulfamate and carboxylic groups with different size, charge and chemical composition. Carboxymethyl dextran benzylamidesulfonate sulfate (CMDBSSu) was designed to mimic heparin-like properties offering the benefit of no potential risk of virus contamination and with a relatively simple synthesis [281]. It

Table 14 Composition, anticoagulant (antithrombic), and anticomplementary activities of dextran derivatives with different DS of carboxymethyl (CM), benzylamide (B) and sulfonate/sulfate (S) functions

Samples	DS CM ±0.01	B ±0.01	S ±0.01	NIH units mg^{-1} [a]	μg/ 10^7 EAC4b,3b [b]
CMD	0.69	–	–	–	120
CMDB	0.51	0.14	–	–	120
CMDSu	0.45	–	0.04	15	13
CMDSu	0.48	–	0.05	25	9
CMDSu	0.64	–	0.05	18	11
CMDSu	0.72	–	0.10	40	3
CMDSu	0.75	–	0.14	65	1
CMDBSSu	0.40	0.12	0.03	3	60
CMDBSSu	0.60	0.14	0.05	16	10
CMDBSSu	0.43	0.04	0.11	23	7.5
CMDBSSu	0.37	0.15	0.45	1	60
CMDBSSu	0.37	0.11	0.90	1	45
CMDBSSu	0.50	0.03	0.10	22	4
CMDBSSu	0.68	0.04	0.12	31	4

[a] Specific anticoagulant activity is expressed as the number of NIH units of human thrombin (1094 NIH units mg^{-1}) inactivated by 1 mg of dextran derivative

[b] The anticomplementary activity was expressed as the input of dextran derivative that inhibits 50% convertase formation on 10^7 EAC4b,3b

can delay the coagulation of plasma by catalysing the inactivation of thrombin [107, 282–284]. Thrombin is a multifunctional serine proteinase that activates platelets, converts fibrinogen into clottable fibrin, and amplifies blood coagulation by activating factors V, VIII, XI. The most potent inhibitor of thrombin in plasma is AT, forming an inactive covalent complex with thrombin and other serine proteinases. Thrombin is the only clotting proteinase that is inhibited by HCII [283]. CMDBSSu also displays anticomplementary activity both in vitro [285] and in vivo [286] and can modulate the proliferation of vascular cells [287–289].

The synthesis of CMDBSSu involves the random carboxymethylation of hydroxyl groups on the glucose units, the benzylamidation of some of the carboxylic groups with benzylamine in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) to carboxymethyl dextran benzylamide (CMDB), the sulfonation of phenyl rings and sulfation of remaining hydroxyl groups at the polymer backbone (Fig. 39, [108, 222]).

The synthesis steps described may also lead to carboxymethyl dextran sulfate (CMDSu) and carboxymethyl dextran benzylamide sulfate (CMDBSu, see Fig. 39). The anticoagulant activities depend on the proportion and distribution of the substituents. CMDBSSu represents a family of soluble

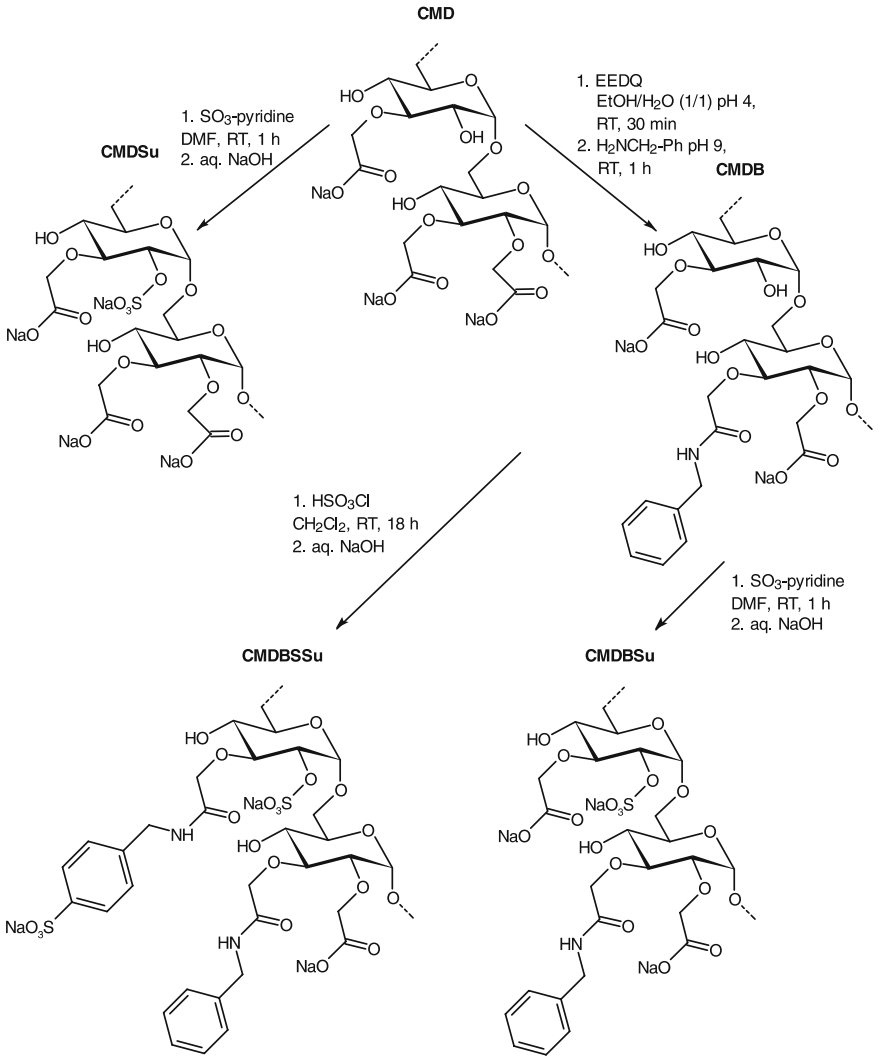


Fig. 39 Synthesis of carboxymethyl dextran sulfate (CMDSu), carboxymethyl dextran benzylamide sulfate (CMDBSu) and carboxymethyl dextran benzylamide sulfonate sulfate (CMDBSSu), respectively

macromolecular compounds, designed for interaction with specific protein domains. The results in Table 14 show that CMD and CMDB have no anticoagulant ability, whereas the CMDBSSu and CMDSu samples reveal a significant activity. A pronounced effect is observed when the carboxymethyl group content is beyond a DS of 0.40 and activity can be increased both by higher degree of sulfation and benzylsulfonation, expressed as S content (Fig. 40, [107, 281]).

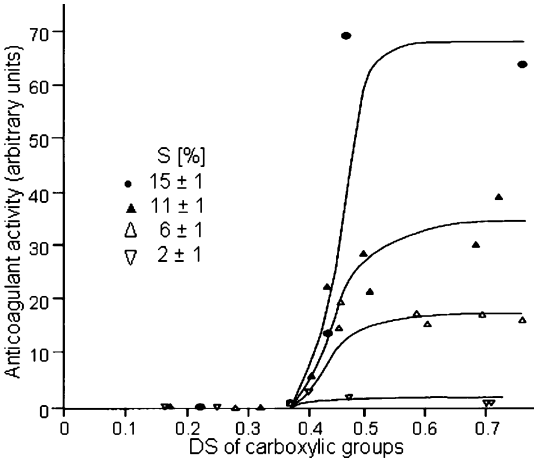


Fig. 40 Anticoagulant activity of CMDBSSu in relation to the carboxymethyl group and sulfonate/sulfate content (adapted from [107, 281])

The anticoagulant activity of dextran derivatives were assessed by measuring the thrombin clotting time (ThNIH units) of freshly prepared platelets from plasma in the presence of the CMD, CMDB, CMDBSSu and CMDSu polymers and of human thrombin (1094 NIH units mL^{-1}). The anticomplementary activity was expressed as the amount of polymer that inhibits 50% formation of the alternative and classical pathway C3 convertase [220, 290, 291].

A prerequisite for CMDBSSu activity is the availability of active sites (OH –, $CH_2COOH(Na)$, SO_3Na and $CH_2CONHCH_2C_6H_5SO_3Na$), capable of binding thrombin and catalysing interactions with its inhibitors. Highly substituted CMDBSu with a DS of carboxymethyl moieties up to 1.1, of benzylamide up to 0.35, and of sulfate up to 1.5 was synthesised by benzylamidation of carboxymethyl groups with benzylamine in the presence of water-soluble 1-cyclohexyl-3-(2-morpholino-ethyl)carbodiimide and subsequent partial sulfation of remaining hydroxyl groups with SO_3-pyridine in DMF [292]. The anticoagulant activity of CMDBSu increases with the sulfate content and reaches a heparin activity of 20% at a DS of sulfate of 1.3. The presence of *O*-sulfate groups is essential for the biological activity (compare Sect. 4.1.2). Furthermore, the presence of benzylamide groups improves the activity.

For CMDBSSu, the anticoagulant and anticomplementary activities increase with increasing molecular weight and reach a plateau at about 40 000 g mol^{-1} [285]. Heparin [293, 294] and synthetic heparinoids derived from *cis*-1,4-polyisoprene containing carboxylic and sulfonate groups [295] show a comparable plateau. For CMDBSSu with a high degree of substitution (DS of CM 0.72–1.09, DS of benzylamide 0.14–0.66, DS of sulfonate $\leq$ 0.09, DS of sulfate 0.03–0.76), the anticoagulant activity increases in the range of molecular weights 10 000–80 000 g mol^{-1} [283].

In addition to anticoagulant activities, CMDBSSu has the capacity to inhibit smooth muscle cells (SMC) and stimulate endothelial cells (EC) proliferation. The proliferative nature is not related to the anticoagulant properties [109, 288]. Some CMDB derivates exhibit an inhibitory effect on human breast cancer lines [296–298]. The inhibitory effect on cell growth appears to be independent of the sulfate and sulfonate groups but depends rather on the benzylamide content.

A CMDBSSu (DS of CM 1.1, DS of benzylamide 0.025, DS of sulfonate/sulfate 0.37) can be applied as tissue repair agent, efficient at protecting the heparin-binding growth factors FGF2 and TGFβ1 against pH, thermal and trypsin degradation [299, 300]. A test with an in vivo wound-healing model of colonic anastomosis indicated that the polymer effected after 48 h a twofold increase of the anastomosis resistance to leakage compared to untreated controls.

CMD and mixed derivatives based on CMD may be used for the fixation of antibiotics and enzymes on a polysaccharide matrix. A procedure was developed for amidation of CMD and its ethyl ester (CMDEE) with various aromatic amines in dioxane (100 °C), ethanol (78 °C), n-propanol (97 °C) and 2-propanol (82 °C) in the presence of a small amount of water [301]. CMDEE reacts with aliphatic and aromatic amines but in the case of amino acids the degree of amidation depends on the position of the amino moieties in the acids [302, 303]. The DS decreases from δ-, γ-, β, to α-amino acids. For the reaction with α-amino acids, carboxymethyl and carboxyethyl dextran azides are used [304].

5.2.2.2 CMD as Coating Material

The cytocompatible CMDs are able to stabilise iron oxide-based nanoparticles by coating. The commonly used synthesis route to obtain dextran-coated iron oxide-based nanoparticles is the alkaline precipitation of magnetite-like compounds (Fe^{2+} and Fe^{3+} salt solutions) in water in the presence of the colloid stabilising CDM [305, 306]. The separation from unbound polysaccharide is carried out by gel filtration chromatography. The coated superparamagnetic iron oxide particles are more stable in biological media because the CMD coating separates the iron oxide core from solutes and minimises protein adsorption on the nanoparticle surface. According to the synthesis conditions, the structure of the CMD coating and the persistence time in the blood can be influenced.

These nanoparticles can interact cell-type-specifically with viable human cells [307]. The functional groups are accessible to subsequently covalent fixation of bioactive molecules such as oligonucleotide (Fig. 41). Such oligonucleotide-functionalised magnetic nanoparticles can be taken up intracellularely by endocytosis [308].

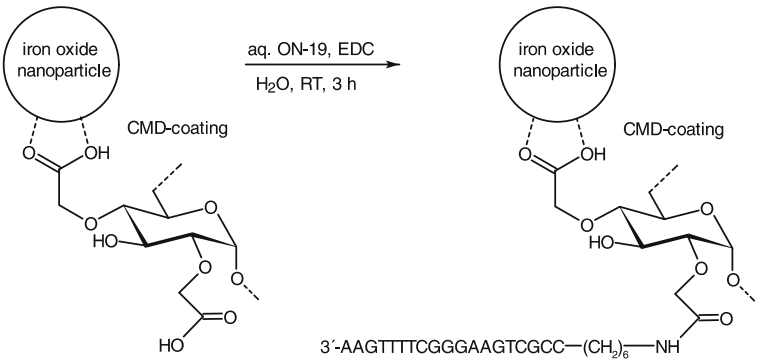


Fig. 41 Coupling of the oligonucleotide ON-19 with carboxymethyl dextran-coated iron oxide nanoparticles

Nanoparticles may be applied in tumour diagnosis and therapy [309]. The nanoparticles have several advantages such as high drug encapsulation, efficient drug protection against chemical or enzymatic degradation, unique ability to create a controlled release of drugs, cell internalisation and the ability to reverse the multidrug resistance of tumour cells [310]. The dextran-coated nanoparticles show prolonged blood flow time and allow targeting to specific tissue, such as lymph nodes or brain tumour [88].

5.2.2.3 CMD Complexes

CMD and dicarboxymethyl dextran (DCMD) as biocompatible and water-soluble polymeric carriers can react with *cis*-dihydroxo(cyclohexane-*trans*-L-1,2-diamine)platinum II (Dach-Pt) via a complex-type bond (Fig. 42). Low molecular weight platinum complexes are poorly water soluble. Their cytotoxic activity decreases in the bloodstream because of ligand exchange reactions with proteins, amino acids and other amino compounds. The immobilisation of Dach-Pt to DCMD by a chelate-type coordination bond is stable to maintain the cytotoxic activity in contrast to a single coordination of Dach-Pt to CMD, where the cytotoxic activity decreases gradually with the preincubation time. DCMD/Dach-Pt should also show a longer half-life in the body and a larger accumulation at the inflammatory tumour site than low molecular weight platinum complexes [311].

5.2.2.4 CMD for Protein Immobilisation

Multiple funtional improvements in β-lactoglobulin (β-LG) could be achieved by covalent binding to CMD ($M_n \sim 10\,000\,g\,mol^{-1}$) using the water-soluble

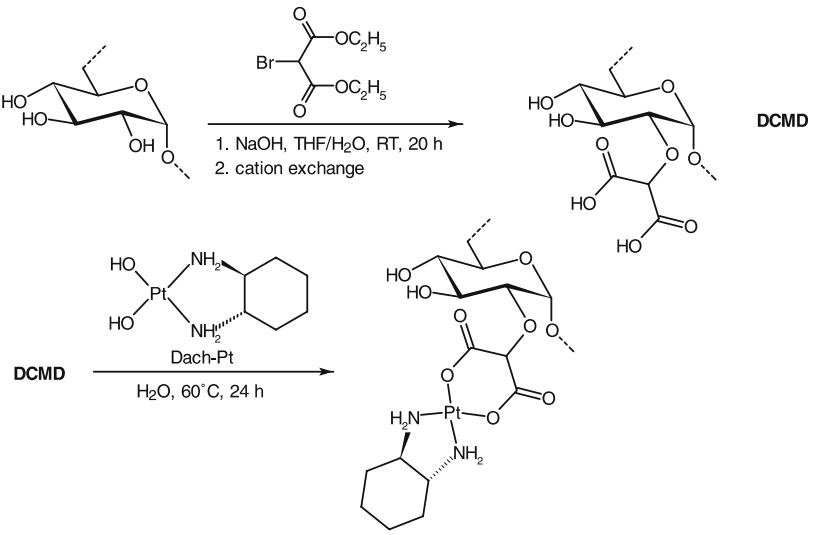


Fig. 42 Synthesis of dicarboxymethyl dextran (DCMD)/*cis*-dihydroxo(cyclohexane-*trans*-L-1,2-diamine)platinum II (Dach-Pt) complex

1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) as coupling reagent [312, 313]. β-LG is a predominant whey protein and known as a potent allergen of milk allergy (82% of milk allergy patients are sensitive to β-LG). The modification of the protein by covalent binding to CMD leads to reduced allergenicity, improved heat stability and emulsifying properties of the protein by maintaining the retinol binding activity [314]. The emulsifying properties of β-LG can be markedly improved with the molecular weight of the CMD used ($M_n \sim 40\,000$ g mol^{-1}, $\sim 70\,000$ g mol^{-1}, $\sim 162\,000$ g mol^{-1}), especially in the pH region lower than 7, in the presence of salt, and after heating. It is assumed that the physical properties of CMD (such as hydrophilicity, negative charge and viscosity) influence the emulsifying activities rather than the effect of the conformational changes [315]. The immunogenicity of β-LG also depends on the molecular weight of the CMD. The higher the molecular weight of CMD ($M_n \sim 40\,000$ g mol^{-1}, molar ratio of β-LG to CMD 8 : 1 and $M_n \sim 162\,000$ g mol^{-1}, molar ratio of β-LG to CMD 7 : 1) the lower is the immunogenicity of β-LG [316, 317]. By means of intrinsic fluorescence spectra, circular dichroism spectra and enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies, it can be shown that the surface of β-LG is covered by CMD with retention of the native conformation. Local conformational changes of β-LG by covalent binding of CMD were evaluated by competitive ELISA. Whereas the conformation around ^{125}Thr–^{135}Lys (α-helix) and the regions near the epitope 61B4 maintain their native form, the conformations around ^{15}Val – ^{29}Ile (β-sheet) and ^{8}Lys – ^{19}Trp (random coil, β-sheet and short helix) are changed. The suppression of the generation of T cell epitopes

by covalent binding of CMD is important for the explanation of the reduced immunogenicity of β-LG. The substances could be applied for hypoallergenic formulations and hypoallergenic emulsifying agents also suitable for novel foods with low allergenicity.

5.2.2.5 Drug Support

One of the serious problems in chemotherapy is the limited selectivity of most of the common anticancer drugs. Because of the low cell selectivity, they do not only interfere with tumour cells but also with healthy cells, and lead therefore to serious side effects. New macromolecular prodrugs were synthesised that are composed of CMD, Gly – Gly – Gly linker, and camptothens (CPTs), one of the most promising classes of anticancer drugs [318].

T-2513 (7-ethyl-10-(3-aminopropyloxy)-camptothecin, Fig. 43), which exhibits a good hydrophobic/hydrophilic balance, can be bound to the polymer via an amide bond to the peptidyl linker. The macromolecular prodrug is stable and pharmacologically inactive during circulation in the bloodstream but becomes active after reaching the target site. The entry of macromolecules into the cells is restricted to endocytosis and pinocytosis that can be highly cell specific. A comparable macromolecular chemotherapeutic with tumour targeting capability is methotrexate, covalently bound via peptide linker (Pro – Val – Gly – Leu – Ile – Gly) onto CMD [319].

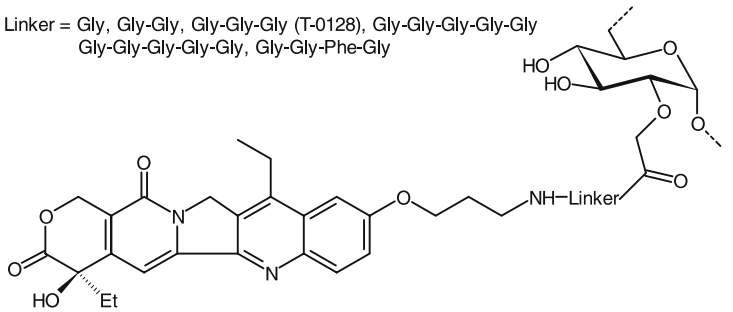


Fig. 43 Chemical structure of T-2513 covalently bonded to CMD via linker [318]

5.2.2.6 Stimuli-Responsive Hydrogels Based on CMD

Stimuli-responsive homo- and copolymeric hydrogels with pH, temperature, ionic strength, solvation, electrical field, or magnetic field-sensitive pendent groups have been produced for drug, peptide and protein delivery devices, for biosensors and for tissue engineering. Their swelling behaviour, net-

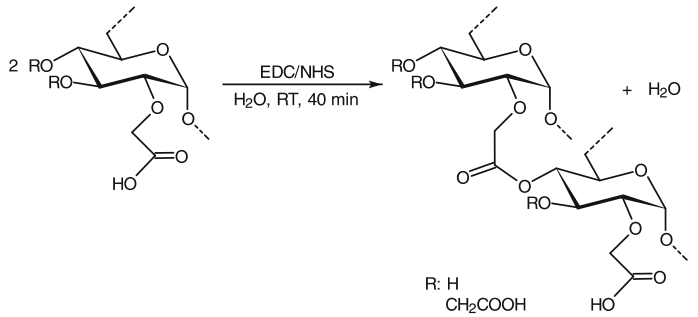


Fig. 44 pH- and ionic strength-sensitive hydrogel synthesised by inter- and intramolecular cross-linking of carboxymethyl dextran

work structure, permeability and mechanical strength changes in response to external stimuli [320–324]. The pH and ionic-strength sensitive hydrogel membranes for drug delivery and tissue engineering applications can be synthesised by intermolecular cross-linking of CMD in the presence of EDC and *N*-hydroxysuccinimide (NHS) (Fig. 44) [325]. The synthesis method is similar to the procedure described in [326]. EDC/NHS is not incorporated into the hydrogel in contrast to conventional cross-linking agents because the water-soluble urea derivatives formed can be simply washed out [327].

Both the DS of carboxymethyl functions and the concentration of the cross-linking reagent control the degree of cross-linking and the charge density of the hydrogel. Hence, the sensitivity of this hydrogel is tailored for specific applications. CMD of low DS shows no pH sensitivity. With increasing DS, the sensitivity arises and, therefore, the porosity of the hydrogel can be reversibly changed in response to changes in environmental pH. This response can control the transport of proteins through the membrane. At high pH, the COOH groups are dissociated inducing electrostatic repulsion, expanding the conformation and increasing the porosity of the hydrogel. Similar behaviour has been observed with pH-sensitive methacrylated dextran hydrogels, polypeptide hydrogels and chitosan-polyvinyl pyrrolidone hydrogels [324, 328, 329]. The structure of the hydrogel membrane is also sensitive to changes in ionic strength and the protein diffusion rates. At low ionic strengths, the protein diffusion rate increases with the ionic strengths; however, above a certain value the expanded matrix changes to a more compact one and the diffusion rate drops with further increase of ionic strengths.

5.2.3
2-(Diethylamino)ethyl (DEAE) Dextran

2-(Diethylamino)ethyl dextran (DEAE dextran) is synthesised by reaction of dextran with (2-chloroethyl)diethylammonium chloride in the presence of $NaBH_4$ in an alkaline solution at 85–90 °C (Fig. 45).

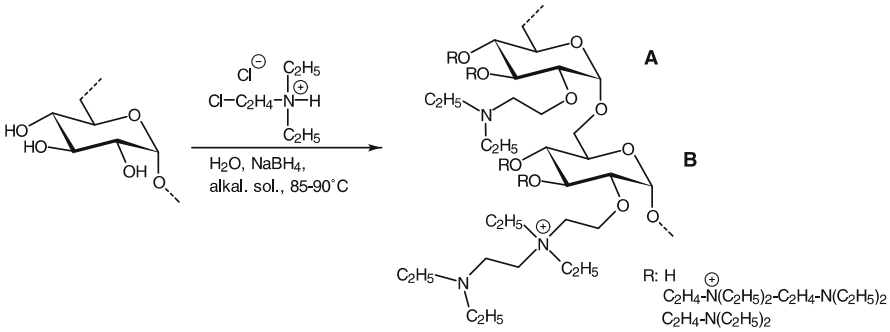


Fig. 45 Synthesis and structure of DEAE dextran containing 2-(diethylamino)ethyl (*A*) and 2-[[2-(diethylamino)ethyl]diethylammonium]ethyl (*B*) groups

DEAE dextran contains three basic groups with different pK_a values (Fig. 45). One (2-(diethylamino)ethyl, A) consists of a tertiary amino group with a pK_a of 9.5. The other two moieties are (2-[[2-(diethylamino)ethyl]-diethylammonium]ethyl, B) a tertiary amino group with a pK_a of 5.7, and the strong basic quaternary ammonium group with a pK_a of 14 [242, 243]. The pH value and ionic strength influences the dissociation behaviour and the conformational change of DEAE dextran.

DEAE dextran is a biocompatible derivative with pharmacological and therapeutic properties [330]. In particular, DEAE dextran is suitable for binding bile acids and reducing the absorption of dietary cholesterol and fatty acids in the gastrointestinal lumen. A hypocholesterolaemic effect [331, 332] and reduction of triglycerides in presence of DEAE dextran have been proved [333]. Furthermore, DEAE dextran has the ability to enhance viral infectivity in cell cultures and is suitable as an agent for transfection because it enhances the uptake of proteins and nucleic acids by cells. Cultured human lymphocytes can be utilised for the synthesis of DNA in the presence of DEAE dextran deoxynucleotides [334]. The DNA synthesis in cells, treated with DEAE dextran, resembles DNA synthesis in vivo [244]. Plasmid DNA mixed with DEAE dextran is taken up by colon epithelial cells. This transfection technique is useful in gene therapy, e.g. for colon disease [335–338]. Vacuum-dried enzymes and enzymes in solution can be stabilised by DEAE dextran. Thus, the activity of vacuum-dried glycerol kinase is preserved in the presence of DEAE dextran and lactitol [339].

Water-insoluble PEC were prepared by mixing oppositely charged polyelectrolytes, e.g. DEAE dextran with CMD [340, 341], sodium dextran sulfate, poly(styrene sulfonate) (NaSS) [342], poly(sodium *L*-glutamate) (PSLG), poly(vinyl alcohol)sulfate [343], or potassium metaphosphate (MPK) [240]. They are useful as membranes or in biomedical applications [343, 344].

5.2.3.1 Hydrophobically Modified DEAE Dextran

In contrast to the hydrophilic DEAE dextran, new hydrophobically modified polycationic dextran (HMPC) was synthesised, carrying pendant *N*-alkyl-*N*,*N*-dimethyl-*N*-(2-hydroxypropyl)ammonium chloride groups, with alkyl being an octyl, dodecyl or cetyl moiety (Fig. 46, [193]).

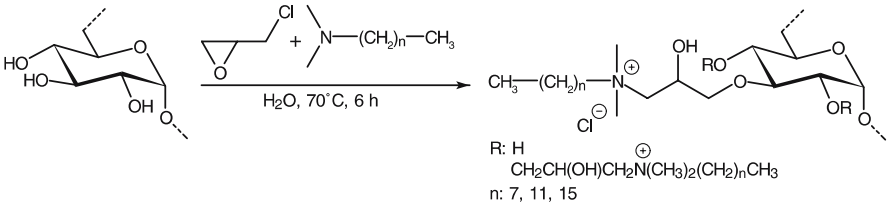


Fig. 46 Synthesis of amphiphilic polycationic dextran derivatives

These polymers show self-assembly into intramolecular (hydrophobic interactions in the same polymer chain) or intermolecular (between different chains) micelle-like clusters. Below the CAC, the polymer is extended because of electrostatic repulsion that does not allow a significant association between the hydrophobic pendant groups. Above the CAC, the higher ionic strength changes the polymer conformation to a less extended form, resulting in association of the hydrophobic side chains. The properties of the micelles depend significantly on the length of the hydrophobic side chains and the degree of substitution. The micelles with dodecyl- and cetyl alkyl side groups are formed mainly intramolecularly, whereas octyl side groups lead to intra- and intermolecular interactions. The association of hydrophobically modified polycationic dextran derivatives with oppositely charged surfactants like sodium octyl, dodecyl and tetradecyl sulfate leads to aqueous solutions with complex phase behaviour and self-assembling morphologies in solution [247–250]. These properties, e.g. the spontaneous formation of hydrophobic cores in aqueous solution, exhibit a great potential in drug/gene delivery research and for other biomedical applications. The mechanism and strength of the interaction between HMPC and oppositely charged surfactants depend on the structure of the surfactants, their hydrophobic part, the charge density, DS, chain conformation, hydrophobicity and molecular weight of the HMPC. In particular, the effect depends on the molar ratio of surfactant and HMPC.

6
Miscellaneous Dextran Derivatives

6.1
Dextran Conjugates

An enormous amount of publications have appeared over the last three decades dealing with the preparation of so-called dextran conjugates. These are either dextrans carrying bioactive molecules such as pharmaca, enzymes, proteins and hormones or dextrans modified with fluorescent dyes. These conjugates usually have low DS values and structural data are not accessible. A number of different paths for the covalent coupling are known. The preparation of ester-type conjugates mainly prodrugs is described in Sect. 4.2. In addition, binding via carbonate and carbamate moieties, using cyanogen bromide activation of the dextran, periodate oxidised dextran and binding as Schiff base, and the application of spacer molecules are all broadly exploited techniques. In this section, the chemistry of dextran conjugates is briefly discussed for representative examples. For more detailed information on the application of conjugates, the excellent reviews by DeBelder [45], Larsen [178] and Mehvar [179] are recommended. In addition, the importance and the influence of the linker between the polymer and the drug on the biological interactions and the release mechanism was reviewed [345].

6.1.1
Coupling via Formation of a Schiff Base

The most versatile method for the introduction of bioactive molecules with terminal NH_2 functions such as proteins and enzymes is the coupling via formation of a Schiff base (Fig. 47). It is necessary to oxidise the dextran backbone in the first step to obtain reactive aldehyde moieties. The Schiff base formed between the amine group of the bioactive molecule and the oxidised dextran is usually reduced in a separate step to obtain a stable conjugate.

The activity of proteins is not significantly diminished. Complex structures such as monoclonal and polyclonal antibodies [346] and bacterial proteins usable in the tumour-specific delivery of oncolytic drugs [347] can be covalently attached. Recently, such a system was used for immunodetection. Thus, proteins covered with dialdehyde dextran have been exploited as carrier molecules to detect the immunoresponse against an aminated hapten [348]. In addition, dialdehyde dextran has been exploited to detect protein–protein interactions. A very simple strategy, based on the intermolecular cross-linking of associated proteins by using dialdehyde dextran, has been proposed [349]. Dextran dialdehyde is applied as coating material for magnetic nanoparticles and immobilisation of enzymes such as glucose oxidase on the particle surface [350].

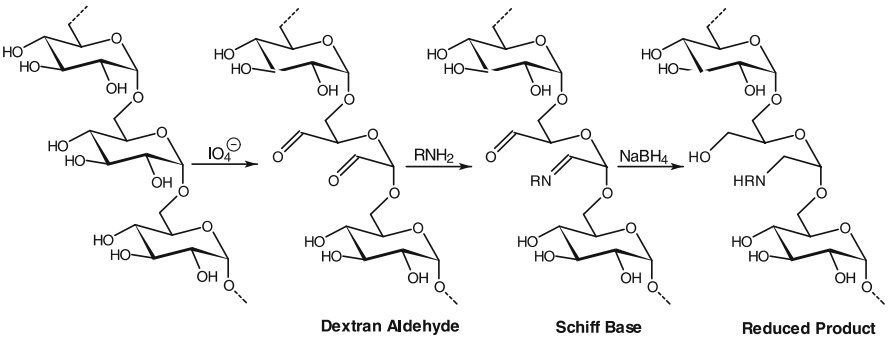


Fig. 47 Covalent binding of bioactive molecules with terminal NH_2 functions to dextran via oxidation of the dextran, formation of a Schiff base, and reduction

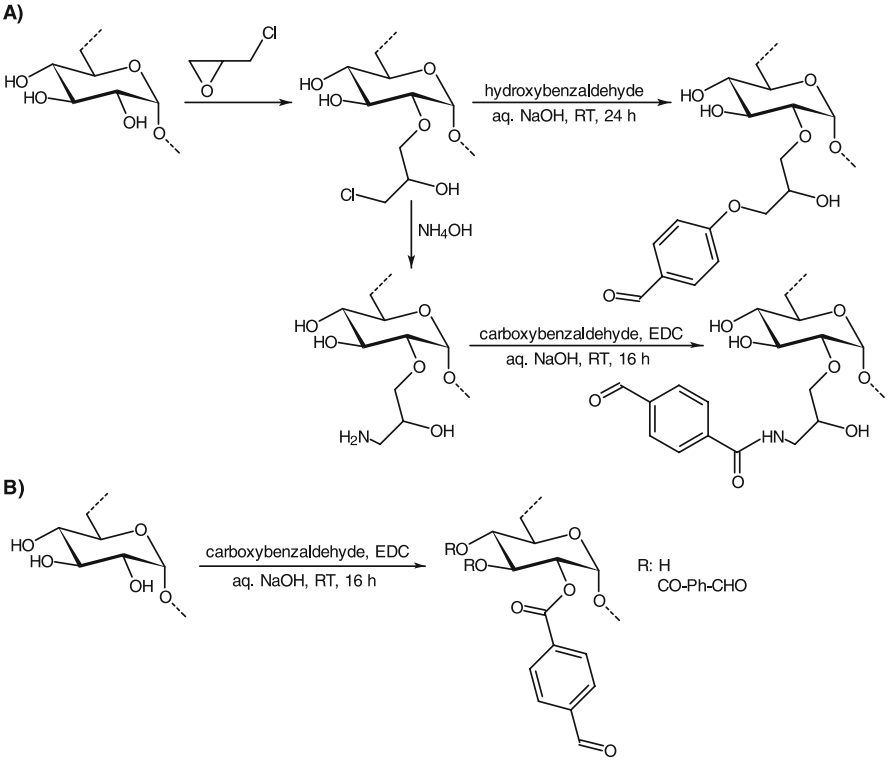


Fig. 48 Introduction of aldehyde functions by conversion of 3-chloro-2-hydroxypropyl dextran (activated dextran) with hydroxybenzaldehyde *A* or carboxybenzaldehyde (*A*, *lower part*, *B*)

It should be mentioned that introduction of a reactive aldehyde function can also be achieved by activation of dextran with epichlorhydrin and subsequent conversion with carboxybenzaldehyde or hydroxybenzaldehyde

(Fig. 48) [351]. The reaction via the aminated dextran (path A lower part) exhibits almost complete conversion of the amine functions, resulting in a fairly high amount of aldehyde moieties. Nevertheless, the amount of aldehyde is in the range of one to seven aldehyde groups per 100 mol anhydroglucose units.

6.1.2
Cyanogen Bromide as Coupling Agent

A method with the same focus, i.e. conjugation of amine-containing drugs and proteins to dextran, is the activation of dextran with cyanogen halides (Fig. 49). This procedure was among the first synthesis tools exploited for the covalent binding of peptides and proteins to polysaccharides [352]. Cyanogen bromide is the most efficient coupling agent. Despite its popularity, the precise total structure of the cyanogen bromide-activated polysaccharides and the endogenous functional groups are obscure. The most reasonable intermediate is shown in Fig. 49 [353].

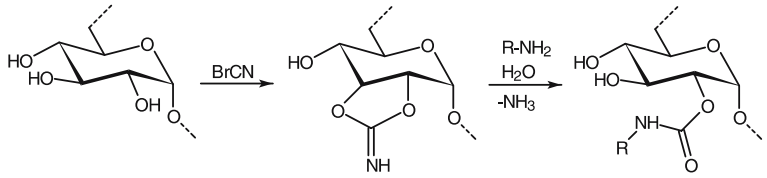


Fig. 49 Use of cyanogen bromide for the conjugation of peptides and proteins to polysaccharides

The technique is also used to introduce diene moieties, which enable the polymer for a highly efficient modification with dienophile components via Diels–Alder reaction [354]. Therefore, methyl octa-4,6-dienoate is converted to octa-4,6-dienoic acid hydrazide, which is bound to dextran via reaction with BrCN. The maleimide-modified protein (albumin) is easily attached to the polymer backbone by Diels–Alder reaction (Fig. 50).

6.1.3
Carbonates and Carbamates

First attempts to use the carbonate or the carbamate function for the covalent binding of amino acids and proteins applying the activation of dextran with phosgene showed that this approach is limited due to the fact that it is combined with a number of side reactions (Fig. 51) [355].

More useful is the conversion of dextran with ethyl chloroformate or 4-nitrophenyl chloroformate carried out in DMF/LiCl to give the corresponding carbonates [356]. Analysis of the total carbonate content and the content of 4-nitrophenyl carbonate moieties during the course of the reac-

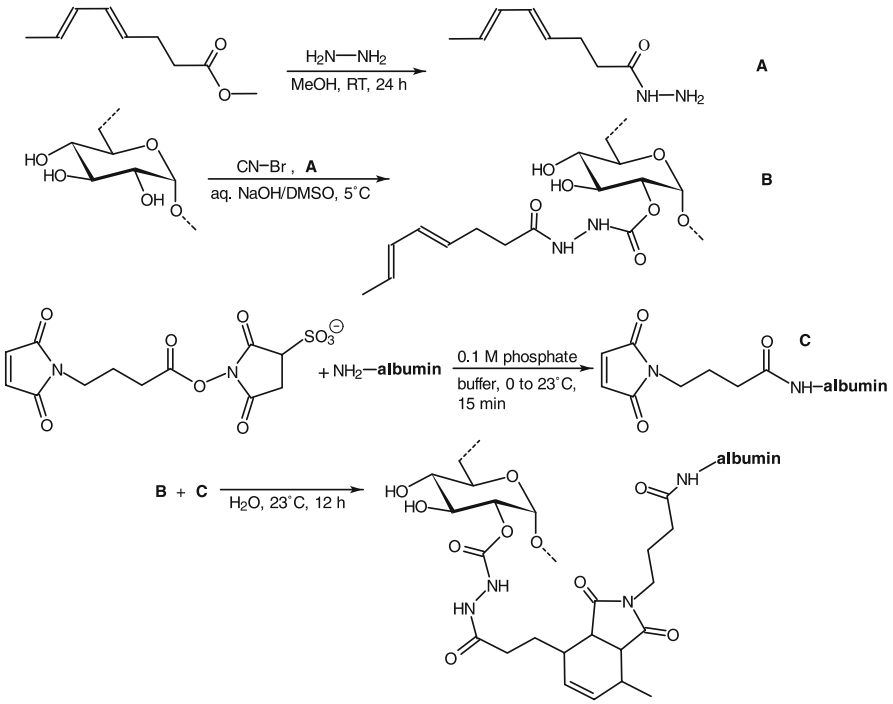


Fig. 50 Synthesis path applied for the binding of a maleimide-modified protein, e.g. albumin, to dextran via Diels–Alder reaction after introduction of a dienophil component using BrCN

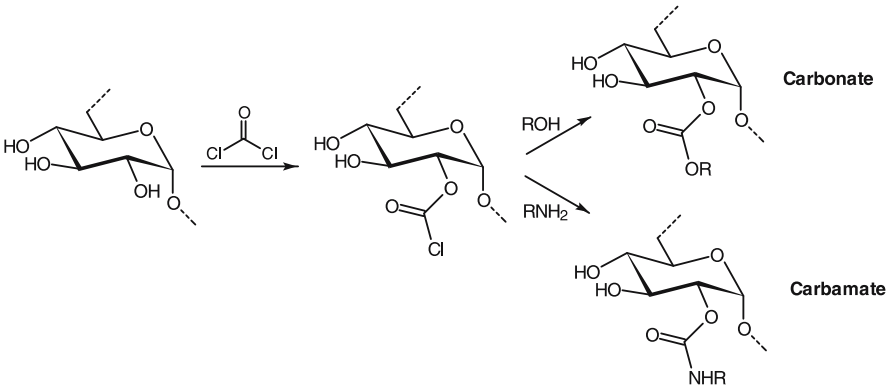


Fig. 51 Preparation of dextran carbonates and carbamantes after phosgene treatment

tion demonstrates the formation of different types of carbonate moieties. The 4-nitrophenyl carbonate moieties are transformed into other carbonate structures, most likely by reaction with neighbouring polymeric hydroxyl groups as revealed by NMR spectroscopic studies. The formation of cyclic carbon-

ates is discussed, which could be inter- or intramolecular (Fig. 52). The data suggest that the process occurs predominantly within the polymer chains. It is strongly enhanced by addition of a strong base. The maximum total DS reached is in the region of 0.8 after 40 min. If longer reaction times are applied, the DS values decrease because of the attack of a neighbouring hydroxyl group of the dextran [357]. In model experiments with phenylamine and tyramine it was shown that conversion to carbamate moieties can be achieved (Fig. 52).

A useful approach for the introduction of a carbonate function is the reaction with *N*,*N*′-carbonyldiimidazole as discussed in Sect. 4.2.2.3 (Fig. 29), which was applied for the preparation of a new class of polymerisable dextrans [177].

More often, carbamates of dextran are prepared by one-step conversion of the polysaccharide with isocyanates or isothiocyanates. This path is especially useful for the conjugation of fluorescent dyes. Fluorescent-marked dextrans are commercial products today. Fluorescent dextran derivatives with different molecular weights and substituents are available as invaluable tracers for studies on microcirculation and vascular permeability in health and disease [8]. Fluorescein isothiocyanate (FITC) dextran (actually the thiocarbamate) has been well established in this area of research since the 1970s [358]. The isothiocyanate of fluorescein is covalently bound to dextran leading to

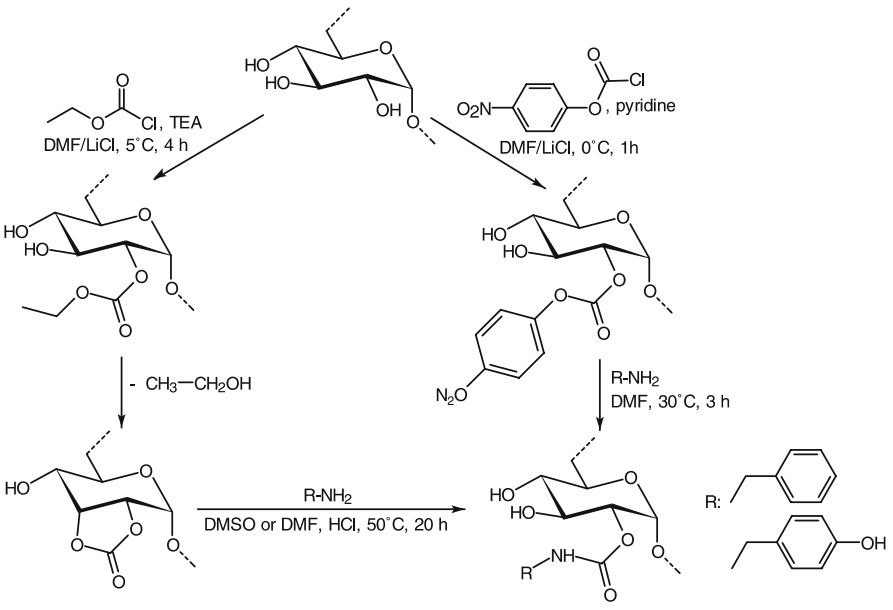


Fig. 52 Binding of phenylamine and tyramine to dextran via functionalisation with 4-nitrophenyl carbonate and subsequent formation of a carbamate

a low substituted fluorescein-labelled dextran (DS 0.004–0.01), as schematically shown in Fig. 53 [359].

An alternative method for the synthesis of different dextran conjugates with fluorescent dyes is the reaction of a water-soluble amino dextran with the succinimidyl ester of the corresponding dye [360]. The amide linkage is more stable than the corresponding thiocarbamate formed with isothiocyanates. This method is displayed schematically in Fig. 54 for the preparation of 5- (and 6-) carboxynaphthofluorescein-labelled dextran from amino dextran (Fig. 47, [361, 362]).

Fluorophores (including coumarin, cascade blue, tetramethylrhodamine and texas red), pH and Ca^{2+} indicators (fura, indo, calcium green-1) and coenzymes (biotine) are coupled to dextran by using the appropriate technique [360]. The investigation of hydrodynamic properties of the cytoplasmic matrix [363], the observation of uptake and internal processing of exogenous materials by fluid-phase endocytis [364], and the in vivo tracing of cell lineage [365] are examples of the importance of dextran conjugates

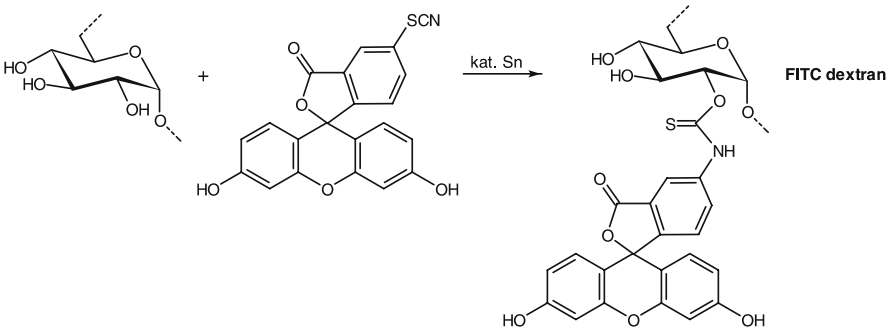


Fig. 53 Preparation of fluorescein-labelled dextran (FITC dextran) by the isothiocyanate technique [359]

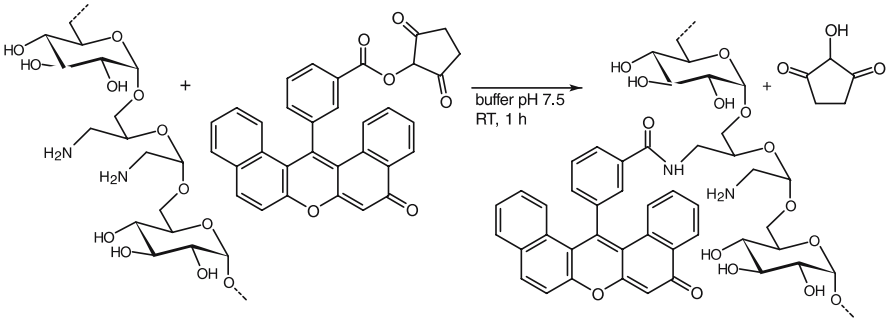


Fig. 54 Preparation of 5-carboxynaphthofluorescein-labelled dextran by the succinimide technique [361]

in medical research. One further application is the use as a size exclusion probe for determining relative pore sizes in both synthetic and natural membranes [366].

6.2 *p*-Toluenesulfonic Acid Ester of Dextran

Chemical functionalisation of polysaccharides directly at the C-atoms of the anhydrosugar unit may be carried out by nucleophilic displacement reactions (S_N). An indispensable prerequisite for S_N is the transformation of the hydroxyl moieties to a leaving group, which can be achieved by sulfonic acid ester formation, especially by introduction of a tosyl group. It has been shown that tosylation of dextran under homogeneous conditions is an efficient modification yielding polymers with a broad spectrum of solubility (Table 15). Preliminary studies on the S_N reaction of tosyl dextran (DS 1.39) with NaN_3 revealed that a polymer with DS_{Azide} 0.62 and DS_{Tosyl} 0.66 can be synthesised, i.e. almost 45% of the tosyl moieties were substituted [367].

Table 15 Conditions and results for the reaction of dextran dissolved in DMAc/LiCl with *p*-toluenesulfonic acid chloride in the presence of triethylamine for 24 h at 8 °C

Molar ratio [a]	Elemental analysis (%) S	Cl	DS [b] DS_{Tosyl}	DS_{Cl}	Solubility
1 : 0.5	1.54	0.32	0.08	0.03	DMSO, DMA, DMF
1 : 1.5	8.63	0.21	0.75	0.02	DMSO, DMA, DMF, THF
1 : 5.0	11.83	0.22	1.39	0.02	DMSO, DMA, DMF, THF, acetone, $CHCl_3$

[a] Glucopyranosyl unit: *p*-toluenesulfonic acid chloride, 2 mol triethylamine per mol *p*-toluenesulfonic acid chloride were used as base

[b] Degree of substitution of *p*-toluenesulfonic acid ester (index Tosyl) and chlorodeoxy (index Cl) groups

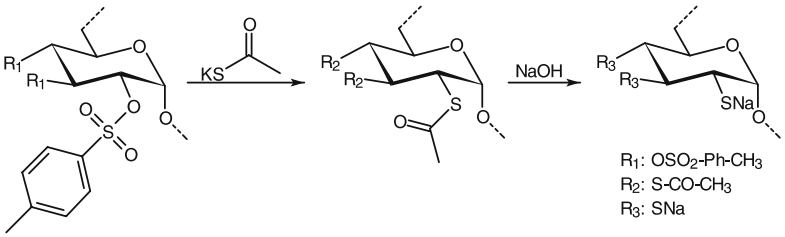


Fig. 55 Direct thiolation of the dextran backbone accessible via S_N reaction starting from tosylates of dextran [368]

In addition, dextran tosylates may be used for the preparation of thiol-containing derivatives usable for self-assembly structures (Fig. 55) [368].

6.3 Thiolated Dextran

Besides the direct thiolation of the polysaccharide backbone accessible via S_N reaction starting from tosylates of dextran, as discussed above, a number of paths for the introduction of spacer-bound thiol functions are described. Early attempts towards thiolation of dextran applied acetylthiosuccinic anhydride yielding the mercaptosuccinyl derivative of dextran (Fig. 56). The reaction was carried out by addition of acetylthiosuccinic anhydride to a solution of the macromolecule while the pH was maintained at 8 by addition of NaOH and subsequent treatment of the product with Amberlite [369].

More recently, thiolated dextran was synthesised by modification of dextran with 4-nitrophenyl chloroformate in DMSO/pyridine in the presence of DMAP at 0 °C yielding a carbonate (content 6%), which can be substituted with cystamine. Subsequent reduction yields the thiolated dextran containing between 1 and 4% thiol moieties (Fig. 57) [370].

This thiolated dextran may be immobilised by chemisorption on a metal surface such as silver and can thus be used as an inhibitor of non-specific protein adsorption. Surface plasmon resonance (SPR) was employed to monitor BSA adsorption from a flowing buffered solution, revealing a significant reduction of BSA adsorption for coated silver compared to the uncoated surface. The results obtained by SPR have been complemented and validated by ellipsometry, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) analysis. The amount of thiol functions and the molecular weight of the polymer affect the protein resistant performance of the dextran layer. By increasing the proportion of thiol substituents, a greater coverage of the silver surface is observed, conferring improved protein resistance. Higher molecular weight dextran derivatives (M_w ranging from 5000 to 500 000 g mol^{-1}) are shown to pack less efficiently to produce layers with less effective protein resistance [370, 371]. In addition, SPR has been employed to

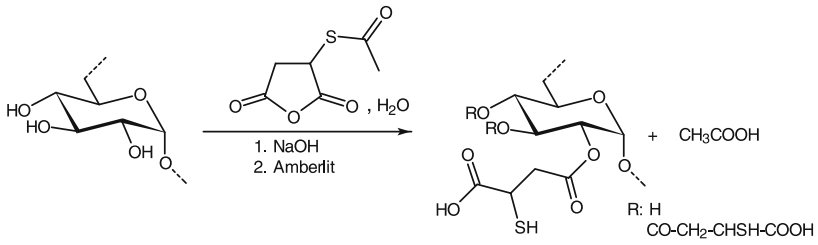


Fig. 56 Synthesis of thiol-functionalised dextran by conversion of dextran with acetylthiosuccinic anhydride

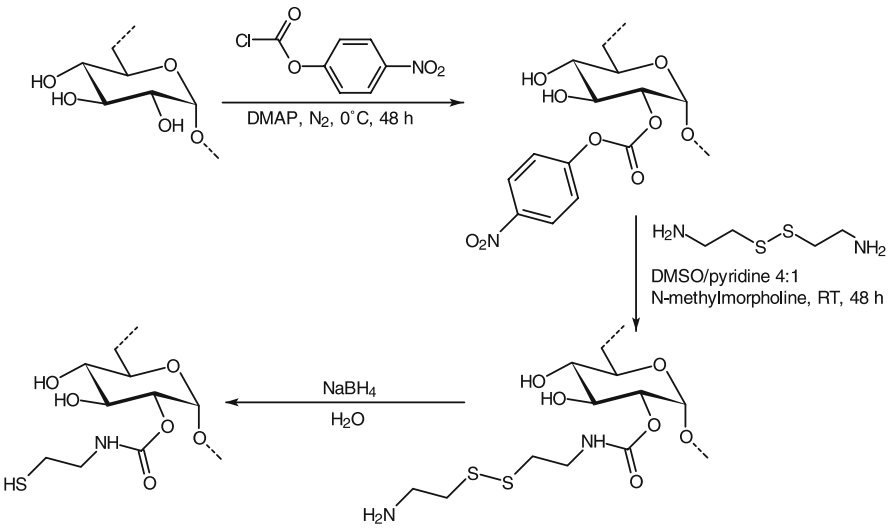


Fig. 57 Synthesis of thiolated dextran via formation of a nitrophenyl carbonate [370]

investigate the hydrolytic degradation of thiolated dextran monolayers on silver by the enzyme dextranase. It was demonstrated that dextranase does not completely remove the thiolated dextran monolayers, even at the enzyme's most active pH. A significant protein resistance is still found after degradation [372]. AFM measurements in liquid environment have demonstrated the ability to measure corresponding changes in both monolayer morphology and elasticity due to the hydration state of the dextran derivative [373].

Two methods for the derivatisation of dextran with boron-containing substituents via sulfur bridges were developed for substances useful in boron neutron capture therapy (BNCT). One method comprises activation of dextran with 1-cyano-4-(dimethylamino)pyridine (CDAP) with subsequent coupling of 2-aminoethylpyridyl disulfide (Fig. 58, method A). The thiolated dextran could couple the boron-containing molecule ($Na_2B_{12}H_{11}SH$, BSH) with a terminal thiol function in a disulfide exchange reaction. In another procedure, dextran was derivatised to an allyl dextran (Fig. 58, method B), which reacted with BSH in a free-radical-initiated addition reaction. The amount of functionalisation is rather low. For method A, 10–20 boron-containing functions were determined per dextran molecule (starting dextran was T70) and 100–125 units per dextran chain in the case of method B. Nevertheless, the derivatives are useful for tumour targeting applications [374].

The binding of proteins such as tetanus toxoid (TTd) onto dextran can also be achieved via thiolation. Therefore, dextran is converted selectively at the terminal reducing ends. This is possible either after amination and modification of the terminal amino function with 2-iminothiolan hydrochlo-

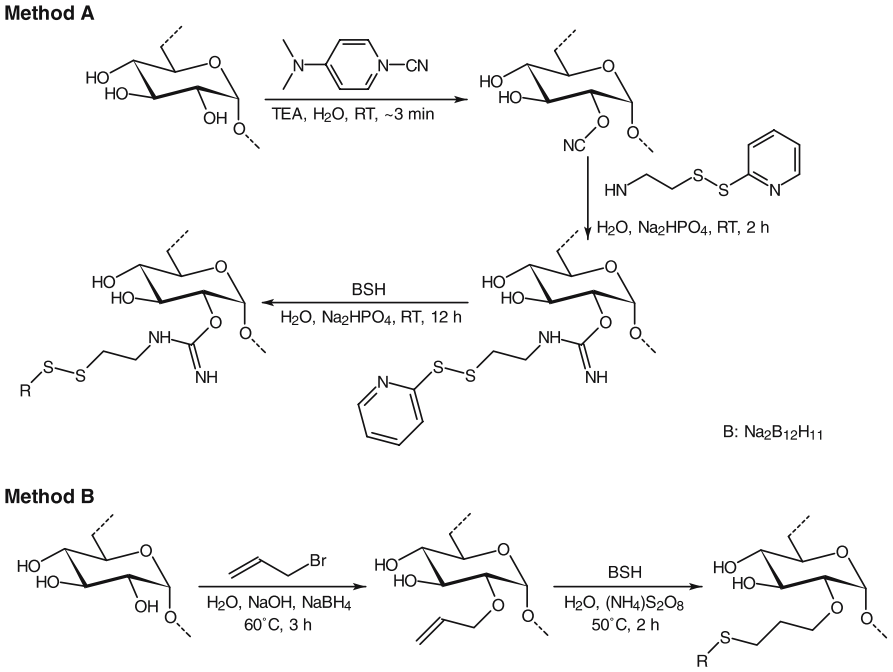


Fig. 58 Methods for the derivatisation of dextran with boron-containing substituents via sulfur bridges developed for boron neutron capture therapy (BNCT)

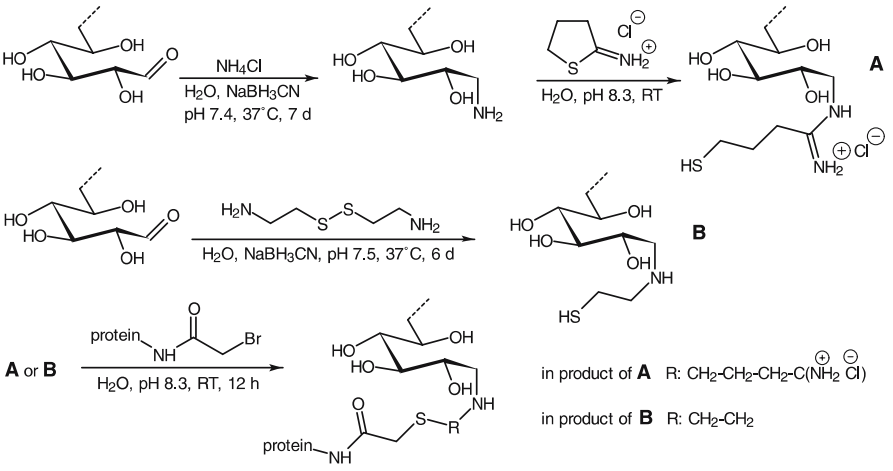


Fig. 59 Selective binding of proteins onto dextran by activation of the terminal reducing ends

ride (Fig. 59, path A) or by direct treatment of dextran with cystamine (Fig. 59, path B). The molecular weight of the dextran investigated for modification was in the range of 500 to 150 000 g mol^{-1}. The conjugation of the protein (e.g. TTd) onto the polysaccharide was performed after activation with *N*-hydroxysuccinimide ester of bromoacetic acid (Fig. 59). In contrast to random activation of the polysaccharide, this method avoids cross-linking leading to conjugates that induce strong antipolysaccharide T-helper-cell dependent immune responses. Unlike direct reductive amination, the 2-iminothiolan based conjugation technique is fast and made it possible to couple fairly large polysaccharides to TTd [375].

6.4 Silyl Dextran

Silylation reactions on polysaccharides with chlorosilanes and silazanes were attempted more than 50 years ago resulting in hydrophobic silyl ethers with both increased thermal stability and solubility in organic solvents [376]. The silylation reaction for the protection of hydroxyl groups in mono- and polysaccharides exhibits many advantages, e.g. fast silylation, solubility of silylether in organic solvents suitable for subsequent derivatisation, stability of the resulting silylether under basic conditions but easy deprotection of the silyl moieties by acid hydrolysis or nucleophilic agents like fluoride and cyanide ions [377]. The partial and complete silylation of dextran was studied in detail by Ydens and Nouvel [215–217].

The partial silylation was carried out with 1,1,1,3,3,3,-hexamethyldisilazane (HMDS) in DMSO at 50 °C (Fig. 60). The hydroxyl groups react with HMDS resulting in trimethylsilyl dextran and $(CH_3)_3SiNH_2$, which further reacts with hydroxyl groups to silyl ether and NH_3. The silylation reaction starts ho-

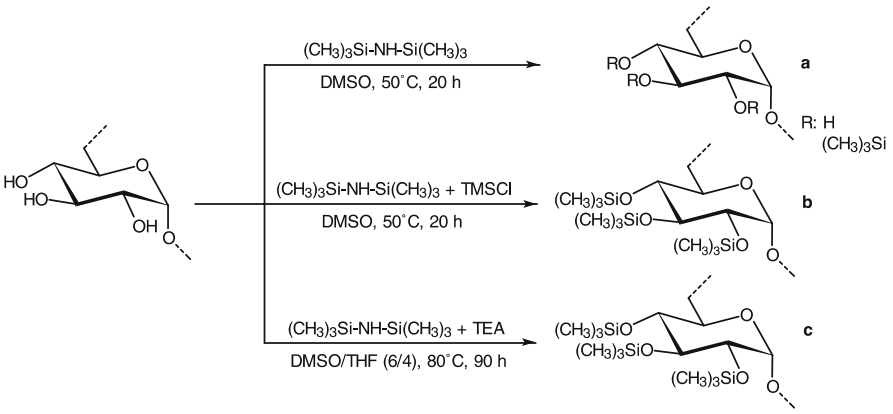


Fig. 60 Partial **a** and complete silylation (**b**, **c**) of dextran

mogeneously, but with increasing degree of silylation the solubility in DMSO decreases. Highly substituted products are soluble in THF, chloroform or toluene. The DS of silylation increases with reaction time, temperature and HMDS/OH ratio and decreases with the molecular weight of dextran; a complete silylation is not reached under these conditions (samples 1–5 and 13 in Table 16). Although acidic catalysts like HCl, *p*-toluenesulfonic acid or trifluoroacetic acid can increase the reactivity of HMDS, they are excluded due to their degrading potential.

Table 16 shows the influence of reaction conditions and of activation reagents such as TMSCl, hexamethylphosphoric triamide (HMPA), *N*-methylimidazole (NMI), saccharin and tetrabutylammonium fluoride (TBAF) on the DS of silyl dextran. For complete silylation, a mixture of 1.8 mol HMDS and 0.2 mol chlorotrimethylsilane (TMSCl) per mol OH groups have to be used. The more reactive TMSCl reacts in a first step with hydroxyl groups to trimethylsilyl dextran and HCl. Subsequently, the HCl cleaves HMDS in TMSCl and NH_3 [218]. Complete silylation of dextran could also be carried

Table 16 Influence of HMDS/OH molar ratio, co-solvent and activation reagent on the degree of substitution (DS_{Silyl})

Dextran	Sample	Medium	Molar ratio [HMDS]/[OH]	Activation reagent [a] (mol)	Temp. (°C)	Time (h)	DS_{Silyl}
T40	1	DMSO	0.25	–	50	20	1.1
	2	DMSO	0.50	–	50	20	2.0
	3	DMSO	2.0	–	50	20	2.3
	4	DMSO	2.0	–	50	16	2.1
	5	DMSO	5.0	–	50	20	2.3
	6	DMSO/THF (6/4)	4.0	0.2 NEt_3	60	48	3.0
	7	DMSO/toluene (1/1)	2.0	–	50	20	2.5
	8	DMSO	1.8	0.2 TMSCl [b]	50	20	3.0
	9	DMSO	2.0	0.1 HMPA [c]	50	20	2.5
	10	DMSO	2.0	0.1 NMI [d]	50	20	2.3
	11	DMSO	2.0	0.1 Saccharine	50	20	2.7
	12	DMSO	2.0	0.1 TBAF [e]	50	20	2.6
T10	13	DMSO	2.0	–	50	16	2.3
	14	DMSO/THF (5/2)	4.0	0.2 NEt_3	60	48	2.8
	15	DMSO/THF (6/4)	4.0	0.2 NEt_3	80	90	3.0

[a] With respect to mol HMDS
[b] Chlorotrimethylsilane
[c] Hexamethylphosphoric triamide
[d] *N*-Methylimidazole
[e] Tetrabutylammonium fluoride

out in DMSO using THF as co-solvent in a ratio of 6 : 4 and 0.2 mol triethylamine as activation agent with respect to mol HMDS and a HMDS to OH molar ratio of 4 : 1. Co-solvents such as THF and toluene guarantee homogeneous conditions for the whole conversion. At 50 °C, silylation of dextran with HMDS proceeds without chain degradation independent of the reaction times, co-solvents, or activation reagents. Nevertheless, degradation of dextran was observed at 80 °C. More reactive silylation reagents such as *N*,*O*-*bis*(trimethylsilyl)-acetamide lead to significant chain degradation even at 50 °C.

The degree of silylation can be determined by ^{1}H NMR in $CDCl_3$ or in DMSO-d_6. In the case of slightly silylated dextran, the analysis of the ^{1}H NMR spectra in D_2O and DMSO-d_6 give similar results. A precise assignment of the NMR signals was achieved by 2D ^{1}H – ^{1}H – and ^{1}H – ^{13}C techniques (COSY 45° and HMQC, Fig. 61).

The silylation yield can be calculated using the equation:

$$\text{Yield}(\%) = \frac{\text{A}(\text{OSiMe}_3)}{\text{A}(\text{anomeric H})} \times \frac{100}{27} .$$

$\text{A}(\text{OSiMe}_3)$ is the area of the trimethylsilyl group at 0.15 ppm in $CDCl_3$ or 0.18 ppm in DMSO-d_6. The area of the anomeric protons A(anomeric H) is centred at 4.7 ppm and the area of the glucosidic protons A(glycosidic H) is

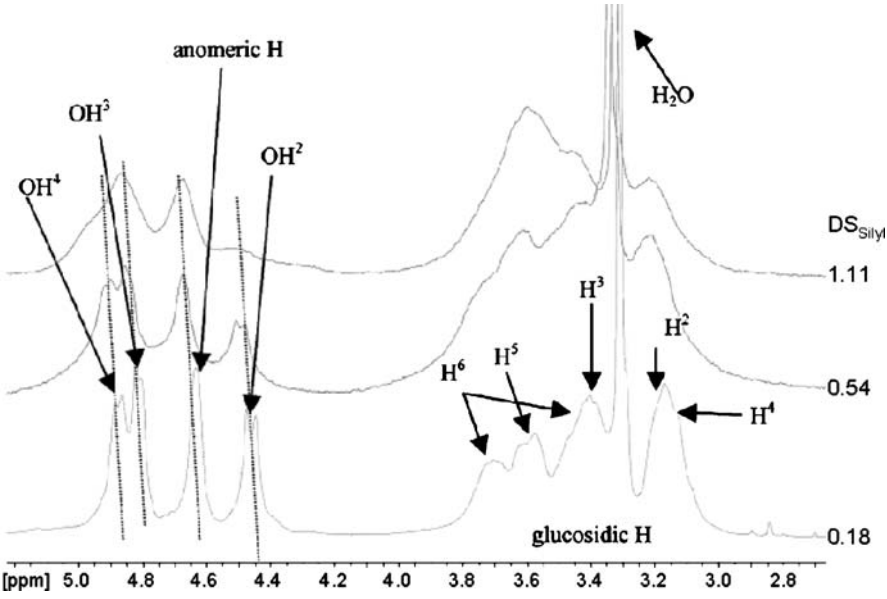


Fig. 61 ^{1}H-NMR spectra of silylated dextrans in DMSO-d_6 with different DS_{Silyl} (adapted from [217])

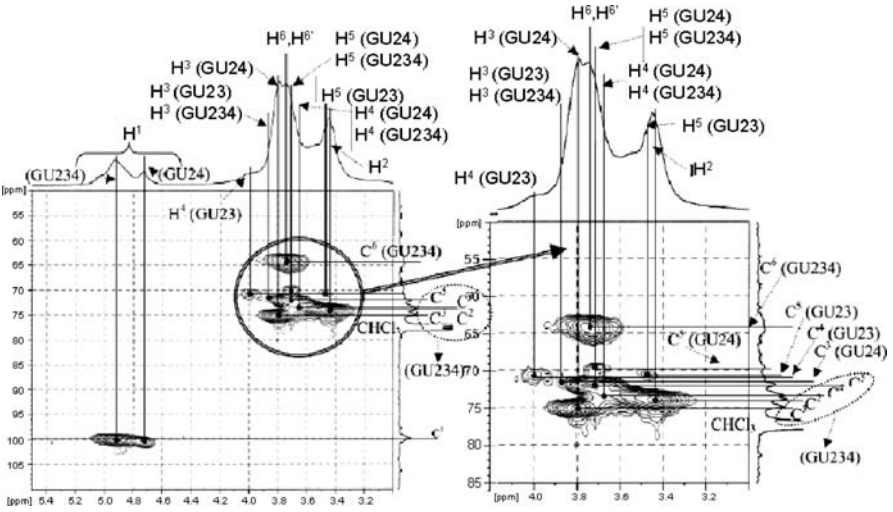


Fig. 62 HMQC spectrum of silylated dextran (DS_{Silyl} = 2.85, adapted from [217])

situated from 3.2 to 4.2 ppm in $CDCl_3$, and from 3.0 to 4.0 ppm in DMSO-d_6 (Fig. 61).

The DS can also be determined by subsequent reaction of the remaining OH groups with phenylisocyanate [378] or with trichloroacetylisocyanate [379] to the carbamate. The calculation from NMR data can be carried out either before or after hydrolysis of the TMS groups by using the ratio of the A(NH) of the urethane protons at 8.5–9.5 ppm to the total of A(anomeric H) and A(glycosidic H) between 3 and 5 ppm. Both methods give comparable values. The analyses of the NMR spectra in Fig. 62 allow determination of the different reactivity of the OH groups. The rapid decrease of the hydroxyl proton signal at position 2 means that OH2 is the most reactive group. Nouvel found the following order of reactivity for the silylation of dextran: OH2 > OH4 > OH3 [217]. The HMQC spectrum of silyl dextran with a DS of 2.85 (Fig. 62) confirms the presence of 2,3,4-tri-*O*-; 2,3-di-*O*-; and 2,4-di-*O*-silylated glucose units.

6.4.1
Poly(ε-Caprolactone)-grafted (PCL) Dextran Copolymers

The combination of biodegradable hydrophobic polyesters with biodegradable hydrophilic dextran leads to completely biodegradable amphiphilic polymer architectures with promising properties, useful as environmentally friendly surfactants or as a matrix in drug delivery systems. For instance, the synthesis of PLA-grafted dextran copolymers obtained by ring-opening polymerisation (ROP) of lactide with dextran in presence of stannous octoate

was described [380–384]. A controlled synthesis of the amphiphilic PLA-grafted dextran copolymers was achieved by partial silylation of the hydroxyl functions in dextran, with subsequent ROP of the lactide with the remaining hydroxyl groups and, finally, the deprotection of the silyl ether [216, 217, 385, 386]. A three-step procedure was also suggested by Ydens [215] and Nouvel [216, 217] for the controlled synthesis of amphiphilic PCL-grafted copolymers of dextran (Fig. 63).

The remaining hydroxyl groups of partially silylated dextran may initiate the ring-opening polymerisation of ε-caprolactone (Cl). Through a controlled silylation reaction, the number of hydroxyl groups available for further ROP of ε-caprolactone is adjusted. The hydroxyl groups along the silylated dextran backbone are catalytically activated by $AlEt_3$ or $Al(O\text{-}isoPr)_3$ at 60 °C in toluene or $Sn(Oct)_2$ at 100 °C (Table 17).

Assuming that each hydroxyl group initiates the ring-opening polymerisation of ε-caprolactone (well-known as "living" character of the "coordination–insertion" mechanism), high efficiency of grafting is observed, including the control of the average length of every PCL branch (DP_{PCL}) and the average number of grafts per glucosidic unit (N_g) (Table 17).

The third step is the deprotection, i.e. splitting off the trimethylsilyl groups under mild conditions (THF, aqueous HCl). Both the silylated and the deprotected PCL-grafted dextran copolymers are semicrystalline with a melting temperature of 57 °C, attributed to the PCL grafts. The PCL-grafted dextran copolymers with low PCL content are soluble in water but insoluble in organic solvents such as toluene or THF. For high PCL content, the solubility in water is limited. The amphiphilic copolymers show a decreased surface tension compared to dextran. An aqueous solution of dextran T10 (M_n 6600 g mol^{-1}) has a surface tension in the range of water (73 mN m^{-1}). PCL-grafted dextran copolymer with a PCl weight fraction (F_{PCl}) of 0.20 and a DP_{PCL} of 6.6 displays a surface tension of 54 mN m^{-1} in aqueous solution (5 g L^{-1}).

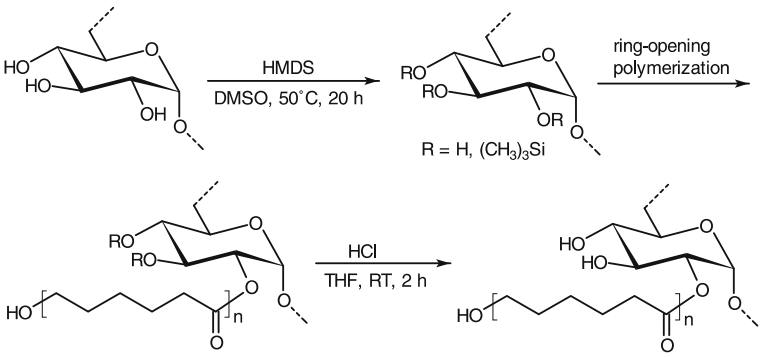


Fig. 63 Controlled synthesis of poly(ε-caprolactone) grafted onto dextran via partially silylated dextran

Table 17 Dependence of the weight fraction of poly(ε-caprolactone) (PCL) grafted on silyldextran on the catalysator and the initial weight fraction of ε-caprolactone by ROP in toluene

Silyldextran DS	M_n	[Cl]/[OH]	Catalyst [a]	f_{Cl} [b]	F_{PCl} [c]	AL_{PCL} [d]	M_n	M_w/M_n
2.8[e]	10 100	20	$Al(Et)_3$	0.64	0.41	13	34 700	1.4
2.8[e]	10 100	20	$Al(OiPr)_3$[g]	0.64	0.20	6.6	11 600	1.2
2.6[e]	12 600	20	$Al(OiPr)_3$	0.77	0.73	18	31 300	1.4
2.6[e]	12 600	30	$Al(OiPr)_3$	0.83	0.77	30	28 300	1.3
2.8[e]	10 100	20	$Sn(Oct)_2$	0.64	0.50	17	36 500	1.5
2.7[f]	32 900	5	$Sn(Oct)_2$	0.32	0.26	5	34 300	1.6
2.7[f]	32 900	10	$Sn(Oct)_2$	0.50	–	–	43 100	1.7

[a] Molar ratio of [catalyst]/[OH] = 0.05
[b] Initial weight fraction of ε-caprolactone
[c] Weight fraction of ε-caprolactone in the copolymers determined by gravimetry
[d] Average length of every PCL branch
[e] 60 °C, 68 h, dextran T10
[f] 100 °C, 44 h, dextran T40
[g] Molar ratio = 0.02

Well defined amphiphilic PCL-grafted dextran copolymers have been used to prepare nanoparticles by the nanoprecipitation technique in water. The copolymers were first dissolved in DMSO at 50 °C and then added drop-wise under vigorous stirring into a large volume excess of water thermostated at 50 °C. The suspension turned opalescent as a result of colloidal nanoparticle formation and was dialysed against distilled water to separate DMSO. The nanoparticle suspension was finally concentrated and is stable over a period of 3 weeks. The apparent mean diameter of the nanoparticles is close to 200 nm with a narrow size distribution. Such behaviour suggests a core-shell structure consisting of a hydrophobic PCL core surrounded by a hydrophilic dextran shell, which prevents rapid agglomeration [387, 388].

7 Outlook

Dextran is a unique polysaccharide because of its structure (only glucose units), purity, defined branching pattern depending on the microbial sources and defined molecular weight. Today, it is produced on a commercial scale resulting from optimised biotechnological processes for the biosynthesis of dextran using preferably *Leuconostoc mesenteroides*.

The commercial availability and the outstanding features of this important biopolymer make it a desired starting material for products in biological and medical applications where reproducible properties and biocompatibility are essential prerequisites. In addition, dextran is of growing interest for the construction of supramolecular architecture because of the flexibility of the $1 \rightarrow 6$ linked polymer backbone. The use of dextran and its derivatives is focused on the synthesis of advanced materials and bioactive compounds. An additional aspect of dextran is its conformity with the principles of green chemistry because it calls for the design of biodegradable end products, principally by employing starting materials from renewable resources. Together with other polysaccharides, dextran will become an important starting material; however, it requires detailed understanding of various aspects including:

- Biosynthesis to obtain dextran with controlled and reproducible molecular structure and molecular weight
- Controlled biosynthesis to design the material with controlled reactivity and in a special shape, e.g. as nanoparticles
- Solubility in simple, non-toxic media and understanding of activation prior to a modification reaction by methods that meet the principles of green chemistry
- Details of the reaction steps both by chemical and biochemical (enzyme-catalysed) conversions
- Functionalisation of the polymer backbone through reaction of the hydroxyl groups (esterification and etherification), nucleophilic displacement reactions, oxidation and selective reactions at the terminal reducing ends
- Modification of the biopolymer with naturally occurring molecules in order to obtain completely bio-based materials.

A huge variety of functionalisation reactions on dextran have been carried out over the last three decades. In comparison to other polysaccharides such as cellulose and starch, the esters of simple carboxylic acids and short chain alkyl ethers did not find significant interest. In contrast, the sulfuric acid halfesters of dextran (dextran sulfates) are among the most promising polysaccharide derivatives in the pharmaceutical field. A variety of highly functionalised dextran sulfates are commercial products. They exhibit pronounced anticoagulating, cancerostatic, antiviral and even antiprion activity. Nevertheless, the chemical structure of the polysaccharide derivative was not specifically modified for most of the investigations towards the bioactivity of dextran sulfates, i.e. it was not considered as a parameter of influence for their effectiveness. From the authors' point of view, this is one of the weaknesses of the majority of the studies in the field. The comprehensive analysis of the derivatives and a defined variation of structural features, such as the amount of sulfate groups and their distribution within the basic units and along the polymer chain, should be used for the establishment of the structure–

property relationship. The need for selective synthesis and analysis is even more pronounced in the case of mixed derivatives such as carboxymethylated dextran sulfates, which are investigated as heparin mimics. Only the exploitation of structure–property relationships can lead to products with tailored biological activity.

The search for new synthesis tools for the binding of different chemical functionalities onto dextran is stimulated by the growing number of poorly water soluble pharmaceuticals, which can be bound to the polymer to increase their bioavailability and to protect them from fast metabolism. There is still a need for efficient reagents that can realise the conjugation of the polymer with the bioactive molecule without pronounced side reactions and side products. For most of the modification methods, the detailed mechanisms and structures are poorly understood, as in case of the broadly used conjugation reactions with cyanogen halides. Esterification reactions of dextran after in situ activation of the corresponding carboxylic acids with efficient, non-toxic reagents such as CDI have demonstrated its usefulness. These approaches guarantee fast and complete conversion to structurally homogeneous derivatives giving only non-toxic and easily removable by-products. In addition to the binding of drugs, they are also applied to the introduction of photo cross-linkable substituents. The preparation of hydrogels and nanostructured materials is achieved. The fine-tuning of the chemical structure results in compounds with an adjusted hydrophilic–hydrophobic balance or with environmental sensitivity. Moreover, modified dextran is of growing interest in the field of surface modification. In this case, ionic substituents are introduced to yield self assembly via electrostatic interactions or specific binding on a surface, initiated by using substituents capable of recognition effects such as thiol moieties.

All in all, it is obvious that dextran will gain increasing importance as a carrier material in pharmaceutical applications, as a basis for bioactive derivatives and as a nanostructured device. Dextran and modified dextrans should always be considered as a biocompatible material with a high structure-forming potential.

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