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Studies on the molecular flexibility of novel dendronized carboxymethyl cellulose derivatives

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ABSTRACT

Water-soluble deoxy-azido cellulose derivatives were synthesized by heterogeneous carboxymethylation, applying 2-propanol/aqueous NaOH as slurry medium. The novel, carboxymethyl deoxy-azido cellulose provides a convenient starting material for the selective dendronization of cellulose via the copper-catalyzed Huisgen reaction yielding water-soluble carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) cellulose derivatives of first (degree of substitution, DS 0.51), second (DS 0.44) and third generation (DS 0.39). The novel biopolymer derivatives were characterized by FT-IR and NMR spectroscopy, intrinsic viscosity, sedimentation coefficient and weight average molar mass. Solution conformation and flexibility were estimated qualitatively using conformation zoning and quantitatively (persistence length) using the combined global method. Sedimentation conformation zoning showed a semi-flexible coil conformation and the global method applied to each carboxymethyl deoxy-azido cellulose and carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) cellulose derivative yielded persistence length all within the range of 2.8–4.0 nm with no evidence of any change in flexibility with dendronization.

1. Introduction

The synthesis of advanced and highly engineered functional biomaterials based on polysaccharides is of increasing interest due to the need to replace products and materials derived by the petrochemical industry. In this regard, cellulose as the most widely available biopolymer provides excellent prospects as a starting biopolymer due to its high structural uniformity, being a β-1 → 4-linked glucan and due to the high number of functional groups (three reactive OH moieties per repeating unit) [1–3]. A promising approach in this context is the addition of dendritic structures to the polymeric backbone. Thus, cellulose and cellulose derivatives decorated with dendrons of different functions in the periphery were synthesized and characterized [4–12]. Many possible application areas can be envisioned with these novel dendronized cellulose derivatives, e.g., in cosmetics, as biosensors, and for controlled drug release. In these application areas, aqueous systems are preferred; therefore, the dendronized cellulose derivatives should be soluble in water. An appropriate procedure is carboxymethylation, affording water-soluble products based on cellulose even with a rather low degree of substitution (DS) of 0.4 [13]. Carboxymethyl cellulose (CMC) has been well investigated in part due to being the most important commercial ionic cellulose ether [13]. Water-soluble dendronized cellulose was synthesized by the conversion of the carboxyl moieties of CMC with amino-triester dendrons (Behera's amine) by divergent polymer analogue synthesis [5,6]. The products obtained were characterized by thermogravimetric analysis and

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intrinsic viscosity. However, the decrease of intrinsic viscosity found after the attachment of the dendrons is attributed to the conversion of the sodium carboxylate moieties, resulting in a decrease of charge of the polyelectrolyte. Another reason for the decrease in intrinsic viscosity could be the strong decrease of the degree of polymerization due to the high number of reaction steps. Thus, the influence of the dendritic structures on the hydrodynamic behaviour of the cellulose derivatives has not been clarified. In addition, due to the divergent synthesis of the dendritic structures at the polymeric backbone, no complete conversion can be ensured and thus, the number of imperfections increases resulting in uncontrolled properties [14].

Our studies are focused on alternative paths including nucleophilic displacement (S_N)- and 1,3-dipolar cycloaddition reactions that afford novel products, which are not accessible via the most commonly used reactions, namely esterification and etherification [15,16]. In this regard, 6-deoxy-6-azido cellulose, obtained via a S_N reaction starting from *p*-toluenesulfonic acid esters of cellulose (tosyl cellulose), was modified with polyamidoamine (PAMAM) dendrons possessing an ethynyl focal moiety via the copper-catalyzed Huisgen reaction (1,3-diploar cycloaddition) both under homogeneous and heterogeneous conditions [9,10]. Furthermore, the introduction of different functional groups, e.g., azide and carboxymethyl moieties in the cellulose backbone should allow a simple method to design water-soluble dendronized cellulose derivatives.

In the present study, water-soluble carboxymethylated deoxy-azido cellulose derivatives were selectively functionalized with propargyl-polyamidoamine dendrons via the copper-catalyzed Huisgen reaction. The novel water-soluble dendronized cellulose derivatives were characterized in detail by FT-IR and NMR spectroscopy. Information on the influence of dendronization of cellulose on the hydrodynamic behaviour and the overall conformation was obtained by sedimentation velocity in the analytical ultracentrifuge, size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS) and intrinsic viscosity measurements.

2. Experimental part

2.1. Materials

Cellulose (Modo 500, Modo Paper Domsjö, Sweden, degree of polymerization, DP 500) was dried under vacuum at 110 °C for 3 h before use. *p*-Toluenesulfonyl chloride was purchased from Merck. Dimethyl sulfoxide (DMSO), LiCl, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, sodium hydroxide, ethylenediamine, methylacrylate, 2-propanol and propargyl amine were Acros products. *N,N*-Dimethyl acetamide (DMA), sodium chloroacetate and sodium azide were obtained from Alfa Aesar. All reagents were used without further purification.

2.2. Measurements

FT-IR spectra were recorded on a Nicolet Avatar 370 spectrometer using the KBr-technique. The ^1H and ^{13}C NMR spectra were acquired on a Bruker AVANCE 400 spectrometer in DMSO-d_6 at 60 °C. For ^1H NMR spectra 16 scans and for ^{13}C NMR spectra up to 65,000 scans were accumulated. Elemental analysis (EA) was performed with a Vario EL III from Elementar Analysensysteme, Hanau, Germany.

2.3. Size exclusion chromatography coupled to multi-angle laser light scattering and a differential pressure viscometer (SEC-MALLS-DPV)

Analytical fractionation was carried out using a series of SEC columns TSK G6000PW, TSK G5000PW and TSK G4000PW protected by a similarly packed guard column (Tosoh Bioscience, Tokyo, Japan) with online MALLS (Dawn-Heleos-II), differential pressure viscometer (Viscostar-II) and refractive index (Optilab rEX) detectors (all instruments from Wyatt Technology, Santa Barbara, USA). The eluant (phosphate-buffered saline 0.1 M, pH 6.8 at 30 °C) was pumped at 0.80 ml/min (PU-1580, Jasco Corporation, Great Dunmow, UK) and the injected volume was 100 µl for each sample. Absolute molar masses were calculated for each sample using a Debye first-order model (where the refractive index increment, $dn/dc = 0.150$ ml/g) incorporated into the ASTRA® (Version 5.3.2.17) software (Wyatt Technology, Santa Barbara, USA).

2.4. Sedimentation velocity in analytical ultracentrifuge

Sedimentation velocity experiments were performed using the Beckman Optima XLI Analytical Ultracentrifuge (Palo Alto, USA). Sample solutions (390 µl) of different concentrations (0.1–3.0 mg/ml) and phosphate-buffered saline 0.1 M, pH 6.8 (400 µl) were injected into the solution and reference channels, respectively, of a double sector 20 mm optical path length cell. Samples were centrifuged at 45,000 rpm at a temperature of 20.0 °C. Concentration profiles and the movement of the sedimenting boundary in the analytical ultracentrifuge cell were recorded using the Rayleigh interference optical system and converted to concentration (in units of fringe displacement relative to the meniscus, j) versus radial position, r [17]. The data was then analyzed using the $\text{ls} - g^*(s)$ model incorporated into the SEDFIT program [18]. This software, based on the numerical solutions to the Lamm equation, follows the changes in the concentration profiles with radial position and time and generates an apparent distribution of sedimentation coefficients in the form of $g^*(s)$ versus $s_{T,b}$, where the * indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects [17].

As sedimentation coefficients are temperature- and solvent-dependent, it is conventional to convert sedimentation coefficients (or their distributions) to the standard conditions of 20.0 °C and water using the following equation [19].

$$S_{20,w} = s_{T,b}\left[\frac{(1-\bar{v}\rho_{20,w})\eta_{T,b}}{(1-\bar{v}\rho_{T,b})\eta_{20,w}}\right] \tag{1}$$

where $\bar{v}$ is the partial specific volume of the cellulose derivative [20] (0.715 ml/g) and $\eta_{T,b}$ and $\rho_{T,b}$ are the viscosity and density of the experimental solvent (phosphate-buffered saline 0.1 M, pH 6.8) at the experimental temperature (20.0 °C) and $\eta_{20,w}$ and $\rho_{20,w}$ are the viscosity and density of water at 20.0 °C.

To account for hydrodynamic non-ideality (co-exclusion and backflow effects), the apparent sedimentation coefficients ($s_{20,w}$) were calculated at each concentration and extrapolated to infinite dilution using the following equation [19,21,22]

$$\frac{1}{s_{20,w}} = \frac{1}{s^{0}_{20,w}}(1 + k_s c) \tag{2}$$

where k_s (ml/g) is the sedimentation concentration dependence or "Gralén" coefficient [21].

2.5. Methods

The *p*-toluenesulfonic acid esters of cellulose (tosyl cellulose, degree of substitution, DS 0.58, 0.83, and 1.04) were synthesized homogenously in DMA/LiCl according to Ref. [23]. 6-Deoxy-6-azido cellulose samples (DS 0.58, 0.81, and 1.01) were obtained by a nucleophilic displacement reaction as described in Ref. [15]. The preparation of the propargyl-polyamidoamine (PAMAM) dendrons of first to third generation was carried out as described in Ref. [24].

2.6. Carboxymethyl deoxy-azido cellulose: general procedure

9.25 g of NaOH (231 mmol) in 42 ml of water were added to a suspension of 7 g of a deoxy-azido cellulose (**2b**, DS_{Azide} 0.81, 38 mmol) in 140 ml of 2-propanol. After stirring for 1 h at ambient temperature, 27.2 g of sodium chloroacetate (233 mmol) was added and the mixture was allowed to react for 5 h at 60 °C. Subsequently, sodium chloroacetate (10 g, 85.5 mmol) was added and stirring was continued for 1 h. After cooling to room temperature, the polymer was collected by filtration and washed with 600 ml of ethanol, twice with an ethanol/water mixture (8:2, 400 ml) and again with ethanol (2× 300 ml). Purification was carried out by reprecipitation of carboxymethyl deoxy-azido cellulose from water (200 ml) into ethanol (600 ml), washing twice with an ethanol/water mixture (8:2, 400 ml) and subsequently twice with ethanol (250 ml). Drying in vacuum at 45 °C afforded product **3b**.

DS_{Azide} 0.81 (calculated from N content determined by elemental analysis, EA).

DS_{CM} 1.25 (analyzed by conductometric titration).

FT-IR (KBr): ν (cm^{-1}) = 3432 (OH), 2921 (CH_2), 2113 (azide), 1602 (COO^-Na^+), 1065 (C—O—C).

^{13}C NMR (D_2O): δ (ppm) = 179.2 to 177.4 (C=O, C-8, 10 and 12), weak 131.0 to 128.3 (C_{aryl}, toluenesulfonyl, tosyl), 103.6–102.7 (C-1 and 1′, anhydroglucose unit, AGU), 84.0 (C-2s, AGU), 82.8 (C-3s, AGU), 81.3 to 69.4 (C-2–C-5, AGU), 71.8 to 71.0 (CH_2, C-7, 9 and 11) 70.3 (C-6_{CM}, AGU), weak 66.0 (C-6_{Tosyl}, AGU), 60.7 (C-6_{OH}, AGU), 51.1 (C-6_{Azide}, AGU).

^{1}H NMR (D_2O): δ (ppm) = weak 8.27 to 7.94 (H_{aryl}, tosyl), 5.30 to 3.42 (H, AGU), 4.63 to 4.30 (H-7, 9 and 11), weak 2.45 (CH_3, tosyl).

2.7. Carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-polyamidoamine) cellulose of first generation

To a solution of a carboxymethyl 6-deoxy-6-azido cellulose (**3b**, DS_{Azide} 0.81, DS_{CM} 1.25, 1 g, 3.72 mmol) in 100 ml water, $CuSO_4$ pentahydrate (0.2 g, 0.8 mmol), sodium ascorbate (0.4 g, 2 mmol) and first generation of propargyl-polyamidoamine dendron (**4**, 2.54 g, 11.2 mmol) were added. The mixture was stirred at ambient temperature for 24 h and 5 g of sodium diethyldithiocarbamate trihydrate was added in order to remove the copper catalyst. Isolation took place by precipitation in 700 ml of acetone, washing three times with acetone (300 ml) and drying in vacuum at 45 °C (**5**).

DS_{Dend} 0.51 (calculated from N content determined by EA).

FT-IR (KBr): ν (cm^{-1}) = 3342 (OH), 2924 (CH_2), 2114 (azide), 1717 (C=O, methyl ester), 1598 (COO^-Na^+), 1050 (C—O—C).

^{13}C NMR (D_2O): δ (ppm) = 175.6 to 173.3 (C=O, C-15, 17, 19 and 12), 124.4 (C-7, triazole), 102.4 to 100.7 (C-1 and 1′, AGU), 83.0 to 69.4 (C-2–C-5, AGU), 71.8 to 71.4 (CH_2, C-14, 16 and 18), 70.3 (C-6_{CM}, AGU), weak 66.1 (C-6_{Tosyl}, AGU), 60.3 (C-6_{OH}, AGU), 51.0 (C-6_{Azide} and C-6_{Dend}, AGU), 48.7 (C-13, methyl ester), 46.9 (C-10), 44.9 (C-9), 29.2 (C-11).

^{1}H NMR (D_2O): δ (ppm) = weak 8.78 (H-7, triazole), 5.28 to 3.62 (H, AGU), 4.71 to 4.31 (H-14, 16 and 18), 3.61 (CH_3), 3.98 to 2.92 (CH_2, dendron).

2.8. Carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-polyamidoamine) cellulose of second generation

Carboxymethyl 6-deoxy-6-azido cellulose (**3b**, DS_{Azide} 0.81, DS_{CM} 1.25, 1 g, 3.72 mmol) was dissolved in 100 ml of water and allowed to react with 4.75 g (7.4 mmol) of second generation of propargyl-PAMAM dendron (6) in the presence of $CuSO_4$ pentahydrate (0.2 g, 0.8 mmol) and sodium ascorbate (0.4 g, 2 mmol). After stirring the mixture for 48 h at ambient temperature, 5 g of sodium diethyldithiocarbamate trihydrate was added for complete removal of the catalyst and the polymer was precipitated in 600 ml of acetone and collected by filtration. The polymer was then washed four times with acetone (250 ml), dialyzed against water for 24 h and dried in a vacuum oven at 45 °C to yield product **7**.

DS_{Dend} 0.44 (calculated from N content determined by EA).

FT-IR (KBr): ν (cm^{-1}) = 3324 (OH), 3081 (NH, shoulder), 2954 (CH_2), 2 109 (azide), 1728 (C=O, methyl ester), 1651 to 1535 (C=O amide and COO^-Na^+), 1050 (C—O—C).

^{13}C NMR (D_2O): δ (ppm) = 179.9 to 178.6 (C=O, C-20, 22 and 24), 173.7 (C=O, C-12 and 17), 143.3 (C-8, triazole), 127.2 (C-7, triazole), 103.1 to 102.5 (C-1 and 1′, AGU), 82.3 to 71.9 (C-2–C-5, AGU), 71.9 to 71.4 (CH_2, C-19, 21 and 23), 62.9 (C-6_{OH}, AGU), 53.2 to 53.6 (C-6_{Azide} and C-6_{Dend}, AGU), 52.0 to 32.0 (CH_2, C-14–C-16, dendron), 51.1 (C-18, methyl ester).

^{1}H NMR (D_2O): δ (ppm) = weak 8.83 (H-7, triazole), 8.52 (NH, amide), 5.31 to 3.61 (H, AGU), 4.70 to 4.28 (H-19, 21 and 23), 3.61 (CH_3), 3.99 to 2.45 (CH_2, dendron).

2.9. Carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-polyamidoamine) cellulose of third generation

Carboxymethyl 6-deoxy-6-azidocellulose (**3b**, DS_{Azide} 0.81, DS_{CM} 1.25, 1 g, 3.72 mmol) dissolved in 150 ml water was converted with 9.72 g (7.44 mmol) of third generation of propargyl-PAMAM dendron (**8**) in the presence of $CuSO_4$ pentahydrate (0.2 g, 0.8 mmol) and sodium ascorbate (0.4 g, 2 mmol) and allowed to react at 25 °C for 72 h. After the removal of the copper catalyst by addition of 5 g of sodium diethyldithiocarbamate trihydrate the polymer was isolated by precipitation into 700 ml of acetone, filtration and washing four times with 250 ml of acetone. Purification of the polymer was carried out by dialyzing against water for 48 h and drying under vacuum at 45 °C (**9**).

DS_{Dend} 0.39 (calculated from N content determined by EA).

FT-IR (KBr): ν (cm^{-1}) = 3384 (OH), 3082 (NH, shoulder), 2960 to 2852 (CH_2 and CH_3), 2114 (azide), 1734 (C=O, methyl ester), 1644 to 1541 (C=O amide and COO^-Na^+), 1058 (C—O—C).

^{13}C NMR (D_2O): δ (ppm) = 178.3 to 177.9 (C=O, C-25, 27 and 29), 175.1 to 174.9 (C=O, C-12, 17 and 22), 143.3 (C-8, triazole), 127.2 (C-7, triazole), 103.1 to 102.7 (C-1 and 1′, AGU), 83.7 to 71.7 (C-2–C-5, AGU), 72.6 to 71.7 (CH_2, C-24, 26 and 28), the carbon atom of C-6_{OH}, is not visible, weak 52.7 (C-6_{Azide} and C-6_{Dend}, AGU), 52.1 to 30.7 (CH_2, C-14–C-21, dendron), 51.4 (C-23, methyl ester).

^1H NMR (D_2O): δ (ppm) = weak 8.44 (NH, amide), 5.15 to 3.61 (H, AGU), 4.75 to 4.57 (H-24, 26 and 28), 3.61 (CH_3), 4.08 to 2.88 (CH_2, dendron).

3. Results and Discussion

Deoxy-azido cellulose is soluble in polar aprotic solvents, e.g., in *N,N*-dimethyl formamide, *N,N*-dimethyl acetamide and dimethyl sulfoxide, but it is completely insoluble in water. It is very well known that carboxymethylation of cellulose yields water-soluble carboxymethyl ethers [13]. Thus, it seems to be appropriate that water solubility of deoxy-azido cellulose by carboxymethylation of the remaining hydroxyl groups may be reached that was experimentally realized for the first time in this work (Scheme 1).

3.1. Carboxymethyl deoxy-azido cellulose

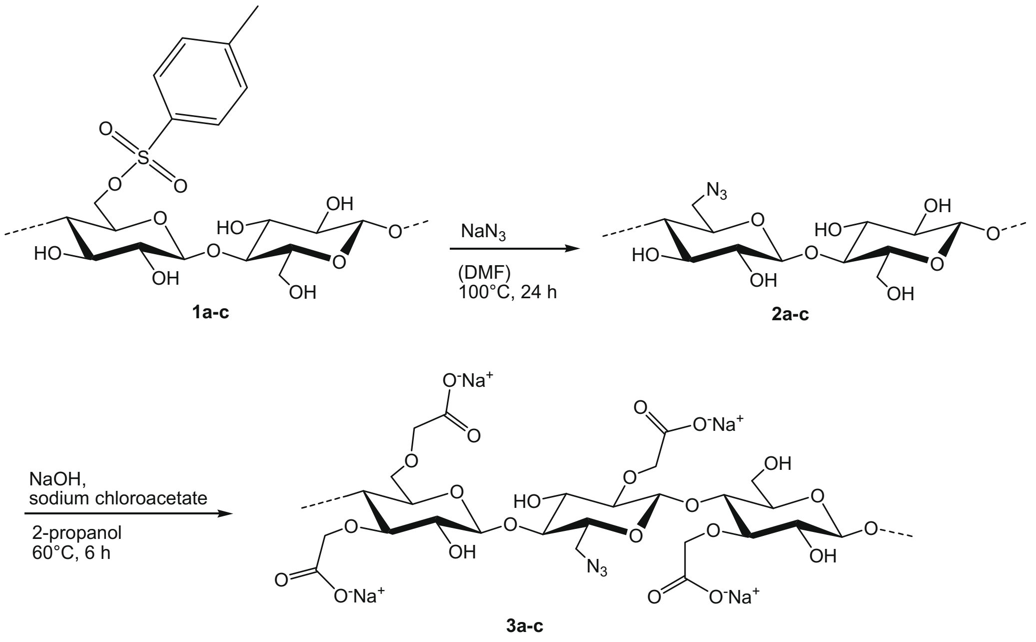
Prior to etherification, the preparation of deoxy-azido celluloses (**2a**–**c**) with degree of substitution (DS) in the range from 0.58 to 1.01 was carried out by nucleophilic displacement (S_N) reaction of *p*-toluenesulfonic acid esters of cellulose (tosyl cellulose) with azide ions under usual conditions. By adjusting the DS of tosyl moieties lower than 1, the tosylation reaction occurred mainly at position 6 [23] and thus, regioselective deoxy-azido cellulose derivatives can be obtained. Subsequently, the deoxy-azido cellulose samples were activated with 22 wt% aqueous sodium hydroxide and allowed to react with 8.4 mol sodium monochloroacetate per mol anhydroglucose unit (AGU) under heterogeneous conditions applying 2-propanol as slurry medium (Williamson etherification, Scheme 1).

A decrease of both the concentration of NaOH and the molar ratio of sodium monochloroacetate to AGU yielded water insoluble products. The determination of the DS of carboxymethyl moieties introduced was carried out by conductometric acid–base titration [25]. It can be seen in Table 1 that the DS values of carboxymethyl moieties increase with decreasing DS of azide groups. It is known that by carboxymethylation, the OH group at position 3 possesses the lowest reactivity. Furthermore, by increasing the concentration of NaOH higher than 20 wt%, the reactivity of O-6 is higher than that of O-2 [26]. Thus, the more OH at O-6 is accessible (decrease of DS_{Azide}) the more carboxymethyl moieties can be attached. The highest DS of carboxymethyl moieties of 1.35 was achieved starting from deoxy-azido cellulose with a DS_{Azide} of 0.58 (**3a**, total DS 1.92). It should be pointed out that under comparable reaction conditions, the total DS (DS of the carboxymethyl- and deoxy-azido moieties) is in the range of 2 (Table 1). Carboxymethylation of a deoxy-azido cellulose with a DS_{Azide} of 0.81 yielded a water-soluble product with a DS_{CM} of 1.25 (sample **3b**, total DS 2.08). Moreover, the DS of carboxymethyl moieties of 1.01 was obtained by carboxymethylation of deoxy-azido cellulose with a DS value of 1.01 (**3c**, total DS 2.02).

The determination of the structure of the water-soluble carboxymethyl deoxy-azido cellulose derivatives (**3a**–**c**) was carried out by FT-IR and NMR spectroscopy. The carbonyl signal of the carboxymethyl group (sodium salt) can be observed at 1602 cm^{-1}. The increasing in the signal at 2921 cm^{-1} is due to the increasing number of CH_2 moieties resulting from the carboxymethyl moieties. The azide moiety gives a signal at 2113 cm^{-1} in the FT-IR spectrum. A typical, well-resolved ^{13}C NMR spectrum of carboxymethylated deoxy-azido cellulose **3b** recorded in D_2O at 60 °C is shown in Fig. 1. The peaks for the carbonyl moieties of the sodium carboxylate (C-8, 10, and 12) are visible between 179.2 to 177.4 ppm. The weak peaks at 131.0 and 128.3 ppm indicate the presence of a small number of remaining tosyl moieties due to incomplete conversion with sodium azide by S_N reaction. The signal of the carbon atom of C-1 of the AGU is split to 103.6 and 102.7 ppm due to carboxymethylation at position 2. Besides the resonances of the carbon atoms 2–5 of the AGU (84.0 to 69.3 ppm), signals for the CH_2 moieties of the carboxymethyl groups introduced can be observed in the range of 71.8 to 71.0 ppm (C-7, 9, and 11). The peak of the carbon atom of the unmodified C-6 is visible at 60.7 ppm. Furthermore, the modified carbon atoms at position 6 bearing either a carboxymethyl or deoxy-azide moiety are attributed to the resonances at 70.2 (C-6_{CM}) and 51.0 ppm (C-6_{Azide}). Additionally, the weak peaks at 66.0, 131.0 and 128.3 ppm indicate the presence of a small number of remaining tosyl moieties due to incomplete conversion with sodium azide by S_N reaction.

3.2. Carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-polyamidoamine) celluloses

Homogeneous synthesis of water-soluble dendronized cellulose derivatives of different generations bound via 1,4-disubstituted 1,2,3-triazole linker was investigated.



Scheme 1. Etherification of deoxy-azido celluloses (**2a–c**), obtained by nucleophilic displacement reaction from toluenesulfonyl cellulose ester (**1a–c**), with sodium monochloroacetate in the presence of aqueous NaOH.

Table 1
Degree of substitution (DS) of carboxymethyl moieties (determined by conductometric titration) of carboxymethyl deoxy-azido cellulose derivatives.

Carboxymethyl deoxy-azido cellulose	DS_{Azide}	DS_{CM}	Total DS
3a	0.58	1.35	1.93
3b	0.81	1.25	2.06
3c	1.01	1.01	2.02

The first to third generation of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) (PAMAM) cellulose (**5**, **7** and **9**) were obtained by the conversion of carboxymethyl 6-deoxy-6-azido cellulose (**3b**) with the propargyl-PAMAM dendron of first to third generation (**4**, **6** and **8**) in water in the presence of $CuSO_4$ pentahydrate and sodium ascorbate. Scheme 2 shows the general reaction pathway exemplified by the synthesis of the first

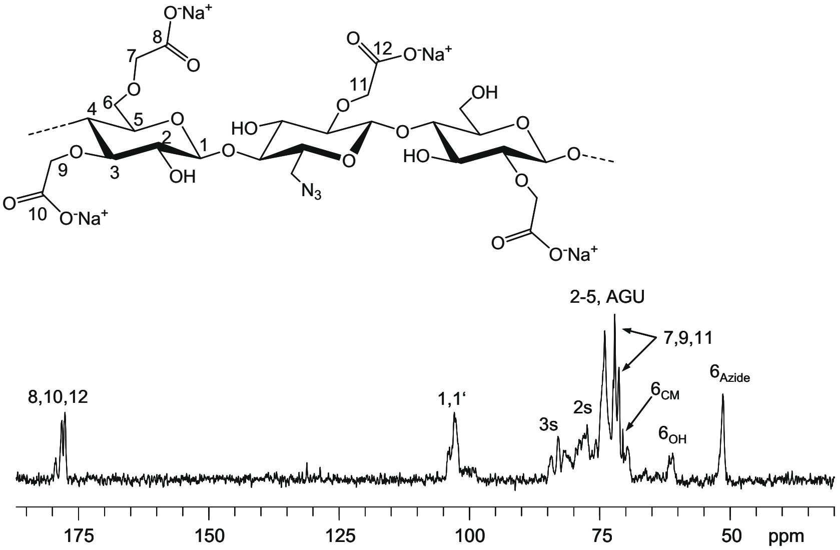


Fig. 1. ^{13}C NMR spectrum of carboxymethyl deoxy-azido cellulose (**3b**, DS_{Azide} 0.81, DS_{CM} 1.25), recorded in D_2O at 60 °C.

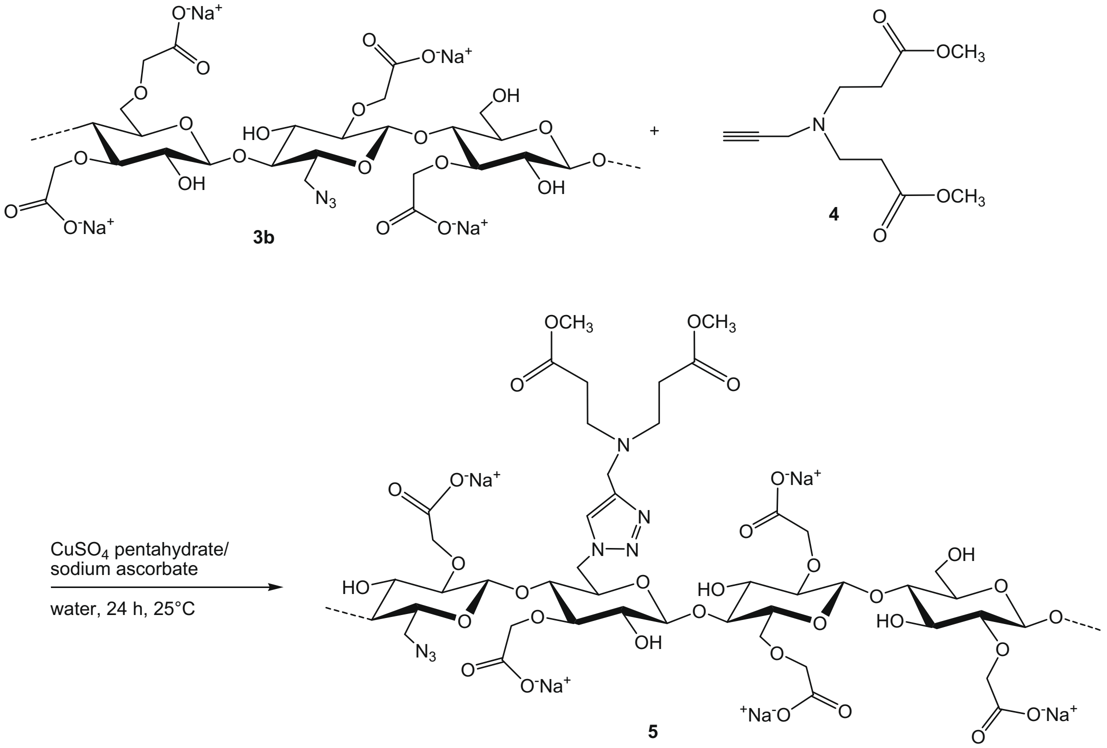
generation carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose. The stirred reaction mixtures were maintained at ambient temperature for 24 h for dendron (**4**), whilst a prolongation of time to 48 h for the larger (**6**) and to 72 h for the largest dendron (**8**) was necessary. The products obtained after purification are soluble in water and possess DS values of 0.51 (first generation, **5**), 0.44 (second generation, **7**) and 0.39 (third generation, **9**). The decrease of the DS value with increasing dendritic generation and thus, increasing bulkiness and steric hindrance despite the prolongation of the reaction time, agrees with previous results of dendronization of cellulose [9,11].

It is known that decomposition of PAMAM dendrimers will occur after several months upon standing due to the presences of different copper species [27,28]. In order to avoid fragmentation of the PAMAM-system, the removal of the Cu(II) catalyst is required [27,28]. Therefore, sodium diethyldithiocarbamate trihydrate was applied, which is a very efficient complexing agent. The removal of the copper-based catalyst was proven by ICP-OES analyses (Perkin-Elmer, Optima 2000 DV) possessing a detection limit concerning copper of 5 ppm. Only traces of 12 ppm for the first (**5**), 14 ppm for the second (**7**) and 10 ppm for the third generation (**9**) of remaining copper were found in the purified products.

The structure of the different dendronized cellulose derivatives (**5**, **7** and **9**) was confirmed by FT-IR (not shown) and ^{13}C NMR spectroscopy. Due to structural problems known from their dendrimer counterparts [27,28], it is necessary to discuss the structural elucidation of the different dendronized cellulose derivatives in detail.

3.2.1. First generation carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-PAMAM) cellulose

Characteristic bands of the dendritic structure appear in the FT-IR spectrum at 1717 cm^{-1} for the carbonyl moiety of the methyl ester. The methyl group of the peripheric ester is visible at 2924 cm^{-1}. Fig. 2 shows the ^{13}C NMR spectrum of the first generation of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose acquired in D_2O at 60 °C. The characteristic signals for the carbon atoms 1–5 of the AGU are visible between 102.4 and 69.4 ppm. The resonance for the peripheric methyl moiety (C-13) occurs at 48.7 ppm and for the carbon atom of the corresponding carbonyl function at 173.3 ppm (C-12). The carbon atom of the 1,4-disubstituted 1,2,3-triazole linker (C-7) gives a signal at 124.4 ppm. Furthermore, the peaks at 70.3, 60.3 and 51.0 ppm are attributed to the different types of C-6 bearing either carboxymethyl, hydroxyl, azide, or triazole moieties. The resonances of the CH_2 moieties are assigned at 46.9, 44.9 and 29.2 ppm. It should be pointed out that the conversion carried out in aqueous media at a pH value of 8.5 (pH results from the carboxylate sodium salt) resulted in some saponification of methyl ester indicated by small signals at 30.9 (C-11′) and 46.7 (C-10′ overlapped with C-10).



Scheme 2. Homogeneous conversion of carboxymethyl 6-deoxy-6-azidocellulose (**3b**, DS_{Azide} 0.81, DS_{CM} 1.25) with first generation of propargyl-polyamidoamine dendron (**4**) via the copper-catalyzed Huisgen reaction.

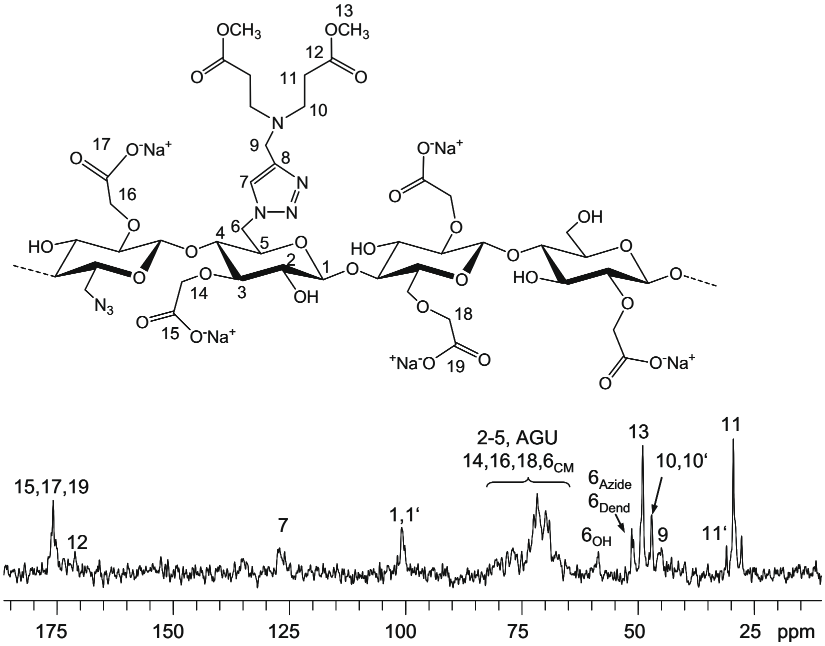


Fig. 2. ^{13}C NMR spectrum of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose of first generation (**5**, DS_{Dend} 0.51) recorded in D_2O at 60 °C.

3.2.2. Second generation carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-PAMAM) cellulose

The characteristic signals in the FT-IR spectrum appear at 3081 cm^{-1} (NH of the amide), at 2954 to 2861 cm^{-1} (CH_2 and CH_3) and at 1728 and 1651 cm^{-1} of the carbonyl functions of both the methyl ester and the amide for propargyl-PAMAM dendron. A weak signal at 2109 cm^{-1} reveals the existence of remaining azide moieties. The ^{13}C NMR spectrum of the second generation carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose (**7**) recorded at 60 °C in D_2O (Fig. 3) shows the characteristic signals of the AGU carbon atoms 1–5 are visible between 103.1 and 71.8 ppm. The carbon atoms of the 1,4-disubstituted 1,2,3-triazole linker formed give signals at 143.3 and 127.2 ppm. The peak at 175.7 ppm is attributed to the overlapping resonances of the carbonyl functions of the amide moieties and the peripheric methyl ester groups of the dendron. The corresponding methyl moiety of the ester can be observed at 51.1 ppm. The peaks for the modified position 6 (C-6_{Azide} and C-6_{Dend}) are found at 53.2 and 53.6 ppm and for the unmodified C-6 at 62.9 ppm. Furthermore, the resonances of the CH_2 moieties of the dendron are assigned to the signals between 52.0 and 32.0 ppm. Peaks due to saponified methyl ester groups are visible in the ^{13}C NMR spectrum at 36.6 (C-16′) and at 47.2 ppm (C-15′).

3.2.3. Third generation carboxymethyl 6-deoxy-(1-N-[1,2,3-triazolo]-4-PAMAM) cellulose

In the FT-IR spectrum, new characteristic bands appear due to the introduced dendron at 3082 cm^{-1} (NH, amide) and at 1734 and 1644 cm^{-1} for the carbonyl moieties. Furthermore, signals the methyl groups of the peripheric ester are visible at 2960 cm^{-1}. The intensity of the signals of the carbon atoms of the repeating unit C-2–C-5 and the triazole linker decreases dramatically due to the large number of branches and the corresponding carbon atoms. In the case of the third generation, the carbon atoms of the AGU could be observed as small and broad "signals" between 83.7 and 71.7 ppm. Nevertheless, the ^{13}C NMR proves the structure of the third generation of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose (Fig. 4). Resonances appear at 175.1 to 174.9 ppm that are assigned to the carbonyl moieties of both the methyl ester and the amide. The signal at 51.4 ppm is attributed to the C atoms of the peripheric methyl moieties. Furthermore, the carbon atoms of the 1,4-disubstituted 1,2,3-triazole linker give peaks at 143.3 and 127.2 ppm. The modified carbon atom of position C-6 (C-6_{Azide} and C-6_{Dend}) of the AGU can be observed as a weak signal at 52.7 ppm. The resonances between 52.1 and 30.7 ppm are due to the CH_2 moieties of the introduced dendron. In addition, peaks resulting from saponification due to basic reaction medium can be observed at 39.2 (C-21′) and 49.7 ppm (C-20′).

To get information on the influence of the dendritic structures introduced on the chain stiffness of the polymers and on the solution behaviour (conformation in solution), size exclusion chromatography coupled to multi-angle laser light scattering and differential pressure viscometer (SEC-MALLS-DPV) and analytical ultracentrifugation were applied.

3.3. Size exclusion chromatography coupled to multi-angle laser light scattering and a differential pressure viscometer (SEC-MALLS-DPV)

Weight average molecular weight, M_w and intrinsic viscosity, $[\eta]$ are summarized in Table 2. It is known that at

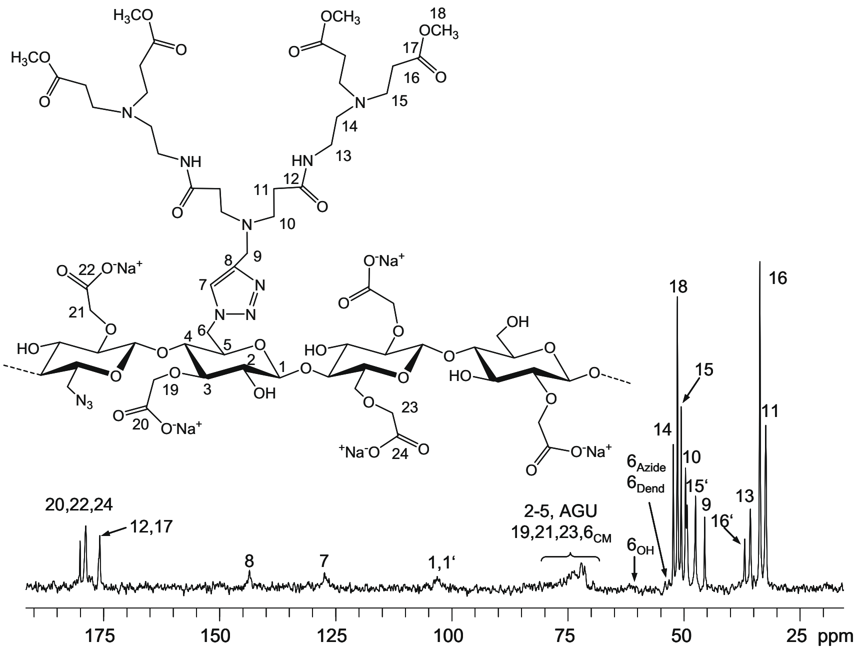


Fig. 3. ^{13}C NMR spectrum of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose of second generation (**7**, DS_{Dend} 0.44) recorded in D_2O at 60 °C.

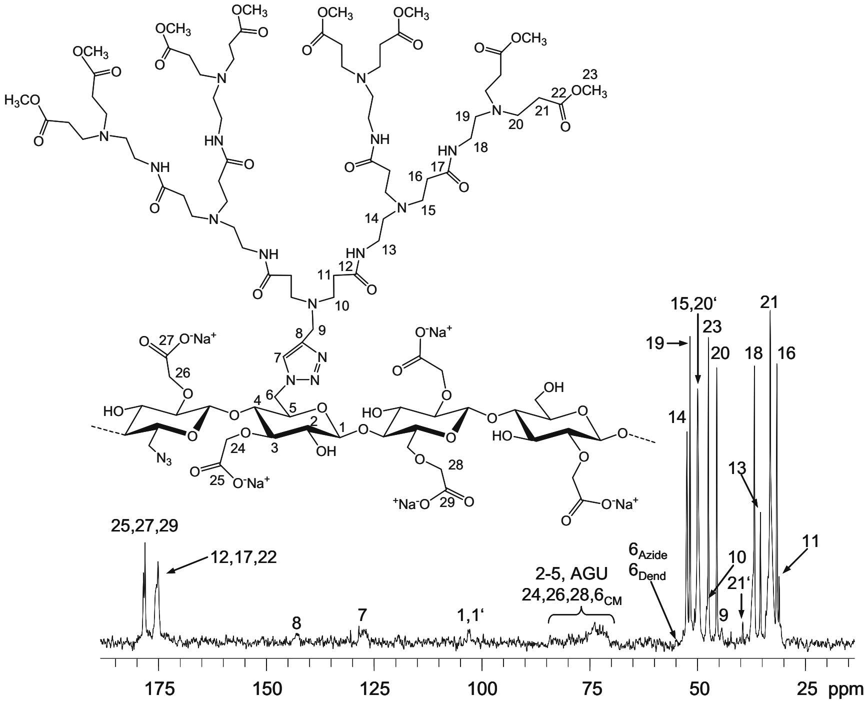


Fig. 4. ^{13}C NMR spectrum of carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose of third generation (**9**, DS_{Dend} 0.39) recorded in D_2O at 60 °C.

low polyelectrolyte concentrations the intrinsic viscosity is increased due to polyelectrolyte effect [29,30]. However, under the influence of salt ions or at high polyelectrolyte concentration, coil expansion due to the polyelectrolyte effect is reduced, since, on the one hand, the charges are screened and, on the other hand, there is a reduction in the degree of dissociation of the carboxylate group. Thus, in order to get direct information on the influence of the introduced dendritic structures on the conformation in solution of the polymers and on the chain stiffness, a 0.1 mol buffered saline was used as solvent.

It is interesting that dendronization leads to polymers of decreased intrinsic viscosity, which was not expected. Due to the increase of bulkiness with increasing generation an increase of the intrinsic viscosity was assumed, according to results presented in literature [14]. In the carboxymethyl deoxy-azido (**3a–c**) series, an increase of M_w from 72,000 g/mol for **3a** (DS_{Azide} 0.58, DS_{CM} 1.35) to 105,000 g/mol for **3c** (DS_{Azide} 1.01, DS_{CM} 1.01) is reflected by an increase in intrinsic viscosity indicating similar conformations. However, for the dendronized carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose series (first to third generation), no such correlation can be seen. The intrinsic viscosity is essentially independent of M_w, possibly indicating a compact structure.

3.4. Sedimentation velocity in analytical ultracentrifuge

Sedimentation coefficients, ($s^0_{20,w}$) and their concentration dependencies (k_s) are shown in Table 2. Due to the low concentration of sedimenting materials, it was impossible to estimate k_s for samples **3a**, 3c and **9;** however, for the purposes of further analyses it was assumed that **3a** and **3c** have the same k_s as **3b** (195 ± 15 ml/g) and that **9** has an average value (185 ± 45 ml/g).

The trend in molecular weight dependence of sedimentation coefficient is the opposite of that observed for intrinsic viscosity. In carboxymethyl deoxy-azido cellulose series (**3a–c**), there is little or no molecular weight dependence, whilst for the carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose series (**5**, 7 and **9**), the sedimentation coefficient increases with molecular weight.

3.5. Conformational analysis

Knowledge of molecular weight, sedimentation coefficient (and its concentration dependence) and intrinsic viscosity allows the estimation of gross solution conformation using various different methods including:

(1) The translational frictional ratio, *f/f₀* [31],
(2) Combined Analysis method (HYDFIT) [32],
(3) Sedimentation conformation zoning [33].

Table 2
Hydrodynamic data for carboxymethyl deoxy-azido cellulose derivatives (**3a–c**) and dendronized carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) celluloses (**5**, **7** and **9**).

Sample	$s^0_{20,w}$ (S)	k_s (ml/g)	$[\eta]$ (ml/g)	M_w (g/mol)
3a	1.95 ± 0.10	(195 ± 15)[a]	98 ± 10	72,000 ± 7000
3b	1.96 ± 0.07	195 ± 15	118 ± 3	79,000 ± 15,000
3c	1.85 ± 0.10	(195 ± 15)	159 ± 3	105,000 ± 4000
5	2.05 ± 0.07	155 ± 35	33 ± 1	60,000 ± 3000
7	2.73 ± 0.07	200 ± 65	41 ± 2	172,000 ± 5000
9	2.93 ± 0.10	(185 ± 45)	34 ± 2	235,000 ± 12,000

[a] Results in brackets are estimated.

3.6. The translational frictional ratio, f/f₀

The translational frictional ratio [31] *f/f₀* is a parameter that depends on molecular weight, conformation and molecular expansion through hydration effects. It can be measured experimentally from the sedimentation coefficient and molecular weight:

$$\frac{f}{f_0} = \frac{M_w(1-\overline{v}\rho_{20,w})}{(N_A 6\pi\eta_{20,w}s^0_{20,w})}\left(\frac{4\pi n_A}{3\overline{v}M_w}\right)^{1/3} \tag{3}$$

where the partial specific volume for a cellulose derivative is $\overline{v} = 0.715$ ml/g.

The frictional ratio (Table 3) varies from 3 to 5 indicating either a random coil or semi-flexible coil conformation for each of the six polymers. It is likely that the differences in frictional ratios are due to differences in molecular weight and not conformation as in general the higher frictional ratios correlate with higher molecular weights.

3.7. Combined analysis method (HYDFIT)

The linear flexibility of polymer chains is represented quantitatively in terms of the persistence length, L_p of equivalent *worm-like chains* [34]. The persistence length is defined as the average projection length along the initial direction of the polymer chain. In theory for a perfect random coil $L_p = 0$, and for the equivalent extra-rigid rod $L_p = \infty$, although in practice limits of ~1 nm (e.g., pullulan) and 200 nm (e.g., DNA) are more appropriate [35]. Chain persistence lengths, L_p can be estimated using several different approaches using either intrinsic viscosity [36–39] or sedimentation coefficient [40,41] measurements. For example the Bohdanecky relation [37]:

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3}\left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2} \tag{4}$$

where Φ is the Flory–Fox constant (2.86×10^{23} mol^{-1}) and A_0 and B_0 are tabulated coefficients [37], and the Yamakawa–Fujii equation [40]:

$$s^0 = \frac{M_L(1-\overline{v}\rho_0)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \cdots\right] \tag{5}$$

Table 3
Conformational parameters for carboxymethyl deoxy-azido cellulose derivatives (**3a–c**) and dendronized carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) celluloses (**5**, **7** and **9**), Zone C = semi-flexible conformation.

Sample	f/f_0	L_P (nm)	M_L (g/mol nm)	Conformation zone
3a	3.4 ± 0.4	3.0 ± 1.4	330 ± 100	C
3b	3.6 ± 0.6	3.0 ± 2.0	310 ± 130	C
3c	4.6 ± 0.4	3.6 ± 0.3	290 ± 20	C
5	2.9 ± 0.2	2.8 ± 0.2	470 ± 30	C
7	4.3 ± 0.2	3.7 ± 0.2	450 ± 30	C
9	5.0 ± 0.3	4.0 ± 0.4	420 ± 50	C

Yamakawa and Fujii showed that A_2 can be considered as $-\ln(d/2L_p)$ and $A_3 = 0.1382$ if the L_p is much higher than the chain diameter, d [40]. Difficulties arise if the mass per unit length is not known, although both relations have now been built into an algorithm Multi_HYDFIT [32] which estimates the best values – or best range of values of L_p and M_L based on minimization of a target function Δ. An estimate (0.8 nm) for the chain diameter d is also required.

$$d = \left(\frac{4M_L\overline{v}}{\pi N_A}\right)^{1/2} \tag{6}$$

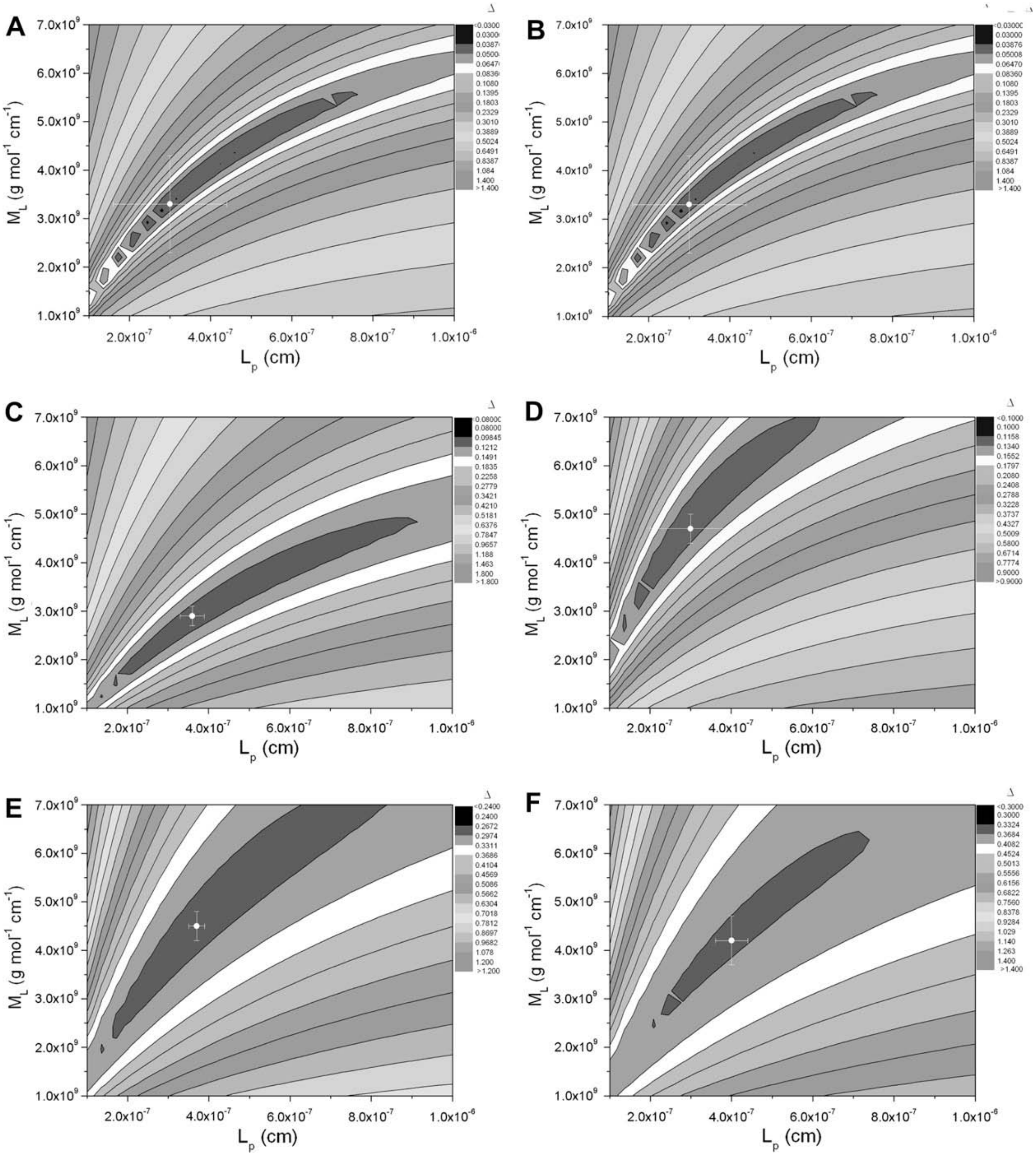


Fig. 5. HYDFIT analysis for carboxymethyl deoxy-azido celluloses (**3a–c**) and dendronized carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose derivatives (**5**, **7** and **9**). Solutions of the Bohdanecky and Yamakawa–Fujii relations for L_p with M_L allowed to float. The target function, △ is then calculated over a range of values for M_L and L_p. (A) Contour plot from HYDFIT analyses for **3a**. (B) Contour plot from HYDFIT analyses for **3b**. (C) Contour plot from HYDFIT analyses for **3c**. (D) Contour plot from HYDFIT analyses for **5**. (E) Contour plot from HYDFIT analyses for **7**. (F) Contour plot from HYDFIT analyses for **9**.

where the partial specific volume, $\overline{v}$ and the mass per unit length, M_L for each cellulose derivative were estimated to be 0.715 ml/g and 380 g/mol nm, respectively. Extensive simulations have shown that the results returned for L_p are relatively insensitive to the value chosen for d.

In this procedure as defined in references [32], Δ is calculated using equivalent radii (or the ratio of equivalent radii), where an equivalent radius is defined as the radius of an equivalent sphere having the same value as the determined property. These 'determined properties' include the translational frictional coefficient, f (calculated from either the diffusion or sedimentation coefficients), intrinsic viscosity, $[\eta]$, radius of gyration, $R_{g,z}$ or the rotational relaxation time, τ.

In this study, it was of interest to get information about the equivalent radii resulting from the sedimentation coefficient, i.e., from translational frictional coefficient (a_T) and from the intrinsic viscosity (a_I).

$$a_T = \frac{f}{6\pi\eta_0} \tag{7}$$

where η_0 is the viscosity of water at 20.0 °C, and

$$a_I = \left(\frac{3[\eta]M_w}{10\pi N_A}\right)^{1/3} \tag{8}$$

The target function, Δ can be evaluated from this relation:

$$\Delta^2 = \frac{1}{N_s}\sum_{i=1}^{N_s}\left[\left(\sum_T W_T\right)^{-1}\sum_T W_T\left(\frac{a_{T(cal)} - a_{T(\exp)}}{a_{T(\exp)}}\right)^2\right] \tag{9}$$

and this one

$$\Delta^2 = \frac{1}{N_s}\sum_{i=1}^{N_s}\left[\left(\sum_I W_I\right)^{-1}\sum_I W_I\left(\frac{a_{I(cal)} - a_{I(\exp)}}{a_{I(\exp)}}\right)^2\right] \tag{10}$$

where N_s is the number of samples in multi-sample analysis, W_T and W_I are the statistical weights for equivalent radii a_T and a_I (from translation frictional coefficient and intrinsic viscosity data, respectively) and the subscripts cal and exp represent values from calculated and experimental values, respectively. Δ is thus a dimensionless estimate of the agreement between the theoretical calculated values for the translational frictional coefficient (consequently the sedimentation coefficient) and the intrinsic viscosity for a particular persistence length, L_p and mass per unit length, M_L and the experimentally measured parameters [32].

The optimum value for both the persistence length, L_p and the mass per unit length, M_L are indicated in Fig. 5(A–F). There is good general agreement between the persistence length and the translational frictional ratio again indicating either a random coil or semi-flexible coil conformation (Table 3). The large errors for **3a** and **3b** are due to the fact that there are a large number of possible conformations, all of which could possibly account for the experimental measurements where the ratio of L_p/M_L remains constant.

3.8. Sedimentation conformation zoning

The sedimentation conformation zone [33] plot k_sM_L versus $[s]/M_L$ enables an estimate of the "overall" solution conformation of a macromolecule in solution ranging from Zone A (extra-rigid rod) to Zone E (globular or branched). The parameter $[s]$ related to the sedimentation coefficient by the relation

$$[s] = \frac{s^0_{20,w}\eta_{20,w}}{\left(1 - \overline{v}\rho_{20,w}\right)} \tag{11}$$

and M_L the mass per unit length for each derivative is taken as calculated by HYDFIT.

Both the translational frictional ratio and the persistence length indicate that the carboxymethyl deoxy-azido cellulose series (**3a**–**c**) and the dendronized carboxymethyl

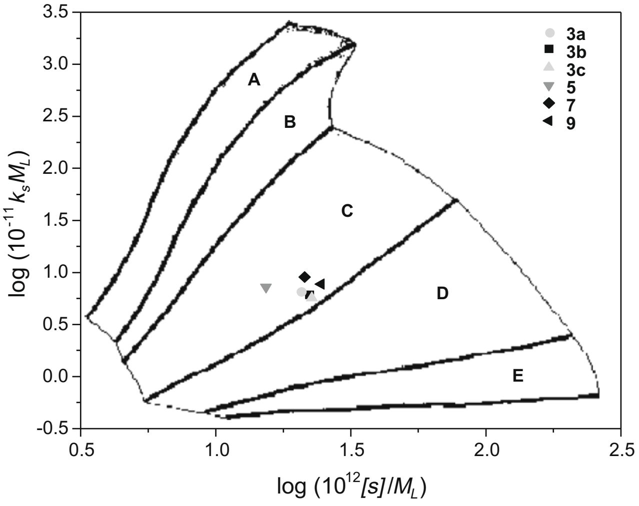


Fig. 6. The sedimentation conformation zoning plot (adapted from [33]). Zone A, extra-rigid rod; Zone B, rigid rod; Zone C, semi-flexible; Zone D, random coil and Zone E, globular or branched. Individual cellulose derivatives are marked: **3a** (●), **3b** (■), **3c** (▲), **5** (▼), **7** (♦) and **9** (◀)

6-deoxy-(1-*N*-[1,2,3-triazolo]-4-PAMAM) cellulose series (**5**, **7** and **9**) have either a random coil or semi-flexible coil conformation. The sedimentation conformation zoning plot (Fig. 6) confirms that all six samples fall into Zone C (semi-flexible coil) which is consistent with another cellulose derivative – methyl cellulose albeit of much larger persistence length [42].

4. Conclusions

The heterogeneous conversion of deoxy-azido cellulose with sodium monochloroacetate in the presence of aqueous NaOH applying 2-propanol as slurry medium leads to water-soluble carboxymethyl deoxy-azido cellulose. The novel biopolymer derivatives provide an appropriate starting material for the selective dendronization via the copper-catalyzed Huisgen reaction resulting in water-soluble carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) celluloses from first to third generation. It is interesting that dendronization leads to an increase in sedimentation coefficient (with increasing dendritic generation), however, a decrease in intrinsic viscosity. It could be shown from sedimentation conformation zoning that the carboxymethyl deoxy-azido cellulose and the carboxymethyl 6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) cellulose derivatives possess a semi-flexible coil conformation (Zone C). The values of persistence length obtained from combined analysis for these samples also indicate a semi-flexible coil conformation in solution. Thus, dendronization of carboxymethylated deoxy-azido cellulose has no or only a rather small influence on the chain stiffness and the conformation in solution.

The investigations will be continued with regards to characterization of the polymers and to use different synthesis path including the variation of the peripheric groups of the dendron. Studies on the application of these novel biopolymer derivatives are under investigation.

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