Regioselectively Oxidized Cellulose Ethers

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Summary: 3-Mono-*O*-(2-methoxyethyl) cellulose (MEC) was selectively oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)/NaBr/ NaOCl. According to 1D and 2D NMR measurements, the primary hydroxyl group was completely oxidized without affecting the secondary one at position 2. Size-exclusion chromatography revealed high tendency of 3-mono-*O*-(2-methoxyethyl)-6-carboxyl cellulose (MECO) to aggregate. Viscosity measurement and gelation of MECO upon interaction with chitosan and Cu^{2+} ions confirmed the introduction of anionic ions into the polymer backbone. Both MEC and MECO expressed the property of surface activity hence decreasing the surface tension of water (72 mN/m), being 45.0 and 56.8 mN/m, respectively.

Keywords: amphiphiles; biopolymer; 3-mono-*O*-(2-methoxyethyl) cellulose; oxidation; surfactants

Introduction

The introduction of ionic groups into polysaccharides is an approach to obtain biopolymer-based polyelectrolytes. In addition to the commercially produced carboxymethyl ethers, this can also be achieved by oxidation of hydroxyl- into carboxyl groups. Hydroxyl groups on positions 2, 3, and 6 of the repeating unit are available for oxidation. The secondary OH groups can be oxidized with $NaIO_4$, which causes glycol cleavage and hence formation of aldehyde functions.[1] The treatment of a cellulose solution in dimethyl sulfoxide/ paraformaldehyde with acetic anhydride yields derivatives bearing carbonyl groups at position 2 and 3.[2] In contrast, oxidation of the primary hydroxyl group is more difficult due to the fact, that both aldehyde- and carboxy groups can be formed and conversion of the secondary ones may also occur. As shown in Table 1, there are several oxidation systems for the primary hydroxyl group.

1) Nitroxides, e.g. NO_2 and N_2O_4, have been successfully used to selectively oxidize primary hydroxyl group of cellulose.[3] The toxicity of the oxidant and depolymerization as side reaction are the main drawbacks of the method.
2) Homogeneous oxidation with $NaNO_2$ or $NaNO_3$ in 85% H_3PO_4 solution is another choice for oxidation of primary hydroxyl groups. In this system, 95% primary hydroxyl and about 10% secondary hydroxyl group could be oxidized simultaneously and $NaBH_4$ should be used after oxidation to stabilize the product.[4]
3) Stoichiometric oxidation with $NaBrO_3$, $NaClO_3$, or $NaClO_2$ homogeneously in 85% H_3PO_4 solution also could be applied to modify primary hydroxyl group. Depolymerization and ring cleavage (20–30%) occur and ketones formed in side reactions can be reduced with $NaBH_4$.[5]

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Table 1.
Reagents for the oxidation of polysaccharides.

Oxidant	Medium	Oxidation of the OH group at	Remarks	Reference
$NaIO_4$	H_2O	2 and 3	Glycol cleavage	[1]
NO_2/N_2O_4		6	Highly toxic	[3]
$NaNO_2/NaNO_3$	85% H_3PO_4	6(2 and 3)	Depolymerization	[4]
$NaBrO_3/NaClO_3$	85% H_3PO_4	6	Depolymerization	[5]
TEMPO[a)]	H_2O	6	Comparably mild	[6]

[a)] 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO) in combination with, e.g., NaBr and NaOCl as oxidants.

4) At present, 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)/ NaBr/ NaOCl is the most popular oxidation system for primary hydroxyl groups of polysaccharides because of its high reaction rate and yield, high selectivity, use of catalytic amount of TEMPO and NaBr, and modest depolymerization of the polysaccharide.[6]

The properties of cellulose derivatives are determined by the type of substituent, its degree and its distribution within the repeating unit. Therefore, cellulose derivatives with a defined functionalization pattern are a prerequisite for the investigation of structure-property-relationships. Regioselective introduction of ether moieties is usually carried out applying blocking groups or direct selective functionalization.[7] Moreover, methods for selective oxidation have been developed as well. Such polymers containing both hydrophilic and hydrophobic moieties are a potential interest for emulsifiers, liposomes, nanoparticles, and drug delivery devices through formation of supramolecular structures (Figure 1).

Thus, it was of interest to study the combination of two regioselective approaches in the field of cellulose functionalization, namely the selective introduction of an ether function at position 2 and/or 3 of the repeating unit into cellulose and the consecutive selective oxidation of the remaining CH_2OH group. The structure of the polymers was characterized by means of NMR spectroscopic techniques. Moreover, molecular mass distribution, surface activity, and viscosity behaviour were determined.

Experimental Part

General Methods

Microcrystalline cellulose (Merck, microcrystalline cellulose, DP 419) was dried at 105 °C in vacuum over KOH for 24 h. LiCl (Fluka) was dried at 130 °C in vacuum over KOH for 24 h. Anhydrous tetrahydrofuran (THF) over molecular sieves was obtained from Fluka. 1-Bromo-2-methoxyethane was purchased from Aldrich. Sodium hydride (60% in mineral oil, Fluka) was washed with n-hexane and pentane and dried at room temperature in vacuum. *N,N*-Dimethyl acetamide (DMA, Acros Organics), thexyldimethylchlorosilane (TDMS-Cl, ABCR Karlsruhe), tetrabutylammonium fluoride trihydrate (TBAF, Fluka), 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO, Fluka), NaBr, NaClO and anhydrous $CuSO_4$ were used as received.

Characterization and Measurements

FTIR spectra were recorded with a Nicolet Avatar 370 DTGS spectrometer using the

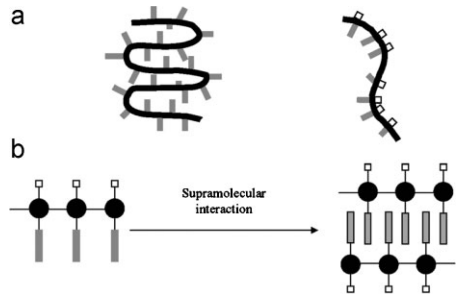

Figure 1.
Schematic representation of the structure in solution (a) and supramolecular interaction (b) of polymers bearing hydrophilic (□) and hydrophobic (▬) moieties.

KBr technique. 1D and 2D NMR spectra were acquired with Bruker Avance 250 (250 MHz) and Avance 400 (400 MHz) spectrometers in $CDCl_3$ or D_2O (sample concentration, 5–10%) at a temperature up to 70 °C using standard pulse sequences for ^1H-, ^{13}C-, DEPT 135-, and 2D (COSY, HSQC/DEPT) NMR spectra. The scan number was 51200 for ^{13}C NMR spectra. Size exclusion chromatography (SEC) in aqueous 0.1 M $NaNO_3$ containing 0.1% NaN_3 was measured with a JASCO SEC system (degasser DG 980-50, pump PU 980, UV detector 975 ($\lambda = 254$ nm), refractive index detector 930, columns Suprema 1000+ and Suprema 100 (Kromatek, Great Dunmow, Essex, UK), with eluent flow rate 1.000 ml/min). Weight average- (M_w) and number average molecular mass (M_n) as well as polydispersity index (PDI) were calculated. The surface tension of aqueous solutions (1%, w/v) was determined with a tensiometer K100 (KRÜSS, Hamburg, Germany) using the plate method with a platinum plate and taken after 6 h. The intrinsic viscosities were determined with an automatic viscometer (Lauda PVS 1/2) equipped with a dilution Ubbelohde viscometer (Schott Instruments, Mainz, Germany) in a thermostated water bath (Lauda E 200, Lauda-Königshofen, Germany) at 20 °C. An automatic burette (Metrohm Dosimat 765, Filderstadt, Germany) was used to dilute the solution automatically.

Preparation of 3-Mono-*O*-(2-methoxyethyl) Cellulose

3-mono-*O*-(2-methoxyethyl) cellulose (MEC) was prepared as described elsewhere.[8] Briefly, cellulose (Avicel, **1**) is homogeneously silylated with thexyldimethylchlorosilane (TDMS-Cl) in *N,N*-dimethyl acetamide (DMA/LiCl) in the presence of imidazole for 24 h at 100 °C, leading to 2,6-di-*O*-TDMS cellulose (sample **2**). **2** was alkylated with excess of 1-bromo-2-methoxyethane in THF in the presence of NaH as base for 72 h at 50 °C to obtain 3-mono-*O*-methoxyethyl-2,6-di-*O*-TDMS cellulose **3**. Deprotection of **3** was carried out by conversion with TBAF in THF for 24 h at 50 °C and subsequent with TBAF in dimethyl sulfoxide for 24 h at 50 °C yielding water-soluble 3-mono-*O*-methoxyethyl cellulose.

Yield: 3.0 g (sample **4**).

Degree of substitution: 1.02 (DS, determined by means of ^1H NMR spectroscopy of the peracetylated derivative.

^1H NMR ($CDCl_3$, ppm): δ4.79 (H-2), 4.46 (H-1), 4.35, 4.13 (H-6), 3.90, 3.57 ($OC\mathbf{H}_2CH_2OCH_3$), 3.62 (H-4), 3.46 (H-3,5), 3.36 ($OCH_2C\mathbf{H}_2OCH_3$), 3.31 ($OCH_2CH_2OC\mathbf{H}_3$).

^{13}C NMR (D_2O, ppm): 102.8 (C-1), 83.3 (C-3), 75.8 (C-5), 75.4 (C-4), 73.5 (C-2), 60.3 (C-6), 72.0 ($O\mathbf{C}H_2\mathbf{C}H_2OCH_3$), 58.3 ($OCH_2CH_2O\mathbf{C}H_3$).

$M_w = 42100$ g/mol, $M_n = 10067$ g/mol, PDI 3.944.

The polymer is soluble in water.

Preparation of 3-Mono-*O*-methoxyethyl-6-carboxy Cellulose Sodium Salt

1.0 g (4.5 mmol) 3-mono-*O*-MEC **4** was dissolved in 50 ml of distilled water in a 100 ml beaker at room temperature. 66 mg (0.64 mmol, 0.142 mol/mol anhydroglucose unit, AGU) NaBr and 9.5 mg (0.061 mmol, 0.0135 mol/mol AGU) TEMPO were added under stirring at room temperature. The beaker was cooled in an ice bath at 1 °C before aqueous NaClO (10%, w/v) was carefully added. The pH value was monitored and maintained at about 10 through addition of NaClO or 0.1 M NaOH, while keeping the reaction temperature lower than 4 °C. After the addition of 5.5 ml NaClO within 40 min, 10% NaOH was used to adjust the pH at about 7 to quench the reaction. The reaction mixture was dialyzed in a dialysis tube (molecular weight cut-off 3500 g/mol) against distilled water at room temperature for 48 h and then freeze-dried.

Yield: 0.79 g (sample **5**).

DS of methoxyethyl groups: 1.

Degree of oxidation: 1 (according to NMR spectroscopy).

^{13}C NMR (D_2O, ppm): 102.7 (C-1), 83.3 (C-3), 79.0 (C-5), 75.4 (C-4), 73.2 (C-2), 72.0($O\mathbf{C}H_2\mathbf{C}H_2OCH_3$), 60.3 (C-6), 58.3 ($OCH_2CH_2O\mathbf{C}H_3$).

$M_w = 115{,}200$ g/mol, $M_n = 62{,}960$ g/mol, PDI 1.830.

The polymer is soluble in water.

Results and Discussion

3-Mono-*O*-methoxyethyl cellulose (3-mono-*O*-MEC) with DS 1 was synthesized starting from microcrystalline cellulose (Avicel, Figure 2).[8]

Thus, a cellulose (**1**) solution in DMA/LiCl was treated with thexyldimethylchlorosilane (TDMS-Cl) in the presence of imidazole to afford 2,6-di-*O*-TDMS cellulose **2** with DS 2. Subsequent conversion with 1-bromomethoxyethane in the presence of sodium hydride yielded 3-mono-*O*-methoxyethyl-2,6-di-*O*-TDMS cellulose **3** that was finally treated with tetrabutylammonium fluoride trihydrate in tetrahydrofuran and dimethyl sulfoxide to cleave off the TDMS groups. The resulting 3-mono-*O*-MEC **4** dissolves in water.

2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)/NaClO/NaBr is a highly efficient mixture for selective oxidation of primary hydroxyl groups of water-soluble polysaccharides, with almost 100% degree of oxidation, and without affecting the secondary hydroxyl groups (Figure 2). The oxidation was carried out in aqueous solution. NaClO was slowly added to the reaction mixture containing 3-mono-*O*-MEC (**4**), NaBr, and TEMPO, while maintaining the temperature below 4 °C and the pH at about 10. 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** was obtained after dialysis and freeze drying.

The structure characterization of samples **4** and **5** was carried out by means of 1D and 2D NMR spectroscopy at room temperature in D_2O. The ^{13}C signals in the DEPT135 NMR spectrum of **4** (Figure 3) were assigned as follows: 102.8 ppm (C-1), 83.3 ppm (C-3), 75.8 ppm (C-5), 75.4 ppm (C-4), 73.5 ppm (C-2), 72.0 ppm (C-7, 8), 60.3 ppm (C-6), 58.3 ppm (C-9). Only one peak was found for every C-atom, thus regioselective alkylation of position 3 occurred. After oxidation, the signal of the methylene group at position 6 disappeared in the DEPT 135 NMR spectrum of sample **5**, indicating a complete oxidation of the primary hydroxyl group. The signal corresponding to C-5 is

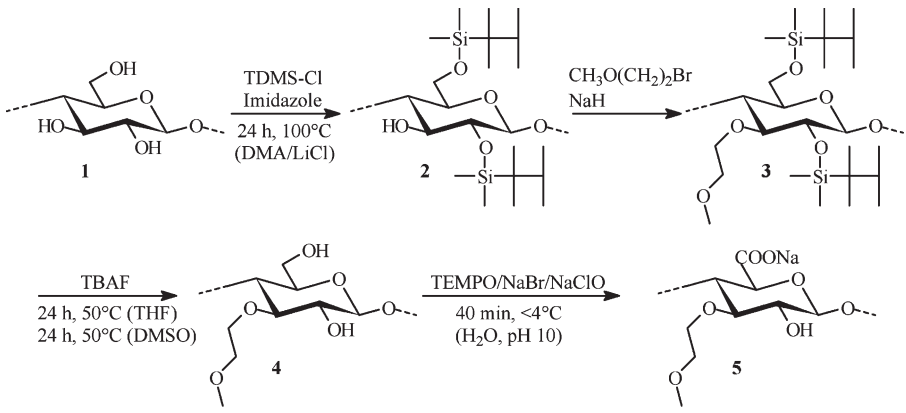

Figure 2.
Reaction scheme for the preparation of 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5**.

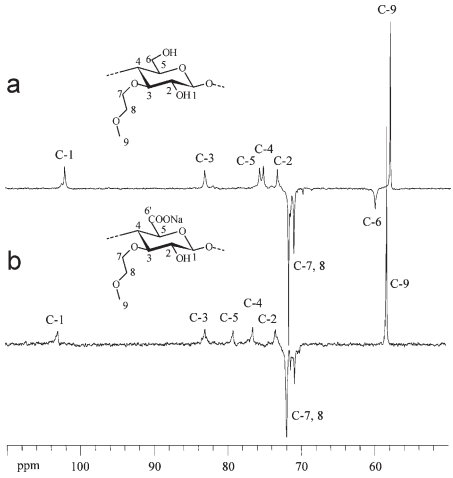

Figure 3.
Dept 135 NMR spectra of (A) 3-mono-*O*-methoxyethyl cellulose **4** and (B) 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** recorded at room temperature in D_2O.

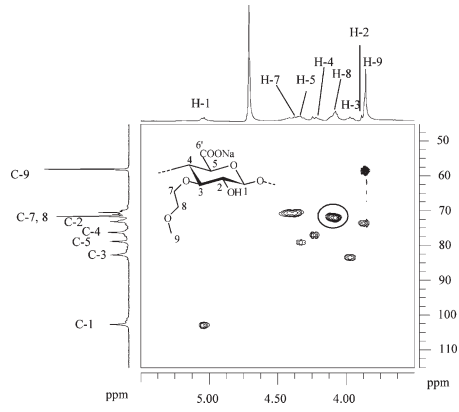

Figure 5.
HSQC/DEPT NMR spectrum of 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** recorded in D_2O at 70 °C. Methylene groups are marked in the spectrum.

shifted about 3.2 ppm to higher field after oxidation, while the chemical shift of the other signals is not significantly influenced (Figure 3). In the ^{13}C NMR spectrum of **5**, a peak at 175.0 ppm becomes obvious, which results from the carboxylate moiety (Figure 4). The absence of additional peaks in the range from 170 to about 200 ppm, which are caused by ketones, clearly proofs the selective oxidation of position 6 without affection of position 2 and 3.

The structure of 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** was characterized by HSQC/DEPT NMR spectroscopy in D_2O at 70 °C (Figure 5). The following cross-peaks were detected: 102.8 ppm/5.0 ppm (position 1), 83.3 ppm/4.0 ppm (position 3), 79.0 ppm/4.3 ppm (position 5), 75.4 ppm/4.2 ppm (position 4), 73.1 ppm/3.9 ppm (position 2), 72.0 ppm/4.4 ppm (position 7), 72.0 ppm/4.1 ppm (position 8), 58.0 ppm/3.9 ppm (position 9). The HSQC/DEPT NMR spectrum further confirmed the assignment of ^{13}C NMR spectrum.

The molar mass of the novel biopolymer derivatives was determined by means of SEC in 0.1 M $NaNO_3$ solution. A mass average molar mass (M_w) of 42,100 g/mol and a number average molar mass (M_n) of 10,067 g/mol were determined for sample **4** (Table 2). The M_n value corresponds to a

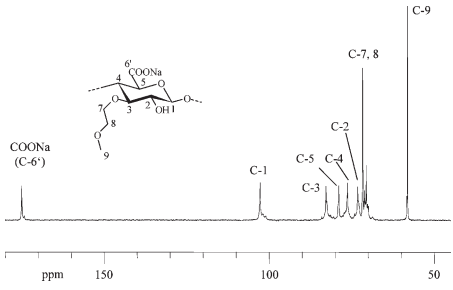

Figure 4.
^{13}C NMR spectrum of 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** recorded in D_2O at room temperature.

Table 2.
SEC and surface tension of 3-mono-*O*-methoxyethyl cellulose **4** and 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5**.

Sample	Molecular mass (g/mol)		PDI[c]	DP_n[d]	Surface tension[e] (mN/m)
	M_w[a]	M_n[b]			
4	42100	10067	3.944	46	45.0
5	115200	62960	1.830	246	56.8

[a] Weight average molar mass;
[b] Number average molar mass;
[c] Polydispersity index;
[d] Number average degree of polymerization calculated for a degree of substitution of 1 and degree of oxidation of 1;
[e] Surface tension of 1% aqueous solution was taken at 6 h.

number average degree of polymerization (DP_n) of 46. For sample **5**, M_w and M_n were determined to be 115,200 g/mol and 62,960 g/mol, which results in a DP_n of 246 assuming DS_{MOE} of 1 and degree of oxidation of 1. In addition, polymer degradation in the system of TEMPO/NaOCl/NaBr due to β-elimination must also be considered. Obviously, sample **5** tends to aggregate in aqueous solution as concluded from the SEC data. The SEC curves of samples **4** and **5** appear with a unimodal distribution, which does not show evidence of polymer fractionation, i.e. the appearance of further peaks (Figure 6).

Aqueous solutions of **4** and **5** were investigated by tensiometry to evaluate the influence of the carboxylate moiety on the surface activity of the polymer. Obviously, it took a long time to reach the equilibrium of constant surface tension σ (Figure 7). Therefore, the values were taken after 6 h, where σ appeared nearly constant. Both **4** and **5** lowered the surface tension of water (72 mN/m), being 45.0 (sample **4**) and 56.8 mN/m (sample **5**), respectively (Table 2). The introduction of carboxylate moieties reduces the surface activity of 3-mono-*O*-methoxyethyl cellulose leading to a higher value of σ.

The surfactant is adsorbed at the surface of the solution, which results in lowering the surface tension. Compared to **4**, sample **5** seems to be dissolved in water rather than being absorbed at the water/air interface.

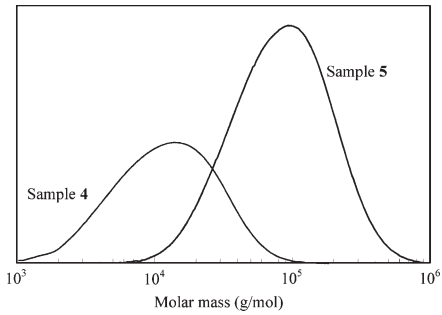

Figure 6.
SEC of 3-mono-*O*-methoxyethyl cellulose **4** and 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** using 0.1 M $NaNO_3$ as eluent.

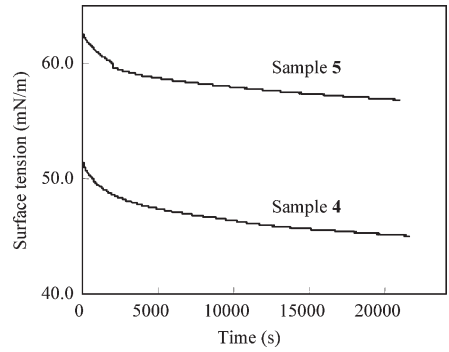

Figure 7.
Plots of surface tension of aqueous solutions of 3-mono-*O*-methoxyethyl cellulose **4** and 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** versus measuring time.

The methoxyethyl moieties of the aggregates are surrounded by hydrophilic carboxylate moieties and hence are not effectively absorbed at the water/air interface. The phenomena are same as those of hydroxyethyl cellulose bearing both alkyl chains and sulfonate groups.[9]

Aqueous solutions of **4** and **5** were studied by capillary viscometry in the concentration range from 0.004 to 0.01 g/ml (Figure 8). For sample **4**, a linear correlation between concentration and reduced viscosity was observed leading to an intrinsic viscosity [η] of 85.5 ml/g.

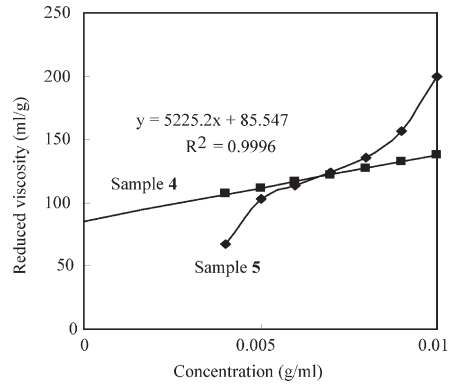

Figure 8.
Plot of the reduced viscosity of 3-mono-*O*-methoxyethyl cellulose **4** and 3-mono-*O*-methoxyethyl-6-carboxy cellulose **5** versus concentration of their aqueous solutions at 20 °C.

For the oxidized sample **5**, the typical non-linear correlation of polyelectrolytes was observed. The polyelectrolyte behaviour of **5** was qualitatively confirmed by reaction of the aqueous polymer solution with the cationic polyelectrolyte chitosan or with aqueous $CuSO_4$ solution. A gelation occurred in both cases, which is caused by this reaction.

Conclusion

3-Mono-*O*-methoxyethyl cellulose was oxidized with TEMPO/NaClO/NaBr, to obtain 3-mono-*O*-methoxyethyl-6-carboxy cellulose in a regioselective manner without formation of side-structures. The compounds were comprehensively characterized by means of NMR spectroscopy, SEC, viscometry, and tensiometry. The typical polyelectrolyte behaviour of the final product could be evidenced. Further studies should be carried out to elucidate the aggregation behaviour in solution. Moreover, it became obvious that the introduction of carboxylate groups did not increased the surface activity of the polymer. Consequently, to study the influence of the non-ionic substituents, i.e., variation of the alkyl chain length is of interest.

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