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Grafting of poly(ethylene glycol) to chitosan at C(6) position of glucosamine units via "click chemistry" reactions

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N-Phthaloyl chitosan derivatives containing azide or propargyl moieties at C(6) position of glucosamine units were synthesized for the first time. The products obtained are useful as precursors for modification of chitosan via "click chemistry" reactions. New chitosan–MPEG derivatives containing intermediate triazolyl moieties at C(6) position of glucosamine units were prepared by coupling via 1,3-dipolar cycloaddition between azide and propargyl groups of chitosan and poly(ethylene glycol) monomethyl ether (MPEG). Comb copolymers were characterized by FT-IR and ^{1}H NMR spectroscopy, intrinsic viscosity, elemental and functional group analysis. The degree of chitosan substitution in new copolymers depended on the ratio of azide and propargyl groups and was in the range 20 to 65%, proving an efficient control of the graft density. The intrinsic viscosity of the chitosan derivatives was low, evidencing a significant degradation of the chitosan backbone during modification.

Key words: chitosan, poly(ethylene glycol), click chemistry, graft copolymer, azidated chitosan

INTRODUCTION

Chitosan is a cationic natural biopolymer produced by alkaline *N*-deacetylation of chitin, the most abundant natural polymer after cellulose. Chitosan and its derivatives are used in various fields, such as biomedicine, cosmetics, food industry, agriculture, etc. [1, 2]. However, applications of chitosan are limited by its poor solubility. It is soluble in acidic aqueous solutions only where the amino groups are protonated. Chemical modifications of chitosan are widely used to obtain its derivatives, expecting that the derivatives will preserve the original physicochemical and biochemical properties of chitosan and get new properties depending on the nature of the introduced groups [3]. Among the various possible modifications (e. g. phosphorylation, sulfation, acylation, Schiff's base formation, alkylation [4–6]), graft copolymerization is expected to be one of the most promising [7]. Recently prepared comb-like chitosan derivatives containing methoxy poly(ethylene glycol) (MPEG) grafts [5, 8–10] may find application in household and personal care products. PEG'ylated chitosans are also interesting as dispersing agents, solubilization aids, surface conditioners, and drug carriers [11]. There are many publications concerning chitosan modification by MPEG through amino groups [7, 8, 11–16]. The number of *O*-derivatives of chitosan is much less because of problems related to the protection of more reactive amino groups during modification. Modification of polysaccharides through their hydroxyl groups is usually done by esterification and etherification reactions. Oxidation and nucleophilic displacement reactions are utilized to a significantly lower extent.

Recently Sharpless et al. [17] introduced the term "click chemistry" which qualifies the reaction as modular and wide in scope. "Click chemistry" is a general term that identifies a class of chemical transformations with a number of attractive features including excellent functional-group tolerance, high yields and good selectivity under mild experimental conditions. Among these reactions, the Huisgen 1,3-dipolar cycloaddition has been receiving increasing interest following the emergence of an enormous improvement in regioselectivity and yields in the presence of a copper(I)-based catalyst [18]. The Cu-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) has been established as one of the most reliable

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means for the covalent assembly of complex molecules [19]. It has enabled a number of applications in synthesis, medicinal chemistry, molecular biology, and materials science. The overall reaction is a cycloaddition of an alkyne and an organic azide to give a five-membered 1,2,3-triazole. Azides and alkynes are essentially inert to most biological and organic conditions, including highly functionalized biological molecules, molecular oxygen, water and the majority of common reaction conditions in organic synthesis [20]. CuAAC has been used for a variety of selective conversions, e. g. the synthesis of dendrimers or the modification of proteins [18], biological polymers such as nucleic acids or polysaccharides, for the preparation of polymer bioconjugates [21]. More complex biological entities such as proteins, enzymes, viruses, bacteria, and cells may also be transformed using azide–alkyne chemistry. Moreover, the copper-catalyzed azide – alkyne cycloaddition can be performed in various solvents (including water) and in the presence of numerous other functional groups [21]. There are several publications concerning "click chemistry" on polysaccharides such as cellulose [22], potato starch [23], and (1,3)-β-d-glucans [24]. No publications were found, however, on attachment of MPEG to chitosan by "click chemistry".

The present work focuses, for the first time, on the synthesis of chitosan-*C(6)*-MPEG copolymers by "click chemistry". Huisgen's 1,3-dipolar cycloaddition between azidated or propargyl-terminated *N*-phthaloyl chitosan and an inverse group containing MPEG was used to control the composition of the copolymers and to reduce the problems related to purification of the products. It had been found in earlier studies that separation of chitosan comb copolymers from unreacted oligomeric MPEG was complicated and time-consuming [23, 25].

EXPERIMENTAL

Materials

Chitosan (medium molecular weight with deacetylation degree (DD) 72%), poly(ethylene glycol) monomethyl ether (MPEG) (M_r 2000), propargyl bromide (80 wt. % in toluene), sodium azide and sodium dodecyl sulfate (SDS) were obtained from Fluka. Sodium hydride (60% dispersion in mineral oil) was obtained from Aldrich. All solvents were of puriss grade and used without further purification.

Synthetic procedures

N-Phthaloyl chitosan was prepared by the method described elsewhere [9].

Anal. calcd. for $[C_{14}H_{13}O_6N]_{72}[C_8H_{13}O_5N]_{28}$: C 55.50%, H 6.10%, N 5.27%. Found: C 54.06%, H 5.63%, N 5.18%.

^{1}H NMR spectrum (DMSO-d_6, ppm): δ = 1.8 (CH_3 in acetamide), δ = 2.7–4.4 (pyranose ring), δ = 7.5–7.8 (aromatic ring).

C(6)-Tosyl-N-phthaloyl chitosan was synthesized according to the procedure described before [2].

Anal. calcd. for $[C_{21}H_{19}O_8SN]_{72}[C_{15}H_{19}O_7SN]_{28}$: C 55.15%, H 4.52%, N 3.33%. Found: C 54.84%, H 4.31%, N 3.48%.

Synthesis of C(6)-azidated chitosan via N-phthaloyl chitosan. To a solution of *C(6)*-tosyl-*N*-phthaloyl chitosan (1 g, 2.4 mmol) in a mixture of 35 ml DMF and 10 ml water, sodium azide (0.78 g, 12 mmol) was added, and the reaction mixture was stirred at 100 °C for 24 h. The product was isolated by precipitation to 400 ml of ice water and filtration. It was washed several times with water, then with ethanol and dried at 50 °C under vacuum to give 0.55 g of the product (yield 73%).

FT-IR (KBr): ν (cm^{-1}) = 3417 (OH), 2930 (C–H), 2112 (N_3), 1777 and 1717 (phthalimide), 1076 (C–O).

Preparation of SDS / chitosan complexes. SDS / chitosan complexes (SCC) were prepared by mixing acidic solutions of chitosan and SDS according to the procedure described elsewhere [26]. Chitosan (3 g, 17 mmol) and SDS (5 g, 17 mmol) were dissolved in 200 ml and 70 ml of 2% acetic acid, respectively. SDS solution was poured into the chitosan solution under vigorous stirring, and the mixture was gently stirred for 2 h at room temperature. The resulting precipitates were filtered off, washed three times with distilled water and finally freeze-dried to give 6.2 g of a white product (yield 98%).

^{1}H NMR spectrum (in DMSO d-6, ppm): δ = 0.85 (CH_3 in SDS), δ = 1.25 (-$(CH_2)_9$- in SDS), δ = 1.49 (-$\underline{CH_2}CH_2$-O in SDS), δ = 1.87 (CH_3 in acetamide of chitosan), δ = 2.88 (H-2 of chitosan), δ = 3.2–3.5 (H-3 – H-6 of chitosan (pyranose ring)), δ = 3.69 (-$CH_2\underline{CH_2}$-O in SDS).

FT-IR (KBr): ν (cm^{-1}) = 3600–3100 (O–H, N–H), 2930–2830 (CH_2, SDS), 1640 (imide I), 1530 (imide II), 1250 (S = O, SDS), 1150–950 (C–O, pyranose), 807 (C–O–S, SDS).

"Activation" of SCC using toluene-4-sulfonyl chloride (TsCl). Tosylation of SCC was performed in 4% LiCl solution in *N,N*-dimethylacetamide (DMA) according to the known procedure [2].

"Activation" of SCC using N-bromosuccinimide (NBS). To a solution of SCC (1.5 g, 4.1 mmol) in 4% LiCl solution in DMA (150 ml) at 4 °C, *N*-bromosuccinimide (7.12 g, 40 mmol) and triphenylphosphine (10.5 g, 40 mmol) were added, and the reaction mixture was stirred at 4 °C for 15 min, then heated to 80 °C and left under stirring for 2 h. The product was isolated by precipitation into 500 ml of aqueous Na_2CO_3 solution and filtration. It was washed several times with water, then with ethanol and dried at 50 °C under vacuum to give 0.72 g of the product (yield 42%).

"Activation" of SCC using 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride). To a solution of SCC (1.5 g, 4.1 mmol) in 4% LiCl solution in DMA (150 ml) at 4 °C, Na_2CO_3 (3.61 g, 34.1 mmol) and cyanuric chloride (4.4 g, 34.1 mmol) in DMA (30 ml) were added, and the reaction mixture was stirred at 80 °C for 40 h. The product was isolated by precipitation into 400 ml of acetone and filtration. It was washed several times with a mixture of water and acetone (1/1, v/v), then with water, and dried at 50 °C under vacuum to give 1.0 g of the product (yield 73%).

Synthesis of C(6)-azidated chitosan via "activated" SCC. SCC "activated" with *N*-bromosuccinimide (0.5 g) was dissolved in DMSO (50 ml), and sodium azide (0.5 g, 7.7 mmol) was added. The reaction was carried out at 100 °C for 24 h. The solution was dialyzed against water for 48 h, concentrated by rotary evaporation and vacuum-dried at room temperature to give 0.25 g of the product (yield 56%).

Synthesis of C(6)-propargyl chitozan. N-phthaloyl chitosan (0.8 g, 3.0 mmol) was dissolved in DMF (30 ml). The solution was cooled to 0 °C in an ice bath and NaH (60% w/w in mineral oil, 0.27 g, 9.0 mmol) was added. After stirring for 30 min, propargyl bromide (80% in toluene, 0.65 ml, 4.5 mmol) was added slowly, and the mixture was stirred at 0 °C for 2 h and at 60 °C for 22 h. The excess of NaH was destroyed by the addition of water (10 ml), and the product was precipitated by pouring into acetone (300 ml), filtered, washed several times with acetone and vacuum-dried at room temperature to give 0.77 g of the product (yield 84%).

Synthesis of propargyl-terminated MPEG. To a solution of MPEG-2000 (5 g, 2.5 mmol) in 20 ml of dichloromethane (DCM), NaH (60% w/w in mineral oil, 0.15 g, 6.25 mmol) was added at 0 °C under stirring. After stirring for 30 min, propargyl bromide (80% in toluene, 0.4 ml, 2.75 mmol) was added slowly, and the mixture was stirred at 0 °C for 2 h and then warmed to room temperature and stirred with a magnetic stirrer for 22 h. Then the reaction mixture was dispersed in water and extracted with DCM. The DCM extract was evaporated, dissolved in 20 ml of benzene, and the product was precipitated to diethyl ether (200 ml). The precipitate was filtered off, washed several time with diethyl ether and vacuum-dried at room temperature to yield 4.6 g of the product (80%). ^{1}H NMR ($CDCl_3$) δ: 4.22 (d, 2H, J = 2.4 Hz, $O\underline{CH_2}C \equiv CH$), 3.66–3.61 (m, -($OCH_2CH_2$)O-), 3.39 (s, 3H, $\underline{CH_3}OCH_2$-), 2.46 (t, 1H, J = 2.4 Hz, $OCH_2C \equiv \underline{CH}$).

Synthesis of MPEG azide. Methansulfonyl chloride (0.2 ml, 2.5 mmol) solution in 15 ml DCM was added dropwise to an MPEG-2000 (2 g, 1 mmol) solution in 10 ml of pyridine vigorously stirred and cooled to 0 °C. The reaction mixture was allowed to warm to room temperature and stirred with a magnetic stirrer for 18 h. The reaction mixture was concentrated using rotary evaporation to ca. 30% of its original volume, and the residue was washed with a saturated solution of sodium hydrogen carbonate and extracted with DCM. The organic layer was dried over Na_2SO_4, and the product was precipitated into diethyl ether (200 ml), filtered, washed several times with diethyl ether and vacuum-dried at room temperature. The obtained monotosylated MPEG (1.5 g, 0.65 mmol) was dissolved in DMF (10 ml), and sodium azide (0.10 6 g, 1.63 mmol) was added to the solution. The reaction mixture was stirred for 4 h at 105 °C in a nitrogen atmosphere and for 18 h at room temperature. The reaction mixture was concentrated by rotary evaporation and dissolved in 20 ml of benzene. The product was precipitated by pouring into diethyl ether (200 ml), filtered, washed several times with diethyl ether and vacuum-dried at room temperature to give 1.13 g of MPEG azide (yield 56%).

FT-IR (KBr): ν (cm^{-1}) = 2930–2830 (C–H), 2110 (N_3), 1110 (C–O).

Synthesis of chitosan-C(6)-MPEG copolymer by "click chemistry" (an example of the synthesis of a chitosan derivative with DS 50%). To a solution of *C(6)*-azidated chitosan (0.12 g, 0.37 mmol) in 15 ml DMSO, copper(II) sulfate pentahydrate (4.6 mg, 0.012 mmol, in 2 ml of water), sodium ascorbate (7.3 mg, 0.037 mmol, in 2 ml of water), and propargyl-terminated MPEG (0.75 g, 0.37 mmol, in 10 ml of water) were added. The mixture was stirred at 55 °C for 24 h, and then water was removed by rotary evaporation. Hydrazine monohydrate (1 ml) was added to the reaction vessel, and the mixture was heated at 80 °C under magnetic stirring in a nitrogen atmosphere for 2 h. The solution was dialyzed against water for 48 h, concentrated by rotary evaporation and vacuum-dried at room temperature to give 0.39 g of the product (yield 50%).

^{1}H NMR spectrum of the graft copolymer in D_2O containing one drop of DCl: δ = 1.9 (CH_3 in acetamide), δ = 2.99 (H-2), δ = 3.2 (-OCH_3), δ = 3.3–3.8 (pyranose ring and (-O-CH_2-)).

FT-IR (KBr): ν (cm^{-1}) = 3426 (OH, N–H), 2885 (C–H), 1645 (amine, amide-I), 1107 (C–O).

Chitosan-*C(6)*-MPEG copolymer via *C(6)*-azidated SCC was synthesized by the same method. SDS from the copolymer was removed by the use of a strong base of *TRIS*, which acted as a decomplexing agent.

Analytical procedures

Determination of the degree of substitution (DS) of chitosan. The degree of substitution of MPEG by a monosaccharide residue of chitosan (DS, %) was calculated by two independent methods, i. e. according to the content of primary amino groups determined experimentally and from the ^{1}H NMR spectrum.

The content of primary amino groups *a* (%) was determined by potentiometric titration [9]. The DS (%) of chitosan was calculated by the following equation:

$$DS = \frac{(6.66 - a) \cdot 173}{a \cdot 2063} \cdot 100,$$

where 6.66 is the content of amino groups in chitosan (DD 72%), %; 173 is the average ,molecular weight of a monosaccharide residue of chitosan (DD 72%); and 2063 is the molecular weight of the attached MPEG-containing triazole intermediate.

Calculation of the DS of chitosan from ^{1}H NMR spectra was based on the ratio between the peak integration of the protons from the metoxy group at 3.3 ppm for MPEG and the methyl protons of the acetamide group at 1.8 ppm for chitosan.

Spectroscopic measurements. The infrared absorption spectra were recorded with a Perkin Elmer Spectrum BX spectrometer under dry air at 20 °C by the KBr pellet method.

^{1}H NMR spectra of the samples were recorded on a Unity Inova Varian spectrometer (300 MHz, Varian). Samples of chitosan and its derivatives were prepared in D_2O containing one drop of CD_3COOD. Samples of MPEG and its derivatives were prepared in chloroform ($CDCl_3$).

Viscosity measurements. The intrinsic viscosity of copolymer solutions in aqueous 0.1 M CH_3COONa / 0.2 M CH_3COOH at 25 °C was measured using a diluted-type Ubbelohde viscometer.

RESULTS AND DISCUSSION

A serious problem is the purification of chitosan comb copolymers from unreacted MPEG. To avoid this, the method of "click chemistry" (a copper-catalysed Huisgen reaction) was employed, which usually gives nearly quantitative yields of the main products in mild conditions, generating virtually no by-products [22]. To exploit the copper-catalyzed Huisgen reaction, either chitosan or MPEG should contain azide moieties, and the second reagent should contain alkyne moieties.

Synthesis of precursors for "clicking" of MPEG onto chitosan

Poly(ethylene glycol) monomethyl ether (MPEG) derivative containing propargyl moiety was obtained by the use of propargyl bromide in the presence of sodium hydride as shown in Scheme 1, bottom. The progress of the reaction was followed by ^{1}H NMR spectra in which the signals of propargyl group appeared at 4.22 and 2.46 ppm, attributed to $O\underline{CH_2}C \equiv CH$ and $OCH_2C \equiv \underline{CH}$, respectively (Fig. 1). The yield of MPEG alkylation, which was calculated from the ratio between the peak integration of the protons from oxymethyl groups of MPEG at 3.39 ppm and the propargyl group at 2.46 or 4.22 ppm, was over 90%. Propargyl-terminated MPEG was soluble in water and many organic solvents excluding ethers, THF and hexane. The substance was solid at room temperature.

MPEG azide was prepared by MPEG mesylation followed by nucleophilic substitution using sodium azide (Scheme 1, top) [27]. MPEG mesylation proceeded with high yields (≈98). The azidation progress was followed by FT-IR spectroscopy according to the intensity of the absorption band at 2105 cm^{-1}, attributed to the azide group. The degree of MPEG azidation was determined from elemental analysis (nitrogen content) and was 65%.

C(6)-azidated chitosan was prepared through *C(6)*-tosylation of *N*-phthaloyl chitosan. *N*-phthaloyl chitosan, the phthaloyl group of which can be removed to regenerate free amino groups, was prepared as a key intermediate for the modification of chitosan with the grafting site at the hydroxyl group [18, 28]. The *N*-Phthaloyl derivative of chitosan is soluble in several organic solvents such as DMSO, DMF, pyridine and dimethylacetamide (DMA) [28]. The (*p*-tolylsulfonyl) oxy group is one of the most effective leaving groups widely used in carbohydrate chemistry. Treatment of *N*-phthaloyl chitosan with a 10-fold excess of *p*-toluensulfonyl chloride in DMA solution afforded *C(6)*-tosyl-*N*-phthaloyl chitosan in a good yield (68%) with a very high degree of tosylation (Scheme 2) [2]. The degree of tosylation was calculated from nitrogen content (N = 3.38%) determined by elemental analysis and was over 99%.

The FT-IR spectrum of *C(6)*-tosyl-*N*-phthaloyl chitosan showed a characteristic absorption at 1180 cm^{-1} due to tosyl groups. *C(6)*-azidated chitosan was prepared from this key reactive intermediate through displacement of the leaving group at the C(6) position of glucosamine units with a nucleophile including azide (Scheme 2).

The FT-IR spectrum of *C(6)*-azidated chitosan shows a significant absorption at 2110 cm^{-1} typical of the azide moiety (Fig. 2). The degree of chitosan azidation was calculated from the elemental analysis data (nitrogen content, N = 11.6%) and was 57%.

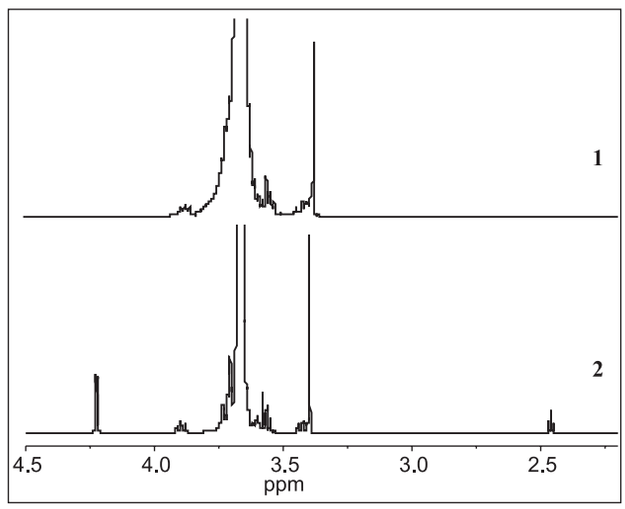
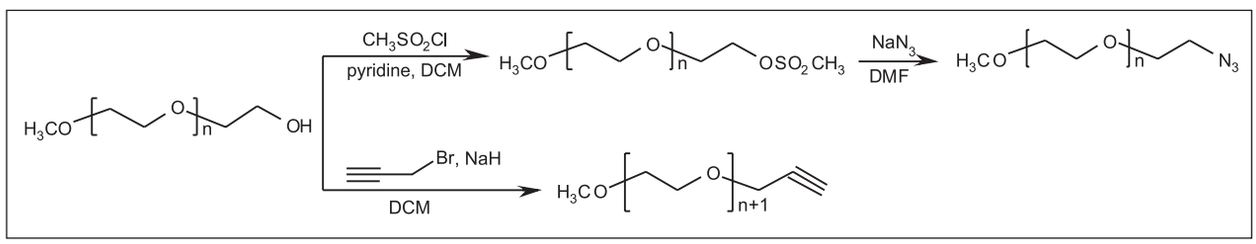
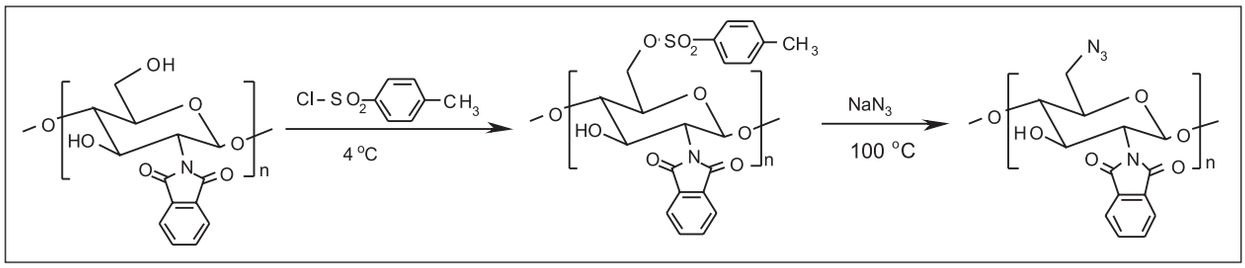


Fig. 1. ^{1}H NMR spectra of MPEG (1) and propargyl-terminated MPEG (2)



Scheme 1. Synthesis of azide- (top) and propargyl- (bottom) terminated MPEG



Scheme 2. Tosylation and azidation of *N*-phthaloyl chitosan

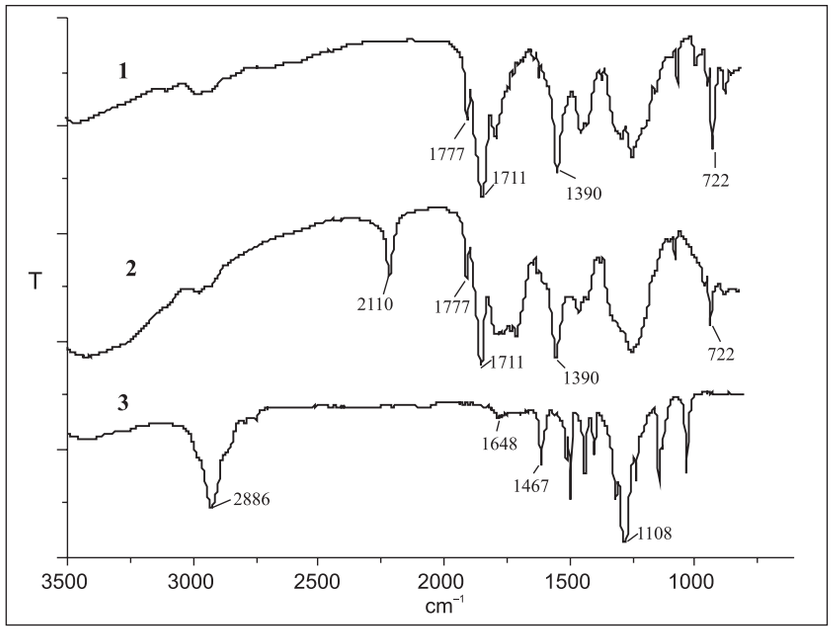


Fig. 2. FT-IR spectra of *N*-phthtaloyl chitosan (1), *N*-phthtaloyl–*C(6)*–azide chitosan (2) and chitosan–*C(6)*–MPEG copolymer (3)

C(6)-propargyl chitosan derivative was prepared by the same method as acetylene-terminated MPEG, i.e. by treating propargyl bromide with *N*-phthaloyl chitosan. Determination of the degree of alkylation of *N*-phthaloyl chitosan from ^{1}H NMR spectra was problematic since the peak of protons of the propargyl group overlapped with wide signals of protons of the chitosan backbone. Taking into account the high reactivity of propargyl halides towards hydroxyl groups [29], we found that the degree of alkylation of *N*-phthaloyl chitosan was high and, like in the case of propargyl-terminated MPEG, exceeded 90%.

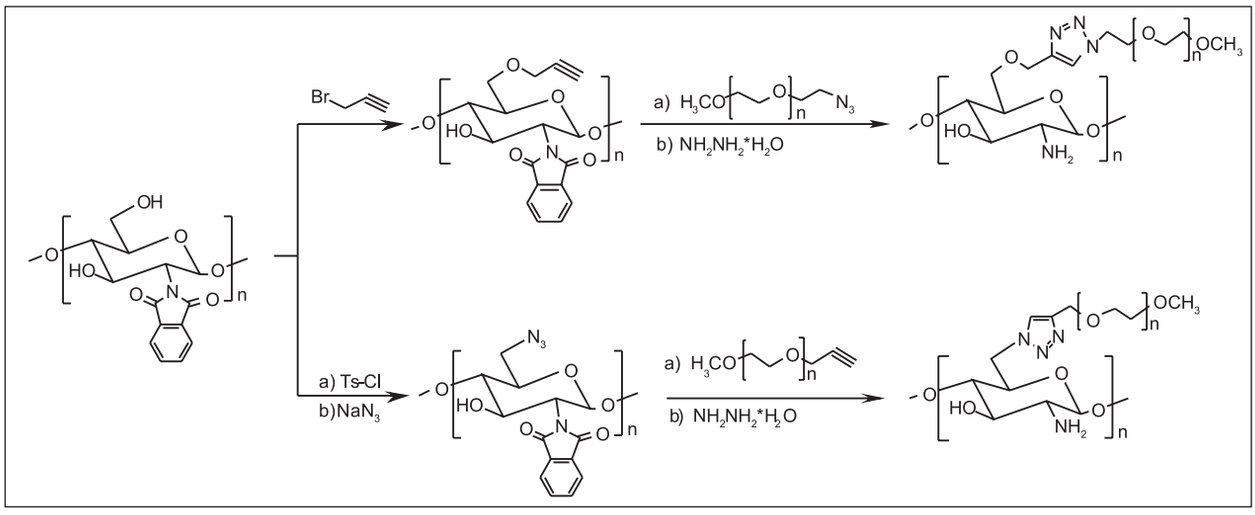
"Clicking" of MPEG onto N-phthaloyl chitosan

A series of chitosan-*C(6)*-MPEG comb copolymers differing in the degree of substitution (DS) of chitosan were synthesized by "click chemistry". Copolymers were obtained via 1,3-dipolar cycloaddition between *C(6)*-azidated chitosan and propargyl-terminated MPEG or *(C6)*-propargyl chitosan and azidated MPEG in mild conditions as shown in Scheme 3.

The "clicking" of MPEG onto chitosan was carried out in a mixture of DMSO and water, using $CuSO_4 \cdot 5H_2O$ as a catalyst and sodium ascorbate as a reductant. The actual catalyst Cu^I was prepared *in situ* by the reduction of the Cu^{II} salt which was proven to have several advantages against Cu^I, including the lower cost and a higher purity of Cu^{II} salts [30]. "Click chemistry" using azides and acetylenes is most effective when performed in water or in mixtures of water and organic co-solvents, such as *tret*-butanol, ethanol, DMSO, THF or acetonitrile [25].

The results of coupling MPEG to chitosan via "click chemistry" are summarized in Table. The DS of chitosan in its MPEG'ylated derivatives synthesized via the use of *C(6)*-azidated chitosan ranged from 39 to 59%, while the intrinsic viscosity of these copolymers was low and varied between 0.109 and 0.126 dL/g. The DS of chitosan in its MPEG'ylated derivatives prepared via *C(6)*-propargyl chitosan ranged within 20 to 64%, while the intrinsic viscosity of these copolymers varied between 0.102 and 0.132 dL/g. One can notice that the characteristics of the reaction products are similar despite the different precursors used in the two pathways.

The FT-IR spectra of chitosan-*C(6)*-MPEG copolymers showed intense bands typical of MPEG at 1108 cm^{-1} (C–O stretching) and 2886 cm^{-1} (C–H stretching), and a band of chitosan at 1648 cm^{-1}, assigned to the amide groups (Fig. 2).



Scheme 3. Synthesis of chitosan–*C(6)*–MPEG copolymers by "click chemistry"

Table. **Characteristics of chitosan–*C(6)*–MPEG copolymers prepared via "click chemistry"**

No.	Molar ratio		NH_2, %	DS(NH_2), %	DS(NMR), %	[η], dL/g
	***C(6)*-azidated chitosan (DA 57%)**	**Propargyl-terminated MPEG (DA 93%)**				
1	1	0.5	1.10	42	39	0.126
2	1	0.8	0.89	54	50	0.117
3	1	1	0.80	61	59	0.109
	***C(6)*-propargyl chitosan (DA 90%)**	**MPEG azide (DA 65%)**				
4	1	0.4	1.72	24	20	0.132
5	1	0.6	1.15	40	36	0.128
6	1	1	0.75	66	64	0.102

Completion of the reaction was assessed by the disappearance of the azide absorption band at 2110 cm^{-1}.

^{1}H NMR spectra confirmed formation of chitosan-MPEG copolymers (Fig. 3). The presence of a weak singlet assigned to the proton of the triazole ring at δ 7.91 evidenced that the attachment of MPEG to chitosan backbone was realized through a triazolyl-containing intermediate. A strong broad signal of the protons of oxymethylene groups of MPEG at 3.2–3.8 ppm prevailed in the spectra and overlapped with the signals of the protons H-3–H-6 of the chitosan backbone. The spectra of chitosan-*C(6)*-MPEG copolymers contained also signals at 1.7–1.9 and 2.9–3.1 ppm, attributed to the protons of residual acetyl groups of the chitosan and to H-2 protons of the chitosan backbone, respectively.

The phthaloylation-dephthaloylation procedure is known to lead to a partial destruction of chitosan [2]. Dephthaloylation of chitosan-*C(6)*-MPEG copolymers prepared via "click chemistry" was studied using hydrazine monohydrate solutions in DMF, DMSO, formamide, pyridine and water. The intrinsic viscosity of the copolymers was shown to depend slightly on the deprotection conditions giving the lowest viscosity in aqueous solutions. The most promising result was achieved using a 1.5 M hydrazine solution in pyridine where a 1.5 times higher intrinsic viscosity was determined compared to that of the products observed for the other solutions of hydrazine.

Dephthaloylation of chitosan-*C(6)*-MPEG copolymers prepared via "click chemistry" was attempted also by the use of methylamine. Methylamine and dimethylaminopropylamine

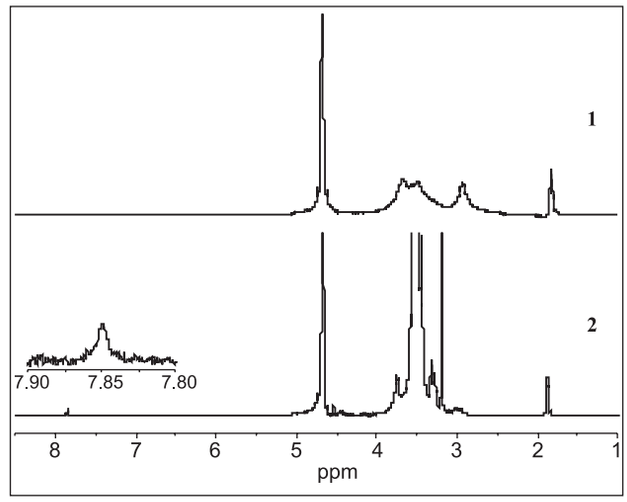
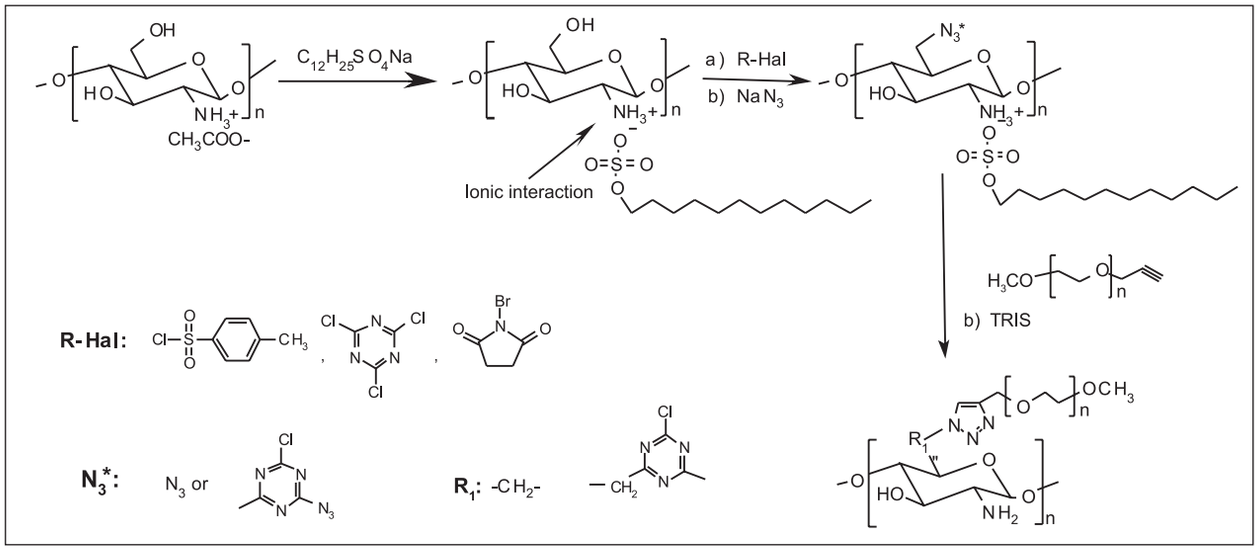


Fig. 3. ^{1}H NMR spectra of chitosan (1) and chitosan–*C(6)*–MPEG copolymer (DS 64%) (2) in D_2O



Scheme 4. "Clicking" of MPEG onto chitosan–SDS complexes

had been used earlier for dephthaloylation of amine functionalities in *N*-aminophthalimide derivatives [31], azetidinone compounds [32], and resins [33]. Our experiments were not successful, however. ^{1}H NMR and FT-IR spectra showed signals attributed to *N*-phthaloyl residues, which confirmed an incomplete removal of the protective groups.

According to the values of intrinsic viscosity in an acetate buffer, the chitosan-*C(6)*-MPEG copolymers were low-molecular-weight products [9]. Surprisingly, chitosan-*C(6)*-MPEG copolymers prepared using "click chemistry" reactions and isolated through rotary evaporation and vacuum drying were insoluble in water irrespective of the DS of chitosan. They were soluble in neutral water, however, when isolated by freeze-drying at –40 °C. All samples were soluble in an acetate buffer (pH 3.7).

"Clicking" of MPEG onto chitosan–SDS complexes

N-Phthaloyl chitosan as a precursor for the synthesis of *C(6)*-derivatives of chitosan has some drawbacks associated with a partial destruction of the products under protection–deprotection procedures [28, 34]. To avoid a significant degradation of the chitosan backbone, a different approach for protection of amino functionality, recently proposed for the regioselective modification of chitosan [26], was employed in the present study. The method is based on the use of sodium dodecyl sulfate – chitosan complexes (SCC) which are soluble in DMSO. SCC were prepared by mixing acidic aqueous chitosan and sodium dodecyl sulfate (SDS) solutions at equimolar amounts of the components (Scheme 4).

SCC was used as an intermediate for the synthesis of *C(6)*-azidated chitosan. The next step was "activation" of SCC,

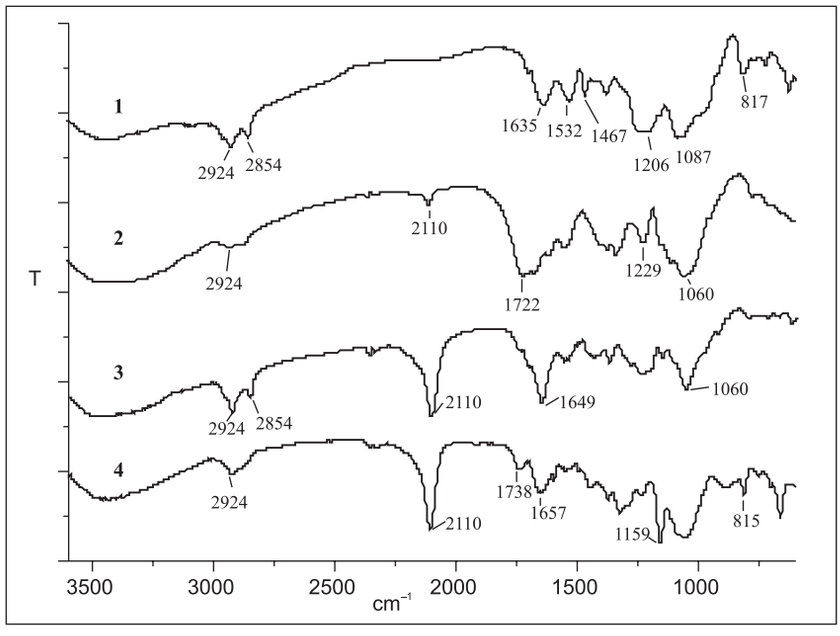


Fig. 4. FT-IR spectra of SCC (1) and SCC azidated through the use of trichlorotriazine (2), *N*-bromosuccinimide (3) and tosylchloride (4)

during which the complex was dissolved in a 4% LiCl / DMA solution and treated with excess *N*-bromosuccinimide, toluene-4-sulfonyl chloride (tosylchloride) or trichlorotriazine. The successful "activation" of SCC (incorporation of an active halide) was confirmed by elemental analysis, FT-IR or ^{1}H NMR spectrometry. The degree of bromination (mol. %) of SCC, calculated [35] from the content of bromine *Br* (%) covalently attached to SCC, was 91%. The degree of tosylation, calculated from ^{1}H NMR spectra comparing the ratio between the peak integration of protons from the tosyl group at 7.5–7.8 ppm and the proton H-2 of chitosan at 2.9 ppm, was 98%. The content of the dichlorotriazine moiety was not determined quantitatively. FT-IR spectra showed a characteristic absorption band at 1548 cm^{-1} (triazine); however, it was weak.

Azidation of "activated" SCC was done by the method used for azidation of "activated" *N*-phthaloyl chitosan. The FT-IR spectra of the azidated products showed an azide absorption band at 2110 cm^{-1}, which was strongest for the chitosan derivative obtained through tosylation of SCC (Fig. 4).

Unfortunately, the characteristic absorption bands at 1159 cm^{-1} and 815 cm^{-1} attributed to tosyl groups did not disappear, implying that the azidation of SCC was not full. Nevertheless, SCC azidated through tosylation was soluble in DMSO and was used for "clicking" with propargyl-terminated MPEG. The "click chemistry" reaction was unsuccessful, giving virtually no chitosan derivative. The unsuccessful "clicking" onto azidated SCC is possibly related to a partial destruction of SCC, which releases dodecyl sulphate anions. These anions interact with Cu ions present both in solution and in catalyst, thus destroying the catalyst or masking its active centers and making "clicking" impossible.

CONCLUSIONS

N-Phthaloyl chitosan derivatives containing azide or propargyl moieties at C(6) position of glucosamine units were synthesized for the first time. They are useful as precursors for modification of chitosan via "click chemistry" reactions. Novel chitosan-MPEG derivatives containing intermediate triazolyl moieties at C(6) position of glucosamine units were prepared by coupling via 1,3-dipolar cycloaddition between azide and propargyl groups of chitosan and poly(ethylene glycol) monomethyl ether (MPEG). The degree of chitosan substitution in novel copolymers was 20 to 65%, proving an efficient control of the graft density. The intrinsic viscosity of the chitosan derivatives was low, evidencing a significant breakdown of the chitosan backbone during modification. "Clicking" of propargyl-terminated MPEG onto azidated chitosan – dodecyl sulphate complexes was unsuccessful, giving virtually no graft copolymer.

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POLIETILENGLIKOLIO SKIEPIJIMAS PRIE CHITOZANO GLIUKOZAMINO GRANDŽIŲ C(6) PADĖTIES VYKDANT „KLIK CHEMIJOS" REAKCIJAS

S a n t r a u k a

Pirmą kartą susintetinti *N*-ftaloilchitozano dariniai, gliukozamino grandžių C(6) padėtyje turintys azido arba propargilo liekanas ir tinkami modifikavimui naudojant „klik chemijos" reakcijas. Vykdant reakciją tarp azido arba propargilo grupes turinčių chitozano ir polietilenglikolio monometileterio (MPEG), susintetinti nauji chitozano–MPEG dariniai, turintys jungiamąjį triazolil fragmentą gliukozamino grandžių C(6) padėtyje. Naujieji chitozano dariniai apibūdinti naudojant FT-IR ir ^{1}H BMR spektroskopiją, elementinę ir funkcinių grupių analizę bei viskozimetriją. Chitozano pakeitimo laipsnis kopolimeruose priklausė nuo azido ir propargilo grupių santykio, kito nuo 20 iki 65 % ir buvo lengvai valdomas. Šių chitozano darinių ribinis klampos skaičius buvo mažas, o tai rodo, kad chitozano modifikavimo metu iš dalies skilo jo pagrindinė grandinė. Buvo išbandytas alternatyvus chitozano–MPEG kopolimerų sintezės „klik chemijos" būdu variantas, azidinant chitozano–dodecilsulfato kompleksus, tačiau jis laukiamų rezultatų nedavė.