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Alkynyl polysaccharides: synthesis of propargyl potato starch followed by subsequent derivatizations

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Abstract—Potato starch propargyl ethers (PgS) with degrees of substitution (DS) from 0.1 to 2.2 have been prepared by etherification of starch with sodium hydroxide or Li dimsyl in Me_2SO and propargyl bromide. DS values and substituent distribution were determined after hydrolysis and acetylation by GC–MS. The order of reactivity was $2 > 6 \gg 3$, with O-3 substitution being preferably observed in the trisubstituted units. Repeated analysis of the starch derivatives revealed that propargyl residues were lost during storage, a phenomenon that was not fully understood until now. Selected PgS were further functionalized: (a) O- and C-methylated to *O*-(2-butynyl)-*O*-methyl starch (BMS), (b) in a Mannich type reaction with diethylamine and formaldehyde to yield *O*-(4-diethylamino)-2-butinyl starch (DEABiS), (c) in a 1,3-dipolar cycloaddition with benzyl azide ('click-chemistry') to a *N*-benzyltriazole derivatized starch (BTrS), and (d) with carbon dioxide to *O*-(3-carboxy)-2-butinyl starch (CBiS). While the yield of carboxylation was only poor, conversion was high or nearly quantitative for reactions a–c. Thus, it is demonstrated that starch propargyl ethers are valuable intermediates for the preparation of functional polysaccharides.

Keywords: Acetylenic carbohydrates; Starch propargyl ethers; Click-chemistry; Substituent distribution

1. Introduction

Functionalized polysaccharides are of interest for a wide field of applications from bulk products for paper and building materials to special applications in medicine or bioanalysis. Besides other structural parameters, the properties are influenced by the degree of substitution (DS) and the location of the substituents. For kinetically controlled base-induced etherifications, this usually depends on activation and solution state of the substrate and reagents, the amount of base, and the spatial requirements of the reagent. Mixed derivatives such as alkylhydroxylalkyl celluloses or amphiphilic starches with ionic and lipophilic substituents can be prepared in a one-pot reaction with the reagents competing for the most reactive positions, or by consecutive modification, the order of which determines the substituent distribution. Transformation of a reactive intermediate into a range of products with various functionalities offers an alternative approach, thus decoupling functional group interconversion from the steric and electronic effects of the polysaccharide backbone. In this way, polysaccharide derivatives with various functionalities, but identical substitution patterns, could become available, since the latter are established and can be influenced in the first step. Unsaturated ethers can serve as such intermediates. Terminal alkynyl groups are very interesting candidates since due to their CH acidity they can be functionalized under retention of their stiff triple bonds, but also modified by addition reactions, the 1,3-dipolar cycloaddition of azides presently being the most prominent reaction.[1,2] Gierlich et al. have, for example, used propargyl functionalized nucleotides to post-modify these with azide containing labels.[3] Terminal alkynes also exhibit metal complexing properties which might be valuable for water clearance.

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Concerning polysaccharides, there are some reports in the literature on various methods of preparation of propargyl celluloses. Masaidova et al. substituted cellulose tosylates with sodium propargyl alcoholate.[4] Another approach, allylation of cellulose followed by bromination and subsequent double dehydrobromination with *tert*-BuOK in Me_2SO, has been reported by Bhatt et al.[5] The polymer was characterized by IR spectroscopy and estimation of acetylenic H by exchange against Ag. Complexation of propargyl celluloses with $AlCl_3$, $BiCl_3$, $AgNO_3$, or $HgCl_2$ has been reported by Avny et al.[6] As an example of propargyl functionalization, aminomethylation was described by Rashidova and Valiev.[7] Further polymer-analogous transformations of propargyl cellulose giving acetonyl cellulose, 2-methoxypropenyl cellulose, or 2-(diethylamino)propenyl cellulose have been reported by Gal'braikh et al.[8] These compounds were not characterized with respect to their substituent distribution.

In a project aiming at the preparation and application of modified propargyl polysaccharides, we have performed model studies with methyl 4,6-*O*-benzylidene-2,3-di-*O*-propargyl-α-D-glucoside and the deprotected methyl 2,3-di-*O*-propargyl-α-D-glucoside, bearing two free OH groups.[9] In a number of reactions acetylide anions were prepared and trapped with various electrophiles to alcohols, aldehydes, carboxy acids and nitrogen containing derivatives up to nearly quantitative conversion and in 40–87% isolated yields. We now report on the preparation of propargyl starches, influence of reaction parameters, their further polymer analogous modification and the analysis of the substituent distribution in the glucosyl units.

2. Results and discussion

2.1. Preparation and substituent distribution of propargyl starches

Potato starch was propargylated with Li dimsyl or sodium hydroxide and propargyl bromide (PgBr) in Me_2SO. Samples were isolated by dialysis and freeze drying. PgS in a DS range of 0.1–2.2 were obtained. To study the influence of the amounts of base and PgBr, equivalents of sodium hydroxide were varied, keeping PgBr constant at 1 equiv/OH. The DS increased up to a maximum at 1.5 equiv/OH (DS 1.6) and then slightly decreased (Fig. 1). When the amount of PgBr was varied while keeping the molar ratio of NaOH/OH constant at 1.25, the DS increased to 1.76 at 1.75 equiv PgBr/OH and then decreased again (Fig. 2). In both cases the

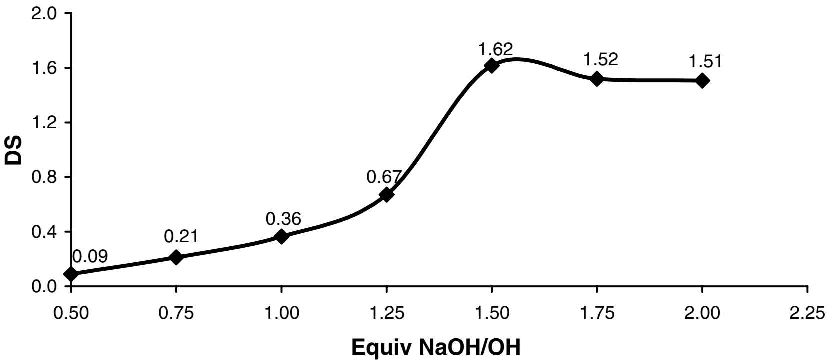


Figure 1. DS of PgS depending on the equivalents of NaOH/OH of starch used. Amount of PgBr was constant at 1 equiv/OH for all reactions.

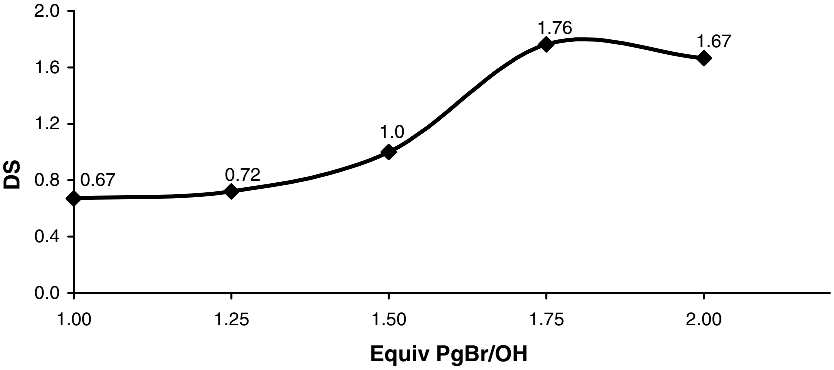


Figure 2. DS of PgS depending on the equivalents of PgBr/OH of starch used. Amount of NaOH was constant at 1.25 equiv/OH for all reactions.

course of the DS is unusual. The only slight increase of DS at low amounts of base and of PgBr and a steeper increase between 1 and 1.5 equiv NaOH/OH might be interpreted as base and also PgBr consumption by a competing side-reaction or equilibrium. With Li dimsyl, highest reagent efficiency (73%) was obtained with 1 equiv/OH of both base and PgBr yielding a DS of 2.2 (PgS 19). PgS were only partially soluble in solvents such as Me_2SO, THF, chloroform, methanol or water. The characteristic absorptions of $\nu(\mathrm{C}{\equiv}C\text{–}H)$ at 3280 cm^{-1}, $\nu(C{\equiv}C)$ at 2110 cm^{-1}, and $\delta(\mathrm{C}{\equiv}C\text{–}H)$ at 630 cm^{-1} are clearly visible in the ATR-IR-spectrum (Fig. 3). A very small absorption band at 1975 cm^{-1} probably indicates some allenyl ether formation by alternative S_N2' reaction or propargyl isomerization. These allenyl residues are lost during acid hydrolysis.[10] PgS were ochre coloured indicating by-products, which could not be separated by dialysis due to their insolubility. Colour was more intense at higher DS and more pronounced for the products obtained with NaOH compared to Li dimsyl. This side reaction, which we interpret as a polymerisation of the alkynyl halide, will be discussed later.

2.2. Analysis of propargyl starches

The DS and substituent distribution of PgS were determined by GLC analysis after hydrolysis and acetylation. When *O*-propargyl glucoses were reduced to glucitol derivatives with $NaBD_4$ in water as commonly performed to reduce the complexity of the analyte mixture, *O*-propyl and *O*-propenyl residues were observed besides residual propynyl groups. GC–MS analysis indicated that hydrogenated C_3-residues contained varying

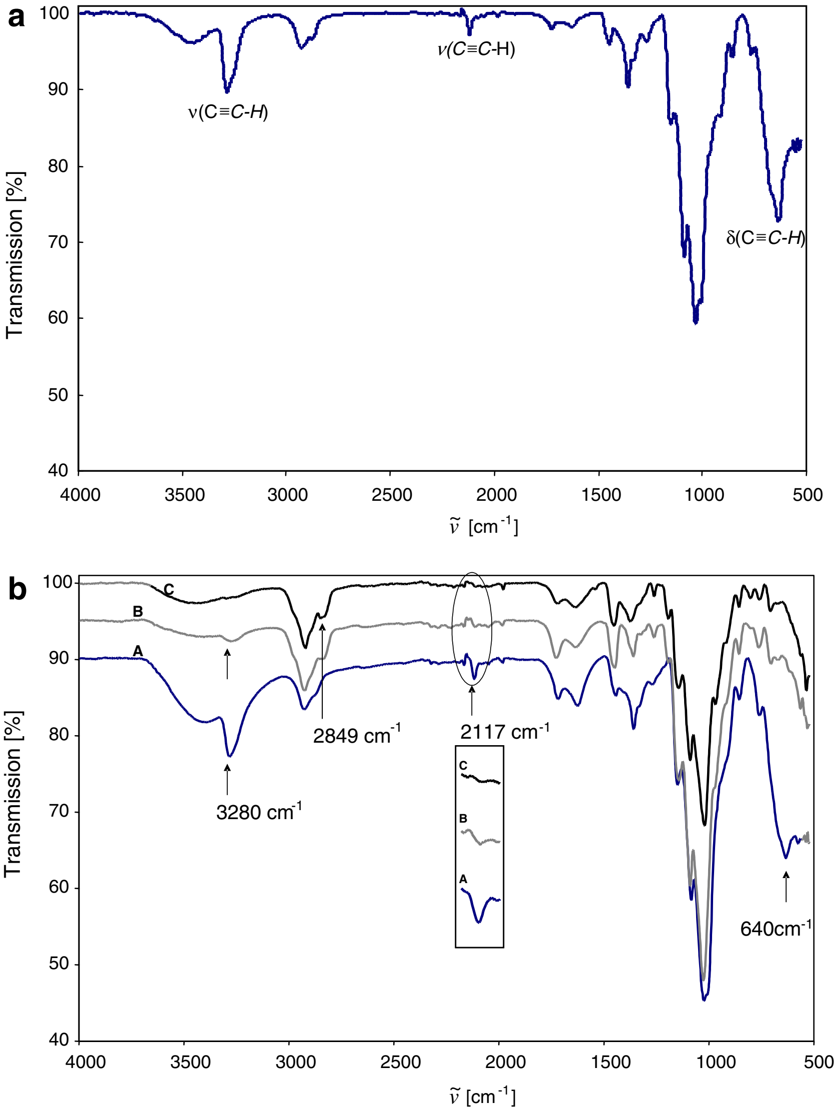


Figure 3. (a) ATR-IR spectrum of PgS 19 (1.0 equiv Li dimsyl/1.0 equiv PgBr, DS 2.2) and (b) ATR-IR spectrum of A: PgS 1 (DS 1.1), B: PgS 1, 1 × methylated, and C: PgS 1, 2 × methylated (BMS).

combinations of H and D. First, it was tried to achieve complete reduction by repeated addition of small portions of sodium borohydride. While finally hydrogenation was complete, up to 90% loss of Pg residues was observed at the same time. Presumably, the 2-propenyl carbanion intermediate isomerizes to its 1-isomer, which is subsequently reduced to the *O*-(1-propenyl) ether. This enol ether is not reduced further but readily hydrolyzed when the reaction is quenched with acetic acid.[10] Cleavage of propargyl ethers was proved with the pure methyl 2,3-di-*O*-propargyl glucoside. When this was submitted to hydrolysis and $NaBD_4$ reduction, a mixture of several products was obtained, which was analyzed by GC–MS. Glucitol (44 mol %), 2-*O*- and 3-*O*-propyl, 2-*O*- and 3-*O*-propenyl (11 mol % 2-, and 19 mol % 3-), and all propyl and propenyl combinations of 2,3-di-*O*-substituted glucitols (Σ26 mol %) were detected, indicating a higher lability of the 2- than the 3-*O*-propargyl residue. This might be interesting with respect to subsequent changes of the substitution pattern in favour of the less reactive positions. Because of these problems, it was tried to transform the propargyl to propyl starches by hydrogenation in the presence of Pd/C. Since the solubility of PgS was poor, starches were submitted to methanolysis prior to hydrogenation. Due to the different mechanism of concerted *cis*-hydrogenation, no isomerization was expected to occur. However, unfortunately partial loss of propargyl residues was also observed under these conditions. Pal et al. reported on the Pd-catalyzed deprotection of propargyl aryl ethers in water/DMF and proposed a mechanism via an allenylpalladium intermediate.[11] Therefore, the reduction step was abandoned and acetylation performed directly after hydrolysis.

Positions of *O*-propargylation in the α-and β-acetates obtained were deduced from their mass spectra. Assignment of 1,4,6-tri-*O*-acetyl-2,3-di-*O*-propargyl-α,β-D-glucose could be confirmed by comparison with the authentic standard. Figure 4 shows a gas chromatogram with the peaks assigned with respect to propargyl location. Regioselectivity of propargylation was $2 > 6 \gg 3$. It was striking to note that nearly all propargyl groups in 3-position of glucose were observed in di- and trisubstituted residues. Minor amounts of 2,3- and 3,6-di-*O*-propargyl glucoses were also detected and unfortunately were not separated, while 3-*O*-propargyl ether only occurred in traces, if at all. By GC–MS, the order of elution was found to be 3,6 > 2,3 > 2,3 > 3,6 for the two α, β peak pairs and characteristic fragments of 2,3-Pg-Glc being more abundant than 3,6-Pg-Glc. These results gave a first indication that reactivity of O-3 is enhanced by primary and secondary propargylation of an individual glucosyl unit. Figure 5 gives an overview on the monomer composition of a series of PgS with increasing DS.

Experimental data were compared with the monomer fractions calculated according to the models of Spurlin,[12] while comparison with the more differentiating model of Reuben[13] was not possible due to the comigration of 2,3- and 3,6-di-*O*-propargyl derivatives. Heterogeneity parameter[14] H_1 is calculated as follows:

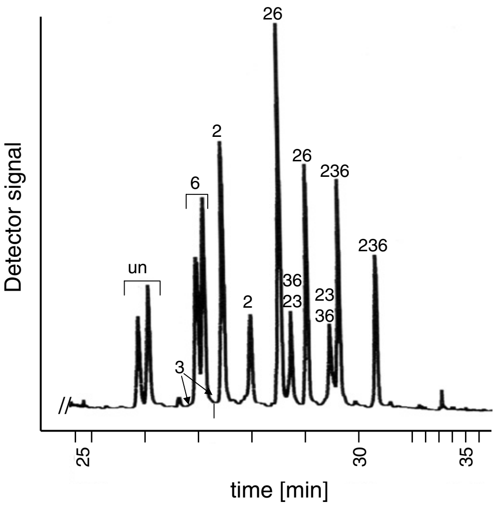


Figure 4. Gas chromatogram of PgS 19 (DS 1.95) after hydrolysis and acetylation. Peaks of α- and β-acetates are assigned with the positions of propargyl groups.

$$H_1 = \sqrt{\sum_{i=0}^{236} \Delta s_i^2} \qquad (1)$$

For PgS 18 reacted with the suspended base NaOH (1.5 equiv NaOH/1.0 equiv PgBr, DS 1.62), H_1 was 13.7. Under these conditions, the polysaccharide is adsorbed on the nanometer-scaled solid sodium hydroxide particles and locally deprotonated to a high extent to form highly alkylated domains.[15,16] Therefore, the observed deviation from the Spurlin model, higher molar ratios of un- and trisubstituted glucosyl residues, while mono- and disubstituted fractions are lower than those calculated by the model, is in agreement with previous results. For PgS 17 prepared with the Me_2SO-soluble base Li dimsyl (1.25 equiv Li dimsyl/1.0 equiv PgBr, DS 1.95), the monomer pattern deviates less from the statistical model, but heterogeneity is still uncommonly high with 10.2 for H_1. This might be caused by the poor state of dissolution of the potato starch used, and consequently improved local accessibility by primary substitution, but possibly also by the known self-association of terminal alkynyl groups, which can act both as a hydrogen bond donator and an acceptor.

2.3. Loss of propargyl residues

When we repeated the monomer analysis of the propargyl starches after several months, we found systematic deviations for all samples. The DS was significantly lowered for all starch derivatives. The relative compositions of 2,3,6-substitution changed in favour of the O-2-posi-

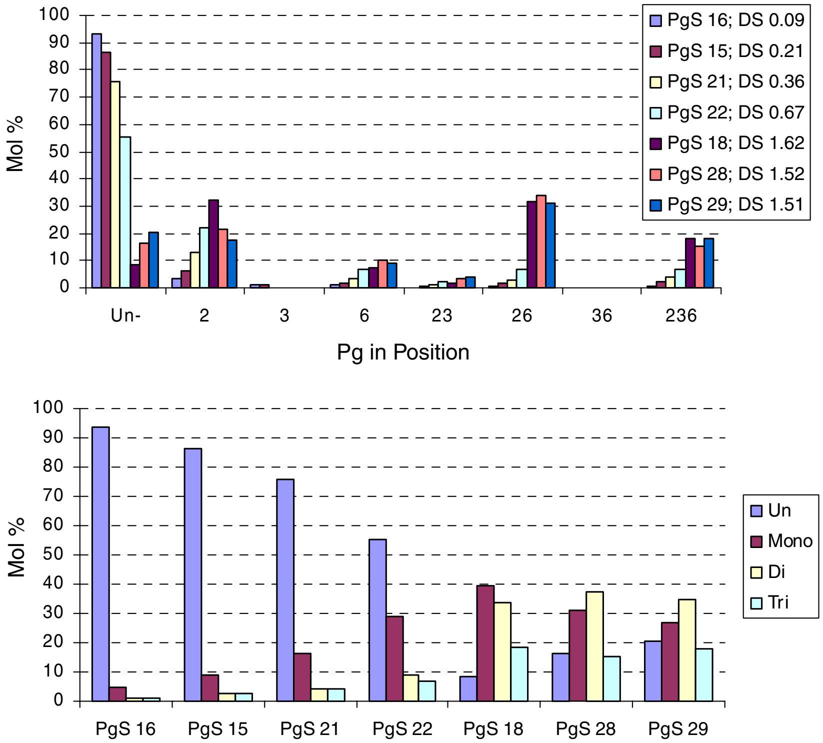


Figure 5. Monomer composition of PgS in the DS range of 0.09–1.51 (top) and fractions of un-, mono-, di- and trisubstituted glucosyl residues (bottom).

tion, which already has been the preferably substituted one. After 13 months of storage at room temperature, only about 65% of the propargyl groups were still found after hydrolysis and acetylation, while after 19 months nearly half of these residues were lost. For example, the DS of PgS 17 has decreased from 1.95 to 1.12, and for PgS 19 from 2.22 to 1.16 during this period. Control by ATR-IR spectroscopy confirmed this change (Fig. 6). Beside a pronounced decrease of the characteristic propargyl absorption bands at 3280, 2113, 1354,

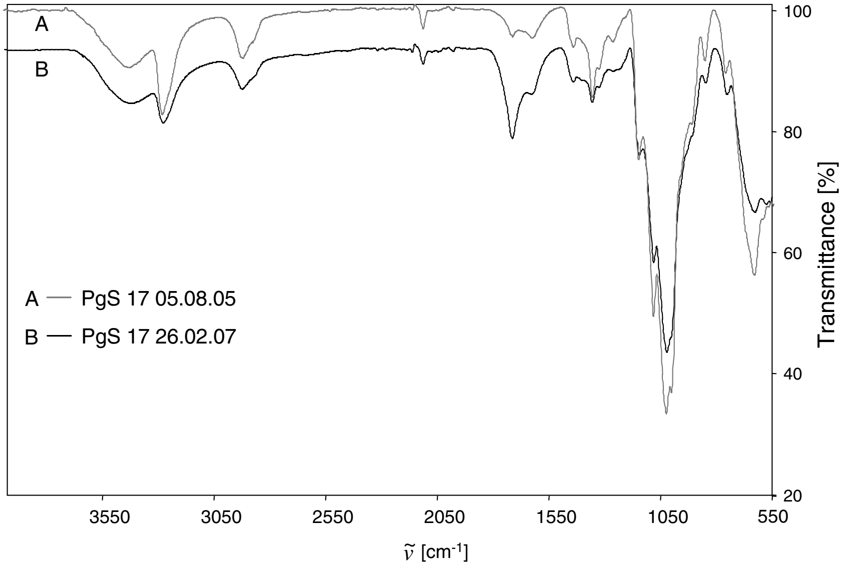
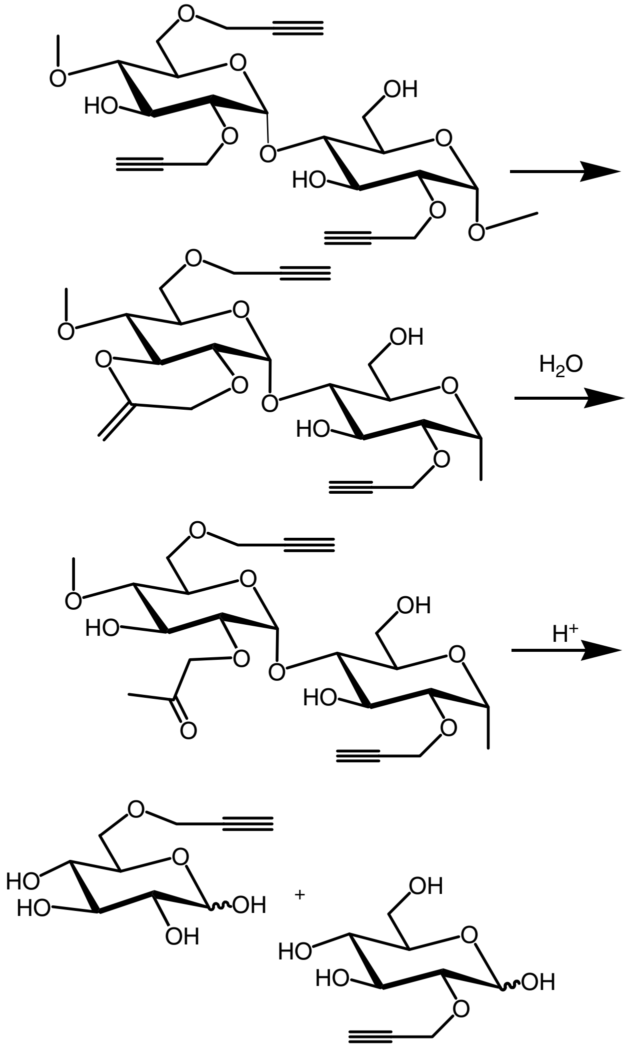


Figure 6. ATR-IR spectra of PgS 17 directly after preparation (DS after hydrolysis: 1.95) and after 19 months of storage at room temperature (DS after hydrolysis: 1.16).

and 630 cm^{-1}, a new absorption band occurred at 1712 cm^{-1}, which should be related to a carbonyl function and would best fit to a saturated ketone. In addition, the small absorption at 1625 cm^{-1} has become more intense during storage. However, OH absorption at 3430 cm^{-1} had not increased significantly, while by cleavage of propargyl ethers an increase would have been expected. We were very astonished by this surprising behaviour since propargyl ethers were expected to be stable as were our monomeric methyl 4,6-*O*-benzylidene-2,3-di-*O*-propargyl-glucoside model compound.[9] We assumed that due to co-operative interactions or by the influence of the remaining side-product in the sample, a reaction was slowly progressing under storage conditions, which usually only takes place in alkaline solution. Propargyl ethers are also used as protecting groups and their cleavage usually requires relatively harsh conditions. Isomerization to allenyl ethers, for example, with potassium *t*-butanoate,[10] enables subsequent cleavage under mild acidic conditions. However, no significant allenyl and no =C–*H* absorption band have been observed in the IR spectra of PgS (expected at ca. 1950 cm^{-1}), neither directly after preparation nor after storage. Therefore, a different mechanism must be responsible. Vasella and co-workers[17] and McDonald and co-workers[18] have described the formation of *exo*- and *endo*-glycals by intramolecular cycloisomerization of OH or other nucleophilic groups to terminal alkynes, especially when favoured five- and six-membered rings can be formed. Usually, this reaction proceeding via vinylidene intermediates is catalyzed by metal complexes such as chromium, tungsten or molybdenum pentacarbonyls. High yields are also reported when performed with NaH in DMF or with NaOMe/MeOH, but these conditions usually require an activation of the triple bond by a diynyl structure or by SiR_3 or bromine substitution and/or pre-positioning.[17] In the starch propargyl ethers, free OH, for example, at C-3, might react with the propargyl group at O-2 to form a six-memberd *exo*-glycal-like structure and vice versa (Scheme 1). This enol ether can be readily hydrolyzed to the corresponding (2-oxopropyl) ethers, which again can enolize to the *O*-(2-hydroxy-prop-1-enyl) ether and thus be cleaved off under the conditions of acid hydrolysis. The (2-oxopropyl) ethers will be in equilibrium with the hemiacetal, which corresponds to the addition of water to the exocyclic double bond. During their studies of cycloisomerization of bishomopropargylic alcohols, Trost and Rhee[19] observed a corresponding ketone as a side-product and discussed its formation via the *exo*-cyclisation product. Strict exclusion of water did not enhance the yield of the desired products. Thus they claimed that the enol ether also decomposes in the absence of water. The changes in the IR-spectra during storage are in agreement with this interpretation. The product of *exo*-cycloisomerization (Scheme 1) is assumed to be presented by the increase of the broad absorption around 1625 cm^{-1}. However, most of these cyclic enol ethers are cleaved to OH/*O*-(2-oxopropyl) derivatives, indicated by the new carbonyl absorption at 1712 cm^{-1}. Repetition of elemental analysis after 19 months of storage showed no significant change of C,H-content. In addition, no smell and no pressure formation in the sample vials were observed during storage, thus also supporting our assumption. To prove this we hydrolyzed the methyl 2,3-di-*O*-propargyl-α-D-glucoside, bearing free 4-OH neighboured to the 3-*O*-propargyl group after 20 months of storage. In contrast to the corresponding fully protected 4,6-*O*-benzylidene glucoside, which exclusively gave the 2,3-di-*O*-propargyl glucose, loss of propargyl residues was observed from position 3, or from O-3 and O-2, but no mono-3-*O*-propargyl glucose was observed. Some 2,3-di-*O*-propargyl derivative was also still present. This result is in accordance with the mechanism described.



Scheme 1. Possible pathway for propargyl loss in propargyl starches by cycloisomerizaton and hydrolysis.

2.4. Polymerization of propargyl bromide in Me_2SO under alkaline conditions

The colour and poor solubility of the PgS prompted us to look for side product formation. Elemental analysis of PgS 8 (DS = 0.5) gave a sulfur content of 1.5% which corresponds to a molar ratio of S:C of about 0.01. For PgS 13 with an original DS of 1.14 sulfur content was only 0.74, corresponding to a molar S:C ratio of 0.006.

When a control experiment was performed with NaOH and PgBr in Me_2SO without starch, brown flakes were instantaneously formed. The material was insoluble in any common solvent. Elemental analysis revealed only traces of bromine and a molar C:H ratio of 1:0.86. About 5% sulfur content corresponding to a molar C:S ratio of 1:0.03 was detected, thus indicating that the Me_2SO or dimsyl anion also participated in the reaction. C, H and S together covered 77% of the material. Polymerization of propargylic compounds has been the subject of several papers.[20–24] The side-reaction occuring during propargylation of starch will be the object of further investigations.

2.5. Polymer-analogous functionalization of propargyl starches

2.5.1. Methylation. In the next step, PgS was submitted to several selected transformation reactions that have been studied for the methyl glucoside model compounds.[9] In a first experiment, PgS 1 (DS 1.1) was methylated with Li dimsyl/methyl iodide in Me_2SO. While Li acetylides are reported to exhibit higher reactivity than Li alcoholates allowing selective C-methylation in the case of di-lithiated propargyl alcohol in liquid ammonia and slowly added methyl iodide,[25] both C-and O-methylation were observed as well in these experiments, yielding *O*-(2-butynyl)-*O*-methyl starch (BMS). Presumably, due to poor solubility, methylation was not complete even after two methylation steps as monitored by ATR-IR spectroscopy (Fig. 3b). However, finally, $\nu(OH)$ at 3450 cm^{-1}, $\nu(C{\equiv}C{-}H)$ at 3280 cm^{-1}, and due to enhanced symmetry of $-CH_2-C{\equiv}C-CH_3$ compared to $-CH_2-C{\equiv}C-H$, $\nu(C{\equiv}C)$ at 2117 cm^{-1} had nearly disappeared, while a new absorption band for $\nu(CH_3)$ occurred at 2850 cm^{-1}. ESIMS of the hydrolyzed sample shows signals at m/z values referring to sodium adducts of glucose without or with 1, 2, or 3 Pg groups in combination with 2, 3, or even 4 methyl groups. Beside terminal glucosyl residues this might indicate C-methylation of the activated 1-methylene group of propargyl residues. The triply methylated glucoses ($[M+Na]^+$ m/z (n_{Pg}): 245 ($n = 0$), 283 ($n = 1$), 321 ($n = 2$) and 359 ($n = 3$)) always presented the main constituent. Taking into account the side reactions of propargyl residues under alkaline conditions,[10,17,25] it must be assumed that propargyl residues were also partly lost during this reaction.

2.5.2. Carboxylation. Although carboxylation was not very efficient during our model studies (40% yield),[9] it was performed due to the interesting properties of this functionality for bioanalytical applications. Carboxy groups should impart water solubility and can be used for formation of bioconjugates via ester or amide linkages. Partial carboxylation was achieved for PgS 19 (DS 2.2) in Me_2SO with Li dimsyl and dried CO_2, as is evident from a new IR-absorption band at 1707 cm^{-1} for the C=O group and at 1588 cm^{-1} for the carboxylate. After methanolysis, $[M+Na]^+$ of methyl glucosides with all combinations of Pg (x) and Pg–COOMe (y) were detected in ESIMS with $x/y = 2/0$, 3/0, 1/1 and 0/2 showing peaks of highest intensity, followed by 0/1, 2/1 and 1/2. The product was not water-soluble.

2.5.3. Diethylaminomethylation. This mild and efficient copper(I)-catalyzed Mannich-type reaction in aqueous solvents[26] is predetermined for polymer analogous reactions on polysaccharides. Since in the model studies conversion of propargyl residues with aqueous formaldehyde and diethylamine in the presence of CuI was quantitative for both the protected and the deprotected methyl glucoside,[9] this reaction was applied to PgS 8 ($DS_{Pg} = 0.5$). Referred to the average molecular mass/AGU for 100% conversion, *O*-(4-diethylamino-2-butynyl)starch (DEABiS) was obtained in 95% yield. After hydrolysis $[M+H]^+$, $[M-H_2O+H]^+$, and $[M+Na]^+$ of mono-, di- and trisubstituted glucose residues were detected by ESIMS (Fig. 7). Due to the increasing ion yield with increasing numbers of basic nitrogen/molecule in electrospray ionization, unsubstituted glucoses are completely suppressed (as will be propargyl glucoses) and higher substituted residues are overestimated. However, from possible higher propargylated glucoses with incomplete aminomethylation only a trace of bifunctionalized tri-*O*-propargyl glucose was observed, indicating a very high degree of conversion. To finally obtain free propargyl amino groups, 4-piperidone hydrochloride hydrate will be employed, which can be deprotected with amines or ammonia.[27]

2.5.4. Cycloaddition to benzyl azide. This reaction, shown in Scheme 2, is also catalyzed by copper(I) and can be performed under aqueous conditions. Due to its high efficiency under mild conditions and its compatibility with many other functional groups, this so-called 'click reaction' is also a method of choice for polymer analogous reactions on polysaccharides. Recently, Binder and Sachsehofer gave a review on its application in polymer and material science.[28] It was performed here on PgS 13 (DS_{Pg} 1.1) with copper(II) sulfate and sodium ascorbate as reducing agent in Me_2SO–water (4:1) at room temperature. After hydrolysis of the dialyzed starch, again mono-, di- and trisubstituted glucose resi-

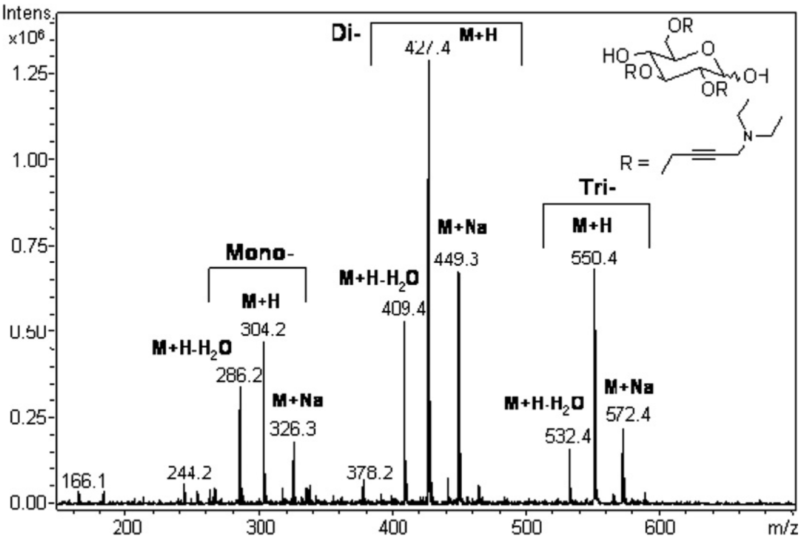
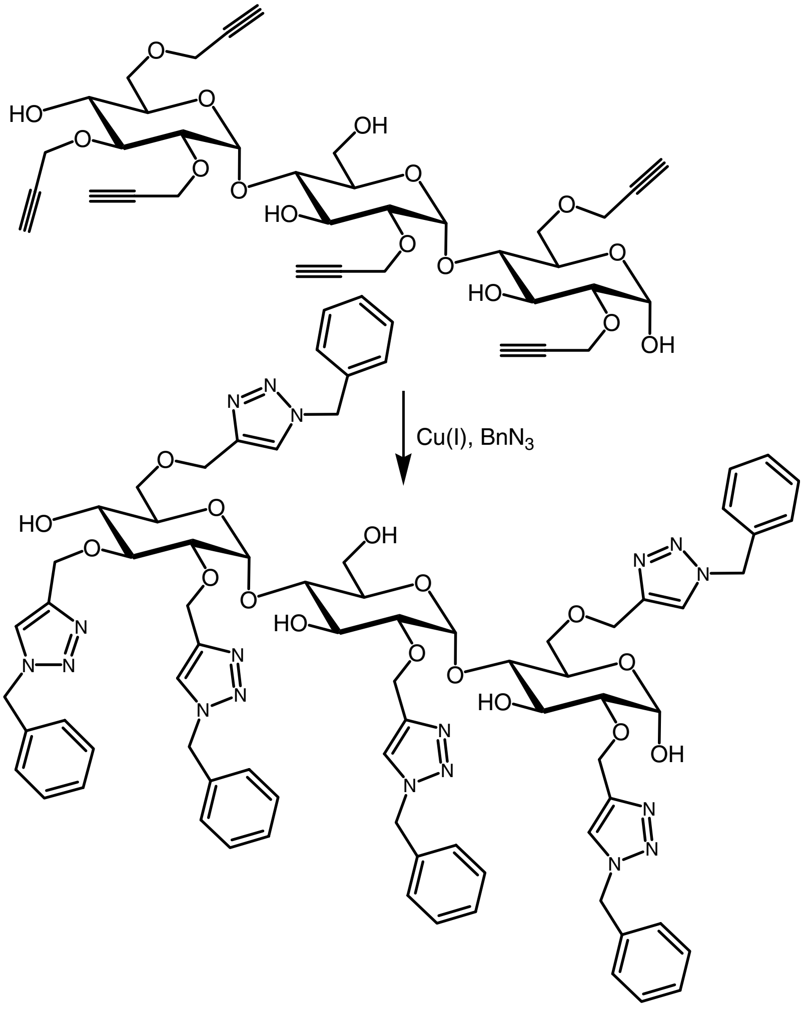
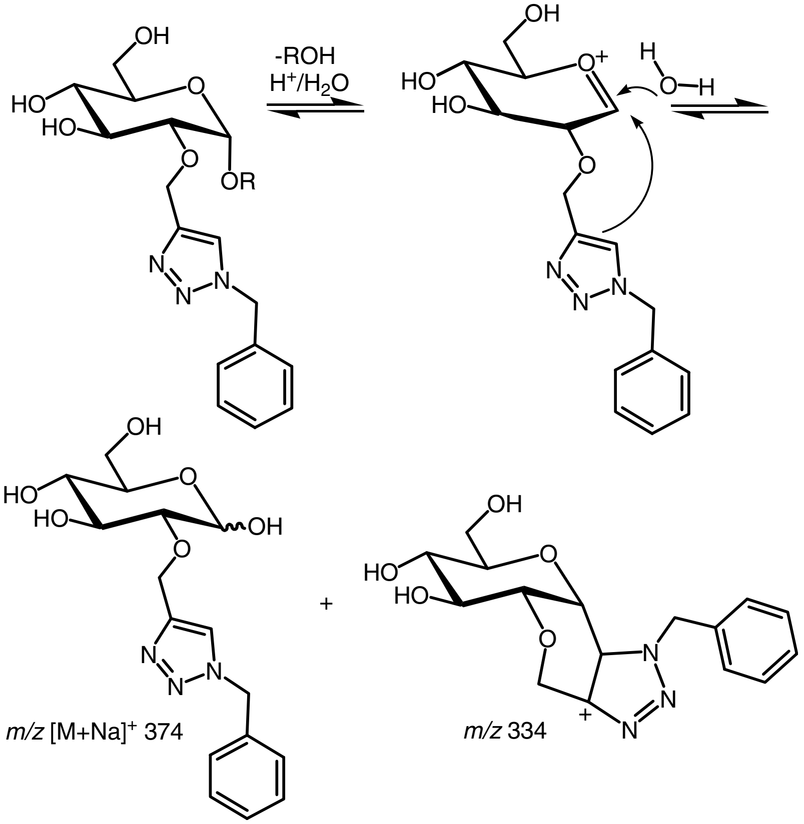


Figure 7. ESI mass spectrum of *O*-(4-*N*,*N*-diethylamino-but-2-ynyl) starch (DEABiS) obtained from PgS 8 (DS 0.5) after hyrolysis. Series of $[M+H]^+$ (*m*/*z* 304, 427, 550), $[M+H-H_2O]^+$ (*m*/*z* 286, 409, 532), and $[M+Na]^+$ (*m*/*z* 326, 449, 572) of mono-, di-, and trisubstituted glucoses are detected.



Scheme 2. 1,3-Dipolar cycloaddition of benzyl azide to PgS. Conditions see Section 4.



Scheme 3. Proposed intramolecular stabilization of the carboxoniumion of 2-*O*-(4-triazolylmethyl)-glucose derivatives in ESIMS as explanation for $[M-18]^+$.

dues were detected in ESIMS as $[M+Na]^+$ pseudo-molecular ions ($m/z = 374$, 545, 716). No di- or tri-*O*-propargyl glucoses with one or two triazole residues were detected, again indicating high conversion. Two additional peak series with m/z −40 and +40 compared to $[M+Na]^+$ are also observed. The first might be explained by loss of water in 2-*O*-substituted glucose residues by intramolecular nucleophilic reaction of the substituent with the carboxonium ion formed during hydrolysis, forming $[M+H]^+$ of the resulting products, as illustrated in Scheme 3. However, after acetylation, only the expected $[M+Na]^+$ ions of un-, mono-, and disubstituted glucose moieties are observed (m/z 413, 542, 671). This reaction shall be used to introduce fluorophores, receptors or bioactive molecules of choice for bioanalytical applications. Use of sulfonic acid azides allows the subsequent elimination of nitrogen from the triazole moiety to achieve sulfonamido derivatives as has been described by Cassidy et al.[29]

3. Conclusion

Propargyl starches (PgS) could be prepared in a wide range of DS values up to 2.2. IR spectra showed the typical absorptions of terminal alkynes. Substitution pattern could be determined after acid hydrolysis and acetylation, while reduction with sodium borohydride and hydrogenation caused significant losses of propargyl residues. The order of reactivity was $2 > 6 \gg 3$, with nearly no detectable 3-monosubstitution, but a relative high amount of 2,3,6-trisubstitution. Comparison of the monomer composition with the model of Spurlin (random) indicated a pronounced deviation, indicating a reactivity-enhancing effect of primary propargylation of a glucosyl moiety. Self-association of propargyl residues which can act both as hydrogen bond donors and acceptors could also be of influence. Favoured by excess of reagents, a brownish and insoluble side product is formed. Changes during storage of PgS were observed by IR spectroscopy resulting in lowered DS values after hydrolysis. This loss is presumably initiated by intramolecular addition of free OH to the triple bond and subsequent hydrolysis of these enolic structures. Methyl (O- and C-), carboxy-, diethylaminobutynyl-, and triazole derivatives could be obtained by subsequent polymer analogous transformations of the propargyl residues.

4. Experimental

4.1. General

All reagents were of highest purity available and purchased from Fluka, Aldrich or Merck. Native potato starch was a gift from Lyckeby, Stärkelsen, Kristianstad, Sweden. Amylose content was 22%, and the degree of branching was 3.1%.

4.2. Instrumentation

All analytical reactions were carried out in 1 mL V-Vials in a heating block with a stirring and an evaporation unit from Barkey, GmbH & Co. KG, Germany.

Infrared spectra were recorded using a Bruker Tensor 27 attenuated total reflectance infrared (ATR-IR) spectroscopy.

Gaschromatographic analysis was carried out with a GLC-FID instrument Carlo Erba GLC 6000 Vega Series 2 with a CPSil 8 column (25 m), a retention gap (1.5 m), and H_2 as carrier gas. Injection was carried out on-column. The temperature program starts at 60 °C for 1 min, heats with 20 °C/min to 130 °C, and heats again with 4 °C min^{-1} to 290 °C, remaining constant for 30 min. Data were recorded with a Merck Hitachi D 2500 Chromato-Integrator. For peak identification GLC–MS with an Agilent 6890 GLC and a TSQ 700, Finnigan MAT mass spectrometer was applied. The GLC was equipped with a HP-5 column (30 m, 0.32 mm ID, 0.25 μm). A split injection port at 250 °C was used for sample application, and the split ratio was set at 5:1. Helium carrier gas was set to 1.6 mL min^{-1} flow rate (constant flow mode). Transfer line was kept at 250 °C. The MS was operating in electron impact ionization (EI) mode at 70 eV with an ion source temperature of 150 °C.

Electrospray ionization mass spectra (ESIMS, positive mode) were recorded on an Esquire LC (Bruker Daltonics, Bremen, Germany). The partially degraded samples were dissolved in MeOH, and introduced directly via a syringe at a flow of 200 μL h^{-1}. Nitrogen was used as drying gas (4 L min^{-1}, 300 °C) and as nebulizer gas (10 psi). The following voltages were used: capillary 4500 V, end plate offset −500 V, capillary exit 120.0 V, skim 1 40.0 V and skim 2 10.0 V.

4.3. Propargylation of starch

Potato starch was dried at 90–100 °C for 6–10 h before use. Dried starch (100–200 mg ≙ 0.62–1.23 mmol AGU) was dispersed under nitrogen in dry $\mathrm{Me_2SO}$ at room or slightly elevated temperature (22–50 °C, ca. 10 mg/mL). 2–3 equiv/AGU of freshly prepared 1.5 M $\mathrm{CH_3SOCH_2{}^-Li^+}$ was added under nitrogen. After 0.5–3 h propargyl bromide (2.8–32 equiv/AGU) was added under ice cooling and subsequently stirred at room temperature for 1.5 bis 3 d. The product was isolated by dialysis (MWCO 12,000) and freeze drying. When water was added, PgS precipitated. Ochre coloured solids were obtained in an apparent yield of 99–145%, calculated on the basis of the determined DS.

Propargylation with NaOH was performed according to Ciucanu and Kerek.[30] NaOH was varied between 0.5 and 2.0 equiv/OH (PgBr: 1 equiv/OH) and in a second series 1.25 equiv NaOH/OH and varying amounts of PgBr (1.0–2.0 equiv/OH) were added. The mixture was stirred for 72 h at room temperature. The products were isolated as described.

4.4. Monomer analysis

PgS (ca. 2 mg) was hydrolyzed with 2 M TFA at 120 °C in a 1 mL V-Vial for 3 h. TFA was evaporated under nitrogen and co-distilled with toluene. The residue was acetylated with 200 μL $\mathrm{Ac_2O}$ and 50 μL pyridine for 3 h at 90 °C. The reaction was quenched with water. The products were extracted with dichloromethane, washed with $\mathrm{NaHCO_3}$, cold 0.1 M HCl (aq), and water. The dried organic phase was used for GLC analysis. Peak areas were corrected according to the ECR concept (factors: un: 1.000, mono: 0.845 (2 and 3) and 0.855 (6); di: 0.731 (2,3) and 0.739 (2,6 and 3,6): tri: 0.650).

4.5. Methanolysis/methyl ester formation

Ca. 2 mg PgS or modified PgS was stirred with 1 mL of 1.5 M methanolic HCl in a 1 mL V-Vial at 90 °C for 3 h and subsequently evaporated at 40 °C in a stream of nitrogen with repeated additions of dry methanol.

4.5.1. *O*-(2-Butynyl)-*O*-methyl starch (BMS). Methylation of PgS was performed according to the procedure described for propargylation with Li dimsyl. PgS 1 (DS = 1.1), 20 mg (0.1 mmol AGU) was dispersed in 2.5 mL dry $\mathrm{Me_2SO}$. 10 equiv/($\mathrm{OH} + {\equiv}\mathrm{CH}$) of $\mathrm{Li^+CH_3SOCH_2{}^-}$ and MeI is used. After two methylation steps, 15 mg of *O*-(but-2-ynyl)-*O*-methyl or *O*-(2-butynyl)-*O*-methyl starch (BMS) was obtained as a pale yellow powder. IR spectroscopy still showed weak OH absorption. ESIMS after hydrolysis: $[\mathrm{M+Na}]^+$ m/z (n(Pg)/n(Me)): 245 (0/3); 259 (0/4); 269 (1/2); 283 (1/3); 297 (1/4); 307 (2/2); 321 (2/3); 359 (3/3).

4.5.2. *O*-(3-Carboxy-prop-2-ynyl) starch (CPS). PgS 19 (DS = 2.2), 50 mg (0.20 mmol AGU) was dispersed in 7 mL THF in an ultrasonic bath. *n*-Butyllithium (0.5 mL) (0.74 mol; 1.6 M in hexane) was added dropwise at −40 °C during 5 min and the reaction mixture was stirred for 2.5 h. After coming to room temperature, $\mathrm{CO_2}$ (s) was added under vigorous stirring. After 4.5 h saturated NaCl solution (12 mL) was added. The product was isolated by dialysis and freeze drying. Due to poor conversion (IR), the product was dispersed in dry $\mathrm{Me_2SO}$ (7 mL) at 80 °C under ultrasonic treatment. Within 10 min 5 equiv/AGU $\mathrm{CH_3SOCH_2{}^-Li^+}$ (2.2 mL;

1.5 M in Me_2SO) was added at room temperature and the mixture stirred for 3 h. Under ice cooling, CO_2 (g) dried over a molecular sieve was passed through the reaction mixture under thorough stirring. After the exothermic reaction had ceased, CO_2 was introduced for a further 1.5 h. The reaction mixture was poured into 20 mL NaCl solution and subsequently dialyzed against distilled water. After freeze drying 56 mg of *O*-(3-carboxy-prop-2-ynyl) starch (CPS) was obtained as a yellowish solid.

IR (diamant-ATR): $\tilde{\nu}$[cm^{-1}] 3383, ν(O–H-Val, b), 3279, ν($\equiv$CH, s), 2924, ν(C–H, aliph., m), 2238, ν (C$\equiv$C, 1,2-disubst. acetylene, w), 2117, ν(C$\equiv$C, monosubst. acetylene, w), 1588, ν(C=O in COO$^-$, carboxylate, s), 1443, 1350, 1150, 1086, 1024, 635. ESIMS (after methanolysis, giving methyl *O*-(3-methoxycarbonyl-prop-2-ynyl glucosides)): $[M+Na]^+$ *m*/*z* (*n*(Pg)/*n*(Pg–COOMe)): 217 (0/0); 255 (1/0); 293 (2/0); 313 (0/1); 331 (3/0); 351 (1/1); 371 (2/1–H_2O); 389 (2/1); 409 (0/2); 429 (1/2–H_2O); 447 (1/2); 505 (0/3). $[M-H_2O+Na]^+$ might be caused by intramolecular lactone formation.

4.5.3. *O*-(4-*N*,*N*-Diethylamino-but-2-ynyl) starch (DEABiS).

PgS 8 (DS(GC) 0.5, 55 mg, 0.3 mmol AGU), diethylamine (0.12 mL, 85 mg, 1.16 mmol), aqueous formaldehyde (35%, 0.6 mL), and Cu(I)-iodide (6 mg, 0.03 mmol) dissolved in 5 mL Me_2SO were stirred at 35–40 °C for 76 h. Three millilitres of 2 M NaOH was added and 65 mg of the brownish product was obtained by dialysis against distilled water and freeze drying corresponding to an apparent yield of 95% (0.29 mmol). CHN found: C, 44.07; H, 6.01; N, 2.46; C/H, 7.34; C/N, 17.91; H/N, 2.44; (calculated for $DS_{Pg} = 0.59$ and $DS_{DEABi} = 0.49$: C/H, 7.35; C/N, 17.89; H/N, 2.43), corresponding to a conversion of 83%.

IR (diamant-ATR): $\tilde{\nu}$[cm^{-1}] 3307 ν(O–H, b), 2970, 2933 ν(C–H_n, aliph., m), 1356 ν(C–N, amine, s). ESIMS after hydrolysis: $[M+H-H_2O]^+/[M+H]^+/[M+Na]^+$: *m*/*z* 286/304/326 (monosubstituted), 409/427/449 (disubstituted), 532/550/572 (trisubstituted). No di- or trisubstituted glucosides with incomplete conversion were detected.

4.5.4. *O*-(*N*-Benzyl-[1,2,3]-triazoyl)-methyl starch (triazole starch, BTrS).

To a solution of PgS 13 (DS 1.1, 53 mg, 0.29 mmol alkynyl) in 4:1 Me_2SO–water (7 mL) and benzyl azide (115 mg, 0.86 mmol), freshly prepared 1 M Na L-ascorbate solution (34 mg, 0.17 mmol) and $CuSO_4{\cdot}5H_2O$ (12 mg, 0.05 mmol) were added. After stirring for 6 h at room temperature, water (20 mL) was added and the mixture cooled to 0 °C. The precipitated brownish solid was filtered off, washed twice with 20 mL of cold water and dried under diminished pressure. Triazole starch (63 mg) was obtained corresponding to 75% yield calculated for 100% conversion.

IR (diamant-ATR): $\tilde{\nu}$[cm^{-1}]: 3388 ν(O–H, b), 2928 ν(C–H, aliph., m), 1497 ν(C=C arom., m), 1456 δ(CH$_n$ aliph., m), 1362, 1332 ν(C–N, m). ESIMS (after hydrolysis): $[M+Na]^+$ *m*/*z* 374 (monosubstituted), 545 (disubstituted), 716 (trisubstituted); additional series corresponding to $[M-H_2O+H]^+$ and $[M+63]^+$ are detected.

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