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Preparation and structural characterisation of *O*-aminopropyl starch and amylose

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

O-Aminopropyl starch was prepared by Michael addition of acrylonitrile and subsequent reduction with freshly prepared cobalt boride and sodium borohydride. In a second approach, the aminopropyl group was introduced via Williamson etherification with *N*-phthalyl-protected 3-bromo-1-propylamine. The protecting group was removed by borohydride reduction and subsequent hydrolysis in acetic acid. The DS of all samples and the degree of reduction of the cyanoethyl groups were estimated from the ^{1}H NMR spectra. Total monomer composition was determined after methanolysis or hydrolysis and trimethylsilylation by GLC and GCMS. While the regioselectivity in the thermodynamically controlled reaction was O-6 > O-2 > O-3 (50:37:13), the kinetically controlled process showed strongly preferred O-2-etherification (up to 94%) followed by O-6- and O-3-substitution. It could be influenced by choice of solvent (water, Me_2SO) and base (NaOH, Li-dimsyl).

Keywords: Starch derivatives; Aminopropyl starch; Substitution pattern; Monomer analysis; Kinetic and thermodynamic control

1. Introduction

Polymers with amino functions are of interest for metal coordination,[1,2] as carriers for contrast agents for magnetic resonance imaging,[3] in waste water flocculation,[4] as additives in paper manufacturing,[5] or as ion exchangers, e.g., diethylaminoethylcellulose (DEAE-cellulose). Formulation of cosmetics and hair conditioners are further potential applications.[6] In contrast to polysaccharides bearing quaternary ammonium groups (''cationic starches'', e.g., *O*-(3-trimethylammonium-2-hydroxypropyl)starch), the charge density and number of nucleophilic functions can be tuned by the pH in the case of primary amino groups. Due to the reactivity of amino groups, they are of interest in biosensor techniques, for biofunctionalisation of surfaces,[7] and for the binding of enzymes or antibodies.[8]

Chitosan, as a natural aminopolysaccharide, has found wide interest and applications.[9] Chemical modification of biopolymers like cellulose and starch presents an interesting alternative to the application of oil-based polymers such as polyamidoamines, since biodegradability can be expected at low degrees of substitution. Berlin et al. prepared amino-modified celluloses by O-6-tosylation and nucleophilic displacement of the primary tosylate with a diamine.[8] Direct etherification of polysaccharides with aminoalkyl halides[3,10] or by addition of ethylenimine (aziridine)[11] does not yield uniform products since amino groups compete with the sugar alcoholates for the alkyl halide. Tandem reactions and polymerisation of the reagent are expected as side reactions. One strategy to avoid these side reactions is the introduction of nitrogen in a higher oxidation state, i.e., as azide, nitro or cyano group. Cyanoethylation has been used to introduce nitrogen by Michael addition.[12–14] For the reduction to aminopropyl functions, hydrogenation with Raney-nickel or Raney-cobalt[15] would be the method of choice, but the cooperation of amino groups was found to deactivate the catalyst.[1] Therefore, Verraest et al.[1]

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used cobalt chloride/sodium borohydride, and reported the reduction with metals in liquid ammonia (Birch reduction) as most effective. Daly and Munir[14] prepared aminopropyl cellulose via cyanoethylation and reduction with diborane dimethylsulfide or tetrahydrofuran complex under reflux. Heeres et al. introduced nitrogen via nucleophilic addition of a nitroalkene, which is formed in situ from 2-nitropropylacetate, however, tandem reaction of the nitroalkyl group and side reactions in the reduction step prevented uniform product formation.[16] Another strategy uses *N*-protection for temporary blocking of its nucleophilicity. Phthalimides have been widely used for the preparation of amino-deoxy sugars by nucleophilic displacement of, e.g., an *O*-tosylate and subsequent hydrazinolysis of the phthalimide.[17]

Dunn et al.[18] reacted chitosan with various *N*-(bromoalkyl)phthalimides to introduce a spacer between the amino groups and the polysaccharide backbone. After hydrazinolysis, the formation of *N*-(aminoethyl)-chitosan was reported.

It is well known that, apart from the type of functionalisation and the degree of substitution (DS), the substitution pattern also determines the properties of polysaccharide derivatives. Therefore, it is of interest to investigate how the method of modification influences the distribution of the substituents. In this paper, we report on the preparation of *O*-(3-amino)propyl amylose and starch via cyanoethylation, and as an alternative by etherification with an *N*-protected amino alkyl halide. DS and monomer composition were determined by ^{1}H NMR spectroscopy and GCMS analysis after hydrolysis or methanolysis and trimethylsilylation.

2. Results and discussion

Introduction of nitrogen in a higher oxidation state.— One approach for the preparation of amino sugars is the introduction of nitrogen in a higher oxidation state, usually as cyano, nitro or azido group. Cyanoethylation of starch and cellulose, and inulin has been studied extensively.[1,12–14] Due to competing side reactions, the equivalents of base and acrylonitrile, the amount of water, reaction time and temperature must be optimised to achieve high reaction efficiency. Undesired reactions are the reversibility of the nucleophilic addition, the addition of water instead of sugar, and the formation of amides and carboxyethyl groups from the nitrile group. The reaction was carried out according to Verraest et al.[12] in a 1 mmol scale in an ultrasonic bath. Samples were purified by dialysis and then freeze-dried. Products from larger scale preparations were isolated by precipitation in ethanol and washing. The DS_{CE} was calculated from the ratio of the ^{1}H NMR signals of the CH_2CN functionality and the summarised H-1 signals of the glucosyl moieties (Eq. (1)).

$$DS_{CE} = \frac{1/2\int CH_2CN}{\int \text{H-1}_{1,4\text{-Glc}+t\text{-Glc}} + \int \text{H-1}_{2\text{-O-CE-Glc}} + \int \text{H-1}_{\text{branched Glc}}} \quad (1)$$

In addition, the partial DS at O-2 can be estimated (Eq. (2)) from the H-1 signals since O-2-etherification causes a downfield shift of 0.3 ppm while O-3- and O-6-substitution do not.

$$\text{2-O-CE (\%)} = \frac{\left(\int \text{H-1}_{2\text{-O-CE-Glc}}\right)\cdot 100}{1/2\int CH_2CN} \quad (2)$$

The proton chemical shifts of CE-starches are listed in Table 1. NMR data for potato starch reported by Nilsson et al.[19] were used as reference data. Results for a series of cyanoethylations are given in Table 2. The reaction efficiency was between 81 and 92%. The relative amount of O-2-substitution can only roughly be estimated from the NMR spectra since integration of the very small and broad H-1 signal for the 2-O-substituted glucosyl units is not exact. The average value is about 39% and therefore in good agreement with those calculated from the monomer analysis (37%).

Reduction of cyano groups can usually be achieved by hydrogenation with Raney-nickel or similar catalysts as Raney-cobalt, but was not successful in the case of cyanoethyl inulin.[1] Good results were obtained by Birch reduction with sodium in liquid ammonia.[1] Daly and Munir reported on quantitative reduction of *O*-cyanoethylcellulose with diborane complexes under reflux.[14] De Brabender-van den Berg and Meijer[15] described the hydrogenation of cyano groups in the synthesis of poly(propylenimin)-dendrimers. In spite of the high density of cyano and subsequent amino groups, the reaction was successful with Raney-cobalt under optimised reaction conditions. Borohydride/transition metal salt systems have also been used for the reduction of organic nitriles, nitro and amide compounds.[20] We applied cobalt chloride in combination with sodium borohydride. In contrast to the mechanism reported by Verraest et al.[1] cobalt boride, instead of cobalt(0), is formed as a black precipitate when the reagents are combined.[21] Then the nitrile is activated by adsorption on the freshly prepared solid, and reduction requires an excess of sodium borohydride. The reaction pathway is given in Scheme 1. By this method, up to 90% reduction was obtained for CES3 with a DS of 0.17. However, reproducibility of the method was unsatisfactory. The degree of reduction was calculated from the ^{1}H NMR data by comparison of the remaining CH_2CN and the new occurring $CH_2CH_2NH_2$ and CH_2NH_2 signals. Only slight losses of substituents were observed

Table 1
Characteristic chemical shifts of *O*-cyanoethyl- (CE), *O*-aminopropyl- (AP), and *O*-(*N*-phthalyl)aminopropylstarch (PAP)

Protons	δ (ppm)		
	O-Cyanoethyl-	*O*-Aminopropyl-	*O*-(*N*-Phthalyl)-aminopropyl-
Glc-H-1,(4→1)-linked and terminal	5.50	5.50	5.52
Glc-*H*-1,1,4,6-branched	5.08	5.08	5.10
Glc-*H*-1,2-*O*-CE	5.82		
Glc-*H*-1,2-*O*-AP		5.75	
Glc-*H*-1,2-*O*-PAP			5.80
Glc-*H*-2,3,4,5,6	3.5–4.3	3.5–4.3	3.5–4.3
C*H*$_2$CN	3.07		
C*H*$_2$CH$_2$NH$_2$		2.26	
CH$_2$C*H*$_2$NH$_2$		3.36	
C*H*$_2$CH$_2$N-Phth			2.02
CH$_2$C*H*$_2$N-Phth			3.23
N-Phth-*m*-*H*			7.64
N-Phth-*o*-*H*			7.80

^{1}H NMR in D_2O (δ *H*DO = 4.80 ppm).

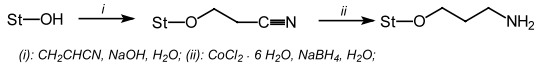
Table 2
Cyanoethylation of starch

Sample	Equiv. CH_2CHCN/AGU	DS (NMR)	Reaction efficiency (%)	2-*O*-CE (%) (NMR)	2:3:6 (GC)
CES1	0.038	0.035	92	45	37.5:14.2:48.3
CES2	0.115	0.094	82	29	n.d.
CES3	0.191	0.170	89	40	34.6:12.6:52.8
CES4	0.267	0.215	81	40	37.8:13.5:48.7
CES5	0.344	0.293	85	38	36.9:13.2:49.9
		Average	86	38	37:13:50

DS values calculated from ^{1}H NMR and from monomer composition (GC).

due to retro-Michael addition (β elimination) under the alkaline reaction conditions. The decrease of the total DS ($DS_{CE} + DS_{AP}$) was between 0 and 19 and 11% on average. Chemical shifts of appropriate signals are also given in Table 1. Cyanoethyl starches showed slightly improved water solubility compared to the original potato starch used. Aminopropyl starches gave a blue precipitate with $CuSO_4$-solution. In contrast to $Cu(OH)_2$, the solid remained blue and did not change to brown.

Monomer analysis.—After acid hydrolysis of the cyanoethyl starch, the glucose derivatives were analysed by GC and GCMS as their *O*-trimethylsilyl ethers/glucosides (Fig. 1(A)). All monosubstituted α- and β-glucosides ($M = 521$) could be assigned by EI-MS. Typical fragments of per-*O*-trimethylsilyl-glucosides[22] are the C_2-ring fragments at m/z 204, which are mainly formed from C-2–C-3, but also from C-3–C-4. If O-2 or O-3 is cyanoethylated, this fragment is partly shifted to m/z 185, since the mass difference of $SiMe_3$ compared to CN is −19. A further valuable diagnostic C_3-fragment at m/z 217 contains C-2–C-3–C-4 and the substituents at positions 2 and 4. That means that this fragment is shifted to m/z 198 only in the case of O-2-substitution, since O-4 is always trimethylsilylated in our case. By rearrangement of the substituent at O-3 to C-1, fragment ion m/z 191 is formed from per-*O*-$SiMe_3$-glucosides, while methyl *O*-$SiMe_3$-glucosides give m/z 133. Therefore, the signal at m/z 172 is characteristic for $SiMe_3$ derivatives, 3-*O*-cyanoethyl glucose. The mass spectra of the two 6-*O*-cyanoethyl regioisomers are very similar to those of the unsubstituted glucosides, because the exocyclic residue is easily lost during fragmentation. None of the ring fragments mentioned above is shifted. Disubstituted glucose ethers were not detected. When



Scheme 1.

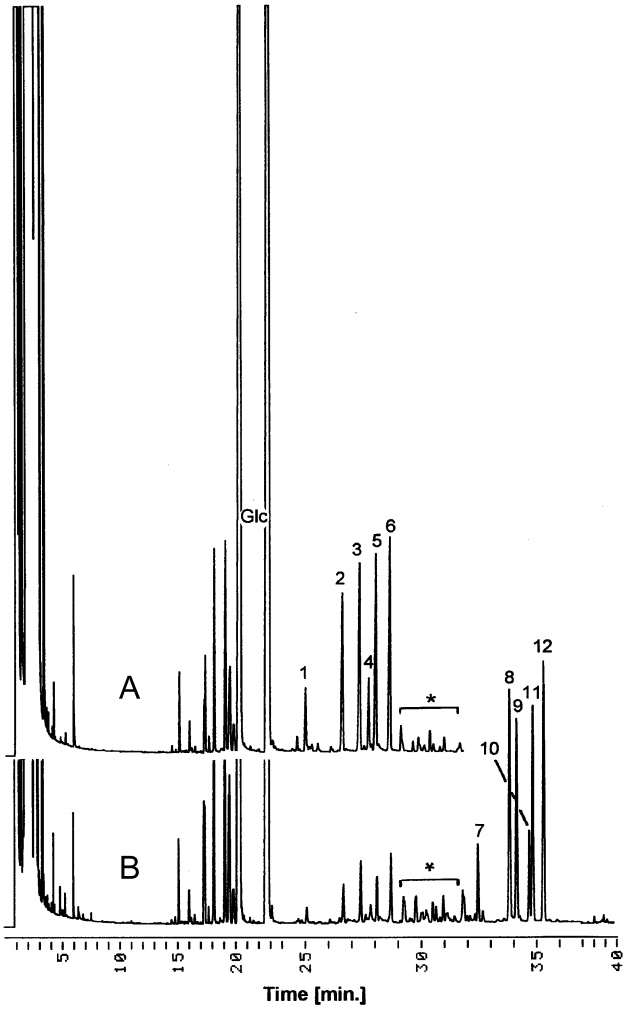


Fig. 1. Gas chromatograms of the constituents obtained by hydrolysis and trimethylsilylation from (A) cyanoethylstarch (CES) and (B) after partial reduction to aminopropylstarch (APS). Glc, trimethylsilyl 2,3,4,6-tetra-*O*-trimethylsilyl-α,β-D-glucosides; 1–6, trimethylsilyl mono-*O*-cyanoethyl-tri-*O*-trimethylsilyl-α,β-D-glucopyranosides; 1 and 4, 3-*O*-CE; 2 and 5, 2-*O*-CE; 3 and 6, 6-*O*-CE; 7–12, trimethylsilyl mono-*O*-(*N*-di-trimethylsilyl)aminopropyl-tri-*O*-trimethylsilyl-α,β-D-glucopyranosides; 7 and 10, 3-*O*-AP; 8 and 11, 2-*O*-AP; 9 and 12, 6-*O*-AP. *, corresponding *O*-carboxyethyl-glucosides as $SiMe_3$ derivatives, formed during hydrolysis from cyanoethyl groups.

methanolysis was applied instead of hydrolysis, significant amounts of methoxycarbonylethyl ethers were detected as side products from methanolysis of the nitrile function. Shift of diagnostic fragments as mentioned above was +14 now. The order of reactivity of cyanoethylation was found to be O-6 > O-2 > O-3. When reaction conditions (time) allowed thermodynamic control, the relative ratio was 37:13:50 (O-2:O-3:O-6) on average. For larger scale preparations, enhanced 2-O-substitution was found, which was in the order of 41–50%, while 35–43% of the substituents were located at O-6 and 15–16% at O-3. Reduction studies were mainly carried out with CES3 (DS 0.17). After reduction, the monomer composition was calculated from the gas chromatogram of the hydrolysed and trimethylsilylated sample (Fig. 1(B)). Unsubstituted, *O*-cyanoethyl, *O*-carboxyethyl, and *O*-aminopropyl glucosides could be detected as their *O*-$SiMe_3$ derivatives. The mass spectra of the amino compounds were dominated by the favoured α-cleavage fragment $[CH_2{=}N(SiMe_3)_2]^+$ at m/z 174. The position of substitution was deduced from the strong discrimination of characteristic ring fragments, while only traces of the corresponding shifted fragments ($\Delta m/z = +129$) were observed, if any. So, m/z 333 and 346 could be detected for the 2-*O*-aminopropyl glucoside, and the fragment at m/z 333 for the 3-*O*-aminopropyl ether as well. Here, the intensity of the normal fragment from rearrangement (m/z 191) was only strongly decreased, while the shifted fragment was not observed. Recovery of the aminopropyl-substituted glucosides was not reproducible and sometimes very poor, especially for the 6-*O*-aminopropyl glucosides which were strongly discriminated. It turned out that the components were adsorbed on the glass surface of the V-vials, as could be shown by a positive ninhydrin reaction. Therefore, all glassware was silylated prior to use to prevent substance losses. The relative ratios of O-6-:2:3-substitution were the same for CE- and AP-ethers within experimental error. This means that the rate of reduction is independent of the position of substitution.

Introduction of nitrogen via etherification with an N*-protected aminoalkyl halide.*—A potential drawback of the strategy just described is the occurrence of nucleophilic amino groups beside electrophilic cyano groups during the reduction step, which might cause side reactions. A second one arises from the difficulties in the reduction of polymer-linked nitrile groups. Therefore, the introduction of nitrogen in the desired oxidation state but blocked with a protecting group, should be the priority. In carbohydrate chemistry, the phthalimide functionality is well known for the preparation of amino-deoxy sugars.[17] We used *N*-(3-bromopropyl)-phthalimide for the etherification of amylose in different systems. In aqueous solution, with sodium hydroxide as base, reactivity was very poor (DS 0.004), presumably due to the bad solubility of the lipophilic reagent in the polar solvent. Reaction of starch with NaH in DMF at room temperature was also ineffective (DS 0.02). Slightly better results were obtained with sodium hydroxide in Me_2SO/tetrabutylammonium bromide (TBAB) (DS up to 0.12), while the highest reactivity was observed for amylose with Li-dimsyl in Me_2SO and TBAB as an additive (DS up to 0.29). It was difficult to purify the product from the excess of reagent and phthalimide side products. Prior to dialysis, the sample was extracted with toluene and dichloro-

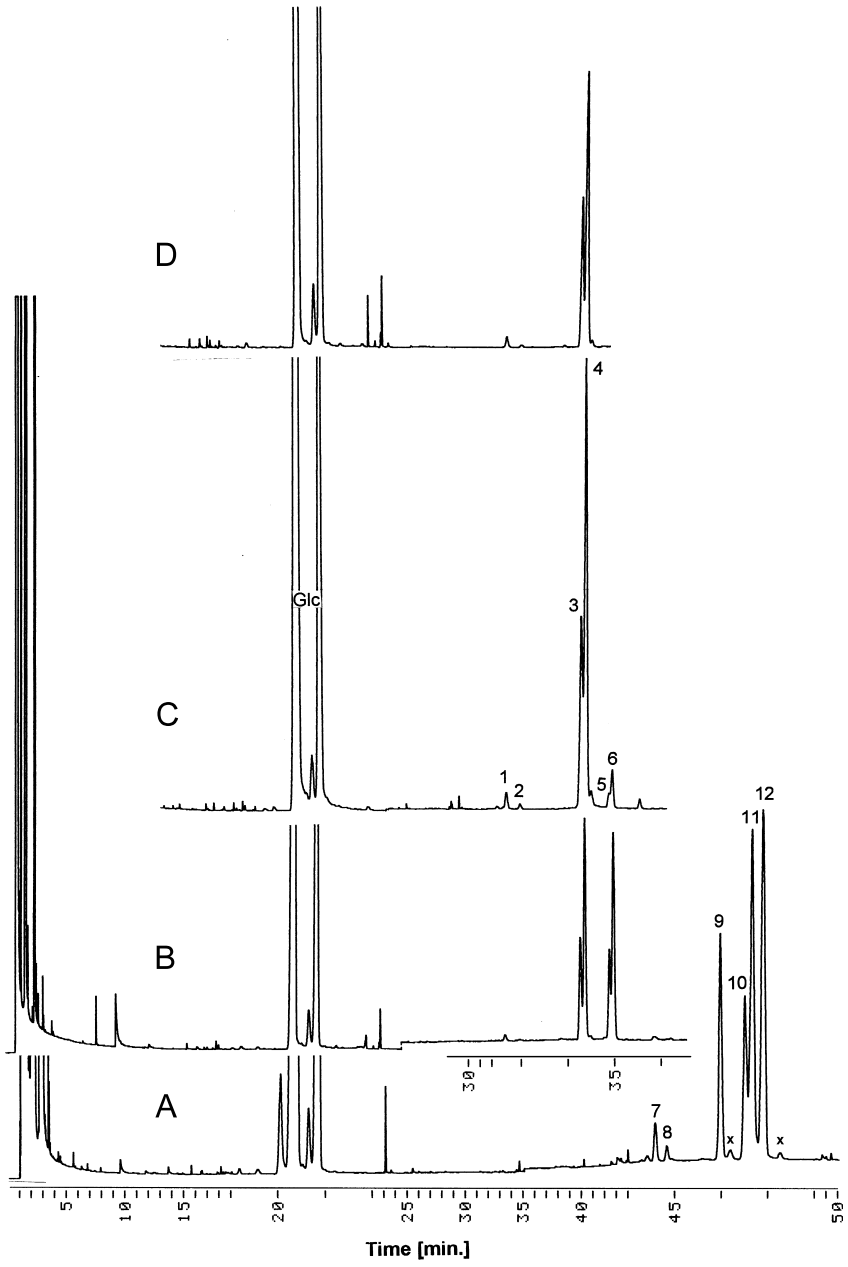


Fig. 2. Gas chromatograms of the constituents obtained by methanolysis and trimethylsilylation from (A) *O*-(*N*-phthalyl)-aminopropyl amylose PAP8; (B) aminopropyl amylose APA8; (C) APA11; and (D) APA5. Glc, methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α,β-D-glucopyranosides; 1–6, methyl mono-*O*-(*N*-di-trimethylsilyl)aminopropyl-tri-*O*-trimethylsilyl-α,β-D-glucosides; 1 and 2, 3-*O*-AP; 3 and 4, 2-*O*-AP; 5 and 6, 6-*O*-AP; 7–12, methyl mono-*O*-(*N*-phthalyl)aminopropyl-tri-*O*-trimethylsilyl-α,β-D-glucopyranosides; 7 and 8, 3-*O*-PAP; 9 and 11, 2-*O*-PAP; 10 and 12, 6-*O*-PAP; ×, probably methyl mono-*O*-(*N*-phthalyl)aminopropyl-tri-*O*-trimethylsilyl glucosides.

methane several times. Products were investigated by ^{1}H NMR. Chemical shifts are listed in Table 1. DS values estimated from NMR are always higher than those calculated from the monomer composition due to the impurities. Therefore, signals of the *N*-alkyl-phthalimido group in NMR are not sufficient to prove the covalent linking of the reagent, especially at low DS values, as it has been done by Dunn et al.[18] However, GC and GCMS analysis after methanolysis and trimethylsilylation of the polymer (Fig. 2(A)) undoubtedly shows whether or not the glucosyl residues are etherified with the protected aminopropyl residues. In the gas chromatogram, two major peaks and up to six minor peaks were observed with $M = 597$ as deduced from CI-MS spectra, corresponding to methyl-mono-*O*-(*N*-phthalyl)aminopropyl-tri-*O*-trimethylsilyl glucosides. Disubstituted compounds were not detected. Although the basic fragments in EI-MS are formed by the substituent (m/z 160

Table 3
Examples for the synthesis of *O*-(*N*-phthalyl)aminopropyl (PAP) starch/amylose (from potato) by etherification with *N*-(3-bromopropyl)-phthalimide in different base/solvent systems

PAP	Educt	Base/solvent	*TBAB*	DS (NMR)	DS (GC)	Substitution in position		
						2	3	6
1	Amylose	$NaOH/H_2O$	+	n.d.	0.004	84	8	8
2	Starch	NaH/DMF	−	0.017	n.d.		n.d.	
3	Starch	$NaOH/Me_2SO$	−	0.021	n.d.		n.d.	
4	Amylose	$NaOH/Me_2SO$	+	n.d.	0.057	92	5	3
5			+	n.d.	0.124	94	5	1
6	Amylose	Li-dimsyl/Me_2SO	−	(0.14)	0.053	72	4	24
7	Starch		−	0.13	n.d.			
8	Amylose	Li-dimsyl/Me_2SO	+	0.27	0.15	48	4	48
9			+	0.37	0.24	66	3	31
10			+	0.25	0.26	90	3	7
11				0.28	0.23	83	3	14

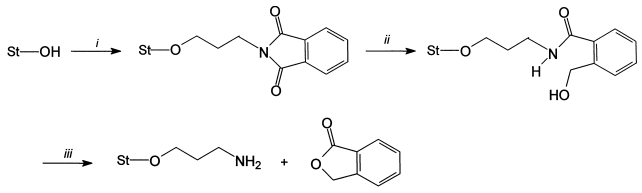
n.d., not determined.

and m/z 188, as the basic fragment), and are therefore not characteristic of the substituted position, some diagnostically valuable fragment shifts allowed the assignment of the regioisomers following the rules for the cyanoethyl ethers as outlined above ($\Delta m/z$ for *N*-phthalyl-aminopropyl compared to $SiMe_3 = +115$). The main products could be unambiguously identified as methyl 2-*O*-(*N*-phthalyl-3-amino)propyl-*O*-trimethylsilyl α- and β-glucosides (partial shift of m/z 204 to 319, shift of m/z 217 to 332). The *O*-(*N*-phthalyl)-aminopropyl amyloses prepared with Li-dimsyl in Me_2SO showed further isomeric peaks, which could be assigned as the 6-O- and the 3-O-substituted methyl glucosides. The 3-O-substituted components showed a partial shift of m/z 204 to 319, and of m/z 133 to 248, while m/z 217 remained unshifted. For the 6-*O*-PAP glucosides, new characteristic fragment ions were observed at m/z 260, 261 and 320. Some minor peaks with typical fragments of the functional groups and similar retention time as the monosubstituted components could not be identified (× in Fig. 1(A)). However, from earlier investigations of cationic and *O*-sulfobutyl ethers of glucans, we know that furanosides might be formed to a higher extent than expected for glucose when the 2-OH is functionalised.[23]

The results of monomer analysis with regard to the reaction conditions are shown in Table 3. With Me_2SO as the solvent and NaOH (1.0–1.3 equiv/OH) as the base, nearly exclusive O-2-substitution was observed (92–94%), followed by O-3 and -6-substitution. This is in agreement with the well-known strongly preferred O-2-reactivity of starch and amylose under kinetic control. For example, tosylation exclusively occurs at O-2, while O-6-tosylation is preferred in (1→4)-β-glucans as cellulose.[24] The highest DS was achieved in Me_2SO/Li-dimsyl/TBAB with a reaction efficiency of about 10%. Although the same proportion of base and phthalimide was used, now only 48–92% of the substituents were located in 2-O-position, 2–6% in 3-O-position and 5–48% at O-6 (not all samples are shown in Table 3). This might be explained by higher effective base concentration in the Li-dimsyl/Me_2SO system, since sodium hydroxide is not really dissolved in Me_2SO, but the reaction occurs at the interphase of solid NaOH particles on which the glucan chains are adsorbed.[25,26] With the stronger base Li-dimsyl, a polyanion is formed in the first step prior to the addition of the electrophile to avoid alkylation of the sulfoxide. In contrast, base and electrophile are present at the same time in the NaOH/RX system. The poor reproducibility is presently not understood and might be influenced by the solution state. It was obvious that the products from a clear and orange reaction solution exhibited a high degree of O-2-substitution (> 80%) followed by O-6 and -3-substitution (Table 3, PAP 10 and 11). If the mixture was rather green at the beginning of the reaction, and dark orange and turbid at the end, the isolated products showed a comparably high rate of 6-O-substitution (PAP6, 8 and 9). Although the reactivity could be improved by addition of TBAB, it is still unsatisfactory, presumably due to the differences in polarity of the hydrophilic amylose and the hydrophobic phthalimide. Microwave irradiation has been reported as a valuable alternative for organic reactions.[27] However, attempts to improve the reactivity by microwave treatment were not successful.

Deprotection of the amino group.—Cleavage of phthalimides is usually performed by hydrazinolysis. Osby et al.[28] reported a mild method including reduction of one imide linkage by borohydride and subsequent hy-

drolysis of the remaining amide in acetic acid under formation of the *o*-hydroxymethylbenzoic acid lactone. This method could be adapted to the requirements for the polymer analogous reaction. Using 6:1 *i*-propanol–water[28] and 9:1 methanol–water[29] and a fivefold excess of sodium borohydride for phthalimide reduction, the samples did not dissolve and coloured brown. No amino function was detected in the liquid phase. The rate of reduction was in the order of 60% but recovery of the amino and phthalimido groups was not satisfactory. In consequence, we used a mixture of 1:3 glycerine–water for complete dissolution of the sample. Major problems occurred in removing the glycerine



Scheme 2. (i): N-3-Br-propyl-Phth, Li-dimsyl, Me_2SO_4; (ii) $NaBH_4$, H_2O/MeOH (7:3, v:v); (iii) add HOAc, reflux.

Table 4
DS and relative substituent distribution of aminopropyl amylose samples prepared via deprotection of the *O*-(*N*-phthalyl)aminopropyl group

APA	Base/solvent	Substitution in position		
		2	3	6
5	$NaOH/Me_2SO$	95	4	1
8	Li-dimsyl/Me_2SO	49	2	49
9		69	3	28
10		90	3	7
11		90	3	7

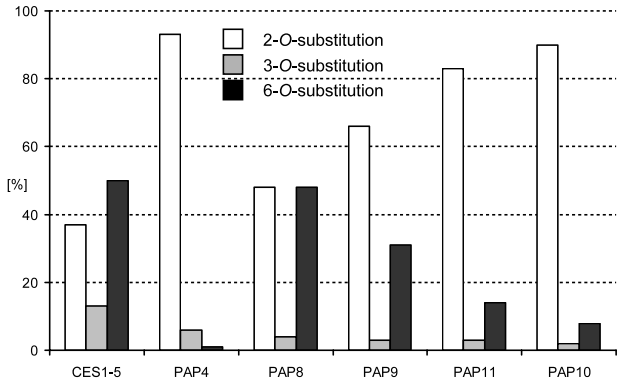


Fig. 3. Regioselectivity of *O*-aminopropyl glucans in dependence on the reaction pathway and the conditions applied. For the cyanoethylation-route (Scheme 1) the average of CES1-5 (Table 2) is given. The numbers of the APA obtained via the phthtalimide-route (Scheme 2) refer to Tables 3 and 4.

without losing the product. Again, reduction was around 60% and recovery was poor. Various solvent combinations in different ratios were tested. The samples were first dissolved, a solution of borohydride in a tenfold excess was added drop-wise and the mixture was stirred overnight at room temperature and subsequently heated to 50 °C for different lengths of times. Reaction was followed by TLC where the amino groups were detected via ninhydrine reaction. Complete deprotection of the amino group with quantitative recovery of the aminopropyl amylose could be achieved in 3:7 methanol–water. The suspension was stirred overnight and heated to 50 °C for 5 h before the addition of acetic anhydride. The reaction pathway is shown in Scheme 2. The aminopropyl amylose obtained was investigated by ^1H NMR spectroscopy and GCMS analysis after methanolysis and trimethylsilylation in silylated V-vials (Fig. 2(B–D)) as described above. By comparison with the retention times of the corresponding products obtained on the cyanoethyl route, peak assignment could be confirmed. The relative ratios of 2:3:6 substitution were in accordance with the composition of the *N*-protected components. While APA8 from the reaction with Li-dimsyl in Me_2SO showed O-2 and -6-substitution in a ratio of 1:1 (Fig. 2(B)), APA11 in contrast showed preferred 2-regioselectivity (Fig. 2(C)), while APA5, prepared with NaOH in Me_2SO was nearly exclusively etherified in position 2 (Fig. 2(D)). Results are given in Table 4.

The monomer composition of the aminopropyl glucans calculated from the analysis of the primary intermediates and obtained by the different reaction routes is summarised in Fig. 3. Nearly exclusive O-2-substitution, but also nearly equal O-2 and -6-aminopropylation can be achieved by the appropriate choice of reaction route and conditions applied.

Relative reactivities in carbohydrate etherification under basic conditions and kinetic control correspond with the acidities of the OH groups. Due to the inductive effect of the neighbour acetal, the 2-OH shows the highest acidity in aldoses. The deprotonation is additionally favoured in the α configuration by interaction of the 2-OH with the axially oriented α-glucosidic oxygen. With increasing amounts of base, the selectivity generally decreases and the order changes in favour of the 6-position. Due to sterical effects, reaction of the primary position is often preferred for bulky reagents. Under thermodynamic control, the primary position is the preferred one as well known from esterification of carbohydrates. If the cyanoethylation is stopped before the substitution pattern has equilibrated, preferred functionalisation of 2-OH is observed, followed by 6 and 3.

With respect to the substituent distribution along the amylose chain, a random pattern with all glucosyl units substituted independently of the status of the neighbour

can be expected as has been found for cellulose sulfates[30] and acetates[31] when prepared under thermodynamic control, also in heterogeneous reaction systems. In contrast, kinetically controlled reactions often show deviations from this independence model,[32] depending on the course of the reaction. These assumptions must still be proven for our starches by further experimental investigations.

3. Experimental

General.—Starch was obtained from Lyckeby Stärkelsen, Kristianstad, Sweden. Amylose (potato) was purchased from Sigma. All reagents were of highest purity available and purchased from Fluka, Aldrich or E. Merck.

GLC.—GLC separations were carried out on a Carlo Erba GC 6000 Vega Series 2 instrument equipped with an on-column injector, a flame ionisation detector (FID), a 25 m capillary column CPSil 8CB (Chrompack) connected with a retention gap (2 m), and an E. Merck Hitachi D-2500 integrator. Hydrogen was used as a carrier gas (80 kPa). Temperature program: 60 °C (1 min isotherm), with 20 °C/min to 130 °C, then with 4 °C/min to 290 °C (hold).

GCMS.—EI (70 eV)-mass spectra were recorded on a Finnigan MAT SSQ 710, the scan interval was 50–650 m/z with a scan time of 1 s. GC separations were carried out on a gas chromatograph Varian 3400 with a 30 m capillary column ZB-5 (Phenomenex) with splitless injection using the same temperature program as above (GC). He was used as carrier gas. A Hewlett–Packard GC 6890 with a 25 m BPX-5 column (SGE), a helium flow of 1 mL/min and splitless injection (15 s, 250 °C) coupled to a MSD 5973 was used for the analysis of some samples. Other spectra were recorded on a TSQ 700 with a ZB1 column of 30 m length, 1:20 split injection and a temperature program starting at 150 °C and heating with a rate of 6–300 °C. Helium was used as a carrier gas (12 psi). For CI, isobutane was used as a reactant gas.

NMR spectroscopy.—Proton NMR spectra were recorded with a Bruker AMX 300 instrument (^{1}H: 300 MHz); solvent D_2O. Chemical shifts were referred to HDO at 4.8 ppm.

O-*Cyanoethyl starch (CES).*—Cyanoethylation of starch was carried out according to the conditions reported for inulin by Verraest et al.[12] Starch (500 mg, 85% dry mass, 2.62 mmol) was added to a solution of NaOH (24 mg, 0.6 mmol) in 900 μL water in a 5-mL V-vial, mixed and homogenised for 15 min at 130 °C. At 40 °C acrylonitrile (0.1–0.9 mmol, see Table 2) was added to the starch paste, mixed and treated by ultrasound for 30 min at 41 °C. The vial was then cooled to rt, and the mixture was neutralised with 2 M HCl. The reaction mixture was evaporated under a stream of nitrogen to remove possible unreacted acrylonitrile. CES was isolated by dialysis against tap water and subsequently distilled water, and freeze-dried. The DS values were estimated from the ^{1}H NMR spectra (see Table 1 and Eqs. (1) and (2)). Reaction efficiency was 81–92%.

Reduction of CES to O-*aminopropyl starch (APS).*—Reduction was carried out with $CoCl_2{\cdot}6\ H_2O$ and $NaBH_4$ in water according to Verraest et al.[12] CES3 (25–30 mg) was stirred and homogenised in water (3–4 mL) overnight in a three-necked-vessel. Solutions of $CoCl_2{\cdot}6\ H_2O$ and $NaBH_4$ in a molar ratio of 3.75:1 were added dropwise and alternatively at rt until the total volume was 25–45 mL. Cobalt boride is formed as a black precipitate. When the addition of reagents was finished, the suspension was stirred for further 1 h, then treated with 2 M HCl until the boride dissolved, dialysed and freeze dried. The degree of reduction (d.r.)[1] was determined by ^{1}H NMR spectroscopy (for chemical shifts see Table 1) and was between 13 and 90%.

O-*(*N-*Phthalyl)-3-aminopropyl starch (PAP) preparation with NaH in DMF.*—Starch (0.55–0.9 mmol was dissolved in DMF in a 5 mL V-vial, NaH (5.0–5.9 equivalents/OH) and then *N*-(3-bromopropyl)-phthalimide (3.3–7.9 equivalents/OH) was added. The suspensions were stirred either at rt or at 50 °C for 24–72 h. Products were isolated by dialysis against tap water and subsequently distilled water. The excess of reagent was washed out with dichloromethane. The starch was finally freeze-dried.

O-*(*N-*Phthalyl)-3-aminopropyl amylose (PAP) preparation with NaOH in water or Me_2SO.*—Amylose (0.62 mmol, 100 mg) was suspended in 3 mL of water in a 5 mL V-vial, pulverised NaOH (0.7–1.3 equiv/OH) was added and the amylose dissolved. *N*-(3-Bromopropyl)-phthalimide (500 mg, 1.86 mmol), and for some entries, TBAB (600 mg, 1.86 mmol) was added and the reaction mixture stirred for 72 h at rt. The colourless suspension was diluted with 30 mL of distilled water and neutralisation was performed with HCl. The mixture was extracted three times with 10 mL of dichloromethane. Isolation was performed via precipitation with EtOH or via dialysis of the water phase and subsequent freeze-drying.

O-*(*N-*Phthalyl)-3-aminopropyl amylose (PAP) preparation with Li-dimsyl in Me_2SO.*—The amylose and all glassware were dried at 103 °C and stored in a desiccator before use. Amylose (0.62 mmol, 100 mg) was dissolved in 1 mL of Me_2SO, and stirred overnight. A

[1] $\text{d.r.} = [1/2(\int CH_2NH_2 + \int CH_2CH_2NH_2)]/[1/2(\int CH_2NH_2 + \int CH_2CH_2NH_2) + \int CH_2CN]\cdot 100.$

third flask was filled with 1.6 mL of dry Me_2SO, stirred, purged with nitrogen and evacuated several times. The flasks were sealed with septa. Methyl-Li (1.6 mL of a 1.6-M solution in diethylether) was added resulting in 2.4 mmol Li-dimsyl ($Li^+CH_3SOCH_2^-$). The ether was evaporated by nitrogen. The clear and colourless Li-dimsyl solution was added to the amylose solution and stirred for 15 min forming a gel of the deprotonated-activated amylose. The gel was cooled in ice water. Then a solution of *N*-3-bromopropyl-phthalimide (500 mg, 1.86 mmol) and TBAB (600 mg, 1.86 mmol) in 1.5 mL of Me_2SO was added and the ice bath removed. The reaction mixture was stirred over night. Work up was performed as described above.

O-*(3-Amino)propyl amylose (APA) by deprotection of* O-*(*N-*phthalyl)-3-aminopropyl amylose*.—Due to adsorption of the amino groups on glassware, the vials and flasks were silylated before use with a mixture of 10% BSTFA in toluene for 1 h at rt and washed with MeOH, CH_2Cl_2 and acetone, successively.

The reduction of the phthalimido groups was performed with $NaBH_4$ (tenfold excess) in 7:3 water–MeOH. PAP amylose (ca. 15 mg) was dissolved in a silylated V-vial in 1.5 mL of solvent. $NaBH_4$ in 1 mL of solvent was slowly added to the amylose solution. The reaction mixture was stirred for 15 h at rt. When heating it to 50 °C for 5 h, the suspension became clear. Afterwards glacial acetic acid (50 μL) was added and the solution was heated for 2 h to 80 °C under reflux. Amine formation was controlled by TLC (detection with ninhydrin). The sample was purified via dialysis with a cellulose membrane (Spectrapor® MWCO 3500) against water and freeze-dried. For larger scale preparation (4 g) reaction flasks were used instead of V-vials.

Monomer analysis.—All reaction steps of sample preparation were carried out in a thermoblock with evaporation equipment (Fa. Barkey, Leopoldshöhe).

Hydrolysis.—The starch or amylose derivative (1–2 mg) was stirred in a 1 mL V-vial with 2 M trifluoroacetic acid (1 mL) at 120 °C for 2 h. Afterwards the acid was removed in a stream of nitrogen. Residues of acid were removed by co-distillation with toluene (three times).

Methanolysis.—The starch or amylose derivative (1–2 mg) was stirred in a 1 mL V-vial with 1.5 M methanolic HCl (0.9 mL) at 90 °C for 90 min or at 100 °C for 2 h. The acid was removed by codistillation with MeOH in a stream of nitrogen.

Trimethylsilylation.—To the evaporated residue of hydrolysis or methanolysis BSTFA (100 μL), trimethylchlorosilane (10 μL) and pyridine (5 μL) were added and the sample was heated to 100 °C for 1 h. The cooled solution was diluted with CH_2Cl_2 and analysed by GC and GCMS.

Quantification.—Monomer composition (mol%) was calculated from the areas of the peaks is the gas chromatogram that have been assigned by GCMS. Areas were corrected with respect to the effective carbon response concept.[33–35] $SiMe_3$ glucosides: F (tetra-O-$SiMe_3$, $M = 540$) $\equiv 1.000$; F (mono-*O*-cyanoethyl-tri-*O*-$SiMe_3$, $M = 521$) $= 1.069$; F (mono-*O*-aminopropyl-per-*O*,*N*-$SiMe_3$, $M = 669$) $= 0.811$. Methyl glucosides: F (tetra-*O*-$SiMe_3$, $M = 482$) $\equiv 1.000$; F (mono-*O*-(*N*-phthalyl)-3-aminopropyl-tri-*O*-$SiMe_3$, $M = 597$) $= 0.827$; F (mono-*O*-aminopropyl-per-*O*,*N*-$SiMe_3$, $M = 611$) $= 0.888$.

4. Conclusion

Aminopropyl starch and amylose with DS values up to 0.30 have been prepared via cyanoethylation and subsequent reduction and via alkylation with an *N*-phthalyl protected aminoalkyl halide and subsequent deprotection. Different substitution patterns were obtained under thermodynamic and kinetic control, which could be further influenced by choice of base and solvent.

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