Synthesis, analysis and reduction of 2-nitropropyl starch

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Abstract

Granular 2-nitropropyl potato starch was synthesized by reaction with 2-nitropropyl acetate in an aqueous suspension. Nitroalkylation occurs preferentially with the amylose fraction of potato starch, as was confirmed by leaching experiments and digestion of the modified starch with α-amylase. The 2-nitropropyl substituent is a mixture of the nitroalkane and nitronic acid tautomer. Some grafting occurs and to a lesser extent additional reactions (formation of carbonyls and oximes) of the nitro group take place. After catalytic hydrogenation of water soluble 2-nitropropyl starch only a small amount of the nitro functionality was reduced to the corresponding amine. Reduction of granular 2-nitropropyl starch with sodium dithionite did not go to completion and led to a complex mixture of starting material, several intermediates and side products (for example sulfamates). © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Several methods are used on an industrial scale to modify starch chemically in order to change properties such as viscosity, water binding capacity, hydrophobicity or hydrophilicity, retrogradation and gelatinization temperature. Modified starches have found widespread applications in both the food and non-food industry. Derivatization of this biopolymer on an industrial scale is generally performed in aqueous slurry reactions,[1] in aqueous solution, in a 'semi-dry process',[2,3] and by means of extruders.[4,5] Several classes of starch derivatives can be distinguished, e.g., starch esters and ethers, cross-linked starches, oxidized starches and graft polymers.

Recently, we have described the synthesis of a novel class of modified starches: the 2-nitroalkyl starch ethers.[6–8] These compounds can be synthesized by Michael additions to 2-nitroalkenes, or precursors for nitroalkenes, such as β-nitro-acyloxy-alkanes and β-nitro-haloalkanes. High efficiencies were obtained especially for Michael additions to 2-nitroalkenes formed in situ from β-nitro-acyloxy alkanes in concentrated aqueous systems (Scheme 1).

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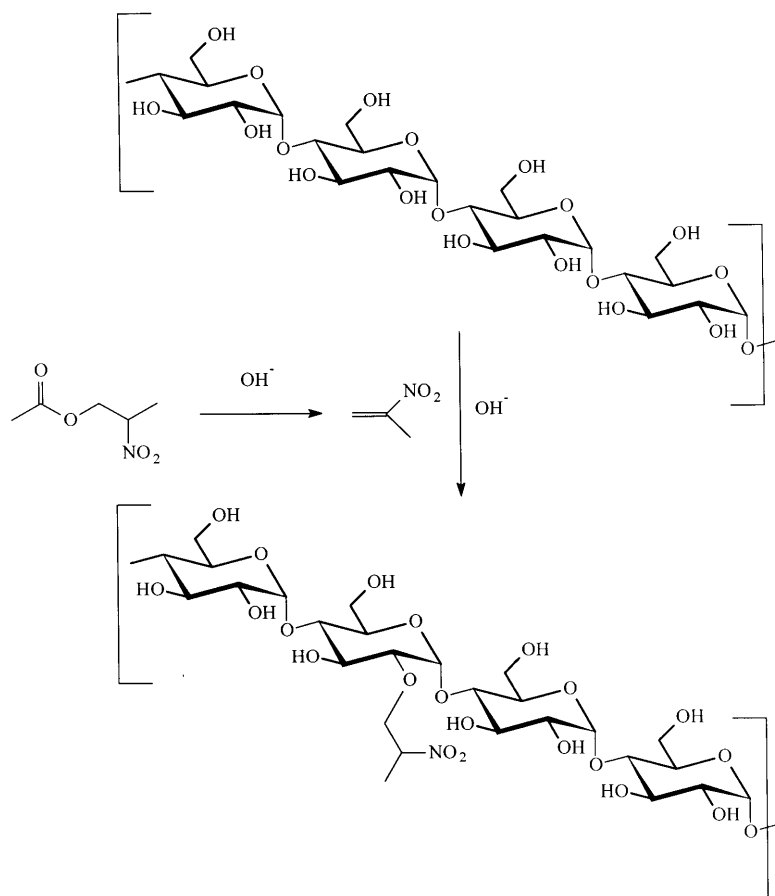
Scheme 1. Synthesis of 2-nitroalkyl starches.

These 2-nitroalkyl starches are interesting compounds in themselves and at the same time serve as precursors for secondary starch derivatisations, for example by means of reduction to 2-aminoalkyl starch ethers.

Analysis of 2-nitroalkyl starches is essential to gain more insight into the relationship between the structure of this modified starch and its physical and chemical properties. Functional properties of 2-nitroalkyl starches, such as retrogradation, gelatinization temperature, viscosity and interaction with other compounds are strongly influenced by the nature of the substituent, the molar substitution and the localization of nitroalkyl groups (topochemistry).

However, the complex supramolecular structure of the starch granule (amylose/amylopectin, crystalline/amorphous regions, branched/linear regions) and the low degrees of substitution of granular 2-nitroalkyl starch ethers complicate both the analysis of this

modified starch as well as the reduced compound.

Here, we report various aspects of the analysis of 2-nitropropyl starch—used as a model system—in order to obtain a better understanding with respect to structure–property relationships of these compounds. Furthermore, attempts are described to reduce 2-nitropropyl starch to the corresponding 2-aminoalkyl compounds.

2. Results and discussion

Analysis of 2-nitropropyl starch
Determination of the molar substitution of 2-nitropropyl starch. —2-Nitropropyl starches were prepared by a Michael addition of starch to 2-nitropropene formed in situ from 2-nitropropyl acetate. The molar substitution (ms), the number of nitropropyl substituents per mole of glucose monomer can easily be deter-

mined from the nitrogen content (N) measured according to the formula:

$$ms = 1.62N/\{14 - 0.01N(Mw_{alkyl\ substituent} - 1)\}$$

For 2-nitropropyl starches ($Mw_{alkyl\ substituent}$ = 88) the formula can be rearranged to:

$$ms = 1.62N/\{14 - 0.87N\}$$

Elemental analysis does not permit distinction between covalent and noncovalent bonding. Noncovalent interactions between the reactant and side products thereof and the starch matrix are possible; if they are not washed out thoroughly, the molar substitution calculated from the nitrogen content will be too high. However, after continuous extraction of gelatinized 2-nitropropyl starch (ms = 0.078) with methanol (96 hours) no decrease in nitrogen content was observed and therefore it can be concluded that the nitrogen containing material is indeed covalently bound to the starch granule.

Topochemical aspects of 2-nitropropyl starch. —The development and utilization of new techniques in starch chemistry have answered many, although not all, questions about the structure and organization of the starch granule.[9,10] In addition to small amounts of non-carbohydrate components (minerals, proteins and phosphates $< 0.1\%$), potato starch granules are composed of about 20% amylose (a polymer of glucose units linked via α-(1 → 4) bonds) and 80% amylopectin (linear chains of glucose units (α-(1 → 4)-linked), which are interconnected via α-(1 → 6) branching points). According to the cluster model of Hizukuri, the fine structure of amylopectin leads to the formation of crystalline domains and amorphous regions.[11,12] The amylose is thought to fill the amorphous regions although some additional crystallization may occur with amylopectin within the crystalline regions.

Potato starch granules suspended in water absorb the solvent and swell reversibly until the water content is about 35–40% of the total weight.[13] It is believed that the amorphous regions of the starch granule are most readily penetrated by water. Owing to the increase in volume, the starch granules are susceptible to penetration by low molecular weight, water soluble substances (< 1000 Dalton).

The organization of the starch granule mainly determines the reactivities of various parts of the starch granule. Most chemical reactions occur, or at least originate, in the amorphous regions of the starch granule. To obtain structural information about the reactivity of the starch granule towards 2-nitropropene (topochemistry), different levels of topochemical aspects of substituent distribution have to be taken into account: granular surface or homogeneous, amylose or amylopectin, crystalline or amorphous,[14,15] branched or linear.[16]

A surface effect for the Michael addition of 2-nitropropene, formed in situ from 2-nitropropyl acetate, is expected if the rate of the reaction is much faster than diffusion into the granule. Surface effects can be studied by transmission electron microscopy,[17] surface gelatinization with $CaCl_2$[18] and by sieving of starch granules. Potato starch granules are well suited for the latter approach because they have a broad distribution of particle sizes (15–75 μm). Large spherical granules have a lower surface to volume ratio than small granules, hence for a surface reaction the molar substitution of the 2-nitroalkyl substituent will be higher for small particles. The results in Table 1 show that the 2-nitropropyl substituent is almost homogeneously distributed over the starch granule. Note, however, that this is not absolute proof that the diffusion is faster than the Michael addition; reversible and retro Michael additions in which 2-nitropropene 'walks' through the granule could provide an additional pathway for homogeneous distribution.

Two approaches can be distinguished for the determination of the substitution pattern of

Table 1
Molar substitution of 2-nitropropyl starch granules (ms = 0.058, synthesis at pH 10.5, 5% Na_2SO_4, water–starch = 1:1, 2-nitropropyl acetate) of different sizes

Fraction (μm)	Ms
> 63	0.057
$45 < x < 63$	0.057
$25 < x < 45$	0.058

Table 2
Molar substitution of branched and linear 2-nitropropyl starch fractions, obtained as described according to Ref. 16

Substituent	Ms	Ms branched	Ms linear	Ratio branched/ linear
2-Nitropropyl	0.078	0.026	0.083	0.31

modified starches;[19] namely the statistical and the selective approach. In the statistical approach the starch derivative is (perdeuterio)methylated and then partly hydrolyzed, and the oligomers are analyzed by mass spectrometry (FABMS or MALDI-TOF-MS). The substitution patterns of the oligomeric mixtures are compared with a calculated statistical distribution of the oligomers for random substitution (the model of Reuben). Due to the acid induced nonuniform decomposition (a.o., Nef reaction) of the substituent this approach was not suitable for 2-nitropropyl starch.

A second and more selective approach consists of selective hydrolysis of the glycosidic bonds of the modified starch matrix. Recently, van der Burgt et al. described the digestion of methylated starches with α-amylase from *Bacillus subtilis*. After precipitation in methanol, fractions enriched in branched and linear D-Glc oligomers were obtained. Methylation of granular starch in aqueous suspension with dimethyl sulfate occurred preferentially at the branched regions of amylopectin. Surprisingly, nitroalkylation of starch with 2-nitropropene takes place mainly in the linear regions of the starch granule (see Table 2).

Separation of the amylose fraction from the amylopectin of 2-nitropropyl starch by means of leaching[20,21] showed an extreme preference for the amylose fraction (see Table 3). This preference is much higher than previously observed for methylated starches.

From these results it can be concluded that 2-nitropropene, formed in situ from 2-nitropropyl acetate, reacts almost completely with the amylose fraction located in the amorphous region of the starch granule.

It is known from the literature that lipids, surfactants, alcohols, iodine and nitro-

alkanes[22] form very stable complexes with amylose. The amylose rearranges itself in a single helix (V-helix) in which the guest molecule is located in the hydrophobic interior of the helix.[23]

Probably, the amylose fraction of granular potato starch forms an inclusion complex with 2-nitropropyl acetate, the precursor of 2-nitropropene. Driving forces could be hydrophobic interactions, van der Waals interactions (dipole–dipole interactions), and hydrogen-bonding between host and guest.[24] Under the alkaline conditions used acetate splits off and the 2-nitropropene formed reacts within the amylose helix.

The exact nature of the 2-nitropropyl substituent. —As the ultimate aim of our research program was the reduction of the 2-nitropropyl substituent to the corresponding 2-aminoalkyl group, solving the exact structure of the 2-nitropropyl functionality and determination of side reactions is essential. ^{1}H NMR spectra (D$_2$O) of water soluble 2-nitropropyl starch (gelatinized) showed small resonances of the methyl functionality between 1.2 and 2.2 ppm, besides resonances of the starch backbone. The complexity of the system is confirmed by a ^{13}C NMR spectrum of this compound. It only showed resonances of the starch backbone (24 hours scanning). Factors that can explain the complexity are: the nitro-functionality of 2-nitropropyl starch is a mixture of nitroalkane and nitronic acid (tautomers) and is partly neutralized after reaction (sodium nitronate);[25] grafting of 2-nitropropene on the starch backbone occurs to some extent;[26] chirality: after reaction with 2-nitropropene a new chiral centre is introduced; different monomer distribution (C-2, C-3 and C-6) over the glucopyranoside monomer.[27]

Substituents prevent the enzymatic hydrolysis of neighbouring glycoside bonds of modified starches and, after hydrolysis of 2-nitropropyl starch (ms = 0.078) with an enzyme cocktail of α-amylase (EC 3.2.1.1), amyloglucosidase (EC 3.2.1.3), and *Pseudomonas* isoamylase (EC 3.2.1.68), glucose and oligomers containing 2-nitropropyl groups were isolated. After separation with gel permeation chromatography the smallest

Table 3
Molar substitution of 2-nitropropyl amylose and 2-nitropropyl amylopectin, obtained by leaching as described in Ref. 21

Substituent	Leaching temperature (°C)	ms amylose	ms amylopectin	Amylose/amylopectin ratio
2-Nitropropyl	60	0.554	0.032	17
2-Nitropropyl	90	0.772	0.015	51
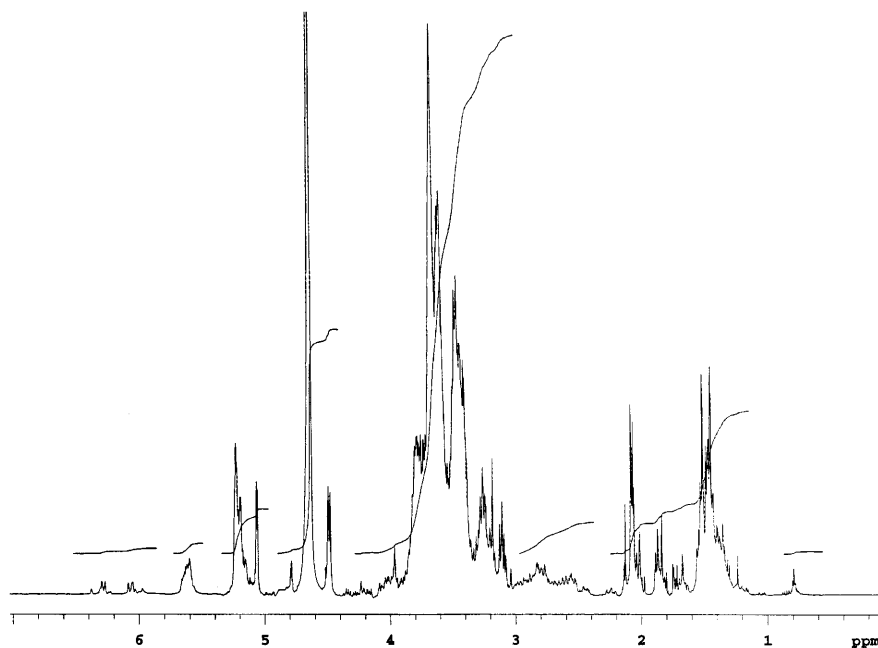
Fig. 1. ^{1}H NMR of the smallest oligomer fraction of a hydrolysate of 2-nitropropyl starch.

oligomer fraction was analyzed with ^{1}H NMR (Fig. 1).

Besides resonances of the carbohydrate backbone of the 2-nitropropyl oligomers, peaks are observed between δ 1.4 and 2.2 ppm (several multiplets, CH$_3$) and less intense ones between δ 2.4 and 3.0 (a broad multiplet). Comparison of the proton NMR spectra with the spectra of the model systems[28] 1-methoxy-2-nitropropane (a), 1-methoxy-2-methyl-2,4-dinitropentane (b) and 3-O-(2-nitropropyl)-α,β-D-glucopyranoside (c) shows that the multiplet at δ 1.50 ppm is in agreement with the chemical shift observed for the methyl group of the 2-nitropropyl ether of the 'normal' nitroalkane tautomer. The multiplet observed at δ 2.4–2.8 ppm is in agreement with the chemical shift of the diastereotopic hydrogen atoms of the methylene group of grafted 2-nitropropene on starch. As estimated from the integrals, the amount of grafted material in

2-nitropropyl starch (ms = 0.078) is about 30–35% of the total molar substitution.

It proved to be very difficult to obtain satisfactory ^{13}C NMR spectra of 2-nitropropyl oligomers. Therefore, we synthesized the ^{13}C-labeled derivative 2-nitropropyl starch from nitroethane-1-^{13}C. The ^{13}C NMR spectrum of gelatinized 2-nitropropyl-2-^{13}C starch (50% enriched), obtained by heating a suspension of the derivative in an NMR tube, clearly shows three peaks for the α-C nitro carbon atom (Fig. 2) at δ 80, δ 90 and δ 92 ppm. Comparing these results to values obtained for model systems and NMR simulations[28] it can be concluded that the high field resonance originates from the CHNO$_2$ carbon atom, one of the resonances at about δ 90 ppm originates from the quaternary C(Me)NO$_2$ carbon atom, formed after grafting of 2-nitropropene onto the starch backbone. The other resonance at about 90 ppm most likely signifies the pres-
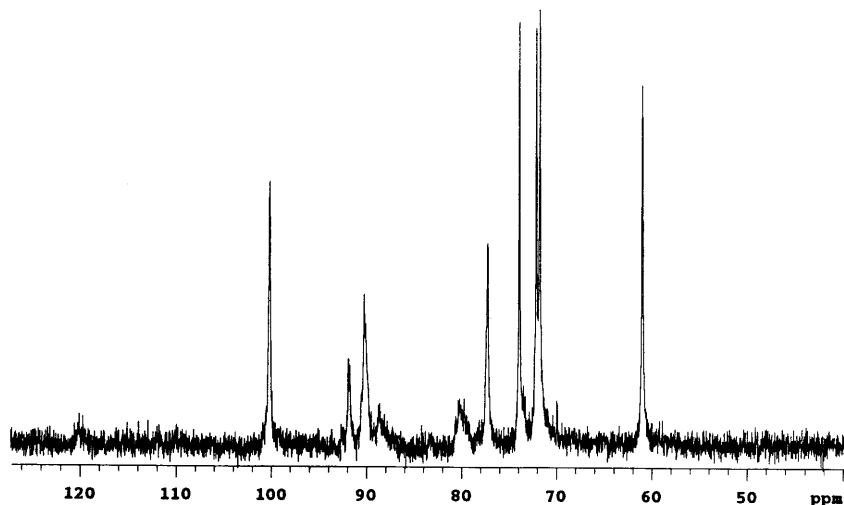
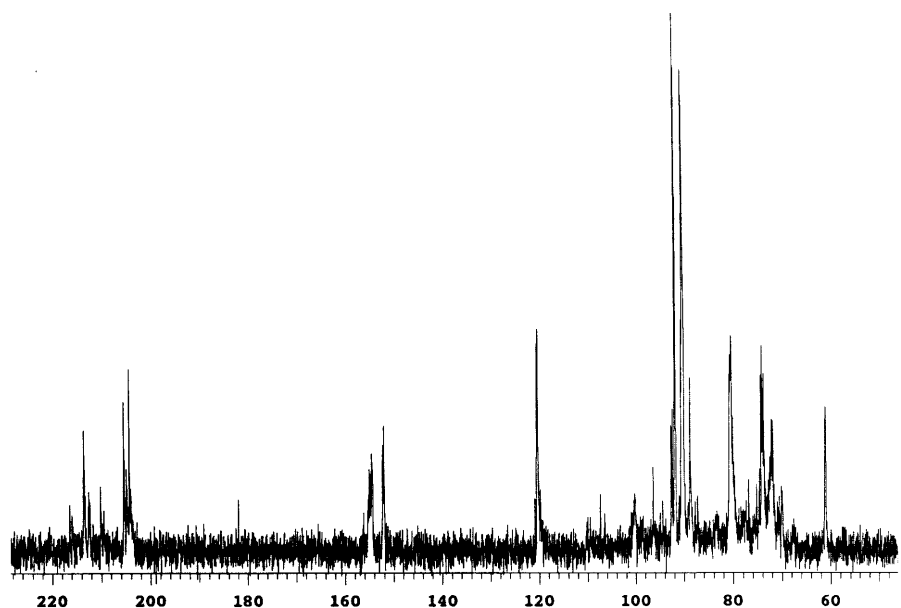Fig. 2. ^{13}C NMR of gelatinized 2-nitropropyl-2-^{13}C starch.

Fig. 3. ^{13}C NMR of the smallest oligomer fraction of a hydrolysate of 2-nitropropyl-2-^{13}C starch.

ence of the nitronic acid tautomer (C=NO$_2$H) of the 2-nitropropyl functionality.

The presence of the nitronic acid (*aci*-compound) was confirmed by the Konolow test reaction.[29] A solution of 2-nitropropyl starch immediately turns red after addition of dilute ferric chloride solution.

After enzymatic hydrolysis of 2-nitropropyl-2-^{13}C starch followed by GPC fractionation, the oligomers not only showed NMR signals for the oligomer backbone, the 'normal' 2-nitropropyl substituent (δ 80 ppm, the grafted 2-nitropropene (δ 90/92 ppm) and *aci*-2-nitropropyl-2-^{13}C (δ 90/92 ppm), but resonances

are also observed at δ 120, δ 152–156 and δ 204–215 ppm. These resonances most likely originate from allyl,[30] oximes[31]/sodium nitronate and carbonyl functionalities (Nef reaction) (Fig. 3).[32]

It can be concluded that the Michael addition of 2-nitropropene to starch leads to a complex mixture of products. Some grafting of 2-nitropropene occurs, and to a lesser extent additional reactions of the nitro group take place.

Reduction of 2-nitropropyl starch.—Up until now a successful route has not been developed for the synthesis of primary or secondary

amino alkyl starch ethers on an industrial scale. The trivial route via reaction of starch with ethylene imine is hampered by the extremely toxic character of this reagent.[33] In principle, primary and secondary aminoalkyl starch ethers are excellent precursors for the synthesis of starch based detergents (reaction with fatty acids) or the coupling of drugs or proteins to starch. A novel route to aminoalkyl starch ethers could be the reduction of 2-nitropropyl starch (see Scheme 2).

From an industrial point of view, catalytic hydrogenation of 2-nitroalkyl starch ethers with a heterogeneous catalyst would be a very

Scheme 2. Synthesis of aminoalkyl starch ethers from 2-nitroalkyl starches.

Table 4
Hydrogenation of 2-nitropropyl starch ($T = 80$ °C, 80 bar, 20 h, Raney Ni)

$\%N$	$\%N$ after reaction	$\%N_{max}$ [a]
1.50	0.79	1.55

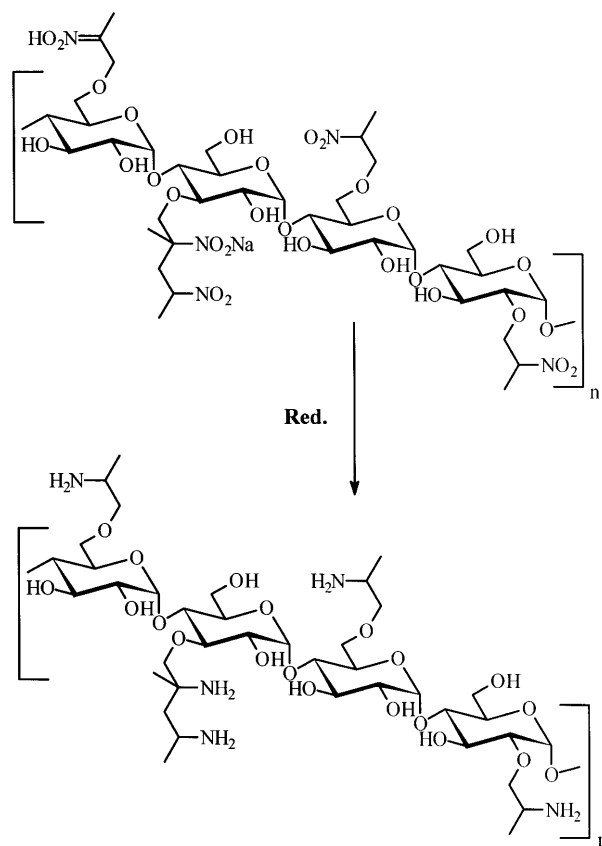[a] Calculated nitrogen content for quantitative conversion to the corresponding amine.

attractive process. The catalyst can easily be recovered in most cases and isolation of the reaction product is often uncomplicated. However, because of the insolubility of granular 2-nitroalkyl starch ethers this method is restricted to water soluble (gelatinized) 2-nitroalkyl starch ethers.

Catalytic hydrogenation of water soluble 2-nitroalkyl starch ethers. Catalytic hydrogenation of aliphatic nitro compounds is widely used for the synthesis of aminoalkyl compounds.[34] In the present study we tested the catalytic hydrogenation of water soluble 2-nitropropyl starch with Raney Ni as the catalyst.

As shown in Table 4, the nitrogen content has decreased considerably after catalytic hydrogenation of 2-nitropropyl starch, most likely due to reverse Michael additions.[‡] IR spectra of the isolated compound show a clear absorbance of the initial nitro functionality (about 1550 cm^{-1}) and it can be concluded that a complete conversion of the nitro group has not been achieved. Positive ninhydrin tests[35] confirm the presence of at least some amine functionalities in the polysaccharide. The results are in agreement with a study of the catalytic hydrogenation of cyanoethyl inulin.[36] The low conversions for catalytic hydrogenation of 2-nitropropyl starch can be explained by catalyst poisoning, absorption of the polysaccharide onto the catalyst surface and/or reduced contact between the catalyst and the polysaccharide compared to small molecules.

Reduction of 2-nitropropyl starch with sodium dithionite ($Na_2S_2O_4$). Sodium dithionite is widely used in the paper (bleaching agent) and textile industries (reduction of certain dyes) and its use in organic chemistry involves the reduction of various nitrogen functionalities[37] and aldehydes and ketones in protic solvents[38] or in organic solvent–water systems.[39]

Solutions of sodium dithionite are not stable but decompose to bisulfite (HSO_3^-) and thiosulfate ($S_2O_3^{2-}$).[40] To prevent rapid decomposition of sodium dithionite we reduced suspensions of granular 2-nitropropyl starch

‡ The pH of the solution increases owing to formation of amines.

Table 5
Reduction of 2-nitropropyl starch (2-nps) with $Na_2S_2O_4(/NaBH_4)$

Conditions (granular)	%N (weight of N)	%NO$_2$	%N other	Yield in nitrogen containing material (in %) [b]
1, (2-nps)	0.64	0.64		
2, T_k	0.57	0.26 [a]	0.31	48
3, T_k, 2 M NaNO$_3$	0.55	0 [a]	0.55	86
4, $T = 35$ °C	0.55	0.22 [a]	0.33	52
5, T_k, NaBH$_4$	0.49 (%S = 0.21%)	0 [a]	0.49	77

One-pot synthesis	%N	%NO$_2$	%N other	Yield in nitrogen containing material (in %) [e]
6, T_k	0.49 (0.71) [c]	0.42 [d]	0.07	10
7, $T = 35$ °C (reduction step)	0.43 (0.76) [c]	0.20 [d]	0.23	30 8
8, T_k, no Na$_2$SO$_4$	0.49 (0.71) [c]	0.43 [d]	0.06	

Conditions (water soluble)	%N	%NO$_2$	%N other	Yield in nitrogen containing material (in %)[b]
9, (2-nps)	0.64	0.64		
10, T_k	0.36	0.19 [a]	0.17	27
11, T_k, NaBH$_4$	0.48	0 [a]	0.48	75

[a] Determined with FTIR ((area 1550/860 cm^{-1} reduced product)/area 1550/860 cm^{-1} 2-nps).

[b] %N other/0.64 (= %N 2-nps).

[c] %N$_{max}$.

[d] Determined with FTIR (area 1550/860 cm^{-1} reduced product)/area 1550/860 cm^{-1}. %N values of the areas of %N$_{max}$ are determined from a calibration curve of 2-nitropropyl starches with different degrees of substitution).

[e] %N other/%N$_{max}$.

and water soluble 2-nitropropyl starch at neutral conditions and added the sodium dithionite in portions to keep the concentration low.

For a complete conversion of a nitro functionality to an amine at least three equivalents of sodium dithionite (six electrons) are necessary. The reduction of nitro compounds with different reducing agents gives a wide range of possible products (nitro-nitroso/oxime-hydroxylamine-amine). Numerous procedures have been developed to obtain the product at any desired reduction stage, but it is often quite difficult to predict a possible outcome because the conversion to a certain stage strongly depends on the reducing agent, the reaction conditions used and the structure of the nitro compound.[41]

In an initial attempt, we added about 5 molar equivalents of sodium dithionite per nitro group to granular 2-nitropropyl starch (**1**, Table 5). The product was analyzed by means of elemental analysis (nitrogen content) and FTIR. As the absorbance of the nitro functionality at 1550 cm^{-1} increases (or decreases) linearly with the molar substitution of the modified starch the yield of new N-containing starch ethers can be determined by combining elemental analysis with IR spectroscopy. The yield (Table 5, fifth column) can be calculated by subtracting the %N present in the form of the nitro groups (Table 5, third column) from the total nitrogen content of the product isolated (Table 5, second column) and dividing this by the nitrogen content of the starting material (2-nps, %N = 0.64).

During the reduction the nitrogen content of the reduced 2-nitropropyl starch decreases to some extent. According to the literature this can be explained by hydrolysis of an oxime or imine intermediate product. Other explanations for the decrease in nitrogen content are: reverse Michael additions and, although less likely, the Nef reaction of the nitronic acid.

The result of the reduction with sodium dithionite (T_k, (**2**)) was moderate, about 48%

of the nitro functionalities were converted into other nitrogen containing starch ethers. The moderate yield probably originates from low accessibility of the nitro functionality in the starch matrix and in this case the decomposition of sodium dithionite competes with the reduction.

The accessibility of the nitro functionalities is increased when the reduction is performed in 2 M sodium nitrate (**3**) in which the starch granules swell and are probably more susceptible to the reducing agent. A similar effect can be achieved if the temperature is raised (**4**). Indeed, higher yields were obtained under these conditions. The effect of addition of sodium borohydride (2 mol equivalents) during the reduction was also tested. It is known that sodium borohydride accelerates the reduction of the nitroso/oxime intermediate to a hydroxylamine and in this case (**5**) an almost complete conversion of the nitro functionality was achieved at T_k.

The results for the synthesis of 2-aminopropyl starch in a one-pot procedure (**6–8**) were disappointing. It is likely that this procedure can be improved by performing the reduction with mixtures of sodium dithionite and sodium borohydride. An attempt in which no swelling inhibitor is added to the nitro alkylation had no effect on the conversion of the nitro functionality (**8**).

The reduction of drum-dried 2-nitropropyl starch is in line with granular 2-nitropropyl starch. The yield of nitrogen-containing material from the reduction of water soluble 2-nitropropyl starch with sodium dithionite (**10**, 27%) is much lower than for sodium dithionite/sodium borohydride (**11**, 75%).
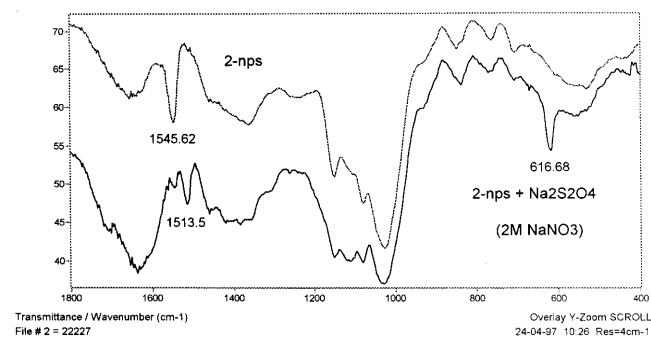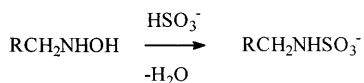
Analysis of reduced 2-nitropropyl starch. As described previously, the complete conversion of the nitro functionality of granular and drum-dried 2-nitropropyl starches can be achieved with sodium dithionite in 2 M sodium nitrate (**3**) or when sodium dithionite is used in combination with sodium borohydride (**5**). This does not necessarily mean that the reduction has proceeded completely to the amine functionality. Intermediates in the reaction (e.g. nitroso, oxime, hydroxylamine) may be stabilized during the reaction or side reactions may occur. The characterization of the reduced 2-nitroalkyl starches is difficult. Owing to the low degrees of substitution no direct information about the products formed can be obtained with FTIR. Nitroso, oxime, hydroxylamines and amines give rise to rather low or medium absorbances in the 1500–1700 cm^{-1} region,[42] and these absorbances cannot be observed clearly due to strong polysaccharide–water absorbances at these wavelengths.

To increase the molar ratio of the substituents we used the same enzymatic procedure as utilized for the analysis of 2-nitropropyl starch. After hydrolysis of compounds synthesized according to procedures **2–8** (Table 5) the smallest oligomer fraction is separated from glucose and higher oligomers by gel permeation chromatography. An example of an FTIR spectrum of one of these oligomers is given in Fig. 4.

Characteristic in the FTIR spectrum of the reduced products is the decrease or disappearance of the absorbance of the nitro functionality at ± 1550 cm^{-1} and the appearance of an absorbance at 1515 cm^{-1}.

Elemental analysis of reduced 2-nitropropyl starch (procedure **5**) revealed the incorporation of sulfur (0.21%) in reduced 2-nitropropyl starch (yield in sulfamate 14%[§]). It has been reported in the literature that reduction of nitro and nitroso-compounds with sodium dithionite may indeed lead to formation of sulfamates if the reduction is performed under neutral or slightly alkaline conditions.[43] Their formation is explained by reaction of the hydroxylamine intermediate with bisulfite

Fig. 4. FTIR spectra of a 2-nitropropyl oligomer and a reduced 2-nitropropyl oligomer (1545 cm^{-1} (NO$_2$)), 1513 cm^{-1} (NO, N=OH, NHOH, NH$_3^+$), 617 (NHSO$_3$Na).

§ Determination: $\%S = 0.21\%$, therefore $\%N$ (sulfamate) = 14/32. $0.21 = 0.9$; yield of sulfamate = 0.09/0.64 (= $\%N$ 2-nps) × 100% = 14%.

$$RCH_2NHOH \xrightarrow[-H_2O]{HSO_3^-} RCH_2NHSO_3^-$$

Scheme 3. Formation of sulfamates from hydroxylamines.
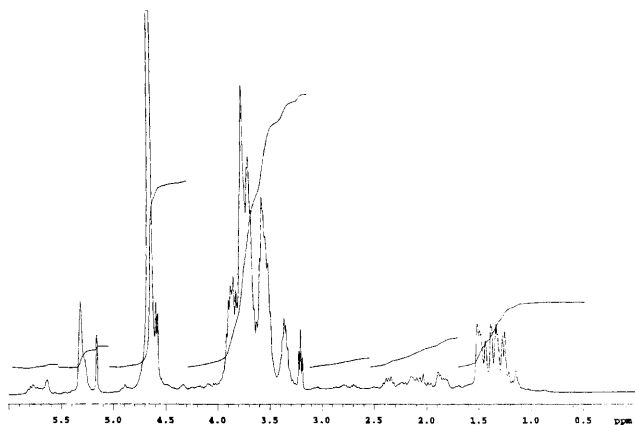
Fig. 5. ^{1}H NMR of the smallest oligomer fraction of reduced 2-nitropropyl starch (Na$_2$S$_2$O$_4$/NaBH$_4$).
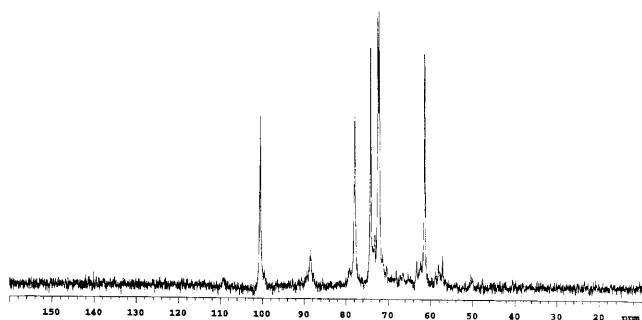
Fig. 6. ^{13}C NMR spectrum of gelatinized reduced 2-nitropropyl-2-^{13}C starch.
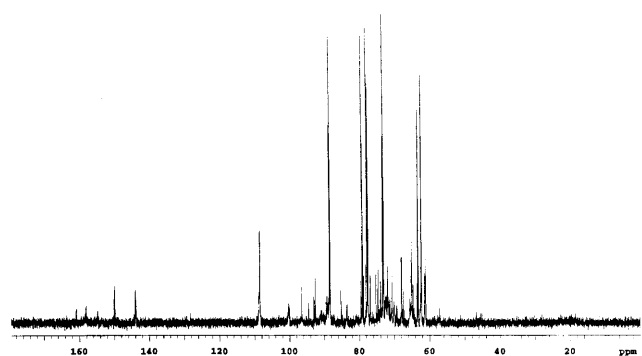
Fig. 7. ^{13}C NMR of the smallest oligomer of reduced granular ^{13}C-labeled 2-nitropropyl-2-^{13}C starch (Na$_2$S$_2$O$_4$/NaBH$_4$).

(HSO$_3^-$, Scheme 3). Bisulfite is formed in large quantities during the decomposition of sodium dithionite and also in the reduction step.

In the ^{1}H NMR spectra of oligomers obtained from the reduced compounds (procedures **2**–**8**), resonances of the methyl functionality (δ 1.1–1.5 ppm) and the diastereotopic methylene protons (δ 1.8–2.4 ppm) of the ether group shift to a higher field (see Fig. 5), compared with the oligomers obtained from 2-nitropropyl starch (methyl δ 1.3–2.2 ppm, methylene δ 2.4–3.1 ppm, see Fig. 1).

In order to obtain more information about the products formed we reduced ^{13}C-labeled 2-nitropropyl-2-^{13}C starch. We compared the ^{13}C NMR spectra of the reduced labeled compound with the reduction of the model systems (a–c) and an ADS NMR simulation program, as well in order to obtain more information about the selectivity of the process.

Reduction of granular ^{13}C-labeled 2-nitropropyl-2-^{13}C starch (50% enriched) with sodium dithionite/sodium borohydride leads, as determined by FTIR, to almost complete (80–90%) conversion of the nitro functionality. The ^{13}C NMR spectrum of the gelatinized product is rather complex (see Fig. 6).

Resonances of the starch backbone are clearly present in the spectrum, as well as some remaining resonances of the labeled methine group of the starting material (grafting + nitronic acid at about δ 90 ppm, CHNO$_2$ at δ 80 ppm). A new resonance for the labeled methine carbon appears at δ 73 ppm and several small resonances are observed in the region δ 50–70 ppm.

The ^{13}C NMR spectrum of the smallest oligomer of reduced granular ^{13}C-labeled 2-nitropropyl-2-^{13}C starch (sodium dithionite/ sodium borohydride, Fig. 7), obtained after enzymatic hydrolysis and purification by gel permeation chromatography, shows that in comparison with the starting material (see Fig. 3) new absorbances for the labeled methine carbon appear at δ 150–144, 73 and 62–70 ppm.

The new resonances observed for reduced gelatinized 2-nitropropyl-2-^{13}C-starch and the smallest oligomer of reduced 2-nitropropyl-2-^{13}C-starch originate most likely from oximes, nitroso compounds, hydroxypropyl substituents and sulfamate, hydroxylamine or

amine functionalities. Unfortunately all the compounds synthesized according to procedures **2**–**8** and **10**, **11** gave negative ninhydrin tests, which excludes the formation of 2-aminopropyl starch.

As already mentioned before, we also reduced some model systems with sodium dithionite in order to get a better insight into the reduction process. An NMR tube reaction of 1-methoxy-2-nitropropane with sodium dithionite proceeded rather selectively (80–90%) and a major product was formed in which a resonance for the methine carbon appeared at δ 48.51 ppm. The chemical shift is in reasonable agreement with the methine carbon of 1-methoxy-2-aminopropane, the corresponding hydroxylamine or the sulfamate.[28] In order to distinguish between these compounds we performed the reaction on a preparative scale. Unfortunately, the compound formed could not be isolated from the water layer by extraction with organic solvents and therefore we could not determine the exact nature of it. Because of the very good water solubility of the product formed it seems likely that the sulfamate is formed under the reaction conditions used (sodium carbonate is used to maintain a constant pH).

3. Conclusions

Suspension reactions of starch with 2-nitropropyl acetate led to accumulation of the 2-nitroalkyl substituent in the amorphous amylose fraction of the starch granule. A possible explanation is that (a helical) inclusion complex between 2-nitropropyl acetate and amylose is formed and after splitting off acetate 2-nitropropene reacts within the amylose helix. This regiospecificity makes the nitroalkylation very suitable for prevention of retrogradation of starch pastes, even at low degrees of substitution.

The nitro functionality of 2-nitropropyl starch is a mixture of the nitroalkane and nitronic acid tautomer. Some grafting of 2-nitropropene occurs, and additional reactions of the nitro group take place, to a lesser extent.

Catalytic hydrogenation of the high molecular weight 2-nitroalkyl starch ethers is not a

useful procedure for the synthesis of aminoalkyl starch ethers and only a small fraction of the nitro functionalities is converted to amines.

The model system 2-nitropropyl starch (granular and water soluble) can be reduced with sodium dithionite or sodium dithionite/sodium borohydride to new starch ethers. However, a complex mixture of products is formed. Owing to the low accessibility of the nitro functionality and/or (some) stabilization of intermediates, the decomposition of sodium dithionite competes with reduction of the nitro group. The reaction does not go to completion and several intermediates (oxime, nitroso, and probably hydroxyl amine) and side products (e.g. sulfamates) are formed.

The absence of amines in the reduction of model systems and 2-nitropropyl starch and the incorporation of sulfur into the polysaccharides make it very likely that the reduction of aliphatic nitroalkanes with sodium dithionite at neutral conditions initially leads to the formation of aliphatic polysaccharide sulfamates. These are formed by the reaction of the hydroxylamine intermediate with bisulfite.

It can be concluded that, owing to the restrictions of our system, sodium dithionite is not a suitable reducing agent for the synthesis of aminoalkyl starch ethers from 2-nitroalkyl starch ethers. Further research in this field is necessary to achieve a complete conversion of the nitro functionality to an aminoalkyl starch ether.

4. Experimental

tropentane (b) and 3-*O*-(2-nitropropyl)-α,β-D-glucopyranoside (c) were prepared according to literature procedures.[28]

^{1}H and ^{13}C NMR spectra were recorded on a 500 Varian MHz spectrometer. FTIR spectra were recorded on a Biorad FTS 135 spectrometer. Fractionation of starch was achieved with sieves from Retsch (pore sizes 63, 45 and 25 μm). High-performance liquid chromatography (HPLC) was performed with a Benson BCX4 column (Ca^{2+}, 250 × 10 mm, $T = 85$ °C, eluent 100 ppm Ca-EDTA). Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Sulfur analyses were performed at the Microanalytical Department of the University of Groningen.

Continuous extraction of 2-nitropropyl starch. 2-Nitropropyl starch was extracted continuously with MeOH in a Soxhlet apparatus. After 96 h the derivative was dried in the air and the nitrogen content was determined again.

Fractionation of 2-nitropropyl starch. 2-Nitropropyl starch (48.50 g, ms = 0.058) was separated into four size groups by using sieves and mechanically agitating (dry product) for 1 h (> 63 μm (13.66 g), 45 μm < x < 63 μm (29.73 g), 25 μm < x < 45 μm (3.96 g), < 25 μm (nd)). The nitrogen content of each fraction was determined again (see Table 1).

Enzymatic hydrolysis of 2-nitropropyl starch. Drum-dried 2-nitropropyl starch (ms = 0.080, 5.00 g) was dissolved in 100 mL water. The pH of the solution was adjusted to 5.0 with a 0.5 M HCl solution. α-Amylase (25 mg, EC 3.2.1.1), amyloglucosidase (25 mg, EC 3.2.1.3) and isoamylase (25 mg, EC 3.2.1.68) were added and after 48 h (pH 5) the solution was heated for 1 h at 80 °C and filtered over a cellulose membrane. The water layer was evaporated to a volume of 5–10 mL and fractionated by Biogel P2 (column 85 × 5 cm, 172 mL/h, fraction size 43 mL). Detection of carbohydrates was effected by spraying plates of silica with 20% H_2SO_4 in MeOH followed by charring. Carbohydrate-containing fractions were analyzed with HPLC and the smallest oligomer (but no glucose) containing fraction was freeze dried. Yield 50 mg. ^{1}H NMR: (see Fig. 1). FTIR: 3410 (s), 2927 (m),

1656 (m), 1547 (s), 1459 (m), 1364 (m), 1235 (w), 1152 (m), 1080 (m), 1027 (s), 849 (w), 746 (w), 706 (w), 529 (w).

Catalytic hydrogenation of water soluble 2-nitropropyl starch. 2-Nitropropyl starch (10.01 g, %N = 1.50%, dry substance = 89.10%) was dissolved in water (200 mL). Raney Ni (about 0.50 g) was added and the hydrogenation was carried out in a Parr apparatus at 80 bar and 80 °C. After 20 h, the pressure was released and after the catalyst was allowed to settle the solution was decanted and acidified to pH 3. After precipitation in MeOH (800 mL), followed by filtration, the residue was dried at $T = 40$ °C. Yield 8.16 g reduced 2-nitropropyl starch (%N = 0.79%, dry substance = 89.43%).

*Reduction of 2-nitropropyl starch with $Na_2S_2O_4$/($NaBH_4$), general example (**2–5**).* Sodium dithionite was added in portions to a suspension of 2-nitropropyl starch (ms = 0.077 (%N = 0.64%), dry substance = 84.50%) in water. The pH was regulated with a pH-stat (pH 7) by adding portions of 1 M NaOH. After 16 h the suspension was filtered, washed thoroughly with water and dried in the air. (For procedure **5** the suspension was acidified to pH 3, stirred for 3 h, filtered, washed thoroughly with water and dried in the air.)

*Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (one-step synthesis, general example **6–8**).* Native starch was suspended in water. The pH of the starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropyl acetate was added dropwise to the suspension. The pH was regulated with a pH-stat by adding portions of 1 M NaOH. After 1 h the reaction was complete (no more acid formation) and the pH was adjusted to 7.0 with a 4 M HCl solution. Sodium dithionite was added in portions to the suspension and the pH was regulated with a pH-stat (pH 7) by adding portions of 1 M NaOH. After 16 h the suspension was filtered, washed thoroughly with water and dried in the air.

*Reduction of water soluble 2-nitropropyl starch with $Na_2S_2O_4$/$NaBH_4$ (general example **10–11**).* Sodium dithionite was added in portions to a dispersion of drum-dried 2-nitropropyl starch (dry substance = 94.82%, %N = 0.64%) in water. The pH was regulated

Table 6
Reduction of 2-nitropropyl starch with $Na_2S_2O_4(/NaBH_4)$

Procedure	2-nps	$Na_2S_2O_4$	$NaBH_4$	$NaNO_3$	H_2O	Yield	%N
2	100.10 g	42.10 g 200 mmol			150 mL	98.30 g	0.57
2a (^{13}C enriched)	2.28 g	1.00 g	0.08 g		6 mL	1.75 g	nd
3	49.96 g	19.72 g 94 mmol		8.50 g 10 mmol	50 mL	44.59 g	0.55
4	100.04 g	42.02 g 200 mmol			150 mL	95.43 g	0.55
5	100.10 g	20.75 g 99 mmol	150 g 40 mmol		100 mL	97.54 g	0.49

One-pot synthesis	Starch	2-npa	Na_2SO_4 [a]	H_2O	$Na_2S_2O_4$	Yield	%N
6	50.91 g	3.30 g 22.4 mmol	2.53 g	50 mL	23.01 g 110 mmol	46.13 g	0.49
7	30.03 g	2.01 g 13.7 mmol	1.51 g	30 mL	14.28 g 68 mmol	28.51 g	0.43
8	50.45 g	3.30 g 22.4 mmol		50 mL	23.05 g 110 mmol	48.80 g	0.49

Water soluble	2-nps	$Na_2S_2O_4$	$NaBH_4$	$NaNO_3$	H_2O	Yield	%N
10	50.12 g	23.00 g 109 mmol			2000 mL	50.15 g	0.36
11	50.05 g	20.75 g 99 mmol	1.50 g 40 mmol		2000 mL	45.01 g	0.48

[a] Swelling inhibitor.

with a pH-stat (pH 7) by adding portions of 1 M NaOH. After 72 h the suspension was dialyzed and freeze-dried (**10**) or concentrated and precipitated in MeOH (**11**) (Table 6).

References

1. Rutenberg, M. W.; Solarek, D. In *Starch Chemistry and Technology*; Whistler, R. L.; BeMiller, J. N.; Paschall, E. F., Eds.; Academic Press: New York, 1984; pp. 312–314.
2. Hellwig, G; Bisschoff, D.; Rubo, A. *Starch/Stärke* **1991**, *44*, 69–74.
3. Gardenier, K. J.; Heimbürger, W. DE 2900073, *Chem. Abstr.* **1981**, *94*, 17391s.
4. Wang, L; Shogren, R. L.; Willet, J. L. *Starch/Stärke* **1997**, *49*, 116–120.
5. Valle, G. D.; Colonna, P.; Tayeb, J. *Starch/Stärke* **1991**, *43*, 300–307.
6. Gotlieb, K. F.; Bleeker, I. P.; van Doren, H. A.; Heeres, A. EP 0710671 A1, 1996.
7. Heeres, A.; van Doren, H. A; Gotlieb, K. F.; Bleeker, I. P.; Kellogg, R. M. In *Carbohydrates as Organic Raw Materials IV*; Praznik, W.; Hüber, A., Eds.; WUV/Üniversitätsverlag, 1998; pp. 264–277.
8. Heeres, A.; van Doren, H. A.; Gotlieb, K. F.; Bleeker, I. P.; Kellogg, R. M. In *Starch 96, The Book*; van Doren, H. A.; Van Swaay, N., Eds.; Zestec/Carbohydrate Research Foundation, 1997; pp. 179–182.
9. French, D. In *Starch Chemistry and Technology*; Whistler, R. L.; BeMiller, J. N.; Paschall, E. F., Eds.; Academic Press: New York, 1984; pp. 183–247.
10. Zobel, H. F. *Starch/Stärke* **1988**, *40*, 44–50.
11. Hizukuri, S. *Carbohydr. Res.* **1985**, *141*, 295–306.
12. Hizukuri, S. *Carbohydr. Res.* **1986**, *147*, 342–347.
13. Morsi, M. K. S.; Sterling, C. *Carbohydr. Res.* **1966**, *3*, 97–101.
14. Steeneken, P. A. M.; Smith, E. *Carbohydr. Res.* **1991**, *209*, 239–249.
15. Steeneken, P. A. M.; Woortman, A. J. J. *Carbohydr. Res.* **1994**, *258*, 207–221.
16. van der Burgt, Y. E. M.; Bergsma, J.; Bleeker, I. P.; Mijland, P. J. H. C.; van der Kerk-van Hoof, A.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1998**, *312*, 201–208.
17. Whistler, R. L.; Goatley, J. L.; Spencer, W. W. *Cereal Chem.* **1959**, *36*, 84–90.
18. Jane, J.-L.; Shen, J. J. *Carbohydr. Res.* **1993**, *247*, 279–290.
19. Mischnick, P. *Macromol. Symp.* **1997**, *120*, 281–290.
20. Young, A. H. In *Starch Chemistry and Technology*; Whistler, R. L.; BeMiller, J. N.; Paschall, E. F., Eds.; Academic Press: New York, 1984; pp. 259–260.
21. van der Burgt, Y. E. M.; Bergsma, J.; Bleeker, I. P.; Mijland, P. J. C. H.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **2000**, *327*, 423–429.
22. Whistler, R. L.; Hilbert, G. E. *J. Am. Chem. Soc.* **1945**, *67*, 1161–1165.

23. Chandrasekaran, R. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 345–346.

24. Kubik, S.; Höller, O.; Steinert, A.; Tolksdorf, M.; Van der Leek, Y.; Wulff, G. In *Carbohydrates as Organic Raw Materials III*; Van Bekkum, H.; Röper, H.; Voragen, F., Eds.; CRF, 1994; pp. 169–187.

25. Patai, S. In *The Chemistry of the Nitro and Nitroso Groups*; Feuer, H., Ed.; John Wiley: New York, 1969; I pp. 1–135, 165–213 and 301–612 and II pp. 1–384.

26. Feuer, H.; Markofsky, S. *J. Org. Chem.* **1964**, *29*, 929–934.

27. Wilke, O.; Mischnick, P. *Starch/Stärke* **1997**, *49*, 453–458.

28. Heeres, A.; Spoelma, F. F.; van Doren, H. A.; Gotlieb, K. F.; Bleeker, I. P.; Kellogg, R. M. *Carbohydr. Polym.* **2000**, *42*, 33–43.

29. Nielsen, A. T. In *The Chemistry of the Nitro and Nitroso groups, Part 1*; Feuer, H.; Patai, S., Eds.; John Wiley: New York, 1969; pp. 470–473.

30. Breuer, E. In *The Chemistry of Amino, Nitroso and Nitro compounds and their derivatives, Part 1*; Patai, S., Ed.; John Wiley: New York, 1982; p. 551.

31. Breuer, E. In *The Chemistry of Amino, Nitroso and Nitro compounds and their derivatives, Part 1*; Patai, S., Ed.; John Wiley: New York, 1982; pp. 546–549.

32. March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*; McGraw-Hill: New York, 1977; p. 808.

33. Jarowenko, W. US 3,354,034, 1967; *Chem. Abstr.* **1967**, *67*, 74683e.

34. Schöter, R. Houben-Weyl, *Methoden der Organischen Chemie*; Müller, E.; Ed.; George Thieme Verlag, 1957; Vol. 11/1, pp. 382–393.

35. Feigl, F. *Spot tests, II, Organic Applications*; Elsevier: New York, 1954; pp. 207–210.

36. Veraest, D. Thesis TU Delft, 1997, 127–133.

37. Schöter, R. Houben-Weyl, *Methoden der Organischen Chemie*, Müller, E., Ed.; George Thieme Verlag, 1957; Vol. 11/1, pp. 437–442.

38. (a) Louis-André, O.; Gelbard, G. *Bull. Soc. Chim. Fr* **1986**, 565–577. (b) de Vries, J. G.; Kellogg, R. M. *J. Org. Chem.* **1980**, *45*, 4126–4129.

39. (a) Park, K. K.; Lee, C. W.; Choi, S. Y. *J. Chem. Soc., Perkin Trans.* **1992**, 601–603. (b) Park, K. K.; Oh, C. H.; Sim, W. J. *J. Org. Chem.* **1995**, *60*, 6202–6204.

40. Wayman, M.; Lem, W. J. *Can. J. Chem.* **1970**, *48*, 782–787.

41. March, J. *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure*; McGraw-Hill: New York, 1977; pp. 1125–1126.

42. Hesse, M.; Meier, H.; Zeeh, B. *Spektroskopischen Methoden in der Organischen Chemie*; 3rd ed., George Thieme Verlag, 1987; pp. 27–62.

43. Pecorari, P; Rinaldi, M.; Costi, M. P.; Antolini, L. *J. Heterocycl. Chem.* **1992**, *29*, 1149–1455.