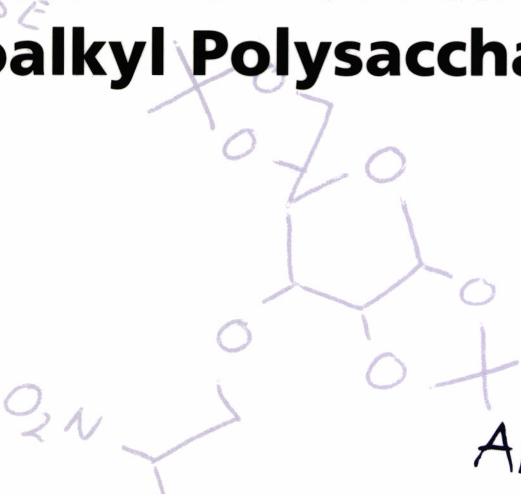
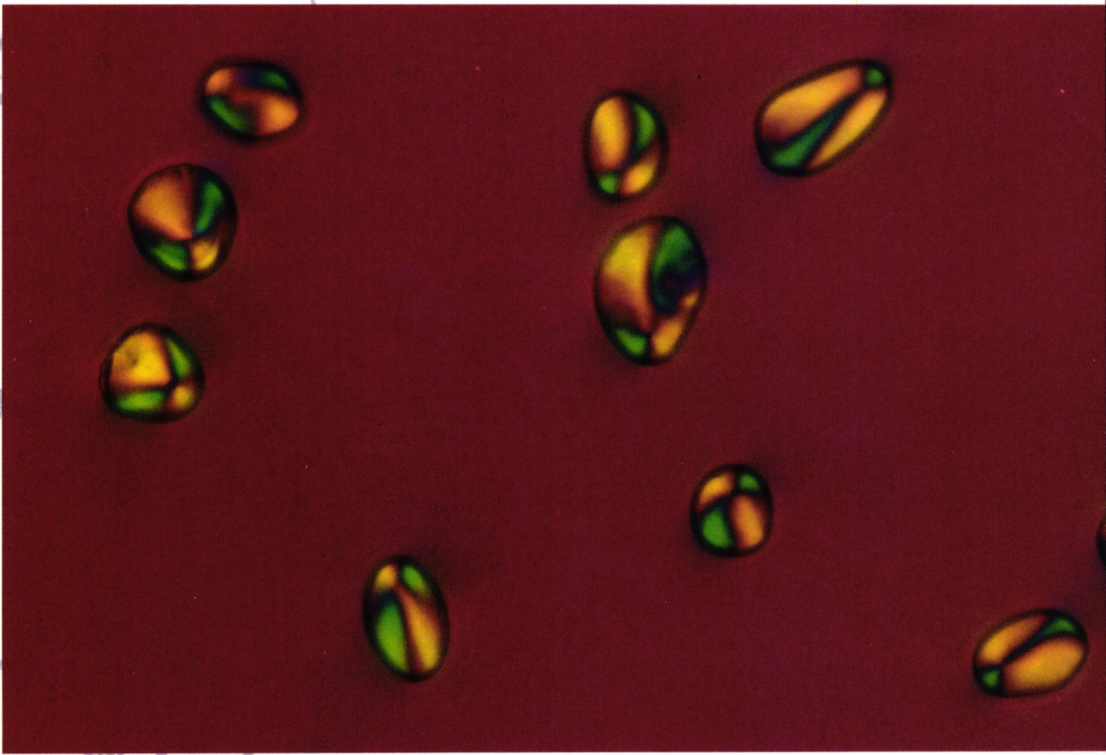
Synthesis and Reduction of 2-Nitroalkyl Polysaccharide Ethers



André Heeres



Synthesis and Reduction of 2-Nitroalkyl Polysaccharide Ethers

This research project was supported by AVEBE B.A., The Netherlands Institute for Carbohydrate Research (NIKO-TNO), and TNO Nutrition and Food Research Institute.

Cover design: Wazoo, Groningen
Photograph: Albert Woortman (TNO Nutrition and Food Research Institute)

RIJKSUNIVERSITEIT GRONINGEN

Synthesis and Reduction of 2-Nitroalkyl Polysaccharide Ethers

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, Dr. D. F. J. Bosscher,
in het openbaar te verdedigen op
vrijdag 20 november 1998
om 16.15 uur

door

André Heeres
geboren op 20 juni 1966
te Nieuwe Pekela

No reprints available
139 pag.

Promotor: Prof. Dr. R. M. Kellogg

Referent: Dr. H. A. van Doren

ISBN 90-367-0995-4

Dankwoord

Na iets meer dan 3 jaar is het proefschrift af. Vanzelfsprekend heb ik het niet alleen gedaan en op deze plaats wil ik dan ook iedereen die meegeholpen heeft bedanken voor hun bijdrage.
Vanuit historisch perspectief bekeken moet ik als eerste Kees Gotlieb bedanken. Hij is degene geweest die mijn onderzoek geïnitieerd heeft en met het voorstel kwam om van het bilaterale onderzoek voor AVEBE B.A., dat ik indertijd uitvoerde op het NIKO-TNO, een promotieonderzoek te maken. Verder wil ik hem ook bedanken voor zijn chemische en vooral morele steun gedurende de laatste jaren. Ido Bleeker en Peter Bruinenberg (beiden werkzaam bij AVEBE B.A.) wil ik bedanken voor de begeleiding. Ik denk dat we alles keurig hebben afgesloten.
Mijn promotor professor R.M. Kellogg bedank ik voor de begeleiding van het onderzoek en het snelle nakijken van artikelen en hoofdstukken. Ik wil de leden van zijn werkgroep bedanken voor de gezellige maar vermoeiende werkweken. Voor het corrigeren van mijn proefschrift en het verlenen van toestemming om mijn proefschrift in het openbaar te verdedigen ben ik de leescommissie, professor J.B.F.N. Engberts, professor B.L. Feringa en professor J.H. Teuben, zeer erkentelijk.
Henk, ik denk dat we ons de afgelopen jaren kostelijk vermaakt hebben. We hebben veel gelachen, hard gewerkt en ik heb veel geleerd. Mede dankzij jou is ook mijn Engels stukken vooruit gegaan. Ik wil je bedanken voor de begeleiding en de vrijheid die ik kreeg voor het doen van wilde experimenten. Ik denk dat ik je vertrouwen niet beschaamd heb.
Goed toeven was het bij mijn zaalgenoten Harry, Elly, Monique en de stagiaires Froukje, Ronald en Jantine. Froukje Spoelma (HLO-stagiaire) heeft meegewerkt aan het deel dat beschreven staat in hoofdstuk 6. Elly en Monique zorgden er met hun kook- en bakkunsten voor dat het gemiddeld gewicht van de mensen op onze zaal gestaag toenam. Harry regelde o.a. de krant. Ik zal onze wandelingen door blubber, sneeuw, langs spoorlijnen en verlaten volkstuinen nooit vergeten.
Alle NIKO'ers en tegenwoordig TNO'ers bedankt voor de hulp op allerlei vlakken (computers, chemie, het aanduwen van mijn auto, etc.). Ik zal het gemoedelijke missen. Albert Woortman wordt bedankt voor het beschikbaar stellen van de foto die op de voor- en achterkant van mijn proefschrift staat.
Jan Herrema en Wim Kruizinga wil ik bedanken voor het opnemen van de NMR spectra. Groot was hun verbazing als ik tevreden knikte bij het zien van spectra die in hun ogen meteen de prullenbak in konden.
Ik wil AVEBE B.A., NIKO-TNO, TNO-Voeding en het Stratingh college bedanken voor hun bijdrage in de kosten voor het drukken van mijn proefschrift. De afdeling analyse van AVEBE B.A. wil ik bedanken voor het bepalen van de stikstofgehaltes van mijn derivaten.
Mijn huisgenoot Michiel wil ik bedanken voor zijn gezelligheid en kookkunsten. Natuurlijk wil ik al mijn vrienden in Groningen en "Pekel" bedanken voor de lol en steun. De voetbalverenigingen Helpman (zaalvoetbal) en P.J.C (veldvoetbal) zorgden tijdens mijn promotieonderzoek voor de nodige in- en ontspanning. Medespelers/supporters: Thanks a lot.

Erik, bedankt voor het corrigeren van de ruwe hoofdstukken. Verder heb je er voor gezorgd dat je broertje de chemie echt leuk is gaan vinden. Mijn ouders wil ik bedanken voor alles wat ze voor me gedaan hebben. Ik kom nog altijd met veel plezier langs. Monique bedankt voor de gezelligheid binnen en buiten het lab. Je bent een succes.

ANDRÉ

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Chapter 1. Introduction

Chapter 1

Introduction

1.1 General introduction

Polysaccharides are naturally occurring carbohydrate polymers (also called glycans or gums) of high molecular weight. They play an essential role in the existence of life on earth. Plants store energy from photosynthesis in the form of polysaccharides, as a food reserve. The structures of these polysaccharides depend on the type of plant. Starch (**1**a, **1**b, Scheme 1) and inulin (**2**) are examples of polysaccharides which are mainly used as an energy source. The biosynthesis of these compounds is not yet fully understood[1]. Polysaccharides, especially starch, have always been an item in the diet of man[2]. Starch is ingested by consuming plants and animals and hydrolyzed in the digestive system. Many animals, including humans, use the polysaccharide glycogen[*] as an energy reserve[3]. This polymer is synthesized enzymatically from glucose.

Not only are polysaccharides of importance as a food reserve, they also play an important structural role in nature. Cell walls of plants contain large amounts of cellulose (**3**)[4], chitin (**4**) is present in the exoskeletal structure of most invertebrates[5]. Polysaccharides also play a fundamental role in intercellular interaction and recognition for transport of substrates through the cell membrane[6].

Nature has provided us with hundreds of polysaccharides as has become clear from recent research. Gums can be extracted from seaweeds, isolated from seeds, and they can be obtained as exudates from plants[7]. Extracellular polysaccharides are produced by micro-organisms[8]. Simple polysaccharides, such as cellulose, which is a straightforward homopolymer of glucose, are known. However, polysaccharides with very complicated branched structures and large repeating units also exist. Several naturally occurring polysaccharides bear nonionic groups, such as acetyl and puryvate substituents (xanthan), anionic groups, such as carboxylic(alginates (**5**)) or sulfate functionalities (keratan sulfate (**6**)) or cationic groups, such as amino groups (chitosan (**7**))[9].

About 130 years B.C., the Egyptians recognized that polysaccharides (starch) were useful products in nonfood applications. By impregnating papyrus with starch, paper with a smooth surface was obtained. Three centuries later the Romans described a procedure for the use of starches in textile products[10]. Nowadays, polysaccharides find many applications in industry. In contrast with petrochemicals, polysaccharides are renewable, biodegradable, nontoxic, and

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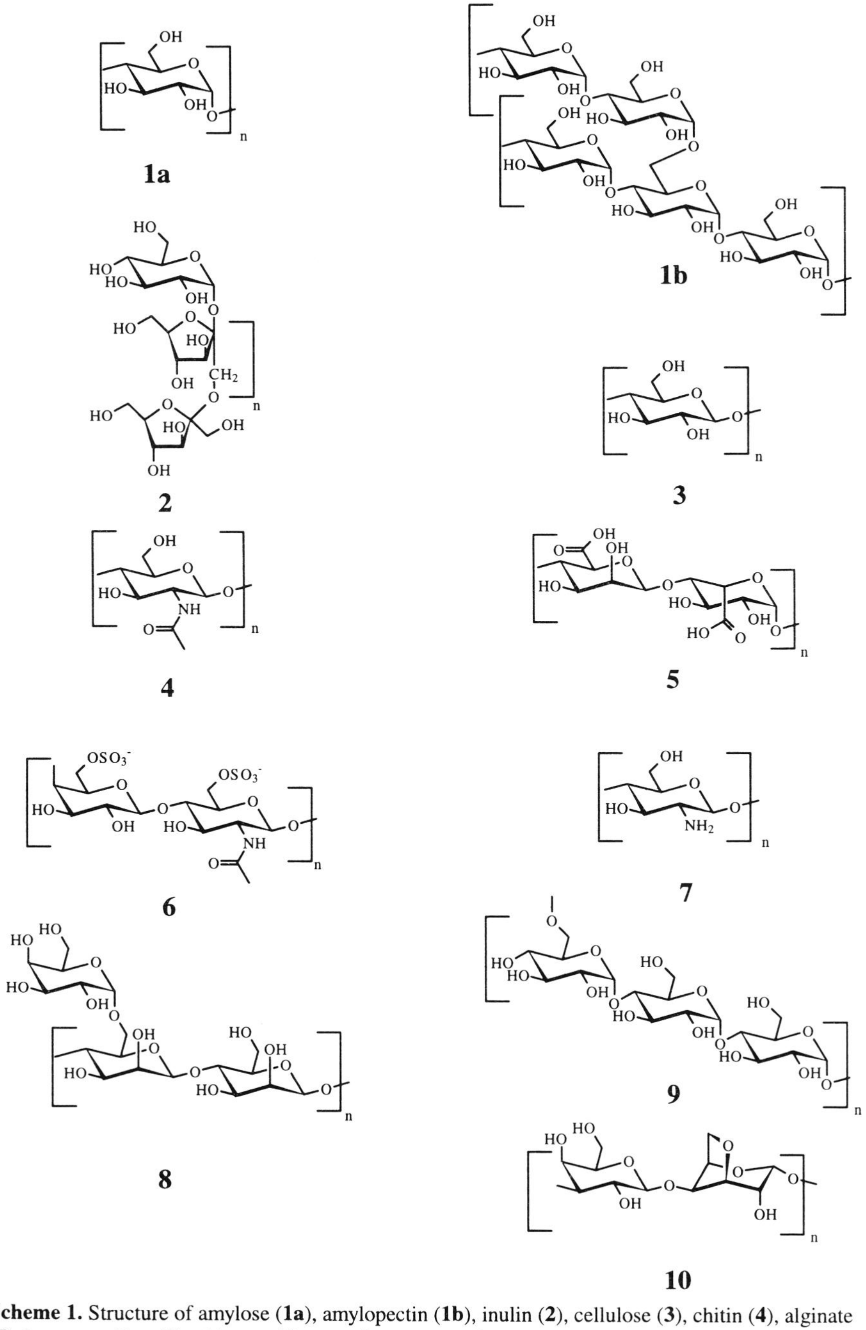
[*] Glycogen resembles amylopectin (**1b**) in its chemical composition but it features fewer branches and has shorter average chain lengths.



Scheme 1. Structure of amylose (**1a**), amylopectin (**1b**), inulin (**2**), cellulose (**3**), chitin (**4**), alginate (**5**), keratan sulfate (**6**), chitosan (**7**), guar (**8**), pullulan (**9**), and agarose (**10**).

they have a neutral CO_2 cycle. It is within the realm of possibility that the plant-based economy, which existed before 1850, will be restored in the future due to the exhaustion of fossil fuels[11]. Research involving polysaccharides is currently stimulated by agriculture. Owing to an excess of agricultural products (*i.a.*, polysaccharides) especially in North America and Western Europe, the search for new applications of existing polysaccharides and modified polysaccharides and the development of new polysaccharide derivatives has intensified.

1.2 Modification of polysaccharides

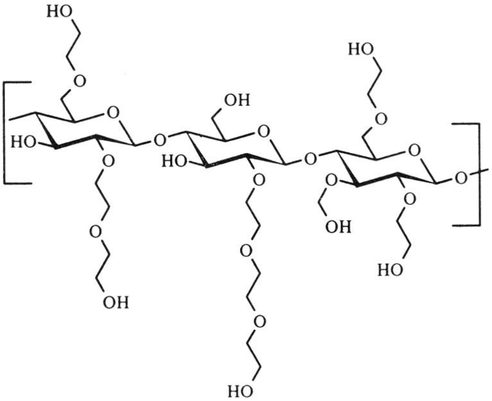
Chemical modification of polysaccharides is useful for altering chemical and physical properties of these biocompounds. Roughly, the modification of polysaccharides can be divided into:

- hydrolysis of polysaccharides to oligo- or monosaccharides.
- modification of the polysaccharide with new functionalities without loss in molecular weight.

The acetal functionality of polysaccharides can be hydrolyzed, enzymatically or with acid, partially to oligomers or completely to their monomers. In this way syrups, cyclodextrins, maltose and glucose are synthesized on a large scale from starch[12]. These products have found a tremendous number of applications in the food industry: glucose is converted on an industrial scale to products such as fructose, gluconic acid, mannose, sorbitol, and vitamin C (also important products for the food industry). Ethanol and lactic acid are produced by fermentation of starch[13].

Another approach for chemical derivatization is modification of the existing polysaccharide without appreciable loss in molecular weight. The incorporation of new functionalities leads to new properties of the polysaccharides[14]. Not only are these modified polysaccharides used in new applications, they can also be designed to replace traditional gums. Traditional gums may suffer from a lack of reproducibility in their properties, purity, supply, and costs. In the confectionary industry gum arabic has partly been replaced by thin boiling (acid modified) starches.

In general, derivatization to a low degree of substitution (ds 0.01-0.10) is sufficient to produce modified polysaccharides with different properties and applications than the original compounds. The maximum degree of substitution per monomer unit for starch and cellulose is three, at which point all three hydroxyl functionalities of every monomeric unit of starch or cellulose are functionalized. At a degree of substitution of 0.1 on average one of 10 glucose monomers of the polysaccharide bears a substituent*. If the reagent employed (for example ethylene oxide or propylene oxide) is capable of reacting with unsubstituted hydroxyl groups, as well as with the functionality introduced (hydroxyethyl or hydroxypropyl groups), a more complicated situation occurs (Scheme 2). Poly(oxyalkylene) side chains are formed and the molar substitution (ms) becomes, in principle, infinite.



Scheme 2. Structure of hydroxyethyl cellulose.

Determination of the degree of substitution (ds) or molar substitution (ms) depends on the nature of the substituents. If the substituents contain other elements than the polysaccharide, the ds or ms can be calculated from elemental analysis. Other methods that may be used are proton, carbon-[13] and solid state NMR[15], IR spectroscopy[16], UV spectroscopy[17], and indirect methods such as gas chromatography[18] and titrimetric methods[19].

Selective chemical modification can be achieved with polysaccharides that contain unique functional groups. An example is the acylation of the amine functionality of chitosan[20]. Selective chemical modification is also accomplished by enzymatic modifications of polysaccharides. Guar is selectivily oxidized at the primary hydroxyl functionality of the galactose unit by the enzyme galactose oxidase[21]. Another example is the chain extension of starch with α-glucose-1-phosphate[22]. Selective chemical modifications can also be achieved by using selective (re)agents. Tosylation or tritylation of cellulose/starch occurs mainly at the primary hydroxyl function[23], oxidation of starch and inulin with TEMPO/NaOCl/NaBr occurs preferentially at the primary hydroxyl groups (Scheme 3)[24] and oxidation of starch with $NaIO_4$ leads to oxidative glycol scission of the vicinal secondary (C2-C3) hydroxyl groups and the formation of dialdehyde starch[25].

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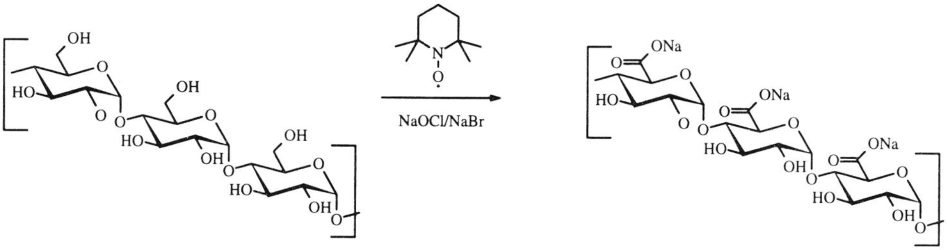
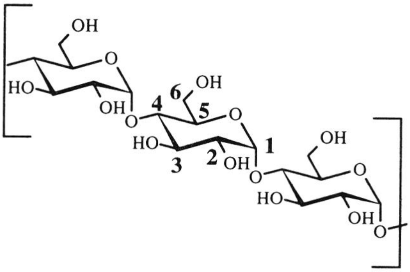
* The substituents are not equally distributed over the monomers; a single monomer unit may bear two or three substituents.



Scheme 3. An example of a selective modification: the oxidation of starch with TEMPO/NaOCl/NaBr (TEMPO = 2,2,6,6-tetramethylpiperidinyloxy).

Most of the chemical modifications of polysaccharides do not go to completion and they often show no exclusive preference for one of the hydroxyl groups. Under homogeneous conditions a random distribution of substituents over the polysaccharide chain is obtained in which the substituent (in the case of etherification or esterification) is divided over the different hydroxyl groups of the monomer/repeating unit. The relative reaction rates of the hydroxyl groups of the monomer/repeating unit are not identical and most often a preference for certain positions is observed, depending on the reaction conditions and the substrate used[26]. The relative reaction rates of reactions of the different hydroxyl groups do not necessarily remain constant during the reaction. For example, substitution at the C-2 position of starch or cellulose influences the reactivity of the C-3 hydroxyl group. The hydrogen atom of the secondary C-2 hydroxyl group is the most acidic proton of starch and cellulose and the primary C-6 hydroxyl group of these polysaccharides shows the least steric hindrance.



Scheme 4. Numbering of the carbon atoms of the glucose monomer of starch.

Under heterogeneous reaction conditions, which are preferable from an economic point of view, a non-uniform substituent distribution is obtained. A huge number of starch and cellulose derivatives are synthesized by heterogeneous slurry processes. In these cases there is a preference for certain domains of the polysaccharide matrix[27]. For heterogeneous cellulose derivatization, the outside of this highly crystalline and water-insoluble polysaccharide is more susceptible to substitution than the less accessible core of the matrix. Thus, products are obtained with regions of relatively high and low degrees of substitution. This explains why polysaccharide derivatives which have the same overall degree of substitution can have completely different physical properties.

The modified polysaccharides (selective and nonselective) can be divided into four classes:

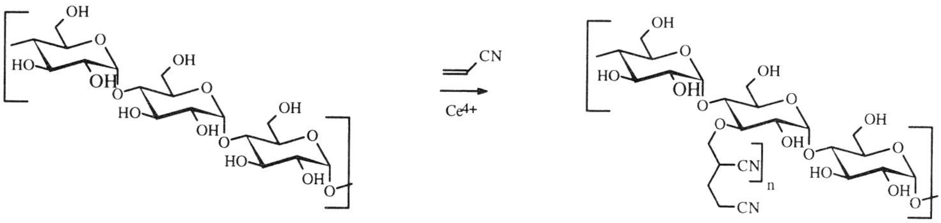
- polysaccharides esters and ethers
- cross-linked polysaccharides
- oxidized polysaccharides
- grafted polysaccharides

Well known polysaccharide esters are acetates, propionates, carbamates, succinates, alkenylsuccinates, palmitates and the inorganic nitrate, phosphate and xanthate esters. Examples of nonionic polysaccharide ethers are methyl, ethyl, allyl, benzyl, hydroxyethyl, hydroxypropyl, cyanoethyl and carbamoethyl ethers. Cationic polysaccharide ethers that have found applications in the paper industry are synthesized by reaction with diethylaminoethyl chloride, 2,3-epoxypropyldiethylamine and 2,3-epoxypropyltrimethylammonium chloride. Anionic polysaccharide ethers are prepared by reaction with chloroacetic acid (carboxymethyl), sulfation with triethylamine-sulfur trioxide (sulfates) or reaction with inorganic phosphate salts[28]. A number of miscellaneous polysaccharide derivatives also have commercial applications[29].

Esterification or etherification of polysaccharides with bifunctional compounds leads to cross-linked polysaccharides. Examples of such bifunctional reagents are adipic acid anhydrides[30], phosphorous oxychloride[31], sodium trimetaphosphate[32], divinyl sulfone[33] and epichlorohydrin[34]. Cross-linking of polysaccharides can also be achieved with formaldehyde[35]. With minor amounts of cross-linking agents, large aggregates with interesting rheological behavior or sometimes insoluble products are obtained.

Oxidation of polysaccharides (starch, inulin, cellulose, pullulan, guar) to polyuronates (C-6 oxidation) can be achieved with nitrogen oxides (NO_x), however, some degradation of the polysaccharide occurs[36]. A better alternative is the oxidation with the elegant catalyst system TEMPO/NaOCl/NaBr (Scheme 2)[27]. Inulin and starch are oxidized selectively at the secondary hydroxyl groups with $NaIO_4$ (preparation of dialdehyde starch) or NaOCl/NaBr (preparation of dicarboxy inulin or starch)[37].

A wide range of polymerization techniques for the synthesis of grafted polymers from polysaccharides are available. These products, which contain a polysaccharide backbone, can be prepared by radical polymerization of vinyl monomers with Ce^{4+}, peroxide-ferrous ion, or ^{60}Co irradiation as radical initiators (Scheme 5)[38].



Scheme 5. Radical polymerization of acrylonitrile to starch; Ce^{4+} is used as the initiator.

Secondary derivatives of polysaccharides are obtained if a pre-existing substituent (primary derivative) of the polysaccharide undergoes a second reaction. Coupling of enzymes, antibodies and antigens to dialdehyde cellulose[39] or the synthesis of hydrophobic polysaccharides by reaction of hydroxyethyl starch with large epoxyalkanes are examples of such secondary derivatives[40].

1.3 Structure, properties, and applications of polysaccharides used in this thesis

We chose the polysaccharides starch, inulin, cellulose, guar, pullulan and agarose as the starting materials, although this thesis mainly focuses on starch. All of these polysaccharides are readily available at low cost. A short description of these materials will be provided in the next paragraphs.

1.3.1 Starch

Starch consists of two glucose polymers: amylose (**1**a, Scheme 1) and amylopectin (**1**b). Amylose is a linear polymer and consists of glucose units linked via α-(1→4) bonds. Amylopectin has a branched structure, consisting of linear chains of glucose units (α-(1→4) linked), which are interconnected *via* α-(1→6) branching points. Starch is isolated in granular form from seeds, tubers or roots of plants where it serves as an energy reserve. The sizes of the granules, the structures and molecular sizes of the amylose and amylopectin as well as the amylose/amylopectin ratio depend on the botanical origin, the genetic background and the moment of harvesting.

Starch granules are insoluble in water. However, when suspensions of starch granules are heated above a critical temperature, the so-called gelatinization temperature, water penetrates the granules and weakens the hydrogen bonds. The starch granules swell up to large "ghosts" in which crystallinity is no longer observed. When shear is imposed (by stirring), the integrity of the ghosts is lost. Amylose, followed by amylopectin, leaks out of the ghost, goes into solution, and a viscous paste is obtained[41].

The gelatinization temperature (56-68°C) of starch granules and the viscosity of the paste obtained after gelatinization also depend on the botanical orgin. These properties of starches make them very attractive for commercial applications, for example, as a thickener in industry. However, these pastes of native starches have some disadvantages, which limit their potential. The linear amylose polymer is prone to parallel orientation in which hydrogen bonds are formed and water molecules are repelled. This process of intermolecular association is called retrogradation, as a result the sol becomes opaque and precipitation of amylose may occur[42]. Amylopectine is less sensitive towards this process because the branches prevent the parallel alignment of the amylopectin chains.

Recently, an amylose-free potato variety was developed, which overcomes the above-mentioned shortcoming of ordinary potato starches[43]. Chemical modification of amylose-containing starches is another way to prevent the process of retrogradation. The attachment of substituents (branches) prevents the retrogradation of amylose and no precipitation occurs.

A wide variety of starch derivatives are produced on an industrial scale by heterogeneous slurry processes with granular starch. After the reaction the granules can be washed with excess water to purify them from salts, side reaction products and remaining reagent(s). Most of these commercially modified starches have a degree of substitution between 0.01 and 0.1. The purest form of starch, obtained from potatoes, is especially attractive for derivatization. The cheaper cereal starches, which contain varying quantities of proteins and lipids, are often used for the synthesis of hydrolyzates.

1.3.2 Inulin

Inulin (**2**, Scheme 1) is a linear polysaccharide of relatively low molecular weight (300-5,000 Daltons), which consists of (2→1)-linked β-D-fructofuranosyl residues and is usually terminated by an α-D-glucopyranosyl unit ((1→2) linked). Inulin is isolated from chicory, dahlias and artichokes. The molecular weight depends on the origin of the inulin[44].

Inulin is produced on an industrial scale and has found applications in the food industry. It is not digestible by the enzymes of the human intestine and arrives in the colon largely intact. Therefore, inulin is used as a fat replacer in foods.

Rapid progress has been made in the derivatization of inulin, but these modified inulins are not yet produced on a large scale. Etherification efficiencies are high (*e.g.* carboxymethyl inulin) when the reactions are performed in concentrated (50%) inulin/water systems[45] Dicarboxy inulin, prepared by oxidation of inulin with NaOCl/NaBr, is an excellent calcium binder. It contains an oxydiacetate structure which is favorable for the complexation of Ca^{2+} and Mg^{2+} ions. Dicarboxy inulin is currently being tested as a sequestering agent in detergents[37c,d].

Scheme 6. Synthesis of dicarboxyinulin.

1.3.3 Cellulose

Cellulose (**3**, Scheme 1) is a linear polymer consisting of β-(1→4) linked glucose units[46]. It is mainly isolated from wood pulp and cotton lint in the form of fibers, although the interest in bacterial cellulose produced by micro-organisms has increased significantly[47]. The fibers isolated from these plants have different morphological structures and have to be purified from hemicelluloses (mostly xylans) and lignin. The molecular weight (about 1,000,000 Daltons) strongly depends on the origin of the cellulose and decreases somewhat during purification.

The linearity of cellulose is, due to the glycoside β-(1→4) linkages, responsible for the rigidity of the polymer chains. This results in partly crystalline regions in which hydroxyl groups form *intra*-chain and *inter*-chain hydrogen bonds[48]. The degree of crystallinity depends on the source of the cellulose. The crystal form of cellulose also depends on the method of treatment of the cellulose. Regenerated cellulose, obtained in the viscose process, has a crystal structure different from native cellulose. The supramolecular structure of cellulose renders it water insoluble.

Certain areas of cellulose are less available to reagents than others. This heterogeneity of the cellulose fibers adds to the complexity of chemical modification of cellulose in slurry processes. Preswelling with acid or NaOH (synthesis of alkali cellulose) is often carried out before derivatization. Fully acylated cellulose derivatives are synthesized under heterogeneous conditions in which the acylated surface dissolves in the reaction medium and a fresh surface is exposed.

In contrast to the starch derivatives, commercially produced cellulose derivatives have high degrees of substitution. Well-known water soluble cellulose derivatives, produced on an industrial scale, are methyl cellulose (ds 1.6-2.0), carboxymethyl cellulose (ds 0.4-1.2), hydroxyethyl cellulose (ms 1.8-3.5), the more hydrophobic hydroxypropyl cellulose (ms 3.5-4.5) and cellulose esters (ds 2.2-3.0). These cellulose derivatives are used, for example as thickeners, water binders, stabilizers, and suspending agents[49].

1.3.4 Guar

Guar (**8**, Scheme 1) is a branched polysaccharide composed of β-(1→4) mannopyranosyl units, which form the linear backbone, and irregularly distributed α-(1→6) linked galactopyranosyl units[50]. The galactose to mannose ratio is approximately 1:2. The closely related endosperm polysaccharide locust bean gum has a lower galactose to mannose ratio (about 1:3-4). Guar has a molecular weight of about 220,000 and is derived from the guar plant (*Cyanoposis tetragonolobus*). The guar plant needs very little surface water and grows excellently in semiarid regions.

Guar solutions in water are highly viscous due to the galactopyranosyl branches of the polysaccharide. Guar is used as a thickener in the food industry. The *cis* hydroxyl groups of the galactose as well as the mannose units of guar promote the hydrogen binding to clay, talc and mineral ores. Therefore guar has found applications as a settling agent in the mining industry and is used as a filter aid to remove, for example, clay particles.

The viscosity of guar solutions can be altered by cross-linking with borate[51]. This cross-linking reaction is reversible. Under alkaline conditions the *cis* hydroxyl groups form complexes with borate and a cohesive structural gel is obtained. The gel breaks up after neutralization. Important guar derivatives are the hydroxyalkylated (ethyl and propyl) compounds and carboxymethyl guar.

1.3.5 Pullulan

Pullulan (**9**, Scheme 1) is a linear polysaccharide obtained from starch by several fungi[52]. It is comprised of mostly (1→6)-α-D-maltotriose units ({→6-α-D-Glcp-1→4-α-D-Glcp-1→4-α-D-Glcp-1}) and has a molecular weight which varies between 10,000 and 400,000 Daltons, depending on the growing conditions. The flexible 1→6 linkage between the maltotriose units makes pullulan more viscous than amylose. Pullulan is nontoxic and nondigestible.

Although numerous applications of this exocellular α-D-glucan have been described in the literature and claims have been made, modifications of pullulan are so far of no commercial importance.

1.3.6 Agarose

Agarose (**10**, Scheme 1) is a linear polysacharide that consists of alternating (1→3)-linked-β-D-galactopyranoside and (1→4)-linked 3,6-anhydro-α-L-galactopyranoside units[53]. It is isolated from several seaweeds by hot water extraction. After cooling down a hot suspension of agarose a gel is formed. Agarose adopts a complex helical structure[54]. The gel matrix formed is ideal for the separation of biopolymers by means of, for example, electrophoresis. Agarose is also used as medium for cultivating bacteria.

Derivatization of agarose is performed under homogeneous and heterogeneous conditions. An important derivative is hydroxyethyl agarose ("Seaplaque"). Modified agaroses have lower gelling temperatures than agarose. These low-melting gels are used for the introduction of living cells onto agarose sols[55]. Owing to its gel forming capacity and the resistance towards micro-organisms agarose is often used for the immobilization of enzymes.

1.4 Aim of this thesis

Although claims have been made and attempts have been described in the literature to synthesize polysaccharides with primary or secondary amino functionalities, none of the routes to these compounds are suitable for large scale synthesis. Modified polysaccharides with primary or secondary amino groups are expected to have interesting properties for a number of reasons:

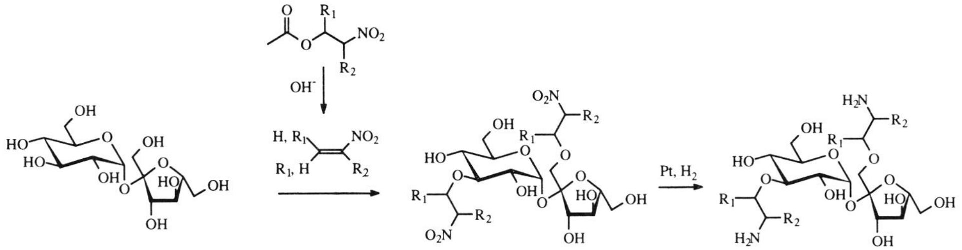
- owing to the basicity of the amino functionality the rheology of these derivatives will be pH dependent.
- The amino functionality, which can easily be converted into other functionalities, makes these compounds excellent precursors for a wide variety of other secondary polysaccharides.
- Aminopolysaccharides have potential for the immobilization of enzymes, as ion-exchange resins, and for complexation of heavy metals in polluted water.

Nature provides us with a primary aminopolysaccharide, chitosan. Unfortunately, the solubility of chitosan in water or alcohols is low and modifications of chitosan are usually performed in exotic solvents, which lowers its attractiveness for industrial scale reactions[56].

When we started our research project we first tried to reduce the nitrile functionality of granular cyanoethyl starch. However, the results were disappointing and no complete conversion to aminoethyl starch was achieved.

A novel route for aminoalkyl sucrose ethers was claimed by Greber and Gruber[57]. After the Michael addition of a nitroalkene (or a precursor thereof) to sucrose, a 2-nitroalkyl sucrose ether is formed. The corresponding 2-aminoalkyl sucrose ether is obtained after reduction of the nitro functionality by catalytic hydrogenation (Scheme 7).

We decided to investigate the potential of this method for the synthesis of 2-aminoalkyl polysaccharide ethers but realized that another reducing agent had to be used. It is well known that catalytic hydrogenation or oxidation of polymers is often complicated and low conversion rates are obtained.



Scheme 7. Synthesis of 2-aminoalkyl sucrose ethers (the distribution of the nitroalkyl substituent over the sucrose molecule is chosen arbitrarily).

The 2-nitroalkylpolysaccharide ethers are interesting compounds themselves. The polar character of this basically nonionic compound* gives polysaccharide derivatives carrying these substituents interesting chemical and physical properties. Also, substituents on the nitroalkene can be varied easily. This allows the synthesis of 2-nitroalkyl polysaccharide ethers with different properties.

1.5 Survey of contents of this thesis

In *Chapter 2* the syntheses of 2-nitroalkyl starch ethers and 2-nitroalkyl polyglucuronates are described. The influence of different precursors of nitroalkenes and different substituents on these unsaturated compounds on the efficiency of the reaction is investigated. The reaction conditions for the Michael addition have been optimized.

In *Chapter 3* the analysis of 2-nitroalkyl starch ethers is described. Physical properties and topochemical aspects (distribution over the starch granule, amylose/amylopectin distribution, nature of the nitroalkyl functionality) of these compounds are outlined. To simplify NMR spectra derived from these starch ethers, a model system, ^{13}C-labeled 2-nitropropyl starch, was synthesized.

Atempts to reduce 2-nitroalkyl starch ethers are described in *Chapter 4*. The high molecular weight of the polysaccharide prevents a successful catalytic hydrogenation. A mixture of products is formed when the cheap and water-soluble sodium dithionite is used (in combination with $NaBH_4$) as the reducing agent.

Cross-linking of starch with bifunctional precursors for 2-nitroalkenes is described in *Chapter 5*. The results obtained for the precursors used are compared with the commercially used epichlorohydrin. The influence of the degree of substitution of the cross-linking agents on the viscosity and swelling capacities is determined. Cross-linked 2-nitroalkyl starch ethers can also be synthesized in a one-pot synthesis.

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* The α-CH proton of aliphatic nitroalkanes has a pK_a 7-10.5.

The syntheses of other 2-nitroalkyl polysaccharide ethers (cellulose, hydroxyethyl cellulose, guar, agarose, pullulan, inulin and polyglucuronate) are described in *Chapter 6.* To obtain more information from ^{13}C-NMR spectra ^{13}C-labeled 2-nitropropyl pullulan was synthesized and used as a model system. The reduction of these compounds (with sodium dithionite in combination with $NaBH_4$) is also described in this chapter.
Finally, the thesis ends with an epilogue (*Chapter 7*). Our results are evaluated and estimates for the costs of 2-nitroalkylpolysaccharide ethers are described in this chapter.

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Chapter 2

Synthesis of 2-nitroalkyl starch ethers*

2.1 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. Derivatization of starch on an industrial scale is performed in aqueous or aqueous/alcoholic slurry reactions[1], in aqueous solution, in a "semi-dry" process[2], and by means of extruders[3]. The slurry process and preparation in solution were used for the synthesis of 2-nitroalkyl starch ethers in our research project. All these methods will be explained briefly.

Upon completion of the reaction in a slurry process, the suspension is neutralized (as most of the modifications of starch are carried out under alkaline conditions), filtered, washed to remove salts, unreacted reagent and by-products, and finally dried. It is essential to prevent gelatinization of the starch granules. This imposes the restriction that the reaction temperature should not exceed 50°C, and, also, high degrees of substitution cannot be obtained using this process. Swelling inhibitors are often added to alkaline suspension reactions to prevent the gelatinization of starch in a batch slurry process. Cheap chemicals such as Na_2SO_4 and NaCl prevent the gelatinization of starch to some extent under neutral as well as alkaline conditions[4].

The above-mentioned restrictions can be avoided by performing the reaction in aqueous solution. This also generates a more uniform product. However, to keep the viscosity of the starch solution below a certain level, the starch concentration has to be low or autoclaves have to be used. The recovery of the starch derivative (*e.g.*, drum drying) is difficult and expensive.

In a "semi-dry" process (a solvent-free reaction) starch granules are mixed with pulverized NaOH and (etherification) reagents are added by spraying or mixing. In this manner, starch ethers can be obtained with high degrees of substitution. No problematic effluents are formed during the syntheses. By-products and residues which stay behind in the starch granules limit the applications and starch derivatives obtained by this process cannot be used in the food industry.

Recently, extruders have been introduced for chemical modification of starch. Reactive extrusion offers the advantage of continuous processing instead of batchwise processing. The

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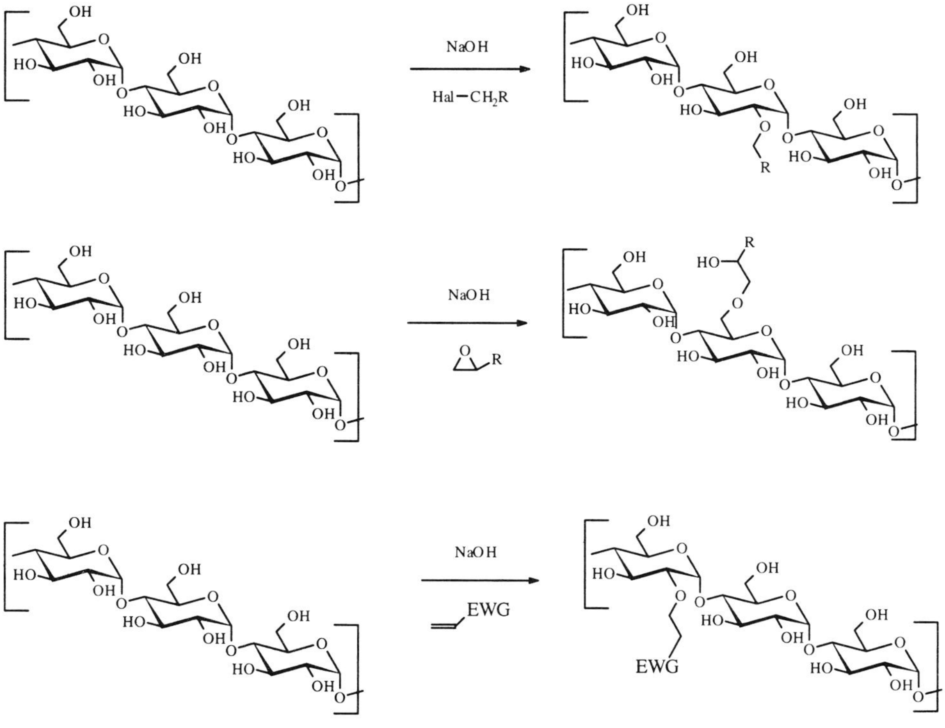
* Part of the work described in this chapter has been patented: K.F. Gotlieb, I.P. Bleeker, H.A. van Doren, A. Heeres, EP 0710671 A1 (1996), and published: A. Heeres, H.A. van Doren, K.F. Gotlieb, I.P. Bleeker, R.M. Kellogg, *Carbohydrates as Organic Raw Materials IV,* W. Praznik, A. Huber (eds.), WUV/Universitätsverlag, (1998) 264-277.

relatively extreme reaction conditions modify the starch physically. The granular structure is destroyed and, depending on the conditions used, in some cases degradation of the polymer may occur by mechanical shear.

Nitromethylation of starch has been achieved by reaction of dialdehyde starch with nitromethane[5]. We were more interested in a route to nitroalkyl starches in which the nitrofunctionality is introduced in starch in a one-step procedure through formation of an ether linkage by reaction of one of the hydroxyl groups of the polysaccharide. In contrast to the starch esters, starch ethers are thermally more stable and less sensitive to acidic and alkaline conditions. Well known methods for the synthesis of starch ethers are the nucleophilic substitution of halo compounds, Michael additions with activated unsaturated compounds, and reactions with epoxides (Scheme 1).



Scheme 1. Different types of reaction for the synthesis of starch ethers (EWG = electron-withdrawing group).

Surprisingly, reports of a Michael addition of granular starch with the best Michael acceptors known in the literature, the nitroalkenes[6], are lacking. Synthesis by means of this addition leads to an essentially nonionic starch derivative with high polarity.

Another approach towards 2-nitroalkyl starch ethers is reaction of starch with epoxides containing a nitro group. A well-suited epoxide which we synthesized for our research project is 1,2-epoxy-3-nitropropane.

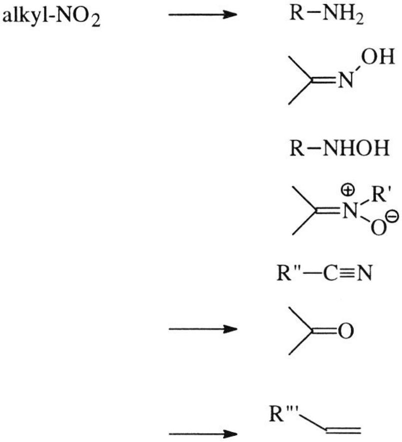
Not only are 2-nitroalkyl starch ethers interesting compounds themselves, they could also be useful starting materials for other starch ethers. We examined the synthesis of granular and water soluble 2-nitroalkyl starch ethers with the above-mentioned substrates in batch processes (slurry and aqueous solution).

Furthermore, the results of the synthesis of 2-nitroalkyl α-polyglucuronates will be presented. Sodium α-polyglucuronate is synthesized very easily by oxidation of the primary hydroxyl group of starch with TEMPO/NaOCl/NaBr. Nitroalkylation and reduction of the nitro group to an amine could lead to interesting amphoteric polysaccharide derivatives.

In this chapter the synthesis of nitroalkyl starches and nitroalkyl α-polyglucuronates is described. In *Chapter 3* the analysis of these compounds will be outlined.

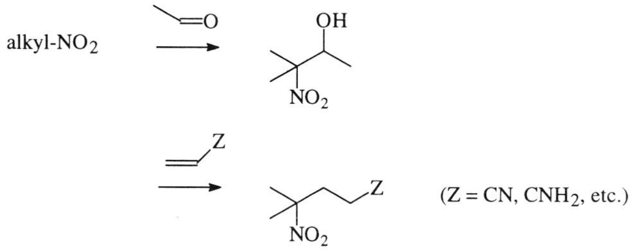
2.2 Results and discussion

2.2.1 Synthesis of (precursors of) nitroalkenes and 1,2-epoxy-3-nitropropane



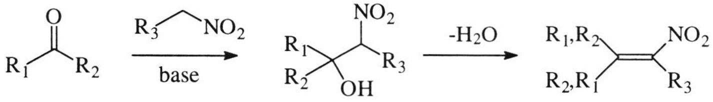
Scheme 2a. Transformation of nitroalkanes (conversion without changes in the carbon skeleton).

Nitroalkenes are versatile intermediates in organic synthesis[7]. Various reactions have been described in the literature in which nitroalkenes are used as dienophiles in Diels-Alder reactions[8], or as substrates for carbon-[9], nitrogen-[10], sulfur-[11] and oxygen-[12]centered nucleophiles. Owing to the relative ease with which nitro functionalities can be transformed into a legion of other functionalities (ketones, oximes, hydroxylamines, amines) and their excellent properties for carbon-carbon coupling reactions (see Scheme 2a/2b) with various compounds makes them very attractive substrates (intermediates) for organic chemists.



Scheme 2b. Transformations of nitroalkanes (reaction at the α-CH carbon atom).

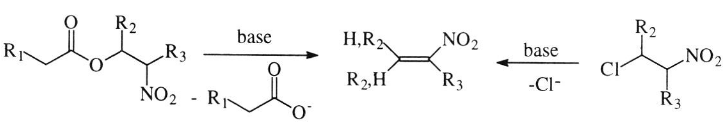
Several routes are available for the synthesis of nitroalkenes[13]. The dehydration of β-nitroalcohols, obtained by an aldol condensation (Henry reaction) of a nitroalkane with a carbonyl compound shows good perspective for large scale synthesis (Scheme 3)[14].



Scheme 3. Synthesis of nitroalkenes by dehydration of β-nitroalcohols.

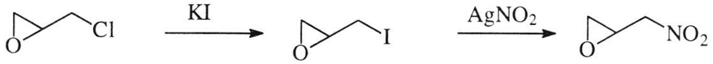
Dehydration can be accomplished with dicyclohexylcarbodiimide[15], acyl chlorides, and anhydrides can also be used for this purpose. After esterification a better leaving group has been introduced which simplifies the dehydration step[16]. An elegant route, which we used for the synthesis of nitroalkenes, is reaction of a β-nitroalcohol with methanesulfonylchloride and subsequent addition of base (triethylamine) to remove the sulfonic acid[17].

The reaction conditions (slightly alkaline) utilized for the synthesis of nitroalkenes from β-nitro-chloroalkanes[18] and β-nitro-acyloxy-alkanes are almost identical to the reaction conditions necessary for the Michael addition. Therefore these precursors, which allow the *in situ* formation of nitroalkenes, were synthesized and tested in the Michael addition to starch (Scheme 4).



Scheme 4. Synthesis of nitroalkenes from β-nitro-acyloxy-alkanes and β-nitro-chloroalkanes.

Another reagent that, in principle, is well suited for the (laboratory scale) synthesis of nitroalkyl starch ethers is the epoxide, 1,2-epoxy-3-nitropropane. This compound, which has also been used as a substrate for cationic polymerization[19], was obtained in acceptable yields (25%) from epichlorohydrin by halogen exchange with KI and subsequent nitration with $AgNO_2$ (Scheme 5) [20].

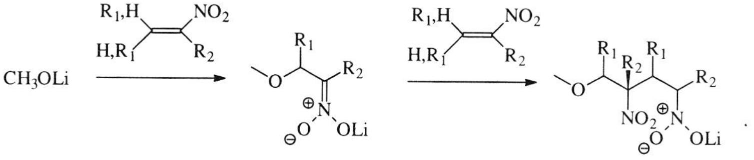


Scheme 5. Synthesis of 1,2-epoxy-3-nitropropane

2.2.2 *Synthesis of 2-nitroalkyl starch ethers*

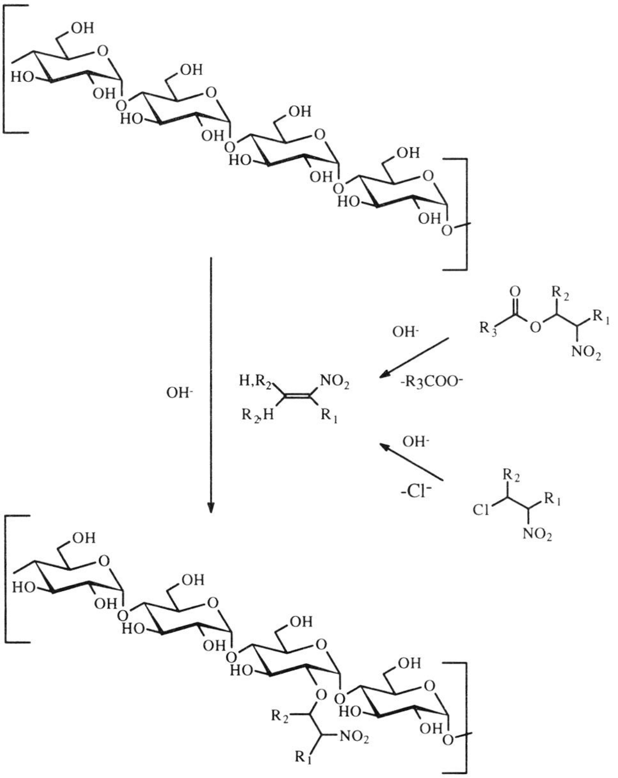
Introduction

In general, the yields of Michael additions of nitroalkenes to oxygen-centered nucleophiles are only moderate[11]. A side reaction is the competing Michael addition of the intermediate nitronate with unreacted nitroalkene, which results in oligomerization (Scheme 6). The oligomerization is suppressed with excess nucleophile and by carrying out the reaction at a low temperature.



Scheme 6. Oligomerizations of nitroalkenes in Michael additions of lithium methoxide to nitroalkenes.

The Michael addition of nitroalkenes to starch is illustrated in Scheme 7. The mixtures of the *cis* and *trans* isomers of the nitroalkenes (for 1-nitroalkenes and disubstituted nitroalkenes) were either added directly to the starch suspension, or precursors for nitroalkenes, such as β-nitro-acyloxy-alkanes and β-nitro-haloalkanes, were used. We varied the structure of the nitroalkene (2-nitro-1-alkenes, 1-nitro-1-alkenes and disubstituted nitroalkenes) in order to investigate the influence on the efficiency of the Michael addition (see Table 1).



Scheme 7. Synthesis of 2-nitroalkyl starch ethers (C-2 substitution is taken as an example)

Synthesis of 2-nitroalkyl starch ethers with (precursors of) nitroalkenes in aqueous slurry

All reactions were performed in concentrated starch/H_2O suspensions at room temperature and Na_2SO_4 was added as swelling inhibitor. The pH was held constant at 10-10.5 (the formation of acidic nitroalkyl groups as well as elimination of HCl or acetic acid from the nitroalkene precursors lower the pH) with a pH-stat apparatus. After reaching a constant pH (no more acid formation) the suspension was neutralized and washed with water. Slightly yellow starch derivatives were isolated, which remained in the original granular structure as confirmed by polarization microscopy*.

The reaction time depends on the structure and the solubility of the nitroalkene (or precursor). Reaction with 2-nitrononyl acetate took 5 days, but the smaller 2-nitropropyl acetate or 2-

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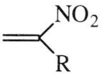
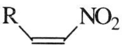
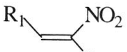
* The analysis of these compounds is described in the next chapter

nitrobutyl acetate reacted within a few minutes, which is quick compared to other Michael additions. We varied the molar ratio nitroalkene/starch (ms_{max}) from 0.06-0.17. At higher nitroalkene/starch ratios (>0.20-0.25) the gelatinization temperature of the products is lowered to such an extent that the granular form is disrupted under the alkaline reaction conditions used.

The results for the Michael addition of nitroalkenes, β-nitro-acetoxy-alkanes and β-nitro-haloalkanes to granular starch are shown in Table 1. Several factors influence the efficiency of the reaction.

Table 1. Synthesis of 2-nitroalkyl starch ethers.

<table>
<tr><td></td><td colspan="4">β-nitro-acetoxy-alkanes (precursor)</td><td colspan="4">β-nitro-halo-alkanes (precursor)</td><td colspan="4">nitroalkenes</td></tr>
<tr><td>nitroalkene formed</td><td colspan="2">R</td><td>ms_{max}</td><td>eff.^{a}</td><td colspan="2">R</td><td>ms_{max}</td><td>eff^a.</td><td colspan="2">R</td><td>ms_{max}</td><td>eff^a.</td></tr>
<tr><td rowspan="6"></td><td colspan="2"></td><td></td><td></td><td colspan="2">H^c</td><td>0.167</td><td>0.22</td><td colspan="2"></td><td></td><td></td></tr>
<tr><td colspan="2">CH_3^b</td><td>0.065</td><td>0.92</td><td colspan="2">CH_3^c</td><td>0.105</td><td>0.55</td><td colspan="2">CH_3^b</td><td>0.076</td><td>0.79</td></tr>
<tr><td colspan="2">$C_2H_5^b$</td><td>0.065</td><td>0.92</td><td colspan="2">$C_2H_5^b$</td><td>0.065</td><td>0.77</td><td colspan="2">$C_2H_5^b$</td><td>0.075</td><td>0.79</td></tr>
<tr><td colspan="2">$C_3H_7^b$</td><td>0.065</td><td>0.87</td><td colspan="2"></td><td></td><td></td><td colspan="2"></td><td></td><td></td></tr>
<tr><td colspan="2">$C_4H_9^b$</td><td>0.062</td><td>0.85</td><td colspan="2"></td><td></td><td></td><td colspan="2"></td><td></td><td></td></tr>
<tr><td colspan="2">$C_7H_{15}^b$</td><td>0.141</td><td>0.38</td><td colspan="2"></td><td></td><td></td><td colspan="2"></td><td></td><td></td></tr>
<tr><td rowspan="2">
(E, Z)</td><td colspan="2">CH_3^c</td><td>0.107</td><td>0.36</td><td colspan="8" rowspan="2"></td></tr>
<tr><td colspan="2">$C_5H_{11}^c$</td><td>0.122</td><td>0.24</td></tr>
<tr><td rowspan="3">
(E, Z)</td><td>R_1</td><td>R_2</td><td></td><td></td><td>R_1</td><td>R_2</td><td></td><td></td><td>R_1</td><td>R_2</td><td></td><td></td></tr>
<tr><td>CH_3^c</td><td>CH_3</td><td>0.062</td><td>0.43</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>CH_3^c</td><td>C_2H_5</td><td>0.115</td><td>0.05</td><td>CH_3^c</td><td>C_2H_5</td><td>0.092</td><td>0.15</td><td>CH_3^c</td><td>C_2H_5</td><td>0.094</td><td>0.34</td></tr>
</table>

a) eff = reaction efficiency (ms/ms_{max}), the ms was calculated by means of the following equation: $ms = 1.62N/\{14-0.01N(Mwt_{alkt}-1)\}$, N = nitrogen content in %N, Mwt_{alk} = molecular weight of the nitroalkyl substituent. b) H_2O/starch 1:1, pH = 10, 5% Na_2SO_4. c) H_2O/starch 2:1, pH = 10.5, 5% Na_2SO_4.

The highest reaction efficiencies (ms/ms_{max}) were obtained when 2-nitro-1-alkenes are used as Michael acceptors. In these cases, the Michael addition is sterically less hindered at the C-1 position of the nitroalkene and subsequently fewer side reactions, such as anionic polymerization, occur. Furthermore, an alkyl group at the C-1 position, as is the case for

1-nitro-1-alkenes and disubstituted nitroalkenes, reduces the electron deficiency of the unsaturated bond and decreases the rate of the Michael addition.

Well-known side reactions of the Michael additions are the already-mentioned anionic polymerization/oligomerization of nitroalkenes under alkaline conditions. Especially the small nitroalkenes are rather sensitive to oligomerization. It is possible that oligomers which are formed during the reaction influence the efficiency of the Michael addition because these compounds compete with reaction with nitroalkenes. As shown in Figure 1, oligomerization of 1-nitroalkenes (R_2 = H) leads to products with *n* reaction centers for subsequent reaction with 1-nitro-1-alkenes. Oligomerization of 2-nitro-1-alkenes or disubstituted alkenes leads to products with *one* reaction centre for subsequent reaction. Thus oligomers obtained from 1-nitro-1-alkenes are more reactive towards nitroalkenes in comparison with oligomers obtained from 2-nitro-1-alkenes and disubstituted nitroalkenes.

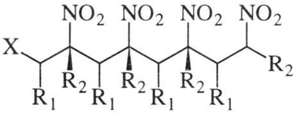


Figure 1. Oligomerization of nitroalkenes (R_2 = H, $R_1CH_2CH(NO_2)R_2$), *etc*: 1-nitroalkenes; R_2 = alkyl: 2-nitroalkenes, disubstituted nitroalkenes, X = for example OH/acyl).

Also relevant is the rate of formation of the carbanion ($R_2C^{\ominus}NO_2$) of the oligomer. Although secondary nitroalkanes are more acidic than primary nitroalkanes* (Figure 2) the rate of salt formation is much lower (the nitroalkane anomaly[21]). This is also in accordance with a higher reactivity of oligomers obtained from 1-nitro-1-alkenes and subsequently lower efficiencies of the Michael addition. These factors are consistent with an increase in efficiency for the Michael addition of nitroalkenes to starch in the order 2-nitro-1-alkenes > disubstituted nitroalkenes > 1-nitro-1-alkenes.

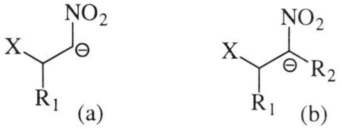


Figure 2. Primary (a) and secondary (b) nitroalkane salts.

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* Nitromethane (pK_a 10.22, K_{OH^-} 27.6 $M^{-1}s^{-1}$), nitroethane (pK_a 8.60, K_{OH^-} 5.19 $M^{-1}s^{-1}$), 2-nitropropane (pK_a 7.74, K_{OH^-} 0.316).

The best results were obtained when the 2-nitro-1-alkenes are formed *in situ* from β-nitro-acetoxy-alkanes. Owing to the relatively slow formation of the 2-nitro-1-alkene, the concentration remains low and side reactions are suppressed.

In Table 1 the molar ratio nitroalkene/starch (ms_{max}) varies from 0.06-0.17. To investigate the efficiency as a function of the nitroalkene/starch ratio we synthesized a series of 2-nitrobutyl starches (ms = 0.018-0.118) with 2-nitrobutyl acetate. As shown in Figure 3, the efficiency of the nitroalkylation seems to be independent of the molar substitution. A higher occupation of nitroalkyl groups in the starch granule does not influence the efficiency at low molar substitutions ($ms \leq 0.12$)

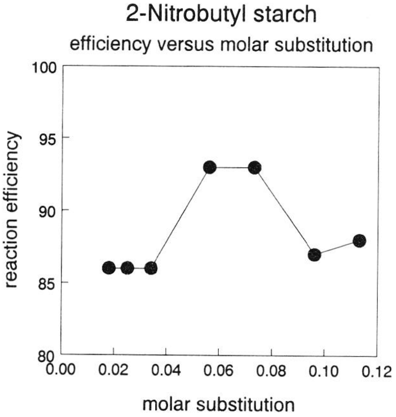
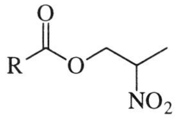


Figure 3. Influence of the degree of substitution of 2-nitrobutyl starch (synthesized with 2-nitrobutyl acetate) on the efficiency (ms/ms_{max})) of the reaction.

Synthesis of 2-nitroalkyl starch ethers with β-nitro-acyloxy-alkanes (acyl ≠ acetate) in aqueous slurry

Remarkably, it was found that the reaction efficiency also depends on the nature of the acyl group (the leaving group) of the β-nitro-acyloxy-alkanes. Almost quantitative yields were obtained when the 2-nitroalkene (2-nitro-1-propene, Table 2) is formed *in situ* from β-nitro-acyloxy-alkanes in which the acyloxy group is an electron-donating group (*n*-Pr, *n*-Bu). No etherification occurs when the acyloxy group contained a strongly electron-withdrawing group, such as a trichloro- or trifluoromethyl group. In these cases, the β-nitro-acyloxy-alkanes are more susceptible to hydrolysis of the ester functionality to the corresponding carboxylic acid and β-nitro-alcohol.

Table 2. Reaction efficiencies of the synthesis of 2-nitropropyl starch with 2-nitropropyl acylates (ms_{max} = 0.065).

2-nitropropyl acylate	R	eff.[a]
	H	0.18
	CH_3	0.92
	C_2H_5	0.99
	C_3H_7	0.99
	$MeOCH_2$	0.69
	CH_2Cl	0.25
	$CHCl_2$	0.09
	CCl_3	0.02
	CF_3	0.01

a) eff = reaction efficiency (ms/ms_{max}), the ms was calculated by means of the following equation: $ms = 1.62N/\{14-0.01N(Mwt_{alkt}-1)\}$, N = nitrogen content in %N, Mwt_{alk} = molecular weight of the nitroalkyl substituent.

Optimization of the synthesis of 2-nitroalkyl starch ethers with β-nitro-acetoxy-alkanes in aqueous slurry

2-Nitropropyl and 2-nitrobutyl starch, synthesized by using the corresponding 2-nitroalkyl acetates, seem most promising for commercial applications. The efficiencies of both reactions are high and these modified starches are synthesized easily from the cheap starting materials nitroethane (nitropropane), formaldehyde, acetic anhydride and starch. As the Michael additions with both 2-nitroalkyl acetates are already very fast at room temperature we did not investigate the influence of the temperature on the efficiency of the reaction but focussed on the variables starch/H_2O ratio and pH.

Optimization of these reactions revealed that the highest reaction efficiency for the synthesis is reached, as expected, in concentrated aqueous systems (starch/H_2O 1:1). The concentration of the oxygen centered nucleophile is high and, consequently, fewer side reactions occur.

The pH optimum lies around 10-10.5. Under less alkaline conditions the balance between the Michael addition and side reactions, such as oligomerization of nitroalkenes and hydrolysis of the 2-nitroalkyl acetates is probably shifted unfavorably. pH Values higher than 11-12 lead to disruption of the starch granule.

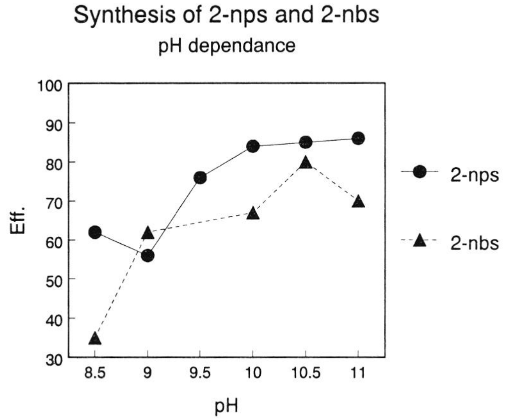


Figure 4a. Influence of the pH on the efficiency of the formation of 2-nitropropyl (2-nps) and 2-nitrobutyl starch (2-nbs).

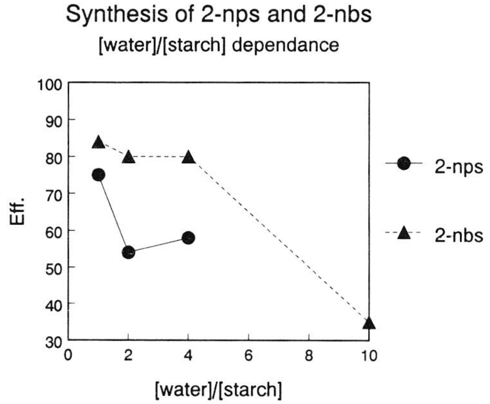


Figure 4b. Influence of the starch/H_2O concentration on the efficiency of the formation of 2-nitropropyl (2-nps) and 2-nitrobutyl starch (2-nbs).

Synthesis of 2-nitroalkyl starch ethers with β-nitro-acyloxy-alkanes in aqueous/alcoholic slurry

The use of water-miscible organic solvents in aqueous alkaline media - so far the reactions were carried out in aqueous suspension - eliminates the requirement for a gelatinization inhibitor and unreacted organic material and organic side products are washed out more easily[22]. High efficiencies were obtained for the preparation of cationic and amphoteric starches in an aqueous alcohol process. The irreversible gelatinization was inhibited by decreasing the effective water concentration[22]. The reaction efficiencies for the synthesis of 2-nitropropyl starch with 2-nitropropyl acetate in H_2O/2-propanol mixtures are shown in Figure 5.

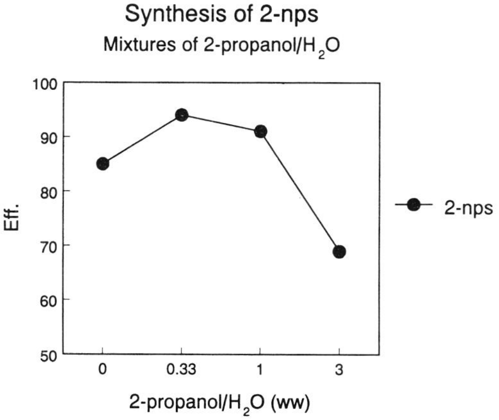


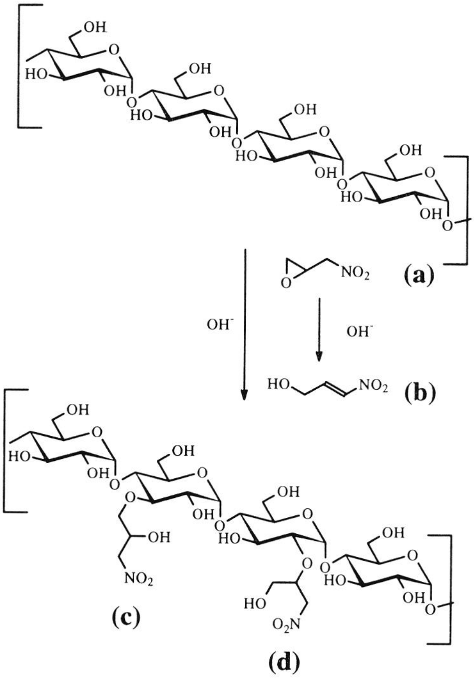
Figure 5. Reaction efficiencies for synthesis of 2-nitropropyl starch with 2-nitropropyl acetate in mixtures of 2-propanol/H_2O (ms_{max} = 0.098).

The selection of 2-propanol as co-solvent was based on reasons of cost, availability, low toxicity, and because a recent study revealed better results for cationization of starch with 2-propanol as the water-miscible solvent compared to MeOH and EtOH[22a]. The efficiency of the reaction was almost 100% when the synthesis was performed in a 1:3 mixture of 2-propanol/H_2O. In this medium the side reactions are suppressed and the formation of 2-nitropropene and subsequent Michael addition are favored.

Synthesis of water-soluble 2-nitroalkyl starch ethers

Although less interesting for industrial applications than slurry reactions, we also synthesized 2-nitroalkyl starch ethers in aqueous solution. The synthesis of 2-nitroalkyl starch ethers in homogeneous solutions may lead, in contrast to slurry reactions, to a more uniform starch polymer. Isolation of the water-soluble starch ether is achieved by pouring the reaction mixture in alcohol in which the modified starch precipitates. The synthesis using β-nitro-acetoxy-alkanes proceeds less efficiently compared to slurry reactions, probably due to the low concentration of starch (2-nitropropyl starch, eff. = 0.48; 2-nitrobutyl starch, eff. = 0.67). Attempts to obtain water-soluble 2-nitropropyl starch by stirring granular 2-nitropropyl starch (ms = 0.078) in a Parr apparatus (15 min at 140°C) leads to water-soluble starch with low viscosity (some degradation of the starch backbone occurs under these conditions). However, the drastic conditions also lead to a reduction in molar substitution (ms = 0.026). A better method to obtain water-soluble 2-nitroalkyl starches is by means of drum drying. By using this technique, 2-nitropropyl starch (ms = 0.080, 2%), solutions of the starch ether were obtained without difficulty. In contrast with the synthesis in homogeneous solutions, the non-uniform substituent distribution caused by the slurry synthesis is retained in this water-soluble starch ether.

Attempts to synthesize 2-hydroxy-3-nitropropyl starch with 1,2-epoxy-3-nitropropane in an aqueous slurry



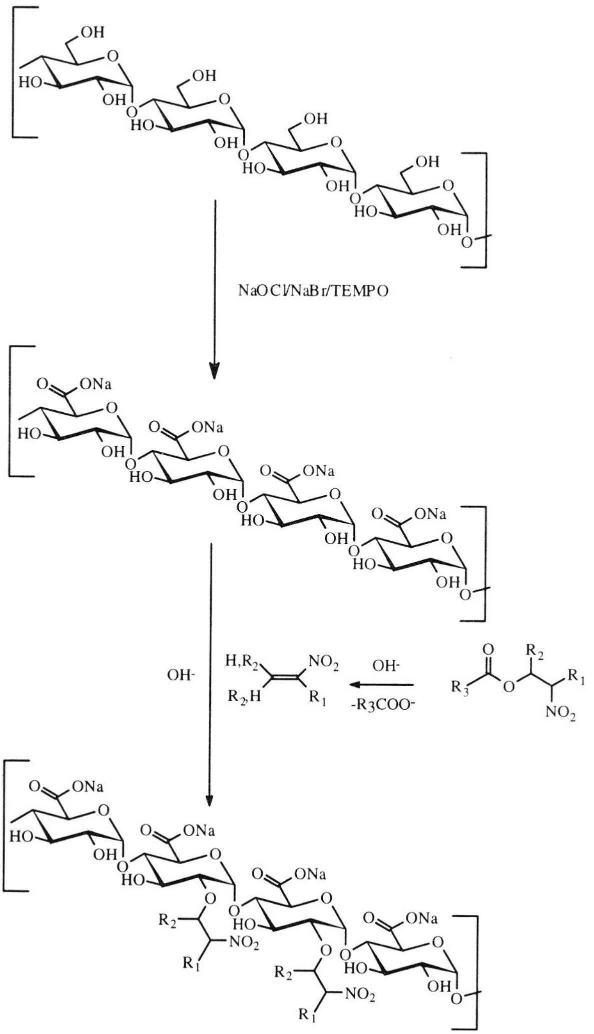
Scheme 8. Reaction of starch with 1,2-epoxy-3-nitropropane (etherification can occur on C-2, C-3 or C-6).

Another route for the synthesis of 2-nitroalkyl starch ethers entails reaction of starch with nitro-containing epoxides, such as for example 1,2-epoxy-3-nitropropane. However, reaction of starch with 1,2-epoxy-3-nitropropane suffered from side reactions. After the reaction a orange-yellow starch derivative was isolated (m.s. = 0.027, eff = 0.30). It is likely that 1,2-epoxy-3-nitropropane isomerizes rapidly to a nitroallylic alcohol (3-hydroxy-1-nitro-1-propene) under the alkaline conditions used[23]. In principle, this nitro-olefin is a substrate for a Michael addition to starch (Scheme 8). Other potential side reactions, besides the Michael addition of the nitroallylic alcohol (**b**, Scheme 8) are polymerization of the nitroallylic alcohol, a reverse Henry addition of the β-nitroalcohol (**c**) to nitromethane and 2-ethanal starch and hydrolysis of the epoxide to a diol. Due to the low efficiency of the epoxide etherification we concentrated on the Michael additions of nitroalkenes to starch.

2.2.3 *Synthesis of 2-nitroalkyl sodium α- polyglucuronate ethers*

After oxidation of 2-nitroalkyl starch ethers or reaction of oxidized starch ethers with nitroalkenes novel anionic nitroalkyl polysaccharide ethers are obtained. The second route, reaction of oxidized starch ethers with nitroalkenes, has the advantage that higher molar substitutions can be obtained than when the compound is synthesized from granular starch. After reduction of the nitroalkyl functionality a new class of amphoteric polysaccharide derivatives could be synthesized.

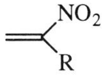
The selective oxidation of starch with the catalyst system NaOCl/NaBr/TEMPO is an elegant route for the synthesis of sodium α-polyglucuronate[24]. Recently, we oxidized several starch ethers, which are predominantly substituted on the C-2 position of the polysaccharide backbone, to the corresponding sodium α-polyglucuronate ethers[25]. Especially the carboxymethyl (obtained after oxidation of carboxymethyl starch or hydroxyethyl starch) α-polyglucuronate has excellent sequestering capacities. These compounds were not only synthesized by oxidation of starch ethers. An alternative, even more selective, route is the etherification of polyglucuronate. The synthesis of 2-nitroalkyl α-polyglucuronate from sodium α-polyglucuronate is described in Scheme 9.



Scheme 9. Synthesis of 2-nitroalkyl polyglucuronates.

Unfortunately, the efficiencies of the nitroalkylation of polyglucuronates with 2-nitroalkyl acetates are rather low (see Table 3). Several factors, such as high water concentrations, highly alkaline (starting) conditions, the absence of the primary alcohol group and/or the negative charge of the uronate can be the origin of the low selectivity of the nitroalkyl etherification.

Table 3. Reaction efficiencies for synthesis of 2-nitroalkyl polyglucuronates with β-nitro-acetoxy-alkanes.

	β-nitro-acetoxy-alkanes (precursor)		
nitroalkene formed	R	ms_{max}	eff.[a]
	CH_3[b]	84	32
	C_2H_5[c]	124	25
	C_3H_7[b]	83	20
	C_4H_9[b]	83	21

a) eff = reaction efficiency (ms/ms_{max}), the ms was calculated by means of the following equation: $ms = 1.98N/\{14-0.01N(Mwt_{alkt}-1)\}$, N = nitrogen content in %N, Mwt_{alk} = molecular weight of the nitroalkyl substituent. b) No quantitative yields, owing to alkaline depolymerization of α-polyglucuronate (R = Me (80%), *n*-Pr (45%), *n*-Bu (60%). c) The ketals of the residual aldehyde functions in the α-polyglucuronate synthesized were reduced with $NaBH_4$ before etherification (R = Et (99%)[24].

2.3 Conclusions

A route to a new class of starch ethers, based on Michael additions of nitroalkenes (formed *in situ*) to starch, has been developed. The efficiency of the etherification with granular starch is especially high when 2-nitroalkenes are formed from β-nitro-acyloxy-alkanes and the starch derivatives are prepared in concentrated starch/water systems (or water/2-propanol mixtures) under alkaline conditions (pH 10.0-10.5). The 2-nitropropyl and 2-nitrobutyl starches seem most promising for commercial applications. These starch ethers can be synthesized from cheap chemicals in a straightforward manner.

Reaction of starch with 1,2-epoxy-3-nitropropane proceeds less efficiently. The epoxide suffers from side reactions such as, for example, isomerization, anionic polymerization, and hydrolysis.

Initial oxidation of granular starch with NaOCl/NaBr/TEMPO and subsequent etherification with 2-nitroalkyl acetates provides 2-nitroalkyl α-polyglucuronates. The yields of the nitroalkylations are moderate.

2.4 Experimental

***General warning:* Nitro compounds, especially nitroalkenes, are explosive materials and should be handled with care. Nitroalkenes are lachrymators.**

General methods. 1-Iodo-octane was purchased from Fluka. Nitromethane (96%), nitroethane (96%), 1-nitrobutane (98%), 1-nitropentane (97%), 2-nitroethanol (85%), 2-nitropropanol (97%), 3-nitro-2-butanol (98%), epichlorohydrin, silver nitrite and TEMPO were purchased from Aldrich. 1-Nitropropane (95%), formaldehyde (37% solution), acetaldehyde, hexanal, acetic anhydride, propionic anhydride, butyric anhydride, trifluoroacetic acid, trichloroacetic acid, dichloroacetic acid, monochloroacetic acid, NaOH pellets, potassium iodide, concentrated HCl, thionylchloride and methanesulfonyl chloride were purchased from Merck. Methoxyacetic acid was purchased from Acros. Diethyl ether, CH_2Cl_2 and EtOH were purchased from Lab-Scan. NaBr was purchased from Sigma. 1-Nitrooctane was synthesized according to a literature procedure[26]. 2-Nitro-1-propanol, 2-nitro-1-butanol, 2-nitro-1-pentanol, 2-nitro-1-hexanol, 2-nitro-1-nonanol, 1-nitro-2-propanol, 1-nitro-2-heptanol and 3-nitro-2-butanol were synthesized according to literature procedures[12b,14a]. 2-Nitropropyl acetate, 2-nitrobutyl acetate, 2-nitropentyl acetate, 2-nitroheptyl acetate, 2-nitrononyl acetate, 2-nitrononyl acetate 1-nitro-2-propyl acetate, 1-nitro-2-heptyl acetate, 3-nitro-2-butyl acetate, 3-nitro-2-pentyl acetate, 2-nitropropyl formiate, 2-nitropropyl propianate, 2-nitropropyl butyrate, 2-nitropropyl methoxyacetate, 2-nitropropyl monochloroacetate, 2-nitropropyl dichloroacetate, 2-nitropropyl trichloroacetate, 2-nitropropyl trifluoroacetate were synthesized according to slightly modified literature procedures[27]. The synthesis of 2-nitropropyl acetate is given as a general example.

Synthesis of 2-nitropropyl acetate. - Acetic anhydride (3850 g, 37.7 mole) and 2-nitropropanol (3600 g, 34.28 mole) were divided into 20 portions of about 300 ml. A few drops of concentrated H_2SO_4 were added to these fractions. After 15 minutes the fractions were collected and stirred for 16 hours. The reaction mixture was poured into Et_2O (15 L) and neutralized with saturated $NaHCO_3$ solution (15 L). After separation of the organic layer, the $NaHCO_3$ solution was washed with Et_2O (5 L) and the combined ether layers were dried over Na_2SO_4. After filtration and rotary evaporation of the solvent, the residue was distilled under reduced pressure (0.1 mm Hg, 75°C) and 4373 g of 2-nitropropyl acetate (29.75 mole, 87%) was isolated. NMR ($CDCl_3$), in ppm): ^1H δ 1.55 (d, 3H, CH_3), δ 2.05 (s, 3H, $O{=}CCH_3$), δ 4.41 (d, 2H, CH_2), 4.77 (m, 1H, CH). ^{13}C δ 15.37 (CH_3), δ 20.26 (CH_3), δ 64.16 (CH_2), δ 80.84 (CH), δ 170.16 (C=O).

1-Chloro-2-nitroethane, 1-chloro-2-nitropropane, 1-chloro-2-nitrobutane, and 2-chloro-3-nitropentyl acetate were synthesized according literature procedures[13e]. 2-Nitro-1-propene, 2-Nitro-1-butene, and 3-nitro-2-pentene were synthesized according to literature procedures[16]. Sodium α-polyglucuronate was synthesized according to a literature procedure[23]. Potato starch, soluble potato starches and NaOCl solutions (140 g/l) were obtained as a gift from AVEBE (Foxhol, The Netherlands).
^1H-NMR and ^{13}C-NMR spectra were recorded on a 300MHz Varian VTR-300 spectrometer. Differential Scanning Calorimetry was performed on a Perkin-Elmer DSC7 PC Series apparatus (heating rate 5.0 K min^{-1}). The pH-stat, a Titration Controller 1200 equipped with a Titronic T110 burette was purchased from Schott Geräte. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Dry substance content of the starch and modified starches were determined after drying for 1.5 h at T = 140°C.

Synthesis of 2-nitropropyl starch (2-nitropropyl acetate). - Native starch (100.31 g, dry substance 86.90%, 538 mmoles) was suspended in 100 ml H_2O which contained 5.00 g Na_2SO_4 (swelling inhibitor). The pH of the starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropyl acetate (5.14 g, 35.0 mmole) was carefully added dropwise to the suspension. The pH was regulated with a pH-stat by adding portions of 1 M NaOH. After 45 minutes the reaction was complete (no more acid formation) and the suspension was neutralized with a 0.5 N HCl solution. After filtration and washing with water the product was dried in the air. Yield: 102.52 g 2-nitropropyl starch (T_{gel} = 50.55°C*, ms = 0.060, dry substance = 85.70%).

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* Swelling temperature (in °C) of the starch derivative, determined by DSC (onset). The T_{gel} of potato starch (starting material) was 59.34°C.

Synthesis of 2-nitrobutyl starch (2-nitrobutyl acetate). - The reaction conditions are identical to the reaction conditions given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however in this case 100.68 g starch and 5.64 g 2-nitrobutyl acetate (35.0 mmole) were employed. Yield 102.73 g 2-nitrobutyl starch (T_{gel} = 51.30°C, ms = 0.060, dry substance = 85.72%).

Synthesis of 2-nitropentyl starch (2-nitropentyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.03 g starch and 6.20 g 2-nitropentyl acetate (35.4 mmole) were employed. Yield 102.23 g 2-nitropentyl starch (T_{gel} = 50.77°C, ms = 0.057, dry substance = 86.53%).

Synthesis of 2-nitrohexyl starch (2-nitrohexyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.51 g starch and 6.24 g 2-nitrohexyl acetate (33.0 mmole) were employed. Yield 103.31 g 2-nitrohexyl starch (T_{gel} = 52.06°C, ms = 0.052, dry substance = 87.05%).

Synthesis of 2-nitrononyl starch (2-nitrononyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 25.01 g starch and 4.24 g 2-nitrononyl acetate (18.4 mmole) were employed. Yield 23.50 g 2-nitrononyl starch (T_{gel} = 54.58°C, ms = 0.054, dry substance = 89.03%).

Synthesis of 1-methyl 2-nitroethyl starch (1-nitro-2-propyl acetate). - Native starch (185.26 g, dry substance 86.90%, 994 mmole) was suspended in 300 ml H_2O which contained 15.24 g Na_2SO_4 (swelling inhibitor). The pH of the starch suspension was adjusted to 10.5 with a 1 M NaOH solution. 1-Nitro-2-propyl acetate (15.71 g, 107 mmole) was carefully added dropwise to the suspension. The pH was regulated with a pH-stat by adding portions of 1 M NaOH. After 2 h the reaction was complete (no more acid formation) and the suspension was neutralized with a 0.5 N HCl solution. After filtration and washing with water the product was dried in the air. Yield 176.53 g 1-methyl-2-nitroethyl starch (T_{gel} = 56.10°C, ms = 0.038, dry substance = 87.75%).

Synthesis of 1-pentyl-2-nitroethyl starch (1-nitro-2-heptyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitroethyl starch (1-nitro-2-propyl acetate) with the understanding that 188.13 g starch and 25.13 g 2-nitrohexyl acetate (123 mmole) were employed. Yield 187.13 g 1-pentyl-2-nitroethyl starch (T_{gel} = 57.55°C, ms = 0.030, dry substance = 87.53%).

Synthesis of 1-methyl-2-nitropropyl starch (3-nitro-2-butyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitropropyl starch (1-nitro-2-propyl acetate), however, in this case 182.50 g starch and 9.80 g 3-nitro-2-butyl acetate (60.9 mmole) were employed. Yield 178.89 g 1-methyl-2-nitropropyl starch (T_{gel} = 56.83°C, ms = 0.027, dry substance = 86.90%).

Synthesis of 1-methyl-2-nitrobutyl starch (3-nitro-2-pentyl acetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitropropyl starch (1-nitro-2-propyl acetate), however, in this case 184.65 g starch and 19.98 g 3-nitro-2-pentyl acetate (114 mmole) were employed. Yield 186.21 g 1-methyl-2-nitrobutyl starch (T_{gel} = nd., ms = 0.006, dry substance = 85.44%).

Synthesis of 2-nitroethyl starch (1-chloro-2-nitroethane). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitropropyl starch (1-nitro-2-propyl acetate), however, in this case 30.22 g starch and 3.03 g 1-chloro-2-nitroethane (27.7 mmole) were employed. Yield 30.25 g 2-nitroethyl starch (T_{gel} = nd., ms = 0.036, dry substance = 86.71%).

Synthesis of 2-nitropropyl starch (1-chloro-2-nitropropane). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitropropyl starch (1-nitro-2-propyl acetate), however, in this case 188.84 g starch and 13.26 g 1-chloro-2-nitropropane (107 mmole) were employed. Yield 188.69 g 2-nitropropyl starch (T_{gel} = 50.15°C, ms = 0.058, dry substance = 87.25%).

Synthesis of 2-nitrobutyl starch (1-chloro-2-nitrobutane). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.62 g starch and 4.83 g 1-chloro-2-nitrobutane (35.1 mmole) were employed. Yield 101.13 g 2-nitrobutyl starch (T_{gel} = 52.89°C, ms = 0.050, dry substance = 86.84%).

Synthesis of 1-methyl-2-nitrobutyl starch (2-chloro-3-nitropentane). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitropropyl starch (1-nitro-2-propyl acetate), however, in this case 99.62 g starch and 7.40 g 2-chloro-3-nitropentane (48.9 mmole) were employed. Yield 178.89 g 1-methyl-2-nitrobutyl starch (T_{gel} = 59.07°C, ms = 0.014, dry substance = 85.80%).

Synthesis of 2-nitropropyl starch (2-nitro-1-propene). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 201.62 g starch and 7.13 g 2-nitropropene (82.0 mmole) were employed. Yield 200.38 g 2-nitropropyl starch (T_{gel} = 48.15 °C, ms = 0.060, dry substance = 85.43%).

Synthesis of 2-nitrobutyl starch (2-nitro-1-butene). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 250.26 g starch and 10.10 g 2-nitrobutene (100 mmole) were employed. Yield 200.38 g 2-nitrobutyl starch (T_{gel} = 48.71°C, ms = 0.059, dry substance = 86.64%).

Synthesis of 1-methyl-2-nitrobutyl starch (3-nitro-2-pentene). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 1-methyl-2-nitroethyl starch (1-nitro-2-propyl acetate), however, in this case 115.26 g starch and 6.70 g 3-nitro-2-pentene (58.2 mmole) were employed. Yield 113.64 g 1-methyl-2-nitrobutyl starch (T_{gel} = 57.49°C, ms = 0.032, dry substance = 89.12%).

Synthesis of 2-nitropropyl starch (2-nitropropyl formiate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.24 g starch and 4.65 g 2-nitropropyl formiate (35.0 mmole) were employed. Yield 101.01 g 2-nitropropyl starch (T_{gel} = 58.74°C, ms = 0.014, dry substance = 86.24%).

Synthesis of 2-nitropropyl starch (2-nitropropyl propionate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.00 g starch and 5.64 g 2-nitropropyl propionate (35.0 mmole) were employed. Yield 100.08 g 2-nitropropyl starch (T_{gel} = 52.12°C, ms = 0.065, dry substance = 87.08%).

Synthesis of 2-nitropropyl starch (2-nitropropyl butyrate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.11 g starch and 6.14 g 2-nitropropyl butyrate (35.0 mmole) were employed. Yield 98.45 g 2-nitropropyl starch (T_{gel} = 50.20°C, ms = 0.065, dry substance = 87.20%).

Synthesis of 2-nitropropyl starch (2-nitropropyl trifluoroacetate, pH = 10). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 50.03 g starch and 3.40 g 2-nitropropyl trifluoroacetate (16.9 mmole) were employed. Yield 45.10 g 2-nitropropyl starch (T_{gel} = nd., ms = 0.002, dry substance = 86.62%).

Synthesis of 2-nitropropyl starch (2-nitropropyl trifluoroacetate, pH = 8.5). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 24.97 g starch and 1.94 g 2-nitropropyl trifluoroacetate (9.65 mmole) were employed and the pH of the suspension was held at 8.5. Yield 22.86 g starch (T_{gel} = nd., ms = 0.001, dry substance = 88.00%).

Synthesis of 2-nitropropyl starch (2-nitropropyl monochloroacetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 50.01 g starch and 3.05 g 2-nitropropyl monochloroacetate (16.8 mmole) were employed. Yield 44.23 g 2-nitropropyl starch (T_{gel} = 54.55°C, ms = 0.016, dry substance = 91.85%, dried at T = 40°C).

Synthesis of 2-nitropropyl starch (2-nitropropyl dichloroacetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 49.97 g starch and 3.65 g 2-nitropropyl dichloroacetate (16.9 mmole) were employed. Yield 44.10 g 2-nitropropyl starch (T_{gel} = 55.85°C, ms = 0.006, dry substance = 92.30%, dried at T = 40°C).

Synthesis of 2-nitropropyl starch (2-nitropropyl trichloroacetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 50.02 g starch and 4.23 g 2-nitropropyl trichloroacetate (16.9 mmole) were employed. Yield 46.71 g 2-nitropropyl starch (T_{gel} = 56.65°C, ms = 0.00, dry substance = 87.66%).

Synthesis of 2-nitropropyl starch (2-nitropropyl methoxyacetate). - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl starch (2-nitropropyl acetate), however, in this case 100.02 g starch and 6.00 g 2-nitropropyl methoxyacetate (33.9 mmole) were employed. Yield 96.89 g 2-nitropropyl starch (T_{gel} = 51.68°C, ms = 0.45, dry substance = 87.66%).

Synthesis of 2-nitropropyl starch (2-nitropropyl acetate, 2-propanol/H_2O, the synthesis is given as a general example for synthesis in 2-propanol/H_2O mixtures). - Native starch (100.18 g, dry substance 86.90%, 538 mmole) was suspended in H_2O (75 ml) and 2-propanol (25 ml). The pH of the starch suspension was adjusted to 10.0 with a 2.5 M NaOH solution. 2-Nitropropyl acetate (5.14 g, 35.0 mmole) was carefully added dropwise to the suspension. The pH was regulated with a pH-stat by adding portions of 2.5 M NaOH (total amount 35 ml) and portions of 2-propanol (total amount 15 ml). After 60 minutes the reaction was complete (no more acid formation) and the suspension was neutralized with a 0.5 N HCl solution. After centrifugation and washing with water the product was dried in a ventilation stove. Yield: 101.35 g 2-nitropropyl starch (T_{gel} = 44.79°C, ms = 0.092, dry substance = 90.06%).

Synthesis of 1,2-epiiodohydrin. - Epichlorohydrin (23.66 g, 256 mmole) was added to a solution of KI (92.52 g, 558 mmole) in 300 ml H_2O. After stirring for 72 hours the aqeous solution was extracted with 3x 100ml Et_2O and the ether layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (T = 90°C, 50 mm Hg) and 23.35 g (127 mmole, 50%) epiiodohydrin was isolated. NMR ($CDCl_3$, in ppm): ^1H δ 2.54 (dd, 1H, CH_2O, H_a), δ 2.89 (dd, 1H, CH_2O, H_b), δ 2.99 (m, 1H, CHO), δ 3.17 (m, 2H, CH_2I). ^{13}C δ 4.81 (CH_2I), δ 50.29 (CH_2O), δ 52.08 (CHO).

Synthesis of 1,2-epoxy-3-nitro-propane. - $AgNO_2$ (22.65 g, 147 mmole) was added in portions to a solution of epiiodohydrin (22.70 g, 123 mmole) in 50 ml Et_2O at T = 0° C in the dark. After stirring for 72 hours the suspension is filtered and washed with 2 x 75 ml Et_2O. After evaporation of the solvent, the residue was distilled under reduced pressure (T = 82°C, 10 mm Hg) and 6.19 g (60 mmole, 49%) of 1,2-epoxy-3-nitropropane was isolated. NMR ($CDCl_3$, in ppm): ^1H δ2.69 (dd, 1H, CH_2O, H_a), δ 2.96 (t, 1H, CH_2O,H_b), δ 3.58 (m, 1H, CHO), δ 4.28 (dd, 1H, CH_2NO_2, H_x),δ 4.64 (dd, 1H, CH_2NO_2, H_y). ^{13}C δ 45.09 (CH_2O), δ 47.00 (CHO), δ 58.82 (CH_2NO_2).

Synthesis of 2-hydroxymethyl-3-nitropropyl starch. - Native starch (101.83 g, dry substance 86.90%, 538 mmole) was suspended in 100 ml H_2O which contained 5.03 g Na_2SO_4 (swelling inhibitor). The pH of the starch suspension was adjusted to 11.0 with a 1 M NaOH solution. 1,2-Epoxy-3-nitropropane (5.02 g, 49 mmole) was added to the suspension dropwise. The pH was regulated with a pH-stat by adding portions of 1 M NaOH. After 20 h the reaction was complete (no more acid formation) and the suspension was neutralized with a 0.5 N HCl solution. After filtration and washing with water, MeOH, acetone and again water the product was dried in the air. Yield 101.25 g 2-hydroxymethyl-3-nitropropyl starch (T_{gel} = 54.90°C, ms = 0.027, dry substance = 85.81%).

Synthesis of 2-nitropropyl starch (water soluble). - Water soluble starch (81.84 g) was added to a solution of NaOH (12 g, 0.3 mole) in 750 ml H_2O. 2-Nitropropyl acetate (20.68 g, 141 mmole) was added dropwise. After stirring for 3 hours the viscous suspension was acidified to pH = 5 with 4 M HCl solution and poured into 2 l EtOH. After sedimentation of the starch derivative at T = 4°C for 16 h the supernatant layer was decanted. The product was washed with aceton/H_2O (3:1) and aceton and finally dried under reduced pressure. Yield 93.07 g watersoluble 2-nitropropyl starch (ms = 0.15±0.01, dry substance not determined[*]).

Synthesis of 2-nitrobutyl starch (water soluble). - Water soluble starch (108.67 g) was added to a solution of NaOH (16 g, 0.4 mole) in 400 ml H_2O. 2-Nitrobutyl acetate (29.65 g, 184 mmole) was added dropwise. After stirring for 16 hours the viscous suspension was acidified to pH = 5 with 4 M HCl solution and poured into 1.5 l MeOH. After sedimentation of the starch derivative at T = 4°C for 16 h the supernatant layer was decanted. The product is washed with aceton/H_2O (3:1) and aceton and finally dried under reduced pressure. Yield 114.42 g watersoluble 2-nitrobutyl starch (ms = 0.22±0.01, dry substance not detected).

Synthesis of 2-nitropropyl α-polyglucuronate. - Sodium α-polyglucuronate (10.02 g, about 50 mmole) was added to 25 ml H_2O which contained 4.00 g NaOH (100 mmole). 2-Nitropropyl acetate (6.17 g, 42.0 mmole) was added dropwise. After stirring for 1.5 h the solution was acidified with 4N HCl solution to pH = 5 and poured into 300 ml of MeOH. After centrifugation, washing with MeOH/H_2O 2:1 and centrifugation the product was dried at T = 40°C in a ventilation stove. Yield 8.00 g 2-nitropropyl polyglucuronate (ms = 0.27).

Synthesis of 2-nitrobutyl α-polyglucuronate. - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl α-polyglucuronate, however, in this case 10.00 g

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[*] Higly substituted 2-nitroalkyl starch ethers are thermally unstable compounds.

α-polyglucuronate[*] (about 50 mmole), 5.02 g NaOH (126 mmol) and 10.00 g 2-nitrobutyl acetate (62.1 mmole) were employed. Yield 9.99 g 2-nitrobutyl α-polyglucuronate (ms = 0.31).

Synthesis of 2-nitropentyl α-polyglucuronate. - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl α-polyglucuronate, however, in this case 1.00 g α-polyglucuronate (about 5 mmole), 0.38 g NaOH (9.50 mmole) and 0.73 g 2-nitropentyl acetate (4.17 mmole) were employed. Yield 0.45 g 2-nitropentyl α-polyglucuronate (ms = 0.17).

Synthesis of 2-nitrohexyl α-polyglucuronate. - The reaction conditions are identical to the reaction conditions as given for the synthesis of 2-nitropropyl α-polyglucuronate, however, in this case 1.00 g α-polyglucuronate (about 5 mmole), 0.38 g NaOH (9.50 mmole) and 0.79 g 2-nitrohexyl acetate (4.18 mmole) were employed. Yield 0.60 g 2-nitrohexyl α-polyglucuronate (ms = 0.17).

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[*] The polyglucuronate is reduced with $NaBH_4$ before the nitroalkylation. Reduction of the ketals of the residual aldehyde functionalities formed during oxidation diminishes depolymerization under alkaline reaction conditions.

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Chapter 3

Analysis of 2-nitroalkyl starch ethers

3.1 Introduction

The development of new analytical techniques has drastically influenced the analysis of modified starches. Traditionally, starch and starch derivatives were analyzed by means of their physical properties (rheology, viscosity, gelatinization behavior etc.) and products for new applications were mainly developed by trial and error. Today, scientists are trying to get a full insight into the parameters that influence the physical behavior of starch and modified starches in order to develop a complete range of tailor-made starch products.

However, traditional methods for the analysis of starch pastes, such as the Brabender Amylograph (a viscometer designed to permit a continuous measurement of the viscosity of a starch water mixture during heating and cooling), the Brookfield viscometer, and various rheometers are still extensively used for the analysis of starch pastes. The physical analysis of 2-nitroalkyl starch ethers is discussed briefly in the first part of this chapter.

The main part of this chapter deals with the chemical analysis of 2-nitroalkyl starch ethers. The analysis of these modified starches is quite complicated in comparison to simple organic compounds. Starch granules are nonuniform and consist of two high molecular weight polymers (amylose and amylopectin). Purification from side products by normal techniques (e.g. crystallization or distillation) is not possible. Several levels of structural information (molar substitution, topochemistry, nature of the nitroalkyl substituent) can be distinguished and will be explained in more detail in section 3.3.

3.2 Physical analysis of 2-nitroalkyl starch ethers

Most of the commercial applications of (modified) starches are based on their ability to form viscous pastes, and a common way to fingerprint starches is by their pasting profile. A granular starch suspension is subjected to a defined heating and cooling program in which the viscosity is recorded as a function of temperature and time. For years the Brabender Amylograph was used for the determination of profiles of (modified) starches, but nowadays it is being replaced, *e.g.*, by Rapid Visco Analyzers (viscometers which also follow a temperature program). The advantages of RVA's over Brabenders are the use of smaller amounts of starch (derivative) and shorter analysis times[1]. The shear imposed by the stirrer in the RVA is recorded during the temperature program. Profiles for starch (5% suspensions) and 2-nitropropyl starch (5% suspensions) are shown in Figure 1.

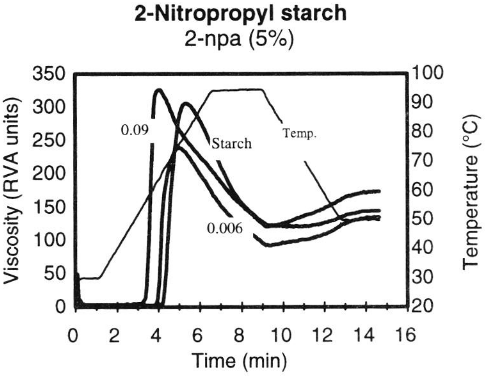


Figure 1. RVA curves of starch and 2-nitropropyl starches (ms = 0.09 and 0.006, 5% suspension)

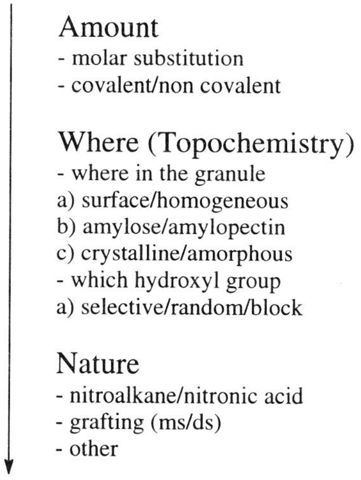
As the temperature is increased, the viscosity starts to rise at the gelatinization temperature of the starch granules. Amylose, followed by amylopectin, leaks out of the swollen granules (ghosts) and goes into solution, consequently the viscosity increases. The viscosity continues to rise until it approaches a peak viscosity where the granules have approached their maximum hydration. Further heating and shear promote the disruption of "ghosts" and the viscosity decreases. As the resulting sol is cooled the viscosity tends to rise again[2]. The exact profile depends on the botanical origin of the starch used.

As shown in Figure 1, the gelatinization temperature of the modified starch is lowered by the introduction of nitroalkyl groups. This phenomenon, also observed for most other starch derivatives, can be explained by the disturbance of the starch matrix by the incorporation of polar nitroalkyl functionalities. The profiles of 2-nitropropyl starches (ms = 0.006 and ms = 0.092, pH = 10.1) deviate slightly from starch. The RVA curves of these compounds and other 2-nitroalkyl starch ethers strongly depend on the pH of the suspension due to the acidic α-CH group of the 2-nitroalkyl ether. In general, gelatinization at higher pH leads to higher peak and end viscosities. It can be concluded that the rheological behavior of 2-nitroalkyl starches depends on the pH, the molar substitution and, although we did not investigate this, the nature (length) of the nitroalkyl group.

3.3 Chemical analysis of 2-nitroalkyl starch ethers

3.3.1 Introduction

Analysis of modified starches is essential to gain more insight into the relationship between the structure of the compound and its chemical and physical properties. The supramolecular structure of starch and the low degrees of substitution of granular 2-nitroalkyl starch ethers complicate the analysis of 2-nitroalkyl starch ethers in comparison to simple organic compounds. Various levels of structural information can be distinguished (Scheme 1).



Amount
- molar substitution
- covalent/non covalent

Where (Topochemistry)
- where in the granule
a) surface/homogeneous
b) amylose/amylopectin
c) crystalline/amorphous
- which hydroxyl group
a) selective/random/block

Nature
- nitroalkane/nitronic acid
- grafting (ms/ds)
- other

Scheme 1. Overview of the decrease of various levels of structural information of 2-nitroalkyl starch ethers.

The average number of substituents per glucopyranoside unit of the 2-nitroalkyl starch ether (molar substitution, ms) is one of the main factors that influence the physical properties. The situation can be rather complex because the nitroalkyl substituent is capable of reacting with another nitroalkene[3]. If grafting dominates, the properties of 2-nitroalkyl starch ethers depend not only on the number of substituents but also on the average chain length (ms:ds) of the side chains.

Because the nonuniformity of modified granular starches is reflected in the starch paste (obtained after gelatinization) it is essential to obtain information as to **where** the nitroalkene reacts within the granule (topochemistry). A surface effect is expected if the Michael addition is much faster than the diffusion of the nitroalkene into the granule. Furthermore, the heterogeneity of starch granules may be the origin of different reactivity of certain regions (amylose/amylopectine, amorphous/crystalline regions) in the granule for nitroalkenes (or precursors thereof).

A more refined parameter is the distribution of substituents over the hydroxyl groups of the glucopyranoside monomer of starch. It is known from the literature that different distributions can alter the chemical properties of a starch derivative[4] but to our knowledge no systematic studies have been performed which describe the effects of different distributions on physical properties of modified starches.

Another factor which influences properties of starch pastes is, of course, the nature of the substituent(s). One section of this chapter focusses on side reactions during the synthesis of 2-nitroalkyl starch ethers, the amount of grafting, and the exact nature (nitroalkane *versus* nitronic acid[5]) of the substituent.

The analysis of 2-nitroalkyl starch ethers as described in this chapter affords the different levels of structural information as shown in Scheme 1.

3.3.2 Results and Discussion

Determination of the molar substitution of 2-nitroalkyl starch ethers by elemental analyses (nitrogen content)

Amount
- molar substitution
- covalent/non covalent

Where (Topochemistry)
- where in the granule
  - a) surface/homogeneous
  - b) amylose/amylopectin
  - c) crystalline/amorphous
- which hydroxyl group
  - a) selective/random/block

Nature
- nitroalkane/nitronic acid
- grafting (ms/ds)
- other

Numerous procedures have been developed for the determination of the degree of substitution or molar substitution of polysaccharide derivatives. If the substituent contains other elements than C, H, O, the degree of substitution or molar substitution can be determined easily from elemental analysis of this other element. Normally, the percentage of an element of a compound is determined by the equation:

$$\%E = a(M_E/M)\cdot 100\%$$

(a = number of atoms of element E, E = the element, M_E = molecular weight of the element and M = the molecular weight of the compound).

For polysaccharide derivatives that are built up of repeating units, the percentage of this element is:

$$\%E = a\cdot M_E\cdot ms/\{A+ms(M_{subst}-1)\}\cdot 100\%$$

(ms = molar substitution, A = molecular weight of an average monomeric unit of the polysacharide and M_{subst} = the molecular weight of the substituent).

After rewriting this equation, the molar substitution of a substituent of a polysaccharide can be expressed as:

$$ms = 0.01\cdot A\cdot \%E/\{aM_E-0.01\cdot \%E(M_{subst}-1)\}$$

(2-nitroalkyl starch ethers: $a = 1$, $A = 162$, $E = N$, $M_E = 14$, 2-nitroalkyl sodium α-polyglucuronates: $a = 1$, $A = 198$, $E = N$, $M_E = 14$, the results are described in chapter 2).

The determination of the molar substitution by elemental analysis does not distinguish between covalent and noncovalent bonding. Noncovalent interactions between oligomers of nitroalkenes or starting materials and the starch matrix are possible; if they are not washed out thoroughly, the ms calculated from the nitrogen content will be too high. To investigate the amount of noncovalent material, 2-nitropropyl starch, (2-nps), 2-nitrobutyl starch (2-nbs), 2-nitropentyl starch (2-npes) and 2-nitrohexyl starch (2-nhs) were extracted continuously with MeOH (4 days).

As shown in Table 1, a decrease in nitrogen content of 0-20% was observed after continuous extraction with MeOH. It can be concluded that the efficiencies given in Chapter 2 are a bit too high (0-20%) but that most of the nitrogen-containing material is indeed covalently bound to the starch granule.

Table 1. Molar substitution (ms) of 2-nitroalkyl starch ethers after continuous extraction with MeOH, gelatinization at pH = 5 and gelatinization at pH = 9.

	starting material	continuous extraction with MeOH	
compound	ms	ms	%
2-nitropropyl starch	0.078	0.078	100
2-nitrobutyl starch	0.050	0.045	90
2-nitropentyl starch	0.052	0.042	81
2-nitrohexyl starch	0.036	0.033	92

Determination of the molar substitution of 2-nitroalkyl starch ethers by FT-IR spectroscopy

The above-mentioned procedure for the determination of the molar substitution is very accurate but has the disadvantage that elemental analysis is a time-consuming (expensive) method. In the literature new techniques have been described to obtain more rapidly the degree of substitution *e.g.* in hydroxypropylated starches[6]. Because nitro functionalities show characteristic absorbances in the infrared region we investigated the possible utility of this technique for analysis of 2-nitroalkyl starch ethers.

In Figure 2, the FT-IR spectra (600-1800 cm^{-1}) of granular potato starch and 2-nitropropyl starch (m.s. = 0.083) are displayed.

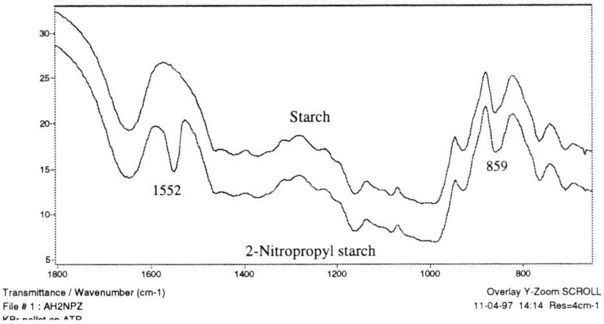


Figure 2. FT-IR spectra of starch and 2-nitropropyl starch.

A characteristic absorption of the nitro functionality of 2-nitropropyl starch appears at 1552 cm^{-1}. This corresponds to the asymmetric stretching frequency of the nitro group[7]. The symmetric stretching frequency is expected at about 1385 cm^{-1}, but due to the low absorbance of this vibration and the low molar substitution, it cannot be observed. Potential absorptions of nitronic acids (1620-1680 cm^{-1})[8] overlap with natural absorption frequencies of starch.

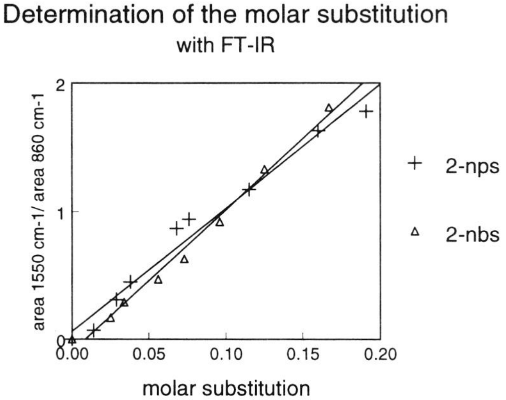


Figure 3. Correlation of the absorption intensity (peak area) in FT-IR with the molar substitution of 2-nitropropyl starch (2-nps, correlation coefficient 0.988) and 2-nitrobutyl starch (2-nbs, correlation coefficient 0.995) samples.

The area of the absorption peak of the N-O vibration at 1552 cm^{-1} was divided by the area of the C-1 (anomeric) vibration of the starch polymer at 859 cm^{-1} to exclude concentration effects of the starch ether in the KBr pellet. In Figure 3, a linear correlation between the absorption of the asymmetric N-O vibration in 2-nitropropyl and 2-nitrobutyl starch and the molar substitution determined by nitrogen analysis is shown. The slope of the graph depends somewhat on the nature of the nitroalkyl group of the starch ether.

The results demonstrate that, after preparing a calibration curve, FT-IR spectroscopy is a feasible technique for the routine ms determination of 2-nitroalkyl starch ethers.

Determination of the molar substitution of 2-nitroalkyl starch ethers by DSC (gelatinization temperature)

Substituents cause a disturbance of the supramolecular structure (hydrogen bonds, crystalline and semi-crystalline regions) of starch. The gelatinization temperature of starch, the temperature at which the irreversible swelling and leakage of amylose and amylopectin into the solution occurs, is lowered when substituents are incorporated into the starch matrix. The decrease depends on the chemical structure of the substituent, and especially anionic and cationic substituents are capable of lowering the gelatinization temperature, even at low degrees of substitution. In these cases, the decrease in the gelatinization temperature is directly proportional to the degree of substitution[9].

Application of this principle would allow us to determine the molar substitution of the polar 2-nitroalkyl starches by measuring the gelatinization temperature with DSC, by microscopy (coupled to a hot stage), or a RVA.

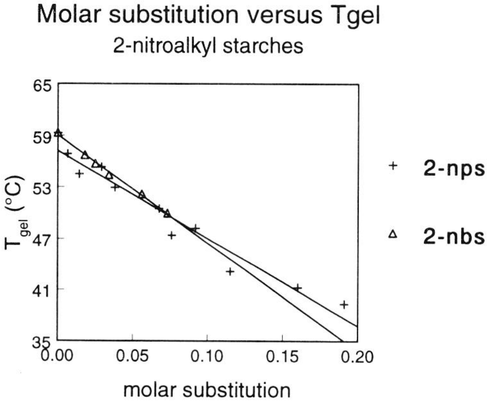


Figure 4. Influence of the molar substitution of 2-nitroalkyl starch ethers on the gelatinization temperature (measured by DSC, T_{onset}; 2nps, correlation coefficient 0.977; 2-nbs, correlation coefficient 0.987).

Figure 4 shows that the decrease in the gelatinization temperature for 2-nitropropyl and 2-nitrobutyl starch (synthesized under identical conditions) is indeed directly proportional to the molar substitution.

Determination of the molar substitution of 2-nitropropyl starch by exothermic decomposition

Nitro-compounds, such as 2,4,6-tri-nitrotoluene, nitroglycerin, and highly substituted starch nitrate, are used commercially as explosives. The explosive efficiency of the starch nitrate is proportional to the nitrogen content; most commercial products contain about 13-13.5% nitrogen[10]. We studied the thermal decomposition of 2-nitroalkyl starch ethers in order to develop a fourth method for determination of the molar substitution of these starch ethers.
Although the 2-nitroalkyl starch ethers contains considerably less nitrogen (0-1.5%) than commercial starch nitrate, DSC showed that 2-nitroalkyl starch ethers are also thermally unstable at high temperatures. A typical DSC scan of 2-nitropropyl starch is shown in Figure 5. The decomposition of the nitroalkyl group is clearly observed as an exothermic peak (T = 165°C, Figure 5).

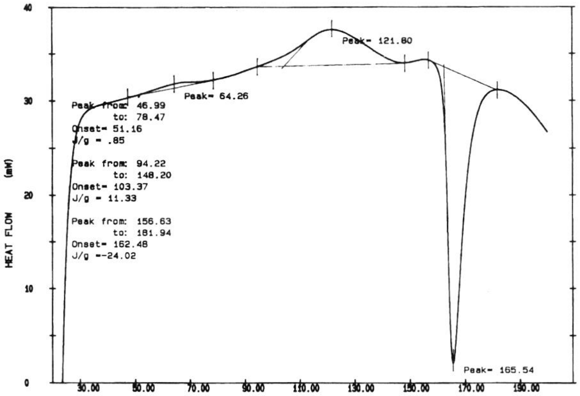


Figure 5. DSC scan of granular 2-nitropropyl starch.

The temperature range of the exothermic peak broadens from 2-nitropropyl via 2-nitrobutyl to 2-nitroalkyl starch ethers with longer chains and in the latter cases the energy of decomposition cannot be determined due to decomposition of starch at these temperatures (>200°C). As for starch nitrate, the energy of the decomposition of 2-nitropropyl starch (synthesized with 2-nitropropyl acetate) is directly proportional to the molar substitution (Figure 6).

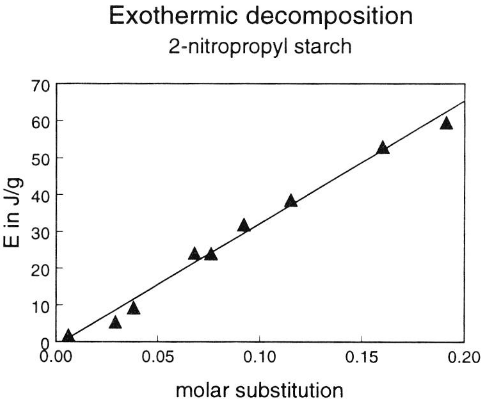


Figure 6. Heat content of the exothermic decomposition of 2-nitropropyl starch plotted as a function of the molar substitution (correlation coefficient 0.994).

Figure 7 shows the exothermic decomposition temperature of 2-nitropropyl starch for different degrees of molar substitution. The decomposition temperature of 2-nitropropyl starch decreases as a function of the molar substitution, but is not linear.

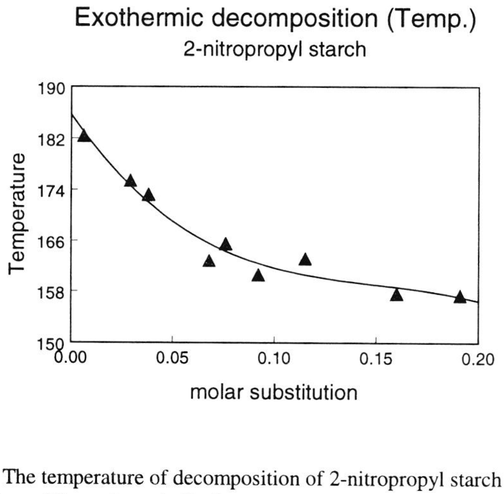


Figure 7. The temperature of decomposition of 2-nitropropyl starch as a function of the molar substitution.

3.3.3 Topochemical aspects of 2-nitroalkyl starch ethers

Introduction

Amount
- molar substitution
- covalent/non covalent

Where (Topochemistry)
- where in the granule
  - a) surface/homogeneous
  - b) amylose/amylopectin
  - c) crystalline/amorphous
- which hydroxyl group
  - a) selective/random/block

Nature
- nitroalkane/nitronic acid
- grafting (ms/ds)
- other

The development and utilization of new techniques (X-ray diffraction[11], electron microscopy[12], high performance-anion-exchange chromatography[13]) in starch chemistry has answered many, although not all, questions about the structure and organization of the starch granule[14].

Besides small amounts of non-carbohydrate components (minerals, proteins, and phosphates, <0.1%), potato starch granules are composed of about 20% amylose and 80% amylopectin. X-ray scattering has shown that potato starch (and other starches) are partly crystalline. The X-ray patterns differ for cereal starches (A-type), tuber starches (B-type), and certain root and seed starches (C-type).

The amylose is not evenly distributed over the starch granule; the highest percentages are found at the periphery. The percentage of amylose increases with increasing granular size[15]. In the granular state it complexes readily with iodine and in solution amylose forms single helical structures with hydrophobic compounds such as alcohols, fatty acids *etc.*[16].

Amylopectin, the main constituent of the potato starch granule, consists of short linear chains (about 20-30 glucopyranoside units) grouped in clusters[17]. A model for the clustered organization, as proposed by Hizukuri, is shown in Figure 8[18].

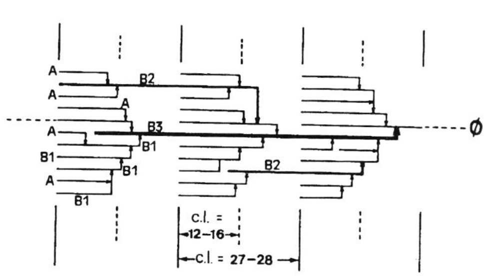


Figure 8. Organization of clusters in an amylopectin molecule (cl = glucopyranoside units).

Several linear chains are distinguished. The C-chain (not shown in the figure) carries the sole reducing group of the molecule. The B-chains are joined through α-1→6 bonds and can connect several clusters (B1 = one cluster, B2 = 2 clusters, etc.). The B-chains are branched with A and other B-chains on C-6 primary hydroxyl groups. The A-chains are relatively small, unsubstituted, and remain in a single cluster. The cluster model can be viewed as a tree, the A chains are the twigs, the B-chains are the branches and the C-chain is the stem.

The fine structure of amylopectin leads to the formation of crystalline domains. Within a cluster, left-handed double helices are formed between A and the exterior parts of the B-chains. As a result, long fine structures of crystalline (about 60Å, A + B chains) and amorphous regions (about 40Å, B-chains), containing the interior parts of the B-chains, are obtained. Evidence for the existence of these regions is obtained by electron microscopy and mild acid hydrolysis, which leads to the formation of highly crystalline Naegeli dextrins[19]. The amylose is considered to fill the amorphous regions although some additional crystallization may occur with amylopectin within the crystalline regions.

The crystalline lamellae form a more or less continuous network of left-handed helices which can intertwine. This leads to a left-handed helical crystal. The crystalline superhelices are arranged in a tetragonal array, and for waxy maize (amylopectin) the starch is considered to have a fishbone-like structure as a projection of all the helical structures (Figure 9*).

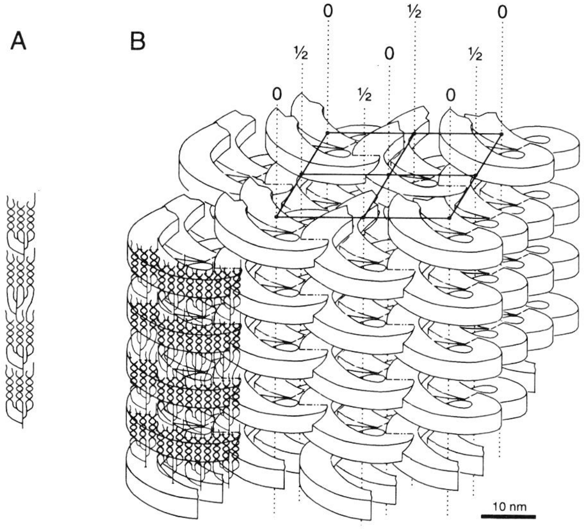


Figure 9. A three-dimensional structure of amylopectin in potato starch.

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* Taken from *Carbohydr. Polym.*, G.T. Oostergetel, E.F.J.van Bruggen, *The crystalline domains in potato starch granules are arranged in a helical fashion*, 7-12 (**21**), copyright 1998, with permission from Elsevier Science.

When potato starch granules are suspended in water, the granules absorb water and swell until the water content is about 35-40% of the total weight. It is believed that the amorphous regions of the starch granules are most readily penetrated by water[20]. The swelling of these regions is reversible. Owing to the increase in volume the starch granules are susceptible to penetration by low molecular weight, water-soluble substances. This is limited to solutes with molecular weights of less then 1000 Daltons. Most chemical reactions take place, or at least start, in the amorphous regions of the starch granule.

To determine the topochemical aspects of 2-nitroalkyl starch ethers we investigated surface effects and chose 2-nitropropyl starch as a model system for the distribution of the 2-nitroalkyl ether over amorphous/crystalline and amylose/amylopectin regions.

Distribution of substituents in 2-nitroalkyl starch granules of different sizes, surface effects

A surface effect for the Michael addition of nitroalkenes to starch is expected if the rate of reaction is much faster than the diffusion of the nitroalkene into the granule. The simplest method to determine whether surface effects are involved is sieving of starch granules. Potato starches are well suited for this approach because they have a broad distribution of particle sizes (15-75 μm). Large spherical granules have a much 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. Other methods described in literature for the determination of surface effects are the use of transmission electron microscopy[21] and surface gelatinization with $CaCl_2$[22].

The results in Table 2 show that the 2-nitropropyl and 2-nitrobutyl substituent, synthesized with the corresponding 2-nitroalkyl acetates (pH = 10-10.5, 5% Na_2SO_4), are distributed almost homogeneously 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 the nitroalkene "walks" through the granule could provide an additional pathway for homogeneous distribution.

Table 2. Molar substitution of 2-nitroalkyl starch ether granules of different size.

	2-nitropropyl starch (ms = 0.058)[a]	2-nitrobutyl starch (ms = 0.081)[b]
> 63 μm	0.057	0.081
45 < x < 63 μm	0.057	0.080
25 < x < 45 μm	0.058	0.083

a) pH = 10.5, 5% Na_2SO_4, H_2O/starch = 1:1, 2-nitropropyl acetate. b) pH = 10, Na_2SO_4, H_2O/starch = 1:1, 2-nitrobutyl acetate.

Distribution of the 2-nitropropyl ether functionality over amorphous/crystalline and amylose/amylopectin regions

Despite the fact that the pattern of substitution of modified starches is one of the (main) factors which influences the properties of these derivatives, few studies on this subject have been reported in the literature[23]. Two approaches can be distinguished. In a statistical approach the derivative is (perdeuterio)methylated and subsequently partly hydrolyzed. The oligomers are analyzed by mass spectroscopy (FAB-MS or MALDI-TOF-MS). The substitution patterns of the oligomeric mixtures are compared with a calculated statistical distribution of the monomers for random subsitution (the model of Reuben[24]).

Methyl amylose prepared under homogeneous conditions shows good agreement with the statistical data, indicating a random distribution. For methyl amylose prepared in a slurry process, deviations from a random distribution were observed. The ratios of oligomers with low and high substitution were enhanced, whereas the fractions with an average degree of substitution decreased. The nonuniformity is a consequence of improved solubility of certain regions causing increased accessibility for reagents during the etherification.

The second and more selective approach consists of selective hydrolysis of the glycosidic bonds of the starch matrix. Unsubstituted starch can be hydrolyzed completely by α-amylases (EC 3.2.1.1) and amyloglucosidases (EC 3.2.1.3). Substituents on the monomeric units inhibit the enzymatic cleavage of the glycosidic bonds. Therefore, treatment with α-amylases and amyloglucosidases affords mixtures of glucose and substituted oligomers.

Using a newly-developed enzymatic method (AVEBE R&D and Bijvoet Center for Biomoleculair Research, Utrecht University)[*], granular methyl and 2-nitropropyl starch were partly hydrolyzed to linear fractions and branched fractions and these fractions were separated by means of gel permeation chromatography. Linear fractions are obtained from amylose and the linear crystalline amylopectin regions, whereas the branched fractions are obtained from the amorphous branched amylopectin regions. As shown in Table 3, the reactivity ratio (RR = ms (ds) branched fractions/ms (ds) linear fractions) of the 2-nitropropyl derivative is the opposite of that of the methylated derivative. The inhomogeneity (RR $\neq$ 1) of both derivatives originates from the organization of the starch granule. The methyl substituent is encountered mainly (RR = 2) in the branched units of the amorphous amylopectin region, the 2-nitropropyl substituent, on the other hand, primarily in the linear regions (RR = 0.3).

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[*] To be published in Carbohydr. Res..

Table 3. Enzymolysis of potato starch, methyl starch and 2-nitropropyl starch

Starch	reagent or substituent	ms	ms branched regions	ms linear regions	branched/ linear (RR)
methyl	dimethyl sulfate[a]	0.077	0.112	0.006	2
2-nitropropyl	2-nitropropyl acetate[a]	0.078	0.026	0.083	0.3

a) Slurry synthesis

2-Nitropropyl acetate has a preference for the amylose fraction of the amorphous regions or the linear chains of the crystalline amylopectin region, although the latter is less likely. To distinguish between both possibilities, the amylose of 2-nitropropyl starch was separated from the amylopectin by leaching experiments[25] (Table 4). The smaller and more mobile amylose molecules diffuse more easily from swollen granules than amylopectin.

Table 4. Leaching of amylose from starch, methyl starch and 2-nitropropyl starch

Starch	reagent	amylose (ms)	amylopectin (ms)	amylose/ amylopectin
methyl	dimethyl sulfate	0.147	0.095	1.5
2-nitropropyl	2-nitropropyl acetate[a]	0.554	0.032	15
2-nitropropyl	2-nitropropyl acetate[b]	0.772	0.015	50

a) Leaching at 60°C b) Leaching at 90°C

For 2-nitropropyl starch the amylose/amylopectin ratio is very high. From the results shown in Tables 3 and 4 it can be concluded that 2-nitropropyl acetate reacts almost completely with the amylose fraction located in the amorphous regions of the starch granule.

As a result the iodine binding capacity of 2-nitropropyl starch, which reflects the amount and properties of the amylose fraction[26], is changed drastically. Owing to the substituents present in the amylose fraction, complexation of iodine is reduced to (nearly) zero (Figure 10).

It is known from the literature that several compounds (such as lipids, surfactants, alcohols, 1- and 2-nitroalkanes[27]) form very stable inclusion complexes with amylose. The amylose rearranges itself in a single helix (V-helix) in which the mandrel or guest molecule is located in the hydrophobic interior of the helix[28]. It is not unlikely that when 2-nitropropyl acetate is added to suspensions of starch granules, the amylose in the amorphous regions of the granule rearranges and forms helices which accommodate the 2-nitropropyl acetate. Such a rearrangement within the granule requires a huge amount of energy. For example, hydrogen bonds between amylose and amylose-amylopectin strands have to be broken. Important driving forces for the formation of an amylose V-helix (comparable to cyclodextrins[29]) with

2-nitropropylacetate are the hydrophobic interactions between the amylose and the substrate, van der Waals interactions (dipole-dipole interactions), and hydrogen-bond formation between host and guest[30].

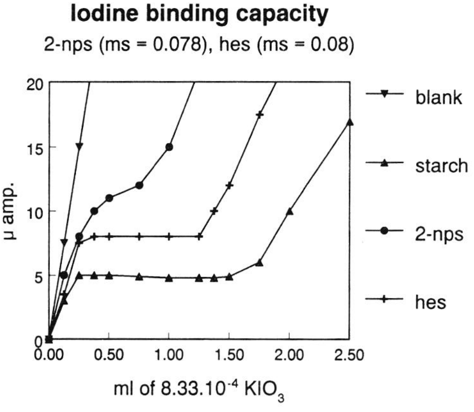


Figure 10. Iodine binding capacity of starch, 2-nitropropyl starch, and hydroxyethyl starch.

The driving force for the hydrophobic interactions between amylose and 2-nitropropyl acetate is the release of the relatively high Gibbs energy of a hydrophobic molecule such as 2-nitropropyl acetate when an inclusion complex is formed[31]. Displacement of the hydration shell around 2-nitropropyl acetate leads to a gain in entropy and subsequent lowering of the Gibbs energy.

As derived from molecular orbital calculations[32], α-cyclodextrin possesses a large dipole moment (13.5 D). It seems reasonable to assume a similar dipole moment for an amylose V-helix. The high dipole moment of a nitro functionality (~3.5 D) suggests that dipole-dipole (van der Waals) interactions are important in stabilizing the inclusion compound, and also in determining the orientation of 2-nitropropyl acetate within the helix.

Nitro compounds are known to be (weak) hydrogen bond acceptors[33]. It is possible that, similar to hydrogen bond formation of α-cyclodextrin and MeOH[34], a hydrogen bond is formed between a disordered primary hydroxyl group and 2-nitropropyl acetate.

Some native starch genotypes show mixed A+V, B+V, and C+V patterns in X-ray diffraction[11b]. These mixed patterns confirm the ability of starch to form an amylose V helix within the starch granule. It is also possible to induce V-structures in cereal starches (which contain naturally occurring fatty acids and phospholipids) through selected heat/moisture treatments. Treatment of granular starch with iodine leads to an amylose V-helix, which is visualized by a blue color. It is presumed that van der Waals interactions (oxygen-iodine charge transfer forces) play an important role in the formation of an amylose helix with iodine[35].

It seems realistic to explain the reactivity of 2-nitropropyl acetate with the following hypothesis: at first an amylose-V helix is formed in the amorphous regions with 2-nitropropyl acetate. Under the alkaline reaction conditions used, acetate splits off and 2-nitropropene is formed within the helix. The nitroalkene reacts with the hydroxyl functionalities of amylose. Another possibility, deacetylation of 2-nitropropyl acetate followed by formation of an amylose-2-nitropropene complex and subsequent Michael addition, seems less likely because 2-nitroalkenes are very reactive compounds and it seems doubtful that complexation would occur before reaction.

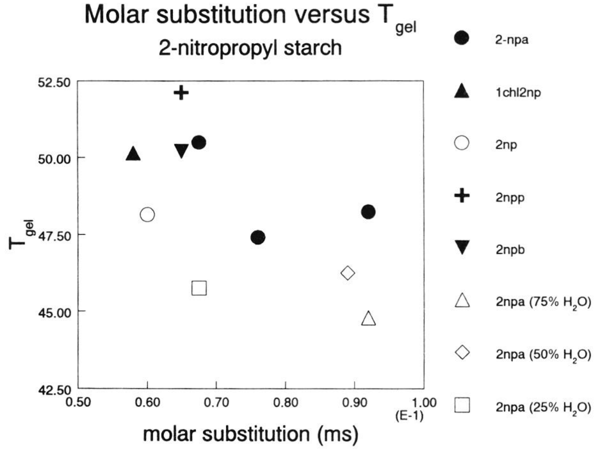


Figure 11. T_{gel} of 2-nitropropyl starches (2npa = 2-nitropropyl acetate, 1chl2np = 1-chloor-2-nitropropane, 2-np = 2-nitropropene, 2npp = 2-nitropropyl propionate, 2-npb = 2-nitropropyl butyrate, (x % H_2O) = synthesized in x% H_2O and (1-x)% 2-propanol.

The formation of an amylose-2-nitropropyl acetate complex depends on the reaction conditions used. Changes in the reaction conditions, for example reaction of 2-nitropropyl acetate with starch in 2-propanol/H_2O mixtures, can lead to 2-nitropropyl starch with a different distribution pattern of substituents over the starch domains. Variations in precursors (2-nitropropyl propionate, 2-nitropropyl butyrate, 1-chloro-2-nitropropane) and direct addition of 2-nitropropene can also lead to different fine structures in the granule. An indication for these different distribution patterns is shown in Figure 11. No firm conclusions can be drawn due to the limited data available. However, the decrease in the gelatinization temperature found for products of the reaction of granular starch with 2-nitropropene and the synthesis in

H_2O/2-propanol mixtures, relative to products with the same ms obtained in the standard slurry reaction with 2-nitropropyl acetate, provide an indication of a larger distortion (substitution of amorphous/crystalline amylopectin fractions) of the granular structure.

Distribution of the 2-nitroalkyl substituents over the hydroxyl groups of the glucopyranoside monomer of starch

A general approach for determining the distribution of ether substituents over the hydroxyl groups of starch and cellulose monomers consists of hydrolysis of the polysaccharide, reduction of the monomer, and finally alkylation or acetylation of the monomer and analysis with gas chromatography and GC-MS[36].

Unfortunately, 2-nitroalkyl starch ethers decompose under the acidic conditions necessary for the hydrolysis of the polysaccharide (CF_3CO_2H, HCl, and H_2SO_4 are commonly used) and a brown/black syrup is obtained. It seems that this process proceeds autocatalytically, starting at the reducing end group of the glucose monomers or the oligosaccharides which are formed during the hydrolysis.

Although we did not investigate the distribution of the 2-nitroalkyl substituents over the hydroxyl groups of the glucopyranoside monomer of starch any further, we predict that the primary hydroxyl group (C-6) of the 2-nitroalkyl starch ether is more substituted than in the case of methyl starch, carboxymethyl starch, and hydroxyethyl starch. Within the amylose-2-nitropropyl acetate helix the hydroxymethyl groups are rather flexible and can rotate in the core of the helix. In this way, they can react much more easily with a nitroalkene formed in the helix than the secondary C-2 (most acidic) and C-3 hydroxyl groups which point away from the helix.

3.3.4 The exact nature of the ether substituent of 2-nitroalkyl starch ethers

Amount
- molar substitution
- covalent/non covalent

Where (Topochemistry)
- where in the granule
  - a) surface/homogeneous
  - b) amylose/amylopectin
  - c) crystalline/amorphous
- which hydroxyl group
  - a) selective/random/block

Nature
- nitroalkane/nitronic acid
- grafting (ms/ds)
- other

Solid state NMR of granular 2-nitroalkyl starch ethers and NMR of water-soluble 2-nitroalkyl starch ethers

The exact nature of the nitroalkyl substituent is not only important for structure/property relationships. Our ultimate aim is to reduction of the nitroalkyl functionality to an aminoalkyl functionality, hence solution of the structure and determination of the nature of side reactions is essential. Direct information about the structure of granular 2-nitroalkyl starch ethers can be obtained from ^{13}C-CPMAS spectroscopy. As shown in Figure 12, the spectra are rather complex. For 2-nitropropyl starch (ms

= 0.073) two resonances for methyl functionalities are visible (about 18 and 28 ppm). 2-Nitrobutyl starch (ms = 0.072) shows the expected resonances of the ethylgroup (about 9 and 26 ppm). Other resonances of the nitroalkyl ether are difficult to see because they are of low intensity or fall under the resonances of starch.

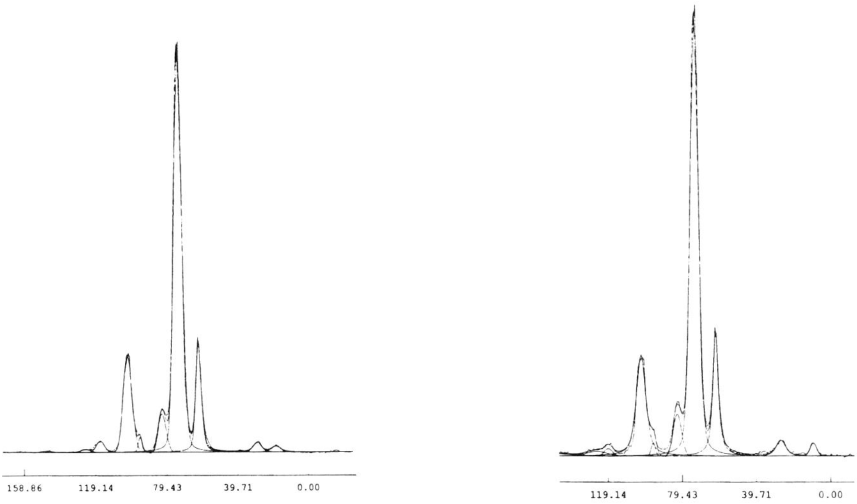


Figure 12. ^{13}C-CPMAS spectra of 2-nitropropyl (ms = 0.073, left) and 2-nitrobutyl starch (ms = 0.072, right) (synthesized with the corresponding 2-nitroalkyl acetates).

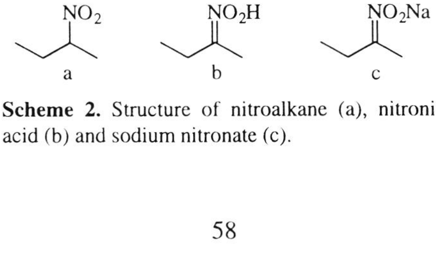
^{1}H-NMR spectra (D_2O) of water soluble 2-nitropropyl starch show very small resonances of the methyl functionalities between 1.2 and 2.2 ppm, besides resonances of the starch backbone. The ^{13}C-NMR spectrum of this compound only showed resonances of the starch backbone (24 hours scanning). Factors that can explain the complexity of the spectra are:

a) distributions (C-2, C-3, C-6) of the 2-nitroalkyl substituents over the hydroxyl groups of the glucopyranoside monomers of starch.

b) chirality. After reaction of 2-nitro-1-alkenes with starch a new chiral centre is introduced.

c) grafting of nitroalkenes to the polysaccharide backbone.

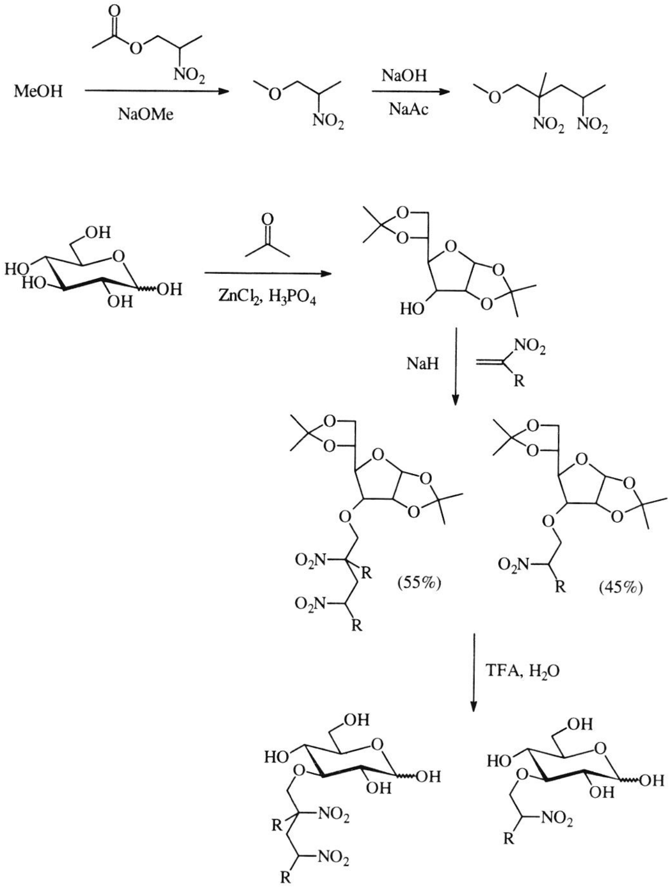
d) the nitro functionality can be present as a "normal" nitroalkane, nitronic acid or sodium nitronate (Scheme 2).



Scheme 2. Structure of nitroalkane (a), nitronic acid (b) and sodium nitronate (c).

In order to gain more information from NMR, we synthesized a number of model compounds and isolated 2-nitroalkyl oligomers from 2-nitropropyl starches by enzymatic hydrolysis and subsequent fractionation of glucose and the nitroalkyl oligomers by gel permeation chromatography.

Synthesis of model compounds



Scheme 3. Synthesis of 1-methoxy-2-nitropropane, 1-methoxy-2-methyl-2,4-dinitropentane, 3-*O*-(2-nitropropyl)-α,β-D-glucopyranoside and 3-*O*-(2-nitrobutyl)-α,β-D-glucopyranoside.

To get more insight in the chemical shifts of specific NMR resonances of 2-nitroalkyl ethers, we synthesized the simple 2-nitroalkyl ethers, 1-methoxy-2-nitropropane and 1-methoxy-2-nitrobutane, and 1-methoxy-2-methyl-2,4-dinitropentane as a model system for grafting reactions (Scheme 3). 1-Methoxy-2-nitropropane and 1-methoxy-2-nitrobutane were synthesized in moderate yields from MeOH and the corresponding 2-nitroalkyl acetates. Reaction of 1-methoxy-2-nitropropane with 2-nitropropyl acetate gave 1-methoxy-2-methyl-2,4-dinitropentane in low yield[37].

Furthermore, we synthesized the specifically substituted glucose derivatives 3-*O*-(2-nitropropyl)-α,β-D-glucopyranose and 3-*O*-(2-nitrobutyl)-α,β-D-glucopyranose (Scheme 3) by Michael additions of 1,2-5,6-diisopropylidene-α-D-glucofuranose[38] with 2-nitropropene or 2-nitro-1-butene and subsequent de-isopropylidenation with trifluoro acetic acid (TFA/H_2O (9:1))[39]. Unfortunately, the "mono" substituted product could not be separated from the grafted "di" substituted product.

Enzymatic hydrolysis of 2-nitroalkyl starch ethers

Starch derivatives such as methyl starch can be hydrolyzed partially to glucose and oligomers which contain one or more methyl groups. Substituents prevent the enzymatic hydrolysis of neighboring α-1→4 glycoside bonds, and when the methyl group is located on the C-2 hydroxyl group it is assumed that the smallest substituted fragment is a maltotriose unit with a methyl group located at the C-2 or C-6 position of the non-reducing end group of the oligomer[23e]. The oligomers and glucose fractions can be separated by GPC.

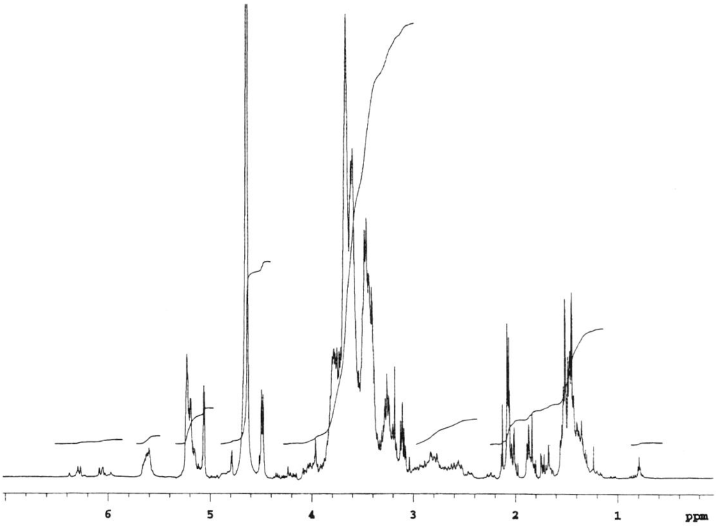


Figure 13a. ^{1}H-NMR of the smallest oligomer fraction of a hydrolysate of 2-nitropropyl starch.

The same procedure was used for the analysis of 2-nitroalkyl starch ethers. After enzymatic hydrolysis and GPC, the smallest oligomer fraction was analyzed with NMR. As expected, complicated ^{1}H-NMR spectra were obtained. Besides resonances of the carbohydrate backbone of 2-nitropropyl oligomers, peaks are observed (Figure 13a) between δ1.4-2.2 ppm (several multiplets, CH_3) and less intense ones between δ2.4-3.0 (a broad multiplet).
For 2-nitrobutyl oligomers (Figure 13b) peaks of the nitroalkyl ether substituent are observed at δ0.75-1.05 ppm (multiplet (CH_3), δ1.8-2.0 ppm (multiplet (CH_2) and less intense ones at δ2.5 ppm (multiplet), and between δ2.1-2.4 ppm (broad multiplet) and δ2.75-3.05 (broad multiplet). Other resonances of the nitroalkyl group are overlapped by resonances of the carbohydrate or H_2O.

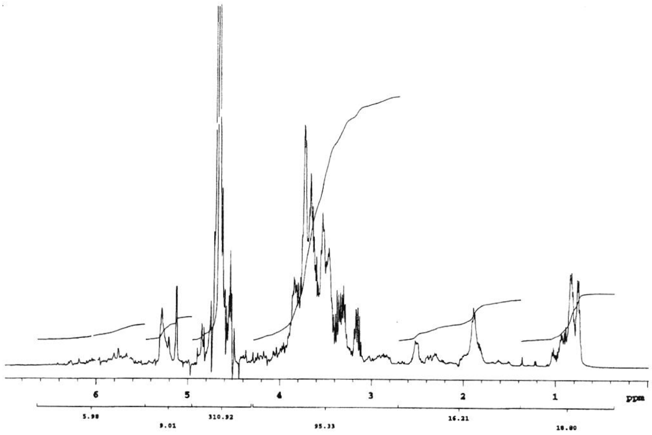
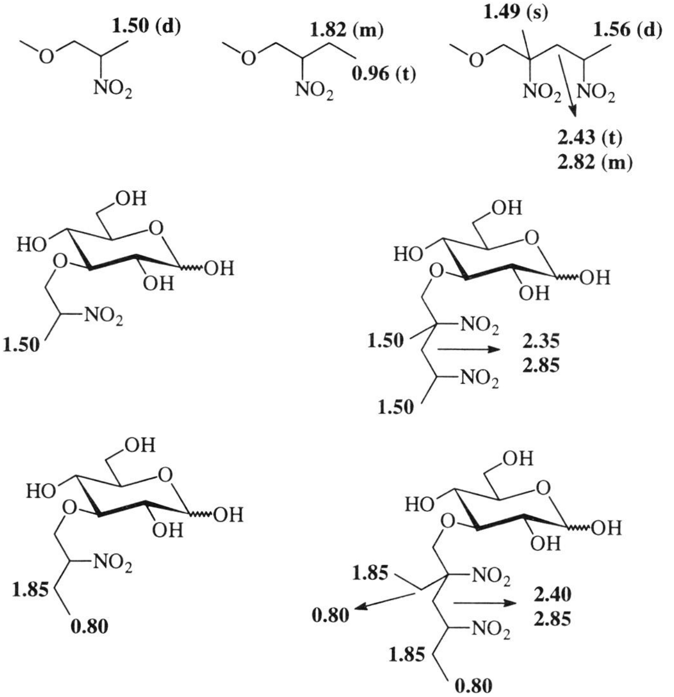


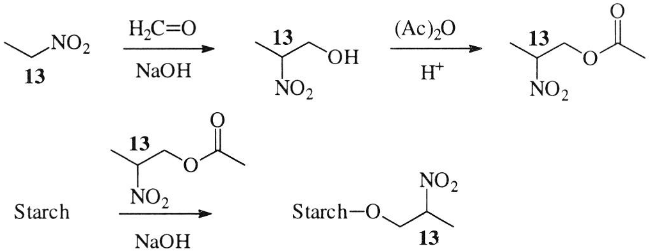
Figure 13b. ^{1}H-NMR spectrum of the smallest oligomer fraction of a hydrolysate of 2-nitrobutyl starch.

Comparison with ^{1}H-NMR spectra of model systems (Scheme 4) shows that for the hydrolyzate of 2-nitropropyl starch the multiplet at δ1.50 ppm is in agreement with the chemical shifts observed for the methyl group of 2-nitroalkyl ethers of the "normal" nitroalkane tautomer. The multiplet observed at δ2.4-2.8 ppm is in agreement with the chemical shift of the diastereotopic hydrogen atoms of the methylene group of grafted nitroalkenes on starch. According to the integrals, the amount of grafted material in 2-nitropropyl starch (ms = 0.078) is about 30-35%. Due to the accumulation of 2-nitropropyl acetate in the amylose fraction, it is likely that grafting occurs even at low overall ms. The ^{1}H-NMR values obtained for the ethyl group of model systems (CH_2 δ1.85, CH_3 δ1.85) for 2-nitrobutyl oligomers are in agreement with the ethyl group of the hydrolysate of 2-nitrobutyl starch (ms = 0.060) in the "normal" nitroalkane form. The amount of grafting seems to be of the same order of magnitude as found for 2-nitropropyl starch.



Scheme 4. High field proton chemical shifts of 1-methoxy-2-nitropropane, 1-methoxy-2-nitrobutane, 1-methoxy-2-methyl-2,4-dinitropentane, 3-*O*-(2-nitropropyl)-α,β-D-glucopyranoside and 3-*O*-(2-nitrobutyl)-α,β-D-glucopyranoside.

Due to the complexity of the system, it is very difficult to obtain satisfactory ^{13}C-NMR spectra of the smallest oligomers of 2-nitropropyl and 2-nitrobutyl starches. Extremely long pulse sequences are required to raise the various signals of the nitroalkyl functionality above the noise level. Therefore, we synthesized the ^{13}C-labeled derivative 2-nitropropyl-2-^{13}C starch from nitroethane-1-^{13}C (Scheme 5, ms = 0.104, calculated with FT-IR).



Scheme 5. Synthesis of 2-nitropropyl-2-^{13}C starch.

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 (Figure 14) at δ80, δ90 and δ92 ppm. Comparing these results to values obtained from the ^{13}C-NMR spectra of the model systems and NMR simulation (Figure 14) it can be concluded that the high field resonance results from the CHNO$_2$ carbon atom.

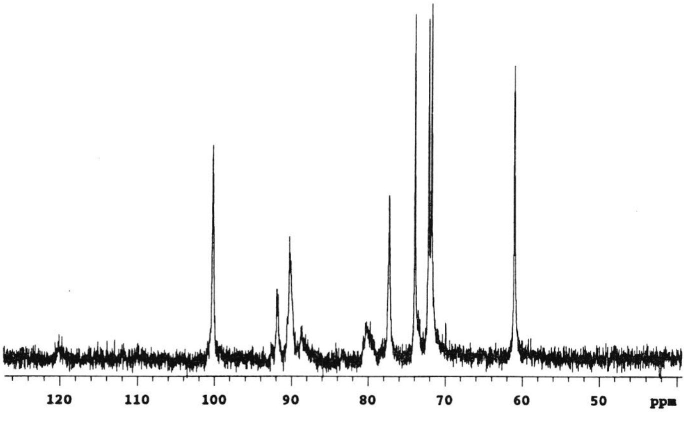
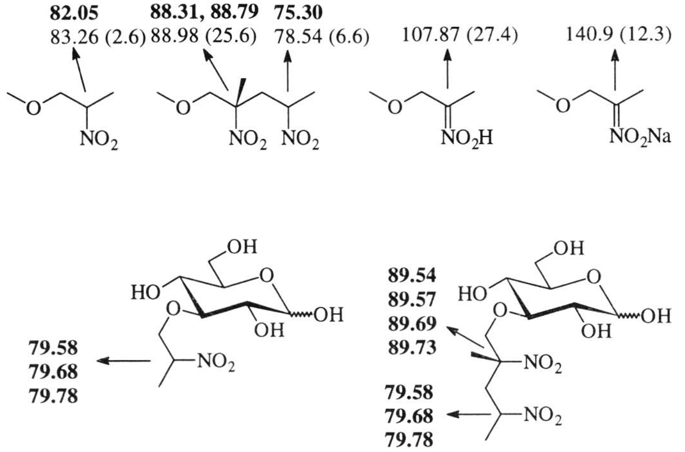


Figure 14. ^{13}C-NMR of gelatinized 2-nitropropyl-2-^{13}C starch.

One of the resonances at about δ90 ppm originates from the quaternary C(Me)NO$_2$ carbon atom, formed after grafting of 2-nitropropene on the starch backbone. The other resonance at about δ90 ppm most likely signifies the presence of the nitronic acid form (the *aci*-compound, C=NO$_2$H) of the 2-nitropropyl functionality.



Scheme 6. ^{13}C-NMR values of modelsystems (bold = measured value, normal = calculated value, ADS program)

As derived from an ADS NMR simulation program the value for the nitronic acid is expected at 107.87 ± 27.4 ppm. The presence of the nitronic acid tautomer was confirmed by the Konowalow test reaction[40]. A solution of 2-nitropropyl starch immediately turns red after addition of dilute ferric chloride solution.

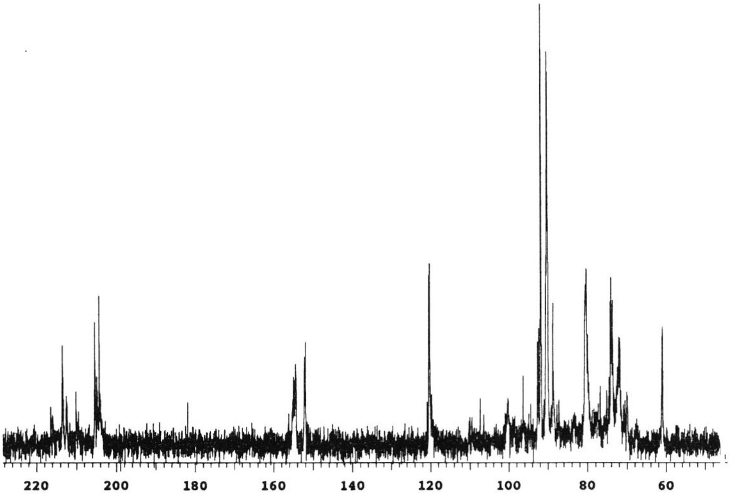
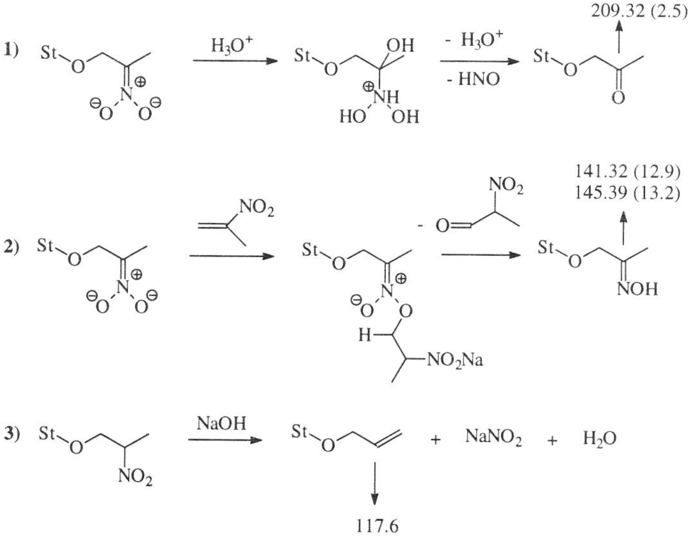


Figure 15. ^{13}C-NMR of the smallest oligomer fraction of a hydrolysate of 2-nitropropyl-2-^{13}C starch.

After enzymatic hydrolysis of 2-nitropropyl-2-^{13}C starch followed by GPC fractionation the oligomers not only show NMR signals for the "normal" 2-nitropropyl" (δ80 ppm), the grafted 2-nitropropene (δ90/92 ppm) and *aci*-2-nitropropyl-2-^{13}C (δ90/92 ppm), but resonances are also observed at δ120, δ152-156, δ204-215 ppm.

These resonances most likely originate from side reactions such as: 1) the Nef reaction in which a carbonyl functionality (209.31 ± 2.5) is formed (which can occur during neutralization of the alkaline suspension, Scheme 7); 2) The formation of an oxime (141.32 ± 12.9, 145.39 ± 13.2). A plausible explanation for the formation of oximes is the reaction of 2-nitropropene with the oxygen nucleophile of the nitro/nitronic acid group to nitronic esters and decomposition to an oxime and 2-nitro-1-propanal[41]; 3) The splitting off of sodium nitrite under the alkaline conditions used (Scheme 7)[42]. The resonances at 152-156 ppm can also originate from a sodium nitronate (140.9 ± 12.3).



Scheme 7. Proposed side reactions in the synthesis of 2-nitropropylstarch (the NMR spectra calculated for the corresponding methoxy compound).

It can be concluded that 2-nitropropyl starch, synthesized in a slurry process with 2-nitropropyl acetate, is a mixture of the *aci* and the normal tautomers. Normally, the equilibrium lies on the side of the thermodynamically more stable "normal" nitro tautomer. However, it is certainly possible that the nitronic acid ("*aci*-structure") is stabilized by hydrogen bonds and a mixture of both tautomers is isolated. Grafting of nitroalkenes occurs to a degree of 30-35% at molar substitutions of about 0.06-0.08. Well-known side products for Michael additions of nitroalkenes (Nef reaction, formation of oximes and splitting off of nitrate) are built into the polymer to a certain degree. However, the amount seems restricted to about 10-15%.

3.5 Analysis of 2-nitroalkyl sodium α-polyglucuronates

Because the synthesis of 2-nitroalkyl sodium α-polyglucuronates by reaction of sodium α-polyglucuronates with 2-nitroalkyl acetates proceeds in solution, a homogeneous distribution over the polyglucuronate backbone is likely to be obtained. Only one topochemical aspect, the distribution of the 2-nitroalkyl substituents over the remaining C-2 and C-3 functionalities of the polysaccharide, has to be taken into consideration. Unfortunately, the degradation of polyglucuronates, which is necessary for analysis, is very cumbersome. Acid degradation proceeds, according to literature reports[43], slowly and leads to undesired side products. To our knowledge, no enzymes have been reported which are capable of hydrolyzing the α-(1→4) bond of polyglucuronates.

In the ^{13}C-NMR spectra (Scheme 8) of 2-nitropropyl sodium α-polyglucuronate the resonances of the α-CNO_2 carbon atoms are observed at about δ83 ppm and δ90 (grafting). No resonances are observed for the *aci*-tautomer of the nitroalkyl group. This is confirmed by a negative Konowalow test. The negative charge of the polyglucuronate influences the neutralization of the sodium nitronate and protonation of the carbon atom is probably preferred over that of the oxygen atom of the nitronate. The same applies to 2-nitrobutyl sodium α-polyglucuronate, however, for 2-nitrobutyl sodium α-polyglucuronate no grafting of 2-nitro-1-butene on the polysaccharide is observed.

Scheme 8. NMR resonsances of the nitroalkyl functionality of 2-nitropropyl and 2-nitrobutyl sodium α-polyglucuronates (bold face ^{1}H NMR, normal type ^{13}C NMR).

3.6 Conclusions

Several methods have been developed for the determination of the molar substitution of 2-nitroalkyl starch ethers. The gelatinization temperature of 2-nitroalkyl starches and the enthalpy of the exothermic decomposition of 2-nitropropyl starch are directly proportional to the molar substitution.

Suspension reactions of starch with 2-nitropropyl acetate lead to accumulation of the 2-nitroalkyl substituent in the amorphous amylose fraction of the starch granule. A possible explanation is that at first an amylose-2-nitropropyl acetate helix is formed and after splitting off acetate the nitroalkene reacts within the amylose helix. The regiospecificity makes the nitroalkylation very suitable for prevention of retrogradation of amylose of starch pastes, even at low degrees of substitution.

The nitro group of 2-nitroalkyl starches is a mixture of the nitroalkane and nitronic acid tautomer. Some grafting of nitroalkenes occurs, and to a lesser extent additional reactions of the nitro group, which lead to the formation of carbonyl groups (Nef reaction) and oximes (decomposition of nitronic esters), take place.

3.7 Experimental

General methods. - MeOH, EtOH, Et_2O, THF, EtOAc were purchased from Lab-Scan. Trifluoroacetic acid, acetic acid, Na_2SO_4, NaH (80% in paraffin oil) and silica gel 60 were purchased from Merck. NaOMe (30%) was purchased from Aldrich. Biogel P2 was obtained from Pharmacia. Cellulose membrane filters (45 μm) were purchased from Sartorius. α-Amylase (EC 3.2.1.1) was purchased from Sigma. Amyloglucosidase (EC 3.2.1.3) was purchased from Merck, *Pseudomonas* isoamylase (EC 3.2.1.68) was purchased from Hayashibara. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose, 2-Nitropropyl acetate[44], 2-nitrobutyl acetate, 2-nitropropene[45], and 2-nitrobutene were prepared according to literature procedures.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 200 MHz Nicolet NT-200 spectrometer, or a 500 Varian MHz spectrometer. Differential Scanning Calorimetry was performed on a Perkin-Elmer DSC7 PC Series apparatus (heating rate 5.0 K min^{-1}). FT-IR spectra were recorded on a Biorad FTS 135 spectrometer. Fractionation of starch was achieved with sieves from Retsch (porous sizes 63 μm, 45 μm, 25 μm). Viscosity profiles were recorded on a Rapid Visco Analyser RVA-3d purchased from Newport Scientific. HPLC was performed with a Benson BCX4 column (Ca^{2+} form, 250x10 mm, eluent 100 ppm CaEDTA, 0.4 ml/min), a Meyvis pump, a Marathon autosampler and a Jasco 830-RI detector. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands).

Continuous extraction of 2-nitroalkyl starches. - 2-Nitropropyl-, 2-nitrobutyl-, 2-nitropentyl- and 2-nitrohexyl starch were extracted continuously with MeOH in a soxhlet apparatus. After 4 days the derivatives were dried in the air and the nitrogen content was determined again. (see Table 1).

Fractionation of 2-nitropropyl starch. - 2-Nitropropyl starch (48.50 g, ms = 0.058) was separated into four size groups by using sieves and mechanical agitation (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 2).

Fractionation of 2-nitrobutyl starch. - 2-Nitrobutyl starch (47.35 g, ms = 0.081) was separated into four size groups by using sieves and mechanically agitating (dry product) for 1 h (> 63 μm (13.55 g) , 45 μm < x < 63 μm (21.37 g), 25 μm < x < 45 μm (11.04 g), < 25 μm (0.02 g)). The nitrogen of each fraction was determined again (see Table 2).

Synthesis of 1-methoxy-2-nitropropane. - 2-Nitropropyl acetate (9.13 g, 62.1 mmole) was added dropwise at T = 0°C to a solution of 160 ml MeOH and 18.5 ml 30% NaOMe (240 mmole). After 24 h stirring and rotary evaporation of the solvent 40 ml H_2O was added. After addition of acetic acid (16 ml) at T = 0°C the water layer was extracted with 3 x 75 ml Et_2O. The combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (15 mm Hg, 80°C) and 2.68 g (22.5 mmole, 36%) 1-methoxy-2-nitropropane was isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^1H δ 1.50 (d, 3H, CH_3), 3.35 (s, 3H, OCH_3), 3.60 (dd, 1H, CH_2, H_a), 3.80 (t, 1H, CH_2, H_b), 4.75 (m, 1H, CH). ^{13}C δ 15.27 (CH_3), 58.94 (OCH_3), 73.37 (CH_2), 82.05 ($CHNO_2$).

Synthesis of 1-methoxy-2-nitrobutane. - 2-Nitrobutyl acetate (7.50 g, 46.6 mmole) was added dropwise at T = 0°C to a solution of 120 ml MeOH and 14 ml 30% NaOMe (182 mmole). After 20 h stirring and rotary evaporation of the solvent 30 ml H_2O was added. After addition of acetic acid (12 ml) at T = 0°C the water layer was extracted with 3 x 75 ml Et_2O. The combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (12 mm Hg, 75°C) and 2.32 g (17.5 mmole, 37%) 1-methoxy-2-nitrobutane was isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^1H δ 0.96 (t, 3H, CH_3), 1.82 (m, 2H, CH_2), 3.33 (s, 3H, OCH_3), 3.58 (dd, 1H, CH_2, H_a), 3.82 (t, 1H, CH_2, H_b), 4.60 (m, 1H, $CHNO_2$). ^{13}C δ 9.79 (CH_3), 23.22 (CH_2), 58.89 (OCH_3), 72.35 (CH_2), 88.75 ($CHNO_2$).

Synthesis of 1-methoxy-2-methyl-2,4-dinitropentane. - 1-Methoxy-2-nitropropane (1.01 g, 8.5 mmole) was added at T = 0°C to a solution of NaOH (0.32 g, 8.0 mmole) and NaAc (0.70 g, 8.5 mmole) in $MeOH/H_2O$ (3:2, 5 ml). After stirring for 1 h at T_k the solution was cooled to T = 0°C and 2-nitropropyl acetate (1.25 g, 8.5 mmole) was added dropwise. After 20 h stirring and rotary evaporation of the solvent 10 ml H_2O was added. The water layer was extracted with 3 x 15 ml Et_2O and the combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (Kugelrohr, 0.1 mm Hg, 100°C) and 0.18 g (0.87 mmole, 10% (not optimalized) of both diastereoisomers of 1-methoxy-2-methyl-2,4-dinitropentane were isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^1H δ 1.49 (s, 3H, CH_3) 1.56 (d, 3H, CH_3), 2.43 (t, 1H, CH_2, H_a), 2.82 (m, 1H, CH_2, H_b) 3.16 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 3.52 (t, 1H, OCH_2, H_a), 3.81 (m, 1H, OCH_2, H_b), 4.78 (m, 1H, $CHNO_2$). ^{13}C δ 19.00, 21.28 ($CH_{3(q)}$), 21.91, 22.87 ($CH_{3(t)}$), 39.00, 39.78 (CH_2), 58.34, 59.03 (OCH_3), 75.30, (OCH_2), 79.55, 79.66 ($CHNO_2$), 88.31, 88.79 (CNO_2).

Synthesis of 3-O-(2-nitropropyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose. - 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (6.58 g, 25.3 mmole) was added to a solution of 0.68 g NaH* in dry THF (70 ml). After stirring for 16 h the solution was refluxed for 1 h. 2-Nitropropene (2.20 g, 25.3 mmole) was added dropwise and after 1.5 h acetic acid (3 ml) was added. After subsequent stirring for 0.75 h the solvent was evaporated en the residue was dissolved in 100 ml EtOAc. The EtOAc layer was extracted with 2 x 100 ml H_2O, dried over $Na_2S_2O_4$ and, after filtration and evaporation of the solvent, the residue was purified over a silica gel column (250 g), eluent $CHCl_3/MeOH$ 100:1). Yield 3.63 g of a mixture of 3-*O*-(2-nitropropyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (m) and 3-*O*-(2-methyl-2,4-dinitropentyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (d). NMR (200 MHz, $CDCl_3$, in ppm): ^1H δ 1.2-1.6 (m, 21.5H, ($C(\mathbf{CH_3})_2$, $CH(NO_2)\mathbf{CH_3}$ (m + d), $C(NO_2)\mathbf{CH_3}$), 2.40

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* NaH (80% suspension in paraffin) was washed with pentane before use.

(m, 1.2H, **CH_2** (d), H_a), 2.80 (m, 1.2H, **CH_2** (d), H_b), 3.80-4.20 (m, 8H, C-2**H**, C-4**H**, C-5**H**, C-6**H_2**, O**CH_2** (m + d)), 4.40 (m, 1H, C-3**H**, 4.80 (m, 1H, **CH**NO_2 (m+d)), 5.80 (m, 1H, C-1**H**). ^{13}C δ 15.10-22.42, $CHNO_2$**CH_3** (m+d),CNO_2**CH_3**, 24.79-26.50 (C(**CH_3**)$_2$), 38.56-39.88 (**CH_2** (d)), 67.16-67.53 (**C**-6), 71.77-77.50 (O**CH_2** (m+d)), 78.36-83.66 (**C**-2, **C**-3, **C**-4, **C**-5, **C**HNO_2 (m+d)), 88.07-88.43 (**C**NO_2 (d), 105.07-105.13 (**C**-1), 108.92-111.83 (**C**(CH_3)$_2$).

Synthesis of 3-O-(2-nitrobutyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose. - 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (10.02 g, 38.5 mmole) was added to a solution of 0.98 g NaH in dry THF (70 ml). After stirring for 16 h the solution was refluxed for 1 h. 2-Nitrobutene (3.89 g, 38.5 mmole) was added dropwise and after 1.5 h acetic acid (5 ml) was added. After subsequent stirring for 45 min the solvent was evaporated en the residue was dissolved in 100 ml EtOAc. The EtOAc layer was extracted with 2 x 100 ml H_2O, dried over $Na_2S_2O_4$ and after filtration and evaporation of the solvent the residue was purified over a silica gel column (450 g, eluent $CHCl_3$/MeOH 100:1). Yield 6.49 g of a mixture of 3-*O*-(2-nitrobutyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (m) and 3-*O*-(2-ethyl-2,4-dinitrohexyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (d). NMR (200 MHz, $CDCl_3$, in ppm): ^1H δ 0.90-1.00 (m, 6.9H, CH_2**CH_3** (m+d), 1.20-1.60 (m, 12H, C(**CH_3**)$_2$, 1.60-2.05 (m, 4.6H, **CH_2**CH_3 (m+d), 2.40 (m, 1.2 H, **CH_2** (d), H_a), 2.80 (m, 1.2H, **CH_2** (d), H_b), 3.80-4.20 (m, 8H, C-2**H**, C-4**H**, C-5**H**, C-6**H_2**, O**CH_2** (m + d)), 4.40 (m, 1H, C-3**H**, 4.70 (m, 1H, **CH**NO_2 (m+d)), 5.80 (m, 1H, C-1**H**). ^{13}C δ 7.53-9.47 (CH_2**CH_3** (m+d), 24.74-26.71 (C(**CH_3**)$_2$, 28.66-29.14 (**CH_2**CH_3 (m+d), 34.85-35.44 (**CH_2** (d)), 67.18-67.58 (**C**-6), 69.79-71.80 (O**CH_2**,(m+d)), 80.90-85.83 (**C**-2, **C**-3, **C**-4, **C**-5, **C**HNO_2 (m+d)), 91.71-92.36 (**C**NO_2 (d)), 105.17 (**C**-1), 109.01-111.78 (**C**(CH_3)$_2$).

Synthesis of 3-O-(2-nitropropyl)-α,β-D-glucopyranoside. - A mixture of 3-*O*-(2-nitropropyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (m) and 3-*O*-(2-methyl-2,4-dinitropentyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (d) (1.01 g) was added to a solution of trifluoroacetic acid/H_2O (9:1, 10 ml). After 20 min stirring the solvent was evaporated and triturated with Et_2O (10 ml, 4x). The residue was purified over a silica gel column (25 g, eluent EtOAc, followed by EtOAc/EtOH/H_2O 12:2:1). Yield 0.23 g of a mixture of 3-*O*-(2-nitropropyl)-α,β-D-glucopyranoside (m) and 3-*O*-(2-methyl-2,4-dinitropentyl)-α,β-D-glucopyranoside (d). NMR (500 MHz, D_2O, in ppm): ^1H δ 1.40-1.60 (m, 7.6H, **CH_3** (m+d)), 2.35 (m, 1.2H, **CH_2** (d), H_a), 2.80 (m, 1.2H, **CH_2** (d), H_b), 3.05-3.80 (m, 8H, C-2**H**, C-3**H**, C-4**H**, C-5**H**, C-6**H_2**, O**CH_2** (m + d)), 4.50 (m, 0.7H, C-1**H** (β)), 4.85 (m, 1H, **CH**NO_2 (m+d)), 5.05 (m, 0.3H, C-1**H** (α)). ^{13}C δ 18.70-20.65 (**CH_3**), 38.68 (**CH_2** (d)), 60.09-60.26 (**C**-6), 68.55-75.47 (**C**-2, **C**-4, **C**-5), 76.28-76.51 (O**CH_2**) 79.58-79.78 (**C**HNO_2 (m+d)) 82.48-84.79 (**C**-3), 89.69 (**C**NO_2 (d)), 91.91-95.52 (**C**-1 (α,β)). FT-IR (in cm^{-1}): 3433 (s), 2941 (m), 1681 (m), 1551 (s), 1459 (m), 1394 (m), 1358 (m), 1295 (w), 1206 (m), 1140 (m), 1080 (m), 1036 (m), 905 (w), 851 (m), 802 (w), 775 (w), 722 (w), 641 (w).

Synthesis of 3-O-2-nitrobutyl-α,β-D-glucopyranoside. - A mixture of 3-*O*-2-nitrobutyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (m) and 3-*O*-2-ethyl-2,4-dinitrohexyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (d) (0.93 g) was added to a solution of trifluoroacetic acid/H_2O (9:1, 10 ml). After 0.33 h stirring the solvent was evaporated and triturated with Et_2O (10 ml, 4x). The residue was purified over a silica gel column (25 g, eluent EtOAc). Yield 0.57 g of a mixture of 3-*O*-2-nitrobutyl-α,β-D-glucopyranoside (m) and 3-O-2-methyl-2,4-dinitrohexyl-α,β-D-glucopyranoside (d). NMR (500 MHz, D_2O, in ppm): ^1H δ 0.70-0.82 (m, 6.2H, **CH_3** (m+d)), 1.70-1.90 (m, 4.2 H, **CH_2** (m+d), 2.40 (dd, 1.1H, **CH_2** (d), H_a), 2.82 (m, 1.1H, **CH_2** (d)), 3.50-3.80 (m, 6.4H, C-2**H**, C-3**H**, C-4**H**, C-5**H**, C-6**H_2**, O**CH_2** (m+d), 4.50 (m, 0.6H, C-1**H** (β)), 4.75 (m (1H, **CH**NO_2 (m+d)), 5.05 (m, 0.4H, C-1**H** (α)). ^{13}C 6.69-8.83 (**CH_3** (m+d), 27.08-28.87 (**CH_2** (m+d), 34.32-35.35 (**CH_2** (d)), 60.14-60.27 (**C**-6), 68.40-75.53 (**C**-2, **C**-4, **C**-5, O**CH_2**), 82.21-95.72 (**C**-3, **C** HNO_2 (m+d), **C**NO_2 (d), **C**-1 (α), **C**-1 (β).

FT-IR (in cm^{-1}) : 3422 (s), 2979 (m), 2944 (m), 1680 (m), 1551 (s), 1465 (m), 1444 (w), 1376 (w), 1206 (m), 1140 (m), 1081 (w), 1036 (w), 917 (w), 851 (m), 801 (w), 772 (w), 721 (w), 642 (w).

Enzymatic hydrolysis and fractionation of 2-nitroalkyl starches (the hydrolysis of 2-nitropropyl starch is given as a general example). - Drum dried 2-nitropropyl starch* (ms = 0.080, 5.00 g) was dissolved in 100 ml H_2O. 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. After 48 h (pH = 5) the solution was heated for 1h at 80°C and filtrated over a cellulose membrane. The water layer was evaporated till 5-10 ml and fractionized by Biogel P2 (column 85x5 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. 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 Scheme 13a). FT-IR: 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).

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[*] 2-Nitrobutyl starch (ms = 0.060) was gelatinized before the enzymes were added.

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Chapter 4

Reduction of 2-nitroalkyl starch ethers

4.1 Introduction

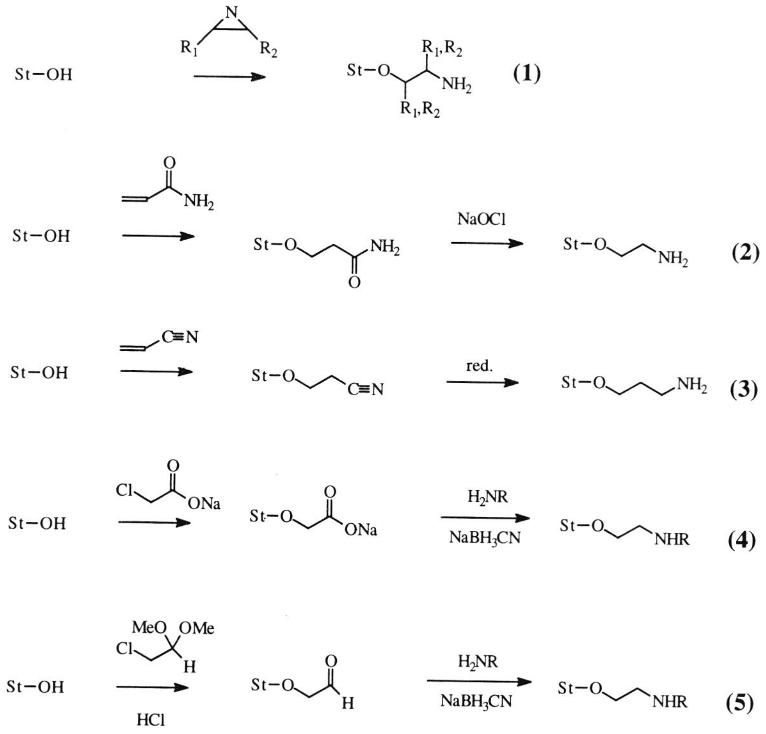
Up to now, no successful route has been developed for the synthesis of primary or secondary amino alkyl starch ethers on an industrial scale. This is in contrast with the synthesis of tertiary aminoalkyl- and quaternary ammonium starch ethers. These starch derivatives are widely used as wet-end additives in the paper industry, as warp sizing agents in textile manufacture and as flocculants for suspensions of inorganic or organic matter having a negative charge[1].

Primary or secondary aminoalkyl starch ethers (which may also fulfil the above-mentioned applications under acidic conditions) are very interesting starting materials for other (secondary) starch derivatives. In principle, primary and secondary aminoalkyl starch ethers are excellent precursors for the synthesis of starch-based detergents (reaction with fatty acids) or for the coupling of drugs or proteins to starch.

The current state of the art for synthesis of primary and secondary aminoalkyl starch ethers is shown in Scheme 1-3. The disadvantages of these routes, which make them unsuitable for application on an industrial (or pharmaceutical) scale, will be explained briefly. The approaches may be divided into three categories:

- aliphatic aminoalkyl starch ethers (Scheme 1)
- aromatic aminoalkyl starch ethers (Scheme 2)
- amino-deoxy starch derivatives (Scheme 3)

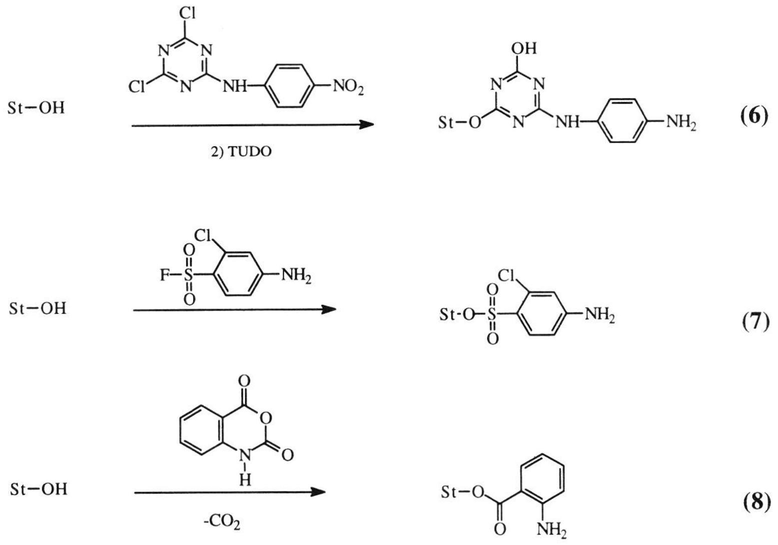
Reaction of starch with ethylene imine (aziridine, Route **1**) suffers from polymerization and grafting of ethylene imine on starch and is not suitable for large-scale synthesis due to the prohibitive costs and the highly toxic character of ethylene imine[2]. The Hoffmann rearrangement[3] of carbamoethyl (amidoethyl) starch (synthesized in high yield by reaction of starch with acrylamide) does not go to completion and a mixture of the intermediate *N*-chlorocarbamoethyl, isocyanate, and the desired aminoethyl groups is isolated after reaction (Route **2**)[4]. The molecular weight of the starch polymer decreases during the process, due to oxidative degradation caused by NaOCl. Despite the drawbacks, the oxidized compound has found applications as wet-end additive in the paper industry[5].The reduction of cyanoethyl starch (synthesized in high yield by a Michael addition of starch with acrylonitrile) is performed in high efficiency with borane (BH_3), but the reaction can only be performed under dry reaction conditions and is not applicable for granular starch (Route **3**)[6]. Other claims for the reduction of the nitrile functionality are rather doubtful and the products obtained have not been insufficiently analyzed[7].



Scheme 1. Routes for the synthesis of aliphatic primary/ secondary aminoalkyl starch ethers.

The same holds for the synthesis of *N*-alkyl aminoethyl starch by reduction of the corresponding amide (formed after reaction of carboxymethyl starch with an amine) with $NaCNBH_3$ (Route **4**)[8]. Most likely, the products obtained are ammonium salts of carboxymethyl starch. The last approach of Scheme 1 (Route **5**), reaction of starch with the acetal of chloro acetaldehyde followed by reductive amination is not applicable on a large scale, owing to the cost of the reagent [9].

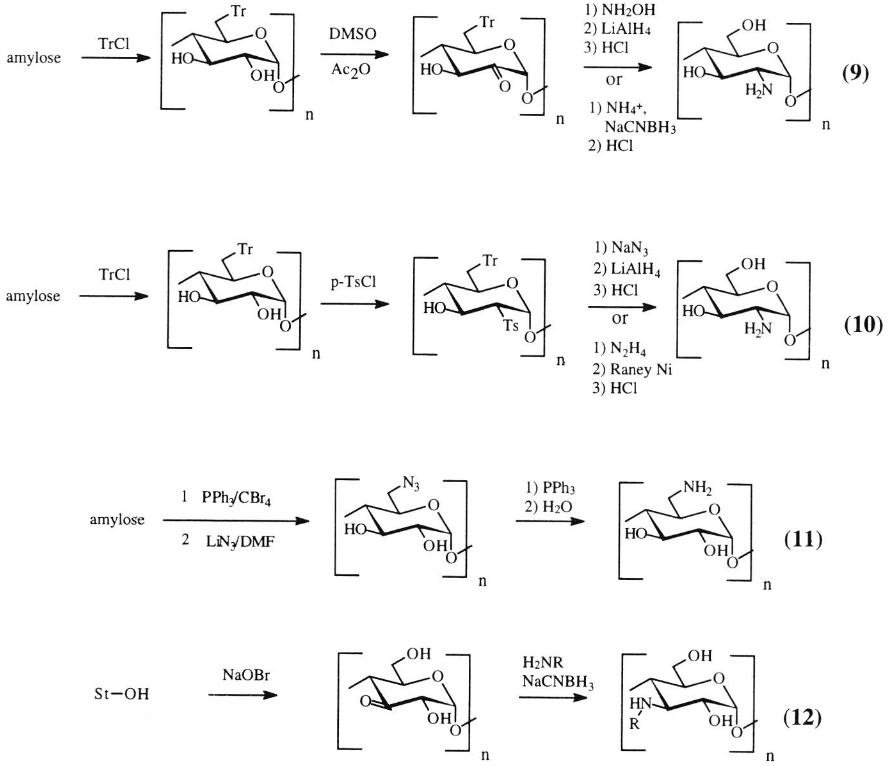
Aromatic aminoalkyl starch derivatives are prepared by reaction of starch with cyanuric chloride (Scheme 2, Route **6**) and subsequent reduction of the aromatic nitrofunctionality with thiourea dioxide (TUDO)[10], reaction with arylamino sulfonyl fluorides (Route **7**)[11], and esterification with isatoic anhydride (Route **8**)[12]. The products obtained have been tested as ion exchangers, and used in the syntheses of starch-based azo dyes. These azo dyes are obtained after diazotization with $NaNO_2$ and subsequent reaction with an appropriate coupling agent (*e.g.*, *m*-phenyl diamine, 2-naphthol).



Scheme 2. Routes for the synthesis of aromatic aminoalkyl starch ethers/esters.

Chitosan-alike derivatives are obtained after oximation of the keto functionality of the oxidized amylose[13] and subsequent reduction or by reductive amination of the oxidized amylose (Route **9**)[14]. The trityl group may be introduced in this procedure as a protecting group to obtain a more selective oxidation. Other methods for syntheses of amino-deoxy amyloses are: tritylation of starch followed by *p*-toluenesulfonylation of a secondary hydroxyl group of starch followed either by: a) reaction with sodium azide, reduction, and detritylation[15] or b) hydrazinolysis, catalytic reduction and detritylation (Route **10**)[16]. Synthesis of 6-azido-6-deoxy amylose is achieved under mild and homogeneous conditions by reaction of amylose with Ph_3P/CBr_4 followed by DMF/LiN_3[17]. 6-Amino-6-deoxy-amylose is formed after the reduction of the azide functionality with Ph_3P and H_2O[18] (Route **11**). Cimeciogli *et al.* are currently investigating a one-step synthesis method by combining the above-mentioned procedures.

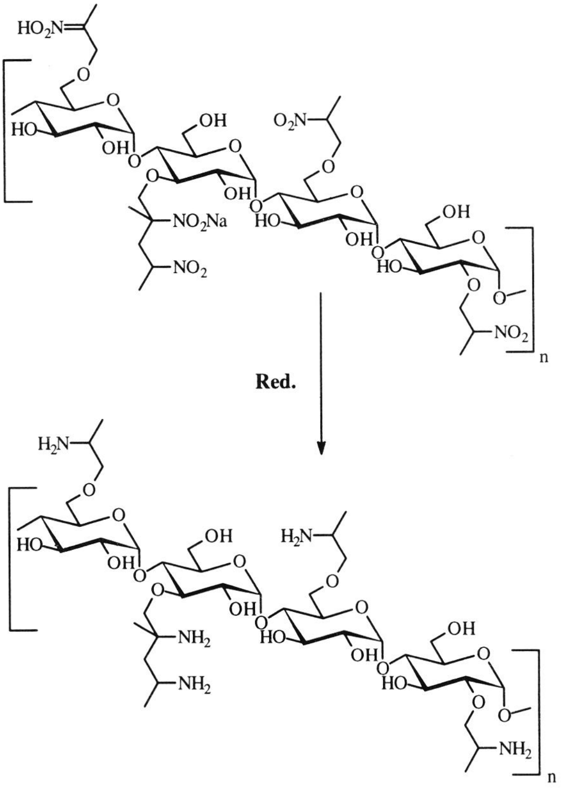
In contrast to the other, more tedious methods the last method in Scheme 3 (Route **12**), oxidation of starch with bromine followed by reductive amination has,, some potential for large scale synthesis[19]. However, oxidation under the conditions used leads to a mixture of 2-oxo, 3-oxo, 2,3-dioxo and 2,3-dicarboxy starch.



Scheme 3. Synthesis of amino-deoxy amylose/starch derivatives.

As already indicated in previous chapters, a novel route to aminoalkyl starch ethers could be the reduction of 2-nitroalkyl starch ethers (Scheme 4). From an economic point of view, a one-pot synthesis in which granular starch is first nitroalkylated and subsequently reduced to the corresponding aminoalkyl derivative, would be highly preferred.

For the reduction of the granular 2-nitroalkyl starch ethers to aminoalkyl starch ethers the reducing agent has to be water soluble, cheap and nontoxic. After reduction, the purification of the aminoalkyl starch has to be facile. Furthermore, the reactions have to be performed at temperatures below the gelatinization temperature and almost neutral conditions to prevent hydrolysis of starch (which takes place under acidic conditions) and reverse Michael additions (under alkaline conditions).



Scheme 4. Synthesis of 2-aminoalkyl starch ethers from 2-nitroalkyl starches.

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

A reducing agent which fits all the above-mentioned requirements and which seems also applicable for granular 2-nitroalkyl starches is sodium dithionite ($Na_2S_2O_4$)[20]. In this chapter, the results for the catalytic hydrogenation of water soluble 2-nitroalkyl starch ethers (propyl and butyl) as well as the reduction of 2-nitropropyl starch (granular and water soluble) with $Na_2S_2O_4$ or a combination of $Na_2S_2O_4/NaBH_4$ are described.

4.2. Results and discussion

4.2.1 Catalytic hydrogenation of water soluble 2-nitroalkyl starch ethers

Catalytic hydrogenation of aliphatic nitro compounds is widely used for the synthesis of aminoalkyl compounds[21]. In the present study we tested the catalytic hydrogenation of water-soluble 2-nitropropyl and 2-nitrobutyl starches with Raney Ni as the catalyst.

Table 1. Hydrogenation of 2-nitropropyl and 2-nitrobutyl starch (T = 80°C, 80 bar, 20 h, Raney Ni).

	%N	%N after reaction	$\%N_{max}$ (eff. 100%)
2-nitropropyl starch	1.50	0.79	1.55
2-nitrobutyl starch	1.66	0.84	1.72

As shown in Table 1, the nitrogen content is lowered after catalytic hydrogenation of 2-nitropropyl and 2-nitrobutyl starch, most likely due to reverse Michael additions*. IR spectra of the isolated compounds show a clear absorbance of the starting nitro functionality and it can be concluded that a complete conversion of the nitro group has not been achieved. Positive ninhydrine tests[22] 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[23]. The low conversions for catalytic hydrogenation of 2-nitroalkyl starch ethers 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. The isolated products are slightly green/brown. This is caused by contamination of the product with the nickel catalyst and confirms the metal chelating properties of aminoalkyl starch ethers.

4.2.2 Reduction of 2-nitropropyl starch with sodium dithionite ($Na_2S_2O_4$)

$Na_2S_2O_4$ is widely used in the paper (bleaching agent) and textile industry (reduction of certain dyes) and its use in organic chemistry involves the reduction of several nitrogen functionalities and aldehydes and ketones in protic solvents[24] or in organic solvent-water systems[25]. In these two-phase systems, viologen (1,1'-dialkyl-4,4'-bipyridyl, V^{2+}) is utilized as an electron-transfer catalyst.

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* The pH of the solution increases owing to formation of amines. Better results are probably obtained if the solution is buffered.

Solutions of $Na_2S_2O_4$ are not stable but decompose to bisulfite (HSO_3^-) and thiosulfate ($S_2O_3^{2-}$, Scheme 5)[26]. The decomposition is accelerated autocatalytically under acidic conditions (formation of HSO_3^-) by H_2S or colloidal sulfur. H_2S And sulfur are probably formed in minor amounts owing to side reactions of the sulfoxylate anion (HSO_2^-). To prevent rapid decomposition of $Na_2S_2O_4$, we reduced suspensions of granular 2-nitropropyl starch and water soluble 2-nitropropyl starch at neutral conditions and added the sodium dithionite in portions to keep the concentration low.

$$S_2O_4^{2-} + H_2O \longrightarrow HSO_2^- + HSO_3^-$$

$$HSO_2^- + S_2O_4^{2-} \longrightarrow HSO_3^- + S_2O_3^{2-}$$

Scheme 5. Decomposition of $Na_2S_2O_4$.

It is still a topic of discussion whether the sulfoxylate anion (HSO_2^-) or a SO_2^-. radical anion, which is detected by electron spin resonance in $Na_2S_2O_4$ solution, is the reducing agent. For complete conversions of a nitro functionality to an amine at least three equivalents of sodium dithionite (6 electrons, see Scheme 6) are necessary (nitro → nitroso/oxime → hydroxylamine → amine). The reduction of nitro compounds with different reducing agents gives a wide range of possible products, the most frequently observed compounds are shown in Scheme 6. 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.

$$HSO_2^- + H_2O \longrightarrow HSO_3^- + 2H^+ + 2e^-$$

$$\begin{tikzcd}
RCH_2NO_2 \arrow[r, "2e^-"] & RCH_2NO \arrow[r, "2e^-"] \arrow[d, shift right] & RCH_2NHOH \arrow[r, "2e^-"] & RCH_2NH_2 \\
 & RCH{=}NOH \arrow[u, shift right] & &
\end{tikzcd}$$

Scheme 6. Reduction of nitroalkanes to aminoalkanes.

In an initial attempt, we added about 5 mole equivalents $Na_2S_2O_4$ per nitro group to granular 2-nitropropyl starch (Table 2, condition 1). The product was analyzed by means of elemental analysis (nitrogen content) and FT-IR. As the absorbance of the nitro functionality at 1550 cm^{-1} changes linearly with the molar substitution of the modified starch (see Chapter 3), the yield of new *N*-containing starch ethers can be determined by combining elemental analysis with IR spectroscopy. The yield (Table 2, fourth column) can be calculated by subtracting the %N present in the form of the nitro groups (Table 2, third column) from the total nitrogen content of the product isolated (Table 2, second column) and dividing this by the nitrogen content of the starting material (2-nps, %N = 0.64). The result of the reduction with $Na_2S_2O_4$ (T_k) was moderate, about 48% of the nitrofunctionality was 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.

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

conditions (granular)	%N	%NO_2	%N other	yield in nitrogen containing material (in %)
1, - (2-nps)	0.64	0.64	-	-
2, T_k	0.57	0.26^{a}	0.31	48^{b}
3, T_k, 2M $NaNO_3$	0.55	0^{a}	0.55	86^{b}
4, T = 35°C	0.55	0.22^{a}	0.33	52^{b}
5, T_k, $NaBH_4$	0.49 (%S = 0.21%)	0^{a}	0.49	77^{b}
one-pot synthesis	%N	%NO_2	%N other	yield in nitrogen containing material (in %)
6, T_k	0.49 (0.71)^{c}	0.42^{d}	0.07	10^{e}
7, T = 35°C (reduction step)	0.43 (0.76)^{c}	0.20^{d}	0.23	30^{e}
8, T_k, no Na_2SO_4	0.49 (0.71)^{c}	0.43^{d}	0.06	8^{e}
conditions (water soluble)	%N	%NO_2	%N other	yield in nitrogen containing material (in %)
9, - (2-nps)	0.64	0.64	-	-
10, T_k	0.36	0.19^{a}	0.17	27^{b}
11, T_k, $NaBH_4$	0.48	0^{a}	0.48	75^{b}

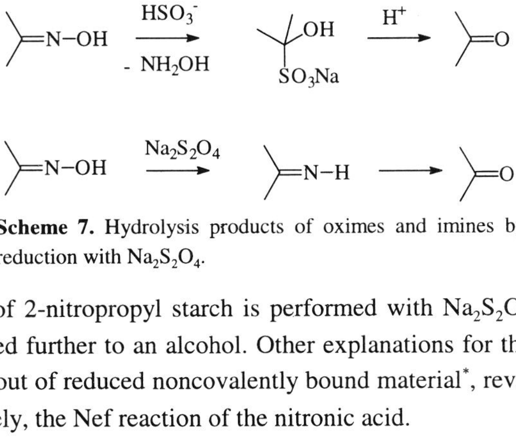
a) Determined with FT-IR ((area 1550/860 cm^{-1} reduced product/area 1550/860 cm^{-1} 2-nps). b) %N other/0.64 (= %N 2-nps) c) %N_{max} d) determined with FT-IR (area 1550/860 cm^{-1} reduced product)/area 1550/860 $cm^{-1}\cdot$ %N_{max}, the values of the areas of %N_{max} are determined from the calibration curve as described in Chapter 3, Scheme 3). e) %N other/%N_{max}.

The accessibility of the nitro functionalities is increased when the reduction is performed in 2 M $NaNO_3$ (entry **3**) in which the starch granules swell and are probably more susceptible to the reducing agent. A similar effect can be achieved when the temperature is raised (**4**). Indeed, higher yields were obtained under these conditions. The effect of addition of $NaBH_4$ (2 mole equivalents) during the reduction was also tested. It is known that $NaBH_4$ 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 room temperature.

The results of 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 $Na_2S_2O_4$ and $NaBH_4$. An attempt in which no swelling inhibitor was added during the nitroalkylation 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 $Na_2S_2O_4$ (**10,** 27%) is much lower than for $Na_2S_2O_4/NaBH_4$ (**11**, 75%).

During the reduction, the nitrogen content of the reduced 2-nitropropyl starch decreases to some extent. According to the literature this decrease can be explained by hydrolysis of an oxime or imine intermediate product (see Scheme 7).



Scheme 7. Hydrolysis products of oximes and imines by reduction with $Na_2S_2O_4$.

When the reduction of 2-nitropropyl starch is performed with $Na_2S_2O_4/NaBH_4$ the carbonyl functionality is reduced further to an alcohol. Other explanations for the decrease in nitrogen content are: washing out of reduced noncovalently bound material*, reverse Michael additions and, although less likely, the Nef reaction of the nitronic acid.

4.2.3 Analysis of reduced 2-nitropropyl starch.

As described in the previous section, the complete conversion of the nitro functionality of granular and drum-dried 2-nitropropyl starches can be achieved with $Na_2S_2O_4$ in 2M $NaNO_3$

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* See Chapter 3.

(**3**) or if $Na_2S_2O_4$ is used in combination with $NaBH_4$ (**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 substition no direct information about the product formation can be obtained from FT-IR. Nitroso, oxime, hydroxylamines, and amines give rise to rather low or medium absorbances in the 1500-1700 cm^{-1} region[27], and these absorbances cannot be observed clearly, due to strong polysaccharide/water absorbances at these wavelenghts.

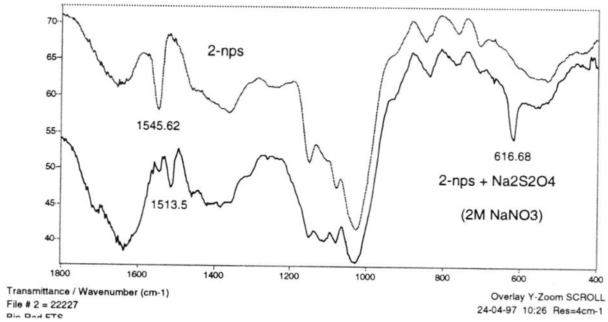
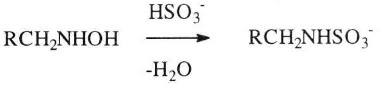


Figure 1. FT-IR spectra of a 2-nitropropyl oligomer and a reduced 2-nitropropyl oligomer (1545 cm^{-1} (NO_2), 1513 cm^{-1} (NO, N=OH, NHOH, NH_3^+), 617 cm^{-1} ($NHSO_3Na$).

To increase the molar ratio of the substituents, we used the same enzymatic procedure as utilized for the analysis of 2-nitroalkyl starch ethers (see Chapter 3). After hydrolysis of compounds synthesized according to procedures **2-8** (Table 2) the smallest oligomer fraction is separated from glucose and higher oligomers by gel permeation chromatography. An example of an FT-IR spectrum of one of these oligomers is given in Figure 1. Characteristic in the FT-IR spectrum of the reduced products is the decrease or disappearance of the absorbance of the nitro functionality at *c.*1550 cm^{-1} and the appearance of an absorbance at 1515 cm^{-1}. Especially in the case of reduction according to procedure **3** ($NaNO_3$) a new absorbance appears at 617 cm^{-1}. The low-region absorbance of the reduced product is in close agreement with an absorbance of Na_2SO_4 (620 cm^{-1})[28] and in reasonable agreement with the sodium salt of sulfamic acid (NH_2SO_3Na, 595 cm^{-1}), and can be explained by the formation of a sulfamate ($RCH(CH_3)NHSO_3Na$).

Elemental analysis of reduced 2-nitropropyl starch (procedure **5**) confirms this hypothesis. Some incorporation of sulfur (0.21%) was found for reduced 2-nitropropyl starch (yield in sulfamate 14%*). It has been reported in the literature that reduction of nitro- and nitroso-compounds with $Na_2S_2O_4$ may indeed lead to formation of sulfamates if the reduction is performed under neutral or slightly alkaline conditions[29]. Their formation is explained by reaction of the hydroxylamine intermediate with bisulfite (HSO_3^- , Scheme 8). Bisulfite is formed in large quantities during the decomposition of $Na_2S_2O_4$ and in the reduction step as well.



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

Scheme 8. Formation of sulfamates from hydroxylamines.

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 higher field (see Figure 2), compared to the oligomers obtained from 2-nitropropyl starch (methyl δ 1.3-2.2 ppm, methylene δ 2.4-3.1 ppm, see Figure 12, Chapter 3).

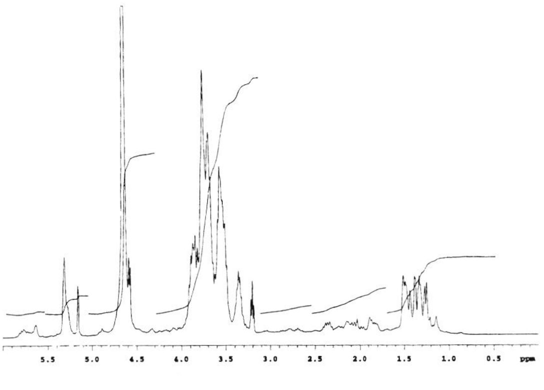


Figure 2. ^{1}H-NMR of the smallest oligomer of reduced 2-nitropropyl starch ($Na_2S_2O_4$/$NaBH_4$ (**5**)).

No proper ^{13}C-NMR spectra were obtained due to the complexity of the system. In order to obtain more information about the product formation, we reduced ^{13}C-labeled 2-nitropropyl-2-^{13}C starch (described in Chapter 3). We compared the ^{13}C-NMR spectra of the reduced labeled compound with the reduction of some model systems in order to obtain more information about the selectivity of the process. For identification purposes, we calculated the ^{13}C-NMR of

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* Analysis: %S = 0.21%, %N = 14/32. 0.21 = 0.09; yield of sulfamate = 0.09/0.64 (= %N 2-nps). 100% = 14%.

possible products with an ADS NMR simulation program as well. The following compounds were used as model systems for the reduction: 1-methoxy-2-nitropropane, 1-methoxy-2-nitrobutane, 3-*O*-(2-nitropropyl)-α,β-D-glucopyranoside and 3-*O*-(2-nitrobutyl)-α,β-D-glucopyranoside, 2-nitropropyl-α-sodium polyglucuronate, and 2-nitrobutyl-α-sodium polyglucuronate.

Reduction of granular ^{13}C-labeled 2-nitropropyl-2-^{13}C starch (50% enriched) with $Na_2S_2O_4$/$NaBH_4$ leads to an almost complete (80-90%) conversion of the nitro functionality, as determined by FT-IR. The ^{13}C-NMR spectrum of the gelatinized product* is rather complex (see Figure 3).

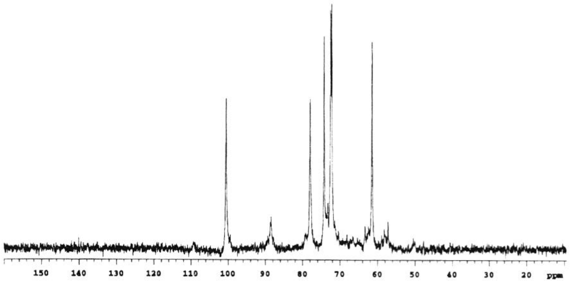

Figure 3. ^{13}C-NMR spectrum of gelatinized reduced 2-nitropropyl-2-^{13}C starch.

Resonances of the starch backbone are clearly present in the spectrum as well as some remaining resonances of the labeled methyne 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 ($Na_2S_2O_4$/$NaBH_4$, Figure 4) obtained after enzymatic hydrolysis and purification by gel permeation chromatography, shows new absorbances for the labeled methine carbon at δ150-144, 73 ppm and 62-70 ppm.

In order to obtain more information about the nature of the products formed, we analyzed the resonance for the methine group with possible reaction products of 1-methoxy-2-nitropropane with an ADS ^{13}C-NMR simulation programme (Scheme 9).

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* The NMR tube was heated to 80° C and subsequently cooled down to T_k

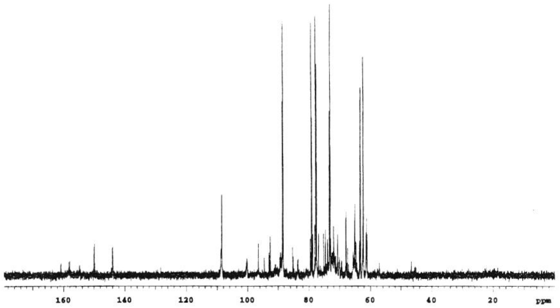
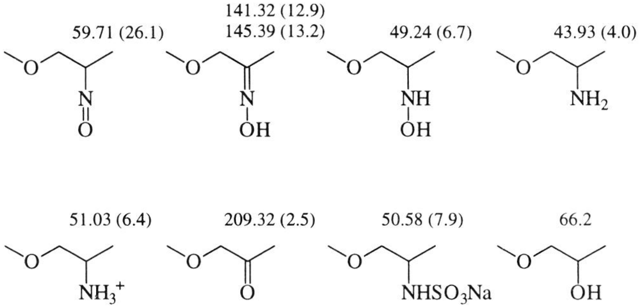


Figure 4. ^{13}C-NMR spectrum of the smallest oligomer of reduced 2-nitropropyl-2-^{13}C-starch.

According to this program, 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 compounds synthesized according to procedures **2-8** and **10,11** gave negative ninhydrine tests which excludes the formation of 2-aminopropyl starch.



Scheme 9. Calculated ^{13}C-NMR values of several reaction products of the methine group of reduced 1-methoxy-2-nitropropane (the calculated deviation is given between brackets).

As already mentioned we reduced some model systems with $Na_2S_2O_4$ as well, in order to get a better insight in the reduction proces. NMR tube reactions of 1-methoxy-2-nitropropane and 1-methoxy-2-nitrobutane with $Na_2S_2O_4$ proceed rather selectively (80-90%) and a major product was formed in which new resonances for the methine carbon appeared at δ 48.51 and 54.13 ppm. The chemical shifts are in reasonable agreement with the chemical shift for the methine carbon of 1-methoxy-2-aminoalkanes, the corresponding hydroxylamines, or the sulfamates (Scheme 8). After preparative reactions, the compounds could not be isolated from the water layer by extraction with organic solvents and therefore, we could not determine the exact nature of the compounds. However, because of the very good water solubility of the product formed it seems likely that sulfamates are formed under these reaction conditions (Na_2CO_3 was used to maintain a constant pH).

NMR tube reactions of 3-O-(2-nitropropyl)-α,β-D-glucopyranose and 3-O-(2-nitrobutyl)-α,β-D-glucopyranose with $Na_2S_2O_4$ lead, according to the ^{13}C-NMR spectra, to a mixture of products. It is likely that the reduction of the anomeric carbon atom of the glucopyranose interferes with the reduction of the nitro functionality.

The reduction of 2-nitroalkyl sodium α-polyglucuronates leads to a small decrease in the nitrogen content. In the ^{13}C-NMR spectra, an almost complete conversion of the nitro functionality is observed and a product was formed which showed new resonances at δ 49 (2-nitropropyl) and δ 54 ppm (2-nitrobutyl). These values are in agreement with resonances observed for the NMR tube reductions of 1-methoxy-2-nitropropane and 1-methoxy-2-nitrobutane. Negative ninhydrin tests exclude the formation of amines. In the FT-IR spectra the absorption of the nitro functionality is decreased and a new absorption is formed at 622 cm^{-1}. This low-region absorbance is in agreement with the formation of sulfamates. From the relatively low sulfur content compared to nitrogen of reduced 2-nitropropyl-sodium-α-polyglucuronate (%S = 0.84%, %N = 1.58%, yield in sulfamate = 21%) it can be concluded that the sulfamate is not the only product formed.

4.3 Conclusions

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 part of the nitro functionality is converted to an amine. As a result of the increasing pH during hydrogenation (amines are formed), reverse Michael additions lower the nitrogen content.

The model system 2-nitropropyl starch (granular and water soluble) can be reduced with $Na_2S_2O_4$ or $Na_2S_2O_4/NaBH_4$ to new starch ethers. However, a complex mixture of products is formed. Due to the low accessibility of the nitro functionality and/or (some) stabilization of intermediates, the decomposition of $Na_2S_2O_4$ hampers the 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 in the polysaccharides makes it very likely that the reduction of aliphatic nitroalkanes with $Na_2S_2O_4$ under 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.

4.4 Experimental

General methods. - See Chapter 3.7 for general remarks. Raney Nickel, $NaBH_4$, Na_2CO_3, and ninhydrin were purchased from Merck. D_2O was purchased from Aldrich. The syntheses of 2-nitropropyl acetate, 2-nitropropyl starch (with 2-nitropropyl acetate), 2-nitropropyl α-polyglucuronate, and 2-nitrobutyl α-polyglucuronate are described in Chapter 2. The syntheses of 1-methoxy-2-nitropropane, 1-methoxy-2-nitrobutane, 3-O)-(2-nitropropyl)-α,β-D-glucopyranoside and 3-O-(2-nitrobuty)-α,β-D-glucopyranoside, 2-nitropropyl-2-^{13}C starch and a method for the enzymatic hydrolysis and isolation of the smallest oligomer fraction of modified starches are described in Chapter 3.

Catalytic hydrogenation of water soluble 2-nitropropyl starch. - 2-Nitropropyl starch (10.01 g, %N = 1.50%, dry substance = 89.10%) was dissolved in H_2O (200 ml). Raney Ni (about 0.50 g) was added and the hydrogenation was carried out in a Parr apparatus under H_2 atmosphere at 80 bar and 80°C. After 20 h, the pressure was released and after the catalyst formed a deposit, 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%).

Catalytic hydrogenation of water soluble 2-nitrobutyl starch. - 2-Nitrobutyl starch (15.21 g, %N = 1.66%, dry substance = 91.11%) was dissolved in H_2O (200 ml). Raney Ni (about 0.50 g) was added and the hydrogenation was carried out in a Parr apparatus under H_2 atmosphere at 80 bar and 80°C. After 20 h, the pressure was released and after the catalyst formed a deposit, the solution was decanted and acidified to pH = 3. After precipitation in MeOH (700 ml), followed by filtration, the residue was dried at T =4 0° C. Yield 10.09 g reduced 2-nitrobutyl starch (%N = 0.84%, dry substance = 90.38%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (T_k, 2). - $Na_2S_2O_4$ (42.10 g, 200 mmole) was added in portions to a suspension of 2-nitropropyl starch (100.10 g, ms = 0.077 (%N = 0.64%), dry substance = 84.50%) in 150 ml H_2O. 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. Yield 98.30 g reduced 2-nitropropyl starch (%N = 0.57%, dry substance = 86.36%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (T_k, 2M $NaNO_3$, 3). - $Na_2S_2O_4$ (19.72 g, 93.9 mmole) was added in portions to a suspension of 2-nitropropyl starch (49.96 g, ms = 0.077 (%N = 0.64%), dry substance = 84.50%) in 50 ml 2 M $NaNO_3$. The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 6 h the suspension was filtered, washed thoroughly with water and dried in the air. Yield 44.59 g reduced 2-nitropropyl starch (%N = 0.55%, dry substance = 87.25%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (T = 35-40°C, 4). - $Na_2S_2O_4$ (42.02 g, 200 mmole) was added in portions to a suspension of 2-nitropropyl starch (100.04 g, ms = 0.077 (%N = 0.64%), dry substance = 84.50%) in 150 ml H_2O at T = 35-40°C. 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. Yield 95.43 g reduced 2-nitropropyl starch (%N = 0.55%, dry substance = 85.82%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$/$NaBH_4$ (T_k, 5). - A mixture of $Na_2S_2O_4$ (20.75 g, 98.8 mmole) and $NaBH_4$ (1.50 g, 40.0 mmole) was added in portions to a suspension of 2-nitropropyl starch (100.10 g, ms = 0.077 (%N = 0.64%), dry substance = 84.50%) in 100 ml H_2O. The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the suspension was acidified to pH =3, stirred for 3 h, filtered, washed thoroughly with water and dried in the air. Yield 97.54 g reduced 2-nitropropyl starch (%N = 0.49%, dry substance = 86.71%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (one step synthesis, T_k, 6). - Native starch (50.91 g, dry substance 86.90%, 273 mmole) was suspended in 50 ml H_2O which contained 2.53 g Na_2SO_4 (swelling inhibitor).The pH of the starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropyl acetate (3.30 g, 22.4 mmole) 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. $Na_2S_2O_4$ (23.01 g, 110 mmole) was added in portions to the suspension and the pH was regulated with a pH-stat (pH = 7) by adding portions of 1M NaOH. After 16 h the suspension was filtered, washed thoroughly with water and dried in the air. Yield 46.13 g reduced 2-nitropropyl starch (%N = 0.49%, dry substance = 87.05%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (one step synthesis, T = 35-40°C, 7). - Native starch (30.03 g, dry substance 86.90%, 161 mmole) was suspended in 30 ml H_2O which contained 1.51 g Na_2SO_4 (swelling inhibitor).The pH of the starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropyl acetate (2.01 g, 13.7 mmole) 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. $Na_2S_2O_4$ (14.28 g, 68.0 mmole) was added in portions to the suspension and the pH was regulated with a pH-stat (pH = 7) by adding portions of 1M NaOH. After 16 h the suspension was filtered, washed thoroughly with water and dried in the air. Yield 28.51 g reduced 2-nitropropyl starch (%N = 0.43%, dry substance = 86.92%).

Reduction of 2-nitropropyl starch with $Na_2S_2O_4$ (one step synthesis, T_k, no swelling inhibitor, 8). - Native starch (50.45 g, dry substance 86.90%, 271 mmole) was suspended in 50 ml H_2O. The pH of the starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropyl acetate (3.30 g, 22.4 mmole) was added dropwise to the suspension. The pH was regulated with a pH-stat by adding portions of 1 M NaOH. After 1.5 h the reaction was complete (no more acid formation) and the pH was adjusted to 7.0 with a 4 M HCl solution. $Na_2S_2O_4$ (23.05 g, 110 mmole) was added in portions to the suspension and the pH was regulated with a pH-stat (pH = 7) by adding portions of 1M NaOH. After 16 h the suspension was filtered, washed thoroughly with water and dried in the air. Yield 48.80 g reduced 2-nitropropyl starch (%N = 0.49%, dry substance = 86.73%).

Reduction of water soluble 2-nitropropyl starch with $Na_2S_2O_4$ (10). - $Na_2S_2O_4$ (23.00 g, 109 mmole) was added in portions to a dispersion of drumdried 2-nitropropyl starch (50.12 g, dry substance = 94.82%, 293 mmole, %N = 0.64%) was suspended in H_2O (2000 ml). The pH was regulated with a pH-stat (pH =7) by adding portions of 1 M NaOH. After 72 h the suspension was dialyzed and freeze-dried. Yield 50.15 g reduced 2-nitropropyl starch (%N = 0.36%, dry substance = 95.11%).

Reduction of water soluble 2-nitropropyl starch with $Na_2S_2O_4$/$NaBH_4$ (11). - $Na_2S_2O_4$ (20.75 g, 99 mmole) and $NaBH_4$ (1.50 g, 40 mmole) were added in portions to a dispersion of drumdried 2-nitropropyl starch (50.05 g, dry substance = 94.82%, 292 mmole, %N = 0.64%) in H_2O (2000 ml). The pH was regulated with a pH-stat (pH =7) by adding portions of 1 M NaOH. After 72 h the suspension was dialyzed and the suspension was concentrated to 750 ml and precipitated in 2 L MeOH. After filtration the residue was washed with acetone and dried in an ventilation stove. Yield 45.01 g reduced 2-nitropropyl starch (%N = 0.48%, dry substance = 92.17%).

Reduction of 2-nitropropyl starch 2-^{13}C with $Na_2S_2O_4$/$NaBH_4$ ($T_{k,}$ 50% enriched). - A mixture of $Na_2S_2O_4$ (1.00 g, 4.76 mmole) and $NaBH_4$ (0.08g, 2.13 mmole) was added in portions to a suspension of 2-nitropropyl starch 2-^{13}C (2.28 g, ms = 0.09[*]) in 6 ml H_2O. The pH was regulated with a pH-stat (pH =7) by adding portions of 1 M NaOH. After 16 h $NaBH_4$ (0.01 g, 2.64 mmole) was added and after 2 h the suspension was acidified to pH =3, stirred for 3 h, filtered, washed thoroughly with water, and dried in the air. Yield 1.75 g reduced 2-nitropropyl starch.

Reduction of 1-methoxy-2-nitropropane with $Na_2S_2O_4$ (NMR tube reaction). - 1-Methoxy-2-nitropropane (0.075 g, 0.62 mmole), Na_2CO_3 (0.16 g, 1.51 mmole) and $Na_2S_2O_4$ (0.50 g, 2.38 mmole) were dissolved in 2 ml D_2O and the reaction is followed with NMR. NMR (after 24 h, 200 MHz, D_2O, in ppm): (major product) ^{1}H δ 1.03 (3H, d, **CH_3**), 3.30 (m, 5H, OCH_3 + OCH_2). ^{13}C δ. 17.08 (**CH_3**), 46.41 (CH), 58.14 (OCH_3), 75.45 (OCH_2), (minor product) ^{1}H δ 1.14 (3H, d, CH_3), 3.30 (m, 5H, OCH_3 + OCH_2). ^{13}C δ 14.22 (CH_3), 46.41 (CH), 58.14 (OCH_3), 72.69 (OCH_2).

Reduction of 1-methoxy-2-nitrobutane with $Na_2S_2O_4$ (NMR tube reaction). - 1-Methoxy-2-nitropropane (0.082 g, 0.62 mmole), Na_2CO_3 (0.16 g, 1.51 mmole) and $Na_2S_2O_4$ (0.50 g, 2.38 mmole) were dissolved in 2 ml D_2O and the reaction is followed with NMR. NMR (after 24 h, 200 MHz, D_2O, in ppm): (major product) ^{1}H δ 0.77 (t, 3H, **CH_3**), 1.42 (m, 2H, **CH_2**), 3.30 (m, 5H, **OCH_3** + **OCH_2**). ^{13}C δ. 9.29 (**CH_3**), 24.16 (**CH_2**), 54.13 (OCH), 58.25 (OCH_3) 73.39 (OCH_2), (minor product) ^{1}H δ 0.84 (d, 3H, CH_3), 1.42 (m, 2H, CH_2), 3.30 (m, 5H, OCH_3 + OCH_2). ^{13}C δ 8.99 (CH_3), 21.90 (CH_2), 51.85 (CH), 58.56 (OCH_3), 70.89 (OCH_2).

Reduction of 2-nitropropyl sodium-α-polyglucuronate with $Na_2S_2O_4$. - $Na_2S_2O_4$ (1.48 g, 7.05 mmole) was added in portions to a solution of 2-nitropropyl sodium-α-polyglucuronate (1.96 g, 9.90 mmole, %N = 1.71%) in H_2O (20 ml). The pH was regulated with a pH-stat (pH =7) by adding portions of 1 M NaOH. After 4 h the solution was acidified with a 0.5 M HCl solution and precipitated in MeOH (300ml). After centrifugation, washing with MeOH/H_2O 2:1 (300 ml), and centrifugation, the product was dried in the air. Yield 1.48 g reduced 2-nitropropyl sodium-α-polyglucuronate (%N = 1.58%, %S = 0.84%). FT-IR (KBr, in cm^{-1}): 3413 (s), 2936 (m), 1731 (m), 1615 (s), 1418 (m), 1362 (m), 1146 (s), 1036 (s), 766 (w), 718 (w), 622 (m).

Reduction of 2-nitrobutyl sodium-α-polyglucuronate with $Na_2S_2O_4$. - $Na_2S_2O_4$ (1.53 g, 7.28 mmole) was added in portions to a solution of 2-nitrobutyl sodium-α-polyglucuronate (1.96 g, 9.90 mmole, %N = 1.32%) in H_2O (20 ml). The pH was regulated with a pH-stat (pH =7) by adding portions of 1 M NaOH. After 16 h the solution was acidified with a 0.5 M HCl solution and precipitated in MeOH (300ml). After centrifugation, washing with MeOH/H_2O 2:1 (300 ml), and centrifugation, the product was dried on air. Yield 1.87 g reduced 2-nitrobutyl sodium-α-polyglucuronate (%N = 1.10%). FT-IR (KBr, in cm^{-1}): 3425 (s), 2937 (m), 1731 (m), 1615 (s), 1419 (m), 1363 (m), 1144 (s), 1035 (s), 719 (w), 622 (m).

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[*] Determined with FT-IR

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Chapter 5

Cross-linking of starch with bifunctional precursors of nitroalkenes

5.1 Introduction

The development of derivatization methods for polysaccharides with various reagents has included reactions with bifunctional compounds that lead to cross-linked polymers. Thus larger molecular aggregates with different rheological behavior, or insoluble products, with a wide range of swelling characteristics may be synthesized. The insoluble cross-linked polysaccharides have found several applications, *e.g.*, in chromatography (gel filtration)[1]. Well known examples of such compounds are cross-linked dextrans (Sephadex) and agaroses (Sepharose)[2].

In the starch industry, the cross-linking of starch granules with bi- or polyfunctional reagents has led to a wide variety of commercial products in the food, textile, and adhesives industries[3]. Not only are the cross-linked starches interesting compounds themselves, but they can also be applied in combination with other chemical modifications such as acetylation, hydroxypropylation, or carboxymethylation. In this way, products with widely different properties and applications have been synthesized.

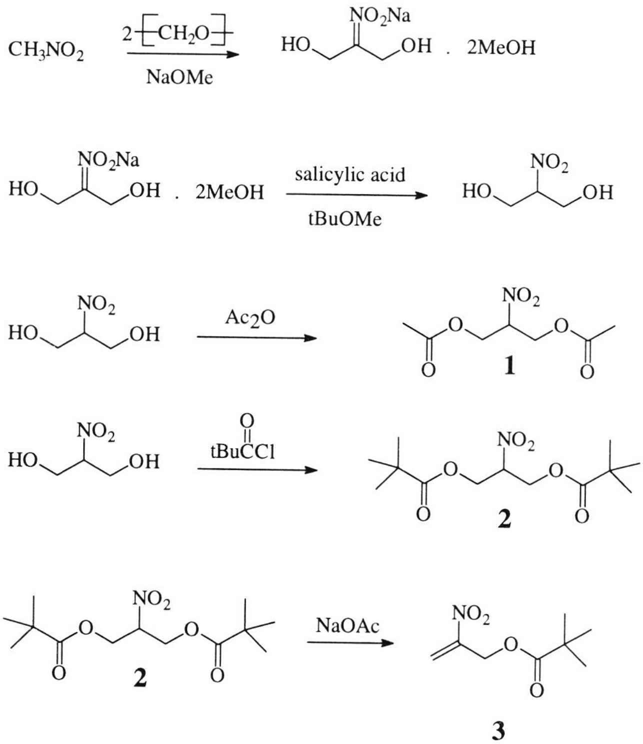
Although a wide variety of cross-linking agents for starch and other polysaccharides have been described in the literature, only a few are of commercial importance. Examples are adipic acid anhydrides[4], phosphorus oxychloride[5], divinylsulfone[6], sodium trimetaphosphate[7], and epichlorohydrin[8]. The cross-linking of starch with these compounds can easily be achieved in aqueous starch suspensions under alkaline reaction conditions. The cross-linked starch adipates and phosphates are used on a large scale in the food industry. A disadvantage of the distarch adipates and phosphates is the instability of the ester moieties under alkaline conditions.

Cross-linking of starch with epichlorohydrin leads to distarch glycerol. Due to the toxicity of epichlorohydrin, these cross-linked starches cannot be applied in the food sector. Distarch glycerol is highly resistant towards shear and owing to the ether linkages the epichlorohydrin cross-linked starch is stable under acidic and alkaline conditions. A slight disadvantage of the reaction is the relatively long reaction time required to achieve satisfactory degrees of conversion.

This chapter describes the efficient cross-linking of granular starch with readily preparable bifunctional precursors of nitroalkenes. Similar to distarch glycerol, these compounds are stable under alkaline and moderately acidic conditions owing to the ether linkages. Due to the excellent Michael acceptor capacities of nitroalkenes, the rate of the cross-linking reaction is high. The synthesis and reduction of cross-linked 2-nitroalkyl starches is also described.

5.2 *Results and discussion*

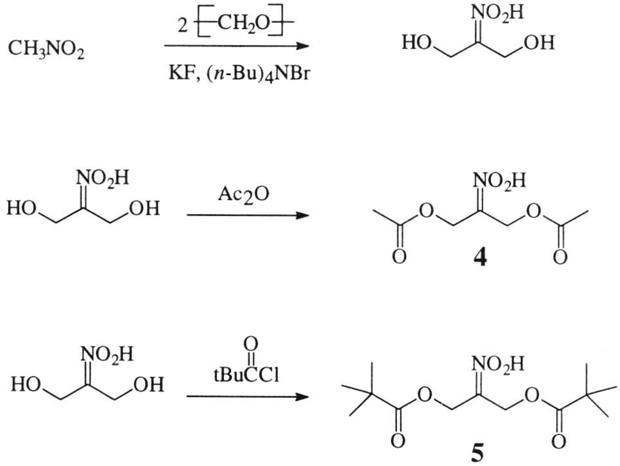
5.2.1 Syntheses of the cross-linking agents.



Scheme 1. Synthesis of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**) and 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**).

The synthesis of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**) and 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**) has been described by Seebach and Knochel[9]. The syntheses of **1** and **2** (Scheme 1) are straightforward, the yields are high and the procedure appears to be applicable to large scale reactions. The synthesis of **3** is achieved by adding a weak base (NaOAc) to **2**. Under these weakly alkaline conditions, one molecule of pivaloic acid splits off, and a nitroalkene is formed. However, the yield of this selective multicoupling reagent is disappointing, the overall yield from formaldehyde, nitromethane, and pivaloyl chloride being only about 25%.

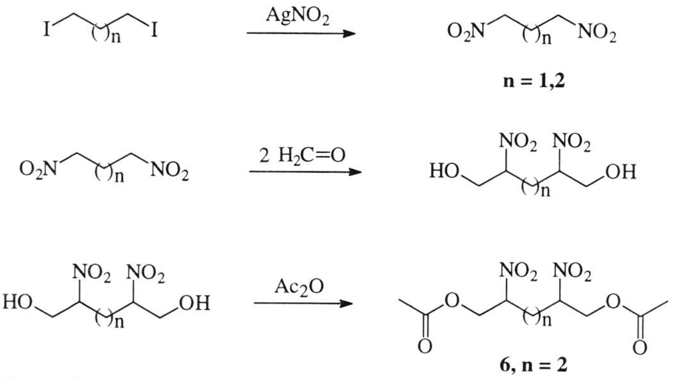
Also described in the literature is the synthesis of an intramolecularly stabilized nitronic acid, *aci*-2-nitro-1,3-propanediol[10]. High yields (73%) of this compound are obtained by reaction of nitromethane with paraformaldehyde in the presence of catalytic amounts of potassium fluoride and tetra-*n*-butyl ammonium bromide. Surprisingly, the *aci*-nitrocompounds 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol (**4**) and 1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol (**5**) are isolated in moderate to good yields after acetylation with acetic acid anhydride or reaction with pivaloyl chloride (see Scheme 2). Attempts to synthesize 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**) from **5** were not successful.



Scheme 2. Synthesis of 1,3-diacetyl-*aci*-2-nitropropane (**4**) and 1,3-dipivaloxy-*aci*-2-nitropropane (**5**).

The synthesis of α,ω-bis-(β-nitroacetoxy)alkanes, in which the β-nitroacetoxy functionalities are separated by a short hydrophobic chain, is complicated. To our knowledge no satisfactory route for the synthesis of α,ω-di-nitroalkanes has been described in the literature. Low to moderate yields are reported for the reaction of α,ω-di-iodoalkanes with silver nitrite[11]. Further reaction of the α,ω-dinitroalkanes with formaldehyde (Henry reaction) followed by acetylation proceeds in good yields for the synthesis of 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**, n = 2, see Scheme 3)[12].

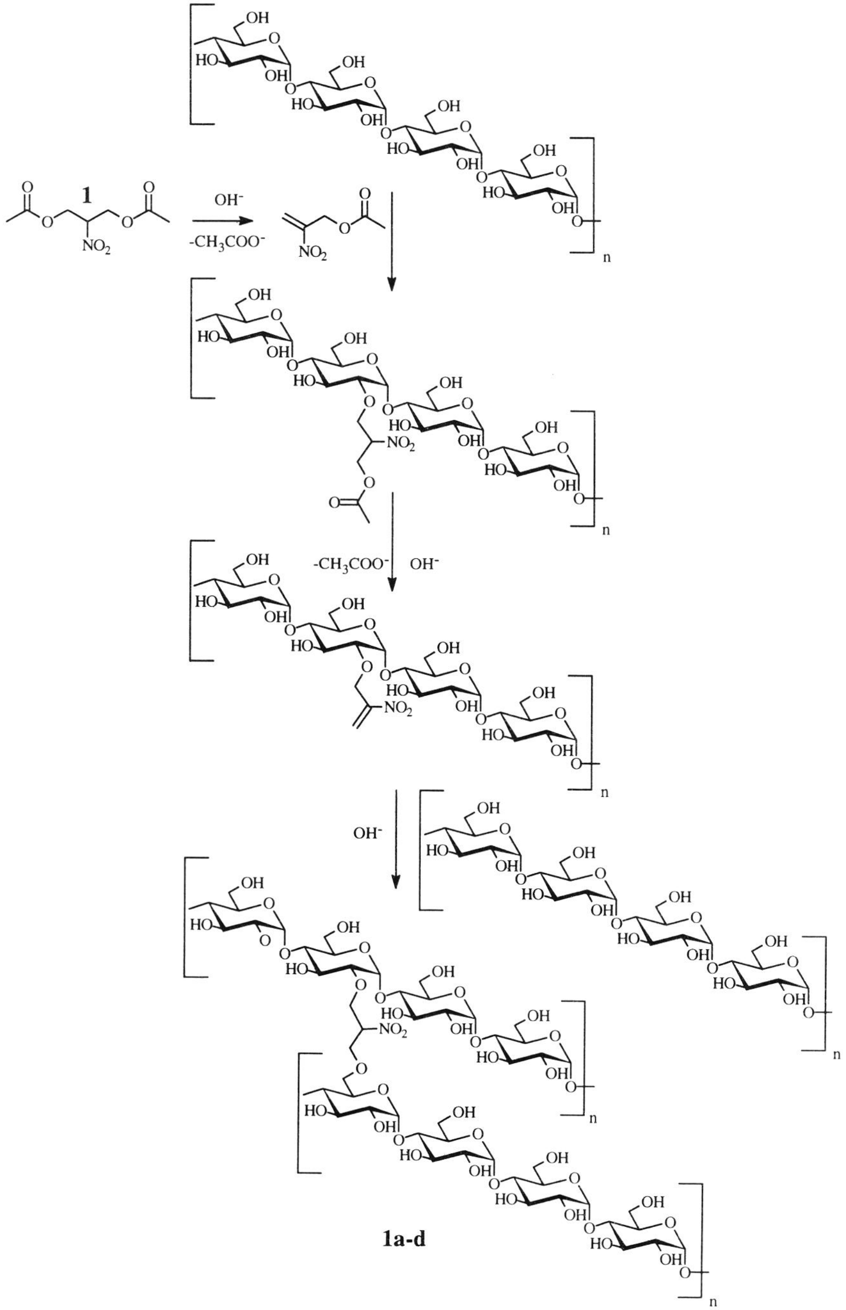
An attempt to synthesize 1,5-di-*O*-acetyl-2,4-dinitro-1,5-pentanediol (variation in the carbon chain) was not successful. The intermediate product (1,3-dinitropropane (n = 1, Scheme 3)) is not stable under the reaction conditions required for the Henry reaction with formaldehyde. Due to the alkaline conditions a reverse aldol condensation probably takes place, in which nitromethane and nitroethene are formed. Nitroethene is known to be very sensitive to anionic polymerization[13].



Scheme 3. Synthesis of α,ω-bis- (β-nitroacetoxy)alkanes.

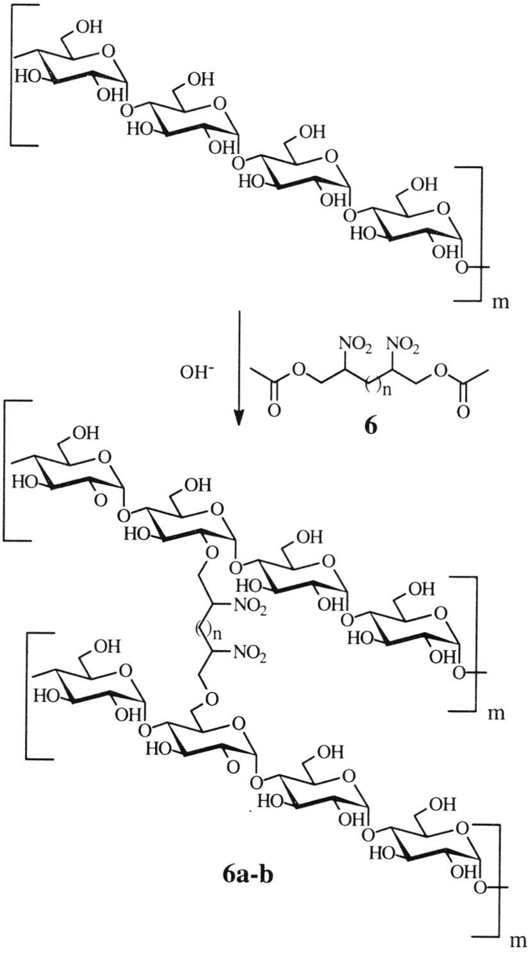
5.2.2 *Cross-linking of granular starch*

The anticipated pathway for cross-linking of granular starch suspensions with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**) is shown in Scheme 4. Under alkaline conditions acetic acid splits off and 2-nitro-3-*O*-acetyl-1-propene-3-ol is formed. It is expected that the *aci*-compound **4** reacts according to a similar pathway. If the pivaloyl derivatives **2** and **5** are used, pivalic acid splits off, which results in the formation of 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**). After a Michael addition of the corresponding nitroalkene with starch, another molecule of acetic acid or pivalic acid is split off and a 2-nitro-3-propene starch ether is formed. A second Michael addition with starch completes the cross-link. It should be noted that the cross-link can be intramolecular within the glucose monomer, intramolecular within the same starch chain and intermolecular between different starch chains. The rheology of gelatinized cross-linked starches is predominantly influenced by intermolecular cross-links.



Scheme 4. Example of the cross-linking reaction; reaction sequence of 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol with starch (reaction may take place with any of the free hydroxyl groups).

The cross-linking of starch with *α,ω*-bis-(β-nitroacetoxy)alkanes (Scheme 5) is comparable to the synthesis of 2-nitroalkylstarch ethers. Formation of a (di)nitroalkene and Michael additions to both sides of the molecule results in a cross-link within the starch granule. In this way, a (short) hydrophobic chain is introduced into the starch matrix.



Scheme 5. Synthesis of distarch *α,ω*-bis (β-nitro)alkanediol (2,5-dinitro-1,6-hexanediol (**6**, n=2).

5.2.3 *Viscosity profiles of cross-linked starches*

Information about the efficiency of the cross-linking reactions can be obtained from viscosity profiles. When suspensions of starch granules are heated above a certain temperature, water penetrates into the granules and weakens the hydrogen bonds in starch segments. The viscosity of the suspension rises but when shear is imposed (by stirring) the integrity of the swollen starch granules is lost and the viscosity of the solution decreases. Amylose, followed by amylopectin, leaks out of the 'ghosts' and goes into solution. After cooling down, the viscosity of the starch paste increases again. This gelatinization behavior can be followed with, *e.g.*, a Rapid Visco Analyser (RVA), which measures the viscosity of the starch suspension while stirring the suspension with a revolving cup driven at constant speed[14]. The suspension passes through a certain temperature profile (1 min, T 30 °C; 5.4 min, T 30 °C → T 95 °C; 2.5 min, T 95 °C; 3.7 min, T 95 °C → T 50 °C; 2.1 min, T 50 °C).

The gelatinization behavior of cross-linked starches depends on the extent of cross-linking[15]. If the number of cross-links is high enough (≥ one cross-link per 100 glucose monomers) and the starch granules are heated above their gelatinization temperature, the crystallinity is lost but the granule shape remains intact. No increase in viscosity is observed in the temperature profile. At low levels of cross-linking, the viscosity of the starch solution is altered. At approximately one cross-link per 500 glucose monomers, the maximum (peak viscosity) and the end viscosity (the viscosity at the end of the temperature profile) are lower than for gelatinized native starch (same concentrations). At approximately one cross-link per 1000-1500 glucose monomers, the maximum viscosity is lower in many cases, but the end viscosity is higher than for native gelatinized starch.

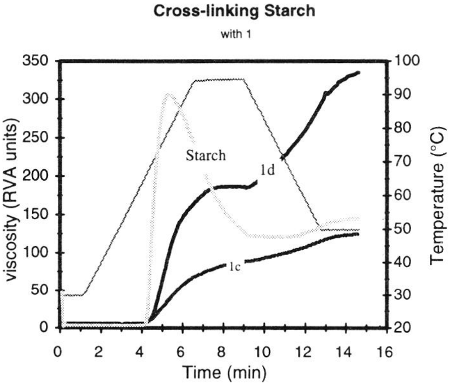


Figure 1. RVA curves of starch and distarch 2-nitropropanediol (5% suspension, **1c**, ds_{max} = 0.0019, **1d**, ds_{max} = 0.00098).

The same trend was observed for viscosity profiles (see Figure 1) of starch cross-linked with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol starch (**1c**, ds_{max} = 0.0019, **1d**, ds_{max} = 0.00098*).

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* ds_{max} = the maximum number of cross-links per mole of glucose monomer of the cross-linked starch.

With increasing ds, the peak viscosity disappears, resulting in a continuing increase in viscosity with prolonged cooking. With even larger numbers of cross-links ($ds_{max} = 0.019$) no increase in viscosity was observed during the temperature programme. The granule form remains intact but the crystallinity is lost (as confirmed by means of polarization microscopy).

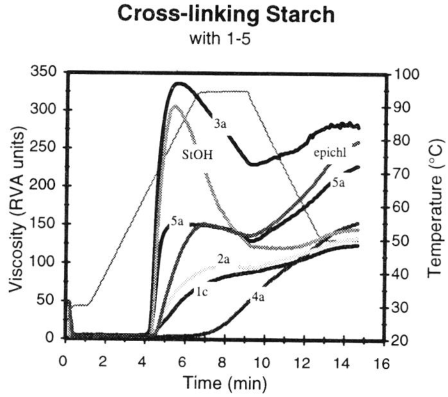


Figure 2. RVA curves of distarch 2-nitropropanediol (5% suspension, synthesized with compounds **1-5** (**1**c-**2**a, **4**a-**5**a, $ds_{max} = 0.0019$, **3**a $ds_{max} = 0.0016$), distarch glycerol (epichl., **7**b, $ds_{max} = 0.0022$) and native starch.

The RVA curves of the products of distarch 2-nitropropanediol ethers prepared with compounds **1-5** (**1**c, **2**a, **4**a, **5**a, $ds_{max} = 0.0019$, **3**a $ds_{max} = 0.0016$), distarch glycerol (epichlorohydrin **7**b, $ds_{max} = 0.0022$) and native potato starch (5% solution) are shown in Figure 2. One can readily conclude that cross-linking of granular starch with compounds **1** (1,3-di-*O*-acetyl-2-nitro-1,3-propanediol), **2** (1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol) and **4** (1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol) leads to a lower viscosity profile than cross-linking with epichlorohydrin (with the same degree of substitution). Cross-linking with compound **5** (1,3-di-*O*-pivaloyl-*aci*-2-nitro-1,3-propanediol) results in products with almost similar peak and end viscosities as epichlorohydrin (**7**b). Under the alkaline reaction conditions, anionic polymerization of the nitroalkene **3** (2-nitro-3-*O*-pivaloyl-1-propene-3-ol) probably leads to less efficient cross-linking for **3**a.

The viscosity profiles of granular starch cross-linked with the α,ω-bis-(β-nitroacetoxyalkane) 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol also fall in the range mentioned above. For relativily highly substituted distarch 2,5-dinitro-1,6-hexanediol (**6**a, $ds_{max} = 0.0065$) no increase in viscosity was observed during the temperature programme. Less substituted distarch 2,5-dinitro-1,6-hexanediol (**6**b, $ds_{max} = 0.0011$) gave a higher end viscosity than granular starch after gelatinization (Figure 3).

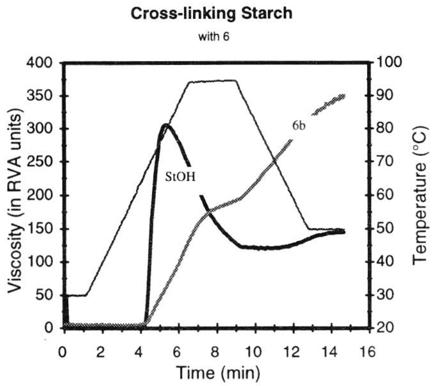


Figure 3. RVA curves of distarch 2,5-dinitro-1,6-hexanediol (**6**b, $ds_{max} = 0.0011$) and starch.

5.2.4 *Swelling properties of cross-linked starches*

Although gelatinized starches are often referred to as solutions, these systems in fact consist of particles[16]. After centrifugation a supernatant phase, which contains dissolved material, and a starch gel are obtained. The volume of the starch particles depends on the botanic source of the starch used[17]. As expected, the cross-linking of starch has a drastic influence on the volume of the centrifuged starch particles. Due to the cross-links, the aggregates are packed tighter and the volume of the gelatinized starch particles is reduced.

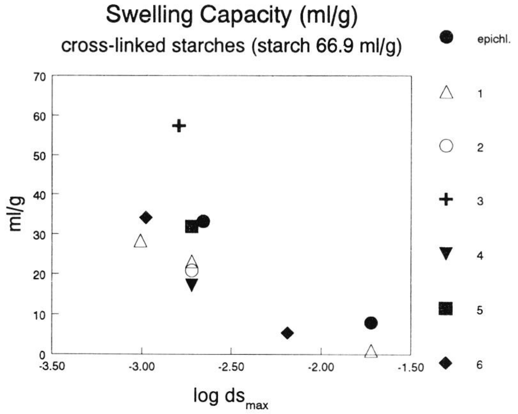


Figure 4. Particle volume (swelling capacity) of starch cross-linked with bifunctional precursors of nitroalkenes.

Comparing the particle volume of the centrifuged cross-linked starch at the same maximum degree of substitution (ds_{max}) gives information about the efficiency of the cross-linking with different cross-linking agents. However, it should be taken into account that topochemical aspects (distribution across the granule, amylose/amylopectine distribution) may also influence the swelling capacities of these aggregates. The particle volumes (swelling properties) of the cross-linked starches synthesized are shown in Figure 4.

As expected, within one series and using the same cross-linking agent, the swelling capacity of the cross-linked starches decreases at higher degrees of substitution. The swelling capacities of starch cross-linked with bifunctional precursors for nitroalkenes (**1**, **2** and **4-6**) are lower than those of starch cross-linked with commercially used epichlorohydrin. This means not only that the reaction rate of cross-linking is considerably higher than in the case of cross-linking with epichlorohydrin, but probably also that the efficiency of the cross-linking with (**1, 2** and **4-6**) is higher. The results obtained are fairly consistent with the RVA curves as shown in Figure 1 and 2. Low end viscosities and swelling capacities were obtained for **1c, 2**a, **4**a and **6**b, moderate end viscosity and swelling capacity was obtained for **5**a, and a high end viscosity and swelling capacity was obtained for **3a**. It can therefore be concluded that a novel series of easily synthesized crosslinkers for starch (and other polysaccharides), based on Michael additions to (di)-nitroalkenes, has been developed.

The cross-linking properties of most of the above-mentioned bifunctional precursors (especially **1**, **2** and **4-6**) are rather remarkable if we presume, comparable to the synthesis of 2-nitropropyl starch, that initially an amylose/cross-linking agent helix is formed. After formation of the nitroalkene, reaction, and subsequent formation of a nitroalkene, an intramoleculair reaction within the helix seems most likely to occur. This should in principle lead to a highly cross-linked amylose fraction with a rather small effect on the viscosity properties. However, the results obtained confirm that intermolecular reactions between amylose and the amorphous amylopectin fractions play an important role during the reaction.

5.2.5 Chemical characterization of cross-linked starches.

Chemical analysis of cross-linked starches is often complicated due to the low degrees of substitution[18]. However, the ratio between the real cross-linking degree and mono functionalization and topochemical aspects are of importance for the physical behavior of these compounds. For the cross-linking of starch with epichlorohydrin the amount of mono functionalization (the monoether glycerol bond) was established by oxidation of the modified starch with $NaIO_4$ and subsequent determination of formaldehyde[19]. Gel permeation chromatograms of gelatinized distarch glycerol (low degrees of crosslinking) are in agreement with cross-links between amylose and amylopectin in the amorphous regions of the starch granule[20].

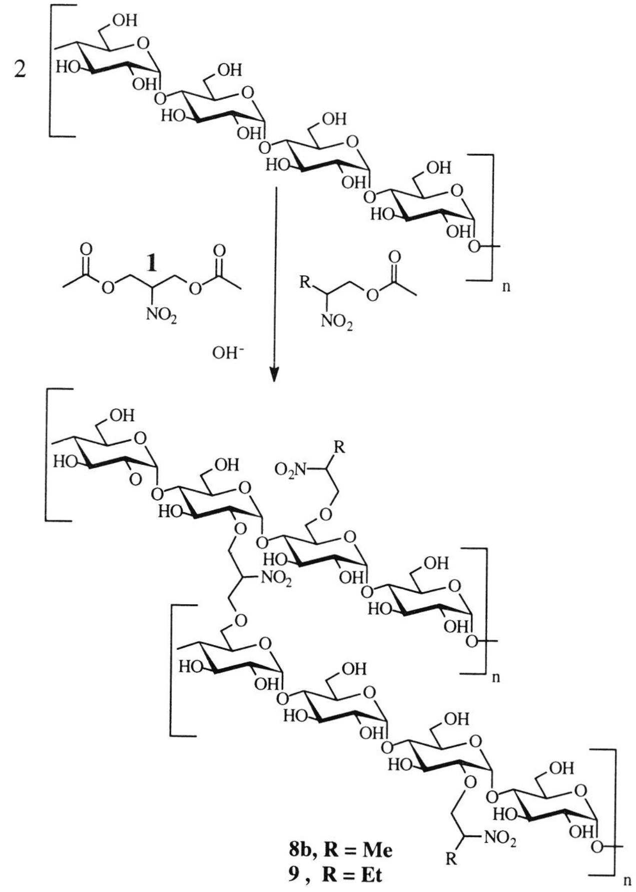
The efficiency of the reaction of starch with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1a**) can be determined from the incorporation of nitrogen and was found to be 85%. The IR absorbance of the nitro functionality (1552 cm^{-1}) is in agreement with values obtained for 2-nitroalkyl starch ethers. We also attempted to determine the amount of mono functionalization, side reactions, and the distribution over the different hydroxyl groups by cross-linking starch with the labeled compound 1,3-di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol-2-^{13}C. After reaction with starch, the labeled distarch 2-nitropropanediol-2-^{13}C (**4**b, ds_{max} = 0.020) was gelatinized and the swollen "granules" were treated with an enzyme cocktail of α-amylase (EC 3.2.1.1), amyloglucosidase (EC 3.2.1.3) and isoamylase (EC 3.2.1.68). Normally, this procedure yields a mixture of glucose and substituted oligomers which can subsequently be separated by GPC and analyzed with ^{13}C-NMR. Unfortunately, the cross-linked material was not sufficiently accessible to the enzymes for hydrolysis to occur.

5.3 Synthesis of 2-nitroalkyl distarch 2-nitropropanediol ethers.

Cross-linking of starch is often used in combination with other chemical modifications[21]. It has been demonstrated that aminoalkyl cross-linked starches have potential for application as ion exchange resins and these compounds are excellent materials for the complexation of heavy metals ions[22]. In principle, primary or secondary aminoalkyl cross-linked starches (or other polysaccharides) are interesting materials for the immobilization of enzymes[23]. Such compounds can be prepared readily in a two-step (or possibly a one-step) procedure: synthesis of 2-nitroalkyl distarch 2-nitropropanediol followed by reduction of the nitro functionalities.

2-Nitroalkyl distarch 2-nitropropanediol (See Scheme 6) was synthesized by nitroalkylation of distarch 2-nitropropanediol with 2-nitropropylacetate (**8**a). The cross-linking and nitroalkylation of granular starch can also be accomplished in a one-pot synthesis. In this way 2-nitropropyl distarch 2-nitropropanediol (**8**b, **10**) and 2-nitrobutyl distarch 2-nitropropanediol (**9**, **11**) were synthesized by addition of 1,3-di-*O*-acetyl-2-nitro-1,3-propaandiol/2-nitroalkylacetate (**8**b, **9**) or 1,3-di-*O*-acetyl-*aci*-2-nitropropane/2-nitroalkyl-acetate (**10**, **11**) to starch.

The efficiency of the nitroalkylation is moderate (for the 2-nitrobutyl ether) to high (for the 2-nitropropyl ether). The nitroalkyl functionality decreases the gelatinization temperature of the cross-linked nitroalkyl starch ethers (native starch T_{gel} = 59.3° C). Owing to the cross-links no viscosity increase is observed for the gelatinized cross-linked 2-nitroalkyl starch derivatives (see Table 1, 5% suspension).



8b, R = Me
9 , R = Et

Scheme 6. One-step synthesis of 2-nitroalkyl distarch 2-nitropropanediol ethers.

Table 1. Synthesis of 2-nitropropyl and 2-nitrobutyl distarch 2-nitropropanediol.

compound	ms (2nps)[a]	RE[b]	T_{gel}[c]	ds_{max}(crl)[d]	RVA[e]
8b	0.062	87%	48.4	0.0034	-[f]
9	0.046	65%	50.3	0.0036	-
10	0.057	71%	48.6	0.0033	-
11	0.043	61%	54.7	0.0032	-

a) See Experimental. b) Reaction efficiency (ms/ms_{max}). c) Gelatinization temperature (in °C), determined using DSC (onset). d) Maximum degree of cross-linking. e) In RVA units. f) No increase in viscosity.

5.4 Reduction of 2-nitroalkyl distarch 2-nitropropanediol ethers ($Na_2S_2O_4$)

Natural polysaccharides (agarose, cellulose, dextran, and starch) are commonly employed as water-insoluble supports for the covalent attachment of enzymes. Immobilized enzymes are reusable and in many cases more stable than "free" enzymes. Well-known methods for immobilization of enzymes on natural supports are the coupling to carboxymethyl cellulose azide, aminoaryl celluloses, *s*-triazinyl celluloses, cyanobromide-activated agarose and dextran, and dialdehyde starch^{20}. Except for the cyanogen bromide method, all these methods involve multistep syntheses.

An alternative for these methods is the reduction of the nitroalkyl functionality of, for example, crosslinked 2-nitroalkyl starches. These compounds also have perspectives for the purification of water polluted with heavy metals. Unfortunately, we were not able to achieve complete conversion of the nitrofunctionality to the corresponding amine (see Chapter 4). After reaction of 2-nitroalkyl distarch 2-nitropropanediol (**8**a, %N = 2.65%) with $Na_2S_2O_4$, the nitrogen content decreased somewhat (**12**, %N = 2.11%). From FT-IR spectra it could be determined that 30% of the nitroalkyl ether remained in its initial form. As described in Chapter 4, the nitrofunctionality is converted to *e.g.* amine, hydroxylamine, oxime, and/or sulfamates.

A one-step procedure to reduced 2-nitroalkyl distarch 2-nitropropanediol (cross-linking and nitroalkylation followed by reduction with $Na_2S_2O_4$) leads to a cross-linked, nitrogen-containing, ether (**13**, %N = 0.32, $\%N_{max} = 0.59\%$) which contains, according to FT-IR, only minor amounts of the initial nitro functionality. The efficiency of the reduction of these cross-linked 2-nitroalkyl starches seems to decrease with larger number of cross-links (**12** $ds_{max} = 0.096$, **13** $ds_{max} = 0.035$).

Although these procedures seem to have perspective for the synthesis of cross-linked 2-aminoalkyl starches, further research is needed for the optimization of the reduction of the nitro functionality.

5.5 Conclusions

Granular starch is cross-linked with 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (**1**), 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**), 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**), 1,3-di-*O*-acetyl-*aci*-2-nitropropane (**4**), 1,3-di-O-pivaloyl-*aci*-2-nitropropane (**5**), and 1,6-di-*O*-acetyl-2,5-dinitrohexane (**6**). The bifunctional precursors for nitro-alkenes **1**, **2** and **3**, **4** are readily synthesized in high yields from nitromethane, paraformaldehyde and acetic acid anhydride (**1**, **3**) or pivaloyl chloride (**2, 4**), respectively. The reaction rates for the cross-linking are high and, for **1** and **3**, the reaction reaches completion within 1 h (at room temperature). The swelling capacities of the products obtained when starch is cross-linked with precursors for nitroalkenes **1-4** and **6** are lower compared to those of epichlorohydrin cross-linked starch. These results indicate a high reaction efficiency at low degrees of substitution. Cross-linked 2-nitroalkyl starch ethers are synthesized in a one-pot synthesis by addition of **1** or **3** and 2-nitroalkylacetates to granular suspensions of starch. Reduction of these compounds with $Na_2S_2O_4$ leads to a new unidentified crosslinked starch ether.

5.6 Experimental

General methods. - 1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (**1**)[9], 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (**2**)[9], 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (**3**)[9], 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (**6**)[12], *aci*-2-nitro-1,3-propanediol[10], *aci*-2-nitro-1,3-propanediol-2-^{13}C[10], 2-nitropropyl acetate[24] and 2-nitrobutyl acetate[24] were prepared according to literature procedures. Formaldehyde (37% solution), Ac_2O, 1-nitropropane (95%), NaOH pellets, KF and tetra-*n*-butylammonium bromide were purchased from Merck. Nitromethane (96%), nitromethane-^{13}C (99% labeled), nitroethane (96%), salicylic acid, $AgNO_2$, NaOMe (30% solution), epichlorohydrin, pivaloyl chloride, and di-iodobutane were purchased from Aldrich. Blue dextran was purchased from Pharmacia Biotech. Potato starch was a gift from AVEBE (Foxhol, The Netherlands). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz Varian VTR-300 spectrometer. FT-IR spectra were recorded on a Biorad FTS 135 spectrometer. Differential Scanning calorimetry was performed on a Perkin-Elmer DSC7 PC Series apparatus (heating rate 5.0 K min^{-1}).Viscosity profiles were recorded on a Rapid Visco Analyser RVA-3d purchased from Newport Scientific. Spectrophotometric measurements were performed with a LKB Novaspec II from Biochrom. The pH-stat, a Titration Controller 1200 equipped with a Titronic T110 burette was purchased from Schott Geräte. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Molar substitution of the 2-nitroalkyl substituent were determined according to the formula $ms = 1.62N/\{14\text{-}0.01N(Mwt_{alkyl\ subst}\text{-}1)\}$ (N = nitrogen content measured in %N and $Mwt_{alkyl\ subst}$ = molecular weight of the nitroalkyl substituent).

Synthesis of 1,3-di-O-acetyl-*aci*-2-nitro-1,3-propanediol (4)*. - A drop of conc H_2SO_4 was added to a stirred solution of *aci*-2-nitro-1,3-propanediol (2.63 g, 21.7 mmol) and acetic acid anhydride (4.45 g, 43.6 mmol). After 3 h CH_2Cl_2 (20 ml) was added and the mixture was poured into sat. $NaHCO_3$ (25 ml). After separation of the organic layer, the $NaHCO_3$ solution was washed with CH_2Cl_2 (20 ml) and the combined CH_2Cl_2 layers were dried over Na_2SO_4. After filtration and concentration, the residue was distilled under reduced pressure (0.1 mm Hg, 200 °C) and **4** was isolated (2.42 g, 55%). NMR ($CDCl_3$): ^{1}H, δ 4.54 (s, 4 H, CH_2O), 2.08 (s, 6 H, CH_3); ^{13}C, δ 169.51 (C=O), 88.01 (CNO_2), 60.49 (CH_2O), 20.43 (CH_3). FT-IR (KBr): 2968 (w), 2905 (w), 1825 (w), 1756 (s), 1559 (s), 1465 (m), 1438 (w), 1382 (s), 1369 (s), 1224 (s), 1126 (m), 1048 (s), 1016 (m), 959 (w), 900 (w), 840 (w), 737 (w), 666 (w) cm^{-1}.

Synthesis of 1,3-di-O-pivaloyl-*aci*-2-nitro-1,3-propanediol (5). - Pivaloyl chloride (10.01 g, 83 mmol) was added to a solution of *aci*-2-nitro-1,3-propanediol (3.25 g, 26.9 mmol) in CH_2Cl_2 (15 ml). After boiling under reflux for 20 h and concentration, the residue was dissolved in CH_2Cl_2 (15 ml) and washed with sat $NaHCO_3$ (3 x 10 ml) and H_2O (2 x 10 ml). The CH_2Cl_2 layer was dried ($Na_2SO_{4)}$ and, after filtration and concentation, crude **5** (6.02 g) was isolated. Part of the residue (3.35 g) was distilled under reduced pressure (0.02 mm Hg, 150 °C) and **5** (2.59 g, 60%) was isolated. NMR

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[*] The synthesis 1,3-di-*O*-acetyl-aci-2-nitro-1,3-propanediol-2-^{13}C is similar to the synthesis of **4**.

($CDCl_3$): ^1H, δ 4.50 (s, 4 H, CH_2O), δ 1.15 (s, 18 H, CH_3); ^{13}C, δ 177.8 (C=O), 88.4 (CNO_2), 60.38 (CH_2), 38.90 (C), 26.64 (CH_3). FT-IR (KBr): 2976 (s), 2939 (s),2912 (s), 2876 (s), 1743 (s), 1556 (s), 1481 (s), 1466 (s), 1399 (m), 1369 (m), 1352 (m), 1282 (s), 1135 (s), 1039 (m), 1014 (w), 999 (w), 985 (w), 944 (w), 922 (w), 865 (m), 845 (w), 813 (w), 798 (w), 784 (w), 767 (m), 665 (w) cm^{-1}.

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1a), ds_{max} = 0.096. - Native starch (100.01 g, 520 mmol) was suspended in H_2O (100 ml). The pH of the H_2O/starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (**1**, 10.25 g, 50 mmol) was added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 16 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at T = 40°C and distarch 2-nitropropanediol (**1a**, 95.54 g, dry substance = 88.44%, %N = 0.68%) was isolated. FT-IR (KBr): 3453 (s), 2928 (m), 1658 (m), 1552 (m),1164 (s), 988 (s),857 (m),763 (m), 573 (m) cm^{-1}.

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1b), ds_{max} = 0.019. - The same procedure was followed as for **1a** with starch (100.70 g, 520 mmol), H_2O (100 ml) and 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (2.05 g, 10.0 mmol). Isolated: 94.26 g distarch 2-nitropropanediol (**1b**, dry substance = 89.27%).

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1c), ds_{max} = 0.0019. - The same procedure was followed as for **1a** with starch (250.02 g, 1.30 mol), H_2O (250 ml) and 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (0.51 g, 2.49 mmol). Isolated: 222.20 g distarch 2-nitropropanediol (**1c**, dry substance = 91.65%).

Cross-linking of starch with 1,3-di-O-acetyl-2-nitro-1,3-propanediol (1d), ds_{max} = 0.00098. - The same procedure was followed as for **1a** with starch (250.88 g, 1.30 mol), H_2O (250 ml) and 1,3-di-*O*-acetyl-2-nitro-1,3-propanediol (0.26 g, 12.7 mmol). Isolated: 229.74 g distarch 2-nitropropanediol (**1d**, dry substance = 91.65%).

Cross-linking of starch with 1,3-di-O-pivaloyl-2-nitro-1,3-propanediol (2a), ds_{max} = 0.0019. - The same procedure was followed as for **1a** with starch (100.33 g, 520 mmol), H_2O (100 ml) and 1,3-di-*O*-pivaloyl-2-nitro-1,3-propanediol (0.29 g, 1.00 mmol). After stirring for 48 h distarch 2-nitropropanediol (**2a**, 91.08 g, dry substance = 90.94%) was isolated.

Cross-linking of starch with 2-nitro-3-O-pivaloyl-1-propene-3-ol (3a), ds_{max} = 0.0016. - The same procedure was followed as for **1a** with starch (100.04 g, 520 mmol), H_2O (100 ml) and 2-nitro-3-*O*-pivaloyl-1-propene-3-ol (0.16 g, 0.86 mmol). After stirring for 16 h distarch 2-nitropropanediol (**3a**, 90.28 g, dry substance = 91.11%) was isolated.

Cross-linking of starch with 1,3-di-O-acetyl-*aci*-2-nitro-1,3-propanediol (4a), ds_{max} = 0.0019. - The same procedure was followed as for **1a** with starch (100.02 g, 520 mmol), H_2O (100 ml) and 1,3-diacetyl-*aci*-2-nitro-1,3-propanediol (0.20 g, 1.0 mmol). After stirring for 16 h distarch 2-nitropropanediol (**4a**, 89.60 g , dry substance = 91.73%) was isolated.

Cross-linking of starch with 1,3-di-O-acetyl-*aci*-2-nitro-1,3-propanediol-2-^{13}C (4b), ds_{max} = 0.020. - The same procedure was followed as for **1a** with starch (10.01 g, 52.0 mmol), H_2O (12 ml) and 1,3-diacetyl-*aci*-2-nitro-1,3-propanediol-2-^{13}C (0.21 g, 1.02 mmol in 1 ml 2-propanol). After stirring for 16 h distarch 2-nitropropanediol-2-^{13}C (**4b**, 9.13 g) was isolated.

Cross-linking of starch with 1,3-di-O-pivaloyl-*aci*-2-nitro-1,3-propanediol (5a), ds$_{max}$ = 0.0019. - The same procedure was followed as for **1**a with starch (100.02 g, 520 mmol), H_2O (100 ml) and 1,3-di-*O*-pivaloyl-*aci*-2-nitropropane (0.29 g, 1.0 mmol). After stirring for 48 h distarch 2-nitropropanediol (**5**a, 90.16 g, dry substance = 91.16%) was isolated.

Cross-linking of starch with 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (6a), ds$_{max}$ = 0.0065. - The same procedure was followed as for **1**a with starch (10.0 g, 52 mmol), H_2O (10 ml) and 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (0.10 g, 0.34 mmol). After stirring for 20 h distarch 2,5-dinitrohexanediol (**6**a, 9.99 g, dry substance = 83.78%, air dried) was isolated.

Cross-linking of starch with 1,6-di-O-acetyl-2,5-dinitro-1,6-hexanediol (6b), ds$_{max}$ = 0.0011. - The same procedure was followed as for **1**a with starch (10.0 g, 52 mmol), H_2O (10 ml) and 1,6-di-*O*-acetyl-2,5-dinitro-1,6-hexanediol (0.016 g, 0.055 mmol). After stirring for 20 h distarch 2,5-dinitrohexanediol (**6**b, 9.98 g, dry substance = 81.19%, air dried) was isolated.

Cross-linking of starch with epichlorohydrin (7a), ds$_{max}$ = 0.019. - Native starch (250.02 g, 1.30 mol) was suspended in H_2O (250 ml). The pH of the H_2O/starch suspension was adjusted to 11.0 with 1 M NaOH and heated to T = 35 °C. Epichlorohydrin (2.28 g, 25 mmol) was added and after 48 h the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at T = 40 °C and 235.35 g distarch glycerol (**7**a, dry substance = 86.09%) was isolated.

Cross-linking of starch with epichlorohydrin (7b), ds$_{max}$ = 0.0022. - The same procedure was followed as for **7**a with starch (150.02 g, 0.78 mol), H_2O (150 ml) and epichlorohydrin (0.16 g, 1.73 mmol). After stirring for 48 h distarch glycerol (**7**b, 134.76 g, dry substance = 90.84%) was isolated.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (8a). - Cross-linked starch (**1**a, 25.00 g, about 145 mmol) was suspended in H_2O (50 ml). The pH of the H_2O/cross-linked starch suspension was adjusted to 10.0 with a 1 M NaOH solution. 2-Nitropropylacetate (10.27 g, 69.9 mmol) was added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 16 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried to air and 26.50 g 2-nitropropyl distarch 2-nitropropanediol (**8**a, %N = 2.65%, dry substance not determined due to decomposition of the material) was isolated. FT-IR (KBr): 3448 (s), 2930 (s), 1655 (m), 1554 (s), 1459 (m), 1364 (m), 1164 (s), 1083 (s), 988 (s), 933 (m), 850 (m), 763 (m), 709 (m), 573 (m), 523 (m) cm^{-1}.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-2-nitro-1,3-propanediol, one pot synthesis (8b)). - Native starch (49.96 g, 260 mmol) was suspended in a solution of H_2O (50 ml), which contained Na_2SO_4 (2.53 g, 17.8 mmol). The pH of the H_2O/starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (0.18 g, 0.88 mmol) and 2-nitropropylacetate (2.72 g, 18.5 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 1 hour the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at T = 40°C and 45.19 g 2-nitropropyl distarch 2-nitropropanediol (**8**b, dry substance = 92.65%, %N = 0.52%) was isolated. FT-IR (KBr): 3456 (s), 2928 (s), 1658 (m), 1550 (m), 1465 (m), 1164 (s), 1083 (m), 983 (s), 928 (m), 859 (m), 764 (m), 709 (m), 573 (m) cm^{-1}.

Synthesis of 2-nitrobutyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-2-nitro-1,3-propaandiol, one pot synthesis (9)). - Native starch (50.10 g, 260 mmol) was suspended in H_2O (50 ml), which contained Na_2SO_4 (2.52 g, 17.8 mmol). The pH of the H_2O/starch suspension was adjusted to 10.0

with 1 M NaOH. 1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (0.19 g, 0.93 mmol) and 2-nitrobutylacetate (3.00 g, 18.6 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 2 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at T = 40 °C and 45.39 g 2-nitrobutyl distarch 2-nitropropanediol (**9**, dry substance = 92.61%, %N = 0.39%) was isolated. FT-IR (KBr): 3451 (s), 2930 (s), 1654 (m), 1546 (m), 1464 (m), 1163 (s), 986 (s), 928 (m), 859 (m), 764 (m), 709 (m), 573 (m) cm^{-1}.

Synthesis of 2-nitropropyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-aci-2-nitro-1,3-propanediol, one pot synthesis (10)). - Native starch (100.01 g, 520 mmol) was suspended in H_2O (100 ml), which contained Na_2SO_4 (5.04 g, 35.6 mmol). The pH of the H_2O/starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-*O*-acetyl-*aci*-2-nitro-1,3-propanediol (0.35 g, 1.70 mmol) and 2-nitropropylacetate (5.45 g, 37.1 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 3 h the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After filtration and washing with water the product was dried at T = 40 °C and 93.54 g 2-nitrobutyl distarch 2-nitropropanediol (**10**, dry substance = 91.49%, %N = 0.48%) was isolated. FT-IR (KBr): 3434 (s), 2930 (s), 1654 (m), 1548 (m), 1464 (m), 1164 (s), 986 (s), 859 (m), 764 (m), 709 (m) cm^{-1}.

Synthesis of 2-nitrobutyl distarch 2-nitropropanediol (with 1,3-di-O-acetyl-aci-2-nitro-1,3-propanediol, one pot synthesis (11)). - Native starch (75.03 g, 389 mmol) was suspended in H_2O (75 ml), which contained Na_2SO_4 (3.77 g, 26.6 mmol). The pH of the H_2O/starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-*O*-acetyl-aci-2-nitro-1,3-propanediol (0.26 g, 1.26 mmol) and 2-nitrobutylacetate (4.42 g, 27.5 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 3 h the reaction was complete (no more acid formation) and the suspension was neutralized with a 0.5 M HCl solution. After filtration and washing with water the product was dried at T = 40 °C and 68.85 g 2-nitrobutyl distarch 2-nitropropanediol (**11**, dry substance = 91.08%, %N = 0.36%) was isolated. FT-IR (KBr): 3468 (s), 2929 (s), 1653 (m), 1545 (m), 1464 (m), 1163 (s), 985 (s), 928 (m), 859 (m), 764 (m), 708 (m), 573 (m) cm^{-1}.

Reduction of 2-nitropropyl distarch 2-nitropropanediol (12). - 2-Nitropropyl distarch 2-nitropropanediol (8a) was suspended in H_2O (50 ml). After heating to 75° (0.5 h) and cooling to T_k $Na_2S_2O_4$ (17.71 g, 102 mmol) was added in portions. The pH (= 7) was controled with a pH-stat by adding portions of a 5 M NaOH. After 72 h the reaction was complete (no more acid formation) and the suspension was filtered, washed with water, dried at T = 40°C and 9.65 g reduced 2-nitropropyl distarch 2-nitropropanediol (**12**, %N = 2,11%) was isolated. FT-IR (KBr): 3405 (s), 2931 (s), 1646 (m), 1546 (m), 1511 (m), 1420 (m), 1368 (m), 1155 (m), 1082 (m), 1025 (s), 933 (m), 851 (m), 761 (m), 708 (m) cm^{-1}

Synthesis and reduction of 2-nitropropyl distarch 2-nitropropanediol, one pot synthesis (13). - Native starch (49.98 g, 260 mmol) was suspended in a solution of H_2O (50 ml), which contained Na_2SO_4 (2.52 g, 17.8 mmol). The pH of the H_2O/starch suspension was adjusted to 10.0 with 1 M NaOH. 1,3-Di-*O*-acetyl-2-nitro-1,3-propanediol (0.19 g, 0.93 mmol) and 2-nitropropylacetate (2.16 g, 14 mmol) were added dropwise and the pH was controlled with a pH-stat by adding portions of 1 M NaOH. After 1 hour the reaction was complete (no more acid formation) and the suspension was neutralized with 0.5 M HCl. After heating to 75° (0.5 h) and cooling to T_k $Na_2S_2O_4$ (20.04 g, 117 mmol) was added in portions. The pH (= 7) was controled with a pH-stat by adding portions of a 1 M NaOH. After 16 h the reaction was complete (no more acid formation) and the suspension was filtered, washed with water and aceton, dried at T = 40°C and 43.78 g reduced 2-nitropropyl distarch 2-nitropropanediol (**12**, dry substance = 93.80 %, %N = 2,11%) was isolated. FT-IR (KBr): 3451 (s), 2928 (m), 1655 (m), 1547 (w), 1461 (w), 1161 (m), 1083 (m), 990 (w), 851 (m), 762 (m), 708 (m) cm^{-1}.

Determination of the swelling capacity.-The swelling capacities of the cross-linked starches **1-7**b were determined by a slightly modified procedure of Evans[16]. 2.00 g Blue dextran (0.3%) was added to a suspension of cross-linked starch (40-400mg) in 8.00 g H_2O. The closed tubes were rotated for 45 minutes at 90-95°C in a ventilating fan. After cooling down the tubes were centrifuged (15 min, 3000 rpm (swing out rotor)). The supernatant liquid (1.5 ml) was filtered and the absorbance of the initial and the obtained supernatant solutions were measured in a spectrophotometer at 620 nm. The swelling capacity (SC) was determined by the formula: SC = 10*(A-A0/A)/w*ds mL/g (A = extinction cross-linked strarch, A0 = extinction blue dextran (control), w = weight cross-linked starch, ds = dry substance cross-linked starch).

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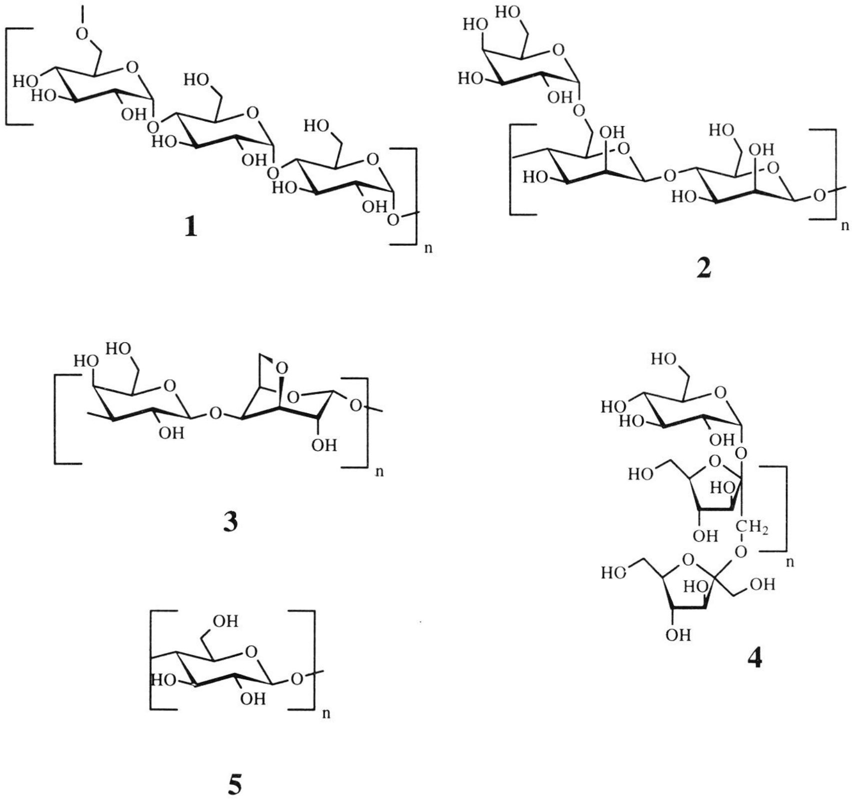
Chapter 6

Synthesis and reduction of 2-nitroalkyl polysaccharide ethers

6.1 Introduction

The use of renewable raw materials for non-food applications has increased rapidly in the last decade. The surplus of agricultural products and the growing demand of consumers for natural products have stimulated this growth. Therefore, we decided to investigate the scope of the Michael addition of nitroalkenes to polysaccharides other than starch and extended the reaction to pullulan (**1**), guar (**2**), agarose (**3**), inulin (**4**), and cellulose (**5**, Scheme 1). The second part of this chapter describes the attempts to reduce these modified polysaccharides to the corresponding aminoalkyl polysaccharide ethers.



Scheme 1. Structure of pullulan (1), guar (2), agarose (3), inulin (4), and cellulose (5).

A short description of the chemical and physical properties and industrial importance of these polysaccharides and their derivatives was already given in Chapter 1.

Etherification of polysaccharides **1-5** with nitroalkenes could in principle lead to products with emulsifying or dispersing properties. Furthermore, the rheological behavior of these compounds is expected to be pH-dependent, owing to the weakly acidic nitro functionality. The polar nitro functionality ($N^{\oplus}$-$O^{\ominus}$) makes these polysaccharide derivatives attractive for use in the paper (wet-end additive) and textile industries. Not only are 2-nitroalkyl polysaccharides interesting compounds themselves, they can also be excellent starting materials for other compounds, *e.g.*, aminoalkyl starch ethers (see Chapter 4).

This chapter describes the synthesis of the 2-nitropropyl and 2-nitrobutyl ethers of the polysaccharides **1-5** and the first attempt to reduce these compounds with $Na_2S_2O_4$ and $Na_2S_2O_4/NaBH_4$.

6.2 Results and Discussion

6.2.1 Synthesis of 2-nitroalkyl polysaccharide ethers

Although various methodologies (based on organic solvents) for chemical derivatization of polysaccharides have been developed in recent years[1], modifications using water as reaction medium are the most attractive for industrial reactions. Proper conditions for the alkaline etherification of polysaccharides depend on both the reagent and the polysaccharide. Published procedures for the derivatization of the individual compounds are briefly described in the following sections.

The best results for etherification of the water-soluble polysaccharides inulin and pullulan are obtained if the reactions are performed under alkaline conditions in highly concentrated aqueous systems[2].

Guar is mainly modified under heterogeneous conditions[3]. The synthesis is performed by addition of concentrated NaOH solutions to dry guar and subsequent addition of the etherifying agent. Guar and agarose can also be derivatized in slurry reactions in water/alcohol (3b)[4]. Homogeneous etherification of agarose is performed at temperatures at which the agrose is completely dissolved and no gel formation occurs[5].

Cellulose ethers are prepared predominantly in slurry systems[6]. The polysaccharide is often activated (alkalization with aqueous NaOH) before the reaction to achieve a better availability of the insoluble polysaccharide for the etherification reagents. Most reactions are conducted in organic solvent/water systems to produce a slurry from which the solid product can easily be recovered. The heterogeneous conditions for etherification of cellulose (and agarose and guar as well) lead, as already described in previous chapters, to non-uniform substitution during etherification.

We performed the Michael addition of compounds **1-5** to 2-nitroalkenes in concentrated homogeneous (pullulan, inulin) or heterogeneous (agarose, guar, cellulose) water systems. 2-Nitropropylacetate and 2-nitrobutylacetate were used as precursors for the corresponding nitroalkenes and 2 mole equivalents of NaOH per β-nitro acetoxyalkane were added to maintain alkaline conditions. All reactions were performed at room temperature and the modified polysaccharides were purified by dialysis or precipitation in organic solvents. The results for the nitroalkylation of polysaccharides **1-5** are shown in Table 1.

Table 1. Nitroalkylation of polysaccharides **1-5**

	2-nitropropyl acetate[a]		2-nitrobutylacetate[a]	
	ms_{max}	eff[b].	ms_{max}	eff.[b]
pullulan (**1**)[c]	0.29	>0.86	0.30	>0.61
guar (**2**)[c]	0.30	>0.67	0.30	>0.62
agarose (**3**)	0.27	0.93	0.27	0.67
inulin (**4**)	0.28	0.66[d,e]	0.30	1.00[d,e]
cellulose (**5**)	0.28	0.11	0.28	0.18
hydroxyethyl cellulose (**6**)	0.55	0.45[f]	-	-

a) H_2O/polysaccharide 2:1, 2 eq NaOH per mole 2-nitroalkyl acetate. b) eff. = reaction efficiency (ms/ms_{max}), the ms was calculated by means of the following formula: $ms = 1.62N/\{14-0.01N(Mwt_{alk}-1)\}$ or for agarose $ms = 1.53N/\{14-0.01N(Mwt_{alk}-1)\}$. c) the starting materials were contaminated with enzymes and other nitrogen containing material, pullulan %N = 0.28 (%), guar %N = 0.78% . d) No quantitative yields, owing to the low molecular weight and alkaline depolymerization (recovered yields: 2-nitropropyl inulin (42%), 2-nitrobutyl inulin (58%). e) H_2O/polysaccharide 1:1. f) H_2O/polysaccharide 4:1.

High reaction efficiencies are obtained for the synthesis of 2-nitropropyl- pullulan, guar, and agarose. The reaction of **1-3** with 2-nitrobutyl actetate proceeds less efficiently, but still satisfactorily.

The efficiency of the nitroalkylation of inulin cannot be determined properly, because during the work up procedure (precipitation in organic solvents or dialysis against MWCO 500) material was lost. Most likely, the low molecular inulin suffers from alkaline depolymerization during etherification. The β-(2→1)-linkage between the fructose units of inuline is rather labile and peeling off the polysaccharide chains from the reducing end thus formed results in the formation of lactates and glucosaccharinates[7]. An experiment in which the inulin was dialyzed before the etherification suffered from the same loss of material during the work-up procedure. This supports the above-mentioned hypothesis.

The efficiency of the nitroalkylation of the highly crystalline cellulose is low. Higher efficiencies are obtained for hydroxyethyl (HE) cellulose (**6**, ms = 3.23). Maximalization of the accessibility of cellulose by hydroxyethylation has a positive effect (eff. = 0.45) on the etherification reaction compared to unsubstituted cellulose, but the efficiency is still rather low in comparison with many other polysaccharides.

Attempts to modify (nitroalkylation) agarose in aqueous/organic solvents were discouraging. The efficiency of a slurry synthesis of agarose in 2-propanol/H_2O mixtures (2-propanol/H_2O 13:1, 2-nitropropylacetate: $ms_{max} = 0.23$, eff. = 24%, 2-nitrobutyl acetate: $ms_{max} = 0.24$, eff. = 11%) was rather low.

As expected, in more dilute systems lower efficiencies for nitroalkylation are obtained, *e.g.*, agarose (**3**), H_2O/agarose 15:1, 2-nitropropyl: $ms_{max} = 0.19$, eff. = 52%; 2-nitrobutyl: $ms_{max} = 0.26$, eff. = 39%). Side reactions, such as hydrolysis of the β-nitroacetoxy-alkanes, become more important under these conditions.

6.2.2 Analysis of 2-nitroalkyl polysaccharides

The incorporation of nitrogen (elemental analysis) and the appearance of a nitro absorbance in FT-IR spectra (about 1550 cm^{-1}) in all the modified polysaccharides confirm the presence of NO_2 groups in the polysaccharides and imply a successful Michael addition. It has been shown for 2-nitroalkyl starches that the nitro functionality is a mixture of nitroalkane, nitronic acid, and sodium nitronate. Grafting of nitroalkenes occurs to some extent and the products of side reactions are also incorporated in starch. This is also expected to be the case for compounds **1-6**.

Despite the higher molar substitutions of the 2-nitroalkyl polysacharide ethers compared to granular 2-nitroalkyl starches, satisfactory ^{13}C-NMR spectra could not be obtained for water-soluble 2-nitroalkyl pullulan ethers and 2-nitroalkyl inulines. Proton NMR spectra confirmed the complexity of the system and for both derivatives several multiplets were observed for the methyl protons (from the 2-nitropropyl group) and methylene and methyl protons of 2-nitrobutyl ethers, respectively. Resonances between δ 2.2-3.0 (diastereotopic hydrogens of the methylene group) are consistent with some grafting of nitroalkenes onto the polysaccharide.

In order to obtain more information about the structure of the polysaccharide derivatives, we synthesized labeled 2-nitropropyl-2-^{13}C pullulan (see Chapter 3). The ^{13}C-NMR spectrum of this compound is presented in Figure 1.

The values obtained are in close agreement with the ^{13}C-NMR of the smallest oligomer of hydrolyzed 2-nitropropyl-2-^{13}C starch. As expected, the nitro functionality is a mixture of the nitroalkane (δ 80-84 ppm), nitronic acid (δ 89-92 ppm), and sodium nitronate (δ 154-155). Grafting (δ 89-92 ppm) occurs and side products, such as allyl (resulting from the splitting off of nitrite, allyl pullulan δ 120 ppm) and carbonyl functionalities (Nef reaction, δ 204-205) are incorporated into the polysaccharide to some extent (see Scheme 2). The formation of the nitronic acid is confirmed by a positive Konowalow test[8].

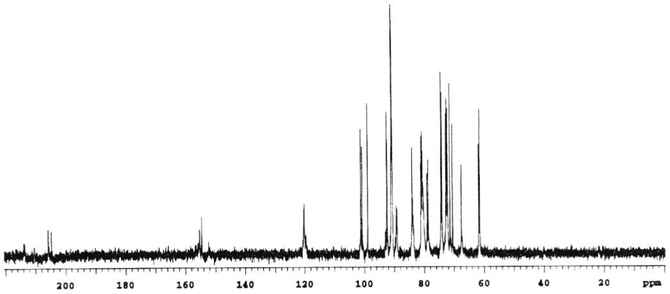
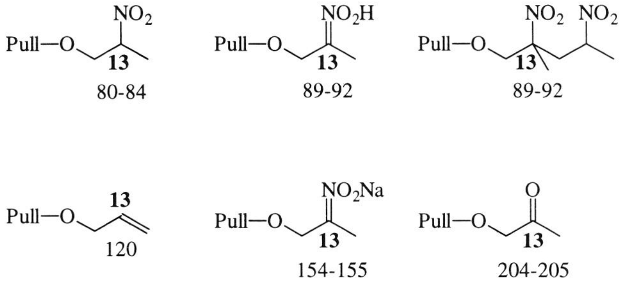


Figure 1. ^{13}C-NMR spectrum of 2-nitropropyl-2-^{13}C pullulan (50% enriched, $ms_{max} = 0.25$).

No other ^{13}C-labeled polysaccharides were synthesized in this project owing to the high cost of 2-nitropropyl acetate. However, the results obtained for 2-nitropropyl-2-^{13}C pullulan and 2-nitropropyl-2-^{13}C starch suggest the formation of a very similar mixture of products for the other polysaccharides.



Scheme 2. Products obtained in the synthesis of 2-nitropropyl pullulan.

6.2.3 Reduction of 2-nitroalkyl polysaccharide ethers with $Na_2S_2O_4$ ($NaBH_4$)

Introduction

Although the nitroalkyl polysaccharide ethers synthesized in section 6.2.1 have interesting physical properties (hydrophilicity, macromolecular solubility, ability to associate) we were mainly interested in the chemical properties of these compounds. As already described in Chapter 3, the reduction of the nitro-functionality to an amine group is of special interest.

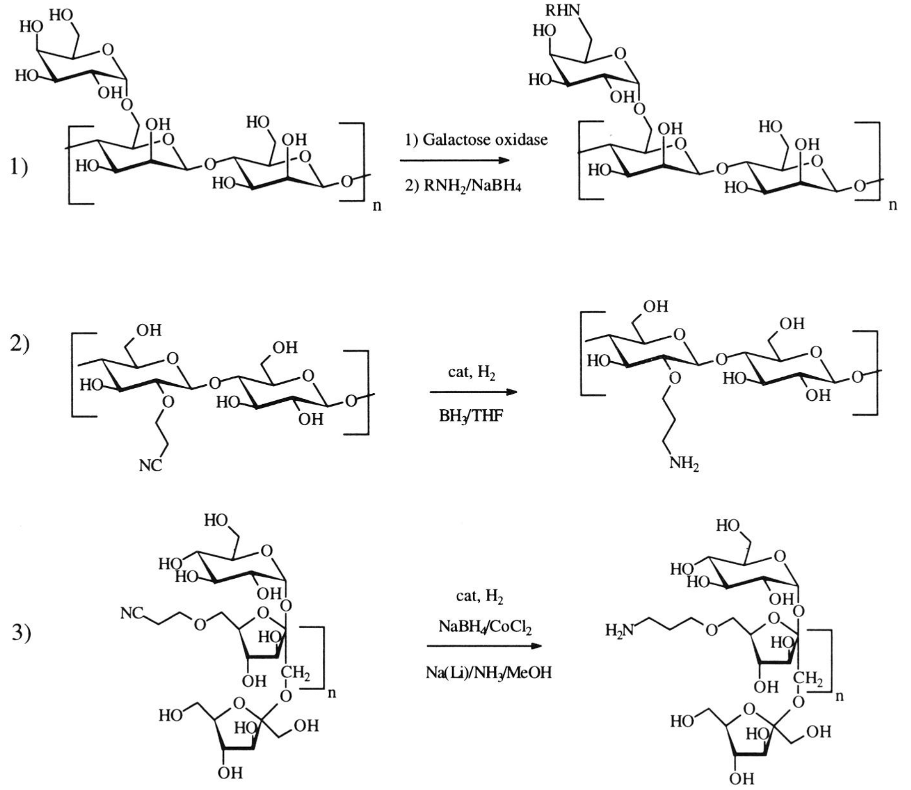
Amino alkyl polysaccharide ethers have potential for, *e.g.*, the immobilization of enzymes (agarose[9], cellulose[10]), use as an ion-exchange resin (cellulose[11]), for drug targeting (inulin[12]), and utilization as metal chelating agents (cellulose[13]). These compounds are also interesting materials for the paper industry (wet-end additive), textile industry (warp-sizing agent) and cosmetics (hair conditioners). Furthermore, aminoalkyl polysaccharide ethers are interesting starting materials for a large variety of other products (*e.g.*, hydrophobic polysaccharides).

Nature has provided us with an amino-functionalized polysaccharide: chitosan. Unfortunately, the utilization of the naturally occurring *N*-containing polysaccharide chitosan is restricted due to its insolubility in practical solvents[14]. Several reports deal with the synthesis of amino functionalized polysaccharide derivatives from compounds **1-6**[15]. The methods are identical to[16] or slightly modified procedures[17] of, the synthesis of amino starch derivatives (see Chapter 4). Most of these procedures (or procedures developed for the synthesis of amino dextran derivatives[18]) are not commercially attractive routes. The routes described in scheme 3 have some potential.

The enzymatic oxidation of guar with galactose oxidase (E.C. 1.1.3.9) and catalase is particularly attractive because of its specificity and the overall simplicity of the reaction[19]. It introduces an aldehyde group at the C-6 position of the pendant galactose units of guar gum. Subsequent reductive amination with any primary or secondary amine and $NaBH_4$ ($NaBH_3CN$) yields a polymer that bears a substituent on the primary alcohol of the galactose unit of the branched polysaccharide. Yields (not optimized) of the *in situ* reaction vary between 60-70%. The reaction is restricted to galactose-containing polysaccharides having free C-6 hydroxyl groups.

Catalytic hydrogenation of cyanoethyl cellulose (synthesized by reaction of cellulose with acrylonitrile) requires extreme reaction conditions (150 °C, 100 bar)[20]. Degradation probably accompanies this procedure. The patent describing this procedure does not mention any physical properties or analysis of the product obtained and it seems questionable whether the procedure is correct. A milder procedure is the reduction of the nitrile functionality (ds = 0.84, 1.84) with $BH_3.THF$ (or $BH_3.SMe_2$) in refluxing THF[21].

The catalytic hydrogenation of cyanoethyl inulin (50 °C, 100 bar) is slow, incomplete, and suffers from reverse Michael additions[22]. Reduction with $NaBH_4/CoCl_2$ gives a complete conversion of the nitrile functionality[23]. However, the conversion requires large amounts of reactants. A novel alternative is the reduction of the nitrile group with metals (Na, Li) in liquid ammonia in which an alcohol is used as the proton source. Although this process suffers from reverse Michael additions, 70% of the cyanoethyl functionality is selectively converted into aminopropyl groups.



Scheme 3. Methods for the synthesis of amino functionalized polysaccharides.

As we were interested in a cheap, preferably one-step, synthesis of 2-aminoalkyl polysaccharide ethers, efforts were made to reduce these compounds in water. Because of the rather disappointing results obtained for catalytic hydrogenation of water-soluble 2-nitropropyl starches and cyanoethyl inulin no attempts were made to reduce the 2-nitroalkyl polysaccharide ethers of compounds **1-6** *via* this route. The next section describes the reduction of 2-nitroalkylpolysaccharides (from **1-6**) in water with the cheap reducing agent $Na_2S_2O_4$ and combinations of $Na_2S_2O_4/NaBH_4$.

Reduction of 2-nitroalkyl polysaccharide ethers with $Na_2S_2O_4/(NaBH_4)$

The reductions were performed at room temperature and the pH was maintained at 7 by addition of 1 M NaOH. About 5 equivalents of $Na_2S_2O_4$ (and 2 equivalents of $NaBH_4$) were added to achieve a complete conversion of the nitro group. Sodium dithionite was added in portions to prevent decomposition (see Chapter 4). The results of the reduction of the 2-nitropropyl and 2-nitrobutyl derivatives of **1-6** are given in Table 2-4.

Table 2. Reduction of 2-nitropropyl polysaccharides with $Na_2S_2O_4$

2-nitropropyl	%N_{st}	%N (after red.)	%NO_2[a]	%N other	yield in nitrogen containing material (in %)[b]
1a pullulan	2.21	1.82	<0.05	1.82	82
2a guar	2.32	2.00	1.24	0.76	33
3a agarose	2.00	1.78	0.45	1.33	67
4a inulin	1.40	0.54	nd	nd	-
6a HE cellulose	1.09	0.87	0.40	0.47	43

a) determined with FT-IR, see Chapter 4 (**1** 860 cm^{-1}, **2** 872 cm^{-1}, **3** 932 cm^{-1}, **4** nd., **6** 890 cm^{-1}).
b) %N_{other}/%N_{st} (N_{st} =%N starting matertiał).

Table 3. Reductions of 2-nitrobutyl polysaccharides with $Na_2S_2O_4$

2-nitrobutyl	%N_{st}	%N (after red.)	%NO_2[a]	%N other	yield in nitrogen containing material (in %)[b]
1b pullulan	1.71	1.54	<0.05	1.54	90
2b guar	2.22	1.77	0.16	1.61	73
3b agarose	1.47	1.59	0.74	0.85	58
4b inulin	2.22	0.38	nd	nd	-
5b cellulose	0.43	0.40	0.17	0.23	53

a) determined with FT-IR, see Table 2.

Table 4. Reductions of 2-nitropropyl polysaccharides with $Na_2S_2O_4/NaBH_4$

2-nitropropyl	%Nst	%N (after red.)	%NO_2[a]	%N other	yield in nitrogen containing material (in %)[b]
1a pullulan	2.21	1.75	0.31	1.44	65
2a guar	2.32	2.47	0.19	2.28	98
3a agarose	2.00	1.66	<0.05	1.66	83
6a HE cellulose	1.09	0.76	<0.05	0.76	70

a) determined with FT-IR, see Table 2.

The yield in nitrogen-containing material for the reduction of 2-nitropropyl and 2-nitrobutyl starch (determined by FT-IR spectroscopy and elemental analysis) is moderate to high. The low efficiency for the reduction of 2-nitropropyl guar (**2**a) is probably an artefact. The effect of simultaneous addition of $NaBH_4$ is rather small and does not improve the conversion of the nitro functionality significantly.

Analysis of reduced 2-nitroalkyl polysaccharides

The almost complete conversion of the nitro functionality for the reduction of the 2-nitroalkyl polysaccharides described in Tables 2-4 does not necessarily imply that the corresponding aminoalkyl polysaccharides are formed. It is likely that the reductions of these nitroalkyl polysaccharides proceed in a way similar to that determined for the reduction of water-soluble 2-nitroalkyl starches and 2-nitroalkyl sodium-α-polyglucuronates. At the time we performed these experiments we had not analyzed the starch derivatives completely as we were still synthesizing 2-nitropropyl-2-^{13}C-starch and were thus unaware of the results of Chapter 4 in which we obtained a mixture of products.

Negative ninhydrin tests for **1a**-**6a** exclude the formation of amino functionalities[*]. In order to get a better insight into the reduction process, 2-nitropropyl-2-^{13}C-pullulan (50% enriched) was reduced with $Na_2S_2O_4$ and $Na_2S_2O_4/NaBH_4$. In the ^{1}H-NMR spectra the resonance of the methyl group of the 2-nitropropyl ether (δ 1.6-2.2 ppm) shifts to higher field (δ 1.2-1.8 ppm). The ^{13}C-NMR of reduced 2-nitropropyl-2-^{13}C-pullulan ($Na_2S_2O_4/NaBH_4$) is shown in Figure 2.

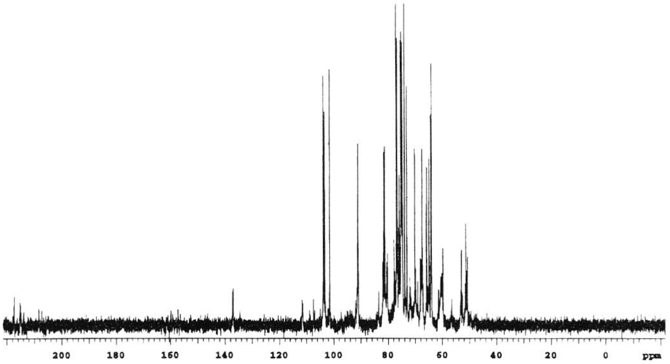


Figure 2. ^{13}C-NMR spectrum of reduced 2-nitropropyl-2-^{13}C-pullulan ($Na_2S_2O_4/NaBH_4$).

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[*] A ninhydrin test of the aminoalkyl polysaccharide chitosan gave a characteristic blue coloring.

Compared to the starting material (Figure 1) new resonances appear at δ 48-54 ppm and δ 60-68 ppm. The resonances at δ 120 (allyl), δ 155-157 (sodium nitronate/oxime) and δ 204-205 ppm (carbonyl) have dissappeared and the intensities of the resonances at δ 80-82 (nitroalkane) and δ 90-92 (nitronic acid/grafting) have become smaller. Resonances of the carbonyl and oxime remain present in the reduced product if no $NaBH_4$ is added during the reduction. Based on model systems and calculated NMR shifts (ADS program, see Chapter 4) the new resonances most likely originate from nitroso compounds, hydroxylamines, hydroxypropyl substituents and sulfamates.

It can be concluded that the reduction of 2-nitroalkyl polysaccharides with $Na_2S_2O_4$ or $Na_2S_2O_4/NaBH_4$ does not go to completion and several intermediates (probably stabilized during the reaction) are formed. It is likely that a complete reduction with excess $Na_2S_2O_4$ initially leads to the formation of a sulfamate, formed by the reaction of the hydroxylamine with bisulfite (see Chapter 4).

6.3 Conclusions

2-Nitroalkyl polysaccharide ethers are synthesized by Michael additions of 2-nitroalkenes, formed *in situ* from β-nitro-acetoxy-alkanes. The efficiency of the reaction depends on the accessibility of the polysaccharide for the substrate. High efficiencies (60-95%) are obtained for pullulan, guar, agarose, and inulin when the Michael additions are performed under alkaline conditions in highly concentrated homogeneous/heterogeneous systems (H_2O).

According to the ^{13}C-NMR spectrum of ^{13}C enriched 2-nitropropyl pullulan the nitro functionality exists as a mixture of the nitroalkane, nitronic acid, and sodium nitronate. Grafting occurs and products of side reactions *e.g.*, the Nef reaction (carbonyl), the splitting off of nitrite (allyl) are incorporated into the polysaccharide.

Reduction of 2-nitroalkyl polysaccharides with $Na_2S_2O_4$ (5 equivalents) or a mixture of $Na_2S_2O_4/NaBH_4$ leads to complex polysaccharide ethers which are most likely mixtures of nitro and nitroso compounds, hydroxylamines, hydroxypropyl ethers and sulfamates. Further research is needed to achieve a selective conversion of the nitro functionality to aminoalkyl polysaccharides.

6.4 Experimental

General methods. - 2-Nitropropyl acetate, 2-nitropropylacetate-2-^{13}C and 2-nitrobutylacetate were prepared according to literature procedures[24]. Pullulan and guar were gifts from AVEBE (Foxhol, The Netherlands). Agarose and cellulose were purchased from Sigma. Hydroxyethyl cellulose was a gift from Aqualon (Zwijndrecht, The Netherlands). Inulin was a gift from Sensus (Roosendaal, The Netherlands). Nitroethane (96%) was purchased from Aldrich. Nitroethane-1-^{13}C was obtained from Campro Scientific (Veenendaal, The Netherlands). Formaldehyde (37% solution), acetic acid

anhydride and 1-nitropropane (95%) were purchased from Aldrich. NaOH pellets and $NaBH_4$ were purchased from Merck. $Na_2S_2O_4$ was obtained from Fluka.
^{1}H-NMR and ^{13}C-NMR were recorded on a 500 Varian MHz spectrometer. FT-IR spectra were recorded on a Biorad FTS 135 spectrometer. The pH-stat, a Titration Controller 1200 equipped with a Titronic T110 burette was purchased from Schott Geräte. Spectra/Por membranes (MWCO: 2000 and MWCO: 500, for dialysis) were obtained from Spectrum Medical Industries. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Sulfur analyses were performed at the micro analytrical department of the University of Groningen.

Synthesis of 2-nitropropyl pullulan (1a). - 2-Nitropropyl acetate (2.40 g, 16.3 mmol) was added dropwise to a viscous solution of pullulan (10.01 g, 55.5 mmol, dry substance 89.89%) and NaOH (1.35 g, 33.8 mmol) in 20 ml H_2O. After 3 h stirring 250 ml H_2O was added and the solution was neutralized with 4.0 N HCl. After dialysis (MWCO: 2000) and freeze-drying 2-nitropullulan (**1**a, 8.39 g, %N = 2,21%) was isolated.

Synthesis of 2-nitropropyl-2-^{13}C pullulan. - The same procedure was followed as for **1**a using 2-nitropropyl acetate (0.206 g, 1.40 mmol; (mixture of 0.108 g 2-nitropropyl acetate (0.73 mmol) and 0.098 2-nitropropyl-2-^{13}C acetate (0.67 mmol)), pullulan (1.00 g, 5.55 mmol), NaOH (0.11 g, 2.75 mmol) and H_2O (2.5 ml). Isolated: 0.85 g 2-nitropropyl-2-^{13}C pullulan.

Synthesis of 2-nitrobutyl pullulan (1b). - The same procedure was followed as for **1**a using 2-nitrobutyl acetate (2.68 g, 16.6 mmol), pullulan (10.04 g, 55.7 mmol), NaOH (1.34 g, 33.5 mmol) and H_2O (20 ml). Isolated: 8.83 g 2-nitrobutyl pullulan (**1**b, %N = 1.71%).

Synthesis of 2-nitropropyl guar (2a). - The same procedure was followed as for **1**a using 2-nitropropyl acetate (2.40 g, 16.3 mmol), guar (10.00 g, 55.0 mmol, dry substance 89.16%), NaOH (1.33 g, 33.3 mmol) and H_2O (20 ml, heterogeneous reaction mixture). Isolated: 9.46 g 2-nitropropyl guar (**2**a, %N = 2.32 %).

Synthesis of 2-nitrobutyl guar (2b). - The same procedure was followed as for **1**a using 2-nitrobutyl acetate (2.61 g, 16.2 mmol), guar (10.13 g, 55.8 mmol), NaOH (1.34 g, 33.5 mmol) and H_2O (20 ml). Isolated: 9.04 g 2-nitrobutyl guar (**2**b, %N = 2.22%).

Synthesis of 2-nitropropyl agarose (3a). - The same procedure was followed as for **1**a using 2-nitropropyl acetate (2.40 g, 16.3 mmol), agarose (10.00 g, 60.6 mmol, dry substance 92.68%), NaOH (1.35 g, 33.7 mmol) and H_2O (20 ml, the reaction mixture was heterogeneous). Isolated: 10.07 g 2-nitropropyl agarose (**3**a, %N = 2.00%).

Synthesis of 2-nitrobutyl agarose (3b). - The same procedure was followed as for **1**a using 2-nitrobutyl acetate (2.69 g, 16.7 mmol), agarose (10.07 g, 61.0 mmol), NaOH (1.34 g, 33.5 mmol) and H_2O (20 ml). Isolated: 9.88 g 2-nitrobutyl agarose (**3**b, %N = 1.47%).

Synthesis of 2-nitropropyl agarose (diluted system). - The same procedure was followed as for **1**a using 2-nitropropyl acetate (2.30 g, 15.6 mmol), agarose (13.86 g, 84.1 mmol), NaOH (1.35 g, 33.8 mmol), and 145 ml H_2O. Isolated: 12.90 g 2-nitropropyl agarose (%N = 0.83%).

Synthesis of 2-nitropropyl agarose (2-propanol/H_2O). - Agarose (5.51 g, 35.5 mmol) was stirred in a blender with 2-propanol (130 ml) for 0.5 h. NaOH (0.67 g, 16.8mmol) in H_2O (10 ml) was added in portions and the suspension was stirred for 1 h. 2-Nitropropyl acetate (1.26 g, 8.60 mmol) was added dropwise. After 1.5 h, acetic acid (1 ml) was added, the suspension was filtered, washed with MeOH/H_2O 8:2 (250 ml) and dried in the air. Isolated: 4.96 g 2-nitropropyl agarose (%N = 0.48%).

Synthesis of 2-nitropropyl inulin (4a). - The same reaction procedure was followed as for **1**a using 2-nitropropyl acetate (6.00 g, 40.8 mmole), inulin (25.00 g, 148 mmol, dry substance 96.49%), NaOH (3.35 g, 8.4 mmole) and H_2O (25 ml). After precipitation in acetone (50 ml), filtration and washing with acetone/H_2O 4:1 (150 ml) and aceton (100 ml) the product was dried in the air. Isolated: 10.47 g 2-nitropropyl inulin (**4**a, %N = 1.40%).

Synthesis of 2-nitrobutyl inulin (4b). - The same procedure was followed as for **4**a using 2-nitrobutyl acetate (6.30 g, 39.1 mmol), inulin (24.87 g, 148 mmol), NaOH (3.36 g, 84 mmol) and H_2O (25 ml). Isolated: 15.78 g 2-nitrobutyl inulin (**4**b, %N = 1.63%).

Synthesis of 2-nitropropyl cellulose (5a). - The same reaction procedure was followed as for **1**a using 2-nitropropyl acetate (2.42 g, 16.4 mmol), cellulose (9.98 g, 59.0 mmol, dry substance 95.83%), NaOH (1.36 g, 34.0 mmol) and H_2O (20 ml). After precipitation in MeOH (50 ml), filtration and washing with MeOH/H_2O 3:1 (150 ml) the product was dried in the air. Isolated: 5.52 g 2-nitropropyl cellulose (**5**a, %N = 0.27%).

Synthesis of 2-nitrobutyl cellulose (5b). - The same procedure was followed as for **5**a using 2-nitrobutyl acetate (2.70 g, 16.8 mmol), cellulose (10.20 g, 60.3 mmol), NaOH (1.35 g, 33.8 mmol) and H_2O (20 ml). Isolated: 8.88 g 2-nitrobutyl cellulose (**5**b, %N = 0.46%).

Synthesis of 2-nitropropyl hydroxyethyl cellulose (6a). - The same procedure was followed as for **1**a using 2-nitropropyl acetate (2.40 g, 16.3 mmol), hydroxyethyl cellulose (10.00 g, 29.6 mmol, ms = 3.23, dry substance 89.91%), NaOH (1.37 g, 34.3 mmol) and H_2O (20 ml). Isolated: 9.17 g 2-nitropropyl hydroxyethyl cellulose (**6**a, %N = 1.09%).

Reduction of 2-nitropropyl pullulan with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.69 g, 22.3 mmol) was added in portions to a solution of 2-nitropropyl pullulan (**1**a, 2.32 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 2.06 g reduced 2-nitropropyl pullulan (%N = 1.82%).

Reduction of 2-nitropropyl-2-^{13}C pullulan with $Na_2S_2O_4$. - $Na_2S_2O_4$ (0.90 g, 4.28 mmol) was added in portions to a solution of 2-nitropropyl-2-^{13}C pullulan (0.50 g) in H_2O (10 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 0.45 g reduced 2-nitropropyl-2-^{13}C pullulan.

Reduction of 2-nitrobutyl pullulan with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.83 g, 23.0 mmol) was added in portions to a solution of 2-nitrobutyl pullulan (**1**b, 2.16 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 1.86 g reduced 2-nitrobutyl pullulan (%N = 1.54%).

Reduction of 2-nitropropyl guar with $Na_2S_2O_4$. - $Na_2S_2O_4$ (5.02 g, 23.9 mmol) was added in portions to a solution of 2-nitropropyl guar (**2**a, 2.57 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 2.39 g reduced 2-nitropropyl guar (%N = 2.00%).

Reduction of 2-nitrobutyl guar with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.69 g, 22.0 mmol) was added in portions to a solution of 2-nitrobutyl guar (**2b**, 2.33 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 1.86 g reduced 2-nitrobutyl guar (%N = 1.77%).

Reduction of 2-nitropropyl agarose with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.65 g, 22.1 mmol) was added in portions to a solution of 2-nitropropyl agarose (**3a**, 2.46 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the suspension was dialyzed and freeze-dried. Isolated: 1.58 g reduced 2-nitropropyl agarose (%N = 1.78%).

Reduction of 2-nitrobutyl agarose with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.30 g, 20.5 mmol) was added in portions to a solution of 2-nitrobutyl agarose (**3b**, 2.47 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the suspension was dialyzed and freeze-dried. Isolated: 2.36 g reduced 2-nitrobutyl agarose (%N = 1.59%).

Reduction of 2-nitropropyl inulin with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.35 g, 20.7 mmol) was added in portions to a solution of 2-nitropropyl inulin (**4a**, 2.35 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 1.20 g reduced 2-nitropropyl inulin (%N = 0.54%).

Reduction of 2-nitrobutyl inulin with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.63 g, 22.0 mmol) was added in portions to a solution of 2-nitrobutyl inulin (**4b**, 2.35 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 1.53 g reduced 2-nitrobutyl inulin (%N = 0.38%).

Reduction of 2-nitrobutyl cellulose with $Na_2S_2O_4$. - $Na_2S_2O_4$ (5.08 g, 24.2 mmol) was added in portions to a solution of 2-nitrobutyl cellulose (%N = 0.43%, 2.94 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the suspension was dialyzed and freeze-dried. Isolated: 2.40 g reduced 2-nitrobutyl cellulose (%N = 0.40%).

Reduction of 2-nitropropyl hydroxyethyl cellulose with $Na_2S_2O_4$. - $Na_2S_2O_4$ (4.48 g) was added in portions to a solution of 2-nitropropyl hydroxyethyl cellulose (**6a**, 2.50 g) in H_2O. The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried. Isolated: 2.09 g reduced 2-nitropropyl hydroxyethyl cellulose (%N = 0.87%).

Reduction of 2-nitropropyl pullulan with $Na_2S_2O_4/NaBH_4$. - $Na_2S_2O_4$ (4.50 g, 21.4 mmol) and $NaBH_4$ (0.30 g, 8.0 mmol) were added in portions to a solution of 2-nitropropyl pullulan (**1a**, 2.48 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h $NaBH_4$ (0.05 g, 1.3 mmol) was added and after 2 h the solution was acidified with 4 N HCl to pH = 3. MeOH (10 ml) was added and after concentration to about 20 ml the solution was dialyzed and freeze-dried. Isolated: 2.07 g reduced 2-nitropropyl pullulan (%N = 1.56%).

Reduction of 2-nitropropyl-2-^{13}C pullulan with $Na_2S_2O_4/NaBH_4$. - $Na_2S_2O_4$ (0.36 g, 1.71 mmol) and $NaBH_4$ (0.025 g, 0.67 mmol) were added in portions to a solution of 2-nitropropyl-2-^{13}C pullulan (0.19 g) in H_2O (10 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was acidified to pH = 3 with 0.5 HCl. MeOH (3 ml) was added and after concentration to about 5 ml the solution was dialyzed and freeze-dried. Isolated: 0.17 g reduced 2-nitropropyl-2-^{13}C pullulan.

Reduction of 2-nitropropyl guar with $Na_2S_2O_4/NaBH_4$. - $Na_2S_2O_4$ (4.50 g, 21.4 mmol) and $NaBH_4$ (0.30 g, 8.0 mmol) were added in portions to a solution of 2-nitropropyl guar (**2**a, 2.52 g) in H_2O (200 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h $NaBH_4$ (0.05 g, 1.3 mmol) was added and after 2 h the solution was acidified to pH = 3 with 4 N HCl. MeOH (10 ml) was added and after concentration to about 20 ml the solution was dialyzed and freeze-dried. Isolated: 1.96 g reduced 2-nitropropyl guar (%N = 2.47%).

Reduction of 2-nitropropyl agarose with $Na_2S_2O_4/NaBH_4$. - $Na_2S_2O_4$ (4.50 g, 21.4 mmol) and $NaBH_4$ (0.30 g, 8.0 mmol) were added in portions to a suspension of 2-nitropropyl agarose (**3**a, 2.53 g) in H_2O (50 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h $NaBH_4$ (0.05 g, 1.3 mmol) was added and after 2 h the solution was acidified to pH = 3 with 4 N HCl. MeOH was added and after concentration to about 20 ml the suspension was dialyzed and freeze-dried. Isolated: 1.99 g reduced 2-nitropropyl agarose (%N = 1.66%).

Reduction of 2-nitropropyl hydroxyethyl cellulose with $Na_2S_2O_4/NaBH_4$. - $Na_2S_2O_4$ (4.50 g, 21.4 mmol) and $NaBH_4$ (0.30 g, 8.0 mmol) were added in portions to a solution of 2-nitropropyl hydroxyethyl cellulose (**6**a, 2.51 g) in H_2O (100 ml). The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h $NaBH_4$ (0.05 g, 1.3 mmol) was added and after 2 h the solution was acidified to pH = 3 with 4 N HCl. MeOH was added and after concentration to about 20 ml the solution was dialyzed and freeze-dried. Isolated: 2.25 g reduced 2-nitropropyl hydroxyethyl cellulose (%N =0.76%).

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Chapter 7

Epilogue

7.1 Introduction

Attempts to develop a route for the large scale synthesis of aminoalkyl polysaccharide ethers have led to a novel class of polysaccharide derivatives; the 2-nitroalkyl polysaccharide ethers. The work described in the preceding chapters focusses mainly on the synthesis, a complete analysis and reduction of these compounds. In this chapter we will evaluate the results obtained and correlate these with the original aims of our research project. Some concluding remarks and suggestions for further research and an estimate for the price of 2-nitroalkyl polysaccharides are presented as well.

7.2 Goals, achievements and concluding remarks

7.2.1 Applications of 2-nitroalkyl polysaccharide ethers

Because the ultimate aim of our research project entailed the synthesis of aminoalkyl polysaccharide ethers we did not pay much attention to potential applications of the easily accessible 2-nitroalkyl polysaccharide ethers. We investigated the rheological behavior of 2-nitroalkyl starches in water briefly (see Chapter 3), but did not look further into the emulsifying or dispersing (or other) properties of this new class of polysaccharide derivatives. 2-Nitroalkyl starch ethers appear to have potential for the protection against retrogradation and as wet-end additives in the paper industry.

The instability of starch dispersions/solutions is caused by the intermolecular association between amylose molecules. This process (retrogradation) leads to the formation of opaque dispersions and finally to precipitation. Retrogradation is usually prevented by the introduction of a variety of substituent groups (0.5-2.5%[1], acetyl, hydroxyethyl, etc.) in the starch matrix. In principle, this makes the reagents described in this thesis useful for protection against retrogradation, and therefore attractive for utilization in non-food areas in view of the observed preferential nitroalkylation[*] of the amylose fraction in 2-nitropropyl starch.

Cationic starches are widely used as wet-end additives in the paper industry. They are very effective for improving the strength of the paper. Addition of these compounds leads to a better retention of fillers (clay, $CaCO_3$, TiO_2). Furthermore, the water drainage of the fiber mat

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[*] Suspension reaction of starch with 2-nitropropyl acetate, see Chapter 3.

on a paper machine is improved[2]. Cyanoethyl starch, although nonionic, is also retained tightly onto slightly anionic cellulosic substrates such as paper. The retentive properties are attributed to the polar character of the nitrile functionality[3]. Therefore, we intended to test the even more polar 2-nitropropyl starch (ms = 0.078) as a wet-end additive in the paper industry. Unfortunately, the molar substitution of this particular derivative proved to be too high. Phase separation occurred and a clear solution, which is necessary for the process, could not be obtained. It is not clear whether the phase separation originates from the highly substituted amylose fraction or if it can be attributed to hydrogen bonding between hydroxyl groups of starch and the nitro group. Better results may be obtained with 2-nitropropyl starch with a lower degree of substitution.

We have only performed some preliminary experiments. It is obvious that the range of potential applications of 2-nitroalkyl starches and other 2-nitroalkyl polysaccharide ethers, easily synthesized in moderate to high yields, is fully open and needs to be explored in the future.

7.2.2 Reduction of the nitro functionality

A large number of methods are known for conversion of simple aliphatic nitroalkanes to aminoalkanes. Most of these methods are, however, not suitable for the reduction of 2-nitroalkyl polysaccharides, due to the polysaccharide matrix. The route we have chosen, reduction with the cheap, water soluble $Na_2S_2O_4$ does not go to completion and initially leads to the formation of polysaccharide sulfamates under neutral conditions. Consecutive cleavage of sulfamates to obtain amines requires rather extreme alkaline or acidic conditions and will most likely lead to depolymerization of the polysaccharide[4]. Despite our efforts, we did not reach the ultimate aim of our research project, *i.e.*, development of a simple route for the synthesis of aminoalkyl polysaccharide ethers on a large scale.

Alternative routes for the synthesis of aminoalkyl polysaccharides are reduction of 2-nitroalkyl polysaccharides with $N_2H_4.H_2O$[5] (or N_2H_4/Raney Ni[6]) or $NaBH_4/CoCl_2$[7]. However, more promising methods for the synthesis of aminoalkyl polysacharide ethers have recently been developed by Verraest *et al.* (reduction of the nitrile functionality of cyanoethyl inulin with metals in liquid ammonia[8]) and for cellulose derivatives, by Daly (reduction of cyanoethyl cellulose with $BH_3.SMe_2$ or $BH_3.THF$[9]).

7.2.3 Other conversions of the nitro functionality of 2-nitroalkyl polysaccharide ethers

Not only are simple aliphatic nitro compounds excellent starting materials for amines, they can also be transformed in many other functionalities[10]. Modified Nef reactions of 2-nitroalkyl polysaccharide ethers to 2-ketoalkyl polysaccharide ethers (*i.a.*, H_2O_2/K_2CO_3*, $NaNO_2/C_3H_7ONO$) appear to have potential for large scale reactions[11].

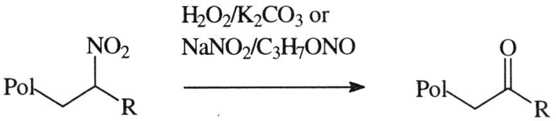

Fig 1. Modified Nef reactions of 2-nitroalkyl polysaccharide ethers.

The carbonyl functionality of this slightly hydrophobic compound most likely forms inter- and intramolecular hemiacetals. This leads to a product with interesting rheological behavior. Furthermore, this compound is an interesting starting material for other starch derivatives, *e.g.* hydrophobic polysaccharides which can probably be obtained easily by reductive aminations with aliphatic amines and $NaBH_4$.

Other examples of potential chemical modification of 2-nitroalkyl polysaccharide ethers are carbon-carbon coupling reactions such as Henry reactions with aldehydes, Michael additions with unsaturated compounds and various Mannich reactions (see Chapter 2). A wide variety of new starch derivatives (hydrophobic, anionic, cationic, zwitterionic) could be synthesized in this way.

7.3 Estimation of the cost prices of 2-nitropropyl and 2-nitrobutyl polysaccharides

7.3.1 Introduction

The price/performance of a compound determines in most cases if a successful commercial introduction in the market can be achieved. As a first approximation the variable chemical costs for 2-nitropropyl and 2-nitrobutyl polysaccharides were calculated. Evidently, the total manufacturing costs will be significantly higher and also contain items such as fixed costs and capital charge. The variable chemical costs and estimated bulk prices of 2-nitroalkyl polysaccharides will be compared with some other commercially available polysaccharide derivatives.

7.3.2 Estimation of the cost prices of 2-nitropropyl and 2-nitrobutyl acetate

As shown in Chapter 2, 2-nitroalkyl polysaccharides can be synthesized with several nitroalkenes and precursors thereof. High efficiencies are obtained for Michael additions of

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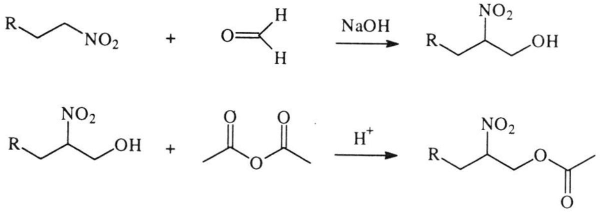
* Some oxidation of the polysaccharide occurs as well.

starch with 2-nitroalkenes, formed *in situ* from β-nitro-acyloxy-alkanes. The β-nitro-acyloxy-alkanes 2-nitropropyl and 2-nitrobutyl acetate are commercially attractive because they can be synthesized from cheap nitroalkanes (nitroethane and 1-nitropropane), formaldehyde and acetic acid anhydride. Estimates for the variable chemical costs are shown in Table 1.

Table 1. Variable chemical costs for 2-nitropropyl- (R = H) and 2-nitrobutyl acetate (R = Me).



Synthesis of 2-nitropropanol (yield 75%)

chemicals	price.kg^{-1} (in Hfl)[12]	throughput (kg/kg 2-nitropropanol)	contribution (Hfl/kg 2-nitropropanol)
nitroethane	11.22	0.95 kg (12.7 mole)	10.66
formaldehyde (37% solution)	0.52	1.15 kg (13.3 mole)	0.60
NaOH (50%)	0.66	0.50 kg (12.7 mole)	0.33
acetic acid	1.58	0.85 kg (14.3 mole)	1.26
		variable chemical costs	12.85 (1.35 mole^{-1})

Synthesis of 2-nitrobutanol (yield 75%)

chemicals	price.kg^{-1} (in Hfl)	throughput (kg/kg 2-nitrobutanol)	contribution (Hfl/kg 2-nitrobutanol)
1-nitropropane	8.80	1.00 kg (11.2 mole)	8.80
formaldehyde (37% solution)	0.52	1.05 kg (12.0 mole)	0.55
NaOH (50%)	0.66	0.48 kg (12.0 mole)	0.32
acetic acid	1.58	0.78 kg (13.0 mole)	1.23
		variable chemical costs	10.90 (1.30 mole^{-1})

We estimated the selling price (variable chemical costs + profit + manufacturing costs) for multiton production to be about 14-16 Hfl kg^{-1} for 2-nitropropanol and 12-14 Hfl kg^{-1} for 2-nitrobutanol.

Synthesis of 2-nitropropyl acetate (yield 85%)			
chemicals	price.kg^{-1} (in Hfl)	throughput (kg/kg 2-nitropropanol)	contribution (Hfl/kg 2-nitropropyl acetate)
2-nitropropanol	15	0.84 kg (8.0 mole)	12.60
acetic anhydride	2.18	0.87 kg (8.5 mole)	1.90
		variable chemical costs	14.50 (2.13 mole^{-1})

Synthesis of 2-nitrobutyl acetate (yield 85%)			
chemicals	price.kg^{-1} (in Hfl)	throughput (kg/kg 2-nitropropanol)	contribution (Hfl/kg 2-nitropropyl acetate)
2-nitrobutanol	13	0.87 kg (7.3 mole)	11.30
acetic anhydride	2.18	0.79 kg (7.7 mole)	1.70
		variable chemical costs	13.00 (2.09 mole^{-1})

It seems realistic to assume a multiton price for 2-nitropropyl- and 2-nitrobutyl acetate of about 15-20 Hfl kg^{-1}.

7.3.3 Estimation of the cost prices of 2-nitropropyl and 2-nitrobutyl polysaccharides

Important factors that determine the selling price of polysaccharide derivatives are the bulk prices of the polysaccharides used, the bulk prices and amount (molar substitution) of reagents used and the manufacturing costs. The commercially available polysaccharides used in this thesis that gave moderate to high yields for nitroalkylation with 2-nitropropyl- and 2-nitrobutyl acetate are potato starch (0.70 Hfl kg^{-1})[13], guar (3.00 Hfl kg^{-1})[12] and inulin (3.50 Hfl kg^{-1})[14]. Estimations of selling prices of 2-nitropropyl and 2-nitrobutyl polysaccharides are shown in Table 2. The bulk prices are compared with some other commercially available polysaccharide derivatives.

The influence of the molar (or degree of) substitution on the price of some commercially available potato starch derivatives is shown in Table 3 and Figure 2 (bulk price = 0.70 Hfl.kg^{-1}, dry substance = 85%, the manufacturing costs for suspension reactions of starch are about 0.50 Hfl.kg$^{-1.}$

Table 2. Estimated bulk prices of 2-nitropropyl and 2-nitrobutyl polysaccharide ethers and other commercially available polysaccharide ethers.

Reagent	price.kg^{-1} (Hfl)	Mwt	price.mole^{-1} (Hfl)	price.kg^{-1} polysaccharide (ms or ds <0.1, Hfl)[*]
2-nitropropyl acetate	15-20	147	2.50	$w + 15.42 \cdot x \cdot y \cdot ms + z$[a]
2-nitrobutyl acetate	15-20	161	2.50	$w + 15.42 \cdot x \cdot y \cdot ms + z$
ethylene oxide	2.46[12]	44	0.11	$w + 0.67 \cdot x \cdot y \cdot ms + z$
propylene oxide	2.81[12]	58	0.16	$w + 1.01 \cdot x \cdot y \cdot ms + z$
CHPTMAC[b]	6.00[15]	188	1.13	$w + 6.96 \cdot x \cdot y \cdot ms + z$
acrylonitrile	2.33[12]	53	0.12	$w + 0.76 \cdot x \cdot y \cdot ms + z$
acetic anhydride	2.18[12]	102	0.22	$w + 1.37 \cdot x \cdot y \cdot ds + z$

a) w = bulk price polysaccharide (potato starch 0.70 Hfl.kg^{-1}, guar 3.00 Hfl.kg^{-1}, inulin 3.50 Hfl.kg^{-1}), x = efficiency of the reaction, y = dry substance of the polysaccharide (starch ~85%, guar ~90%, inulin ~95%), z = manufacturing costs. b) 3-chloro-2-hydroxypropyl-trimethylammonium chloride.

Table 3. Bulk prices of potato starch derivatives.

Reagent	efficiency	price.kg^{-1} starch (ms or ds <0.1, Hfl)
2-nitropropyl acetate	85%	$1.20 + 11.0 \cdot ms$
2-nitrobutyl acetate	85%	$1.20 + 11.0 \cdot ms$
ethylene oxide	75%	$1.20 + 0.4 \cdot ms$
propylene oxide	70%	$1.20 + 0.6 \cdot ms$
CHPTMAC (c)	85%	$1.20 + 5.0 \cdot ds$
acrylonitrile	85%	$1.20 + 0.6 \cdot ms$
acetic anhydride	85%	$1.20 + 1.0 \cdot ds$

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[*] Assuming that the dry substance and the molecular weight of the isolated derivative are in the same order. At low ms (ds) the deviations will be minor.

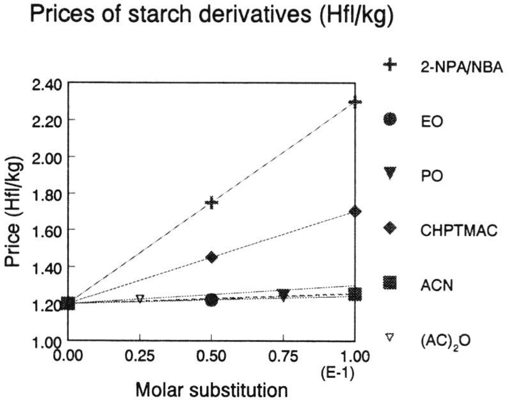

Fig. 2. Estimated bulk prices of starch derivatives (2-NPA = 2-nitropropyl acetate, NBA = 2-nitrobutyl acetate, EO = ethylene oxide, PO = propylene oxide, CHPTMAC = 3-chloro-2-hydroxypropyl-trimethyl ammonium chloride, ACN = acrylonitrile, $(AC)_2O$ = acetic anhydride.

It can be concluded that at moderate molar substitution, (ms = 0.1) 2-nitroalkyl starch ethers are more expensive than the hydroxyalkyl starch ethers and starch acetate. Therefore, for commercial applications it is essential that the nitroalkyl functionality of these starches, and also of course for other 2-nitroalkyl polysaccharide ethers, provides some additional value. The fact that nitroalkylation takes place almost exclusively on amylose implies that a much smaller degree of substitution may already induce a similar inhibition of retrogradation. The effective price difference could therefore be significantly smaller.

The synthesis of the 2-nitroalkyl starch ethers has been patented by AVEBE B.A.[16].

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Samenvatting

Polysachariden zijn essentieel voor het leven op aarde. Deze polymeren van koolhydraten worden bijvoorbeeld gebruikt voor de opslag van energie, de opbouw van het skelet van bepaalde diersoorten en het geven van stevigheid aan planten. Kort voor het begin van de christelijke jaartelling ontdekten de Egyptenaren dat polysachariden niet alleen geschikt waren voor voedingsdoeleinden maar dat het polysacharide zetmeel ook bruikbaar is voor het verbeteren van de kwaliteit van papier. In de loop der eeuwen zijn talloze andere niet-levensmiddelen (non-food) toepassingen voor polysachariden ontwikkeld.

In de 19e en 20e eeuw zijn veel van deze non-food applicaties van polysachariden vervangen door producten uit de petrochemische industrie. De eigenschappen van de petrochemische producten zijn over het algemeen beter te sturen en deze verbindingen kunnen in veel gevallen goedkoper geproduceerd worden. De laatste jaren groeit het besef dat er toch belangrijke voordelen aan het gebruik van polysachariden ten opzichte van petrochemische producten zitten. Polysachariden zijn hernieuwbaar, de natuur kan deze verbindingen steeds weer aanmaken (elk jaar kunnen er weer aardappelen verbouwd en geoogst worden). Petrochemicaliën worden verkregen uit een eindige bron. Ook vanuit het oogpunt van het milieu heeft het gebruik van polysachariden de voorkeur: polysachariden zijn over het algemeen goed biodegradeerbaar, niet toxisch en ze hebben een neutrale CO_2 cyclus. Het overschot aan landbouwgewassen (polysachariden) in de Westerse wereld is op dit moment waarschijnlijk de belangrijkste drijfveer voor verder onderzoek naar industriële toepassingen van deze verbindingen.

De natuur heeft ons bedeeld met een breed scala aan polysachariden met verschillende chemische en fysische eigenschappen. Nieuwe eigenschappen kunnen worden verkregen door bestaande polysachariden chemisch te modificeren. Meerdere strategieën kunnen worden onderscheiden. Het polysacharide kan bijvoorbeeld gesplitst worden in kleinere eenheden die andere eigenschappen hebben dan het oorspronkelijke polymeer. Het bekendste voorbeeld is waarschijnlijk de synthese van glucose/glucosestropen uit zetmeel. Een andere manier is het modificeren van polysachariden waarbij het molecuulgewicht niet afneemt. De inbouw van nieuwe functionele groepen leidt tot nieuwe eigenschappen en daarmee mogelijk tot nieuwe commerciële toepassingen.

In dit proefschrift is de laatste strategie gebruikt voor de synthese van een nieuwe klasse van gemodificeerde polysachariden: de 2-nitroalkylpolysaccharide-ethers. Veel onderzoek is verricht naar de analyse van deze gemodificeerde polysachariden, tevens is getracht om 2-nitroalkylpolysacharide-ethers te reduceren tot 2-aminoalkylpolysacharide-ethers. Amino gefunctionaliseerde polysachariden zijn in principe goede ionenwisselaars, geschikt voor het immobiliseren van enzymen, het binden van zware metalen (water zuivering) en ze kunnen gebruikt worden als uitgangsstoffen voor de synthese van een grote verscheidenheid aan andere producten.

De synthese van gesubstitueerde 2-nitroalkylzetmeelethers (molaire substitutiegraad < 0.2) verloopt zeer efficiënt voor Michael addities van zetmeel aan 2-nitro-1-alkenen die in basisch milieu *in situ* gevormd worden uit 2-nitroalkylacylaten (hoofdstuk 2). De rendementen (80-90%) zijn bijzonder hoog in geconcentreerde zetmeel suspensies (pH = 10) en de reacties verlopen voor kleine, redelijk wateroplosbare 2-nitro-1-alkenen vrijwel instantaan. Het gebruik van andere precursors (β-nitro-chlooralkanen) en rechtstreeks toevoegen van 2-nitro-1-alkenen aan zetmeel heeft een verlaging van het rendement tot gevolg.

Door nevenreacties is het rendement van de Michael addities van zetmeel aan 1-nitro-1-alkenen en digesubstitueerde nitroalkenen (eventueel *in situ* gevormd) over het algemeen lager. Ook de Michael addities van wateroplosbaar zetmeel en natrium-α-polyglucuronaat (homogene synthese) met 2-nitropropyl- en 2-nitrobutylacetaat verlopen minder efficiënt dan voor natief zetmeel.

Factoren die de uiteindelijke eigenschappen van 2-nitroalkylzetmeelethers bepalen zijn: de molaire substitutie graad (ms, de hoeveelheid substituent die ingebouwd is), de topochemie (waar reageert het 2-nitro-1-alkeen in de zetmeelkorrel), de structuur van de substituent (nitroalkanen kunnen in de *aci*-vorm voorkomen) en de mate waarin neven- en vervolgreacties plaatsvinden.

In hoofdstuk 3 staan verschillende mogelijkheden voor de bepaling van de molaire substitutiegraad beschreven (element analyse, FT-IR en DSC).

Uit de identieke molaire substitutiegraad van kleine en grote zetmeelkorrels kan geconcludeerd worden dat de nitroalkyl substituent homogeen over de zetmeelkorrel verdeeld is. Verrassend is dat vooral de amylosefractie van de zetmeelkorrel gesubstitueerd is. Een mogelijke verklaring is dat 2-nitropropylacetaat de vorming van een amylose-2-nitropropylacetaat complex bewerkstelligt waarbij 2-nitropropylacetaat in een amylose helix zit. Na afsplitsing van acetaat reageert het *in situ* gevormde 2-nitro-1-propeen in de helix met de hydroxylgroepen van amylose.

De supramoleculaire structuur van natief zetmeel maakt de analyse van gemodificeerd zetmeel in vergelijking met simpele organische verbindingen complex. Met behulp van NMR, FT-IR, de synthese van een aantal modelverbindingen en gelabeld 2-nitropropyl-2-^{13}C zetmeel en een ^{13}C-NMR simuleringsprogramma is de structuur van de zetmeelether opgelost. De nitrogroep blijkt een mengsel te zijn van het nitroalkaan, het *aci*-tautomeer en het nitronaat. De nitroalkyl groep is in staat om verder te reageren met een nitroalkeen waardoor strengen van nitroalkyl substituenten ontstaan ("grafting"). Verder treden in beperkte mate vervolgreacties op waarbij de nitrogroep omgezet wordt in carbonyl of oxim functionaliteiten.

Pogingen om 2-nitroalkylzetmeelethers te reduceren tot 2-aminoalkylzetmeelethers staan beschreven in hoofstuk 4. Katalytische hydrogenering van wateroplosbare 2-nitroalkylzetmeelethers leidt, waarschijnlijk door het geringe contact tussen de katalysator (Raney Ni) en het gemodificeerde polysaccharide, slechts tot omzetting van een klein deel van de nitroalkylgroepen in de overeenkomstige aminoalkylgroepen. Bij suspensiereacties van 2-nitroalkylzetmeelethers met natriumdithioniet ($Na_2S_2O_4$), eventueel in combinatie met natriumboorhydride ($NaBH_4$), vindt een redelijke tot goede omzetting van de nitrogroep plaats. Helaas blijkt uit analyse van de gevormde producten dat de gereduceerde verbindingen een mengsel zijn van uitgangsstof, verschillende intermediairen van de reductie stap (nitroso, oxim, hydroxyl amine) en sulfamaat. Het sulfamaat wordt waarschijnlijk gevormd door reactie van het hydroxylamine met bisulfiet dat tijdens de reductie gevormd wordt uit natriumdithioniet.

Derivatisering van polysachariden met bifunctionele reagentia leidt tot verknoopte polymeren. Door de verknoping verandert de reologie en, afhankelijk van de verknopingsgraad, kunnen er grote wateronoplosbare aggregaten gesynthetiseerd worden. Bifunctionele precursors van nitroalkenen blijken uitstekende verknopers van zetmeel te zijn (hoofdstuk 5). De reactiesnelheid is hoog en het zwelvermogen van deze zetmeelderivaten is in bepaalde gevallen lager dan van met epichloorhydrine verknoopt zetmeel. Dit duidt op een hoge efficiëntie. Verknoopte 2-nitroalkylzetmeelethers kunnen in één stap worden gesynthetiseerd door de bifunctionele precursor en 2-nitroalkylacetaten gelijktijdig toe te voegen.

In hoofstuk 6 staan de resultaten van de synthese van 2-nitroalkylpolysacharide-ethers anders dan zetmeel beschreven. De efficiëntie van de reactie is afhankelijk van het polysaccharide. Redelijk hoge tot hoge rendementen worden gehaald voor pullulaan, guar, agarose en inuline. Het kristallijne cellulose is minder toegankelijk voor nitroalkenen en in dit geval treden in belangrijke mate zijreacties op. Het rendement van de nitroalkylering kan verbeterd worden door de reactie uit te voeren met hydroxyethylcellulose.

Pogingen om deze gemodificeerde polysachariden te reduceren met natriumdithioniet (eventueel in combinatie met natriumboorhydride) leveren dezelfde resultaten op als voor zetmeel. De nitrogroep wordt gedeeltelijk omgezet in nitroso, oxim, hydroxylamine en sulfamaat.

Het proefschrift besluit met een epiloog waarin enkele voorstellen voor verder onderzoek staan beschreven. De kostprijzen van 2-nitropropyl- en 2-nitrobutylacetaat zijn berekend (Hfl 15-20/kg) en de prijs van eenvoudig te bereiden 2-nitroalkylzetmeelethers (afhankelijk van de gewenste molaire substitutiegraad) is vergeleken met die van commercieel verkrijgbare zetmeelethers.

Stellingen

behorende bij het proefschrift van André Heeres, 20 November 1998

1 De formules die Kweon *et al.* en Khalil *et al.* gebruiken voor het berekenen van molaire substitutiegraden van gemodificeerde polysachariden leiden tot een onjuiste uitkomst voor de reactierendementen.
Starch/Stärke **1997**, *49*, 59 en *Starch/Stärke* **1998**, *50*, 267.

2 Het beïnvloeden van de wereldmedia is een essentieel onderdeel van oorlogvoering geworden.

3 Op dit moment heeft een belegger meer aan een cursus psychologie dan een studie bedrijfskunde.

4 Het Bosman arrest heeft geleid tot een daling van het spelpeil op de Europese voetbalvelden.

5 In vergelijking met enkele nonionische amylose derivaten kan het verminderde complexerend vermogen van carboxymethyl amylose voor jodium niet *a priori* verklaard worden door een, niet correcte, andere verdeling van substituenten over het glucose monomeer.
Carbohydr. Res. **1998**, *307*, 19.

6 In het buitenland geldt: Drs. = n · Dr. ($n \geq 2$). In dit licht bezien houdt een promotie een degradatie in.

7 In tegenstelling tot wat veel scholieren beweren kun je alleen studeren aan de universiteit.

8 Stoplichten die zeer kort op groen staan nodigen uit tot verkeersovertredingen.

9 Industrieën die promotieplaatsen bekostigen lijken soms onvoldoende te beseffen dat een AIO meer is dan alleen een goedkope arbeidskracht.

0,15 g NaOH (3,85

0,38 g KAc (3,85

2 mL H_2O

8 mL MeOH

- T = 0°C, 1,00 g diisopropy (3,85 mmol)
- op laten warmen tot Tk (

T = 0°C, 0,57 g 2-nitropropy

laten warmen tot Tk

2-3 uur

ont