Tetrahedron Vol. 41, No. 15, pp. 2957 to 3020, 1985
Printed in Great Britain.

0040–4020/85 $5.00 + .00
© 1985 Pergamon Press Ltd.

TETRAHEDRON REPORT NUMBER 188

A SURVEY OF RECENT ADVANCES IN SELECTIVE CHEMICAL AND ENZYMIC POLYSACCHARIDE MODIFICATIONS

MANSSUR YALPANI
Domtar Research Centre, P.O. Box 300, Senneville, Quebec, Canada H9X 3L7

(*Received in USA* 1 *October* 1984)

CONTENTS

1. INTRODUCTION

1.1. *Scope of Review*

Considerable attention has been attributed in recent years to various physicochemical,[1-18] biochemical,[19-22] biomedical[23-27] and industrial[28-33] aspects of native and derivatized polysaccharides as evidenced by numerous monographs and reviews. Similarly, the sources, purification, and analytical applications of polysaccharases have been summarized.[34-37] There has, however, been no review to date which addresses methods for selective modifications of polysaccharides. Various comprehensive accounts of polysaccharides provide only brief summaries of several selective derivatization reactions in the context of their applications to structural elucidations.[2,38-42] A recent review of cyclodextrins contains a short section on this topic,[43] while a previous article contains information about a few selectively modified cyclodextrin derivatives.[44]

The objective of this review is to provide a general survey of recent developments in the area of selective chemical and enzymic modifications of polysaccharides. These methods have been classified according to the most frequently encountered functional groups and important polysaccharide residues rather than by polysaccharide type. No distinction has been made here between specific methods, i.e. those involving the exclusive modification of one particular type of residue or functional group, and selective ones, i.e. modifications which affect one functionality under conditions that minimize the rates of competing reactions such that one product predominates. Such distinctions are in most cases difficult to assess, as reactions involving reagents or enzymes which are expected to act specifically, are often found to be limited by factors associated with the secondary or tertiary structure of the polymer.

While classical synthetic methods for the selective degradation of polysaccharides have generally been limited to structural elucidation applications, the salient features of some of these methods have been included here to draw attention to their scope and potential utility for the preparation of modified polysaccharides. Similarly, the treatment of cyclodextrin modifications in this account is not comprehensive, in light of the availability of previous reviews.[43] Inclusion of the cyclodextrins is primarily intended to highlight the sharp contrast between these polymers and most other polysaccharides in their susceptibility to selective derivatization methods.

It should be noted that for the purposes of this review, selective methods do not include those commonly employed in the removal of sulfate, acetyl, pyruvate, and similar labile groups, as these are adequately described elsewhere.[37,45-48] Similarly, reactions involving the non-selective modification of vicinal diols or other alcohol functions, such as periodate oxidation,[49] cyanogen bromide activation,[45] or the formation of cyclic carbonate residues,[50] have, with certain exceptions, not been included. No reference has been made here to the effects of the structural modifications on the physical properties of polysaccharides, as this topic will be addressed elsewhere. The coverage of the literature extends to May 1984.

1.2. *Background*

After many years of neglect, the area of polysaccharide chemistry is presently experiencing a renaissance of unprecedented proportions. This is, in part, a consequence of advances in our

understanding of the fundamental role of these biopolymers in various biological processes, such as immunochemical recognition, and, on the other hand, a reflection of the growing appreciation of their functional versatility and immense commercial utility.

Thus, while traditional interest in polysaccharides was mostly confined to the usage of products such as cellulose and starch in the pulp and paper, food and allied industries, considerable efforts are now being directed at applications of animal and particularly bacterial polysaccharides[9,21,51] in industries ranging from adhesives, textiles, detergents, agriculture, paints, cosmetics, and pharmaceuticals, to explosives, drilling fluids, and enhanced oil recovery.[28–33,52] Many of these areas constitute large volume, high growth rate markets with annual sales of tens and hundreds of million dollars.[28] It can be anticipated that the low cost, rich abundance and broad spectrum of functional properties of polysaccharides will lead to an even wider utilization in the future. There are, for example, already indications of incursions by polysaccharides into markets which are presently dominated by petrochemical-based polymers.[53,54]

While until recently the prevailing approach to polysaccharide modifications involved "statistical" chemistry, i.e. the use of non-selective chemical reagents which afford randomly derivatized polymers, there is now a growing trend toward selective methods which either exploit differential chemical reactivities of available functional groups of the polysaccharide, such as carboxyl, hydroxyl, or amine functions, or which, in the absence of such sites, rely on the selective activation or introduction of appropriate reactive functions by way of chemical or enzymic methods. Some of the pioneering work in this area has been performed by Horton, Kochetkov, Lindberg, Wolfram and their co-workers.

Among the many advantages of selective over non-selective modifications are the choice, in many cases, of milder reagents and reaction conditions which help to eliminate or minimize the formation of side products and/or the partial degradation or depolymerization of the native polymer, as is often associated with non-selective derivatizations. Typical products obtained by the latter methods include the alkyl, hydroxyalkyl, carboxyalkyl, and triphenyl ethers, and the halogen, nitrate, phosphate, sulfate, sulfonate, and xanthate esters of various polysaccharides,[17,29,33] although, as will be seen in the subsequent discussion (see Section 2.1.2), a certain degree of selectivity can be achieved for some of the above derivatives under carefully controlled reaction conditions. An equally important advantage derives from the fact that selective derivatizations facilitate product characterization to a much greater extent than random methods, by eliminating uncertainties about the distribution of derivatized sites in the products. The location of substituents in partially derivatized polysaccharides can pose a formidable task in product characterizations.[55,56]

In addition to these practical considerations, selective modification techniques may play a pivotal role in the establishment of fundamental knowledge about polysaccharide properties. Despite the substantial advances in this field, the understanding of the important structure/function relationship of these polymers is extremely limited,[4,5,57–59] particularly in comparison to other classes of biopolymers. This is partly a reflection of their intrinsic complexity, such as their heterogeneity in terms of structure, molecular weight distribution, etc. In addition, the chemical and physicochemical properties of many polysaccharides often vary drastically, depending on their source and the pretreatment and extraction methods used. Another factor that distinguishes polysaccharides from other types of polymers is their unique chemical reactivity. Some routine organic derivatization reactions can either not be applied to polysaccharides without resorting to pretreatments or elaborate synthetic strategies, or else, proceed with only very low yield. Product formation is, on the other hand, in some cases observed under reaction conditions which have no formal analogy in organic chemistry. Other complications arise from the inadequacies of traditional analytical and synthetic methodologies. There is consequently a need for the development of facile methods for the preparation of model polysaccharides for a systematic evaluation of these aspects.

1.3. *Uses and Potential Applications of Selectively Modified Polysaccharides*

Concomitant with the expanding commercial utilization of polysaccharides, there is a growing demand for synthetic methods which facilitate selective structural modifications in order to affect, or ideally tailor, product properties such as viscosity,[60] hydrophilicity/hydrophobicity,[15,61] polyelectrolyte characteristic,[62] gelation[33] and metal chelating[11,63] capacity.

One of the most important applications of selective modifications is the synthesis of analogues of natural polysaccharides, such as dextran,[9,64] heparin[19] or xanthan gum,[9,65] for purposes of reducing

polymer cost, improving physical properties, or as the ultimate method of obtaining structural proof for the native polymer. In the biomedical context, selective chemical procedures are of interest in areas such as structure/activity elucidations or activity modifications of biologically active (e.g. antitumor[23] and anticoagulant[29,66]) polysaccharides. For the preparation of conjugates of polysaccharides and biological substrates[25,27,67] (enzymes, proteins, etc.), these methods may avoid or minimize cross-linking and extraneous chemical reactions, and afford better-defined products for applications such as affinity partition[68–70] and immunology.[26]

Selective modification is also of interest in polysaccharide applications involving the preparation of selectively permeable membranes,[71] matrices for drug delivery[25,27] and controlled release[72] formulations, as well as in various processes relating to the recovery of biological materials, such as in the preparation of substrates for affinity chromatography[45] and partitioning,[68] electrophoresis,[73] enzyme and cell immobilization,[74] etc.

The availability of selective modification methods is also of considerable value for the synthesis of branched polysaccharides, a particularly important category of polymers.[75,76] Thus, versatile strategies which would allow the synthesis of branched polymers, whose branch type, stereochemistry, and length, as well as degree of branching is readily controllable, could facilitate systematic investigations of the structure/function relationship of these biopolymers. Considerable efforts have already been directed at the conversion of linear polysaccharides into branched-chain analogues which by themselves are of interest for a variety of other reasons,[77,78] including the investigation of lectin–carbohydrate reactions,[79] and the preparation of model compounds in the fields of allergy,[80] enzymology,[81] and immunology.[82] Previous studies have, for example, applied to cellulose, amylose, alginic acid, and other polysaccharides various synthetic routes including (a) copolymerization,[83] (b) reaction with orthoesters,[84] acetobromosugars,[85] or hydrazones,[86] and (c) enzymic glycosylations.[87] These procedures, however, suffer from various limitations since they require (a) specific protection of the linear polysaccharide, such as in the reaction of 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose, (b) activation and/or partial protection of the saccharide residue which is to form the side chain and removal of the protecting groups subsequent to the reaction, (c) reaction conditions which may lead to partial or extensive degradation reactions, or (d) enzymes which are presently either specific for a limited number of carbohydrate substrates or not readily available on a large scale. Most of the above reactions are also laborious, costly, and low yielding, all reasons which mitigate against routine or industrial-scale applications.

2. HYDROXYL MODIFICATIONS

2.1. *Primary Alcohol Functions*

2.1.1. *Conversion to aldehydes and carboxyl functions*

2.1.1.1. *Oxidations.* The introduction of carbonyl groups into polysaccharides constitutes one of the most important synthetic tasks, because it affords reactive intermediates which are amenable to further modifications, such as oximation or reductive amination, epimerization or isotope (deuterium or tritium) labelling via reduction, and conversion into deoxy or branched derivatives via reaction with Grignard, Wittig, or other addition reagents. Selective oxidations are furthermore of interest in the evaluation of the stability of oxidized polysaccharides in various industrial processes, such as in the bleaching and aging of cellulose-containing materials.

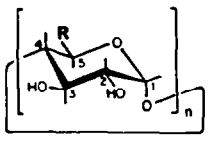
The complete oxidation of all primary hydroxyl functions of α- and β-cyclodextrin **1** and **2** has been reported by Casu *et al.*[88] to afford the corresponding hexakis- and heptakis(5-carboxy-6-deoxy-5-demethyl)cyclodextrin derivatives (**3** and **4**), using either nitrogen dioxide (N_2O_4) or catalytic (O_2/Pt) oxidations.

In contrast to the facile cyclodextrin modifications, there is, at present, no simple and generally applicable method available for selectively converting the C-6 hydroxyl functions of other types of polysaccharides to the corresponding aldehydes or carboxylic acids. Most oxidation procedures result in the formation of mixtures of aldehyde and acid residues and degradation products.[89] Considerable efforts have, for example, been directed at the oxidation of cellulose using nitrogen dioxide either in the gas phase or dissolved in carbon tetrachloride.[90] It has been shown that the predominant reaction is the conversion of D-glucose (**5**) to D-glucuronic acid (**6**) residues.[90] However, this is accompanied by some oxidation of secondary hydroxyl functions. Quantitative oxidations at C-6 can furthermore not be accomplished without concomitant depolymerization.

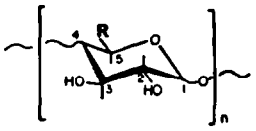
Painter[91] has described an improved synthesis of C-6-oxycellulose using nitrogen dioxide in the presence of sodium nitrate and phosphoric acid. The product contained 87.5% D-glucuronic acid residues and about 6% 2- and 3-keto groups, and exhibited higher degrees of polymerization than that obtained by previous methods. In a study of the blood-anticoagulant activities of heparin analogues, Hoffman *et al.*[92] have applied this method for the preparation of C-6-carboxyl derivatives of amylose, cellulose, guaran, and locust bean gum with uronic acid residue contents of between 20 and 60%. The application of this method has more recently been extended to amylose.[93] The reaction was, however, accompanied by nitrogen incorporation and severe depolymerization. Luzakova *et al.*[94] studied oxidations of bleached beech sulphate pulp with gaseous nitrogen dioxide and found initial introduction of up to 70 mmol carboxyl function per 100 g cellulose and subsequent formation of carbonyl function, the latter being associated with a sharp decrease in the degree of polymerization. The authors also found a stabilizing effect of manganese cations on the alkali resistance of oxidized cellulose samples.

Deneault *et al.*[95] have reported on catalytic oxidations of kraft pulp cellulose with ruthenium tetroxide which afforded oxy-cellulose products containing carbonyl and carboxyl groups. Their results seem to contradict an earlier claim[96] that this oxidant affords quantitative transformation of cellulose into C-6 carboxyl-cellulose. Snyder *et al.*[97] have reported that oxidation of cotton with dimethyl sulfoxide–acetic anhydride (DMSO–Ac_2O) and subsequent treatment with chlorous acid gave products with 47–62% carboxyl residues, the remainder being 2- or 3-keto functions.

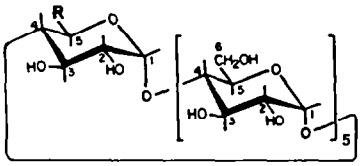
Aspinall and co-workers[98,99] have reported that terminal, primary hydroxyl functions of non-reducing residues, of side chains, and of otherwise sterically favourable positions can be preferentially oxidized to carboxyl groups via catalytic oxidation. This procedure introduces furanosyluronic and pyranosyluronic acid residues into the polysaccharide, but has found only limited application to date. Aspinall and co-workers have demonstrated this method for two highly branched plant polysaccharides, namely rye-flour arabinoxylan[98] and the arabinogalactan from European larch.[99] Treatment of these polysaccharides with oxygen, Adams catalyst and sodium hydrogen carbonate for 4 and 14 days, respectively, resulted in the formation of 4 and 7.5% uronic acid residues. Similarly, Heyns and Beck[100] found that application of this method to 1 → 4 linked polysaccharides containing primary hydroxyl functions in each repeat unit gave only very low yields of oxidation due to apparent steric hindrance factors. Adams[101] has applied this method to an arabinogalactan.



	R	n
1	CH_2OH	6
2	CH_2OH	7
3	CO_2H	6
4	CO_2H	7

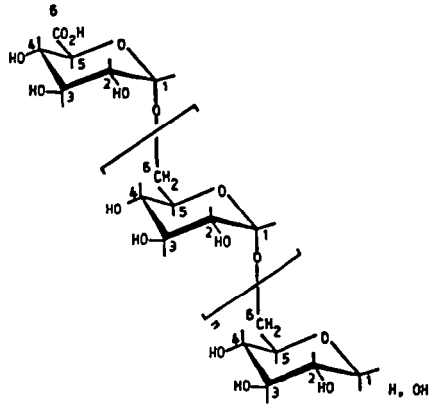


	R
5	CH_2OH
6	CO_2H



	R
12	CHO
13	CH_2OTs
14	CH_2N_3
15	CH_2NH_2

Catalytic oxidation appears to be best suited only for highly branched polysaccharides containing 1 → 6 linked backbones. A number of workers have applied catalytic oxidations to different (branched and virtually unbranched) dextrans (see Section 7.1.2).[102,103] Typical reaction conditions involved oxidation at 70° for 23–30 days.[102] Oxidative conversions to the carboxyl derivative **7** of up to 80–85% have been reported by Abbott *et al.*[102] and by Miyah *et al.*[103]

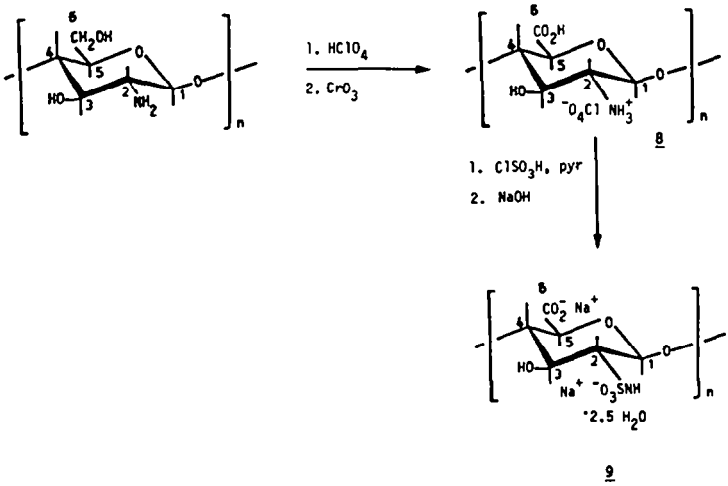


7

Horton and Just[104] treated chitosan with perchloric acid to obtain the ammonia perchlorate salt and utilized the bulkiness of this group for protection of the C-3 hydroxyl group in order to achieve selective oxidations at C-6 (Scheme 1). Using chromium trioxide they obtained the C-6 carboxyl derivative of chitosan (**9**) with a degree of substitution (d.s.) of 1.0, i.e. in quantitative yield, and with apparent retention of polymer integrity (average molecular weight 580,000). Similarly, a partially 2-trifluoroacetamide-substituted amylose derivative was selectively oxidized at the primary alcohol function using catalytic oxidation (O_2/Pt) to produce, after sulfation, a heparin analogue **11** (d.s. CO_2H 0.46, Scheme 2).[105] Defaye[106] has recently reported the selective oxidation of unprotected amylose using hydrogen peroxide. However, the same reagent could apparently not be applied successfully to cellulose.

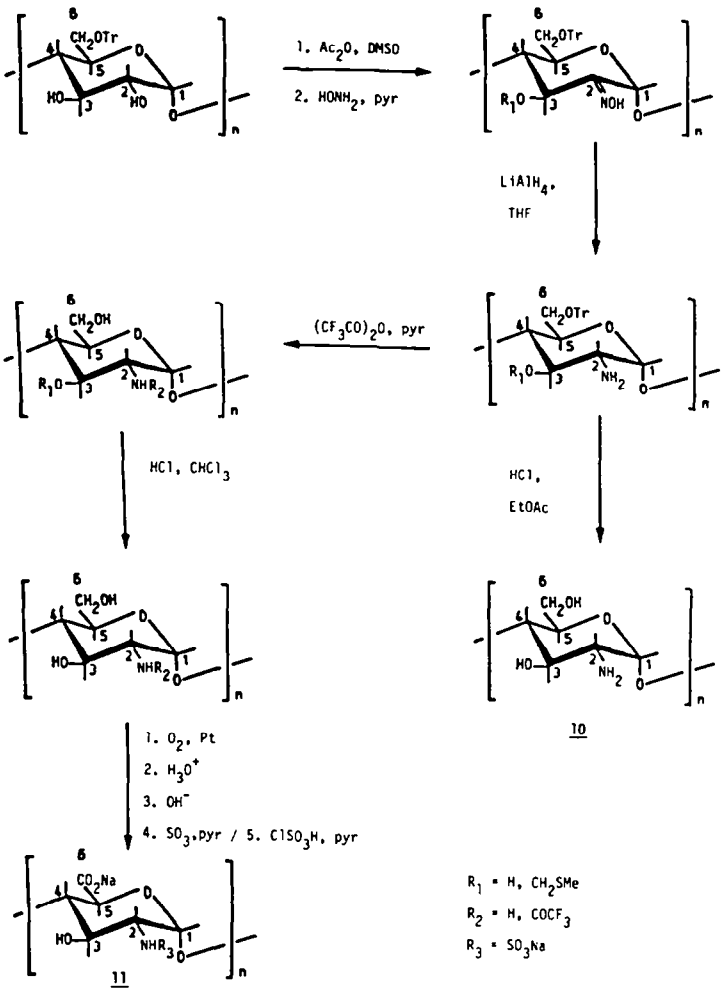
Recent ESCA studies of chromium trioxide-treated wood surfaces have shown that oxidations occur mainly at primary hydroxyl functions, which are initially converted to the corresponding carboxylic acid functions.[107] Decarboxylation occurs subsequently as evidenced by carbon dioxide evolution.

2.1.1.2. *Other chemical routes.* A mono-6-carboxaldehyde derivative of α-cyclodextrin (**12**) has been prepared by Slessor and co-workers[108,109] via two routes. The first involved the conversion of the lyophilized mono-6-*O*-tosyl-α-cyclodextrin intermediate (**13**) into the corresponding mono-6-azide



8 **9**

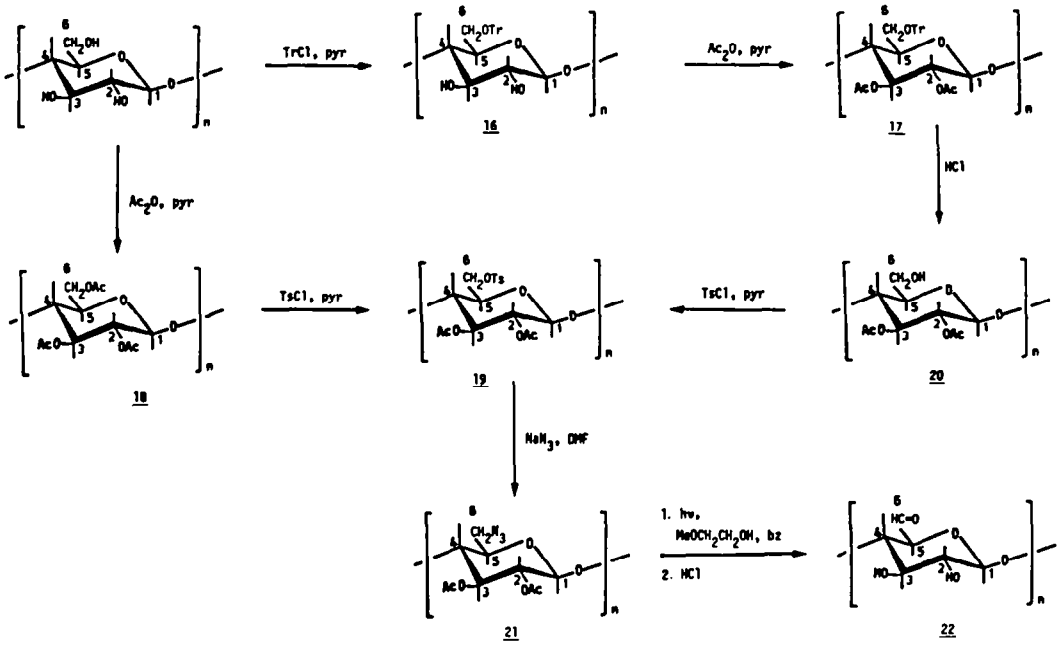
Scheme 1. Synthesis of heparin analogue from chitosan (Ref. 104).



Scheme 2. Synthesis of heparin analogue from amylose (Ref. 105).

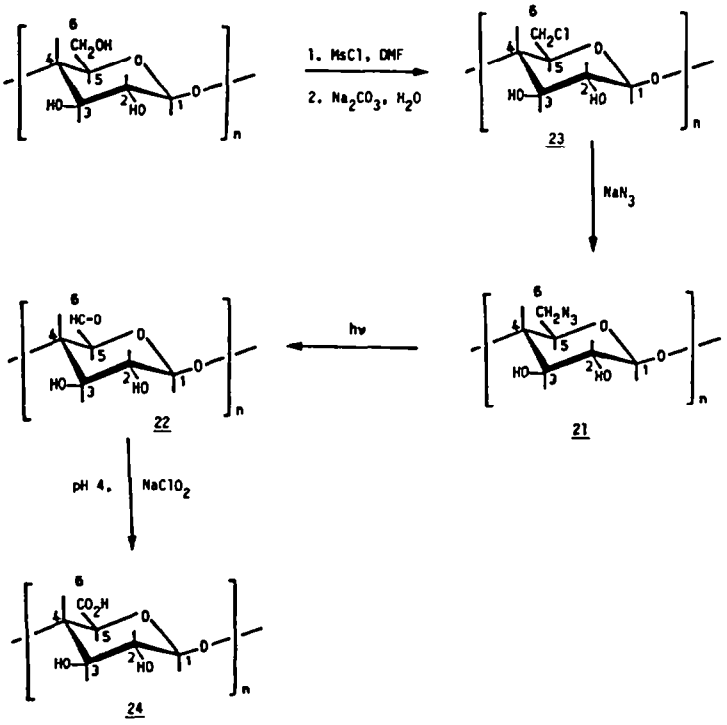
(**14**) using sodium azide, and subsequent photolysis to the mono-(5-formyl-5-demethyl-6-deoxy)-α-cyclodextrin product.[109] The second route relied on catalytic reduction of the above mono-6-azide to obtain the mono-6-amine **15**, which was then oxidatively deaminated by treatment with ninhydrin in aqueous sodium bicarbonate.[108]

The first synthesis of C-6 aldehyde cellulose (**22**) was reported by Clode and Horton[110] and involved some six chemical reactions (Scheme 3). The aldehyde derivative (d.s. 0.45) was obtained via (i) 6-*O*-triphenylmethylation (**16**), (ii) acetylation of the secondary hydroxyl functions (**17**), (iii) removal of the triphenylmethyl groups (**20**), (iv) 6-*O*-toluenesulfonation (**19**), (v) azidation at C-6 (**21**) and, (vi) photolysis of the azido group to yield the 6-carboxaldehyde-2,3-diacetyl cellulose derivative. The degree of polymerization (d.p.) of the product was not reported. Horton *et al.*[111] subsequently reported the preparation of 6-aldehyde cellulose via heterogeneous chlorination. Their synthetic strategy involved sequential chlorination using methanesulfonyl chloride in *N*,*N*-dimethylformamide to obtain 6-chloro-6-deoxy-cellulose (**23**), and conversion into the corresponding 6-azido-6-deoxy derivative using sodium azide as shown in Scheme 4. The d.s. values of the C-6-aldehyde product varied between 0.03 and 0.45, depending on the type of cellulose starting material employed. Thus, from a series of products derived from cotton, paper, and regenerated cellulose, the latter had the highest substitution values. The formation of the 6-chloro and subsequent products was accompanied by mild to substantial depolymerization and discoloration, and some crosslinking. The percentage aldehyde incorporation was determined by a deuteration-mass spectrometry method and by copper number. Similar techniques have been applied in the synthesis of C-6-aldehyde derivatives of amylose.[112]

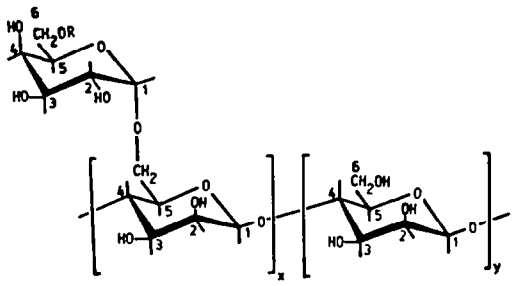


Scheme 3. Synthesis of C-6 aldehyde cellulose (Ref. 110).

Various workers have described the specific transformation of C-6 aldehyde functions of the galactose residues of oxidized guaran (**26**) (see Section 8.2) into carboxyl functions (**27**) using either mild bromine, iodine or other halogen-based oxidations.[113,114] Several galactomannans have been transformed into galacturomannan derivatives using these procedures.[115,116] Aspinall and Chaudhari[117] converted the terminal galactopyranosyl residues of *Pneumococcus* type 14 polysaccharide into D-galactosyluronic acid residues by sequential galactose oxidase and hypoiodide oxidations.



Scheme 4. Synthesis of C-6 aldehyde cellulose (Ref. 111).



	R
25	CH_2OH
26	CHO
27	CO_2H
28	$CH_2NH_2CH_2CO_2H$

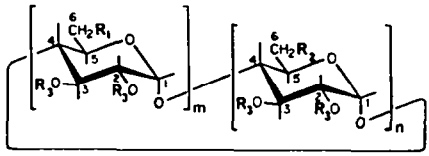
An alternative approach for the introduction of carboxyl functions has been demonstrated recently for galactose oxidase-treated guar gum.[118] The guar gum aldehyde derivative was reductively aminated with glycine to afford the corresponding cationic derivatives **28**.

2.1.1.3. *Enzymic oxidations.* The efficient, stereospecific transformation of the primary alcohol functions of galactose-containing polysaccharides to the corresponding C-6 aldehyde derivatives will be discussed in Section 8.2.

2.1.2. *Triphenylmethyl ethers, toluenesulfonyl esters and related derivatives*

Selective modifications of the primary hydroxyl functions of cyclodextrins can be performed to yield mono-, di-, tri- tetra-, and poly-C-6 substituted products, as well as a range of mixed C-6 substituted products.[43,44]

The preparations of many monoalkyl cyclodextrins have relied on the corresponding mono-6-triphenylmethyl (trityl) intermediates **29**, whose synthesis was originally described by Melton and Slessor.[109] Similarly, 6-*O*-monotoluenesulfonyl-α-cyclodextrin **30** has been prepared by esterification of lyophilized α-cyclodextrin in pyridine.[119] Boger and co-workers[120] have reported the synthesis of a



	R_1	R_2	R_3	m	n
29	OTr	OH	H	1	5
30	OTs	OH	H	1	5
31	OMs	OH	Me	3	3
32	OTr	OH	Me	4	3
33	OTr	OH	H	4	3
34	ODMTr	OH	H	4	3
35	NH_2	NH_2	H	3	3
36	OMe	OMe	H	3	3
37	N_3	N_3	OAc	4	3

Tr = $C(C_6H_5)_3$

Ts = $SO_2C_6H_4CH_3$

Ms = SO_2CH_3

Me = CH_3

Ac = $COCH_3$

ODMTr = 4,4'-dimethoxytriphenylmethane

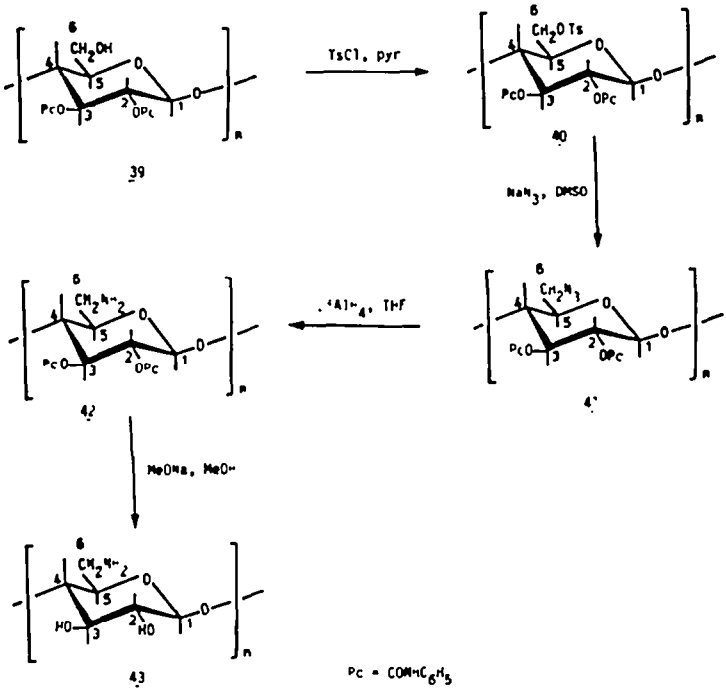
6-*O*-trimethanesulfonate (mesyl) derivative, $6^A, 6^C, 6^I$-tri-*O*-mesyl-$2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 3^E, 3^F, 6^A, 6^C, 6^E$-pentadeca-*O*-methyl-α-cyclodextrin (**31**), which was derived from the corresponding tri-trityl derivative, $6^A, 6^C, 6^E$-tri-*O*-trityl-α-cyclodextrin **32**. The latter compound, in turn, had been chromatographically isolated from a mixture of di-, tri-, and tetra-trityl derivatives. Cramer *et al.* have described the synthesis of a tetrakis-6-*O*-trityl-β-cyclodextrin **33** and an analogous tetrakis-[-6-*O*-(4,4'-dimethoxytrityl)]-β-cyclodextrin derivative **34**.[119] Derivatives of α- and β-cyclodextrins in which all primary hydroxyl functions are substituted by tosyl-, mesyl-, or other arylsulfonyl esters have been reported.[119]

Boger *et al.*[44] have prepared α- and β-cyclodextrin derivatives whose primary hydroxyl groups were selectively modified by two methods. One involved selective activation of the primary hydroxyl groups via a bulky triphenyl phosphonium salt and subsequent substitution by azide ion. The other, indirect approach, involved perbenzoylation of the hydroxyl functions and subsequent selective deprotection of the primary alcohol functions. Using these techniques, hexakis-(6-amino-6-deoxy)-α-cyclodextrin **35**, hexa-(6)-*O*-methyl-α-cyclodextrin **36** and heptakis-(6-azido-6-deoxy)-β-cyclodextrin-tetradeca-(2,3)-acetate **37** were synthesized.

The partly substituted cyclodextrin derivatives mentioned above have been employed for the synthesis of a variety of amino-,[120,121] azide-,[44] and halo-[119,122] derivatives as well as for cyclodextrin products with pendant imidazole,[123] nucleobase,[124] porphyrin,[125] pyridine,[126] and sulfur-containing[119,127] functional groups by nucleophilic reactions with the appropriate reagents.

Preferential substitution of the primary hydroxyl groups of cellulose and amylose can be accomplished under controlled reaction conditions using trityl chloride[128,129] or, to a more limited extent, *p*-toluenesulfonyl (tosyl) chloride.[130,131] However, quantitative substitutions of primary positions cannot be achieved without some concomitant derivatization of secondary positions. For example, 6-*O*-trityl cellulose with a d.s. of 0.98 can be obtained having a minimum 2-*O*- and 3-*O*-substitution level of d.s. 0.07, while 6-*O*-substitution of d.s. 0.99 is associated with 10% substitution at positions 2 and 3.[128] The tritylation of cellulose proceeds initially 58 times faster at the primary than the secondary positions. However, with increasing substitution this ratio eventually decreases to about unity.[128,130]

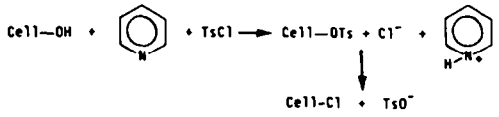
A 6-*O*-phenyl cellulose product **38** with d.s. 0.75 has been derived from 6-*O*-tosyl cellulose and sodium phenoxide which reportedly contained no substituents at secondary positions.[128,132] The synthesis of 6-*O*-tosylated starch has also been reported.[133]



Scheme 5. Synthesis of 6-amino-6-deoxy cellulose (Ref. 142).

Comparative rates have been measured for the esterification of the 6-*O*-, 2-*O*- and 3-*O*-positions of ethylcellulose and cellulose acetate, respectively, using tosylchloride;[134,135] the relative ratios of the first-order rate constants were found to be 15 : 2.3 : 0.07 and 23 : 2.2 : 0.11, respectively. Similarly, it has been observed that the heterogeneous tosylation of cellulose proceeds 5.8 times faster at the primary than the secondary positions.[130,134] In contrast, the relative reactivities of *O*-6, *O*-2, and *O*-3 with respect to carboxymethylation were determined by Croon and Purves to be 2.5 : 2 : 1.[136,137]

It should be emphasized, however, that both the tritylation and tosylation reactions are associated with side reactions, particularly at elevated temperatures. These can include the formation of: (i) intra-residue cyclic ethers, e.g. 3,6-anhydroproducts from the corresponding 6-*O*-tosyl derivatives;[130] (ii) chlorinated polysaccharide products in the presence of pyridine;

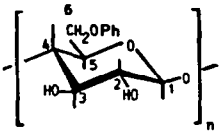


(1)

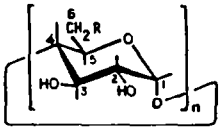
and (iii) quaternary pyridinium salts of polysaccharides.[130] Furthermore, the removal of tosyl groups usually involves competing reactions, and trityl group migration from primary to secondary positions has been observed during the tritylation of cellulose.[129,138] The loss of trityl groups has been reported to occur under alkaline conditions,[139] or during subsequent derivatization reactions.[140] Lastly, a certain degree of depolymerization is also associated with the tritylation as well as with the detritylation by acid treatment.[128,138]

2.1.3. *Other* C-6 *substituted derivatives*

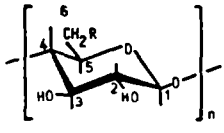
A variety of halogenated and other types of C-6 derivatives have been prepared by nucleophilic substitution of the corresponding trityl or tosyl intermediates.[141] Thus, 6-amino-6-deoxy cellulose (**43**) has been prepared by Teshirogi *et al.*[142] via the 6-*O*-tosyl, and 6-azido intermediates in low yield (Scheme 5). The same group[143] has also reported the synthesis of a predominantly C-6 substituted amino starch product using a tosylated precursor (yield 14%).



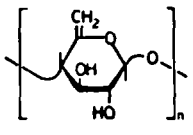
38



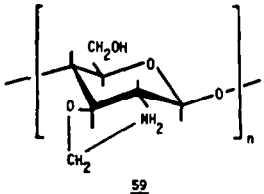
	R	n
44	Br	6
45	Br	7
46	Br	8
47	H	6
48	H	7
49	H	8



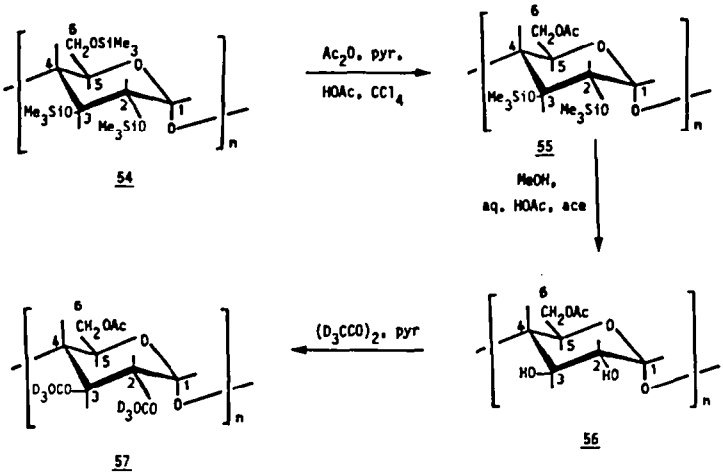
	R
50	OH
51	Br
52	Cl
53	I



58



59



Scheme 6. Synthesis of 6-*O*-acetylamylose (Ref. 147).

Takeo *et al.*[144] prepared 6-bromo-6-deoxy derivatives (**44–46**) and 6-deoxy derivatives (**47–49**) of α-, β- and γ-cyclodextrin and amylose (**50, 51**) in high yields. Selective brominations were achieved using methanesulfonyl bromide and DMF for 18–20 hr at 65°. The resulting brominated and formylated products were deformylated with sodium methoxide in methanol to afford 6-bromo-6-deoxy derivatives of the cyclodextrins (95–98% yield, d.s. 1.0) and amylose (91% yield, d.s. 0.92). These derivatives were used as precursors for the preparation of the corresponding 6-deoxy products. The latter synthesis involved conversion of the brominated products to 2,3-di-*O*-acetates, reductive debromination with sodium borohydride in DMSO (70°, 2 hr), and deacetylation with sodium methoxide in methanol. The 6-deoxy products were obtained in 89–95% yields.[144]

Wolfram *et al.*[145] treated amylose with sulfurylchloride to obtain the 6-chloro-6-deoxy-amylose product **52**. Guerrera and Weill[146] have reported the synthesis of 6-iodo-6-deoxy-amylose **53**.

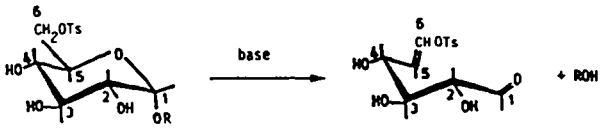
In a different approach, 6-*O*-acetylated amylose has been prepared by Horton and Lehmann[147] using a pertrimethylated starting material (**54**), which was treated with pyridine–acetic anhydride–aqueous acetic acid in carbon tetrachloride to yield, depending on reaction duration, products with different degrees of acetylation (Scheme 6). Thus, reaction for five days at 45–50° gave 6-*O*-acetylated products (**56**), whereas, after 14 days, a quarter of the secondary hydroxyl groups were also acetylated. Removal of the *O*-trimethylsilyl groups with methanol–aqueous acetic acid in acetone gave 6-*O*-acetyl-amylose with d.s. 1.0. The procedure was reported to be applicable to amylose samples from different sources without appreciable changes in the d.s. values of the products.

Ishii *et al.*[148] and others have reported on the chlorination of cellulose. Treatment of hardwood dissolving pulp dissolved in chloral/dimethylformamide with mesyl chloride at 75° produced initially 6-chloro-6-deoxy-cellulose up to d.s. levels of about 1.0.[148] Further chlorination gave rise to additional chlorination at C-3 which was accompanied by Walden inversion to yield 3,6-dichloro-dideoxy-cellulose. Prolonged chlorination resulted in polymer degradation reactions. Such halogenated cellulose products are useful intermediates as demonstrated by the hydrazination studies of Machida and Sueyoshi.[149] These authors obtained hydrazinedeoxy-cellulose with d.s. 0.6, and presented evidence that residual chlorine residues were hydrolyzed to the corresponding primary alcohols during the reaction. The products were used for selective metal chelation.

6-Halogeno-6-deoxycellulose derivatives have been prepared by reaction of 2,3-di-*O*-acetate cellulose with *N*-halogenosuccinimides, and subsequent deacetylation.[150] Dehydroiodination of 6-iodo-6-deoxycellulose with alcoholic potassium hydroxide or piperidine afforded the unsaturated 5,6-cellulosene derivative **58** which was used as starting material for the synthesis of several metal (lead or tin)-containing 6-deoxycellulose derivatives.[150]

Whistler[151] has prepared 6-*O*-sulfated chitosan using either chlorosulfonic acid/pyridine or sulfur trioxide/dimethylformamide treatments.

The susceptibility of glycosidic linkages of hexapyranosides containing 6-*p*-toluenesulfonyl-6-deoxy groups to base catalyzed β-elimination reactions[2,40–42] (Scheme 7) has been employed for the selective degradation of several polysaccharides. In view of the fact that, for example, in dextran only



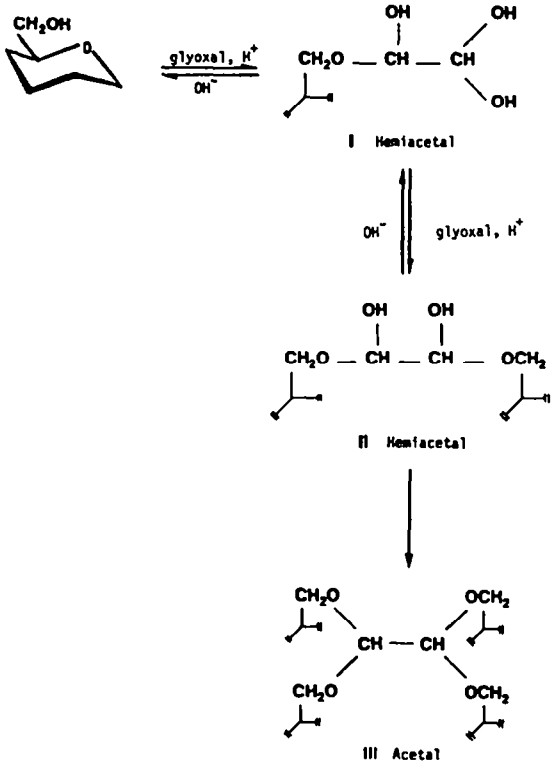
Scheme 7. β-Elimination of aryl sulfonate derivatives.

the terminal glucosyl residues of the backbone and of the branches bear unsubstituted primary hydroxyl functions, Larm *et al.*[152] could achieve the selective removal of these terminal glucosyl residues using tosylated derivatives. These methods could be of utility in selective, low-level substitution reactions, as will be discussed in Section 7.

The treatment of polysaccharides with various aldehydes, such as glyoxal, chloral, or paraformaldehyde, has been shown to result in preferential formation of hemiacetals at the C-6 position[153] (Scheme 8). Such reversible substitution reactions have been successfully exploited in selective modifications of secondary hydroxyl functions.

Thus, Nicholson and co-workers[154,155] have reported on the homogeneous carboxymethylation and methylation of cellulose using the dimethyl sulfoxide/paraformaldehyde solvent system. Their results indicated a preferential alkylation at the secondary hydroxyl groups, which were found to be 1.5 times more reactive than the primary hydroxyls due to the predominant formation of C-6-methylol cellulose. This finding was supported by the fact that partial removal of the methylol substituents by heat treatment resulted in products with higher degrees of C-6 alkylation. On the other hand, carboxymethylation of cellulose in *N*-methylmorpholine-*N*-oxide/DMSO (1:9) was shown to be primarily directed at the C-6 position.[155]

Petrapavlovsky *et al.*[156] have investigated the chemical modification of cellulose by solid state reaction with glyoxal. They found that glyoxylation proceeds mainly at the primary alcohol functions and obtained d.s. values of up to 0.58.



Scheme 8. Glyoxal-mediated hemiacetal and acetal formation (Ref. 153).

Sandford *et al.*[153] and others[157–159] have shown that treatment of polysaccharides, such as xanthan gum, with glyoxal affords products with improved dispersibility and solubility characteristics, due to hemiacetal and acetal formation of the primary alcohol functions. Bosso *et al.*[160] have utilized the DMSO/paraformaldehyde solvent system for selective oxidations of the C-3 hydroxyl groups of amylose and cellulose, as will be discussed in greater detail in the subsequent section.

On the other hand, Dutkiewicz[161] has reported that treatment of dilute chitosan solutions in acetic acid with formaldehyde leads to formation of products (**59**) in which the added formaldehyde is involved in intramolecular reactions between secondary hydroxyl groups and amine functions.

In the synthesis of cellulose sulfates by homogeneous transesterification of cellulose nitrite with *N,N*-dimethylformamide–sulfur trioxide, Schweiger has observed that the sulfation proceeds preferentially at secondary positions when the product d.s. is kept below 1.0.[162] Thus, for cellulose sulfate products with d.s. values of 0.45, 0.73, 0.95, and 1.05, the levels of C-6 substitution as a percentage of total substitution were found to be 0.13, 14.4 and 17.8%, respectively.[162] By contrast, the ratio of primary to secondary substitution in products derived from heterogeneous derivatizations, such as carboxymethyl cellulose, usually approaches unity.[136]

2.2. *Secondary Alcohols*

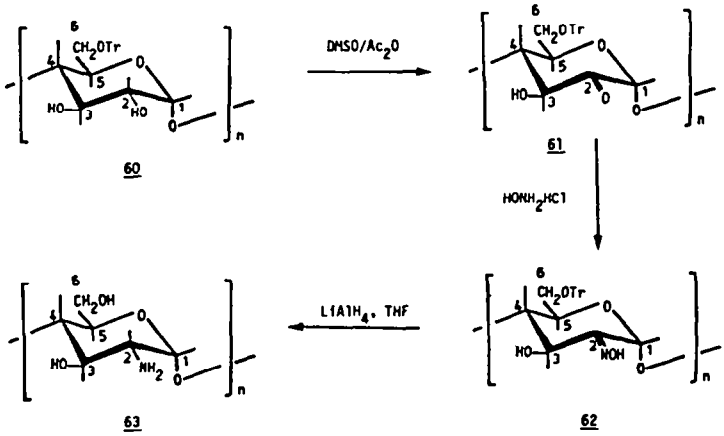
2.2.1. *Oxidations*

Perhaps the most significant advances in the selective modification of polysaccharides have been witnessed in the area of secondary alcohol derivatizations. Several groups of workers have developed mild oxidation procedures for various polysaccharides.

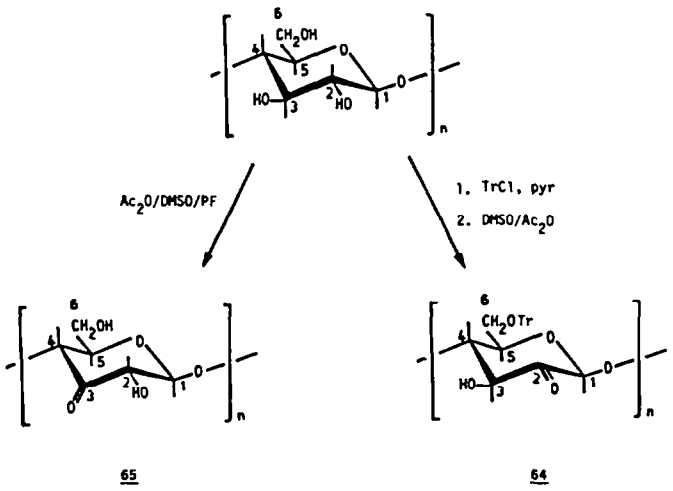
The first such study involved the oxidation of the hydroxyl function at C-2 of amylose.[163] Starting with 6-*O*-trityl amylose, Wolfram and Wang used dimethyl sulfoxide–acetic anhydride (DMSO/Ac_2O) to produce 2-oxy-amylose, which after subsequent oximation, reduction, and detritylation, was converted into the corresponding 2-amino-2-deoxy derivative **63** with d.s. 0.8 (Scheme 9).[163]

Horton and Usui[164] reported that oxidation of 6-*O*-tritylamylose with dicyclohexylcarbodiimide/DMSO afforded 2-oxyamylose when appropriate concentrations of oxidant were employed.

Brederick subsequently observed that oxidation of regenerated cellulose with DMSO/Ac_2O yielded mixtures of 2-oxy-, 3-oxy-, and 2,3-dioxy-cellulose products.[165] The author also used the Pfitzner Mofatt reagent (DMSO/dicyclohexylcarbodiimide/pyridine/trifluoracetic acid) as oxidant without, however, providing a comparison of the efficiency of the two oxidants. Subsequently, Bichoreva *et al.* concluded in 1974 that oxidation of 6-*O*-tritylcellulose with DMSO/Ac_2O did not constitute a viable method for selective oxidations, since a mixture of carbonyl, carboxyl, and aldehyde functions was generated.[166] These conclusions were, however, contradicted by results obtained by Defaye and co-workers, who employed this method for the selective oxidation of 6-*O*-tritylcellulose to obtain the corresponding 2-oxy-cellulose derivative (**64**, Scheme 10).[167,168] Further developments were achieved by the same group following the discovery of the DMSO/paraformaldehyde solvent system for cellulose.[160] They found that 3-oxy-cellulose (**65**) could be obtained in yields of 60–70% without prior C-6 protection of the native polymer, using the DMSO/Ac_2O oxidation system in



Scheme 9. Synthesis of 2-amino-2-deoxy-amylose (Ref. 163).



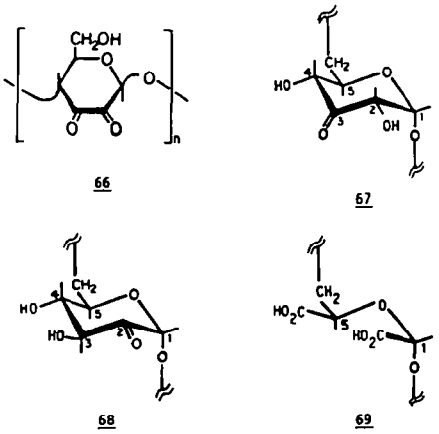
65 64

Scheme 10. Synthesis of 2-oxy- and 3-oxy-cellulose derivatives.

combination with the DMSO/paraformaldehyde solvent[169] (Scheme 10). Detailed studies of the oxidation products obtained from 6-*O*-tritylcellulose, 6-*O*-tritylamylose, and 6-*O*-acetyl-cellulose were performed using ^{13}C-NMR, deuteration and GLC methods. The NMR studies showed that formaldehyde substitution occurred initially at the C-6 and C-2 positions of amylose and methyl-4-*O*-methyl-α-D-glucopyranoside, and with increasing paraformaldehyde (PF) concentration also at C-3. While oxidation of unprotected cellulose with DMSO/Ac_2O/PF proceeds exclusively at position C-3, it was found that in the case of amylose some 10% oxidation had also occurred at C-2 at similar overall levels of oxidations (degree of oxidation, d.o., 0.6–0.7). For 6-*O*-tritylcellulose, they found a greater proportion of 2-oxy-(56%) than 3-oxy-(36%) products. On the other hand, oxidation occurred exclusively at C-2 for 6-*O*-tritylamylose, but 56% at C-2 and 30% at C-3 in the case of 6-*O*-acetyl-amylose (d.o. 0.7). These data would tend to indicate that the selectivity of C-2 oxidation is not related to the bulkiness of the C-6 substituent; although the authors quote unpublished results by Horton and Usui, who reportedly found exclusive C-2 oxidation for 6-*O*-acetylamylose. It can be concluded from the work of Bosso *et al.*[160] that the selective oxidation of C-3 positions of unprotected amylose and cellulose is due to the reversible, covalent formation of hydroxymethyl and poly(oxymethylene)ol groups at positions C-2 and C-6.

Defaye and Gadelle have examined the effect of various magnesium salts on the resistance of 2- and 3-oxy-celluloses to alkaline-oxygen degradation.[168,169] The authors found that magnesium salts, particularly its carbonate, exert a stabilizing effect on the partially oxidized celluloses. A mechanism involving chelates of magnesium with the tautomeric 2,3-enediol form of the hexulopyranoside units was suggested to account for these observations. Confirmatory evidence was subsequently obtained from ^{1}H- and ^{13}C-NMR experiments of model monosaccharides and oxy-celluloses.

DeBelder *et al.*[170] oxidized dextran-2,4-diphenylboronate with DMSO/Ac_2O and obtained products (**67**, **68**) with C-2/C-3 oxidation ratios of 0.7, but with no oxidation occurring at C-4. The d.o. values for C-2 and C-3 were 0.08 and 0.115, respectively.



66 67

68 69

It should be noted that oxidations involving the use of DMSO are usually accompanied by the introduction of *O*-methyl thiomethyl groups, which may have undesirable consequences such as odour development.[97] However, these groups can readily be removed by mild acid treatment.[92]

Larm and co-workers[172–179] have examined the use of aqueous bromine for the selective oxidation of several polysaccharides. In contrast to previous applications of this oxidant,[180,181] their methods do exhibit varying degrees of selectivity for different polysaccharides and do not, in all cases (see subsequent discussions for exceptions), give rise to undesired side reactions, such as overoxidation and polymer degradation. For example, bromine oxidation of starch and starch derivatives had previously been employed to obtain mainly aldehyde derivatives.[180] However, some of the side reactions observed included oxidation of the primary hydroxyl groups to carboxyl functions, and cleavage of the polymers.[182]

By contrast, the mild bromine oxidation of Sepharose (crosslinked agarose) was found to proceed at the secondary C-4 hydroxyl function.[178] For alginic acid, this procedure was somewhat less selective, giving rise to oxidation at C-2, but also at other positions.[173] Similarly, mixed oxidation products were obtained for cellulose and curdlan.[175] On the other hand, bromine oxidation of dextran resulted mainly in oxidations at C-2 and C-4, when equimolar concentrations of oxidant and glucopyranoside were employed. For total oxidation levels of 50%, the relative d.o. for C-2, C-4 and C-3 were found to be 0.215, 0.25 and 0.04, respectively, with the oxidations at C-3 arising in all likelihood from isomerization of 2-oxy- and 4-oxy-products. Overoxidation resulted in an additional small percentage of acidic, ring cleavage products (**69**).

Ziderman and Bel-Ayche[183] have observed that treatment of amylose suspensions with dilute aqueous bromine at 30° introduced carbonyl groups, if the reaction was conducted at pH 6–7, whereas at pH 8 both carbonyl and carboxyl groups were produced in equal amounts. Interestingly, for oxidations of waxy maize starch (100% amylopectin) at pH 6–8 the ratio of carbonyl : carboxyl groups formed was 2 : 1. The overall degrees of oxidation (carbonyl and carboxyl) for amylose were in the range of 0.54–0.68. The authors concluded from IR evidence that amylose oxidation at pH 8 introduced aldehyde functions at C-6 and suggested that the helical conformation of the polymer may be responsible for rendering O-6 more reactive towards bromine than O-2 and O-3.[183]

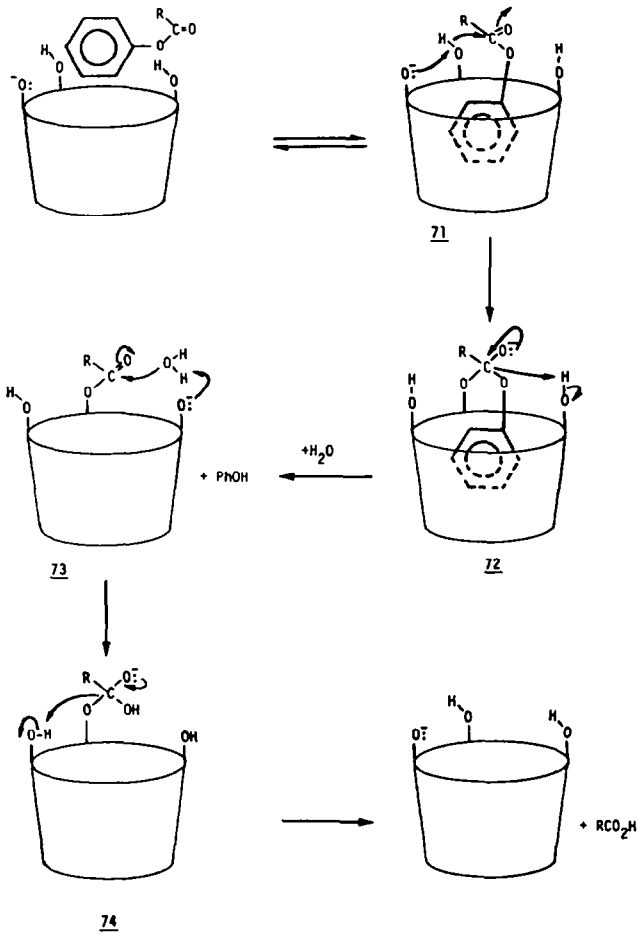
Some disadvantages observed for the bromine oxidation of Sepharose CL-4B and Sephadex G50[178] include (a) low overall d.o. levels: 8.5–9.4 mol percent/hexose residue and 18–30 mol percent/hexose residue for Sepharose and Sephadex, respectively, with Sephadex losing its rigidity at the upper d.o. levels; and (b) introduction of substantial amounts of carboxyl functions: 5.6–11.2 mol percent/hexose residue and 3–11.6 mol percent/hexose residue for Sepharose and Sephadex, respectively, at the above d.o. levels.

A potentially important modification of the bromine oxidation has recently been developed by Augustinsson and Scholander.[179] Exploiting the fact that carbohydrates containing *cis*-oriented vicinal diols form borate complexes in aqueous solution, the authors performed bromine oxidations of dextran (T40) in the presence of sodium metaborate in order to avoid oxidative cleavage to dicarboxylic acid residues. They found for various bromine concentrations, that although the relative amounts of 2- and 4- (and traces of 3-) glycosylulose residues introduced remained unaffected by the presence of borate ions, the latter caused a drastic increase in the ratio of glycosylulose to dicarboxylic acid residues. Using, for example, a concentration of 1 mol oxidant per glusosyl unit in the presence of borate, the yields of glycosylulose and dicarboxylic acid residues were *ca* 65% and less than 3%, respectively. The comparable yields in the absence of borate were 43 and 11%, respectively.

Lastly, in this context it should be mentioned that selective oxidations of secondary hydroxyl groups can in some instances be accomplished using sodium periodate. Selectivity in these cases can rely on either differential oxidation rates of *cis* and *trans* diols, on different degrees of steric accessibility, or on other structural uniqueness.

It has been found that, in general, periodate oxidations of *cis*-1,2-diols proceed at faster rates than the corresponding *trans*-isomers.[49,184] Thus, Ebisu *et al.*[185] have reported that the limited periodate oxidation of the *Pneumococcus* S-14 polysaccharide could lead, via Smith degradation, to the selective removal of β-D-galactopyranosyl branch groups without affecting the 1 → 4-linked β-D-glucopyranosyl residues of the backbone. Similar results have also been reported for other bacterial polysaccharides.[182,186]

Painter and co-workers have collected data for the initial second-order rate constants of periodate oxidations of some 18 polysaccharides, including amylose, dextran, guaran and lichenan.[188] These



Scheme 11. Mono-*O*-acylation of cyclodextrin (Ref. 43).

data provide a useful means of determining reaction conditions for selective periodate oxidations. The authors have also delineated the role of several reaction parameters, such as the ionic strength of the reaction medium, in achieving selectivity in the oxidation of various types of polysaccharides.

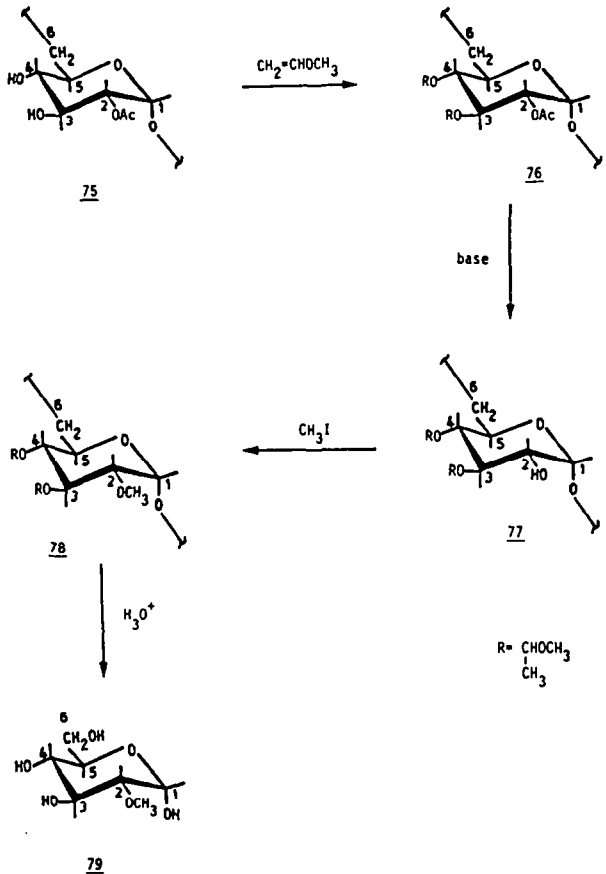
Further applications of the selective periodate oxidation of branch residues in guar gum, locust bean gum, and scleroglucan are discussed in Section 7.2.1.

2.2.2. *Other chemical modifications*

Monoacylations of the secondary hydroxyl groups of cyclodextrins have been accomplished by a method developed by Bender and co-workers.[10,189] This method involves the formation of a cyclodextrin inclusion complex with an aryl ester guest molecule whose ester carboxylate contains the desired acyl residue (Scheme 11). Covalent catalysis by the secondary hydroxyl (O-2 or O-3) groups of the host affords a covalent mono-2- (or -3-) substituted cyclodextrin product (**73**) which can be often isolated. A variety of monoacyl derivatives of α- and β-cyclodextrin have been prepared in this fashion, including derivatives containing unsaturated side chains,[190] ferrocenyl moieties,[191] and nitroxide spin label residues.[192]

Breslow and Czarnik have selectively attached pyridoxamine to the C-3 position of β-cyclodextrin, using pyridoxamine thiol and a tosylate precursor.[193]

DeBelder and Norrman[194] found variations in the distribution of *O*-acetyl residues in dextrans which were prepared by two acetylatious procedures. The authors employed methyl vinyl ether as protective reagent for the replacement of *O*-acetyl groups by stable *O*-methyl groups in a strategy outlined in Scheme 12. The resulting *O*-methyl-*O*-(1-methoxyethyl) dextran derivative (**78**) was then hydrolyzed, facilitating the elucidation of acetyl substitution patterns. Using this method, it was shown that the preparation of partially *O*-acetylated dextrans (d.s. ~ 0.6–0.7) with acetic anhydride in aqueous alkali leads to an essentially random distribution of C-2, C-3, and C-4 substituents, whereas



Scheme 12. Synthesis of *O*-methyl-*O*-(1-methoxyethyl) dextran (Ref. 194).

acetylation in pyridine results in preferential C-2 substitution, with the ratio of percentage molar C-2 : C-3 : C-4-substitution being 22.2 : 9.3 : 8.9.[194]

Mansson and Westfelt[195] used cellulose trinitrite ester as starting material for the homogeneous acetylation of cellulose. The reaction was conducted in DMF by distillation (which facilitated a gradual regeneration of the cellulose) and simultaneous addition of isopropanol under acetylation conditions (acetic anhydride–pyridine). This method resulted in the apparent removal of N_2O_4, while the acetylation proceeded primarily at C-2 for cellulose acetates of low d.s. values.

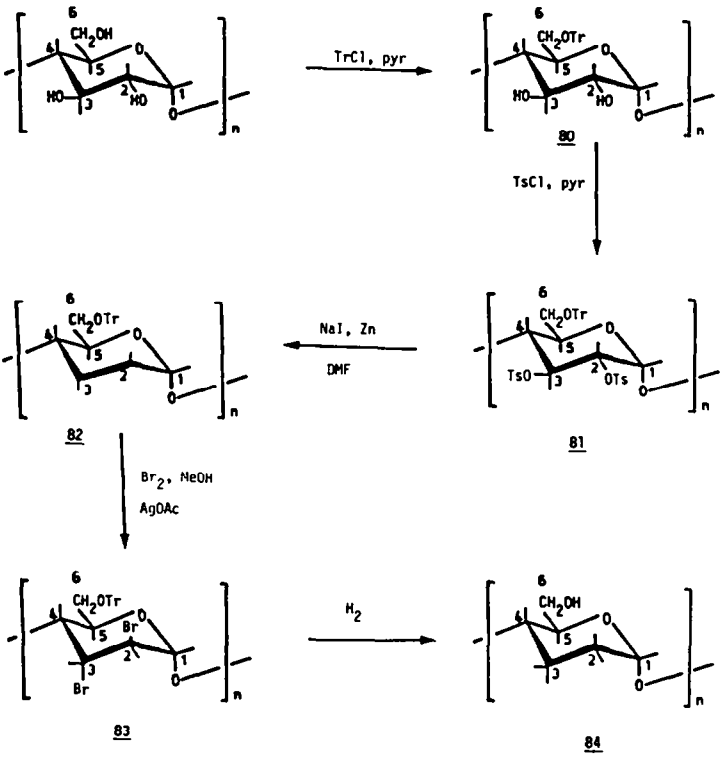
Horton and Meshreki[196] have reported the synthesis of 2,3-unsaturated polysaccharides from amylose (d.s. 0.75) and xylan using 2,3-di-*O*-tosylated precursors (**81**) and sodium iodide and zinc dust in DMF. The unsaturated amylose derivative **82** was subsequently converted into a 2,3-dibromo-dideoxy product (**83**) and a saturated 2,3-dideoxy derivative (**84**) as outlined in Scheme 13.

3. CARBOXYL MODIFICATIONS

3.1. *Esterifications*

Alkyl esters of polyuronides and glycosaminoglycuronans have been prepared in various ways, using alkylene oxides,[197] methanolic hydrogen chloride,[198,199] diazomethane,[198,200] acid chloride[201] or other types[202,203] of intermediates.

In many cases the efficiency of the esterification reaction depends critically on the methods used for activation or pretreatment of the polysaccharides, particularly if maintenance of structural integrity is a concern. For example, for alginic acid, partial methylation of the carboxyl groups can be achieved only with considerable difficulty and under conditions which effectively destroy its colloidal properties.[204] Well dried alginic acid is a horny material which may be difficult to dissolve and derivatize. Complete esterification is rather difficult to accomplish. Thus, treatment with methanolic hydrogen chloride at ambient temperature for 1, 2, 5 and 13 days resulted in 21, 37, 51, and 60% esterification, respectively.[199]



Scheme 13. Synthesis of 2,3-substituted amylose derivatives (Ref. 196).

Similar treatment of pectic acid gave 1, 3, 9, and 12% esterification, respectively.[199] In order to overcome these problems, various methods of pretreatment have been employed,[206,207] including the use of activated (dissolved and reprecipitated) material,[207,209] solvent exchange,[210] e.g. using glacial acetic acid, and partial neutralization of the carboxyl functions prior to reaction.

Diazomethane has been shown to be a satisfactory methylating agent for alginic acid affording relatively high degrees of substitution with little polymer degradation, but with some side product formation. Lucas and Stewart[198] found that treatment of a regenerated alginic acid with diazomethane for 10 days gave products with between 84 and 92% of the carboxyl groups methylated. However, some 26–34% of the total methylation had taken place on the secondary alcohol functions.

The only commercially available alginic esters are derived from the condensation of alginic acid in aqueous medium with propylene oxide at slightly elevated temperatures (<75°). Under these conditions, the esterification rate is found to exceed that of the hydrolysis of the oxide.[202] Examination of the relationship between the molar concentration of added propylene oxide and the resultant degree of alginic acid esterification reveals that while 50–75% esterification is readily obtainable, higher conversion yields are rather difficult to achieve. The reaction product is thought to be primarily 2-hydroxypropyl alginate (**85**), with possible formation of minor quantities of the 1-hydroxy-isomer.

Similar alginic esters have been prepared using ethylene oxide (**86**), butylene oxide (**87**), pentylene oxide (**88**) (with the d.s. values of these water soluble products ranging from 0.60 to 0.80), 1,3-epoxides (trimethylene oxide) (**89**), and long chain 1,2-epoxides of hexane to dodecane (with d.s. values of 0.3–0.6).[202] It has been shown that the reaction mechanism of these epoxide esterifications involves the alginate ion, rather than the acid. Although the latter does undergo reaction, only negligible conversion into the ester takes place even after 1 day. On the other hand, when 10–20% or more of the carboxyl functions are neutralized with base prior to reaction, reasonable yields are obtained within a few hours. Mono- and divalent ions have been shown to catalyze the esterification.[202]

Alginic acid has been partially dehydrated by treatment with glacial acetic acid and then esterified using 4-hydroxy-2,2,6,6-tetramethylpiperidene-1-oxyl in aqueous acetone yielding the nitroxide product **90** with d.s. 0.05.[209]

Esters of carboxylic acid containing polysaccharides have been employed as precursors for the preparation of the corresponding amides, using primary amines (see Section 3.2.2), or for conversion into carboxyl-reduced heparins (see Section 3.3).

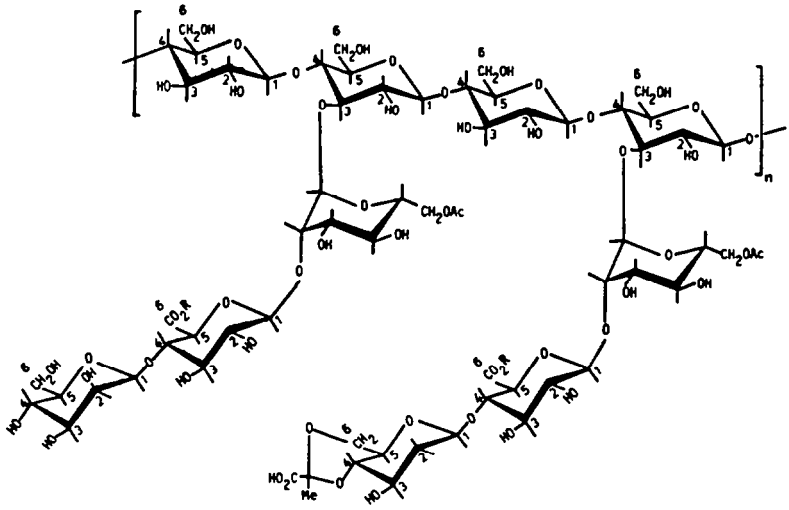
3.2. *Amidations*

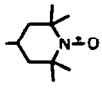
3.2.1. *Via carbodiimide-mediated couplings*

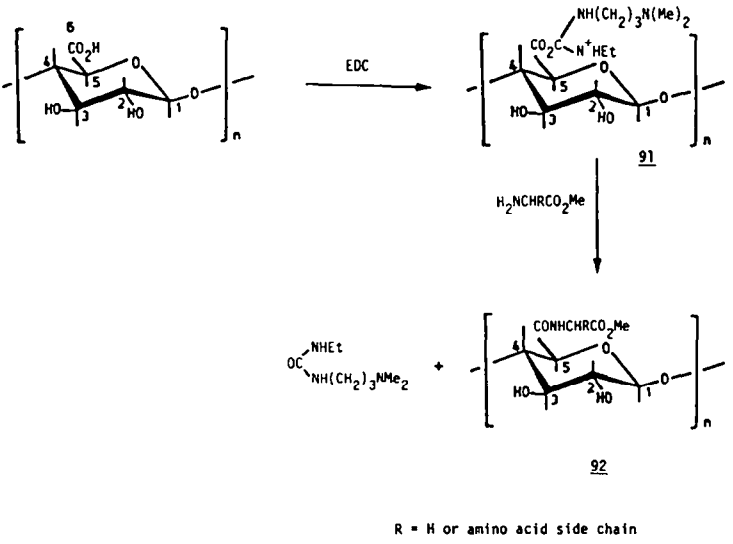
In a comprehensive study of carbodiimide-mediated couplings, Danishevsky and Siskovic[211] have prepared amide derivatives of heparin, heparin sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, hyaluronic acid, and alginic acid using *N*-ethyl-*N*-dimethylaminopropyl carbodiimide (EDC) and labelled amino acids (Scheme 14). The d.s. values of the resulting products (**92**) ranged between 0.33 and 0.87, with heparin and heparin sulfate having the highest yields (d.s. 0.7–1.0). Amidations were conducted at pH 4.75, since higher pH conditions lead to considerable reductions in reaction rates, while at lower pH the carbodiimide was relatively unstable. The authors found also no evidence of hydrolysis of the sulfate groups during the reaction. The specificity of the reagents for carboxyl groups and the absence of other side reactions was supported by data obtained from the electrometric titration experiments which allowed distinction between the acidic sulfate and carboxyl groups of heparin. These experiments confirmed the absence of carboxyl groups in the products, whereas the saponified heparin-glycine methyl ester product revealed the presence of free carboxyl functions.

The authors ascribed the higher esterification yields of heparin and heparin sulfate to a greater accessibility of the carboxyl groups in the α-D-(1-4) glycosyluronic linkage of these polymers than those in the β-D- (or α-L-) linkages of the chondroitin sulfates and hyaluronic acid (dermatan sulfate).[211]

Amide derivatives of alginic acid have been prepared by carbodiimide-mediated coupling in either organic or mixed aqueous-organic solvents.[209] It was found, however, that only partially dehydrated alginic acid was reactive. Amidations with 4-amino-2,2,6,6-tetramethylpiperidene-1-oxyl and either *N*,*N*-dicyclohexylcarbodiimide (DCC) in dimethylformamide, or EDC in aqueous acetone afforded the spin-labelled algin amides (**93**) with d.s. values of 0.1 and 1.0, respectively.[209] Similar spin-labelled amide derivatives of xanthan gum (**95**) have been prepared using EDC (d.s. 0.43).[209] Lasker *et al.*[212] have synthesized spin-labelled heparins (**97**) using equivalent methods for a study of the complex formation with antithrombin. They found that the anticoagulant activity of the labelled heparin product resembled that of the unlabelled heparin. Motional correlation times of the nitroxide moieties in the heparin–antithrombin complex suggested no involvement of the carboxyl functions.

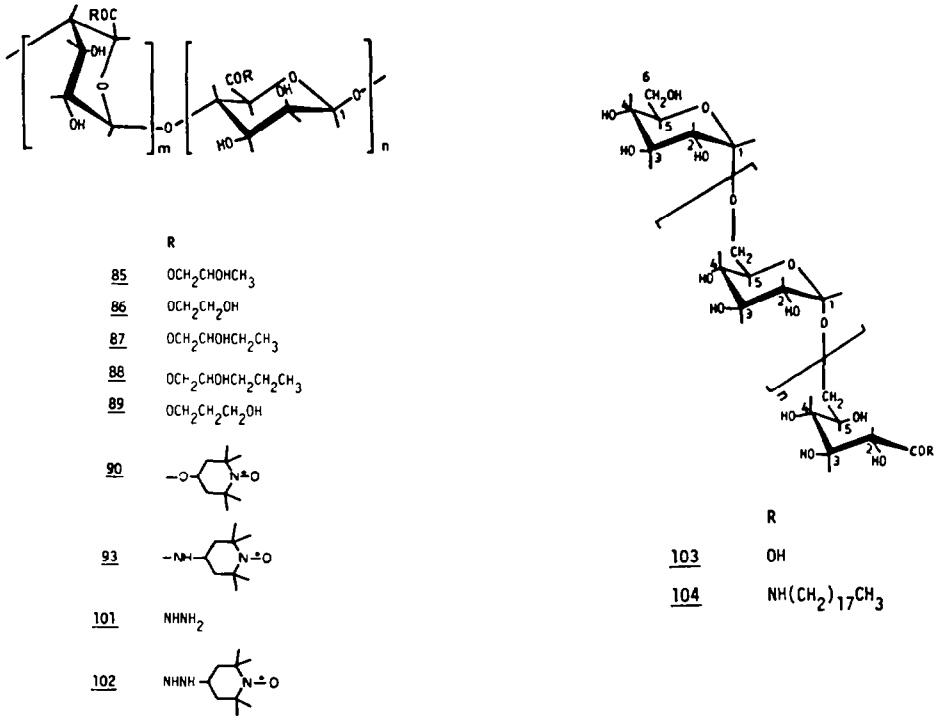


	R
94	OH
95	
96	$NH(CH_2)_{17}CH_3$



Scheme 14. Carbodiimide-mediated amidation of polyuronides (Ref. 211).

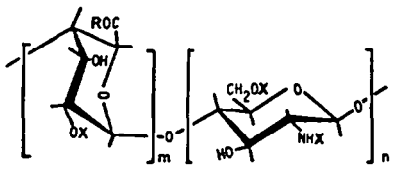
The carboxyl functions of heparin have been linked via EDC-mediated amidation to amino-Sepharose 4B (**98**).[213] Similarly, heparin has been employed for the immobilization of chymotrypsin.[214]



A dextran derivative bearing a terminal carboxyl group (**103**) has been converted into the corresponding ^{14}C-labelled octadecyl amide **104** (d.s. 0.33) using DCC.[69]

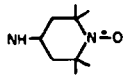
Nagasawa and co-workers[215,216] have synthesized fluorescent derivatives (**99**) of all known glycosaminoglycuronans using 5-amino fluorescein and EDC in pyridine–hydrogen chloride (pH 4.75). The d.s. values of the products ranged from 0.008 to 0.040 and the various labelled and unlabelled polymer fractions were separated by chromatography on octyl Sepharose CL-4B.[215] Compounds with comparable degrees of substitution were obtained under identical labelling conditions for all glycosaminoglycuronans. It was observed that the d.s. values were not affected by reversal of the order of carbodiimide and amino label addition and that no appreciable desulfation occurred under the

labelling conditions. Quantitative hydrolysis of the amide linkages could be achieved using 1 M HCl for 12 hr at 100°.

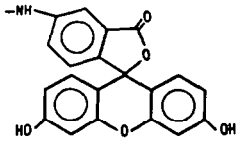


X = SO_3^-

R

97 

98 NH—Sepharose

99 

100 $NHNH_2$

Turley and Roth[217] have attached chondroitin-6-sulfate and hyaluronic acid to sulfonamide resin beads via EDC-mediated coupling. The reaction yields were monitored by using tritiated chondroitin sulfates which were prepared by galactose oxidase oxidation followed by sodium borotritide reduction. The authors found spontaneous agglutination of hyaluronic acid-derivatized beads with chondroitin sulfate-derivatized beads, although each bead type alone showed no self-agglutination.

Another major application of EDC couplings involves structural studies of mucopolysaccharides, where blocking of the carboxyl functions results in a considerable decrease in the acid resistance of the glycosyluronic linkages, thereby facilitating selective degradation experiments[218] (see Section 3.3).

3.2.2. *Via nucleophilic substitution of esters*

Propylene glycol esters of alginic acid (PGA) have been condensed with a wide range of amines, such as primary and secondary aliphatic, cycloaliphatic, aromatic and diamines to afford the corresponding amides in high yields.[219–221] PGA with different degrees of esterification have been converted into spin-labelled amides in dimethylformamide in the presence of small amounts of water.[209]

Similarly, reactive alginate intermediates have been prepared using PGA and hydrazine hydrate and the resulting alginate hydrazides (**101**) have been further derivatized (**102**).[209,222]

Shacklee and Conrad[223] have converted the uronic acid residues of heparin into their hydrazide derivatives (**100**). They found that unsubstituted L-iduronic acid residues reacted much more slowly than unsubstituted D-glucoronic acid or 2-*O*-sulfated L-iduronic acid residues. The chemical modification of the carboxyl function was also accompanied by a low rate of C-5 epimerization of the uronic acid residues and by partial depolymerization of heparin. The hydrazide derivatives could be converted back into uronic acid residues by treatment with nitric acid.[223]

3.2.3. *Via direct condensation*

A Polish patent describes the esterification of 6-carboxycellulose in organic solvents with epichlorohydrin at 80–150° using either triethylamine or stannous 2-ethylhexoate catalyst.[224]

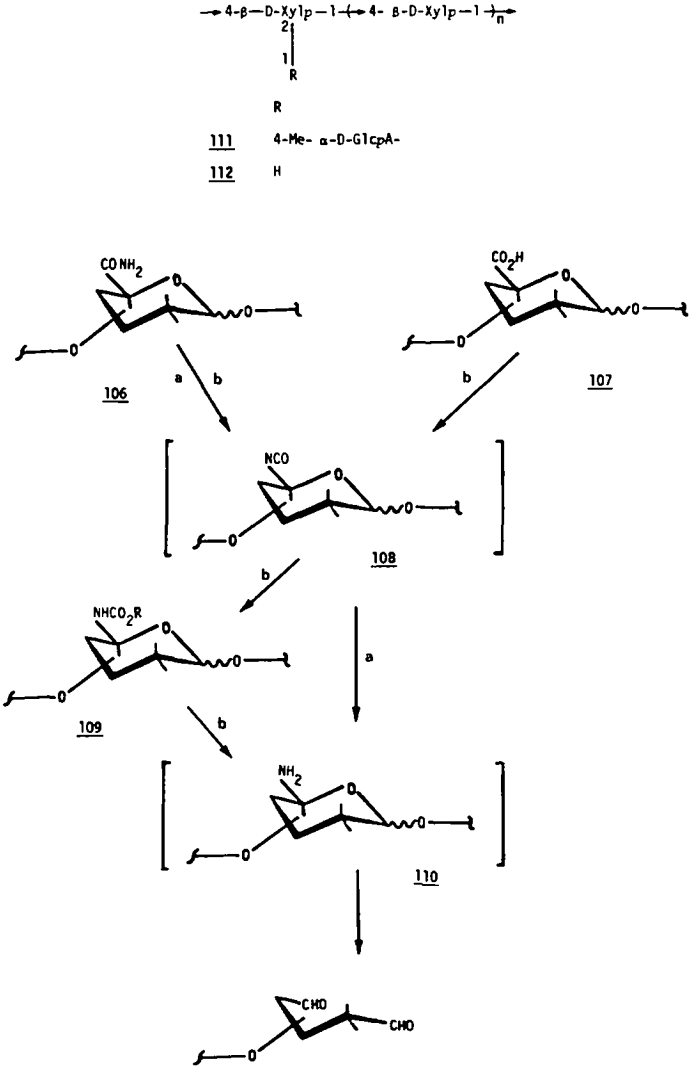
A procedure has been described in the literature for the preparation of "amine" alginates which involves the condensation of amines with solid alginate in the presence of small amounts of water.[225] However, this report contained no further information about the actual structure of these products. It would appear that the covalent products derived from amines and hexuronide carboxylates should be

more appropriately classified as amides. Yalpani and Hall[209] subsequently adapted this procedure to xanthan gum using an amine spin label. The ESR spectrum of the resulting product **105** confirmed the formation of a covalent conjugate, suggesting amide formation. A diamagnetic xanthan octadecyl amide (**96**) with d.s. of about 1.0 was also prepared.[209]

3.2.4. *Hofman–Weerman modifications*

Amides of various polysaccharides have been prepared for the purposes of structural elucidations in conjunction with the Hofman degradation reaction.[42,226] This procedure was developed by Kochetkov *et al.*[227] and involves the sequential conversion of the carboxylic acid function of hexuronides into the corresponding amides, Hofman–Weerman reaction using sodium chlorite at pH 13 to obtain the 5-aminopentopyranoses (**109**), and lastly, hydrolysis of the polymer with dilute acid, as outlined in Scheme 15. The Hofman–Weerman reaction has been applied to polysaccharides containing uronic acid residues in the side chain or in the main chain, resulting in the specific removal of side chains or the selective degradation into oligosaccharide fractions, respectively. Thus, amides of gum arabic and polysaccharides of the glucuronoxylan type, as well as extracellular glurunomannans from *Lipomyces lipofer* have been prepared.[228–230]

Kochetkov *et al.*[226,231] have modified the 4-*O*-methyl-D-glucuronic acid branch residues of white birch xylan (**111**) to introduce amide and amine functions prior to branch elimination to obtain derivative **112**. However, the synthetic potential of this method remains to be exploited.



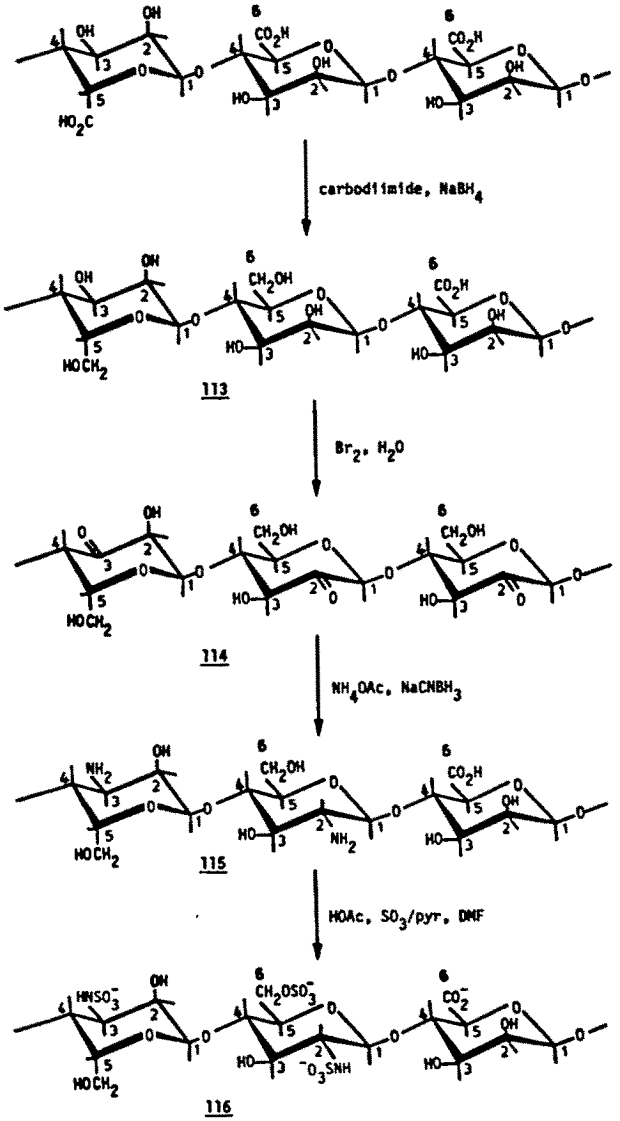
Scheme 15. Modification and degradation of polyuronamides and polyuronides (a) via Hofman–Weerman rearrangement and (b) carbonate (R = t-butyl or benzyl) intermediates (Ref. 2).

3.3. *Reductions*

The reduction of carboxyl functions has been extensively employed in selective polysaccharide degradation studies.[232] For example, Wolfram and co-workers have reduced the carboxyl functions of *N*- and *O*-desulfated heparins using diborane.[233,234]

Taylor and Conrad[218] reacted the uronic acid residues of heparin with EDC and reduced the resulting product with tritiated sodium borohydride. They suggested that the reduction with EDC involves a lactone or intramolecular ester intermediate, which reacts with borohydride to form the carboxyl-reduced product. However, Inoue and Nagasawa[216] subsequently demonstrated that EDC activation of *N*-acetylchondrosine-6-sulfate, chondroitin-6-sulfate, and heparin produces the corresponding, relatively stable *O*-acylisoureas. The latter are then reduced with borohydride to give the carboxyl-reduced polysaccharides. A smaller proportion of the acyl group of the *O*-acylisourea migrates to either nitrogen atom of the 3-ethyl-1-(3-dimethylaminopropyl) ureido residue of the compound to give stable *N*-acylureas under mildly alkaline conditions.

Barker *et al.*[235] have reduced the carboxyl groups of heparin and converted the polymer into its pyridinium salt, in order to obtain enhanced solubility in DMSO for subsequent methylations.



Scheme 16. Synthesis of heparin analogue from alginate (Ref. 116).

A polyuronide from a seaweed (*Sargassum fulvellum*) has been transformed into a D-manno-L-gulonoglycan by a reaction sequence involving protection of the hydroxyl functions with propionic acid, borohydride reduction, and deprotection of the hydroxyl groups with sodium hydroxide.[236]

Hoffman *et al.*[237] prepared a heparin analogue (**116**) from alginic acid by partially (50%) reducing the carboxyl functions using the EDC/borohydride method, followed by selective bromine oxidation (see Section 2.2.1), reductive amination using ammonium acetate, and sulfation, as outlined in Scheme 16. It was found that the heparin analogue was partially degraded and exhibited low anticoagulant activity. These methods have also been applied in the conversion of seaweed-derived (*S. fulvellum*) polyuronides to D-manno-L-gulonoglycans, which showed activity as neoplasm inhibitors.[175]

Unger[238] has recently methylated the extracellular polysaccharide elaborated by *Rhinocladiella elatior* (2-acetamido-2-deoxy-D-glucuronic acid polymer) prior to reduction, in order to increase the solubility of the native polymer. The reduction was performed first with sodium borohydride in ethanol/dioxane, followed by reduction according to Taylor and Conrad's method.[218] However, application of the latter method to the reduction of carboxyl groups of the capsular polysaccharides from *Streptococcus pneumoniae* types S12A and S12F was unsuccessful.[239]

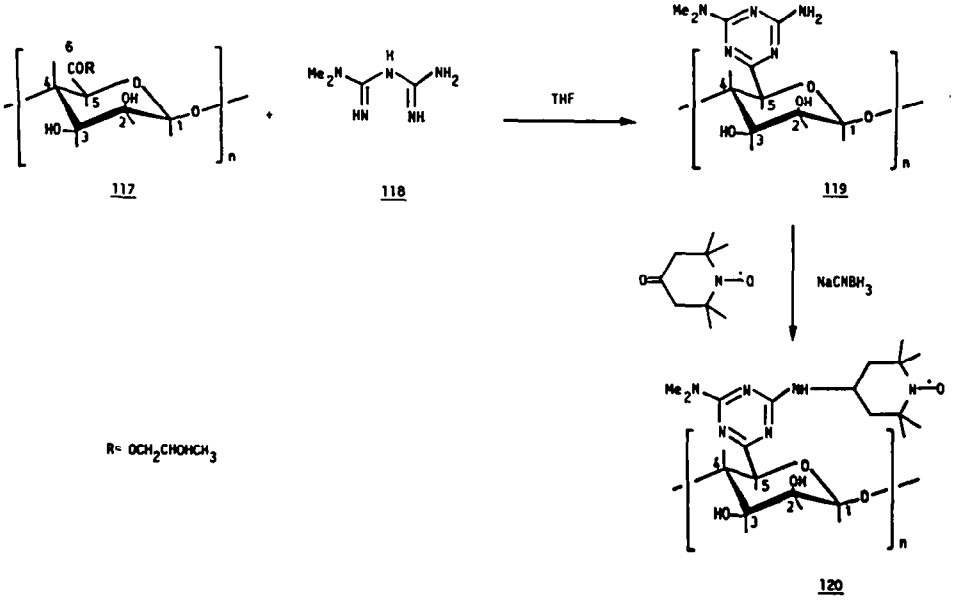
Whistler and Towle[240] have partially reduced the pectic acid propionate derivative (d.s. 1.9) with gaseous diborane to yield, after removal of the propionyl protective functions, a series of polysaccharides with varying carboxyl content (18–82%). The products were examined for their anticoagulant activity.

Hatton *et al.*[241] have reported the borohydride reduction of the terminal reducing groups of heparin without concomitant reduction of the carboxyl functions.

Reduction of the uronic acid–carboxyl groups of mucopolysaccharides has also been accomplished, as mentioned above, by conversion into the methyl ester derivatives and subsequent reduction to the primary alcohol functions using borohydride.[218] This method, however, also results in partial desulfation and some polymer degradation.

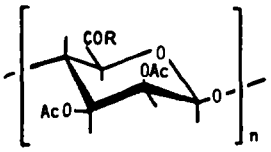
3.4. *Other Modifications*

Lee and Maekawa[242] have reported a method for incorporating the carboxyl groups of polyuronides into *s*-triazine rings using dimethylbiguanidine hydrochloride (**118**) (Scheme 17). Following this method, propylene glycol alginate has been converted into the corresponding *s*-triazine product **119**, and subsequently spin labelled using 4-oxy-2,2,6,6-tetramethylpiperidene-1-oxyl and sodium cyanoborohydride.[209] The *s*-triazine type derivatives may be of interest for applications in which a partial masking of the carboxylate groups of polyuronides is desired.



117 118 119 120

Scheme 17. *s*-Triazinyl derivatives of alginic acid (Refs. 209, 242).



	R
121	Cl
122	CH_2NH_2
123	CH_2Cl
124	CH_2Br

Acid chlorides of glycosaminoglycans, such as chondroitin and its sulfates, have been prepared by treatment with sulfonyl chloride in pyridine–dimethyl formamide solvent.[243] These acid chlorides were subsequently converted into prodrug ester derivatives by reaction with chloramphenicol.

Wypych has prepared a diazoketone derivative (**122**) of acetylalginic acid by treatment of the corresponding acid chloride precursor (**121**) with diazomethane.[244] The product was then treated with gaseous hydrogen chloride or hydrogen bromide to afford α-haloketone products, containing 9% Cl (**123**) and 15% Br (**124**), respectively.

Holzworth prepared fluorescent xanthan gum derivatives by isocyanide coupling of 5-amino fluorescein to the carboxyl groups of the polymer.[245] DeBelder and Wik[246] and others[247] had previously labelled hyaluronate with either isothiocyanato fluorescein, by way of thiocarbamoyl linkages, or 5-amino fluorescein using the isocyanide coupling method.[247]

4. AMINE MODIFICATIONS

4.1. *Acylations*

Selective *N*-acylations have been extensively performed for chitosan and, to a lesser extent, for heparin and other glycosaminoglucoranans.

Hirano's group, for example, has established procedures for the preparation of a variety of selectively *N*-acylated chitosan derivatives (**125**) by reaction of chitosan with alkyl and aryl monocarboxylic anhydrides.[248–258] Exploiting the fact that chitosan dissolves in a number of organic acids, such as formic and acetic acid, the acylations are conveniently conducted in these media together with the corresponding anhydride (2–3 mol equivalents) at room temperature, affording the following products with high d.s. (0.82–1.00) and in high yields (77–96%): formyl, acetyl, propionyl, butyryl, hexanoyl, octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, and benzoyl chitosan. The absence of *O*-acetyl substituents in these products was ascertained by IR spectroscopy. On the other hand, partially *N*- and *O*-acylated products could be obtained by slight modifications of the reaction conditions, i.e. by using a greater excess (10 mol equivalents) of the anhydride reagent.

Hirano and Kondo[254] have also prepared *N*-haloacyl chitosan derivatives by reaction of halocarboxylic acid solutions of chitosan with the corresponding halocarboxylic anhydride. The *N*-, *O*-acyl derivatives thus obtained (d.s. 1.2–3.0), were subsequently *O*-deacylated by alkali treatment to afford *N*-trifluoroacetyl (**126**), *N*-chloroacetyl (**127**), and *N*-formyl (**128**) chitosans (d.s. 1.0) in 75–80% yields.

The coenzymic activity of lipoamide has been utilized by Kijima *et al.*[259] who prepared a chitosan conjugate (**129**) (d.s. 0.43) for applications as a polymeric reducing catalyst. The cleavage of *O*-benzyl hydroxylamine to benzyl alcohol and ammonia was accomplished using lipoyl chitosan and sodium borohydride in the presence of ferrous ions.

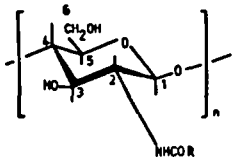
Yamaguchi *et al.*[260] reported the synthesis of partially *N*-succinylated chitosan and glycol chitosan. The d.s. values obtained (0.11–0.80) for these products were low in comparison to *N*-acyl derivatives derived from monobasic carboxylic anhydrides.[260] The partially *N*-succinylated glycol chitosan derivatives were water soluble, whereas the corresponding *N*-succinylated chitosan derivatives swelled in water at d.s. levels of less than 0.2, and dissolved in water at d.s. levels of 0.3–0.6. Introduction of low

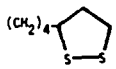
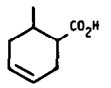
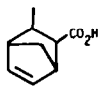
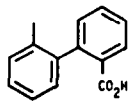
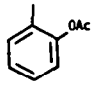
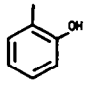
levels of crosslinks (0.5–1.0%) into several of the water soluble *N*-succinylated derivatives resulted in the formation of transparent gels which were employed as matrices for enzyme immobilization.

Hirano and Moriyasu[253] have prepared a series of *N*-carboxyacyl chitosan derivatives using intramolecular dicarboxylic anhydrides, such as maleic (**130**), icatonic (**131**), (acetyl thio) succinic (**132**), *cis*-tetrahydrophthalic (**133**), 5-norbornene-2,3-dicarboxylic (**134**), and diphenic anhydrides (**135**). The product yields (isolated as esters or salts 56–76%) and d.s. values (0.45–0.80) were lower than in the case of simpler *N*-acyl products, presumably as a result of the bulkiness of the substituents. The unsubstituted chitosan amino functions of *N*-[(acetylthio) succinyl] chitosan (d.s. 0.45) were selectively acetylated to produce a copolymer consisting of *N*-acetylated and *N*-[(acetylthio)] succinylated glucosamine residues.

A German patent[261] describes *N*-acylations of chitosan with saturated and unsaturated dibasic acid anhydrides, the latter being amenable to further modifications, such as addition reactions with ammonia, aliphatic amines, or diamines.

N-Acylations of *N*-desulfated heparins have been performed in aqueous alkaline solutions using acid anhydrides or halides, or by the reaction of the heparin hyamine complex in organic solvents in the presence of amines. Some of the *N*-acylated derivatives reported include *N*-acetyl,[262,263] *N*-succinyl,[264] *N*-benzoyl,[265] *N*-(3,5-dimethylbenzoyl),[265,267] and *N*-sulfo- and *N*-disulfobenzoyl[264,268,269] heparin. Hirano and Ohashi[270] synthesized a series of fatty acyl derivatives (up to C_{18}), **138** of heparin using three procedures: (i) acid anhydride–formamide (no catalyst); (ii) acid anhydride–formamide–pyridine; and (iii) acid anhydride–anion exchange resin–water. The products had d.s. values of between 0.28 and 1.00, with method (i) affording the highest yields, together with some



	R
125	ALKYL, ARYL
126	CF_3
127	CH_2Cl
128	CH_3
129	
130	$CH{=}CHCO_2H$
131	$C(=CH_2)CH_2CO_2H$
132	$CH_2CH(SAc)CO_2H$
133	
134	
135	
136	
137	

O-acylation with acetic anhydride but not with the higher (C_4–C_{18}) fatty acid anhydrides. Method (iii) was applicable only to the synthesis of lower ($<C_{10}$) acyl derivatives.

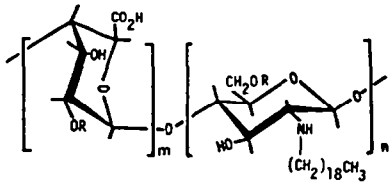
Moore and Roberts[271] have examined the effect of the solubility parameter (δ) of the reaction medium on the rate of chitosan *N*-acylations. They found maximum *N*-acetylation rates in the range $13.1 < \delta < 13.5$ Hildebrandts for chitosan films in binary mixtures of ethanol/methanol and methanol/formamide at 25°. Under these conditions, they observed acylation of 80–95% of the amine groups after 30 min. A considerable shortening of the reaction periods (6-fold reduction) could be achieved by pre-steeping the chitosan films in the solvent mixtures prior to reaction. The authors prepared a series of linear and branched aliphatic (up to decanoyl) and some aromatic *N*-acyl substituents on subsequent *O*-acylation reactions. They found that an increasing size of the linear and branched *N*-acyl substituents (within a certain limit) permitted more rapid *O*-acylations.[271]

Various other acylation procedures have been reported. Thus, Kurita and co-workers[272,273] have developed a method which involves the use of partially (*ca* 40%) *N*-deacetylated, water soluble chitin and aromatic acid anhydrides, such as phthalic, trimellitic, and pyromellitic anhydrides in aprotic organic solvents. The resulting amic acid chitin derivatives (**139–141**) were subsequently heated to induce imidization and simultaneous *O*-deacylation, affording selectively *N*-acylated derivatives which displayed improved solubility in organic solvents and aqueous alkaline media. The same approach could be employed to accomplish quantitative *N*-acetylation of partially *N*-deacetylated chitins in 3 min at room temperature.

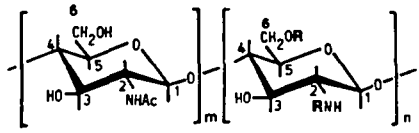
Mrachkovskaya *et al.*[274] have prepared carboxyl-containing chitosan derivatives in a two-step process which is based on *N*-acylations with dicarboxylic anhydrides at pH 4–5.5 using low anhydride concentrations (0.5–1.0 mol equivalents/amine mol equivalent), and subsequent treatment of the resulting polyampholyte with higher concentrations of anhydride (0.5–2.0 mol equivalents/amine mol equivalent) at pH 7.5–8.0. A Japanese patent describes the preparation of *N*-acylated chitosan derivatives using a preactivation of the intractable polymer by regeneration.[275]

Hirano and Ohe have recently described the synthesis of *N*-(2′-acetoxybenzoyl) [aspirin] chitosan **136** (d.s. 0.65) and *N*-(2′-hydroxybenzoyl) chitosan **137** (d.s. 0.81).[276] They studied the release of aspirin and salicylic acid from these products, and suggested their use for drug delivery applications.

N-Acyl derivatives of chitosan are of interest because of their reported ability for selective

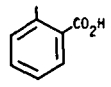


138 R = SO_3^-

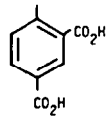


R

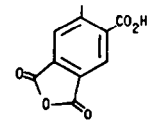
139



140



141



aggregation of certain cancer cells,[277,278] and their potential use for artificial kidney membranes,[279,280] and enzyme immobilization support media.[281]

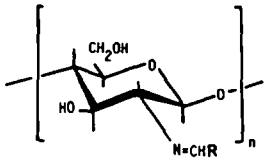
4.2. N-*Alkylations and Schiff's-base Formations*

Nud'ga *et al.*[282] have synthesized quaternary alkyl ammonium derivatives of chitosan with d.s. 0.52–0.78, using regenerated chitosan and alkyl iodides in the presence of organic bases such as pyridine and triethylamine. The resulting tri-*N*-alkyl chitosan iodides were water soluble. Similarly, Okimasu has prepared *N*-methylated hydroxyethyl chitosan.[283]

A substantial number of *N*-alkylidene and *N*-arylidene derivatives of chitosan have been prepared using formaldehyde, gluteraldehyde, saturated and unsaturated alkyl aldehydes, and aromatic mono- and di-aldehydes.[284] For example, Nud'ga *et al.*[285] synthesized a series of aromatic Schiff's-base derivatives for the purpose of reversibly protecting the chitosan amine functions in *O*-alkylation reactions. Thus, salicylidene chitosan was subjected to sulfoethylation (with 2-chloroethanesulfonate) and carboxymethylation. Subsequent acid treatment produced the corresponding *N*-deprotected derivatives (d.s. 0.31 and 1.00, respectively), for which some depolymerization was observed.

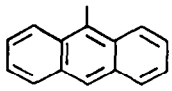
Hirano and Osaka[286] have used a similar approach for the synthesis of *O*-stearyl chitosans (d.s. 0.82–1.91; 62–89% yields) via a series of *N*-alkylidene and *N*-arylidene chitosan derivatives (d.s. 0.8–1.0). The latter products exhibited characteristic C=N IR absorptions at 1640–1650 cm^{-1}. Moore and Roberts[287] employed the Schiff's-base intermediate technique for the preparation of di-*O*-acetyl-*N*-acetyl chitosan via *N*-acetylation of di-*O*-acetyl chitosan. They found that this method overcame previously experienced difficulties in synthesizing fully *O*-acetylated products from either *N*-acetyl chitosan[271] or Schiff's-base derivatives.[288]

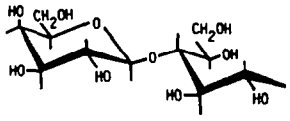
Hirano and Takeuji[289] prepared chitosan Schiff's-base derivatives (d.s. 0.6–1.0) using *o*-, *m*-, and *p*-

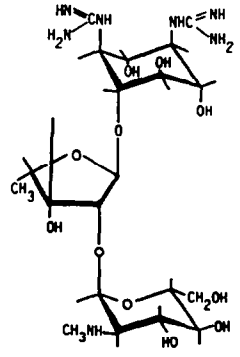


R

142 

143 

144 

145 

phthalaldehyde. The products contained both *N*-formylbenzylidene structures and intermolecular crosslinks in various proportions. A fluorescent chitosan Schiff's-base derivative (**143**, d.s. 0.77) has been prepared using 9-anthraldehyde.[290]

Most of the *N*-alkylidene and *N*-arylidene chitosan derivatives mentioned above are obtained as gels from the reaction in alcoholic acetic acid and the isolated products are insoluble in common aqueous or organic solvents, although some *N*-arylidene derivatives swell in organic solvents.[257] These products are of interest for their membrane forming ability and their porous ultrastructure which may find applications for gel filtration.[291,292]

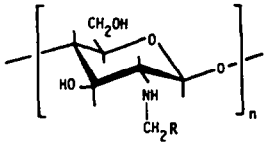
Another type of Schiff's-base derivative has been derived from condensation of chitosan with carbonyl-containing carbohydrates, such as lactose **144** (d.s. 0.1), streptomycin sulfate **145** (d.s. 0.08), and others.[293] These derivatives will be discussed in greater detail in Section 4.3.

The hydrolytic lability of the imine linkages of the Schiff's-base products may be detrimental to the synthesis of high d.s. products. It has been noted, for example, that for a given concentration of aldehyde reagent and otherwise identical reaction conditions, Schiff's-base products have lower (*ca* 8 times) d.s. values than analogous amine products obtained by reductive alkylation (see Section 4.3). This disadvantage may, however, be of value for certain applications, such as drug release formulations, as illustrated by Hirano and Ohe[276] for the chitosan aspirin derivative **136**.

4.3. *Reductive Alkylations*

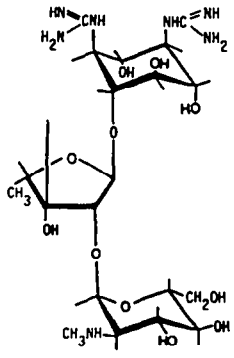
Although Schiff's-base derivatives are of value for certain areas, it is desirable for most polysaccharide applications to obtain hydrolytically stable derivatives. The reductive alkylation procedure[294] (for reductive amination methods see Section 5.2) using sodium cyanoborohydride offers one of the most convenient routes for the synthesis of stable amine derivatives. The method can be employed under a fairly broad range of experimental conditions, including aqueous and organic solvents, acid, neutral or alkaline pH, etc. (for reviews see Refs. 295 and 296). One of the major advantages of this method in the context of polysaccharides derives from the fact that it leads to no or little polymer degradation in comparison to some other methods, e.g. $LiAlH_4$ or $NaBH_4$ reductions, presumably because it does not require an acid treatment for the removal of excess reducing agent.

The utility of the reductive alkylation procedure has been recently demonstrated for the synthesis of new types of chitin and chitosan derivatives.[293] These inexpensive, and widely abundant aminopolysaccharides have, until recently been underutilized, mainly as a consequence of their intractability. Reductive alkylation of chitosan, dissolved in methanolic aqueous acetic acid, with generally small molar excesses of reducing sugars, such as lactose, at ambient temperatures transformed the linear polymer into a branched, water soluble derivative in high yields, as indicated in

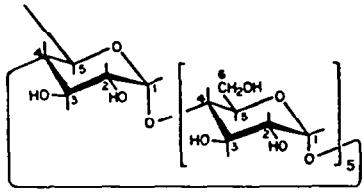


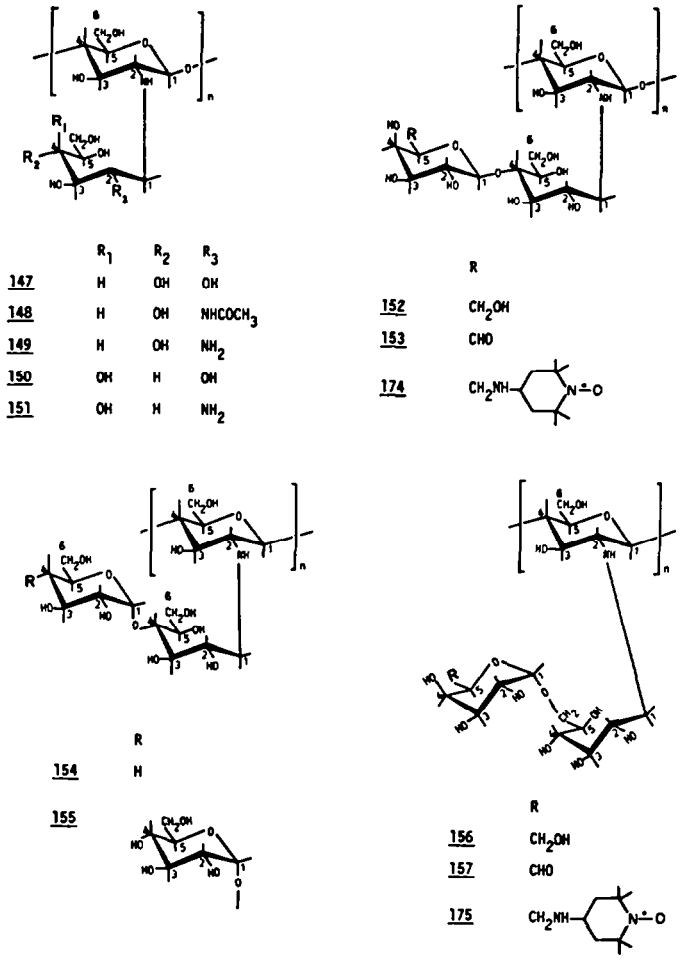
R

159

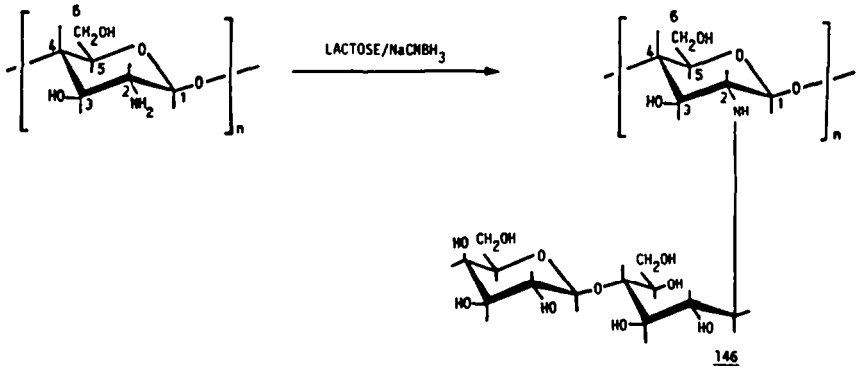


160





Scheme 18. Using this approach and some 14 reducing and other carbonyl-containing mono-, di-, and higher oligo-saccharides, a series of stable, branched chitosan derivatives (**146–157**) were obtained with d.s. values ranging from 0.54 (for maltotriose, **155**) to 0.97 (for glucose, **147**, glucosamine, **149**, *N*-acetylglucosamine, **148**, and lactose, **146**). Almost all of these reductive alkylation reactions were accompanied by the formation of gels, and the reaction rates decreased in proportion to the size of the saccharidic residue. Facile alkylation reactions were also performed with ketoses, such as fructose **158** (d.s. 0.7), with streptomycin sulfate **159**, selectively oxidized β-cyclodextrin **160**, and dextran **171** (M_w *ca* 10,000, d.s. *ca* 0.15). Reductive alkylation of the small percentage (*ca* 10%) of free amine functions of chitin with lactose gave products (d.s. *ca* 0.09) with substantially modified physical properties. 1-Deoxylactit-1-yl chitin **172**, unlike its chitosan analogue with equivalent d.s., was not water soluble, but was found to form transparent sols in water, DMSO, and several other organic solvents.

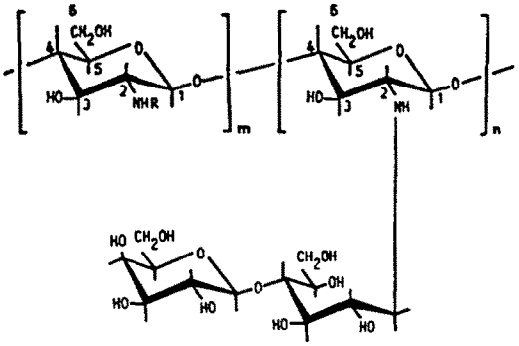


Scheme 18. Synthesis of branched chitosan derivatives (Ref. 293).

All of the branched chitosan derivatives were soluble in either neutral or slightly acidic (pH 5–6) aqueous medium. Water solubility was achievable at even relatively low levels of substitution (d.s. 0.14). Some of the derivatives were compatible with a variety of salt solutions.

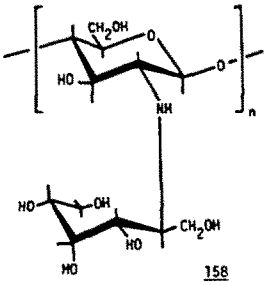
The versatility of this procedure was also demonstrated by post-modification reactions of some of these branched chitosan derivatives.[293] Thus, specific derivatizations of the terminal branch residues of the 1-deoxylactit-1-yl and 1-deoxymelibiit-1-yl derivatives could be achieved via oxidation of the galactosyl primary hydroxyl functions (see Section 8.1) and subsequent reductive amination of the resulting aldehyde groups (**153, 157**) (see Section 5.3). Incorporation of other types of branch residues bearing suitable functional groups, e.g. the 2-amino-2-deoxy groups of glucosamine or galactosamine, would allow similar selective chemical derivatizations.

The solubility, hydrophobicity, and other product properties of the chitosan products could also be readily modified by co-reacting various nonsaccharidic carbonyl compounds in admixture with lactose.[293] Thus, reductive alkylation of chitosan with a mixture of lactose and propionaldehyde (1 : 1.3) gave a water soluble product **173**, which in contrast to the corresponding derivative bearing only lactityl substituents, was also compatible with several organic solvents. Other examples of such mixed derivatives were given.



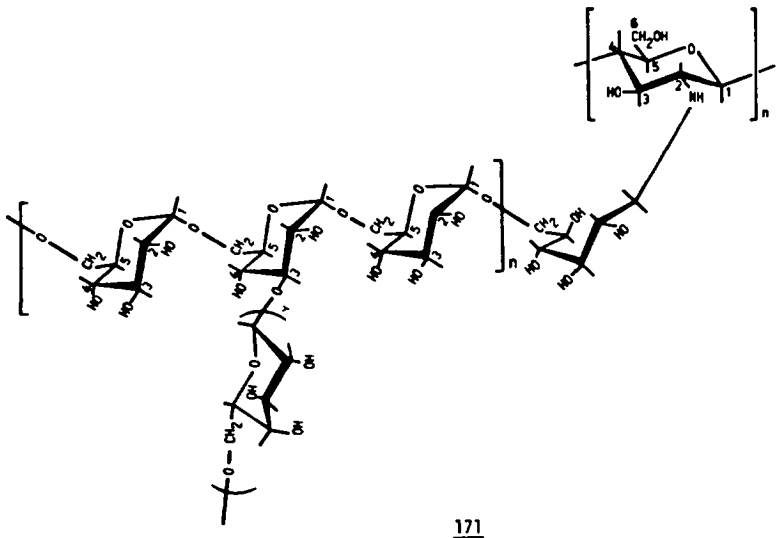
	R
172	$COCH_3$
173	$CH_2CH_2CH_3$

200



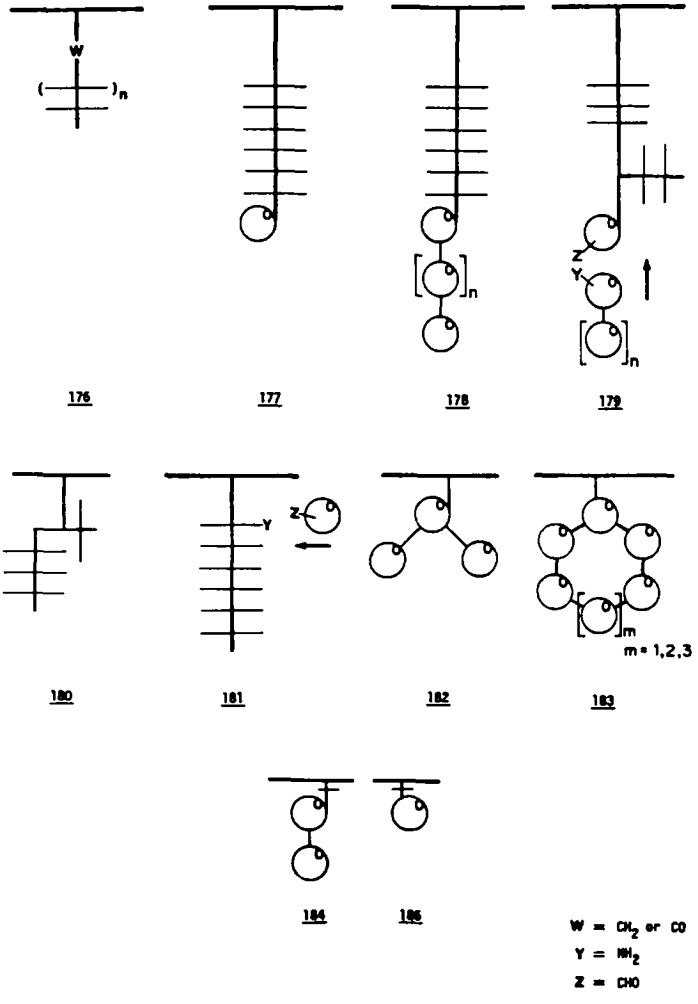
158

The most important implication from the study of branched chitosan derivatives is that the chemical approach employed could be instrumental in systematic polysaccharide structure/function investigations, by virtue of its efficiency and simplicity, and its potential for systematic variations of a host of branch parameters. As illustrated in Scheme 19, the method allows the synthesis of polymer derivatives with varying branch length (**176–179**) and types (**180, 182, 183**), for which the stereochemistry at almost all carbon centres can be modified by suitable choice of stereoisomers. Similarly, medium-sized (C_{12}–C_{18} residues) or longer branches may be derived from appropriate di- or oligo-saccharides or polysaccharides or by extension of preformed branches (**179, 181**) via, e.g. the



171

selective oxidation/reductive amination method mentioned above. Other structural parameters which can be modified include the extent of branching and the overall charge, which can be altered by incorporation or derivatization of suitable functional groups, such as aldehydes, amines or carboxylic acids.



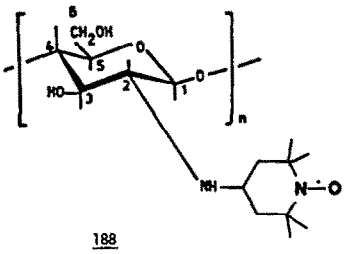
176 177 178 179

180 181 182 183

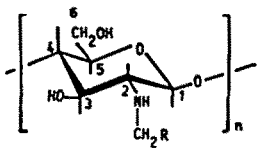
184 185

Scheme 19. Branched chitosan derivatives (see text for explanation, Ref. 293).

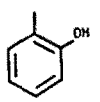
The reductive alkylation procedure has also been applied to chitosan using various aliphatic and aromatic aldehydes and ketones.[297–299] For example, treatment of chitosan in methanolic acetic acid with 2 mol equivalents of salicylaldehyde afforded the salicylidene chitosan **170** (d.s. 0.6), which exhibited enhanced metal chelating capacities in comparison to both the native polymer as well as the Schiff's-base analogue **142**.[297] Similar condensations of chitosan with *m*-fluorobenzaldehyde,[300] 9-anthraldehyde,[290] and 4-oxy-2,2,6,6-tetramethylpiperidene-1-oxy[293,301] afforded the corresponding fluorinated **186** (d.s. 0.9), fluorescent **187** (d.s. 0.77), and spin-labelled **188** (d.s. 0.1) derivatives,

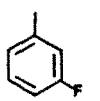


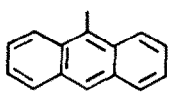
188

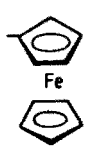


R

170 

186 

187 

189 

190 $-(OCH_2CH_2)_n-OCH_2CH_2OH$

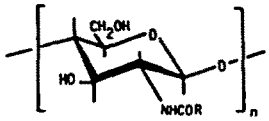
respectively. Other workers have employed this method for the preparation of metal chelating derivatives using glyoxilic acid,[302] ascorbic acid[303] and *o*-phthalaldehyde.[304]

Reductive alkylation of chitosan with ferrocenecarboxaldehyde resulted in the formation of ferrocenyl chitosan **189**, a new type of covalent organometallic polysaccharide derivative (d.s. 0.46) which may find application in catalysis.[299]

Another type of new polysaccharide conjugate **190** has been obtained by the reductive alkylation of chitosan with an aldehyde derivative of poly(ethylene) glycol (M_w 8000).[304]

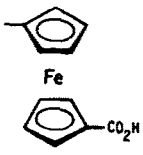
A similar reductive alkylation approach has been employed for the derivatization of aminodeoxy cellulose derivatives (**193**), for guar gum derivatives carrying 6-amino-6-deoxy-galactosyl residues (**194**), and for reducing-end modified 1-amino-1-deoxy-dextran derivatives (**195**).

Another recent paper describes the synthesis of a number of spin-labelled chitosan derivatives.[301] Reductive alkylation of chitosan was accomplished either by derivatization in methanolic acetic acid using the keto spin label **196** or alternatively, via a moderately soluble *N*-sulfate chitosan intermediate

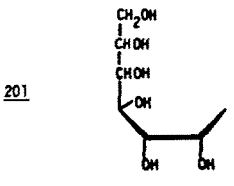


R

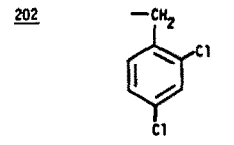
191 $CH(CH_2)_3CH(NH_2)CO_2H$



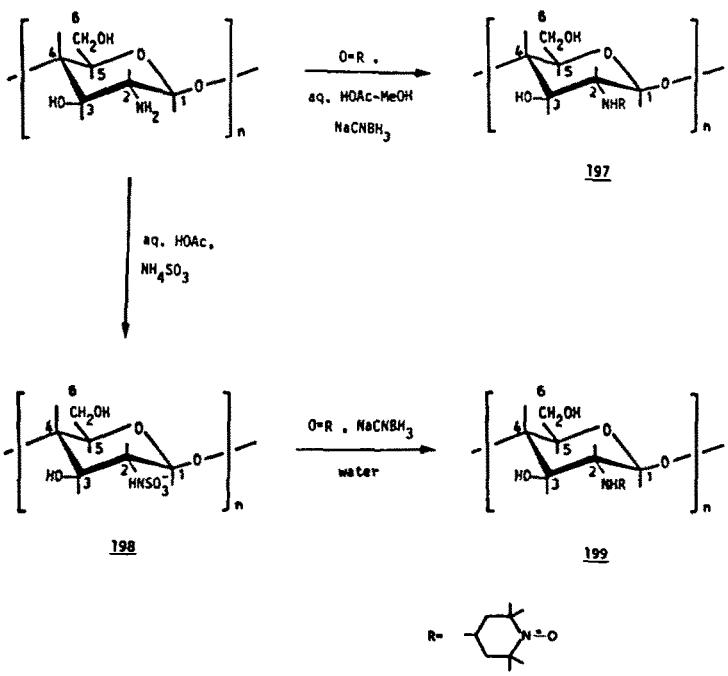
192



201



202



198 in order to avoid degradation of the acid-sensitive spin label reagent (Scheme 20). In both cases the spin-labelled derivatives (**197–199**) (d.s. 0.1 and 0.45, respectively) were found to form gels in aqueous or methanolic media. Selective broadening experiments involving interaction of the labelled chitosan derivatives with varying concentrations of nickel ion probe molecules suggested structural heterogeneities for some products. In a different study, the residual amino groups (*ca* 20%) of a water

Scheme 20. Synthesis of spin-labelled chitosan derivatives (Ref. 301).

soluble 1-deoxylactit-1-yl chitosan derivative (**146**, d.s. 0.8) were reductively alkylated in aqueous solution using the keto spin label to afford the corresponding copolymer product **200**.

4.4. *Amidations*

Various amidation reactions involving the chitosan amine functions and carboxylic acid or lactone derivatives have been performed. Thus, reaction of chitosan in methanolic acetic acid with α-glucoheptonic acid–γ-lactone for 6 days gave the corresponding water soluble amide derivative **201** with d.s. 0.77.[293] Carbodiimide-mediated coupling of chitosan with diaminopimellic acid afforded *N*-(2,6-diamino)-6-carboxyhexanoyl chitosan **191** (d.s *ca* 0.75), which could serve as a reactive intermediate for further derivatizations.[293] A similar approach was employed in the synthesis of a ferrocenyl chitosan derivative **192** (d.s. 0.4), using ferrocene carboxylic acid,[299] and a 2,4-dichlorophenoxyacetamido chitosan derivative **202** (d.s. 0.77).[306]

Funahashi *et al.*[213] have linked the amine functions of heparin to carboxylated Sepharose 4B using EDC.

Emmerling and Pfannemüller[307,308] have also prepared a series of branched chitosan derivatives using aldonic acid lactones of glucose (d.s. 1.0), maltose (d.s. 0.81), and cellobiose. They used chitosan hydrochloride salts as starting materials and ethylene glycol or ethylene glycol–glycerin (1 : 1) mixtures as solvents in the presence of triethylamine at 70°. The presence of the triethylamine acid scavenger, however, led to the formation of chitosan gels during the reaction and the isolated products (hydrochloride salts) were found to be water insoluble in contrast to some of the previously mentioned branched derivatives.[293]

4.5. *Other Modifications*

The selective cleavage of *N*-deacetylated 2-amino-2-deoxyglycosidic linkages by nitrous acid treatment has been applied to numerous aminopolysaccharides.[38,42,309,310] The deamination of 2-amino-2-deoxy-D-glucopyranosides (**203**) usually proceeds via selective scission of the glycosidic bonds with formation of 2,5-anhydromannose derivatives (**206**) and liberation of the aglycone, as illustrated in Scheme 21 (path 1). However, the existence of an alternative mechanism, leading to the formation of 2-C-formyl pentofuranosides (**208**) and liberation of any *O*-3 substituent (Scheme 21, path 2), has been demonstrated in a number of cases. The details of this degradation technique have been reviewed by Aspinall[38,42] and Williams.[309]

Deaminations resulting in polymer degradation have been reported for heparin[311,312] and various other polysaccharides.[313,314]

The deamination of mannosamine-containing polysaccharides proceeds in a different manner, resulting in the predominant formation of D-glucose residues (**211**), without cleavage of glycosidic linkages, as demonstrated for the case of the *N*-deacetylated, carboxyl-reduced 2-acetamido-2-deoxy-D-mannurono-D-glucan **210** from *Micrococcus lysodeikticus*[315] (Scheme 22).

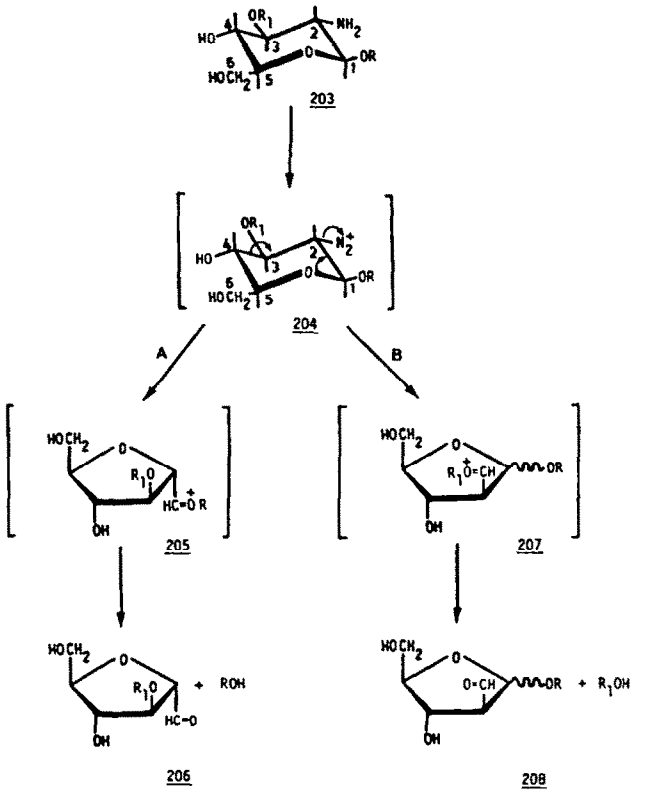
5. ALDEHYDE AND CARBONYL MODIFICATIONS

5.1. *Aminations*

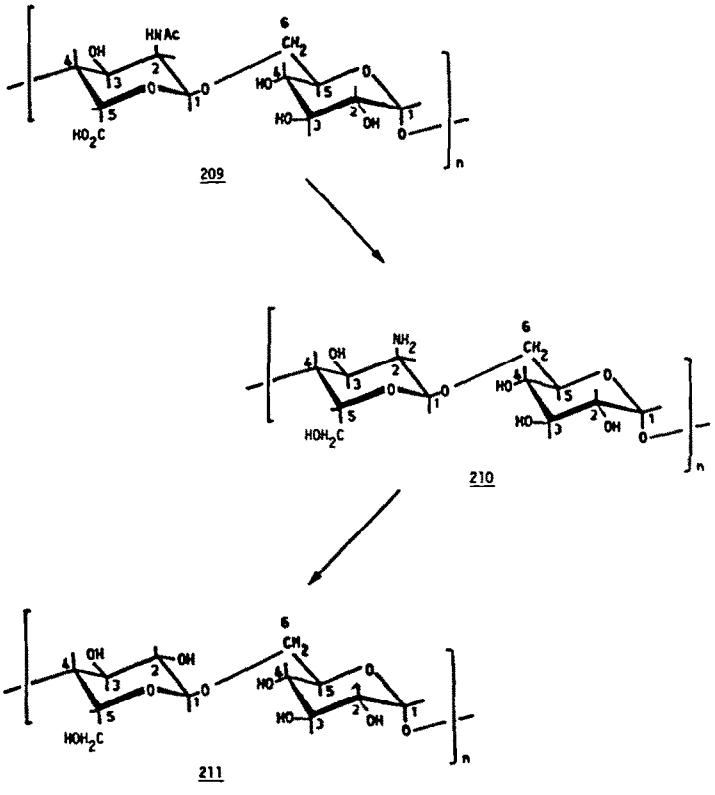
5.1.1. *Reductive aminations*

The merits of this procedure have already been discussed in Section 4.2. A number of recent publications have demonstrated the potential of this method for the selective modification of carbonyl-containing polysaccharides.

Hoffman *et al.*,[316] for example, have prepared heparin fragments with terminal 2,5-anhydro-D-mannose reducing residues of $M_w \simeq 10{,}000$ by deaminative depolymerization (see Section 4.5) of the native polysaccharide. These heparin fragments were then coupled through their aldehyde functions to 6-aminohexyl-Sepharose by reductive amination in aqueous solutions at pH 7 using $NaCNBH_3$. The amino-Sepharose substrate itself was obtained by bromine oxidation of Sepharose, followed by reductive amination with 1,6-hexanediamine. A 6-amino-curdlan derivative was obtained essentially in the same fashion, and then condensed with the heparin fragments. The heparinized gels thus prepared contained 20 and 55% heparin by weight, respectively, and were employed for binding studies



Scheme 21. Nitrous acid-deamination reactions leading to (a) selective glycoside cleavage and formation of 2,5-anhydro-D-mannose derivatives, and (b) formation of 2-C-formyl derivatives (Ref. 2).

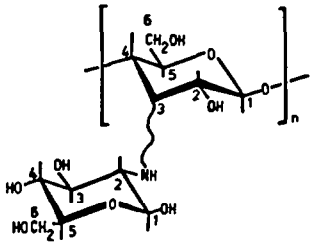


Scheme 22. Deamination of glucan from *Micrococcus lysodeikticus* (Ref. 211).

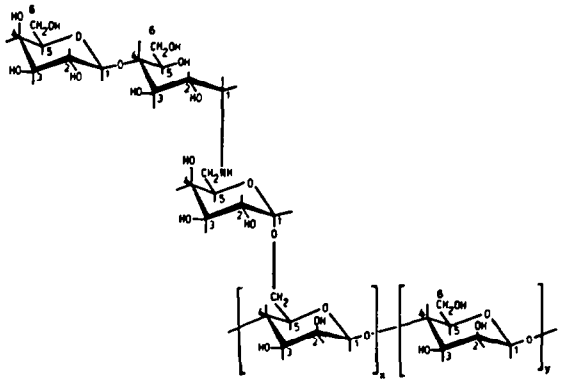
with antithrombin. The same group has previously reported on the coupling of proteins (human serum albumin), enzymes (urokinase), and other amines (1,6-hexanediamine, 1-aminodecane) to oxidized Sepharose, Sephadex and cellulose samples via reductive amination.[173,178] The coupling yields were generally high and the biological activity of the coupled enzyme was unaltered. The authors evaluated the effects of concentration of reductant, reaction times, and concentration of substrates on the efficiency of the couplings.

Larm *et al.*[174] have reductively aminated a partially carboxyl-reduced, bromine-oxidized alginic acid derivative (**114**, Scheme 16), using ammonium acetate and obtained a mixture of partially aminated products (**115**) whose exact composition was not determined.

Selectively oxidized guar gum and locust bean gum (see Section 8.2) have been chemically modified in several ways, including via reductive amination (Scheme 23).[118] These reactions were conducted either *in situ* following oxidation, or after isolation of the C-6-aldehyde products. The reactions proceeded generally in high yields (60–90%) affording a number of stable amine derivatives, including hydroxypropylamine guar **212** (d.s. 0.8), an anionic glycine guar derivative **28** (d.s. 0.54), a cationic aminoguar **216** (d.s. 0.56), a bovine serum albumin guar conjugate **217**, and a 6-*N*-(4-amino)-5-imidazolecarboxamide guaran derivative **214** (d.s. 0.14) (see Scheme 24). The spin-labelled guar **218** and locust bean gum (**219**) derivatives were used in ESR structural studies of the distribution of galactosyl side chains in these polymers. Some of the guar gum derivatives exhibited unusual rheological behaviour and compatibility with various salts.

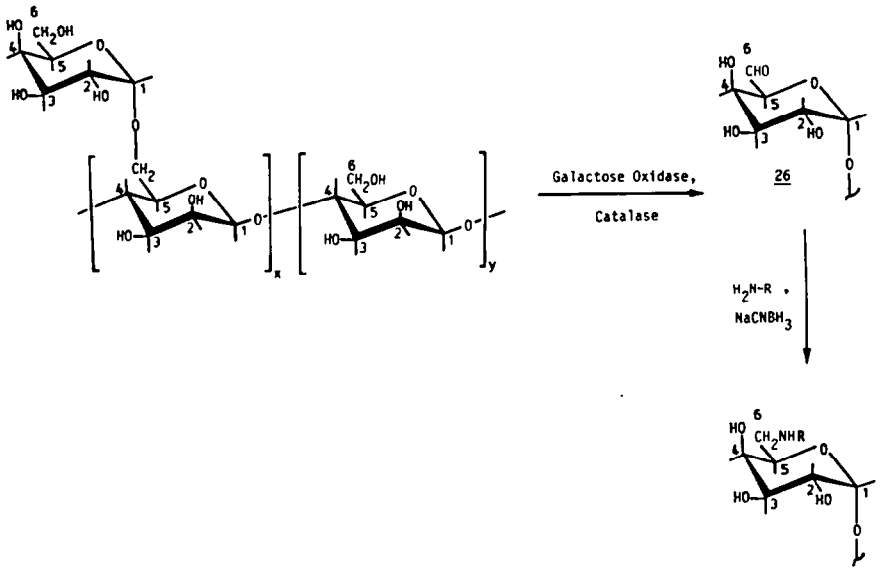


193



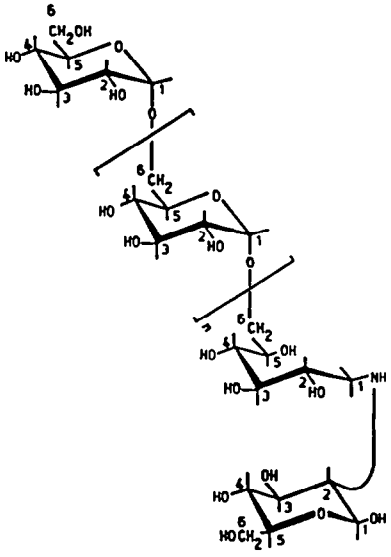
194

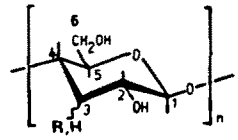
Similarly, spin labelling has been performed for two branched chitosan products which were activated by selective oxidation of the primary hydroxyl functions of the terminal galactose residues.[301] The reductively aminated products **174** and **175** had d.s. values of 0.7 and 0.15, respectively.



Scheme 23. Oxidation and reductive amination of galactomannans (Ref. 118).

A recent paper describes the synthesis of a series of cellulose amine derivatives from selectively oxidized cellulose precursors using the reductive amination procedure.[317] Nitroxide labelled cellulose derivatives (**221**, **222**) were prepared from 3-oxy-cellulose (**65**) and 2-oxy-6-*O*-triphenylmethylcellulose (**64**) in methanolic medium in yields of 28 and 9%, respectively. The low d.s. value of the C-2 derivative **222** was ascribed to steric hindrance arising from the bulky C-6 trityl group. The ESR spectra of the labelled derivatives reflected solubility differences stemming from the presence or absence of the hydrophobic trityl group, respectively. Diamagnetic derivatives were obtained using various amine reagents. Thus, reductive amination of 3-oxy-cellulose with glucosamine afforded a branched, water soluble cellulose product **193**. Similarly, a cationic 3-amino-3-deoxy-cellulose derivative **220** (d.s. 0.3) was obtained using ammonium acetate. Reductive amination of 2-oxy-cellulose with *p*-toluidine chromium tricarbonyl and 1,10-diazo-18-crown-6 produced two new types of covalent organometallic (**223**) (d.s. 0.25) and crown ether (**224**) (d.s. 0.06) derivatives, respectively. The merits of employing

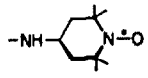


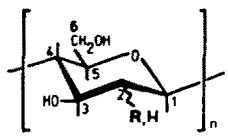


R

220 $-NH_2$

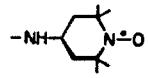
221



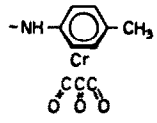


R

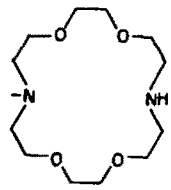
222



223

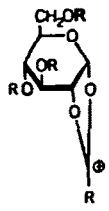


224

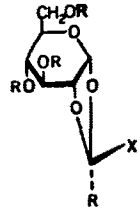


strategies involving either 6-*O*-protected or unprotected oxy-cellulose intermediates were discussed in terms of reaction yields and product properties.

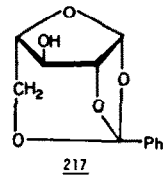
Various recent reports describe the reductive amination of the reducing end group of dextran, heparin and other polysaccharides (see Section 7.1.1.2).



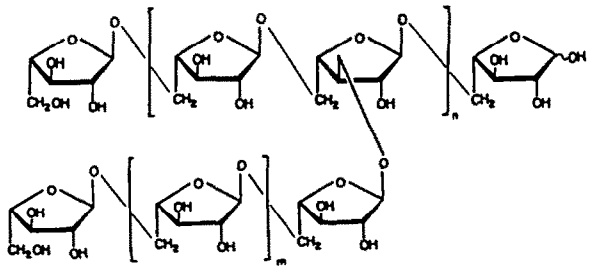
213



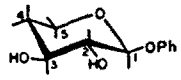
214



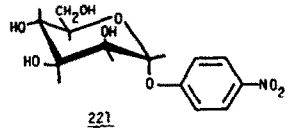
217



218



222



221

5.1.2. *Aminations via oximation/reduction*

In a detailed study, Wolfram and Wang[163] have reported on the preparation of 2-amino-2-deoxy-amylose with d.s. 0.8. Their method involved oxidation of 6-*O*-tritylamylose with DMSO/Ac_2O, oximation in pyridine, reduction with $LiAlH_4$ in tetrahydrofuran, and detritylation (Scheme 9). The aminated product was obtained as a completely white, odourless powder of which 60% was non-dialyzable (i.e. indicating substantial depolymerization during the reaction). The reduction of the oximated 6-*O*-tritylamylose was found to proceed with retention of the D-gluco configuration. At the same time the reduction of the oxime also resulted in the formation of minor quantities of keto groups. The authors reported that the oxidation did not lead to the formation of any appreciable (methylthio)methyl ether by-product (only traces of sulfur were found). The maximum extent of amination (d.s. 0.8) could not be improved by an additional oxidation step.

Teshirogi *et al.*[318] used the above approach for the synthesis of 2-amino-2-deoxy-cellulose. The maximum d.s. value obtained in this case was 0.37. The product contained minor amounts of other aminosugars. The authors reported retention of the D-gluco configuration during the reduction step as for the case of amylose. Other workers have also utilized the oximation/reduction method for the preparation of aminodeoxy polysaccharides.[164,319]

5.2. *Other Modifications*

Various other modifications of aldehyde- and carbonyl-containing polysaccharides are discussed in Sections 7.1.1.1 and 8.2.

6. MISCELLANEOUS MODIFICATIONS

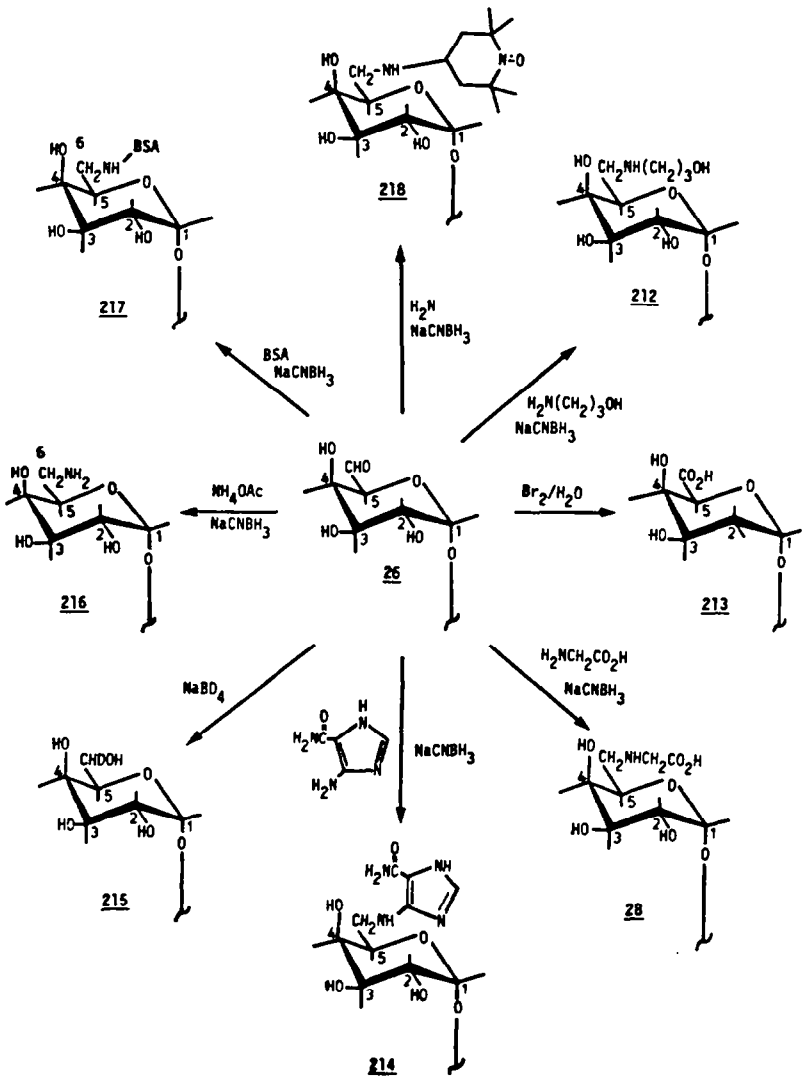
6.1. *Synthesis of Branched Polysaccharides*

There is considerable interest in the synthesis and modification of branched polysaccharides, for reasons already alluded to in the Introduction (for reviews and recent articles on the synthesis of linear polysaccharides, see Refs. 6, 7, 18, 83, 320–322). Several methods are available for the preparation of branched polymers, including the *de novo* synthesis using various types of polymerization processes, and the conversion of linear polysaccharides into branched derivatives by enzymic or chemical methods.[323] In the former category, Kochetkov and other workers developed methods based on Koenigs–Knorr type glycoside synthesis,[324] and on techniques which rely on the intermediary bicyclic acyloxonium ions **213**, derived from 1,2-orthoesters **214** (X = OAlk) or 1,2-thioorthoesters of sugars **215** (X = SAlk or SAr), or from 1,2-cyanoethylidene derivatives **216** (X = CN).[7,18,325] However, both synthetic approaches show no absolute regio- and stereospecificity and suffer from the other disadvantages mentioned in Section 1.3. The following selected syntheses typify the scope of these techniques.

The orthoester **217** has been used for the synthesis of a highly branched arabinan **218** with an average degree of polymerization (d.p.) of 60.[326]

Kochetkov *et al.*[327] also reported the synthesis of a β-D-glucopyranosyl-branched cellulose derivative (d.p. 30–60) via the cyclic orthoester glycosylation method, using 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(t-butyl orthoacetate) and randomly substituted cellulose diacetate. The branch residues were found to be attached mainly to secondary hydroxyl positions.

The German group of Hüsemann, Richter, and Pfannemüller have reported the synthesis of a number of branched amylose and cellulose derivatives.[84–86,328–332] The primary hydroxyl functions of the respective polymers were substituted with peracetylated glycosylbromides of glucose, maltose, and maltodextrins (up to heptasaccharides—derived from acetobrominolytic cleavage of β-cyclodextrin acetate) using either 6-trityl-2,3-dicarbanilate derivatives (**219**) of the polysaccharides in nitromethane-*p*-dioxane in the presence of silver perchlorate catalyst,[85,328] or the corresponding 6-*O*-detritylated polysaccharide carbanilate derivatives (**220**) in acetonitrile-*p*-dioxane and mercuric bromide or cyanide catalyst[85] (Scheme 25). The preferential formation of α-(1 → 6) linkages, with no concomitant depolymerization, was observed for the case of the detritylated derivatives, while use of the former reagents led to preferential formation of β-(1 → 6) linkages with extensive depolymerization arising from the tritylation reactions.[85,330] For the glucosylated amylose and cellulose derivatives

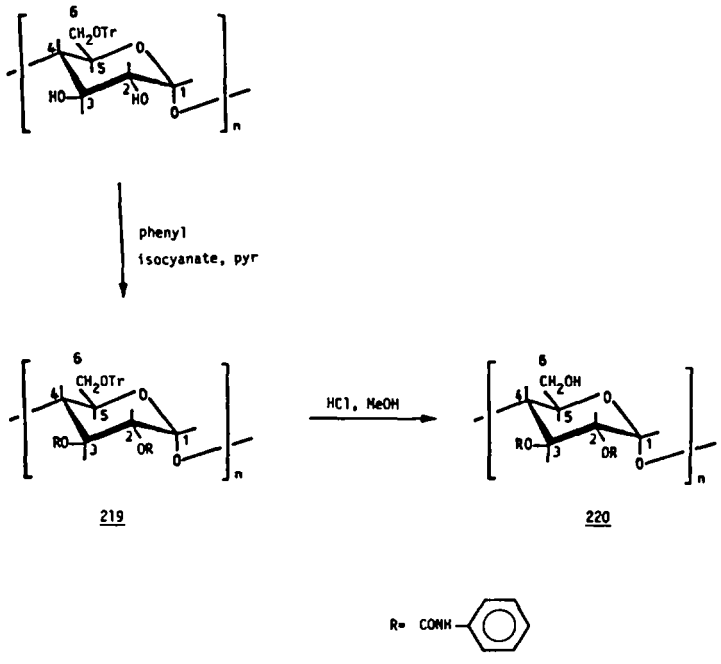


Scheme 24. Derivatives obtained from C-6 aldehyde guar gum and locust bean gum (Ref. 118).

degrees of substitution of 0.21–0.44 and 0.09–0.13, respectively, were obtained with yields of about 50–85%. Much lower d.s. values (0.01–0.04) were observed for the higher maltodextrin substituents.[330] The latter derivatives were also examined for their ability to act as acceptors of potato phosphorylase-catalyzed side chain extensions.[329] Some of the branched amylose derivatives have been studied by proton and ^{13}C-NMR.[333]

The same group also employed 1,2-(t-butyl orthoacetate) and 1,2-(ethyl orthoacetate) derivatives of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose and 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose in the preparation of the corresponding branched derivatives (d.s. 0.25–0.30) bearing mainly β-(1 → 6) linked (with small proportion of α-(1 → 6) linked) residues.[84] Similar branched polymers (d.s. 0.05–0.20) were obtained from 1,2-(ethyl orthoacetate) derivatives of maltose, maltotetrose, and maltohexose.[84]

Subsequent studies of these workers involved the coupling of glucose and malto-oligomers to hydrazide derivative of 6-carboxycellulose, 6-carboxyamylose, alginic acid, pectic acid, and carboxymethyl cyclodextrin which resulted in the formation of branched Schiff 's-base products.[86] The maltooligosaccharide branch residues of these derivatives (d.s. 0.2–1.03) were then extended by way of phosphorylitic synthesis yielding products with variable branch lengths. A similar synthetic approach has been recently employed by this group for the preparation of branched chitosan derivatives[307] (see Section 4.4).



219 220

Scheme 25. Synthesis of carbanilate derivatives of amylose (Ref. 85).

Lindberg and Svensson[334] have used aryl mannoside (**221**) and aryl xyloside (**222**) derivatives in the preparation of branched dextran derivatives.

In a different approach, Ito and Schuerch synthesized a series of branched dextran analogues **230** by copolymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-but-2-enyl-α-D-glucopyranose **223** with 1,6-anhydro-2,3,4-tri-*O*-benzyl-α-D-glucopyranose **224**, as indicated in Scheme 26.[83] Removal of the crotyl protective functions from *O*-3 of the glucopyranosyl residues of the resulting high molecular weight products **225**, afforded a material which, after α-D-glucosidation with 6-*O*-(*N*-phenylcarbamoyl)-2,3,4-tri-*O*-(*p*-methylbenzyl)-1-*O*-tosyl-D-glucopyranose **227**, and subsequent decarbanilation and debenzylation, gave a series of dextrans with different degrees of branching. The products were characterized by NMR, rheological and other methods.

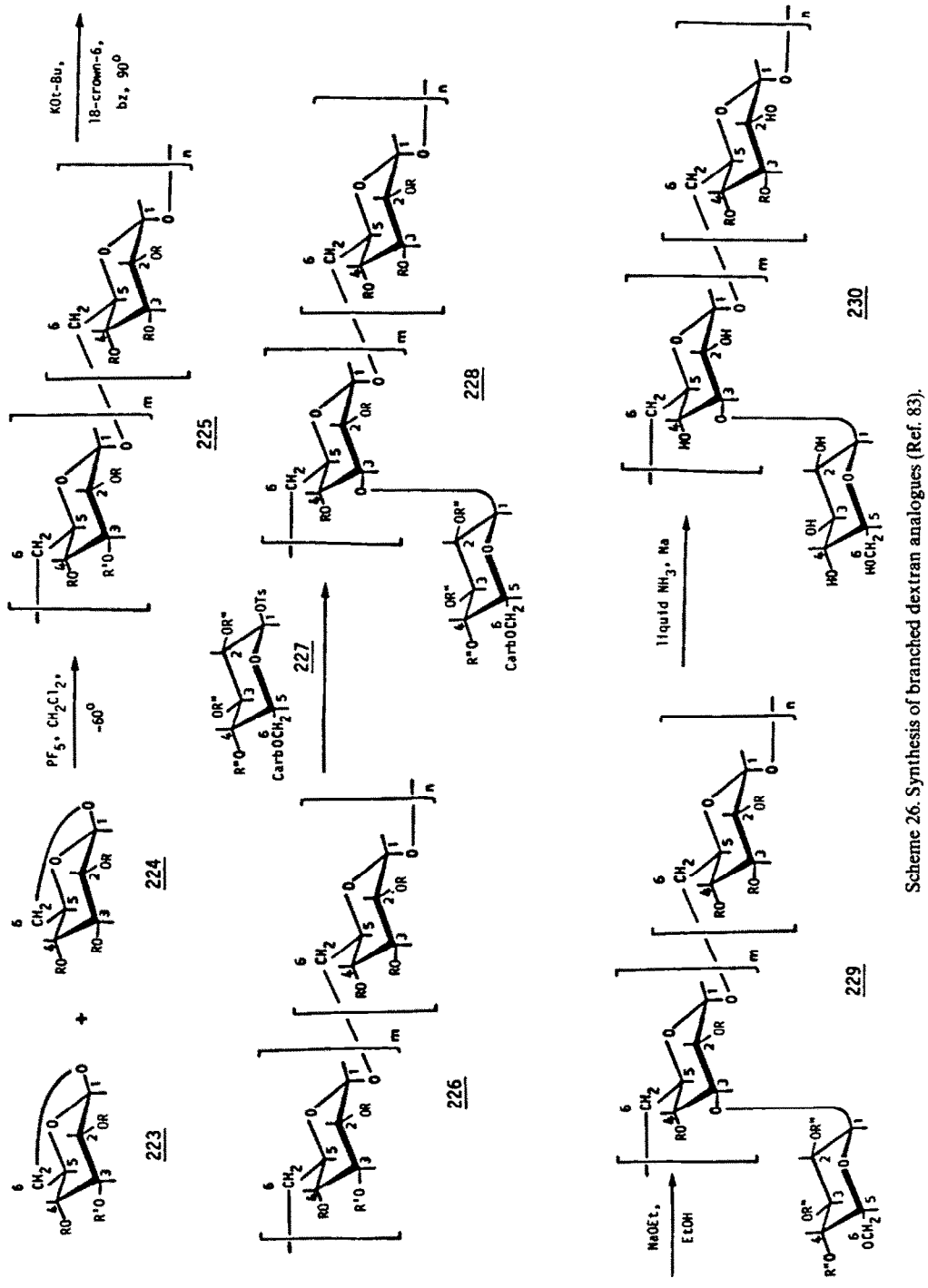
Cationic polymerization of perbenzylated 1,6-anhydromaltose and 1,6-anhydrocellobiose derivatives has been used by Schuerch *et al.* for the preparation of highly stereoregular 4-*O*-α-D-glucopyrano-(1 → 6)-α-D-glucopyran **231** and 4-*O*-β-D-glucopyrano-(1 → 6)-α-D-glucopyran (**232**) derivatives, respectively, of low d.p. values (14–32).[335,336]

Various syntheses of branched polysaccharides derived by chemical and/or enzymic modifications of cellulose, chitin, chitosan, dextran, guar gum, etc. have been discussed in connection with the reductive alkylation and amination methods (see Sections 4.3, 5.1.1, and 7.2.). Selected branched polysaccharides were characterized by NMR,[300] ESR,[118,209,301] steady-state viscosity,[118,337,339] and other methods.[118] A number of enzymatically synthesized branched cyclodextrin, cellulose, chitin, pullulan and other derivatives are discussed in Section 8.2.

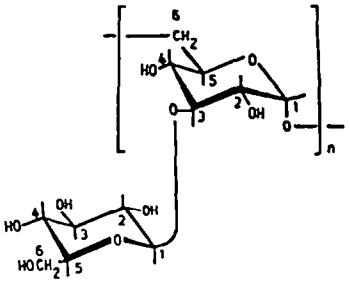
6.2. *Synthesis of Cyclic Anhydro Derivatives*

In view of the unusual properties of several polysaccharides containing 3,6-anhydro rings (e.g. agarose, carrageenans), a number of workers have developed synthetic methods for the introduction of 3,6-anhydro residues.[339]

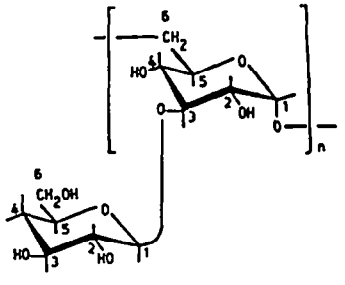
The preparation of 3,6-anhydroamylose **234** has been accomplished via internal nucleophilic displacement of a good leaving group in a five-step procedure involving tritylation, acetylation, detritylation, *p*-toluenesulfonylation, and saponification.[340] The product had a d.s. of 0.85.



Scheme 26. Synthesis of branched dextran analogues (Ref. 83).



231



232

Gal'braikh and co-workers[12,341] have prepared 3,6-anhydrocellulose derivatives with 50–70 mol-% anhydro ring substitution by saponification of 6-*O*-tosyl-2,3-di-*O*-acetylcellulose derivatives. Similarly, 2,3-anhydrocellulose products **236** with 0.60 α-oxide rings per repeat unit were synthesized from 2(3)-*O*-tosyl-cellulose **235**.[12] Treatment of the latter (or of cellulose epoxide derivatives) with ammonia afforded product mixtures (**207**, **208**) containing aminodeoxy functions at secondary positions (Scheme 27).[342]

In an attempt to prepare analogues of agarose, Misaki and Tsumuraya[343] have used a dimethyl sulfoxide–sulfur trioxide treatment to obtain *O*-6 sulfation of elsinan (repeat unit **239**). The partially sulfated glucan (**240**) was then converted into a 3,6-anhydro derivative **241** (d.s. *ca* 0.5) using alkali treatment at 80° (Scheme 28). The modification procedures resulted in reductions in molecular weight (from 300,000 to 200,000) and intrinsic viscosity (from 1.86 to 0.029) of the products.

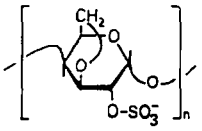
Rees[344] has similarly introduced 3,6-anhydro residues into the galactosyl units of kappa-carrageenan (**242**) via alkali treatment; the 2-*O*-sulfate group performed a stabilizing effect in the product formation.

Perceival and Wold[345] obtained 2,3-epoxide derivatives of L-rhamnose-containing polysaccharides (**243**) by alkali treatment of the native polymer.

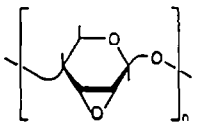
Other examples of the selective introduction of 3,6-anhydro residues into polysaccharide branch residues will be presented in Section 7.2.2.

6.3. *Other Modifications*

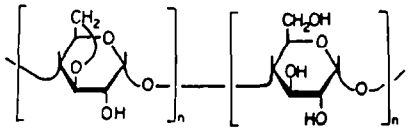
The preparation of several incompletely characterized aminodeoxy cellulose and aminodeoxy dextran derivatives has been described. The syntheses involve the use of *p*-tosyl cellulose[346] or fluorodeoxycellulose[347] precursors, electron beam or X-ray irradiation,[348] and ammonia-treatment of dextran tosylates.[349] Wolfram *et al.*[350] prepared 2(3),6-di-*O*-*p*-tolylsulfonyl amylose for subsequent conversion into the corresponding (mixed) aminated derivatives via hydrazination and reduction.



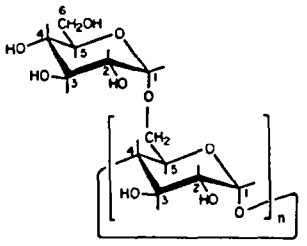
242



243

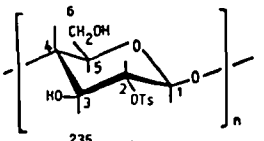


234

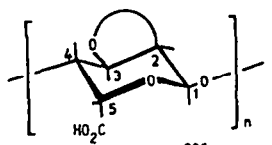


251 n = 5

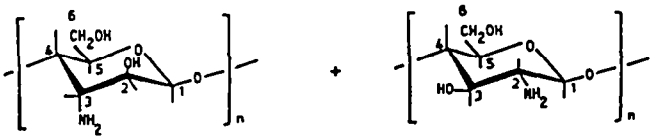
252 n = 6



235

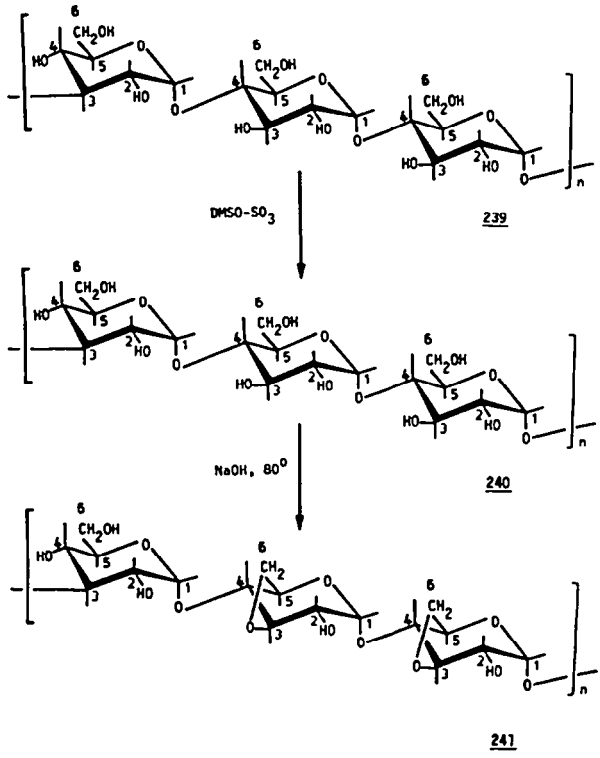


236



237 238

Scheme 27. Synthesis of 2(3)-aminocellulose (Ref. 342).



Scheme 28. Synthesis of 3,6-anhydroelsinan (Ref. 343).

7. MODIFICATION OF SELECTED POLYSACCHARIDE RESIDUES

7.1. *End Group Modifications*

Most studies of polysaccharide end group modifications to date have been concerned with structural elucidations. The role of terminal groups as loci for chemical modification or attachment of other molecules is only recently emerging.

The modification of polysaccharide end groups offers a convenient route for low-level substitution reactions. Such derivatizations may be advantageous for a number of applications, including spectroscopic studies requiring the incorporation of radioisotopes or various other probe molecules, or in the preparation of polysaccharide conjugates of pharmaceuticals or biological substrates, e.g. proteins, enzymes, etc. End group coupling methods are, in many respects, superior to non-selective chemical techniques, as they avoid or minimize undesirable phenomena, such as the formation of crosslinkages (interpolymer and/or between polymers and substrate) and other extraneous reactions (involvement of non-targeted substrate functional groups in binding, etc.).[70] End group modifications also do not contribute to, or cause only minimal structural perturbation of the polymer, affording well-defined products which may be of greater utility in the interpretation of complex biological systems.

Monofunctional derivatives have been successfully employed in the synthesis of polysaccharide-based immunogens, affinity ligands, etc.

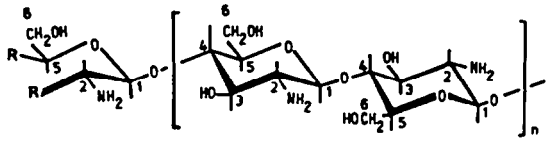
7.1.1. *Reducing end groups*

7.1.1.1. *Oxidations and reductions.* Sodium borohydride reductions of polysaccharide reducing end groups have been commonly employed in molecular weight determinations and similar applications.[351]

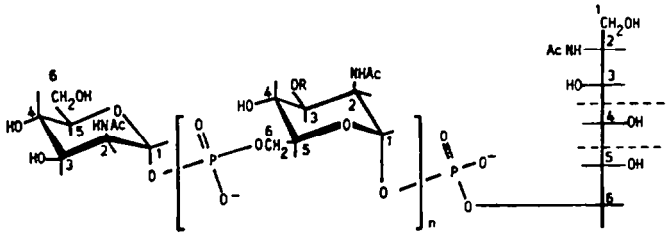
A study of heparin revealed that only a small proportion (*ca* 4–6%) of the polymer molecules possessed reducing end groups.[241] The reduced heparin product seemed to retain its biological activity as evidenced by affinity chromatography on thrombin- and antithrombin-Sepharose. The selective

borohydride reduction of terminal aldehyde functions in the presence of in-chain carboxylic groups has been described for heparin.[352]

In order to facilitate chemical differentiation between the reducing and non-reducing end groups of the meningococcal group A polysaccharide **244** (M_w 25,000), for subsequent derivatization reactions, Jennings and Lugowski reduced the reducing *N*-acetylmannosamine end group of this polymer with borohydride.[353] This transformation rendered the resulting acyclic *N*-mannosaminitol residues more susceptible to periodate oxidation than the other non-*O*-acetylated internal residues (see also Section 7.1.2). The reduced end group was then periodate oxidized to introduce a reactive aldehyde function at C-4 or C-5, as indicated in structure **244**. The combined reduction/oxidation procedure did not result in any appreciable reductions in molecular weight. The polysaccharide was subsequently attached to tetanus toxoid via reductive amination. The molar ratio of polysaccharide to tetanus toxoid in the resulting conjugate was 0.4 : 1.0.



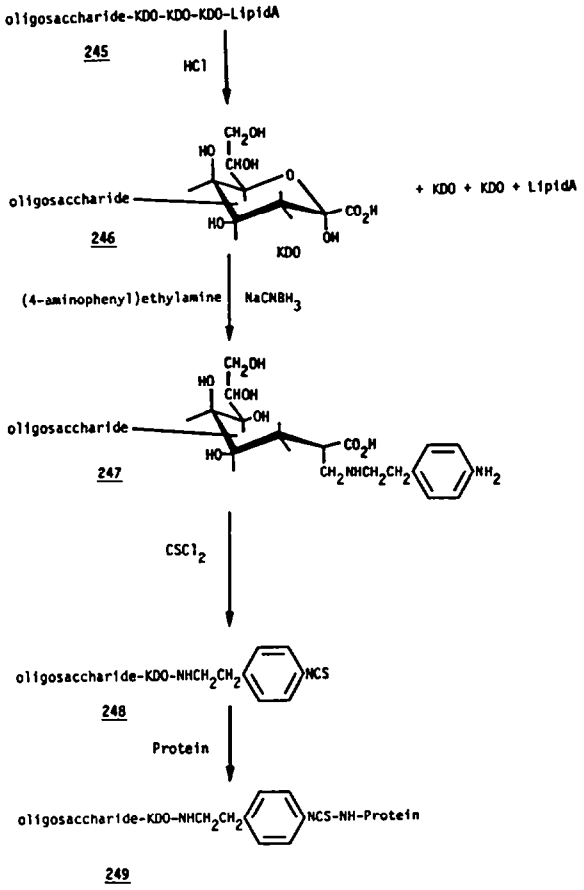
	R
261	CHO
262	CH_2OH



244 R = H or Ac

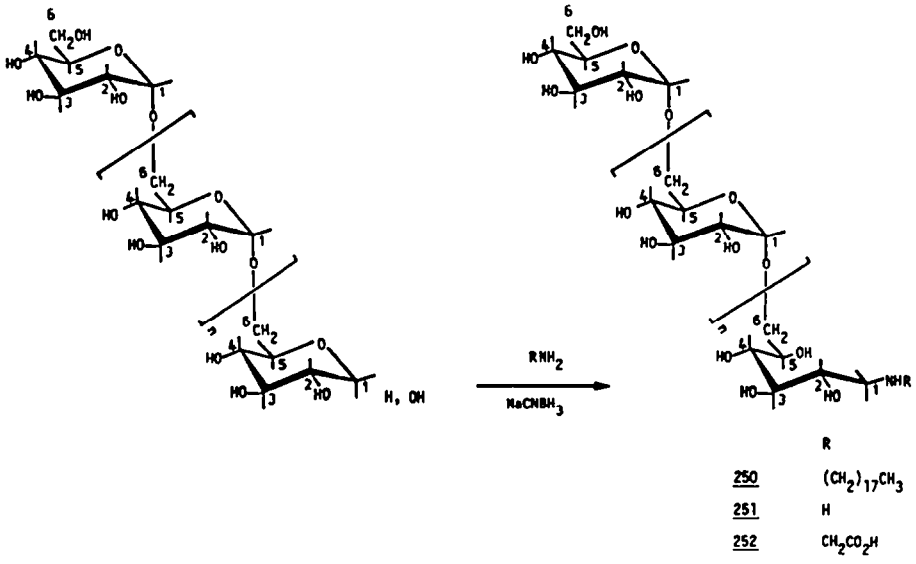
7.1.1.2. *Reductive aminations.* In addition to the example mentioned above, the reductive amination procedure (see Section 5.2) has been used in several recent studies involving the coupling of polysaccharides or polysaccharide fragments to various substrates. Thus, heparin has been conjugated via its reducing end group to amino-Sepharose **80**.[307] In another recent investigation, heparin was partially depolymerized by deaminative cleavage with nitrous acid to afford oligosaccharide fragments, half of which had 2,5-anhydro-D-mannose residues as reducing terminals.[316] This reactive heparin fraction was then covalently linked to 6-aminohexyl substituted curdlan and Sepharose, as mentioned previously.

Jennings *et al.* have used the reductive amination approach in the preparation of oligosaccharide–tetanus toxoid conjugates (**249**) (Scheme 29).[354] The oligosaccharides (M_w *ca* 1500), obtained by mild acid hydrolysis of a meningococcal lipopolysaccharide, were dephosphorylated and attached to BSA, tetanus toxoid, and an aminoethyl Bio-gel column either directly, by reductive amination through the hemiketal of their terminal reducing 2-keto-3-deoxyoctonic acid (KDO) residues, or indirectly, through 2-(4-aminophenyl)-ethylamine spacers (**247**).[354] For the tetanus toxoid conjugates, an incorporation of between 18 and 38 oligosaccharide residues was achieved. The reductive amination procedure was found to be less efficient than the indirect method. Similar methods have been employed by other workers.[355–358]



Scheme 29. Synthesis of oligosaccharide–tetanus toxoid conjugates (Ref. 354).

More recently, a series of end group modifications have been described for dextran, guar gum and locust bean gum using reductive amination methods (Scheme 30).[69] Octadecylamine derivatives **250** of various dextran molecular weight fractions (M_w 10,000–500,000) were synthesized using DMSO, DMF, or aqueous alcohol solutions containing 10% sodium chloride as reaction medium and temperatures of 80–95°. The presence of salt, which induces a more expanded dextran solution



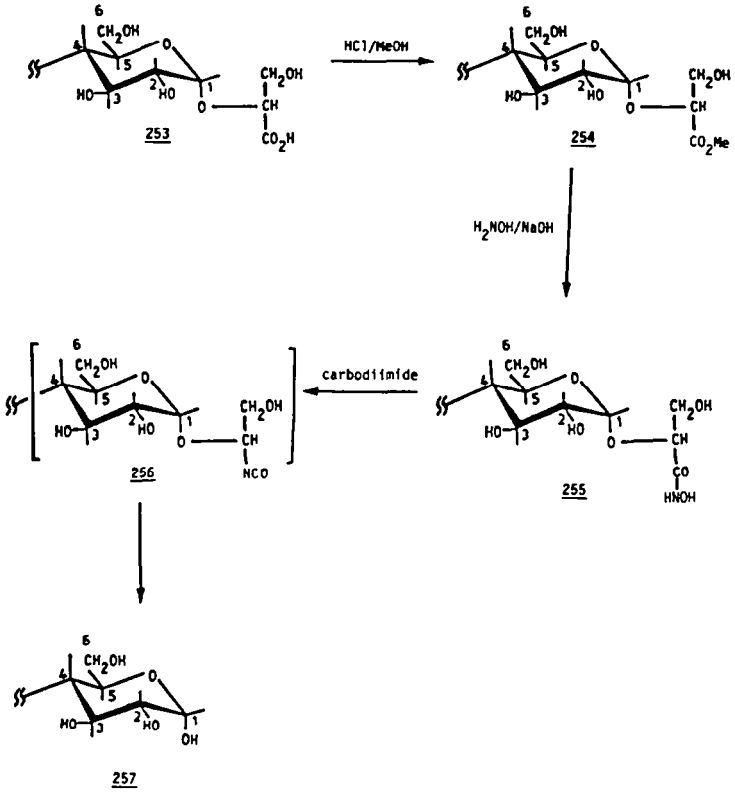
Scheme 30. Reductive aminations of dextran (Ref. 69).

conformation, was found to significantly affect the yields (generally between 45 and 60%). The dextran derivatives were employed as polymeric affinity ligands for partition studies in dextran–ficoll and dextran–polyethylene glycol two phase systems.[70] For this purpose, it was important that the derivative possessed a single affinity ligand per polymer molecule in order to minimize the possibility of inducing cell aggregation. Following the same synthetic approach, a series of functional derivatives were obtained using sodium cyanoborohydride and ammonium acetate (**251**), glycine (**252**), and glucosamine (**195**), respectively. Chain extension of dextran by a single ^{14}C-labelled glucosamine residue offered the possibility of obtaining reductively aminated products in essentially the same manner as for the native polymer, with simultaneous incorporation of a label. Other reducing end modifications included streptomycin, nitroxide spin label, and aminopropyl-activated controlled pore glass derivatives.

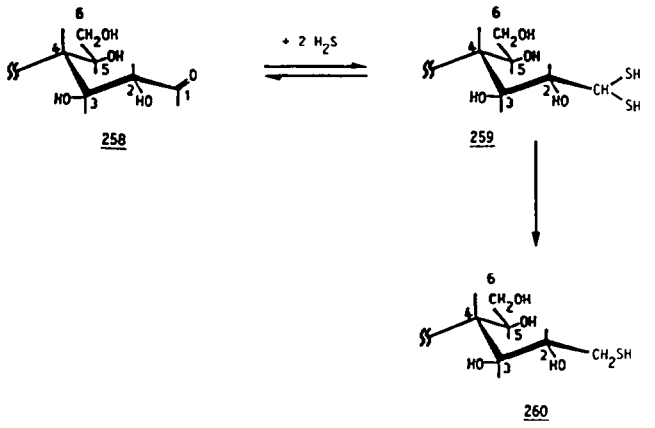
7.1.1.3. *Other reducing end group modifications.* Isbell[359] developed an efficient method for the synthesis of ^{14}C-labelled polysaccharides, which is based on treatment of the aldehyde function of the reducing end group with sodium cyanide and subsequent hydrolysis of the polysaccharide nitrile to the corresponding carboxylic acid derivative. Quantitative yields are obtainable, but require extended reaction periods (7 days). A ^{14}C-labelled carboxyl dextran derivative (**103**) is commercially available and has been used for carbodiimide-mediated amidation with octadecylamine (**104**).[69]

Yabusaki and Ballou[360] have coupled tryptophan via amide linkages to the glyceric acid carboxyl groups at the reducing end of two mycobacterial polymethylated polysaccharides, and in another study,[361] selectively removed a terminal glyceric acid residue via carbodiimide-mediated Lossen rearrangement from a polysaccharide of *Mycobacterium phlei* (Scheme 31).[361]

Various studies have been concerned with the effects of chemicals, such as sodium borohydride, hydrogen sulfide, anthraquinone, etc., on the stability of cellulosic materials under alkaline pulping conditions.[362–364] In the case of hydrogen sulfide for example, stabilization of cellulose and soft wood glucomannans seems to rely on the conversion of the reducing terminals to alkali-resistant 1-thio-D-glucitol moieties **260** (Scheme 32).[362]



Scheme 31. Selective removal of terminal glyceric acid residues (Ref. 361).

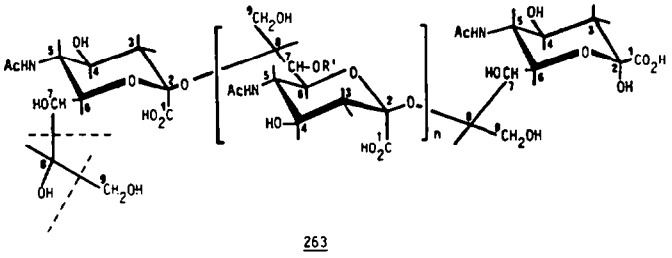


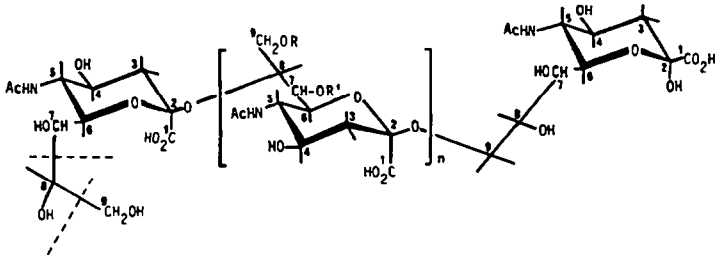
Scheme 32. Reaction of glucomannans with hydrogen sulfide (Ref. 362).

7.1.2. *Non-reducing end group modifications*

7.1.2.1. *Oxidations.* In a study of the susceptibility of chitin and chitosan derivatives to enzymic degradation, Hirano and Yagi[365] have employed substrates with modified non-reducing terminal groups. The rates of chitinase and lysozyme hydrolysis of such oxidized *N*-acetyl chitosan derivatives (**261, 262**) were found to be higher than for the unoxidized polymer.

Aldehyde groups have been selectively introduced into terminal non-reducing sialic acid residues of meningococcal group B (**263**) and group C (**264**) polysaccharides by periodate oxidation without concomitant depolymerization.[353]





264

A R = R' = H

B R = R' = Ac

C R = H, R' = Ac

D R = Ac, R' = H

The selective conversion of the primary hydroxyl functions of terminal non-reducing D-glucopyranosyl residues (in the polymer backbone and branches) of dextrans into carboxylic acid residues (**7**) has been accomplished by catalytic oxidation, as mentioned previously.[102] Oxidation yields of up to 80–85% have been reported.[102,103]

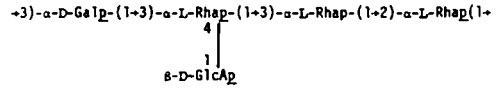
7.1.2.2. *Reductive aminations.* Periodate-oxidized meningococcal group B (M_w 10,000) and group C (M_w 40,000) polysaccharides have been attached to tetanus toxoid, BSA, and lysine by reductive amination.[353] Molar incorporation ratios of polysaccharide to tetanus toxoid and BSA ranged between 1.1 : 1.0 and 2.5 : 1.0, respectively.

7.1.2.3. *Other modifications.* The presence of only a limited number of primary hydroxyl functions at the non-reducing terminals of dextran, has been exploited for selective modifications. Thus, for a dextran derived from *Leuconostic mesenteroides* NCIB 2206, successive tritylation, methylation, and detritylation afforded a product whose secondary hydroxyl functions were methylated.[366] The free primary alcohol functions were then *p*-toluenesulfonylated and the resulting derivative hydrolyzed. Similar techniques have been employed for the stepwise removal of single glucopyranosyl residues from dextran, as previously discussed.[152]

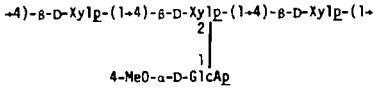
Base catalyzed β-elimination reactions of terminal, non-reducing uronic acid residues (or of uronic acid residues derived from other types of residues) have been employed in structure elucidation studies.[38,40–42] For polysaccharides containing 4-*O*-substituted uronic acid residues, this method relies on the preferential splitting of a β-glycosidic linkage to the C-4 position of glucuronic acid residues (Scheme 33). Since the β-elimination reaction is less complex and proceeds smoother when the polysaccharide hydroxyl functions are substituted, methylated derivatives are usually employed.

These eliminations can be accomplished through various chemical intermediates which have been previously reviewed in detail.[38,40–42,367] Although practical considerations such as generally low yields, the requirement of strictly anhydrous reaction conditions, and the (usually) irreversible formation of etherified (methylated) products, impose limitations on the utility of this method for chemical modification applications, several examples will be cited here to illustrate its scope.

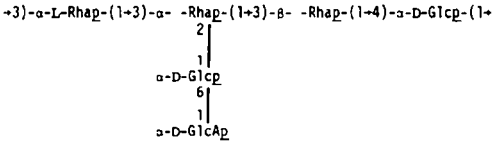
β-Eliminations of terminal glucuronic acid residues have been accomplished for *Klebsiella* type 9 (**267**),[368] birch xylan (**268**),[368] *pneumococcus* type 2 (**269**),[239] and various other polysaccharides. Lindberg *et al.*[370] eliminated the terminal D-glucuronic acid residues of a permethylated *Kelbsiella* type 59 capsular polysaccharide (repeat unit **270**). The resulting free hydroxyl functions on the newly exposed terminal residues were then oxidized to carbonyl groups by treatment with chlorine–DMSO in methylene chloride (45°, 6 hr) to give derivative **271**.



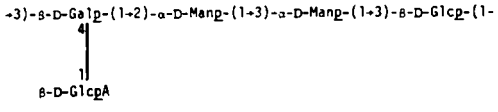
267



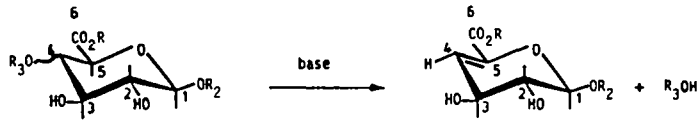
268



269



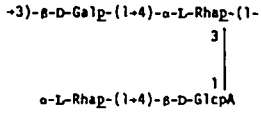
270



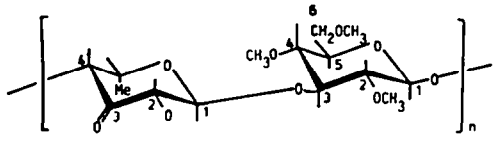
265 266

Scheme 33. β-Elimination reaction of polyuronides.

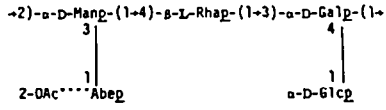
Terminal non-reducing galactosyl residues may be converted into the corresponding galactosyluronic acid residues by treatment with galactose oxidase (see Section 8.2) and subsequent hypoiodide oxidation.[371] The resulting oxidation products may then be selectively degraded as described above.



272



273



276

The β-elimination reaction can also be applied to polysaccharides in which the glucuronic acid residues form part of the side chain, as in the case of *Klebsiella* type 47 polysaccharide (repeat unit **272**). The debranched polymer was subsequently oxidized at C-3 of the rhamnopyranosyl residue to yield the disaccharide repeat unit **273**.[372]

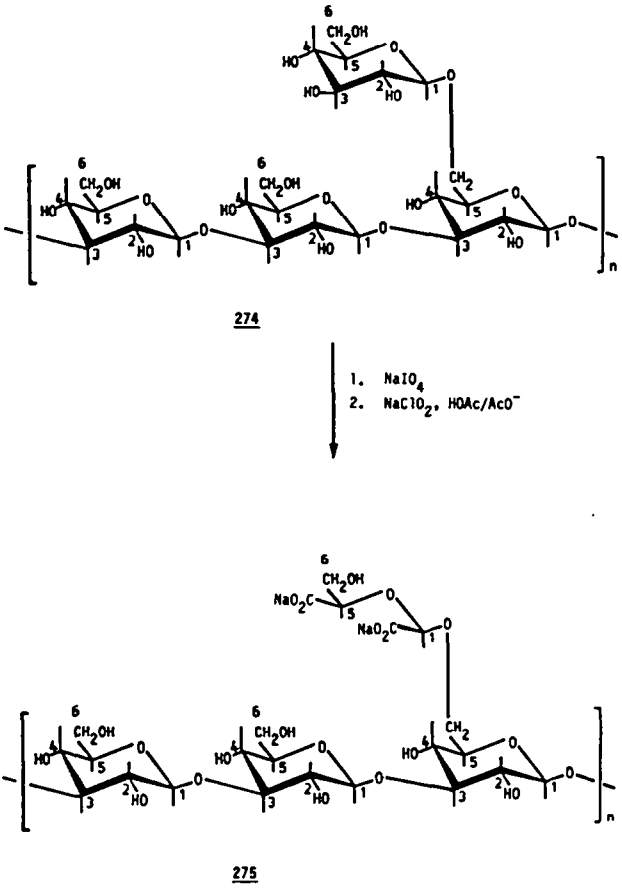
7.2. *Branch Residue Modifications*

7.2.1. *Oxidations*

A high degree of selectivity has been reported by Opie and Keen[373] in the preferential oxidation of the galactopyranosyl *cis*-diol residues of galactomannans, such as guar gum and locust bean gum. Using 0.25 mol $NaIO_4$ per mol of guaran hexose residue, they found that 77% of the oxidation had occurred at the galactosyl residues and the remainder at the mannan backbone.

This method was subsequently adopted to the oxidation of guaran and locust bean gum, using 0.19 and 0.14 mol $NaIO_4$ per mol of galactomannan hexose unit, respectively.[118] These concentrations of oxidant corresponded to 14 and 12%, respectively, of the theoretical requirement and would afford maximum degrees of oxidation of 0.53 and 0.70, respectively, of oxidized galactopyranosyl residues, assuming no oxidation of backbone residues. The products were spin labelled for structural studies.

In a recent study Crescenzi *et al.*[374] have oxidized the side chains of scleroglucan, a β-1 → 3-linked glucan which bears β-1 → 6-linked glucopyranosyl residues at every third glucopyranosyl unit (**274**).



274

275

Scheme 34. Oxidation of scleroglucan (Ref. 374).

Quantitative periodate oxidation of the branch residues afforded a dialdehyde product which was further treated with sodium chlorite. After conversion into the sodium salt, a derivative (**275**) was thus obtained which had two carboxylate functions per repeat unit (Scheme 34). The physical properties of this new anionic polymer were examined.

The enzymic oxidation of the primary alcohol functions of terminal galactofuranose and galactopyranose residues will be discussed in Section 8.2.

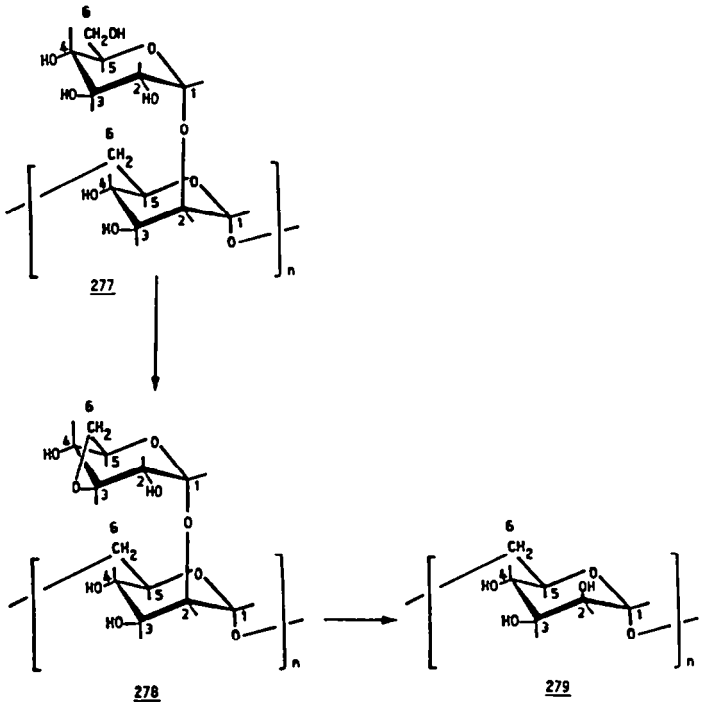
7.2.2. *Other modifications*

Many of the previously discussed β-elimination reactions (see Section 7.1.2.2) involve the modification of branch residues. The selective elimination of the 4-*O*-methyl-D-glucuronic acid residues of white birch xylan by the Hofman degradation technique has also already been mentioned (see Section 3.4).

The α-abequopyranosyl branch residue (as well as the lipid component) of the permethylated lipopolysaccharide from *Salmonella typhimurium* LT2 (repeat unit **276**) have been selectively removed by mild acid hydrolysis.[375] The generated free hydroxyl functions of the mannopyranosyl backbone residues were subsequently oxidized to carbonyl functions with ruthenium tetroxide affording a trisaccharide repeat unit (**241**).[375]

Gorin and Spencer[376] have eliminated the α-D-glucopyranosyl (and other) side chains of a *Ceratocystis brunnea* glucomannan **277** by means of selective introduction of 3,6-anhydro rings in the branch units **278** (see Scheme 35). This was accomplished in a six-step modification process, involving tritylation, acetylation, detritylation, *p*-toluenesulfonylation, and successive treatments with sodium methoxide and acid, to afford **279**.

Enzymic methods for the removal of branch residues are discussed in Section 8.4.



Scheme 35. Branch elimination of *Ceratocystis brunnea* glucomannans (Ref. 376).

7.3. *In-chain Residue Modifications*

The modification or selective cleavage of polysaccharides in which uronic acid or 2-amino-2-deoxy residues constitute a part of the polymer backbone, can be accomplished using the methods (including β-elimination, deamination, etc.) discussed in Sections 3 and 4, respectively. Aspinall and Puvanesarajah[377,378] have also recently applied a combined modified Curtius rearrangement/carbamate degradation technique to permethylated gum arabic (see Scheme 15).

8. ENZYMIC MODIFICATIONS

8.1. *Introduction*

Considerable efforts are currently being directed in industry at the development and improvement of enzymic processes for the production of various microbial exopolysaccharides, e.g. curdlan, dextran, pullulan, and xanthan gum,[9,22,34] the conversion of polysaccharides into fuels, solvents[379] and other useful chemicals such as sucrose, and the converse transformation of simple chemicals, such as methanol, to polysaccharides[380] for use in microbially enhanced oil recovery,[381] as well as for the isolation of new types of lipopolysaccharides with emulsifying properties.[53]

Over 40 polysaccharases are presently available[34–37] which can be classified in three major categories according to (i) depolymerizing, (ii) debranching, and (iii) synthetic activity. Polysaccharide depolymerizations can be mediated by enzymes in category (i) via hydrolysis (hydrolases, E.C.3.2.–.–) or transelimination (lyases, E.C.4.2.–.–) reactions in either *endo* (random action along the polymer chain) or *exo* (sequential degradation initiated at the non-reducing end of the polymer) fashion. Cleavage of polysaccharide C—O bonds by lyases produces olefinic bonds at the non-reducing end of the oligosaccharide fragments. The activity of hydrolases can be associated with transglycosylation reactions, i.e. the transfer of oligosaccharides from the polysaccharide to the hydroxyl functions of other oligosaccharides instead of water.

Debranching enzymes selectively remove intact branch residues, such as the 1 → 6 linked branches of (1 → 4), (1 → 6) α-D-glucans.

The activity of synthetic enzymes may involve (a) the transfer of oligosaccharides, e.g. the attachment of branches, (b) the cyclization of oligosaccharide sections, e.g. the formation of cyclodextrins, (c) the modification of functional groups, such as oxidations of hydroxyl groups,

or (d) the transfer of non-carbohydrate moieties, e.g. sulfate functions. As with many of the chemical methods discussed above, most enzymic polysaccharide modifications have so far been concerned with analytical rather than synthetic uses.

The purpose of the discussion in this section is to provide an introduction to a selected number of enzymes which have been employed, or are of potential utility for selective polysaccharide modifications. Other aspects of polysaccharases, such as their source, purification, mode of action, and analytical uses have been comprehensively described in several recent monographs, articles and periodical accounts.[34–37,382,383]

8.2. *Oxidations*

The oxidation of D-galactose-containing polysaccharides by galactose oxidase (D-galactose: oxygen 6-oxidoreductase, E.C.1.1.3.9) is perhaps one of the most widely used enzymic modification methods. This enzyme exists as a single polypeptide chain with a copper ion as its sole co-factor and is derived from fungal species, such as *Dactylium dendroides*.[113,384]

Galactose oxidase is particularly attractive for the high specificity and overall simplicity of the reaction it catalyses (Scheme 23), in which the *pro*-S hydrogen is abstracted from the C-6 position of galactose residues to afford the corresponding aldehyde derivatives **26**.[385]

Since its discovery by Avigad *et al.*[113] in 1962, it has been established that this enzyme displays a much greater affinity for polymeric than monomeric substrates, but its exact metabolic function remains obscure. Nevertheless, the reaction of galactose oxidase has been successfully applied to a number of carbohydrate systems, including guar gum,[113,114,118,386] locust bean gum,[118] agarose[115,387] and cell surface glycoproteins.[388] The enzyme is apparently inactivated only by C-4 substitution for galactose and galactosides and by C-3 substitution for 2-amino-2-deoxygalactose derivatives.[113,386,389] Oxidations of both the pyranosyl and furanosyl forms of galactose have been reported.

Galactose oxidase oxidations are performed in the presence of catalase (E.C. 1.11.1.6, which consumes the product-inhibiting H_2O_2 formed in the reaction)[390] in buffered solutions (pH *ca* 7) for periods of 1 or 2 days, depending on the substrate. The oxidized polysaccharides can either be derivatized *in situ*, or after isolation (by, e.g. precipitation).[118]

Very high efficiencies have been reported for the enzymic oxidation process, as evidenced by various direct and indirect methods,[113,388] including the nitrogen incorporation of reductively aminated products (60–70% yields for aminated guar gum, and 70–90% for aminated locust bean gum derivatives).[118]

The introduction of reactive aldehyde functions into the polysaccharide matrix provides a convenient approach to the synthesis of Schiff 's-base or stable amine derivatives. The latter type can be obtained via the reductive amination procedure, as discussed previously (see Section 5.2). Various spectroscopic and other probes, such as deuterium,[391] tritium,[388] fluorine,[392] fluorescent,[393] and nitroxide spin labels[118] have been incorporated by these procedures into polysaccharides and biological substrates of different complexity. The oxidation has also been applied to branch modifications of native and derivatized polysaccharides, as indicated earlier (see Section 5.2).

The substantial utility of galactose oxidase oxidations can be fully appreciated by considering equivalent chemical methods for the introduction of C-6 aldehyde functions into polysaccharides, as exemplified by the case of the multi-step synthesis of C-6 aldehydo amylose and cellulose[110,111] (see Section 2.1.1.2).

This modification method promises therefore to be of substantial importance in future industrial applications. Particularly, when one considers the present availability of efficient production methods for the enzyme. Using, for example, *Dactylium dendroides*, 280 mg of homogeneous enzyme can be obtained per 100 litres' growth medium in 9 days. The commercial viability of galactose oxidase modifications has not yet been tested, but should be achievable if immobilization techniques are used.

Unfortunately, no equivalent enzyme systems are presently known for the selective oxidation of the primary hydroxyl functions of glucose or other common carbohydrate residues.

8.3. *Transglycosylations*

A substantial number of studies have been concerned with synthetic oligo- and polysaccharide modifications based on the utilization of carbohydrate transferases (*exo*-glycosidases)[394] which

proceed according to eqn (2):

$$\underset{\text{donor}}{R{-}O{-}R'} + \underset{\text{acceptor}}{R''OH} \rightleftharpoons \underset{\text{product}}{R{-}O{-}R''} + \underset{\text{by-product}}{R'OH}. \quad (2)$$

The transfer may involve any acceptor hydroxyl function, but for the case of aldohexoses, the primary hydroxyl group is preferred.

The phosphorylitic modification of polysaccharides bearing maltooligosaccharide branch residues has been discussed in Section 6.1.

A dextran sucrase form *Leuconostoc mesenteroides* has been employed to transfer sucrose-derived α-glucopyranosyl residues to cellulose powder, wood pulp, and chitin.[87] The products exhibited improved tensile strength and other properties, but were not characterized in terms of their structure.

A Japanese patent[395] describes the preparation of α-glycosylated pullulan derivatives using cyclodextrin glycosyltransferase or isoamylase (E.C. 3.2.1.68) and maltosyl oligosaccharides obtained via hydrolysis with pullulanase.

Treatment of the mother liquors derived from the large-scale preparation of cyclodextrins with amyloglucosidase from *Aspergillus niger* has been reported to afford α-1 → 6-glucosyl derivatives of α- and β-cyclodextrin (**251**, **252**).[396,397]

8.4. *Debranchings*

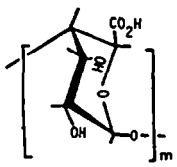
A small number of debranching enzymes are known which can be classified as hydrolases of *O*-glycosyl bonds. Most commonly, their action involves attack at 1 → 6 linkages of (1 → 4)(1 → 6) linked α-D-glucans, e.g. amylopectin.

Slodki and Cadmus[398] have isolated a *Bacillus* species which produces an enzyme complex with debranching activity for xanthan gum. The enzyme complex removes the trisaccharide side chains from the native polymer and affords a product with a reduced viscosity profile.

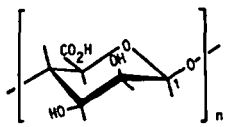
In their investigations of the interaction properties of galactomannans with other polysaccharides, such as agarose and xanthan gum, McCleary and co-workers[399-401] have employed highly purified α-D-galactosidase (E.C.3.2.1.22) from germinating guar seeds for the removal of the galactosyl branch residues of guar gum. These preparations were devoid of chain-splitting β-D-mannanase, as indicated by viscosity measurements. The enzymic treatment produced galactose-depleted derivatives whose D-galactose content was reduced from 39 to 14%. The debranched products formed more viscous solutions than the native polymer. A recent patent[402] exploits this method for the production of viscous solutions and gels.

8.5. *Other Modifications*

Haug and Larsson[403] have affected the conversion of mannuronic acid residues (**280**) to guluronic acid residues (**281**) by treatment of alginate with an epimerase from *Azobacter vinelandii*.



281



280

Pectin esterase (E.C.3.1.1.11) selectively cleaves methyl and ethyl but not other types of ester groups off pectin.[404,405] The enzymes attack from the reducing end of the polysaccharide or adjacent to free carboxyl functions, and then act along the molecule.

9. OUTLOOK

From the foregoing discussion, it is evident that a considerable arsenal of chemical and enzymic methods is now at hand for the selective transformation of many polysaccharides. In view of the inadequacies of most of these techniques, e.g. in terms of the achievable degrees of selectivity and

conversion efficiencies, further advances are obviously required, particularly in the area of new, mild oxidants, acylation and alkylating reagents, low-cost reversible protecting agents, etc. Greater efforts will also have to be directed at the development of new chemical catalysts and methods for the enhancement of polysaccharide reactivity and solubility.

The enzymic modification approach will play an increasingly prominent role in future, particularly with improvements in the availability and cost of enzymes. An additional impetus to this trend will derive from the extension of current studies on the effects of chemical modifications on the activity of polysaccharases.

In view of the recent advances in the synthetic strategies for the preparation of new types of branched polysaccharides, it appears that polysaccharide chemists are now poised to achieve a significant breakthrough in the development of model polysaccharides for the establishment of a systematic understanding of the fundamental structure/property relation.

Acknowledgements—The author wishes to express his gratitude to Drs I. C. M. Dea, J. M. Harris, and B. McKague for their comments, to Drs G. O. Aspinall, N. K. Matheson, B. V. McCleary, and S. Paoletti for preprints of their publications and to the National Science and Engineering Research Council of Canada and the Science Council of B.C. for partial financial support.

REFERENCES

[1] W. Pigman and D. Horton (Editors), *Carbohydrates, Chemistry and Biochemistry*, 2nd edn., Vol. II A (1978) and Vol. II B (1980). Academic Press, New York.
[2] G. O. Aspinall (Editor), *The Polysaccharides*, Vol. I (1982) and Vol. II (1983). Academic Press, New York.
[3] J. F. Kennedy and C. A. White, *Compreh. Org. Chem.* **5**, 755 (1979).
[4] D. A. Rees, *Polysaccharide Shapes*. Chapman & Hall, London (1977).
[5] E. R. Morris and I. T. Norton, *Stud. Phys. Theor. Chem.* **26**, 549 (1983).
[6] N. K. Kochetkov, *Pure Appl. Chem.* **42**, 327 (1975).
[7] A. F. Bochko and G. E. Zaikov, *Chemistry of the O-Glycosidic Bond*. Pergamon Press, New York (1979).
[8] R. M. Rowell and R. A. Young (Editors), *Modified Cellulosics*. Academic Press, New York (1978).
[9] P. A. Sandford and A. Laskin (Editors), *Extracellular Microbial Polysaccharides*, Vol. 45. ACS Symp. Ser. (1977).
[10] M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*. Springer, Berlin (1978).
[11] R. A. A. Muzzarelli, *Chitin*. Pergamon Press, New York (1977).
[12] L. S. Gal'braikh and Z. A. Rogovin, *Adv. Polym. Sci.* **14**, 87 (1974).
[13] R. L. Whistler, A. A. Bushway and P. P. Singh, *Adv. Carbohydr. Chem. Biochem.* **32**, 235 (1976).
[14] C. M. Sturgeon, *Carbohydrate Chemistry* **14** (II), 375 (1983).
[15] D. A. Brant (Editor), *Solution Properties of Polysaccharides*, Vol. 150. ACS Symp. Ser. (1981).
[16] R. G. Schweiger (Editor), *Carbohydrate Sulfates*, Vol. 77. ACS Symp. Ser. (1978).
[17] R. L. Whistler (Editor), *Methods Carbohydr. Chem.* **5** (1965).
[18] N. K. Kochetkov, *Soviet Sci. Rev.* Sect. B, **4**, 1 (1982).
[19] R. L. Lundblad, W. V. Brown, K. G. Mann and H. R. Roberts, *Chemistry and Biology of Heparin*. Elsevier/North-Holland, New York (1980).
[20] D. J. Candy, *Biological Functions of Carbohydrates*. Wiley, New York (1980).
[21] P. A. Sandford and K. Matsuda (Editors), *Fungal Polysaccharides*, Vol. 126. ACS Symp. Ser. (1980).
[22] J. W. Cottrell, *The Chemistry and Biochemistry of Fungal Polysaccharides*, Vol. 251, p. 126. ACS Symp. Ser. (1980).
[23] V. N. Nigam and A. Cantero, *Adv. Cancer Res.* **17**, 1 (1973).
[24] J. M. Mishler, *Pharmacology of Hydroxyethyl Starch, Use in Therapy and Blood Banking*. Oxford University Press, New York (1982).
[25] R. Arnon and E. Hurioitz, *Targeted Drugs* (Edited by E. P. Goldberg), p. 23. Wiley, New York (1983).
[26] H. J. Jennings, *Adv. Carbohydr. Chem. Biochem.* **41**, 155 (1983).
[27] M. J. Poznansky and L. G. Cleland, *Drug Delivery Systems* (Edited by R. L. Juliano), p. 253. Oxford University Press, New York (1980).
[28] P. A. Sandford and J. Baird, *The Polysaccharides* (Edited by G. O. Aspinall), Vol. 2, p. 411. Academic Press, New York (1983).
[29] R. L. Whistler (Editor), *Industrial Gums*, 2nd edn. Academic Press, New York (1973).
[30] M. Glicksman (Editor), *Food Hydrocolloids*, Vol. 1. CRC Press, Inc., Boca Raton (1982).
[31] J. A. Radley, *Starch and its Derivatives*, 4th edn. Chapman & Hall, London (1968).
[32] R. W. James, *Industrial Starches*. Noyes Data Corporation, Park Ridge, New Jersey (1974).
[33] Y. L. Meltzer, *Water-soluble Resins and Polymers*. Noyes Data Corporation, Park Ridge, New Jersey (1976).
[34] R. C. W. Berkeley, G. W. Gooday and D. C. Ellwood (Editors), *Microbiol Polysaccharides and Polysaccharases*. Academic Press, New York (1979).
[35] J. F. Kennedy, *Carbohydrate Chemistry* **14** (II), 230 (1983) and previous reviews in this series.
[36] M. E. Bushell (Editor), *Progr. Ind. Microbiol.* **18** (1983).
[37] R. L. Whistler and J. N. BeMiller, *Methods Carbohydr. Chem.* **8** (1980).
[38] G. O. Aspinall, *Pure Appl. Chem.* **45**, 1105 (1977).
[39] K. Jann and B. Jann, *Methods Enzymol.* **50C**, 251 (1978).
[40] B. Lindberg and J. Lönngren, *Methods Enzymol.* **50C**, 3 (1978).
[41] B. Lindberg, J. Lönngren and S. Svensson, *Adv. Carbohydr. Chem. Biochem.* **31**, 185 (1975).
[42] G. O. Aspinall, *The Polysaccharides* (Edited by G. O. Aspinall), Vol. 1, p. 36. Academic Press, New York (1982).
[43] A. P. Croft and R. A. Bartsch, *Tetrahedron* **39**, 1417 (1983).
[44] J. Boger, R. J. Corcoran and J. M. Lehn, *Helv. Chim. Acta* **61**, 2190 (1978).
[45] J. H. Pazur, *Adv. Carbohydr. Chem. Biochem.* **39**, 405 (1981).

[46] J. Porath, *Methods Enzymol.* **34**, 13 (1974).
[47] E. Perceival, *Methods Carbohydr. Chem.* **8**, 281 (1980).
[48] A. H. Haines, *Adv. Carbohydr. Chem. Biochem.* **39**, 13 (1981).
[49] A. S. Perlin, *The Carbohydrates, Chemistry and Biochemistry* (Edited by W. Pigman and D. Horton), Vol. IIB, p. 1167. Academic Press, New York (1980).
[50] W. M. Doane, B. S. Shasha, E. I. Stout, C. R. Russell and C. E. Rist, *Carbohydr. Res.* **11**, 321 (1969).
[51] L. Anderson and F. M. Unger (Editors), *Bacterial Lipopolysaccharides, Structure, Synthesis, and Biological Activities*, Vol. 231. ACS Symp. Ser. (1983).
[52] F. J. Fayers (Editor), *Enhanced Oil Recovery*. Elsevier, Amsterdam (1981).
[53] N. Basta, *High Technology* 66, February (1984).
[54] *Commercial Biotechnology: An International Analysis*. Office of Technology Assessment, U.S. Government Printing Office, Washington, D.C. (1984).
[55] H. G. Geniesser, D. Gabel and B. Jastorff, *J. Chromatogr.* **215**, 235 (1981).
[56] R. A. Gelman, *XIth Int. Carbohydr. Symp.*, Abstr. III-7, Vancouver, August 22–28 (1982).
[57] E. R. Morris, *Techniques and Applications of Fast Reactions in Solution* (Edited by W. J. Gettins and E. Wyn-Jones), p. 379. D. Reidel, Boston (1979).
[58] D. A. Rees, E. R. Morris, D. Thom and J. K. Madden, *The Polysaccharides* (Edited by G. O. Aspinall), Vol. 1, p. 195. Academic Press, New York (1982).
[59] V. Crescenzi, *Supramolecular Structure and Function* (Edited by G. Pifat and J. N. Herak), p. 59. Plenum Press, New York (1983).
[60] E. R. Morris and S. B. Ross-Murphy, *Techniques in Carbohydrate Metabolism*, p. 1. Elsevier, Amsterdam (1981).
[61] A. J. Wicken and K. W. Knox, *Biochim. Biophys. Acta* **604**, 1 (1980).
[62] Y. Shigeno, K. Kondo and K. Takemoto, *Angew. Makromol. Chem.* **91**, 55 (1980).
[63] T. C. Yang and R. R. Zall, *I.E.C. Product Res. Develop.* **23**, 168 (1984).
[64] P. A. Sandford, *Adv. Carbohydr. Chem. Biochem.* **36**, 265 (1979).
[65] J. K. Baird, P. A. Sandford and I. W. Cottrell, *Biotechnology* 778, November (1983).
[66] J. Folkman, R. Langer, R. J. Linhardt, C. Handenschild and S. Taylor, *Science* **221**, 719 (1983).
[67] L. Molteni, *Drug Carriers in Biology and Medicine* (Edited by C. G. Gregoriadis), p. 107. Academic Press, New York (1979).
[68] P. A. Albertsson, *Partition of Cells, Particles and Macromolecules*, 2nd edn. Wiley, New York.
[69] M. Yalpani and D. E. Brooks, Submitted for publication.
[70] J. M. Harris and M. Yalpani, *Partitioning in Aqueous Two Phase Polymer Systems: Theory, Methods, Uses, and Applications to Biotechnology* (Edited by H. Walters, D. E. Brooks and E. Fisher). Academic Press, New York (1985), in press.
[71] K. Kobayashi and H. Sumitomo, *Polym. Bull.* **1**, 121 (1978).
[72] C. L. McCormick and D. K. Lichatowich, *J. Polym. Sci. Polym. Lett.* **17**, 479 (1979).
[73] A. Frigeno and L. Renoz (Editors), *Recent Developments in Chromatography and Electrophoresis*. Elsevier, Amsterdam (1979).
[74] J. F. Kennedy and J. M. S. Cabral, *Appl. Biochem. Bioeng.* **4**, 189 (1983).
[75] W. A. Szarek, *MTP Int. Rev. Sci.: Org. Chem. Ser. One.* **7**, 80 (1973).
[76] A. F. Bochkov and G. E. Zaikov, *Chemistry of the O-Glycosidic Bond*. Pergamon Press, New York (1979).
[77] W. W. Graessley, *Acc. Chem. Res.* **10**, 332 (1977).
[78] H. Grisebach and R. Schmidt, *Angew. Chem. Int. Ed. Engl.* **11**, 159 (1972).
[79] I. J. Goldstein, R. D. Poretz, L. L. So and Y. Yang, *Arch. Biochem. Biophys.* **127**, 787 (1968).
[80] S. F. Grappel, *Experimentia* **27**, 329 (1971).
[81] E. T. Reese and F. W. Parrish, *Biopolymers* **4**, 1043 (1966).
[82] J. Cisar, E. A. Kabat, M. M. Dorner and J. J. Lia, *J. Exp. Med.* **142**, 435 (1975).
[83] H. Ito and C. Schuerch, *J. Am. Chem. Soc.* **101**, 5797 (1979).
[84] B. Pfannemüller, G. C. Richter and E. Hüsemann, *Carbohydr. Res.* **56**, 139 (1977).
[85] B. Pfannemüller, G. C. Richter and E. Hüsemann, *Carbohydr. Res.* **47**, 63 (1976).
[86] H. Andresz, G. C. Richter and B. Pfannemüller, *Makromol. Chem.* **179**, 301 (1978).
[87] W. B. Neely, U.S. Patent 3,133,850, 1964 (*Chem. Abstr.* **61**, 5899h, 1964).
[88] B. Casu, G. Scovenna, A. J. Cifonelli and A. S. Perlin, *Carbohydr. Res.* **63**, 13 (1968).
[89] T. C. Allen and J. A. Cuculo, *J. Polym. Sci.: Macromol. Rev.* **7**, 189 (1973).
[90] T. P. Nevell, *Methods Carbohydr. Chem.* **3**, 95 (1977).
[91] T. J. Painter, *Carbohydr. Res.* **55**, 95 (1977).
[92] J. Hoffman, O. Larm, K. Larsson, L. O. Andersson, E. Holmer and G. Söderström, *Carbohydr. Polym.* **2**, 115 (1982).
[93] S. Paoletti, Private communication.
[94] V. Luzakova, T. Marcincinova and A. Blazej, *Cellulose Chem. Technol.* **17**, 227 (1983).
[95] C. Deneault, B. V. Kokta and H. Cheradame, *J. Wood Chem. Technol.* **3**, 459 (1983).
[96] J. H. Arendt, J.-P. Sacheto, J.-P. Carriere and P. A. Bouchez, Brit. Pat. 1,401,824, 1975 (*Chem. Abstr.* **83**, 175,686, 1975).
[97] S. L. Snyder, T. L. Vigo and C. M. Welch, *Carbohydr. Res.* **34**, 91 (1974).
[98] G. O. Aspinall and I. M. Cairncross, *J. Chem. Soc.* 3998 (1960).
[99] G. O. Aspinall and A. Nicolson, *J. Chem. Soc.* 2503 (1960).
[100] K. Heyns and M. Beck, Cited as unpublished results in K. Heyns and H. Paulsen, *Adv. Carbohydr. Chem. Biochem.* **17**, 194 (1962).
[101] G. A. Adams, *Pulp Paper Magazine Canada* **65**, T13 (1964).
[102] D. Abbott, E. J. Bourne and H. Weigel, *J. Chem. Soc.* 827 (1966).
[103] H. Miyah, A. Misaki and M. Toru, *Carbohydr. Res.* **31**, 277 (1973).
[104] D. Horton and K. Just, *Carbohydr. Res.* **29**, 173 (1973).
[105] D. Horton and K. Just, *Carbohydr. Res.* **30**, 349 (1973).
[106] J. Defaye, Private communication.
[107] R. S. Williams and W. C. Feist, *Colloids Surf.* **9**, 253 (1984).
[108] A. R. Gibson, L. D. Melton and K. N. Slessor, *Can. J. Chem.* **52**, 3905 (1974).
[109] L. D. Melton and K. N. Slessor, *Carbohydr. Res.* **18**, 29 (1971).
[110] D. M. Clode and D. Horton, *Carbohydr. Res.* **19**, 329 (1971).
[111] D. Horton, A. E. Luetzow and O. Theander, *Carbohydr. Res.* **26**, 1 (1973).
[112] D. M. Clode and D. Horton, *Carbohydr. Res.* **17**, 365 (1971).

[113] G. Avigad, D. M. Amaral, C. Asensio and B. L. Horecker, *J. Biol. Chem.* **237**, 2736 (1962).
[114] J. K. Rogers and N. S. Thompson, *Carbohydr. Res.* **7**, 665 (1968).
[115] W. Jack and R. J. Sturgeon, *Carbohydr. Res.* **49**, 335 (1976).
[116] J. S. Myers and O. Gabriel, *Carbohydr. Res.* **67**, 223 (1978).
[117] G. O. Aspinall and A. S. Chaudhari, *Can. J. Chem.* **53**, 2189 (1975).
[118] M. Yalpani and L. D. Hall, *J. Polym. Sci.: Polym. Chem. Edn.* **20**, 3399 (1982).
[119] F. Cramer, G. Mackenson and K. Sensse, *Chem. Ber.* **102**, 494 (1969).
[120] J. Boger, D. G. Brenner and J. R. Knowles, *J. Am. Chem. Soc.* **101**, 7630 (1979).
[121] K. Takeo and T. Kuge, *Staerke* **28**, 226 (1976).
[122] Y. Matsui, T. Yokoi and K. Mochida, *Chem. Lett.* 1037 (1976).
[123] F. Cramer and G. Mackensen, *Chem. Ber.* **103**, 2138 (1970).
[124] K. Nagai, K. Hayakawa and K. Kanetmatsu, *J. Org. Chem.* **49**, 1022 (1984).
[125] H. Tsuchida, Jpn. Kokai Tokkyo Koho JP 58,113,99, 1983 (*Chem. Abstr.* **99**, 212,881, 1983).
[126] R. Breslow, M. Hammond and M. Lauer, *J. Am. Chem. Soc.* **102**, 421 (1980).
[127] B. Siegel, *J. Inorg. Nucl. Chem.* **41**, 609 (1979).
[128] J. W. Green, *Methods Carbohydr. Chem.* **3**, 327 (1963).
[129] D. Horton, *Carbohydr. Res.* **30**, 349 (1973).
[130] E. Pacsu, *Methods Carbohydr. Chem.* **3**, 251 (1963).
[131] H. J. Roberts, *Methods Carbohydr. Chem.* **4**, 299 (1964).
[132] J. J. Honeyman, *Chem Soc.* 169 (1947).
[133] Z. A. Rogovin and T. V. Vladimirova, *Khim. Nauka; Prom.* **2**, 527 (1957) (*Chem. Abstr.* **52**, 4167, 1958).
[134] J. F. Mahoney and C. B. Purves, *J. Am. Chem. Soc.* **64**, 9 (1942).
[135] E. Heuser, M. Heath and W. H. Shockley, *J. Am. Chem. Soc.* **72**, 670 (1950).
[136] J. W. Green, *Methods Carbohydr. Chem.* **3**, 322 (1963).
[137] I. Croon and C. B. Purves, *Svensk. Papperstidn.* **62**, 876 (1959).
[138] W. M. Hearon, G. D. Hiatt and C. R. Fordyce, *J. Am. Chem. Soc.* **65**, 2449 (1943).
[139] P. P. Shorygin, A. E. Veitsman and N. N. Makarova-Zemlyanskaya, *Zh. Obshch. Khim.* **7**, 430 (1937) (*Chem. Abstr.* **31**, 4809, 1938).
[140] R. F. Schwenker and L. Lifland, *Text. Res. J.* **33**, 107 (1963).
[141] E. Pacsu, *Methods Carbohydr. Chem.* **3**, 259 (1963).
[142] T. Teshirogi, H. Yamamoto, M. Sakamoto and H. Tonami, *Sen-I Gakkaishi* **34**, T510 (1978).
[143] T. Teshirogi, H. Yamamoto, M. Sakamoto and H. Tonami, *Sen-I Gakkaishi* **35**, T479 (1979).
[144] K. Takeo, T. Sumimoto and T. Kuge, *Staerke* **26**, 111 (1974).
[145] M. L. Wolfram, K. C. Gupta, K. K. De, A. K. Chatterjee, T. Kinoshita and P. Y. Wang, *Staerke* **2**, 39 (1969).
[146] J. Guerrera and C. E. Weill, *Carbohydr. Res.* **27**, 471 (1973).
[147] D. Horton and J. Lehmann, *Carbohydr. Res.* **61**, 553 (1978).
[148] T. Ishii, A. Ishizu and J. Nakano, *Carbohydr. Res.* **59**, 155 (1977).
[149] S. Machida and Y. Sueyoshi, *Angew. Makromol. Chem.* **49**, 171 (1976).
[150] L. S. Gal'braikh and Z. A. Rogovin, *Cellulose Chem. Technol.* **2**, 375 (1968).
[151] R. L. Whistler and M. Kosik, *Arch. Biochem. Biophys.* **142**, 106 (1971).
[152] O. Larm, B. Lindberg and S. Svensson, *Carbohydr. Res.* **20**, 39 (1971).
[153] P. A. Sandford, J. Baird and I. W. Cottrell, *Solution Properties of Polysaccharides*, Vol. 150, p. 31. ACS Symp. Ser. (1981).
[154] M. D. Nicholson and D. C. Johnson, *Cellulose Chem. Technol.* **11**, 349 (1977).
[155] M. D. Nicholson, D. C. Johnson and F. C. Haigh, *Appl. Polym. Symp.* **28**, 931 (1976).
[156] G. A. Petrapavlovsky, E. I. Larina and I. T. Borisova, *Cellulose Chem. Technol.* **14**, 683 (1980).
[157] P. Falcoz, P. Celle and J.-C. Campagne, U.S. Pat. 4,095,991 (1978).
[158] F. J. Maske and R. Nordgren, U.S. Pat. 4,028,127, 1976 (*Chem. Abstr.* **87**, 89531, 1977).
[159] H. Ziche, Ger. Offen. 2, 415, 556, 1974 (*Chem. Abstr.* **84**, 1976).
[160] C. Bosso, J. Defaye, A. Gadelle, C. C. Wong and C. Pederson, *J. Chem. Soc. Perkin Trans. I* 1579 (1982).
[161] J. Dutkiewicz, *J. Macromol. Sci.-Chem.* **A20**, 877 (1983).
[162] R. G. Schweiger, *Carbohydr. Res.* **70**, 185 (1979).
[163] M. L. Wolfram and P. Y. Wang, *Carbohydr. Res.* **12**, 109 (1970).
[164] D. Horton and T. Usui, *Carbohydrate Sulfates* (Edited by R. G. Schweiger), Vol. 77, p. 95. ACS Symp. Ser. (1978).
[165] K. Brederick, *Tetrahedron Lett.* 695 (1967).
[166] G. A. Bichoreva, L. S. Gal'braikh and Z. A. Rogovin, *Cellulose Chem. Technol.* **8**, 115 (1974).
[167] J. Defaye, H. Driguez and A. Gadelle, *Appl. Polym. Symp.* **28**, 955 (1976).
[168] J. Defaye and A. Gadelle, *Carbohydr. Res.* **56**, 411 (1977).
[169] J. Defaye and A. Gadelle, *Pulp Paper Canada* **75**, 50 (1974).
[170] A. N. DeBelder, B. Lindberg and S. Svensson, *Acta Chem. Scand.* **22**, 949 (1968).
[171] C. E. Weill and J. Guerrera, *Carbohydr. Res.* **27**, 451 (1973).
[172] O. Larm, K. Larsson, E. Scholander, B. Meyer and J. Thiem, *Carbohydr. Res.* **91**, 13 (1981).
[173] O. Larm and E. Scholander, *Carbohydr. Res.* **58**, 249 (1977).
[174] O. Larm, K. Larsson, E. Scholander, L. O. Andersson, E. Holmer and G. Söderström, *Carbohydr. Res.* **73**, 332 (1979).
[175] L. O. Andersson, J. Hoffman, E. Hohner, E. Larm, K. Larsson and G. Söderström, *Thromb. Res.* **28**, 741 (1982).
[176] J. Hoffman, O. Larm and E. Scholander, *Carbohydr. Res.* **117**, 328 (1983).
[177] E. Johnson, R. Seljelid, J. Bogwald, O. Larm and E. Scholander, *Scand. J. Immunol.* **15**, 205 (1982).
[178] M. Einarsson, B. Forsberg, O. Larm, M. E. Riqueline and E. Scholander, *J. Chromatogr.* **215**, 45 (1981).
[179] B. Augustinsson and E. Scholander, *Carbohydr. Res.* **126**, 162 (1984).
[180] F. F. Farley and R. M. Hixon, *Ind. Eng. Chem.* **34**, 677 (1942).
[181] V. Syniewski, *Ann.* **441**, 277 (1925).
[182] M. E. McKillican and C. B. Purves, *Can. J. Chem.* **32**, 312 (1954).
[183] I. Ziderman and J. Bel-Ayche, *Carbohydr. Res.* **27**, 341 (1973).
[184] L. S. Forsberg and J. H. Pazur, *Carbohydr. Res.* **75**, 129 (1979).
[185] S. Ebisu, J. Lönngren and I. J. Goldstein, *Carbohydr. Res.* **58**, 187 (1977).
[186] J. K. Sloneker, D. G. Orentas, C. A. Knutson, P. R. Watson and A. Jeanes, *Can. J. Chem.* **45**, 3353 (1968).

[187] J. H. Pazur, A. Cepure, J. A. Kane and C. G. Hellerquist, *J. Biol. Chem.* **248**, 279 (1973).
[188] K. M. Aalmo, M. I. Ishak and T. J. Painter, *Carbohydr. Res.* **63**, C3 (1973).
[189] R. L. Van Etten, G. A. Cower, J. F. Sebastian and L. Bender, *J. Am. Chem. Soc.* **89**, 3253 (1967).
[190] A. Harada, M. Furne and S. Nazakura, *Macromolecules* **10**, 676 (1977).
[191] M. F. Czarniecki and R. Breslow, *J. Am. Chem. Soc.* **100**, 7771 (1978).
[192] R. M. Paton and E. T. Kaiser, *J. Am. Chem. Soc.* **92**, 4273 (1970).
[193] R. Breslow and A. W. Czarnik, *J. Am. Chem. Soc.* **105**, 1390 (1983).
[194] A. N. DeBelder and B. Norrman, *Carbohydr. Res.* **8**, 1 (1968).
[195] P. Mansson and L. Westfelt, *Cellulose Chem. Technol.* **14**, 13 (1980).
[196] D. Horton and M. H. Meshreki, *Carbohydr. Res.* **40**, 345 (1975).
[197] A. B. Steiner and W. H. McNeely, U.S. Pat. 2,494,912, 1950 (*Chem. Abstr.* **44**, 4496, 1950).
[198] H. J. Lucas and W. T. Stewart, *J. Am. Chem. Soc.* **62**, 1070 (1940).
[199] E. F. Jansen and R. Jang, *J. Am. Chem. Soc.* **68**, 1475 (1946).
[200] K. Morell and C. Link, *J. Biol. Chem.* **108**, 763 (1935).
[201] Z. Wypych, *Rocz. Chem.* **43**, 1616 (1969).
[202] A. B. Steiner and W. H. McNeely, *Ind. Eng. Chem.* **43**, 2073 (1951).
[203] R. H. McDowell, *Properties of Alginates*, 2nd edn. Alginate Industries, London (1961).
[204] A. Wassermann, *Nature* **158**, 271 (1946).
[205] R. H. McDowell, *J. Soc. Cosmet. Chem.* **21**, 441 (1970).
[206] N. H. Chamberlain, G. E. Cunningham and J. B. Speakman, *Nature* **158**, 553 (1946).
[207] T. Takahashi, K. Kimoto and Y. Takamo, *J. Chem. Soc. Jpn.* **54**, 536 (1951).
[208] R. G. Schweiger, *J. Org. Chem.* **27**, 1786 (1962).
[209] M. Yalpani and L. D. Hall, *Can. J. Chem.* **59**, 3105 (1981).
[210] R. Lespieau, *Bull. Soc. Chim.* **7**, 254 (1940).
[211] I. Danishevsky and E. Siskovic, *Carbohydr. Res.* **16**, 199 (1971).
[212] S. E. Lasker, I. Pullman, R. A. Peckauskas and C. Patel, *Chemistry and Biology of Heparin* (Edited by R. L. Lundblad, W. V. Brown, K. G. Mann and H. R. Roberts), p. 81. Elsevier, Amsterdam (1981).
[213] M. Funahashi, I. Matsumoto and N. Seno, *Anal. Biochem.* **126**, 414 (1982).
[214] V. P. Torchilin, *Biorg. Khim.* **4**, 566 (1978).
[215] A. Ogamo, K. Matsuzaki, H. Uchiyama and K. Nagasawa, *Carbohydr. Res.* **105**, 69 (1982).
[216] Y. Inoue and K. Nagasawa, *Carbohydr. Res.* **111**, 113 (1982).
[217] E. A. Turley and S. Roth, *Nature* **283**, 268 (1980).
[218] R. L. Taylor and H. E. Conrad, *Biochemistry* **11**, 1383 (1972).
[219] K. W. Kirby, Ph.D. Thesis, Purdue University (1958).
[220] R. Köhler and W. Dierichs, U.S. Pat. 2,881,161, 1959 (*Chem. Abstr.* **53**, 14387, 1959).
[221] Henkel & Cie, G.m.b.H., Brit. Pat. 768,309, 1955 (*Chem. Abstr.* **51**, 13267, 1957).
[222] H. Andresz, G. C. Richter and B. Pfannemüller, *Makromol. Chem.* **179**, 301 (1978).
[223] P. N. Shacklee and H. E. Conrad, *Biochem. J.* **217**, 187 (1984).
[224] R. T. Sikorski and J. Kokocinski, Pol. PL 113,337, 1979 (*Chem. Abstr.* **98**, 162680, 1979).
[225] *Kelco Algin*, 2nd edn., p. 41. Kelco Co., San Diego, California (1976).
[226] N. K. Kochetkov, O. S. Chizov and A. F. Sviridov, *Methods Carbohydr. Chem.* **8**, 123 (1980).
[227] N. K. Kochetkov, O. S. Chizov and A. F. Sviridov, *Carbohydr. Res.* **14**, 277 (1970).
[228] T. E. Timell, *Adv. Carbohydr. Chem.* **19**, 282 (1964).
[229] A. F. Sviridov, O. D. Gikia, S. E. Gorin, O. S. Chizhov, I. P. Bab'eva and N. K. Kochetkov, *Biorgan. Khim. USSR* **3**, 232 (1977).
[230] N. K. Kochetkov, S. E. Gorin, A. F. Sviridov, O. S. Chizhov, V. I. Golubev, I. P. Bab'eva and A. Ya. Podel'ko, *Izv. Akad. Nauk SSSR, Ser. Chim.* 2304, 1975 (p. 2245 in Transl.).
[231] N. K. Kochetkov, O. S. Chizhov and A. F. Sviridov, *Izv. Akad. Nauk SSSR, Ser. Chim.* 2089, 1968 (p. 1983 in Transl.).
[232] J. W. Green, *The Carbohydrates, Chemistry and Biochemistry* (Edited by W. Pigman and D. Horton), Vol. 1B, p. 989. Academic Press, New York (1980).
[233] M. L. Wolfram, H. Tomometsu and W. A. Szarek, *J. Org. Chem.* **31**, 1173 (1966).
[234] M. L. Wolfram, J. R. Vercellotti and D. Horton, *J. Org. Chem.* **29**, 540 (1964).
[235] S. A. Barker, R. E. Hurst, J. Settine, F. P. Fish and R. L. Settine, *Carbohydr. Res.* **125**, 291 (1984).
[236] Wakamoto Pharmaceutical Co. Ltd., Jpn. Kokai Tokkyo Koho 81 79, 101, 1981 (*Chem. Abstr.* **95**, 175786, 1981).
[237] J. Hoffman, O. Larm, K. Larsson, L. O. Andersson, E. Holmer and G. Soederstroem, *Carbohydr. Polym.* **2**, 115 (1982).
[238] P. Unger, Chem. Commun. Univ. Stockholm, No. 1 (1983).
[239] K. Leontein, Chem. Commun. Univ. Stockholm, No. 3 (1983).
[240] R. L. Whistler and G. A. Towle, *Arch. Biochem. Biophys.* **138**, 39 (1970).
[241] M. W. C. Hatton, L. R. Berry, R. Machovich and E. Regoeczi, *Anal. Biochem.* **106**, 417 (1980).
[242] C. S. Lee and K. Maekawa, *Agric. Biol. Chem.* **40**, 785 (1976).
[243] A. Walton, R. V. Sparer and N. N. Ekwuribe, PCT Int. Appl. WO 83 00, 150, 1983 (*Chem. Abstr.* **98**, 166902, 1983).
[244] Z. Wypych, *Roczniki Chemii* **43**, 1619 (1969).
[245] G. Holzworth, *Carbohydr. Res.* **66**, 173 (1978).
[246] A. N. DeBelder and K. O. Wik, *Carbohydr. Res.* **44**, 251 (1975).
[247] K. Nagasawa and H. Uchiyama, *Biochim. Biophys. Acta* **544**, 430 (1978).
[248] S. Hirano and R. Yamaguchi, *Biopolymers* **15**, 1685 (1976).
[249] S. Hirano, Y. Ohe and H. Ono, *Carbohydr. Res.* **47**, 315 (1976).
[250] S. Hirano, K. Tobetto, M. Hasegawa and N. Matsuda, *J. Biomed. Mater. Res.* **14**, 477 (1980).
[251] R. Yamaguchi, Y. Arai, T. Itoh and S. Hirano, *Carbohydr. Res.* **88**, 172 (1981).
[252] S. Hirano, K. Tobetto and Y. Noishiki, *J. Biomed. Mater. Res.* **15**, 903 (1981).
[253] S. Hirano and T. Moriyasu, *Carbohydr. Res.* **92**, 323 (1981).
[254] S. Hirano and Y. Kondo, *J. Chem. Soc. Jpn.* 1622 (1982).
[255] H. Saito, R. Tabeta and S. Hirano, *Chem. Letters Jpn.* 1479 (1981).
[256] S. Hirano, *Agric. Biol. Chem.* **42**, 1939 (1978).
[257] S. Hirano, N. Matsuda, O. Miura and H. Iwaki, *Carbohydr. Res.* **71**, 339 (1979).
[258] S. Hirano, N. Matsuda, O. Miura and T. Tanaka, *Carbohydr. Res.* **71**, 344 (1979).

[259] M. Kijima, Y. Nambu, T. Endo and M. Okawara, *J. Polym. Sci.: Polym. Chem. Edn.* **22**, 821 (1984).
[260] R. Yamaguchi, Y. Arai, T. Kaneko and T. Itoh, *Biotechnol. Bioeng.* **24**, 1081 (1982).
[261] G. Vanlergerghe and H. Sebag, Germ. Pat. 2,222,733, 1972 (*Chem. Abstr.* **78**, 47665, 1973).
[262] M. L. Wolfram and R. Montgomery, *J. Am. Chem. Soc.* **72**, 2859 (1950).
[263] I. Danishefsky, H. B. Eiber and J. J. Carr, *Arch. Biochem. Biophys.* **90**, 114 (1960).
[264] I. B. Cushing, U.S. Pat. 3,118,816, 1964 (*Chem. Abstr.* **60**, 1159, 1964).
[265] L. Velluz, G. Nominé and A. Pierdet, *C.R. Acad. Sci.* **247**, 1521 (1958).
[266] C. Platka and R. Jequier, *Arch. Int. Pharmocodyn. Ther.* **126**, 140 (1960).
[267] L. Velluz, C. Platka and G. Nominé, *C.R. Acad. Sci.* **247**, 2203 (1958).
[268] Roussel-UCLAF, Belg. Pat. 635,463, 1964 (*Chem. Abstr.* **61**, 14770, 1964).
[269] G. Nominé and R. Bucourt, U.S. Pat. 3,118,817, 1964 (*Chem. Abstr.* **60**, 12098, 1964).
[270] S. Hirano and W. Ohashi, *Carbohydr. Res.* **59**, 285 (1977).
[271] G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.* **3**, 292 (1981).
[272] K. Kurita, T. Sannan and Y. Iwakura, *Makromol. Chem.* **178**, 2595 (1977).
[273] K. Kurita, H. Ichikawa, S. Ishizeki, H. Fujisaki and Y. Iwakura, *Makromol. Chem.* **183**, 1161 (1982).
[274] T. A. Mrachkovskaya, A. I. Gamzazade and S. V. Rogozhin, U.S.S.R. Pat. 802,290, 1981 (*Chem. Abstr.* **94**, 158,671, 1981).
[275] Agency of Industrial Sciences and Technology, Jpn. Kokai Tokkyo Koho JP 58 29, 801, 1983 (*Chem. Abstr.* **99**, 55340, 1983).
[276] S. Hirano and Y. Ohe, *Carbohydr. Polym.* **4**, 15 (1984).
[277] A. E. Sirica and R. J. Woodman, *Fed. Proc.* **29**, 681 (1970).
[278] A. E. Sirica and R. J. Woodman, *Natl. Cancer Inst.* **47**, 377 (1971).
[279] I. Koshino, M. Matsushita, H. Sakamoto, T. Kawamata, M. Kondo, T. Komai and Y. Kasai, *Proc. 2nd Meeting Int. Soc. Artificial Organs*, p. 262 (1979).
[280] T. Komai, Jpn. Kokai Tokkyo Koho JP 57 192, 563, 1981 (*Chem. Abstr.* **98**, 78186, 1981).
[281] S. Hirano and O. Miura, *Biotechnol. Bioeng.* **21**, 711 (1979).
[282] L. A. Nud'ga, E. A. Plisko and S. N. Danilov, *Zh. Obshch. Khim.* **43**, 2756, 1973 (p. 2733 in Transl.).
[283] S. Okimasu, *Bull. Agr. Chem. Soc. Jpn.* **20**, 29 (1956).
[284] R. A. A. Muzzarelli, *Carbohydr. Polym.* **3**, 53 (1983).
[285] L. A. Nud'ga, E. A. Plisko and S. N. Danilov, *Zh. Obshch. Khim.* **43**, 2752, 1973 (p. 2729 in Transl.).
[286] S. Hirano and T. Osaka, *Agric. Biol. Chem.* **47**, 1389 (1983).
[287] G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.* **4**, 246 (1982).
[288] G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.* **3**, 337 (1981).
[289] S. Hirano and M. Takeuji, *Int. J. Biol. Macromol.* **5**, 373 (1983).
[290] M. Yalpani and L. D. Hall, *Can. J. Chem.* **59**, 2934 (1981).
[291] S. Hirano, R. Yamaguchi and N. Matsuda, *Biopolymers* **16**, 2752 (1977).
[292] L. D. Hall, M. Yalpani and N. Yalpani, *Biopolymers* **20**, 1413 (1981).
[293] M. Yalpani and L. D. Hall, *Macromolecules* **17**, 272 (1984).
[294] R. F. Borch, M. D. Bernstein and H. D. Durst, *J. Am. Chem. Soc.* **88**, 1024 (1966).
[295] C. F. Lane, *Synthesis* 135 (1975).
[296] R. O. Hutchins and N. R. Natale, *Org. Preps. Procs. Int.* **11**, 201 (1979).
[297] L. D. Hall and M. Yalpani, *Carbohydr. Res.* **83**, C5 (1980).
[298] L. D. Hall and M. Yalpani, U.S. Pat. 4,424,346, 1984 (*Chem. Abstr.* **100**, 156,910, 1984).
[299] M. Yalpani and L. D. Hall, in preparation.
[300] L. D. Hall and M. Yalpani, *Carbohydr. Res.* **91**, C1 (1981).
[301] M. Yalpani and L. D. Hall, *Can. J. Chem.* **62**, 975 (1984).
[302] R. A. A. Muzzarelli, *Polymers in Medicine, Biomedical and Pharmacological Applications* (Edited by E. Chiellini and P. Giusti), p. 359. Plenum Press, New York (1982).
[303] R. A. A. Muzzarelli, F. Tanfani and M. Emanuelli, *Carbohydr. Polym.* **4**, 137 (1984).
[304] R. A. A. Muzzarelli, F. Tanfani, S. Mariotti and M. Emanuelli, *Carbohydr. Polym.* **2**, 145 (1982).
[305] J. M. Harris, E. C. Struck, M. G. Case, M. S. Paley, M. Yalpani, J. M. Van Alstine and D. E. Brooks, *J. Polym. Sci.: Polym. Chem. Edn.* **22**, 341 (1984).
[306] M. Yalpani, unpublished results.
[307] B. Pfannemüller and W. N. Emmerling, *Staerke* **35**, 298 (1984).
[308] W. N. Emmerling and B. Pfannemüller, *Makromol. Chem.* **184**, 1441 (1983).
[309] J. M. Williams, *Adv. Carbohydr. Chem. Biochem.* **31**, 9 (1975).
[310] Q. P. Peniston and E. L. Johnson, U.S. Pat. 3,922,260, 1975 (*Chem. Abstr.* **84**, 32922, 1976).
[311] U. Lindahl and P. Axelsson, *J. Biol. Chem.* **246**, 74 (1971).
[312] A. S. Perlin, N. M. Ng Ying Kin, S. S. Bha Hacherjee and L. F. Johnson, *Can. J. Chem.* **50**, 2437 (1972).
[313] C. Erbing, L. Kenne, B. Lindberg, G. Naumann and W. Nimmich, *Carbohydr. Res.* **56**, 371 (1977).
[314] J. Arnarp, P. J. Garegg, B. Lengstad and J. Lönngren, *J. Carbohydr. Res.* **83**, 394 (1980).
[315] S. Hase and J. Matsushima, *J. Biochem. Tokyo* **72**, 1117 (1972).
[316] J. Hoffman, O. Larm and E. Scholander, *Carbohydr. Res.* **117**, 328 (1983).
[317] M. Yalpani, L. D. Hall, J. Defaye and A. Gadelle, *Can. J. Chem.* **62**, 260 (1984).
[318] T. Teshirogi, H. Yamamoto, M. Sakamoto and H. Tonami, *Sen-I Gakkaishi* **36**, T501 (1980).
[319] D. Horton and E. K. Just, *Carbohydr. Res.* **30**, 349 (1973).
[320] K. Kobayashi and H. Sumitomo, *Macromolecules* **16**, 710 (1983).
[321] T. Uryu, J. Yamanouchi, T. Kato, S. Higuchi and K. Matsuzaki, *J. Am. Chem. Soc.* **105**, 6865 (1983).
[322] T. Uryu, K. Hatanaka, K. Matsuzaki and H. Kuzuhara, *Macromolecules* **16**, 853 (1983).
[323] N. K. Kochetkov, *Bacterial Lipopolysaccharides, Structure Synthesis, and Biological Activities*, Vol. 231, p. 67. ACS Symp. Ser. (1983).
[324] I. J. Goldstein and T. L. Hullar, *Adv. Carbohydr. Chem.* **21**, 431 (1966).
[325] N. K. Kochetkov and A. F. Bochkov, *Recent Developments in the Chemistry of Natural Carbon Compounds*, Vol. 4, p. 77. Akademia Kiado, Budapest (1971).
[326] A. F. Bochkov, V. N. Chernetsky and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.* 465 (1975).
[327] N. K. Kochetkov, A. F. Bochkov and T. A. Sokolovskaya, *Carbohydr. Res.* **19**, 1 (1971).
[328] B. Pfannemüller, G. C. Richter and E. Hüsemann, *Carbohydr. Res.* **43**, 151 (1975).

[329] B. Pfannemüller, G. C. Richter and E. Hüsemann, *Carbohydr. Res.* **56**, 147 (1977).
[330] B. Pfannemüller and A. Berg, *Makromol. Chem.* **180**, 1183 (1979).
[331] B. Pfannemüller and W. Emmerling, *Mechanisms of Saccharide Polymerization/Depolymerization* (Edited by J. J. Marshall), p. 313. Academic Press, New York (1980).
[332] W. N. Emmerling and B. Pfannemüller, *Makromol. Chem.* **179**, 1627 (1978).
[333] F. R. Seymour and R. D. Knapp, *Carbohydr. Res.* **81**, 67 (1980).
[334] B. Lindberg and S. Svensson, *Acta Chem. Scand.* **24**, 711 (1970).
[335] B. Veruovic and C. Schuerch, *Carbohydr. Res.* **14**, 199 (1970).
[336] V. Masura and C. Schuerch, *Carbohydr. Res.* **15**, 65 (1970).
[337] M. Yalpani, L. D. Hall, M. A. Tung and D. E. Brooks, *Nature* **302**, 812 (1983).
[338] M. Yalpani and D. E. Brooks, unpublished results.
[339] T. R. Ingle and R. L. Whistler, *Methods Carbohydr. Chem.* **5**, 411 (1965).
[340] R. L. Whistler and S. Hirase, *J. Org. Chem.* **26**, 4600 (1961).
[341] L. G. Krylova, L. S. Gal'braikh and Z. A. Rogovin, *Khim. Prirodn. Soedin.* **2**, 11 (1967).
[342] S. I. Polukhina, L. S. Gal'braikh and Z. A. Rogovin, *Vysokomolekul. Soedin.* **B11**, 270 (1969).
[343] A. Misaki and Y. Tsumuraya, *Fungal Polysaccharides*, Vol. 126, p. 211. ACS Symp. Ser. (1980).
[344] D. A. Rees, *J. Chem. Soc.* 1821 (1963).
[345] E. Perceival and J. K. Wold, *J. Chem. Soc.* 5459 (1963).
[346] J. Peska, J. Stamberg and J. Hradil, *Angew. Makromol. Chem.* **53**, 73 (1976).
[347] B. N. Gorbunov, P. A. Protopopov and A. P. Khardin, U.S.S.R. Pat. 473,724, 1975 (*Chem. Abstr.* **82**, 133746, 1975).
[348] O. Turunen, L. Mandell, V. Eklund, K. Ekman and J. I. Huttunen, PCT Int. Appl. WO 8302,278, 1981 (*Chem. Abstr.* **99**, 123320, 1983).
[349] V. A. Snezhko, K. P. Khomyakov, V. P. Komar, V. V. Osipova, A. D. Virnik, R. G. Zhbankov and Z. A. Rogovin, *Zh. Prikl. Khim.* **48**, 1540 (1975) (*Chem. Abstr.* **83**, 195619, 1975).
[350] M. L. Wolfram, M. I. Taha and D. Horton, *J. Org. Chem.* **28**, 3553 (1963).
[351] J. W. Green, *Methods Carbohydr. Chem.* **3**, 49 (1963).
[352] I. Danishefsky, H. B. Eiber and E. Langholtz, *J. Biol. Chem.* **237**, 1413 (1962).
[353] H. J. Jennings and C. Lugowski, *J. Immunol.* **127**, 1011 (1981).
[354] H. J. Jennings, C. Lugowski and F. E. Ashton, *Infect. Immun.* **43**, 407 (1984).
[355] S. B. Svenson and A. A. Lindberg, *J. Immunol. Methods* **25**, 323 (1979).
[356] A. A. Lindberg, R. Wollin, G. Bruse, E. Ekwell and S. B. Svenson, *Bacterial Lipopolysaccharides, Structure, Synthesis, and Biological Activities*, Vol. 231, p. 83. ACS Symp. Ser. (1983).
[357] K. Jann, *Bacterial Lipopolysaccharides, Structure, Synthesis, and Biological Activities*, Vol. 231, p. 171. ACS Symp. Ser. (1983).
[358] P. R. Lambden and J. E. Heckels, *J. Immunol. Methods* **48**, 233 (1982).
[359] H. S. Isbell, *Methods Carbohydr. Chem.* **5**, 249 (1965).
[360] K. K. Yabusaki and C. E. Ballou, *Proc. Natl. Acad. Sci. USA* **75**, 691 (1978).
[361] C. E. Ballou, *Acc. Chem. Res.* **1**, 366 (1968).
[362] V. L. Chiang and K. V. Sarkanen, *J. Wood Chem. Technol.* **4**, 1 (1984).
[363] G. P. Donnini, T. J. Blain, H. H. Holten and G. W. Kutney, *J. Pulp Paper Sci.* TR 134, November (1983).
[364] L. Löwendahl and O. Samuelson, *TAPPI* **61**, 19 (1978).
[365] S. Hirano and Y. Yagi, *Carbohydr. Res.* **92**, 319 (1981).
[366] D. A. Rees, N. G. Richardson, N. J. Wright and E. Hirst, *Carbohydr. Res.* **9**, 451 (1969).
[367] J. Kiss, *Adv. Carbohydr. Chem. Biochem.* **29**, 229 (1974).
[368] B. Lindberg, J. Lönngren and J. L. Thompson, *Carbohydr. Res.* **28**, 351 (1973).
[369] L. Kenne, B. Lindberg and S. Svensson, *Carbohydr. Res.* **40**, 69 (1975).
[370] B. Lindberg, J. Lönngren, U. Ruden and W. Nimmich, *Carbohydr. Res.* **42**, 83 (1975).
[371] B. Lindberg, J. Lönngren and D. A. Powell, *Carbohydr. Res.* **58**, 177 (1977).
[372] J. Kenne, J. Lönngren and S. Svensson, *Acta Chem. Scand.* **27**, 3692 (1973).
[373] J. W. Opie and J. L. Keen, Germ. Pat. 1,262,756, 1968 (*Chem. Abstr.* **68**, 88358, 1968).
[374] V. Crescenzi, A. Gamini and G. Paradossi, *Carbohydr. Polym.* **3**, 273 (1983).
[375] C. C. Hellergvist, B. Lindberg, S. Svensson, T. Holme and A. A. Lindberg, *Carbohydr. Res.* **9**, 237 (1969).
[376] P. A. J. Gorin and J. F. T. Spencer, *Carbohydr. Res.* **13**, 339 (1970).
[377] G. O. Aspinall and V. Puvanesarajah, *Can. J. Chem.* **61**, 1858 (1983).
[378] G. O. Aspinall and V. Puvanesarajah, *Can. J. Chem.* **61**, 1864 (1983).
[379] J. M. Nystrom, C. G. Greenwald, F. G. Harrison and E. D. Gibson, *Chem. Engng Prog.* **80**, 68 (1984).
[380] I. J. Higgins, D. J. Best, R. C. Hammond and D. Scott, *Microbiol. Rev.* **45**, 556 (1981).
[381] V. Moses, *First International Conference on the Commercial Applications and Implications of Biotechnology*, p. 415. Online Publications, Northwood, U.K. (1983).
[382] N. K. Matheson and B. V. McCleary, *The Polysaccharides* (Edited by G. O. Aspinall), Vol. III (1985), in press.
[383] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.* **6**, 283 (1984); and previous articles in this series.
[384] D. Amaral, L. Bernstein, D. Morse and B. L. Horecker, *J. Biol. Chem.* **238**, 2281 (1963).
[385] A. Maradufu, G. M. Cree and A. S. Perlin, *Can. J. Chem.* **50**, 768 (1971).
[386] R. A. Schlegel, C. M. Gerbeck and R. Montgomery, *Carbohydr. Res.* **7**, 193 (1968).
[387] M. W. C. Halton and E. Regoeczi, *Biochim. Biophys. Acta* **438**, 339 (1976).
[388] C. G. Gahmberg, *Methods Enzymol.* **50C**, 205 (1978).
[389] A. Maradufu and A. S. Perlin, *Carbohydr. Res.* **32**, 43 (1974).
[390] G. A. Hamilton, P. K. Adolf, J. de Jersey, G. C. Du Bois, G. R. Drykaca and R. D. Libby, *J. Am. Chem. Soc.* **100**, 1899 (1978).
[391] M. A. Bernstein, L. D. Hall and W. E. Hull, *J. Am. Chem. Soc.* **101**, 2744 (1979).
[392] B. J. Marvedel, D. J. Kosman, R. D. Bereman and R. J. Kurland, *J. Am. Chem. Soc.* **103**, 268 (1981).
[393] G. Abraham and P. S. Low, *Biochim. Biophys. Acta* **597**, 285 (1980).
[394] J. J. Marshall, *Mechanism of Saccharide Polymerization and Depolymerization*. Academic Press, New York (1980).
[395] Hayashibara Biochemical Laboratories, Inc., Jpn. Kokai Tokkyo Koho JP 81, 147,801, 1981 (*Chem. Abstr.* **96**, 179449, 1981).
[396] P. M. Taylor and W. J. Whelan, *Arch. Biochem. Biophys.* **113**, 500 (1966).
[397] J. H. Carter and E. Y. C. Lee, *Anal. Biochem.* **39**, 521 (1971).
[398] M. E. Slodki and M. C. Cadmus, *Proc. 1982 Int. Conf. Microbial Enhanced Oil Recovery*, pp. 3–5. DOE Conf. 8205140.

[399] B. V. McCleary, R. Amando, R. Waibel and H. Neukom, *Carbohydr. Res.* **92**, 269 (1981).
[400] B. V. McCleary, *Phytochemistry* **22**, 649 (1983).
[401] B. V. McCleary, I. C. M. Dea, J. Windust and D. Cooke, *Carbohydr. Polym.* **4**, 253 (1984).
[402] R. L. Whistler, U.S. Pat. 4,332,894, 1982 (*Chem. Abstr.* **97**, 108547, 1982).
[403] A. Haug and B. Larsson, *Carbohydr. Res.* **17**, 297 (1971).
[404] C. J. Brady, *Aust. J. Plant Physiol.* **3**, 163 (1976).
[405] J. M. V. Blanshard and J. R. Mitchell, *Polysaccharides in Food*, p. 109. Butterworths, London (1979).