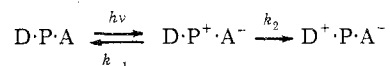


## Intensity Dependence of PSII Reactions

The simple D·P·A model



accounts for most of the photochemical activities attributable to the PSII reaction centers. However, the light intensity dependence for these reactions is not readily accommodated by this model. The simple model of independent reaction centers would predict that the rate of photoreduction of C-550 should be proportional to light intensity and that the photooxidation of cytochrome  $b_{559}$  should saturate at a maximal rate determined by  $k_2$ . Measurements of the quasi rate constants (reciprocal half-times) for the photoreduction of C-550 and the photooxidation of cytochrome  $b_{559}$  at  $-196^\circ$ <sup>40</sup> showed that the rates of both reactions were linear with light intensity at low intensities, below  $200 \mu\text{W}/\text{cm}^2$ , but fell off in a parallel fashion at higher intensities. The falling-off of the rate of C-550 photoreduction at the higher light intensities suggests a decrease in the apparent quantum yield of that reaction. Such a decrease in the quantum yield would occur if the back reaction,  $k_{-1}$ , were stimulated by light. It is possible that  $P_{680}^+$  absorbs light ( $P_{680}^+$  is postulated to quench fluorescence) and that the back reaction is facilitated when  $P_{680}^+$  is excited. Such a photostimulation of the back reaction should be favored at higher light intensities because of the greater accumulation of  $P_{680}^+$ , but there is no independent evidence for such

a mechanism. Siggel, *et al.*,<sup>29</sup> presented evidence that one DCMU molecule inactivated two PSII reaction centers. If these D·P·A reaction centers actually function in pairs, the back reaction might be more probable at the higher light intensities which would favor higher degrees of charge accumulation in the reaction center pairs. While such speculations should probably be restrained, to prevent their overproliferation, the anomalous intensity dependence of the photoreaction at low temperatures holds the promise that further investigations of the kinetics of the primary PSII reactions may provide new insights into the nature of the PSII reaction centers and the primary photochemical reactions.

### C-550 vs. Q

The absorbance measurements of C-550 provide a more direct indication of the primary electron acceptor of PSII than the fluorescence yield measurements attributed to Q. Since fluorescence yield appears to be affected by both the primary electron acceptor and the primary donor, the use of the symbol Q, which represents quencher, for the primary electron acceptor could be ambiguous in certain cases. It seems preferable to replace Q with C-550 as indicated in Figure 1 and to relate the fluorescence yield changes attributable to the acceptor to changes of C-550. However, C-550 is an operational term dependent on a specific absorbance change, and in many cases where that absorbance change is not being measured or discussed a more generic symbol such as A (or  $A_{II}$ ) might be more appropriate to use for the primary electron acceptor of PSII.

# Systematic Approaches to the Chemical Synthesis of Polysaccharides

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Living organisms exist as structures with mechanical strength and some degree of extensibility, with the ability to grow, move, and reproduce, to localize foods and fluids, and to manipulate information. The organic structural elements that mediate these functions within living organisms for the most part belong to three classes of macromolecules—the proteins, the nucleic acids, and the polysaccharides.

The functions of proteins and nucleic acids are

widely recognized, but the roles played in living tissue by polysaccharides and oligosaccharidic side chains of other macromolecules are less appreciated. Polysaccharides are found as major portions of the exoskeletons of insects and arthropods and cell walls of plants and microbes. In many organisms they act as reserve foodstuffs and important components of intercellular, synovial, and ocular fluids, mucous secretions, blood serum, etc. They also constitute protective capsules of some of the most virulent microorganisms, capsules which, nevertheless, carry information which activate mammalian defenses: the immune, interferon, and properdin systems.

In a sulfated form some polysaccharides inhibit blood clotting. Oligosaccharidic side chains of glyco-

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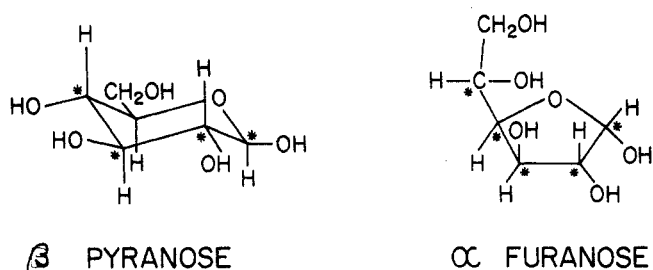


Figure 1. Types of monosaccharide structure.

proteins on erythrocytes induce an antibody-like response which is the molecular basis of the ABO blood-typing system. Changes in the structure of cell wall polysaccharides, furthermore, have been correlated with the derangement of control mechanisms which govern the passage of growth regulators into the cell and with the development of cancer.

It is thus apparent that a knowledge of many important physiological processes at a molecular level will require a knowledge of the conformation of polysaccharides in solution and their interactions with ions, small molecules, and macromolecules. Since in essence these interactions involve the subject matter of entire disciplines—enzymology, immunology, allergy, hematology—small chemical advances can have far-ranging implications.

In systems of such complexity, it is often productive to deal with models and model systems. However, until recently all physiological and biochemical studies of polysaccharides have been carried out on natural products with the possibility for error introduced by the presence of trace structural variations or biologically active impurities. Recently a few simple model polysaccharides have become available from chemical synthesis and are being used for biochemical and physicochemical investigations. These, however, are modest successes since there are sixteen possible stereoregular linear polysaccharides from a single common hexose like D-glucose, and a nearly infinite variety of branched and copolymeric structures.

The basic repeating unit of polysaccharides is, of course, a furanose (five membered) or pyranose (six membered) monosaccharide ring linked by one of two possible ( $\alpha$  or  $\beta$ ) acetal or glycosidic links to an alcoholic oxygen atom on the next unit (Figure 1). For synthesis selection of ring size and linkage site can, in principle, be controlled by the use of appropriate blocking groups during the glycoside formation, but in each glycoside chain-forming reaction step an asymmetric center must be formed stereospecifically on C-1. This reaction site is adjacent to a ring oxygen which tends to destabilize equatorial electronegative leaving groups and to stabilize an adjacent carbonium ion. This does nothing to aid reaction stereospecificity. In addition there are flexible rings, severe steric restraints, and neighboring group influences to contend with. No similar problems arise in the synthesis of proteins or nucleic acids.

It is thus perfectly clear that there will be no general solution to polysaccharide synthesis, and the dimensions of the problems involved can be appreciated if one remembers that a glycoside-forming reac-

tion which is 99.7% stereoselective will produce average flawless chains of only 330 sugar units. Molecules of this size are near the minimum that retain many physical properties characteristic of polymers. In contrast, even in disaccharide syntheses today, mixtures of anomers and by-products are obtained, yields of 20–50% are not uncommon and reaction times for a single coupling are usually 12–18 hr. Syntheses of tri-, tetra-, and pentasaccharides are rarely attempted, and the results become progressively worse with increasing molecular weight. Nevertheless it is possible to suggest some possible approaches and solutions to these difficulties.

First we can separate our problem into two general areas: (1) the stepwise synthesis of complex oligosaccharides and polysaccharides by combining several sugars in a controlled sequence with glycosidic bonds of known anomeric configuration linked to specific hydroxyl groups (*cf.* protein synthesis); (2) the development of ring-opening propagation reactions and condensation reactions suitable for the synthesis of homopolysaccharides and polymers of simple repeating or randomly distributed sugar units (*cf.* the Leuch's anhydride polypeptide synthesis).

### Problems of Stepwise Synthesis

In the stepwise synthesis of ordered sequences of different sugars each monomer must be a sugar derivative with a reactive leaving group at C-1, one hydroxyl group protected by an easily removable "temporary" blocking group, and the remaining hydroxyls protected by "persistent" blocking groups.<sup>1</sup> Following each coupling of C-1 to hydroxyl group, the temporary blocking group can be removed to liberate a new hydroxyl which can then be allowed to react with the anomeric center (C-1) of another sugar derivative (Figure 2).<sup>2</sup>

Unfortunately the reactivity of C-1 and the stereoselectivity of its reactions depend in a complex way on the leaving group, the blocking groups, the configuration of the sugar, and the structure of the molecule bearing the entering hydroxyl function. For example, the methanolysis of fully etherified  $\alpha$ -D-glucopyranosyl chloride (cis-1,2 configuration) to etherified methyl  $\beta$ -D-glucopyranoside (trans-1,2 configuration) is the glycoside-forming reaction at its simplest, a nearly stereospecific inversion at C-1 complicated slightly by racemization.<sup>3</sup> Since in most cases electronegative leaving groups are only stable enough for practical synthesis in the  $\alpha$  or axial configuration (because of dipolar interaction), it is usually possible to obtain only the  $\beta$ -glycoside in this manner. The same reaction on a corresponding  $\alpha$ -D-bromide results primarily in racemization because the nature of the intermediate ion pair is different, or because more rapid interchange between  $\alpha$  and  $\beta$  ion pairs occurs, primarily by inversion with halide ion.<sup>4</sup> Racemization is also favored in solvolysis of etherified  $\alpha$ -D-mannosyl halides (trans-1,2 configuration) due to steric compression in the transition state.<sup>3</sup> Race-

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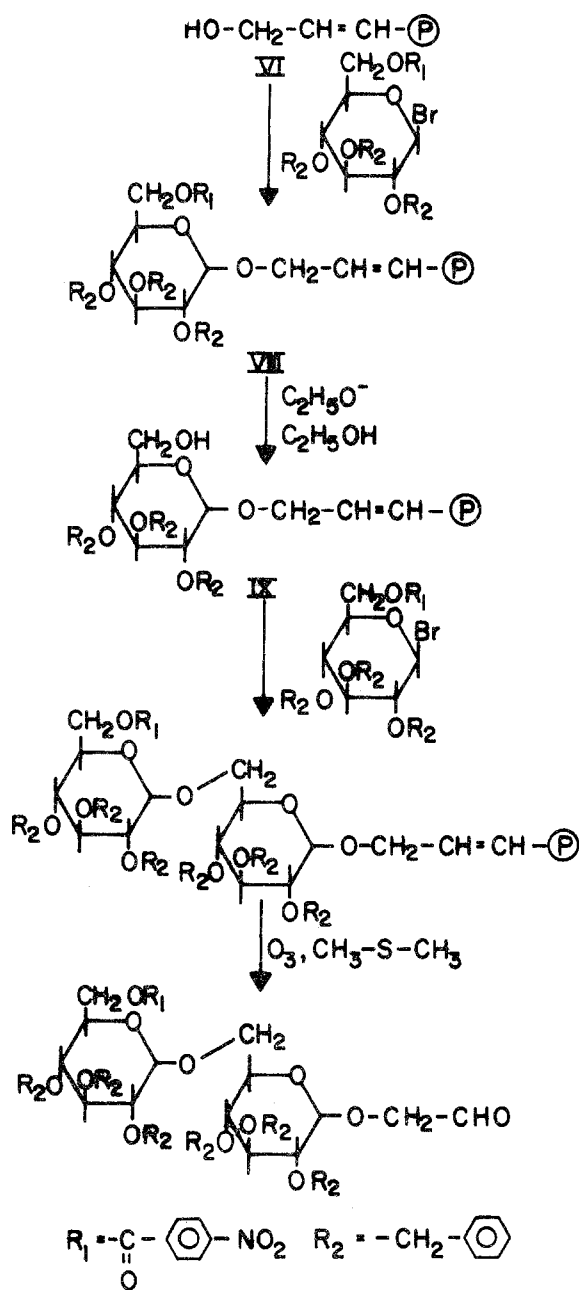


Figure 2. Solid-phase synthesis of a disaccharide.

mization usually becomes more serious with alcohols of increasing molecular weight as would be used in oligosaccharide synthesis.<sup>5</sup>

Solvolyses of derivatives with participating groups on C-2, such as the benzoylated glucosyl or mannosyl bromides, tend to give high trans-1,2 stereospecificities by neighboring group interaction<sup>6</sup> (Figure 3). Thus reasonably pure cis-1,2 glycosides are not generally obtainable from sugars with either participating or nonparticipating substituents on C-2, although a few examples are known.<sup>7-9</sup>

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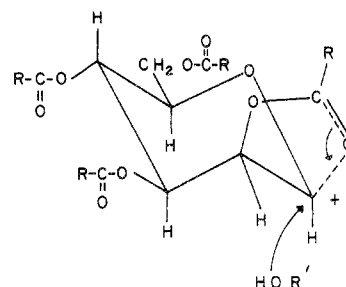


Figure 3. Neighboring group participation in glycoside synthesis.

Another problem of glycoside synthesis by simple alcoholysis of glycosyl halides is that of slow rates. The glycosyl chlorides react much more slowly than the bromides in solvolysis, and compounds bearing carbanilate<sup>10</sup> or ester functions react more slowly than the corresponding ether derivatives. This difficulty is compounded under conditions appropriate for solid-phase<sup>10,11</sup> oligosaccharide synthesis, where alcohol concentrations are very low compared to those in solvolyses.

The traditional method still used to enhance reaction rates in glycoside synthesis is by using silver salts either in solution or as solids. Both of these techniques were described by Koenigs and Knorr<sup>12</sup> in 1901, and although the method has been greatly extended, it has been improved only moderately since.<sup>13</sup> The mechanisms of these reactions are extremely complex,<sup>14-17</sup> but the role of silver is predominantly to assist halide elimination, not to act as an acid acceptor as is sometimes stated. The steric result is determined predominantly by a C-2 participating group if present.<sup>13</sup> When only nonparticipating groups are present, the overall steric outcome is extremely variable and may be either largely inversion or retention.<sup>9,14,15,18,19</sup> Apparently retention is favored at low alcohol concentrations,<sup>19</sup> and a number of systems have recently been described in which  $\alpha$ -enriched products have thus been obtained. Virtually none of these are stereoselective enough to be considered truly satisfactory for stepwise oligomer or polymer formation. In addition, the most commonly used reagent—silver carbonate—leads to water formation and side reactions.

Only a few glycoside syntheses have been carried out on glycosyl halides bearing nonparticipating groups on C-2 and one or more ester functions elsewhere.<sup>1,4,11,20-24</sup> Molecules of this structure bearing

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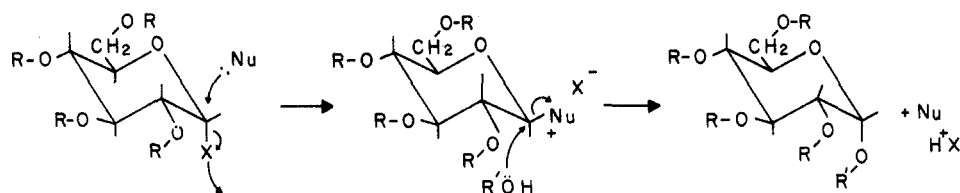


Figure 4. Cis-1,2 glycoside synthesis by double inversion.

removable but persistent blocking groups—benzyl ethers—and temporary blocking groups—esters—are ideal models for systematic stepwise synthesis. One of the more interesting examples has been found by Ishikawa and Fletcher.<sup>4</sup> They noted that a number of benzylated  $\alpha$ -D-glucopyranosyl bromides with a *p*-nitrobenzoate group on C-6 produced over 90% of the corresponding methyl  $\alpha$ -glucosides on methanolysis.

Flowers<sup>23,24</sup> has used similar glycosyl derivatives in syntheses of  $\alpha$ -linked oligosaccharides under Koenigs-Knorr conditions, while Frechet<sup>11</sup> found that  $\beta$ -glucosides are formed when *p*-methoxybenzoate is used in lieu of *p*-nitrobenzoate as the C-6 substituent. It has been suggested that these surprising results involve carbonyl participation<sup>11,23,24</sup> from C-6 and that the degree of steric control may be sufficient for syntheses of simple 1,6-linked oligosaccharides. The reaction is, however, sensitive to reaction conditions—alcohol concentration, choice of silver salt, blocking groups, solvent, etc.—and limited in scope. In view of the possible interconversion of anomeric halides prior to alcoholysis,<sup>3,4</sup> further investigation of its mechanism and that of other metal-assisted reactions<sup>25</sup> is needed and is in progress.<sup>19</sup>

We have recently begun a new approach to the synthesis of cis-1,2 glycosides.<sup>26</sup> In the limited tests so far given, it has shown some promise and may be a more general solution. This research began with our conviction that glycoside-forming reactions of highest stereoselectivity would be achieved by inversion of C-1 with loss of a leaving group from its thermodynamically preferred configuration. Otherwise extensive anomerization of C-1 would probably occur before displacement. Dipole interaction with ring oxygen is known to favor an axial configuration for electronegative substituents. Therefore dipole effects as well as steric effects should favor an equatorial configuration for electropositive substituents. The “reverse anomeric effect” of Lemieux<sup>27</sup> suggests that this is so. A positively charged substituent on C-1 should, therefore, be the preferred leaving group for synthesis of axial glycosides. Positive substituents have the further advantage that they should make C-1 susceptible to nucleophilic attack.

It is possible, therefore, to conceive of  $\alpha$ -glycoside synthesis *via* a double inversion mechanism from an axial or  $\alpha$ -glycosyl halide with a nonparticipating substituent on C-2 (Figure 4). Reaction with a nucleophile that does not readily lose proton or other positive species should result in the formation of a  $\beta$  or equatorial positive substituent. Nucleophilic at-

tack on this species with alcohol should give an axial glycoside. Reactions have been carried out on glycosyl halides with disubstituted sulfur and trisubstituted nitrogen and phosphorus as the first nucleophile. The positively charged intermediates are then allowed to react with simple alcohols. This sequence shows promise. Reactivity and electronic balance in the intermediate can be altered widely with variations in the structure of the initial nucleophile, thus providing an unusually versatile approach to cis-1,2  $\alpha$ -glycoside synthesis. Improvements could also be used in the synthesis of trans-1,2 glycosides.

Since the same problems of isolation, purification, and crystallization of intermediates are present in stepwise oligosaccharide synthesis as in protein and polypeptide synthesis, it is natural to think of the application of the Merrifield solid-phase technique<sup>28</sup> (Figure 2). However, systematic investigation of the application of this method to oligosaccharide synthesis is only beginning.<sup>1,2,10,11,22,29,30</sup> Furthermore, until the stereoselectivity of glycoside synthesis is improved, the method will be practicable only for relatively small oligomers.

### Condensation and Ring-Opening Reactions

We can now turn to our second problem, that of preparing stereoregular polysaccharides of simple repeating or randomly mixed sugar units. A number of attempts have been made to prepare polysaccharides by self-condensation of sugar derivatives<sup>31-34</sup> (Table I, rows a-d). These suffer from the fact that only oligomers and very low polymers can be produced unless the reaction goes essentially to completion. The monomers that have been used have had ester functions as blocking groups which are susceptible to migration, so that some isomeric structures are present in the formed polymer. The glycoside-forming reactions are subject to side reactions that lead to chain termination, and the degrees of polymerization obtained are generally quite low.

The orthoacetate reaction of Kochetkov demonstrates the superiority of ring-opening reactions to condensation reactions for polymer synthesis.<sup>35,36</sup> When a sugar having both an alkyl orthoester and a

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Table I  
Polymerization of Sugar Derivatives<sup>a,†</sup>

Monomer type	Catalyst	Polymerization type	Product	Derivative product		Free polysaccharide		Ref
				Yield, <sup>b</sup> %	Maximum <sup>b</sup> DP <sub>n</sub>	Maximum <sup>b</sup> DP <sub>n</sub>	[α] <sup>b</sup> , deg.	
a. Glucosyl bromide, acetylated	Ag <sub>2</sub> O	Condensation	(1→6)-β-D-Glucopyranan	<1		9	-28	31
b. 1,2,3,4-Tetra- <i>O</i> -acetylglucose	ZnCl <sub>2</sub> or C <sub>7</sub> H <sub>7</sub> SO <sub>3</sub> H <sup>c</sup>	Condensation	(1→6)-α,β-D-Glucopyranan <sup>c</sup>	0.5		9	+4.8	32
c. Tetra- <i>O</i> -acetylglucoses	ZnCl <sub>2</sub> or C <sub>7</sub> H <sub>7</sub> SO <sub>3</sub> H <sup>c</sup>	Condensation	(1→2)-, (1→3)-, (1→4)-α,β-D-glucopyranans	Low				33
d. Glucose 2,3,6-tricarbanilate	P <sub>2</sub> O <sub>5</sub>	Condensation	(1→4)-β-D-Glucopyranan		640	60	0.0	34
e. Orthoester, disaccharide	HgBr <sub>2</sub>	Condensation	(1→6)-α-D-Galactopyranan	4.3		Low	+112	37
f. Orthoester, cyclic <sup>d</sup>	HgBr <sub>2</sub>	Ring opening	(1→5)-α-L-Arabinofuranan <sup>d</sup>	50		59.5	-90	38
g. Orthoester, cyclic <sup>e</sup>	HgBr <sub>2</sub>	Ring opening	(1→5)-α-L-Arabinofuranan <sup>e</sup>	20		23	-85	39
h. Orthoester, cyclic trimer	HgBr <sub>2</sub>	Ring opening	(1→3)-β-D-Glucopyranan	13-20		30	-9	40
i. 1,6-Anhydroglucose <sup>f</sup> methyl and ethyl ether	ClSO <sub>3</sub> H BF <sub>3</sub> , PF <sub>5</sub>	Ring opening	(1→6)-α-D-Glucopyranan	90	200 <sup>g</sup>			45-47, 49
j. 1,6-Anhydroglucose <sup>f</sup> benzyl ether	PF <sub>5</sub>	Ring opening	(1→6)-α-D-Glucopyranan	>95	930	260	202 ± 4	47, 48, 50, 60, 61
k. 1,6-Anhydromannose benzyl ether	PF <sub>5</sub>	Ring opening	(1→6)-α-D-Mannopyranan	>90	2200	350	122 ± 1	51, 65, 66
l. 1,6-Anhydrogalactose benzyl ether		Ring opening	(1→6)-α-D-Galactopyranan	80-85	~400	150		50, 52, 65
m. 1,6-Anhydromaltose benzyl ether	PF <sub>5</sub>	Ring opening	4-α-D-Glucopyranosyl-(1→6)-α-D-glucopyranan	76	32 <sup>h</sup>		+174	53
n. 1,6-Anhydrocellulose benzyl ether	PF <sub>5</sub>	Ring opening	4-β-D-Glucopyranosyl-(1→6)-α-D-glucopyranan	70	14 <sup>h</sup>		+77.8	54
o. 1,6-Anhydroglucose and galactose benzyl ethers	PF <sub>5</sub>	Ring opening	Copolymers					56
p. 1,6-Anhydroglucose and maltose benzyl ethers	PF <sub>5</sub>	Ring opening	Copolymers					59

<sup>a</sup> This table is restricted to reports claiming reasonably stereospecific products. <sup>b</sup> Yield is of highest  $\overline{DP}$  fraction reported. The specific rotation values are not entirely comparable. The solvents are not the same, and the polymers contain varying amounts of water. <sup>c</sup> ZnCl<sub>2</sub> as catalyst produces polysaccharides of  $\overline{DP}$  up to 9, but poor stereoregularity. *p*-Toluenesulfonic acid gives lower  $\overline{DP}$  products of higher stereoregularity. <sup>d</sup> Monomer β-L-arabinofuranose 1,3,5-*O*-orthobenzoate. Product branched (1→3); 70% (1→5). <sup>e</sup> Monomer 3-*O*-benzoyl-β-L-arabinofuranose 1,3,5-*O*-orthobenzoate. Product reported to contain some (1→2) linkages by Kochetkov at the Fifth International Symposium on Carbohydrate Chemistry, Paris, Aug 1970. <sup>f</sup> Polymerization of 1,2- and 1,4-anhydro sugar derivatives and esterified 1,6-anhydro sugars leads to nonstereospecific products. See ref 44, 48, 72, and 74. <sup>g</sup> Less-soluble fractions also formed. <sup>h</sup>  $\overline{DP}$  is based on anhydroglucose, not the disaccharide. Higher fractions were obtained at lower yields. <sup>†</sup> See also E. Husemann, E. El-Kari, and B. Pfannemüller, *Monatsh. Chem.*, 103, 1669 (1972).

free hydroxyl group is used as monomer, the yield and molecular weight of the product are low<sup>37</sup> (Figure 5, Table I, row e). However, when cyclic orthoesters without free hydroxyls are used, both yields and molecular weights are improved.<sup>38-40</sup> (Figure 6, Table I, rows f-h). In the first case, the mechanism of polymerization is analogous to condensation polymerization. Reaction proceeds by elimination of a

small molecule and by combination of monomers, dimers, trimers, etc., as they are formed. A most probable distribution of molecular weights results and remains very low until the reaction is forced nearly to completion. In the second instance, growth starts with an alcohol initiator, the molecular weight is determined, to a first approximation at least, by the monomer/initiator ratio, and the polymerization rate is strongly dependent on the amount of catalyst present. These results are typical of chain propagation *via* an activated monomer, as pointed out by Szwarc<sup>41</sup> for the related ring opening polymerization

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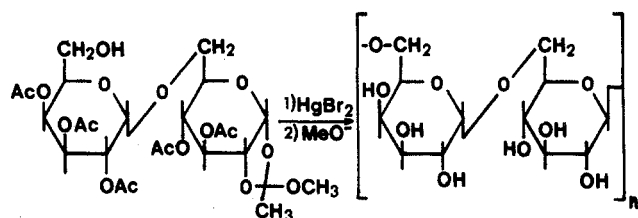


Figure 5. Orthoester condensation polymerization.

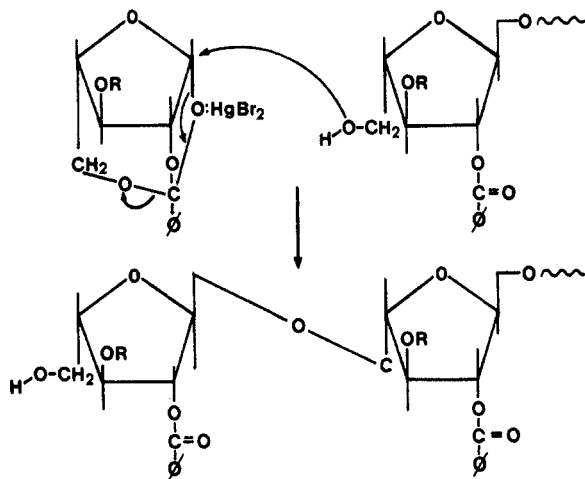


Figure 6. Orthoester ring opening polymerization

of Leuch's anhydrides. Increasing the concentration of catalyst greatly enhances the rate of propagation of each growing polymer chain by increasing the concentration of activated monomer. If an appropriate catalyst with low chain-transfer characteristics can be found, best results in these polymerizations can be expected at high catalyst concentrations. At the present state of development, this polymerization has given products with trans-1,2 configurations in moderate yields with degrees of polymerization of 30-60. A few fortuitous links are found in the polysaccharides.

A second ring-opening polymerization has proven most successful in producing very high yields of high molecular weight polysaccharides. Historically, the development derives from Pictet's<sup>42</sup> early discovery that 1,6-anhydro- $\beta$ -D-glucopyranose (levoglucosan) could be polymerized to (in our terms) a multi-branched polymer of mixed configurations and from Irvine and Oldham's early unsuccessful attempts to polymerize a fully substituted derivative to a linear polymer.<sup>43</sup> The first successful polymerizations of fully substituted derivatives of levoglucosan were by Hutten and Brederick,<sup>44</sup> who produced polysaccharides of mixed configuration by initiating with carbonium ion perchlorates, and by Korshak<sup>45</sup> and co-workers who treated 2,3,4-tri-*O*-methyl-1,6-anhydro- $\beta$ -D-glucopyranose (levoglucosan trimethyl ether) with boron trifluoride etherate at ambient temperatures. The discovery that the latter polymerization was stereospecific<sup>46</sup> justified a concerted attempt to improve and exploit the reaction, and the results can

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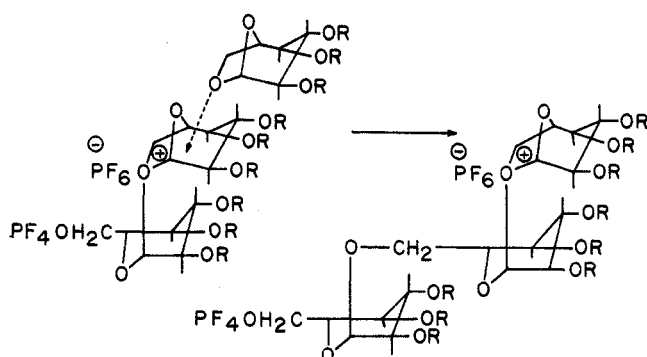
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Figure 7. Anhydro ring opening polymerization.

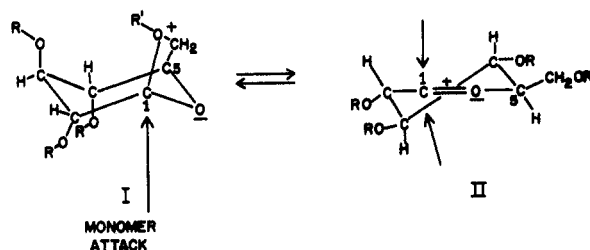


Figure 8. Ionic intermediates.

be readily explained by analogy with other ring-opening polymerizations.

These reactions proceed by an activated chain polymerization like that of tetrahydrofuran,<sup>47</sup> in contrast to the orthoester polymerization. The propagation process presumably involves attack by the bridge oxygen of a monomer on C-1 of a growing trialkyloxonium ion with simultaneous opening of the oxonium ion's ring (Figure 7). However, some role is also played by the accompanying anion for the range of temperatures within which stereospecific polymerization occurs is a function of the Lewis acid catalyst used.<sup>47,48</sup> Boron trifluoride operates stereospecifically at temperatures from perhaps  $-20^\circ$  to ambient, while in this range phosphorus pentafluoride produces random products of low molecular weight and high molecular weight stereoregular products at  $-55$  to  $-78^\circ$ . In general, best results to date have been obtained with fluorine-containing Lewis acids, especially phosphorus pentafluoride (*cf.* tetrahydrofuran polymerization), but chlorosulfonic acid has also produced stereoregular polymers.<sup>49</sup> The nonregular products obtained under more drastic conditions of temperature or Lewis acidity must be generated *via* a carbonium ion, presumably of half-chair conformation<sup>48</sup> (Figure 8). That a different intermediate is involved in atactic polymerization is also suggested by the higher transfer or termination rates and much lower molecular weights achieved.

Molecular weights of the stereoregular products are highest at low temperatures, and vary only little with conversion. These relationships are typical of chain propagation with termination by transfer processes. Transfer may in part be to catalyst because even extreme precautions in monomer and solvent purification lead to only limited improvement<sup>50</sup> in

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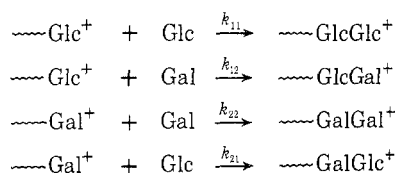
molecular weight, and higher catalyst concentrations result in lower viscosity products.

If esters of levoglucosan are used in lieu of ethers, the reactivity of both monomer and intermediate are decreased—temperatures above  $-25^\circ$  are needed for polymerization and stereospecificity is lost, presumably because of neighboring group participation.<sup>48</sup>

The tribenzyl ethers of 1,6-anhydro- $\beta$ -D-manno-<sup>51</sup> and -galactopyranose<sup>52</sup> also polymerize readily. The molecular weights of the products are highest from the monomers exhibiting highest rates of propagation. Apparently the rates of chain transfer in the three cases are more similar than the rates of propagation. The mannose derivative has the highest degree of polymerization, glucose around 900 and galactose about 480.<sup>50</sup> The polymerization of derivatives of 1,6-anhydromaltose<sup>53</sup> and -cellobiose<sup>54</sup> are much slower and require high catalyst and "cocatalyst" concentrations. The comb-shaped polysaccharides formed average only 14–30 saccharide units and the stereospecificity of these reactions is much more sensitive to reaction conditions.

The driving force for the ring-opening polymerizations is clearly derived predominantly from release of steric and conformational strain.<sup>47</sup> In 1,6-anhydro- $\beta$ -D-glucopyranose all substituents and in the mannose and galactose series all but one substituent are axial. The parent ring compound 6,8-dioxabicyclo[3.2.1]octane, in contrast, polymerizes only sluggishly.<sup>55</sup>

Copolymerization of 1,6-anhydro- $\beta$ -D-glucopyranose (Glc) and 1,6-anhydro- $\beta$ -D-galactopyranose (Gal) benzyl ethers appears to follow classical copolymerization theory<sup>66</sup> and is explicable in terms of four propagation reactions.



The two reactivity ratios  $r_1 = k_{11}/k_{12} = 1.36 \pm 0.05$  and  $r_2 = k_{22}/k_{21} = 0.14 \pm 0.04$  are approximately what would be expected from the homopolymerizations; that is, the faster polymerizing monomer (glucose) is incorporated more rapidly into the copolymer. Apparently the two cross-propagation constants  $k_{21}$  and  $k_{12}$  are not greatly different and the copolymerization is dominated by the homopolymerization rates.

There is no reason to expect that the copolymerization would be less stereospecific than the homopolymerizations, and, therefore, one would expect that the optical rotations of the copolymers would be a linear function of the copolymer composition.<sup>57,58</sup> In

fact, the rotations of the copolymers in the intermediate range of compositions  $\sim 30$ – $70\%$  glucose are about 15% less than would be expected on this basis. We suggest that this probably reflects a modest degree of preference for a particular conformational order in the two homopolymers which is disrupted in the copolymer.

It is, of course, possible to calculate the weight fraction of different sequence lengths of structure  $\text{Gal}\beta\text{-}\alpha(\text{Glc}\beta)_n\text{-}\alpha\text{Glc}$  as a function of copolymer composition from copolymerization theory. It is thus possible to show that reasonable amounts of oligomers of that structure with  $n = 1 \rightarrow 11$  should be obtained if an endo  $\alpha$ -galactosidase is allowed to degrade debenzylated copolymers of appropriate composition.<sup>56</sup>

The same general features are also observed in the copolymerization of the benzyl ethers of 1,6-anhydro- $\beta$ -D-glucopyranose and 1,6-anhydromaltose.<sup>59</sup> The reactivity ratios at 10 mol % catalyst concentration are  $r_1 = 1.91 \pm 0.35$  and  $r_2 = 0.28 \pm 0.25$ . The product should be a stereoregular 1,6- $\alpha$ -D-glucopyranan with randomly distributed 1,4- $\alpha$ -linked glucopyranoside side chains. However, because of the sluggishness of the disaccharide polymerization and the sensitivity of the stereospecificity to the reaction conditions, this structure is not certain.

#### Properties of Synthetic Glycopyranans

The free polysaccharides can be readily obtained from the homopolymers on debenzylation by means of sodium in liquid ammonia.<sup>60,61</sup> The resultant linear galactan,<sup>52</sup> mannan,<sup>51</sup> and glucan<sup>61</sup> have degrees of polymerization of about 150–350. It can be calculated that only about one chain break occurs in the removal of 900 benzyl groups. The polydispersity,  $M_w/M_n$ , of the benzylated mannan, galactan, and glucan and the free glucan ranges from 1.5 to 3.0.<sup>62,63</sup> The stereoregularity of the three polysaccharides has been proven by a number of physical, chemical, and enzymic tests. The presence of a single anomeric proton peak is found at an appropriate chemical shift for equatorial hydrogen.<sup>51,61</sup> The specific rotation of the glucan is about  $200^\circ$ , essentially the same as natural dextran.<sup>61</sup> The circular dichroism bands of natural dextran<sup>64</sup> and synthetic glucan,<sup>64</sup> mannan, and galactan<sup>65</sup> acetates are centered at 210 nm and the intensity of the glucose polymer bands is sensitive to the presence of other than  $\alpha$ -1,6 linkages. The synthetic glucan and galactan acetate bands are very similar and substantially higher than that of the natural dextran.<sup>65</sup> That of mannan acetate is of opposite sign and lesser magnitude.<sup>65</sup>

The X-ray diffraction pattern of the synthetic glucan<sup>60,61</sup> shows a higher degree of order than the natural product which is slightly branched. Periodate

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oxidation of the glucan,<sup>61</sup> mannan,<sup>51</sup> and galactan<sup>52</sup> give theoretical values of periodate consumption and formic acid liberation. The final specific rotations of the three oxidized polysaccharides are identical, as they should be, since the configurational differences at C-2 and C-4 are destroyed on oxidation. Neither the glucan nor the mannan precipitates with conalbumin A, and therefore both must be linear.<sup>66,67</sup> The glucan has been subjected to enzymic analysis and is reported to be 100%  $\alpha$  and 97-98%  $\alpha$ -1,6 linked.<sup>68</sup> A high molecular weight sample of the mannan has been degraded by an *exo*- $\alpha$ -mannanase to the extent of 42-44%. This result indicates that the product is about 99%  $\alpha$ -D-mannopyranan.<sup>69</sup>

In spite of the great similarity in structures differing as they do only in the configuration of a single carbon atom per sugar unit, these polysaccharides are greatly different in solubility. The glucan is water soluble,<sup>61</sup> the mannan dispersible in water and soluble in dimethyl sulfoxide,<sup>51</sup> while the galactan is insoluble in almost all solvents effective for hydroxyl-bearing polymers.<sup>52</sup> Only two unusual complexing solvents were found to be effective in dissolving this polymer. Because of its extreme insolubility, the galactan resists enzymic attack.<sup>70</sup>

The  $\alpha$ -1,6-linked glucan and mannan structures are found in a number of biologically significant polysaccharides produced by microorganisms, lichen, fungi and higher plants.<sup>71</sup> They include the multi-branched bacterial dextran used clinically for osmotic pressure control, blood volume and viscosity control in surgery, and extracorporeal circulation. A similar dextran is present in dental plaque. Related glucans and mannans induce various allergic reac-

tions and activate the interferon system (a natural defense against virus attack) and are reported to be active against neoplastic growths including sarcomas. The synthetic glucan and mannan have been tested in a few biological systems.<sup>71</sup>

### Summary

It is unfortunate that ring-opening reactions are not generally applicable to the formation of polysaccharides with other linkages. Ethers of two 1,4-anhydroglycopyranoses apparently have too great ring strain and polymerize to give products with a mixture of anomeric linkages and both pyranose and furanose rings.<sup>72</sup> 1,6-Anhydro-2,3,5-tri-*O*-benzyl- $\alpha$ -D-galactofuranose appears to have little conformational or steric strain and polymerizes sluggishly to very low molecular weight products,<sup>73</sup> so high molecular weight 1,6-linked glycofuranoses are probably not available from this reaction. The only accessible 1,2-anhydro sugar, "Brigl's anhydride," contains ester functions that complicate its polymerization enormously.<sup>74</sup> It will, therefore, be necessary to look to other reactions for the synthesis of many other polysaccharides.

At its present state of development, the chemical synthesis of polysaccharides allows us to make simple correlations between molecular structure and physical properties and provides us with a few well-defined substrates for biological testing and biochemical investigation. Perhaps their greatest utility is in encouraging us to believe that the synthesis of many more of these important natural polymers is not beyond the reach of organic chemistry.

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## New Amino-Protecting Groups in Organic Synthesis

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The synthesis of molecules of ever-increasing sensitivity or complexity demands the availability of a

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variety of protective groups to ensure the survival of common reactive functional groups such as the hydroxyl and amino functions. Early workers made considerable use of simple acyl functions with removal at an appropriate stage ("deblocking") by hydrolytic techniques. In many situations of current interest such deblocking conditions are far too drastic; therefore much recent effort has been devoted to the development of new protective groups capable of removal under exceptionally mild and/or highly specific conditions.<sup>1</sup> Deblocking techniques cover a wide spectrum of conditions, depending on the remainder