Copolymerization of 1,6-Anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-β-D-glucopyranose and 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose. Possible Application to the Synthesis of Stereoregular Heteropolysaccharides and Oligosaccharides

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ABSTRACT: 1,6-Anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-β-D-glucopyranose (M_1, TXGL) has been copolymerized with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose (M_2, TBGA). Reactivity ratios have been calculated from data at several molar ratios by means of Lewis and Mayo's integrated copolymer composition equation and are found to be $r_1 = 1.36 \pm 0.05$ and $r_2 = 0.14 \pm 0.04$. The reactivity of these monomers in copolymerization thus generally follows their reactivity in homopolymerization and azeotropic copolymerization is not observed. However, fairly homogeneous copolymers can be obtained at moderate conversions in batch processes when high mole fractions of the more reactive monomer, M_1, are present in the feed. The utility of copolymerization for the preparation of stereoregular polysaccharides of controlled properties and for the preparation of higher oligomers is discussed. A mathematical expression is derived for the weight fraction of sequences of TXGL of length *n* in the copolymer. The specific rotations of the copolymers vary with copolymer composition in a manner that suggests a preferred conformation of the homopolymers in solution.

Stereoregular homopolysaccharides and heteropolysaccharides are important constituents of animals, plants, and microorganisms. The ability to synthesize such polysaccharides chemically as model compounds should be useful especially for the fields of immunology, serology, and medicine.[1,2] Although many efforts had been made in the past, the successful chemical synthesis of stereoregular (1→6)-α-D-glucopyranan,[3] -mannopyranan,[4] and -galactopyranan[5] has only been developed recently. The synthetic scheme for the homopolysaccharides involved cationic polymerization of tribenzyl ethers of 1,6-anhydroglycopyranoses with PF_5 and debenzylation of the polymer with sodium in liquid ammonia.

This method should also be useful in the synthesis of stereoregular heteropolysaccharides and oligosaccharides which might possess interesting physical and chemical properties. Heteropolysaccharides which contain galactose units interspersed with glucose residues might be more soluble than stereoregular (1→6)-α-D-galactopyranan which is insoluble in almost all solvents.[5] The successful preparation of water-soluble or swellable glucogalactans should allow one to perform enzymic and immunological studies on the (1→6)-α-D-galactopyranosyl unit in polysaccharides.

The preparation of an homologous series of oligosaccharides is customarily achieved by controlled hydrolysis of a natural polysaccharide, and the same method could be applied to synthetic polysaccharides. However, optimum conditions must be determined empirically and there is, of course, no end point to the reaction. It has occurred to us that an alternative method of preparing an homologous series of heterooligosaccharides of structure $\mathrm{Glcp}\overset{\alpha}{-}(6\mathrm{Glcp})_n\overset{\alpha}{-}6\mathrm{Galp}$ might be developed by copolymerization of the appropriate monomers, debenzylation, and enzymic cleavage as in Scheme I. Successful debenzylations such as the above have been carried out repeatedly on homopolymers, but the indicated enzymic degradation is still speculative. However in principle, there would be a definite end point to the enzymic degradation and a copolymer of a particular average sequence length $\bar{n}$ could be obtained in maximum yield by proper control of the monomer feed concentrations. Enzymatic degradation of the heteropolysaccharides would require an endo enzyme, α-D-galactosidase,[6-8] to obtain oligosaccharides which contain *n* glucose units and one reducing galactose unit. The synthetic scheme described above is analogous to the

Scheme I

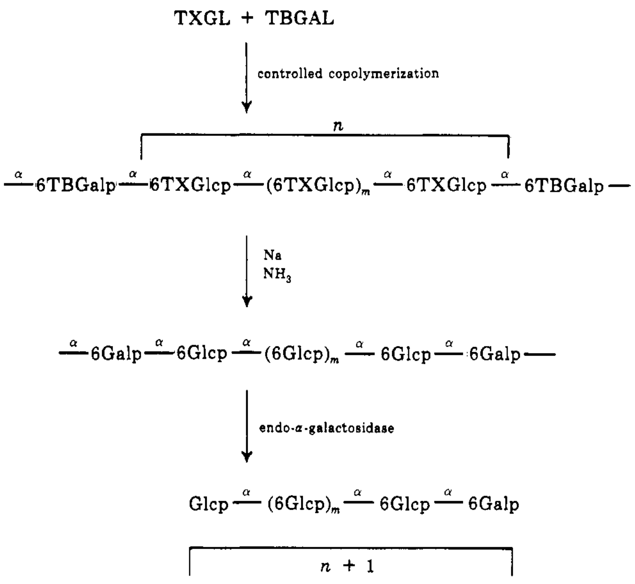

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Table I
Copolymerization of 1,6-Anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-β-D-glucopyranose (TXGL) and 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose (TBGA) at −60°[a]

No.	TXGL Feed, g (mol %)	TBGA Feed, g (mol %)	Polymerization Time (min)	Unreacted Monomers Wt (g)	TXGL in Unreacted Feed (mol %)	Copolymer Wt, g (% Yield)	TXGL in Copolymer (mol %)	Av TXGL in Copolymer (mol %)	$[\alpha]^{25}\text{D}^c$ (deg)	$[\eta]$,[c] dl/g
1	0.53406 (89.06)	0.05978 (10.94)	13.5	0.3848	87	0.2102 (35.4)	92.6 91.2[b]	91.9	101.5	0.34
2	0.47806 (75.97)	0.13775 (24.03)	13.0	0.4002		0.2095 (29.4)	82.4	82.4	94.3	0.42
3	0.4220 (70.07)	0.1643 (29.93)	15.5	0.3502	65.3	0.2525 (43.0)	78.67, 79.40 76.5[b]	78.2	88.1	0.25
4	0.35833 (60.00)	0.21772 (40.00)	18.0	0.3970	53.9	0.1810 (31.4)	72.8 70.9[b]	71.85	88.0	0.23
5	0.29915 (47.63)	0.2997 (52.37)	33.0	0.3423		0.2595 (43.3)	62.8	62.8	88.8	0.27
6	0.17894 (29.68)	0.3863 (70.32)	88	0.3290	15.9	0.2330 (41.3)	48.5 49.98[b]	49.24	87.0	0.25
7	0.12073 (19.97)	0.44105 (80.03)	110	0.3793	9.4	0.1802 (32.1)	42.0 42.6[b]	42.3	87.1	0.25
8	0.06056 (10.00)	0.49665 (90.00)	229	0.3609	2.9	0.1980 (35.5)	24.17 23.02[b]	23.6	97	0.33

[a] Methylene chloride (1 ml) and *p*-chlorobenzenediazonium hexafluorophosphate (15.8 mg; ~4.3 mol %) were used. [b] The lettered value in each case was calculated from the mole % of TXGL in unreacted feed mixture. [c] Determined in chloroform at 25°.

approach of Harwood and Kanakkanatt[9] in their measurement of sequence distribution in synthetic L-tyrosine–L-glutamic acid copolymers. Homooligosaccharides with exactly *n* glucose units (*n*-mers) could be obtained if a specific method of removing the reducing end group were available.

In this paper, we report the results of copolymerization of TXGL and TBGA including calculations of their reactivity ratios r_1 and r_2 and treat mathematically the mer sequence distribution in the copolymer and the feasibility of oligosaccharide synthesis.

Results and Discussion

Copolymerization of 1,6-anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-β-D-glucopyranose (TXGL) and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose (TBGA) was carried out under high vacuum at −60° in anhydrous methylene chloride with ~4.3 mol % PF_5. The polymerizations were terminated at −60° with methanol at a yield of 30–40%. Low conversion experiments were avoided because they required large quantities of monomers. The results are shown in Table I. By preparing a derivative of levoglucosan with *p*-methylbenzyl groups it was possible to determine the copolymer composition by nmr since the *p*-methyl groups of xylyl units could be very easily distinguished from the rest of the protons in the copolymer. The integration ratios of *p*-methyl:aromatic protons, *p*-methyl:nonaromatic protons, and *p*-methyl:all protons were used on both copolymer and unreacted monomer mixtures in the determination of copolymer composition. The average values obtained from data on both substrates agreed with each other and thus indicated that no substantial experimental error was made in the determination of copolymer composition (Table I). In all cases, the mole percentage of TXGL in the copolymer was higher than that of TXGL in the initial monomer feed. The results thus indicated that TXGL is more reactive than TBGA. This implication was consistent with the observation that at higher molar ratio of TBGA:TXGL, the rate of polymerization was lower. The copolymers obtained from both high and low molar ratios of TXGL:TBGA in the feed had somewhat higher intrinsic viscosities than those in the intermediate range. Since the polymerization time, yield, and copolymer composition varied from one experiment to another, the significance of this fact is not clear.

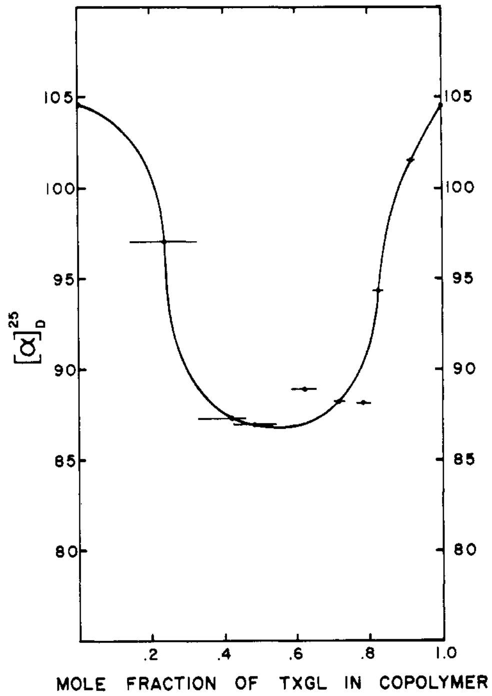

Figure 1. Optical rotations of copolymers.

Figure 1 shows that the optical rotatory power of the two homopolymers are the highest of all the products ($[\alpha]^{25}$D 104.7°) and that of TXGL:TBGA copolymer decreases as the randomness of the copolymer structure increases. The specific rotation reaches a minimum value when the copolymer contains approximately equal molar quantities of TXGL and TBGA. This result may be due to differences

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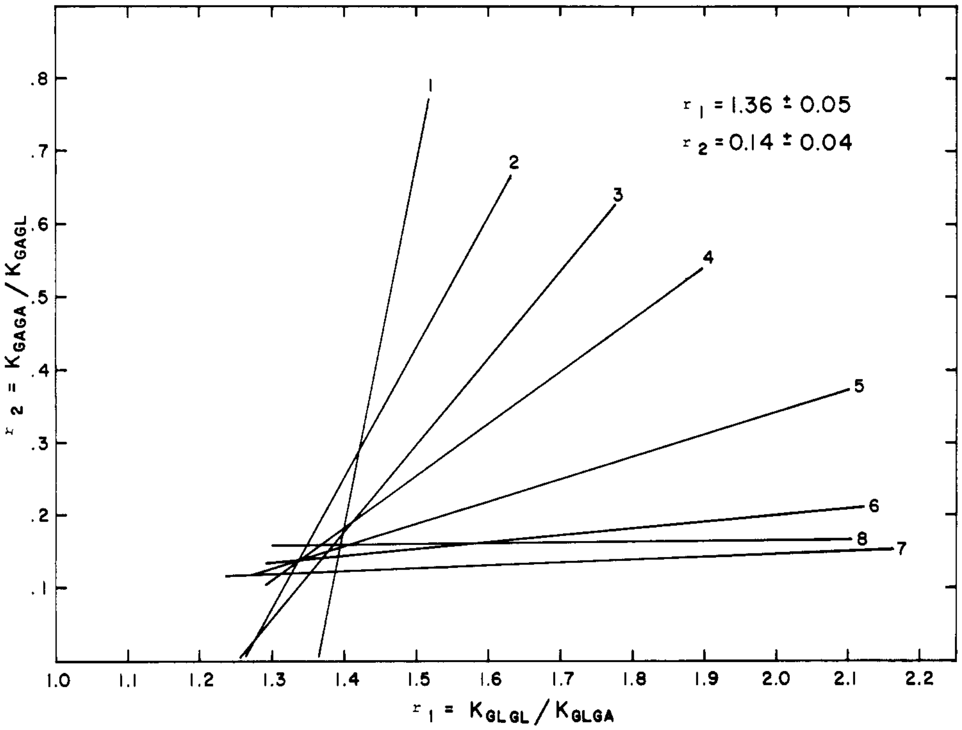

Figure 2. Estimation of reactivity ratios by method of Mayo and Lewis.[14]

in the conformations of the homopolymers and copolymers in solution. The relationship between the optical rotatory activity of stereoregular polymers and polymer conformation in solution has been studied extensively.[10,11] In copolymers of (*S*)-4-methyl-1-hexene and (inactive) 4-methyl-1-pentene, Carlini, Ciardelli, and Pino[12] found that at each copolymer composition the specific rotation of the copolymer was higher than that of a mixture of the reference homopolymers having the same composition. They concluded that the stereoregular copolymer possessed a left-handed helical conformation which allowed contributions of optical rotatory power from both monomer units, (*S*)-4-methyl-1-hexene and 4-methyl-1-pentene. With copolymers having low stereoregularity or the asymmetric center more distant from the main chain,[13] the optical rotation was linearly dependent on the content of the optically active comonomer because a specific conformation was not favored. In our case, the copolymer is most probably stereoregular and asymmetric centers are on the main polymer chain. It is possible that the tribenzyl ethers of the two homopolysaccharides possess ordered but somewhat different conformations in chloroform solution. By introducing a foreign mer into the structure, a structural flaw is produced in the copolymer chain, the ordered conformation is disturbed and the optical rotatory power of the polymer is decreased. Other explanations for the nonlinear relationship may be possible but are not immediately obvious.

For the evaluation of reactivity ratios of TXGL and TBGA, the procedure of Mayo and Lewis[14] was employed with the usual premises assumed to apply to our cationic copolymerization[15] system. The average copolymer compositions obtained from nmr analysis were used in the calculation. A computer program designed to use the integrated composition equation of Mayo and Lewis was written. The input data consisted of molecular weights of monomers, an approximate p value ($p = 1 - r_1/(1 - r_2)$), the initial weights of monomers in feed, the mole fraction of TXGL in copolymer, and the total weight of copolymer formed. The approximate p value was estimated from reactivity ratios (r_1 and r_2) which were obtained by the differential method of Fineman and Ross.[16,17] By varying the initial p value several sets of r_1 and r_2 values were obtained for each experiment and plotted, essentially as straight lines, in Figure 2. The lines associated with high molar concentrations of TXGL had higher slopes and were very sensitive to experimental error in copolymer composition. The intersections of lines the slopes of which differ by 10° or less were excluded in the final evaluation of reactivity ratios. The reactivity ratios of TXGL (M_1) and TBGA (M_2) were found to be $r_1 = 1.36 \pm 0.05$ and $r_2 = 0.14 \pm 0.04$ and were used in the construction of the instantaneous copolymer composition curve (Figure 3). The length of the horizontal lines in Figure 3 represents the range of monomer concentration change during copolymerization while the length of the vertical lines represents the maximum variation of instantaneous copolymer composition in each experiment. The position of the horizontal lines corresponds to the average copolymer composition obtained from nmr analysis (Table I). At high molar ratios of the more reactive monomer to the less reactive: TXGL:TBGA (1 and 2 in Table I), the copolymerization approaches azeotropic copolymerization and only small changes were observed for both the monomer feed concentration and the copolymer composition during copolymerization. As shown in Figure 3, the copolymers formed at high concentration of TXGL are quite homogeneous even though the conversion was high in a batch process. The

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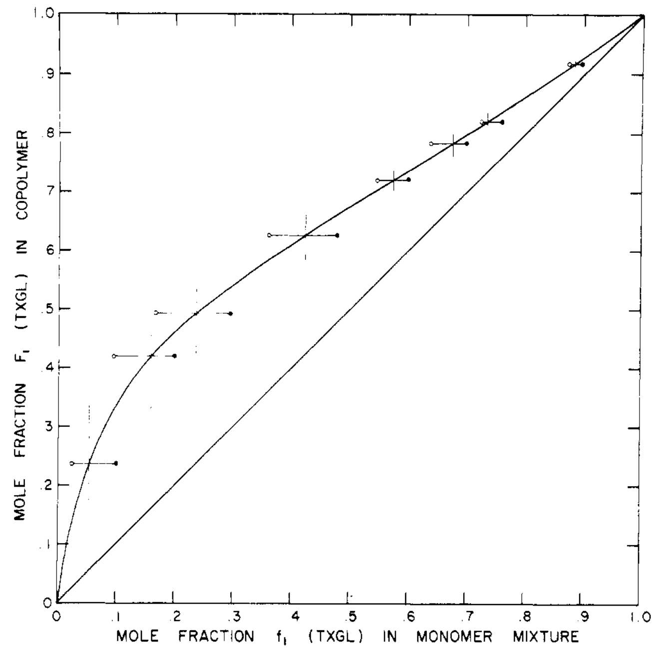

Figure 3. Copolymer composition: (●) initial monomer concentration; (○) final monomer concentration. The vertical line represents the theoretical range of copolymer composition.

sequence distribution of the copolymers containing largely glucose units and a small percentage of galactose units can, therefore, be controlled and reasonable yields of nearly homogeneous copolymer with a calculable sequence distribution can be obtained for the synthesis of desirable heterosaccharides and oligosaccharides.

Theoretical Considerations

In order to explore the feasibility of oligosaccharide preparation by copolymerization and enzymic degradation, a mathematical derivation of the weight fraction of a given sequence of TXGL of length n was attempted. Let us consider a macromolecule which consists of a sequence of A's (TXGL) bounded by B's (TBGA).

$$\cdots\text{B-}\underbrace{\text{A-A-A}\cdots\cdots\text{A}}_{n}\text{-B}\cdots$$

It has been shown that the probability[18,19] of a sequence of n units of monomer A is

$$P_{\mathrm{A}_n} = P_{\mathrm{AA}}^{\,n-1}P_{\mathrm{AB}} \qquad (1)$$

where $P_{\mathrm{AA}} = r_{\mathrm{A}}[\mathrm{A}]/(r_{\mathrm{A}}[\mathrm{A}] + [\mathrm{B}])$ and $P_{\mathrm{AB}} = [\mathrm{B}]/(r_{\mathrm{A}}[\mathrm{A}] + [\mathrm{B}])$. P_{A_n} is also the fraction of all "A" sequences, the length of which is n. The number average sequence length of A units is

$$\bar{n}_{\mathrm{A}} = \sum_{n=1}^{n=\infty} nP_{\mathrm{A}_n} = \frac{1}{P_{\mathrm{AB}}} = \frac{1}{1-P_{\mathrm{AA}}} \qquad (2)$$

Of the total number of moles of A in the copolymer, the mole fraction of A's in a given sequence of length n is

$$m_{\mathrm{A}} = \frac{nP_{\mathrm{A}_n}}{\sum_{n=1}^{n=\infty} nP_{\mathrm{A}_n}} = nP_{\mathrm{AA}}^{\,n-1}P_{\mathrm{AB}}^{\,2} \qquad (3)$$

The mole fraction of A's in a given sequence of length n based on all *(A + B)-mer* units in the copolymer is

$$M_{\mathrm{A}} = F_{\mathrm{A}}m_{\mathrm{A}} \qquad (4)$$

where F_{A} is the mole fraction of A in the copolymer. The weight fraction of A's in a given sequence of length n in the copolymer can be expressed as follows

$$W_{\mathrm{f}} = \frac{M_{\mathrm{A}}(\mathrm{MW})_{\mathrm{A}}}{F_{\mathrm{A}}(\mathrm{MW})_{\mathrm{A}} + F_{\mathrm{B}}(\mathrm{MW})_{\mathrm{B}}} = \frac{F_{\mathrm{A}}nP_{\mathrm{AA}}^{\,n-1}P_{\mathrm{AB}}^{\,2}(\mathrm{MW})_{\mathrm{A}}}{F_{\mathrm{A}}(\mathrm{MW})_{\mathrm{A}} + F_{\mathrm{B}}(\mathrm{MW})_{\mathrm{B}}} \qquad (5)$$

where $(\mathrm{MW})_{\mathrm{A}}$ and $(\mathrm{MW})_{\mathrm{B}}$ are the molecular weights of monomers A and B, respectively. Since $F_{\mathrm{A}}P_{\mathrm{AB}} = F_{\mathrm{B}}P_{\mathrm{BA}}$, the above equation can be transformed to the following

$$W_{\mathrm{f}} = \frac{nP_{\mathrm{AA}}^{\,n-1}P_{\mathrm{AB}}^{\,2}P_{\mathrm{BA}}(\mathrm{MW})_{\mathrm{A}}}{(\mathrm{MW})_{\mathrm{A}}P_{\mathrm{BA}} + (\mathrm{MW})_{\mathrm{B}}P_{\mathrm{AB}}} \qquad (6)$$

The weight fraction of the benzyl ethers of oligosaccharide, $\mathrm{A}_n\mathrm{B}$ is

$$W_0 = W_{\mathrm{f}}\,\frac{n(\mathrm{MW})_{\mathrm{A}} + (\mathrm{MW})_{\mathrm{B}}}{n(\mathrm{MW})_{\mathrm{A}}} \qquad (7)$$

To determine that sequence of length n which constitutes the largest weight fraction one sets $\mathrm{d}W_{\mathrm{f}}/\mathrm{d}n = 0$. The sequence length at maximum weight fraction is

$$\bar{n}_{\max} = \frac{-1}{\ln P_{\mathrm{AA}}} \qquad (8)$$

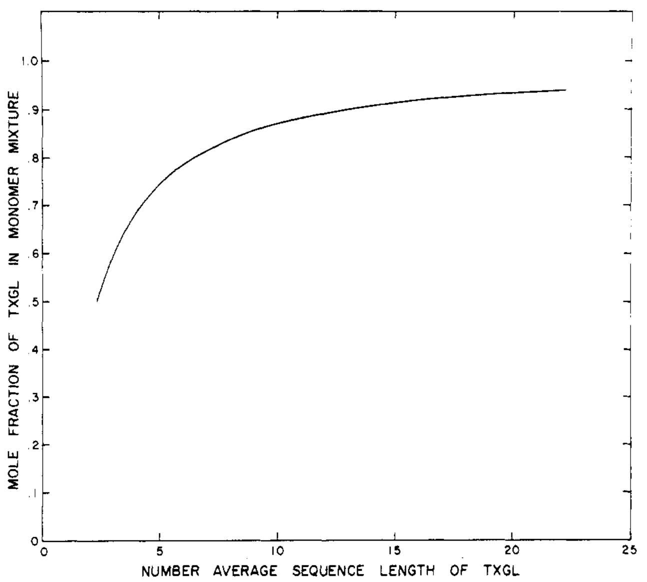

Figure 4. Calculated number-average sequence length of TXGL as a function of feed concentration.

A computer program was written to calculate the weight fraction of A's in sequence of length $n = 1 \rightarrow 40$ in different copolymers, based on eq 5, monomer feed compositions, and reactivity ratios. The program also calculated the probabilities of A-A, A-B, B-A, and B-B linkages, $\bar{n}_{\max}$ the sequence length at maximum weight fraction, weight fraction of A's in $\bar{n}_{\max}$, and the number average sequence length ($\bar{n}$) at different monomer feed concentrations. Some of the results are shown in Figures 4 and 5. Figure 4 shows the relationships between the mole fraction of monomer (TXGL) in the feed and the number-average sequence length of TXGL in the instantaneous copolymer. As can be seen, at high monomer (TXGL) mole fractions, the number-average sequence length, $\bar{n}$, changes greatly with respect to the feed concentration. At low concentration of TXGL, the $\bar{n}$ value for A sequences is somewhat less sensitive to the change of feed concentration.

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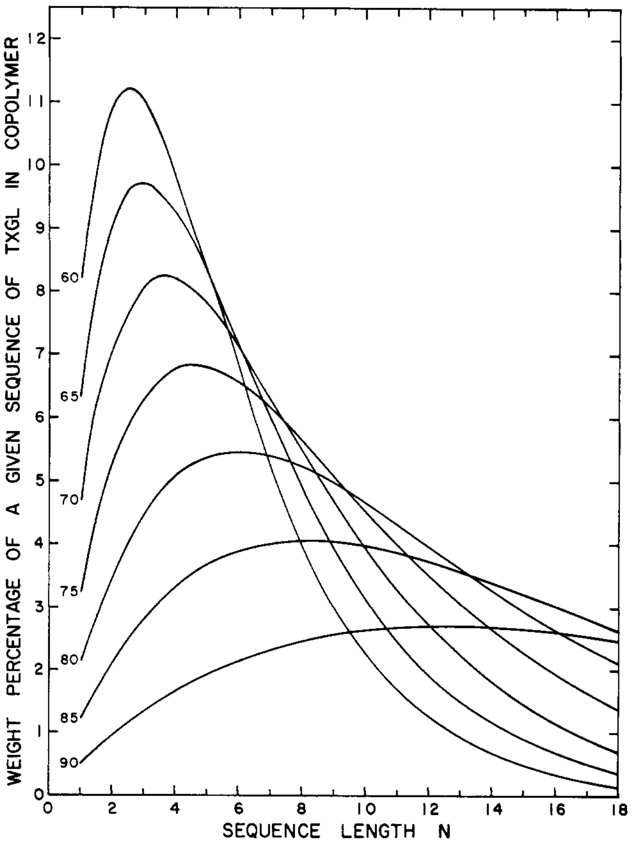

Figure 5. Calculated weight percentage of various sequences of TXGL in copolymers produced by different feed concentrations (mole %) of TXGL.

Figure 5 shows the relationship between the weight fraction in the instantaneous copolymer of a given sequence of TXGL of a length *n* (*cf.* eq 5) and the number of TXGL units, *n*, in the sequence. The calculated yield of a particular sequence containing *n* glucose units and a galactose unit in the copolymer can be determined from Figure 5 by applying a correction factor of $(n(\mathrm{MW})_A + (\mathrm{MW})_B)/n(\mathrm{MW})_A$ to the weight fraction W_f. Thus, one can design copolymerization experiments so that an optimum and predictable yield of the desired range of unsubstituted oligosaccharides is obtained after the removal of benzyl groups of copolymer, enzymatic degradation, and fractionation.

Different weight distribution curves are shown for different initial monomer feed concentrations of TXGL. The intersections of the weight distribution curves indicate that in some cases a constant weight fraction of "TXGL" in a given sequence of TXGL can be obtained even with some drift in monomer (TXGL) feed concentrations. For example, about 6.5-7.5% weight percentage (W_f) of the total copolymer would have a sequence length of six glucose units if the mole % of the monomer feed was maintained between 60 and 70%. Likewise, sequences of ten glucose units would constitute about 4-4.5% of the total copolymer weight if the molar feed concentration of TXGL was in the range of 70-85%. Finally it seems probable that this approach can be used not only for the synthesis of other (1→6)α-linked oligosaccharides but also to demonstrate the relationship between monomer feed concentrations and sequence length distributions in binary copolymers.[9]

Experimental Section

Optical rotations were determined in a Perkin-Elmer Model 141 polarimeter using a jacketed 1-dm cell. Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer in deuterated chloroform with tetramethylsilane as internal standard. Viscosities were measured in a Cannon-Ubbelohde semimicro viscometer at a constant temperature. Melting points were recorded without correction.

Synthesis of Monomers. 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose (TBGA) was prepared according to the procedures described previously.[5] The product had a melting point of 55-56°, $[\alpha]^{25}\mathrm{D}$ −46.8° (*c* 1.2, $CHCl_3$). The TBGA monomer was recrystallized several times from 2-propanol and finally recrystallized from anhydrous ether-naphtha solvent four times.

1,6-Anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-β-D-glucopyranose (TXGL) was prepared by a modification of the method of Zemplen, Csürös, and Angyal.[20] Triacetyllevoglucosan (25 g) was dissolved in 250 ml of freshly distilled α-chloro-*p*-xylene and powdered potassium hydroxide (65 g) was added to this solution with mechanical stirring. The reaction mixture was heated at 105° for 3.5 hr under nitrogen. After removal of α-chloro-*p*-xylene at reduced pressure, the cake-like reaction mixture was washed with water and extracted with chloroform. The concentrated chloroform solution was steam distilled at reduced pressure to remove volatile impurities. Then the product was extracted with chloroform and dried over Na_2SO_4. After removal of solvent, the product was crystallized from absolute alcohol to give 25.5 g (68% yield) of TXGL. The TXGL monomer was recrystallized several times from 2-propanol and finally recrystallized three times from naphtha solvent. It had mp 66-67°, $[\alpha]^{25}\mathrm{D}$ −29.95° (*c* 0.9, $CHCl_3$). The nmr spectrum (given in δ) had nine protons at 2.3 (s, methyl), twelve protons at 7.18 (q, aromatic), seven protons at 4.7-4.44, five protons at 4.0-3.26 (multiplets), and one proton at 5.45 (s, anomeric).

Polymerization. The high-vacuum polymerization technique used in this work was described previously.[3,21] Methylene chloride (1 ml) and *p*-chlorobenzenediazonium hexafluorophosphate (15.8 mg) were used. Polymerization was terminated at about 30-40% yield judging by the mobility of the polymer solution. The polymer solution was worked up as usual. The polymer was precipitated three times by pouring the chloroform solution into naphtha solvent and isolated by freeze-drying from benzene. After the polymer was thoroughly dried under high vacuum, it was weighed and analyzed by nmr. The mixture of unreacted monomers in the supernatant was isolated from naphtha solvent and analyzed by nmr. The nmr spectrum of each sample was integrated several times and the average integration heights were used in determining the integrations ratios of p-CH_3 protons:aromatic protons, p-CH_3 protons:nonaromatic protons, and p-CH_3 protons:total protons. Three values of copolymer compositions were obtained for each copolymer or monomer mixture. The average copolymer compositions were listed in Table I.

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