potential effects. No contamination of the substrate solution by chloride was observed during the typical analysis time of less than 30 min. The stationary platinum wire anode (6 × 0.57 mm) was preconditioned according to the following procedure before each sample was examined: (1) 30-s wash with 1 M chromium trioxide in concentrated sulfuric acid, then distilled water rinse; (2) 30 s wash with 1 M ferrous ammonium sulfate in 1 M sulfuric acid, water rinse, and dry; (3) 60 s with 0.00 V vs. SCE applied to the electrode immersed in the electrolyte solution with stirring followed by 60 s without stirring. The last treatment was repeated before each run. Each reported half-wave potential is an average of at least four runs using a sweep rate of 100 mV/s.

No cathodic waves were observed at scan rates up to 20V/s.[18]

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Synthesis of α-(1→3)-Branched Dextrans by Copolymerization and α-D-Glucosidation

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Abstract: A family of stereoregular (1→6)-α-D-glucopyranans with randomly distributed 3-*O*-(α-D-glucopyranosyl) side chains has been synthesized as models of microbial dextrans, by the following series of reactions: 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-but-2-enyl-β-D-glucopyranose (DBCGL, M_1) has been copolymerized with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBGL, M_2) to give a series of polymers of very high molecular weight. Crotyl groups are removed quantitatively and side chains introduced by reaction of the hydroxylated polymers with 6-*O*-(*N*-phenylcarbamoyl)-2,3,4-tri-*O*-(*p*-methylbenzyl)-1-*O*-tosyl-D-glucopyranose. Decarbanilation and debenzylation gave a family of dextrans with different degrees of branching. The polymers have been characterized by viscosity, optical rotation, circular dichroism, molecular weight, and ^1H and ^{13}C NMR. Weight fractions and sequence length distributions of the branched unit have been calculated from copolymerization data. Comparisons of structure and ^{13}C NMR spectra are made with dextrans from various strains of *Leuconostoc mesenteroides*.

Introduction

Stereoregular homo- and heteropolysaccharides have been synthesized by cationic ring opening polymerization of 2,3,4-tri-O-substituted 1,6-anhydrosugars followed by removal of the substituents with sodium in liquid ammonia.[1] The resulting products have been used to investigate lectin-carbohydrate reactions[2,3] and as model compounds in the fields of allergy,[4,5] enzymology,[6] and immunology.[7,8]

The α-(1→6)-linked glucopyranan formed by polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBGL) corresponds to the backbone of most natural dextrans

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but differs from them in being unbranched,[9,10] as evidenced by its failure to precipitate with concanavalin A.[2,3] It is, therefore, of interest to prepare similar glucans with well-defined structural features in order to determine their influence on the physiological and physicochemical properties of the polysaccharide.

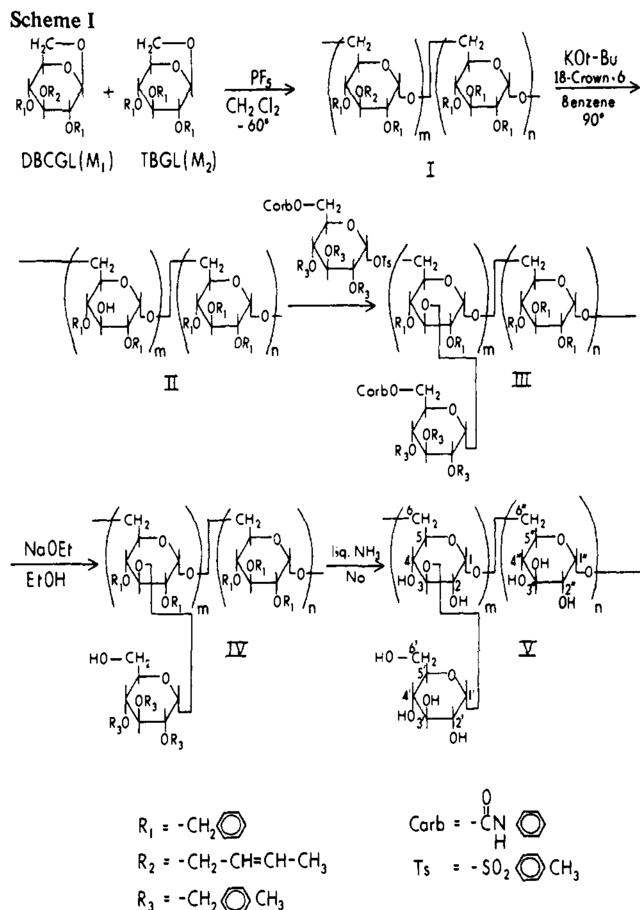
Leuconostoc mesenteroides NRRL B-512 dextran, which is used for clinical purposes, has 95% α-(1→6)- and 5% α-(1→3)-D-glucopyranosidic linkages.[11-13] Lindberg and associates have estimated, from the results of a novel degradation sequence, that 40% of the branches on this dextran consist of single α-D-glucopyranosyl units linked to the 3 position of glucose units in the backbone and around 45% of the branches consist of 3-O-α-linked isomaltose units.[14] Solution properties[15-17] and enzymic degradation[18] of this dextran demonstrate the presence of some long chains as well. *Leuconostoc mesenteroides* NRRL B-1375 (Birmingham strain) dextran has been shown to contain 26% of α-(1→3)-D-glucopyranosidic linkages,[19] all of which form branch points, and the branches consist mainly, if not exclusively, of single glucose units.[20,21]

The purpose of this research is to demonstrate a method of introducing a known number of short chains at a known position and distribution and to prepare a family of (1→6)-α-D-glucopyranans with various amounts of 3-*O*-(α-D-glucopyranosyl) side chains as models of these and other related natural dextrans. Synthesis of randomly branched polysaccharides requires copolymerization of two different anhydro sugar derivatives. The amounts and sequence distribution of the individual mer units in the polymer chain can be predicted from the reactivity of the monomers used and prior knowledge of the copolymerization process. Derivatives of 1,6-anhydro-β-D-gluco-, manno-, and galactopyranose have been shown to copolymerize even though varying 100-fold in reactivity.[22-25] The reaction has been shown by the method of Kelen and Tüdös[26,27] in all cases to follow classical binary copolymerization theory.[28] Reactivity ratios can be determined by NMR when benzyl and *p*-methylbenzyl substituents are used on the two monomers, and it is known that the *p*-methyl group does not significantly influence the propagation reaction.[28] Monomer reactivity follows an order observed in similar cationic reactions[29] and is strongly influenced by conformational changes during propagation.[23]

Two methods can be used to introduce side chains; the first is the homo-[30,31] and copolymerization[32] of anhydrodisaccharide derivatives. Anhydrodisaccharide derivatives are relatively unreactive, however, and high molecular weight polymers are not obtained by homopolymerization[30,31] or copolymerization with high mole fractions of the disaccharide monomer.[32] This method is also not readily applicable to the formation of polysaccharides with longer side chains. We, therefore, elected a second method: to copolymerize TBGL with a second 1,6-anhydro-β-D-glucopyranose derivative with a temporary blocking group on C-3, to remove the C-3 substituent, and to allow the resulting hydroxyl group to react in an α-D-glucosidation reaction to introduce the side chain. The sequence selected is shown in Scheme I.

In preliminary experiments in this laboratory, H. F. Vernay prepared a number of derivatives of the known 1,6-anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose and tested their polymerizability. An ester function on C-3 tended to make monomers of this class less reactive,[33-35] and the polymers produced were relatively low in molecular weight.[33-35] Gelation appeared to be a problem with the 3-*O*-prop-2-enyl (allyl) derivative and, therefore, 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-but-2-enyl-(crotyl)-β-D-glucopyranose (DBCGL) was chosen. The crotyl group can be removed selectively with *tert*-butoxide anion in nonprotonic solvents. The α-D-glucosidation reaction chosen was a modification of one recently developed in this laboratory[36] and shown to be suitable for the synthesis of α-isomaltooligosides with high stereoselectivity.[36,37] It thus permits the further extension of the side chains if desired.

Scheme I



It has been necessary to discover appropriate conditions and reagents which allow deblocking and α-glycosidation reactions to proceed to completion with an unavoidable minimum of chain degradation.

Results and Discussion

Polymerization. Polymerization and copolymerization of DBCGL(M_1) and TBGL(M_2) were carried out in vacuo at −60 °C in anhydrous methylene chloride with 1 mol % PF_5 (Table I). Highly purified DBCGL proved to be more reactive than TBGL, for in 10 min a 70% yield of homopolymer (polymer no. I 32) was obtained with a higher intrinsic viscosity (1.25 dL/g) than has ever been obtained from TBGL.[35,38-44] As shown in Table I, introduction of DBCGL resulted in formation of copolymers with very high molecular weights (polymer no. I 37, I 17, and I 39), even when the concentration of the DBCGL monomer in the feed was low (10%, polymer no. I 39). The polymerization is, however, highly sensitive to impurities, for less pure or older samples of monomer, even though stored under nitrogen in a refrigerator, gave lower yields and intrinsic viscosities (cf. no. I 26, I 28, and I 38) than comparable runs with pure fresh monomer (cf. no. I 29, I 32, I 34, I 37, and I 39).

Although some scatter of physical properties is evident due to the use of two different batches of DBCGL, both the specific rotation and intrinsic viscosity of copolymers appear to increase linearly with the mole fraction of DBCGL in the copolymer (cf. no. I 29, I 31, I 32, I 34, I 37, and I 39). A similar linear relationship of these physical constants has been observed in copolymers from perbenzylated and perxylylated 1,6-anhydro-β-D-glucopyranose (TBGL and TXGL).[28] In contrast, three heteropolysaccharides from three different sugars (Glc, Gal, Man) show minima of one or both properties,[22,23,25] a

Table I. Copolymerization of 1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-crotyl-β-D-glucopyranose (DBCGL) with 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBGL) at −60 °C

polym no.	mole fraction of DBCGL in feed	Polym time, min	copolymer yield, %	mole fraction of DBCGL[i] in copolymer	mole fraction of DBCGL[i] in recovered monomer	$[\alpha]^{25}_{D}$,[j] deg	$[\eta]$,[k] dL/g	k'[l]	$\overline{M_n}$[m]	$\overline{dp_n}$
110[a,f]	1.00	210	66.6	1.000	1.000	118.2	0.74	0.42		
132[a,g]	1.00	10	70.2	1.000	1.000	118.2	1.25	0.38		
129[b,g]	0.85	5	44.1	0.946	0.849	118.4	1.23	0.35		
128[b,f]	0.70	5	13.4	0.810	0.711	117.9	0.43	0.41		
134[b,g]	0.70	5	74.8	0.764	0.549	121.1	1.13	0.33		
138[b,h]	0.65	15	8.8	0.740	0.581	115.0	0.44	0.34		
126[b,f]	0.55	5	20.2	0.661	0.818	113.6	0.52	0.56		
137[b,g]	0.50	5	61.0	0.544	0.400	116.8	1.12	0.36	792 000	1910
117[b,f]	0.40	5	46.4	0.469	0.368	115.0	1.00	0.51	723 200	1730
116[b,f]	0.30	5	33.7	0.362	0.236	112.0	0.98	0.35		
115[b,f]	0.20	8	48.3	0.255	0.115	112.1	0.89	0.40		
114[b,f]	0.10	12	43.8	0.167	0.068	113.5	0.87	0.52		
139[c,g]	0.10	40	94.3	0.122	0.088	116.5	1.05	0.32	687 400	1603
131[d]	0	13	66.3	0	0	115.3	1.02	0.32	389 600	900
135[d]	0	60	92.1	0	0	113.8	0.91	0.32	384 500	890
158[e]	0	60	97.2	0	0	114.6	1.02	0.32	423 000	980

[a] Total monomer, 1.0×10^{-3} mol; *p*-chlorobenzenediazonium hexafluorophosphate, 2.8 mg, 1.0×10^{-5} mol; methylene chloride, 1.0 mL. [b] Total monomer, 2.5×10^{-3} mol; *p*-chlorobenzenediazonium hexafluorophosphate, 7.1 mg, 2.5×10^{-5} mol; methylene chloride, 2.5 mL. [c] Total monomer, 1.0×10^{-2} mol; *p*-chlorobenzenediazonium hexafluorophosphate, 28.4 mg, 1.0×10^{-4} mol; methylene chloride, 10.0 mL. [d] Total monomer, 1.0×10^{-3} mol; *p*-chlorobenzenediazonium hexafluorophosphate, 2.9 mg, 1.0×10^{-5} mol; methylene chloride, 1.0 mL; mp of TBGL, 89.5–91.0° (ref 28). [e] Total monomer, 2.0×10^{-2} mol; *p*-chlorobenzenediazonium hexafluorophosphate, 57.0 mg, 2.0×10^{-4} mol; methylene chloride, 20.0 mL; mp of TBGL, 87–88 °C. [f] The first batch DBCGL was used, mp of DBCGL, 33–34 °C. [g] The second batch DBCGL was used, mp of DBCGL, 38–39 °C. [h] Mixture of the first and second batches of DBCGL was used. [i] Mole fractions of DBCGL in the copolymers and in the unreacted feed mixtures were determined by 100-MHz NMR spectroscopy and corrected by multiplying by 1.094. [j] Specific rotations were determined in chloroform at 25 °C. [k] Intrinsic viscosities were determined in chloroform (1 g/100 mL) at 25 °C. [l] Huggins constant. [m] Number-average molecular weights were determined by membrane osmometry in toluene at 25 °C. [n] Gel formation: polymn no. 132 (1.0%), 128 (2.2%), 134 (0.5%), 138 (1.4%), 126 (0.3%).

phenomenon which suggests possible conformational differences between the copolymers and homopolymers.

The ^{13}C NMR spectra of the polymers were nearly identical except for those peaks which could be clearly identified with structural differences in the substituents. The absence of observable β-anomeric carbon signals proved the high stereospecificity of the polymerization.

The mole fractions of DBCGL units in the copolymers (Table I) were determined from the proton resonances of the methyl and vinyl groups which appear, well separated from other peaks, at 1.68 and 5.64 ppm, respectively. Mole fractions of the DBCGL monomer in the residual monomer mixture were determined only from the methyl signal intensity, rather than from the vinyl resonance, which was close to that of the anomeric proton. (The highest component of the vinyl quartet was at 5.49 ppm and the anomeric proton at 5.46 ppm.) This method of determining copolymer composition is somewhat less accurate than using methyl resonances on a fully xylylated mer, as has been done previously.[22-25,28,32] Therefore, in determining monomer reactivity ratios, some scatter of the data points was observed. Nevertheless, good agreement was found between the Fineman-Ross method[45] ($r_1 = 1.63 \pm 0.21$, $r_2 = 0.83 \pm 0.22$), the linear Kelen-Tüdös method[26,27] ($r_1 = 1.63$, $r_2 = 0.83$), the Kelen-Tüdös method using average monomer concentrations[46] ($r_1 = 1.82$, $r_2 = 0.74$), and the Mayo-Lewis integration method[47] ($r_1 = 1.75 \pm 0.40$, $r_2 = 0.70 \pm 0.35$). Since no curvature was observed in the Kelen-Tüdös plots, it can be assumed that the polymerization follows classical binary copolymerization theory.[26-28,46]

The crotylated monomer DBCGL(M_1) is thus more reactive than TBGL(M_2). This result is consistent with the observations made during homopolymerization and with the mechanistic model previously proposed.[23] Since the substituent on C3 shields the anhydro ring oxygen on the monomer as it approaches the C1 carbon of the growing polymer chain end, it is understandable that the monomer having a smaller group

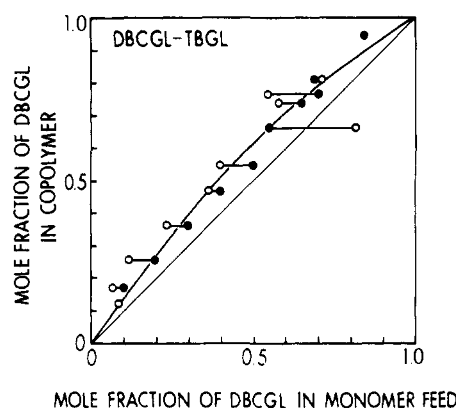


Figure 1. Copolymer composition vs. monomer feed of DBCGL and TBGL: (●) initial monomer concentration; (○) final monomer concentration.

on C3 is more easily accessible to the propagating cation. In addition to the ease of approach, the conformational change from the chair-like to the boat-like form may require less energy in the case of DBCGL, since the monomer has a smaller substituent on C3. The corresponding anhydromaltose monomer HBMA[30,32] has a bulky glucopyranosyl residue linked to C4 and is considerably less reactive than TBGL.

In Figure 1 is shown the instantaneous copolymer composition curve calculated from the reactivity ratios. The position of the horizontal lines corresponds to the copolymer composition obtained from ^{1}H NMR analysis. The length of the horizontal line represents the range of the monomer composition change during polymerization.

Although the copolymerizations were terminated at high conversion with a few exceptions, neither the copolymer composition nor the monomer mixture composition varied

Table II. Mole Fractions of DBCGL in Copolymer and in Unreacted Monomer Mixture Calculated for High-Conversion Copolymerizations

polym no.	mole fraction of DBCGL in the copolymer		mole fraction of DBCGL in the recovered monomer
	av	range	
128	0.791	0.796-0.785	0.686
138	0.751	0.755-0.748	0.641
126	0.654	0.666-0.641	0.523
137	0.570	0.618-0.505	0.392
117	0.479	0.514-0.437	0.331
116	0.377	0.399-0.352	0.261
115	0.247	0.274-0.216	0.156
114	0.127	0.141-0.112	0.079
139	0.104	0.141-0.044	0.031

much with conversion because the reactivity ratios of the monomers are not greatly different. The mole fractions of each monomer in the copolymer and in the recovered monomer mixture were calculated from the Mayo-Lewis reactivity ratios at intervals of 0.1% conversion increment to the final conversion, by means of a computer program similar to Harwood's[48] and used previously.[22-24] As shown in Table II, the found and the calculated values obtained for both the copolymer composition and the final monomer composition show good agreement. Number-average sequence lengths of DBCGL and TBGL in the copolymers were calculated from the reactivity ratios for instantaneous copolymerization based on the differential copolymerization equations and are shown in Figure 2.[71] In Table III are summarized probabilities that one monomer follows another monomer, number-average sequence lengths of DBCGL and TBGL, and number-average lengths at maximum weight fraction, calculated from the reactivity ratios using a computer program developed previously for instantaneous copolymerization.[22-24]

Decrotylation. Decrotylation of the copolymers was incomplete under customary reaction conditions with potassium *tert*-butoxide in dimethyl sulfoxide[49] even at 90 °C with freshly prepared base in anhydrous conditions. However, the use of freshly prepared potassium *tert*-butoxide complexed with the cyclic polyethylene oxide derivative,[50] 18-crown-6, in benzene at 90 °C removed all crotyl groups as evidenced by IR and ^{1}H and ^{13}C NMR analyses. The decrotylation caused about 0.7 ppm upfield shift of the anomeric carbon resonance, which then appeared at 96.9 ppm. The shift allowed us to determine the mole fraction of glucose units containing an hydroxyl group on C3 in the copolymers (II 17 and II 37) by comparing the relative peak heights of the two anomeric carbon resonances. The results showed good agreement with the values obtained for the corresponding crotylated copolymers by ^{1}H NMR analyses.

Physical properties of the decrotylated copolymers are summarized in Table IV. Intrinsic viscosities of II 17 and II 37 were dramatically reduced by the decrotylation. Comparison of the degree of polymerization ($\overline{dp}_n$) before and after the decrotylation reaction indicates that one main chain scission took place during formation of II 39, four to five breaks for **II** 17, and seven breaks for II 37. The number of main chain scissions appears to be linearly related to the crotyl group mole fraction, or perhaps in actuality to the *tert*-butoxide concentration used. Optical rotations of the decrotylated polymers are not linearly related to the mole fraction of glucose units containing an hydroxyl group, but gradually increase with increasing hydroxyl group concentration in the polymer.

α-D-Glucosidation. The reaction of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoly)-1-*O*-tosyl-α-D-glucopyranose with alcohols in ether solvent has been developed in this laboratory for the preparation of α-D-glucosides and oligosaccharides in high yield with high stereoselectivity and relatively rapid rate.[36,37] For the synthesis of α-(1→3)-branched (1→6)-α-D-glucopyranans, the corresponding 2,3,4-tri-*O*-*p*-methylbenzyl derivative was prepared in order to permit the determination of extent of reaction and mole fraction of branches by means of ^{1}H NMR spectroscopy.[22-25,28,32] Model glucosidations with methanol in diethyl ether and dimethoxyethane (DME) gave only about 80% of α-D-glucoside, but with the bulkier cyclohexanol the α stereoselectivity was about 95% in both solvents. Because DME is a much better solvent for the polymers, coupling reactions with the polymeric alcohols were carried out in this solvent at room temperature overnight with the usual vacuum rack techniques. In contrast to reactions on alcohols of small molecular weight,[36,37] a large excess of tosyl derivative (about 10 equiv) was required to carry the reaction to completion. The much slower glycosidation rate may have been due to the lower concentration of hydroxyl groups in the polymer solutions, compared to prior reactions on monomers and oligomers.

By comparing the integrated areas of the three methyl proton absorptions at δ 2.10, 2.24, and 2.32 ppm with the nonaromatic and with aromatic and NH proton areas, it was possible to calculate the mole fraction of branches. These results and the physical constants of the coupled polymers are summarized in Table V. The mole fraction of branched units in early experiments proved to be less than the analytical values for crotylated and decrotylated units in the polymers from which the branched polymers were derived. For example, III 39B was coupled to about 61% of theoretical. Later experiments on the same scale under improved conditions gave polymers III 17, III 37, and III 32 with degrees of branching corresponding to complete glycosidation (cf. Tables I, IV, and V). Mechanical difficulties in transferring reactant in a larger scale preparation resulted in only 29% coupling (III 39A) but, on a second glucosidation, reaction went to completion (III 39C.) Because of limited amounts of the xylylated glucose derivative, 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-α-D-glucopyranose was used in the second glucosidation of III 39A to III 39C. For analysis of the substituents on III 39C, therefore, a strong band at 233 nm in the circular dichroism (CD) spectrum due to the carbonyl group of the *N*-phenylcarbamate substituent was used. A calibration curve using data from the other polymers was prepared (Table V), and the mole fraction of branched glucose units was estimated to be 0.11 as compared to 0.12 of crotylated units in the original polymer.

^{13}C NMR spectra at this stage are rather complex and were not analyzed in detail. However, the side chain C-6 resonance could be observed somewhat upfield from the main chain C-6 resonance and permitted a rough estimate of the degree of branching. The anomeric carbon resonance appeared at 97.5 ppm as a rather broad singlet and the amount of β-anomeric carbon was below the level of detection on all polymers. The intrinsic viscosities and $\overline{dp}_n$ values of the polymers were very close before and after coupling except for the $\overline{dp}_n$ of III 39C (777 to 439) (Tables IV and V). We assume, therefore, that few chain breaks occur during glycosidation. Specific rotation of the coupled polymers was less than that of the decrotylated polysaccharides from which they were derived.

Decarbamoylation. Decarbamoylation was carried out with freshly prepared sodium ethoxide in absolute ethanol.[37] Complete removal of the byproduct ethyl *n*-phenylcarbamate was accomplished by three precipitations into petroleum ether. Disappearance of the carbamate residue was monitored by IR, CD, and ^{13}C NMR.

After removal of the carbamoyl group, the side chain C6 resonance shifted about 2 ppm to higher field, leaving the main chain C6 resonance unchanged. The shift permitted us to approximate the degree of the branching by comparing the C6

Table III. Probabilities of Dimeric Sequences, Number-Average Sequence Lengths, and Number-Average Sequence Lengths of DBCGL(M_1) at Maximum Weight Fractions

mole fraction of DBCGL(M_1) in feed	probabilities				number-average sequence lengths		number-average sequence lengths of DBCGL (max wt fractions)	
	M_1M_1	M_1M_2	M_2M_1	M_2M_2	M_1	M_2		
0.1	0.163	0.837	0.137	0.863	1.19	7.30	0.55	(0.114)
0.2	0.304	0.696	0.263	0.737	1.44	3.80	0.84	(0.127)
0.3	0.429	0.571	0.380	0.620	1.75	2.63	1.18	(0.125)
0.4	0.538	0.462	0.488	0.512	2.17	2.05	1.62	(0.116)
0.5	0.636	0.364	0.588	0.412	2.75	1.70	2.21	(0.101)
0.6	0.724	0.276	0.682	0.318	3.63	1.47	3.10	(0.083)
0.7	0.803	0.197	0.769	0.231	5.08	1.30	4.57	(0.063)
0.8	0.875	0.125	0.851	0.149	8.00	1.18	7.49	(0.042)
0.9	0.940	0.060	0.928	0.072	16.75	1.08	16.24	(0.021)

Table IV. Decrotylation and Physical Constants of Decrotylated Polymers[a]

polym no.	starting polym no.	yield, %	mole fraction of hydroxylated glucose unit	$[\alpha]^{25}_D$, deg	$[\eta]$, dL/g	k'	$\overline{M}_n$	$\overline{dp}_n$
I139	139	96		115.6			329 200	777
I117	117	83	0.42	120.8	0.45	0.66	120 200	303
I137	137	86	0.54	122.5	0.39	0.56	92 550	239
I132	132	~100	1.0	124.3				

[a] Mole fractions of hydroxylated glucose unit in the polymer were determined by ^{13}C NMR. Specific rotations were determined in chloroform at 25 °C. Intrinsic viscosities were determined in chloroform (1 g/100 mL) at 25 °C. Number-average molecular weights were determined by membrane osmometry in chloroform at 25 °C.

Table V. Coupling Reaction and Physical Constants of Coupled Polymers[a]

polym no.	starting polym no.	mole fraction of branched glucose unit in main chain	$[\alpha]^{25}_D$, deg	$[\eta]$, dL/g	k'	$\overline{M}_n$	$\overline{dp}_n$	specific ellipticity at 233 nm, deg
II139A	I139	0.035	116.4	0.66	0.39			206
II139B	I139	0.075	114.8	0.65	0.39			393
II139C	II139A	0.11	112.9	0.59	0.34	210 600	439	500
II117	I117	0.42	110.1	0.45	0.49	116 700	263	1388
II137	I137	0.52	113.0	0.40	0.33	162 560	238	1673
II132	I132	0.97	113.9	0.12	0.62	31 600	34	2077

[a] Mole fractions of branched glucose unit were determined by 100-MHz NMR, except II139C. The mole fraction of the branched glucose unit in II139C was determined by CD. Specific rotations were determined in chlforform at 25 °C. Intrinsic viscosities were determined in chloroform at 25 °C. Number-average molecular weights were determined in chloroform at 25 °C by membrane osmometry. $\overline{dp}_n$ means approximate number of glucose units in the main chain. Specific ellipticities were measured in dioxane at room temperature.

Table VI. Decarbamoylation and Physical Constants of Decarbamoylated Polymers[a]

polym no.	starting polym no.	yield, %	$[\alpha]^{25}_D$, deg	$\overline{M}_n$	$\overline{dp}_n$
IV39C'[b]	II139C		113.2	280 400	590
IV39C[c]	II139C	91	110.9	189 100	398
IV17	II117	63	103.1	110 600	180
IV37	II137	64	99.2	104 700	164
IV32	II132	80	101.1	28 600	35

[a] Specific rotations were determined in chloroform at 25 °C. Number-average molecular weights were determined in chloroform at 25 °C by membrane osmometry. $\overline{dp}_n$ means approximate number of glucose units in the backbone. [b] Preliminary small-scale reaction. [c] Large-scale reaction.

peak areas, more accurately than for the carbamoylated polymers. The fully coupled polymer IV 32 had in fact two peaks of equal intensity at 61.7 and 66.3 ppm and the higher field peak was less in intensity for those with lesser amounts of branched units. Anomeric carbon resonance was observed only at 97.6 ppm, and again no resonances due to β anomeric configurations were detected. Little change in number-average molecular weight occurred during decarbamoylation (cf. Tables V and VI) but, surprisingly, some decrease in specific rotation was evident.

Debenzylation. Debenzylation was carried out with sodium in liquid ammonia at reflux (−33 °C).[43,44,51] The polymers were freeze-dried from water after dialysis and further dried under high vacuum but retained water tenaciously for the apparent yields were over 100% in most cases. The water content in previous cases has ranged from approximately one water molecule per two anhydroglucose units for linear glucan[41] to one water molecule per single anhydroglucose unit for branched glucan.[30] The water contents in natural dextrans have been determined gravimetrically by drying preweighed

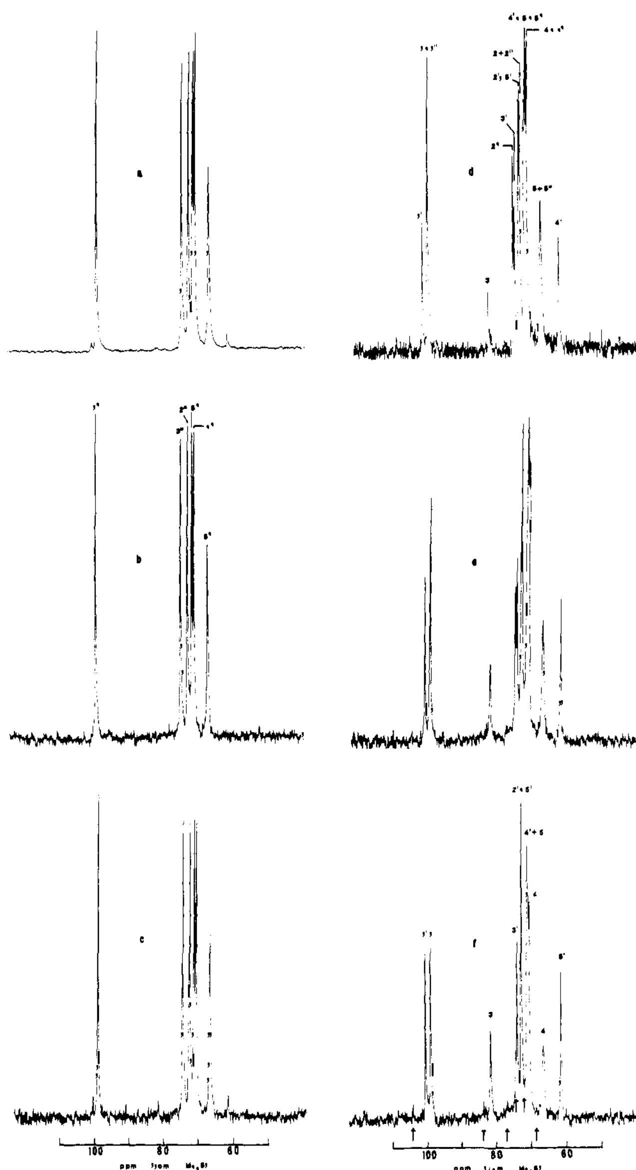


Figure 3. 25-MHz ^{13}C NMR spectra of natural and synthetic dextrans in deuterium oxide at 27 °C. (a) Natural dextran T 70, (b) synthetic linear dextran V58, (c) synthetic branched dextran V39, (d) synthetic branched dextran V17, (e) synthetic branched dextran V37, and (f) synthetic branched dextran V32. (†) shows resonances due to β-(1 → 3) linkages.

samples for 36 h at 120 °C and ranged from 5.0 to 9.5%.[52] The amount of bound water of dextran calculated by Gekko and Noguchi[53] from sound velocity measurements amounted to a constant value of 1.5 H_2O/glucose unit for Mn > 2000, but the amount of hydration increased with decreasing molecular weight for Mn < 2000. Since these results have been somewhat variable, physical constants are given without correction in this article. Specific rotations of these polymers are very high, although generally slightly lower than natural dextrans[9] and somewhat variable (Table VII). The most probable source of variability may be in water content, but a minor percentage of β-(1→3) linkages was found to be present in the debenzylated products by ^{13}C NMR spectroscopy and is described below.

Debenzylation of IV 39C caused only about one scission per molecule, giving a synthetic dextran with 11-12% branch points and of number-average molecular weight 38 800, comparable in size to a clinical dextran and previous samples of synthetic linear dextran. Previous debenzylations of linear dextran have usually resulted in three to four scissions per molecule.[42,44] It is conceivable that these represent an occasional random alkali-labile linkage, which in this case was cleaved during decrotylation.

Characterization of Dextrans. The synthetic dextrans were characterized primarily by ^{13}C NMR spectroscopy in deuterium oxide at 27 °C, and their spectra were compared with that of B-512 dextran (Figure 3). The chemical shifts due to the carbons of unbranched units of the dextran backbone are already established. Initially Colson, Jennings, and Smith reported assignments of ^{13}C chemical shifts of various polymers and oligomers of glucose including a linear α-(1→6)-D-glucan synthesized in this laboratory, but left the assignments of C4 and C5 uncertain.[54] Gagnaire and Vignon[55] achieved unambiguous assignments of C4 and C5 resonances by selective irradiation of protons on C4. However, a number of minor inconsistencies or gaps have been evident in prior assignments of resonances on branching units and side chains. We, therefore, assumed that the shifts induced in the α-D-glucopyranose spectrum by 3-*O* substitution of an α-D-glucopyranosyl unit (to form α-nigerose) would be duplicated in the linear dextran spectrum on like substitution. This application of the additivity rule[56,57] proved valid as evidenced by the observed and calculated chemical shifts and assignments shown in Table VIII. The changes in peak intensities at 100.4, 81.7, 74.1, 72.9, 71.3, and 61.6 ppm with increased branching are satisfactorily explained by these data.

Prior assignments of ^{13}C chemical shifts of the branching units of the backbone and of the terminal branch units could also be readily correlated with a number of these values. Usui et al.[58] assigned a peak at 83.0 ppm to the C3 involved in the α-(1→3) linkages of *Leuconostoc mesenteroides* NRRL B-1299S dextran which they say contains about 60% of α-(1→6), 33% of α-(1→2), and 7% of α-(1→3) linkages. This value was obtained at 90 °C, and using the temperature correction of Seymour et al.[59] a value of about 82.0 ppm at 27 °C is obtained. This is consistent with our additivity calculations (81.6) and observations (81.7). Seymour et al. did not, however, observe this peak in a sample of the same dextran but observed it at 81.55 and 81.60 ppm in the spectra of *Leuconostoc mesenteroides* NRRL B-1351S and B-1355S dextrans, respectively, at 27 °C. Colson, Jennings, and Smith[54] reported ^{13}C chemical shifts of α- and β-(1→3)-glucans, the former only at pD 14. They estimated that a pD change to 7 would result in an upfield shift of about 1 ppm for the carbons involved in a linkage. On this basis they assigned a peak at 81.9 ppm to the C3 resonance of a linear α-(1→3)-linked D-glucopyranose unit of *Leuconostoc mesenteroides* NRRL B-742 dextran.[54] This is not greatly different from the value we observe for C3 resonance on the branching unit. Their values for the anomeric carbon involved in α-(1→3) linkage and unbound C6 resonances are also close to those found in nigerose and in our branched polymers.

B-512 dextran has been treated as a linear unbranched glucan in ^{13}C NMR studies,[52,55,60] because it contains only about 5% of α-(1→3)-D-glucopyranoside linkages. However, minor signals due to α-(1→3) linkages were detected by Benesi and Gerig,[52] but only the unlinked C6 of these was identified.[52] As shown in Figure 3, the ^{13}C spectrum of B-512 dextran (a) is very similar to that of synthetic linear dextran V 58 (b), but contains additional minor peaks at 61.6, 81.7, and 100.4 ppm which are also found in the spectrum of the 12% branched dextran V 39 (c). Quantitatively the spectrum of V 39 (c) is more similar to that of B-1351S dextran reported by Seymour et al.[59] This contains about 11% of α-(1→3) linkages involved in the branches.[61] The only difference is the presence of a minor peak at 67.54 ppm in the C6 resonance region in their spectrum which is not present in our spectra or indeed in their spectrum of *Leuconostoc mesenteroides* NRRL B-1355S dextran. They ascribe this peak erroneously to C6 of the 1,3,6-tri-*O*-substi-

Table VII. Physical Properties of Synthetic Dextrans[a]

dextran no.	starting polym no.	yield, %	mole fraction of branched glucose unit		(1→3) linkages, %		$[\alpha]^{25}_{D}$, deg	$[\eta]$, dL/g	k'	$\overline{M}_n$	$\overline{dp}_n$	diad fraction			number av seq length	
			^1H	^{13}C	^1H	^{13}C						$F_{\underset{G}{\underset{\vert}{G}}\underset{G}{\underset{\vert}{G}}}$	$F_{\underset{G}{\underset{\vert}{G}}G} = F_{G\underset{G}{\underset{\vert}{G}}}$	F_{GG}	$\underset{G}{\underset{\vert}{G}}$	G
V58	158		0	0	0	0	189.2	0.46	0.61	43 700	270	0	0	1.00		
V39	IV39C	95.0	0.11	0.12	10	11	189.1	0.36	0.72	38 830	213 (240)	0.01	0.09	0.80	1.15	9.88
V17	IV17	101.6	0.33	0.43	25	30	184.4					0.24	0.24	0.28	2.02	2.20
V37	IV37	107.6	0.50	0.54	33	25	168.2					0.34	0.23	0.20	2.46	1.86
V32	IV32	106.5	0.71	1.04	42	50	186.0					1.00	0	0		

[a] Mole fractions of the branched glucose unit were determined by ^1H and ^{13}C NMR in deuterium oxide. Percentages of (1→3) linkages were calculated by (mole fraction of branched glucose units)/(1 + mole fraction of branched glucose units), which mean the (1→3) linkage % of the total glucosidic linkages, and this expression has been used in references on dextrans. Specific rotations were determined in water at 25 °C and are shown without correcting retained water content. Number-average molecular weights were determined in Me_2SO–water at 25 °C by membrane osmometry. Intrinsic viscosities of V58 and V39 were determined in Me_2SO and water, respectively, at 25 °C. $\overline{dp}_n$ of V39 in parentheses was calculated by $\overline{M}_n/162$. $\overline{dp}_n$ 213 corresponds to the number of the glucose units in the backbone. Diad fractions and number-average sequence lengths were calculated for high-conversion copolymerizations of DBCGL–TBGL using the monomer reactivity ratios r_{DBCGL} = 1.75 and r_{TBGL} = 0.70.

Table VIII. ^{13}C Chemical Shifts of α-(1→3)-Branched Dextrans[a]

carbon	obsd	calcd	ref
C1	99.0	99.3	
C1′	100.4		100.2
C1″	99.0		99.0
C2	72.6	72.4	
C2′	72.9		72.9
C2″	72.6		72.5
C3	81.7	81.6	
C3′	74.1		74.1
C3″	74.6		74.5
C4	70.7	70.2	
C4′	71.3		71.2
C4″	70.7		70.3
C5	71.3	71.5	
C5′	72.9		72.9
C5″	71.3		71.3
C6	66.7	66.6	
C6′	61.6		61.7
C6″	66.7		66.7

[a] Refer to Scheme I. To calculate ^{13}C chemical shifts of the branched glucose unit, ^{13}C chemical shifts of α-D-glucose, α-(1→6)-glucan, and nigerose were used from ref 54. Chemical shifts were corrected to the values reported by Colson et al. (ref 54).

tuted branching residue.[59] Since substitution of an α-D-glucopyranosyl unit on C3 of a dextran causes a shift of C6 resonance of only 0.1 ppm, this minor peak must be an impurity or may be caused by instrumental noise. (Another minor inconsistency in their data is the location of peak F at 61.4 ppm in the text when in fact their Figures 1 and 2 place it around 66.6 ppm.)

The 27 °C spectrum of B-1355S dextran reported by Seymour and co-workers[59] is almost identical with that of our fully branched synthetic dextran V 32 (Figure 3f). The proportions of α-(1→6) and α-(1→3) linkages in these two dextrans are very similar but the detailed structures are quite different. B-1355S dextran has 47% of α-(1→6) and 35% of α-(1→3) linkages in the main chain and 11% of α-(1→3)-branch linkages,[61] while the synthetic dextran has no α-(1→3) linkages in the backbone. When the spectrum of B-1355S dextran was taken at 90 °C, however, two additional peaks appeared in the region 69–74 ppm, which may reflect the difference in structure of the two dextrans. Their unexplained observation, that the intensity of the unbound C6 peak (at 61.38 ppm) of dextran B-1355S is much greater than the corresponding peak for dextran B-1351S,[59] is understandable since the unbound C6

Table IX. Predicted ^{13}C Chemical Shifts of β-(1→3)-Branched Dextrans[a]

carbon	chemical shift	carbon	chemical shift
C1′	104.0	C5 + C5″	71.1-71.3
C1 + C1″	99.0	C4′ + C4″	70.3-70.7
C3	84.3	C4	68.9
C3′ + C5′	76.7	C6 + C6″	66.7-67.0
C2′ + C3″	74.6	C6′	62
C2 + C2″	72.1-72.5		

[a] To calculate ^{13}C chemical shifts, reported chemical shift values of α-D-glucose, α-(1→6)-glucan, and laminaribiose were used (ref 54).

carbon on α-(1→3)-linked glucose units in the backbone resonates at nearly the same frequency as terminal α-D-glucopyranosyl units in the branches at 61.4–61.7 ppm (cf. dextran B-742).[54]

Careful examination of the ^{13}C spectra of the highly branched synthetic dextrans (Figures 3d–f), especially fully branched dextran V 32 (Figure 3f), revealed that some minor peaks are present, which should be ascribed to β-(1→3)-linked D-glucopyranosyl residues. The ^{13}C chemical shifts of β-(1→3)-branched dextrans were calculated by the previously described method using reported chemical shift values for α-D-glucopyranose, laminaribiose, and α-(1→6)-D-glucopyranan.[54] These are shown in Table IX. Of these, the anomeric carbon resonance involved in the β-(1→3) linkage was detected at 104.1 ppm, the branch point C3 at 83.9 ppm, C3 and C5 of the side chain at 76.9 ppm, C2 of the side chain at 74.6 ppm, C2 of the branch point at 72.2 ppm, and C4 of the branch point at 68.7 ppm. The β-(1→3) linkages were estimated to be 5–8% of the total (1→3) linkages in the polysaccharides, and the stereoselectivity of the glycosidation reaction, therefore, almost the same as that achieved with cyclohexanol.

Specific comment is required on the quantitative analysis of branching in the synthetic dextrans. The percent branching was calculated as the ratio of terminal branch units to the sum of unbranched and branching units in the backbone. On this basis, percent branching ranges from 12.2% for V 39 to 100% for V 32. The usual values from degradative experiments are based on the total number of units in the polysaccharide and thus would be 10.9% for V 39 and 50% for V 32.

Spectrometric analysis at each step of the synthesis gave good agreement with expected values calculated on the assumption of complete reaction. This agreement was achieved after decrotylation, α-D-glucosidation, and decarbamoylation.

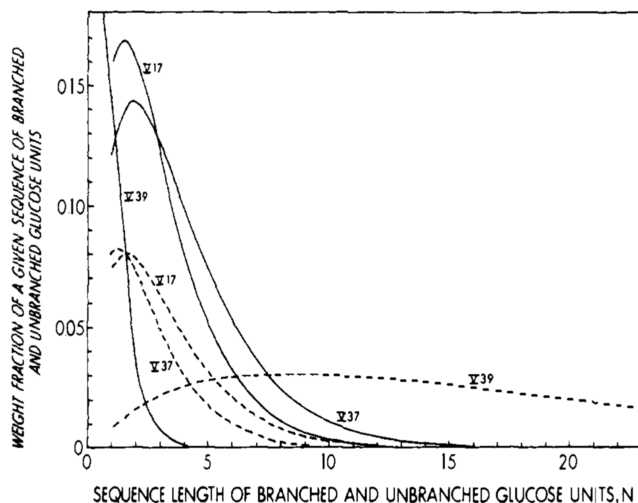


Figure 5. Weight fractions of a given sequence lengths of branched (—) and unbranched (---) glucose units in dextrans.

Analyses on the free dextrans, following debenzylation with sodium in liquid ammonia, also showed general agreement but greater scatter. It is difficult to believe that relatively selective elimination of glucose units linked to secondary positions would occur under these conditions, which cleave very few primary glycosidic linkages in the main chain; so presumably the branches present in each dextran correspond closely to the number of crotyl groups in the original copolymers.

This was confirmed generally by the results of ^{13}C NMR analysis. Peak heights and peak areas were compared for C1 of the branches and the sum of the two resonances corresponding to C1 of the substituted and unsubstituted units in the backbone. The same comparison was made for C3 and C6 resonances. In general the best agreement for narrow resonances was with peak heights and for broader resonances with peak areas. The results for fully branched dextran V 32 were best, varying from 99 to 111% branching; dextran V 37, 54.4% theory, 53-68% measured; dextran V 17, 46.6% theory, 38-44% measured; V 39, 12.2% theory, 7.0-12.3% measured.

Proton magnetic resonance (Figure 4)[71] was of no assistance in improving the precision of these results. Two prior comparisons have been made of the determination of α-(1→3) branching in dextrans by means of ^{1}H NMR and by methylation techniques. In the two cases, ^{1}H NMR gave lower values for the degree of branching.[62,63] In our experience, a similar observation was made. Usually ^{1}H NMR values were lower than ^{13}C NMR values. In the case of fully branched dextran V 32 a continuous-wave spectrum at 27 °C gave equal amounts of α-(1→3) and α-(1→6) linkages but a Fourier transform spectrum failed to do so.

The proton magnetic resonance spectra of a number of natural dextrans have been described.[55,58,62-65] The positions of the anomeric protons at the α-(1→3), α-(1→6), α-(1→4), and α-(1→2) have been established.[64,65] The initial assignment of the C2, C3, C4, C5, and C6 protons[64] has recently been revised from data obtained on a 250-MHz ^{1}H NMR spectrum.[55] Because of the paucity of comparative data on branched dextrans, the quantitative interpretation of these spectra should be accepted with caution. However, it appears probable that the calculated values of branching on the synthetic dextrans should be very close to the correct values and no other structural aspects are in doubt.

Figure 2[71] gives the instantaneous number-average sequence length of the DBCGL-TBGL copolymers based on the differential copolymer composition equation. A computer program developed by Lin,[22] and modified for high conversion copolymer,[24] was used to calculate from the same data number-average sequence lengths, diad fractions, and weight fractions of branched and unbranched sequences in the backbone of the final dextrans (Table VII and Figure 5). These data permit a more complete interpretation of the structures of the dextrans.

In dextran V 39 only 1 wt % of all mers fall in diad sequences in which two branching D-glucopyranosyl residues are adjacent, and only about 11 wt % of all mers are in sequences of six unbranched units or less. Covacevich and Richards[66] have interpreted an enzymic degradation of dextran B-512 as showing that almost all branch points are at least six α-(1→6)-linked units apart. The sequence distributions in V 39 and B-512 dextrans may, therefore, not be dissimilar.

Dextran B-1375 contains 26% of α-(1→3) linkages as branches.[19] Enzymic[20] and Smith degradations[21] of the dextran indicated that the branches are mainly single glucose units, randomly distributed along the backbone,[67] and that more than 17% of all branches are joined to adjacent α-(1→6)-linked glucose units.[21] On the same analytical basis, synthetic dextran V 17 contains 32% of α-(1→3) linkages in the structure of which 50% are linked to adjacent units in the backbone (Table VII). The branch points are randomly distributed along the backbone (Figure 5), and number-average sequence lengths of both branched and unbranched units are about two.

It appears clear that it is possible to synthesize a family of α-(1→6)-linked linear glucans with a randomly distributed sequence of α-(1→3)-linked D-glucopyranosyl units as branches which can serve as well-characterized models for related natural dextrans.

Experimental Section

General. NMR spectra of monomers and polymers were recorded on a Varian XL-100 spectrometer in deuteriochloroform with tetramethylsilane as internal standard, except those of debenzylated polymers, which were measured in deuterium oxide without any standard. The ^{13}C chemical shifts in the latter were corrected to the values of Colson et al.[54] Optical rotations were determined in a Perkin-Elmer Model 141 polarimeter using a jacketed 1-dm cell. Circular dichroism (CD) spectra were recorded on a JASCO optical rotatory dispersion recorder Model ORD/UV-5 with Sproul Scientific SS-107 CD modification in the wavelength region from 300 to 230 nm at room temperature. Infrared (IR) spectra were obtained with a Perkin-Elmer 137 sodium chloride spectrophotometer. Number-average molecular weights were determined in toluene, chloroform, or dimethyl sulfoxide (Me_2SO)-water at 25 °C by means of a Hewlett-Packard high-speed membrane osmometer Model 503 equipped with a variable temperature controller. The CDC-3200 computer system was used for calculation of copolymerization parameters.

The natural dextran (*Leuconostoc mesenteroides* NRRL B-512) was Dextran T-70 obtained from Pharmacia Fine Chemicals, Uppsala, Sweden ($\overline{M}_w$ = 70 000, $\overline{M}_n$ = 42 500).

Polymerization. 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBGL) was synthesized and purified according to the method previously described,[22-25,28,30-32,35,38-44] mp 89.5-91.0 °C[28] (lit. 89.5-90.5,[35] 90-91.5 °C[44]), $[\alpha]^{25}{}_D$ −30.8°[28] (*c* 1, $CHCl_3$) (lit. −30.8,[40] −31.6°[44]) 1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-crotyl-β-D-glucopyranose (DBCGL) was synthesized by H. F. Vernay from 1,6-anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose.[68] The structure proof of this compound by Zemplén[68] has been confirmed by its synthesis from 1,6:3,4-dianhydro-2-*O*-benzyl-β-D-galactopyranose and from 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-mannopyranose by reaction with benzyl alcohol.[69] ^{13}C NMR studies in this laboratory were consistent with this distribution of substituents.

According to the method of Gent et al.,[49] a mixture of 1,6-anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose (7.8 g, mp 100-103.5 °C, lit. 103 °C [49]), sodium hydride (1.44 g), and crotyl bromide (6.0 mL, 7.9 g) was refluxed in benzene, followed by quenching with a small amount of methanol, washing with water, and drying over magnesium sulfate. A small amount of BDCGL was crystallized from ethanol-water. The remainder was passed through a silica gel column, eluted with chloroform-methylene chloride. A saturated solution of the compound was seeded and allowed to stand in a freezer, giving white

crystals (4.5 g), mp 33–34, 38–39 °C, $[\alpha]^{25}_D$ −30.9° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.64; H, 7.19.

^{13}C ($CDCl_3$): CH_3, 17.75; C6, 65.62; OCH_2, 71.07, 71.42, and 72.01; C5, 74.63; C2, C3, C4, 76.23, 77.07, and 77.52; Cl, 100.88; vinyl 127.6 and 129.76; aromatic C2, C3, C4, 128.00 and 128.60; aromatic C1, 138.24 ppm.

Methylene chloride and *p*-chlorobenzenediazonium hexafluorophosphate were purified in the usual manner and copolymerization was carried out under high vacuum at −60 °C in anhydrous methylene chloride with 1 mol % PF_5 as described previously.[22-25,28,32] Total monomer was 2.5 × 10^{-3} mol, except polymers no. I 10, I 32, I 39, and I 58. Polymerization conditions are shown in Table I. Polymerization was terminated at −60 °C by adding a small volume of cold methanol. A small amount of gel formed was filtered out, dried, and weighed. The polymer was precipitated three times by pouring a chloroform solution into petroleum ether and isolated by freeze-drying from benzene. The combined supernatant petroleum ether solution was concentrated on a flash evaporator to dryness, and the residue dried under vacuum, weighed, and used for NMR analyses.

Mole fractions of DBCGL in copolymers and in recovered monomers were determined from integration ratios of methyl/aromatic and methyl/nonaromatic proton resonances.

Approximate reactivity ratios were obtained by the method of Fineman–Ross[45] and also by the Kelen–Tüdös methods,[26,27,46] using a computer program similar to those previously developed.[22-25,28,32] Final reactivity ratio values were determined by the integration method of Mayo–Lewis.[47]

^{13}C ($CDCl_3$): PDBCGL CH_3, 17.8, C6, 65.8; C2, C3, C4, C5, OCH_2, 71–82; Cl, 97.6; vinyl 127 and 129; aromatic C2, C3, C4, 127.4, 127.8, 128.4; aromatic C1, 139 ppm.

Decrotylation. Decrotylation of the polymers was ineffective when attempted with commercial potassium *tert*-butoxide in $(CH_3)_2$-SO–1,2-dimethoxyethane (DME) mixture at about 90 °C.[49] Reaction with freshly prepared potassium *tert*-butoxide in $(CH_3)_2SO$–DME mixture under high vacuum resulted in partial decrotylation. However, the following conditions were satisfactory for complete decrotylation. The polymers and 18-crown-6[50] (10 equiv to the crotyl group, Aldrich Chemical Co., Inc., used without further purification) were dried under high vacuum and dissolved in anhydrous benzene. Potassium *tert*-butoxide was prepared by the reaction of potassium metal (10 equiv to the crotyl group) with carefully dried *tert*-butyl alcohol on a high-vacuum line and thoroughly dried and the decrotylation reaction was carried out in a sealed tube under high vacuum in the benzene solution stirred overnight in a steam bath. After water was added, the product was extracted with chloroform, washed several times with water, and dried over anhydrous sodium sulfate. The polymer was precipitated three times in petroleum ether and freeze-dried from benzene, except II 32, which was freeze-dried from dioxane, because the solubility of this polymer was poor in benzene, yield 82–96%.

Kobayashi et al. recently reported ^1H and ^{13}C NMR spectra of poly-2,4-di-*O*-benzyl-(1→6)-α-D-glucopyranose derived from a polymer obtained by polymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-acetyl-β-D-glucopyranose.[34] ^{13}C chemical shifts of the decrotylated homopolymer (II 32) are shown below: ^{13}C ($CDCl_3$) C 6, 66.0; C 2, C3, C4, C5, OCH_2, 70–80; C1, 96.9; aromatic C2, C3, C4, 127.8–128.6; aromatic C1, 138.4 138.9 ppm.

Completeness of the reaction was confirmed by the disappearance of the methyl and vinyl proton resonances and the appearance of a newly formed hydroxyl group on C3, the proton of which resonates at 2.3–2.4 ppm. The decrotylation was confirmed to be complete by IR for the absorption at 1670 cm^{-1} due to double bond stretching disappeared and hydroxyl absorption appeared after the reaction.

Coupling Reaction. 2,3,4-Tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-α-D-glucopyranose in ether has been shown to give α-D-glucoside with high stereoselectivity.[36] In order to be able to determine by ^1H NMR how many glucose units are linked to the polymer, 2,3,4-tri-*O*-(*p*-methylbenzyl)-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-α-D-glucopyranose was prepared from 1,6-anhydro-2,3,4-tri-*O*-(*p*-methylbenzyl)-α-D-glucopyranose by the same procedures.[36] Since, as reported elsewhere,[70] the transesterification reaction of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-(*p*-methylbenzyl)-D-glucopyranose with sodium ethoxide gave a mannose derivative as a byproduct, acid hydrolysis was employed to convert 1,6-diacetate to diol.

1,6-Di-*O*-acetyl-2,3,4-tri-*O*-(*p*-methylbenzyl)-D-glucopyranose had mp 70–78 °C, $[\alpha]^{25}_D$ 42.4–43.9° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{34}H_{40}O_8$: C, 70.83; H, 6.94. Found: C, 70.80; H, 7.00.

2,3,4-Tri-*O*-(*p*-methylbenzyl)-D-glucopyranose had mp 127–130°, 129–132 °C, $[\alpha]^{25}_D$ 12.2° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{30}H_{36}O_6$: C, 73.15; H, 7.37. Found: C,73.27; H, 7.33.

1,6-Di-*O*-(*N*-phenylcarbamoyl)-2,3,4-tri-*O*-(*p*-methylbenzyl)-D-glucopyranose. The α anomer had mp 135–141, 131–137 °C, $[\alpha]^{25}_D$ 37.6° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{44}H_{46}N_2O_8$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.13; H, 6.30; N, 3.86. The β anomer had mp 191–195 °C, $[\alpha]^{25}_D$ −1.3° (*c* 1, $CHCl_3$). Anal Calcd for $C_{44}H_{46}N_2O_8$: C, 72.32; H, 6.34; N, 3.83. Found: C, 72.50; H, 6.49; N, 3.84.

Coupling reactions with methanol, cyclohexanol, and polymers were carried out under high vacuum according to the method previously described.[36,37] In the case of the polymer-coupling reaction, about 10 equiv of the monomer to the hydroxyl group in a minimum quantity of anhydrous DME was used to complete the reaction. After a workup procedure, purification of the coupled polymer was achieved by column chromatography and reprecipitation in petroleum ether. The resulting polymer was freeze-dried from benzene. α stereoselectivities were determined by ^1H and ^{13}C NMR for methanol and cyclohexanol, respectively.

Mole fractions of the branched glucose unit were calculated from the integration ratios of *p*-methyl/(aromatic + NH) and *p*-methyl/nonaromatic proton signals on all samples except III 39C in which the side chains were partly substituted with benzyl rather than *p*-methylbenzyl groups. To determine the mole fraction of branched glucose units on III 39C, the specific ellipticity of a strong band at 233 nm in the CD spectrum was plotted against mole fraction of branched units in the several samples and a calibration curve was drawn. This absorption, due to the C6 carbonyl group, was much stronger than the positive band due to TXGL (280–231 nm)[23] or TBGL units (275–230 nm), which were negligible in comparison.

In the ^{13}C NMR spectra comparison of the intensities of the side chain C6 carbon resonance and the lower field main chain C6 carbon resonance gave an approximate value for branching. ^{13}C chemical shifts of the fully coupled polymer III 32 are shown below.

^{13}C ($CDCl_3$): CH_3, 21.1; side chain C6, 64.0; main chain C6, 66.1; C2, C3, C4, C5, OCH_2, 70–83; C1, 97.5; aromatic C2, C4 on a side chain C6, 118.7 123.3; aromatic C2, C3, C4 on a main chain, aromatic C2, C3 on side chain xylyl groups, and aromatic C3 on a side chain C6, 128–130; aromatic quaternary carbons on a side chain (7 peaks), 135–138; aromatic C1 on a main chain, 139.0; C=O, 153.3 ppm.

Decarbamoylation and Debenzylation. The 6-*O*-(*N*-phenylcarbamoyl) group of the coupled polymers was removed by the reaction with sodium ethoxide in absolute ethanol on a steam bath for 3–4 h.[37] After a few drops of acetic acid was added, the solution was concentrated. The resulting syrup was dissolved in chloroform, washed with water, and dried over anhydrous magnesium sulfate. The polymer was precipitated three times in petroleum ether and freeze-dried from benzene.

After the reaction, the carbonyl absorption at 1730 cm^{-1} in the IR spectra and the CD band at 233 nm disappeared. The resonances due to the carbonyl and aromatic groups on C6 of the side chain were not detected in the ^{13}C spectra of the decarbamoylated polymers, yield 80–90%.

^{13}C chemical shifts of the decarbanilated polymer IV 32 are as follows. ^{13}C ($CDCl_3$): CH_3, 21.1; side chain C6, 61.7; main chain C6, 66.3; C2, C3, C4, C5, OCH_2, 71–83; C1, 97.6; aromatic C2, C3, C4 on a main chain and aromatic C2, C3 on a side chain, 127–130; aromatic quaternary carbons on a side chain, 135–139; aromatic C1 on a main chain, 139.1 ppm.

Debenzylation of the polymers was carried out in toluene with sodium–liquid ammonia at −33 °C according to the method previously described.[43,44,51] After washing with methylene chloride, the solution was dialyzed with water and the polymer was freeze-dried from water (or, for ^1H NMR spectra, several times from deuterium oxide on a high-vacuum line).

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Supplementary Material Available: Figure 2, number-average sequence lengths in DBCGL-TBGL copolymers, and Figure 4, 100-MHz ^{1}H NMR spectra of natural and synthetic dextrans (2 pages). Ordering information is given on any current masthead page.

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