of a compound the sample was oxidized and then duplicate weighings and counting of each product benzoic acid were carried out. The per cent rearrangements reported are the average of these duplicate weighings and countings.

Kinetics and Per Cent Rearrangement Determinations. Titrimetric rate constants were determined at 90 and 115° at 0.5 *M* 2-phenylethyl *p*-toluenesulfonate (inactive) in 99.7% acetic acid (no buffer) in sealed ampoules in a constant-temperature bath according to the method of Winstein and coworkers.[8]

For the determination of per cent rearrangement in a given compound from a reaction the sealed ampoule method (above) was used with the conditions being specified in Tables I, II, and III (the data in Tables II and III represent runs with no added buffers and an initial concentration of 0.5 *M* 2-phenylethyl-1-^{14}C *p*-toluenesulfonate in 99.7% acetic acid). Sample size was usually 22 ml of 0.5 *M* 2-phenethyl-1-^{14}C *p*-toluenesulfonate solution. After the appropriate reaction time the ampoules were removed from the constant-temperature bath, cooled in ice, and opened. The contents were diluted with ice water, and the acetic acid was neutralized with a slight excess of sodium carbonate (cold). The basic mixture was extracted with two portions of 100 ml of ether, and the ether solutions were combined, washed with water, and dried. The ether was then removed and the remaining oil was heated with 70 ml of petroleum ether. After cooling, the radioactive 2-phenylethyl *p*-toluenesulfonate which had crystallized was collected by filtration and recrystallized from petroleum ether (bp 30–60°, mp 39–40°), prior to oxidation. The petroleum ether solution (containing the radioactive 2-phenylethyl acetate) from which the tosylate originally crystallized was concentrated and the resulting oil was purified by preparative gas chromatography or by distillation through a short-path column to yield the radioactive 2-phenylethyl acetate, bp 107–108° (12 mm) (lit.[5a] bp 109° at 18 mm), which was oxidized.

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Chemical Synthesis and Structure Proof of a Stereoregular Linear Mannan, Poly-α-(1→6′)-anhydro-D-mannopyranose[1]

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Abstract: Stereoregular poly-α-(1→6′)-anhydro-D-mannopyranose has been synthesized by the phosphorus pentafluoride initiated polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-mannopyranose at −78° in methylene chloride, followed by debenzylation of the polymeric derivative with sodium in liquid ammonia. The polymer, isolated as a hemihydrate, $[\alpha]^{30}D$ 121.5–122.8° (1% in DMSO), forms cloudy solutions in water and clear solutions in dimethyl sulfoxide. On periodate oxidation, this polymer consumes the theoretical amount of oxidant and produces the theoretical amount of formic acid. After oxidation the optical rotation of both the synthetic mannan and a synthetic stereoregular poly-α-(1→6′)-anhydro-D-glucopyranose are identical. Less stereoregular mannans have been synthesized from the corresponding triacetate and after oxidation show lower positive rotations.

Of all natural polymers, only the polysaccharides must be synthesized by a propagation or stepwise coupling process under complete steric control. Recent publications from this laboratory have described the chemical synthesis of the linear backbone of a bacterial dextran,[2-4] the complete stereoregularity of which has been confirmed independently by enzymic analysis (100% α, ~98% α-(1→6′)).[5] We wish now to demonstrate that the stereoregularity of this polymerization is duplicated with the corresponding mannose derivative in spite of the different configuration of the carbon atom (C_2) adjacent to the reaction site.

The monomer, 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-mannopyranose (mp 60–61°, $[\alpha]^{25}D$ −31.2–32° (1% in chloroform)), was synthesized *via* conventional benzylation of 1,6-anhydro-β-D-mannopyranose. The latter was prepared by pyrolysis of ivory nut meal[6] and isolated as the triacetate. Polymerization was accomplished using high vacuum technique in methylene chloride at −78° and initiation with 4–25 mol % phosphorus pentafluoride to monomer and 33–66 w/v % monomer to solvent ratios. The polymers produced had intrinsic viscosities in chloroform at 25° of 0.40–2.4 dl/g, proper elementary analyses, and specific rotations of $[\alpha]^{30}D$ +56–57° (1% in chloroform). An estimate of molecular size from a viscosity–number average molecular weight relationship derived for the corresponding tribenzylglucan[7] suggests that the benzylated mannan may have been prepared at $\overline{DP}_n$ values up to ~1050 or $\overline{M}_n$ values up to 450,000.

The highest viscosities obtained were about six times those previously reported for the glucan derivative,[2,3] and were obtained by taking pains to transfer only phosphorus pentafluoride from the decomposition products of *p*-chlorobenzenediazonium hexafluoro-

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Table I. Polymerization of 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-mannopyranose (TBM) and of 1,6-Anhydro-2,3,4-tri-*O*-acetyl-β-D-mannopyranose (TAM)

No.	Monomer	Scale, g	[I] × 100/[mono]	Monomer: solvent ratio	Temp, °C	Time, hr	% yield	$[\alpha]_D$, deg	ϕ, deg	$[\eta]^{25}$, dl/g	k'
1[a]	TBM	1.0	20	33	−78	24	65	55	238	0.65	0.35
2[a]	TBM	1.0	20	33	−78	30	84	56	242	0.72	0.31
3[a]	TBM	1.5	20	33	−78	80	92	56	242	0.63	0.25
4[a]	TBM	1.5	21	33	−78	120	81	56	242	0.53	0.35
5[b]	TBM	0.5	10	50	−78	75	85	56.5	244	0.97	0.36
6[b]	TBM	1.5	16.5	40	−78	18	81	56.5	244	0.80	0.23
7[b]	TBM	1.5	20	33	−78	75	91	57.5	248	0.82	0.30
8[b]	TBM	0.8	20	40	−78	82	85	57.5	248	2.40	0.41
9[c]	TBM	1.5	20	33	−78	130	92	52	225	0.23	0.32
10[c]	TBM	1.5	22	34	−78	240	85	55	238	0.40	0.33
11[c]	TBM	0.5	4	42	−78	48	90	56	242	0.70	0.27
12[d]	TBM	1.0	10	66	−78	72	76	55	238	0.41	0.16
13[e]	TBM	0.8	25	40	−78	82	74	53.5	231	0.41	0.20
14	TAM	0.5	20	33	−78	96	0	...	...	...	...
15	TAM	0.5	20	50	−25	120	0	...	...	...	...
16	TAM	0.5	20	33	0	96	49	92.5	266.5	0.07	0.41
17	TAM	0.5	20	33	0	120	51	92	265	0.065	0.37
18	TAM	1.0	20	28	0	144	54	94	271	0.075	0.49
19	TAM	1.5	20	33	0	72	59	96	276.5	0.073	0.56
20	TAM	2	20	40	0	96	67	92	265	0.078	0.47

[a] No special care taken to transfer all catalyst. Some *p*-chlorofluorbenzene remained in catalyst tube. [b] *p*-Chlorofluorobenzene condensed in Vigreux column of catalyst tube; not transferred into polymerization ampoule. [c] All *p*-chlorofluorobenzene transferred into polymerization ampoule. [d] All *p*-chlorofluorobenzene transferred; 0.15 ml of *p*-chlorofluorobenzene added. [e] All *p*-chlorofluorobenzene transferred; 0.5 ml of *p*-chlorofluorobenzene added.

phosphate. In contrast, when our customary polymerization techniques with a 20% molar catalyst to monomer ratio are used, a large amount of liquid impurity is transferred into the polymerization ampoule. The method employed was to interpose a Vigreux column between the polymerization tube and the tube in which catalyst was decomposed. By this method most of the *p*-chlorofluorobenzene remained in the column. The larger reaction system and the possible solubility of phosphorus pentafluoride in *p*-chlorofluorobenzene also resulted in less complete transfer of the initiator. It seems probable, therefore, that either a lower initiator concentration or the absence of a chain transfer agent, or both of these factors, favor higher molecular weights. However, if *p*-chlorofluorobenzene acts as the chain transfer agent, its chain transfer constant must be low since the addition of a substantial quantity of this compound did not result in the expected drop in viscosity. Direct comparison of our present techniques suggests that the difference in molecular size of mannan and glucan may be primarily due to a greater rate of propagation in the case of the mannose derivative.

Ruckel's technique[2,4] of debenzylation with sodium in liquid ammonia at −78° and his work-up procedure of dialysis and freeze drying was modified only slightly to give 88% yields of poly-α-(1→6′)-anhydro-D-mannopyranose. This new polysaccharide has properties strikingly similar to Ruckel's stereoregular dextran model. After drying 12 hr (10^{-5} mm), it still retained one molecule of water per two anhydromannose units. *Anal.* Calcd for $(C_6H_{10}O_5)_2H_2O$: C, 42.10; H, 6.48. Found: C, 41.87–42.25; H, 6.27–6.66. It dissolves with difficulty in cold water to from opalescent solutions, the viscosities and optical rotations of which are difficult to determine. It forms clear solutions in dimethyl sulfoxide and dimethyl sulfoxide–water mixtures. In DMSO–water (68:32), these products had $[\eta]^{25}$ 0.28–0.41 dl/g. In the same solvent mixture or in pure DMSO, specific rotations were $[\alpha]^{30}D$ 121.5–122.8° (corrected for 5.55% water content of polymer). By way of contrast, the α-linked trisaccharide, Man-(1→6)-Man-(1→6)-Man, is reported to have a specific rotation in water of $[\alpha]_D$ +67°.[8] A variety of α-linked branched mannans containing 1→6′, 1→2′, and 1→3′ linkages have also been isolated from bacteria[8,9] and yeasts,[10–18] and their specific rotations range from +47 to 95°. No naturally occurring mannan appears to have been found with so high a specific rotation. (However, their optical rotations are not reported in dimethyl sulfoxide solution.)

The 60-Mc nmr spectrum of the polymeric tribenzyl ether showed the correct ratio of aliphatic and aromatic protons (13:15). Nmr spectra of the free mannan were taken in D_2O after freeze drying three times from the same solvent and showed a 1:6 ratio of anomeric (δ 4.92) to ring protons (δ_M 4.0–3.82) using DSS as internal standard. If any β linkages were present, the

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Table II. Debenzylation of Poly-α-(1 → 6′)-anhydro-2,3,4-tri-*O*-benzyl-D-mannopyranose

Mannan designation[a]	% yield	[α]D, deg cor[b]	$[\eta]^{25}$, dl/g	k'
4	88	122.8	0.315	0.19
6	89.5	121	0.412	0.31
7	90	122.5	0.401	0.28
9	60	122	...	...
10	88	121.5	0.295	0.28

[a] Mannan designation numbers correspond to polymerization number of Table I. [b] Corrected for 5.55% water content.

α-anomeric proton might have been located between δ 4 and 4.92, a region masked by the DOH peak (δ 4.62) at the usual operating temperature of 40°. At 10°, however, the DOH peak moved downfield to the region of the β-anomeric proton and no peak was observable between anomeric and ring proton regions. With deuterated DMSO as solvent and temperatures of 20–60°, the nmr spectrum gave comparable results (Figure 1). On integration, the appropriate 1:6 ratio of anomeric to ring protons was again found and no peak was observed between anomeric and ring proton regions. The details of individual polymerizations and the properties of the products obtained are outlined in Tables I and II.

At the present time it is not possible to test the structure and stereoregularity of these polymers by enzymic analysis and one is, therefore, limited to chemical methods. The simplest criterion that suggests itself is that of periodate oxidation. The oxidant consumption and formic acid liberation are accurate indicators of the structural regularity, and the specific rotation of the oxidized polymer should be identical with that of an oxidized stereoregular α-1,6-linked synthetic dextran since the different configuration of C_2 is destroyed by the oxidation. We, therefore, synthesized stereoregular poly-α-(1→6′)-anhydro-D-glucopyranose from synthetic monomer.[19] This monomer should be free of any pyrolysis impurities such as those which may have resulted in the incorporation of 2% of structural flaws in Ruckel's polymers. Polymerization conditions for 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose were similar to Ruckel's with an initiator to monomer molar ratio of 10–25% and monomer to solvent ratio of 20–33% g/ml. The polymers had intrinsic viscosities in chloroform at 25° of 0.59–0.63 and specific rotations +112–113° (1% in chloroform), as high as Ruckel's best value. Similarly the dextran had specific rotations of +197.5–198° (1% in DMSO). Samples of both polymers were oxidized at low temperature under standard conditions. The theoretical values of 2 mol of periodate consumption/mol of anhydro sugar residue and of 1 mol/mol of anhydro sugar for formic acid production were obtained within experimental error and there is no measurable difference between the specific rotation ([α]D 132.5 ± 1.5°) of oxidized glucan and mannan (Figure 2).

It is also possible to synthesize nonstereoregular 1→6′-linked polysaccharides from anhydro sugar derivatives. Hutten and Bredereck[20] have prepared polyanhydroglucoses of mixed configuration by cationic polymerization of the triacetate and tribenzyl ether of

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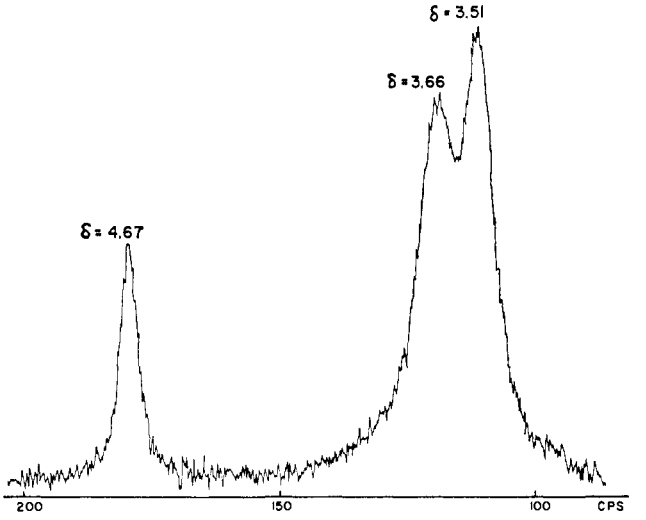

Figure 1. Expanded nmr spectrum of the synthetic *O*-deuterated mannan; solvent DMSO-d_6, 50°, sweep width 250 cps, offset 100 cps.

levoglucosan, and Zachoval[21] has made a comprehensive investigation of the factors which determine stereoregularity or irregularity at the glycosidic position. Following these methods, we have also synthesized a few 1→6′-linked mannans of mixed configuration and low molecular weight by polymerization of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-mannopyranose, and deacetylation. The details of these polymerizations and the products obtained are also described in Table I.

The 60-Mc nmr spectrum of the nonstereospecific mannan taken in D_2O after freeze drying three times from the same solvent showed a 0.85:6 to 0.7:6 ratio of anomeric (δ 5.45) to ring protons (δ 4.33–4.51) using TMS as external standard. When these polymers were oxidized with periodate under the same conditions as those used on the stereoregular polymers, the optical rotations of the products were substantially less and those polymers having the lowest specific rotation before oxidation had the lowest specific rotation after oxidation (Figure 2). The difference in optical rotation was larger for the oxidized products than for the unoxidized irregular mannans.

A stereoregular linear poly-α-(1→6′)-anhydro-D-mannopyranose of high molecular weight has, therefore, been synthesized in which no structural or configurational flaws have been discovered either with nuclear magnetic resonance or more significantly by periodate oxidation. It appears doubtful that more than 3% of a structural or configurational flaw would escape detection. These polysaccharides are being tested elsewhere for their immunological characteristics.

Other interesting approaches to the stereospecific polymerization of carbohydrate derivatives have been reported from the laboratories of Husemann[22] and Kochetkov.[23]

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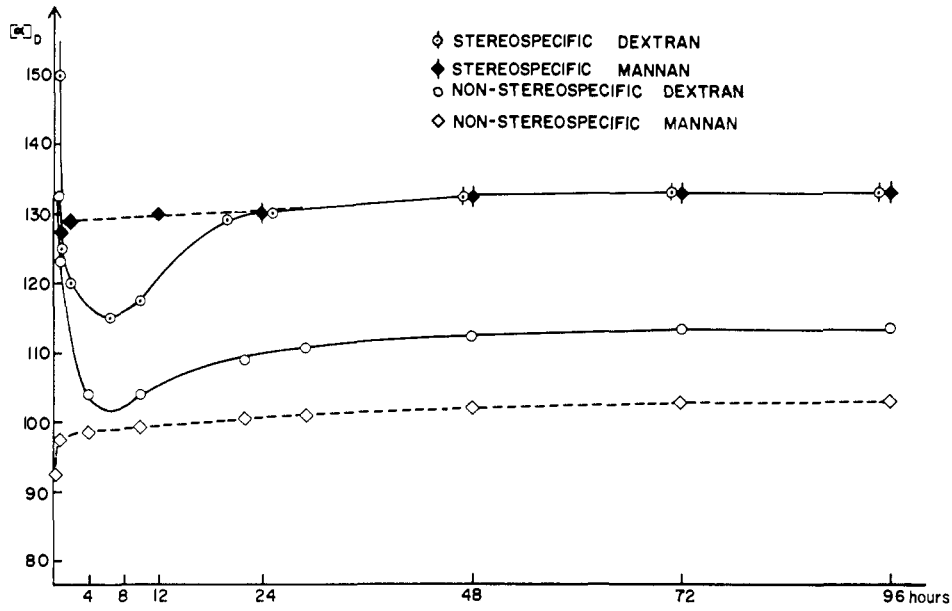

Figure 2. Change in specific rotation during oxidation of mannans and dextrans with sodium metaperiodate. (Specific rotation is based on dry weight of starting material.)

Experimental Section

Polymerizations. All polymers were prepared under high vacuum in a polymerization apparatus resembling closely that described by Ruckel[3] with the exception of the initiator ampoule which was replaced by a breakseal attached to a 15–20-cm Vigreux column. The solvent, initiator, and monomer were thoroughly dried as described by Ruckel.[3] After condensation of all solvent in the polymerization ampoule, the initiator breakseal was broken and the *p*-chlorobenzenediazonium hexafluorophosphate heated to 160°. Phosphorus pentafluoride was condensed in the polymerization ampoule while most of the *p*-chlorofluorobenzene remained in the Vigreux column. The initiator tube was then melted off at the constriction and the polymerization ampoule placed in a bath of the appropriate temperature.

For both the perbenzyl and the peracetyl monomers, polymerization was stopped at polymerization temperature by addition of an excess of methanol; the mixture was diluted with chloroform, warmed to ~10°, and neutralized with an excess of sodium bicarbonate. The organic layer was next washed with distilled water, treated with Darco, and dried over magnesium sulfate.

The polymer solution was then concentrated and the polymer precipitated by dropwise addition into 10 vol % of naphtha solvent for the tri-*O*-benzyl-substituted mannan or into 10 vol % of absolute ethanol for the tri-*O*-acetyl-substituted mannan. After reprecipitation, the polymers were freeze dried from benzene.

The details of polymerizations and results of characterization are reported in Table I.

Debenzylation of Poly-α-(1→6′)-anhydro-2,3,4-tri-*O*-benzyl-D-mannopyranose. Ruckel's technique[2,4] of debenzylation with sodium and liquid ammonia was employed. Best results were obtained at −78° using a 3:1 mixture of toluene and dimethoxyethane as a solvent. The mannans were isolated by 72–96-hr dialysis against running distilled water followed by freeze drying from the same solvent. During the dialysis a substantial amount of the polymer precipitated but both the water-insoluble and water-soluble fractions were collected and no fractionation was made prior to freeze drying. The mannans were obtained in ~88% yield. Physical constants are given in Table II.

Deacetylation of Poly-(1→6′)-anhydro-2,3,4-tri-*O*-acetyl-D-mannopyranose. To a slurry of 500 mg of polymer in 10–15 ml of methanol was added ~0.05 g of sodium. The mixture was refluxed 30 min and dialyzed 72 hr against running distilled water. The mannan solution was then concentrated *in vacuo* and freeze dried, yield 30–60%.

Periodate Oxidations. All periodate oxidations were carried out in the dark at temperatures ranging between 12 and 15° using sodium metaperiodate. About 50 mg of sample (0.3 mmol) was placed in a 100-ml volumetric flask and dissolved in 50 ml of water and 50 ml (1.2 mmol) of a periodate solution was added. At the same time a blank containing the same amount of periodate was prepared.

Oxidant consumption was determined by the method of Malaprade,[24] as described by Marder and Schuerch.[25] The formic acid liberated during the oxidation was estimated by the total acidity of the solutions according to the iodometric procedure of Halsall, Hirst, and Jones.[26]

Periodate oxidations were also carried out in 1-dm polarimetric cells kept in the dark at 5°. More concentrated (0.2–0.3%) solutions of both dextran and mannan were used. Optical rotations were measured at intervals. Final rotations for both the stereoregular mannan and dextran ranged from +131 to +134°.

Physical Methods. Optical rotations were determined in a 1-dm cell with Perkin-Elmer Model 141 polarimeter; nmr spectra were determined with a Varian A-60A analytical nuclear magnetic resonance spectrometer.

Viscosities of the peracetyl- and perbenzylmannans were determined in chloroform with an Ubbelohde dilution viscometer immersed in a constant-temperature bath at 25°.

Viscosities of the mannans were determined in a DMSO–water system at 25°. A system containing 32% water and 68% DMSO was used since its viscosity was less affected by a change in per cent humidity than pure DMSO. Specific viscosities, $[\eta]$, Huggin's viscosity constants, k', and molecular rotations, ϕ [(= $[\alpha]_D \times$ molecular weight)/100], are recorded in Table I.

Acknowledgment. The present work has been supported by Research Grant GM06168 of the Division of General Medical Sciences, National Institutes of Health.

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