5. *The Structure of Starch. The Ratio of Non-terminal to Terminal Groups.*

By F. Brown, T. G. Halsall, E. L. Hirst, and J. K. N. Jones.

An assay of the proportion of terminal groups present in various starches has been made by measuring the amount of formic acid liberated on oxidation of the polysaccharide by potassium periodate. The results obtained are in good agreement with those derived by measurement of the amount of tetramethyl methylglucoside resulting from the methanolysis of the corresponding methylated starch derivatives. Few, if any, glucose residues linked solely through carbon atoms 1 and 6 can therefore be present in starch. The amylose component of certain starches has been examined and shown to contain approximately one non-reducing end-group for every 250 glucose residues. The yield of formic acid produced on oxidation of the whole starch with potassium periodate, combined with an estimate of the amylopectin content of the starch obtained iodometrically, has enabled a calculation to be made of the ratio of terminal to non-terminal groups in the amylopectin component. For many varieties of amylopectin this ratio is 20 : 1, but there are exceptions, for example, the amylopectin component of potato, sweet potato, and arrowroot starches, where the ratio is higher.

The application to the glycogen series of Hudson and Jackson's method of oxidation of glycosides by periodic acid has been described in a previous paper (Halsall, Hirst, and Jones, *J.*, 1947, 1399). In the present communication we record results obtained in the course of work on the structural chemistry of the starches. In the first place, evidence has been sought concerning the nature of the linkages in starch. It has been established that methylglycosides of the pyranose series yield 1 mol. of formic acid on oxidation with the periodate ion, and from this it follows that in a polysaccharide molecule which has no appreciable proportion of reducing end-groups any pyranose sugar residue which is linked to other residues only through C_1 (and is therefore an end-group) or only through C_1 and C_6 (main-chain groups), will give rise to 1 mol. of formic acid. If, therefore, the proportion of end-groups determined by the methylation technique is the same as that estimated from the amount of formic acid liberated in the periodate oxidation, it is clear that the polysaccharide can contain no sugar residues linked only through C_1 and C_6. On the other hand, the excess of formic acid would give a measure of the proportion of pyranose residues linked solely through C_1 and C_6. The importance of this lies in the fact that the estimations of formic acid can be readily carried out whereas the separation and quantitative estimation of a mixture of 2 : 3 : 6-trimethyl glucose and 2 : 3 : 4-trimethyl glucose are by no means easy tasks especially when the latter sugar is present in small amount. The new method, therefore, provides a delicate test for the presence in starch and cellulose of main-chain glucose residues linked through C_1 and C_6 in place of the normal C_1 and C_4 linkages. The occurrence in starch of branched chains involving linkages at C_1, C_4, and another carbon atom, including C_6, does not involve the liberation of any additional formic acid, since no grouping of the type –CH(OH)·CH(OH)·CH(OH)– is then present in the residue concerned. In the course of the present experiments many duplicate series of end-group determinations have been performed by both the methylation method and the periodate method, and in no case has any significant discrepancy been observed between the two sets of values. It follows, therefore, that in these starches there can be no appreciable occurrence of glucose residues linked only through C_1 and C_6.

Another problem which has been investigated is concerned with the size of the repeating unit in the amylopectin portion of the various starches. Information about this is required in order to decide whether or not the amylopectins of different starches possess the same type of structure, and whether samples of the same starch, differing in maturity or in respect of the botanical variety of the plant from which it was derived, have similar proportions of end group. The average size of the repeating units in some amylopectins can be estimated directly, both by the methylation technique and by the periodate method. These include waxy maize starch, which contains a negligible proportion of the amylose component, and amylopectins which have been isolated from the starch granule and separated from the amylose component by one of the

processes recently devised for this purpose (see, for example, Schoch, "Advances in Carbohydrate Chemistry", 1945, **1**, 347; Baldwin, *J. Amer. Chem. Soc.*, 1930, **52**, 2907; Whistler and Hilbert, *ibid.*, 1945, **67**, 1161; Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19; Higginbotham and Morrison, *Chem. and Ind.*, 1947, 45). Unfortunately, the separation of pure amylopectin is at best a lengthy and difficult process, and we record results in this paper only for one such purified amylopectin, which was very kindly put at our disposal by Mr. R. S. Higginbotham of the British Cotton Industry Research Association. Estimates of the average sizes of the repeating units of other amylopectins were arrived at indirectly by the following method. First, the relative proportions of amylose and amylopectin present in whole starch were estimated by the iodine-binding power method of Bates, Rundle, and French (*J. Amer. Chem. Soc.*, 1943, **65**, 142). The proportion of end-group in the whole starch was then determined by periodate titration. The amount of formic acid liberated by the long unbranched molecule of amylose, even after allowing for oxidation at a possible reducing end-group, is small in comparison with that given by the numerous end-groups in amylopectin, and to a first approximation it may be neglected. It is possible, therefore, to calculate the proportion of end-groups present in the amylopectin component. A similar calculation can be made using the figure for the proportion of end-groups estimated by the methylation method. In this case the influence of the amylose content of the whole starch on the final result is still less, and it is significant that the end-group figures for amylopectins so obtained agree within the limits of experimental error with those derived from the periodate oxidation experiments.

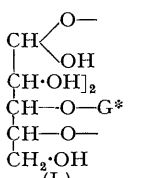
Source of starch.	Amylose content, %.	Wt. of starch oxidised, mg.	Yield of formic acid, mg.	Average no. of glucose residues per non-reducing end-group: (*a*) By periodate.	(*b*) By methylation.	Calc. no. of glucose residues per non-reducing end-group in the amylopectin fraction.
			Starches.			
1. Arrowroot	20	497	4·6	31	—	25
2. Banana	21	494	5·2	27	26 [1]	21
3. Maize	23	491	5·6	25	—	20
4. Pearl manioc ...	16	1002	11·9	24	—	20
5. Potato	18	1003	9·1	31	25, 28	24—26
6. Rice (I)	14	529	6·5	23	—	20
7. Rice (II)	15	485·5	6·0	24	25, 28	21
8. Rice (III) [2, 3] ...	—	—	—	—	30 [2]	—
9. Sago	26	1004	11·9	24	—	18
10. Sweet potato ...	18	512	4·5	32	28, 34	26
11. Tapioca	19	988	11·2	25	—	20
12. Wheat	19	1036	11·3	26	—	21
			Amylopectins.			
13. Waxy maize starch	0—1	1036	14·7	20	18	20
14. Potato amylopectin	0—1	514·5	6·1	24	—	24
			Amyloses.			
15. Sago amylose ...	100	—	—	—	250±100	—
16. Potato amylose (I)	90	2544	7·2	500±100 (for amylose component)	—	—
17. Potato amylose (II)	80	—	—	—	200±100 (for amylose component)	—

Samples 1, 3, 4, 5, 7, 9, 11, and 12 were commercial samples kindly supplied by Messrs. Elliott & Crabtree Ltd., Manchester.
Sample 2 was prepared by Hawkins, Jones, and Young (*J.*, 1940, 390).
Samples 6 and 8 were commercial samples from British Drug Houses Ltd.
Sample 10 was a commercial sample kindly supplied by The Laurel Starch Plant, Laurel, Miss., U.S.A.
Samples 14 and 15 were kindly supplied by Mr. R. S. Higginbotham of the British Cotton Industry Research Association.
Sample 16 was kindly supplied by Dr. Peat of Birmingham University and was separated by the "thymol" method (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19).
Sample 17 was prepared by the *n*-butanol–*iso*amyl alcohol precipitation method of Schoch.

[1] Hawkins, Jones, and Young (*J.*, 1940, 390).
[2] Hirst and Young (*J.*, 1939, 1471).
[3] Bell (*J.*, 1946, 476) has found one non-reducing end-group per 26—28 glucose residues in a sample of methylated rice starch.

The results are collected in the Table, from which it is evident that the amylopectin component of several starches has a repeating unit of average chain-length of 20 glucose residues. The evidence as it stands, however, does not warrant generalisations regarding amylopectins from different sources. It will be seen that some of the starches examined gave rather higher figures for the average size of the repeating unit of their amylopectin component, and it is unlikely that the discrepancies can be attributed entirely to experimental error. Included amongst these are the amylopectins from arrowroot (25 units), potato (24—26), and sweet potato (26). Furthermore, a highly purified sample of potato amylopectin prepared by Mr. R. S. Higginbotham gave directly, by the periodate method, a value of 24 units. Rice starch presents a further problem. We have examined two separate samples by both methods of assay and find for the whole starch 23—24 glucose residues (cf. Bell, *J.*, 1944, 473) per end-group, giving for the amylopectin component a value of 20 residues per end-group. On the other hand, another sample which we had examined previously (Hirst and Young, *J.*, 1939, 1471) gave for the whole starch 30—32 residues per end-group. In view of the divergence we re-investigated the original sample of the methylated derivatives of this starch and confirmed the earlier results (30—32). It is possible, therefore, that starches may vary from sample to sample in respect of either the proportions of amylose and amylopectin or the actual size of the repeating units, or both, the variation being dependent upon the maturity or the precise botanical origin of the starch. This introduces a serious additional problem into starch chemistry, some insight into which we are attempting to gain by experiments carried out in collaboration with Dr. F. W. Sansome of the Horticultural Research Station of Manchester University. The exact nature of the relationship in the starch granule of the amylose and amylopectin components is not yet clear, and the possibility cannot be ruled out that forms intermediate between the two may exist. Nevertheless, it is clear that all starches which have been examined up to the present give values within the range of 20—26 glucose residues per end-group for their amylopectin components. The gap is not a wide one, and further experiments may be expected to reveal whether or not this implies the occurrence of a variety of amylopectins in Nature.

The present results show also that the methylation method and the periodate method yield end-group values substantially in agreement with one another and thus rule out the hemi-acetal type of linkages proposed by Pacsu and Hiller (*Text. Res. J.*, 1946, **16**, 243) in their novel formulæ for starch and cellulose. Pacsu and Hiller's structure involves essentially long chains of 1 : 4-linked α-glucopyranose residues joined together by groups of the type shown diagrammatically in (I), the number of these being such that the end-group proportions as determined by the methylation process can be accommodated. The structure, however, provides additional points of attack for periodate ions, and additional quantities of formic acid would be liberated per end-group. The detailed formula pictured by these authors would, indeed, necessitate a yield of formic acid 100% or more greater than that required by a structure of the laminated type, yet in our experiments we have invariably found agreement between the end-group values estimated by the two methods. For these and other reasons which have been briefly indicated elsewhere (Halsall, Hirst, and Jones, *Nature*, 1947, **159**, 97) we regard it as very unlikely that hemi-acetal linkages of this type find any appreciable place in the structure of starch.



(I.)

(G* = A terminal glucose residue.)

The experimental error in the values given above for the average size of the repeating unit of the amylopectins is of the order ± 2 glucose residues, and the figure is not materially affected by the presence of some amylose, for which suitable correction can easily be made. It is much more difficult, however, to assess accurately the proportion of end-groups in amylose itself. In the first place, the very small proportion of end-group in the molecule renders exact analyses difficult by any method, but the uncertainties are much increased if corrections have to be made for amylopectin present as impurity, since in this case a very small proportion of amylopectin provides end-groups equal in amount to those in the amylose. Even when the proportions of amylose and amylopectin in the sample are known accurately—no easy matter in itself—assay by the methylation process would be liable to error should the preparation and purification of the methylated derivative involve even a slight change in the relative proportions of the two components. The periodate method avoids the last difficulty but has others of its own. It is not known with certainty whether or not the amylose molecule possesses a reducing end-group in addition to the non-reducing end-group, and when working with a highly purified amylose from sago we met an unexpected difficulty in that we failed to dissolve the amylose in the periodate solution, presumably owing to retrogradation of the polysaccharide. For these reasons the figures now given can be regarded only as approximate, but they do nevertheless

show that the molecular size of amylose is large, probably involving some hundreds of glucose residues. Similar high figures have been recorded previously by Haworth, Heath, and Peat (*J.*, 1942, 55), by Myrbäck (*J. pr. Chem.*, 1943, **162**, 29) and by Hassid, Cori, and McCready (*J. Biol. Chem.*, 1943, **148**, 89) for synthetic amylose prepared by Hanes's method, and for potato amylose and the amylose from maize starch (Meyer, Westheim, and Bernfeld, *Helv. Chim. Acta*, 1941, **24**, 378). A direct determination of the tetramethyl glucose obtained on hydrolysis of the methylated derivative of a specially pure (99%) sample of sago amylose, prepared by Mr. R. S. Higginbotham, indicated a chain length of 200 (± 100) glucose residues. Another sample of amylose was prepared by Schoch's method from potato starch (*J. Amer. Chem. Soc.*, 1942, **64**, 2954). This contained some 20% of amylopectin as estimated by the iodine-binding power (Bates, Rundle, and French, *loc. cit.*). It dispersed more readily in water, and the amount of tetramethyl glucose liberated on hydrolysis of the methylated derivative indicated a chain length of 250 (± 100) glucose residues. Approximately the same figure was obtained by the periodate method for a sample of potato amylose prepared by the thymol process of Haworth, Peat, and Sagrott, to whom we are indebted for a gift of this material.

Experimental.

Potato Starch.—The potato starch used was a commercial sample. Iodometric titration (Bates, Rundle, and French, *loc. cit.*; Hudson, Schoch, and Wilson, *J. Amer. Chem. Soc.*, 1943, **65**, 1380) showed the presence of 18% of amylose. In the calculation of the amylose content the uptake of iodine by pure amylose was taken as 21·5 g. per 100 g. of amylose (Higginbotham and Morrison, *loc. cit.*).

Determination of percentage of non-reducing terminal glucose residues by the periodate method. (The following details are typical for all the oxidations carried out, cf. Halsall, Hirst, and Jones, *loc. cit.*)

The starch (1003 mg.) was suspended in water (90 ml.) and potassium chloride (5 g.), and a solution of sodium metaperiodate (30 ml.; 0·275M) added. (For details of purity of reagents see Halsall, Hirst, and Jones, *J.*, 1947, 1427). The mixture was shaken, and at intervals samples (20 ml., free from starch granules) were withdrawn. Excess of ethylene glycol was added and the solution titrated with 0·1N-barium hydroxide [Found, in ml. of 0·01N-barium hydroxide per 1003 mg. of starch: 19·3 (168 hours); 20·0 (192 hours); 21·3 (264 hours), and 21·9 (336 hours)]. As the titration proceeded a concentration of starch occurred in residual aqueous mixture, and corrections are therefore necessary for each of the last three titrations. These titres correspond to the formation of 9·06 mg. formic acid after 150 hours' oxidation—the time required to oxidise completely β-methylmaltoside under these conditions (see Halsall, Hirst, and Jones, *loc. cit.*). This corresponds to the presence of one non-reducing terminal residue per 31 glucose residues.

Potato starch (504 mg.) was oxidised as above. After 191 hours' oxidation the uptake of periodate was estimated by the arsenite method (Found: One glucose residue took up 1·03 mol. of periodate). Potato starch (500·5 mg.) was oxidised as above. After oxidation (191 hours) excess of ethylene glycol was added to the reaction solution which was then continuously extracted with ether. The acid extracted was equivalent to 9·80 ml. of 0·01N-sodium hydroxide. This figure agrees well with that obtained by direct titration after 192 hours' oxidation in the first experiment described above.

An attempt was made to determine the amount of formic acid in the neutralised ether extract by the calomel method, but the yield of calomel was only about one-third of that expected. Pirie (*Biochem. J.*, 1946, **40**, 100) in a discussion of methods for the determination of small amounts of formic acid has pointed out that the calomel method gives low results with small quantities of formic acid.

Methylated potato starch was prepared in the usual manner in an atmosphere of nitrogen (Hirst and Young, *loc. cit.*). The methylated starch (3·16 g., OMe 42·6%) was boiled with methyl-alcoholic hydrogen chloride under the usual conditions; the solution was then neutralised with N-sodium hydroxide and the glucosides were isolated in the usual manner. The tetramethyl methyl-*d*-glucoside was concentrated by a preliminary extraction of the mixed glucosides from aqueous solution with purified light petroleum (b. p. 40—60°). The resulting syrup was dissolved in purified light petroleum (b. p. 60—80°) and chromatographed on active alumina by the method of Brown and Jones (*J.*, 1947, 1344), giving tetramethyl methyl-*d*-glucoside (150 mg., $n_D^{16°}$ 1·4440). This yield of end-group corresponds to one non-reducing terminal residue per 28 glucose residues (assuming a recovery of 93% of " end-group ").

In a second experiment the methylated starch (0·864 g., OMe, 42·6%) was converted into a mixture of glucosides by boiling with methyl-alcoholic hydrogen chloride. The solution was neutralised with diazomethane and immediately concentrated under reduced pressure to the syrupy mixture of glucosides. This material was dissolved in water (50 ml.) and extracted continuously with purified light petroleum (b. p. *ca.* 40°) in the usual manner {Found: tetramethyl methyl-*d*-glucoside, 38 mg., n_D^{16} 1·4430, $[\alpha]_D^{20°}$ + 39° (in water), OMe, 61·2%}. This yield corresponds to one non-terminal reducing per 28 glucose residues.

Potato Amylose.—A sample of potato amylose (amylopectin content 20%) prepared by the butanol method of Schoch from the potato starch used for methylation and oxidative studies was methylated and the product isolated in the usual manner (yield 80%). It was assumed that during the methylation the proportions of amylose and amylopectin did not alter owing to preferential experimental loss of one component.

The methylated derivative (4·0 g., OMe, 44%) was converted into the methylglucosides by boiling it under reflux with methyl-alcoholic hydrogen chloride. The resultant mixed glucosides were separated by extraction and counter-extraction with light petroleum (b. p. 40—60°) (Found: tetramethyl methyl-*d*-glucoside, 66 mg., $n_D^{16°}$ 1·4443, $[\alpha]_D^{20°}$ + 79°, OMe, 61%). This material on hydrolysis with 2N-hydrochloric acid gave crystalline 2 : 3 : 4 : 6-tetramethyl *d*-glucose.

If it is assumed that the amylopectin fraction (20%) of the polysaccharide mixture has an average repeating unit of 24 glucose residues, it can be calculated that the amylose component contains one non-reducing terminal residue per 200 ± 100 glucose residues.

A second sample of potato amylose separated from a different sample of potato starch by the thymol method by Haworth, Peat, and Sagrott (*Nature*, 1946, **157**, 19) (amylose content 90%) on oxidation with aqueous potassium periodate solution gave a yield of formic acid corresponding to the presence of one non-reducing terminal residue per 500 ± 100 glucose residues after allowing for formic acid produced by the amylopectin fraction (10%) and by the reducing end of the amylose molecule.

Rice Starch.—This was a commercial sample and contained 15% of amylose. Oxidation of the starch with potassium periodate solution under standard conditions gave a yield of formic acid corresponding to the presence of one non-reducing terminal group per 24 ± 2 glucose residues.

The starch was converted into the methylated derivative by the method of Hirst and Young (*loc. cit.*). The product was purified by extraction with boiling water and by precipitation from chloroform with ether (OMe, 44%). A portion of the methylated derivative (3·66 g.) was dissolved in methyl-alcoholic hydrogen chloride and boiled under reflux until hydrolysis was complete. The glucosides were isolated in the usual manner and the fully methylated derivative was concentrated by extraction from aqueous solution with light petroleum (b. p. 40—60°). The resultant syrupy glycosides were then chromatographed on alumina (Found: tetramethyl methyl-*d*-glucoside, 174 mg., $n_D^{16°}$ 1·4440). This corresponds to the presence of one non-reducing terminal residue per 25 ± 2 glucose residues.

In a second experiment, the methylated derivative (2·03 g., OMe, 44·3%) on methanolysis gave a yield of methylglucosides (2·07 g.) which on partition extraction yielded tetramethyl methyl-*d*-glucoside {72 mg., $n_D^{16°}$ 1·4440, $[\alpha]_D^{20}$ + 81° (in water)} corresponding to one non-reducing terminal per 28 ± 2 glucose residues.

Methylated "disaggregated" rice starch (part of Fraction 3, Hirst and Young, *J.*, 1939, 1471) (4·11 g.) was hydrolysed with boiling 1% methyl-alcoholic hydrogen chloride, and the methanolysis products isolated after neutralisation with silver carbonate. The tetramethyl methyl-*d*-glucoside was concentrated by extraction from aqueous solution with light petroleum, and the concentrate chromatographed on alumina (Found: tetramethyl methyl-*d*-glucoside, 163 mg., $n_D^{16°}$ 1·4455). This corresponds to one non-reducing terminal residue per 29 ± 2 glucose residues.

The methylated "disaggregated" starch (3·145 g.) was converted into the glucosides (3·642 g.) and the tetramethyl methyl-*d*-glucoside isolated by preferential extraction with light petroleum (b. p. 40—60°) {Found: tetramethyl methyl-*d*-glucoside, 116 mg., $n_D^{16°}$ 1·4441, $[\alpha]_D^{20°}$ + 80° (in water)}. This corresponds to one non-reducing terminal group per 33 ± 2 units. On hydrolysis with boiling 2N-hydrochloric acid the glucoside gave crystalline 2 : 3 : 4 : 6-tetramethyl *d*-glucose, m. p. 81°.

Amioca "Waxy" Maize Starch.—This work was made possible by gifts of starch from the British Cotton Industry Research Association and from Dr. Fred Smith of the University of Birmingham. This starch was coloured blue by the first drop of iodine and then red by any further addition. It contained a very small amount (*ca.* 1%) of amylose. Oxidation of the starch with potassium periodate solution under standard conditions gave formic acid corresponding to one non-reducing terminal group per 20 glucose residues.

The starch was methylated in a nitrogen atmosphere by portionwise addition of sodium hydroxide (30%) and methyl sulphate to its solution in 30% sodium hydroxide. Yield, 80% (after 4 methylations).

The methylated derivative (3·39 g., OMe, 44%) was boiled with methyl-alcoholic hydrogen chloride and the glucoside isolated in the usual manner. The tetramethyl methyl-*d*-glucoside was concentrated by extraction from water with light petroleum (b. p. 40—60°), and the concentrate chromatographed on alumina (Found: tetramethyl methyl-*d*-glucoside, 215 mg., $n_D^{16°}$ 1·4438, OMe, 61·4%). This corresponds to one non-reducing terminal group per 18 ± 2 glucose residues.

Sago Amylose.—This material was prepared by the British Cotton Industry Research Association, and potentiometric titrations indicated that it contained 99—100% of amylose (iodine-binding power 21·6). This amylose was methylated in the usual manner, and a practically quantitative yield (2·29 g.) of the methylated derivative was isolated {$[\alpha]_D^{20°}$ + 200° (in chloroform), OMe, 44%}. The properties of this substance were very similar to those of a methylated slightly degraded cellulose.

The methylated amylose (2·19 g.) was submitted to methanolysis and the resultant mixed glucosides were fractionated by extraction from water with purified light petroleum (b. p. 40—60°). Eventually, a fraction (35 mg., $n_D^{16°}$ 1·4531) was isolated. This fraction could not be further separated owing to the experimental difficulties involved. It was calculated, however, from the n_D values that it contained approximately 12 mg. of tetramethyl methyl-*d*-glucoside, corresponding to one non-reducing terminal group of about 250 ± 100 glucose residues.

Sweet Potato Starch.—The sweet potato starch was provided by The Laurel Starch Plant, Laurel, Miss., U.S.A., to whom our best thanks are due. The starch was a white powder very similar in general properties to potato starch. Iodometric titration showed the presence of some 18% of amylose.

The starch (50 g.) was methylated in an atmosphere of nitrogen (Hirst and Young, *loc. cit.*). After 5 methylations the crude dry product (40 g.), which had been purified by boiling with water and then with ether, was dissolved in chloroform and fractionated by the portionwise addition of light petroleum (b. p. 40—60°). Four fractions were obtained. Fraction (I), 4·2 g. (OMe, 43%); Fraction (II), 18·7 g. (OMe, 44·2%); Fraction (III), 12·1 g. (OMe, 44·3%); and Fraction (IV), 1·8 g. (OMe, 44·1%). These fractions were grey in colour and on standing became insoluble in solvents, swelling only in chloroform. For these reasons observations of optical activity were not possible. Fraction (II) (6·38 g.) was hydrolysed with methyl-alcoholic hydrogen chloride and the mixed glucosides (6·5 g.) were isolated after neutralisation with silver carbonate followed by filtration and removal of the solvent under reduced pressure. The syrup was dissolved in water (50 c.c.) and all the tetramethyl methyl-*d*-glucoside and some trimethyl methyl-*d*-glucoside were separated by continuous extraction for 6 hours with purified light petroleum (b. p. 40—60°). The syrupy mixture was dissolved in light petroleum (b. p. 60—80°) and chromatographed on active alumina, giving tetramethyl methyl-*d*-glucoside (259 mg., n_D^{16} 1·4440,

OMe, 61·2%). This corresponds to an average repeating unit of 28 ± 2 glucose residues (assuming a recovery of 93% of end-group).

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