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Cycloheptaamylose as a model for starch in the pyrolysis of polysaccharides*

Todd L. Lowary and Geoffrey N. Richards†
Wood Chemistry Laboratory, University of Montana, Missoula MT 59812 (U.S.A.)

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ABSTRACT

The pyrolysis of cycloheptaamylose has been studied as a model for starch. 1,6-Anhydro-β-D-glucopyranose (levoglucosan, LG, **7**) and its furanose isomer are major products from vacuum pyrolysis at 280, 300, and 320°, with combined yields ranging from 38 to 50% of the substrate dependent on temperature. Pyrolysis in methyl sulfoxide at 150° produced LG and glucose as well as gluco-oligosaccharides of d.p. up to 7, with both reducing and 1,6-anhydro end-groups. A mechanism is postulated in which the first step is the heterolytic scission of a glucosidic linkage to form a linear, seven-membered oligosaccharide having a glucosyl cation in place of the reducing end-group. The cation is stabilized either by intramolecular attack of O-6 on the C-1 cation or by intermolecular transglycosylation. The former product subsequently yields LG upon scission of a terminal glucosidic linkage.

INTRODUCTION

The pyrolysis or thermal degradation of polysaccharides has been extensively studied and comprehensively reviewed[1]. However, while a great deal is known about the nature and yields of the pyrolysis products of polysaccharides, there are still no exact conclusions concerning chemical mechanisms, except in the specific case of (1→3)-glycopyranans[2].

Our investigations are directed toward determining, unambiguously, the mechanisms of polysaccharide pyrolysis[3,4]. We recently[5] proposed that the heterolytic scission of a glucosidic linkage, analogous to acid hydrolysis, is the initial step in the pyrolysis of pure cellulose. This initial scission results in the formation of two shorter cellulose chains, one with a short-lived resonance-stabilized glucosyl cation in place of a 'normal' reducing end-group. The glucosyl cation gives rise, *via* intramolecular nucleophilic attack by O-6, to a 1,6-anhydro-β-D-glucopyranose (levoglucosan, LG) end-group. The predominant final product, LG, is liberated by any subsequent scission of the terminal glucosidic linkage and, due to its relatively high volatility, is able to leave the pyrolysis site. In this mechanism the glucosyl cation effectively enters a 'stability sink' by forming the 1,6-anhydride at the chain end. This 'sink' is postulated as the key to high-yield pyrolysis (such as 60% of LG from cellulose).

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† Corresponding author.

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Interest in the formation of pyrodextrins (British gums) has led to the study of low-temperature pyrolyses of amylose[6-8] and starch[9-11]. The chemical structure of these pyrodextrins has been elucidated independently in several laboratories[6-8,12]. Of particular interest is the work of Thompson and Wolfrom[7,8] which showed, in addition to an increase in chain branching and a decrease in d.p., that LG end-groups were present in a pyrodextrin from amylose. These end-groups were formed, they proposed, by the attack on the C-1 carbon by O-6 in a concerted reaction, resulting in chain scission.

In the light of Thompson and Wolfrom's experiments and in order to test our hypothesis outlined here, we now describe an investigation of the pyrolysis of cycloheptaamylose (**1**, in protonated form). We consider this cyclic oligosaccharide to be an appropriate model compound for the study of starch pyrolysis. In addition to having the desired stereochemistry, **1** has the advantage (over the starch polysaccharides amylose and amylopectin) that the postulated initial reaction-product (a seven-membered linear oligosaccharide having an LG end-group, **4**) may be readily analyzed by l.c. Furthermore, the cyclic oligosaccharide has no potential aldehyde end-group, and hence we eliminate the relatively facile and complex pyrolytic reactions which originate from that functionality, but which have little relevance to the major reaction channels in polysaccharide pyrolysis. It has previously been shown that **1** yields LG and 1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose on pyrolysis[13].

RESULTS AND DISCUSSION

Thermogravimetry in nitrogen at 10°/min from 110 to 440° showed a single derivative weight-loss peak at 333° with weight loss commencing (2%) at 309°. In pyrolyzing the cycloheptaamylose therefore, relatively low-temperature conditions under vacuum were used in order to gain maximum insight concerning the pyrolysis mechanisms. This is in contrast to the alternative approach of using higher temperatures or flash pyrolysis in order to obtain maximum yields of pyrolysis oils.

Table I shows that for untreated **1** an increase in pyrolysis temperature results in an increase of total volatiles (pyrolyzates I and II) and a decrease in carbonaceous char formation. This result can be explained in terms of more rapid formation and removal of volatile products at elevated temperatures, thereby precluding the complex, nonspecific dehydration and recombination reactions which lead to char formation in the pyrolysis zone.

Major components of pyrolyzate **I** (*i.e.*, room temperature condensates), are shown in Table II together with g.l.c. retention times and relative detector response factors of the tri-*O*-methylsilylated (Me_3Si) components using glucitol as internal standard. Only LG and its furanose isomer were detected by Me_3Si–g.l.c. in the pyrolyzate **I** fraction, the combined yield ranging from 58 to 75% of the pyrolyzate. Obviously, therefore, some major components of the pyrolyzate are not detected by g.l.c. of Me_3Si ethers, presumably because they do not pass through the column. There is little doubt that much of the material not accounted for consists of oligosaccharides with LG end-groups. Thus Pouwels and coworkers[14] have detected such oligomers up to

d.p. 6 in volatiles from cellulose pyrolysis. The lower yield of levoglucosan from the 280° pyrolysis appears to be associated with a relative increase in contribution of dehydration reactions leading to char.

The formation of levoglucosan (**7**) may be envisaged as the result of an initial scission of the ring to form a seven-membered open-chain oligosaccharide having the reducing end-group replaced by a glucosyl cation (**2**). Stabilization of this cation occurs by intramolecular addition of O-6 to the C-1 carbocation to yield **4**. The LG is liberated upon any subsequent scission of its adjacent glucosidic linkage, simultaneously producing another glucosyl cation (**5**). LG is also likely to be produced by scission of the glycosidic linkage of a 'normal' non-reducing end-group to liberate another glucosyl cation (**6**) with subsequent addition of O-6 to C-1. This latter channel for conversion of terminal glycose units to the 1,6-anhydride is analogous to the known facile pyrolysis of pendant arabinofuranose units of polysaccharides to produce the anhydroarabinose[15]. Furthermore, after the initial, ring-opening scission, mid-chain scission can occur producing oligosaccharides of lower d.p., some of which may be sufficiently volatile to enter the distillate. The mechanism whereby the furanose isomer is formed requires further investigation. Presumably it requires an unsubstituted C-4 hydroxyl group, but it seems to us unlikely that LG itself will isomerize after formation, since in our system it distils away very rapidly from the heated zone.

Effect of sodium chloride. — In previous studies of cellulose pyrolysis[5] it has been shown that 'neutral' salts have a dramatic effect upon the nature and amount of pyrolysis products, even at extremely low levels. For example, in vacuum pyrolysis, the addition of 0.05% sodium chloride to cellulose decreased the yield of LG from 55 to 9% and increased the glycolaldehyde ('hydroxyacetaldehyde') yield from <0.1 to 4.0%.

Similar effects were observed when sodium chloride-treated cycloheptaamylose was pyrolyzed, as shown in Tables I–III. The addition of 1% sodium chloride to the substrate decreased the pyrolyzate **I** yield fourfold and increased the pyrolyzate **II** yield by over three times, while only slightly increasing the char yield. The proportion of pyrolyzate **I** accounted for by Me_3Si–g.l.c. also decreased dramatically, by nearly threefold. In addition to a decrease in LG yield, the production of trihydroxybenzenes is also seen. We have previously reported[16] a similar observation with cellulose, where the

TABLE I

Yields of products from pyrolysis of cycloheptaamylose at 266 Pa

Pyrolysis				
Temp.	*Time (min)*	**I**[a]	**II**[b]	*Char (%)*
280	120	65.5	11.7	21.5
300	60	65.1	12.6	15.8
320	30	66.0	18.9	11.9
300[c]	60	16.7	46.4	17.5

[a] Room temperature condensates. [b] Condensates at −80°. [c] Sodium chloride (1%) added.

TABLE II

Major components of pyrolysate I from pyrolysis at 266 Pa.

Pyrolysis		*% Yield (% Pyrolyzate I)*			*Pyrolyzate I accounted for (%)*
temp.	*time (min)*	*Levoglucosan*[a]	*1,2,3-THB*[b]	*1,2,4-THB*[c]	
280°	120	38.1 (58.1)	[d]	[d]	58.1
300°	60	48.2 (74.0)	[d]	[d]	74.0
320°	30	49.9 (75.4)	[d]	[d]	75.4
300°	60[e]	1.8 (10.8)	0.4 (2.5)	1.1 (6.4)	19.7
Relative response factor[f]		0.72	0.98	0.98	
G.l.c. retention time (min)		10.3	7.4	8.4	

[a] Combined pyranose and furanose isomers. [b] 1,2,3-Trihydroxybenzene. [c] 1,2,4-Trihydroxybenzene [d] None detected. [e] Sodium chloride (1%) added. [f] Relative to glucitol (r.t. = 14.2 min) as internal standard.

TABLE III

Major components (other than water) of pyrolyzate II from pyrolysis at 266 Pa

Pyrolysis		*% Yield*			
temp	*time (min)*	*Glycolaldehyde*	*Hydroxyacetone*	*Acetic acid*	*Formic acid*
280°	120	<0.2	<0.1	<0.1	<0.1
300°	60	3.1	<0.2	0.3	<0.3
320°	30	2.1	<0.1	<0.1	<0.3
300°	60[a]	12.8	1.6	3.7	1.3

[a] Sodium chloride (1%) added.

addition of sodium chloride resulted in a 0.5% yield of 1,2,4-trihydroxybenzene and a 0.4% yield of 1,2,3-trihydroxybenzene, whereas neither were detected in untreated cellulose. While no definite mechanisms are known for formation of these products, some speculations exist[16].

Table III indicates a substantial increase in the production of low-molecular-weight volatiles upon treatment with sodium chloride. Most notable is the fourfold increase of glycolaldehyde. Earlier study[17] on the ^{1}H-n.m.r. spectra of glycolaldehyde showed that, in an equilibrated deuterium oxide solution, 70% of the glycolaldehyde is in the hydrated *gem*-diol form of the monomer. Our own study of an equilibrated solution in deuterium indicated that 78% of the glycolaldehyde existed as the hydrated monomer. The peaks (δ 3.44 and 3.50 p.p.m.) used for quantification were those corresponding to this species, and therefore the values in Table III have been corrected to account for the 21.8% of the glycolaldehyde present in forms other than the hydrate. While no mechanism for glycoaldehyde formation in pyrolysis of polysaccharides has been established, some hypotheses have been advanced[18,19].

Pyrolysis in methyl sulfoxide. — In order to obtain a more-detailed understanding

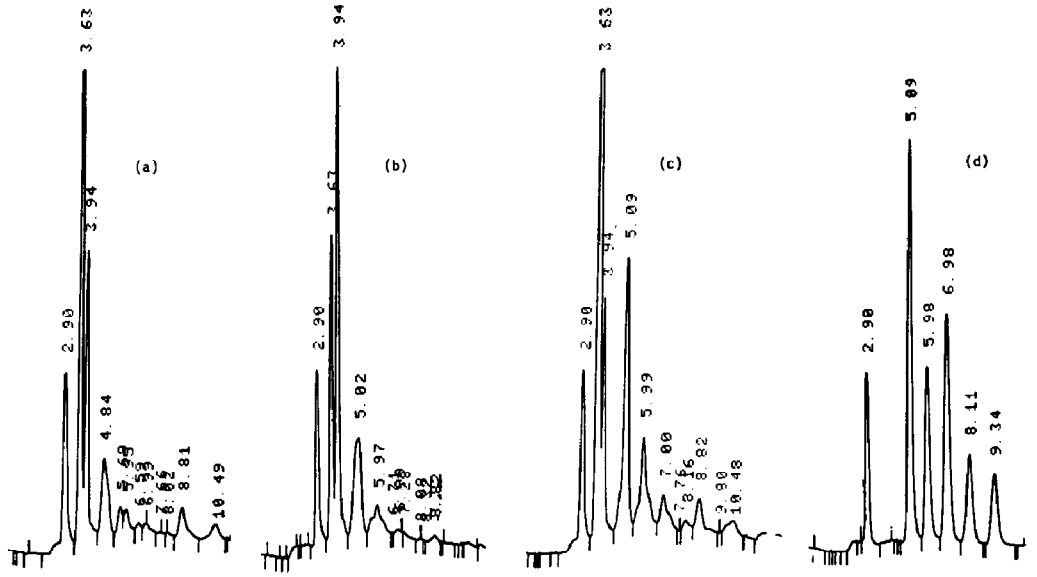


Fig. 1. L.c. of products from heating cycloheptaamylose in methyl sulfoxide at 150°: (*a*) heated for 2 h; (*b*) 10mM sulfuric acid, heated 1 h; (*c*) 5% water added, heated 2 h; (*d*) reference solution of glucose and malto-oligosaccharides, d.p. 2–5.

of the initial steps in the pyrolytic reaction, we investigated the reaction at 150° in dry methyl sulfoxide. Fig. 1 shows l.c. traces of pyrolysis reaction-mixes under various conditions as well as a standard mix of glucose and malto-oligosaccharides having d.p. 2–5. Levoglucosan ran at 3.94 min, while the peaks at 2.90, 3.63, and 8.81 min are water (solvent), methyl sulfoxide and cycloheptaamylose, respectively. Comparing Fig. 1a and 1b, it is evident that the addition of acid accelerates both the reactions leading to LG and the loss of starting material. The acid no doubt acts as a catalyst by protonating a glucosidic oxygen atom (**1**) and thus facilitating the initial rate-determining step, namely the scission of a glucosidic bond.

Previous study[20] on the effect of γ-radiation on an aqueous solution of cycloheptaamylose showed that the substrate was converted into linear malto-oligosaccharides having d.p. between three and seven, the principal product being maltohexaose. The required accompanying product for maltohexaose production, namely glucose, was not present, and the authors accounted for this discrepancy by proposing that the glucose had been decomposed by 'localized pyrolysis' during the initial scission of the cycloheptaamylose.

Methylation analysis was performed on the components corresponding to three of the peaks present in Fig. 1a (retention times 3.94, 8.81, and 10.49 min) after isolation by preparative l.c. The results are outlined in Table IV. The 3.94-min peak gave two products, namely the 2,3,4 and 2,3,5-trimethylglucitol acetates, which are derived respectively from LG and its furanose isomer. The peak at 8.81 min yielded predominantly the 2,3,6-trimethylglucitol acetate, from cycloheptaamylose. Also in this frac-

tion, however, were small, approximately equal amounts of the 2,3,4,6-tetramethylglucitol acetate and the 2,3-dimethylglucitol acetate. These products must arise from a linear oligosaccharide having LG as the end-group and we propose, in consideration of the results from the methylation of the slowest eluting peak (see later), that this linear product is the pentaglucosyl levoglucosan, which coelutes with the cycloheptaacetate peak. The shoulder on the trailing edge of the peak at 8.81 min in the l.c. trace is probably this linear anhydrooligosaccharide. The peak at 10.49 min gave 2,3,4,6-tetramethylhexitol acetate, 2,3,6-trimethylhexitol acetate, and 2,3-dimethylhexitol acetate in the approximate corrected[21] ratio of 1:5:1. This analysis confirms the presence of a seven-membered linear oligosaccharide (**2**) having a 1,6-anhydro end-group, the initial reaction-product proposed in Scheme 1. Furthermore, a sample of cycloheptaamylose solution pyrolyzed for 30 min at 150° showed only two peaks, namely unchanged starting material and this seven-membered linear anhydrooligosaccharide.

Components eluting between LG and cycloheptaamylose were not isolated in pure form nor subjected to methylation analysis. However, it may be inferred, by comparison with the standard mix profile in Fig. 1d, that they correspond to glucose and to linear oligosaccharides, both reducing and anhydro, of d.p. 2–5. L.c. at constant flow-rate (2 mL/min) of the reaction solution, followed by plotting the logarithm of the elution time after the solvent front *versus* d.p. produced two linear curves, one of which coincided with that given by the malto-oligosaccharide standard mix. We conclude that a linear anhydrooligosaccharide of *n* glucosyls plus one LG unit elutes just in front of the linear reducing oligosaccharide of *n* glucosyl residues. Hence in Fig. 1a the peak at 5.95 min is maltose and the peak at 5.69 min is the anhydrotrisaccharide (diglucosyl-levoglucosan). Similarly the inflexion on the trailing edge of the 4.84 min peak in Fig. 1a is glucose and the major component of this peak is glucosyl-LG, while conversely, the shoulder on the leading edge of the peak at 5.09 min in Fig. 1c is glucosyl-levoglucosan and the main 5.09 peak is glucose.

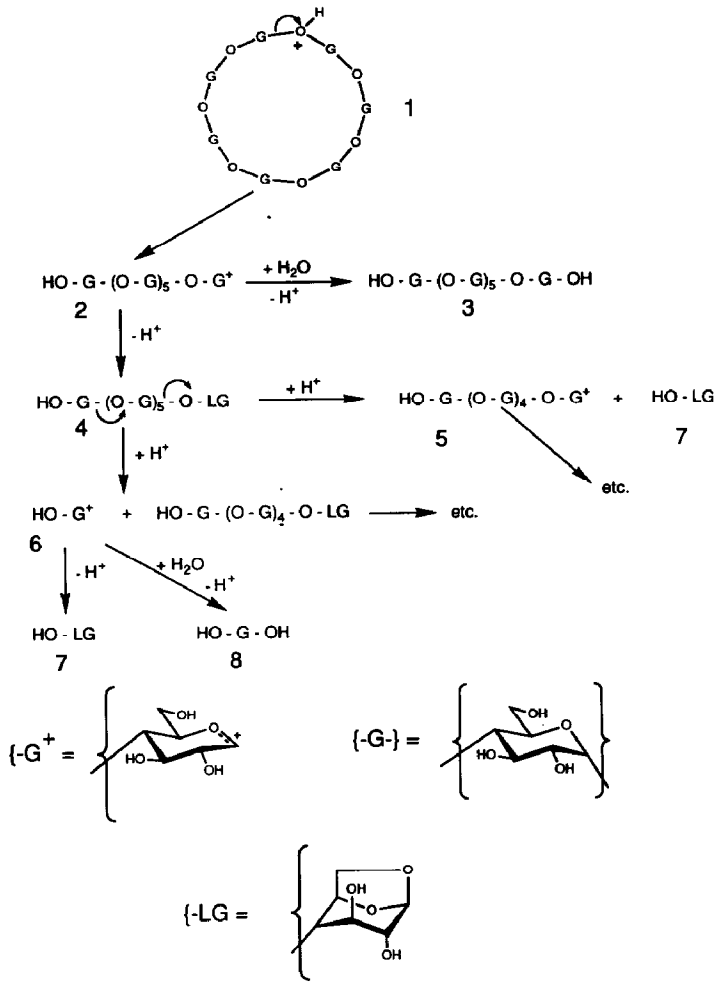
Theander and Westerlund[12] have studied the heat processing of starch and wheat flour and, in accordance with most earlier workers, have posited that after formation, *the 1,6-anhydro end-group* can react with hydroxyl groups on adjacent polymer chains to

TABLE IV

Relative amounts of methylated alditol acetates obtained from oligosaccharides[a]

L.c. retention time (min)	*Position of methylation*								
	2,3,4,6	*2,4,6*	*3,4,6*	*2,3,4*	*2,3,6*	*2,3,5*	*2,4*	*2,3*	*3,4*
3.94	[b]	[b]	[b]	4.25	[b]	1.00	[b]	[b]	[b]
8.81	1.00	0.504	0.195	0.783	8.654	[b]	0.257	1.521	[b]
10.49	1.00	0.307	0.140	0.404	5.076	[b]	0.127	1.207	[b]

[a] Corrected area ratios[25] relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol when present. [b] None detected.



transglycosylate and to form a more-branched structure. They based this conclusion upon the observations that, at longer heating times, the starch became more soluble in 80% ethanol and that trifluoroacetic acid hydrolysis gave a higher value for glucose content than amylolytic enzymic treatment. While these observations do indicate that fragmentation and chain branching occur, they offer no insight into the role of 1,6-anydro end-groups in these processes. We consider it more likely that chain branching results from the *addition of the glucosyl cation* (produced by a glucosidic scission) to a hydroxyl group on an adjacent polysaccharide chain, as an alternative reaction channel to anhydride formation, that is, stabilization of the glucosyl cation occurs by trans-glucosylation, rather than intramolecular nucleophilic addition. Certainly the chain branching is always accompanied by chain fragmentation and clearly more study of these competing reactions, namely anhydride formation and transglycosylation, is needed.

The presence of reducing sugars, even in rigorously dried methyl sulfoxide

solutions, may be explained by noting that non-specific competing dehydration reactions also occur and that these reactions, which are evidenced by the darkening of the reaction solution, provide the water necessary to attack glucosyl cations, thereby producing reducing sugars. In comparing Figs. 1a and 1c, the similar yields of unchanged starting material suggest that the addition of water to the reaction mix has little effect upon the rate-controlling reaction (that is, scission of a glycosidic linkage). However, there is a shift in emphasis from the production of anhydro to reducing oligosaccharides (**4** and **3** in Scheme 1) as evidenced for example by the increased formation of glucose (5.09 min, **8**) at the expense of levoglucosan (**7**). This implies of course the addition of hydroxyl ion to the glucosyl cation.

In an earlier paper[3] on the pyrolysis of cellobiitol, we showed that the heating of a cellobiitol melt at 171° produced no levoglucosan. We proposed that the absence of LG was a result of the increased tendency (compared with vacuum pyrolysis) of the glucosyl cation to undergo transglycosylation reactions in place of the intramolecular addition-reaction. The presence of levoglucosan in the pyrolysis of cycloheptaamylose in methyl sulfoxide may be explained similarly by the assumption that the transglycosylation reactions are slowed relative to the intramolecular addition-reaction by the solvent acting as a diluent, thus separating the reactant molecules.

In addition to end-group ring closing to produce the linear anhydrooligosaccharide, it is conceivable that the glucosyl cation arising from the initial scission could react intramolecularly with a hydroxyl group on one of the glucosyl residues to form another cyclodextrin ring. Although steric factors render unlikely the formation of a ring having fewer than six glucosyl residues[22], six-or seven-membered rings are possible. The most likely reaction would occur between O-6 on a glucose unit six or seven residues away from the glucosyl cation to form either a seven-membered ring or a six-membered ring having a pendant glucopyranosyl unit. Such a compound, termed "iso-α-cyclodextrin", a six-membered cyclodextrin having five α-(1→4) linkages and one external α-(1→6) linkage, has been synthesized[23]. However, we say no evidence of such secondary cyclodextrin formation and therefore conclude that these possible reaction pathways do not occur in our situation.

EXPERIMENTAL

Materials. — Dry methyl sulfoxide was stored over CaH_2. All other reagents were used as received in the purest commercially available grade.

General methods. — All ^{1}H-n.m.r. spectra were recorded at room temperature with a Jeol FX-90Q instrument at 89.56 MHz. G.l.c. was carried out using flame-ionization detection and digital integration. Pyrolyzate I (room-temperature condensate) was converted into Me_3Si ethers using bis(trimethylsilyl)trifluoroacetamide in pyridine (1:1, v/v) after addition of glucitol as internal standard. G.l.c. of the Me_3Si ethers of pyrolyzate I from vacuum pyrolysis was performed on a nickel column (2.2 mm i.d. × 2.4 m) packed with 3% SE52 on Gas Chrom Q (100–120 mesh) using N_2 as the carrier gas and a linear temperature-program from 130 to 250° at 6°/min. All l.c.

analyses were done on a Waters μ-Bondapak-NH_2, 8 mm × 10 cm radial-compression column at room temperature with detection by differential refractometry. Acetonitrile–water (13:7) was the eluent and a flow program of 1 to 2 mL/min over 5 min then 2 to 4 mL/min over 4 min followed by 4 to 6 mL/min over 2 min was used. Vacuum pyrolyses were conducted on a 0.5-g scale at 266 Pa under a nitrogen flow (sufficient to decrease the original vacuum by 133 Pa) as described previously[24]. Pyrolysis times were adjusted according to temperature to ensure completion of the rapid weight-loss phase of the pyrolysis. Pyrolyses in methyl sulfoxide were conducted by heating a measured volume (100 μL) of a 10% solution of cycloheptaamylose in a sealed glass tube in a thermostatted air oven (±1°). Where required, water and acid (a standard solution of conc. H_2SO_4 in Me_2SO) were added. Samples containing NaCl were prepared by dissolving the salt and the cycloheptaamylose in a minimum amount of water followed by freeze-drying. Pyrolyzate **II** components (−80° condensates) were analyzed by ^{1}H-n.m.r. in D_2O using 2,2-dimethylpropan-1-ol as the internal standard. Yields of individual products were then determined by integration of peak areas. Identification of products was achieved by comparison with chemical shifts as reported previously[19] (see discussion). Solutions from pyrolyses in Me_2SO were analyzed by l.c. after drying overnight under vacuum at 60° to remove the Me_2SO, followed by redissolution in water (200 μL). Successive methylation, hydrolysis, reduction, and acetylation of oligosaccharides obtained by preparative l.c. and g.l.c.–m.s. were carried out as previously reported[25].

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