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SYNTHESIS OF 6-DEOXYMALTOOLIGOSACCHARIDES AND A STUDY OF THEIR LIPID-BINDING PROPERTIES*

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ABSTRACT

Amylose reacted in *N,N*-dimethylformamide with crystalline bromomethylenedimethylammonium bromide (Vilsmeier bromide) to give 6-bromo-6-deoxyamylose with a high degree of substitution. Reduction of the bromoamylose with sodium borohydride yielded 6-deoxyamylose that, following controlled acetolysis, gave 6-deoxymaltooligosaccharides. Such oligosaccharides, of appropriate chain-length, formed stable complexes with long-chain fatty acids that have a dissociation constant of about 2μM in their interaction with parinaric acid (*trans*-9,11,13,15-octadecatetraenoic acid). Treatment of 6-bromoamylose with sodium methoxide in dimethyl sulfoxide produced a 3,6-anhydroamylose that contains few unaltered D-glucose units. This anhydroamylose also bound to a palmitoyl-resin column, suggesting that the polymer may be partially helical and somewhat lipophylic. Finally, reaction of 6-bromoamylose with methylvinyl ether led to the 2,3-di(methoxyethyl) ether that, on reaction with sodium methoxide, gave the 6-methyl ether from which 6-*O*-methylamylose was obtained by mild acid hydrolysis.

INTRODUCTION

Amylose is known to assume a variety of helical conformations in the

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crystalline state[1], of which the ordered structure appears to be partially retained in solution, this depending on the solvent[2,3]. In the presence of small nonpolar substances, inclusion complexes form readily and lead to more tightly ordered helical conformations[2,4,5], but this complexing ability appears to be greatly reduced or lost in low-molecular-weight fragments of amylose[6,7]. On the other hand, the cyclodextrins that have fixed helical conformations interact weakly (K_d 3mM) with alkyl and aromatic compounds[8]. Mycobacteria have evolved a novel way to enhance the lipophylicity of short α-D-(1→4)-glycans by synthesizing polymethylated forms, exemplified by the 6-*O*-methyl-D-glucose lipopolysaccharides[9] and the 3-*O*-methyl-D-mannose polysaccharides[10] These polymethylpolysaccharides form tight inclusion complexes (K_d 0.4μM) with long-chain fatty acids[11,12], and they appear to function as lipid carriers in the cell[11].

Many interesting and practical uses of the cyclodextrins have been reported[13], and it is possible that synhetic analogs of the polymethylpolysaccharides might have even more utility owing to the potential for a tighter and more specific interaction with lipids. We have reasoned that the role of the 6-*O*-methyl group in the mycobacterial polysaccharide might be duplicated by a 6-deoxy group and that 6-deoxymaltooligosaccharides should have good lipid-binding properties. Here, we show that such compounds, produced by partial acetolysis of 6-deoxyamylose prepared from 6-bromo-6-deoxyamylose[14], when of an appropriate chain length are water-soluble and form tight complexes with long-chain fatty acids.

6-Bromo-6-deoxyamylose is also a potential source of 3,6-anhydroamylose[15] and 6-*O*-methylamylose[16], and we describe new and improved syntheses of these polymers with higher degrees of modification than previously reported. All of these studies were facilitated by the development of an improved synthesis of 6-bromo-6-deoxyamylose using pure, crystalline bromomethylenedimethylammonium bromide as the brominating agent[17].

EXPERIMENTAL

Materials — 6-Deoxyamylose was synthesized initially according to Takeo *et al.*[14] by treating "activated" amylose with methanesulfonyl bromide[18] in dry *N,N*-dimethylformamide, and then reducing the 6-bromo-6-deoxyamylose with $NaBH_4$ in dimethyl sulfoxide. This bromination procedure, which presumably involves the *in situ* formation of bromomethylenedimethylammonium bromide (Vilsmeier bromide), is accompanied by some methanesulfonylation of the amylose. Therefore, the procedure was modified by use of the pure, crystalline Vilsmeier bromide, which was synthesized from triphenylphosphine and bromine[17], and allowed to react with amylose in *N,N*-dimethylformamide.

Amylose (Type II; from potato) was from Sigma Chemical Co , whereas all other reagents and solvents were of the highest purity that is available commercially. *N,N*-Dimethylformamide was dried by distillation from P_2O_5 and was stored over molecular sieve 4Å, pyridine was dried by distillation from CaH_2,

and dimethyl sulfoxide was dried with molecular sieve 4Å. Palmityl aminoalkylsilyl silicate, used in the isolation of lipophylic oligosaccharides, was from an earlier study[19]. Bio-Gel P-4 (−400 mesh) and Bio-Gel P-60 (100–200 mesh) were from Bio-Rad Laboratories, and Sephadex G-25 (fine) was from Sigma. Methyl vinyl ether was from the Matheson Division of Searle Medical Products.

General methods. — Carbohydrate was determined by the phenol–sulfuric acid procedure[20], D-glucose by D-glucose oxidase using the Glucostat reagent, and D-glucose, 6-deoxy-D-glucose, 6-*O*-methyl-D-glucose, and 3,6-anhydro-D-glucose by g.l.c. of the alditol acetates[21]. Liquid chromatography (l.c.) under elevated pressure was performed on a Waters Associates model ALC/GPC 201 chromatograph equipped with two pumps and a model 680 gradient controller. A reversed-phase, analytical column (Technicon LC18) was eluted with CH_3CN–water gradient (Program 4) from 3:7 to 9:1 (v/v), and the acetylated oligosaccharides were detected by absorbance at 206 nm. Oligosaccharides having the ability to bind to the palmityl-affinity column were separated partially by gel filtration in water on Bio-Gel P-4 (−400 mesh), Bio-Gel P-60 (100–200 mesh), or Sephadex G-25 (fine) columns (2 × 190 cm), and the recovered material was fractionated further by l.c. after acetylation in dry pyridine with acetic anhydride.

^{1}H-N.m.r. spectra were recorded for solutions in D_2O with a Bruker 500 MHz spectrometer in the Department of Chemistry, University of California (Berkeley). Chemical shifts are referenced to an internal standard of acetone (δ 2.217). The mass spectra were recorded at Imperial College with a VG Analytical ZAB HF mass spectrometer equipped with a fast-atom-bombardment source and an M-Scan FAB gun.

Oligosaccharides having lipid-binding ability were recovered from acetolysis reactions by passing a water solution of the deacetylated products through a column containing palmityl aminoalkylsilyl silicate (250 g; 4 × 45 cm packed bed). After the column had been washed with water until the effluent was free of carbohydrate, the bound material was eluted with 50% ethanol. The capacity of the column was ~250 mg of carbohydrate[19]. Qualitative detection of polysaccharide–lipid interaction was done in a similar manner with a 1 × 10-cm column of palmityl aminoalkylsilyl silicate. As a negative control, a column of acetyl aminoalkylsilyl silicate was used, which does not bind mycobacterial polymethylpolysaccharides[12]. Quantitative evaluation of polysaccharide–lipid interaction was done by fluorimetric titration with *trans*-parinaric acid[7]. Fluorescence emission was recorded at 410 nm after excitation at 328 nm, and the quantum yield was measured by comparison with anilinonaphthalenesulfonate, Q 0.37 in absolute ethanol[22].

2,3-Di-O-acetyl-6-bromo-6-deoxyamylose. — To a stirred suspension of "active" amylose (7.50 g, 46 mmol of anhydroglucose) in dry *N,N*-dimethylformamide (300 mL) at 65–70° was added bromomethylammonium bromide (101 g, 465 mmol) under conditions that excluded moisture and at a rate that maintained the temperature at 65–70°. The mixture was stirred at this temperature for 48 h, after which the dark solution was concentrated *in vacuo* to~1/3 vol. and the pH

adjusted to 9–10 by the addition of 3M sodium methoxide in methanol (175 mL) The solution was kept at room temperature for 20 min to destroy the formate esters formed in the reaction, after which it was poured into vigorously stirred ice–water. The precipitate that formed was collected by vacuum filtration, washed well with water, and dried. The solid was redissolved in *N,N*-dimethylformamide and reprecipitated by pouring the solution into stirred ice–water. The dry product weighed 9.66 g (95%).

The 6-bromoamylose (6.0 g, 26.5 mmol of bromodeoxyanhydroglucose) was acetylated in dry pyridine (90 mL) and acetic anhydride (60 mL) with stirring at room temperature. After 48 h, the viscous solution was poured into ice–water and the solid that formed was collected by vacuum filtration and washed with water. The dry solid weighed 7.42 g (90%). A sample was washed with hot 95% ethanol and dried for analysis, $[\alpha]_D^{22}$ +153° (*c* 0.3, dimethyl sulfoxide); lit.[14] $[\alpha]_D^{25}$ +148° (*c* 1, chloroform).

Anal. Calc. for $(C_{10}H_{13}BrO_6)_n$: C, 38.87; H, 4.24; Br, 25.86. Found: C, 38.80; H, 4.21; Br, 25.75.

Deacetylation of 2,3-di-*O*-acetyl-6-bromo-6-deoxyamylose in *N,N*-dimethylformamide–methanol–sodium methoxide yielded 6-bromo-6-deoxyamylose, which was precipitated by pouring the reaction mixture into water. The product was dried at 100° *in vacuo* for analysis, $[\alpha]_D^{22}$ +90° (*c* 0.2, pyridine); lit.[14] $[\alpha]_D^{25}$ +130° (*c* 0.1, pyridine).

Anal. Calc. for $(C_6H_9BrO_4)_n$: C, 32.01; H, 4.00; Br, 35.50. Found: C, 31.20; H, 3.88; Br, 34.70.

Takeo *et al.*[14] reported a product with 33.4% bromine. The fact that the deacetylated polymer described here contained slightly less than the calculated proportion of bromine suggests that a small amount of debromination may occur during deacetylation, possibly by 3,6-anhydride formation (see below).

*2,3-Di-*O*-acetyl-6-deoxyamylose.* — This compound was prepared by reduction of 2,3-di-*O*-acetyl-6-bromo-6-deoxyamylose with sodium borohydride according to Takeo *et al.*[14]. The acetylated product was recovered by pouring the reaction mixture into water.

Anal. Calc. for $(C_{10}H_{14}O_6)_n$: C, 52.17; H, 6.14. Found: C, 51.00; H, 5.95.

6-Deoxyamylose was obtained by deacetylation of the diacetate as described for the bromo derivative. Monosaccharide analysis of an acid hydrolyzate by g.l.c. showed >90% of 6-deoxy-D-glucose along with small amounts of D-glucose and an unidentified peak.

Anal. Calc. for $(C_6H_{10}O_4)_n$: C, 49.30; H, 6.84. Found: C, 49.52; H, 6.17.

6-Deoxymaltooligosaccharides. — 6-Deoxyamylose (250 mg) was acetylated for 30 min at room temperature with 1:2 (v/v) acetic acid–trifluoroacetic anhydride (5 mL), after which the excess reagent was evaporated *in vacuo*. To the residue was added the acetolysis reagent 10:10:1 (v/v) acetic anhydride–acetic acid–conc. H_2SO_4 (6 mL), the content of the tube was mixed rapidly to ensure a uniform solution, and then it was warmed at 40° in a water bath for exactly 25 min

Alternatively, the acetylated polysaccharide was acetolyzed with 40:10:1 (v/v) trifluoroacetic anhydride–acetic acid–H_2SO_4 at room temperature (21°) for 1 min.

Dichloromethane (20 mL) and water (20 mL) were added to the mixture, which was shaken in a separatory funnel, and the organic layer recovered and washed repeatedly with water. The dichloromethane extract was dried (Na_2SO_4) and the acetylated oligosaccharides were deacetylated with sodium methoxide in methanol. After removal of the organic solvents by evaporation, the oligosaccharide mixture was dissolved in water and passed through the palmityl-affinity column. Unbound material (~40% of the applied carbohydrate) was eluted with water, after which the bound oligosaccharides (60% of the applied carbohydrate) were eluted with 50% ethanol.

The unbound oligosaccharide fraction consisted of mono- to penta-saccharide as assessed by paper chromatography and by l.c. separation of the polyacetates, and this material was not further studied. The bound oligosaccharides were fractionated first by gel filtration (Fig. 1) into several size groups, and each was then separated into individual oligomers by l.c. of the polyacetates (Fig. 2 and Table I).

3,6-Anhydroamylose. — To a stirred solution of 6-bromo-6-deoxyamylose (0.90 g, 4 mmol) in dry dimethyl sulfoxide (15 mL) was added 3M sodium methoxide–methanol (6 mL, 18 mmol) in dry dimethyl sulfoxide (5 mL). A gel formed and the mixture was stirred for 3 h at room temperature (22°), during which the precipitate dissolved. The clear solution was poured into water (60 mL), and the mixture dialyzed against water overnight and then lyophilized to yield a water-soluble material (0.36 g) giving a negative Beilstein test for halogen, $[\alpha]_D^{21}$ +51.4° (*c* 0.1, water). Following acid hydrolysis, reduction, and acetylation, a peak having R_T of 3,6-anhydroglucitol acetate was observed that represented ~90% of the sample. After being dried at 100° *in vacuo,* the polysaccharide regained weight and analysis showed the presence of a monohydrate.

Anal. Calc. for $(C_6H_8O_4 \cdot H_2O)_n$: C, 44.44; H, 6.17. Found: C, 44.05; H, 4.85.

A synthesis of 3,6-anhydroamylose with up to 85% conversion of the D-glucose units has been reported, but analytical data were not given for the polymer[15]. The similarity of the specific rotation of methyl α-D-glucopyranoside (+189°) to amylose (+220°), and of methyl 3,6-anhydro-α-D-glucopyranoside[23] (+56°), to the 3,6-anhydroamylose described here (+51.4°) supports the structural assignment.

A water solution of the 3,6-anhydroamylose was passed through the palmityl-affinity column, to which all of the carbohydrate bound. The bound material was eluted quantitatively with 50% ethanol. If the column was overloaded with polysaccharide, the material that passed through was enriched in D-glucose, whereas that which bound was composed almost solely of the 3,6-anhydride. Thus, the homopolymer bound more tightly than the material that still contained some D-glucose.

A sample of 3,6-anhydroamylose was acetylated with acetic anhydride in dry

pyridine with 4-dimethylaminopyridine as a catalyst for 30 min at 90° and 24 h at 23°, the solution was poured into water, and the precipitate that formed was collected by centrifugation, washed with water, and dried to give a white solid (93%), $[\alpha]_D^{21}$ +63° (*c* 0.3, dichloromethane).

Anal. Calc. for $(C_8H_{10}O_5)_n$: C, 51.66; H, 5.38. Found: C, 48.83; H, 5.13.

6-Bromo-6-deoxy-2,3-di-O-(1-methoxyethyl)amylose. — 6-Bromo-6-deoxyamylose (1.0 g, 4.4 mmol) in ice-cold dry dimethyl sulfoxide (23 mL) containing *p*-toluenesulfonic acid (0.04 g) was treated with methyl vinyl ether[24] (2.15 g, 35.6 mmol). The solution was allowed to warm to room temperature (21°) and was kept for 2.5 h. The mixture was poured into water (500 mL), which contained some $NaHCO_3$ to neutralize the acid, and the precipitate that formed was collected by filtration, washed on the funnel with water, and dried to give 1.48 g (98%) of the ether derivative, $[\alpha]_D^{21}$ +85° (*c* 0.3, *N,N*-dimethylformamide).

Anal. Calc. for $(C_{12}H_{21}BrO_6)_n$: C, 42.26; H, 6.16; Br, 23.43. Found: C, 41.92; H, 6.20; Br, 23.52.

2,3-Di-O-(1-methoxyethyl)-6-O-methylamylose. — 6-Bromo-6-deoxy-2,3-di-*O*-(1-methoxyethyl)amylose (0.20 g, 0.59 mmol) was heated under reflux for 18 h with 3M sodium methoxide in methanol (5 mL). The solvent was evaporated and the residue washed several times with water by centrifugation to yield 0.15 g (88%) of a product that had a negative Beilstein test for halogen, $[\alpha]_D^{21}$ +98° (*c* 0.3, *N,N*-dimethylformamide). Monosaccharide compositional analysis by g.l.c. indicated that the polysaccharide contained >75% of 6-*O*-methyl-D-glucose. A significant contamination by 3,6-anhydro-D-glucose probably reflects incomplete substitution during reaction with methyl vinyl ether or hydrolysis of the ether during work-up of the reaction product. Attempts to circumvent this problem were unsuccessful.

Anal. Calc. for $(C_{13}H_{24}O_7)_n$: C, 53.42; H, 8.21. Found: C, 53.72; H, 7.83.

2,3-Di-O-acetyl-6-O-methylamylose. — 2,3-Di-*O*-(1-methoxyethyl)-6-*O*-methylamylose (0.20 g) was dissolved in glacial acetic acid (2 mL), and the solution was heated at 100° for 10 min and then evaporated to dryness *in vacuo*. The residue was dissolved in dry pyridine (2 mL), acetic anhydride (1.3 mL) and 4-dimethylaminopyridine (10 mg) were added, and the mixture was kept at room temperature for 17 h. The solution was poured into water, and the precipitate that formed was collected by centrifugation and washed several times with water and dried; the yield was 0 12 g (70%).

Anal. Calc for $(C_{11}H_{16}O_5)$: C, 50.76; H, 6.15. Found: C, 50 36; H, 5.48.

6-O-Methylmaltooligosaccharides. — 2,3-Di-*O*-(1-methoxyethyl)-6-*O*-methylamylose (60 mg) was allowed to dissolve, and undergo deacetalation and acetylation in 2:1 (v/v) trifluoroacetic anhydride–acetic acetic acid (1.5 mL) at room temperature (21°) for 30 min. The solution was evaporated to dryness *in vacuo* and an acetolysis reagent (10:10:1, v/v, acetic anhydride–acetic acid–H_2SO_4, 0.6 mL) was added to the residue. After 5 min at 21°, the reaction was mixed with ice–water containing $NaHCO_3$, and the acetylated oligosaccharides were extracted into dichloromethane, which was washed with water and dried (Na_2SO_4). The product

was deacetylated in dichloromethane by addition of a 0.5M sodium methoxide in methanol solution. After evaporation of the solvents, the residue was dissolved in water and decationized with Dowex 50(H^+). The acetolysis products could be separated into homologs by gel filtration on a Bio-Gel P-4 column, but they were not studied further.

RESULTS AND DISCUSSION

Preparation of 6-deoxyamylose oligosaccharides. — Acetolysis of 6-deoxyamylose occurs at least 20 times faster than that of amylose, and reaction with 10:10:1 acetic anhydride–acetic acid–sulfuric acid at 40° for 25 min or 40:10:1 trifluoroacetic anhydride–acetic acid–sulfuric acid at 21° for 1 min yielded a mixture of fragments that, after deacetylation, were water-soluble and had an average size of about 10 hexose units. A water solution of the product was fractionated on the palmityl-affinity column with ~40% of the carbohydrate passing through the column in water and 60% binding, which could be eluted with 50% ethanol.

After elution of the bound material, the solution was evaporated to dryness and the product was fractionated on a Bio-Gel P-60 column in water. Because clear

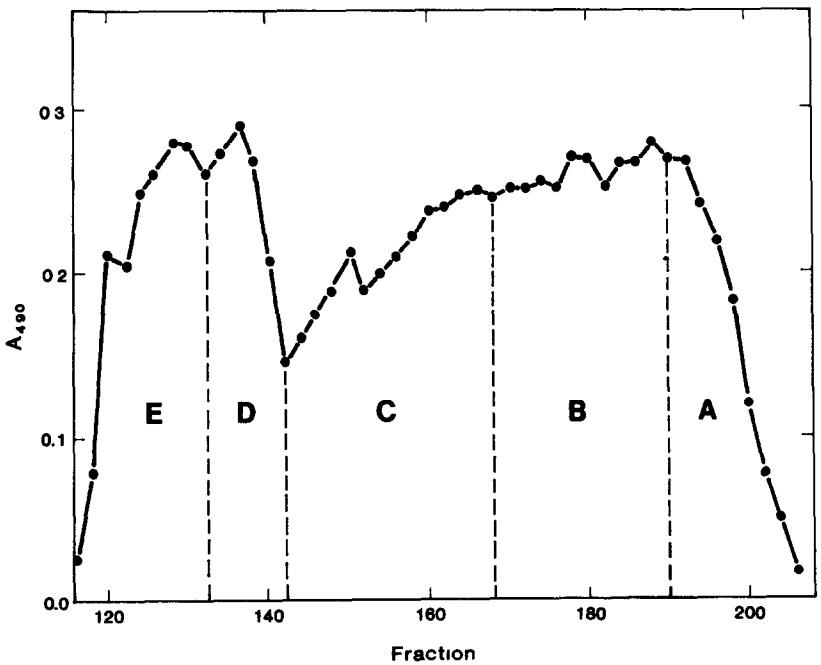


Fig 1 Gel filtration profile of 6-deoxymaltooligosaccharides The deacetylated acetolysis mixture was separated on a Sephadex G-25 (fine) column (2 × 190 cm) by elution with water in 0 8-mL fractions, and carbohydrate was determined (A_{490}) Tubes were combined into five fractions as indicated, and each was acetylated and then fractionated by l c. on a reversed-phase column An example of one such separation is shown in Fig 2

separation of homologs was not obtained, the fractions were combined arbitarily and individual fractions were analyzed by f.a.b.–m.s. Oligosaccharides obtained from 6-deoxyamylose prepared according to Takeo *et al.*[14] showed some ions for 6-deoxymaltooligosaccharides with a methylsulfonyl group that were not observed in oligosaccharides obtained from the 6-deoxyamylose prepared from the pure Vilsmeier bromide. Such ions were recognized as the sodium-cationized form of the free oligosaccharide at m/z $[M + Na + 78]^+$ when the methylsulfonyl group was on a secondary hydroxyl group, so that the primary position could be reduced, or at m/z $[M + Na + 94]^+$ when it apparently was at OH-6, so that reduction to the 6-deoxyhexose was not possible. Because such undesired derivatives became enriched in the longer-chain oligosaccharides owing to an enhanced resistance to

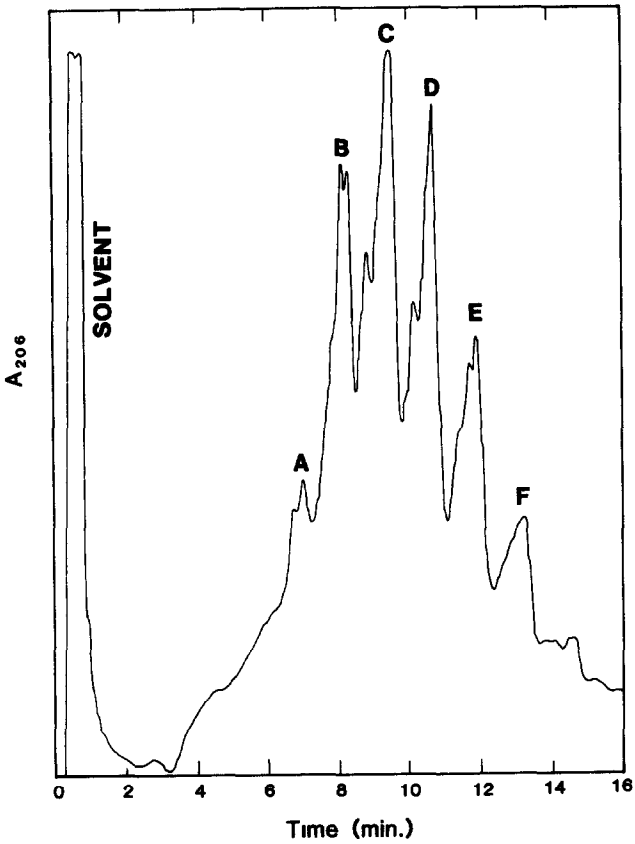


Fig 2 L c separation of acetylated 6-deoxymaltooligosaccharides Fraction A from Fig 1 was acetylated and fractionated on a reversed-phase column by elution with an acetonitrile–water gradient from 3 7 to 9 1 (v/v) by use of program 4 of the Waters 680 gradient controller The compounds were detected by absorbance at 206 nm and peak A corresponds to the tetrasaccharide, whereas each subsequent peak is one hexose larger Retention times, sizes, and K' values for the major peaks from fractions A, B, C, and D are listed in Table I along with similar data for the unbound material

TABLE I

FRACTIONATION OF ACETYLATED 6-DEOXYAMYLOOLIGOSACCHARIDES BY LIQUID CHROMATOGRAPHY

Nonbinding fraction[a] *(retention time)*[b]	*Binding fraction*[a,b,c] *(retention time)*				*log K'*[d]	*Molecular ion*[e]	*Polymer size*
	A	*B*	*C*	*D*			
5 67					0 58	503	2
6 83					0.68	733	3
8 17					0 78	963	4
9 33	9 33				0 84	1193	5
	10 08				0 88	1423	6
	11 30	11 27			0.94	1653	7
	12 43	12 20			0 97	1883	8
		13.35			1 02	2113	9
		14 55	14.60		1 06	2343	10
		15.82	15.78		1 10	2573	11
			16 78		1 12	2803	12
			18 12		1 16	3033	13
			19 07		1 18	3263	14
				20.50	1.22	3493	15
				22.17	1 25	3723	16
				24 33	1 30	2953	17
				26 67	1 34	4183	18

[a]When passed in water through a palmityl aminoalkylsilyl silicate column [b]Retention time (min) on a reversed-phase column eluted with an acetonitrile–water gradient (see Methods) [c]Separated into size groups by gel filtration on a Sephadex G-25 (fine) column. [d]K' = (retention time of solute − retention time of solvent)/retention time of solvent. [e]By f.a.b.–m.s. The major chracteristic positive ion in each example resulted from the loss of an acetoxy group (mass 59) from a peracetate with the general structure [(2,3-di-*O*-acetyl-6-deoxyglucosyl)$_x$(Ac,OAc)] and a molecular weight of [(230)$_x$ + 102], where x = number of hexose units.

acetolysis of the glycosidic bond, the method of Takeo *et al.*[14] for preparation of 6-deoxyamylose was abandoned.

Oligosaccharides obtained by acetolysis of the 6-deoxyamylose prepared from the pure Vilsmeier reagent were isolated on the affinity column and then separated according to size on a Sephadex G-25 column (2 × 200 cm). Again, distinct fractions were not obtained, so tubes were combined arbitrarily to give five fractions representing material that ranged from the smallest to the largest sizes (Fig. 1). The material in each fraction was acetylated and fractionated by reverse-phase l.c. (Fig. 2), and material from the peak tubes was analyzed by f.a.b.–m.s. (Table I). The major ions observed in the spectra corresponded to the carbonium ions of the peracetates resulting from loss of acetoxy anion, without or with adsorbed sodium cation, m/z $[M - 59]^+$ and $[M - 59 + 23]^+$. Only the former ions are listed in the Table and the composition of the fractions revealed that the oligosaccharides ranged from penta- to octadeca-saccharide. The pattern in Fig. 2 and the results in Table I suggest that the pentasaccharide is the smallest of the oligomers with good fatty acid-binding properties, although some of this oligosaccharide is also found in the nonbinding fraction when the affinity column is overloaded. A plot of log K' against polymer size gave the curve in Fig. 3.

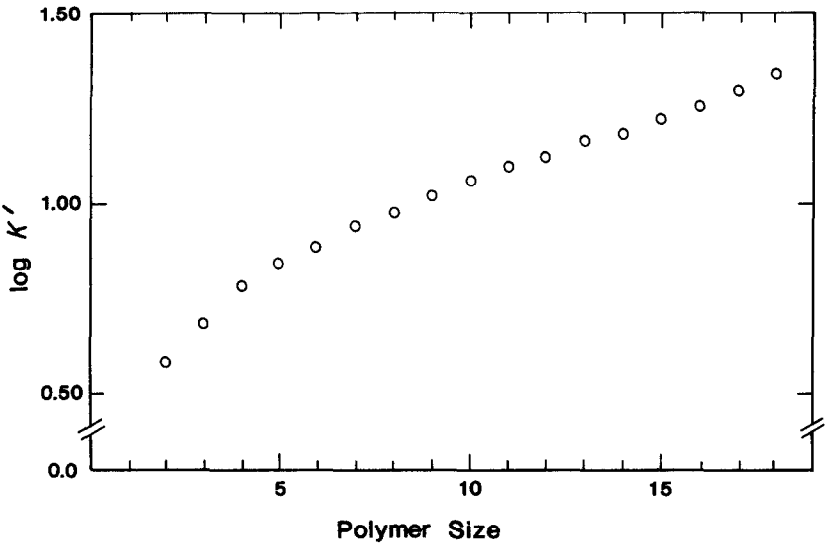
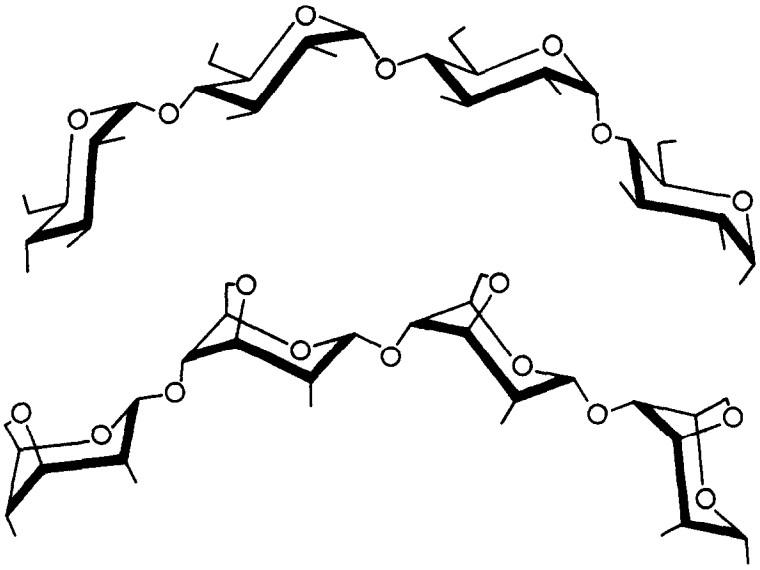


Fig 3 L c elution position of acetylated 6-deoxymaltooligosaccharides as a function of the degree of polymerization. The values of K' listed in Table I are plotted against the molecular sizes determined by mass spectrometry

Preparation of 3,6-anhydroamylose and 6-O-methylamylose. — Treatment of 6-bromo-6-deoxyamylose with sodium methoxide in methanol led, in excellent yield, to 3,6-anhydroamylose that contains only a small proportion of D-glucose and 6-*O*-methyl-D-glucose. The product is water-soluble, is very labile to acid hydrolysis and to acetolysis and, surprisingly, had the ability to bind to the palmityl-affinity column. The material could be fractionated into strongly and weakly binding polymers, the former being a pure homopolymer whereas the latter was enriched in D-glucose and 6-*O*-methyl-D-glucose. Thus, a regularity of structure appears to enhance the lipid interaction. The 3,6-anhydroamylose failed to bind to acetyl aminoalkylsilyl silicate, which suggests that the interaction is of a specific nature.

The ability of 3,6-anhydroamylose to bind to the fatty acid column may reflect the presence of a partially helical conformation, which is feasible because the *cis* relation between the linkages at O-1 and -4 are retained when the rings invert from the $^4C_1$(D) to the $^1C_4$(D) conformation to yield the anhydroamylose[25] (Scheme 1). It is obvious on inspection, however, that the inner surface of such a postulated helical polymer would not have the hydrophobicity of a helical amylose or 6-deoxyamylose.

Interaction of modified amyloses with iodine. — In contrast to amylose, which gives a blue solution with iodine–potassium iodide (λ_{max} 625 nm), water-soluble oligosaccharides obtained by acetolysis of 6-deoxyamylose gave a reddish brown color with a strong u.v end absorption. 6-Deoxyamylose itself, which is insoluble in water, absorbed iodine from an aqueous solution to give a brown solid residue



Scheme 1 Illustrations of the coiled conformation of an α-(1→4)-linked D-glucan and an α-(1→4)-linked 3,6-anhydro-D-glucan Note that conversion to the 3,6-anhydride changes each ring from $^4C_1$(D) to the $^1C_4$(D) conformation, but the *cis* relation between the linkages at O-1 and -4 are retained so that both molecules can assume a helical conformation[25].

when kept overnight in suspension. 3,6-Anhydroamylose is water-soluble and showed no immediate reaction with iodine, but in a few seconds a flocculent brownish precipitate formed that suggests the occurrence of some kind of interaction.

Quantitative assessment of oligosaccharide–lipid interaction. — The all-*trans* form of parinaric acid is an excellent probe for the measurement of polysaccharide–lipid interaction since the fatty acid has a low fluorescence in a polar environment, such as water, that increases more than 10-fold in a nonpolar environment[22]. Two aspects of the interaction are of interest, the quantum yield (Q), which reflects the environment of the parinaric acid in the complex, and the dissociation constant (K_d), which indicates the tightness of the interaction. Studies of the mycobacterial *O*-methyl-D-mannose and *O*-methyl-D-glucose polysaccharides showed that the complexes with parinaric acid had saccharide K_d values of about 0.4 μM and quantum yields of 0.004–0.005, whereas the Q value for *trans*-parinaric acid in methanol was 0.011 (ref. 22).

Compared with the mycobacterial polymethylpolysaccharides, a novel feature of the 6-deoxymaltooligosaccharides is that the relatively short oligomers bind lipid fairly well. In a complex with parinaric acid, the pentasaccharide would not be able to enclose the whole fatty acid chain unless higher-order complexes were formed, so the quantum yield is expected to be low even though the interaction might be strong The titration curves illustrated in Fig. 4 support this

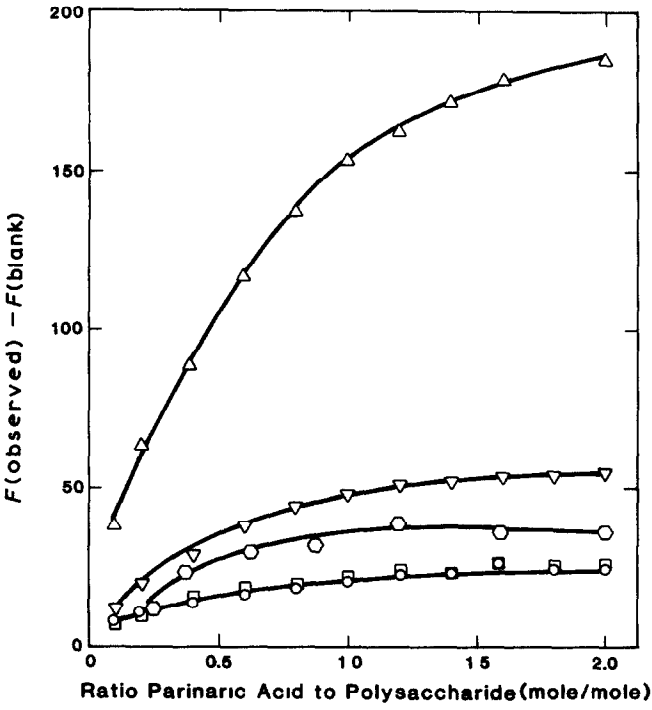


Fig 4. Fluorimetric titration of oligosaccharide fractions from Fig 1. Solutions of oligosaccharide fraction A (○), B (□), C (▽), and D (△) (each at 5μM concentration based on average size) and amylase-digested *O*-methyl-D-glucose polysaccharide (○) (at 8μM concentration) in 10mM phosphate buffer, pH 7.0, were titrated by adding increasing amounts of parinaric acid in methanol The observed fluorescences were corrected for the fluorescence of the polysaccharides and parinaric acid, each determined alone in buffer. Although the longer-chain oligosaccharides gave the greatest fluorescence enhancement, an evaluation of the parinaric acid concentration that gave half the maximum fluorescence increase in each titration yielded K_d values of 1 5 to 3μM for all oligosaccharides

conclusion in that there is little correlation between relative fluorescence and K_d. All of the oligosaccharides that are able to bind to the affinity column appeared to interact with parinaric acid with a K_d of 1.5–3μM, although the maximum fluorescence observed in each titration was dependent on the length of the polysaccharide (Fig. 5). The data suggest that 1:1 molar complexes were formed and that the longer-chain oligosaccharides create a more nonpolar environment for the included parinaric acid than do the shorter-chain ones, thus increasing the quantum yield. This result is somewhat different from the *O*-methyl-D-mannose–parinaric acid interaction in which a significant polysaccharide-chain-length dependence was observed[7]. This is probably related to the observation that the mycobacterial polysaccharides bind parinaric acid approximately 10-fold greater than these synthetic homologs.

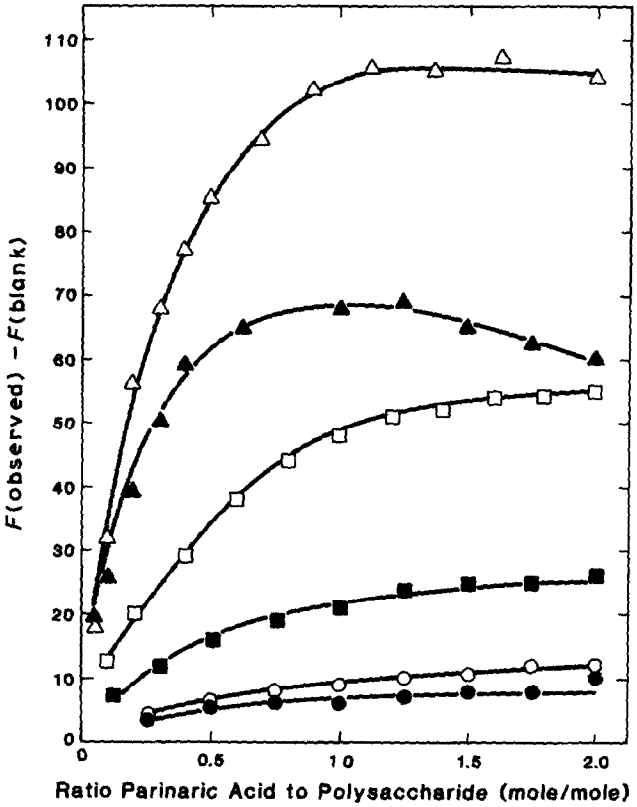


Fig. 5 Fluorimetric titration of oligosaccharide fractions at various concentrations 6-Deoxymaltooligosaccharide fractions B (closed symbols) and C (open symbols) from Fig 1 were titrated at 2 (●, ○), 5 (■, □), and 10 (▲, △) μM concentrations. From the different titrations, K_d values from 1 to 3μM were estimated

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