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Selective Alkylation of Cycloheptaamylose

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The verification of a number of assumptions made about cycloamylose structure, substrate binding and catalysis is dependent on the ability to 3-*O*-alkylate selectively these oligosaccharides. In the presence of barium oxide and barium hydroxide octahydrate, cycloheptaamylose reacts with 3-bromopropene in dimethylformamide and dimethyl sulfoxide to produce a high yield of tetradecakis-2,6-*O*-allylcycloheptaamylose, a compound which can serve as a highly versatile intermediate in the preparation of heptakis-3-*O*-alkylated cycloheptamyloses. Depending on the nature of the substituent to be introduced at the C-3 hydroxyl position, the intermediate allyl ether can be 3-*O*-alkylated and the allyl ether can be isomerized to the vinyl ether and cleaved; or, alternatively, the allyl ether can be first subjected to isomerization followed by alkylation, and then cleavage.

Bloch has shown the fatty acid synthetase complex isolated from *Mycobacterium smegmatis* (*1*) to be stimulated by both the methylmannose containing polysaccharide and the methylglucose lipopolysaccharides isolated from the same organism. In a series of studies employing modified cycloamyloses as models, we were able to demonstrate some of the structural features of these cellular polysaccharides responsible for this stimulation (*2*). In the course of these investigations, we developed a simple and potentially flexible procedure for selectively alkylating the 3-hydroxyl positions of cycloheptaamylose.

This selective 3-*O*-alkylation makes it possible to verify a number of assumptions made about cycloamylose structure, substrate binding and catalysis (*3*). For example, hydrogen bonding between the 2-hydroxyl oxygen of one glucose ring and the 3-hydroxyl hydrogen of an adjacent ring has been suggested to account in part for the conformational rigidity of cycloamylose in solution. Removal of the 3-hydroxyl hydrogen by alkylation will permit confirmation of this suggestion. Furthermore, with the 3-hydroxyl positions selectively blocked, definitive proof that the 2-hydroxyl and not the 3-hydroxyl groups are the catalytic sites in hydrolysis is now possible. Finally, whether or not the enhanced binding of 2,6-peralkylated cycloamyloses to aromatic substrates requires alkylation at both faces of the oligosaccharides now can be determined. For these reasons, and owing to the continued and widespread interest in these oligosaccharides, we feel compelled to report on this sequence (*4–6*).

Inspection of the literature revealed the alkylation products of cycloamyloses fall into two categories: (a) those with complete position refunctionalization (as the heptakis-3-*O*-methylcycloheptaamylose to be described (*7–8*), and (b) those with partial position refunctionalization, e.g., carboxymethylcyclohexaamylose (*3, 9*). Of the seven possible substitution modes of compounds in category (a), i.e., complete alkylation at position C-2, C-3, or C-6; at C-2, 3, C-2, 6, or C-3, 6; or at C-2, 3, 6, only C-2, 3, 6 and C-2, 6 have been reported (*8*).

Although alkylation at C-2, 3, 6 does not require any directed synthesis (use of protective groups), selective alkylation at C-2, 6 might be expected to require the application of blocking groups. Methylation, however, of cycloheptaamylose with dimethyl sulfate in dimethyl sulfoxide and dimethyl formamide with barium hydroxide octahydrate and barium oxide as base, requires 48 hr for complete C-2,6-*O*-alkylation and an additional 72 hr before measurable C-3 alkylation even begins (*8*). The combined inductive effects of the neighboring hydroxyls coupled with alkoxide ion stabilization by intramolecular hydrogen bonding have been offered as explanations for the relative hydroxyl acidities and thus for the slow methylation of the C-3 hydroxyl (*3*). This rather unusual selectivity in alkylation has allowed us to design a procedure for selectively 3-*O*-alkylating cycloheptaamylose.

The idea was simply to block the C-2 and C-6 oxygens of cycloheptaamylose, alkylate the C-3 oxygens, and then remove the protecting groups. Our requisites for these protecting groups were clear: They should be affixed and removed in high yield, and the removal step should be mild enough so as not to disrupt any groups introduced at the C-3–OH positions during alkylation. Furthermore, the intermediate (2–*O*, 6–*O* protected cycloamylose) should be stable and easy to purify.

Both the benzyl (*10*) and allyl ether (*11*) protecting groups were investigated. The benzylation reactions, however, proceeded in low yield; the products were difficult to purify; and the removal step required rather harsh conditions, *i.e.*, lithium and ethylamine. Ultimately, we found that tetradecakis 2,6-*O*-allylcycloheptaamylose satisfied our requirements. The allylation reaction was effected with 3-bromopropene under conditions identical with the above described methylation procedures, followed by silica gel chromatography ($CHCl_3$/EtOAc) of the crude product. This procedure provided a stable white crystalline solid in 81% yield. The presence of the allyl ether was indicated by a doublet at 4.4 δ, and two complex multiplets centered at 5.3 and 5.9 δ. The allyl ether was quantitatively methylated with a 5 M excess of methyl iodide in dimethylformamide in the presence of NaH at room temperature for 24 hr. The nmr of the product clearly revealed a methyl singlet (3.67 δ), and the ir revealed the absence of any hydroxyl stretching.

The allyl ether was isomerized to the corresponding vinyl analog with potassium tertiary butoxide in dimethyl sulfoxide at 100°C (*11*). The progress of the reaction was followed by nmr analysis: The 4.4 δ methylene doublet disappeared, and a 1.63 δ methyl doublet developed along with two multiplets centered at 5.3 and 6.15 δ. After 6 hr, integration of the signal at 1.6 δ was constant. The vinyl ether generated was surprisingly impervious to cleavage by both basic permanganate oxidation (*12*) and acid hydrolysis at pH 5 (*13*). Although the ether could be cleaved with stronger acid, this was concomitant with glycosidic cleavage. The deprotection was finally accomplished with a mixture of mercuric oxide, mercuric chloride in aqueous acetone at

room temperature (*11*), and time studies revealed that all 14 vinyl groups were removed in less than 2 min. The methylated product was desalted and purified on Sephadex G-25-150 in 80% yield.

Proton nmr spectra (D_2O) indicated, in addition to the expected complex multiplet centered at 4.05 δ and the characteristic broad anomeric proton signal at 5.2 δ, a clear methyl signal at 3.95 δ. Because of the C_7 symmetry of cycloheptaamylose, all of the glucopyranose residues are chemically and physically equivalent, and the C-13 nmr spectrum is thus very simple. Furthermore, the carbon assignments have been made for cycloheptaamylose itself in DMSO (CS_2 reference): C-1 (89.7), C-2 (118.5), C-3 (119.2), C-4 (110.1), C-5 (119.6), C-6 (131.6) (*14*).

With these facts available, assignment of the C-13 signals in heptakis-3-*O*-methylcycloheptaamylose is straightforward. In D_2O (upfield from CS_2 in ppm) C-1 (91.8), C-4 (110.2), C-3 (115.1), C-6 (132.5), and 6-OCH_3 (133.3) are of approximately equal intensity. However, while 3-*O*-methylation has caused C-3 to move downfield 4.10 ppm from C-3 in simple cycloheptaamylose, C-2 has moved upfield resulting in apparent C-2, C-5 superposition at 120.8 ppm, thereby producing a signal of approximately twice the intensity of the other signals.

As a final proof of structure, the methylated oligosaccharide was subjected to acidic methanolysis followed by silylation with trimethylsilyl chloride and hexamethyldisilazane in pyridine. The resulting product was identical on glc (3% OV-1) with the silyl ether of 3-*O*-methylglucose.

Clearly the most serious shortcoming in this procedure is the allyl to vinyl ether isomerization step. The conditions for this migration are extremely severe and would not permit the introduction of any base-labile substituent at the 3-hydroxyl position. The application of Corey's $RhCl(PPh_3)_3$ reagent (*15*) in the allyl to vinyl ether isomerization generated mixtures of products and severe separation problems. We were finally able to overcome this difficulty by isomerizing the allyl to the vinyl ether followed by methylation and vinyl ether cleavage. The above sequence, then, allows for generation of selectively 3-*O*-alkylated cycloheptaamylose in good yield. Furthermore, it should be possible to extend this procedure to cyclohexaamylose by carefully monitoring the allylation reaction and being somewhat cautious about 3-*O*-allylation. Figure 1 summarizes the sequences described above. A single unit of the cyclic oligosaccharide is used to demonstrate the transformations.

Experimental

Cycloheptaamylose was obtained from Aldrich Chemical Company, Inc., and purified by the method of Cramer (*16*). The nmr spectra were taken on either a Varian A-60 or a Varian XL-100. When nmr's were run in D_2O, a capillary tube containing TMS was inserted as a reference. Because of the complex nature of the cycloamylose proton spectra, we have included both the center of multiplets followed by several defining characteristics of these envelopes when there is clear definition.

Tetradecakis-2,6-O-allylcycloheptaamylose. Cycloheptaamylose 3 g (2.3 mmole) is allowed to react with an excess of allyl bromide, 21 g (0.31 mole), and with barium oxide, 15 g (0.09 mole), barium hydroxide octahydrate 15 g (0.09 mole) in dimethyl sulfoxide, 75 ml and dimethyl formamide, 75 ml at room temperature for 48 hr under

nitrogen. The reaction mixture was cooled, and 60 ml of ammonium hydroxide was added slowly with continual stirring. After 20 min this mixture was added to 500 ml of chloroform and the inorganic salts completely precipitated by addition of hexanes. The organic phase was washed with water 5 × 50 ml, dried over sodium sulfate; the solvent stripped under vacuum, and the resulting oil applied to a silica gel column,

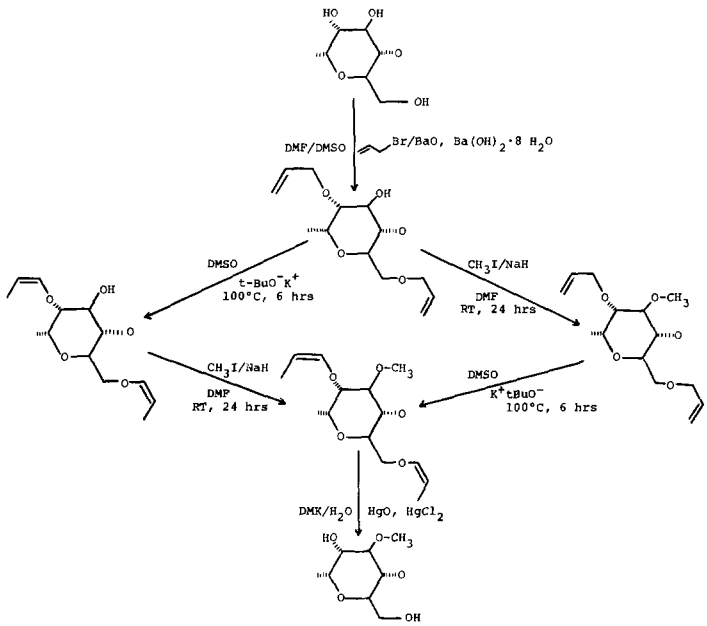

FIGURE 1

300 g (70 × 4.0 cm), and eluted with ethyl acetate: chloroform (100% $CHCl_3$, 20% 40%, 60%; v/v). This produced 3.2 g of a white solid, 71% yeield.

nmr ($CDCl_3$): 4.0 δ m (3.55), (3.73), (3.87), (4.00), (4.10), (4.28), (4.38), (4.46), 5.20 δ m (4.06), (5.13), (5.28), (5.41) 5.83 m.

ir ($CHCl_3$): 3450 (OH), 2945 (C–H).

Heptakis-3-O-methyl-tetradecakis-2,6-O-allylcycloheptaamylose. The allyl ether 1.86 g (1.0 mmole) is allowed to react with excess methyl iodide 2.84 g (0.02 mole) in dimethyl formamide in the presence of sodium hydride 0.24 g (10 mmole) at room temperature for 24 hr under nitrogen. The reaction mixture was cooled and water added slowly until gas evolution ceased. This mixture was taken up in 300 ml of chloroform and washed with 5 × 25 ml water, the organic phase dried over sodium sulfate, filtered, and stripped leaving 1.80 g (92% yield) of a clear oil that crystalized from a hexane ether mixture.

nmr ($CDCl_3$): 3.80 δ (m), (3.57), (4.00), (4.08), (4.18), (4.25), 5.26 δ (m), (5.1), (5.15), (5.23), (5.26), (5.43), 5.9 δ (m).

ir ($CHCl_3$): (no OH), 2945 (C–H).

Heptakis-3-O-methyl-tetradecakis-2,6-O-prop-1-enylcycloheptaamylose. The allyl ether 0.97 g (0.5 mmole) is allowed to react with potassium-*t*-butoxide 1.6 g (150 mmole) in 20 ml of dimethyl sulfoxide at 100°C for 5 hr under nitrogen. The reactants

were cooled and taken up in 150 ml of diethyl ether and washed with water 5 × 50 ml, the ether phase dried over sodium sulfate, filtered and stripped leaving 0.94 g of an amber oil that was unstable to silica gel and alumina chromatography. However, nmr indicated clear and quantitative allyl to vinyl ether isomerization.

nmr ($CDCl_3$): 1.62 δ (d), 4.01 δ (m), (3.55), (3.83), (4.01), (4.33), (4.43), (4.55), (5.33), 6.16 δ (m).

Vinyl ether cleavage. The vinyl ether 0.98 g (0.5 mmole) is allowed to react with mercuric oxide 0.720 g (3.3 mmole) and mercuric chloride 0.720 g (2.6 mmole) in 16 ml of water and 8 ml of acetone by shaking for 8 min at room temperature. The mixture is centrifuged and decanted, the aqueous phase washed with diethyl ether to remove acetone and other soluble organic materials.

The aqueous phase is run through a Sephadex G-25-250 column and the polysaccharide fractions determined by α-naptholsulfuric acid reagent.

This yielded 0.56 g (80% yield) of the heptakis-3-*O*-methylcycloheptaamylose.

nmr (D_2O): 4.05 δ (m), (3.95, 3–OCH_3), 5.46 δ (broad, anomeric proton). The carbon 13 nmr was run in D_2O with sodium formate as the reference (0.1 m); the shifts were then adjusted to CS_2 for comparison with the literature values and are reported in ppm: C-1 (91.8), C-4 (110.2), C-3 (115.1), C-2, 5 (120.8), C-6 (132.5), OCH_2 (133.3).

Methanolysis and Silylation of Heptakis-3-O-methylcycloheptaamylose. The polysaccharide 100 μg is dissolved in 3 ml of methanolic HCl (0.75 *M* HCl) and heated in a sealed tube at 80°C for 18 hr. The reactants are cooled to room temperature and neutralized with $AgCO_3$. The AgCl is allowed to settle out. The methanolic solution is transferred to a ½-dram vial and evaporated in a hot water bath under a stream of nitrogen. The residue is reacted with trimethylsilylating reagent (1 ml pyridine, 0.4 ml hexamethyldisilazane, 0.2 ml trimethylchlorosilane). After 10 min at room temperature 2 to 3 μl are injected onto a 6 ft × 1/8-in. glass column of 3% OV-1 at 150°C. The glc profile was identical with that obtained by putting 3-*O*-methylglucose through the same sequence.

Tetradecakis-2,6-O-prop-1-enylcycloheptaamylose. The tetradecakis-2,6-*O*-allylcycloheptaamylose was isomerized and worked up exactly the same as was the heptakis-3-*O*-methyltetradecakis-2,6-*O*-allylcycloheptaamylose (77% yield).

nmr ($CDCl_3$): 1.66 δ (d), 4.16 δ (m), (4.16), (4.45), (4.55), (4.66), 3.17 δ (m), 3.75 δ (m).

3-O-methylation of Tetradecakis-2,6-O-prop-1-enylcycloheptaamylose. The alkylation procedure was identical with that for tetradecakis 2,6-*O*-allylcycloheptaamylose and the nmr of product was identical with the nmr of heptakis-3-*O*-methyl tetradecakis-2,6-*O*-prop-1-enylcycloheptaamylose. The yield was quantitative.

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