Preparation of Amylose Derivatives Selectively Modified at C-6. 6-Amino-6-deoxyamylose

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ABSTRACT: The synthesis of various amylose derivatives selectively modified at C-6 leading to the preparation of 6-amino-6-deoxyamylose (4) was carried out under homogeneous conditions. Amylose was initially halogenated directly at the primary carbon either by using methanesulfonyl chloride in dimethylformamide/lithium chloride as solvent, giving 6-chloro-6-deoxyamylose (1) or with triphenylphosphine and *N*-bromosuccinimide in dimethylformamide/lithium bromide, giving 6-bromo-6-deoxyamylose (2). Several of these derivatives with different degrees of substitution were prepared. C-6 chlorinated amyloses were then converted to the corresponding 6-azido-6-deoxyamylose analogs (3) by chloride displacement with azide ion in dipolar aprotic media. Triphenylphosphine facilitated reduction of these intermediates in dimethyl sulfoxide gave 6-amino-6-deoxyamyloses with the same degrees of substitution as the C-6 chlorinated precursors. Products were characterized in terms of the site and the extent of modifications using ^{13}C NMR, FTIR, HPLC, and elemental analyses.

Introduction

Over the last decade, there has been a growing interest in selective chemical modification of polysaccharides under homogeneous conditions. Such procedures offer several advantages over classical derivatization methods that are often conducted in a heterogeneous manner, resulting in nonuniform substitutions. Selective approaches, on the other hand, allow control of the degree and distribution of the intended modifications. This facilitates systematic studies of effects of the type and extent of modification on structure/property relationships and on the biological properties of the products. A large volume of literature now available in this field has been compiled in several recent reviews.[1-3]

Site-selective modifications often exploit the reactivity differences between the functional groups on the sugar residues of polysaccharides. This is relatively straightforward when large differences exist, for example, carboxyl and amine functionalities in the presence of hydroxyl groups. When, however, all functionalities are the same, such as hydroxyls, as is often the case, more elaborate procedures are needed. These usually require several stages involving sequential protection and deprotection techniques. This has been one major disadvantage associated with selective methods, and it has hampered their more wide scale utilization. The more abundant and technologically important polysaccharides, such as cellulose and starch, have nevertheless received considerable attention. The basis of the regiospecific derivatization in these polysaccharides lies in the higher reactivity of the C-6 primary hydroxyl group. Steric accessibility of this site either lends itself to direct derivatization or facilitates its protection and deprotection, thus enabling further chemistry at the secondary positions. The synthesis of several selectively modified cellulose and amylose derivatives has been summarized by Horton.[4] Among these, regiospecific halogenation is one of the most effective routes for activation of the primary site, facilitating further transformations at this position. Earlier work has often employed the displacement of a good leaving group, such as tosyl, by the halide ion.[5] Recently, several methods, developed in carbohydrate chemistry for the direct replacement of the primary hydroxyl by a halide, have also found applications in polysaccharide modification. One of the most facile of these was described by Evans *et al.*[6] and involved the use of methanesulfonyl chloride in *N,N*-dimethylformamide (DMF). This procedure was later effectively extended to cellulose and amylose.[4] Initial use of this reaction with amylose reported direct displacements of the primary hydroxyl by chlorine up to a DS of 0.9, in spite of the heterogeneous conditions[7] (DS is defined here based on substitution at C-6 per anhydroglucose unit). Nearly complete bromination of amylose at C-6 was also reported by Takeo et al.[8] using the analogous methanesulfonyl bromide in DMF. These C-6 halogeno amyloses were then employed to prepare other derivatives such as 6-deoxy, 6-azido-6-deoxy-, and 6-aldehydoamylose. Recently, similarly modified pullulans were prepared during an investigation of the effects of substitution at C-6 on the enzymatic degradation of this polysaccharide.[9]

We have been studying regiospecific C-6 modifications of amylose with particular emphasis on the preparation of its 6-amino-6-deoxy derivative. 6-Azido-6-deoxyamylose was the intermediate of choice since it appeared to provide a convenient route to the amino derivative by way of reduction. Even though this route is regularly employed in monosaccharide[10] and cyclodextrin chemistry,[11] its utilization for polysaccharides has been rare. While the preparation of 2-amino-2-deoxyamylose with varying degrees of substitution has been reported,[12-14] preparation of the 6-amino analog met with limited success.[15] 6-Amino-6-deoxyamylose with a high degree of substitution could have very interesting properties and potential uses as a cationic polysaccharide. In this article, we report the preparation of several C-6 modified amylose derivatives at varying DS's up to near complete substitution (DS = 1), including 6-amino-6-deoxyamylose.

Experimental Section

Materials. Amylose (type III from potato), methanesulfonyl chloride (Sigma), triphenylphosphine (Ph_3P), *N*-bromosuccinimide (NBS), lithium chloride, lithium bromide, sodium azide,

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Table 1. ^{13}C NMR Chemical Shifts[a] of C-6 Modified Amyloses

polymer[b]	solvent	C-1	C-2[c]	C-3[c]	C-4	C-5	C-6
amylose	DMSO-d_6	100.0	71.8	71.8	78.6	73.1	60.4
1	DMSO-d_6	99.9	71.6	72.4	79.8	69.9	45.3
2	DMSO-d_6	99.9	71.8	72.2	81.0	69.6	35.2
3	DMSO-d_6	100.0	71.5	72.5	79.6	69.7	51.1
4	CD_3COOD[d]	97.1	71.1	71.7	76.6	66.9	40.6

[a] Ppm from TMS. [b] See Experimental Section for preparation conditions. [c] Tentative assignments. [d] 2% in D_2O.

and lithium azide (Aldrich) were used without further purification. Reaction solvents *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and *N*-methylpyrrolidone (NMP) (Aldrich) were anhydrous grade; other solvents were reagent grade, and all were used as received.

Analyses. ^{13}C NMR spectra (50.3 MHz) were recorded with a Varian XL-200 spectrometer from DMSO-d_6 solutions with tetramethylsilane (TMS) as the internal standard and occasionally from D_2O and 2% CH_3COOD in D_2O solutions with external TMS standard. IR spectra were obtained from KBr discs using a Nicolet 20SXB, fourier transform spectrometer. HPLC analyses of the hydrolysates were performed on a Waters Associates Model 6000A liquid chromatograph equipped with a YMC-Pack PA 13-5 (250 × 6 mm i.d.) column and a R401 differential refractometer detector. Acetonitrile/water (75/25, v/v) was the mobile phase at a flow rate of 2 mL/min. 6-Chloro-6-deoxyglucose and glucose were used as standards. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN.

Syntheses. 6-Chloro-6-deoxyamylose (1). Amylose (20 g, 0.12 mol as anhydroglucose) and lithium chloride (25 g) (dried overnight at 100 °C in vacuum) were dissolved by stirring in DMF (700 mL) at 75 °C (bath temperature) under nitrogen (~2 h). To this homogeneous solution, maintained at 75 °C, methanesulfonyl chloride (100 mL, 1.3 mol) was added dropwise over a 15-min period. A white swollen mass precipitated about halfway through the addition. Stirring was continued at this temperature for 2.5 h during which time the mixture became homogeneous and yellowish-brown. After cooling to room temperature, the solution was slowly poured into 3 L of crushed ice–water with vigorous stirring, and the pH of the mixture was adjusted to 8–9 by slow addition of solid sodium carbonate. The suspension was stirred overnight, and the precipitate was collected by filtration, washed extensively with water and finally with ethanol, and dried overnight at 60 °C in vacuum: yield 20 g (93% based on 6-chloro-6-deoxyamylose).

A small amount of the product (~25 mg) was hydrolyzed by boiling with 2 N aqueous HCl (25 mL) under reflux. The hydrolysate, analyzed by HPLC, contained 6-chloro-6-deoxyglucose with no significant quantities of glucose, indicating nearly complete C-6 substitution, DS = ~1 was also confirmed by the ^{13}C NMR spectrum (Figure 1) and elemental analysis given in Table 2.

Partially C-6 Chlorinated Amyloses (1a and 1b). The above described procedure was repeated twice under slightly differing conditions as follows. A homogeneous solution of amylose (8.1 g, 50 mmol as anhydroglucose) and LiCl (10 g) in DMF (200 mL), obtained initially by heating to 80 °C for 2 h under nitrogen, was treated by dropwise addition of methanesulfonyl chloride (39 mL, 0.5 mol) at room temperature. A slight exotherm developed during the addition step and some precipitation also took place as mentioned above. The resulting mixture was stirred at room temperature for 2 days, and the product was recovered and analyzed as described above: **1a**, yield 5.87 g, DS = 0.37, from HPLC of the hydrolysate.

A second reaction, also performed at room temperature under similar conditions using amylose (10.4 g, 64 mmol as anhydroglucose), LiCl (12 g), DMF (300 mL), and methanesulfonyl chloride (50 mL, 0.64 mol) for 4 days, gave **1b**: yield 10.9 g, DS = 0.22, from HPLC of the hydrolysate.

6-Bromo-6-deoxyamylose (2). Amylose (5.1 g, 31.5 mmol as anhydroglucose) and lithium bromide (6 g) (dried at 100 °C overnight in vacuum) were dissolved in DMF (200 mL) at 70 °C with stirring under a nitrogen atmosphere until homogeneous (~2 h). The solution was cooled to room temperature, and a 2-fold excess/anhydroglucose unit of NBS (11.2 g, 63 mmol) was added. The resulting yellow solution was then treated by dropwise addition over a 15-min period of a solution of Ph_3P (16.5 g, 63 mmol, 2-fold excess/anhydroglucose unit) in DMF (30 mL). A gelatinous precipitate formed at this stage, and the mixture progressively darkened in color. After stirring at room temperature for 30 min, the temperature was raised to 70 °C (bath temperature), and stirring was continued for a further 2 h. The brown and homogeneous mixture was then cooled to

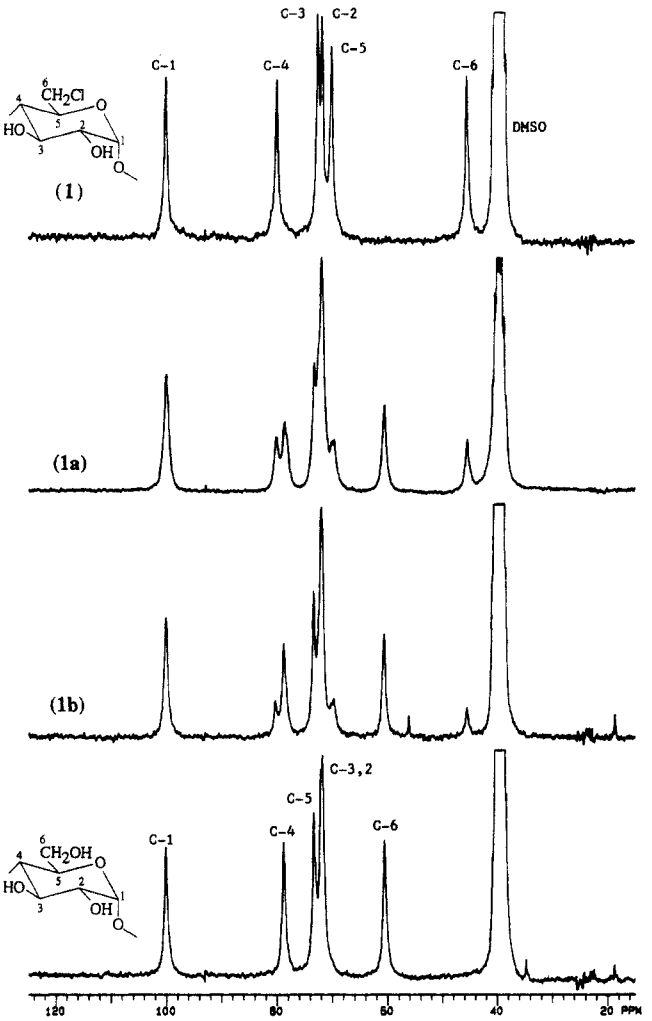


Figure 1. ^{13}C NMR spectra of amylose, 6-chloro-6-deoxyamylose (1), and partially C-6 chlorinated amylose derivatives (**1a** and **1b**) (in DMSO-d_6).

Table 2. Elemental Analyses of C-6 Modified Amyloses

	found[b,c]			
polymer[a]	%C	%H	%N	%Br/Cl
1	37.59	5.34		18.41
	(39.91)	(5.02)		(19.63)
1a	39.51	6.28		7.94
1b	40.53	6.43		4.90
2	33.17	4.36		28.61
	(32.02)	(4.03)		(35.51)
2a	34.08	4.62		20.82
2b	37.40	5.31		17.94
3	37.47	5.16	20.47	
	(38.51)	(4.85)	(22.45)	
3a	38.18	6.41	8.30	
3b	39.55	6.39	4.70	
4	42.27	7.23	7.35	
	(44.72)	(6.88)	(8.69)	
4a	37.29	6.75	3.65	
4b	40.00	6.57	2.44	

[a] See Experimental Section for preparation conditions. [b] For amylose, calcd %C 44.45, %H 6.22. [c] Values in parentheses are that of calculated values for the corresponding completely C-6 substituted derivatives.

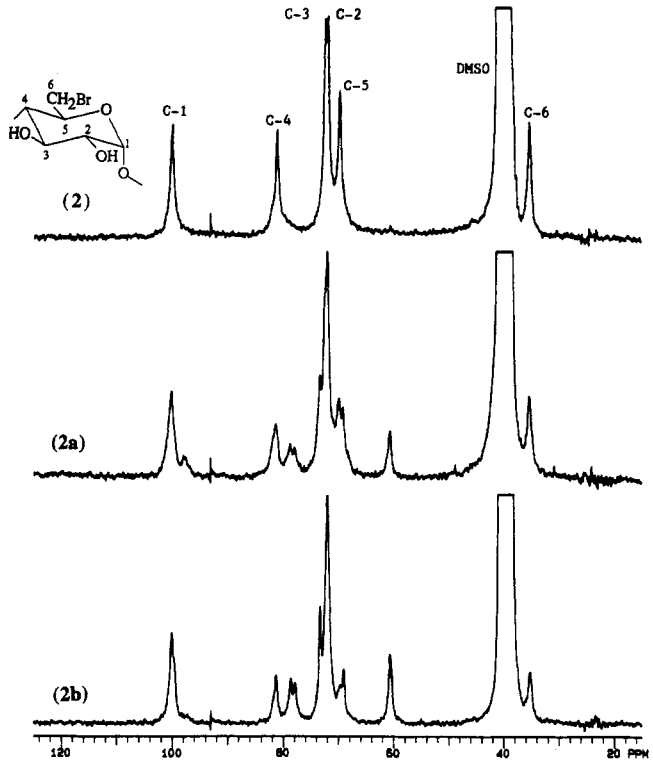


Figure 2. ^{13}C NMR spectra of 6-bromo-6-deoxyamylose (**2**) and partially C-6 brominated amylose derivatives (**2a** and **2b**) (in DMSO-d_6).

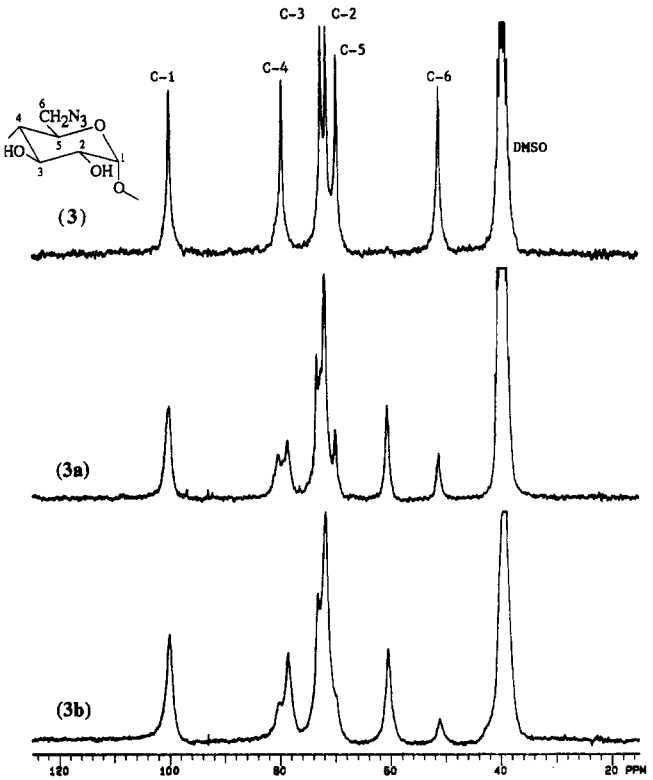


Figure 3. ^{13}C NMR spectra of 6-azido-6-deoxyamylose (**3**) and partially C-6 azidated amylose derivatives (**3a** and **3b**) (in DMSO-d_6).

room temperature and slowly poured into 2 L of ice–water containing 50 g of Na_2CO_3. After stirring overnight, the light brown precipitate was collected by filtration, rinsed extensively with water and finally with ethanol, and dried in vacuum at 70 °C: yield 6.5 g (92% based on 6-bromo-6-deoxyamylose).

Partially C-6 Brominated Amyloses (2a and 2b). The above described bromination of amylose was repeated twice using different molar ratios of reactants as follows. **2a**: amylose (5.6 g 34.5 mmol as anhydroglucose), NBS (6.14 g, 34.5 mmol), Ph_3P (9.05 g, 34.5 mmol), LiBr (6 g), and DMF (220 mL of total volume); yield 6.2 g. **2b**: amylose (5.8 g, 35.8 mmol as anhydroglucose), NBS (3.18 g, 17.9 mmol), Ph_3P (4.69 g, 17.9 mmol), LiBr (6 g), and DMF (220 mL of total volume); yield 3.2 g.

6-Azido-6-deoxyamylose (3). To a solution of 1 (4 g, 22 mmol) in *N*-methylpyrrolidone (150 mL) was added lithium azide (10 g, 0.2 mol), and the mixture (LiN_3 was partially soluble) was stirred at 70 °C for 65 h under nitrogen. The cooled mixture was slowly poured into ice–water, and the tan colored precipitate was collected by filtration, washed with water and ethanol, and dried in vacuum at 70 °C overnight: yield 3.9 g (95% based on 6-azido-6-deoxyamylose). The ^{13}C NMR spectrum (Figure 3) and elemental analysis (Table 2) indicated that complete substitution by azide ion had been achieved.

Partial C-6 Azidodeoxyamyloses (3a and 3b). In a slightly different approach from the above procedure, DMSO solutions (25 and 200 mL) of **1a** and **1b** (2 and 5 g, respectively) were treated with sodium azide (5 g) at 70–75 °C for 48 h. The resulting solutions were diluted with water and dialyzed for 48 h, and the resulting suspensions were lyophilized. **3a**: yield 1.5 g. DS of the product was the same as the parent **1a** (0.37) confirmed by the absence of a signal for CH_2Cl in the ^{13}C NMR spectrum (Figure 3) and by the elemental analysis (Table 2). **3b**: yield 4.6 g; DS = 0.22, as in precursor **1b**, from ^{13}C NMR (Figure 3) and the elemental analysis (Table 2).

6-Amino-6-deoxyamylose (4). A DMSO solution (120 mL) of **3** (3.6 g, 19.2 mmol) was treated with a 2-fold molar excess of Ph_3P (10 g, 38.5 mmol) at room temperature for 24 h with stirring. Steady N_2 evolution subsided after about 2 h. Water (1 mL) was then added, and the slightly turbid solution was stirred for a further 24 h. The product was recovered by precipitation into ethanol, filtered off, Soxhlet extracted with ethanol for 3 days, and dried in vacuum at 60 °C: yield 3.1 g (100% based on 6-amino-6-deoxyamylose). The ^{13}C NMR spectrum (Figure 4) and elemental analyses (Table 2) confirmed complete reduction of the azido group.

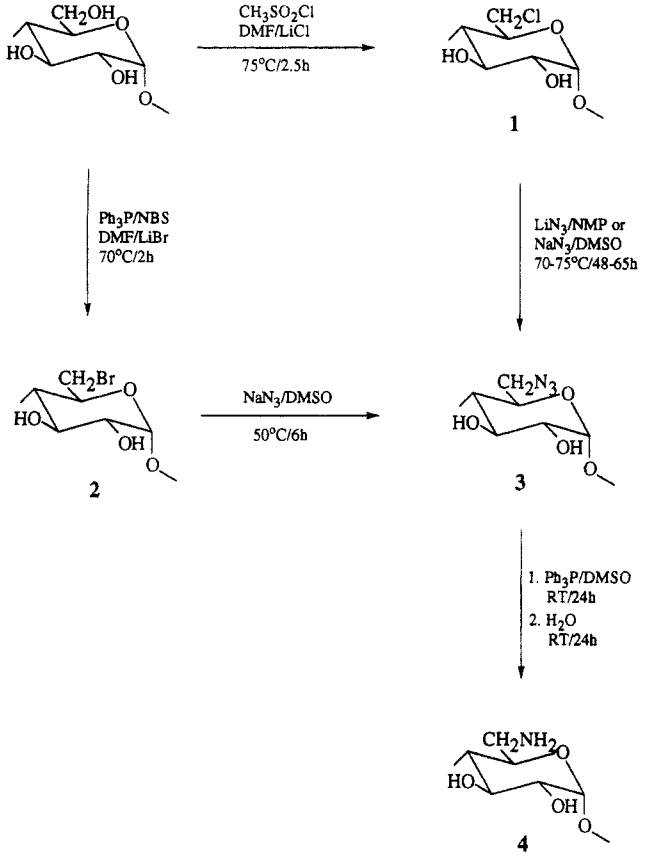
Partial C-6 Aminodeoxyamyloses (4a and 4b). The above described reduction with Ph_3P was repeated for **3a** and **3b** under similar conditions, except that the initial Ph_3P treatment was restricted to 3 h. The products were recovered by dialysis (48 h) and freeze dried. Each was finally purified by Soxhlet extraction with ethanol for 3 days. **4a:3a** (0.6 g) in DMSO (25 mL) was treated with Ph_3P (0.8 g): yield 0.53 g, DS = 0.37 as in precursors **1a** and **3a** from ^{13}C NMR (Figure 4) and elemental analysis (Table 2). **4b:3b** (4.4 g) in DMSO (150 mL) was treated with Ph_3P (4 g): yield 4.1 g, DS = 0.22 as in precursors **1b** and **3b** confirmed by ^{13}C NMR (Figure 4) and elemental analysis (Table 2).

Results and Discussion

During the synthesis of 6-amino-6-deoxyamylose (**4**), several C-6 modified intermediates were prepared. The route taken is illustrated in Scheme 1. The first stage involved halogenation at the primary carbon to give 6-chloro- and 6-bromodeoxyamyloses (**2**, **2a** and **2b**) with different degrees of substitution. The 6-chloro derivatives (**1**, **1a**, and **1b**) underwent nucleophilic substitution to give the 6-azido analogs (**3**, **3a**, and **3b**, respectively), and, finally, the reduction of this set yielded the 6-amino-6-deoxyamyloses (**4**, **4a**, and **4b** respectively) which had essentially the same DS as the parent chlorinated derivatives.

Chlorination at the primary carbon of amylose was selected as the initial activation step since it has been shown to proceed with a high degree of selectivity. This is unlike other routes such as tosylation and mesylation which at high degrees of substitution have been found to suffer in selectivity and also involve reactions in secondary positions.[4] While the literature on direct C-6 chlorination of amylose is limited, there are several examples for cellulose.[16-20] The reagents commonly employed are thionyl chloride, methanesulfonyl chloride, chlorodi-

Scheme 1. The Synthesis of C-6 Modified Amylose Derivatives



methylformimidium chloride, and *p*-toluenesulfonyl chloride. DMF has been the solvent used almost exclusively. Most of these reactions, however, were carried out under heterogeneous conditions that undoubtedly resulted in nonrandom modification, except at the highest degree of substitution (which is often unattainable due to the heterogeneous conditions). Not until solvent systems capable of dissolving cellulose such as *N,N*-dimethylacetamide (DMA)/LiCl or DMF/chloral has it been possible to effect these halogenations under homogeneous conditions.[18-20] To our knowledge, however, no similar attempts were made with amylose. In this respect, we have decided to perform the C-6 modifications of amylose in a DMF/LiCl solvent system. When up to 5 wt% of amylose was treated with DMF containing 4–5 wt% LiCl at 70–80 °C, homogeneous solutions were obtained within 2 h, which upon cooling to room temperature, remained clear. Initially, these solutions were treated by dropwise addition during 10–20 min of a 10-fold molar excess/anhydroglucose residue of methanesulfonyl chloride. A slight exotherm developed and, depending on the rate of addition of the reagent, a highly swollen gelatinous mass precipitated, usually halfway through the addition step. The mixtures were then stirred at room temperature for several days and gave partially substituted products **1a** and **1b**. A third reaction, carried out at 75 °C throughout, was observed to rehomogenize within 1.5–2 h after the addition of the reagent and was discontinued after 2.5 h, giving the product **1**. Upon hydrolysis of these products only glucose and 6-chloro-6-deoxyglucose was obtained. Quantitative HPLC analysis of the hydrolysates indicated DS's of 1, 0.37, and 0.22 for **1**, **1a**, and **1b**, respectively. These values were also tentatively confirmed by elemental analyses given in Table 2. The slightly lower elemental compositions observed were attributed to significant moisture content of the samples which is often difficult to remove even after extensive drying. Additional confirmation of the selective C-6 chlorination was found in the ^{13}C NMR spectra shown in Figure 1. Upon replacement of the primary hydroxyl with chlorine the C-6 resonance shifts upfield to δ = 45.3 ppm from δ = 60.4 ppm (Table 1). While the partial, but increasing chlorination level was indicated in Figure 1 for **1b** and **1a**, no significant resonance corresponding to the primary CH_2-OH was visible in the spectrum of **1**. This, together with HPLC analysis, confirmed the near complete C-6 substituted nature of **1**. The very high degree of modification was also reflected in the solubility behavior of **1**, since it was soluble in most dipolar aprotic solvents such as DMSO, DMF, DMA, and NMP as well as pyridine. Partially chlorinated derivatives **1a** and **1b**, on the other hand, showed more complex solubility characteristics. The former was partially soluble in water, and the latter was not, which is believed to account for the lower yield obtained from the reaction giving **1a**. Similar solubility changes for the partially C-6 chlorinated amyloses were also reported by Horton *et al.*[7] Different DS's exhibited by the products **1a** and **1b**, even though they were prepared under essentially the same conditions, i.e., at room temperature, can probably be explained by the mechanism of this type of reaction which is well understood.[20] Following the reaction of methanesulfonyl chloride with DMF to give *O*-(methanesulfonyl)-*N,N*-dimethylformiminium salt, $CH_3SO_3CH{=}NMe_2^+Cl^-$, two subsequent routes are possible. The first yields the amylose, *N,N*-formimidium chloride, Amy-O-$CH{=}NMe_2^+Cl^-$, by reaction with an amylose hydroxyl, or the second which yields chloro *N,N*-dimethylformiminium chloride, $ClCH{=}NMe_2^+Cl^-$, by chloride ion displacement. An amylose hydroxyl can then displace chlorine in the latter case; again yielding Amy-O-$CH{=}NMe_2^+Cl^-$. This intermediate ionic product is believed to be the precipitate that forms during methanesulfonyl chloride addition in our reactions. Two further routes are then possible: hydrolysis with water to give the formyl ester or chloride ion displacement at elevated temperature to give chlorodeoxyamylose. Thus the different DS's observed with **1a** and **1b** which were obtained after 2–4 days at room temperature are most probably attributable to the fluctuations in temperature during the long course of these treatments and, perhaps, to a lesser extent, to the partially heterogeneous nature of the reactions.

For various reasons it was also desirable to have a better leaving group than chlorine at the primary position of amylose. 6-Bromo-6-deoxyamylose was the obvious choice, provided that it could be prepared as selectively as the chloro derivative. The only mention of **2** in the literature was made by Takeo *et al.*[8] who used methanesulfonyl bromide in DMF to successfully brominate amylose to high DS (0.92). This reagent was not, however, readily accessible, which prompted us to seek an alternative procedure. Of the several methods surveyed in carbohydrate chemistry for the direct replacement of primary hydroxyl groups, the use of Ph_3P in the presence of *N*-halosuccinimides in DMF has been shown to be one of the most facile in terms of the selectivity.[21] The reaction proceeds to high yields under essentially neutral conditions.[21] A similar approach was used for 6-halogeno-6-deoxycellulose preparations with *N*-halosuccinimides where secondary hydroxyls were protected during the reaction.[22] Apart from this report, no systematic use of these reagents appears to have been made in polysaccharide modifications. Boger *et al.*[23] have, nevertheless, successfully employed Ph_3P with carbon tetrabromide in the presence of LiN_3 to prepare 6-azido-6-deoxy-α- and β-cyclodextrins

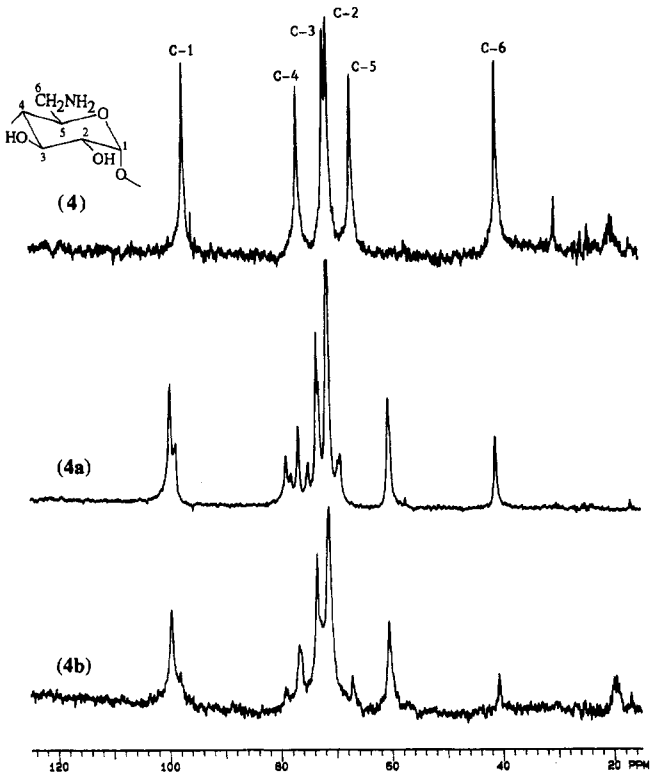


Figure 4. ^{13}C NMR spectra of 6-amino-6-deoxyamylose (**4**) and partially C-6 aminated amylose derivatives (**4a** and **4b**) (**4** and **4b** in 2% CD_3COOD in D_2O, **4a** in D_2O).

with high degrees of selectivity.

As in the case of LiCl, the use of LiBr in DMF also facilitated the dissolution of amylose at reasonable concentrations (<5 wt%). These solutions were then treated with different molar equivalents of Ph_3P and NBS according to the procedure described in the Experimental Section. Products **2**, **2a**, and **2b** were obtained by treatment with 2-fold, equivalent and $^1/_2$-fold molar proportion of the reagents per anhydroglucose unit, respectively. ^{13}C NMR spectra of the products are given in Figure 2, and the elemental analyses are listed in Table 2. It was apparent that selective bromination of the primary carbon occurred as the CH_2OH resonances at δ = 60.4 ppm were replaced by a new peak at δ = 35.2 ppm (Table 1). As inferred from its ^{13}C NMR spectrum, nearly complete C-6 bromination of amylose (product **2**) resulted by utilizing a 2-fold molar excess of the reagents. Elemental analyses of this particular sample, however, had significantly lower bromine content than expected. This is believed to be a consequence of a small amount of gelatinous residue that was often observed during the dissolution of the sample in DMSO. Therefore, it is conceivable that a side reaction might have come into effect at the higher substitution attempt, leading to, perhaps, some cross-linking and hence resulting in the observed lower bromine content of the sample. This was not the case for **2a** and **2b**. However, in light of the slightly lower than expected elemental analyses obtained from these polymers, due to the reasons mentioned earlier, at this stage only a rough estimation of the DS of these samples was possible. Thus, the C-6 bromination levels in **2a** and **2b** are estimated to be around 60 and 40%, respectively. It was also noted that the ^{13}C spectrum of **2a** (Figure 2) contained an additional shoulder in the anomeric carbon region at ca. δ = 97 ppm. This resonance is believed to be due to a small amount of 3,6-anhydro ring formation, which most likely occurred during the workup of the polymer. 3,6-Anhydro ring formation is a common occurrence in carbohydrates containing a leaving group in the primary position (or the secondary positions for 2,3- and other anhydro derivatives). Since slightly basic conditions were employed during the workup (see Experimental Section), the presence of bromine on C-6 presumably increased the likelihood of internal cyclization even under heterogeneous conditions. However, as was the case for **2b**, this could be avoided by careful control of the pH of the supernatant during precipitation. Otherwise the reactions proceeded smoothly, giving somewhat more discolored products at higher bromination levels. An intermediate precipitation and redissolution during the addition stage also occurred for **2**. This was much less pronounced for **2a** and was absent in case of **2b**. The precipitate is believed to be the alkoxyphosphonium bromide salt, Amy-O-$PPh_3^+Br^-$, from which triphenylphosphine oxide is displaced by the bromide upon heating, as described elsewhere in the mechanistic analysis of similar reactions.[24] It thus appears that the Ph_3P/NBS/DMF/LiBr system is equally effective for the selective halogenation at the primary carbon of amylose and should also be applicable to other polysaccharides.

6-Chloro-6-deoxyamylose derivatives, **1**, **1a**, and **1b** discussed earlier, were converted to the corresponding 6-azido analogs, **3**, **3a**, and **3b**, using well established reactions. Two routes employed at this stage involved either the use of LiN_3 in NMP or NaN_3 in DMSO. While the former reaction was heterogeneous due to the partial solubility of LiN_3 in NMP, the latter was homogeneous throughout. Products, analyzed by ^{13}C NMR spectroscopy (Figure 3, CH_2N_3; δ = 51.1 ppm, Table 1), and elemental analyses (Table 2) were found to be completely substituted by the azide with no significant amounts of CH_2Cl groups remaining. The IR spectrum of **3** (Figure 5) also confirmed the conversion, as the C–Cl stretch at 662 cm^{-1} was replaced by the C–N_3 stretching vibration at 2107 cm^{-1}. 6-Azido-6-deoxyamylose derivatives were also accessible through C-6 brominated precursors (**2**, **2a**, and **2b**) via treatment with NaN_3 in DMSO, as illustrated in Scheme 1. In this case, reactions proceeded under milder conditions, at 50 °C, and were complete within 6 h. However, due to the lesser degree of certainty concerning the extent of modification of the C-6 brominated intermediates, this route was not exploited any further at this stage.

In the final stage of the sequences illustrated in Scheme 1, 6-azido derivatives were reduced to the corresponding 6-amino-6-deoxyamylose analogs **4**, **4a**, and **4b**. Traditionally, the amino derivatives of polysaccharides are usually prepared by catalytic reduction of oxime or azide precursors. Difficulties in these reactions are often encountered due to limited solubilities of the polysaccharides which can result in incomplete conversion or in low overall yields. Teshirogi *et al.*[15] have reported the synthesis of 6-amino-6-deoxystarch by sequential tosylation, azide displacement, acetylation, and $LiAlH_4$ reduction steps. Even though a DS of 0.92 was obtained, the overall yield was only 14%. For our reduction, we elected to attempt a relatively little used procedure, at least in carbohydrate chemistry, which involves the reaction of a primary azide with Ph_3P followed by the hydrolysis of the iminophospharene formed, according to the following scheme.

$$RN_3 + Ph_3P \xrightarrow{-N_2} RN{=}PPh_3 \xrightarrow{H_2O} RNH_2 + Ph_3PO$$

Also known as the Staudinger reaction,[25] this method has recently been demonstrated to promote quantitative reduction of primary azides to the corresponding primary

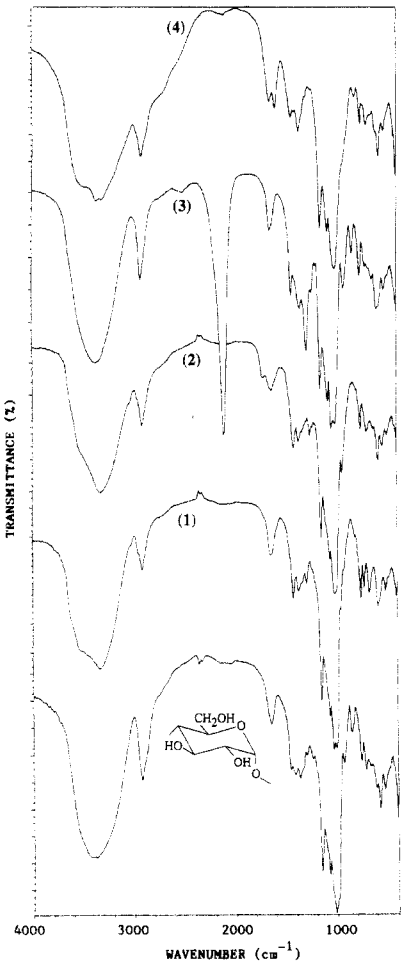


Figure 5. FTIR spectra of amylose, 6-chloro-6-deoxyamylose (1), 6-bromo-6-deoxyamylose (2), 6-azido-6-deoxyamylose (3), and 6-amino-6-deoxyamylose (4).

amines in polar solvents such as THF and at room temperature.[26] Earlier Boger *et al.*[23] utilized this reaction to effect the reduction of hexakis(6-azido-6-deoxy)-α-cyclodextrin to its 6-amino analog in quantitative yield in dioxane. Our initial attempts at the reduction of **3** in THF and NMP was only partially successful primarily due to the heterogeneous reaction in the former solvent and to a somewhat reluctant transformation even after prolonged treatment in the latter case. When these two solvents were used together (1:1 v/v), better results were obtained, but the IR spectrum of the product still indicated the presence of azide. In all cases, further characterization of the products was hampered by their insolubility, once recovered, in any solvent, even though they did swell highly in dilute acetic acid solutions. However, when a solution of **3** (*ca.* 3 wt%) in DMSO was treated with a 2 molar excess of Ph_3P, as described in the Experimental Section, an immediate evolution of gas bubbles occurred which subsided after 2–3 h. The product (**4**) recovered, after hydrolysis, was insoluble in all solvents, swelling in DMSO and water. It, nevertheless, dissolved in 2% acetic acid up to 1 wt%. Concentrations above this level gave either extremely viscous solutions or gelled. The IR spectrum (Figure 5) of **4** no longer contained the azide stretching band at 2107 cm^{-1}. Products **3a** and **3b** were also treated in a similar manner to give **4a** and **4b**. While **4a** was soluble in water, **4b** was not, but it dissolved in dilute acetic acid and DMSO. The ^{13}C NMR spectra (Figure 4), showed a new resonance at δ = 40.6 ppm (Table 1), and in all cases the peaks corresponding to the primary azido carbon (δ = 51.1 ppm, Figure 3) were absent. Elemental analyses (Table 2) also indicated that reduction of the azides had taken place. The DS's of the products were, therefore, essentially the same as those of the parent 6-azido and 6-chloro derivatives, i.e., ~1, 0.37, and 0.22 for **4**, **4a**, and **4b**, respectively. Furthermore, as also described in the literature,[26] two stage treatment of the azide could be avoided in our system by adding Ph_3P and water simultaneously, each in slight excess, to the DMSO solution of the azide.

Ph_3P facilitated reduction of primary azides, demonstrated here for the preparation of 6-amino-6-deoxyamylose, was found to be extremely facile and proceeded to completion under neutral conditions in DMSO at room temperature. The great utility of this method should no doubt find further application in the amination of other polysaccharides.

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