A Direct Regioselective Route to 6-Azido-6-deoxy Polysaccharides under Mild and Homogeneous Conditions

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Received August 27, 1996

Introduction

We have recently reported the preparation of C-6 aminodeoxyamylose derivatives with varying degrees of substitution via triphenylphosphine (Ph_3P) facilitated reduction of the corresponding C-6 azidodeoxy precursors.[1] Known as the Staudinger reaction,[2] the ease of this reduction step, which proceeds at room temperature under neutral conditions in dimethyl sulfoxide (DMSO), prompted us to seek alternative and simpler approaches to synthesize the C-6 azido intermediates. This is usually achieved by displacement of a leaving group such as chloride, bromide, or tosyloxy with the azide ion.[3] Recently, several additional new reports described the highly selective C-6 chlorination and bromination of some polysaccharides in *N*,*N*-dimethylformamide (DMF) or *N*,*N*-dimethylacetamide (DMA)/LiCl or LiBr solvent systems with Ph_3P and *N*-chloro- or bromosuccinimide.[4−6] The latter system was also employed by us for the synthesis of 6-bromo-6-deoxyamylose.[1] As was found in our case,[7] however, such initial activation of the primary position can involve severe reaction conditions, sometimes causing significant degradation of the polysaccharide.[4−6] Therefore, a milder and direct procedure to C-6 azidodeoxy derivatives would be a useful synthetic tool in accessing the C-6 aminodeoxy analogs.

Almost two decades ago Boger et al.[8] reported that when conducted in the presence of a large excess of lithium azide (LiN_3), the reaction of Ph_3P/carbon tetrabromide (CBr_4) with cyclodextrins in DMF at room temperature yielded 6-azido-6-deoxy-α- and -β-cyclodextrins directly with high degrees of selectivity. However, no attempt to use this reaction appears to have been made for polysaccharide modification, presumably due to the poor solubility of these high molecular weight substrates. In this article, we report our preliminary results on the extension of this approach (Scheme 1) to amylose and pullulan.

Experimental Section

Materials. Amylose (Type III from potato, Sigma), carbon tetrabromide (CBr_4), lithium azide (LiN_3), triphenylphosphine (Ph_3P), and *N*,*N*-dimethylformamide (DMF) (anhydrous grade, Aldrich) were used without further purification. Pullulan was supplied by Hayashibara Laboratories, Okayama, Japan.

Procedure. All reactions were carried out using the following general procedure: The polysaccharide (0.98 g, 6 mmol as anyhdroglucose; dried in vacuum at 100 °C overnight) and LiN_3 (1.47−2.93 g, 30−60 mmol) were dissolved in DMF (30 mL) at 80 °C under a nitrogen atmosphere. Homogeneous solutions resulted within 1−1.5 h. After cooling to room temperature, Ph_3P (1.57−3.14 g, 6−12 mmol) was added and allowed to dissolve. A freshly prepared solution of CBr_4 (1.99−3.98 g, 6−12 mmol) in DMF (5 mL) was then rapidly introduced dropwise with vigorous stirring, during which time the solution turned orange-yellow in color and a slight exotherm developed. The homogeneous solutions were stirred overnight (∼18 h) at room temperature and kept under a nitrogen atmosphere throughout. Methanol (5 mL) was added, and the products were recovered by precipitation into ethanol. They were washed in ethanol overnight, filtered, and finally washed with ethanol and ethanol/water and dried at 60 °C overnight in vacuum. Yields were generally quantitative. Specific molar ratios of reactants employed and elemental analyses of the products are given in Table 1.

Scheme 1

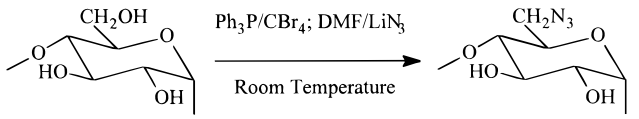

Table 1. Reaction of Ph_3P/CBr_4 with Amylose and Pullulan in DMF/LiN_3

polysaccharide	molar stoichiometry of reactants[a]	elemental analysis[b]		
amylose	1:1:1:5	39.40	5.88	10.44
amylose	1:1.5:1.5:7.5	37.76	5.59	16.63
amylose	1:2:2:10	37.54	5.13	20.31
pullulan	1:1.5:1.5:7.5	37.96	5.51	13.54

[a] Anhydroglucose unit: Ph_3P:CBr_4:LiN_3. [b] Calc for amylose: C, 44.45; H, 6.22. Calc for 6-azido-6-deoxyamylose: C, 38.51; H, 4.84; N, 22.45. Calc for 6-azido-6-deoxypullulan: C, 40.30; H, 5.26; N, 15.66.

Analyses. ^{13}C NMR spectra (50.3 MHz) were recorded in DMSO-d_6 solutions (∼5%) with tetramethylsilane (TMS) internal standard using a Varian XL-200 spectrometer. IR spectra were obtained from KBr pellets with a Nicolet 20SXB Fourier transform spectrometer. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN.

Results and Discussion

As in the case of LiCl and LiBr described earlier,[1] LiN_3 also facilitated the dissolution of amylose in DMF. Homogeneous solutions up to 5 wt % were obtained within 1.5 h at 80 °C, and these remained clear upon cooling to room temperature. Treatment of these solutions with Ph_3P/CBr_4, as described in the Experimental Section, caused a slight exotherm and a color change to orange-yellow. The reaction mixture otherwise remained homogeneous throughout. Table 1 lists the conditions employed as well as elemental analyses of the products. LiN_3 was kept at a 5-fold molar excess with respect to CBr_4 in all cases. The initial attempt, carried out using equimolar quantities of Ph_3P/CBr_4 per anhydroglucose residue, gave a product whose IR spectrum contained a strong C−N_3 stretching vibration at 2107 cm^{-1}. The highly selective nature of the substitution was then confirmed by ^{13}C NMR spectroscopy (Figure 1A), indicating a new C-6 resonance at $\delta = 51.1$ ppm, corresponding to $-CH_2N_3$[1] in addition to $\delta = 60.4$ ppm for $-CH_2OH$. The C-4 resonance at $\delta = 80$ ppm corresponds to the shift when C-6−OH becomes C-6−N_3. There was no evidence of substitution at C-2 or C-3 in the spectrum. The DS of the product (defined here as the degree of substitution at C-6 per anhydroglucose unit) was estimated to be ca. 0.45 from the NMR spectrum and elemental analysis. Two other reactions on amylose (Table 1) were then carried out using a 1.5- and 2-fold molar excess of the reagents. ^{13}C NMR spectra of these products are given in Figures 1B,C, respectively. An increase in DS to ca. 0.80 was clearly evident in the spectrum of the former case. Near complete substitution was achieved in the latter, as the spectrum of this product (Figure 1C) contained only a

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S0024-9297(96)01282-X CCC: $14.00

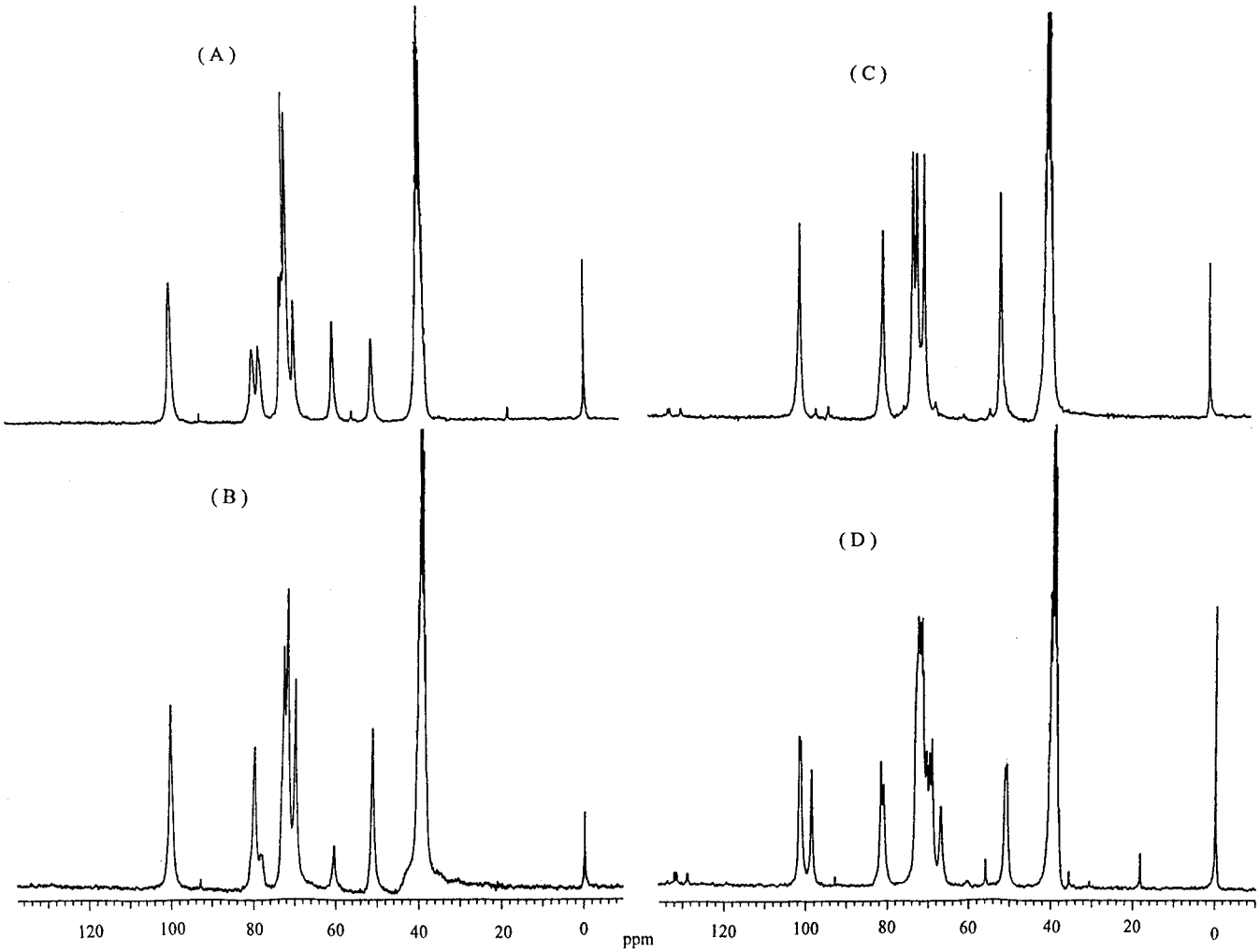

Figure 1. ^{13}C NMR spectra of (A) and (B) partially substituted C-6 azidodeoxyamylose derivatives, (C) 6-azido-6-deoxyamylose, and (D) 6-azido-6-deoxypullulan (DMSO-d_6).

weak $-CH_2OH$ resonance. Again, no clear indication of any side reactions was present in these spectra. Elemental analyses of the products also agreed with the theoretical values. Slightly lower % C and % N were attributed to equilibrium moisture in the samples since this was very difficult to remove completely.

The reaction was also successfully extended to pullulan as the substrate, as illustrated in Table 1 and Figure 1D. In this case a 1.5-fold excess of reagents was sufficient to achieve near complete azidation at C-6 without any side reaction at C-4.

Substituting CCl_4 for CBr_4 gave somewhat sluggish reactions, products of which could not be recovered by precipitation into ethanol. They showed poor solubility in DMSO and had low substitution at C-6, presumably due to much lower reaction rates with the chloride ion. It is conceivable, however, that in this case some reduction to corresponding amino derivatives might also have taken place, and this could account for the poor solubility of the products in DMSO. It was also observed that, in reactions employing CBr_4, excess Ph_3P should be avoided, as it leads to some reduction of the azide, often evidenced as difficult to recover and insoluble products. Indeed, we have preliminary indications that, upon completion of the azidation, as described in the Experimental Section, reduction can be effected by dilution of the reaction mixture with DMSO (~40 mL) followed by further treatment with Ph_3P and H_2O, as described earlier.[1] These products were insoluble and swelled in DMSO and acetic acid, and their IR spectra contained a weak $C-N_3$ stretching band.

The use of Ph_3P/CBr_4 in DMF/LiN_3 was found to be a facile route to the preparation of C-6 azidodeoxy derivatives of amylose and pullulan. The reaction is regiospecific to C-6 and proceeds readily under homogeneous conditions at room temperature and should also be applicable to other polysaccharides. Coupled with Ph_3P-facilitated reduction of such azides in DMSO,[1] it thus provides a simple two-step procedure to the C-6 aminodeoxy derivatives of polysaccharides carried out entirely under mild, homogeneous conditions at room temperature. With further refinement, this approach should allow direct access to C-6-aminated polysaccharides in a one-pot method without necessitating recovery of the intermediate azides.

References and Notes

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MA961282J