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N-Deacetylation of 2-Acetamido-2-deoxy-hexose-containing Oligosaccharides and Polysaccharides by Calcium in Liquid Ammonia

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_N_-Deacetylation of 2-acetamido-2-deoxy-hexose residues is accomplished in liquid ammonia containing calcium. Oligosaccharides, lacto-_N_-fucopentaose II and lacto-_N_-difucohexaose I, containing 3,4-disubstituted _N_-acetylhexosamine residues are quantitatively _N_-deacetylated. When applied to polysaccharides, however, only partial _N_-deacetylation was achieved.

Amino sugars, generally *N*-acetylated, are common components of natural carbohydrates. After *N*-deacetylation, the resulting amino sugar residues are starting points for specific degradations such as deamination using nitrous acid [1]. Oligosaccharides containing such groups are also suitable starting materials for other chemical modifications.

The methods used for *N*-deacetylation of carbohydrates all involve drastic conditions. The reaction can be carried out using strong base, sodium hydroxide in water or water-dimethylsulfoxide, or with hydrazine in the presence of hydrazine sulfate [1]. Trifluoroacetolysis involves treatment with trifluoroacetic anhydride and trifluoroacetic acid, and hydrolysis of the resulting *N*-trifluoroacetyl groups under mild, alkaline conditions [2].

The temperature and time needed to obtain complete *N*-deacetylation using these methods vary considerably. 2-Acetamido-2-deoxy-hexose residues linked through *O*-3 have proved to be especially difficult to *N*-deacetylate, and the *N*-deacetylation is often accompanied by severe degradation.

A method for *N*-deacetylation, using calcium in liquid ammonia, has been reported [3, 4]. We now report the use of this method on oligo- and polysaccharides.

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Materials and Methods

Materials

The human milk oligosaccharides, lacto-*N*-fucopentaose II (LNF II) and lacto-*N*-difucohexaose I (LND I) were obtained from BioCarb Chemicals (Lund, Sweden). The bacterial polysaccharides from *Escherichia coli* 06, 055; *Salmonella thompson* and *Streptococcus pneumoniae* type 4 were isolated as previously described [5-8].

Gel filtration was carried out on a Bio-Gel P-2 (Bio-Rad, Richmond, CA, USA) column (2.6 × 80 cm) eluted with pyridinium acetate buffer (0.1 M; pH 5.0), and a differential refractometer (R 403, Waters Ass.) was used for monitoring the effluents. Calcium turnings were purchased from Aldrich (Milwaukee, WI, USA) and ammonia 4.5 from AGA (Stockholm, Sweden).

Spectroscopic Methods

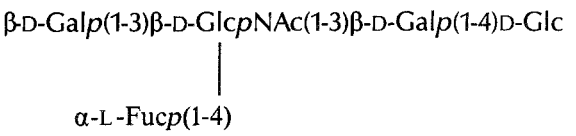
^{1}H-NMR Spectroscopy was performed on a JEOL GX-400 instrument with 2% ^2H_2O solutions at 30°C using sodium 3-trimethylsilyl-tetradeuteriopropanoate (TSP) as internal standard. For fast atom bombardment (FAB) mass spectroscopy (positive ion mode) a VG ZAB SE instrument was used. Solutions of samples in glycerol were loaded on the stainless-steel target which was bombarded with xenon atoms with a kinetic energy of 8 keV.

N-Deacetylation

A round-bottom two-neck flask fitted with a dry ice condenser was used. The flask was flushed with nitrogen and cooled with dry-ice in ethanol to -50°C. Ammonia (20 ml) was condensed in the flask and freeze-dried oligo- or polysaccharide (10 mg) was added under reflux and stirring until a clear solution was obtained. Calcium (25 mg) was added under vigorous stirring and small pieces (5 mg) were further added at intervals to maintain the dark blue colour. After 2 h, ethanol was added dropwise until the blue colour had disappeared. The ammonia was evaporated under a stream of nitrogen and the residue dissolved in 50% aqueous ethanol. Aqueous hydrogen chloride (0.5 M) was added dropwise under stirring to pH 5. The solution was concentrated and purified by gel chromatography on a Bio-Gel P-2 column to give the *N*-deacetylated product.

Results and Discussion

The human milk oligosaccharides, lacto-*N*-fucopentaose II (**1**), and lacto-*N*-difucohexaose I (**2**), both containing 3,4-disubstituted *N*-acetylglucosamine residues, were dissolved in ammonia under reflux and calcium was added to the solution. The best results were obtained when calcium was added in small pieces, just enough to maintain the dark blue colour. The reaction was terminated by addition of ethanol, the solution was concentrated to dryness and the products were purified by gel chromatography. The degree of *N*-deactylation was monitored by ^{1}H-NMR by comparing the intergrals of signals given by the *N*-acetyl group and the methyl group(s) of the fucosyl residue(s). After 2 h the reaction was complete but prolonged reaction time did not cause any degradation of the reducing oligosaccharides. The yield of the pure *N*-deacetylated



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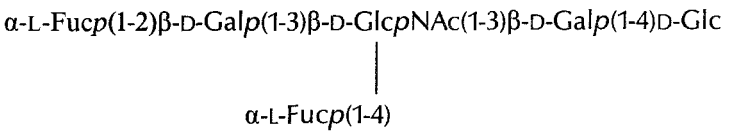

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products were almost quantitative. Their structures were confirmed by ^{1}H-NMR and FAB-MS. The FAB mass spectra of the products from LNF II and LND I gave protonated molecular ions of m/z 812.3 and m/z 958.3 respectively, these values being consistent with *N*-deacetylated products. No ions indicating unchanged starting materials were observed.

The method was also applied to some polysaccharides containing 2-acetamido-2-deoxy-hexose residues; the *O*-specific polysaccharides from *Escherichia coli* 06 [5], 055 [6], *Salmonella thompson* [7] and *Streptococcus pneumoniae* type 4 [8]. For all these polysaccharides the *N*-deacetylation was incomplete (50-90%) even when the reaction was prolonged or the treatment repeated. The same result was obtained with a decasaccharide, containing two 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, obtained by phage-enzyme catalysed hydrolysis of the O-specific polysaccharide from *S. thompson* [7]. A possible explanation of these results is that the partially *N*-deacetylated material precipitates and is thereby withdrawn from further reaction.

The method described in this paper is suitable for *N*-deacetylation of reducing oligosaccharides, whereas other methods cause severe degradation.

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