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Enzymic Regioselective Hydrolysis of Peracetylated Reducing Disaccharides, Specifically at the Anomeric Centre: Intermediates for the Synthesis of Oligosaccharides.

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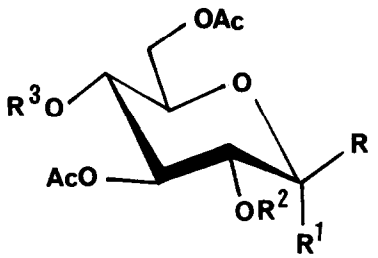
Abstract: An enzyme-catalysed reaction of peracetylated reducing disaccharides **1**, **4** and **6** gave exclusively either the corresponding heptaacetates **2**, **5**, and **7**, with the free hydroxyl group at C-1, or the hexaacetates **3** and **8**, with the hydroxyl groups at C-1,2, from the corresponding peracetates **1** and **6**.

The oligosaccharide moieties of glycoproteins and glycolipids are components of cell membranes and are known to be involved in biological processes such as cell-cell interaction and cell-virus recognition. The physiological functions of free, uncomplexed oligosaccharides are less well understood but there is a growing body of information indicating that they possess potent biological activities. This has stimulated considerable interest in the synthesis of a variety of oligosaccharides. The major challenges in oligosaccharide syntheses remain in the methodologies to generate glycosidic linkages with the desired configurations, and in devising a suitable protective-group strategy for the hydroxyl groups.[1]

The use of enzymes in the protection and deprotection of carbohydrates is now recognised.[2] In continuation of our work on enzyme-catalysed selective acetylation and deacetylation of carbohydrates and their derivatives,[3-5] we now report facile and useful enzymic reactions of peracetylated reducing disaccharides which lead to the corresponding heptaacetates, with the free hydroxyl group at the anomeric position (C-1), and the hexaacetates with the free hydroxyl groups at C-1,2 positions. The latter compounds provide potentially useful intermediates for the synthesis of 1,2-linked oligosaccharides and for functionalization at the C-2 position.

Treatment of ß-cellobiose octaacetate (**1**), ß-maltose octaacetate (**4**), and α/ß-lactose octaacetate (**6**) with a lipase from *Aspergillus niger* (Lipase A Amano 6) in a mixture of phosphate buffer (pH 7) and organic solvents at room temperature for 0.5 h afforded exclusively the heptaacetates **2**, **5**, and **7**, respectively. The products were isolated by extraction with ether or ethyl acetate to give, after removal of the solvent, crystalline **2** or **5**. In the case of **7**, the syrupy product was purified by elution from a column of silica gel with ethyl acetate. When the reaction of **1** and **6** was performed for a longer period of time, hydrolysis of a second

ester group occurred to give the corresponding hexaacetates **3** and **8**, respectively, with hydroxyl groups at C-1,2 positions (see Table). The efficacy of the enzyme was confirmed by blank experiments.



1 R = OAc; R^1 = H; R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-glucopyranosyl
2 R = R^1 = H,OH; R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-glucopyranosyl
3 R = R^1 = H,OH; R^2 = H; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-glucopyranosyl
4 R = OAc; R^1 = H; R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl
5 R = R^1 = H,OH; R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl
6 R = R^1 = H,OAc R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-galactopyranosyl
7 R = R^1 = H,OH; R^2 = Ac; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-galactopyranosyl
8 R = R^1 = H,OH; R^2 = H; R^3 = 2,3,4,6-tetra-*O*-acetyl-ß-D-galactopyranosyl

Compounds **2**, **5** and **7** have been synthesised from their corresponding octaacetates using piperidine.[6] However, the chemical method is not as efficient as the enzymic process described in this communication. In addition, prolonged treatment with piperidine leads to the corresponding 1-piperidyl-2-hydroxy-hexaacetate derivative.[6]

The physical constants of the heptaacetates **2**, **5** and **7** were in agreement to those reported in the literature.[6] The structures of the hepta- and the hexa-acetates were confirmed by 2D n.m.r. experiments. The fact that the resonances due to H-1 in **2**, **5** and **7**, and the H-1,2 resonances in **3** and **8** were shifted upfield on deacetylation, indicated that the free hydroxyl groups were located at C-1 in the heptaacetates and C-1,2 in the hexaacetate derivatives .

Table: Enzymic Regioselective Hydrolysis of Peracetylated Reducing Disaccharides using LAA[a]

Substrate	Solvent System	Reaction time	Products	Isolated yield (%)
β-Cellobiose octaacetate (**1**)	b	30 min	**2**	93
(**1**)	b	24 h	**3** (major) **2** (minor)	51 32
α/β Lactose octaacetate (**6**)	b	30 min	**7**	75
(**6**)	b	48 h	**8** (major) **7** (minor)	40 -
β-Maltose octaacetate (**4**)	b	45 min	**5**	70
(**4**)	c	30 min	**5**	95

[a] LAA = Lipase A Amano 6 from *Aspergillus niger* (donated by Amano Enzyme Europe Limited, Milton Keynes, UK), for 2 g substrate, 2 g of the enzyme was used.

[b] The total volume was 312 ml, THF: Acetone: PO_4^{-2} buffer (1:1:8.4).

[c] The total volume was 81 ml, DMF: PO_4^{-2} buffer (1:10.6).

A typical procedure for selective enzyme-catalysed deacetylation of reducing disaccharide peracetates is as follows: a solution of, for example cellobiose octaacetate (**1**, 2 g) in a mixture of acetone and THF (1:1) was added dropwise to a suspension of lipase A Amano 6 in a phosphate buffer (250 ml, pH7, 0.1M, containing 3 mM $CaCl_2$ and 0.2 mM NaCl).The reaction mixture was stirred at room temperature for 30 min. T.l.c. (ethyl acetate) revealed complete conversion to a slower moving product. The reaction mixture was extracted with ethyl acetate (2x100 ml), the organic layer dried (Na_2SO_4), concentrated and crystallized from ethanol to afford the heptaacetate **2**. When the enzymic reaction was performed for 24 h, t.l.c. showed a major product which moved slower than the heptaacetate. The reaction mixture after work up as previous described gave, after chromatography on silica gel, the hexaacetate **3** as the major and the heptaacetate **2** as the minor product (see Table).

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