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Enzymatic regioselective deprotection of peracetylated mono- and disaccharides

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Abstract

Selective enzymatic hydrolysis of the peracetylated disaccharides, namely cellobiose, lactose, maltose and melibiose, with lipase from *Asperilligus niger* in aqueous buffer and organic solvent for 30 min afforded exclusively the corresponding heptaacetates with a free hydroxyl group at C-1 in high yield. Prolonged reaction of the β-1,4 linked cellobiose and lactose peracetates afforded selectively their hexaacetates with free hydroxyl groups at C-1,2, whereas the α-1,4 linked disaccharides maltose and melibiose peracetate gave a complex mixture of products. The reaction of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetylglucopyranose (**11**) for 22 h afforded as the major product the diacetate **12** with free hydroxyl groups at C-1,4.

Keywords: Asperilligus niger lipase; Regioselective deacetylation; Disaccharide octaacetates; Disaccharide 1-hydroxy-hepta- and 1,2-dihydroxy-hexa-acetates; 2-Acetamido-2-deoxy-3,6-di-*O*-acetylglucopyranose

1. Introduction

Oligosaccharide residues of glycoproteins and glycolipids are components of biological membranes and are known to be involved in various biological processes. This has stimulated interest in the synthesis of biologically active oligosaccharides [1]. In this context, the major challenges remain in methodologies to generate glycosidic linkages with specific configurations, and in the regioselective protection and deprotection of the hydroxyl groups.

Selective acetylation and deacetylation of carbohydrates have been achieved using chemical [2–6] and enzymatic processes [7]. We have recently described the use of hydrazine hydrate for selective removal of the acetate group from C-1 position in mono- and disaccharide peracetates [6]. However, the reagent was not found to be selective for deacetylation at positions other than C-1. The enzyme-catalysed esterifica-

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tion of carbohydrates has been reported to occur mainly at primary hydroxyl groups [8–10]. Under forcing conditions acetylation at a secondary position has also been observed. For example, treatment of sucrose with isopropenyl acetate in pyridine at 60°C in the presence of Lipase P Amano gave 6-*O*-acetyl-(33%) and 4′,6-di-*O*-acetyl-(8%) sucrose [11].

The enzymatic deacetylation reactions of carbohydrate peracetates have been studied by Hennen et al. [12]. Lipases from *Aspergillus niger* was found to selectively remove the anomeric acetate group from 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose. Little or no deacetylation occurred at other positions. Peracetylated methyl furanosides were monodeacetylated at the primary position by using *Candida cylindraceae* lipase. The acetate group at the anomeric position in 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**11**) was hydrolysed by lipase from porcine pancreas but with low reaction rate. No appreciable deacetylation was observed with peracetylated *N*-acetylglucosammine having the anomeric position glycosylated [12]. Maltose and cellobiose octaacetates, using a series of 10 hydrolases, have been reported to give the corresponding heptaacetates with the free hydroxyl group at the anomeric position [13,14], lipase from *A. niger* leading to the highest reaction rate and selectivity.

Following our preliminary communication [13], we now report the full work on enzyme-catalysed deacetylation of peracetylated reducing disaccharides to afford 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 functionalisation at the C-2 position. It is noteworthy that the selectivity of lipase from *A. niger* towards the secondary ester group of some β-1,4 linked disaccharides at the C-2 position, has not been reported previously, since other studies have indicated that the primary ester at the 6 position reacts faster in other peracetylated sugars [14]. In addition, we report a facile method for regioselective deprotection of peracetylated 2-acetamido-2-deoxy-D-glucopyranose leading to the corresponding diacetate with free hydroxyl groups at C-1,4 positions. Such a compound would be potentially useful for the synthesis of $1 \rightarrow 4$ linked oligosaccharides, for example, *N*-acetyllactosamine.

2. Experimental

2.1. Materials and methods

Lipase Amano A6 from *A. niger* (EC 3.1.1.3) was donated by the Amano Pharmaceutical Milton Keynes, UK. The efficacy of the enzyme was confirmed by blank experiments. In all the experiments, the enzyme was employed dissolved in 0.1 M phosphate buffer pH 7 containing 3 mM $CaCl_2$ and 0.2 mM NaCl. Spectra

Table 1
Experimental conditions and results of enzymatic deacetylation

Substrate	Products	Time (h)	Organic cosolvent	Solvent/buffer (v/v)	Isolated yield (%)
1	**2**	0.5	Acetone–THF[a]	1:5	93
1	**3**	24	Acetone–THF[a]	1:5	51
	2				
4	**5**	0.5	Acetone–THF[a]	1:5	75
4	**6**	6	CH_3CN	1:10	42
	5				
7	**8**	1	DMF	1:10	95
9	**10**	0.75	DMF	1:10	54
11	**12**	22	CH_3CN	1:10	41

[a](1:1 v/v).

Table 2
Melting point and optical rotation of products

Products	**2**	**3**	**5**	**6**	**8**	**10**	**12**
Melting point (°C)	206–209	196–198	86–87	87–90	188–190	196–197	syrup
$[\alpha]_D^{22}$ ($CHCl_3$)	+27.0° (c = 1.0)	+45.2 (c = 1.0)	+32.8° (c = 1.0)	+41.4° (c = 1.1)	+81.9° (c = 1.1)	+92.2° (c = 1.0)	−14.9° (c = 1.1)

were recorded at 297 K at 200.13 and 300.13 MHz, respectively using Bruker AC 200 and AM 300 WB spectrometers. Spectra were recorded in $CDCl_3$ For the assignment of the signals in ^{1}H NMR spectra, 2D homonuclear correlated spectroscopy COSY-45, 1D TOCSY experiments and homodecoupling were used.

Optical rotations were measured using a Perkin-Elmer 141 polarimeter in 1 dm tubes at 22°C. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Mono- and disaccharide peracetates were prepared by acetylation of the parent sugar with acetic anhydride and sodium acetate. Peracetylated disaccharides, and 2-acetamido-2-deoxyglucopyranose tetraacetate were crystallised from acetone–diethyl ether.

2.2. Enzymatic deacetylation

In a typical reaction 1.0 g of peracetylated sugar dissolved in organic solvent was added dropwise to a phosphate buffer solution containing the lipase (1.0 g), the final volume of the reaction mixture being of 155 ml. The ratios of organic cosolvent and phosphate buffers are indicated in Table 1. The reaction mixture was stirred at room temperature and monitored by thin layer chromatography (ethyl acetate) with detection by charring with ethanolic 5% H_2SO_4.

Reaction products were separated by extracting the reaction mixtures with ethyl acetate (products **2**, **8**, **10**) or diethyl ether (products **3**, **5**, **6**). The combined extracts were dried over Na_2SO_4, and the solvent was evaporated. Products **2** and **8** were directly crystallised from acetone–diethyl ether. Compound **3** was purified by silica gel column chromatography using acetone–CH_2Cl_2 (4:1 v/v) as eluent and crystallised from acetone–diethyl ether. Product **5** and **6** were purified by silica gel column chromatography using petroleum ether–acetone (3:2 v/v) as eluent. Compound **10** was purified by silica gel chromatography (acetone–petroleum

Table 3
^{1}H NMR chemical shift and coupling constant data of acetylated derivatives of 2-acetamido-2-deoxy D-glucose

δ

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	NH	OH	CH_3–CO–
11	6.13	4.45	5.19	5.19	3.96	4.21	4.03	5.63		2.16, 2.05, 2.02, 2.01, 1.90
12α	5.21	4.14	5.18	3.75	4.17	4.40	4.40	[a]	[a]	2.15, 2.12, 2.00
12β	4.90	3.82	5.03	3.75	3.78	4.35	4.29	[a]	[a]	2.15, 2.12, 1.98

Coupling constants J/Hz

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6,6b}$	J_{2NH}
11	3.6	10.5	[a]	[a]	4.0	2.4	12.5	9.1
12α	3.5	10.4	9.4	10.2	[a]	[a]	[a]	[a]
12β	8.5	10.5	9.0	[a]	[a]	[a]	[a]	[a]

[a] Not determined.

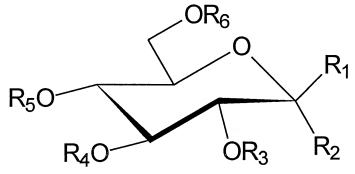
ether 1:2 v/v) and crystallised from acetone–diethyl ether.

The physical constants and ^{1}H NMR data for products **2**, **3**, **5**, **6**, **8**, and **10** were identical to those reported in the literature [6].

In case of product **12**, the acetonitrile was removed from the reaction mixture under reduced pressure at room temperature. The resulting aqueous solution was lyophilised and the product was purified by eluting the dry residue through a column of silica gel with acetone–petroleum ether (1:2 v/v). The ^{1}H NMR spectrum showed upfield shifts of 0.93, 1.24, and 1.44 for resonances H-1α, H-1β and H-4 consistent with deacetylation at C-1,4 and identifying the products as the 1,4-dihydroxy diacetate **12** (Tables 2 and 3).

3. Results and discussion

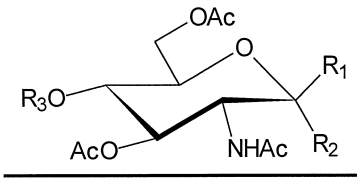
Treatment of the octaacetates of cellobiose (**1**), lactose (**4**), maltose (**7**) and melibiose (**9**) with a lipase from *A. niger* (Lipase A Amano 6) in a mixture of phosphate buffer (pH 7) and organic solvents at room temperature for 30 min caused the selective removal of the acetate at the anomeric (C-1) position to give exclusively the heptaacetates **2**, **5**, **8**, and **10**, respectively in high yield. This may have been predicted as the anomeric acetoxy groups would be expected to be the most reactive towards hydrolysis. Surprisingly, when the reactions of the β-1,4 linked disaccharides **1** and **4** were performed for a longer period of time, further deacetylation occurred exclusively in the reducing sugar moiety



Compound		R1	R2	R3	R4	R5	R6
Cellobiose octaacetate	(1)	OAc	H	Ac	Ac	[a] β-D-Glc*p*2,3,4,6Ac$_4$	Ac
" heptaacetate	(2)	H	OH	Ac	Ac	[a] β-D-Glc*p*2,3,4,6Ac$_4$	Ac
" hexaacetate	(3)	H	OH	H	Ac	[a] β-D-Glc*p*2,3,4,6Ac$_4$	Ac
Lactose octaacetate	(4)	OAc	H	Ac	Ac	[b] β-D-Gal*p*2,3,4,6Ac$_4$	Ac
" heptaacetate	(5)	H	OH	Ac	Ac	[b] β-D-Gal*p*2,3,4,6Ac$_4$	Ac
" hexaacetate	(6)	H	OH	H	Ac	[b] β-D-Gal*p*2,3,4,6Ac$_4$	Ac
Maltose octaacetate	(7)	OAc	H	Ac	Ac	[c] α-D-Glc*p*2,3,4,6Ac$_4$	Ac
" heptaacetate	(8)	H	OH	Ac	Ac	[c] α-D-Glc*p*2,3,4,6Ac$_4$	Ac
Melibiose octaacetate	(9)	H	OAc	Ac	Ac	Ac	[d] α-D-Gal*p*2,3,4
" heptaacetate	(10)	H	OH	Ac	Ac	Ac	[d] α-D-Gal*p*2,3,4

[a] β-D-Glc*p*2,3,4,6Ac$_4$ = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl; [b] β-D-Gal*p*2,3,4,6Ac$_4$ = 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl; [c] α-D-Glc*p*2,3,4,6Ac$_4$ = 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl; [d] α-D-Gal*p*2,3,4,6Ac$_4$ = 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl

Scheme 1.



	R1	R2	R3
***N*-acetylglucosamine tetraacetate (11)**	H	OAc	Ac
" **triacetate (12)**	H	OAc	H

Scheme 2.

at C-2 to give **3** and **6**, respectively, and not at either of the less hindered primary positions (C-6,6′) (Scheme 1).

Maltose (**7**) and melibiose (**9**) octaacetates, being α-1,4 and β-1,6 linked disaccharides, respectively, did not show the same selectivity. On prolonged treatment, they gave a complex mixture of products. The nature of the glycosidic linkage and the presence of the free 1-OH group in the disaccharide appear to play an important role in the interaction of the enzyme with the substrate and in the mechanism of deacetylation reaction. A possible mechanism for the formation of the 1,2-hydroxy compounds **3** and **4** from the β-1,4 linked disaccharides **1** and **5**, could be that, in the initial step of the enzymatic reaction, the 1-OH group binds in close proximity to the receptor site which then allows the acetate group at the C-2 position to interact with the receptor site and then to exchange the acetyl group ($CH_3C=O$) with a proton.

The reaction of 2-acetamido-2-deoxy-β-D-glucopyranose tetraacetate (**11**) with lipase for 2 h gave the corresponding 1-OH deprotected derivative, as previously reported [12], whereas after 22 h afforded the corresponding 1,4-dihydroxy compound **12** as the major product (Table 1) (Scheme 2).

It is of interest to note that the formation of the expected 3,4-di-*O*-acetyl-2-acetamido-2-deoxy-α/β-D-glucopyranose was not observed. However, in this case, it is possible that initial deacetylation occurred at C-6 followed by 4 → 6 acetyl migration; such an acetyl migration is well-known to occur under acidic or basic conditions in organic reactions of carbohydrate acetates [15,16].

4. Conclusions

Reducing peracetates **1**, **4**, **7**, and **9** in aqueous buffer solution using *A. niger* lipase for 30 min gave products exclusively deacetylated at C-1. On prolonged treatment, unlike the α-1,4 (**7**) and β-1,6 (**9**) linked disaccharides, the β-1,4 linked disaccharides **1** and **4** showed further selectivity i.e., the second deacetylation in the reducing sugar moiety occurred at C-2.

Peracetylated *N*-acetylglucosamine (**11**) was deacetylated by *A. niger* lipase preferentially at the C-1 and C-4 positions after 22 h reaction.

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