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# Enzymatic procedure catalysed by lipase from *Candida antarctica* for the regioprotection–deprotection of glucosamine

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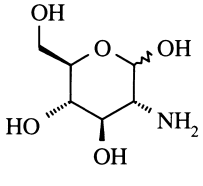
**Abstract**

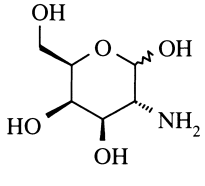
Both peracetyl α-glucosamine **3** and its β-epimer **4** undergo alcoholysis with butanol in THF, catalysed by lipase from *Candida antarctica* (Novozym 435) to afford the triester **6** and diester **9**, respectively. Esterification of these compounds, using vinyl acetate in the presence of Novozym 435, gives the partial esters **10** and **11**, bearing a deprotected hydroxyl function at C-4, ready for inversion of configuration to open a route in the preparation of galactosamine. 

## 1. Introduction

Aminosugars are an important class of natural compounds occurring as subunits in many biological mediators, including heparin, an anticoagulant agent present in blood,[1] or endotoxins, lipopolysaccharides of bacterial origin responsible for pathological effects, such as the 'lethal septic shock'.[2] The naturally occurring 2-aminosugars are glucosamine **1** and the less widespread galactosamine **2**.




**1**




**2**

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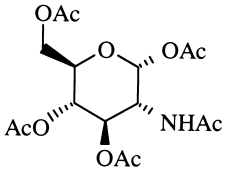
Hydrolases are known to be valid catalysts in the selective hydrolysis or esterification of carbohydrates.[3] Nevertheless, only in a few cases has this methodology been applied to aminosugars. Among their successful uses, we recall here the hydrolysis of the ester function at C-1 for both peracetyl β-glucosamine and peracetyl β-mannosamine by the use of porcine pancreas lipase in DMF/phosphate buffer.[4] Hydrolysis of the acetate group at C-6 has been obtained for peracetyl α-glucosamine, in the presence of an esterase obtained from *Rodosporidium toruloides* but not commercially available.[5] On the other hand, esterification at the same position has been obtained for *N*-acetyl-D-mannosamine working with various proteases in organic solvents.[6]

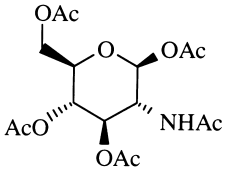
In order to have access to a series of selectively protected derivatives of glucosamine **1** we examined alcoholysis of the relevant peracetates using Novozym 435, a commercially available lipase from *Candida antarctica* immobilized on matrix, possessing good stereoselectivity.

## 2. Results and discussion

In a preliminary screening, peracetyl glucosamine [mixture of (+)-α-(**3**) and (+)-β-(**4**)] was subjected to alcoholysis with butanol in THF for 24 h, in the presence of one of the following lipases, namely from *Aspergillus niger*, *Aspergillus orizae*, *Candida antarctica* type A, *Candida rugosa*, *Mucor javanicus*, *Mucor miehei*, *Pseudomonas cepacia*, *Rizophus javanicus* and porcine pancreas lipase. Among them, only lipase from *Candida antarctica* (Novozym 435) proved to be active and was therefore selected for a closer examination of the regioselectivity of the reaction on the glucosamine epimers, peracetyl α-glucosamine **3** and peracetyl β-glucosamine **4**.




**3**

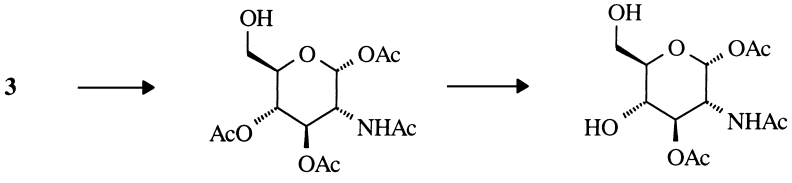



**4**

### 2.1. Enzymatic alcoholysis of peracetyl α-glucosamine *3*

Ester **3**, subjected to Novozym catalyzed alcoholysis with butanol, afforded a single product after 48 h which was isolated by chromatography in 35% yield, and subsequently identified by $^1$H NMR spectroscopy as 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside **5**.

When alcoholysis was protracted for 96 h, along with **5** (yield 36%), a second compound was isolated (yield 24%), and identified as 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside **6**. Since we have never observed the presence, among the reaction products, of the partial ester deriving from hydrolysis at C-4, it is apparent that **6** originates from partial ester **5**, in which position C-4 is more accessible following the removal of the acetate group at C-6.

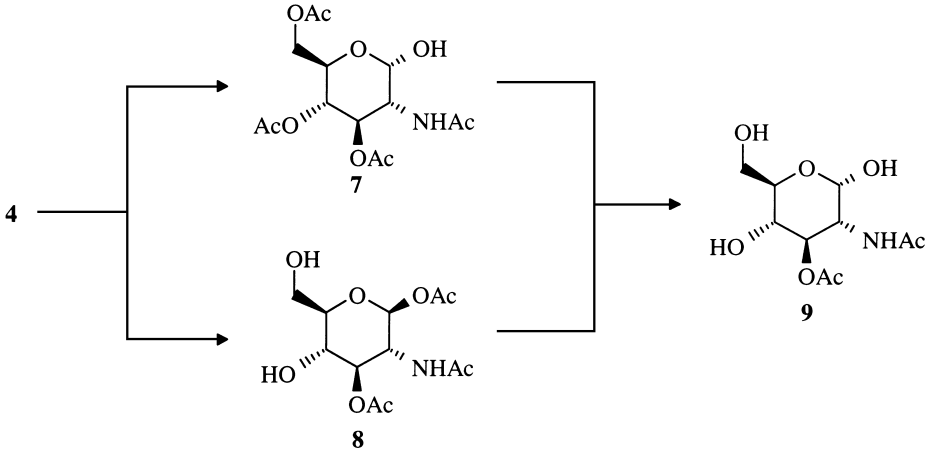



**5** **6**

Further prolongation of the reaction time (10 days) resulted in the complete conversion of the starting material into **6**, isolated by column chromatography in 96% yield. Hydrolysis does not proceed beyond the formation of **6** even after longer incubation.

### 2.2. *Enzymatic alcoholysis of peracetyl β-glucosamine **4***

When the alcoholysis was carried out on **4** under the same conditions used for the epimer **3**, compound **7** was isolated after 48 h incubation as the main product (yield 32%), along with trace amounts of minor products. The $^1$H NMR spectrum of **7**, whose FABMS spectrum shows a quasi-molecular ion at 348 *m*/*z*, contained signals for four acetate groups. The H-1 resonance showed a remarkable shift ($\Delta\delta$=0.43 ppm), as compared to the corresponding signal of **4**; in addition, a coupling constant value of $J_{1,2}$=3.4 Hz, only compatible with an axial–equatorial coupling,[7] was measured, thus indicating that an inversion of the configuration at C-1 had occurred. These data revealed a regioselective deprotection at C-1, establishing **7** as 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside.




When the incubation time was prolonged to four days, two additional compounds, **8** (yield 10%) and **9** (yield 10%), were isolated in addition to **7** (yield 31%) from the reaction mixture. Spectroscopic analysis (FABMS and $^1$H NMR) established **8** as an isomer of **5**, arising from deprotection of the ester functions at C-4 and C-6 of the parent compound, and therefore it was assigned the structure of 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside.

In the $^1$H NMR spectrum of the second minor product, **9**, decidedly more polar than **7** and **8**, only two signals for acetate groups were present and in the low-field region significant shifts were seen for the signals of H-1 ($\Delta\delta$=0.61 ppm), H-4 ($\Delta\delta$=1.51) and H-6 ($\Delta\delta$=0.39 ppm) in comparison with the corresponding peaks in the spectrum of **4**. Moreover, the coupling constant measured for the H-1 resonance (4.99 ppm, d, $J_{1,2}$=3.5 Hz) also indicated in this case an inversion of the configuration at C-1 and consequently **9** could be assigned the structure of 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside.

From the data above it can be inferred that in the alcoholysis of **4**, Novozym has a moderate regioselectivity and two compounds **7** and **8** are initially formed. These, which bear ester functions on carbons of suitable configuration, become substrates for the enzyme and are converted into **9**. This was the only product (yield 95%) when the reaction was protracted for 10 days.

Table 1
Enzymatic alcoholysis with butanol of **3** and **4**[a]

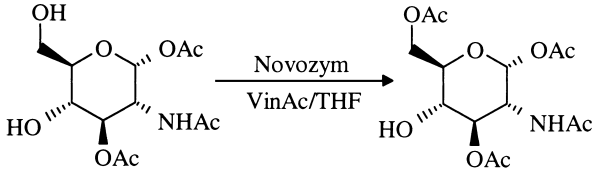
| Substrate | Time (h) | Recovered product (yield %) | Deprotected Position |
|---|---|---|---|
| Peracetyl α–glucosamine **3** | 48 | **5** (α) (35) | 6 |
| " | 96 | **5** (α) (36)<br>**6** (α) (24) | 6<br>4, 6 |
| " | 240 | **6** (α) (95) | 4, 6 |
| Peracetyl β–glucosamine **4** | 48 | **7** (α) (32) | 1 |
| " | 96 | **7** (α) (31)<br>**8** (β) (10)<br>**9** (α) (10) | 1<br>4, 6<br>1, 4, 6 |
| " | 240 | **9** (α) (96) | 1, 4, 6 |

[a] Substrate: 1 g; Enzyme 3 g: Butanol 3 mL; THF 40 mL; T 45°C; 300 rpm

For the sake of an easy comparison, the pertinent data for the enzymatic alcoholysis of **3** and **4** are summarised in Table 1.

### 2.3. *Enzymatic esterification of **6** and **9***

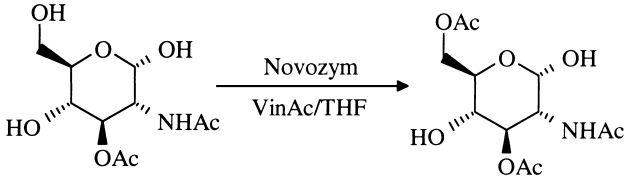
In order to prepare a partial ester of glucosamine with a single unprotected hydroxyl at C-4, not accessible by alcoholysis, compound **6** was esterified in THF with vinyl acetate in the presence of Novozym. The reaction was very fast, being completed in 15 min, to give the desired 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside **10**, whose structure was confirmed by spectroscopic analysis.




**6** **10**

Formation of peracetyl α-glucosamine was not observed, even after protracted incubation. This can be explained by two concurrent factors: lower reactivity of the secondary hydroxyl and steric hindrance after the esterification of the primary hydroxyl.

Under the conditions above, compound **9** undergoes a rapid (15 min) conversion, affording a single product (yield 95%) which, on the basis of spectral data, was assigned the structure of 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside **11**. In this case the α-configuration of the C-1 hydroxyl does not allow enzymatic recognition, and a derivative with both C-1 and C-4 free hydroxyl is obtained.




**9** **11**

Compounds **10** and **11** can undergo inversion of configuration at C-4 according to standard procedures[5,8] and are useful synthetic precursors of galactosamine, a compound more valuable than its epimer, glucosamine.

## 3. Conclusions

The results obtained show that the commercially available lipase *Candida antarctica* (Novozym 435) can be used profitably to catalyse alcoholysis with butanol of both peracetyl α-glucosamine and peracetyl β-glucosamine. The reaction is regioselective and its course is related to the configuration of the C-1 carbon atom. The α-epimer **3**, and the β-epimer **4**, undergo preferential alcoholysis of the ester group at C-6 and C-1, respectively. Under forcing conditions the reaction causes complete removal of the acetates at C-4,6 for compound **3**. The epimer **4**, subjected to prolonged incubation, undergoes partial deprotection at C-4,6 and at C-1,4,6, this latter product being the only one when the reaction is prolonged up to the exhaustion of the substrate.

The primary hydroxyl in partial esters **6** and **9** can be easily acetylated with vinyl acetate in the presence of Novozym to give compounds **10** and **11**, useful as starting materials for the preparation of galactosamine.

## 4. Experimental

### 4.1. Materials and methods

$^1$H NMR spectra were recorded at 250 MHz on a Bruker AC 250 instrument. Mass spectra (FAB) were recorded on a Kratos MS 50 instrument using dinitrobenzyl alcohol as matrix. Optical rotations were measured at 25°C on a Jasco 135 instrument. Melting points were determined using a Kofler apparatus. All reactions were monitored by thin-layer chromatography (TLC) carried out on Merck 60 $F_{254}$ plates and using anisaldehyde–sulfuric acid[9] as chromogenic reagent. Column chromatography was on a Si gel (Merck, 0.063–0.200 mm, gradient of THF in $Et_2O$ as the eluent).

Lipases from *Aspergillus niger, Aspergillus orizae, Candida rugosa, Mucor javanicus, Rizophus javanicus* and porcine pancreas lipase were purchased from Sigma. *Pseudomonas cepacia* lipase was a gift from Amano Co. *Candida antarctica* and *Mucor miehei* lipases were a gift from Novo Nordisk.

### 4.2. Preparation of peracetyl α-glucosamine *3* and peracetyl β-glucosamine *4*

The epimeric peracetyl derivatives **3** and **4** were prepared according to standard procedures.[10] The products were recrystallised from ethanol to give pure peracetyl α-glucosamine **3**, mp 135–136°C (lit.[5] 136–137°C); $[\alpha]_D$ +89.4 (*c* 1, $CHCl_3$) (lit.[11] $[\alpha]_D$ +92.0), and peracetyl β-glucosamine **4**, mp 185–188°C (lit.[10] mp 187°C), $[\alpha]_D$ +1.2 (*c* 1, $CHCl_3$) (lit.[10] $[\alpha]_D$ +1.0).

### 4.3. Enzymatic alcoholysis of *3* and *4*

Butanol (3 mL) and Novozym 435 (3 g) were added to a solution of the substrate (1 g of **3** or **4**) in THF (40 mL) and the mixture was shaken (300 rpm) at 45°C. At the end of the incubation (96 h) reaction was quenched by filtering off the enzyme and the filtrate was evaporated in vacuo.

*4.3.1. Chromatographic purification of the residues from **3** gave **5** and **6***

(+)-2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside **5**: oil (320 mg, yield 36%); $[\alpha]_D$ +61.7 (*c* 1, $CHCl_3$) (lit.[5] $[\alpha]_D$ +57.0). FABMS and $^1H$ NMR data are in agreement with those reported in the literature.[5] (+)-2-Acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside **6**: white solid (186 mg, yield 24%), mp 167–170°C, $[\alpha]_D$ +80.1 (*c* 1, $CHCl_3$). FABMS and $^1H$ NMR data are in agreement with those reported in the literature.[5]

*4.3.2. Chromatographic purification of the residues from **4** gave **7**, **8** and **9***

(+)-2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside **7**: oil (276 mg, yield 31%), $[\alpha]_D$ +52.2 (*c* 1, $CHCl_3$). FABMS and $^1H$ NMR data are in agreement with those reported in the literature.[4] (−)-2-Acetamido-1,3-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside **8**: oil (80 mg, yield 10%) $[\alpha]_D$ −8.8 (*c* 1, $CHCl_3$). $^1H$ NMR (250 MHz, $CDCl_3$): 1.92 (s, 3H, NHAc), 2.09 (s, 6H, 3-Ac and 4-Ac), 2.11 (s, 3H, 1-Ac), 3.58 (m, 1H, H-5), 3.77–3.88 (m, 3H, H-4 and H-6), 4.35 (ddd, *J*=10.0, 9.6, 8.7 Hz, 1H, H-2), 5.10 (dd, *J*=10.0, 9.5 Hz, 1H, H-3), 5.69 (d, *J*=8.7 Hz, 1H, H-1), 6.52 (d, *J*=9.6 Hz, 1H, NH). FABMS: $[M+H]^+$ at *m*/*z* 306. Anal. calcd for $C_{12}H_{19}NO_8$: C, 47.21; H, 6.27; N, 4.59; found: C, 47.10; H, 6.22; N, 4.60. (+)-2-Acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside **9**: solid (70 mg, yield 10%), mp 155–157°C, $[\alpha]_D$ +22.4 (*c* 1, EtOH). $^1H$ NMR (250 MHz, $CD_3OD$): 1.93 (s, 3H, NHAc), 2.01 (s, 6H, 3-Ac and 4-Ac), 3.58 (dd, *J*=9.7, 9.6 Hz, 1H, H-4), 3.78 (m, 2H, H-6), 3.86 (m, 1H, H-5), 4.06 (dd, *J*=10.7, 3.5 Hz, 1H, H-2), 5.08 (d, *J*=3.5 Hz, 1H, H-1), 5.20 (dd, *J*=10.7, 9.7 Hz, 1H, H-3). FABMS: $[M+H]^+$ at *m*/*z* 264. Anal. calcd for $C_{10}H_{17}NO_7$: C, 45.63; H, 6.51; N, 5.32; found: C, 44.50; H, 6.45; N, 5.36.

*4.4. Enzymatic esterification of **6** and **9***

Novozym 435 (700 mg) was added to a solution of the substrate (200 mg, 0.07 mmol of **6**; or 0.07 mmol of **9**) in THF (20 mL) containing vinyl acetate (0.4 mL, 0.5 mmol) and the suspension was kept at 45°C under stirring (300 rpm). After 15 min the reaction was quenched, the enzyme filtered off, and the filtrate was taken to dryness.

*4.4.1. Chromatographic purification of the residues from **6** gave **10***

(+)-2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside **10**: oil, (215 mg, yield 96%), $[\alpha]_D$ +60.3 (*c* 1, $CHCl_3$) (lit.[5] $[\alpha]_D$ +62.0). FABMS and $^1H$ NMR data are in agreement with those reported in the literature.[5]

*4.4.2. Chromatographic purification of the residues from **9** gave **11***

(+)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside **11**: oil (220 mg, yield 95%), $[\alpha]_D$ +33.5 (*c* 1, EtOH). $^1H$ NMR (250 MHz, $CD_3OD$): 1.92 (s, 3H, NHAc), 2.03 (s, 6H, 3-Ac and 4-Ac), 2.05 (s, 3H, 1-Ac), 3.53 (dd, *J*=9.8, 9.3 Hz, 1H, H-4), 4.02–4.09 (m, 2H, H-2 and H-5), 4.22 (dd, *J*=11.8, 5.2 Hz, 1H, H-6), 4.35 (dd, *J*=11.8, 2.2 Hz, 1H, H-6), 5.03 (d, *J*=3.5 Hz, 1H, H-1), 5.18 (dd, *J*=10.7, 9.3 Hz, 1H, H-3). FABMS: $[M+H]^+$ at *m*/*z* 306. Anal. calcd for $C_{12}H_{19}NO_8$: C, 47.21; H, 6.27; N, 4.59; found: C, 47.08; H, 6.24; N, 4.63.

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