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ESTERASE CATALYZED REGIOSELECTIVE HYDROLYSES OF ACETYLATED MONOSACCHARIDES

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Abstract: Deacylations of fully acylated D-glucopyranoses, methyl D-glucopyranosides, 2-acetamido-2-deoxy-D-glucopyranoses and methyl 2-acetamido-2-deoxy-D-glucopyranosides catalyzed by rabbit serum or the esterase isolated from rabbit serum were investigated. Depending on the structure of the acyl protecting group a high degree of regioselectivity is observed. Products of enzymic hydrolysis followed by intramolecular migrations of acetyl groups are also described.

INTRODUCTION

Due to the synthetic challenge that multifunctional sugars offer, enzymatic techniques for the introduction of blocking groups into carbohydrates and/or their subsequent removal have been recently developed[1,2]. Thus it was shown that esterases and lipases may be used not only as catalysts in regioselective acylations[3-11] and deacylations[12-15] of sugars but that chemoselective deprotections could be carried out as well[16,17].

In continuation of our work on enzymic regioselective deacylations of acylated sugars with esterases from mammalian sera[18-23] we prepared a series of ^{14}C-labelled and unlabelled acetylated monosaccharides and used them as novel substrates for esterases in rabbit serum (Chart 1.). Acetyls are commonly used as protective groups in carbohydrate chemistry since they are easily introduced into the molecule and in high yields. The disadvantage of this group is that it is labile under basic conditions, prone to intramolecular migrations and that a very low degree of selectivity is observed during the deprotection process. The aim of this work was to study esterase catalyzed regioselective deacetylations of fully acetylated monosaccharides. Furthermore, the possible selectivity in hydrolysis reactions of substrates containing a combination of acetyl and other acyl groups was also studied.

RESULTS AND DISCUSSION

Preparation of the starting materials

The peracetylated α-D- and β-D-glucopyranoses (**1**), methyl α-D-glucopyranoside (**4α**) and methyl 2-

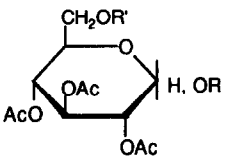
acetamido-2-deoxy-α-D-glucopyranoside (**5α**) were prepared by a standard acetylation procedure with acetic anhydride in pyridine (1:1). Compounds protected with a combination of acetyl and pivaloyl groups (**2**, **14** and **16**) were prepared according to previously published results[23,24].

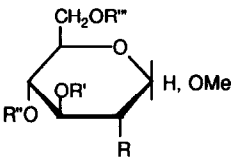
^{14}C-labelled compounds were synthesized starting from [U-^{14}C]glucose and using a modification of the procedure reported for ^{14}C-labelled pivalates[18,21].

Esterase catalyzed deacylations

As previously reported mammalian sera possess various enzymic activities and can catalyze the deesterification of *O*-pivaloyl monosaccharides[18,19,21] as well as cause intramolecular transesterification of partially pivaloylated substrates[20]. Isolation and partial purification of esterases with specificity for sugar substrates from rabbit serum was also recently reported[22]. Whole rabbit serum or the esterase isolated from rabbit serum were also used in this work as sources of enzymic activity.

Chart 1





1	R = R' = Ac
2	R = Piv; R' = Ac
3	R = H; R' = Ac

4	R = OAc; R' = R'' = R''' = Ac
5	R = NHAc; R' = R'' = R''' = Ac
6	R = OAc; R' = R''' = Ac; R'' = H
7	R = OAc; R' = H; R'' = R' = Ac
8	R = OAc; R' = R''' = Ac R'' = Piv
9	R = OAc; R' = Piv; R'' = R''' = Ac
10	R = NHAc; R' = R''' = Ac; R'' = H
11	R = NHAc; R' = H; R'' = R''' = Ac
12	R = NHAc; R' = R''' = Ac; R'' = Piv
13	R = NHAc; R' = Piv; R'' = R''' = Ac
14	R = NHAc; R' = R'' = Ac; R''' = Piv
15	R = NHAc; R' = R'' = H; R''' = Ac
16	R = NHAc; R' = R'' = Piv; R''' = Ac

Both anomers of the peracetylated D-glucopyranose **1** were deacetylated by rabbit serum (RS) or the esterase isolated from rabbit serum (RE), primarily at the anomeric centre, producing the tetraacetates **3** in 55-75% yield. The ^{14}C-labelled pentaacetates **1** were used in time-dependence studies using again RS or RE as sources of enzymic activity (Table 1).

Table 1. Esterase-Catalyzed Deacetylations of **1β**[a]

Time (h)	Serum			Esterase		
	1β	**3**	Other	**1β**	**3**	Other
1	53	31	16	73	16	11
2	25	60	15	63	25	12
3	9	75	16	50	34	16
8	0	35	65	23	52	25
24	0	5	95	8	38	54

[a] Analysis based on radioactivity and given as a percentage of the total recovered after t.l.c.

1β = 1,2,3,4,6-penta-*O*-acetyl-β-**D**-[U-^{14}C]glucopyranose

3 = 2,3,4,6-tetra-*O*-acetyl-**D**-[U-^{14}C]glucopyranose

Other = mixture of partially and totally deacetylated products

The main product of hydrolysis catalyzed by whole serum after 3 hrs was isolated by column chromatography and submitted to pivaloylation. After chromatography the product was shown to be (by ^{1}H-NMR, t.l.c and comparison to independently synthesized product) a 2:1 mixture of 2,3,4,6-tetra-*O*-acetyl-β- and α-**D**-glucopyranoses. No significant differences in product ratios were observed when the α-**D**-pentaacetate (**1α**) was used as a substrate instead of the β-anomer in enzyme catalyzed hydrolyses described in Table 1. Again, preferential formation of **3** was observed but even more in favor of the β-anomer (β:α = 16:1). These results indicate that preferential hydrolysis occurs at the anomeric centre thus giving a product with a free 1-OH under mild, neutral reaction conditions. Therefore, this may be a reaction of choice for preparing some sugar conjugates at the anomeric centre followed by further chemical or enzymic deacetylation of the whole sugar part.

Under the same reaction conditions the substrate protected with a combination of different acyl groups, i.e. acetyls and pivaloyls (**2β**) underwent again preferential hydrolysis at the anomeric centre. Thus, the 1-*O* Piv group in **2β** was hydrolyzed in 5h at 37°C in PBS with rabbit serum as a source of catalytic activity to produce the 2,3,4,6-tetra-*O*-acetyl-**D**-glucopyranose (**3**) in 66% yield. The same product was isolated when a purified esterase was used as a catalyst, in 33% yield within 12 h.

Previous results showed that, independent of the enzyme source or the type of acyl protecting group the anomeric ester is generally cleaved most readily in the presence of both primary and secondary esters[1]. These

results are in accordance with data presented so far in this work. Furthermore, it was also found that selective hydrolysis of the esters of *N*- and *O*-glycosides occurs predominantly at the primary position with only a few exceptions[1], all of them on acetylated sugars. The result of these anomalous hydrolyses was the cleavage of the 3-*O* Ac group[3] . We therefore proceeded by experimenting with peracetylated methyl glycopyranosides **4** and **5.** Unexpectedly, hydrolysis of these products with rabbit serum or the esterase from rabbit serum led to the isolation of a mixture of products, none of them showing the primary hydroxyl group (6-OH) to be free. Thus, hydrolysis of **4α** followed by pivaloylation gave a mixture of the 4-*O* Piv (**8α**) and 3-*O* Piv (**9α**) triacetates and hydrolysis of **5α** followed by pivaloylation resulted in a mixture of the 4-*O* Piv (**12α**) and 3-*O* Piv (**13α**) diacetates. An example of the effect of reaction time on the ratio of hydrolysis products is given in Table 2, using [U-^{14}C]-labelled **4α** as a substrate in rabbit serum and esterase catalyzed reactions.

Taking into account the fact that acetyl groups have a tendency to migrate we proceeded to determine whether the isolated compounds were a result of enzymic hydrolysis only, or a result of hydrolysis followed by immediate intramolecular migration. For this purpose we used three different substrates, the ^{14}C-labelled peracetylated methyl α-D-glucopyranoside (**4α**),unlabelled peracetylated methyl 2-acetamido-2-deoxy-α-D-

Table 2. Esterase-Catalyzed Deacetylations of **4α**[a]

Time	Serum				Esterase			
	4α	**6α**	**7α**	Other	**4α**	**6α**	**7α**	Others
1	44	17	37	2	75	10	14	1
3	10	38	46	6	49	19	28	4
6	2	16	23	59	16	32	45	7
24	0	2	1	97	0	15	21	64

[a]Analysis based on radioactivity and given as a percentage of the total recovered after t.l.c.

4α = methyl 2,3,4,6-tetra-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

6α = methyl 2,3,6-tri-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

7α = methyl 2,4,6-tri-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

Other = mixture of partially and totally deacetylated products

-glucopyranoside (**5α**), and unlabelled methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (**14**). Thus, the ^{14}C-labelled **4α** was incubated in the first part of the experiment in PBS with rabbit serum as a source of enzymic activity. Proteins were removed and the mixture of some unreacted **4α** and

hydrolysis products **6α** and **7α** thus obtained was further incubated in PBS at 37° without any addition of a catalyst (Table 3). It was shown, by t.l.c., that the ratio of triacetates **6α** and **7α** changes with time in favor of **7α** with its free 3-OH group. The results presented in Table 3 imply that the 3-*O* Ac migrates to the free 4-OH. Prolongation of reaction times (44 h) most probably leads to further migration of the 2-*O* Ac to the free 3-OH resulting in diminuition of **7α**. Further prolongation of reaction times shows that some slow medium induced hydrolysis occurs most probably of the 6-*O* Ac followed by intramolecular migration of the 4-*O* Ac to the free 6-OH, and resulting thus in further diminuition of **7α** and formation of a mixture of other, highly deprotected hydrolysis products. On the contrary, no further hydrolysis of **6α** occurred and its quantities, after reaching equilibrium remained the same. Furthermore, no medium induced hydrolysis of the starting **4α** occurs at any time giving further proof that the original deacylation was catalyzed by the esterase from rabbit serum.

Table 3. Solvent mediated acetyl migration and hydrolysis of **4α**[a]

Time	**4α** (%)	**6α** (%)	**7α** (%)	Others (%)
0	15	40	25	20
20	15	25	40	20
44	13	30	32	25
68	13	36	18	33
100	14	37	11	38

[a]Analysis based on radioactivity and given as a percentage of the total recovered after t.l.c.

4α = methyl 2,3,4,6-tetra-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

6α = methyl 2,3,6-tri-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

7α = methyl 2,4,6-tri-*O*-acetyl-α-D-[U-^{14}C]glucopyranoside

Others = mixture of partially and totally deacetylated products

As we previously described, esterase from rabbit serum catalyzed preferentially deacylation of the pivaloyl group in the presence of acetyl groups (substrate **2**). To give further proof of initial enzymic 6-acyl group hydrolysis followed by acetyl migration we proceeded by incubating the 6-*O* Piv substrate **14α** with rabbit serum in PBS. As expected a mixture of 3,6- and 4,6-diacetates (**10α** and **11α**) resulted proving that hydrolysis of the primary 6-*O* Piv group occurs in the first step followed by acetyl migration to the 6-OH which was freed in the process.

Further experiments with peracetylated **5α** using rabbit serum or the esterase from rabbit serum, led to the same mixture of diacetates. Further enzymic hydrolysis of the diacetate **11α** induced by longer incubation times

led to the methyl 6-*O*-acetyl-2-acetamido-2-deoxy-α-**D**-glucopyranoside (**15α**). According to previous results it is to be expected that further hydrolysis of the 6-*O* Ac occurs followed by intramolecular migration of the 4-*O* Ac and 3-*O* Ac to the 6-OH resulting thus in the monoacetate **15α**. Further prolongation of the reaction time resulted finally in complete deprotection leading to methyl 2-acetamido-2-deoxy-α-**D**-glucopyranoside.

Taking into consideration all the results presented in this work two conclusions can be made. Firstly, peracylated substrates undergo enzymic hydrolysis with RS and RE, primarily at the anomeric centre. Peracetylated methyl glycosides (**4** and **5**) gave anomalous products with free 3- and 4-OH groups. A reasonable assumption can be made, based on the data presented, to rationalize the mechanism for formation of these products. All results point to the fact that hydrolysis in these substrates occurs at the primary position only, 6-*O* Ac is deacetylated in the process, stepwise intramolecular migrations of acetyl groups towards the 6-OH follow, and the process continues to result finally in complete deprotection of the sugar ring. The products isolated are partially in accord with products described in the work of other authors[3] who used enzymes from other sources. Whether the previously reported data are the result of the enzyme catalyzed anomalous hydrolyses or of enzymic hydrolyses followed by migrations of acetyl groups is not quite clear. Medium mediated intramolecular migrations of acetyls in some partially acetylated sugars were reported by other authors as well[25] and whether this process occurs generally in all partially acetylated monosaccharides remains to be seen.

EXPERIMENTAL SECTION

General methods

Column chromatography was performed on silica gel (Merck) and t.l.c. on Kieselgel G (Merck) with A, ethyl acetate-benzene (proportions are given in the text); B, ethyl acetate-benzene-ethanol (10 :2 :1.5); C, acetonitrile-water (5:1), D, ethanol, and detection was effected by charring with sulphuric acid. ^{1}H-NMR spectra (100 MHz, $CDCl_3$, internal Me_4Si) were recorded with a Jeol JNM FX-100 F.t. spectrometer. Radioactivity was measured by using a Beckman LS-100 liquid scintillation counter and Aquasol (NEN) as a scintillation cocktail.

1,2,3,4,6-Penta-O-acetyl-β-D-[U-^{14}C]glucopyranose (1β).

A solution of **D**-[U-^{14}C]glucopyranose (7.4 MBq) and **D**-glucopyranose (25 mg) in pyridine (2mL) and acetic anhydride (2 mL) was stirred at room temperature for 16 h. Solvents were evaporated under reduced pressure and water was distilled thrice from the residue in order to remove traces of acetic anhydride. The residue in ethyl acetate (1 mL) was applied to a column of silica gel and eluted with solvent A (1:2) to give chromatographically homogeneous **1β** (54 mg, 96%), R_F 0.5 (solvent A, 1:2) and 0.8 (solvent A, 2:1), specific activity 29.6 MBq/mmol.

Methyl 2,3,4,6-tetra-O-acetyl-α-D-[U-^{14}C]glucopyranoside (4α).

A solution of **D**-[U-^{14}C] glucopyranose (7.4 MBq) and **D**-glucopyranose (20 mg) in dry methanol was boiled under reflux for 14 h in the presence of catalytic quantities of hydrogen chloride (0.3 mL of 3.3M HCl in methanol). The solvent was removed under reduced pressure and methanol was distilled thrice from the residue in order to remove traces of HCl. T.l.c (solvent C) indicated that the residue consisted mainly of methyl **D**-glucopyranoside (98% based on radioactivity).

A solution of crude methyl **D**-glucopyranoside in pyridine (2 mL) and acetic anhydride (2 mL) was stirred at room temperature for 16 h. Solvents were evaporated at reduced pressure and water was distilled thrice from the residue. The residue in ethyl acetate (1 mL) was applied to a column of silica gel and eluted with solvent A (2:1) to give chromatographically homogeneous **4α** (30 mg, 75% based on glucose), R_F 0.6 (solvent A, 2:1), specific activity 24.5 MBq/mmol, and a small quantity of chromatographically homogeneous **4β** (4 mg, 10% based on glucose), R_F 0.7 (solvent A, 2:1), specific activity 29.4 MBq.

Enzymic hydrolyses of substrates 1, 4 and 5: general procedure.

Solutions of **1** and **2** in a 10:1 mixture of PBS (0.01 M, pH 7.2) and DMSO, and suspensions of **4** and **5** in PBS were incubated with rabbit serum (RS) and/or the esterase isolated from rabbit serum (RE, 5.5 mg protein/ mL) at 37° for various periods of time. The pH was maintained by the addition of 0.1 M NaOH and each reaction was monitored by t.l.c.. Incubations were stopped by the addition of ethanol, proteins were removed by centrifugation, and the supernatants concentrated under reduced pressure. If necessary (for identification purposes or the prevention of acyl migrations) the products were pivaloylated *in situ* with an excess of pivaloyl chloride in pyridine. Thus obtained crude products were subjected to column chromatography on silica gel.

Enzymic hydrolysis of 1,2,3,4,6-penta-O-acetyl-β-D-[U-^{14}C]-glucopyranose (1β).

1β (50 mg) and ^{14}C-labelled **1β** (2.5 mg, specific activity 29.6 MBq/mmol) were incubated in PBS (10 mL) and DMSO (1 mL) with RS (1 mL) or RE (100 μL) as enzyme sources. The reactions were monitored by t.l.c. (Solvent A, 2:1) at time intervals given in Table 1, with unlabelled **1β** (R_F 0.8) and **3** (R_F ~ 0.5) as references. Highly deacetylated products had R_F values of ~ 0-0.2. The reference compounds were detected by charring with sulfuric acid. The absorbent containing each product (**1β**, **3** and others) of enzymic action was scraped off, suspended in a mixture of 2 mL of aqueous 75% ethanol and 2 mL of scintillation cocktail, and the radioactivity was measured and expressed as a percentage of the total radioactivity (Table 1).

2,3,4,6-Tetra-O-acetyl-D-glucopyranose (3).

a) The tetraacetate was obtained in 6 h from 1,2,3,4,6-penta-*O*-acetyl-α-**D**-glucopyranose **1α** (100 mg) in PBS (20 mL) and DMSO (2 mL) with RS (2 mL) as enzyme source. Column chromatography (Solvent A, 2:1) produced chromatographycally homogeneous **3β** (49 mg, 55%), R_F 0.56(solvent A, 2:1) and **3α** (3 mg, 3.4%), R_F 0.42 (solvent A, 2:1).

b) The tetraacetate was obtained in 16 h from **1α** as in a) with RE (200 μL) as enzyme source. Column chromatography produced homogeneous **3β** (40 mg, 45%). No **3α** was isolated.

c) The tetraacetate was obtained in 3 h from 1,2,3,4,6-penta-*O*-acetyl-β-**D**-glucopyranose (**1β**, 100 mg) in PBS (20 mL) and DMSO (2 mL) with RS (2 mL) as enzyme source. Column chromatography produced homogeneous **3** (64 mg, 72%), R_F ~ 0.5. Pivaloylation of **3** (64 mg) with pivaloyl chloride (75μL) in dry pyridine (1 mL) for 16 h at ambient temperature, followed by the removal of the solvents and column chromatography (Solvent A, 1:1) afforded a mixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-pivaloyl-α- and β-**D**-glucopyranose (**2α** and **2β**)[23] in a ratio of 1:2 (as indicated by ^{1}H-NMR); (52 mg, 65%), R_F 0.7 (Solvent A, 1:1).

d) The tetraacetate was obtained in 8 h as in c) with RE (200 μL) as enzyme source.Column chromatography (Solvent A, 2:1) produced **3** (56 mg, 50%).

e) The tetraacetate was obtained in 5 h from 2,3,4,6-tetra-*O*-acetyl-1-*O*-pivaloyl-β-**D**-glucopyranose (**2β**, 43 mg) in PBS (10 mL) and DMSO (1 mL) with RS (1 mL) as source of enzymic activity. Column chromatography afforded **3** (23 mg, 66%).

f) The same procedure as in e) but with RE (200 μL) as enzyme source produced **3** (12 mg, 33%)

Enzymic hydrolysis of methyl 2,3,4,6-tetra-O-acetyl-α-D-[U-^{14}C]glucopyranoside (4α).

4α (50 mg) and ^{14}C-labelled **4α** (2.5 mg, specific activity 24.5 MBq/mmol) were incubated in PBS (10 mL) with RS (1 mL) or RE (100 μL) as enzyme sources. The reactions were monitored by t.l.c. (Solvent A, 2:1) at time intervals indicated in Table 2, with unlabelled **4α** (R_F 0.6), **6α** (R_F 0.5) and **7α** (R_F 0.4) as references. The work-up procedure was the same as described for enzymic hydrolysis of 1,2,3,4,6-penta-*O*-acetyl-β-**D**-[U-^{14}C]-glucopyranose (**1β**). The dependence of product ratios on reaction times is shown in Table 2.

Methyl 2,3,6- and 2,4,6-tri-O-acetyl-α-D-glucopyranosides (6α and 7α).

a) The triacetates were obtained in 3 h from methyl 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranoside (**4α**, 50 mg) in PBS (10 mL) with RS (1 mL) as enzyme source. Column chromatography produced **6α** (10 mg, 22%); R_F 0.5 and **7α** (20 mg, 44%), RF 0.4 (Solvent A, 2:1). Pivaloylations of **6α** (10 mg) with pivaloyl chloride (50μL) in dry pyridine (1mL) for 16 h at ambient temperature produced methyl 2,3,6-tri-*O*-acetyl-4-*O*-pivaloyl-α-**D**-glucopyranoside (**8α**, 10 mg, 81%), R_F 0.6 (Solvent A, 1:3). Following the same procedure **7α** gave methyl 2,4,6-tri-O-acetyl-3-*O*-pivaloyl-α-**D**-glucopyranoside (**9α**, 11 mg, 89%), R_F 0.6 (Solvent A, 2:1). Change of solvent did not induce the separation of **8α** and **9α** on t.l.c (R_F 0.6, Solvent A, 1:3).

8α, ^{1}H-NMR ($CDCl_3$) δ 3.43 (s, 3H, *O*Me), 2.1, 2.08, 1.99 (3s, 9H, Ac*O*-6, Ac*O*-2, Ac*O*-3), 1.16 (s, 9H, Piv*O*-4).

8β, ^{1}H-NMR ($CDCl_3$) δ 4.44 (d, $J_{1,2}$ 8 Hz, H-1), 3.50 (s, 3H, *O*Me), 2.09, 2.05, 1.98 (3s, 9H, Ac*O*-6, Ac*O*-3, Ac*O*-6), 1.16 (s, 9H, Piv*O*-4).

9α, ^{1}H-NMR ($CDCl_3$) δ 3.40 (s, 3H, *O*Me), 2.11, 2.05, 2.03 (3s, 9H, Ac*O*-2, Ac*O*-6, Ac*O*-4), 1.13 (s, 9H, Piv*O*-3).

b) The triacetates were obtained in 3 h from **4α** (200 mg), in PBS (35 mL) with RS (3 mL) as enzyme source. The residue, after protein removal and evaporation of the solvents, was immediately submitted to pivaloylation with pivaloyl chloride (200 μL) in dry pyridine (5 mL). Column chromatography (Solvent A, 1:3), following work-up of the residue, afforded a mixture of **8α** and **9α** (as indicated by ^{1}H-NMR), 150 mg (68%), R_F 0.6 .
c) The triacetates were obtained in 8 h from **4α** as described in b) with RE (300 μL) as enzyme source. A mixture of **8α** and **9α** resulted (110 mg, 50%).

Methyl 2-acetamido-3,6- and 4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosides (10α and 11α)

a) The diacetates were obtained in 20 h from methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-**D**-glucopyranoside (**5α**, 108 mg) in PBS (18 mL) and with RS (3 mL) as enzyme source. Column chromatography (Solvent B) produced **10α** (70 mg, 24%), R_F 0.51 (Solvent B) and **11α** (27 mg, 9.5 %), R_F 0.38 (Solvent B). Pivaloylation of **10α** (10 mg) with pivaloyl chloride (50 μL) in dry pyridine (1 mL) for 16 h at ambient temperature produced methyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-pivaloyl-α-**D**-glucopyranoside (**12α**, 10 mg, 89%), R_F 0.33 (Solvent A, 1:1). Following the same procedure **11α** gave methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-pivaloyl-α-**D**-glucopyranoside[24] (**13α**,9 mg, 85%), R_F 0.40 (Solvent A, 1:1).

10α; ^{1}H-NMR ($CDCl_3$) δ 4.69.(d, 1H, $J_{1,2}$ 3.80 Hz, H-1), 3.39 (s, 3H, *OMe*), 2.14 and 2.09 (2s, 6H, Ac*O*-6 and Ac*O*-3), 1.96 (s, 3H, NAc).

11α, ^{1}H-NMR ($CDCl_3$) δ 4.73 (d, 1H, $J_{1,2}$ 3.52 Hz, H-1), 3.40 (s, 3H, *O*Me), 2.12 (s, 6H, Ac*O*-4 and Ac*O*-6), 2.05 (s, 3H, NAc).

12α, ^{1}H-NMR ($CDCl_3$) δ 4.72 (d, 1H, $J_{1,2}$ 3.81 Hz, H-1), 3.41 (s, 3H, *O*Me), 2.10 (s, 3H, Ac*O*-6), 1.99 and 1.96 (2s, 6H, Ac*O*-3 and NAc), 1.16 (s, 9H, Piv*O*-4).

13α, ^{1}H-NMR ($CDCl_3$) δ 4.72 (d, 1H, $J_{1,2}$ 3.00 Hz, H-1), 3.41 (s, 3H, *O*Me), 2.11 (s, 3H, Ac*O*-6), 2.00 (s, 3H, Ac*O*-4), 1.93 (s, 3H, NAc), 1.13 (s, 9H, Piv*O*-3).

b) The diacetates were obtained in 30 h from **5α** (36 mg) in PBS (6 mL) with RE (500 μL) as enzyme source. Column chromatography (Solvent B) produced **10α** (7.5 mg, 10%) and **11α** (5.7 mg, 18%).

c) Incubation of **11α** (15 mg) in PBS (3 mL) with RS (1 mL) for 4 days followed by pivaloylation of the crude product with pivaloyl chloride (50 μL) in pyridine (1 mL) produced methyl 2-acetamido-6-*O*-acetyl-3,4-di-*O*-pivaloyl-α-**D**-glucopyranoside[22] (**16**, 12 mg, 70%), R_F 0.6 (Solvent D). ^{1}H-NMR ($CDCl_3$) δ 4.72 (1H, $J_{1,2}$ 3.52 Hz, H-1), 3.41(s, 3H, *OMe*), 2.1, 1.93 (2s, 6H, Ac*O*-6, NAc), 1.15 (s, 9H, Piv*O*-4), 1.12 (s, 9H, Piv*O*-3).

Hydrolysis of methyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-pivaloyl-α-D-glucopyranoside (14α)[24]

a) Compound **14α** (100 mg) was incubated 8h in PBS (16 mL) with RS (2 mL) as enzyme source. Column chromatography (Solvent B) produced **10α** (23 mg, 29%) and **11α** (20 mg, 25%).

b) Compound **14α** (20 mg) was incubated for 41 h in PBS (10 mL) with RE (500 μL) as enzyme source. Column chromatography produced **10α** (2.9 mg, 17%) and **11α** (1.0 mg , 6.3%).

References

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