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Original Scientific Paper

Syntheses and Enzymatic Hydrolyses of Acylated Methyl α-D-Mannopyranosides

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Selective pivaloylations of methyl α-D-mannopyranoside under various reaction conditions have been studied. The structures of the products were established by ^{1}H-NMR spectroscopy and acetylation of partially pivaloylated compounds. The order of reactivity of hydroxyl groups was established and found to be 6-OH>3-OH>2-OH>4-OH. The newly synthesized acylated monosaccharides were subjected to the hydrolysis by rabbit serum and esterases isolated from rabbit serum. Various degrees of regioselectivity were observed in these reactions, depending on the sugar structure.

INTRODUCTION

Regioselective acylation of sugars is rarely carried out with efficiency.[1] Therefore, the ability of pivaloyl chloride to acylate sugars selectively has been exploited in the synthesis of partially and totally pivaloylated simple glycosides[2–4] and disaccharides.[5,6] Chemical deacylations of such compounds present some difficulties, and can be achieved only in rather strongly basic conditions. To avoid such drastic reaction conditions enzymatic techniques for the removal of pivaloyl groups have been developed. Thus, it was shown that *O*-pivaloyl derivatives from the D-glucose and 2-acetamido-2-deoxy-D-glucose series of monosaccharides are good substrates for esterases in mam-

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malian sera, and regioselective enzymatic hydrolyses of these compounds can be easily achieved.[3,4,7–9]

In continuation of our work on selective formations and hydrolyses of pivaloylated and acetylated[10] monosaccharides, we prepared a series of acylated methyl α-D-mannopyranosides. Furthermore, we used some of these novel compounds as substrates for rabbit serum and esterases isolated from rabbit serum to effect deprotection in very mild neutral conditions.

RESULTS

Preparation of acylated monosaccharides

Unless noted otherwise, the pivaloylations described in this work were conducted in dry pyridine at room temperature. Acetylations were peformed using a standard procedure, with an excess of acetic anhydride in pyridine (1:1)

Treatment of methyl α-D-mannopyranoside (**1**) with 2 equivalents of pivaloyl chloride for 2 hrs, followed by column chromatography on silica gel produced the 3,6-dipivalate **6** as a major isolated product (42%). A mixture of **6** and the 2,6-dipivalate **5** followed (9%). A small quantity of pure **5** (6%) was isolated next. The last product to be isolated was the 6-monopivalate **2** (31%). Further attempts to resolve the mixture of **5** and **6** failed. However, acetylation of this mixture produced a mixture of diacetates **15** and **16,** the chromatography of which resulted in isolation of pure **15** and **16**. The 6-monopivalate **2** obtained in the 2 h pivaloylation was also acetylated to give the triacetate **12**.

Treatment of **1** with 3 equivalents of pivaloyl chloride for 24 hrs followed by chromatography, gave the 2,3,4,6-tetrapivalate **10** (3%), 2,3,6-tripivalate **7** (12%), 2,4,6-tripivalate **8** (1%), 3,4,6-tripivalate **9** (8%) and 3,6-dipivalate **6** as the major product isolated (22%). Acetylation of **7** and **9** produced the monoacetates **17** and **18**, respectively.

Treatment of **1** with 5 equivalents of pivaloyl chloride for 45 hrs, followed by chromatography, gave the tetrapivalate **10** (42%) and some 2,3,6-tripivalate **7** (16%).

Conventional acetylation of **1** produced the peracetylated compound **11** (89%).

Enzymatic hydrolysis of the 3,6-dipivalate **6** with rabbit serum, followed by column chromatography produced the 6-pivalate **2** (38%) and the 3-pivalate **3** (20%). Some unreacted **6** was recovered (5%) as well as the unresolved mixture of **2** and **3** (16%). Incubation with the esterase isolated from rabbit serum (E II) gave pure products **2** and **3** in 16% and 7% yields, respectively. Unreacted **6** was recovered (3%) as well as some unresolved mixture of monopivalate products (24%).

Enzymatic hydrolysis of the 2,3,6-tripivalate **7** gave the 2,3-dipivalate **4** as the only isolated reaction product, both with serum (36%) and the esterase isolated from rabbit serum (23%). Some unreacted starting material was recovered, as well, in the reaction with rabbit serum (21%) and with E II (47%).

Conventional acetylation of the 2,3-dipivalate **4** produced the diacetate **14**.

DISCUSSION

Monosaccharides, oligosaccharides, and various other glycoconjugates generally have a multitude of hydroxyl groups of comparable chemical reactivity. Consequently, for the directed formation of carbohydrate derivatives, these functional groups must be manipulated selectively, generally making cumbersome protection and deprotection steps necessary. Numerous chemical techniques are available for that purpose.[11–13] In addition, due to the synthetic challenge that the multifunctional carbohydrates offer, enzymatic techniques for the introduction of protective groups into sugars and/or their subsequent removal have been recently developed.[14,15] Towards this goal, numerous commercially available lipases and some esterases have been used. The effectivness of enzymatic acylations and deacylations of various organic substrates, including sugars, was increased by the discovery that enzymes may catalyze reactions not only in aqueous media but in organic solvents as well.[16,17] It was also reported that in some cases, enzymatic regioselective as well as chemoselective deprotections of sugars may be achieved.[18,19]

In continuation of our efforts towards regioselective protection and deprotection of acylated monosaccharides, we now wish to report on selective chemical pivaloylations and enzymatic hydrolyses of pivaloylated sugars from the D-mannopyranose series.

On the basis of synthetic studies and ^{1}H-NMR data which are in perfect accord with previously published data on some other acylated mannopyranoses,[20,21] it could be concluded that in the case of methyl mannopyranoside the reactivity for secondary hydroxyl groups follows the growing order of 3-OH>2-OH>4-OH. As expected, acylation at the primary hydroxyl group (6-OH), occurs first. Thus, the 2 h pivaloylation produces the 6-monopivalate **2** which is the result of the primary OH acylation. 2,6- and 3,6-dipivalates (**5** and **6**) also form but the ratio is in favour of the 3,6-derivative **6**. No acylation of 4-OH occurs under these reaction conditions. Prolongation of the reaction time (24 hrs) and an increase of pivaloyl chloride (from 2 to 3 equiv.) produces a complex mixture of products, including small quantities of tetrapivalate **10**, the 2,3,6- and 3,4,6-tripivalates (**7** and **9**), traces of the 2,4,6-tripivalate **8**, and the 3,6-dipivalate **6** as the major reaction product.

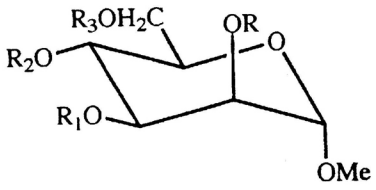
Further prolongation of reaction times and an excess of pivaloyl chloride (5 equiv.) produce the tetrapivalate **10**, but even after 45 hrs the reaction is not complete and some 2,3,6-tripivalate **7** together with unreacted starting material were recovered, as well. These data are in accord with the previously published data on selective acylations of mannopyranosides using benzoyl chloride in pyridine.[22–24] The low reactivity of the 4-hydroxyl group may be attributable to steric hindrance through interactions with 3-OH and the 6-ester group (acylation at the primary hydroxyl group being assumed to occur first). The 3-hydroxyl group suffers smaller steric interactions. Furthermore, any possible activation of the 2-OH produced by its proximity to the anomeric centre, as observed in α-D-glucopyranosides and explained by the presence of the *cis*-2-OH-1-OMe grouping favouring intramolecular hydrogen bonding, is apparently counteracted by its sterically unfavourable, axial orientation.

Enzymatic hydrolyses of pivaloylated methyl α-D-glucopyranosides[9], D-glucopyranoses[4] and methyl 2-acetamido-2-deoxy-D-glucopyranosides[3] were previously described and led to the conclusion that various degrees of regioselectivity could be obtained depending on the substrate structure, enzyme source, and the degree of purification of the enzyme used. Rabbit serum was shown to be a good source of enzyme, the purification obtained for the esterase isolated from rabbit serum was tenfold, and either native serum or purified esterase II (E II) could be used in hydrolysis studies.[9] Both the native serum and E II were used in this work. Hydrolysis of the 3,6-dipivalate **6** led to an approx. 2:1 mixture of the 6- and 3-monopivalates (**2** and **3**, respectively) showing that the 3-*O*Piv undergoes faster hydrolysis than the 6-*O*Piv. In contrast, the hydrolysis of the 2,3,6-tripivalate **7** led, almost exclusively, to the 2,3-dipivalate **4** showing that, in the more crowded substrate, the 6-*O*Piv group is hydrolyzed at faster rates. Product **4**, with its 2-*O*Piv in an unfavorable axial orientation and a neighbouring equatorial 3-*O*Piv, is of special interest since its non-enzymatic synthesis would involve numerous reaction steps.

EXPERIMENTAL

General methods

Column chromatography was performed on silica gel (Merck) and TLC on Kieselgel G (Merck) with A, benzene-ethyl acetate (proportions given in the text). Detection was effected by charring with sulfuric acid. The ^{1}H-NMR spectra (300 MHz, $CDCl_3$, internal Me_4Si) were recorded with a Varian Gemini spectrometer, and the data are given in Table I. Rabbit serum and esterase II (E II) were prepared as previously described. Methyl α-D-mannopyranoside was prepared by a conventional treatment of D-mannopyranose (SIGMA) with methanolic hydrogen chloride[25] and was used as starting material.



1	$R = R_1 = R_2 = R_3 = H$	**10**	$R = R_1 = R_2 = R_3 = Piv$
2	$R = R_1 = R_2 = H$; $R_3 = Piv$	**11**	$R = R_1 = R_2 = R_3 = Ac$
3	$R = R_2 = R_3 = H$; $R_1 = Piv$	**12**	$R = R_1 = R_2 = Ac$; $R_3 = Piv$
4	$R_2 = R_3 = H$; $R = R_1 = Piv$	**13**	$R = R_2 = R_3 = Ac$; $R_1 = Piv$
5	$R_1 = R_2 = H$; $R = R_3 = Piv$	**14**	$R_2 = R_3 = Ac$; $R = R_1 = Piv$
6	$R = R_2 = H$; $R_1 = R_3 = Piv$	**15**	$R_1 = R_2 = Ac$; $R = R_3 = Piv$
7	$R_2 = H$; $R = R_1 = R_3 = Piv$	**16**	$R = R_2 = Ac$; $R_1 = R_3 = Piv$
8	$R_1 = H$; $R = R_2 = R_3 = Piv$	**17**	$R_2 = Ac$; $R = R_1 = R_3 = Piv$
9	$R = H$; $R_1 = R_2 = R_3 = Piv$	**18**	$R = Ac$; $R_1 = R_2 = R_3 = Piv$

TABLE I

^{1}H-NMR data ($CDCl_3$), 300 MHz for acyl derivatives of methyl α-D-mannopyranoside

Comp	H-1,d	Chemical shifts δ (ppm)								
		PivO				AcO				MeO
		2	3	4	6	2	3	4	6	1
2	4.77				1.20					3.38
3	4.71		1.24							3.40
4	4.68	1.24	1.19							3.39
5	4.67	1.22			1.25					3.38
6	4.78		1.23		1.25					3.40
7	4.64	1.25	1.19		1.23					3.40
8	4.72	1.23		1.23	1.26					3.40
9	4.76		1.19	1.17	1.23					3.42
10	4.68	1.27	1.12	1.16	1.25					3.42
11	4.72					2.11	2.05	2.00	2.16	3.41
12	4.71				1.25	2.13	2.05	2.00		3.41
13	4.70		1.13			2.12		2.02	2.14	3.41
14	4.70	1.27	1.12					2.01	2.10	3.42
15	4.69	1.24			1.25		2.03	2.01		3.41
16	4.69		1.12		1.25	2.11		2.02		3.40
17	4.68	1.25	1.12		1.24			2.01		3.41
18	4.70		1.11	1.17	1.24	2.11				3.41

*Selective pivaloylations of methyl α-D-mannopyranoside (**1**)*

(a) To a solution of **1** (193 mg, 1 mmol) in dry pyridine (2 mL), pivaloyl chloride (250 μL, 2 mmol) was added. The mixture was stirred at ambient temperature for 2 hrs, EtOH (2 mL) was added, the mixture was concentrated and traces of pyridine were removed by successesive evaporations of water and toluene from the residue. Column chromatography (solvent A, 1:1) gave, first, crystalline methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**6**, 152 mg, 42%); R_F 0.66 (solvent A, 1 : 1), 0.77 (solvent A, 1:2), 0.81 (solvent A, 1:7); m.p. 96–97 °C. A mixture (31 mg, 9%) was eluted next that was shown by TLC (solvent A, 1:1) to consist of **6** and methyl 2,6-di-*O*-pivaloyl-α-D-mannopyranoside (**5**, R_F 0.45, solvent A, 1:1). Methyl 2,6-di-*O*-pivaloyl-α-D-mannopyranoside followed (oil, **5**, 23 mg, 6%), R_F 0.45 (solvent A, 1:1), 0.64 (solvent A, 1:2), 0.75 (solvent A, 1:7). Last to be eluted was methyl 6-*O*-pivaloyl-α-D-mannopyranoside as oil (**2**, 85 mg, 31%); R_F 0.19 (solvent A, 1:1), 0.21 (solvent A, 1:2) and 0.29 (solvent A, 1:7).

Conventional treatment of the mixture of **5** and **6** (59 mg, 0.16 mmol) with acetic anhydride-pyridine for 16 hrs at ambient temperature afforded, after column chromatography (solvent A, 5:1) crystalline methyl 2,4-di-*O*-acetyl-3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**16**, 25 mg, 34%); R_F 0.55 (solvent A, 5:1); m.p. 95–96°C, followed by methyl 3,4-di-*O*-acetyl-2,6-di-*O*-pivaloyl-α-D-mannopyranoside as oil (**15**, 21 mg, 29%); R_F 0.49 (solvent A, 5:1).

Conventional acetylation of **2** (122 mg, 0.44 mmol), followed by column chromatography (solvent A, 2:1) gave methyl 2,3,4-tri-*O*-acetyl-6-*O*-pivaloyl-α-D-mannopyranoside (**12**,103 mg, 59%); R_F 0.66 (solvent A, 2:1).

(b) To a solution of **1** (194 mg, 1 mmol) in dry pyridine (3 mL), pivaloyl chloride (400 μL, 3 mmol) was added. The mixture was stirred at ambient temperature for 24 hrs. After the work-up procedure as in (a), followed by column chromatography (solvent A, 3:1), the first isolated product was crystalline methyl 2,3,4,6-tetra-*O*-pivaloyl-α-D-mannopyranoside (**10**, 15 mg, 3%); R_F 0.84 (solvent A, 3:1), 0.66 (solvent A, 5:1); m.p. 104–105 °C. Methyl 2,4,6-tri-*O*-pivaloyl-α-D-mannopyranoside as oil was eluted next (**8**, 5 mg, 1%); R_F 0.65 (solvent A, 3:1). A mixture of **7** and **8** followed (8 mg, 2%). Next to be eluted was crystalline 2,3,6-tri-*O*-pivaloyl-α-D-mannopyranoside (**7**, 53 mg, 12%), R_F 0.56 (solvent A, 3:1), followed by a mixture of **7** and methyl 3,4,6-tri-*O*-pivaloyl-α-D-mannopyranoside **9** (9 mg, 2%), and pure **9** as oil (37 mg, 8%), R_F 0.48 (solvent A, 3:1). The 3,6-dipivalate **6** (80 mg, 22%), R_F 0.33 (solvent A, 3:1) was eluted last.

Acetylation of **7** (100 mg, 0.22 mmol), followed by column chromatography (solvent A, 5:1) gave crystalline methyl 4-O-acetyl-2,3,6-tri-*O*-pivaloyl-α-D-mannopyranoside (**17**, 60 mg, 55%); R_F 0.57 (solvent A, 5:1); m.p. 66–67 °C.

Acetylation of **9** (20 mg, 0.044 mmol), followed by column chromatography (solvent A, 5:1), gave methyl 2-*O*-acetyl-3,4,6-tri-*O*-pivaloyl-α-D-mannopyranoside as oil (**18**, 10 mg, 46%); R_F 0.53 (solvent A, 5:1).

(c) To a solution of **1** (485 mg, 2.5 mmol) in dry pyridine (10 mL), pivaloyl chloride (2 mL, 12.5 mmol) was added. The mixture was stirred at ambient temperature for 45 hrs. After the work-up procedure, followed by column chromatography (solvent A, 10:1), the first eluted product was crystalline methyl 2,3,4,6-tetra-O-pivaloyl-α-D-mannopyranoside (**10**, 554 mg, 42%); R_F 0.78 (solvent A, 10:1); m.p. 104–105 °C. Eluted next was **7** (182 mg, 16%); R_F 0.50 (solvent A, 10:1)

Conventional acetylation of **1** (1 g, 5.2 mmol), followed by recrystallization from hot water-ethanol gave crystalline methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**11**, 1.7 g, 89%); R_F 0.62 (solvent A, 1:1); m.p. 64–65 °C.

Enzymatic Deacylations

1. Enzymatic deacylation of methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**6**)

(a) A suspension of **6** (100 mg, 0.27 mmol) in methylsulfoxide (DMSO, 2 mL) and phosphate-buffered saline (PBS, 10 mL, pH 7.2) was incubated (with constant mixing) with rabbit serum (600 μL) for 22 hrs at 37 °C. The pH was periodically adjusted to 7.2 by addition of 0.1 M NaOH and the reaction was monitored by TLC (solvent A, 1:7). The reaction was stopped by adding ethanol (5 mL), the precipitated proteins were removed by centrifugation, the solvent was evaporated, and the residue subjected to column chromatography (solvent A, 1:7). The first isolated product was the unreacted **6** (5 mg, 5%); R_F 0.81 (solvent A, 1:7), followed by pure methyl 3-*O*-pivaloyl-α-D-mannopyranoside (**3**, 15 mg, 20%), R_F 0.35 (solvent A, 1:7). The unresolved mixture of **3** and methyl 6-*O*-pivaloyl-α-D-mannopyranoside (**2**, 12 mg, 16%) followed. Pure **2** (30 mg, 38%); R_F 0.29 (solvent A, 1:7) was isolated last.

(b) A suspension of **6** (100 mg, 0.27 mmol) in DMSO (2 mL) and PBS (10 mL) was incubated with E II (350 μL, protein content 6.2 mg/mL), for 22 hrs at 37 °C. After the work-up procedure and column chromatography as described in (a) unreacted **6** was isolated (3 mg, 3%), followed by the 3-pivalate **3** (5 mg, 7%), the mixture of **3** and **2** (18 mg, 24%), and pure 6-pivalate **2** (12 mg, 16%).

2. Enzymatic deacylation of methyl 2,3,6-tri-*O*-pivaloyl-α-D-mannopyranoside (**7**)

(a) A suspension of **7** (55 mg, 0.12 mmol) in DMSO (1 mL) and PBS (5 mL) was incubated with rabbit serum (600 μL) for 24 hrs at 37 °C. After the work-up procedure as in 1(a), the residue was submitted to column chromatography (solvent A, 1:2) to give the unreacted **7** as the first product (12 mg, 26%), R_F 0.9 (solvent A, 1:2), followed by methyl 2,3-di-*O*-pivaloyl-α-D-mannopyranoside (**4**, 16 mg, 36%), R_F 0.54 (solvent A, 1:2).

(b) A suspension of **7** (55 mg, 0.12 mmol) in DMSO (1 mL) and PBS (5 mL) was incubated with E II (400 μL, protein content 6.2 mg/mL) for 24 h at 37 °C. After the work-up procedure and column chromatography as in 2(a), the unreacted **7** was isolated (26 mg, 47%), followed by **4** (10 mg, 23%).

Conventional acetylation of **4** (16 mg, 0.035 mmol), followed by column chromatography (solvent A, 3:1) produced methyl 4,6-di-*O*-acetyl-2,3-di-*O*-pivaloyl-α-D-mannopyranoside (**14**, 10 mg, 51%), R_F 0.59 (solvent A, 3:1).

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SAŽETAK

Sinteze i enzimske hidrolize aciliranih metil-α-D-manopiranozida

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Proučene su reakcije selektivnog pivaloiliranja metil-α-D-manopiranozida u različitim reakcijskim uvjetima. Strukture produkata određene su pomoću ^{1}H-NMR spektroskopije i acetiliranja djelomično pivaloiliranih spojeva. Utvrđen je redoslijed reaktivnosti hidroksilnih skupina 6-OH>3-OH>2-OH>4-OH.

Novo sintetizirani spojevi podvrgnuti su hidrolizi kataliziranoj kunićjim serumom kao i esterazama izoliranima iz kunićjeg seruma. Regioselektivnost tih reakcija ovisila je o strukturi šećernih molekula.