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Enzymatic Preparation of Isomerically Pure 1,4:3,6-Dianhydro-D-glucitol Monoacetates - Precursors for Isoglucitol 2- and 5-mononitrates

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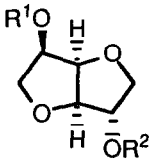
Abstract: Using a highly selective lipase from *Pseudomonas sp.* (SAM II) the isomerically pure title compounds **3** and **4** were prepared either by enzymatic hydrolysis (alcoholysis) of the diacetate **2** or by enzymatic esterification of the diol **1**, respectively. They were futher converted into the pharmaceutically important, isomerically pure 1,4:3,6-dianhydro-D-glucitol-2- and 5-nitrates **6** and **7**.

1,4:3,6-Dianhydro-D-glucitoldinitrate (Isoglucitoldinitrate), the corresponding 2-nitrate **6** and 5-nitrate **7** are well known for their vasodilatory effects[1-3].

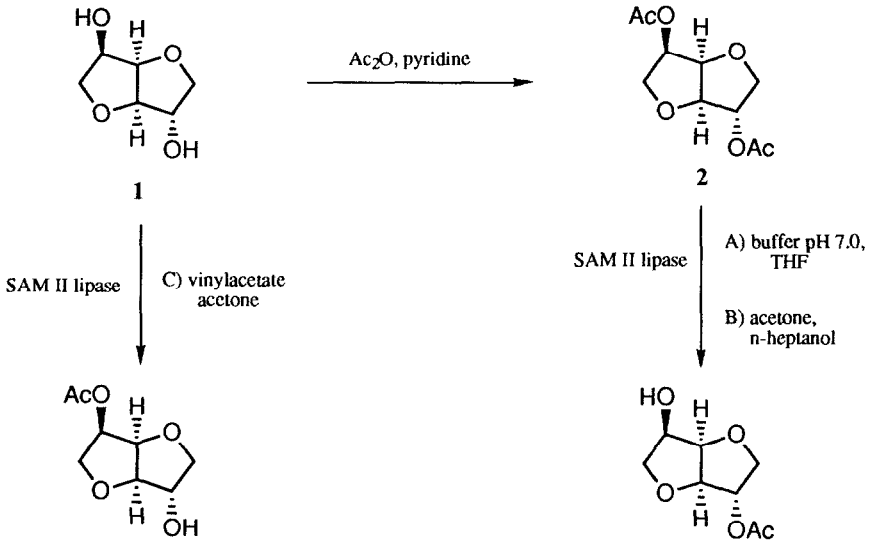


	R^1	R^2
1	H	H
2	CH_3CO	CH_3CO
3	H	CH_3CO
4	CH_3CO	H
5	O_2N	CH_3CO
6	H	O_2N
7	O_2N	H

In view of the rapid metabolism of Isoglucitoldinitrate *in vivo* leading to the mononitrates **6** and **7** with improved bioavailability as well as half life time these molecules, in particular **7** are used preferentially in pharmaceutical preparations. Key intermediates for the preparation of **6** and **7** are the corresponding monoesters (e.g. **3** and **4**, R = acyl) which can be prepared by a number of methods displaying variable selectivities regarding the isomeric purities of the resulting products[4-6]. Unfortunately, none of the methods leads to isomerically pure monoesters and additional purification steps of these key intermediates are always required.

Esterhydrolases (esterases, lipases) have become well known for their capability to differentiate with high regioselectivity between hydroxy groups in carbohydrate derivatives, both by enzymatic esterification[7-11] or hydrolyses of acylated derivatives[12-15]. They therefore seemed to be highly attractive also for the differentiation of the 2- and 5-hydroxy groups in 1,4:3,6-Dianhydro-D-glucitol **1** (Scheme 1).

Scheme 1: Enzymatic transformations of **1** and **2**



This was particularily true for a lipase from *Pseudomonas sp.* (SAM II)[16], which, in the past, proved to be a highly suitable biocatalyst for the enantioselective transformation of numerous molecules with secondary alcohol substructure of a wide structural variety[17-21] including cyclopentane derivatives.

Enzymatic hydrolysis of the diacetate **2** (Scheme 1, Route A) proved to be quite difficult at first due to the insolubility of the crystalline material in the aqueous reaction medium[22]. Fortunately, the problem can be overcome by the addition of a cosolvent like THF.

In typical experiments (Scheme 1, Route A) 10 mmol of **2** was dissolved in 5 ml abs. THF and 20 ml phosphate buffer pH 7.0. After addition of 400 mg lipase from *Pseudomonas sp.* (SAM II) the decreasing pH was kept constant by continuous addition of 1 M sodium hydroxide solution from an autoburette. After the consumption of one equivalent of NaOH, corresponding to the hydrolysis of one ester group the reaction rate decreased markedly, indicating a selective transformation. The resulting 1,4:3,6-Dianhydro-D-glucitol5-acetate **3** was simply isolated by extraction of the reaction medium (EtOAc) in excellent yield and isomerically pure (≥ 99% d.e.) as determined by glc (OV 17 capillary column). The structure of **3** was secured by the correlation of NMR spectra and optical rotation with literature data[23].

As an alternative to enzymatic hydrolyses in aqueous or biphasic reaction media a selective enzymatic removal of ester functions can occassionally be achieved by alcoholysis in organic reaction media, e.i. by employing alkanols instead of water as nucleophiles [24-26].

In a typical experiment (Scheme 1, Route B) 10 mmol of **2** was dissolved in a mixture of 15 ml acetone and 5 ml n-heptanol. After addition of 200 mg lipase SAM II the mixture was stirred at room temperature while the reaction progress was monitored *via* glc. After complete conversion of **2** into **3** (12 days), the enzyme was

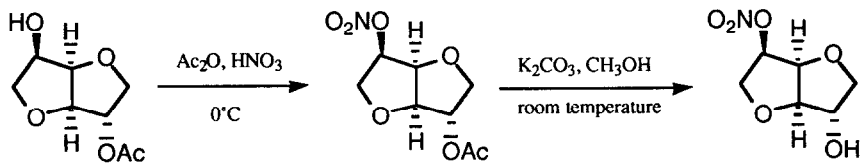
simply filtered off, all volatile materials removed under reduced pressure leading to isomerically pure (≥ 99% d.e.) **3** in excellent yield (91%).

While in both reaction modes enzymatic hydrolysis (A) and alcoholysis (B) the same product was obtained in comparable yields and identical isomeric purities, for practical reasons hydroysis is preferred over the much slower alcoholysis reaction.

In addition to their capability of catalysing the hydrolysis of esters in a highly selective way, esterhydrolases are equally well known for their ability to catalyze the corresponding reverse reactions, e.i. ester syntheses under the conditions of reversible and irreversible acyl transfer. . Due to the identical selectivities towards the absolute configuration of the parent molecule, products of complementary stereochemistry are obtained. Adopting this concept towards the esterification of 1,4:3,6-dianhydro-D-glucitol **1** one would expect that in this case the C(5) hydroxy group will be esterified leading exclusively to the 5-acetate **4.**

In typical experiments 10 and 100 mmol, respectively , of **1** were dissolved in 20 ml (200 ml) acetone[27] and 30 mmol (300 mmol) vinyl acetate. After addition of 200 mg (500 mg) lipase SAM II the mixtures were stirred at room temperature while the reaction progress was monitored by glc. After total conversion of **1** (3d and 5d, respectively) the enzyme was filtered off, the solvent removed *in vacuo* and the resulting monoester **4** isolated directly in excellent yields (90%) and isomerically pure (≥ 99% d.e.) The stereochemistry of **4** was secured both by 2D-NMR and the correlation of spectroscopic and optical data with the literature[23].

In summary, using enzyme catalyzed hydrolyses (alcoholyses) and esterifications in a complementary fashion both regioisomerically pure monoacetates **3** and **4** can be obtained directly and conveniently without the need of additional purification steps.



3 **5** **7**

The monoacetates **3** and **4** can be converted easily into the physiologically active mononitrates **6** and **7**, repectively following literature procedures[2,3]. Thus nitration of **3** (HNO_3, Ac_2O) at 0°, followed by the removal of the acetate function (K_2CO_3, MeOH, r.t.) leads to **7**.

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