Title: PROCESS FOR THE PREPARATION OF 3,6-DI-O-ACETYL-D-GLYCALYS

Abstract: There is described a process for the preparation of 3,6-di-O-acetyl-D-glycalys by C4→C6 acyl migration by treatment of corresponding 3,4-di-O-acetyl-D-glycalys with a buffer at pH of from 8.6 to 9.5. The starting 3,4-di-O-acetyl-D-glycalys may be prepared in situ by enzymatic hydrolysis of corresponding peracetylated D-glycalys in a buffer at a pH of from 3 to 5.
"Process for the preparation of 3,6-di-O-acetyl-D-glycals"

FIELD OF THE INVENTION

The present invention concerns a process for the preparation of 3,6-di-O-acetyl-D-glucal or 3,6-di-O-acetyl-D-galactal by regioselective migration of an acetyl group from the position 4 to the position 6 of corresponding 3,4-di-O-acetyl-D-glycals.

More particularly, the invention refers to a process for preparing 3,6-di-O-acetyl-D-glycals starting from the corresponding 3,4,6-tri-O-acetyl-D-glycal by enzymatic regioselective deprotection by deacetylation in position 6 and subsequent controlled migration of the 4-O-acetyl group toward the position 6.

BACKGROUND OF THE INVENTION


The D-glycals, peracetylated and monodeprotected in the positions 6, 4 or 3, are synthons useful for chemical manipulation leading to final product of industrial interest. The chemical methods employed to obtain said synthons involve long protection and deprotection procedures. On the contrary, enzymatic methods proved to be particularly interesting because of the regioselectivity shown by enzymes.

PRIOR ART

The acetylation of D-glucal and D-galactal and the regioselective deacetylation of the triacetylated compounds has been described by E.W. Holla [Holla 1, EP 0337920]. In particular, this document describes the transacetylation of D-glucal and D-galactal in a non aqueous medium on vinyl esters, catalyzed by Pseudomonas fluorescens lipase to obtain the corresponding 3,6-di-O-acetyl derivatives in a 92% and 75%
yield, respectively.
The same Author describes the regioselective deacetylation of tri-O-acetyl-D-glucal in aqueous solution by catalysis with *Pseudomonas fluorescens* in a weakly buffered solution without pH control to give 4,6-di-O-acetyl-D-glucal in a 90% yield, while in the case of tri-O-acetyl-D-galactal the same reaction affords a mixture of 4,6-di-O-acetyl-D-galactal and of several di- and mono-acetates [E.W. Holla Angew. Chem. Int. Ed. Engl. 28 (1989) 220-221 – Holla 2].

Still the same Author describes the preparation of 6-O-acetyl-, 3-methoxyacetyl- (or phenoxyacetyl-, or benzoylpropionyl-, or phenylacetetyl-, or N-substituted-glycyl)-glucal and of 6-O-phenylacetyl-3-O-acetylglucal by lipase acyl transfer via vinyl esters [E.W. Holla, Journal of Carbohydrate Chemistry 1990, 9(1), 113-119 – Holla 3].

The regioselective deprotection of tri-O-acetil-D-galactal is described by R. Pfau et al. [Synlett 11 (1999) 1817 – Pfau et al.] who used pig liver esterases (PLE-A, Pig Liver Esterase A) at 37°C and at pH 7 for 30 minutes to give a sufficiently selective formation of the 6-O-deblocked galactal in 65% yield, however, with incomplete conversion of the triacetylated starting material.

The regioselective deprotection of tri-O-acetyl-D-glucal is described by P. Crotti et al. [Tetrahedron 58 (2002) 6069-6091 – Crotti et al.] who incubated the starting material with lipase CCL in isopropanol, phosphate buffer and acetone for 16 hours to obtain pure 3,4-di-O-acetyl-D-glucaal in a 92% yield.

**SUMMARY OF THE INVENTION**

It has now been found that, by treating a 3,4-di-O-acetyl-D-glycal in aqueous medium buffered at a pH of from 8.0 to 9.5, a selective C-4→C-6 acyl migration occurs, giving the corresponding 3,6-di-O-acetyl-D-glycal, characterized by the free hydroxyl in position 4.

It has also been found that the starting 3,4-di-O-acetylglycal may be obtained in yields of from 95% to 100% from the corresponding 3,4,6-tri-O-acetylglycal by selective 6-O-deprotection in a buffered aqueous medium at a pH of from 3 to 5 by catalysis with immobilized lipases. The 3,4-di-O-acetyl-D-glycal thus obtained may be isolated in a practically quantitative (95-100%) yield.
Alternatively, the solution containing the 3,4-di-O-acetyl-D-glycal thus obtained may be subjected, in situ, after removal of the immobilized enzyme, to the selective C-4→C-6 acyl migration affording the corresponding 3,6-di-O-acetyl-D-glycal in a yield in the range of from 80% to 95%.

Thus, according to the present invention, starting from peracetylated D-glycals, in their turn obtained from the corresponding, known D-glucal e D-galactal, it is possible to manufacture, by enzymatic route, the corresponding 6-deacetyl derivatives which can be converted, in situ, into the corresponding 3,6-di-O-acetyl-D-glucal and 3,6-di-O-acetyl-D-galactal, namely into the corresponding 4-deprotected, peracetylated D-glycals by C-4→C-6 acyl migration according to the present invention.

In the present description, unless otherwise specifically defined, the term "D-glycal", in the singular or plural form, globally designates D-glucal and D-galactal.

The bond "±" which is present in the structural formulas herein below represents either the α configuration or the β configuration of the substituent in the 4-position of the glycal, said glycal being enantiomerically pure.

DETAILED DESCRIPTION

Thus, it is an object of the present invention to provide a process for the preparation of a 3,6-di-O-acetyl-D-glycal of formula I

\[ \text{CH}_2\text{OAc} \]

\[ \text{HO} \]

\[ \text{O} \]

\[ \text{OAc} \]

\[ (I) \]

wherein Ac represent an acetyl group, which comprises treating a corresponding 3,4-di-O-acetyl-D-glycal of formula II

\[ \text{CH}_2\text{OH} \]

\[ \text{O} \]

\[ \text{AcO} \text{OAc} \]

\[ (II) \]

wherein Ac is as defined above, with a buffer at a pH of from 8.0 to 9.5.

The migration process is carried out in aqueous medium buffered for example with TRIS buffer or, preferably, with phosphate buffer (the expression "phosphate buffer"
designates a KH₂PO₄ buffer in a concentration varying from 10 to 100 mM and will preferably be about 25 mM), at a pH value of from 8.0 to 9.5, advantageously from 8.6 to 9.0, preferably from 8.6 to 8.8, optionally in the presence of an organic co-solvent, preferably acetonitrile or acetone, at a concentration up to 50%, preferably of from 10 to 30%, at a temperature of from 0°C to 25°C.

Advantageously, the solution containing the 3,4-di-O-acetyl-D-glycal of formula I is brought to a pH from 8.0 to 9.5, advantageously from 8.6 to 9.0 preferably from 8.6 to 8.8 and kept at the chosen pH for 2-6 hours at a temperature of from 0°C to 25°C. The 3,6-di-O-acetyl-D-glycal thus obtained is isolated according to conventional methods, for example by adjusting the pH to acidic values, for example to a value of from 3 to 5, in order to block the migration, by removing the organic solvent, if any, by extracting the final product from the aqueous solution, for example with ethyl acetate and recovering the 3,6-di-O-acetyl-D-glycal, for example by column chromatography.

As mentioned above, the 3,6-di-O-acetyl-D-glycals of formula I are known in the literature, but the described methods for their preparation are carried out under non-advantageous experimental conditions, in particular in a non-aqueous solvent.

According to the present invention, the starting 3,4-di-O-acetyl-D-glycals of formula II may be prepared and isolated by treating a corresponding 3,4,6-tri-O-acetyl-D-glycal with an immobilized lipase in an aqueous medium buffered at a pH of from 3 to 5. By catalysis with the immobilized lipase, the 3,4,6-tri-O-acetyl-D-glycals are thus regioselectively deprotected in position 6 in yields which may vary in the range of from 90% to 100%.

Advantageously, without isolating the obtained 3,4-di-O-acetyl-D-glycal, at the end of the treatment with the immobilized lipase the subsequent filtration of the enzyme and the regulation in situ of the pH to values of from 8.0 to 9.5, advantageously from 8.6 to 9.0, preferably from 8.6 to 8.8, allows the selective C-4→C-6 acyl migration to give the desired 3,6-di-O-acetyl-D-glycal of formula I characterized by the free hydroxyl in the position 4, in yields which may vary from l'80% to 95%.

Thus it is another object of the present invention to provide a process for the preparation of a di-O-acetyl-D-glycal of formula I°
wherein one of X and X' is hydrogen and the other is Ac, and Ac is an acetyl group, which comprises
(a) submitting a 3,4,6-tri-O-acetyl-D-glycal of formula III

wherein Ac is as defined above, to a selective hydrolysis of the acetyl group in the position 6 with an immobilized lipase, in a buffer at a pH of from 3 to 5, whereby a 3,6-di-O-acetyl-D-glycal of formula II

wherein Ac is as defined above, is obtained; and, optionally,
(b) treating said 3,6-di-O-acetyl-D-glycal of formula II with a buffer at a pH of from 8.0 to 9.5, whereby a 3,6-di-O-acetyl-D-glycal of formula I

wherein Ac is as defined above.

The lipase used as a catalyst for the selective hydrolysis of step (a) is generally obtainable from a micro-organism, for example of the genus *Rhizomucor, Candida* or *Pseudomonas*. Advantageous microbial lipases are those obtainable from microorganisms of the *Candida* or *Pseudomonas* genus.
The lipase from *Candida* may be obtained from *Candida rugosa*, *Candida antarctica*, or *Candida lipolytica*, that from *Candida rugosa*, preferably immobilized as described in WO 03/057894, being preferred.
The lipase from *Pseudomonas* may be obtained from *Pseudomonas putida*, *Pseudomonas cepacia* or, advantageously, from *Pseudomonas fluorescens*, preferably immobilized as described in WO 03/057894.

The immobilization of the lipase is normally made on solid hydrophobic supports. Advantageously, the immobilization of lipase may be made on a silicon matrix consisting of an organosilicon compound, namely of a compound containing at least a Si-C bond (US 6,080,402). More advantageously, the immobilization may be made on a octyl agarose gel such as Octyl Sepharose® CL-4B, or on polymetacrylate resins with butyl character such as Sepabeads FP-BU or with decaoctyl character such as Sapebeads FP-RPOD which are already totally derivatized with hydrophobic groups, i.e. butyl and, decaoctyl chains, respectively. Preferably, the hydrophobic immobilizing support is octyl-agarose or decaoctyl-Sapebeads.

Alternatively, the immobilization may be made on a silica or silicates macroporous matrix (EP 444092), on a matrix consisting of adsorbing, optionally reticulated acrylic-type resins such as Amberlite® XAD-8 or Lewatit® E 2001/85 (EP 529 424), of an amphiphilic support containing lipophilic chains (US 5,182,201), on a styrene and divinylbenzene matrix optionally containing epoxy groups such as Lewatit® R 259 K or R 260 K or Diaion® HP-40, on a polycrylic resin containing epoxy groups such as FP 4000, on a polymethacrylic resin containing epoxy groups such as Sepabeads® FP-EP or Eupergit® C, suitably derivatized with hydrophobic groups.

In practice, according to the process of the present invention, the peracetylated D-glycal is dissolved in a buffer at a pH of from 3 to 5, preferably of from 4 to 5, for example in a 50 mM phosphate buffer containing 20-30% acetonitrile or acetone kept at the desired pH value. The solution thus obtained is treated with a preferably immobilized lipase from *Candida rugosa* or from *Pseudomonas fluorescens* immobilized for example on octyl-agarose as described in WO 03/57894 and left to incubate for a period of time from 3 to 72 hours, by controlling the hydrolysis reaction by HPLC.

At the end of the selective hydrolysis of the acetyl group in position 6, the obtained 3,4-di-O-acetyl-D-glycal may be isolated according to known methods, for example by flash chromatography.
Alternatively, step (b) is performed. After removal of the enzyme, for example by filtration, the solution containing the 3,4-di-O-acetyl-D-glycal of formula II is brought to a pH from 8.0 to 9.5, advantageously from 8.6 to 9.0, preferably from 8.6 to 8.8 and kept at the chosen pH at a temperature of from 0°C to 25°C for 2-6 hours as described herein above and the 3,6-di-O-acetyl-D-glycal thus obtained is isolated by conventional methods, for example by adjusting the pH to acidic values, for example at values in the range of from 3 to 5 in order to block the migration, by removing the organic solvent, if any, from the aqueous solution, by extracting the end product from the aqueous solution, for example with ethyl acetate and recovering the 3,6-di-O-acetyl-D-glycal for example by column chromatography.

According to an advantageous embodiment, the present invention provides a process for the preparation of a 3,6-di-O-acetyl glycal of formula I, which comprises submitting a corresponding 3,4,6-tri-O-acetyl-D-glycal of formula III to a selective hydrolysis in position 6 with an immobilized lipase, in a buffer at a pH of from 3 to 5, removing the enzyme, adjusting the pH of the solution containing the corresponding 3,4-di-O-acetyl-D-glycal of formula II thus obtained to a value of from 8.0 to 9.5, advantageously from 8.6 to 9.0, preferably from 8.6 to 8.8, and isolating the corresponding 3,6-di-O-acetyl glycal of formula I.

The acyl migration under controlled conditions and in an aqueous medium has shown to be a simple and rapid method, characterized by mild conditions, reduced percent amounts of organic solvents, if any, in the aqueous solution and low temperatures, for obtaining up to quantitative (70-100%) yields in 3,6-di-O-acetyl-D-glycals of formula I characterized by a free hydroxyl in position 4, in remarkably abbreviated reaction times in respect of the heretofore known reactions.

The following examples illustrate the invention. The control of the pH during the acyl migration was made with an automatic pH-Stat, 718 Stat Tritino by Metrohm (Herisau, Switzerland). The HPLC analyses were carried out by using a HPLC Kontron of Biotek Kontron Instruments S.p.A. (Milano, Italy), equipped with a HPLC 422A pump, with a UV HPLC 535 detector and with an injection valve with a 20 μL loop. A column Kromasil RP C18 (250 x 4.6 mm; 5 μm) by Akzo Nobel Eka Chemicals AB (Bohus, Sweden) was used. The analyses were carried out at room
temperature at a wave length of 220 nm. In monitoring the acyl migration reactions
the mobile phase was of 20% acetonitrile in 10mM KH₂PO₄ buffer at pH 4; the
mobile phase was filtered and de-gased before the use; the flux was of 1 mL/minute.
Monitoring during the reactions and during the purification operations was made
using TLC on silica gel 60 (0.25 mm, E. Merck, Darmstadt, Germany).
The purified regioisomers were identified by ¹H-NMR and COSY 2D NMR analyses
registered in CDCl₃-d₆ (δ=ppm) using an instrument Bruker AMX 400.

Example 1

3,4-Di-O-acetyl-D-gluca

To a 20 mM solution of 3,4,6-tri-O-acetyl-D-gluca (commercial: Lancaster) in 50
mM phosphate buffer containing 20% acetonitrile at a pH kept constant at a 4.0
value, 1 g of candida Rugosa lipase immobilized on octyl-agarose gel as described
in Preparation I of WO 03/057894 was added. The solution was let to stand under
mechanical stirring at room temperature, by controlling its course by HPLC and
keeping the pH of 4 constant by automatic titration. After a 4-hour incubation, 100%
conversion of the substrate was observed. The organic solvent was removed by
evaporation under reduced pressure. The product was extracted from the aqueous
solution with ethyl acetate. After evaporation of the collected organic extracts under
reduced pressure, the residue was purified on a silica gel chromatographic column by
using a n-hexane/ethyl acetate 1/1 (v/v) mixture as eluent. The 6-hydroxy derivative
was isolated in pure form and characterized by COSY 2D NMR studies, in order to
determine the correct position of the hydrolyzed acetyl group. Thus, the 3,4-di-O-
acetyl-D-gluca was obtained in a 100% yield. The product, isolated by column
chromatography, was identical to the compound described by Crotti et al.

Example 2

3,4-Di-O-acetyl-D-galacta

To a 20 mM solution of 3,4,6-tri-O-acetyl-D-galacta (commercial: Sigma-Aldrich)
in 50 mM phosphate buffer containing 20% acetonitrile at a pH kept constant at a 4.0
value, 1 g of Candida Rugosa lipase immobilized on octyl-agarose gel as described
in Preparation I of WO 03/057894 was added. The solution was let to stand under
mechanical stirring at room temperature, by controlling its course by HPLC and
keeping the pH of 4 constant by automatic titration. After a 5-hour incubation, 90% conversion of the substrate was observed. The organic solvent was removed by evaporation under reduced pressure. The product was extracted from the aqueous solution with ethyl acetate. After evaporation of the collected organic extracts under reduced pressure, the residue was purified on a silica gel chromatographic column by using a n-hexane/ethyl acetate 4/6 (v/v) mixture as eluent. The 6-hydroxy derivative was isolated in pure form and characterized by COSY 2D NMR studies, in order to determine the correct position of the hydrolyzed acetyl group. Yield: 100%.

$^1$H-NMR: in CDCl$_3$ (δ=ppm): 6.40 (dd, J=6.2Hz, 1.6Hz; 1H-1), 5.48 (m, J=1.7Hz; 1H-4), 4.75 (m, J=2.4Hz; 1H-2), 4.15-4.30 (m, J$_{5,6}$= 7.4Hz, J$_{6,6}$=12.2Hz; 3H-5/6a,b), 4.10-4.15 (m, J=4.7Hz; 1H-3), 2.11 (CH$_3$, s, 3H), 2.03(CH$_3$, s, 3H).

**Example 3**

3,6-Di-O-acetyl-D-glucaL

A solution of 20 mM of 3,4-di-O-acetyl-D-glucaL in 50 mM KH$_2$PO$_4$ buffer containing 20% acetonitrile at a pH 4.0 was brought to a temperature of 4°C and a pH 9.6 by rapid addition of NaOH and by controlling the pH value by pH-stat. After a 60-minute standing under mechanical stirring, the pH of the solution was brought pH 4 by addition of aqueous HCl in order to block the migration and the organic solvent was removed by evaporation under reduced pressure. The product was extracted from the aqueous solution with ethyl acetate. After evaporation of the collected organic extracts under reduced pressure, the residue was purified on a silica gel chromatographic column by using a n-hexane/ethyl acetate 1/1 (v/v) mixture as eluent. The 4-hydroxy derivative was isolated in pure form and characterized by COSY 2D NMR studies, in order to determine the correct position of the migrated acetyl group. Thus, the 3,6-di-O-acetyl-D-glucaL was obtained. The product, isolated by column chromatography, was identical to that described by Holla 3. Yield: 94%.

**Example 4**

3,6-Di-O-acetyl-D-galactaL

A solution of 20 mM of 3,4-di-O-acetyl-D-galactaL in 50 mM KH$_2$PO$_4$ buffer containing 20% acetonitrile at a pH 4.0 was brought to a temperature of 4°C and to pH 9.5 by rapid addition of NaOH under the control of the pH value by pH-stat.
After a 90-minute standing under mechanical stirring, the pH of the solution was brought to a value of 4 by addition of aqueous HCl in order to block the migration and the organic solvent was removed by evaporation under reduced pressure. The product was extracted from the aqueous solution with ethyl acetate. After evaporation of the collected organic extracts under reduced pressure, the residue was purified on a silica gel chromatographic column by using a n-hexane/ethyl acetate 1/1 (v/v) mixture as eluent. The 4-hydroxy derivative was isolated in pure form and characterized by COSY 2D NMR studies, in order to determine the correct position of the migrated acetyl group. Thus, the 3,6-di-O-acetyl-D-galactal was obtained. The product, isolated by column chromatography, was identical to that described by Pfau et al. Yield: 80%.

**Example 5**

3,6-Di-O-acetyl-D-glucal

To a 20 mM solution of 3,4,6-tri-O-acetyl-D-glucal (commercial: Lancaster) in 50 mM phosphate buffer containing 20% acetonitrile at a pH kept constant at a 4.0 value, 1 g of *Candida Rugosa* lipase immobilized on octyl-agarose gel as described in Preparation 1 of WO 03/057894 was added. The solution was let to stand under mechanical stirring at room temperature, by controlling its course by HPLC and keeping the pH of 4 constant by automatic titration. After a 4-hour incubation, 100% conversion of the substrate was observed. The immobilized enzyme was removed by filtration and the solution thus obtained was rapidly brought to a temperature of 4°C and pH 9.6 by addition of a solution of NaOH and under the control of the pH by pH-stat. After a 60-minute standing under mechanical stirring, the pH of the solution was brought to pH 4 by addition of an aqueous solution of HCl in order to block the migration and the organic solvent was removed by evaporation under reduced pressure. The product was extracted from the aqueous solution with ethyl acetate. After evaporation of the collected organic extracts under reduced pressure, the residue was purified on a silica gel chromatographic column by using a n-hexane/ethyl acetate 1/1 (v/v) mixture as eluent. The 4-hydroxy derivative was isolated in pure form and characterized by COSY 2D NMR studies, in order to determine the correct position of the migrated acetyl group. Thus, the 3,6-di-O-
acetyl-D-glucal (Formula I, R₁ = α-hydroxy, R₂ = β-hydrogen was obtained. Global yield 90%.

1H-NMR: in CDCl₃ (δ=ppm): 6.45 (dd, J=6, 1.2 Hz; 1H-1), 5.31 (dt, J=6,7, 2,2 Hz 1H-3), 4.76 (dd, J=3,6, 2.4Hz, 1H-2), 4.38-4.57 (2 dd, 2H-6A,B), 4.01 (m, H-5) 3.84(bt,J=9.6 1H-4), 3.54(bs, 1H-OH) 2.14-2.16 (2s, 6H).

Example 6

3,6-Di-O-acetyl-D-galactal

By operating as described in Example 5, starting from a 20 mM solution of 3,4,6-tri-O-acetyl-D-galactal (commercial: Sigma-Aldrich) in 50 mM phosphate buffer containing 20% acetonitrile at a pH maintained constant at a value of 4 and from 1 g of Candida rugosa lipase, a 90% conversion to 3,4-di-O-acetyl-D-galactal was observed. After removal of the immobilized enzyme by filtration, the obtained solution was brought to 4°C and, rapidly, to pH 9.5 by rapid addition of a NaOH solution, then it was treated as described in Example 2. Thus, 3,6-di-O-acetyl-D-galactal was obtained in a 70% global yield.

1H-NMR: in CDCl₃ (δ=ppm): 6.43 (dd, J=6,3, 1.2 Hz; 1H-1), 5.37 (m, J=4.7 Hz 1H-3), 4.65 (ddd, J=3,8, 1.7 Hz, 1H-2), 4.30 (m, H-5), 4.15-4.32 (2 m, 2H-6A,B), 4.15 (m 1H-4), 2.41(bs, 1H-OH) 2.08-2.06 (2s, 6H).
CLAIMS

1. A process for the preparation of a 3,6-di-O-acetyl-D-glycal of formula I

   \[ \text{CH}_2\text{OAc} \]

   \[ \text{HO} \text{Ac} \]

   \[ \text{(I)} \]

   wherein Ac represents an acetyl group, which comprises treating a corresponding

   3,4-di-O-acetyl-D-glycal of formula II

   \[ \text{CH}_2\text{OH} \]

   \[ \text{AcO} \text{Ac} \]

   \[ \text{(II)} \]

   wherein Ac is as defined above, with a buffer at a pH of from 8.0 to 9.5.

2. The process of claim 1, wherein said buffer consists of phosphate buffer.

3. The process of claim 1, wherein said buffer consists of phosphate buffer
   and of an organic co-solvent.

4. The process of claim 1, wherein said buffer consists of phosphate buffer
   containing 10-30% acetonitrile at a pH of from 8.6 to 9.0.

5. The process of claim 1, wherein the starting 3,4-di-O-acetyl-D-glycal
   of formula II is prepared by treating a corresponding 3,4,6-tri-O-acetyl-D-glycal
   with an immobilized lipase in an aqueous medium buffered at a pH of from 3 to 5.

6. The process of claim 1, wherein the starting 3,4-di-O-acetyl-D-glycal
   of formula II is prepared by treating a corresponding 3,4,6-tri-O-acetyl-D-glycal
   with an immobilized lipase in an aqueous medium buffered at a pH of from 3 to 5
   and, after removal of the immobilized enzyme, converted in situ into the
   corresponding 3,6-di-O-acetyl-D-glycal of formula I by adjustment of the pH to a
   value of from 8.0 to 9.5.

7. The process of claim 6, wherein said buffer consists of phosphate buffer.

8. The process of claim 6, wherein said buffer consists of phosphate buffer
   and of an organic co-solvent.

9. The process of claim 6, wherein said buffer consists of phosphate buffer
buffer containing 10-30% of acetonitrile or acetone.

10. A process according to anyone of claims 5 to 9, wherein said lipase is from *Candida rugosa* and is immobilized on a hydrophobic support.

11. The process of claim 10, wherein the immobilizing hydrophobic support is octyl-agarose.


\[
\text{CH}_2\text{OX} \\
\text{X'O} \quad \text{OAc} \\
\text{(I°)}
\]

wherein one of X and X' is hydrogen and the other is Ac, and Ac is an acetyl group, which comprises

10 (a) submitting a 3,4,6-tri-O-acetyl-D-glycal of formula III

\[
\text{CH}_2\text{OAc} \\
\text{AcO} \quad \text{OAc} \\
\text{(III)}
\]

wherein Ac is as defined above, to a selective hydrolysis of the acetyl group in the position 6 with an immobilized lipase, in a buffer at a pH of from 3 to 5, whereby a 3,6-di-O-acetyl-D-glycal of formula II

\[
\text{CH}_2\text{OH} \\
\text{AcO} \quad \text{OAc} \\
\text{(II)}
\]

wherein Ac is as defined above, is obtained; and, optionally,

(b) treating said 3,6-di-O-acetyl-D-glycal of formula II with a buffer at a pH of from 8.0 to 9.5, whereby a 3,6-di-O-acetyl-D-glycal of formula I

\[
\text{CH}_2\text{OAc} \\
\text{HO} \quad \text{OAc} \\
\text{(I)}
\]

wherein Ac is as defined above.