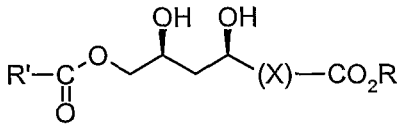
PROCESS FOR THE PREPARATION OF DIHYDROXY ESTERS AND DERIVATIVES THEREOF

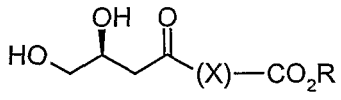
The present invention concerns a stereoselective process for the preparation of dihydroxy esters, and derivatives thereof.

According to a first aspect of the present invention, there is provided a process for the preparation of a compound of formula (1)



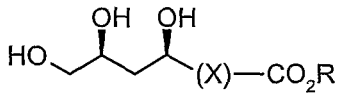
Formula 1

which comprises either a) the stereoselective reduction of a compound of formula (2)



Formula 2

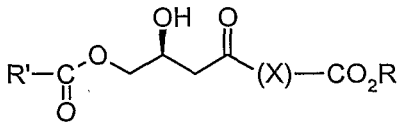
to produce a compound of formula (3), and



Formula 3

b) Esterification of the compound of formula (3) in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (1); or

c) Esterification of a compound of formula (2) in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (4), and



Formula 4

d) the stereoselective reduction of a compound of formula (4) to produce a compound of formula (1)

wherein

X represents an optionally substituted hydrocarbyl linking group

R and R" each independently represent an optionally substituted hydrocarbyl group, and R' represents an optionally substituted hydrocarbyl, preferably an optionally substituted alkyl group.

Hydrocarbyl groups represented by X, R, R' or R" may be substituted by one or more substituents, and may be per-substituted, for example perhalogenated. Examples of substituents include halo, especially fluoro and chloro, alkoxy, such as C_{1-4}alkoxy, and oxo.

Preferably, X represents a group of formula $-(CH_2)_n-$ where n is from 1 to 4, and most preferably X represents a group of formula $-CH_2-$.

R" may be an alkyl group, such as a C_{1-6} alkyl group, or an alkylcarbonyl group, such as a C_{1-6}alkylcarbonyl group, for example a $CH_3(C=O)-$ or $CF_3(C=O)-$ group. R" is most preferably a vinyl or isopropenyl group.

R preferably represents a C_{1-6} alkyl group, which may be linear or branched, and may be substituted by one or more substituents. Most preferably, R represents a t-butyl group.

R' may represent a substituted alkyl, often C_{1-6} alkyl group, such as a CF_3- or CF_3CH_2- group, but is preferably an unsubstituted C_{1-6} alkyl group, and most especially a methyl group.

The stereoselective reduction of the compounds of formulae (2) or (4) preferably employ chemical or microbial reduction methods, such as hydrogenation, transfer hydrogenation, metal hydride reduction or dehydrogenases. Examples of a suitable hydrogenation process such as that described in Helv. Chim. Acta 69, 803, 1986 (incorporated herein by reference) include the use of between 0.01 and 10% (w/w) of catalysts such as platinum, palladium or rhodium on heterogeneous supports such as carbon, alumina, silica using molecular hydrogen at between 1 and 10 bar in a solvent such as methanol, ethanol, t-butanol, dimethylformamide, t-butylmethylether, toluene or hexane. Alternatively homogenous hydrogenation catalysts such as those described in EP0583171 (incorporated herein by reference) may be used.

Examples of suitable chemical transfer hydrogenation processes include those described in Zassinovich, Mestroni and Gladiali, Chem. Rev. 1992, 92, 1051 (incorporated herein by reference) or by Fuji et al in J. Am. Chem. Soc. 118, 2521, 1996 (incorporated herein by reference). Preferred chemical transfer hydrogenation processes employ chiral ligated complexes of transition metals, such as ruthenium or rhodium, especially chiral diamine-ligated neutral aromatic ruthenium complexes. Preferably, such

a chemical transfer hydrogenation employs an acid, especially a formate salt such as triethylammonium formate, as the hydrogen source.

Metal hydride reagents such as those described in Tet.1993, 1997, Tet. Asymm. 1990,1, 307, (incorporated herein by reference), or J. Am. Chem. Soc. 1998, 110, 3560 (incorporated herein by reference) can be used.

Examples of suitable microbial reductions include contacting the compound of formula (2) or (4) with an organism possessing the properties of a microorganism selected from Beauveria preferably Beauveria bassiana, Pichia preferably Pichia angusta or Pichia pastoris, trehalophila, haplophila or membranefaciens, Candida preferably Candida humicola, solani, guillermondii, diddenssiae or friedrichii, Kluyveromyces preferably Kluyveromyces drosophilarum, or Torulaspora preferably Torulaspora hansenii. The reduction may be achieved by contacting the compounds of formulae (2) or (4) with an enzyme extracted from the foregoing microorganisms. Most preferably, the compounds of formulae (2) or (4) are contacted with a microorganism selected from Pichia angusta, Pichia pastoris, Candida guillermondii, Saccharomyces carlsbergensis, Pichia trehalophila, Kluyveromyces drosopliarum and Torulospora hansenii, or an extract from the foregoing organisms.

The invention preferably comprises producing a compound of formula (3) by selectively reducing a compound of formulae (2) using whole cells of or extracts from the aforementioned microorganisms, preferably Pichia angusta, Pichia pastoris, Candida guillermondii, Saccharomyces carlsbergensis, Pichia trehalophila, Kluyveromyces drosopliarum and Torulospora hansenii.

The invention is most preferably carried out using whole cells of the organisms as this avoids the need to separate the desired enzyme and provides co-factors for the reaction.

Any of the above species may be used but in many embodiments, it has been found that high conversions and high selectivity can be achieved by the use of the enzyme or whole cells of Pichia angusta.

In general a co-factor, normally NAD(P)H (nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate) and a system for re-generating the co-factor, for example glucose and glucose dehydrogenase, are used with the enzymes to drive the reaction. As suitable co-factors and reduction mechanisms are present in the whole cells it is preferred to use the whole cells in a nutrient medium which preferably contains a suitable carbon source, which may include one or more of the following: a sugar, e.g. maltose, sucrose or preferably glucose, a polyol e.g. glycerol or sorbitol, citric acid, or a lower alcohol, for example methanol or ethanol.

If whole cells are intended to grow during the reaction nitrogen and phosphorus sources and trace elements should be present in the medium. These may be those normally used in culturing the organism.

The process may be carried out by adding a compound of formula (2) or (4) to a culture of the growing organism in a medium capable of supporting growth or to a suspension of the live cells in a medium which preferably contains a carbon source but which lacks one or more nutrients necessary for growth. Dead cells may also be used providing the necessary enzymes and co-factors are present; if necessary they may be added to the dead cells.

If desired the cells may be immobilised on a support which is contacted with compound of formula (2) or (4) preferably in the presence of a suitable carbon source as previously described.

The pH is suitably 3.5 to 9, for example 4 to 9, preferably at most 6.5 and more preferably at most 5.5. Very suitably a pH of 4 to 5 is used. The process may suitably be carried out at a temperature of 10 to 50°C, preferably 20 to 40°C and more preferably 25 to 35°C. It is preferred to operate under aerobic conditions if live whole cells of the aforesaid organisms are present. An aeration rate equivalent to 0.01 to 1.0 volumes of air measured at standard temperature and pressure per volume of the culture medium per minute is suitably employed at the aforesaid conditions of pH and temperature but it will be appreciated that considerable variation is possible. Similar pH, temperature and aeration conditions may be used during growth of the organisms if this is carried out separately from the process.

Purified enzymes may be isolated by known means suitably by centrifuging a suspension of disintegrated cells and separating a clear solution from debris, separating the desired enzyme from the solution for example by ion exchange chromatography suitably with elution from the column with liquid of increasing ionic strength and/or by selective precipitation by the addition of an ionic material, for example ammonium sulphate. Such operations may be repeated if desired to enhance purity.

The microbial reduction of compounds of formula (2) or (4) is particularly preferred, and this process forms a second aspect of the present invention.

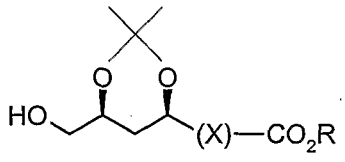
In the esterification of compounds of formula (2) or (3) it is preferred to transesterify with another ester, which is present in at least mole equivalence with respect to the alcohol and is suitably a vinyl ester (as the by-product, acetaldehyde is not involved in a back-reaction). Alternatively an anhydride such as acetic anhydride or trifluoroacetic anhydride, or an ester such as ethylacetate or a fluorinated ester such as trifluoroethylacetate may be used. It is preferred that the regiospecific esterification reaction be carried out in an organic solvent containing less than 1% (w/w) water such as acetonitrile, ethylacetate, tetrahydrofuran, tert-butylmethylether, toluene, butanone, pentanone or hexanone at a temperature of preferably 20 to 75°C, more preferably 25 to 50°C. The esters are preferably esters of lower alkanoic acids having 2 to 8 carbon atoms, or substituted derivatives thereof. Optionally an inert atmosphere may be employed, for example a flow of nitrogen may be passed through the solution.

The enzymes may be provided as such or as whole cells comprising them. It is preferred that they be immobilised so as to facilitate their separation from the product and, if desired, re-use.

Preferred enzymes include lipases such as Porcine pancreatic lipase, Candida cylindracea lipase, Pseudomonas fluorescens lipase, Candida antarctica fraction B such as that available under the trade mark Chirazyme L2, those from Humicola lanuginosa for example that sold under the Trade Mark Lipolase or those from Pseudomonas for example that sold under the Trade Mark SAM II and more preferably those from Candida antarctica, for example that sold under the Trade Mark Chirazyme.

Compounds of formula (1) wherein R' is CH_3, R is optionally substituted hydrocarbyl, X is $-(CH_2)_n-$ and n is 1 to 4 form a third aspect of the present invention. Preferably, R is t-butyl and most preferably, X is $-CH_2-$.

Compounds of formula (1) are useful intermediates for the preparation of pharmaceutical compounds. Commonly, they are reacted with a protecting group for 1,3-dihydroxy moieties such as 2,2-dimethoxypropane to form an acetonide as described in Synthesis 1998, 1713. The group R'-(C=O)- may then be selectively removed by treatment with weakly basic alcoholic solution eg K_2CO_3 solution as described in US5,278,313 or a lipase either in aqueous solution, or in organic solution containing sufficient water to support hydrolysis, to form a compound of formula (5):



Formula 5

This process for the preparation of compounds of formula (5) forms a fourth aspect of the present invention.

Example 1

Preparation of (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate.

To a stirred 250ml round bottom flask 20ml acetonitrile, 0.405g (0.662mmoles) of di-mu-chlorobis[(p-cymene)chlororuthenium (II)], and 0.492g (1.34mmoles) (1S,2S)-(+)-N-(4-toluenesulfonyl)-1,2-diphenylethylenediamine were charged. The solution was de-oxygenated by sparging with nitrogen and thereafter maintaining a trickle. A de-oxygenated solution of 26g (0.119mol) optically pure (5S) t-butyl 3-keto-5,6-dihydroxyhexanoate in 15ml acetonitrile was charged to the reaction vessel and the solution stirred at ambient temperature for 20 minutes. 65ml of a 5:2 (mol/mol) mixture of distilled formic acid and triethylamine were then added over a period of 10 minutes and

the reaction mixture stirred at ambient temperature for 48 hours. To this solution 80ml dichloromethane and 120ml saturated sodium bicarbonate were slowly added. 70g ammonium chloride was charged to the aqueous layer and the organic layer separated. The aqueous layer was washed thrice more with 90ml ethylacetate, the organic fractions combined, dried over sodium sulfate and the solvent removed to give 21.1g of a crude oil containing mainly (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate. The ratio of diastereomers was determined by ^{13}CNMR to be 5.2 : 1 (3R:5S) : (3S:5S). The material was used crude in the next reaction but could be purified by column chromatography.

Preparation of (3R,5S) t-butyl 6-acetoxy-3,5-dihydroxyhexanoate.

To a stirred 1l round bottom flask 700ml tetrahydrofuran and 70.7g (0.32mol) of (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate, 41ml (0.46mol) vinylacetate and 6.3g of the supported lipase Chirazyme L2 ™ were charged. After 3 hours stirring at ambient temperature the lipase was removed by screening and the volatiles removed by distillation under vacuum. The mass of crude oil was 78.7g and the major component was determined to be (3R,5S) t-butyl 6-acetoxy-3,5-dihydroxyhexanoate. This material was used directly in the next stage.

Preparation of (4R,6S)-6-[(acetyloxy)methyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid, 1,1-dimethylethylester.

To a stirred 1 litre round bottom flask 78.7g (3R,5S) t-butyl 6-acetoxy-3,5-dihydroxyhexanoate, 800ml of 2,2-dimethoxypropane and 5.7g p-toluenesulfonic acid were charged. After 35 minutes thee reaction mass was concentrated to half its volume and 300ml of dichloromethane and 300ml of 1M sodiumbicarbonate added. The organic layer was separated and the aqueous layer washed thrice more with 150ml ethylacetate. The organic fractions were combined, dried over sodium sulfate and the volatiles removed by distillation under vacuum. 92g of a crude oil was obtained. This was purified first by passing through a short column of flash silica and eluting with hexane and then hexane:ethylacetate 85:15 (v/v), and then crystallising the material 3 times from hexane to give 22.17g (4R,6S)-6-[(acetyloxy)methyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid, 1,1-dimethylethylester which by chiral GC was determined to be 99.9%de.

Preparation of (4R,6S)-6-(hydroxymethyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid, 1,1-dimethylethylester.

To a 500ml stirred round bottom flask 22.17g of (4R,6S)-6-[(acetyloxy)methyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid, 1,1-dimethylethylester, 250ml methanol and 5.05g crushed potassium carbonate were charged. The reaction was stirred for 35 minutes until the hydrolysis was complete, then the potassium carbonate was removed by screening, the reaction mass concentrated and 150ml 5% (w/w) brine and 150ml toluene added. The

organic layer was separated and the aqueous washed twice more with 250ml toluene. The organic layers were combined, washed three times with 15% (w/w) brine and the solvent removed by vacuum distillation to give 17.78g of clear oil, which was determined to be >99% (4R,6S)-6-(hydroxymethyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid, 1,1-dimethylethylester.

Example 2

Preparation of (5S) tert-butyl 6-acetoxy-5-hydroxy-3-ketohexanoate.

To a stirred 250ml round bottom flask were charged 2.32g (0.0106moles) (5S) tert-butyl 5,6-dihydroxy-3-ketohexanoate, 40ml tetrahydrofuran, 0.98ml (0.0106 moles) vinyl acetate and 0.22g of the supported lipase Chirazyme L2 ™. After 20 minutes the lipase was removed by screening and the volatiles removed by distillation under vacuum, to give 2.96g of a crude oil that was characterised by NMR as (5S) tert-butyl 6-acetoxy-5-hydroxy-3-ketohexanoate.

Example 3

Preparation of (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate.

Pichia angusta NCYC R230 (deposited under the provisions of the Budapest Treaty on May 18th, 1995) was grown in a Braun Biostat Q multi-fermenter system in the following medium (per litre): glucose 40 g; $MgSO_4$, 1.2 g; K_2SO_4, 0.21 g; KH_2PO_4, 0.69 g; H_3PO_4 (concentrated), 1 ml; yeast extract (Oxoid), 2 g; $FeSO_4.7H_2O$, 0.05 g; antifoam (EEA 142 Foammaster), trace elements solution, 1 ml (this solution contained per litre $CuSO_4.5H_2O$, 0.02 g; $MnSO_4.4H_2O$, 0.1 g; $ZnSO_4.7H_2O$, 0.1 g; $CaCO_3$, 1.8 g.

Each of 4 fermenters were charged with 250 ml of medium and sterilised by autoclaving. The pH was adjusted to 4.5 using 7 molar ammonium hydroxide solution, the temperature was set to 28°C, the air flow set at 300 ml/minute and the agitator speed set to 1200 rpm. Fermenters were inoculated with cells taken from agar plates (2% agar) comprising the same medium as described above except that the glucose concentration was 20 g/litre. Following 22 hours growth in the fermenters the bioreduction reaction was started by the addition of (5S) t-butyl 3-keto-5,6-dihydroxyhexanoate; two of the fermenters were charged with 3.75 ml each and the other two charged with 5 ml each.

The reaction was continued for a further 78 hours until 100% conversion of substrate. During this period the culture was fed with a 50% solution of glucose at a rate of 1-3 grams glucose/litre culture/hour to maintain cell viability and provide a source of reducing power. Reactions were terminated by removal of the cells by centrifugation. To the recovered cell-free supernatant was added sodium chloride to a final concentration of 20% w/v and the mixture extracted three times with an equal volume of acetonitrile. The

pooled acetonitrile extracts were dried with anhydrous sodium sulfate and the solvent removed under reduced pressure in a rotary evaporator (water bath temperature 45°C) to yield a viscous pale yellow oil. The identity of the product from each reaction was confirmed as (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate and the diastereomeric excess of each of the samples is given in the table below.

experiment	diastereomeric excess (%)
1	99.6
2	99.6
3	99.4
4	99.6

Example 4

Preparation of (3R,5S) t-butyl 3,5,6-trihydroxyhexanoate.

Pichia angusta NCYC R320 (deposited under the provisions of the Budapest Treaty on May 18th, 1995) was grown in a Braun Biostat Q multi-fermenter system in the following medium (containing, per litre): glucose, 20 g; ammonium sulfate, 10 g; Yeast extract (Oxoid), 2 g; $MgSO_4.7H_2O$, 1.2 g; KH_2PO_4, 0.69 g; K_2SO_4, 0.21 g; $FeSO_4.7H_2O$, 0.05 g; H_3PO_4 (concentrated), 1 ml; EEA 142 "foammaster" antifoam, 0.5 ml; trace elements solution, 1 ml (this solution contained per litre $Ca(CH_3CO_2)_2$, 2.85g; $ZnSO_4.7H_2O$, 0.1 g; $MnSO_4.H_2O$ 0.075 g $CuSO_4.5H_2O$, 0.02 g; sulphuric acid (concentrated), 1 ml).

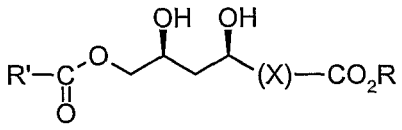
One fermenter was charged with 250 ml medium and sterilised by autoclaving. The pH was adjusted to 5.0 using 2 molar sodium hydroxide solution. The temperature was set to 28°C, the air flow was set to 250 ml $minute^{-1}$ and the agitator speed set to 1200 rpm. The fermenter was inoculated with 2.5ml of a suspension of cells in sterile deionised water prepared from an agar plate of *Pichia angusta* NCYC R320. Following 17 hours growth the bioreduction was started by the addition of 6.36g 5(S) t-butyl 3-keto-5,6-dihydroxyhexanoate as an aqueous solution. At the same time a glucose feed to the fermenter was started at a rate of 2g glucose L^{-1} h^{-1}.

The reaction was continued for a further 78 hours at which point 96% conversion of the substrate had been achieved. Starting material and product were detected by HPLC (Hichrom S5 CN-250A column, temperature 35°C, mobile phase: aqueous TFA (0.1%) : acetonitrile 95:5, flow rate 1 ml min^{-1}, injection volume 5 ml, refractive index detector).

The reaction was terminated by the removal of cells by centrifuging at 4000 x g for 20 minutes. The pH of the recovered cell-free supernatant was adjusted to 7.5 using 2M NaOH. $MgSO_4.1.6H_2O$ (15 % w/v based on anhydrous) was dissolved in the cell-free supernatant and the resulting solution was extracted twice with an equal volume of 2-pentanone. The solvent phases were collected and the solvent removed under reduced pressure in a rotary evaporator at 45°C yielding an orange viscous oil. This was re-dissolved in 50 ml dry, distilled 2-pentanone and again the solvent was removed by rotary evaporation to afford t-butyl 3,5,6-trihydroxyhexanoate (5.08 g, 80 % isolated yield). Diastereomeric excess was determined as follows; a sample of t-butyl 3,5,6-trihydroxyhexanoate (30 mg) was derivatised by reaction for at least 10 minutes at room temperature in an excess of trifluoroacetic anhydride, excess anhydride was removed under a stream of dry nitrogen and the residual oil diluted with dichloromethane (1 ml). The sample was analysed using a Chiralcel Dex CB column (25 metre) at a temperature of 140°C (isothermal). The diastereomers eluted at 14.4 minutes (3R,5S diastereomer) and 15.7 minutes (3S,5S diastereomer). The diastereomeric excess of the sample was found by this method to be 99.7%.

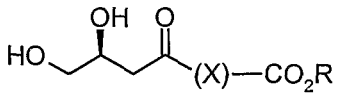
CLAIMS

1. A process for the preparation of a compound of formula (1)



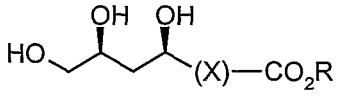
Formula 1

which comprises either a) the stereoselective reduction of a compound of formula (2)



Formula 2

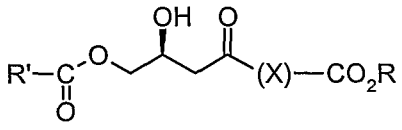
to produce a compound of formula (3), and



Formula 3

b) Esterification of the compound of formula (3) in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (1); or

c) Esterification of a compound of formula (2) in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (4), and



Formula 4

d) the stereoselective reduction of a compound of formula (4) to produce a compound of formula (1)

wherein

X represents an optionally substituted hydrocarbyl linking group

R and R" each independently represent an optionally substituted hydrocarbyl group, and R' represents an optionally substituted hydrocarbyl, preferably an optionally substituted alkyl group.

2. A process according to claim 1, wherein X represents a group of formula $-CH_2-$.

3. A process according to either preceding claim, wherein R" represents a vinyl or isopropenyl group.

4. A process according to any preceding claim, wherein R' represents a substituted or unsubstituted C_{1-6} alkyl group.

5. A process according to claim 4, wherein R' represents a methyl group.

6. A process according to any preceding claim, wherein the compounds of Formulae (2) or (4) are reduced by contact with an organism possessing the properties of a microorganism selected from Beauveria, Pichia, Candida, Kluyveromyces or Torulaspora genera or an enzyme extracted therefrom.

7. A process according to claim 6, wherein the compounds of formulae (2) or (4) are reduced by contact with an organism selected from the group consisting of Pichia angusta, Pichia pastoris, Candida guillermondii, Saccharomyces carlsbergensis, Pichia trehalophila, Kluyveromyces drosopliarum and Torulospora hansenii, or an enzyme extracted therefrom.

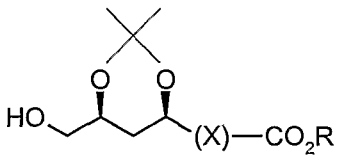
8. A process according to either of claims 6 or 7, wherein whole cells are employed.

9. A process according to any one of claims 6, 7 or 8, wherein the compounds of formulae (2) or (4) are reduced at a pH of from 4 to 5.

10. A process according to any preceding claim, wherein the compounds of formulae (2) or (3) are esterified in the presence of an enzyme selected from the group consisting of Porcine pancreatic lipase, Candida cylindracea lipase, Pseudomonas fluorescens lipase, Candida antarctica fraction B and lipase from Humicola lanuginosa.

11. A process according to any preceding claim, wherein the compound of formula R"-O-COR' is vinyl acetate.

12. A process for the preparation of a compound of formula (5)



Formula 5

which comprises:

a) preparing a compound of formula (1) by a process according to any preceding claim; b) reacting the compound of formula (1) with 2,2-dimethoxypropane to form an acetonide; and

c) removing the group R'-(C=O)-;

wherein

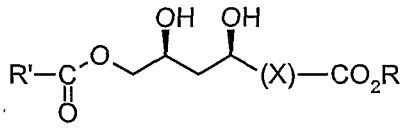
X represents an optionally substituted hydrocarbyl linking group

R represents an optionally substituted hydrocarbyl group, and

R' represents an optionally substituted hydrocarbyl, preferably an optionally substituted alkyl group.

13. A process according to claim 12, wherein the R'-(C=O)- is removed by treatment with a basic, alcoholic solution.

14. A compound of formula (1):

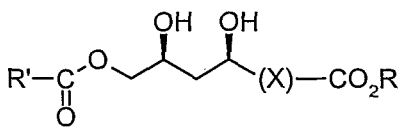


Formula 1

wherein R' is CH_3 , R is optionally substituted hydrocarbyl, X is $-(CH_2)_n-$ and n is 1 to 4.

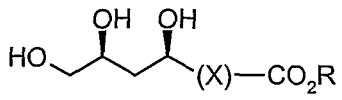
15. A compound according to claim 14, wherein R is t-butyl and X is $-CH_2-$.

16. A process for the preparation of a compound of formula (1)



Formula 1

which comprises esterification of a compound of formula (3)



Formula 3

in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (1);

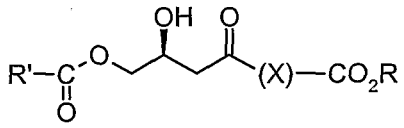
wherein

X represents an optionally substituted hydrocarbyl linking group

R and R" each independently represent an optionally substituted hydrocarbyl group, and

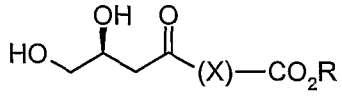
R' represents an optionally substituted hydrocarbyl, preferably an optionally substituted alkyl group.

17. A process for the preparation of a compound of formula (4)



Formula 4

which comprises esterification of a compound of formula (2)



Formula 2

in the presence of a compound of formula R"-O-COR' and a lipase or hydrolase enzyme thereby to form the compound of formula (4);

wherein

X represents an optionally substituted hydrocarbyl linking group

R and R" each independently represent an optionally substituted hydrocarbyl group, and

R' represents an optionally substituted hydrocarbyl, preferably an optionally substituted alkyl group.

18. A process according to either of claims 16 or 17, wherein R' is CH_3, R is t-butyl and X is $-CH_2-$.

19. A process according to any one of claims 16, 17 or 18, wherein R" is a vinyl or isopropenyl group.

20. A process according to any one of claims 16 to 19, wherein the enzyme is selected from the group consisting of Porcine pancreatic lipase, Candida cylindracea lipase, Pseudomonas fluorescens lipase, Candida antarctica fraction B and lipase from Humicola lanuginosa.