FORM 1

612986

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

Pfizer Inc., of 235 East 42nd Street, New York, State of New York, UNITED STATES OF AMERICA, hereby apply for the grant of a standard patent for an invention entitled:

Processes for Tigogenin Beta-Cellobioside

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
365,588	US	13 June 1989

The address for service is:-

Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia

DATED this ELEVENTH day of JUNE 1990

Pfizer Inc.

By: J. Gibson.

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 129951
S&F CODE: 60031

S 015410 120690

5845/7

~~PC 7600/RKB~~

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA
THE PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

AUSTRALIA CONVENTION STANDARD & PETTY PATENT DECLARATION

In support of the Convention Application made for a patent for an invention entitled:

Title of Invention

PROCESSES FOR TIGOGENIN BETA-CELLOBIOSIDE

Full name(s) and address(es) of Declarant(s)

I/~~We~~ J. Trevor Lumb

of Pfizer Inc., A Corporation Organized Under The Laws Of The State Of Delaware. United States Of America Of 235 East 42nd Street, New York, State Of New York, United States Of America.

do solemnly and sincerely declare as follows:-

Full name(s) of Applicant(s)

1. ~~I am/We are the applicant(s) for the patent~~

(or, in the case of an application by a body corporate)

1. I am/~~We are~~ authorised by PFIZER INC.

the applicant(~~s~~) for the patent to make this declaration on its/~~their~~ behalf.

2. The basic application(~~s~~) as defined by Section 141 of the Act was/~~were~~ made

Basic Country(ies)

in United States of America

Priority Date(s)

on June 13, 1989

Basic Applicant(s)

by Frank John Urban

3. ~~I am/We are the actual inventor(s) of the invention referred to in the basic application(s)~~

Full name(s) and address(es) of inventor(s)

(or where a person other than the inventor is the applicant)

3. Frank John Urban

of 12 Twin Lakes Drive, Waterford, State of Connecticut, United States of America

is/~~are~~ the actual inventor(~~s~~) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows: (~~respectively~~)

Set out how Applicant(s) derive title from actual inventor(s) e.g. The Applicant(s) is/are the assignee(s) of the invention from the inventor(s)

The said applicant is the assignee of the actual inventor.

4. The basic application(~~s~~) referred to in paragraph 2 of this Declaration was/~~were~~ the first application(~~s~~) made in a Convention country in respect of the invention(~~s~~) the subject of the application.

Declared at Groton, Connecticut, USA this 23rd day of April 1990

J. TREVOR LUMB
DIRECTOR OF PATENTS
CENTRAL RESEARCH

Signature of Declarant(s)

SFP4 To: The Commissioner of Patents 1/81

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(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 619986

(54) Title
PROCESSES FOR TIGOGENIN BETA-CELLOBIOSIDE

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(43) Publication Date : 01.08.91

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(71) Applicant(s)
PFIZER INC.

(72) Inventor(s)
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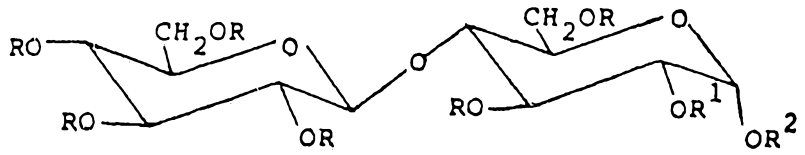
(74) Attorney or Agent
SPRUSON & FERGUSON , GPO Box 3898, SYDNEY NSW 2001

(56) Prior Art Documents
AU 30977/84 C07J 71/00
US 4602005

(57) Claim

1. A compound of the formula

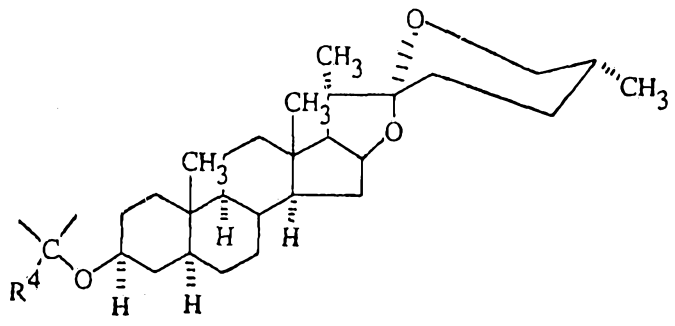


(I)

wherein

R is R^4CO;

R^1 and R^2 are taken together and are



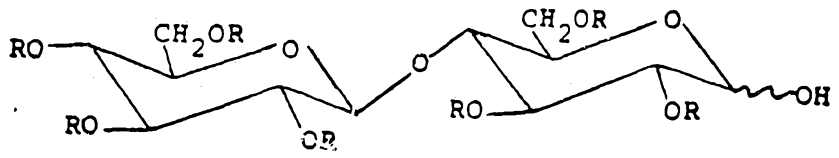
; and

R^4 is (C^1-C^4)alkyl.

3. A process for the synthesis of tigogenin beta-cellobioside which comprises the steps of

.../2

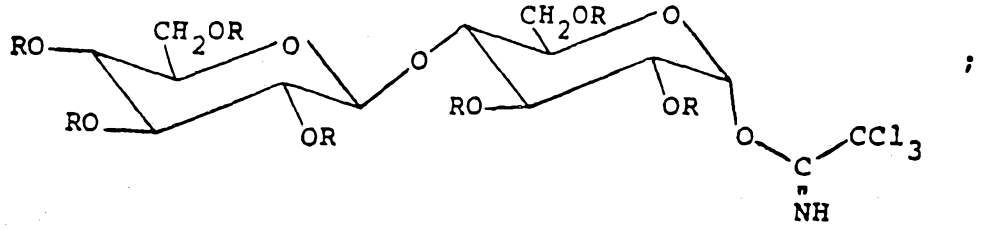
(a) reacting a cellobiose heptaalkanoate of the formula



(II)

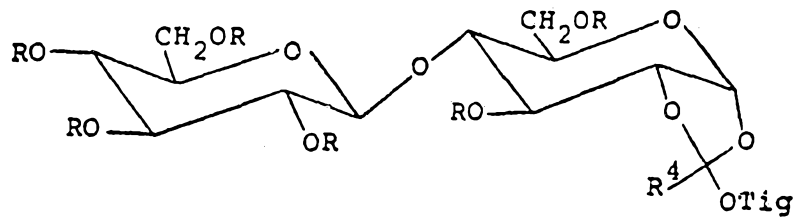
wherein

R is R^4CO and R^4 is (C_1-C_4)alkyl, with trichloroacetonitrile in the presence of a catalytic amount of cesium carbonate in a reaction-inert solvent at or about ambient temperature to form an imidate of the formula



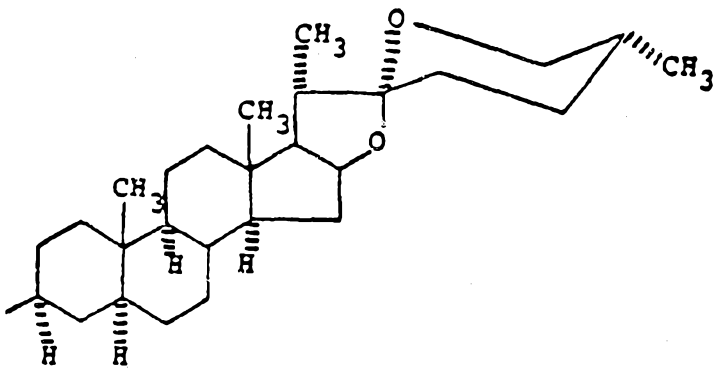
(III)

(b) reacting said imidate with tigogenin in the presence of zinc bromide or magnesium bromide etherate in the same or another reaction-inert solvent at or about ambient temperature to form an orthoester of the formula



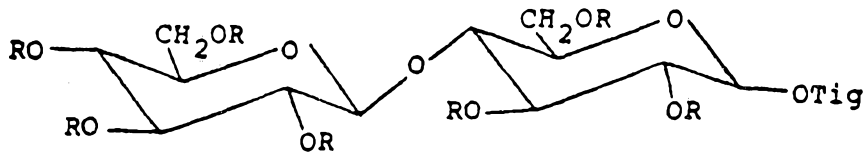
(IV)

wherein Tig is



;

(c) heating said orthoester in the same or another reaction-inert solvent to form a tigogenin beta-cellobioside heptaalkanoate of the formula



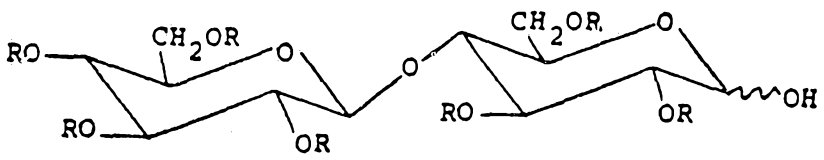
; and

(V)

(d) conventionally hydrolyzing said tigogenin beta-cellobioside heptaalkanoate to form said tigogenin beta-cellobioside.

5. A process for the synthesis of tigogenin beta-cellobioside which comprises the steps of

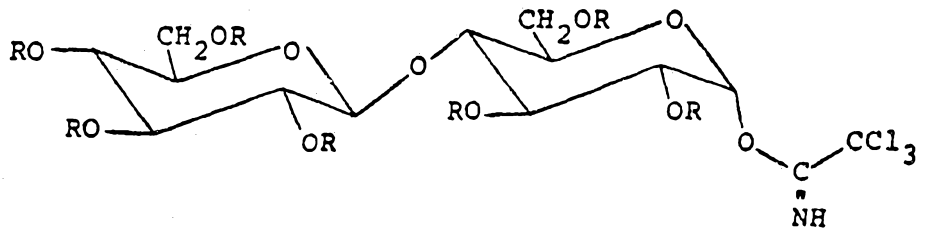
(a) reacting a cellobiose heptaalkanoate of the formula



,

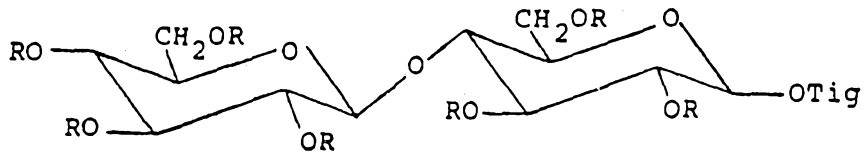
(II)

wherein R is (C_2-C_5)alkanoyl, with trichloroacetonitrile in the presence of a catalytic amount of cesium carbonate in a reaction-inert solvent at or about ambient temperature to form an imidate of the formula



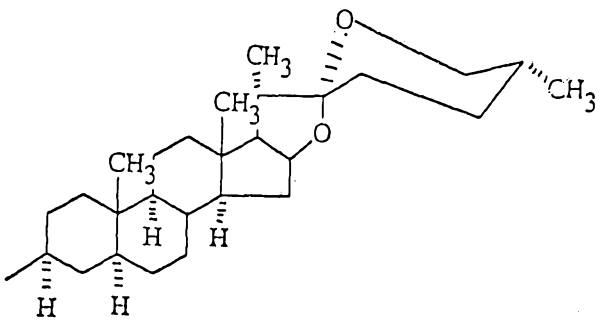
(III)

(b) reacting said imidate with tigogenin in the presence of boron trifluoride etherate in the same or another reaction-inert solvent at or about ambient temperature to form a tigogenin beta-cellobioside heptaalkanoate of the formula



(V)

wherein Tig is



; and

(c) conventionally hydrolyzing said tigogenin beta-cellobioside heptaalkanoate to form said tigogenin beta-cellobioside.

S & F Ref: 129951

FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

619986

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

Name and Address of Applicant: Pfizer Inc.
235 East 42nd Street
New York State of New York
UNITED STATES OF AMERICA

Address for Service: Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower, 31 Market Street
Sydney, New South Wales, 2000, Australia

Complete Specification for the invention entitled:

Processes for Tigogenin Beta-Cellobioside

The following statement is a full description of this invention, including the best method of performing it known to me/us

5845/8

PATENT
PC7600/RKB

PROCESSES FOR TIGOGENIN BETA-CELLOBIOSIDE

Abstract

Improved processes for the synthesis of tigogenin beta-cellobioside, a known hypocholesterolemic agent, using cellobiose heptaacetate and tigogenin as starting materials.

PROCESSES FOR TIGOGENIN BETA-CELLOBIOSIDE

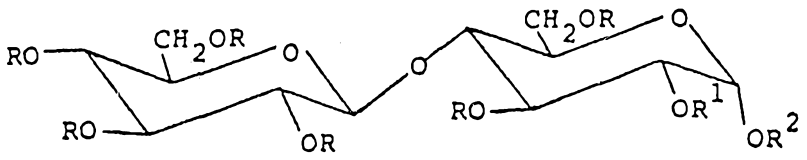
The present invention is directed to novel and advantageous processes for the synthesis of tigogenin beta-cellobioside and to certain novel intermediates used in these processes.

Tigogenin beta-cellobioside is a known compound having utility in the treatment of hypercholesterolemia and atherosclerosis (Malinow, U.S. Patents 4,602,003 and 4,602,005; Malinow et al. Steroids, vol. 48, pp. 197-211, 1986). Each patent discloses a different synthesis of this compound from beta-cellobiose octa-acetate; the first via the glycolyl bromide heptaacetate which is coupled with tigogenin in the presence of silver carbonate, and finally hydrolyzed; and the second direct stannic chloride catalyzed coupling of the octa-acetate with tigogenin in methylene chloride, again followed by hydrolysis. In Malinow et al., reaction of cellobiose octaacetate with titanium tetrabromide gave the glycosyl bromide heptaacetate, which was coupled with tigogenin by means of mercuric cyanide, and then hydrolyzed. All of these methods have serious drawbacks for producing bulk material. A desirable goal, met by the present invention, has been to devise synthetic methods which avoid toxic and/or expensive reagents, and which cleanly produce the desired tigogenin beta-cellobioside, avoiding tedious and expensive purification steps.

Schmidt, Angew. Chem. Int. Ed. Engl., v. 25, pp. 212-235 (1986) has reviewed the synthesis and reactions of O-glycosyl trichloroacetimidates formed by the reaction of sugars possessing a 1-OH group (but with other hydroxy groups protected, e.g., by benzyl or acetyl) with trichloroacetonitrile in the presence of a base.

There is preferential formation of the alpha-anomer when NaH is used as base, and preferential formation of the beta-anomer when the base is K_2CO_3. The alpha anomer of tetrabenzylglucosyl trichloroacetimidate when coupled with cholesterol gave anomeric mixtures which varied with catalyst (p-toluene-sulphonic acid or boron trifluoride etherate) and temperature (-40 to +20°C). On the other hand, both the alpha and beta anomers of tetracetylglucosyl analog reportedly yield exclusively beta-anomeric products.

The present invention is directed to intermediate compounds of the formula

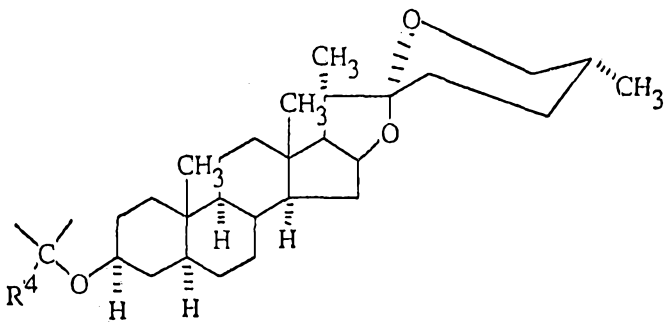


(I)

wherein

R is R^4CO;

R^1 and R^2 are taken together and are



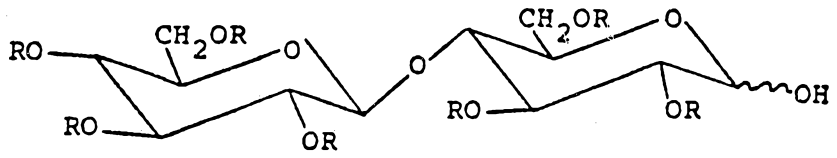
; and

R^4 is (C_1-C_4)alkyl.

Of particular value are those compounds wherein R^4 is methyl, i.e., R is acetyl.

The present invention is also directed to over-all processes and certain individual process steps used for the present syntheses of tigogenin beta-cellobioside, as follows:

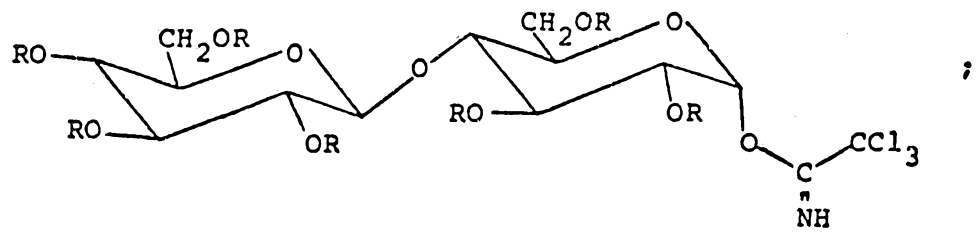
(a) reacting a cellobiose heptaalkanoate of the formula



(II)

wherein

R is R^4CO and R^4 is (C_1-C_4)alkyl, with trichloroacetonitrile in the presence of a catalytic amount of cesium carbonate in a reaction-inert solvent at or about ambient temperature to form an imidate of the formula (I) wherein R^1 and R^2 are taken together, i.e., of the formula

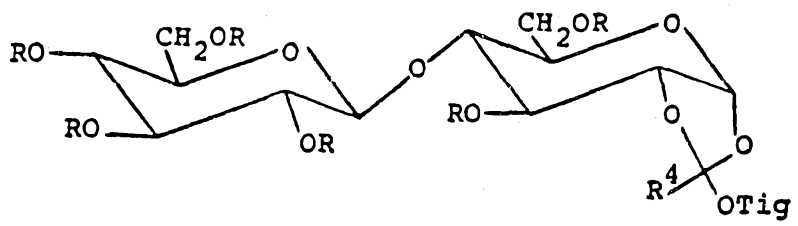


(III)

either

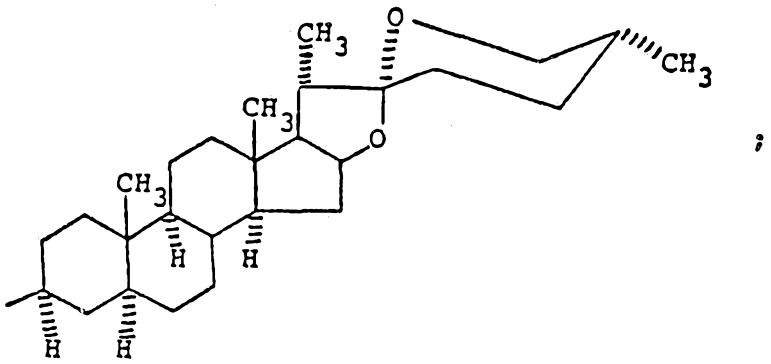
(b) reacting said imidate with tigogenin in the presence of zinc bromide or magnesium bromide etherate in the same or another reaction-inert solvent at or

about ambient temperature to form an orthoester of the formula (I) wherein R^1 and R^2 are taken together, i.e., of the formula

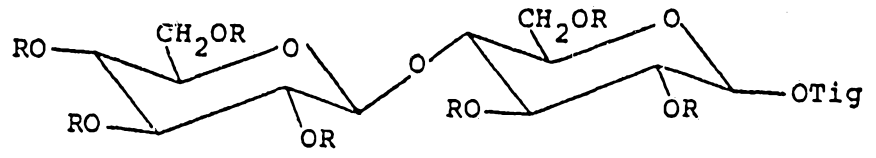


(IV)

wherein Tig is



followed by heating said orthoester in the same or another reaction-inert solvent to form a tigogenin beta-cellobioside heptaalkanoate of the formula



(V)

or

(b') reacting said imidate of the formula (III) with tigogenin in the presence of boron trifluoride etherate in the same or another reaction-inert solvent

at or about ambient temperature to form a said tigogenin beta-cellobioside heptaalkanoate of the formula (V); and

(c) conventionally hydrolyzing said tigogenin beta-cellobioside heptaalkanoate to form said tigogenin beta-cellobioside.

Again, the preferred value of R^4 is methyl, i.e., R is acetyl.

As used above and elsewhere herein, the expression "reaction-inert solvent" refers to a solvent which does not interact with starting materials, reagents, intermediates or products in a manner which adversely affects the yield of the desired product. In general, said solvent can comprise a single entity, or contain multiple components.

One key to the present invention is the stereospecific conversion of cellobiose heptaalkanoate (II) to a key intermediate, viz., the alpha-acetimidate of the formula (III). In this conversion, the cellobiose heptaalkanoate is reacted with at least one molar equivalent (preferably a 1-10 fold molar excess) of trichloroacetonitrile in a reaction-inert solvent such as methylene chloride in the presence of a catalytic amount of cesium carbonate (e.g., about 5 mol% relative to cellobiose heptaacetate). Temperature is not critical, but the reaction is preferably carried out at or near ambient temperature so as to avoid the cost of heating or cooling. The present stereospecific formation of the alpha-anomer with this catalyst is most surprising, since Schmidt, particularly expert in this type of transformation, recommends another alkali metal carbonate, viz., potassium carbonate as catalyst for selective formation of the undesired beta-anomer.

The resulting alpha-imidate (III) is coupled with tigogenin in a reaction-inert solvent in the presence of boron trifluoride etherate in analogy to the method of Schmidt, cited above. This coupling step, which is also conveniently accomplished at or about ambient temperature, produces known tigogenin beta-cellobioside heptaacetate (V).

We have presently discovered that use of either zinc bromide or magnesium bromide etherate as catalyst under otherwise similar conditions leads to the clean formation of an intermediate orthoester of the formula (IV). If desired, this ortho ester can be isolated. However, it is preferred to simply heat the reaction mixture to accomplish rearrangement of this ortho ester to intermediate tigogenin beta-cellobioside heptaacetate (V). It is convenient to replace any alkanoyl groups lost in this process by reaction with the appropriate alkanoic acid anhydride prior to isolation of this intermediate.

In the final step, the heptaacetate of the formula (V) is conventionally hydrolyzed or solvolyzed, e.g., according to the method of Malinow, cited above; or by the method specifically exemplified below.

The present invention is illustrated by the following examples. However, it should be understood that the invention is not limited to the specific details of these examples.

EXAMPLE 1

alpha-O-Cellobiosyl Trichloroacetimidate Heptaacetate (III, R = acetyl)

Under N_2, cellobiose heptaacetate (10 g, 0.0157 mol; prepared from the octaacetate according to the method of Excoffier et al., Carbohydrate Res., v. 39, pp. 368-373, 1975) was dissolved in 100 ml CH_2Cl_2 in a flame dried flask and cooled to 0-5°C. Trichloroacetonitrile (4 ml) was added by syringe and then Cs_2CO_3 (0.52 g, 0.00158 mol) was added as a finely ground powder. The mixture, which was immediately allowed to warm to room temperature, was stirred for 5 hours, then clarified by filtration over diatomaceous earth, and the filtrate stripped, taken up in hexane/ethyl acetate and restripped to yield 11 g of title product. Recrystallization from ethyl acetate/hexane gave 6.1 g of purified title product, m.p. 192-194°C; ^{1}H-NMR($CDCl_3$, 300 MHz)delta(ppm) 8.63 (s, 1H), 6.45 (d, 1H), 5.50 (t, 1H), 5.1 (m, 3H), 4.9 (t, 1H), 4.52 (m, 2H), 4.37 (dd, 1H), 4.07 (m, 3H), 3.82 (t, 1H), 3.65 (m, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 1.97 (m, 15H).

Analysis: C 43.02, H 4.49, N 1.81;
Calculated: C 43.06, H 4.65, N 1.79.

EXAMPLE 2

Orthoester Derived From alpha-O-Cellobiosyl Trichloroacetimidate Heptaacetate and Tigogenin (IV, $R^4 = CH_3$)

Title product of the preceding Example (1.2 g, 1.54 mmol), tigogenin (0.5 g, 1.2 mmol) and molecular sieves (0.5 g, 3A type) were combined in 20 ml of CH_2Cl_2 at room temperature. After stirring for 10 minutes, $ZnBr_2$ (0.21 g, 0.93 mmol) was added and the mixture stirred for 1.25 hours, filtered over diatomaceous earth, the filtrate washed with 0.5M HCl, H_2O and

brine, dried over $MgSO_4$, stripped, and the residue slurried in hexane to yield present title product as a white solid, 0.55 g, m.p. 187.5-188.6°C; tlc Rf 0.3 (3:1 $CHCl_3$:ethyl acetate).

Analysis: C, 61.14; H, 7.54.
Calculated: C, 61.49; H, 7.60.

Alternatively, title product was simply formed *in situ* by the same method, omitting the filtration and subsequent isolation steps. The formation of title product was monitored by tlc.

This ortho ester product was also produced when magnesium bromide etherate was used in place of $ZnBr_2$.

EXAMPLE 3

Tigogenin beta-Cellobioside Heptaacetate (V, R = acetyl)

Method A

Title product of the preceding Example was formed in 20 ml of CH_2Cl_2 from title product of Example 1 (1.15 g, 1.47 mmol) according to the procedure of the preceding Example. Monitoring by tlc demonstrated complete conversion to the orthoester within 2 hours. The ortho ester was then converted to present title product by heating the reaction mixture at reflux for 18 hours, then cooling to room temperature, adding acetic anhydride and allowing the reaction to stir for 3 hours to replace partially lost acetyl groups. To isolate and purify title product, the reaction mixture was filtered, and the filtrate washed with H_2O and brine, dried ($MgSO_4$), stripped and the residue chromatographed on silica gel using 4:1 $CHCl_3$:ethyl acetate as eluant. The yield of purified title product was 0.8 g (59%), identical with the known product.

Alternatively, following treatment with acetic anhydride, the reaction mixture was filtered, washed with 0.5N HCl, water and brine, dried ($MgSO_4$), stripped to an oil and the residue crystallized from isopropyl

ether, 0.46 g (34%). Additional product (0.09 g, 7%) was obtained from mother liquors by stripping and chromatography according to the preceding paragraph.

Method B

A mixture of tigogenin (4.7 g, 0.0113 mol) and flame dried molecular seives (3A type, 10 g) and 100 ml hexane was added to a solution of title product of Example / (0.014 mol) in 100 ml of CH_2Cl_2, and the mixture stirred 18 hours at room temperature, then cooled to 0-5°C. $BF_3 \cdot (C_2H_5)O$ (0.43 ml, 0.0055 mol) in 10 ml CH_2Cl_2 was added dropwise over 30 minutes. After 2 hours solid $NaHCO_3$ (5 g) was added, and the mixture stirred for 10 minutes, filtered, the filtrate washed 2x saturated $NaHCO_3$ and 1x brine, dried ($MgSO_4$) and stripped to solids which were twice recrystallized from absolute alcohol to yield 5.32 g of purified title product.

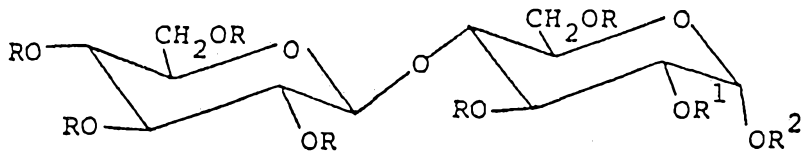
EXAMPLE 4

Tigogenin beta-Cellobioside

Under N_2, and under anhydrous conditions, title product of the preceding Example (7.8 g, 7.53 mmol) was dissolved in 78 ml of CH_3OH:tetrahydrofuran 1:1 by volume. Sodium methoxide (0.020 g, 0.37 mmol) was added in one portion and the mixture heated to reflux for 1 hour. Tetrahydrofuran was removed by distillation to a head temperature 62°C. Fresh methanol (80 ml) was added and distillation continued to a head temperature of 65°C. Water (8 ml) was added and the mixture reheated to reflux, seeded, digested at reflux for 2.5 hours, cooled slowly with stirring to room temperature, stirred overnight and present title product recovered by filtration, 4.21 g, identical with the known product.

The claims defining the invention are as follows:

1. A compound of the formula

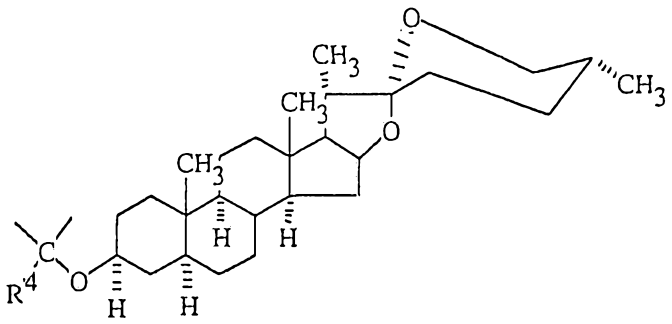


(I)

wherein

R is R^4CO;

R^1 and R^2 are taken together and are



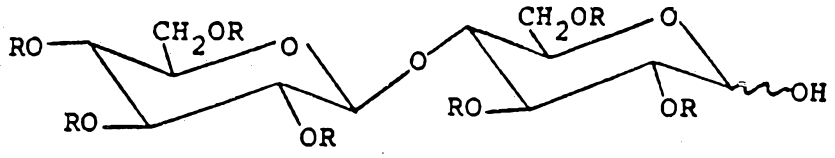
; and

R^4 is (C^1-C^4)alkyl.

2. The compound of claim 1 wherein R^1 and R^2 are taken together, R is acetyl, and R^4 is methyl.

3. A process for the synthesis of tigogenin beta-cellobioside which comprises the steps of

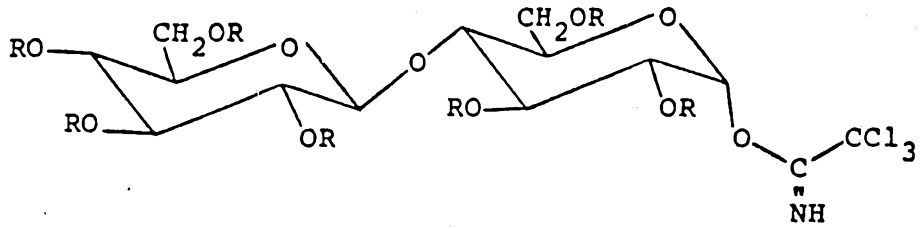
(a) reacting a cellobiose heptaalkanoate of the formula



(II)

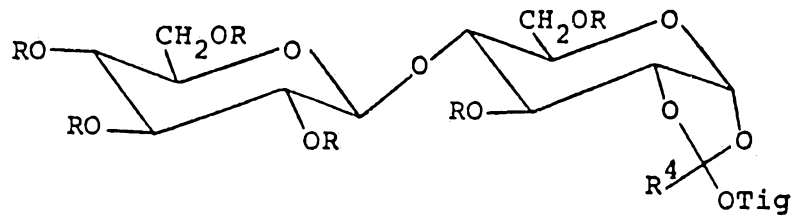
wherein

R is R^4CO and R^4 is (C_1-C_4)alkyl, with trichloroacetonitrile in the presence of a catalytic amount of cesium carbonate in a reaction-inert solvent at or about ambient temperature to form an imidate of the formula



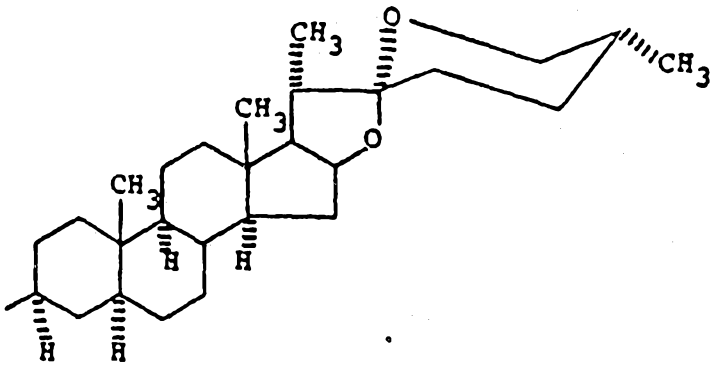
(III)

(b) reacting said imidate with tigogenin in the presence of zinc bromide or magnesium bromide etherate in the same or another reaction-inert solvent at or about ambient temperature to form an orthoester of the formula



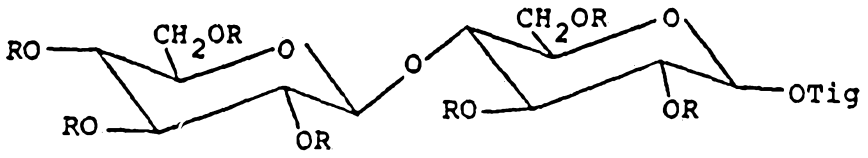
(IV)

wherein Tig is



;

(c) heating said orthoester in the same or another reaction-inert solvent to form a tigogenin beta-cellobioside heptaalkanoate of the formula



; and

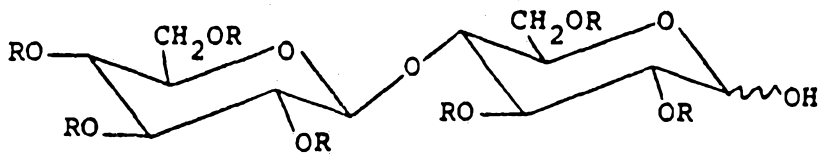
(V)

(d) conventionally hydrolyzing said tigogenin beta-cellobioside heptaalkanoate to form said tigogenin beta-cellobioside.

4. A process of claim 3 wherein R is acetyl and R^4 is methyl.

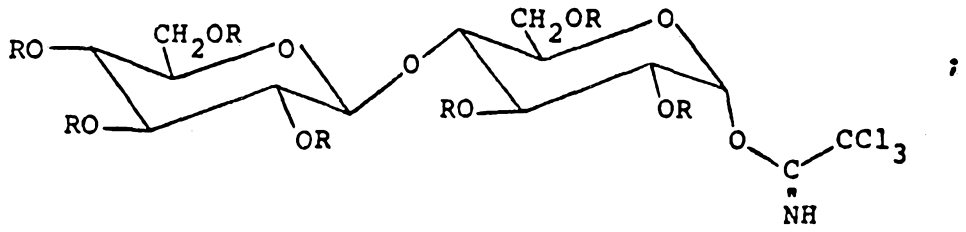
5. A process for the synthesis of tigogenin beta-cellobioside which comprises the steps of

(a) reacting a cellobiose heptaalkanoate of the formula



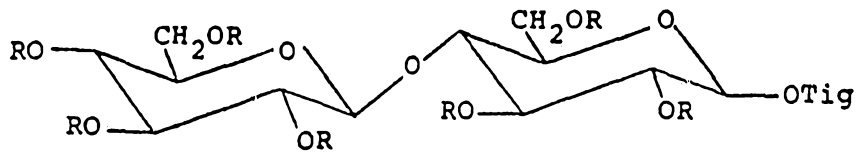
(II)

wherein R is (C_2-C_5)alkanoyl, with trichloroacetonitrile in the presence of a catalytic amount of cesium carbonate in a reaction-inert solvent at or about ambient temperature to form an imidate of the formula



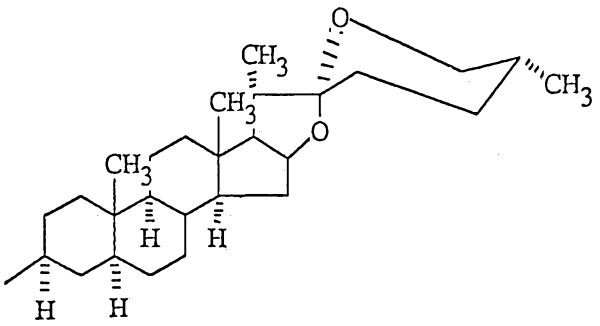
(III)

(b) reacting said imidate with tigogenin in the presence of boron trifluoride etherate in the same or another reaction-inert solvent at or about ambient temperature to form a tigogenin beta-cellobioside heptaalkanoate of the formula



(V)

wherein Tig is



; and

(c) conventionally hydrolyzing said tigogenin beta-cellobioside heptaalkanoate to form said tigogenin beta-cellobioside.

6. A process of claim 5 wherein R is acetyl.

7. A celiobiose alkanoate derivative, substantially as hereinbefore described with reference to Example 2.

8. A process for the synthesis of tigogenin beta-cellobioside substantially as hereinbefore described with reference to Example 4.

9. A tigogenin beta-cellobioside whenever prepared by the process of any one of claims 3 to 6 or 8.

DATED this TWENTY-FIRST day of OCTOBER 1991

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Patent Attorneys for the Applicant

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MTS