Abstract:
The present invention relates to cannabinoid glycoside prodrugs suitable for site- and tissue-specific delivery of cannabinoid molecules. The present invention also relates to methods of forming the cannabinoid glycoside prodrugs through glycosyl transferase mediated glycosylation of cannabinoid molecules.
CANNABINOID GLYCOSIDE PRODRUGS AND METHODS OF SYNTHESIS

FIELD OF THE INVENTION

[001] The present invention pertains to the field of drug development and in particular to novel cannabinoid glycoside prodrugs and methods for their production by enzyme-mediated carbohydrate transfer.

BACKGROUND

[002] Phytocannabinoids from Cannabis sativa have long been used for altering mental states, but recent findings have illuminated the potential of specific cannabinoid compounds for treatment and maintenance of various diseases and conditions. Of particular importance is the non-psychotropic molecule cannabidiol (CBD) which has potential therapeutic application as an anti-psychotic, a neuroprotectant, and has potential for treatment of numerous other maladies (Zuardi 2012, Iuvone 2009, for review Mechoulam 2002, respectively). One shortcoming of CBD is that it is easily oxidized to THC and CBN derivatives by light, heat, and acidic or basic conditions, and another detrimental attribute to CBD is that its extremely hydrophobic nature makes it difficult for formulation and delivery. Additionally, current pharmaceutical compositions of CBD and THC have unpleasant organoleptic properties, and their hydrophobic nature results in a lingering on the palate.

[003] Cannabinoids are extremely hydrophobic in nature, complicating their use in drug formulations. Non-covalent methods have been found to improve the solubility of cannabinoids by utilizing carrier carbohydrates such as cyclized maltodextrins (Jarho 1998). Covalent chemical manipulations have produced novel CBD prodrugs with improved solubility (WO2009018389, WO 2012011112). Even fluorine substituted CBD compounds have been created through synthetic chemical manipulations in an effort to functionalize CBD (WO2014108899). The aforementioned strategies were somewhat successful in improving the solubility of CBD, but they create unnatural compositions which alter the composition and will release the unnatural prodrug moieties upon hydrolysis.

[004] A growing body of evidence shows that glycosides are capable of acting as prodrugs and also to have direct therapeutic effects. Glycoside prodrugs may enable improved
drug bioavailability or improved drug pharmacokinetics including more site-specific or tissue-specific drug delivery, more consistent levels of drug in the plasma, and sustained or delayed release of the drug. Site-specific delivery of steroid glycosides to the colon has previously been demonstrated (Friend 1985, Friend 1984), and could enable treatment of local disorders such as inflammatory bowel disease. Glycosylation of steroids enabled survival of stable bioactive molecules in the acidic stomach environment and delivery into the large intestine, where the aglycones were liberated by glycosidases produced by colonic bacteria, and then absorbed into the systemic circulation. Glycosidases are also present universally in different tissues (Conchie 1959), so delivery of glycosides by methods that bypass the digestive tract and colon, such as intravenous delivery, will enable targeted delivery to other cells and tissues that have increased expression of glycosidases. In addition, the distribution of alpha-glycosidase and beta-glycosidase enzymes differ throughout the intestinal tract and other tissues, and different forms of glycosides may therefore provide unique pharmacokinetic profiles, including formulations that target delivery of specific diseased areas, or targeted release at locations that can promote or restrict systemic absorption of the cannabinoids and other compounds described herein. Many biologically active compounds are glycosides, including members of classes of compounds such as hormones, antibiotics, sweeteners, alkaloids, and flavonoids. While it is generally accepted that glycosides will be more water-soluble than the aglycones, literature reviews have analyzed structure-activity relationships and determined that it is nearly impossible to define a general pattern for the biological activities of glycosides across different classes of compounds (Kren 2008).

[005] As with synthetic chemistry, in vivo detoxification strategies serve as another model for improving the solubility of cannabinoids. CBD is glucuronidated in humans by the liver glucosyltransferases, but to date only minor activity has been demonstrated with UGT1A9 and UGT2B7 in in vitro assays (US Patent No. 8,410,064). In vitro assays showed that cannabino (CBN) is efficiently glucuronidated by the Human UGT1A10 (US Patent No. 8,410,064). The glucuronidation of CBD is one mechanism to increase CBD solubility and facilitate removal and excretion through the kidneys. Searching for glucosyltransferase activity towards cannabinoids, cannabino was found to be glycosylated when incubated with in vitro cell culture of Pinellia ternata (Tanaka 1993). Similarly, cannabidiol was shown to be glycosylated when incubated with tissue cultures from Pinellia ternata and Datura inoxia, yielding CBD-6'-0^-D-glucopyranoside and CBD-(2',6')-0^-D-diglucopyranoside (Tanaka 1996). These biotransformation studies demonstrate the potential for limited glycosylation of these two compounds to occur by unknown plant glucosyltransferases, and for them to be produced in
minute quantities, but to date, no specific plant glucosyltransferase proteins capable of
glycosylation of cannabinoids have been identified, no cannabinoid glycosides been produced
in large, purified quantities, and the biological activity or pharmaceutical properties of
cannabinoid glycosides have never been characterized.

[006] Cannabinoids contain a hydroxylated hydrophobic backbone, similar to the steviol
backbone of steviol glycosides found in the Stevia rebaudiana plant. UGT76G1 is a
glucosyltransferase from Stevia that is capable of transferring a secondary glucose to the 3C-
hydroxyl of the primary glycosylation on both C13-OH and C19-COOH position of the steviol
glycoside, and thus its substrates include steviolmonoside, stevioside, rubusoside, RebA,
RebD, RebG, RebE, etc. (Richman et al. 2005, Stevia First Corp unpublished work). The
substrate recognition site of UGT76G1 is capable of binding and glycosylating multiple steviol
glycosides, but it was previously not known to have glycosylation activity towards any other
glycosides, and there previously was no established activity of UGT76G1 towards any aglycone
compounds at all. As UGT76G1 is capable of glycosylating steviol glycosides on the primary
sugar located on both C13 hydroxyl group and the C19 carboxyl group it demonstrates bi-
functional glycosylation. Cyclodextrin glucanotransferase (CGTase, Toruzyme 3.0L,
Novozymes Inc.) is a member of the amylase family of enzymes and is best known for its ability
to cyclize maltodextrin chains. A lesser known activity of CGTase is disproportionation of linear
maltodextrin chains and transfer to an acceptor sugar molecule (Li 2012).

[007] There are no known cannabinoid glycosides available as cannabinoid prodrugs. Nor is
there a known method for the efficient regioselective production of cannabinoid glycosides,
which is necessary in order to produce large, purified quantities of individual glycosides and to
assess their pharmaceutical properties, including evaluation of in vivo drug pharmacokinetics
and pharmacodynamics. To solve the aforementioned problem, screening of glucosyltransferase enzymes from various organisms has been conducted to identify
candidates for the glycosylation of cannabinoids, and to identify cannabinoid glycosides as
potential prodrugs of cannabinoids, and as novel cannabinoid compositions with novel
properties and functions.

[008] This background information is provided to reveal information believed by the applicant
to be of possible relevance to the present invention. No admission is necessarily intended, nor
should be construed, that any of the preceding information constitutes prior art against the
present invention.
SUMMARY OF THE INVENTION

[009] The present invention relates to novel cannabinoid glycoside prodrugs and methods for their production by enzyme-mediated carbohydrate transfer.

[0010] An object of the present invention is to provide a cannabinoid glycoside prodrug. In accordance with an aspect of the present invention, there is provided a cannabinoid glycoside prodrug compound having formula (I):

\[
\begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{RO} \\
\text{OR'}
\end{array}
\]

wherein \( R \) is H, \( \beta \)-D-glucopyranosyl, or 3-0\(^{-}\)-D-glucopyranosyl\(^{-}\)-D-glucopyranosyl; \( R' \) is H or \( \beta \)-D-glucopyranosyl, or 3-0\(^{-}\)\( \beta \)-D-glucopyranosyl\(^{-}\)-D-glucopyranosyl; and \( A \) is an aglycone moiety formed through reaction of a hydroxyl group on a cannabinoid compound, an endocannabinoid compound, or a vanilloid compound, or a pharmaceutically compatible salt thereof.

[0011] In accordance with another aspect of the present invention, there is provided a method for the site-specific delivery of a cannabinoid drug to a subject, comprising the step of administering a cannabinoid glycoside prodrug in accordance with the present invention to a subject in need thereof.

[0012] In accordance with another aspect of the present invention, there is provided a method of producing a cannabinoid glycoside, comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.

[0013] Further aspects of the technology described herein will be brought out in the following portions of the specification, wherein the detailed description is for the purpose of fully disclosing preferred embodiments of the technology without placing limitations thereon.
BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1A illustrates aglycones employed in the glycosylation methods of the present invention. Figure 1B illustrates the possible points of glycosylation on the aglycones.

[0015] Figure 2 illustrates possible products of the glycosylation of cannabidiol (CBD).

[0016] Figure 3 illustrates possible products of the glycosylation of cannabidivarin (CBDV).

[0017] Figure 4 illustrates possible rotational products of the glycosylation of cannabidiol (CBD).

[0018] Figure 5 illustrates possible rotational products of the glycosylation of cannabidivarin (CBDV).

[0019] Figure 6 illustrates the proposed superpositioning of the substrate cannabidiol (CBD) in the catalytic site of UGT76G1.

[0020] Figure 7 illustrates possible products of the glycosylation of tetrahydrocannabinol (Δ9-THC).

[0021] Figure 8 illustrates possible products of the glycosylation of cannabinol (CBN).

[0022] Figure 9 illustrates possible products of the glycosylation of arachidonoyl ethanolamide (AEA).

[0023] Figure 10 illustrates possible products of the glycosylation of 2-arachidonoyl ethanolamide (2-AG).

[0024] Figure 11 illustrates possible products of the glycosylation of 1-arachidonoyl ethanolamide (1-AG).

[0025] Figure 12 illustrates possible products of the glycosylation of N-docosahexaenoyl ethanolamine (DHEA).

[0026] Figure 13 illustrates possible products of the glycosylation of capsaicin.

[0027] Figure 14 illustrates possible products of the glycosylation of vanillin.


[0029] Figure 16 is an HPLC linetrace of the reaction products of the glycosylation of CBD.

[0030] Figure 17 is an HPLC linetrace of the reaction products of the glycosylation of CBDV.

[0031] Figure 18 is an HPLC linetrace of the reaction products of the glycosylation of A9-THC.

[0032] Figure 19 is an HPLC linetrace of the reaction products of the glycosylation of CBN.

[0033] Figure 20 is an HPLC linetrace of the reaction products of the glycosylation of 1-AG and 2-AG.

[0034] Figure 21 is an HPLC linetrace of the reaction products of the glycosylation of synaptamide (DHEA).
Figure 22 is an HPLC linetrace of the reaction products of the glycosylation of AEA.

Figure 23 is an HPLC linetrace of the reaction products of the glycosylation of vanillin.

Figure 24 is an HPLC linetrace of the reaction products of the glycosylation of capsaicin.

Figure 25 is an HPLC linetrace of the reaction products of the glycosylation of CBDgl (VB1 04) with the glycosyltransferase UGT76G1.

Figure 26 is an HPLC linetrace of the reaction products of the glycosylation of CBDgl (VB1 04) with the glycosyltransferase Os03g0702000.

Figure 27 is a ¹NMR spectrum of an isolated product, VB1 04, of the glycosylation of CBD.

Figure 28 is a ¹NMR spectrum of an isolated product, VB1 10 of the glycosylation of CBD.

Figure 29 is a plot of C18 retention times vs cLogP values for selected cannabinoids and cannabinoid glycosides.

Figure 30A is a graphical presentation of the results of the analysis of the small intestine extracts of a bioavailability assay.

Figure 30B is a graphical presentation of the results of the analysis of the large intestine extracts of a bioavailability assay.

DETAILED DESCRIPTION OF THE INVENTION

The following abbreviations are used throughout:

CB Cannabinoid
CBD Cannabidiol
CBDV Cannabidivarin
CBG Cannabigerol
A9-THC or THC Tetrahydrocannabinol
CBN Cannabinol
CBNV Cannabinavarin
CBDA Cannabidiolic acid
THCV Tetrahydrocannabivarin
UGT UDPG-dependent glucosyltransferase
UDPG Uridine diphosphoglucose
UDP Uridine diphosphate
AEA Arachidonoyl ethanolamide (aka, anandamide)
2-AG 2-Arachidonoyl ethanolamide.
1-AG 1-Arachidonoyl ethanolamide.,
DHEA N-Docosahexaenoylethanolamine (aka, synaptamide)
SUS Sucrose synthase.

[0046] The term “glucopyranoside” is used for naming molecules and is shorthand for a β-D-glucose attached through the hydroxyl at the 1-position (the anomeric carbon) of the glucose to the aglycone.

[0047] The term "aglycone" is used in the present application to refer to the non-glycosidic portion of a glycoside compound.

[0048] The term "prodrug" refers to a compound that, upon administration, must undergo a chemical conversion by metabolic processes before becoming an active pharmacological agent.

[0049] The term "cannabinoid glycoside prodrug" refers generally to the glycosides of cannabinoid compounds, endocannabinoid compounds and vanilloid compounds. The cannabinoid glycoside prodrug undergoes hydrolysis of the glycosidic bond, typically by action of a glycosidase, to release the active cannabinoid, endocannabinoid or vanilloid compounds to a desired site in the body of the subject. The cannabinoid glycoside prodrug of the present invention may also be referred to using the term "cannaboside".

[0050] The term "cannabinoid" is used in the present application to refer generally to compounds found in cannabis and which act on cannabinoid receptors. "Cannabinoid" compounds include, but are not limited to, cannabidiol (CBD), cannabidivarin (CBDV), cannabigerol (CBG), tetrahydrocannabinol (A9-THC or THC), cannabinol (CBN), cannabidiolic acid (CBDA), and tetrahydrocannabivarin (THCV). Particularly preferred cannabinoids compounds are CBD, CBDV, THC and CBN.

[0051] The term "endocannabinoid" is used in the present application to refer to compounds including arachidonoyl ethanolamide (anandamide, AEA), 2-arachidonoyl ethanolamide (2-AG), 1-arachidonoyl ethanolamide (1-AG), and docosahexaenoyl ethanolamide (DHEA, synaptamide), oleoyl ethanolamide (OEA), eicsapentanoyl ethanolamide, prostaglandin
ethanolamide, docosahexaenoyl ethanolamide, linolenoyl ethanolamide, 5(Z),8(Z),11(Z)-eicosatrienoic acid ethanolamide (mead acid ethanolamide), heptadecanoyl ethanolamide, stearoyl ethanolamide, docosaenoyl ethanolamide, nervonoyl ethanolamide, tricosanoyl ethanolamide, lignoceroyl ethanolamide, myristoyl ethanolamide, pentadecanoyl ethanolamide, palmitoleoyl ethanolamide, docosahexaenoic acid (DHA). Particularly preferred endocannabinoids are AEA, 2-AG, 1-AG, and DHEA.

[0052] The term "vanilloid" is used in the present application to refer to compounds comprising a vanillyl group and which act on vanilloid receptors like TRPV1. "Vanilloid" compounds include, but are not limited to, vanillin, capsaicin and curcumin.

[0053] As used herein, the term "about" refers to a +/-10% variation from the nominal value. It is to be understood that such a variation is always included in a given value provided herein, whether or not it is specifically referred to.

[0054] The term "subject" or "patient" as used herein refers to an animal in need of treatment. In one embodiment, the animal is a human.

[0055] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0056] In accordance with the present invention, cannabinoids, endocannabinoids and vanilloids are employed as substrates for glucosyltransferases to which one or more sugar molecules are attached to create novel cannabinoid glycoside prodrugs. The resulting cannabinoid glycoside prodrugs demonstrate site-specific or tissue-specific delivery, improved aqueous solubility for improved pharmacological delivery, and/or sustained or delayed release of the cannabinoid, endocannabinoid and vanilloid drug molecules.

[0057] Also in accordance with the present invention, the cannabinoid glycoside prodrugs are converted upon hydrolysis of the glycosidic bond to provide the active cannabinoid, endocannabinoid and vanilloid drug. Accordingly, the present invention has demonstrated that glycosides with a hydrophobic aglycone moiety undergo glucose hydrolysis in the gastrointestinal tract or in tissues having increased expression of glycosidases, yielding the hydrophobic cannabinoid compound in the targeted tissue or organ.
The glucose residues of glycosides are commonly acid-hydrolyzed in the stomach or cleaved by glycosidase enzymes in the intestinal tract, including by alpha-glycosidases and beta-glycosidases, which are expressed by intestinal microflora across different regions of the intestine. Accordingly, glycosides are hydrolyzed upon ingestion to release the desired compound into the intestines or target tissues.

In one embodiment, glycosylation of cannabinoid drugs provides cannabinoid glycoside prodrugs capable of persisting in the acidic stomach environment upon oral administration, thereby allowing delivery of the prodrug into the large intestine, where the cannabinoid aglycones can be liberated by glycosidases produced by colonic bacteria.

In one embodiment, glycosylation of cannabinoid drugs provides cannabinoid glycoside prodrugs suitable for targeted delivery to tissues having increased expression of glycosidases. Upon parenteral administration of the cannabinoid glycoside prodrug formulation to the subject, the cannabinoid aglycones are liberated by the glycosidases in the target tissues.

It is also within the scope of the present invention that the cannabinoid glycoside prodrug are also useful as pharmaceutical agents without glucose cleavage, where they exhibit novel pharmacodynamic properties compared to the parent compound alone. The increased aqueous solubility of the cannabinoid glycoside prodrugs of the present invention also enables new formulations for delivery in transdermal or aqueous formulations that would not have been achievable if formulating hydrophobic cannabinoid, endocannabinoid and vanilloid molecules.

In one embodiment of the present invention, there are provided cannabinoid glycoside prodrug compounds having formula (I):

or a pharmaceutically compatible salt thereof, wherein R is H, β-D-glucopyranosyl, or 3-0- β-D-glucopyranosyl-D-glucopyranosyl; R' is H or β-D-glucopyranosyl, or 3-0-D-glucopyranosyl-
β-D-glucopyranosyl; and A is an aglycone moiety formed through reaction of a hydroxyl group on a cannabinoid compound, an endocannabinoid compound, or a vanilloid compound.

[0063] In accordance with one embodiment of the present invention, A is A’, A” or A’’;

wherein A’ is:

wherein A’” is:

wherein A’” is:
and wherein $A''$ is:

![Chemical Structure 1]

wherein $G$ is $H$, $\beta$-D-glucopyranosyl, 3-0^-D-glucopyranosyl^-D-glucopyranosyl, or $\beta$-D-glucopyranosyl-(1 $\rightarrow$ 3)$^\land$-D-glucopyranosyl-(1 $\rightarrow$ 3)-D-glucopyranosyl; or a pharmaceutically compatible salt thereof.

[0064] In accordance with one embodiment of the present invention, the cannabinoid glycoside prodrug is a glycoside of a cannabinoid, wherein the prodrug has the formula ($I'$):
wherein R is H, β-D-glucopyranosyl, or 3-0- β-D-glucopyranosyl- β-D-glucopyranosyl; R’ is H, β-D-glucopyranosyl, or 3-0- β-D-glucopyranosyl- β-D-glucopyranosyl; and wherein A’ is:

wherein G is β-D-glucopyranosyl, 3-0^-D-glucopyranosyl^-D-glucopyranosyl, or β-D-glucopyranosyl-(1-3)-β-D-glucopyranosyl-(1-3)-D-glucopyranosyl.

[0065] Compounds of Formula (I’) include the compounds listed in Tables 1 to 4.

[0066] Exemplary cannabidiol (CBD)-glycosides falling within the scope of Formula (I’), produced by the glycosylation of CBD (VB1 0 1) in accordance with the present invention, include:
Exemplary cannabidivarin (CBDV)-glycosides falling within the scope of Formula (I'), produced by the glycosylation of CBDV (VB201) in accordance with the present invention, include:

- VB118
- VB119
- VB202
- VB204
- VB206
Exemplary tetrahydrocannabinol (A9-THC)-glycosides falling within the scope of Formula (I), produced by the glycosylation of Δ9-THC (VB301) in accordance with the present invention, include:
Exemplary cannabinol (CBN)-glycosides falling within the scope of Formula (I'), produced by the glycosylation of CBN (VB401) in accordance with the present invention, include:
[0070] In accordance with one embodiment of the present invention, the cannabinoid glycoside prodrug is a glycoside of an endocannabinoid, the prodrug having the formula (I"):

\[
\text{R} = \text{H, } \beta-\text{D-glucopyranosyl, or } 3-0- \beta-\text{D-glucopyranosyl-} \beta-\text{D-glucopyranosyl;}
\]
\[
\text{R}' = \text{H, } \beta-\text{D-glucopyranosyl, or } 3-0- \beta-\text{D-glucopyranosyl-} \beta-\text{D-glucopyranosyl; and}
\]

wherein A" is:
Compounds of Formula (I") include the compounds listed in Tables 5 to 8.

Exemplary arachidonoyl ethanolamide (AEA)-glycosides falling within the scope of Formula (I"), produced by the glycosylation of AEA (VB501) in accordance with the present invention, include:

- VB502
- V8503
Exemplary 2-arachidonoyl ethanolamide (2-AG)-glycosides falling within the scope of Formula (I"), produced by the glycosylation of 2-AG (VB601) in accordance with the present invention, include:
VB605

VB607

VB608

VB610

VB609

and
Exemplary 1-arachidonoyl ethanolamide (l-AG)-glycosides falling within the scope of Formula (I"), produced by the glycosylation of 1-AG (VB701) in accordance with the present invention, include:

[Chemical Structures Diagram]
VB708

VB707

VB710

VB709

and
Exemplary N-docosahexaenylethanolamine (DHEA)-glycosides falling within the scope of Formula (I''), produced by the glycosylation of DHEA (VB801) in accordance with the present invention, include:

VB715

VB802

VB803
In accordance with one embodiment of the present invention, the cannabinoid glycoside prodrug is a glycoside of a vanilloid, the prodrug having the formula (I""):
wherein
R is H, β-D-glucopyranosyl, or 3-O-β-D-glucopyranosyl-β-D-glucopyranosyl;
R' is H or β-D-glucopyranosyl, or 3-0-β-D-glucopyranosyl-β-D-glucopyranosyl; and,
wherein A''' is:

[0077] Compounds of Formula (H) include the compounds listed in Tables 9 to 11.

[0078] Exemplary capsaicin-glycosides falling within the scope of Formula (H), produced by the glycosylation of capsaicin (VB901) in accordance with the present invention, include:
Exemplary vanillin-glycosides falling within the scope of Formula (H), produced by the glycosylation of vanillin (VB1001) in accordance with the present invention, include:

VB902, VB903, and VB904.
Exemplary curcumin-glycosides falling within the scope of Formula (H), produced by the glycosylation of curcumin (VB1 1 0 1 ) in accordance with the present invention, include: [0080] VB1002, VB1003, VB1004, VB1005, VB1006, and VB1102.
In one embodiment, there is provided a method for the site-specific delivery of a cannabinoid drug to a subject, comprising the step of administering to a subject in need thereof one or more cannabinoid glycoside prodrugs in accordance with the present invention. In one embodiment, the site of delivery is the large intestine. In one embodiment, the site of delivery is the rectum. In one embodiment, the site of delivery is the liver. In one embodiment, the site of delivery is the skin.

In one embodiment, there is provided a method for facilitating the transport of a cannabinoid drug to the brain through intranasal, stereotactic, or intrathecal delivery, or delivery across the blood brain barrier of a subject comprising administering a cannabinoid glycoside prodrug in accordance with the present invention to a subject in need thereof.

In accordance with the present invention, the cannabinoid glycoside prodrugs are useful in the treatment of conditions that benefit from or can be ameliorated with the administration of a cannabinoid drug. Conditions that can be treated or ameliorated through the administration of cannabinoid glycoside prodrugs of the present invention, include but are not limited to, inflammatory bowel disease including induction of remission from Crohn's disease, and colitis and induction of remission from ulcerative colitis. Among the benefits that can be achieved through the administration of cannabinoid glycoside prodrugs of the present invention are decreased inflammation of the intestines and rectum, decreased pain in the intestines, rectum, as well as decrease in neuropathic pain and abdominal pain, and inhibition of proliferation or cytotoxicity against colorectal cancer. Additional treatment indications, effects, or applications for cannabinoids or cannabinoid glycosides may include but are not limited to anorexia, nausea,

[0084] In one embodiment, the cannabinoid glycoside prodrug is administered in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. In one embodiment, the pharmaceutical compositions comprise one or more cannabinoid glycoside prodrugs and one or more pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants. For administration to a subject, the pharmaceutical compositions can be formulated for administration by a variety of routes including but not limited to oral, topical, rectal, parenteral, and intranasal administration.

[0085] The pharmaceutical compositions may comprise from about 1% to about 95% of a cannabinoid glycoside prodrug of the invention. Compositions formulated for administration in a single dose form may comprise, for example, about 20% to about 90% of the cannabinoid glycoside prodrug of the invention, whereas compositions that are not in a single dose form may comprise, for example, from about 5% to about 20% of the cannabinoid glycoside prodrug of the invention. Non-limiting examples of unit dose forms include tablets, ampoules, dragees, dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs. Such compositions can be prepared according to standard methods known in the art for the manufacture of
pharmaceutical compositions and may contain one or more agents selected from the group of
sweetening agents, flavouring agents, colouring agents and preserving agents in order to
provide pharmaceutically elegant and palatable preparations. Tablets contain the active
ingredient in admixture with suitable non-toxic pharmaceutically acceptable excipients including,
for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium
phosphate or sodium phosphate; granulating and disintegrating agents, such as corn starch, or
alginic acid; binding agents, such as starch, gelatine or acacia, and lubricating agents, such as
magnesium stearate, stearic acid or talc. The tablets can be uncoated, or they may be coated
by known techniques in order to delay disintegration and absorption in the gastrointestinal tract
and thereby provide a sustained action over a longer period. For example, a time delay
material such as glyceryl monostearate or glyceryl distearate may be employed to further
facilitate delivery of the drug compound to the desired location in the digestive tract.

[0087] Pharmaceutical compositions for oral use can also be presented as hard gelatine
capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium
carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active
ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

[0088] Pharmaceutical compositions formulated as aqueous suspensions contain the active
compound(s) in admixture with one or more suitable excipients, for example, with suspending
agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose,
sodium alginate, polyvinylpyrrolidone, hydroxypropyl-\(\alpha\)-cyclodextrin, gum tragacanth and gum
acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example,
lecithin, or condensation products of an alkylene oxide with fatty acids, for example,
polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic
alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene
oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene
sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from
fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The
aqueous suspensions may also contain one or more preservatives, for example ethyl, or \(n\)-
propyl \(p\)-hydroxy-benzoate, one or more colouring agents, one or more flavouring agents or one
or more sweetening agents, such as sucrose, stevia, or saccharin.

[0089] Pharmaceutical compositions can be formulated as oily suspensions by suspending the
active compound(s) in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut
oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0090] The pharmaceutical compositions can be formulated as a dispersible powder or granules, which can subsequently be used to prepare an aqueous suspension by the addition of water. Such dispersible powders or granules provide the active ingredient in admixture with one or more dispersing or wetting agents, suspending agents and/or preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavouring and colouring agents, can also be included in these compositions.

[0091] Pharmaceutical compositions of the invention can also be formulated as oil-in-water emulsions. The oil phase can be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixture of these oils. Suitable emulsifying agents for inclusion in these compositions include naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate. The emulsions can also optionally contain sweetening and flavouring agents.

[0092] Pharmaceutical compositions can be formulated as a syrup or elixir by combining the active ingredient(s) with one or more sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations can also optionally contain one or more demulcients, preservatives, flavouring agents and/or colouring agents.

If desired, other active ingredients may be included in the compositions. In one embodiment, the glycoside prodrugs may be combined with other ingredients or substances that have glycosidase activity, or that may in other ways alter drug metabolism and pharmacokinetic profile of various compounds in vivo, including ones in purified form as well as such compounds found within food, beverages, and other products. In one embodiment, the cannabinoid glycoside prodrug is administered in combination with, or formulated together with, substances that have direct glycosidase activity, or that contribute to modifications to the gut microflora that
will alter the glycosidase activity in one or more regions of the intestines. Examples of such compositions include, but are not limited to, yogurt, prebiotics, probiotics, or fecal transplants.

[0093] In a further preferred embodiment, the pharmaceutical compositions are formulated for parenteral administration. The term "parenteral" as used herein includes subcutaneous injections, intravenous, intramuscular, intrathecal, intrasternal injection or infusion techniques.

[0094] Parenteral pharmaceutical compositions can be formulated as a sterile injectable aqueous or oleaginous suspension according to methods known in the art and using one or more suitable dispersing or wetting agents and/or suspending agents, such as those mentioned above. The sterile injectable preparation can be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that can be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples include, sterile, fixed oils, which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including, for example, synthetic mono- or diglycerides. Fatty acids such as oleic acid can also be used in the preparation of injectables.

[0095] Due to the highly lipophilic nature of cannabinoids, these molecules are typically poorly absorbed through membranes such as the skin of mammals, including humans, and the success of transdermally administering therapeutically effective quantities of cannabinoid to a subject in need thereof within a reasonable time frame and over a suitable surface area has been substantially limited. It is therefore proposed that the cannabinoid glycoside prodrugs of the present invention, through conjugation of the hydrophobic cannabinoid aglycone to the hydrophilic glycosidic moieties, provide a molecule having an amphipilic character favourable for passive diffusion which should be more readily absorbed through the skin.

[0096] Accordingly, in one embodiment, the pharmaceutical compositions are formulated for topical administration. Such topical formulations may be presented as, for example, aerosol sprays, powders, sticks, granules, creams, liquid creams, pastes, gels, lotions, ointments, on sponges or cotton applicators, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion.
Topical pharmaceutical compositions can be formulated with thickening (gelling) agents. The thickening agent used herein may include anionic polymers such as polyacrylic acid (CARBOPOL® by Noveon, Inc., Cleveland, Ohio), carboxypolymethylene, carboxymethylcellulose and the like, including derivatives of Carbopol® polymers, such as Carbopol® Ultrez 10, Carbopol® 940, Carbopol® 941, Carbopol® 954, Carbopol® 980, Carbopol® 981, Carbopol® ETD 2001, Carbopol® EZ-2 and Carbopol® EZ-3, and other polymers such as Pemulen® polymeric emulsifiers, and Noveon® polycarbophils. Thickening agents or gelling agents are present in an amount sufficient to provide the desired rheological properties of the composition.

Topical pharmaceutical compositions can be formulated with a penetration enhancer. Non-limiting examples of penetration enhancing agents include C8-C22 fatty acids such as isostearic acid, octanoic acid, and oleic acid; C8-C22 fatty alcohols such as oleyl alcohol and lauryl alcohol; lower alkyl esters of C8-C22 fatty acids such as ethyl oleate, isopropyl myristate, butyl stearate, and methyl laurate; di(lower)alkyl esters of C6-C22 diacids such as diisopropyl adipate; monoglycerides of C8-C22 fatty acids such as glyceryl monolaurate; tetrahydrofurfuryl alcohol polyethylene glycol ether; polyethylene glycol, propylene glycol; 2-(2-ethoxyethoxy)ethanol; diethylene glycol monomethyl ether; alkylaryl ethers of polyethylene oxide; polyethylene oxide monomethyl ethers; polyethylene oxide dimethyl ethers; dimethyl sulfoxide; glycerol; ethyl acetate; acetoacetic ester; N-alkylpyrrolidone; and terpenes.

The topical pharmaceutical compositions can further comprise wetting agents (surfactants), lubricants, emollients, antimicrobial preservatives, and emulsifying agents as are known in the art of pharmaceutical formations.

Transdermal delivery of the cannabinoid glycoside prodrug can be further facilitated through the use of a microneedle array drug delivery system.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "Remington: The Science and Practice of Pharmacy" (formerly "Remington's Pharmaceutical Sciences"); Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, PA (2000).

The pharmaceutical compositions of the present invention described above include one or more cannabinoid glycoside prodrugs of the invention in an amount effective to achieve
the intended purpose. Thus the term "therapeutically effective dose" refers to the amount of the cannabinoid glycoside prodrug that improves the status of the subject to be treated, for example, by ameliorating the symptoms of the disease or disorder to be treated, preventing the disease or disorder, or altering the pathology of the disease. Determination of a therapeutically effective dose of a compound is well within the capability of those skilled in the art. In one embodiment, cannabinoid glycosides can be combined to enable simultaneous delivery of multiple cannabinoids in a site-specific manner, including THC and CBD, whose effects in some ways may be synergistic (Russo 2006). Accordingly, in one embodiment, the pharmaceutical composition comprises one or more CBD-glycosides and one or more THC-glycosides formulated together in a single dosage form.

[00103] The exact dosage to be administered to a subject can be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide desired levels of the cannabinoid glycoside prodrug and/or the cannabinoid drug compound obtained upon hydrolysis of the prodrug. Factors which may be taken into account when determining an appropriate dosage include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Dosing regimens can be designed by the practitioner depending on the above factors as well as factors such as the half-life and clearance rate of the particular formulation.

[00104] In accordance with the present invention, there is provided a method of producing a cannabinoid glycoside, comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.

[00105] In one embodiment, the one or more glycosyltransferases is a UGT76G1 or UGT76G1-like glucosyltransferase. In one embodiment, the one or more glycosyltransferases comprise a UGT76G1 or UGT76G1-like glucosyltransferase and a Os03g0702000 or Os03g0702000-like glucosyltransferase.

[00106] In one embodiment, the one or more sugar donors are selected from the group consisting of UDP-glucose, UDP-glucuronic acid, UDP-mannose, UDP-fructose, UDP-xylose, UDP-rhamnose, UDP-fluoro-deoxyglucose, and combinations thereof. In a preferred embodiment, the sugar donor is UDP-glucose.
[00107] In accordance with the present invention, the cannabinoid aglycone is a cannabinoid, an endocannabinoid, or a vanilloid. In a preferred embodiment, the cannabinoid glycoside prodrug produced by the methods of the present invention is a compound of the Formula (I).

[00108] In one embodiment, the method of producing a cannabinoid glycoside comprises incubating a cannabinoid aglycone with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation.

[00109] In one embodiment, the method of producing a cannabinoid glycoside comprises incubating a cannabinoid aglycone with one or more sugar donors in the presence of a first glucosyltransferase and a second glucosyltransferase under conditions which allow for glycosylation. In one embodiment, sugar donor is UDP-glucose, the first glucosyltransferase is a UGT76G1 or UGT76G1-like glucosyltransferase, and the second glucosyltransferase is a Os03g0702000 or Os03g0702000-like glucosyltransferase.

[00110] In one embodiment, the method of producing a cannabinoid glycoside comprises incubating a cannabinoid aglycone with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and Os03g0702000 or Os03g0702000-like glucosyltransferase under conditions which allow for glycosylation.

[00111] In one embodiment, the method of producing a cannabinoid glycoside comprises incubating a cannabinoid aglycone with maltodextrin, in the presence of a cyclodextrin glucanotransferase under conditions that allow for glycosylation.

[00112] In one embodiment, the method of producing a cannabinoid glycoside comprises incubating a cannabinoid aglycone with UDP-glucose and maltodextrin in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and cyclodextrin glucanotransferase under conditions which allow for glycosylation.

[00113] In a preferred embodiment, the glucosyltransferase employed in the methods of producing the cannabinoid glycoside is UGT76G1 or UGT76G1-like glucosyltransferase. In one embodiment, the UGT76G1 or UGT76G1-like glucosyltransferase comprises the sequence as set forth in SEQ ID NO:1, 3, 5 or 7.
[001 14] In one embodiment, the glycosyltransferase employed in the methods of producing the cannabinoid glycoside is Os03g0702000 or Os03g0702000-like glycosyltransferase. In one embodiment, the Os03g0702000 or Os03g0702000-like glycosyltransferase comprises the sequence as set forth in SEQ ID NO:9.

[001 15] In one embodiment, the method of producing the cannabinoid glycoside further comprises incubating with sucrose synthase. In one embodiment, the sucrose synthase comprises the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

[001 16] In one embodiment, the method for the production of a cannabinoid glycoside prodrug comprises expressing one or more of the glycosyltransferases in a cell or plant which produces the cannabinoid aglycone and isolating the cannabinoid glycoside prodrug.

[001 17] Glycosylation of cannabinoids improves solubility in aqueous solutions, as demonstrated by accelerated elution from an C18 analytical HPLC column, indicating that the new cannabinoid-glycosides require far less organic solvent for elution from the hydrophobic chromatography column. This improved solubility was further demonstrated by testing the aqueous solubility of purified solid cannabinoids, where solutions were successfully prepared up to 500mg/ml (50% mass/volume) with a mixture of higher glycoside forms of cannabinoids. Given the markedly improved solubility and novel secondary and tertiary glycosylation on cannabinoids, glycosylated cannabinoids can act as efficient prodrugs for selective delivery of cannabinoids to desired tissues where the glucose molecules can be hydrolyzed to release the aglycone cannabinoids. Additionally the glycosylations promote stability of CBD and CBDV by protecting them from oxidation and ring-closure of the C6'-hydroxyl group, which prevents degradation into A9-THC or A9-THCV, respectively, and subsequently into cannabinol (CBN) or cannabnavarin (CBNV), respectively.

[001 18] Increasing the diversity and complexity of sugar attachments to cannabinoids, and administration of a mixture of glycosides will provide altered prodrug delivery kinetics, thus providing an extended release formulation of the drug. The primary detoxification mechanism for cannabinoids in humans is CYP450 mediated hydroxylation of the C7 methyl group of CBD and CBDV, or the C11 methyl group of THC and CBN, glycosylation of the acceptor hydroxyl groups of the cannabinoid resorcinol ring may afford protection from C7/C11 hydroxylation and subsequent elimination from the body due to steric hindrance preventing the cannabinoid-glycoside from binding in the CYP450 active site. In fact, the hydroxyl groups of CBD are
thought to facilitate the binding to the detoxification cytochrome P450 CYP3A4 in the epithelium of the small intestine (Yamaori 2011). Reduced degradation or metabolism in the stomach and small intestine due to these effects could also lead to higher total bioavailability of any glycosylated product upon oral delivery.

[0019] In some cases, removal of the sugar from glycosides in the body may be required in order for the compounds to exert their primary biological activity. Therefore, glycoside prodrugs may enable stable drug formulations that are resistant to abuse, due to the potential for their primary biological effects to only occur after oral ingestion. As most abuse-deterrent compounds are simply mixing or formulation based deterrents, they can still be compromised by simple physical and chemical methods. As one example, the beta-glycosides described herein will only release the aglycone upon the action of beta-glycosidase enzymes. Beta-glycosidases are known to be secreted by microbes that occupy the large intestines of mammals, therefore upon oral ingestion the glycoside prodrugs will remain glycosylated until they reach the large intestine. A similar approach may be used for abuse-resistant, abuse-deterrent, and site-specific delivery of other compounds through glycosylation. It has been found that the UGT76G1 enzyme (SEQ ID N0.1) from Stevia rebaudiana transfers a glucose molecule from the sugar donor UDP-glucose (UDPG) to the hydroxyl groups of CBD to create novel CBD-O-glycosides (Table 1, Figures 2 & 4). The UDPG is inverted by UGT76G1 to produce β-D-glucose residues covalently linked through the to the hydroxyl acceptor sites on CBD. To improve the catalytic efficiency UGT76G1 open reading frame (ORF) codon optimization was performed (SEQ ID NOs. 4 and 6) for expression in Pichia pastoris. Similar to its activity towards steviol glycosides, UGT76G1 is highly productive and has an equilibrium constant (Keq) for CBD of -24. Through experimentation and analysis it was determined that UGT76G1 has the unique ability to apply multiple glucose moieties to the CBD molecule. Upon prolonged incubation of CBD with UGT76G1 and UDPG, HPLC analysis of the reaction mixture yielded 8 glycoside product mobility groups, suggesting that UGT76G1 is able to glycosylate both the C2' and C6' hydroxyl groups on CBD, as well as glycosylating the primary glucose residues with a secondary and tertiary glucose moieties. The secondary and tertiary glycosylations by UGT76G1 occurs at the C3 hydroxyl group of the recipient sugar (3→1 connectivity), as would be suggested by its activity in Stevia, creating 0-(3-1)-glycosides, and the subsequent products. The CBD-glycoside product mobility groups also suggest that CBD can dock in the UGT76G1 active site both forwards and backwards creating a cis-like-conformation for the glycosylations relative to the cannabinoid backbone (mechanism depicted in Figure 3), or possibly the rotational freedom about the bond at C1' (C6 described by Mazur
2009) allows the hydroxyl group to rotate after glycosylation, placing the other hydroxyl group adjacent to the UDPG in the active site and creating a trans-like-conformation for the glycosylations on the cannabinoid backbone (mechanism depicted in Figure 4). Potential CBD molecular docking in the active site of UGT76G1 is depicted in Figure 6 where CBD is superpositioned over the bi-functional substrate for UGT76G1, Rebaudioside E (RebE) (Figure 6).

[00120] As CBD was successfully glycosylated by UGT76G1, CBDV was incubated with UGT76G1 and UDPG to test for glycosylation activity. CBDV depletion was observed upon HPLC analysis, in addition to the appearance of four additional product peak mobility groups, which were dependent on addition of both UGT76G1 and UDPG. The four new products formed displayed the same absorbance characteristics as CBDV and were determined to be the primary glycosides CBDV-2'-0-glucopyranosides, CBDV-6'-0-glucopyranosides, and the secondary glycosides CBDV-2'-0-(3-1)-diglucopyranoside, and CBDV-6'-0-(3-1)-diglucopyranoside (compounds VB202, VB206, VB204 and VB208, respectively, Table 2). With additional reaction time it was determined that higher order glycoside products were also formed. CBDV-glycoside production was similar to CBD-glycosides from UGT76G1 (Table 2), and proceeded to completion with a K_{eq} -24. Given the number of CBDV-glycoside products, UGT76G1 transfers multiple glucose molecules onto CBDV on both C2' and C6' hydroxyl groups, as well as onto the primary and secondary glycosylations.

[00121] When the cannabinoid A9-THC was incubated with UGT76G1 and UDPG, HPLC analysis of the reaction mixture showed three main product peak mobility groups. The three products were identified as A9-THC-1-0-glucopyranoside, A9-THC-1-0-(3-1)-diglucopyranoside, and A9-THC-1-0-(3-1,3-1)-triglucopyranoside (formal pyran numbering, Table 3, Figure 7). Given that the rigid structure of A9-THC does not have the same rotational freedom as CBD around the C1' resorcinol ring attachment, the cannabinoid backbone is recognized in the active site of UGT76G1 with the A9-THC C1 hydroxyl group situated towards the UDPG sugar donor (pyran numbering, Figure 1B).

[00122] As UGT76G1 demonstrated glycosylation activity for all other phytocannabinoids analyzed, it was also tested for glycosylation activity against cannabinol (CBN). Effective glycosylation of CBN by UGT76G1 was observed, in a similar pattern to A9-THC, as both share a single hydroxyl recipient group at the C1 position of the resorcinol ring. The activity seen with UGT76G1 is consistent with a broad recognition of cannabinoids by the enzyme active site.
Alternative cannabinoid substrates may be inserted into this UGT76G1 glycosylation reaction infrastructure to generate novel cannabinoid-glycosides, given they possess hydroxyl groups in similar positions on the cannabinoid backbone. Ideal candidates are cannabigerol (CBG), cannabichromene (CBC), cannabidiol hydroxyquinone (CBDHQ), HU-331, other isomers of A9-THC such as Δ9-THC, etc., and synthetic analogues of A9-THC such as HU-210.

Similar to the secondary 3→1 glycosylation activity of UGT76G1, it was determined that following a primary glycosylation by UGT76G1, the UGT enzyme Os03g0702000 (SEQ ID NO.9) from Oryza sativa is also capable of transferring an additional glucose moiety from UDP-glucose onto the C2-hydroxyl of the primary sugar (Tables 1 - 11, Figures 7-9 & 12 - 14). This glycosylation activity is consistent with the activity of UGT Os03g0702000 towards steviol glycosides in establishing C2-hydroxyl secondary glycosylations (2→1 connectivity) on existing primary glucose residues. This secondary glycosylation was observed with CBDV (Table 2, Figure3), and THC (Table 2, Figure 7), generating novel CBDV and A9THC-1,0-(2-1)-diglucopyranoside species, respectively. Consistent with broad substrate recognition and reactivity, this activity of Os03g0702000 was further demonstrated for the remainder of the substrates identified in Figure 1.

In addition to the UDPG-dependent glucosyltransferase activity, cyclodextrin-glucanotransferase (CGTase, Toruzyme 3.0L, trademark of Novozymes Inc.) is capable of transferring a short α-(1-4)-maltodextrin chain onto the hydroxyl groups of cannabinoids. The CGTase is also capable of glycosylating primary and secondary glycosylations established by UGT76G1 and Os03g0702000, resulting in carbohydrate attachments that start with β-D-glucose molecules, but terminating in α-D-glucose molecules termed β-primed-a-glucosyl (Tables 1-11). α-glycosylation by cyclodextrin glucanotransferase mediated maltodextrin transfer can occur on any of the hydroxyl groups of the primary or secondary sugars covalently linked to the cannabinoid. One skilled in the art will appreciate that this makes possible any number of conformations of a-glycosyl chains linked to the glycosides listed in Tables 1-11.

Alternative enzymes with homology to UGT76G1 and Os03g0702000 may be used to produce the same glycosylation of cannabinoids. Suitable enzymes for establishing the primary glycosylation similar to UGT76G1 are additional members of the UGT76 clade such as UGT76G2 or UGT76H1. BLAST results with the UGT76G1 protein sequence yield a maximum homology of 49% identity, as much as 66% positives (similar identity). Ideal candidates may have low overall peptide identity or similarity, but will likely have conserved amino acids at the
opening adjacent to the UDPG catalytic site. This sequence is exemplified by a leucine at position 379, and a broader peptide sequence of SDFGLDQ (AA’s 375 to 381 of UGT76G1). Suitable enzymes for producing the secondary glycosylation of Os03g0702000 are members of the UGT91 clade, including UGT91 D1 and UGT91 D2.

[00127] The glycosylation reactions performed herein included UDP-glucose as the nucleotide sugar donor, however there is some cross-reactivity amongst UGTs that allows for use of alternative nucleotide sugars such as UDP-glucuronic acid, etc. Glucuronic acid is the predominant nucleotide sugar utilized by phase-II detoxification UGTs in the liver, and cannabinoid-glucuronides are a common detoxification product. Additional nucleotide sugars which could be used to donate carbohydrate moieties to create novel glycosides with similar properties include UDP-glucuronic acid, UDP-mannose, UDP-fructose, UDP-xylene, UDP-rhamnose, UDP-fluorodeoxyglucose, etc. In addition, nucleotide sugars can also be used in combination to create glycosides that contain multiple types of residues on the same aglycone backbone. Alternative strategies to further improve the solubility and delivery of cannabinoids and other compounds described herein include their glycosylation and then functionalizing the sugar moieties with additional ligands or modifications. Examples of this include sulfation, myristoylation, phosphorylation, acetylation, etc.

[00128] The endocannabinoid system has recently been the subject of intense research efforts due to its demonstrated role in and impact on a broad range of clinical pathologies. As UGT76G1 has been determined to recognize a broad class of phytocannabinoids, it was hypothesized that the same enzyme would also recognize and glycosylate endocannabinoids, which are the endogenous signaling molecules recognized by the cannabinoid receptors in Humans. Upon testing a sample of four prototypic endocannabinoids including arachidonylethanolamide (anandamide, AEA), 2-arachidonylethanolamide (2-AG), 1-arachidonylethanolamide (1-AG), and docosahexaenoyl ethanolamide (DHEA, synaptamide), it was found that UGT76G1 effectively glycosylated each endocannabinoid (Tables 5-8, Figures 9-12). Glycosylation of endocannabinoids enables the creation of endocannabinoid-glycosides and other fatty acid neurotransmitter-glycosides, representing a new method of targeted delivery of endocannabinoids.

[00129] As endocannabinoids such as AEA, 2-AG, 1-AG, and synaptamide are glycosylated by UGT76G1, it is hypothesized that similar endocannabinoids will also be suitable substrates for glycosylation by UGT76G1. Other endocannabinoid candidates that are likely to be glycosylated by UGT76G1 include oleoyl ethanolamide (OEA), eicsapentaenoyl ethanolamide, prostaglandin
ethanolamide, docosahexaenoyl ethanolamide, linolenoyl ethanolamide, 5(Z),8(Z),11(Z)-
eicosatrienoic acid ethanolamide (mead acid ethanolamide), heptadecanoyl ethanolamide, stearoyl ethanolamide, docosaenoyl ethanolamide, nervonoyl ethanolamide, tricosanoyl ethanolamide, lignoceroyl ethanolamide, myristoyl ethanolamide, pentadecanoyl ethanolamide, palmitoleoyl ethanolamide, docosahexaenoic acid (DHA), and similar compounds. These glycolipids may have a wide range of commercial uses, ranging from pharmaceutical use as a novel endocannabinoid drug with improved solubility and pharmacokinetic properties, to use as an antibacterial agent, to use as a detergent similar to other glycolipids, etc.

[00130] It has been characterized that AEA and CBD are full agonists of the toll-like vanilloid receptor type 1 (TRPV1), which is the receptor for capsaicin. In addition, other cannabinoids and botanical extracts, including but not limited to CBD, CBN, cannabigerol (CBG), and various propyl homologues of CBD, THC, and CBG have been demonstrated to bind and have activity towards transient receptor potential channels (TRPs) (De Petrocellis 2011). This includes stimulating and desensitizing TRPV1, as well as TRPA1, TRPV2, and also antagonism of TRPM8. Although stimulation of TRPV1 leads to vasodilation and inflammation, capsaicin and its analogues act to desensitize the receptors to stimulants, and provide potent anti-inflammatory effects (Bisogno 2001). Analogous effects may occur with TRPA1 in addition to other TRPs. For CBD, this may occur at concentrations that are lower than what is required for binding of cannabinoid receptors, and at concentrations that are within the range of those typically attained in human clinical testing and use. In addition to acting as a direct agonist of the TRPV1 receptor, CBD has been shown to inhibit fatty acid amide hydroxylase (FAAH), the enzyme responsible for facilitating the metabolism of the endocannabinoid anandamide (Watanabe, 1998; De e Petrocellis 2010). Given that these phytocannabinoids act as ligands of diverse TRPs, it was postulated that UGT76G1 would be capable of glycosylating many different ligands of the same TRPs, including TRPM8, TRPV2, TRPA1, and TRPV1. Capsaicin is capable of contorting into a CBD-like structure (Bisogno 2001), therefore it was postulated that capsaicin was likely to be a suitable substrate for glycosylation by UGT76G1. To this end, it was shown that UGT76G1 is capable of glycosylating the vanilloid moiety of capsaicin in a structurally identical way to PaGT3 from *Phytolacca americana* (Noguchi 2009). As the glycosylated structure of capsaicin is the vanilloid head, it was further hypothesized that UGT76G1 would be capable of glycosylation of the minimal vanilloid, i.e., vanillin, as well as many analogues. Consistent with this hypothesis, through HPLC analysis it was determined that UGT76G1 created multiple glycoside products of vanillin (Figure 14, Table 10). Seeking to test the ability of UGT76G1 to glycosylate vanilloids more broadly, curcumin, the well characterized
vanilloid found in turmeric spice, isolated from the ginger *Curcuma longa* was applied as a substrate in the glycosylation reaction. Consistent with the glycosylation of vanillin, UGT76G1 effectively glycosylated curcumin, creating multiple glycosylation product peaks, suggesting a bifunctional recognition and glycosylation by UGT76G1 similar to that seen with CBD and steviol glycosides (Figures 15A & 15B, Table 11).

[00131] Cannabinoid glycosides may also have direct bioactive and therapeutic effects, beyond their utility a prodrug for their aglycone form. Quercetin is an antioxidant flavonoid that is ubiquitous in vegetables and often present both in its aglycone and glycosylated forms. It has been demonstrated through *in vitro* studies that quercetin glucuronides act as a bioactive agent as well as a precursor molecule to aglycone quercetin (Terao 2011). In many cases, including with glycosides that exert antibacterial and antitumor effects, the glycosidic residues are crucial to activity (Kren & Rezanka 2008).

[00132] Glycosides have also been demonstrated to receive facilitated transport across the blood brain barrier (BBB) by the glucose transporter GLUT1. A prime example is the glycoside of ibuprofen achieving a significant increase of ibuprofen aglycone concentration in the brain (Chen 2009). Similar to these glycosides, glycosides of cannabinoids and other compounds described herein may benefit from enhanced facilitated transport across the BBB or other barriers. Glucose transporters are a wide group of membrane proteins encoded by the human genome and that are found not only in the BBB but across many different cells and tissues, including brain, erythrocytes, fat, muscle, kidney, liver, intestine, and pancreas, so glycosylation will be tailored to provide site-specific delivery to any of these tissues. Accordingly, in one embodiment, there is provided a method for facilitating the transport of a cannabinoid drug across the blood brain barrier of a subject comprising administering to the subject a cannabinoid glycoside prodrug in accordance with the present invention.

[00133] Delivery of cannabinoids and cannabidiol to the brain may be especially useful because of oligodendrocyte protective (oligoprotective) and general neuroprotective effects. It has been demonstrated that cannabinoid signaling is involved with both oligodendrocyte differentiation (Gomez 2010) and that cannabinoids promote oligodendrocyte progenitor survival (Molina-Holgado 2002). Drug formulations that include cannabidiol as a major ingredient have been approved to treat muscle spasticity and pain from multiple sclerosis, a neurodegenerative disorder that causes loss of myelin and oligodendrocyte progenitor cells. The effects of cannabidiol have been demonstrated to mediate oligoprotective effects through attenuation of endoplasmic reticulum stress pathways (Mecha 2012). Cannabidiol has also
been studied extensively for its antipsychotic effects, however the exact role in protection of oligodendrocytes and promotion of remyelination has not yet been described (Zuardi 2012). Despite the correlation between the clinical symptoms of psychosis with neuropathological analysis that indicates dysmyelination is involved, the role of dysmyelination as a driver or cause of schizophrenia and other psychoses remains controversial (Mighdoll 2015). Remyelination has also been described as potentially useful for treatment of Alzheimer's disease and other forms of dementia (Bartzokis 2004). Therefore, delivery of cannabinoids to the brain may be especially useful for its established neuroprotective and oligoprotective effects. Cannabinoid glycoside drug formulations co-administered in combination with other agents that influence other aspects of repair or regeneration, such as oligodendrocyte progenitor differentiation or remyelination, may also prove to be beneficial. This includes compounds such as anti-LINGO-1 monoclonal antibodies, guanabenz, sephinn, benzatropine, clemastine, polyunsaturated fatty acids, etc.

[00134] In the course of the present work, it was discovered that UGT76G1, Os03g0702000 and cyclodextrin glucanotransferase (CGTase) were capable of primary, secondary and tertiary glycosylations of steviol glycosides and aglycone products of diverse chemical structure, including cannabinoids, endocannabinoids, vanillin, curcumin, and capsacin.

[00135] In the screening and analysis methods described by Dewitte 2016, a 50mm HPLC separation column combined with a high solvent flow rate was used limiting the separation and overall detection of glycoside products. Thus, the interpretation of the glycosylation reaction products for many compounds is speculative, yet still reinforces the significance of the present finding that UGT76G1 has broad substrate specificity. Clearly, the work described herein demonstrate that UGT76G1 can glycosylate not only steviol glycosides, but other forms of glycosides, and novel aglycone compounds such as cannabidiol as well. Internal studies that used an improved separation methodology involving a 150mm length C18 column coupled with a low solvent flowrate also enabled the clear detection of secondary and tertiary glycosides. These compounds were unable to be detected by the methods described in Dewitte 2016, and provide additional verification of the ability of UGT76G1 to not only glycosylate compounds with diverse chemical structures, but also to perform multiple higher order glycosylations on glycosides of these same compounds.

[00136] The reactions described herein take place in vitro using recombinant enzymes and all necessary cofactors, and the expression of UGT76G1 enzyme within the cells of a Cannabis
plant is possible for the *in vivo* biotransformation of cannabinoids prior to extraction of cannabinoids from plant tissue. As UGT76G1 is an enzyme from the plant *Stevia rebaudiana*, it will be compatible with expression in the genus Cannabis. The ideal strategy for expression of UGT76G1 within the Cannabis plant is to genetically engineer the UGT76G1 open reading frame under a promoter element that is specific for the same tissue that cannabinoids are produced in, namely the secretory trichomes of the plant. Suitable promoter elements include the promoter for the cytosolic 0-acetylserine(thiol)lyase (OASA1) enzyme from *Arabidopsis thaliana* (Gutierrez-Alcala 2005). Candidates for transformation with UGT76G1 include *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. A similar approach may be used with UGT76G1 and similar enzymes for *in planta* production of glycosylated secondary metabolites within many other different plant species, and may be especially useful when plant species already produce large quantities of the desired aglycone product or known enzyme substrate.

[00137] In the course of performing phytocannabinoid glycosylation reactions CBD and THC displayed noticeable antimicrobial activity, even preventing large-scale reaction mixtures from becoming contaminated after failure of the sterile filter apparatus. Prior pilot-scale glycosylation reaction utilizing steviol glycosides as substrates during enzymatic processing were quite susceptible to infection in the absence of strict sanitation techniques. CBD and THC pilot-scale reactions remained aseptic for over a week in the same reaction vessels with very limited ongoing maintenance or care. To this end, the use of the aglycone cannabinoids and their respective glycosides is proposed as efficient antimicrobial agents. Accordingly, in one embodiment, there is provided an antimicrobial agent comprising an effective amount of a cannabinoid glycoside prodrug in accordance with the present invention.

[00138] Similarly, upon the production of large quantities of cannabinoid-glycosides and formulation in aqueous solutions, it was observed that multiple cannabinoid-glycosides in water had foaming properties similar to detergents. This is consistent with other glycoside detergents like 8-octylglycoside, 8-octylthioglycoside, and similar, and establishes a potential use for cannabinoid-glycosides as a detergent. Accordingly, in one embodiment, there is provided a detergents agent comprising an effective amount of a cannabinoid glycoside prodrug in accordance with the present invention.
Nucleic Acids

[00139] The present invention provides for nucleic acids comprising nucleotide sequences encoding a glycosyltransferase. The glycosyltransferases of the present invention are capable of primary, secondary, tertiary glycosylations or a combination thereof. In certain embodiments, the glycosyltransferases are capable of primary, secondary and tertiary glycosylations. In other embodiments, the glycosyltransferases are capable of secondary and tertiary glycosylations. In certain embodiments, the nucleic acids encode a glucosyltransferase, including but not limited to a UDP-glucosyltransferase. The glucosyltransferases include but are not limited to a Stevia rebaudiana UDP-glucosyltransferase, such as UGT76G1 or UGT74G1 or an Oryza sativa glucosyltransferase, such as Os03g0702000. In other embodiments, the invention provides for nucleic acids comprising nucleotide sequences encoding a cyclodextrin glucanotransferase. Also provided are nucleic acids comprising nucleotide sequences that encode a sucrose synthase.

[00140] Nucleic acids include, but are not limited to, genomic DNA, cDNA, RNA, fragments and modified versions, including but not limited to codon optimized versions thereof. For example, the nucleotide sequences may be codon optimized for expression in Pichia pastoris or E. coli. The nucleic acids may include the coding sequence of the glycosyltransferase or sucrose synthase, in isolation, in combination with additional coding sequences (e.g., including but not limited to a purification tag).

[00141] In certain embodiments, the nucleic acid comprises a sequence encoding UGT76G1 or UGT76G1-like glucosyltransferase. UGT76G1-like glucosyltransferase include for example, other members of the UGT76G1 clade such as UGT76G2 or UGT76H1. In certain embodiments, the nucleic acid comprises a sequence encoding an UGT76G1 glucosyltransferase having the amino acid sequence as set forth in any one of SEQ ID NOs:1, 3, 5 and 7 and listed below or fragments and variants thereof.

[00142] SEQ ID NO:1 (UGT76G1 (native protein sequence))

MENKTETVRRRRIILFPVPFOGHINPILQANVLKFSITIFHTNFKPKNFTSNYPTFRFL DNDPQDERISNLPTHGPLAGMRIPINEHGADELREELLELMASEEDEEVSLIDALWYFAQS VAQSLNRRLRLMTSSLNFHAHVSLQPFDELGYLPDDKDREEEAQASGFPMYVKTIRKAYSQ NQILKIEILGKMKQTAKASSGVWNSFKELEESELETVIAREPFLPLPQKHLTASSSSLLHDTVFQWLDQPPSSVLKGYSTSEDEVKDFLEIRGLVDSKQSFLLVRPGFVKGSTWVEELP DGFLGERGRIVKVWPQVEVLAGAGAAFWTHSGWNSLLESVCEGVPVIIFSDFGLDQPLNARY MSDVLYGVYLENGWEIANGAIARRVMMDEEGEYIRQVARNLKQKADVSLMKGSSYESLE SLVSYISSL
In certain embodiments, the nucleic acid comprises a sequence encoding UGT76G1 glucosyltransferase and comprising the nucleotide sequence as set forth in any one of SEQ ID NOs: 2, 4, 6 and 8 and listed below, or fragments and variants thereof.
SEQ ID NO:2 (UGT76G1 native nucleic acid sequence)

ATGGAAAATAAAACGGGAGACCCACGTTCGCGCGCGGAGAATAAATATTATCCCCTGGTA
CCATTCTAACGGCACCATTACCCCATTTCTCAGCTGGCAATGTGTTGACTCTCAAGGATT
CAGATTCACCCTCTTTCACCAACTTCACAAACCCCAATTACTTACTCATCTCACTTCA
CTTTCAAGTTCCATCCTTCACAACTTCAACCAACAGCTTACACACTTCAATCTGGAGGAT
CTGGTCCGCTTGCTGATGGTTGGGATTCCGGATATTCAAGCAGACGGAGCTGGCAATACG
ACGGGACTGAACTGGTATCTGGATAGTACTGTTGACTGGAAGAGAAGATGATGTTGTTTA
TACACGGGATGCTTTTGGCAGTCTTCACTTTACCTCGGCAAATCCTACGGGCTTTG
TTTTGATGACAAGGCTTGTTGAAATTATTCTCACTTCATGCTGCTGCTGATGGGTACAT
GTTGGATGGTGGTCTTATGCCAGCTTATCCATTTTCAAAACACCTTTCAGAATTGAGAAG
TAAGATTTGGTTTCTTCATCTCCCAGTTTCAAGCAGCTGTTCTTTGATGACTATGATGT
ACGGTGGGACCCGCGCAACTTACCTCCACGTTCGAGTTGTACAGTGGGATATTACATGT
ATGCACCACCATCACCAGGCTTCTCGTGAAAAACAAAATCTAAAATGATGTAAGAAAG
GAAGAAGAATTTTGTATTTCGAGTCTACACACAGCAGCTATGTTGTTGACTCTCAACTT
GCCAATTGTCAATGTGAAAAGATATGAAATCTTCTCAGATTTTGGGAATATATATGATG
AAAGATCTATCTCCAGCTATCCATCTTCTCAGATTATTTTGGAATATATATGATGATG
AGATATCTTATTCTTCAGCTATCTTCTCATTTTGGGAAAATGATGATGATGATGAGATT
GAAAGAAGAATTTTGGAATACATGTTTCTGCAGGCTGATGATGATGATGAGATTGAAG
ATGGATGTTTGATTCAGCTTGATGATGATGATGAGATTGAAG

SEQ ID NO:3 (Sequence encoding SEQ ID NO:2 codon optimized for expression in Pichia pastoris)

ATGCACTGACACATCCACCCAGCTTCTCGTGAAAAACAAAATCTAAAATGATGTAAGAAAG
GAAGAAGAATTTTGTATTTCGAGTCTACACACAGCAGCTATGTTGTTGACTCTCAACTT
GCCAATTGTCAATGTGAAAAGATATGAAATCTTCTCAGATTTTGGGAATATATATGATG
AAAGATCTATCTCCAGCTATCTTCTCATTTTGGGAAAATGATGATGATGATGAGATT
GAAAGAAGAATTTTGGAATACATGTTTCTGCAGGCTGATGATGATGATGAGATTGAAG
ATGGATGTTTGATTCAGCTTGATGATGATGATGAGATTGAAG

SEQ ID NO:4 (Sequence encoding SEQ ID NO:3 codon optimized for expression in Pichia pastoris)

ATGCCACCACCATCACCAGGCTTCTCGTGAAAAACAAAATCTAAAATGATGTAAGAAAG
GAAGAAGAATTTTGTATTTCGAGTCTACACACAGCAGCTATGTTGTTGACTCTCAACTT
GCCAATTGTCAATGTGAAAAGATATGAAATCTTCTCAGATTTTGGGAATATATATGATG
AAAGATCTATCTCCAGCTATCTTCTCATTTTGGGAAAATGATGATGATGATGAGATT
GAAAGAAGAATTTTGGAATACATGTTTCTGCAGGCTGATGATGATGATGAGATTGAAG
ATGGATGTTTGATTCAGCTTGATGATGATGATGAGATTGAAG

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TAGACAAATGCTAGAGTTTTGAAACAAAAAGCTGATGTTTCTTTGATGAAGGGTGGATCTTCTTATGAATCTTTGGAATCTTTGGTTTCTTACATTTCTTCTCTTTAA

[00149] SEQ ID NO: 6 (Sequence encoding SEQ ID NO: 5 codon optimized for expression in *Pichia pastoris*)

ATGCAATCAACATCAACACCAATCTGGAATCTATGGGAAGAAGAAGACTGCAATCTGGATCTATGGAGAACAAGACCGAGACTACAGTTAGAA
GAAGAAGAAGAATAATCCTGCTTCTTCCGGAGTCTTCTTATCAATTTCTTCTTCTCTTTAA

[00150] SEQ ID NO: 8 (Sequence encoding SEQ ID NO: 7 codon optimized for expression in *Escherichia coli*)

ATGAAAATAAAACCGAAACCACCGGCAATTTGCGGACTCTGCTCCTCCTTCTGTCTCTGAAAATGTTGGGAGGGTTGGATCCGCTGCCGAAACATCTGACCGCGTCATCGAGCTCTCTGCTGGATCACGACCGTACGGTGTTTCAGTGGCTGGATCAGCAACCGCCGAGTTCCGTGCTGTACGTTAGCTTCGGTAG

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In certain embodiments, the nucleic acid molecule encodes an UGT76G1 glucosyltransferase and comprises the nucleotide sequence as set forth in GenBank Accession number AY345974.1 or a variant or fragment thereof.

In certain embodiments, the nucleic acid comprises a sequence encoding UGT76G2 glucosyltransferase. In specific embodiments, the nucleic acid comprises a sequence encoding UGT76G2 glucosyltransferase having the amino acid sequence as set forth in SEQ ID NO:27 and listed below or variants and fragments thereof.

SEQ ID NO:27
MENKTETTVRRRIILFVPVQGHINPILQLANVLTKFSITIFHTNFKNPKTNSYNPHFTFRFIL DNDPODVRISNLPTHGLPTVMRILINEHADELQREELLMLASEEDGEVSCLTDQIWYFTQSD VADSLNLRLVLMTSSLNFHAHSLVPLQDELGYLPDKTRLEEQASGFPMLKVDIKCGFS MWKQGKEIFENITQTKASSGVIWNSFKELESEELTVIREIPAPSLPLPHTLASSLDH DRTVFVPWLDQPSVLVSFGSATEVADKDFLEIARGLVDKQSFLWVRPGFVKGSTWVE LPDGFLGERGRIVKWPQEVLAHGAIGAFWTHSNGWSTLESCEGVPMIFSAAFDQPLNA RYMSDVLKVGYVLENWGEREINAIRRVVMDEEGGYIRQNASVLQKADVSLMKGGSSYES LESLVAYISSL

In specific embodiments, the nucleic acid comprises a sequence encoding UGT76G2 glucosyltransferase and having the nucleic acid sequence as set forth in SEQ ID NO:28 and listed below or variants and fragments thereof.

SEQ ID NO:28
ATGGAAAATAAAACGGAGACCCCGTTGCCCGCGCGCCGAGAAATAATATTATTCCTCCCGTA CCAGTCATGGCCACATTTAACACAAATTTCTCTGAGTCCGAATGTTGTTGCTACTCAGAGGAT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT TCAGATCATCATCTTTCACCAACCATCTTCAAAACCAACATCTAATTACTTCCAAGAGGT
In certain embodiments, the nucleic acid comprises a sequence encoding UGT76H1 glucosyltransferase. In specific embodiments, the nucleic acid comprises a sequence encoding UGT76H1 glucosyltransferase having the amino acid sequence as set forth in SEQ ID NO:29 and listed below or variants and fragments thereof.

```
MLQLATYLHSGISITAIQYPNFNSPDSSNHPELTFLPLSSGNSLAVADISGGFQFTQ1NHNCKP
HFREYLVMSSDDKESIVIIRDLNLMFFAGEIAGEGLPSILRGSNAVMLTASDIIQPLHQEGRF
PPPDSLLEETIPELVPFRYKDFYKIPHIQTEFSITMMTPKSPASAILINTLEFQSALTQIHD
YKVPVFITIGPLHIVTRTSILEEEDTSCINWLDKQSKSVYVSLGLAKLDEKVASEMACGLA
MSNHKFLWVRP6MVHGEVWVEFLPDSLVEGEMKARYLGWKAPQTTVLHANVGGFWSHC
GWNSIECLESQVPMCMQPPFADDQLLNNARYSDWVKTGFIEVIEGAIKRVLDEEGEEM
RQRAEIKEKVIANDGSSYDSFKDLVAFISSL
```

In specific embodiments, the nucleic acid comprises a sequence encoding UGT76H1 glucosyltransferase and having the nucleic acid sequence as set forth in SEQ ID NO:30 and listed below or variants and fragments thereof.

```
ATGCTTCAGCTTGCAGCCATCTCCTCATTCAAGGGATTCTTCAATAACCATCGTCATGCC
CAACTTCAACCTCGCGATTTCTCAATCTACCCGAGGAACCTACATCCAGAACTACCTCCTCC
ACTCCATCGGGAGCATCTCCCGGCCTTTTCAAGTCTCATCCTACCCTATATCCATTTACCA
ATAACTGCAACCATCTTCGGAGAATCTCTTCTGAGAATCCTATTGCTATCGGTCAGGAA
ATCAATCTTATACCGTATGATTGTCTTCTTCTCCGGAGAAACTGCCGGCGAGCTG
GGTGTGCTTCTCGATACATTGTTGAAGGTGACTCGACCTGCAACTCGGAGACTCG
TCCCTCAGCTCATTCAAGGGATTCTTCAATAACCATCGTCATGCC
```

In certain embodiments, the nucleic acid comprises a sequence encoding *Oryza sativa* Os03g0702000 or Os03g0702000-like glucosyltransferase. Os03g0702000-like glucosyltransferase include for example, other members of the UGT91clade such as UGT91 D1 or UGT91 D2. In certain embodiments, the nucleic acid comprises a sequence encoding Os03g0702000 glucosyltransferase having the amino acid sequence as set forth in SEQ ID NO: 9 and listed below or a variant or fragment thereof.

<table>
<thead>
<tr>
<th>SEQ ID NO:9</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHQHQQSGSMDSGYSSYYAAGAMHVICPWLAFGHELLPCLDLAQLRASRGRHVRSFVSTP</td>
</tr>
<tr>
<td>RNISRLPPVPRPALPLVAFLVAPLPRVEGLPDGAESTNVDHRDPDMVHELHRAFGLLAEAFSE</td>
</tr>
<tr>
<td>FLGTACADWIVIDVHHWAAAALAEHKVPCAMMLGSAHMIAISAIERRERETEPAAGQG</td>
</tr>
<tr>
<td>RPAAAPTVEFVARMLRTGKGSGLAERFSLTSRLSSKVGRCFVPEGFPETVPLSTLRPKT</td>
</tr>
<tr>
<td>FLGLMPPLHEGRREDGADVWLDAQPANSVYVVALGSEVPLGEVKHELALGLELAGTRFL</td>
</tr>
<tr>
<td>WALKRPGTVDSDALPPAGFEERTRGVRATRVWVQMISHSAAVGAFLTHCGWNSLIEGLM</td>
</tr>
<tr>
<td>FGHPLMLIPFIDQPNARLRIEAKNAGLQVARNDGSDRFREGVAAAIRAVEEESKVFQAK</td>
</tr>
<tr>
<td>AKKLQIEIVADMACHERYIDFGIQQLRSSYK</td>
</tr>
</tbody>
</table>

In certain embodiments, the nucleic acid molecule encodes Os03g0702000 glucosyltransferase and comprises a nucleotide sequence as set forth in SEQ ID NO: 10 and as detailed below or a variant or fragment thereof.

<table>
<thead>
<tr>
<th>SEQ ID NO:10</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATGCATCAGCACCACCATCAGCCGTTATCTATGACTCCGGTGACTCTCTCTCTCTACGCC</td>
</tr>
<tr>
<td>GCCGCCGCCGATGACGTGACTCTTCCGCTTGCCCGTGGCTGCTCCGGCCACCTGTCTCC</td>
</tr>
<tr>
<td>GTGGGCGCACCCTGCGCCTGCGCTGGCGCCACCGTGCCGGCGCCACCGTGCCGGCGCC</td>
</tr>
<tr>
<td>CTGTGGGGCGCCTGCCGCTGCCGCCCTGCAGGGCCGCCGCCGCCGCGCCGCCGCGCC</td>
</tr>
<tr>
<td>AAGGCCAGTCCAACACAGCAACAGCCGCCACATGGCTGAGCTCCGCCAGGAGGCCTTCAGG</td>
</tr>
<tr>
<td>GCTGGCCGCCTCTCCGAGTCTTGGGCACTGGCGGCCCCAGGGCGCTGCTGCCGCTGCCGG</td>
</tr>
<tr>
<td>ACCGCCCTGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCC</td>
</tr>
<tr>
<td>AAGTGTTGGGCTCTGGACATGATCGCTCCATTACAGACAGACAGCGCTCAGGCAGCCG</td>
</tr>
</tbody>
</table>

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In certain embodiments, the nucleic acid molecule encodes Os03g0702000 glucosyltransferase and comprises the sequence as set forth in GenBank Accession number XM_015773655 or a variant or fragment thereof.

In certain embodiments, the nucleic acid comprises a sequence encoding UGT91 D1 glucosyltransferase. In certain embodiments, the nucleic acid comprises a sequence encoding UGT91 D1 glucosyltransferase having the amino acid sequence as set forth in SEQ ID NO:31 and listed below or a variant or fragment thereof.

SEQ ID NO:32

In certain embodiments, the nucleic acid molecule encodes UGT91 D1 glucosyltransferase and comprises a nucleotide sequence as set forth in SEQ ID NO:32 and as detailed below or a variant or fragment thereof.

SEQ ID NO:32
In certain embodiments, the nucleic acid comprises a sequence encoding UGT91 D2 glucosyltransferase. In certain embodiments, the nucleic acid comprises a sequence encoding UGT91 D2 glucosyltransferase having the amino acid sequence as set forth in SEQ ID NO: 33 and listed below or a variant or fragment thereof.

SEQ ID NO:33

MATSDIVDDRKQLHLVATFPWLAFGHILPYLQSKLIAEKGKHVSFLSTTRNIQRSSHISPLNIVVQLTLPRVERPEADADTVHPEDIPYLLKASDGQOLETFQHSPDPIIYDHYWLPISA

In certain embodiments, the nucleic acid molecule encodes UGT91 D2 glucosyltransferase and comprises a nucleotide sequence as set forth in SEQ ID NO: 34 and as detailed below or a variant or fragment thereof.

SEQ ID NO:34
In certain embodiments, the nucleic acid comprises a sequence encoding Stevia rebaudiana UDP-glycosyltransferase 74G1. In certain embodiments, the nucleic acid comprises a sequence encoding Stevia rebaudiana UDP-glycosyltransferase 74G1 which comprises the amino acid sequence as set forth in SEQ ID NO: 13 and as listed below or a variant or fragment thereof.

SEQ ID NO: 13

MAEQQKIKKSPHVLLIPFPLQGHINPFIQFGKRLISKGVKTTLVTTIHTLNSTLNHSNTTSSIEIOA ISDGCEDEGGFSAGSYLETFIQVQVGSKLALDLKIKLQSEGTTIAVDSTETWVLVDAIEFGID GGSFSTQCQVVSQHKLISLPGETSVGVPGFLQVREWTLQNLQHEQISPSQMLMGQQFANIDQARWFTNSFYYKEELIEVHRTKINWKLVIQPPLPSMYLJKRLDDDNSNGFNYKANHHECMNWLDKPKESVYLYAVFSGVHKHPFQVEETIALUDSVNFLVWIKHKEEKNPLSEIKVTGKQLAWCQVDLVAQHSVECFVHTCQFNSLNEAGLQVPAQFDQOQTTQNNKDEILGTVGVRRVADENGIVRRGNLASCIIIIMEMEERGVIIKNAVWKKDLAKAVVHEGGSSNDI VEFVSEIKA

In certain embodiments, the nucleic acid encodes Stevia rebaudiana UDP-glycosyltransferase 74G1 and comprises a nucleotide sequence as set forth in SEQ ID NO: 14 and as listed below or a variant or fragment thereof.
In certain embodiments, the nucleic acid molecule encodes *Stevia rebaudiana* UDP-glycosyltransferase 74G1 and comprises the sequence as set forth in GenBank Accession number AY345982 or a variant or fragment thereof.

In other embodiments, the invention provides for nucleic acids comprising nucleotide sequences encoding a cyclodextrin glucanotransferase (WO1 996033267; US6271010).

Also provided are nucleic acids comprising nucleotide sequences that encode a sucrose synthase. Accordingly, in certain embodiments, the nucleic acid comprises a sequence encoding sucrose synthase which comprises the amino acid sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25 and listed below or a variant or fragment thereof.

SEQ ID NO:15 {*Stevia rebaudiana* SUS1 isoform)
MAERVLTRVHSLRERLDSLTHNilRHEILFSHGSGLKHPQVMTEFEAICKEDQSKLSDG AFYEVLKCTQEAIVQPVWALIRLPVGYWEYRVNNVLVEELVSPEYLHKFKEELVNGTSN GNFVLJELDFPFTASFRPRLTSGNSIGVEFNLRLSLAKMFHDKSMHPVLLDRTLHYYKGTKM MLNRDQNLNSLQSRKLASEYLSLDTAATPSEFEHKFQIEGLERGWGDAEVMEMHML DLEEPADACTLEKFLGRIQMVNFVLISPHGFAENVLGYPDTPGQVYILDQVPALEMLK RIEQGQDLIPRILVTRLAVGVTCLQGRLVFKAGHSLILRVPNTRFEGKILRWWWRFEDVW YIETFTEVDVKEVTAELQKAPDILIGNYSEGLNLASSLLAHKLGVTOCTIAHAALEKTYPSDIYWK NFEEXKFQFTADLAMNNHTDFIISTTFQEIAGSKDTVGQYESHTAMFGLYRWGHIVGDFD PKFNIVSPGADMGIYYSYTEKEKRALTHELPEIDELLFSEVNEELHVCLDKSKPILTFMLADRDNV KNLTGLWEYAKNDRLRELVNLVGDSRKESSLDEEQAMQKMHELIETKLYNQFRWIS SQMNRVRRNGELYRVIAADTRGAIFQPAFYAEFLGTVEAMTCGLPTFATLHGGPAAEIIVGHKSGF HIDPYHGQTVELLVNFEEKTKQDPGHWEAISKGLQRIRIEKYTWQIYSRDLTLAVGYSFGWK HVSKLRDLREIRRLMYFALKYREALESVPLAVEDE

[00180]  SEQ ID NO:1 7 {Stevia rebaudiana SUS2 isoform)

MATSKLSRTHSRVEEETLSAHRNEILSSRLYVAQGKAQILQPHQILHELIENIIDGVTSRQKLT DGPGFDALKTAQCEELVPFFALAVPRPPRGYWYEVYRDAYQLSVEQTVSYELFTKEELVES NSSSLMLDEFPLPFSQFRPSSTNGSNGILNRLSSMRKSDPCGFLFDLRTHRHNGH VMMLNDRTSMTRQLQSLKVAEYLSLPSDTYSEFQYELQGMGFERGWWNAAERIEEMMLHL LSDLQAPDSLEFLRIPMVNFVQANVLPGLDTQVYLYILQVRALENEMKL LKHQGQLDKPRILVTRLPDAGKTCNQRLLERGSTEHTHILRVPFRTEKGLRRWWWRFEDVWPF LEKFTQDAOASEIASELHGTPLDIGNYSQNVLANLALSYMKMGTVQCIHAELEKTYPSDIYWK KFDEKHYFQCTADLAMNNHTDFIISTTFQEIAGSKDTVGQYESHTAMFGLYRWGHIVGDFD PKFNIVSPGADMISYFYTEKEKRALTHELPEIDELLFSEVNEELHVCLDKSKPILTFMLADRDNV NITGLWEYAYKDLRELVNLVGDSRKESSLDEEQAMQKMHELIETKLYNQFRWIS SQMNRVRRNGELYRVIAADTRGAIFQPAFYAEFLGTVEAMTCGLPTFATLHGGPAAEIIVGHKSGF HIDPYHGQTVELLVNFEEKTKQDPGHWEAISKGLQRIRIEKYTWQIYSRDLTLAVGYSFGWK HVSKLRDLREIRRLMYFALKYREALESVPLAVEDE

[00181]  SEQ ID NO:1 9 {Stevia rebaudiana SUS4 isoform)

MATPKLTRTPSMRERLEETLSAHRNDIVSRLVQDIQGAILQPHHLEIDEINFIGQDQCNQQKL DAALSFLGEILSKAEGIQPPIPYYTVLAVPRPGWFDLRVNLVDELSEQLTVEYELSFKEELVDGSQ NPFVELEDPFNATTPRSMRSSLQSGIGQVNLNRHLSSMRKSDPCGFLFDLRHAKHGKYA MMLNRDQNMSSLRLESLAKELSKLPETPSEFCYELQGFMGFWGDCERLGMLMHML LSDLQAPDSLEFLKLGKMPMFNQVNLISHYGFGQANVLPDLTQGQVYLYILDQVLSENEMKL LKLHQQGLDKIPLVTRLPNAGKTCQRLKSGTEETYLRFVTREFKIGLKWLSRFWDI PWYLEAFFTDASEIAAEILHGVPDLLIGNYSDGLNALSLLNLGVTQCNIAHALEKTYPSDLY WKKFEDKYFQCSQTADLAMNAFFDIYSTFQEIAGKTVQVENHSSFPLGTYLRVGHIDG VFDFPKFNIVQGADMIAFDSKQRLELYSGLYSEYTVLQGHEIKEEEDSNSPSNIKMLHML DHLKVNTIGFEVCYANKNKRELHANLXGAYNDKSSREEIAEIKMHNLIQKLYLDGQMW IRAQTNRNARRQEFYRHYADGRGQVFQPAFYAEFLGTVEAMTCGLPTFATCGGPEIELGDSV GHVIDPYHPDMSTTDLADFFQKCKEPESSYWYGKISDGGLKRISERYWTKIYSRMLTLAGYSFHKY VsKVLERETRRELYMFLKFRDLKSVVSVPVATDEAA

[00182]  SEQ ID NO:21 {Stevia rebaudiana SUS4 isoform)
MASASSSIMKRSESIVDTMPEALKQSRYHKMCCFLKYVEKGIRMKRHHLIQQEMETAIKDKEK AQULLDGLLLGYLCTTQEAAVPVCVAFAIRNPQGFWEFVKVNSDLSVDRAMTADYLKFKEMID ETWAKDENALEIDFGLMDNPLNMLSCSINGVNFSTKFTCITKLYQAQQSCQQLLVYDDLSSLN HQENLMINDALNSVLSLRAASLSSPLNDTPYQSFELRFKEFKWEKGWGDNAERARET IRFLLELQAPDINPALEALFSRIPNIFVNLFIYHGFQVNSNLPLTDGQVYVYLDQVWAMEEE LLMRLIKQGGQNFQPLIVTQTRLPDAGTKCNQVEPLVNTKSHILRVPFTRDGKLVRKWSSR FDITYPIYFENFTQDASAKIEMMEKPDLPIGNYTDGKVSLMSLKVGTTLGTIAHAEKLYEDS DMNWKQYDHCFQFTADMAMISSADFTFSTOEIAGSDKDRPQYESHFATLPGLYRVR SGINVFDPKFNASPDADQTGVFPYETKTRKTAFAQPIEELFSKVENEHYLEDKTPKIF SMLARLTVKNTLGEWFKERNRLSLRVNLNLVAGFDPKSKDREAMEEIKHMLHIKYQLKG QIRWIAAQTDKMNRELSRELIADSGKAFQVQALYAFGLTVIAMNCGLPTFATNQGGPAAIEIVD GVSGFQIDPFDGQSSNQLVGLPDTGGQVYVYLDQVWAMEE EEEELRIKQGGLQPSLEVTIRQVTRLPLDAKGETQSQVLEPLVTKSHILRVPFRTEKGLRWV SRFDIYPLEKFTQDASAKIEMMEKPDLQIGNYTDGKVSLMSLKVGTTLGTIAHAEKLYEDS DSDMKWKHLQDKYHSCQFTADMAMISSADDFTOFEIAGSDKDRPQYESHFATLPGLYRVR SGINVFDPKFNASPDADQTGVFPYETKTRKTAFAQPIEELFSKVENEHYLEDKTPKIF SMLARLTVKNTLGEWFKERNRLSLRVNLNLVAGFDPKSKDREAMEEIKHMLHIKYQLKG QIRWIAAQTDKMNRELSRELIADSGKAFQVQALYAFGLTVIAMNCGLPTFATNQGGPAAIEIVD GVSGFQIDPFDGQSSNQLVGLPDTGGQVYVYLDQVWAMEE EEEELRIKQGGLQPSLEVTIRQVTRLPLDAKGETQSQVLEPLVTKSHILRVPFRTEKGLRWV SRFDIYPLEKFTQDASAKIEMMEKPDLQIGNYTDGKVSLMSLKVGTTLGTIAHAEKLYEDS DSDMKWKHLQDKYHSCQFTADMAMISSADDFTOFEIAGSDKDRPQYESHFATLPGLYRVR SGINVFDPKFNASPDADQTGVFPYETKTRKTAFAQPIEELFSKVENEHYLEDKTPKIF SMLARLTVKNTLGEWFKERNRLSLRVNLNLVAGFDPKSKDREAMEEIKHMLHIKYQLKG QIRWIAAQTDKMNRELSRELIADSGKAFQVQALYAFGLTVIAMNCGLPTFATNQGGPAAIEIVD GVSGFQIDPFDGQSSNQLVGLPDTGGQVYVYLDQVWAMEE EE
In certain embodiments, the nucleic acid molecule encodes sucrose synthase and comprises a nucleotide sequence as set forth in SEQ ID NO: 16, 18, 20, 22, 24 or 26 and listed below or a fragment or variant thereof.

SEQ ID NO:16 (encodes SUS1 isoform)

ATGGCGGAACGTGACTACACTGACTGTTTCCACAGCTGACGGCCTGGATATCCACACTTCTCG
CAACTCATGCGTTATGAATTCAAGCTGACGACGGCTCCTTCTTCTTCTTCTATGCAGATTGAAAGCTGAGGC
TTGAAAGCCTCATCAAGTTATAGCTGAATTTGAGCTATCTGCAAAGAGATGAGAGATCTGACAGCCTC
TCTCTGATGGTGCTTTTTATGAAGTTCTTAAATGCACACAGGAAGCAATAGTGCAACCTCCAAGGTTGG
TCACTCGCACTCACTGCTGTGTTTGAGAGCTATTTTGACTTTCTGACAATGACTGCTGCTGCTGCTGCT
ATGCGGCACTCGAGTCACCAGTCACTCGGCTGCTGGTCGTCGGTGGTGAC

SEQ ID NO:18 (encodes SUS2 isoform)

ATGGCGGAACGTGACTACACTGACTGTTTCCACAGCTGACGGCCTGGATATCCACACTTCTCG
CAACTCATGCGTTATGAATTCAAGCTGACGACGGCTCCTTCTTCTTCTTCTATGCAGATTGAAAGCTGAGGC
TTGAAAGCCTCATCAAGTTATAGCTGAATTTGAGCTATCTGCAAAGAGATGAGAGATCTGACAGCCTC
TCTCTGATGGTGCTTTTTATGAAGTTCTTAAATGCACACAGGAAGCAATAGTGCAACCTCCAAGGTTGG
TCACTCGCACTCACTGCTGTGTTTGAGAGCTATTTTGACTTTCTGACAATGACTGCTGCTGCTGCTGCT
ATGCGGCACTCGAGTCACCAGTCACTCGGCTGCTGGTCGTCGGTGGTGAC
ATGGCGACAAGTAAGTTGAGCAGAACGCATAGTATGCGTGAGCGTGTTGAAGAAACTCTTCTCCGCTCATCGCAACGAAATCGTTTCTCTTCTTTCTAGGTATGTGGCTCAGGGGAAGGCGATATTGCAGCCGCATCAGATACTCCATGAACTTGAGAATATCATCGGTGATGTTACTTCGCGCCAAAAGCTTACAGATGGTCCGTTTGGAGATGCGTTGAAGACAGCACAGGAATGTATAGTTCTACCTCCATTTGTAGCTTTAGCAGTTCGTCCAAGACCTGGTGTTTGGGAATACGTGCGCGTGGATGCATATCAACTAAGTGTGGAACAACTAACTGTTTCAGAGTATCTTACCTTCAAAGAAAGAACTTGTTGGAGAGTCTAATAGTTCTTTAATGCTCGAGTTGGATTTTGAGCCATTTAATGCTTCGTTTCCTAGACCAACCCGTTCTTCATCCATTGGCAATGGAGTTCAGTTCCTGAATCGCACACCTGTCGTCAAGCATGTTTCGCAGCAAAGATTGTTTAGAACCGCTTCTGGATTTCCTACGACACACAGACATAATGGACATGTAATGATGTTAAATGACCGCATAACAAGCATGACTAGACTTCAATCTTCTTTGGTCAAAGCAGAGGAATATCTTTCTAAACTACCATCTGATACAGACTACTCAGATCTTTCAATATGAAGTTGGAATGGGTTTTGAAAGAGGATGGGGAAACAATGCTGAAAGAATCATTGAGATGATGCATCTTCTCTCAGACATTCTACAAGCTCCAGATCCTTCCAATTTGGAATCTTTTCTTGCTAGAATACCTATGGTGTTTAATGTTGTTATATTATCAATACATGGCTACTTTGGGCAAGCAAATGTTTTGGGTTTGCCAGATACTGGTGGCCAGATTGTATATATATTGGATCAAGTCCGTGCATTGGAAAATGAGATGCTTCTTAAATTAAAGCACCAAGGACTATCAACAGACTCGGTTAATACCTGATGCAAAAGGTACTTCATGTAACCAACGACTGGAAAGAGTCAGTGGAACTGAACACACACATATACTTCGTGTTCCTTTATGAACCGAGAAAGGAATTCTTCGTAAATGGATCTCAAGGTTTGATGTATGGCCTTTTTTGAGAAATTTACACAGGATGCAGCAAGTGAAATTTCTGCTGAGTTGCATGGTACTCCAGATCATTATAATTGGAAATTATAGTGATGGCAATCTTGTTGCCTCTTTATTATCTTACAAAATGGGA
TAACCCAGTGAAACATTGCTCATGTACTTAGAAGAAGAACGATATCGCAGATTCTGCTTTTTATTTTATGGAAGAAATTGATGAGAAATATCACTTTTCTTGTCAATTTACTGCTGATCTTTTAGGATGAAACATTCGAGATGAAATGATGGAGATGTTTTATATTCTTAAGTTCCGTGATCTGGTAAAATCTGTTCCAGTGGCTACTGATGATGAGGCTTAG

[00188] SEQ ID NO:20 (encodes SUS3 isoform)
AACCGTCACTCTCGTCAATTATGTTGGCAGATCGATCCGTTTCTTGATTT
CCTTCGTGCTCATAAACATAAAGGATACGCGATGATGTTGAATGATCGGATACAAACAATG
TCTAGATGGTTACTTTGGTCAGGCTAATGTTTTGGGTTTGCCGGATACCGGTGGTCAGGTT
GATATATTGGTACACTCAGTCTCTTGGGAGAATGGAAATGTTACTTGAAGTATAGCTGT
CCCTTTAGGACAGAGAAAGCGGATTTGATAAGTTCTAAGGGTATATGATGCGCT
TTTTGGAGAATGAAATGTTACTTAAATTAAGGCATCA
AGGACTTGATATCAAACCCAAGATTCTAATTGTAAAACATTGAAAGACAATCCCAAGGAAT
GGTATTATGACTTACATCTTACGGTGATGGGATACTGCCACAGATTACTTGAAGTT
CAAGGAAATGATCGTAGATGAGACATGGGCTAAAGATGAAAATGCATTGGAGATTGACTTT
GGATCGATGGACTTTAACCTACCAAACATGAGTTTATCTTGTTCGATTGGAAATGGTGTTAA
CTTCACATCATCATCTTGATAAGATCGACTAGCTTGATGAGGATCAAGTGGTAGCTATGGAAGAAGAACTACTCATGAGGATC

[00189]  SEQ ID NO:22 (encodes SUS4 isoform)

ATGGCATCTGCTTCAAGTTCTATCATGAAACGGTCTGAATCAATAGTTGACACCATGCCAG
AAGCCTTAAAGCAGAGCCGCTATCATATGAAAAAATGTTTTCTAAAATATGTAGAAAAAGGA
ATTCGCATGATGAAAAGACATCATTTGATACAAGAAATGGAGACCGCAATTGAAGACAAGG
GCTCAGCTTCTAGATGGCTTACTTGGCTACATCTTGTGCACAACTCAGGAAGC
AGCCGTTGTTCCTCCTCTTGGGCTATATGAAAGACCATATTGGGAGGACTTGGTGAGTT
GTAAAGTCATACCTAATGTACATCTCGGTATGAGGATAACTGACACAGATTACCTGAGTT
CAAGGAAATGATCGACTAGCTTACATCTTGATAAGATCGACTAGCTTGATGAGGATCAAGTGGTAGCTATGGAAGAAGAACTACTCATGAGGATC

CTTGATGAGGATCAAGTGGTAGCTATGGAAGAAGAACTACTCATGAGGATC
AAACAACAAGGACTCAACTTCAAGCCTCAAATTCTTGTGGTGACCCGACTTCTTCCTGATGCTAAAGGGACCAAGTGTAATCAGGTGTTGGAACCAGTTCTGAACACGAAACATTCGCATAT...
TACGAAGATTCCAGACATGAAATGGAAACATTTGGACACAAAATATCACTTTTCTTGTCAATT
TACAGCTGATATGATAGCAATGAATTCAGCAGATTTCATCATCACTAGTACTTTCCAA ... TACACCGAGAAGGAGAAACGGTTAACGGATTTTCATCCCGCAATTAAAGAACTACTTTTC
CAACGAACAAGACAATGACGAGCATATGGGATACCTCGCGGATGTAACCAAACCGATAATA

[00191] (encodes SUS6 isoform)

ATGGATTTCGGTGATACGAGAGACCTTGGGACAGGCTTACCACTTGGTTATACATGGGAGAAGCTTATGATCAATGAGACAATAGATACAGTTTCAAAGCTCCAGAAAGCAT
TAATTGCTGTATAGCAGCTGTATACCTTCTGCAACCCGCAAAGACGACAATATCAACCCCTAGAG
CCCAAGCTTAAAAGAATGGGGATTTGAGAAAGGATGGGGAGATACTGCTGAAAGAGTTAGAGACAATGAAAATGCTTTCGGAGATTCTTCAAGCACCCGACCCGATTAACATGCAATCGT
CTTTACAGTGGTCTTCTGATACCTGATACCCGAGAAGGAGAAACGGTTAACGGATTTTCATCCCGCAATTAAAGAACTACTTTTC
CAACGAACAAGACAATGACGAGCATATGGGATACCTCGCGGATGTAACCAAACCGATAATA

CATCGAAGAGATTTGAGCGAAAAAGAATGGGAAACATTTGGACACAAAATATCACTTTTCTTGTCAATT
TACAGCTGATATGATAGCAATGAATTCAGCAGATTTCATCATCACTAGTACTTTCCAA ... TACACCGAGAAGGAGAAACGGTTAACGGATTTTCATCCCGCAATTAAAGAACTACTTTTC
CAACGAACAAGACAATGACGAGCATATGGGATACCTCGCGGATGTAACCAAACCGATAATA
TTCTCAATGGCGAGGCTCGATACGGTGAAGAACATAACAGGGTTAACCGAGTGGTTCGGTAAAGAACAAACGACTTAGAAGTCTTGTAAACTTGGTTGTTGTCGCGGGGTTCTTCGATCCATTTCTCAATGGCGAGGCTCGATACGGTGAAGAACATAACAGGGTTAACCGAGTGGTTCGGTAAAGAACAAACGACTTAGAAGTCTTGTAAACTTGGTTGTTGTCGCGGGGTTCTTCGATCCA... 8, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 and 34 or the complement thereof under conditions of low, moderate

[00192] In other embodiments, there is provided a nucleic acid comprising a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of the sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 and 34 and fragments thereof or the complement thereof.

[00193] In other embodiments, there is provided a nucleic acid encoding a polypeptide comprising a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% percent identity to any one of the sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 13, 15, 17, 19, 21, 23, 25, 27, 29, 30, 31 and 33 and fragments thereof. A worker skilled in the art would readily appreciate that overall sequence identity or similarity may be less than 50% but regions of the enzyme (such as the catalytic site or areas adjacent to the catalytic site) may have conserved amino acids. For example, there are conserved amino acids at the opening adjacent to the UDPG catalytic site. In particular, a leucine at position 379 of UGT76G1 is conserved. In certain embodiments, the nucleic acid encodes an UDP-glucosyltransferase having the sequence SDFGLDQ at a position corresponding to amino acid residues 375 to 381 of the UGT76G1 set forth in SEQ ID NO:1.

[00194] In certain embodiments, fragments are at least 10, at least 20, at least 50 nucleotides in length. The fragments may be used, for example, as primers or probes.

[00195] Also provided are nucleic acids that hybridize to the nucleic acids of the present invention or the complement thereof. In certain embodiments, there is provided a nucleic acid that hybridizes to any of the sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 and 34 or the complement thereof under conditions of low, moderate
or high stringency. A worker skilled in the art readily appreciates that hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. Such a worker could readily determine appropriate stringent (see, for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) pp. 9.50-51, 11.48-49 and 11.2-1 1.3).

Typically under high stringency conditions only highly similar sequences will hybridize under these conditions (typically >95% identity). With moderate stringency conditions typically those sequence having greater than 80% identity will hybridize and with low stringency conditions those sequences having greater than 50% identity will hybridize.

A non-limiting example of "high stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5XSSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4·H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1XSSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed. A non-limiting example of "medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5XSSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4·H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0XSSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed. A non-limiting example "Low stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 degree. C. in a solution consisting of 5XSSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4·H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5XSSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

The polynucleotides include the coding sequence polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters (including inducible promoters, tissue-specific promoters (such as root-specific or leaf specific promoters), enhancers, terminators,
and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

[00199] Appropriate additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), non-coding sequences (e.g. regulatory elements such as promoters (including inducible promoters, tissue-specific promoters (such as root-specific or leaf specific promoters), enhancers, terminators, and the like), and vectors for use in prokaryotic such as *E. coli* and eukaryotic cells, including but not limited to yeast and plant cells are known in the art.

Polypeptides

[00200] The present invention provides for glucosyltransferases. The glucosyltransferases of the present invention are capable of primary, secondary and/or tertiary glycosylations. In certain embodiments, the glucosyltransferases are capable of primary, secondary and tertiary glycosylations. In other embodiments, the glucosyltransferases are capable of secondary and/or tertiary glycosylations. In certain embodiments, the glucosyltransferases is a glucosyltransferase, including but not limited to a UDP-glucotransferase. The glucosyltransferases include but are not limited to a *Stevia rebaudiana* UDP-glucosyltransferase, such as UGT76G1 or UGT74G1 or an *Oryza sativa* glucosyltrasferase, such as Os03g0702000. In other embodiments, the invention provides for a cyclodextrin glucanotransferase. Also provided are sucrose synthases.

[00201] In certain embodiments, there is provided an UGT76G1 or UGT76G1-like glucosyltransferase. UGT76G1-like glucosyltransferase include for example, other members of the UGT76G1 clade such as UGT76G2 or UGT76H1. Accordingly, in certain embodiments, there is provided an UGT76G1 comprising the amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 5 and 7 or fragments and variants thereof. In certain embodiments, there is provided an UGT76G1 encoded by the nucleic acid molecule comprising the sequence as set forth in any one of SEQ ID NOs: 2, 4, 6 and 8.

[00202] In certain embodiments, there is provided an UGT76G2 comprising the amino acid sequence as set forth in SEQ ID NO: 27 or fragments and variants thereof. In certain embodiments, there is provided an UGT76G1 encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 28.
In certain embodiments, there is provided a UGT76H1 comprising the amino acid sequence as set forth in SEQ ID NO: 29 or fragments and variants thereof. In certain embodiments, there is provided an UGT76G1 encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 30.

In certain embodiments, there is provided an Os03g0702000 or Os03g0702000-like glucosyltransferase. Os03g0702000-like glucosyltransferase include for example, other members of the UGT91 clade such as UGT91D1 or UGT91D2. Accordingly, in certain embodiments, there is provided an Os03g0702000 comprising an amino acid sequence as set forth in SEQ ID NO: 9 or fragments and variants thereof. In certain embodiments, there is provided an Os03g0702000 encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 10.

In certain embodiments, there is provided an UGT91D1 comprising the amino acid sequence as set forth in SEQ ID NO: 31 or fragments and variants thereof. In certain embodiments, there is provided an UGT91D1 encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 32.

In certain embodiments, there is provided an UGT91D2 comprising the amino acid sequence as set forth in SEQ ID NO: 33 or fragments and variants thereof. In certain embodiments, there is provided an UGT76G1 encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 34.

In certain embodiments, there is provided a Stevia rebaudiana UGT74G1. Accordingly, in certain embodiments, the UGT74G1 comprises the amino acid sequence as set forth in SEQ ID NO: 13 or fragments and variants thereof. In certain embodiments, the UGT74G1 is encoded by the nucleic acid molecule comprising the sequence as set forth in SEQ ID NO: 14.

In other embodiments, the invention provides for a cyclodextrin glucanotransferase. Cyclodextrin-glucanotransferase is commercially available (CGTase, Toruzyme 3.0L, trademark of Novozymes Inc.).

In certain embodiments, there is provided sucrose synthase. Accordingly, in certain embodiments, the sucrose synthase comprises the amino acid sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25 or fragments and variants thereof. In certain embodiments, the polypeptide comprises an amino acid sequence encoded by the nucleic acid
molecule comprises comprising the sequence as set forth in SEQ ID NO: 16,18, 20, 22, 24 or 26.

[00210] In other embodiments, there is provided a polypeptide comprising a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% percent identity to any one of the sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31 and 33 and fragments thereof. A worker skilled in the art would readily appreciate that overall sequence identity or similarity of related enzymes may be less than 50% but regions of the enzyme (such as the catalytic site or areas adjacent to the catalytic site) may have conserved amino acids and therefore the related enzymes have similar activity. For example, there are conserved amino acids at the opening adjacent to the UDPG catalytic site. In particular, a leucine at position 379 of UGT76G1 is conserved. In certain embodiments, the nucleic acid encodes an UDP-glucosyltransferase having the sequence SDFGLDQ at a position in certain embodiments, fragments are at least 10, at least 20, at least 50 amino acids in length. In certain embodiments, the polypeptide sequences contain heterologous sequences including but not limited to purification tags such as a HIS tag. In certain embodiments, there is provided a polypeptide comprising a 6X HIS tag at the N-terminus. In other embodiments, there is provided a polypeptide comprising a 6X HIS tag at the C-terminus.


Cells and Plants

[00212] The present invention further provides cells and plants which express one or more of the polypeptides of the present invention. The cells and plants may naturally express one or more of the polypeptides of the present invention or have been modified to express one or more the polypeptides of the present invention. The cells may be prokaryotic or eukaryotic
cells and include but are not limited to, *E. coli*, yeast such as *Pichia pastoris*, *Stevia rebaudiana*, *Phytolacca Americana*, *Cannabis* including but not limited to *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*.

[00213] In certain embodiments, there is provided a cell which expresses an UGT76G1 or UGT76G1-like glucosyltransferase (such as UGT76G2 and UGT76H1). Accordingly, in certain embodiments, there is provided a cell which expresses an UGT76G1 glucosyltransferase comprising a sequence encoding the amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 5 and 7. In certain embodiments, there is provided a cell which expresses an UGT76G1-like glucosyltransferase comprising a sequence encoding the amino acid sequence as set forth in SEQ ID NO: 27 or 29. The cell may further express further glucosyltransferases, such as Os03g0702000 or Os03g0702000-like glucosyltransferase (such as UGT91 D1 and UGT91 D2) and/or a sucrose synthase, such as the sucrose synthase comprising the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

[00214] Accordingly, in certain embodiments, there is provided a cell which expresses UGT76G1 glucosyltransferase comprising a sequence encoding the amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 5 and 7 and Os03g0702000 glucosyltransferase comprising the sequence as set forth in SEQ ID NO: 10. The cell may further express a sucrose synthase comprising the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

[00215] In certain embodiments, there is provided a cell which expresses an Os03g0702000 or Os03g0702000-like glucosyltransferase. Accordingly, in certain embodiments, there is provided a cell which expresses Os03g0702000 glucosyltransferase comprising a sequence encoding the amino acid sequence as set forth in SEQ ID NO: 10. The cell may further express a sucrose synthase, such as the sucrose synthase comprising the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

[00216] Transgenic cells and plants (including plant cells, or plant explants, or plant tissues) can be produced by a variety of well established techniques. Following construction of a vector, most typically an expression cassette, including a polynucleotide of the invention, standard techniques can be used to introduce the polynucleotide into cell or a plant. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

[00217] In a certain embodiments, there is provided *Cannabis* plants genetically engineered to express one or more of the proteins of the invention. A worker skilled in the art
would readily appreciate appropriate vectors and promoters for genetically engineering *Cannabis* plants. For example, a tissue specific promoter, such as a secretory trichomes specific promoter may be used such that the proteins of the invention are expressed in the same tissue that cannabinoids are produced in, namely the secretory trichomes of the plant. Suitable promoter elements include the promoter for the cytosolic 0-acetylserine(thiol)lyase (OASA1) enzyme from *Arabidopsis thaliana* (Gutierrez-Alcala 2005).

[00218] Transformation and regeneration of plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence into a plant in a manner to cause stable or transient expression of the sequence.

[00219] Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Pat. Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

[00220] Following transformation, plants may be selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

**Methods**

[00221] The present invention further provides methods for the production of cannabinoid glycoside prodrugs and the cannabinoid glycosides prodrugs produced by the methods. The methods may be *in vitro* or *in vivo* (in a cell system or *in planta*). In certain embodiments, there is provided a method of producing cannabinoid glycoside prodrugs, said method comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.
The aglycones include but are not limited to: cannabinoids, including but not limited to cannabidiol, cannabidivarin, cannabigerol, tetrahydrocannabinol, cannabinoil and cannabidiolic acid, endocannabinoids including but not limited to arachidonoylthanolamide (anandamide, AEA), 2-arachidonoylthanolamide (2-AG), 1-arachidonoylthanolamide (1-AG), and docosahexaenoil ethanolamide (DHEA, synaptamide); and vanilloids including but not limited to vanillin, curcumin, and capsaicin.

A worker skilled in the art would readily appreciate that the one or more sugar donors will be dependent on the one or more glycosyltransferases used in the method and/or the desired end products. For example, for UDP-glycosyltransferases, the sugar donors include but are not limited to UDP-glucose, UDP-glucuronic acid, UDP-mannose, UDP-fructose, UDP-xylose, UDP-fluorodeoxyglucose, and UDP-rhamnose. For cyclodextrin glucanotransferase, the sugar donor includes maltodextrin.

In certain embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating an aglycone with a sugar donor in the presence of a glycosyltransferase. Also provided are the cannabinoid glycosides produced by the above method. In specific embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating an aglycone with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation. In other specific embodiments, there is provided a method of producing a glycoside prodrug, said method comprising incubating an aglycone with maltodextrin, in the presence of a cyclodextrin glucanotransferase under conditions that allow for glycosylation.

An exemplary method for producing cannabinoid-glycosides comprises incubating a cannabinoid, with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions which allow for glycosylation. Also provided are cannabinoid-glycosides produced by the above method.

A further exemplary method for producing cannabinoid-glycosides comprises incubating a cannabinoid with maltodextrin in the presence of a cyclodextrin glucanotransferase under conditions which allow for glycosylation. Also provided are cannabinoid-glycosides produced by the above method.

In certain embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating an aglycone with one or more sugar donors in the
presence of a first glycosyltransferase and a second glycosyltransferase under conditions which allow for glycosylation. Also provided are cannabinoid glycosides produced by the above method.

[00228] A worker skilled in the art would readily appreciate that the first glycosyltransferase and a second glycosyltransferase may be provided concurrently or added sequentially. In addition, if more than one sugar donor is used, the sugar donors may be provided concurrently or added sequentially. Such a worker would further appreciate that the structure of the resulting cannabinoid glycoside may be dependent on the order the glycosyltransferases are provided. In addition, the ratio of first to second glycosyltransferase may impact the resulting products. A worker skilled in the art would further appreciate that the activity levels of the glycosyltransferases may dictate the ratios and the ratios could be readily determined by a worker skilled in the art. For example, the ratios first to second glycosyltransferase include but are not limited to 1:1, 1:2, 1:10, 1:50 and vice versa.

[00229] In specific embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating an aglycone with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and Os03g0702000 or Os03g0702000-like glucosyltransferase under conditions which allow for glycosylation. In alternative specific embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating an aglycone with UDP-glucose and maltodextrin in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and cyclodextrin glucanotransferase under conditions which allow for glycosylation. Also provided are cannabinoid glycosides produced by the above methods.

[00230] An exemplary method for producing cannabinoid-glycosides comprises incubating cannabinoid, including but not limited to cannabidiol, cannabidivarin, canabigerol, tetrahydrocannabinol, cannabinol and cannabidiolic acid, with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and Os03g0702000 or Os03g0702000-like glucosyltransferase under conditions which allow for glycosylation. Also provided are cannabinoid-glycosides produced by the above method.

[00231] A further exemplary method for producing cannabinoid-glycosides comprises incubating cannabinoids with UDP-glucose and maltodextrin in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and and cyclodextrin glucanotransferase under conditions
which allow for glycosylation. Also provided are cannabinoid-glycosides produced by the above method.

[00232] It is within the scope of the present invention that each of the above described glycosylation methods may be applied to a lower order cannabinoid glycoside to form a higher order cannabinoid glycoside. For example, a cannabinoid monoglycoside may be glycosylated using any of the glycosylation methods of the present invention to form a diglycoside, or a cannabinoid diglycoside may be glycosylated to form a triglycoside, etc.

[00233] Methods of purifying the cannabinoid glycosides are known in the art and include for example solid phase extraction, such as column purification.

[00234] The invention also provides cell culture and in planta methods for the production of cannabinoid glycosides. The methods comprise expressing one or more of the glycosyltransferases in a cell or plant which produces the aglycone and isolating the cannabinoid glycosides. In certain embodiments, one or more sucrose synthases are also expressed. Appropriate vectors and genetic engineering methods are known in the art.

[00235] The invention also provides methods for the conversion of UDP to UDPG utilizing the sucrose synthases of the present invention. Accordingly, in certain embodiments of the methods of producing cannabinoid glycosides which utilize UDP-glucose as a sugar donor, the methods further comprise the use of sucrose synthase to recycle UDP. In certain embodiments, there is provided a method of producing a cannabinoid glycoside, said method comprising incubating aglycone with UDP-glucose, in the presence of a UGT76G1 glucosyltransferase and a sucrose synthase under conditions that allow for glycosylation.

[00236] The invention will now be described with reference to specific examples. It will be understood that the following examples are intended to describe embodiments of the invention and are not intended to limit the invention in any way.

**EXAMPLES**

**Example 1: Conversion of cannabinoids to cannabinoid glycoside prodrugs**

[00237] Glycosylation reactions consisted of 50mM KP0₄ pH 7.2, 3mM MgCl₂, 0.005% CBD, 2.5% UGT76G1 purified enzyme preparation, and 2.5mM UDP-glucose. Buffers were
degassed and tubes were purged with nitrogen, reactions were protected from light and incubated at 28°C with 180rpm agitation for 18 hours. Reactions were then extracted 3x with an equal volume of ethyl acetate, evaporated to dryness, and dissolved in a half volume of HPLC grade methanol. 50 microliters was injected on a reverse phase C18 column and eluted with a gradient of acetonitrile starting at 10% and increasing to 99%. UGT76G1 was produced through expression in *Pichia pastoris* and purified through standard molecular biology techniques. The UGT76G1 enzyme was found to glycosylate CBD in a UDP-glucose dependent manner. This activity was also proportional to the amount of UDP-glucose present. Incubation temperature was 28°C, and an acceptable range would be 20°C to 30°C as high temperatures can cause significant degradation of CBD. Reactions were carried out in the dark to prevent photo-degradation of the substrates. Gentle agitation from 120 to 200rpm were used to mix the reactions in an inert atmosphere.

[00238] Substrate CBD in the reactions was replaced with A9THC and CBDV and performed in an identical fashion with similar results. Enzyme combinations needed to create various products are listed in Table 4 for CBD-glycosides, Table 5 for CBDV-glycosides, and Table 6 for A9THC-glycosides.

[00239] Other enzymes screened for activity towards CBD were the *Stevia rebaudiana* UGT74G1, UGT85C2, UGPase, *E.coli* Maltodextrin phosphotransferase (MalP), and *O.sativa* Os03g0702000 (SEQ ID NO. 9). No primary glycosylation activity was seen with any other tested enzyme other than UGT76G1.

**Example 2: 2-0 glycosylation of CBD-monoglucoside**

[00240] Enzymatic reactions are performed as described in Example 1 but with the inclusion of recombinant Os03g0702000 enzyme at a 1:2 ratio relative to UGT76G1. Samples were extracted and analyzed as in Example 1. Recombinant Os03g0702000 enzyme was codon optimized and expressed in *E. coli* BL21-DE3 cells and purified by immobilized metal ion chromatography.

**Example 3: Conversion of CBD to alpha-glycoside linked CBD compounds.**

[00241] Recombinant cyclodextrin glucanotransferase (CGTase, Toruzyme 3.0L trade name, Novozymes Inc.) was added to reactions as indicated in Example 1 but without UDPG or UGT76G1. Maltodextrin was used at 0.05% final concentration, and Toruzyme 3.0L was used at 0.1%. Samples were extracted and analyzed as in example 1. Additionally, reactions from
Example 1 were carried out to convert cannabinoids to cannabinoid-glycosides, and then CGTase and maltodextrin were added and given adequate time to incubate with the cannabinoid-glycosides. The resulting products contain a β-glycosylation on the cannabinoid backbone, and α-glycosylations emanating from the primary sugar. This additional treatment created a new category of compounds termed β-primed, α-glycosylated cannabinoids.

**Example 4: Purification of cannabinoid glycosides**

[00242] Glycoside products were generated through the aforementioned biocatalytic reactions and purified to homogeneity by C18 solid phase extraction. 100mg Hypersep C18 columns (Thermo) were hydrated in methanol, rinsed with 50% methanol in water, rinsed with water, glycosylation reaction passed through the column, washed with water, washed with 10%, 20%, and 30% methanol, and the glycoside products were eluted with 45 and 60% methanol in water. Eluates were dried and extracted with ethyl acetate, and dried to completion to yield >95% pure cannabinoid -glycosides for further analysis and testing.

**Example 5: HPLC analysis of cannabinoid glycoside prodrugs**

The HPLC linetraces of the reaction products of glycosylation reactions of the cannabinoid aglycones CBD, CBDV, A9-THC, CBN, 1-AG and 2-AG, DHEA, AEA, capsaicin, and vanillin, are provided in Figures 16 to 24, respectively. Enzymatic reactions were performed as described in Example 1. The solid lines indicate the elution profile of the starting aglycone and the dashed lines indicate the elution profile of the glycosylation reaction product mixture.

[00243] In Figure 16, the CBD aglycone retention time is 13.65 minutes, and product peaks are observed at 8.87, 9.02, 9.97, 10.33, and 10.37 min.

[00244] In Figure 17, the CBDV aglycone retention time is 12.75 minutes, and product peaks are observed at 8.53, 9.70, and 10.01 min.

[00245] In Figure 18, the THC aglycone retention time is 14.45 minutes, and product peaks are observed at 9.46, 10.67, 10.97, 11.28, 11.67, and 12.49 min.

[00246] In Figure 19, the CBN aglycone retention time is 14.32 minutes, and product peaks are observed at 10.87, 11.50, and 12.25 min.
In Figure 20, the 1-AG aglycone retention time is 14.18 minutes and the 2-AG aglycone retention time is 14.32 minutes, and product peaks are observed at 11.40, 11.78, 11.83, 11.97, 12.53, 12.92, 13.07, and 13.35 min.

In Figure 21, the DHEA aglycone retention time is 13.78 minutes, and product peaks are observed at 10.09 and 12.43 min.

In Figure 22, the AEA aglycone retention time is 13.87 minutes, and product peaks are observed at 12.47 min.

In Figure 23, the vanillin aglycone retention time is 1.95 minutes and product peaks are observed from 1.25 to 1.35 min.

In Figure 24, the capsaicin aglycone retention time is 11.73 minutes, and product peaks are observed at 10.23 min.

**Example 6A: LCMS analysis of CBD glycosides**

As shown in the HPLC linetrace of Figure 16, input CBD aglycone (VB101, 13.65') has been depleted to 5% of original quantity after +65 hours of incubation time. The CBD-glycosides elute off the HPLC column at 8.87, 9.02, 9.97, 10.33, and 10.37 min. The glycosylated products were identified by LCMS analysis. The glycosylated product "g1" is a monoglycoside, "g2" is a diglycoside, "g3" is a triglycoside, and "g4" is a tetraglycoside. LC-LRMS was performed on a Shimadzu LC-MS 2010 EV instrument. The LC column used was a Silia Chrom XDB C18 5um, 150A, 4.6X50 mm. The method was 12 min 5 to 95 H2O:ACN gradient. For LRMS electrospray ionization (ESI) was performed in positive mode.

**VB101 (CBD aglycone) MS data:** LC/ESI-LRMS. \([M + H]^+ (C_{21} H_{31} O_2)\) Calcd: \(m/z = 315\). Found: \(m/z= 315\).

**VB101 (CBDgl) MS data:** LC/ESI-LRMS. \([M + H]^+ (C_{27} H_{41} O_7)\) Calcd: \(m/z = All\). Found: \(m/z = All\).

**VB104 (CBDg2) MS data:** LC/ESI-LRMS. \([M + H]^+ (C_{33} H_{51} O_{12})\) Calcd: \(m/z = 639\). Found: \(m/z = 639\).

**VB110 (CBDg2) MS data:** LC/ESI-LRMS. \([M + H]^+ (C_{33} H_{51} O_{12})\) Calcd: \(m/z = 639\). Found: \(m/z = 639\).
**Example 6B: LCMS analysis of Δ9-THC glycosides**

[00259] In a manner similar to that carried out in Example 6A, the products of the glycosylation reaction of Δ9-THC (shown in the HPLC linetrace of Figure 18) were identified by LCMS analysis.

[00260] **VB301 (THC aglycone) MS data:** LC/ESI-LRMS. [M + H]+ (C_{21}H_{31}O_{2}) Calcd: m/z = 315. Found: m/z = 315. [M + 3ACN + 2H]^{2+} (C_{27}H_{41}N_{3}O_{2}) Calcd: m/z = 314. Found: m/z = 314.

[00261] **VB304 (THCg2) MS data:** LC/ESI-LRMS. [M + H]+ (C_{33}H_{51}O_{12}) Calcd: m/z = 639. Found: m/z = 639.

[00262] **VB308 (THCg3) MS data:** LC/ESI-LRMS. [M + H]+ (C_{39}H_{61}O_{17}) Calcd: m/z = 801. Found: m/z = 801. [M + Na]+ (C_{39}H_{62}O_{17}Na) Calcd: m/z = 823. Found: m/z = 823. [M + K + H]+ (C_{41}H_{63}NO_{17}) Calcd: m/z = 860. Found: m/z = 860.

**Example 7: NMR analysis of cannabinoid glycosides**

[00263] Figure 27 depicts the 1H NMR spectra of isolated VB104 and Figure 28 depicts the 1H NMR spectra of isolated VB110. Each of these products was isolated from the reaction mixture produced by the glycosylation reaction of CBD. The 1H NMR spectra of 10 mg/ml solutions of each compound prepared in CD_{3}OD were obtained on a Bruker Avance II 400 MHz instrument using TopSpin acquisition and processing software.

**Example 8: Solubility Analysis**
C18 retention times were empirically determined on a linear ramp of increasing acetonitrile on a Phenomenex Kinetex 2.6u 100A C18 column, on a Dionex HPLC equipped with Diode Array Detector. CLogP values in Table A were predicted by ChemDraw (CambridgeSoft). Reference cannabinoids were analyzed by HPLC and established logP values (http://pubchem.ncbi.nlm.nih.gov/) and used to create a calibration line as depicted in Figure 29. The predicted cLogP values correlated with the reference calibration line. C18 reverse phase HPLC retention times were plotted against the cLogP values presented in Table A, as depicted in Figure 29. Data point numbering correlates with table numbering. Open diamonds indicate novel cannabinoid glycosides, filled diamonds indicate reference cannabinoids and derivatives. ClogP values were predicted by ChemDraw (CambridgeSoft). Linear regression was performed on all data points (R2 = 0.9455).

**Table A: CLogP values for select cannabinoid glycosides and reference cannabinoids**

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<th>#</th>
<th>Compound</th>
<th>Retention Time</th>
<th>ClogP</th>
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<tbody>
<tr>
<td>1</td>
<td>VB110</td>
<td>8.967</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>11-COOH-Tetrahydrocannabinol Glucuronide</td>
<td>9.347</td>
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<tr>
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<td>VB104</td>
<td>10.720</td>
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<tr>
<td>4</td>
<td>VB304</td>
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</tr>
<tr>
<td>5</td>
<td>VB302</td>
<td>11.688</td>
<td>5.7</td>
</tr>
<tr>
<td>6</td>
<td>11-COOH-Tetrahydrocannabinol</td>
<td>12.910</td>
<td>5.7</td>
</tr>
<tr>
<td>7</td>
<td>Cannabidivarin</td>
<td>13.017</td>
<td>5.6</td>
</tr>
<tr>
<td>8</td>
<td>11-OH-Tetrahydrocannabinol</td>
<td>13.037</td>
<td>5.9</td>
</tr>
<tr>
<td>9</td>
<td>Cannabidiol</td>
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</tr>
<tr>
<td>10</td>
<td>Cannabinol</td>
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<td>Tetrahydrocannabinol</td>
<td>14.487</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Example 9: Bioavailability Assay**

In order to investigate the effectiveness of glycosylation to effect site-specific drug delivery, VB1 10 was administered to three mice by oral gavage and the animals sacrificed at 30, 60, and 90 minutes. Eight week old male Swiss mice were fasted for 12 hours prior to administration of 120mg/kg VB1 10 in 10% Ethanol USP, 10% Propylene Glycol USP, 0.05% Sodium Deoxycholate USP, 79.95% Saline USP. Following termination and tissue harvest, the intestinal contents were then extracted and analyzed by C18 reverse phase HPLC. As shown in Figure 30A, the small intestinal contents showed intact VB1 10, but no decoupled CBD. As shown in Figure 30B, the large intestinal contents contained both VB1 10 and CBD in the 60 and 90 minute time points. This decoupling of VB1 10 is consistent with the large intestinal
decoupling seen for sennoside beta-glycosides, and is the result of secreted beta-glycosidases from the large intestinal microflora.

Example 10: Analysis of Large Intestine Contents Upon Administration of CBD and CBD Glycosides

[00266] In order to investigate the metabolism and decoupling of CBD-glycosides in the large intestine, an aqueous solution of a mixture of CBD-glycosides was administered to a mouse by oral gavage. As a control, a solution of CBD in cremophor, ethanol, and saline was administered to a second mouse. The animals were each sacrificed at 2 hours. Following termination and tissue harvest, the intestinal contents were then extracted and analyzed by C18 reverse phase HPLC. The mice employed in this example were eight week old male Swiss mice fasted for 12 hours prior to administration of the solutions.

[00267] The resulting extracts were analyzed by LCMS performed using a Shimadzu LC-MS 2010 EV. LC separation was carried out using a Silia Chrom XDB C18 5um, 150A, 4.6X50 mm. The method was 12 min, 5 to 95 H2O:ACN gradient elution. Low resolution MS was performed in negative mode via electrospray ionization (ESI). Acetic acid and formic acid were used as sample additives during analysis, and the injection volume was 20 µl.

[00268] Analysis of the large intestinal contents of animals administered a mixture of oral CBD-glycosides indicated that both aglycone and glycosides were present, along with hydroxy metabolites of each:

[CBD - H], [2CBD - H] and [CBD*20H + Formic acid - H] MS data: LC/ESI-LRMS. [M - H]- (C21H29O2) Calcd: m/z = 313. Found: m/z = 313. [2M - H]- (C42H59O4) Calcd: m/z = 627. Found: m/z = 627. [IVhoH + Formic acid - H]- (C22H31O6) Calcd: m/z = 391. Found: m/z = 391.

[CBDgl - H], [CBDgl + Cl] and [2CBDgl - H] MS data: LC/ESI-LRMS. [Mg1 - H]- (C27H39O7) Calcd: m/z = 475. Found: m/z = 475. [Mg1 + Cl]- (C27H40O7Cl) Calcd: m/z = 511. Found: m/z = 511. [2Mg1 - H]- (C54H79O14) Calcd: m/z= 951. Found: m/z = 951.

[CBDg2 - H] and [CBDg2 + Acetic acid - H] MS data: LC/ESI-LRMS. [Mg2 - H]- (C33H49O12) Calcd: m/z = 637. Found: m/z = 637. [Mg2 + Acetic acid - H]- (C35H53O14) Calcd: m/z = 697. Found: m/z = 697.
[CBDg3 - H], [CBDg3*OH - H] and [CBDg3*OH - 2H] MS data: LC/ESI-LRMS. [Mg3 - H]-
Found: m/z = 815. [Mg3O H - 2H]- (CsgHssOis) Calcd: m/z= 407. Found: m/z = 407.

[00269] Analysis of the large intestinal contents of animals administered oral CBD indicated
that hydroxy metabolites of CBD were present:

[CBD*20H + Formic acid - H] and [2CBD*30H + Acetic acid - H] MS data: LC/ESI-LRMS.

[M+2OH + Formic acid —H]- (C22H31O6 -) Calcd: m/z = 391. Found: m/z = 391. [2M+3OH + Acetic acid
- H]- (C44H63O2 -) Calcd: m/z= 783.9. Found: m/z= 784.

[00270] The plasma and brains from the same animals were also extracted and analyzed by
HPLC for the presence of CBD-glycosides and CBD. CBD was only present in the control
animal that received CBD aglycone (data not shown). The contents of the small intestines from
the same animals were also extracted and analyzed by HPLC for the presence of CBD-
glycosides and CBD, but no CBD aglycone was present in the small intestines (data not shown,
consistent with THC decoupling data shown in example 11). The presence of the CBD aglycone
in the large intestinal contents indicates the successful delivery of CBD-glycosides, and the
subsequent hydrolysis of the glycosides by beta-glycosidase enzymes only present in the large
intestine. The presence of decoupled CBD in the large intestine, but not in the small intestine,
indicates that glycoside decoupling only occurs upon transit to the large intestine. The presence
of CBD detoxification metabolite CBD-20H is also consistent with delivery of CBD and
absorption into the intestinal epithelium where CBD begins to be metabolized. This example
illustrates the potential to administer CBD-glycosides, safely transit the CBD-glycosides through
the small intestine without absorption, transit to the large intestine where the sugars can be
decoupled to release CBD locally, avoiding systemic absorption and delivery of the CBD to
other tissues where it can have unwanted effects.

Example 11: Analysis of Large Intestine Contents Upon Administration of THC-
Glycosides

[00271] In order to investigate the metabolism and decoupling of THC-glycosides in the large
intestine, an aqueous solution of a mixture of THC-glycosides was administered to two mice by
oral gavage. The first animal was sacrificed at 2 hours and the second animal was sacrificed at
4 hours. Following termination and tissue harvest, the intestinal contents were then extracted
and analyzed by C18 reverse phase HPLC. The mice employed in this example were eight week old male Swiss mice fasted for 12 hours prior to administration of the solutions.

[00272] The resulting extracts were analyzed by LCMS under the same conditions employed in Example 10.

[00273] Analysis of the large intestinal contents from mice administered THC glycosides after 2 hours indicated that both THC aglycone and THC glycosides were present, along with hydroxy metabolites of each:


[THCgl + Cl], [THCgl + Acetic acid - H], [2THCgl - H], and [2THCgl + Acetic acid - H] MS data: LC/ESI-LRMS. [Mg1 + Cl]- (C27H40O7Cl) Calcd: m/z= 511. Found: m/z= 511. [Mg1 + Acetic acid - H]- (C29H43O9) Calcd: m/z = 535. Found: m/z = 535. [2Mg1 - H]- (C54H79O14) Calcd: m/z = 951. Found: m/z = 951. [2Mg1 + Acetic acid - H]- (C35H51O14) Calcd: m/z = 1011. Found: m/z = 1011.

[THCg2 - H], [THCg2 + Acetic acid - H] and [THCg2*OH + Formic acid - H] MS data: LC/ESI-LRMS. [Mg2 - H]- (C33H49O12) Calcd: m/z = 637. Found: m/z = 637. [Mg2 + Acetic acid - H]- (C35H53O14) Calcd: m/z = 697. Found: m/z = 697. [Mg2*H + Acetic acid - H]- (C34H51O15) Calcd: m/z = 699. Found: m/z= 699.

[THCg3 - H], [THCg3 + Acetic acid - H], [CBDg3*OH - H] and [CBDg3*OH - 2H] MS data: LC/ESI-LRMS. [Mg3 - HKC39H59O17] Calcd: m/z = 799. Found: m/z = 799. [Mg3 + Acetic acid - H]- (C41H63O19) Calcd: m/z = 859. Found: m/z = 859. [Mg3O - H]- (C39H59O18) Calcd: m/z = 815. Found: m/z= 815. [Mg3O - 2H]2 (C39H58O18) Calcd: m/z= 407. Found: m/z = 407.

[00274] Analysis of the THC glycosides mixture extract after 4 hours indicated that both THC aglycone and THC glycosides were confirmed, along with hydroxy metabolites of each:

[THC - H], [THC*OH + Acetic acid - H], [2THC*30H + Acetic acid - H] and [THC*20H + Formic acid - H] MS data: LC/ESI-LRMS. [M - H]- (C21H29O2) Calcd: m/z = 313. Found: m/z =
3.13. [MOH + Acetic acid - H]- (C23H33O5-)

Calcd: m/z = 389. Found: m/z = 389. [2M+3OH + Acetic acid - H]- (C44H63O12-)

Calcd: m/z = 783.9. Found: m/z = 784. [4H+ + Formic acid - H]- (C22H31O6)

Calcd: m/z = 391. Found: m/z = 391.

[THCgly1 + Cl], [THCgly1 + Acetic acid - H], [2THCgly1 - H], and [2THCgly1 + Acetic acid - H] MS data: LC/ESI-LRMS. [Mgly1 + Cl]- (C27H40O7CI-)

Calcd: m/z = 511. Found: m/z = 511. [Mgly1 + Acetic acid - H]- (C29H43O9-)

Calcd: m/z = 535. Found: m/z = 535. [2Mgly1 - H]- (C54H79O14)

Calcd: m/z = 951. Found: m/z = 951. [2Mgly1 + Acetic acid - H]- (C58H83O15-)

Calcd: m/z = 1011. Found: m/z = 1011.

[THCgly2 - H] and [THCgly2 + Acetic acid - H] MS data: LC/ESI-LRMS. [Mgly2 - H]- (C33H49O12)

Calcd: m/z = 637. Found: m/z = 637. [Mgly2 + Acetic acid - H]- (C35H53O14-)

Calcd: m/z = 697. Found: m/z = 697.

[THCgly3 - H], [THCgly3 + Acetic acid - H], [CBDgly3*OH - H], [CBDgly3*OH - 2H] and [CBDgly3*OH + Acetic acid - 2H] MS data: LC/ESI-LRMS. [Mgly3 - H]- (C39H59O18)

Calcd: m/z = 799. Found: m/z = 799. [Mgly3 + Acetic acid - H]- (C41H63O19-)

Calcd: m/z = 859. Found: m/z = 859. [Mgly3H - H]- (C39H59O18-)

Calcd: m/z = 815. Found: m/z = 815. [Mgly3H - 2H]- (C39H59O18-)

Calcd: m/z = 407. Found: m/z = 407. [Mgly3H + Acetic acid - 2H]- (C41H63O19-)

Calcd: m/z = 467. Found: m/z = 467.

[00275] The plasma and brains from the same animals were also extracted and analyzed by HPLC for the presence of THC-glycosides and THC, but neither compound was seen in these tissues (data not shown). The contents of the small intestines from the same animals were also extracted and analyzed by HPLC for the presence of THC-glycosides and THC, but no THC aglycone was observed (data not shown, consistent with CBD decoupling data shown in Example 10). The presence of the THC aglycone in the large intestinal contents at 2 and 4 hours indicates the successful delivery of THC-glycosides, and their subsequent hydrolysis of the glycosides by beta-glycosidases in the large intestine. The presence of decoupled THC in the large intestine, but not in the small intestine, indicates that glycoside decoupling only occurs upon transit to the large intestine. The presence of THC detoxification metabolites in the large intestine is further proof that the THC aglycone is present and being absorbed by the intestinal epithelium where it begins to be metabolized. This example illustrates the potential to administer THC-glycosides orally, transit the THC-glycosides through the small intestine without absorption, transit to the large intestine where the sugars can be decoupled to release THC.
locally, avoiding systemic absorption and delivery of the THC to the central nervous system where it can have unwanted psychoactivity.

Example 12: Discovery of novel sucrose synthase isoforms from Stevia rebaudiana

A number of research groups have utilized simple UDP to UDPG recycling systems to decrease the amount of UDPG needed for product formation (Hardin 2004, Bungarang 2013). These studies have characterized the primary sucrose synthase isoforms found in leaf tissue, which presumably carry out the synthesis of sucrose by reacting fructose with UDPG, producing sucrose and spent UDP.

As plants are known to contain numerous isoforms of the sucrose synthase enzyme, identification of alternative SUS enzymes from the Stevia rebaudiana plant with enhanced activity for the back reaction of UDP + sucrose → UDPG + fructose was carried out. As steviol glycosides occur at a high level in Stevia leaves, it was postulated that a sucrose synthase from the leaves of Stevia would have improved ability to catalyze the back reaction that recycles UDP to UDPG. Six sucrose synthase isoforms were identified within the stevia transcriptome, all having similar homology to the 6 isoforms found in Arabidopsis thaliana and named in conjunction with their homologues. These transcripts were cloned as described in materials and methods with the corresponding sequence ID information listed herein.

Enzymatic activities were tested and assayed for their ability to enhance UGT reactions with decreased UDPG input. The best isoform, SrSUS4, was capable of recycling UDP to UDPG with sucrose, in concert with the steviol 19-O-glucosyltransferase SrUGT74G1 mediated glycosylation of steviol bioside to stevioside.

Targeted mutagenesis was performed to mutate a serine residue at the N-terminus that is commonly phosphorylated in planta to prevent dimerization (Hardin 2004). SrSUS1-S13D mutants were created by mutating serine at position 13 to an aspartic acid residue (S13D), thus forming a phospho-mimetic protein. Additionally, the creation of SrSus1-S13R,L14I was created to replace the serine with an arginine, a large charged residue, also to prevent dimerization and inactivation of the enzyme. Sucrose synthase mutants showed improved UDPG production activity compared to their native counterparts. SrSUS5 (SEQ ID NOs. 19 and 20) was identified in the Stevia transcriptome and primers designed (SEQ ID NOs. 67 and 68), but was not able to be amplified from cDNA. SrSus4 showed an impressive UDPG recycling activity with a 20% improvement over the activity seen in SrSus1. It is proposed that
SrSus4 is the ideal isoform for carrying out the back reaction of converting UDP to UDPG in the presence of sucrose. For midi-scale purification of cannabinoid glycosides the use of C18 flash chromatography columns were employed. Biotage flash C18 columns with 33g of resin were washed, loaded, washed, and eluted using peristaltic pumps to achieve the similar separation and purification as the gravity fed Hypersep columns listed previously.

[00280] Relative activity for UDPG production with SUS isoforms is as follows:

SrSus4 > SrSus1 -Untagged > SrSus6 > SrSus2 > SrSus1 > 6xHis-SrSus1 > SrSus3

Example 13: Improved in vitro catalysis of cannabinoid-glycosides

[00281] As the formation of cannabinoid glycosides via UGT enzyme requires the nucleotide sugar donor UDP in stoichiometric amounts, it is advantageous to recycle or recapture the spent UDP following a glycosylation reaction. Utilizing the SUS4 isoform from Stevia rebaudiana, cannabinoid glycosides were successfully produced using only UMP as the input nucleotide.

[00282] A two step reaction took place, first to produce UDP from UMP, and second to produce UDPG from the UDP in tandem with the UGT reaction. First, a 5L reaction containing 50mM KP04 pH7.2, 200mM UMP disodium salt, 200mM ATP disodium salt, 1M MgCl2, 10% UMPK recombinant enzyme in 50% glycerol was prepared. The reaction was incubated at 28C with stirring for > 24hours. The 5L reaction 1 was filtered at 0.45microns to remove precipitate then applied to a 50L reaction containing 50mM KP04 pH7.2, 50mM MgCl2, 300mM Sucrose, 200mg of CBD in 200ml DMSO, 5L UGT76G1 in 50% glycerol, 2.5L SrSUS4 in 50% glycerol. The main 50L reaction was then mixed and allowed to react. An additional 200mg of CBD in 200ml DMSO was added after the reaction went to completion, and allowed to continue incubating at the same conditions. After the remaining CBD was consumed by the reaction, the mixture was filtered by tangential flow filtration with a ultrafiltration membrane at 5kDa to remove enzymes and particulate, and then concentrated using nanofiltration membrane at 500Da. The nanofiltration retentate containing the cannabinoids was then applied to hydrated C18 flash columns, washed with 10-30% methanol, and eluted with 40-65% methanol. The eluate was then concentrated by rotary evaporation to remove all solvent, shell-frozen in a vacuum beaker and lyophilized to dryness. The powdered cannabinoids produced were then collected and stored at -20C in sealed vials. Sucrose should be sterile filtered to avoid
carmelization or sugar breakdown, as autoclaving sucrose stock solutions greatly decreases reaction activity.
<table>
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<th>2*2-O-</th>
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<th>2*2-O-</th>
<th>2*3-O-</th>
<th>3*3-O-</th>
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**Table 1: Cannabidiol-glycoside compositions by R-group**

R-group location is as depicted in Figure 1B.
### Table 2: Cannabidivarin-glycoside compositions by R-group

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<th>3° Position</th>
<th>4° Position</th>
<th>Name</th>
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<th>2° Enzyme</th>
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Table 3: AS-Tetrahydrocannabinol-glycoside compositions by R-group

R-group locations as depicted in Figure 1B

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Table 4: Cannabinol-glycoside compositions by R-group

R-group locations as depicted in Figure 18

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Table 5: Anandamide (AEA) glycoside Compositions by R-group

R-group location is as depicted in Figure 1B

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<th>Name</th>
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<th>2° Enzyme</th>
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Table 6: 2-Arachidonoylycerol (2-AG)-glycoside compositions by R-group

R-group location is as depicted in Figure 1B

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<th>2° Enzyme</th>
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Table 7: 1-Arachidonoylglucero! (1-AGJ-glycoside compositions by R-group

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<td>CTase</td>
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Tabie 7: 1-Arachidonoylglucero! (1-AGJ-glycoside compositions by R-group
R-group location is as depicted in Figure 1.
Table 8: Docosahexaenoyl ethanolamide (DHEA) glycoside compositions by R-group

R-group location is as depicted in Figure 18

<table>
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<th>VB#</th>
<th>1° 1-O Position</th>
<th>2° 2-O-</th>
<th>3° 3-O-</th>
<th>Name</th>
<th>1° Enzyme</th>
<th>2° Enzyme</th>
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<td>β-D-glucose</td>
<td>DHEA-1-O-(3-1)-diglucopyranoside</td>
<td>UGT76G1</td>
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<td>β-D-glucose</td>
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<td>UGT76G1</td>
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<td>VB805</td>
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<td>H</td>
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Table 9: Capsaicin glycoside compositions by R-group

R-group location is as depicted in Figure 18

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<th>1° 1-O Position</th>
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<th>3° 3-O-</th>
<th>Name</th>
<th>1° Enzyme</th>
<th>2° Enzyme</th>
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<td>β-D-glucose</td>
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### TableO: Vanillin glycoside compositions by R-group

R-group location is as depicted in Figure 1B

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<th>1(^{st}) Enzyme</th>
<th>2(^{nd}) Enzyme</th>
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</table>
It is obvious that the foregoing embodiments of the invention are examples and can be varied in many ways. Such present or future variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

REFERENCES


Production of the Endocannabinoid 2-Arachidonoylglycerol Participates in Oligodendrocyte Differentiation. Glia. 58:1913-1 927.


Cannabinoid Receptors and Phosphatidylinositol-3-Kinase/Akt Signaling. J. Neurosci. 22(22):9742-9753.


WE CLAIM:

1. A cannabinoid glycoside prodrug compound having formula (I):

   ![Formula Image]

   wherein
   
   \( R \) is \( H, \beta-D\text{-glucopyranosyl}, \) or \( 3-0^-\text{-D-glucopyranosyl}^-\text{-D-glucopyranosyl}; \)
   
   \( R' \) is \( H \) or \( \beta-D\text{-glucopyranosyl}, \) or \( 3-0^-\text{-D-glucopyranosyl}^-\text{-D-glucopyranosyl}; \) and
   
   \( A \) is an aglycone moiety formed through reaction of a hydroxyl group on a cannabinoid compound, an endocannabinoid compound, or a vanilloid compound, or a pharmaceutically compatible salt thereof.

2. A compound according to claim 1, wherein \( A \) is \( A', A'' \) or \( A''' \);

   wherein \( A' \) is:

   ![Diagram Image]

   wherein \( A'' \) is:

   ![Diagram Image]
and

wherein A" is:
wherein G is H, β-D-glucopyranosyl, 3-O^-D-glucopyranosyl^-D-glucopyranosyl, or β-D-glucopyranosyl-(1\rightarrow3)^-D-glucopyranosyl-(1 \rightarrow3)-D-glucopyranosyl.

3. A compound according to claim 2, wherein A is A'.

4. A compound according to claim 3, wherein A' is:

wherein G is as defined above.

5. A compound according to claim 4, selected from:
6. A compound according to claim 3, wherein $A'$ is:

![Chemical Structure](image)

wherein $G$ is as defined above.

7. A compound according to claim 6, selected from:
8. A compound according to claim 3, wherein $A'$ is:

![Chemical Structure]

9. A compound according to claim 8, selected from:

- VB302
- VB303
- VB304
10. A compound according to claim 3, wherein \( A' \) is:

\[ \text{Structure Image} \]

11. A compound according to claim 10, selected from:

\[ \text{VB305} \quad \text{and} \quad \text{VB308} \]

\[ \text{VB402} \quad \text{VB403} \quad \text{VB404} \]
12. A compound according to claim 2, wherein A is A".

13. A compound according to claim 12, wherein A" is:

14. A compound according to claim 13, selected from:

VB502, VB503
15. A compound according to claim 12, wherein A" is:

![Chemical structure of A"](image)

wherein G is as defined above.

16. A compound according to claim 15, selected from:

![Chemical structures of VB506, VB602, and VB603](image)
VB605

VB607

VB608

VB610

VB609

and
17. A compound according to claim 12, wherein A" is:

```
O

or
```

wherein G is as defined above.

18. A compound according to claim 17, selected from:

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VB702

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VB703
19. A compound according to claim 12, wherein \( A \) is:

![VB715](image)

20. A compound according to claim 19, selected from:

![VB802](image)
21. A compound according to claim 2, wherein A is A''.

22. A compound according to claim 21, wherein A'' is:

23. A compound according to claim 22, selected from:

and
24. A compound according to claim 21, wherein A" is:

![](image)

25. A compound according to claim 24, selected from:

![](image)
26. A compound according to claim 21, wherein A'' is:

\[
\begin{align*}
\text{H}_2\text{CO} & \quad \text{O} \quad \text{O} \quad \text{OCH}_3 \\
\text{\text{\text{-}}}_2 \quad \text{\text{-}}_2 & \quad \text{\text{\text{-}}}_2 \quad \text{\text{\text{-}}}_2 \\
\text{O} & \quad \text{O} \\
\text{G} & \quad \text{G}
\end{align*}
\]

wherein G is as defined above.

27. A compound according to claim 26, selected from:

\[
\begin{align*}
\text{H}_2\text{CO} & \quad \text{O} \quad \text{O} \quad \text{OCH}_3 \\
\text{\text{\text{-}}}_2 \quad \text{\text{-}}_2 & \quad \text{\text{\text{-}}}_2 \quad \text{\text{\text{-}}}_2 \\
\text{O} & \quad \text{O} \\
\text{G} & \quad \text{G}
\end{align*}
\]

VB1102
VB1123

VB1126

VB1127

, and

29. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 28 and a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant.

30. A method for the site-specific delivery of a cannabinoid drug to a subject, comprising the step of administering a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28 to a subject in need thereof.
31. The method of claim 30, wherein the cannabinoid glycoside prodrug is formulated for oral administration.

32. The method of claim 30, wherein the cannabinoid glycoside prodrug is formulated for parenteral administration.

33. The method of claim 30, wherein the cannabinoid glycoside is formulated for transdermal administration.

34. A method for the site-specific delivery of a cannabinoid drug to a subject, comprising the step of administering a pharmaceutical composition as defined in claim 29 to a subject in need thereof.

35. The method of claim 34, wherein the pharmaceutical composition is formulated for oral administration.

36. The method of claim 34, wherein the pharmaceutical composition is formulated for parenteral administration.

37. The method of claim 34, wherein the pharmaceutical composition is formulated for transdermal administration.

38. A method for facilitating the transport of a cannabinoid drug across the blood brain barrier of a subject comprising administering a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28 to a subject in need thereof.

39. An antimicrobial agent comprising an effective amount of a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28.

40. Use of an effective amount of a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28 as an antimicrobial agent.

41. A detersive agent comprising an effective amount of a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28.
42. Use of an effective amount of a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28 as a detersive agent.

43. A method of producing a cannabinoid glycoside, comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.

44. The method of claim 43, wherein the one or more glycosyltransferases is a UGT76G1 or UGT76G1-like glucosyltransferase.

45. The method of claim 44, wherein the one or more glycosyltransferases further comprise a Os03g0702000 or Os03g0702000-like glucosyltransferase.

46. The method of any one of claims 43 to 45, wherein the one or more sugar donors are selected from the group consisting of UDP-glucose, UDP-glucuronic acid, UDP-mannose, UDP-fructose, UDP-xylose, UDP-rhamnose, UDP-fluoro-deoxyglucose and combinations thereof.

47. The method of claim 46, wherein the sugar donor is UDP-glucose.

48. A method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation.

49. A method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of a first glycosyltransferase and a second glycosyltransferase under conditions which allow for glycosylation.

50. The method of claim 49, wherein the sugar donor is UDP-glucose, the first glycosyltransferase is a UGT76G1 or UGT76G1-like glucosyltransferase, and the second glycosyltransferase is a Os03g0702000 or Os03g0702000-like glucosyltransferase.

51. A method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and Os03g0702000 or Os03g0702000-like glucosyltransferase under conditions which allow for glycosylation.
52. The method of any one of claims 43 to 51, wherein the cannabinoid aglycone is a cannabinoid, an endocannabinoid, or a vanilloid.

53. The method of any one of claims 43 to 51, wherein the cannabinoid glycoside produced by the method is a compound of the Formula (I) as defined in any one of claims 1 to 28.

54. A method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with maltodextrin, in the presence of a cyclodextrin glucanotransferase under conditions that allow for glycosylation.

55. A method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with UDP-glucose and maltodextrin in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and cyclodextrin glucanotransferase under conditions which allow for glycosylation.

56. The method of any one of claims 44, 48, 51, 52 and 55, wherein the UGT76G1 or UGT76G1-like glucosyltransferase comprises the sequence as set forth in SEQ ID NO: 1, 3, 5 or 7.

57. The method of any one of claims 45, 50 and 51, wherein the Os03g0702000 or Os03g0702000-like glucosyltransferase comprises the sequence as set forth in SEQ ID NO: 9.

58. The method of any one of claims 43 to 57, further comprising incubating with sucrose synthase.

59. The method of claim 58, wherein the sucrose synthase comprises the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

60. A method for the production of a cannabinoid glycoside comprising expressing one or more of the glucosyltransferases in a cell or plant which produces a cannabinoid aglycone and isolating the cannabinoid glycoside.
61. A method of producing a higher order cannabinoid glycoside, comprising incubating a lower order cannabinoid glycoside with one or more sugar donors in the presence of one or more glycosyltransferases.

62. The method of claim 61, wherein the one or more glycosyltransferases is a UGT76G1 or UGT76G1-like glucosyltransferase.

63. The method of claim 62, wherein the one or more glycosyltransferases further comprise a Os03g0702000 or Os03g0702000-like glucosyltransferase.

64. The method of any one of claims 61 to 63, wherein the one or more sugar donors are selected from the group consisting of UDP-glucose, UDP-glucuronic acid, UDP-mannose, UDP-fructose, UDP-xylose, UDP-rhamnose, UDP-fluoro-deoxyglucose and combinations thereof.

65. The method of claim 64, wherein the sugar donor is UDP-glucose.

66. A method of producing a higher order cannabinoid glycoside comprising incubating a lower order cannabinoid glycoside with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation.

67. A method of producing a higher order cannabinoid glycoside comprising incubating a lower order cannabinoid glycoside with one or more sugar donors in the presence of a first glycosyltransferase and a second glycosyltransferase under conditions which allow for glycosylation.

68. The method of claim 67, wherein the sugar donor is UDP-glucose, the first glycosyltransferase is a UGT76G1 or UGT76G1-like glucosyltransferase, and the second glycosyltransferase is a Os03g0702000 or Os03g0702000-like glucosyltransferase.

69. A method of producing a higher order cannabinoid glycoside comprising incubating a lower order cannabinoid glycoside with UDP-glucose in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and Os03g0702000 or Os03g0702000-like glucosyltransferase under conditions which allow for glycosylation.
70. The method of any one of claims 61 to 69, wherein the lower order cannabinoid glycoside is a lower order cannabinoid glycoside, a lower order endocannabinoid glycoside, or a lower order vanilloid glycoside.

71. The method of any one of claims 61 to 70, wherein the higher order cannabinoid glycoside produced by the method is a compound of the Formula (I) as defined in any one of claims 1 to 28.

72. A method of producing a higher order cannabinoid glycoside comprising incubating a lower order cannabinoid glycoside with maltodextrin, in the presence of a cyclodextrin glucanotransferase under conditions that allow for glycosylation.

73. A method of producing a higher order cannabinoid glycoside comprising incubating a lower order cannabinoid glycoside with UDP-glucose and maltodextrin in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase and cyclodextrin glucanotransferase under conditions which allow for glycosylation.

74. The method of any one of claims 62, 66, 69, 70 and 73, wherein the UGT76G1 or UGT76G1-like glucosyltransferase comprises the sequence as set forth in SEQ ID NO:1, 3, 5 or 7.

75. The method of any one of claims 63, 68 and 69, wherein the Os03g0702000 or Os03g0702000-like glucosyltransferase comprises the sequence as set forth in SEQ ID NO:9.

76. The method of any one of claims 61 to 75, further comprising incubating with sucrose synthase.

77. The method of claim 76, wherein the sucrose synthase comprises the sequence as set forth in SEQ ID NO: 15, 17, 19, 21, 23 or 25.

78. A method for the production of a higher order glycoside comprising incubating a lower order glycoside with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation, wherein the lower order glycoside is other than a steviol glycoside.
79. A method for the production of a glycoside comprising incubating an aglycone with UDP-glucose, in the presence of a UGT76G1 or UGT76G1-like glucosyltransferase under conditions that allow for glycosylation.
FIG. 7
**FIG. 30**
**INTERNATIONAL SEARCH REPORT**

International application No. PCT/US 16/53122

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: 52-53, 56, 58-59, 70-71, 74-77 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see supplemental pages -

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-11, 28-29, 39-51, 54-55, 57, 60-69, 72-73 and 78-79

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

**Remark on Protest**

□ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (January 2015)
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

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<thead>
<tr>
<th>IPC(8)</th>
<th>CPC</th>
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<tr>
<td>A61P 3/10, A61K 31/70 (2017.01)</td>
<td>C07F 9/65586, C07D 405/10</td>
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</table>

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

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<tr>
<td>A61P 3/10, A61K 31/70 (2017.01)</td>
<td>C07F 9/65586, C07D 405/10</td>
</tr>
</tbody>
</table>

Documented search other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patent, Google Web

Search terms used - glycosyltransferases synthesis cannabinoid Tetrahydrocannabinol glucose ether maltodextrin Os03g0702000 Pubchem substructure search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 8,410,064 B2 (Radominska-Pandya et al.) 02 April 2013 (02.04.2013); col 2, in 31-33, col 3, in 11-14, 25-50, col 12, in 61-62, 64, col 13, in 2-5, Fig 5A-E</td>
<td>43, 46/43, 49, 61, 64/61, 67</td>
</tr>
<tr>
<td>WO 2014/122227 A2 (EVOLVA SA) 14 August 2014 (14.08.2014); para [0009], [0022], [00103], [00217], [00329]</td>
<td>78-79</td>
</tr>
<tr>
<td>US 5,627,270 A (Kahne et al.) 06 May 1997 (06.05.1997); col 5, in 37-44, Fig. 14</td>
<td>1-11, 28-29, 39-42</td>
</tr>
<tr>
<td>US 2014/029851 1 A1 (Lewis et al.) 02 October 2014 (02.10.2014); para [0075]</td>
<td>6-7, 29/(6-7)</td>
</tr>
<tr>
<td>US 5,292,899 A (Tius et al.) 08 March 1994 (08.03.1994); entire document</td>
<td>1-11, 28-29, 39-51, 54-55, 57, 60-69, 72-73, 78-79</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

- **Special categories of cited documents:**
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed
  - "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search: 31 January 2017

Date of mailing of the international search report: 17 FEB 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I: Claims 1-29 and 39-42, directed to cannabinoid glycoside compounds having the general formula of claim 1, and to pharmaceutical/antimicrobial/detergent compositions containing the same. The compound of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by the first formula of claim 1, wherein R is H, R' is H, and A is an aglycone moiety formed through reaction of a hydroxyl group on a cannabinoid compound. It is believed that claims 1-11, 28-29 and 39-42 read on this first named invention, and thus these claims will be searched without fee to the extent that they encompass the first species of claim 1. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the -"+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a compound of claim 1, wherein R is H, R' is H; and A is an aglycone moiety formed through reaction of a hydroxyl group on a vanillloid compound (i.e., claims 1-2, 21-29 and 39-42).

Group II: Claims 30-38, directed to method for the site-specific delivery of a cannabinoid drug/facilitating the transport of a cannabinoid drug across the blood brain barrier to a subject, comprising the step of administering a cannabinoid glycoside prodrug as defined in any one of claims 1 to 28 to a subject in need thereof.

Group III: Claims 43-57, 60-69, 72-73 and 78-79, directed to a method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I includes the technical feature of a unique cannabinoid glycoside compound of claim 1, which is not required by any other invention of Group I.

Group II includes the technical feature of a method for the site-specific delivery of a cannabinoid drug facilitating the transport of a cannabinoid drug across the blood brain barrier to a subject, not required by Group I and III.

Group III includes the technical feature of a method of producing a cannabinoid glycoside comprising incubating a cannabinoid aglycone with one or more sugar donors in the presence of one or more glycosyltransferases.

Common technical features:

The inventions of Group I share the technical feature of a cannabinoid glycoside compound of claim 1.

Groups I and II share the technical feature of a cannabinoid glycoside compound of claim 1.

Groups I, II and III share the technical feature of a cannabinoid glycoside compound.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by a document entitled "Pubchem CID 6452133" (hereinafter Pubchem-133) Create Date: 19 April 2006, which teaches a cannabinoid glycoside compound of formula (I), wherein R is H, R' is H, and A is an aglycone moiety formed through reaction of a hydroxyl group on a vanillloid compound (vanilillin) (pg. 3, compound listed).

As said compound and compositions were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I, II and III.

The inventions of Group I, II and III thus lack unity under PCT Rule 13.

Note: claims 58-59, 70-71 and 74-77 determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Note: claims 52-53 and 56 are also determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a); and were not identified as such previously upon submitting notice of LOU.