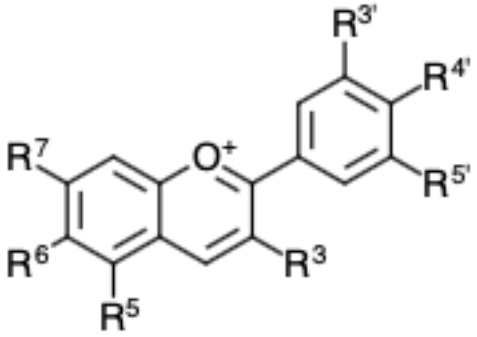
in vivo (typically through protein overexpression in hosts such as bacteria), isolated via chromatography, and then added into a bioreactor with a substrate (starting material). The enzymes transform the substrate in the same way that occurs in plants but without the complexity of the organism. In this way, natural products can begin to be created without the plant, cell, or chemical synthesis.

BRIEF SUMMARY

It is an objective of the present disclosure to provide a cell-free method that allows for the production of anthocyanidins (cyanidin glycosides, such as cyanidin-3-glycosides, cyanidin-5-glycosides, etc.) starting from anthocyanidins via a cell-free biosynthesis platform. Examples of the present disclosure can be freely combined with each other if they are not mutually exclusive. In one aspect, the present disclosure provides a cell-free method of producing a product anthocyanin from an anthocyanidin, the method comprising:

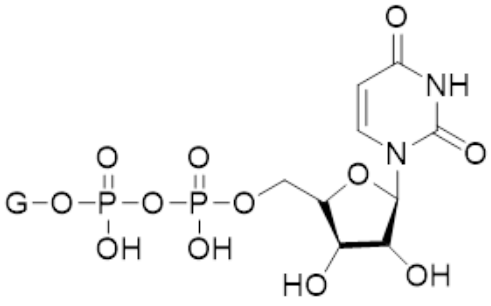
a) adding to a mixture comprising a suitable solvent (i) an anthocyanidin of Formula I:



Formula I

wherein each of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ are independently selected from the group consisting of H, OH, and OCH_3, wherein at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH or OCH_3 and at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH;

(ii) a glycosylated uridine-5'-diphosphate of Formula II:



Formula II

wherein G is a glycosyl residue selected from the group consisting of 5- and 6-

membered monosaccharides; and

(iii) a glycosyltransferase enzyme to form a reaction mixture;

b) removing the supernatant from the reaction mixture from (a); and

c) isolating the product anthocyanin

wherein the reaction mixture has a starting pH, wherein the starting pH is 4 to 6.

In another aspect, the present disclosure provides a cell-free method of producing an anthocyanin from an anthocyanidin, the method comprising:

a) in a cell-free vessel, providing a glycosyltransferase enzyme;

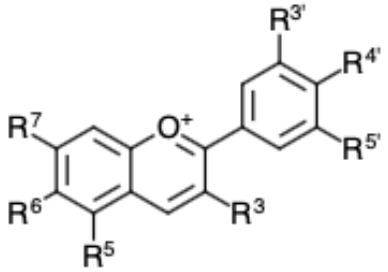
b) adding an anthocyanidin and a glycosylated uridine-5'-diphosphate to the cell-free vessel to form the product anthocyanin; and

c) removing the anthocyanin from the cell-free vessel.

wherein the cell-free vessel has a starting pH of 4 to 6.

In yet another aspect, the present disclosure provides a cell-free method of producing a product anthocyanin from an anthocyanidin, the method comprising:

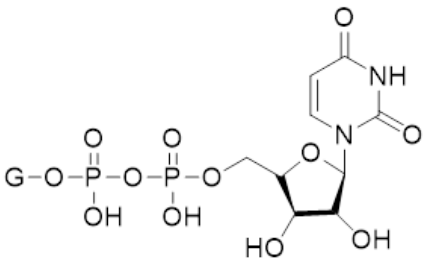
a) adding an anthocyanidin or anthocyanin of Formula I:



Formula I

wherein each of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ are independently selected from the group consisting of H, OH, OCH_3, and a sugar, wherein at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH;

a glycosylated uridine-5'-diphosphate of Formula II:



Formula II

wherein G is a glycosyl residue selected from the group consisting of 5- and 6-membered monosaccharides; and

a glycosyltransferase enzyme to form a reaction mixture;

b) removing the supernatant from the reaction mixture from (a); and

c) isolating the product anthocyanin

wherein the reaction mixture has a starting pH of 4 to 6.

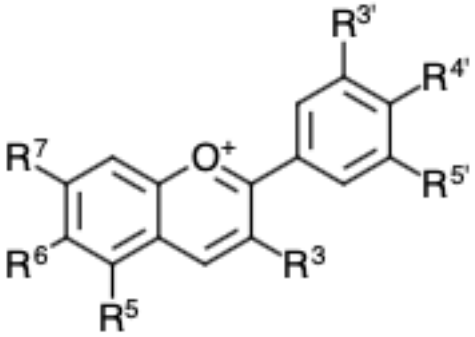
The present disclosure uses glycotransferase enzymes to convert anthocyanidins to several possible anthocyanidin glycosides, such as cyanidin-3-glycosides, depending on the glycosyl donor.

The presently claimed process uses a controlled enzymatic step to increase product titer in short reaction times. In some examples, the controlled enzymatic step may increase product titer by a factor of at least five. Additionally, enzyme immobilization may be used to enhance anthocyanidin conversion to corresponding anthocyanins. For example, enzyme immobilization was used to improve cyanidin conversion to cyanidin-3-glucoside (C3G) by avoiding enzyme precipitation, inactivation, and the unreliability of the non-immobilized system.

A unique and inventive technical feature of the present disclosure is the use of a cell-free system for the production of anthocyanins (anthocyanidin glycosides), such as cyanidin-3-glycoside. Without wishing to limit the disclosure to any theory or mechanism, it is believed that the technical feature of the present disclosure advantageously provides for higher reaction concentrations of starting materials, reagents, and enzymes, resulting in higher concentrations of final products. Additionally, the present disclosure eliminates the complications of cell walls, thereby eliminating a barrier to product and substrate diffusion. Furthermore, the present disclosure eliminates competition for carbon flux, which limits the efficiency of cell-based synthesis methods, and thus greatly reduces byproduct formation. Also, because there is no cell, the present disclosure is not vulnerable to degradation by cell death due to the formation of toxic compounds. The adaptability of the system to accommodate different starting anthocyanidins and glycosylated UDPs (UDP-sugars) lends the system flexibility to produce a multitude of products and the ability to use various solvents, such as organic solvents, to permit higher concentrations of solutes without

CLAIMS

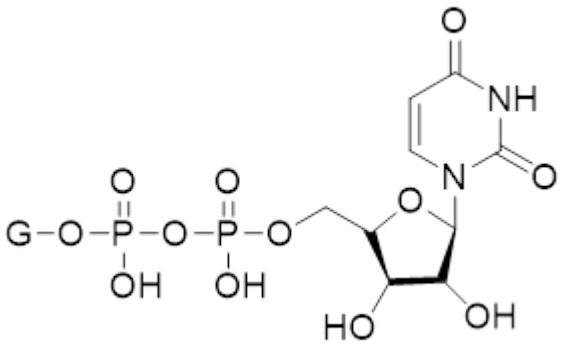
1. A cell-free method of producing a product anthocyanin from an anthocyanidin, the method comprising:
    a) adding to a mixture comprising a suitable solvent (i) an anthocyanidin of Formula I:



Formula I

wherein each of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ are independently selected from the group consisting of H, OH, and OCH_3, wherein at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH or OCH_3 and at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH;

(ii) a glycosylated uridine-5'-diphosphate of Formula II:



Formula II

wherein G is a glycosyl residue selected from the group consisting of 5- and 6-membered monosaccharides; and

(iii) a glycosyltransferase enzyme to form a reaction mixture;

    b) removing the supernatant from the reaction mixture from (a); and
    c) isolating the product anthocyanin

    wherein the reaction mixture has a starting pH, wherein the starting pH is 4 to 6.

2. A cell-free method of producing an anthocyanin from an anthocyanidin, the method comprising:
    a) in a cell-free vessel, providing a glycosyltransferase enzyme;

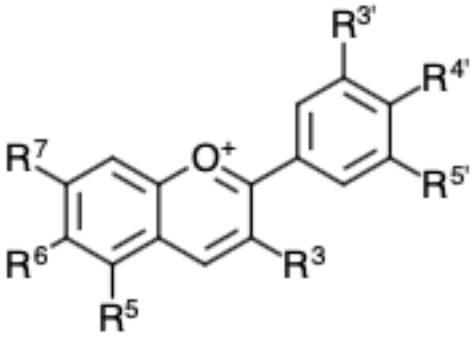
b) adding an anthocyanidin and a glycosylated uridine-5’-diphosphate to the cell-free vessel to form the product anthocyanin; and

c) removing the product anthocyanin from the cell-free vessel

wherein the cell-free vessel has a starting pH of 4 to 6.

3. A cell-free method of producing a product anthocyanin from an anthocyanidin, the method comprising:

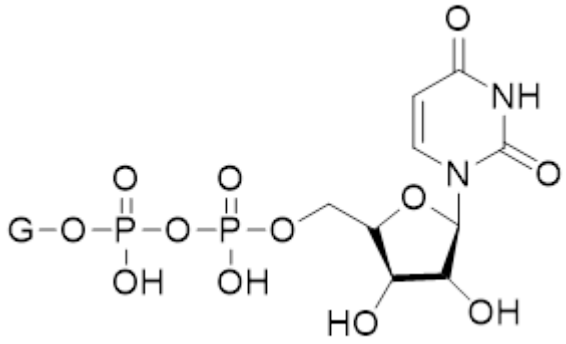
a) adding an anthocyanidin or anthocyanin of Formula I:



Formula I

wherein each of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ are independently selected from the group consisting of H, OH, OCH_3, and a sugar, wherein at least one of R^3, R^5, R^6, R^7, $R^{3'}$, $R^{4'}$, and $R^{5'}$ is OH;

a glycosylated uridine-5’-diphosphate of Formula II:



Formula II

wherein G is a glycosyl residue selected from the group consisting of 5- and 6-membered monosaccharides; and

a glycosyltransferase enzyme to form a reaction mixture;

b) removing the supernatant from the reaction mixture from (a); and

c) isolating the product anthocyanin

wherein the reaction mixture has a starting pH of 4 to 6.

4. The method of claim 1 or claim 2, wherein the anthocyanidin is selected from the group consisting of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin.

5. The method of claim 3, wherein the anthocyanidin is selected from the group consisting of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin, a glycosylated cyanidin, a glycosylated delphinidin, a glycosylated malvidin, a glycosylated pelargonidin, and a glycosylated peonidin.

6. The method of claim 3, wherein the anthocyanidin is selected from the group consisting of a glycosylated cyanidin, a glycosylated delphinidin, a glycosylated malvidin, a glycosylated pelargonidin, and a glycosylated peonidin.

7. The method of any one of claims 1 to 6, wherein the glycosylated uridine-5'-diphosphate has an initial concentration of 1 mM to 30 mM.

8. The method of any one of claims 1 to 7, wherein the glycosyltransferase enzyme is a 3-O-glycosyltransferase or a 5-O-glycosyltransferase.

9. The method of any one of claims 1 to 7, wherein the glycosyltransferase enzyme is 3-O-glucoside-6"-O-rhamnosyltransferase (3RT), 3-O-glucoside-2"-O-glucotransferase (3GGT), 3'-O-glycosyltransferase, or 7-O-glycosyltransferase.

10. The method of any one of claims 1 to 9, wherein the sequence for the glycosyltransferase enzyme is one of SEQ ID NO 1-5.

11. The method of any one of claims 1 to 9, wherein the sequence for the glycosyltransferase enzyme has at least 95%similarity to one of SEQ ID NO 1-5.

12. The method of any one of claims 1 to 11, wherein the glycosyltransferase enzyme is non-immobilized.

13. The method of any one of claims 1 to 11, wherein the glycosyltransferase enzyme is immobilized.

14. The method of any one of claims 1 to 13, wherein the glycosyltransferase enzyme is housed in a continuous reactor system.

15. The method of any one of claims 1 to 14, wherein R^3 is OH, and the glycosyltransferase enzyme is 3-O-glycotransferase (3GT).

16. The method any one of claims 1 to 14, wherein R^5 is OH, and the glycosyltransferase enzyme is 5-O-glycotransferase (5GT).

17. The method of any one of claims 1 to 16, wherein the glycosylated uridine-5'-diphosphate is selected from the group consisting of UDP-arabinose, UDP-galactose, UDP-glucose, UDP-rhamnose, and UDP-xylose.

18. The method of any one of claims 1 to 17, wherein the starting pH is 4.5 to 5.5.

19. The method of any one of claims 1 to 18, wherein the starting pH is 5.

20. The method of any one of claims 1 to 19, wherein the anthocyanidin, glycosylated

uridine-5’-diphosphate, and the glycosyltransferase enzyme comprise one of the combinations:

Anthocyanidin	Enzyme	Glycosyl Donor Molecule	Anthocyanin
Cyanidin	3GT	UDP-arabinose	cyanidin-3-O-arabinoside
Cyanidin	3GT	UDP-galactose	cyanidin-3-O-galactoside
Cyanidin	3GT	UDP-glucose	cyanidin-3-O-glucoside
Cyanidin	3GT	UDP-rhamnose	cyanidin-3-O-rhamnoside
Cyanidin	3GT	UDP-xylose	cyanidin-3-O-xyloside
Cyanidin	5GT	UDP-arabinose	cyanidin-5-O-arabinoside
Cyanidin	5GT	UDP-galactose	cyanidin-5-O-galactoside
Cyanidin	5GT	UDP-glucose	cyanidin-5-O-glucoside
Cyanidin	5GT	UDP-rhamnose	cyanidin-5-O-rhamnoside
Cyanidin	5GT	UDP-xylose	cyanidin-5-O-xyloside
Delphinidin	3GT	UDP-arabinose	delphinidin-3-O-arabinoside
Delphinidin	3GT	UDP-galactose	delphinidin-3-O-galactoside
Delphinidin	3GT	UDP-glucose	delphinidin-3-O-glucoside
Delphinidin	3GT	UDP-rhamnose	delphinidin-3-O-rhamnoside
Delphinidin	3GT	UDP-xylose	delphinidin-3-O-xyloside
Delphinidin	5GT	UDP-arabinose	delphinidin-5-O-arabinoside
Delphinidin	5GT	UDP-galactose	delphinidin-5-O-galactoside
Delphinidin	5GT	UDP-glucose	delphinidin-5-O-glucoside
Delphinidin	5GT	UDP-rhamnose	delphinidin-5-O-rhamnoside
Delphinidin	5GT	UDP-xylose	delphinidin-5-O-xyloside
Malvidin	3GT	UDP-arabinose	malvidin-3-O-arabinoside
Malvidin	3GT	UDP-galactose	malvidin-3-O-galactoside
Malvidin	3GT	UDP-glucose	malvidin-3-O-glucoside
Malvidin	3GT	UDP-rhamnose	malvidin-3-O-rhamnoside
Malvidin	3GT	UDP-xylose	malvidin-3-O-xyloside
Malvidin	5GT	UDP-arabinose	malvidin-5-O-arabinoside
Malvidin	5GT	UDP-galactose	malvidin-5-O-galactoside
Malvidin	5GT	UDP-glucose	malvidin-5-O-glucoside

Anthocyanidin	Enzyme	Glycosyl Donor Molecule	Anthocyanin
Malvidin	5GT	UDP-rhamnose	malvidin-5-O-rhamnoside
Malvidin	5GT	UDP-xylose	malvidin-5-O-xyloside
Pelargonidin	3GT	UDP-arabinose	pelargonidin-3-O-arabinoside
Pelargonidin	3GT	UDP-galactose	pelargonidin-3-O-galactoside
Pelargonidin	3GT	UDP-glucose	pelargonidin-3-O-glucoside
Pelargonidin	3GT	UDP-rhamnose	pelargonidin-3-O-rhamnoside
Pelargonidin	3GT	UDP-xylose	pelargonidin-3-O-xyloside
Pelargonidin	5GT	UDP-arabinose	pelargonidin-5-O-arabinoside
Pelargonidin	5GT	UDP-galactose	pelargonidin-5-O-galactoside
Pelargonidin	5GT	UDP-glucose	pelargonidin-5-O-glucoside
Pelargonidin	5GT	UDP-rhamnose	pelargonidin-5-O-rhamnoside
Pelargonidin	5GT	UDP-xylose	pelargonidin-5-O-xyloside
Peonidin	3GT	UDP-arabinose	peonidin-3-O-arabinoside
Peonidin	3GT	UDP-galactose	peonidin-3-O-galactoside
Peonidin	3GT	UDP-glucose	peonidin-3-O-glucoside
Peonidin	3GT	UDP-rhamnose	peonidin-3-O-rhamnoside
Peonidin	3GT	UDP-xylose	peonidin-3-O-xyloside
Peonidin	5GT	UDP-arabinose	peonidin-5-O-arabinoside
Peonidin	5GT	UDP-galactose	peonidin-5-O-galactoside
Peonidin	5GT	UDP-glucose	peonidin-5-O-glucoside
Peonidin	5GT	UDP-rhamnose	peonidin-5-O-rhamnoside
Peonidin	5GT	UDP-xylose	peonidin-5-O-xyloside
Petunidin	3GT	UDP-arabinose	petunidin-3-O-arabinoside
Petunidin	3GT	UDP-galactose	petunidin-3-O-galactoside
Petunidin	3GT	UDP-glucose	petunidin-3-O-glucoside
Petunidin	3GT	UDP-rhamnose	petunidin-3-O-rhamnoside
Petunidin	3GT	UDP-xylose	petunidin-3-O-xyloside
Petunidin	5GT	UDP-arabinose	petunidin-5-O-arabinoside
Petunidin	5GT	UDP-galactose	petunidin-5-O-galactoside
Petunidin	5GT	UDP-glucose	petunidin-5-O-glucoside

Anthocyanidin	Enzyme	Glycosyl Donor Molecule	Anthocyanin
Petunidin	5GT	UDP-rhamnose	petunidin-5-O-rhamnoside
Petunidin	5GT	UDP-xylose	petunidin-5-O-xyloside

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6"-acetyl)-galactoside	uridine-5'-diphosphate-galactose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-sambubioside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(3"-malonyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6"-malonyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6"-dioxaloyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	cyanidin-3-O-rutinoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	cyanidin-3-O-xylosylrutinoside	uridine-5'-diphosphate-glucose, uridine-5'-diphosphate-rhamnose, uridine-5'-diphosphate xylose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Cyanidin	3-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	cyanidin-3-glucosylrutinoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3,5-O-diglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	cyanidin-3-O-sophoroside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6-O-p-coumaroyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6-O-malonylglucoside)	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-3'',6''-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-(6-p-caffeoyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3,5-O-dihexoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-lathyroside	uridine-5'-diphosphate-galactose & uridine-5'-diphosphate-xylose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Cyanidin	3-O-glycosyltransferase; 3'-O-glycosyltransferase	cyanidin-3,3'-diglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-xylosyl-galactoside	uridine-5'-diphosphate-galactose & uridine-5'-diphosphate-xylose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6"malonylglucoside)	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-malonylglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-succinylglucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-sambubioside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3,5-O-diglucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	delphinidin-3-O-rutinoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3,5-O-diglucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Delphinidin	3-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	delphinidin-3-O-sophoroside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-sambubioside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-(6"-O-malonyl-glucoside)	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-(6-caffeoylglucoside)	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-(6-p-coumaroyl)-glucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 7-O-glycosyltransferase	delphinidin-3,7-O-diglucoside	uridine-5'-diphosphate-glucose
Malvidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	malvidin-3,5-O-diglucoside	uridine-5'-diphosphate-glucose
Malvidin	3-O-glycosyltransferase	malvidin-3-O-acetylglucoside	uridine-5'-diphosphate-glucose
Malvidin	3-O-glycosyltransferase	malvidin-3-p-coumaroylglucoside	uridine-5'-diphosphate-glucose
Malvidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	malvin	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3,5-O-diglucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Pelargonidin	3-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	pelargonidin-3-O-rutinoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-acetylglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-malylglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-(6-p-coumaroyl)-glucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-(6''-O-malonylglucoside)	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	pelargonidin-3-O-sophoroside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-(6-caffeoylglucoside)	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	peonidin-3-O-rutinoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Peonidin	3-O-glycosyltransferase	peonidin-3-O-acetylglucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase	peonidin-3-p-coumaroylglucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Peonidin	3-O-glycosyltransferase	peonidin-3-O-(6"-malonylglucoside)	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase	peonidin-3-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Petunidin	3-O-glycosyltransferase	petunidin-3-O-(6-O-p-coumaroyl glucoside)	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3,5,3'-O-triglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-O-(6-O-p-coumaroyl)-glucoside-5-O-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	cyanidin-3-O-rutinoside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(6"-O-malonyl-2"-O-glucuronyl)-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-glucoside-5-caffeoylglucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	malonylshisonin	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-O-sambubioside-5-O-rhamnoside	uridine-5'-diphosphate-glucose, uridine-5'-diphosphate-xylose, uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	cyanidin-3-O-sophoroside-5-O-rhamnoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-O-sambubioside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(caffeoyl)-diglucose	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(cis-p-coumaroyl)-diglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-(trans-p-coumaroyl)-diglucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-O-diglucoside-5-O-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-O-xylosyl-glucosyl-galactoside	uridine-5'-diphosphate-glucose,

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
			uridine-5'-diphosphate-xylose, uridine-5'-diphosphate-galactose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-O-malonylglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	cyanidin-3-(caffeoyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	cyanidin-3-O-sophoroside-5-O-glucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-sambubioside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-(caffeoyl)-diglucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6"-O-	delphinidin-3-O-(caffeoyl)-rutinoside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
	rhamnosyltransferase (3RT)		
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	delphinidin-3-O-(feruloyl)-rutinoside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-glucoside-5-caffoyl-glucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-glucoside-5-O-(6-coumaroylglucoside)	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 3'-O-glycosyltransferase	delphinidin-3-O-(6''-O-malonyl)-glucoside-3'-O-glucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase	delphinidin-3-O-3'',6''-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 3'-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3,5,3'-O-triglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	pelargonidin-3-O-rutinoside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-(p-coumaroyl)-diglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase	pelargonidin-3-O-3",6"-O-dimalonylglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-O-sambubioside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-xylose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-O-glucoside-5-O-(6-coumaroylglucoside)	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-glucoside-5-caffeoylglucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-O-(6-caffeoylglucoside)-5-O-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	peonidin-3-(6"-p-coumaroyl-glucoside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	peonidin-3-O-sophoroside-5-O-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-xylosyl-glucosyl-galactoside-coumaric acid	uridine-5'-diphosphate-glucose, uridine-5'-diphosphate-xylose, uridine-5'-

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
			diphosphate-galactose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-xylosyl-glucosyl-galactoside-ferulic acid	uridine-5'-diphosphate-glucose, uridine-5'-diphosphate-xylose, uridine-5'-diphosphate-galactose
Cyanidin	3-O-glycosyltransferase	cyanidin-3-xylosyl-glucosyl-galactoside-sinapic acid	uridine-5'-diphosphate-glucose, uridine-5'-diphosphate-xylose, uridine-5'-diphosphate-galactose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	cyanidin-3-(caffeoyl-feruloyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	cyanidin-3-(caffeoyl-p-hydroxybenzoyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	cyanidin-3-(dicaffeoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	cyanidin-3-(feruloyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	cyanidin-3-(p-hydroxybenzoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(caffeoyl)(p-coumaroyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(sinapoyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	delphinidin-3-O-(trans-coumaroyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Delphinidin	3-O-glycosyltransferase; 3'-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3,3'-diglucoside-5-(6-caffeoyl)-glucoside	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	delphinidin-3-O-(p-coumaroyl)-rutinoside-5-O-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Malvidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	malvidin-3-(feruloyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Malvidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-O-(6-O-caffeoylglucoside)-5-O-(6-O-malonylglucoside) (monodemalonylsalvianin)	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	peonidin-3-(caffeoyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	peonidin-3-(feruloyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	peonidin-3-(p-hydroxybenzoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6"-O-rhamnosyltransferase (3RT)	peonidin-3-(p-coumaroyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Petunidin	3-O-glycosyltransferase; 5-	petunidin-3-(feruloyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
	O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)		& uridine-5'-diphosphate-rhamnose
Petunidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-6''-O-rhamnosyltransferase (3RT)	petunidin-3-(p-coumaroyl)-rutinoside-5-glucoside	uridine-5'-diphosphate-glucose & uridine-5'-diphosphate-rhamnose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(caffeoyl-sinapoyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(p-coumaroyl)(sinapoyl)-triglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(feruloyl)(sinapoyl)-triglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(p-coumaroyl)(sinapoyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(feruloyl)(sinapoyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(sinapoyl)(sinapoyl)-diglucoside-5-glucoside	uridine-5'-diphosphate-glucose
Cyanidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	cyanidin-3-(sinapoyl)-diglucoside-5-(sinapoyl)-glucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
Delphinidin	3-O-glycosyltransferase; 3'-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-glucosyl-5-O-caffeoylglucoside-3'-O-caffeoylglucoside (gentiodelphin)	uridine-5'-diphosphate-glucose
Delphinidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	delphinidin-3-O-(6-O-(trans-p-coumaroyl)-glucosyl)-5-O-(6-O-malonylglucoside) (malonylawobanin)	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-(caffeoyl-feruloyl sophoroside)-5-O-glucoside	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	monardaein	uridine-5'-diphosphate-glucose
Pelargonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	pelargonidin-3-O-(6-O-caffeoylglucoside)-5-O-(4-O,6-O-dimalonylglucoside) (salvianin)	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-glucotransferase (3GGT)	peonidin-3-(caffeoyl-feruloyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2"-O-	peonidin-3-(caffeoyl-p-coumaroyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
	glucotransferase (3GGT)		
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	peonidin-3-(caffeoyl-p-hydroxybenzoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	peonidin-3-(dicaffeoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	peonidin-3-(feruloyl-p-coumaroyl sophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase; 3-O-glucoside-2''-O-glucotransferase (3GGT)	peonidin-3-(feruloyl-p-hydroxybenzoylsophoroside)-5-glucoside	uridine-5'-diphosphate-glucose
Peonidin	3-O-glycosyltransferase; 5-O-glycosyltransferase	peonidin-3-(2-(6-(3-(glucosyl)caffeoyl)glucosyl)-6(4-(6-(3-(glucosyl)caffeoyl)glucosyl)caffeoyl)glucoside)-5-	uridine-5'-diphosphate-glucose

Anthocyanidin	Enzyme	Anthocyanin Product	Glycosyl Donor Molecule(s)
		glucoside (heavenly blue anthocyanin)	

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21. The method of any one of claims 1 to 20, wherein the anthocyanin is selected from the group consisting of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin; the glycosylated uridine-5'-diphosphate is selected from the group consisting of UDP-arabinose, UDP-galactose, UDP-glucose, UDP-rhamnose, and UDP-xylose; and the glycosyltransferase enzyme is selected from the group consisting of 3-O-glycotransferase (3GT) and 5-O-glycotransferase (5GT).

22. The method of any one of claims 1 to 21, wherein the anthocyanin is cyanidin, the glycosylated uridine-5'-diphosphate is UDP-glucose, and the glycosyltransferase enzyme is 3-O-glycotransferase (3GT).