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(54) Title: METHODS AND CELLS FOR PRODUCTION OF TAXOL PRECURSORS

(57) Abstract: The present invention relates to the field of plant diterpenoid synthesis, and in particular host cells, methods and uses therefor. More specifically, the invention relates to production of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, as well as enzymes and host cells useful for such production.

METHODS AND CELLS FOR PRODUCTION OF TAXOL PRECURSORS

Technical field

5 The present invention relates to the field of plant diterpenoid synthesis, and in particular host cells, methods and uses therefor. More specifically, the invention relates to production of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, as well as enzymes and host cells useful for such production.

Background

10 Paclitaxel, also known as taxol, is one of the most effective anticancer drugs ever developed. It is used for the treatment of melanoma, ovarian, breast, bladder, prostate and esophageal cancer (Cragg, G. M., 1998).

15 However, its current production is particularly costly, pressing for the development of sustainable and cost-effective production methods. Production of paclitaxel in microbial or plant cell factories will reduce its cost, increase its availability, and enable the efficient synthesis of derivatives with improved pharmacological properties. Nevertheless, progress in this direction has been hampered by incomplete knowledge of the biosynthetic pathway in the native host, the yew tree (*Taxus* spp.).

20 One of the structures that is essential to paclitaxel (taxol) anti-cancer activity is hydroxy-group on the C2' position of the C13 side chain. However, an efficient enzyme that catalyses this reaction has not yet been reported and therefore complete biosynthesis of taxol in a heterologous host has not been achieved.

25

Summary

Hitherto taxol has been semi-synthesised due incomplete understanding of the biosynthesis, lack of or ineffective catalytic enzymes hampering *de novo* heterologous biosynthesis. The inventors of the present disclosure have realised efficient
30 heterologous production of the immediate taxol precursor N-debenzoyl-taxol and a precursor thereof, namely 10-deacetyl-N-debenzoyl-taxol, both comprising the important hydroxy-group (OH-group) on the C2' position of the C13 side chain. This hydroxylation is of great importance to the anti-cancer activity of taxol. Thus, the invention allows for efficient heterologous production of taxanes comprising a hydroxy-

group on the C2' position of the C13 side chain, in other words taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety.

Thus, the invention allows for heterologous production of taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety, such as the immediate precursor(s) of taxol, such as N-debenzoyl-taxol and/or 10-deacetyl-N-debenzoyl-taxol. Thereby efficient production of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, taxol and/or other taxanes is enabled, which may both reduce production costs and environmental footprint of producing these bioactive compounds. Heterologous production using microorganisms may furthermore allow for controlled production processes and potentially reduce the level of purification and associated resources and costs therefor for obtaining safe-to-consume medicaments.

In particular, the present disclosure provides novel host cells, enzymes, methods and uses for biosynthesis of taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety, for example the immediate precursor(s) of taxol, such as N-debenzoyl-taxol and/or 10-deacetyl-N-debenzoyl-taxol.

It is a main aspect of the present disclosure to provide host cells comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity thereto. The host cells of the invention are preferably capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably wherein said taxane comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or paclitaxel (taxol).

A further main aspect is to provide methods of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:

- i. providing a host cell described herein;
 - ii. cultivating said host cell in a cultivation medium,
- thereby producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. Preferably said taxane having a side chain comprising a

hydroxylated β -phenylalanine moiety is produced in the presence of baccatin III, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol. Production in the presence of aforementioned compounds may be obtained in multiple
5 ways. Baccatin III, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol may be present in the cultivation medium, e.g. said compounds may be added to the cultivation medium. Alternatively, said host cells are capable of producing baccatin III, 10-deacetyl-baccatin III, β -phenylalanine, β -
10 phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

It is also an aspect of the disclosure to provide use of an oxidase from *Taxus chinensis*, such as a 2-oxoglutarate-dependent oxygenase from *T. chinensis* or a 2-oxoglutarate-
15 dependent dioxygenase from *T. chinensis*, preferably a 2-oxoglutarate-dependent dioxygenase from *T. chinensis*, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. In preferred embodiments, said *T. chinensis* oxidase is OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 71% sequence identity,
20 such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, such as at least 76% sequence identity, such as at least 77% sequence identity, such as at least 78% sequence identity, such as at least 79% sequence identity, such as at least 80% sequence identity, such as at least 81% sequence identity, such as at least 82%
25 sequence identity, such as at least 83% sequence identity, such as at least 84% sequence identity, such as at least 85% sequence identity, such as at least 86% sequence identity, such as at least 87% sequence identity, such as at least 88% sequence identity, such as at least 89% sequence identity, such as at least 90% sequence identity, such as at least 91% sequence identity, such as at least 92%
30 sequence identity, such as at least 93% sequence identity, such as at least 94% sequence identity, such as at least 95% sequence identity, such as at least 96% sequence identity, such as at least 97% sequence identity, such as at least 98% sequence identity, such as at least 99% sequence identity thereto.

The terms “2-oxoglutarate-dependent oxygenase”, “2-oxoglutarate-dependent dioxygenase”, “Fe(II) and 2-oxoglutarate-dependent dioxygenases”, “Fe(II) and α -ketoglutarate-dependent dioxygenases”, “ α -ketoglutarate-dependent dioxygenases” and “ α -ketoglutarate-dependent oxygenases” are interchangeable.

5

It is also an aspect of the present disclosure to provide a nucleic acid construct for expression in a host cell, comprising a nucleic acid encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 8 or a homologue thereof having at least 70% sequence identity, for example at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity thereto.

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Provided is also an isolated polypeptide as set forth in SEQ ID NO: 3 or a functional homologue or variant thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity thereto, such as at least 85% sequence identity, such as at least 90% sequence identity thereto, such as at least 95% sequence identity, or more.

20

Furthermore, provided is also vectors comprising one or more of the above nucleic acid constructs, as well as host cells comprising said nucleic acid constructs and/or vectors.

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Also provided herein is a kit of parts comprising a host cell as described herein, and/or a nucleic acid construct as described herein, and/or a vector as described herein and optionally a host cell to be modified, and further optionally instructions for use.

30

Also provided is the use of the nucleic acid constructs, vectors and/or host cells for production of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

Provided herein is also a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-taxol obtained by a method, a host cell and/or a use described herein.

- 5 Provided is also cell cultures obtained by the methods and/or comprising the host cells described herein.

Furthermore, also provided is a fermentation liquid comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said fermentation
10 liquid is obtained by a method, comprised in a cell culture, and/or comprised within and/or secreted by a host cell described herein.

Provided is also compositions comprising a fermentation liquid described herein, and/or a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, N-
15 debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol obtained by a method described herein.

Further provided is methods for treating a disorder such as cancer, comprising administration of a medicament comprising a composition obtained by a method, a host
20 cell, and/or a use described herein, said composition comprising of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. Preferably, said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol.

25 Description of Drawings

Figure 1. a) Biosynthesis of N-debenzoyl-taxol in tobacco by expressing a coenzyme A ligase mutant (CoAL(A312G, SEQ ID NO: 2 encoded by SEQ ID NO: 7) from *Penicillium chrysogenum* (CoAL(A312G)), an amino phenylpropanoyl transferase from *Taxus cuspidata* (BAPT, SEQ ID NO: 1 encoded by SEQ ID NO: 6) and an oxidase
30 from *Taxus chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8). b) UPLC-HRMS chromatograms (EIC, positive mode) of methanol extracts of tobacco (*Nicotiana benthamiana*) leaves transiently expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6) and OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) reveal the production of N-debenzoyl-taxol
35 (compound 3, $[M+H]^+$ 750.3120 $\pm$ 0.01, C₄₀H₄₇NO₁₃). The extract of tobacco leaves only

expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6) was used as negative control and reveal no production of compound 3. Instead, N-debenzoyl-2'-deoxy-taxol (compound 2, $[M+H]^+$ 734.3171 $\pm$ 0.01, $C_{40}H_{47}NO_{12}$), the substrate of OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8), was accumulated. 200mg/l β -phenylalanine and 200mg/l baccatin III (compound 1) were fed to tobacco leaves after agro-infiltration. Chemically synthesized compounds (compound 2 and 3) or commercially purchased compound 1 were used as standard. P19 (SEQ ID NO: 5 encoded by SEQ ID NO: 10) was co-expressed in all samples to help suppress gene silencing.

Figure 2. a) Production of N-debenzoyl-taxol in *S. cerevisiae* (baker's yeast) by expressing an A312G mutant (CoAL(A312G), SEQ ID NO: 2 encoded by SEQ ID NO: 7) of coenzyme A ligase from *Penicillium chrysogenum* (CoAL), an amino phenylpropanoyl transferase from *Taxus cuspidata* fused with MBP (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9) and an oxidase from *Taxus chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8). BAPT was fused with a maltose binding protein from *E.coli* (MBP) on the N terminus to increase BAPT solubility. b) UPLC-HRMS chromatograms (EIC, positive mode) of ethyl acetate extracts expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), MBPig3BAPT (SEQ ID NO: 4 encoded by SEQ ID NO: 9) and OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) revealed the production of N-debenzoyl-taxol (compound 3, $[M+H]^+$ 750.3120 $\pm$ 0.01, $C_{40}H_{47}NO_{13}$). The extract of yeast cultures only expressing CoAL(A312G) and MBPig3BAPT was used as negative control and revealed no production of compound 3. Instead, N-debenzoyl-2'-deoxy-taxol (compound 2, $[M+H]^+$ 734.3171 $\pm$ 0.01, $C_{40}H_{47}NO_{12}$), the substrate of OD3 accumulated. Chemically synthesized compounds (2 and 3) or commercially purchased compound 1 were used as standard.

Figure 3. a) Production of 10-deacetyl-N-debenzoyl-taxol in tobacco by expressing an A312G mutant (CoAL(A312G), SEQ ID NO: 2 encoded by SEQ ID NO: 7) of coenzyme A ligase from *Penicillium chrysogenum* (CoAL), an amino phenylpropanoyl transferase from *Taxus cuspidata* (BAPT, SEQ ID NO: 1 encoded by SEQ ID NO: 6) and an oxidase from *Taxus chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8). b) UPLC-HRMS chromatograms (EIC, positive mode) of methanol extracts of tobacco (*Nicotiana benthamiana*) leaves transiently expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6) and

OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) revealed the production of 10-deacetyl-N-debenzoyl-taxol (compound 6, $[M+H]^+$ 708.3015 $\pm$ 0.01, $C_{38}H_{45}NO_{12}$). The extract of tobacco leaves only expressing CoAL(A312G) and BAPT was used as negative control and revealed no production of compound 6. Instead, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, $[M+H]^+$ 692.3065 $\pm$ 0.01, $C_{38}H_{45}NO_{11}$), the substrate of OD3 accumulated. 200mg/l β -phenylalanine and 200 mg/l 10-deacetyl-baccatin III (compound 4) were fed to tobacco leaves after agro-infiltration. Chemically synthesized compounds (5 and 6) or commercially purchased compound 4 were used as standards. P19 (SEQ ID NO: 5 encoded by SEQ ID NO: 10) was co-expressed in all samples to help suppress gene silencing.

Figure 4. a) Production of N-debenzoyl-2'-deoxy-taxol and N-debenzoyl-taxol in tobacco by expression of DBAT from *Taxus cuspidata* (SEQ ID NO: 21 encoded by SEQ ID NO: 23). b) UPLC-HRMS chromatograms (EIC, positive mode) of methanol extracts of tobacco (*Nicotiana benthamiana*) leaves transiently expressing DBAT from *Taxus cuspidata* (SEQ ID NO: 21 encoded by SEQ ID NO: 23) revealed production of N-debenzoyl-taxol (compound 3, $[M+H]^+$ 750.3120 $\pm$ 0.01, $C_{40}H_{47}NO_{13}$) and N-debenzoyl-2'-deoxy-taxol (compound 2, $[M+H]^+$ 734.3171 $\pm$ 0.01, $C_{40}H_{47}NO_{12}$) when substrates 10-deacetyl-N-debenzoyl-taxol (compound 6, $[M+H]^+$ 708.3015 $\pm$ 0.01, $C_{38}H_{45}NO_{12}$) and 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, $[M+H]^+$ 692.3065 $\pm$ 0.01, $C_{38}H_{45}NO_{11}$) were fed individually. Tobacco plants not expressing DBAT were used as negative control, and the fed substrates 10-deacetyl-N-debenzoyl-taxol (compound 6, $[M+H]^+$ 708.3015 $\pm$ 0.01, $C_{38}H_{45}NO_{12}$) and 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, $[M+H]^+$ 692.3065 $\pm$ 0.01, $C_{38}H_{45}NO_{11}$) were not acetylated.

Figure 5. Production of N-debenzoyl-taxol ($[M+H]^+$ 750.3120 $\pm$ 0.01, $C_{40}H_{47}NO_{13}$) in *E. coli*.

a). Biochemical pathway for the production of N-debenzoyl-taxol (compound 3) from baccatin III (BACIII, compound 1) in *E. coli* by expressing a coenzyme A ligase mutant (CoAL(A312G); SEQ ID NO: 2 encoded by SEQ ID NO: 7), an amino phenylpropanoyl transferase from *Taxus cuspidata* fused with a maltose binding protein through a four amino acid linker IG3 (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9), and an oxidase from *Taxus chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8).

b). UPLC-HRMS chromatograms (EIC, positive mode) of ethyl acetate extracts of *E. coli* cells expressing different enzymes of the N-debenzoyl-taxol biosynthetic pathway and supplied with 100 mg/L baccatin III (compound 1) and 100 mg/L β -phenylalanine.

Chromatogram I: The extract of *E. coli* cells (strain ETL001) expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and BAPT fused with maltose binding protein MBP using an IG3 amino acid linker (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9) revealed no production N-debenzoyl-taxol (compound 3, $[M+H]^+$ 750.3120 $\pm$ 0.01, C₄₀H₄₇NO₁₃). Instead, N-debenzoyl-2'-deoxy- taxol (compound 2) accumulated.

Chromatogram II: Analysis of *E. coli* cells (strain ETL002), co-expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT fused with maltose binding protein MBP through an IG4 amino acid linker (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9), and OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8), revealed the production of N-debenzoyl-taxol (compound 3) and the consumption of the OD3 substrate N-debenzoyl-2'-deoxyl-taxol (compound 2).

Chromatogram III: Chemically synthesized compounds 2 and 3 or commercially available compound 1 and taxol were used as standards.

Figure 6. Production of 10-deacetyl-N-debenzoyl-taxol ($[M+H]^+$ 708.3015 $\pm$ 0.01, C₃₈H₄₅NO₁₂) in *E. coli*.

a. Biochemical pathway for the production of 10-deacetyl-N-debenzoyl-taxol (compound 6) from 10-deacetyl-baccatin III (10-DAB, compound 4) in *E. coli* by expressing a coenzyme A ligase mutant (CoAL(A312G), SEQ ID NO: 2 encoded by SEQ ID NO: 7), an amino phenylpropanoyl transferase from *Taxus cuspidata* fused with a maltose binding protein through a four amino acid linker IG3 (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9), and an oxidase from *T. chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8).

b) UPLC-HRMS chromatograms (EIC, positive mode) of ethyl acetate extracts of *E. coli* cells expressing different enzymes of the N-debenzoyl-taxol biosynthetic pathway and supplied with 100 mg/L 10-deacetyl-baccatin III (compound 4) and 100 mg/L β -phenylalanine.

Chromatogram I: Extract of *E. coli* cells (strain ETL001) expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and BAPT fused with maltose binding protein MBP using an IG3 amino acid linker (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9) revealed no production 10-deacetyl-N-debenzoyl-taxol (compound 6,

[M+H]⁺708.3015±0.01, C₃₈H₄₅NO₁₂). Instead, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5) accumulated.

Chromatogram II: Analysis of *E. coli* cells (strain ETL002) co-expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT fused with maltose binding protein MBP through an IG3 amino acid linker (SEQ ID NO: 28) (MBPig3BAPT, SEQ ID NO: 4 encoded by SEQ ID NO: 9), and OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) revealed the production of 10-deacetyl-N-debenzoyl-taxol (compound 6) and consumption of the OD3 substrate 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5).

Chromatogram III: Chemically synthesized compounds 5 and 6 or commercially available compound 4 and taxol were used as standards.

Figure 7. OD3 variants at amino acid positions 169 and 195 retain their ability to synthesize N-debenzoyl-taxol.

a) Biochemical pathway for the production of N-debenzoyl-taxol (compound 3) from baccatin III (BACIII, compound 1) in tobacco by expressing a coenzyme A ligase mutant (CoAL(A312G), SEQ ID NO: 2 encoded by SEQ ID NO: 7), an amino phenylpropanoyl transferase from *Taxus cuspidata* BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6), and an oxidase from *Taxus chinensis* (OD3, SEQ ID NO: 3 encoded by SEQ ID NO: 8).

b) UPLC-HRMS chromatograms (EIC, positive mode) of methanol extracts of tobacco leaves transiently expressing different enzymes of the N-debenzoyl-taxol biosynthetic pathway and supplied with 200 mg/L baccatin III (compound 1) and 200 mg/L β-phenylalanine after agro-infiltration. P19 (SEQ ID NO: 5 encoded by SEQ ID NO: 10) was co-expressed in all samples to help suppress gene silencing.

Chromatogram I: The extract of tobacco leaves only expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6) was used as negative control and revealed no production of compound 3. Instead, N-debenzoyl-2'-deoxy-taxol (compound 2, [M+H]⁺ 734.3171±0.01, C₄₀H₄₇NO₁₂), the substrate of OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8), accumulated.

Chromatogram II: The extract of tobacco leaves expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6), and OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) from *Taxus chinensis* reveal production of compound 3 and almost full consumption of compound 2, the substrate of OD3.

Chromatogram III-IV: The extract of tobacco leaves expressing CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7), BAPT (SEQ ID NO: 1 encoded by SEQ ID NO: 6),

and two mutants of OD3 (SEQ ID NO: 3), OD3(N195A) (SEQ ID NO: 24 encoded by SEQ ID NO: 25) or OD3(Y169F) (SEQ ID NO: 26 encoded by SEQ ID NO: 27) individually from *Taxus chinensis* reveal production of compound 3 and almost full consumption of compound 2, the substrate of OD3.

- 5 *Chromatogram V*: Chemically synthesized compounds (compound 2 and 3) or commercially purchased compound 1 were used as standard.

Detailed description

Definitions

- 10 The term “as set forth in” is herein used as equivalent to “as set out in”, “as described in”, “as depicted in”, “being the same as”, and/or “according to”. For example “[.] OD3 as set forth in SEQ ID NO: 3” herein means, that OD3 has the sequence of SEQ ID NO: 3. Another example is “[.] taxol as set forth in structure (VI)”, which herein implies that structure (VI) is the structure of the compound taxol. It follows that “as set forth in”
15 denotes that something “is the same as” and is therefore to be construed as “being limited to”.

- The term “in the presence of” with respect to a compound herein refers to that said compound is available to a host cell, either because it is provided to the host cell and/or
20 because it is synthesised by said host cell. For example, “a host cell cultivated in the presence of baccatin III” herein implies that baccatin III is either provided to the cell, such as supplied to or comprised in the cultivation medium, or that the host cell is producing or is capable of producing baccatin III.

- 25 As used herein, the singular forms “a”, “an” and “the” include plural referents unless the context clearly states otherwise.

- The terms “incubating” and “cultivating” are used interchangeably herein, and refers to maintaining host cells under culture conditions, which allow the cells to grow.
30 Preferably, said culture conditions allow expression of the enzyme(s) encoded by the heterologous gene(s) contained in said host cells. Preferably, the host cells are incubated under culture conditions allowing said host cells to a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol. In embodiments where the host cell is
35 contained within a multicellular organism (e.g. a plant), “cultivating” or “incubation”

refers to maintaining said multicellular organisms under conditions allowing said multicellular organism to grow. In embodiments where the host cell is a unicellular organism, "cultivating" or "incubating" refers to maintaining said unicellular organism under conditions allowing said unicellular organism to grow and/or multiply.

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The term "enzyme" as used herein refers to proteins or polypeptides, which are capable of catalysing biochemical reactions. Further, unless context dictates otherwise, as used herein "enzyme" includes protein fragments that retain the relevant catalytic activity, and may include artificial enzymes synthesized to retain the relevant catalytic activity.

10

The terms "functional homologue" or "variant" of an amino acid sequence, refers to a polypeptide comprising said amino acid sequence with the proviso that one or more amino acids are substituted, deleted, added, and/or inserted, and which polypeptide has (qualitatively) the same enzymatic functionality for substrate conversion. The terms "functional homologue" or "homologue" of a nucleic acid encoding a polypeptide, refers to a nucleic acid comprising said nucleic acid sequence with the proviso that one or more nucleobases are substituted, deleted, added, and/or inserted, and which nucleic acid encodes a polypeptide, which polypeptide has (qualitatively) the same enzymatic functionality for substrate conversion as the polypeptide encoded by said nucleic acid.

15

20

Nucleic acids or nucleic acid sequence may also be referred to as polynucleotides.

Preferably, a functional homologue or a variant shares at least 70% sequence identity, preferably at least 80%, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at

25

least 98% sequence identity to said amino acid sequence. Preferably, a functional homologue, homologue or variant shares at least 70% sequence identity, preferably at least 80%, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to the amino acid sequence encoded by said nucleic acid sequence.

30

Preferably, a functional homologue or homologue shares at least 70% sequence identity, preferably at least 80%, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to said nucleic acid sequence.

The term "heterologous nucleic acid" refers to a nucleic acid, which has been inserted into a host cell or into a progenitor of the host cell, e.g. by recombinant or transgenic

35

methods. The respective protein or RNA encoded by a heterologous nucleic acid is also referred to as "heterologous". The heterologous nucleic acid may be part of a non-integrated nucleic acid, e.g. a vector, including but not limited to a plasmid. Preferably, the heterologous nucleic acid(s) are integrated into the host cell genome.

5

The expressions "nucleic acid encoding" and "nucleic acid sequence encoding" are used interchangeably herein.

10

The term "host cell" refers to a cell, which comprises one or more heterologous nucleic acids.

15

The term "polypeptide" as used herein refers a sequential chain of amino acids linked together via peptide bonds. The term is used to refer to an amino acid chain of any length. As is known to those skilled in the art, polypeptides may be processed and/or modified, and the term polypeptide may refer to both unmodified or modified polypeptides.

20

The term "sequence identity" as used herein describes the relatedness between two amino acid sequences or between two nucleotide sequences, i.e. a candidate sequence (e.g. a mutant sequence) and a reference sequence (such as a wild type sequence) based on their pairwise alignment. For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277,), preferably version 5.0.0 or later (available at https://www.ebi.ac.uk/Tools/psa/emboss_needle/). The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of 30 BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

30

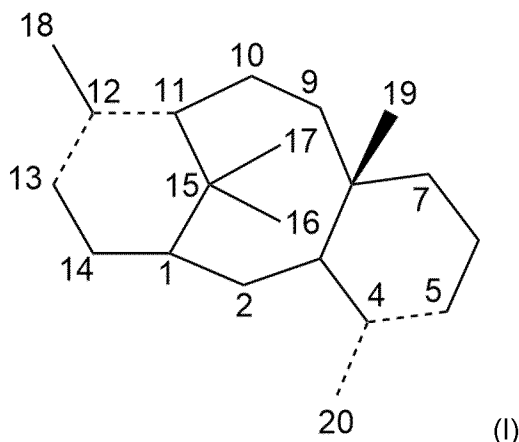
$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

The Needleman-Wunsch algorithm is also used to determine whether a given amino acid in a sequence other than the reference sequence corresponds to a given position in a reference sequence.

For purposes of the present invention, the sequence identity between two nucleotide
5 sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the DNAFULL
10 (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment - Total Number of Gaps in Alignment). Sequence identity is calculated over the entire length of the reference
15 sequence.

The term "taxane" herein comprises a class of diterpenoid compounds comprising a common core skeleton set forth in (I):



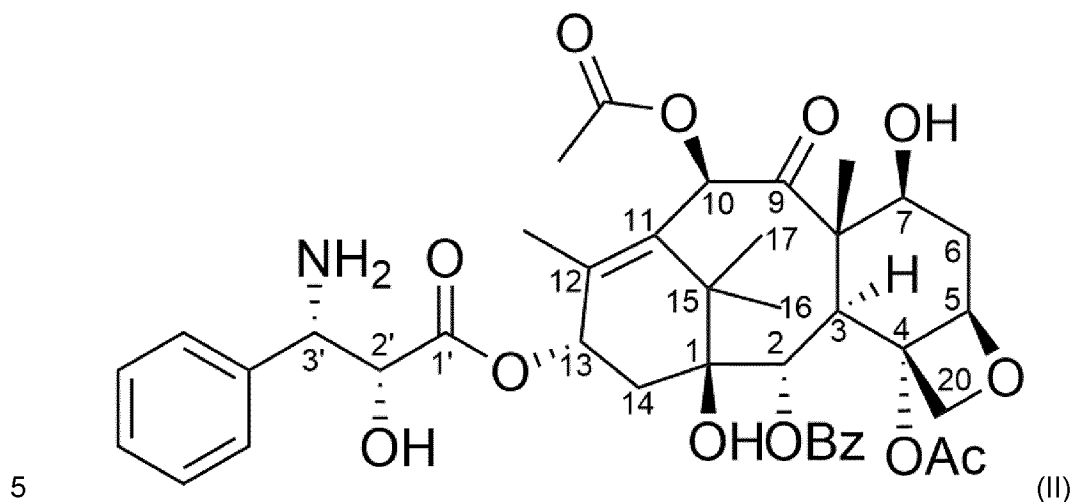
and wherein the core may further be substituted, preferably the core may be further
20 substituted at positions 1, 2, 4, 5, 7, 9, 10, 11, 13, and/or 20. With regards to the structure set forth in (I), the dashed lines indicates either a single or a double bond, with the proviso that only one of the bonds between position 11 and 12 or position 12 and 13 may be a double bond simultaneously and only one of the bonds between position 4 and 5 or position 4 and 20 may be a double bond simultaneously. The atom
25 at position 20 may be carbon (C) or oxygen (O) and may further be substituted. In particular, the atoms 4, 5 and 20 may be connected to form a ring, preferably an

oxetane ring. Taxoids are taxadiene-derived diterpenoids. A preferred type of taxanes are taxoid compounds and/or taxoids. Taxanes have various structures and may be substituted with different groups, such as different functional groups. Non-limiting examples of taxanes of structure (I) are taxol (paclitaxel, VI), N-debenzoyl-taxol (II), N-debenzoyl-2'-deoxy-taxol (III), 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (IV), 10-deacetyl-N-debenzoyl-taxol (V), and/or baccatin III. Unless otherwise specified the numbering of atoms of taxanes, such as a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety and/or a taxane having a side chain comprising a β -phenylalanine moiety, used herein is as indicated in formula (I).

In agreement with the above definition, the term "taxane having a side chain comprising a β -phenylalanine moiety" herein refers to a taxane of structure (I) substituted with a side chain comprising a β -phenylalanine moiety, preferably at position 13, i.e. at C13. Herein β -phenylalanine moiety refers to β -phenylalanine covalently bound to another moiety. The structure of free β -phenylalanine is provided in Figure 1a. The terms side chain comprising a β -phenylalanine moiety, side chain comprising β -phenylalanine, and β -phenylalanine side chain, are used interchangeably herein. Furthermore, the terms C13 side chain, C13 side chain comprising β -phenylalanine and C13 side chain comprising a β -phenylalanine moiety all refer to a side chain comprising a β -phenylalanine moiety positioned at C13 of a taxane of structure (I). If nothing else is specified, the term "taxane side chain" is also used to refer to a C13 side chain comprising a β -phenylalanine moiety. In accordance with this, the term "taxane having a side chain comprising a hydroxylated β -phenylalanine moiety" herein refers to a taxane of structure (I) substituted with a side chain comprising a hydroxylated β -phenylalanine moiety, preferably at position 13, i.e. at C13, of structure (I), preferably wherein position 2', i.e. C2' or 2'C, of said side chain is hydroxylated. Thus, the terms side chain comprising a hydroxylated β -phenylalanine moiety, side chain comprising hydroxylated β -phenylalanine, hydroxylated β -phenylalanine side chain, and/or hydroxylated taxane side chain all refer to a side chain comprising a hydroxylated β -phenylalanine moiety, preferably positioned at C13 of a taxane of structure (I), preferably position 2', i.e. C2' or 2'C, of said side chain is hydroxylated. Preferably said side chain comprising a hydroxylated β -phenylalanine moiety is a side chain comprising a 2'C hydroxylated β -phenylalanine moiety or a side chain comprising a 3'N-benzoylated-2'C hydroxylated β -phenylalanine moiety. Furthermore, the terms hydroxylated C13 side chain, C13 side chain comprising

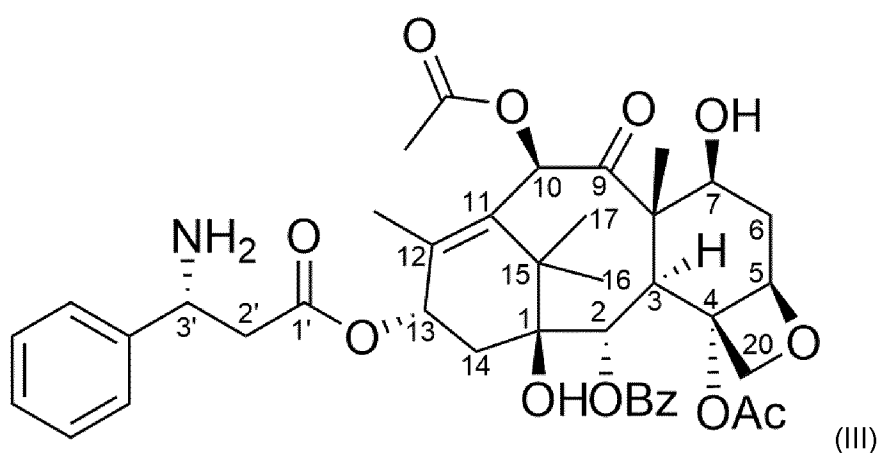
hydroxylated β -phenylalanine, and/or C13 side chain comprising a hydroxylated β -phenylalanine moiety all refer to a side chain comprising a hydroxylated β -phenylalanine moiety positioned at C13 of a taxane of structure (I).

The term “N-debenzoyl-taxol” refers to the chemical structure as set forth in (II):



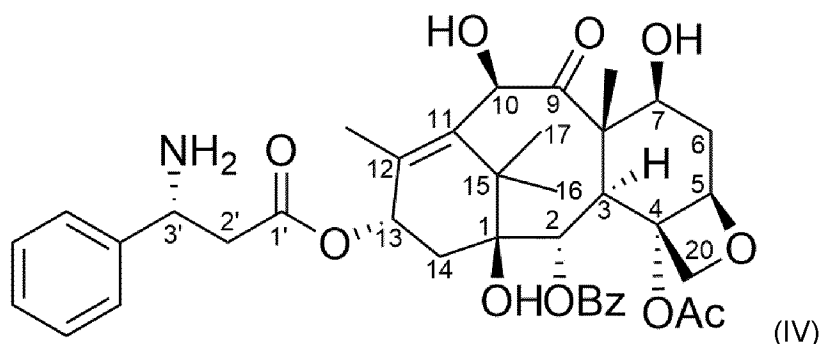
“Bz” of structure (II) denotes a benzoyl group and “Ac” denotes an acetyl group. N-debenzoyl-taxol may also be referred to as compound 3 or (3) herein, and the terms are used interchangeably.

10 The term “N-debenzoyl-2'-deoxy-taxol” refers to the chemical structure as set forth in (III):



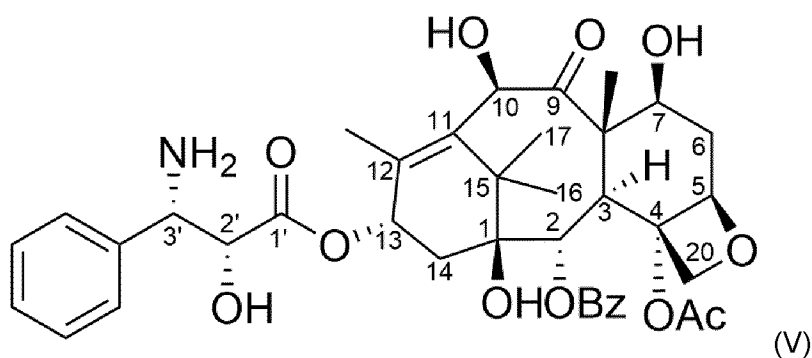
“Bz” of structure (III) denotes a benzoyl group and “Ac” denotes an acetyl group. N-debenzoyl-2'-deoxy-taxol may also be referred to as compound 2 or (2) herein, and the terms are used interchangeably.

The term “10-deacetyl-N-debenzoyl-2’-deoxyl-taxol” refers to the chemical structure as set forth in (IV):



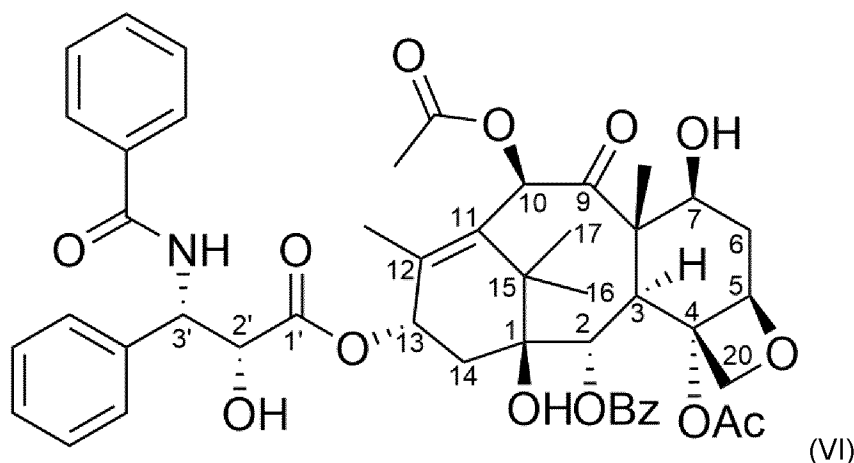
“Bz” of structure (IV) denotes a benzoyl group and “Ac” denotes an acetyl group. 10-deacetyl-N-debenzoyl-2'-deoxy-taxol may also be referred to as compound 5 or (5) herein, and the terms are used interchangeably.

The term “10-deacetyl-N-debenzoyl-taxol” refers to the chemical structure as set forth in (V):



10 “Bz” of structure (V) denotes a benzoyl group and “Ac” denotes an acetyl group. 10-deacetyl-N-debenzoyl-taxol may also be referred to as compound 6 or (6) herein, and the terms are used interchangeably.

The term “taxol” herein refers to the chemical structure as set forth in (VI):



“Bz” of structure (VI) denotes a benzoyl group and “Ac” denotes an acetyl group. Taxol may also be referred to as paclitaxel and the terms are used interchangeably.

5 The term “capable of producing” with reference to a host cell comprising certain enzymes, herein implies that in the presence of suitable/appropriate substrate(s)/precursor(s), the host cell also converts the substrate(s)/precursor(s) to the intended product(s) of the catalyzed reaction(s), when the cell expresses the required enzymes as described herein.

Oxidase

10 The present disclosure provides host cells, methods, uses and polypeptides for production of diterpenoids, notable taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety, such as N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol. The host cell to be used with the present disclosure comprises a heterologous nucleic acid encoding an enzyme capable of hydroxylating

15 C2' of the C13 side chain, i.e. the so-called taxane side chain, or the side chain comprising a β -phenylalanine moiety, of taxanes.

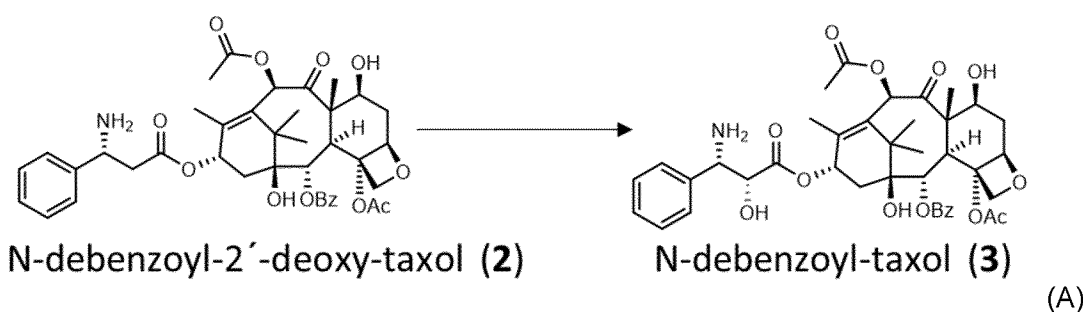
20 Production of taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety, for example N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol, may be obtained by expression of an oxidase enzyme, either alone or together with one or more polypeptides of different activity as described herein, for example below in the sections “Host cell” or “Polypeptides and nucleic acids”, in a host cell of the present disclosure. The oxidase preferable have the enzyme activity described in this section.

The oxidase described herein is capable of catalysing formation of hydroxyl-group (OH) at position 2' of the side chain comprising a β -phenylalanine moiety, i.e. said oxidase is capable of hydroxylating position 2' of the C13 side chain. In other words, said oxidase is capable of catalysing formation of taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety from taxanes having a side chain comprising a β -phenylalanine moiety.

OD3

The host cell to be used with the present disclosure may preferably comprise a heterologous nucleic acid encoding an enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. The invention also provides such enzyme as well as method using same.

It is preferred that said enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is capable of catalysing formation of N-debenzoyl-taxol as set forth in structure (II). In other words, it is preferred that said enzyme is capable of catalysing the following reaction (A):

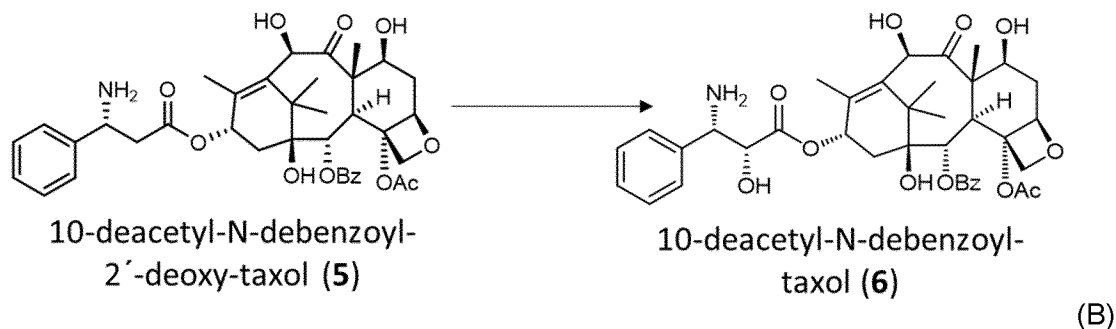


In other words, the oxidase capable of catalysing hydroxylation of position 2' of the C13 side chain of taxanes may be capable of catalysing reaction A outlined above. Said reaction may also be referred to as 2' α hydroxylation.

N-debenzoyl-2'-deoxy-taxol may also be referred to as 3'-N-dehydroxydebenzoyltaxol, β -phenylalanoyl baccatin III and/or 13-O- β -phenylalanoylbaccatin III, and the names may be used interchangeably.

In another preferred embodiment, the enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is capable of catalysing formation of 10-deacetyl-N-debenzoyl-taxol as set forth in

structure (V). In other words, it is preferred that said enzyme is capable of catalysing the following reaction (B):



- 5 In other words, the oxidase capable of catalysing hydroxylation of position 2' of the C13 side chain of taxanes may be capable of catalysing reaction B outlined above.

In preferred embodiment, said enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is capable of catalysing both reactions A and B outlined above.

Thus, a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be N-debenzoyl-taxol as set forth in structure (II) and/or referred to as compound 3 herein. In other embodiments, a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be 10-deacetyl-N-debenzoyl-taxol as set forth in structure (V) and/or referred to as compound 6 herein. In further embodiments, a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be taxol as set forth in structure (VI).

20 The enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be any useful enzyme with above-mentioned activities, in particular said enzyme may be an oxidase. In some embodiments, the enzyme is a 2-oxoglutarate-dependent dioxygenase. The enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be derived from any suitable source, but in a preferred embodiment, said enzyme is an enzyme from *Taxus chinensis*. Thus, the enzyme capable of catalysing formation of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be an oxidase enzyme from *Taxus chinensis*. The oxidase enzyme may be a 2-oxoglutarate-dependent dioxygenase from *T. chinensis*.

In preferred embodiments of the present disclosure, the host cell comprises a heterologous nucleic acid encoding the oxidase enzyme OD3. Said OD3 is preferably OD3 of SEQ ID NO: 3 or a functional homologue thereof. The person skilled in the art will appreciate that OD3 or a functional homologue thereof preferably have the ability to convert N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-taxol as well as 10-deacetyl-N-debenzoyl-2'-deoxy-taxol to 10-deacetyl-N-debenzoyl-taxol as outlined in reaction A and B, respectively, depicted herein above.

10 A functional homologue of OD3 of SEQ ID NO: 3 preferably has at least 70% sequence identity, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, such as at least 76% sequence identity, such as at least 77% sequence identity, such as at least 78% sequence
15 identity, such as at least 79% sequence identity, preferably at least 80% sequence identity, such as at least 81% sequence identity, such as at least 82% sequence identity, such as at least 83% sequence identity, such as at least 84% sequence identity, preferably at least 85% sequence identity, such as at least 86% sequence identity, such as at least 87% sequence identity, such as at least 88% sequence
20 identity, such as at least 89% sequence identity, preferably at least 90% sequence identity, such as at least 91% sequence identity, such as at least 92% sequence identity, such as at least 93% sequence identity, such as at least 94% sequence identity, preferably at least 95% sequence identity, such as at least 96% sequence identity, such as at least 97% sequence identity, more preferred at least 98% sequence
25 identity thereto, preferably at least 99% sequence identity thereto.

A functional homologue of OD3 may be identified by expressing said homologue in a host cell, and either purifying the produced enzyme to perform an *in vitro* enzyme activity assay or by performing an *in vivo* enzyme activity assay, in order to measure
30 the conversion of N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-taxol and/or the conversion of 10-deacetyl-N-debenzoyl-2'-deoxy-taxol to 10-deacetyl-N-debenzoyl-taxol using standard techniques. For example, the activity may be measured as described in Example 1 to 4. Non-limiting examples of functional homologues of OD3 of SEQ ID NO: 3 are OD3(Y169F) as set forth in SEQ ID NO: 26 and OD3(N195A) as
35 set forth in SEQ ID NO: 24.

The heterologous nucleic acid encoding OD3 of SEQ ID NO: 3 or a functional homologue thereof, may have any sequence encoding said OD3. In some embodiments, the nucleic acid encoding OD3 is a nucleic acid as set forth in SEQ ID NO: 8, or a functional homologue thereof encoding a functional homologue of OD3 having at least 70% sequence identity to the OD3 encoded by SEQ ID NO: 8.

Host cells

The present disclosure relates to host cells comprising one or more heterologous nucleic acids encoding enzymes of the biosynthetic pathway towards taxol, i.e. taxoid pathway.

A main aspect of the present disclosure is to provide host cells comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 3.

Thus, in a preferred embodiment the host cell comprises a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 3.

A heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto may preferably be "a heterologous nucleic acid encoding a protein comprising or consisting of SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto". Similarly, a nucleic acid sequence encoding an enzyme of a sequence disclosed herein or a functional homologue thereof, may preferably be a nucleic acid encoding a protein comprising or consisting of said sequence or a functional homologue thereof.

35

In another preferred embodiment, the host cell comprises a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto, wherein said host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably wherein said taxane comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or paclitaxel (taxol).

In other embodiments, the host cell comprises a heterologous nucleic acid sequence encoding OD3(N195A) as set forth in SEQ ID NO: 24 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 24. In some embodiments, the host cell comprises a heterologous nucleic acid sequence encoding OD3(Y169F) as set forth in SEQ ID NO: 26 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 26.

In other embodiments, the host cell comprises a heterologous nucleic acid sequence encoding OD3(N195A) (SEQ ID NO: 24) or OD3(Y169F) (SEQ ID NO: 26), or functional homologues thereof having at least 70% sequence identity to SEQ ID NO: 24 or SEQ ID NO: 26, respectively, wherein said host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably wherein said taxane comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or paclitaxel (taxol).

In some embodiments, the host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety in the presence of baccatin III, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol. Said precursor compounds may be provided to the host cell, such as in the cultivation medium or with respect to plant cells be infiltrated in the leaves. In other embodiments, the host cell is capable of producing baccatin III, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-

debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol. Host cells capable of producing said compounds are described in the sections herein below.

- Thus, the product of the reaction catalysed by OD3 is to some extent determined by the available substrate(s)/precursor(s). For example, in the presence of 2'-deoxy-N-debenzoyl-taxol, the host cell comprising a heterologous nucleic acid sequence encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, may produce N-debenzoyl-taxol. In the presence of 10-deacetyl-N-debenzoyl-2'-deoxy-taxol the host cell comprising a heterologous nucleic acid sequence encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, may produce 10-deacetyl-N-debenzoyl-taxol. The produced N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol may be converted into taxol and 10-deacetyl-taxol, respectively, either enzymatically or by chemical synthesis upon purification of the precursors of said compounds, e.g. N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol. Similarly, the substrates 2'-deoxy-N-debenzoyl-taxol and 10-deacetyl-N-debenzoyl-2'-deoxy-taxol may be produced by the host cell via enzymatic catalysis or may be provided to the host cell, such as in the cultivation medium.
- Hence, in addition to the heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof, the host cell may also comprise one or more nucleic acids encoding one or more of the following:
- an amino phenylpropanoyl transferase (EC: 2.3.1) capable of converting baccatin III and β -phenylalanoyl-CoA into N-debenzoyl-2'-deoxy-taxol as well as 10-deacetyl-baccatin III and β -phenylalanoyl-CoA into 10-deacetyl-N-debenzoyl-2'-deoxy-taxol;
 - a coenzyme A ligase (CoAL, EC: 6.2.1) capable of converting β -phenylalanine to β -phenylalanoyl-CoA;
 - a 10-deacetyl-baccatin III-10-O-acetyltransferase (DBAT, EC: 2.3.1) capable of converting 10-deacetyl-N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-2'-deoxy-taxol, and/or 10-deacetyl-N-debenzoyl-taxol to N-debenzoyl-taxol, and/or 10-deacetyl-baccatin III to baccatin III; and/or
 - a 3'-N-debenzoyl-2'-deoxytaxol-N-benzoyltransferase (DBTNBT, EC: 2.3.1) described in the section "DBTNBT" herein.

These enzyme activities and host cells comprising said activities are described further herein below.

5 It is preferred that the host cell is capable of producing GGPP, baccatin III, 10-deacetyl-baccatin III, and/or β -phenylalanine.

10 In some embodiments the host cell may further comprise one or more of the heterologous nucleic acids described in patent application EP23386045.1 or in a patent application claiming priority thereof, such as PCT/EP2024/065772 (published as WO2024/251968).

Organisms

15 In some embodiments of the present disclosure, said host cell is selected from the group of plant cells, yeast cells, bacterial cells and fungal cells.

In some embodiments the host cell is comprised within a multicellular organism. In such embodiments, only some of the cells of said multicellular organism may comprise heterologous nucleic acid(s) and/or heterologous polypeptide(s). It is however preferred that all cells of said multicellular organism are host cells that comprise the same heterologous nucleic acids(s) and/or heterologous polypeptide(s).

20

In some embodiments of the present disclosure, said host cell is plant cells, such as plant cells comprised within a plant, within a part of a plant and/or within the seeds of said plant. Preferably, all cells of said plant or part thereof are host cells comprising the same heterologous nucleic acid(s) and/or polypeptide(s).

25

In some embodiments of the present disclosure, the host cells are plant cells, such as plant cells from a species of *Nicotiana*, such as *Nicotiana benthamiana* or *Nicotiana tabacum*. The person skilled in the art will appreciate that a "plant cell" as used within the present invention refers to a structural and physiological unit of a plant, e.g. a tobacco plant. The plant cell may be in form of a protoplast without a cell wall, an isolated single cell or a cultured cell, or as a part of higher organized unit such as but not limited to, plant tissue, a plant organ, or a whole plant.

30

In some embodiments of the present disclosure, the host cell is a yeast cell, such as yeast cell belonging to the genus of *Saccharomyces*, *Pichia*, *Candida*, *Cryptococcus*, *Pichia* (*Komagataella*), *Lipomyces*, *Pseudozyma*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Trigonopsis*, *Yarrowia* or *Saccharomycopsis*, such as a yeast cell of the species *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Hansenula polymorpha* (*Ogataea polymorpha*), *Rhodotorula toruloides* or *Pichia pastoris* (*Komagataella phaffii*).

The person skilled in the art will appreciate that a fungi or fungal cell(s) as used herein refers to any cell present within or derived from an organism belonging to the Kingdom Fungi. The methods are applicable to all fungi and fungal cells that are susceptible of genetic modifications. Furthermore, the person skilled in the art will appreciate that a “yeast cell” is herein defined to include the group consisting of small, unicellular organisms capable of growth and reproduction through budding or direct division (fission), or by growth as simple irregular filaments (mycelium). The yeast cell may be transformed or transfected with a heterologous vector for expression of a nucleic acid sequence inserted into the heterologous vector. Examples of a yeast cell include, but are not limited to *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Hansenula polymorpha* (*Ogataea polymorpha*), *Rhodotorula toruloides* and/or *Pichia pastoris* (*Komagataella phaffii*), commonly used for transfection and expression of heterologous proteins.

In some embodiments of the present disclosure, the host cell is a bacterial cell, such as a bacterial cell belonging to the genus of *Escherichia*, *Bacillus*, *Corynebacterium*, *Pseudomonas* or *Streptomyces*, such as a bacterial cell of the species *Escherichia coli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Pseudomonas putida* or *Streptomyces sp.*

The person skilled in the art will appreciate that a bacterial cell includes prokaryotic cells that may be propagated in culture. The bacterial cell may act as a host cell for the recombinant expression of heterologous polypeptide(s). The bacterial cell may be transformed, transfected or infected with a vector for expression of a nucleic acid sequence inserted into the vector. Examples of suitable bacterial cells include, but are not limited to *E. coli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Pseudomonas putida* and/or *Streptomyces sp.*

The host cell or a progenitor thereof may be prepared by any useful method available to the skilled person. For example, the heterologous nucleic acid(s) may be inserted into a cell by direct uptake, transduction, f-mating, transfection, transformation, bacterial infiltration or any other methods known in the art useful for creating recombinant host cells.

In preferred embodiments, the host cell is a yeast cell, such as a yeast cell of the species *S. cerevisiae*, or a bacterial cell, such as a bacterial cell of the species *E. coli*.

Amino phenylpropanoyl transferase

The host cell comprising a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof, may further comprise a nucleic acid encoding an amino phenylpropanoyl transferase as described in this section and elsewhere.

Said amino phenylpropanoyl transferase may be an amino phenylpropanoyl transferase falling within EC no. 2.3.1. The amino phenylpropanoyl transferase may be capable of converting baccatin III and β -phenylalanoyl-CoA to N-debenzoyl-2'-deoxy-taxol. Said amino phenylpropanoyl transferase may also be capable of converting 10-deacetyl-baccatin III and β -phenylalanoyl-CoA to 10-deacetyl-N-debenzoyl-2'-deoxy-taxol. Hence, a host cell comprising a nucleic acid encoding said amino phenylpropanoyl transferase may be capable of producing N-debenzoyl-2'-deoxy-taxol and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

In some embodiments, said amino phenylpropanoyl transferase is native to a *Taxus* cell, such as a *Taxus cuspidata* cell. In other embodiments, said amino phenylpropanoyl transferase is BAPT as set forth in SEQ ID NO: 1 or MBPig3BAPT as set forth in SEQ ID NO: 4, or functional homologues thereof having at least 70% sequence identity to any of the SEQ ID NO: 1 or SEQ ID NO: 4, respectively.

The nucleic acid encoding BAPT (SEQ ID NO: 1) or a functional homologue may be the nucleic acid as set forth in SEQ ID NO: 6 or a homologue thereof having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto. The a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or a functional homologue thereof may be the nucleic

acid as set forth in SEQ ID NO: 9 or a homologue thereof having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.

5 In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively. In the
10 presence of β -phenylalanoyl-CoA and baccatin III, said host cell is capable of producing N-debenzoyl-taxol from β -phenylalanoyl-CoA and baccatin III. If instead of baccatin III then 10-deacetyl-baccatin III is present, said host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol from β -phenylalanoyl-CoA and 10-deacetyl-baccatin III. Preferably, said host cell is capable of producing β -phenylalanoyl-CoA,
15 baccatin III and/or 10-deacetyl-baccatin III, but said compounds may also be supplied to the host cell, such as in the cultivation medium, wherein said host cell is cultivated.

CoAL

20 The host cell comprising a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof, may further comprise a nucleic acid encoding a coenzyme A ligase (CoAL) as described in this section and elsewhere. Preferably, said host cell further comprises a nucleic acid encoding an amino phenylpropanoyl transferase as described herein above.

25 Said CoAL may be a CoAL falling within EC no. 6.2.1. The CoAL may be capable of converting β -phenylalanine to β -phenylalanoyl-CoA. Thus, a host cell comprising a nucleic acid encoding said CoAL may be capable of producing β -phenylalanoyl-CoA.

30 In some embodiments, said CoAL is a CoAL native to a *Penicillium* cell, such as a *P. chrysogenum* cell or a CoAL native to a *Taxus* cell, such as a *T. chinensis* cell. In other embodiments, said CoAL is CoAL(A312G) as set forth in SEQ ID NO: 2 or TchiAAE5 as set forth in SEQ ID NO: 22, or functional homologues thereof having at least 70% sequence identity to any of the SEQ ID NO: 2 or SEQ ID NO: 22, respectively. In other embodiments, said CoAL is AAE-867.5 as set forth in SEQ ID NO: 29 or a functional
35 homologue thereof having at least 70% sequence identity thereto.

Disclosed herein is also a host cell comprising a heterologous nucleic acid sequence encoding TchiAAE5 as set forth in SEQ ID NO: 22 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 22. In some embodiments, the host cell comprises a heterologous nucleic acid sequence encoding TchiAAE5 as set forth in SEQ ID NO: 22 or a functional homologue thereof having at least 70% sequence identity thereto, wherein said host cell is capable of producing β -phenylalanoyl-CoA. Preferably, said host cell is capable of converting β -phenylalanine to β -phenylalanoyl-CoA.

In some embodiments, said CoAL is the CoAL as set forth in GenBank accession no.: XP_002569052.1 or a functional homologue thereof having at least 70% sequence identity thereto.

CoAL(A312G) may also sometimes be referred to as CoAL_A312G and/or CoALA312G and the terms may be used interchangeably.

The nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof may be the nucleic acid as set forth in SEQ ID NO: 7 or a homologue thereof having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto. The nucleic acid encoding TchiAAE5 (SEQ ID NO: 22) or a functional homologue thereof may be a nucleic acid encoding the polypeptide set forth in SEQ ID NO: 22 or a functional homologue thereof having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and further comprises a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) and a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and further comprises a

nucleic acid encoding TchiAAE5 (SEQ ID NO: 22) and a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and further comprises a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) and a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In the presence of β -phenylalanine and baccatin III, said host cell is capable of producing N-debenzoyl-taxol from β -phenylalanine and baccatin III. If instead of baccatin III then 10-deacetyl-baccatin III is present, said host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol from β -phenylalanine and 10-deacetyl-baccatin III. Preferably, said host cell is capable of producing β -phenylalanine, baccatin III and/or 10-deacetyl-baccatin III, but said compounds may also be supplied to the host cell, such as in the cultivation medium, wherein said host cell is cultivated.

DBAT

The host cell comprising a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof, may further comprise a nucleic acid encoding a 10-deacetyl-baccatin III-10-O-acetyltransferase (DBAT) as described in this section and elsewhere herein. Preferably, said host cell further comprises a nucleic acid encoding CoAL and/or amino phenylpropanoyl transferase as described herein above.

Said DBAT may be a DBAT falling within EC no. 2.3.1. The DBAT may be capable of acetylating 10-deacetyl-N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-2'-deoxy-taxol.

Said DBAT may be or may also be capable of acetylating 10-deacetyl-N-debenzoyl-taxol to N-debenzoyl-taxol. Said DBAT may be or may also be capable of acetylating 10-deacetyl-baccatin III to baccatin III. Said DBAT may be or may also be capable of acetylating 10-deacetyl-taxol to taxol. Hence, a host cell comprising a nucleic acid encoding said DBAT may be capable of producing baccatin III, taxol, N-debenzoyl-2'-deoxy-taxol and/or N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III, 10-deacetyl-taxol, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol and/or 10-deacetyl-N-debenzoyl-taxol, respectively. Said DBAT may be *Tcu*DBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 21.

In some embodiments, said DBAT is native to a *Taxus* cell, such as a *Taxus cuspidata* cell. In other embodiments, the DBAT is TcuDBAT as set forth in SEQ ID NO: 21 or a functional homologue thereof having at least 70% sequence identity thereto.

- 5 The activities of producing baccatin III from 10-deacetyl-baccatin III, and taxol from 10-deacetyl-taxol, by TcuDBAT has been described elsewhere (Li et al., 2016 and Walker et al., 2000).

- 10 The nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof may be the nucleic acid as set forth in SEQ ID NO: 23 or a homologue thereof having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.

- 15 For example, a host cell comprising a nucleic acid encoding DBAT, such as TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto, is capable of producing N-debenzoyl-taxol in the presence of 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

- 20 In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and further comprises a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In the presence of β -phenylalanoyl-CoA and 10-deacetyl-baccatin III, said host cell is capable of producing N-debenzoyl-taxol from β -phenylalanoyl-CoA and 10-deacetyl-baccatin III. Preferably, said host cell is capable of producing β -phenylalanoyl-CoA and/or 10-deacetyl-baccatin III, but said compounds may also be supplied to the host cell, such as in the cultivation medium, wherein said host cell is cultivated.

- 30 In other embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:
- a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto;

- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- 5 iii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- 10 i. a nucleic acid encoding TchiAAE5 (SEQ ID NO: 22) or a functional homologue thereof having at least 70% sequence identity thereto;
- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- 15 iii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- 20 i. a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) or a functional homologue thereof having at least 70% sequence identity thereto;
- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- 25 iii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

In the presence of β -phenylalanine and 10-deacetyl-baccatin III, said host cell is capable of producing N-debenzoyl-taxol from β -phenylalanine and 10-deacetyl-baccatin III. Preferably, said host cell is capable of producing β -phenylalanine and/or 10-deacetyl-baccatin III, but said compounds may also be supplied to the host cell, such as in the cultivation medium, wherein said host cell is cultivated.

DBTNBT

The host cell comprising a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof, may further comprise a nucleic acid encoding a 3'-N-debenzoyl-2'-deoxytaxol-N-benzoyltransferase (DBTNBT) as described in this section and elsewhere. Preferably, said host cell further comprises a nucleic acid encoding DBAT, CoAL and/or amino phenylpropanoyl transferase or a nucleic acid encoding CoAL and/or amino phenylpropanoyl transferase, as described herein above.

Said DBTNBT may be a DBTNBT falling within EC no. 2.3.1. The DBTNBT may be capable of converting N-debenzoyl-taxol to taxol and/or converting N-debenzoyl-2'-deoxy-taxol to 2'-deoxy-taxol. In other words, DBTNBT is capable of substituting in a benzoyl group at the amine group attached at position 3' in the side chain comprising a β -phenylalanine moiety or at position 3' in the side chain comprising a hydroxylated β -phenylalanine moiety. Thus, a host cell comprising a nucleic acid encoding said DBTNBT may be capable of producing taxol (also known as paclitaxel). DBTNBT preferably uses benzoyl-CoA as co-substrate, when benzoylating its substrate(s), such as N-debenzoyl-taxol and N-debenzoyl-2'-deoxy-taxol. Said benzoyl-CoA may be comprised within the cultivation medium or be produced by the host cell.

In some embodiments, said DBTNBT is native to a *Taxus* cell, such as a *Taxus canadensis* cell. In other embodiments, said DBTNBT is TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto.

The activity of TcaDBTNBT has been described elsewhere (Walker, K. et al., 2002, Long, RM. Et al., 2009).

N-debenzoyl-taxol may also be referred to as 3'-N-debenzoyltaxol, N-debenzoyltaxol, N-debenzoyltaxol A, 3'-N-debenzoyltaxol, N-debenzoylpaclitaxel, 3'-N-debenzoylpaclitaxel, 3'-debenzoylpaclitaxel, Baccatin III 13-[β (S)-amino- α (R)-hydroxyphenylpropionate], 13-O-3-phenylisoserinoyl baccatin III, and the names may be used interchangeable.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1);
 - 5 and
 - ii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT as set forth in SEQ ID NO: 20,
- or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

In other embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2);
 - 15 ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1);
 - and
 - iii. a nucleic acid encoding a DBTNBT, such as TcaDBTNBT (SEQ ID NO: 20),
- or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding TchiAAE5 (SEQ ID NO: 22);
 - 25 ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1);
 - and
 - iii. a nucleic acid encoding a DBTNBT, such as TcaDBTNBT (SEQ ID NO: 20),
- or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

In some embodiments, the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29);

- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1);
and
iii. a nucleic acid encoding a DBTNBT, such as TcaDBTNBT (SEQ ID NO: 20),
or functional homologues of any of the aforementioned having at least 70% sequence
5 identity thereto.

In the presence of β -phenylalanine and baccatin III, said host cell is capable of
producing taxol from β -phenylalanine and baccatin III. Preferably, said host cell is
capable of producing β -phenylalanine and baccatin III, but said compounds may also
10 be supplied to the host cell, such as in the cultivation medium, wherein said host cell is
cultivated.

If the host cell comprising a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3),
and further a nucleic acid encoding TchiAAE5 (SEQ ID NO: 22), AAE-867.5 (SEQ ID
15 NO: 29) or CoAL(A312G) (SEQ ID NO: 2), a nucleic acid encoding MBPig3BAPT (SEQ
ID NO: 4) or BAPT (SEQ ID NO: 1), and a nucleic acid encoding a DBTNBT, such as
TcaDBTNBT (SEQ ID NO: 20), also comprises a nucleic acid encoding TcuDBAT
(SEQ ID NO: 21), or functional homologues of any of the aforementioned having at
least 70% sequence identity thereto,
20 said host cell may be capable of producing taxol in the presence of 10-deacetyl-
baccatin III and β -phenylalanine.

Enzyme activities for *de novo* baccatin III or 10-deacetyl-baccatin III production

The host cell may also comprise nucleic acids encoding enzyme activities for
25 production of other taxoid compounds, such as baccatin III from the common diterpene
precursor geranylgeranyl diphosphate (GGPP), in order to obtain complete
biosynthesis of taxol.

In other words, the host cell may comprise enzyme activities enabling *de novo*
30 production of said taxane having a side chain comprising a hydroxylated β -
phenylalanine moiety from GGPP. Enzyme activities required for complete biosynthesis
of baccatin III from GGPP may include one terpene synthase, such as taxadiene
synthase, two acetyltransferases, a benzoyltransferase, an epoxidase and seven
cytochrome P450. Enzyme activities required for complete biosynthesis of 10-deacetyl-
35 baccatin III from GGPP may include one terpene synthase, such as taxadiene

synthase, a acetyltransferases, a benzoyltransferase, an epoxidase and seven cytochrome P450s.

5 In particular, it may be the enzymes described in Zhang, YJ. et al., 2023 and/or McClune, CJ. et al., 2024.

Methods

10 In another main aspect, the present disclosure concerns methods for preparing producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. The methods of the invention generally comprises the steps of:

- i. providing a host cell as described herein;
- ii. cultivating said host cell in a cultivation medium,

thereby producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

15

Provided is also a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety and/or a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:

- 20
- i. providing a host cell as described herein;
 - ii. cultivating said host cell in a cultivation medium, thereby obtaining a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety; and
 - 25 iii. optionally recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety,

thereby producing a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety and/or said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

30 Provided is also a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety and/or a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:

- i. providing a host cell as described herein;

- ii. incubating and optionally propagating said host cell in a cultivation medium, thereby obtaining a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety; and
- iii. optionally recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety,
- thereby producing a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety and/or said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

The host cell may be any of the host cells described herein, for example herein above in the section "Host cells".

Thus, in some embodiments, the method is a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:

- i. providing a host cell comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity to SEQ ID NO: 3;
- ii. cultivating said host cell in a cultivation medium, thereby producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

As described herein above, non-limiting examples of functional homologues of OD3 of SEQ ID NO: 3 are OD3(Y169F) of SEQ ID NO: 26 and OD3(N195A) of SEQ ID NO: 24, and OD3 of SEQ ID NO: 3 may be exchanged with these functional homologues in the methods described herein, in particular for production of N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol.

In preferred embodiments, said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol.

Thus, the method may be a method for producing N-debenzoyl-taxol, said method comprising the steps of:

- 5 i. providing a host cell comprising a heterologous nucleic acid sequence encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 3;
- ii. cultivating, and optionally propagating, said host cell in a cultivation medium, thereby producing N-debenzoyl-taxol. OD3 or functional homologues thereof are capable of converting N-debenzoyl-2'-deoxy-taxol into N-debenzoyl-taxol. The
10 cultivation medium may comprise N-debenzoyl-2'-deoxy-taxol, however preferably the host cell is capable of producing N-debenzoyl-2'-deoxy-taxol.

The method may be a method for producing 10-deacetyl-N-debenzoyl-taxol, said method comprising the steps of:

- 15 i. providing a host cell comprising a heterologous nucleic acid sequence encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 3;
- ii. cultivating and optionally propagating said host cell in a cultivation medium, thereby producing 10-deacetyl-N-debenzoyl-taxol. OD3 or functional homologues
20 thereof are capable of converting 10-deacetyl-N-debenzoyl-2'-deoxy-taxol into 10-deacetyl-N-debenzoyl-taxol. The cultivation medium may comprise 10-deacetyl-N-debenzoyl-2'-deoxy-taxol, however preferably the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

25 Cultivating

The step of cultivating and/or incubating the host cell in a cultivation medium may be under conditions enabling growth may be performed by any method known to the skilled person. The term "growth" of a host cell or a multicellular organism comprising a host cell should be understood as a reference to proliferation, multiplication,
30 differentiation and/or maintenance of viability of the subject host cell, or multicellular organism.

If the host cell is comprised in a multicellular organism, said conditions are usually conditions enabling maintenance of viability and/or growth of the multicellular organism.
35 Thus, if the host cells are plant cells comprised in a plant, the cultivation conditions are

conditions suitable for maintenance and/or growth of said plant. That could e.g. be sowing seeds or other regenerative parts of the said plant in a field or in a green house. Cultivation may further comprise watering and/or fertilising.

- 5 If the host cell is a unicellular organism, such as a bacterium or yeast cell, cultivation may be incubation in a medium, such as a cultivation medium, comprising at least a carbon source and a nitrogen source at a temperature suitable for growth of said unicellular organism. The carbon source may e.g. be a carbohydrate, such as sugars or polysaccharides. The nitrogen source may for example be amino acids or
- 10 polypeptides. The skilled person is well able of selecting a suitable cultivation medium based on the particular host cell.

Culture conditions

- 15 In some embodiments, said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is produced in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

- 20 Cultivating and/or incubating said host cell in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol may be obtained in several manners. For example, said compounds may be added to the host cell. If the host cell is a microorganism, then said compounds may be added to the cultivation medium of said microorganism. If the host organism, such as the host
- 25 cell, is a plant, then said compounds may be added to the soil of the plant or it may be introduced into the plant by infiltration. Thus, if the heterologous nucleic acid(s) are introduced into the plant by infiltration, then said compounds may be co-infiltrated together with the heterologous nucleic acid(s).

- 30 In other words, in some embodiments, the cultivation medium is suitable for producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably said cultivation medium comprises baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

Thus, in some embodiments, the method is a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said step of cultivating said host cell is performed in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

Thus, the method may further comprise a step of supplying said baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol to the host cell, such as incubating said host cell in a cultivation medium comprising said compounds.

It is also comprised within this disclosure, that the host cell may be capable and/or is capable of producing baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol. Host cells capable of producing said compounds are described elsewhere herein, for example in section "Host cells" herein above. In such embodiments incubating said host cell in the presence of taxadiene simply requires cultivating said host cell.

Thus, in other embodiments, the method is a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said host cell is capable of producing baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

Methods of producing N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol

The methods disclosed herein are in particular useful for producing N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, taxol and/or derivatives thereof. Host cells useful for producing said compounds are also described in the different sections herein, in particular "Host cells" herein above. This section discloses examples of methods for producing N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol from various substrates/precursors and examples of suitable host cells therefor. Depending on which enzymes the host cell comprises, it may preferably be cultivated in the presence of suitable enzyme substrates for production of the products of the reaction catalysed

by the enzyme or the host cell may be capable itself of producing said enzyme substrates. Suitable substrates for the enzymes disclosed herein, are described both herein above as well as in this section.

- 5 For example, in some embodiments, the method is a method for producing N-debenzoyl-taxol and said cultivation medium comprises baccatin III. In other embodiments, the method is a method for producing N-debenzoyl-taxol and said host cell is producing baccatin III and/or is capable of producing baccatin III.
- 10 In some embodiments, the method is a method of producing N-debenzoyl-taxol, and:
- i. said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol and/or the host cell is producing N-debenzoyl-2'-deoxy-taxol and/or is capable of producing N-debenzoyl-2'-deoxy-taxol;
 - ii. said cultivation medium comprises baccatin III and/or the host cell is producing
15 baccatin III or is capable of producing baccatin III;
 - iii. said cultivation medium comprises baccatin III and the host cell is producing β -phenylalanoyl-CoA or is capable of producing β -phenylalanoyl-CoA;
 - iv. said cultivation medium comprises baccatin III and the host cell is producing β -phenylalanine or is capable of producing β -phenylalanine;
 - 20 v. said cultivation medium comprises baccatin III and/or β -phenylalanoyl-CoA, and/or the host cell is producing baccatin III and/or β -phenylalanoyl-CoA or is capable of producing baccatin III and/or β -phenylalanoyl-CoA; and/or
 - vi. said cultivation medium comprises baccatin III and/or β -phenylalanine, and/or the host cell is producing baccatin III and/or β -phenylalanine or is capable of producing
25 baccatin III and/or β -phenylalanine.

In other embodiments, the method is a method of producing 10-deacetyl-N-debenzoyl-taxol, and:

- i. said cultivation medium comprises 10-deacetyl-N-debenzoyl-2'-deoxy-taxol
30 and/or the host cell is producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol;
- ii. said cultivation medium comprises 10-deacetyl-baccatin III and/or the host cell is producing 10-deacetyl-baccatin III or is capable of producing 10-deacetyl-baccatin III;

- iii. said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA, and/or the host cell is producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA or is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA;
 - 5 iv. said cultivation medium comprises 10-deacetyl-baccatin III, and the host cell is producing β -phenylalanoyl-CoA or is capable of producing β -phenylalanoyl-CoA;
 - v. said cultivation medium comprises 10-deacetyl-baccatin III, and the host cell is producing β -phenylalanine or is capable of producing β -phenylalanine; and/or
 - 10 vi. said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanine, and/or the host cell is producing 10-deacetyl-baccatin III and/or β -phenylalanine or is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanine.
- 15 In some embodiments, the method is a method of producing N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the method is a method of producing
- 20 N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and a nucleic acid encoding BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the method is a method of producing N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol, and the host cell
- 25 comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) and a nucleic acid encoding BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the method is a method of producing N-debenzoyl-taxol or 10-deacetyl-N-debenzoyl-taxol, and the host cell comprises a
- 30 heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) or TchiAAE5 (SEQ ID NO: 22) and a nucleic acid encoding BAPT (SEQ ID NO: 1), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.
- 35 In some embodiments, the method is a method of producing N-debenzoyl-taxol, and:

- i. said cultivation medium comprises 10-deacetyl-N-debenzoyl-2'-deoxy-taxol and/or the host cell is producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol;
- 5 ii. said cultivation medium comprises 10-deacetyl-baccatin III and/or the host cell is producing 10-deacetyl-baccatin III or is capable of producing 10-deacetyl-baccatin III;
- iii. said cultivation medium comprises 10-deacetyl-baccatin III, and the host cell is producing β -phenylalanoyl-CoA or is capable of producing β -phenylalanoyl-CoA;
- 10 iv. said cultivation medium comprises 10-deacetyl-baccatin III, and the host cell is producing β -phenylalanine or is capable of producing β -phenylalanine;
- v. said cultivation medium comprises 10-deacetyl-baccatin III and β -phenylalanoyl-CoA, and/or the host cell is producing 10-deacetyl-baccatin III and β -phenylalanoyl-CoA or is capable of producing 10-deacetyl-baccatin III and β -phenylalanoyl-CoA; and/or
- 15 vi. said cultivation medium comprises 10-deacetyl-baccatin III and β -phenylalanine, and/or the host cell is producing 10-deacetyl-baccatin III and β -phenylalanine or is capable of producing 10-deacetyl-baccatin III and β -phenylalanine.

20

In some embodiments, the method is a method of producing N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21), or functional homologues of any of the aforementioned

25 having at least 70% sequence identity thereto. In some embodiments, the method is a method of producing N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding BAPT (SEQ ID NO: 1) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21), or functional homologues of any of the aforementioned having at least 70% sequence identity

30 thereto. In some embodiments, the method is a method of producing N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21), or functional homologues of any of the aforementioned having at least 70% sequence

35 identity thereto. In some embodiments, the method is a method of producing N-

debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), a nucleic acid encoding BAPT (SEQ ID NO: 1) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In some embodiments, the method is a method of producing N-debenzoyl-taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) or TchiAAE5 (SEQ ID NO: 22), a nucleic acid encoding BAPT (SEQ ID NO: 1) and a nucleic acid encoding TcuDBAT (SEQ ID NO: 21), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

In some embodiments, the method is a method of producing taxol, and:

- i. said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol and/or the host cell is producing N-debenzoyl-2'-deoxy-taxol or is capable of producing N-debenzoyl-2'-deoxy-taxol; and/or
- ii. said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol and/or benzoyl-CoA, and/or the host cell is producing N-debenzoyl-2'-deoxy-taxol and/or benzoyl-CoA or is capable of producing N-debenzoyl-2'-deoxy-taxol and/or benzoyl-CoA; and/or
- iii. said cultivation medium comprises baccatin III and/or the host cell is producing baccatin III or is capable of producing baccatin III;
- iv. said cultivation medium comprises 10-deacetyl-baccatin III and/or the host cell is producing 10-deacetyl-baccatin III or is capable of producing 10-deacetyl-baccatin III;
- v. said cultivation medium comprises baccatin III and the host cell is producing β -phenylalanine or is capable of producing β -phenylalanine;
- vi. said cultivation medium comprises baccatin III and the host cell is producing β -phenylalanoyl-CoA or is capable of producing β -phenylalanoyl-CoA;
- vii. said cultivation medium comprises baccatin III and β -phenylalanoyl-CoA and/or the host cell is producing baccatin III and/or β -phenylalanoyl-CoA or is capable of producing baccatin III and/or β -phenylalanoyl-CoA; and/or
- viii. said cultivation medium comprises baccatin III and β -phenylalanine and/or the host cell is producing baccatin III and/or β -phenylalanine or is capable of producing baccatin III and/or β -phenylalanine.

When producing taxol by the methods disclosed herein, benzoyl-CoA is preferably present. Benzoyl-CoA may be produced by the host cell or be comprised in the cultivation medium.

5 In some embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

10

In other embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4), and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional homologues of any of the
15 aforementioned having at least 70% sequence identity thereto. In some embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding BAPT (SEQ ID NO: 1), and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional homologues of any of the aforementioned having at
20 least 70% sequence identity thereto. In other embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4), and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional homologues of any of the
25 aforementioned having at least 70% sequence identity thereto. In some embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), a nucleic acid encoding BAPT (SEQ ID NO: 1), and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or
30 functional homologues of any of the aforementioned having at least 70% sequence identity thereto. In other embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) or TchiAAE5 (SEQ ID NO: 22), a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4), and a nucleic acid encoding a
35 DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional

homologues of any of the aforementioned having at least 70% sequence identity thereto. In some embodiments, the method is a method of producing taxol, and the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3), a nucleic acid encoding AAE-867.5 (SEQ ID NO: 29) or TchiAAE5 (SEQ ID NO: 22), a nucleic acid encoding BAPT (SEQ ID NO: 1), and a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20), or functional homologues of any of the aforementioned having at least 70% sequence identity thereto.

In some embodiments, the host cell may further comprise a nucleic acid encoding a DBAT, such as TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

Isolation

The method may further comprise a step of isolating and/or recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

The compounds may be isolated and/or recovered through any useful method known to the skilled person. For example, recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be isolated and/or recovered by a method comprising one or more of the following:

- extraction, e.g. solvent extraction;
- precipitation; and/or
- chromatography, e.g. liquid chromatography (LC).

In some embodiments of the present disclosure, the step of isolating and/or recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises a step of extraction, such as extraction with a solvent, for example methanol (MeOH) and/or ethyl acetate (EtOAc).

In other embodiments, the step of isolating and/or recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises a step of chromatography, such as liquid chromatography (LC), for example column chromatography or preparative/semi-preparative high performance LC (HPLC).

Polypeptides and nucleic acids

In addition to the methods and host cells, the invention also provides enzymes useful in the production of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

5

Provided herein is a nucleic acid construct for expression in a host cell, comprising a nucleic acid encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 8 or a homologue thereof having at least 70%, for example at least 65% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity thereto. Furthermore, provided is a nucleic acid encoding OD3(N195A) as set forth in SEQ ID NO: 24 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 25 or a homologue thereof having at least 70% sequence identity, for example at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity to SEQ ID NO: 25. Provided is also a nucleic acid encoding OD3(Y169F) as set forth in SEQ ID NO: 26 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 27 or a homologue thereof having at least 70%, such as at least 80% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity to SEQ ID NO: 27.

25 In some embodiments, said nucleic acid construct comprising a nucleic acid encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof, such as SEQ ID NO: 8 or a homologue thereof having at least 70% sequence identity thereto, further comprises one or more of:

- 30 i. a nucleic acid encoding BAPT as set forth in SEQ ID NO: 1 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 6;
- ii. a nucleic acid encoding MBPig3BAPT as set forth in SEQ ID NO: 4 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 9;

- iii. a nucleic acid encoding CoAL(A312G) as set forth in SEQ ID NO: 2 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 7;
- iv. a nucleic acid encoding TchiAAE5 as set forth in SEQ ID NO: 22 or a functional homologue thereof having at least 70% sequence identity thereto;
- v. a nucleic acid encoding AAE-867.5 as set forth in SEQ ID NO: 29 or a functional homologue thereof having at least 70% sequence identity thereto;
- vi. a nucleic acid encoding TcuDBAT as set forth in SEQ ID NO: 21 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 23; and/or
- vii. a nucleic acid encoding TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto, or homologues of any of the aforementioned having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.

The functional homologue of OD3 (SEQ ID NO: 3) may be OD3(N195A) of SEQ ID NO: 24 or OD3(Y169F) of SEQ ID NO: 26. The nucleic acid encoding OD3(N195A) (SEQ ID NO: 24) may be as set forth in SEQ ID NO: 25 or a homologue thereof having at least 70% sequence identity thereto, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95% sequence identity to SEQ ID NO: 25. The nucleic acid encoding OD3(Y169F) (SEQ ID NO: 26) may be as set forth in SEQ ID NO: 27 or a homologue thereof having at least 70% sequence identity thereto, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95% sequence identity to SEQ ID NO: 27.

In some embodiments, the nucleic acid construct further comprises a promotor, such as a constitutive promoter and/or an inducible promoter, operably linked to any one or more of the nucleic acid sequences.

Provided herein is also an isolated polypeptide as set forth in SEQ ID NO: 3 or functional homologue thereof having at least 70% sequence identity, such as at least 71%, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity to SEQ ID NO: 3.

Furthermore, provided herein is an isolated polypeptide as set forth in SEQ ID NO: 24 or a functional homologue thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity to SEQ ID NO: 24.

Provided is also an isolated polypeptide as set forth in SEQ ID NO: 26 or a functional homologue thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity to SEQ ID NO: 26.

Also provided herein is a vector comprising at least one of the nucleic acid constructs described herein, for example a vector comprising a nucleic acid encoding OD3 (SEQ ID NO: 3), OD3(N195A) (SEQ ID NO: 24) or OD3(Y169F) (SEQ ID NO: 26), or functional homologues thereof having at least 70% sequence identity to SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, respectively. Thus, in some embodiments, the vector comprises at least one nucleic acid construct comprising or consisting of the nucleic acid sequence of SEQ ID NO: 8, SEQ ID NO: 25 or SEQ ID NO: 27, or homologues thereof having at least 70%, for example at least 65% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity to SEQ ID NO: 8, SEQ ID NO: 25 or SEQ ID NO: 27.

Provided is also a host cell as described elsewhere herein, comprising the nucleic acid construct described herein in this section, or a vector according to this section. Preferably, said host cell is a host cell as described in the section "Host cells" herein above.

Provided is also a kit of parts comprising:

- i. the host cell described herein in this section, and optionally instructions for use, and/or
 - ii. one or more nucleic acid constructs described herein or said vector described herein, and optionally instructions for use, and further optionally a host cell to be modified,
- preferably wherein the host cell is selected from the group consisting of plant cells, yeast cells, bacterial cells and fungal cells.

Uses

Discloses herein is also the use of an oxidase from *Taxus chinensis*, such as a 2-oxoglutarate-dependent oxygenase from *T. chinensis* or a 2-oxoglutarate-dependent dioxygenase from *T. chinensis*, preferably a 2-oxoglutarate-dependent dioxygenase from *T. chinensis*, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. Preferably, said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol.

In preferred embodiments, said *T. chinensis* oxidase is OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity thereto. Hence, disclosed is the use of OD3 of SEQ ID NO: 3, or a functional homologue thereof having at least 70% sequence identity thereto, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. Similarly, disclosed herein is also the use of OD3(N195A) of SEQ ID NO: 24 or OD3(Y169F) of SEQ ID NO: 26, or functional homologues thereof having at least 70% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 24 or SEQ ID NO: 26, respectively, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. In some embodiments, the method is a method described herein, for example in the section "Method".

In other embodiments, the polypeptide or oxidase comprises the sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, with the exception that at the most 30 residues are mutated, such as at the most 29 residues, such as at the most 28 residues, such as at the most 27 residues, such as at the most 26 residues, such as at

the most 25 residues, such as at the most 20 residues, such as at the most 15 residues, such as at the most 10 residues, such as at the most 5 residues, or less residues are mutated.

5 In some embodiments, said use comprises expressing the polypeptide as set forth in SEQ ID NO: 3, or a functional homologue thereof having at least 70% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 3, in a host cell, preferably wherein the host cell is as described herein, for example in the section "Host
10 cells".

Compounds, compositions and pharmaceutical use

The host cells, methods and/or uses described herein can be used to produce different plant diterpenoid compounds efficiently and/or with high titer. In particular, the present
15 disclosure provides host cells, methods and/or uses for production of one or more taxanes having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably wherein said taxane comprises N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol. Said methods are described elsewhere herein, for example in the sections "Oxidase" and "Methods" herein above. Said host cells are
20 described elsewhere herein, for example in the sections "Oxidase" and "Host cells" herein above. Said uses are described elsewhere herein, for example in the sections "Oxidase" herein above and "Uses" herein below.

Furthermore, provided in this section is a fermentation liquid, cell culture and/or
25 composition, comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said taxane having said side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol.

30 Compounds

Thus, provided herein is composition comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety obtained by a method, a host cell and/or a use described herein. Provided is also N-debenzoyl-taxol obtained by a method, a host cell and/or a use described herein. Also provided herein is 10-deacetyl-
35 N-debenzoyl-taxol obtained by a method, a host cell and/or a use described herein.

Provided is also N-debenzoyl-2'-deoxy-taxol obtained by a method, a host cell and/or a use described herein. Also provided is N-debenzoyl-taxol obtained by a method, a host cell and/or a use described herein.

5 Cell culture, fermentation liquid and titers

Herein provided is also a cell culture obtained by a method described herein. Provided is also a cell culture, comprising a host cell described herein, and optionally a cultivation medium.

10 Further provided herein is a fermentation liquid comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. In some embodiments, said fermentation liquid is obtained by a method and/or a use described herein. In other
15 embodiments, the method may further comprise a step of obtaining a fermentation liquid, optionally wherein said fermentation liquid comprise the host cell as described herein and/or a taxane having a side chain comprising a hydroxylated β -phenylalanine
20 moiety. In some embodiments, said fermentation liquid is comprised in the cell culture described herein. In further embodiments, said fermentation liquid is comprised within and/or secreted by a host cell described herein, for example to the fermentation liquid, cultivation medium or broth. In some embodiments, the fermentation liquid comprises
25 host cells described herein, and at least 50% of the host cells are lysed, such as at least 75%, such as at least 95%, such as at least 99% of the host cells are lysed. In other embodiments, at least 50% of solid cellular material has been separated from the liquid, such as at least 75%, such as at least 95%, such as at least 99% of solid cellular material has been separated from the liquid.

30 As described above, the taxane having a side chain comprising a hydroxylated β -phenylalanine moiety may be secreted by the host cell, and thus be present in the extracellular fraction (supernatant), or it may be retained in the host cell, and thus be present in the intracellular fraction. The total titer of a compound is the sum of the
35 intracellular titer and extracellular titer of the compound. In some embodiments, the host cell may be capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety with a titer of at least 50 $\mu\text{g/L}$, such as at least 0.75 $\mu\text{g/L}$, for example at least 100 $\mu\text{g/L}$, such as at least 250 $\mu\text{g/L}$, for example 500 $\mu\text{g/L}$, such as at least 750 $\mu\text{g/L}$, for example at least 900 $\mu\text{g/L}$, such as at least 1000

µg/L, for example at least 2.5 mg/L, such as at least 5 mg/L, for example at least 7.5 mg/L, for example at least 10 mg/L, or more.

5 In some embodiments, the yield of a taxane having a side chain comprising a hydroxylated β-phenylalanine moiety is at least 0.2 µg/mg wet weight.

Methods for determining titers of the plant diterpenoid compounds are known in the art. For example, the titers may be determined by UPLC-HRMS, as in the Examples of the present disclosure.

10

Compositions

The diterpenoid compounds, and more particular the taxanes having a side chain comprising a hydroxylated β-phenylalanine moiety obtainable by the present methods and/or uses may be useful for obtaining compositions comprising any of said
15 compounds produced by the host cell of the present disclosure.

Thus, provided herein is a composition comprising one or more of a taxane having a side chain comprising a hydroxylated β-phenylalanine moiety, such as N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol, and/or N-debenzoyl-2'-deoxy-taxol
20 and/or N-debenzoyl-taxol, obtained by a method and/or a use described herein, and optionally one or more agents, additives and/or excipients. In particular, provided herein is a composition comprising a taxane having a side chain comprising a hydroxylated β-phenylalanoyl moiety obtained by a host cell, method and/or use described herein. Disclosed is also a composition comprising taxol obtained by a host
25 cell, method and/or use described herein, and optionally one or more agents, additives and/or excipients.

Provided is also a composition comprising the fermentation liquid described herein above.

30

In some embodiments, said composition have been processed into in a semi-dry or dry solid form, optionally in form of a powder, tablet, capsule, chewable, gel and/or gum. In other embodiments, said composition is in a liquid form, optionally in a stabilized liquid form.

35

Pharmaceutical use

The plant diterpenoid compounds, and more particular the taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, obtainable by the present methods and/or with the present host cells may be useful for manufacturing pharmaceuticals compounds and/or compositions, in particular taxoid compounds, such as N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or paclitaxel (taxol).

Thus, the methods may further comprise a step of producing a medicament and/or composition from any of the compounds produced by the host cell of the present disclosure.

Provided is also a method for treating a disorder such as cancer, comprising administration of a medicament comprising a composition obtained by a method, a host cell, and/or a use described herein, said composition comprising of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety. Preferably, said method of treating a disorder comprises administration of a therapeutic sufficient amount of said medicament. Preferably, said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol and/or taxol.

Non-limited examples of such cancer are melanoma cancer, ovarian cancer, breast cancer, bladder cancer, prostate cancer and/or esophageal cancer.

Examples

Example 1: Materials and Methods for Examples 2, 3 and 4 to 9

This example comprises the materials and methods for examples 2-4 and 5-9 herein below.

CDS obtaining and gene cloning

CoAL(A312G), BAPT, and MBP (maltose-binding protein) from *E. coli* were synthesized from Thermo Fisher and codon optimized for *S. cerevisiae*. OD3 was synthesized from TWIST as a native cDNA sequence.

Vectors for both *N. benthamiana* (tobacco) transient expression and yeast expression were produced using USER cloning with specific primers (USER-GeneName-FP and

USER-GeneName-RP, **Table 1**). All of the primers were ordered from TAG Copenhagen, Denmark. pLIFE33 vector (Forman et al., 2022) was used in tobacco transient expression.

- 5 **Table 1.** Primers used for construction of vectors for gene expression in tobacco and yeast.

Primer	SEQ ID NO:
US_BAPT_F	11
US_BAPT_R	12
US_BAPT_F_IG3	13
US_MBP_F	14
US_MBP_IG3_R	15
US_CoAL_F	16
US_CoAL_R	17
US_OD3_F	18
US_OD3_R	19
US_DBAT_F	30
US_DBAT_R	31

Transient co-expression of genes of interest in *N.benthamiana* leaves

- 10 Constructs for tobacco transient expression were electro-transformed into agrobacteria (*A. tumefaciens* strain AGL-1- GV3850). The overnight cultures of engineered agrobacteria strains were used for agro-infiltration. Briefly, OD₆₀₀ of 1 culture mix was used with equal ratio to every agrobacterial strain. For infiltration, four-week-old tobacco leaves were used. After infiltration, the plants were kept in the greenhouse (16 h light at 20°C, 8 h dark at 19 °C) for 2 days before substrate feeding. Substrates were dissolved
- 15 in 5% methanol.

Heterologous expression of candidate genes in yeast

- The *S. cerevisiae* strain EGY48 was used as the parent strain for the gene expression. Yeast transformation was carried out using a lithium-acetate protocol. To prepare for the
- 20 detection of produced compounds, the yeast strains were cultivated overnight in selective glucose media at 30°C and 150 rpm and used as seed cultures. To induce the taxol derivative production, each seed culture was washed three times with sterilized MQ water and transferred into a 100 mL glass flask containing 10 mL galactose/raffinose

media. The yeast cultures were incubated (20°C and 150 rpm) for 3 days before ethyl acetate extraction.

5 Yeast media: Yeast glucose media: 2% (w/v) glucose, 0.13% (w/v) Yeast Synthetic Dropout Medium Supplements (all essential amino acids), 0.67% (w/v) Yeast Nitrogen Base w/o AA. Yeast galactose/raffinose medium: 2% (w/v) galactose, 1% (w/v) raffinose, 0.13% (w/v) Yeast Synthetic Dropout Medium Supplements (all essential amino acids), 0.67% (w/v) Yeast Nitrogen Base w/o AA (Y2025, US Biologicals). Yeast pH buffered media: yeast galactose/raffinose media containing 10% phosphate buffer solution (1M) and the pH adjusted to 7.

10

Table 2. Yeast strains. Abbreviations: MBP; maltose-binding protein, IG3: IGGG-linker (SEQ ID NO: 28). SEQ ID NOs of nucleic acids and/or polypeptides comprised in the yeasts are indicated as well.

S. <i>cerevisia</i> e	Characteristics	SEQ ID NOs of heterologou s polypeptide s	SEQ ID NOs of heterologou s nucleic acids
EGY48	MATalpha <i>ura3 his3 trp1</i> 6xLexAop:: <i>LEU2</i>		
TL001	EGY48, pHUS_P _{GAL1} _MBPig3BAPT_CoAL(A312 G)	SEQ ID NO: 2 and SEQ ID NO: 4	SEQ ID NO: 7 and SEQ ID NO: 9
TL002	EGY48, pHUS_ P _{GAL1} _MBPig3BAPT_CoAL(A312G); pWUS_ P _{GAL1} _OD3	SEQ ID NO: 2, 3 and SEQ ID NO: 4	SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9

15

Heterologous expression of candidate genes in *E.coli*

The *E.coli* strain BL21 (DE3) (Studier et.al, 1986) was used for the gene expression. *E. coli* transformation was carried out using a heat shock protocol. To prepare for the detection of produced compounds, the *E. coli* strains were cultivated overnight in LB

Broth (Miller) at 37°C and 150 rpm and used as seed cultures. To induce production, each seed culture was diluted into fresh LB Broth (Miller) with 1 to 100 ratio and cultivate under 37°C for 1-2 hours until the OD600 reaching 0.5. At this point, production was induced by adding 200µM isopropyl β-D-1-thiogalactopyranoside (IPTG), 100mg/l baccatin III and 100mg/l β-phenylalanine. The *E. coli* cultures were incubated (18°C and 150 rpm) for 1 day before ethyl acetate extraction.

Table 3. *E. coli* strains. Abbreviations: MBP; maltose-binding protein, IG3: IGGG-linker (SEQ ID NO: 28). SEQ ID NOs of nucleic acids and/or polypeptides comprised in the bacteria are also indicated.

<i>E. coli</i> strain name	Characteristics	SEQ ID NOs of heterologous polypeptides	SEQ ID NOs of heterologous nucleic acids
BL21 (DE3)	F- <i>ompT hsdSB</i> (rB-, mB-) <i>gal dcm</i> (DE3)		
ETL001	BL21 (DE3); pRSET-P _{TT} -CoAL(A312G)-P _{trc} -MBPig3BAPT	SEQ ID NO: 2 and SEQ ID NO: 4	SEQ ID NO: 7 and SEQ ID NO: 9
ETL002	BL21 (DE3); pRSET-P _{TT} -CoAL(A312G)-P _{trc} -MBPig3BAPT; P _{trc} -OD3	SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4	SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9

Sample extraction and UPLC-HRMS analysis

For tobacco metabolite extraction, two leaf discs (Ø3 cm, 1 disc per leaf) were used for each sample and ground in liquid nitrogen before extracting with 1 mL methanol (MeOH). For yeast samples, 5 mL ethyl acetate (EtOAc) was added to each induced yeast culture (10 mL cultures in 100 mL flasks). The mixtures were centrifuged at 4000 RPM for 10 min. Afterwards, 1 mL EtOAc supernatant was dried by blowing nitrogen and the dried metabolites were re-dissolved in 100 µL MeOH. All the samples were filtered with a 0.22 µM filter before Ultra-Performance-Liquid-Chromatography-High-Resolution-Mass-Spectrometry (UPLC-HRMS) analysis. The final samples were diluted 200 times before HPLC-HRMS analysis.

UPLC-HRMS analysis was performed on the 731 Dionex UltiMate® 3000 Quaternary Rapid Separation UHPLC focused system (Thermo Fisher Scientific, 732 Germering, Germany) equipped with a Phenomenex Kinetex XB-C18 column (100 mm × 2.1 mm i.d., 1.7 µm 733 particle size, 100 Å pore size) (Phenomenex, Inc., Torrance, CA, USA).
5 The column was operated at 40 °C, and the flow rate was maintained at 0.3 mL min⁻¹. The mobile phases were water (A) and 100% acetonitrile (B), both acidified with 0.05% formic acid. Separations were performed using the following gradient profile: 0 min, 20% B; 11 min, 80% B; 21 min, 90% B; 22 min, 100% B; 27 min, 100% B; 28 min, 20% B. The column outlet was connected to a Bruker Daltonics Compact QqTOF mass
10 spectrometer equipped with an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany). Mass spectra were acquired in positive ion mode, using a capillary voltage of 4000V, an end plate offset of -500V, a drying temperature of 220 °C, a nebulizer pressure of 2.0 bar, and a drying gas flow of 8 L min⁻¹. Sodium formate solution (internal standard) was injected at the beginning of each chromatographic run, and the
15 UPLC-HRMS raw data was calibrated against these sodium clusters using the Data Analysis 4.3 (Bruker Daltonics) software program.

Example 2: Production of N-debenzoyl-taxol in tobacco

The present example demonstrates that N-debenzoyl-taxol is synthesized *in vivo* by
20 expressing BAPT, CoAL(A312G), and OD3, and feeding the host cell with β-phenylalanine and baccatin III.

See Example 1 herein above for Material and Methods.

25 *Agrobacterium*-mediated transient gene expression in tobacco (*Nicotiana benthamiana*) is an efficient and reliable method for producing terpenoid compounds. To reconstruct the bioreaction pathway to produce N-debenzoyl-taxol, we infiltrated tobacco leaves with agrobacteria carrying nucleic acids encoding BAPT (SEQ ID NO: 1), CoAL(A312G) (SEQ ID NO: 2), OD3 (SEQ ID NO: 3) and P19 (SEQ ID NO: 5). P19 was used to suppress
30 gene silencing. Prior to the co-expression, the nucleic acid sequence encoding BAPT from *Taxus cuspidata* (SEQ ID NO: 6) and the A312G mutant (CoAL(A312G), SEQ ID NO: 7) of CoAL from *Penicillium chrysogenum* was codon optimized to *S. cerevisiae* codon usage and synthesized by Thermo Fisher. The nucleic acid encoding OD3 from *Taxus chinensis* (SEQ ID NO: 8) was synthesized from TWIST without codon
35 optimization. Two days after agro-infiltration, 200mg/l β-phenylalanine and 200mg/l

baccatin III dissolved in 5% methanol were injected to leaves which were infiltrated with corresponding agrobacteria earlier.

5 UPLC-HRMS of methanol extracts of tobacco (*N. benthamiana*) leaves transiently expressing CoAL(A312G) (SEQ ID NO: 2 and encoded by 7), BAPT (SEQ ID NO: 1 and encoded by 6) and OD3 (SEQ ID NO: 3 and encoded by 8) revealed production of N-debenzoyl-taxol (compound 3, $[M+H]^+$ 750.3120 $\pm$ 0.01, C₄₀H₄₇NO₁₃). The extract of tobacco leaves only expressing CoAL(A312G) (SEQ ID NO: 2 and encoded by 7) and BAPT (SEQ ID NO: 1 and encoded by 6) was used as negative control and revealed no
10 production of N-debenzoyl-taxol (compound 3). Instead, N-debenzoyl-2'-deoxy-taxol (compound 2, $[M+H]^+$ 734.3171 $\pm$ 0.01, C₄₀H₄₇NO₁₂), the substrate of OD3 (SEQ ID NO: 3 and encoded by 8), accumulated. See the drawing description of **Figure 1** herein above for further details.

15 Conclusion: The experiment shows that OD3 (SEQ ID NO: 3 and encoded by 8) expression together with BAPT (SEQ ID NO: 1 and encoded by 6) and CoAL(A312G) (SEQ ID NO: 2 and encoded by 7) are sufficient to synthesize the taxol intermediate N-debenzoyl-taxol (compound 3) in tobacco (**Figure 1**).

20 *Example 3: Production of N-debenzoyl-taxol in yeast*

The present example demonstrates that N-debenzoyl-taxol is synthesized *in vivo* by expressing BAPT, CoAL(A312G), and OD3, and feeding β -phenylalanine and baccatin III in yeast (*Saccharomyces cerevisiae*).

25 See Example 1 herein above for Material and Methods.

To investigate whether OD3 (SEQ ID NO: 3 and encoded by SEQ ID NO: 8) can catalyze the hydroxylation of C2' position on the C13 sidechain of taxol intermediate in another heterologous bio-production system, we introduced OD3 in the *S. cerevisiae* strain TL00, expression CoAL(A312G) (SEQ ID NO: 2 and encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 and encoded by SEQ ID NO: 9) that is engineered for
30 producing C13 side chain non-hydroxylated taxol intermediates to obtain strain TL002 (**Table 2**).

UPLC-HRMS analysis showed that *S. cerevisiae* TL002 cells produce N-debenzoyl-taxol (compound 3) in different yeast media, but TL001 cells, which lack OD3 (SEQ ID NO: 3 and encoded by SEQ ID NO: 8), do not produce compound 3 (**Figure 2**). Instead, TL001 accumulates the OD3 substrate N-debenzoyl-2'-deoxy-taxol (compound 2, **Figure 1**).

5 See the drawing description of **Figure 2** herein above for further details.

Conclusion: These results demonstrate that OD3 (SEQ ID NO: 3 and encoded by SEQ ID NO: 8) expression together with CoAL(A312G) (SEQ ID NO: 2 and encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 and encoded by SEQ ID NO: 9), is sufficient
10 to synthesize the taxol intermediate N-debenzoyl-taxol in yeast, here *S. cerevisiae* (baker's yeast) (**Figure 2**). BAPT was fused with a maltose binding protein from *E. coli* (MBP) on the N terminus to increase solubility. The extract of yeast cultures only expressing CoAL(A312G) and MBPig3BAPT was used as negative control and revealed
15 no production of compound 3, but instead N-debenzoyl-2'-deoxy-taxol (compound 2) accumulated.

Example 4. Production of 10-deacetyl-N-debenzoyl-taxol in tobacco

The present example demonstrates that 10-deacetyl-N-debenzoyl-taxol is synthesized in vivo in tobacco by expressing BAPT, CoAL(A312G) and OD3, and feeding β -phenylalanine and 10-deacetyl-baccatin III.
20

See Example 1 herein above for Material and Methods.

To synthesize 10-deacetyl-N-debenzoyl-taxol in tobacco, we infiltrated tobacco leaves
25 with agrobacteria carrying nucleic acids encoding BAPT, CoAL(A312G), OD3 and P19, which was used to suppress gene silencing. Prior to the co-expression, the nucleic acid sequence encoding BAPT from *Taxus cuspidata* (SEQ ID NO: 1 and encoded by 6), CoAL(A312G) (SEQ ID NO: 2 and encoded by 7) derived from CoAL from *Penicillium chrysogenum* was codon optimized to *S. cerevisiae* codon usage and synthesized from
30 Thermo Fisher. OD3 from *Taxus chinensis* (SEQ ID NO: 3 and encoded by 8) was synthesized from TWIST and without codon optimization. 2 days after agro-infiltration, 200 mg/l β -phenylalanine and 200 mg/l 10-deacetyl-baccatin III dissolved in 5% methanol were injected to leaves which were infiltrated with corresponding agrobacteria earlier.

35

UPLC-HRMS analysis of methanol extracts of tobacco (*N. benthamiana*) leaves transiently expressing CoAL(A312G) (SEQ ID NO: 2 and encoded by 7), BAPT (SEQ ID NO: 1 and encoded by 6) and OD3 (SEQ ID NO: 3 and encoded by 8) revealed production of 10-deacetyl-N-debenzoyl-taxol (compound 6, $[M+H]^+$ 708.3015 $\pm$ 0.01, C₃₈H₄₅NO₁₂). The extract of tobacco leaves only expressing CoAL(A312G) and BAPT was used as negative control and reveal no production of 10-deacetyl-N-debenzoyl-taxol (compound 6). Instead, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, $[M+H]^+$ 692.3065 $\pm$ 0.01, C₃₈H₄₅NO₁₁), the substrate of OD3, was accumulated. See drawing description of **Figure 3** herein above for further details.

Conclusion: These results demonstrate that N-debenzoyl-2'-deoxy-taxol (compound 2) can be produced in tobacco by expressing a A312G mutant (CoAL(A312G), SEQ ID NO: 2 and encoded by 7) of CoAL from *P. chrysogenum*, BAPT from *T. cuspidata* (SEQ ID NO: 1 and encoded by 6) and OD3 from *T. chinensis* (SEQ ID NO: 3 and encoded by 8).

Example 5: Production of N-debenzoyl-2'-deoxy-taxol and N-debenzoyl-taxol in tobacco

The present example demonstrates that N-debenzoyl-2'-deoxy-taxol and N-debenzoyl-taxol is synthesized *in vivo* in tobacco by expressing *TcuDBAT* (SEQ ID NO: 21 encoded by SEQ ID NO: 23) and feeding 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or 10-deacetyl-N-debenzoyl-taxol, respectively.

See Example 1 herein above for Material and Methods.

TcuDBAT, catalysing C₁₀ acetylation, takes various substrates, including 10-deacetyl-baccatin III, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol and 10-deacetyl-N-debenzoyl-taxol. We infiltrated tobacco leaves with agrobacteria carrying nucleic acids encoding DBAT and P19, which was used to suppress gene silencing. Prior to the co-expression, *TcuDBAT* (SEQ ID NO: 21 encoded by SEQ ID NO: 23) was synthesized from Thermo Fisher and was codon optimized to *S. cerevisiae* codon usage. 2 days after agro-infiltration, 100 mg/l 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or 100mg/l 10-deacetyl-N-debenzoyl-taxol dissolved in 5% methanol were injected to leaves which were infiltrated with the corresponding agrobacteria (see above).

UPLC-HRMS analysis of methanol extracts of tobacco (*N. benthamiana*) leaves transiently expressing *TcuDBAT* (SEQ ID NO: 21 encoded by SEQ ID NO: 23) revealed production of N-debenzoyl-2'-deoxy-taxol (compound 2, [M+H]⁺ 734.3171±0.01, C₄₀H₄₇NO₁₂) when 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, [M+H]⁺ 692.3065±0.01, C₃₈H₄₅NO₁₁) was supplied. The extract of tobacco leaves only supplied with 10-deacetyl-N-debenzoyl-2'-deoxy-taxol, without expressing *TcuDBAT*, was used as negative control, and revealed no production of N-debenzoyl-2'-deoxy-taxol (compound 2, [M+H]⁺ 734.3171±0.01, C₄₀H₄₇NO₁₂). Instead, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound 5, [M+H]⁺ 692.3065±0.01, C₃₈H₄₅NO₁₁), remained unconsumed in the control. See drawing description of **Figure 4** herein for further details.

UPLC-HRMS analysis of methanol extracts of tobacco (*N. benthamiana*) leaves transiently expressing *TcuDBAT* (SEQ ID NO: 21 encoded by SEQ ID NO: 23) revealed production of N-debenzoyl-taxol (compound 3, [M+H]⁺ 750.3120±0.01, C₄₀H₄₇NO₁₃) when 10-deacetyl-N-debenzoyl-taxol (compound 6, [M+H]⁺ 708.3015±0.01, C₃₈H₄₅NO₁₂) was supplied. The extract of tobacco leaves only supplied with 10-deacetyl-N-debenzoyl-taxol, without expressing *TcuDBAT*, was used as negative control, and revealed no production of N-debenzoyl-taxol (compound 3, [M+H]⁺ 750.3120±0.01, C₄₀H₄₇NO₁₃). Instead, 10-deacetyl-N-debenzoyl-taxol (compound 6, [M+H]⁺ 708.3015±0.01, C₃₈H₄₅NO₁₂), remained unconsumed in the control. See drawing description of **Figure 4** herein for further details.

Conclusion: These results demonstrate that N-debenzoyl-taxol (compound 3) can also be produced in tobacco by using 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or 10-deacetyl-N-debenzoyl-taxol as substrates. Therefore the biosynthetic routes or reactions are multiple. See drawing description of **Figure 4**, in particular **Figure 4a**, herein for further details.

Example 6: Production of N-debenzoyl-taxol in E. coli

The present example demonstrates that OD3 can be used to synthesize N-debenzoyl-taxol in *E. coli* cells.

See Example 1 herein above for Material and Methods.

To investigate whether OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) can catalyze the oxidation of the C13 side chain of taxol pathway intermediates in *E. coli*, we introduced OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) in *E. coli* cells together with CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 encoded by SEQ ID NO: 9) to obtain strain ETL002 (**Table 3**). *E. coli* cells expressing only CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 encoded by SEQ ID NO: 9) were also constructed and used as a negative control (designated as strain ETL001). Strain ETL002 (**Table 3**) was used to test the function OD3 in *E. coli* cells.

Results:

UPLC-HRMS analysis showed that *E. coli* ETL002 cells supplied with 100 mg/L baccatin III (compound **1**) and 100 mg/L β -phenylalanine produced N-debenzoyl-taxol (compound **3**). ETL001 cells, which lack OD3, did not produce **3** when supplied with 100 mg/L baccatin III (compound **1**) and 100 mg/L β -phenylalanine (**Figure 5**). Instead, in ETL001 cells, N-debenzoyl-2'-deoxy-taxol (compound **2**), the substrate of OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8), accumulated.

Conclusion:

These results demonstrate that OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) is sufficient to synthesize N-debenzoyl-taxol (compound **3**) from N-debenzoyl-2'-deoxy-taxol (compound **2**) in *E. coli*.

Example 7: Production of 10-deacetyl-N-debenzoyl-taxol in *E. coli*

The present example demonstrates that OD3 can be used to synthesize 10-deacetyl-N-debenzoyl-taxol in *E. coli* cells.

See Example 1 herein above for Material and Methods.

To investigate whether OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) can catalyze the oxidation of the C13 side chain of taxol intermediates in *E. coli*, we introduced OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) in *E. coli* cells together with CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 encoded by SEQ ID NO: 9) to obtain strain ETL002 (**Table 3**). *E. coli* cells expressing only CoAL(A312G) (SEQ ID NO: 2 encoded by SEQ ID NO: 7) and MBPig3BAPT (SEQ ID NO: 4 encoded

by SEQ ID NO: 9) were also constructed and used as a negative control (designated as strain ETL001). Strain ETL002 (**Table 3**) was used to test the function OD3 in *E. coli* cells.

5 Results:

UPLC-HRMS analysis showed that *E. coli* ETL002 cells supplied with 100 mg/L 10-deacetyl-baccatin III (compound **4**) and 100 mg/L β -phenylalanine produced 10-deacetyl-N-debenzoyl-taxol (compound **6**). ETL001 cells, which lack OD3, did not produce **6** when supplied with 100 mg/L 10-deacetyl-baccatin III (compound **4**) and 100
10 mg/L β -phenylalanine (**Figure 6**). Instead, in ETL001 cells, 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound **5**), the substrate of OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8), accumulated.

Conclusion:

15 These results demonstrate that OD3 (SEQ ID NO: 3 encoded by SEQ ID NO: 8) is sufficient to synthesize 10-deacetyl-N-debenzoyl-taxol (compound **6**) from 10-deacetyl-N-debenzoyl-2'-deoxy-taxol (compound **5**) in *E. coli* (**Figure 6**).

Example 8: Other T. chinensis OD enzymes cannot produce N-debenzoyl-taxol.

20 This example demonstrates that other 2-oxoglutarate-dependent dioxygenase (OD) enzymes from *T. chinensis* sharing up to 71% identity with OD3 cannot produce N-debenzoyl-taxol in tobacco.

See Example 1 herein above for Material and Methods.

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To investigate if *Taxus* plants possess another OD enzyme able to produce N-debenzoyl-taxol using 2'-deoxy-N-debenzoyl-taxol as substrate, we chose eight OD candidates from *Taxus chinensis* (**Table 4**). The eight OD candidates shared from 18% to 71% sequence identity with OD3 (SEQ ID NO: 3) at amino acid level. However, none
30 of the OD candidates could produce N-debenzoyl-taxol in tobacco. Furthermore, OD candidate 8 shares 92% sequence identity to KP178205 (TB328) of Ramírez-Estrada K., et al., 2016.

Table 4. Amino acid sequence identity of 8 candidate ODs to OD3 (SEQ ID NO: 3).

Name of OD candidate	Amino acid sequence identity to OD3 (SEQ ID NO: 3)
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OD candidate 1	18%
OD candidate 2	42%
OD candidate 3	43%
OD candidate 4	46%
OD candidate 5	47%
OD candidate 6	49%
OD candidate 7	54%
OD candidate 8	71%

Results: Neither the candidate, OD candidate 8, with the highest identity to OD3, nor the remaining OD candidates 1 to 7 could produce detectable levels of N-debenzoyl-taxol in tobacco.

5

Conclusion: These results show that while OD3 is able to synthesize N-debenzoyl-taxol, other enzymes of the same family sharing up to 71% amino acid sequence identity to OD3 cannot catalyse production of N-debenzoyl-taxol to any significant extent.

10 *Example 9: OD3 variants at amino acid positions 169 and 195 retain their ability to synthesize N-debenzoyl-taxol.*

This example demonstrates that OD3 variants at amino acid positions 169 and 195 retain their ability to synthesize N-debenzoyl-taxol (3).

15 See Example 1 herein above for Material and Methods.

Variants of OD3 were obtained by mutating amino acid residues Y169 and N195 into phenylalanine (F) and alanine (A), respectively, resulting in the variants OD3(Y169F) (SEQ ID NO: 26 encoded by SEQ ID NO: 27) and OD3(N195A) (SEQ ID NO: 24 encoded by SEQ ID NO: 25).

20

Results: We tested the ability of these two variants to produce N-debenzoyl-taxol (3) in tobacco. To this end, we transiently expressed CoAL(A312G) and BAPT with either wild-type OD3 (SEQ ID NO: 3), or OD3(Y169F) (SEQ ID NO: 26), or OD3(N195A) (SEQ ID NO: 24) (**Figure 7**) in tobacco through agro-infiltration and supplied with 200 mg/L β -phenylalanine and 200 mg/L baccatin III (BACIII) two days after agro-infiltration. After 5 days of bioconversion, we extracted metabolites from the tobacco leaves and carried out

25

LC-qTOF analysis to determine the activity of the expressed enzymes. The results showed that both OD3(Y169F) and OD3(N195A) have similar catalytic activity to wild-type OD3, since they produce a similar amount of N-debenzoyl-taxol (3) as wild-type OD3 (Figure 7).

5

Conclusion:
These results show that OD3 variants at amino acid positions 169 or 195 retain the ability to synthesize N-debenzoyl-taxol (3) from β -phenylalanine and baccatin III (BACIII).

10 **Sequence overview**

SEQ ID NO:	Characteristics	Sequence
1	BAPT, GenBank accession no.: Q8H2B5.1, <i>Taxus cuspidata</i> , polypeptide	MKKTGSFAEFHVNMIERVMPCLPSPKTILPLSAIDNM ARAFSNVLLVYAANMDRVSADPAKVIREALSKVLVYYYP FAGRLRNKENGEELECTGQGVLFLEAMADSDLSVLTD LDNYPNPSFQQLIFSLPQDIEDLHLLIVQVTRFTCGGFV VGANVYGSACDAKGFQFLQSMAMARGEVKPSIEPIW NRELVKLEHCMPFRMSHLQIIHAPVIEEFVQTSLVINFEII NHIRRRIMEERKESLSSFEIVAALVWLAKIKAFQIPHSENV KLLFAMDRLRRSFNPPLPHGYGNAFGIACAMDNVHDLLS GSLRLTIMIIKKSKFSLHKELNSKTMSSSVVDVNTKFED VVSISDWRHSIYYEVDGFWGDAMNVSTMLQQQEHEKSL PTYFSFLQSTKNMPDGIKMLMFPPSKLKKFKIEIAMIK KYVTKVCPSKL
2	CoAL(A312G), synthetic construct, polypeptide	MPTIYRSPYPDLDIQSVDLVSYLFSNPFNTPLDRPMYINAI SGEQYTFGDVVQRTRSLSNGLRQSIGLKPNVVALFSP NTIDYPVVCHAIVGSRAIVAPTSAALELNAQLKTSGA RFIVVHSTLLETAQKAAGTSVEKVLLIDGQTPVNGQPTC NYLANTFAPDDLTVDPAEADRQPTFICFSSGTSGAAGK VITTHQNITSNLQQWRQHMLLESGLPSQRPRRQSAIAFLP FSHIYGLNLFMCQCLIWGTTVVVMPRFDLDLYLSCIQKY RPDELALVPPIALMLVKDPRVSKYDLSSVRKIMSAGAPLT IELSSALEAKFTEICKTEVFCTQSWGLTETSPMATAVPND RMDKRNTGVGCIAPNMQLRFVDPESMKDAAVKPDGST EPAEIWCRGPNVVMGYNNNEKATKEAFHVEDGTRWF RTGDIGTIDGDGYVTIQDRIKEMIKYKGLQVIPSELEGKLV DHPDVEDAAVTGMWVDDMATELPVGFVVLSPQAKDRD QKAVLDGIHAWLNERIANHKRLRGGIHVLSQIPKSPSGKI LRRQLRDLLKSQAPKARL
3	OD3, <i>Taxus chinensis</i> , polypeptide	MSEFNLPQIDISQFQQGIEGDHIQYHAELAKLREACKEW GVFRVVNHGITPELVQEVSQVTDLLSMPAEVKDRVTT NPTESYVRLPEFEMFYLLGMPNPDSIQEIALKIWPEGNK NFCDAIGKFSLLLSDLWTKIISILLSLGLDATKFYQSDFEK CKANLQINGYSSHGNCMGDVVFPVSHADVECLTILYNDE HAGLQFRSKQGWFSVKPQPDSEFVVNIGDCLQAWTNG

		RYCSPHHRVVYTGWTNRISLPFSIIFPHDDQIWAPEELVD EDNPRRYKPFTYSAILNESSSYQGSQGDAEIPKEIERIAG I
4	MBPig3BAPT, synthetic construct, polypeptide	MIEEGKLVWINGDKGYNGLAEVGGKFEKDTGIKVTVEH PDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAE ITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNK DLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTW PLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVD LIKXKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNID TSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKEL AKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKD PRIAATMENAKQGEIMPNIQMSAFWYAVRTAVINAASG RQTVDEALKDAQIGGGKKTGSFAEFHVNMIERVMVRPC LPSPKTILPLSAIDNMARAFSNVLLVYAANMDRVSADPAK VIREALSKVLVYYYPFAGRLRNKENGELEVECTGQGVLF LEAMADSDLSVLTDLNYPNPSFQQLIFSLPQDIEDLHL LIVQVTRFTCGGFVVGANVYGSACDAKGFGQLQSMAG MARGEVKPSIEPIWNRELKLEHMCMPFRMSHLQIIHAPVI EEKFVQTSLVINFEIINHRRRIMEERKESLSSFEIVAALVW LAKIKAFQIPHSENVKLLFAMDLRNPNPLPHGYGNAF GIACAMDNVHDLGSGSLLRTIMIKKSKFSLHKELNSKT MSSSVVDVNTKFEDVVSISDWRHSIYYEVDGFWGDAMN VSTMLQQQEHEKSLPTYFSFLQSTKNMPDGIKMLMFMP PSKLKKFKIEIAMIKKYVTKVCP SKL
5	P19, Tobacco virus 1 isolate AnHui, GenBank accession no.: AKT94764.1, polypeptide	MLSDYEVGSNNSSHYLAIAVVCSENSDELLMFESEDSNE NAIFFEDSCVISKSPSGGALNCAENYYLTSNAGLLDFLKS TNFKSPKPLPVANRCSNGPNHVDFHMRPLHLGLIFRVNL SLKSLDDLDTFQDSL VKIKGFWNNGAQHQSMFHYLSNFI NYEKYSIVSFEYFKL
6	BAPT, synthetic construct, nucleic acid	ATGAAGAAAAC TGGTTCCTTCGCTGAATTTACGTCAA CATGATTGAAAGAGTTATGGTCAGACCTTGTGGCCAT CTCCAAAAC TATTTTGCCATTGTCCGCCATTGATAAC ATGGCTAGAGCTTTTCTAACGTCTTGTGGTTTACGC TGCTAACATGGACAGAGTTTCTGCTGATCCAGCTAAG GTTATTAGAGAAGCTTTGTCCAAGGTTTGTGCTACTA TTATCCATTCTGCTGGTAGATTGAGAAACAAAGAAAACG GTGAATTGGAAGTTGAATGTACCGGTCAAGGTGTTTT GTTTTTGGAAGCTATGGCTGATTCCGATTTGTCTGTTT TGACTGATTTGGACAAC TACAACCCATCCTTCCAACAA TTGATTTTCTCATTGCCACAAGACACCGATATCGAAGA TTTGCATTTGTTGATCGTTCAAGTCACCAGATTCACTT GTGGTGGTTTTGTTGTTGGTGCTAATGTTTATGGTTCT GCTTGTGATGCTAAAGGTTTCGGTCAATTCTTGCAATC AATGGCTGAAATGGCCAGAGGTGAAGTTAAGCCATCT ATTGAACCTATCTGGAACAGAGAATTGGTCAAGTTGG AACATTGCATGCCATT CAGAATGTCCCACTTGCAAATT ATTCATGCCCCAGTTATCGAAGAAAAGTTCTGTTCAAAC TTCCTTGGTCATCAACTTCGAAATCATCAACCACATTA GAAGAAGAATCATGGAAGAAAGAAAAGAATCCTTGTC CTCCTTCGAAATTGTCGCTGCTTTGGTTTGGTTGGCTA

		AGATTAAGGCTTTCCAAATTCCCACTCCGAAAACGTT AAGTTGTTGTTGCTATGGACTTGAGAAGATCTTTTAA TCCACCATTCGCTCATGGTTACTACGGTAATGCTTTTG GTATTGCTTGTGCCATGGATAACGTTACGATTTGTTG TCTGGTTCTTTGTTGAGAACCATCATGATCATCAAGAA GTCCAAGTTTTCTTGCACAAAGAATTGAACTCCAAGA CCGTTATGTCCTCCTCTGTTGTTGATGTTAACACCAAG TTCGAAGATGTCGTCAGTATTTCTGATTGGAGACACTC TATCTACTACGAAGTTGACTTTGGTTGGGGTGATGCTA TGAATGTTTCTACCATGTTACAACAACAAGAACACGAA AAGTCTTTGCCAACCTACTTCTCCTTTTTACAATCCAC TAAGAACATGCCAGACGGTATCAAGATGTTAATGTTCA TGCCACCATCCAAGTTGAAGAAGTTCAAGATTGAAATC GAAGCCATGATTAAGAAGTACGTTACCAAGGTTTGTC CATCTAAGTTGTAA
7	CoAL(A312G), synthetic construct, nucleic acid	ATGCCGACCATTTATCGTAGCCCGTATCCGGATCTGG ATATTCAGAGCGTTGATCTGGTTAGCTACCTGTTTAGC AATCCGTTTAATACACCGCTGGATCGTCCGATGTATAT TAACGCAATTAGCGGTGAACAGTATACCTTTGGTGAT GTTGTTTCAGCGTACCCGTAGCCTGAGCAATGGTCTGC GTCAGAGCATTGGTCTGAAACCGAATGATGTTGTGGC ACTGTTTAGCCCGAATACCATTGATTATCCGGTTGTTT GTCATGCCATTGTTGGTAGCCGTGCAATTGTTGCACC GACCAGCGCAGCACTGACCGCACTGGAAGTGAATGC ACAGCTGAAAACCGCGGTGCACGTTTTATTGTTGTT CATAGCACCCCTGCTGGAAACCGCACAGAAAGCAGCAA AAGGCACCAGCGTTGAAAAAGTTCTGCTGATTGATGG TCAGACACCGGTTAATGGTCAGCCGACCTGTAATTAT CTGGCAAATACCTTTGCACCGGATGATCTGCTGACCG TTGATCCGGCAGAAGCAGATCGCCAGCCGACATTTAT TTGTTTTAGCAGCGGTACAAGCGGTGCAGCCAAAGGT GTTATTACCACACATCAGAATATTACCAGCAATCTGCA GCAGTGGCGTCAGCACATGCTGGAAAGCGGTCTGCC GAGCCAGCGTCCGCGTCGTCAGAGCGCAATTGCATTT CTGCCGTTTAGCCATATTTATGGCCTGAACCTGTTTAT GTGTCAGTGTCTGATTTGGGGCACCACCGTTGTTGTT ATGCCTCGTTTTGATCTGGATCTGTATCTGAGCTGCAT TCAGAAATATCGTCCGGATGAACTGGCACTGGTTCCG CCTATTGCACTGATGCTGGTTAAAGATCCGCGTGTTA GCAAATATGATCTGAGCAGCGTTTCGAAAATTATGAG CGCAGGCGCACCGCTGACAATTGAACTGAGCAGTGC CCTGGAAGCAAAATTTACCGAAATTTGCAAAACCGAA GTGTTTTGTACCCAGAGCTGGGGTCTGACCGAAACCA GTCCGATGGCAACCGCAGTTCCGAATGATCGTATGGA TAAACGTAATACCGGTGTTGGTTGATTGCACCGAATA TGCAGCTGCGTTTTGTTGATCCTGAAAGCATGAAAGA TGCAGCAGTTAAACCGGATGGTAGCACCGAACCTGCA GAAATTTGGTGTCTGTTCCGAATGTTGTGATGGGCT ATTATAACAATGAGAAGGCAACCAAGAAGCCTTTTAC GTTGACGAAGATGGCACCCGTTGGTTTCGCACCGGT GATATTGGCACCATTGATGGTGATGGTTATGTTACCAT TCAGGATCGCATCAAAGAGATGATCAAATACAAAGGC

		CTGCAGGTTATTCCGAGCGAACTGGAAGGTAACTGG TTGATCATCCTGATGTTGAAGATGCCGCAGTGACCGG TATGTGGGTTGATGATATGGCCACCGAACTGCCGGTT GGTTTTGTTGTTCTGAGTCCGCAGGCAAAAGATCGTG ATCAGAAAGCCGTTTCTGGATGGTATTCATGCATGGCT GAATGAACGTATTGCCAATCATAAACGTCTGCGTGGT GGCATTTCATGTTCTGAGCCAGATTCCGAAAAGCCCGA GCGGTAAAATTCTGCGTCGCCAGCTGCGTGACCTGCT GAAAAGCCAGGCACCGAAAGCACGTCTGTAA
8	OD3, synthetic construct, nucleic acid	ATGTCTGAATTTAATCTTCCTCAGATTGATATTTACAA TTTCAACAGGGAATTGAGGGAGATCATATTCAATATCA TGCTGAACTAGCCAAGCTCAGAGAAGCATGCAAAGAA TGGGGTGTTTTCCGCGTGGTGAATCATGGAATTACAC CAGAACTTGTGCAAGAGGTTGTGTCTGTTACCCAAGA TTTACTGTCCATGCCCCGCGGAGGTTAAAGACAGAGTC ACAACTTCTAATCCCACGGAGAGTTACGTGCGCTTGC CAGAATTTGAAATGTTCTACTTGTTGGGCATGCCCAAT CCGGATTCAATTCAGGAAATTGCTCTCAAGATATGGC CCGAAGGAAACAAGAATTTCTGCGACGCGATTGGCAA ATTCAGTTTACTTCTGTCAGATCTGTGGACTAAGATCA TTATATCTATTCTTCTAAGCCTTGTTTAGATGCCACG AAATTCTACCAATCTGATTTGAAAAATGCAAAGCCAA TTTGCAAATAAACGGCTATTCATCTCATGAAATTGTA TGGGAGATGTGGTTTTTCCCTCTCACGCAGATGTCGA ATGTTTGACAATCCTGTACAACGATGAACATGCAGGT CTCCAGTTTCGATCCAAACAAGGAAAGTGTTTCAGCG TCAAACCTCAACCAGATTCATTGTTGTTAACATAGGG GATTGCCTTCAGGCATGGACTAATGGCAGGTAAGTCA GCCACATCACCGAGTTGTTTATACAGGGTGGACGAA TCGTATTTGCTTCCCTTTTCTATAATTTCCACACGA TGACCAAATTTGGGCTCCTGAGGAGCTCGTGATGAA GATAATCCACGACGCTACAAGCCTTTTACATACTCAGC AATTCTGAATGAAAGTTCAAGTTACCAAGGTTCCCAAG GGGATGCGGAAATTCCCAAGGAGATCGAACGAATTGC AGGTATATAG
9	MBPig3BAPT, synthetic construct, nucleic acid	ATGATCGAAGAAGGTAACTGGTAATCTGGATTAACG GCGATAAAGGCTATAACGGTCTCGCTGAAGTCGGTAA GAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTT GAGCATCCGGATAAACTGGAAGAGAAATTCCACAGG TTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTG GGCACACGACCGCTTTGGTGGCTACGCTCAATCTGG CCTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAG GACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTT ACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGA AGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGA ACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGG ATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGAT GTTCAACCTGCAAGAACCGTACTTCACCTGGCCGCTG ATTGCTGCTGACGGGGTTATGCGTTCAAGTATGAAA ACGGCAAGTACGACATTAAAGACGTGGGCGTGGATAA CGCTGGCGCGAAAGCGGGTCTGACCTTCTGTTGA CCTGATTAAAAACAACACATGAATGCAGACACCGATT

		ACTCCATCGCAGAAGCTGCCTTTAATAAAGGCGAAAC AGCGATGACCATCAACGGCCCGTGGGCATGGTCCAA CATCGACACCAGCAAAGTGAATTATGGTGTAAACGGTA CTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTTCG TTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTC CGAACAAAGAGCTGGCAAAAGAGTTCTCGAAAATA TCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAA GACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTAC GAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCC ACCATGGAAAACGCCCAGAAAGGTGAAATCATGCCGA ACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCG TACTGCGGTGATCAACGCCGCCAGCGGTGCTCAGAC TGTCGATGAAGCCCTGAAAGACGCGCAGATTGGAGG AGGTAAGAAAACCTGGTTCCTTCGCTGAATTCACGTCA ACATGATTGAAAGAGTTATGGTCAGACCTTGTTCGCCA TCTCCAAAACTATTTTGCCATTGTCCGCCATTGATAA CATGGCTAGAGCTTTTTCTAACGTCTTGTTGGTTTACG CTGCTAACATGGACAGAGTTTCTGCTGATCCAGCTAA GGTTATTAGAGAAGCTTTGTCCAAGGTTTTGGTCTACT ATTATCCATTTCGCTGGTAGATTGAGAAACAAAGAAAAC GGTGAATTGGAAGTTGAATGTACCGGTCAAGGTGTTT TGTTTTTGGAAGCTATGGCTGATTCCGATTTGTCTGTT TTGACTGATTTGGACAACTACAACCCATCCTTCCAACA ATTGATTTTCTCATTGCCACAAGACACCGATATCGAAG ATTTGCATTTGTTGATCGTTCAAGTCACCAGATTCACT TGTGGTGGTTTTGTTGTTGGTGCTAATGTTTATGGTTC TGCTTGTGATGCTAAAGGTTTCGGTCAATTCTTGCAAT CAATGGCTGAAATGGCCAGAGGTGAAGTTAAGCCATC TATTGAACCTATCTGGAACAGAGAATTGGTCAAGTTG GAACATTGCATGCCATTCAAGATGTCCCACTTGCAAAT TATTCATGCCCCAGTTATCGAAGAAAAGTTTCGTTCAA CTTCCTTGGTCATCAACTTCGAAATCATCAACCACATT AGAAGAAGAATCATGGAAGAAAAGAAAAGAATCCTTGT CCTCCTTCGAAATTGTCGCTGCTTTGGTTTGGTTGGCT AAGATTAAGGCTTTCCAAATCCACACTCCGAAAACGT TAAGTTGTTGTTGCTATGGACTTGAGAAGATCTTTTA ATCCACCATTGCCTCATGGTTACTACGGTAATGCTTTT GGTATTGCTTGTGCCATGGATAACGTTACGATTTGTT GTCTGGTTCCTTGTTGAGAACCATCATGATCATCAAGA AGTCCAAGTTTTCTTGCACAAAGAATTGAACTCCAAG ACCGTTATGTCCTCCTCTGTTGTTGATGTTAACACCAA GTTTGAAGATGTCGTCAGTATTTCTGATTGGAGACACT CTATCTACTACGAAGTTGACTTTGGTTGGGGTGATGC TATGAATGTTTCTACCATGTTACAACAACAAGAACACG AAAAGTCTTTGCCAACCTACTTCTCCTTTTTACAATCC ACTAAGAACATGCCAGACGGTATCAAGATGTTAATGTT CATGCCACCATCCAAGTTGAAGAAGTTCAAGATTGAA ATCGAAGCCATGATTAAGAAGTACGTTACCAAGGTTT GTCCATCTAAGTTGTAA
10	<i>p19</i> , Tobacco virus 1 isolate AnHui,	ATGTTAAGTGATTACGAAGTGGGATCCAATAATTCATC ACACTACTTGGCTATTGCCGTAGTGTGTTCCGAAAATT CCGATGAATTACTAATGTTTGAATCGGAAGACAGCAA

	GenBank accession no.: KT203917.1, nucleic acid	CGAAAACGCAATTTTCTTTGAAGACAGTTGCGTTATTT CTAAGTCACCGTCCGGCGGTGCTTTAAATTGCGCTGA AAATTATTACTTGACTAGTAACGCAGGTTTGTTAGATT TTTTAAAATCTACTAATTTTAAATCACCCAAGCCTTTAC CCGTGGCTAACAGATGCAGTAACGGACCTAACCACGT TGACTTTTCATATGAGACCTCTTCATTTAGGGCTTATAT TCCGGGTGAACCTATCTTTGAAGTCTCTCGACGATCTT GACACTTTTCAGGATTCTTTAGTTAAGATAAAAGGTTT TTGGAATAACGGTGCGCAGCACCAGTCTATGTTTCAC TATCTATCTAACTTCATAAACTACGAAAAGTATTCTATA GTGTCCTTTGAGTATTTTAAGTTATGA
11	US_BAPT_F, synthetic construct, nucleic acid	ATCAACGGGUAAAATGAAGAAAAGTGGTTCCTTCGC
12	US_BAPT_R, synthetic construct, nucleic acid	CGTGCGAUTTACAACCTTAGATGGACAAACCTTG
13	US_BAPT_F_IG 3, synthetic construct, nucleic acid	ATTGGAGGAGGUAAAGAAAAGTGGTTCCTTCGC
14	US_MBP_F, synthetic construct, nucleic acid	ATCAACGGGUAAAATGATCGAAGAAGGTAAACTGG
15	US_MBP_IG3_ R, synthetic construct, nucleic acid	ACCTCCTCCAAUCTGCGC
16	US_CoAL_F, synthetic construct, nucleic acid	ATCAACGGGUAAAATGCCGACCATTATTCG
17	US_CoAL_R, synthetic construct, nucleic acid	CGTGCGAUTTACAGACGTGCTTTCGG
18	US_OD3_F, synthetic construct, nucleic acid	ATCAACGGGUAAAATGTCTGAATTTAATCTTCCTCAGA TTG
19	US_OD3_R, synthetic construct, nucleic acid	CGTGCGAUCTATATACCTGCAATTCGTTTCGATC
20	TcaDBTNBT, GenBank accession no.: Q8LL69.1, <i>Taxus</i>	MEKAGSTDFHVKKFDPVMVAPSLPSPKATVQLSVVDSL TICRGIFNTLLVFNAPDNISADPVKIIREALSKVLVYFPLA GRLRSKEIGELEVECTGDGALFVEAMVEDTISVLRDLDD LNPSFQQLVFWHPLDTAIEDLHLVIVQVTRFTCGGIAVG VTLPHSVCDGRGAAQFVTALAEMARGEVKPSLEPIWNRE

	<i>canadensis</i> , polypeptide	LLNPEDPLHLQLNQFDSICPPPMLEELGQASFVINVDIE YMKQCVMEECNEFCSSFEVVAALVMIARTKALQIPHTEN VKLLFAMDLRKLFPNPLPNGYYGNAIGTAYAMDNVQDLL NGSLLRAIMIIKKAKADLKDNYSRVVTNPYSLDVNKKK DNILALSDWRRLLGFYEADFGWGGPLNVSSLQRLNGLP MFSTFLYLLPAKNKSDGIKLLLSMPPTTLKSFKIVMEAMI EKYVSKV
21	TcuDBAT, GenBank accession no.: Q9M6E2, <i>Taxus cuspidata</i> , polypeptide	MAGSTEFVVRSLERVMVAPSQPSPKAFLQLSTLDNLPG VRENIFNTLLVYNASDRVSVDPKAVIRQALS KVLVYYSPF AGRRLRKKENG DLEVECTGEGALFVEAMADTDL SVLGDL DDYSPSLEQLLFCLPPD TDIEDI HPLVVQVTRFTCGGFVV GVSFCHGICDGLGAGQFLIAMGEMARGEIKPSSEPIWKR ELLKPEDPLYRFQYYHFQLICPPSTFGKIVQGS LVTSETI NCIKQCLREESKEFCSAFEVVSALAWIARTRALQIPHSEN VKLIFAMDMRKLFNPPLSKGYG NFGVTVCAMD NVKDL LSGSLLRVVRIKKAKVSLNEHFTSTIVTPRSGSDESINYE NIVGFGDRRRLGFDEVD FGWGHADNVSLVQHGLKDVS VVQSYFLFIRPPKNNPDGIKILSFMPPSIVKSFKFEMETM TNKYVTKP
22	TchiAAE5, <i>Taxus chinensis</i> , polypeptide	MEDLKRCPANYPPLTPIGFIERAATVYRDCTSLVYNTTRF TWSQTFNRCLKMASALSSRNISRGDVVS VVAPNVPAIYE MHFAVPMAGAVLNNVNIRLDARTMATQFIHCEPKFLFVD YQFLPLVREALSKIGNKPCVVVIEELDYGREIATSTGLTY EGMIGEGDPKFEIRWPEDEWQAAVLNYTSGTTSAPKGV VHCHRG LYSMAMDNLLMWGLTTRPVYLWTLAMFHANG WCFPWTLAAVGGTNICLRKFDAKIIFDALAEHGVTHMCA APVVL SMMLNAHPSERKPMANRVEILTAGSPPPAAILRK GEEMGFFVTHGYGLTETAGLVVSCAWKA EWDNLSAEE RARLKARQGV RNLSLTEVDVKDPASMASVARDGVQIGE VMLRGASVMKGYLKNEQMTAQAMDGGWFHTGDVAVL HPDGYLEIKDRSKDIIISGGENISSVEVESVLYSHPLIMEA AVVARPDPFWGETPCAFVSINND SKDVLSEAQIISFCKE RTAHFMAPKSVIFMKELPKTSTGKVQK FVLREMAKNLAQ PRSKI
23	TcuDBAT, synthetic construct, nucleic acid	ATGGCTGGTTCTACTGAATTTGTTGTTAGATCCTTGGA AAGAGTTATGGTTGCTCCATCTCAACCATCTCCAAAG GCTTTTTTACAATTGTCCACCTTGGATAACTTGCCAGG TGTTAGAGAAAAACATCTTCAACACCTTGTTGGTTTACA ACGCCTCTGATAGAGTTTCTGTTGATCCAGCTAAGGTT ATTAGACAAGCCTTGTCAAAGGTTTTGGTCTACTATTC TCCATTGCTGGTAGATTGAGAAAGAAAGAAAACGGT GATTTGGAAGTTGAATGTACTGGTGAAGGTGCTTTGTT TGTTGAAGCTATGGCTGATACTGATTTGTCCGTTTTGG GTGACTTGGATGATTACTCTCCATCTTTGGAACAATTA TTGTTTTGCTTGCCACCAGATACCGACATCGAAGATAT TCATCCATTGGTTGTTCAAGTCACCAGATTCACTTG TG GTGGTTTTGTTGTTGGTGTTCCTTCTGCCATGGTATC TGTGATGGTTTGGGTGCTGGTCAATTTTGATTGCTAT GGGTGAAATGGCTAGAGGTGAAATCAAACCATCTTCT GAACCTATCTGGAAGAGAGAATTATTGAAGCCAGAAG ATCCATTATACAGATTCCAATATTACCACTTCCAATTGA TCTGTCCACCATCTACTTTTGGTAAGATCGTTCAAGGT

		TCCTTGGTTATTACTTCCGAAACCATTAACCTGCATCAA GCAATGTTTGAGAGAAGAATCCAAAGAATTTTGCTCC GCCTTCGAAGTTGTTTCTGCTTTGGCTTGGATTGCTAG AACTAGAGCTTTACAAATCCCACACTCTGAAAACGTCA AGTTGATTTTCGCTATGGACATGAGAAAGTTGTTCAAC CCACCATTGTCTAAAGGTTACTACGGTAATTTTCGTTGG TACTGTTTGTGCTATGGATAACGTTAAGGATTTGTTGT CCGGTTCCTTGTTGAGAGTTGTTAGAATTATCAAGAAG GCCAAGGTTTCTTTGAACGAACATTTCACTTCTACCAT CGTTACTCCAAGATCTGGTTCTGATGAATCCATCAACT ACGAAAACATCGTTGGTTTTCGGTGACAGAAGAAGATT GGGTTTTGACGAAGTTGACTTTGGTTGGGGTCATGCT GATAATGTTTCTTTGGTTCAACACGGTTTGAAGGATGT CTCTGTTGTTCAATCTTACTTCTTGTTTCATCAGACCAC CAAAGAACAATCCAGATGGTATTAAGATCTTGTCTTC ATGCCACCATCAATCGTTAAGTCTTTCAAGTTCGAAAT GGAAACCATGACCAACAAGTACGTTACTAAGCCATGA
24	OD3(N195A), synthetic construct, polypeptide	MSEFNLQIDISQFQQGIEGDHIQYHAELAKLREACKEW GVFRVNVNHGITPELVQEVSVTQDLLSMPAEVKDRVTS NPTESYVRLPEFEMFYLLGMPNPDSIQEIALKIWPEGNK NFCDAIGKFSLLLSDLWTKIIISILLSLGLDATKFYQSDFEK CKANLQINGYSSHGNCMGDVVFP SHADVECLTILYADEH AGLQFRSKQGWFSVKPQPD SFVNI GDC LQAWTNGR YCSPHHRVVYTGWTNRISLPFSIIFPHDDQIWAPEELVDE DNPRRYKPTY SAILNESSYQGSQGD AEIPKEIERIAGI
25	OD3(N195A), synthetic construct, nucleic acid	ATGTCTGAATTTAATCTTCCTCAGATTGATATTTACAA TTTCAACAGGGAATTGAGGGAGATCATATTCAATATCA TGCTGAACTAGCCAAGCTCAGAGAAGCATGCAAAGAA TGGGGTGTTTTCCGCGTGGTGAATCATGGAATTACAC CAGAACTTGTGCAAGAGGTTGTGTCTGTTACCCAAGA TTTACTGTCCATGCCCGCGGAGGTTAAAGACAGAGTC ACAACTTCTAATCCCACGGAGAGTTACGTGCGCTTGC CAGAATTTGAAATGTTCTACTTGTGTTGGGCATGCCCAAT CCGGATTCAATTCAGGAAATTGCTCTCAAGATATGGC CCGAAGGAAACAAGAATTTCTGCGACGCGATTGGCAA ATTCAGTTTACTTCTGTGATCTGTGGACTAAGATCA TTATATCTATTCTTCTAAGCCTTGGTTTAGATGCCACG AAATTCTACCAATCTGATTTGAAAAATGCAAAGCCAA TTTGCAAATAAACGGCTATTCATCTCATGAAATTGTA TGGGAGATGTGGTTTTTCCCTCTCACGCAGATGTGCA ATGTTTGACAATCCTGTACGCAGATGAACATGCAGGT CTCCAGTTTTCGATCCAAACAAGGAAAGTGGTTTCAGCG TCAAACCTCAACCAGATTCATTCGTTGTTAACATAGGG GATTGCCTTCAGGCATGGACTAATGGCAGGTACTGCA GCCACATCACCGAGTTGTTTATACAGGGTGGACGAA TCGTATTTTCGCTTCCCTTTTCTATAATTTCCACACGA TGACCAAATTTGGGCTCCTGAGGAGCTCGTGGATGAA GATAATCCACGACGCTACAAGCCTTTTACATACTCAGC AATTCTGAATGAAAGTTCAAGTTACCAAGGTTCCCAAG GGGATGCGGAAATCCCAAGGAGATCGAACGAATTGC AGGTATATAG

26	OD3(Y169F), synthetic construct, polypeptide	MSEFNLPQIDISQFQQGIEGDHIQYHAELAKLREACKEW GVFRVNVHGITPELVQEVS SVTQDLLSMPAEVKDRVTT NPTESYVRLPEFEMFYLLGMPNPDSIQEIALKIWPEGNK NFCDAIGKFSLLLSDLWTKIIISILLSLGLDATKFYQSDFEK CKANLQINGFSSHGNCMGDVVFP SHADVECLTILYNDEH AGLQFRSKQGKWFSVKPQPD SFVVNIGDCLQAWTNGR YCSPHHRVVYTGWTNRISLPFSIIFPHDDQIWAPEELVDE DNPRRYKPFTYSAILNESSSYQGSQGD AEIPKEIERIAGI
27	OD3(Y169F), synthetic construct, nucleic acid	ATGTCTGAATTTAATCTTCCTCAGATTGATATTTACAA TTTCAACAGGGAATTGAGGGAGATCATATTCAATATCA TGCTGAACTAGCCAAGCTCAGAGAAGCATGCAAAGAA TGGGGTGTTTTCCGCGTGGTGAATCATGGAATTACAC CAGAACTTGTGCAAGAGGTTGTGTCTGTTACCCAAGA TTTACTGTCCATGCCCCGCGGAGGTTAAAGACAGAGTC ACAACTTCTAATCCCACGGAGAGTTACGTGCGCTTGC CAGAATTTGAAATGTTCTACTTGTTGGGCATGCCCAAT CCGGATTCAATTCAGGAAATTGCTCTCAAGATATGGC CCGAAGGAAACAAGAATTTCTGCGACGCGATTGGCAA ATTCAGTTTACTTCTGTCAGATCTGTGGACTAAGATCA TTATATCTATTCTTCTAAGCCTTGGTTTAGATGCCACG AAATTCTACCAATCTGATTTGAAAAATGCAAAGCCAA TTTGCAAATAAACGGCTTCTCATCTCATGGAAATTGTA TGGGAGATGTGGTTTTTCCCTCTCACGCAGATGTGCA ATGTTTGACAATCCTGTACAACGATGAACATGCAGGT CTCCAGTTTCGATCCAAACAAGGAAAGTG GTTCAGCG TCAAACCTCAACCAGATTCATTCGTTGTTAACATAGGG GATTGCCTTCAGGCATGGACTAATGGCAGGTA CTGCA GCCACATCACCGAGTTGTTTATACAGGGTGGACGAA TCGTATTTGCTTCCCTTTTCTATAATATTTCCACACGA TGACCAAATTTGGGCTCCTGAGGAGCTCGTGGATGAA GATAATCCACGACGCTACAAGCCTTTTACATACTCAGC AATTCTGAATGAAAGTTCAAGTTACCAAGGTTCCCAAG GGGATGCGGAAATCCCAAGGAGATCGAACGAATTGC AGGTATATAG
28	IG3 or four amino acid linker, synthetic construct, polypeptide	IGGG
29	AAE-867.5, <i>Taxus chinensis</i> , polypeptide	MDAEVVKSVRELGVDDVVQAGLPRHRAEIFYGQLQRAI ADIGGSQTS LWHRVSKELLAPHHPHALHQLMYYSIYKN WDTSENGPPLYWFPTQESARLTNLGWMME TYGPQLLG SSYYNPITSFQSFQQFTVDHPEVYWSLV LKELSVVFHES PRCILDTS DKSRSGGVWLPGSVLNVAESCLSAKESINKT DNSIAIVWREEGRNEYPVNKMTLGELRAKVMRIANALDV VFTKGDAIAIDMPMTVNAVAIYLALILAGYVVVSIADSFVP KEIATRV RVTKAKGIFTQDFILRGGKRIPLYSRVVESGAP KAIVIPAEELGTQLREIDVAWSKFLSFSDHLRSPEYYS VRQPV DARTNILFSSGTS GEPKAIPWTHSPPIRCGSECW SHLDVKAGDIFCWPTNLGWWMGPVLVYSCFLSGATAAIY EGSPLDRGFGKFVQDARVTVLGTVP SMVKTWKSTGCM EGLDWSHIRTFASTGEASSIDDDLWLSSKGWYKPVIELC

		GGTELSACFVHGSLQLQPQALGMFSTPTMTTGFLVDDQ QIPYPNDQPCIGELFPRFFGSSYTLLNADHDAVYFKG MPMYKGMRLRRHGDMIERTVGGYYKAHGRSDDTMNLG GIKTSIEIERVCNRAHEQVLETAASISSSEGGPELLALT VLKDGPTVSMIDLKLAFSKAIQSNLNPLFKVSFVKVISDF PRTASNKIMRRVLRDQIKQEFSLHKSRL
30	US_DBAT_F	ATCAACGGGUAAAATGGCTGGTTCTACTGAATTTGTT G
31	US_DBAT_R	CGTGCGAUTCATGGCTTAGTAACGTACTTGTTG

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5

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Items

1. A host cell comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity thereto.
2. A host cell comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3, OD3(N195A) as set forth in SEQ ID NO: 24 or OD3(Y169F) as set forth in SEQ ID NO: 26, or functional homologues thereof having at least 70% sequence identity, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity thereto.

- 5 3. The host cell according to any one of the preceding items, wherein said host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, preferably wherein said taxane comprises or consists of N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or paclitaxel (taxol).
- 10 4. The host cell according to any one of the preceding items, wherein the host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 15 5. The host cell according to any one of the preceding items, wherein the host cell is capable of producing baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 20 6. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of N-debenzoyl-2'-deoxy-taxol, optionally wherein the host cell is capable of producing N-debenzoyl-2'-deoxy-taxol.
- 25 7. The host cell according to any one of the preceding items, wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol in the presence 10-deacetyl-N-debenzoyl-2'-deoxy-taxol, optionally wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 30 8. The host cell according to any one of the preceding items, wherein said host cell further comprises a nucleic acid encoding an amino phenylpropanoyl transferase (EC: 2.3.1), optionally wherein said amino phenylpropanoyl transferase is native to a *Taxus* cell, such as a *Taxus cuspidata* cell.

9. The host cell according to item 8, wherein said amino phenylpropanoyl transferase is BAPT as set forth in SEQ ID NO: 1 or MBPig3BAPT as set forth in SEQ ID NO: 4, or functional homologues thereof having at least 70% sequence identity to any of the SEQ ID NO: 1 or SEQ ID NO: 4, respectively.
- 5
10. The host cell according to any one of the preceding items, wherein said host cell further comprises a nucleic acid encoding a coenzyme A ligase (CoAL, EC: 6.2.1), optionally wherein said CoAL is a CoAL native to a *Penicillium* cell, such as a *P. chrysogenum* cell or a CoAL native to a *Taxus* cell, such as a *T. chinensis* cell.
- 10
11. The host cell according to item 10, wherein said CoAL is CoAL(A312G) as set forth in SEQ ID NO: 2, AAE-867.5 as set forth in SEQ ID NO: 29 or TchiAAE5 as set forth in SEQ ID NO: 22, or functional homologues thereof having at least 70% sequence identity to any of SEQ ID NO: 2, SEQ ID NO: 29 or SEQ ID NO: 22, respectively.
- 15
12. The host cell according to any one of the preceding items, wherein said host cell further comprises a nucleic acid encoding a 10-deacetyl-baccatin III-10-O-acetyltransferase (DBAT), optionally wherein said DBAT is native to a *Taxus* cell, such as a *Taxus cuspidata* cell.
- 20
13. The host cell according to item 12, wherein the DBAT is TcuDBAT as set forth in SEQ ID NO: 21 or a functional homologue thereof having at least 70% sequence identity thereto.
- 25
14. The host cell according to any one of the preceding items, wherein said host cell further comprises a nucleic acid encoding a 3'-N-debenzoyl-2'-deoxytaxol-N-benzoyltransferase (DBTNBT, EC: 2.3.1), optionally wherein said DBTNBT is native to a *Taxus* cell, such as a *Taxus canadensis* cell.
- 30
15. The host cell according to item 14, wherein said DBTNBT is TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto.
- 35

16. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of baccatin III, optionally wherein the host cell is capable of producing baccatin III.
- 5 17. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of baccatin III and β -phenylalanoyl-CoA, optionally wherein the host cell is capable of producing baccatin III and/or β -phenylalanoyl-CoA.
- 10 18. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of baccatin III and β -phenylalanine, optionally wherein the host cell is capable of producing baccatin III and/or β -phenylalanine.
- 15 19. The host cell according to any one of the preceding items, wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III.
- 20 20. The host cell according to any one of the preceding items, wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III and β -phenylalanoyl-CoA, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA.
- 25 21. The host cell according to any one of the preceding items, wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III and β -phenylalanine, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanine.
- 30 22. The host cell according to any one of the preceding items, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70%
- 35 sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively.

23. The host cell according to any one of the preceding items, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:
- i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto; and
 - ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively.
24. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of 10-deacetyl-N-debenzoyl-2'-deoxy-taxol, optionally wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
25. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III.
26. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III and β -phenylalanoyl-CoA, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA.
27. The host cell according to any one of the preceding items, wherein the host cell is capable of producing N-debenzoyl-taxol in the presence of 10-deacetyl-baccatin III and β -phenylalanine, optionally wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanine.
28. The host cell according to any one of the preceding items, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- 5 ii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

29. The host cell according to any one of the preceding items, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto;
- 10 ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- 15 iii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

30. The host cell according to any one of the preceding items, wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- i. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively;
- 25 ii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto; and
- 30 iii. optionally a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

31. The host cell according to any one of the preceding items, wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

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- i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), or a functional homologue thereof having at least 70% sequence identity thereto;
 - ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively;
 - iii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20) or a functional homologue thereof having at least 70% sequence identity thereto; and
 - iv. optionally a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.
32. The host cell according to any one of the preceding items, wherein said host cell is selected from the group of plant cells, yeast cells, bacterial cells and fungal cells.
33. The host cell according to any one of the preceding items, wherein said host cell is plant cells, such as plant cells comprised within a plant or within a part of a plant.
34. The host cell according to item 33, wherein said plant cells are from a species of *Nicotiana*, such as *Nicotiana benthamiana* or *Nicotiana tabacum*.
35. The host cell according to any one of the preceding items, wherein said host cell is a yeast cell belonging to the genus of *Saccharomyces*, *Pichia*, *Candida*, *Cryptococcus*, *Pichia (Komagataella)*, *Lipomyces*, *Pseudozyma*, *Rhodospiridium*, *Rhodotorula*, *Trichosporon*, *Trigonopsis*, *Yarrowia* or *Saccharomycopsis*, such as a yeast cell of the species *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Hansenula polymorpha (Ogataea polymorpha)*, *Rhodotorula toruloides* or *Pichia pastoris (Komagataella phaffii)*, preferably the host cell is a yeast cell of the species *S. cerevisiae*.
36. The host cell according to any one of the preceding items, wherein said host cell is a bacterial cell belonging to the genus of *Escherichia*, *Bacillus*, *Corynebacterium*, *Pseudomonas* or *Streptomyces*, such as a bacterial cell of the species *Escherichia coli*, *Bacillus subtilis*, *Corynebacterium glutamicum*,

Pseudomonas putida or *Streptomyces sp.*, preferably the host cell is a bacterial cell of the species *E. coli*.

- 5 37. A method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:
- i. providing a host cell according to any one of items 1 to 36 or item 85;
 - ii. cultivating said host cell in a cultivation medium, thereby producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.
- 10 38. A method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:
- i. providing a host cell according to any one of items 1 to 36 or item 85;
 - 15 ii. cultivating said host cell in a cultivation medium, thereby obtaining a fermentation liquid comprising said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety; and
 - iii. optionally recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, thereby producing a fermentation liquid comprising said taxane having a side
- 20 chain comprising a hydroxylated β -phenylalanine moiety and/or said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.
- 25 39. The method according to any one of items 37 to 38, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is produced in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 30 40. The method according to any one of items 37 to 39, wherein said step of cultivating said host cell is performed in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.

41. The method according to any one of items 37 to 40, wherein said host cell is capable of producing baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 5
42. The method according to any one of items 37 to 41, wherein said cultivation medium is suitable for producing said taxane, preferably wherein said cultivation medium comprises baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 10
43. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol and/or wherein the host cell is capable of producing N-debenzoyl-2'-deoxy-taxol.
- 15
44. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises baccatin III and/or wherein the host cell is capable of producing baccatin III.
- 20
45. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises baccatin III and/or β -phenylalanoyl-CoA, and/or wherein the host cell is capable of producing baccatin III and/or β -phenylalanoyl-CoA.
- 25
46. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises baccatin III and/or β -phenylalanine and/or wherein the host cell is capable of producing baccatin III and/or β -phenylalanine.
- 30

47. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises baccatin and wherein the host cell is capable of producing β -phenylalanoyl-CoA.
- 5
48. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-N-debenzoyl-2'-deoxy-taxol and/or wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 10
49. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III and/or wherein the host cell is capable of producing 10-deacetyl-baccatin III.
- 15
50. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA, and/or wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA.
- 20
51. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanine, and/or wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanine.
- 25
52. The method according to any one of items 37 to 42, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises baccatin and wherein the host cell is capable of producing β -phenylalanoyl-CoA.
- 30

53. The method according to any one of items 37 to 52, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively.
54. The method according to any one of items 37 to 53, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:
- a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto; and
 - a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively.
55. The method according to any one of items 37 to 54, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
56. The method according to any one of items 37 to 55, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III or wherein the host cell is capable of producing 10-deacetyl-baccatin III.
57. The method according to any one of items 37 to 56, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA, and/or or wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanoyl-CoA.

58. The method according to any one of items 37 to 57, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin and wherein the host cell is capable of producing β -phenylalanoyl-CoA.

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59. The method according to any one of items 37 to 58, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-baccatin III and/or β -phenylalanine, and/or wherein the host cell is capable of producing 10-deacetyl-baccatin III and/or β -phenylalanine.

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60. The method according to any one of items 37 to 59, wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

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- i. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- ii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

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61. The method according to any one of items 37 to 59, wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

25

- i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto;
- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively; and
- iii. a nucleic acid encoding TcuDBAT (SEQ ID NO: 21) or a functional homologue thereof having at least 70% sequence identity thereto.

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62. The method according to any one of items 37 to 61, wherein said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol or wherein the host cell is capable of producing N-debenzoyl-2'-deoxy-taxol.
- 5 63. The method according to any one of items 37 to 62, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is taxol, and wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises a nucleic acid encoding a
- 10 DBTNBT (EC: 2.3.1), optionally TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto.
64. The method according to any one of items 37 to 63, and wherein said cultivation medium comprises baccatin III, β -phenylalanoyl-CoA and/or β -
- 15 phenylalanine, and/or wherein the host cell is capable of producing baccatin III, β -phenylalanoyl-CoA and/or β -phenylalanine.
65. The method according to any one of items 37 to 64, and wherein said cultivation medium comprises baccatin III and wherein the host cell is capable
- 20 of producing β -phenylalanoyl-CoA.
66. The method according to any one of items 37 to 65, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is taxol, and wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70%
- 25 sequence identity thereto, and further comprises:
- i. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively;
- 30 ii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto.

67. The method according to any one of items 37 to 66, and wherein said cultivation medium comprises baccatin III and wherein the host cell is capable of producing β -phenylalanine.

5 68. The method according to any one of items 37 to 67, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is taxol, and wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:

- 10 i. a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), or a functional homologue thereof having at least 70% sequence identity thereto;
- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively;
- 15 iii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20) or a functional homologue thereof having at least 70% sequence identity thereto.

20 69. The method according to any one of items 37 to 68, further comprising a step of recovering said taxane having a side chain comprising hydroxylated $\alpha\beta$ -phenylalanine moiety.

70. The method according to any one of items 37 to 69, wherein said step of recovering comprises:

25 i. a step of extraction, such as extraction with a solvent, for example methanol (MeOH) and/or ethyl acetate (EtOAc); and/or

ii. a step of chromatography, such as liquid chromatography (LC), for example column chromatography or preparative/semi-preparative high performance LC (HPLC).

30 71. Use of an oxidase from *Taxus chinensis*, preferably a 2-oxoglutarate-dependent oxygenase or a 2-oxoglutarate-dependent dioxygenase from *T. chinensis*, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety,

35 optionally wherein the *T. chinensis* oxidase is OD3 as set forth in SEQ ID NO: 3

or a functional homologue thereof having at least 70% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity thereto.

5 72. Use of an OD3(N195A) as set forth in SEQ ID NO: 24 or OD3(Y169F) as set forth in SEQ ID NO: 26, or functional homologues thereof having at least 70% sequence identity to SEQ ID NO: 24 or SEQ ID NO: 26, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

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73. The use according to any one of items 71 or 72, wherein the polypeptide comprises the sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, or functional homologues thereof having at least 70% sequence identity to SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, with the exception
15 that at the most 30 residues are mutated.

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74. The use according to any one of items 71 to 73, said use comprising expressing the polypeptide as set forth in SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, or functional homologues thereof having at least 70% sequence identity to
20 SEQ ID NO: 3, SEQ ID NO: 24 or SEQ ID NO: 26, in a host cell, preferably wherein the host cell is as defined in any one of items 1 to 36.

20

75. The use according to any one of items 71 to 74, wherein said method is as defined in any one of items 37 to 70.

25

76. A nucleic acid construct for expression in a host cell, comprising:

- i. a nucleic acid encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 8 or a homologue thereof having at least 70% sequence
30 identity, for example at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity thereto.

30

35 77. A nucleic acid construct for expression in a host cell, comprising:

35

- 5 i. a nucleic acid encoding OD3(N195A) as set forth in SEQ ID NO: 24 or or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 25 or a homologue thereof having at least 70% sequence identity, for example at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity to SEQ ID NO: 25.

78. A nucleic acid construct for expression in a host cell, comprising:

- 10 i. a nucleic acid encoding OD3(Y169F) as set forth in SEQ ID NO: 26 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 27 or a homologue thereof having at least 70%, such as at least 80% sequence identity, for example at least 90% sequence identity, such as at least 95% sequence identity, for example at least 99% sequence identity to SEQ ID NO: 27.
- 15

79. The nucleic acid construct according to any one of items 76 to 78, further comprising one or more of:

- 20 i. a nucleic acid encoding BAPT as set forth in SEQ ID NO: 1 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 6;
- ii. a nucleic acid encoding MBPig3BAPT as set forth in SEQ ID NO: 4 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 9;
- 25 iii. a nucleic acid encoding CoAL(A312G) as set forth in SEQ ID NO: 2 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 7;
- iv. a nucleic acid encoding TchiAAE5 as set forth in SEQ ID NO: 22 or a functional homologue thereof having at least 70% sequence identity thereto;
- 30 v. a nucleic acid encoding AAE-867.5 as set forth in SEQ ID NO: 29 or a functional homologue thereof having at least 70% sequence identity thereto;
- vi. a nucleic acid encoding TcuDBAT as set forth in SEQ ID NO: 21 or a functional homologue thereof having at least 70% sequence identity thereto, such as SEQ ID NO: 23; and/or

- vii. a nucleic acid encoding TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto, or homologues of any of the aforementioned nucleic acids having at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.
- 5
80. The nucleic acid construct according to any one of items 76 to 79, further comprising a promoter, such as a constitutive promoter and/or an inducible promoter, operably linked to any one or more of the nucleic acid sequences.
- 10
81. An isolated polypeptide as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.
- 15
82. An isolated polypeptide as set forth in SEQ ID NO: 24 or a functional homologue thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity to SEQ ID NO: 24.
- 20
83. An isolated polypeptide as set forth in SEQ ID NO: 26 or a functional homologue thereof having at least 70% sequence identity thereto, such as at least 71% sequence identity, such as at least 72% sequence identity, such as at least 73% sequence identity, such as at least 74% sequence identity, such as at least 75% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity to SEQ ID NO: 26.
- 25
- 30
84. A vector comprising at least one of the nucleic acid constructs according to any one of items 76 to 80.

85. A host cell according to any one of items 1 to 36, comprising the nucleic acid construct according to any one of items 76 to 80, or a vector according to item 84.
- 5 86. A kit of parts comprising:
- i. the host cell according to item 85, and optionally instructions for use, and/or
 - ii. the nucleic acid construct according to any one of items 76 to 80 or the vector according to item 84, and optionally instructions for use, and
- 10 further optionally a host cell to be modified,
preferably wherein the host cell is selected from the group consisting of plant cells, yeast cells, bacterial cells and fungal cells.
- 15 87. A taxane having a side chain comprising a hydroxylated β -phenylalanine moiety obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
- 20 88. N-debenzoyl-taxol obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
- 25 89. 10-deacetyl-N-debenzoyl-taxol obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
- 30 90. N-debenzoyl-2'-deoxy-taxol obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
- 35 91. N-debenzoyl-taxol obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
92. A cell culture obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75.
93. A cell culture, comprising a host cell according to any one of items 1 to 36 or 85, and optionally a cultivation medium.

94. A fermentation liquid comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said fermentation liquid is:
- i. obtained by the method of any one of items 37 to 70 and/or the use according to any one of items 71 to 75;
 - 5 ii. comprised in the cell culture according to any one of items 92 to 93, and/or
 - iii. comprised within and/or secreted by the host cell according to any one of items 1 to 36 or 85.
- 10 95. The fermentation liquid of item 94, wherein at least 50%, such as at least 75%, such as at least 95%, such as at least 99% of the host cells are lysed.
96. The fermentation liquid according to any one of items 94 to 95, wherein at least 50%, such as at least 75%, such as at least 95%, such as at least 99% of solid
- 15 cellular material has been separated from the liquid.
97. A composition comprising one or more of:
- i. the fermentation liquid according to any one of items 94 to 96;
 - 20 ii. a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety obtained by the method of any one of items 37 to 70 and/or the use according to any one of items 71 to 75,
 - iii. N-debenzoyl-taxol obtained by the method of any one items 37 to 70 and/or the use according to any one of items 71 to 75;
 - iv. N-debenzoyl-2'-deoxy-taxol obtained by a method according to any one
 - 25 of items 37 to 70 and/or the use according to any one of items 71 to 75;
 - v. N-debenzoyl-taxol obtained by a method according to any one of items 37 to 70 and/or the use according to any one of items 71 to 75;
 - vi. 10-deacetyl-N-debenzoyl-taxol obtained by the method of any one of items 37 to 70 and/or the use according to any one of items 71 to 75;
 - 30 and/or
 - vii. taxol obtained by the method of any one of items 37 to 70 and/or the use according to any one of items 71 to 75, and optionally one or more agents, additives and/or excipients.

98. The composition of item 97, wherein the composition have been processed into in a semi-dry or dry solid form, optionally in form of a powder, tablet, capsule, chewable, gel and/or gum.
- 5 99. The composition of item 97, wherein the composition is in a liquid form, optionally in a stabilized liquid form.
- 10 100. A method for treating a disorder such as cancer, comprising administration of a medicament comprising a composition obtained by the method of any one of items 37 to 70 and/or the use according to any one of items 71 to 75, said composition comprising of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.
- 15 101. The host cell, the method, the use, the fermentation liquid, the cell culture and/or the composition according to any one of the preceding items, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol.
- 20 102. The host cell, the method, the use and/or the fermentation liquid according to any one of the preceding items, wherein said OD3 or a functional homologue thereof having at least 70% sequence identity thereto is capable of catalysing conversion of N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-taxol and/or of catalysing conversion of 10-deacetyl-N-debenzoyl-taxol to 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 25

Claims

1. A host cell comprising a heterologous nucleic acid sequence encoding OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 99% sequence identity thereto.
5
2. The host cell according to any one of the preceding claims, wherein the host cell is capable of producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol,
10 optionally wherein the host cell is capable of producing baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
15
3. The host cell according to any one of the preceding claims, wherein said host cell further comprises a nucleic acid encoding an amino phenylpropanoyl transferase (EC: 2.3.1),
20 optionally wherein said amino phenylpropanoyl transferase is native to a *Taxus* cell, such as a *Taxus cuspidata* cell, preferably wherein said amino phenylpropanoyl transferase is BAPT as set forth in SEQ ID NO: 1 or MBPig3BAPT as set forth in SEQ ID NO: 4, or functional homologues thereof having at least 70% sequence identity to any of the SEQ ID NO: 1 or SEQ ID NO: 4, respectively.
25
4. The host cell according to any one of the preceding claims, wherein said host cell further comprises a nucleic acid encoding a coenzyme A ligase (CoAL, EC: 6.2.1),
30 optionally wherein said CoAL is a CoAL native to a *Penicillium* cell, such as a *P. chrysogenum* cell or a CoAL native to a *Taxus* cell, such as a *T. chinensis* cell, preferably wherein said CoAL is CoAL(A312G) as set forth in SEQ ID NO: 2, AAE-867.5 as set forth in SEQ ID NO: 29 or TchiAAE5 as set forth in SEQ ID
35

NO: 22, or functional homologues thereof having at least 70% sequence identity to any of the SEQ ID NO: 2, SEQ ID NO: 29 or SEQ ID NO: 22, respectively.

5. The host cell according to any one of the preceding claims, wherein said host cell further comprises a nucleic acid encoding a 10-deacetyl-baccatin III-10-O-acetyltransferase (DBAT),
optionally wherein said DBAT is native to a *Taxus* cell, such as a *Taxus cuspidata* cell,
preferably wherein the DBAT is TcuDBAT as set forth in SEQ ID NO: 21 or a functional homologue thereof having at least 70% sequence identity thereto.
6. The host cell according to any one of the preceding claims, wherein said host cell further comprises a nucleic acid encoding a 3'-N-debenzoyl-2'-deoxytaxol-N-benzoyltransferase (DBTNBT, EC: 2.3.1),
optionally wherein said DBTNBT is native to a *Taxus* cell, such as a *Taxus canadensis* cell, preferably wherein said DBTNBT is TcaDBTNBT as set forth in SEQ ID NO: 20 or a functional homologue thereof having at least 70% sequence identity thereto.
7. The host cell according to any one of the preceding claims, wherein said host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:
- a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2) or a functional homologue thereof having at least 70% sequence identity thereto; and
 - a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively.
8. The host cell according to any one of the preceding claims, wherein the host cell comprises a heterologous nucleic acid encoding OD3 (SEQ ID NO: 3) or a functional homologue thereof having at least 70% sequence identity thereto, and further comprises:
- a nucleic acid encoding CoAL(A312G) (SEQ ID NO: 2), or a functional homologue thereof having at least 70% sequence identity thereto;

- ii. a nucleic acid encoding MBPig3BAPT (SEQ ID NO: 4) or BAPT (SEQ ID NO: 1), or a functional homologue thereof having at least 70% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 1, respectively;
- iii. a nucleic acid encoding a DBTNBT (EC: 2.3.1), optionally TcaDBTNBT (SEQ ID NO: 20) or a functional homologue thereof having at least 70% sequence identity thereto.
9. The host cell according to any one of the preceding claims, wherein said host cell is selected from the group of plant cells, yeast cells, bacterial cells and fungal cells.
10. The host cell according to any one of the preceding claims, wherein said host cell is a yeast cell belonging to the genus of *Saccharomyces*, *Pichia*, *Candida*, *Cryptococcus*, *Pichia (Komagataella)*, *Lipomyces*, *Pseudozyma*, *Rhodospiridium*, *Rhodotorula*, *Trichosporon*, *Trigonopsis*, *Yarrowia* or *Saccharomycopsis*, such as a yeast cell of the species *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Hansenula polymorpha (Ogataea polymorpha)*, *Rhodotorula toruloides* or *Pichia pastoris (Komagataella phaffii)*, preferably the host cell is a yeast cell of the species *S. cerevisiae*.
11. The host cell according to any one of the preceding claims, wherein said host cell is a bacterial cell belonging to the genus of *Escherichia*, *Bacillus*, *Corynebacterium*, *Pseudomonas* or *Streptomyces*, such as a bacterial cell of the species *Escherichia coli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Pseudomonas putida* or *Streptomyces sp.*, preferably the host cell is a bacterial cell of the species *E. coli*.
12. A method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, said method comprising the steps of:
- i. providing a host cell according to any one of claims 1 to 11;
- ii. cultivating said host cell in a cultivation medium, thereby producing said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, optionally further comprising a step of recovering said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.

- 5 13. The method according to claim 12, wherein said step of cultivating said host cell is performed in the presence of baccatin III, benzoyl-CoA, 10-deacetyl-baccatin III, β -phenylalanine, β -phenylalanoyl-CoA, N-debenzoyl-2'-deoxy-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 10 14. The method according to any one of claims 12 to 13, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is N-debenzoyl-taxol, and wherein said cultivation medium comprises N-debenzoyl-2'-deoxy-taxol or baccatin III, and/or wherein the host cell is capable of producing N-debenzoyl-2'-deoxy-taxol or baccatin III.
- 15 15. The method according to any one of claims 12 to 14, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety is 10-deacetyl-N-debenzoyl-taxol, and wherein said cultivation medium comprises 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or 10-deacetyl-baccatin III and/or wherein the host cell is capable of producing 10-deacetyl-N-debenzoyl-2'-deoxy-taxol or 10-deacetyl-baccatin III.
- 20 16. The method according to any one of claims 12 to 15, wherein said step of cultivating said host cell is performed in the presence of baccatin III, and wherein said host cell is as defined in claim 7, whereby N-debenzoyl-taxol is produced, wherein said cultivation medium comprises baccatin III and/or wherein said host cell is capable of producing baccatin III.
- 25 17. The method according to any one of claims 12 to 16, wherein said step of cultivating said host cell is performed in the presence of baccatin III, and wherein said host cell is as defined in claim 8, whereby taxol is produced, wherein said cultivation medium comprises baccatin III and/or wherein said host cell is capable of producing baccatin III.
- 30 18. Use of OD3 as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 75% sequence identity, such as at least 80% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity

thereto, in a method for producing a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety,
preferably wherein said method is as defined in any one of claims 12 to 13.

- 5 19. An isolated polypeptide as set forth in SEQ ID NO: 3 or a functional homologue thereof having at least 75% sequence identity, for example at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90%, such as at least 95%, for example at least 99% sequence identity thereto.
- 10 20. A fermentation liquid comprising a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety, wherein said fermentation liquid is obtained by the method of any one of claims 12 to 17 and/or the use according to claim 18.
- 15 21. A method for treating a disorder such as cancer, comprising administration of a medicament comprising a composition obtained by the method of any one of claims 12 to 17 and/or the use according to claim 18, said composition comprising of a taxane having a side chain comprising a hydroxylated β -phenylalanine moiety.
- 20 22. The host cell, the method, the use and/or the fermentation liquid according to any one of the preceding claims, wherein said taxane having a side chain comprising a hydroxylated β -phenylalanine moiety comprises or consists N-debenzoyl-taxol, 10-deacetyl-N-debenzoyl-taxol, and/or taxol.
- 25 23. The host cell, the method, the use and/or the fermentation liquid according to any one of the preceding claims, wherein said OD3 or a functional homologue thereof having at least 75% sequence identity thereto is capable of catalysing conversion of N-debenzoyl-2'-deoxy-taxol to N-debenzoyl-taxol and/or of catalysing conversion of 10-deacetyl-N-debenzoyl-taxol to 10-deacetyl-N-debenzoyl-2'-deoxy-taxol.
- 30

a

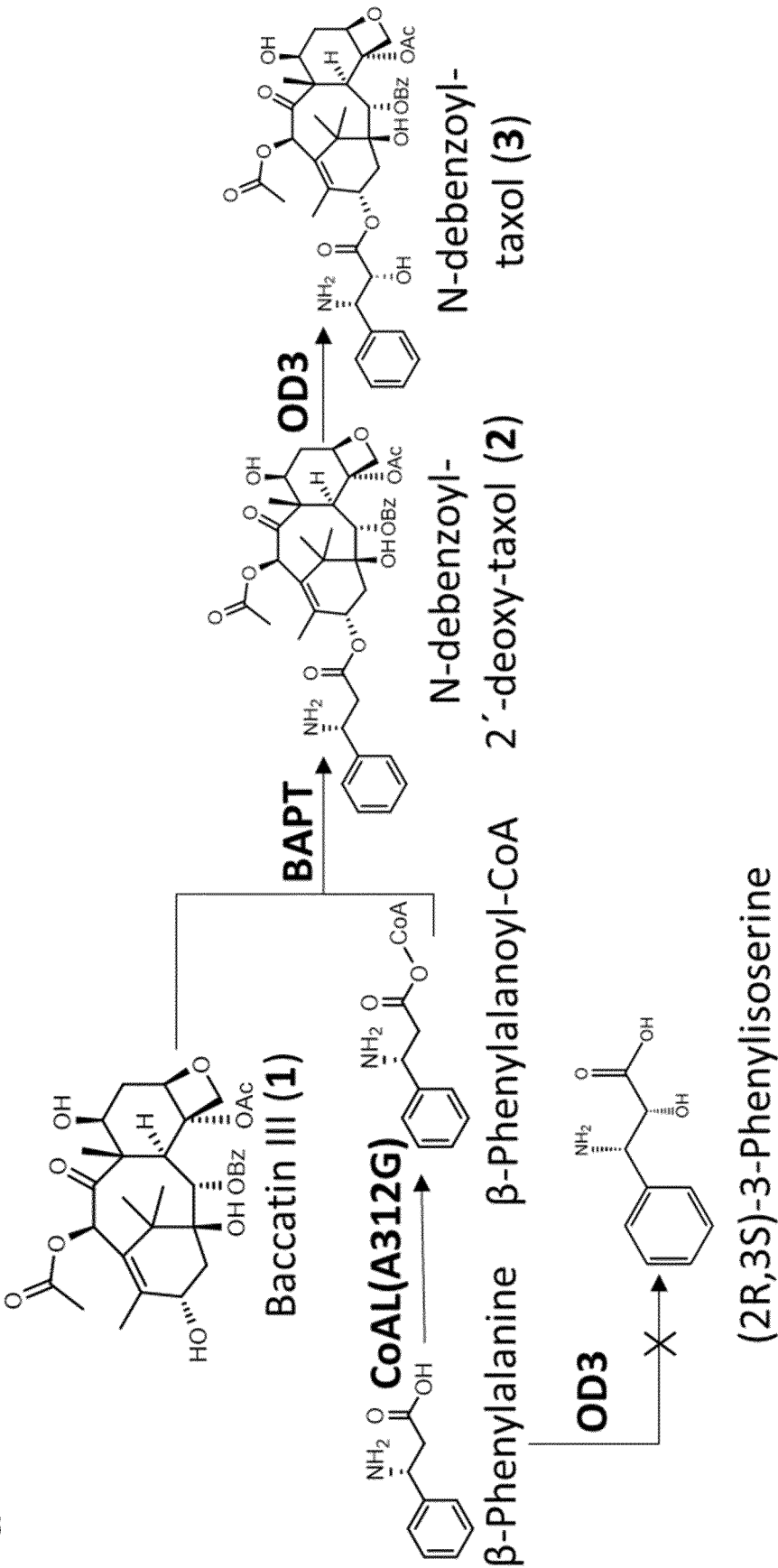


Figure 1a

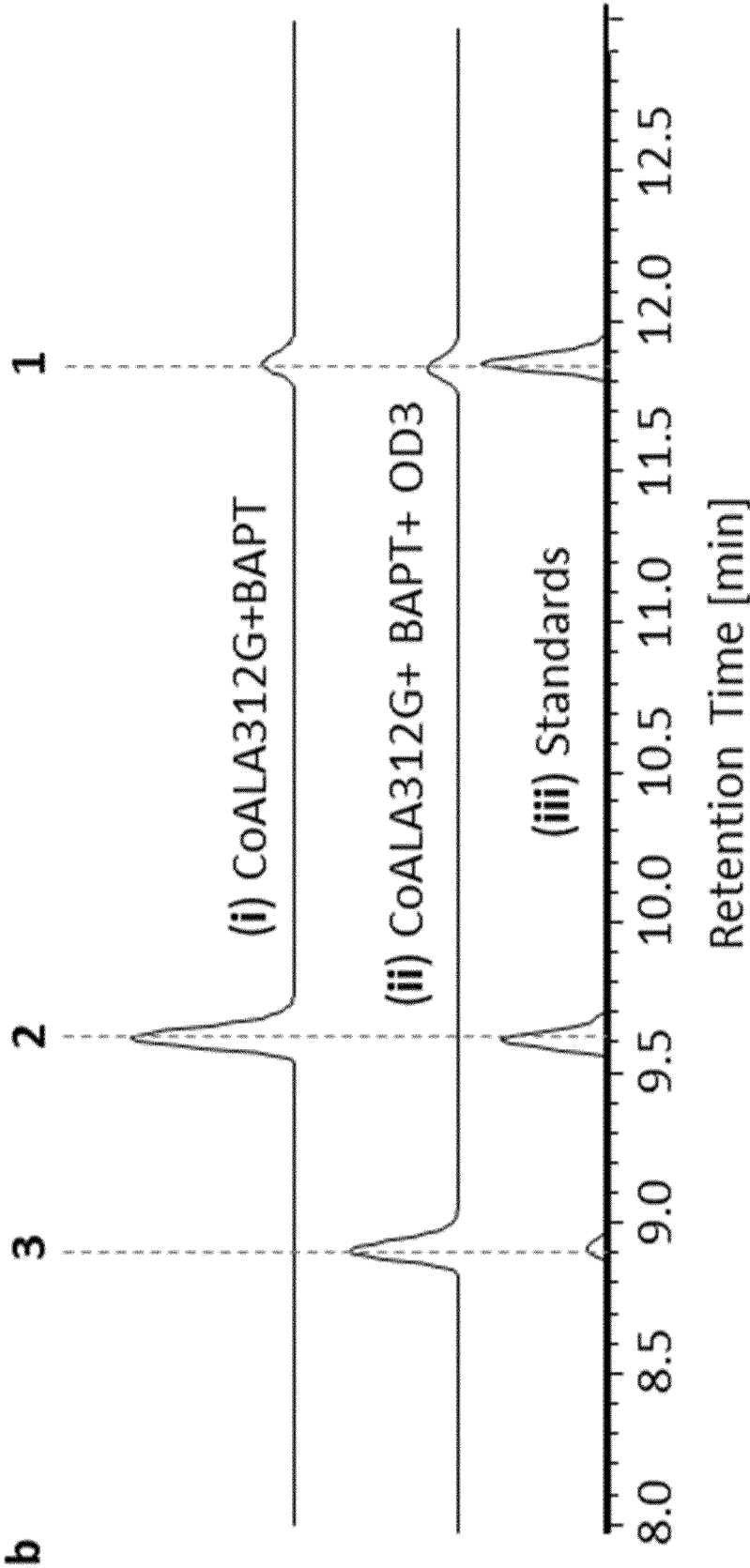


Figure 1b

a

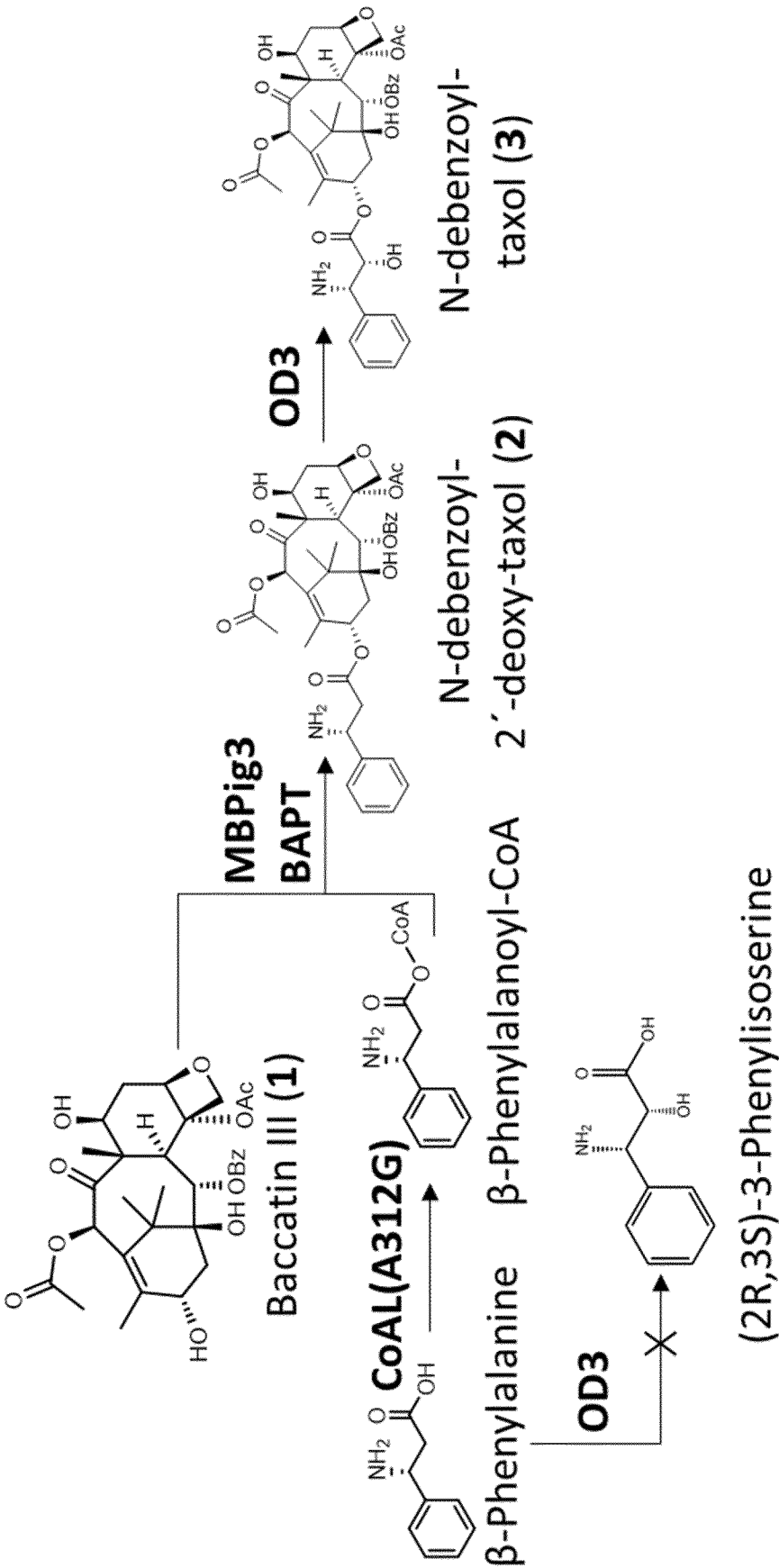


Figure 2a

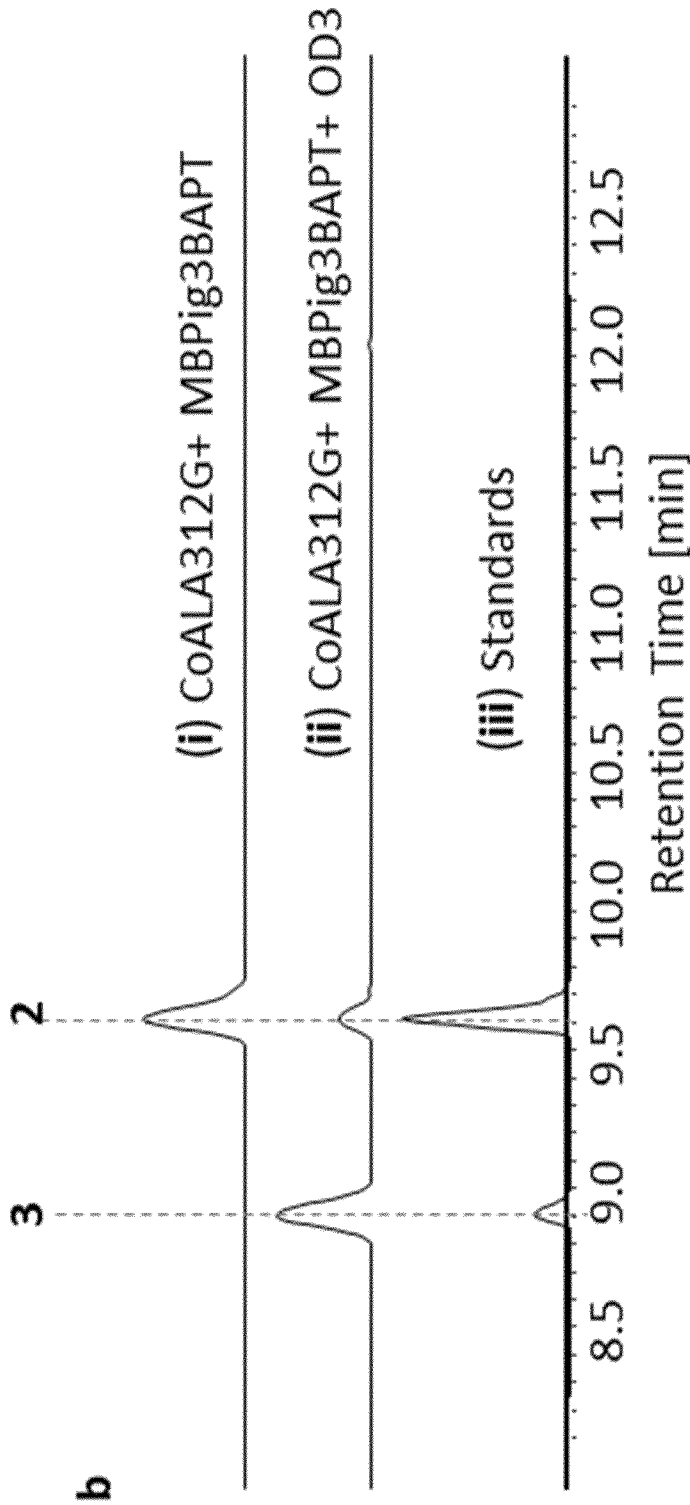


Figure 2b

a

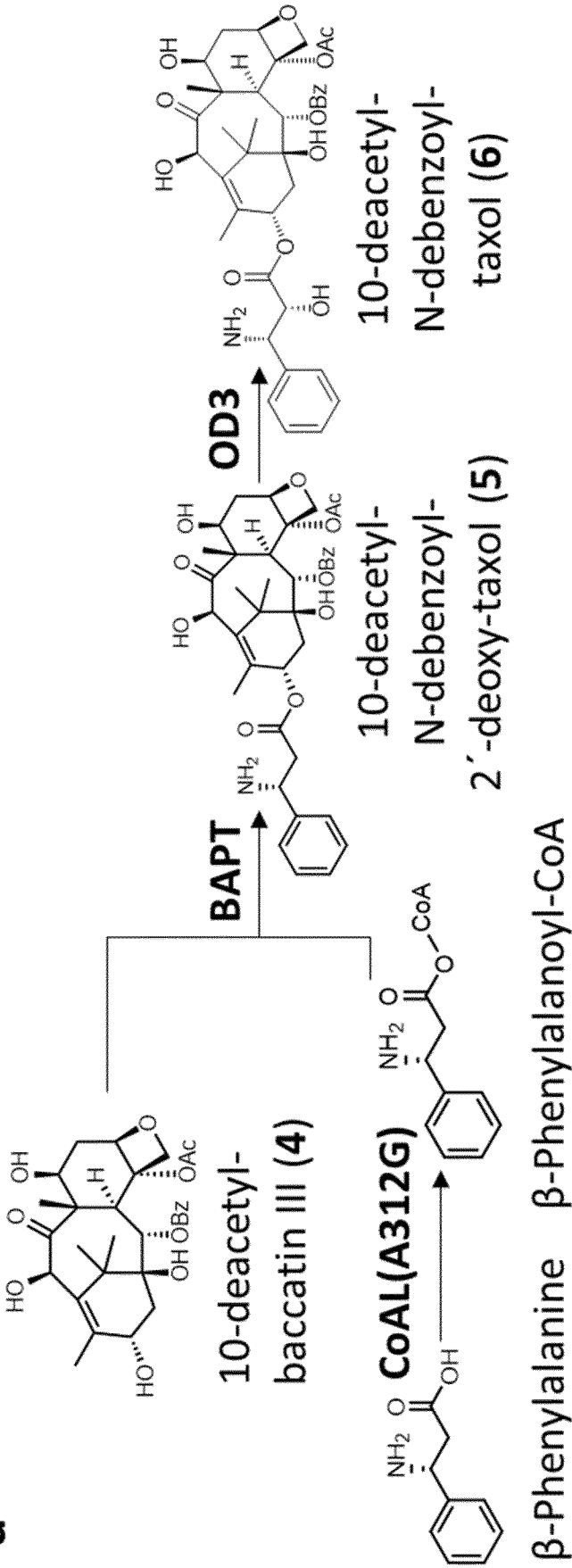


Figure 3a

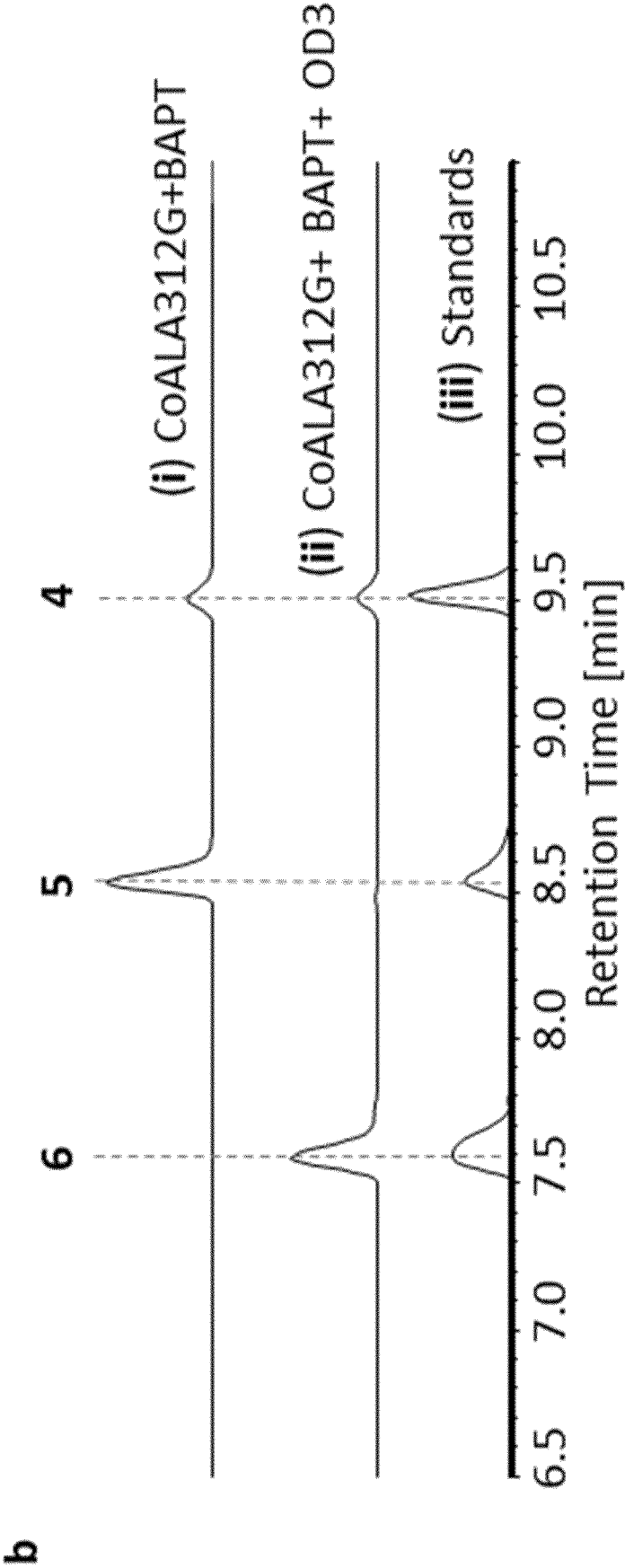


Figure 3b

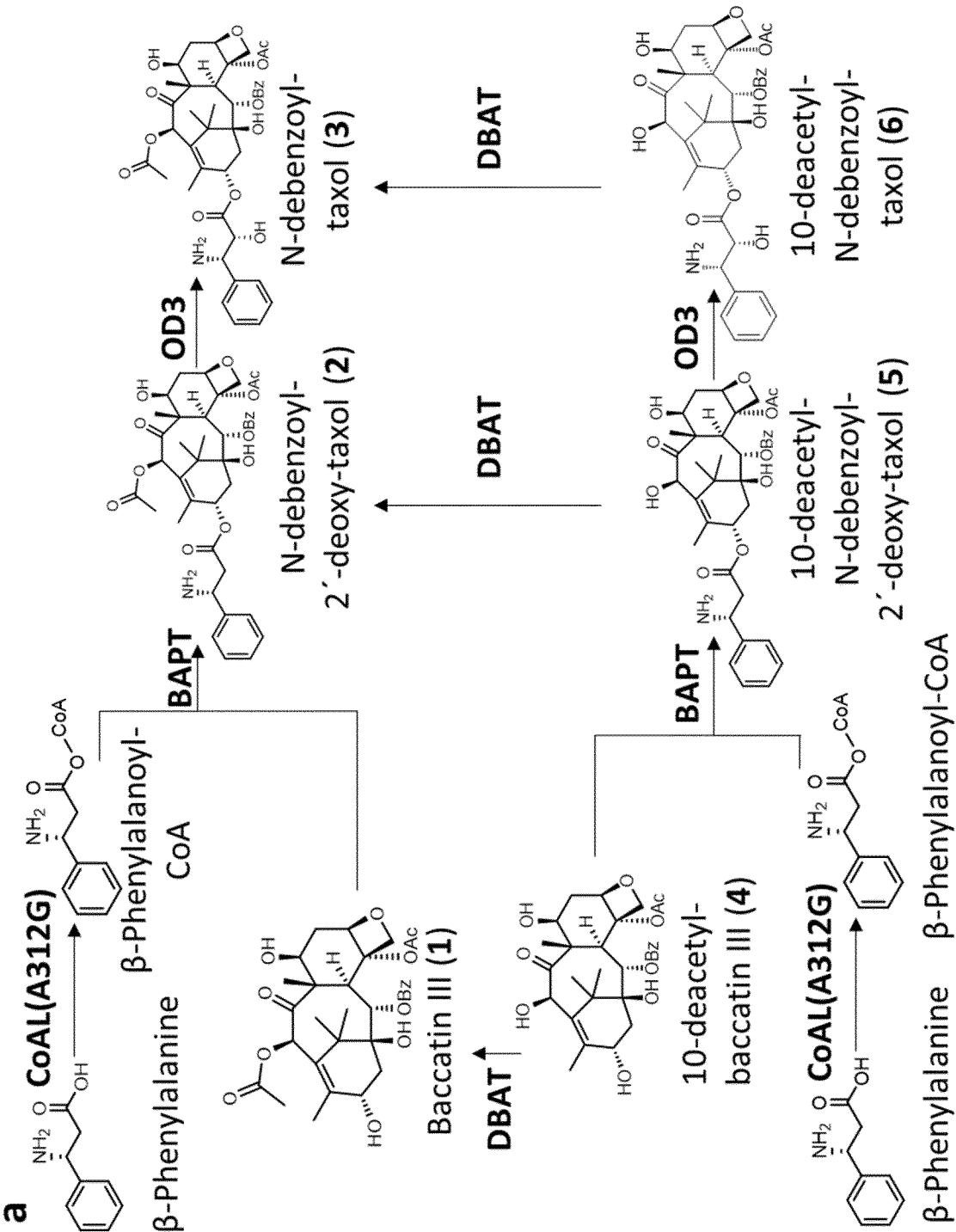


Figure 4a

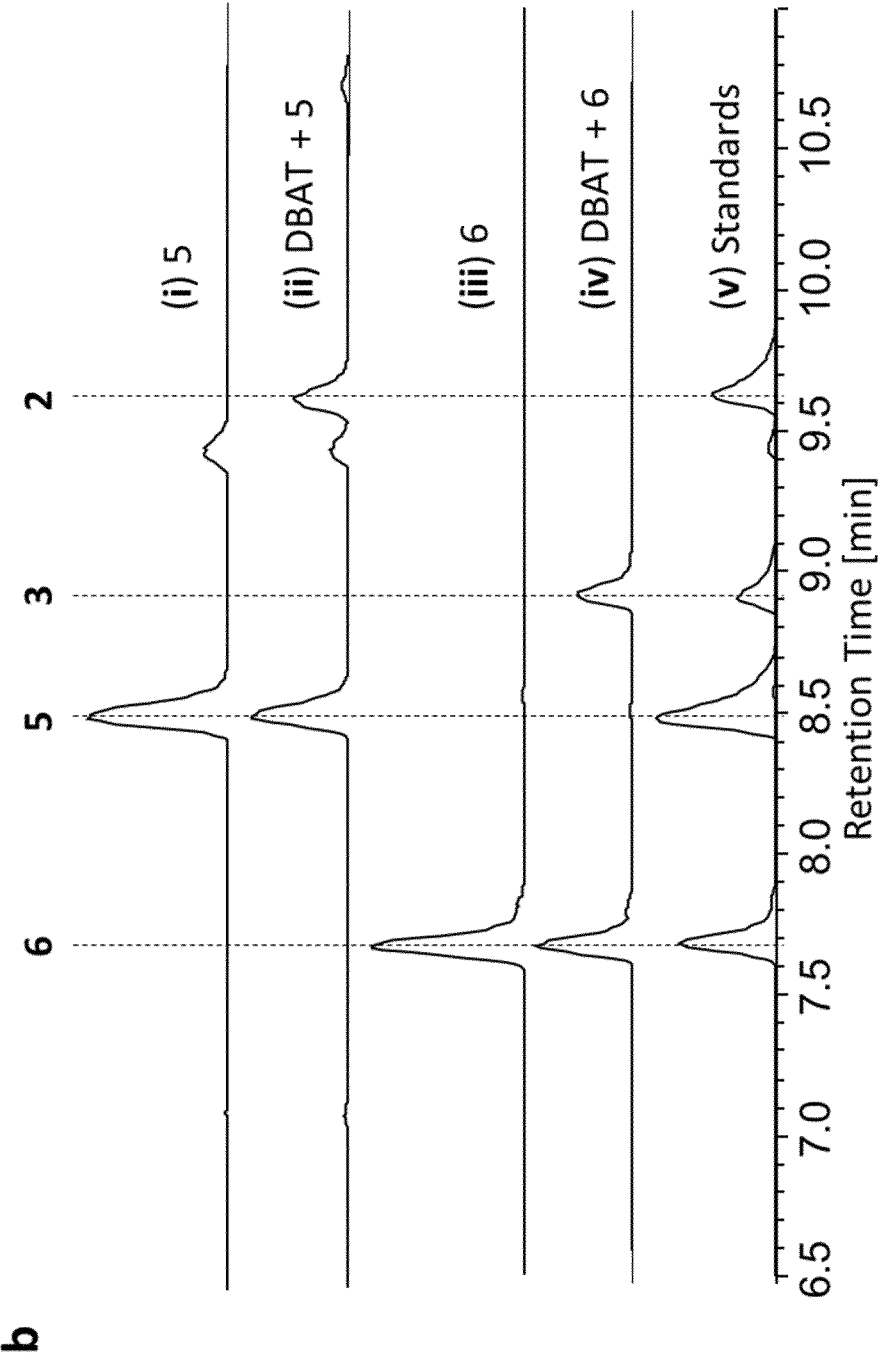


Figure 4b

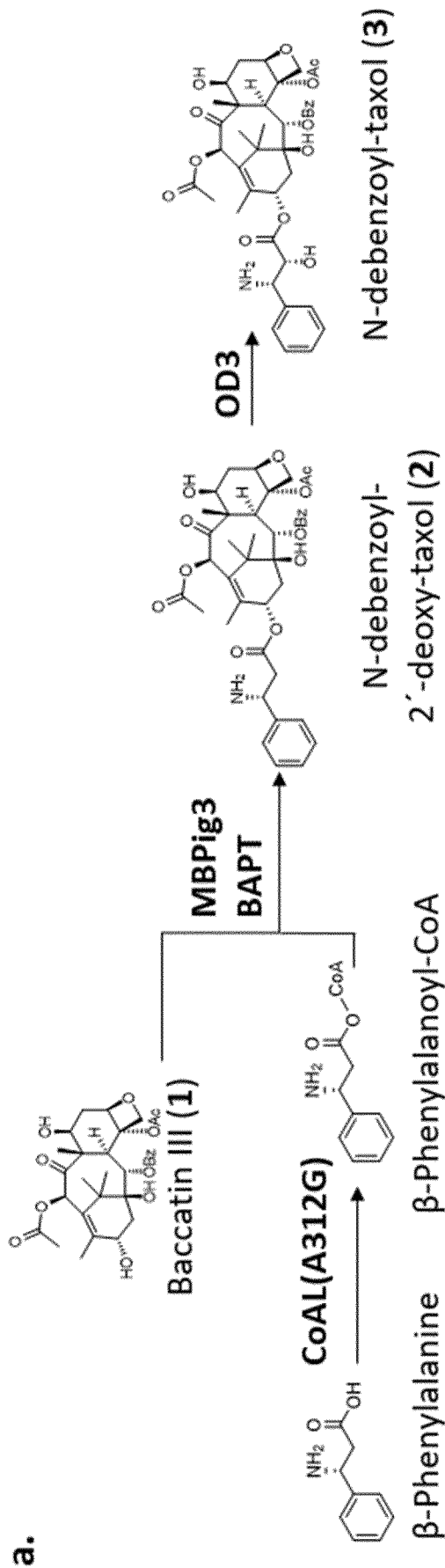


Figure 5a

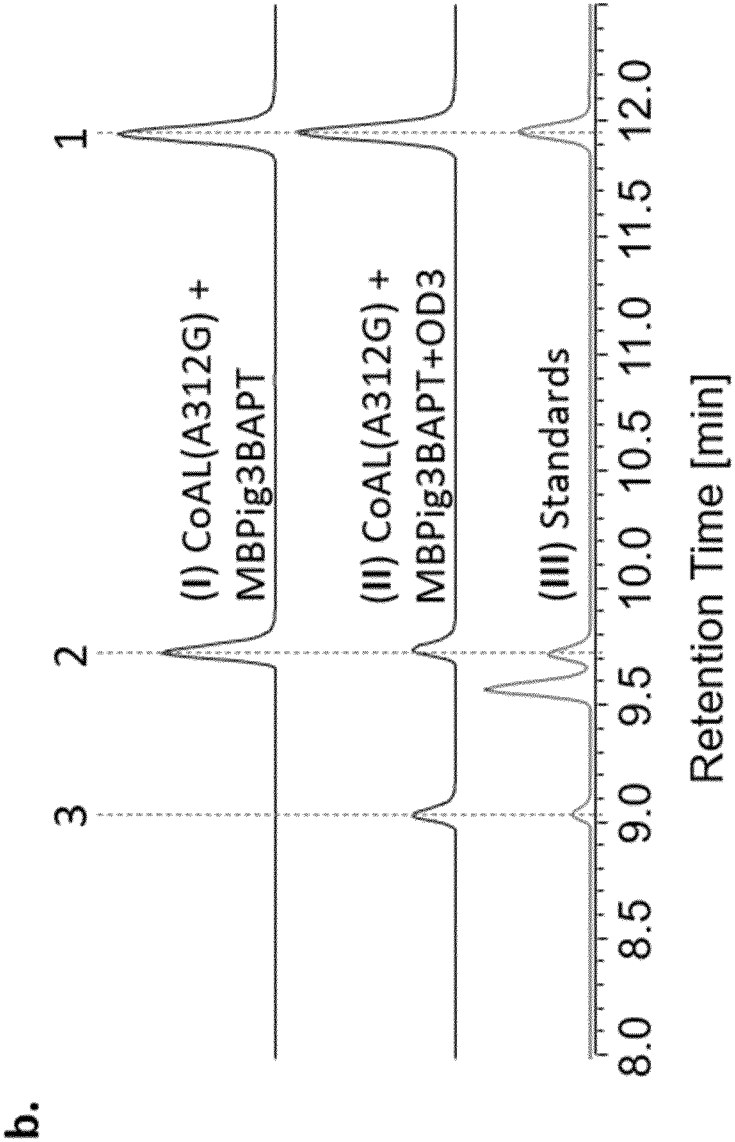


Figure 5b

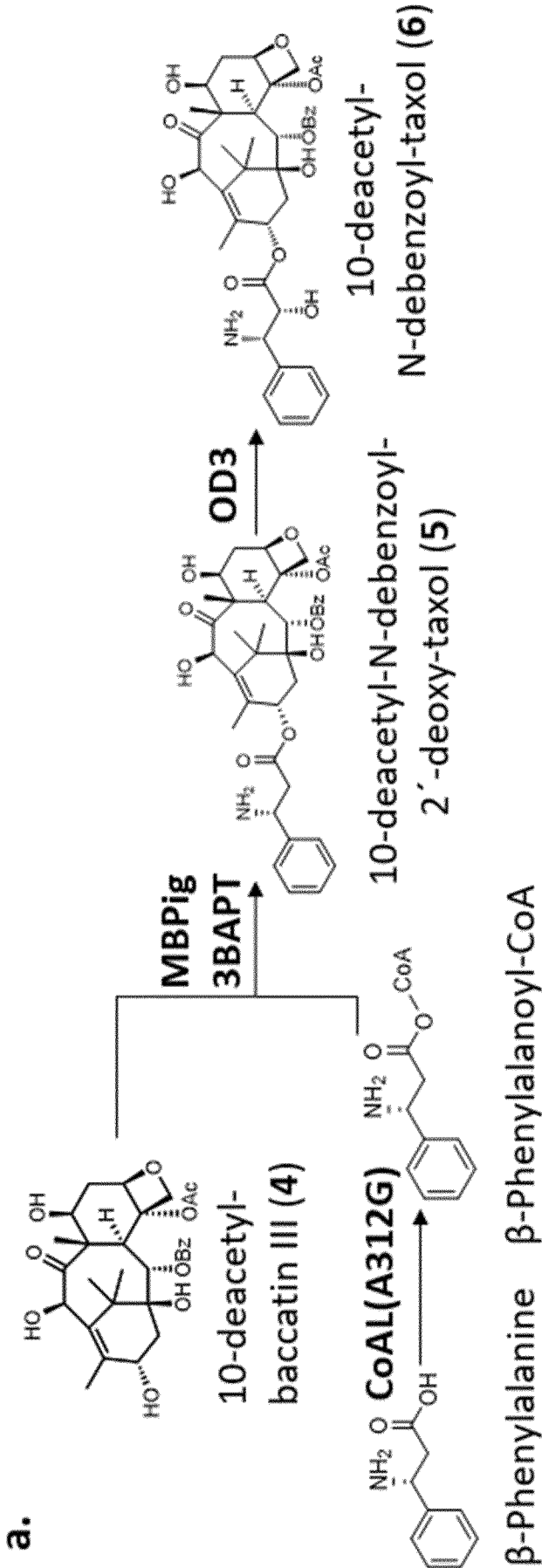


Figure 6a

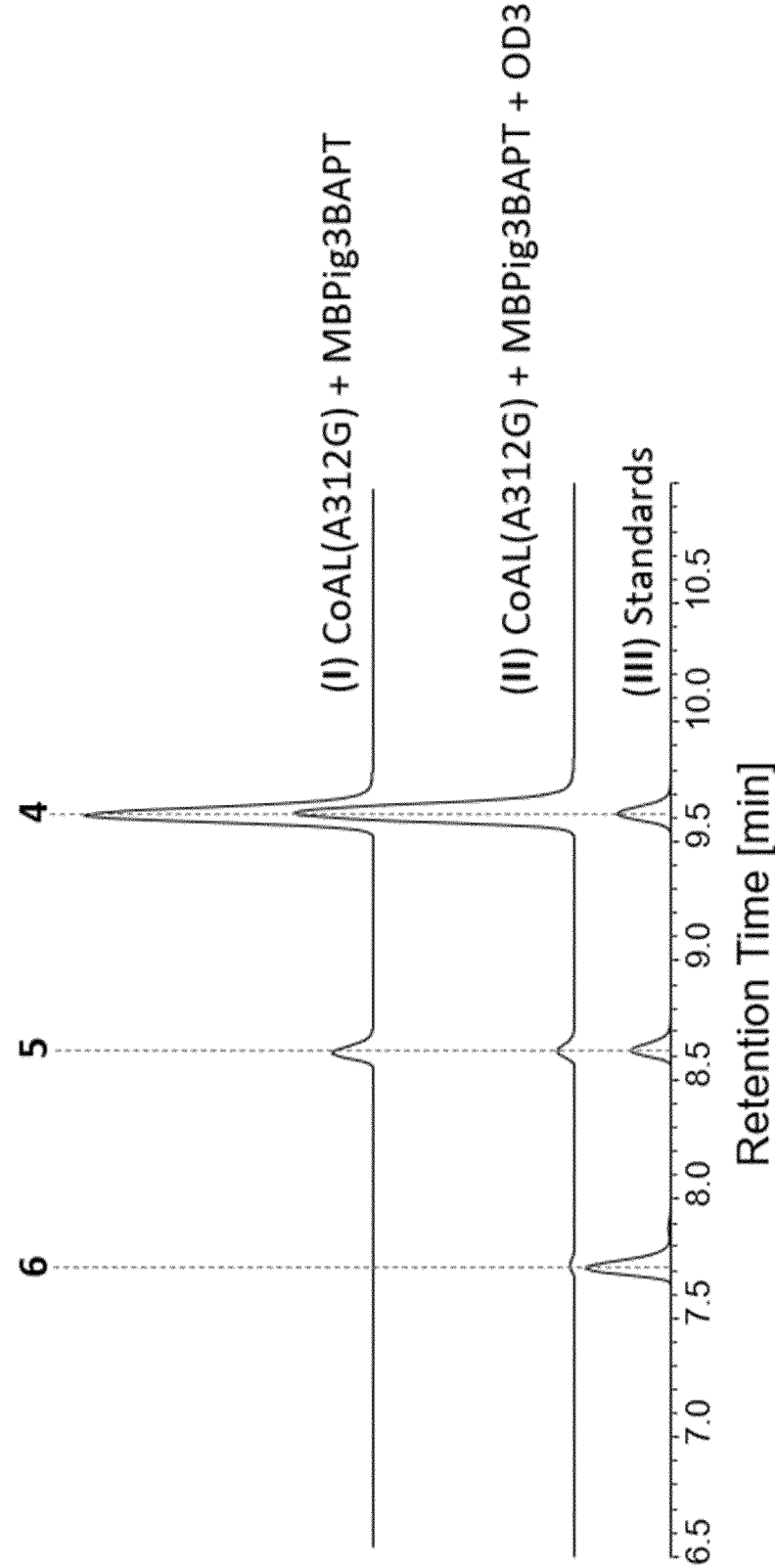


Figure 6b

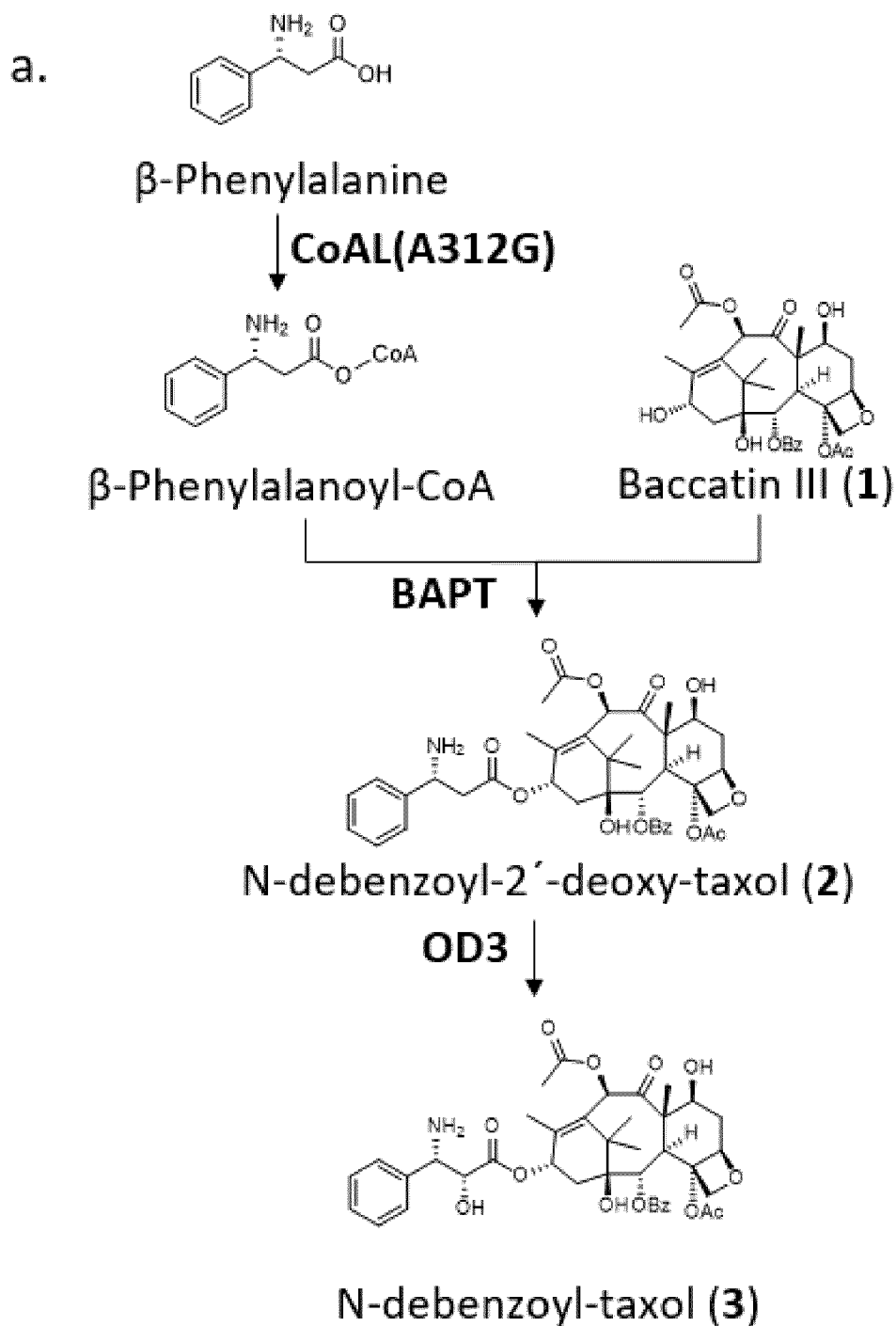


Figure 7a

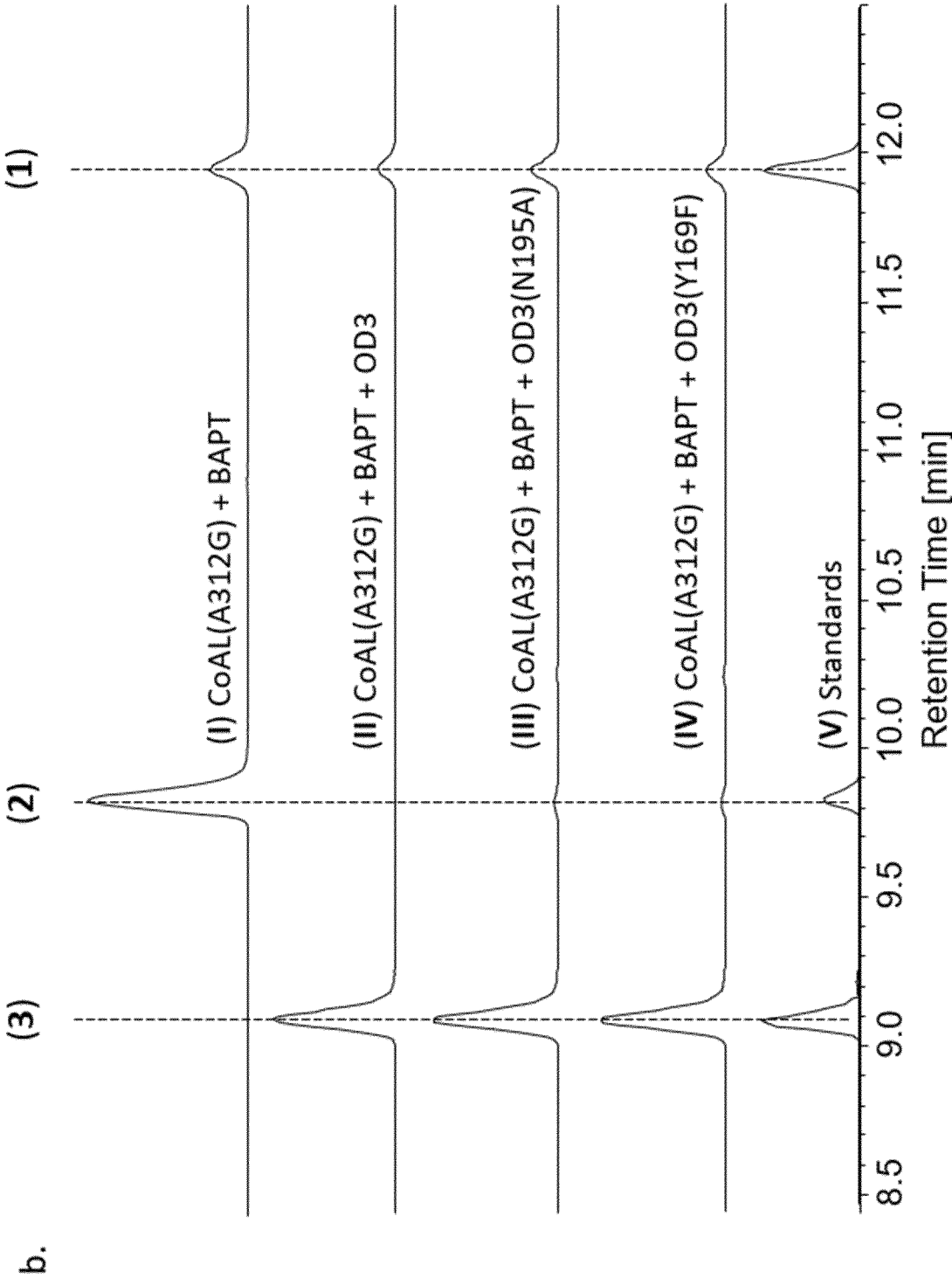


Figure 7b

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/088095

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/088095

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/02 A61K31/337 C07D305/14 C12N15/82 C12P17/02
ADD.

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)

C12N C07D C12P A61K

Documentation searched 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)

EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAMÍREZ-ESTRADA KARLA ET AL: "Transcript profiling of jasmonate-elicited Taxus cells reveals a [beta]-phenylalanine-CoA ligase", PLANT BIOTECHNOLOGY JOURNAL , vol. 14, no. 1 21 April 2015 (2015-04-21), pages 85-96, XP093080562, GB ISSN: 1467-7644, DOI: 10.1111/pbi.12359 Retrieved from the Internet: URL:https://api.wiley.com/onlinelibrary/tdm/v1/articles/10.1111%2Fpbi.12359	21
A	abstract; pages 85, 92, 93; Figure 1; Table 1 -/-	1-20, 22, 23



Further documents are listed in the continuation of Box C.



See patent family annex.

* 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

"&" document member of the same patent family

Date of the actual completion of the international search

18 March 2025

Date of mailing of the international search report

28/03/2025

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Kurz, Birgit

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/088095

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	- & DATABASE UniProt [Online] 22 July 2015 (2015-07-22), "SubName: Full=Putative epoxidase {ECO:0000313 EMBL:AKH04260.1}"; XP093171148, retrieved from EBI accession no. UNIPROT:A0A0F7J1F5 Database accession no. A0A0F7J1F5 the whole document	1-23
A	- & DATABASE GenBank [Online] 18 May 2015 (2015-05-18), Anonymous: "Taxus baccata putative hydroxylase mRNA, complete cds - Nucleotide - NCBI", XP093171158, Database accession no. KP178208 the whole document -----	1-23
A	SANCHEZ-MUÑOZ RAUL ET AL: "A Novel Hydroxylation Step in the Taxane Biosynthetic Pathway: A New Approach to Paclitaxel Production by Synthetic Biology", FRONTIERS IN BIOENGINEERING AND BIOTECHNOLOGY, vol. 8, 13 May 2020 (2020-05-13), pages 1-14, XP093171026, CH ISSN: 2296-4185, DOI: 10.3389/fbioe.2020.00410 abstract; Figure 1; pages 2, 3, 8, 9, 11 -----	1-23
A	ZHANG YOUJUN ET AL: "Synthetic biology identifies the minimal gene set required for paclitaxel biosynthesis in a plant chassis", MOLECULAR PLANT, vol. 16, no. 12, 28 October 2023 (2023-10-28), pages 1951-1961, XP093171142, ISSN: 1674-2052, DOI: 10.1016/j.molp.2023.10.016 cited in the application abstract; Figures 1 and 5; pages 1953, 1956, 1957 ----- -/-	1-23

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/088095

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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