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# ELECTRONIC PAYMENT RECEIPT

| APPLICATION # | RECEIPT DATE / TIME | ATTORNEY DOCKET # |
|---|---|---|
| **63/898,319** | **10/13/2025 05:20:05 PM Z ET** | **38584-30001.00** |

## Title of Invention

PSILOCYBIN AND PSILOCYBIN ANALOGS, AND ENZYMATIC METHODS OF PRODUCTION RELATED THERETO

## Application Information

| | | | |
|---|---|---|---|
| APPLICATION TYPE | Utility - Provisional Application under 35 USC 111(b) | PATENT # | - |
| CONFIRMATION # | 8147 | FILED BY | Nkem Nnam |
| PATENT CENTER # | 72681219 | AUTHORIZED BY | Anita Choi |
| CUSTOMER # | 20872 | FILING DATE | - |
| CORRESPONDENCE ADDRESS | - | FIRST NAMED INVENTOR | Bryan Quoc Le |

## Payment Information

| PAYMENT METHOD | PAYMENT TRANSACTION ID | PAYMENT AUTHORIZED BY |
|---|---|---|
| **DA / 031952** | **E20250CH21077905** | **Nkem Nnam** |

| FEE CODE | DESCRIPTION | ITEM PRICE($) | QUANTITY | ITEM TOTAL($) |
|---|---|---|---|---|
| 2005 | PROVISIONAL APPLICATION FILING FEE | 130.00 | 1 | 130.00 |
| | | | **TOTAL AMOUNT:** | **$130.00** |

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

**New Applications Under 35 U.S.C. 111**

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

**National Stage of an International Application under 35 U.S.C. 371**

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C.

371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

**New International Application Filed with the USPTO as a Receiving Office**

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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# ELECTRONIC ACKNOWLEDGEMENT RECEIPT

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| CUSTOMER # | 20872 | FIRST NAMED INVENTOR | Bryan Quoc Le |
| CORRESPONDENCE ADDRESS | - | AUTHORIZED BY | Anita Choi |

## Documents

**TOTAL DOCUMENTS: 5**

| DOCUMENT | | PAGES | DESCRIPTION | SIZE (KB) |
|---|---|---|---|---|
| 1 - 38584-30001.00 - Application Data Sheet.pdf | | 8 | Application Data Sheet | 1226 KB |
| 2 - 38584-30001.00 SPEC.pdf | | 28 | - | 291 KB |
| 2 - 38584-30001.00 SPEC-SPEC.pdf | (1-14) | 14 | Specification | 136 KB |
| 2 - 38584-30001.00 SPEC-CLM.pdf | (15-27) | 13 | Claims | 101 KB |
| 2 - 38584-30001.00 SPEC-ABST.pdf | (28-28) | 1 | Abstract | 15 KB |
| 3 - 38584-30001.00_FIGS.pdf | | 2 | Drawings-other than black and white line drawings | 661 KB |

## Digest

| DOCUMENT | MESSAGE DIGEST(SHA-512) |
|---|---|
| 1 - 38584-30001.00 - Application Data Sheet.pdf | B30DF9E0B73A64F1493F9D9E0E623B30B470497D9A7A431DD937BEBCAF353C40974464CF1EDD5C29A7CA9611C440025506AB8A846A403F3E5DBC25FDFCF6B001 |
| 2 - 38584-30001.00 SPEC.pdf | 9B0355829507C1606750281FB946E811CB6BC2F175469C790573AA1CA548560BD587D62998B1E5ADDB56F1DACF2725B95DF6E35DBA35B70B426686ED2291A534 |
| 2 - 38584-30001.00 SPEC-SPEC.pdf | BB29564DE8E800AD68A8D149F72EBFED2C20615248D4838E030FBF70B861370AB943FA7D1C57D27EAF9AAD7248EF9AE490B7341AF175A59F89D6699C0332EBE7 |
| 2 - 38584-30001.00 SPEC-CLM.pdf | 54052C7EDC6DA6BC01C06BC76FDB15B1D5E58314346A3386293D240ED03887469BA2C58D5D5EB90F4D06FD1978BDA21FCDB368EDE6F1ACA3AD464A0BEC6249BA |
| 2 - 38584-30001.00 SPEC-ABST.pdf | C14CB7010709065A7329171B9FE83296CDD429146D8FF7BA18CD4BC4E4DC1DA8D49A1E9A23EFC94057070F1B07798F1C7879C24E6654B2C65393F3DA394E0C0E |
| 3 - 38584-30001.00_FIGS.pdf | 582FCF23F55718D01AE56EDB1407A2EFFA07290FBC024CBBB139CCC54BF4713F593A44ACD5E145315AB93BBF5A969911BE0DDC9F4F52E2DBC684749BA06A2EE4 |

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**New Applications Under 35 U.S.C. 111**

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

**New International Application Filed with the USPTO as a Receiving Office**

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PTO/AIA/14 (02-18)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 38584-30001.00 |
|---|---|---|
| | Application Number | |
| Title of Invention | PSILOCYBIN AND PSILOCYBIN ANALOGS, AND ENZYMATIC METHODS OF PRODUCTION RELATED THERETO | |

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

## Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

## Inventor Information:

**Inventor 1** Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Bryan | Quoc | Le | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | Mendocino | State/Province | CA | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| | | | |
|---|---|---|---|
| **Address 1** | 10451 Nichols Lane | | |
| **Address 2** | | | |
| **City** | Mendocino | **State/Province** | CA |
| **Postal Code** | 95460 | **Country** | US |

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

## Correspondence Information:

**Enter either Customer Number or complete the Correspondence Information section below.**
**For further information see 37 CFR 1.33(a).**

☐ **An Address is being provided for the correspondence Information of this application.**

| | | | |
|---|---|---|---|
| **Customer Number** | 20872 | | |
| **Email Address** | PatentDocket@mofo.com | Add Email | Remove Email |

## Application Information:

| | | | |
|---|---|---|---|
| **Title of the Invention** | PSILOCYBIN AND PSILOCYBIN ANALOGS, AND ENZYMATIC METHODS OF PRODUCTION RELATED THERETO | | |
| **Attorney Docket Number** | 38584-30001.00 | **Small Entity Status Claimed** | ☒ |
| **Application Type** | Provisional | | |
| **Subject Matter** | Utility | | |
| **Total Number of Drawing Sheets (if any)** | 2 | **Suggested Figure for Publication (if any)** | |

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## Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

| Application number of the previously filed application | Filing date (YYYY-MM-DD) | Intellectual Property Authority or Country |
|---|---|---|
| | | |

## Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

## Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

| Please Select One: | ◉ Customer Number | ○ US Patent Practitioner | ○ Limited Recognition (37 CFR 11.9) |
|---|---|---|---|
| Customer Number | 20872 | | |

## Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

| Prior Application Status | | | Remove |
|---|---|---|---|
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| | | | |

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

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## Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

| Application Number | Country[i] | Filing Date (YYYY-MM-DD) | Access Code[i] (if applicable) |
|---|---|---|---|
| | | | |

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

## Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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# Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

**NOTE**: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

**1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)**

**A. Priority Document Exchange (PDX)** - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

**B. Search Results from U.S. Application to EPO** - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

**2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)**

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

**NOTE:** Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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| | Application Number | |
| Title of Invention | PSILOCYBIN AND PSILOCYBIN ANALOGS, AND ENZYMATIC METHODS OF PRODUCTION RELATED THERETO | |

## Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

**Applicant 1**

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

| ◉ Assignee | ○ Legal Representative under 35 U.S.C. 117 | ○ Joint Inventor |
|---|---|---|
| ○ Person to whom the inventor is obligated to assign. | ○ Person who shows sufficient proprietary interest | |

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

| Organization Name | Mendosyn Bio, Inc. |
|---|---|

**Mailing Address Information For Applicant:**

| **Address 1** | 10451 Nichols Lane | | |
|---|---|---|---|
| Address 2 | | | |
| **City** | Mendocino | **State/Province** | CA |
| **Country** | US | Postal Code | 95460 |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Applicant Data may be generated within this form by selecting the Add button.

## Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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| | Application Number | |
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**Assignee 1**

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

| Prefix | **Given Name** | Middle Name | **Family Name** | Suffix |
|---|---|---|---|---|
| | | | | |

**Mailing Address Information For Assignee including Non-Applicant Assignee:**

| | | | |
|---|---|---|---|
| **Address 1** | | | |
| Address 2 | | | |
| **City** | | **State/Province** | |
| **Country** i | | Postal Code | |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

## Signature:

**NOTE:** This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

| **Signature** | /Anita Choi/ | | | Date (YYYY-MM-DD) | 2025-10-13 |
|---|---|---|---|---|---|
| First Name | Anita | Last Name | Choi | Registration Number | 71,792 |

Additional Signature may be generated within this form by selecting the Add button.

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

# Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

# PSILOCYBIN AND PSILOCYBIN ANALOGS, AND ENZYMATIC METHODS OF PRODUCTION RELATED THERETO

## FIELD

**[0001]** The present disclosure relates generally to psilocybin and psilocybin analogs, and more specifically to immobilized enzymatic approach to alkylating tryptamines for production of such compounds.

## BACKGROUND

**[0002]** Psilocybin, the psychoactive compound found in various members of the *Psilocybe* genus colloquially known as 'magic mushrooms', has regained a resurgence in the medical research community in the last decade as a potential treatment for major depressive disorder (MDD) and treatment-resistant depression (TRD). Research into the compound's potential therapeutic benefits was greatly stifled by legislative restrictions that set psilocybin and other psychedelic compounds as Schedule I substances during the 1960s. However, recent advancements have led to the United States Food and Drug Administration granting two "Breakthrough Therapy" designations to psilocybin. Clinical trials for the use of psilocybin for substance use disorders and existential distress from cancer are underway as well.

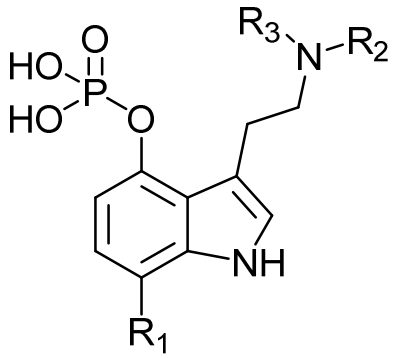
**[0003]** With these new developments, the demand for psilocybin, that conforms to Current Good Manufacturing Practices (cGMP) for pharmaceutical purposes, is on the rise. The majority of pure psilocybin used in clinical trials have been sourced from methodologies relying on multi-gram to kilogram-scale organic syntheses. However, there are challenges in sustaining increased scale in the protocols. Such challenges include, for example, a) the need for strong kinetic control over reaction conditions to limit side reactions, b) difficulties in workup conditions due to instability of intermediates, c) the use of toxic reagents and their associated byproducts, and d) limited yield.

**[0004]** Thus, what is desired in the art are alternative methods to produce psilocybin and psilocybin analogs.

## BRIEF SUMMARY

**[0005]** In some aspects, provided is an immobilized enzyme pathway for constructing structural analogs of psilocybin at the dimethylamine moiety.

**[0006]** In certain aspects, provided is a psilocybin analog of formula (P):




(P),

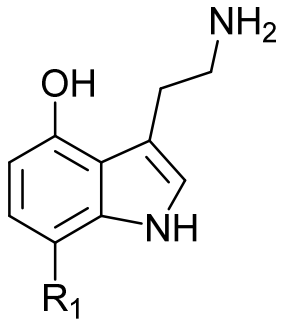
or a salt thereof, wherein $R_1$, $R_2$ and $R_3$ are as described herein.

**[0007]** In other aspects, provided is a method of producing a psilocybin analog of formula (P), or a salt thereof. In some embodiments, the method comprises:

a) providing a main column comprising a co-immobilized enzyme cascade system, wherein kinase PsiK is co-immobilized with methyltransferase PsiM; and

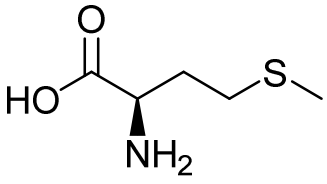
b) flowing a solution comprising:

- 4-hydroxy-7-R1-tryptamine analog of formula (T3):




(T3), or a salt thereof;

- L-methionine (S1):

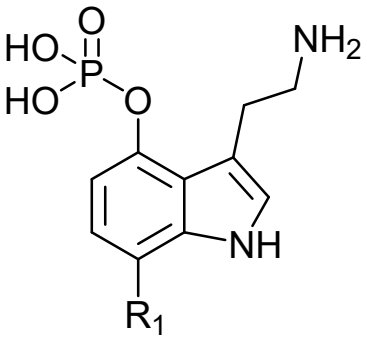



(S1), or a salt thereof;

- HS-$R_2$ and HS-$R_3$, wherein $R_2$ and $R_3$ are the same or different;
- adenosine triphosphate (ATP); and
- at least one coenzyme,

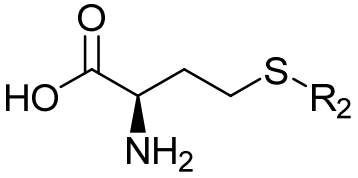
through the main column. In some embodiments, the aforementioned steps lead to:

i) producing 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) from T3:

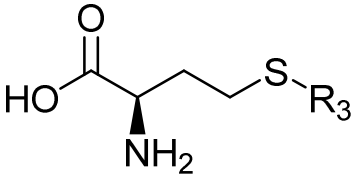



(T4), or a salt thereof,

ii) producing 2-amino-4-($R_2$-thio)butanoic acid (S2-$R_2$) and 2-amino-4-($R_3$-thio)butanoic acid (S2-$R_3$) from S1:

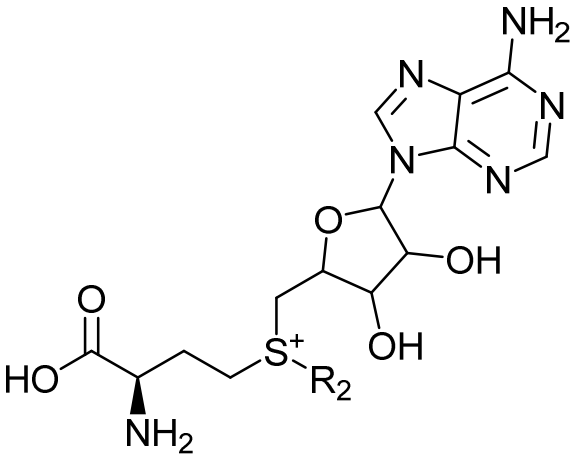



(S2-$R_2$) and

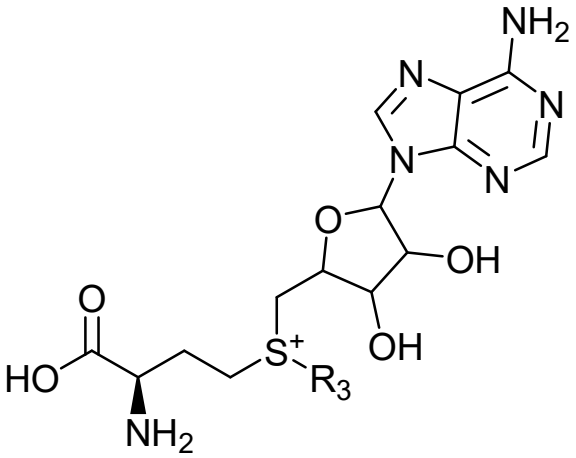



(S2-$R_3$), or a salt thereof;

iii) producing S-adenosyl-L-methionine analog ($SAR_2$) and S-adenosyl-L-methionine analog ($SAR_3$):

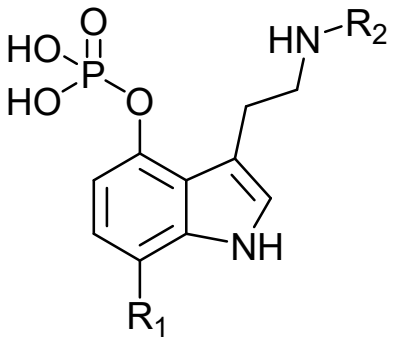


(SAR$_2$) and

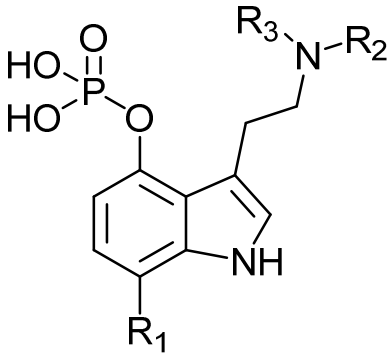


(SAR$_3$), or a salt thereof;

iv) alkylating the 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) in the presence of SAR$_2$ and SAR$_3$ to produce 4-phosphoryloxy-7-R1-N-R2-tryptamine analog of formula (T5) and the psilocybin analog of formula (P):



(T5) and



(P), or a salt thereof.

**[0008]** In other embodiments, the method comprises:

i) providing 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) as described herein, or a salt thereof;

ii) providing S-adenosyl-L-methionine analog ($SAR_2$) and S-adenosyl-L-methionine analog ($SAR_3$) as described above; and

iii) alkylating T4 in the presence of $SAR_2$ and $SAR_3$ to produce 4-phosphoryloxy-7-R1-N-R2-tryptamine analog of formula (T5) as described herein and the psilocybin analog of formula (P), or a salt thereof.

## DESCRIPTION OF THE FIGURES

**[0009]** The present application can be understood by reference to the following description taken in conjunction with the accompanying figures.

**[0010]** FIG. **1** depicts an exemplary scheme to produce psilocybin and structural analogs of psilocybin via a tryptamine analog.

**[0011]** FIGS. **2A** and **2B** depict exemplary schemes to produce S-adenosyl-L-methionine analogs, which are used as co-factors in the synthesis of psilocybin and structural analogs of psilocybin via a tryptamine analog, including as depicted in FIG. **1** above.

**[0012]** FIG. **3** depicts an exemplary scheme to produce a tryptophan analog that serves as the first building block in the exemplary scheme depicted in FIG. **1**.

## DETAILED DESCRIPTION

**[0013]** The following description sets forth exemplary methods, parameters, compounds and compositions, and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

**[0014]** Psilocybin is produced by various species of the *Psilocybe* genus, with special focus on high-yielding species *Psilocybe cubensis* and *Psilocybe cyanescens*, through a multi-step cascade involving a four-enzyme pathway. Namely, L-tryptophan first undergoes a decarboxylation reaction via putative decarboxylase PsiD to form tryptamine. This intermediate

undergoes hydroxylation via putative hydrolase PsiH to generate 4-hydroxytryptamine, followed by phosphorylation by putative kinase PsiK to norbaeocystin. Two methylation steps achieved by methyltransferase PsiM yields psilocybin.

**[0015]** Methyltransferase PsiM relies on the presence of endogenous co-factor S-adenosyl-L-methionine (SAM) to provide the needed methyl groups to undergo the final methylation step. Modification of SAM, either biochemically or synthetically, can yield analogs that transfer alkyl groups beyond the methyl group.

**[0016]** In some embodiments, the production methods provided herein use a co-immobilized enzyme cascade system in which aromatic aldehyde synthase IasA from *Psilocybin mexicana*, gene edited at Phe329 into tyrosine, is co-immobilized with kinase PsiK and methyltransferase PsiM. Additionally, *S*-adenosyl-L-homocysteine nucleosidase (MtnN) and adenine deaminase (Ade) are also immobilized onto the column to remove *S*-adenosyl-L-homocysteine, a byproduct of SAM methylation that inhibits PsiM activity. Methionine-coupling enzyme, *O*-acetyl-L-homoserine sulfhydrolase (OAHS), and L-methionine adenosyltransferase (MAT) are additionally immobilized onto the column to generate the L-methionine analog and *S*-adenosyl-L-methionine analog, respectively. A solution of precursors and cofactors of the desired alkyl thiol analog, 4-hydroxy-L-tryptophan, L-methionine, adenosine triphosphate (ATP), and pyroxidal phosphate (PLP) flow through the immobilized column and yield the desired psilocybin analog.

**[0017]** In some variations, the immobilized enzyme system provides a clean, simple means in which to separate the desired product from the milieu, using magnetic beads as the core of the immobilization substrate that can be removed using a magnet and later washed for future reactions.

**[0018]** In certain embodiments, the production methods further include the separate enzymatic production and isolation of the L-methionine analog via L-methionine, the alkyl thiol analog, and the OAHS to avoid co-reaction of L-methionine in the enzyme cascade to form psilocybin as a side product. The L-methionine analog is then used as a precursor for the above immobilized enzyme column reaction, without OAHS as one of the immobilized enzymes.

**[0019]** Additionally, in certain embodiments, the SAM analog is constructed in a separate immobilized enzyme cascade containing the two enzymes OAHS and MAT and isolated, purified, and then reacted in the above immobilized enzyme embodiment, without OAHS and MAT. In one embodiment, the desired SAM analog may be obtained from any commercially available sources or chemically synthesized according to methods known in the art as the precursor for the enzymatic cascade.

**[0020]** Furthermore, in some embodiments, a set of gene cassettes or a polycistronic gene embedded in a single mRNA strand is inserted into a workhorse microorganism (*e.g.*, *Escherichia coli, Saccharomyces cerevisiae*, *Aspergillus nidulans*, etc.) to express the above enzyme systems with the accompanied production of the psilocybin analog. In such embodiment, the *Psilocybe* cytochrome P450 oxidase *psiH* gene for tryptamine 4-hydroxylation is included in the gene cassettes or polycistronic gene system to endogenously form 4-hydroxy-L-trytophan.

**[0021]** This enzyme is excluded from the above immobilized enzyme cascades due to the inherent insolubility and poor activity of PsiH. Additionally, in other embodiments, the desired alkyl thiol analog is added to the culture media such that the workhorse species uptakes the thiol and incorporates it into the formation of the L-methionine analogs and subsequent psilocybin biosynthesis.

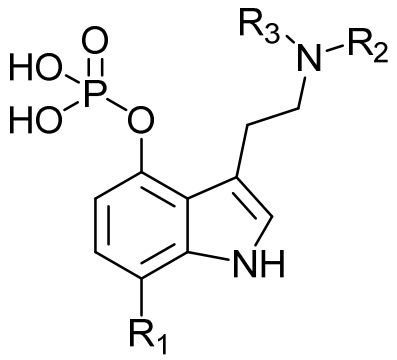
**[0022]** In other embodiments, the production methods further involve incorporating tryptophan synthetase (TrpB) into the immobilized enzyme cascade, and using L-serine as a building block to produce the tryptophan analog. FIG. **3** represents an exemplary scheme to produce the tryptophan analog, that serves as the first building block in the exemplary scheme depicted in FIG. **1**.

**[0023]** It should be understood that to produce 7-$R_1$-tryptophan analog as depicted in FIG. **1**, a suitable $R_1$-substituted indole may be used with L-serine in accordance with the scheme in FIG. **3**. Following the biosynthesis of psilocybin derivatives substituted at the more facile 6 or 7 positions with the halogen series (*e.g.*, fluorine, chlorine, bromine, iodine) or the methoxy substituent with point mutation or directed evolution of TrpB to accommodate the additional bulk of these substituents, this may additional analogs for each dimethylamine dialkyl variant driven by the alkyltransferase OAHS/MAT system. In some variations, these enzymes can either

be expressed, purified, and immobilized into a single biosynthetic system or inserted into a workhorse microorganism for bioreactor scale up and production.

**[0024]** The immobilized enzyme system described herein provides an avenue for rapid scale-up of the specific psilocybin analogs suitable for viable post-screening, for future animal studies and clinical trials, in a cGMP-compliant manner.

**[0025]** In some aspects, the methods described herein may be employed to produce a psilocybin analog of formula (P):




(P),

or a salt thereof, wherein:

$R_1$ is alkyl, haloalkyl, alkoxy, haloalkoxy, or halo;

each $R_2$ and $R_3$ is independently an aliphatic or aromatic moiety,

wherein the aliphatic or aromatic moiety is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$,

wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl), or

wherein the aliphatic moiety is substituted with an aromatic moiety, wherein the aromatic moiety is unsubstituted or substituted as described above.

**[0026]** In some embodiments, $R_1$ is alkyl. In one variation, $R_1$ is methyl. As used herein, "alkyl" refers to a monoradical unbranched or branched saturated hydrocarbon chain. In some embodiments, alkyl has 1 to 20 carbon atoms (*i.e.*, $C_{1-20}$ alkyl), 1 to 8 carbon atoms (*i.e.*, $C_{1-8}$ alkyl), 1 to 6 carbon atoms (*i.e.*, $C_{1-6}$ alkyl), or 1 to 4 carbon atoms (*i.e.*, $C_{1-4}$ alkyl). Examples of

alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, and 3-methylpentyl. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons may be encompassed; thus, for example, "butyl" can include n-butyl, sec-butyl, isobutyl and t-butyl; "propyl" can include n-propyl and isopropyl. In some embodiments, "$C_{1+}$alkyl" refers to alkyl having at least 1 carbon atom, and includes, for example, methyl, ethyl, propyl, etc. Similarly, "$C_{2+}$alkyl" refers to alkyl having at least 2 carbon atoms, and includes, for example, ethyl, propyl, butyl, etc. In one variation, the alkyl is a $C_{1-4}$ alkyl.

**[0027]** In other embodiments, $R_1$ is alkoxy. In one variation, $R_1$ is methoxy.

**[0028]** In other variations, the alkyl present in the alkyl, haloalkyl, alkoxy, or haloalkoxy of $R_1$ is a $C_{1-10}$, or $C_{1-4}$ alkyl. In one variation, the alkyl present in the alkyl, haloalkyl, alkoxy, or haloalkoxy of $R_1$ is methyl.

**[0029]** In yet other embodiments, $R_1$ is halo. In certain embodiments, $R_1$ is F, Cl, Br or I.

**[0030]** In yet other embodiments, $R_1$ is haloalkyl or haloalkoxy. "Haloalkyl" refers to an unbranched or branched alkyl group as defined above, wherein one or more hydrogen atoms are replaced by a halogen. For example, where a residue is substituted with more than one halogen, it may be referred to by using a prefix corresponding to the number of halogen moieties attached. Examples of a haloalkyl group include difluoromethyl ($-CHF_2$) and trifluoromethyl ($-CF_3$). It should be understood that trifluoromethyl ($-CF_3$) may also be referred to as perfluoromethyl.

**[0031]** In some embodiments, $R_2$ and $R_3$ are the same. In other embodiments, $R_2$ and $R_3$ are different.

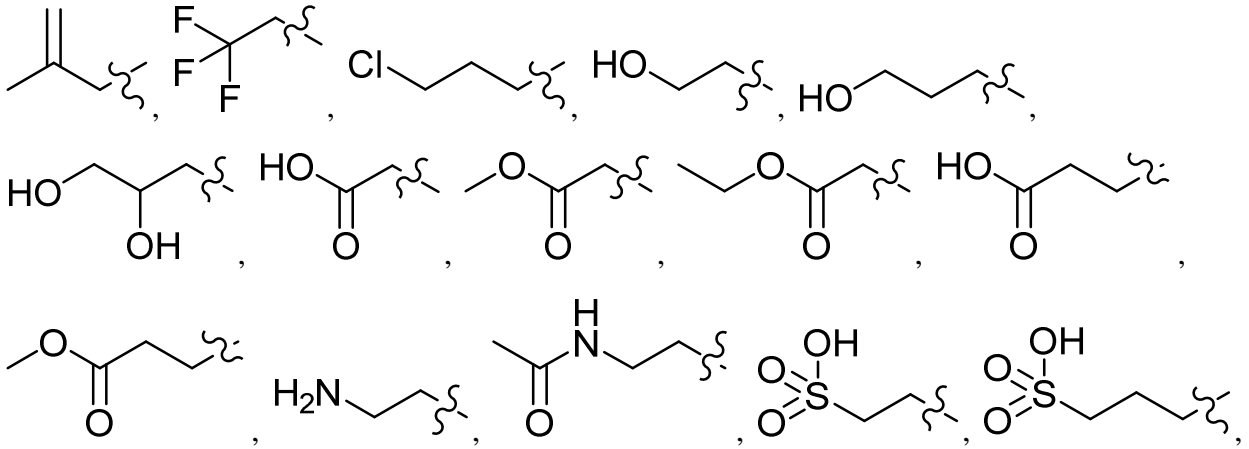
**[0032]** In some embodiments, each $R_2$ and $R_3$ is independently an aliphatic moiety. "Aliphatic" refers to an unbranched or branched group that does not have an aromatic ring. Aliphatic moieties may include alkyl (single bonds), alkenyl (double bonds) or alkynyl (triple bonds). In certain embodiments, each $R_2$ and $R_3$ is independently alkyl or alkenyl. In some variations, the foregoing, each $R_2$ and $R_3$ is unsubstituted or substituted with one or more substituents.

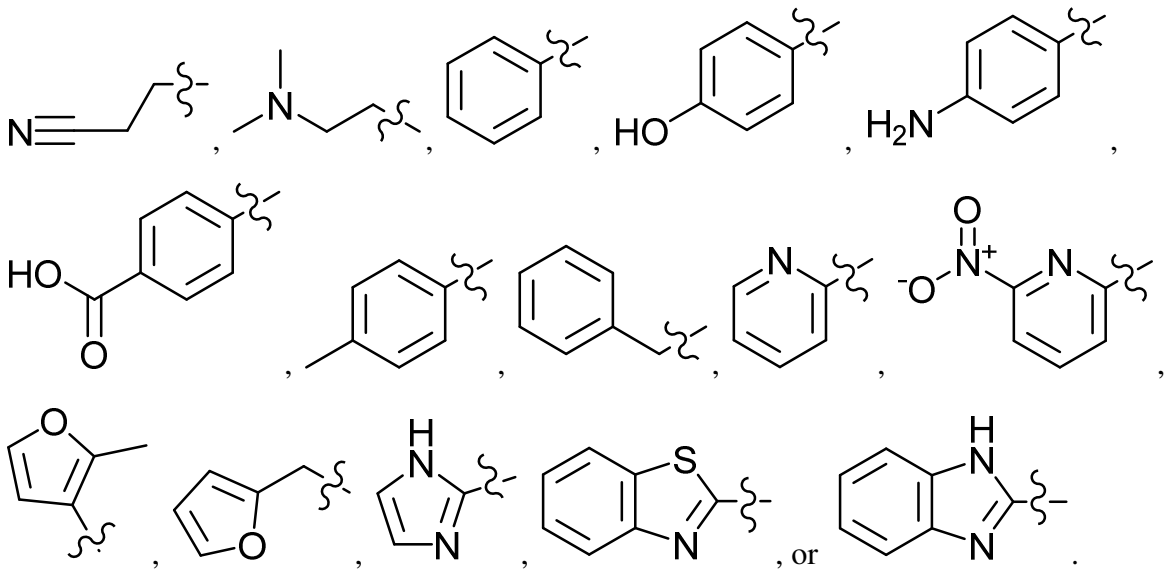
**[0033]** In other embodiments, each $R_2$ and $R_3$ is independently an aromatic moiety. "Aromatic moiety" includes monocyclic and polycyclic ring systems in which at least one of the rings is aromatic. In some variations, the aromatic ring system may be fused or unfused, in the case of a polycyclic ring system. In some variations, an aromatic ring system may include one or more heteroatoms in one or more rings, and the aromatic ring system is a heteroaromatic ring system. In certain embodiments, each $R_2$ and $R_3$ is independently phenyl, pyridinyl, furanyl, imidazolyl, benzothiazolyl, benzoimidazolyl.

**[0034]** In certain embodiments, each $R_2$ and $R_3$ is independently alkyl substituted with an aromatic moiety which may be optionally substituted as described above. In some variations, the aromatic moiety is unsubstituted. In variations, the aromatic moiety is substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$, wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl).

**[0035]** The term "substituted", in some variations, means that any one or more hydrogen atoms on the designated atom or group is replaced with a moiety other than hydrogen, provided that the designated atom's normal valence is not exceeded. In some variations, suitable substituents include -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$, wherein each of $R^a$ and $R^b$ is independently H or alkyl.

**[0036]** In some embodiments, each $R_2$ and $R_3$ is independently:





**[0037]** The symbol ~~~ whether as a bond or displayed perpendicular to a bond indicates the point at which the displayed moiety is attached to the remainder of the molecule.

**[0038]** In some variations of the foregoing, the psilocybin analog of formula (P) is other than psilocybin or a salt thereof. In other variations of the foregoing, $R_2$ and $R_3$ are not both methyl.

**[0039]** In another aspect, provided is a psilocybin analog selected from any of the compounds in Table 1 below, or a salt thereof. Table 1 also specifies the corresponding core, amine substituent(s), substituent formula and precursor.

**[0040]** In certain aspects, provided is a pharmaceutical composition comprising a psilocybin analog of formula (P) as described herein, or a pharmaceutically acceptable salt thereof; and at least one pharmaceutically acceptable excipient. The term "pharmaceutically acceptable salts" includes salts of the active compounds prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein.

**Table 1. Exemplary Compounds**

| Chemical Name | Indole Core | Amine Substituent(s) | Substituent Formula | Precursor |
|---|---|---|---|---|
| 4-phosphoryloxy-N,N-di(methallyl)tryptamine | tryptamine | Methallyl | $H_2C=C(H_3C)CCH_2$- | Methallyl Mercaptan |
| 4-phosphoryloxy-N,N-di(2,2,2-trifluoroethyl)tryptamine | tryptamine | 2,2,2-Trifluoroethyl | $F_3CCH_2$- | 2,2,2,-Trifluoroethanethiol |
| 4-phosphoryloxy-N,N-di(3-chloropropyl)tryptamine | tryptamine | 3-Chloropropyl | $ClCH_2CH_2CH_2$- | 3-Chloro-1-propanethiol |
| 4-phosphoryloxy-N,N-di(2-hydroxyethyl)tryptamine | tryptamine | 2-Hydroxyethyl | $HOCH_2CH_2$- | 2-Mercaptoethanol |
| 4-phosphoryloxy-N,N-di(3-hydroxypropyl)tryptamine | tryptamine | 3-Hydroxypropyl | $HOCH_2CH_2CH_2$- | 3-Mercaptopropanol |
| 4-phosphoryloxy-N,N-di(3,2-dihydroxypropyl)tryptamine | tryptamine | 3,2-Dihydroxypropyl | $HOCH_2CH(OH)CH_2$- | 1-Thioglycerol |
| 4-phosphoryloxy-N,N-di(2-carboxymethyl)tryptamine | tryptamine | Carboxymethyl | HOOC-$CH_2$- | Thioglycolic Acid |
| 4-phosphoryloxy-N,N-di(methyl 2-carboxymethyl)tryptamine | tryptamine | (Methyl carboxy)methyl | $H_3COOC$-$CH_2$- | Methyl Thioglycolate |
| 4-phosphoryloxy-N,N-di(ethyl 2-carboxymethyl)tryptamine | tryptamine | (Ethyl carboxy)methyl | $H_3CH_2COOC$-$CH_2$- | Ethyl Thioglycolate |
| 4-phosphoryloxy-N,N-di(3-carboxyethyl)tryptamine | tryptamine | Carboxyethyl | HOOC-$CH_2CH_2$- | 3-Mercaptopropionic Acid |
| 4-phosphoryloxy-N,N-di(methyl 3-carboxyethyl)tryptamine | tryptamine | (Methyl carboxy)ethyl | $H_3COOC$-$CH_2CH_2$- | Methyl 3-Mercaptopropionate |
| 4-phosphoryloxy-N,N-di(2-aminoethyl)tryptamine | tryptamine | 2-Aminoethyl | $H_2NCH_2CH_2$- | Cysteamine |
| 4-phosphoryloxy-N,N-di(N-acetyl-2-aminoethyl)tryptamine | tryptamine | N-Acetyl-2-aminoethyl | $H_3CCONHCH_2CH_2$- | N-Acetylcysteamine |
| 4-phosphoryloxy-N,N-di(2-sulfonylethyl)tryptamine | tryptamine | 2-Sulfonylethyl | $O_3SCH_2CH_2$- | Sodium 2-Mercaptoethanesulfonate |
| 4-phosphoryloxy-N,N-di(3-sulfonylpropyl)tryptamine | tryptamine | 3-Sulfonylpropyl | $O_3SCH_2CH_2CH_2$- | Sodium 3-Mercapto-1-Propanesulfonate |
| 4-phosphoryloxy-N,N-di(2-cyanoethyl)tryptamine | tryptamine | 2-Cyanoethyl | $N\#CCH_2CH_2$- | 3-Mercaptopropanenitrile |
| 4-phosphoryloxy-N,N-di((2-(dimethylamino)ethyl)tryptamine | tryptamine | 2-(Dimethylamino)ethyl | $(CH_3)_2NCH_2CH_2$- | 2-(Dimethylamino)ethanethiol |
| 4-phosphoryloxy-N,N-diphenyltryptamine | tryptamine | Phenyl | $C_6H_5$- | Thiophenol |
| 4-phosphoryloxy-N,N-di(4-hydroxyphenyl)tryptamine | tryptamine | 4-Hydroxyphenyl | $HOC_6H_4$- | 4-Mercaptophenol |
| 4-phosphoryloxy-N,N-di(4-aminophenyl)tryptamine | tryptamine | Aminophenyl | $H_2NC_6H_5$- | 4-Aminothiophenol |
| 4-phosphoryloxy-N,N-di(4-carboxyphenyl)tryptamine | tryptamine | Carboxyphenyl | $HOOCC_6H_5$- | 4-Mercaptobenzoic Acid |
| 4-phosphoryloxy-N,N-di(4-methylphenyl)tryptamine | tryptamine | Methylphenyl | $H_3CC_6H_5$- | 4-Methylbenzenethiol |
| 4-phosphoryloxy-N,N-dibenzyltryptamine | tryptamine | Benzyl | $C_6H_5CH_2$- | Benzyl Mercaptan |

| | | | | |
|---|---|---|---|---|
| 4-phosphoryloxy-N,N-di(2-pyridinyl)tryptamine | tryptamine | Pyridinyl | $C_5H_4N$- | 2-Mercaptopyridine |
| 4-phosphoryloxy-N,N-di(3-nitropyridinyl)tryptamine | tryptamine | 3-Nitropyridinyl | $O_2NC_5H_4N$- | 3-Nitropyridine-2-Thiol |
| 4-phosphoryloxy-N,N-di(2-methylfuranyl)tryptamine | tryptamine | 2-Methylfuranyl | $H_3CC_4OH_2$- | 2-Methyl-2-Furanthiol |
| 4-phosphoryloxy-N,N-difurfuryltryptamine | tryptamine | Furfuryl | $C_4OH_3CH_2$- | Furfuryl Mercaptan |
| 4-phosphoryloxy-N,N-di(imidazolyl)tryptamine | tryptamine | Imidazolyl | $C_3N_2H_3$- | 2-Mercaptoimidazole |
| 4-phosphoryloxy-N,N-di(benzothiazolyl)tryptamine | tryptamine | Benzothiazolyl | | 2-Mercaptobenzothiazole |
| 4-phosphoryloxy-N,N-di(benzimidazolyl)tryptamine | tryptamine | Benzimidazolyl | | 2-Mercaptobenzimidazole |

## EXAMPLES

**[0041]** The presently disclosed subject matter will be better understood by reference to the following Examples, which are provided as exemplary of the invention, and not by way of limitation.

### Example 1

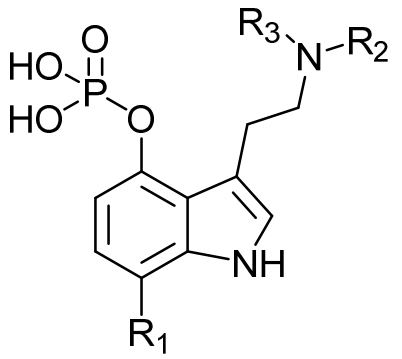
**[0042]** The enzymes IsaA, PsiK, PsiM, MtnN, Ade, OAHS, and MAT are individually expressed in *E. coli*. The enzymes are isolated from each recombinant *E. coli*¸ and immobilized onto substrate beads. A 1 mL solution composed of 50 mM TRIS-HCl buffer (pH 8), 100 µM ATP, 250 µM, 300 µM SAM, 300 µM alkylmercaptan (according to the desired analog as described herein), 5 µM PLP, and 50 µM 4-hydroxy-L-tryptophan is prepared. To the mixture is added 20 µL suspension of the immobilized enzyme beads. The reaction proceeds for 8 hours and shaken at 200 rpm at room temperature. The suspension is then centrifuged and decanted to isolate the supernatant. The supernatant is frozen and lyophilized, with the impure solutes redissolved in 100 µL methanol.

**[0043]** The methanolic solution containing the desired products is analyzed for the desired analog using an appropriate UHPLC-MS instrument equipped with a diode array detector (DAD) and linked to a quadruple mass detector. The chromatograph is fitted with a guard column and a column with the following dimensions: 100 x 2.1 mm, 2.7 µm particle size. The column is heated to 35 degrees Celsius and charged with a linear gradient flow of 0.6 mL per minute, using a solvent solution of 0.1% aqueous formic acid (Solvent A) and neat methanol (Solvent B). The initial 0-4 minutes targets 2% of Solvent B as a linear gradient, followed by a linear gradient target at 100% of Solvent B after 10 minutes.

CLAIMS

What is claimed is:

1. A method of producing a psilocybin analog of formula (P):



(P),

or a salt thereof, wherein:

$R_1$ is alkyl, haloalkyl, alkoxy, haloalkoxy, or halo;

each $R_2$ and $R_3$ is independently an aliphatic or aromatic moiety,

wherein the aliphatic or aromatic moiety is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$,

wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl), or

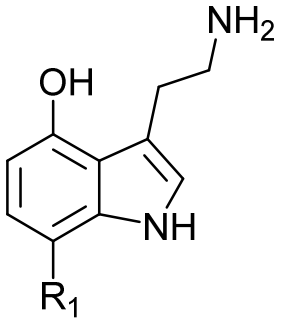
wherein the aliphatic moiety is substituted with an aromatic moiety, wherein the aromatic moiety is unsubstituted or substituted as described above,

the method comprising:

a) providing a main column comprising a co-immobilized enzyme cascade system, wherein kinase PsiK is co-immobilized with methyltransferase PsiM; and

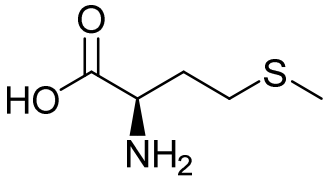
b) flowing a solution comprising:

- 4-hydroxy-7-R1-tryptamine analog of formula (T3):




(T3), or a salt thereof;

- L-methionine (S1):

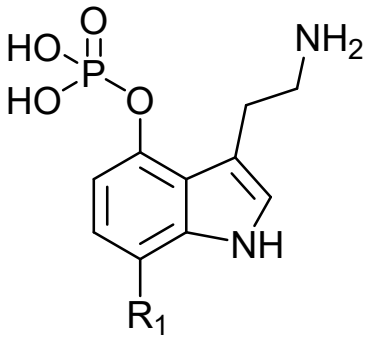



(S1), or a salt thereof;

- HS-$R_2$ and HS-$R_3$, wherein $R_2$ and $R_3$ are the same or different;
- adenosine triphosphate (ATP); and
- at least one coenzyme,

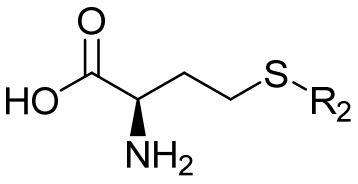
through the main column, thereby:

v) producing 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) from T3:

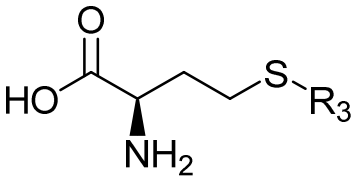



(T4), or a salt thereof,

vi) producing 2-amino-4-($R_2$-thio)butanoic acid (S2-$R_2$) and 2-amino-4-($R_3$-thio)butanoic acid (S2-$R_3$) from S1:

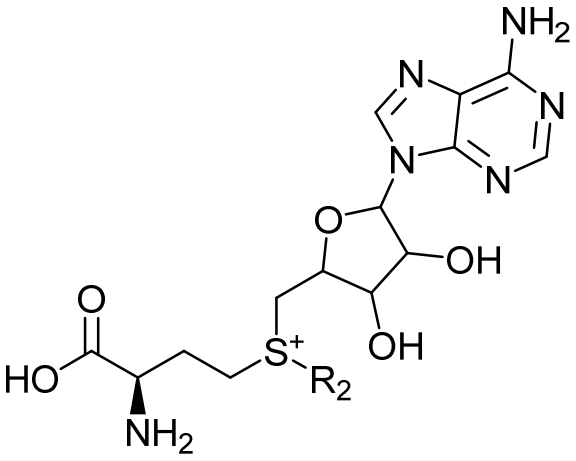



(S2-$R_2$) and

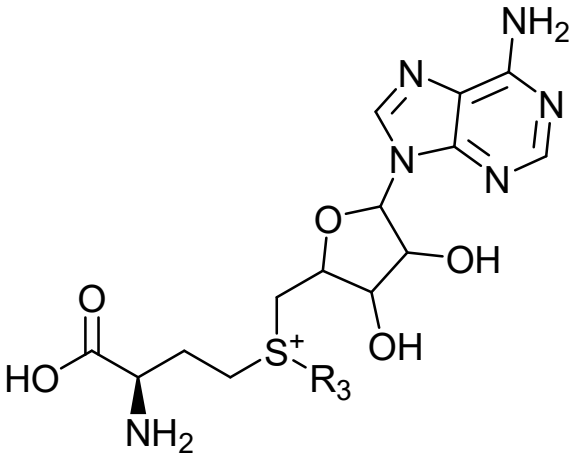



(S2-$R_3$), or a salt thereof;

vii) producing S-adenosyl-L-methionine analog ($SAR_2$) and S-adenosyl-L-methionine analog ($SAR_3$):

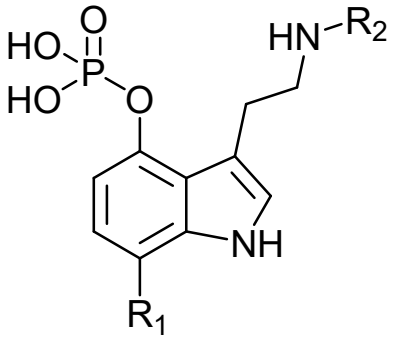



($SAR_2$) and

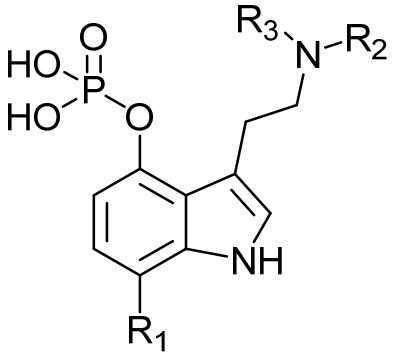



($SAR_3$), or a salt thereof;

viii) alkylating the 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) in the presence of $SAR_2$ and $SAR_3$ to produce 4-phosphoryloxy-7-R1-N-R2-tryptamine analog of formula (T5) and the psilocybin analog of formula (P):




(T5) and




(P), or a salt thereof.

2. The method of claim 1, wherein (iii) *S*-adenosyl-L-homocysteine nucleosidase (MtnN) and (iv) adenine deaminase (Ade) are further immobilized onto the main column to remove *S*-adenosyl-L-homocysteine.

3. The method of claim 1 or 2, wherein (v) *O*-acetyl-L-homoserine sulfhydrolase (OAHS) and (vi) L-methionine adenosyltransferase (MAT) are further immobilized onto the main column to generate the L-methionine analog and *S*-adenosyl-L-methionine (SAM) analog, respectively.

4. The method of claim 1 or 2, wherein (v) *O*-acetyl-L-homoserine sulfhydrolase (OAHS) and (vi) L-methionine adenosyltransferase (MAT) are provided in a side column comprising a separate immobilized enzyme cascade to produce the SAM analog, and the method further comprises:

isolating the SAM analog from the side column; and

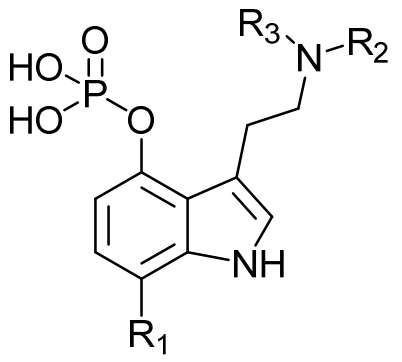
flowing the isolated SAM analog the through the main column to produce the psilocybin analog of formula (P), or a salt thereof.

5. The method of claim 1, wherein the co-immobilized enzyme cascade system uses magnetic beads as the immobilization substrate.

6. The method of claim 1 or 2, further comprising separating enzymatic production and isolation of the L-methionine analog via L-methionine, the *S*-adenosyl-L-methionine (SAM) analog, and the OAHS to minimize or avoid co-reaction of L-methionine in the enzyme cascade to form psilocybin as a side product.

7. The method of claim 4, further using the isolated L-methionine analog as a precursor for the co-immobilized enzyme cascade system, without OAHS as one of the immobilized enzymes.

8. A method of producing a psilocybin analog of formula (P):




(P),

or a salt thereof, wherein:

$R_1$ is alkyl, haloalkyl, alkoxy, haloalkoxy, or halo;

each $R_2$ and $R_3$ is independently an aliphatic or aromatic moiety,

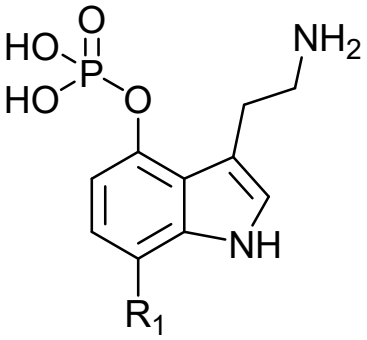
wherein the aliphatic or aromatic moiety is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$,

wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl), or

wherein the aliphatic moiety is substituted with an aromatic moiety, wherein the aromatic moiety is unsubstituted or substituted as described above,

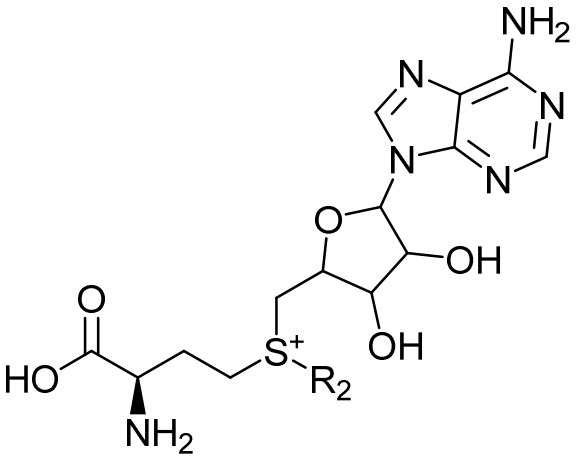
the method comprising:

i) providing 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4):

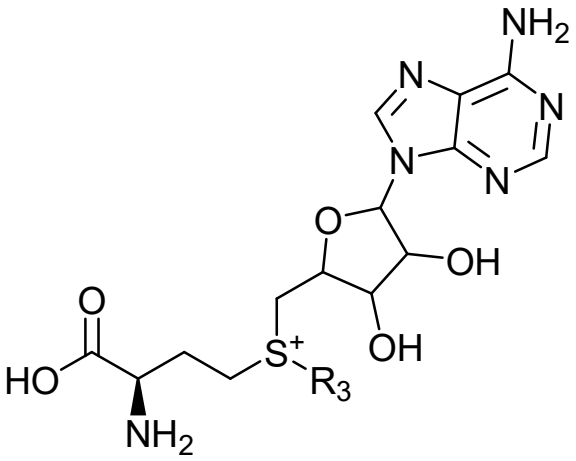


(T4), or a salt thereof,

ii) providing S-adenosyl-L-methionine analog ($SAR_2$) and S-adenosyl-L-methionine analog ($SAR_3$):

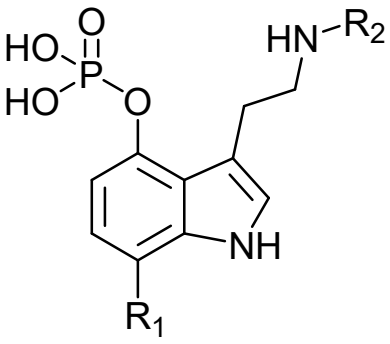


($SAR_2$) and

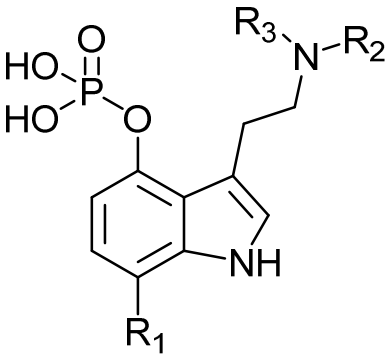


($SAR_3$), or a salt thereof; and

iii) alkylating T4 in the presence of $SAR_2$ and $SAR_3$ to produce 4-phosphoryloxy-7-R1-N-R2-tryptamine analog of formula (T5) and the psilocybin analog of formula (P):

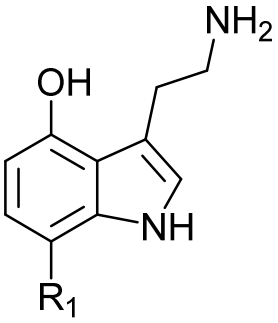



(T5) and




(P), or a salt thereof.

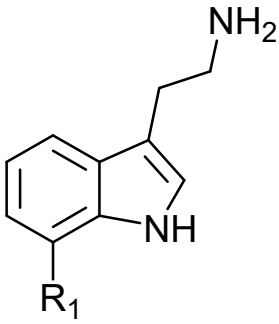
9. The method of claim 8, wherein T4 is produced by phosphorylating 4-hydroxy-7-R1-tryptamine analog of formula (T3):




(T3), or a salt thereof,

in the presence of PsiK and ATP.

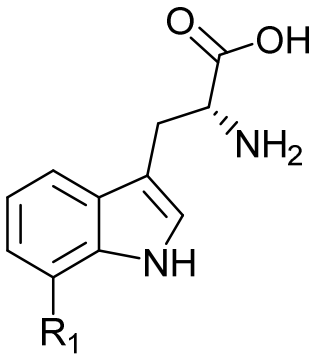
10. The method of claim 9, wherein T3 is produced by oxidizing 7-$R_1$-tryptamine analog of (T2):




(T2), or a salt thereof,

in the presence of PsiH.

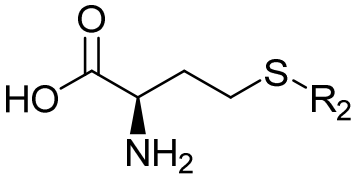
11. The method of claim 10, wherein T2 is produced by decarboxylating 7-$R_1$-tryptophan analog of formula (T1):



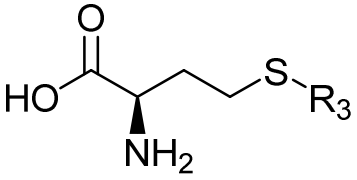
(T1), or a salt thereof,

in the presence of PsiD or IasA.

12. The method of any one of claims 8 to 11, wherein $SAR_2$ and $SAR_3$ are produced from 2-amino-4-($R_2$-thio)butanoic acid (S2-$R_2$) and 2-amino-4-($R_3$-thio)butanoic acid (S2-$R_3$):



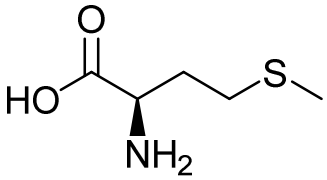
(S2-$R_2$) and



(S2-$R_3$), or a salt thereof;

in the presence of L-methionine adenosyltransferase (MAT) and ATP.

13. The method of claim 12, wherein the 2-amino-4-($R_2$-thio)butanoic acid (S2-$R_2$) and 2-amino-4-($R_3$-thio)butanoic acid (S2-$R_3$) are produced from L-methionine (S1):



(S1), or a salt thereof,

in the presence of (i) HS-$R_2$ and HS-$R_3$, wherein $R_2$ and $R_3$ are the same or different; and (ii) *O*-acetyl-L-homoserine sulfhydrolase (OAHS).

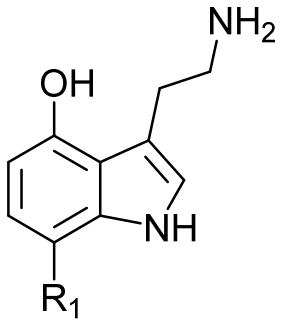
14. A co-immobilized enzyme cascade system comprising:

kinase PsiK is co-immobilized with methyltransferase PsiM,

wherein the system is configured to:

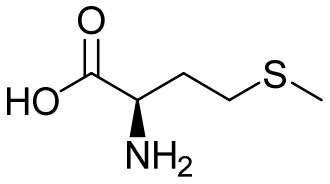
i) receive a solution comprising:

- 4-hydroxy-7-R1-tryptamine analog of formula (T3):




(T3), or a salt thereof,

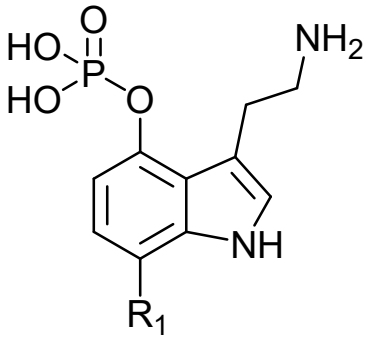
- L-methionine (S1):




(S1), or a salt thereof;

- HS-$R_2$ and HS-$R_3$:
- adenosine triphosphate (ATP); and
- at least one coenzyme; and

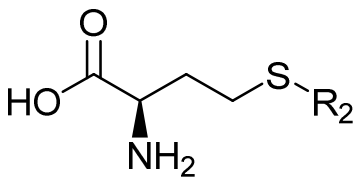
ii) produce 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) from T3:



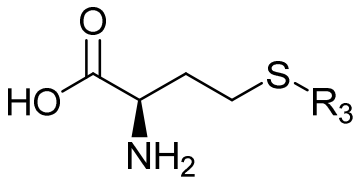
(T4), or a salt thereof,

wherein $R_1$ is alkyl, haloalkyl, alkoxy, haloalkoxy, or halo;

iii) produce 2-amino-4-($R_2$-thio)butanoic acid (S2-$R_2$) and 2-amino-4-($R_3$-thio)butanoic acid (S2-$R_3$) from S1:



(S2-$R_2$) and



(S2-$R_3$), or a salt thereof,

wherein:

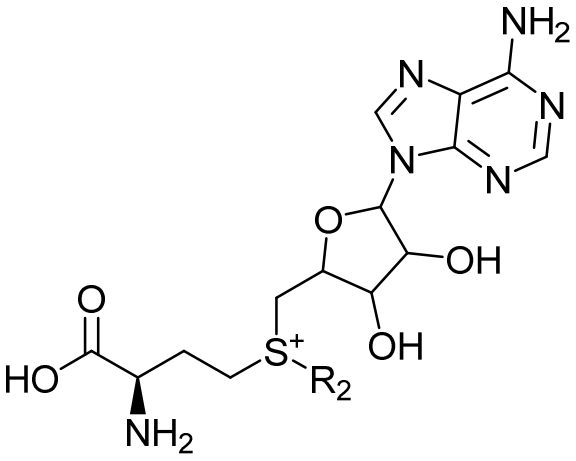
each $R_2$ and $R_3$ is independently an aliphatic or aromatic moiety,

wherein the aliphatic or aromatic moiety is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), -$SO_3$H, -CN, -$NR^aR^b$, and -$NO_2$,

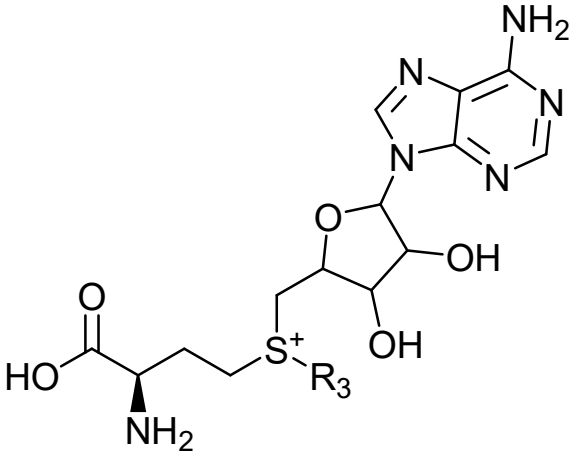
wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl), or

wherein the aliphatic moiety is substituted with an aromatic moiety, wherein the aromatic moiety is unsubstituted or substituted as described above;

iv) produce S-adenosyl-L-methionine analog ($SAR_2$) and S-adenosyl-L-methionine analog ($SAR_3$):

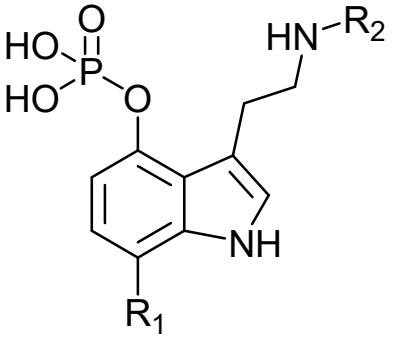



($SAR_2$) and

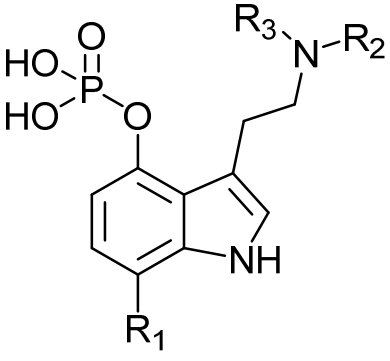



($SAR_3$), or a salt thereof;

v) alkylate the 4-phosphoryloxy-7-R1-tryptamine analog of formula (T4) in the presence of $SAR_2$ and $SAR_3$ to produce 4-phosphoryloxy-7-R1-N-R2-tryptamine analog of formula (T5) and the psilocybin analog of formula (P):

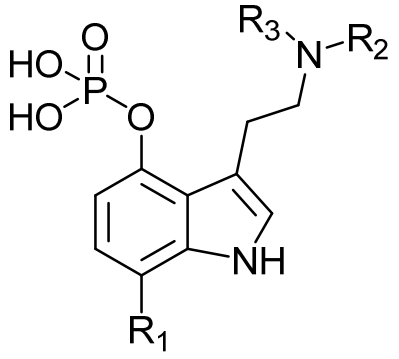



(T5) and




(P), or a salt thereof.

15. A compound of formula (P):




(P),

or a salt thereof, wherein:

$R_1$ is alkyl, haloalkyl, alkoxy, haloalkoxy, or halo;

each $R_2$ and $R_3$ is independently an aliphatic or aromatic moiety,

wherein the aliphatic or aromatic moiety is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH, -COO(alkyl), $-SO_3H$, -CN, $-NR^aR^b$, and $-NO_2$,

wherein each of $R^a$ and $R^b$ is independently H, alkyl, -C(=O)H, or -C(=O)(alkyl), or

wherein the aliphatic moiety is substituted with an aromatic moiety, wherein the aromatic moiety is unsubstituted or substituted as described above,

$R_2$ and $R_3$ are the same or different;

provided that the compound of formula (P) is other than psilocybin or a salt thereof, and

provided that $R_2$ and $R_3$ are not both methyl.

16. A compound selected from the group consisting of:

4-phosphoryloxy-N,N-di(methallyl)tryptamine;

4-phosphoryloxy-N,N-di(2,2,2-trifluoroethyl)tryptamine;

4-phosphoryloxy-N,N-di(3-chloropropyl)tryptamine;

4-phosphoryloxy-N,N-di(2-hydroxyethyl)tryptamine;

4-phosphoryloxy-N,N-di(3-hydroxypropyl)tryptamine;

4-phosphoryloxy-N,N-di(3,2-dihydroxypropyl)tryptamine;

4-phosphoryloxy-N,N-di(2-carboxymethyl)tryptamine;

4-phosphoryloxy-N,N-di(methyl 2-carboxymethyl)tryptamine;

4-phosphoryloxy-N,N-di(ethyl 2-carboxymethyl)tryptamine;

4-phosphoryloxy-N,N-di(3-carboxyethyl)tryptamine;

4-phosphoryloxy-N,N-di(methyl 3-carboxyethyl)tryptamine;

4-phosphoryloxy-N,N-di(2-aminoethyl)tryptamine;

4-phosphoryloxy-N,N-di(N-acetyl-2-aminoethyl)tryptamine;

4-phosphoryloxy-N,N-di(2-sulfonylethyl)tryptamine;

4-phosphoryloxy-N,N-di(3-sulfonylpropyl)tryptamine;

4-phosphoryloxy-N,N-di(2-cyanoethyl)tryptamine;

4-phosphoryloxy-N,N-di((2-(dimethylamino)ethyl)tryptamine;

4-phosphoryloxy-N,N-diphenyltryptamine;

4-phosphoryloxy-N,N-di(4-hydroxyphenyl)tryptamine;

4-phosphoryloxy-N,N-di(4-aminophenyl)tryptamine;

4-phosphoryloxy-N,N-di(4-carboxyphenyl)tryptamine;

4-phosphoryloxy-N,N-di(4-methylphenyl)tryptamine;

4-phosphoryloxy-N,N-dibenzyltryptamine;

4-phosphoryloxy-N,N-di(2-pyridinyl)tryptamine;

4-phosphoryloxy-N,N-di(3-nitropyridinyl)tryptamine;

4-phosphoryloxy-N,N-di(2-methylfuranyl)tryptamine;

4-phosphoryloxy-N,N-difurfuryltryptamine;

4-phosphoryloxy-N,N-di(imidazolyl)tryptamine;

4-phosphoryloxy-N,N-di(benzothiazolyl)tryptamine; and

4-phosphoryloxy-N,N-di(benzimidazolyl)tryptamine,

or a salt thereof.

# ABSTRACT

The present disclosure relates generally methods for producing psilocybin and psilocybin analogs using an immobilized enzymatic approach to alkylating tryptamines.

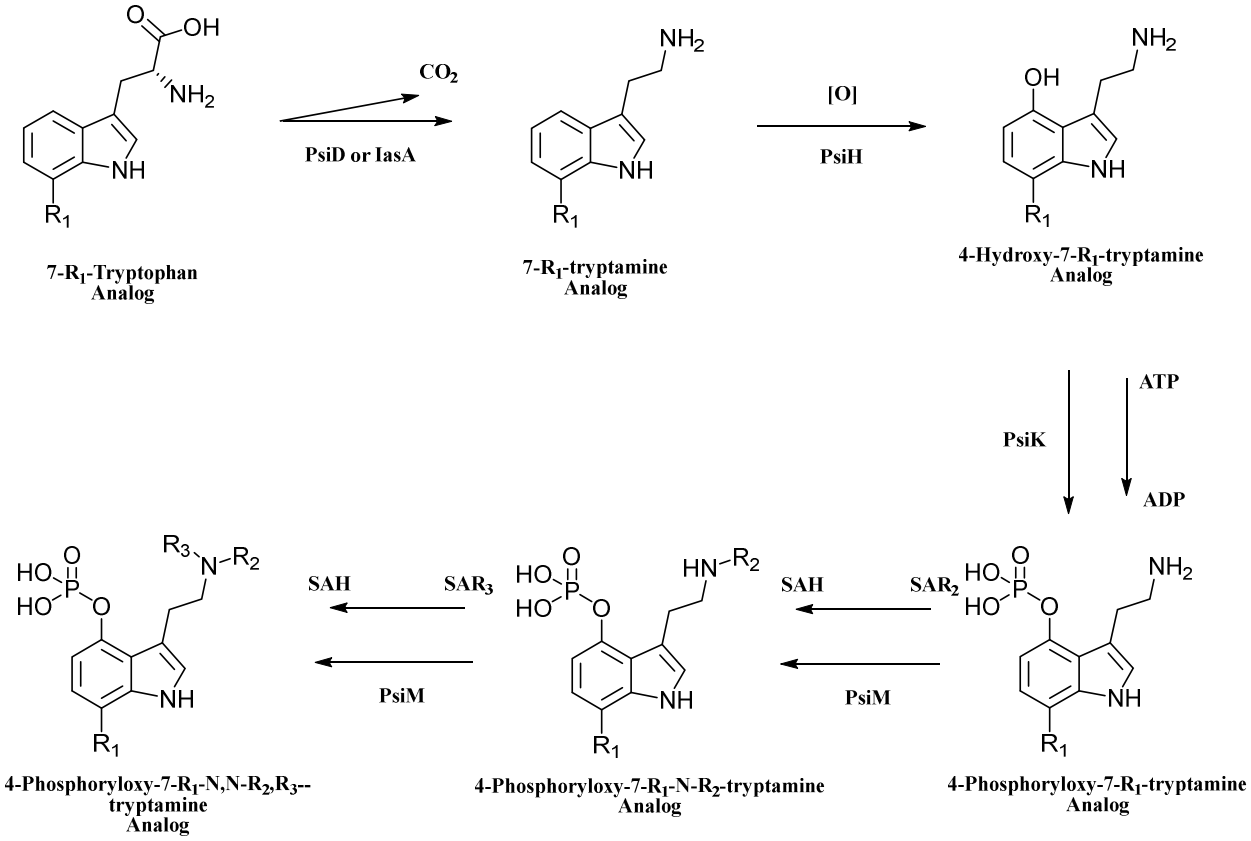

7-R1-Tryptophan Analog
CO2
PsiD or IasA
7-R1-tryptamine Analog
[O]
PsiH
4-Hydroxy-7-R1-tryptamine Analog
ATP
PsiK
ADP
4-Phosphoryloxy-7-R1-tryptamine Analog
SAH
SAR2
PsiM
4-Phosphoryloxy-7-R1-N-R2-tryptamine Analog
SAH
SAR3
PsiM
4-Phosphoryloxy-7-R1-N,N-R2,R3--tryptamine Analog

FIG. 1

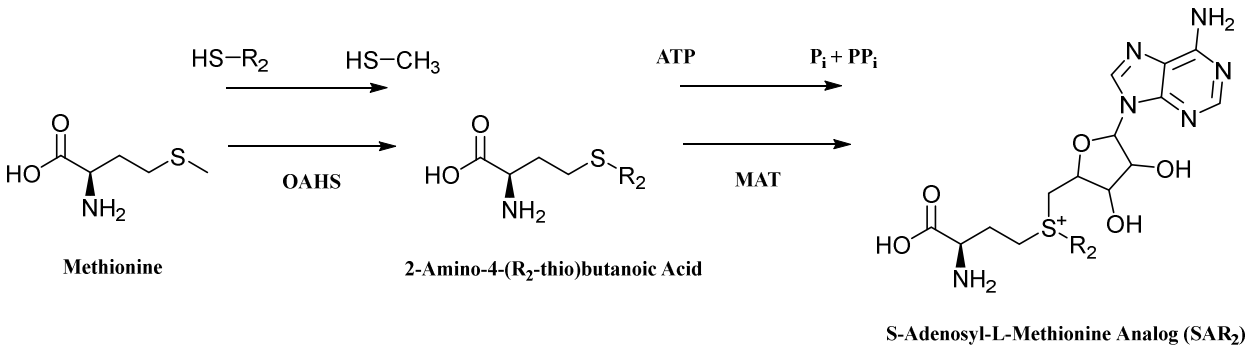

HS−R2
HS−CH3
OAHS
Methionine
2-Amino-4-(R2-thio)butanoic Acid
ATP
Pi + PPi
MAT
S-Adenosyl-L-Methionine Analog (SAR2)

FIG. 2A

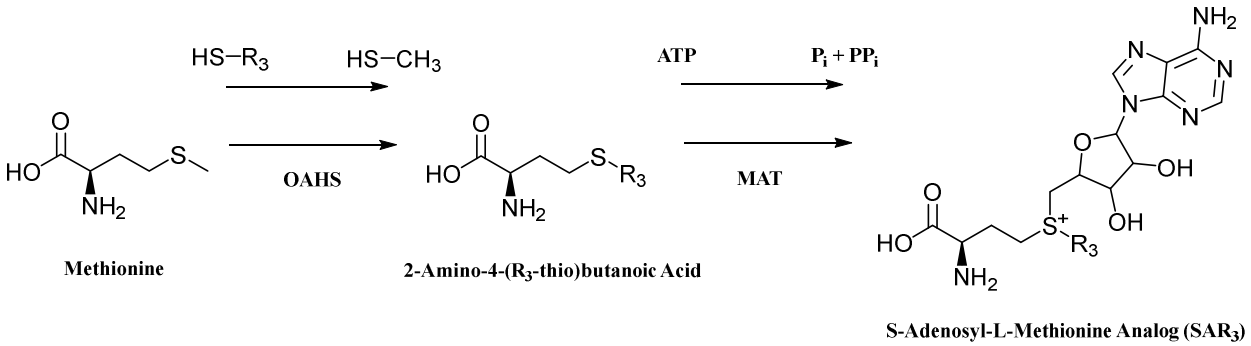

HS−R3
HS−CH3
ATP
Pi + PPi
OAHS
MAT
Methionine
2-Amino-4-(R3-thio)butanoic Acid
S-Adenosyl-L-Methionine Analog (SAR3)

FIG. 2B

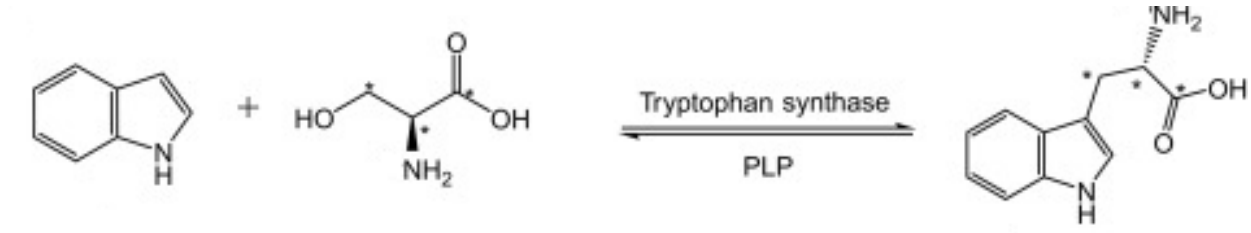

Tryptophan synthase
PLP

FIG. 3