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Article

# Synthesis and Biological Evaluation of Tryptamines Found in Hallucinogenic Mushrooms: Norbaeocystin, Baeocystin, Norpsilocin, and Aeruginascin

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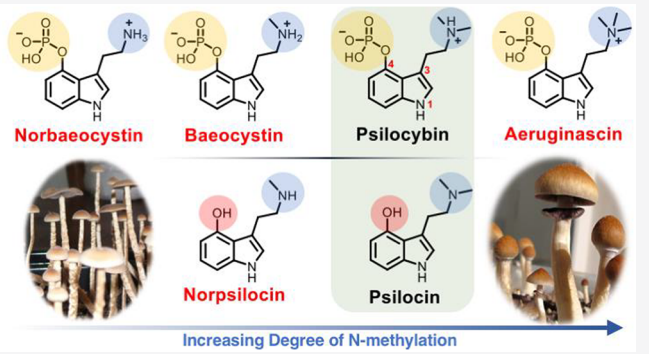
**ABSTRACT:** A general synthetic method was developed to access known tryptamine natural products present in psilocybin-producing mushrooms. *In vitro* and *in vivo* experiments were then conducted to inform speculations on the psychoactive properties, or lack thereof, of the natural products. In animal models, psychedelic activity by baeocystin alone was not evident using the mouse head twitch response assay, despite its putative dephosphorylated metabolite, norpsilocin, possessing potent agonist activity at the 5-$HT_{2A}$ receptor.






The natural product psilocybin (**1**) and its active psychedelic metabolite psilocin (**2**) have been the major focus of research on psychedelic mushrooms (Figure 1).[1] To date, little additional research has been conducted on several related tryptamines also present to varying degrees in the many species of psilocybin-producing mushrooms.[2−9] Recently, Fricke et al. elucidated the enzymatic pathways to **1** and **2** in mushrooms, confirming the roles of 4-hydroxytryptamine (**3**), norbaeocystin (**4**), and baeocystin (**5**) as known enzymatic substrates,[10] and later norpsilocin (**6**) was characterized by Lenz et al. (Figure 1).[11] The quaternary ammonium species aeruginascin (**7**) has been previously identified in a single species of psilocybin-producing mushrooms.[7,9] Trace amounts of β-carbolines with known monoamine oxidase-inhibiting properties were also recently identified in several *Psilocybe* species.[12] The tryptamine products can be generalized as possessing either a hydroxy or phosphate substitution at the 4-position of the indole core and containing sequentially increasing degrees of *N*-methylation at the terminal nitrogen, with the phosphorylated compounds **1**, **4**, **5**, and **7** existing in predominately zwitterionic form at approximately pH 3−6.

| Name (#) | $R^1$ | $R^2$ |
|---|---|---|
| Psilocybin (**1**) | $-OPO_3H^-$ | $-NH(CH_3)_2^+$ |
| Psilocin (**2**) | -OH | $-N(CH_3)_2$ |
| 4-hydroxytryptamine (**3**) | -OH | $-NH_2$ |
| Norbaeocystin (**4**) | $-OPO_3H^-$ | $-NH_3^+$ |
| Baeocystin (**5**) | $-OPO_3H^-$ | $-NH_2CH_3^+$ |
| Norpsilocin (**6**) | -OH | - $NHCH_3$ |
| Aeruginascin (**7**) | $-OPO_3H^-$ | $-N(CH_3)_3^+$ |

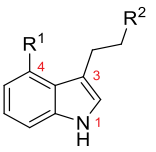



**Figure 1.** Structural variation of tryptamines found in psilocybin-producing fungi.

Some speculation has been made toward the potential biological relevance of these structurally similar minor indole alkaloids and their contribution to the effects induced by psilocybin/psilocin in the consumption of psychedelic mushrooms.[13] However, few studies have been conducted to evaluate pharmacological activity of these tryptamines, largely due to lack of availability of the compounds. Anecdotally, individuals experienced with the use of psychedelics have claimed variability in effects produced when consuming different species of mushrooms with assumed varying levels of the tryptamines outlined in Figure 1 and have speculated that baeocystin (**5**) or norpsilocin (**6**) could potentially contribute to variable subjective effects.

Baeocystin has been detected alongside psilocybin in some varieties of mushrooms in concentrations substantial enough to be potentially physiologically relevant, up to about one-third the relative concentration of psilocybin.[14] Additionally, several anecdotal reports exist describing temporary muscle weakness or paralysis following the consumption of certain psychedelic

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mushrooms. The reported effect has been associated with consumption of *Psilocybe azurescens* and *Psilocybe cyanescens*, but not the more common *Psilocybe cubensis*. Speculation has been made that the quaternary ammonium product aeruginascin (**7**), which has only been identified in *Inocybe aeruginascens* to date, could be responsible for these observed toxicological effects. The speculations on these activities are largely unsubstantiated, though the assumption is tempting given the structural similarity of aeruginascin to bufotenedine (**8**), a toad toxin known to be a potent peripherally active 5-$HT_3$ agonist (Figure 2).[15−17] However, the pharmacological

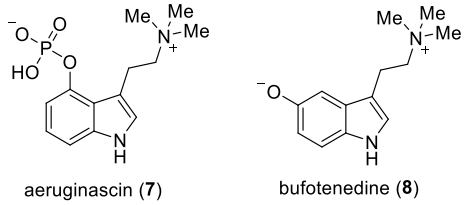


**Figure 2.** Aeruginascin (**7**) compared to bufotenedine (**8**), a potent agonist at the 5-$HT_3$ receptor present in toad skin.

activity of aeruginascin remains unexplored. Finally, the polar primary amine norbaeocystin (**4**) would not be expected to reach the central nervous system (CNS) and contribute to psychedelic effects due to poor blood−brain barrier penetrability[18] and/or rapid metabolism by monoamine oxidase (MAO),[19] especially considering the structural similarity of its putative dephosphorylated metabolite, 4-hydroxytryptamine, to serotonin.[20] Nevertheless, the availability of **4** may have utility in further elucidation and characterization of the enzymatic pathways involved in the biosynthesis of tryptamines in mushrooms as a reference standard. Given the breadth of scientific interest in tryptamines **4**−**7** and their lack of availability in pure form, a general synthetic approach capable of producing useful amounts of these natural products along with full analytical characterization of each has become desirable.

## RESULTS AND DISCUSSION

To provide access to sufficient quantities of **4**−**7** to facilitate more rigorous insight into their potential pharmacological actions, a general synthetic approach avoiding chromatographic purification at the final step for the phosphorylated compounds was preferable. Previous methods used to study compounds **4**−**7** have typically required either indirect instrumental observation on crude mushroom extracts or multistep isolation processes providing small quantities of purified material. The difficulty in purification arises in part due to the zwitterionic nature of **1**, **4**, **5**, and **7** resulting in either very high retention on normal-phase silica gel or very low retention on traditional reversed-phase media such as $C_{18}$ silica.

The first synthesis of **5** was reported 30 years ago and required six steps with multiple rounds of reversed-phase preparative chromatographic purification ultimately to provide 15 mg of target compound.[21] Later, Jensen provided an alternate synthetic procedure to compounds **5**−**7** as part of a doctoral dissertation published in 2004; however, the syntheses also required chromatography of zwitterionic products, and the final isolated yields of the milligram-scale reactions were not reported, with no additional biological characterization conducted.[22] In the current work, access to **4**−**7** was informed by previously reported methods utilizing the classical Speeter−Anthony tryptamine synthesis as well as by unpublished methods developed by our organization for the kilogram-scale synthesis of **1** for clinical use.

Several key modifications from previously reported procedures allowed for scalability. Historically, a challenge in the synthesis of psilocybin (**1**) has been the installation of the phosphate group at the 4-position. According to all available procedures, access to **1** has proceeded through dibenzyl-protected intermediate **9** (Figure 3).[23,24] Handling intermedi-

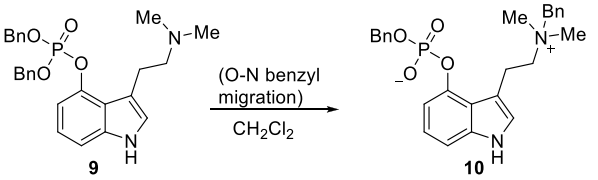


**Figure 3.** Benzyl migration encountered with the use of a benzyl-protected phosphate group in the previously reported synthesis of **1**.

ate **9** is complicated by a spontaneous benzyl migration to zwitterion **10**, which was first characterized by Shirota.[25] Under ideal circumstances, the poor solubility of **10** in methylene chloride may be exploited to provide the compound as a filterable solid. In our hands, control of the migration and further purification of the crude zwitterion **10** was generally challenging.

Given the challenges associated with benzyl migration in **9**, the use of an alternative phosphorylating reagent was most desirable in the synthesis of phosphorylated tryptamines **4**, **5**, and **7**. Furthermore, we envisaged that the variable alkyl substitutions at the terminal nitrogen on the intermediates would potentially affect the migratory aptitude of the benzyl group and/or the solubility of the resulting zwitterions. The reagent *ortho*-xylenyl phosphoryl chloride (*o*-xPCl, **11**) was identified as an attractive alternative protected phosphorylating reagent that may not be as susceptible to intramolecular rearrangement.[26,27]

*Psilocybe* tryptamines (**4**−**6**) were synthesized by the route depicted in Scheme 1. Acyl chloride **12** was synthesized from 4-acetoxyindole (**13**) and oxalyl chloride in methyl tert-butyl ether (MTBE) using a modified version of the Shirota procedure and was a common intermediate for all final products.[25] **12** was immediately treated with either *N*-benzylmethylamine or dibenzylamine to give ketoamide **14** or **15**, respectively. The ketoamides were reduced with lithium aluminum hydride (LAH) in THF and 2-methyltetrahydrofuran (2-Me-THF). The use of 2-MeTHF enabled a slightly higher reflux temperature, although despite extended reflux time, the reactions typically stalled with 5−10% of an expected *β*-hydroxy intermediate as the main impurity, which was detectable as a relatively polar spot on TLC and when monitoring the reactions by LCMS. Removal of this reactive impurity was critical, as it is prone to forming dimers in subsequent synthetic manipulations.[28] The polar intermediate was readily removed by filtration through a silica pad at workup to give amine **16** or **17** with sufficient purity to be used in the next step without additional purification.

In previously reported approaches to psilocybin synthesis, butyllithium was typically employed in the deprotonation of the phenolic hydroxy group in psilocin for the reaction with


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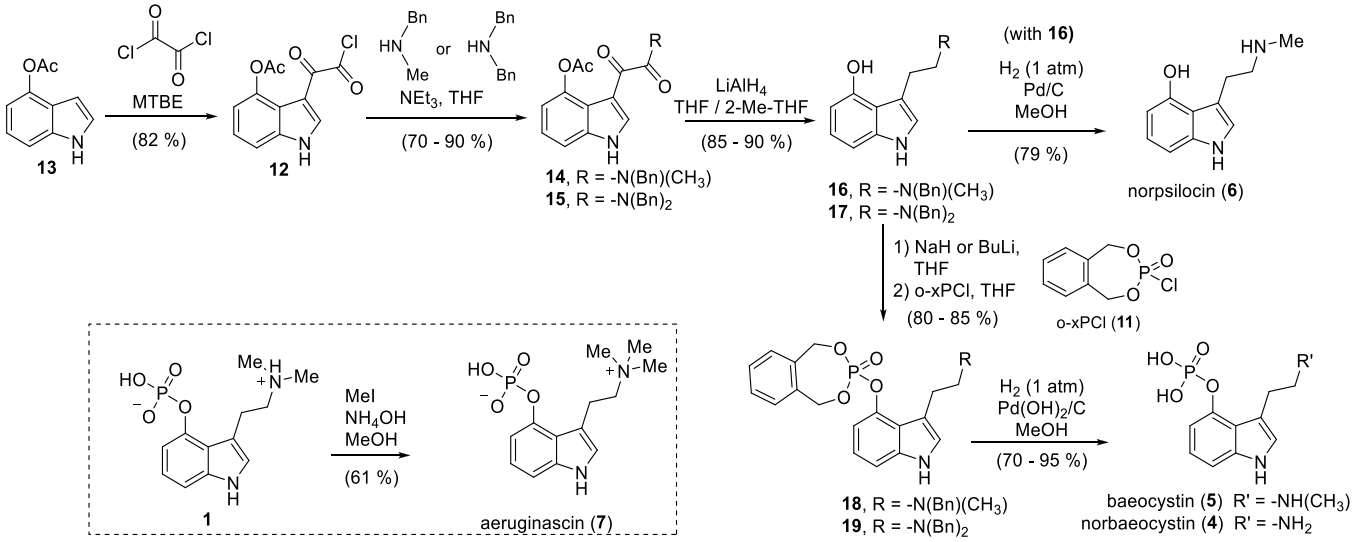
**Scheme 1. General Synthetic Approach to *Psilocybe* Tryptamines 4−7**




the phosphorylation reagent, either tetrabenzylpyrophosphate[23,25] or dibenzylphosphoryl chloride.[24] Butyllithium, when used with *o*-xPCl, led to significant poly phosphorylated side products and required careful control of cryogenic reaction conditions with syringe pump addition of *o*-xPCl to achieve **19** in acceptable yield. Alternatively, a slight excess of sodium hydride and *o*-xPCl in THF was found to be a more operationally simple approach to this phosphorylation, giving phosphorylated intermediate **18** in good yield.

Norbaeocystin (**4**), baeocystin (**5**), and norpsilocin (**6**) were revealed by catalytic hydrogenolysis at 1 atm using either palladium on carbon or palladium hydroxide catalyst. A key development in the effort to isolate the zwitterionic products **4** and **5** in pure form without the need for chromatography was the use of acetone as an antisolvent to precipitate the products from pH-adjusted aqueous solutions. With the pH between 4 and 5, the products were expected to be isoelectric and have minimal water solubility; the anti-solvent-mediated precipitation allowed rapid preparative-scale isolation of the products. The precipitated solids typically exceeded 98% purity by UPLC and could be used for further study without additional purification.

Finally, aeruginascin (**7**) was synthesized by quaternization of psilocybin (**1**) using excess methyl iodide in methanol treated with aqueous ammonium hydroxide. Under these conditions, aeruginascin (**7**) precipitated from the reaction mixture and was collected by filtration. Washing with methanol was sufficient to remove a small amount of unreacted psilocybin and ammonium iodide.

Of the known tryptamines found in mushrooms, baeocystin (**5**) and its putative dephosphorylated metabolite norpsilocin (**6**) would be the most likely candidates to induce psychedelic-like effects similar to psilocybin and its analogous metabolite psilocin. Although psilocin is metabolized in the body through several pathways, it appears to be relatively resistant to deamination by MAO compared to the structurally similar short-acting psychedelic compounds dimethyltryptamine (DMT) and 5-methoxydimethyltryptamine (5-MeO-DMT), which have virtually no oral activity due to rapid metabolic deamination by MAO.[22,29−32] The oral activity and relatively long rate of clearance of psilocin would imply that 4-hydroxy substitution may impart some physical characteristics to the drug providing increased CNS bioavailability and/or resistance to metabolic degradation.[33] An impetus for this work was to determine if, by analogy, baeocystin or norpsilocin would also benefit from 4-hydroxy substitution and possess CNS bioavailability and psychedelic effects.

The head-twitch response (HTR) in mice has proven to be a reliable behavioral proxy for human hallucinogenic effects because it can distinguish hallucinogenic vs nonhallucinogenic 5-$HT_{2A}$ agonists.[34,35] Baeocystin was assayed in comparison to psilocybin for its ability to induce HTR in male C57BL/6J mice (Figure 4). While psilocybin induced the HTR in a dose-dependent fashion ($ED_{50}$ = 0.40 (95% CI 0.22−0.72) mg/kg), baeocystin was indistinguishable from saline, indicating that baeocystin alone would likely not induce 5-$HT_{2A}$ receptor-mediated psychoactive effects *in vivo*.

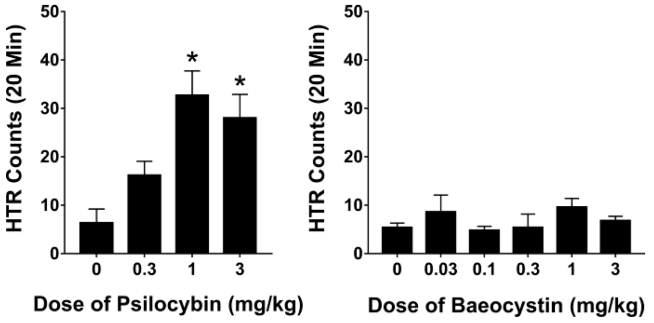



**Figure 4.** Comparison of psilocybin (**1**) and baeocystin (**2**) in the mouse head-twitch response (HTR) assay. Data are the mean ± SEM of HTR counts. Mice were injected i.p. with psilocybin or baeocystin, and then HTR behavior was assessed continuously for 20 min using a head-mounted magnet and a magnetometer coil.[35] *$p$ < 0.01 vs vehicle, Tukey's test.

When consumed, the prodrug psilocybin is rapidly dephosphorylated by alkaline phosphatase in the gut to psilocin, which is capable of CNS penetration and interaction with 5-$HT_{2A}$ receptors to produce psychedelic effects.[36] To further test if the lack of observed *in vivo* activity of baeocystin was primarily related to pharmacokinetic or pharmacodynamic effects, its analogous dephosphorylated metabolite, norpsilocin (**6**), was evaluated at the 5-$HT_{2A}$ receptor for activation of $G_q$-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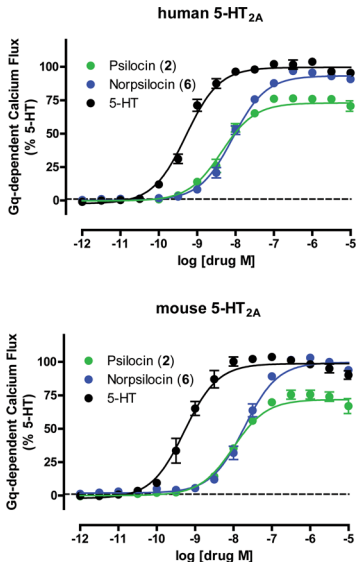


| | Psilocin | | Norpsilocin | | 5-HT | |
|---|---|---|---|---|---|---|
| | $EC_{50}$, nM ($pEC_{50} \pm SEM$) | Emax % 5-HT | $EC_{50}$, nM ($pEC_{50} \pm SEM$) | Emax % 5-HT | $EC_{50}$, nM ($pEC_{50} \pm SEM$) | Emax % 5-HT |
| h5-$HT_{2A}$ | 4.3 (8.37 ± 0.09) | 73 ± 2 | 8.4 (8.08 ± 0.05) | 93 ± 2 | 0.51 (9.29 ± 0.05) | 100 |
| m5-$HT_{2A}$ | 9.9 (8.01 ± 0.08) | 72 ± 2 | 19 (7.72 ± 0.06) | 99 ± 2 | 0.53 (9.28 ± 0.07) | 100 |

**Figure 5.** Comparison of psilocin (**2**) and norpsilocin (**6**) relative to serotonin (5-HT) in the human and mouse 5-$HT_{2A}$ $G_q$-dependent calcium flux assay.

mediated calcium flux. Compared to psilocin, norpsilocin was nearly a full agonist at the human 5-$HT_{2A}$ receptor in the assay ($EC_{50}$ = 8.4 nM, $E_{max}$ = 93%, $N$ = 3), with similar activity at the mouse 5-$HT_{2A}$ receptor (Figure 5). Norpsilocin was thus as potent if not more efficacious compared to psilocin in $G_q$-calcium flux at the 5-$HT_{2A}$ receptor. Given that secondary amines tend to be degraded more readily by MAO enzymes compared to tertiary amines,[37] these data would support the hypothesis that baeocystin and norpsilocin may be rendered inactive by metabolism before reaching targets in the central nervous system. Additionally, studies of commercial CNS drugs have shown that blood−brain barrier (BBB) penetration is optimal when the LogP values are in the range of 1.5−2.7.[18,38] Psilocin, with a higher cLogP (1.4), likely provides enhanced brain penetrance, whereas norpsilocin (cLogP = 1.0) may not be able to cross the BBB.[33] Taken collectively, the HTR data and 5-$HT_{2A}$ functional experiments would imply that baeocystin's lack of CNS activity is possibly related to pharmacokinetics but not lack of activity at 5-$HT_{2A}$ receptors.

In summary, a general synthetic method to known tryptamines in psychedelic mushrooms was developed, highlighted by an operationally simple and novel approach to the isolation of useful amounts of zwitterionic phosphorylated compounds. The synthesis provided materials for systematic pharmacological screening. The *in vivo* data combined with assessment of the pharmacological liabilities suggest it is unlikely that baeocystin or its putative metabolite norpsilocin contribute significantly to centrally mediated psychedelic effects, likely due to rapid degradation by MAO or inability to cross the blood−brain barrier. Though unexplored in this report, baeocystin could potentially exert a synergistic effect with psilocin/psilocybin by competing for MAO, effectively increasing psilocin concentration in the blood. Additionally, if rapid degradation by MAO is not the predominate route of inactivation, the interaction of baeocystin/norpsilocin with peripheral serotonin receptors may be possible. The availability of practical synthetic methods described here will allow further pharmacological screening of tryptamines **4**−**7**.

## EXPERIMENTAL METHODS

**General Experimental Methods.** Reactions were performed using commercially obtained solvents. Unless otherwise stated, all commercially obtained reagents were used as received. Reactions were monitored by thin-layer chromatography (TLC) using EMD/Merck silica gel 60 F254-precoated plates (0.25 mm). Flash column chromatography was performed using prepackaged RediSepRf columns on a CombiFlash Rf system (Teledyne ISCO Inc.). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 400 (at 400 and 101 MHz, respectively) and are reported relative to internal $CHCl_3$ ($^1$H, $\delta$ = 7.26) and $CDCl_3$ ($^{13}$C, $\delta$ = 77.0). Data for $^1$H NMR spectra are reported as follows: chemical shift ($\delta$ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent. Analytical UPLC was performed with a Waters Acquity I-Class UPLC utilizing a Waters HSS T3 column (2.5 $\mu$m, 2.1 mm × 30 mm) run in gradient mode with $H_2O$ (0.1% formic acid) and acetonitrile (0.1% formic acid) mobile phases at 0.6 mL/min. Samples were diluted in acetonitrile or water to approximately 1 mg/mL and 0.1 $\mu$L was injected. Chromatographic peaks were detected by a diode array detector at 269 nm. High resolution mass spectra were acquired inline with UV on a Waters Xevo G2-XS QTof in ESI-positive mode. Low and high collision energy mass spectra were acquired using a Waters MSe experiment.

**5-$HT_{2A}$ Receptor Functional Assays.** 5-$HT_{2A}$ functional experiments (measuring $G_q$-mediated endoplasmic reticulum calcium flux) were performed with Flp-In T-REx 293 cells (Invitrogen, Carlsbad, CA, USA) expressing either human or mouse 5-$HT_{2A}$ cDNA under the tetracycline repressor protein. One day before the assay, T-REx cells were treated with 2 $\mu$g/mL tetracycline to induce receptor expression and plated into black 384 clear-bottom, tissue culture plates in 40 $\mu$L of DMEM containing 1% dialyzed fetal bovine serum (FBS) at a density of approximately 10−15 000 cells per well. On the day of the assay, media were decanted and replaced with 20 $\mu$L per well of drug buffer (HBSS, 20 mM HEPES, pH 7.4) containing Fluo-4 Direct dye (Invitrogen) and incubated for 1 h at 37 °C. Compounds were diluted in drug buffer (HBSS, 20 mM HEPES, 0.1% bovine serum albumin, 0.01% ascorbic acid, pH 7.4). Approximately 15−20 min before the experiment, plates were allowed to equilibrate to room temperature and calcium flux was measured using a FLIPR$^{TETRA}$ (Molecular Devices, Sunnyvale, CA, USA). Plates were read for fluorescence initially for 10 s (1 read per second) to establish baseline fluorescence and then challenged with compounds diluted in a range


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of 32 μM to 1 pM or buffer and read for an additional 120 s. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose−response function of GraphPad Prism 5.0. Psilocin source: Cayman Chemical, 11864.

**Synthesis and Characterization.** *3-(2-Aminoethyl)-1H-indol-4-yl Dihydrogen Phosphate (**4**, Norbaeocystin).* Intermediate **19** (500 mg, 0.93 mmol) was dissolved in MeOH (20 mL) with 4 drops of concentrated HCl. Then 20% $Pd(OH)_2/C$ was added (200 mg), and the flask was sealed then purged with hydrogen gas via a balloon. After stirring overnight, the reaction was checked by LCMS to reveal only monobenzyl intermediate (*m*/*z* 347.1155). Additional catalyst (200 mg) was added, and the reaction stirred for an additional 72 h when it was determined to be complete by LCMS. The reaction was diluted with 5 mL of water and filtered on a small pad of Celite. The flask and filter cake were washed with an additional 5 mL of water. The filtrate was concentrated to about 2−3 mL, and the pH was adjusted to 4−5 with 4 N NaOH (about 8 drops). The product began to crystallize spontaneously. Acetone (50 mL) was added to complete the precipitation. The suspension was filtered to collect a fine white solid (225 mg, 94%): mp 210−212 °C; $^1$H NMR (400 MHz, $D_2O$) δ 7.16−7.02 (3H, m), 6.96 (1H, d, *J* = 7.7 Hz), 3.20 (4H, m); $^{13}$C NMR (100 MHz, $D_2O$) δ (ppm) 146.66, 138.51, 123.84, 122.55, 118.64, 108.98, 108.69, 106.97, 41.04, 23.84; HRMS (ESI) calcd for $C_{10}H_{14}N_2O_4P^+$ 257.0686 $[M + H]^+$, found 257.0686; UPLC purity 97%, $t_R$ 0.32 min.

*3-(2-(Methylamino)ethyl)-1H-indol-4-yl Dihydrogen Phosphate (**5**, Baeocystin).* Intermediate **18** (2.00 g, 4.32 mmol) was dissolved in MeOH (40 mL). Then 20% $Pd(OH)_2/C$ was added (600 mg) and the flask was sealed and then purged with hydrogen gas via balloon. After stirring overnight (17 h) the reaction was checked by LCMS, revealing product and monobenzyl intermediate (*m*/*z* 361) in about 1:1 ratio. Additional catalyst (200 mg) and 2 drops of concentrated HCl were added. The reaction was stirred for an additional 24 h and was then determined to be complete by LCMS. The reaction was diluted with 15 mL water, then filtered on a pad of Celite. The filter cake was washed with an additional 5 mL of water. The filtrate was concentrated under reduced pressure to about 10 mL. Then 4 N NaOH was added dropwise (about 4 drops) to bring the pH to 4−5, which resulted in baeocystin crystallizing out of solution spontaneously. Acetone (50 mL) was added, and the resulting precipitate was collected by filtration to provide the target compound as a fine white solid (0.82 g, 70%): mp 242−244 °C (dec); $^1$H NMR (400 MHz, $D_2O$) δ 7.19 (1H, d, *J* = 8.1 Hz), 7.13 (1H, s), 7.09 (1H, t, *J* = 7.9 Hz), 6.97−6.91 (1H, m), 3.29 (2H, t, *J* = 6.9 Hz), 3.21 (2H, t, *J* = 6.9 Hz), 2.61 (3H, s); $^{13}$C NMR (100 MHz, $D_2O$) δ 145.76, 138.64, 124.20, 122.56, 118.51, 108.92, 108.40, 107.72, 50.51, 32.73, 22.85; HRMS (ESI) calcd for $C_{11}H_{16}N_2O_4P^+$ 271.0842 $[M + H]^+$, found 271.0843; UPLC purity 99%, $t_R$ 0.49 min.

*3-(2-(Methylamino)ethyl)-1H-indol-4-ol (**6**, Norpsilocin).* Intermediate **16** (100 mg, 3.57 mmol) was dissolved in MeOH (20 mL). Then 10% Pd/C was added (150 mg), and the flask was sealed, then purged with hydrogen gas via balloon. After stirring for 24 h the reaction was determined to be complete by TLC. The suspension was filtered on a pad of Celite and washed with MeOH (100 mL). The blue-gray filtrate was concentrated, then purified by flash chromatography with 10:100:1 $MeOH/CHCl_3/NH_4OH$ to provide the target compound as a white solid (0.54 g, 79%): mp 184−186 °C (dec); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, s), 7.08 (1H, t, *J* = 7.9 Hz), 6.93−6.84 (2H, m), 6.60 (1H, d, *J* = 7.6 Hz), 3.02 (2H, dt, *J* = 5.2, 3.1 Hz), 2.99−2.92 (2H, m), 2.50 (3H, s); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 151.91, 138.87, 123.54, 120.99, 118.23, 113.90, 106.89, 102.76, 52.78, 35.92, 26.81; HRMS (ESI) calcd for $C_{11}H_{16}N_2O^+$ 191.1179 $[M + H]^+$, found 191.1181; UPLC purity 98%, $t_R$ 0.61 min.

*3-(2-(Trimethylammonio)ethyl)-1H-indol-4-yl Hydrogen Phosphate (**7**, Aeruginascin).* Psilocybin[22] (200 mg, 0.70 mmol) was suspended in MeOH (2 mL); then concentrated $NH_4OH$ (about 10 drops) was added dropwise until solids dissolved and the pH of the solution was 9−10. Methyl iodide (0.44 mL, 7.0 mmol) was added, and the reaction was protected from light and stirred overnight. A white precipitate formed over the course of the reaction. LCMS analysis indicated aeruginascin as the major product (71%) with psilocybin starting material remaining (25%). The pH was adjusted back to ∼9−10 by dropwise addition of ammonium hydroxide. After stirring for 40 h, LCMS indicated that the starting material was only 10% and the pH was no longer changing. The precipitated solid was collected by filtration and washed sparingly with methanol. LCMS analysis of the filter cake proved to be about 94% aeruginascin and 6% psilocybin. The collected solid was slurried in MeOH (2 mL) containing 3 drops of ammonium hydroxide.* The resulting crystalline solid was collected by filtration to provide aeruginascin (130 mg, 61%, 97% UPLC purity). A second crop of crystals from the mother liquors was isolated to give an additional 24 mg of white crystalline solid (154 mg, 73% yield overall): mp 176−178 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.15−6.97 (4H, m), 3.55−3.45 (2H, m), 3.34−3.25 (2H, m), 3.08 (9H, t, *J* = 2.4 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 1146.96, 138.38, 123.58, 122.70, 118.33, 108.61, 108.17, 106.59, 67.52, 53.03, 20.30; HRMS (ESI) calcd for $C_{13}H_{20}N_2O_4P^+$ 299.1155 $[M + H]^+$, found 299.1158; UPLC purity 98%, $t_R$ 0.50 min.

*In the absence of ammonium hydroxide modifier, the solid collected from the washes tended to be deliquescent and difficult to filter.

*3-(2-Chloro-2-oxoacetyl)-1H-indol-4-yl Acetate (**12**).* A solution of 1*H*-indol-4-yl acetate (20 g, 114 mmol) in MTBE (125 mL) was added dropwise over 30 min to a stirring solution of oxalyl chloride (12 mL, 140 mmol, 1.2 equiv) in MTBE (75 mL) under nitrogen maintaining an internal reaction temperature of 0−10 °C. After the addition was complete the bright yellow suspension was stirred for 2 h at 0−10 °C. A heptane mixture (100 mL) was added at a steady rate over 10 min at 0−10 °C, and the precipitated bright yellow solid was collected by suction filtration and washed with a 1:1 MTBE/heptane mixture (50 mL); suction filtration continued for 15 min under a gentle stream of nitrogen to air-dry the solid. The collected yellow crystalline solid (25 g, 82%), mp 107−110 °C (dec), was used immediately without further characterization.

*3-(2-(Benzyl(methyl)amino)-2-oxoacetyl)-1H-indol-4-yl Acetate (**14**).* To a dry round-bottomed flask (RBF) was added **12** (10.0 g, 37.6 mmol). The flask was sealed and purged with nitrogen; then the solid was dissolved in THF (110 mL). The solution was cooled to 0−10 °C, and a solution of *N*-methylbenzylamine in THF (2 M, 22.6 mL, 45.2 mmol) was added dropwise over 1 h to the stirring solution. A white precipitate was observed during the addition. Once the addition was complete, a solution of triethylamine (6.5 mL, 46.3 mmol) in THF (40 mL) was added dropwise at 0−10 °C. The reaction was warmed to ambient temperature and stirred for 3 h. A heptane mixture (200 mL) was added to the stirring suspension at a steady rate over 10 min at ambient temperature. The suspension was cooled back to 0−10 °C, stirred for 30 min, and vacuum-filtered to collect a crude product as an off-white solid. The solid was washed with a heptane mixture (2 × 20 mL), and suction filtration continued for 15 min to air-dry the solid. The collected solid (∼20 g) was recrystallized from boiling 1:1 water/isopropanol to provide the target compound as a shiny white solid (12 g, 74%, > 98% UPLC purity): mp 145−148 °C; $^1$H NMR* ($CDCl_3$, 400 MHz) δ 10.31 (1H, s), 7.57 (1H, d, *J* = 3.3 Hz), 7.42−7.33 (5H, m), 7.14 (2H, t, *J* = 7.9 Hz), 6.94 (1H, d, *J* = 3.9 Hz), 4.65 (2H, s), 2.86 (3H, s), 2.53 (3H, s) (major rotamer); $^{13}$C NMR* ($CDCl_3$, 100 MHz) δ 185.11, 170.97, 168.51, 144.25, 139.18, 138.13, 135.97, 128.91, 128.12, 127.81, 124.75, 118.26, 115.99, 113.51, 110.83, 50.04, 35.00, 21.60 (major rotamer); HRMS (ESI) calcd for $C_{20}H_{19}N_2O_4^+$ 351.1340 $[M + H]^+$, found 351.1342. *Note: NMR spectra indicative of a ∼1:1.2 mix of rotamers, signals reported for major rotamer where possible.

*3-(2-(Dibenzylamino)-2-oxoacetyl)-1H-indol-4-yl Acetate (**15**).* To a dry RBF was added 3-(2-chloro-2-oxoacetyl)-1*H*-indol-4-yl acetate (5.0 g, 18.8 mmol). The flask was sealed and purged with nitrogen; then the solid was dissolved in THF (55 mL). The mixture was cooled to 0−10 °C, and a solution of dibenzylamine in THF (2 M, 11.3 mL, 22.6 mmol) was added dropwise over 1 h to the stirring


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solution. A white precipitate was observed during the addition. Once the addition was complete, a solution of triethylamine (3.2 mL, 23.2 mmol) in THF (20 mL) was added dropwise at 0−10 °C. The reaction was warmed to ambient temperature and stirred for 3 h. The product did not precipitate from solution, as typically observed for analogous substrates. The solvent was distilled to provide a golden honey-like oil, which was taken up in methylene chloride and filtered over a pad of silica (ca. 50 g). The pad was washed with 500 mL of 10% MeOH/$CH_2Cl_2$. The filtrate was concentrated to a yellow solid (8.0 g, 99%, 98% UPLC purity): mp 172−175 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 10.16 (1H, d, $J$ = 10.6 Hz), 7.67 (1H, d, $J$ = 3.3 Hz), 7.41−7.25 (10H, m), 7.09 (1H, t, $J$ = 7.9 Hz), 7.02 (1H, d, $J$ = 8.1 Hz), 6.90 (1H, dd, $J$ = 7.6, 0.9 Hz), 4.59 (2H, s), 4.38 (2H, s), 2.40 (3H, s); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 185.19, 170.96, 168.70, 144.21, 139.07, 137.93, 136.11, 135.33, 128.88, 128.68, 128.34, 128.30, 127.99, 127.76, 124.75, 118.24, 115.96, 113.87, 110.83, 50.50, 46.17, 21.42.; HRMS (ESI) calcd for $C_{26}H_{23}N_2O_4^+$ 427.1653 [M + H]$^+$, found 427.1677.

*3-(2-(Benzyl(methyl)amino)ethyl)-1H-indol-4-ol (**16**).* To a solution of **14** (6.0 g, 17 mmol) in 2-MeTHF (160 mL) was added LAH (13 mL, 4 M in $Et_2O$) at 0 °C dropwise. The yellow mixture was heated at reflux (~78 °C) under nitrogen for 3 h. The reaction was monitored by LCMS periodically: at 1 h, LCMS analysis revealed a 1:3 mix of a $\beta$-hydroxy intermediate ($m/z$ 297.1604) and product (no other significant peaks were present); at 2 h, no additional change was observed, so an additional 1 equiv of LAH (4.3 mL) was added. The reaction was left to stir overnight. The reaction was analyzed after refluxing for a total of 17 h and was found to still contain about 9% of the hydroxy intermediate. The reaction was cooled to 5 °C, and Glauber's salt ($Na_2SO_4$·$10H_2O$) was added in portions; the temperature was not allowed to rise above 15 °C. The suspension was left to stir for 1 h, then filtered to give an initially clear filtrate that quickly turned purple-brown. The filtrate was passed through a plug of silica (ca. 40 g) and washed with 10% MeOH/dichloromethane (DCM) (400 mL). The resulting tan filtrate was concentrated to a brown oil that became a foam under high vacuum and ultimately solidified (4.1 g, 85%): mp 51−53 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.07 (1H, d, $J$ = 9.2 Hz), 7.33 (2H, dq, $J$ = 5.4, 3.0 Hz), 7.29 (3H, dd, $J$ = 5.1, 2.2 Hz), 7.11 (1H, t, $J$ = 7.9 Hz), 6.89 (1H, d, $J$ = 8.1 Hz), 6.79 (1H, d, $J$ = 2.3 Hz), 6.69 (1H, d, $J$ = 7.6 Hz), 3.68 (2H, s), 3.01−2.94 (2H, m), 2.83−2.76 (2H, m), 2.37 (3H, s); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm) 151.70, 138.96, 135.41, 130.27, 128.35, 127.75, 123.42, 121.01, 117.87, 114.32, 106.50, 102.94, 62.82, 59.20, 42.19, 25.01; HRMS (ESI) calcd for $C_{18}H_{21}N_2O^+$ 281.1649 [M + H]$^+$, found 281.1651.

*3-(2-(Dibenzylamino)ethyl)-1H-indol-4-ol (**17**).* To a solution of **15** (3.65 g, 8.54 mmol) in 2-MeTHF (100 mL) was added LAH (8.54 mL, 34.15 mmol, 4 M in $Et_2O$) at 0 °C dropwise. The yellow mixture was heated at reflux (~78 °C) under nitrogen for 6 h. The reaction stalled containing about 9% of the hydroxy intermediate evidenced by LCMS. The reaction was cooled to 5 °C, and Glauber's salt ($Na_2SO_4$·$10H_2O$) was added in portions; the temperature was not allowed to rise above 15 °C. The suspension was left to stir for 1 h, then filtered to give an initially clear filtrate, which darkened quickly. The filtrate was passed through a plug of silica (ca. 40 g) and washed with 20% MeOH/DCM (200 mL). The resulting filtrate was concentrated to a brown oil that became a foam under high vacuum and ultimately solidified to an off-white solid (2.7 g, 88%): mp 52−55 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.82 (1H, s), 7.37 (5H, dd, $J$ = 7.5, 1.9 Hz), 7.32−7.21 (6H, m), 7.10 (1H, t, $J$ = 7.8 Hz), 6.88 (1H, d, $J$ = 8.0 Hz), 6.75 (1H, d, $J$ = 2.3 Hz), 6.69 (1H, d, $J$ = 7.6 Hz), 3.77 (4H, s), 2.92−2.81 (4H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 151.41, 138.74, 135.82, 130.41, 128.29, 127.54, 123.41, 120.57, 117.88, 114.52, 106.46, 102.98, 59.35, 55.00, 25.20; HRMS (ESI) calcd for $C_{24}H_{25}N_2O^+$ 357.1962 [M + H]$^+$, found 357.1966.

*3-((3-(2-(Benzyl(methyl)amino)ethyl)-1H-indol-4-yl)oxy)-1,5-dihydrobenzo[e][1,3,2]dioxaphosphepine 3-Oxide (**18**).* To a suspension of NaH (60%, 268 mg, 6.71 mmol) in dry THF (15 mL) at 0 °C under Ar was added **16** (1.71 g, 6.10 mmol) in dry THF (20 mL). The robin's egg blue suspension was stirred at 0 °C for 15 min; then *o*-xPCl[37] (1.47 g, 6.71 mmol) in dry THF (30 mL) was added dropwise. The reaction was warmed to ambient temperature and stirred for 30 min. TLC (2% MeOH/$CHCl_3$) indicated complete consumption of starting material with a large more polar product spot and a smaller less polar spot (later revealed to be about 6% diphosphorylated product). The reaction mixture was dumped into ice water (100 mL) and extracted with 3 × 100 mL of $CHCl_3$. The extract was washed with saturated sodium bicarbonate, then dried over sodium sulfate, filtered, and concentrated. The resulting tan solid was purified by flash chromatography (2% MeOH/$CHCl_3$/0.2% $NH_4OH$) to provide the target compound as a shiny off-white solid (2.3 g, 82%): mp 135−139 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.73 (1H, s), 7.39 (2H, dt, $J$ = 7.2, 3.7 Hz), 7.29 (7H, dd, $J$ = 5.8, 3.1 Hz), 7.11 (2H, t, $J$ = 8.5 Hz), 7.02 (1H, t, $J$ = 7.9 Hz), 6.85 (1H, d, $J$ = 2.5 Hz), 5.41 (2H, t, $J$ = 13.3 Hz), 5.25 (2H, dd, $J$ = 19.5, 13.6 Hz), 3.58 (2H, s), 3.16 (2H, t, $J$ = 7.8 Hz), 2.83−2.74 (2H, m), 2.27 (3H, s); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 144.22, 139.24, 138.85, 135.17, 129.30, 129.08, 129.07, 128.19, 126.89, 122.59, 121.93, 118.76, 113.07, 108.85, 108.63, 68.96, 62.19, 59.07, 42.08, 24.78; HRMS (ESI) calcd for $C_{26}H_{28}N_2O_4P^+$ 463.1781 [M + H]$^+$, found 463.1784.

*3-((3-(2-(Dibenzylamino)ethyl)-1H-indol-4-yl)oxy)-1,5-dihydrobenzo[e][1,3,2]dioxaphosphepine 3-Oxide (**19**).* To a solution of **17** (2.2 g, 6.2 mmol) in anhydrous THF (50 mL) under Ar at −78 °C was added a BuLi solution (2.5 M in THF, 2.4 mL, 6.0 mmol) dropwise. The blue-gray solution stirred for 15 min at −78 °C then warmed to −30 to −40 °C. A solution of *o*-xPCl[37] (1.62 g, 7.41 mmol) in THF (25 mL) was added dropwise by syringe pump over 1 h while maintaining a reaction temperature of about −35 °C. The reaction was warmed to 0 °C and quenched by the addition of aminopropyl silica gel (5 g). The suspension was filtered and washed with EtOAc (~100 mL). The resulting tan filtrate was concentrated to an off-white solid, which was purified by flash chromatography (2% MeOH/$CHCl_3$/0.2% $NH_4OH$) to provide the target compound as a tan solid (2.7 g, 81%): mp 152−155 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.53 (1H, s), 7.44−7.31 (7H, m), 7.31−7.16 (8H, m), 7.06 (2H, t, $J$ = 8.4 Hz), 7.02−6.95 (1H, m), 6.66 (1H, s), 5.31 (2H, t, $J$ = 13.3 Hz), 5.18 (2H, dd, $J$ = 19.7, 13.6 Hz), 3.67 (4H, s), 3.17 (2H, t, $J$ = 7.6 Hz), 2.84 (2H, t, $J$ = 7.6 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm) 144.18, 140.07, 138.76, 135.14, 129.30, 129.05, 128.72, 128.16, 126.71, 122.43, 121.81, 118.81, 113.09, 108.82, 108.59, 77.42, 77.10, 76.78, 68.87, 58.17, 54.64, 24.64; HRMS (ESI) calcd for $C_{32}H_{32}N_2O_4P^+$ 539.2094 [M + H]$^+$, found 539.2091.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01061.

$^1$H and $^{13}$C NMR spectra for all final products and intermediates; UPLC chromatograms and low/high collision energy HRMS spectra for **4**−**7** (PDF)

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### Notes

The authors declare no competing financial interest.

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