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# *De Novo* Biosynthesis of β-Valienamine in Engineered *Streptomyces hygroscopicus* 5008

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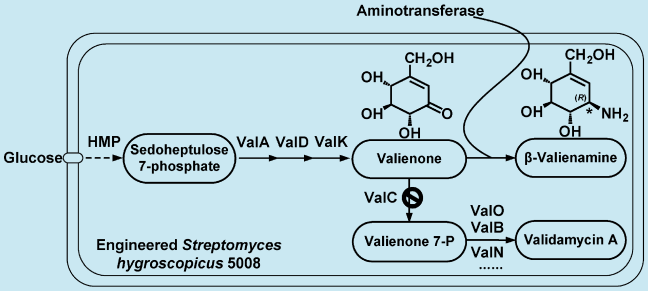
Supporting Information

**ABSTRACT:** The $C_7N$ aminocyclitol β-valienamine is a lead compound for the development of new biologically active β-glycosidase inhibitors as chemical chaperone therapeutic agents for lysosomal storage diseases. Its chemical synthesis is challenging due to the presence of multichiral centers in the structure. Herein, we took advantage of a heterogeneous aminotransferase with stereospecificity and designed a novel pathway for producing β-valienamine in *Streptomyces hygroscopicus* 5008, a validamycin producer. The aminotransferase BtrR from *Bacillus circulans* was able to convert valienone to β-valienamine with an optical purity of up to >99.9% enantiomeric excess value *in vitro*. When the aminotransferase gene was introduced into a mutant of *S. hygroscopicus* 5008 accumulating valienone, 20 mg/L of β-valienamine was produced after 96 h cultivation in shaking flasks. This work provides a powerful alternative for preparing the chiral intermediates for pharmaceutical development.




**KEYWORDS:** *β-valienamine, valienone, aminotransferase, validamycin, biosynthesis*

$C_7N$ aminocyclitols remain increasing interest in the biochemistry of glycosidase inhibitors for their significant bioactivities.[1,2] For instance, β-valienamine, (1*S*,2*S*,3*R*,6*R*)-6-amino-4-(hydroxymethyl)cyclohex-4-ene-1,2,3-triol, is an optical building block for synthesizing the inhibitors of β-glycosidases.[2−4] Its two derivatives, *N*-octyl-β-valienamine and *N*-octyl-4-*epi*-β-valienamine, have been proven to be promising chemical chaperone therapeutic agents for lysosomal storage diseases including Gaucher, $G_{M1}$-gangliosidosis, and Morquio B disease caused by the disorder of β-glycosidase.[5−8] The chemical synthetic routes of this compound have been investigated since 1980s.[9−15] However, the presence of multiple chiral centers in β-valienamine is quite challenging due to the insufficient stereospecificity of the chemical catalysts. Moreover, the multisteps, harsh reactions (lower than −70 °C), and chemical pollutions also cause synthetic and economic hurdles. Biosynthesis has emerged as a powerful alternative,[16,17] especially for chiral amines in asymmetric synthesis.[18] Recently, instead of using traditional rhodium, an aminotransferase was found to be remarkably able to convert prositagliptin ketone into sitagliptin with enantiomeric excess (e.e.) value >99.9%.[19] However, there is no similar report using aminotransferase for the biosynthesis of β-valienamine. It is also valuable to construct a biosynthetic pathway for producing β-valienamine *in vivo*.

In our previous work, 2-*epi*-5-*epi*-valiolone, 5-*epi*-valiolone and valienone were found to be the intermediates in the biosynthetic pathway of validamycin A in *Streptomyces hygroscopicus* 5008 (Figure 1, 1−3).[20,21] It is noteworthy that valienone has a similar structure with β-valienamine except that the former has a carbonyl group, while the latter has an amino group on C6. Such similarity suggests that valienone would be an ideal precursor for certain aminotransferase to produce β-valienamine directly by a transamination reaction (Figure 1). Therefore, we focused on screening aminotransferases that can catalyze the conversion of valienone into β-valienamine in a desired stereospecific manner, and we designed an artificial biosynthetic pathway in *S. hygroscopicus* 5008 for microbial synthesis of β-valienamine.

The key challenge in designing this biosynthetic pathway is the selection of an aminotransferase for the bioconversion of valienone into β-valienamine. To identify suitable enzyme candidates, we used an integrative platform (RxnIP)[22] to collect enzyme information, especially for enzymes recognizing cyclitol substrates, which is similar to valienone in structure. The sugar aminotransferases (SATs) convert keto-sugars into amino-sugars in the biosynthesis of macrolide antibiotics and belong to the subgroup DegT_DnrJ_EryC1 in the Pfam database.[23] We chose SATs that typically use *scyllo*-inosose and NDP-4-keto-sugar as substrates for further analysis. As shown in Figure 2, four main evolutionary branches existed in a maximum likelihood phylogenetic tree, and one aminotransferase was therefore selected from each branch. These aminotransferases were BtrR[24] from *Bacillus circulans* catalyzing the transamination of 2-deoxy-*scyllo*-inosose and 2-deoxy-3-amino-

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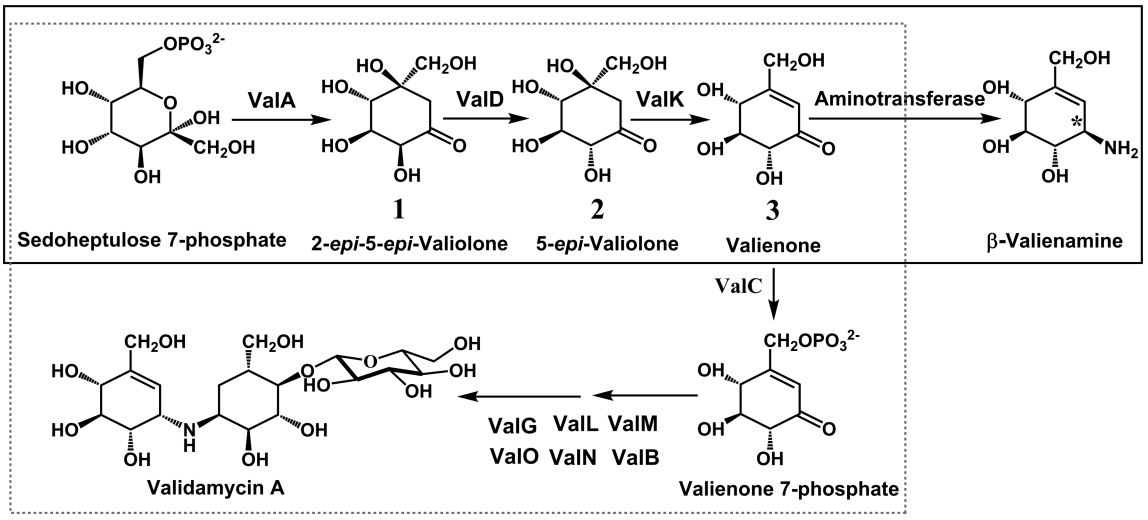


**Figure 1.** Biosynthetic pathway of validamycin A (dashed frame) and *de novo* aminotransferase-catalyzed route for *β*-valienamine biosynthesis (solid frame) in *S. hygroscopicus* 5008.

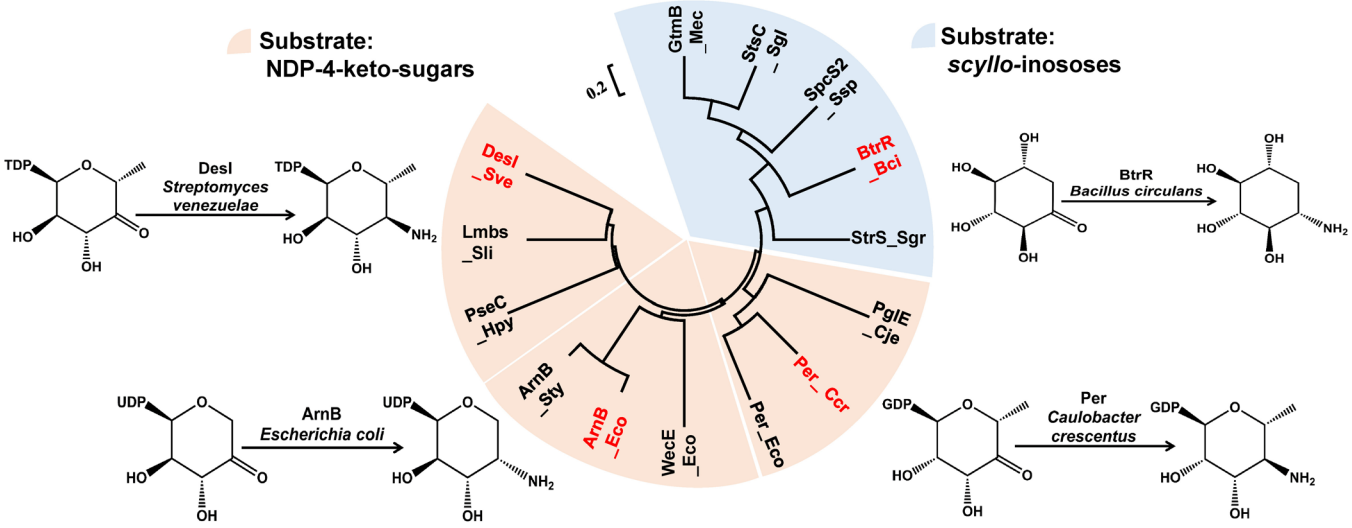


**Figure 2.** Phylogenetic analysis of SATs and the reactions catalyzed by the selected (red) candidates. The multiple alignments were performed by ClustalX, and the phylogenetic tree was generated using MEGA6. BtrR_Bci (CAD41947), SpcS2_Ssp (ABW87804), StsC_Sgl (AIR96275), GtmB_Mec (CAE06512), StrS_Sgr (CAA68523), WecE_Eco (AAC76796), ArnB_Eco (AAM92146), ArnB_Sty (NP_461239), Per_Eco (AAG57096), Per_Ccr (AAK22996), LmbS_Sli (CAA55764), DesI_Sve (AAC68684), PseC_Hpy (AAD07433), PglE_Cje (AAD09304).

*scyllo*-inosose in butirosin A biosynthesis, DesI[25] from *Streptomyces venezuelae* in the biosynthesis of TDP-4-amino-4,6-dideoxyglucose, Per[26] from *Caulobacter crescentus* for GDP-4-amino-6-deoxy-mannose biosynthesis, and ArnB[27] from *Escherichia coli* for UDP-4-amino-arabinose biosynthesis. The four candidate genes were amplified by PCR or chemically synthesized, cloned into plasmid pET28a, and overexpressed in *E. coli* BL21 (DE3) to yield soluble proteins. The recombinant proteins with the *N*-terminal His-tag were purified by Ni-NAT columns and confirmed to be a homogeneous band by SDS-PAGE (Figure S1).

To assess the feasibility of these aminotransferases to convert the non-natural substrate valienone into *β*-valienamine, we checked both the catalytic activity and enantio-preference of these enzymes *in vitro*. The aminotransferase activity assay was performed at 37 °C in sodium phosphate buffer (pH 7.5) with valienone and cofactor pyridoxal 5′-phosphate (PLP). To identify the appropriate amino donors, L-glutamine and L-glutamate were tested. As shown in Figure S2a, L-glutamine showed a higher activity for all of the selected enzymes and was therefore used for further enzyme kinetic assays. Quantitative analysis and the stereopurity of *β*-valienamine were detected through precolumn derivatization with ortho-phthaldialdehyde (OPA) using high-performance liquid chromatography (HPLC) with fluorescence detection. The derivatives of standards valienamine (*S*-configuration) and *β*-valienamine (*R*-configuration) were separated through HPLC by an Eclipse XDB-C18 column with retention times of 5.1 and 5.7 min, respectively (Figure 3a).

The mixture of the reaction was analyzed with the same condition. The results indicated that BtrR, Per, and ArnB showed detectable activity after 3 h of incubation (Figure 3c–e). More interestingly, BtrR and Per demonstrated a stringent stereospecificity with >99.9% e.e. value, while ArnB had low stereospecificity with 61% e.e. value for its *β*-valienamine product. Moreover, BtrR showed the highest activity among


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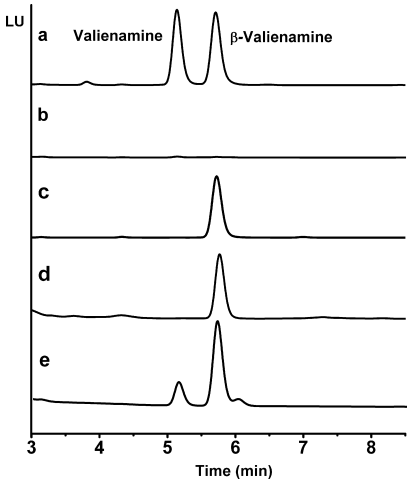
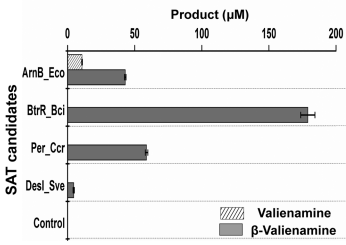



**Figure 3.** HPLC analysis of the reaction mixture *via* precolumn derivatization using OPA. (a) Valienamine and *β*-valienamine standards. (b) Reaction mixture without enzyme as the blank control. (c–e) Reactions catalyzed by BtrR, Per, and ArnB, respectively.




| | e.e. value (%) | $K_m$ (μM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($mM^{-1}min^{-1}$) |
|---|---|---|---|---|
| ArnB_Eco | 61.3 | -- | -- | -- |
| BtrR_Bci | > 99.9 | 43.11±0.01 | 0.043±0.01 | 1.0037 |
| Per_Ccr | > 99.9 | 255.58±0.1 | 0.012±0.2 | 0.0485 |
| Desl_Sve | -- | -- | -- | -- |
| Control | -- | -- | -- | -- |

**Figure 4.** Enzymatic conversion and kinetic properties of selected SATs. (Column) Enzymatic conversion of valienone to *β*-valienamine by selected SATs. Data were collected from three measurements with 1 mg/mL enzymes incubated with 5 mM valienone, 5 mM L-glutamine, and 0.3 mM PLP at 37 °C in sodium phosphate buffer (pH 7.5); (Table) Kinetic parameters of different SATs; 0.5 U L-GDH was added into the reaction system as a coupling enzyme with 5 mM ammonium chloride and 0.5 mM NADH.

these three candidates and converted 5 mM valienone into *β*-valienamine with 76% conversion after 12 h at the enzyme concentration of 5 mg/mL for sample preparation.

The molecular weight and the absolute configuration of the purified amine product were further verified by high-resolution mass spectrometry (HR-MS) with positive mode, $^1$H NMR, NOE, NOESY, and $^{13}$C NMR spectra. The product gave an ion peak at *m/z* 176.0936 $[M + H]^+$ (Table S1 and Figure S3), and its OPA derivative gave an ion peak at *m/z* 374.1041 $[M + Na]^+$ (Table S2 and Figure S6a), matching the molecular formula of *β*-valienamine and its OPA derivative well. $^1$H NMR analysis of the substrate (valienone) and the product (*β*-valienamine) showed that the chemical shifts of all of the protons moved to a higher magnetic field, especially the adjacent protons (*δ* of H-5 from 6.20 to 5.50 ppm and *δ* of H-1 from 4.16 to 3.27 ppm), and a new signal appeared at *δ*3.35 (s, 1H, H-6), which is consistent with the chemical shift of a methylene that is attached to an amino group. The signal of H-1 was changed from a doublet to a triplet with a coupling constant of 9.8 Hz, indicating that both C-2 and C-6 have the same relative configuration (Figure S4a,b). Further NOE and NOESY analysis showed that there was a correlation between H-2 (*δ* 3.50 ppm) and H-6, confirming the configuration of the aminated C-6 as the *R*-configuration (Figure S4c,d). Meanwhile, the transamination also can be observed through the chemical shifts in $^{13}$C NMR spectra (Figure S4e,f).

Further comparison of the catalytic efficiency of BtrR and Per toward valienone (Figure 4) was performed by Michaelis–Menten kinetic analysis with L-glutamate dehydrogenase (L-GDH) as a coupling enzyme (Figure S2b).[28] The $K_m$ value of BtrR is only one-sixth that of Per toward valienone, indicating its stronger substrate-binding capacity. Considering the native substrates of all candidates, the BtrR prefers small *scyllo*-inosose substrates (2-deoxy-*scyllo*-inosose and 2-deoxy-3-amino-*scyllo*-inosose), whereas others prefer large NDP-4-keto-sugars. Possibly, this is the reason why BtrR shows a higher binding affinity for valienone. Additionally, the $k_{cat}$ value of BtrR was 3.6 folds of that of Per, resulting in an overall 13-fold higher catalytic efficiency ($k_{cat}/K_m$) than Per. Combining e.e. values and kinetic parameters summarized in Figure 4, BtrR was proven to be the best catalytic candidate to transfer an amino group from L-glutamine to valienone for the production of chiral *β*-valienamine *in vitro*.

The biosynthetic pathway of validamycin A has been thoroughly studied,[20,21,29,30] and its producer, *S. hygroscopicus* 5008, gives a stable yield of validamycin A. To achieve the acquisition of *β*-valienamine in the microbe *via* a fermentation process, we designed a novel pathway by introducing the *btrR* gene into an engineered *S. hygroscopicus* 5008 to generate a new pathway for the biosynthesis of *β*-valienamine. We have identified that the $C_7$ cyclitol kinase ValC in the validamycin A biosynthetic pathway is responsible for the phosphorylation of valienone.[29] Obviously, the presence of ValC is supposed to consume valienone and form a competitive reaction with BtrR. Therefore, the gene *valC* was knocked out using REDIRECT Technology.[29] The mutant Δ*valC* and wild-type 5008 were cultured in the fermentation media for 72 h, and the production of valienone and validamycin A were analyzed by HPLC. The results showed that the production of validamycin A was abolished (Figure S5a) and valienone was accumulated in the Δ*valC* mutant (Figure 5b, S5b). Subsequently, the *btrR* gene was cloned on the integrative vector pPM927 under the control of the strong *PvalA* promoter[30] and integrated into the Δ*valC* mutant. Thus, a ValA-ValD-ValK-BtrR pathway was constructed for the biosynthesis of *β*-valienamine in the mutant Δ*valC*::*btrR*. Mutant Δ*valC*::*btrR* and Δ*valC* were cultured with 50 mL of fermentation medium for 5 days in shaking flasks, and the productivity of *β*-valienamine was monitored by HPLC with OPA-derivatization for every 12 h. As shown in Figure 5a, the characteristic peak of *β*-valienamine with a retention time of 5.7 min was observed in the fermentation broth of the mutant Δ*valC*::*btrR* but absent in mutant Δ*valC*, which was confirmed by further HR-MS analysis (data not shown). The accumulation of *β*-valienamine was increased with prolonged incubation time and reached the highest of 20 mg/L at 96 h (Figure 5b). The fermented *β*-valienamine product was also verified by HR-MS with precolumn derivatization using OPA. The spectrum matched well with that of the standard *β*-valienamine and showed the characteristic ion peak at *m/z* 374.1038 $[M + Na]^+$ (Table S2 and Figure S6b). The above data showed that the designed ValA-ValD-ValK-BtrR pathway for *β*-valienamine biosynthesis is functional, and the engineered strain is able to produce the unnatural product *β*-valienamine.

In summary, we successfully designed and constructed an artificial pathway for generating highly stereospecific *β*-valienamine both *in vitro* and *in vivo*. Compared with the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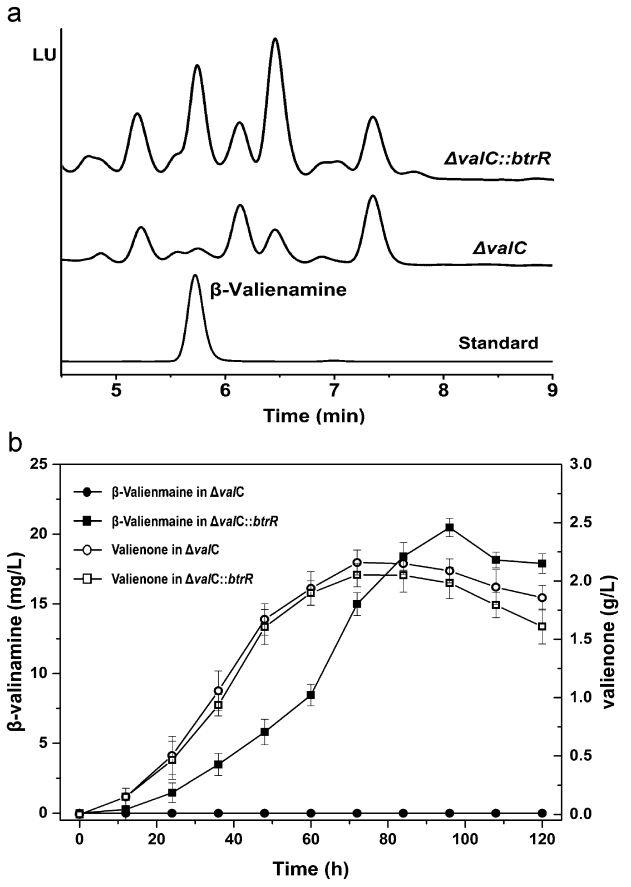


**Figure 5.** Production of *β*-valienamine in the Δ*valC* mutant integrated with the heterogeneous aminotransferase gene *btrR*. (a) HPLC analysis of 96-h fermentation broths with precolumn derivatization using OPA. (b) Time course of *β*-valienamine and valienone titer in Δ*valC* and Δ*valC::btrR* mutant.

current chemical methods for *β*-valienamine synthesis, our biological strategy represents a promising alternative by integrating a heterogeneous aminotransferase gene and engineering host metabolic flux due to its sustainable and efficient process. To further increase *β*-valienamine production, we are investigating the engineering of BtrR for improved catalytic activity on valienone, by *in silico* design based on the protein structure analysis and systematically optimizing the pathway flux of the host microbe. Our research not only builds a new and efficient route to synthesize *β*-valienamine, but also facilitates further synthesis of its active derivatives with glycosidase inhibitory activity for treating related diseases.

## MATERIALS AND METHODS

**Chemicals.** Valienone was synthesized by WuXi AppTec Company (Wu Xi, China). *β*-Valienamine was prepared and identified after bioconversion with BtrR *in vitro* in our lab. Valienamine was kindly provided by Prof. Yuguo Zheng (Zhejiang University of Technology). Other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

**Molecular Phylogenetic Analysis by Maximum Likelihood.** For phylogenetic analysis, full-length amino acid sequences were aligned by ClustalX (Des Higgins *et al.*, Conway Institute UCD Dublin) with the following parameters: protein weight matrix = Gonnet; gap open = 10; gap extension = 0.2. Maximum likelihood analysis was employed by MEGA6 software with the JTT matrix-based model.

**Expression and Purification of SATs.** Candidate SAT genes were amplified and ligated into the pET28a plasmid. Expression plasmids were transformed into *E. coli* BL21 (DE3). Transformants were grown in LB medium with 100 *μ*g/mL kanamycin at 37 °C until the cell density at 600 nm reached 0.3−0.5 followed by induction with 0.8 mM isopropyl-*β*-D-thiogalactopyranoside for 10 h to produce *N*-terminal His6-tagged recombinant proteins. The cells were harvested by centrifugation, resuspended in 20 mM potassium phosphate buffer (pH 7.5), and disrupted by sonication. The fusion proteins were purified by Ni-NTA resin column with 20 mM potassium phosphate buffer (pH 7.5) containing different concentrations of imidazole. The purified proteins were detected by SDS-PAGE.

**Enzymatic Activity and the Kinetic Parameter Assays.** To measure the activity of sugar aminotransferases (SATs), 1 mg/mL purified SAT was incubated with 5 mM valienone as amino acceptor and 5 mM L-glutamine as an amino donor in 20 mM potassium phosphate (pH 7.5) containing 0.3 mM pyridoxal 5′-phosphate as a cofactor at 37 °C for 3 h followed by precolumn ortho-phthaldialdehyde derivatization and fluorescence detection.

The kinetic parameters were determined at 37 °C using NADH-dependent L-glutamate dehydrogenase (L-GDH) as a coupling enzyme with a reaction system involving 0.5 U L-GDH, 5 mM ammonium chloride and 0.5 mM NADH in addition to the normal reaction system. The reduction in the absorbance of NADH ($\varepsilon_{340}$ = 6220 $M^{-1}$ $cm^{-1}$) was monitored continuously at 340 nm by a multiscan spectrum microplate spectrophotometer (Spectra Max, Molecular Devices). One unit of enzyme activity was defined as 1 *μ*mol of NADH consumed per min.

**Stereopurity Determination of *β*-Valienamine by HPLC with Precolumn Derivatization Using OPA.** Stereometric purity of *β*-valienamine was determined by online fluorescence derivatization of ortho-phthaldialdehyde on Agilent 1200 Infinity LC System. *β*-Valienamine and its (*S*)-enantiomer standards were derivatized by 0.15 M OPA in the 0.40 M (pH 9.0) borate buffer. The separation of the derivative mixture was performed using an Eclipse XDB-C18 (5 *μ*m, 4.6 × 150 mm) column eluted with 22% acetonitrile at 30 °C for 18 min under the detection with the fluorescence detector at 445 nm emission and 340 nm excitation wavelengths with medium sensitivity. *β*-Valienamine and valienamine showed the retention times at 5.7 and 5.1 min, respectively.

***β*-Valienamine Purification from the Reaction Mixture and the Fermentation Broth of Δ*valC*::*btrR*.** A mixture of the reaction was filtered through a Microcon YM-10 (Millipore) to remove protein (>10 000 $M_W$) and then purified by Dowex 1 × 2 ($OH^-$ form) (0.5 × 15 cm) eluted with water. Isolation of *β*-valienamine from fermentation broth of Δ*valC*::*btrR* through two-column chromatography system was performed including Dowex 50 × 8 ($H^+$ form) eluted with 0.5 M ammonia−water and Dowex 1 × 2 ($OH^-$ form) eluted with water.

**High Resolution-Mass Spectrometry (HR-MS) and Nuclear Magnetic Resonance Spectrometry (NMR).** High-resolution mass spectral analysis was performed using an Agilent 1290-MS 6230 TOF-MS system with positive model. For NMR identification, the purified samples were dried and redissolved in $D_2O$ solution, and one-dimensional $^1H$, $^{13}C$


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and two-dimensional $^{1}H-^{1}H$ COSY, NOESY NMR spectra were obtained using Bruker Avance III 400 MHz spectrometer.

**Construction of Δ*valC*::*btrR* Mutant.** To construct the Δ*valC*::*btrR* mutant, a 0.9 kb XhoI/NdeI *PvalA* promoter fragment and a 1.3 kb NdeI/XbaI fragment containing the *btrR* gene were cloned into pBluescript II SK(+) plasmid digested by XhoI and XbaI. Subsequently, the fragment containing *PvalA* and *btrR* was cleaved with *Eco*RI from the recombinant plasmid and cloned into the integrative vector pPM927. The resulting plasmid was introduced into the Δ*valC* mutant by intergeneric conjugation mediated by *E. coli* ET12567 (pUZ8002) and confirmed by PCR amplification.

**Fermentation of *S. hygroscopicus* 5008 and Its Mutants.** *S. hygroscopicus* 5008 and its mutants were cultivated for 72−120 h at 37 °C at 220 rpm in the fermentation medium (gram per liter: corn powder 100, soybean flour 25, yeast extract 5, NaCl 1, and $KH_2PO_4$ 1.5). Samples were collected every 12 h. The supernatant of the fermentation broth was extracted with chloroform and centrifuged at 12 000 rpm for 10 min before HPLC analysis.

**Validamycin A Analysis by HPLC.** The fermentation broth of *S. hygroscopicus* 5008 and Δ*valC* mutant were separated through Eclipse XDB-C18 (5 μm, 4.6 × 150 mm) column, eluted with $CH_3CN/H_2O$ (3:97) as mobile phase at 30 °C for 18 min and monitored at 210 nm by diode array detector.

**Valienone Analysis by HPLC with DNPH Precolumn Derivatization.** The accumulation of valienone in the fermentation broth of the Δ*valC* mutant was evaluated by HPLC with a precolumn derivatization using 2,4-dinitrophenylhydrazine. The reaction was performed with 7.57 mM DNPH in 5% $H_3PO_4$ solution. Analysis was performed using an Eclipse XDB-C18 (5 μm, 4.6 × 150 mm) column, eluted with 50% acetonitrile at 30 °C for 18 min, and detected at 380 nm by diode array detector.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.5b00138.

Additional figures as described in the text. (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Mahmud, T. (2003) The $C_7N$ aminocyclitol family of natural products. *Nat. Prod. Rep. 20*, 137−166.

(2) Ogawa, S., Kanto, M., and Suzuki, Y. (2007) Development and medical application of unsaturated carbaglycosylamine glycosidase Inhibitors. *Mini-Rev. Med. Chem. 7*, 679−691.

(3) Suzuki, Y., Ogawa, S., and Sakakibara, Y. (2009) Chaperone therapy for neuronopathic lysosomal diseases: competitive inhibitors as chemical chaperones for enhancement of mutant enzyme activities. *Perspect. Med. Chem. 3*, 7−19.

(4) Higaki, K., Ninomiya, H., Suzuki, Y., and Nanba, E. (2013) Candidate molecules for chemical chaperone therapy of $G_{M1}$-gangliosidosis. *Future Med. Chem. 5*, 1551−1558.

(5) Suzuki, Y. (2013) Chaperone therapy update: Fabry disease, $G_{M1}$-gangliosidosis and Gaucher disease. *Brain & Development 35*, 515−523.

(6) Luan, Z., Li, L., Ninomiya, H., Ohno, K., Ogawa, S., Kubo, T., Iida, M., and Suzuki, Y. (2010) The pharmacological chaperone effect of *N*-octyl-β-valienamine on human mutant acid β-glucosidases. *Blood Cells, Mol., Dis. 44*, 48−54.

(7) Suzuki, Y., Ichinomiya, S., Kurosawa, M., Matsuda, J., Ogawa, S., Iida, M., Kubo, T., Tabe, M., Itoh, M., Higaki, K., Nanba, E., and Ohno, K. (2012) Therapeutic chaperone effect of *N*-octyl 4-*epi*-beta-valienamine on murine $G_{(M1)}$-gangliosidosis. *Mol. Genet. Metab. 106*, 92−98.

(8) Matsuda, J., Suzuki, O., Oshima, A., Yamamoto, Y., Noguchi, A., Takimoto, K., Itoh, M., Matsuzaki, Y., Yasuda, Y., Ogawa, S., Sakata, Y., Nanba, E., Higaki, K., Ogawa, Y., Tominaga, L., Ohno, K., Iwasaki, H., Watanabe, H., Brady, R. O., and Suzuki, Y. (2003) Chemical chaperone therapy for brain pathology in $G_{(M1)}$-gangliosidosis. *Proc. Natl. Acad. Sci. U. S. A. 100*, 15912−15917.

(9) Ogawa, S., Toyokuni, T., and Suami, T. (1980) Synthesis of penta-*N*,*O*-acetyl-DL-valienamine and its related branched-chain unsaturated aminocyclitols and cyclitols. *Chem. Lett.*, 713−716.

(10) Ogawa, S., Chida, N., and Suami, T. (1983) Synthetic studies on the validamycins. 5.[1,2] Synthesis of DL-hydroxyvalidamine and DL-valienamine. *J. Org. Chem. 48*, 1203−1207.

(11) Ogawa, S., Ashiura, M., Uchida, C., Watanabe, S., Yamazaki, C., Yamagishi, K., and Inokuchi, J. (1996) Synthesis of potent β-D-glucocerebrosidase inhibitors. *Bioorg. Med. Chem. Lett. 6*, 929−932.

(12) Ogawa, S., Kobayashi, Y., Kabayama, K., Jimbo, M., and Inokuchi, J. (1998) Chemical modification of beta-glucocerebrosidase inhibitor *N*-octyl-β-valienamine: synthesis and biological evaluation of *N*-alkanoyl and *N*-alkyl derivatives. *Bioorg. Med. Chem. 6*, 1955−1962.

(13) Cumpstey, I., Gehrke, S., Erfan, S., and Cribiu, R. (2008) Studies on the synthesis of valienamine and 1-*epi*-valienamine starting from D-glucose or L-sorbose. *Carbohydr. Res. 343*, 1675−1692.

(14) Kuno, S., Takahashi, A., and Ogawa, S. (2013) Concise syntheses of potent chaperone drug candidates, *N*-octyl-4-*epi*-β-valinenamine (NOEV) and its 6-deoxy derivative, from (+)-proto-quercitol. *Carbohydr. Res. 368*, 8−15.

(15) Li, Q. R., Kim, S. I., Park, S. J., Yang, H. R., Baek, A. R., Kim, I. S., and Jung, Y. H. (2013) Total synthesis of (+)-valienamine and (−)-1-*epi*-valienamine *via* a highly diastereoselective allylic amination of cyclic polybenzyl ether using chlorosulfonyl isocyanate. *Tetrahedron 69*, 10384−10390.

(16) Lee, S. Y. (2012) Metabolic engineering and synthetic biology in strain development. *ACS Synth. Biol. 1*, 491−492.

(17) Niu, W., and Guo, J. (2015) Stereospecific microbial conversion of lactic acid into 1,2-propanediol. *ACS Synth. Biol. 4*, 378−382.

(18) Brenna, E. (2013) *Synthetic Methods for Biologically Active Molecules: Exploring the Potential of Bioreductions*, Wiley-VCH, Weinheim.

(19) Savile, C. K., Janey, J. M., Mundorff, E. C., Moore, J. C., Tam, S., Jarvis, W. R., Colbeck, J. C., Krebber, A., Fleitz, F. J., Brands, J., Devine, P. N., Huisman, G. W., and Hughes, G. J. (2010) Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. *Science 329*, 305−309.

(20) Yu, Y., Bai, L., Minagawa, K., Jian, X., Li, L., Li, J., Chen, S., Cao, E., Mahmud, T., Floss, H. G., Zhou, X., and Deng, Z. (2005) Gene cluster responsible for validamycin biosynthesis in *Streptomyces*




DOI: 10.1021/acssynbio.5b00138
*ACS Synth. Biol.* 2016, 5, 15−20

*hygroscopicus* subsp. *jinggangensis* 5008. *Appl. Environ. Microbiol. 71*, 5066−5076.
(21) Bai, L., Li, L., Xu, H., Minagawa, K., Yu, Y., Zhang, Y., Zhou, X., Floss, H. G., Mahmud, T., and Deng, Z. (2006) Functional analysis of the validamycin biosynthetic gene cluster and engineered production of validoxylamine A. *Chem. Biol. 13*, 387−397.
(22) Hu, Q. N., Deng, Z., Hu, H., Cao, D. S., and Liang, Y. Z. (2011) RxnFinder: biochemical reaction search engines using molecular structures, molecular fragments and reaction similarity. *Bioinformatics 27*, 2465−2467.
(23) Hwang, B. Y., Cho, B. K., Yun, H., Koteshwar, K., and Kim, B. G. (2005) Revisit of aminotransferase in the genomic era and its application to biocatalysis. *J. Mol. Catal. B: Enzym. 37*, 47−55.
(24) Tamegai, H., Nango, E., Kuwahara, M., Yamamoto, H., Ota, Y., Kuriki, H., Eguchi, T., and Kakinuma, K. (2002) Identification of L-glutamine: 2-deoxy-*scyllo*-inososе aminotransferase required for the biosynthesis of butirosin in *Bacillus circulans*. *J. Antibiot. 55*, 707−714.
(25) Burgie, E. S., and Holden, H. M. (2007) Molecular architecture of DesI: a key enzyme in the biosynthesis of desosamine. *Biochemistry 46*, 8999−9006.
(26) Cook, P. D., Carney, A. E., and Holden, H. M. (2008) Accommodation of GDP-linked sugars in the active site of GDP-perosamine synthase. *Biochemistry 47*, 10685−10693.
(27) Breazeale, S. D., Ribeiro, A. A., and Raetz, C. R. (2003) Origin of lipid A species modified with 4-amino-4-deoxy-L-arabinose in polymyxin-resistant mutants of *Escherichia coli*. An aminotransferase (ArnB) that generates UDP-4-deoxyl-L-arabinose. *J. Biol. Chem. 278*, 24731−24739.
(28) Akabayashi, A., and Kato, T. (1989) One-step and two-step fluorometric assay methods for general aminotransferases using glutamate dehydrogenase. *Anal. Biochem. 182*, 129−135.
(29) Minagawa, K., Zhang, Y., Ito, T., Bai, L., Deng, Z., and Mahmud, T. (2007) ValC, a new type of $C_7$-Cyclitol kinase involved in the biosynthesis of the antifungal agent validamycin A. *ChemBioChem 8*, 632−641.
(30) Wu, H., Qu, S., Lu, C., Zheng, H., Zhou, X., Bai, L., and Deng, Z. (2012) Genomic and transcriptomic insights into the thermo-regulated biosynthesis of validamycin in *Streptomyces hygroscopicus* 5008. *BMC Genomics 13*, 337−350.



DOI: 10.1021/acssynbio.5b00138
*ACS Synth. Biol.* 2016, 5, 15−20