

1 Supporting Information

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3 **Table S1.** Predicted and measured ions of of BtrR's product *in vitro*.

Ion	Predicted m/z	Measured m/z	Error (ppm)
[M+H] ⁺	176.0923	176.0936	7.38
[M+Na] ⁺	198.0743	198.0755	6.06
[2M+H] ⁺	351.1768	351.1778	2.85
[2M+Na] ⁺	373.1508	373.1606	4.82

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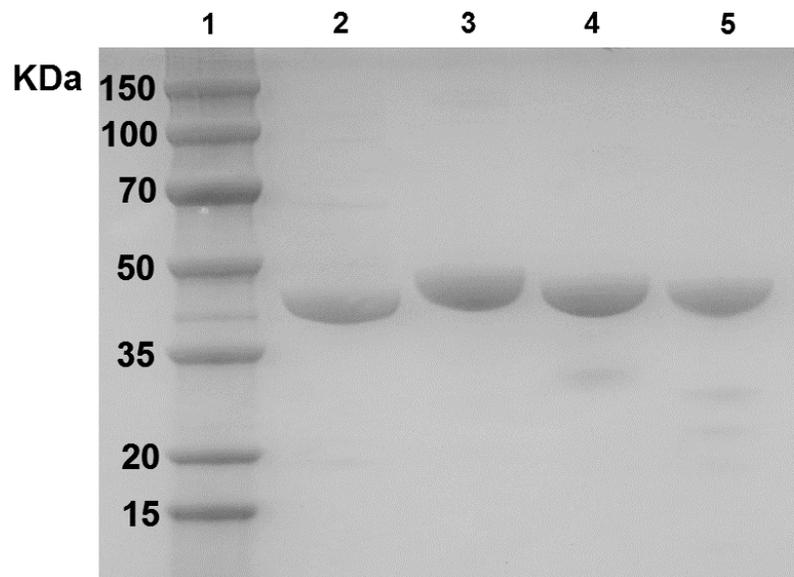
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6 **Table S2.** Predicted and measured ions of the derivated β -valienamine standard
7 and the fermented product by OPA.

[M+Na] ⁺	Predicted m/z	Measured m/z	Error (ppm)
Standard	374.1038	374.1041	0.8
Fermented product	374.1038	374.1038	0

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2 **Figure S1.** SDS-PAGE analysis of heterologous overexpressed SATs purified by
3 Ni-NAT columns. (Lane1) Molecular weight marker. (Lane 2-5) Purified Per, BtrR,
4 DesI, or ArnB.

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1 **a**

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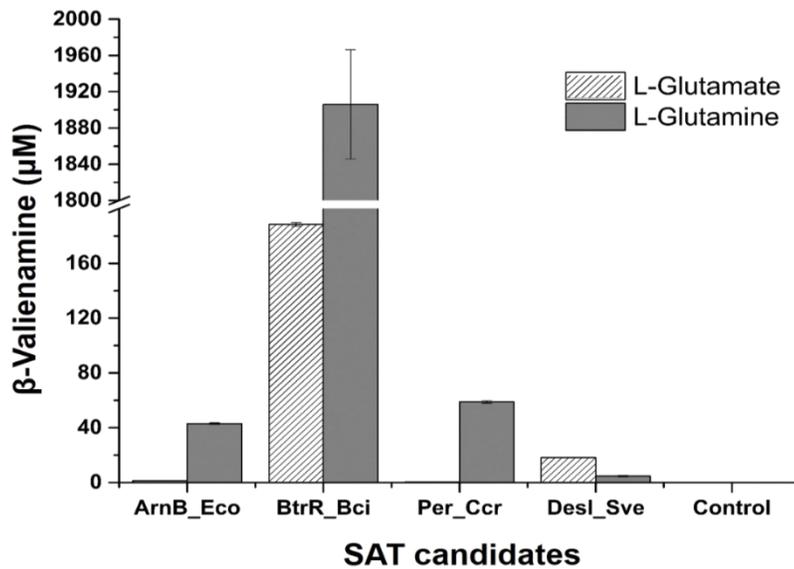
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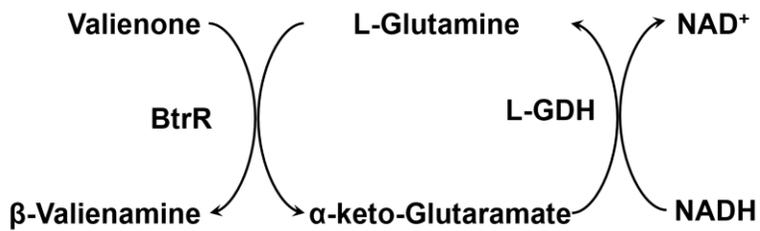
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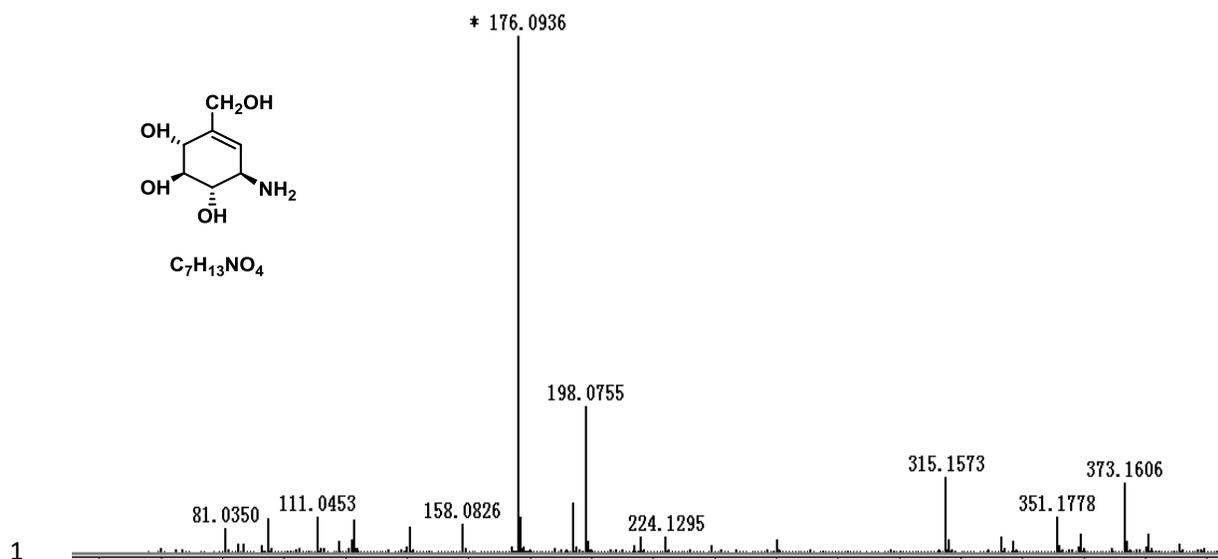
9 **b**



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11 **Figure S2.** (a) Determination of amino donors for selected SATs through
12 enzymatic assays; (b) Schematic diagram of L-GDH coupled enzymatic assay
13 system used in the kinetic analysis for BtrR.

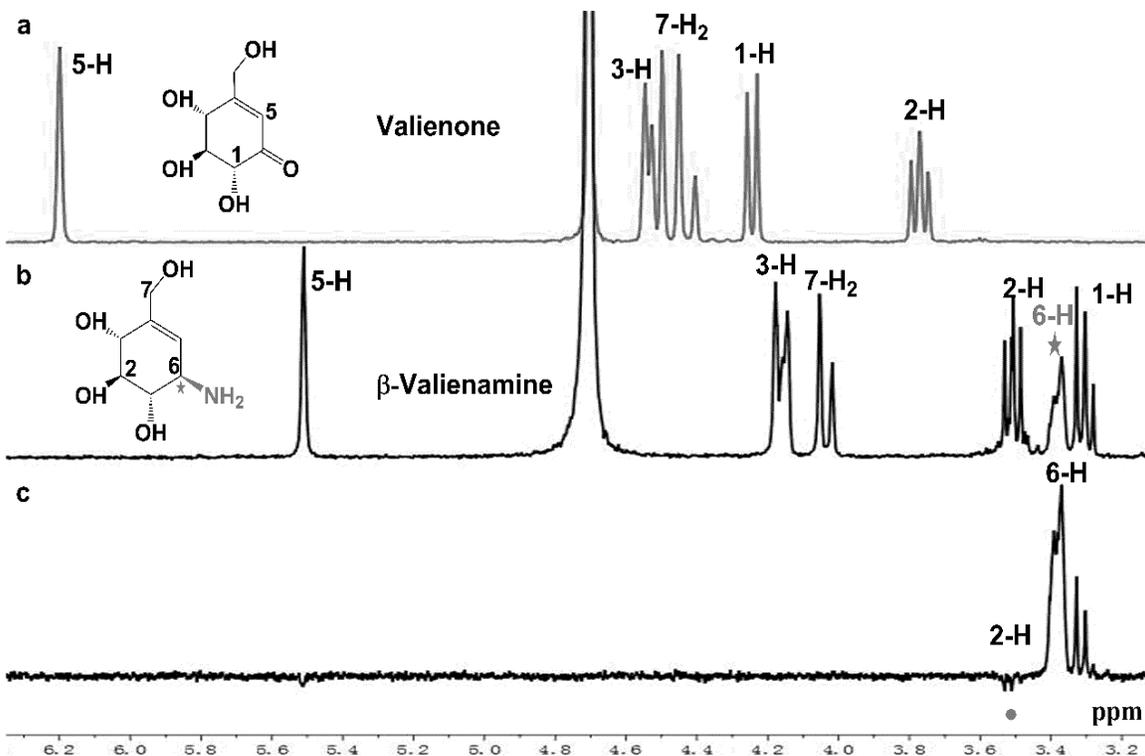
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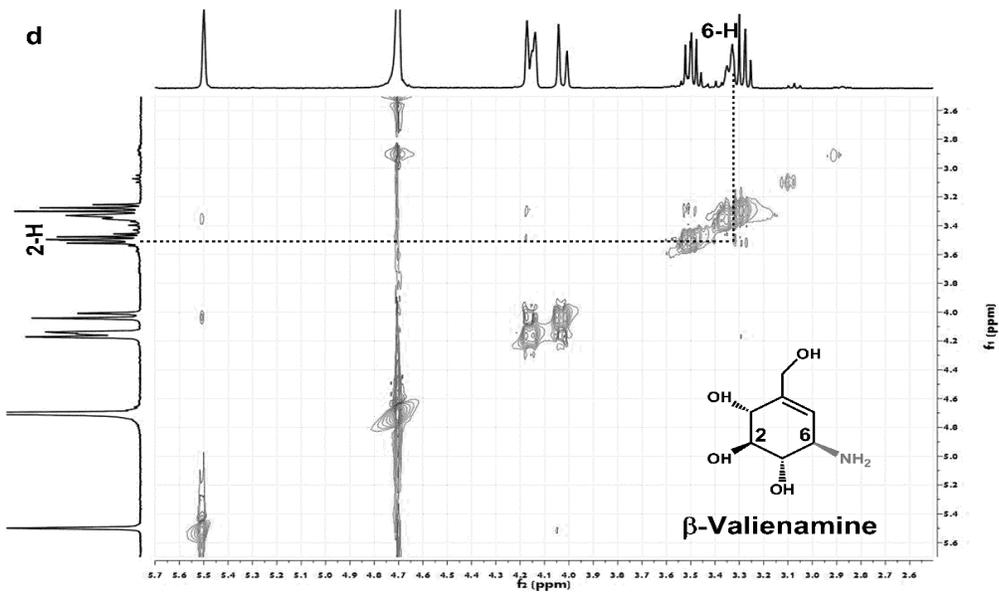
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2 **Figure S3.** HR-MS analysis of the reaction product catalyzed by BtrR.

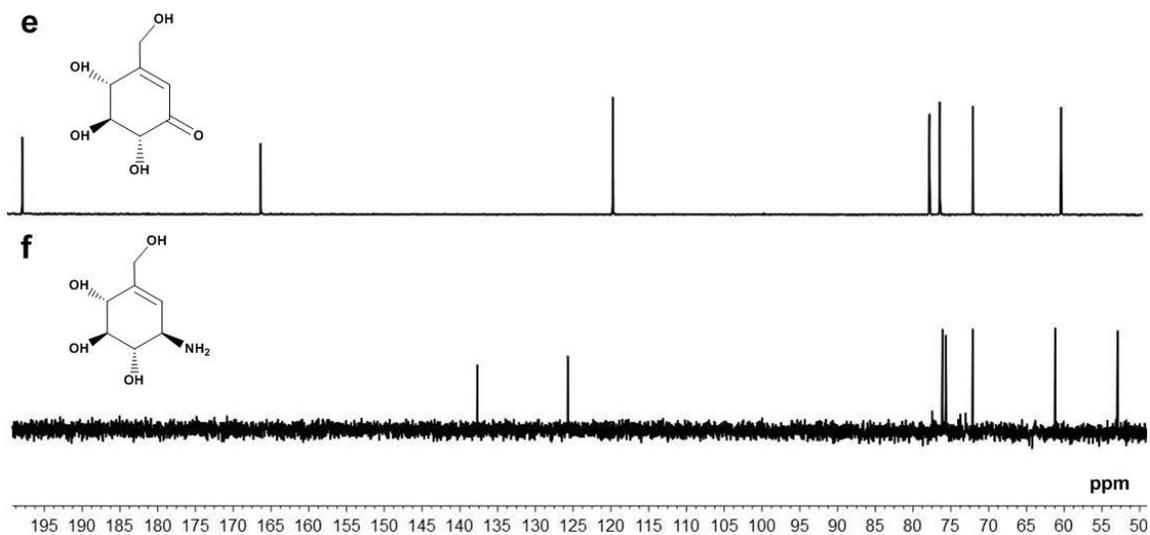
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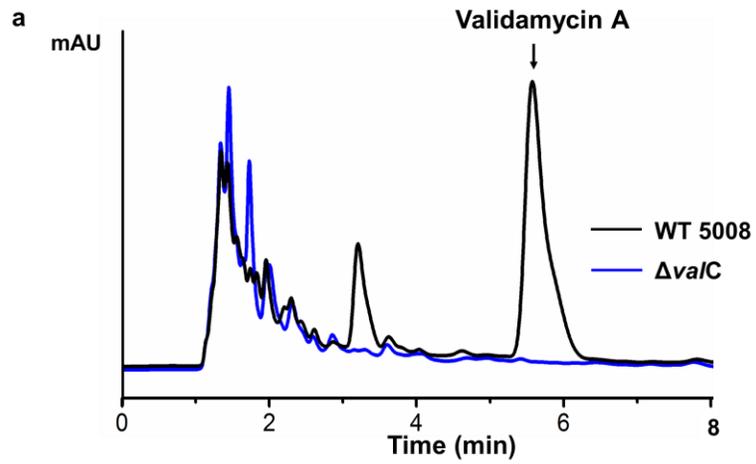
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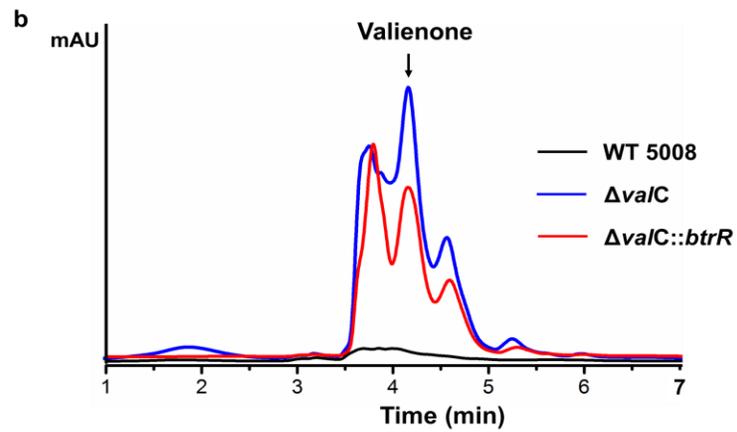
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2 **Figure S4.** Structure verification of BtrR's product by NMR analysis. (a) ^1H NMR
 3 spectrum of valienone; (b-d) ^1H NMR, NOE, and NOESY spectra of BtrR's
 4 product; (e) ^{13}C NMR spectrum of valienone; (f) ^{13}C NMR spectrum of BtrR's
 5 product.

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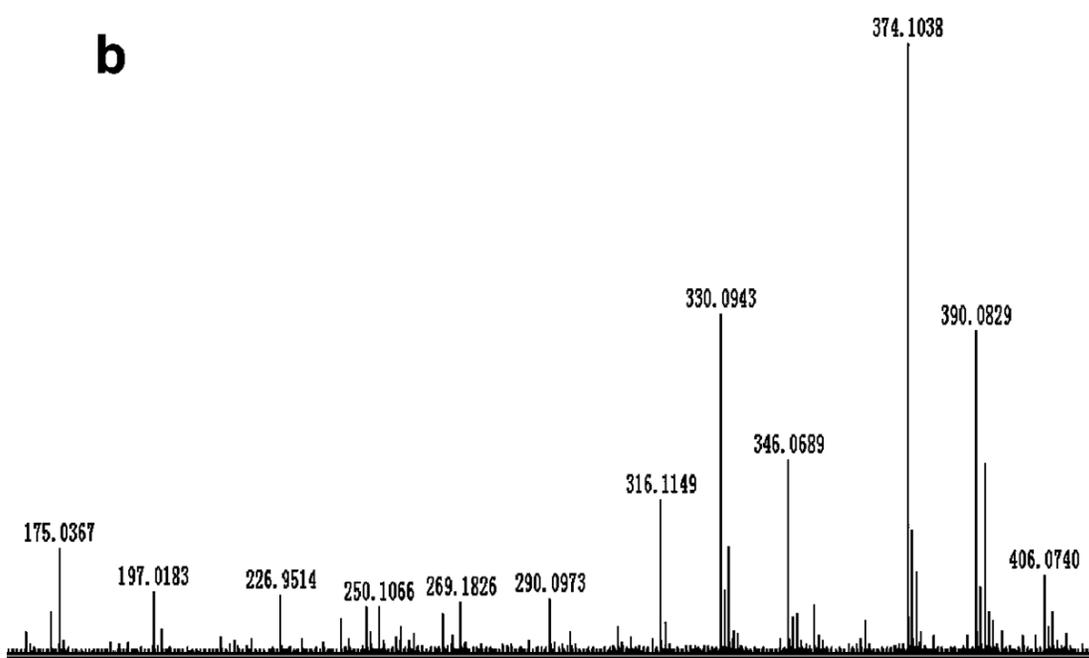
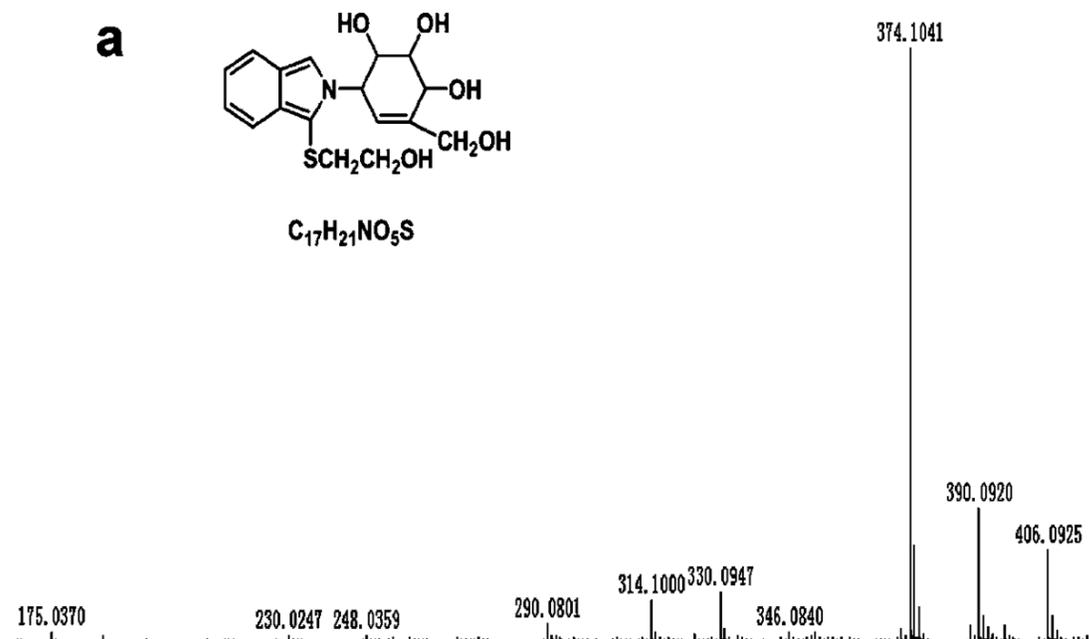


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3 **Figure S5.** (a) HPLC analysis of validamycin A in wild-type 5008 and $\Delta vaIC$
 4 mutant; (b) Detection of valienone by HPLC with pre-column derivatization using
 5 DNPH.



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2 **Figure S6.** (a) HR-MS spectrum of β -valienamine standard and (b) the fermented
 3 product in $\Delta valC::btrR$ mutant with pre-column derivatization using OPA.