Supporting Information for:

A Role for Old Yellow Enzyme in Ergot Alkaloid Biosynthesis

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Supporting Information

Table of Contents

S1. General materials and methods

General recombinant DNA cloning procedures were performed using pGEM-T vector (Promega) propagated in *E. coli* Top10 (Invitrogen). Protein expression was conducted in *E. coli* BL-21(DE3) (Invitrogen). PCR amplification utilized Platinum Taq DNA Polymerase (Invitrogen). Recombinant DNA plasmids were prepared using Qiaprep Spin Miniprep and Qiaquick Gel Extraction kits (Qiagen). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs. Primers for cloning were synthesized by Integrated DNA Technologies and DNA sequencing was conducted by the MIT Biopolymers Laboratory (Cambridge, MA).

LC-MS analysis was conducted using an Acquity Ultra Performance BEH C18 column with a 1.7 mm particle size, 2.1 x 100 mm dimension, with an acetonitrile/0.1% trifluoroacetic acid in water mobile phase. The column elution was coupled to MS analysis carried out using a Micromass LCT Premier TOF Mass Spectrometer with an ESI source (Waters). Exact mass data were acquired on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). ^{1}H-NMR was taken using a 500MHz Varian Inova NMR Spectrometer. A Varian Cary 50 Bio Scanning Spectrometer was used to acquire UV-Vis spectra. HPLC analysis was conducted on a Beckman Coulter System Gold 125 HPLC with a

model 168 photodiode array detector using a Hibar RT 250-4LiChrosorb C18 column (Merck). Preparative HPLC was performed with a Grace Vydac C18 column.

S2. Cloning, overexpression, and purification of EasA

The *A. fumigatus easA* gene was PCR amplified using *A. fumigatus* cDNA. To clone the desired gene, total RNA was extracted from *A. fumigatus* mycelia tissue using the Trizol RNA extraction procedure (Invitrogen).[1] Using Creator SMART MMLV reverse transcriptase (Clontech), cDNA was constructed from the extracted total RNA. Primers were designed based on the nucleotide sequence of *easA* from the NCBI database (XM_751040). A pair of oligonucleotide primers were used to amplify the *easA* gene: forward primer 5'-TTAGATCTGGCGAATTCGGC**CATATG**CGAGAAG AACCGTCCTCTGCTCAGC-3' (with NdeI restriction site in bold) and reverse primer 5'-GA**CTCGAG**TTAAAGCTTGCCGCTAGCGACGGGGAAATTATGCAATGC CATA-3' (XhoI restriction site in bold). (Additional restriction sites were incorporated into the primers to allow for cloning of *easA* as either an N-His_6 or C-His_6 construct.) The PCR amplified *easA* gene was inserted into pGEM-T vector (Promega) for propagation and sequencing. Subsequently, the *easA* sequence was excised from pGEM-T by restriction digest and ligated into the NdeI/XhoI site of pET-28a(+) (Novagen) expression vector as an N-His_6 construct.

Expression was carried out in LB media with Kanamycin (50 μg/mL). *E. coli* BL-21(DE3) cells were grown to an OD_{600} of 0.7 prior to induction with IPTG (5 μM) and grown for 60 hours at 15°C prior to harvesting. EasA enzyme was purified by Ni-NTA agarose (Qiagen). The yield of active (holo) EasA was estimated to be 2.5 mg per liter of culture as measured by UV absorbance of flavin at 446 nm. Fractions containing pure EasA, as demonstrated by SDS-PAGE, were collected and exchanged with dialysis buffer (50 mM K_2HPO_4, 100 mM NaCl, 10% (v/v) glycerol, pH = 7.0) (Figure S1).

S3. Characterization of the bound EasA flavin

The flavin cofactor that co-purifies with EasA was identified as non-covalently bound flavin mononucleotide (FMN). The bound FMN was readily released from EasA by denaturation of the enzyme solution with 0.2% sodium dodecyl sulfate (SDS).[2] Precipitated protein was removed by centrifugation at 17,000 x g, and a UV-visible spectra from 250 nm to 550 nm was taken of the free flavin in solution and compared to the spectra of FMN standard (Figure S2). The released flavin from EasA exhibits the same absorbance maxima at 373 nm and 446 nm as the FMN standard.

The identity of the released flavin was further verified by co-migration with FMN standard by HPLC (Figure S3). The samples were chromatographed using a Hibar 250-4 LiChrosorb RP-Select B 5μm column (Merck) with an acetonitrile/0.1% trifluoroacetic acid in water mobile phase (12:88 to 95:5 from 0-15 min, 95:5 to 12:88 from 15−23 min, at a constant flow rate of 1 mL/min) with the UV detection range set at 280nm.

The extinction coefficient of the purified FMN bound EasA was calculated by comparing the absorbance of the purified EasA versus the absorbance of the flavin released after denaturation at 446 nm.[2] Using the known extinction coefficient of 12,200 $M^{-1}cm^{-1}$ for free FMN at 446 nm, the extinction coefficient for the EasA bound FMN was determined to be 11,500 $M^{-1}cm^{-1}$. Total purified EasA from each expression (holo plus apo-enzyme) was determined by Bradford Assay.

S4. Isolation and HPLC purification of chanoclavine-I aldehyde 3

Chanoclavine-I aldehyde **3** was extracted from 14 day old surface cultures of *A. fumigatus easA* knockout strain (Coyle, C. M.; Cheng, J. Z.; O'Connor, S. E.; Panaccione, D. G. *submitted.*) with a mixture of methanol-water (4-1). This *easA* knockout strain accumulated higher amounts of chanoclavine-I aldehyde than the wild type *A. fumigatus* strain. Chanoclavine-I aldehyde **3** was identified in the extracts by LC-MS analysis (gradient of acetonitrile/0.1% trifluoroacetic acid in water mobile phase, 10:90 to 90:10 from 0-9 min, 90:10 to 10:90 from 9-10 min, at a constant flow rate of 0.5 mL/min). Based on the polarity of **3** exhibited on the LC-MS gradient, a preparative HPLC method was optimized to isolate chanoclavine-I aldehyde **3**. Chanoclavine-I aldehyde **3** was isolated by a Grace Vydac preparative C18 column with an isocratic elution (15:85 acetonitirile/0.1% trifluoroacetic acid in water, constant flow rate at 4.0 mL/min, 228 nm detection). Under these conditions, pure chanoclavine-I aldehyde **3** eluted at 25-26min (Figure S4). Subsequent LC-MS analysis of the preparative HPLC purified compound demonstrated that chanoclavine-I aldehyde was not contaminated with any other compounds from the extract (Figure S5).

Purified chanoclavine-I aldehyde **3** was analyzed by ^{1}H-NMR (500MHz Varian Inova). The spectra matched the ^{1}H-NMR data previously reported by Floss and coworkers (Figure S6).[3] ^{1}H-NMR (500 MHz, CD_3OD) 2.03 (s, 3H), 2.77 (s, 3H), 3.45-3.49 (m, 2H), 3.84 (d, J = 4.5Hz, 1H), 4.58 (dd, J = 4.5, 10Hz, 1H) , 6.52 (d, J = 10Hz, 1H), 6.87 (d, J = 7.0Hz, 1H), 7.16 (d, J = 8.0Hz, 1H), 7.19 (d, J = 8.5Hz ,1H), 7.34 (d, J = 8.0 Hz, 1H), 9.40 (s, 1H).

S5. Conversion of chanoclavine-I aldehyde 3 to 5 by EasA

To test for substrate turnover, purified EasA (1.5 μM) was incubated with chanoclavine-I aldehyde (1mM) and NADPH (1.5 mM) in 100 mM K_2HPO_4 (pH = 7.0)[4] at 25^{o}C for 1 hour. The product identity was confirmed by low and high resolution mass spectrometry (Table S1), as well as by comparison to an authentic standard and ^{1}H NMR spectroscopy of the enzymatically produced product (see sections **6** and **7** below).

S6. Catalytic hydrogenation of agroclavine 1 to yield festuclavine 2

Festuclavine **2** was prepared from agroclavine **1** following a previously published protocol. Agroclavine (1 mg, 4.2 μmol) in 2 mL of methanol was placed over 1 mg of Pt-black at 1 atm of hydrogen at 25^{o}C.[5, 6] After 2 hours, the catalyst was filtered and the filtrate was evaporated in vacuo. The identity of the resulting festuclavine product was verified by LC-MS and ^{1}H-NMR. (Figure 3, Figure S6). NMR analysis of the hydrogenated product indicated that only one major diastereomer was present. Previous reports have demonstrated that the major diastereomer resulting from hydrogenation of agroclavine **1** under these conditions is the festuclavine **2** isomer.[5] ^{1}H-NMR (500 MHz, CD_3OD) 0.92 (m,1H), 1.17 (d, J=6.5Hz, 3H), 1.38 (m, 1H), 2.21 (m, 1H), 2.83-2.95 (m, 3H), 3.09 (s, 3H), 3.27 (m,1H), 3.60 (dd, J=2.0, 12.5Hz, 1H), 3.70 (dd, J=3.5, 14.5Hz, 1H), 6.94 (d, J=7.0Hz, 1H), 7.04 (d, J=1.5Hz, 1H), 7.12 (t, J=7.0Hz, 1H), 7.21 (d, J=8.0Hz, 1H)

Agroclavine **1** starting material (Sigma): ^{1}H-NMR (500 MHz, CD_3OD) 1.80 (s, 3H), 2.49 (s, 3H), 2.73 (t, J=13.7Hz, 1H), 2.98 (d, J=16.3Hz, 1H), 3.26 (d, J=16.2Hz, 1H), 3.36 (dd, J=4.10, 14.2Hz, 2H), 3.68 (d, J=8.50Hz, 1H), 6.23 (s, 1H), 6.89-6.92(m, 2H), 7.05 (t, J=7.10Hz, 1H), 7.13 (d, 1.6Hz, 1H)

S7. Large scale enzymatic preparation of 5 and synthetic reduction for characterization of festuclavine 2

A total of 7 x 1 mL reactions of chanoclavine-I aldehyde (1 mM), NADPH (5 mM), and EasA (6 μM) were allowed to react for 10 minutes prior to quenching with $NaCNBH_3$ (5 mM). The reaction solutions were combined and extracted three times with ethyl acetate 1:1 (v/v), and were centrifuged at 17,000 x g for 15 minutes to pellet protein precipitates. The combined organic fractions were filtered and evaporated to dryness. The concentrated reduced enzymatic product festuclavine was purified by preparative HPLC. The ^{1}H-NMR of the reduced enzymatic product correlated with the festuclavine standard (Figure S6).

S8. Steady state kinetic analysis of EasA

EasA (2.5 nM final concentration), and varying concentrations of chanocalvine aldehyde **3** and NADPH were incubated in 100 mM K_2HPO_4 at pH = 7.0 in 50 μL volumes at 25^{o}C. The k_{cat} and K_m values for chanoclavine-I aldehyde **3** and NADPH were obtained by LC-MS, monitoring the initial rate of chanoclavine-I aldehyde **3** decrease over time. Chanoclavine-I aldehyde **3** was quantified by peak area response for mass $[M+H]^+$ 255. A standard curve of chanoclavine-I aldehyde plotted against peak response area is shown in Figure S10. Aliquots of the reaction were quenched in 0.1% formic acid (containing 500 nM yohimbine as an internal standard) over a 3 minute time course at 30 second intervals. Reactions contained varying substrate ranges of chanoclavine-I aldehyde **3** (0.5- 10 μM) and NADPH (10-500 μM). Linear regression analysis was used to determine the initial rate of each assay. Experiments were repeated in triplicate and data were fit using software SigmaPlot 9.0. Mass spectrometry using electrospray ionization has been previously used as an assay method for kinetic characterization of a variety of enzymes.[7] The stability of chanoclavine-I aldehyde **3** under assay conditions was determined by setting up a reaction containing boiled (inactive) EasA (2.5 nM), chanoclavine-I aldehyde (5 μM), and NADPH (500 μM). Aliquots of the reaction were quenched in 0.1% formic acid (plus internal standard) over time and analyzed by LC-MS. The concentration of chanoclavine-I aldehyde **3** was maintained over a 60 minute time course, suggesting that the disappearance of the substrate during the 3 minute enzyme assay is due solely to enzyme turnover (Figure S11).

S9. Supporting Table and Figures S1-S10

Table S1. Exact Mass of Compounds Determined by High Resolution MS

Compound	Observed Mass	Theoretical Mass	Molecular Formula
Chanoclavine-I Aldehyde **3** $[M+H]^+$	*m/z* 255.1500	*m/z* 255.1497	$C_{16}H_{19}N_2O$
Cyclized Iminium Intermediate **5** $[M]^+$	*m/z* 239.1551	*m/z* 239.1548	$C_{16}H_{19}N_2$
Festuclavine **2** $[M+H]^+$	*m/z* 241.1704	*m/z* 241.1705	$C_{16}H_{21}N_2$

Figure S1. SDS-PAGE of EasA (*A. fumigatus*) (42kDa).
(1) Invitrogen BenchMark Pre-Stained Protein Ladder. Ni-NTA column washes (2) 10 mM imidazole elution, (3) 25 mM imidazole elution, (4) 50 mM imidazole elution, (5) 100 mM imidazole elution 1, (6) 150 mM imidazole elution (used in enzyme assays).

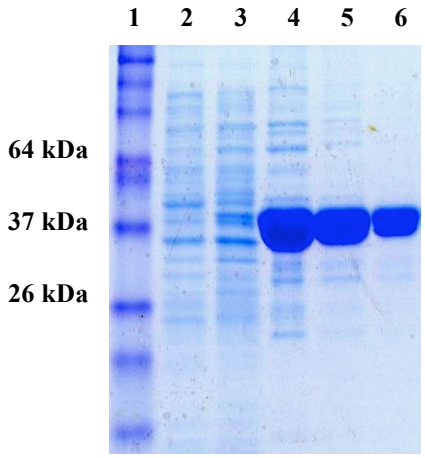


Figure S2. Characteristic flavin absorbance spectrum (300 nm to 550 nm). Native EasA (blue), supernatant from EasA denatured with 0.2% SDS (pink), FMN Standard (green).

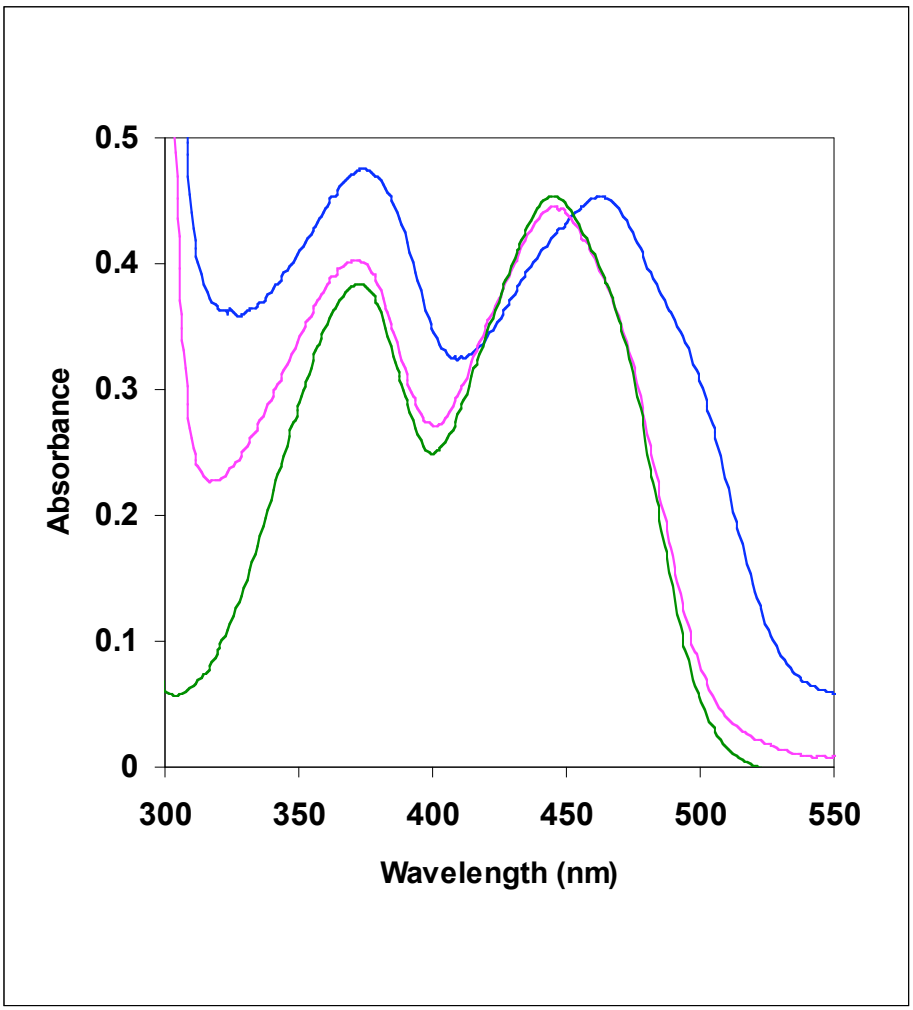
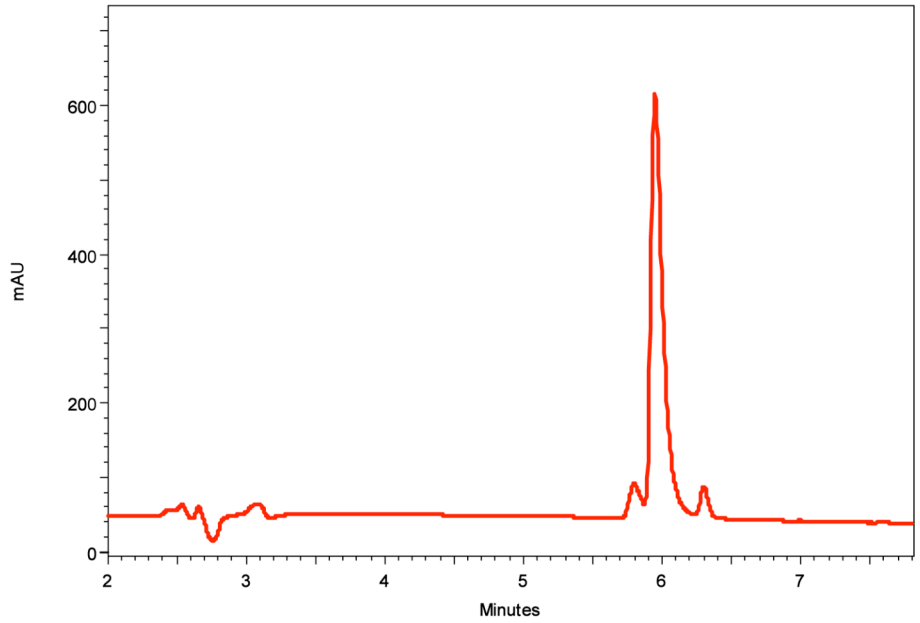


Figure S3. Released flavin from EasA identified as FMN by HPLC.
a. HPLC chromatogram (280 nm) of supernatant from denatured EasA with FMN standard. FMN elutes at 5.95 min.
b. UV-Vis spectrum of peak maxima at 5.95 min characteristic of FMN (see Figure S2).

a.



b.

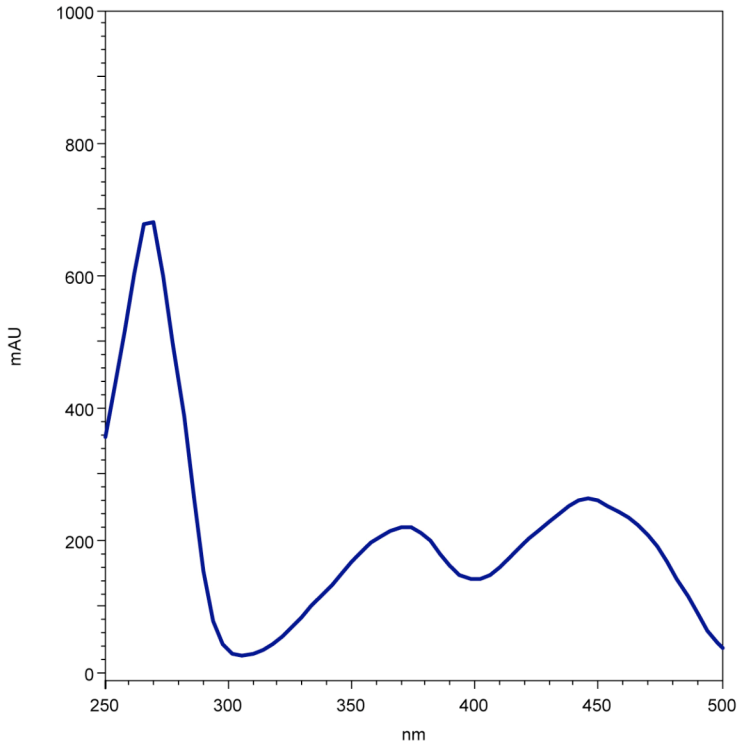
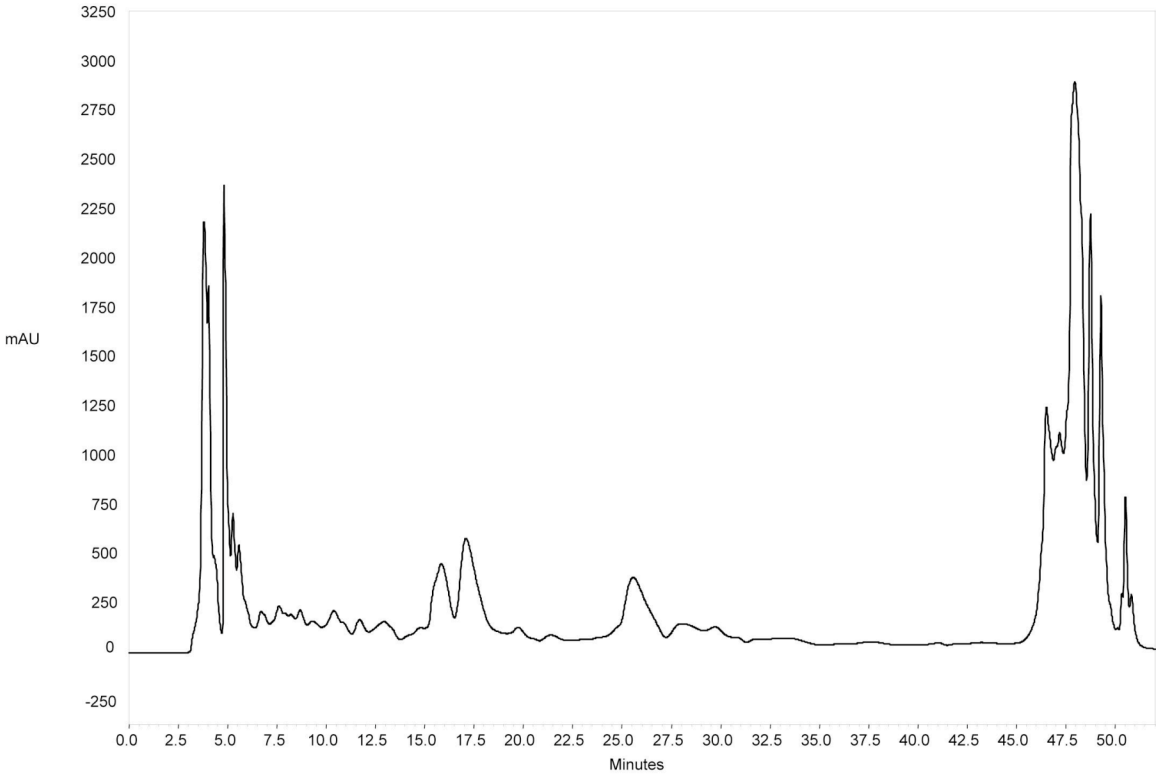


Figure S4.

a. Preparative HPLC chromatogram of crude *A. fumigatus* organic extract. Chanoclavine-I aldehyde **3** elutes at 25 minutes (225 nm detection).



b. UV-Vis spectrum of chanoclavine-I aldehyde **3** (17 μM) in methanol.

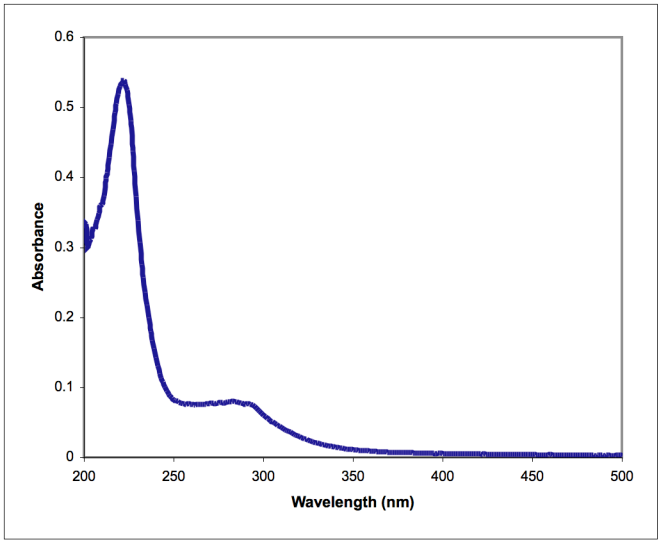


Figure S5. Total ion counts of LC-MS chromatograms of crude *A. fumigatus* extract (top, green) and purified chanoclavine-I aldehyde 3 (bottom, red).

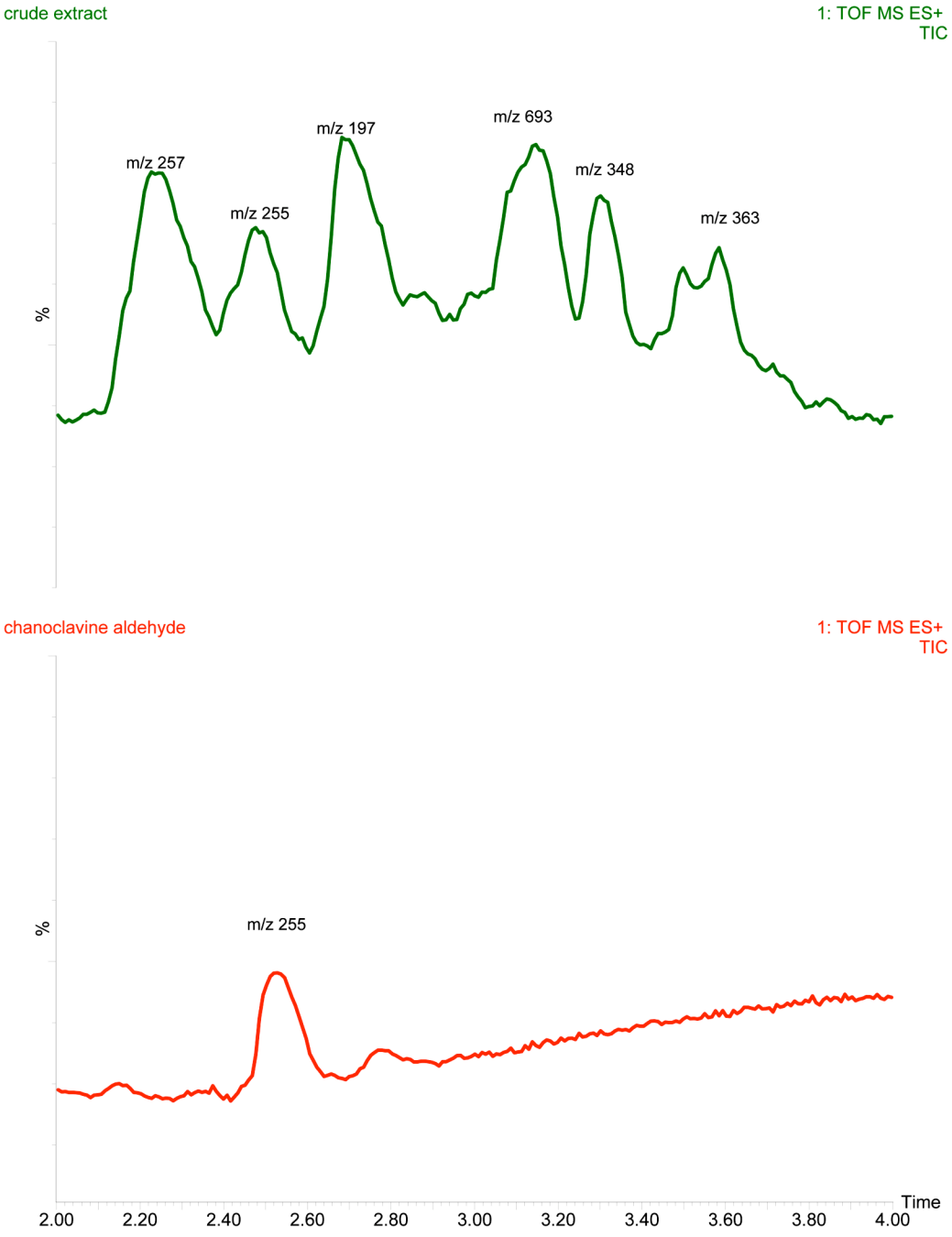
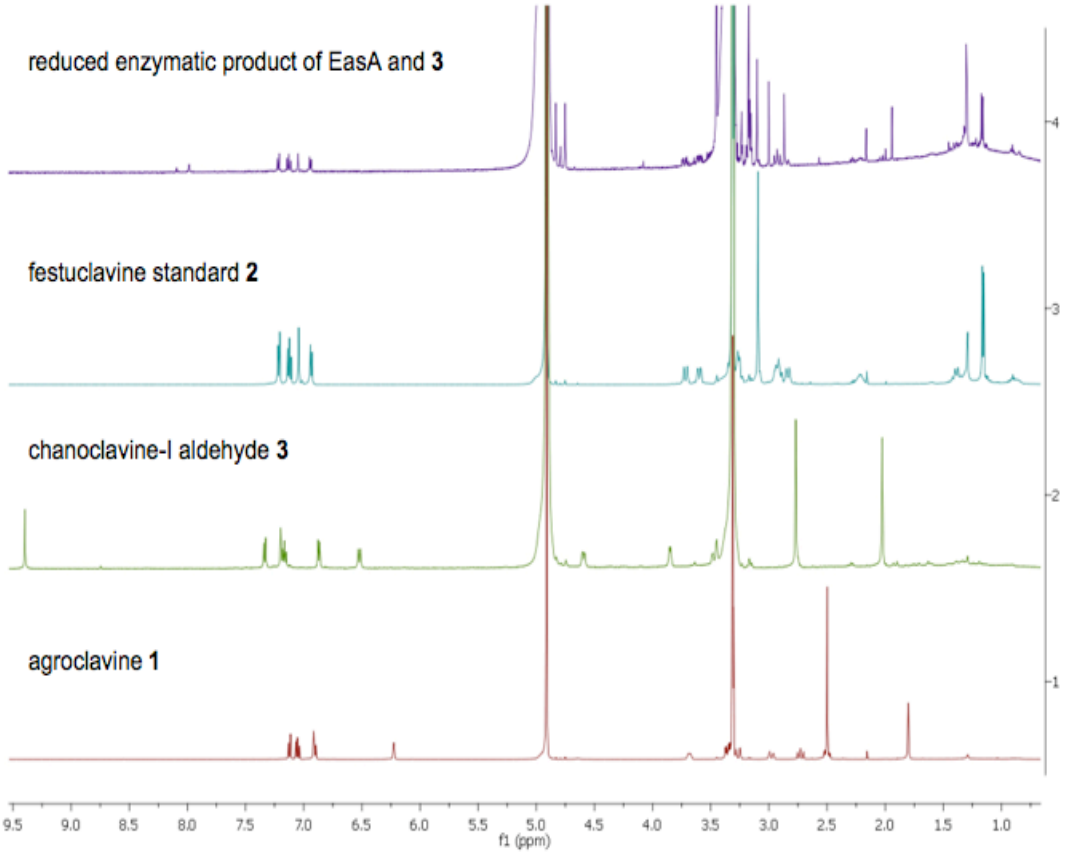
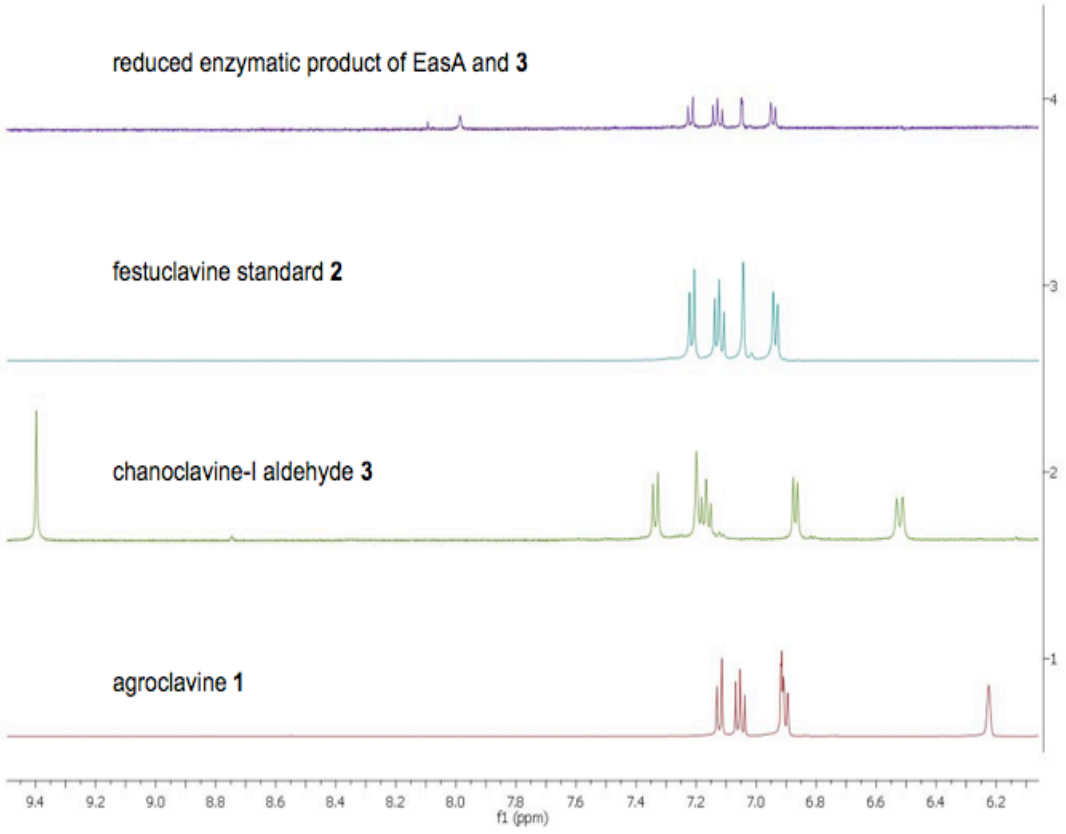


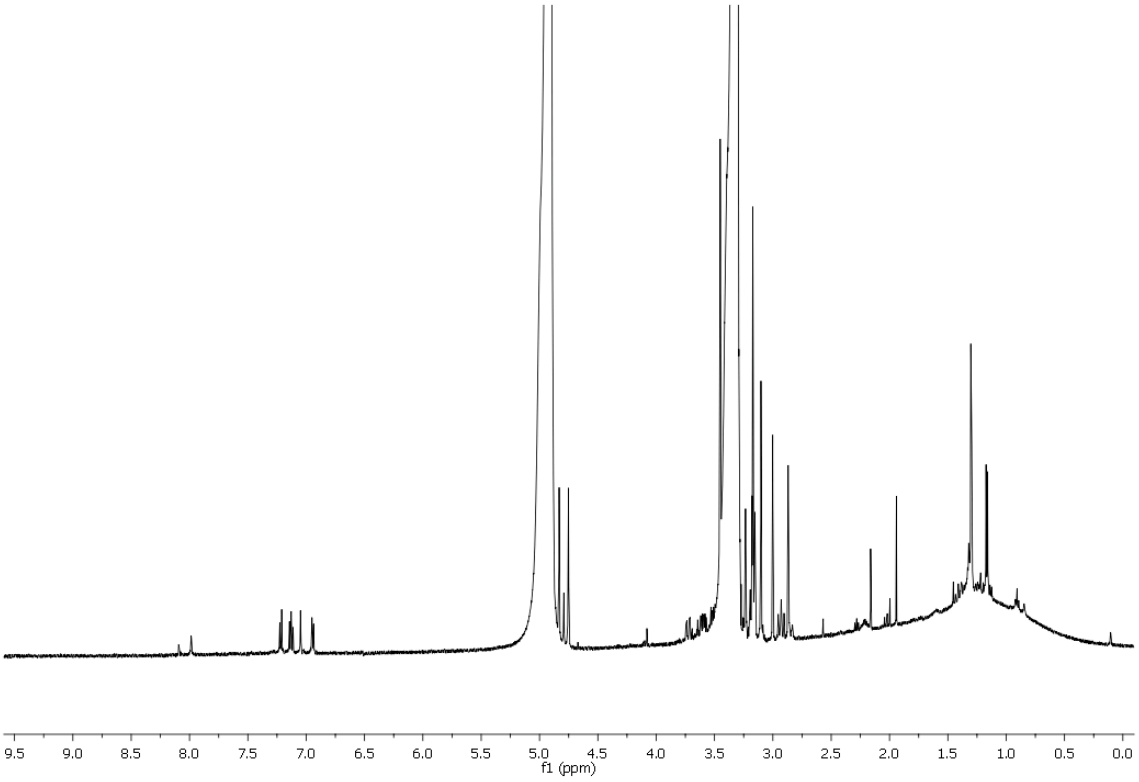
Figure S6. ^{1}H-NMR spectra of compounds used in this study.
a. Full spectra



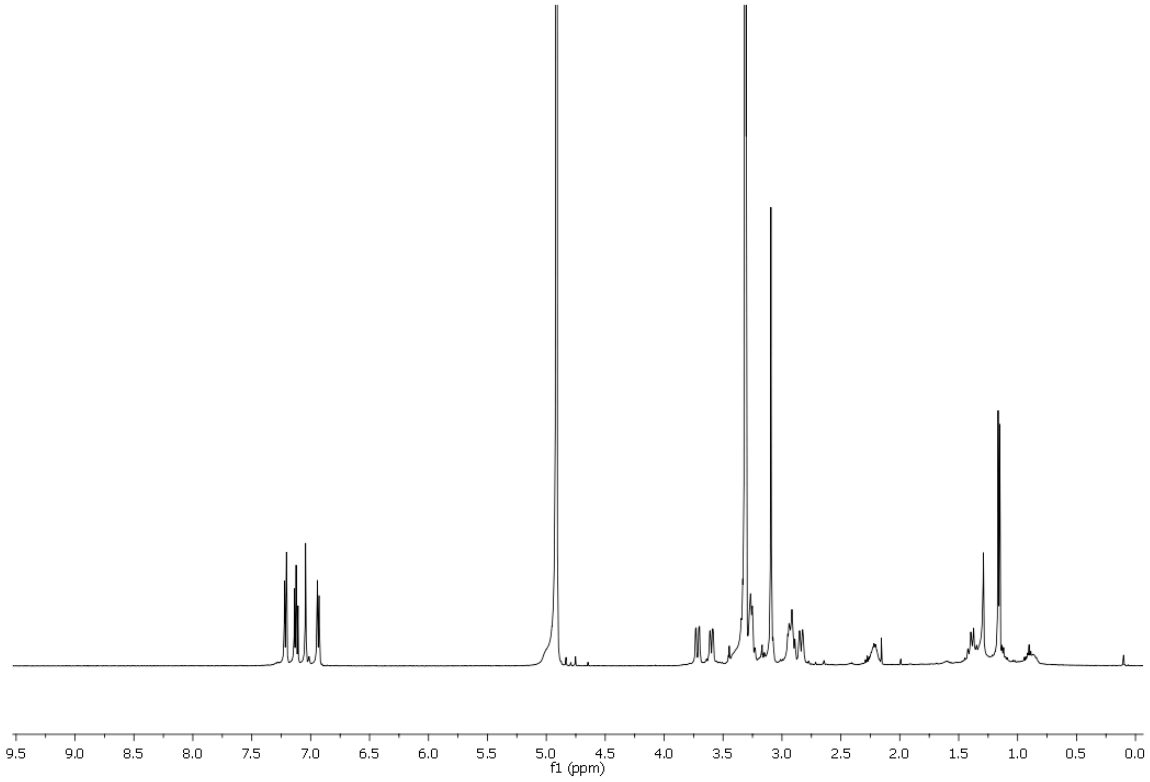
b. Aromatic region of ^{1}H NMR spectra.



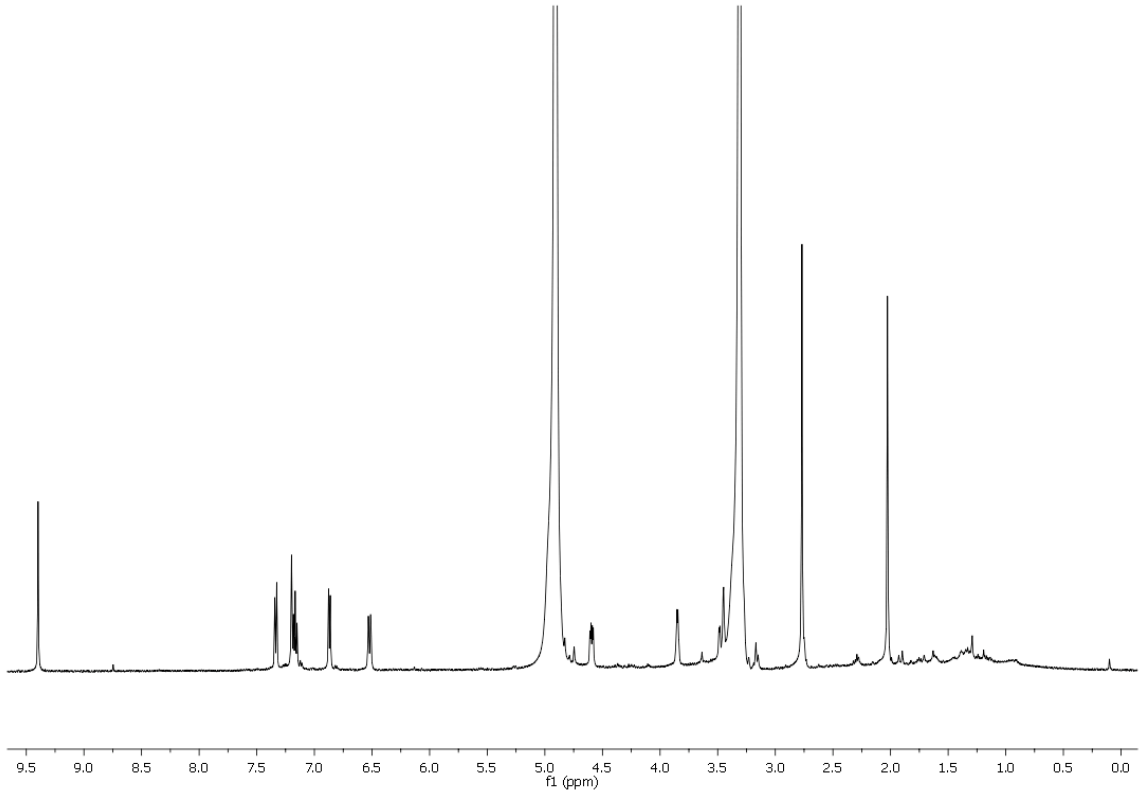
c. Enzymatic product of EasA and **3** reduced with $NaCNBH_3$.



d. Festuclavine standard **2**.



e. Chanoclavine-I aldehyde **3**.



f. Agroclavine **1**.

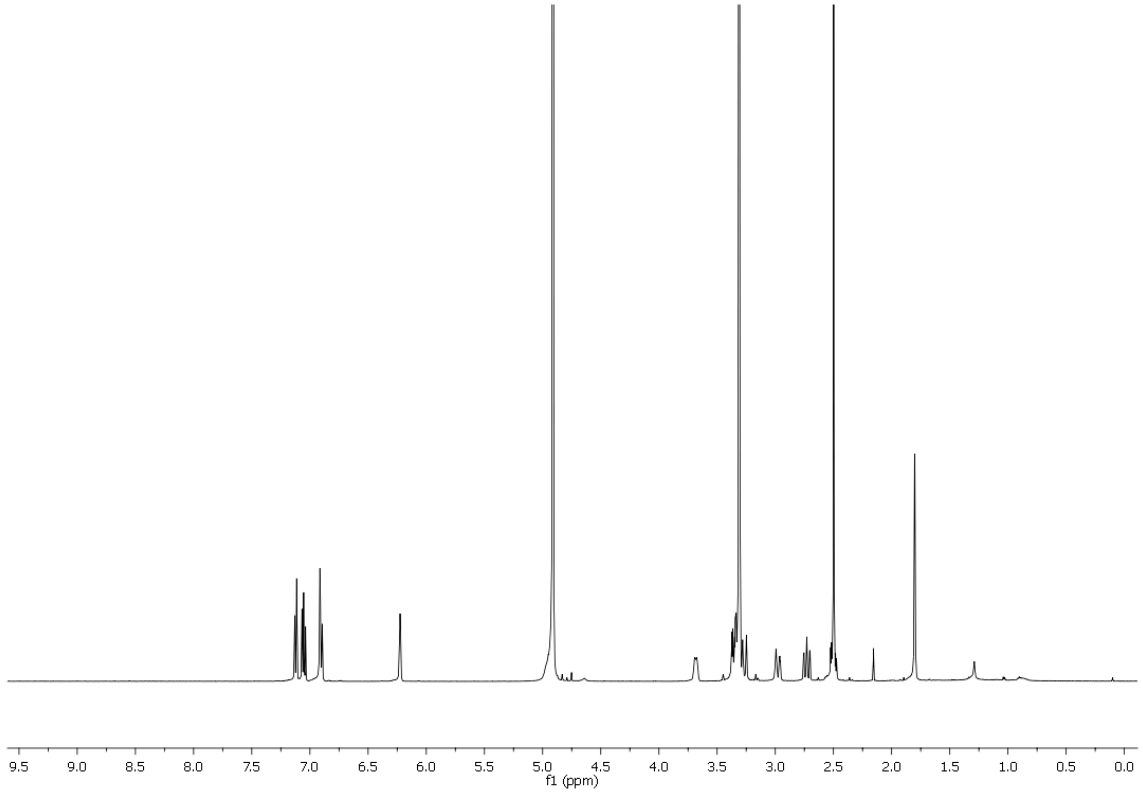


Figure S7. Possible mechanisms for racemization of the dehydrochanoclavine aldehyde product **4** and **5**.

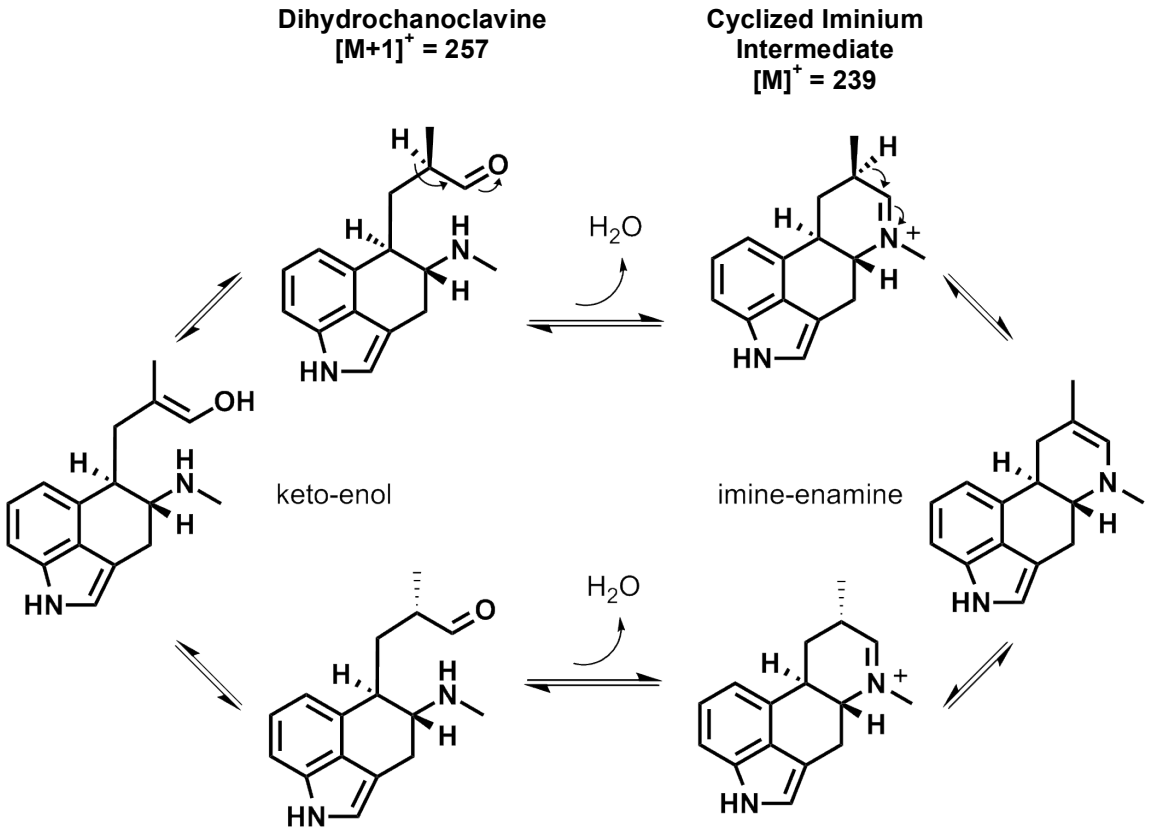


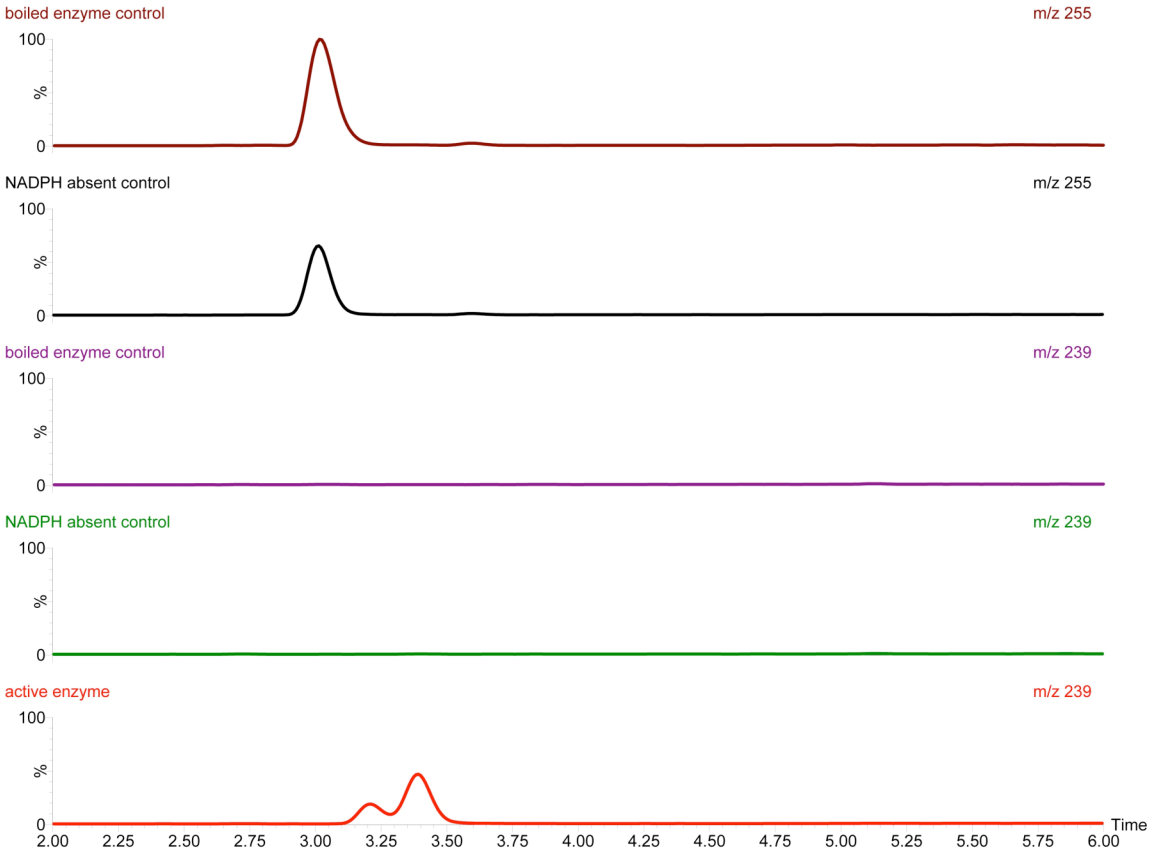
Figure S8. Protein sequence alignments with EasA and Old Yellow Enzyme homologs. Amino acid residues implicated in the OYE mechanism are highlighted in red.

```
OYE1_Q02899                     MSFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRALHPGNIPN 50
OYE2_AAA83386                   MPFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRAQHPGNIPN 50
OYE3_P41816                     MPFVKGFEPISLRDTNLFEPIKIGNTQLAHRAVMPPLTRMRATHPGNIPN 50
NADPHoxidase_kluyv_AAA98815     MSFMN-FEPKPLADTDIFKPIKIGNTELKHRVVMPALTRMRALHPGNVPN 49
easA_afumigatus_XP_756133       -------MREEPSSAQLFKPLKVGRCHLQHRMIMAPTTRFRADGQG-VPL 42
                                        .:::*:*:*:*. .* ** ::.. **:**   * :*

OYE1_Q02899                     RDWAVEYYTQRAQRPGTMIITEGAFISPQAGGYDNAPGVWSEEQMVEWTK 100
OYE2_AAA83386                   RDWAVEYYAQRAQRPGTLIITEGTFPSPQSGGYDNAPGIWSEEQIKEWTK 100
OYE3_P41816                     KEWAAVYYGQRAQRPGTMIITEGTFISPQAGGYDNAPGIWSDEQVAEWKN 100
NADPHoxidase_kluyv_AAA98815     PDWAVEYYRQRSQYPGTMIITEGAFPSAQSGGYDNAPGVWSEEQLAQWRK 99
easA_afumigatus_XP_756133       P-FVQEYYGQRASVPGTLLITEATDITPKAMGYKHVPGIWSEPQREAWRE 91
                                  :.  ** **:. ***::***.:  :.:: **.:.**:**: *   * :

OYE1_Q02899                     IFNAIHEKKSFVWVQLWVLGWAAFPDNLARDGLRYDSASDNVFMDAEQEA 150
OYE2_AAA83386                   IFKAIHENKSFAWVQLWVLGWAAFPDTLARDGLRYDSASDNVYMNAEQEE 150
OYE3_P41816                     IFLAIHDCQSFAWVQLWSLGWASFPDVLARDGLRYDCASDRVYMNATLQE 150
NADPHoxidase_kluyv_AAA98815     IFKAIHDNKSFVWVQLWVLGRQAFADNLARDGLRYDSASDEVYMGEDEKE 149
easA_afumigatus_XP_756133       IVSRVHSKKCFIFCQLWATGRAADPDVLA--DMKDLISSSAVPVEEK--- 136
                                *.  :*. :.* : ***  *  : .* **  .::   :*. * :

OYE1_Q02899                     KAKKANNPQHSLTKDEIKQYIKEYVQAAKNSIAAGADGVEIHSANGYLLN 200
OYE2_AAA83386                   KAKKANNPQHSITKDEIKQYVKEYVQAAKNSIAAGADGVEIHSANGYLLN 200
OYE3_P41816                     KAKDANNLEHSLTKDDIKQYIKDYIHAAKNSIAAGADGVEIHSANGYLLN 200
NADPHoxidase_kluyv_AAA98815     RAIRSNNPQHGITKDEIKQYIRDYVDAAKKCIDAGADGVEIHSANGYLLN 199
easA_afumigatus_XP_756133       -----GPLPRALTEDEIQQCIADFAQAARNAINAGFDGVEIHGANGYLID 181
                                     .   :.:*:*:*:* : :: .**::.* ** ******.*****::

OYE1_Q02899                     QFLDPHSNTRTDEYGGSIENRARFTLEVVDALVEAIGHEKVGLRLSPYGV 250
OYE2_AAA83386                   QFLDPHSNNRTDEYGGSIENRARFTLEVVDAVVDAIGPEKVGLRLSPYGV 250
OYE3_P41816                     QFLDPHSNKRTDEYGGTIENRARFTLEVVDALIETIGPERVGLRLSPYGT 250
NADPHoxidase_kluyv_AAA98815     QFLDPISNKRTDEYGGSIENRARFVLEVVDAVVDAVGAERTSIRFSPYGV 249
easA_afumigatus_XP_756133       QFTQKSCNHRQDRWGGSIENRARFAVEVTRAVIEAVGADRVGVKLSPYSQ 231
                                ** :  .* * *.:**:*******.:**. *::::::* ::..:::***.

OYE1_Q02899                     FNSMSGGAETGIVAQYAYVAGELEKRAKAGKRLAFVHLVEPRVTNPFLTE 300
OYE2_AAA83386                   FNSMSGGAETGIVAQYAYVLGELERRAKAGKRLAFVHLVEPRVTNPFLTE 300
OYE3_P41816                     FNSMSGGAEPGIIAQYSYVLGELEKRAKAGKRLAFVHLVEPRVTDPSLVE 300
NADPHoxidase_kluyv_AAA98815     FGTMSGVSDPVLVAQFAYVLAELEKRAKAGKRLAYVDLVEPRVTSPFQPE 299
easA_afumigatus_XP_756133       YLGMG--TMDELVPQFEYLIAQMRR-----LDVAYLHLANSRWLD----- 269
                                :  *.  :   ::.*: *: .::.:       :*::.*.:.*  .

OYE1_Q02899                     GEGEYEGGSNDFVYSIWKG--PVIRAGNF-ALHPEVVREEVKDK---RTL 344
OYE2_AAA83386                   GEGEYNGGSNKFAYSIWKG--PIIRAGNF-ALHPEVVREEVKDP---RTL 344
OYE3_P41816                     GEGEYSEGTNDFAYSIWKG--PIIRAGNY-ALHPEVVREQVKDP---RTL 344
NADPHoxidase_kluyv_AAA98815     FEGWYKGGTNEFVYSVWKG--NVLRVGNY-ALDPDAAITDSKNP---NTL 343
easA_afumigatus_XP_756133       EEKPHPDPNHEVFVRVWGQSSPILLAGGYDAASAEKVTEQMAAATYTNVA 319
                                 *  :   .:..   :*     :: .*.: *  .: .  :       ..

OYE1_Q02899                     IGYGRFFISNPDLVDRLEKGLPLNKYDRDTFY-QMSAHGYIDYPTYEEAL 393
OYE2_AAA83386                   IGYGRFFISNPDLVDRLEKGLPLNKYDRDTFY-KMSAEGYIDYPTYEEAL 393
OYE3_P41816                     IGYGRFFISNPDLVYRLEEGLPLNKYDRSTFY-TMSAEGYTDYPTYEEAV 393
NADPHoxidase_kluyv_AAA98815     IGYGRAFIANPDLVERLEKGLPLNQYDRPSFY-KMSAEGYIDYPTYEEAV 392
easA_afumigatus_XP_756133       IAFGRYFISTPDLPFRVMAGIQLQKYDRASFYSTLSREGYLDYPFSAEYM 369
                                *.:** **:.***  *:  *: *::*** :**  :* .** ***   * :

OYE1_Q02899                     KLGWDKK 400
OYE2_AAA83386                   KLGWDKN 400
OYE3_P41816                     DLGWNKN 400
NADPHoxidase_kluyv_AAA98815     AKGYKK- 398
easA_afumigatus_XP_756133       ALHNFPV 376
```

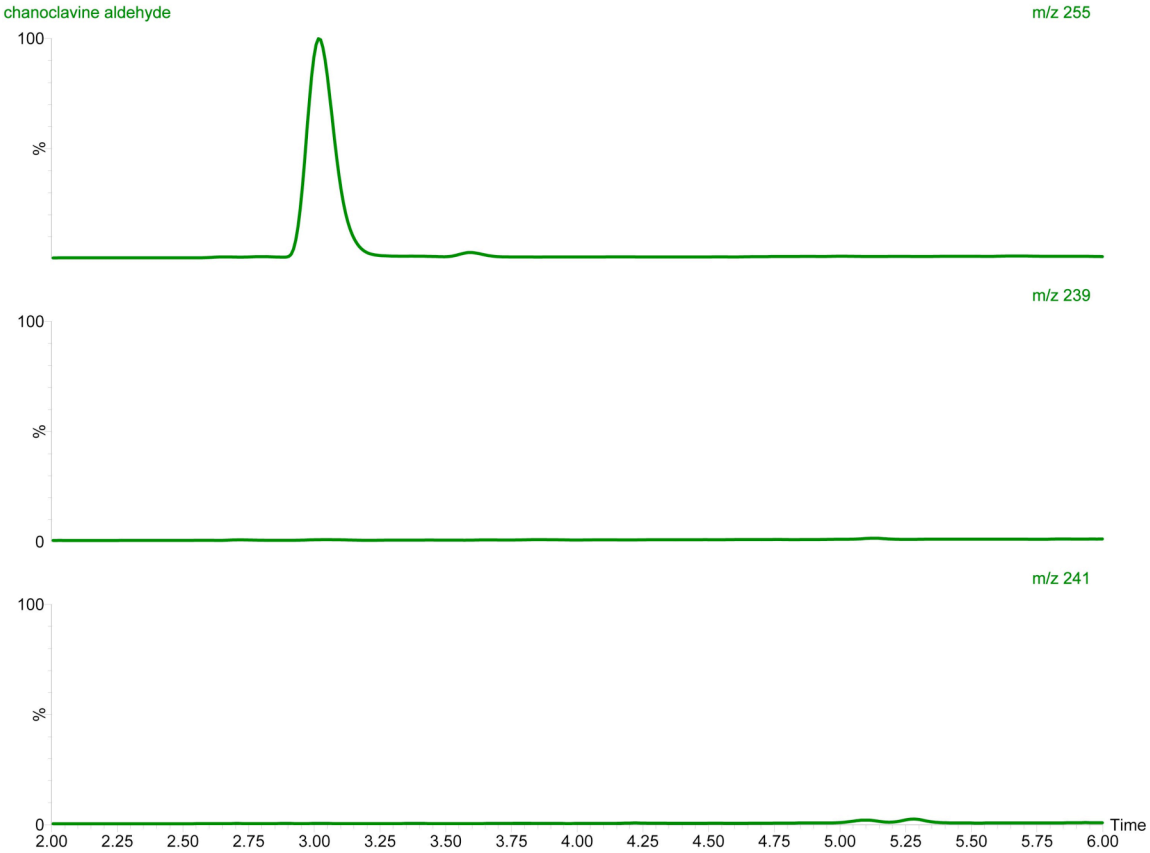
Protein Sequence Alignments with *A. fumigatus EasA*

OYE1 ID = 40% Positives = 58%
OYE2 ID = 41% Positives = 58%
OYE3 ID = 40% Positives = 57%
Kluyveromyces ID = 39% Positives = 55%

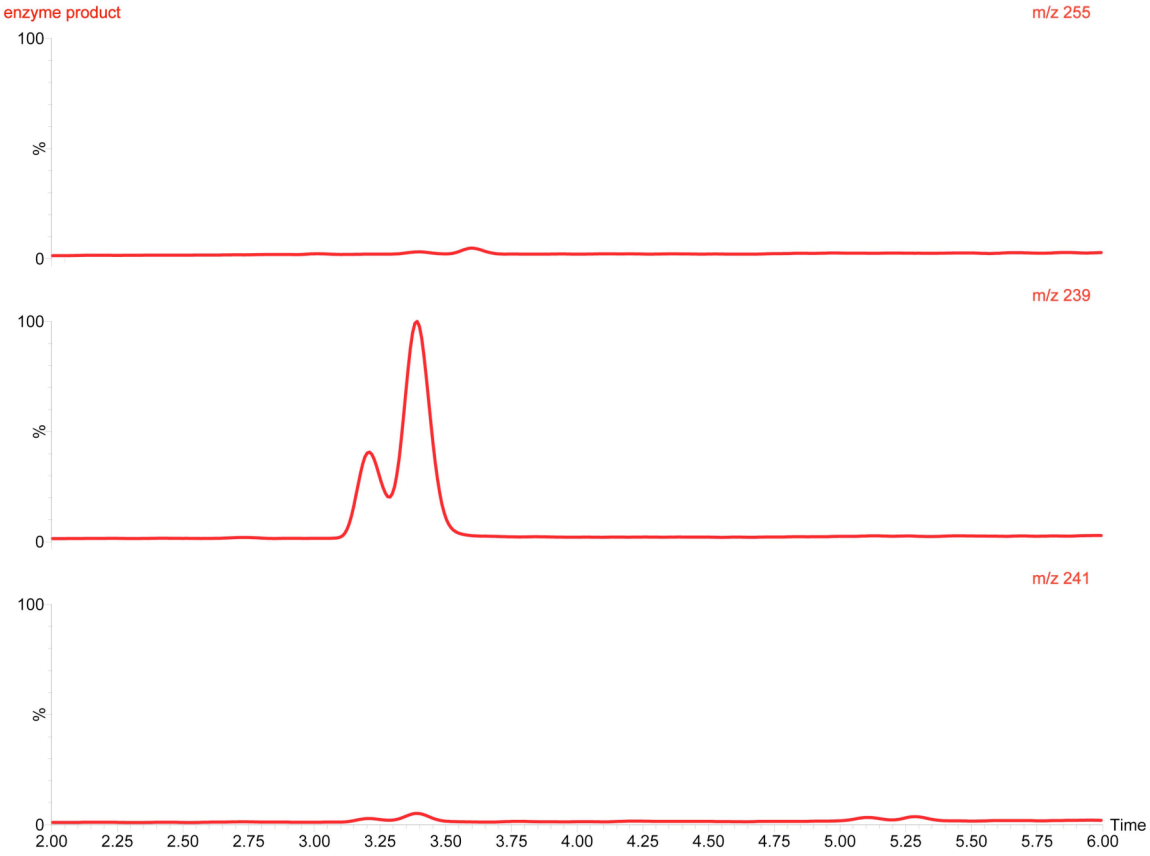
Figure S9. LC-MS chromatograms of control reactions.



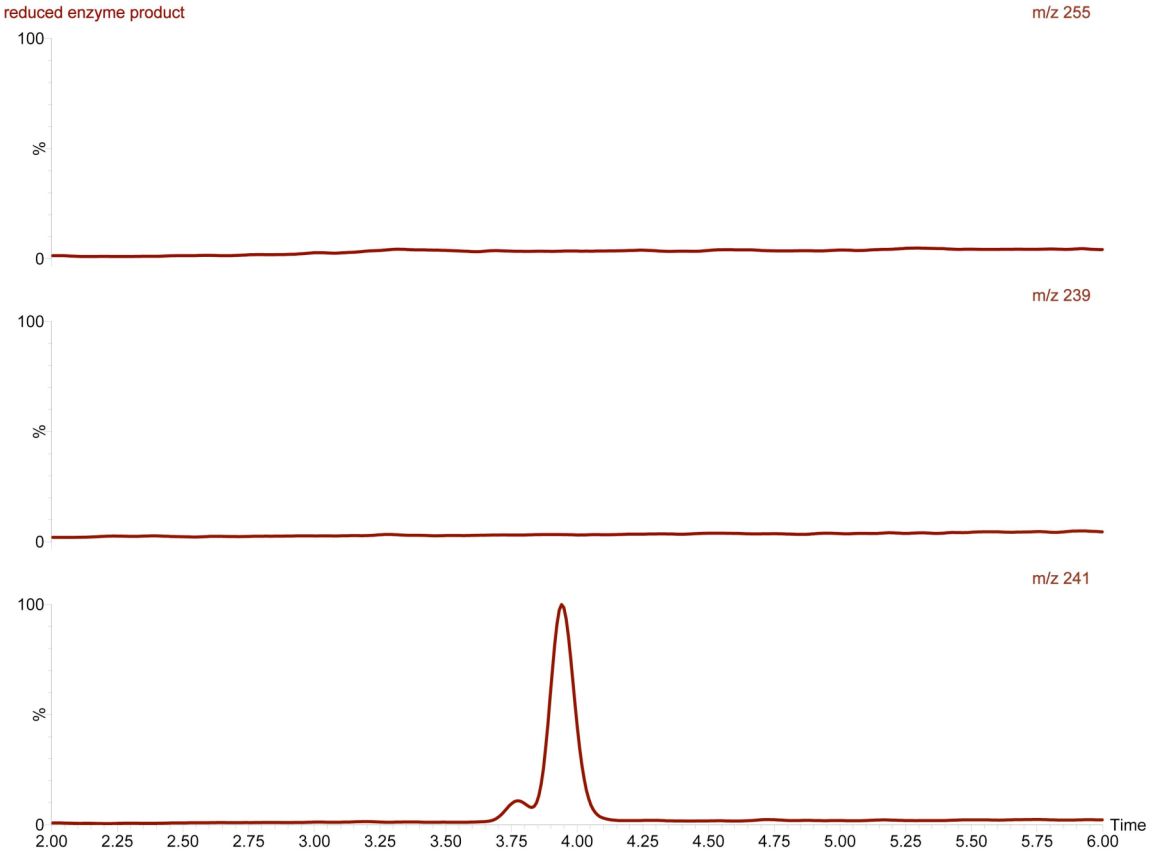
a. Selected ion monitoring shows that starting material chanoclavine-I aldehyde **3** (*m/z* 255) remains in enzyme assays with boiled, denatured EasA enzyme (brown trace) or in assays lacking NADPH (black trace). The iminium ion enzymatic product **5** (*m/z* 239) is not observed with boiled enzyme (purple trace) or when NADPH is lacking (green trace). Product **5** is observed with active EasA and NADPH (red trace).



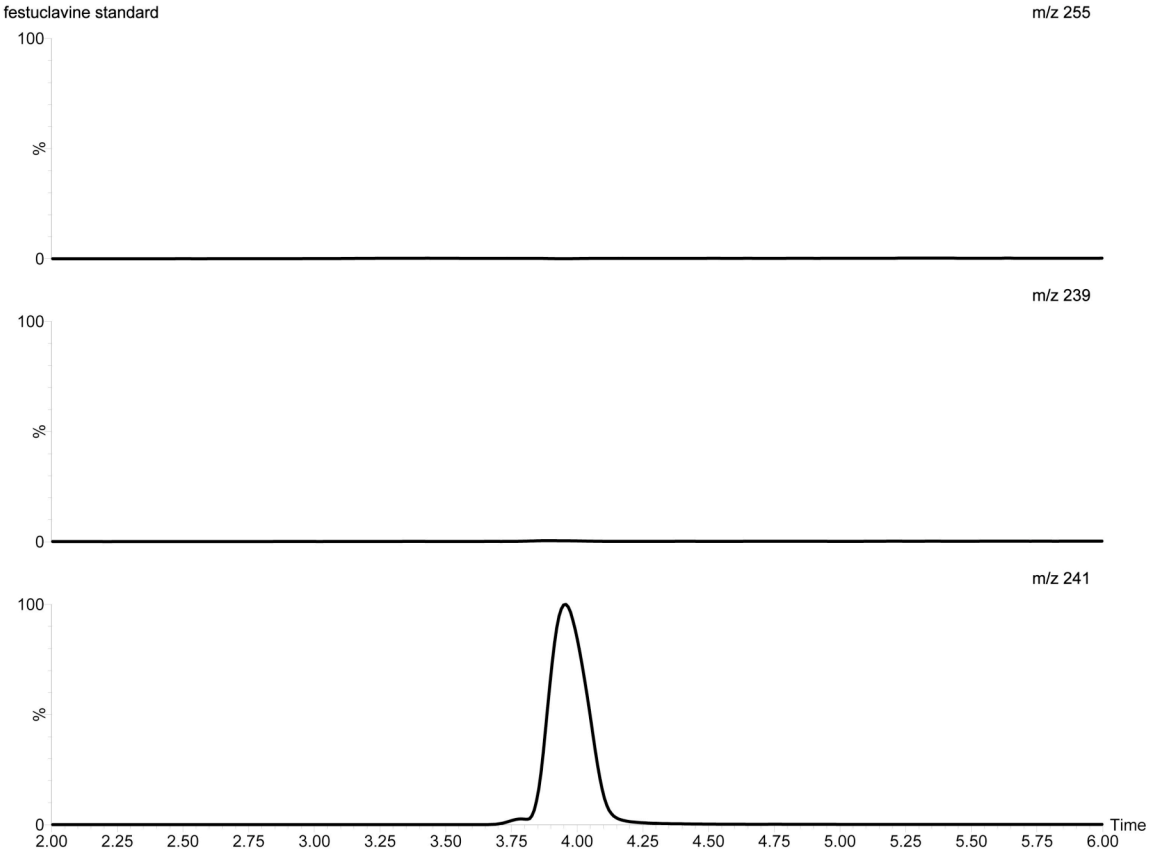
b. Selected ion monitoring of substrate chanoclavine-I aldehyde **3** (*m/z* 255) at *m/z* 239 and 241 demonstrates that there are no contaminants with *m/z* 239 and 241 in the purified substrate.



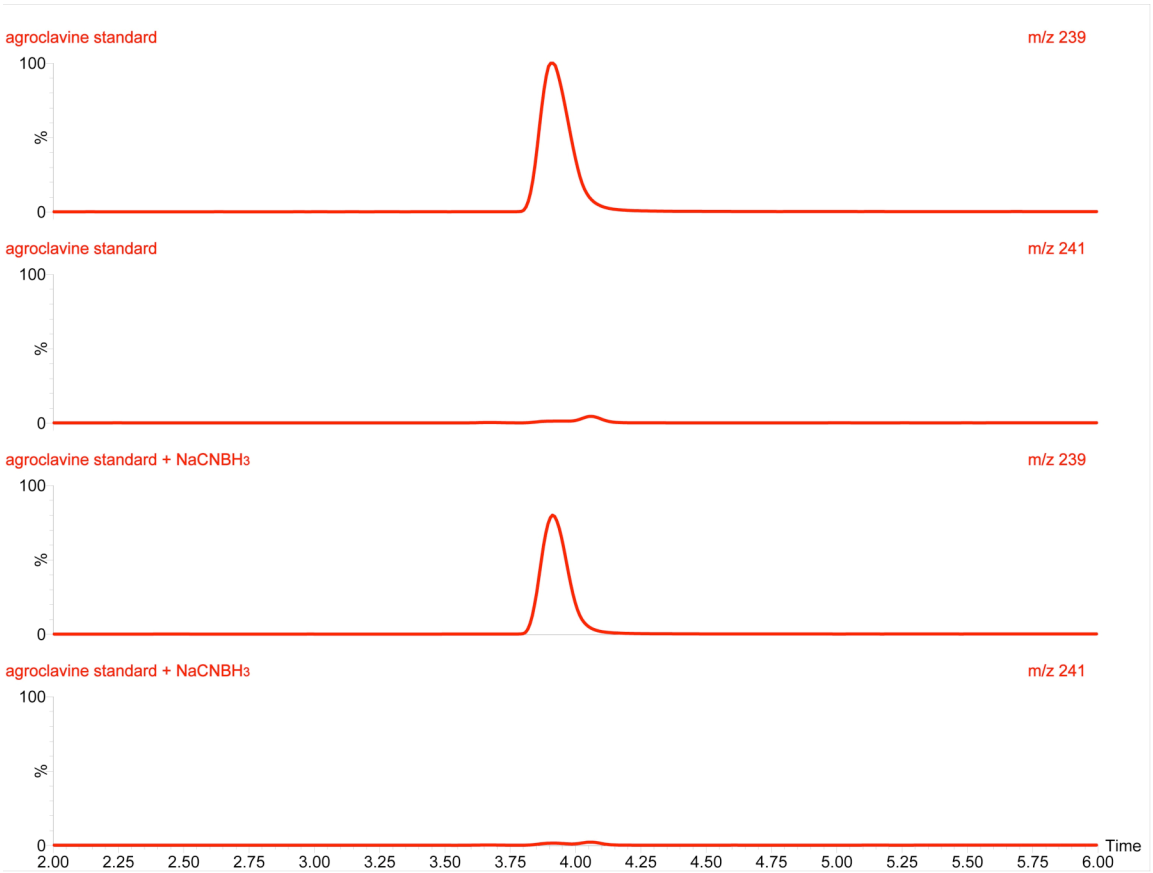
c. Active EasA enzyme with NADPH and chanoclavine-I aldehyde **3** monitored at *m/z* 255 to show complete consumption of starting material **3**, *m/z* 239 to show formation of product **5**, and at *m/z* 241 to demonstrate that the reduced product festuclavine **2** only forms upon addition of $NaCNBH_3$.



d. EasA product after incubation with $NaCNBH_3$. Monitoring at *m/z* 255 demonstrates complete consumption of starting material **3** by EasA, monitoring at *m/z* 239 demonstrates complete reduction of iminium ion **5** by $NaCNBH_3$, and monitoring at *m/z* 241 shows formation of the reduced product **2**.



e. Selected ion monitoring of festuclavine **2** standard (*m/z* 241) at *m/z* 255 and 239 demonstrates that there are no contaminants with *m/z* 255 or 239 in the purified compound.



f. Selected ion monitoring of agroclavine **1** (*m/z* 239) at *m/z* 239 and 241. Agroclavine was also incubated with $NaCNBH_3$ to demonstrate that this compound does not react, as expected, with this reducing agent.

Figure S10. Standard curve for chanoclavine-I aldehyde concentration and peak area response observed by LC-MS. The concentration of the standard was calculated based on UV absorbance at 225 nm using the extinction coefficient (30,900 M^{-1} cm^{-1} at 225 nm).The concentrations 100-500 nM represent the full range of chanoclavine-I aldehyde substrate that was analyzed by mass spectrometry (after dilution of the enzyme reaction for analysis).

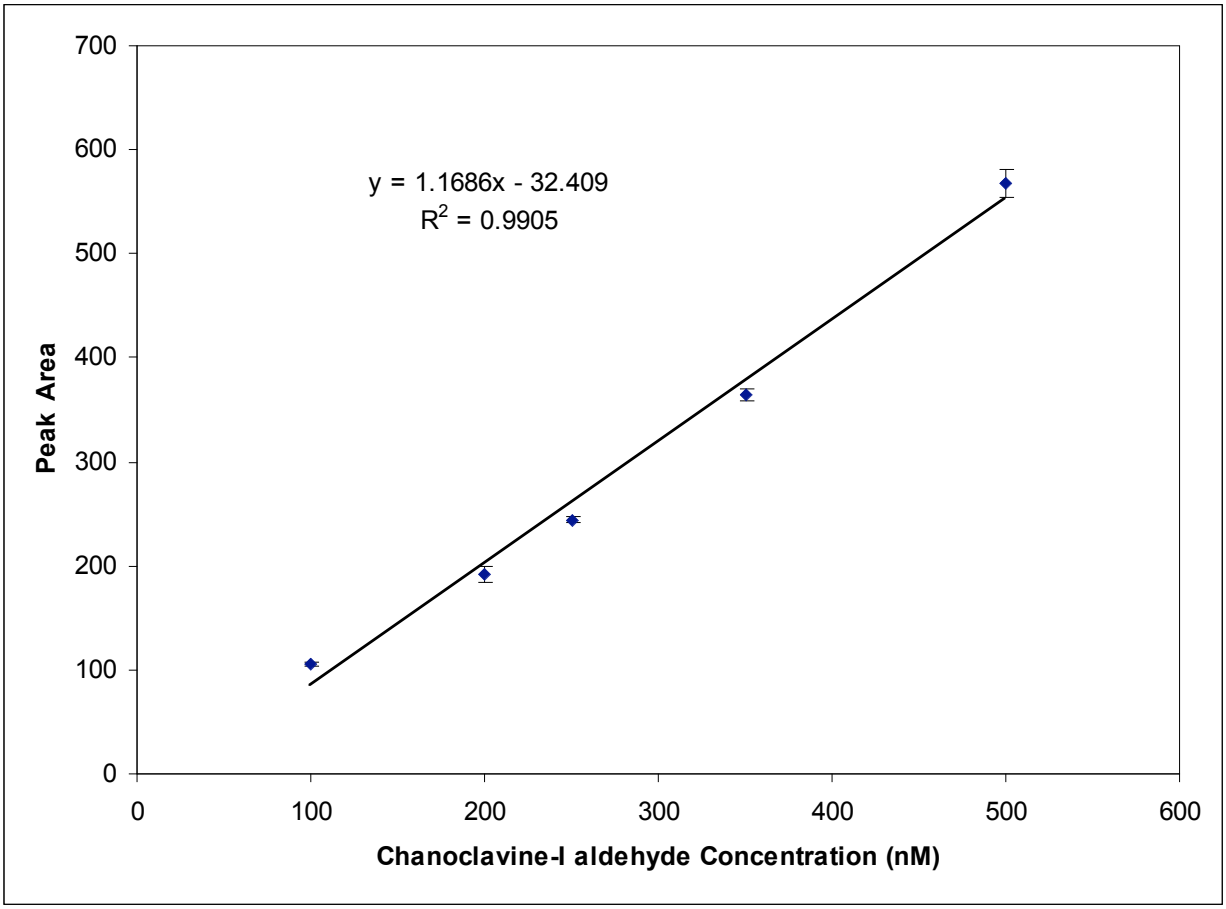
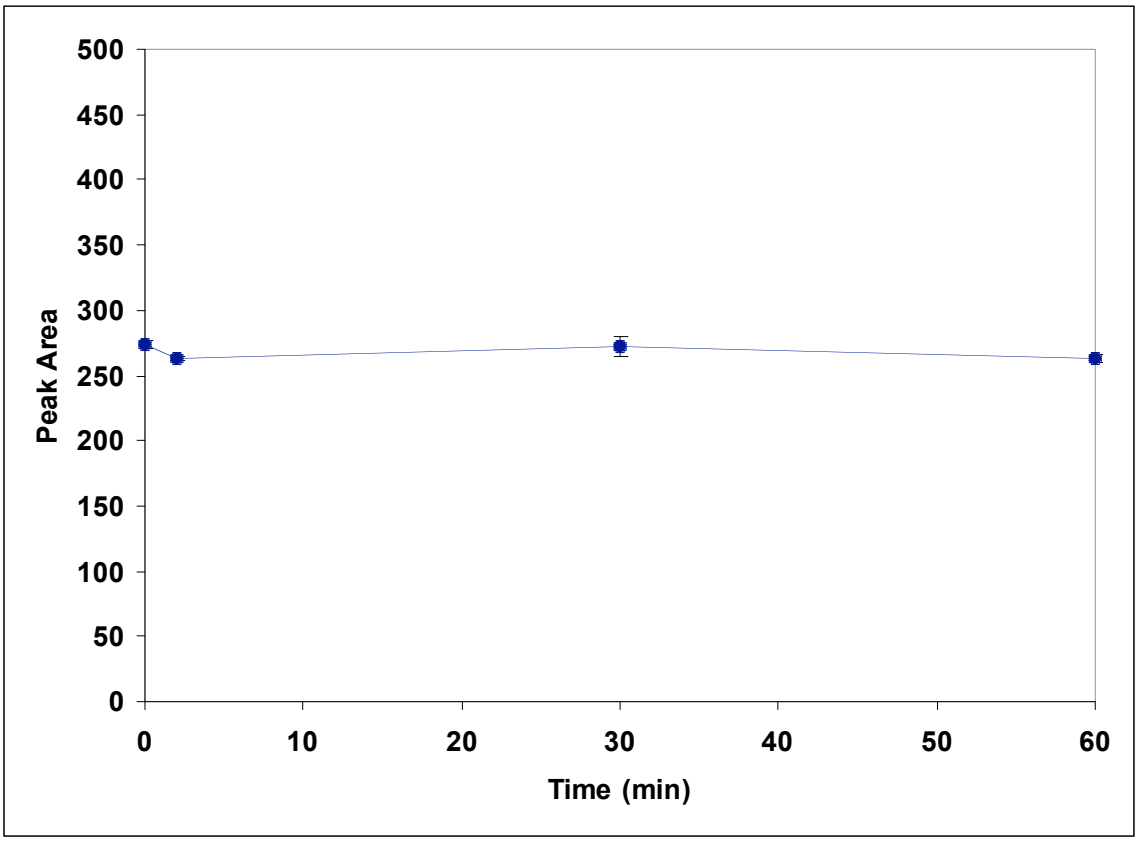


Figure S11. Chanoclavine-I aldehyde **3** concentration measured over time under assay conditions by LC-MS.



S10. References

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