Experimental Methods:

1. Fungal strains and culture conditions

Aspergillus amoenus and *Aspergillus protuberus* spores were generated on YPD agar plates over the course of seven days. Each plate of spores was harvested into 5 mL sterile water by gently scraping the surface of the culture with a sterile inoculating loop. Spores were stored at -80°C prior to genomic DNA extraction. Genomic DNA was harvested using Wizard Genomic DNA Purification Kit from Promega.

2. cDNA preparation and cloning of *notI* and *notI′*

Total RNA was extracted from the mycelia of *Aspergillus protuberus* using the Invitrogen PureLink RNA Mini Kit and corresponding plant tissue processing protocol. The culture was grown statically on liquid medium (50% seawater with 2.0% malt extract and 0.5% peptone) at 28°C and dried by filtration on the 17th day (roughly 500 mg fungal mat). RNA was treated with DNase I, and cDNA was generated using Invitrogen Superscript First Strand Synthesis. PCR was used to amplify *notI* from the cDNA template. Additionally, a codon optimized construct was purchased from GeneArt, Life Technologies and used in all enzymology studies. The codon optimized *notI* was inserted into the *pMCSG7* vector by ligation independent cloning (LIC). To generate *notI′*, introns were predicted by analysis using Softberry Fgenesh-M. Further analysis was performed by comparison with the *notI* sequence, which has 81% DNA sequence identity. The *notI′* gene was amplified from genomic DNA using overlapping PCR with primers in Table S1. The amplified gene was cloned into a pET28b vector with an MBP tag using restriction enzyme digest and ligation. Plasmids were transformed into *E. coli* DH5α for screening and plasmid maintenance.

3. Overexpression and purification of protein for enzymology

The *Escherichia coli* BL21 (DE3) transformant containing *pMCSG7-notI* and Takara chaperone plasmid *pGKJE8* was grown at 37°C overnight in LB media containing 50 μg/mL of ampicillin and 25 μg/mL of chloramphenicol. 25 mL of culture were used to inoculate 1 L of TB media containing the aforementioned concentrations of antibiotic and 4% glycerol, and cultures were supplemented with 0.5 mg/mL L-arabinose and 5 ng/mL tetracycline to induce chaperone expression. Cells were grown at 37°C for roughly 4 hours until A_{600} reached 0.6-1.0, and isopropyl β-D-thiogalactoside (IPTG, 0.2 mM) and riboflavin (50 μM) were added to induce protein overexpression overnight at 18°C. The *Escherichia coli* BL21 *pRARE* transformant containing *pET28b-MBP-notI′* and Takara chaperone plasmid *pTf16* was grown at 37°C overnight in LB media containing 50 μg/mL of kanamycin, 25 μg/mL of chloramphenicol, and 100 μg/mL of spectinomycin. 5 mL of culture was used to inoculate 1 L of TB media containing the aforementioned concentrations of antibiotic and 4% glycerol, and cultures were supplemented with 0.5 mg/mL L-arabinose to induce chaperone expression. Cells were grown at 37°C for roughly 4 hours until A_{600} reached 0.6-1.0, and isopropyl β-D-thiogalactoside (IPTG, 0.2 mM) and riboflavin (50 μM) were added to induce protein overexpression overnight at 18°C.

All purification steps were conducted at 4°C. Briefly, 1 L of expression culture was spun down at 5,500 rpm. The harvested cell pellet was resuspended in 35 ml of lysis buffer (10 mM imidazole pH 8, 50 mM NaH_2PO_4, 300 mM NaCl, 10% v/v glycerol, adjusted to pH 8) with the addition of 10 mg lysozyme, 4 mg DNase, 50 μM flavin adenine dinucleotide (FAD), 2 mM $MgSO_4$ and lysed by sonication. Insoluble material was removed by centrifugation at 20,000 rpm for 30 minutes, and the supernatant was filtered. NotI and NotI′ were purified through metal affinity chromatography with Ni^{2+}-NTA resin (Novagen) that was equilibrated with lysis buffer. The protein-bound resin was washed with 50 mL of lysis buffer, 50 mL of wash buffer (20 mM imidazole pH 8, 50 mM NaH_2PO_4, 300 mM NaCl, 10% v/v glycerol, adjusted to pH 8), and finally 10 mL of elution buffer (250 mM imidazole pH 8, 50 mM NaH_2PO_4, 300 mM NaCl, 10% v/v glycerol, adjusted to pH 8). Protein in the eluate was exchanged into storage buffer (50 mM NaH_2PO_4, 1 mM EDTA, 0.2 mM DTT, 10% v/v glycerol, pH 7.3) using a PD-10 column. Samples were then flash frozen with liquid N_2 and stored at -80°C. A 1L expression culture yielded ~ 42 mg of NotI and ~ 15 mg of NotI′.

4. Enzymatic assays and Q-TOF LC-MS analysis of NotI and NotI′ reactions with deoxybrevianamide E (13), 6-OH-deoxybrevianamide E (14), notoamide S (15), notoamide E (1), (+)/(-)- notoamide T (16/17).
The standard enzyme assay containing 0.5 mM substrate, 2.5 mM NADH, and 20 μM enzyme in 100 μL reaction buffer (50 mM NaH_2PO_4, 1 mM EDTA, 0.2 mM DTT, 10% v/v glycerol, pH 7.3) was performed at 28°C overnight. Each reaction was extracted three times with 200 μL chloroform, and the extract was dried down under N_2 gas. The product was resuspended in 100 μL methanol for Q-TOF LC-MS analysis. The samples were analyzed by QTOF LC-MS using a ZORBAX Eclipse Plus C18 reverse phase column (3.5 μm, 4.6 x 150 mm), monitoring wavelengths 240 nm and 280 nm and scanning 200 to 1200 m/z with the following time program: solvent A: water + 0.1% formic acid and solvent B: 95% acetonitrile in water + 0.1% formic acid; flow rate: 20% B over 2 minutes, 20-100% B over 10 minutes, 100% B over 5 minutes, 100-20% B over 1 minutes, 20% B over 5 minutes. The flow rate was 0.8 mL/min. The large-scale reaction to produce notoamide TI (**18**) was performed under the same reaction conditions with 0.5mM racemic notoamide T (**16**/**17**) on a 10 mL scale to produce 2 mg of final product. The reaction was extracted with chloroform, as described above, and purified by reversed phase HPLC.

5. Enzymatic assays and HPLC analysis of NotI and NotI′ reactions with (+)-stephacidin A (9), (-)-stephacidin A (4), (+)-6-*epi*-stephacidin A (6), and (-)-6-*epi*-stephacidin A (*7*).
The standard enzyme assay containing 200 μM FAD, 50 μM substrate, 5 mM NADH, and 40 μM enzyme in 50 μL reaction buffer (50 mM NaH_2PO_4, 1 mM EDTA, 0.2 mM DTT, 10% v/v glycerol, pH 7.3) was performed at 28°C for 2 hours. The reactions were quenched and extracted three times by the addition of 100 μL ethyl acetate. The ethyl acetate was removed by evaporation under N_2, and the purified reaction mixture was resuspended in 50 μL LC-MS grade methanol. The samples were compared to product standards on a Shimadzu HPLC using a Phenomenex Lux 5 μm Cellulose-3 LC column (250 x 4.6 mm) with the following time program: 30% acetonitrile for 2 minutes, 30-60% acetonitrile over 15 minutes, 60% acetonitrile for 1 minute, 60-30% acetonitrile over 1 minute, and 30% acetonitrile for 8 minutes. The flow rate was 1 mL/min and the reactions were monitored at 240nm.

6. NotI kinetic assays and Q-TOF LC-MS analysis
The reactions were performed with varying substrate concentrations (10-500 μM (-)-stephacidin A (**4**)) on a 250 μL scale containing 2.5 μM NotI, 5 mM NADH, and 0.1 mM FAD in reaction buffer (50 mM NaH_2PO_4, 1 mM EDTA, 0.2 mM DTT, 10% v/v glycerol, pH 7.3). The reactions were pre-warmed at room temperature for 5 minutes and initiated with addition of 5 mM NADH. At varying time points, the reactions were quenched with an equal volume methanol, vortexed vigorously, and placed on ice. The samples were then centrifuged at 17,000 xg at 4°C for 25 minutes to pellet the precipitated protein, and the supernatant was used for Q-TOF LC-MS analysis. The samples were analyzed using a ZORBAX Eclipse Plus C18 reverse phase column (3.5 μm, 4.6 x 150 mm), monitoring at 240 nm/280 nm and scanning 200 to 1200 m/z using the following time program: solvent A: water + 0.1% formic acid, solvent B: 95% acetonitrile in water + 0.1% formic acid; flow rate: 0.8 mL/min; mobile phase: 50% B over 2 minutes, 50-55% B over 5 minutes, 55-50% B over 30 seconds, 50% B over 2.5 minutes. All experiments were performed in duplicate. Quantitative analysis was performed through integration of the extracted ion chromatograms corresponding to substrate and product masses. The data were fit to the Michaelis-Menten model.

7. NotI epodixation efficiency reactions
To measure the rate of NADH oxidation during the NotI/NotI′-catalyzed conversion of (-)-stephacidin A (**4**), 50 μL reactions were run in a 384-well plate and monitored at 340 nm at various timepoints over 45 minutes. The reaction components consisted of 2.5 μM FAD, 2.5 μM NotI, 200 μM (-)-stephacidin A (**4**), and 200 μM NADH. Separate aliquots were quenched at 10, 20, 30, and 40 minutes and analyzed by the above described HPLC method to determine product formation under identical conditions to the NADH assays.

8. Determination of FAD incorporation in NotI and NotI′

Each protein was denatured by boiling and centrifugation and the cofactor identification was monitored by HPLC with a Phenomenex LUNA phenyl-hexyl 100A LC column (250 x 4.6 mm). The samples were monitored at 448nm using the following method for separation: 5%B for 2 minutes, 5-30%B over 10 minutes, hold 30%B for 2 minutes, 30-100%B over 1 minute, hold 100%B for 2 minutes, 100-5%B over 2 minutes, where solvent A = water + 0.1% formic acid and B = acetonitrile + 0.1% formic acid with a flow rate of 1mL/min. FAD incorporation was determined through the use of a standard curve for FAD, from which the concentration of the cofactor was determined and compared to the concentration of the enzyme.