

## Biosynthesis of an Anti-Addiction Agent from the Iboga Plant

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## MATERIALS AND METHODS

### Chemicals and molecular biology reagents

All solvents used for extractions, chemical synthesis and preparative HPLC were HPLC grade, while solvents for UPLC/MS were MS grade. All solvents were purchased from Fisher Scientific. (–)-Ibogamine (17), (–)-ibogaine (1) and (–)-voacangine (3) were gifts from Dr. Kenneth Alper (New York University, USA). Authentic (–)-coronaridine (2) was extracted and purified from *Tabernaemontana divaricata* as previously described<sup>6</sup>. (+)-Coronaridine (5) was synthesized by Professor Corey Stephenson and Rory McAtee (University of Michigan, USA). (+)-Catharanthine (4) was purchased from Sigma Aldrich. Tabersonine (12) was obtained from Ava Chem Scientific. Authentic (–)-vincadifformine (11) was provided by Dr. Vincent Courdavault (University of Tours, France) and Dr. Rod Andrade (Temple University, USA). Stemmadenine was purified by Professor Ivo J. Curcino Vieira as previously described<sup>5</sup>. Stemmadenine acetate (6) was synthesized from stemmadenine by chemical acetylation as previously described<sup>5</sup>.

Precondylocarpine acetate (7) was prepared enzymatically using the following conditions: 250 µg of stemmadenine acetate (6), 5 µg of CrPAS (GenBank MH213134) and 50 µg FAD were combined in a total reaction volume of 500 µL Tris-HCl buffer (50 mM, pH 8.5) and incubated at 37 °C for 2 h with shaking (1000 rpm). Reactions were quenched by addition of 5-volumes 90:9:1 methanol (MeOH):H<sub>2</sub>O:Formic acid (FA), and produced precondylocarpine acetate (7) was purified as previously described<sup>5</sup>. Compound 9 was prepared enzymatically using the following conditions: 250 µg precondylocarpine acetate (7), 15 µg CrDPAS (GenBank KU865331) and 1 mg NADPH were combined in a total reaction volume of 500 µL Tris-HCl buffer (50 mM, pH 8.5) and incubated at 37 °C for 1 h with shaking (1000 rpm). Assays were quenched by addition of 5-volumes 90:9:1 MeOH:H<sub>2</sub>O:FA. Compound 9 was purified using a Dionex ultimate 3000 HPLC system equipped with a YMC-Pack Pro C18 column (250 x 10 mm, 5 µm, 12 nm) and UV detector monitoring 330 nm and was eluted in a gradient of 0.1 % FA and acetonitrile (MeCN) and immediately dried under vacuum using a Büchi roto-vap. Due to the unstable nature of 9, assays were conducted immediately after purification.

Kanamycin sulfate, carbenicillin and gentamycin were from Formedium, and rifampicin was purchased from Sigma. All gene amplifications were performed using Platinum Superfi DNA polymerase (Thermo Fisher) and colony PCR was performed using Phire II master mix (Thermo Fisher). PCR product purification was performed using the Macherey-Nagel PCR clean-up kit. Plasmid purification was performed using the Promega Wizard miniprep kit. cDNA was prepared using Superscript IV VIL0 master mix and Turbo DNase (Thermo Fisher).

### Expression and purification of proteins

TiCorS, TiTabS, TiDPAS1, TiDPAS2, and TiPNAE1 expression in *E. coli*.

*T. iboga* cDNA was isolated as previously described<sup>6</sup>. Full-length TiCorS, TiTabS, TiDPAS1, TiDPAS2, and TiPNAE sequences were amplified from *T. iboga* cDNA using the primers listed in Table S1. PCR products were purified from an agarose gel and ligated into the BamHI and KpnI restriction sites of the pOPINF vector<sup>18</sup> using the In-Fusion kit (Clontech Takara). Constructs were transformed into chemically competent *E. coli* Stellar cells and recombinant colonies were selected on LB agar + carbenicillin (100 µg/mL). Positive

colonies were identified by colony PCR using primers listed in Table S1 and grown overnight at 37 °C for plasmid isolation and sequence confirmation using Eurofins sequencing service (Table S2).

Chemically competent SoluBL21 *E. coli* cells (Amsbio) were transformed with appropriate plasmids by heat shock at 42 °C for 1 min. Transformed cells were selected on LB agar + carbenicillin (100 µg/mL). Single colonies were used to inoculate starter cultures of 50 mL 2 x YT medium supplemented with carbenicillin (100 µg/mL). Cultures were grown overnight at 37 °C with shaking (200 rpm). Starter cultures were used to inoculate 1 L of 2 x YT medium containing carbenicillin (100 µg/mL), grown to OD<sub>600</sub>=0.6 and then transferred to 16 °C for 30 min before inducing protein expression by addition of IPTG (0.3 mM). Protein expression was carried out for 16 h (18 °C, 200 rpm) where after cells were harvested by centrifugation (4000 x g) and re-suspended in 50 mL Buffer A (50 mM Tris-HCl pH 8, 50 mM glycine, 500 mM NaCl, 5% glycerol, 20 mM imidazole) with EDTA-free protease inhibitors (Roche Diagnostics Ltd.) and 10 mg lysozyme. Cells were lysed at 4 °C using a cell disruptor (25 KPSI) and centrifuged (35,000 x g) to remove insoluble debris. The supernatant was filtered through a 0.45 µm glass syringe filter (Sartorius) and applied to a HisTrap HP 5 mL column (GE Healthcare) with the assistance of an ÄKTA pure FPLC (GE Healthcare). Lysate was loaded at a flow rate of 2 mL/min and washed with Buffer A before being eluted with Buffer B (50 mM Tris-HCl pH 8, 50 mM glycine, 500 mM NaCl, 5% glycerol, 500 mM imidazole). Eluted proteins were further purified on a Superdex Hiload 16/60 S200 gel filtration column (GE Healthcare) at a flow rate of 1 mL/min using Buffer C (20 mM HEPES pH 7.5, 150 mM NaCl).

### TiN100MT and TiI10H

Cloning and expression of N100MT and I10H was carried out as previously described<sup>6</sup>.

### Recombinant TiPAS from *N. benthamiana*

TiPAS1-3 full-length sequences were cloned into a modified TRBO vector<sup>19</sup> in which the cloning cassette of the pOPINF vector was inserted in the NotI restriction site. The vector was further modified by removing the N-terminal HisTag using PacI and SapI. PCR products were ligated into the modified vector upstream of the C-terminal HisTag using the In-fusion kit. Constructs were transformed into *E. coli* Stellar cells by heat shock (42 °C) and recombinant colonies were selected on LB agar + kanamycin (100 µg/mL). Positive colonies were screened by colony PCR using the primers listed in Table S1 and sequences were confirmed using Eurofins sequencing service (Table S2). Sequence confirmed constructs were transformed into electrocompetent *Agrobacterium tumefaciens* strain GV3101 by electroporation. Recombinant colonies were selected on LB agar + rifampicin (100 µg/mL), gentamycin (50 µg/mL) and kanamycin (100 µg/mL). Single colonies were grown in 10 mL LB with antibiotics for 48 h at 28 °C and shaking (200 rpm). Cells were pelleted by centrifugation (4000 x g) and re-suspended in 10 mL infiltration buffer (10 mM NaCl, 1.75 mM CaCl<sub>2</sub> and 100 µM acetosyringone). After incubation at room temperature for 3 h, cultures were diluted to OD<sub>600</sub>=0.1 and used to infiltrate 3-4-week old *N. benthamiana* plants. Leaves were harvested 5-days post-infiltration, and proteins were extracted from 30 g of tissue (fresh weight). Total proteins were extracted from pulverized leaf tissue with 100 mL ice-cold Tris-HCl buffer (50 mM, pH 8.0) containing EDTA-free protease inhibitors and 1 % PVPP.

After incubation on ice for 1 h with intermittent vortexing, samples were filtered through miracloth and centrifuged (4000 x g) for 10 min to remove insoluble PVPP and tissue debris. The supernatant was further centrifuged (35,000 x g) for 20 min. Supernatants were collected and incubated with Ni-NTA agarose beads (Qiagen) equilibrated with Tris-HCl buffer (50 mM, pH 8.0) for 1 h. Samples were centrifuged (1,000 x g) for 1 min to pellet Ni-NTA agarose beads and washed 3 times with 10 mL Tris-HCl buffer (50 mM, pH 8). Proteins were eluted twice with 600  $\mu$ L Tris-HCl (50 mM, pH 8) containing 500 mM imidazole. Protein was buffer exchanged into HEPES (pH 7.5, 50 mM NaCl) and stored at -80 °C.

To confirm the presence of TiPAS1-3, proteins were subjected to trypsin digestion and LC/MS/MS analysis on a nano LC-orbitrap (see Data S1) with adaptations to <sup>20</sup>. Protein samples were dissolved in 2.5 % sodium deoxycholate (Sigma, 30970-25G), pH 8.0, incubated with 10 mM DTT for 30 min at 65 °C followed by incubation with 20 mM iodoacetamide (IAA) at room temperature in the dark for 30 min, and quenched with 10 mM DTT (both in 50 mM TEAB). The samples were digested with sequencing grade trypsin (Promega) (1:50 w/w) and incubated at 50 °C for 8 h. Peptides were desalted using C18 tips, and aliquots were analyzed by nano LC-MS/MS on an Orbitrap Fusion Tribrid Mass Spectrometer coupled to an UltiMate 3000 RSLC nano LC system (Thermo Scientific, Hemel Hempstead, UK). The samples were loaded and trapped using a pre-column which was then switched in-line to the analytical column for separation. Peptides were separated on a nanoEase M/Z column (HSS C18 T3, 100 Å, 1.8  $\mu$ m; Waters, Wilmslow, UK) using a gradient of MeCN at a flow rate of 0.25  $\mu$ L/min with the following steps of solvents A (water, 0.1 % FA) and B (80 % MeCN, 0.1 % FA): 0-4 min 3 % B (trap only); 4-15 min increase B to 13 %; 15-77 min increase B to 38 %; 77-92 min increase B to 55 %; followed by a ramp to 99 % B and re-equilibration to 3 % B.

Data dependent analysis was performed using parallel CID and HCD fragmentation with the following parameters: positive ion mode, orbitrap MS resolution = 120k, mass range (quadrupole) = 300-1800 m/z, MS2 top20 in ion trap, threshold 1.9e4, isolation window 1.6 Da, charge states 2-5, AGC target 1.9e4, max inject time 35 ms, dynamic exclusion 1 count, 15 s exclusion, exclusion mass window  $\pm$  5 ppm. MS scans were saved in profile mode while MS2 scans were saved in centroid mode.

Recalibrated peak lists were generated with MaxQuant 1.6.3.4 <sup>21</sup> using T. iboga and N. benthamiana databases. The final database search was performed with the merged HCD and CID peak lists from MaxQuant using in-house Mascot Server 2.4.1 (Matrixscience, London, UK). The search was performed on the T. iboga and N. benthamiana protein sequences and the MaxQuant contaminants databases. For the search a precursor tolerance of 6 ppm and a fragment tolerance of 0.6 Da was used. The enzyme was set to trypsin/P with a maximum of 2 allowed missed cleavages. Oxidation (M) and deamidation (N/Q) were set as standard variable modifications and carbamido-methylation (CAM) of cysteine as fixed modification. The Mascot search results were imported into Scaffold 4.4.1.1 (www.proteomesoftware.com) using identification probabilities of 99 % for proteins and 95 % for peptides.

#### In vitro enzyme assays

##### TiPAS assays

In vitro assays with TiPAS1-3 were performed in 50 mM CHES buffer (pH 9.5), 50  $\mu$ M FAD and 50  $\mu$ M stemmadenine acetate (6). Assays using the isolated dehydrosecodine isomer were

sluggish in more acidic conditions, presumably because the isolated substrate must isomerize to the correct dehydrosecodine isomer (**9b**). Thus, all enzyme assays were performed at pH 9.5 for consistency. Due to low expression of recombinant TiPAS1-3, the amount of protein in these assays was not determined, however, 5  $\mu$ L of HisTrap enriched protein was added to each assay. After TiPAS1-3 activity was demonstrated (Figure S1), all subsequent assays were performed with purified CrPAS, for which an optimized expression system had already been established <sup>5</sup>.

#### Coupled assays

Coupled assays were performed in 50 mM CHES buffer (pH 9.5), with CrPAS, 50  $\mu$ M FAD and 50  $\mu$ M stemmadenine acetate (6) (Figure S2-4,8-10). In assays containing no CrPAS, and only TiDPAS1 or TiDPAS2 and TiCorS, 50  $\mu$ M precondylocarpine acetate (7) was used in place of stemmadenine acetate (6) (Figure S5-6,11-12). TiDPAS1 and TiDPAS2 require NADPH for activity. In assays producing putative dehydrosecodine isomer 9, equimolar concentrations of NADPH were added (Figure S2), whereas in assays producing (-)-vincadifformine (11) or (-)-coronaridine (2), a 1:8 ratio of substrate:NADPH was used (Figure S3-6,9-12,15). The concentration of enzyme in coupled reactions was optimized as: 420 nM CrPAS; 1  $\mu$ M TiDPAS1 or TiDPAS2; 5  $\mu$ M TiCorS or TiTabS. In assays commencing with TiCorS (Figure S15A), 50  $\mu$ M ca. of dehydrosecodine isomer 9 was added as substrate and incubated with 5  $\mu$ M TiCorS for 20 min at 37 °C and shaking (1000 rpm), which yielded a product 13 with m/z 337 as determined using the UPLC/MS method described below (Fig S15A). Following analysis, 13 was isolated in partially purified form – though its instability prevented characterization – using the methods described for 9 and then added to TiDPAS1 or TiDPAS2 (1  $\mu$ M) and NADPH (8 equivalents) for a further 20 min, resulting in the formation of (-)-coronaridine (2) (Figure S15B). Assays were quenched by addition of 5-volumes 90:9:1 MeOH:H<sub>2</sub>O:FA and analyzed immediately using the UPLC/MS method described below.

For D<sub>2</sub>O experiments, assays were conducted in 50 mM CHES (pH 9.5) where ca. 50  $\mu$ M dehydrosecodine (9) (that was isolated from reaction of precondylocarpine acetate (7) with DPAS), TiDPAS2 (1  $\mu$ M) and TiCorS (5  $\mu$ M) or TS or CS were added with excess NADPH (8-equivalents) to a total D<sub>2</sub>O or H<sub>2</sub>O reaction volume of 100  $\mu$ L. Assays were incubated at 37 °C and shaking (1000 rpm) for 30 min and then quenched with 2-volumes 90:9:1 MeOH:H<sub>2</sub>O:FA. Assays were analyzed using the HR-MS method described below.

#### CD spectroscopy

Enzymatically prepared (-)-coronaridine (2) and (-)-vincadifformine (11) were generated using coupled assays in 50 mM CHES buffer (pH 9.5) for circular dichroism (CD) spectroscopy. A total of 10 mg stemmadenine acetate (6) was converted to (-)-coronaridine (2) or (-)-vincadifformine (11) using the coupled assay protocol described above, by pooling reactions in which 250  $\mu$ g of stemmadenine acetate was used as starting material. Reactions were quenched by addition of 2 reaction volumes 90:9:1 MeOH:H<sub>2</sub>O:FA, and compounds were purified using an Agilent 1260 Infinity II HPLC system equipped with an Acquity BEH C18, 1.7  $\mu$ m (2.1 x 50 mm) column. Separation was performed at 40 °C using a flow rate of 0.6 mL/min and 0.1 % ammonia as mobile phase A and MeCN as mobile phase B. Separation began with a 1 min isocratic step at 85 % A and 15 % B followed by a linear gradient from 15 % to 60 % B in 2 min and 60 to 85 % B in 7.5 min. Conditions were then changed immediately to 100 % B for a 1 min wash

followed by a return to initial conditions for a 1 min re-equilibration period prior to the next injection. Elution of compounds was monitored at 284 and 330 nm using an Agilent 1290 Infinity II PDA detector and fractions were collected using the fraction collector. Relevant fractions were pooled and dried under vacuum and stored at -80°C until analysis. Due to the unstable nature of intermediates under in-vitro assay conditions, recoveries of only ca. 50 µg each of 2 and 11 was achieved.

Products and standards were prepared to a final concentration of ≈200 µM in MS grade MeOH. Spectra were recorded in 1 nm steps with a 0.5 s averaging time on a Chirascan Plus spectropolarimeter (Applied Photophysics) at 20°C in a 0.5 mm cuvette. Measurements were collected in triplicates, averaged and background subtracted with MeOH.

Enzymatic synthesis of (-)-voacangine and semi-synthesis of (-)-ibogaine.

Cultures of *S. cerevisiae* (1 mL) expressing I10H or empty vector were grown overnight at 30 °C with shaking (1000 rpm) in SC-leu media containing 2 % glucose. A 100 µL aliquot of this overnight culture was added to 900 µL buffered SC-leu media (pH 7.5, 50 mM HEPES) containing 2 % galactose to induce protein expression. Enzymatically prepared (-)-coronaridine (2), authentic isolated (-)-coronaridine (2) or authentic synthetic (+)-coronaridine (5) was added to separate I10H or empty vector expressing cultures to a final concentration of 50 µM. Reactions proceeded for 24 h at 30 °C and shaking (1000 rpm), where after cells were pelleted by centrifugation (4000 x g) and the supernatant was collected in a 15 mL falcon tube. Cell pellets were rinsed with 500 µL MeOH, sonicated and centrifuged (4000 x g) again to pellet debris. Supernatants were pooled and diluted to 10 mL with ultrapure water. Samples were purified on OASIS HLB cartridges (60 mg, 3 cc) that were activated and equilibrated with 3 mL MeOH and ultrapure water, respectively. Samples were loaded by gravity and washed with 3 mL ultrapure water followed by 3 mL 10 % MeOH. Compounds were eluted with 50:50 MeOH:MeCN v/v and dried under vacuum in a Genevac. Compounds were resuspended in 500 µL CHES buffer (50 mM, pH 9) and reacted with 100 ng of purified N100MT and 1000 µM SAM for 2 h at 37 °C and shaking (1000 rpm). Purified TiPNAE1 (100 ng) was then added to these reactions and incubated under the same conditions for an additional 2 h. Reactions were quenched with 2-volumes MeOH and cleaned using the HLB protocol above. Eluents were dried under vacuum in a Genevac and analyzed using the UPLC/MS method (A) below.

#### Liquid-chromatography mass spectrometry analysis

##### UPLC/MS analysis of enzyme assays

Method A. For in vitro enzyme assays, compounds were separated on an Acquity BEH C18, 1.7 µm (2.1 x 50 mm) column (60 °C) using a Waters UPLC. Separation was performed using a flow rate of 0.6 mL/min and 0.1 % FA as mobile phase A and MeCN as mobile phase B. A linear gradient from 2 to 25 % B in 5 min and 25 to 62.5 % B in 7.5 min was applied for compound separation followed by an increase to 100 % B at 7.6 min. Conditions remained constant for 1 min and immediately changed to 2% B for a 1.3 min re-equilibration step prior to the next injection. Eluting compounds were subjected to positive ESI and analyzed on a Waters Xevo TSQ using optimized source conditions: Capillary voltage, 3.0 kV; source temperature, 150 °C; desolvation temperature, 450 °C; cone gas, 50 L/h; and desolvation gas, 800 L/h. Unit resolution was applied to each

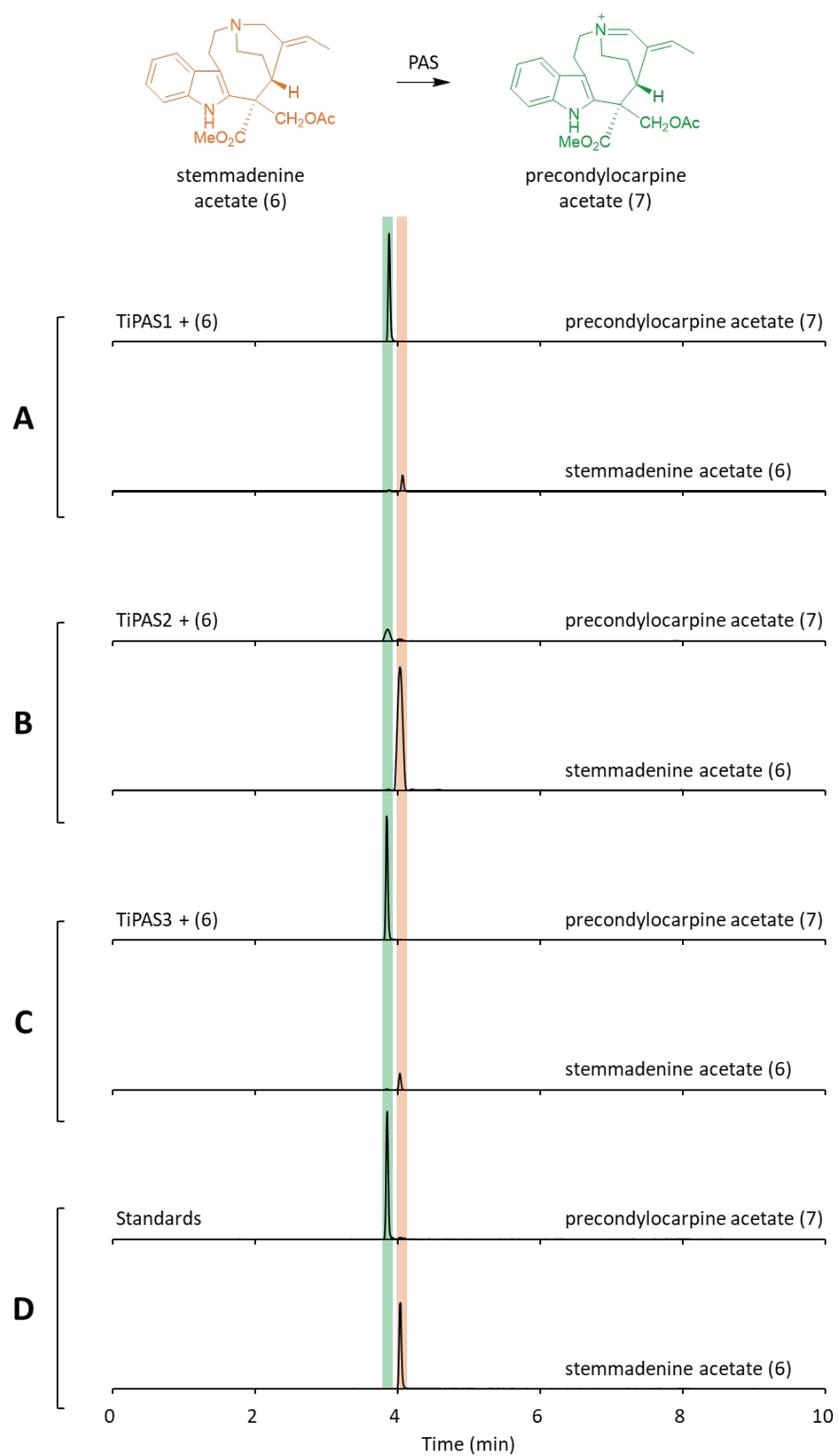
quadrupole and MRM transitions used for analysis are reported in Table S3.

##### UPLC/MS assay of temperature-dependent (-)-voacangine decarboxylation

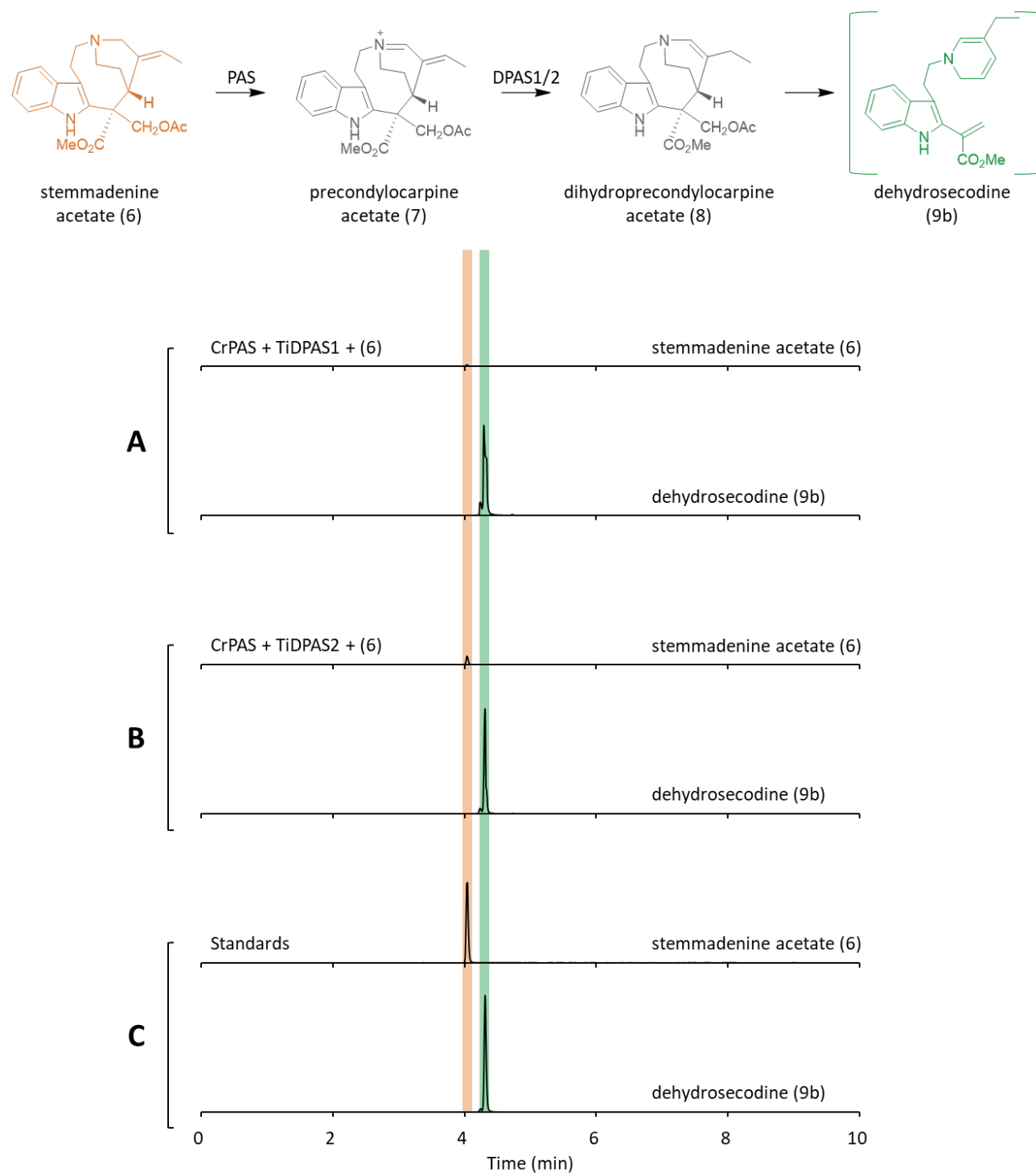
The effect of temperature on the decarboxylation of de-ester-(-)-voacangine (15) was carried out with modifications to UPLC/MS method (A) by increasing the column temperature in 5 °C increments at the beginning of each injection, up to 60 °C (Figure S23).

##### HR-MS

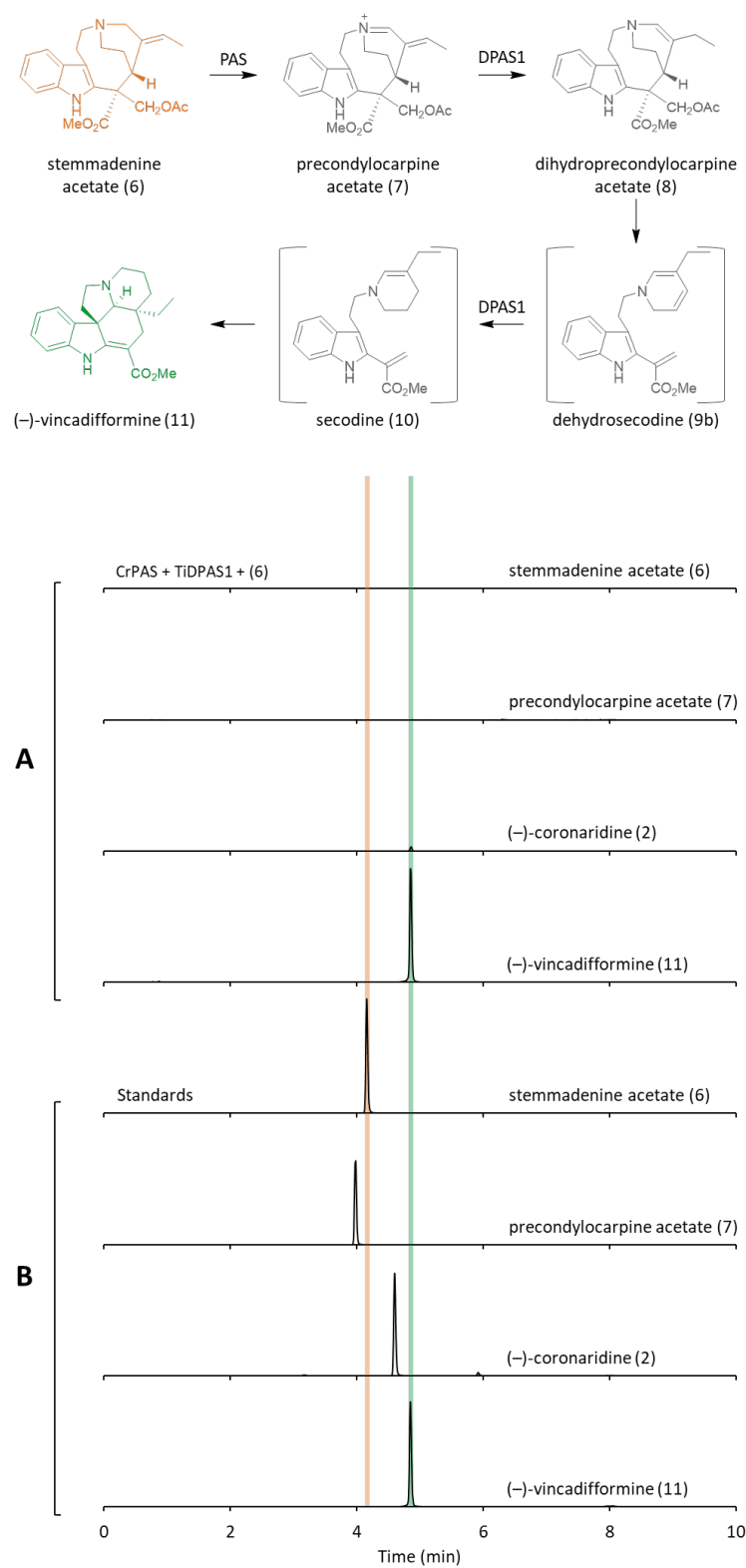
For high resolution MS analysis (Figure S16), compounds were separated on a Waters Acquity UPLC BEH C18 column (1 x 100 mm, 1.7 µm) in a gradient of 0.1% FA and MeCN and injected onto a Synapt G2 HDMS mass spectrometer (Waters) calibrated using a sodium formate solution. Samples were analyzed using positive ESI and monitored in the mass range of 50-1200 m/z. Capillary voltage was 3.5 V, cone voltage 40 V, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 800 L/h. Leu-enkephaline peptide (1 ng/µL) was used to generate a dual lock-mass calibration with  $[M+H]^+ = 556.2766$  and  $m/z = 278.1135$  measured every 10 sec. Spectra were generated in MassLynx 4.1 by combining a number of scans with background subtraction.



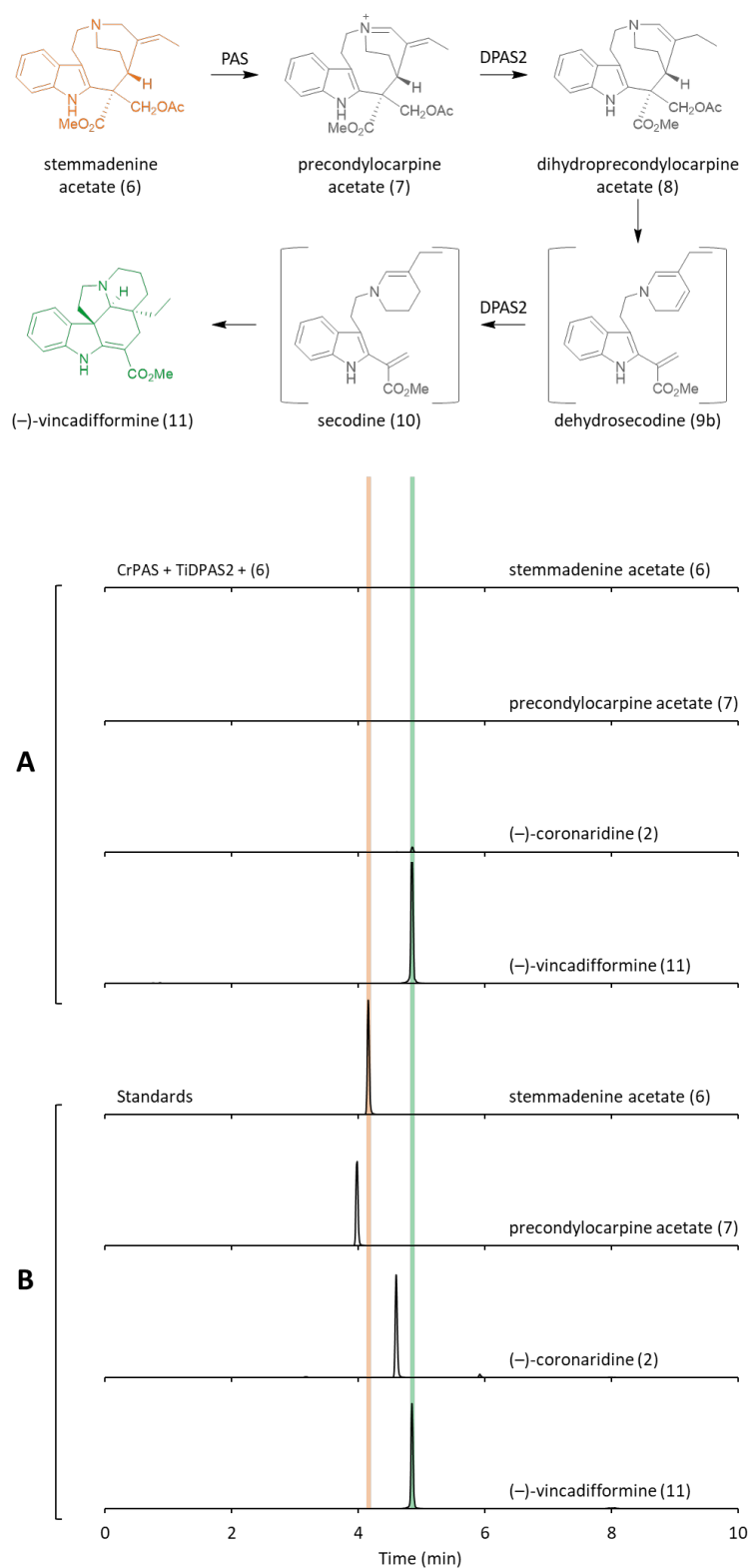
**Figure S1.** Formation of precondylocarpine acetate from stemmadenine acetate with TiPAS. A. UPLC/MS chromatograms illustrating the formation of precondylocarpine acetate (7, green) from stemmadenine acetate (6, orange) in assays with TiPAS1, B. TiPAS2, or C. TiPAS3. D. Authentic standards.



**Figure S2.** Formation of dehydrosecodine isomer from CrPAS, TiDPAS and stemmadenine acetate. A. UPLC/MS chromatograms illustrating the formation of putative dehydrosecodine isomer (9, green) from stemmadenine acetate (6, orange) in assays with CrPAS and TiDPAS1 or B. CrPAS and TiDPAS2. C. Authentic standards. Dehydrosecodine was not characterized due to low isolation yield and lability.

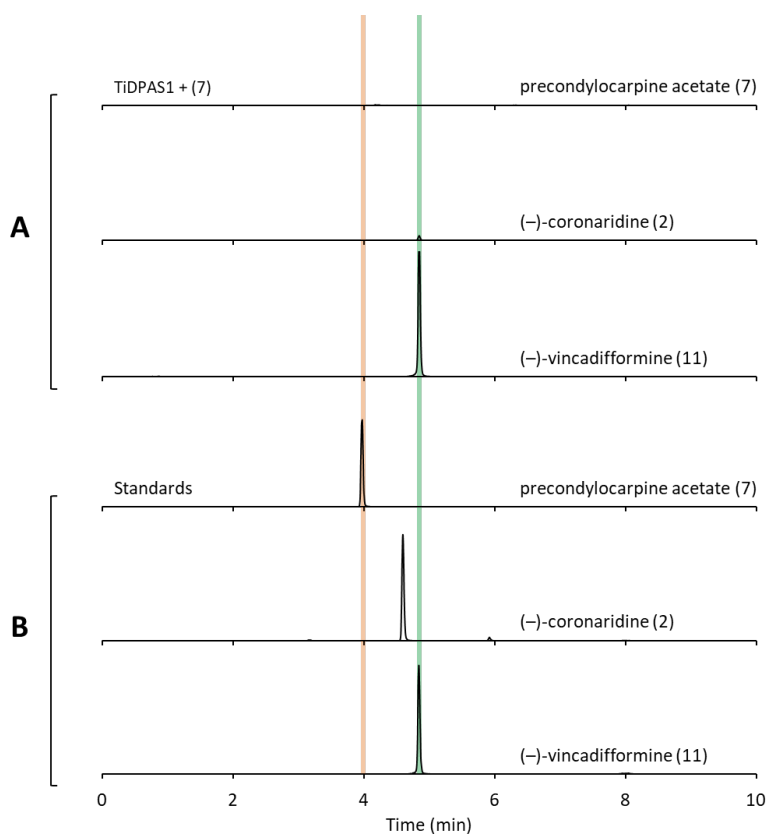
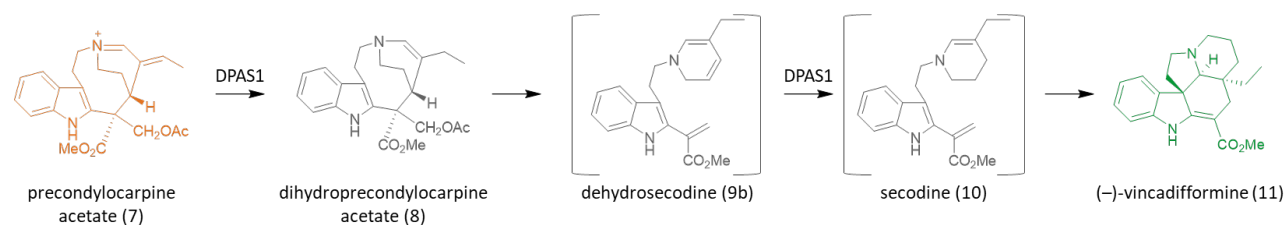


**Figure S3.** Formation of vincadifformine from stemmadenine acetate, CrPAS and TiDPAS1. A. UPLC/MS chromatograms illustrating the formation of (-)-vincadifformine (11, green) from stemmadenine acetate (6, orange) in assays with CrPAS and TiDPAS1. B. Authentic standards.

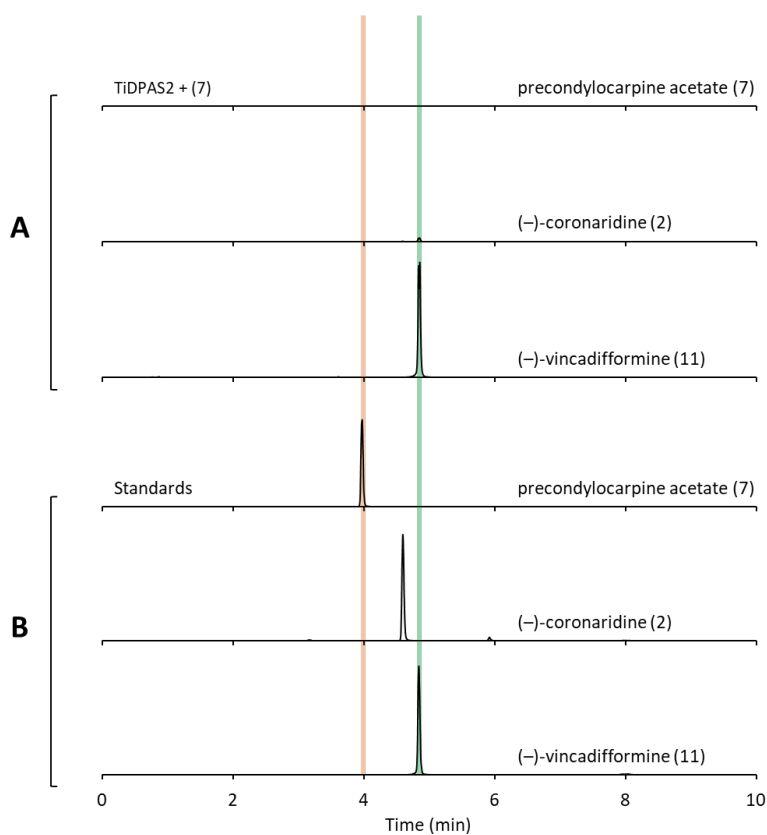
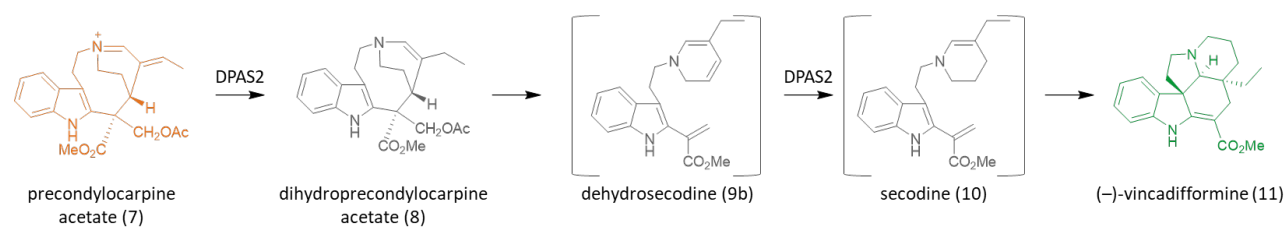


**Figure S4.** Formation of vincadifformine from stemmadenine acetate, CrPAS and TiDPAS2. A. UPLC/MS chromatograms illustrating the formation of vincadifformine (11, green) from stemmadenine acetate (6, orange) in assays with CrPAS and TiDPAS2. B. Authentic standards.

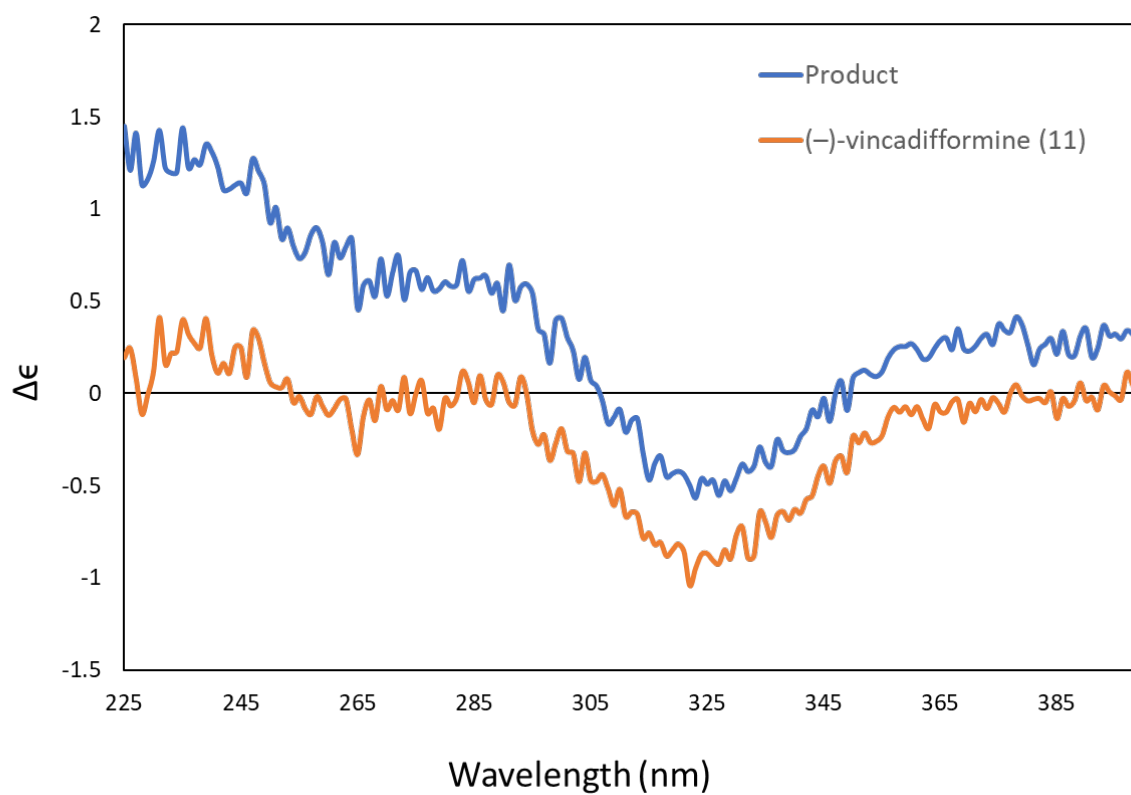




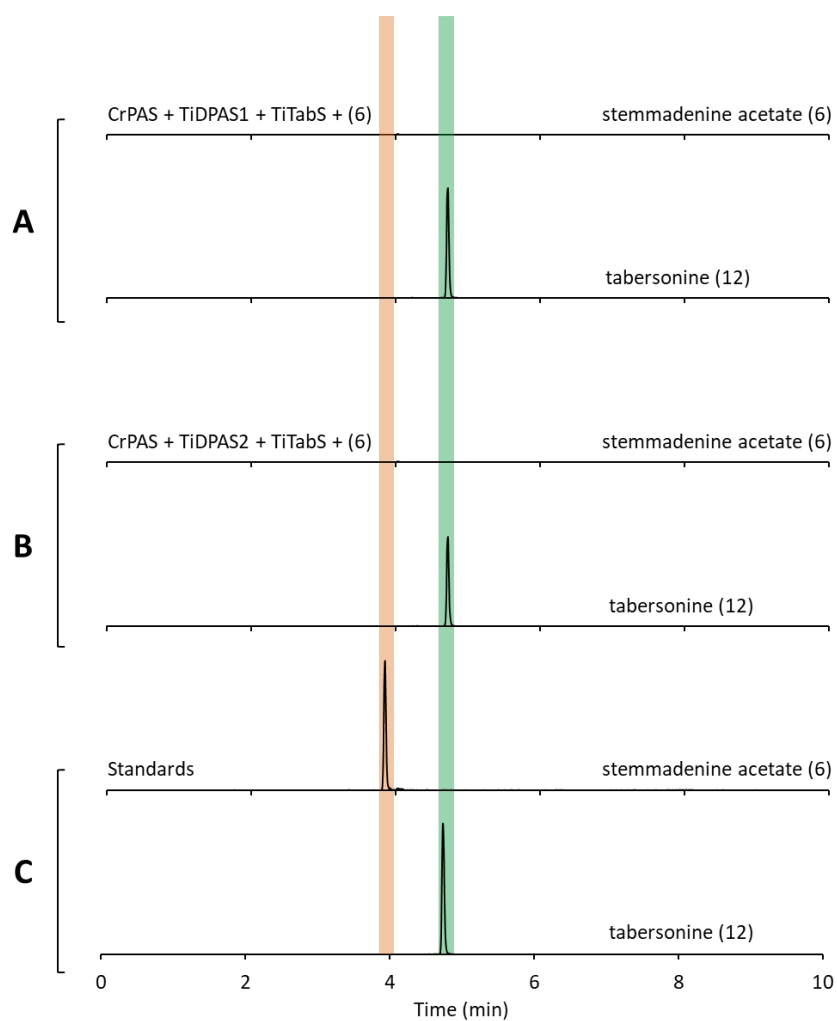
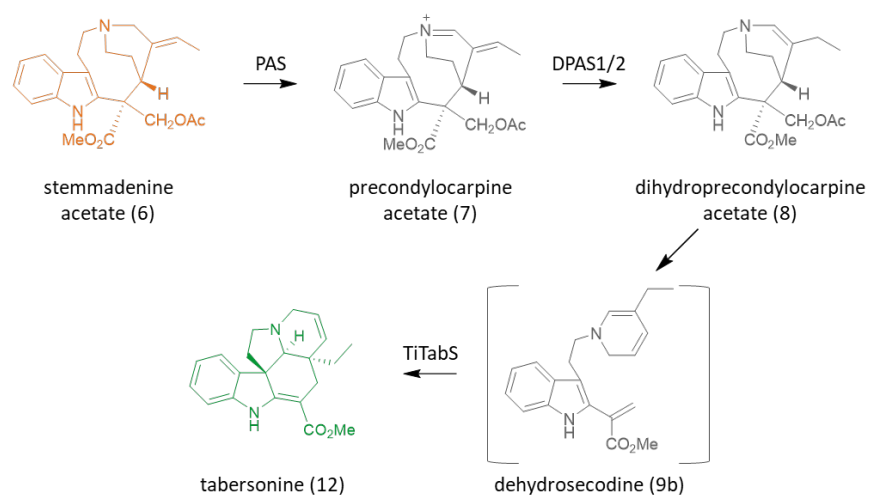
**Figure S5.** Formation of vincadifformine from precondylocarpine acetate and TiDPAS1. A. UPLC/MS chromatograms illustrating the formation of vincadifformine (11, green) from precondylocarpine acetate (7, orange) in assays with CrPAS and TiDPAS1. B. Authentic standards.



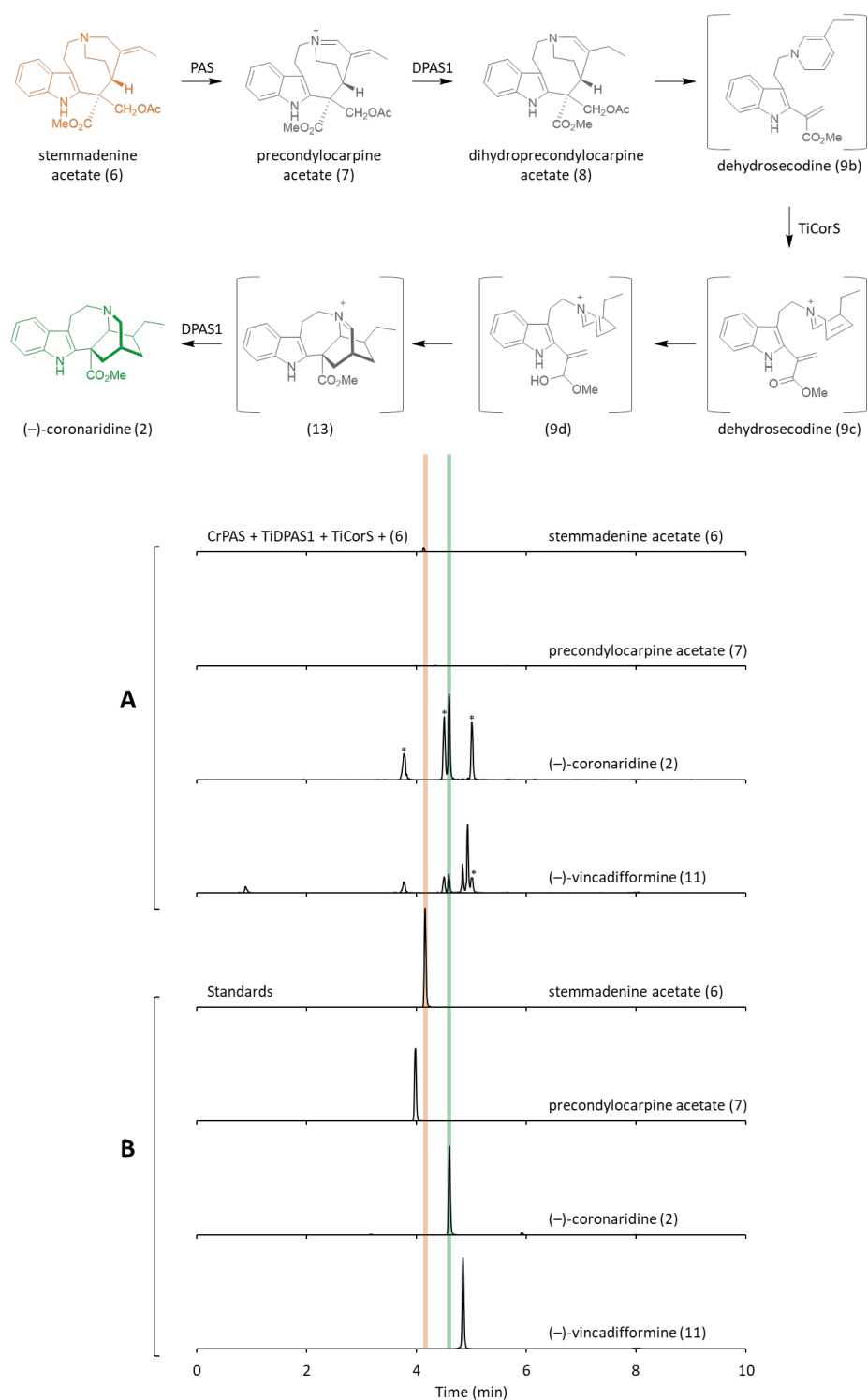
**Figure S6.** Formation of vincadifformine from precondylocarpine acetate and TiDPAS2. A. UPLC/MS chromatograms illustrating the formation of vincadifformine (11, green) from precondylocarpine acetate (7, orange) in assays with CrPAS and TiDPAS2. B. Authentic standards.



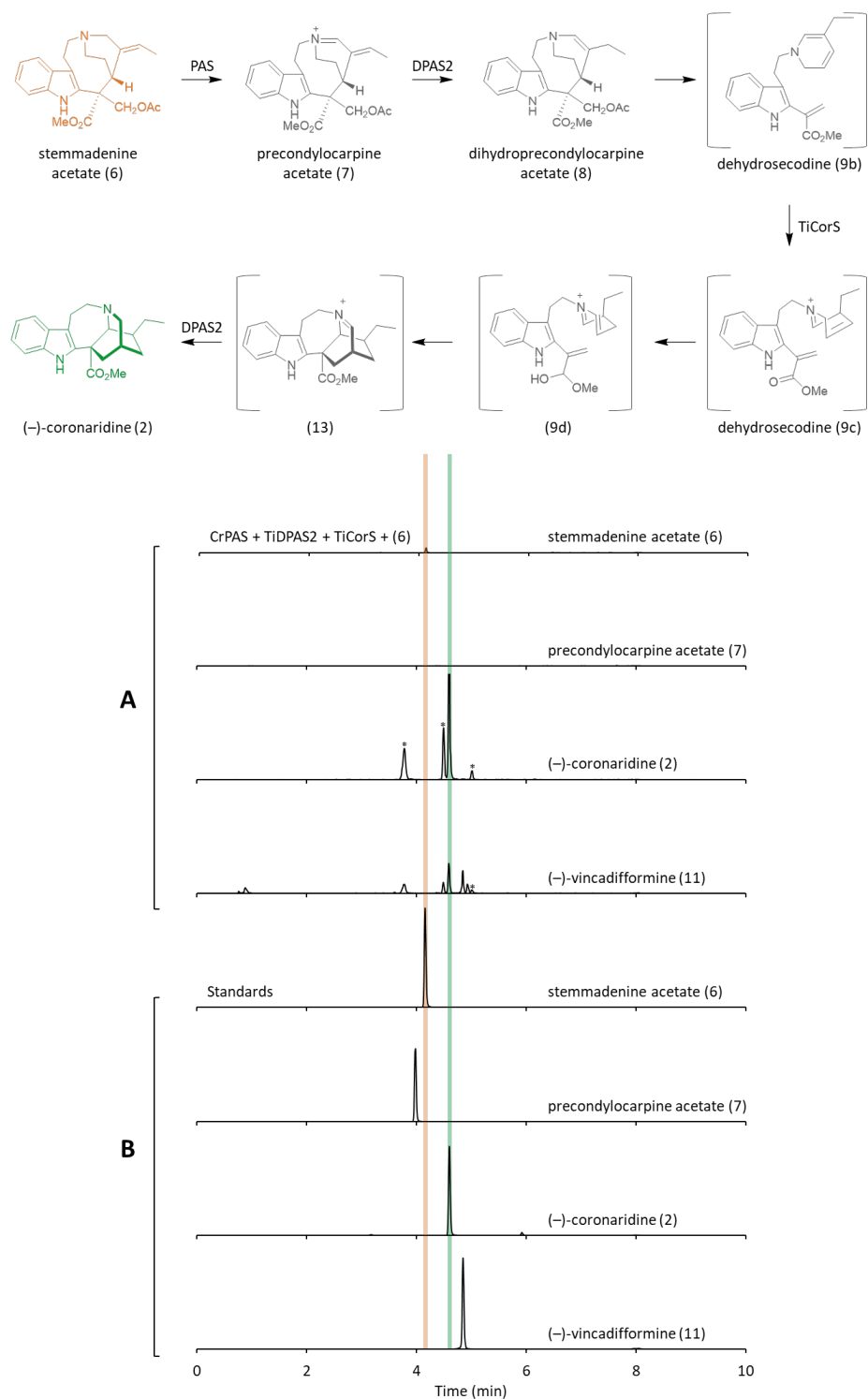
**Figure S7.** Stereochemistry of vincadifformine observed in enzyme assays. Stereochemical assignment of enzymatically produced vincadifformine (11, blue) using CD spectroscopy and comparison to an authentic (–)-vincadifformine standard (11, orange). Spectra were acquired in MS grade MeOH at a concentration of  $\approx 200 \mu\text{M}$ .



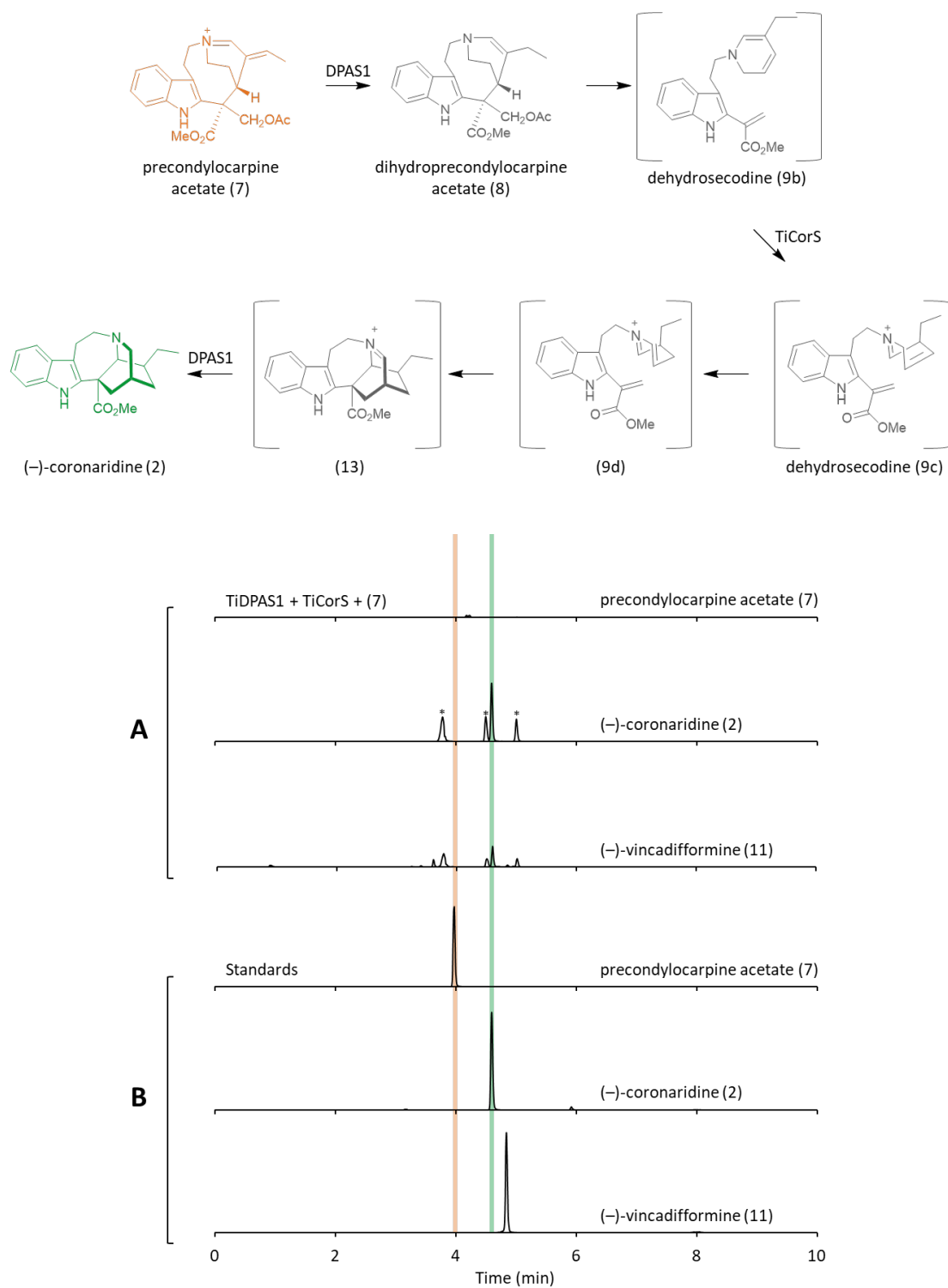
**Figure S8.** Formation of tabersonine from stemmadenine acetate. A. UPLC/MS chromatograms illustrating the formation of tabersonine (12, green) from stemmadenine acetate (6, orange) in assays with CrPAS, TiDPAS1 and TiTabS, or B. CrPAS, TiDPAS2 and TiTabS. C. Authentic standards.



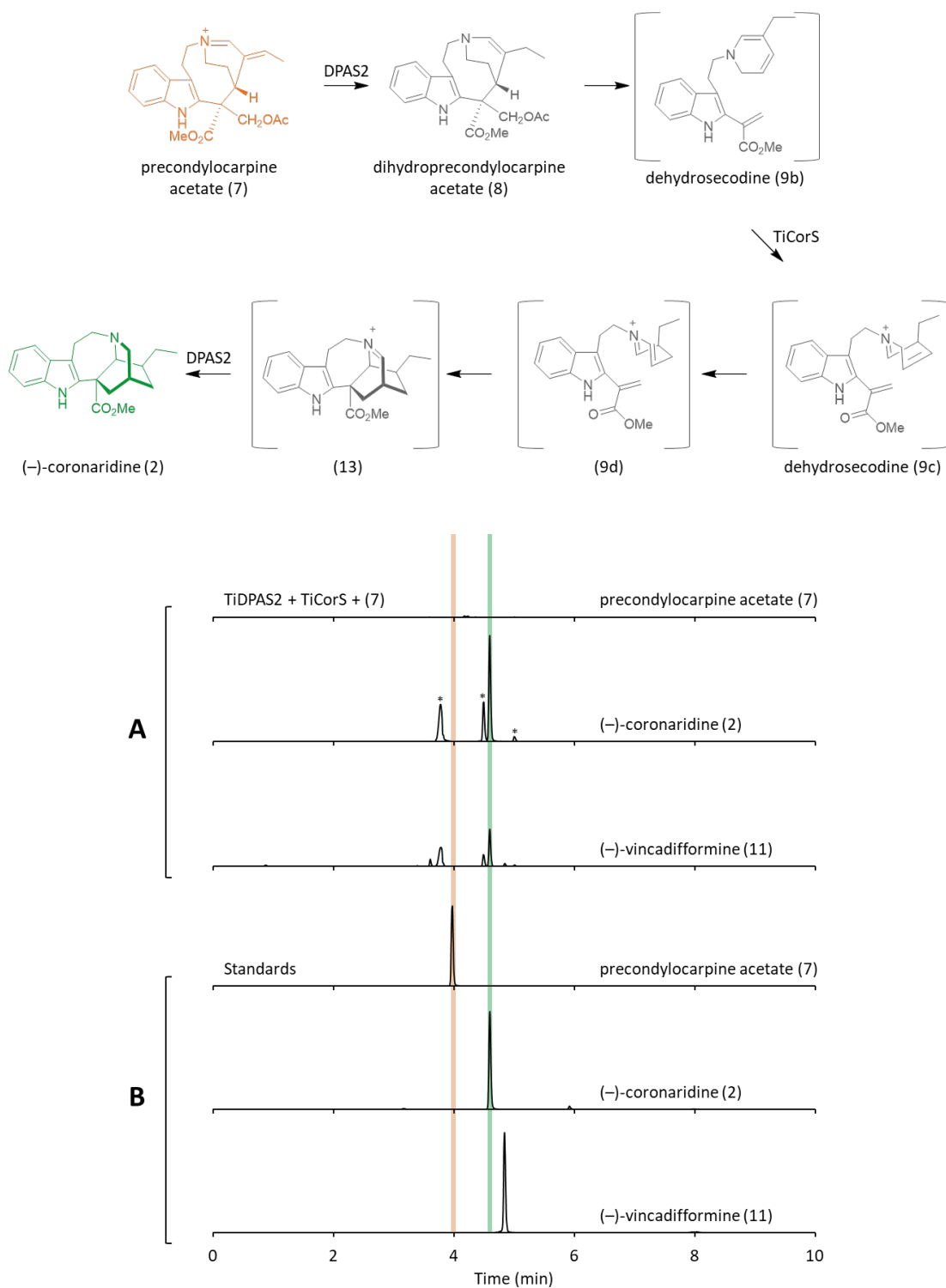
**Figure S9.** Formation of (-)-coronaridine from stemmadenine acetate (TiDPAS1). A. UPLC/MS chromatograms illustrating the formation of (-)-coronaridine (2, green) from stemmadenine acetate (6, orange) in assays with CrPAS, TiDPAS1, and TiCorS. Peaks marked with \* were labile, decomposed during isolation attempts and thus were not characterized. Trace quantities of vincadifformine (11) were detected. B. Authentic standards.



**Figure S10.** Formation of (-)-coronaridine from stemmadenine acetate (TiDPAS2). A. UPLC/MS chromatograms illustrating the formation of (-)-coronaridine (2, green) from stemmadenine acetate (6, orange) in assays with CrPAS, TiDPAS2 and TiCorS. Peaks marked with \* were labile and not characterized. Trace quantities of vincadifformine (11) were detected. B. Authentic standards.

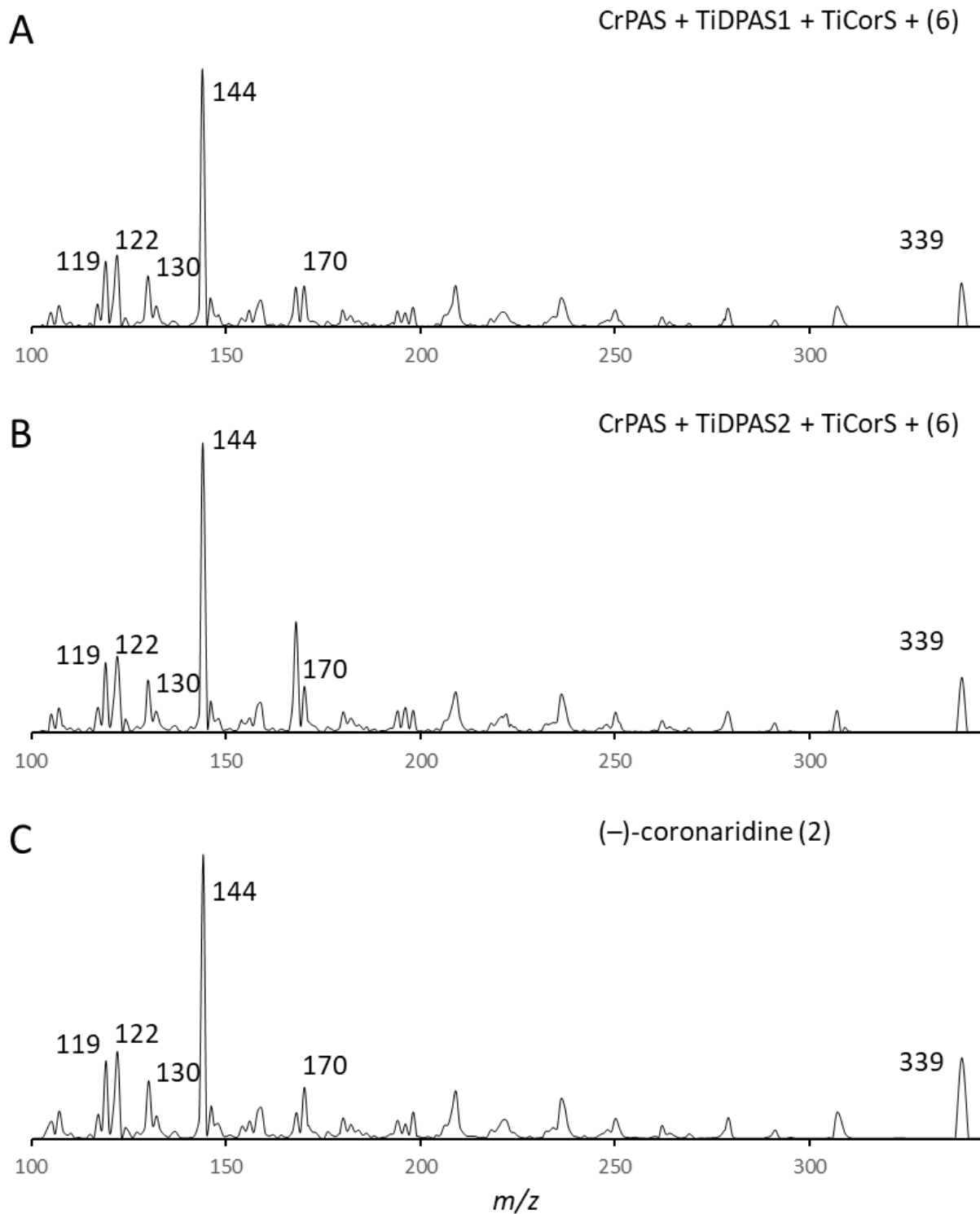


**Figure S11.** Formation of (-)-coronaridine from precondylocarpine acetate (TiDPAS1). A. UPLC/MS chromatograms illustrating the formation of coronaridine (2, green) from precondylocarpine acetate (7, orange) in assays with TiDPAS1 and TiCorS. Peaks marked with \* were labile and not characterized. Trace quantities of vincadifformine (11) were detected. B. Authentic standards.

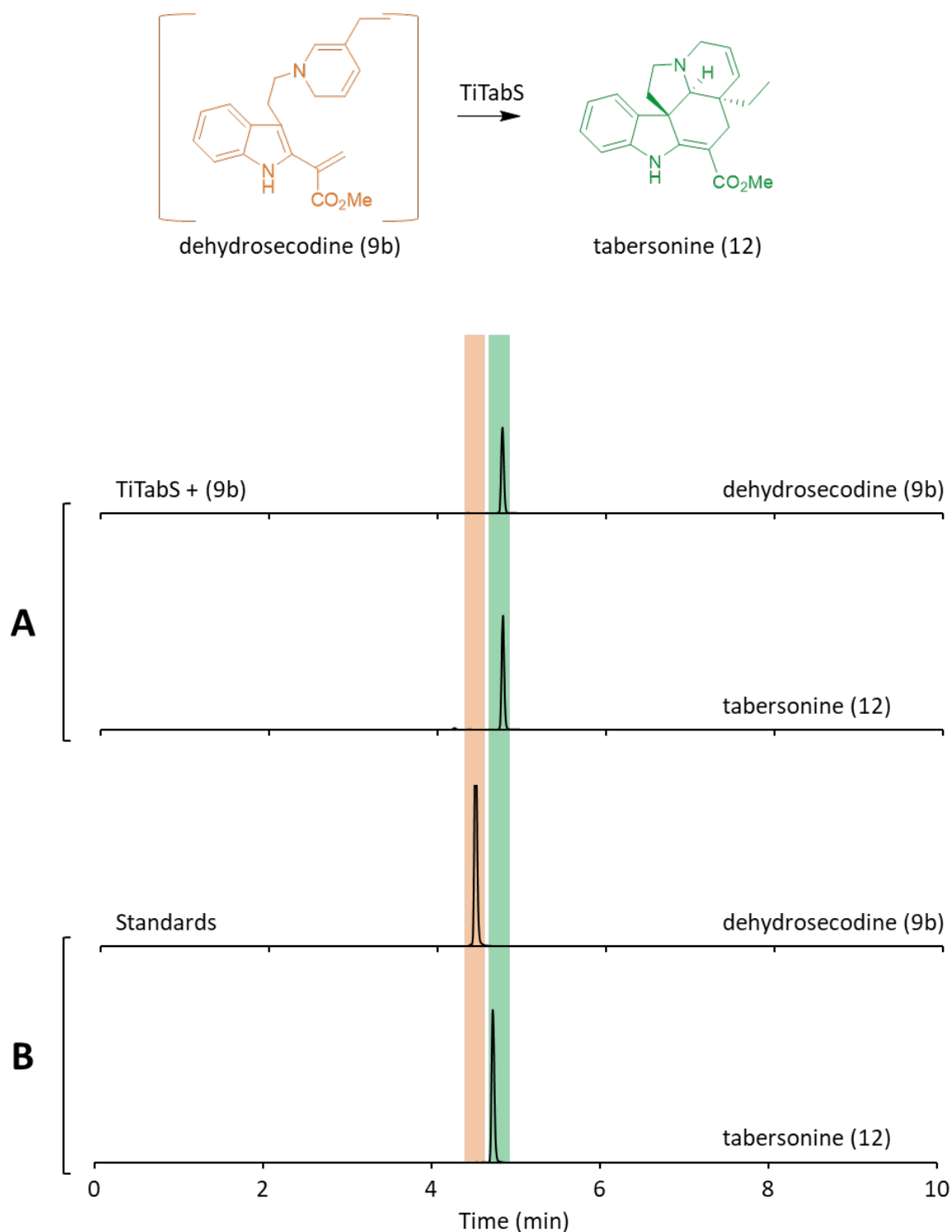


**Figure S12.** Formation of (-)-coronaridine from precondylocarpine acetate (TiDPAS2). A. UPLC/MS chromatograms illustrating the formation of coronaridine (2, green) from precondylocarpine acetate (7, orange) in assays with TiDPAS2 and TiCorS. Peaks marked with \* were labile and not characterized. Trace quantities of vincadifformine (11) were detected. B. Authentic standards.

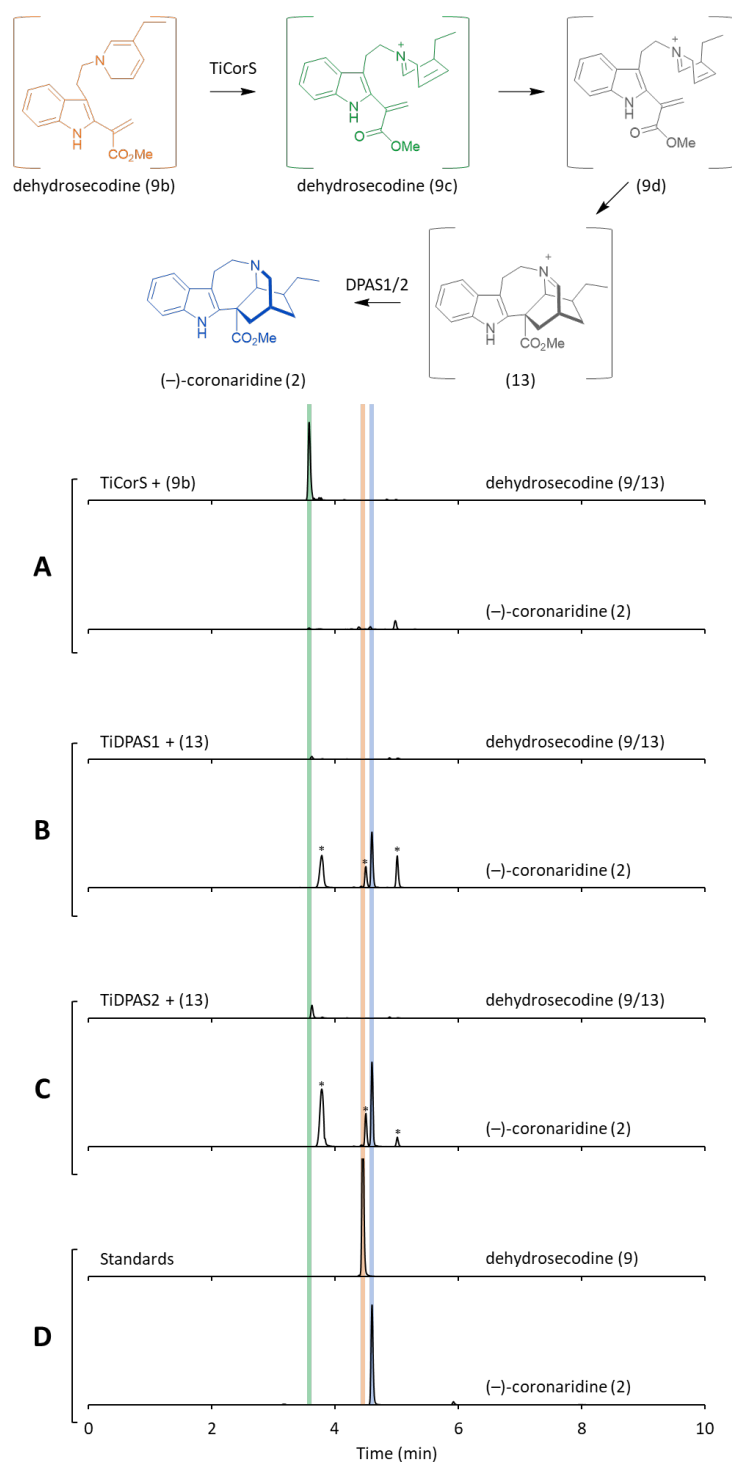




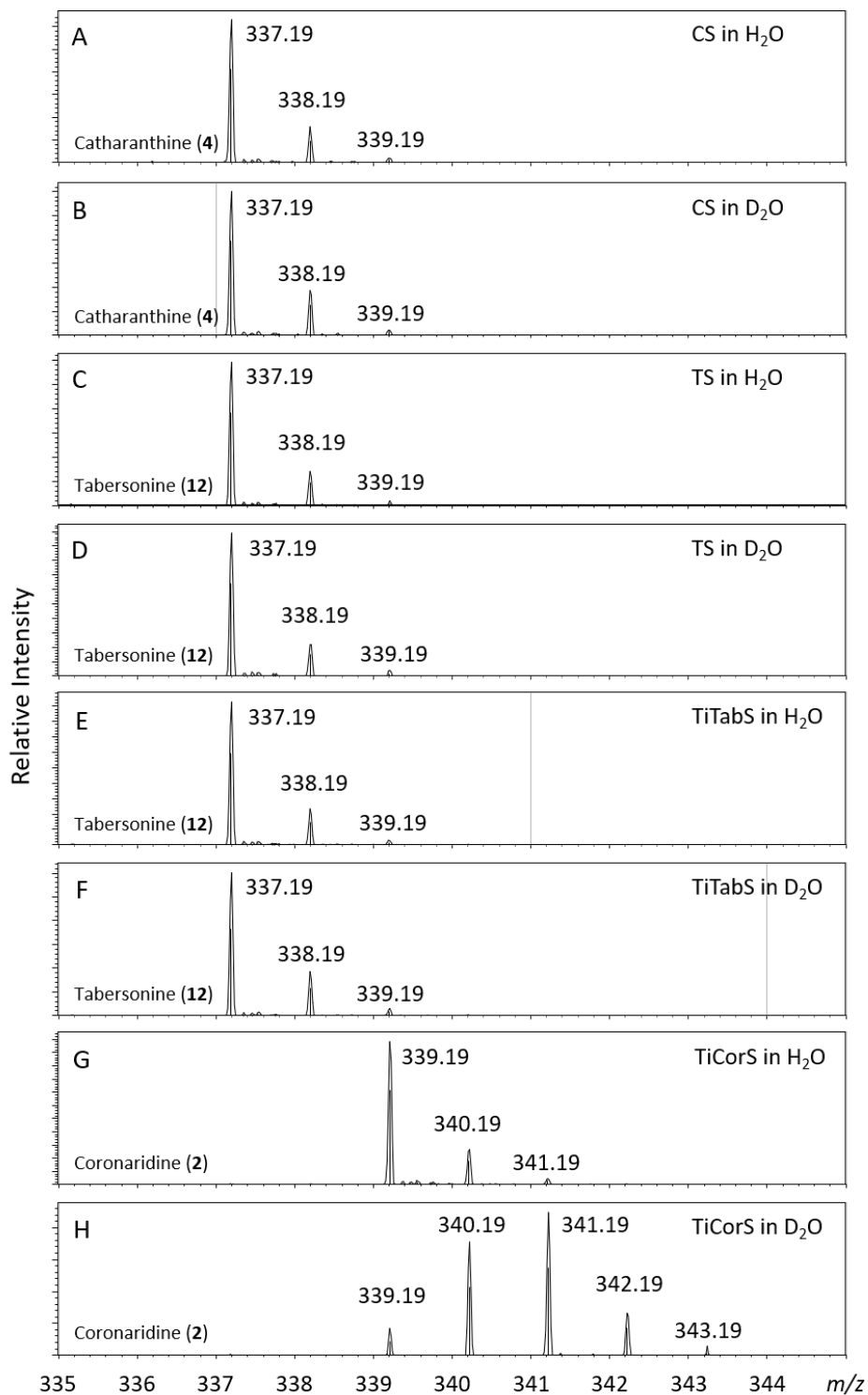
**Figure S13.** Product ion spectra of (–)-coronaridine. A. MS/MS product ion spectra of enzymatically produced coronaridine (2) from assays containing CrPAS, TiDPAS1 and TiCorS, or B. CrPAS, TiDPAS2 and TiCorS. C. Product ion spectra of authentic (–)-coronaridine (2) standard.



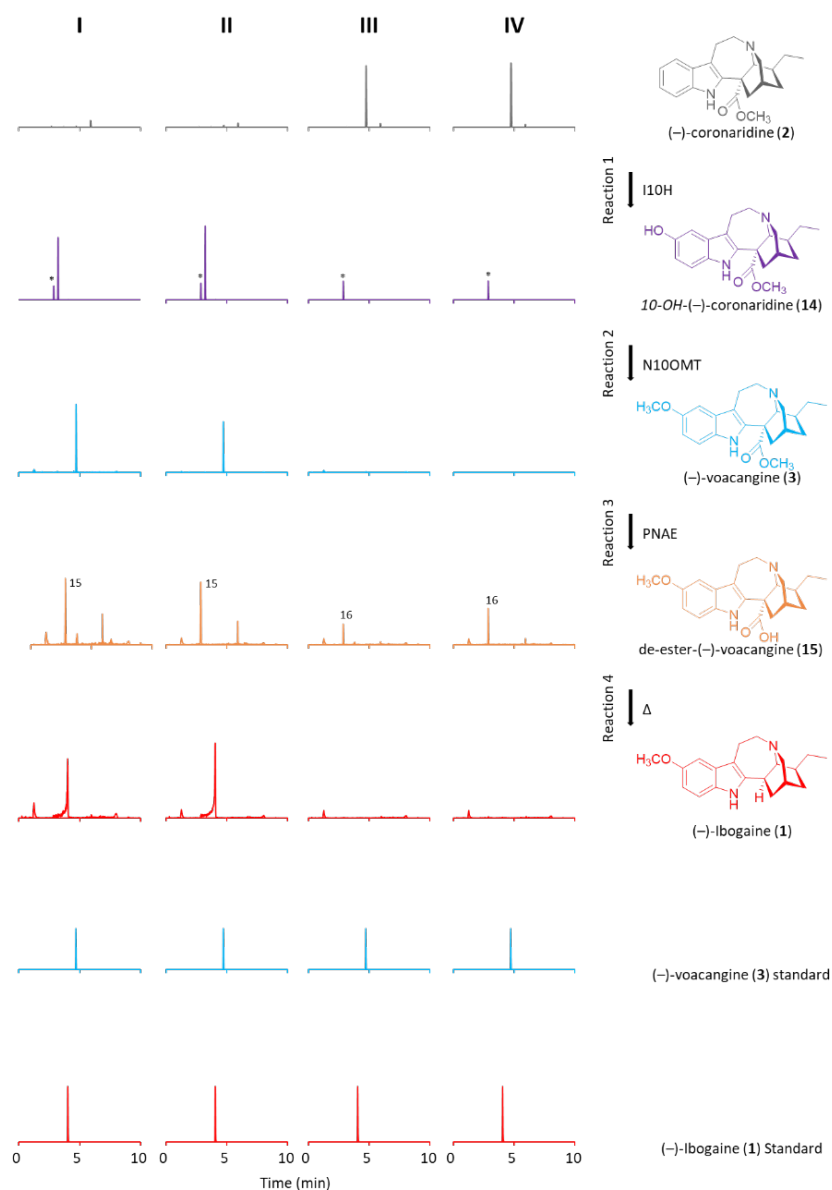
**Figure S14.** Activity of TiTabS from intermediate 9. A. UPLC/MS chromatograms illustrating the formation of tabersonine (12, green) from putative dehydrosecodine isomer 9 (orange), isolated from reaction of precondylocarpine acetate (7) and TiDPAS1 in assays with TiTabS. B. Authentic standards of 9 (isolated from reaction of 7, TiDPAS1 and 1 eq. NADPH) and tabersonine (12).



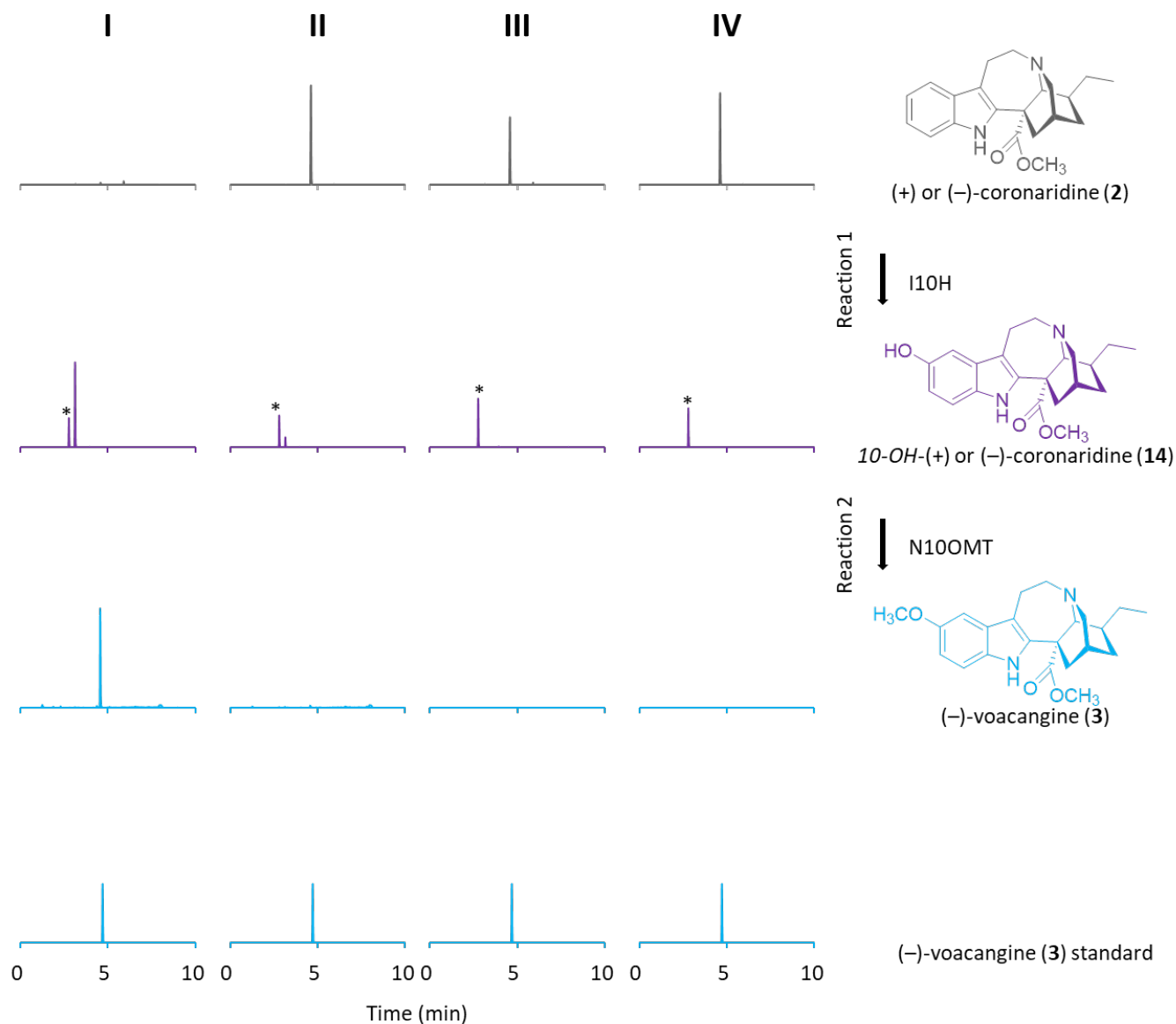
**Figure S15.** Intermediates 9 and 13 in (-)-coronaridine biosynthesis. A. UPLC/MS chromatograms illustrating the formation of 13 (green) from dehydrosecodine isomer 9 (orange) in assays with TiCorS. After 20-minute incubation with TiCorS with 9 to produce 13 (green), either TiDPAS1 (B) or TiDPAS2 (C) was added to partially purified 13 and NADPH to yield coronaridine (2, blue). Authentic standards are shown in (D). Peaks marked with \* were decomposed during isolation attempts and could not be characterized.



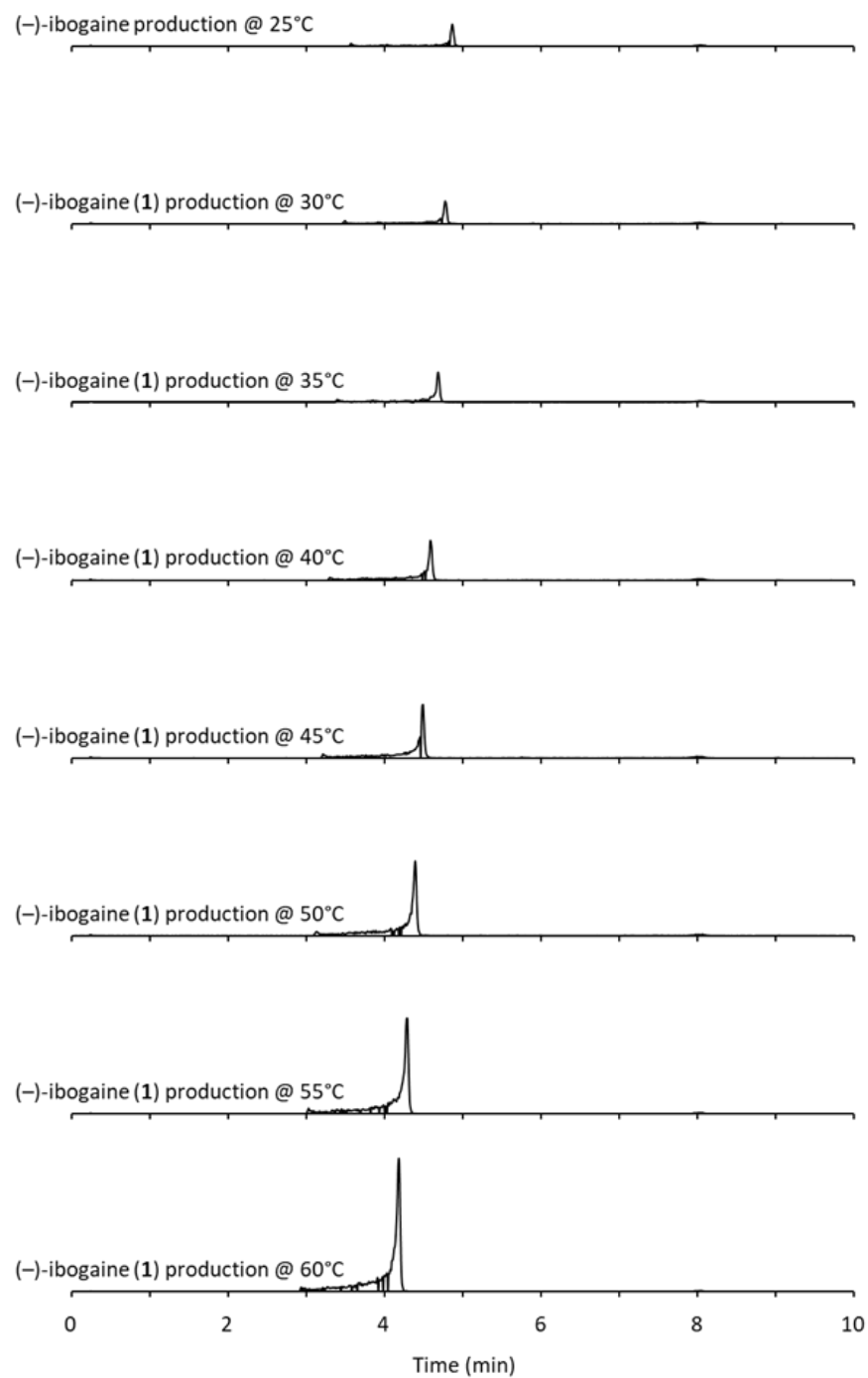
**Figure S16.** Isotopic labeling of (-)-catharanthine by reaction in D<sub>2</sub>O. High resolution MS spectra of enzymatically produced (A) catharanthine (4), (C,E) tabersonine (12) and (G) coronaridine (2) produced in H<sub>2</sub>O. High resolution MS spectra of enzymatically produced (B) catharanthine (2), (D,F) tabersonine (12) and (H) coronaridine (2) produced in D<sub>2</sub>O. Panel H shows coronaridine (2) with 1 (m/z 340.2) and 2 (m/z 341.2) deuterium atoms incorporated.



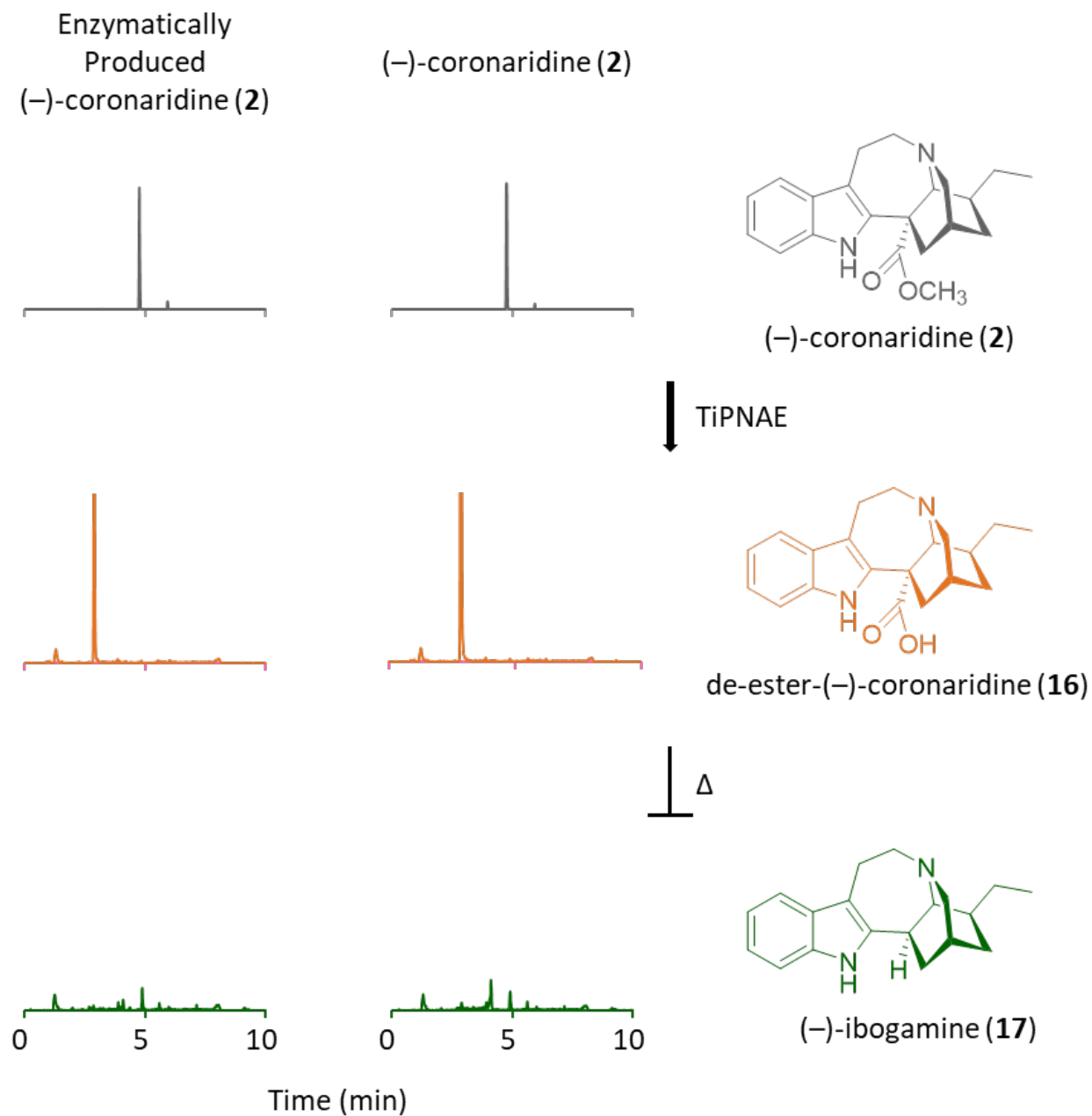
**Figure S17.** Biocatalytic production of ibogaine. Enzymatically produced coronaridine (2) (column I) and authentic (-)-coronaridine (2) (column II) incubated with I10H (reaction 1), yields a compound with a mass consistent with (-)-10-OH-coronaridine (14). Addition of N100MT (reaction 2) to these reactions yields (-)-voacangine (3). Subsequent de-esterification of (-)-voacangine (3) by TiPNAE (reaction 3) yields (-)-de-ester-voacangine (15), and after heating, (-)-ibogaine (1). No products were formed when enzymatically produced coronaridine (2) (column III) or authentic (-)-coronaridine (2) (IV) were incubated with empty vector controls. Peaks marked with an \* were products of endogenous yeast enzymes in feeding assays with I10H. De-ester-(-)-coronaridine (16) produced via TiPNAE in control reactions. These co-elute with de-ester-(-)-voacangine (15) and are detected due to MRM inter-channel cross-talk.



**Figure S18.** Specificity of I10H and N100MT for (-)-coronaridine. Enzymatically produced coronaridine (2) (column I) incubated with I10H (reaction 1), yields a compound with a mass consistent with 10-OH-(-)-coronaridine (14) (purple). (+)-Coronaridine (column II) incubated with I10H (reaction 1), yields only trace quantities of 10-OH-(+)-coronaridine (14) (purple). Addition of N100MT (reaction 2) to I and II yields (-)-voacangine (3) only in I (light blue), showing the specificity of N100MT for the (-)-coronaridine (2) optical series. No products were formed when enzymatically prepared coronaridine (column III) or (+)-coronaridine (5) (IV) were incubated with empty vector controls. Peaks marked with \* were products of endogenous yeast enzymes present in feeding assays with I10H.



**Figure S19.** Decarboxylation of (-)-voacangine. After incubation of (-)-voacangine (3) with TiPNAE1, the resulting de-esterified (-)-voacangine (15) was subjected to increasing temperature on a UPLC column. The decarboxylated product (-)-ibogaine (1) increases with increasing temperature.



**Figure S20.** Decarboxylation of coronaridine. Enzymatically produced coronaridine (2) and authentic (-)-coronaridine (2) are de-esterified by TiPNAE to yield a compound with a mass consistent with de-ester-(-)-coronaridine (16); however, heating this product even to 60 °C does not decarboxylate to yield (-)-ibogamine (17).



**Table S1.** Primer sequences used in this study.

Primers used for Full-length Gene Amplification			
Gene	Strand	In-Fusion Overhangs	Primer Sequence (5'-3')
TiPAS1	Forward	AGTTCGTGTTCTTGTCAATTAATTAATG	TATACTACTGAAGTTCGCAAAG
TiPAS1	Reverse	GATGGTGATGAGAAGAACCAGAACC	AAGTTCGTCCTTGGAAGGAAG
TiPAS2	Forward	AGTTCGTGTTCTTGTCAATTAATTAATG	GTTGAAGTCTCTAAAGTTCCTTC
TiPAS2	Reverse	GATGGTGATGAGAAGAACCAGAACC	CGATGATTCGTCTTGTGAAGTG
TiPAS3	Forward	AGTTCGTGTTCTTGTCAATTAATTAATG	TTAGCAGAAGTCTCCAAAG
TiPAS3	Reverse	GATGGTGATGAGAAGAACCAGAACC	CAATTCATCATGTAAAGTTAGAGGC
TiDPAS1	Forward	AAGTTCTGTTTCAGGGCCCG	GCTGTAAAATCACCTGAAGCAGAG
TiDPAS1	Reverse	ATGGTCTAGAAAGCTTTA	TTCCGGTGGAGTTAGTGTGTTTC
TiDPAS2	Forward	AAGTTCTGTTTCAGGGCCCG	GCAGGAAAATCACCAGAAGAGGAAC
TiDPAS2	Reverse	ATGGTCTAGAAAGCTTTA	CGGTTCTGGCGGAGGAGTTA
TiTabS	Forward	AAGTTCTGTTTCAGGGCCCG	GCTTCTTCAACTGAAAGCTC
TiTabS	Reverse	ATGGTCTAGAAAGCTTTA	CTCCTTGTTGATGAAAGACG
TiCorS	Forward	AAGTTCTGTTTCAGGGCCCG	GCTAATTCAACTGCAAACCTCTG
TiCorS	Reverse	ATGGTCTAGAAAGCTTTA	CTCCTTGTTGATGAAATCGC
TiPNAE1	Forward	AAGTTCTGTTTCAGGGCCCG	GCAACACAAAATTCCTCCTCC
TiPNAE1	Reverse	ATGGTCTAGAAAGCTTTA	ATATTTTCATTGCTACTTCGAGCAAATTG
TiPNAE2	Forward	AAGTTCTGTTTCAGGGCCCG	GCAACACAAAATTCCTCCTCC
TiPNAE2	Reverse	ATGGTCTAGAAAGCTTTA	GTAATATTTTCATTGCTACTTCGAGC
TiPNAE3	Forward	AAGTTCTGTTTCAGGGCCCG	CCCACAGAAAATTCCTCCTC
TiPNAE3	Reverse	ATGGTCTAGAAAGCTTTA	GAAATATTTTCATTGTTACTTGGATCAG
Primers used for Colony PCR			
Name	Strand	Vector	Primer Sequence (5'-3')
GAL10	Forward	pESC-leu2Δ	GGTGGTAATGCCATGTAATATG
GAL10	Reverse	pESC-leu2Δ	GGCAAGGTAGACAAGCCGACAAC
pOPINF	Forward	pOPINF	TAATACGACTCACTATAGGG
pOPINF	Reverse	pOPINF	TAGCCAGAAGTCAGATGCT
pTRBO	Forward	pTRBO	CGATGATGATTCGGAGGCTAC
pTRBO	Reverse	pTRBO	GCACCACGTGTGATTACGGA

**Table S2.** Gene sequences used in this study. Underlined bases indicate mutation sites.

<p>&gt;TiPAS1 (GenBank:MK840850)</p> <p>ATGTATACTACTGAAGTTCGCAAAGTTTTCATATCTTACTGCTTCTCCTTCTTGTCTCACAATGCCACTGCTTCATTCTCTGAATCTTTTCATCAGTTGTCTTTCCAAAGCAATTTCCATCGGATGAACCCATATTCAGCGTTCTGCATGATCGCCGTAATGCTTCATATCAATCTGTCTGGAATCTAATCTTCAGAATCTTAGATTTCTCAAATCAGCAAAACCATTTGGCTATCATCACTCCTCTCCTTTACTCCCATGTCCAAGCTGCTGTTGTCTGTTGCAAACGAGCTGGACTACAAATCAGAATCCGCAGTGGTGGCAATGACTATGAAGGCTTGTTCATATCGTTCTGAGGTTCCATATATCATTTCTAGACCTTCAAATCTTAGGTCAATCATGGTTGACATTGACGACAACAGTGTCTGGGTTGAGTCAGGAGCAACTATTGGTGAAGTGTATTACGAGATAGCTGAGAAGAGTCTGTTCATGCCTTTCTGCCGGTGTCTATCCGACCGTTGGCGTTGGCGGGCACTTAAGTGGTGGTGGGCTTGGTACTATGCTCAGAAAATATGGACTTGTCTGCCGATAACATCCTTGATGCTCATATTGTTGATGCTGAAGGAAGACTTCTCAATAGGGAATCCATGGGAACAGATTGTTTTGGGCCATCAGAGGAGTGGAGGAGCAAGTTTGGTGTCTAGATTGCCTGGAAAATCAAACCTTGTGCATGTTCTCCTCAGTAGTCACAGTATTTGATTTGGCCAAGACTTTGGAGGAAGGAGCCATAGATCTTACTCACAAATGGCAAACCGTCGGACCCAATCTCAATGAAGATGCATTTCTTGCCGCTAGTATTATGGCAGATCCATCAAGTGAACATAAACAACCTCTGGCAGGTTTCTTTTCATTGTTCTTGGTACAGCTGACCAACTCTGAAAGAAATGGGGGAAAGCTTCCCCGAGCTCGGCCTACGAAAAGAGCATTGTTTGAGATGAGTTGGATCAAAGCAGCACTGCAATTTTTCAGGGTATGAATCTGGTGAACAGTATATGCACCTTCAAAATCGAAAGCCTCTCAACCGAAGCATTGCAATCAGTAAGGTCAGATTTTATTCAAGAACCTCTATCCCTGCCCGCATTAGACAAGTTGTGGAAGTTTTTATCAGAGGAAGAGATCCTCCCATAATTGCCATGCTTCTCATGGCGGAATGATGAGCAAAATATCAGAAACGGAATTCATACCCGTACAGAGAAGGTGTGATATATAGCTTTTCTACGAGTTAATTTGGGATTGTGAGGACGATTCTTGTCTGAAAGATACGTTAGTGCATTGACAAAGGCTCTATGACCATATGACTCCTTATGTGCTGAAACATCCAAGAGGTAGTTTTTTGAACATGAGAAGCCTCGAAATTGGCAAGACGATGATTATGGGACAACCTATTCAAAGCTGAGGAATGGGGATTGAAGTATTTCAAGAATAACTTCAAGAGGTTGGCCATTACCAAAGGTGCAGTTGATCCAGATAAATCTTTTACTTTCGAGCAGAGCATTCCCCCTCTTGCTTCCAAGGACGAACTT</p>
<p>&gt;TiPAS2 (GenBank:MK840851)</p> <p>ATGGTTGAAGTCTCTAAAGTTCCTTCATGTTTCTGCTTTCTTCTTCTCCTCTCATTGTCTCATGGCTTGATTCTGAGGCTTTTATCTACTTGTATTTCCAGGAATTTGAGTCATATGAATCCATTCTTAGGGTTCTGCATGATCCCGCAATTCTTCTACCATTCAGTGCTGCAATCAAGAATTCAAAATCTCAGATTTCTCAAGTCACCCAAACCATTTGGCTATTATCACTCCTCTACTTTACTCCCATGTCAGGGTGTATTTGTTGTAGCAAACAGGTGGGATTACAAATTAGAATCCGCAGTGGAGGAAATGACTATGAAGGGTTGTCTGATCGTTCTGAGGTTCCATTTATCCTTCTAGACCTCCATAACCTTAGATCCATTGCGGTTGACATTGAAGACAACAGCGCATGGGTTGAGTCAGGAGCAACCATTTGGCGAAGTATATTATGAGATTGCCAGAAAAGTCTATTTCATGCCTTCTCCTGCGGGGTGTATGCAACCGTTGGCGTTGGCGGGCACTTCAGCGGTGGTGGCTTTGGTACGATGCTCAGGAAATATGGACTAGCAGCTGACAATGTGATTGACGCTTATATTGTTGATGCCAGAGGTAGACTTCTGGATAGGGAATCAATGGGAGAAGATTTGTTTTGGGCCATCAGGGGAGGTGAGGAGCAAGTTTTGGCGTCATAGTTTTCATGGAAGATCAAACCTTGTGCATGTTCTCCTCAGTGGTCACTGTTTTTGACATGCCCAAGACTTTAGAGCAAGGAGCCTTGGATCTTCTGAACAAATGGCAATATATAGGACACAAGGTGAGTGAAGATCTATTCTTCTGTCTAAGTATCATGGCAGATACATCTGGTGGAAATAAACTCTTATGGCAGGTTTCAACTCGTTGTTTCTCGGCACAGCTGACCAGCTCTTGAAAGAAATGGCAGAAAGCTTCCCCGAGCTAGGGTTGAGAAAAGAACATTGCTCCGAGATGGGTGGATCAAGGCAGCAATGCATTTTTCTGGATATCCAAGTGTAGAAACACATCTCCACTTAAAAGTCGAGAACCTCCTCTACCCAAGACTTGCATTGCGACCAAGTCAGACTTCATTCAAGAACCTCTATCCCTGCCCGCATTAGAGAAGTTGTGGAAGTTGTTATGGGAGGAAGAGAATACTCCCATCATTCTCATGCTTCTCATGGCGGAATGATGAGCAAAATATCAGAATGGGAACCTTCATTCCCATACAGACAAGATGTGATATACAGCATGATCGATGAAGTAGTATGGGATTGTGAAGACGATTACTCCTCCGAAGAGCACATTAGTGGACTGAGAAAGCTCTATAGCTTTATGACGCTTATGTGTGAAACACCCAAGAGGTACTTTTCTAAACATGAGAAACCTTGACACGGGTAGGAACGGTGAATATTATGGCACAACCTTATTCAAAGCCAAGGAATGGGGATTGAAGTATTTCAAGAACAACCTTTCGAAAGTTGGCCATTACCAAGGGTGCAGTTGATCCAGATAAATCTTTTACTTTCGAGCAGAGCATTCGCGCTCTCACTTCACAAGACGAATCATCGGGTTCTGGTTCTTCTCATCACCATCACCATCACTGA</p>
<p>&gt;TiPAS3 (GenBank:MK840852)</p> <p>ATGTTAGCAGAAGTCTCAAAGTCTTTTCATGTTTCTACTTTCTTGTCTACTCTCAACATCGCATGGCTTGATTCTCTGAAGCTTTATTAATTTGTGTTTCCAGAAATTTGTGTGCGATCAATCTATTTTATGACATTCTGCATGGTCCCGGAAATTTCTCTATCATTCGTGTGCTGCAATCTAGAATTCAAAATCTTAGATTTCTCAAGTCACCCAAACCATTTGGCTATTATCACTCCGCTACTTTACTCTCATGTCCAAGCCGCTGTGTTTTGTAGCAAACAGGTGGGACTACAAATTAGAATCCGCAGTGGAGGGGCCGACTATGAAGGGTTGTCAATCGTTCTGAGGTTCTTTTGTCTACTAGACCTCCAAAATCTTAGATCCATTGCGGTTGACATTGAGGACAACAGCGCTTGGGTTGAGTCAGGAGCAACCTTGGCGAAGTGTATTATGAGATCGGTGAGAAAAGTCCGATTTCATGCCTTTCTGCAAGGGCCCTGTGCAACTGTAGGCGTTGGCGGGCACTTCAGTTGTGGTGGTTTTGGTACGATGCTCAGAAAATATGGACTGGCATCTGACAATGTAATGTATGCTTATATCGTTGATGCCACAGGTAGACTTCTGGATAAGGAATCAATGGGAGAAGATTTGTTTTGGGCCATCAGAGGAGGTGGAGGAGAAAGTTTTGGCGTCATAGTTTCTGTGAAGATCAAACCTTGTGTATGTTCTCCTCAGTGGTCACTGTTTTTGACTTGCCCAAGACTTTGGAGCAGGGAGCCTTAGATCTTCTTAACAAATGGCAGTATATAGGATACAAGCAAAGTGAAGATCTATTCTTGTGTGAAGTATCATGGCAGATACATCTGCTGGAAATAAAACCTTATGGCAGGTTTCACTCGTTGTTTCTGGGTACAGCTGACCAACTCTCAAGGAAATGCATGAGAGCTTCCCAGAGCTTGGCTTGGAGAAAGAATCTGCTCCGAGATGAGTTGGATCAAGGCAGCAATGCATTTTTCTGGATATCCAAGTGCAGAAACCATATCTGCACTGAGAAAATCAAGATCCTCCTCTACCCAAGACTTGCATTGCGACGACCAAGTCAGACTTCATTCAAGAACCTCTACCCTGGCAGCATTAGAGAAGTTGTGGAAGTTCTCATCGGACGAAGAGAATATCCCAATAATTCTCATGCTTCTCATGGCGGAATGATGAGCAAAATATCAGATTCTGAAACTCCATTCCCATACAGACAAGGTGTGATCTACAGCATGATCGAAGAAGTAGTTTGGGATTGTCAAGACGATTACTCCTCCGAAGAGCACGTTAGTGGACTGAGAAGGCTGTATGACCTTATGGCGCCTTATGTGTGAAACAGCCAAGAGGTACTTTTCAACCATCAGAAACCTCGATACCGGTAGAAATAATGATTCCGGGCACAACCTATTACAGCGGCAAAGGAATGGGGTTGAAGTATTTCAAGAACAACCTTTCGAAAGCTAGCCATTACCAA</p>

GGGTGCAGTTGATCCAGAAAACCTCTTTTATAATGAACAAAGCATTCCGCCTCTAACTTTACATGATGAATTGGGTTCTGGTTC TTCTCATCACCATCACCATCACTGA
>TiTabS (GenBank:MK840853) ATGGCTTCTTCAACTGAAAGCTCTGATGAGATTATTTTTGATCTTCCTCCATACATTAGAGTCTTTAAGGATGGAAGAGTAGAG AGACTCCACTCCTCACCATATGTTCCACCATCACTAGATGATCCGCAACCGGCGTATCCTGGAAAGACGTCCCAATTTTCATCAG AGGTTTCGGCTAGAATCTACCTCCCAAAGATAAGCCAAAAGGAAAAGGAAAAGCTTCCCATTGTGGTCTATTTCCATGGTGCAG GCTTCTGTCTGGAATCCGCCTACAAGTCATTTTCCACACTTATGTCAAGCACTTTGCAGCCGAGGCCAAAGCAATTCAGTTTC GGTTGAGTTTCAAGGCTCTCCCCAGAGCACCACCTGCCTGCAGCTTATGAAGATTGCTGGACTGCCCTTCAGTGGGTGGCTTCACAT GTAGATGTTGACAACTCCAGCCTCAAGAATGCTATAGATAAAGAGCCTTGATAATCAACCATGGAGACTTTGACAAGATCTAC TTATGGGGTGACAGTACGGGTGCCAATATTGTGCACAACGTACTCATCAGAGCTGGTAATGAGAGCTTGCATGGCGGAGTGAAA ATCGTGGGTGCAATTTCTTTATTACCCATATTTCTTGATCAGGACAAGCTCCAGACAGAGCGATTATATGGAGAACGAGTACAGA GCATACTGGAAGCTGGCTTATCCATCTGCTCCAGGTGGGAACGACAACCCGATGATAAACCCCGTAGCTGAGAACGCTCCTGATT TGGCTGGATATGGATGTTTCGAGGCTGCTGGTATCCATGGTGGCAGACGAGGCCAGAGACATAACCCCTTCTCTACATCGAGGCAG TGAAGAAGAGTGGGTGGAAAGGTGAATTGGAGGTGGCTGATTTTGAAGGAGATTACTTTGAAAATTCAGCCCAGAACTGAG ACAGGCAAGAACAAGGTCAAACGTTTAACTGCTTTCATCAACAAGGAGTAA
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>TiDPAS1 (GenBank:MK840855) ATGGCTGTAAAATCACCTGAAGCAGAGCACCCAGTGAAGGCTGTAAAATCACCTGAAGAAGAGCACCCAGTGAAGGCATACGGA TGGGCTATCAAAGACAGAACATCTGGCATTCTTTCCCTTCAAGTTTTCCAGAAGGGCAACAGGAGATGAAGACGTTTCAATA AAGATCCTCTGTTGCGGAGTTTGTACACGGATCTCAGCTACCAAGAATGAATACGAGTTTCTTTTCATATCCTCTAGTGCCTG GGTTGGAGACTGTGCGAATAGCGACAGAGGTGCGGAAGCAAAAGTCACAAAAGTAAAAGTTGGTGAAAAAGTAGCAGTGGCAGCC TATTTGGGCACCTTGTGGCAATGCCACAATTGTCTAAATGACCAAGAGAATTACTGTCCGAAGTGATCATTAGCTACGGCACA CCATATCAGACGGAACAATCAACTACGGAGGCTTCTCGAATGAGACGGTCGTAAATGAGCGCTTCTGTTCTTCATTTTCTGAA AAGCTTTCACCTTCTGGTGGTGACCGCTACTCAGCGCAGGAAGCACCGCTTACAGTGCAATAAGAAATCAAGGCCTTGACAAA CCCGGTATCCACTTGGGAGTGTGCGGCTTGGTGGACTTGGTCATCTGGCTGTGAAGTTTGCCAAGGCTTTTGGTGTCAAGGTGA CAGTGATTAGTTCCACTCCCAGCAAGAAGGATGAAGCCATCAAGAGCCTTGGTGCCGATGCGTTCTTGTTCAGTCTGATGATG AACAAATGAAGGCCGCTATTGGAACCTTTCGATGCAATCATAGATACTATTGCAGTCGCTCATCCTCTTGCGCCATTACTTGATCT ACTAAGGAGTCACTGAAAAATTTTGGTTCGGGGCACCGACCACCCCACTTGAGGTGCCAGTTATTCTTTAGTAGCAGGTGG GAAATCGATTACTGGATGCGTAGTTGGAAATTTGAAGCAAACTCAAGAAATGCTTGAATTTGCTGCAGAACACAACATCACTGC AAACGTTGAGGTTATTTCAATGGATTACATAAACACTGCAATGGAACGTTTAGAAAAAGGTGATGTTAGATATAGATTTGTAA TTGATATTGGAACACACTAACTCCACCGGAATAG
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>TiPNAE1 (GenBank:MK840857) ATGCCCACAGAAAATTCCTCCTCTGTAGACATTTCTGTGCTTGTTCATGGAGGTAATATTGGTGGCTGGTGGTACAAGTTG AAGATATTGTTAGAAGAAGCAGGGCACAATGTCAACCGCTTTCGATCTGGCTGCCTCAGGTGCGAATCTCGAGCCGTTGAACAAC

<p>CTTCAAACCATTTGACGACTACCATGAGCCTTTGTA</p> <p>CTATATGGCTGCCCTAGCTGCCAAGGATAGGGTCATTTTGGTTGGCC</p> <p>ATAGCTCCGGTGGATACGCAGTATGCTCTGCCATGGAGAGGTTTCCTTCCAAAATTGCTCTTGCTGTCTTTGTGACAGCAGGCAT</p> <p>GGTTGGTCCTAAATTTGACTGCTGATCAGATTCAGGAAAAAATATCAAGCCTTGAGAGACCAGACGACATTGAAGATAACATATT</p> <p>CACAGTTTATCCCAATGGGAATATAGATCTCCTTCTCGGGCCAGAATTCATGATACACAAAATGTATAACCAATCACCACCTGA</p> <p>GGACTATACTCTTGCAAGACTGTTAAACAAGAGTAATCCGAACATTCAATGACGAGAAAATCAAGACTTGAGCTCTTAGTTTCAA</p> <p>AGAGAGGTACGGATCAGTTCTCGAGCCTACTTCATTGCTACAGAGGACAATCTATTTTCTATAGAGATAACAACGTTGGTTGAT</p> <p>AGAAACTAATCCTCCAAATGAGGTTCGGGAAAATTAGAGGTGCAGATCACATGGTGATGTTCTCTAAACCGTCGGAGTTATGCCA</p> <p>CAATCTGATCCAAGTAACAATGAAATATTTCTAA</p>
<p>&gt;TiPNAE2 (GenBank:MK840858)</p> <p>ATGGCAACACAAAATTCCTCCTCCTGTAGACACTTCGTGCTGGTTCACGGAGCTAATCATGGAGCATGGTGCTGGTACAAGTTG</p> <p>AAGATACTGTTGGAAGAAGCAGGGCACAAAGTCACTGCTATCGACCTGGCTGCCCTCAGGTCAGAATGCTGAGCCGCTGAACAAC</p> <p>CTTCAAACCATTTGACGACTACCATGAGCCTCTATACTCATAACATGGCAGCCCTAGCTGCCGACTATACTGTTATTTTGGTTGGCC</p> <p>ACAGCTATGGTGGCTACGCTGTTTCCTCTGCCATGGAGAGGTTTCCTTCCAAAATTGCCCTTGCTGTCTTTGTGACTGCTGCCAT</p> <p>GGTTGGTCCTGATTTGAATGTGGCTCAGCTACAGGAAAAGATATCAAGTCTTGTTGGACCAGATTACGCTGAAGATAGCATATC</p> <p>TACCATTTATCCCGACGGGAATATCGATGACCTAGTTGGGCCAGAATTCATAGTACACAAAATGTATAACTTATCGTCACTTGA</p> <p>GGATTATACTCTTGCCATATTGTTAAAAAGAGTAATCGAATATTCTATGACGAGAAAATCAAGACTTCAACTCTTCGTTTCAA</p> <p>GAAGAGGTACGGATCAGTTCTCGAGCCTTCTTCATTGCCACAGAGGACAAGGCATGTCCTGTAGAGTTACAGCGTTGGTTGAT</p> <p>AGAACTAATCCTCCAAATGAGGTTCGGGAAAATTAGGGGCGCAGATCACATGGTGATGTTCTCTAAACCGCTGGAGTTATGCCA</p> <p>CAATTTGCTCGAAGTAGCAATGAAATATTAA</p>
<p>&gt;TiPNAE3 (GenBank:MK840859)</p> <p>ATGGCAACACAAAATTCCTCCTCCTGTAGACATTTTCGTGCTGGTTCATGGAGCCAATCATGGAGCATGGTGCTGGTACAAGTTG</p> <p>AAGACACTGTTGGAAGAAGCAGGGCATAAAGTCACCGCCGTCGATCTGGCTGCCCTCAGGTCAGAATCTTGTCGCCGCTGAACGAC</p> <p>CTTCAAACCATGGACGACTACCATAAGCCTTTGTA</p> <p>CTATATGGCAGCCCTTGCTGCTGAGGATAGGGTCATTTTGGTTGGA</p> <p>CATAGCTACGGTGGGTTAGCAGTATCCTCTGCCATGGAGAGGTTTCCTTCCAAAATTGCTCTTGCTGTCTTTGTGACAGCTGGCA</p> <p>TGGTTGGTCCTAATTTCACTGCGAATGAGCTTTGGGAAAAGATGTCAAGCGTTGAGGGACCGGACTTTTTTGAAGATAGCGTAA</p> <p>CCACAGTTTATCCCAATGAGAATATGGATCTCGAGGCTGGGCCAGAATTCCTGAAACACAAGTTGTATAACCTATCACCCTTG</p> <p>AGGATTATACTCTTGCAAGATTGTTAAACAAGAGCAACCCGAATATTCTGTGACAACAAATCAATACTTGACCCTTAGTTTCAA</p> <p>AGGAGAGGTATGGAACAGTTCTCGAGCCTACTTCATTGTTACAGATGACAAGGCAACGTCTGAAGAGATAACAACGCGTCATGA</p> <p>TAGAAGCTACTCCTCCAAATGAGATCCGGGAAAATTAGAGGTGCAGATCACTTTGTGATGCTCTCTAAACCGCTGGAGTTATGCC</p> <p>ACAATTTGCTCGAAGTAGCAATGAAATATTACTAA</p>

**Table S3.** MRM transitions used for metabolite detection with UPLC/MS method.

Compound	Precursor ion ( <i>m/z</i> )	Product ion ( <i>m/z</i> )	Collision Energy (CE)
Ibogamine (17)	281.2	122.1 144.0	36 36
Ibogaine (1)	311.2	122.1 173.8	30 30
De-ester-(–)-coronaridine (16)	325.2	144.2 237.2	30 30
Dehydrosecodine/iminium (9/13)	337.2	122.0 228.0	20 20
Catharanthine (4)	337.2	173.1 165.1 144.1	16 20 20
Tabersonine (12)	337.2	305.2 228.2 168.1	22 22 36
Vincadifformine (11)	339.2	307.2	27
Coronaridine (2/5)	339.2	144.2 307.2	30 30
10-OH-(–)-coronaridine (14)	355.2	146.1 160.1	40 40
De-ester-(–)-voacangine (15)	355.2	144.1 209.1	40 24
Voacangine (3)	369.2	309.3 337.3	24 24
Precondylocarpine acetate (7)	395.2	234 228.1 196.1	38 22 32
Stemmadenine acetate (6)	397.2	337.1 228.1 168.0	18 24 40

**Data S1.** (separate file)Proteomic analysis of TiPAS1-3 expressed in *N. benthamiana*.

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