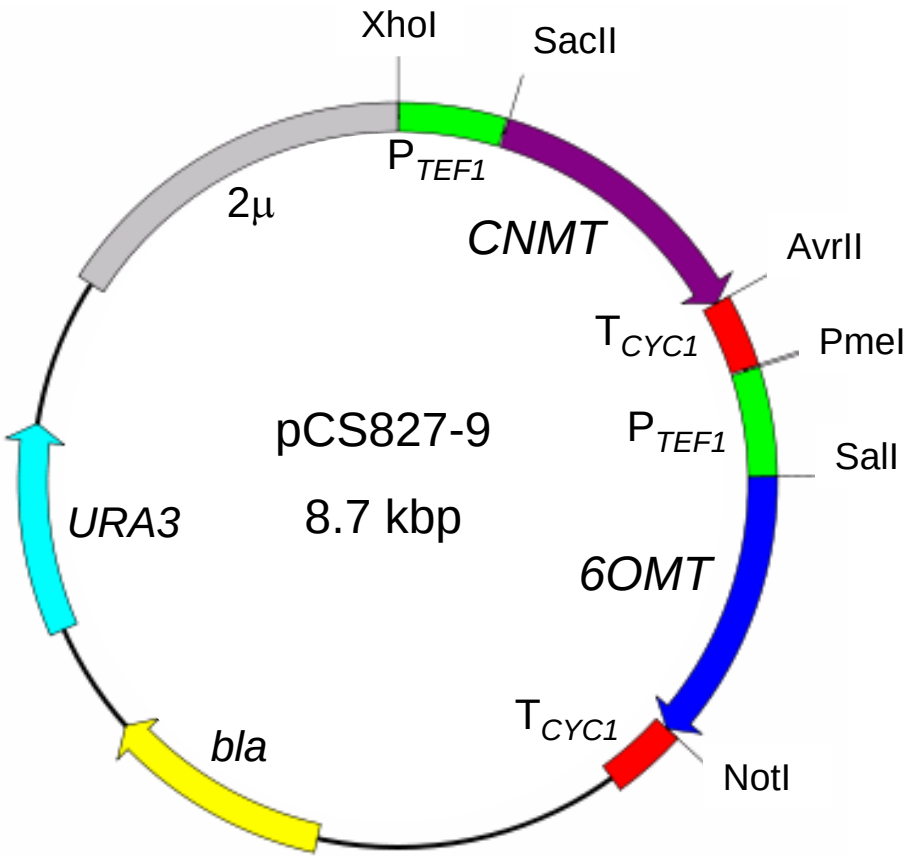
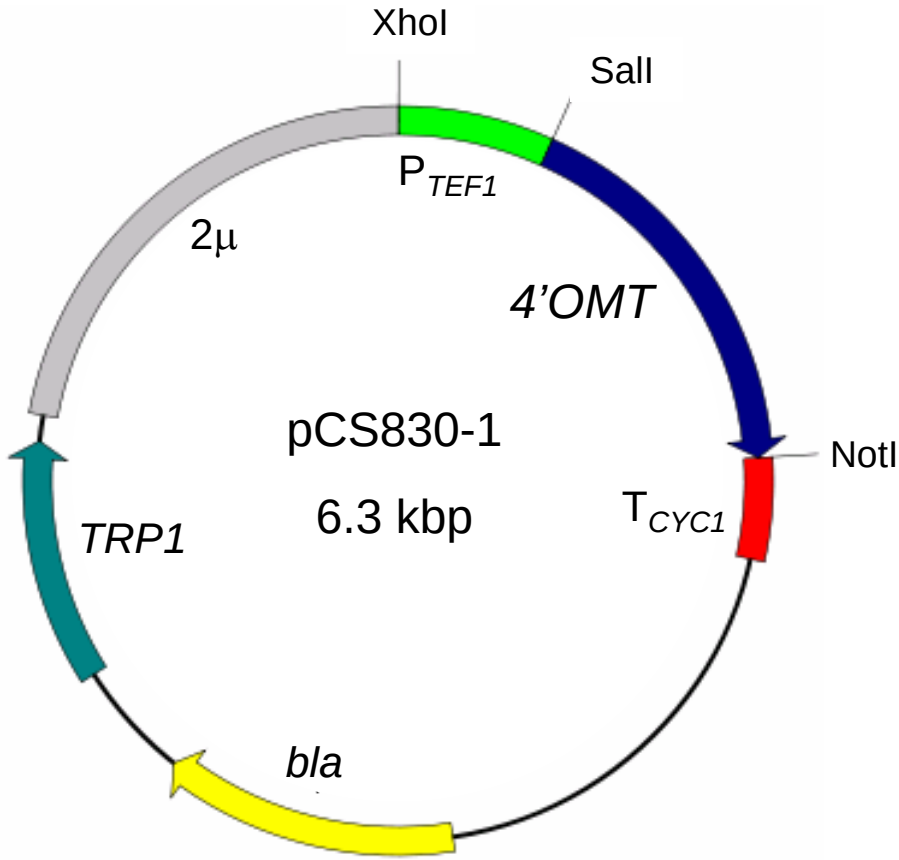
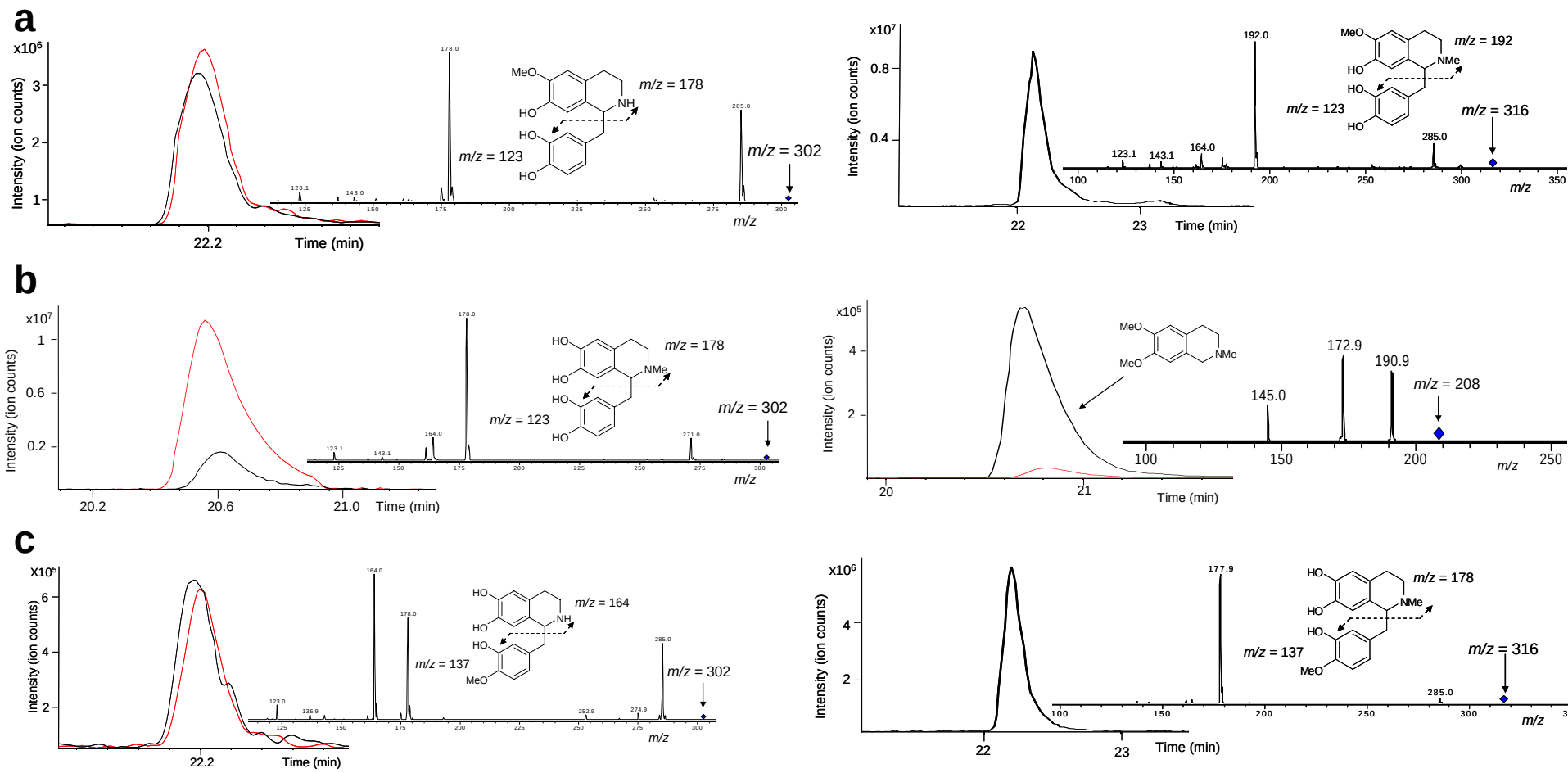
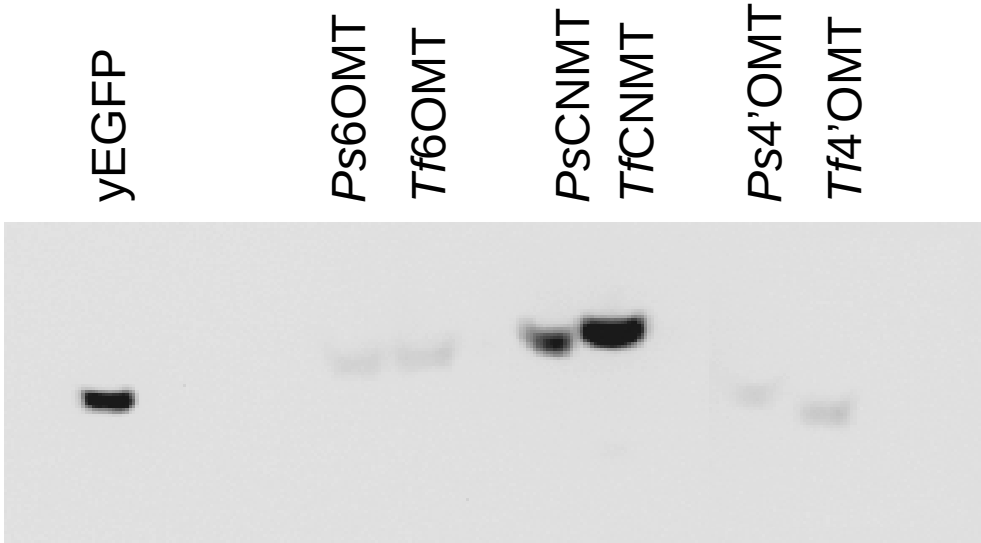




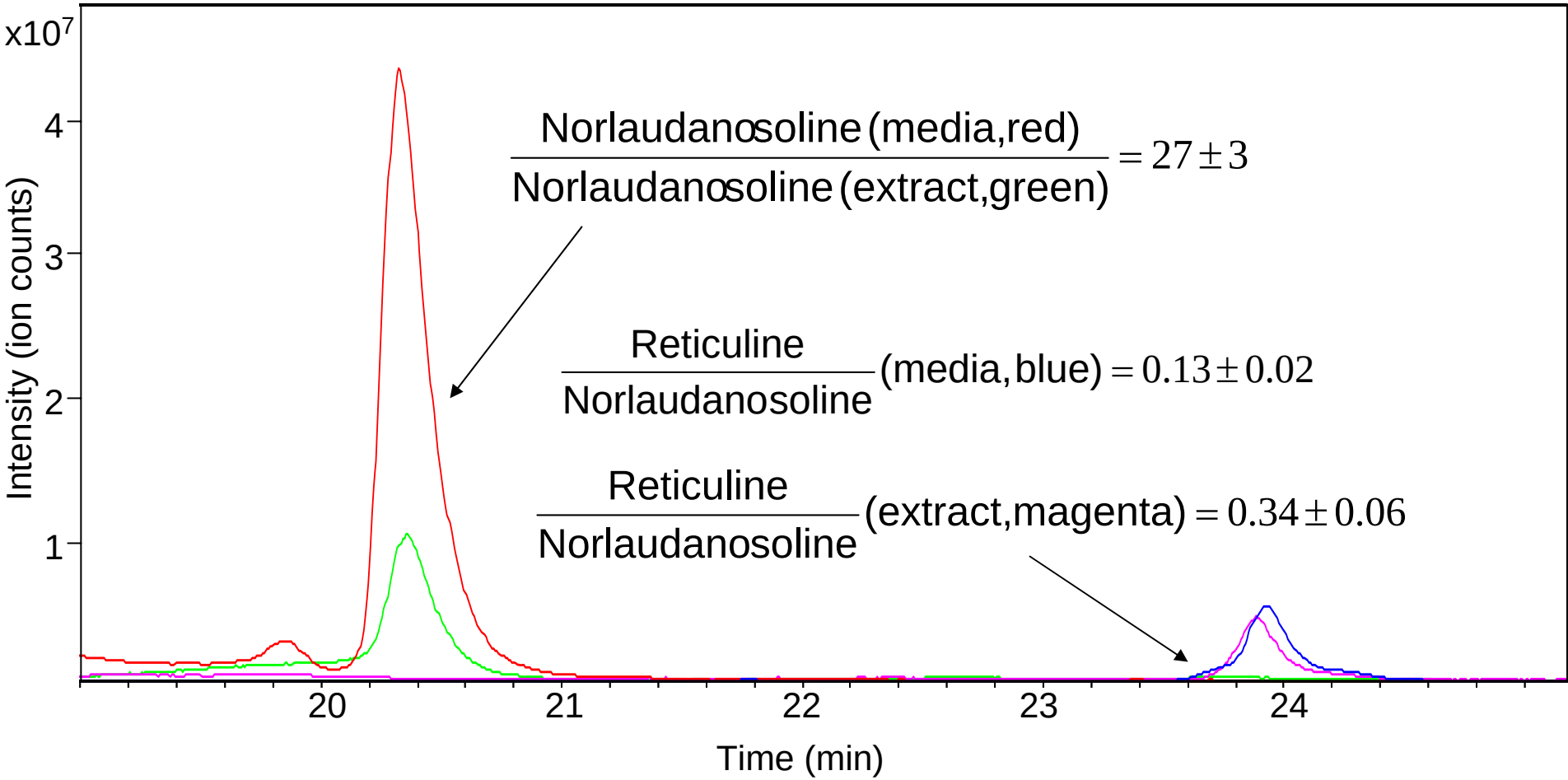
Supplementary Figure 1 Plasmid maps of BIA expression constructs. Heterologous cDNA sequences are expressed in yeast from constitutive TEF1 promoters on high-copy plasmids with different selection markers. The expression systems are used for the combinatorial expression of 6OMT, CNMT, and 4'OMT enzyme variants.



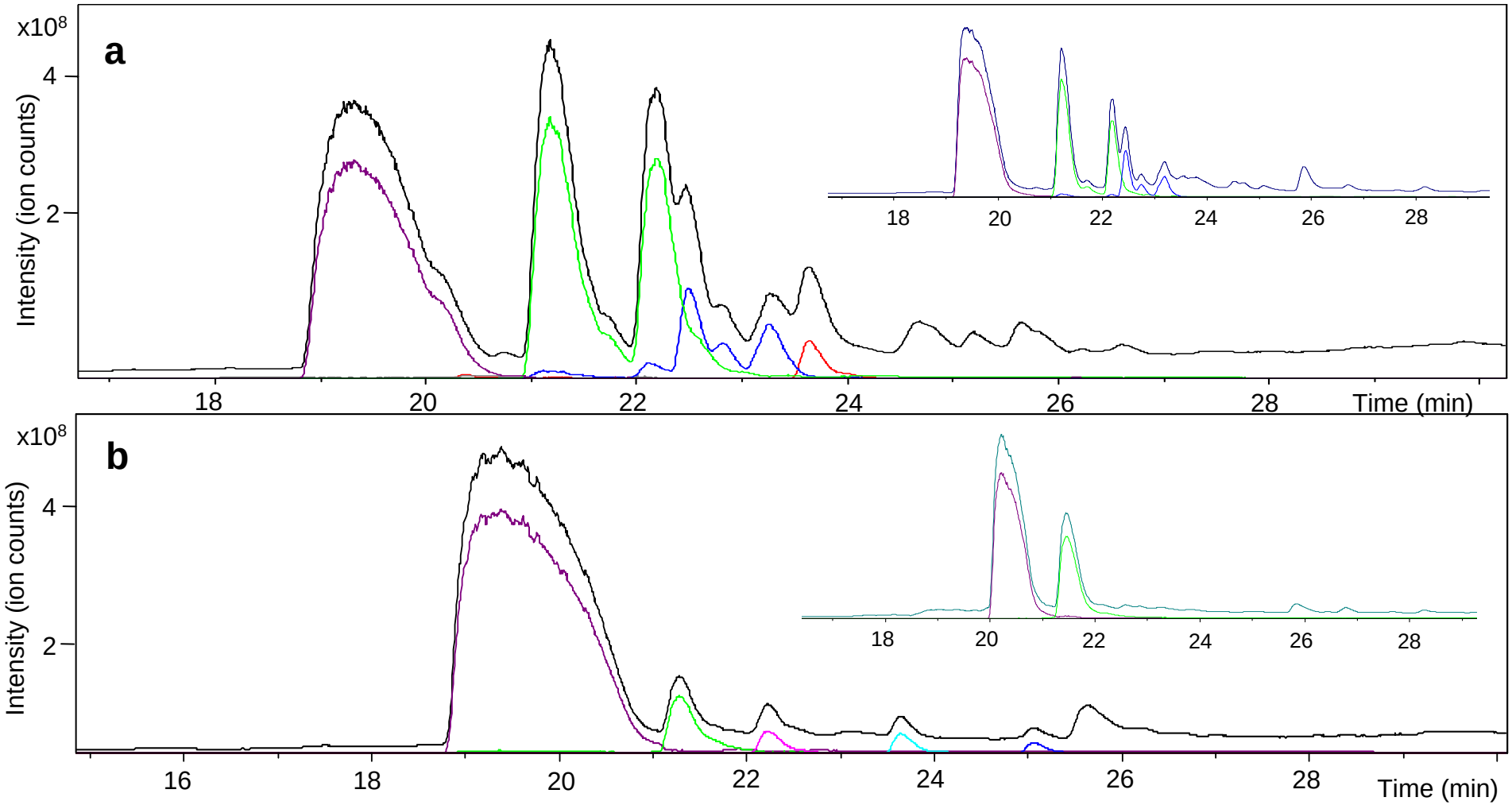
Supplementary Figure 2 LC-MS/MS analysis of the growth media of engineered yeast strains supplemented with 1 mM fed substrate and grown for 48 h confirms individual enzyme activities. (**a**) Characterization of 6OMT fed norlaudanosoline (left) and laudanosoline (right). Left, extracted ion chromatograms for *m/z* = 302 are shown for CSY450 (red) and CSY451 (black). Right, extracted ion chromatogram for *m/z* = 316 is shown for CSY450 and is representative of CSY451. (**b**) Characterization of CNMT fed norlaudanosoline (left) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (right). Left, extracted ion chromatograms for *m/z* = 302 are shown for CSY452 (red) and CSY453 (black). Right, extracted ion chromatograms for *m/z* = 208 are shown for CSY452 (red) and CSY453 (black). (**c**) Characterization of 4'OMT fed norlaudanosoline (left) and laudanosoline (right). Left, extracted ion chromatogram for *m/z* = 302 are shown for CSY454 (red) and CSY455 (black). The 4'OMT reaction product (*m/z* = 302) appears to contain a mixture of norlaudanosoline methylated at both the 6-*OH* and 4'-*OH* positions with major fragments of *m/z* = 164 and 178 observed. The unexpected *m/z* = 178 ion is believed to be due to a co-eluting impurity in the norlaudanosoline with the parent ion *m/z* = 314. Right, extracted ion chromatogram for *m/z* = 316 is shown for CSY454 and is representative of CSY455. The fragmentation pattern of the 4'OMT reaction product does not show evidence of non-specific methylation.



Supplementary Figure 3 Western blot analysis confirms protein expression of 6OMT, CNMT, and 4'OMT enzymes. Enzyme levels are comparable to levels of a yeast-enhanced GFP variant (yEGFP) expressed from the same construct (within ~2-3-fold signal). All proteins were expressed behind the TEF1 promoter on high-copy plasmids with a C-terminal V5 epitope tag. The blot was incubated with the Anti-V5 HRP antibody and a chemiluminescent assay was used for signal detection. Each lane was loaded with ~50 μg total protein. The CNMT variants are the most highly expressed and/or stable of the recombinant enzymes. At higher concentrations, degradation products were visible from the *Tf*CNMT, indicating that this protein may be subject to proteolysis in yeast, although the full-length protein still dominates.



Supplementary Figure 4 BIA metabolites accumulate in the growth media. Analysis of norlaudanosoline (substrate) and reticuline (product) concentrations in cell extracts and growth media support a passive diffusion transport mechanism for BIA metabolites. Data shown is a 1:10 dilution of the growth media (norlaudanosoline, red; reticuline, blue) and a ~1:2 dilution of the cell extract (norlaudanosoline, green; reticuline, magenta) of CSY288 supplemented with 1 mM norlaudanosoline and grown for 48 h. Ratios of product to substrate and extracellular versus intracellular norlaudanosoline concentrations are shown as calculated by LC-MS peak areas. Chromatograms and calculations are representative of two independent experiments ± s.d.



Supplementary Figure 5 LC-MS chromatograms of the growth media of (**a**) CSY288 supplemented with 2 mM norlaudanosoline and (**b**) CSY410 supplemented with 2 mM laudanosoline and grown for 48 h showing impurities and degradation products in the sample. (**a**) The total ion chromatogram is shown in black and the extracted ion chromatogram for norlaudanosoline (MS 288) is shown in purple. The major impurities are MS 314 shown in green and MS 316 shown in blue; reticuline (MS 330) is shown in red. The same 314 and 316 ions are observed in the wild-type strain supplemented with norlaudanosoline (inset). (**b**) The total ion chromatogram is shown in black and the extracted ion chromatogram for laudanosoline (MS 302) is shown in purple. The major impurity is MS 300 shown in green; the intermediate methyl-laudanosoline (MS 316) is shown in magenta; the intermediate reticuline (MS 330) is shown in cyan; and the intermediate tetrahydrocolumbamine (MS 342) is shown in blue. The same 300 ion is observed in the wild-type strain supplemented with laudanosoline (inset). All detectable BIA metabolites elute in this time frame with the rest of the spectra relatively flat.

Supplementary Table 1. A description of engineered yeast strains used in this work.

Strain	Plasmid(s)	Integrated constructs	Plasmid-based constructs
CSY288		*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	
CSY307	pCS827, pCS830		P_{TEF1}-*Ps6OMT*, P_{TEF1}-*PsCNMT*, P_{TEF1}-*Ps4'OMT*
CSY308	pCS828, pCS830		P_{TEF1}-*Ps6OMT*, P_{TEF1}-*TfCNMT*, P_{TEF1}-*Ps4'OMT*
CSY309	pCS829, pCS830		P_{TEF1}-*Tf6OMT*, P_{TEF1}-*PsCNMT*, P_{TEF1}-*Ps4'OMT*
CSY310	pCS772, pCS830		P_{TEF1}-*Tf6OMT*, P_{TEF1}-*TfCNMT*, P_{TEF1}-*Ps4'OMT*
CSY311	pCS827, pCS831		P_{TEF1}-*Ps6OMT*, P_{TEF1}-*PsCNMT*, P_{TEF1}-*Tf4'OMT*
CSY312	pCS828, pCS831		P_{TEF1}-*Ps6OMT*, P_{TEF1}-*TfCNMT*, P_{TEF1}-*Tf4'OMT*
CSY313	pCS829, pCS831		P_{TEF1}-*Tf6OMT*, P_{TEF1}-*PsCNMT*, P_{TEF1}-*Tf4'OMT*
CSY314	pCS772, pCS831		P_{TEF1}-*Tf6OMT*, P_{TEF1}-*TfCNMT*, P_{TEF1}-*Tf4'OMT*
CSY325		*his3*::$P_{GAL1-10}$-*Ps6OMT*-*loxP*-Kan^R, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*, *gal2*::*HIS3*	
CSY326		*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::$P_{GAL1-10}$-*Ps4'OMT*-*loxP*-*LEU2*, *gal2*::*HIS3*	
CSY327		*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::$P_{GAL1-10}$-*PsCNMT*-*loxP*-*URA3*, *ura3*::P_{TEF1}-*Ps4'OMT*, *gal2*::*HIS3*	
CSY328		*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::$P_{GAL1-10}$-*Tf4'OMT*-*loxP*-*LEU2*, *gal2*::*HIS3*	
CSY329		*his3*::$P_{GAL1-10}$-*Tf6OMT*-*loxP*-*LEU2*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*, *gal2*::*HIS3*	
CSY334		*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	
CSY336	pCS1018	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*PsBBE*
CSY337	pCS1070	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*PsBBE*, P_{TEF1}-*TfS9OMT*
CSY338	pCS1018	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*PsBBE*
CSY339	pCS1070	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*PsBBE*, P_{TEF1}-*TfS9OMT*
CSY399	pCS1018, pCS953, pCS1058	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*PsBBE*, P_{TEF1}-*TfS9OMT*, P_{TEF1}-*TfCYP719A*, P_{TEF1}-*AtATR1*
CSY400	pCS1018, pCS953, pCS1058	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*PsBBE*, P_{TEF1}-*TfS9OMT*, P_{TEF1}-*TfCYP719A*, P_{TEF1}-*AtATR1*
CSY410	pCS1018, pCS953	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*, *trp1*::P_{TEF1}-*AtATR1*-*loxP*-Kan^R	P_{TEF1}-*PsBBE*, P_{TEF1}-*TfS9OMT*, P_{TEF1}-*TfCYP719A*
CSY424	pCS782	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*yCYP2D6*
CSY425	pCS782	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*, *trp1*::P_{TEF1}-*AtATR1*-*loxP*-Kan^R	P_{TEF1}-*yCYP2D6*
CSY428		*ura3*::P_{TEF1}-*yEGFP3-loxP-LEU2*	
CSY429		*ura3*::$P_{GAL1-10}$-*yEGFP3-loxP-LEU2, gal2*::Kan^R	
CSY448		*his3*::$P_{TEF7mut}$-*Ps6OMT*, *leu2*::$P_{TEF7mut}$-*PsCNMT*, *ura3*::$P_{TEF7mut}$-*Ps4'OMT*	
CSY449		*his3*::$P_{TEF7mut}$-*Ps6OMT*, *leu2*::$P_{TEF7mut}$-*PsCNMT*, *ura3*::$P_{TEF7mut}$-*Tf4'OMT*	

CSY450	pCS650		P_{TEF1}-*Ps6OMT*
CSY451	pCS677		P_{TEF1}-*Tf6OMT*
CSY452	pCS597		P_{TEF1}-*PsCNMT*
CSY453	pCS678		P_{TEF1}-*TfCNMT*
CSY454	pCS830		P_{TEF1}-*Ps4'OMT*
CSY455	pCS831		P_{TEF1}-*Tf4'OMT*
CSY456		*his3*::P_{TEF1}-*Tf6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	
CSY458		*his3*::$P_{TEF7mut}$-*Tf6OMT*, *leu2*::$P_{TEF7mut}$-*PsCNMT*, *ura3*::$P_{TEF7mut}$-*Tf4'OMT*	
CSY463	pCS781	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*hCYP2D6*
CSY464	pCS1320	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Tf4'OMT*	P_{TEF1}-*yCYP2D6:yCPR1* (fusion protein)
CSY465	pCS781, pCS1035	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*hCYP2D6*, P_{TEF1}-*yCPR1*
CSY466	pCS781, pCS1058	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*hCYP2D6*, P_{TEF1}-*AtATR1*
CSY489	pCS781	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*, *trp1*::P_{TEF1}-hCPR-*loxP*-*LEU2*	P_{TEF1}-*hCYP2D6*
CSY490	pCS1421	*his3*::P_{TEF1}-*Ps6OMT*, *leu2*::P_{TEF1}-*PsCNMT*, *ura3*::P_{TEF1}-*Ps4'OMT*	P_{TEF1}-*hCYP2D6:hCPR1* (fusion protein)

Supplementary Table 2. Primer sequences used to make stable integrations of enzyme constructs, *GAL2* knockouts, and qRT-PCR. Restriction sites are underlined.

Primer name	Sequence
TEF.fwd	ATAAAAGCTTACATATGCATAGCTTCAAAATGTTTCTACTCC
TEF.rev	TGCTAGCGTTTAAACGAATTCTGGATCCATCCTAGGAAAACTTAGATTAGATTGCTATGC
CYC1.fwd	ATGGATCCAGAATTCGTTTAAACGCTAGCACCCGGGGAGGGCCGCATCATGTAATTAGTT
CYC1.rev	ATAACTGCAGAGTCGACGGCCGCAAATTAAAGCCTTCGA
HISint.fwdA	TATACTAAAAAATGAGCAGGTGTCGGGGCTGGCTTAACTATGCGGCATCA
HISint.revA	TATATATATCGTATGCTGCAGCTCTGGCTTATCGAAATTAATACGACTCACTA
HISint.fwdB	ATTGGCATTATCACATAATGAATTATACATTATATAAAGTAATGTGATTTCTTCGAAGAATATACTAAAAAATGAGCAGG
HISint.revB	AGTATCATACTGTTCGTATACATACTTACTGACATTCATAGGTATACATATATACACATGTATATATATCGTATGCTGCAGCT
LEUint.fwdA	AGCAATATATATATATATATTGTCGGGGCTGGCTTAACTATGCGGCATCA
LEUint.revA	AGTTTATGTACAAATATCATAACTGGCTTATCGAAATTAATACGACTCACTA
LEUint.fwdB	TTTTCCAATAGGTGGTTAGCAATCGTCTTACTTTCTAACTTTTCTTACCTTTTACATTTCAGCAATATATATATATATAT
LEUint.revB	TACCCTATGAACATATTCCATTTTGTAATTTCGTGTCGTTTCTATTATGAATTTCATTTATAAAGTTTATGTACAAATATCATAA
URAint.fwdA	ACTGCACAGAACAAAAACCTTGTCGGGGCTGGCTTAACTATGCGGCATCA
URAint.revA	AGTTTAGTATACATGCATTTACTGGCTTATCGAAATTAATACGACTCACTA
URAint.fwdB	GGTATATATACGCATATGTGGTGTTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCT
URAint.revB	AATCATTACGACCGAGATTCCCGGGTAATAACTGATATAATTAAATTGAAGCTCTAATTTGTGAGTTTAGTATACATGCATTTA
GAL.fwd	ATAAAGCTTACATATGTCTAGAAAATTCCTTGAATTTTCAAAAATT
GAL.rev	ATACCTAGGTTTTTTCTCCTTGACGTTAAAGTATAGAG
GAL2ko.fwd	CATTAATTTTGCTTCCAAGACGACAGTAATATGTCTCCTACAATACCAGTGTCGGGGCTGGCTTAACTATGCGGCATCA
GAL2ko.rev	TATATGTACACAAATAATAGGTTTAGGTAAGGAATTTATATAATCGTAAGCTGGCTTATCGAAATTAATACGACTCACTA
GAL2sc.fwd	TGTGCATGTTATCTATATCCTTCTTTATATAGATGCTGTT
GAL2sc.rev	ATTAATTGTATGTTAGCTCAGGAATTCAACTGGAAGAAAG
RTGFP.fwd	GCCATGCCAGAAGGTTATGT
RTGFP.rev	ACCATTCTTTTGTTTGTCAGCC
RT6OMT.fwd	CTCATTAGCTCCACCAGCTAAGTA
RT6OMT.rev	GGTCAAACCGTCGCCTAAAT
RTCNMT.fwd	CAAAGTGCGGGTTACTACACTG
RTCNMT.rev	TCCACAGAACTTGTAACTCCAGT
RT4'OMT.fwd	GGTGCCGATGATACTAGGCA
RT4'OMT.rev	CCATTCCTTCATTAAACAACTGGC
ACT1.fwd	GGCATCATACCTTCTACAACGAAT
ACT1.rev	GGAATCCAAAACAATACCAGTAGTTCTA

Supplementary Table 3. Summary of yields of benzylisoquinoline alkaloids. Yields are reported from the supernatant of growth cultures of the appropriate strains fed 5 mM norlaudonosoline.

BIA compound	yield (μM)	yield (mg l^{-1})
(*R*, *S*)-reticuline	500	164.5
(*S*)-scoulerine	200	65.4
(*S*)-tetrahydrocolumbamine	200	68.2
(*S*)-tetrahydroberberine	100	33.9
salutaridine	75	24.5

SUPPLEMENTARY METHODS

Construction of BIA expression vectors. Briefly, we constructed BIA expression vectors from the yeast shuttle vectors pCM185 and pCM180[1], which harbor an ampicillin resistance marker for maintenance in *E. coli*, *URA3* and *TRP1* selection markers, respectively, and a centromeric (CEN4/ARS1) yeast origin of replication. To construct exemplary vectors (**Supplementary Fig. 1**), the TEF1 promoter and the CYC1 terminator were amplified from p413-TEF[2] and pCM190[1], respectively, and assembled with the *P. somniferum CNMT* coding sequence using a PCR-based assembly strategy[3]. Primers that fused the DNA fragments included unique restriction sites to facilitate replacement of the coding sequences, and end primers included restriction sites for cloning into the plasmid backbones. We cloned the first promoter-gene-terminator DNA fragment into XhoI/BamHI sites of pCM185. A second DNA fragment containing the TEF1 promoter and *P. somniferum 6OMT* gene was assembled using similar PCR-based methods and cloned into PmeI/NotI sites preceding a CYC1 terminator on the plasmid backbone. The XhoI/NotI fragment containing dual promoter-gene-terminator sequences was excised from this vector and ligated into pCM180 to construct an analogous vector with a *TRP1* selection marker. We replaced the low copy origin of replication with the 2 μM origin from pCM190 using standard cloning procedures. We cloned coding sequences for various BIA enzymes into these standard vectors and altered restriction sites on the plasmid backbone(s) as necessary using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Single-gene constructs were made by excising one of the genes using appropriate restriction enzymes and blunting the ends with Klenow prior to ligation as needed, or cloning the desired coding sequence between the first promoter and second terminator in the standard vectors using SacII/NotI sites.

Chromosomal integration of enzyme constructs. The plasmids pUG6, pUG27, pUG72, and pUG73[4] (EUROSCARF) contain geneticin resistance, *S. pombe his5*$^{+}$, *Kluyveromyces lactis URA3,* and *K. lactis LEU2* genes, respectively, flanked by *loxP*

sites and were used in the construction of the integration cassettes. We amplified and assembled the TEF1 promoter and CYC1 terminator using PCR-based methods and the primers TEF.fwd, TEF.rev, CYC1.fwd, and CYC1.rev (**Supplementary Table 2**). The assembled DNA insert contained a multi-cloning site and was subcloned upstream of the selection marker in each construct. We cloned cDNAs into the multi-cloning site and amplified the entire integration cassette in two PCR steps using "A" and "B" primer sets to add ~80 nt of homology (**Supplementary Table 2**). We gel purified integration cassettes using the Zymoclean Gel DNA Recovery Kit (Zymo Research) according to the manufacturer's instructions prior to transformation into the appropriate yeast strain. Integration of the cassettes into the correct locus was confirmed by PCR analysis of the targeted region of the chromosome. To remove all selection markers from the final strains, we transformed cells with the plasmid pSH63 which expresses a GAL-inducible Cre recombinase[4]. Cells harboring the plasmid were induced with galactose for 24 h and then transferred to YPD media to remove the selection pressure for plasmid maintenance. We isolated and restreaked single colonies on appropriate media to verify the loss of selection markers.

Enzyme titration studies. We built template plasmids for chromosomal integrations for the enzyme titration studies by cloning the GAL1-10 promoter (amplified from pRS314-GAL[5] using primers GAL.fwd and GAL.rev) in place of the TEF1 promoter in pUG-based plasmids. Construction of strains using the GAL1-10 promoter to control expression of single enzymes was analogous to that described for the TEF1 promoter strains with a final step to replace the *GAL2* locus with the *his5*$^+$ selection marker using a cassette amplified from pUG27 with primers GAL2ko.fwd and GAL2ko.rev. We confirmed integration into the targeted site with primers GAL2sc.fwd and GAL2sc.rev (**Supplementary Table 2**).

For the enzyme titration assays, GAL-inducible strains were grown overnight in synthetic complete media containing all amino acids with 2% raffinose (w/v), 1% sucrose (w/v) as a sugar source. Galactose was added to the media at the appropriate concentration from a 10X stock at the time of back dilution and the norlaudanosoline

substrate was added to a final concentration of 1 mM following an induction period of 4 h. We sampled aliquots of the growth media for LC-MS/MS analysis 24 h after substrate addition. Data is reported as reticuline production measured by LC-MS peak area.

Fluorescence quantification. For fluorescence measurements of CSY428 and CSY429 (**Fig. 4b** and **Supplementary Table 1**), overnight cultures were grown in synthetic complete media with either 2% dextrose or 2% raffinose and 1% sucrose (noninducing-nonrepressing), respectively. Cells were backdiluted into fresh media containing either dextrose (CSY428) or noninducing-nonrepressing media supplemented with the appropriate galactose concentration from a 10X stock (CSY429). We measured fluorescence (excitation 485 nm, emission 515 nm) and OD_{600} values on a fluorescence plate reader (TECAN, Safire). Fluorescence values were normalized using the OD_{600} reading and values from a no stain control (wild-type cells) were subtracted. We used the resulting trend between relative fluorescence and galactose concentration as a guideline to estimate relative protein expression levels in our titratable strains.

Analysis of protein levels through Western blotting. We constructed plasmids for Western blotting experiments by cloning the C-terminal epitope tag(s) from pYES-NT/A (Invitrogen) into a standard BIA expression vector followed by subcloning of the enzyme of interest. We transformed individual plasmids into wild-type yeast cells using a standard lithium acetate protocol. Overnight cultures were grown and backdiluted 1:100 into 100 ml cultures. Cells were grown to OD_{600} ~1.2 and pelleted by centrifugation. The media was removed and cells were washed in 1 ml PBS, pelleted, and resuspended in 0.5 ml Y-PER plus HALT protease inhibitor (Pierce). Cells were vortexed for ~20 min and the lysate separated by centrifugation. We estimated total protein using the Coomassie Plus Protein Assay Reagent (Pierce) and loaded ~50 μg of each sample onto a protein gel. We used NuPage 4-12% Bis-Tris gels with MES running buffer and transfer buffer according to the manufacturer's instructions (Invitrogen). Proteins were blotted onto a nitrocellulose membrane (Whatman) using a semi-dry transfer cell (Bio-Rad) for 20 min at 15 V. We incubated the membrane with the Anti-V5 HRP antibody (Invitrogen) according to the manufacturer's instructions (Invitrogen) with 5% nonfat milk as the

blocking agent. Proteins were detected with the West Pico Super Signal Detection kit (Pierce) and imaged on a ChemiDoc XRS system (Bio-Rad). The image shown is representative of at least two independent experiments.

Analysis of transcript levels through qRT-PCR. We extracted total RNA from yeast cells grown in 10 ml cultures to OD_{600} ~0.5 using standard acid phenol extraction procedures[6]. Briefly, cells were pelleted, frozen in liquid nitrogen, and resuspended in a 50 mM NaOAc (pH 5.2) and 10 mM EDTA buffer. Cells were lysed by adding SDS to a final concentration of 1.6% and equal volume of acid phenol and incubating for 10 min at 65°C with intermittent vortexing. Following cooling on ice, the aqueous phase was extracted and further extraction was carried out with an equal volume of chloroform. RNA samples were ethanol precipitated and resuspended in water, and total RNA was quantified on a NanoDrop according to the manufacturer's instructions. RNA samples were treated with DNase (Invitrogen) according to the manufacturer's instructions.

We performed cDNA synthesis using gene-specific primers (**Supplementary Table 2**) and Superscript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions with 5 μg total RNA used in each reaction. Relative transcript levels were quantified from the cDNA samples by employing an appropriate primer set and the iQ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions on an iCycler iQ qRT-PCR machine (Bio-Rad). We analyzed the resulting data with the iCycler iQ software according to the manufacturer's instructions. We normalized transcript levels to that of the endogenous *ACT1* gene[7] using *ACT1*-specific primers. Data are reported as the mean ± s.d. from at least two independent experiments.

Analysis of stereoisomer forms of BIA metabolites through capillary electrophoresis-based chiral separation. We performed chiral analysis of norlaudanosoline, laudanosoline, and reticuline using the P/ACE MDQ Capillary Electrophoresis (CE) system (Beckman-Coulter). A bare fused silica capillary (50 μm i.d.) with 31.0 cm effective length (41 cm total) was used. We tested buffer solutions

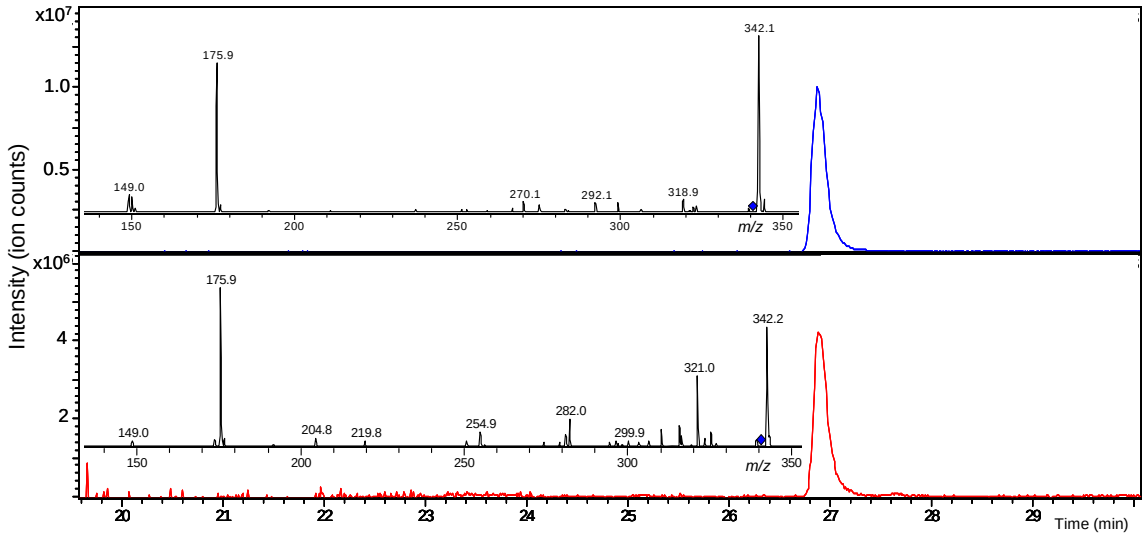
from the chiral test kit (Beckman) with the substrates norlaudanosoline and laudanosoline and optimized conditions using the HS-β-CD separation buffer at 5% (w/v) according to the manufacturer's instructions. Injection was for 5 psi for 10 sec and voltage separation was performed at 15 kV. A diode array detector (PDA) module was used with the spectra presented taken at 280 nm. Representative data is shown from at least two independent experiments.

For analysis of reticuline and other BIA metabolites, we developed HPLC methods to use water as the aqueous buffer rather than 0.1% acetic acid to avoid unnecessary noise in the CE spectra. Samples were run on a ZORBAX SB-Aq 3 x 250 mm, 5 μm column with a gradient elution from 10% B to 90% B between 0 and 30 min where solvent B is methanol and solvent A is water. Reticuline elutes at 13 min using this method as confirmed by MS data. We collected fractions from 13.0-13.2 min and verified purity by re-running the sample(s) on LC-MS. Multiple fractions were collected and concentrated ~4 to 5-fold prior to CE analysis.

Preparation of cell extracts. We prepared cell extracts by washing the cell pellets three times with PBS, adding 1 ml of cold methanol at -40°C, and vortexing with glass beads to disrupt the cells. Cell debris and glass beads were pelleted by centrifugation. The methanol was transferred to a clean tube and concentrated by evaporation to a final volume approximately equal to that of the original cell mass.

Co-elution experiments with a known chemical standard. We performed co-elution experiments for the final metabolite synthesized by yeast in the sanguinarine and berberine branch. Authentic DL-canadin or (*R, S*)-tetrahydroberberine (Apin Chemicals Ltd) co-eluted with the product identified as (*S*)-tetrahydroberberine synthesized by engineered yeast cells at 26.9 min as determined by LC-MS/MS analysis. The *m/z* = 340 extracted ion chromatograms are shown below for the known standard (50 mM; blue) and for the yeast growth media of CSY410 supplemented with 4 mM norlaudanosoline and

grown for 48 h (red). MS/MS on the 340 ions showed the major ions *m/z* = 149 and *m/z* = 175 in common.



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