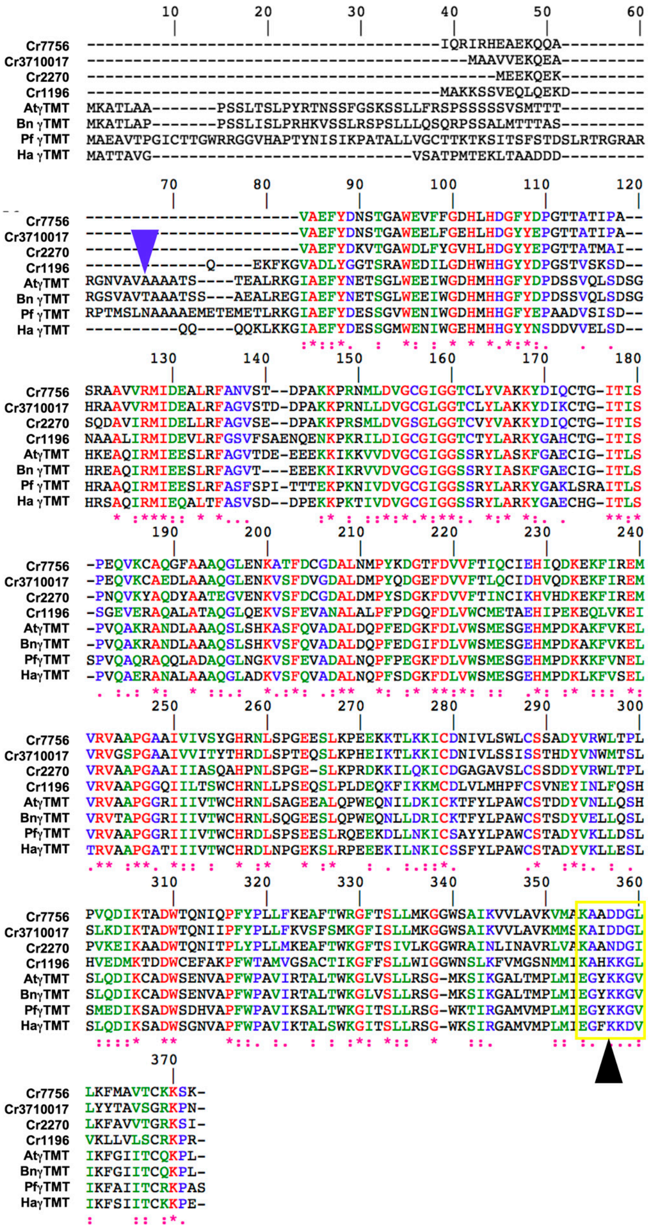
Supporting Information

Liscombe et al. 10.1073/pnas.1009003107



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                   10        20        30        40        50        60
                    |         |         |         |         |         |
Cr7756     --------------------------------------IQRIRHEAEKQQA---------
Cr3710017  -----------------------------------------MAAVVEKQEA---------
Cr2270     --------------------------------------------MEEKQEK---------
Cr1196     --------------------------------------MAKKSSVEQLQEKD--------
AtγTMT     MKATLAA-------PSSLTSLPYRTNSSFGSKSSLLFRSPSSSSSVSMTTT---------
Bn γTMT    MKATLAP-------PSSLISLPRHKVSSLRSPSLLLQSQRPSSALMTTTAS---------
Pf γTMT    MAEAVTPGICTTGWRRGGVHAPTYNISIKPATALLVGCTTKTKSITSFSTDSLRTRGRAR
Ha γTMT    MATTAVG----------------------------VSATPMTEKLTAADDD---------

                   70        80        90       100       110       120
                    |         |         |         |         |         |
Cr7756     -----------------------VAEFYDNSTGAWEVFFGDHLHDGFYDPGTTATIPA--
Cr3710017  -----------------------VAEFYDNSTGAWEELFGEHLHDGYYEPGTTATIPA--
Cr2270     -----------------------VAEFYDKVTGAWDLFYGVHLHDGYYEPGTTATMAI--
Cr1196     -------------Q----EKFKGVADLYGGTSRAWEDILGDHWHHGYYDPGSTVSKSD--
AtγTMT     RGNVAVAAAATS----TEALRKGIAEFYNETSGLWEEIWGDHMHHGFYDPDSSVQLSDSG
Bn γTMT    RGSVAVTAAATSS---AEALREGIAEFYNETSGLWEEIWGDHMHHGFYDPDSSVQLSDSG
Pf γTMT    RPTMSLNAAAAEMETEMETLRKGIAEFYDESSGVWENIWGDHMHHGFYEPAADVSISD--
Ha γTMT    ----------QQ----QQKLKKGIAEFYDESSGMWENIWGEHMHHGYYNSDDVVELSD--
                                  :*::*.  :  *: : * * *.*:*:.   .  .

                  130       140       150       160       170       180
                    |         |         |         |         |         |
Cr7756     SRAAVVRMIDEALRFANVST--DPAKKPRNMLDVGCGIGGTCLYVAKKYDIQCTG-ITIS
Cr3710017  HRAAVVRMIDEALRFAGVSTD-DPAKKPRNLLDVGCGLGGTCLYLAKKYDIKCTG-ITIS
Cr2270     SQDAVIRMIDELLRFAGVSE--DPAKKPRSMLDVGSGLGGTCVYVAKKYDIQCTG-ITIS
Cr1196     NAAALIRMIDEVLRFGSVFSAENQENKPKRILDIGCGIGGTCTYLARKYGAHCTG-ITIS
AtγTMT     HKEAQIRMIEESLRFAGVTDE-EEEKKIKKVVDVGCGIGGSSRYLASKFGAECIG-ITLS
Bn γTMT    HREAQIRMIEESLRFAGVT---EEEKKIKRVVDVGCGIGGSSRYIASKFGAECIG-ITLS
Pf γTMT    HRAAQIRMIEESLRFASFSPI-TTTEKPKNIVDVGCGIGGSSRYLARKYGAKLSRAITLS
Ha γTMT    HRSAQIRMIEQALTFASVSD--DPEKKPKTIVDVGCGIGGSSRYLARKYGAECHG-ITLS
              * :***:: * *...       :* : ::*:*.*:**:. *:* *:. .    **:*

                  190       200       210       220       230       240
                    |         |         |         |         |         |
Cr7756     -PEQVKCAQGFAAAQGLENKATFDCGDALNMPYKDGTFDVVFTIQCIEHIQDKEKFIREM
Cr3710017  -PEQVKCAEDLAAAQGLENKVSFDVGDALDMPYQDGEFDVVFTLQCIDHVQDKEKFIREM
Cr2270     -PNQVKYAQDYAATEGVENKVSFDCGDALDMPYSDGKFDVVFTINCIKHVHDKEKFIREM
Cr1196     -SGEVERAQALATAQGLQEKVSFEVANALALPFPDGQFDLVWCMETAEHIPEKEQLVKEI
AtγTMT     -PVQAKRANDLAAAQSLSHKASFQVADALDQPFEDGKFDLVWSMESGEHMPDKAKFVKEL
BnγTMT     -PVQAKRANDLAAAQSLSHKVSFQVADALDQPFEDGIFDLVWSMESGEHMPDKAKFVKEL
PfγTMT     SPVQAQRAQQLADAQGLNGKVSFEVADALNQPFPEGKFDLVWSMESGEHMPDKKKFVNEL
HaγTMT     -PVQAERANALAAAQGLADKVSFQVADALNQPFSDGKFDLVWSMESGEHMPDKLKFVSEL
            . :.: *:  * ::.:  *.:*: .:**  *: :* **:*: ::  .*: :* ::: *:

                  250       260       270       280       290       300
                    |         |         |         |         |         |
Cr7756     VRVAAPGAAIVIVSYGHRNLSPGEESLKPEEKKTLKKICDNIVLSWLCSSADYVRWLTPL
Cr3710017  VRVGSPGAAIVVITYTHRDLSPTEQSLKPHEIKTLKKICDNIVLSSISSTHDYVNWMTSL
Cr2270     VRVAAPGAAIIIASQAHPNLSPGE-SLKPRDKKILQKICDGAGAVSLCSSDDYVRWLTPL
Cr1196     VRVAAPGGQIILTSWCHRNLLPSEQSLPLDEQKFIKKMCDLVLMHPFCSVNEYINLFQSH
AtγTMT     VRVAAPGGRIIIVTWCHRNLSAGEEALQPWEQNILDKICKTFYLPAWCSTDDYVNLLQSH
BnγTMT     VRVTAPGGRIIIVTWCHRNLSQGEESLQPWEQNLLDRICKTFYLPAWCSTSDYVELLQSL
PfγTMT     VRVAAPGGRIIIVTWCHRDLSPSEESLRQEEKDLLNKICSAYYLPAWCSTADYVKLLDSL
HaγTMT     TRVAAPGATIIIVTWCHRDLNPGEKSLRPEEEKILNKICSSFYLPAWCSTADYVKLLESL
           .** :**. *:: :  * :*   * :*   : . :.::*.       .*  :*:. : .

                  310       320       330       340       350       360
                    |         |         |         |         |         |
Cr7756     PVQDIKTADWTQNIQPFYPLLFKEAFTWRGFTSLLMKGGWSAIKVVLAVKVMAKAADDGL
Cr3710017  SLKDIKTADWTQNIIPFYPLLFKVSFSMKGFISLLMKGGWSAIKVVLAVKMMSKAIDDGL
Cr2270     PVKEIKAADWTQNITPLYPLLMKEAFTWKGFTSIVLKGGWRAINLINAVRLVAKAANDGI
Cr1196     HVEDMKTDDWCEFAKPFWTAMVGSACTIKGFFSLLWIGGWNSLKFVMGSNMMIKAHKKGL
AtγTMT     SLQDIKCADWSENVAPFWPAVIRTALTWKGLVSLLRSG-MKSIKGALTMPLMIEGYKKGV
BnγTMT     SLQDIKCADWSENVAPFWPAVIRTALTWKGLVSLLRSG-MKSIKGALTMPLMIEGYKKGV
PfγTMT     SMEDIKSADWSDHVAPFWPAVIKSALTWKGITSLLRSG-WKTIRGAMVMPLMIEGYKKGV
HaγTMT     SLQDIKSADWSGNVAPFWPAVIKTALSWKGITSLLRSG-WKSIRGAMVMPLMIEGFKKDV
            ::::*  **     *::. :.  : : :*: *::  *   ::.      :: :.  ..:

                  370
                    |
Cr7756     LKFMAVTCKKSK-
Cr3710017  LYYTAVSGRKPN-
Cr2270     LKFAVVTGRKSI-
Cr1196     VKLLVLSCRKPR-
AtγTMT     IKFGIITCQKPL-
BnγTMT     IKFGIITCQKPL-
PfγTMT     IKFAIITCRKPAS
HaγTMT     IKFSIITCKKPE-
           :    :: :*.
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Fig. S1. Aligment of *Catharanthus roseus* γ-tocopherol methyltransferase-like proteins and validated γ-tocopherol methyltransferases. The residue color scheme is as follows: red, identical; green, highly similar; blue, weakly similar; black, different. A blue arrow denotes the transit peptide cleavage site in AtγTMT, as predicted by ChloroP (1). The Rubisco large subunit methyltransferase (RubLSMT) consensus motif (2) is indicated by a yellow box, and a black arrow denotes lysine residue of γ-tocopherol methyltransferases that is methylated by RubLSMT.

1. Emanuelsson O, Nielsen H, von Heijne G (1999) ChloroP, a neural networkbased method for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci* 8:978–984.
2. Magnani R, Nayak NR, Mazarei M, Dirk LM, Houtz RL (2007) Polypeptide substrate specificity of PsLSMT. A set domain protein methyltransferase. *J Biol Chem* 282:27857–27864.

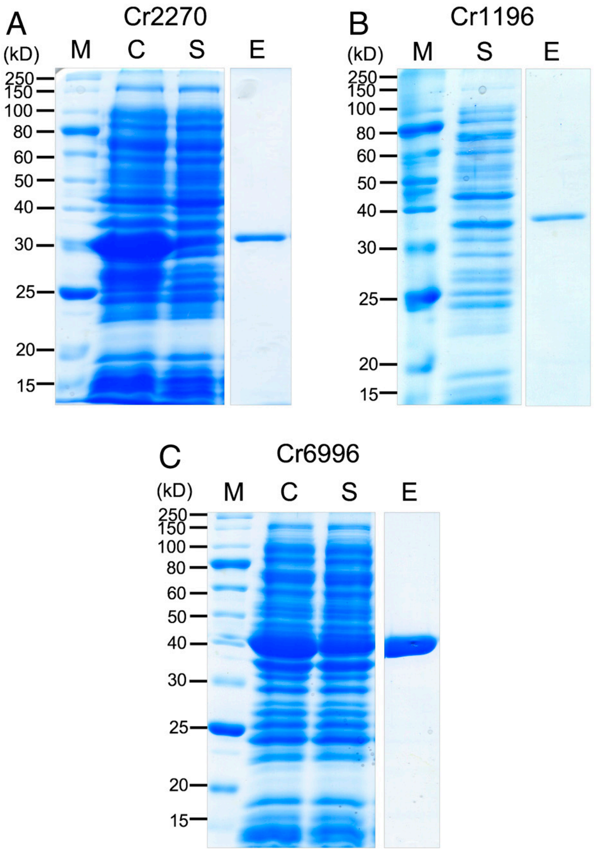

Fig. S2. SDS-PAGE analysis of purified recombinant proteins used in assays. (*A*) Cr2270, (*B*) Cr1196, and (*C*) Cr6996. Lanes are labeled as follows: M, marker; C, crude total protein; S, crude soluble protein; E, pure protein elution.

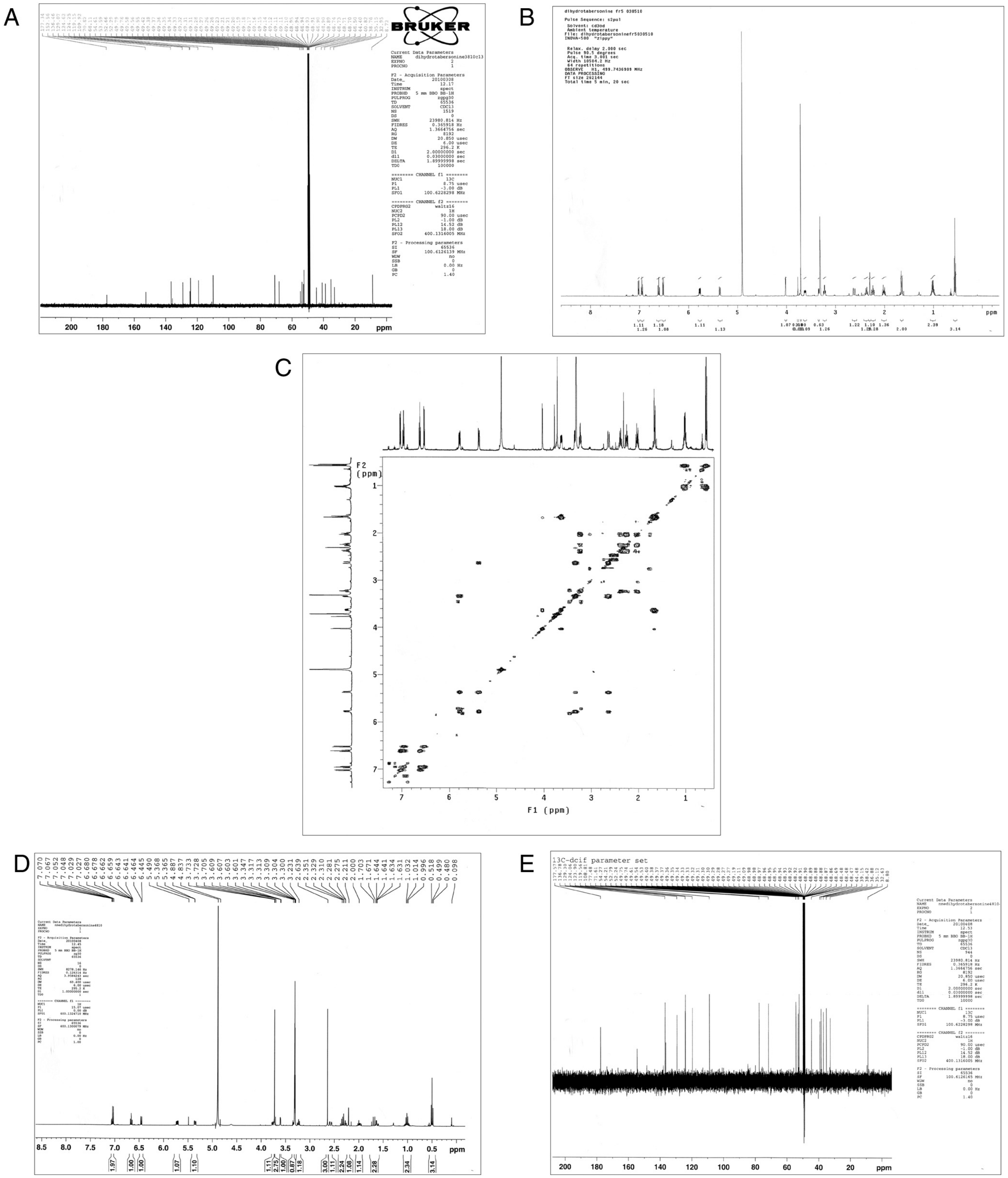

Fig. S3. NMR spectra for synthetic substrates and standards. (*A*) ^{1}H-NMR for (2*R*,3*S*)-2,3-Dihydrotabersonine (DHT) **10**; (*B*) ^{13}C-NMR for DHT **10**; (*C*) COSY for DHT **10**; (*D*) ^{1}H-NMR for *N*-methyl-DHT **11**; (*E*) ^{13}C-NMR for *N*-methyl-DHT **11**.

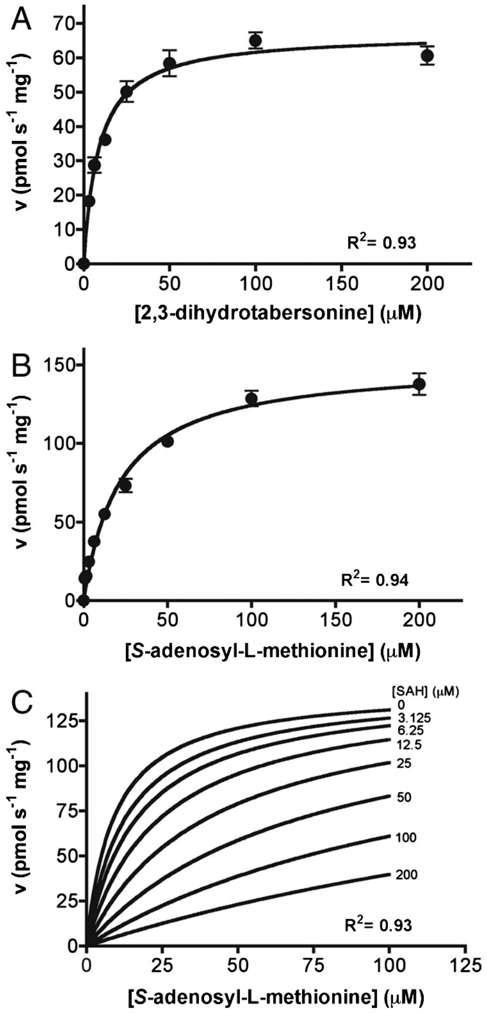

Fig. S4. Michaelis–Menten plots of kinetic data for purified, recombinant Cr2270. Nonlinear regression analysis of kinetic data were used to estimate kinetic parameters for Cr2270 with substrates (*A*) 2,3-dihydrotabersonine (DHT), and (*B*) *S*-adenosyl-L-methionine (SAM). (*C*) Global fit analysis of inhibition by *S*-adenosyl-L-homocysteine (SAH) for estimation of K_i.

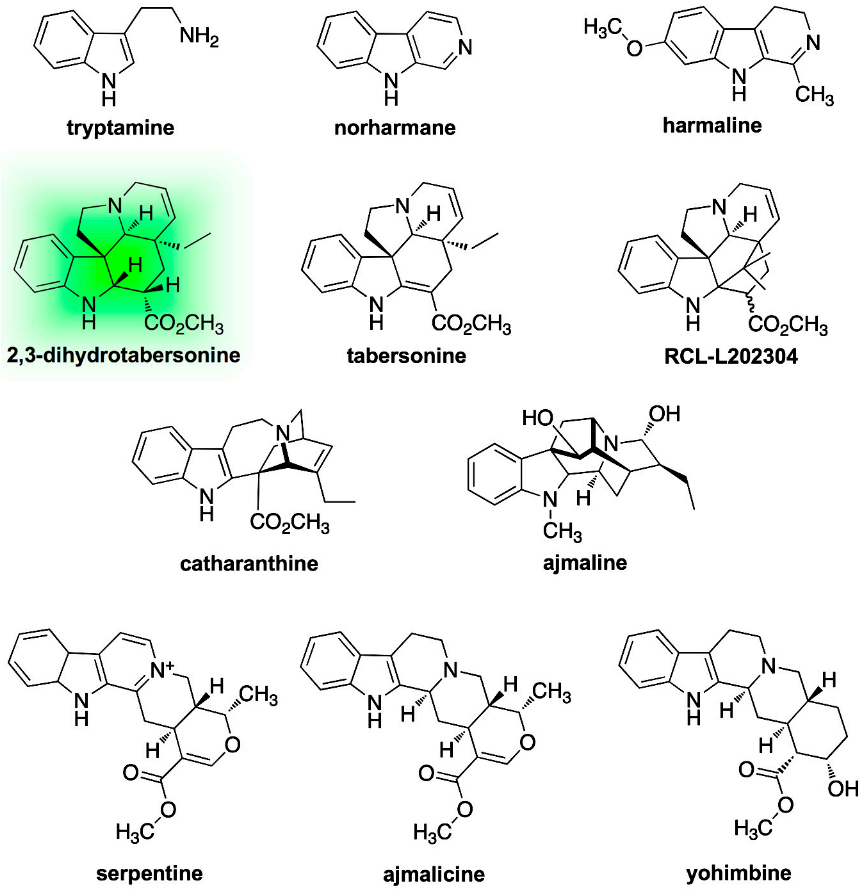

Fig. S5. Substrate specificity of purified, recombinant Cr2270. Structures of aspidosperma-, iboga-, and corynanthe-type alkaloids, along with a beta-carboline and a simple indole, tested as substrates with Cr2270. Only 2,3-dihydrotabersonine (DHT, highlighted in green) was accepted as a substrate.

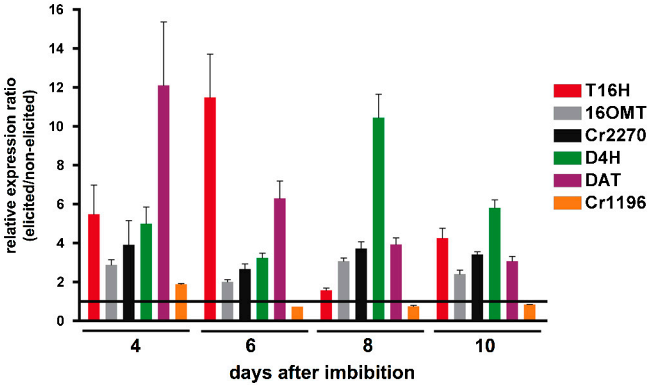

Fig. S6. Quantitative real-time PCR analysis of vindoline biosynthetic gene expression in *C. roseus* seedlings after receiving methyl jasmonate treatment 2 d after imbibition. Data are displayed as a relative expression ratio compared to untreated seedlings. Error bars represent SEM from at least three experiments. Abbreviations: T16H, tabersonine 16-hydroxylase; 16OMT, 16-hydroxytabersonine 16-*O*-methyltransferase; D4H, desacetoxyvindoline 4-hydroxylase; DAT, deacetylvindoline acetyltransferase.

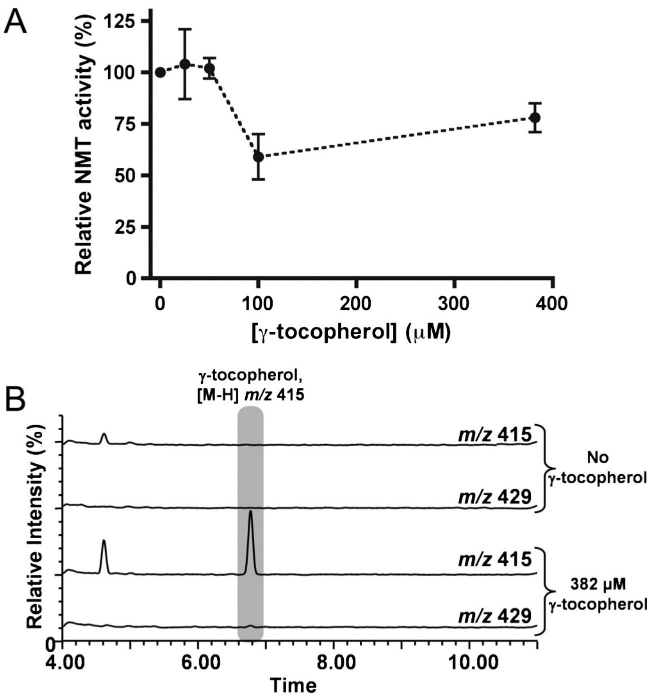

Fig. S7. γ-Tocopherol inhibits DHT **10** methylation by recombinant Cr2270, but is not turned over by the enzyme. (*A*) Cr2270-mediated DHT **10** methylation is inhibited by γ-tocopherol ([DHT] = 100 μM; [SAM] = 200 μM); (*B*) liquid chromatography-mass spectrometry analysis of the assays in (*A*) shows that γ-tocopherol ([M-H] *m/z* 415) is not converted to α-tocopherol ([M-H] *m/z* 429) by Cr2270.

Table S1. *N*-Methyltransferase candidates identified from BLAST searches of *Catharanthus roseus* EST assemblies

PUT ID	Similar to functionally characterized protein	GenBank Accession #	%ID	Predicted full-length ORF?	EST origin*
Cr1189	Resveratrol O-methyltransferase [*Vitis vinifera*]	CAQ76879	64	Yes	Root
Cr1196	γ-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882	52	No	Root
Cr2270	γ-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882	42	No†	Leaf
Cr2551	Caffeic acid *O*-methyltransferase [*Catharanthus roseus*]	AAK20170	52	No	Root, flower bud
Cr5804	loganic acid methyltransferase [*Catharanthus roseus*]	ABW38009	43	No	Root
Cr6424	Coclaurine *N*-methyltransferase [*Coptis japonica*]	BAB71802	51	No	Leaf
Cr6996	Putative *O*-methyltransferase [*Catharanthus roseus*]	AAR02422	100	Yes	CSC
Cr7756	γ-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882	45	No†	Root
Cr7789	Putative *O*-methyltransferase [*Catharanthus roseus*]	AAR02418	100	Yes	CSC
Cr8458	Flavonoid 4′-*O*-methyltransferase [*Mentha piperita*]	AAR09602	50	No	Leaf
Cr3710017	γ-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882	47	No	Root, leaf
Cr3910022	γ-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882	52	No	Root

www.plantgdb.org.

Abbreviations: CSC, cell suspension culture.

*As per assembly details available at plantgdb.org.

†Only missing 5′ end predicted to encode a transit peptide.

Table S2. Oligonucleotide primers used in this study

Primer name	Sequence (5′ to 3′)	Purpose
1196-F1	AAA CTG CCG AGC ACA TCC	3′-RACE
2551-F1	TGT GGA CAA AGC ACA CTT CG	3′-RACE
1196-F2	CAG ATA ATT TTA ACG TCT TGG TGC C	3′-RACE (nested)
2551-F2	CAC TTG AAA GAT GCA ATT CTT GAG GG	3′-RACE (nested)
3′-RACE PCR	GGC CAC GCG TCG ACT AGT AC	3′-RACE reverse
1196-F3	AAA GGA TCC ATG GCA AAA AAA TCA TCA GTA GAA CAA C	Cr1196 ORF cloning
1196-R2	AAA AAG CTT TTA TCT GGG TTT TCG GCA CG	Cr1196 ORF cloning
2270F	AAC ATT AGG ATC CAA AAC ATA CAA CAA TAC AAT GG	Cr2270 ORF cloning
2270R	TTG TAA TCT CGA GTC ATA TTG ATT TTC GTC CC	Cr2270 ORF cloning
6996F	AAC ATT AGG ATC CAT GGA AGT TCA ATC AGC C	Cr6996 ORF cloning
6996R	TTG TAA TCT CGA GTC AAG GAT AAA CTT CAA TAA GAC TCC	Cr6996 ORF cloning
7789F	AAC ATT AGG ATC CAT GGA AGT TCA ATC AGT C	Cr7789 ORF cloning
7789R	TTG TAA TCT CGA GTT AAG GAT AAA CCT CAA TAA TAC TCC	Cr7789 ORF cloning
cDNA cloning	GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT TV	First strand cDNA synthesis for cloning ORFs
2270-qF1	TGA CAA AGT AAC CGG AGC ATG GGA	qPCR
2270-qR1	ATC CGA ATG ACG GCA TCT TGG CTA	qPCR
Cr16OMT_qF1	GCC AAT CCT GCT CTT TCA TAA C	qPCR
Cr16OMT_qR1	AGC TTT AGA AGG GTG AAC TGG	qPCR
CrD4H_qF1	TAC CCT GCA TGC CCT CAA CC	qPCR
CrD4H_qR1	TTG AAG GCC GCC AAT TTG AT	qPCR
CrDAT_qF1	GAC CTA GTC CTT CCC AAA CG	qPCR
CrDAT_qR1	CCT CCA TCA GCA ACT TTG TG	qPCR
CrEF1a_qF1	TCA GGA GGC TCT TCC TGG TGA	qPCR
CrEF1a_qR1	AGC TCC CTT GGC AGG GTC AT	qPCR
CrT16H_qF1	AGG ACC TTG TTG ATG TGC TAC	qPCR
CrT16H_qR1	CAT TGC CCA ATC GAC TGT TG	qPCR
Rbps9_qF1	TTG AGC CGT ATC AGA AAT GC	qPCR
Rbps9_qR1	CCC TCA TCA AGC AGA CCA TA	qPCR

Table S3. Accession numbers for sequences used to construct neighbor-joining tree

Abbreviation	Annotation [organism]	Accession #
AbPNMT	Putrescine *N*-methyltransferase 1 [*Atropa belladonna*]	BAA82264
AtγTMT	Gamma-tocopherol methyltransferase [*Arabidopsis thaliana*]	AAD02882
AtSMT1	Sterol 24-*C*-methyltransferase 1 [*Arabidopsis thaliana*]	NP_001078579
AtSMT2-	Sterol methyltransferase 2 [*Arabidopsis thaliana*]	NP_173458
AtSMT3	Sterol 24-*C*-methyltransferase 3 [*Arabidopsis thaliana*]	NP_177736
AtUCMT	Uroporphyrinogen III methyltransferase [*Arabidopsis thaliana*]	AAB92677
BnγTMT	Gamma-tocopherol methyltransferase [*Brassica napus*]	ACJ54674
BvCFAPS	Cyclopropane-fatty-acyl-phospholipid synthase [*Burkholderia vietnamiensis*]	YP_001120490
CaDMXNMT	3,7-dimethylxanthine *N*-methyltransferase [*Coffea arabica*]	BAC75663
CaMXNMT	7-methylxanthine *N*-methyltransferase [*Coffea arabica*]	BAB39216
CbSAMT	Salicylic acid carboxyl methyltransferase [*Clarkia breweri*]	34809619
CjCNMT	Coclaurine *N*-methyltransferase [*Coptis japonica*]	BAB71802
CjCoOMT	Columbamine *O*-methyltransferase[*Coptis japonica*]	Q8H9A8
CjSOMT	Scoulerine 9-*O*-methyltransferase [*Coptis japonica*]	BAA06192
Cr16OMT	16-hydroxytabersonine *O*-methyltransferase [*Catharanthus roseus*]	ABR20103
CrLAMT	Loganic acid *O*-methyltransferase [*Catharanthus roseus*]	ABW38009
CsCafSyn	Caffeine synthase [*Camellia sinensis*]	BAB12278
DsPNMT	Putrescine *N*-methyltransferase [*Datura stramonium*]	CAE47481
GmSMT1	Sterol 24-*C*-methyltransferase 2-1 [*Glycine max*]	ACS93763
GmSMT2	Sterol 24-*C*-methyltransferase 2-2 [*Glycine max*]	ACS93764
HaγTMT	Gamma-tocopherol methyltransferase [*Helianthus annuus]*	ABB52798
LePEANMT	Phosphoethanolamine *N*-methyltransferase [*Solanum lycopersicum*]	AAG59894
LlBANMT	Beta-alanine *N*-methyltransferase [*Limonium latifolium*]	AAP03058
MlCFAPS	Cyclopropane-fatty-acyl-phospholipid synthase [*Mesorhizobium loti*]	BAB53730
MtPcaA	Mycolic acid synthase (cyclopropane synthase) [*Mycobacterium tuberculosis*]	CAE55286
PfγTMT	Gamma-tocopherol methyltransferase [*Perilla frutescens*]	AAL36933
PiOMT1	Ipecac alkaloid *O*-methyltransferase 1 [*Psychotria ipecacuanha*]	BAI79243
Ps4′OMT2	3′-hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase 2 [*Papaver somniferum*]	AAP45314
Ps6OMT	(*R,S*)-norcoclaurine 6-*O*-methyltransferase [*Papaver somniferum*]	AAQ01669
Ps7OMT	(*R,S*)-reticuline 7-*O*-methyltransferase [*Papaver somniferum*]	AAQ01668
PsCNMT	Coclaurine *N*-methyltransferase [*Papaver somniferum*]	AAP45316
PsN7OMT	Norreticuline-7-*O*-methyltransferase [*Papaver somniferum*]	ACN88562
PsTNMT	(*S*)-tetrahydroprotoberberine-*cis*-*N*-methyltransferase [*Papaver somniferum*]	AAY79177
RgANMT	Anthranilate *N*-methyltransferase [*Ruta graveolens*]	ABI93949
SoPEANMT	Phosphoethanolamine *N*-methyltransferase [*Spinacia oleracea*]	AAF61950
TfCNMT	Coclaurine *N*-methyltransferase [*Thalictrum flavum*]	AAU20766
ZmUCMT	Uroporphyrinogen III methyltransferase [*Zea mays*]	BAA11909