Supplemental Information for

Discovery of a P450-catalyzed step in vindoline biosynthesis: a link between the aspidosperma and eburnamine type alkaloid scaffolds

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A. Experimental Procedures

Analysis of Catharanthus roseus expression profile data

For this analysis, a *C. roseus* expression data set[1] was employed (ftp.plantbiology.msu.edu/pub/data/MPGR/Catharanthus_roseus/). In an initial filtering step, those contigs with an expression value (log2 FPKM) lower than 1.0 in flower, mature leaf, young leaf, and three seedling tissues were eliminated. Since methyl jasmonate is a known elicitor of MIA biosynthesis,[2] expression values of contigs in non-elicited seedlings were compared to the corresponding expression value in seedlings after 12 days of elicitation with methyl jasmonate. Only contigs having a ratio greater than 1.0 (elicited/non-elicited) were retained. After these two filtering steps, 38 contigs annotated as cytochrome P450 were found within the remaining 6538 contigs. From these 38 contigs, five (2442, 1753, 4293, 1227 and 7549, Supplemental Table 1) were chosen for further analysis based on their high response to methyl jasmonate or tight co-expression with various MIA biosynthetic genes. All co-expresssion analyses used six *C. roseus* tissues (flower, mature leaf, young leaf, non-elicited seedling, seedling elicited with methyl jasmonate for 5 days and seedling elicited with methyl jasmonate for 12 days) using Pearson correlation (average linkage clustering) in the MultiExperiment Viewer (MeV v.4.8).

Virus induced gene silencing (VIGS)

The *C. roseus* variety "SunStorm Apricot" was used for all VIGS experiments. Plants were grown in a growth chamber at 25°C for a 12 h day/12 h night regime. For each of the five contigs tested, a fragment of approximately 300 bp was cloned into the USER compatible VIGS plasmid pTRV2u as previously described (Supplemental Table 2).[3] VIGS experiments were conducted as described previously.[4] Briefly, eight *C. roseus* seedlings (12 weeks old) were infiltrated per construct. Additionally, eight seedlings were infiltrated with pTRV2 lacking an insert (empty vector negative control) and four plants were infiltrated with a vector containing a fragment of the protoporphyrin IX magnesium chelatase gene (ChlH) (visual marker for a positive control). After 21 days, seedlings infiltrated with the pTRV-ChlH vector displayed substantial yellowing of leaves; the last leaf pair to emerge above the inoculation site was harvested (50-100 mg fresh weight tissue), frozen in liquid nitrogen and homogenized using a cryo bead mill (Retsch). The samples were divided into two equal batches for RNA and metabolite extraction.

Metabolite extraction and analysis of silenced tissues

For analysis of silenced tissues, 25-50 mg of frozen powdered leaves were incubated with 200 µL methanol at 56°C for 45 minutes. All samples were diluted with an equal volume of water and centrifuged prior to injection onto an LCMS, Thermo-Finnigan with Deca XP ion trap detector, Phenomenex Luna column, 3 µ (100 x 2.00 mm, 3 µm) with a binary solvent system consisting of acetonitrile (ACN) and 0.1% formic acid in water. The method began with 12%

ACN for 1 minute, then a 7 minute gradient to 25% ACN followed by a 4 minute gradient to 50% and then a 2 minute gradient to 100% ACN. The method was held for 7 minutes at 100% ACN, followed by a 2 minute gradient to 12% ACN and then 6 minutes at 12% ACN. Caffeine was used as an internal standard and peak area was calculated with the ICIS algorithm of Xcalibur (Finnigan) and normalized to the fresh weight of the sample. Measurements of changes in alkaloid levels in VIGS samples were performed in positive ion mode. Injection volume was 2 μL for all samples.

Gene expression analysis (qRT-PCR) of silenced tissues
RNA was extracted (RNeasy Plant Minikit, Qiagen) from an aliquot of each harvested sample of silenced tissues and converted to cDNA (iScript cDNA Synthesis Kit, Bio-Rad). The 40S Ribosomal Protein S9 (rps9) was used as a reference gene. The primer pair for *16t3o* was designed for the sequence of *16t3o* from the *C. roseus* variety "SunStorm Apricot" (Supplemental Table 2). Quantitative PCR was performed on a CFX96 Real Time PCR Detection System (Bio-Rad). Technical duplicates were measured for all biological samples, and determination of normalized relative expression was calculated as previously reported.[3]

Localization
The subcellular localization of 16T3O was determined according to the procedures described previously.[5] Briefly, the *16t3o* coding sequence was amplified by PCR using specific primers (Supplemental Table 2) and cloned into the SpeI restriction sites of the pSC-A cassette YFPi plasmid in frame with the 5' extremity of the YFP coding sequence. The resulting plasmid was used for transient transformation of *C. roseus* cells by particle bombardment in combination with a plasmid expressing the ER-CFP marker.

Yeast strains
Saccharomyces cerevisiae strains were cultured in YPD or synthetic complete drop-out (SC) media with 2% glucose lacking specific amino acids (Formedium, Hunstanton, UK). *S. cerevisiae* competent cells for the parental strain BY4741 (EUROSCARF, Frankfurt, Germany), strain A described below, and WAT11, a yeast strain harbouring the *A. thaliana* P450 reductase,[6] were prepared as described previously[7] with a modified transformation procedure. For transformation, the cells were incubated at 30°C for 15 minutes (vortexing briefly every 5 minutes) then 42°C for 15 minutes prior to plating on selective media. All primers, plasmids, and yeast strains used are listed in Supplemental Table 2 and Supplemental Table 3.

To construct strain A, T16H,[8] 16OMT,[9] NMT,[10] DAT,[11] D4H[12] and CPR[13] genes were amplified from cDNA isolated from *C. roseus* ("Little Bright Eyes") young leaves. The pXP plasmid set (Addgene, Cambridge, MA)[14] was used as a base to construct additional pXP plasmids with different sets of yeast promoters (TDH3, ADH1) and terminators (ENO2, TPI1, ADH1) (Supplemental Table 2 and Supplemental Table 3).[15] The yeast promoters and terminators were PCR amplified from BY4741 genomic DNA, digested with the appropriate restriction enzymes, and ligated into a similarly digested pXP. The CPR, T16H, 16OMT, NMT, DAT and D4H genes were amplified, digested, and subcloned into pXP plasmids at the SpeI and XhoI restriction sites. The plasmids were used as a template to amplify a linear fragment containing the desired region flanked by 50 bp homologous to either the *S. cerevisiae* genomic DNA integration site or the adjoining linear DNA fragment. The fragments were co-transformed into yeast for selection on appropriate selection media. Correct integration of all six *C. roseus* genes to yield strain A was confirmed by PCR analysis. pXP218 plasmid containing *16t3o* and empty pXP218 plasmid were transformed

into strain A to yield strain_A+p16T3O and strain_A+pEV. The same plasmids were also transformed into *S. cerevisiae* WAT11 to yield strains WAT11+p16t3o and WAT11+pEV, respectively.

*Production, extraction, and purification of 16-methoxytabersonine (**2**)*
For the production of 16-methoxtabersonine (**2**), a 500 mL culture of strain A was grown in YPD media to an OD600 of 0.5 and supplemented with tabersonine (**1**) to a final concentration of 120 μM. Conversion was monitored every 24 hours by UPLC-MS. To monitor reaction progress, samples (100 μL) were taken at 24 and 48 hours after addition of substrate and subsequently centrifuged to separate the cell pellet from the culture supernatant. An equal amount of methanol was added to the supernatant, and the sample was then incubated for 45 minutes at 56ºC, centrifuged again and then directly analyzed by UPLC-MS. An AQUITY UPLC with a Xevo TQ-S Mass-spec equipped with a BEH Shield RP18 1.7 μm column (Waters) was used for analysis. The 11 minute elution program applied a binary solvent system consisting of ACN and 0.1% formic acid in water. The gradient began with 12% ACN for 50 seconds, followed by a 180 second gradient to 25% ACN, a 130 second gradient to 50% ACN, a 50 second gradient up to 100% and then held at 100% ACN for 130 seconds. The gradient was then ramped to 12% ACN over 12 seconds, finishing with a 110 seconds at 12% ACN. Injection volume was 2 μL for all samples. Total ion chromatograms were collected in positive ion mode. After 48 hours, no tabersonine (**1**) could be detected, and the resulting enzymatic product was extracted from the culture supernatant with ethyl acetate (3 x 400 mL). The extract was dried with Na_2SO_4 and concentrated to a yellow oil. The oil was subjected to silica column chromatography using 2:8 ethyl acetate (EtOAc)/hexanes. The product eluted as the salicylic acid salt as judged by NMR. The salt was then dissolved in a small volume of EtOAc and filtered through K_2CO_3 to recover 3.9 mg of **2**.

*Production, extraction, and purification of 16T3O product **9***
The substrate for 16T3O, 16-methoxytabersonine (**2**), was produced using a 500 mL culture of strain A supplemented with 120 μM tabersonine (**1**). This culture was grown for 48 hours, as described above, centrifuged and the culture supernatant containing 16-methoxytabersonine (**2**) was reserved. In parallel, a 500 mL culture of the WAT11+pT3O strain (SC media with 2% galactose) was grown from a starter culture to an OD600 of 0.5, after which the cells were harvested by centrifugation, washed, and then resuspended in the reserved culture supernatant that contained 16-methoxytabersonine (**2**). The culture was supplemented with 2% glucose and grown for a further 24 hours, at which point the 16-methoxytabersonine starting material was completely consumed as observed by UPLC-MS. The oxidized product was extracted from the liquid fraction using EtOAc (3 x 400 mL). The extract was dried with Na_2SO_4 and concentrated to a yellow oil. The oil was subjected to silica column chromatography using first EtOAc and then followed with 10% MeOH in CH_2Cl_2. As judged by UPLC-MS, the product eluted in the latest EtOAc fractions and the initial 10% MeOH fractions. These fractions were combined, concentrated, and subjected to preparative HPLC using a C18 column and an increasing gradient of acetonitrile in 0.1% formic acid/water. All product-containing fractions were pooled and concentrated to give 10 mg of **9**. For mass spectrometry analysis, an equal amount of methanol was added to the supernatant, and the sample was then incubated for 45 minutes at 56ºC, centrifuged again and then directly analyzed by UPLC-MS. An AQUITY UPLC with a Xevo TQ-S Mass-spec equipped with a BEH Shield RP18 1.7 μm column (Waters) was used for analysis. The 11 minute elution program applied a binary solvent system consisting of ACN and 0.1% formic acid in water. The gradient began with 12% ACN for 50 seconds, followed by a 180 second gradient to 25% ACN, a 130 second gradient to 50% ACN, a 50 second gradient up to 100%

and then held at 100% ACN for 130 seconds. The gradient was then ramped to 12% ACN over 12 seconds, finishing with a 110 seconds at 12% ACN. Injection volume was 2 µL for all samples. Total ion chromatograms were collected in positive ion mode.

*Characterization of 16-methoxytabersonine (**2**) and 16T3O product **9***

IR spectra were obtained on a Perkin Elmer Spectrum BX equipped with a Pike technologies MIRacle™ single reflection horizontal ATR accessory possessing a zinc selenide (ZnSe) crystal lens. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz / 54 mm UltraShield Plus, long hold time automated NMR system (at 400 HMz, 100 MHz, and 40 MHz for ^{1}H, ^{13}C, and ^{15}N respectively). ^{1}H and ^{13}C NMR spectra are reported relative to residual $CHCl_3$ at δ 7.26 and 77.16 ppm respectively. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentent, m = multiplet, comp. m = complex multiplet, app. = apparent, br. = broad. ^{15}N NMR was referenced electronically by TOPSPIN. ^{15}N chemical shifts were obtained indirectly through a ^{1}H–^{15}N HMBC. This experiment yielded only the indole nitrogen (N1) and not the aliphatic one (N4). The shift reported for N1 is in a range consistent with that for other previously reported ^{15}N spectra of related vincamine-like alkaloids.[16] The purified product existed in an equilibrium mixture as shown in Figure 2 of the main text, but product **9** could be assigned completely from the 2D spectra.

The NMR solvent $CDCl_3$ (Cambridge Isotope Laboratories, Inc.) was used as received. All other standard solvents were purchased from Fisher Scientific and used as received. Column chromatography (MPLC) was done on neutral silica gel (particle size 35–70 µm) from Fluorochem. TLC was done on Merck silica gel 60 F254 precoated (0.25 mm) glass backed plates, 20 x 20 cm cut down to various sizes for use.

Data for exact mass was obtained on a Shimadzu IT-ToF using the Nexera LC system. The mass spectrometer was set up to collect spectra from *m/z* 250-2000, and data-dependent MS2 of the most abundant ions, at an isolation width of *m/z* 3.0, 50% collision energy, and 50% collision gas. Spray chamber conditions were 250°C curved desorbation line, 1.5 L min^{-1} nebulizing gas, and 300°C heat block. The instrument was calibrated before use, using sodium trifluoroacetate according to the manufacturer's instructions, and additionally background phthalate contaminant ions were used for mass compensation within the run. The identity of 16-methoxytabersonine (**2**) was validated by comparing NMR results (^{1}H and ^{1}H-^{13}C HSQC) to reported literature values.[17] Data for the 16T3O product **9**[18] is reported in the main text.

B. Supporting Tables

contig	vindoline decrease	FPKM for *C. roseus* tissues						seedling MJ 12 days/ seedling no MJ
		flowers	sterile seedlings	sterile seedlings MJ 12 days	sterile seedlings MJ 5 days	mature leaf	immature leaf	
era locus 1753	no	7.67	1.12	2.05	3.82	4.75	4	1.83
era locus 4293	no	8.07	2.16	3.53	2.8	5.69	5.08	1.63
era locus 7549	ves	3	5.67	6.96	7.84	4.18	5.41	1.23
era locus 2442	no	5.06	7.93	9.36	9.97	7.19	8	1.18
era locus 1227	no	5.55	7.11	7.85	6.33	5	5.25	1.10

Table 1. Candidate contigs that were tested by VIGS, with expression values in six tissues (MJ = methyl jasmonate).

For VIGS plasmid construction	
Contig 7549 forward	GGCGCGAUTGAGCAAATTGATGACGTCATGCA
Contig 7549 reverse	GGTTGCGAUAAGGGAATATCAAGGTCAAGATCACCAC
Contig 2442 forward	GGCGCGAUACCAGGTCCAAGAACACTACC
Contig 2442 reverse	GGTTGCGAUGTAATCTCCATACTGAGCAAAGGTAACAC
Contig 1753 forward	GGCGCGAUTTATCAAGTAGAACAATTCTCAATGCAATTC
Contig 1753 reverse	GGTTGCGAUCTTGAGATGAATTTGAATCATAAGTAGCAAAATCT
Contig 4293 forward	GGCGCGAUGCTTCCCTACTTGCAAGCAATAGTTA
Contig 4293 reverse	GGTTGCGAUCCGCCAACTGGACAGTCATG
Contig 1227 forward	GGCGCGAUGCCTAGCTTCAAGAACAGGATGC
Contig 1227 reverse	GGTTGCGAUATCCTAAGTATTGGTCCGAACCTGA
For qPCR analysis	
qT3O forward	GGCAACTCCCAGATGGTTCTACT
qT3O reverse	TCATGCATAGGACGTAGCGATTAAATGAA
qRPS9 forward	TTACAAGTCCCTTCGGTGGT
qRSP9 reverse	TGCTTATTCTTCATCCTCTTCATC
For localization	
T3Oy-for	CTGAGAACTAGTATGGAGTTTCATGAATCTTCTCCCTTC
T3Oy-rev	CTGAGAACTAGTTGCATAGGACGTAGCGATTAATTGAAG
For pXP construction	
adh1p forward	TTAGATTCCATATGTTAAAACAAGAAGAGGGTTGACTACAT
adh1p reverse	ATAAGTATCTCGAGAATATCACTAGTTTGTATATGAGATAGTTGATTGT
tdh3p forward	TTAGATTCCATATGTCAGTTCGAGTTTATCATTATCAATACTG
tdh3p reverse	ATAAGTATCTCGAGAATATCACTAGTTTTGTTTGTTTATGTGTGTTTATTC
eno2t forward	TATCTTATCTCGAGTAAAGTGCTTTTAACTAAGAATTATTAGTCTT
eno2t reverse	CTTAAGATCTGCAGAGGTATCATCTCCATCTCCCATAT
tpi1t forward	TATCTGCTCTCGAGCTAAGATTAATATAATTATATAAAAATATTATC
tpi1t reverse	CTTAAGATCTGCAGCTATATAACAGTTGAAATTTGGATAAGAACATC
adh1t forward	TTAATAATCTCGAGGAGCGACCTCATGCTATACCTGAGAAA

adh1t reverse	AATCTAGTGGATCCCGAATTTCTTATGATTTATGATTTTTAT
t3o forward	TTATTACTACTAGTATGGAGTTTCATGAATCTTCTCCCTTC
t3o reverse	AATCTAATCTCGAGTCATGCATAGGACGTAGCGATTAAA
For gene amplification	
CPR forward	ATATTCATGCTAGCATGGATTCTAGCTCGGAGAAGTT
CPR reverse	TAATTAATGTCGACTCACCAGACATCTCGGAGATACCTT
16OMT forward	TTATTACTACTAGTATGGATGTTCAATCTGAGGAGTTCCGTGGAG
16OMT reverse	AATCTAATCTCGAGTCAAGGATAAACCTCAATGAGACTCCTT
NMT forward	ATACTATTACTAGTATGAAAACATACAACAATACAATGGAAGAG
NMT reverse	TTTAATTCCTCGAGTCATATTGATTTTCGTCCCGTAACTACA
D4H forward	TATTTCTAACTAGTATGCCTAAGTCTTGGCCAATTGTGATAT
D4H reverse	TACTGACTCTCGAGCTAATTGTTTAACCTGAAAGGAGATAAG
DAT forward	TAAATTCTGCTAGCATGGAGTCAGGAAAAATATCGGTT
DAT reverse	AATTCCTTGTCGACTTAATTAGAAACAAATTGAAGTAGCTGTT
For integration fragments	
trpUTRtdh	ATCTAAAAGAGCTGACAGGGAAATGGTCAGAAAAAGAAACGTGCATCAGTTCGAGTTTATCATT
ENO2ADH1rev	TTTCTTCGATCCCCCTCATCGTGATGTAGTCAACCCTCTTCTTGTTTTAAAGGTATCATCTCCATCTCCC
ENO2adh	AAATGCGGGCCACGACCACAGTGATATGCATATGGGAGATGGAGATGATACCTTTAAAACAAGAAGAGGGTTG
trpUTRleulox1	TTGCTTTTCAAAAGGCCTGCATAACTTCGTATAATGTATGCTATACGAAGTTATGGATTTTCTTAACTT
trpUTRleulox2	TTACAGATTTTATGTTTAGATCTTTTATGCTTGCTTTTCAAAAGGCCTGC
adeUTRpgk	ACCAGTATATCATCTCATTTCCGTAAATACCAAATGTATTATATATTGAAAGGCCGCAAATTAAAGCCT
Pgk1tef1reverse1	GGGATTGGGTGTGATGTAAGGATTCGCGGTAGGCATTTGCAAGAATTACT
Pgk1tef1reverse2	GTGTGGGGGATCACTTGTGGGGGATTGGGTGTGATGTAAG
pgktefforward	CGATAGTTCCTCACTCTTTCCTTACTCACGAGTAATTCTTGCAAATGCCTACCGCGAATCCTTACATCAC
Adh1tdh3	TTTACGTATTCTTTGAAATGGCAGTATTGATAATGATAAACTCGAACTGACGAATTTCTTATGATTTATG
adhttdhforward	GAGCGACCTCATGCTATACCTGAGAAAGCAACCTGACCTACAGGAAAGAGTCAGTTCGAGTTTATCATTA
adeUTRloxmet1	TATGTATGTATGTATAATAAATAACTTCGTATAATGTATGCTATACGAAGTTATGTATAGTACTTGTGA
adeUTRloxmet2	GGTAATTATTCCTTGCTTCTTGTTACTGGATATGTATGTATGTATAATAA
For strain confirmation	
Trp1upconfirm	GATATTCCTTATGGCATGTCTGGCGATG
Trp1downconfirm	CCTGTACAATCAATCAAAAAGCCAAATGATTTAGC
Ade2upconfirm	GTTAGCTATTTCGCCCAATGTGTCCAT
Ade2downconfirm	GGTTCTGCATTGAGCCGCCTTATA
TEFconfirm	CACTTCAAAACACCCAAGCACAGCATACTAAATTT
LEU2markerconfirm	TCTGCCCCTAAGAAGATCGTCGTTTT

Table 2. Primer sequences used in this study. Cloning/restriction sites are underlined.

Strain	Description	Source or Reference
S. cerevisiae BY4741	MAT**a;** *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	EUROSCARF
SB1	BY4741 *trp1*::P_{TDH3}-*t16h*-T_{ENO2}-P_{ADH1}-*cpr*-T_{TPI1}-loxPLEU2-d8-loxP	This Study
SB2	SB1 *ade2*:: T_{CYC1}-*16omt*-P_{PGK1}-P_{TEF1}-*nmt*-T_{ADH1}-P_{TDH3}-*d4h*-T_{ENO2}-P_{ADH1}-*dat*-T_{TPI1}-loxP-MET15-loxP	This Study
Plasmid		
pXP218	P_{PGK1}, T_{CYC1}, URA3, 2μ	Fang, 2011
pXP622	$P_{HX7\text{-}391}$, T_{CYC1}, LEU2-d8, 2μ	Fang, 2011
pXP416	P_{TEF1}, T_{CYC1}, TRP1, 2μ	Fang, 2011
pXP(adh1p/tpi1)	pXP622 with P_{ADH1}, T_{TPI1} replacing $P_{HX7\text{-}391}$, T_{CYC1}	This Study
pXP(tdh3/eno2)	pXP622 with P_{TDH3}, T_{ENO2} replacing $P_{HX7\text{-}391}$, T_{CYC1}	This Study
pXP(adh1p/tpi1)MET	pXP(adh1p/tpi1) with loxP-MET15-loxP (from pXP214) replacing the loxP-LEU2-d8-	This Study
pXP(tef/adh1t)	pXP416 with T_{ADH1} replacing T_{CYC1}	This Study
pXP-CPR	pXP(adh1p/tpi1) with *cpr* inserted in XhoI, SpeI sites	This Study
pXP-16OMT	pXP218 with *16omt* inserted in XhoI, SpeI sites	This Study
pXP-NMT	pXP(tef/adh1t) with *nmt* inserted in XhoI, SpeI sites	This Study
pXP-D4H	pXP(tdh3/eno2) with *d4h* inserted in XhoI, SpeI sites	This Study
pXP-DAT	pXP(adh1p/tpi1)MET with *dat* inserted in XhoI, SpeI sites	This Study
pXP-T16H	pXP(tdh3/eno2) with *t16h* inserted in XhoI, SpeI sites	This Study

Table 3. Yeast strains and plasmids used in this study.

C. Supporting Figures

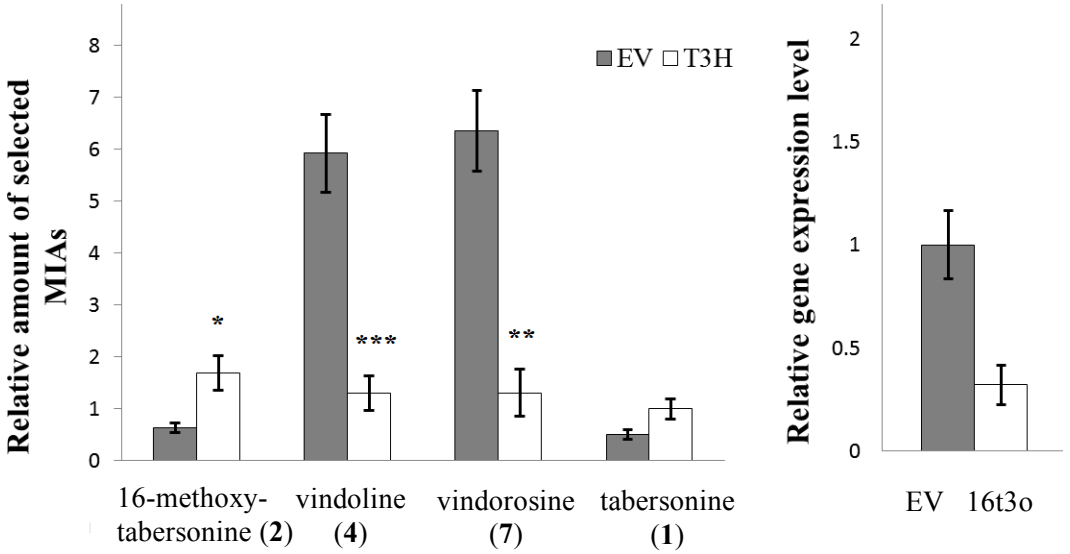


Figure S1. Virus induced gene silencing (VIGS) demonstrates the function of 16T3O *in planta*. A. Silencing of *16t3o* leads to accumulation of the precursor tabersonine (**1**) and 16-methoxytabersonine (**2**) and decreased accumulation of vindoline (**4**) and vindorosine (**7**). Data shown corresponds to average of MIA measurements of 8 plants silenced with construct for gene *16t3o* (white) in comparison to 8 plants silenced with empty vector (dark grey). Error bars indicate standard error. Statistical significance calculated with Student's *t* test (EV in comparison to *16t3o*) is indicated as followed: *P<0.05, **P<0.005, ***P<0.0005. **B.** VIGS results in significantly reduced accumulation of *16t3o* transcripts in *16t3o* silenced tissues compared to empty vector tissues. Values represent average of 6 plants for both constructs, *16t3o* (white) and empty vector (EV) (dark grey).

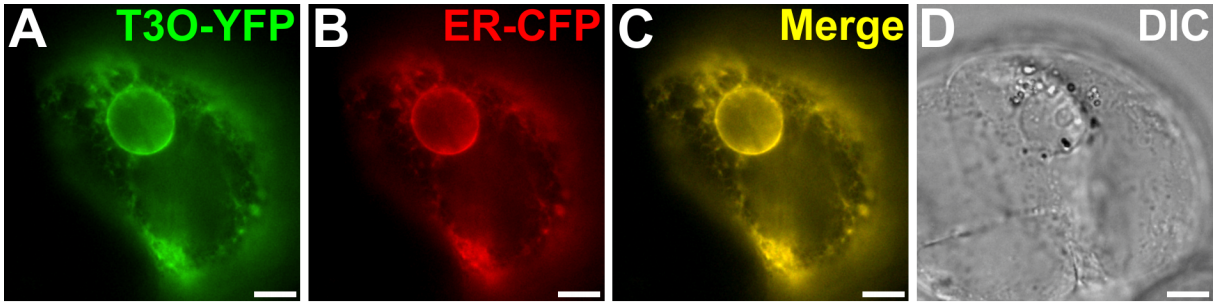


Figure S2. 16T3O is located at the endoplasmic reticulum. *C. roseus* cells were transiently transformed with the 16T3O-YFP expressing vector (T3O-YFP; **A**) in combination with the plasmid expressing an endoplasmic reticulum-CFP marker ("ER"-CFP; **B**). Co-localization of the two fluorescence signals appears on the merged images (**C**). Cell morphology (**D**) was observed with differential interference contrast (DIC). Bars = 10 µm.

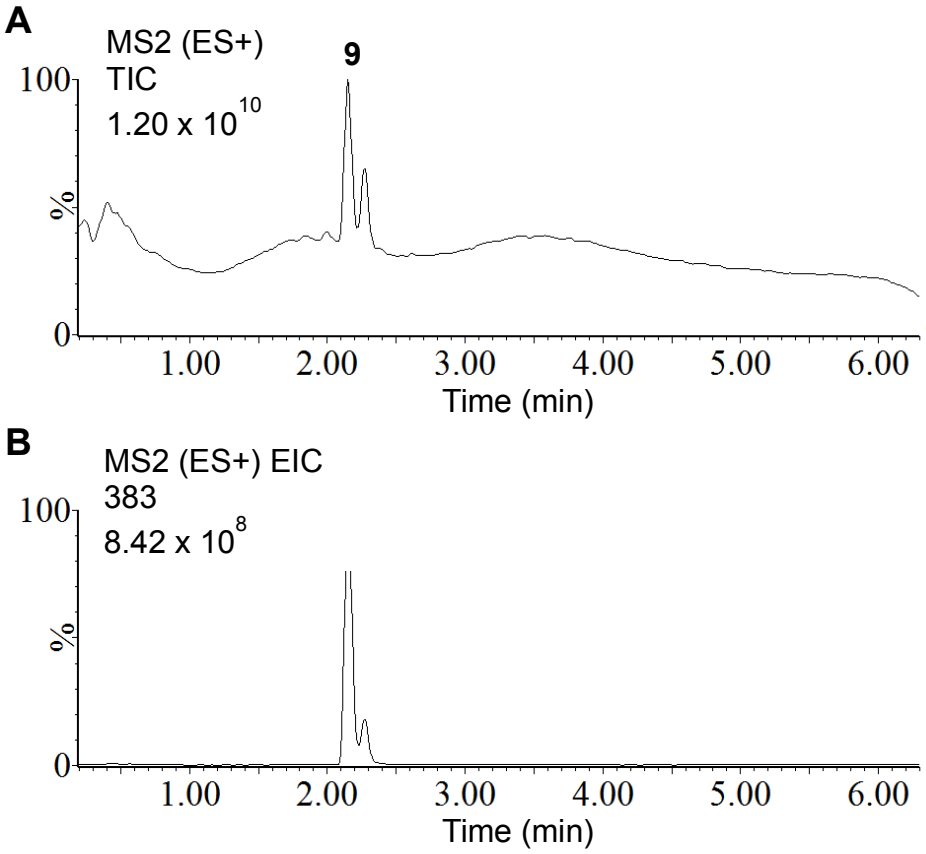


Figure S3. Chromatogram of purified **9**. **A.** Total ion chromatogram. **B.** Selected ion monitoring at *m/z* 383. **C.** Mass spectrum for the major peak at 2.147 minutes. **D.** Mass spectrum for the minor peak at 2.273 minutes. Monitoring was performed by UPLC in tandem with mass spectrometry (UPLC-MS) as described in section "Production, extraction, and purification of 16T3O product **9**" above.

	This study		From supplemental reference 19 (Zhang et al. 2007)		From supplemental reference 19 (Zhang et al. 2007)	
	Compound 9 from isolation		**14,15-didehydro-10,11-dimethoxyvincamine**		**14,15-didehydro-10,11-dimethoxy-14-epivincamine**	
	1H	**13C**	**1H**	**13C**	**1H**	**13C**
indole N(H)	–	–	–	–	–	–
2	–	131.2	–	130.1	–	131.2
3	3.96 (br s, 1H)	57.13	4.06 (br s)	57.6	3.80 (br s)	57.1
alkyl N	–	–	–	–	–	–
5	3.38 (dd, J = 13.9, 7.0 Hz, 1H); 3.25 (dd, J = 11.0, 6.4 Hz, 1H)	49.82	3.43 (dd, 13.9, 7.0); 3.33 (ddd, 13.9, 10.6, 6.1)	49.6	3.38 (dd, 13.8, 6.9); 3.24 (ddd, 13.8, 11.0, 6.3)	49.8
6	3.09–3.02 (comp. m, 1H); 2.48 (ddd, J = 16.3, 6.2, 2.1 Hz, 1H)	16.67	3.05 (m); 2.53 (ddd, 16.1, 6.1, 1.7)	16.8	3.07 (m); 2.49 (ddd, 16.0, 6.3, 1.8)	16.7
7	–	106.4	–	106	–	106.3
8	–	123.22	–	122	–	121.6
9	7.31 (d, J = 8.5 1H)	118.36	6.91 (s)	100.8	6.88 (s)	100.1
10	6.77 (dd, J = 8.6, 2.3 Hz, 1H)	109.36	–	145.4	–	145.2
11	–	156.1	–	146.5	–	146.1
12	7.04 (d, J = 2.3 Hz, 1H)	97.44	6.61 (s)	94.8	7.06 (s)	96.9
13	–	137.7	–	128.2	–	131
14	–	84.07	–	82.1	–	84.1
15	2.63 (d, J = 14.2 Hz, 1H); 2.15 (d, J = 14.2 Hz, 1H)	46	2.38 (d, 14.1); 2.31 (d, 14.1)	43.6	2.57 (d, 14.0); 1.98 (d, 14.0)	45.9
16	–	38.5	–	36.8	–	38.5
17	5.28 (dtd, J = 10.3, 2.3, 1.0 Hz, 1H)	126.89	5.75 (br d, 10.3)	128.1	5.24 (br d, 10.3)	126.6
18	5.48 (dt, J = 10.3, 3.2 Hz, 1H)	125.57	5.60 (ddd, 10.3, 3.8, 2.4)	125.7	5.48 (dt, 10.3, 3.1)	125.8
19	3.05 (dd, J = 3.0, 2.5 Hz, 2H)	43.71	3.11 (ddd, 17.3, 3.8, 2.1); 3.02 (dt, 17.3, 2.4)	43.8	3.01 (br s, 2H)	43.7
20	1.84 (dq, J = 15.0, 7.3 Hz, 1H); 1.54 (dq, J = 14.8, 7.4 Hz, 1H)	35.25	1.95 (dq, 15.1, 7.6); 1.62 (dq, 15.1, 7.6)	34.8	1.76 (dq, 15.0, 7.6); 1.41 (dq, 15.0, 7.6)	35.2
21	0.96 (app. t, J = 7.6 Hz, 3H)	8.44	1.00 (t, 7.6)	8.4	0.91 (app. t, 7.6)	8.4
22	–	172.1	–	173.1	–	172
23	3.50 (s 3H)	52.72	3.87 (s)	54	3.48 (s)	52.6
10-OMe	–	–	3.91 (s)	56.4	3.91 (s)	56.2
11-OMe	3.79 (s 3H)	55.68	3.84 (s)	56.4	3.85 (s)	56.4
14-OH	–	–	3.86 (s)	–	4.24 (br s)	–

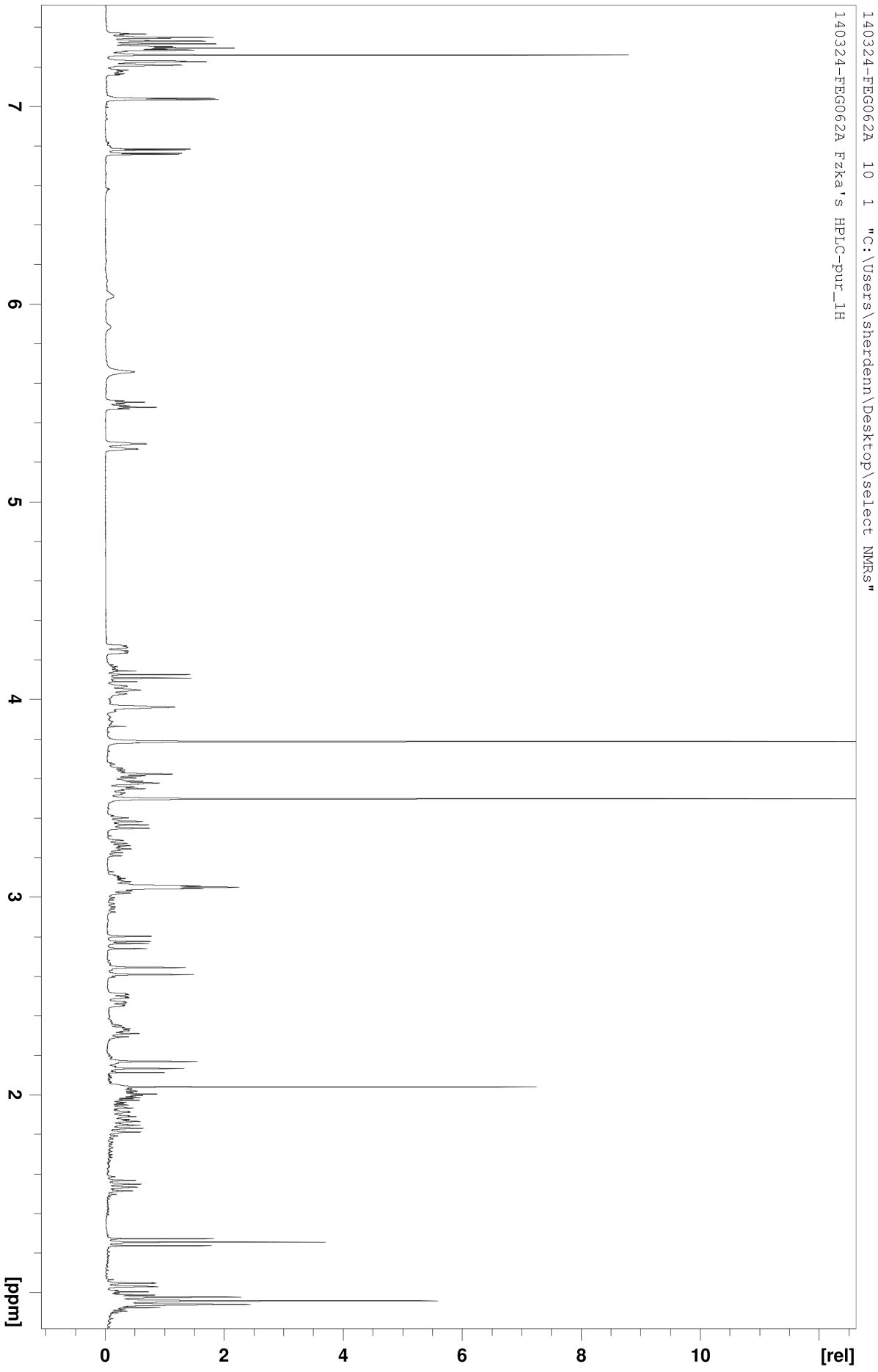
9

14,15-didehydro-10,11-dimethoxyvincamine

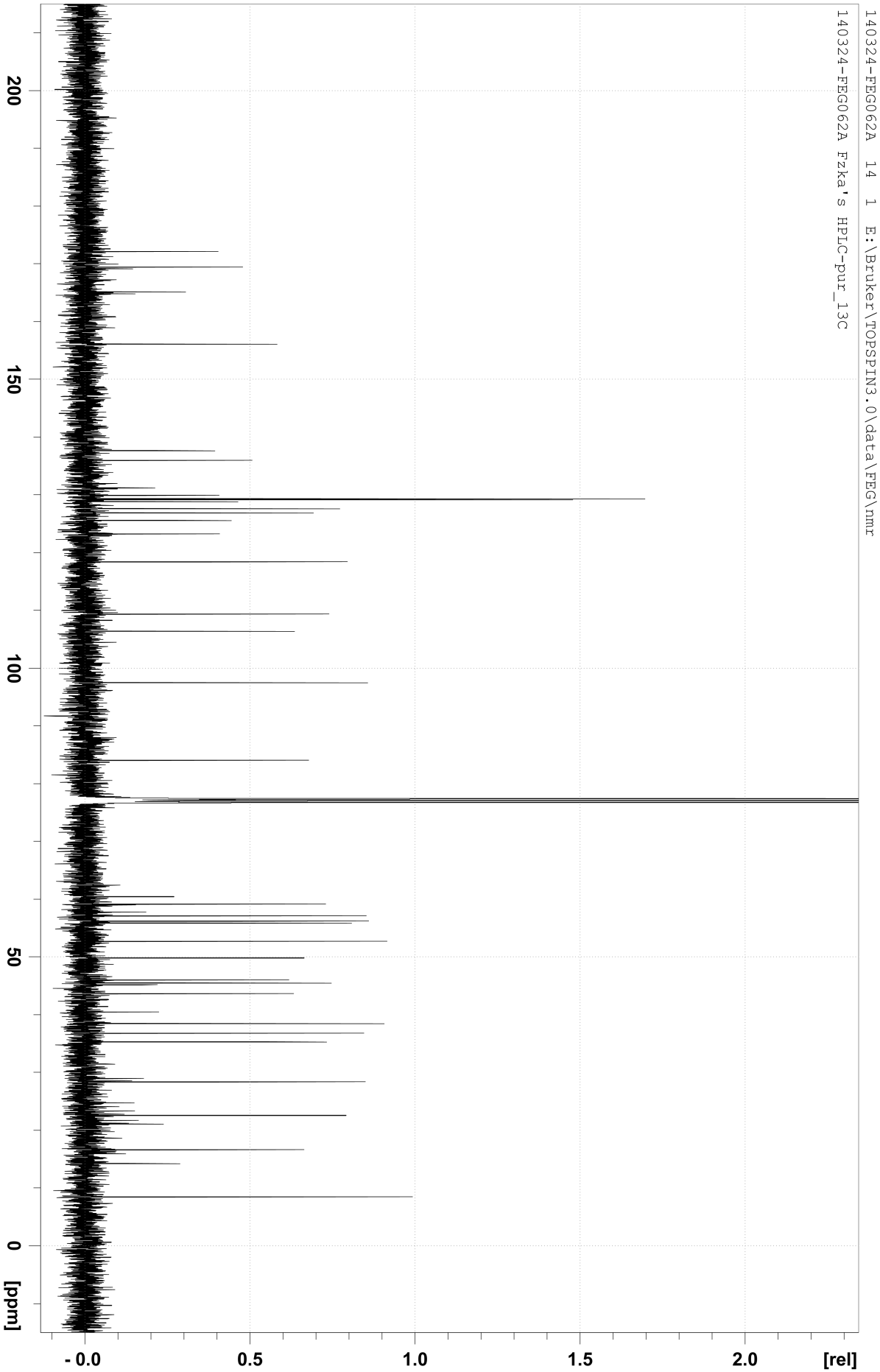
14,15-didehydro-10,11-dimethoxy-16-epivincamine

Comparison of NMR data from compound **9** with NMR data from previously reported compounds having similar structure. See supplemental reference 19.

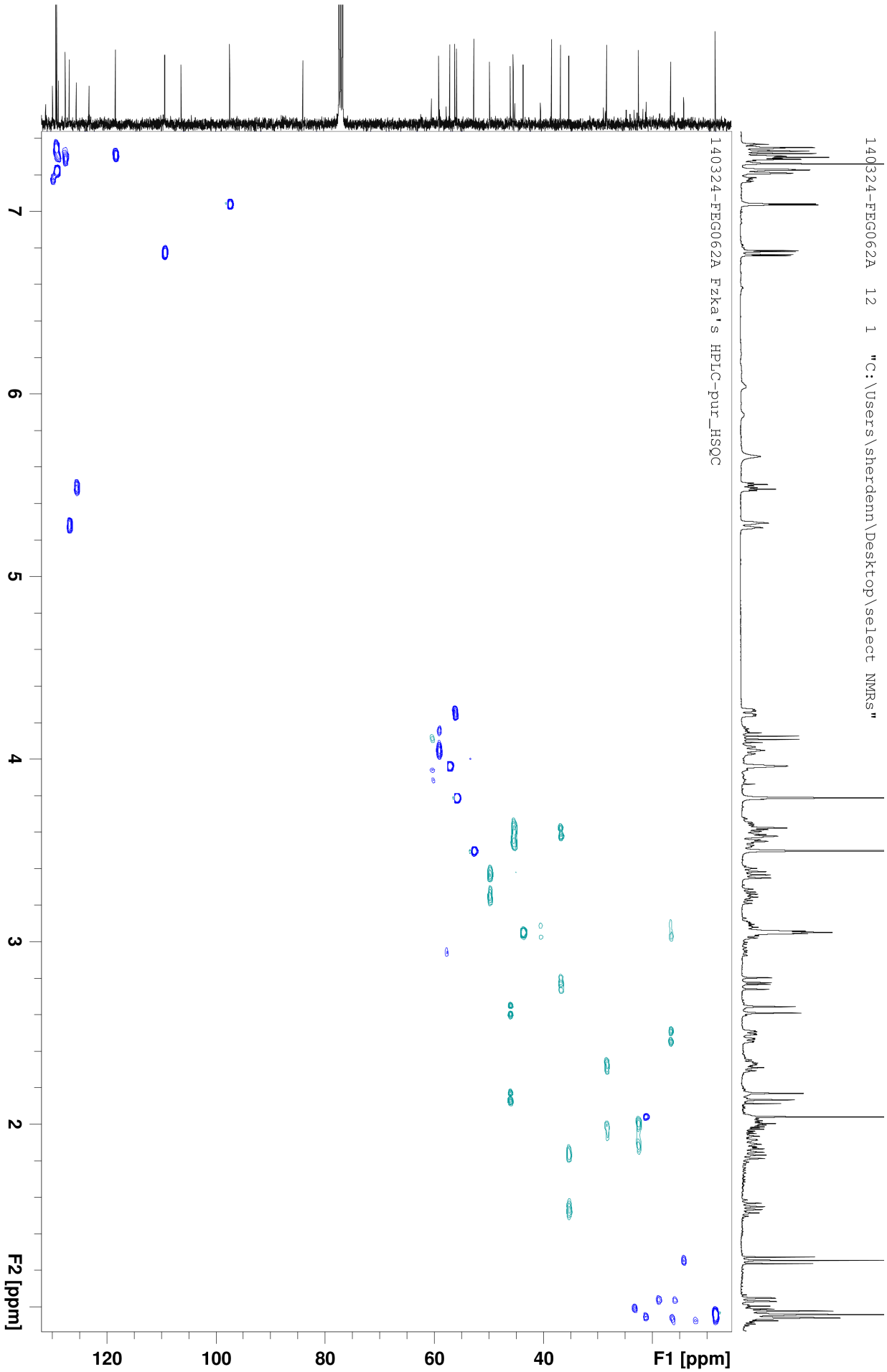
Compound 9 ^{1}H NMR



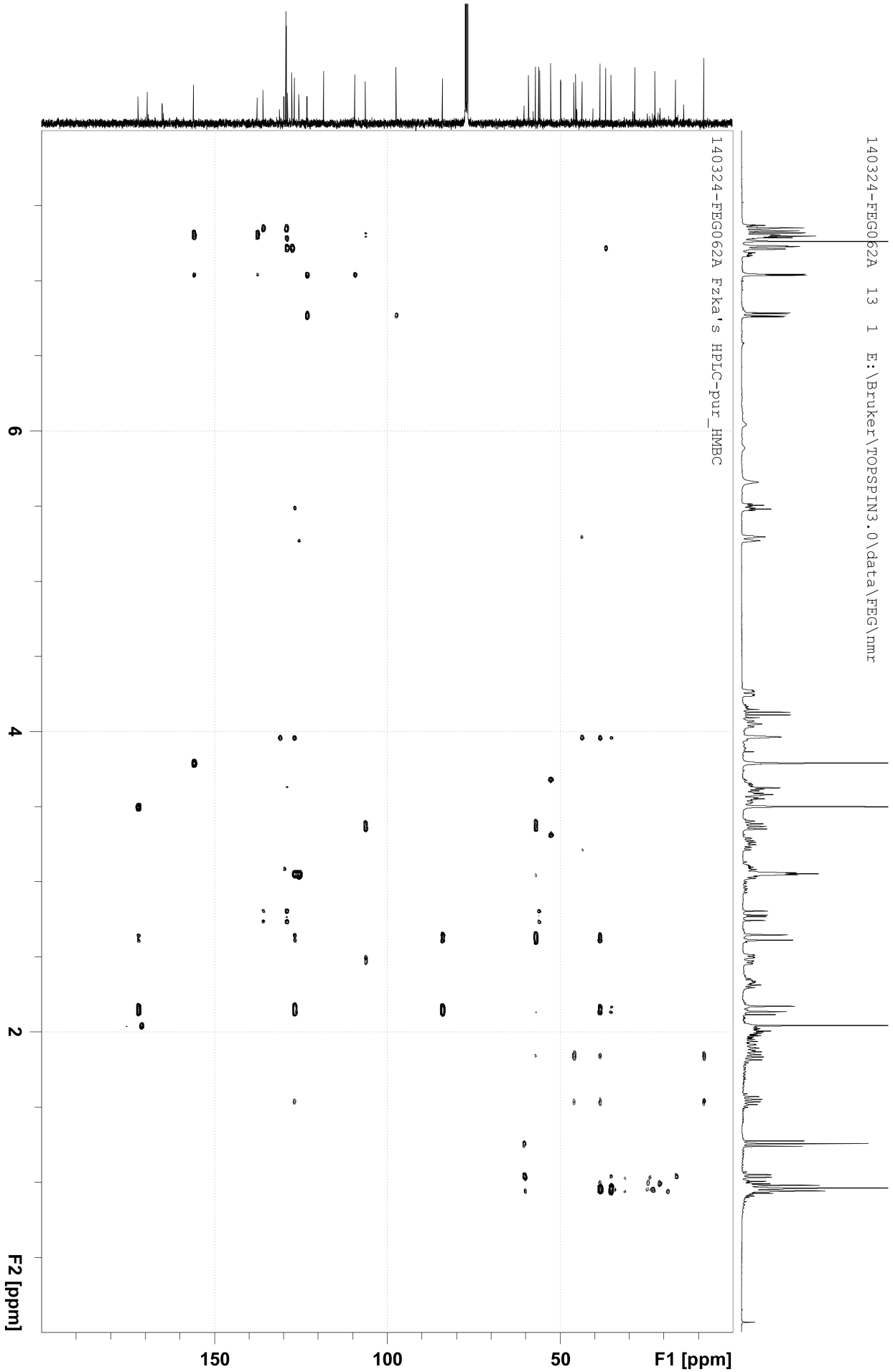
Compound 9 ^{13}C NMR



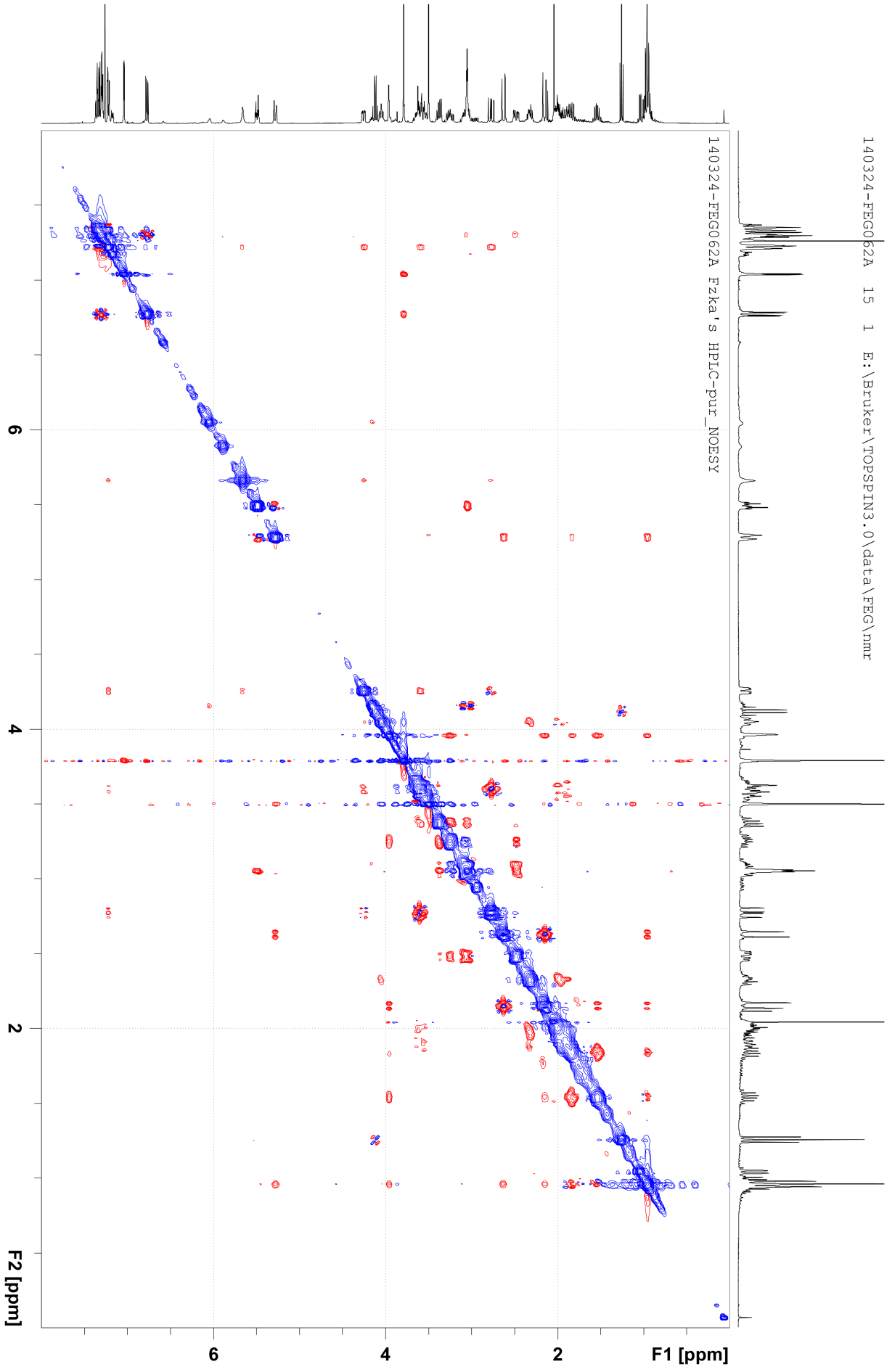
Compound 9 ^{1}H–^{13}C HSQCed



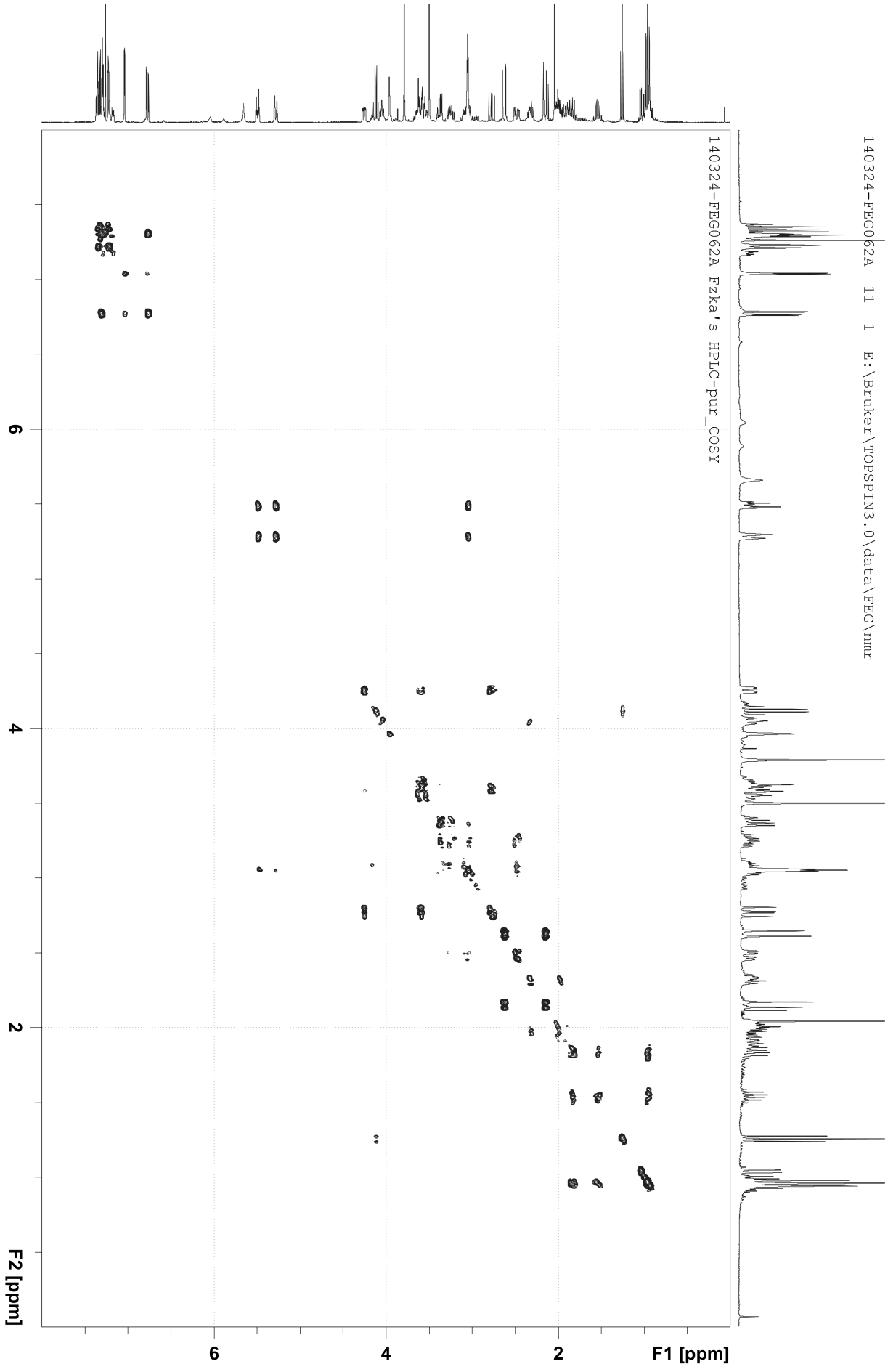
Compound 9 ^{1}H–^{13}C HMBC



Compound 9 ^{1}H NOESY



Compound 9 ^{1}H COSY



Supplemental References

1 E. Góngora-Castillo, K. L. Childs, G. Fedewa, J. P. Hamilton, D. K. Liscombe, M. Magallanes-Lundback, K. K. Mandadi, E. Nims, W. Runguphan, B. Vaillancourt, M. Varbanova-Herde, D. DellaPenna, T. D. McKnight, S. O'Connor and C. R. Buell, *PLoS One*, 2012, **7**, e52506.

2 R. J. Aerts, D. Gisi, E. Carolis, V. Luca and T. W. Baumann, *Plant J.*, 1994, **5**, 635–643.

3 F. Geu-Flores, N. H. Sherden, V. Courdavault, V. Burlat, W. S. Glenn, C. Wu, E. Nims, Y. Cui and S. E. O'Connor, *Nature*, 2012, **492**, 138–142.

4 D. K. Liscombe and S. E. O'Connor, *Phytochemistry*, 2011, **72**, 1969–1977.

5 G. Guirimand, V. Burlat, A. Oudin, A. Lanoue, B. St-Pierre and V. Courdavault, *Plant Cell Rep.*, 2009, **28**, 1215–1234.

6 P. Urban, C. Mignotte, M. Kazmaier, F. Delorme and D. Pompon, *J. Biol. Chem.*, 1997, **272**, 19176–19186.

7 R. D. Gietz and R. H. Schiestl, *Nat. Protoc.*, 2007, **2**, 38–41.

8 G. Schröder, E. Unterbusch and M. Kaltenbach, *FEBS Lett.*, 1999, **458**, 97–102.

9 D. Levac, J. Murata, W. S. Kim and V. De Luca, *Plant J.*, 2008, **53**, 225–236.

10 D. K. Liscombe, A. R. Usera and S. E. O'Connor, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 18793–18798.

11 F. Vazquez-Flota, E. De Carolis, A. M. Alarco and V. De Luca, *Plant Mol. Biol.*, 1997, **34**, 935–948.

12 B. St-Pierre, P. Laflamme, a M. Alarco and V. De Luca, *Plant J.*, 1998, **14**, 703–13.

13 A. H. Meijer, M. I. Lopes Cardoso, J. T. Voskuilen, A. de Waal, R. Verpoorte and J. H. Hoge, *Plant J.*, 1993, **4**, 47–60.

14 F. Fang, K. Salmon, M. W. Y. Shen, K. A. Aeling, E. Ito, B. Irwin, U. P. C. Tran, G. W. Hatfield, N. A. Da Silva and S. Sandmeyer, *Yeast*, 2011, **28**, 123–136.

15 Z. Shao, H. Zhao and H. Zhao, *Nucleic Acids Res.*, 2009, **37**, e16.

16 G. E. Martin, *J. Heterocycl. Chem.*, 1997, **34**, 695–699.

17 S. A. Kozmin, T. Iwama, Y. Huang and V. H. Rawal, *J. Am. Chem. Soc.*, 2002, **124**, 4628–4641.

18 N. Neuss, H. E. Boaz, J. L. Occolowitz, E. Wenkert, F. M. Schell, P. Potier, C. Kan, M. M. Plat and M. Plat, *Helv. Chim. Acta*, 1973, **56**, 2660–2666.

19 H. Zhang, X.-N. Wang, L.-P. Lin, J. Ding, J.-M. Yue, *J. Nat. Prod.* 2007, **70**, 54-59.