

Supplementary data

Identification and Characterization of Two Se6OMTs from *Stephania epigaea* Offer Novel Insights into the Biosynthetic Pathway of Cepharanthine

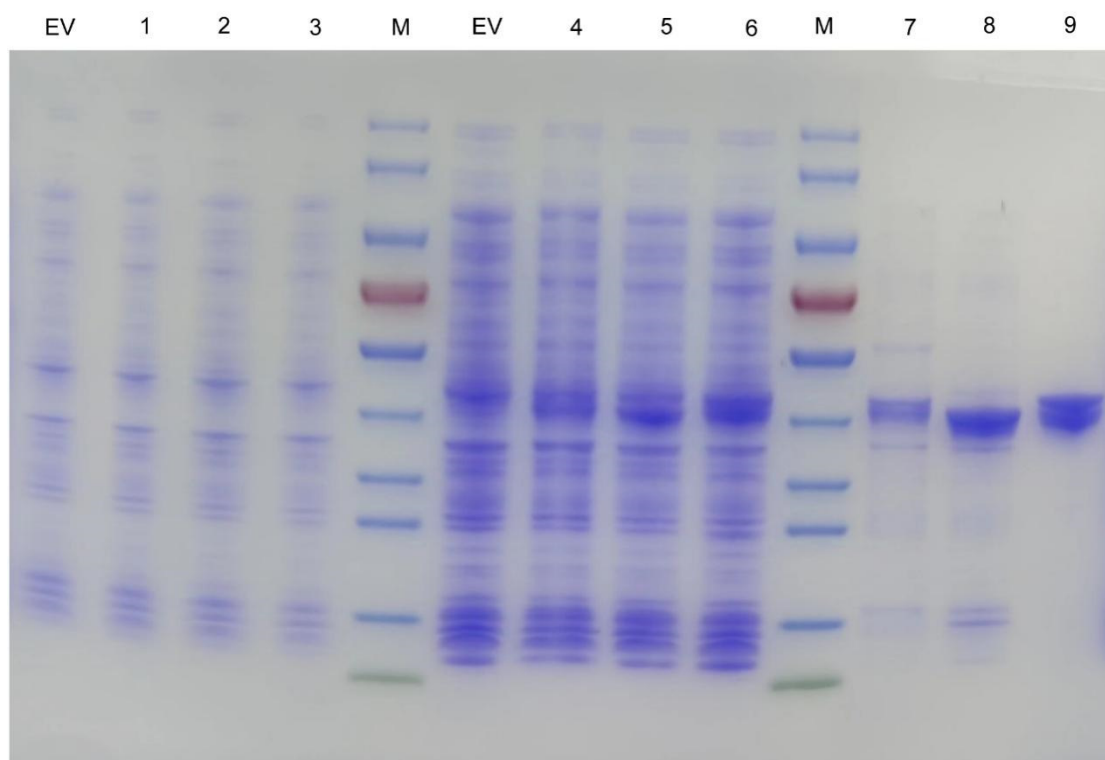


Figure S1. SDS-PAGE analyses was conducted on the purified proteins of St6OMT1-3 from the induced expression of the recombinant plasmid. Lane M: protein ladder, with molecular weights from top to bottom at 180, 130, 100, 70, 55, 40, 35, 25, 15, 10 kDa. Lanes 1-3: cell lysate before induction of St6OMT1-3, respectively. Lanes 2: the supernatant of induced cell lysate of St6OMT1-3. Lanes 3: the purified enzymes of St6OMT1-3. EV denotes the empty vector.

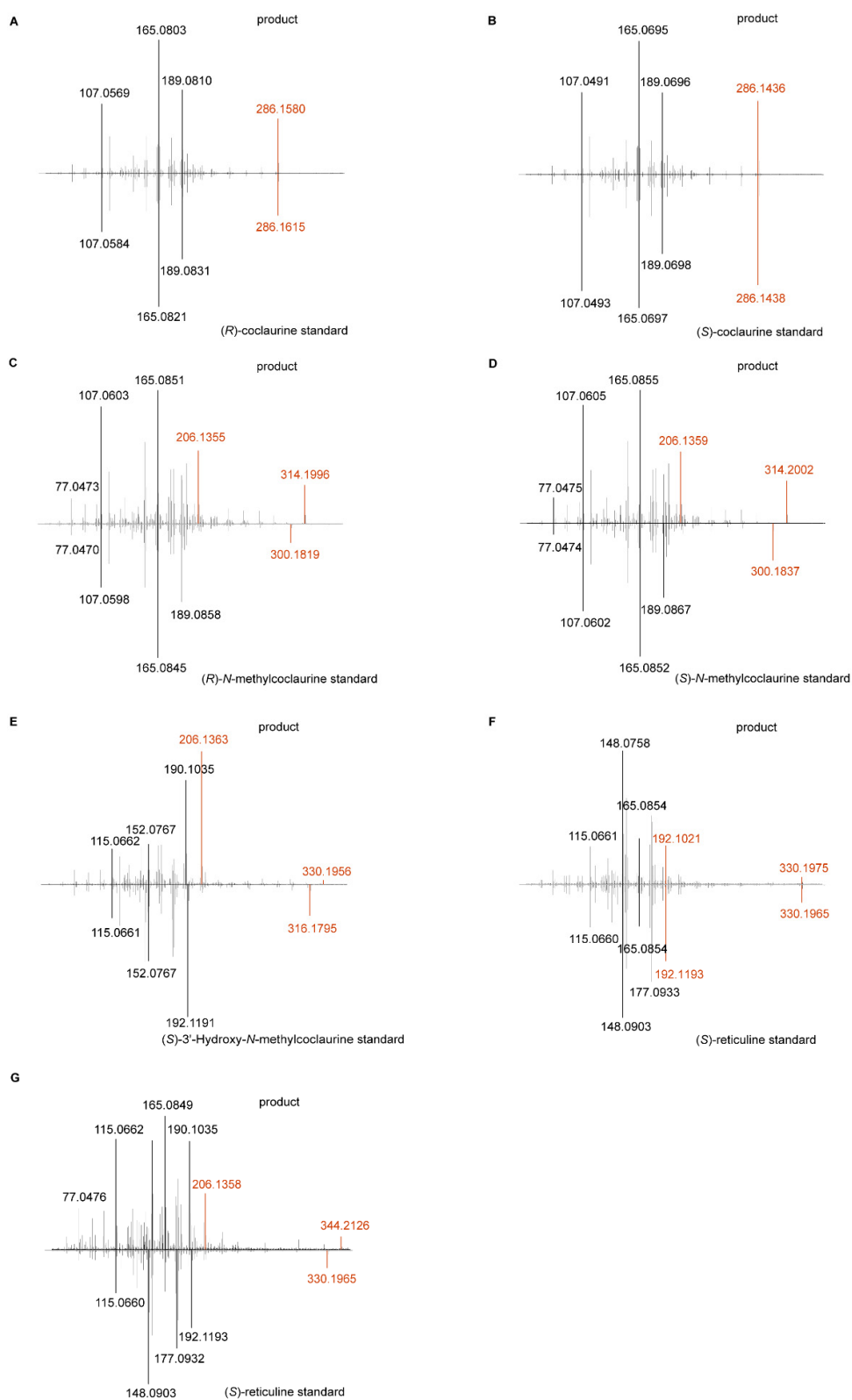
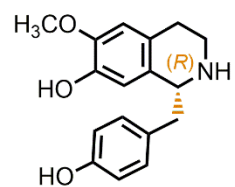
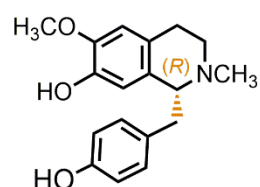


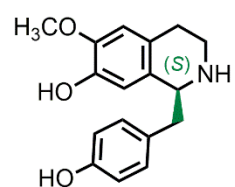
Figure S2. High-Resolution MS/MS spectrum fragmentation of products catalyzed by Se6OMTs. **A**, **B**, Mass spectrum of fragment ions of standard (R)- and (S)-coclaurine (downside) and reaction products of Se6OMT1 and Se6OMT3 with substrate (R)- and (S)-coclaurine (upside), respectively. **C**, **D**, Mass spectrum of fragment ions for standard (R)- and (S)-NMC are depicted in lower side, while the reaction products of Se6OMT3 with substrates (R)- and (S)-NMC are shown in upper side, separately. **E**, the comparison between (S)-HNMC standard and the products of Se6OMT3 with (S)-HNMC as the substrate. **F**, **G**, the comparison between (S)-reticuline standard and two BIAs produced by Se6OMT3 catalyzing (S)-HNMC and (S)-reticuline, respectively.



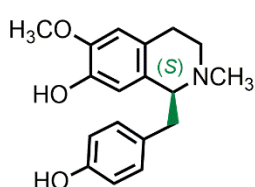
(*R*)-Coclaurine



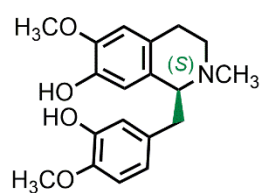
(*R*)-*N*-methylcoclaurine



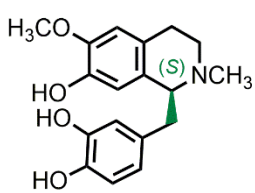
(*S*)-Coclaurine



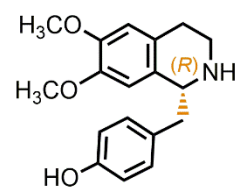
(*S*)-*N*-methylcoclaurine



(*S*)-Reticuline



(*S*)-3'-Hydroxy-*N*-methylcoclaurine



(*R*)-norarmepavine

Figure S3. Seven compound structures of 1-BIAs were tested as potential substrates of Se6OMTs.

Table S1. The full-length nucleic acid sequences of *Stephania epigaea* OMTs in this study.

gene	sequences
<i>Se6OMT1</i>	ATGGCATCAGAAAATCTAAAGCAAGACCAAGCAGCCCAAGCCAAGTTATGGAGCTTCATA- TATGGCTTTGCAGAGTCACTAGTCGTCAAGTCTGTTATTCAGCTTGACATACCTGATATCATTAC AACCATGGCAAGCCCATCTCCCTTTCCAACCTTGCTTCTCGAATTCCTTCTCCGAAC- GGCGCCGTGGATCACGACCGCTTGTTCCGGATCATGCGGTATTTGGTGCACATGAAGCTCTTCA CGAAAGAGTCTATCGACGGCGAAGATCATTATGGTCTTGCGCCGGCCGCGAAGTACCTAG- TGAAGGGGTGGGAGAAGAGCATGGTGCCTTCAATTCTATGTATAACTGATAAGGAGTTCATGTC ACCATGGTACCATCTCAAGGAGGGGTGGGGAACGAGAGCACGACAGCGTTTCGA- GAAGGCGTTGGGGATGAACATATGGGATTACATGTGCAAGAACCCGAAGAAGAACAACATCTT CAATGAGGCCATGGCTTGTGACACTAGGCTACTCATGTCTGCATTGGTGAG- TGAATGCAAGGACATGTTTCAAGGGATTAAGTCGCTAGTCGATGTCGGCGGGGGGACTGGAAC GCAGTTAGGCACATTGCCAAGGCCTACCCTCACATAAATTGCAGTGTC- TATGATCTCCCTCATGTTGCTGCTGATTCTCTACTTACCCTGAAGTGACTCGCATCGAAGGCGA CATGTTCAAGTTCATTCCCAAGCAGATGCCATCCTAATGAAGTG- CATCCTTCATGACTGGGCCGACGACGAATGCATTAAGATTCTAAAGAAGTGCAAGGAAGCGTTG CCGAGTAATGGGGGGAAAGTAATCCTCGTCGAGGTCGTGTTGAATGCGCTTTCGGAG- CATCCATACACAAAGATAAGGATGGTGAGTGACTTGGACATGATGCTGAACTGTGGAGGTAAAG AAAGAACAGAGGGGGAGTGGAAGAACCTCATCTATGCAGCTGGTTTTAGCAGGTACAA- TATCAGGCAACTGGATTTACACAGTATGTTATTGAGGTCTACCCTTGA
<i>Se6OMT2</i>	ATGGCAGCAGAAAATCTAGAGCAACACCAAGCAGCCCAAGCCAAGCTATGGAGC- TACATCTATGGCTATACAGAGTCACTAGTACTCAAGTCTGCTGTTTCACTTGACATCCCTGATATC ATCCACAACCATGGCAAGCCCATCTCCCTCTCCGACCTTGCTCGCCGGATTTCTCACCAA- GCGGCGCCGTGGATCACAAACCGCTTGATCGGATCATGCGCTACTTGGTGTACATGAAGTTCTTC ACGAAGGAGTCCGACGTCAATGGTGAAGATCGATATGGGCTTGACCCGCCCCGCAAAGTAC- CTAGTGAAGGGGTGGGAGAAGAGCATGGTACCTTCAATTCTAAGCCTCACTGATGAGGAATTCA TGTTACCATGGTACCATCTCAAGGATGGTTTGTGCAACAATGGCACAACCCGCGTTAGA- GAAGGCGCTAGGGATGGGCATATGGGAATACATGTGCAAGAACCCCAAGACGAACAGCATCTT CAATGAGGCCATGGCTTGTGACACTAGGCTGGTCATGTCGTCATTGGTGAG- TGAATGCAAGGACATGTTTCAAGGAATTAAGTCACTAGTCGATGTCGGCGGGGGGACTGGGACT GCTGTTAGGTACATTGCCAAGGCCTTCCCTTACATAAATTGCACTGTC- TATGATCTCCCTCATGTTGCTGCTGATTCTCTTACTTACCCCGAAGTGACTCGCGTCGAAGGCGA CATGTTCAAATACATTCTAAAGCTGATGCCATCCTAATGAAGTACATTC- TACATGACTGGGCCGACGAGGAAAGCATTACAGATACTGAAGAAGTGTAAGGAAGCATTGCCGA GTGATGGGGGGAAGGTAATCCTAGTCGATATCGTGTTGAATCCGTTTTTCGGAG- CATCCATACGCGAAGGTGAGGATGGTGAGCGACGTGGACATGATGCAGAGCTGTGGAGGGAAA GAGAGGACAGAGGAGGAGTGGAAGAAGCTCATCTATGCTGCAGGTTTTAG- TAGGTACAACATTAGGCACATAAATACCATACCATCTGTTATTGAGGTTTTTCCTTGA
<i>Se6OMT3</i>	ATGGCAACACAAAATCTAAAGCAAGACCAAGCATCCCAAGCAAAGCTATGGAGCTTCATA- TATGGCTTTGCAGAGTCACTAGTCCTCAAGTCTGCCATTCAACTTGACATACCTGATATCATTAC AACCATGGCAAGCCCATGACCTATCCAACCTTGCTTCTCGAATTCCTTCTCCGAAC- GGCGCCGCGGATCACGACCGCTTGTTCCGGATCATGCGCTATTTGGTGCACATGAATCTCTTCAC GAAAGAGTCTATCGACGGCGAAGATCATTATGGTCTTGCGCCGGCCGCGAAGTACCTAG- TGAAGGGGTGGGAGAAGAGCATGGTGCCTTCAATTCTATGTATAACTGATAAGGAGTTCATGTC ACCATGGTACCATCTCAAGGAGGGGTGGGGAACGAGAGCACGACAGCGTTTCGA- GAAGGCGTTGGGGATGAACATATGGGATTACATGTGCAAGAACCCGAAGAAGAACAACATCTT CAATGAGGCCATGGCTTGTGACACTAGGCTACTCATGTCTGCATTGGTGAG- TGAATGCAAGGACATGTTTCAAGGGATTAAGTCGCTAGTCGATGTCGGCGGGGGGACTGGAAC GCAGTTAGGCACATTGCCAAGGCCTACCCTCACATAAATTGCAGTGTC- TATGATCTCCCTCATGTTGCTGCTGATTCTCTACTTACCCCGAAGTGACTCGCATCGAAGGCGA CATGTTCAAGTTCATTCCCAAGCAGATGCCATCCTAATGAAGTG- CATCCTTCATGACTGGGCCGACGACGAATGCATTAAGATTCTAAAGAAGTGCAAGGAAGCGTTG CCGAGTAACGGGGGGAAGGTAATCCTAGTCGATATCGTGTTGAATGCGTTTTTCGGAG- CATCCATACACGAAGATGAGGATGGTGATGGACTTGGACATGATGCTGAACTGTGGAGGGAAA GAGAGGACAGAGGAGGAGTGGAAGAAGCTCATCTATGCTGCAGGTTTCAGTAGGTACAA- GATTAGGCAAGTGAATGCCATACAGTCTGTTATTGAGGTTTACCCTTACTGA

Table S2. The primer sequences for cloning Se6OMTs-pET-28a.

gene	PET-28a primer sequences
<i>Se6OMT1</i> -F	CAGCAAATGGGTCGCGGATCCATGGCATCAGAAAATCTAAAGCAA
<i>Se6OMT1</i> -R	ACGGAGCTCGAATTCGGATCCTCAAGGGTAGACCTCAATAACAT
<i>Se6OMT2</i> -F	CAGCAAATGGGTCGCGGATCCATGGCAGCAGAAAATCTAGAG
<i>Se6OMT2</i> -R	ACGGAGCTCGAATTCGGATCCTCAAGGAAAAACCTCAATAACAGATG
<i>Se6OMT3</i> -F	CAGCAAATGGGTCGCGGATCCATGGCAACACAAAATCTAAAGCA
<i>Se6OMT3</i> -R	ACGGAGCTCGAATTCGGATCCTCAGTAAGGGTAAACCTCAATAACA

Table S3. The Physical and chemical properties of Se6OMTs in this study.

protein	Se6OMT1	Se6OMT2	Se6OMT3
Molecular weight	39406.59	39605.66	39645.02
Theoretical pI	6.27	6.02	6.60
Total number of negatively charged residues	43	44	43
Total number of positively charged residues	39	39	41
Formula	C ₁₇₆₈ H ₂₇₆₇ N ₄₆₃ O ₅₁₀ S ₂₃	C ₁₇₈₃ H ₂₇₈₀ N ₄₆₀ O ₅₁₉ S ₂₀	C ₁₇₇₆ H ₂₇₈₅ N ₄₆₅ O ₅₁₀ S ₂₆
Total number of atoms	5531	5562	5562
Instability index	41.64	40.76	42.82
Aliphatic index	89.74	88.63	86.47
Grand average of hydropathicity	-0.156	-0.183	-0.184

Table S4. Abbreviations and GenBank accessions numbers for functionally characterized plants OMTs used for phylogenetic analysis.

Gene	Source	Accession
Ps6OMT	<i>Papaver somniferum</i>	AAP45315
Ps4'OMT	<i>Papaver somniferum</i>	AAP45313
Ps7OMT	<i>Papaver somniferum</i>	AAQ01668
PsNMT4	<i>Papaver somniferum</i>	AOR51553
PsTNMT	<i>Papaver somniferum</i>	Q108P1
Cj6OMT	<i>Coptis japonica</i>	BAB08004
St6OMT	<i>Stephania tetrandra</i>	Reference[20]
Cj4'OMT	<i>Coptis japonica</i>	BAB08005
CjSOMT	<i>Coptis japonica</i>	BAA06192
CjCoOMT	<i>Coptis japonica</i>	BAC22084
CjCNMT	<i>Coptis japonica</i>	BAB71802
GflOMT7	<i>Glaucium flavum</i>	AKO60158
NnOMT5	<i>Nelumbo nucifera</i>	XP_010276063
SiCNMT1	<i>Stephania intermedia</i>	MK749412
SiCNMT2	<i>Stephania intermedia</i>	MK749413
SiCNMT3	<i>Stephania intermedia</i>	MK749414
MsChOMT	<i>Medicago sativa</i>	AAB48059
Cc6OMT1	<i>Coptis chinensis</i>	AXC09385