Supporting Information

Minami *et al.* 10.1073/pnas.0802981105

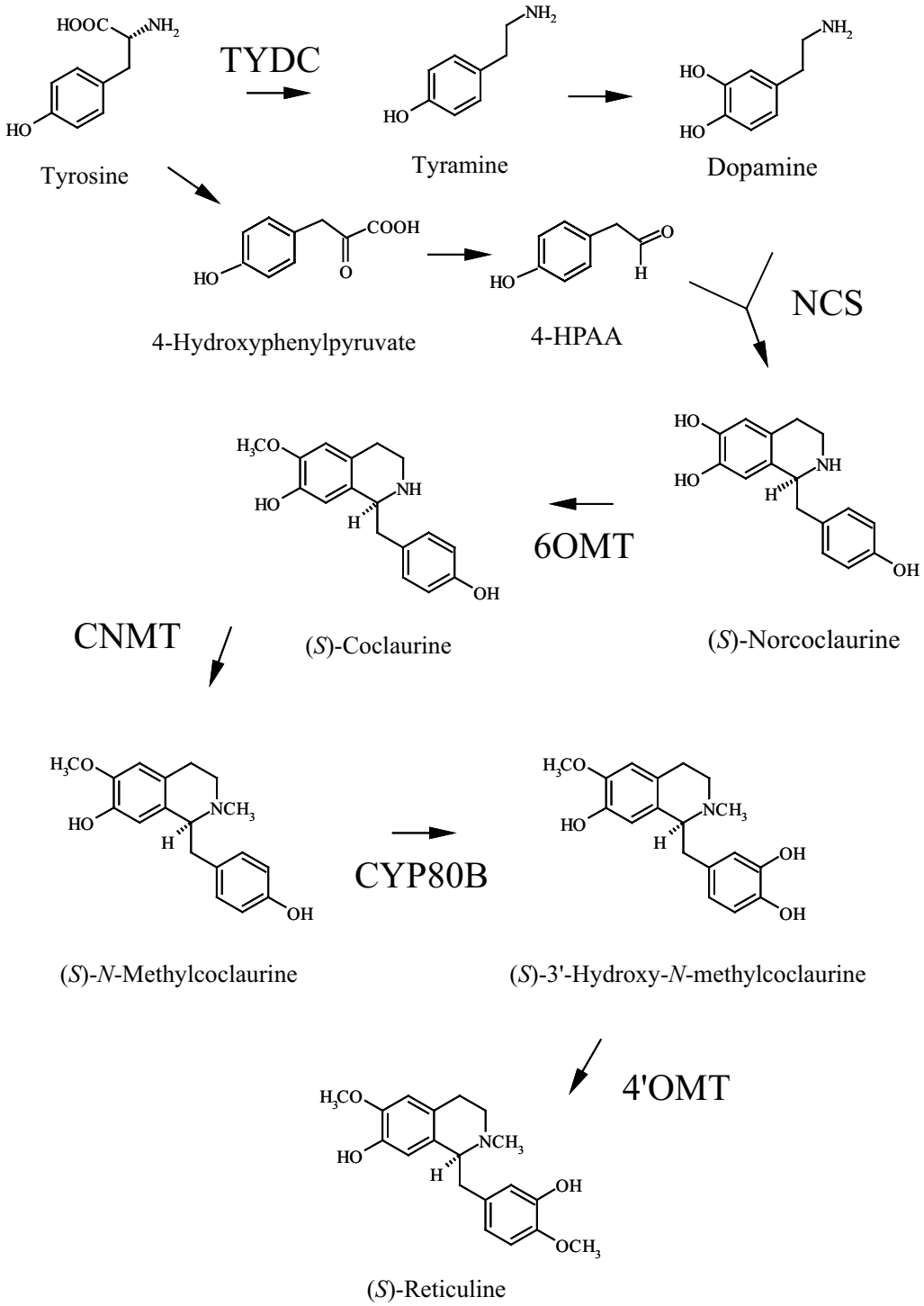
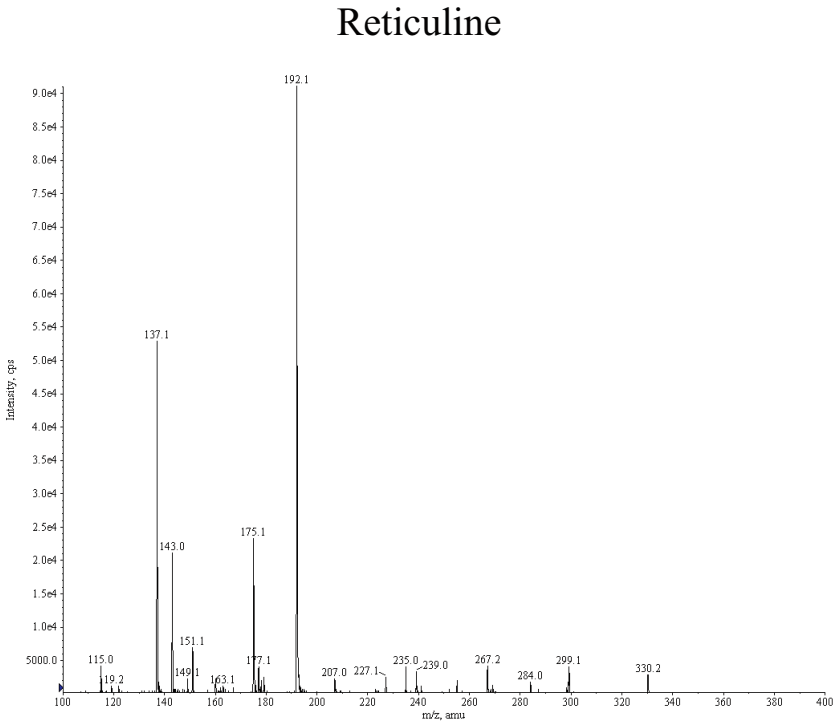


Fig. S1. Biosynthetic pathway of benzylisoquinoline alkaloid and biosynthetic genes. 4′OMT, 3′-hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase; 6OMT, norcoclaurine 6-*O*-methyltransferase; CNMT, coclaurine *N*-methyltransferase; CYP80B, *N*-methylcoclaurine 3′-hydroxylase; NCS, norcoclaurine synthase; TYDC, tyrosine/dopa decarboxylase.

A



B

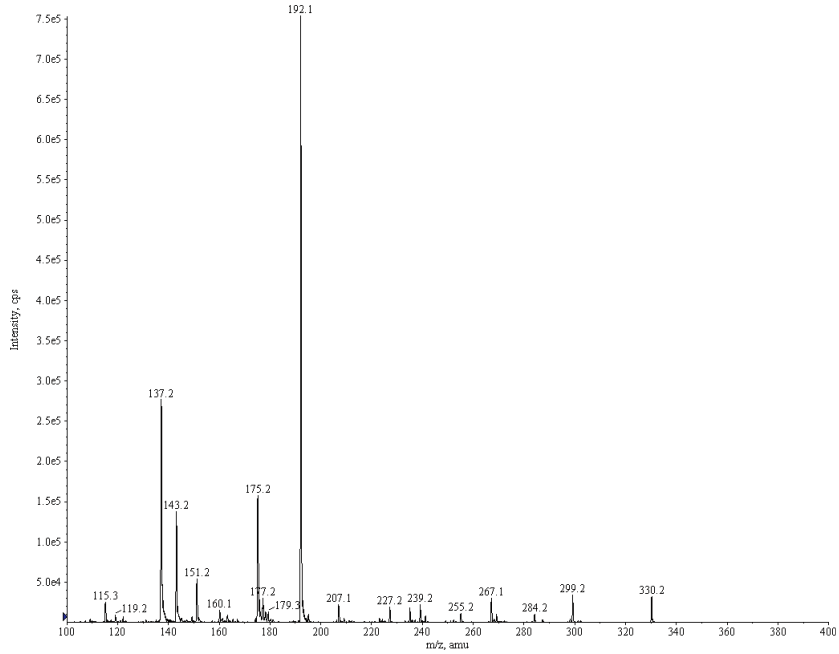


Fig. S2. Collision-induced dissociation (CID) mass spectrum for reticuline (*A*) and the reaction product in our system (*B*).

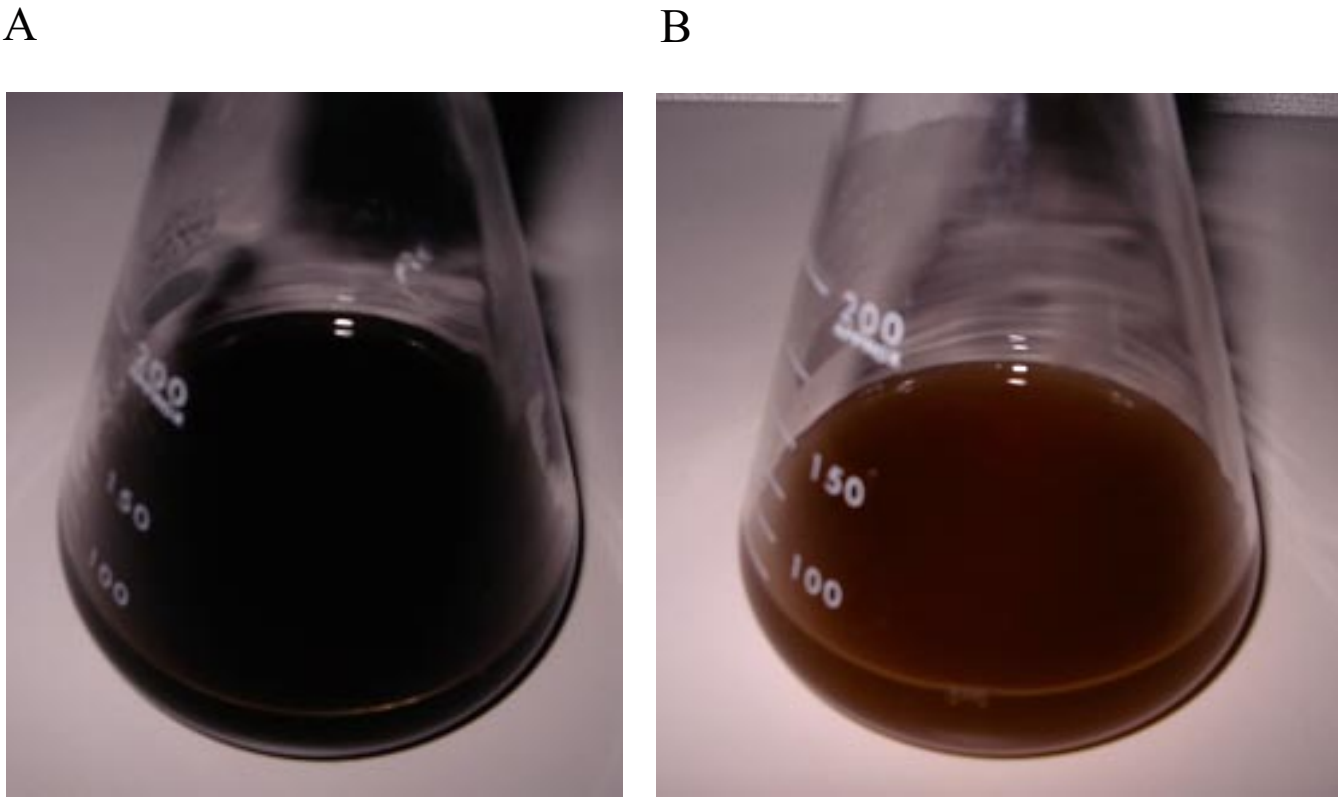


Fig. S3. Culture media of transgenic *E. coli* with empty vectors (*A*) and reticuline biosynthetic genes (*B*). *E. coli* cells were incubated at 25°C for 40 h in medium supplemented with 2 mM dopamine at IPTG induction.

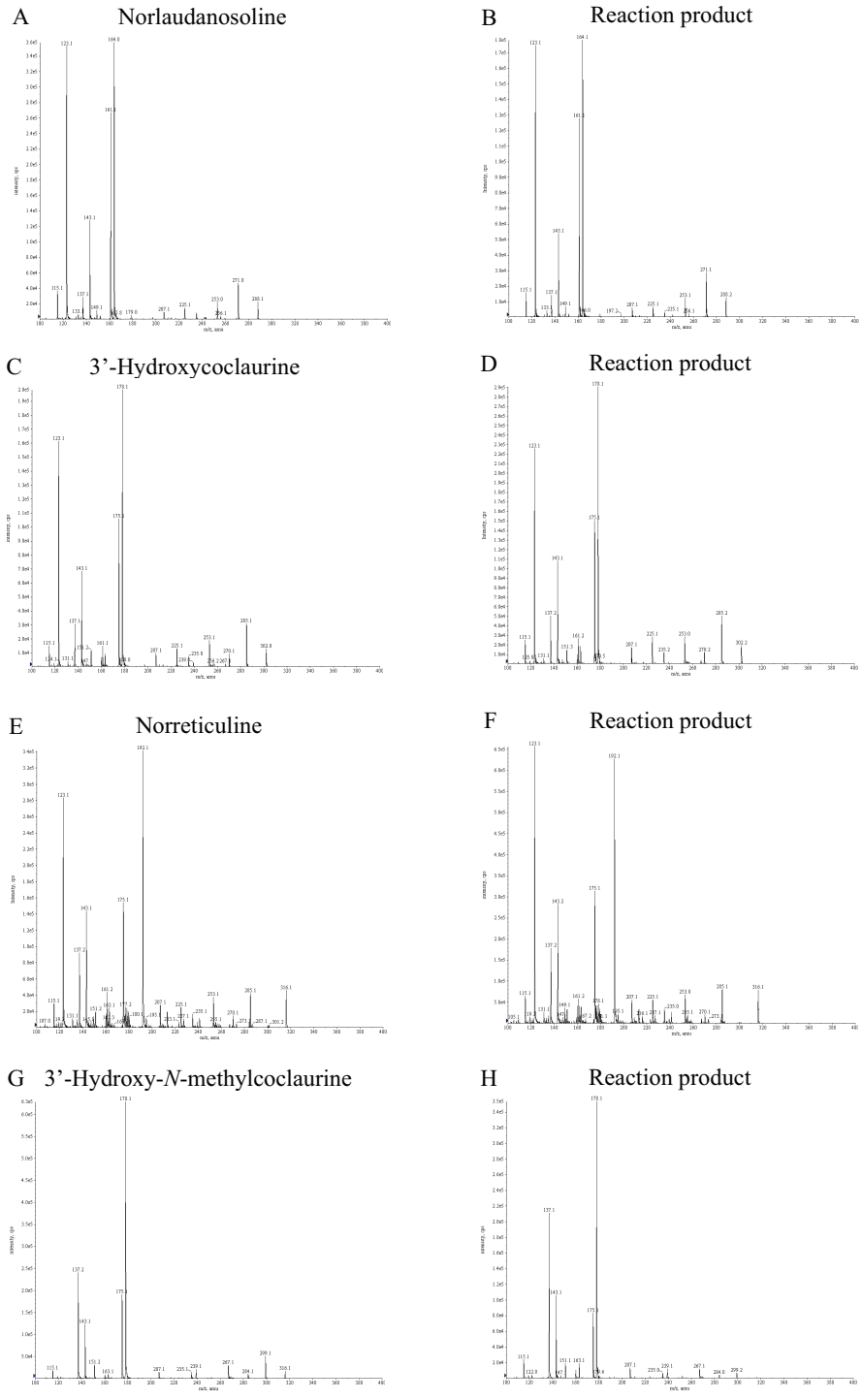


Fig. S4. CID mass spectrum for norlaudanosoline (*A*), reaction product in Fig. 3A (*B*), 3′-hydroxycoclaurine (*C*), reaction product in Fig. 3B (*D*), 3′-hydroxy-*N*-methylcoclaurine (*E*), reaction product in Fig. 3C (*F*), norreticuline (*G*), and reaction product in Fig. 3*D* (*H*).

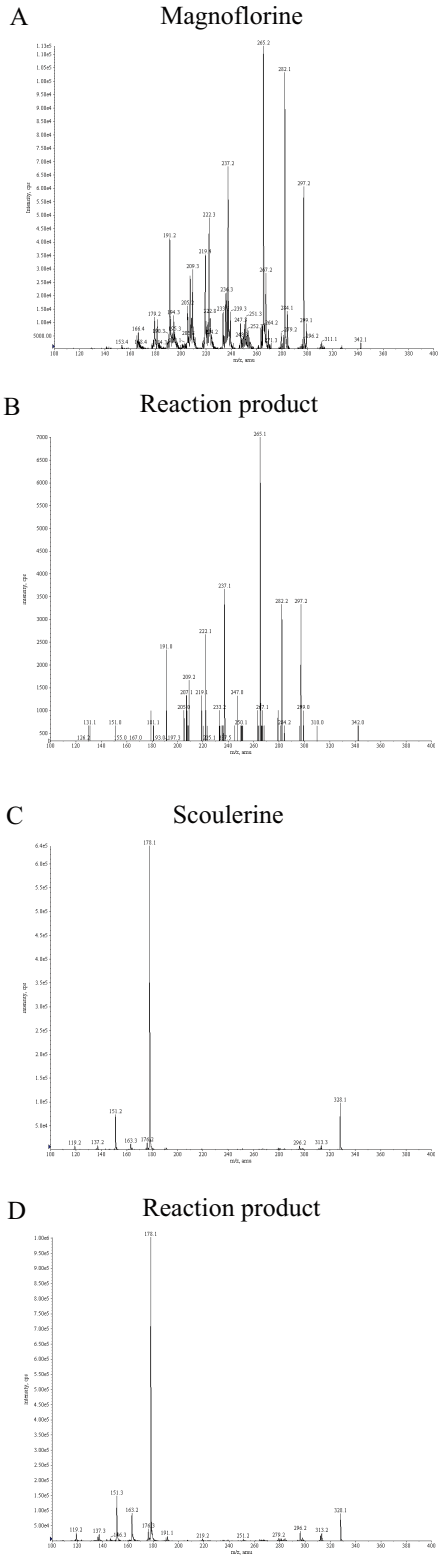


Fig. S5. CID mass spectrum for magnoflorine (*A*), reaction product in Fig. 4*A* (*B*), scoulerine (*C*), and reaction product in Fig. 4*B* (*D*).

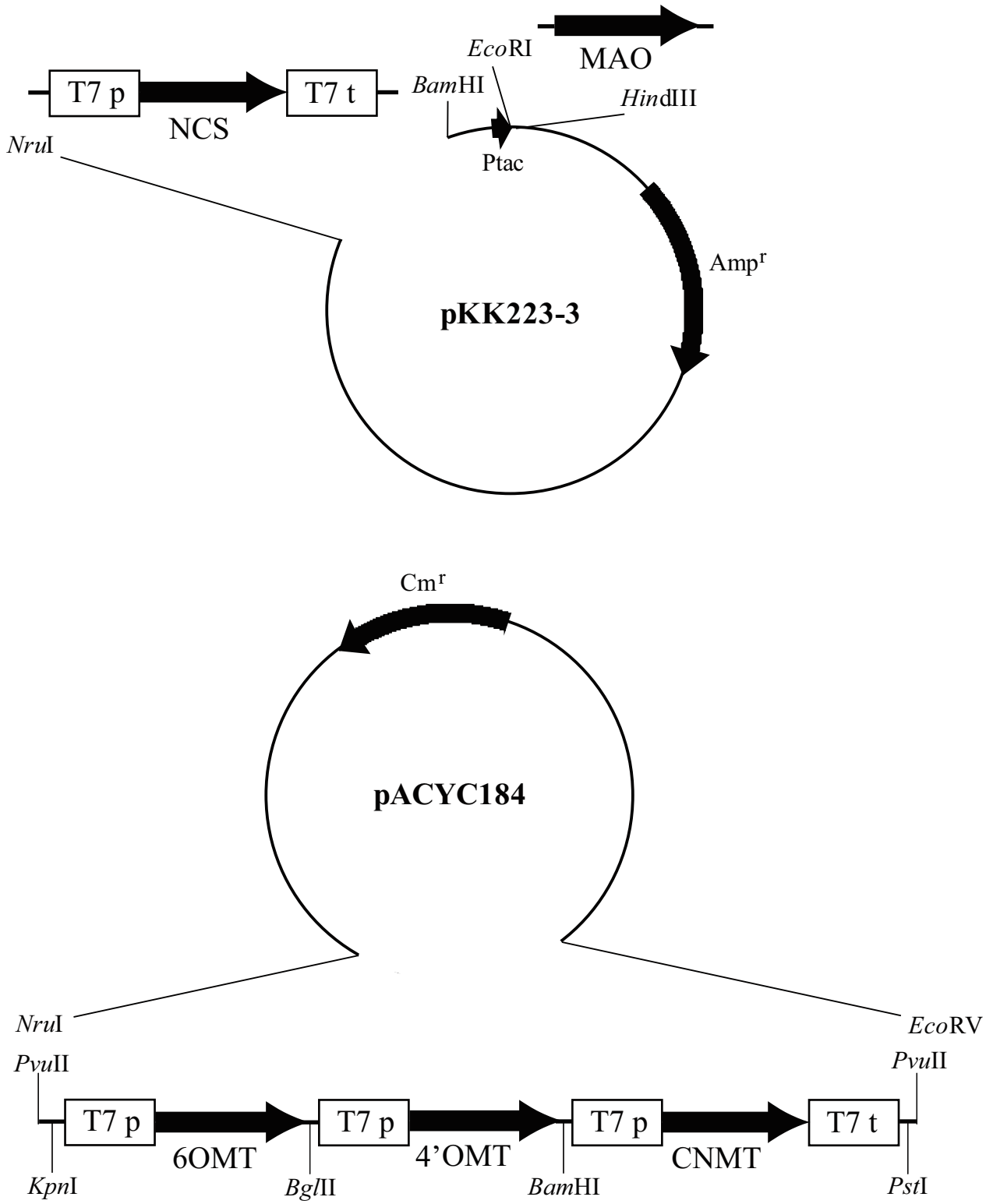


Fig. S6. Structures of plasmids for reticuline synthesis. pKK223–3 contains MAO and NCS genes. pACYC184 contains 6OMT, 4′OMT, and CNMT genes. MAO gene is placed under the control of the tac promoter. NCS, 6OMT, 4′OMT, and CNMT genes are placed under the control of the T7 promoter (T7p). Ptac, tac promoter; T7t, T7 terminator.

Table ST1. DNA sequences of the primers used

Primer name	Sequence
EcoRI-MAO-F	5′-TTGAATTC**ATG**AGCAACCCGCATGTCGTG-3′
(MAO forward primer)	EcoRI
NruI-T7p-F	5′-ACTCGCGATCCCGCGAAATTAATACG-3′
(NCS forward primer)	NruI
KpnI-T7p-F	5′-AGGTACCGATCCCGCGAAATTAATACG-3′
(6OMT forward primer)	KpnI
BglII-T7p-F	5′-CAGATCTGATCCCGCGAAATTAATACG-3′
(4′OMT forward primer)	BglII
BamHI-T7p-F	5′-TGGATCCGATCCCGCGAAATTAATACG-3′
(CNMT forward primer) BamHI	
KpnI-CNMT-F	5′-TATGGTACC**ATG**GCTGTGGAAGCAAAGCAA-3′
(CNMT forward primer)	KpnI
MAO-HindIII-R	5′-CTAAGCTTCAGGCGCGGATGTCCCGGAG-3′
(MAO reverse primer)	HindIII
T7t-BamHI-R	5′-CAGGATCCAGCAAAAAACCCC**TCA**AGAC-3′
(NCS reverse primer)	BamHI
6OMT-BglII-R	5′-CAGAT**CTA**ATATGGATAAGCCTCAATCAC-3′
(6OMT reverse primer)	BglII
4′OMT-BamHI-R	5′-TGGATC**CTA**TGGAAAAACCTCAATGACTG-3′
(4′OMT reverse primer)	BamHI
T7t-PstI-R	5′-ACCTGCAGGCAGCAAAAAACCCCTCAAGAC-3′
(CNMT reverse primer)	PstI
CNMT-SalI-R	5′-CCAGTCGAC**TCA**TTTTTTCTTGAACAGAAC-3′
(CNMT reverse primer)	SalI

The underlined text denotes the restriction site sequences, and bold type indicates the start or stop codons.