

Supplementary Information

Modular assembly of an artificially concise biocatalytic cascade for the manufacture of phenethylisoquinoline alkaloids

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20 **The nucleotide sequences used in this study**

21 **The nucleotide sequence of *TpCAR*.**

22 Codon-optimized nucleotide sequence of *TpCAR* expressed in *E. coli* BL21 (*DE3*) [3318 bp]:

23 ATGAGTATTGAGACCGTACAAAATGGCGTGCCCGCCGAGGGGTCTGTCCCCCAGCCG
24 ACCAACAAACTGAACGTTTGGCCCAAGTTATTGCTCGCATTTTCGCTCAATTTGCGGAC
25 CGCCCCGCATTTGCCACGCGCGAGGCCGGTCCTGGTACCCCTTATGCGACCGTGTCTTA
26 TCGTGAGATTTGGCGTCGCGTCACGGCACTTGTAGCCTCTTGGCAATCTGAGGTAGCC
27 CCAGGTGACTTCGTGGCGATCTTGGGATTCACATCTTCTGATTTTGTACTGTCGATTTA
28 GCTACTACACTTTTAGGAGCTCCAAACGTTCCACTGCAGGCTGGCGCACCAGCCGCGC
29 GCATCGCAACCATCCTTGATGAAACCCGTCCAAAAATTTTGGCCGTTAGCGCCGATCAG
30 GTCGATTTAGCCCAGGAAGCTTTAGCTGAATCAGCTGCCACGCCGCGTGTTGTCGTTTT
31 CGATGGTGAGCGTGACGGCTATGAAGGGATCGAAGCCGATATTTTGTCCGGTAGCGCTT
32 TACCGGCTCCGGAGTTTTTCGCGCCAGAGCCAGGGACTGATCCGCTTGTGACGTTAAT
33 CTACACTTCAGGTTCAACCGGCACGCCGAAGGGCGCCATGTACACAGAGCAGCTGGTA
34 CGTGACGCGTGGTTGAAAGTCGACTCGATTGTTGATATCGACATGCCCGCAGAGTCATT
35 GCTTCATTTTCTGCCCATGTCGCACATGTATGGTCGCAATTGGCTTATCGCTGGCCTTGC
36 GTCTGGAGGGACGGGATACTTCGCCGGGGCATCGGATATGAGTACCTTATTCGATGACC
37 TGGCGGCTGCACGCCCCACCGCCATTGGCTTAGTGCCACGTGTTTGTGAATTAATCCAC
38 CAGCGCTACCTTGCGGTAGAGGCAGATACGGACGCGGAGACGGCTCGCGTAGAGTTG
39 CGTGACCGCGTCCTGGGTGGACGTTTGCAAGCAGCTATGTGTGGAAGTGCTGCGCTGT
40 CATCAGAGTTACAAACATTCATGGAATGGCTTTTAGGCATTGACATTCAAATTGGATAC
41 GGAAGTACCGAAGCGGGGGGTGTCATCCGCGACGGGGTAGTAGTCCGCCCACCGGTG
42 ACAGAGTATAAACTGATCGATGTCCCAGAATTAGGATATTTTGTCACTGACAGCCCTCA
43 TCCGCGCGGGGAGCTTTTGGTGAAATCGACACAGTTGATCCCCGGGTACTATAACAGC
44 GACAAGCGCATCCGTGATGACGAAGGTTTTTATCGTACCGGCGACGTGATGGCTGAAT
45 TGGGACCAGACCGCTTGGAGTACGTCGATCGTCGTAGCAATGTGATCAAGTTGGCACA
46 GGGTGAATTTGTACCGATTGCGCAGTTAGAAGCTATTTATGCGGCAGGACCCGATGTGC
47 ATCAGATCTTTCTTTACGGGACCAGCGAACGCAGCTATTTGATCGGGGTCGTGGTACCC
48 GCGCCAGGCCCGGATGGAGAAACGGACGCGCAAACCCGTACTCGCGTGTTGGACGGA

49 CTGGCGGCTATTGCCCCGCGAAAACGACTTGGCCGCTTATGAAGTTCCCCGTGACGTCT
 50 TGATTGAACGTGATCCCTTTTCGCAGGAGAACGGACTGCGTAGTGGAATCGGTAAGCT
 51 TGTACGCCCTGCACTTATCGCGCGCTACGGTGACCGTCTGCATGATTTATACGCACAAG
 52 CGGACACACGTCAACGCGAAGGCTTACGTGCCTTGGATGCGTCCGGACCAATCATCGA
 53 CACTGTCTTAGGTGCGGCTGCCTTAACCTTAGGCGCTGACATCGCCGATTTTGATGCTG
 54 ATACACGCTTCGGAGACTTGGGTGGGGATTCAATTGTCTGCGCTTAGCTTAGCAACTACA
 55 TTGGAAGGGCTGTACGACGTTCCAGTACCAGTACAAACGATCGTCGGGCCGACAGCA
 56 ACTTTAGGAGGTGTAGCACGTCACATCGAGAAGGCTCGTTCAGGAGGCGTGGCTGCCC
 57 CCACCGCCGACAGTGTGCACGGGGTTGGAGCCAGCGTAGCACGTGCGACGGACTTGA
 58 CATTGGAAAAGTTTATCGATCCAGAACTGCTTGCTTTAGCTCCAACATTGCCGGCCGCG
 59 ACCGGTGAGCCAAATACTGTACTTTTAACAGGTTTCGACGGGTATCTTGGGCGCTTTCT
 60 GTTATTAGATTGGTTGCGTCGTGTGGCGCCACATGGGGGGACTGTTATTGCTTTGGTGC
 61 GTGGGGCGGATGCCGACGACGCACGCCGTCGTGTGACTGCGGGGATCGGAGATTCTG
 62 ACCCCGACCTTACTCAAGAGTTCACGAGTCTGGCTGAGCATCACCTGCATGTGATTGCT
 63 GGCGACTTTGGGTCGCCGGCCTTAGGATTGGATGACGCGACCTGGTCCGATCTTGCAG
 64 GACGTGTAGATCATGTAGTACATTGTGGGGCTCTTGTTAATCATGTCCTGCCATACGACC
 65 AGTTATTTGGGCCAAACGTTGTGCAACTGGTGAGGTTGTCCGTTTAGCGTTGACGAC
 66 ACGCCGCAAGTCAGTCGACTACGTGTCCACTGTCGCTGTCGTTCCCTCAGGATGATGGG
 67 CGTGTCTTGTTGAAGATGATGATGTACGTGAGTTAGGTGCGGAGCGCCGCATCGGCG
 68 CGGACGCTTATGCTAACGGCTACGCCGTGTCGAAATGGGCGGGGGAGGTTCTGTTGCA
 69 TGAGGCAGCAGACTTAGCTGATCTGCCAGTACGCGTGTTCCGCTCCGATATGATTTAG
 70 CGCACTCTCGTTTTACGGTCAATTTAATGAAGTTGACCAATTTACCCGCCTGCTTCTTT
 71 CTATTGCGGAAACTGGGTTAGCTCCCGCCTCTTTCTACACACCTGACCCGAGCGGTCAT
 72 CGTCCGCACTACGATGGTTTACCTGTTGACTTTACCGCTGAGGCAATTACAACTCTGTC
 73 AGCGGCAGGGCGTTCTGGATAACGCACCTTCCACGTTTTGAATGCGAATGATGATGGC
 74 GTGTCACTTGACAGCTTCGTCGATTGGATTGCGGCAAGTGGCCGTAGCATTGAACGTAT
 75 CGACGACTATGATACTTGGTTTGCCCGCTTTGAGCAGGCATTACAACAATTACCCGACG
 76 AAGCGCGCCAGCGTAGCGTGTTACCTCTTTTGACGCGAGTGCGTGAGCCTGCGCCCGC
 77 GGCGGGAACGTCCGCTTTATCGGTGGATCGCTTCCGTGGTGCCGTACGTGAAACTGGT
 78 GTGGGTCCAGGAGACATCCAGTTCTTGACCGTGCACTTATCGAGAAGTATTTGCGTG

79 ATTCGAGACCGCGGGTTGGCTTGCCCCGGGTGCACGTGACTAA

80 **The nucleotide sequence of *BsSfp*.**

81 Codon-optimized nucleotide sequence of *BsSfp* expressed in *E. coli* BL21 (*DE3*) [675 bp]:

82 ATGAAGATTACGGAATTTATATGGACCGCCCGCTTTCACAGGAAGAAAATGAACGGTT
83 CATGTCTTTCATATCACCTGAAAAACGGGAGAAATGCCGGAGATTTTATCATAAAGAAG
84 ATGCTCACCGCACCCCTGCTGGGAGATGTGCTCGTTCGCTCAGTCATAAGCAGGCAGTAT
85 CAGTTGGACAAATCCGATATCCGCTTTAGCACGCAGGAATACGGGAAGCCGTGCATCC
86 CTGATCTTCCCGACGCTCATTTCAACATTTCTCACTCCGGACGCTGGGTCAATTTGCGCG
87 TTTGATTCACAGCCGATCGGCATAGATATCGAAAAACGAAACCGATCAGCCTTGAGAT
88 CGCCAAGCGCTTCTTTTCAAAAACAGAGTACAGCGACCTTTTAGCAAAAGACAAGGA
89 CGAGCAGACAGACTATTTTATCATCTATGGTCAATGAAAGAAAGCTTTATCAAACAGG
90 AAGGCAAAGGCTTATCGCTTCCGCTTGATTCCTTTTCAGTGCGCCTGCACCAGGACGG
91 ACAAGTATCCATTGAGCTTCCGGACAGCCATTCCCCATGCTATATCAAACGTATGAGG
92 TCGATCCCGGCTACAAAATGGCTGTATGCGCCGTACACCCTGATTTCCCGAGGATATC
93 ACAATGGTCTCGTACGAAGAGCTTTTATGA

94 **The nucleotide sequence of *TfNCS*.**

95 Codon-optimized nucleotide sequence of *TfNCS* expressed in *E. coli* BL21 (*DE3*) [549 bp]:

96 ATGCTGCATCACCAAGGGATTATCAACCAAGTCTCAACCGTCACTAAGGTAATCCACCA
97 CGAACTGGAAGTCGCTGCTAGCGCGGACGACATCTGGACCGTGTACTCCTGGCCGGGT
98 CTGGCGAAACACTTACCGGACCTGCTTCCAGGGGCGCTTCGAGAACTTGAGATCATCG
99 GAGACGGGGGGGTGGGACAATCCTGGACATGACTTTCGTACCGGGGGAGTTTCCAC
100 ACGAATATAAGGAAAAGTTCATTCTGGTTGACAACGAACATCGCTTAAAGAAAGTGCA
101 AATGATTGAAGGAGGCTATCTTGACTTAGGTGTAACCTACTATATGGATACGATCCACGT
102 TGTTCCAACCTGGAAAAGACAGTTGTGTCATTAAGTCAAGTACTGAGTATCATGTCAAG
103 CCGGAGTTCGTAAAGATTGTTGAGCCGCTTATTACAACCTGGACCACTGGCCGCTATGGC
104 CGATGCCATTCGAAACTTGTTCTTGAACATAAGTCCAAGTCGAACTCCGACGAGATC
105 GAAGCTGCGATCATCACTGTCTAA

106 **The nucleotide sequence of *Rn*COMT.**

107 Codon-optimized nucleotide sequence of *Rn*COMT expressed in *E. coli* BL21 (*DE3*) [666 bp]:

108 ATGGGAGATACCAAAGAACAGCGCATCTTGCGCTACGTACAGCAGAATGCAAAACCAG
109 GCGACCCTCAGTCAGTGTGGAGGCCATCGACACGTACTGCACCCAGAAAGAATGGG
110 CTATGAACGTTGGTGATGCAAAGGGACAGATCATGGATGCTGTGATCCGCGAATATTCT
111 CCGTCTTTGGTATTAGAATTAGGAGCATACTGTGGTTACTCCGCGGTACGTATGGCGCGT
112 CTTTTGCAACCAGGTGCACGCTTATTGACGATGGAAATGAACCCCGATTATGCCGCGAT
113 TACACAACAAATGTAAATTTTCGCAGGGTTACAAGATAAAGTCACAATTCTTAACGGTG
114 CATCCCAGGATTTAATTCCCAATTAAAAAAGTATGATGTAGACACATTGGATATG
115 GTATTTTTGGACCATTGGAAGGACCGCTATTTACCTGATACATTACTGCTTGAGAAGTGT
116 GGACTTTTACGCAAGGGGACTGTATTATTGGCTGACAATGTAATCGTTCCTGGAACACC
117 AGACTTCTTGGCTTACGTCCGCGGTTCTAGTAGCTTTGAATGCACTCACTACTCGTCTT
118 ATCTTGAGTACATGAAAGTTGTGACGGTCTTGAGAAAGCGATCTACCAAGGTCCTTC
119 TAGCCCTGATAAATCTTAA

120 **The nucleotide sequence of CNMT.**

121 Codon-optimized nucleotide sequence of CNMT expressed in *E. coli* BL21 (*DE3*) [1059 bp]:

122 ATGCAAACAAAAAAGCCGCAATCGTTGAATTGCTGAAGCAGCTTGAATTGGGGCTG
123 GTGCCGTATGACGACATCAAGCAGTTAATCCGCCGTGAGCTGGCGCGCCGCCTGCAGT
124 GGGGGTATAAGCCAACGTATGAAGAGCAAATCGCGGAAATCCAAAATCTGACCCACTC
125 GTTGCGTCAGATGAAGATTGCTACTGAAGTCGAGACGCTTGATAGTCAGTTGTACGAA
126 ATCCCAATTGAGTTTTTGAAGATTATGAACGGAAGCAACTTGAAGGGAAGTTGTTGCT
127 ACTTCAAAGAAGATTCAACCACGTTAGATGAGGCTGAAATCGCTATGTTGGATTGTAC
128 TGTGAACGTGCTCAAATTCAGGACGGGCAAAGTGTGCTGGATCTTGGATGCGGCCAGG
129 GAGCCCTGACGCTTCATGTTGCACAGAAATATAAAAATTGTCGCGTAACGGCTGTCAC
130 AAATTCGTTAGTCAGAAGGAGTACATCGAGGAAGAATCGCGTCGCCGCAACCTGCTG
131 AATGTGGAGGTGAAATTAGCAGACATTACAACGCACGAGATGGCCGAGACTTACGACC
132 GTATTTTAGTAATCGAGCTGTTTGAACACATGAAAACTATGAGCTGTTGCTTCGTAAA
133 ATTTCCGAGTGGATCTCAAAAGATGGACTTTTATTTTATAGAGCATATCTGCCATAAACT
134 TTTGCGTATCATTATGAGCCCTTAGATGATGACGACTGGTTCACGGAGTATGTATTCCT

135 GCAGGAACTATGATTATTCCATCTGCTTCTTTCTTTTTGTATTTCCAGGATGACGTGAGT
 136 GTCGTCAACCATTTGGACTTTGTCAGGTAAACACTTTTCCCGTACAAACGAGGAATGGC
 137 TGAAACGCTTGGATGCCAACCTGGACGTAATTAAACCCATGTTTCGAGACATTAATGGG
 138 GAATGAGGAAGAAGCAGTGAAGTTAATCAACTATTGGCGCGGGTTCTGCTTAAGCGGG
 139 ATGGAGATGTTCCGATATAATAATGGTGAAGAGTGGATGGCATCACATGTTCTTTTCAA
 140 GAAATGA

141 **The nucleotide sequence of *GsOMT1*.**

142 Codon-optimized nucleotide sequence of *GsOMT1* expressed in *E. coli* BL21 (*DE3*) [1086 bp]:

143 ATGGCAAGTGCGCCCATCCACGCTGGAGCTACCCCCGAAGAGGAGGAGGAGAAGGCT
 144 CTTTTTTTGTCTGCAATGCGCCTGACGTCTGCGGCTGGCTTACCCATGGTCGTGAAAGC
 145 TGCCATTGAATTGGACGTGTTAGAAATCATTGCTCGTGCGGGGCCGGGCGCACAACCTT
 146 TCCGCTTCAGAAATTGCATCCCGTATTCCGGGAGCGGCATCATCCGAGGCACCCGCTGT
 147 GTTGGACCGTATGCTGCGTTTCTTGGCGTGCCACTCAGTACTGACGTCCACATCTGTGG
 148 ATGGAAGAGCGTCGCTATGGCCTGGCCCCGTTTGCCGTTTCTTAACCCGTGATAAA
 149 GATGGCGCGTCTATTGGTGATCAGGTTCTGGTAGACCTTGACCCCGTCTTACTTGCGTG
 150 CTGGGAGCAGATGAAGTATTTGGTCTTAGAGGGTATTTCTCCTTTCACCAAAGCGCACG
 151 GTACTACAGCATTTGAATATCACGGCGTAAACCCGCGCATCAATAAAGTTTTCAATAAT
 152 GCGATGGCATCCAATTCTACAATTATTCTTCGTAAAATTCTGGAGATCTACAAAGGTTTT
 153 GAGGGTGTTACTCAGTTGGTGGATGTAGGGGGAGGTTTGGGTATCACTCTGAAAATGA
 154 TTATTTGAAATATCCGCACATCAAAGGGATTAATTATGATTTGCCGCACGTTATTAGTG
 155 ACGCACCTACGTTACCTGGTGTGAGAACATTGGAGGGGATATGTTTCGTTGAAATTCCA
 156 TCAGCTAGCACTATCTTTATGAAATGGATTCTGCATGACTGGAGCGACGAACACTGTCT
 157 GAAGATCCTGAAGAATTGTTGGAAAGCCCTGCCCAATGGCGGTAAAGCGATTATCATG
 158 GAGGCTATTTGCCAGAAACACCAAAAACCGATGCCGCAAGCCAGGAGGCTTTCTATT
 159 CTGACCTTTGCATGTTGATTAGTAACGTCGGTGGCAAAGAACGCACCCGCAACGAGTT
 160 TAAGTCCTTAGCCATGGATGCAGGCTTTACCAAGTTTGAAACCGTGAGCTGCGCCTTG
 161 AATATTTGGGTCATGGAGCTGACAAAATAA

162 **The nucleotide sequence of *MmSAHH*.**

163 Codon-optimized nucleotide sequence of *MmSAHH* expressed in *E. coli* BL21 (*DE3*) [1299 bp]:

164 ATGTCTGACAACTTCCCTATAAGGTAGCCGATATTGGCCTGGCCGCTTGGGGCCGCAA
 165 AGCGCTGGACATCGCCGAGAATGAGATGCCCCGGCCTGATGCGTATGCGTGAAATGTAC
 166 AGCGCCAGTAAACCGCTGAAGGGGGCGCGCATTGCGGGTTGTTTACACATGACCGTAG
 167 AGACCGCTGTACTTATTGAAACCCTTGTTGCATTGGGAGCGGAAGTACGTTGGTCCTCA
 168 TGTAATATCTTCAGCACTCAAGACCATGCAGCTGCCGCAATTGCCAAGGCGGGTATCCC
 169 GGTATTCGCTTGGAAAGGGGAGACAGATGAAGAGTATCTGTGGTGCATTGAACAGACA
 170 TTACATTTCAAGGATGGTCTCTTAACATGATTCTTGATGATGGGGGAGACCTTACGAA
 171 CTTAATCCACACGAAATACCCACAGCTTCTGTCAGGAATCCGTGGAATCTCAGAAGAG
 172 ACAACCACAGGAGTTCACAACTTGTAACAAAATGATGAGCAATGGGATTTTGAAAGTGC
 173 CAGCGATCAACGTGAACGATTCCGTAACTAAGAGCAAATTCGATAACCTGTACGGCTG
 174 CCGTGAATCGTTGATCGACGGAATTAAGCGCGCGACCGATGTGATGATTGCGGGGAAG
 175 GTTGCGGTAGTTGCGGGGTATGGGGACGTAGGTAAGGGGTGCGCGCAAGCACTTCGC
 176 GGTTTTGGAGCTCGTGTAATTATCACAGAGATTGACCCTATTAATGCTCTGCAAGCAGC
 177 GATGGAGGGGTACGAAGTCACGACCATGGATGAGGCGTGCAAGGAGGGTAATATTTTC
 178 GTCACAACCACGGGCTGTGTAGACATCATTTTAGGGCGTCACTTCGAACAAATGAAGG
 179 ATGATGCCATTGTGTGTAACATCGGTCAATTCGATGTGGAGATCGACGTAAAGTGGCTT
 180 AACGAAAATGCAGTAGAAAAAGTCAACATCAAACCACAGGTGGACCGTTATTGGCTTA
 181 AGAATGGGCGTCGTATCATCCTGCTTGCCGAGGGTCGTTTAGTCAACCTTGGGTGTGCG
 182 ATGGGCCACCCCTCTTTCGTAATGTCCAATAGCTTCACAAATCAAGTGATGGCTCAGAT
 183 CGAACTGTGGACGCATCCGGACAAATATCCCGTAGGAGTTCATTTTTTGCCGAAAAAG
 184 TTGGATGAGGCCGTGGCGGAAGCACATCTTGAAAACTGAATGTAAAATTGACTAAAT
 185 TGA CTGAAAAGCAAGCGCAATATTTAGGTATGCCGATCAACGGCCCTTTCAAACCCGA
 186 CCATTATCGTTATTAA

187 **The nucleotide sequence of *Gs*NMTt.**

188 Codon-optimized nucleotide sequence of *Gs*NMTt expressed in *E. coli* BL21 (*DE3*) [894 bp]:
 189 ATGAGTCATGAGGCAGCAGCCGTTGAGGAGGTCAACAAGGGCATCGCGGACCTGTAT
 190 GACGAGATGACCGTTGTCATGGAAACGCTTTGGGGTGATCACATGCACCATGGATTCTA
 191 CGACGTAGGCGTACCTACCCGCTCCTTGCCCGATCACCAGACAGCCCAGATCCGCATG
 192 ATTGAGGAAGCGTTACGTTTTGCCGGAGTTTCAGATGATCCTAGTAAGAAGCCAAAGC

193 GTATTTTAGACGTCGGTTGTGGCATCGGTGGCTCGTCGCTGTACTTAGCAAAAAAGTAC
 194 GGGGCTAAGTGTTCATGGCATCAACTTGTCCCCTTTCCAAATCCAACGTGCTCAGTCTTT
 195 GGCAATGAGTGCGGGTTTAACCGATAAGGTATCCTTTGAAATCGCAGACGCCCAAAAC
 196 CAGCCCTTCCCAGATGGGCACTTCGATTTAGTCTGGGTCTTGGAGACCGCGGAACACA
 197 TGCCGGAAAAATCGAAGTTCATTGGCGAGTTAGCTCGTGTTACTGCGCCCGCGGCAT
 198 TGTGATCATCACTTCCTGGTGCCAGCGCGATTTGTTGCCTAGTGAAGTTTCGTTGCGCC
 199 CCGATGAGGTTTCTCTGTAAACAACTTTAGAAAAGCCACCATTTGCCCAAATGGTG
 200 CTCGCCATCAGAGTACGTTAAGTTGGCTGAATCGTCCAGCTTCAAAGATATTAACCG
 201 CAGACTGGACAGAACATGTTGCCCCACATTGGAACGTAGCAATGCGCCCTGTACTGAC
 202 TTGGAAGGGATTACCTTCATTCTGCGCAGTGGGTGGAAGATGGCGAAGGGTCTTCTT
 203 GCAATGCCCATTTGTGTCCGAAGGACGCGAGAAGAAGATTATTAAATACGCGATCTTGA
 204 CTTTCCGTAAACCGGAGTGA

205 **The nucleotide sequence of PavNMT.**

206 Codon-optimized nucleotide sequence of PavNMT expressed in *E. coli* BL21 (*DE3*) [1047 bp]:
 207 ATGGCAGTAGCCAACTTGATTAAACGTATTGAACATGGGGAAGTTTCAGATGAAGAGA
 208 TTCGCGGAATGATGAAAATTCAAGTGCAGAAGCGTTTAAAATGGGGATACAAGCCGAC
 209 TCATGAACAACAACCTTGACAGCTGGTAACCTTTGCTCAGTCATTAAAGGGGATGGAG
 210 ATGGCGGAGGAGGTGGACACGCTGGATGCCGAGTTGTACGAAATCCCTCTTCCATTCT
 211 TGCACATCATGTGTGGCAAGACCCTTAAGTTCTCACCCGGATATTTCAAAGATGAATCA
 212 ACCACGTTGGATGAGTCAGAAGTCTACATGATGGACTTATATTGCGAACGCGCGCAGAT
 213 CAAAGACGGACAGTCCATCCTTGATCTGGGTTGCGGCCACGGCTCATTAACTTGCAT
 214 GTGGCTCAGAAGTATCGTGGGTGCAAGGTTACCGGAATTACCAACAGCGTCTCCCAGA
 215 AAGAGTTCATTATGGATCAGTGTAATAAATTGGATCTGAGTAATGTGGAAATTATTCTG
 216 GAAGATGTAACCAAGTTTGAGACAGAAATTACCTACGATCGTATCTTCGCAGTCGCATT
 217 AATTGAGCACATGAAGAACTATGAGTTATTTTTAAAGAAGGTCTCCACATGGATTGCCC
 218 AGGATGGCCTTCTGTTTGTAGAGCATCACTGTCATAAAGTCTTCGCTTATCAGTACGAA
 219 CCACTTGACGAAGATGACTGGTATACTGAGTACATTTTTCCTAGCGGCACCCTGGTAAT
 220 GAGCAGTTCCAGCATCTTGCTTTATTTTCAAGAAGACGTGTCGGTCGTCAACCACTGG
 221 ACGCTTTCTGGGAAACATCCATCGCTGGGATTTAAGCAGTGGCTTAAACGTCTGGACG

222 ACAACATCGATGAAGTAAAAGAAATTTTCGAGAGTTTCTATGGTTCAAAAGAAAAGGC
223 CATGAAGTTTATCACGTA CTGGCGCGTGTTTTGCATCGCACACTCTCAGATGTACTCAA
224 CCAATAACGGAGAGGAATGGATGTTGTCCAGGTCTTGTTTAAGAAAAAATGA

225 **The nucleotide sequence of TNMT.**

226 Codon-optimized nucleotide sequence of TNMT expressed in *E. coli* BL21 (*DE3*) [1053 bp]:

227 ATGAAAGAATCTATCGGGGAAATCATGGGCAAACCTTATGCAGGGAGAGATCGGTGACG
228 AAGAGCTTTTCGAAACGTATTAAGGAAATCTTCGGCAAACGCCTTCAGTGGGGCTACAA
229 ACCTACGCATCAACAACAGCTTGCCTTTAACTTGGACTTCATCAAATCGCTTAAAGAGA
230 TGGATATGTCGGGTGAAATTGACACTATGAACGAGGAAACCTATGAGCTGCCAAGCGC
231 ATTCCTTGAAGCCGCGTTTCGGTAAAACCTATCAAGCAGAGTGGTTGCTACTTTAAAGAC
232 GAAACGACTACGATCGATGAAGCGGAGGAGGCCTCACACGAGTTATATTGCGAACGTG
233 CGCAGATTAAGGATGGACAAACAGTGCTGGACATTGGATGCGGACAAGGTGGTTTAGT
234 TTACACATCGCACAAAAATACAAAAACTGCCATGTGACTGGCCTTACTAACTCCAAA
235 GCTCAAAAAAATTATATCCTGATGCAGGCTGAAAAATTGCAACTTTCTAACGTCGATGT
236 CATTCTTGCTGACGTGACAAAGCACGAGAGCGATAAAACGTATGACCGCATCCTTGTC
237 ATCGAGACTATTGAACACATGAAGAACATCCAGCTTTTTATGAAAAAACTTAGCACATG
238 GATGACGGAAGATAGCCTTTTATTCGTCGATCATATCTGCCACAAGACATTCTCTCACCA
239 CTTTGAAGCCATTGATGAAGATGATTGGTATTCCGGTTTTATTTTTCCTAAAGGTTGCGT
240 GACTATTCTGAGCGCGAGTGCACTGTTATATTTTCAGGATGATGTAACCATTCTGGATCA
241 CTGGGTGGTCAACGGCATGCACATGGCACGCTCAGTGGATGCATGGCGTAAAAAACTT
242 GACAAGAATATGGAGTTGGCTCGCGAGATTCTGCTGCCTGGTTTAGGCTCGAAGGAGG
243 CGGTGAATGGAGTCATTACACATATCCGTACATTCTGCATGGGCGGTTACGAACAATTC
244 TCCTATAACAATGGGGAAGAATGGATGGTGGCCCAAATGTTATTTAAGAAGAAATGA

245

246

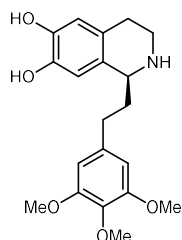
247

248

249

Characterization of substrates and products

(*S*)-1-(3,4,5-trimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol ((*S*)-1b)



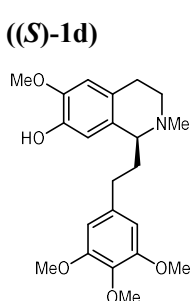
C₂₀H₂₅NO₅: 359.4220 g/mol

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.42 (s, 1H), 9.16 (br, 1H), 8.95 (s, 1H), 6.64 (s, 1H), 6.57 (s, 3H), 4.31 (s, 1H), 3.77 (s, 6H), 3.63 (s, 3H), 3.47 – 3.43 (m, 1H), 3.29 – 3.24 (m, 1H), 2.92 – 2.63 (m, 4H), 2.22 – 2.08 (m, 2H) ppm.

¹³C NMR (101 MHz, DMSO-*d*₆) δ 153.3, 145.5, 144.8, 137.0, 136.2, 123.3, 122.7, 115.7, 113.7, 106.0, 60.4, 56.2, 54.2, 39.3, 36.0, 31.8, 24.7 ppm.

Exact Mass [M+H]⁺: 360.1805 (calculated), 360.1821 (found).

(*S*)-6-methoxy-2-methyl-1-(3,4,5-trimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinolin-7-ol ((*S*)-1d)



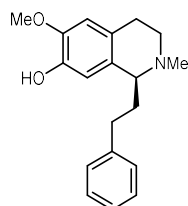
C₂₂H₂₉NO₅: 387.4760 g/mol

¹H NMR (600 MHz, Methanol-*d*₄) δ 6.57 (s, 1H), 6.48 (s, 1H), 6.38 (s, 2H), 3.72 (s, 3H), 3.70 (s, 6H), 3.61 (s, 3H), 3.47 (t, *J* = 5.6 Hz, 1H), 3.15 (td, *J* = 11.1, 10.6, 6.6 Hz, 1H), 2.76 – 2.66 (m, 3H), 2.56 (ddd, *J* = 13.6, 10.4, 6.3 Hz, 1H), 2.46 – 2.41 (m, 4H), 2.04 – 1.98 (m, 1H), 1.97 – 1.91 (m, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 153.0, 146.7, 144.6, 138.4, 135.8, 128.0, 124.1, 113.4, 111.3, 105.3, 62.8, 59.7, 55.2, 55.0, 41.1, 39.0, 35.8, 31.9, 24.4 ppm.

Exact Mass [M+H]⁺: 388.2118 (calculated), 388.2127 (found).

(S)-6-methoxy-2-methyl-1-phenethyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-2d)

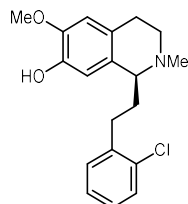


C₁₉H₂₃NO₂: 297.1729 g/mol

¹H NMR (600 MHz, Methanol-*d*₄) δ 7.25 (t, *J* = 7.6 Hz, 2H), 7.19 – 7.13 (m, 3H), 6.68 (s, 1H), 6.59 (s, 1H), 3.82 (s, 3H), 3.61 (t, *J* = 5.4 Hz, 1H), 3.28 – 3.25 (m, 1H), 2.88– 2.78 (m, 3H), 2.71 (ddd, *J* = 13.6, 10.9, 6.0 Hz, 1H), 2.59 (ddd, *J* = 13.7, 10.9, 5.2 Hz, 1H), 2.55 (s, 3H), 2.12 (ddt, *J* = 16.1, 10.7, 5.4 Hz, 1H), 2.02 (ddt, *J* = 14.1, 11.4, 5.9 Hz, 1H) ppm. For ¹H-NMR spectrum see Fig. S21, which is consistent to the literature reported¹.

Exact Mass [M+H]⁺: 298.1802 (calculated), 298.1796 (found).

(S)-1-(2-chlorophenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-3d)



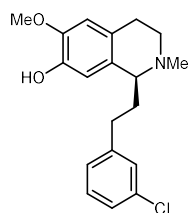
C₁₉H₂₂ClNO₂: 331.1339 g/mol

¹H NMR (600 MHz, Methanol-*d*₄) δ 7.36 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.32 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.25 (td, *J* = 7.4, 1.2 Hz, 1H), 7.21 (td, *J* = 7.6, 1.7 Hz, 1H), 6.82 (s, 1H), 6.69 (s, 1H), 4.31 (t, *J* = 6.0 Hz, 1H), 3.86 (s, 3H), 3.67 (dt, *J* = 13.3, 7.9 Hz, 1H), 3.38 (dt, *J* = 12.5, 5.5 Hz, 1H), 3.10 – 3.07 (m, 2H), 2.93 (s, 3H), 2.91 – 2.88 (m, 2H), 2.29 (ddd, *J* = 16.7, 8.2, 5.3 Hz, 1H), 2.17 – 2.11 (m, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 148.2, 145.6, 138.1, 133.4, 130.3, 129.3, 127.9, 127.1, 123.1, 121.1, 113.6, 111.4, 63.5, 55.1, 46.7, 39.6, 33.3, 29.5, 22.4 ppm.

Exact Mass [M+H]⁺: 332.1412 (calculated), 332.1407 (found).

298 **(S)-1-(3-chlorophenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-4d)**



299

300 **C₁₉H₂₂ClNO₂**: 331.1339 g/mol

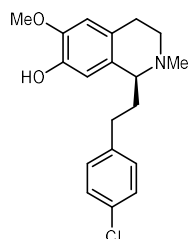
301 **¹H NMR (400 MHz, Methanol-*d*₄)** δ 7.31 – 7.27 (m, 2H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 7.3
302 Hz, 1H), 6.85 (s, 1H), 6.68 (s, 1H), 4.33 (t, *J* = 5.7 Hz, 1H), 3.88 (s, 3H), 3.85 – 3.80 (m, 1H), 3.43
303 – 3.40 (m, 1H), 3.17 – 3.10 (m, 2H), 2.97 (s, 3H), 2.78 (t, *J* = 8.2 Hz, 2H), 2.32 (dq, *J* = 13.7, 7.9
304 Hz, 1H), 2.21 (dq, *J* = 14.2, 7.2 Hz, 1H) ppm.

305 **¹³C NMR (151 MHz, Methanol-*d*₄)** δ 148.3, 145.8, 142.5, 134.1, 129.8, 128.0, 126.5, 126.2, 121.7,
306 120.6, 113.8, 111.5, 63.8, 55.1, 46.0, 39.6, 36.3, 30.9, 21.3 ppm.

307 **Exact Mass [M+H]⁺**: 332.1412 (calculated), 332.1429 (found).

308

309 **(S)-1-(4-chlorophenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-5d)**



310

311 **C₁₉H₂₂ClNO₂**: 331.1339 g/mol

312 **¹H NMR (400 MHz, Methanol-*d*₄)** δ 7.31 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.85 (s,
313 1H), 6.68 (d, *J* = 9.2 Hz, 1H), 4.32 (t, *J* = 6.0 Hz, 1H), 3.88 (s, 3H), 3.85 – 3.78 (m, 1H), 3.42 (dt, *J*
314 = 12.3, 5.2 Hz, 1H), 3.17 – 3.09 (m, 2H), 2.97 (s, 3H), 2.80 – 2.76 (m, 2H), 2.31 (dq, *J* = 14.3, 7.7
315 Hz, 1H), 2.20 (dq, *J* = 15.4, 7.5 Hz, 1H) ppm.

316 **¹³C NMR (151 MHz, Methanol-*d*₄)** δ 152.2, 149.7, 142.9, 135.8, 133.6, 132.3, 125.7, 124.4, 117.7,
317 115.4, 67.7, 59.0, 49.9, 43.5, 40.4, 34.6, 25.3 ppm.

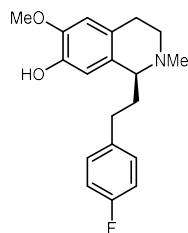
318 **Exact Mass [M+H]⁺**: 332.1412 (calculated), 332.1421 (found).

319

320

321

(S)-1-(4-fluorophenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-6d)



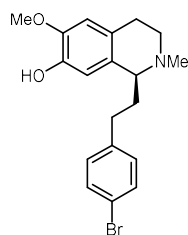
C₁₉H₂₂FNO₂: 315.1635 g/mol

¹H NMR (400 MHz, Methanol-*d*₄) δ 7.24 (s, 2H), 7.02 (t, *J* = 8.2 Hz, 2H), 6.83 (s, 1H), 6.66 (s, 1H), 4.30 (s, 1H), 3.87 (s, 3H), 3.84 – 3.79 (m, 1H), 3.42 – 3.36 (m, 1H), 3.10 – 3.05 (m, 2H), 2.95 (s, 3H), 2.77 (t, *J* = 8.0 Hz, 2H), 2.35 – 2.26 (m, 1H), 2.21 – 2.13 (m, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 162.4, 160.8, 148.3, 145.7, 136.1, 129.7, 121.8, 120.5, 115.0, 114.8, 113.8, 111.4, 63.8, 55.0, 45.9, 39.5, 36.7, 30.5, 21.3 ppm.

Exact Mass [M+H]⁺: 316.1707 (calculated), 316.1693 (found).

(S)-1-(4-bromophenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-7d)



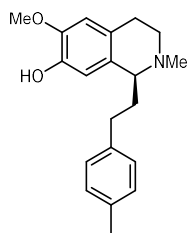
C₁₉H₂₂BrNO₂: 375.0834 g/mol

¹H NMR (400 MHz, Methanol-*d*₄) δ 7.47 (d, *J* = 8.3 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 2H), 6.85 (s, 1H), 6.68 (s, 1H), 4.33 (t, *J* = 5.6 Hz, 1H), 3.89 (s, 3H), 3.85 – 3.78 (m, 1H), 3.44 – 3.38 (m, 1H), 3.14 – 3.10 (m, 2H), 2.97 (s, 3H), 2.77 (t, *J* = 8.3 Hz, 2H), 2.35 – 2.26 (m, 1H), 2.25 – 2.19 (m, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 148.3, 145.8, 139.4, 131.4, 130.0, 121.7, 120.5, 119.8, 113.8, 111.4, 63.8, 55.0, 46.0, 39.5, 36.4, 30.7, 21.3 ppm.

Exact Mass [M+H]⁺: 376.0907 (calculated), 376.0926 (found).

(S)-6-methoxy-2-methyl-1-(4-methylphenethyl)-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-8d)



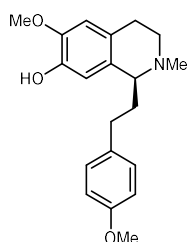
C₂₀H₂₅NO₂: 311.1885 g/mol

¹H NMR (400 MHz, Methanol-*d*₄) δ 7.12 (s, 4H), 6.83 (s, 1H), 6.65 (s, 1H), 4.28 (q, *J* = 8.4, 6.1 Hz, 1H), 3.88 (s, 3H), 3.79 (ddd, *J* = 13.0, 8.8, 6.8 Hz, 1H), 3.43 – 3.38 (m, 1H), 3.17 – 3.08 (m, 2H), 2.94 (s, 3H), 2.74 (t, *J* = 8.2 Hz, 2H), 2.35 – 2.26 (m, 4H), 2.18 (dt, *J* = 14.1, 7.3 Hz, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 152.2, 149.6, 140.9, 139.7, 132.9, 131.8, 125.9, 124.4, 117.7, 115.4, 67.7, 59.0, 49.8, 43.4, 40.7, 34.8, 25.2, 23.6 ppm.

Exact Mass [M+H]⁺: 312.1958 (calculated), 312.1950 (found).

(S)-6-methoxy-1-(4-methoxyphenethyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-9d)



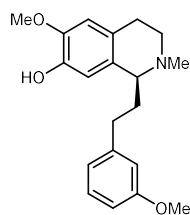
C₂₀H₂₅NO₃: 327.1834 g/mol

¹H NMR (400 MHz, Methanol-*d*₄) δ 7.12 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.72 (s, 1H), 6.61 (s, 1H), 3.84 (s, 3H), 3.78 (t, *J* = 5.8 Hz, 1H), 3.75 (s, 3H), 3.39 (dt, *J* = 13.1, 7.2 Hz, 1H), 3.04 – 2.98 (m, 1H), 2.95 – 2.82 (m, 2H), 2.73 – 2.56 (m, 5H), 2.16 (ddt, *J* = 15.8, 10.7, 5.3 Hz, 1H), 2.03 (ddd, *J* = 14.1, 10.5, 7.0 Hz, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 158.1, 147.2, 144.9, 133.3, 129.0, 126.3, 122.9, 113.6, 113.5, 111.4, 62.9, 55.1, 54.3, 47.1, 40.5, 35.9, 30.7, 23.6 ppm.

Exact Mass [M+H]⁺: 328.1907 (calculated), 328.1913 (found).

371 **(S)-6-methoxy-1-(3-methoxyphenethyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-10d)**



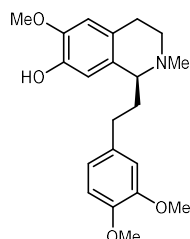
372
373 **C₂₀H₂₅NO₃**: 327.1834 g/mol

374 **¹H NMR (600 MHz, Methanol-*d*₄)** δ 7.19 (t, *J* = 7.6 Hz, 1H), 6.80– 6.74 (m, 4H), 6.63 (s, 1H),
375 4.24 (s, 1H), 3.85 (s, 3H), 3.80 – 3.75 (m, 4H), 3.39 – 3.35 (m, 1H), 3.12 – 3.03 (m, 2H), 2.91 (s,
376 3H), 2.73 (t, *J* = 8.2 Hz, 2H), 2.31 (dq, *J* = 13.4, 7.9 Hz, 1H), 2.16 (dt, *J* = 13.8, 7.2 Hz, 1H) ppm.

377 **¹³C NMR (151 MHz, Methanol-*d*₄)** δ 160.1, 148.2, 145.7, 141.7, 129.4, 121.9, 120.6, 120.3, 113.8,
378 113.7, 111.5, 111.5, 63.8, 55.1, 54.2, 45.8, 39.5, 36.6, 31.3, 21.3 ppm.

379 **Exact Mass [M+H]⁺**: 328.1907 (calculated), 328.1913 (found).

380
381 **(S)-1-(3,4-dimethoxyphenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((S)-**
382 **11d)**



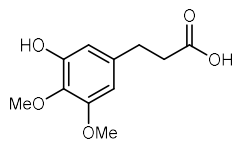
383
384 **C₂₁H₂₇NO₄**: 357.1940 g/mol

385 **¹H NMR (400 MHz, Methanol-*d*₄)** δ 6.90 (d, *J* = 8.1 Hz, 1H), 6.84 (s, 2H), 6.80 (d, *J* = 8.1 Hz,
386 1H), 6.66 (s, 1H), 4.29 (t, *J* = 6.0 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 – 3.78 (m, 4H), 3.44 –
387 3.39 (m, 1H), 3.19 – 3.09 (m, 2H), 2.96 (s, 3H), 2.74 (t, *J* = 8.1 Hz, 2H), 2.37 – 2.28 (m, 1H), 2.26
388 – 2.16 (m, 1H) ppm.

389 **¹³C NMR (151 MHz, Methanol-*d*₄)** δ 149.3, 148.2, 147.8, 145.7, 132.9, 121.9, 120.4, 120.3, 113.8,
390 112.0, 112.0, 111.4, 63.8, 55.2, 55.1, 55.0, 45.9, 39.5, 36.8, 30.9, 21.3 ppm.

391 **Exact Mass [M+H]⁺**: 358.2013 (calculated), 358.2020 (found).

3-Hydroxy-4,5-dimethoxybenzenepropanoic acid (12)

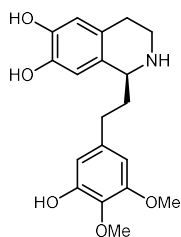


C₁₁H₁₄O₅: 226.2280 g/mol

¹H NMR (400 MHz, DMSO-*d*₆) δ 12.09 (s, 1H), 8.98 (s, 1H), 6.33 (dd, *J* = 16.5, 1.5 Hz, 2H), 3.73 (s, 3H), 3.62 (s, 3H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.47 (d, *J* = 7.6 Hz, 2H) ppm. For ¹H-NMR spectrum see Fig. S31, which is consistent to the literature reported².

Exact Mass [M-H]⁻: 225.0768 (calculated), 225.0769 (found).

(*S*)-1-(3-hydroxy-4,5-dimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol ((*S*)-12b)



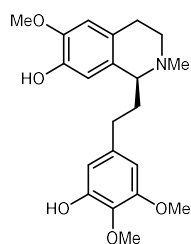
C₁₉H₂₃NO₅: 345.1576 g/mol

¹H NMR (600 MHz, Methanol-*d*₄) δ 6.67 (s, 1H), 6.65 (s, 1H), 6.43 (d, *J* = 1.1 Hz, 2H), 4.40 (dd, *J* = 7.4, 5.2 Hz, 1H), 3.85 (s, 3H), 3.77 (s, 3H), 3.57 (dt, *J* = 12.5, 6.2 Hz, 1H), 3.40 – 3.36 (m, 1H), 3.02 (dt, *J* = 17.0, 6.2 Hz, 1H), 2.95 (dt, *J* = 17.0, 6.3 Hz, 1H), 2.76 – 2.66 (m, 2H), 2.30 (ddt, *J* = 15.5, 10.8, 6.1 Hz, 1H), 2.23 – 2.16 (m, 1H) ppm.

¹³C NMR (151 MHz, Methanol-*d*₄) δ 153.4, 150.3, 145.5, 144.6, 136.3, 134.9, 122.5, 122.3, 114.9, 112.6, 108.7, 103.6, 59.6, 55.0, 54.7, 39.4, 35.7, 31.1, 24.3 ppm.

Exact Mass [M+H]⁺: 346.1649 (calculated), 346.1642 (found).

422 **(S)-autumnaline ((S)-12d)**



423

424 **C₂₁H₂₇NO₅**: 373.1889 g/mol

425 **¹H NMR (400 MHz, Methanol-*d*₄)** δ 6.84 (s, 1H), 6.65 (s, 1H), 6.39 (s, 2H), 4.28 (t, *J* = 6.3 Hz,

426 1H), 3.88 (s, 3H), 3.83 – 3.79 (m, 4H), 3.76 (s, 3H), 3.44 – 3.38 (m, 1H), 3.12 (dd, *J* = 17.9, 8.6 Hz,

427 2H), 2.95 (s, 3H), 2.68 (t, *J* = 8.1 Hz, 2H), 2.35 – 2.26 (m, 1H), 2.20 (dt, *J* = 14.5, 7.6 Hz, 1H) ppm.

428 **¹³C NMR (151 MHz, Methanol-*d*₄)** δ 153.4, 150.3, 148.2, 145.7, 136.1, 134.9, 121.9, 120.5, 113.8,

429 111.4, 108.7, 103.6, 63.8, 59.6, 55.0, 55.0, 45.9, 39.5, 36.6, 31.3, 21.3 ppm.

430 **Exact Mass [M+H]⁺**: 374.1962 (calculated), 374.1972 (found).

431

432

Supplementary Tables

Supplementary Table 1. Kinetic parameters of wild-type *GsOMT1* and *GsOMT1*^{V125Q} towards (*S*)-1-(3-hydroxy-4,5-dimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol ((*S*)-**12b**).

Enzyme	K_m (mM)	k_{cat} (s ⁻¹)	k_{cat} / K_m (mM ⁻¹ ·s ⁻¹)
WT	0.63 ± 0.05	4.56 ± 0.24	7.29 ± 0.23
V125Q	0.33 ± 0.02	8.65 ± 0.26	26.50 ± 0.51

438 **Supplementary Table 2.** The information of the reagents used in this study.

Reagents	Purity (%)	Catalog numbers	Source
Primers			Exsyn-bio (Wuxi, China)
DNA marker		3584A	TaKaRa
Protein marker		3597A	TaKaRa
PrimerSTAR MAX DNA Polymerase		R045Q	TaKaRa
2 × Phanta Flash Master Mix		P510-01	Vazyme
2 × MultiF Seamless Assembly Mix		RK21020	ABclonal (Wuhan, China)
Tryptone		LP0042B	OXOID
Yeast extract		LP0021T	OXOID
NaCl	99.8	10019308	China National Pharmaceutical Group Corp (Shanghai, China)
Acetonitrile (HPLC)	99.9	80988AM	Adamas-Beta (Shanghai, China)
Methanol (HPLC)	99.9	75851AR	Adamas-Beta (Shanghai, China)
3-(3,4,5-trimethoxyphenyl)propanoic acid (1)	98	A011166	Energy Chemical (Shanghai, China)
3-phenylpropanoic acid (2)	98	A010214	Energy Chemical (Shanghai, China)
3-(2-chlorophenyl)propanoic acid (3)	98	A0112270050	Energy Chemical (Shanghai, China)
3-(3-chlorophenyl)propanoic acid (4)	98	A011745	Energy Chemical (Shanghai, China)
3-(4-chlorophenyl)propanoic acid (5)	98	A011140	Energy Chemical (Shanghai, China)
3-(4-fluorophenyl)propanoic acid (6)	98	A010757	Energy Chemical (Shanghai, China)
3-(4-bromophenyl)propanoic acid (7)	98	A010420	Energy Chemical (Shanghai, China)
3-(<i>p</i> -tolyl)propanoic acid (8)	98	A011107	Energy Chemical (Shanghai, China)
3-(4-methoxyphenyl)propanoic acid (9)	98	A010827	Energy Chemical (Shanghai, China)
3-(3-methoxyphenyl)propanoic acid (10)	98	A010503	Energy Chemical (Shanghai, China)
3-(3,4-dimethoxyphenyl)propanoic acid (11)	98	A010228	Energy Chemical (Shanghai, China)
3-hydroxy-4,5-dimethoxybenzaldehyde	>97	45930B	Adamas-Beta (Shanghai, China)
Malonic acid	98	A010411	Energy Chemical (Shanghai, China)

	2-(3,4-Dihydroxyphenyl)ethylamine hydrochloride	98	E080183	Energy Chemical (Shanghai, China)
439				
440				

441 **Supplementary Table 3.** All plasmids used in this study.

Plasmid	Description	Source
pET-21b(+)	T7 promoter, pBR332 ori, Amp ^R	Novagen
pRSFDuet-1	double T7 promoters, RSF ori, Kan ^R , >100 copy number	Novagen
pETDuet-1	double T7 promoters, pBR322 ori, Amp ^R , ~40 copy number	Novagen
pCDFDuet-1	double T7 promoters, CDF ori, Str ^R , 20-40 copy number	Novagen
pEcCas	Native cas9 promoter, rhaB promoter, araBAD promoter, pSC101 ori, Kan ^R	Addgene
pTargetF	pij23119 promoter, pBR332 ori, Spec ^R	Addgene
pTargetF- <i>dkgB</i>	pTargetF carrying sgRNA of gene <i>dkgB</i>	This study
pTargetF- <i>yeaE</i>	pTargetF carrying sgRNA of gene <i>yeaE</i>	This study
pTargetF- <i>yahK</i>	pTargetF carrying sgRNA of gene <i>yahK</i>	This study
pTargetF- <i>yjgB</i>	pTargetF carrying sgRNA of gene <i>yjgB</i>	This study
pTargetF- <i>yqhC</i>	pTargetF carrying sgRNA of gene <i>yqhC</i>	This study
pRSFDuet-1- <i>TpCAR/BsSfp</i>	pRSFDuet-1 carrying <i>TpCAR</i> (6His in N-terminum) and <i>BsSfp</i> (No tag in N/C-terminum)	This study
pET-21b(+)- <i>TjNCS</i>	pET-21b(+) carrying <i>TjNCS</i> (6His in C-terminum)	This study
pET-21b(+)- <i>RnCOMT</i>	pET-21b(+) carrying <i>RnCOMT</i> (6His in C-terminum)	This study
pET-21b(+)- <i>GsOMT1</i>	pET-21b(+) carrying <i>GsOMT1</i> (6His in C-terminum)	This study
pET-21b(+)- <i>CNMT</i>	pET-21b(+) carrying <i>CNMT</i> (6His in C-terminum)	This study
pET-21b(+)- <i>GDH</i>	pET-21b(+) carrying <i>GDH</i> (6His in C-terminum)	This study
pET-21b(+)- <i>GsNMTt</i>	pET-21b(+) carrying <i>GsNMTt</i> (6His in C-terminum)	This study
pET-21b(+)- <i>PavNMT</i>	pET-21b(+) carrying <i>PavNMT</i> (6His in C-terminum)	This study
pET-21b(+)- <i>TNMT</i>	pET-21b(+) carrying <i>TNMT</i> (6His in C-terminum)	This study
pRSFDuet-1- <i>TpCAR/BsSfp-TjNCS</i>	pRSFDuet-1 carrying <i>TpCAR</i> , <i>BsSfp</i> and <i>TjNCS</i>	This study
pETDuet-1- <i>TpCAR/BsSfp-TjNCS</i>	pETDuet-1 carrying <i>TpCAR</i> , <i>BsSfp</i> and <i>TjNCS</i>	This study
pCDFuet-1- <i>TpCAR/BsSfp-TjNCS</i>	pCDFDuet-1 carrying <i>TpCAR</i> , <i>BsSfp</i> and <i>TjNCS</i>	This study
pRSFDuet-1- <i>TpCAR/BsSfp</i>	pRSFDuet-1 carrying <i>TpCAR</i> and <i>BsSfp</i>	This study
pETDuet-1- <i>TpCAR/BsSfp</i>	pETDuet-1 carrying <i>TpCAR</i> and <i>BsSfp</i>	This study
pCDFDuet-1- <i>TpCAR/BsSfp</i>	pCDFDuet-1 carrying <i>TpCAR</i> and <i>BsSfp</i>	This study
pRSFDuet-1- <i>TjNCS</i>	pRSFDuet-1 carrying <i>TjNCS</i>	This study
pETDuet-1- <i>TjNCS</i>	pETDuet-1 carrying <i>TjNCS</i>	This study

pCDFDuet-1- <i>Tj</i> /NCS	pCDFDuet-1 carrying <i>Tj</i> /NCS	This study
pRSFDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT	pRSFDuet-1 carrying <i>Ec</i> MAT and <i>Rn</i> COMT	This study
pETDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT	pETDuet-1 carrying <i>Ec</i> MAT and <i>Rn</i> COMT	This study
pCDFDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT	pCDFDuet-1 carrying <i>Ec</i> MAT and <i>Rn</i> COMT	This study
pRSFDuet-1- CNMT*/ <i>Mm</i> SAHH	pRSFDuet-1 carrying CNMT ^{N92A/F332A} and <i>Mm</i> SAHH	This study
pETDuet-1- CNMT*/ <i>Mm</i> SAHH	pETDuet-1 carrying CNMT ^{N92A/F332A} and <i>Mm</i> SAHH	This study
pCDFDuet-1- CNMT*/ <i>Mm</i> SAHH	pCDFDuet-1 carrying CNMT ^{N92A/F332A} and <i>Mm</i> SAHH	This study
pRSFDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT- <i>Mm</i> SAHH	pRSFDuet-1 carrying <i>Ec</i> MAT, <i>Rn</i> COMT and <i>Mm</i> SAHH	This study
pETDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT- <i>Mm</i> SAHH	pETDuet-1 carrying <i>Ec</i> MAT, <i>Rn</i> COMT and <i>Mm</i> SAHH	This study
pCDFDuet-1- <i>Ec</i> MAT/ <i>Rn</i> COMT- <i>Mm</i> SAHH	pCDFDuet-1 carrying <i>Ec</i> MAT, <i>Rn</i> COMT and <i>Mm</i> SAHH	This study
pRSFDuet-1- CNMT*/ <i>Mm</i> SAHH- <i>Ec</i> MAT	pRSFDuet-1 carrying CNMT ^{N92A/F332A} , <i>Mm</i> SAHH and <i>Ec</i> MAT	This study
pETDuet-1- CNMT*/ <i>Mm</i> SAHH- <i>Ec</i> MAT	pETDuet-1 carrying CNMT ^{N92A/F332A} , <i>Mm</i> SAHH and <i>Ec</i> MAT	This study
pCDFDuet-1- CNMT*/ <i>Mm</i> SAHH- <i>Ec</i> MAT	pCDFDuet-1 carrying CNMT ^{N92A/F332A} , <i>Mm</i> SAHH and <i>Ec</i> MAT	This study

Strain	Description	Source
<i>E. coli</i> BL21 (DE3)	<i>E. coli</i> str. B F ⁻ <i>ompT gal dcm lon hsdS_B (r_B⁻ m_B⁻) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB⁺]_{K-12}(λ^S)</i>	Novagen
IAA	<i>E. coli</i> BL21 (DE3), Δ <i>dkgB</i> , Δ <i>yeaE</i> , Δ(<i>yqhC-dkgA</i>), Δ <i>yahK</i> , Δ <i>yjgB</i>	This study
WT	pRSFDuet-1- <i>TpCAR/BsSfp-TjNCS</i> was expressed in <i>E. coli</i> BL21 (DE3)	This study
M1A	pRSFDuet-1- <i>TpCAR/BsSfp</i> and pETDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1B	pRSFDuet-1- <i>TpCAR/BsSfp</i> and pCDFDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1C	pETDuet-1- <i>TpCAR/BsSfp</i> and pRSFDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1D	pETDuet-1- <i>TpCAR/BsSfp</i> and pCDFDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1E	pCDFDuet-1- <i>TpCAR/BsSfp</i> and pRSFDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1F	pCDFDuet-1- <i>TpCAR/BsSfp</i> and pETDuet-1- <i>TjNCS</i> were co-expressed in IAA	This study
M1G	pRSFDuet-1- <i>TpCAR/BsSfp-TjNCS</i> was expressed in IAA	This study
M1H	pETDuet-1- <i>TpCAR/BsSfp-TjNCS</i> was expressed in IAA	This study
M1I	pCDFDuet-1- <i>TpCAR/BsSfp-TjNCS</i> was expressed in IAA	This study
S1	pRSFDuet-1- <i>EcMAT/RnCOMT</i> and pET-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
S2	pRSFDuet-1- <i>EcMAT/RnCOMT</i> and pCDF-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
S3	pETDuet-1- <i>EcMAT/RnCOMT</i> and pRSF-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
S4	pETDuet-1- <i>EcMAT/RnCOMT</i> and pCDF-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
S5	pCDFDuet-1- <i>EcMAT/RnCOMT</i> and pRSF-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
S6	pCDFDuet-1- <i>EcMAT/RnCOMT</i> and pET-CNMT*/ <i>MmSAHH</i> were co-expressed in IAA	This study
M2A	pRSFDuet-1- <i>EcMAT/RnCOMT-MmSAHH</i> was expressed in IAA	This study
M2B (RnCOMT)	pETDuet-1- <i>EcMAT/RnCOMT-MmSAHH</i> was expressed in IAA	This study
M2B (GsOMT1 ^{V125Q})	pETDuet-1- <i>EcMAT/GsOMT1^{V125Q}-MmSAHH</i> was expressed in IAA	This study
M2B (sumo-GsOMT1 ^{V125Q})	pETDuet-1- <i>EcMAT/sumo-GsOMT1^{V125Q}-MmSAHH</i> was expressed in IAA	This study
M2C	pCDFDuet-1- <i>EcMAT/RnCOMT-MmSAHH</i> was expressed in IAA	This study
M3A	pRSFDuet-1-CNMT*/ <i>MmSAHH-EcMAT</i> was expressed in IAA	This study
M3B	pETDuet-1-CNMT*/ <i>MmSAHH-EcMAT</i> was expressed in IAA	This study
M3C	pCDFDuet-1-CNMT*/ <i>MmSAHH-EcMAT</i> was expressed in IAA	This study

444 **Supplementary Table 5.** All primers used in this study.

Name	Sequence (5'-3')
Duet-F (MCS2)	TAATTAACCTAGGCTGCTGCCAC
CAR-R	ATGTATATCTCCTTCTTAAAGTTTAGTCACGTGCACCCGGG
Sfp-F	TAAGAAGGAGATATACATATGAAGATTTACGGAATTTATATGGACCGC
Sfp-R	AGCAGCCTAGGTTAATTATAAAAGCTCTTCGTACGAGACCATTGTG
NCS-F	ATAAGGAGATATACCATGCTGCATCACCAAGGGATTATC
NCS-R	TTCTGTTGCGACTTAAGCATTAGACAGTGATGATCGCAGCTTCGATC
Duet-R (MCS2)	CATATGTATATCTCCTTCTTATACTTAACTAATACTAAGATGGG
NCS-1-F	GAAGGAGATATACATATGCTGCATCACCAAGGGATTATCAACC
NCS-2-R	AGCAGCCTAGGTTAATTAGACAGTGATGATCGCAGCTTCG
Duet-F (MCS1)	TGCTTAAGTCGAACAGAAAGTAATCGTATTG
Duet-R (MCS1)	CATGGTATATCTCCTTATTAAAGTTAAACAAAATTATTTCTACAGG
MAT-F	TTAATAAGGAGATATACCATGGCAAAACACCTTTTTACGTCCG
MAT-R	CCCATATGTATATCTCCTTCTTAAAGTTcACTTCAGACCGGCAGCATC
COMT-F	GAAGGAGATATACATATGGGAGATACCAAAGAACAGCGCATC
COMT-R	TTCTGTTGCGACTTAAGCATTAAAGATTTATCAGGGCTAGAAGGACCTTG
CNMT-F	TTAATAAGGAGATATACCatgCAAACAAAAAAGCCGCAATCG
CNMT-R	ATGTATATCTCCTTCTTAAAGTTcATTTCCTTGAAAAGAACATGTGATGCC ATCC
SAHH-F	TAAGAAGGAGATATACATATGTCTGACAACTTCCCTATAAGGTAGC
SAHH-R	TTCTGTTGCGACTTAAGCATcAATAACGATAATGGTCGGGTTTGAAAGG
21b(+)-F	CTCGAGCACCACCACCAC
21b(+)-R	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGG
G _s NMTt-F	GAAGGAGATATACATATGAGTCATGAGGCAGCAGCC
G _s NMTt-R	GTGGTGGTGGTGCTCGAGCTCCGGTTTACGGAAAGTCAAGATC
CNMT (L88A)-F	TTGAGTTT GCG AAGATTATGAACGGAAGCAACTTGAAGG
CNMT (L88A)-R	TAATCTT CG CAAACTCAATTGGGATTTTCGTACAACCTGAC
CNMT (M91A)-F	TGAAGATT GCG AACGGAAGCAACTTGAAGGGAAG
CNMT (M91A)-R	TTCCGTT CG CAATCTTCAAAAACTCAATTGGGATTTTCGTACAAC
CNMT (N92A)-F	AGATTATGGCTGGAAGCAACTTGAAGGGAAGTTGTTG
CNMT (N92A)-R	TGCTTCC AGC CATAATCTTCAAAAACTCAATTGGGATTTTCGTACAAC
CNMT (W297A)-F	ACGAGGAAGCTCTGAAACGCTTGGATGCCAACC
CNMT (W297A)-R	GTTTCAG AGC TTCTCTCGTTTGTACGGGAAAAGTGTTTAC
CNMT (W329A)-F	TCAACTAT GCCC GCGGGTTCTGCTTAAGCG
CNMT (W329A)-R	ACCCGCG GGC CATAGTTGATTAACCTCACTGCTTCTTCCTC
CNMT (F332A)-F	GGCGCGGG GCG TGCTTAAGCGGGATGGAGATG
CNMT (F332A)-R	TTAAGCA CG CCCCGCGCCAATAGTTGATTAACCTC
CNMT (C333A)-F	GCGGGTT CG CATTAAGCGGGATGGAGATGTTTCGG
CNMT (C333A)-R	CGCTTAAT TCG GAACCCGCGCCAATAGTTGATTAAC
CNMT (L88G)-F	TTGAGTTT GGG AAGATTATGAACGGAAGCAACTTGAAGG
CNMT (L88G)-R	TAATCTTCCCAAACTCAATTGGGATTTTCGTACAACCTGAC
CNMT (L88S)-F	TTGAGTTT TCG AAGATTATGAACGGAAGCAACTTGAAGG
CNMT (L88S)-R	TAATCTT CG AAAACTCAATTGGGATTTTCGTACAACCTGAC

CNMT (L88V)-F	TTGAGTTT GT CAAGATTATGAACGGAAGCAACTTGAAGG
CNMT (L88V)-R	TAATCTT GAC AAACTCAATTGGGATTTTCGTACAAC TGAC
CNMT (N92V)-F	AGATTAT GGTT GGAAGCAACTTGAAGGGAAGTTGTTG
CNMT (N92V)-R	TGCTTCC AACC ATAATCTTCAAAA ACT CAATTGGGATTTTCGTACAAC
CNMT (F332S)-F	GGCGCGGG TC CTGCTTAAGCGGGATGGAGATGTT C
CNMT (F332S)-R	TTAAGC AGG ACCCGCGCCAATAGTTGATTAA C
CNMT (F332V)-F	GGCGCGGG GT GTGCTTAAGCGGGATGGAGAT G
CNMT (F332V)-R	TTAAGC AGG ACCCGCGCCAATAGTTGATTAA C
GsOMT1(I122N)-F	GGTGATGTGGTTCTGGTAGACCT TG
GsOMT1(I122N)-R	TACCAGAACCACATCACC GTT AGACGCGCCATCTTTATCACGGG
GsOMT1(I122Q)-R	TACCAGAACCACATCACC CTG AGACGCGCCATCTTTATCACGGG
GsOMT1(I122D)-R	TACCAGAACCACATCACC GTC AGACGCGCCATCTTTATCACGGG
GsOMT1(V125N)-F	GTTCTGGTAGACCTTGACCC CG
GsOMT1(V125N)-R	GTCAAGGTCTACCAGAAC GTT ATCACCAATAGACGCGCCATC
GsOMT1(V125Q)-R	GTCAAGGTCTACCAGAAC CTG ATCACCAATAGACGCGCCATC
GsOMT1(V125D)-R	GTCAAGGTCTACCAGAAC ATC ATCACCAATAGACGCGCCATC
GsOMT1(V125E)-R	GTCAAGGTCTACCAGAAC TT CATCACCAATAGACGCGCCATC
GsOMT1(V125H)-R	GTCAAGGTCTACCAGAAC GTC ATCACCAATAGACGCGCCATC
GsOMT1(N181A)-F	TCTACAATTATTCTTCGTAAAATTCTGGAGATCTACAAAGG
GsOMT1(N181A)-R	ACGAAGAATAATTGTAGAG GCG GATGCCATCGCATTATTGAAA ACT TTATTG
GsOMT1(N181Q)-R	ACGAAGAATAATTGTAGAC TG GGATGCCATCGCATTATTGAAA ACT TATTG
GsOMT1(E310V)-F	GCTTTCTATTCTGACCTTTGCATGTTGATT AG
GsOMT1(E310V)-R	AAGGTCAGAATAGAAAGC GAC CTGGCTTGCGGCATCGGTTTT TG
GsOMT1(E310L)-R	AAGGTCAGAATAGAAAGC CA ACTGGCTTGCGGCATCGGTTTT TG
<i>dkgB</i> -KO-F	TTGATCTGACTCTAATCCACGTTTTAGAGCTAGAAATAG C
<i>dkgB</i> -KO-R	GTGGATTAGAGTCAGATCAA ACT AGTATTATACCTAGGACTGAG C
<i>yeaE</i> -KO-F	TTGAGTACGATCTACTCC CCG TTTTAGAGCTAGAAATAGCAAG TT
<i>yeaE</i> -KO-R	GGGGAGTAGATCGTACTCAA ACT AGTATTATACCTAGGACTGAG C
<i>yahK</i> -KO-F	TCATGAAATTGTGGGGCGTGGTTTTAGAGCTAGAAATAGCAAG TT
<i>yahK</i> -KO-R	CACGCCCCACAATTT C ATGAACTAGTATTATACCTAGGACTGAG C
<i>yjgB</i> -KO-F	AAAAGAAGCGGGCGGAGAACGTTTTAGAGCTAGAAATAGCAAG TT
<i>yjgB</i> -KO-R	GTTCTCCGCCCGCTTCTTT ACT AGTATTATACCTAGGACTGAG C
<i>yqhC/yqhD/dkgA</i> -KO-F	TGTACTCGCATAGCCGCAAGTTTTAGAGCTAGAAATAGCAAG TT
<i>yqhC/yqhD/dkgA</i> -KO-R	CTTGCGGCTATGCGAGTACA ACT AGTATTATACCTAGGACTGAG C
<i>dkgB</i> -Up-F	GAAGGAGCATACTGAAGTATGTGACTGAC
<i>dkgB</i> -Up-R	GGAGGAGCTGTCAGAGAGGCGTGATTATGGGCTTCAGACTGTTGAG G
<i>dkgB</i> -Down-F	GCCTCTCTGACAGCTCCT CC
<i>dkgB</i> -Down-R	GCGCTGGTACGTTAACGGAT TC
<i>yeaE</i> -Up-F	CTGTCTTATGCTCACTTTACCCAGTAC G
<i>yeaE</i> -Up-R	TGTTTCCTCCCAGGTATTGCAC
<i>yeaE</i> -Down-F	GTGCAATACCTGGGAGGAAACATGTTGTGTGCCGGATGCAT C
<i>yeaE</i> -Down-R	AAA ACT TGTGAAATCGATCTTGAATCACAT GG
<i>yahK</i> -Up-F	GCAGGATGACGCCAGCTTT G

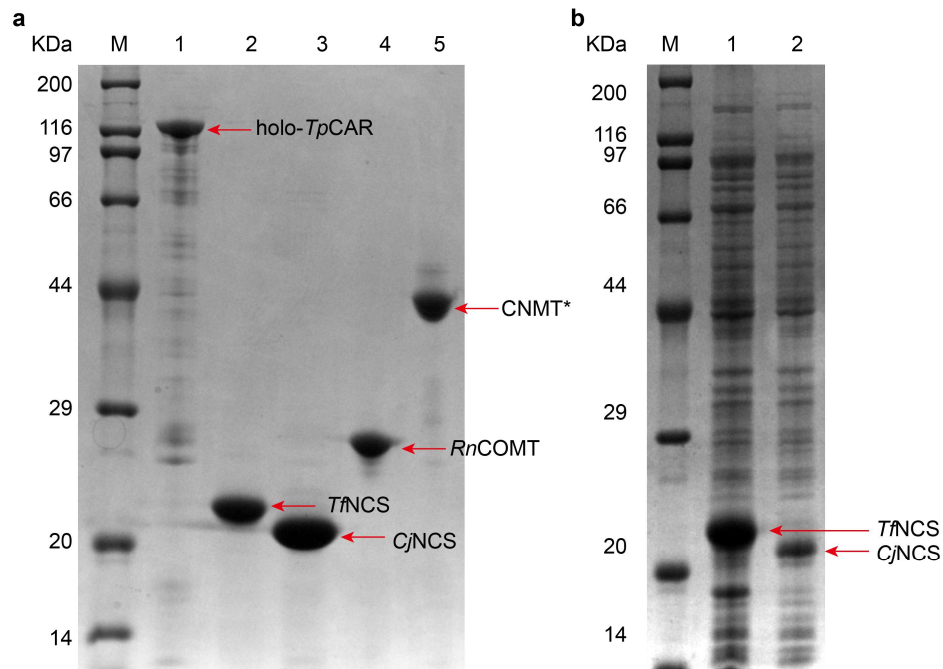
<i>yahK</i> -Up-R	GGCAACGCCTGATATGTCATCG
<i>yahK</i> -Down-F	CGATGACATATCAGGCGTTGCCCCCTGTGGTTTAACATATTAACCTCG CTCTC
<i>yahK</i> -Down-R	GTGAGTGCGTTATCGTCTGACCAC
<i>yjgB</i> -Up-F	TTGGCTGCTCTGACTTTGATATCTG
<i>yjgB</i> -Up-R	TTTTTGGTCTCTCTGCTGTTGTTG
<i>yjgB</i> -Down-F	CAAACAACACCAGAGAAGGACCAAAAAAATCATTTCGAGCGCTGA TC
<i>yjgB</i> -Down-R	TTGCCGCGTTATTATCTGCTAAATTTATTGATG
<i>yqhC/yqhD/dkgA</i> -Up-F	ATTTTCTGCCTACGATTGCGATTCTG
<i>yqhC/yqhD/dkgA</i> -Up-R	TACTTGCTCCCTTTGCTGGGC
<i>yqhC/yqhD/dkgA</i> -Down-F	GCCCAGCAAAGGGAGCAAGTAGCTTTTTACGCCTCAAACCTTCGTTT TC
<i>yqhC/yqhD/dkgA</i> -Down-R	CGAAAGCGCACAAACGGCATC

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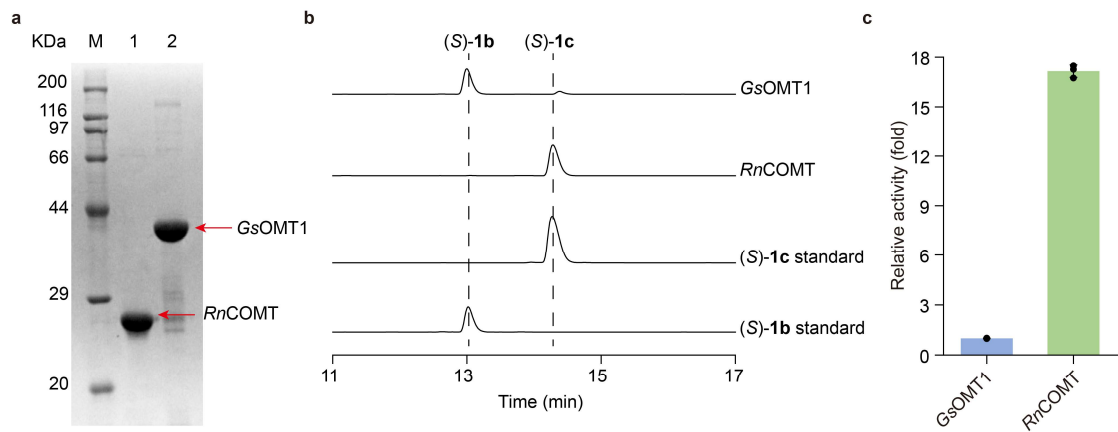
446 **Supplementary Table 6.** The HPLC chromatographic conditions for the preparation of different
 447 final products.

Substrate	Column	Mobile phase	Test conditions
(S)-1b	ZORBAX	Linear gradient 25%-50% acetonitrile	UV 280 nm, 30 °C,
	Eclipse XDB-C18	(0.1% TFA) over 20 min	3 mL min ⁻¹
(S)-1c mixture	ZORBAX	Linear gradient 30%-35% acetonitrile	UV 280 nm, 25 °C,
	Eclipse XDB-C18	(0.1% TFA) over 20 min	1 mL min ⁻¹
(S)-1d	Ultimate XB-phenyl	40%: 60% methanol (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-2d	Ultimate XB-phenyl	30%: 70% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-3d	Ultimate XB-phenyl	35%: 65% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-4d	Ultimate XB-phenyl	35%: 65% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-5d	Ultimate XB-phenyl	40%: 60% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-6d	Ultimate XB-phenyl	27%: 73% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-7d	Ultimate XB-phenyl	30%: 70% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-8d	Ultimate XB-phenyl	27%: 73% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-9d	Ultimate XB-phenyl	27%: 73% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-10d	Ultimate XB-phenyl	28%: 72% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-11d	Ultimate XB-phenyl	20%: 80% acetonitrile (0.1% TFA): ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹
(S)-12b	ZORBAX	Linear gradient 26%-33% acetonitrile	UV 280 nm, 30 °C,
	Eclipse XDB-C18	(0.1% TFA) over 20 min	3 mL min ⁻¹
(S)-12c	ZORBAX	Linear gradient 35%-57% methanol	UV 280 nm, 30 °C,
	Eclipse XDB-C18	(0.1% TFA) over 25 min	3 mL min ⁻¹
(S)-12d	Ultimate XB-phenyl	37%: 63% methanol (0.1% TFA):ddH ₂ O (0.1% TFA)	UV 280 nm, 30 °C, 3 mL min ⁻¹

Supplementary Figures

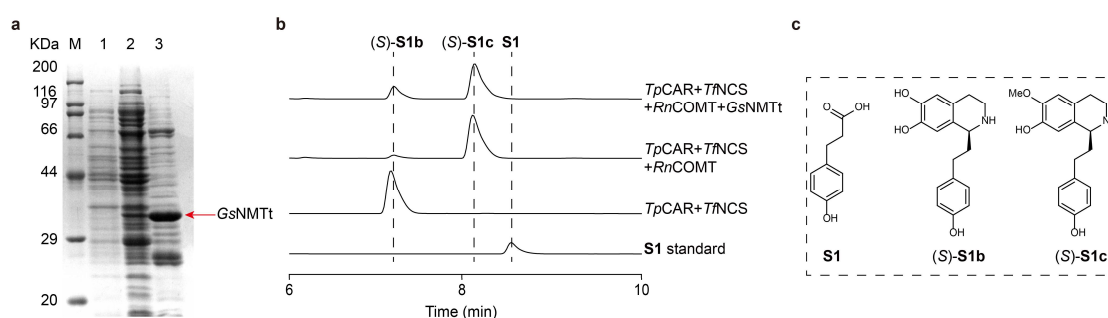


Supplementary Figure 1. SDS-PAGE analysis of purified proteins and protein expression in *E. coli* BL21 (*DE3*). **a** Purified enzymes used for the artificial multi-enzyme cascade *in vitro*. M: marker; lane 1: holo-*TpCAR*; lane 2: *TfNCS*; lane 3: *CjNCS*; lane 4: *RnCOMT*; lane 5: *CNMT*^{N92A/F332A}. **b** Expression of *TfNCS* and *CjNCS* in *E. coli* BL21 (*DE3*). M: marker; lane 1: the supernatant of cell lysates expressing *TfNCS* induced by IPTG; lane 2: the supernatant of cell lysates expressing *CjNCS* induced by IPTG. Source data are provided as a Source Data file.

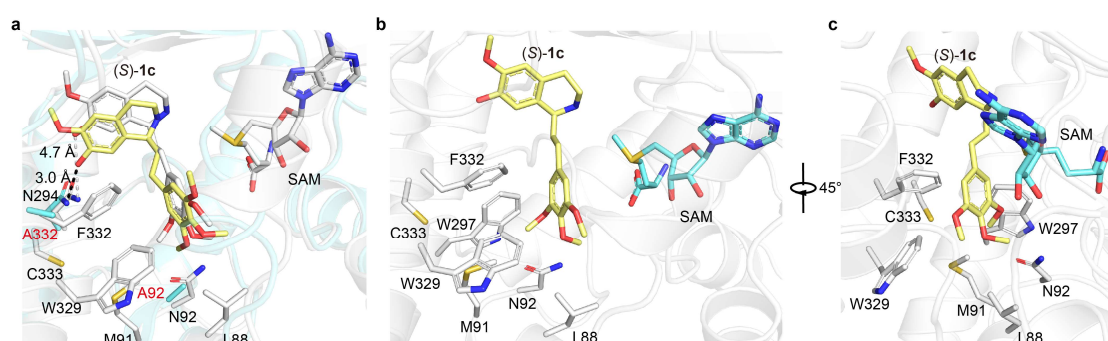


Supplementary Figure 2. Screening of *O*-methyltransferases with high catalytic activity towards (*S*)-**1b**. **a** Purified *RnCOMT* and *GsOMT1* from *E. coli* BL21 (*DE3*). M: marker; lane 1: purified *RnCOMT*; lane 2: purified *GsOMT1*. **b** HPLC analysis of (*S*)-**1b** and (*S*)-**1c** in the reaction systems.

c Relative activity of *Gs*OMT1 and *Rn*COMT towards (*S*)-**1b**. The relative activity was measured by the ratio of (*S*)-**1b** consumption by *Gs*OMT1 to that of *Rn*COMT. All data is presented as mean value of three independent experiments and the error bars indicate $\pm$ sd. Source data are provided as a Source Data file.

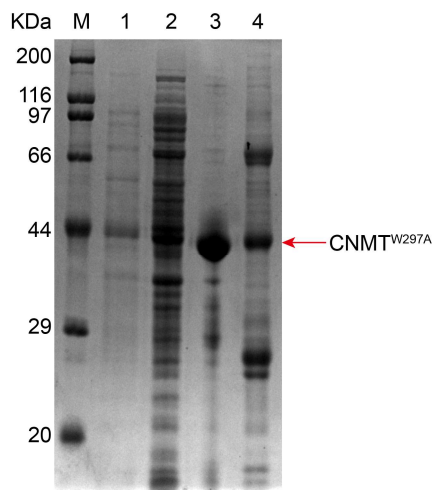


Supplementary Figure 3. Characterization of the *N*-methylation activity of *Gs*NMTt towards (*S*)-**S1c**. **a** SDS-PAGE analysis of *Gs*NMTt expressed in *E. coli* BL21 (*DE3*). M: marker; lane 1: no IPTG induction; lane 2: the supernatant of cell lysates expressing *Gs*NMTt induced by IPTG; lane 3: purified *Gs*NMTt. **b** HPLC analysis of *S1*, (*S*)-**S1b** and (*S*)-**S1c** in a cascade reaction containing the PEIA moiety module, *Rn*COMT and *Gs*NMTt in a one-pot two-step process *in vitro*. **c** The chemical structure of *S1*, (*S*)-**S1b** and (*S*)-**S1c**. Source data are provided as a Source Data file.

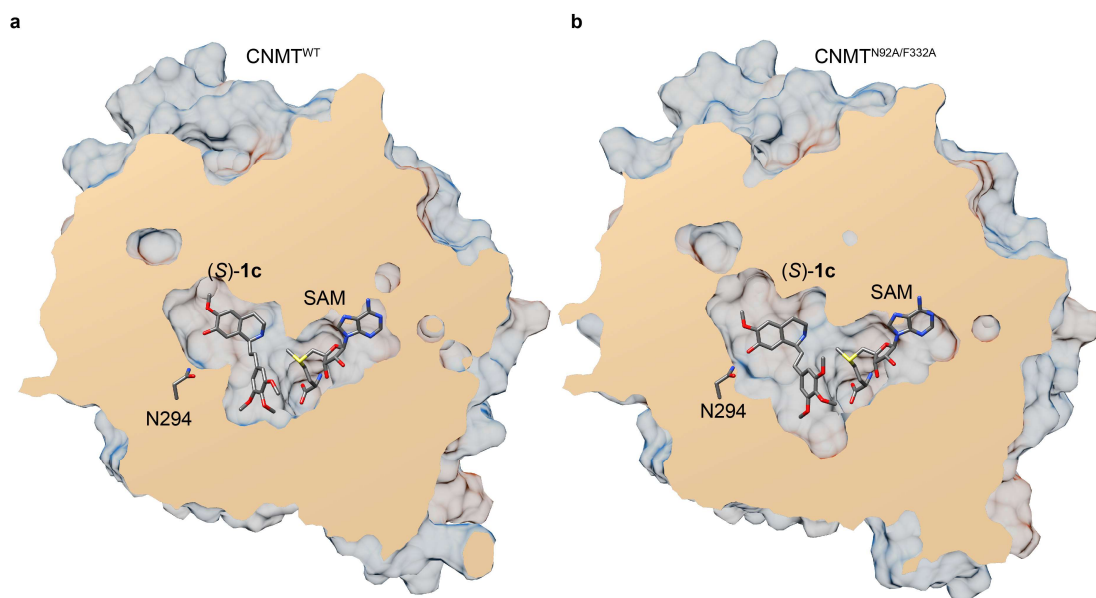


Supplementary Figure 4. Molecular docking analysis of (*S*)-**1c** with CNMT^{WT} and CNMT^{N92A/F332A}. **a** Comparison of the (*S*)-**1c** binding site in CNMT^{WT} and CNMT^{N92A/F332A}. CNMT^{WT} is colored by white while CNMT^{N92A/F332A} is colored by cyan. (*S*)-**1c** in CNMT^{N92A/F332A} is colored by yellow. Residues in CNMT^{WT} located around trimethoxyphenyl group of (*S*)-**1c** within 4.0 Å are indicated by white sticks. The mutated residues in CNMT^{N92A/F332A} were indicated by cyan sticks. The dash line indicates the presence of a hydrogen bond. **b** Substrate (*S*)-**1c**, SAM and the

selected residues with potential interactions with (S)-1c colored by yellow, cyan and white, respectively. Oxygen, nitrogen and sulfur atom are indicated by red, blue and yellow colors, respectively. **c** Close-up views of a 45° rotation along the Y-axis of (a).

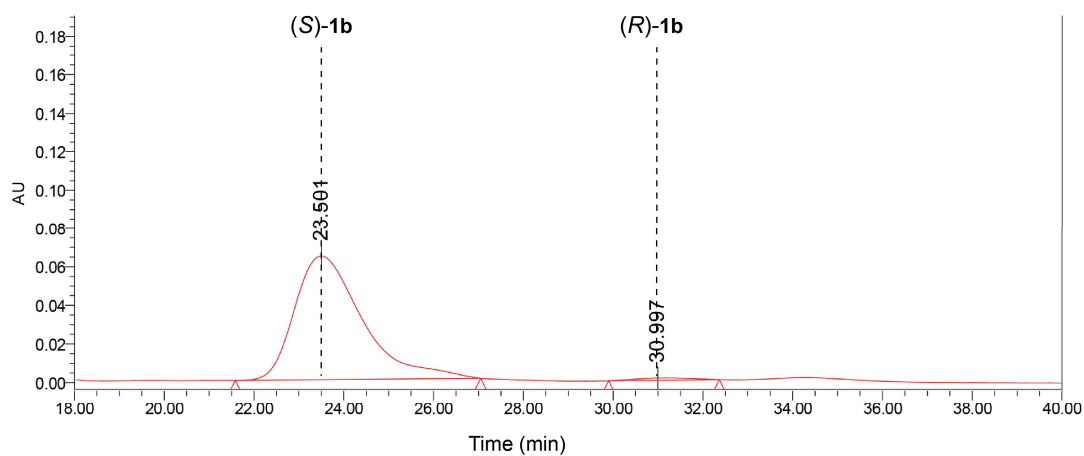


Supplementary Figure 5. SDS-PAGE analysis of CNMT^{W297A} expressed in *E. coli* BL21 (DE3). M: marker; lane 1: no IPTG induction; lane 2: the supernatant of cell lysates expressing CNMT^{W297A} induced by IPTG; lane 3: the pellet; lane 4: purified CNMT^{W297A}. Source data are provided as a Source Data file.

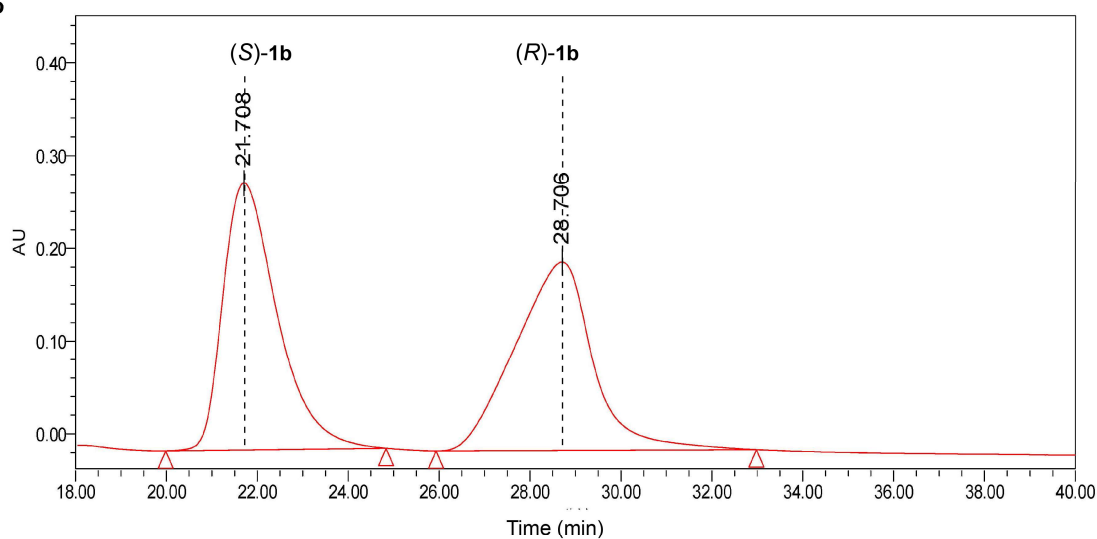


Supplementary Figure 6. Cross-section of the substrate binding pocket. **a** The substrate binding site highlighted in cross-section of CNMT^{WT}. **b** The substrate binding site highlighted in cross-section of CNMT^{N92A/F332A}.

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a

Name	Retention time (min)	Area (μV sec)	Height (μV)	% Area
1	23.501	6672624	63969	98.50
2	30.997	101869	1187	1.50

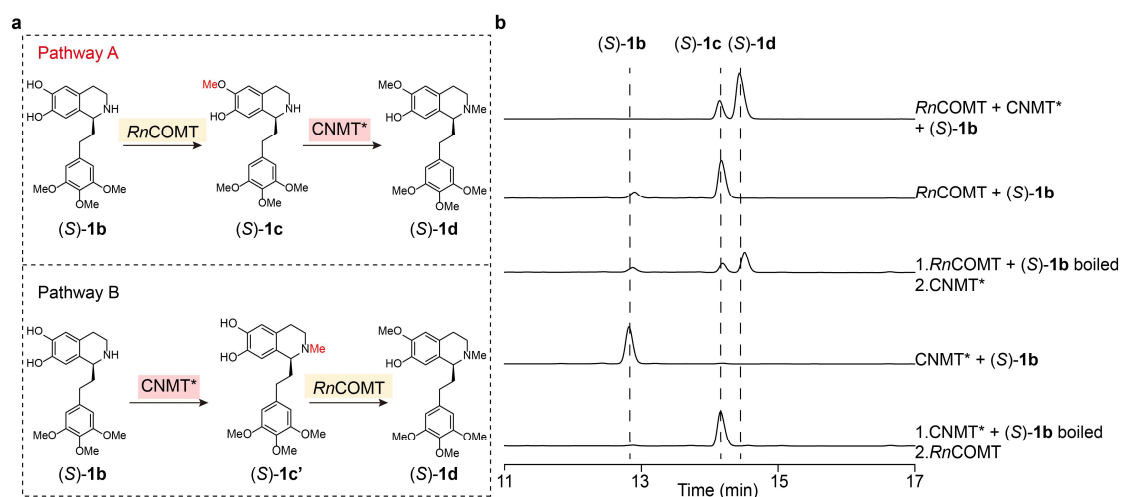
b

Name	Retention time (min)	Area (μV sec)	Height (μV)	% Area
1	21.708	23827994	287725	49.69
2	28.706	24125144	203126	50.31

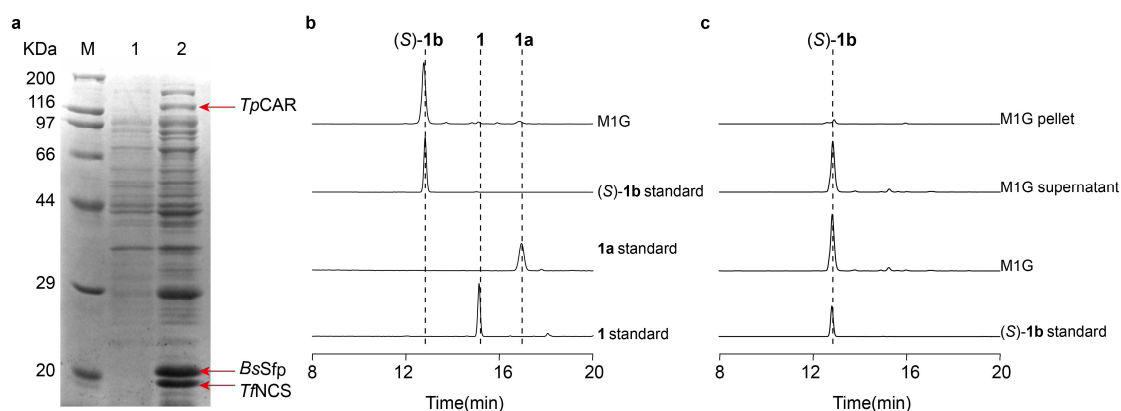
492

493 **Supplementary Figure 7.** Chiral HPLC analysis of (S)-**1b** and (rac)-**1b** synthesized from substrate
 494 **1** and dopamine *in vitro*. **a** Chiral HPLC analysis of (S)-**1b** synthesized by *TpCAR* and *TfNCS* in
 495 HEPES buffer. **b** Chiral HPLC analysis of (rac)-**1b** synthesized by *TpCAR* in KPI buffer. The
 496 enantioselectivities were analyzed via HPLC on a chiral stationary phase.

497

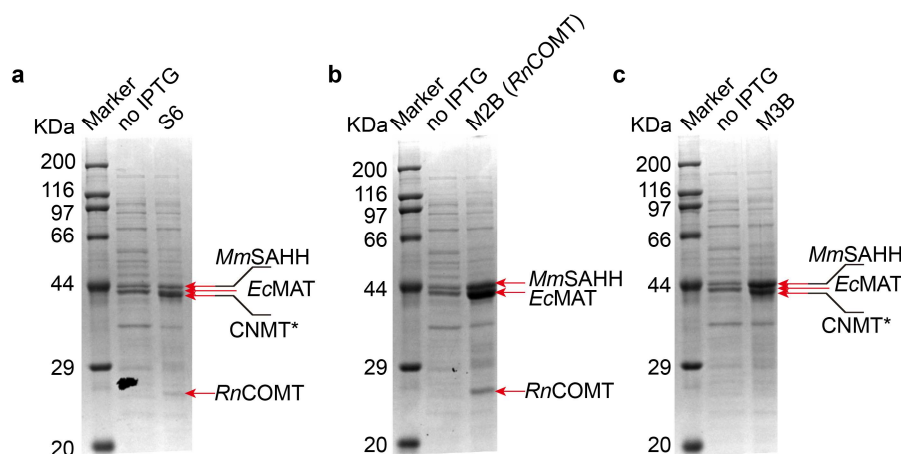


Supplementary Figure 8. Reaction order of *RnCOMT* and *CNMT** towards (*S*)-**1b**. **a** Two proposed reaction pathways from (*S*)-**1b** to (*S*)-**1d**. Pathway A: (*S*)-**1b** was first *O*-methylated by *RnCOMT* and then *N*-methylated by *CNMT**. Pathway B: (*S*)-**1b** was first *N*-methylated by *CNMT** and then *O*-methylated by *RnCOMT*. **b** HPLC analysis of (*S*)-**1b**, (*S*)-**1c** and (*S*)-**1d** in cascade reaction. Method for reaction pathway A: 25 μ M *CNMT**, 2 mM (*S*)-**1b** and 4 mM SAM were incubated in a 200 μ L reaction system for 2 h at 30 $^{\circ}$ C. Next, the above reaction solution was boiled at 95 $^{\circ}$ C for 5 min and centrifuged at 20000 *g* for 5 min. Then, 5 μ M *RnCOMT* was added into the above supernatant to initiate the reaction. Method for reaction pathway B: 5 μ M *RnCOMT*, 2 mM (*S*)-**1b** and 4 mM SAM were incubated in a 200 μ L reaction system for 2 h at 30 $^{\circ}$ C. Next, the above reaction solution was boiled at 95 $^{\circ}$ C for 5 min and centrifuged at 20000 *g* for 5 min. Then, 25 μ M *CNMT** was added into the above supernatant to initiate the reaction. Source data are provided as a Source Data file.

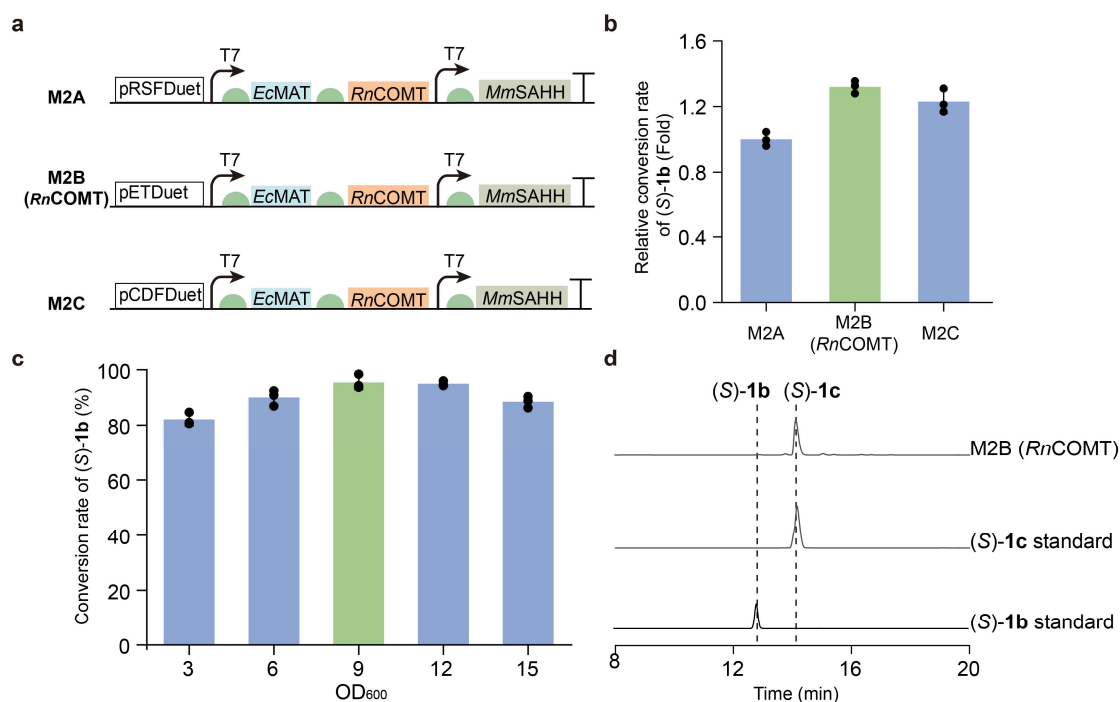


Supplementary Figure 9. Functional verification of the PEIA moiety module in engineered strain IAA (M1G). **a** SDS-PAGE analysis of enzymes of the PEIA moiety module expressed in IAA. M:

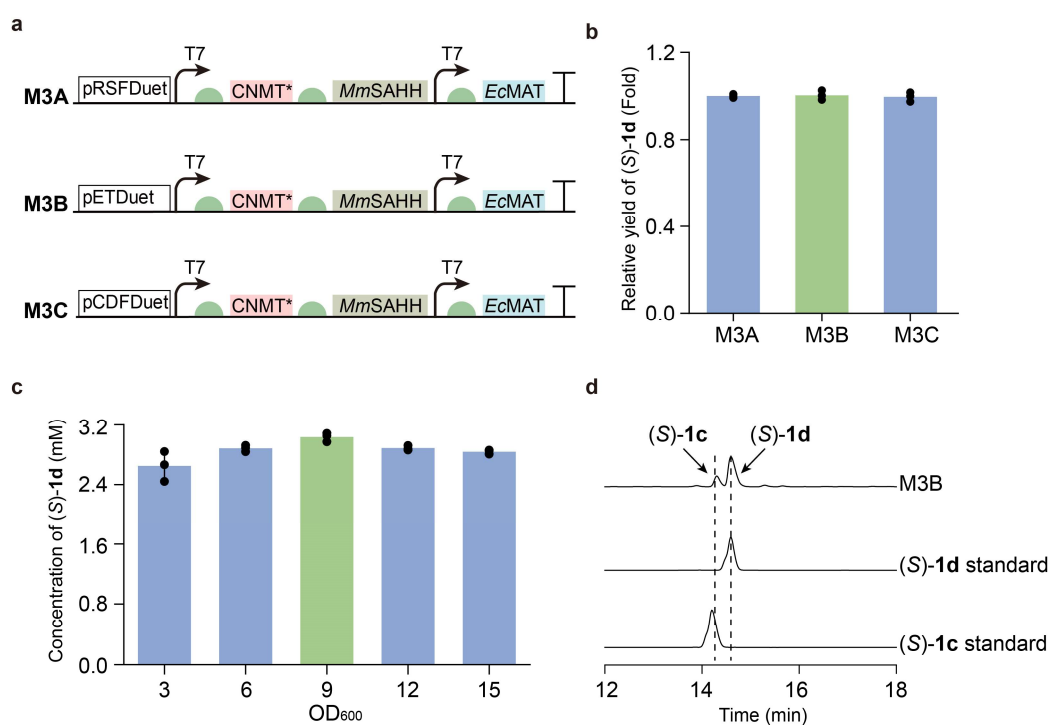
marker; lane 1: no IPTG induction; lane 2: the supernatant of cell lysates expressing the PEIA moiety module in strain M1G induced by IPTG. **b** HPLC analysis of (*S*)-**1b** produced from 5 mM substrate **1** and 5 mM dopamine by strain M1G. **c** Distribution analysis of (*S*)-**1b** in the supernatant and the pellet of strain M1G. Source data are provided as a Source Data file.



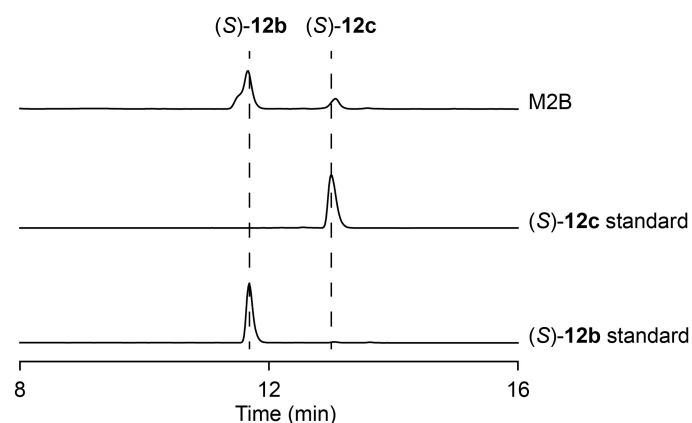
Supplementary Figure 10. SDS-PAGE analysis of protein expression in strain S6, M2B (*RnCOMT*) and M3B. **a** SDS-PAGE analysis of *MmSAHH*, *EcMAT*, *CNMT** and *RnCOMT* expressed in strain S6. **b** SDS-PAGE analysis of *MmSAHH*, *EcMAT* and *RnCOMT* expressed in strain M2B (*RnCOMT*). **c** SDS-PAGE analysis of *MmSAHH*, *EcMAT* and *CNMT** expressed in strain M3B. Source data are provided as a Source Data file.



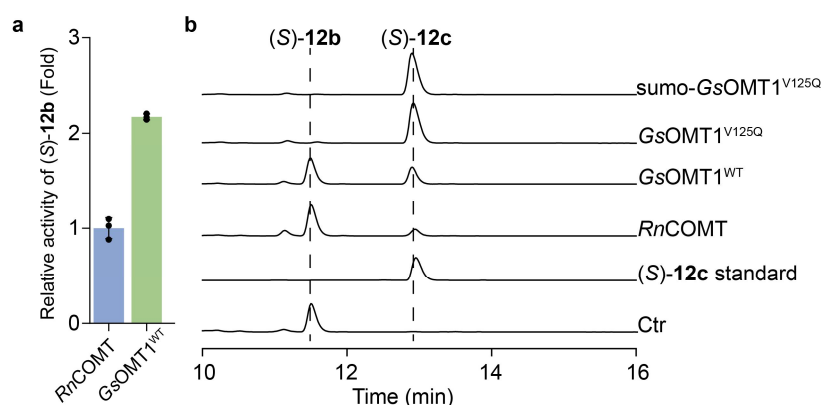
Supplementary Figure 11. Biosynthesis of (*S*)-**1c** in strain IAA containing the OMT module. **a** Construction of three engineered strains using different plasmids to express *RnCOMT*, *EcMAT* and *MmSAHH*. **b** Comparison of the biotransformation efficiency of (*S*)-**1b** using M2A, M2B (*RnCOMT*) and M2C. The conversion rate of (*S*)-**1b** in strain M2A is set as 1. **c** Optimization of the concentration of the strain M2B (*RnCOMT*) to improve the conversion rate of (*S*)-**1b**. **d** HPLC analysis of (*S*)-**1c** synthesized by strain M2B (*RnCOMT*). All data is presented as mean value of three independent experiments and the error bars indicate $\pm$ sd. Source data are provided as a Source Data file.



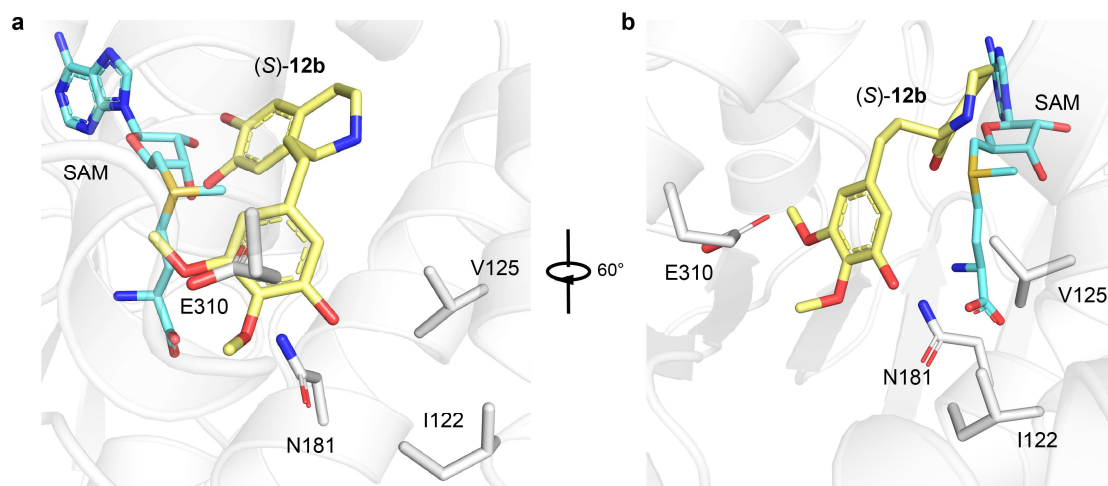
Supplementary Figure 12. Biosynthesis of (*S*)-**1d** in strain IAA containing the NMT module. **a** Construction of three engineered strains using different plasmids to express CNMT*, *EcMAT* and *MmSAHH*. **b** Comparison of the efficiency of the biotransformation of (*S*)-**1c** to (*S*)-**1d** using M3A, M3B and M3C. The yield of (*S*)-**1d** in strain M3A is set as 1. **c** Optimization of the concentration of the strain M3B to improve the titer of (*S*)-**1d**. **d** HPLC analysis of (*S*)-**1d** synthesized by strain M3B. All data is presented as mean value of three independent experiments and the error bars indicate $\pm$ sd. Source data are provided as a Source Data file.



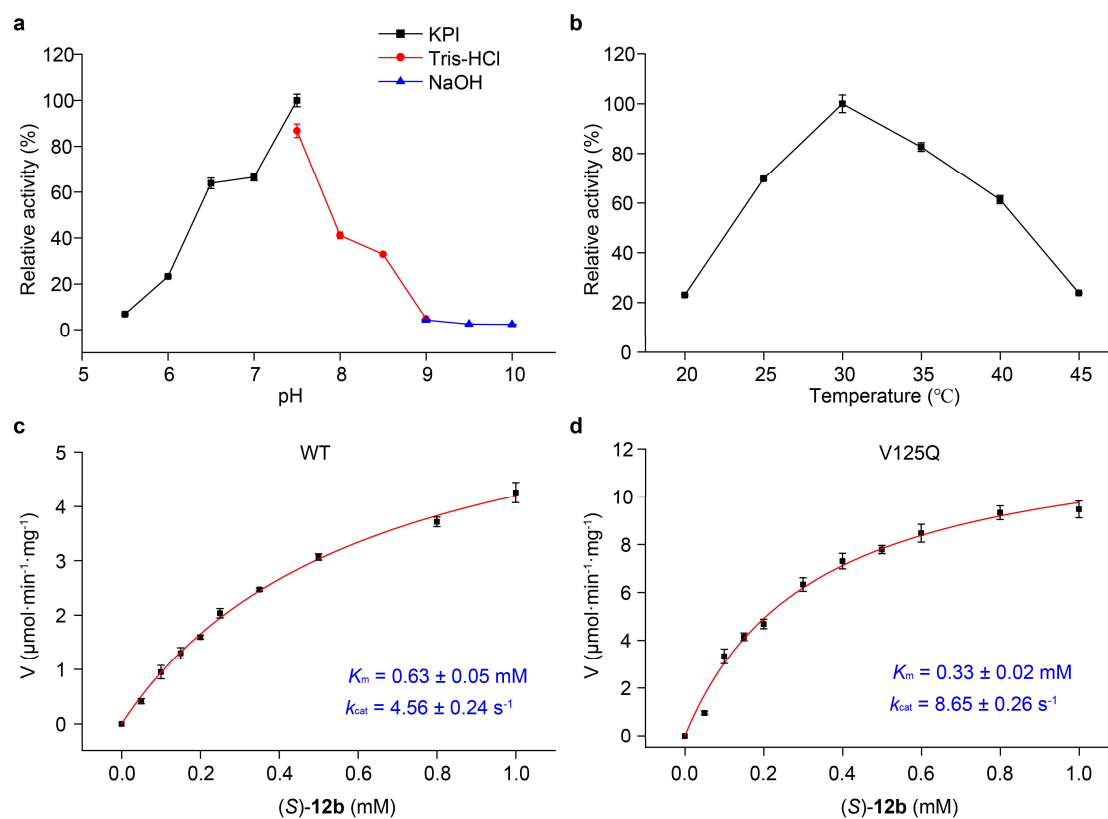
Supplementary Figure 13. HPLC analysis of (S)-12b and (S)-12c in reaction system of strain M2B (*RnCOMT*). Source data are provided as a Source Data file.



Supplementary Figure 14. Screening of *O*-methyltransferases with high catalytic activity towards (S)-12b. **a** Relative activity of *RnCOMT* and *GsOMT1*^{WT} towards (S)-12b. The relative activity was measured by the ratio of the (S)-12c titer of *GsOMT1*^{WT} to that of *RnCOMT*. Green column is the mutant with the highest catalytic activity. **b** HPLC analysis of the catalytic activity of *RnCOMT*, *GsOMT1*^{WT}, *GsOMT1*^{V125Q} and sumo-*GsOMT1*^{V125Q} towards (S)-12b using 1.60 mM (S)-12b, 4 mM SAM and 5 μ M enzymes. Ctr means the reaction system without enzymes. Source data are provided as a Source Data file.

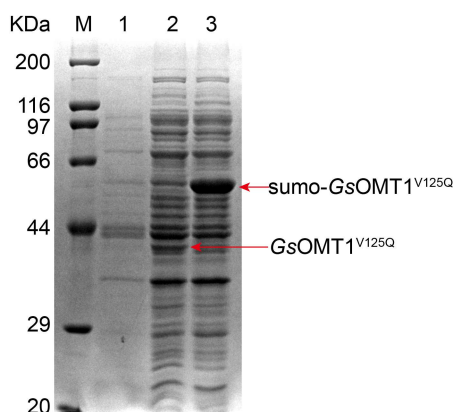


Supplementary Figure 15. Close-up views of the substrate binding pocket of *GsOMT1* for (*S*)-**12b**. (a) Substrate (*S*)-**12b**, SAM and the selected residues with potential interactions with (*S*)-**12b** are colored by yellow, cyan and white, respectively. Oxygen, nitrogen and sulfur atom are indicated by red, blue and yellow colors, respectively. (b) Close-up views of a 60° rotation along the Y-axis of (a).

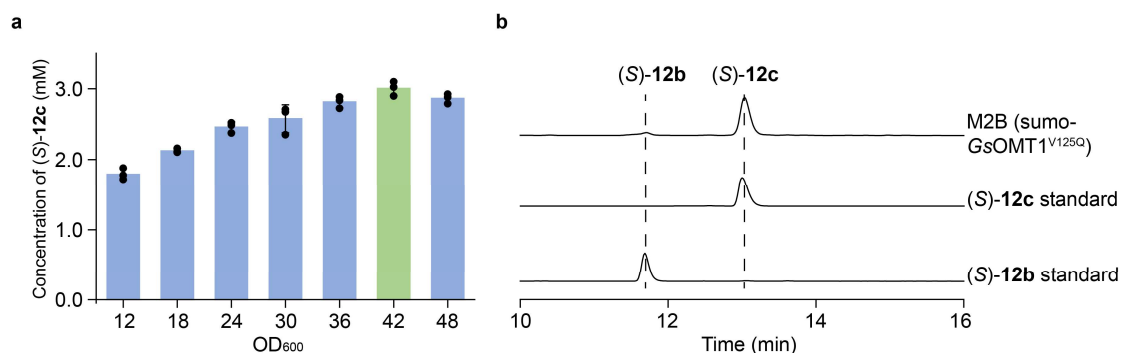


Supplementary Figure 16. The kinetic plots of *GsOMT1*^{WT} and *GsOMT*^{V125Q} towards substrate

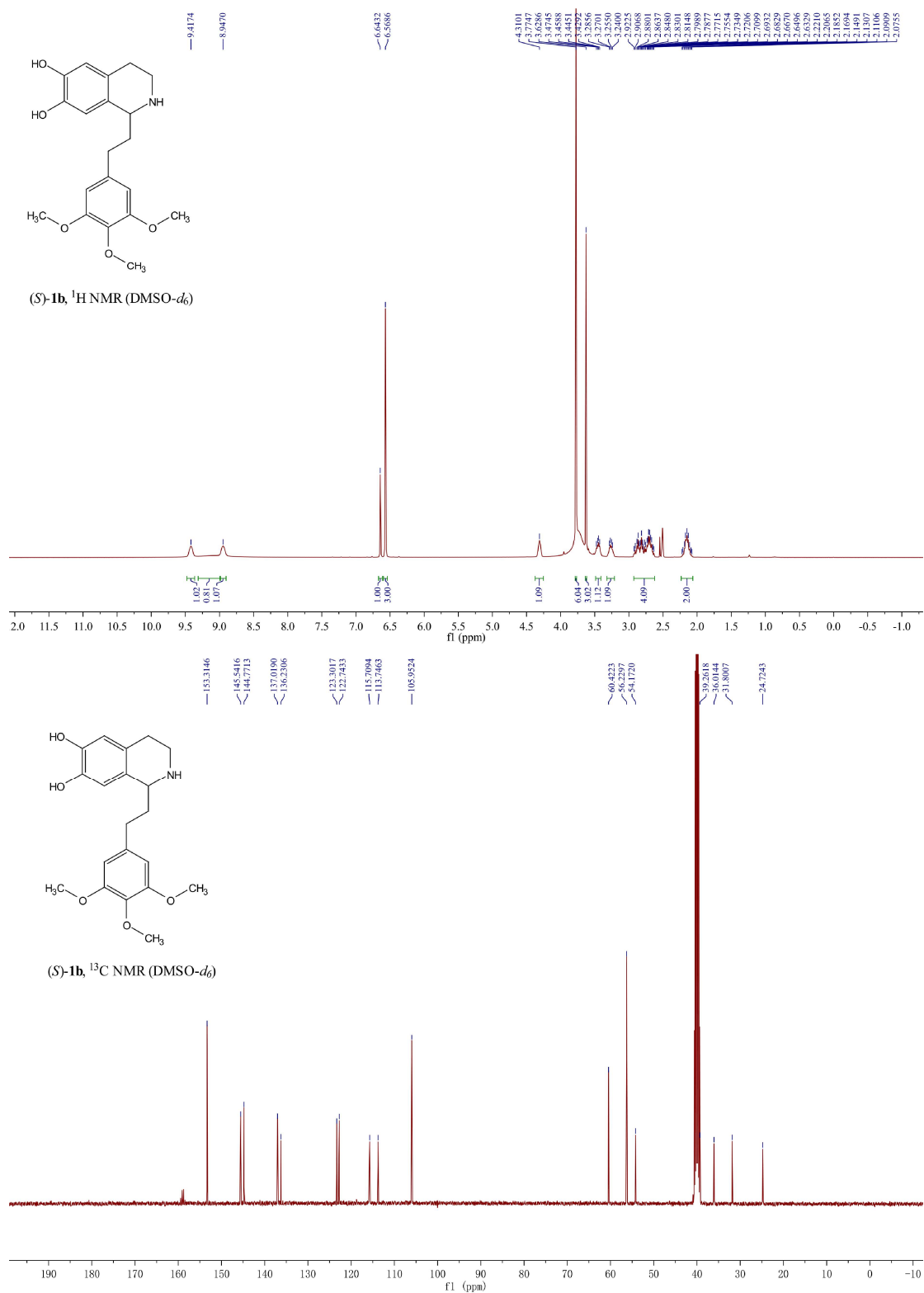
(S)-12b. a Optimization of pH using potassium phosphate buffer (pH 5.5–7.5, 200 mM), Tris-HCl buffer (pH 7.5–9.0, 200 mM) and Glycine-NaOH (pH 9.0–10.0, 200 mM). **b** Optimization of temperature. **c** The kinetic plots of wild-type *GsOMT1*^{WT}. **d** The kinetic plots of *GsOMT1*^{V125Q}. All data is presented as mean value of three independent experiments and the error bars indicate $\pm$ sd. Source data are provided as a Source Data file.



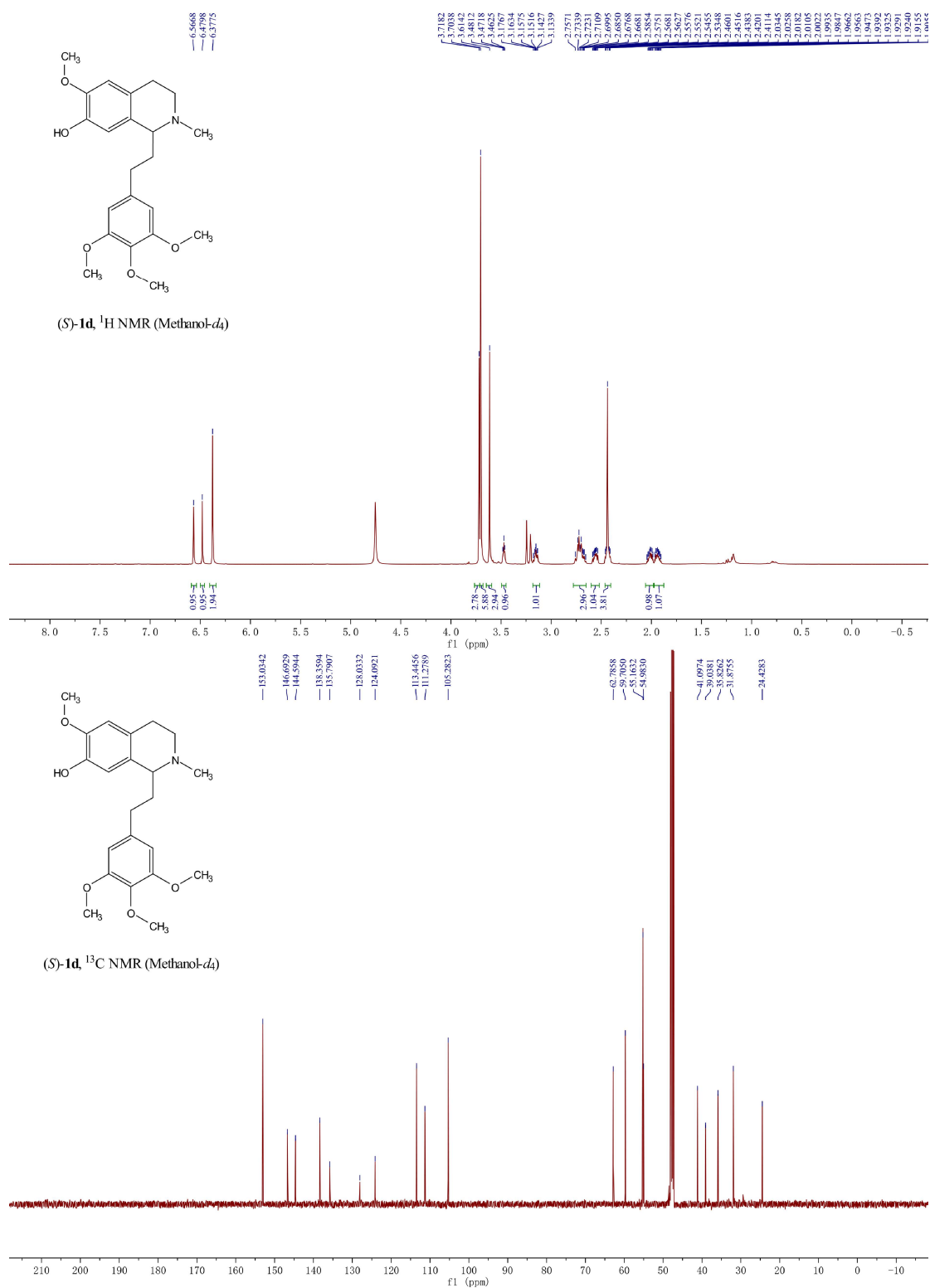
Supplementary Figure 17. SDS-PAGE analysis of *GsOMT1*^{V125Q} and sumo-*GsOMT1*^{V125Q} expressed in *E. coli* BL21 (*DE3*). M: Marker; lane 1: no IPTG induction; lane 2: the supernatant of cell lysates expressing *GsOMT1*^{V125Q} induced by IPTG; lane 3: the supernatant of cell lysates expressing sumo-*GsOMT1*^{V125Q} induced by IPTG. Source data are provided as a Source Data file.



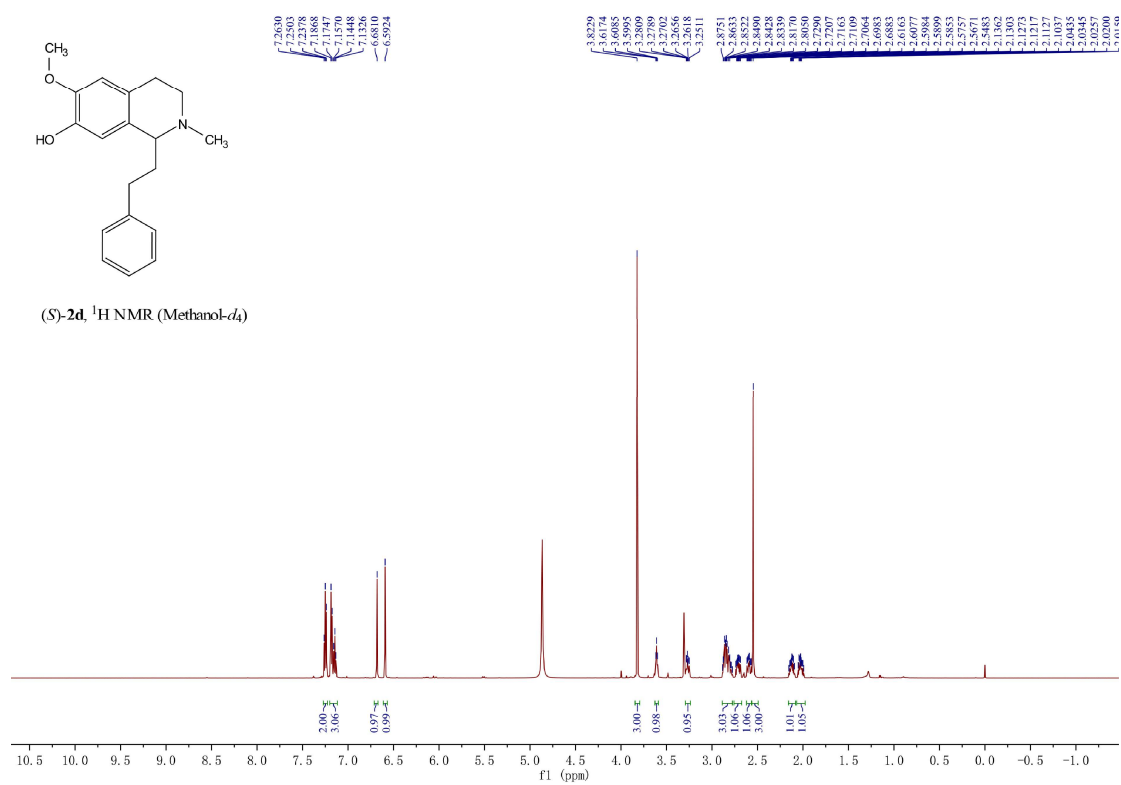
Supplementary Figure 18. Biosynthesis of (S)-12c in strain M2B (sumo-*GsOMT1*^{V125Q}). **a** optimizing the OD₆₀₀ of strain M2B (sumo-*GsOMT1*^{V125Q}) to improve the titer of (S)-12c. **b** HPLC analysis of (S)-12c synthesized by strain M2B (sumo-*GsOMT1*^{V125Q}). All data is presented as mean value of three independent experiments and the error bars indicate $\pm$ sd. Source data are provided as a Source Data file.



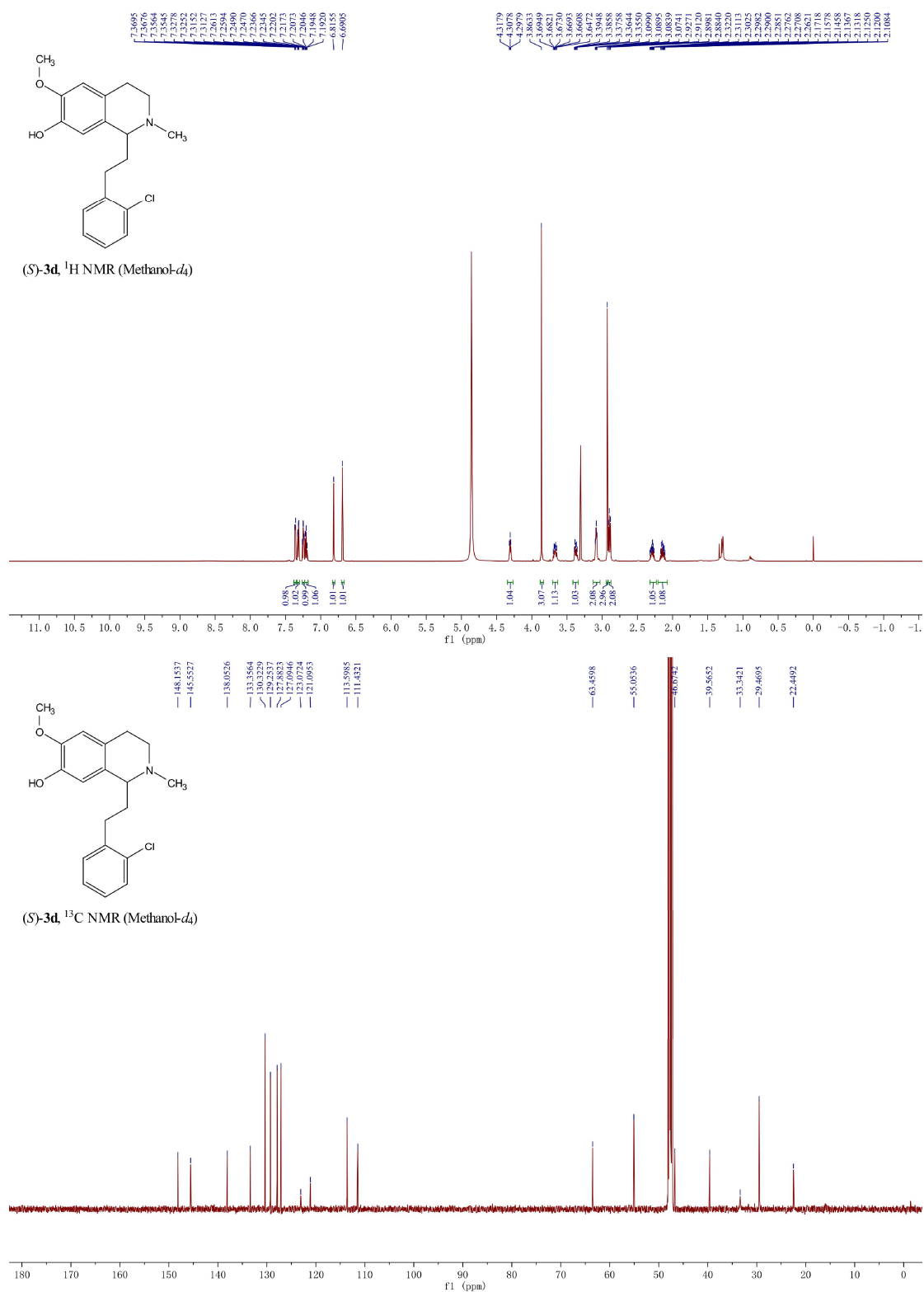
Supplementary Figure 19. ¹H NMR and ¹³C NMR spectra of (S)-1b.



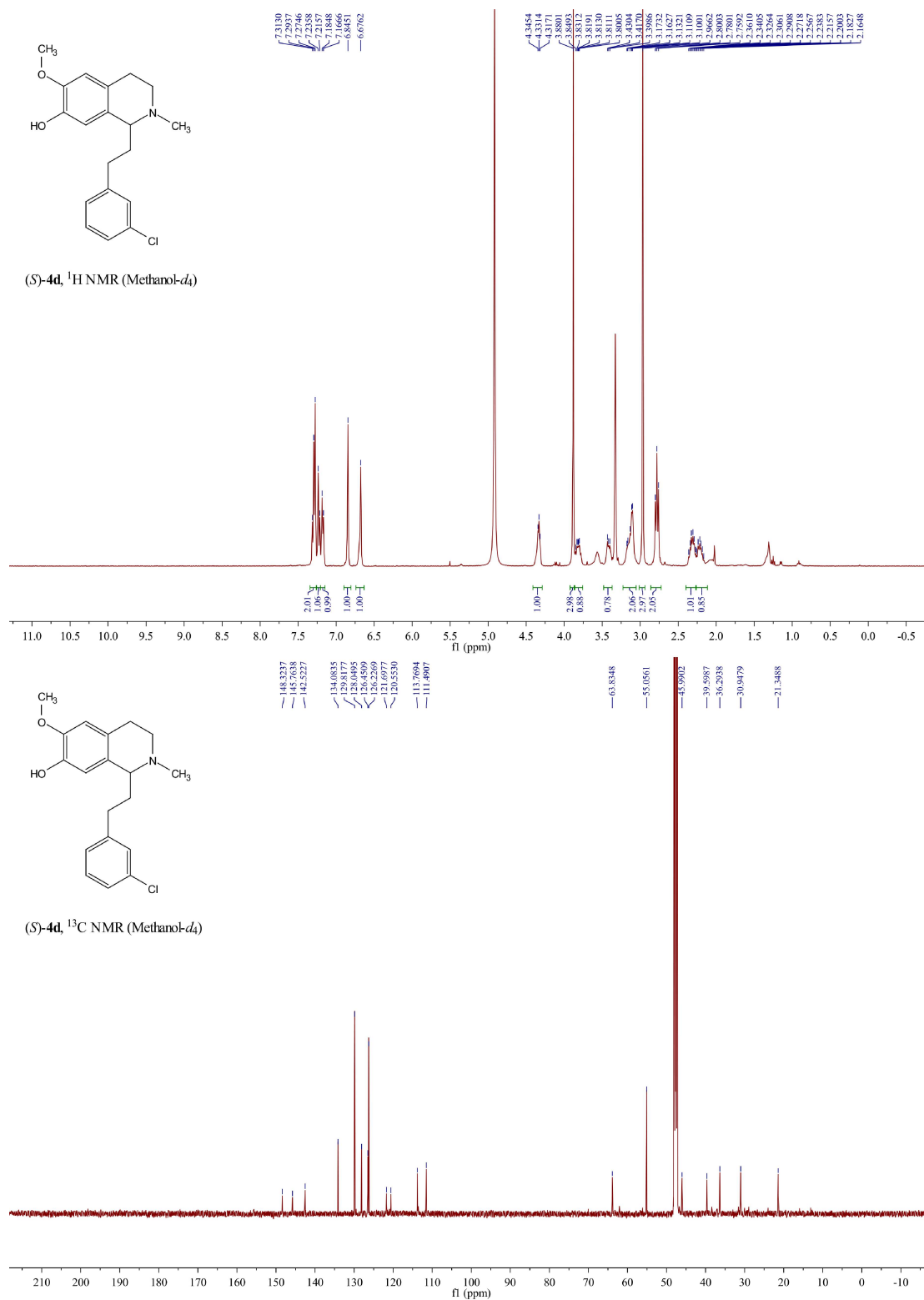
Supplementary Figure 20. ¹H NMR and ¹³C NMR spectra of (S)-1d.



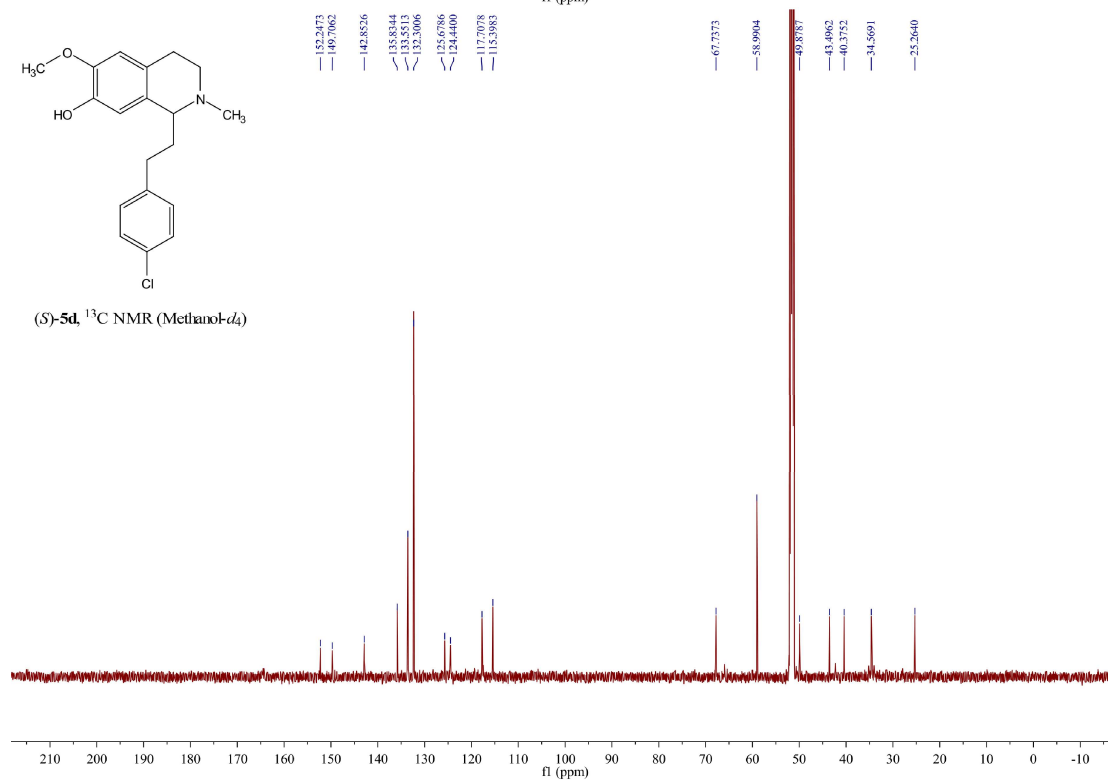
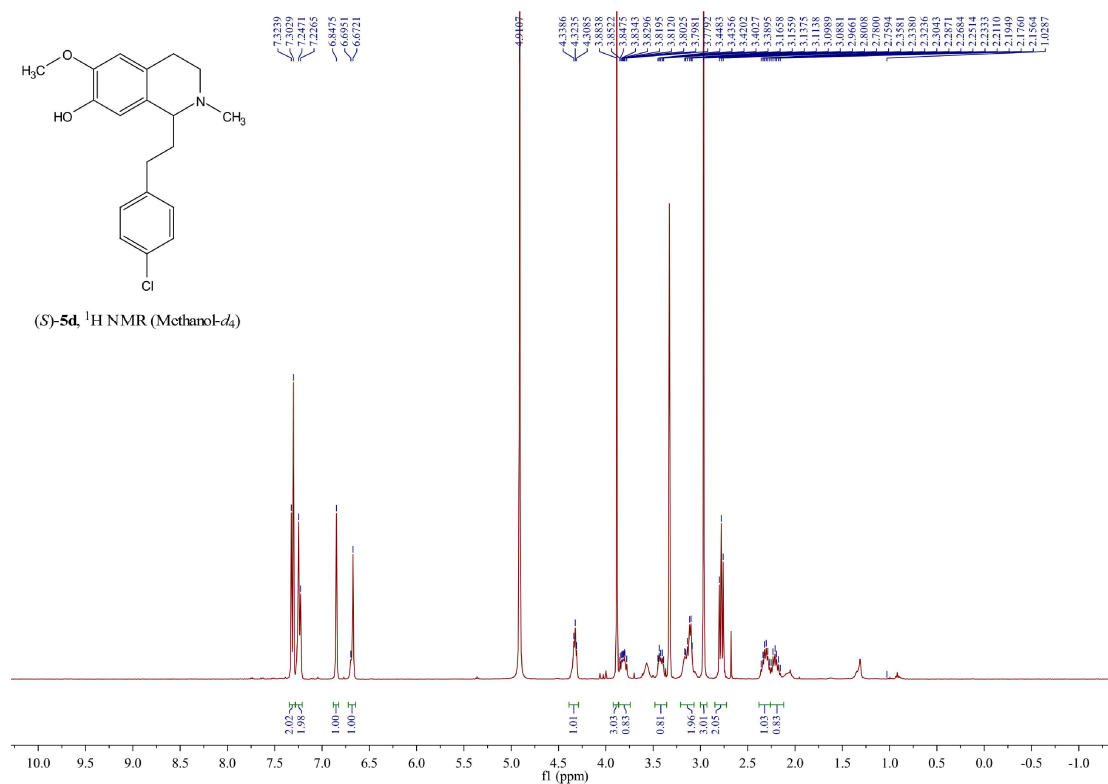
Supplementary Figure 21. ¹H NMR spectra of (S)-2d.

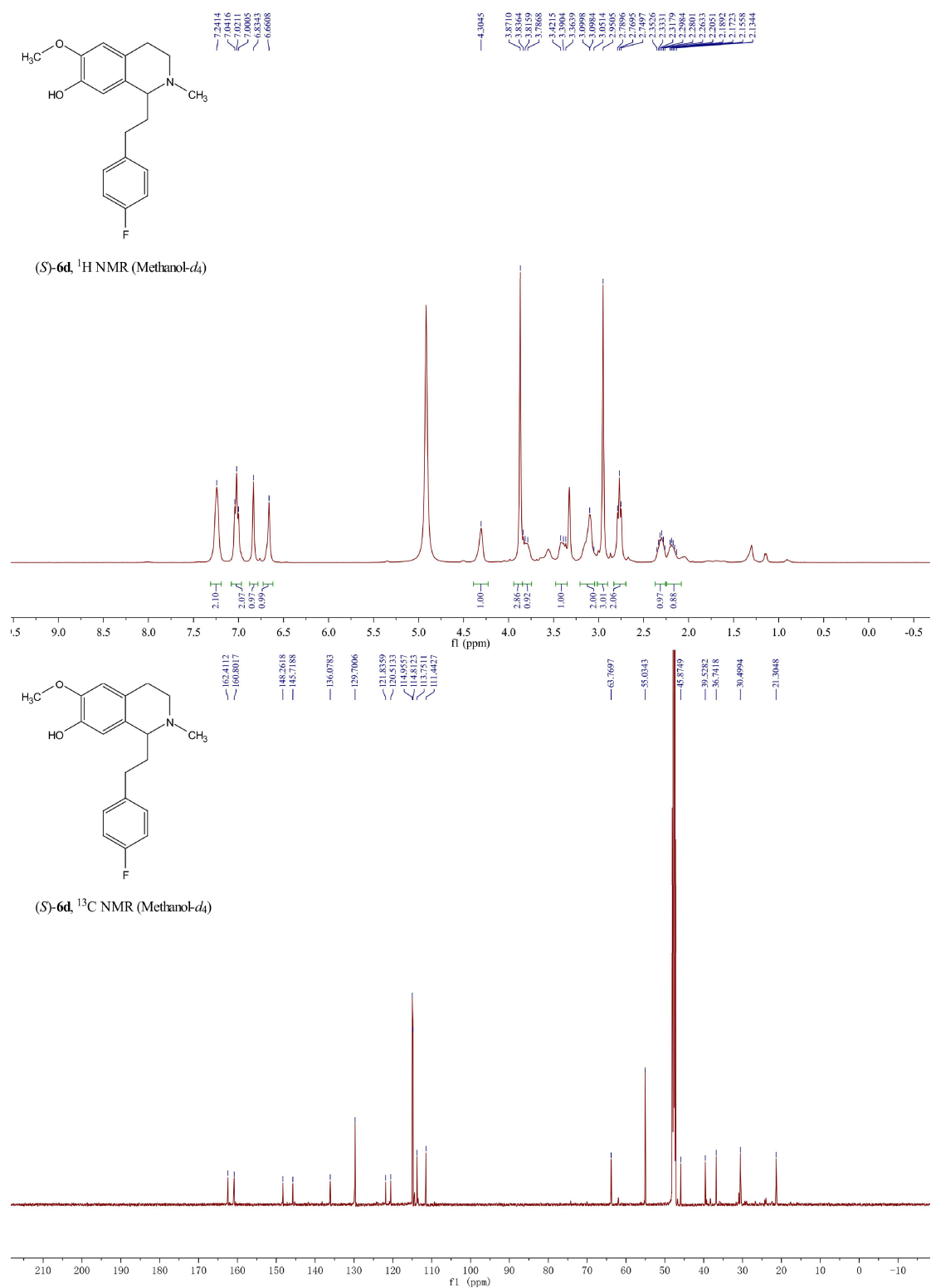


Supplementary Figure 22. ¹H NMR and ¹³C NMR spectra of (S)-3d.

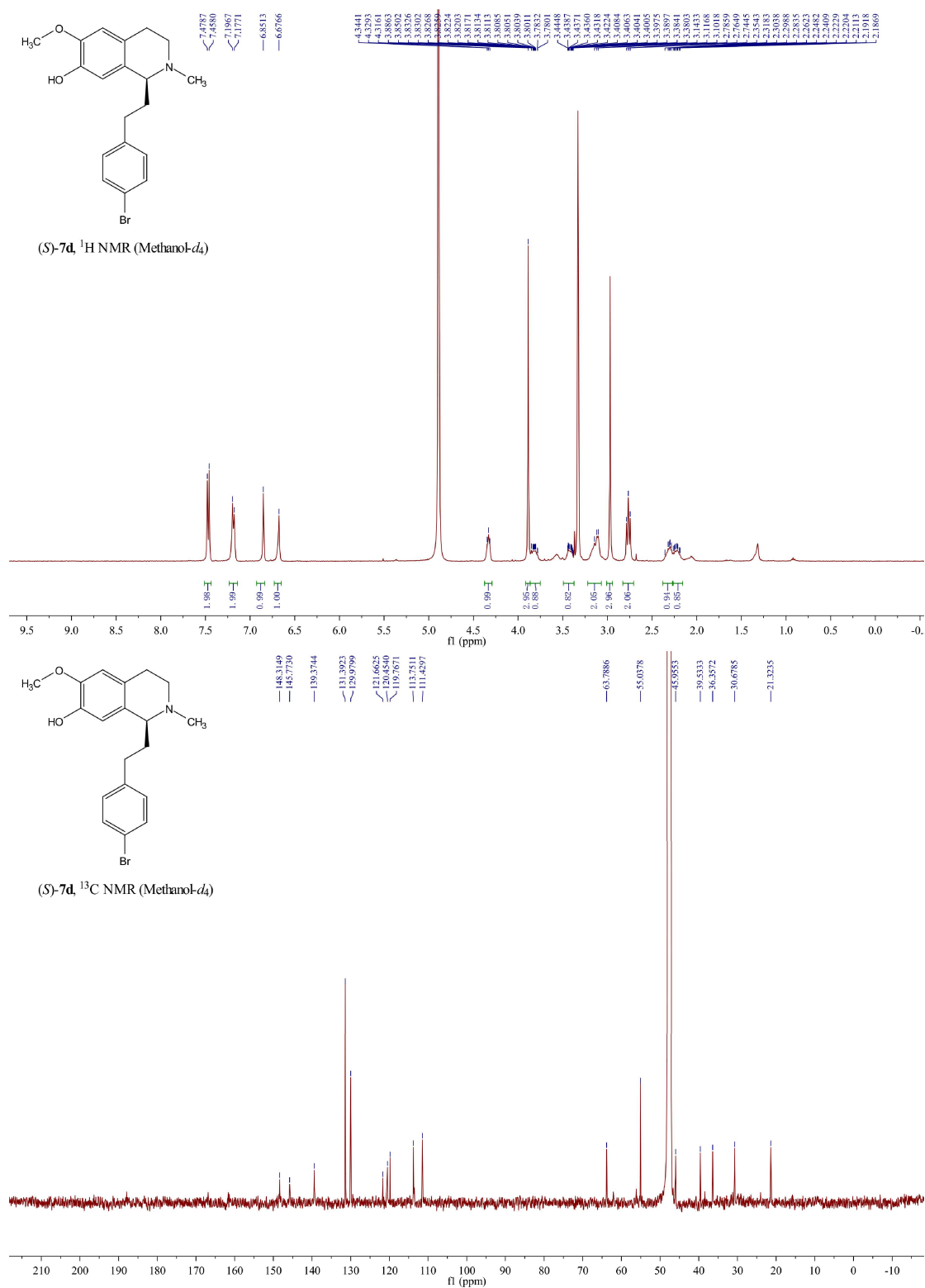


Supplementary Figure 23. ¹H NMR and ¹³C NMR spectra of (S)-4d.

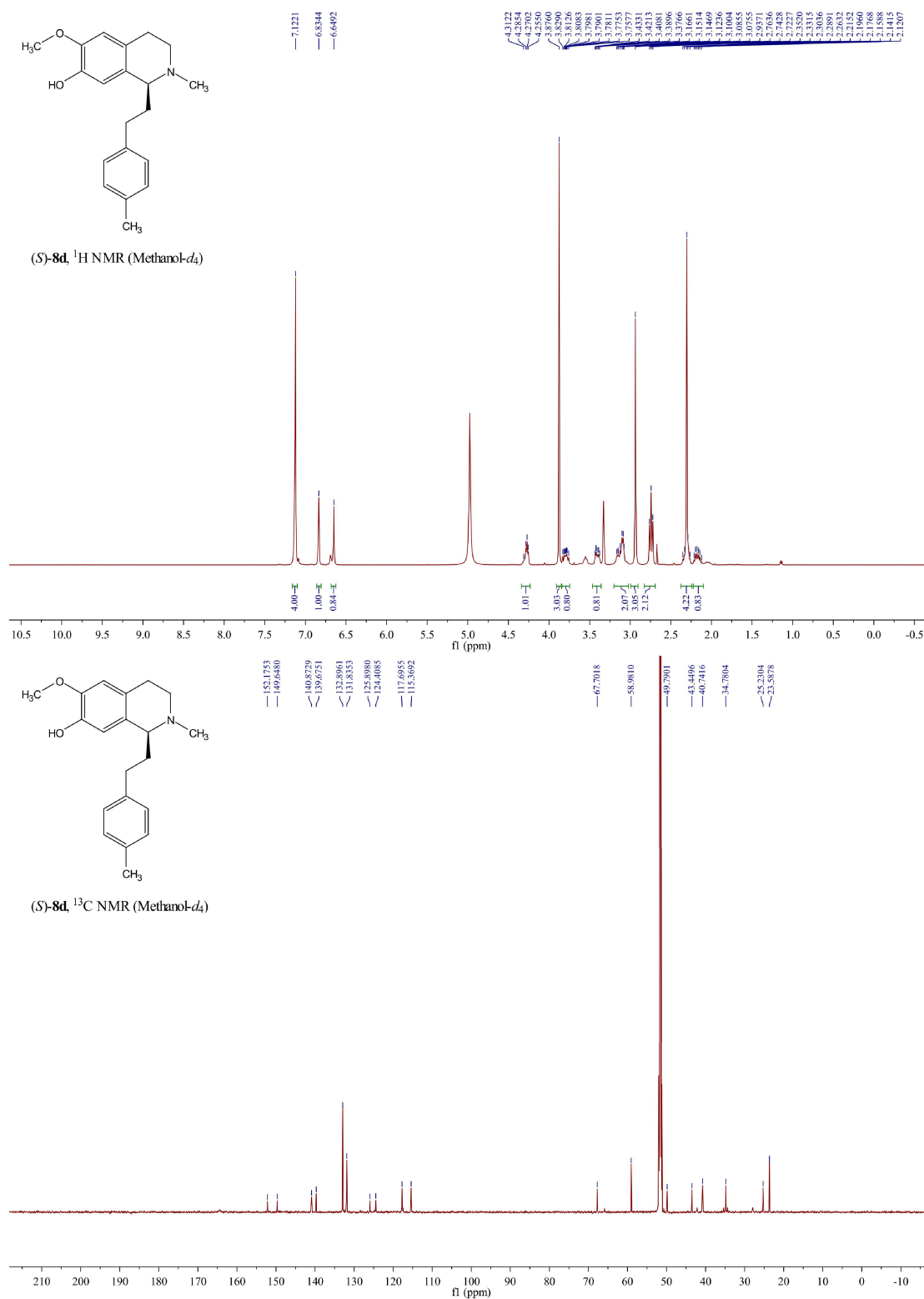




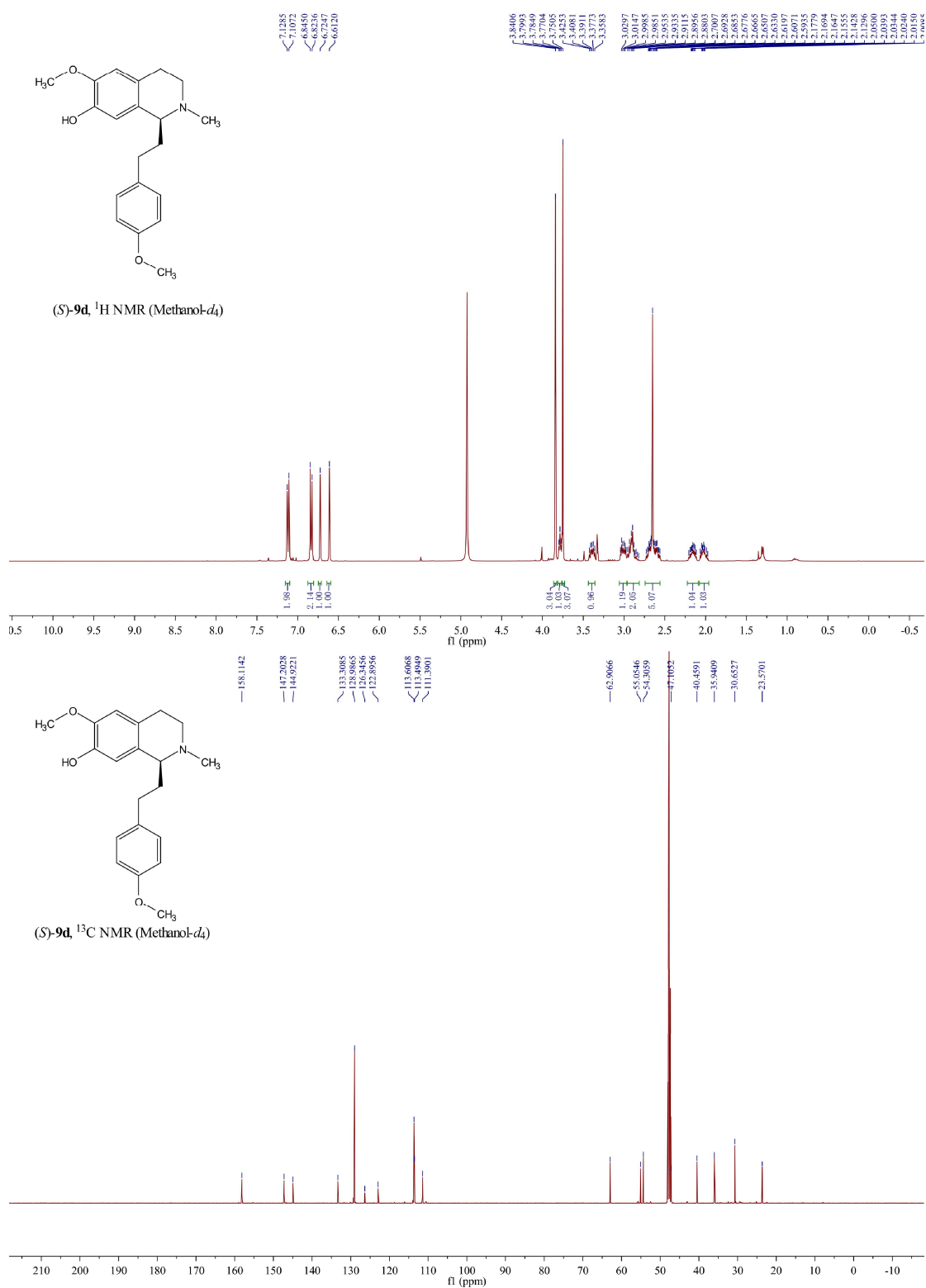
Supplementary Figure 25. ^1H NMR and ^{13}C NMR spectra of (S)-6d.



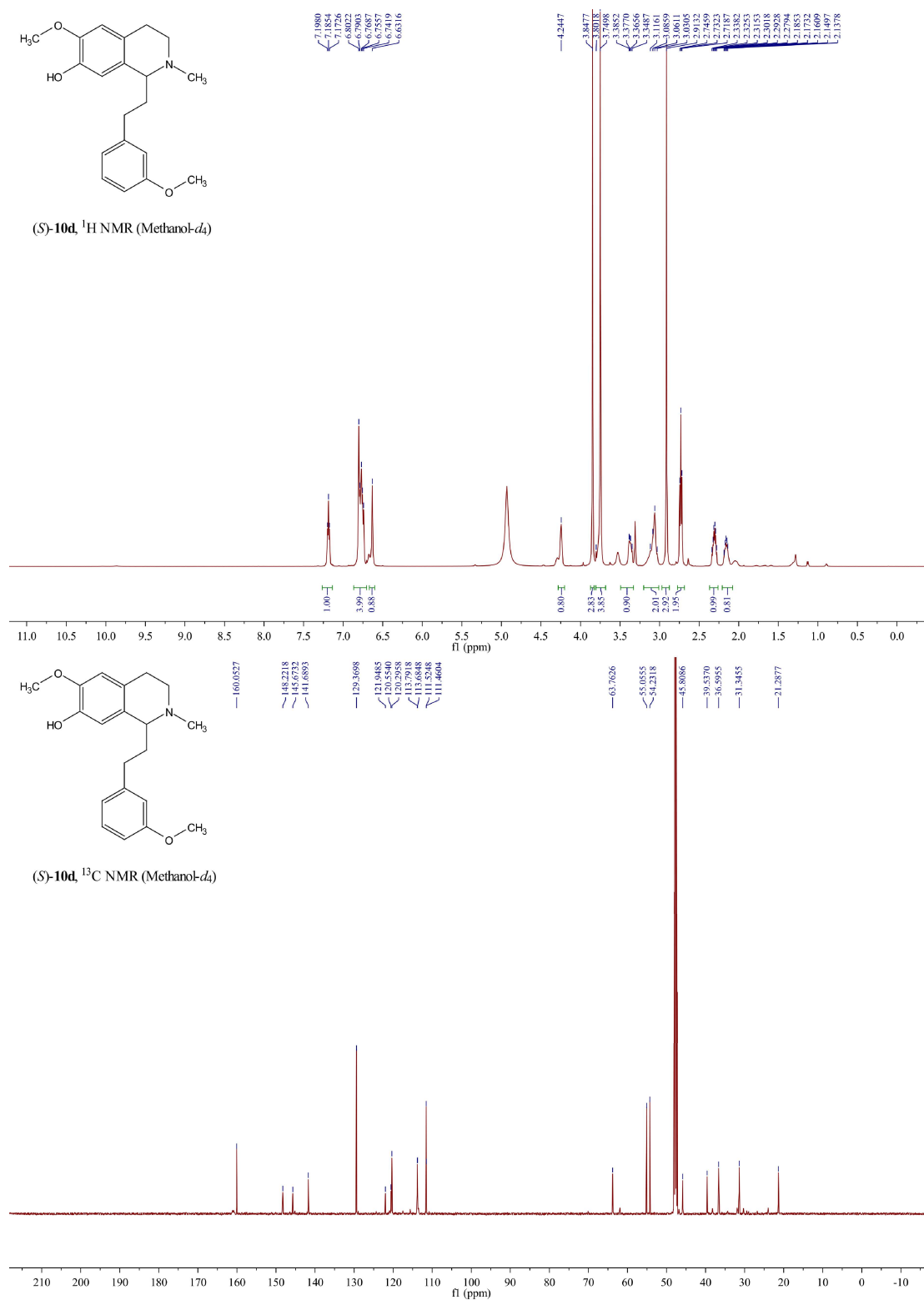
Supplementary Figure 26. ^1H NMR and ^{13}C NMR spectra of (S)-7d.



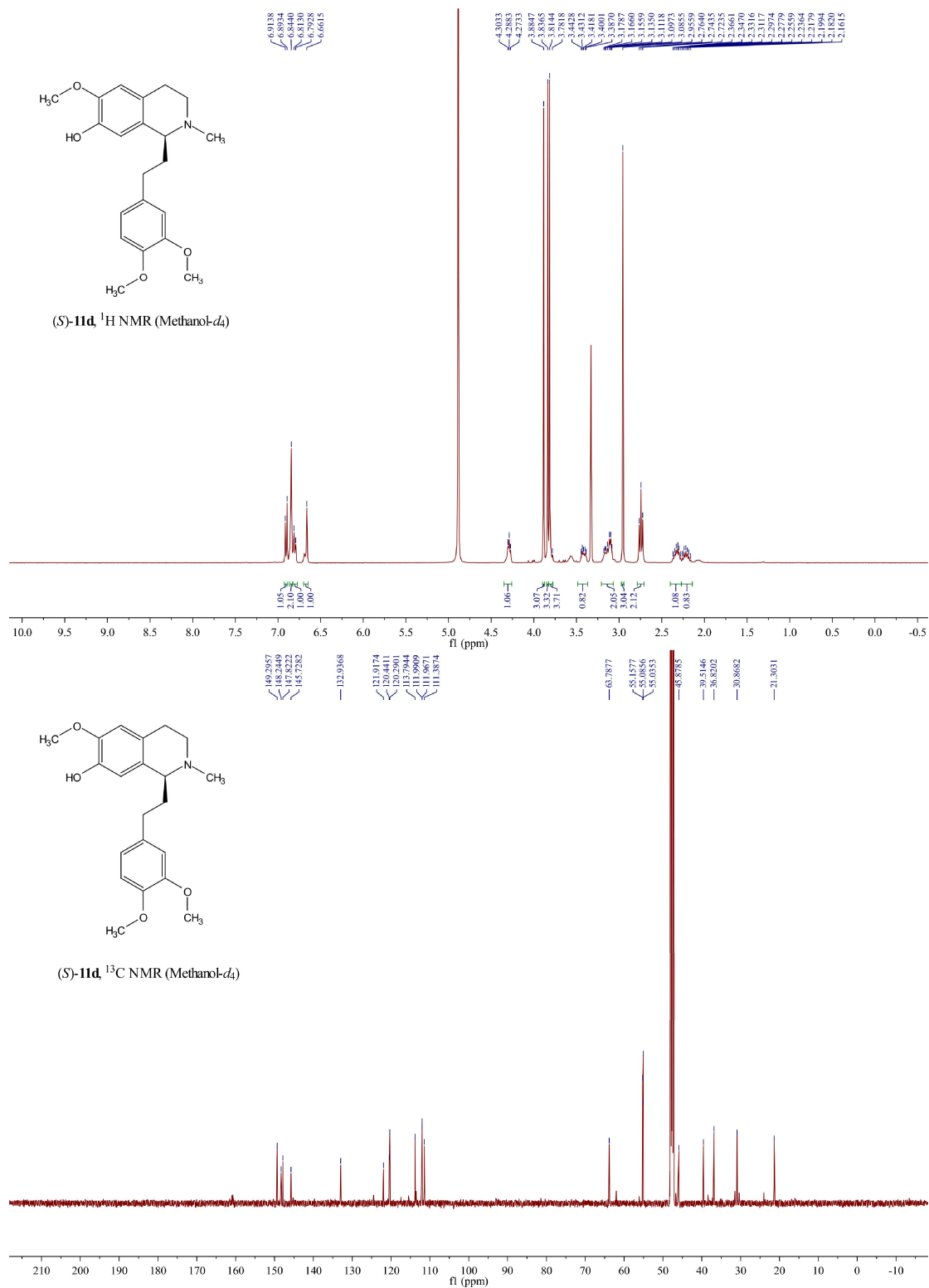
Supplementary Figure 27. ¹H NMR and ¹³C NMR spectra of (S)-8d.



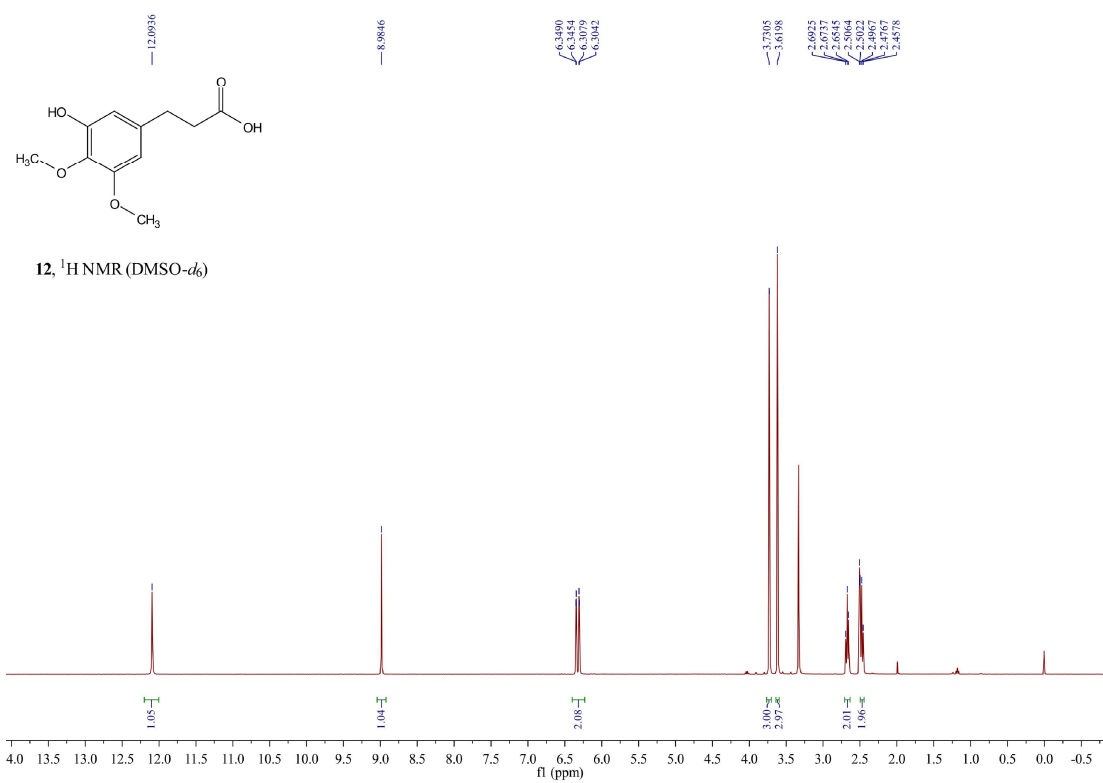
Supplementary Figure 28. ¹H NMR and ¹³C NMR spectra of (S)-9d.



Supplementary Figure 29. ^1H NMR and ^{13}C NMR spectra of (S)-10d.

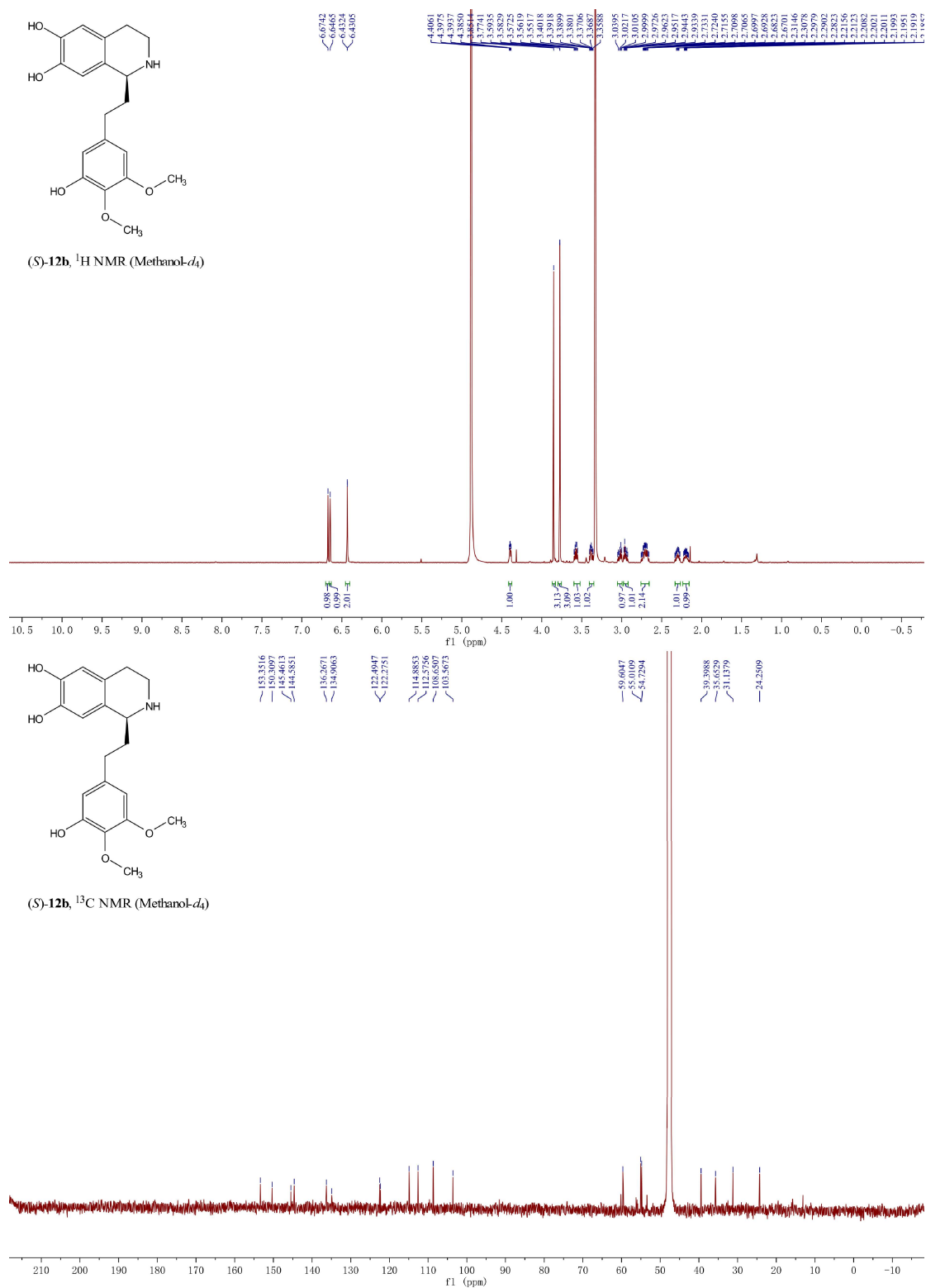


Supplementary Figure 30. ¹H NMR and ¹³C NMR spectra of (S)-11d.

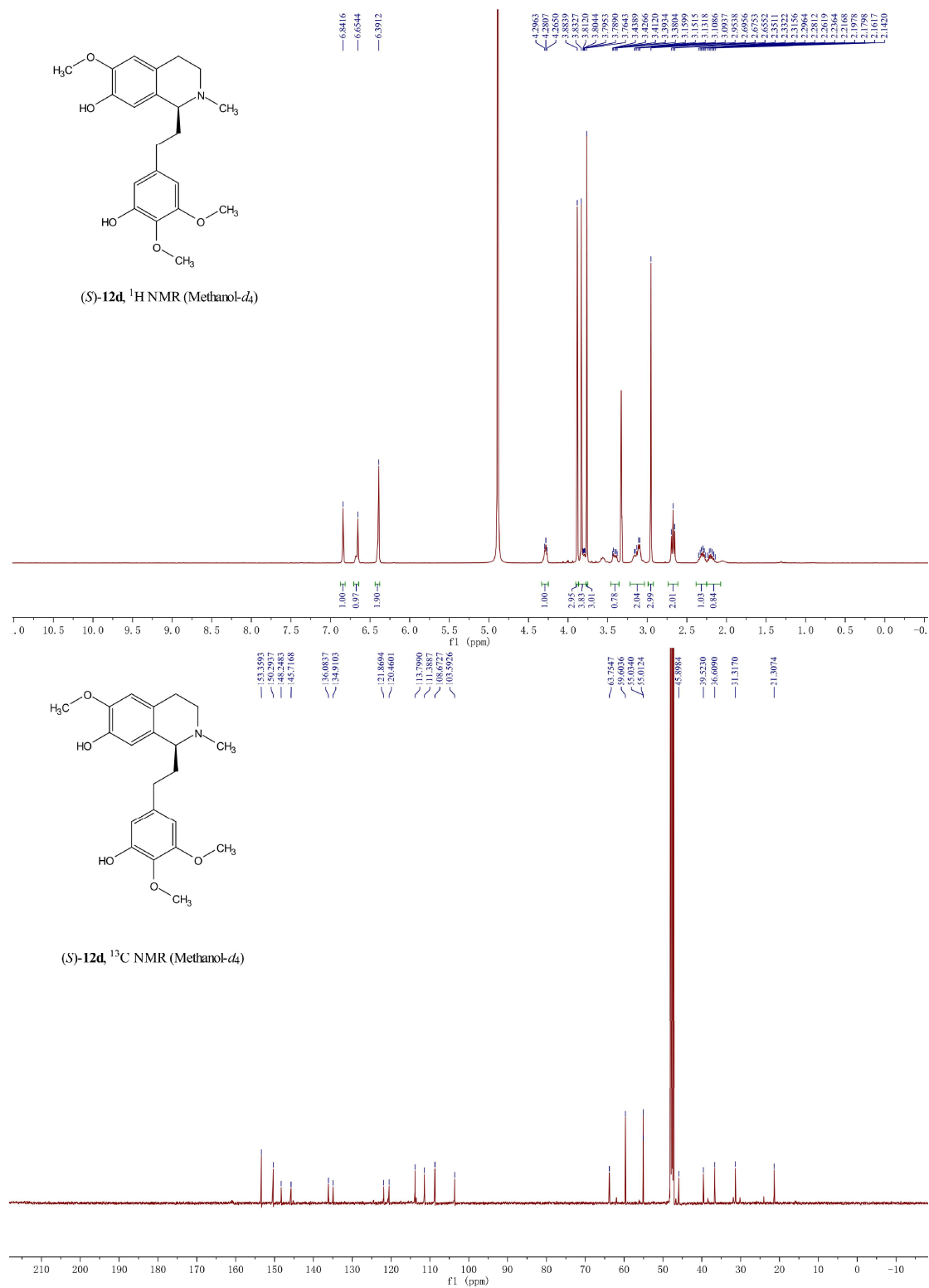


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625 **Supplementary Figure 31.** ^1H NMR spectra of **12**.

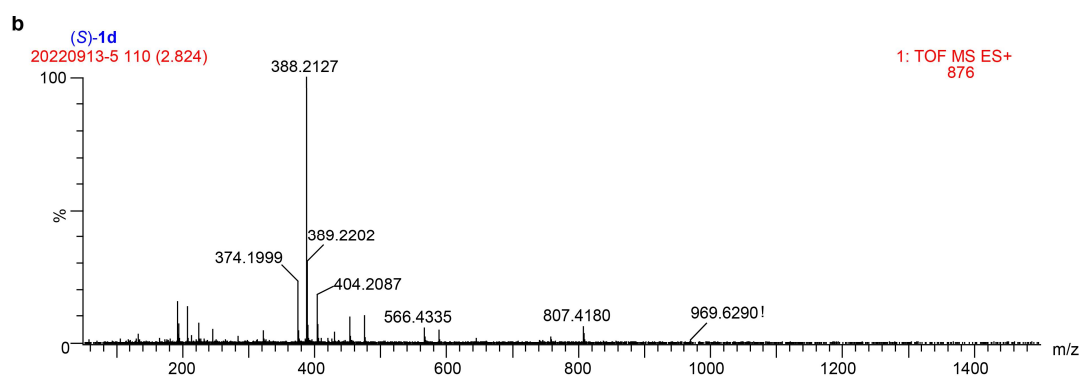
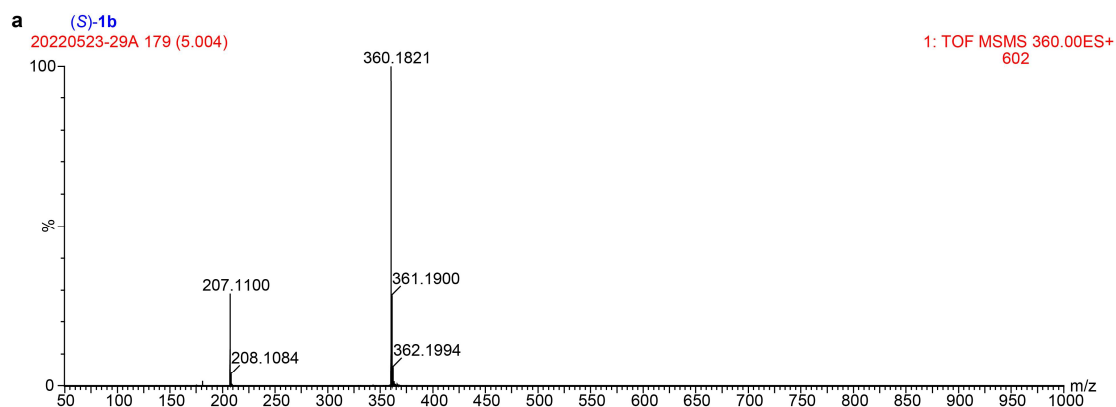


Supplementary Figure 32. ^1H NMR and ^{13}C NMR spectra of (S)-12b.

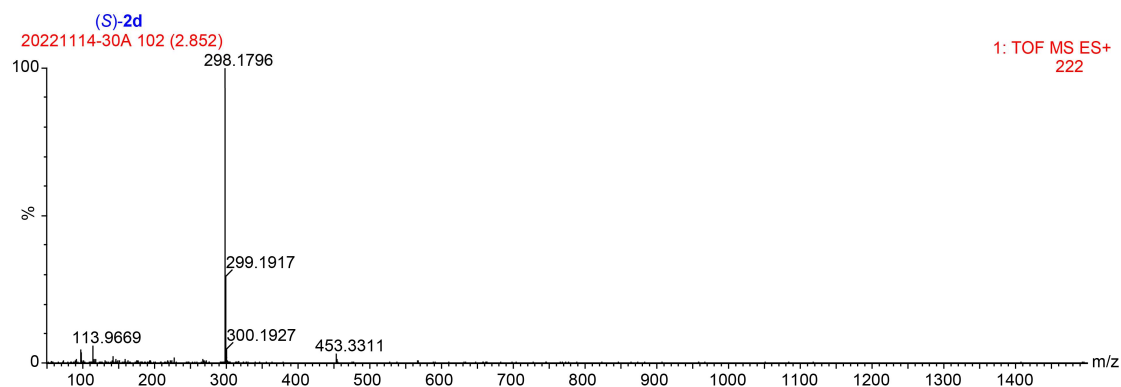


630

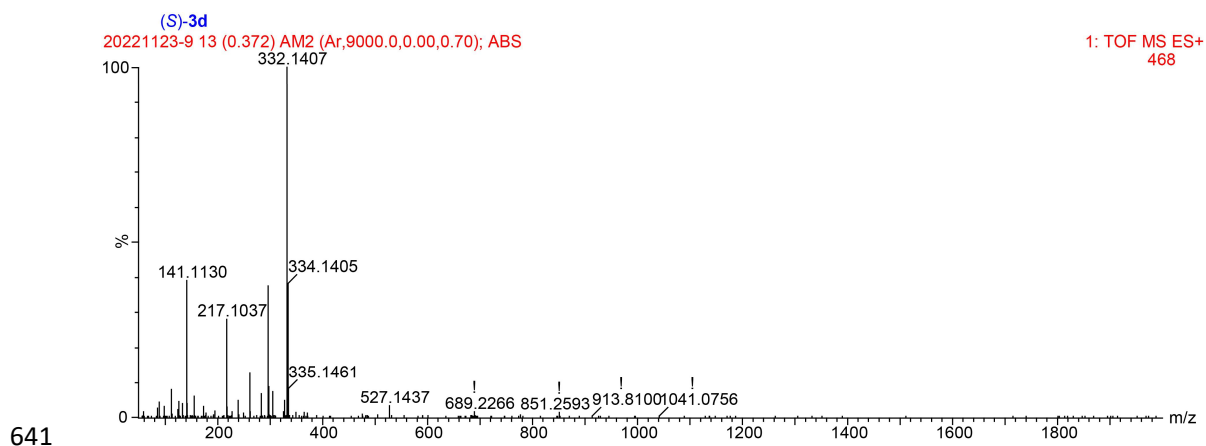
631 **Supplementary Figure 33.** ^1H NMR and ^{13}C NMR spectra of (S)-12d.



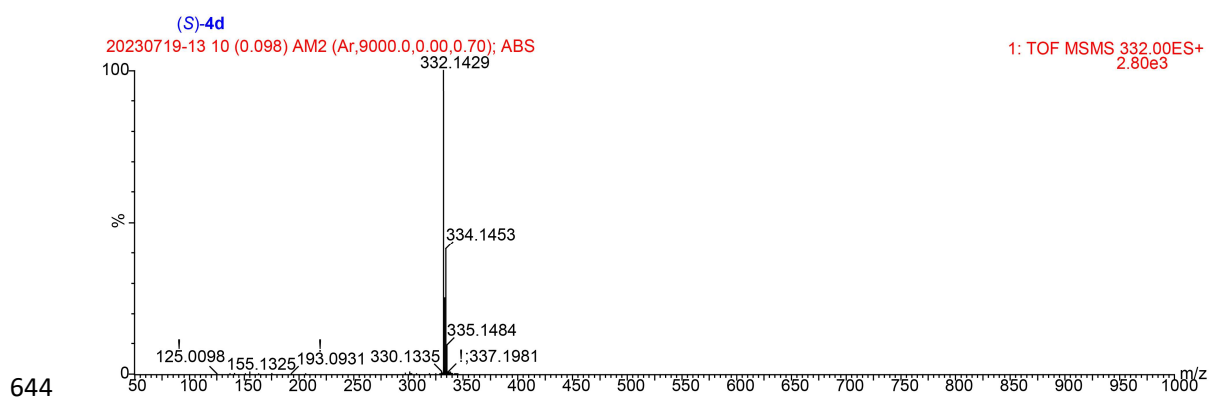
Supplementary Figure 34. HRMS spectra of (S)-1b and (S)-1d. **a** MS spectrum (positive) of (S)-1b showed m/z 360.1821, $[M+H]^+$ calcd. for $C_{20}H_{26}NO_5$, 360.1805. **b** MS spectrum (positive) of (S)-1d showed m/z 388.2127, $[M+H]^+$ calcd. for $C_{22}H_{30}NO_5$, 388.2118.



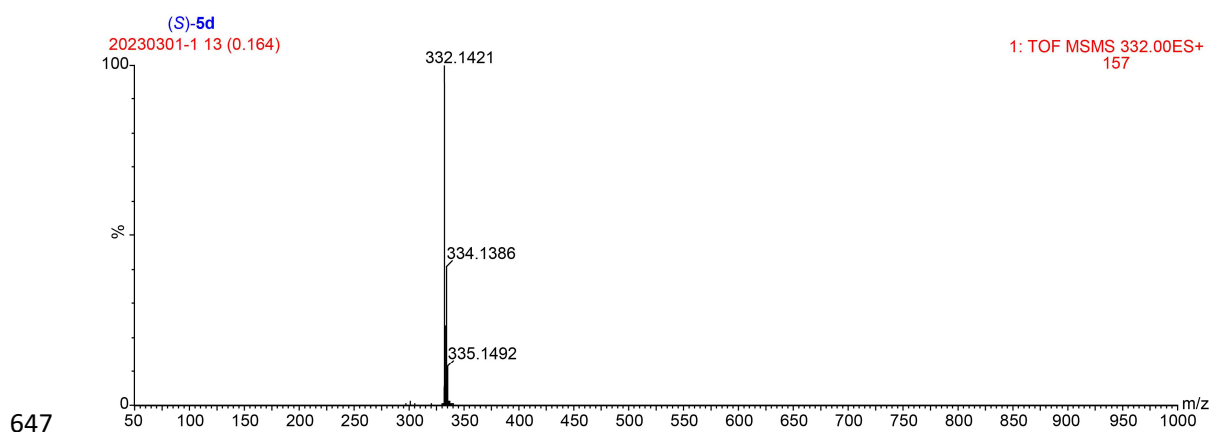
Supplementary Figure 35. HRMS spectra of (S)-2d. MS spectrum (positive) of (S)-2d showed m/z 298.1796, $[M+H]^+$ calcd. for $C_{19}H_{24}NO_2$, 298.1802.



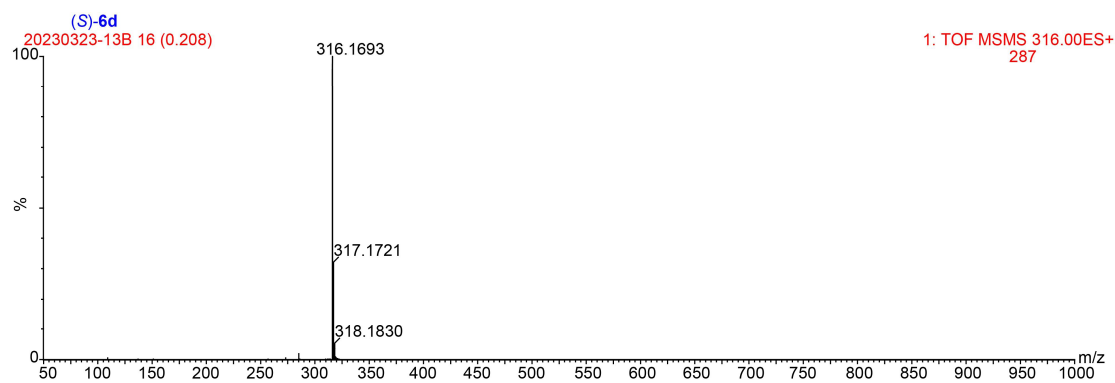
Supplementary Figure 36. HRMS spectra of (S)-3d. MS spectrum (positive) of (S)-3d showed m/z 332.1407, $[M+H]^+$ calcd. for $C_{19}H_{23}ClNO_2$, 332.1412.



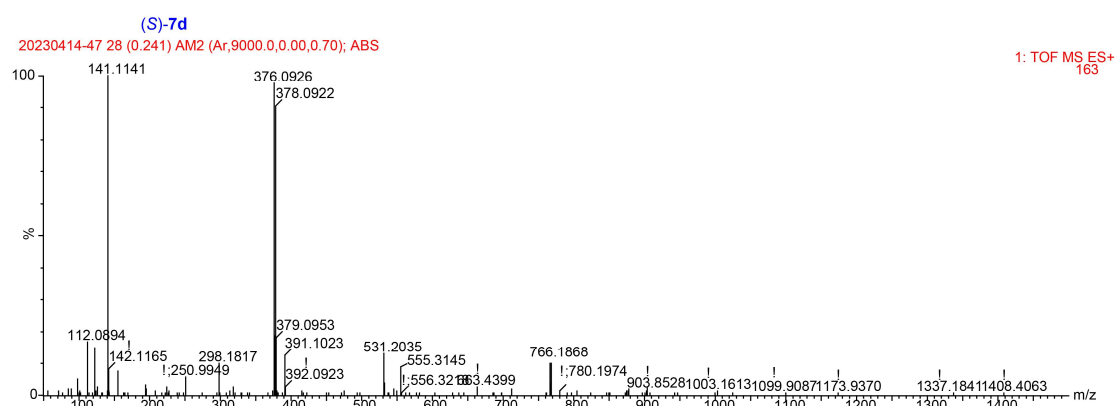
Supplementary Figure 37. HRMS spectra of (S)-4d. MS spectrum (positive) of (S)-4d showed m/z 332.1429, $[M+H]^+$ calcd. for $C_{19}H_{23}ClNO_2$, 332.1412.



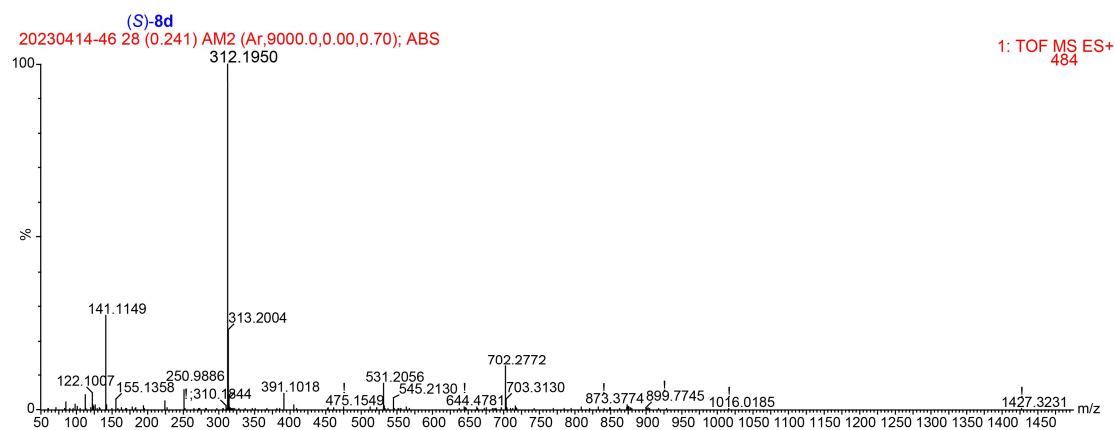
Supplementary Figure 38. HRMS spectra of (S)-5d. MS spectrum (positive) of (S)-5d showed m/z 332.1421, $[M+H]^+$ calcd. for $C_{19}H_{23}ClNO_2$, 332.1412.



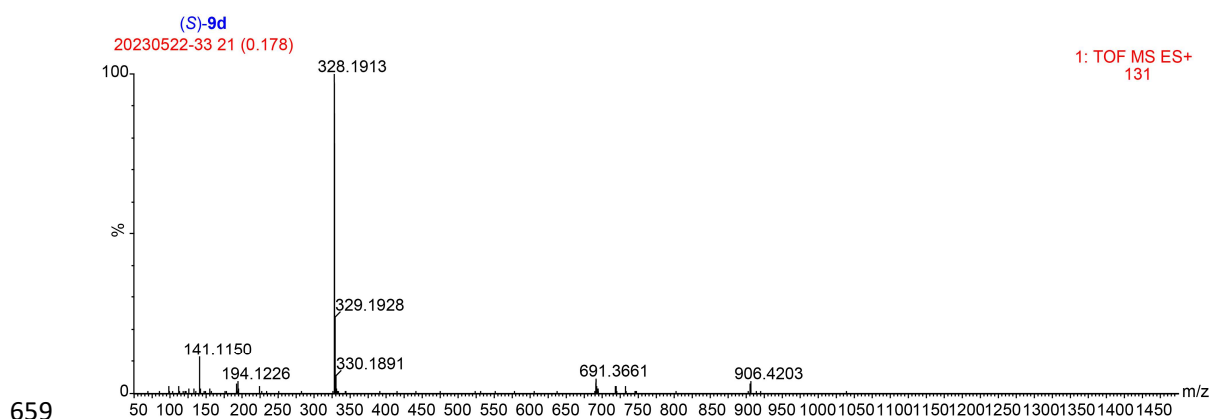
651 **Supplementary Figure 39.** HRMS spectra of (S)-6d. MS spectrum (positive) of (S)-6d showed m/z
 652 316.1693, $[M+H]^+$ calcd. for $C_{19}H_{23}FNO_2$, 316.1707.



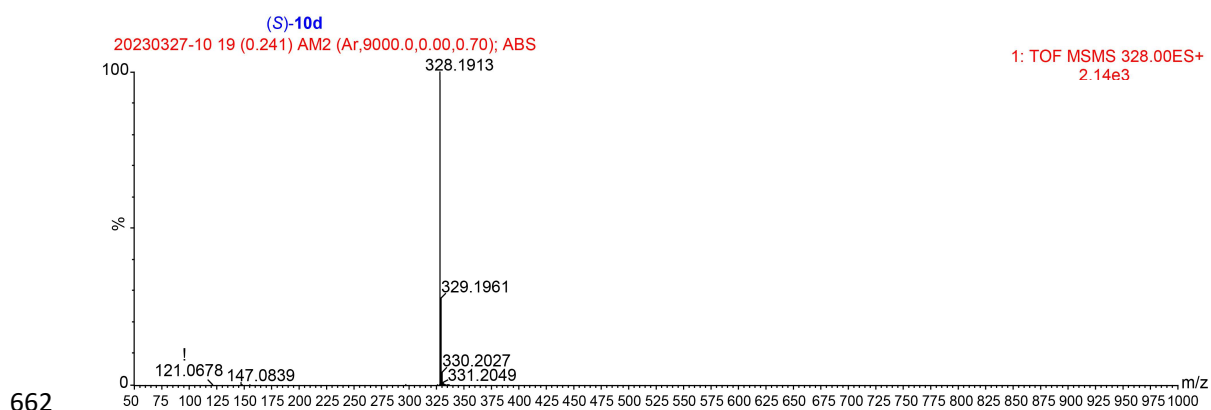
654 **Supplementary Figure 40.** HRMS spectra of (S)-7d. MS spectrum (positive) of (S)-7d showed m/z
 655 376.0926, $[M+H]^+$ calcd. for $C_{19}H_{23}BrNO_2$, 376.0907.



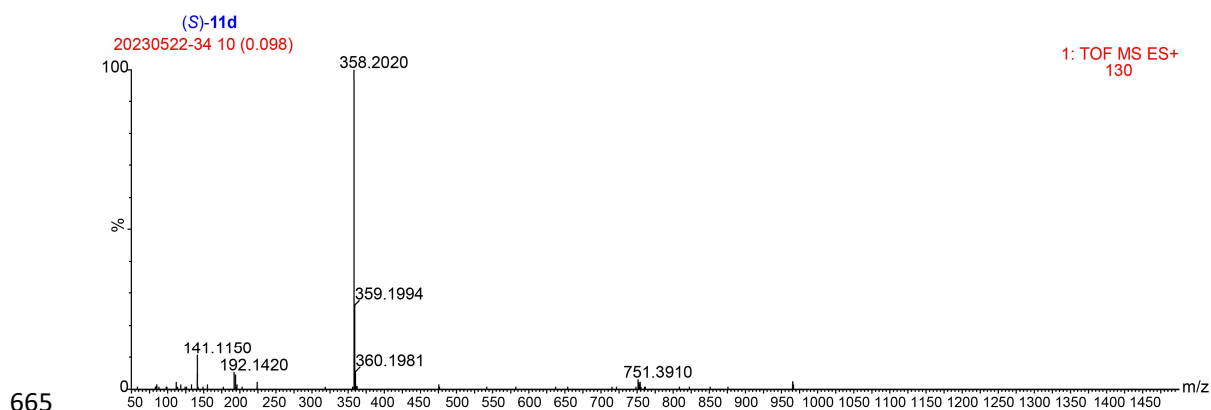
657 **Supplementary Figure 41.** HRMS spectra of (S)-8d. MS spectrum (positive) of (S)-8d showed m/z
 658 312.1950, $[M+H]^+$ calcd. for $C_{20}H_{26}NO_2$, 312.1958.



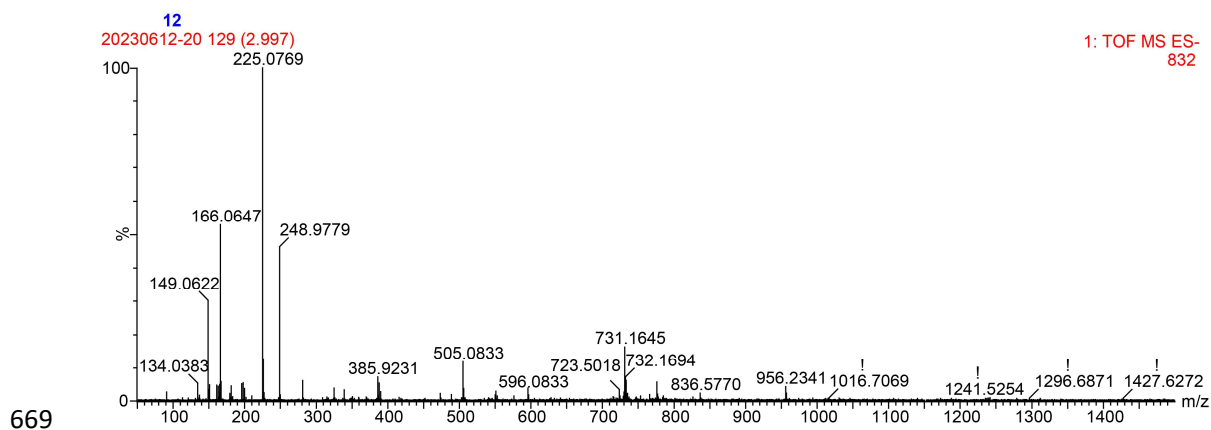
Supplementary Figure 42. HRMS spectra of (S)-9d. MS spectrum (positive) of (S)-9d showed m/z 328.1913, $[M+H]^+$ calcd. for $C_{20}H_{26}NO_3$, 328.1907.



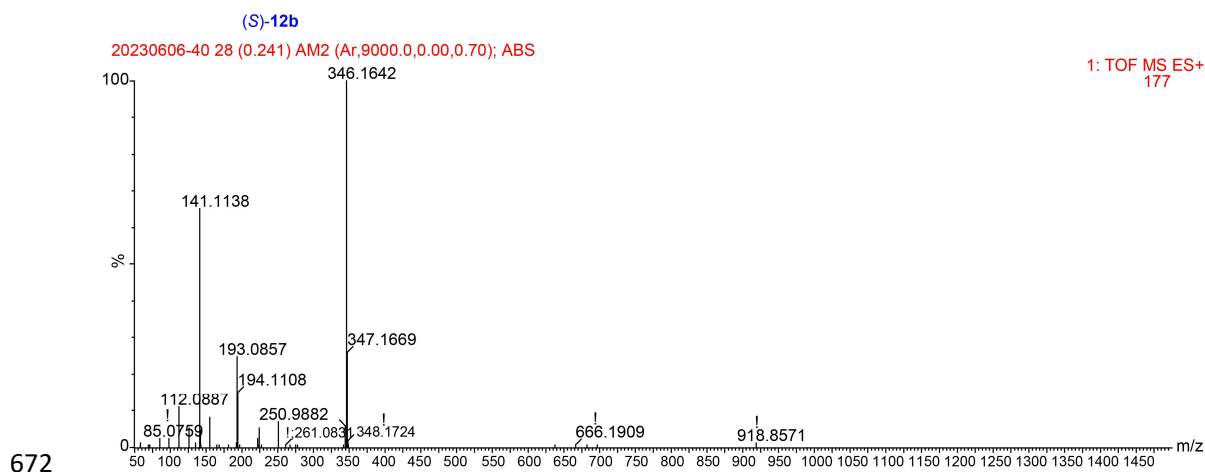
Supplementary Figure 43. HRMS spectra of (S)-10d. MS spectrum (positive) of (S)-10d showed m/z 328.1913, $[M+H]^+$ calcd. for $C_{20}H_{26}NO_3$, 328.1907.



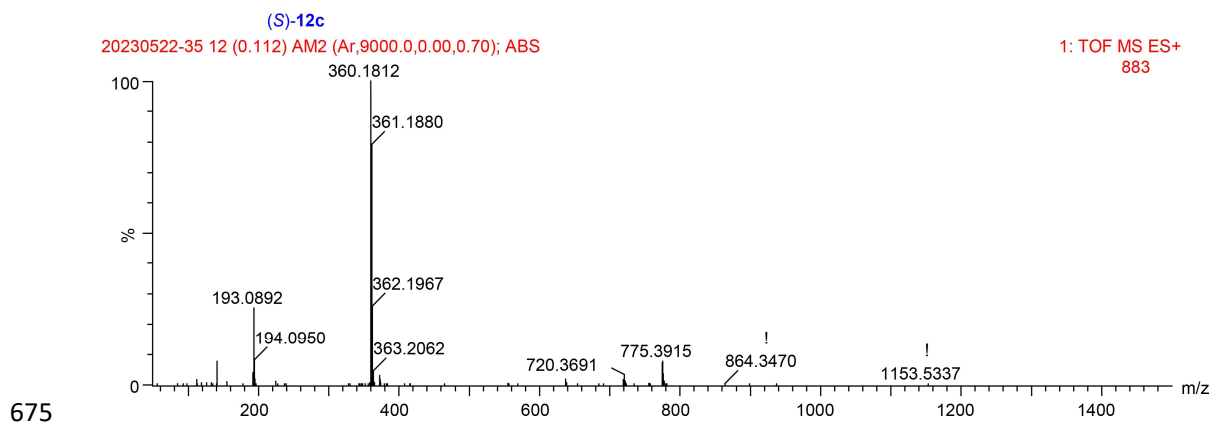
Supplementary Figure 44. HRMS spectra of (S)-11d. MS spectrum (positive) of (S)-11d showed m/z 358.2020, $[M+H]^+$ calcd. for $C_{21}H_{28}NO_4$, 358.2013.



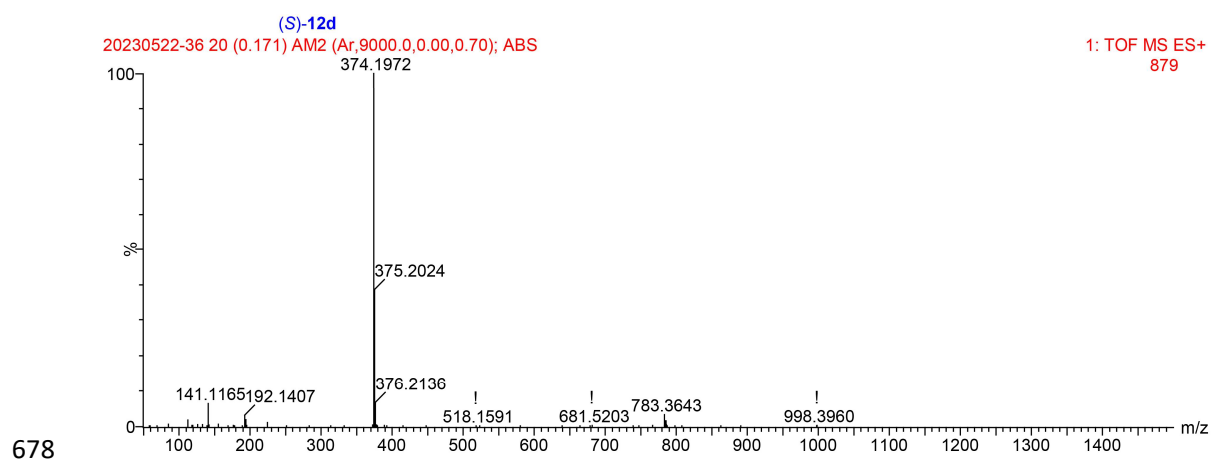
670 **Supplementary Figure 45.** HRMS spectra of **12**. MS spectrum (negative) of **12** showed m/z
 671 225.0769, $[M-H]^-$ calcd. for $C_{11}H_{13}O_5$, 225.0768.



673 **Supplementary Figure 46.** HRMS spectra of **(S)-12b**. MS spectrum (positive) of **(S)-12b** showed
 674 m/z 346.1642, $[M+H]^+$ calcd. for $C_{19}H_{24}NO_5$, 346.1649.



676 **Supplementary Figure 47.** HRMS spectra of **(S)-12c**. MS spectrum (positive) of **(S)-12c** showed
 677 m/z 360.1812, $[M+H]^+$ calcd. for $C_{20}H_{26}NO_5$, 360.1805.



Supplementary Figure 48. HRMS spectra of (S)-12d. MS spectrum (positive) of (S)-12d showed m/z 374.1972, $[M+H]^+$ calcd. for $C_{21}H_{28}NO_5$, 374.1962.

682 **Supplementary Reference:**

- 683 1. Jakobsson, J.E., Gourni, E., Khanapur, S., Brito, B. & Riss, P.J. Synthesis and characterization in rodent brain
684 of the subtype-selective NR2B NMDA receptor ligand [¹¹C]Ro04-5595 as a potential radiotracer for positron
685 emission tomography. *Acs Omega* **4**, 9925-9931 (2019).
686 2. Nimgirawath, S. Synthesis of (±)-isoautumnaline and (±)-dysoxyline. *Aust. J. Chem.* **47**, 957-962 (1994).

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