Supplementary Information for

Stereospecific prenylation of tryptophan by a cyanobacterial post-translational modification enzyme

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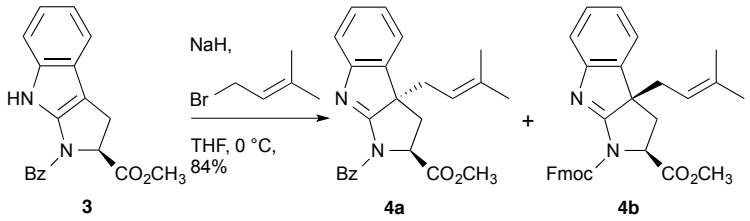
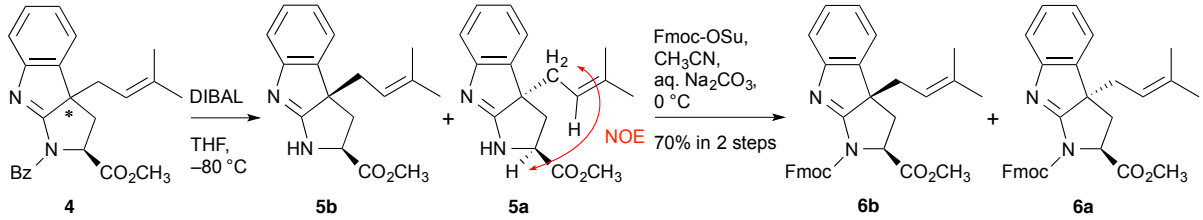
Chemical Synthesis

Synthesis of **4a** and **4b**



According to our previous reports, [1, 2] compound **3** was prepared. To a 0.1 M solution of **3** (1.13 g, 3.54 mmol) in THF at 0 °C under Argon was added sodium hydride (225 mg, 5.63 mmol). After stirring at 0 °C for 1 h, dimethylallyl bromide (0.542 g, 3.64 mmol) was added to the mixture. After stirring for 3 h at 0 °C, the reaction mixture was quenched and neutralized with 0.1 M phosphate buffer (pH 7). The reaction mixture was extracted with diethyl ether, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution was evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to give a mixture of **4a** and **4b** (1.16 g, 2.98 mmol, 84%, α : β = 1.0 : 0.6) as pale yellow oil. The two diastereoisomers could not be separated by column chromatography. ^{1}H-NMR spectrum of the mixture was shown in Figure S2.

Synthesis of **6a** and **6b**



To a 0.1 M solution of **4** (1.16 g, 2.98 mmol) in THF was slowly added a 1.0 M solution of diisobutylaluminium hydride (DIBAL) (3.50 mL) in THF under Argon at –80 °C. After stirring for 3 h at –80 °C, the reaction mixture was poured into 0.1 M phosphate buffer solution (pH 7). The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a celite pad, the solution was evaporated. The residue was purified by silica gel column chromatography (chloroform/methanol) to give a mixture of **5a** and **5b** as a pale yellow oil. The mixture was immediately used in next step without further purification because of instability of **5a**.

For NMR analysis, the mixture was separated by silica gel column chromatography

(hexane/acetone), and each solution was carefully evaporated, but not dried, to replace the solvent by deutrated chloroform. The stereochemistry of **5a** was confirmed by NOEs between the a proton and the olefin and methylene protons of the dimethylalyl group, but no NOEs in **5b**. Unfortunately, conclusive NOEs for determinatiob of the stereochemistry were not observed in **5b**.

NMR spectra were shown in Figure S3-S8.

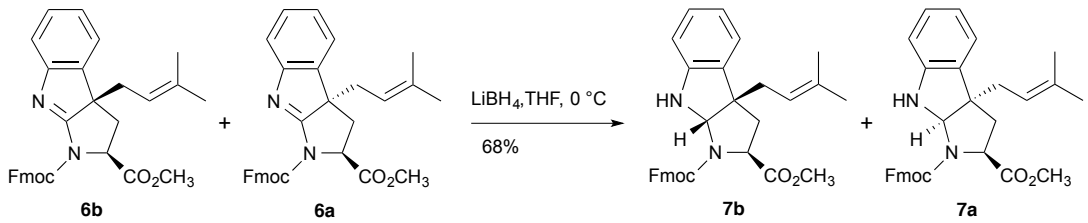
5a ^{1}H-NMR (500 MHz, $CDCl_3$): d 7.16 (t, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.84 (t, *J* = 7.2 Hz, 1H), 5.20 (m, 1H), 4.93 (m, 1H), 3.80 (s, 3H), 2.68 (m, 1H), 2.41 (q, *J* = 7.2 Hz, 1H), 2.23-2.05 (m, 3H), 1.73 (s, 3H), 1.44 (s, 3H), 1.25 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): d 184.5, 173.5, 149.6, 136.0, 128.2, 124.0, 120.7, 118.6, 112.0, 71.0, 60.3, 52.3, 39.4, 34.7, 26.1, 17.8, 14.2.

5b ^{1}H-NMR (500 MHz, $CDCl_3$): d 7.15 (t, *J* = 7.2, Hz, 1H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.90 (d, *J* = 7.2 Hz, 1H), 6.84 (t, *J* = 7.2 Hz, 1H), 5.08 (m, 1H), 4.90 (m, 1H), 3.81 (s, 3H), 2.66 (m, 1H), 2.48-2.29 (m, 2H), 2.22-2.07 (m, 4H), 1.66 (s, 3H), 1.33 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): d 186.3, 174.7, 151.3, 135.7, 128.2, 124.1, 120.8, 118.4, 112.4, 71.1, 59.2, 52.5, 37.0, 36.5, 26.1, 17.8, 14.0.

To a 0.1 M solution of a mixture of **5a** and **5b** in CH_3CN was added a 1.0 M solution of aqueous sodium carbonate (30 mL) and N-(9-fluorenylmethoxycarbonyl)succinimide (Fmoc-OSu) (1.01 g, 2.98 mmol) at room temperature. After stirring for 1 h, the reaction mixture was neutralized with 0.1 M phosphate buffer (pH 7). The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution was evaporated. The residue was purified by silica gel column chromatography (hexane/acetone) to give a mixture of **6a** and **6b** (1.06 g, 2.09 mmol, 70 % in 2 steps). The mixture was immediately used in next step without further purification because of instability.

^{1}H-NMR spectrum of the mixture of **6a** and **6b** was shown in Figure S9.

Synthesis of **7a** and **7b**

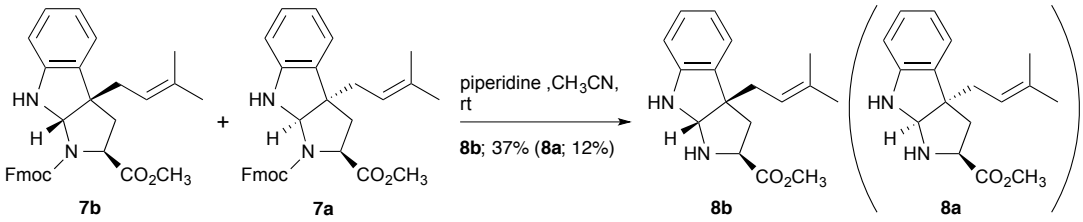


To a solution of the mixture of **6a** and **6b** in THF (20 mL) was added lithium borohydride (26 mg, 1.07 mmol) under Argon at 0 °C. After stirring for 2 h, the reaction mixture was

quenched with 1 % solution of aqueous sodium carbonate. The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution was evaporated. The residue was purified by silica gel column chromatography (hexane/acetone) to give a mixture of **7a** and **7b** (0.713 g, 1.40 mmol, 68%).

^{1}H-NMR spectrum of the mixture of **7a** and **7b** was shown in Figure S10.

Synthesis of **8a** and **8b**



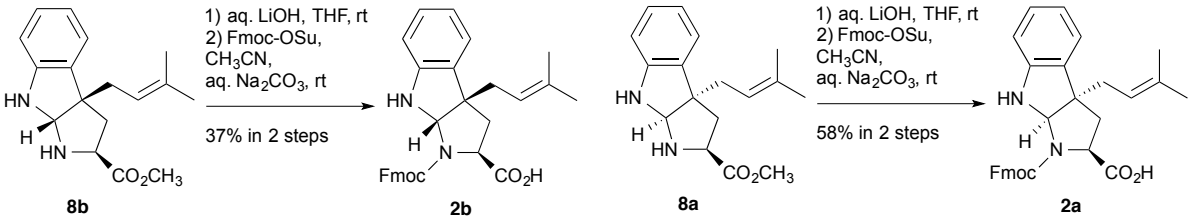
To a solution of the mixture of **7a** and **7b** (0.422 g, 0.830 mmol) in acetonitrile (7.5 mL) was added piperidine (2.5 mL) at room temperature. After stirring for 2 h, the reaction mixture was quenched and neutralized with 0.1 M phosphate buffer (pH 7). The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution wass evaporated. The residue was purified by silica gel column chromatography (hexane/acetone) three times to give **8a** (28.0 mg, 0.0978 mmol, 12%) and **8b** (88.0 mg, 0.307 mmol, 37%) and as pale yellow oil, respectively.

NMR spectra were shown in Figure S11-S14.

8a $[\alpha]^{18}_{D}$= +7.55 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): d 7.02-6.99 (m, 2H), 6.69 (t, *J* = 7.2 Hz, 1H), 6.56 (d, *J* = 7.2 Hz, 1H), 5.12 (m, 1H), 4.80 (m, 1H), 3.87 (q, *J* = 7.6 Hz, 1H), 3.30 (s, 3H), 2.48-2.30 (m, 4H), 1.68 (s, 3H), 1.53 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): d 174.5, 149.5, 134.7, 133.5, 128.2, 123.9, 119.7, 118.9, 109.6, 82.4, 60.1, 57.7, 52.0, 41.2, 36.6, 26.1, 18.1.

8b $[\alpha]^{18}_{D}$= –31.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): d 7.03-7.00 (m, 2H), 6.71 (t, *J* = 7.1 Hz, 1H), 6.54 (d, *J* = 7.1 Hz, 1H), 5.08 (m, 1H), 4.89 (m, 1H), 3.73-3.59 (m, 4H), 2.51-2.28 (m, 3H), 1.99 (m, 1H), 1.66 (s, 3H), 1.54 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): d 174.5, 150.1, 134.7, 133.2, 128.2, 123.7, 119.7, 118.9, 109.1, 82.2, 59.5, 58.8, 52.2, 44.2, 37.0, 26.1, 18.2 .

Synthesis of **2b** and **2a**



To a 0.1 M solution of **8b** (88.0 mg, 0.307 mmol) in THF (5 mL) and MeOH (2mL) was added a 1.0 M solution of aqueous lithium hydroxide (2.0 mL) at room temperature. After stirring for 1 h, the reaction mixture was neutralized with 5 % $KHSO_4$. The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution was evaporated. To a solution of the residue in dioxane (5 mL) was added a 1.0 M solution of aqueous sodium carbonate (2.5 mL) and N-(9-fluorenylmethoxycarbonyl)succinimide (Fmoc-OSu) (117 mg, 0.347 mmol) at room temperature. After stirring for 2 h, the reaction mixture was neutralized with 5 % $KHSO_4$. The reaction mixture was extracted with ethyl acetate, washed with saturated aqueous sodium chloride, and dried with anhydrous sodium sulfate. After filtration through a cotton plug, the solution was evaporated. The residue was purified by silica gel column chromatography (hexane/acetone) three times to give **2b** (56.0 mg, 0.113 mmol, 37 % in 2 step) as pale yellow oil. By a similar method, **2a** (28.0 mg, 0.0566 mmol, 58% in 2 step) was obtained from **8a** (28.0 mg, 0.0978 mmol).

^{1}H-NMR spectra of **2a** and **2b** was observed as 1 : 1 equilibrium mixture between N-conformers, as shown in Figure S15 and S16, respectively.

2a $[\alpha]_D^{21}$ = +36.9 (*c* 1.0, $CHCl_3$), **2b** $[\alpha]_D^{21}$ = –55.1 (*c* 1.0, $CHCl_3$).

References

(1) M. Okada, I. Sato, S. J. Cho, H. Iwata, T. Nishio, D. Dubnau and Y. Sakagami, *Nat. Chem. Biol.*, **2005**, *1*, 23.

(2) M. Okada, I. Sato, S. J. Cho, D. Dubnau and Y. Sakagami, *Tetrahedron*, **2006**, *62*, 8907.

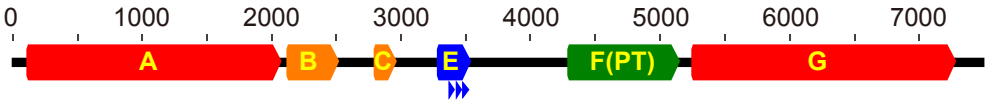


Figure S1. The biosynthetic gene cluster of kawaguchipeptins from *Microcystis aeruginosa* NIES-88.

The 7.4 kb biosynthetic gene cluster consists of six ORFs. The red bars (A and G) indicate genes that encode N-terminal and C-terminal proteases. The blue bar (E) indicates genes that encode precursor peptide of kawaguchipeptins. The amino acid sequences of kawaguchipeptins are shown in blue triangles. The green bar (F) indicates genes that encode prenyltransferases belonging to the ABBA family. The orange bars (B and C) indicate genes that encode unknown proteins.

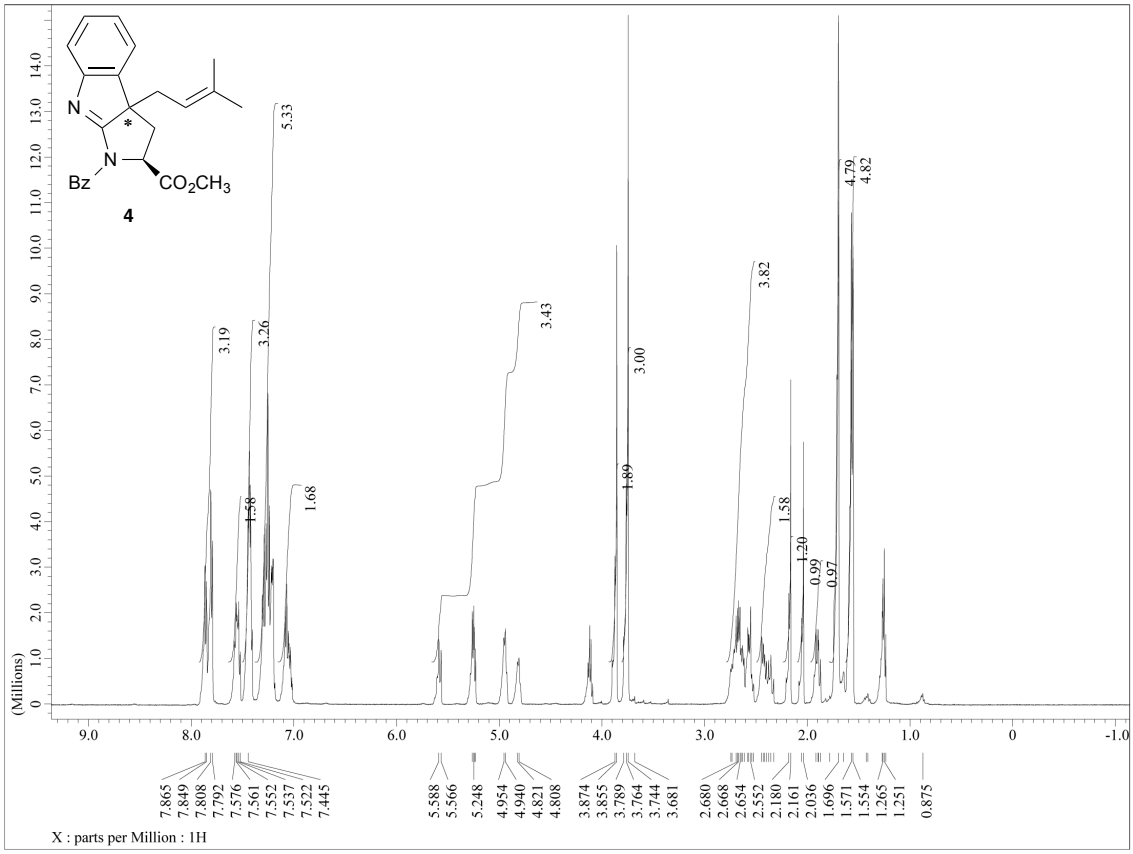


Figure S2. ^{1}H-NMR spectrum of a mixture of **4a** and **4b** ($CDCl_3$, 500 MHz).

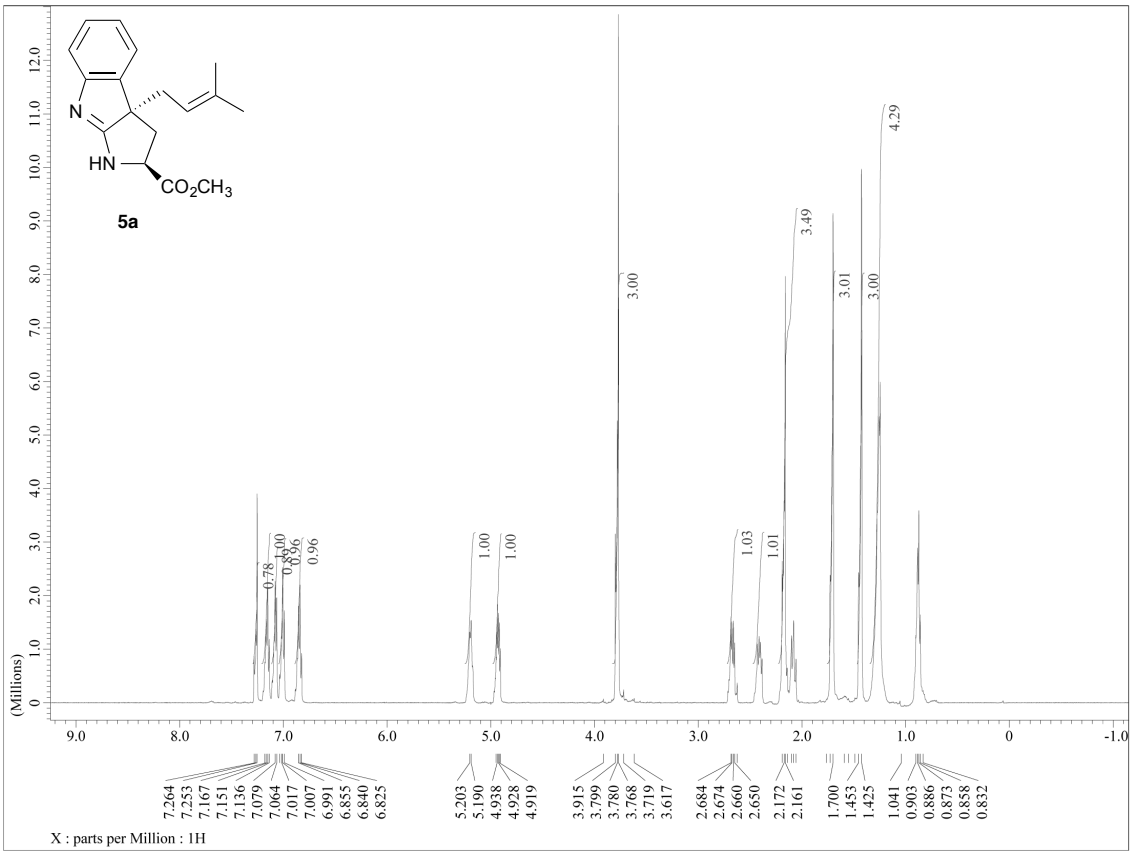


Figure S3. ^{1}H-NMR spectrum of **5a** ($CDCl_3$, 500 MHz).

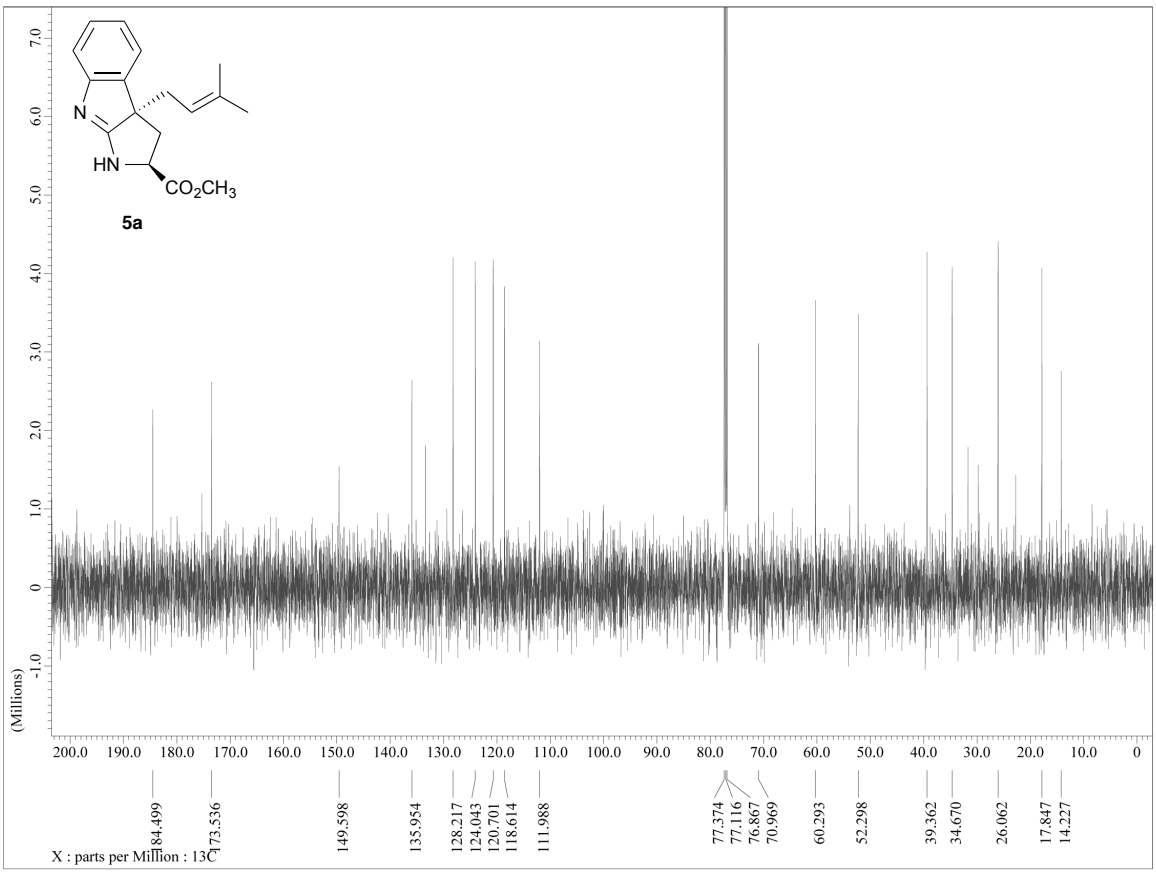


Figure S4. ^{13}C-NMR spectrum of **5a** ($CDCl_3$, 125 MHz).

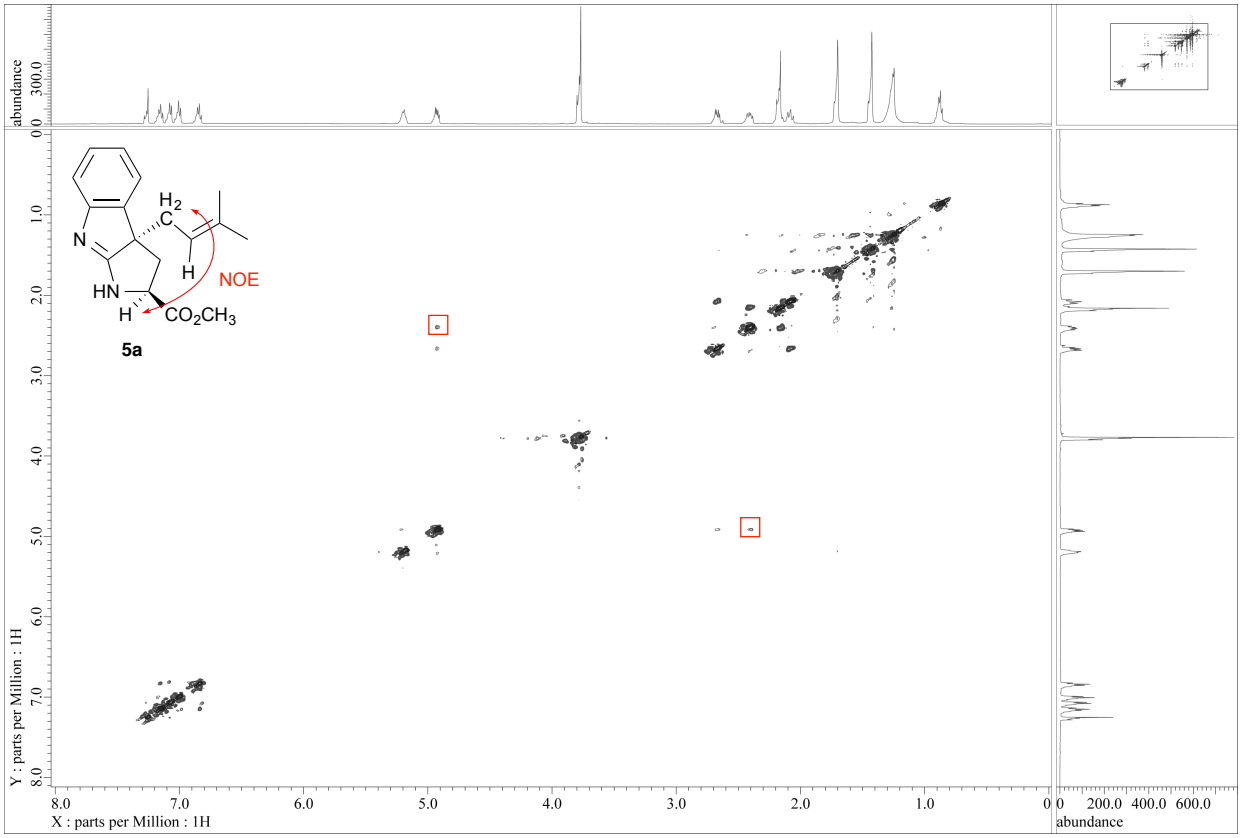


Figure S5. NOESY spectrum of **5a** ($CDCl_3$, 500 MHz).

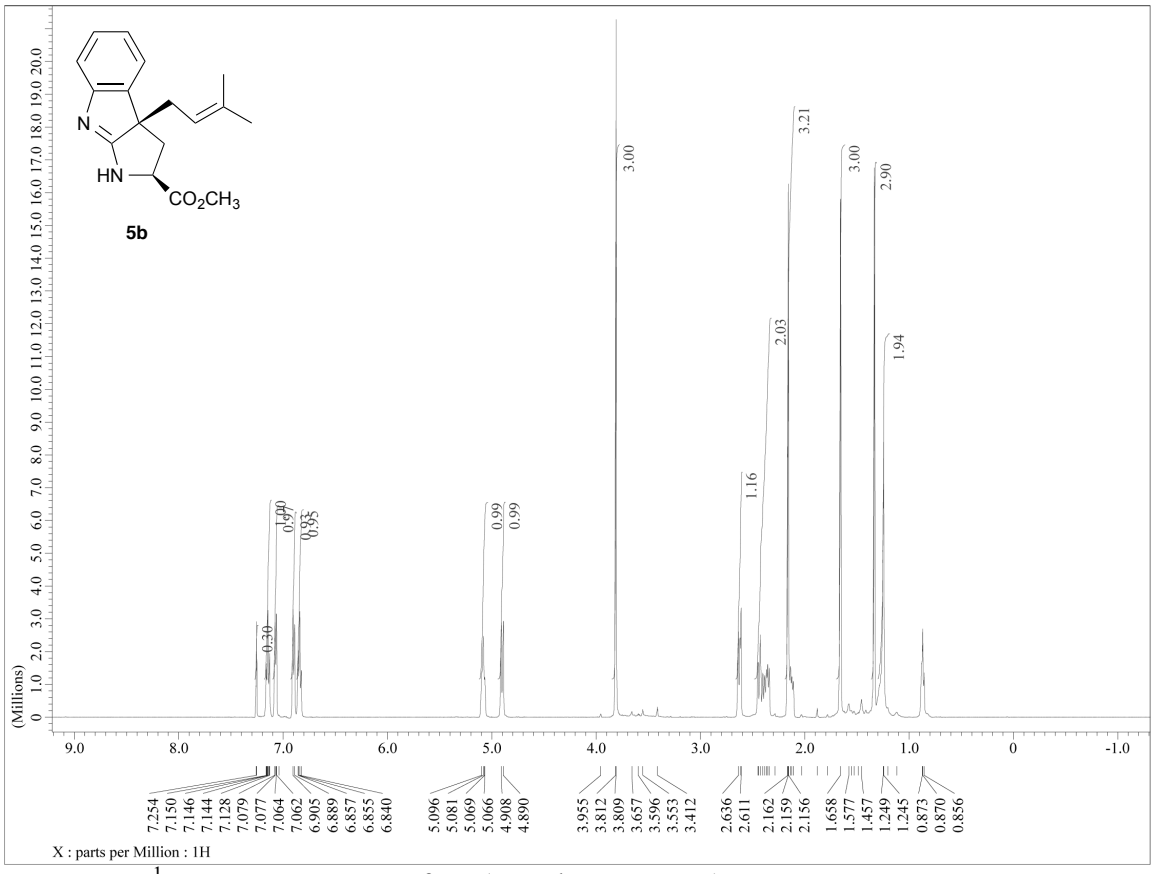


Figure S6. ^{1}H-NMR spectrum of **5b** ($CDCl_3$, 500 MHz).

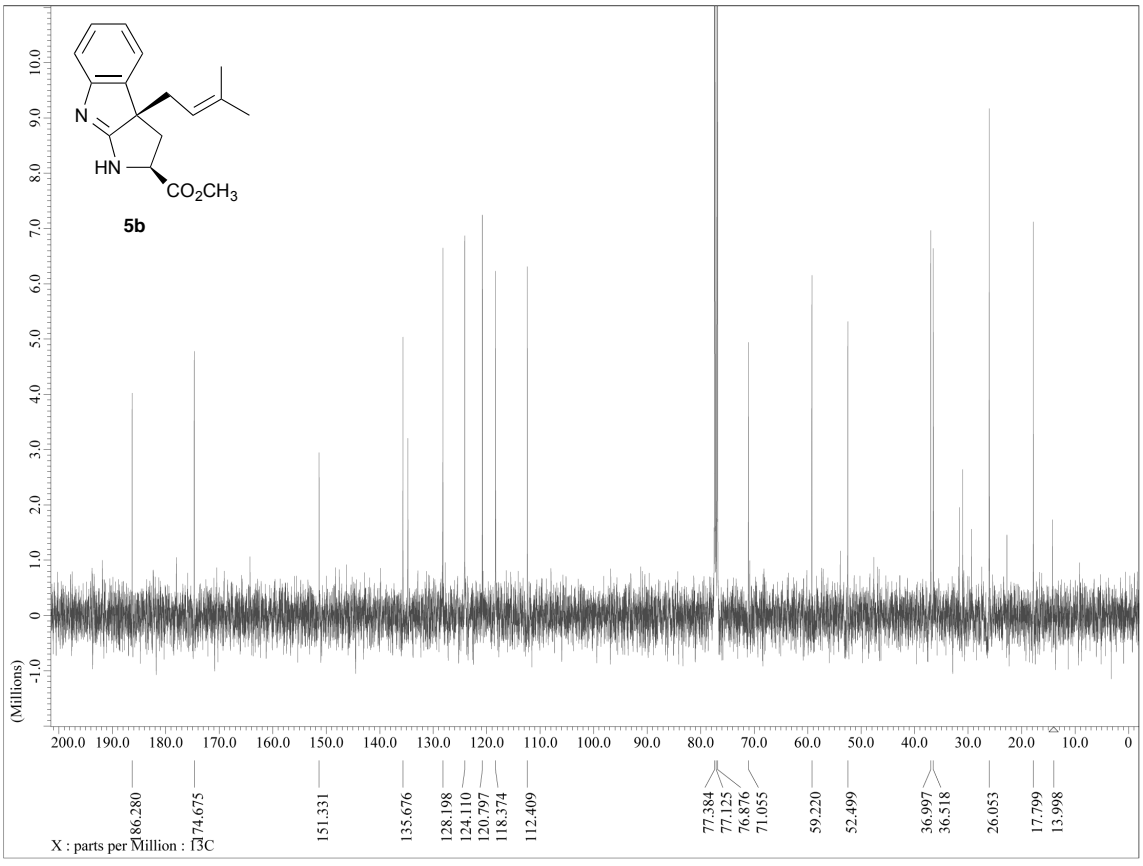


Figure S7. ^{13}C-NMR spectrum of **5b** ($CDCl_3$, 125 MHz).

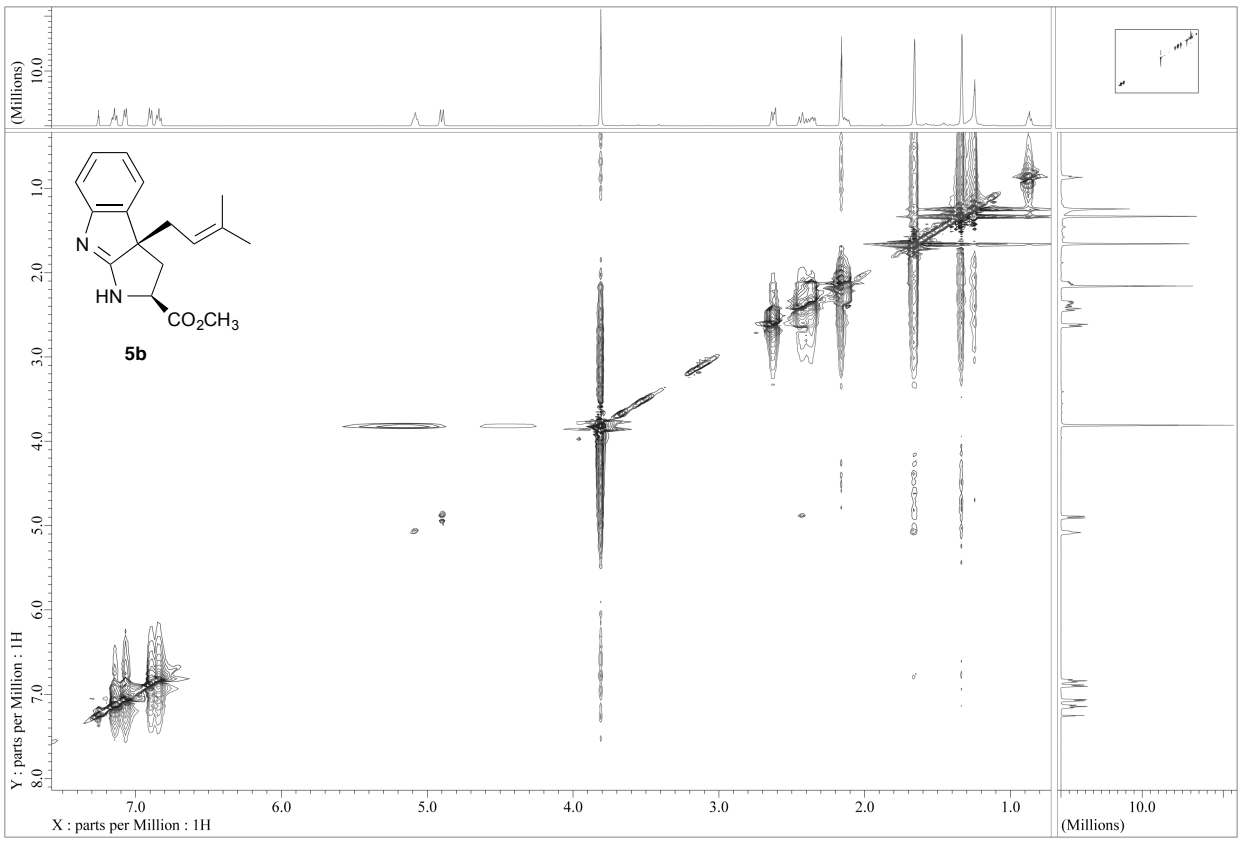


Figure S8. NOESY spectrum of **5b** ($CDCl_3$, 500 MHz).

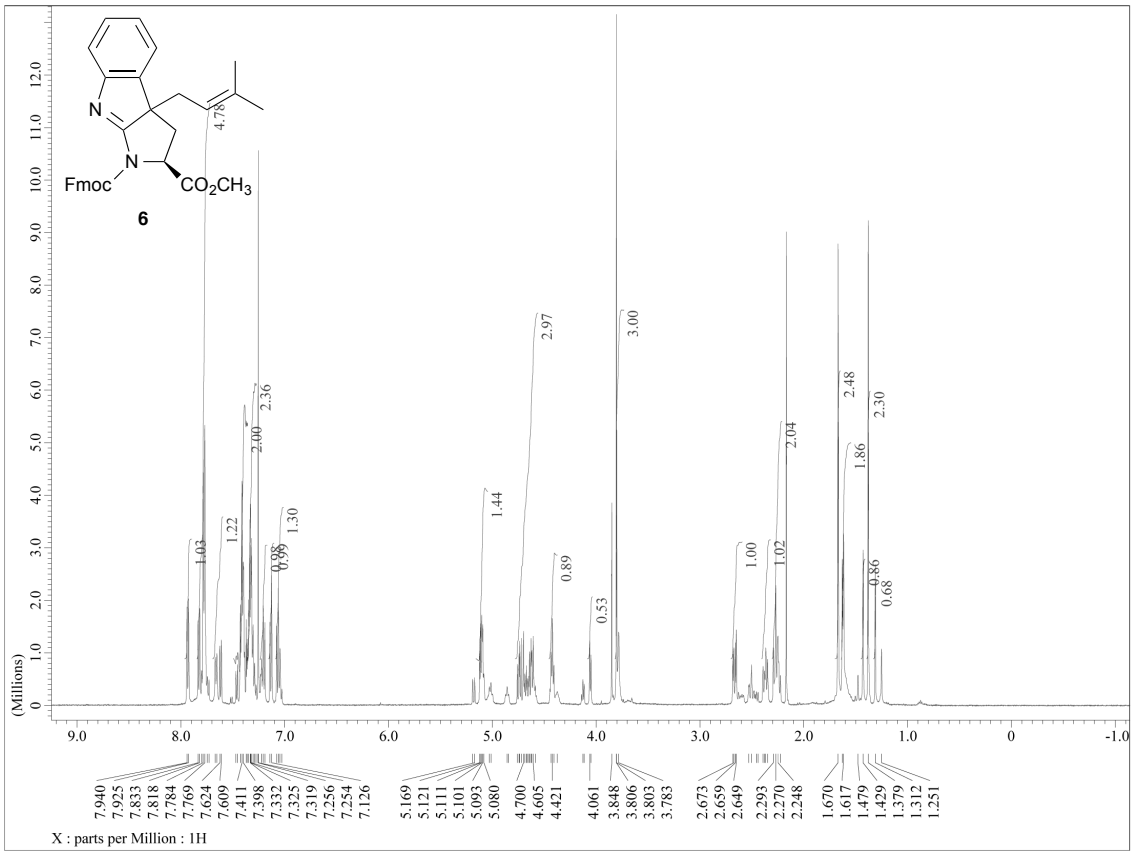


Figure S9. ^{1}H-NMR spectrum of the mixture of **6a** and **6b** ($CDCl_3$, 500 MHz).

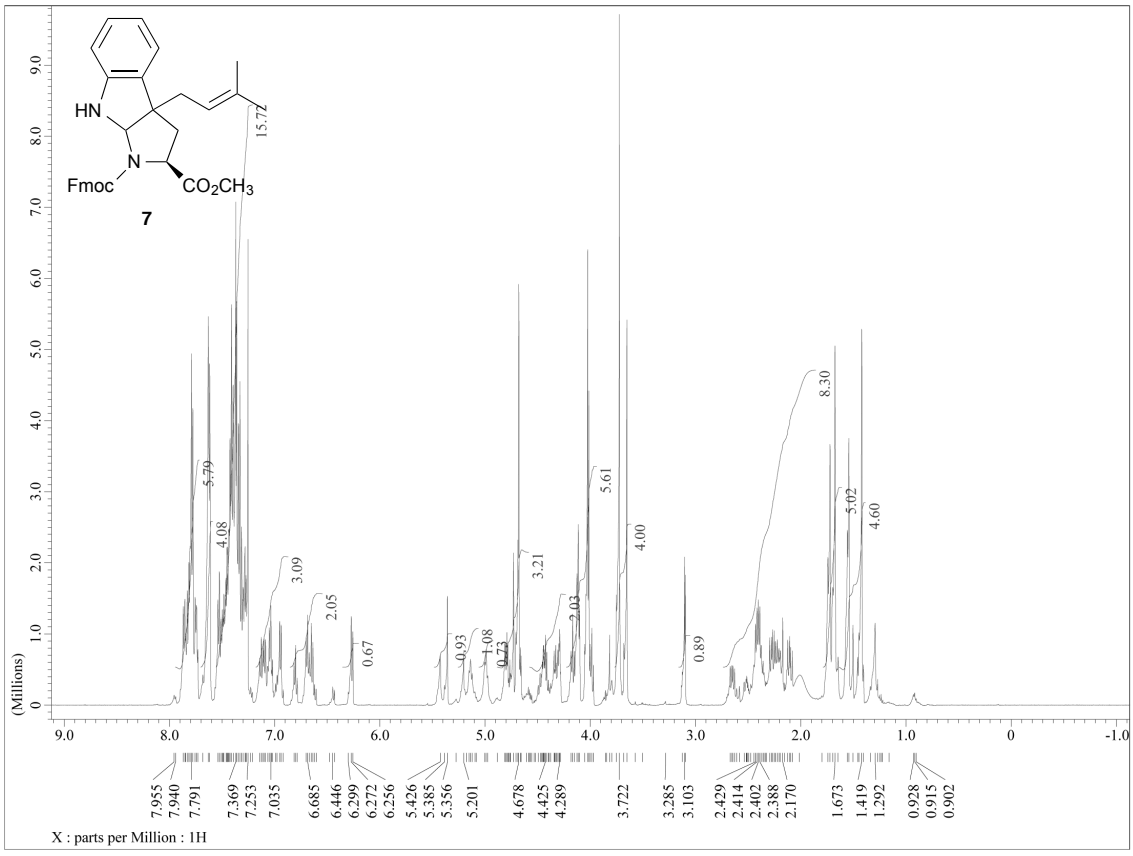


Figure S10. ^{1}H-NMR spectrum of the mixture of **7a** and **7b** ($CDCl_3$, 500 MHz) .

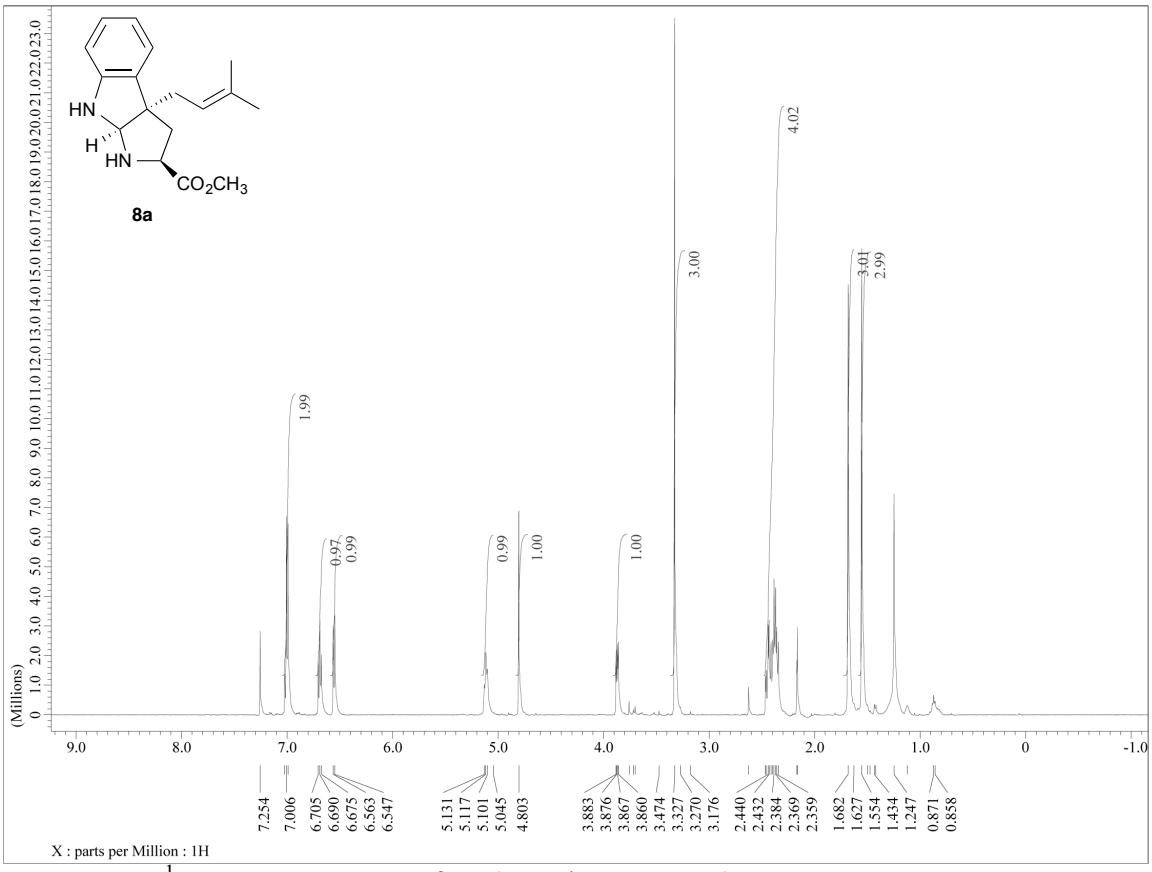


Figure S11. ^{1}H-NMR spectrum of **8a** ($CDCl_3$, 500 MHz).

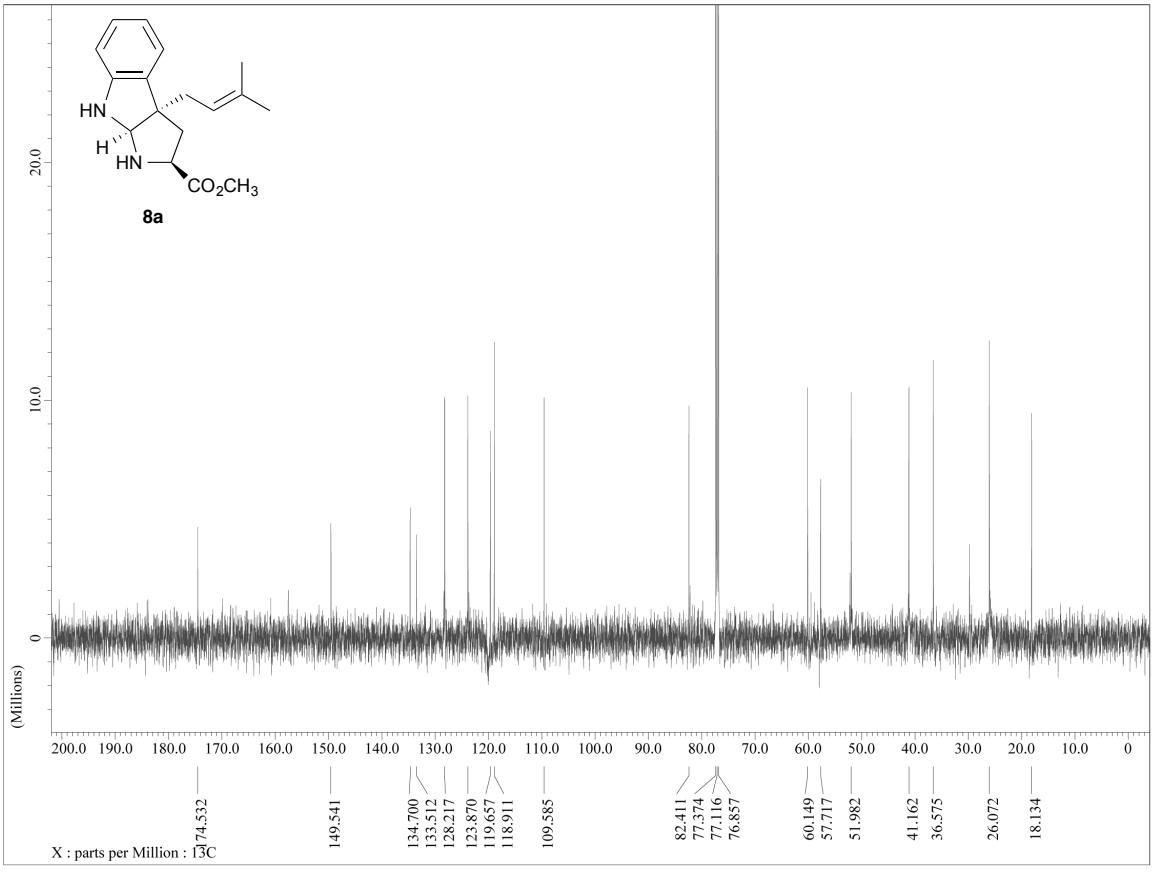


Figure S12. ^{13}C-NMR spectrum of **8a** ($CDCl_3$, 125 MHz).

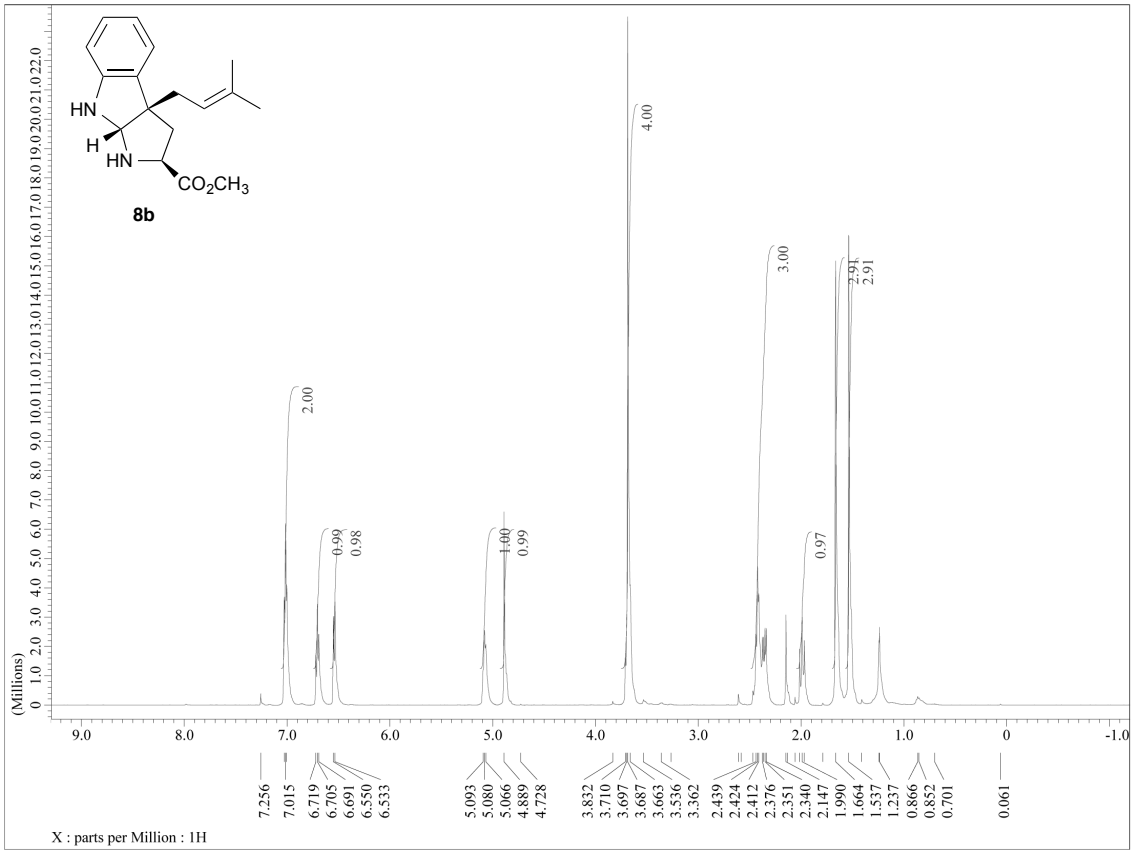


Figure S13. ^{1}H-NMR spectrum of **8b** ($CDCl_3$, 500 MHz).

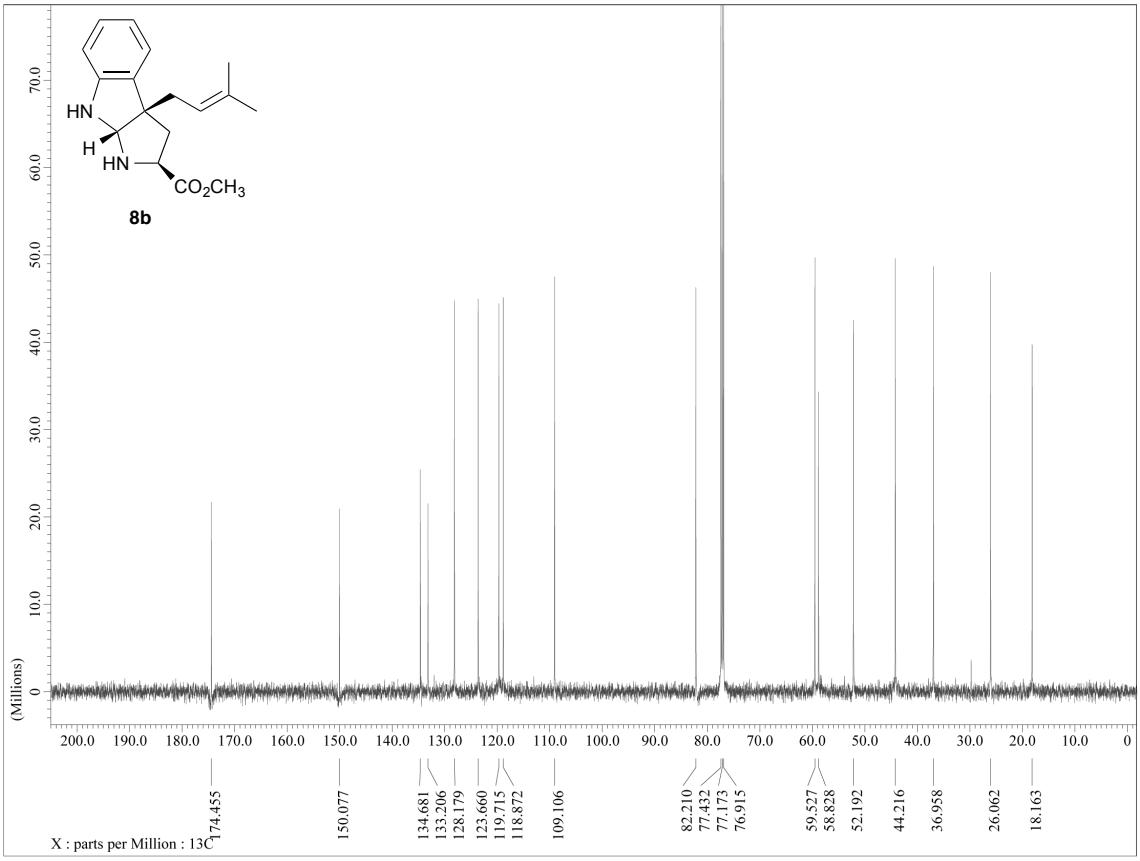


Figure S14. ^{13}C-NMR spectrum of **8b** ($CDCl_3$, 125 MHz).

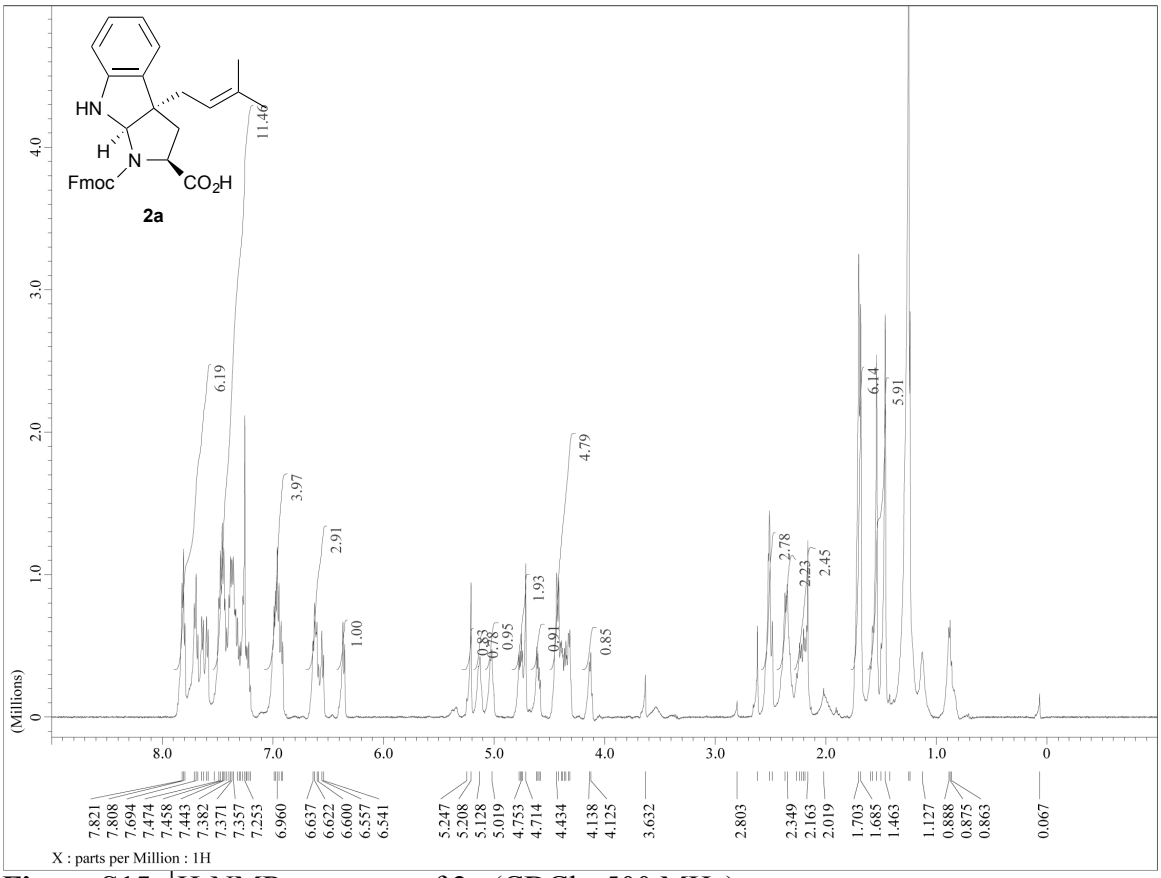


Figure S15. ^{1}H-NMR spectrum of **2a** ($CDCl_3$, 500 MHz).

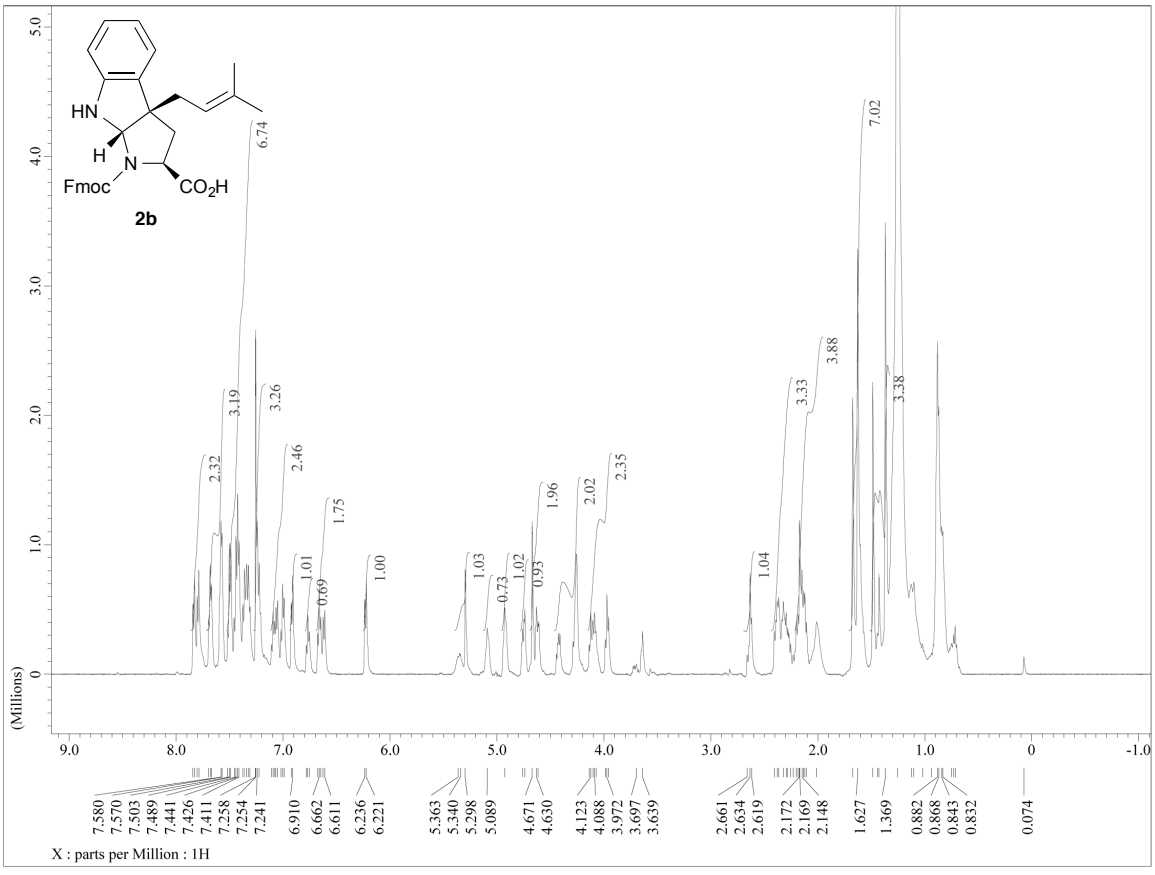


Figure S16. ^{1}H-NMR spectrum of **2b** ($CDCl_3$, 500 MHz).

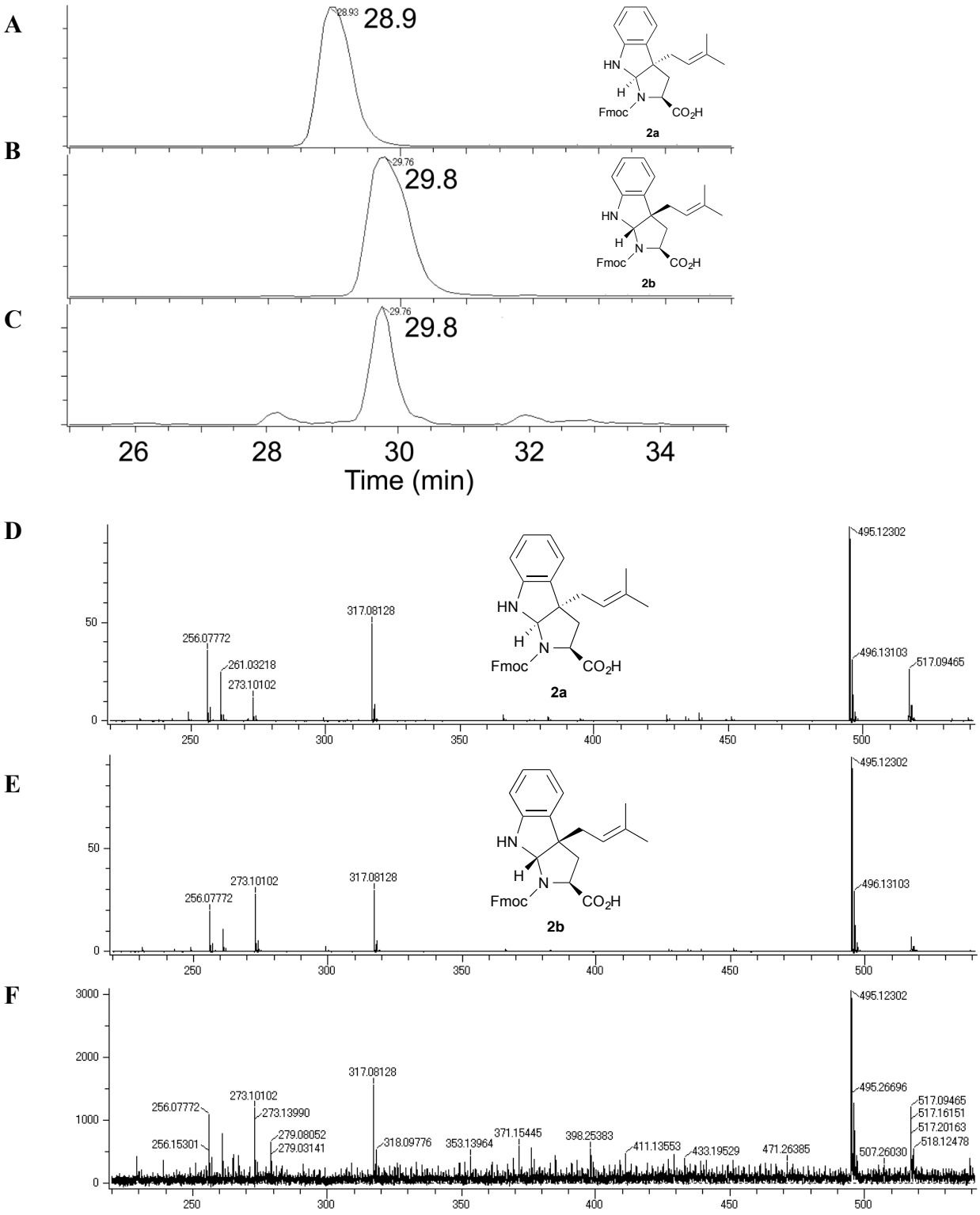


Figure S17. LC-MS analysis of *in vitro* reaction of Fmoc-Trp with KgpF. Enlarged extracted ion chromatograms (positive) at *m/z* 495 of (**A**) synthetic Fmoc-Trp*(α-Pre) (**2a**), (**B**) synthetic Fmoc-Trp*(β-Pre) (**2b**), and (**C**) *in vitro* reaction product. Mass spectrum of (**D**) synthetic Fmoc-Trp*(α-Pre) (**2a**) at 28.9 min, (**E**) synthetic Fmoc-Trp*(β-Pre) (**2b**) at 29.8 min, and (**F**) *in vitro* reaction product at 29.8 min.