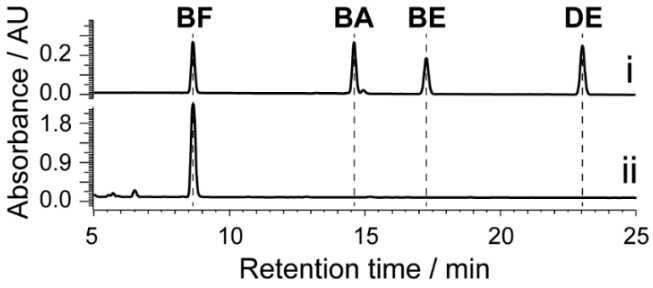
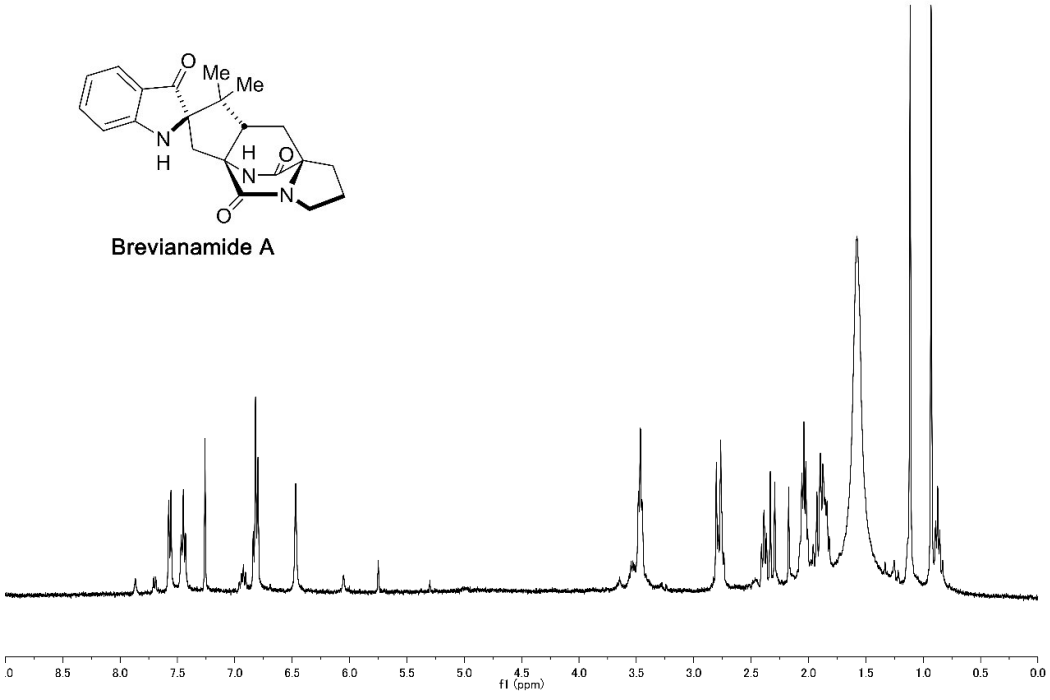
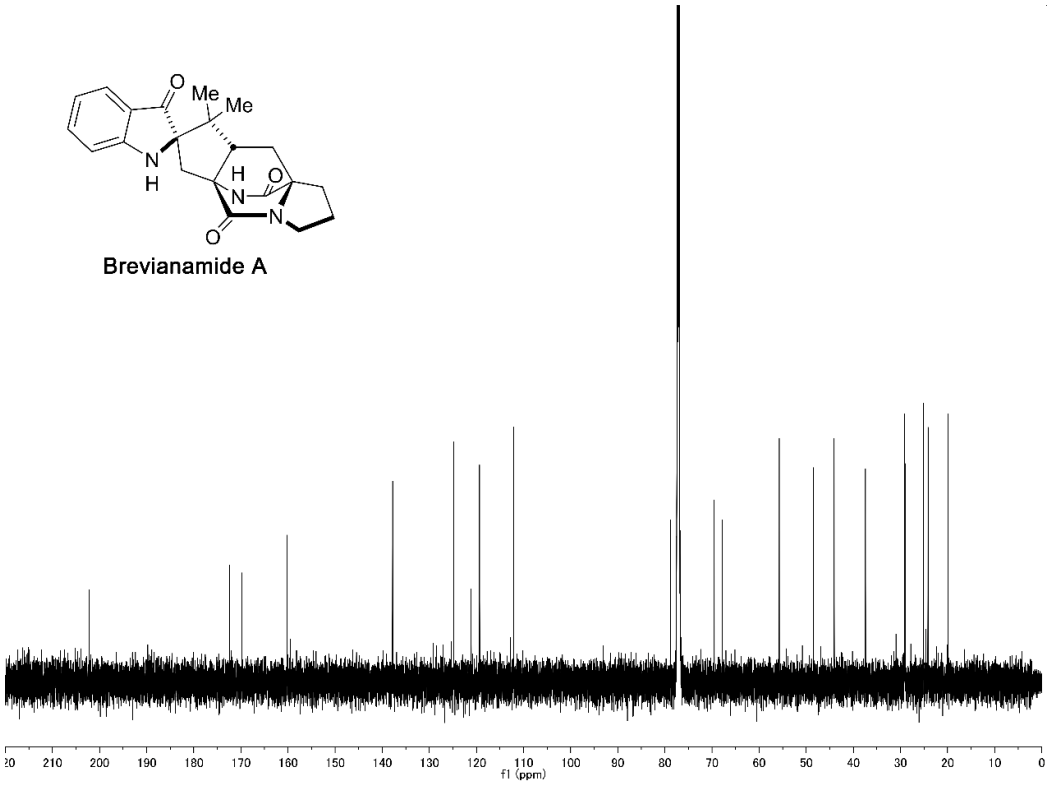
Supplementary Figures



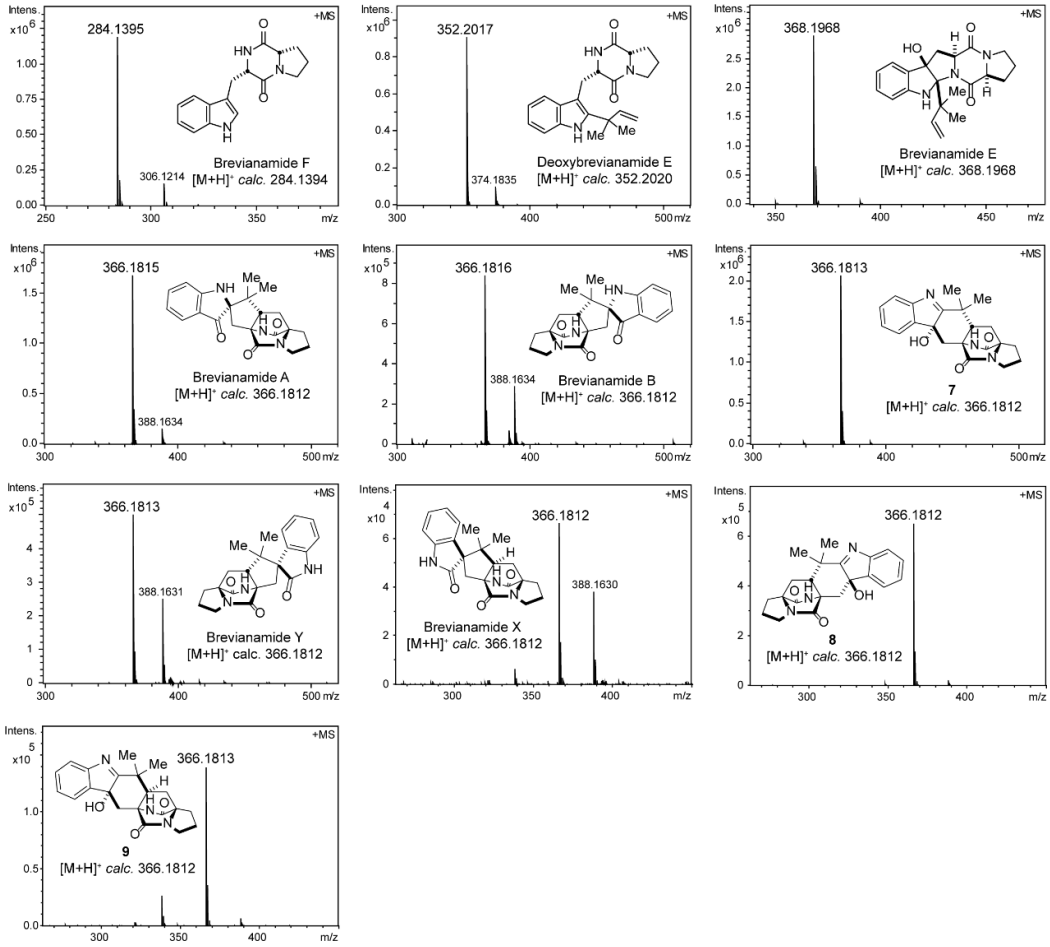
Supplementary Figure 1. HPLC analysis of *Ao-bvnA*. i, Mixed standards; ii, Extract of *Ao-bvnA* fermentation broth.



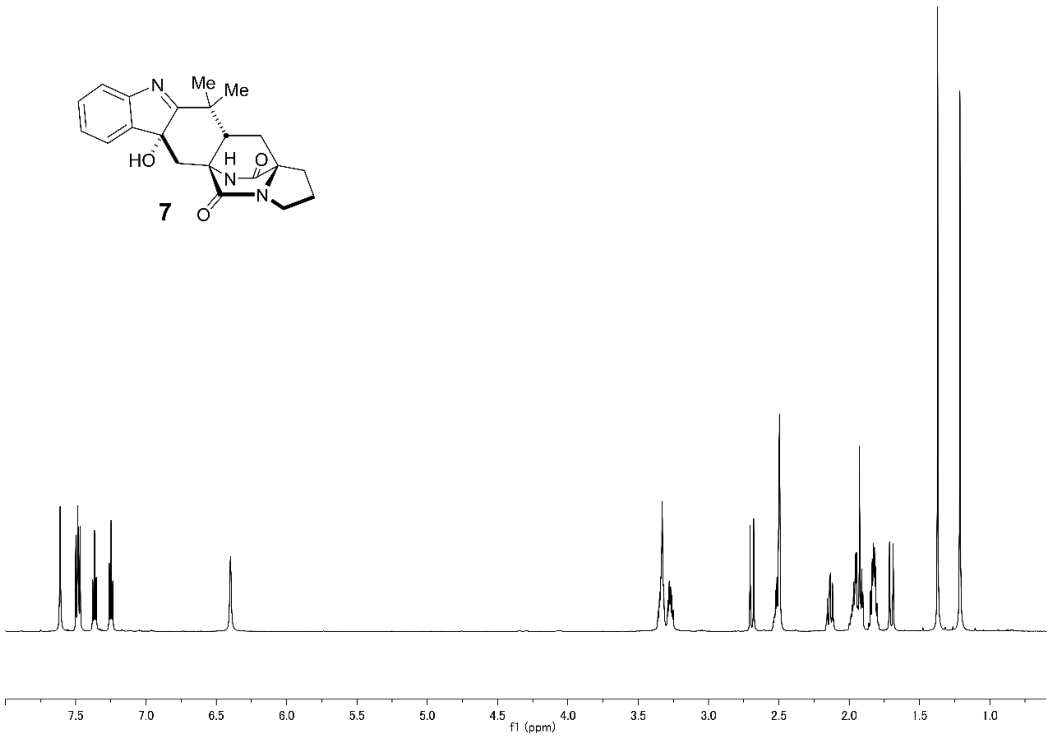
Supplementary Figure 2. ^{1}H NMR spectrum of **BA** in $CDCl_3$.



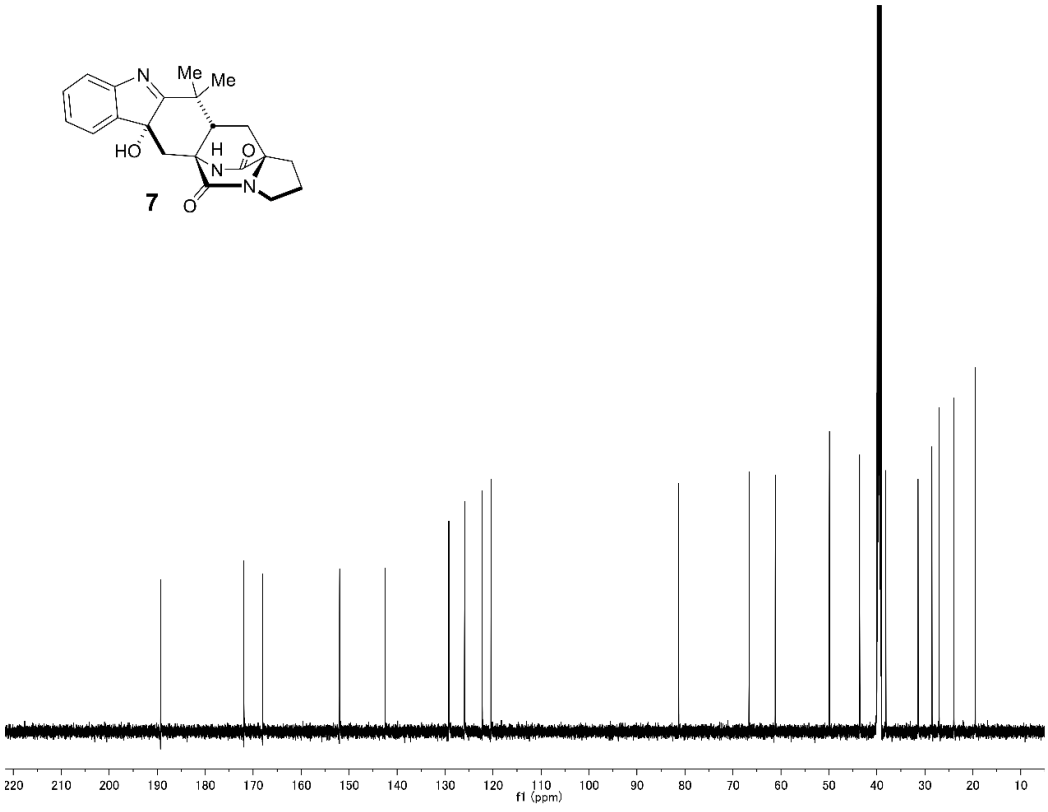
Supplementary Figure 3. ^{13}C NMR spectrum of **BA** in $CDCl_3$.



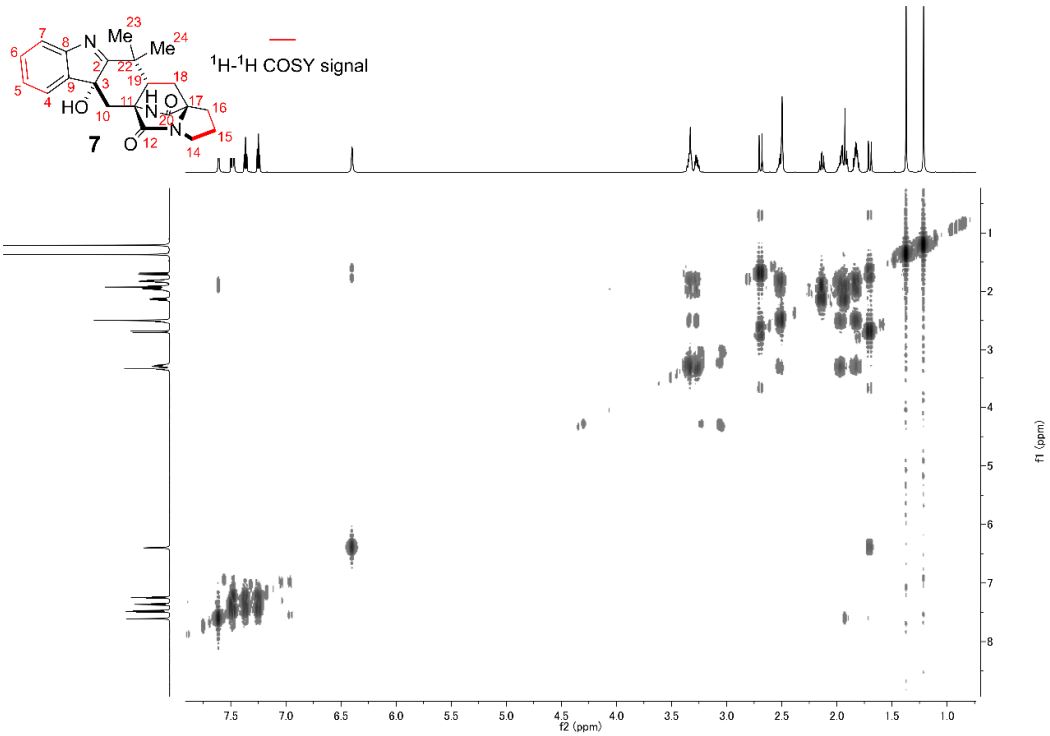
Supplementary Figure 4. HRMS spectra of Brevianamide derivatives.



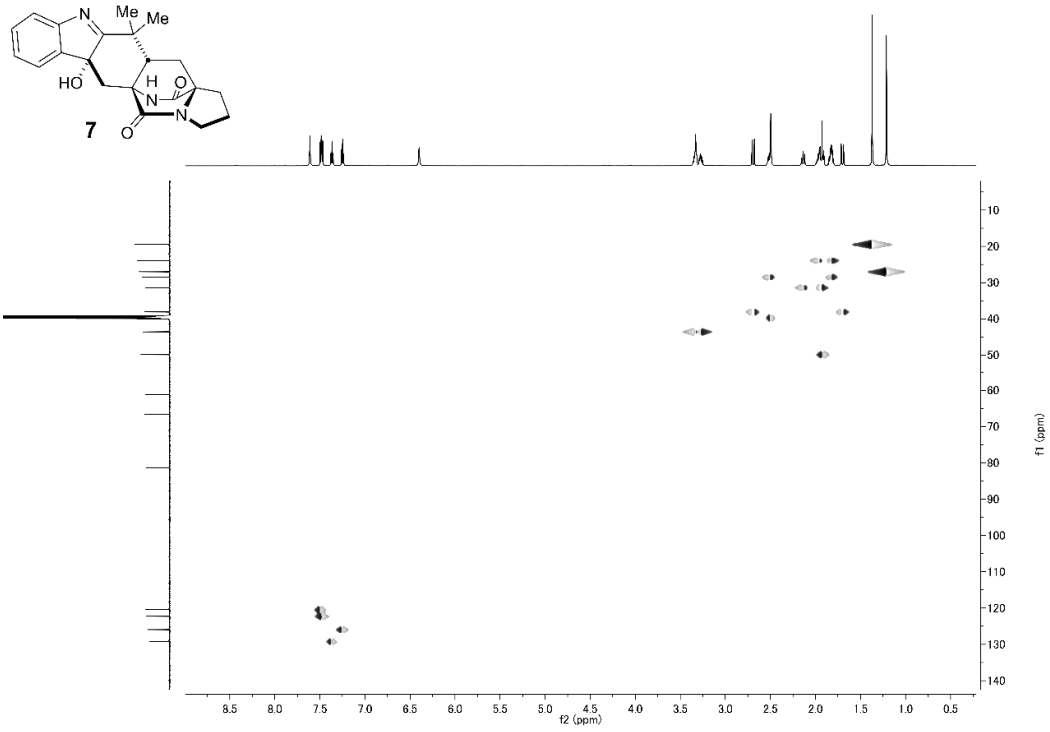
Supplementary Figure 5. ^{1}H NMR spectrum of compound **7** in DMSO-d6.



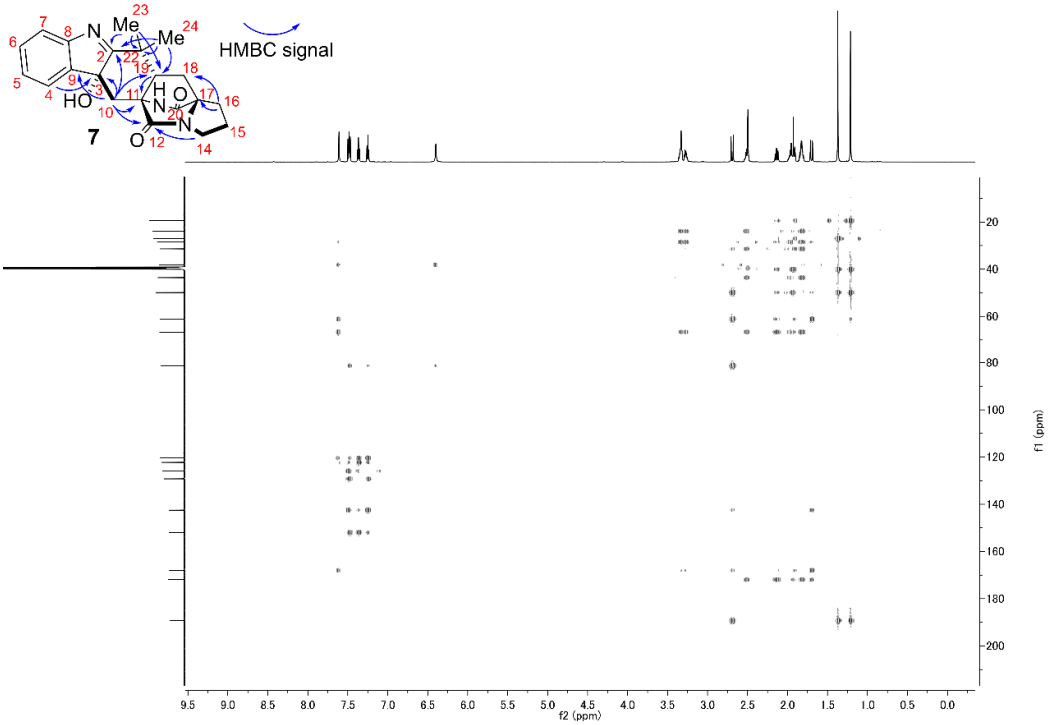
Supplementary Figure 6. ^{13}C NMR spectrum of compound **7** in DMSO-d6.



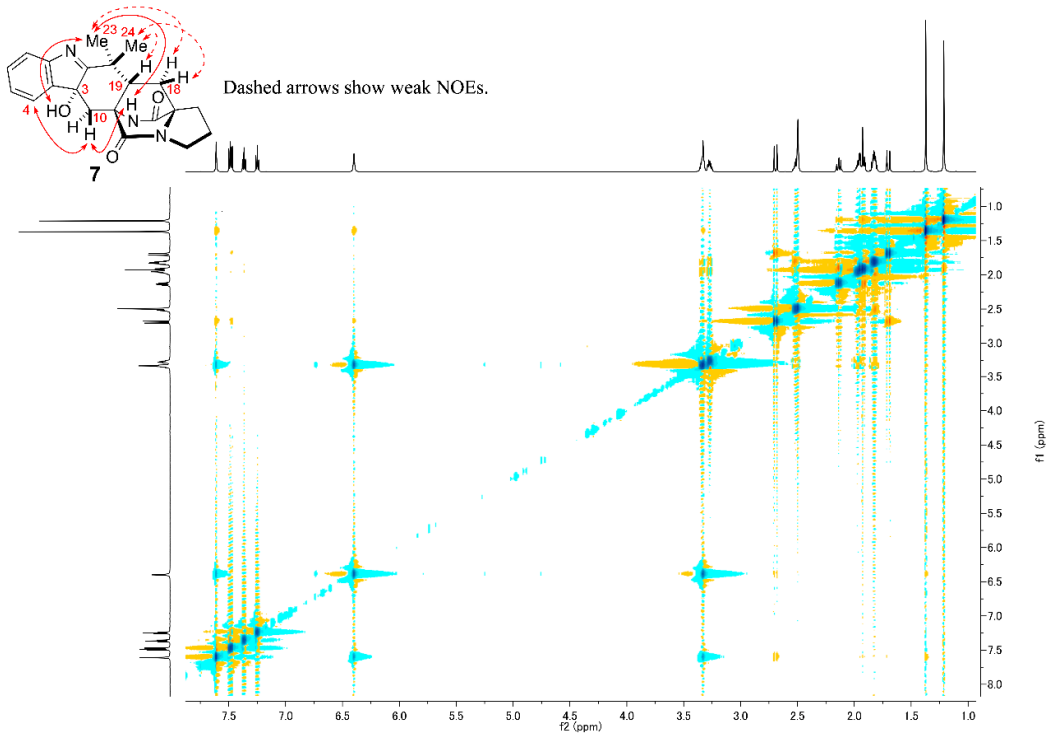
Supplementary Figure 7. ^{1}H-^{1}H COSY spectrum of compound **7** in DMSO-d6.



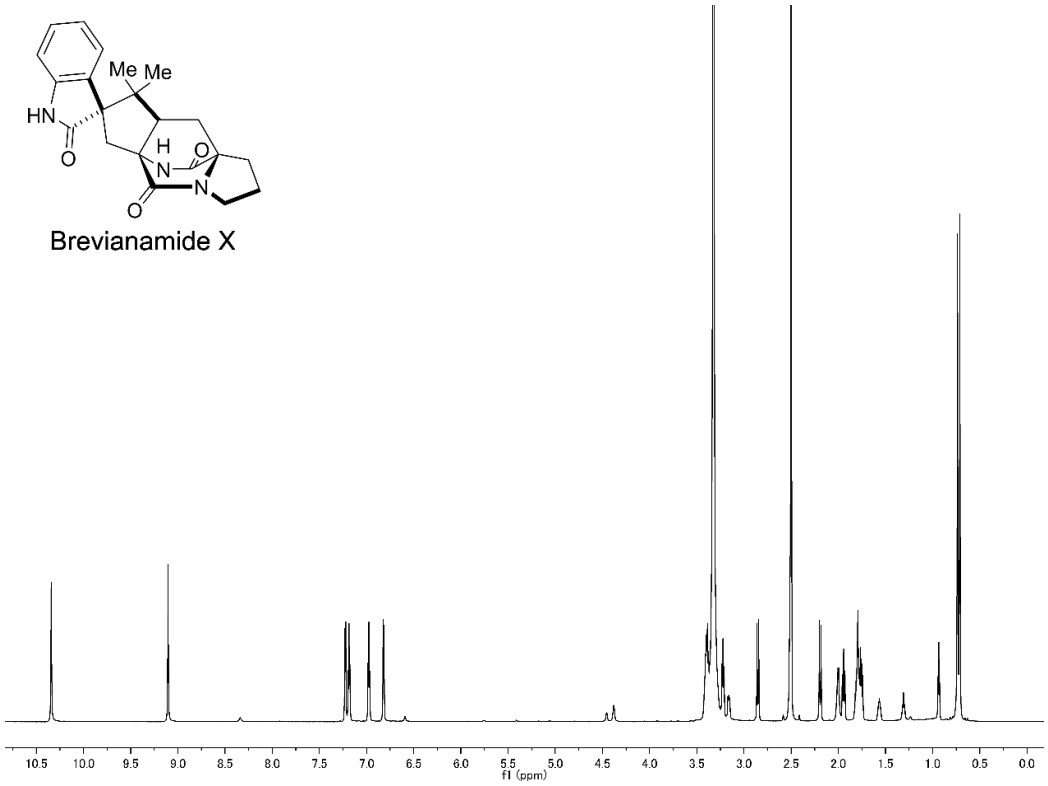
Supplementary Figure 8. HSQC spectrum of compound **7** in DMSO-d6.



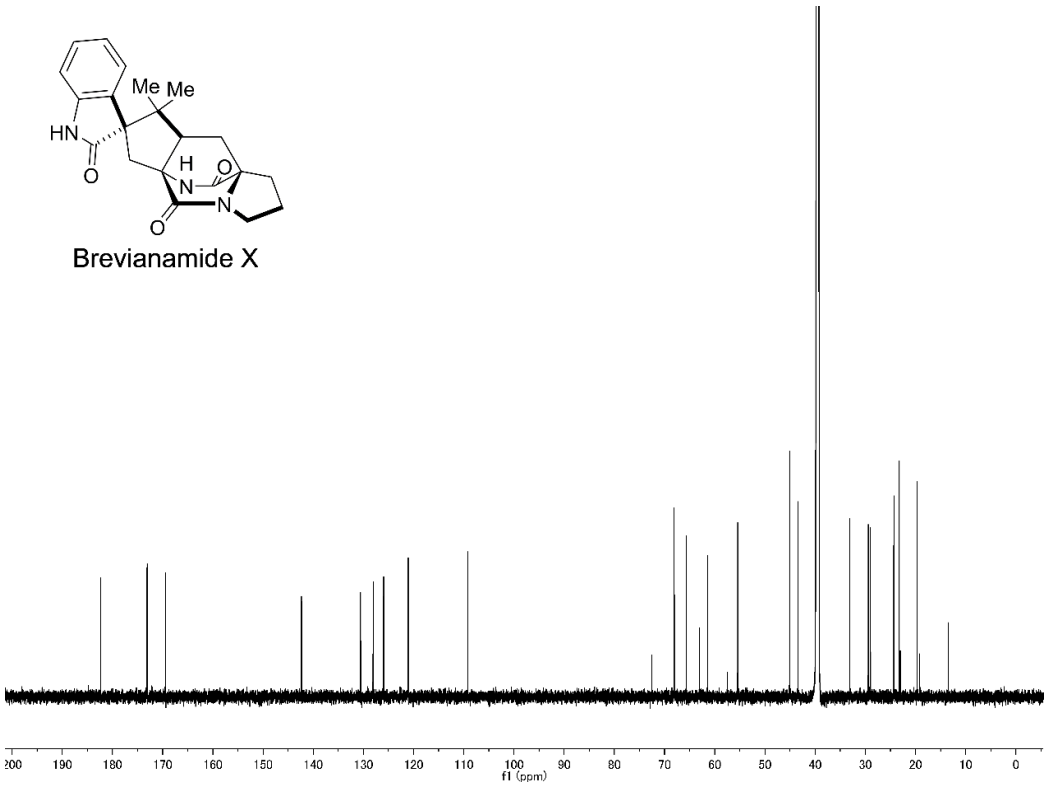
Supplementary Figure 9. HMBC spectrum of compound **7** in DMSO-d6.



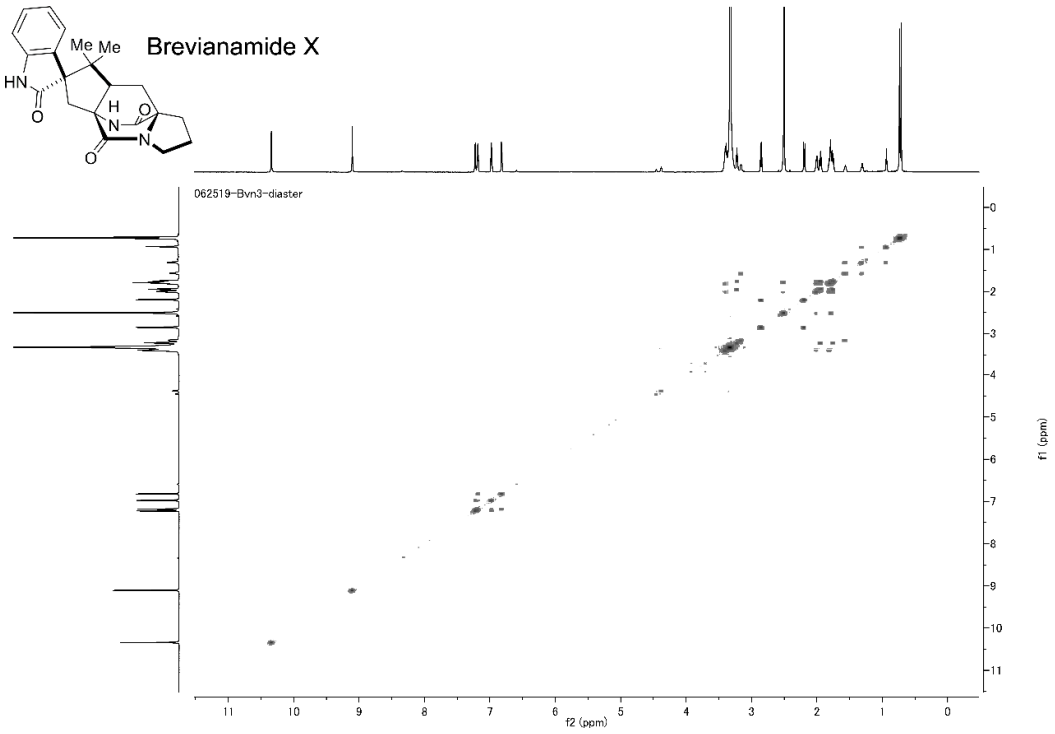
Supplementary Figure 10. NOESY spectrum of compound **7** in DMSO-d6.



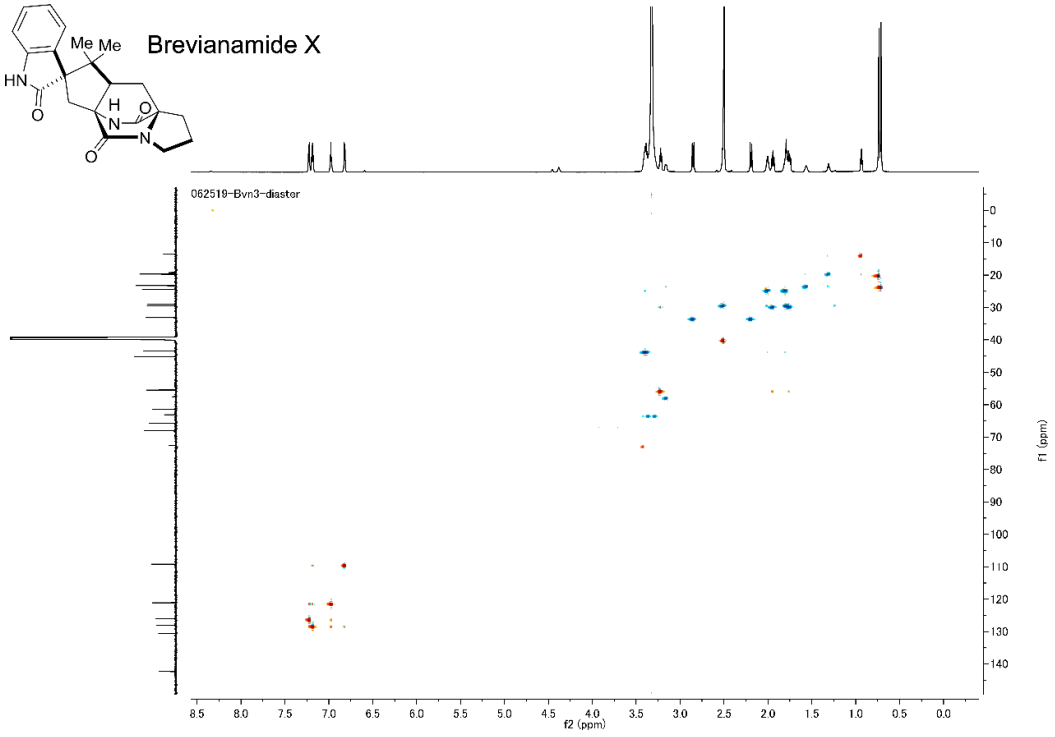
Supplementary Figure 11. ^{1}H NMR spectrum of **BX** in DMSO-d6.



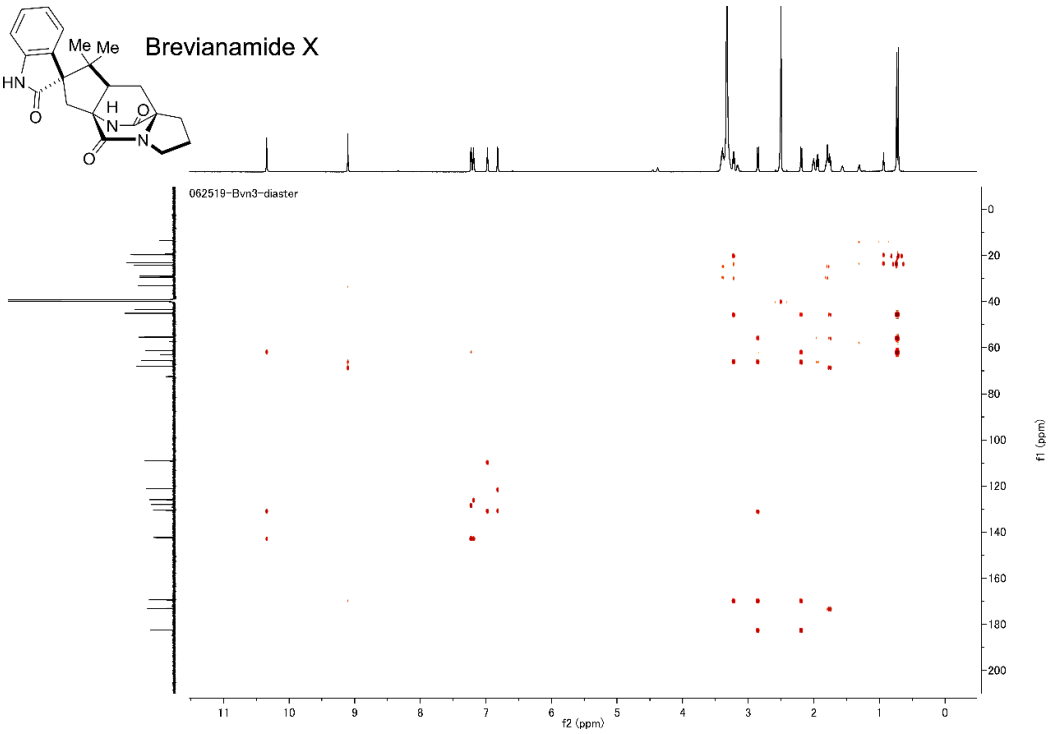
Supplementary Figure 12. ^{13}C NMR spectrum of **BX** in DMSO-d6.



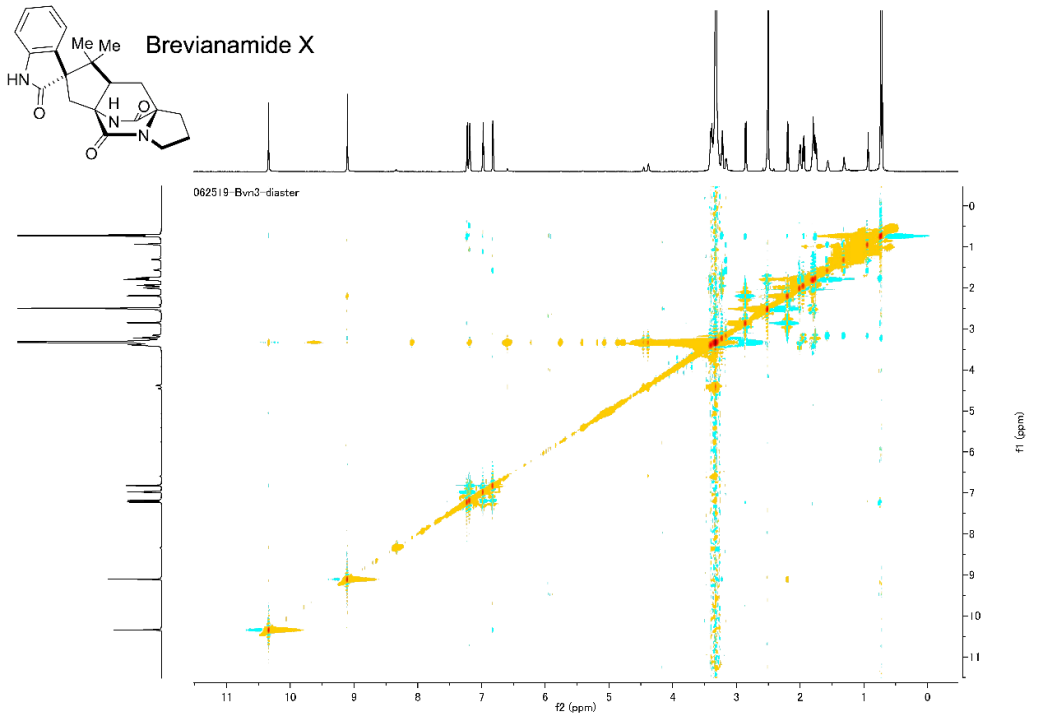
Supplementary Figure 13. ^{1}H-^{1}H COSY spectrum of **BX** in DMSO-d6.



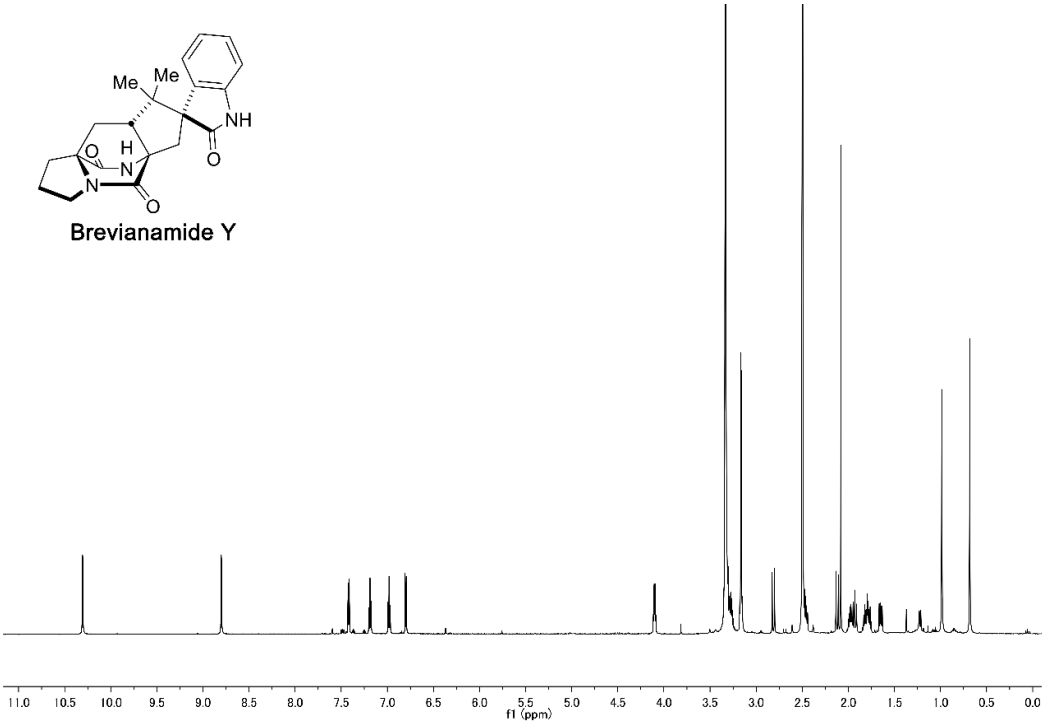
Supplementary Figure 14. HSQC spectrum of **BX** in DMSO-d6.



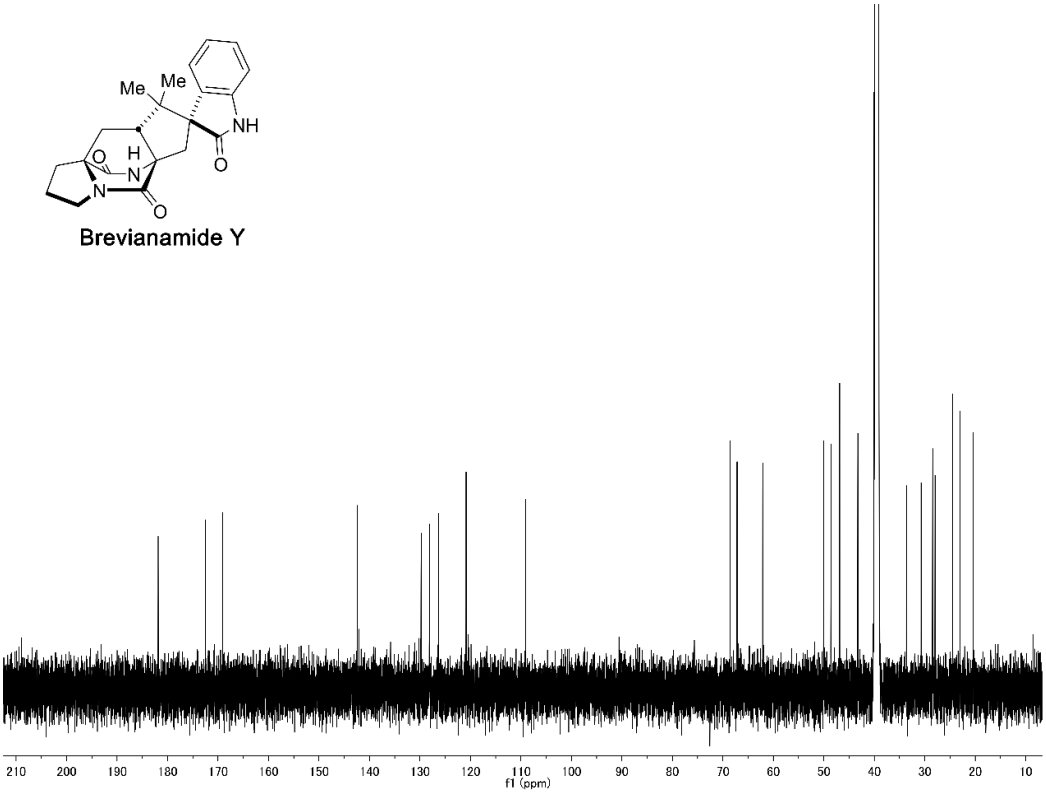
Supplementary Figure 15. HMBC spectrum of **BX** in DMSO-d6.



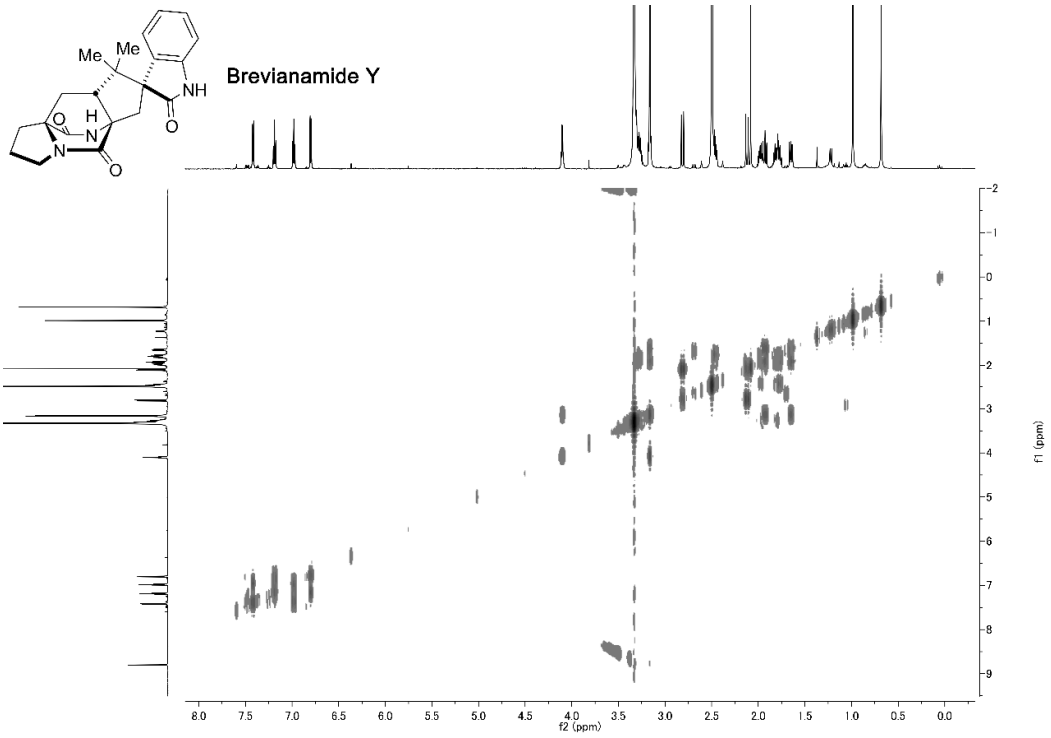
Supplementary Figure 16. NOESY spectrum of **BX** in DMSO-d6.



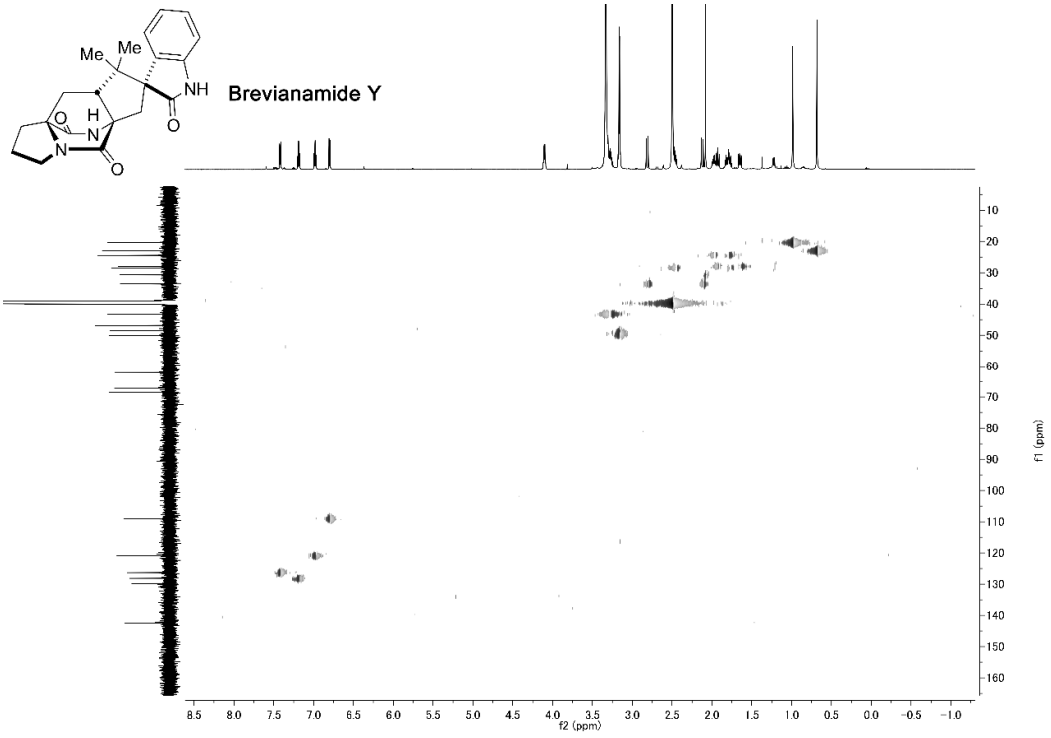
Supplementary Figure 17. ^{1}H NMR spectrum of **BY** in DMSO-d6.



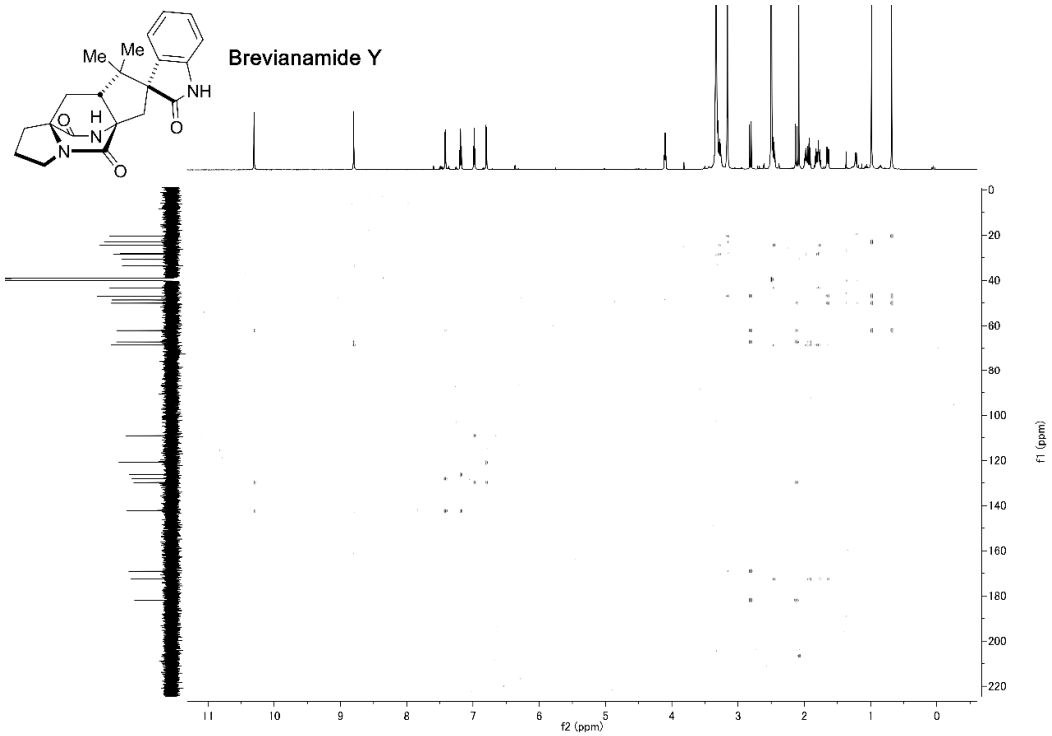
Supplementary Figure 18. ^{13}C NMR spectrum of **BY** in DMSO-d6.



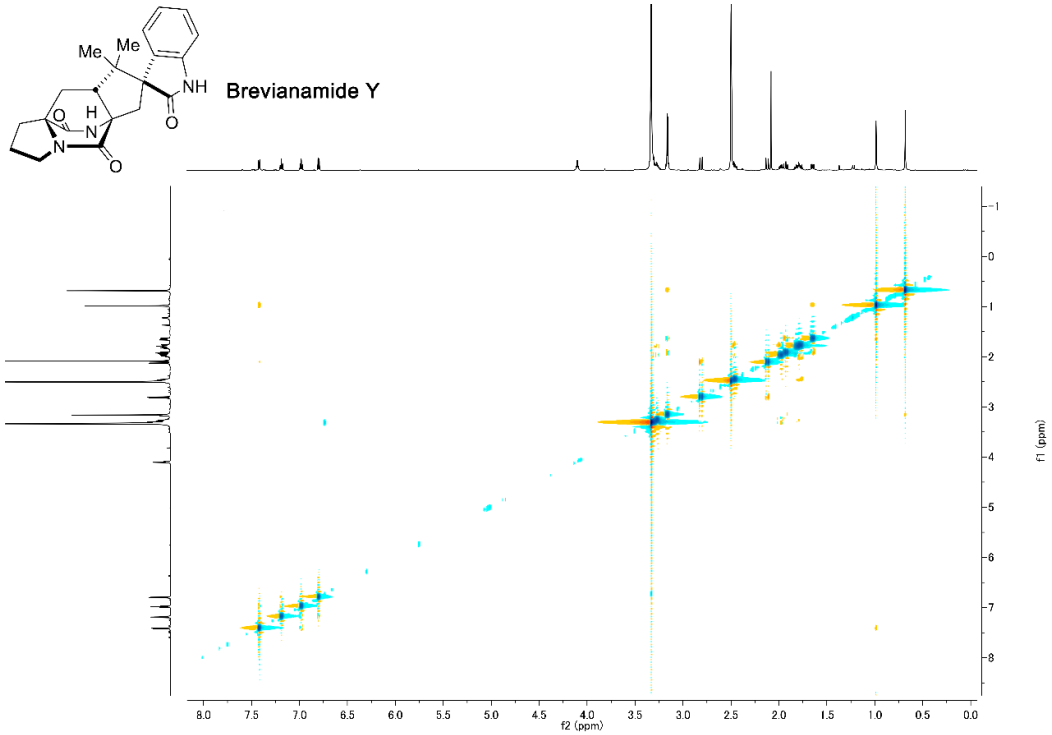
Supplementary Figure 19. ^{1}H-^{1}H COSY spectrum of **BY** in DMSO-d6.



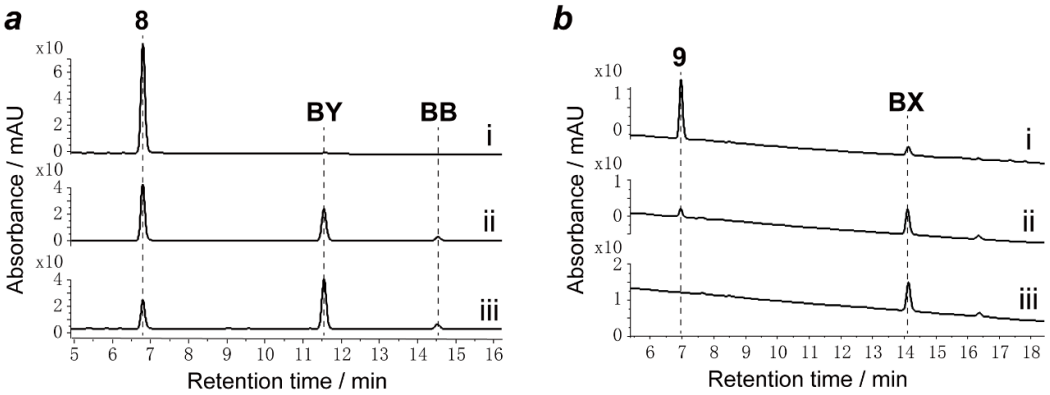
Supplementary Figure 20. HSQC spectrum of **BY** in DMSO-d6.



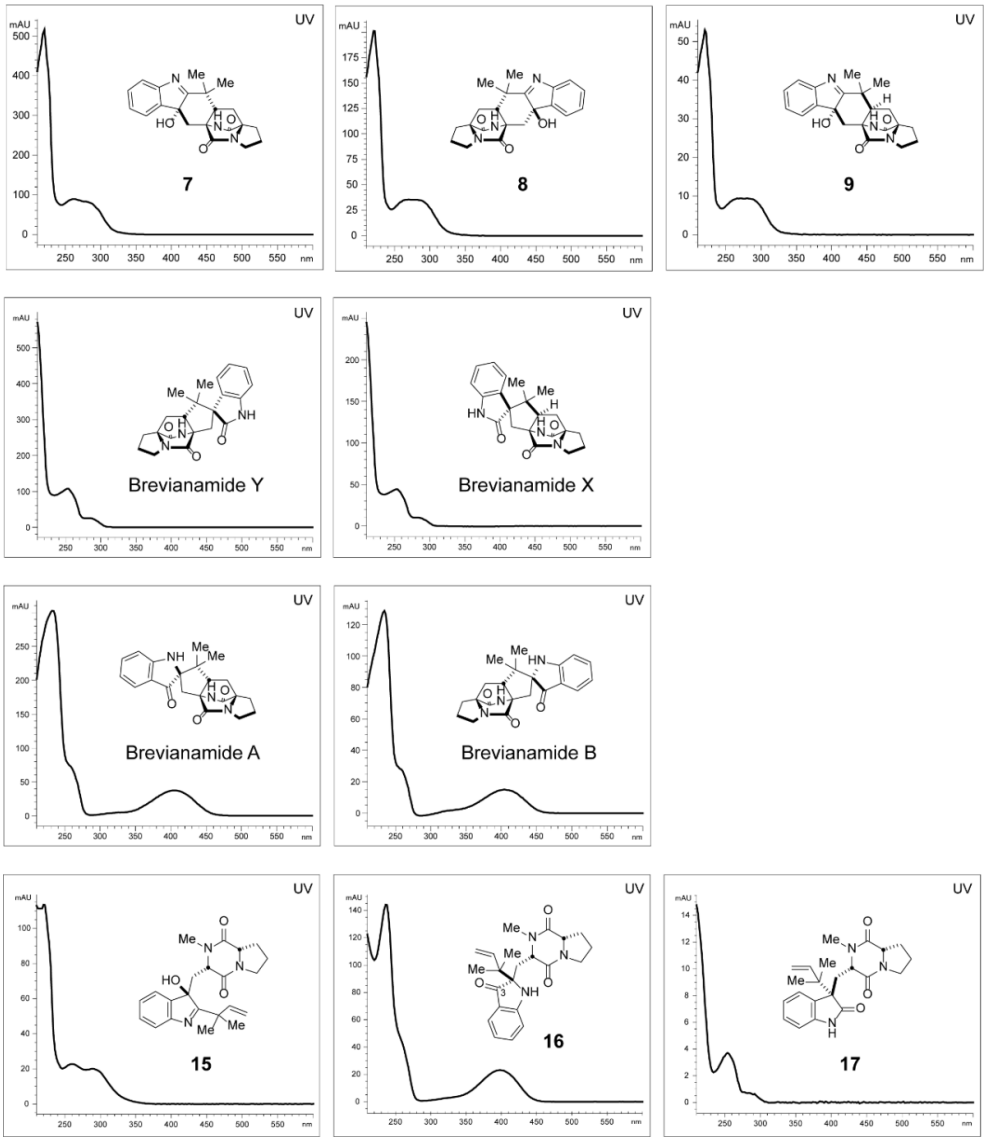
Supplementary Figure 21. HMBC spectrum of **BY** in DMSO-d6.



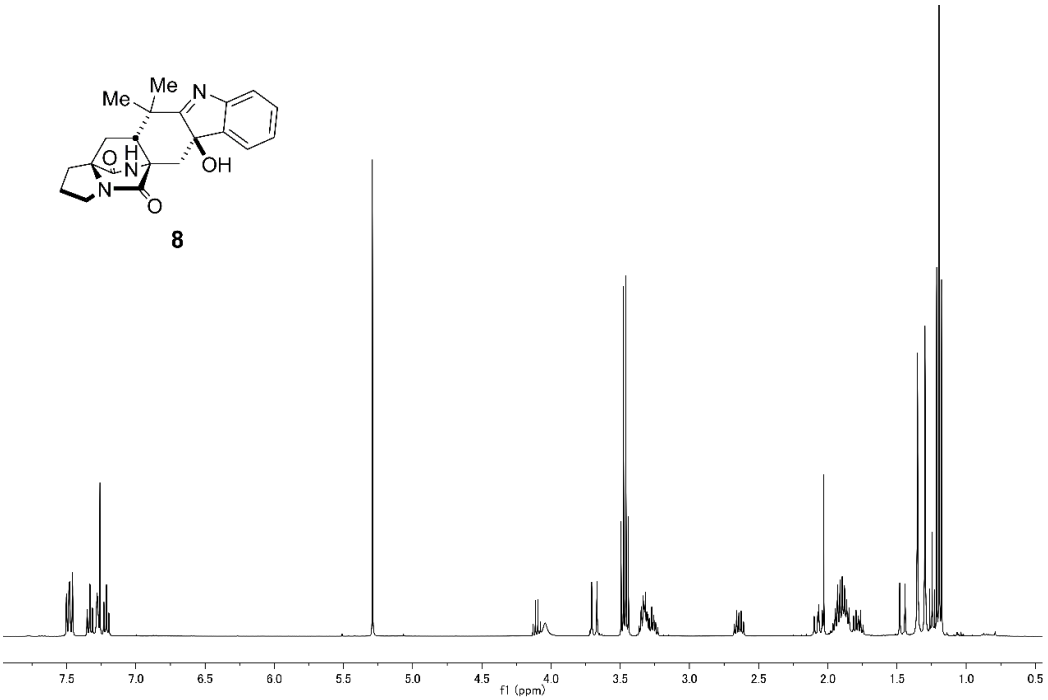
Supplementary Figure 22. NOESY spectrum of **BY** in DMSO-d6.



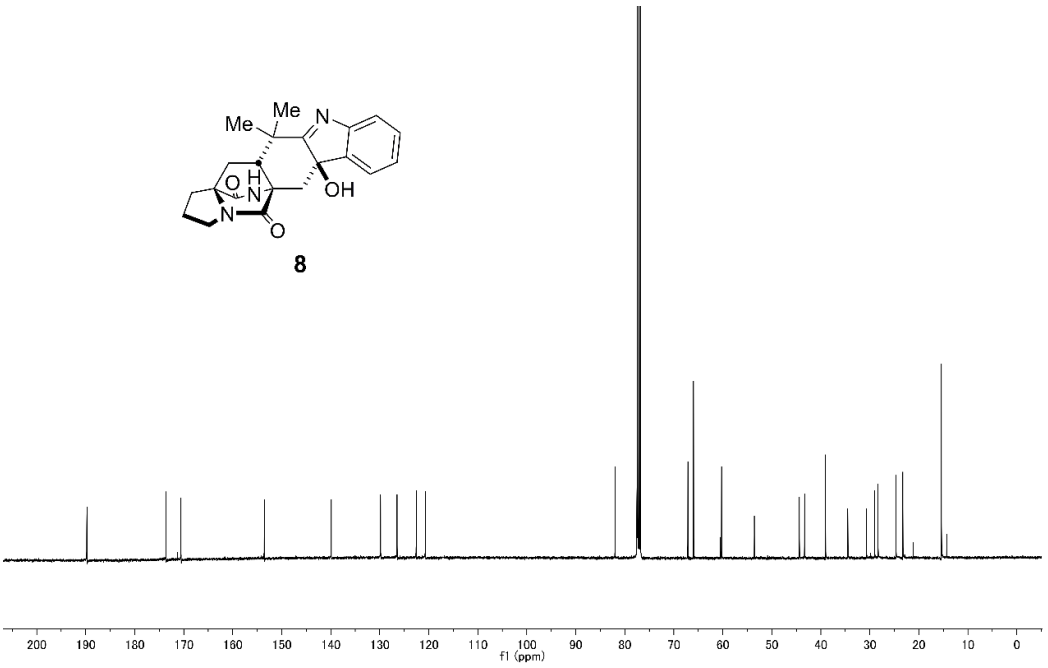
Supplementary Figure 23. Stability assays of **8** (**a**) and **9** (**b**) in 30% methanol-water solution. HPLC analysis of **8** and **9** after 30 min (i), 24h (ii), and 48h (iii).



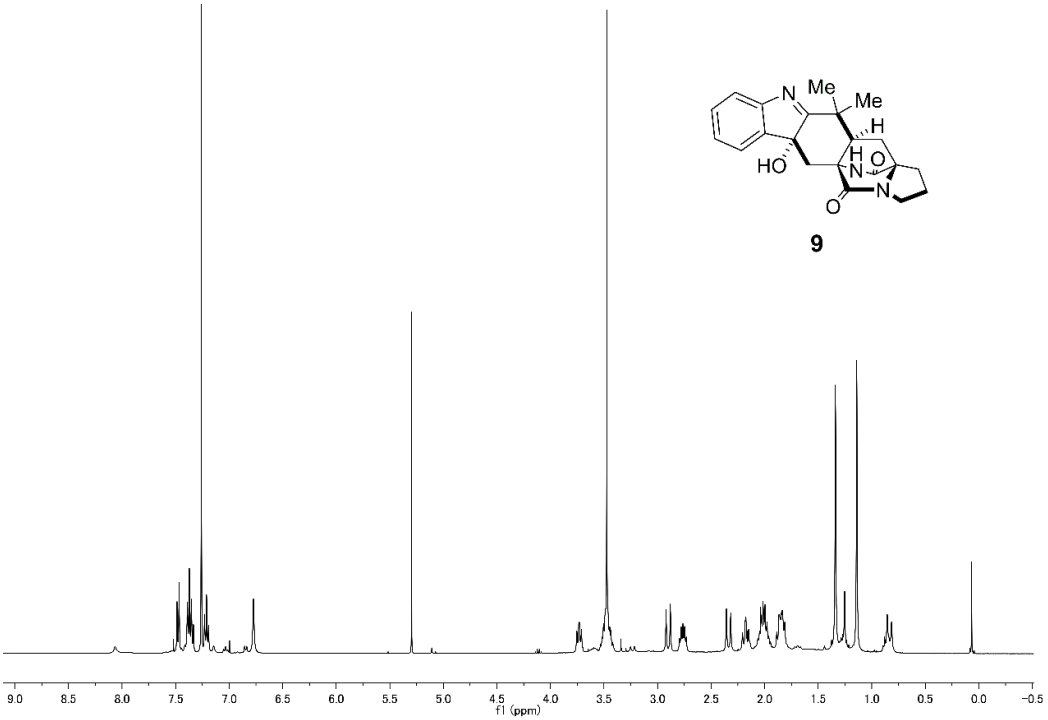
Supplementary Figure 24. UV spectra of different Brevianamide derivatives.



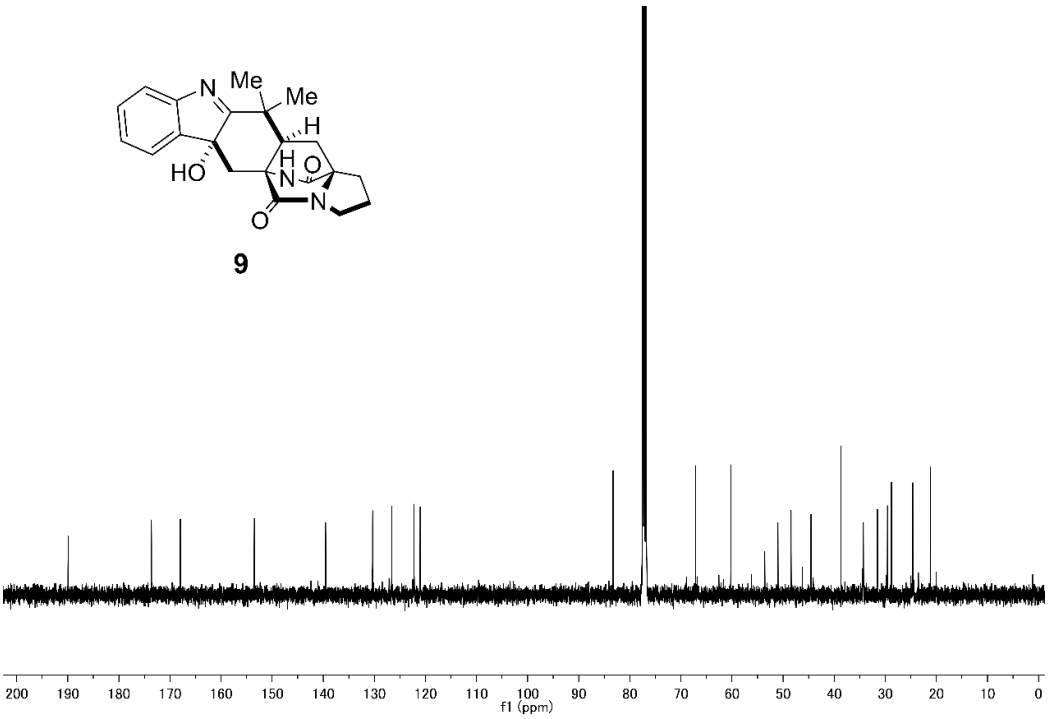
Supplementary Figure 25. ^{1}H NMR spectrum of compound **8** in Chloroform-*d*.



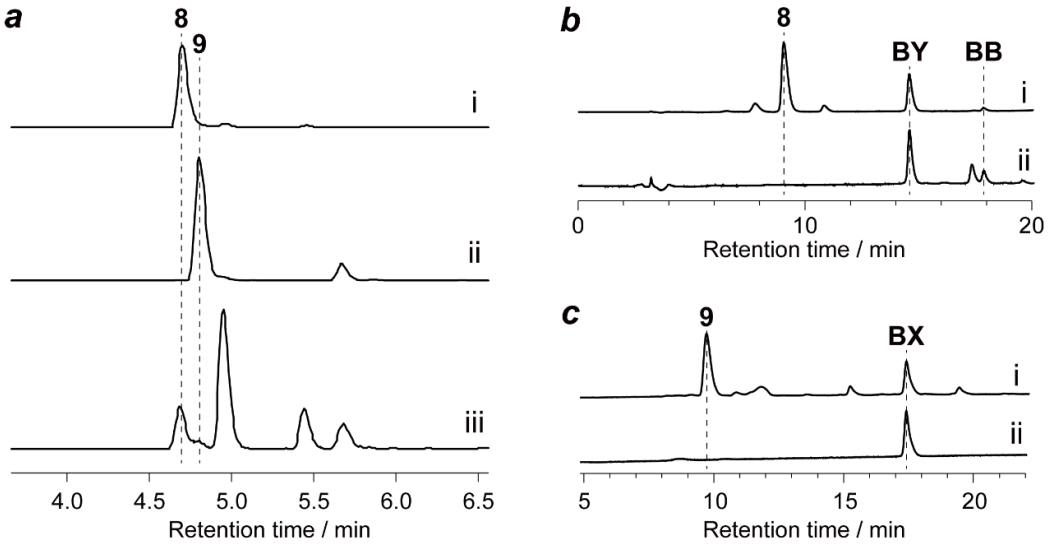
Supplementary Figure 26. ^{13}C NMR spectrum of compound **8** in Chloroform-*d*.



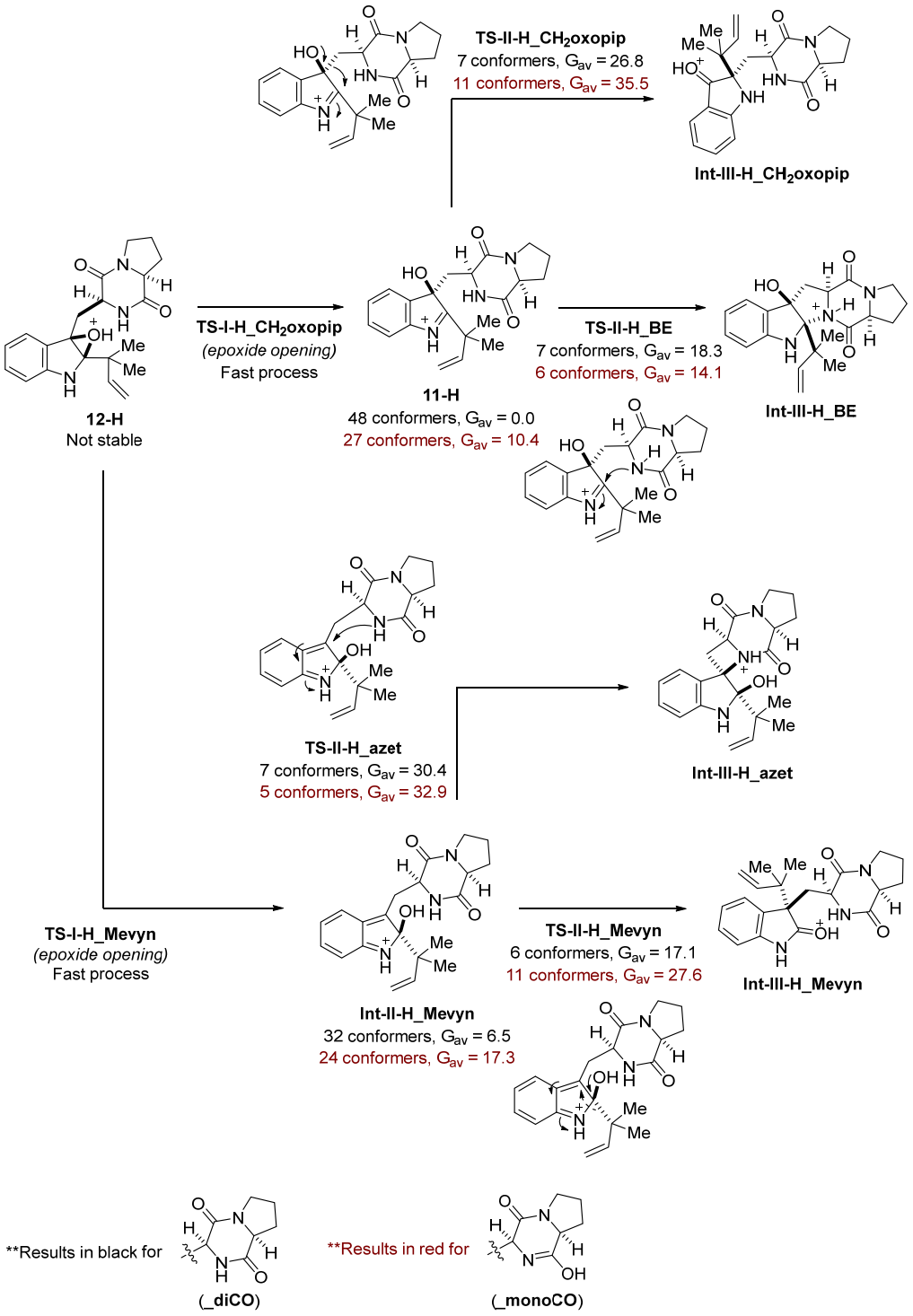
Supplementary Figure 27. ^{1}H NMR spectrum of compound **9** in Chloroform-*d*.



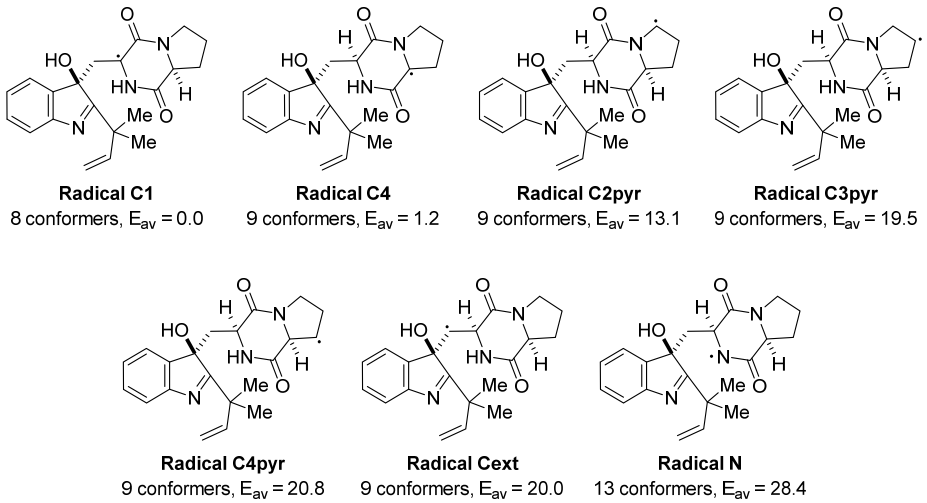
Supplementary Figure 28. ^{13}C NMR spectrum of compound **9** in Chloroform-*d*.



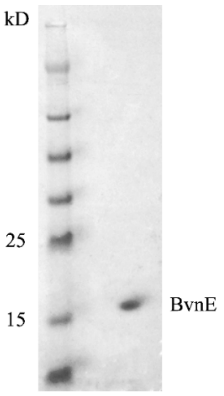
Supplementary Figure 29. HPLC analysis (230 nm) of chemically synthesized **8** and **9**. **a**, Comparison of synthetic **8** (i) and synthetic **9** (ii) with the *Pb-bvnE*-KO profile (iii). **b**, Stability assays of **8** (i, synthetic **8** in 30% methanol-water solution after 24 h; ii, authentic standards of **BY**, **BA** and **BB**). **c**. Stability assays of **9** (i, synthetic **9** in 30% methanol-water solution after 24 h; ii, the authentic standard of **BX**).



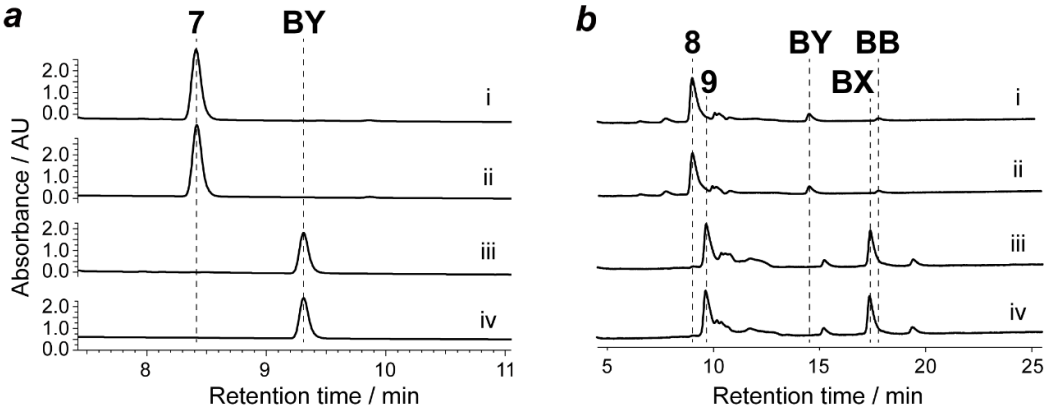
Supplementary Figure 30. Pathways from protonated compound **12** (**12-H**) to form **BE** including the number of conformers found for each reaction step. **12-H** was used instead of **12** for calculation because the activation barrier for **BE** formation was unreasonably high with neutral or deprotonated **12** (data not shown).



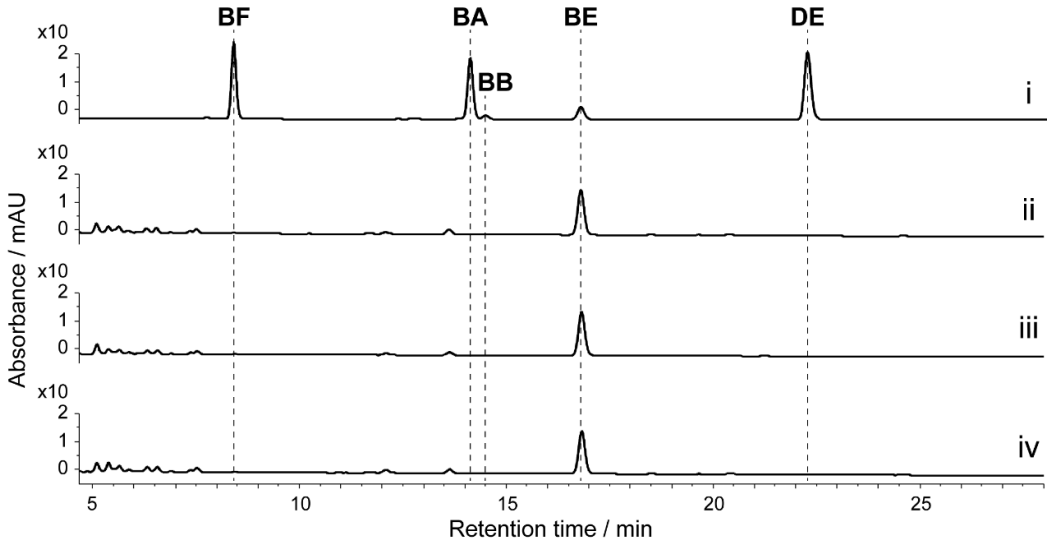
Supplementary Figure 31. Relative Boltzmann weighted electronic energies of different radicals formed from **11**.



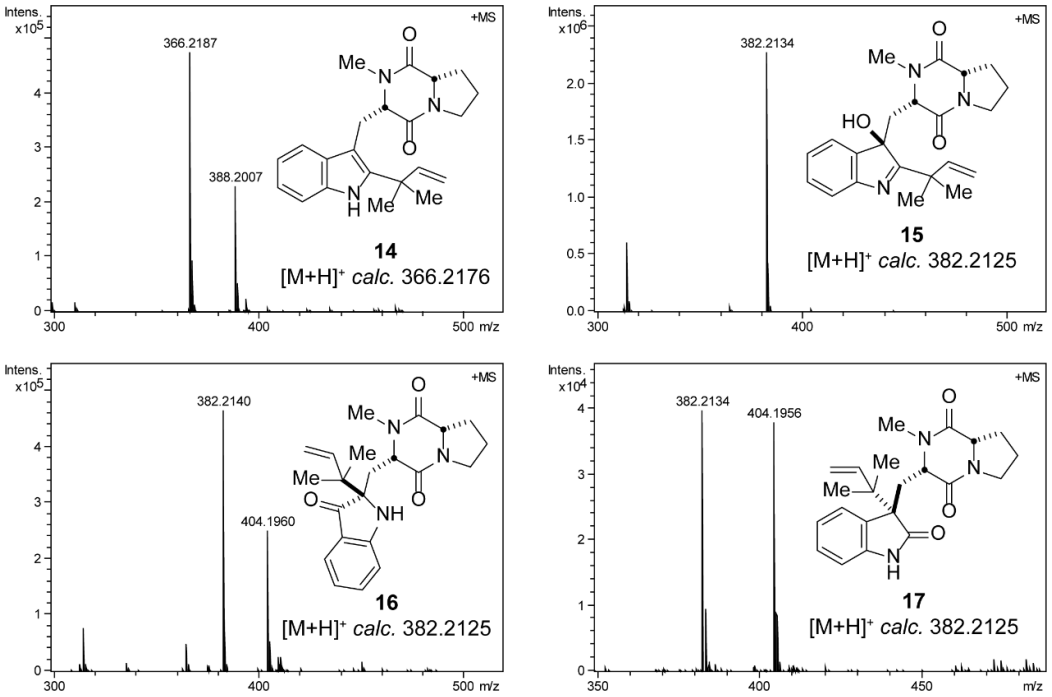
Supplementary Figure 32. SDS-PAGE analysis of purified *N*-His_6-tagged BvnE enzyme.



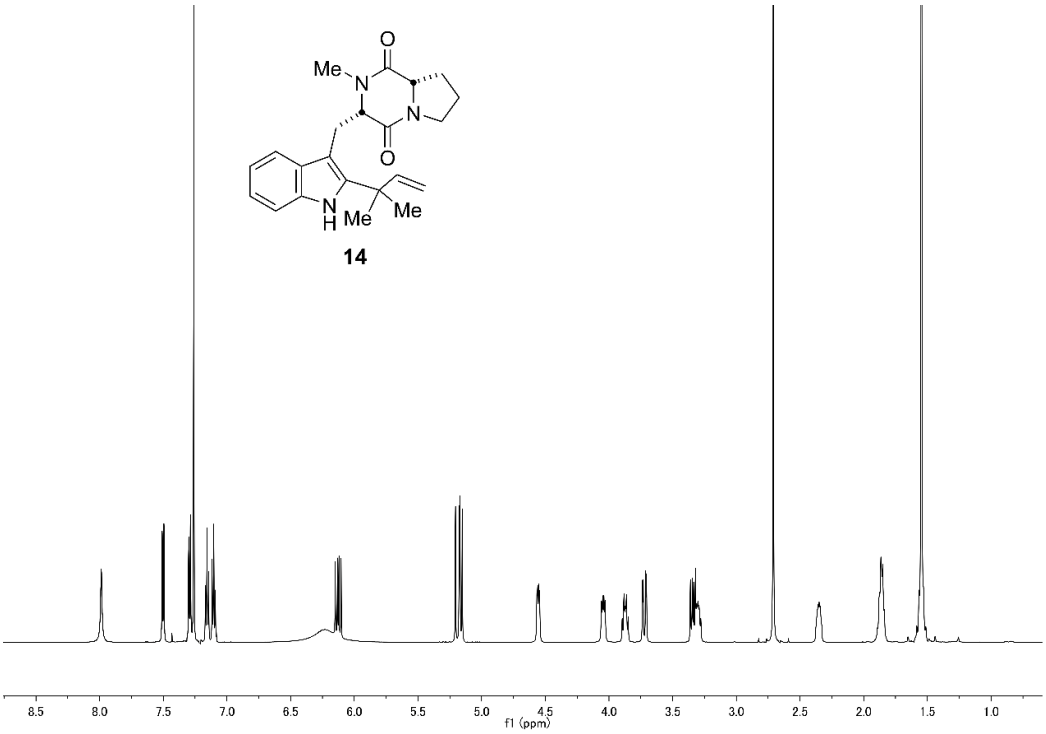
Supplementary Figure 33. HPLC analysis (230 nm) of BvnE *in vitro* reactions with compounds **7**, **BY**, **8** and **9** as potential substrates. **a**, i, BvnE + **7**; ii, boiled BvnE + **7** as a control of i; iii, BvnE + **BY**; iv, boiled BvnE + **BY** as a control of iii. **b**. i, BvnE + **8**; ii, boiled BvnE + **8** as a control of i; iii, BvnE + **9**; iv, boiled BvnE + **9** as a control of iii. Compound **8** and **9** can spontaneously collapse to **BY**/**BB** and **BX**, respectively.



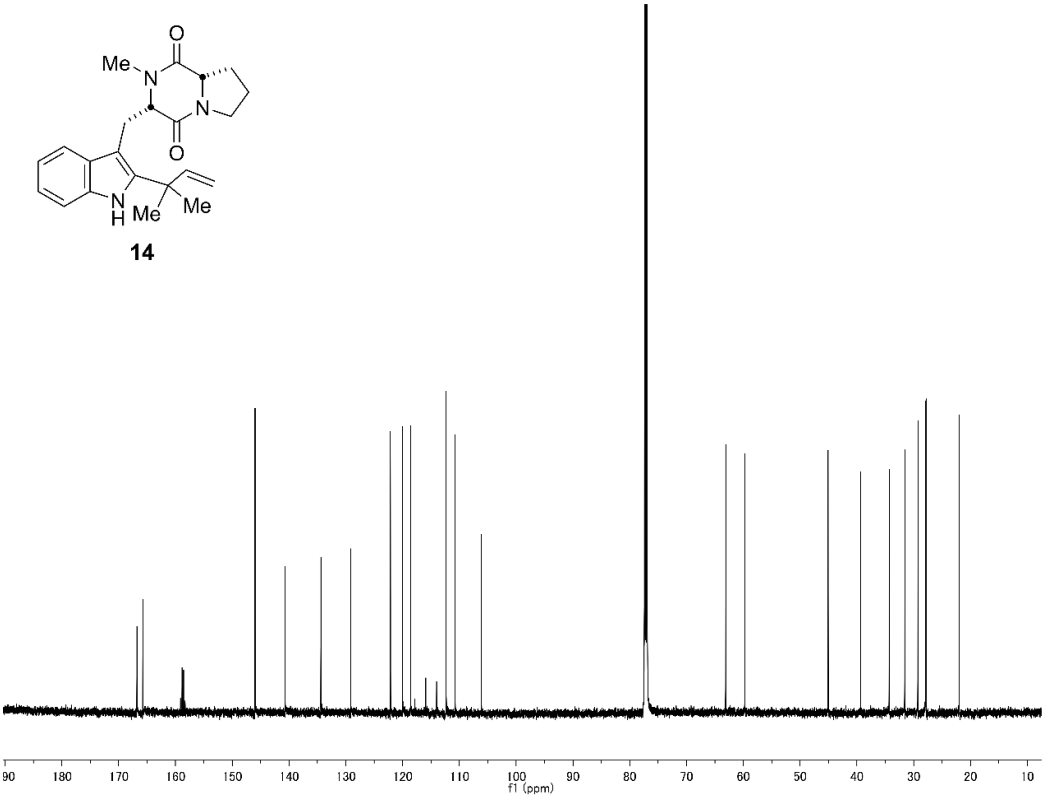
Supplementary Figure 34. HPLC analysis (230 nm) of different *Ao* transformants upon feeding of **BE**. i, standards; ii, *Ao-bvnD* + **BE**; iii, *Ao-bvnDE* + **BE**; iv, *Ao*-WT + **BE**.



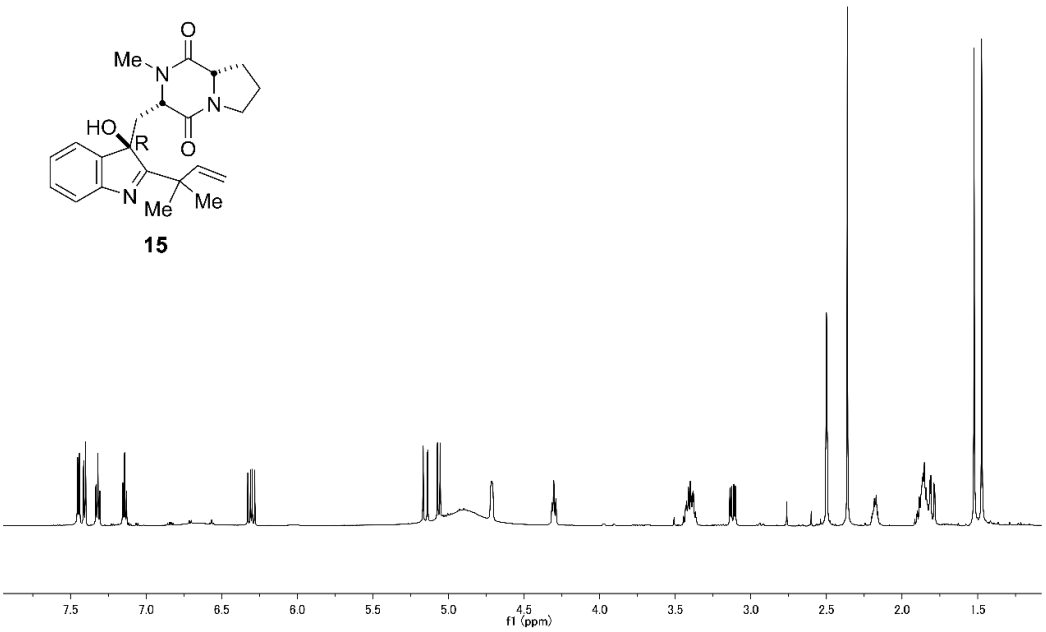
Supplementary Figure 35. HRMS spectra of the *N*-methylated derivatives.



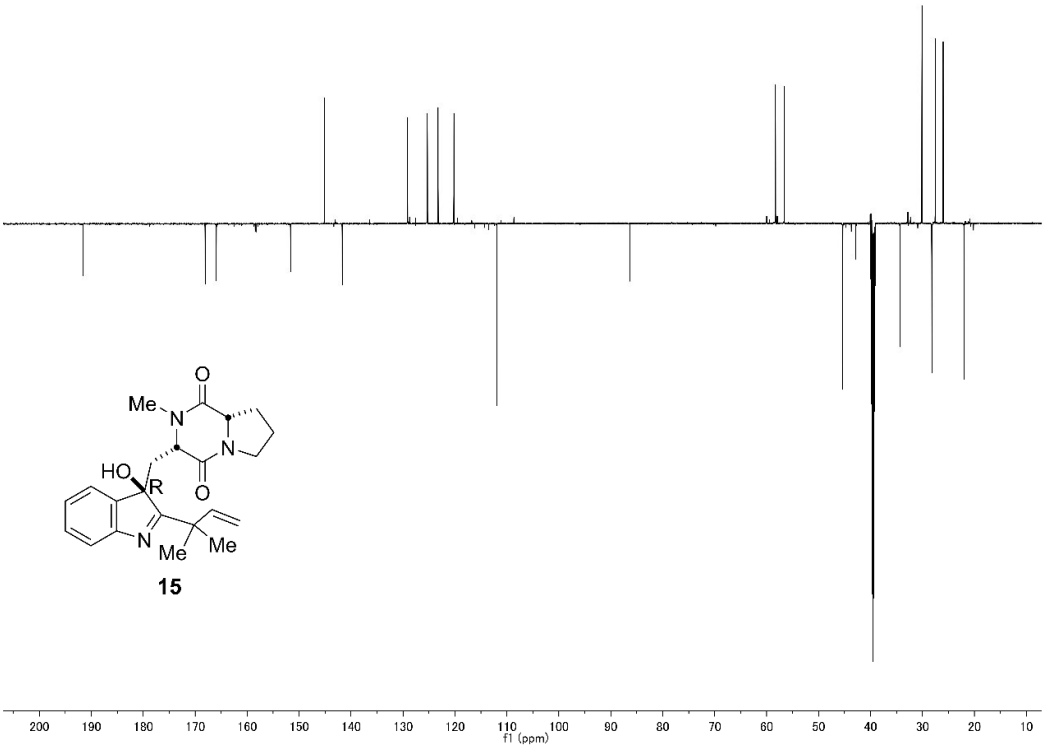
Supplementary Figure 36. ^{1}H NMR spectrum of compound **14** in $CDCl_3$.



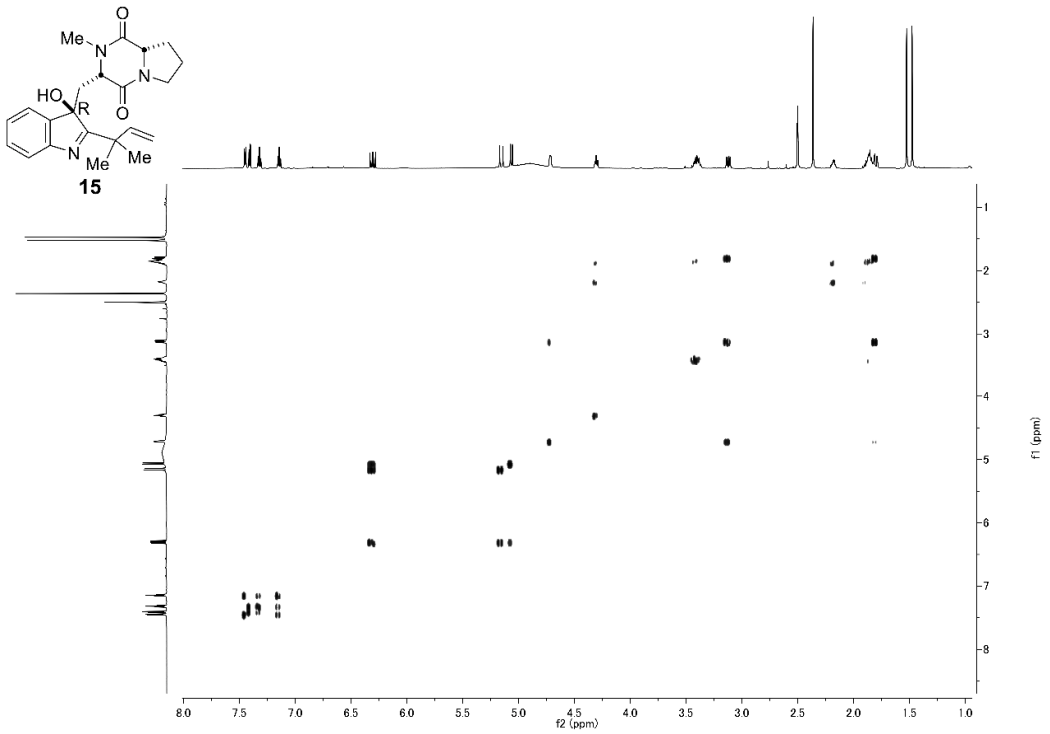
Supplementary Figure 37. ^{13}C NMR spectrum of compound **14** in $CDCl_3$.



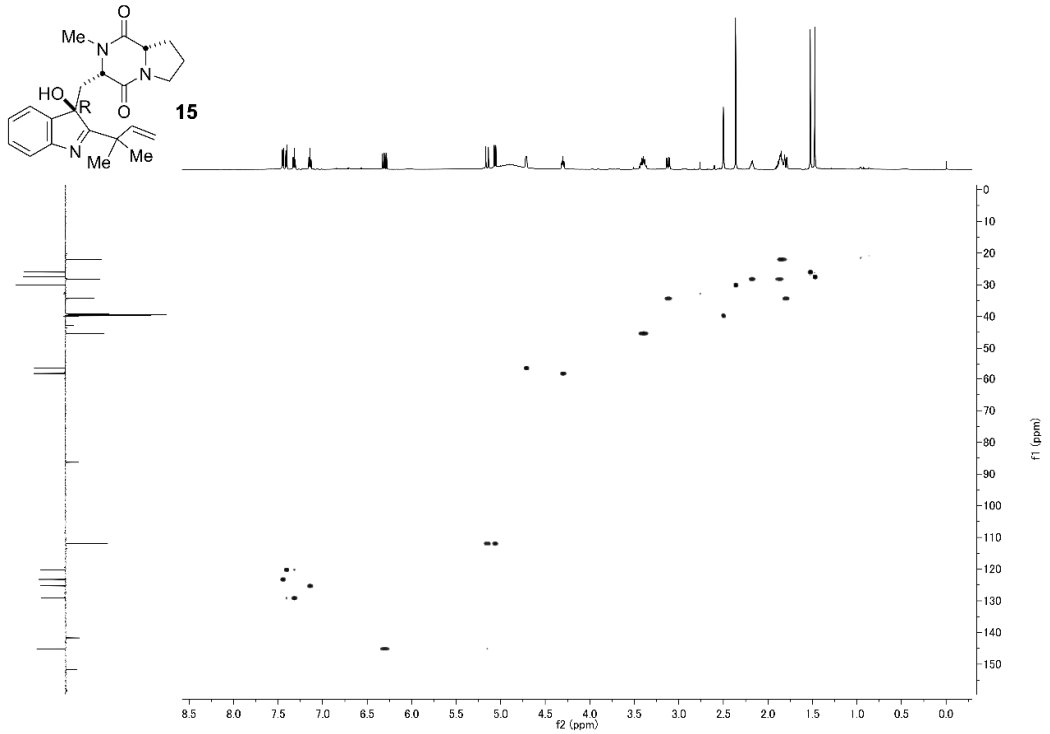
Supplementary Figure 38. ^{1}H NMR spectrum of compound **15** in $CDCl_3$.



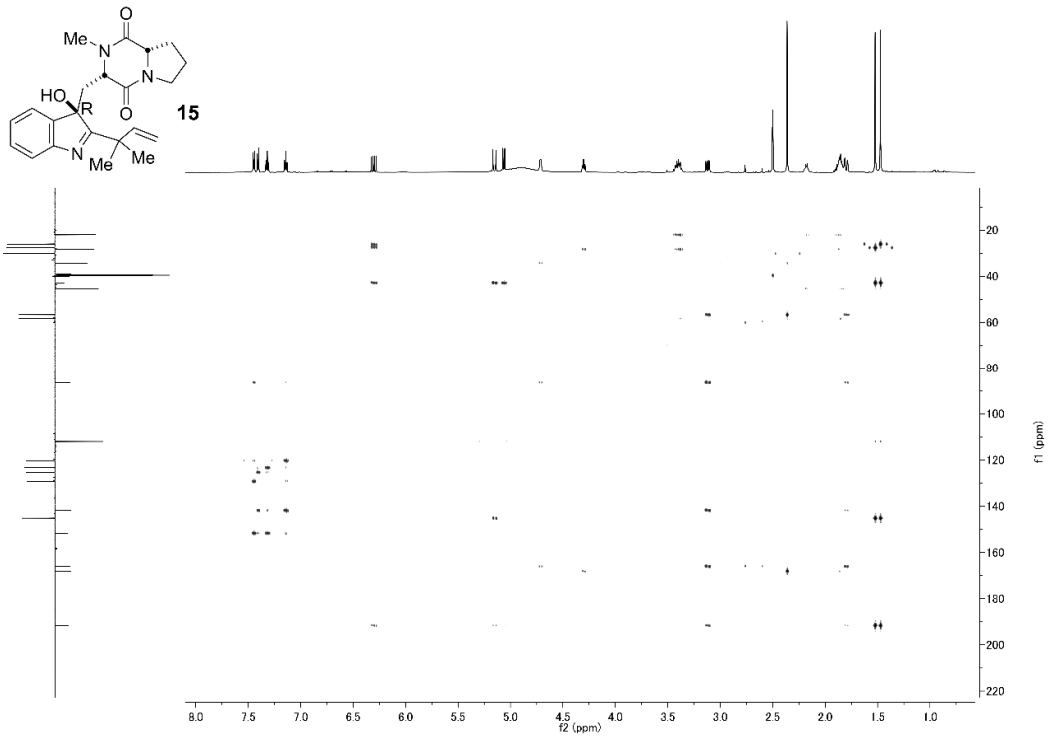
Supplementary Figure 39. DEPT ^{13}C spectrum of compound **15** in $CDCl_3$.



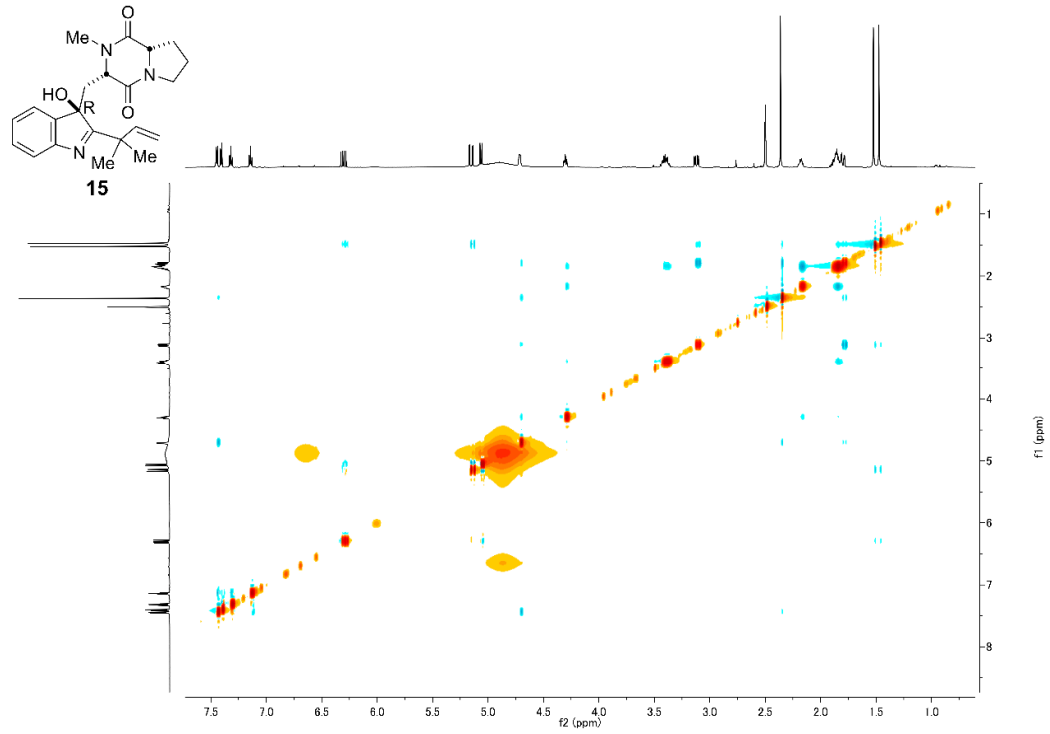
Supplementary Figure 40. ^{1}H-^{1}H COSY spectrum of compound **15** in $CDCl_3$.



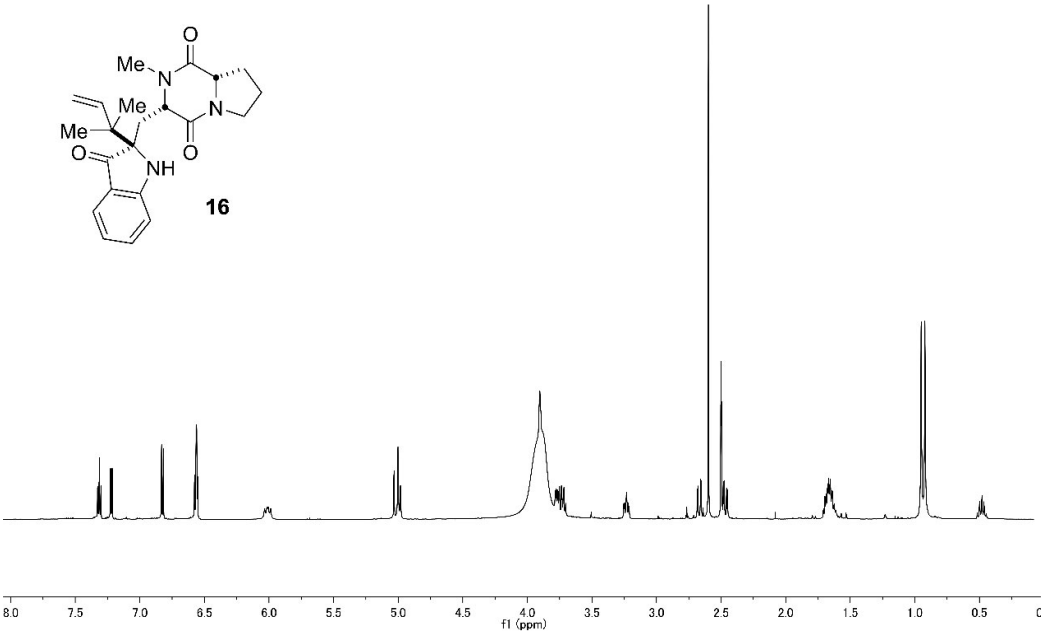
Supplementary Figure 41. HSQC spectrum of compound **15** in $CDCl_3$.



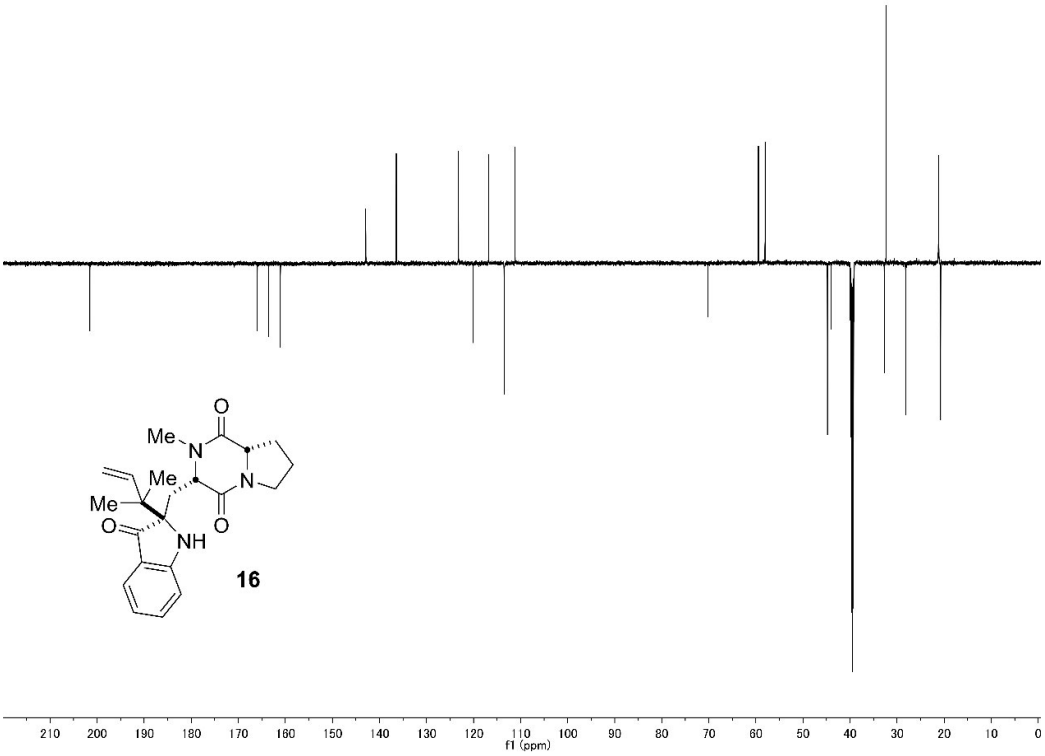
Supplementary Figure 42. HMBC spectrum of compound **15** in $CDCl_3$.



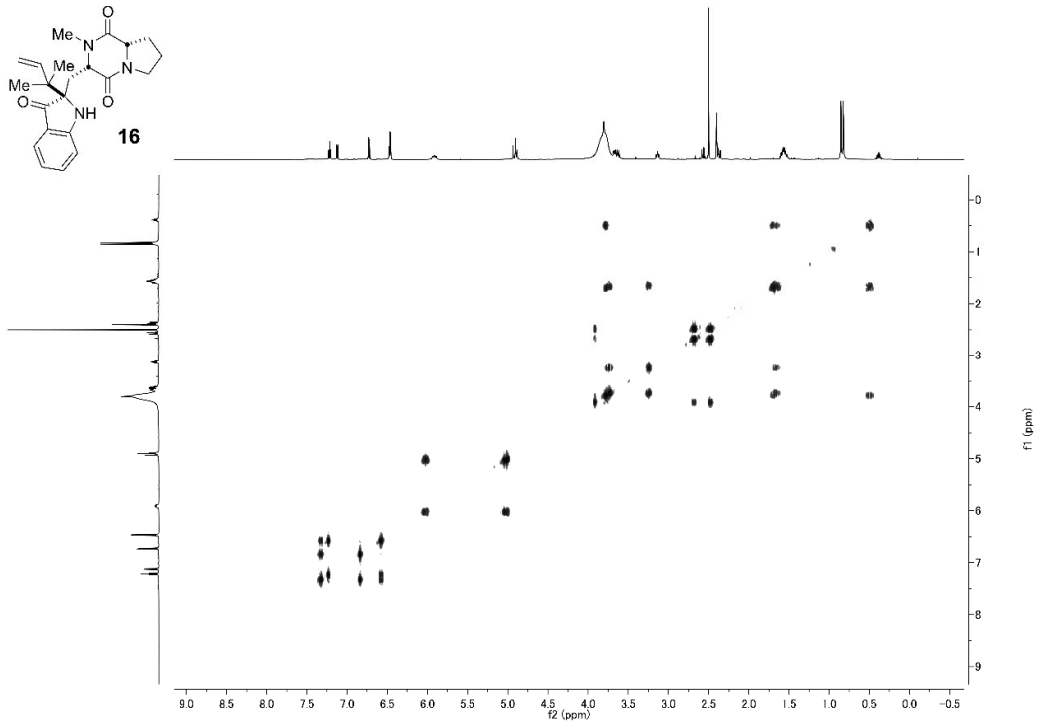
Supplementary Figure 43. NOESY spectrum of compound **15** in $CDCl_3$.



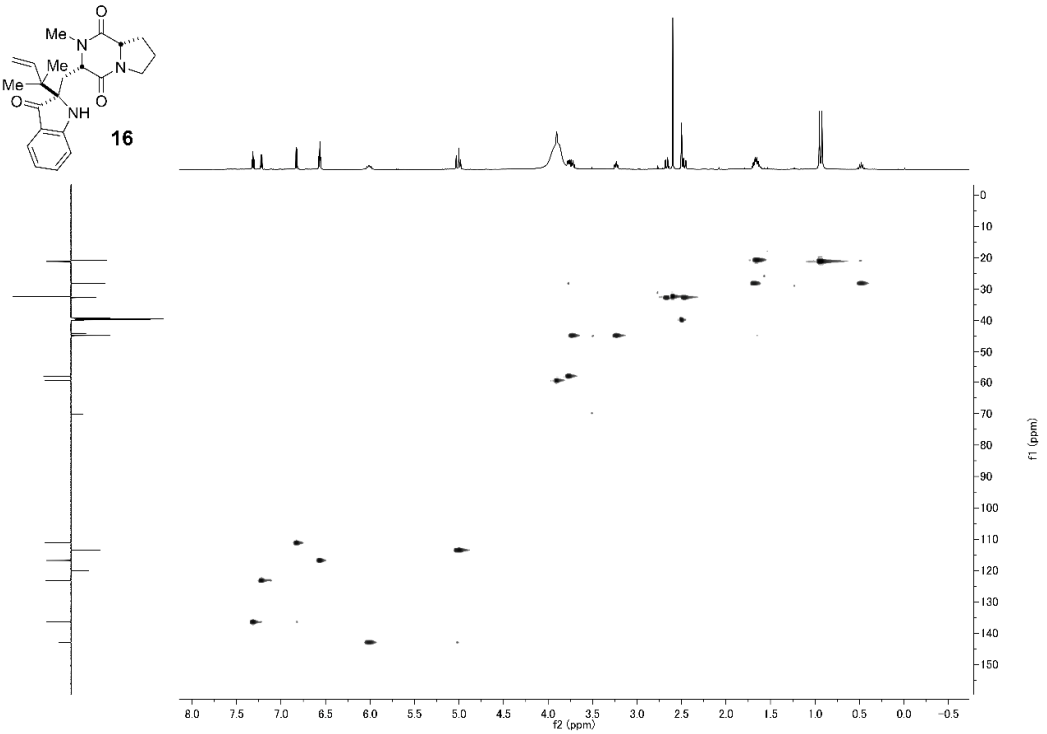
Supplementary Figure 44. ^{1}H NMR spectrum of compound **16** in $CDCl_3$.



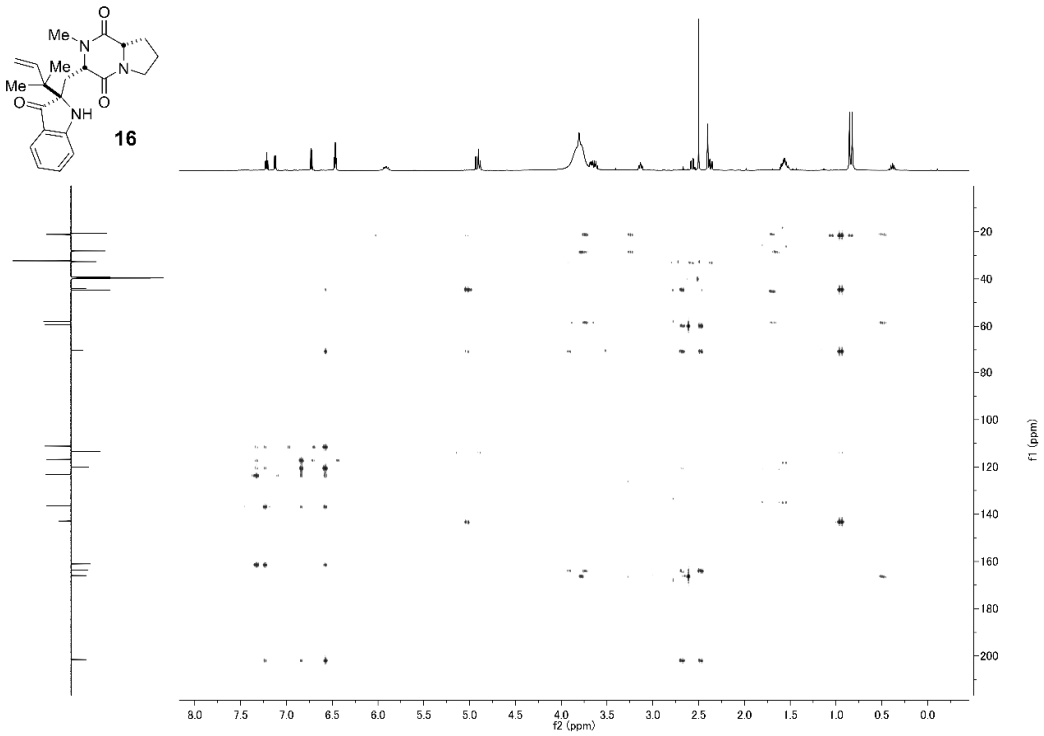
Supplementary Figure 45. DEPT ^{13}C spectrum of compound **16** in $CDCl_3$.



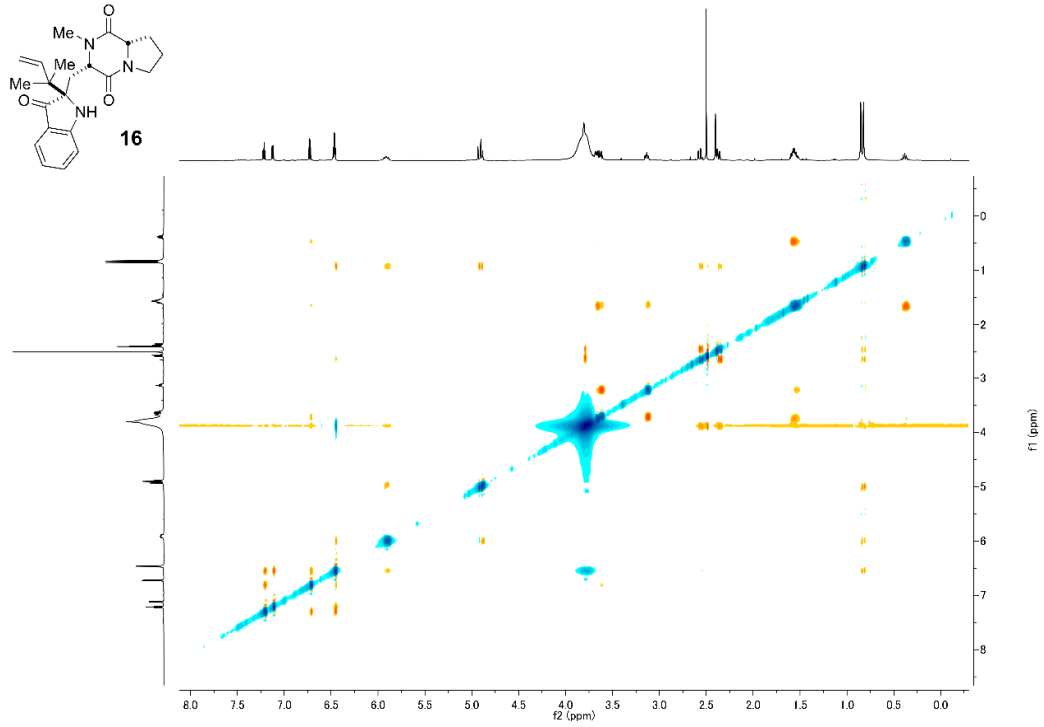
Supplementary Figure 46. ^{1}H-^{1}H COSY spectrum of compound **16** in $CDCl_3$.



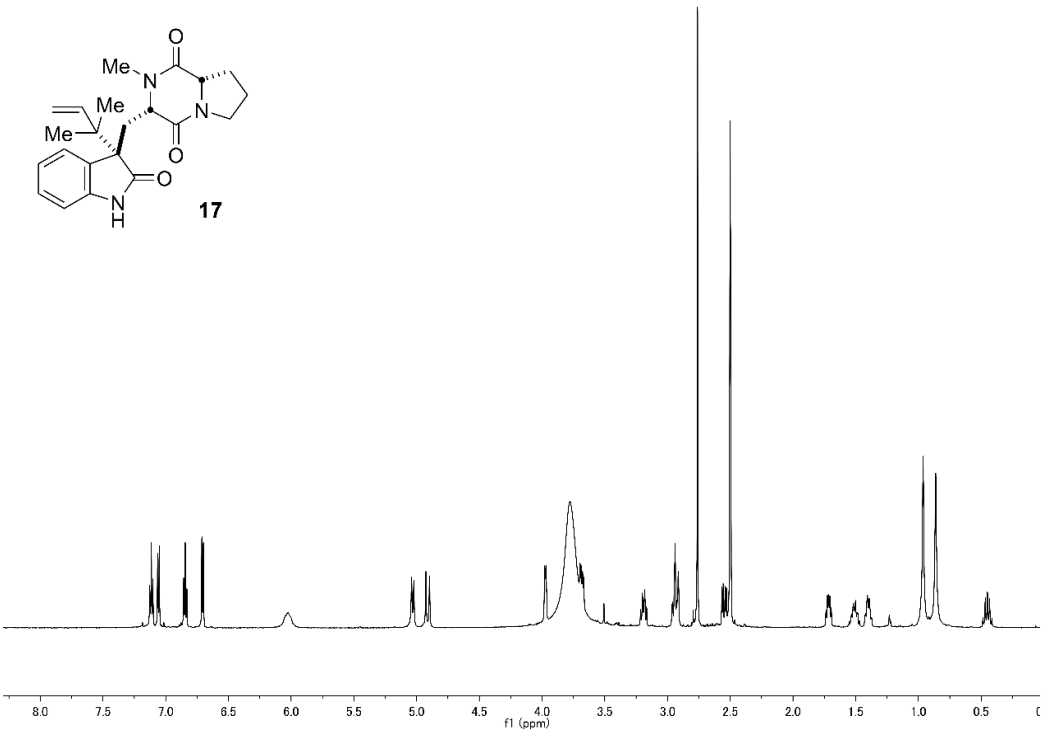
Supplementary Figure 47. HSQC spectrum of compound **16** in $CDCl_3$.



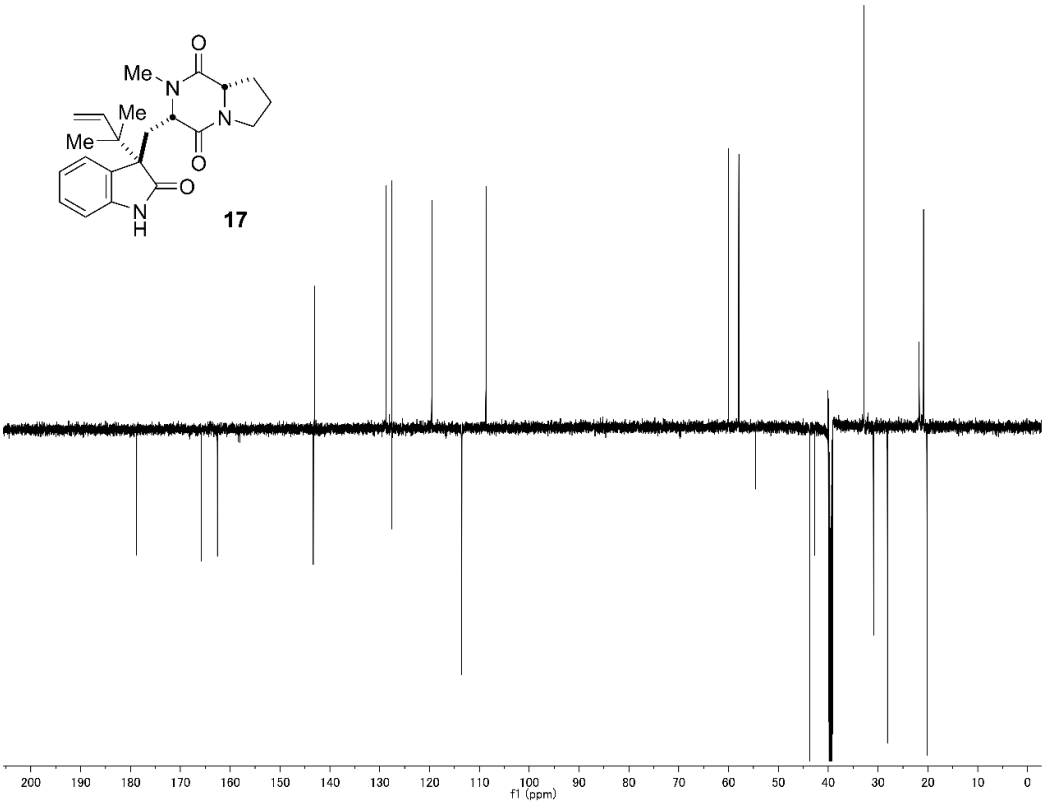
Supplementary Figure 48. HMBC spectrum of compound **16** in $CDCl_3$.



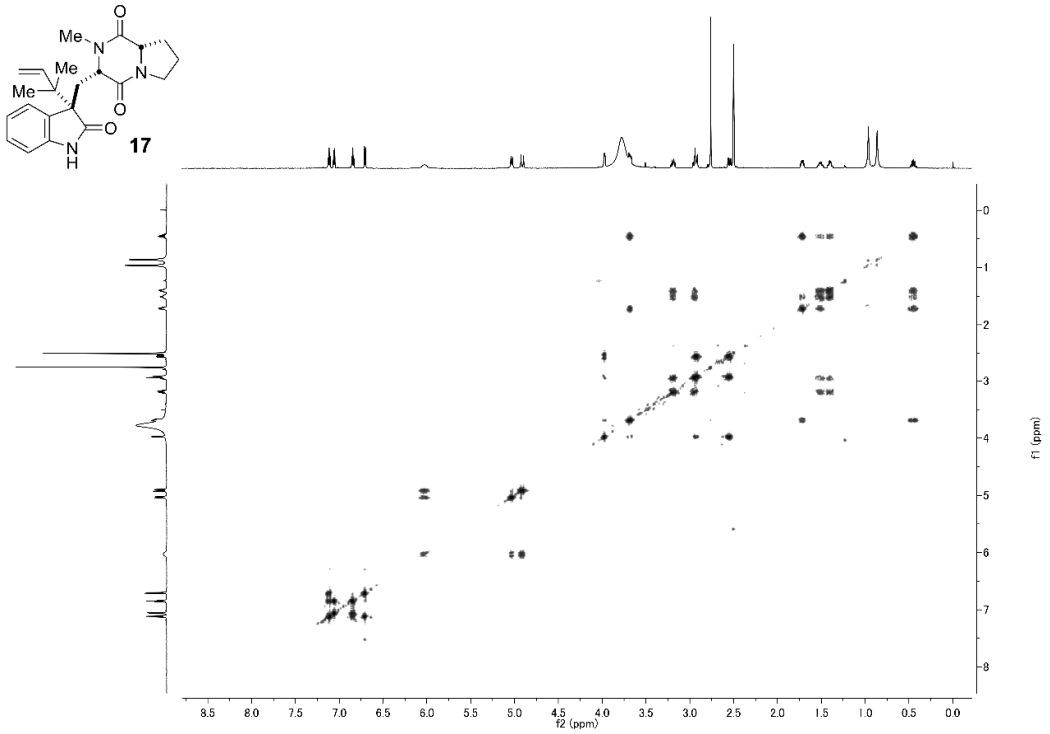
Supplementary Figure 49. NOESY spectrum of compound **16** in $CDCl_3$.



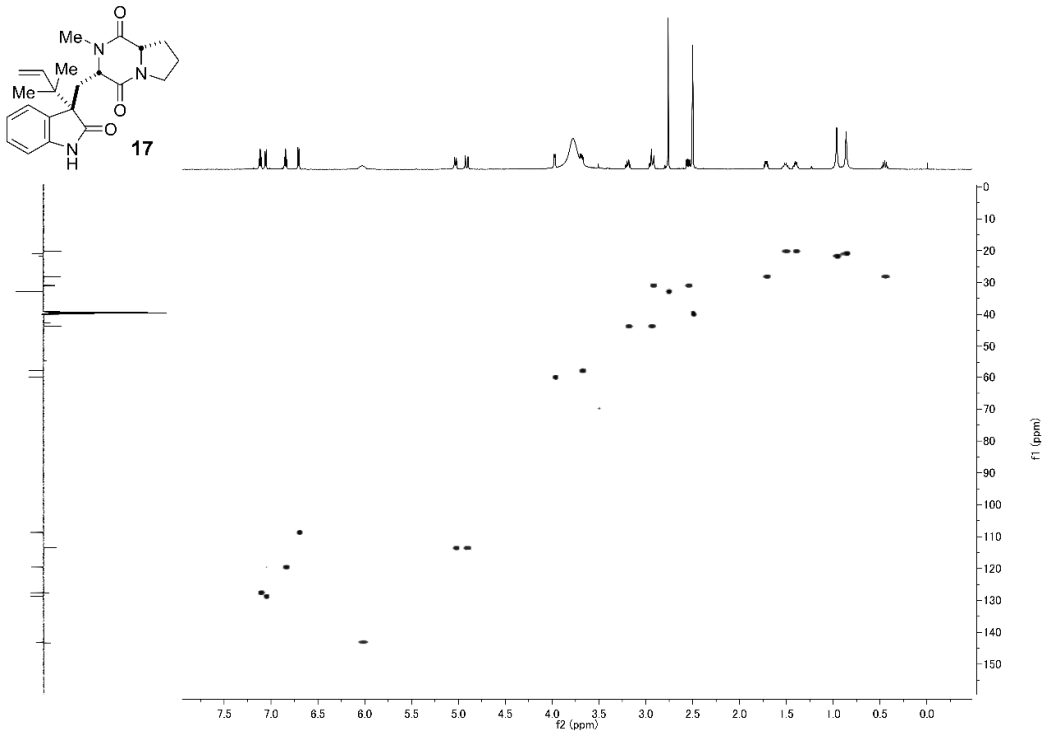
Supplementary Figure 50. ^{1}H NMR spectrum of compound **17** in $CDCl_3$.



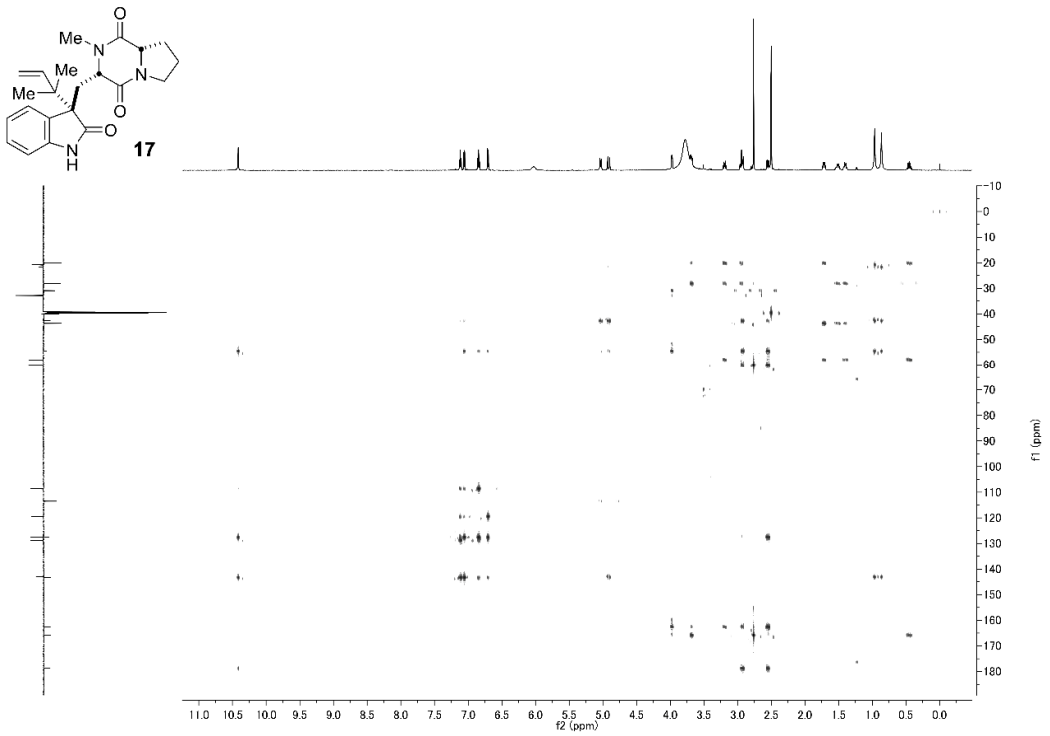
Supplementary Figure 51. DEPT ^{13}C NMR spectrum of compound **17** in $CDCl_3$.



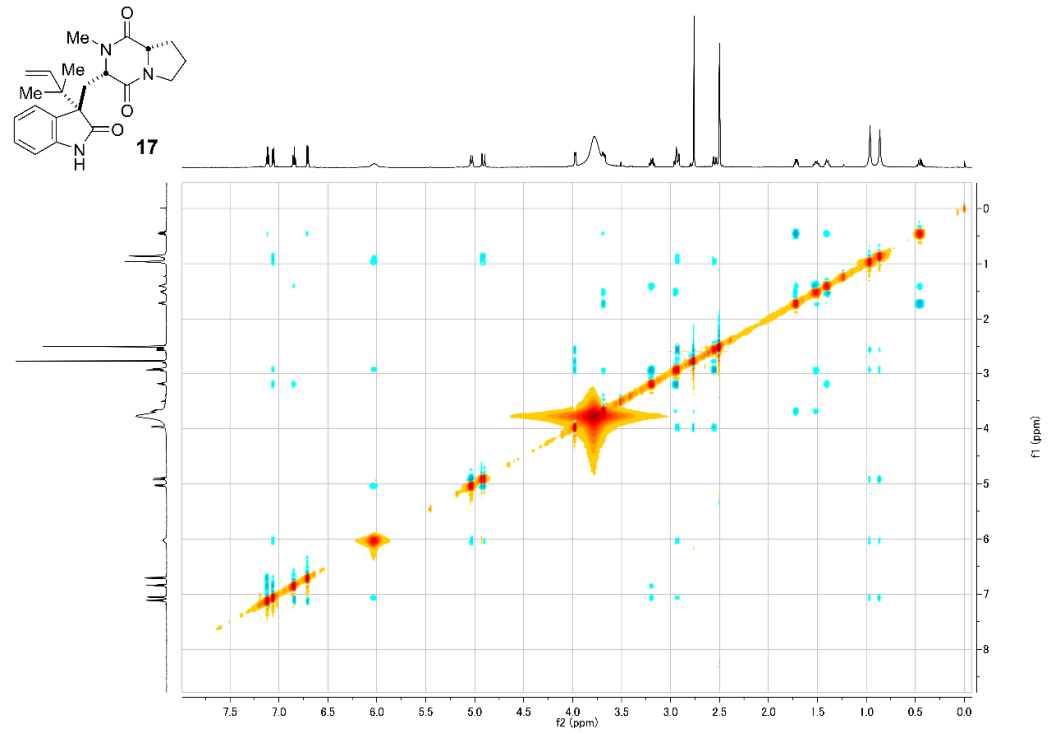
Supplementary Figure 52. ^{1}H-^{1}H COSY spectrum of compound **17** in $CDCl_3$.



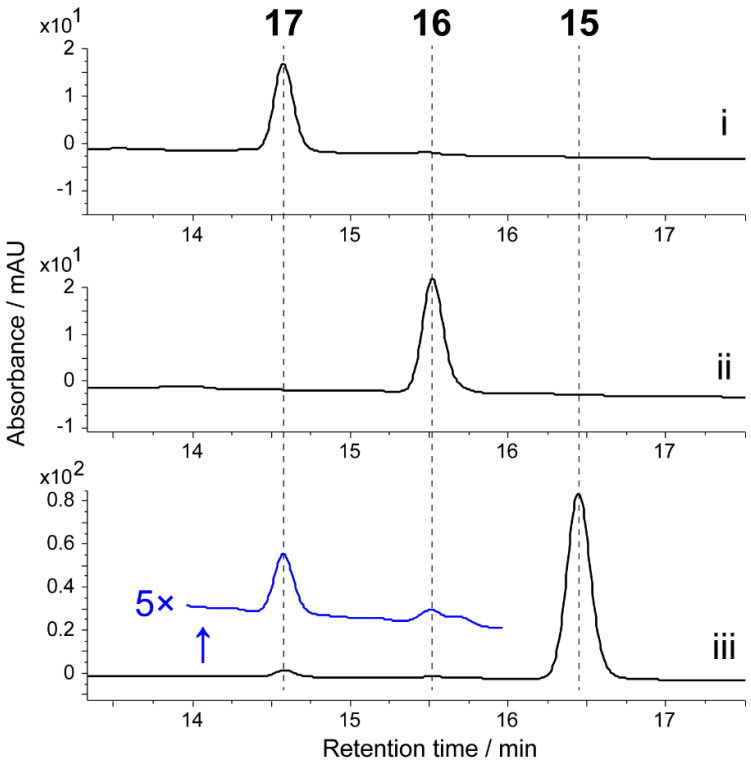
Supplementary Figure 53. HSQC spectrum of compound **17** in $CDCl_3$.



Supplementary Figure 54. HMBC spectrum of compound **17** in $CDCl_3$.

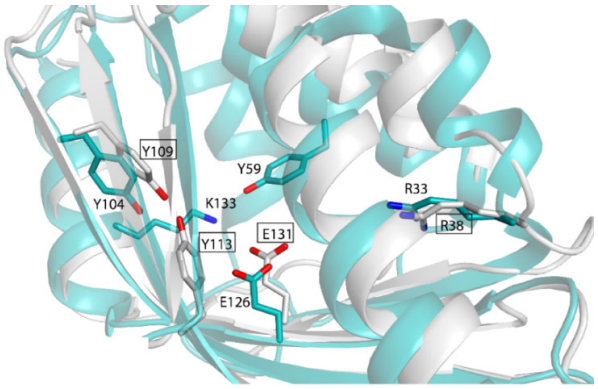


Supplementary Figure 55. NOESY spectrum of compound **17** in $CDCl_3$.

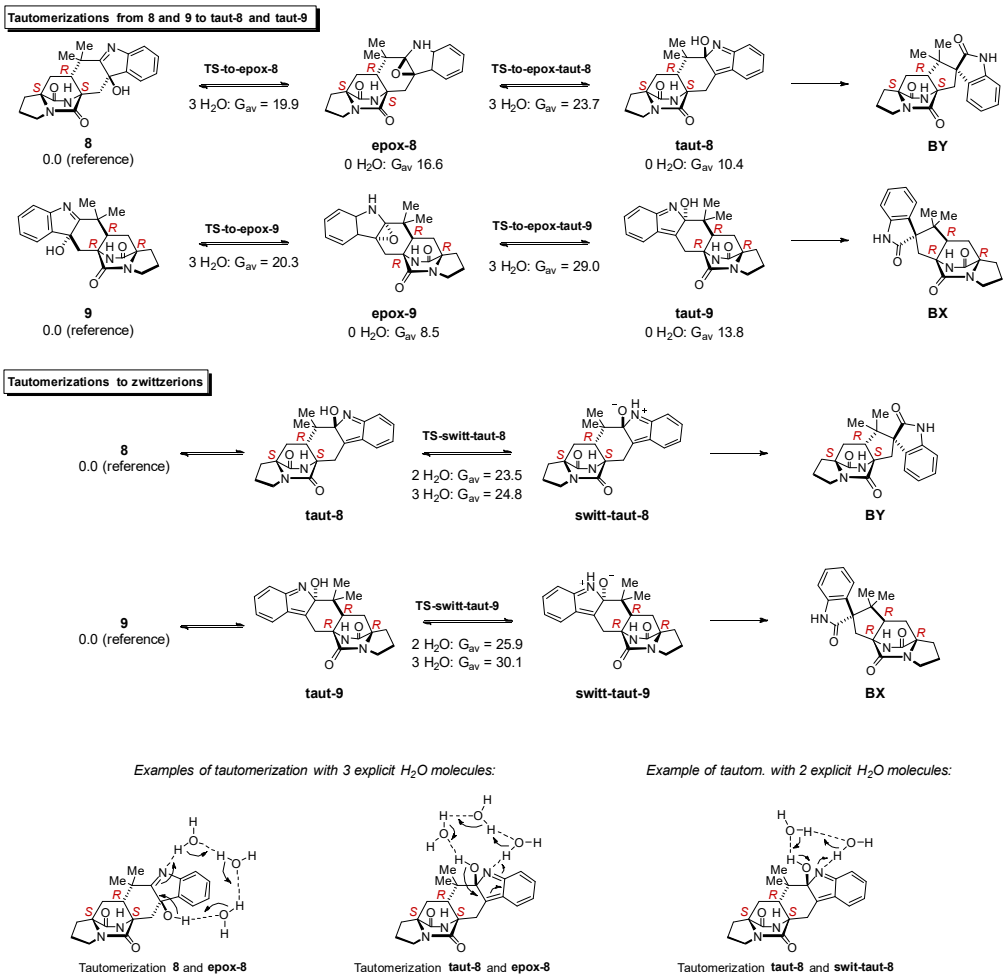


Supplementary Figure 56. Stability analysis of compounds **17** (i), **16** (ii) and **15** (iii). Compounds were dissolved in enzyme reaction buffer and incubated at 30 °C for 24 h before HPLC analysis.

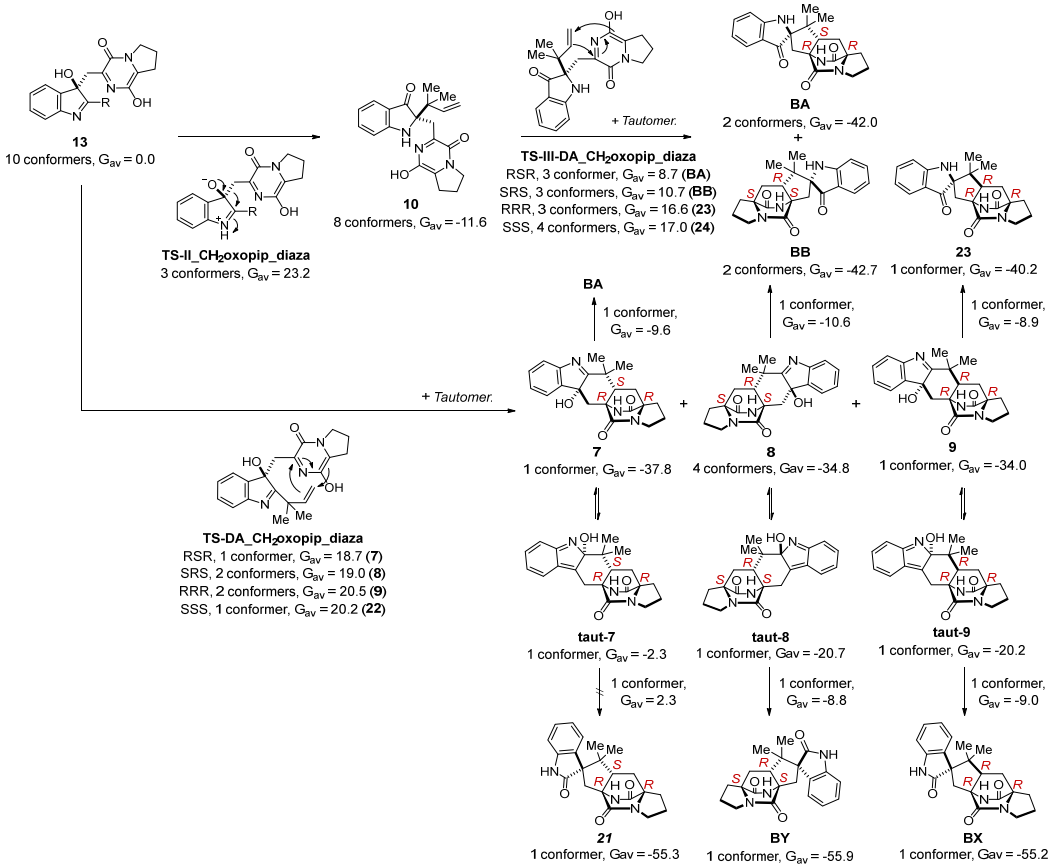
Supplementary Figure 57. BvnE cartoon structure (grey) superimposed with PrhC (PDB ID #: 5x9j, cyan).



Supplementary Figure 58. The superimposed view of candidate catalytic acid and base residues from BvnE (grey cartoon and residues, with squares around the residue numbers) and from PrhC (cyan cartoon and residues, no squares around the residue numbers).

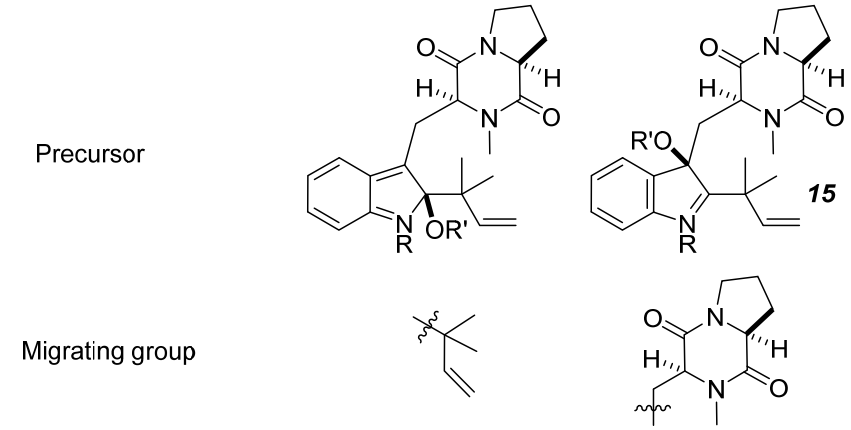


Supplementary Figure 59. Possible tautomerization processes that occur from compounds **8** and **9**. Alternative analogous mechanisms might take place more favorably involving higher ordered H_2O clusters that are very difficult, if not impossible, to model computationally for these reactions. Experimentally, the conversion from compounds **8** and **9** to **BY** and **BX**, respectively, requires approximately 0.5 hours at room temperature.



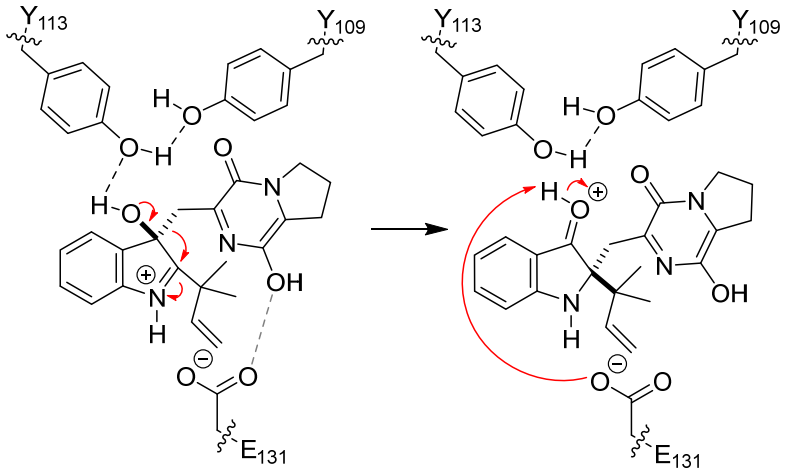
Supplementary Figure 60. Boltzmann weighted G of the main reaction pathways from compound **13** to form **BA**, **BB**, **BX** and **BY**, as well as compound **7**, including the number of conformers found for each reaction step.

a

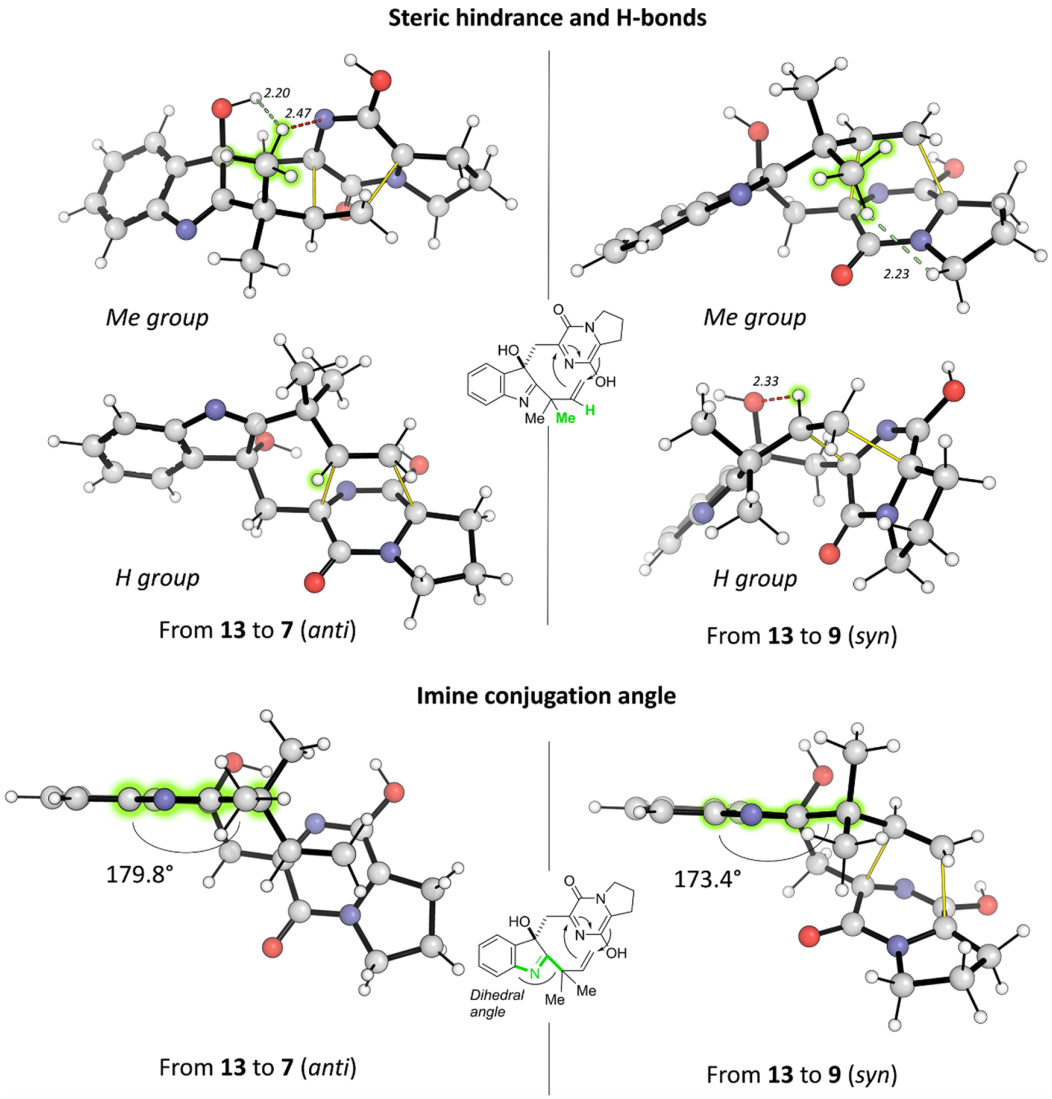


Condition	*ΔG≠ of migration (kcal/mol)*	
Acid	**18.7**	27.5
Neutral	**29.7**	36.0
Basic	19.2	**18.5**
Experimental selectivity	**2**	1
Experimental selectivity with BvnE	1	**20**

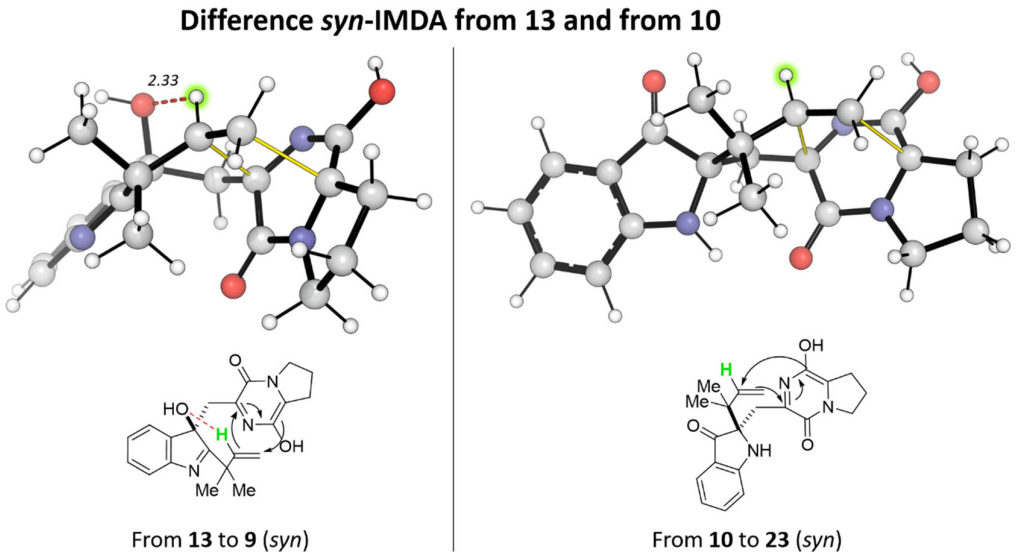
b **BvnE active site**
(docking studies)



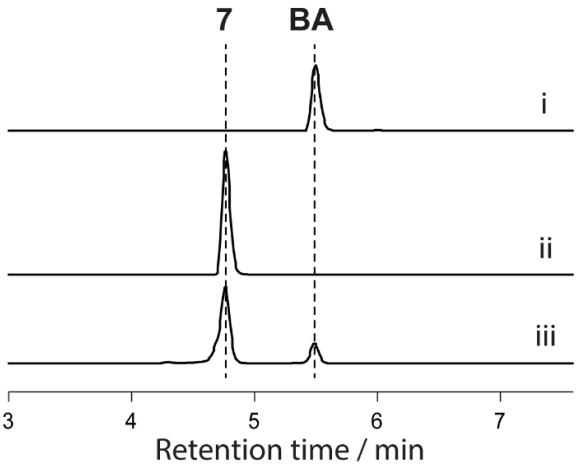
Supplementary Figure 61. a, Activation barriers in kcal/mol for the migration of the reverse-prenyl group and the -CH_2-dioxopiperazine group of **15** when using acid (R = H^+, R' = H), neutral (R = lone electron pair, R' = H) and basic (R = lone electron pair, R' = negative charge) catalysis. Also, the experimental results of selectivity obtained from **15** with and without BvnE are shown. **b**, Results from docking studies of the active site of BvnE. The calculations suggest that the reactivity switch might be promoted by basic groups of BvnE that are able to interact with the OH group involved in the migration. Based on the results of docking calculations, possible candidate group that trigger the reactivity switch are Y_{113} or Y_{109} residue of BvnE.



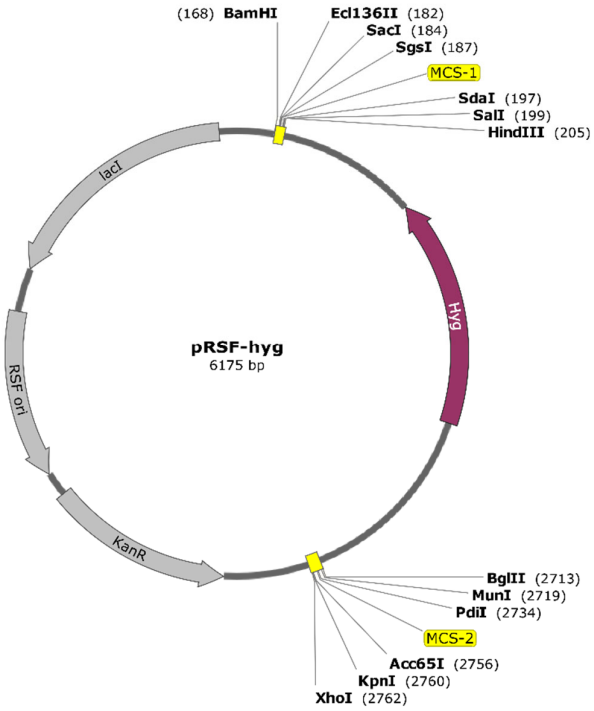
Supplementary Figure 62. Main differences in the *anti*- and *syn*-IMDA transition states from compound **13**. The H and Me substituents that show relevant differences between the systems are highlighted in green. Relevant steric effects and hydrogen bonds are represented as green and red dashed lines, respectively.



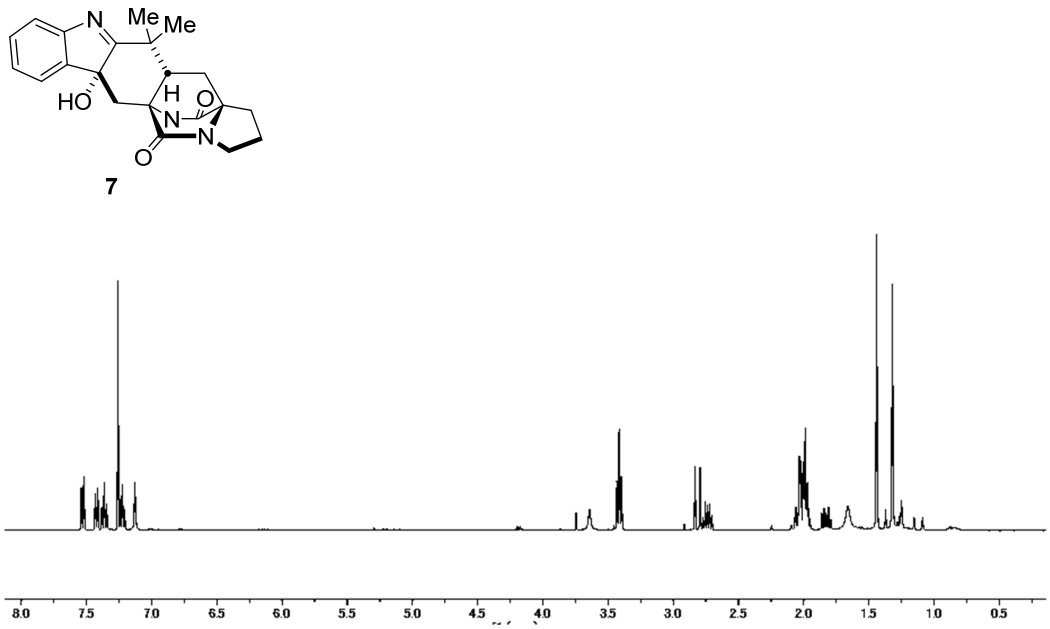
Supplementary Figure 63. Comparison of *syn*-IMDA transition states from compounds **13** and **10**. The key hydrogen bond formed in the IMDA from compound **13** is represented with a red dashed line.



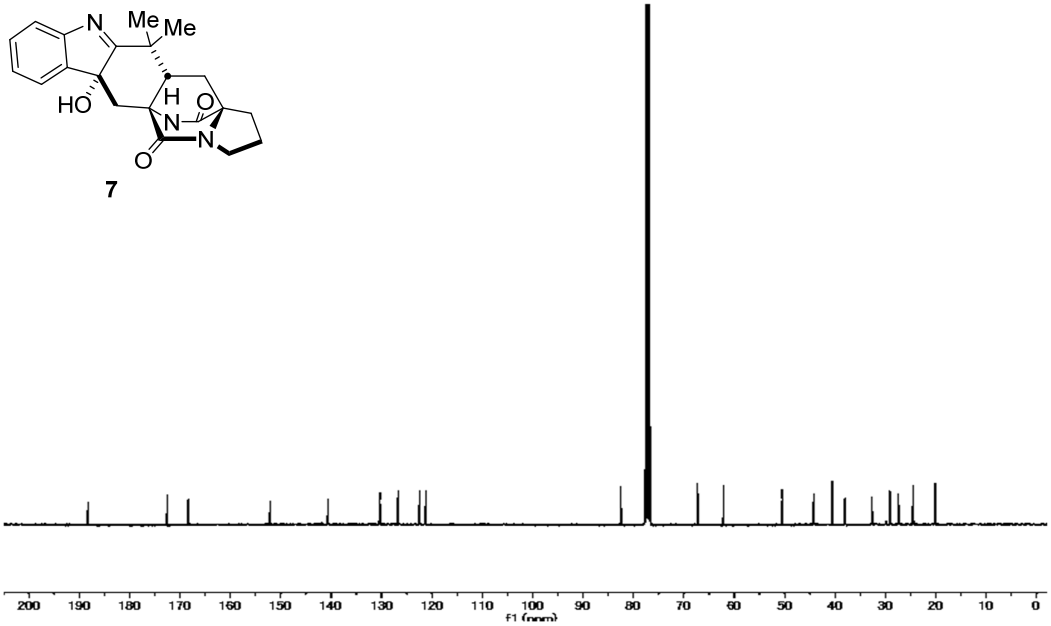
Supplementary Figure 64. HPLC analysis of base treatment of compound **7** as previously described[40]. i, **BA** standard; ii, **7** treated with 0.5 M NaOH for 0 h; iii, **7** treated with 0.5 M NaOH for 1 h.



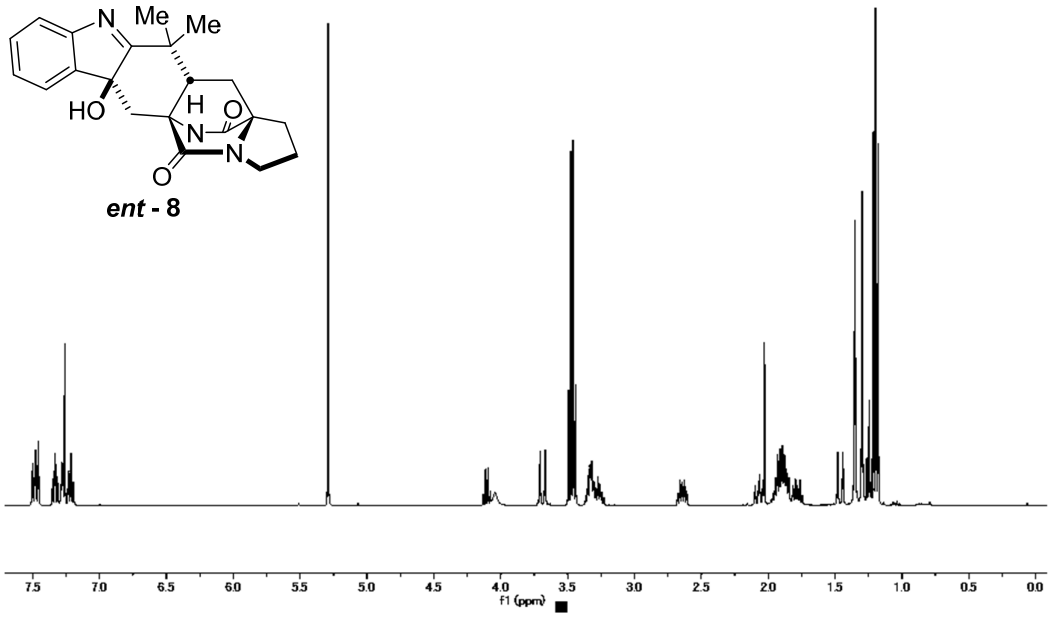
Supplementary Figure 65. Plasmid map of pRSF-hyg (the backbone vector for construction of fungal gene knockout cassettes).



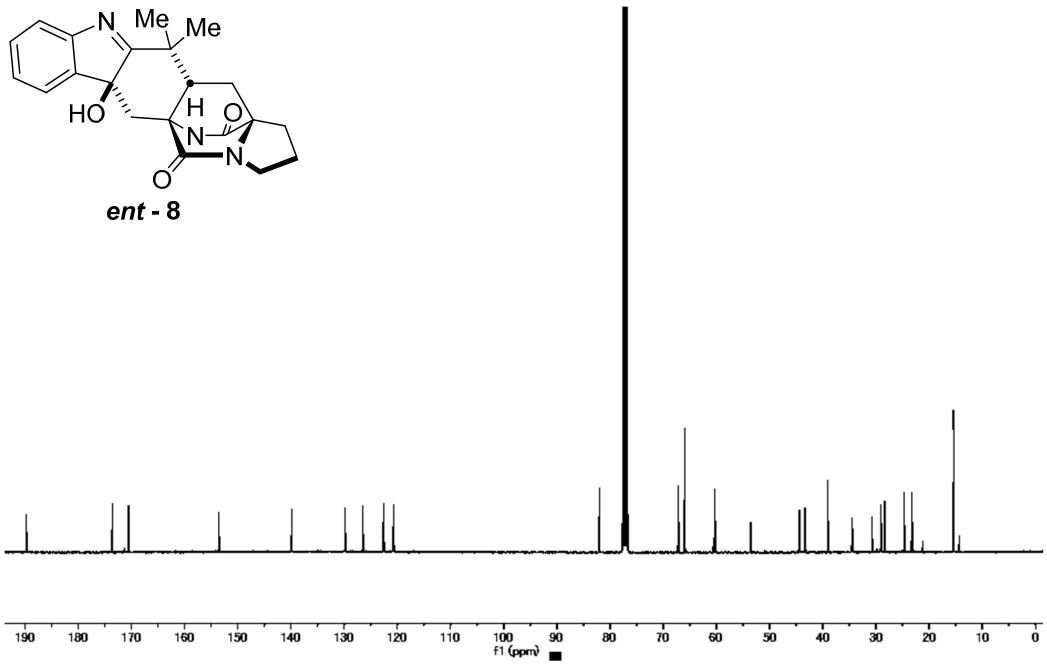
Supplementary Figure 66. ^{1}H NMR of synthetic **7**.



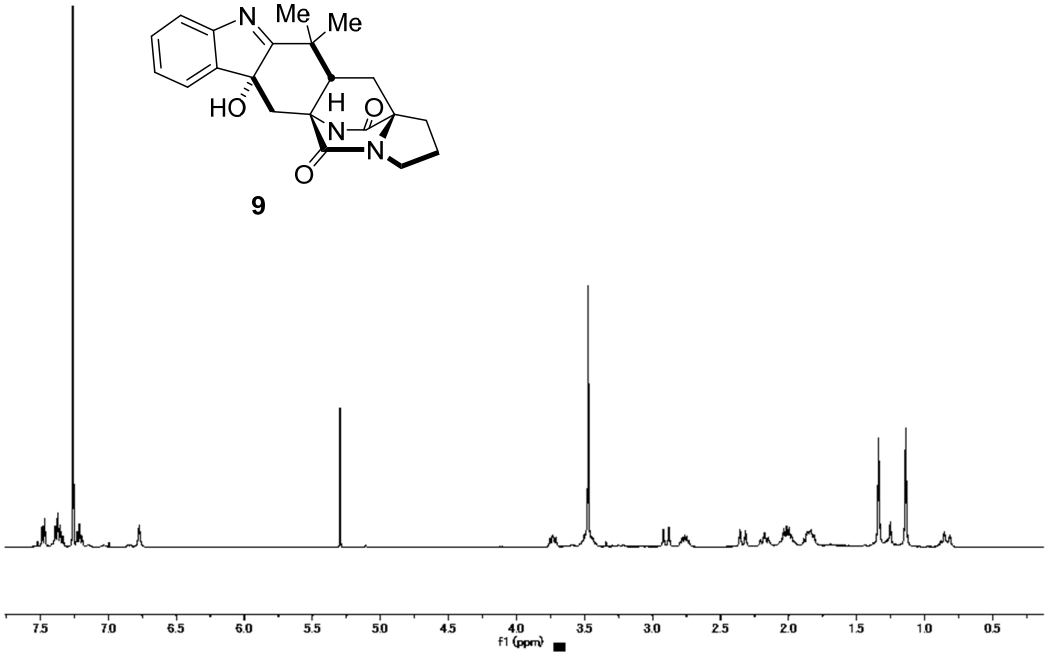
Supplementary Figure 67. ^{13}C NMR of synthetic **7**.



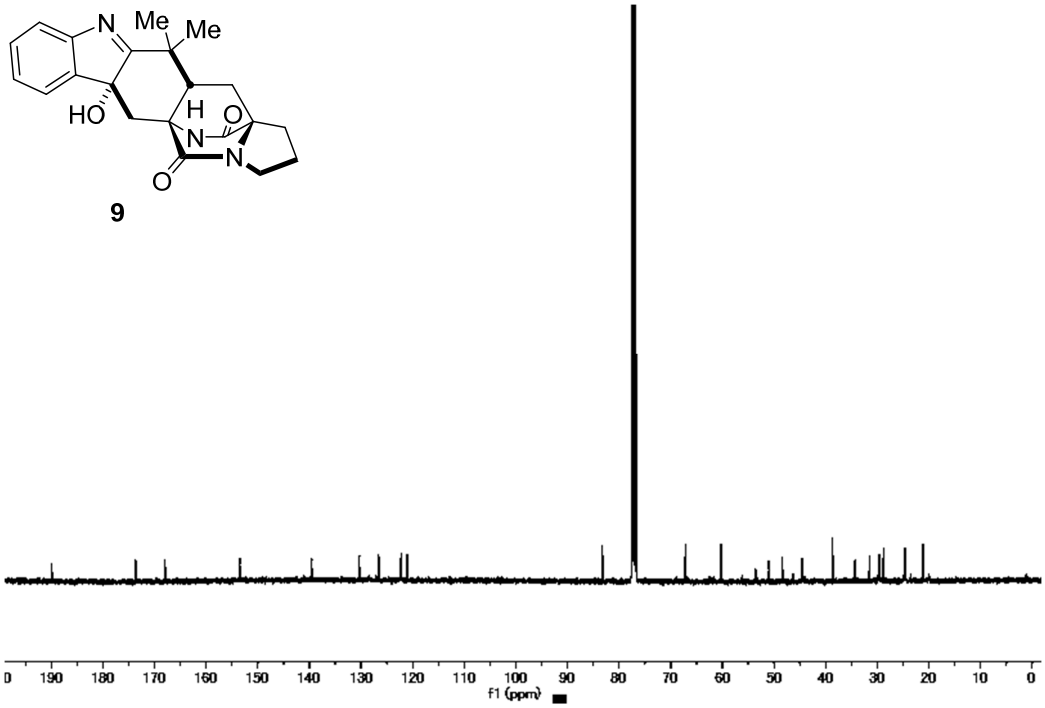
Supplementary Figure 68. ^{1}H NMR of synthetic *ent*-**8**.



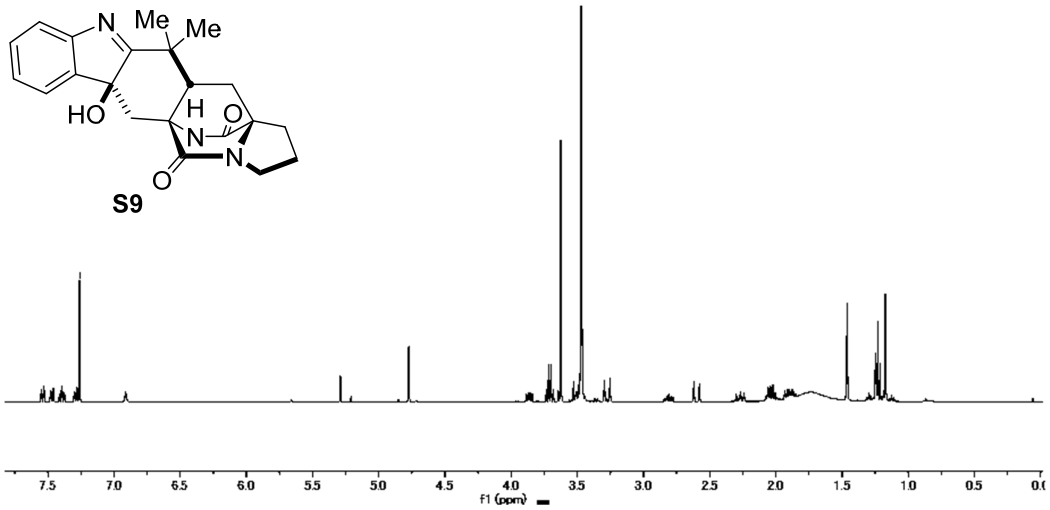
Supplementary Figure 69. ^{13}C NMR of synthetic *ent*-**8**.



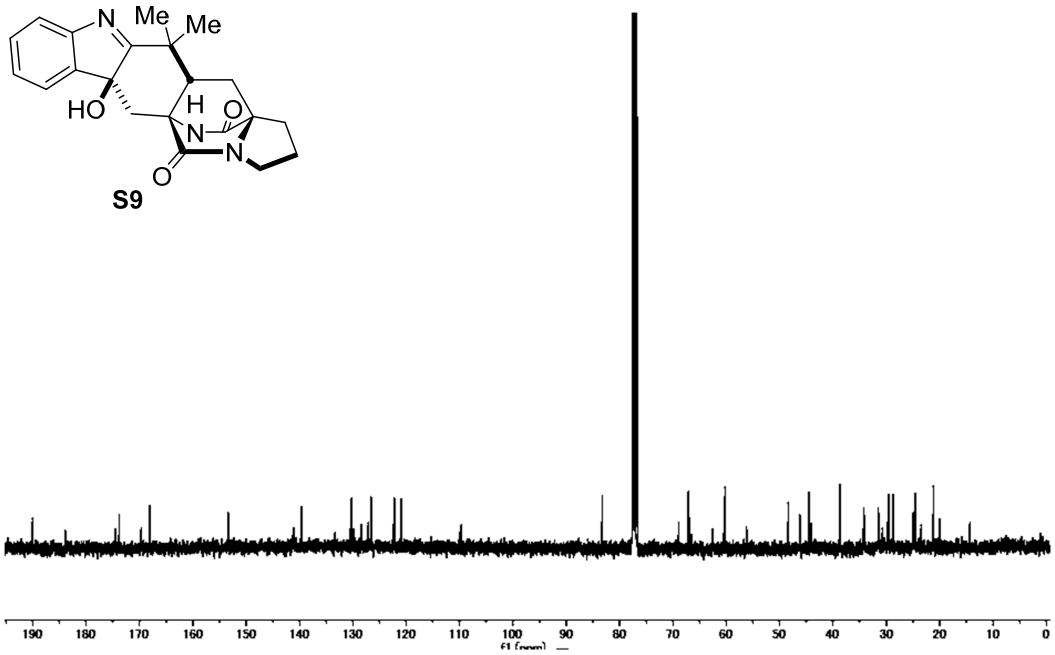
Supplementary Figure 70. ^{1}H NMR of synthetic **9**.



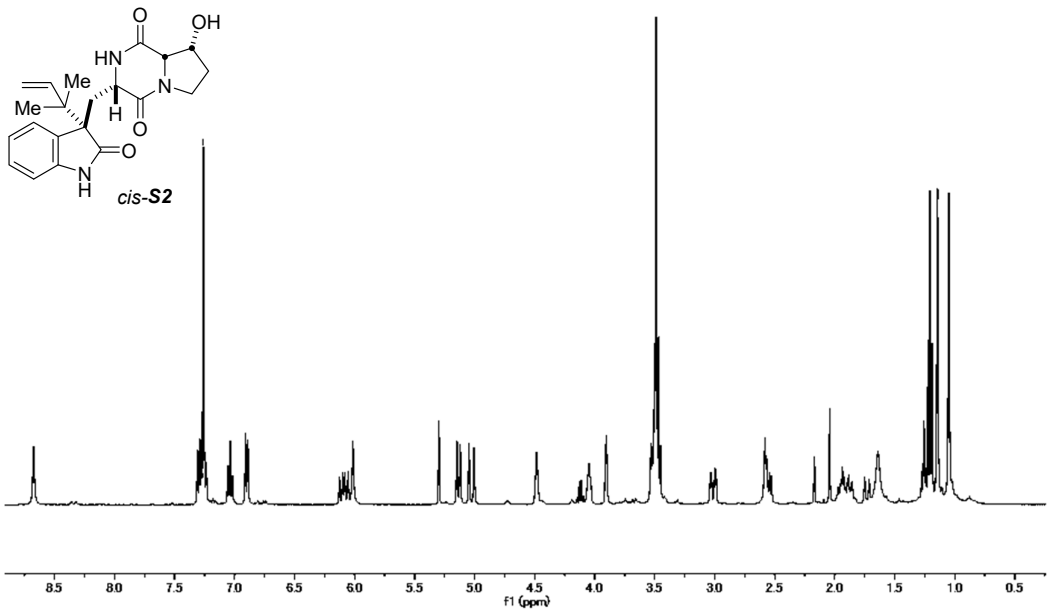
Supplementary Figure 71. ^{13}C NMR of synthetic **9**.



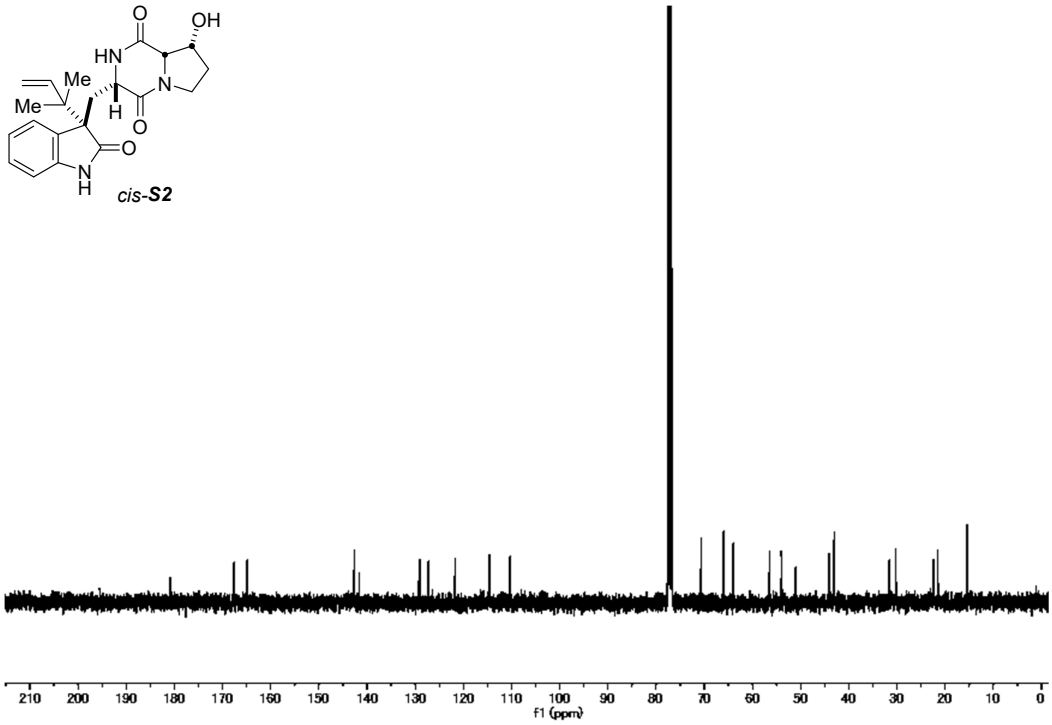
Supplementary Figure 72. ^{1}H NMR of synthetic **S9**.



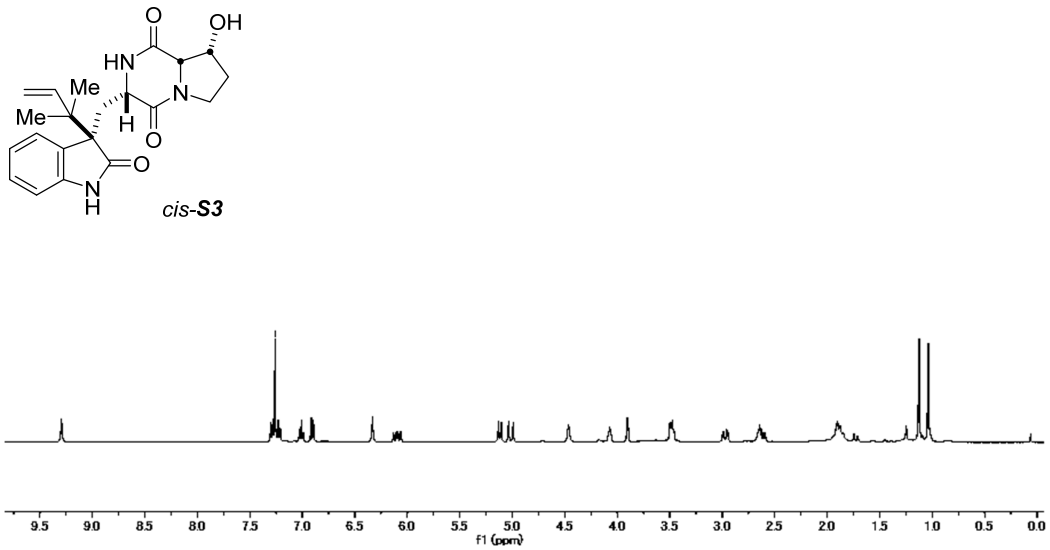
Supplementary Figure 73. ^{13}C NMR of synthetic **S9**.



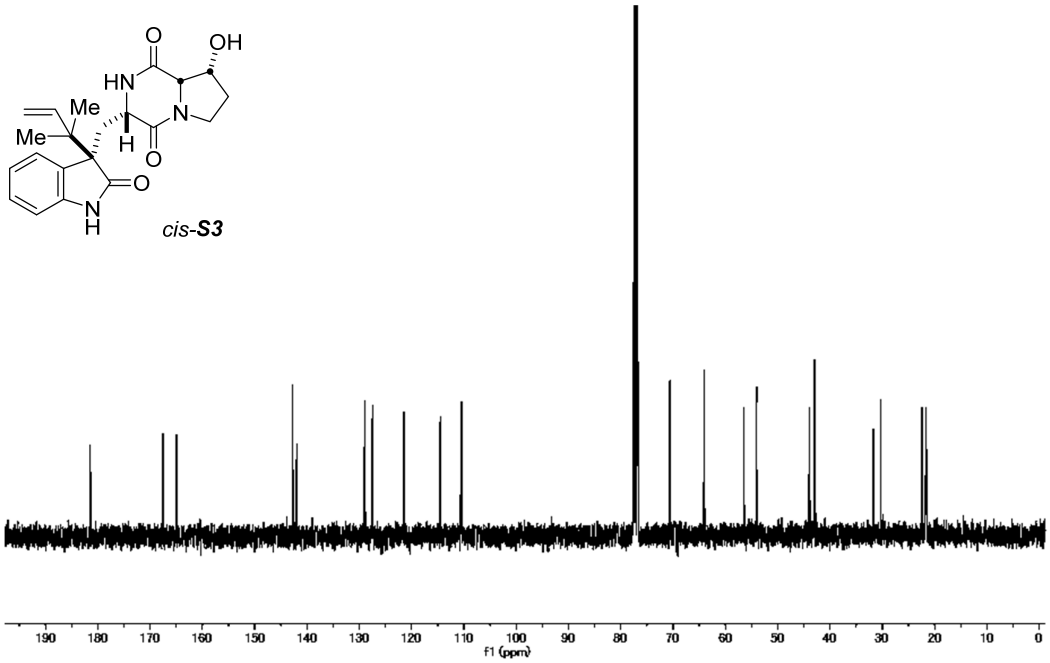
Supplementary Figure 74. ^{1}H NMR of synthetic *cis*-**S2**.



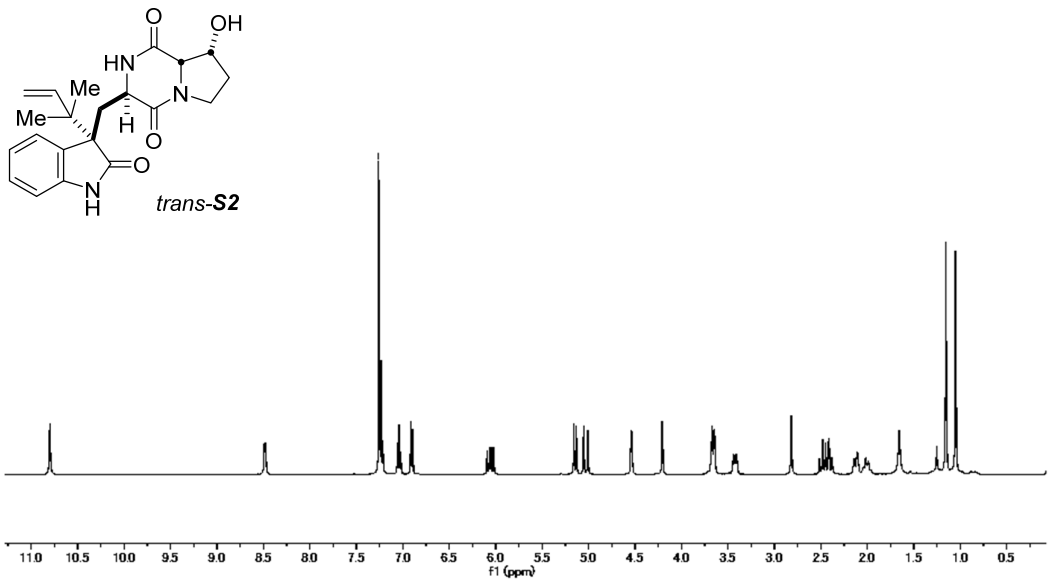
Supplementary Figure 75. ^{13}C NMR of synthetic *cis*-**S2**.



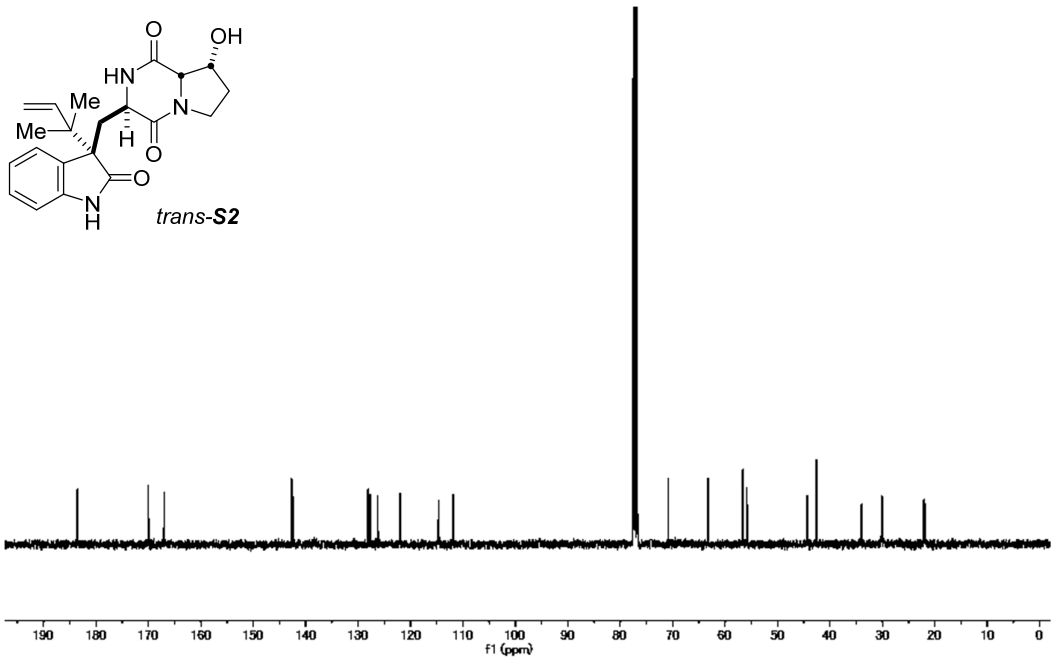
Supplementary Figure 76. ^{1}H NMR of synthetic *cis*-**S3**.



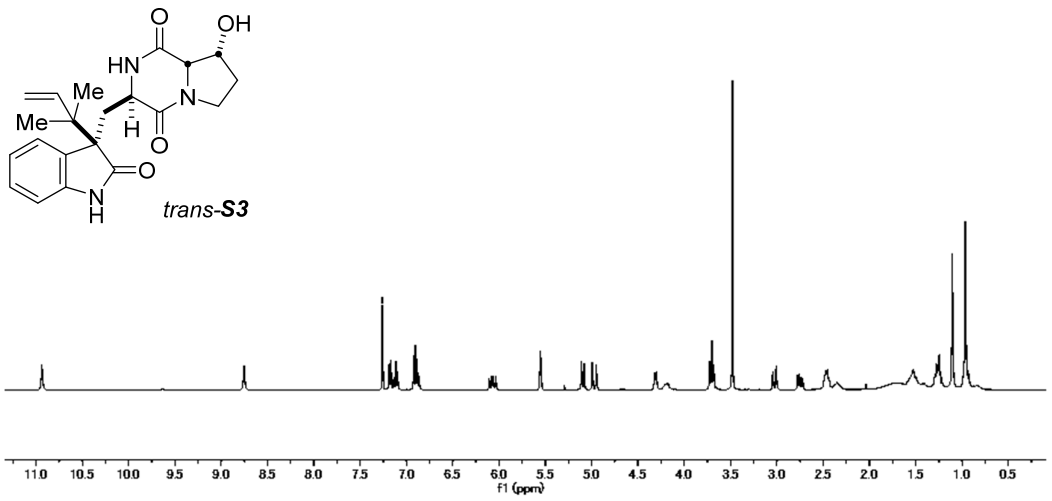
Supplementary Figure 77. ^{13}C NMR of synthetic *cis*-**S3**.



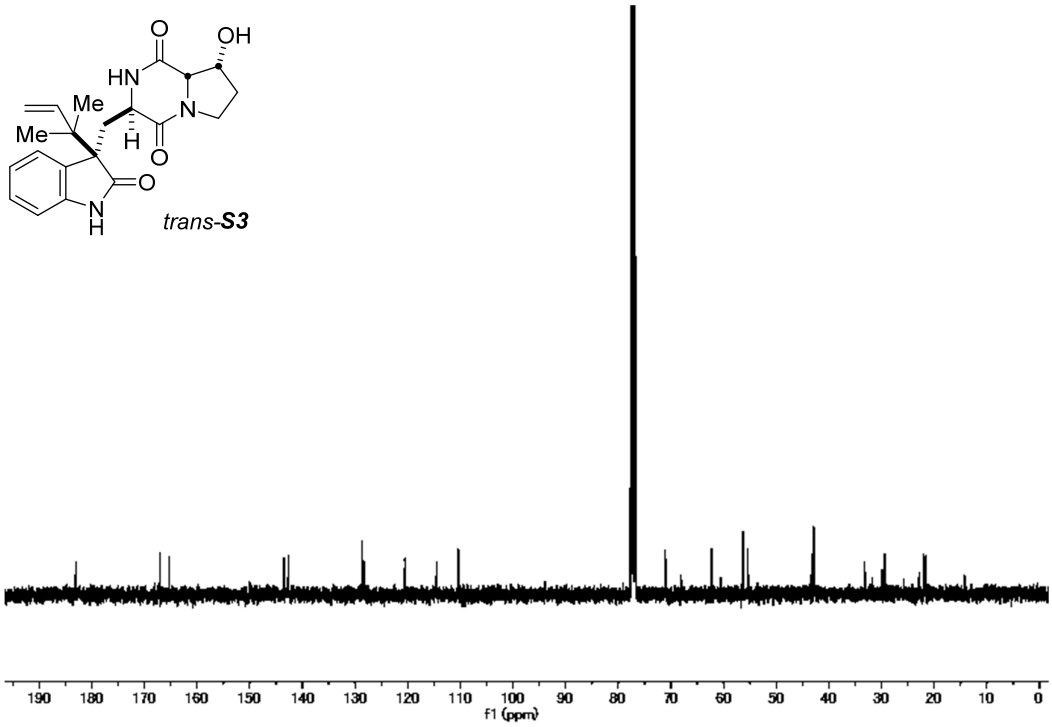
Supplementary Figure 78. ^{1}H NMR of synthetic *trans*-**S2**.



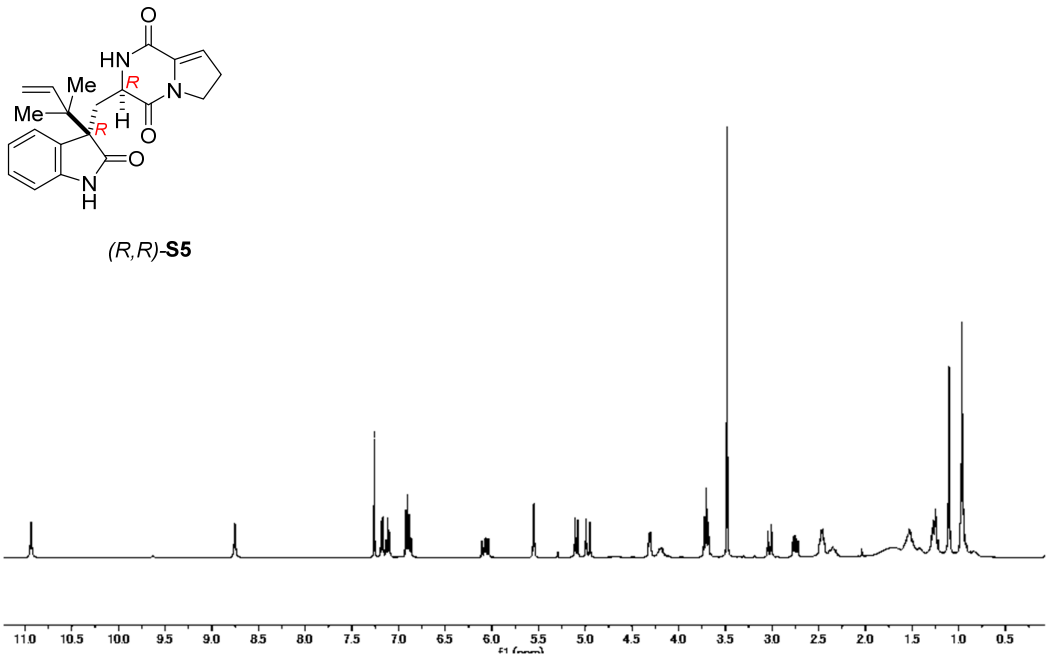
Supplementary Figure 79. ^{13}C NMR of synthetic *trans*-**S2**.



Supplementary Figure 80. ^{1}H NMR of synthetic *trans*-**S3**.

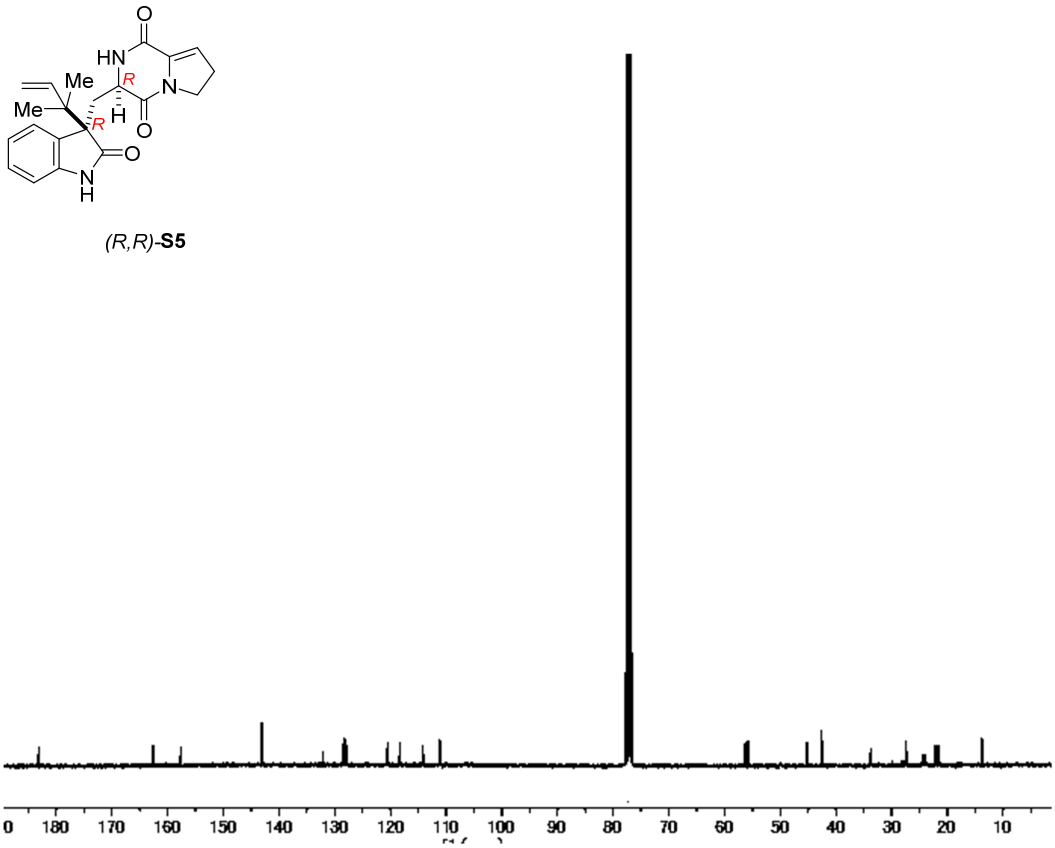


Supplementary Figure 81. ^{13}C NMR of synthetic *trans*-**S3**.



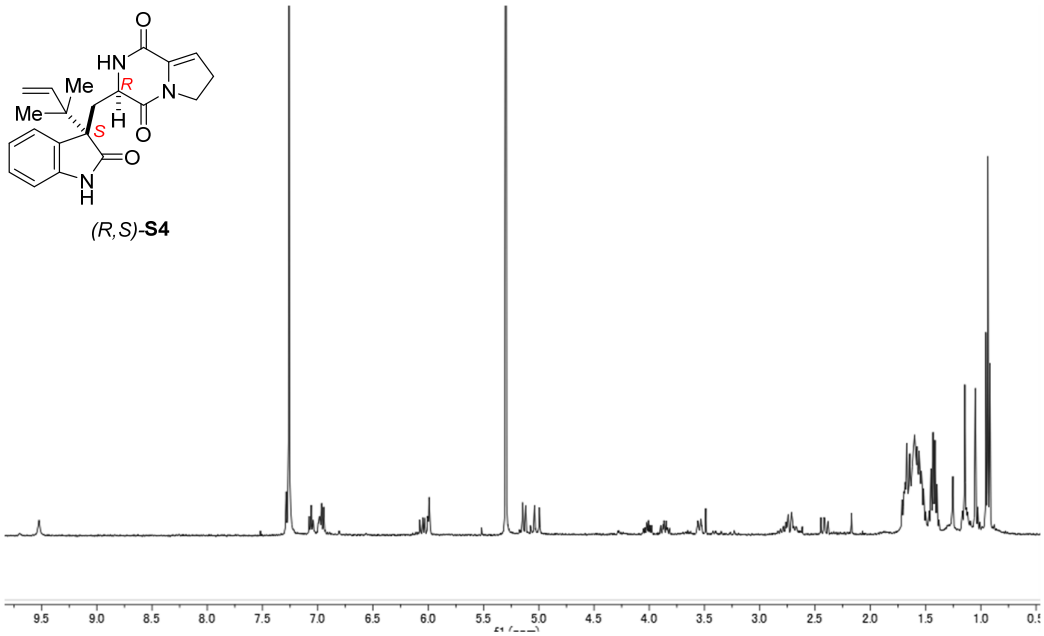
(R,R)-**S5**

Supplementary Figure 82. ^{1}H NMR of synthetic *(R,R)*-**S5** and (S,S)-**S4**.

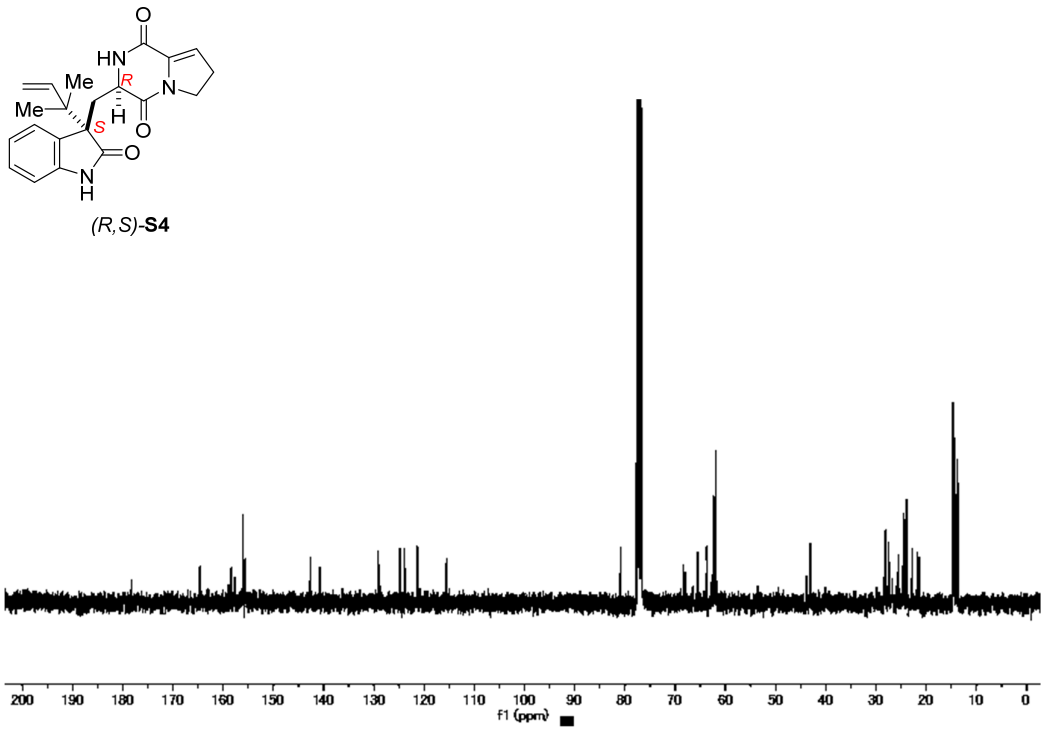


(R,R)-**S5**

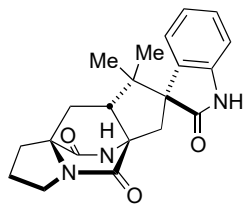
Supplementary Figure 83. ^{13}C NMR of synthetic *(R,R)*-**S5** and (S,S)-**S4**.



Supplementary Figure 84. ^{1}H NMR of synthetic *(R,S)*- **S4** and (*S,R*)-**S5**.

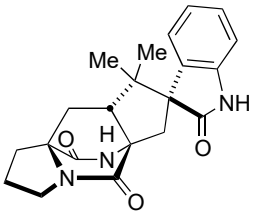


Supplementary Figure 85. ^{13}C NMR synthetic *(R,S)*- **S4** and (*S,R*)-**S5**.



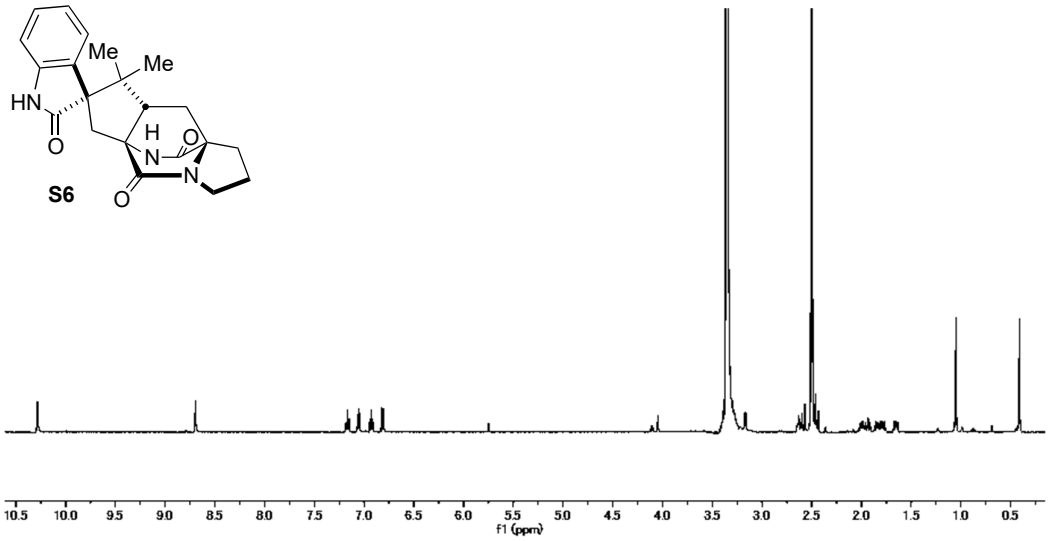
Brevianamide Y

Supplementary Figure 86. ^{1}H NMR of synthetic **BY**.

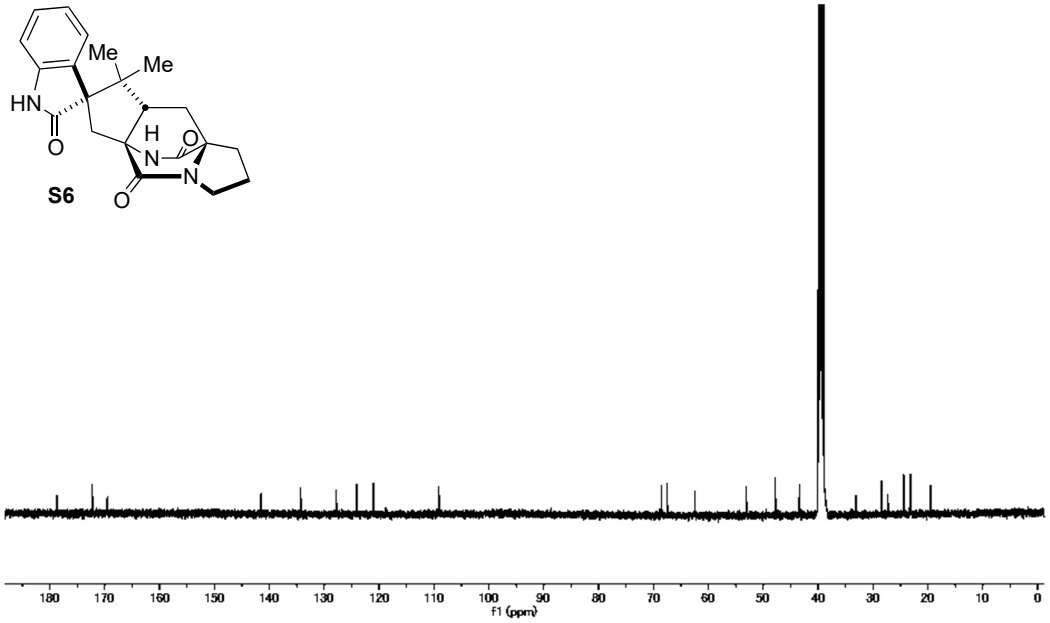


Brevianamide Y

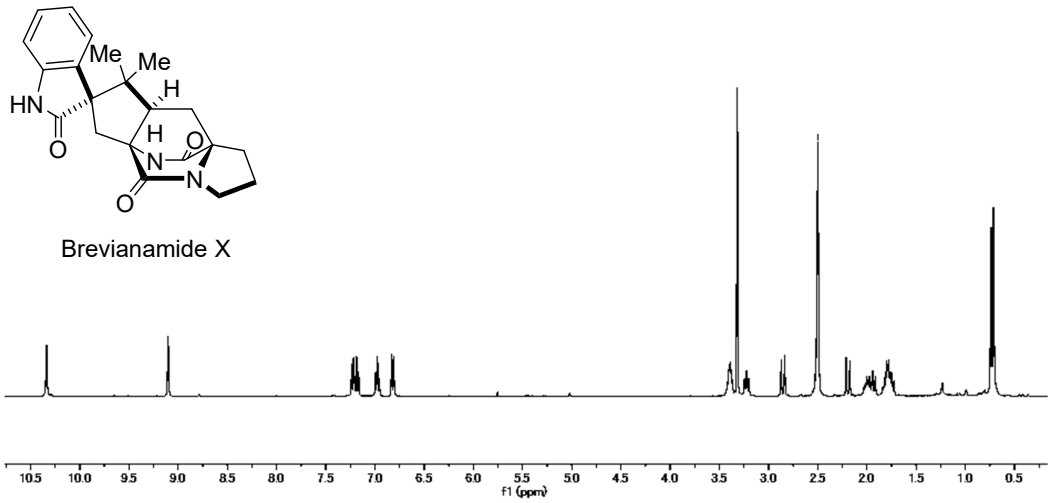
Supplementary Figure 87. ^{13}C NMR of synthetic **BY**.



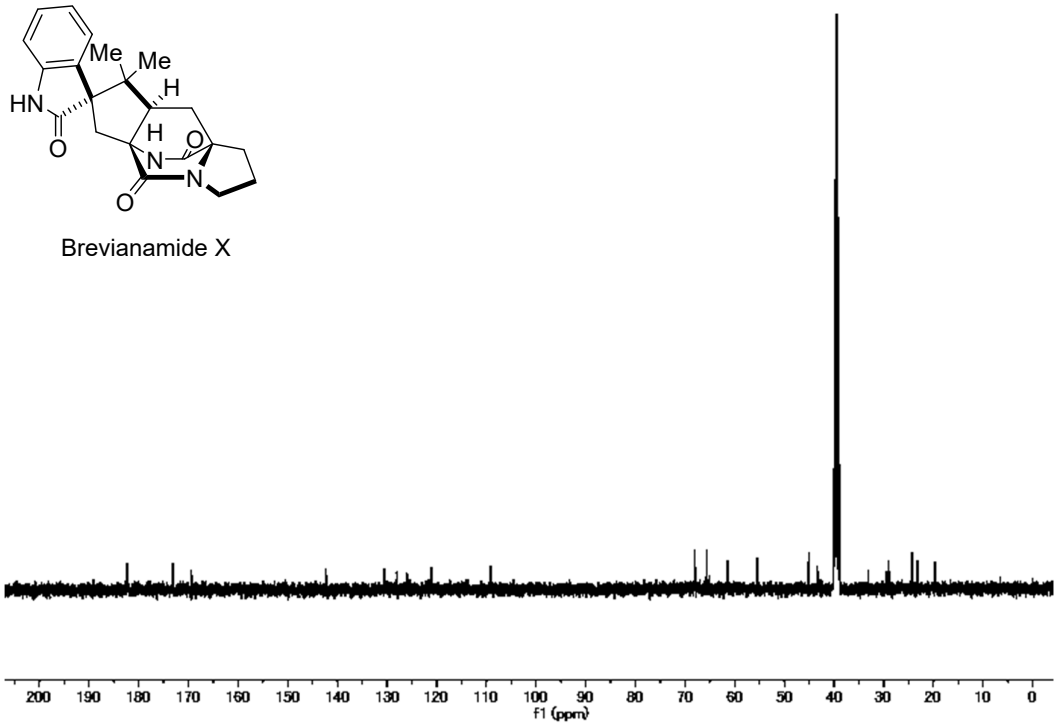
Supplementary Figure 88. ^{1}H NMR of synthetic **S6**.



Supplementary Figure 89. ^{13}C NMR of synthetic **S6**.



Supplementary Figure 90. ^{1}H NMR of synthetic **BX**.



Supplementary Figure 91. ^{13}C NMR of synthetic **BX**.