

Supplementary Material

Functional Characterization of the Cyclomarin/Cyclomarazine Prenyltransferase CymD Directs the Biosynthesis of Unnatural Cyclic Peptides

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Relative yield of known and novel cyclomarin and cyclomarazine analogs

Supplementary Figures 1 and 2..... S2

N-(1,1-dimethyl-1-allyl)-tryptophan (**2**) produced synthetically, enzymatically, and via fermentation

Supplementary Figure 3. ¹H NMR comparison of (**2**)^a..... S3

Structure determination of cyclomarazine P

Supplementary Figure 4. ¹H NMR spectrum of (**7**)..... S4

Supplementary Figure 5. HSQC spectrum of (**7**)..... S5

Supplementary Figure 6. HMBC spectrum of (**7**)..... S6

Structure determination of cyclomarazine M

Supplementary Figure 7. ¹H NMR spectrum of (**6**)..... S7

Supplementary Figure 8. HSQC spectrum of (**6**)..... S8

Supplementary Figure 9. HMBC spectrum of (**6**)..... S9

Structure determination of cyclomarin P

Supplementary Figure 10. ¹H NMR spectrum of (**12**)..... S10

Supplementary Figure 11. HSQC spectrum of (**12**)..... S11

Supplementary Figure 12. HMBC spectrum of (**12**)..... S12

Structure determination of cyclomarin M

Supplementary Figure 13. ¹H NMR spectrum of (**11**)..... S13

Supplementary Figure 14. HSQC spectrum of (**11**)..... S14

Supplementary Figure 15. HMBC spectrum of (**11**)..... S15

Structure determination of *N*-(1,1-dimethyl-1-allyl)-tryptophan and *N*-(1-propargyl)-tryptophan

Supplementary Figure 16. ¹H NMR spectrum of (**2**)^a..... S16

Supplementary Figure 17. ¹³C NMR spectrum of (**2**)..... S17

Supplementary Figure 18. ¹H NMR spectrum of (**15**)^d..... S18

Supplementary Figure 19. ¹³C NMR spectrum of (**15**)^d..... S19

Supplementary Figure 20. ¹H NMR spectrum of (**4**)..... S20

Supplementary Figure 21. ¹³C NMR spectrum of (**4**)..... S21

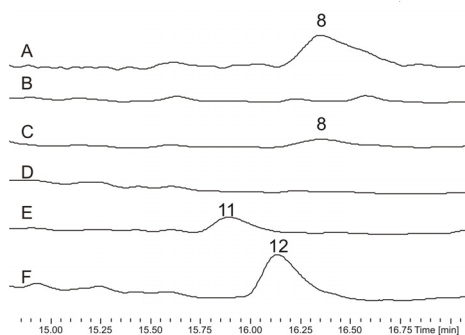
^aall *N*-(1,1-dimethyl-1-allyl)-tryptophan and *N*-(1-propargyl)-tryptophan spectra recorded in D₂O unless specified

^ball cyclomarazine P and M spectra recorded in DMSO-*d*₆

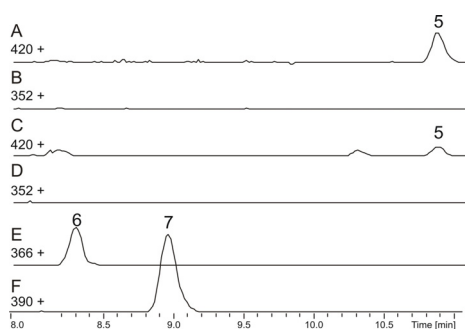
^call cyclomarin P and M spectra recorded in CDCl₃

^drecorded in CDCl₃

Relative yield of known and novel cyclomarzin and cyclomarazine analogs

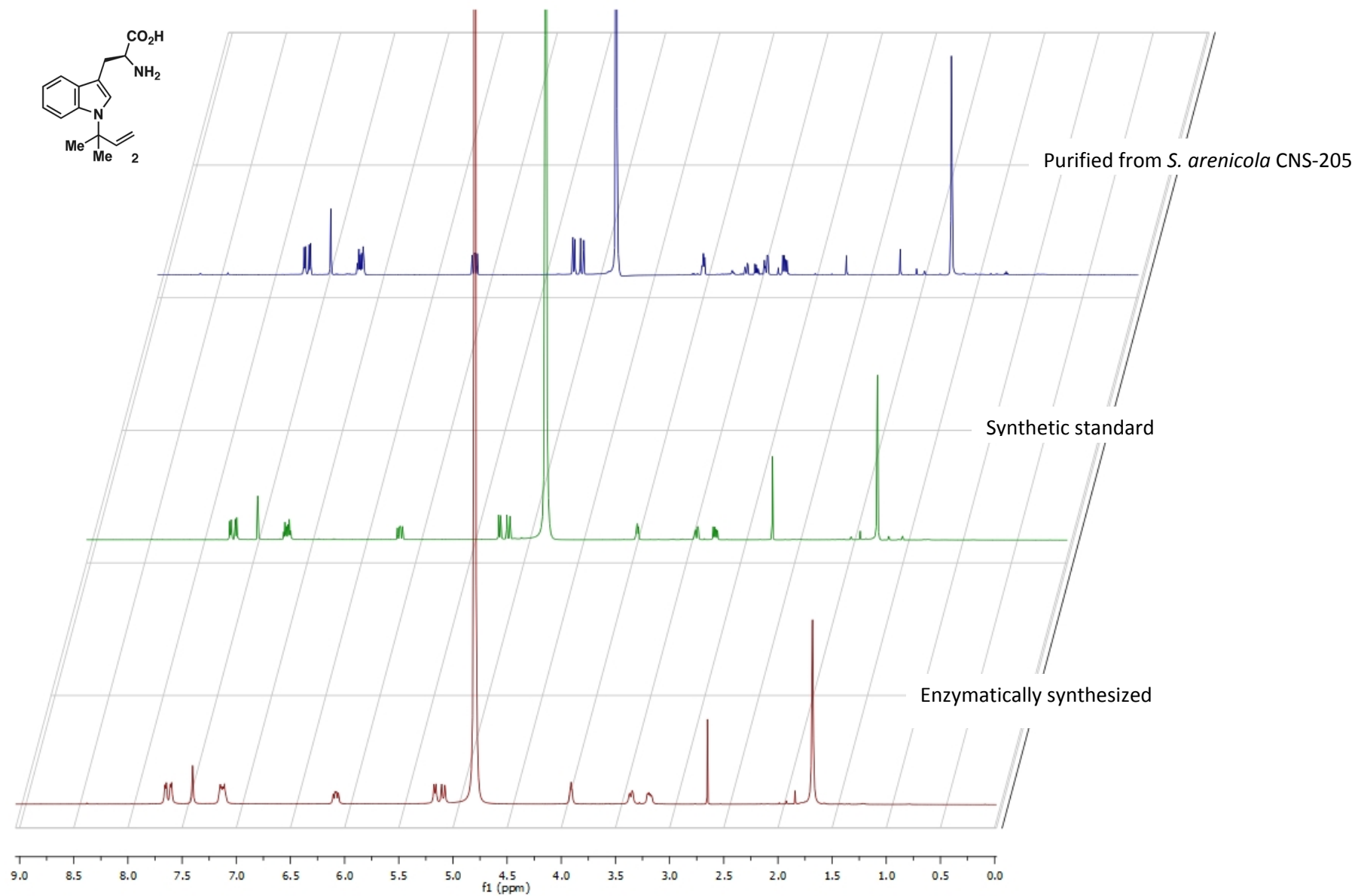


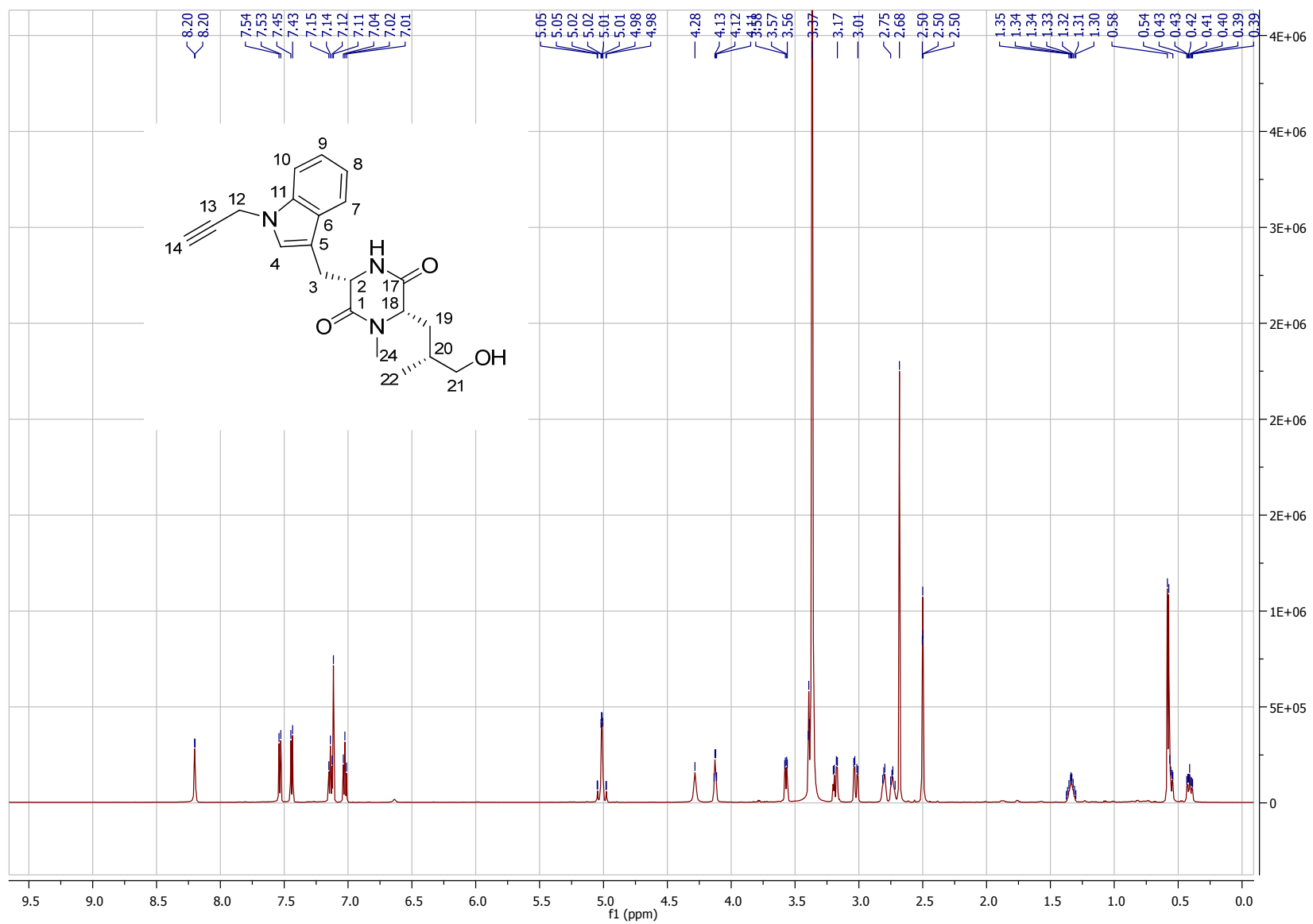
Supplementary Figure 1. Analysis of relative yield of cyclomarzin analogs in *Salinispora arenicola* CNS-205 wild type and *cymD*- mutant. Trace A: Wild type *S. arenicola* CNS-205. Trace B-F, *S. arenicola cymD* mutant. Traces C-F represent cultures supplemented with 80 mg/L tryptophan analogs. Trace C: *N*-(1,1-dimethyl-1-allyl)-tryptophan, D: tryptophan, E: *N*-(1-methyl)-tryptophan, F: *N*-(1-propargyl)-tryptophan. Chromatograms recorded at 210 nm. Note similar production levels of cyclomarzin M (**11**, trace E) and cyclomarzin P (**12**, trace F) in comparison to cyclomarzin A (**8**) in trace A and C. Desprenylcyclomarzin C (**10**, RT=15.2 min) is produced at levels below the detection limit of this analysis in the *cymD* mutant (trace B), and did not increase to a detectable level with the addition of tryptophan (trace D).



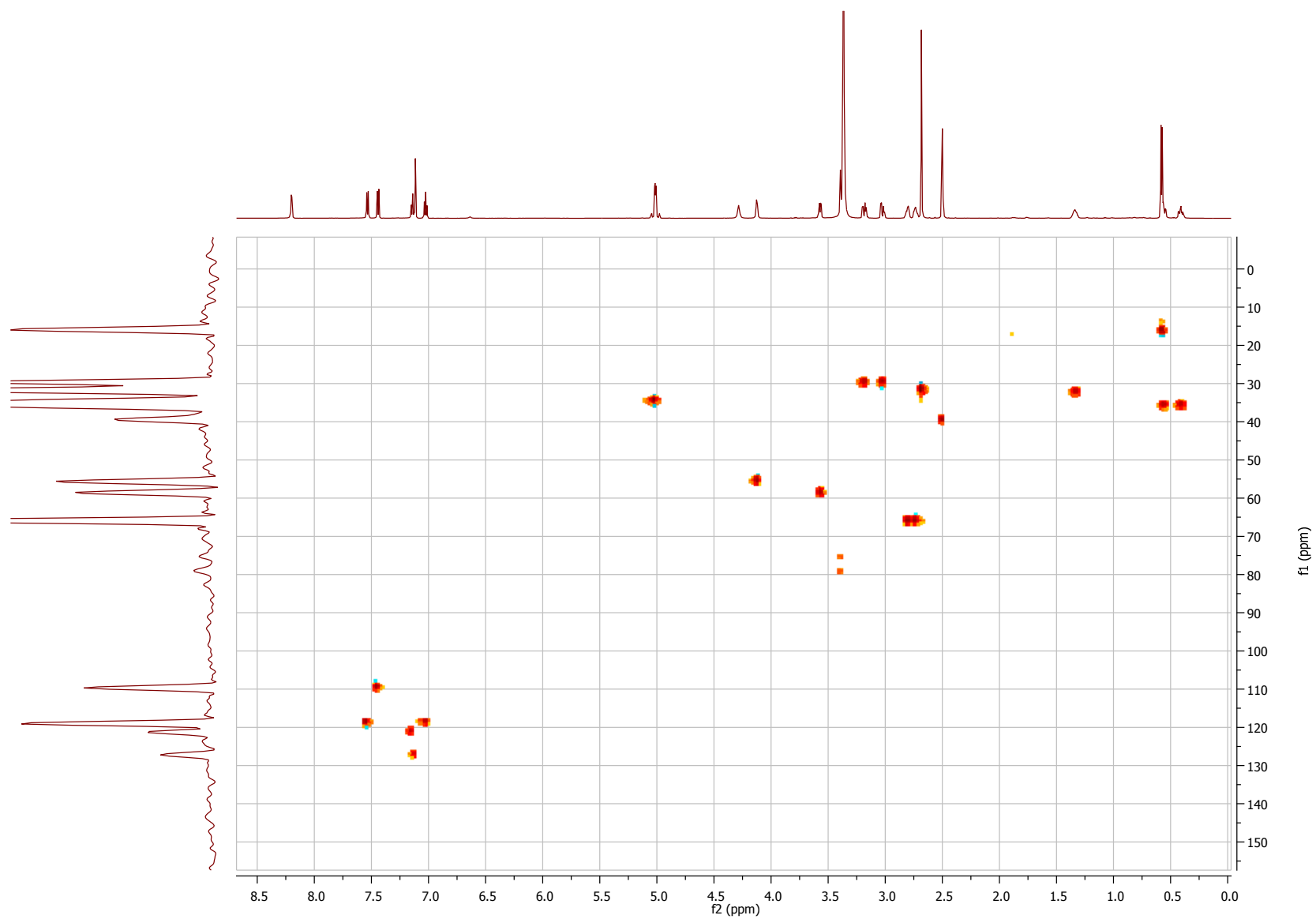
Supplementary Figure 2 Analysis of production levels of cyclomarazine analogs in *Salinispora arenicola* CNS-205 wild type and *cymD*- mutant. Trace A: Wild type *S. arenicola* CNS-205. Trace B-F, *S. arenicola cymD* mutant. Traces C-F represent cultures supplemented with 80 mg/L tryptophan analogs. Trace C: *N*-(1,1-dimethyl-1-allyl)-tryptophan, D: tryptophan, E: *N*-(1-methyl)-tryptophan, F: *N*-(1-propargyl)-tryptophan. Chromatograms represent the ESI-MS extracted ion for the $[M+Na]^+$ species as labeled. Note similar production levels of cyclomarazine M (**6**, trace E) and cyclomarazine P (**7**, trace F) in comparison to cyclomarazine A (**5**) in trace A and C. Production of desprenylcyclomarazine (expected $[M+Na]^+ = 353$) in the *cymD* mutant has never been observed utilizing our assay conditions.

Supplementary Figure 3. $^1\text{H-NMR}$ comparison of *N*-(1,1-dimethyl-1-allyl)-tryptophan (**2**)

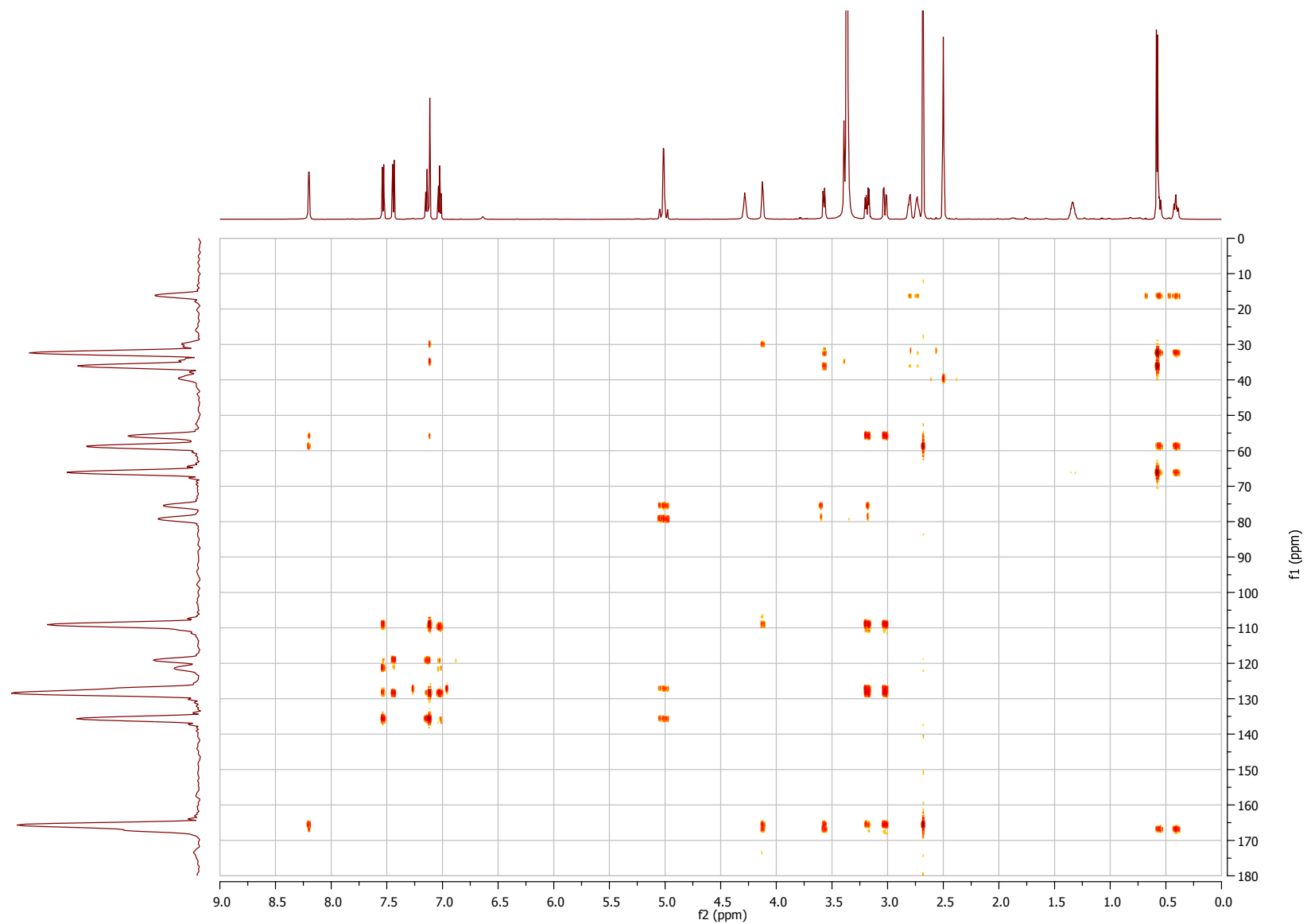


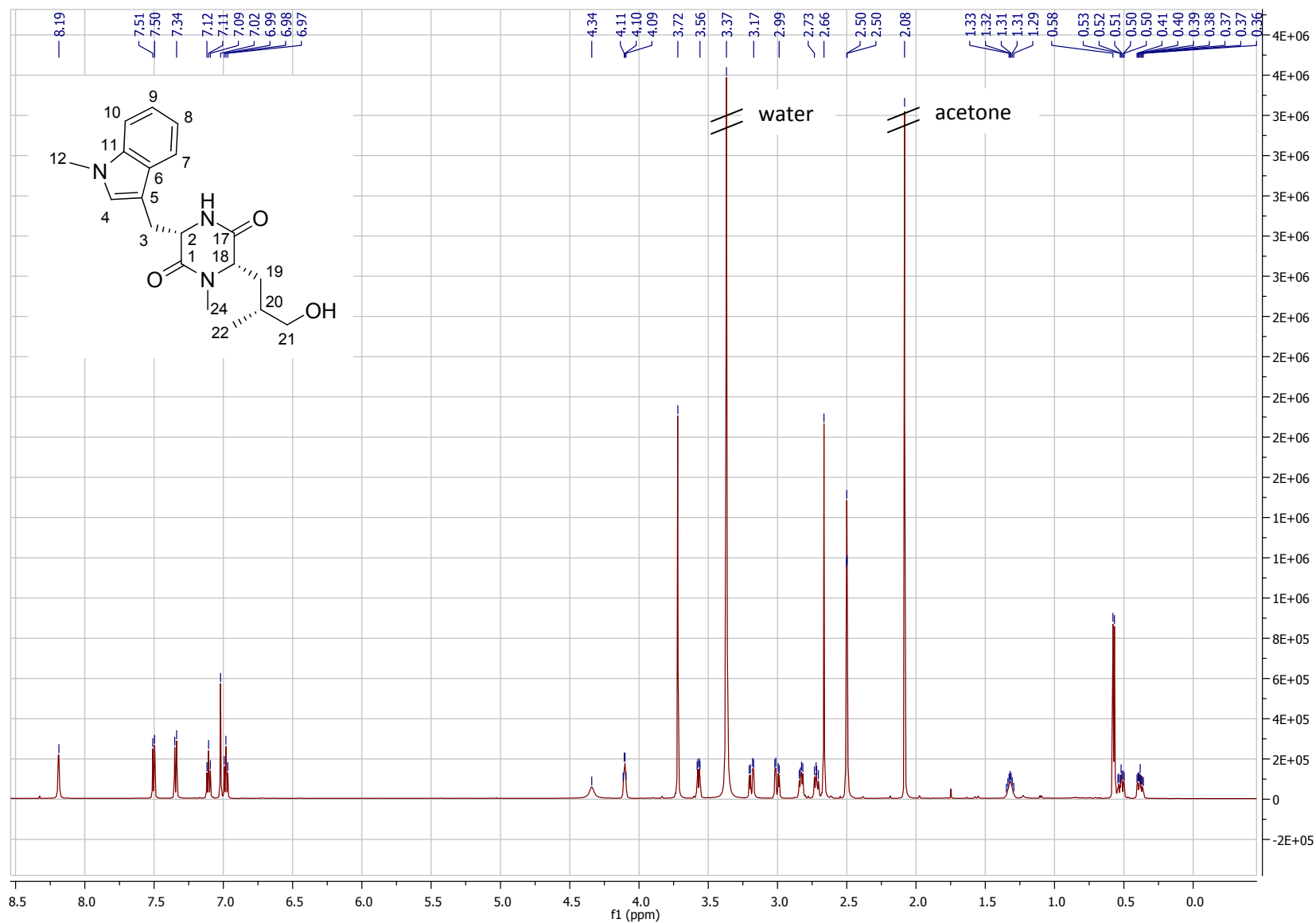
Supplementary Figure 4. ¹H-NMR of Cyclomarazine P (7)

Supplementary Figure 5. HSQC NMR of Cyclomarazine P (7)

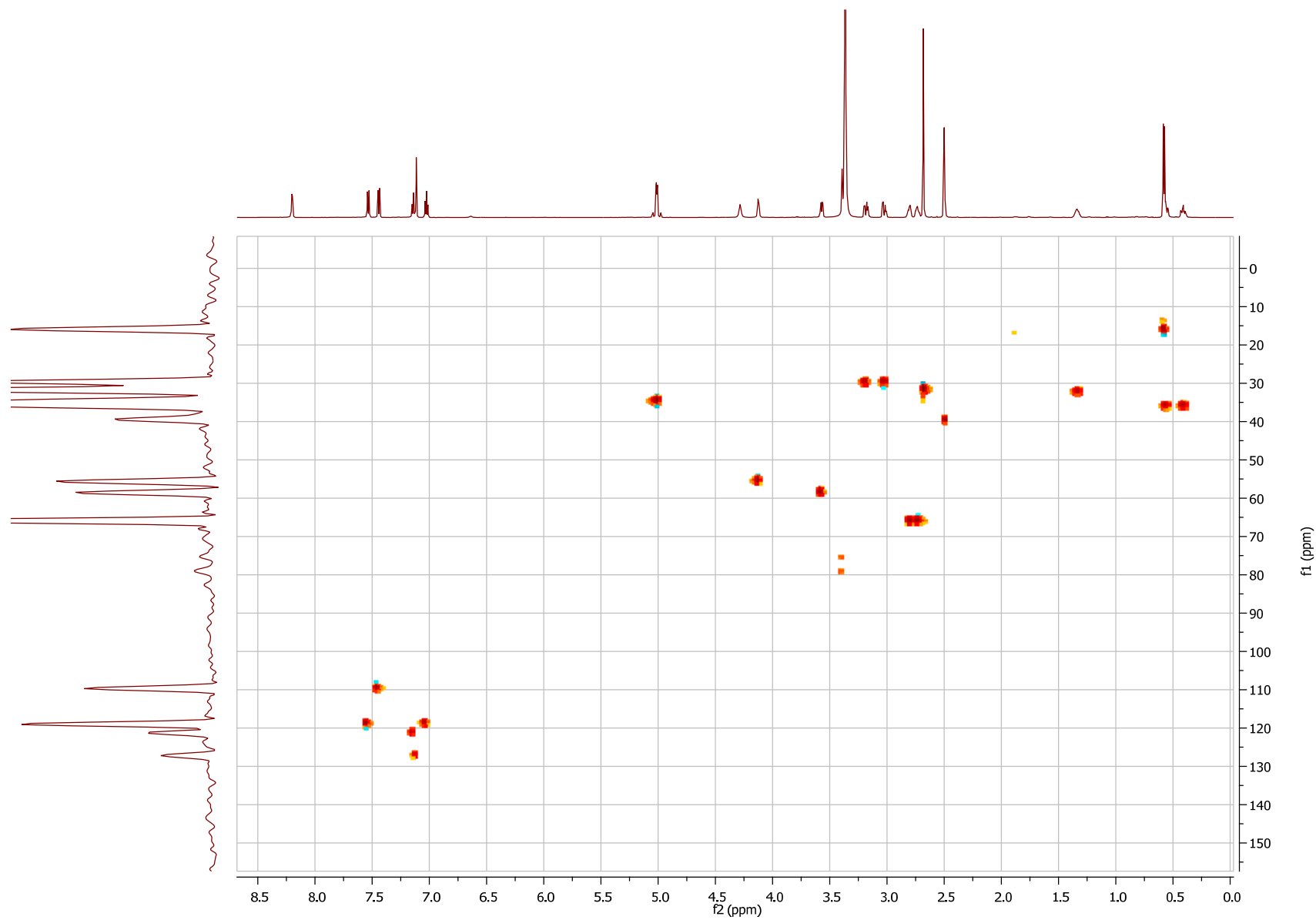


Supplementary Figure 6. HMBC NMR of Cyclomarazine P (7)

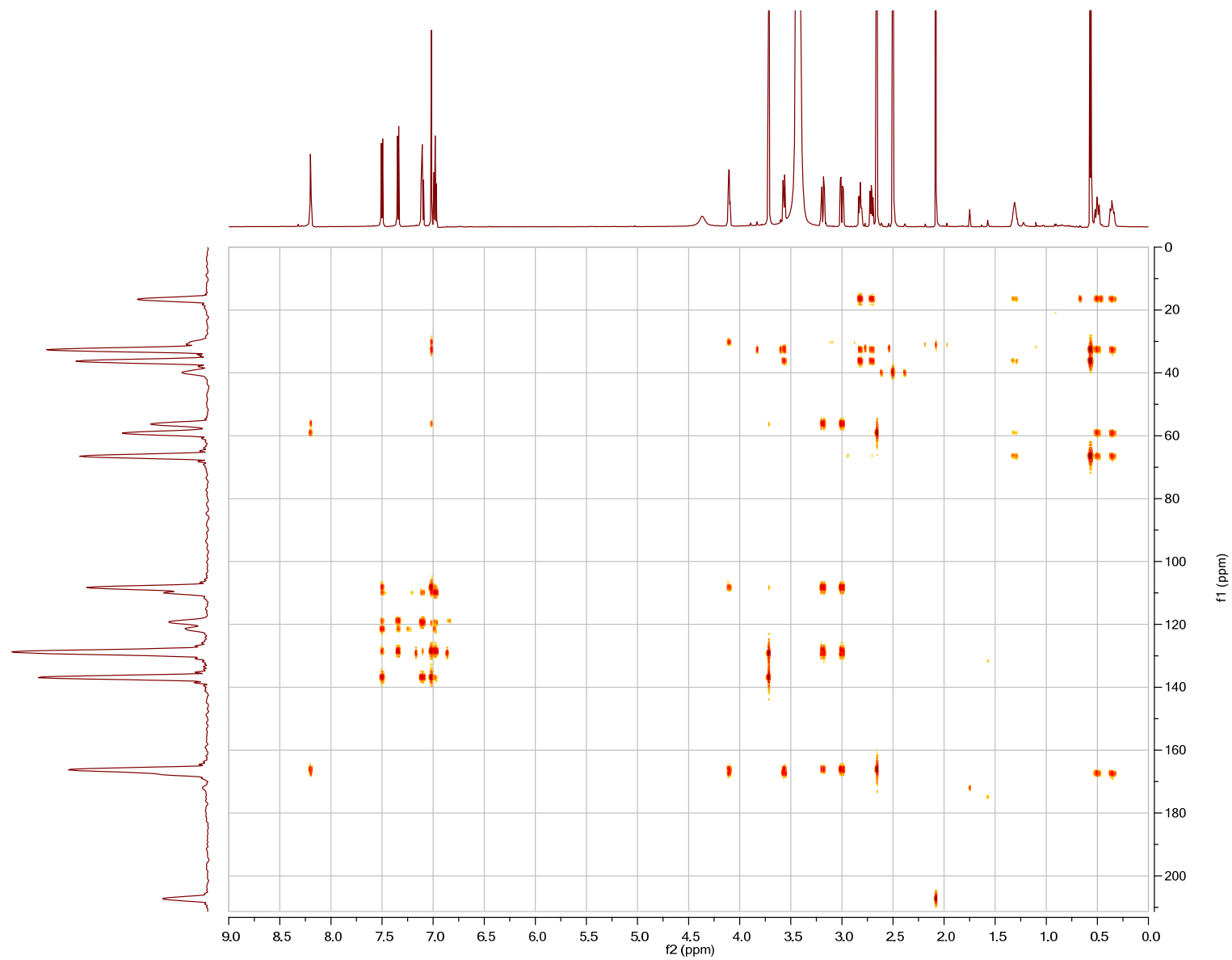


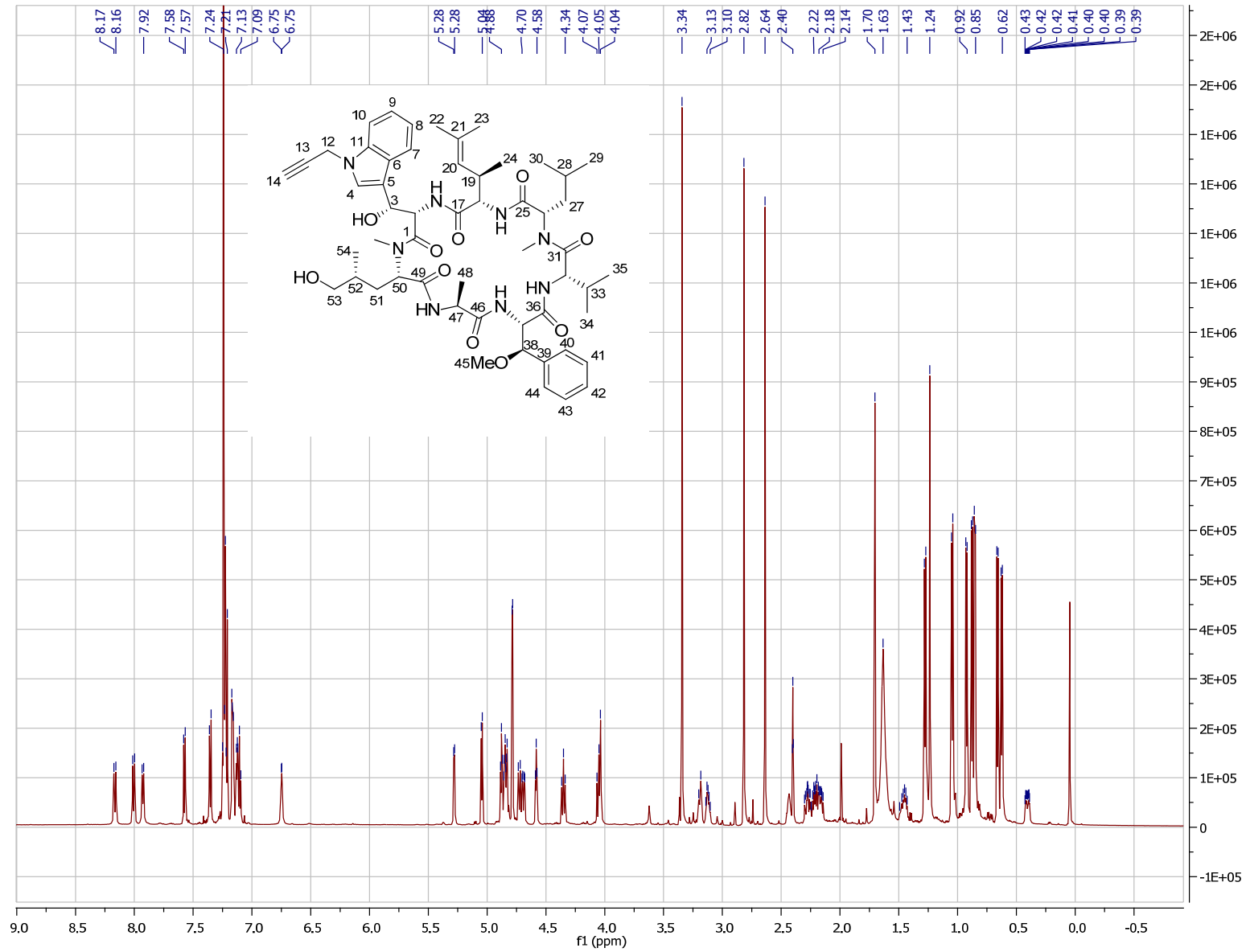
Supplementary Figure 7. $^1\text{H-NMR}$ of Cyclomarazine M (6)

Supplementary Figure 8. HSQC NMR of Cyclomarazine M (6)

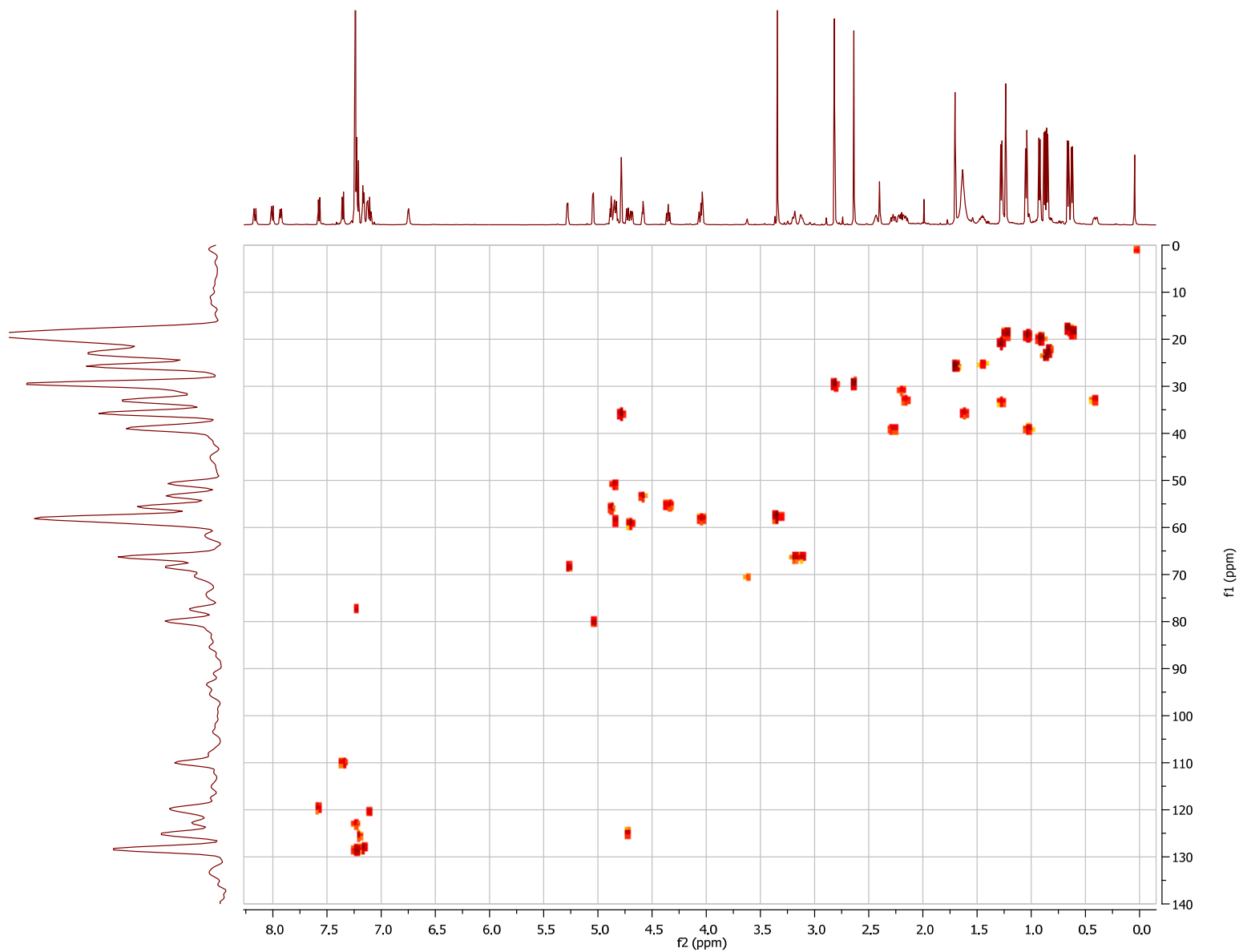


Supplementary Figure 9. HMBC NMR of Cyclomarazine M (6)

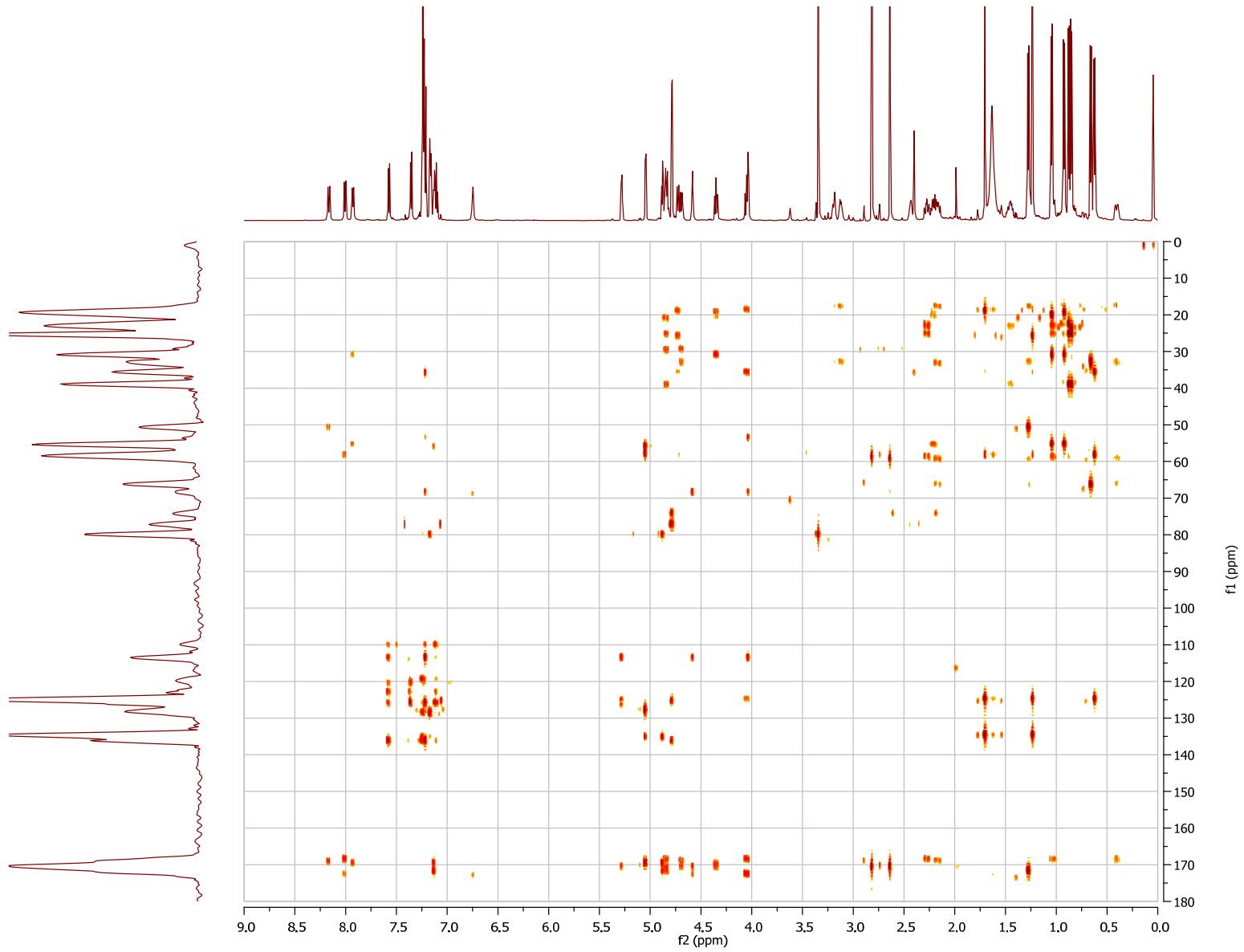


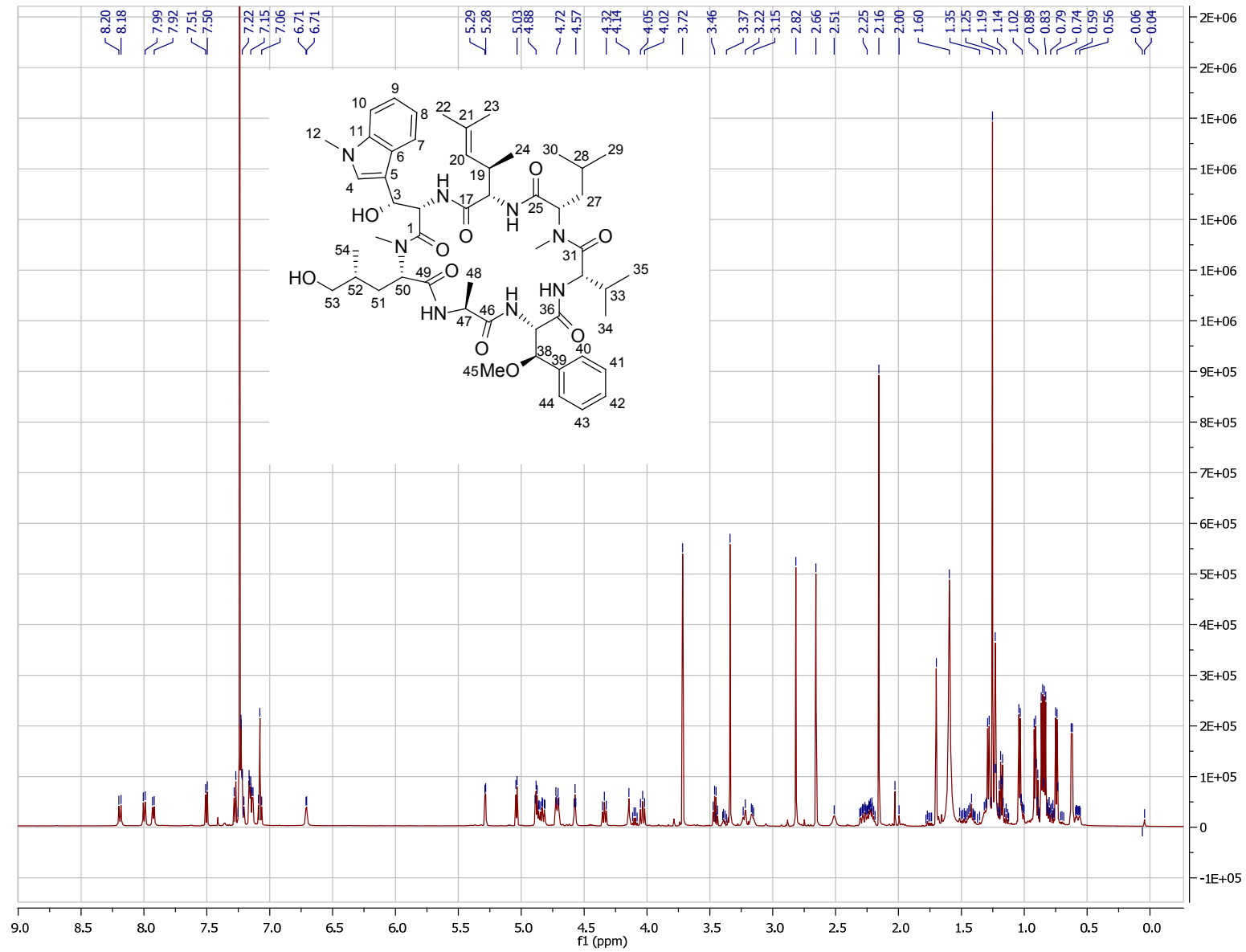
Supplementary Figure 10. ¹H-NMR of Cyclomarín P (12)

Supplementary Figure 11. HSQC NMR of Cyclomarin P (12)

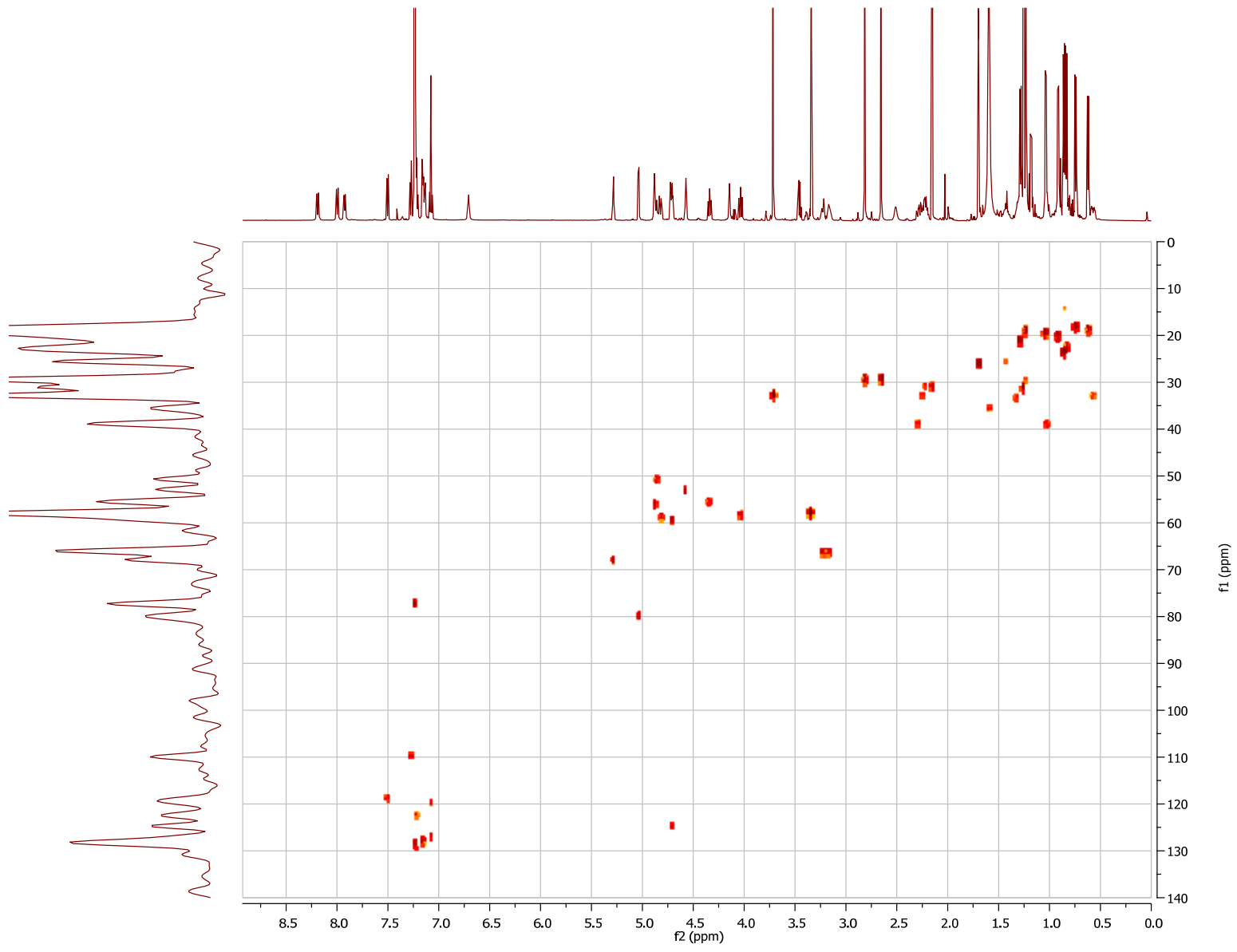


Supplementary Figure 12. HMBC NMR of Cyclomarin P (12)

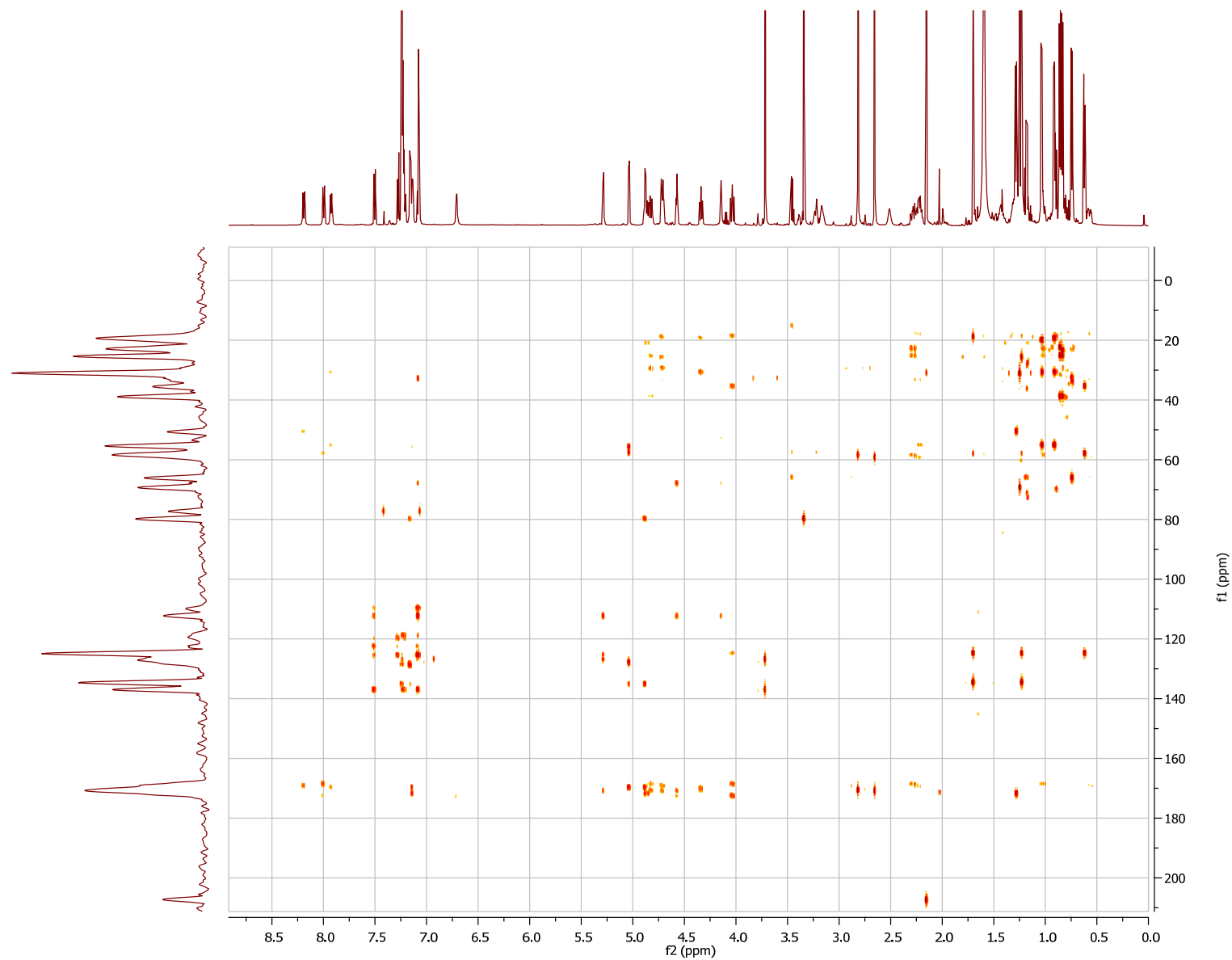


Supplementary Figure 13. ¹H-NMR of Cyclomarín M (11)

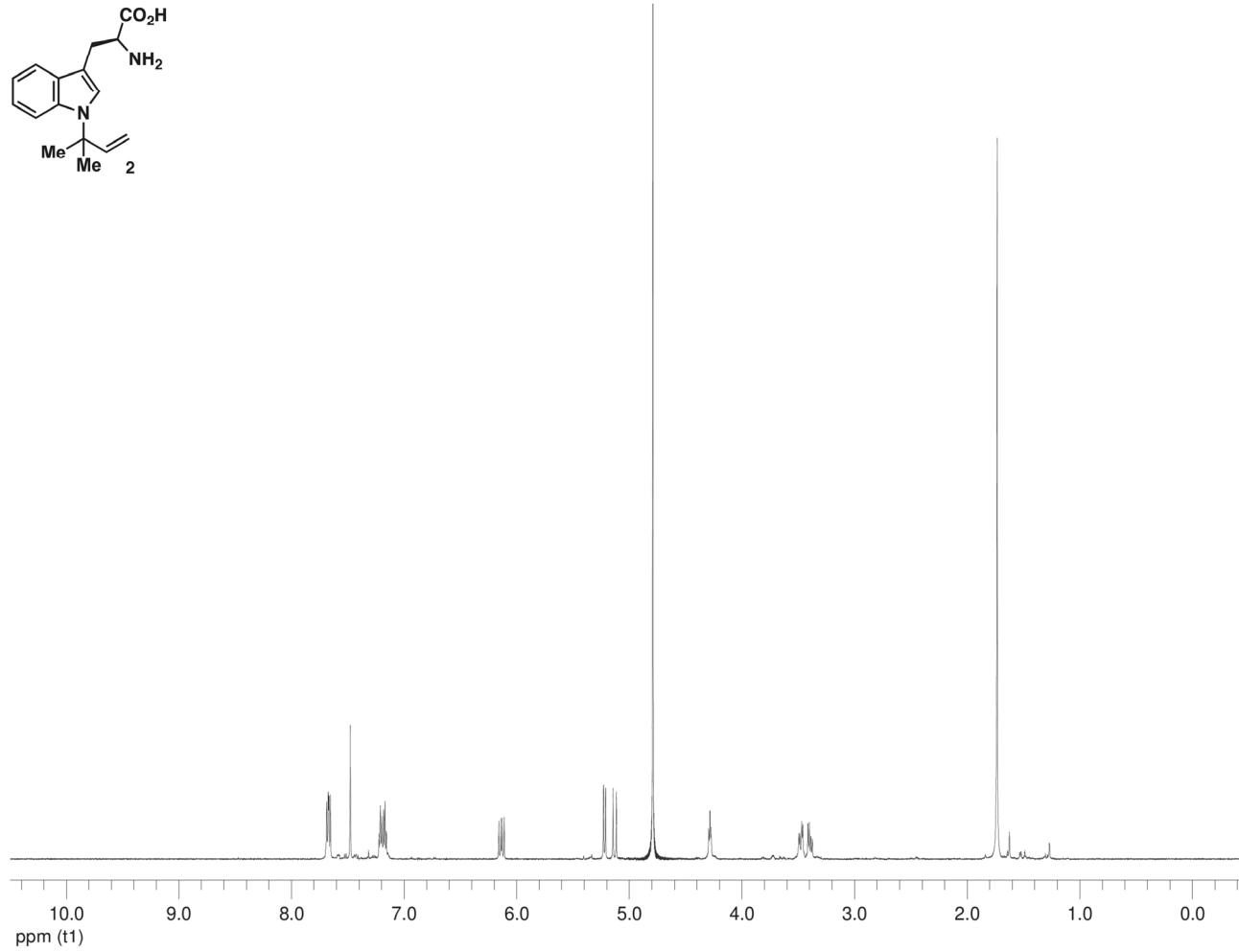
Supplementary Figure 14. HSQC NMR of Cyclomarín M (11)



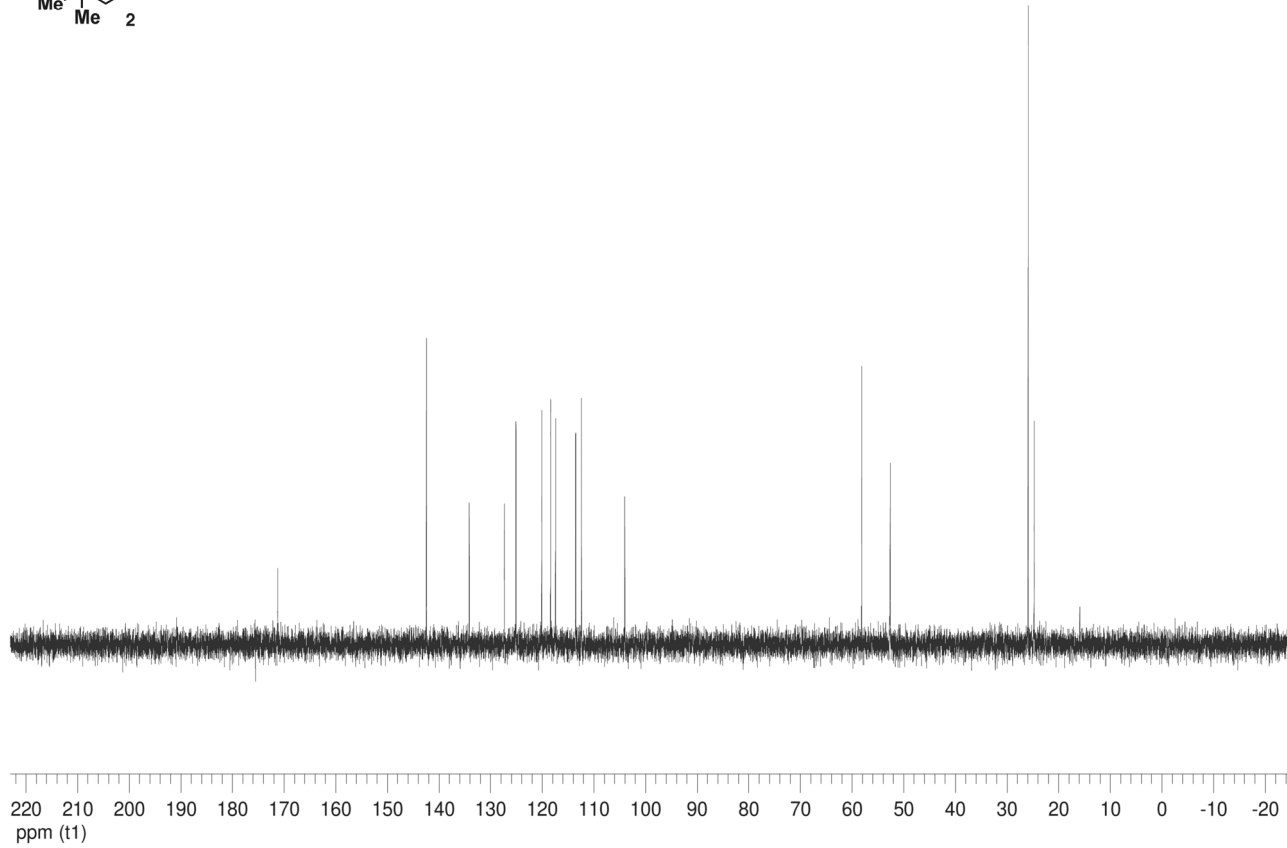
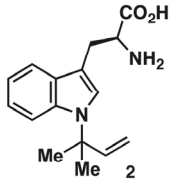
Supplementary Figure 15. HMBC NMR of Cyclomarín M (11)



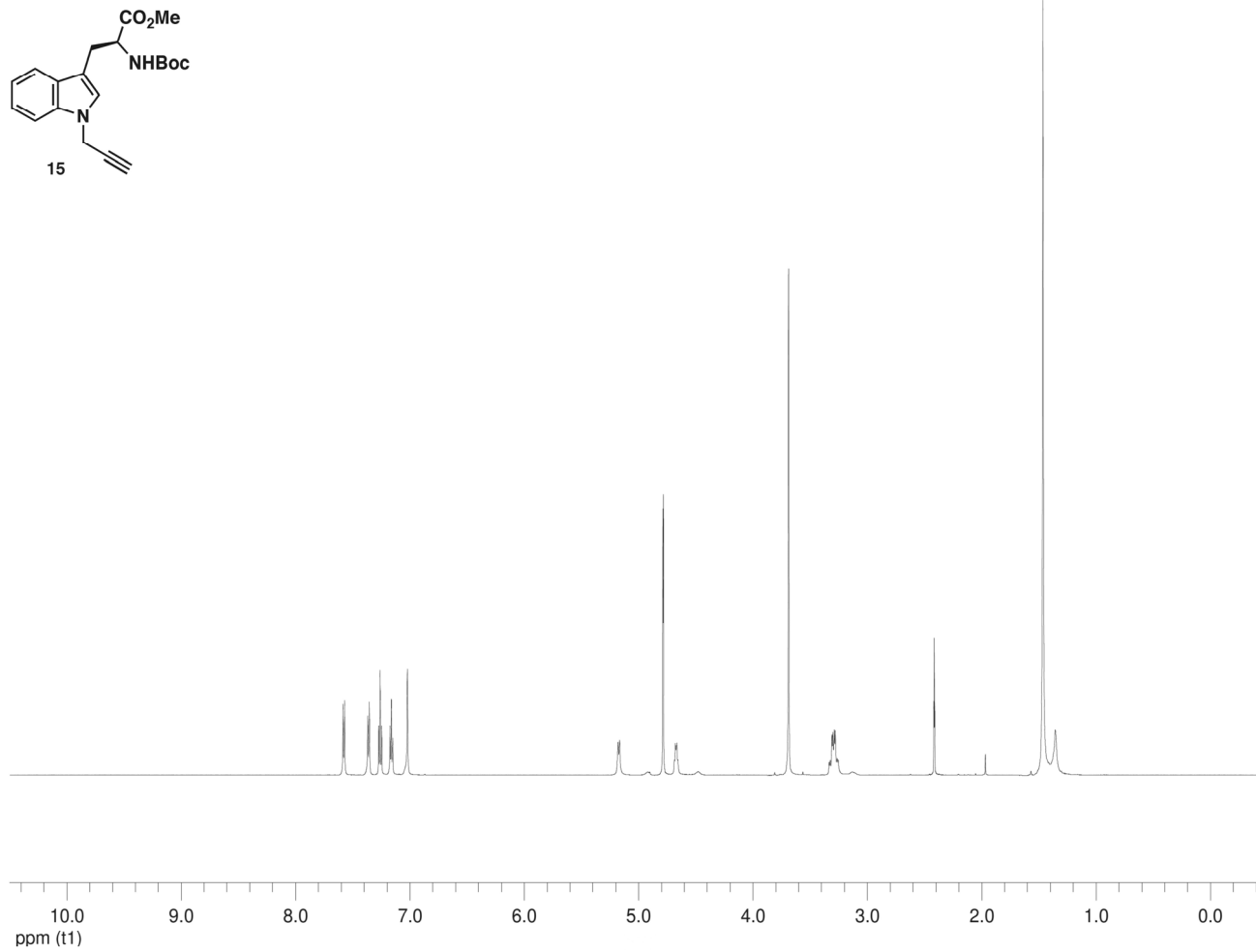
Supplementary Figure 16. ^1H NMR spectrum of (2)



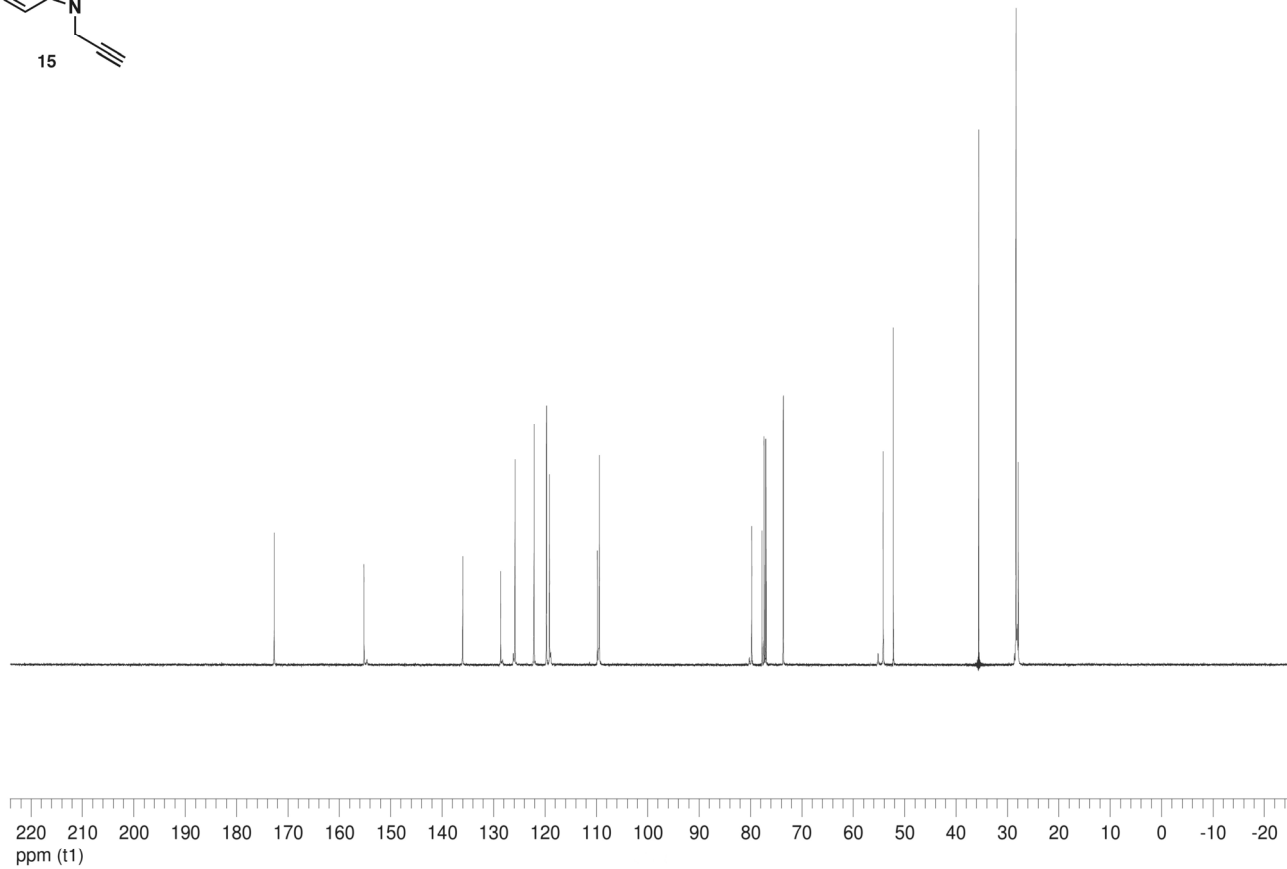
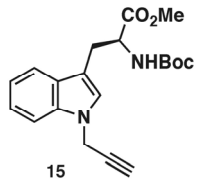
Supplementary Figure 17. ^{13}C NMR spectrum of (2)



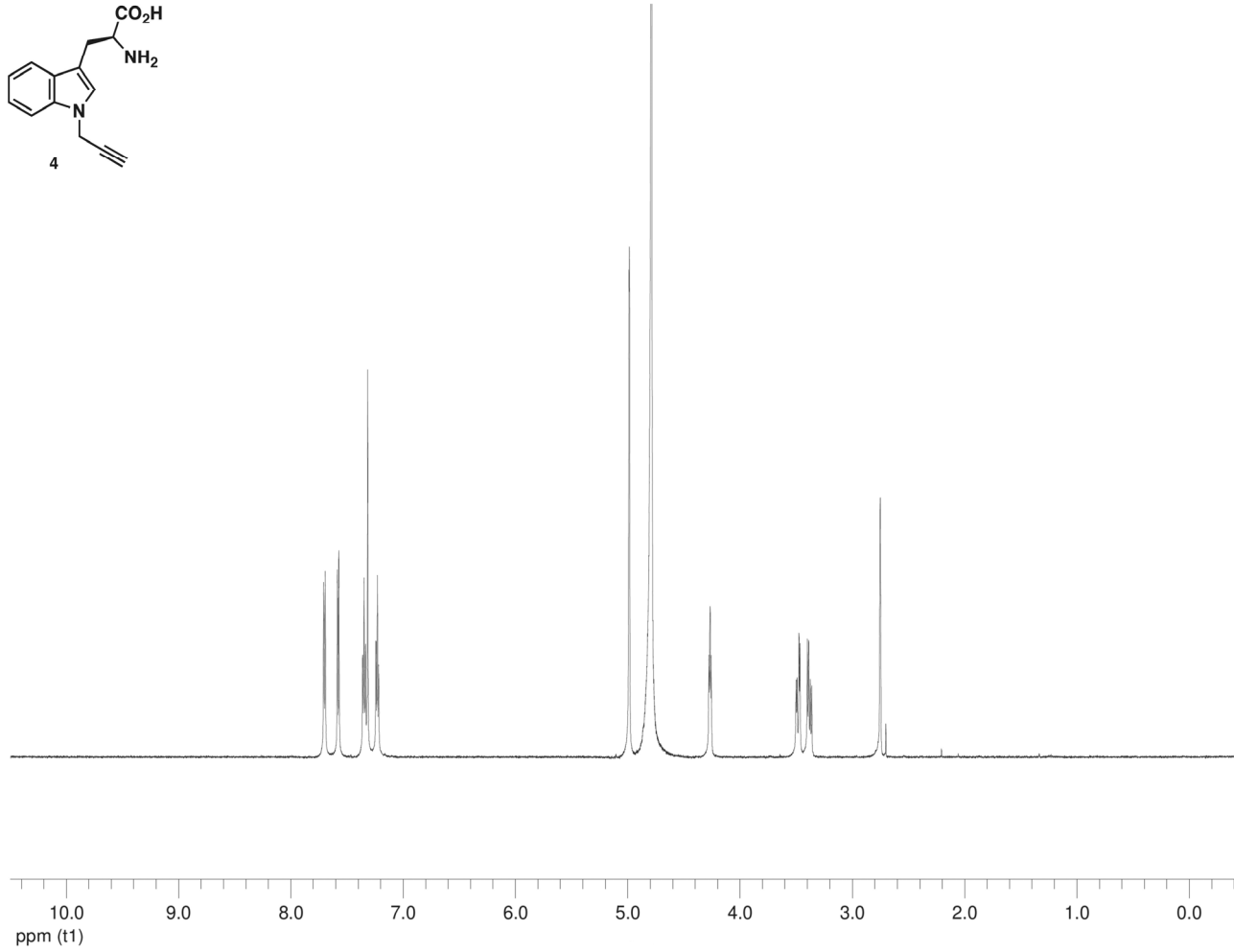
Supplementary Figure 18. ^1H NMR spectrum of (15)



Supplementary Figure 19. ^{13}C NMR spectrum of (15)



Supplementary Figure 20. ^1H NMR spectrum of (4)



Supplementary Figure 21. ^{13}C NMR spectrum of (4)

