Supporting information for:

Boholamide A, an APD-Class, Hypoxia-Selective Cyclodepsipeptide

Figure S1. Validation of Ca^{2+} assay for boholamide. Top: Response of extract 3158H.R.1.a.03 (in green trace) showing increase of cytoplasmic calcium ions over time in comparison to controls. Bottom: Validation of extract 3158H.R.1.a.03 response using calcium imaging. Red arrows indicate reagent and sample used to test cell responsiveness while a line indicates the duration at which the cells are exposed to the extract. Each trace is a response coming from a single cell.

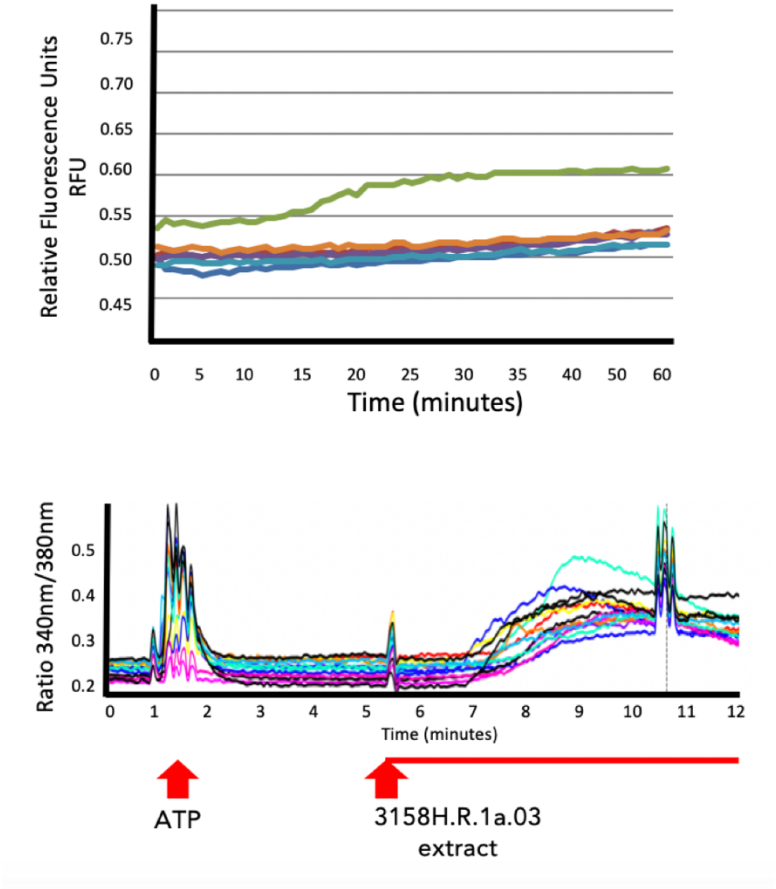


Figure S2. ^{1}H NMR spectrum of compound 1 in DMSO-d_6.

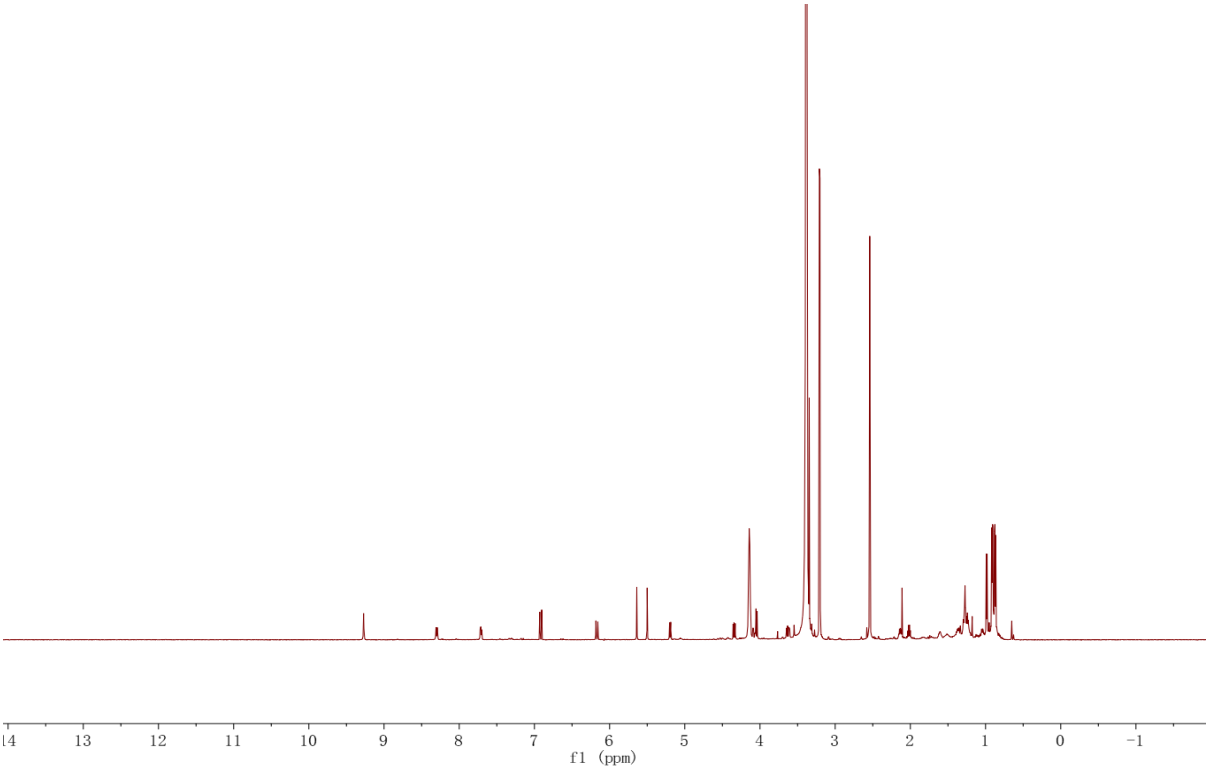


Figure S3. HSQC spectrum of compound 1 in DMSO-d_6.

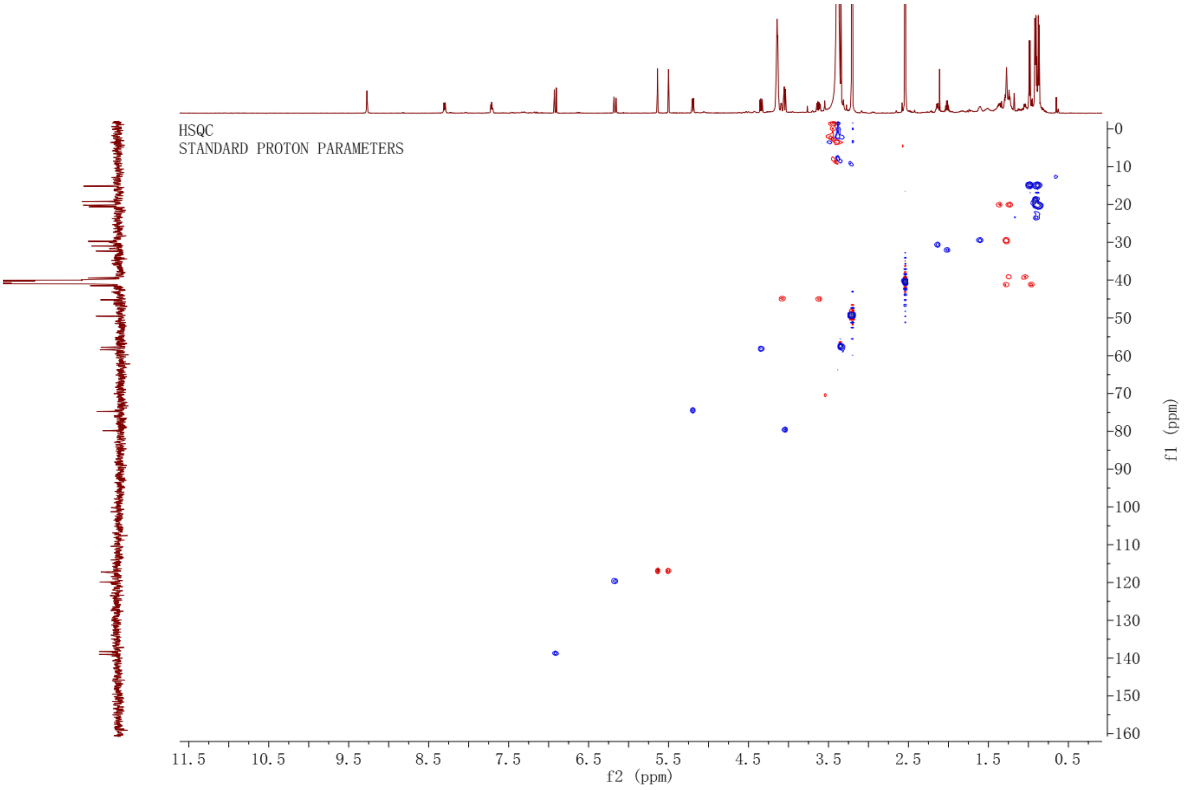


Figure S4. HMBC spectrum of compound 1 in DMSO-d_6.

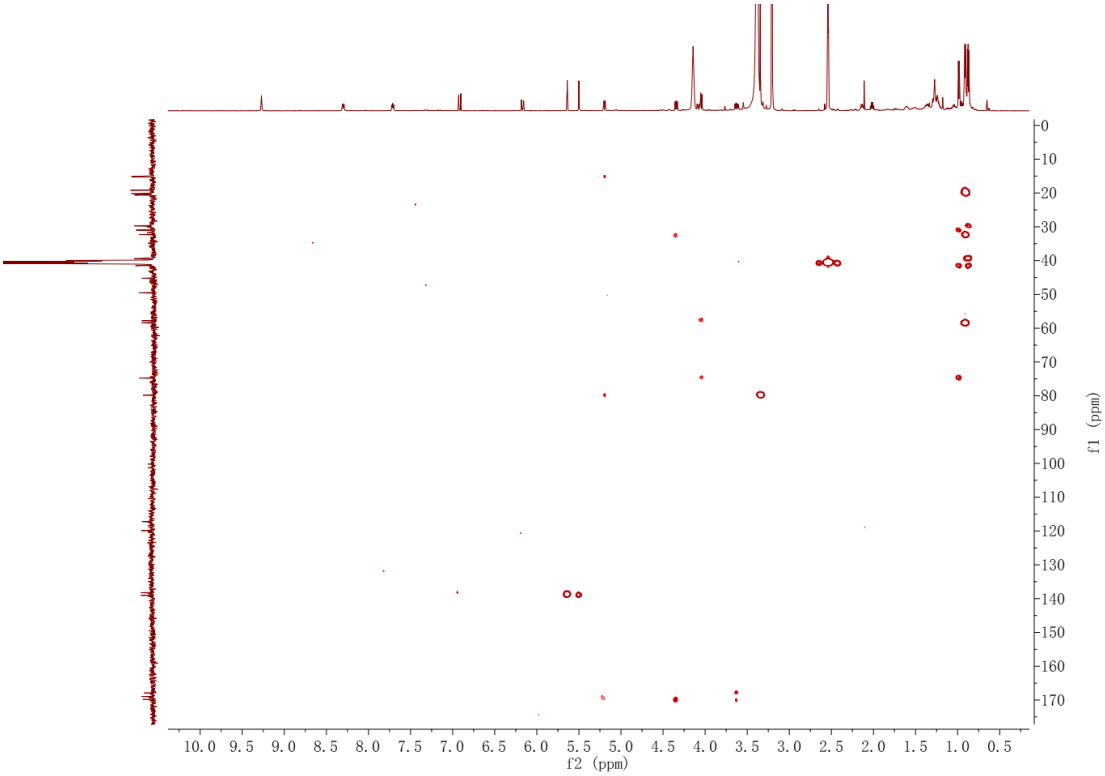


Figure S5. COSY spectrum of compound 1 in DMSO-d_6.

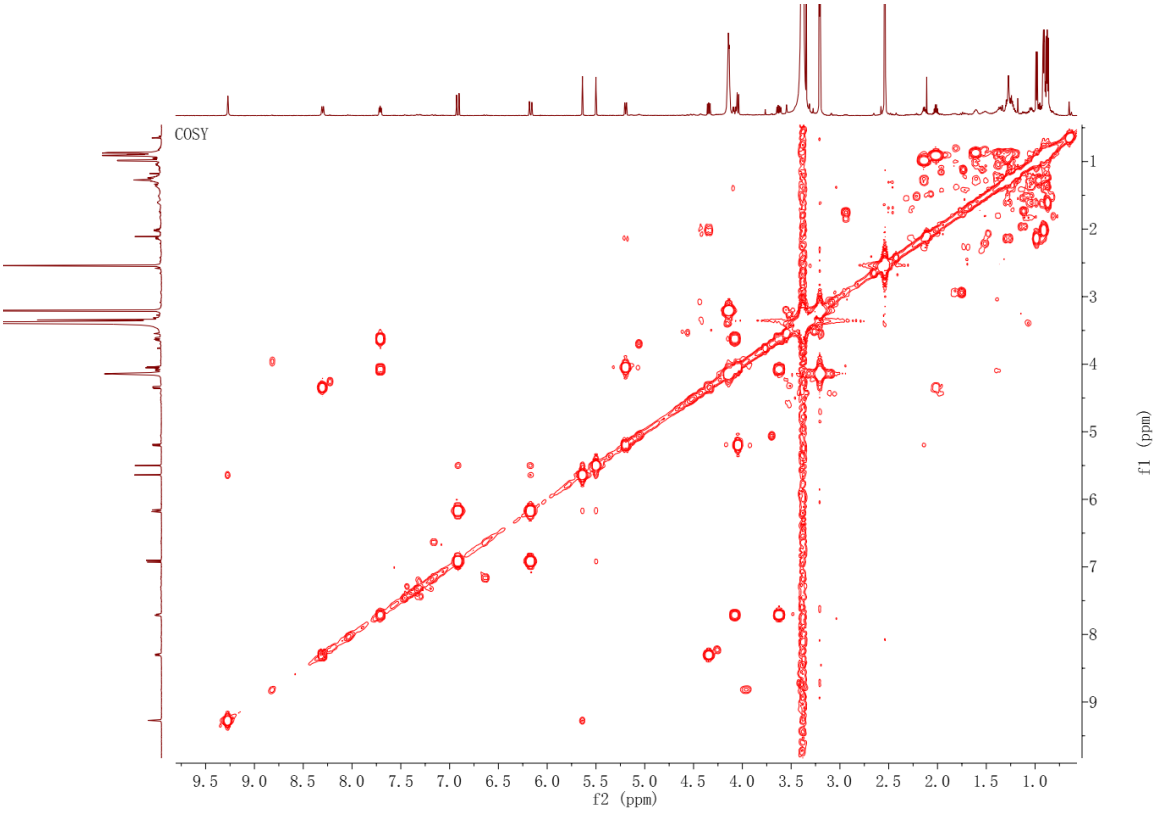


Figure S6. ROESY spectrum of compound 1 in DMSO-d_6.

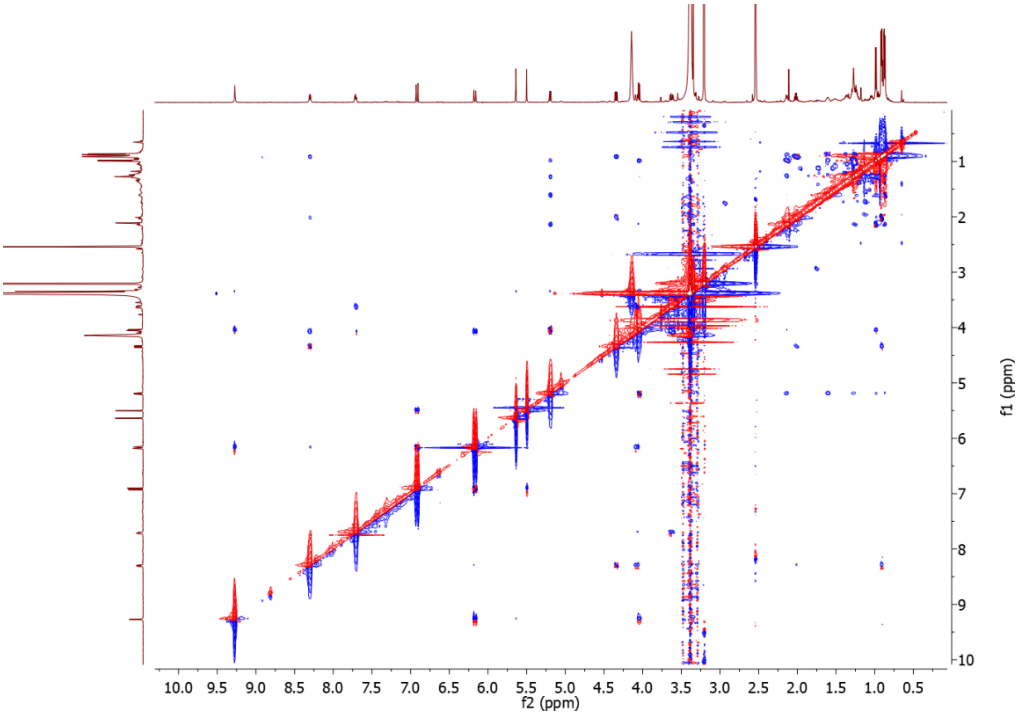


Figure S7. ^{13}C NMR spectrum of compound 1 in DMSO-d_6.

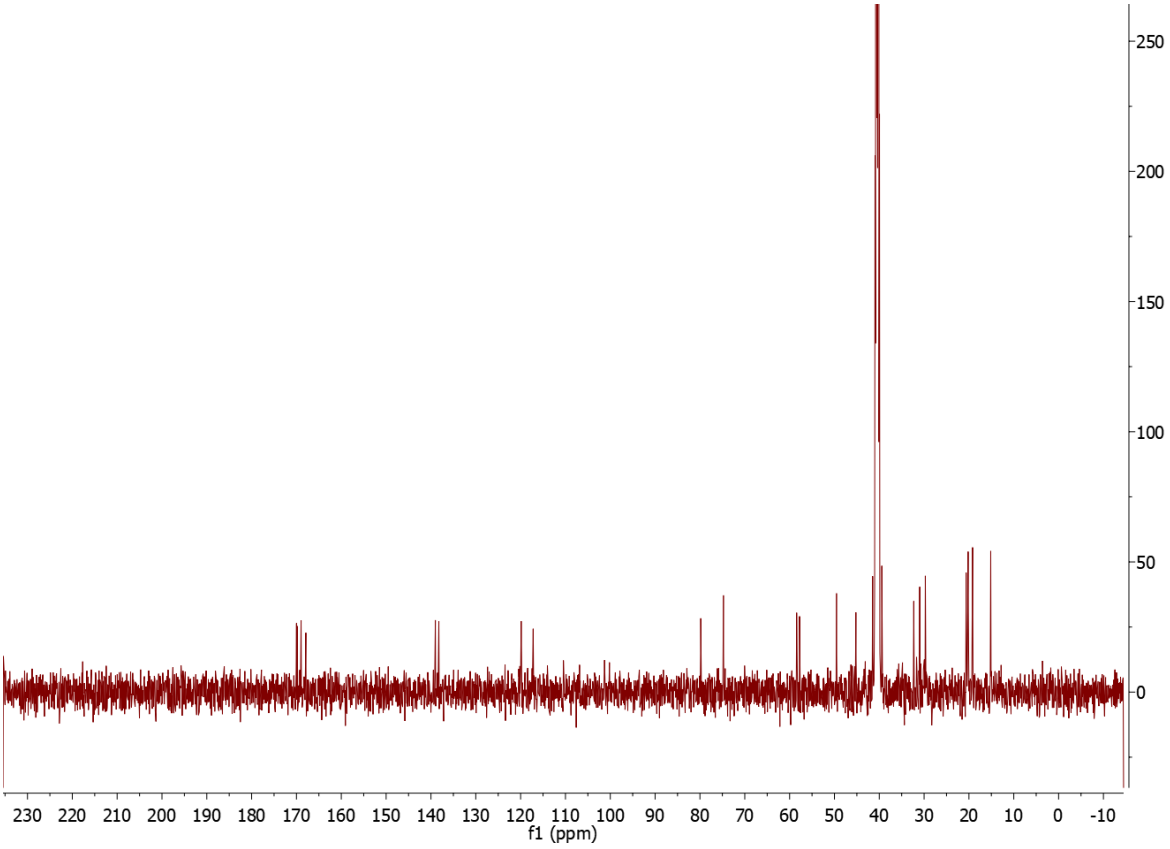


Figure S8. Comparison of the NOESY signal and the coupling constant pattern in the macrocyclic ring between boholamide A (1) and rakicidin A(*1*) (2).

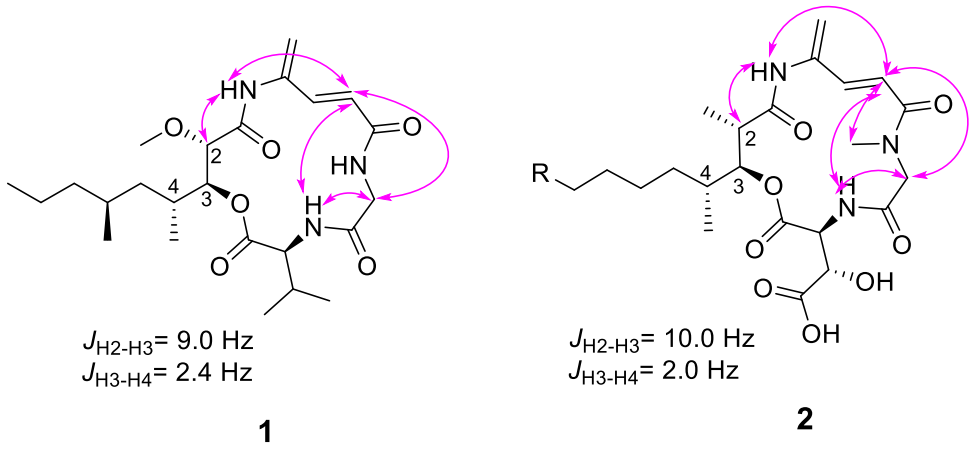


Figure S9. ^{1}H NMR spectrum of 3a/3b in DMSO-d_6.

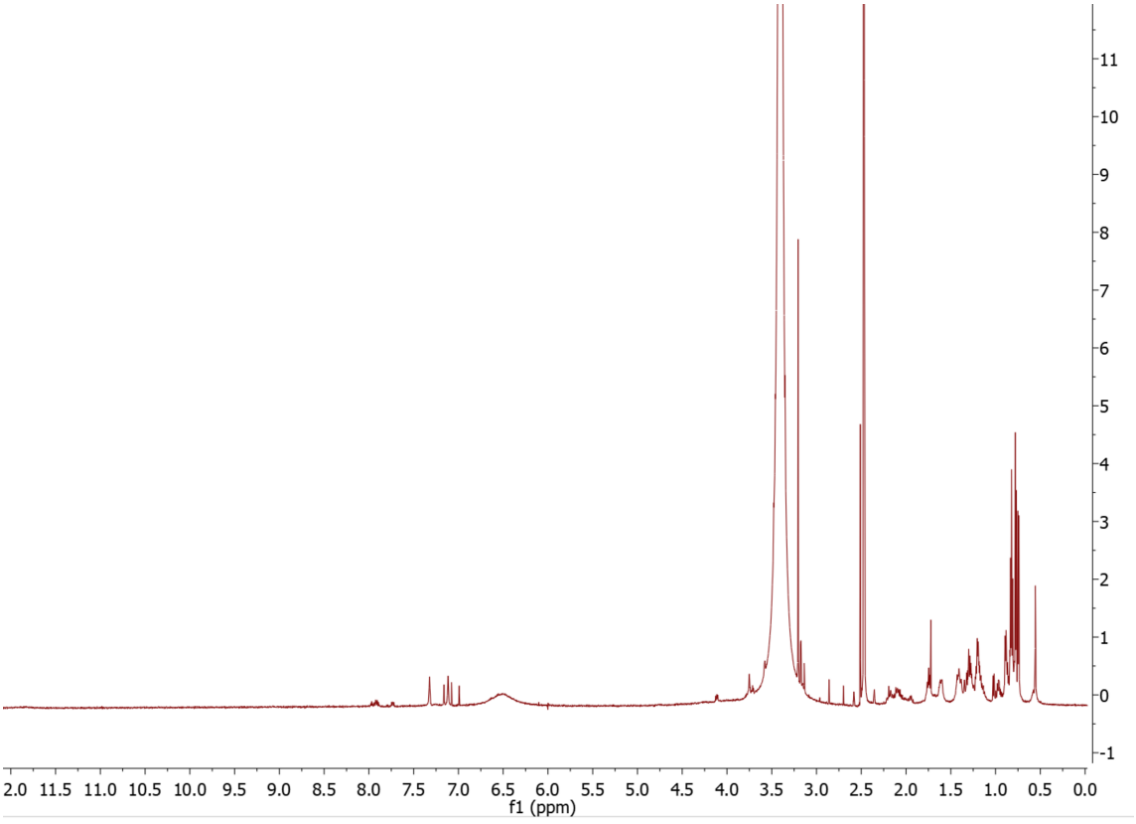


Figure S10. HSQC of 3a/3b in DMSO-d_6.

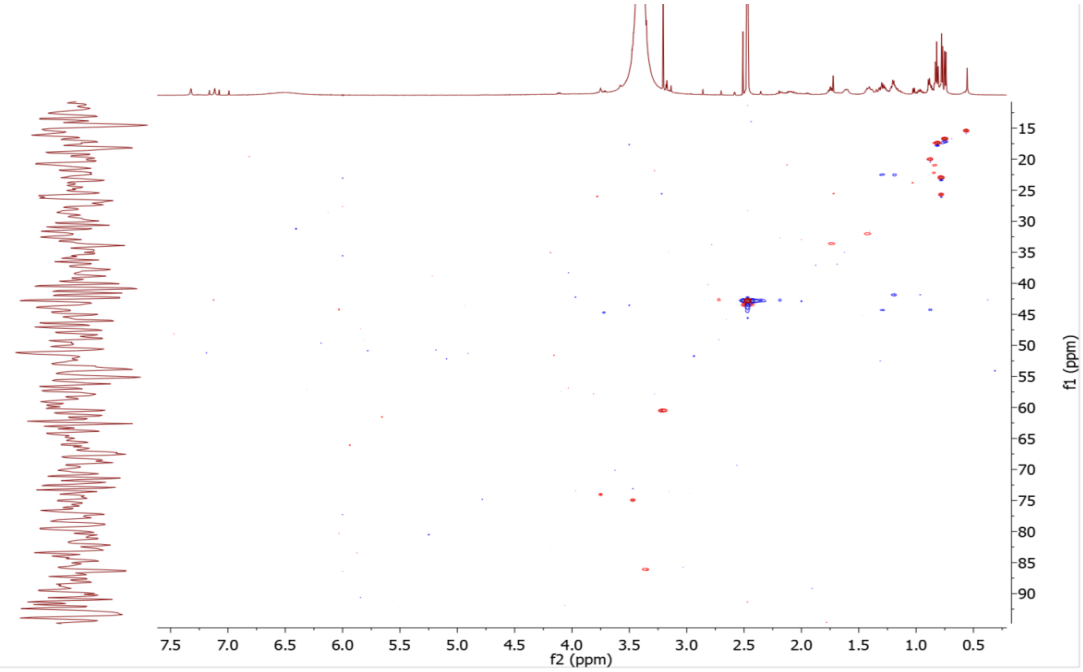


Figure S11. COSY of 3a/3b in DMSO-d_6.

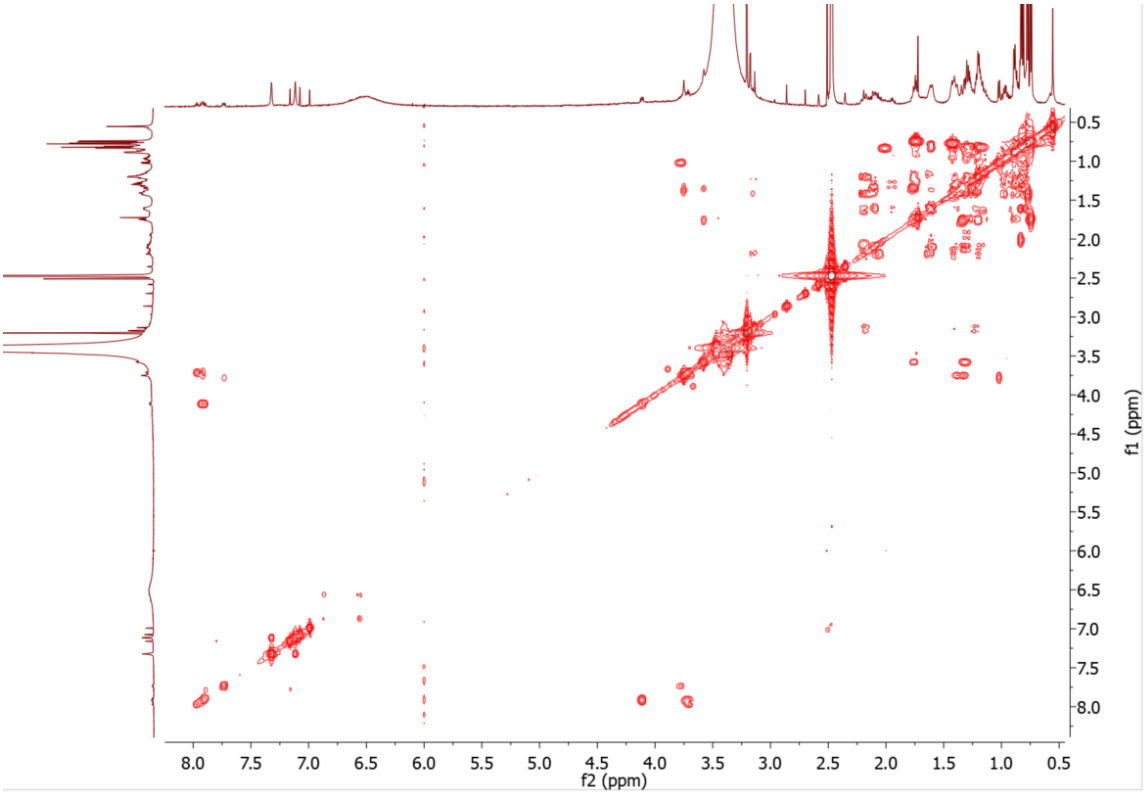
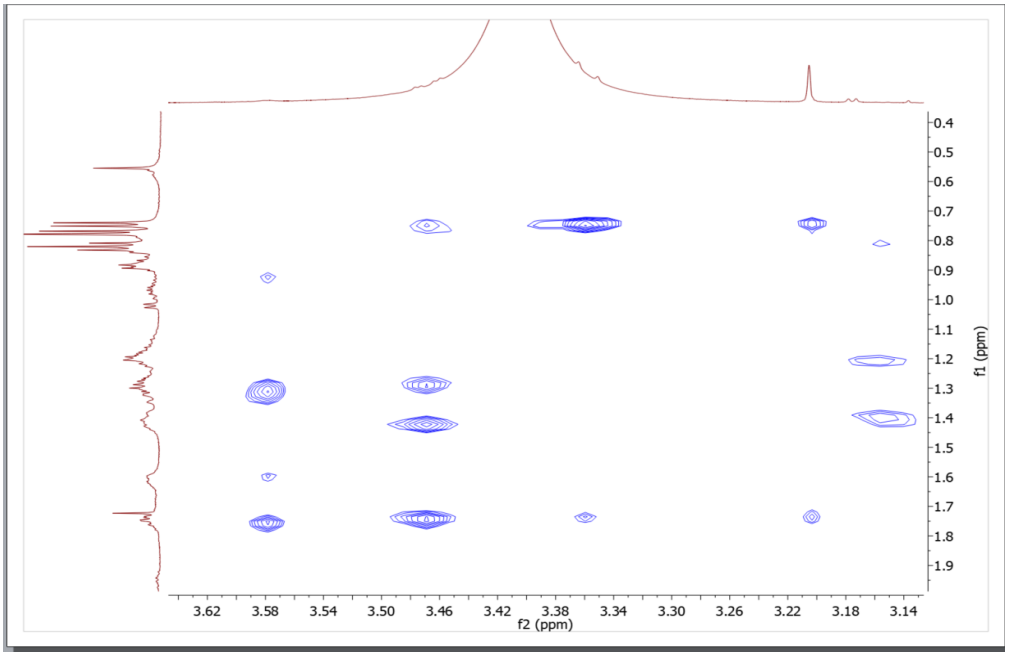


Figure S12. ROESY of 3a/3b in DMSO-d_6.



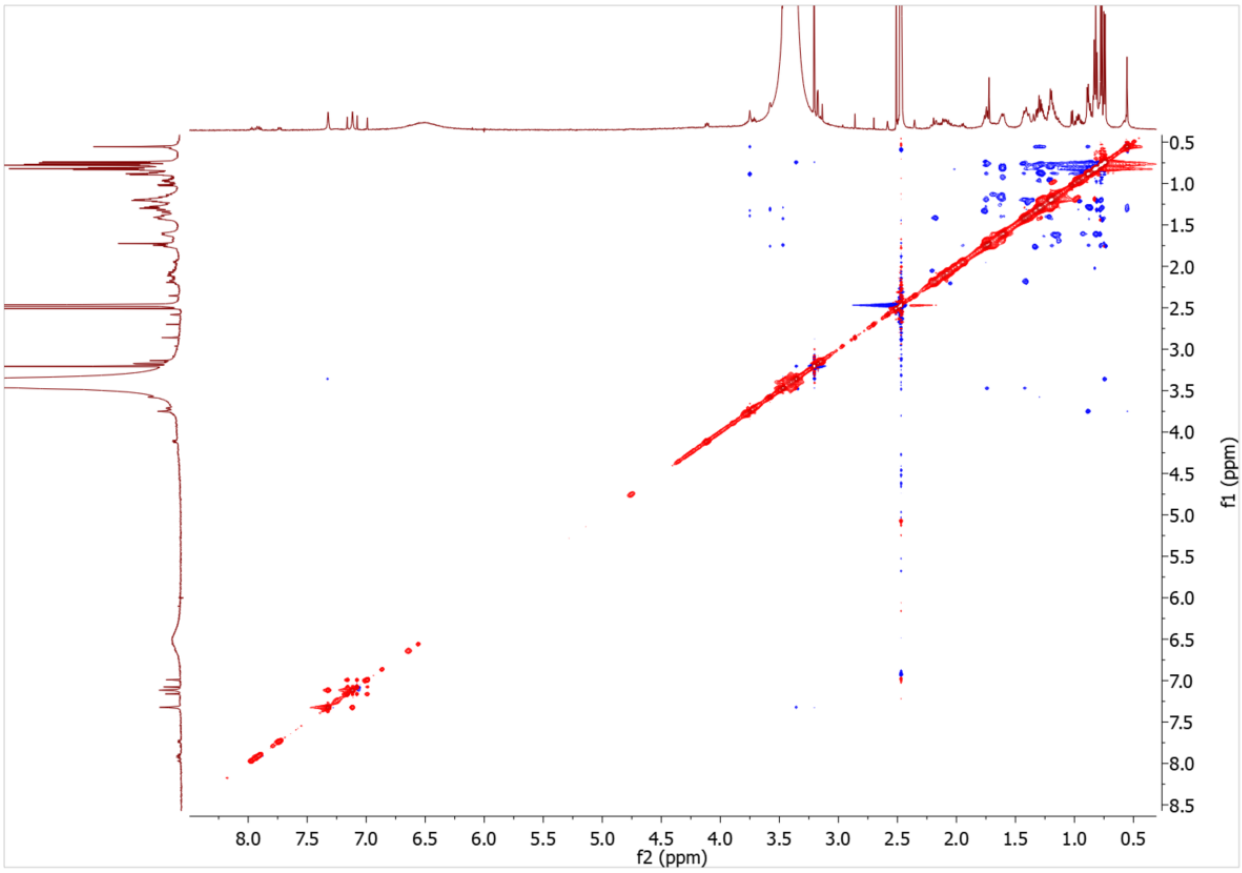


Figure S13. ESI-MS2 spectrum of compound 3a.

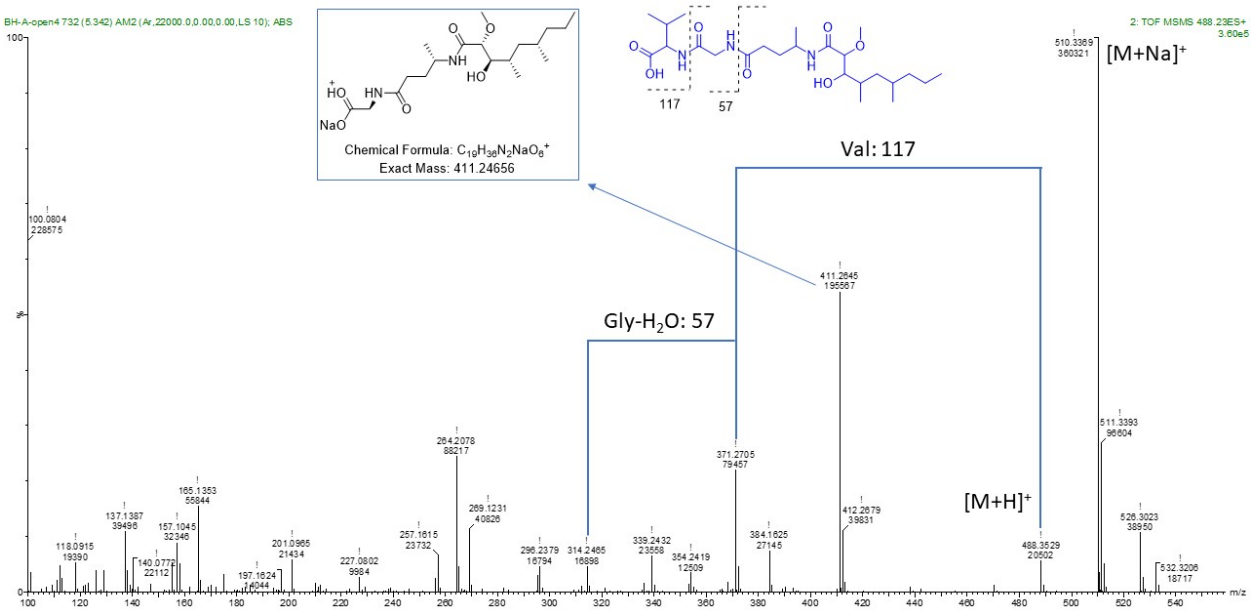


Figure S14. Marfey's analysis of 1. Top: D-Val standard. Mid: L-Val standard. Bottom: Hydrolysate of **1**.

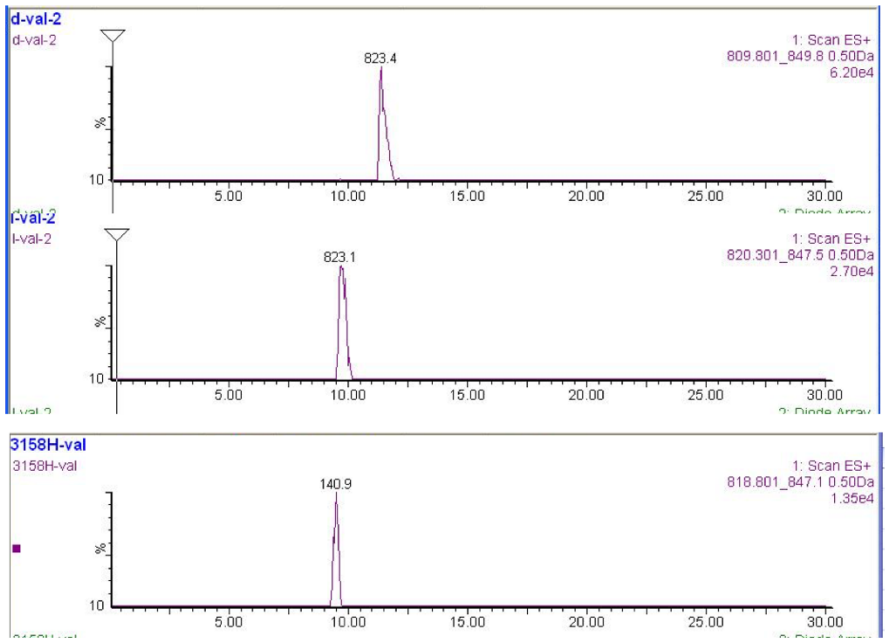
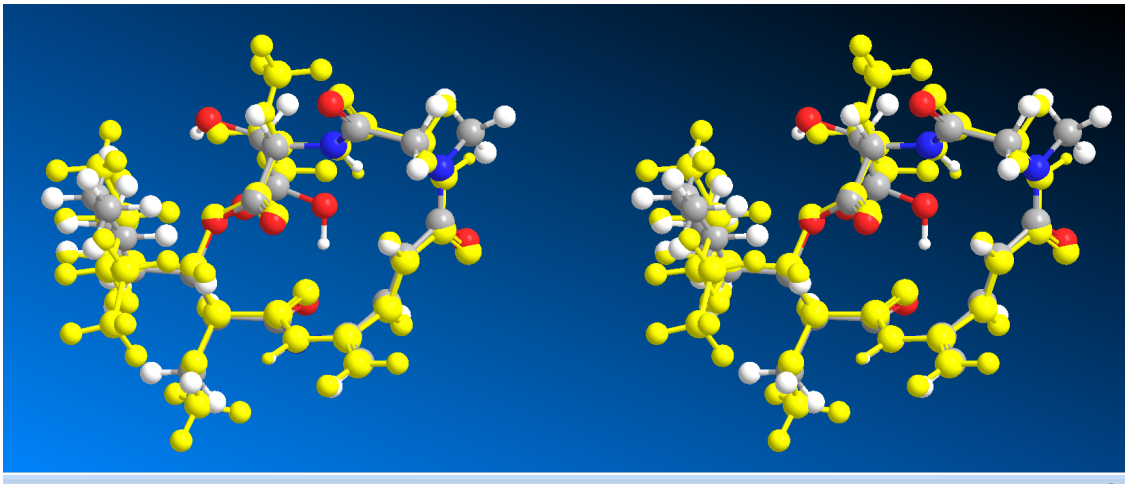
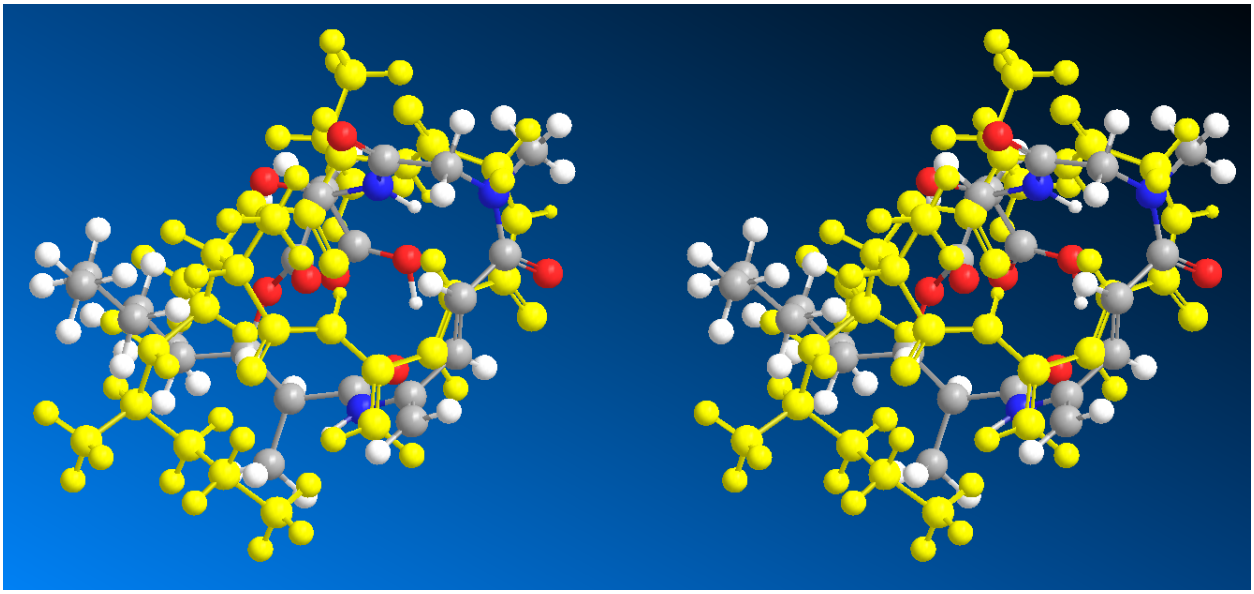


Figure S15. Stereoview of the preferred conformations of boholamide A (yellow) aligned with those for rakicidin (gray), predicted by ConfBuster(*2*). The modeled conformation shown in panel A agreed with the experimental NOESY data and the coupling constant pattern, whereas models B-D significantly deviated and did not give the same predicted NOESY and coupling data.

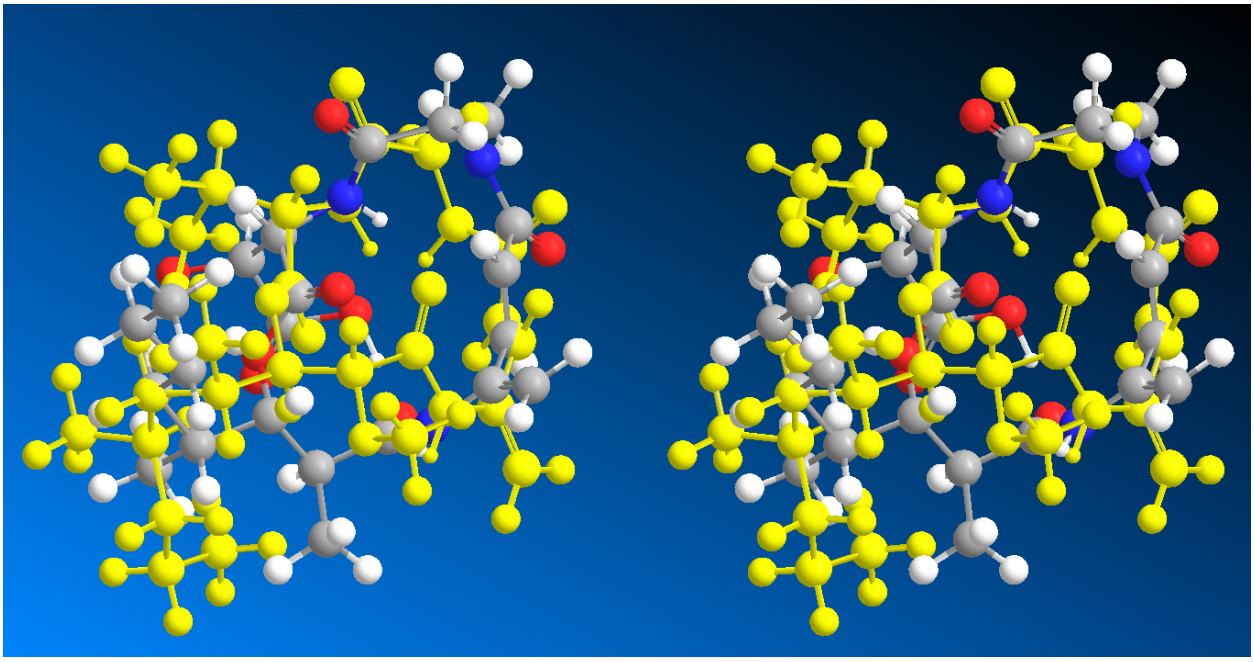
A. 2*S*,3*S*,4*R*,5*S*-boholamide A aligned with rakicidin provides a near-identical fit.



B. 2*S*,3*S*,4*R*,5*R*-boholamide A aligned with rakicidin.



C. 2*R*,3*R*,4*S*,5*R*-boholamide A aligned with rakicidin.



D. 2*R*,3*R*,4*S*,5*S*-boholamide A aligned with rakicidin.

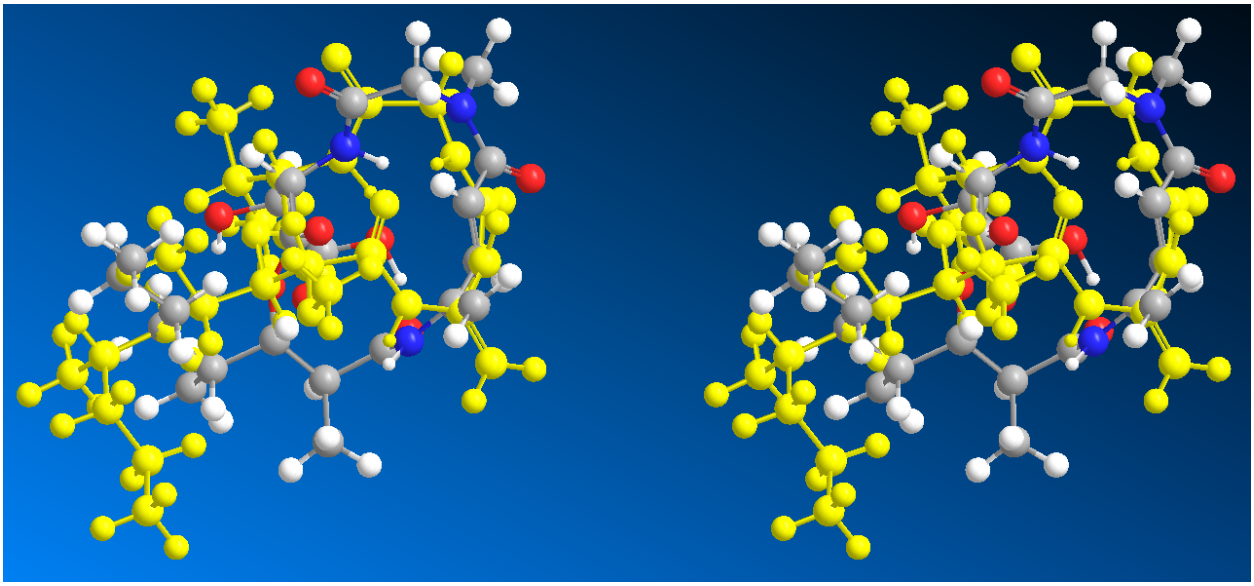


Figure S16. Comparison of calculated and experimental ECD spectra of compound 1.

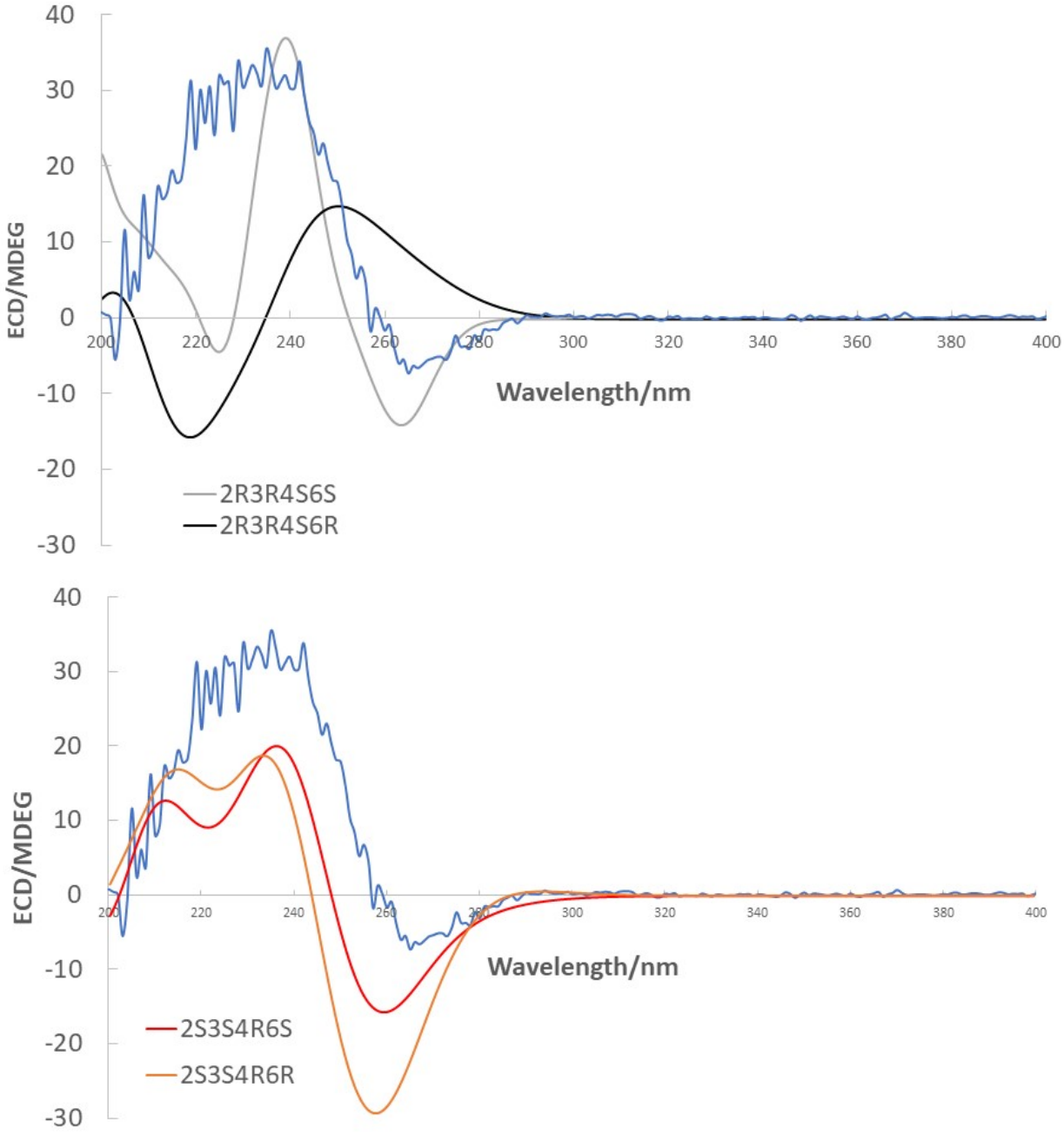


Figure S17. DRG assay with boholamide A (1) using a 15-second pulse. Each trace indicates the response of an individual cell, shown at left (out of approximately 2,000 cells in the visual field). Traces indicate increasing relative fluorescence due to accumulating cytoplasmic Ca^{2+}. Pulses of different solutions are made at the times shown, with A = AITC and C = capsaicin.

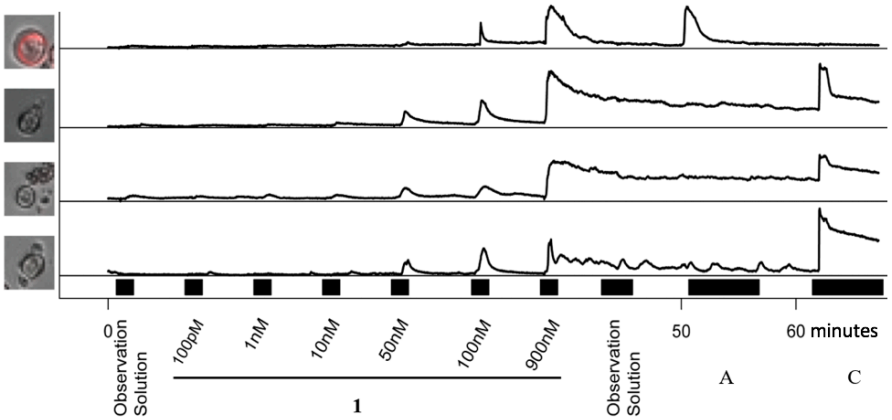


Figure S18. Light micrographs showing R13 and N15 DRG neurons from the experiment shown in Figure 7. The panels show individual DRG neurons from a total of 1125 imaged in this experiment. At 1 μM concentration, compound **1** caused vesiculation in R13 and other neurons, but not in N15 neurons. “START” and “END” refer to the same neurons imaged before the application of compounds and at the end of the experiment, after compounds have been applied.

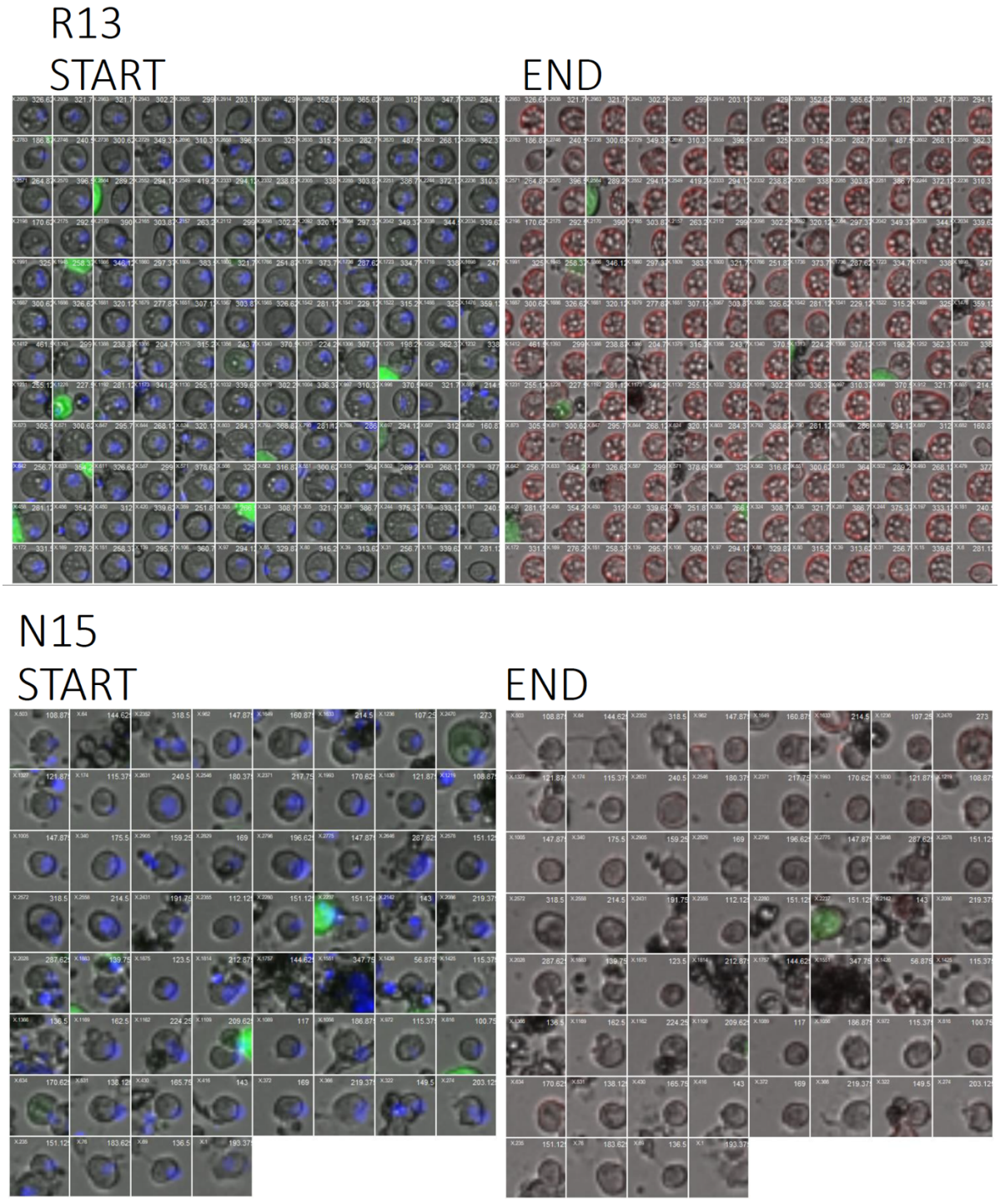


Figure S19. Zebrafish developmental assay with boholamide A (1). Zebrafish larvae were treated at 6 hours post fertilization with 1. Images were taken at 1-day post fertilization. Scale bar = 10 um.

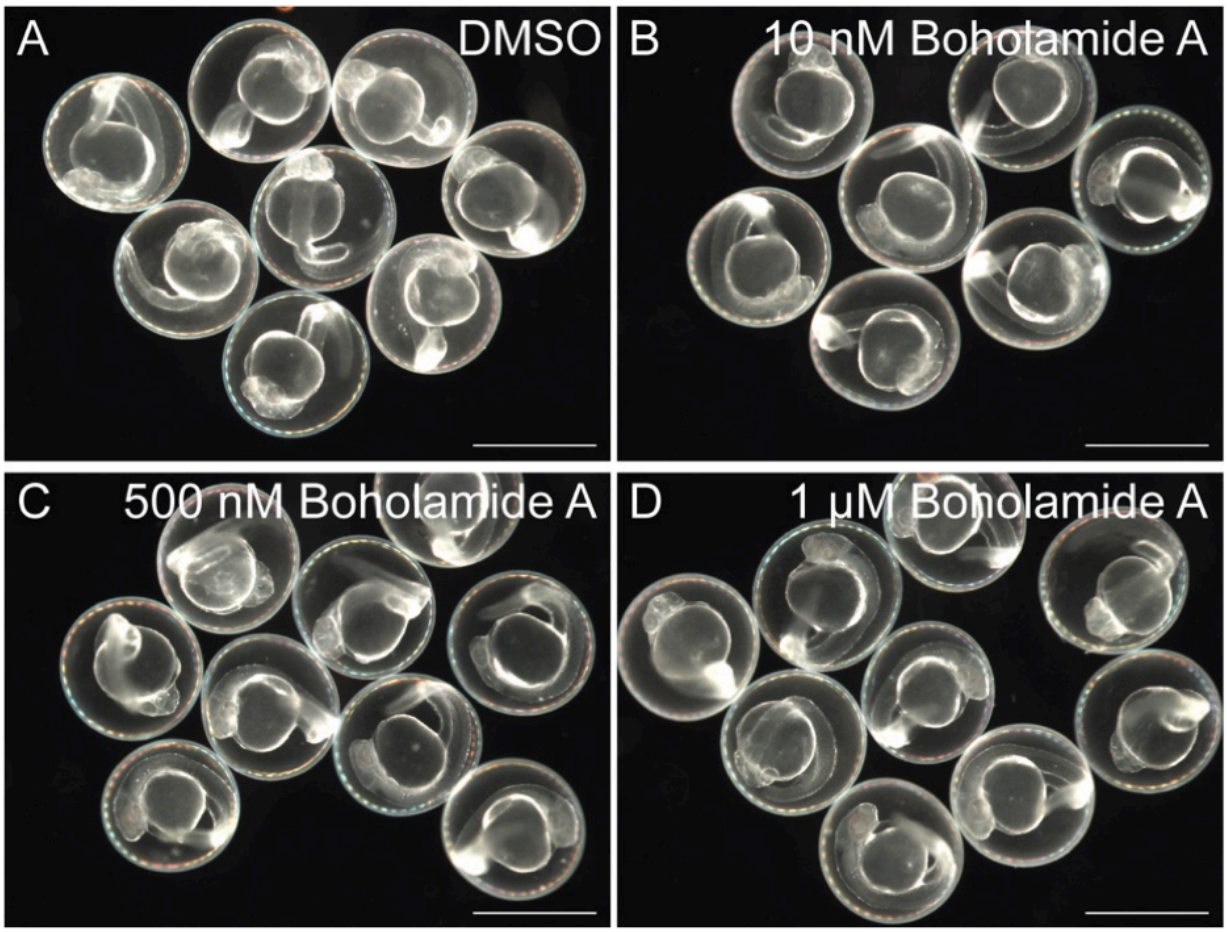
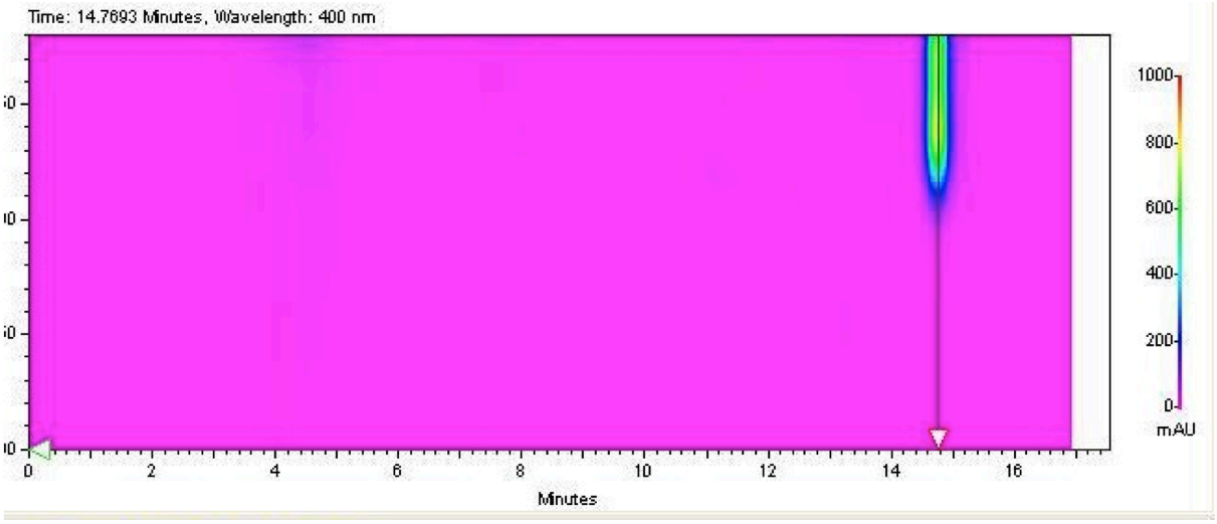


Figure S20. Purity of boholamide A (1) assessed by analytical HPLC.



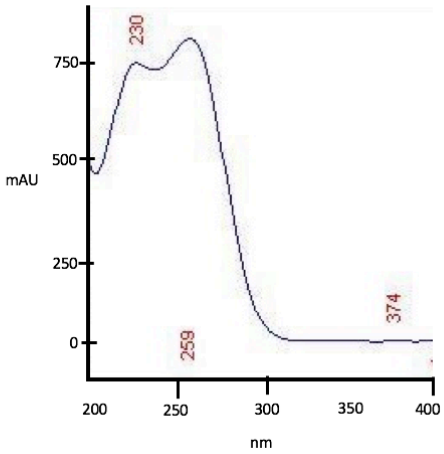


Table S1. NMR data of the HDMN in 3a/3b.

	δ_C, type	δ_H, (J in Hz)
2	86.1, CH	3.35, d (8.2)
3	74.9, CH	3.46, dd (8.2, 2.8)
4	33.6, CH	1.74, m
5	22.5, CH_2	1.28, m; 1.18, m
6	32.0, CH	1.43, m
7	41.2, CH_2	1.19, m
10	16.8, CH_3	0.74, d (6.6)
11	16.7, CH_3	0.77, d (6.5)
12	60.5, CH_3	3.2, s