Supporting Information

Identification and Characterization of a Peptide from the Stony Coral *Heliofungia actiniformis*

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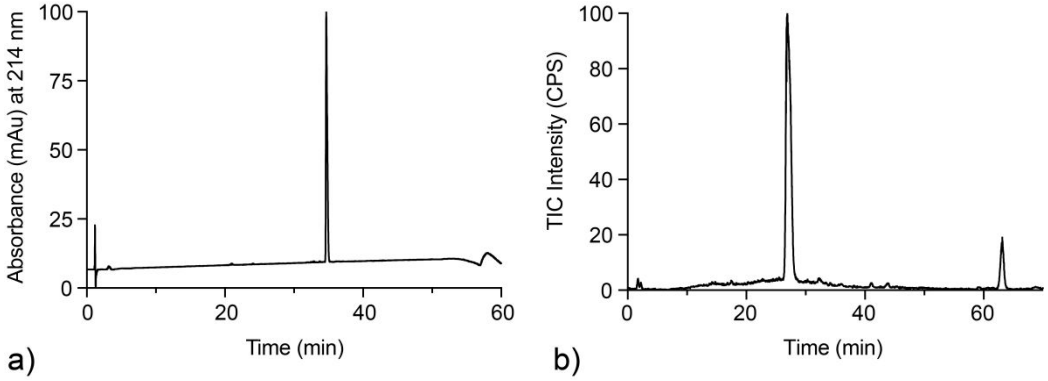

Figure S1. Chromatographic analysis of native and synthetic Hact-1. 1a) RP-HPLC chromatogram of purified synthetic Hact-1 (Phenomenex Aeris PEPTIDE XB-C18 150 x 4.6 mm, 3.6 µm column; 1 mL/min flow rate; Solvent A H_2O/0.05% TFA, Solvent B 90% MeCN/H_2O/0.045% TFA; 0-60% Solvent B in 120 min, 60-90% Solvent B in 5 min, 90% Solvent B for 10 min, and 90–0% Solvent B in 5 min; absorbance at 214 nm). 1b) LCMS chromatogram of coelution of native and synthetic Hact-1. The small peak at ~64 minutes is a contaminate. (Phenomenex Aeris 150 x 2.1 mm 3.6 µm PEPTIDE XB-C18 100Å peptide column; 0.25 mL/min flow rate; Solvent A 0.1% formic

acid/ H_2O, Solvent B 90% MeCN/0.09% formic acid/ H_2O; 0-25% Solvent B in 10 minutes, 25-40% in 60 minutes, 40-90% in 5 minutes, 90% for 5 minutes, and 90-0% in 5 minute; scan range 250-2000 m/z). The chromatogram was recorded at 214 and 218 nm with similar clarity of the peak coelution to that observed with the TIC. The chromatograms shown in a) and b) were done on different instruments with different conditions resulting in the different retention times.

Marine bacteria screen

Experimental procedures

The bacteria tested in the assay were cultured from different coral species, and included *Alteromonas macleodii*, *Pseudoalteromonas shioyasakkiensis*, *Vibrio harveyi*, *Vibrio shilonii*, *Tenacibaculum skagerrakense*, and *Vibrio coralliilyticus.* A microdilution assay similar to the human pathogen antimicrobial screen was used. Cultures of bacteria were grown for 24 or 48 hours, depending on growth, in 2216 Marine Broth medium (BD-DIFCO 279110, NSW, Australia). Mid-log phase cultures (100 µL; OD_{600} 0.4-0.6) were inoculated into 10 mL of 2216 Marine Broth medium containing 0.01 mg/mL cycloheximide. Bacterial suspension (180 µL) was added to the wells with 20 µL of suspended Hact-1 at 27°C for

6 or 20 hours with no shaking. Serial dilutions of Hact-1 included 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL, resulting in approximate final concentrations of 0.1 mg/mL, 0.05 mg/mL and 0.025 mg/mL of Hact-1 being tested. OD_{600} readings were measured on a FLUOstar Omega microplate reader (BMG Labtech) at end timepoints. Kanamycin was used as a positive control, and sterile Milli-Q H_2O was used as a negative control. All experiments were performed in triplicate and repeated. The end point was determined as bacteria growth with an OD_{600} reading between 0.4 and 0.6.

Results

Hact-1 was tested against several marine bacteria commonly associated with a variety of coral species. Because of the complex nature of the microbiome in corals, a sensitive balance of bacteria is necessary to maintain the health of the coral.[1-2] For this reason it has been suggested that corals may possess natural antimicrobial agents in order to resist the possibility of an overgrowth on one specific bacteria and risk of infection.[3] However, consistent with the human pathogens tested, the peptide did not appear to have any inhibitory activity against the marine bacteria tested (Figure S2).

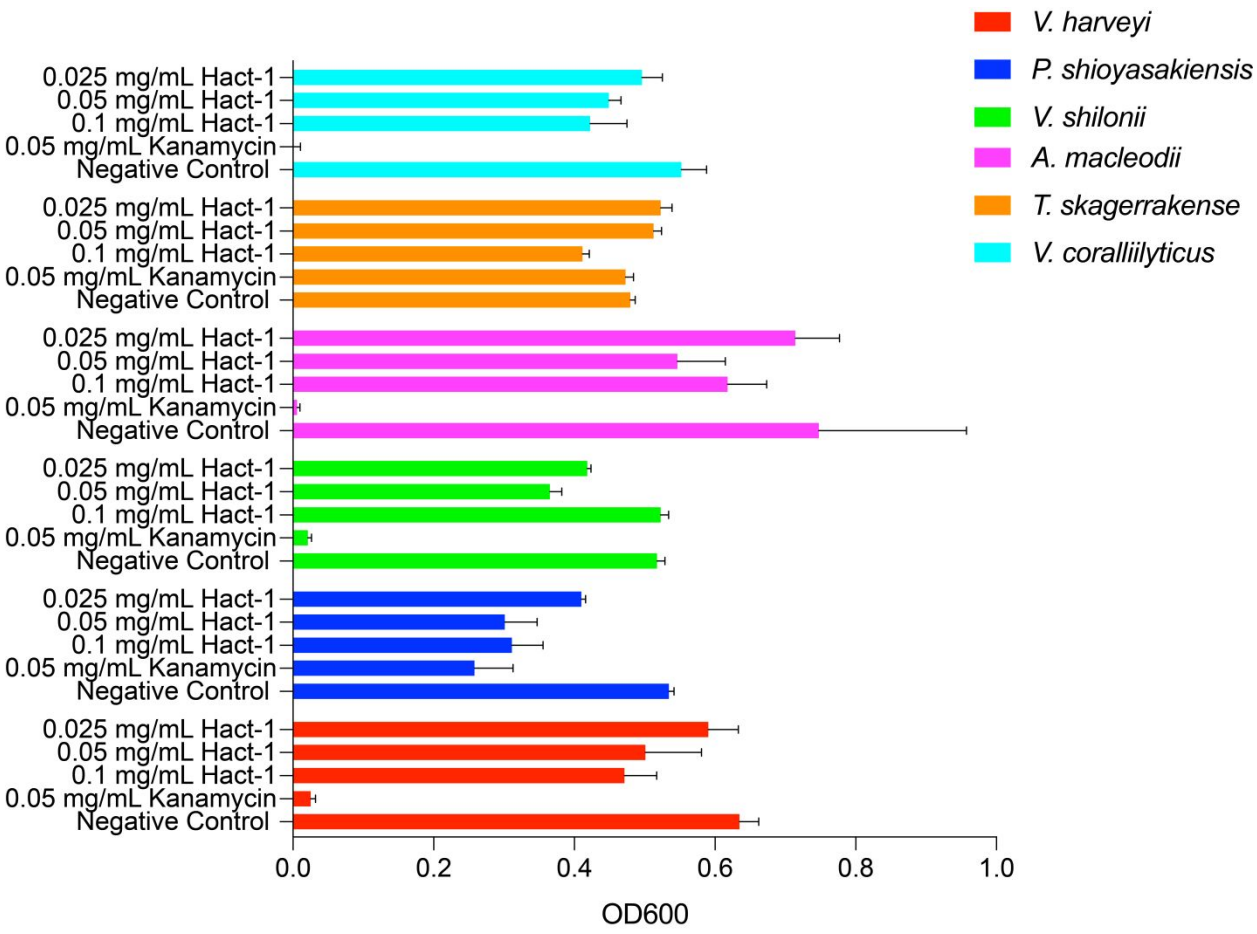

Figure S2. Marine bacteria antimicrobial screen of Hact-1.

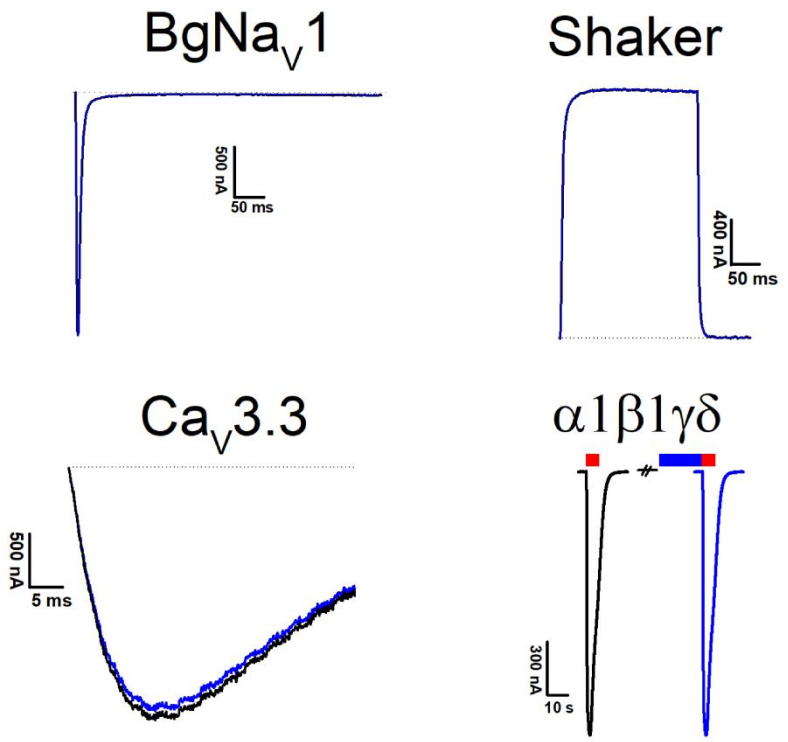

Figure S3. Differential effects of 25 µM Hact-1 on Na_V, K_V, Ca_V and nAChR isoforms expressed in *Xenopus laevis* oocytes. Representative whole cell ion current traces of oocytes expressing cloned BgNav1, Shaker IR, Cav3.3 and α1ß1γδ isoforms are shown. The dotted line indicates the zero-current level. The black line indicates the current in control conditions and the blue line indicates the steady-state current in the presence of 25 µM Hact-1. In the last panel, the red bar indicates application of 200 µM ACh, the blue bar indicates the application of 25 µM Hact-1.

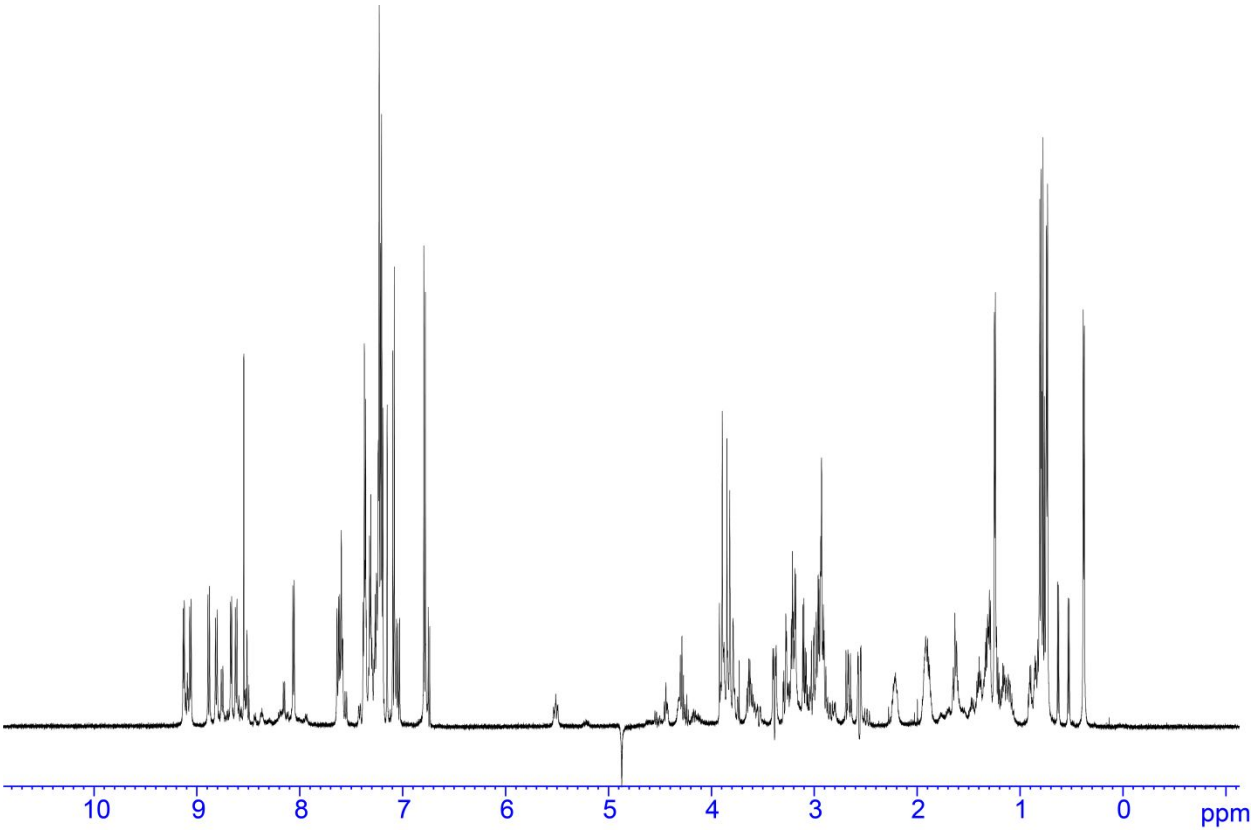

Figure S4. ^{1}H NMR (600 MHz, 90% H_2O/10% D_2O v/v) spectrum of synthetic Hact-1.

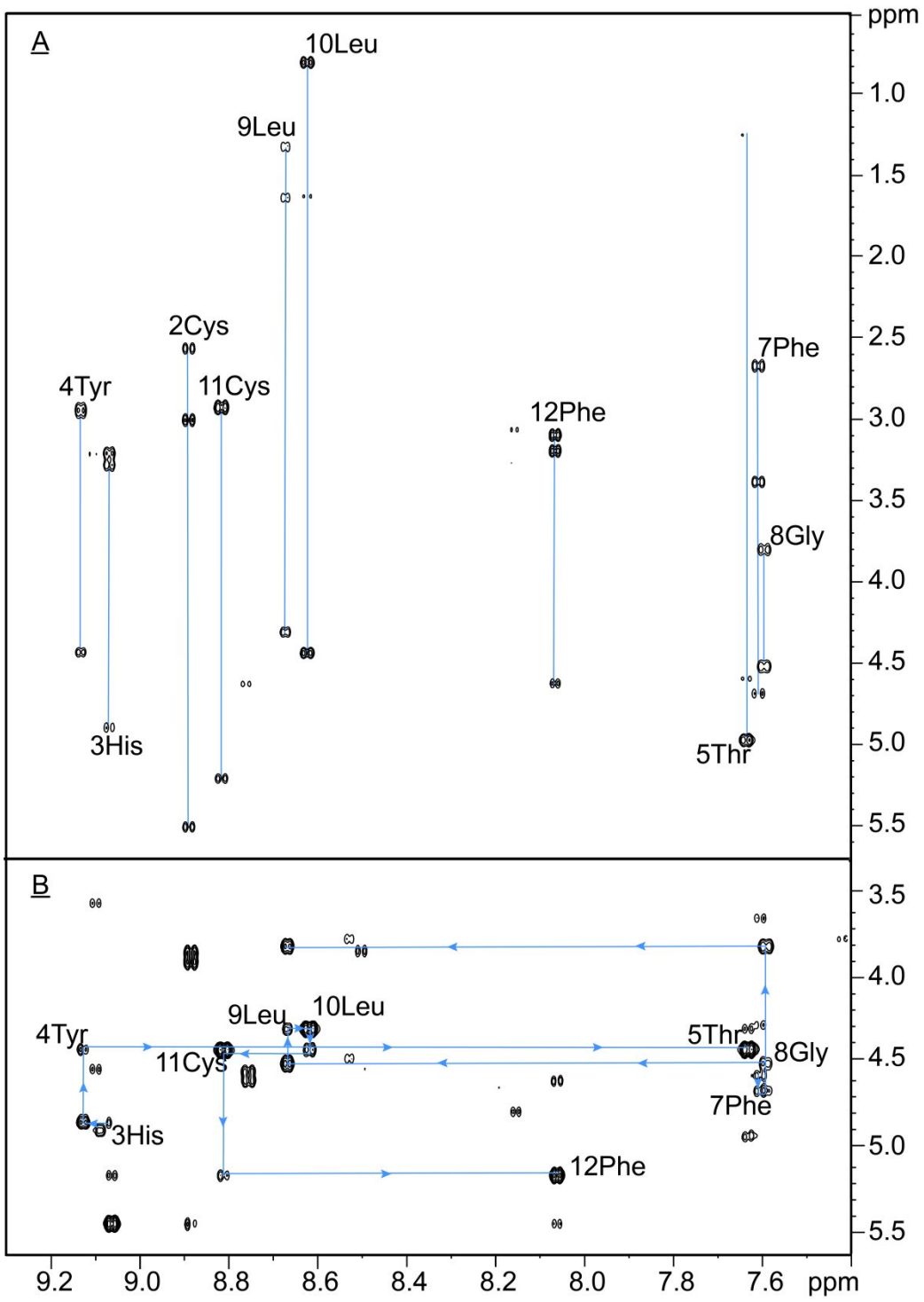

Figure S5. Regions of the TOCSY(a) and NOESY(b) spectra of synthetic Hact-1 dissolved in 90% H_2O/10% D_2O (v/v) (100 μM),

recorded at 290 K. Residue 6 is not present in the amide region as it is a proline and the N-terminal Gly is also not present.

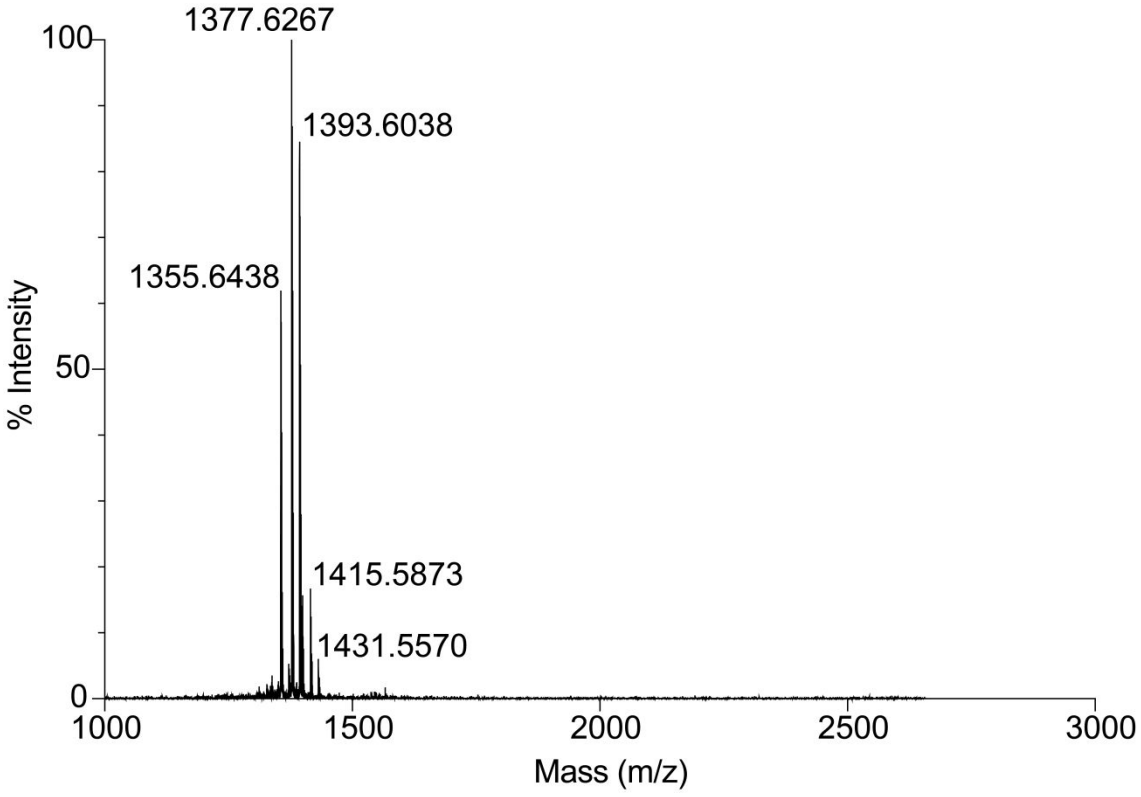

Figure S6. MALDI-TOF MS spectrum of purified, synthetic Hact-1. The correct mass for Hact-1 being $[M+H]^+$ 1355.6438 Da with the $[M+Na]^+$ (1377.6267 Da), $[M+K]^+$ (1393.6038 Da), $[M+K+Na]^+$ (1415.5873 Da), $[M+K+2Na]^+$ (1431.5570 Da) adducts present.

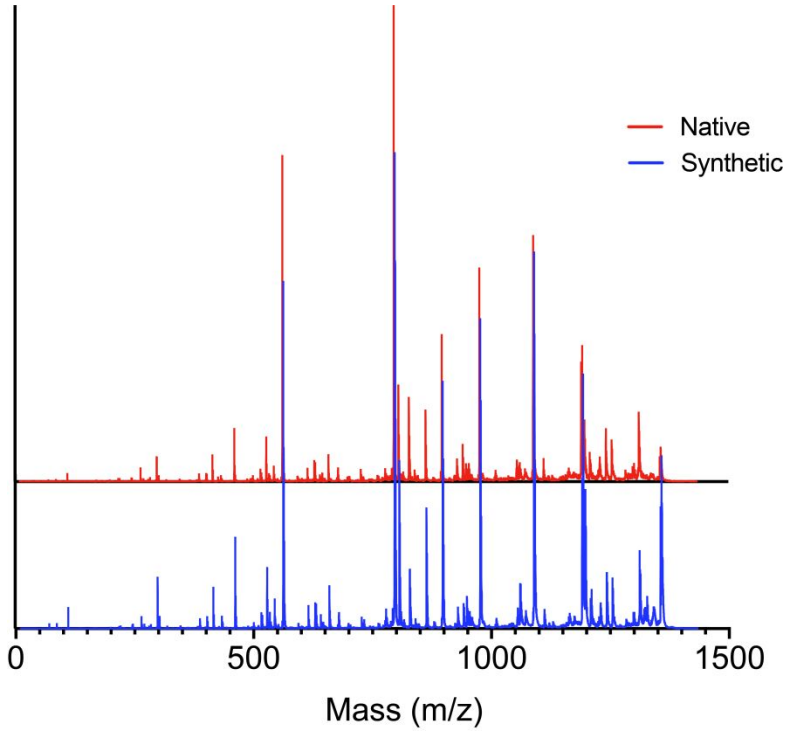

Figure S7. Overlay of MALDI-TOF MS/MS spectra from reduced, synthetic and native Hact-1.

References:

1. Bourne, D. G.; Morrow, K. M.; Webster, N. S., Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annu Rev Microbiol* **2016**, *70*, 317-+.

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