Supporting Information

Codinaeopsin, an antimalarial fungal polyketide

R. Kontnik and J. Clardy*

List of Supporting Information

Materials and Methods

General experimental procedures. All NMR experiments were carried out on a Varian INOVA 600 MHz spectrometer. Codinaeopsin was purified from active fractions on an Agilent 1100 series HPLC (Agilent Technologies) using a semi-preparative Discovery HS-C18 column (Supelco, 25 cm x 10 mm, 10 μm particle size). IR spectrum was obtained on a Perkin Elmer 1600 series FT-IR spectrometer. $[\alpha]_D$ measurements were obtained using a Jasco DIP-0181 digital polarimeter with a sodium lamp.

Culturing and extraction. CR127A was isolated at the National Biodiversity Institute (INBio) in Costa Rica. The fungus was grown on yeast malt plates supplemented with 30 μg/mL streptomycin and 12 μg/mL chlortetracycline. Agar plugs taken from these plates were used to inoculate rich media seed cultures (5 g tryptone peptone, 10 g dextrose, 3 g yeast extract and 10 g malt extract per liter, pH 6.2), which were incubated at 25 ºC with shaking at 150 rpm for 5 days. The contents were then poured into 2% malt extract broths, and these cultures were again incubated at 25 ºC with shaking at 150 rpm.

Following fermentation, an equal volume of 95% ethanol was added to the cultures, and the mixture blended to homogeneity. The resulting solution was sonicated for 20 minutes, then filtered. The filtrate was diluted with an equal volume of water, then extracted three times with ethyl acetate. Organic phases were combined, dried over Na_2SO_4, and concentrated under vacuum. The crude extract was fractionated by silica gel flash column chromatography using 1:1 ethyl acetate:hexanes. Fractions were pooled based on TLC analysis, and the active fraction was further purified by reverse-phase C-18 HPLC (using an 80-100% acetonitrile gradient) to yield pure codinaeopsin, $[\alpha]_D$ –15.6 (*c* 0.54, $CHCl_3$).

Antimalarial Screen. The antimalarial screen was carried out as previously described.[1] Briefly, extracts dissolved in DMSO were arrayed in dilution series in 384-well plates. The solutions were pin-transferred to assay plates containing red blood cells parasitized with *Plasmodium falciparum*. A fluorescent DNA stain (DAPI) was added after a 72-hour incubation period, and the plates imaged to quantify levels of parasitic nuclei.

Sequencing and species identification. For identification by internal transcribed spacer (ITS) sequencing, CR127A was cultured in potato dextrose broth for 5 days. The mycelium was then retrieved by filtration and ground to a fine powder in liquid N_2. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), and large subunit rDNA was amplified by PCR using primers LR5 (5'-TCCTGAGGGAAACTTCG-3') and LROR (5'-ACCCGCTGAACTTAAGC-3'). PCR products were transformed into *E. coli* TOP10 cells using a TOPO TA Cloning Kit (Invitrogen), according to manufacturer's protocols. Transformed plasmids were isolated and sequenced at the Dana-Farber/Harvard Cancer Center DNA Sequencing Facility. The following consensus sequence was used in a BLAST search against deposited sequences:

5'-CAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCCCCAGGCCCG
AGTTGTAATTTGCAGAGGATGCTTCTGGCGCGGTGCCTTCCAAGTCCCCTGGAACGGGGC
GCCATAGAGGGTGAGAGCCCCGTCAGGTCGGCCGCCAAGCCTGTGTGAAGCTCCTTCGA
CGAGTCGAGTAGTTTGGGAATGCTGCTCTAAACGGGAGGTAGACCCCTTCTAAAGCTAA
ATACCGGCCAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTT
GAAAAGAGGGTTAAACAGTACGTGAAATTGTTGAAAGGGAAGCGCTTGTGACCAGACGT
GCACCGGGTGTTCAGCGGGCGTTCTCGCCCGTGCATTCGCCCCGGTGCAGGCCAGCGTCG
GTTCGGGCGGGGGGATAAAGGCATCAGGAACGTGGCTCCCCCGGGAGTGTTATAGCCTG
GCGCGTCATGCCCCCGCCTGGACCGAGGTTCGCGCTCCGCAAGGACGCTGGCGTAATGG
TCCCCAGCGGCCCGTCTTGAAACACGGACCAAGGAGTCGAGGTTTTACGCGAGTGTCTG
GGTGTCAAACCCGCACGCGTAATGAAAGTGAACGTAGGTGGGAGCTTCGGCGCACCACC
GACCGATCCTGATGTTTTCGGATGGATTTGAGTA-3'

[1] Baniecki, M. L.; Wirth, D. F.; Clardy, J. *Antimicrob. Agents Chemother.* 2007, *51*, 716-723.

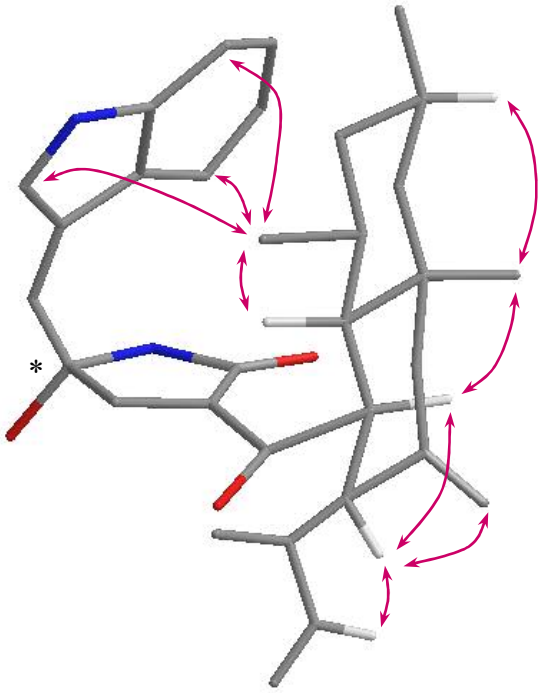


Figure S1. Key ROESY correlations observed in codinaeopsin, used to determine relative stereochemistry. Stereochemistry at the indicated C-2' position (*) is arbitrary.

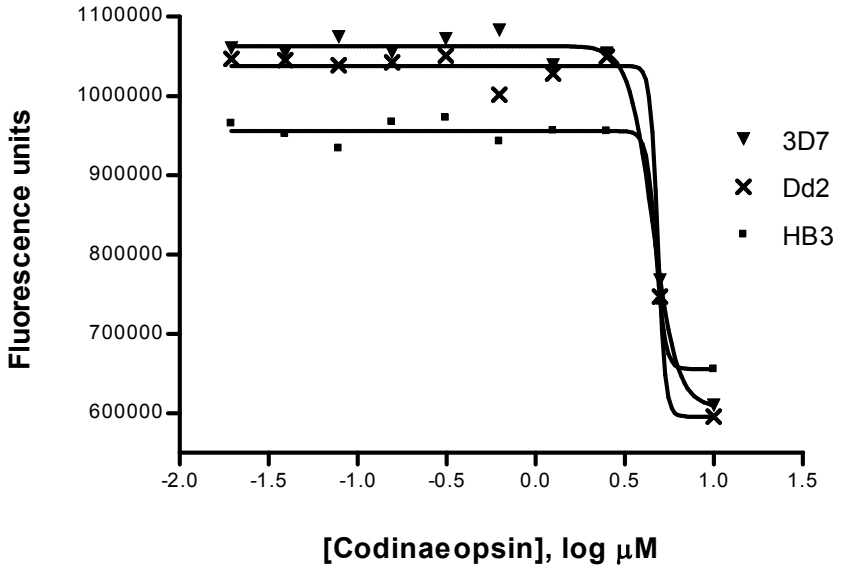
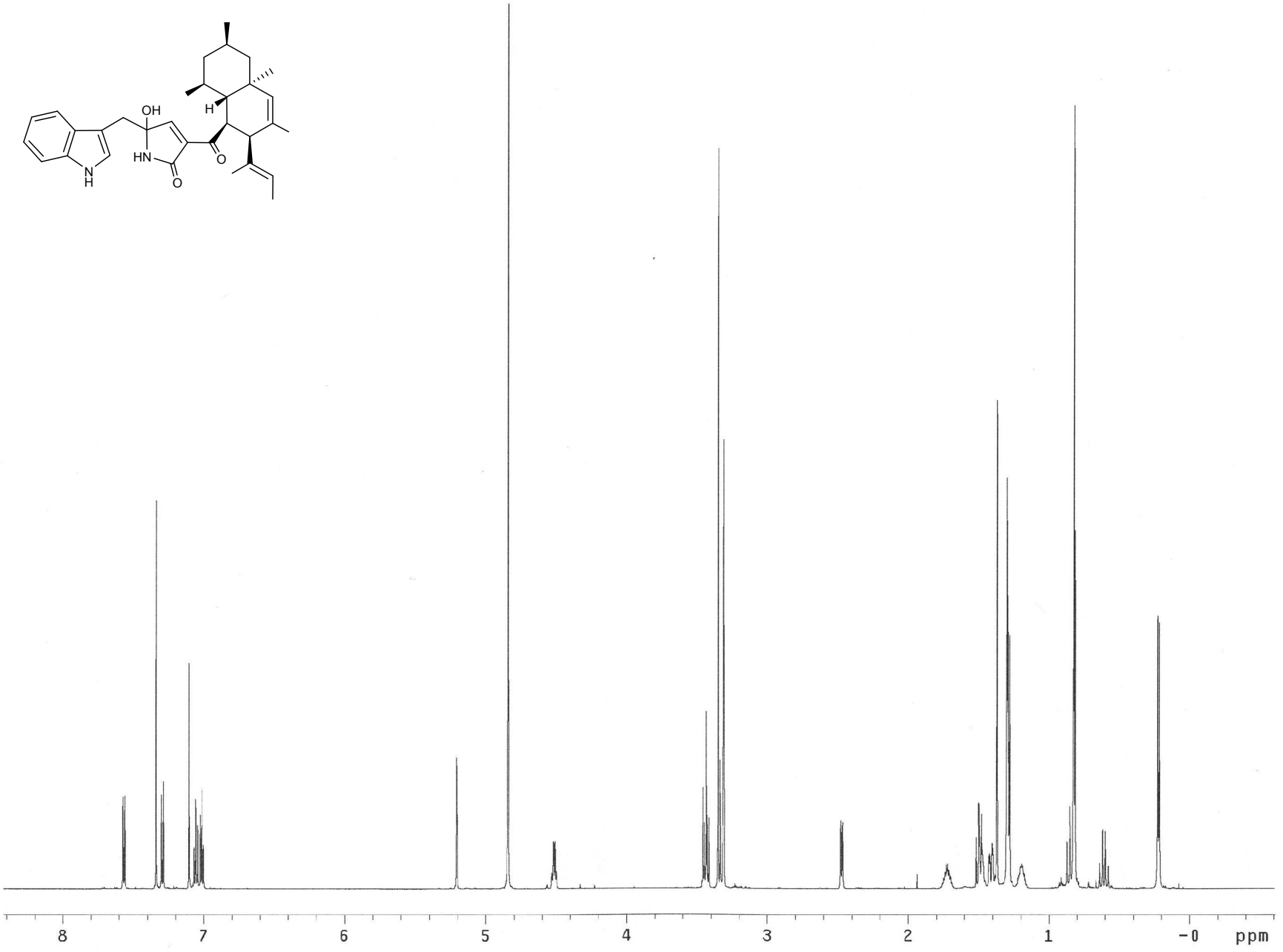
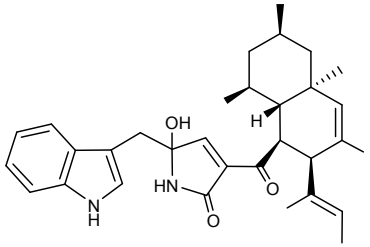


Figure S2. Efficacy of codinaeopsin against *Plasmodium falciparum* strains 3D7 (sensitive to all drugs), HB3 (resistant to pyrimethamine and methloquine), and Dd2 (resistant to chloroquine, pyrimethamine, and methloquine) was assessed. The dose-response curve obtained from cultures of *P. falciparum*-infected erythrocytes incubated with increasing concentrations of codinaeopsin illustrates similar IC_{50} values against all three strains (3D7: 4.66 μM; Dd2: 4.84 μM; HB3: 4.73 μM).

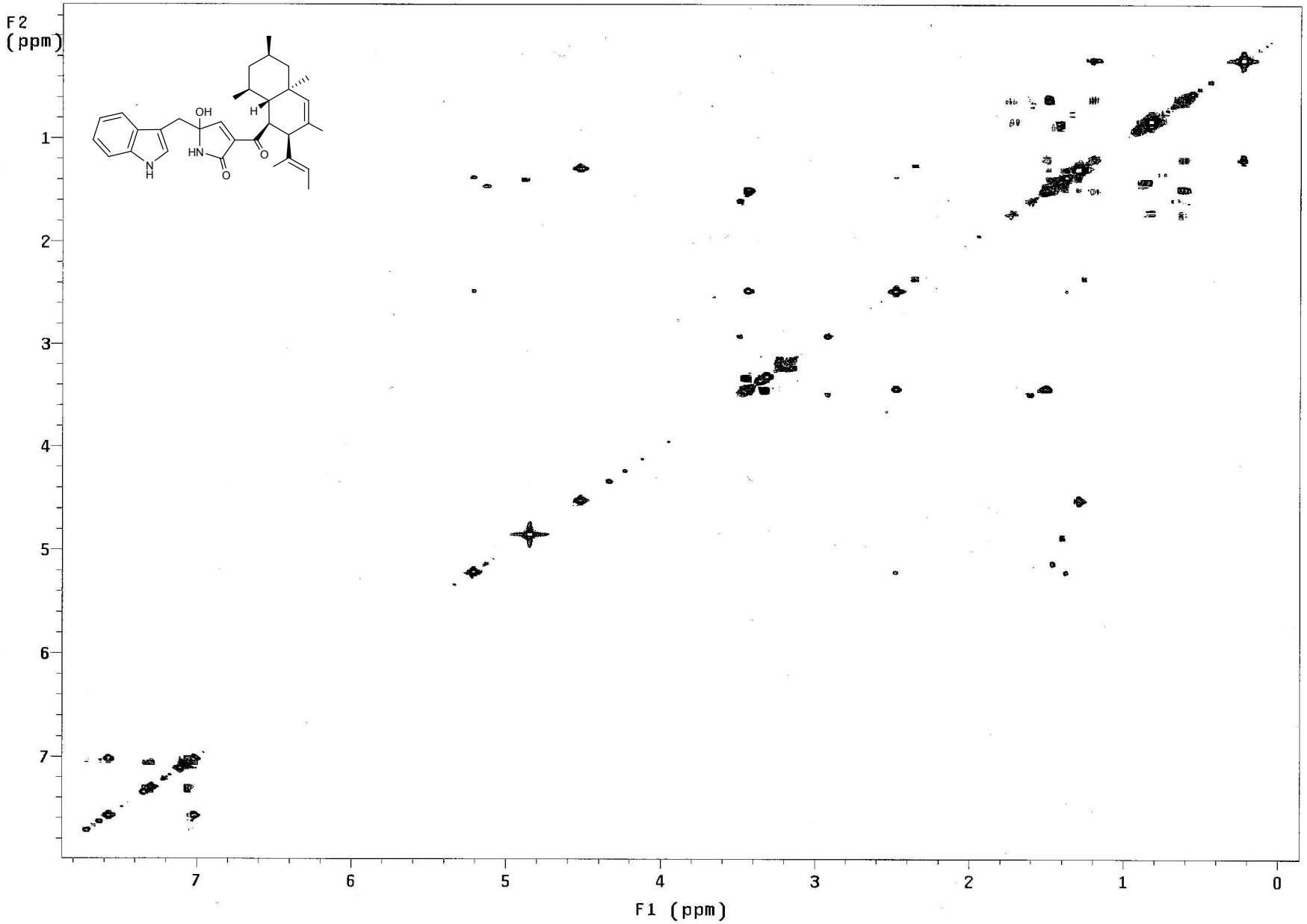
Codinaeopsin – ^{1}H NMR



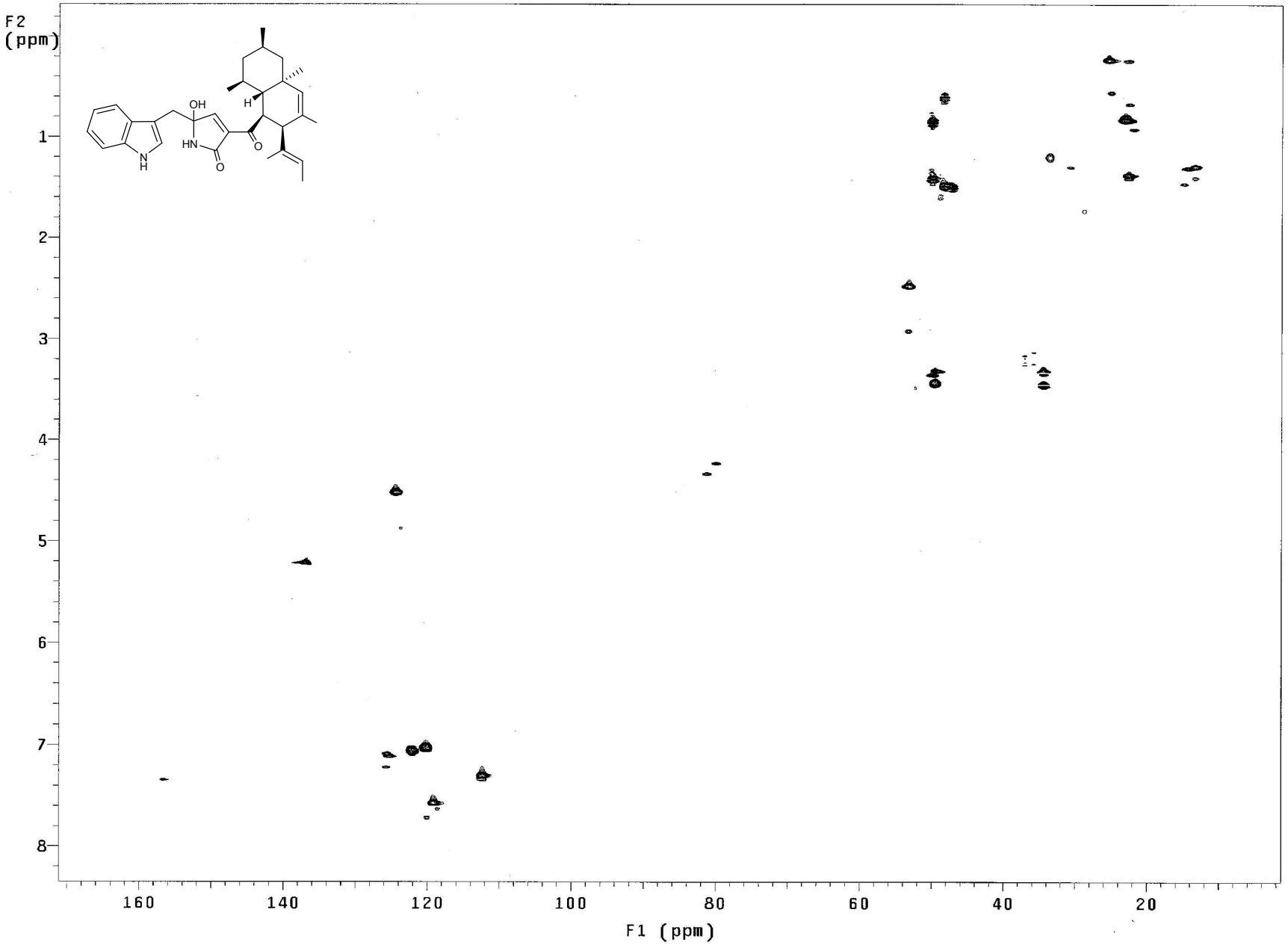
Codinaeopsin – ^{13}C NMR



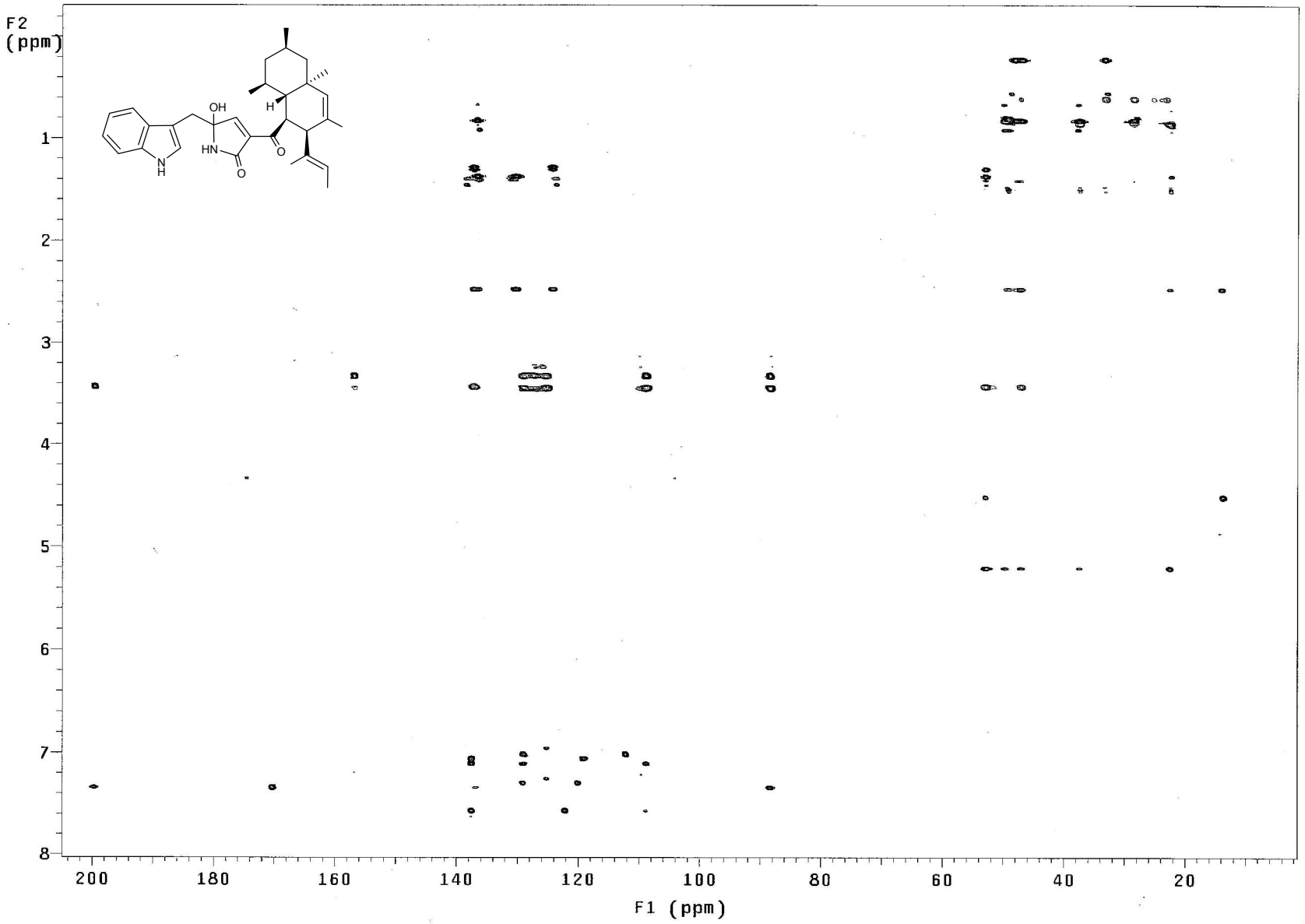
Codinaeopsin – gCOSY



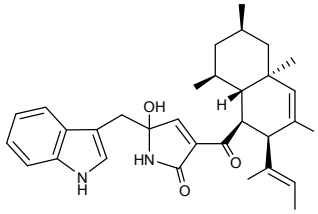
Codinaeopsin – gHMQC

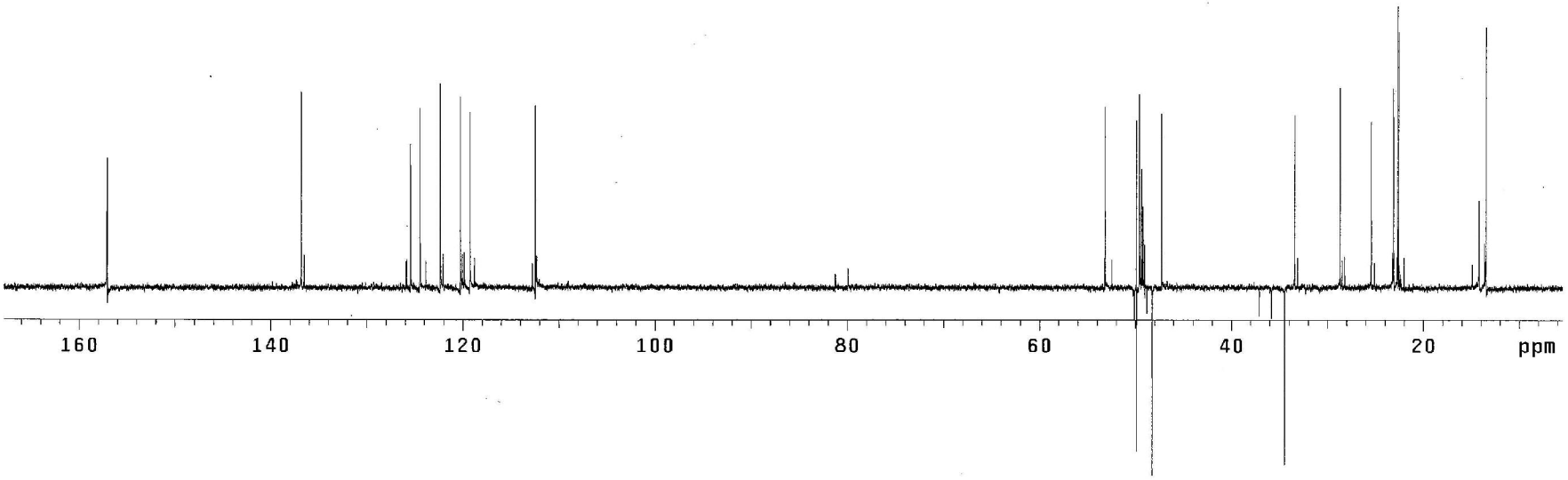


Codinaeopsin – gHMBC



Codinaeopsin – DEPT





Codinaeopsin – IR