

# Lodopyridone, a Structurally-Unprecedented Alkaloid from a Marine Actinomycete.

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## Experimental Procedures

**General Experimental Procedures:** All NMR spectra were measured using a Varian Oxford AS500 spectrometer (5 mm double-resonance inverse broadband probe). Offline processing was conducted using Mestre-C NMR Software (Mestrelab Research, A Coruña, Spain; www.mestrec.com).  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of **1** were referenced with the DMSO solvent peaks at  $\delta$  2.50 and  $\delta$  39.4, respectively. The IR spectrum of **1** was recorded on a Nicolet IR100 FTIR spectrometer (Thermo). The UV spectrum of **1** was recorded on a Beckman Coulter DU800 spectrophotometer. High resolution mass spectra were run by the Scripps Center for Mass Spectrometry. HPLC was carried out on a Beckman System Gold liquid chromatograph (126) equipped with a Shimadzu diode array detector (SPD-M10AVP). All HPLC separations were done using an Ultracarb ODS (30) column (Phenomenex, 250 x 10.0 mm, 5  $\mu\text{m}$  particle size) with a 3mL/min flow rate. Column chromatography was carried out on silica gel (Selecto Scientific, particle size 63-200).

**Culture Conditions:** A 2 mL frozen stock of CNQ490 was used to inoculate 25 mL A1 medium (10 g starch, 4 g yeast extract, 2 g peptone in 1 L seawater) in a 125 mL Erlenmeyer flask and shaken at 80 °F. The 3-day-old seed culture was then used to inoculate a one-liter culture in A1bfe+c (A1 media described above, with the addition of 1 g  $\text{CaCO}_3$ , 5 mL of a 2% (w/v) KBr stock solution, and 5 mL of a 0.8% (w/v)  $\text{Fe}_2(\text{SO}_4)\cdot 4\text{H}_2\text{O}$  stock solution). Lastly, 25 mL aliquots of the 3-day-old one-liter culture were used to inoculate each of 40 one-liter cultures in 2.8 L Fernbach flasks. CNQ490 was allowed to grow with shaking for 7 days at 80 °F prior to extraction.

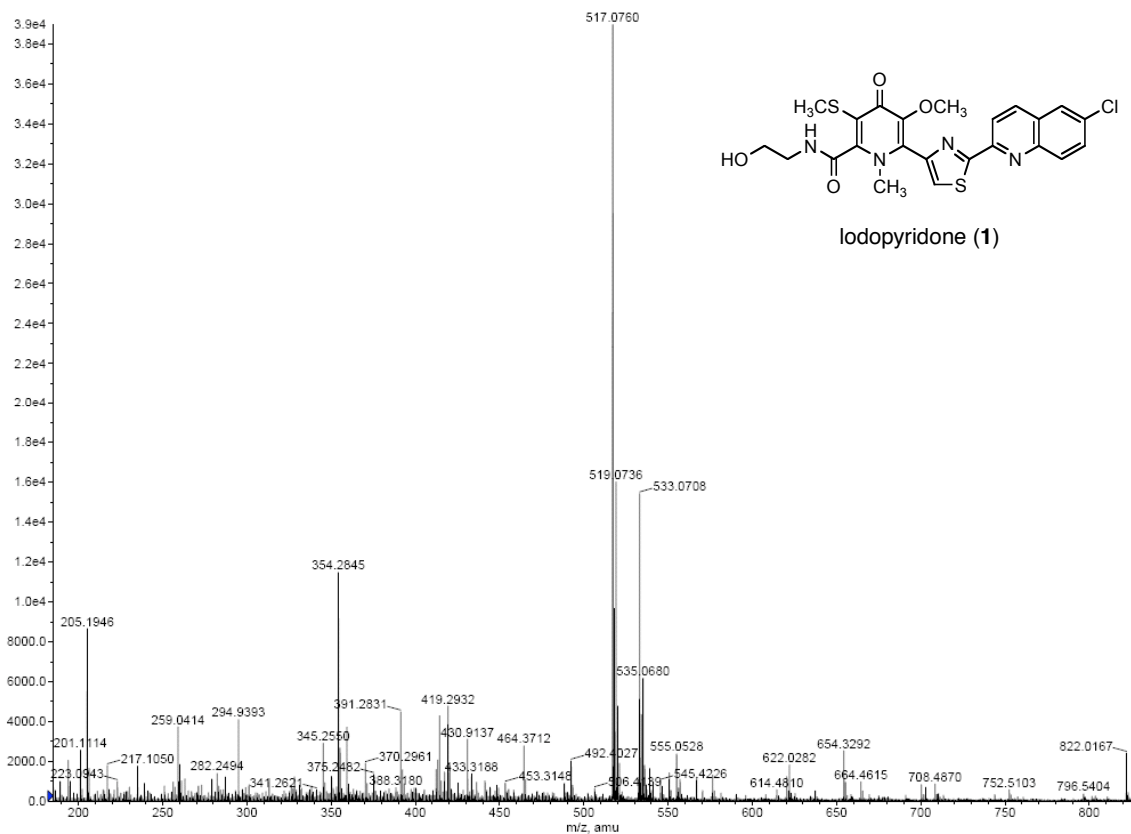
**Isolation of Lodopyridone:** A 40 liter culture of CNQ490 was extracted using XAD-7 resin eluted with acetone (2 $\times$ ). The acetone was removed by evaporation, and the remaining water extracted 3 $\times$  with ethyl acetate. The obtained extract was fractionated twice on silica gel (first with hexanes:ethyl acetate:methanol eluent, then with dichloromethane:methanol eluent). Fractions containing chlorinated compounds (as observed by their characteristic isotope pattern in the LCMS) were subjected to HPLC (50% aqueous acetonitrile eluent) to give lodopyridone.

**X-ray Crystallographic Data Acquisition and Processing:** A colorless block 0.10 x 0.04 x 0.04 mm in size was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using phi and omega scans. Crystal-to-detector distance was 60 mm and exposure time was 10 seconds per frame using a scan width of 0.5°. Data collection was 98.2% complete to 67.00° in  $\theta$ . A total of 9700 reflections were collected covering the indices,  $-10 \leq h \leq 10$ ,  $-10 \leq k \leq 10$ ,  $-37 \leq l \leq 37$ . 3859 reflections were found to be symmetry independent, with an  $R_{\text{int}}$  of 0.0378. Indexing and unit cell refinement indicated a primitive, monoclinic lattice. The space group was found to be P2(1)/c (No. 14). The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SIR-2004) produced a complete heavy-atom phasing model consistent with the proposed structure. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97.

# HR-ESI mass spectrum of Iodopyridone (1)

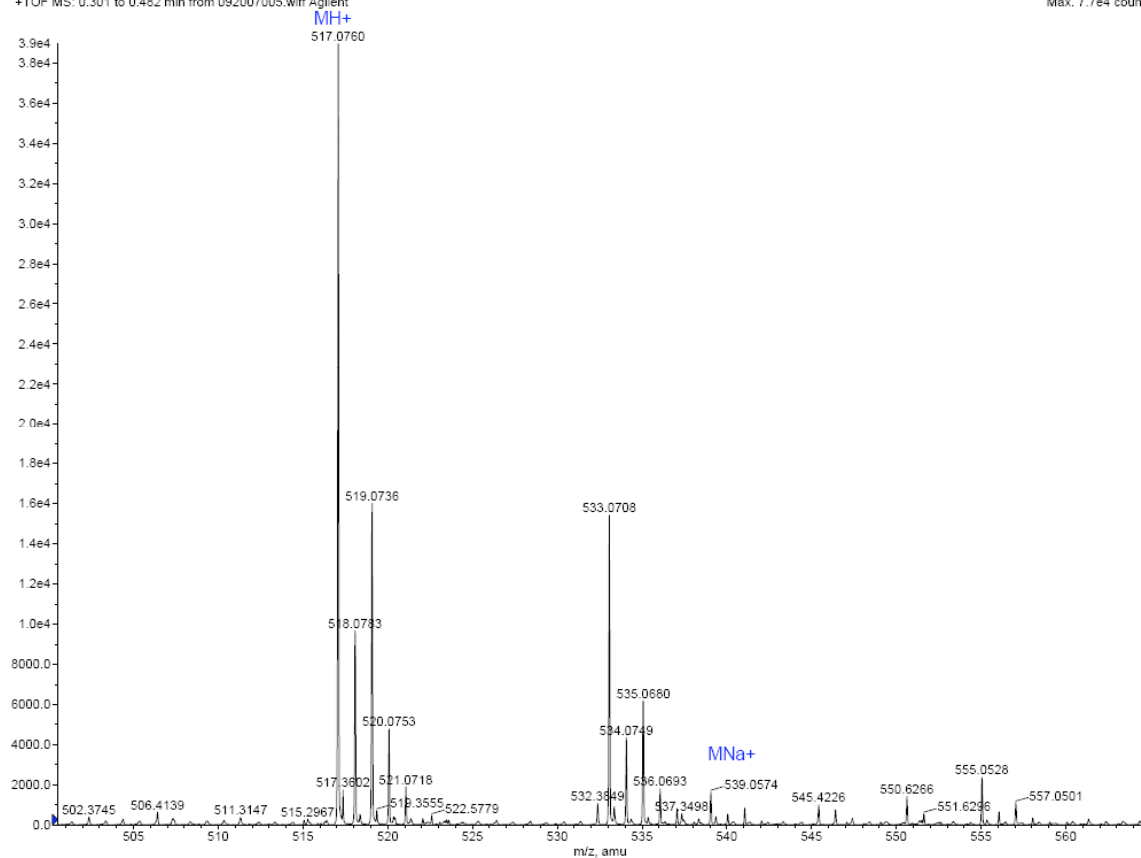
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Max. 7.7e4 counts

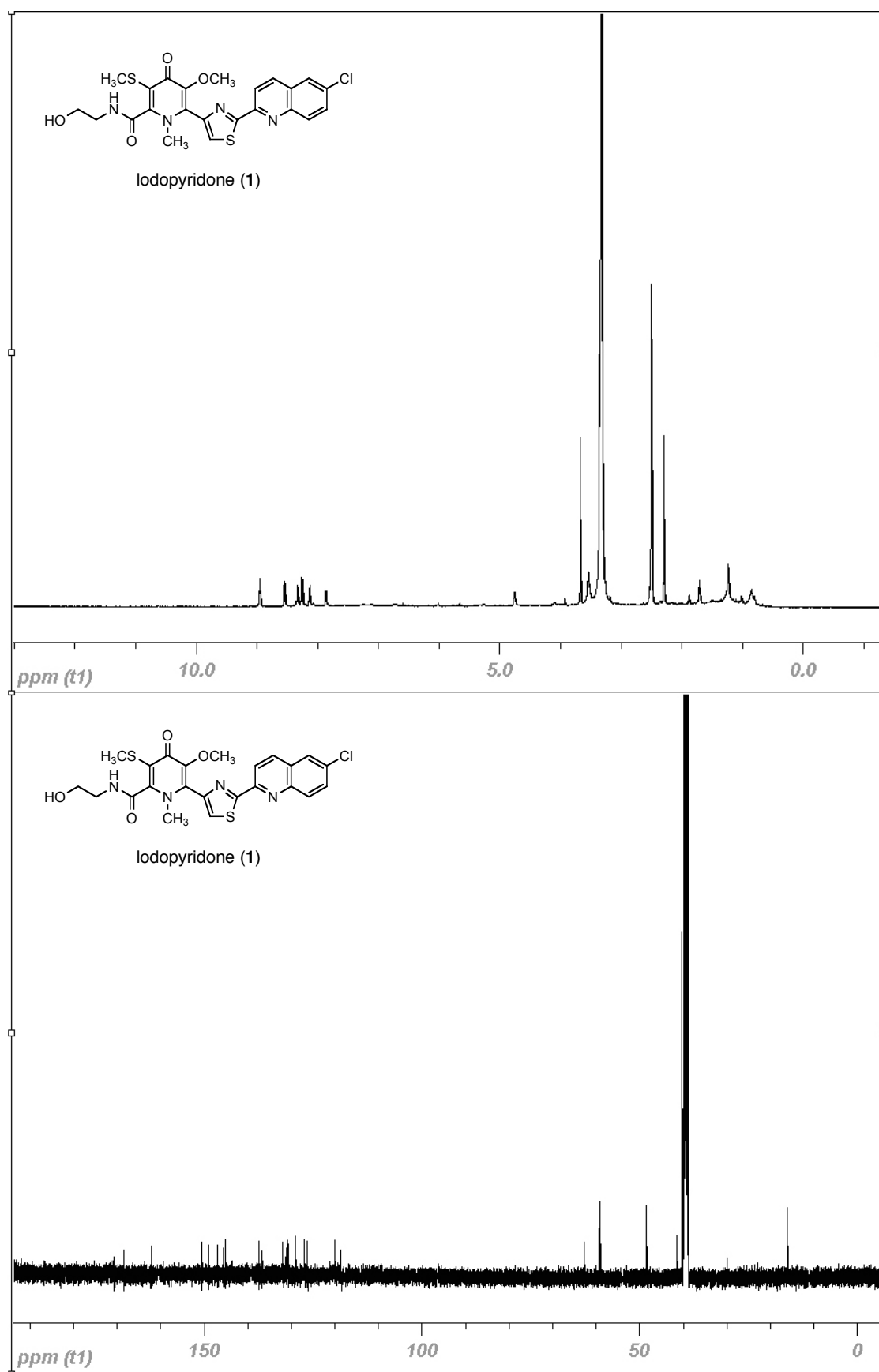


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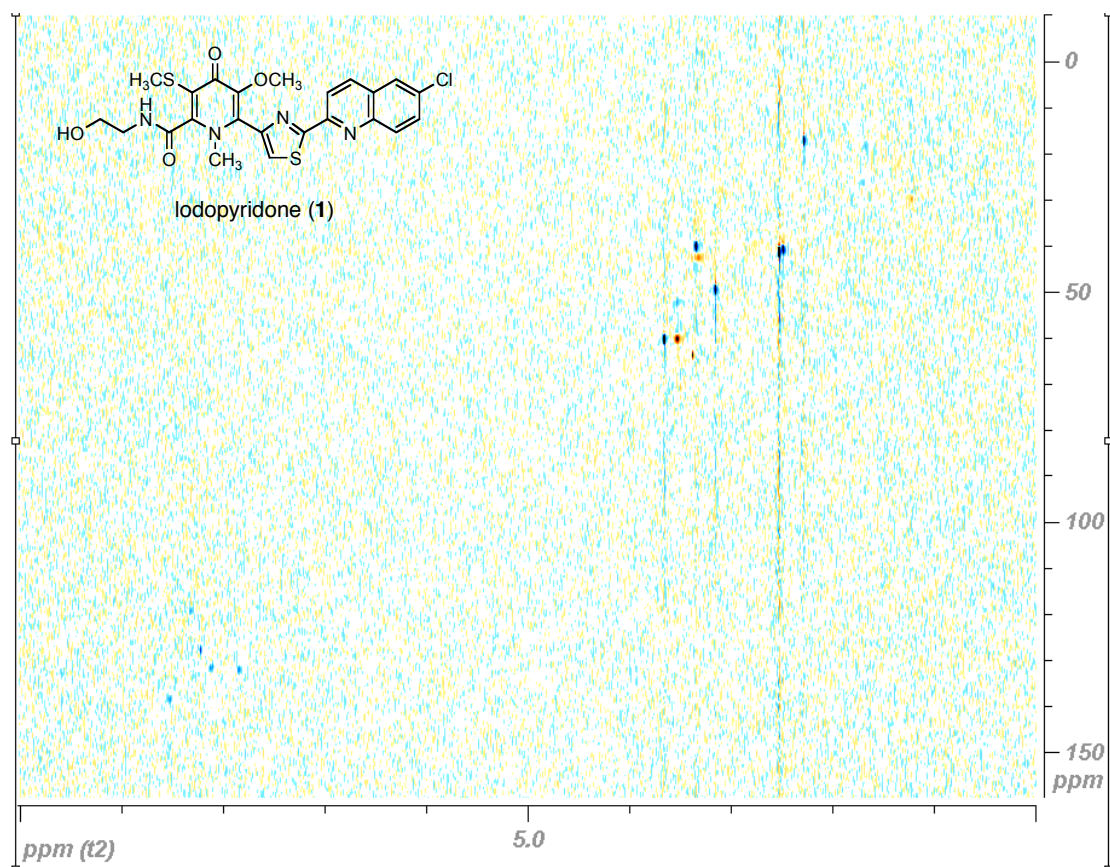
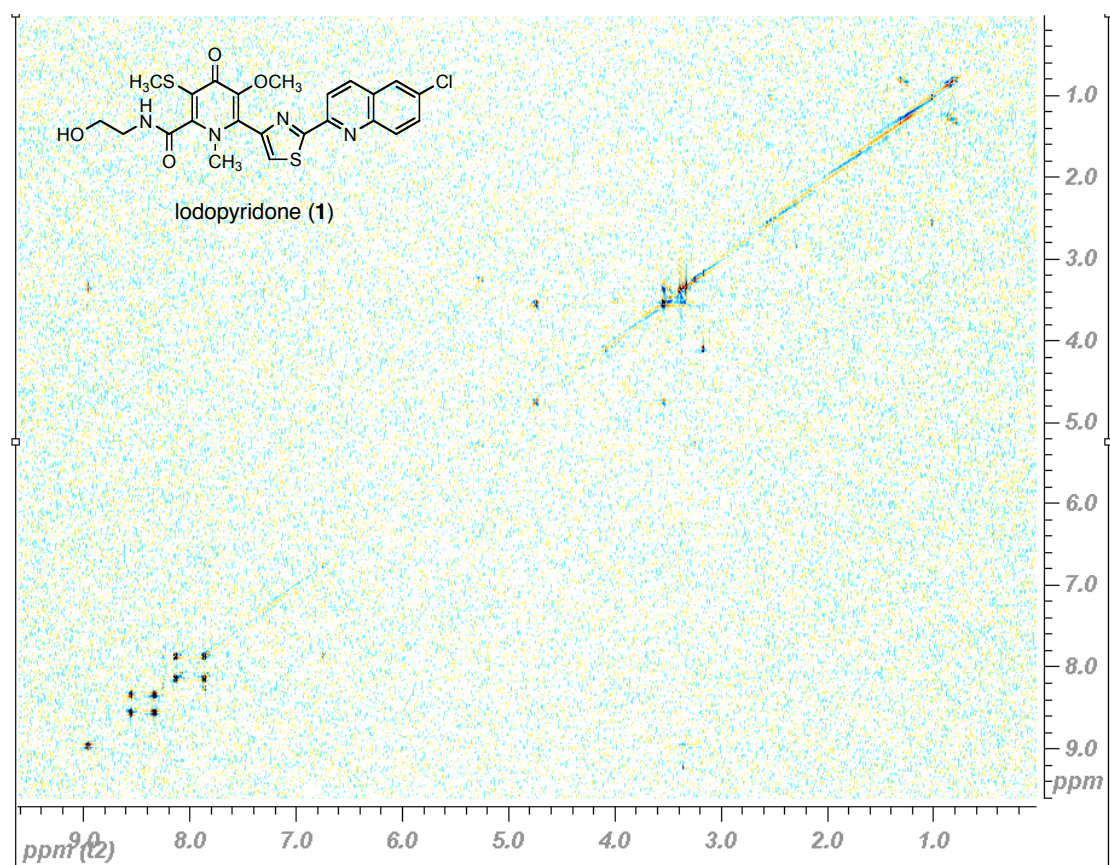
Max. 7.7e4 counts



$^1\text{H}$  (top) and  $^{13}\text{C}$  (bottom) NMR spectra of Iodopyridone (**1**)



$^1\text{H} - ^1\text{H}$  dqfCOSY (top) and HSQC (bottom) NMR spectra of Iodopyridone (**1**)



# $^1\text{H}$ - $^{13}\text{C}$ HMBC NMR spectrum of Iodopyridone (1)

