Supporting information

Inducamides A–C, Chlorinated Alkaloids from a RNA Polymerase Mutant Strain of *Streptomyces* sp.

Peng Fu,[†] Matthew Jamison,[†] Scott La, and John B. MacMillan*

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, United States

[†]Contributed equally to this work.

john.macmillan@utsouthwestern.edu

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I. Experimental details

General Procedures. Optical rotations were recorded with an AUTOPOL AP IV-6W polarimeter equipped with a halogen lamp (589 nm). UV spectra were recorded on a Shimadzu UV-1601 UV–VIS spectrophotometer. 1H and 2D NMR spectral data were recorded at 600 MHz in CD_3OD or DMSO-d_6 solution on Varian System spectrometer, and chemical shifts were referenced to the corresponding residual solvent signal (δ_H 3.31/δ_C 49.00 for CD_3OD, and δ_H 2.50/δ_C 39.52 for DMSO-d_6.). ^{13}C NMR spectra were acquired at 100 MHz on a Varian System spectrometer. High resolution ESI-TOF mass spectra were provided by The Scripps Research Institute, La Jolla, CA. Low-resolution LC/ESI-MS data were measured using an Agilent 1200 series LC/MS system with a reversed- phase C_{18} column (Phenomenex Luna, 150 mm × 4.6 mm, 5 μm) at a flow rate of 0.7 mL/min. Preparative HPLC was performed on an Agilent 1200 series instrument with a DAD detector, using a Phenyl-Hexyl column (Phenomenex Luna, 250 × 10.0 mm, 5 μm). Sephadex LH-20 (GE Healthcare, Sweden) and ODS (50 mm, Merck) were used for column chromatography.

Collection and phylogenetic analysis of strain SNC-109. *Streptomyces* sp. strain SNC-109 was isolated from a sediment sample collected from a mangrove in Vava'u, Tonga (18º38'6'' S, 133º56'6'' W). The sediment was dessicated and stamped onto agar plates using a starch media containing cadaverine and spermidine (10 g starch, 10 μM cdaverine, 10 μM spermidine, 1 L seawater, 15 g agar). Bacterial colonies were selected and streaked to purity using the same agar media. Analysis of the strain by 16S rRNA revealed 99.7% identity to *Streptomyces koyangensis*. The sequence is deposited in GenBank under accession no. KM502784.

Mutants from SNC-109. Mutants were generated in liquid culture through the use of rifampicin. Rifampicin was added to four 50 mL cultures of growth phase *Streptomyces* sp. strain SNC-109 to create a final concentration of 0.1, 0.2, 0.5, and 1 μM. At days 4 and 6, 200 μL culture aliquots were spread on A1+C agar plate (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40mg $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg KBr, 1L seawater, 15 g agar) containing a matching concentration of rifampicin. Colonies were detected from the cultures treated with 0.2 mM (day 4) and 0.5 mM (day 4, day 6) rifampicin, giving rise to mutant strains M1 – M3. All three strains had a dramatic change in resistance to rifampicin, with resistance > 0.5 mM. Resistance was determined by highest concentration which showed no inhibition by disk diffusion assay. In order to determine if resistance was due to mutations in the B subunit of bacterial RNA polymerase (*rpoB*), primers designed by the Ochi lab were used to amplify a section of *rpoB* in SNC-109 and the three mutant strains. Strains M1-M3 all had mutations in *rpoB* as shown in Figure 2b of the manuscript. Strain SNC-109-M3 was verified to originate from strain SNC-109 by comparison of 16S rRNA sequences, with the sequence of SNC-109-M3 being submitted to GenBank under the accession

no. KM974915

Cultivation and extraction. Mutant strain SNC-109-M3 was cultured in 20 × 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40mg $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg KBr) and shaken at 200 rpm at 27 °C. After seven days of cultivation, sterilized XAD-7-HP resin (20 g/L) was added to adsorb the organic products, and the culture and resin were shaken at 200 rpm for 2 h. The resin was filtered through cheesecloth, washed with deionized water, and eluted with acetone. The acetone-soluble fraction was dried *in vacuo* to yield 7.4 g of extract.

Isolation. The extract (7.4 g) was partitioned with EtOAc and MeOH/H_2O. The EtOAc soluble layer (760 mg) was fractionated by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H_2O (20:80–100:0), and 40 fractions (Fr.1–Fr.40) were collected. Fractions 15–18 (60.4 mg) were combined and separated into three fractions (Fr.15.1–Fr.15.3) on Sephadex LH-20, eluting with MeOH. Fr.15.1 (30.2 mg) was purified by reversed phase HPLC (Phenomenex Luna, Phenyl-Hexyl, 250 × 10.0 mm, 2.5 mL/min, 5 μm, UV = 254 nm) using a gradient solvent system from 40% to 100% CH_3CN (0.1% formic acid) over 15 min to afford compound **2** (16.3 mg, t_R = 10.0 min). Fractions 21–24 (68.1 mg) were combined and purified by Sephadex LH-20 eluting with MeOH to give compound **1** (24.1 mg). Fr.25 (7.6 mg) was purified by reversed phase HPLC (Phenomenex Luna, Phenyl-Hexyl, 250 × 10.0 mm, 2.5 mL/min, 5 μm, UV = 210 nm) using a gradient solvent system from 20% to 100% CH_3CN (0.1% formic acid) over 15 min to afford compound **3** (2.5 mg, t_R = 14.9 min).

Inducamide A (1): white solid, $[\alpha]_D^{24}$ –18 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε): 228 (4.6), 287 (3.8) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 233 (–10.5), 205 (+4.6) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 405.0413 $[M - H]^-$ (calcd for $C_{19}H_{15}N_2O_4Cl_2$, 405.0414).

Inducamide B (2): white solid, $[\alpha]_D^{24}$ –10 (*c* 0.05, MeOH); UV(MeOH) λ_{max} (log ε): 222 (4.6), 290 (3.8) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 228 (–5.2), 205 (+4.2) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 373.0948 $[M + H]^+$ (calcd for $C_{19}H_{18}N_2O_4Cl$, 373.0950).

Inducamide C (3): white solid, $[\alpha]_D^{24}$ –28 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε): 225 (4.8), 284 (4.1) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 307 (+5.2), 286 (–5.5), 234 (+56.8), 222 (–8.7) nm; ^{1}H and ^{13}C NMR, see Table S1; HRESIMS *m/z* 403.0271 $[M - H]^-$ (calcd for $C_{19}H_{13}N_2O_4Cl_2$, 403.0258).

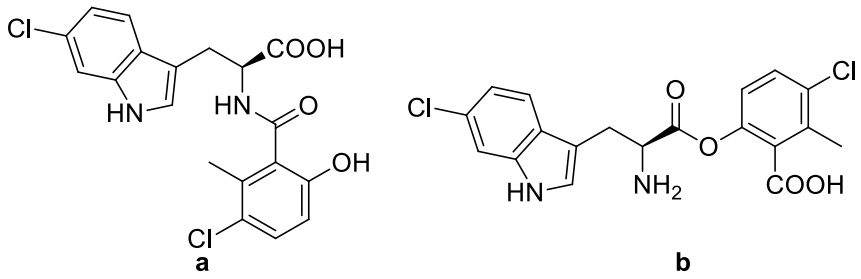
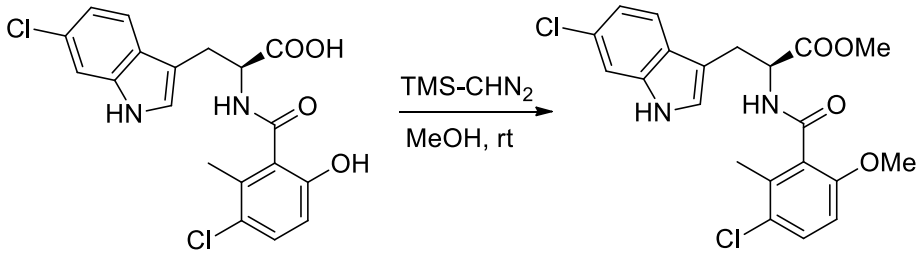


Figure S1. The possible structures of **1**

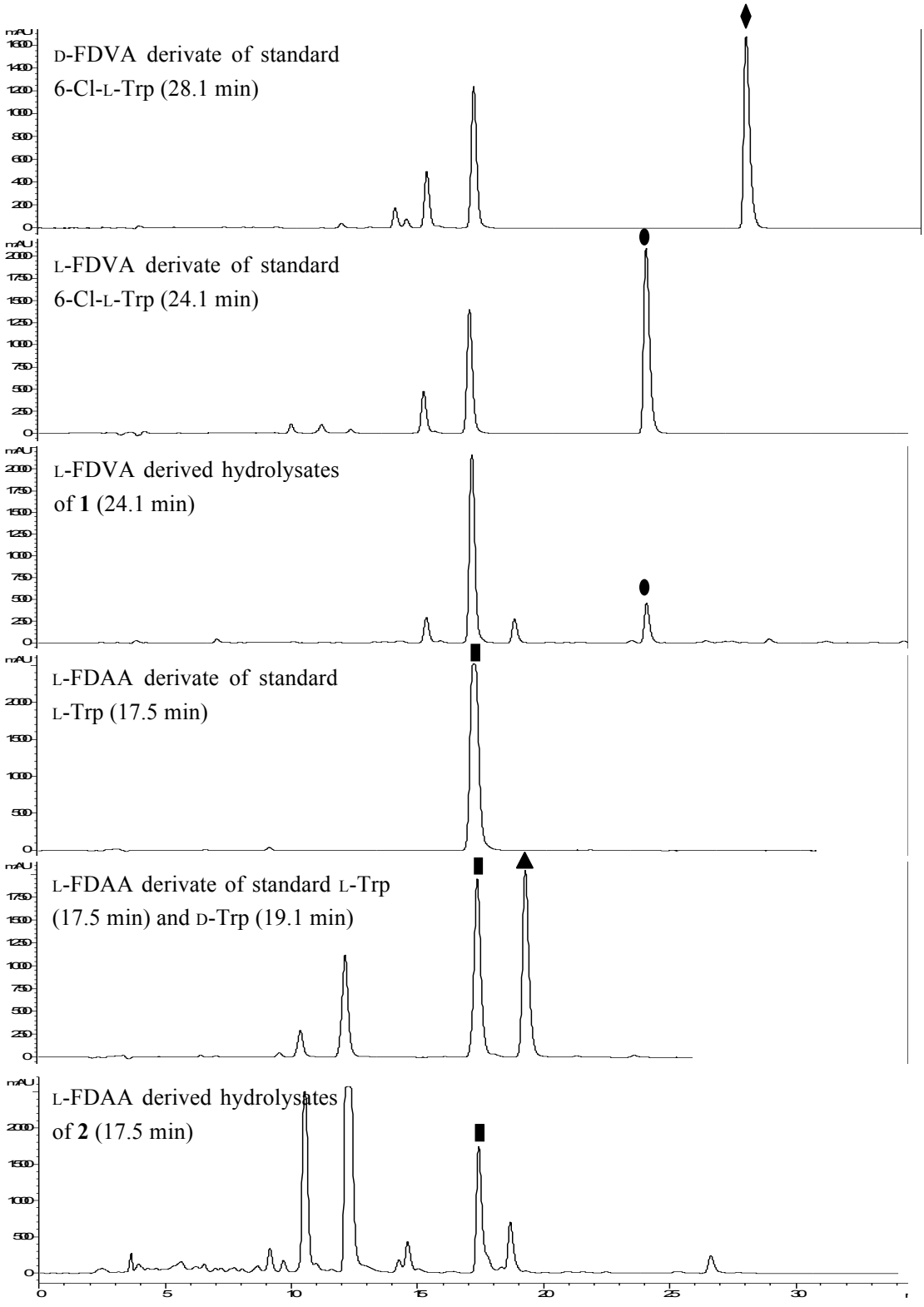
Methylation of 1 with TMS-CHN$_2$. A solution of compound **1** (0.2 mg) in MeOH (anhydrous, 0.4 mL) was added 100 μL of TMS-CHN$_2$ (2.0 M in Et$_2$O). After allowing to stir for 1 h, solution was removed *via* N$_2$ and the reaction mixture was analyzed by LC-MS. The result showed the presence of two methyl groups corresponding to formation of a methyl ester at C-20 and a C-15 methyl ether (Scheme S1).

Scheme S1. Methylation of **1** with TMS-CHN$_2$



Absolute configuration determination of 1 and 2 by Marfey's method. A solution of **1** (0.5 mg) in 6 M HCl (0.5 mL) was heated at 110 °C for 19 h. The solution was then evaporated to dryness and redissolved in H$_2$O (250 μL). 100 μL of the acid hydrolysates solution was then placed in a 4 mL vial and treated with 1% solution of L-FDVA (100 μL) in acetone followed by 1.0 M NaHCO$_3$ (40 μL). The reaction mixture was heated at 45 °C for 1 h, cooled to room temperature, and then acidified with 1.0 M HCl (40 μL). In a similar fashion, standard 6-Cl-L-Trp was derivatized with L-FDVA and D-FDVA. The derivatives of the hydrolysates and standard amino acid were subjected to HPLC analysis (Phenomenex Luna C$_{18}$ column; 5 μm, 4.6 × 150 mm; 0.7 mL/min) using the following gradient program: solvent A, water + 0.1% FA; solvent B, MeCN + 0.1% FA; linear gradient: 0 min 25% B, 40 min 60% B; UV detection at 340 nm. The retention times for the L-FDVA derivatives of hydrolysates of **1** and standard 6-Cl-L-Trp were all 24.1 min, while the retention time for the D-FDVA derivative of standard 6-Cl-L-Trp was 28.1 min (Figure S1). Compound **2** was hydrolyzed and then derivatized with L-FDAA by the same procedure. Standard L-Trp and DL-Trp were also derivatized with L-FDAA. HPLC analysis showed that the retention times for L-FDAA derivatives of hydrolysates of **2**, standard L-Trp and standard D-Trp were 17.5, 17.5 and 19.1 min, respectively.

Figure S2. The determination of the absolute configurations of **1** and **2**



Antibiotic assays. The antibiotic activities against *Pseudomonas aeruginosa* and *Bacillus subtilis* were evaluated by an agar dilution method. The tested strains were cultivated in LB agar plates at 37 °C. Compounds **1**–**3** and positive control (erythromycin) were dissolved in MeOH at different concentrations from 100 to 0.1 μg/mL by the continuous 10-fold dilution methods. A 10 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded.

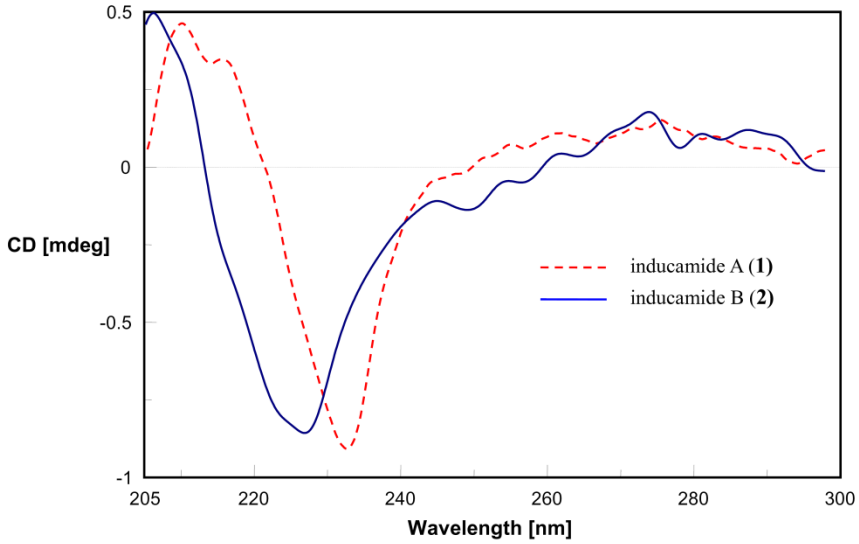


Figure S3. CD spectra of inducamides A and B (**1** and **2**) in MeOH at 0.049 and 0.054 mM, respectively.

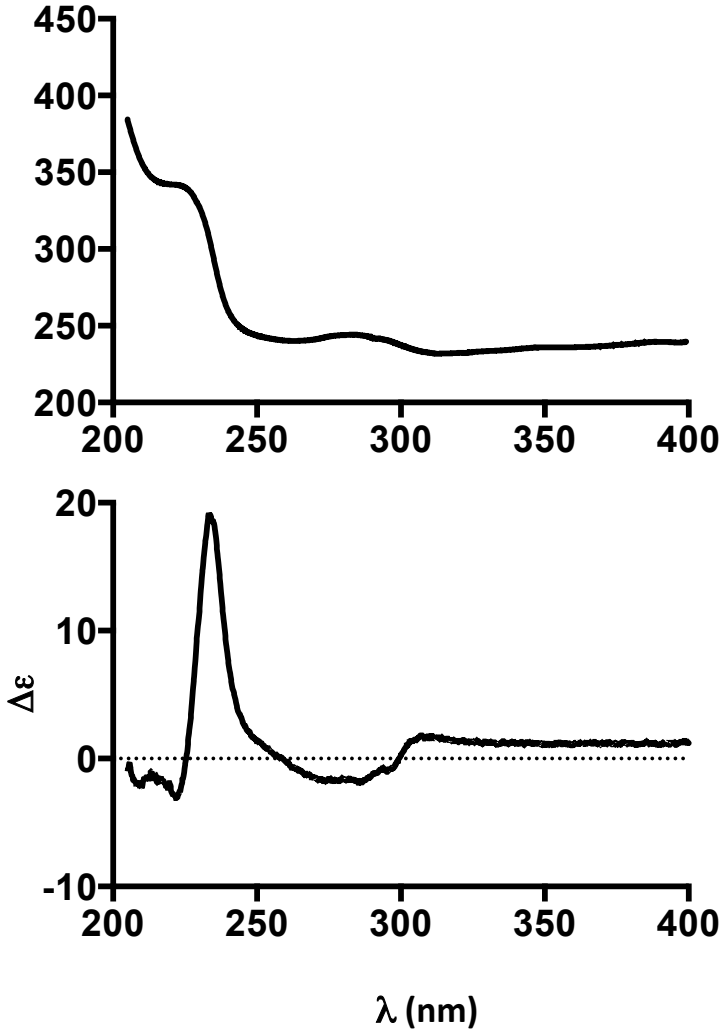


Figure S4. UV and CD spectra of inducamide C (**3**) at 0.1 mM in MeOH.

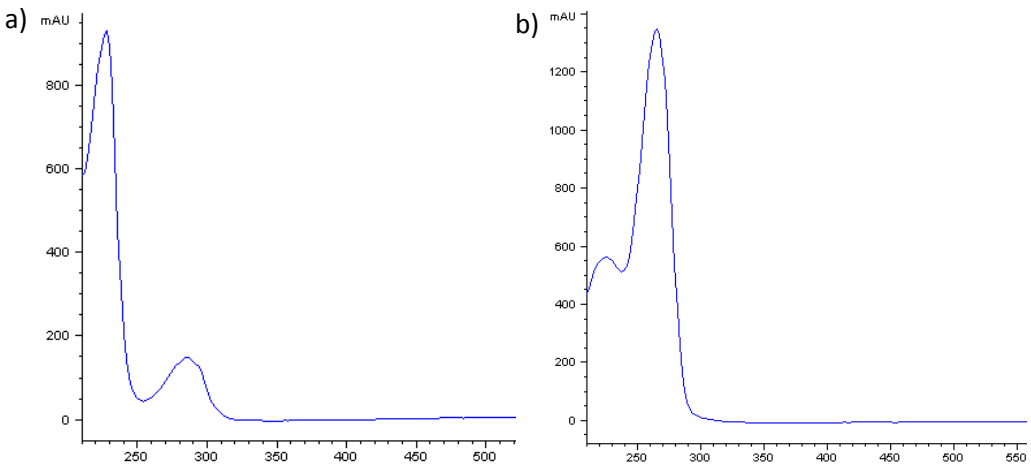


Figure S5. UV profiles of a) inducamide B. b) major peak at 7.5 minutes in the LC-MS trace of wild type SNC-109 referred to in figure 2c. This peak is also present in mutant strain SNC-109-M3.

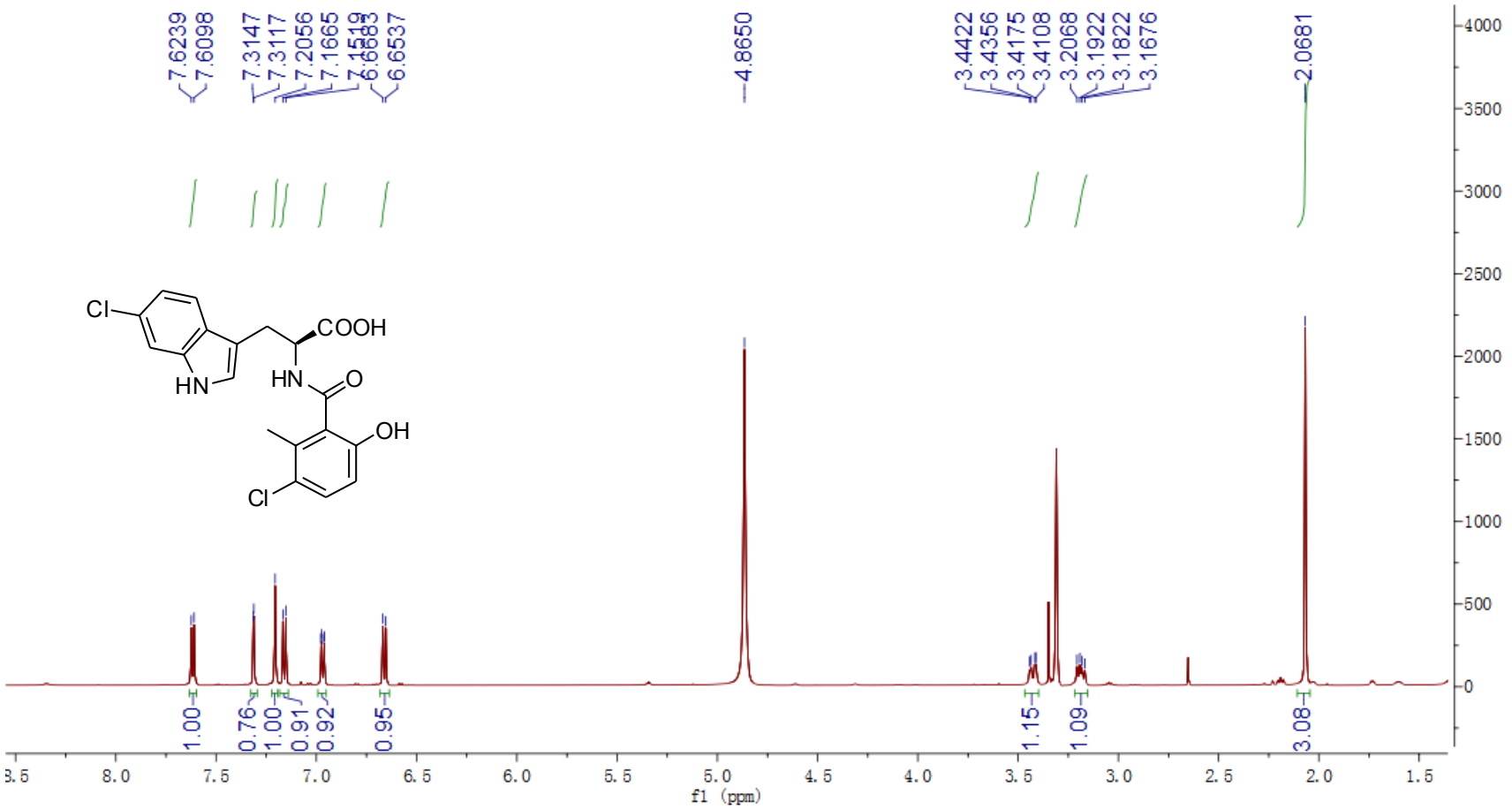
II. NMR table for inducamide C (3)

Table S1. ^{1}H (600 MHz) and ^{13}C (100 MHz) NMR Data for Compound **3** in DMSO-d_6

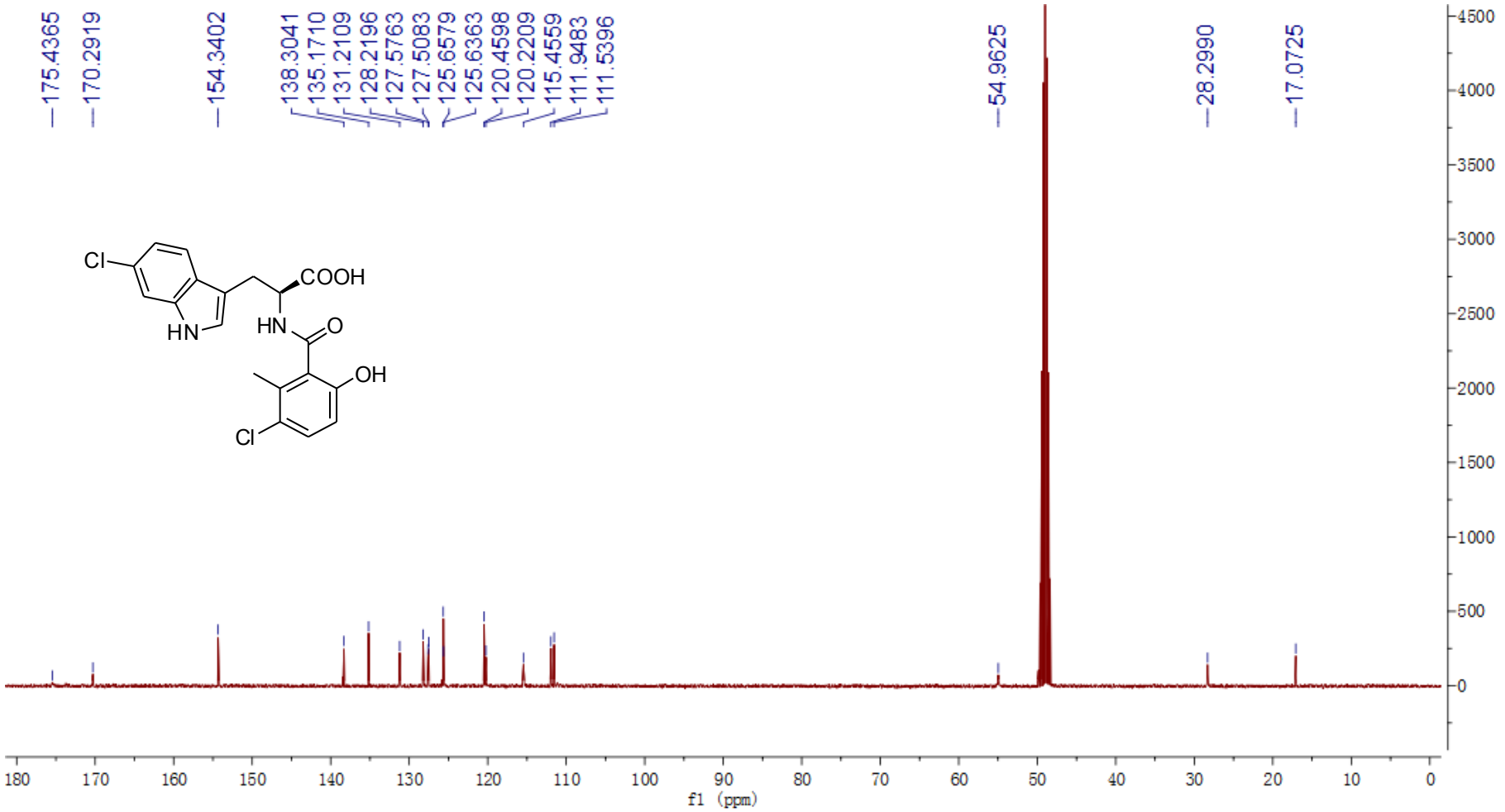
no.	δ_C	δ_H, mult. (J in Hz)
2	126.3, CH	7.26, s
3	113.0, C	
4	152.4, C	
5	109.2, CH	6.45, s
6	125.2, C	
7	107.7, CH	7.10, s
8	139.5, C	
9	118.4, C	
10	32.7, CH_2	3.33, dd (13.8, 4.6); 2.90, dd (13.8, 8.8)
11	59.2, CH	4.30, dd (8.8, 4.6)
13	164.1, C	
14	134.5, C	
15	154.7, C	
16	123.9, CH	7.54, d (7.1)
17	130.4, CH	7.57, d (7.1)
18	130.8, C	
19	130.0, C	
20	170.8, C	
21	16.9, CH_3	2.19, s
1-NH		11.24, s
12-NH		8.01, s

III. NMR spectra for inducamides A–C (1–3)

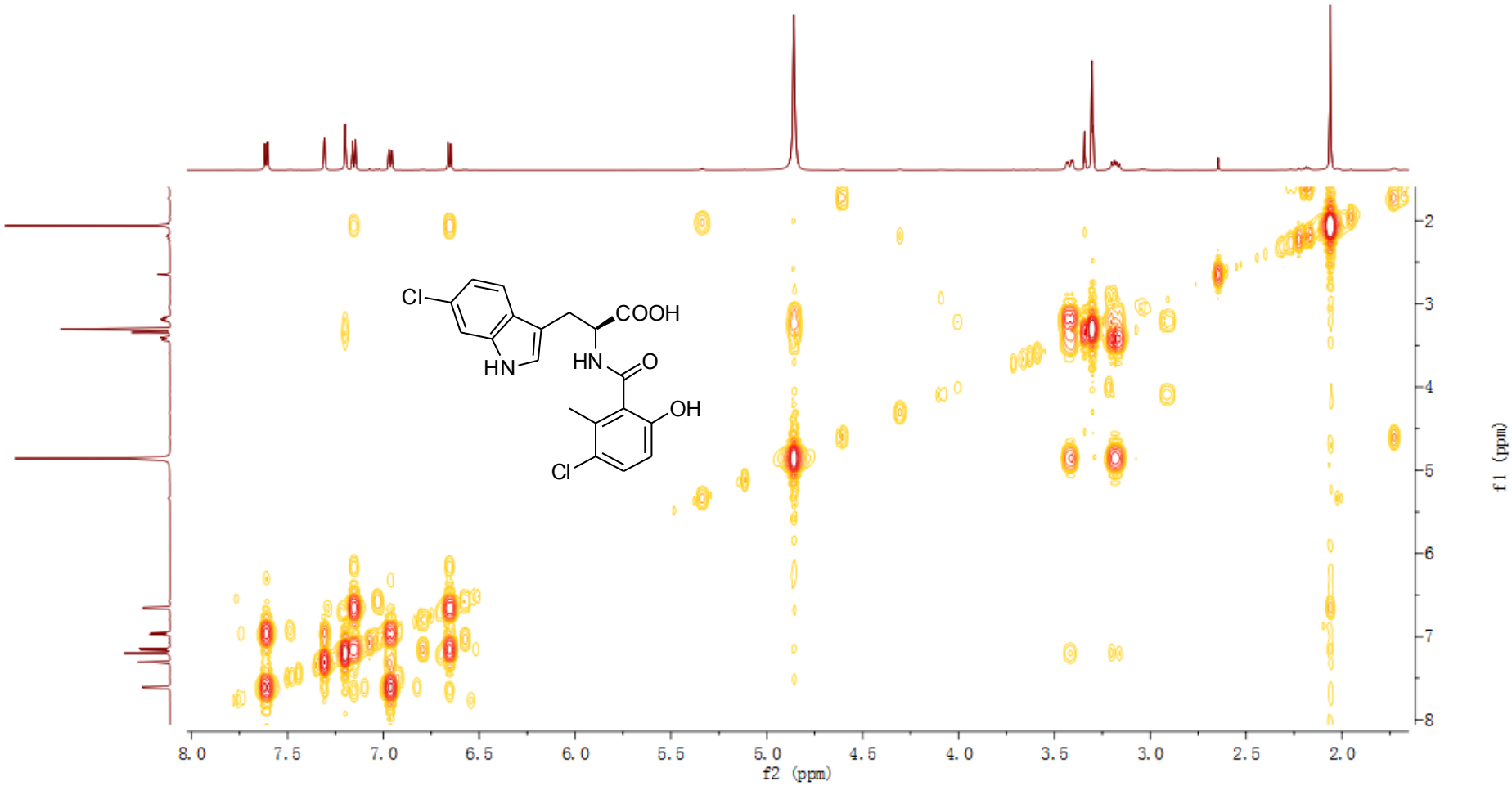
^{1}H NMR spectrum of inducamide A (**1**) in CD_3OD (600 MHz)



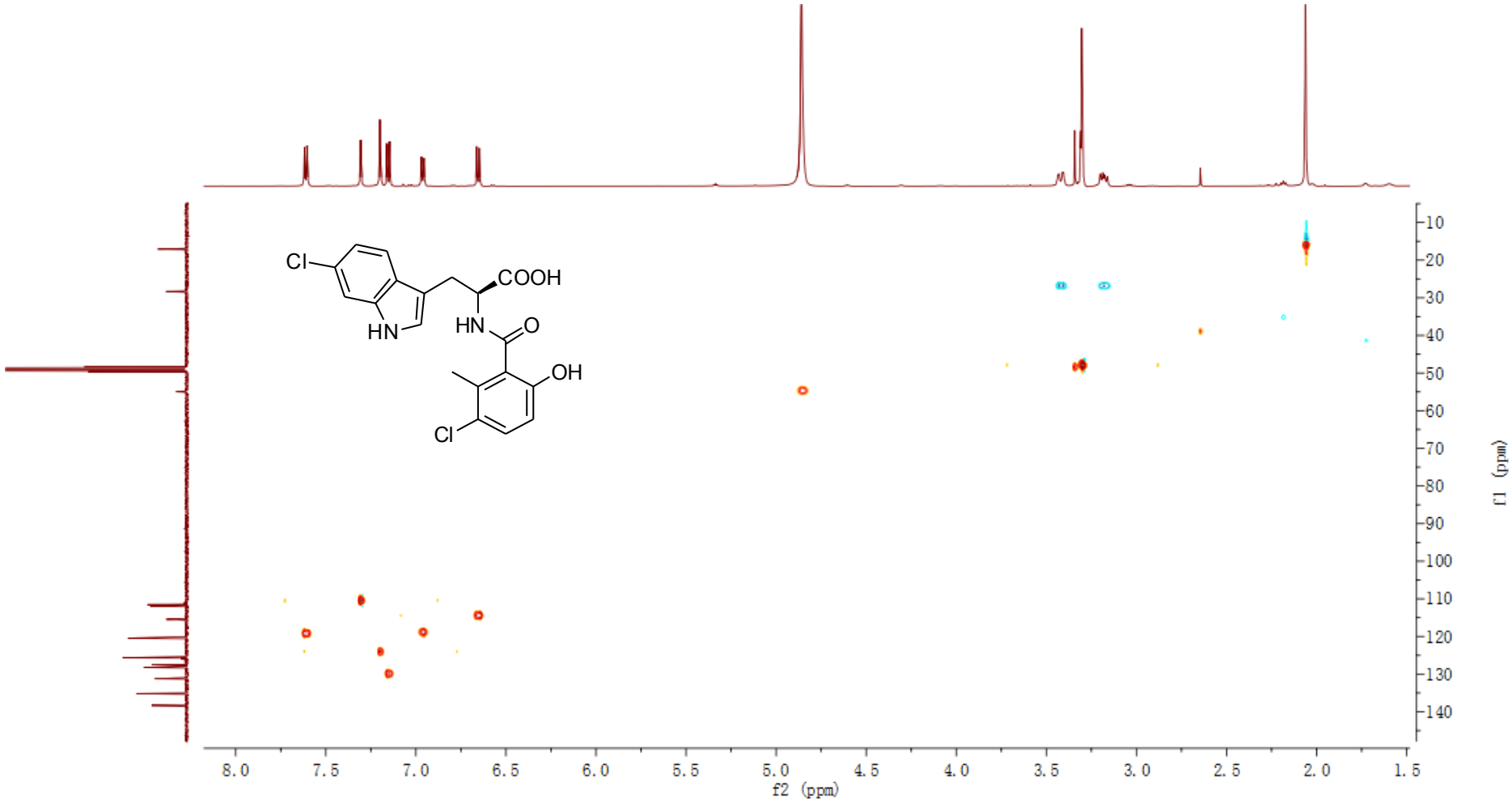
^{13}C NMR spectrum of inducamide A (**1**) in CD_3OD (100 MHz)



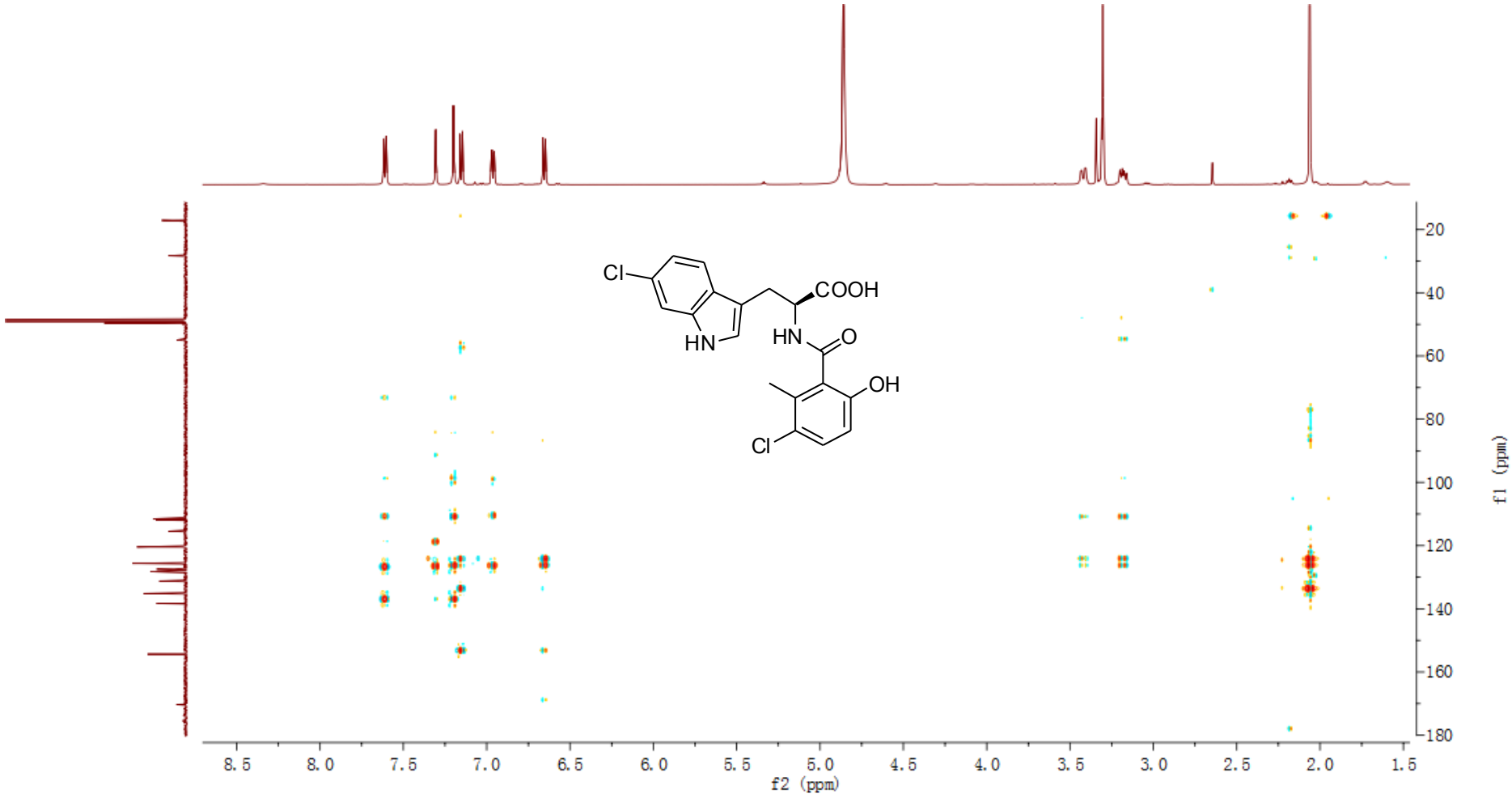
^{1}H-^{1}H COSY spectrum of inducamide A (**1**) in CD_3OD (600 MHz)



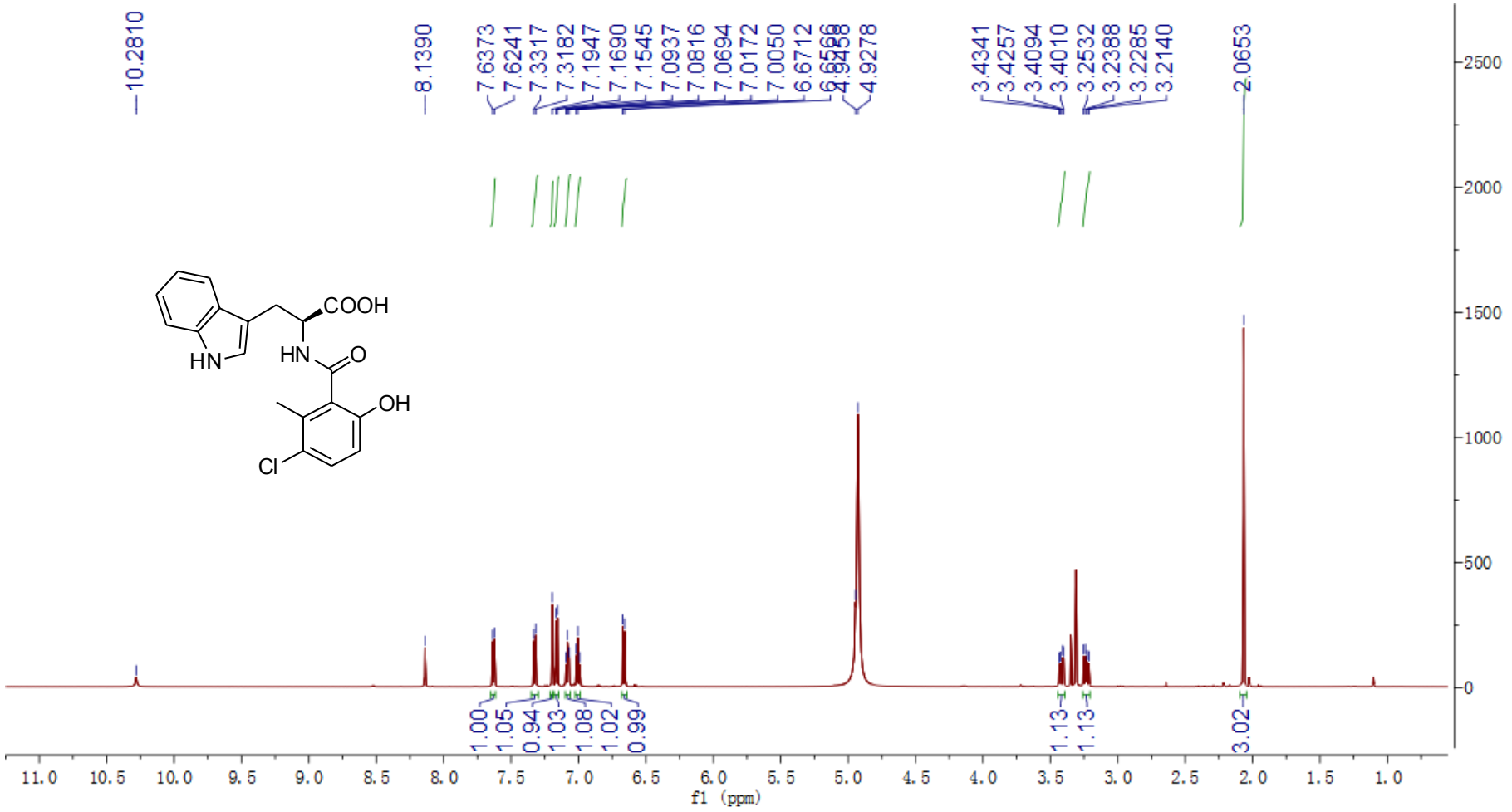
HSQC spectrum of inducamide A (**1**) in CD_3OD (600 MHz)



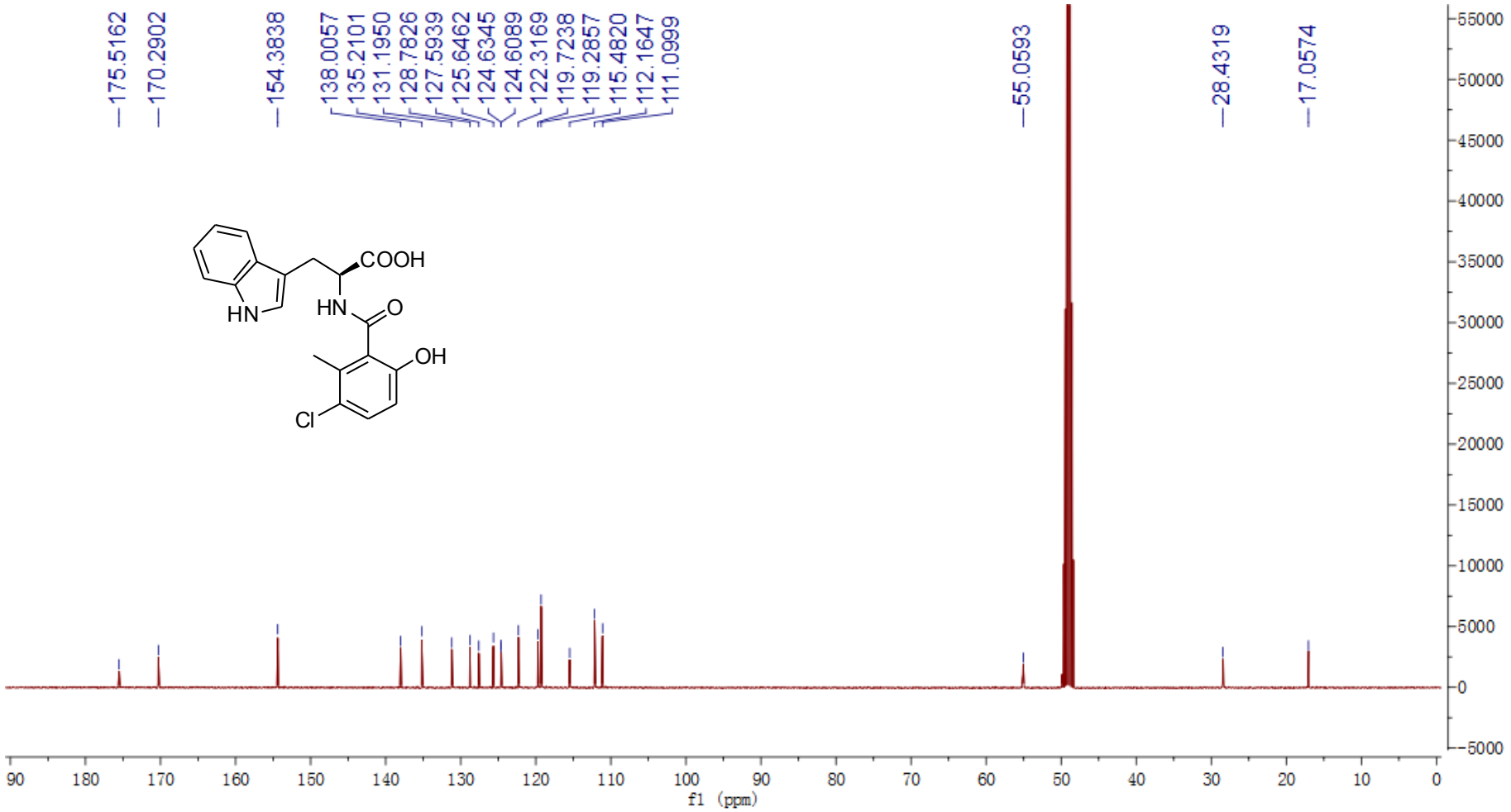
HMBC spectrum of inducamide A (**1**) in CD_3OD (600 MHz)



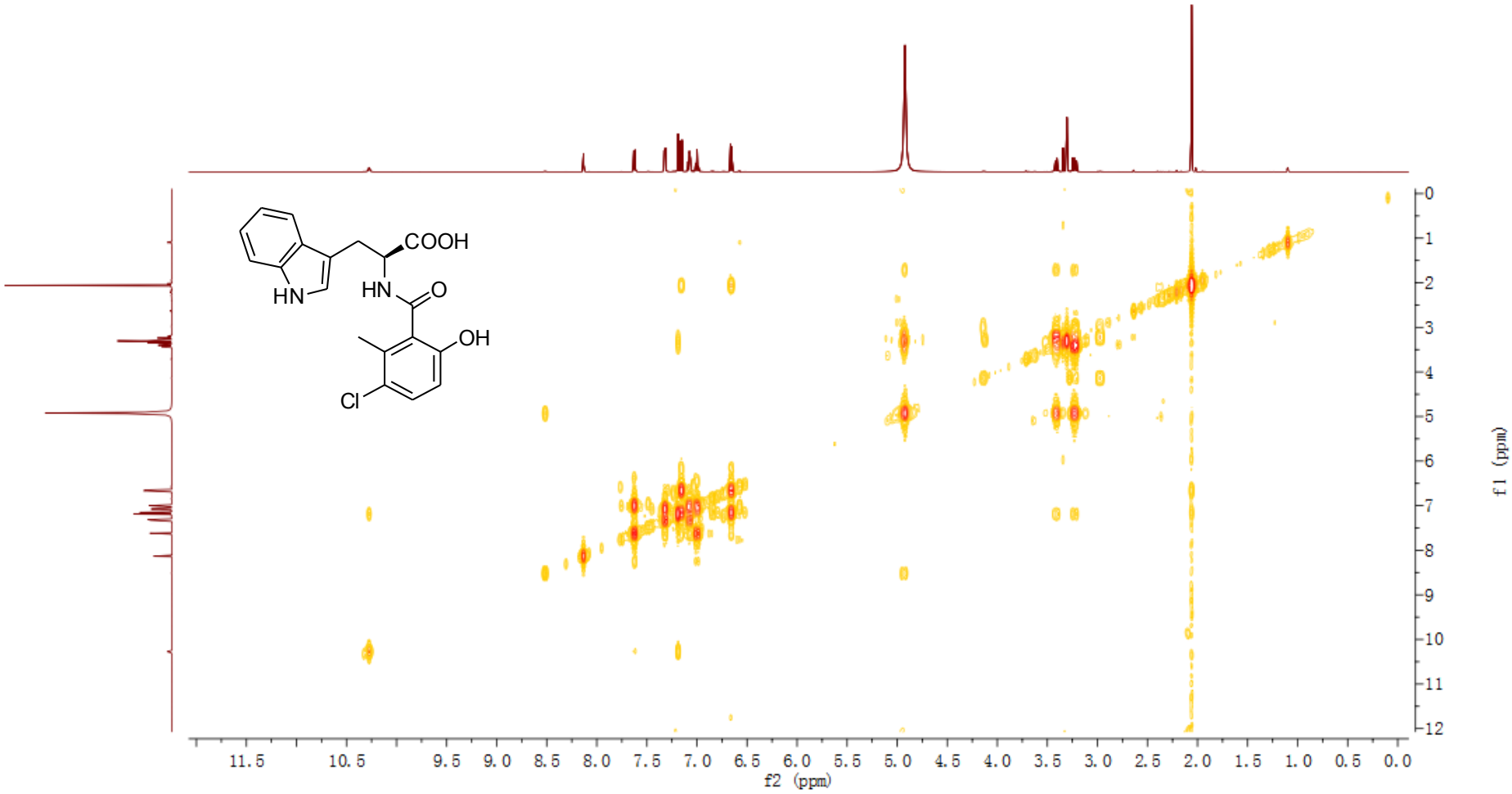
^{1}H NMR spectrum of inducamide B (**2**) in CD_3OD (600 MHz)



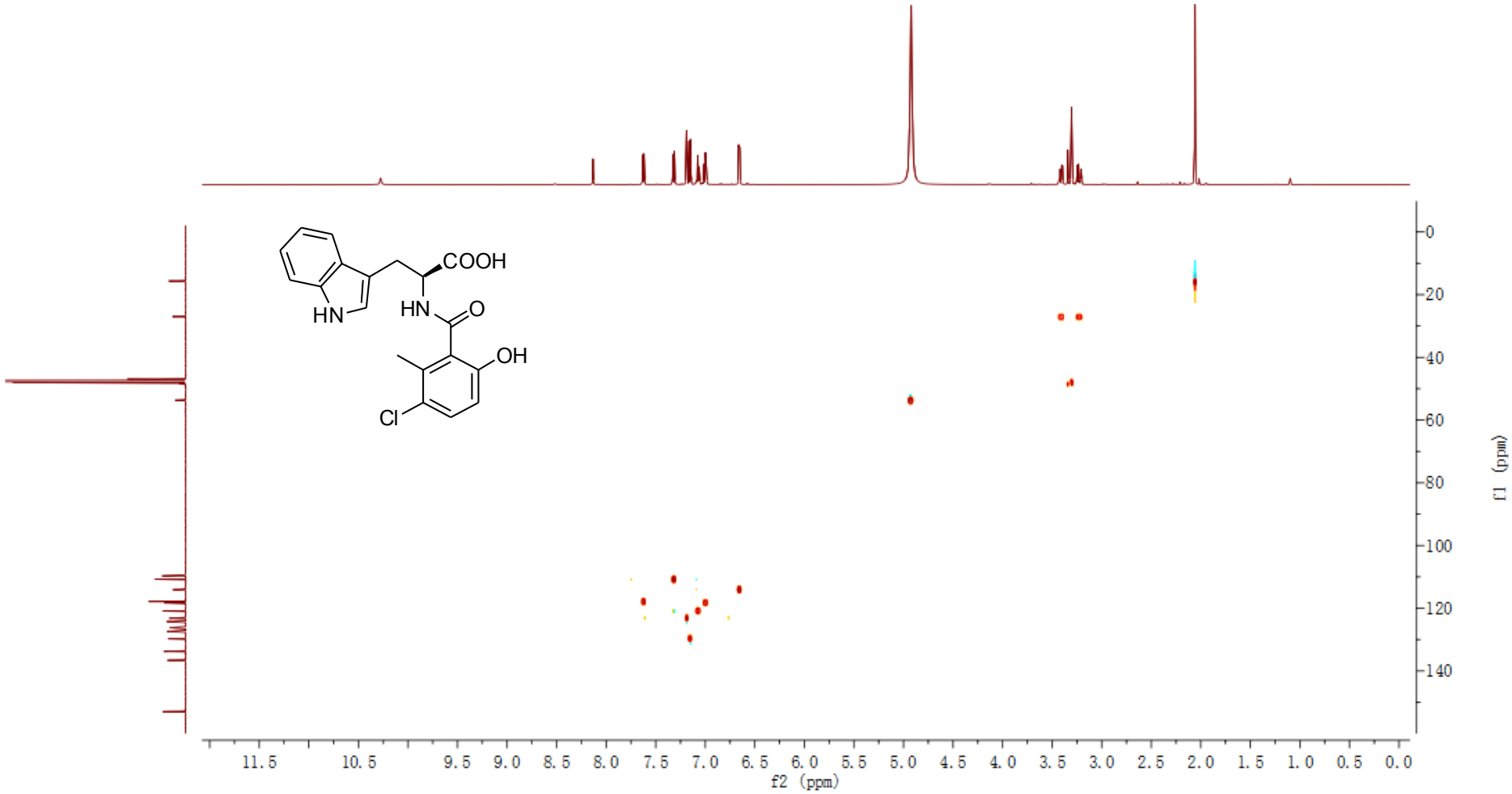
^{13}C NMR spectrum of inducamide B (**2**) in CD_3OD (100 MHz)



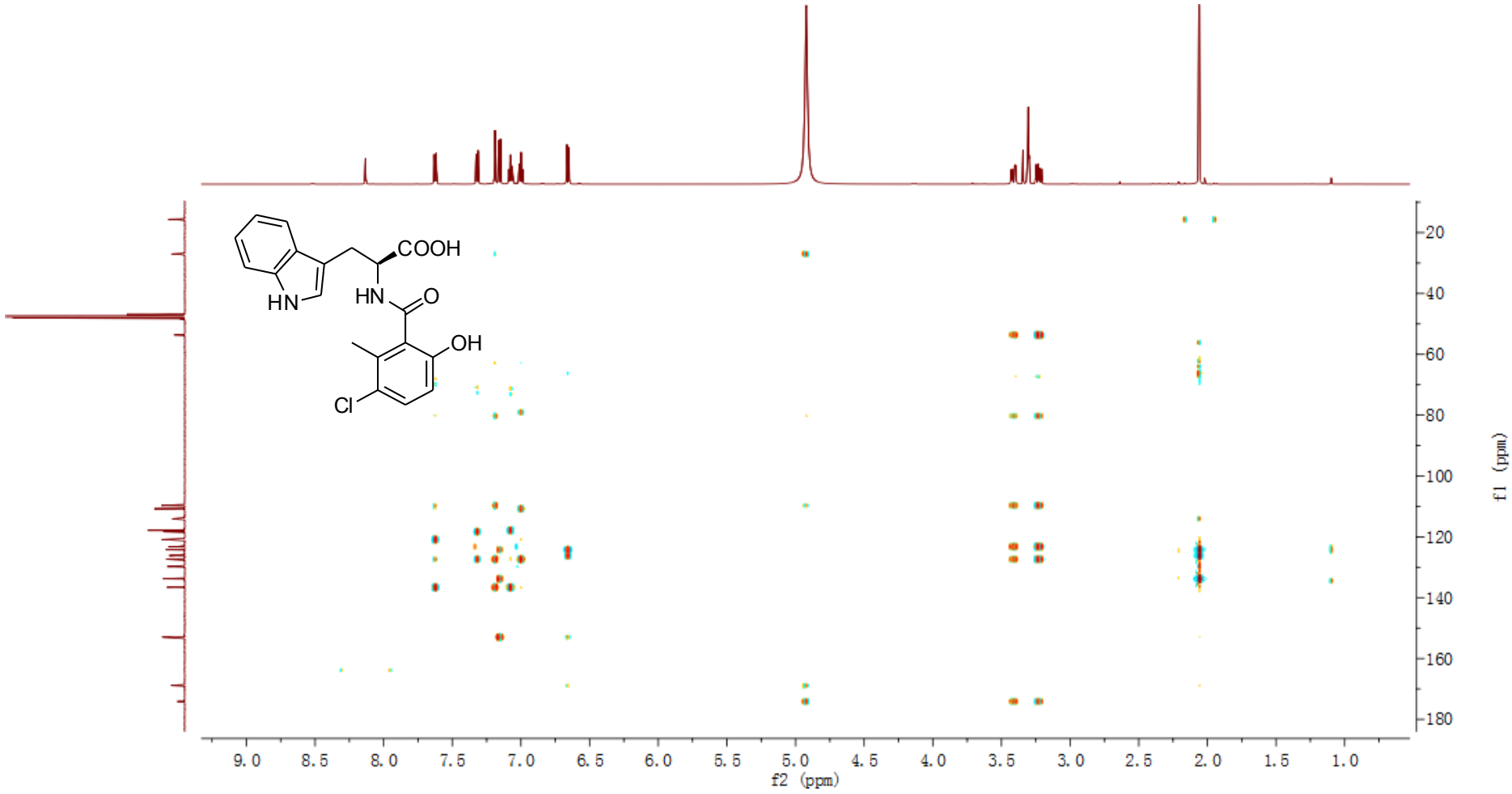
^{1}H-^{1}H COSY spectrum of inducamide B (**2**) in CD_3OD (600 MHz)



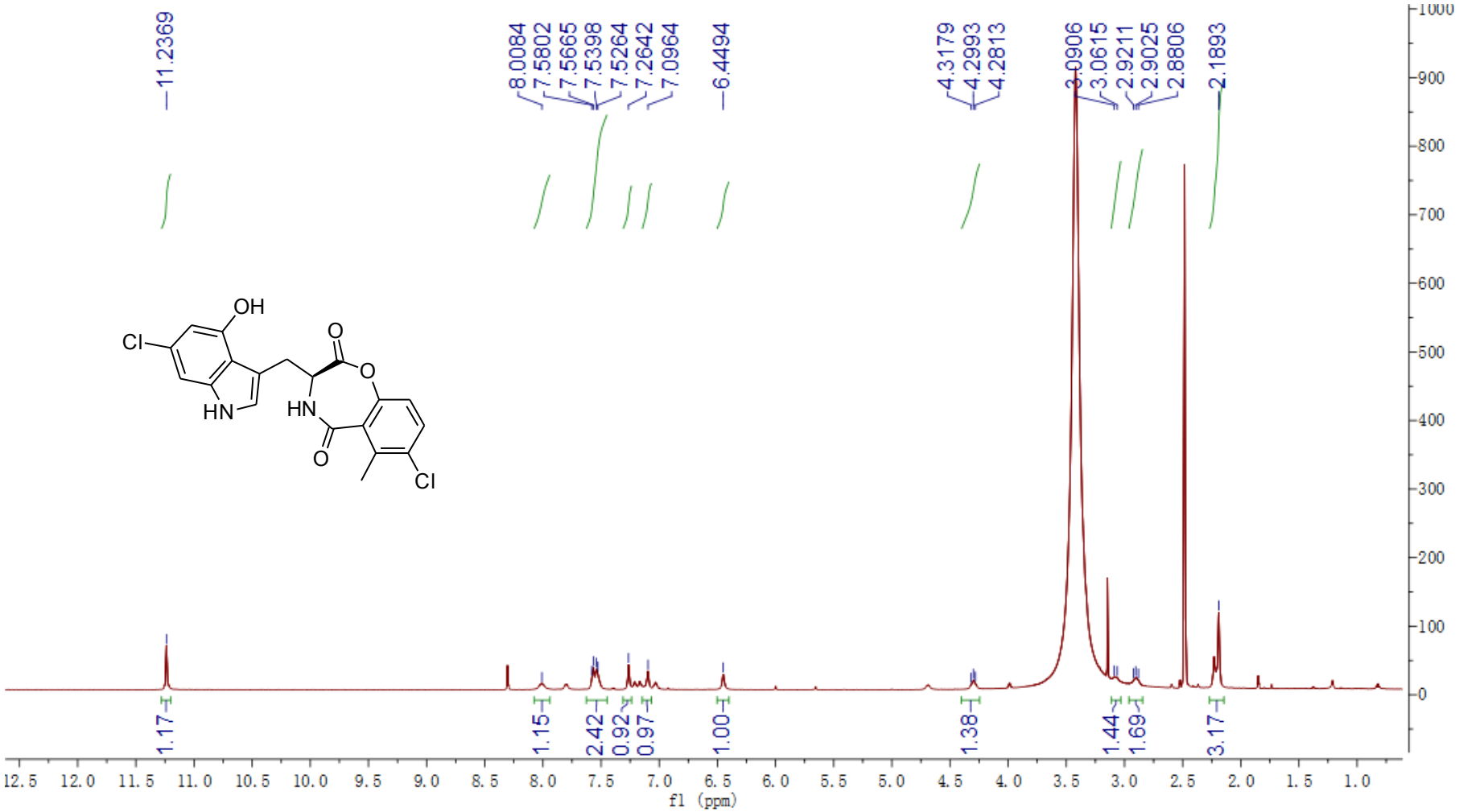
HSQC spectrum of inducamide B (**2**) in CD_3OD (600 MHz)



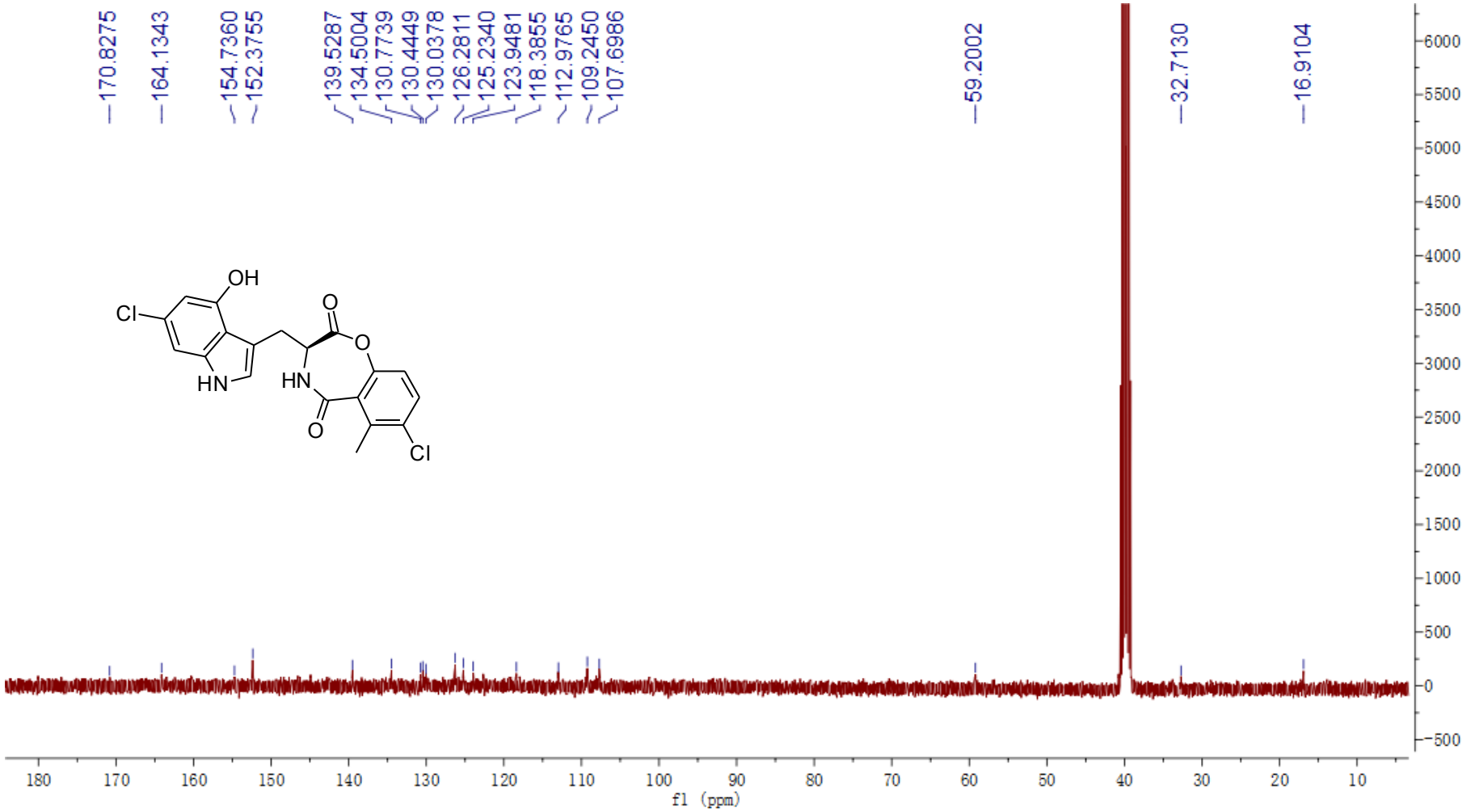
HMBC spectrum of inducamide B (**2**) in CD_3OD (600 MHz)



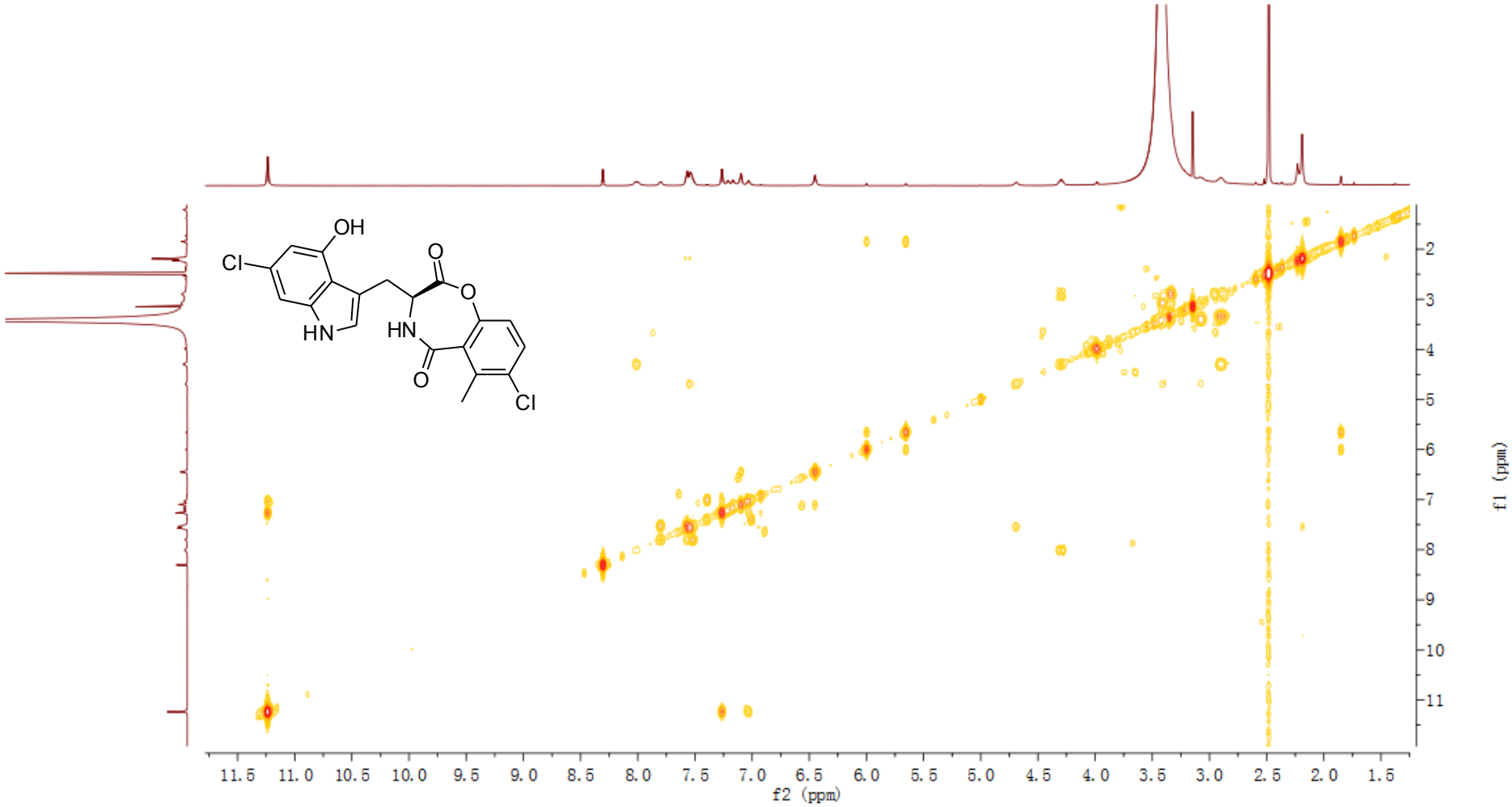
^{1}H NMR spectrum of inducamide C (**3**) in DMSO-d_6 (600 MHz)



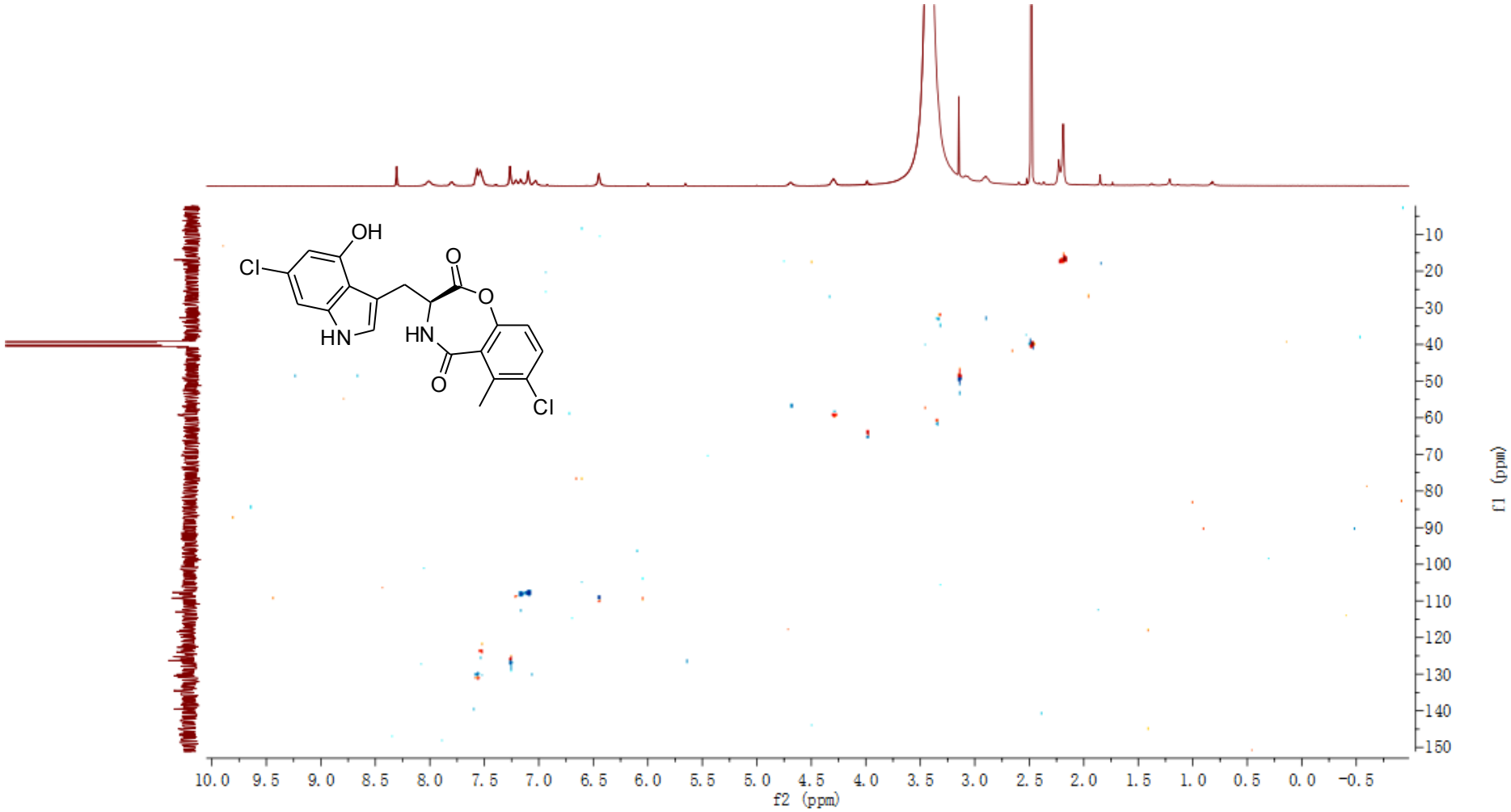
^{13}C NMR spectrum of inducamide C (**3**) in DMSO-d_6 (100 MHz)



^{1}H-^{1}H COSY spectrum of inducamide C (**3**) in DMSO-d_6 (600 MHz)



HSQC spectrum of inducamide C (**3**) in DMSO-d_6 (600 MHz)



HMBC spectrum of inducamide C (**3**) in DMSO-d_6 (600 MHz)