SUPPORTING INFORMATION

Two Novel Triterpenoids from *Dysoxylum Hainanense*

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S1. Experimental section.

S1.1 General Experimental Procedures.

Melting points were measured with an SGW X-4 apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were made on a Perkin-Elmer 577 spectrometer with KBr disks. 1D and 2D NMR spectra of **1** and **2** were recorded on a Varian Mercury Plus 400 spectrometer (except that the ROESY of **1** was performed on a Varian Unity-Inova 600 spectrometer) in $CDCl_3$ with TMS as internal standard. EI-MS (70 eV) was measured on a Finnigan MAT 95 mass spectrometer in *m/z* (rel. %). All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People′s Republic of China). Silica gel (200-300 mesh), silica gel H60 (Qingdao Haiyang Chemical Co. Ltd. Qingdao, People′s Republic of China), Sephadex LH-20 (Amersham Biosciences), C_{18} reversed-phase silica gel (150-200 mesh, Merck), and MCI gel (CHP20P, 75-150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography. Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, People′s Republic of China) were used for TLC.

S1.2 Plant Material.

The twigs and leavies of *Dysoxylum hainanense* Merr. were collected in September of 2005 from Hainan Province of P. R. China. The plant was authenticated by Prof. S. M. Huang, Department of Biology, Hainan University of China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences (access number: DHTS-2005-1Y).

S1.3 Extraction and Isolation.

The air-dried powder of the plant material (2 kg) was percolated with 95% EtOH three times (each 5 L) to give 105 g of crude extract, which was then suspended in water (1 L) and partitioned successively with petroleum ether and EtOAc. The EtOAc soluble fraction (35 g) was subjected to a MCI gel column ($MeOH/H_2O$, 0:10 to 10:0) to give five fractions 1—5. Fraction 4 (6 g) was separated on a silica gel column (petroleum ether/acetone 100:1 to 3:1) to give seven subfractions 4a—4g. Fraction 4c (710 mg) was extensively separated over silica gel, RP-18 silica gel, and Sephadex LH-20 to obtain compound **1** (10 mg) and **2** (6 mg).

S1.4 Crystal data and structure refinement for Dysoxyhainanin B (2).

Empirical formula	$C_{28}H_{44}O_4$
Formula weight	444.63
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)
Unit cell dimensions	a=6.7687(9) Å, α= 90 °
	b=14.2706(19) Å, β= 108.7020(10) °
	c=13.3312(17) Å, γ= 90 °
Volume	1276.8(3) $Å^3$
Z	2
Calculated density	1.157 Mg/m^3
Absorption coefficient	0.075 mm^{-1}
F(000)	488
Crystal size	0.451 ×0.412 ×0.155 mm^3
Theta range for data collection	1.54 to 27.00 °
Limiting indices	-8<=h<=8, -14<=k<=18, -17<=l<=14
Reflections collected / unique	7480 / 2889 [R(int) = 0.0824]
Completeness to theta = 26.49	99.6 %
Absorption correction	Empirical
Max. and min. transmission	1.0000 and 0.8286
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2889 / 2 / 301
Goodness-of-fit on F2	0.948
Final R indices [I>2σ(I)]	R1 = 0.0485, wR2 = 0.1027
R indices (all data)	R1 = 0.0636, wR2 = 0.1077
Absolute structure parameter	10(10)
Largest diff. peak and hole	0.156 and -0.185 e. Å-3

Crystal data were obtained on a Bruker SMART CCD detector employing graphite monochromated Mo-Kα radiation (λ=0.71073 Å) at 293 K and operating in the ϕ-ω scan mode. The structure was solved by direct methods SHELXS-97[1] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[2] Crystallographic data for Dysoxyhainanin B (**2**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-656630). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data

Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].

References

(1) Sheldrick, G. M. *SHELXS-97*: *Program for Crystal Structure Resolution*; University of Göttingen: Göttingen, Germany, 1997.

(2) Sheldrick, G. M. *SHELXL-97*: *Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.

S1.5 Antimicrobial Tests.

The antibacterial tests against *H. pylori* strains (*Hp*-SS1 or ATCC 43504 strain) in vitro were carried out according to the protocols described in our previous literature.[1] To the well-prepared agar plates, *H. pylori* (*Hp*-SS1 or ATCC 43504) cells suspended in saline at the density of 10^8 CFU/mL were inoculated and incubated at 37 °C for 96 h under an atmosphere of 5% O_2, 10% CO_2 and 85% N_2. The blank controls and the positive controls were incubated under the same condition. All the tests were conducted in triplicate under the same condition. The MIC was defined as the lowest concentration of test samples, at which the visible growth was completely inhibited.

The in vitro antibacterial activities against *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *M. luteus* (ATCC 9341), *E. coli* (ATCC 25922), and *B. subtilis* (CMCC 63501) were conducted by following the manipulations described in our previous literature.[2] The microbial cells were suspended in Mueller Hinton broth to form a final density of $5\times10^{-5}-10^{-6}$ CFU/mL and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds, which have been dissolved in DMSO. The blank controls of microbial culture were incubated with limited DMSO under the same condition. DMSO was determined not to be toxic at a limited amount under the experimental conditions.

The in vitro antifungal activity against *C. albicans* (ACTT 1600) was completed according to the protocols described in our previous literature.[3] The fungui were incubated

in Sabouraud dextrose broth at 37 °C for 48 h with the respective compounds and the positive control dissolved in DMSO. The blank controls of fungi cultures were incubated with limited DMSO under the same condition.

Compounds **1** and **2** were tested for their antimicrobial potentials against 8 microorganisms in vitro and the results were summarized as follow.

Table . Antimicrobial activities of compounds **1** and **2**.

Microorganism	MICs (μg/ml)[a]		Positive controls[b]		
	1	**2**	A	B	C
H. pylori-SS1	>50	>50	0.5		
H.pylori-ATCC 43504	>50	>50	128		
S. aureus	12.5	>50		12.5	
S. epidermidis	6.25	>50		6.25	
M. luteus	12.5	>50		6.25	
B. subtilis	6.25	>50		12.5	
E. coli	>50	>50		>50	
C. albicans	>50	>50			6.25

[a] MIC was defined as the lowest concentration that inhibited visible growth; all the tests were conducted in triplicate; the MIC>50 μg/ml was defined to be inactive. [b] A–C representing metronidazole, magnolol and pseudolaric acid B, respectively, were applied as the positive controls.

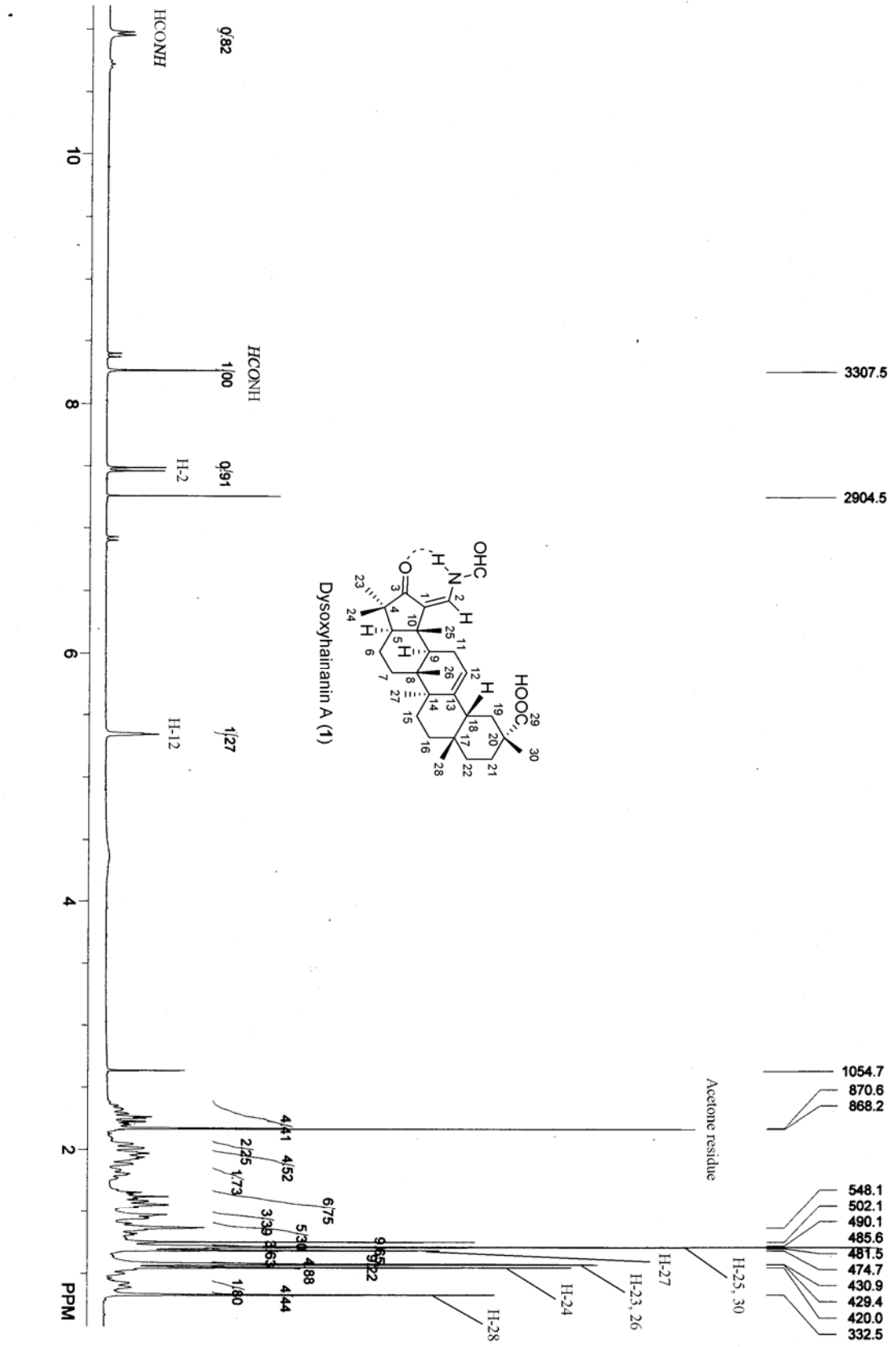
References

(1) Yin, S.; Fan, C. Q.; Dong, L.; Yue, J. M. *Tetrahedron* **2006**, *62*, 2569–2575.

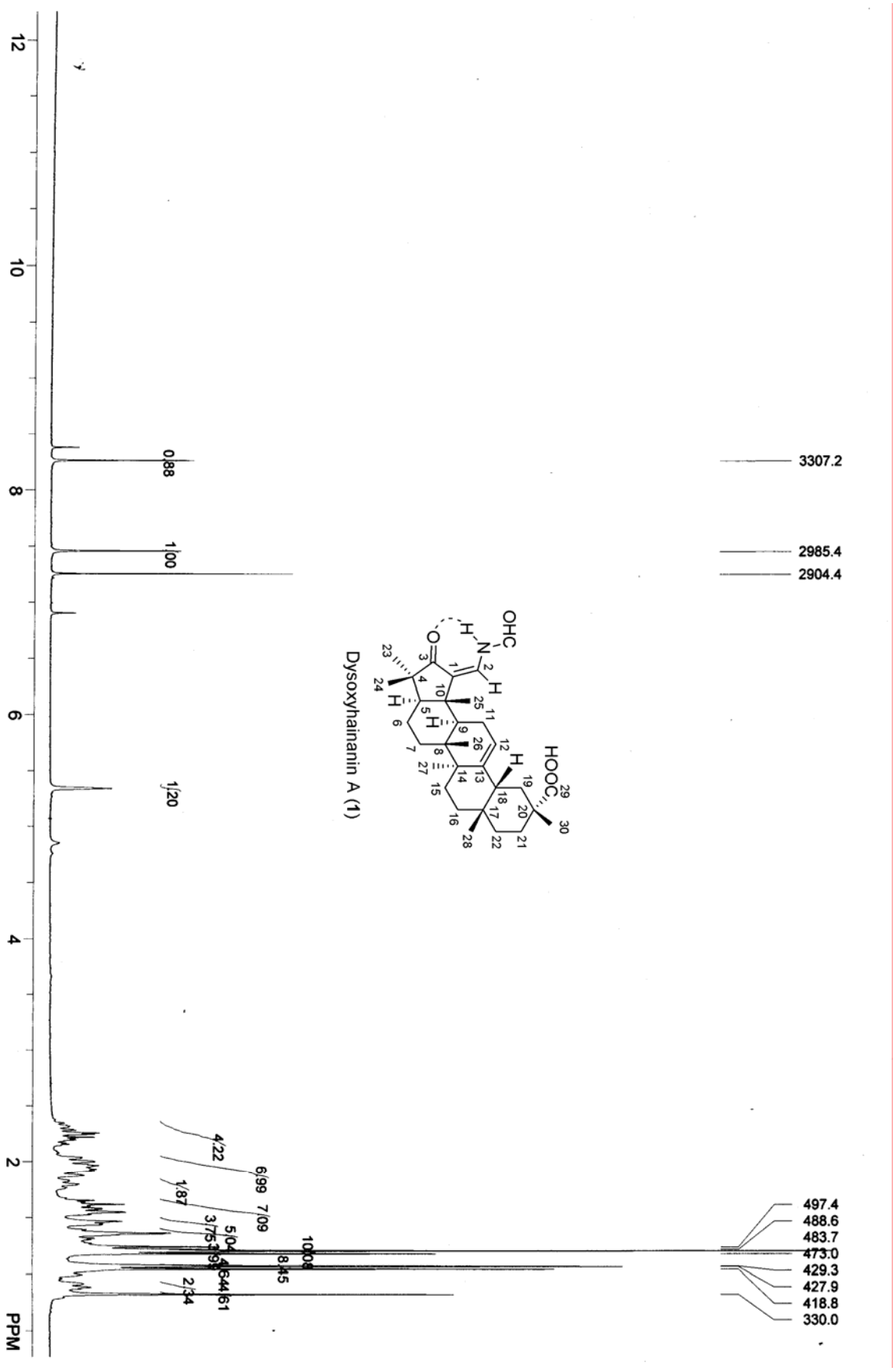
(2) Yin, S.; Fan, C. Q.; Wang, Y.; Dong, L.; Yue, J. M. *Bioorg. Med. Chem.* **2004**, *12*, 4387–4392.

(3) Yang, S. P.; Dong, L.; Wang, Y.; Wu. Y.; Yue, J. M. *Bioorg. Med. Chem.* **2003**, *11*, 4577–4584.

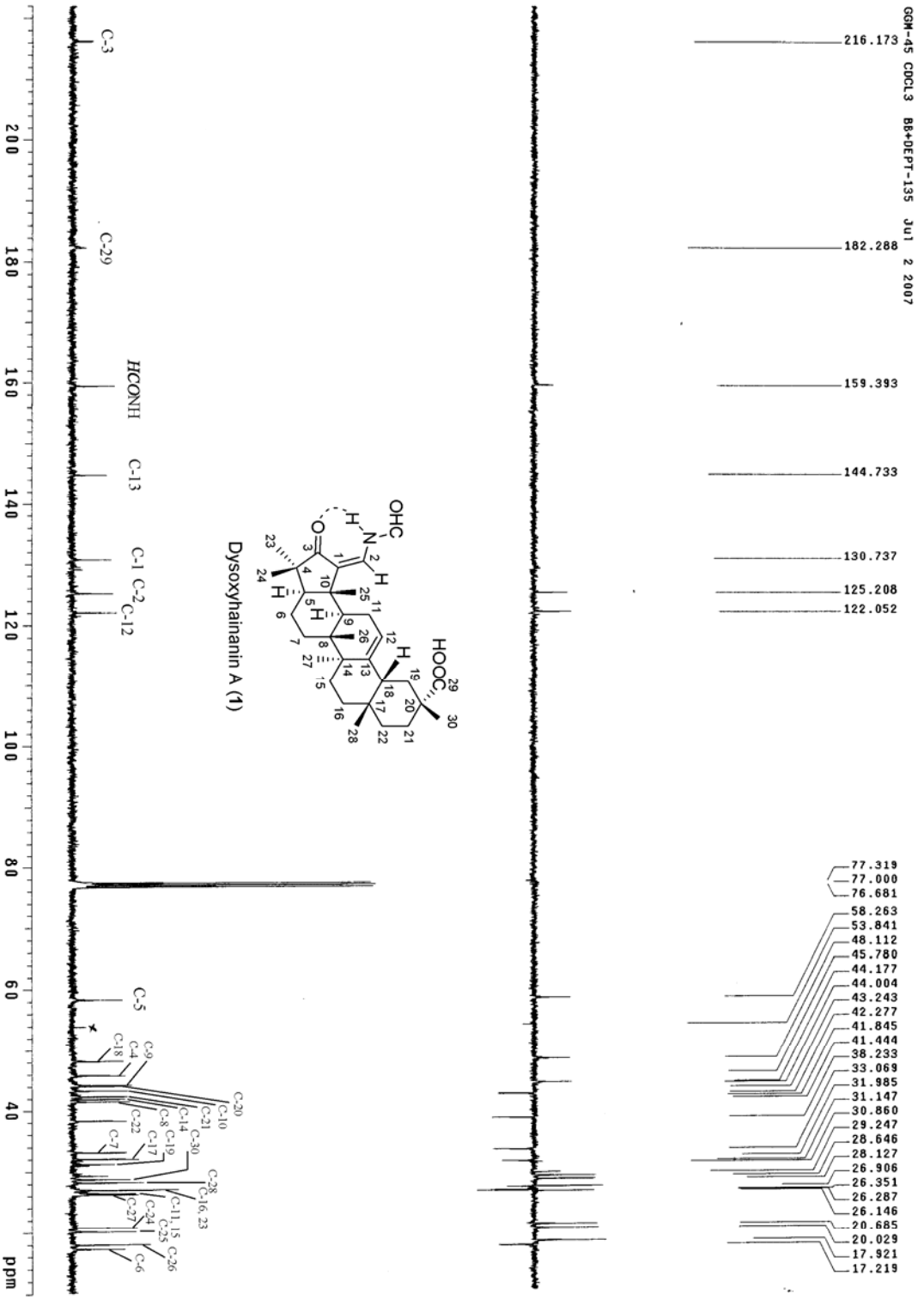
S2. ^{1}H NMR spectrum (400 MHz) of Dysoxyhainanin A (1) in $CDCl_3$.



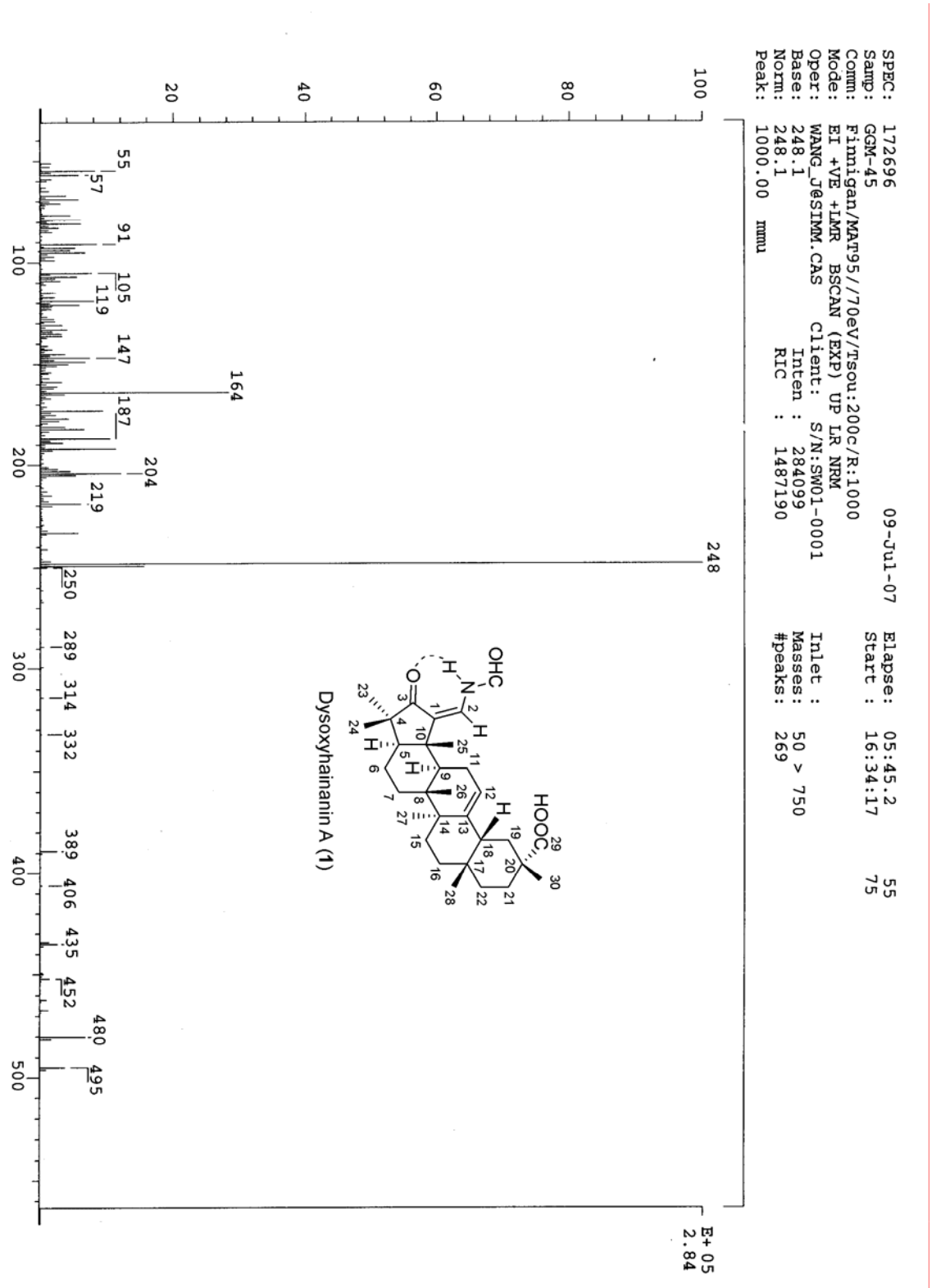
S3. ^{1}H NMR spectrum (400 MHz) of Dysoxyhainanin A (1) for D_2O exchange experiment in $CDCl_3$.



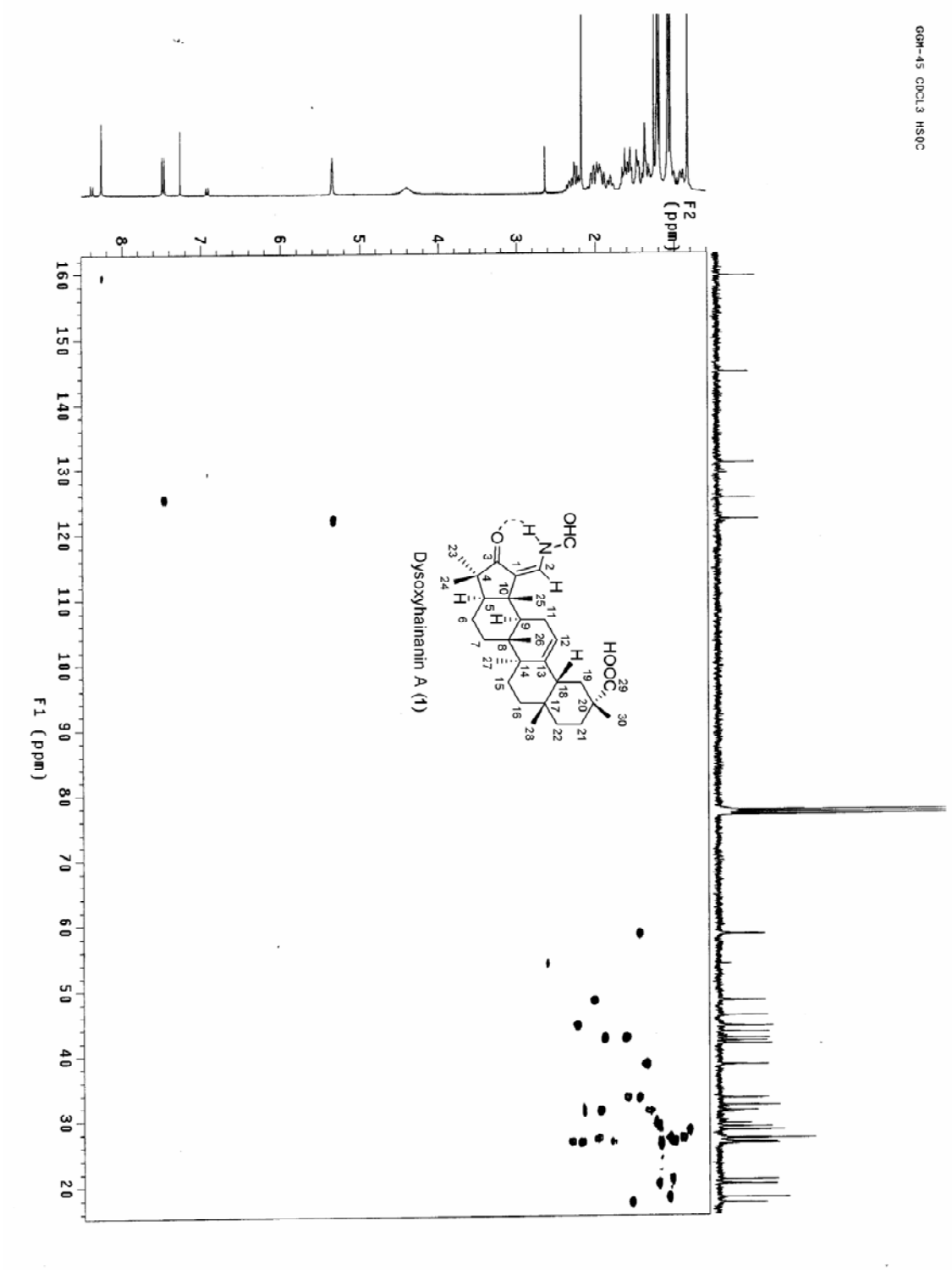
S4. ^{13}C NMR spectrum (100 MHz) of Dysoxyhainanin A (1) in $CDCl_3$



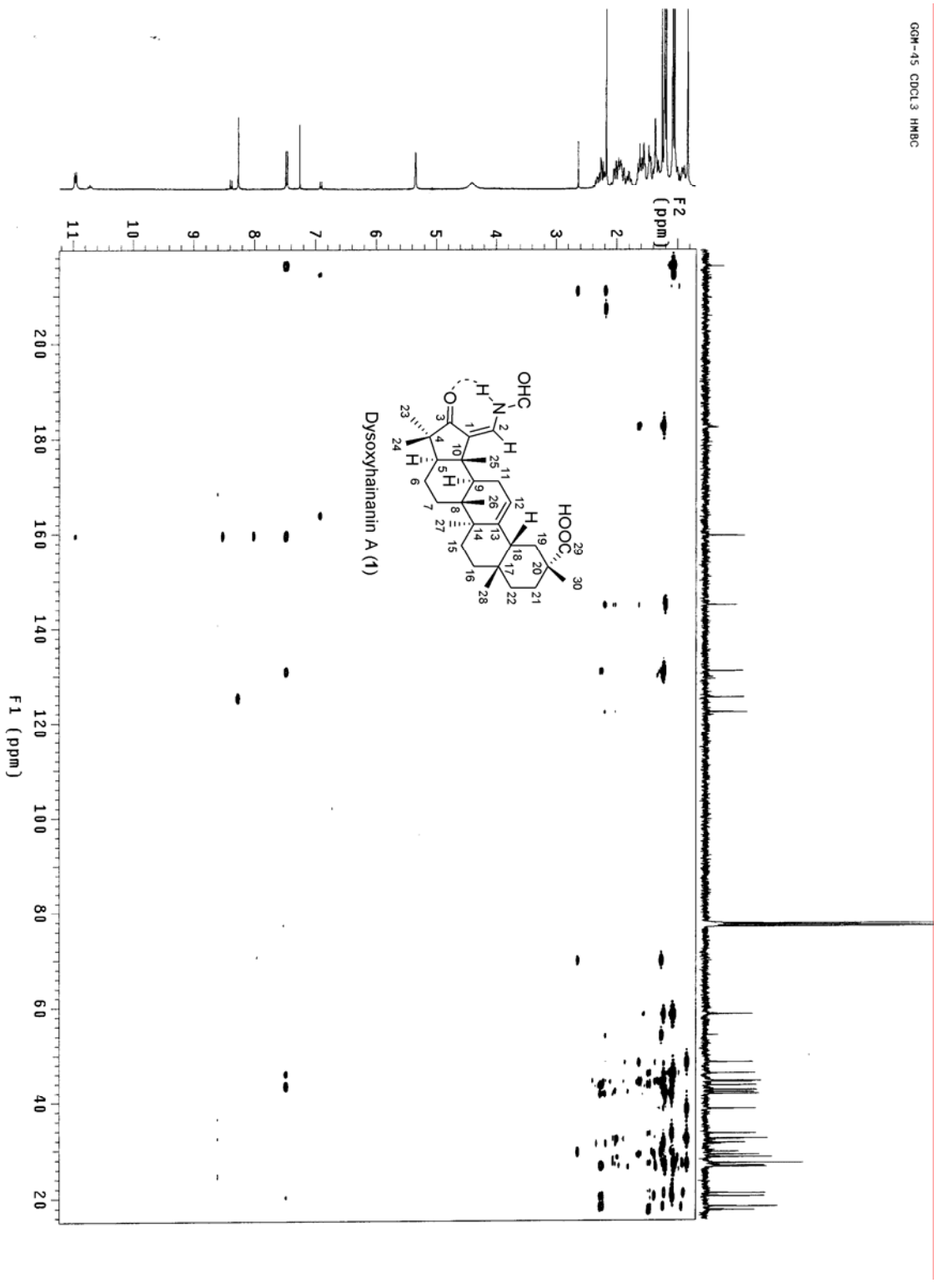
S5. EIMS spectrum of Dysoxyhainanin A (1).



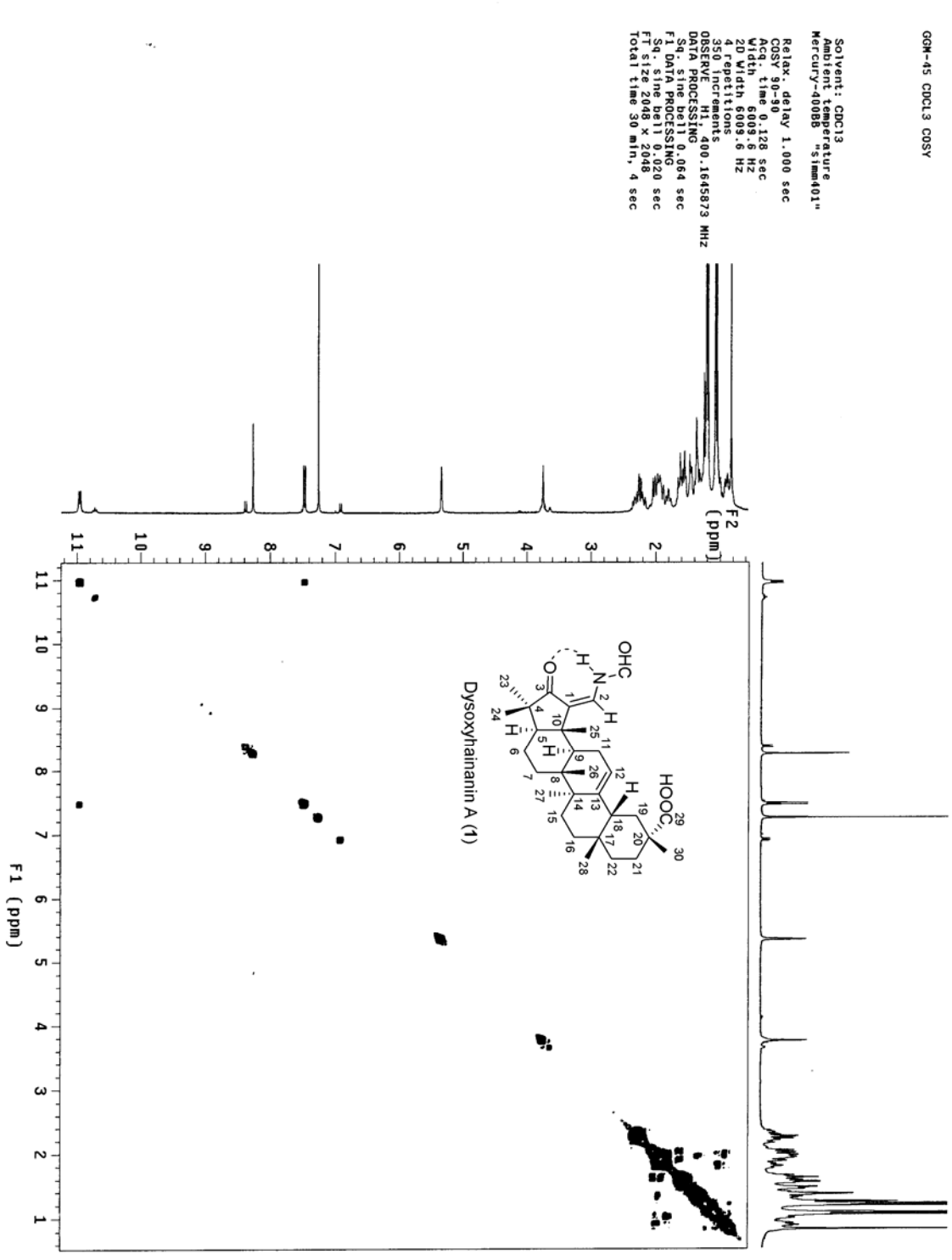
S6. HSQC spectrum (400 MHz) of Dysoxyhainanin A (1) in $CDCl_3$.



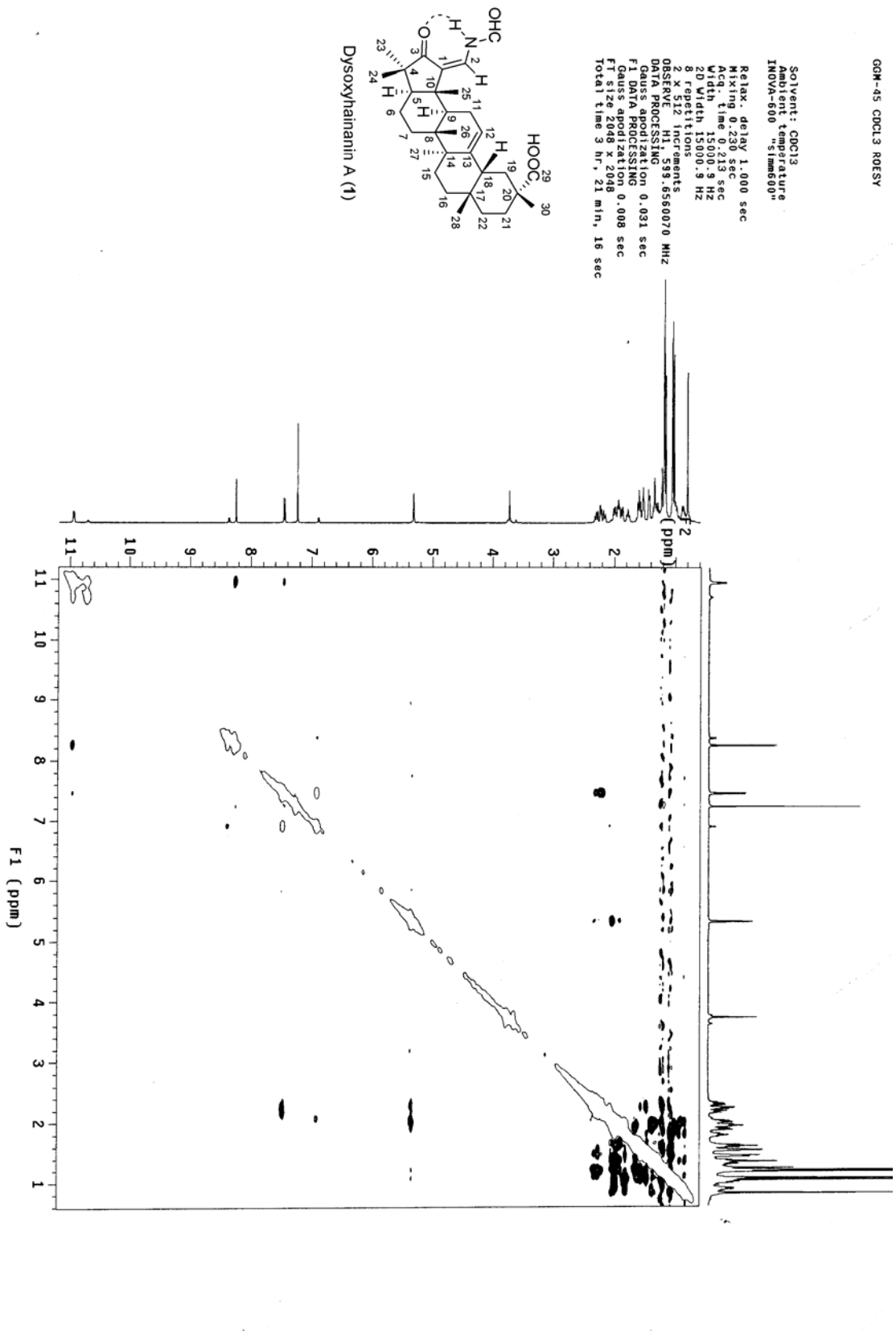
S7. HMBC spectrum (400 MHz) of Dysoxyhainanin A (1) in $CDCl_3$.



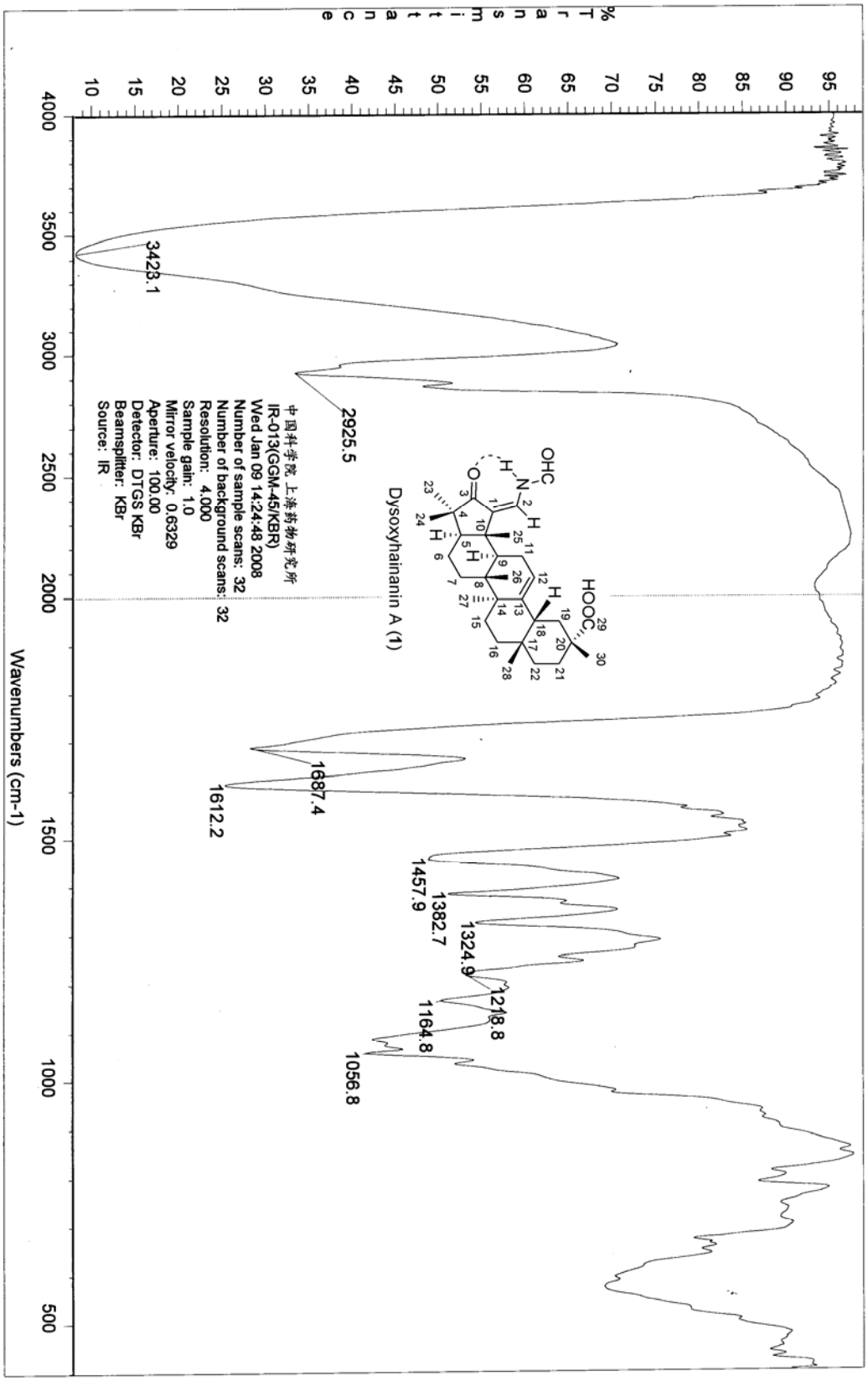
S8. ^{1}H-^{1}H COSY spectrum (400 MHz) of Dysoxyhainanin A (1) in $CDCl_3$.



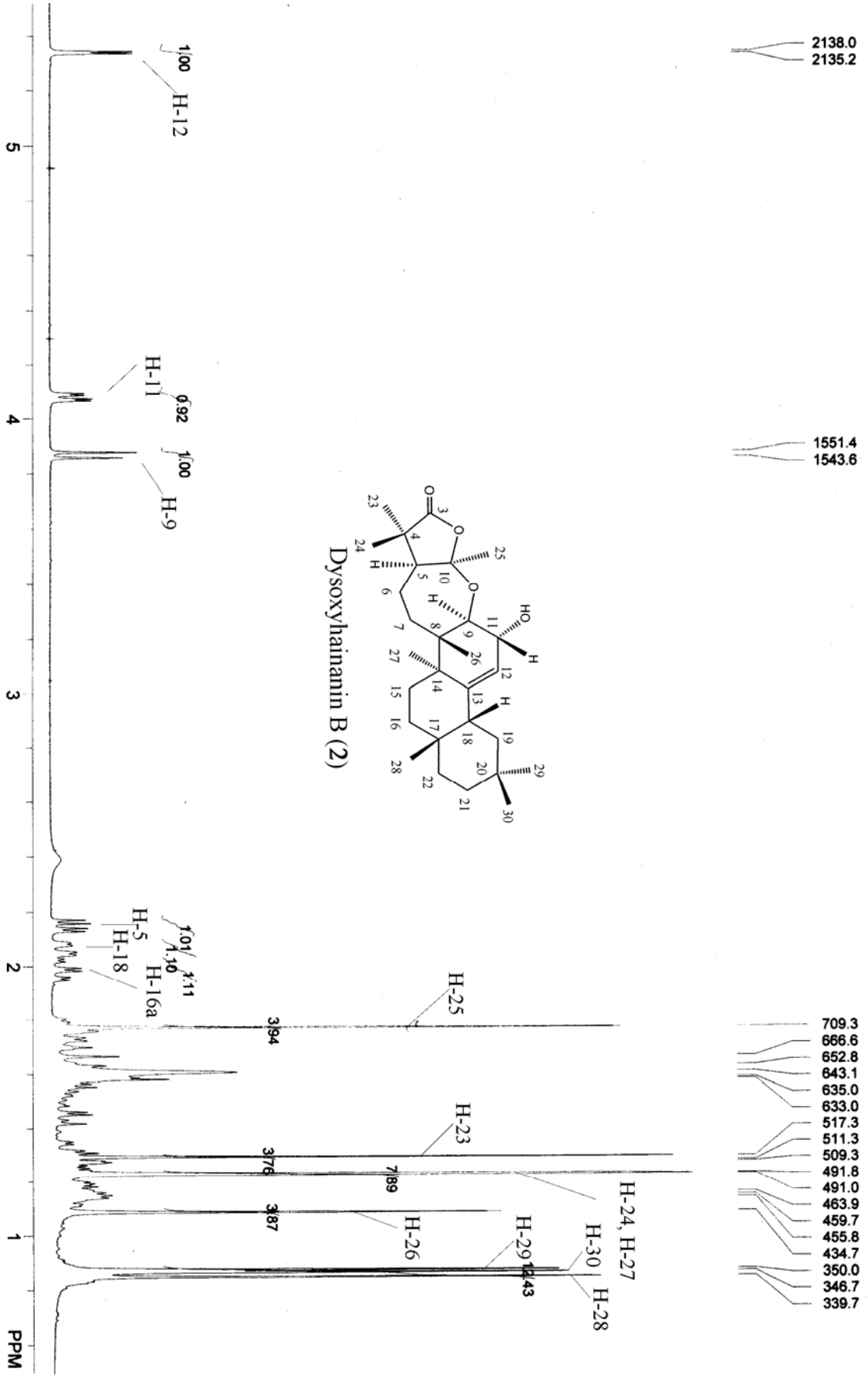
S9. ROESY spectrum (600 MHz) of Dysoxyhainanin A (1) in $CDCl_3$.



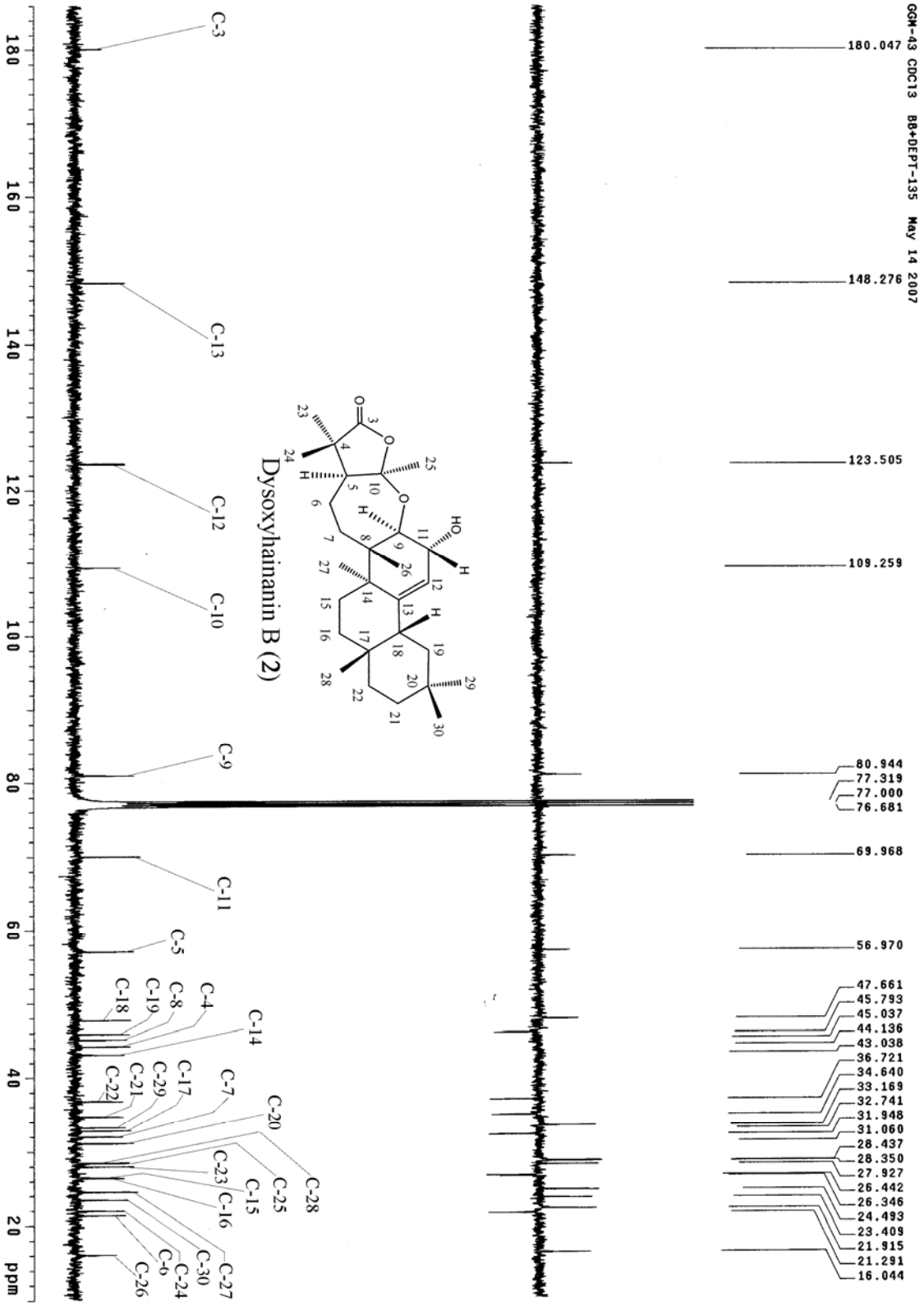
S10. IR (KBr disc) spectrum of Dysoxyhainanin A (1).



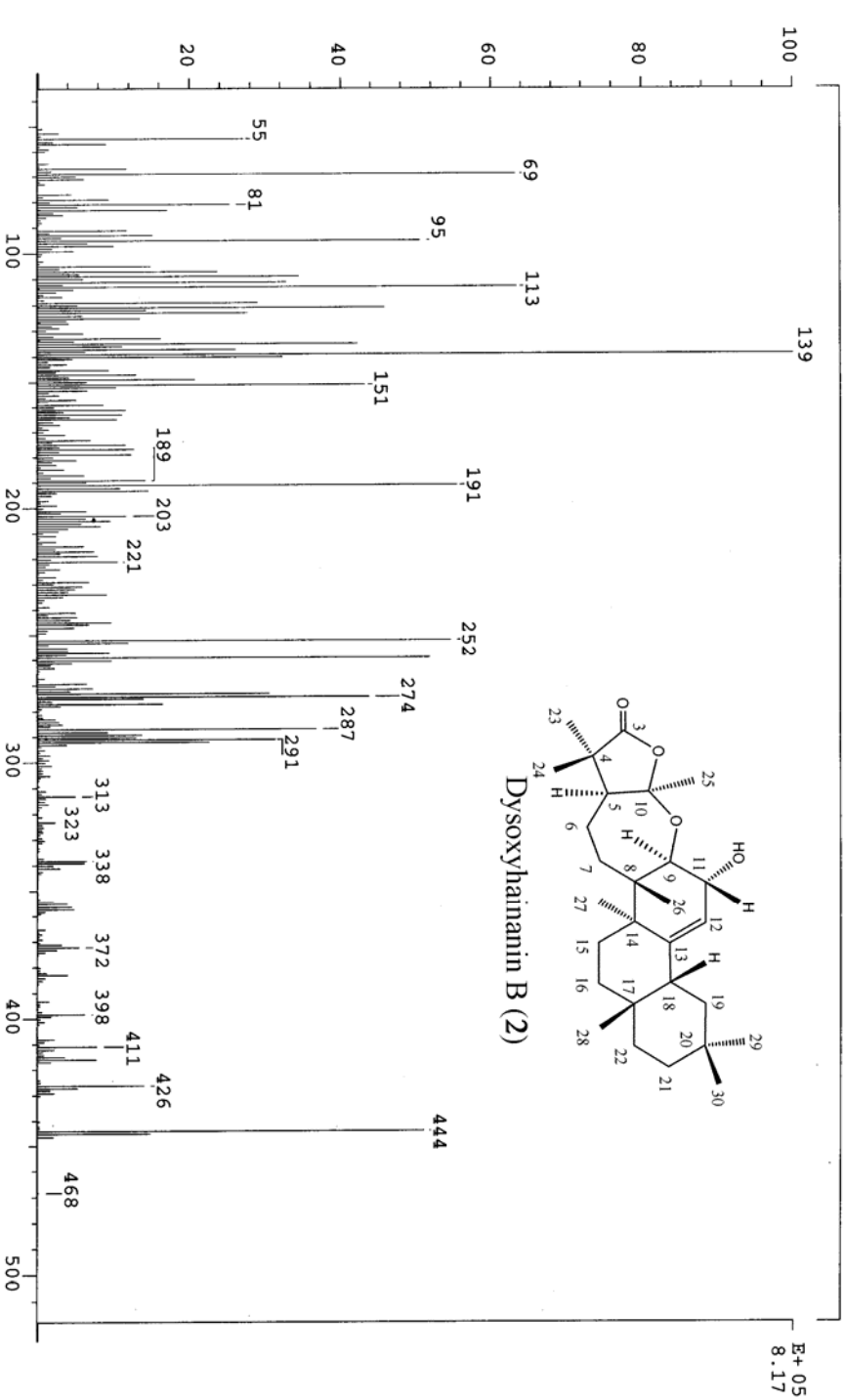
S11. ^{1}H NMR spectrum (400 MHz) of Dysoxyhainanin B (2) in $CDCl_3$.



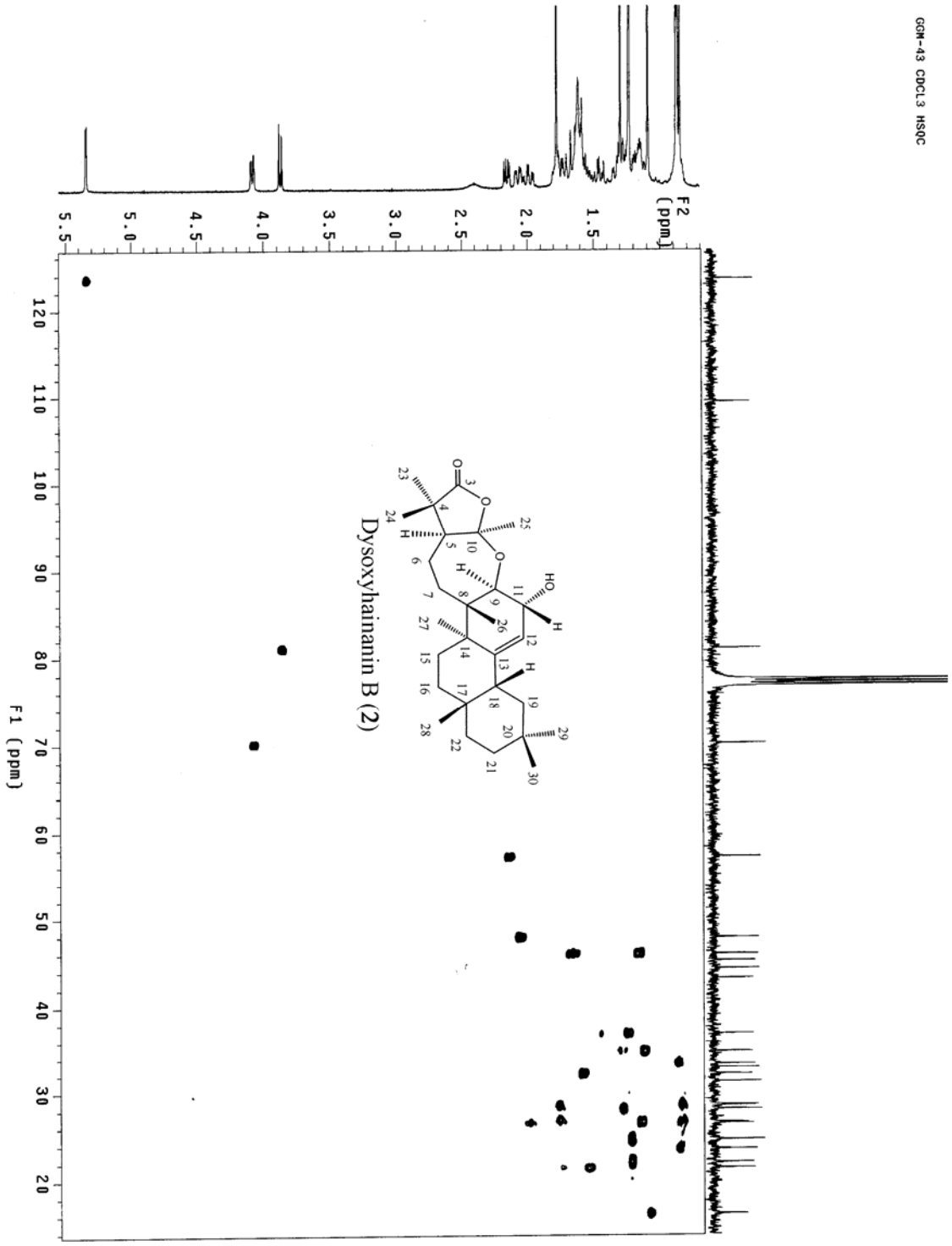
S12. ^{13}C NMR spectrum (100 MHz) of Dysoxyhainanin B (2) in $CDCl_3$.



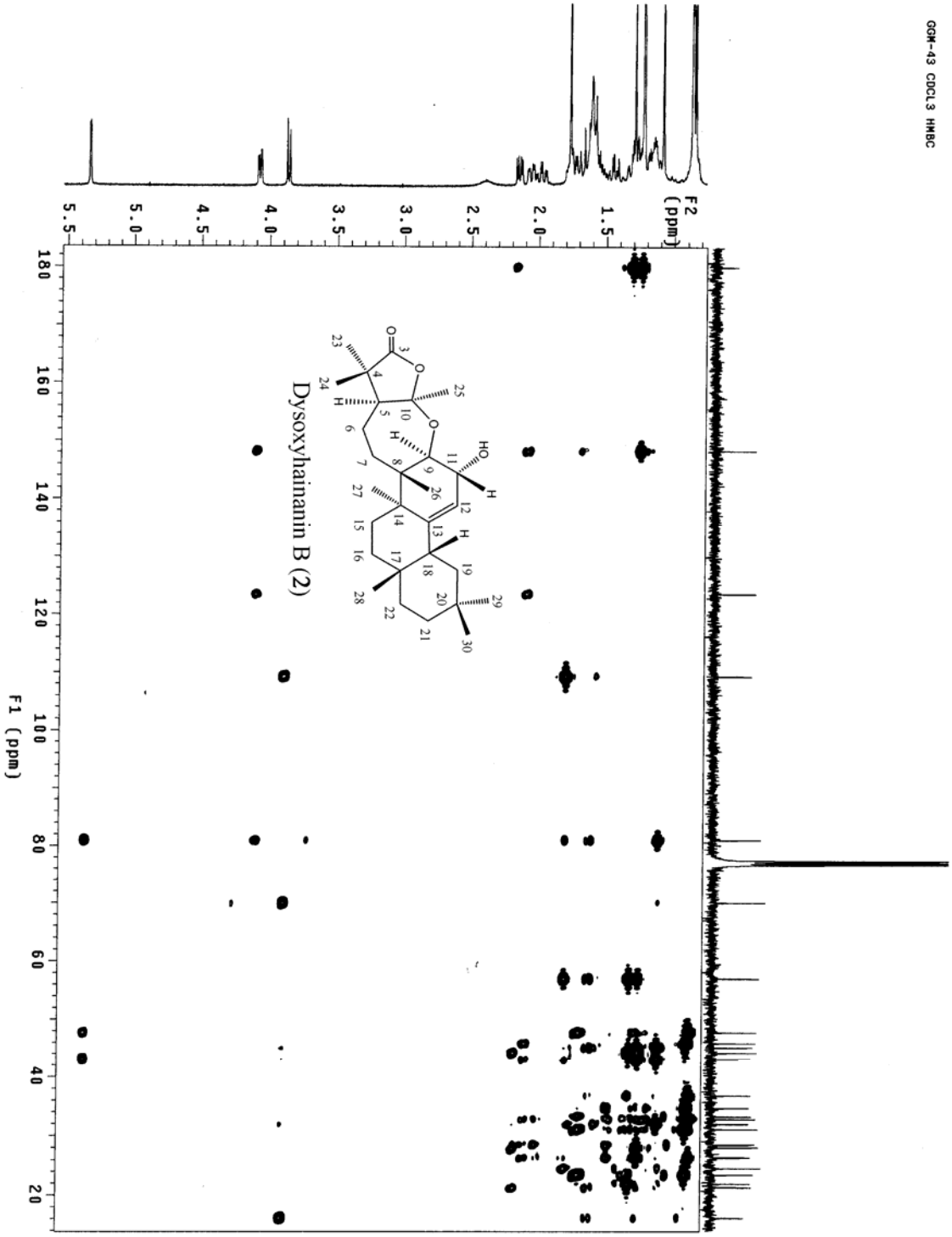
S13. EIMS spectrum of Dysoxyhainanin B (2).



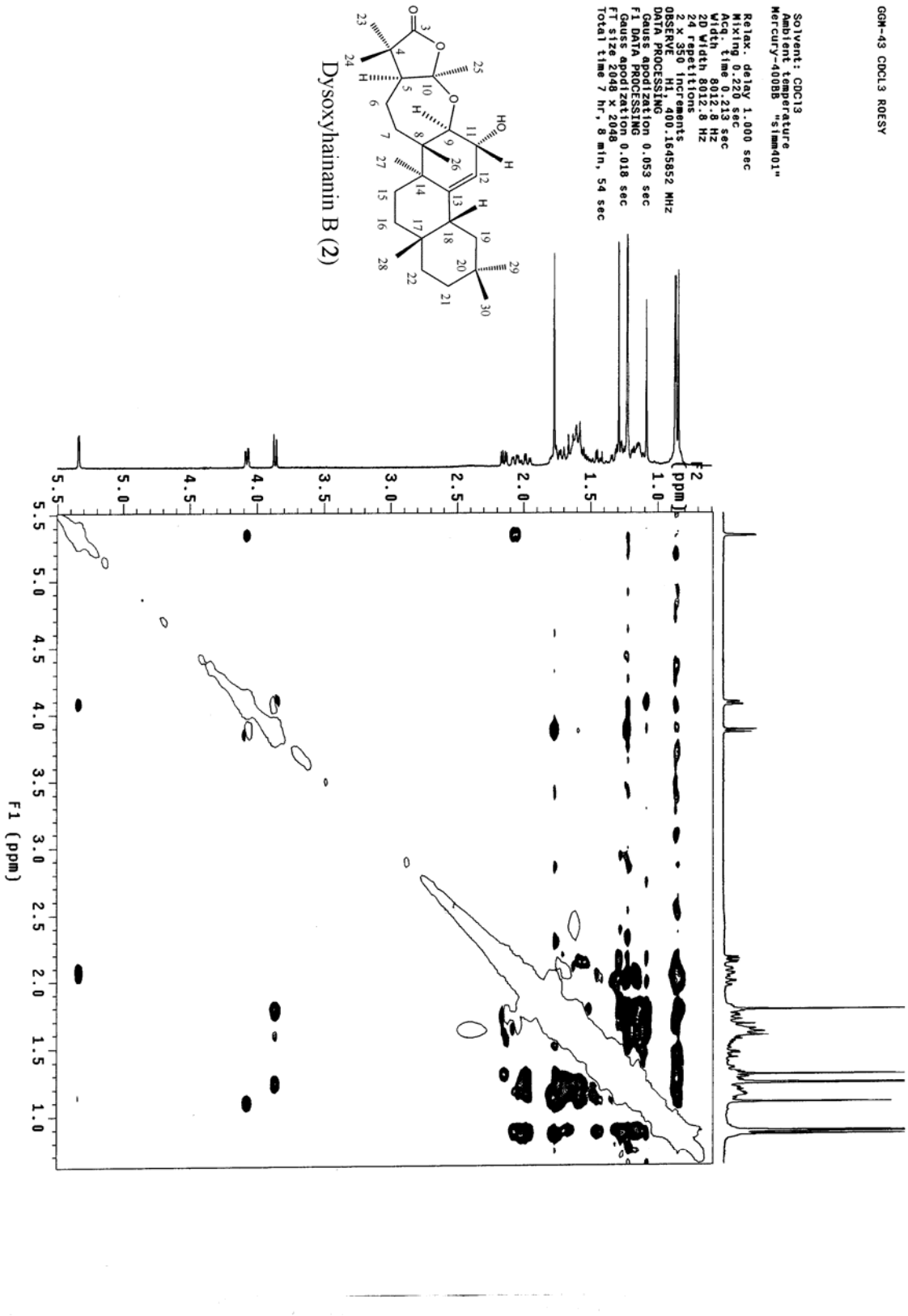
S14. HSQC spectrum (400 MHz) of Dysoxyhainanin B (2) in $CDCl_3$.



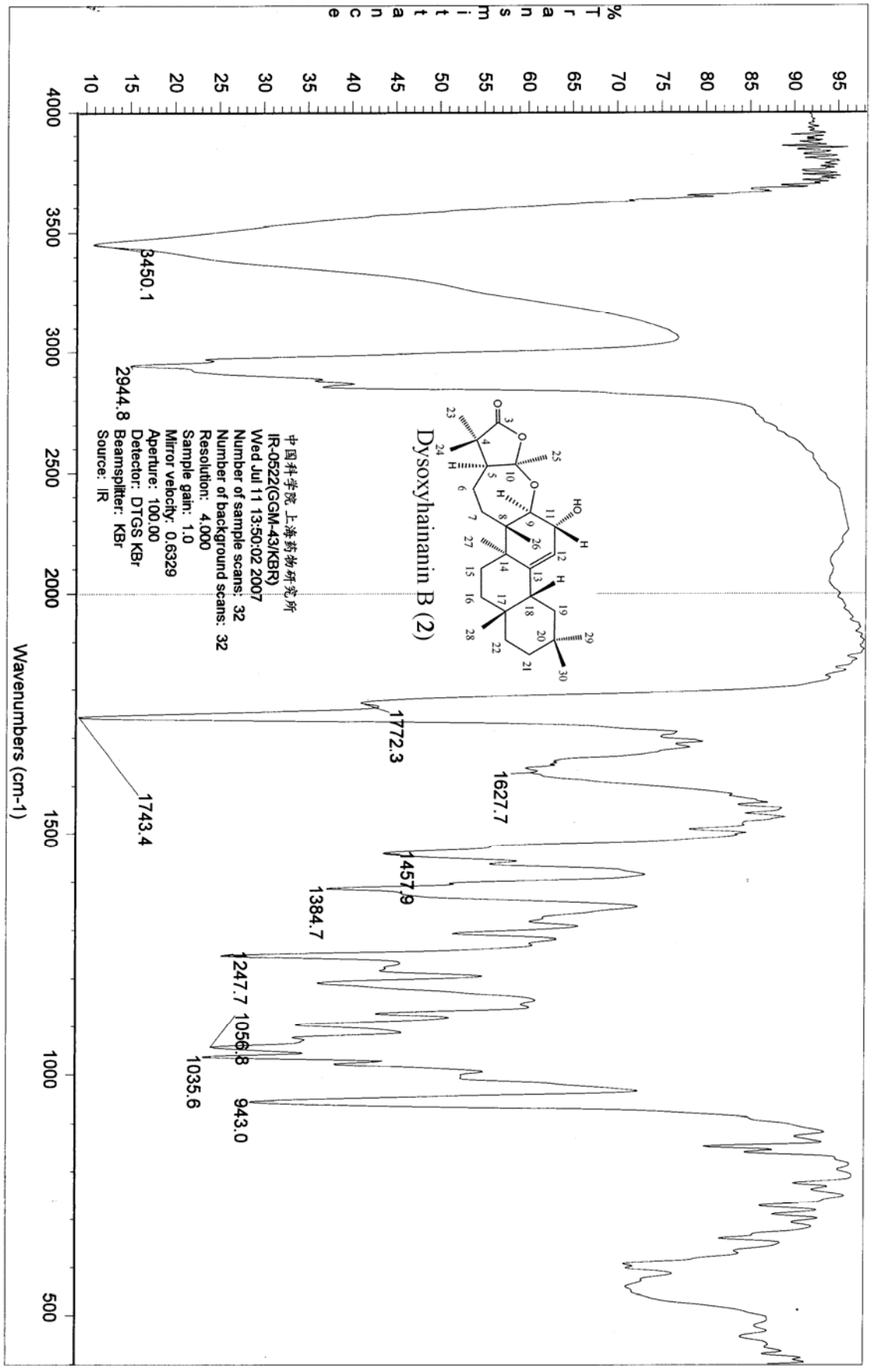
S15. HMBC spectrum (400 MHz) of Dysoxyhainanin B (2) in $CDCl_3$.



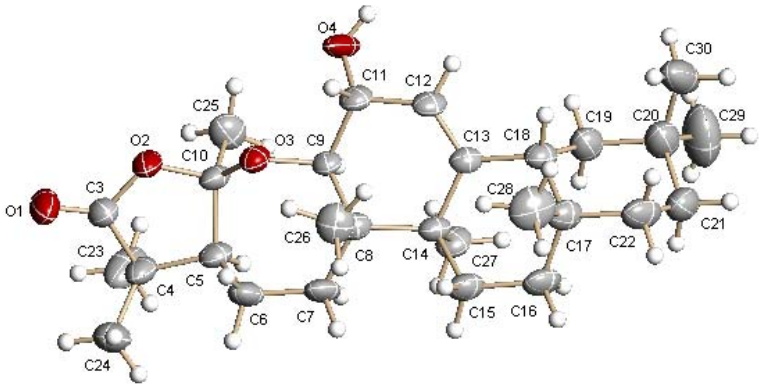
S16. ROESY spectrum (400 MHz) of Dysoxyhainanin B (2) in $CDCl_3$



S17. IR (KBr disc) spectrum of Dysoxyhainanin B (2).



S18. X-ray Structure of Dysoxyhainanin B (2).



S19. Crystal Packing (Stacking) of 2 viewed along the a-axis.