The Aignopsanes, A New Class of Sesquiterpenes from Selected Chemotypes of the Sponge *Cacospongia mycofijiensis*

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[Supporting Information]

[Contents]

General Experimental Procedures.

Optical rotation and CD measurements were obtained on a digital polarimeter and CD spectrophotometer. The NMR spectra were recorded in $CDCl_3$ on 500 and 600 spectrometers operating at 500 and 600 MHz for ^1H and 125.6 and 150.0 MHz for ^{13}C, respectively. Semi preparative HPLC was performed using a 5 μm C_{18} ODS column using an evaporative light scattering detector (ELSD) for compound detection. High resolution mass measurements were obtained from a ESI-TOF mass spectrometer.

Biological Material, Collection and Identification.

Specimens of *Cacospongia mycofijiensis*, 0.8 kg and 0.9 kg (coll. no. 07327 and 07327A-O), were collected using scuba in 2007 off the coast of New Britain (Kimbe Bay), Papua New Guinea at depths of 30-40 m. Complete taxonomic identification of coll. no. 07327A was done by Nicole J. de Voogd of The National Museum of Natural History, The Netherlands. Voucher specimens and underwater photos are available upon request.

Extraction and Isolation. Samples were preserved in the field according to our standard laboratory procedures[6] and stored in a cold room at +4 ˚C until extraction was performed. Initially, a 0.8 kg bottle of pooled specimens of *C. mycofijiensis* was processed by traditional extraction using a modified Kupchan partition scheme[6] and profiled using LCMS-ELSD. (see Chart S1, Supporting information) In effort to further probe for additional new chemotypes, 10g samples of 15 individual sponges (coll. no 07327A – O, 0.9 kg) were rapidly extracted with hexanes (XFH), dichloromethane (XFD) and methanol (XFM) using a pressurized solvent extraction system under high pressure (1700 psi) at 110 ºC. The XFD fractions were immediately subjected to LCMS-ELSD analysis and profiled for any unidentified masses. Samples 07327A and G displayed a series of previously unreported low molecular masses. (Chart S2, in Supporting Information). A second scale up extraction was then performed on the remaining sample of the sponge coll. no. 07327A (138 g) using the aforementioned modified Kupchan extraction scheme. A 33 mg portion of the 07327A dichloromethane extract (FD) was then fractionated using repeated semi preparative reversed-phase isocratic HPLC with an evaporative light scattering detector (ELSD) under conditions of 70% CH_3CN:H_2O for 30 minutes to give nine fractions. Fraction H4 (6.3 mg) was the source of **1**, while H5 (1.6 mg) afforded **3**, and H7 (1.4 mg) yielded pure **2**. The remaining fractions H2 (1.1 mg), H6 (2.3 mg) and H8 (1.9 mg) yielded latrunculol A,[5] fijianolide B[3] and latrunculin A,[5] respectively. (see Chart S3, and the physical properties section in the Supporting Information)

***Trypanosoma brucei brucei* Assay**

Trypanosoma brucei brucei strain 221 was grown in complete HMI-9 medium containing 10% FBS, 10% Serum Plus medium and 1X penicillin/streptomycin. The trypanosomes were diluted to $1x10^5$ per ml in complete HMI-9 medium. 95 µL per well of the diluted trypanosomes was added to sterile Greiner 96-well flat white opaque culture plates that contained 5 µL of test samples (in 10% DMSO). Control wells contained 95 µL of the diluted trypanosomes and 5 µL of 10% DMSO while control wells for 100% inhibition contained 95 µL of the diluted trypanosomes and 5 µL of 1mM Thimerosal (in 10% DMSO). Trypanosomes were incubated with test samples for 48 h at 37°C with 5% CO2 before monitoring viability. Trypanosomes were then lysed in the wells by adding 50 µl of CellTiter-GloTM. Lysed trypanosomes were placed on an orbital shaker at room temperature for 2 min. The resulting ATP-bioluminescence of the trypanosomes in the 96-well plates was measured at room temperature using an Analyst HT plate reader. All IC_{50} curve fittings were performed with Prism 4 software.

Aignopsanoic acid A (1): white amorphous powder and colorless crystal; UV (MeOH) λ_{max} 224 nm (ε 7231). $[\alpha]^{23}_D$ 42.0 (*c* 0.14, MeOH); ^1H and ^{13}C NMR data in Table 1 and Tables S1 and Figures S1-S9 in Supporting Information. LRESITOFMS *m/z* 273.1 $[M+Na]^+$, 251.1 $[M+H]^+$, 233.1 $[M-H_2O+H]^+$, HRESITOFMS *m/z* 273.15606 $[M+Na]^+$ (calc'd for $C_{15}H_{22}O_3Na$, 273.15102). For further information regarding the crystallization method of **1** see Methods and X-ray Crystallography below.

Methyl aignopsonoate A (2): white amorphous powder; UV (MeOH) λ_{max} 222 nm (ε 7369). $[\alpha]^{23}_{D}$ -60.4 (*c* 0.09, MeOH); ^{1}H and ^{13}C NMR data in Table 1 and Table S2 and Figures S10-S16 in Supporting Information. LRESITOFMS *m/z* 287.1 $[M+Na]^+$, 265.1 $[M+H]^+$, 233.1 $[M\text{-}MeOH+H]^+$ HRESITOFMS *m/z* 287.16271 $[M+Na]^+$ (calc'd for $C_{16}H_{24}O_3Na$ 287.16177).

Isoaignopsanoic acid A (3): white amorphous powder; UV (MeOH) λ_{max} 224 nm (ε 7308). $[\alpha]^{23}_{D}$ -45.0 (*c* 0.08, MeOH); ^{1}H and ^{13}C NMR data in Table 1 and Table S3 and Figures S17-S23 in Supporting Information. LRESITOFMS *m/z* 273.1 $[M+Na]^+$, 255.1 $[M\text{-}H_2O+Na]^+$, 233.1 $[M\text{-}H_2O+H]^+$**,** HRESITOFMS *m/z* 273.15443 $[M + Na]^+$ (calc'd for $C_{15}H_{22}O_3Na$, 273.15361).

Latrunculol A: white powder; LRESITOFMS *m/z* 420 $[M\text{-}H_2O+H]^+$; LCMS-ELSD,^{1}H and ^{13}C NMR ($CDCl_3$) data were in agreement with literature values.[5]

Fijianolide B: white powder; LRESITOFMS *m/z* 515 $[M+H]^+$; LCMS-ELSD, ^{1}H and ^{13}C NMR (C_6D_6) data were in agreement with literature values.[3]

Latrunculin A: yellow oil; LRESITOFMS *m/z* 404 $[M\text{-}H_2O+H]^+$; LCMS-ELSD, ^{1}H and ^{13}C NMR ($CDCl_3$) data were in agreement with literature values.[5]

Crystallization method of Aignopsanoic acid A (1). Crystals suitable for X-ray analysis of **1** were obtained by slow evaporation in 70:30 CH_3CN, H_2O over 7 days. The sample crystallizes as colorless plate-like crystals (mp 110 -115 ºC). Crystallographic data of **1** has been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 722754). Copies of these data can be obtained free of charge via www.ccdc.cam.an.uk/conts/retrieving.html.

X-ray crystallography of 1.

The compound crystallizes as colorless plate-like crystals. There are two molecules of the compound and associated waters of crystallization in the unit cell of the primitive, acentric, monoclinic space group $P2_1$. The stereochemistry was assigned based on the relative configurations at C4, C5 and C10.

The compound consists of a cyclohexane ring fused with a cyclohexanone ring. Methyl groups are located in the 4, 5 and 10 positions around the bicyclic system. An ethylene carboxylic acid group is located at the 8 position and the ketone functionality, from the cyclohexanone ring mentioned above, is at the 9 position.

The carboxylic acid group H-bonds to the water of crystallization within the lattice (O2...O10 distance = 2.644 Å, see Table of Hydrogen-bonds). The water in turn, forms H-bonds to the ketone oxygen, O1 of a nearby molecule and to the carbonyl oxygen, O3, of another, different nearby carboxylic acid. These H-bonds are related by the crystallographic 2_1 screw-axis parallel to the *b*-axis (see Figures). Thus, there are one-dimensional chains of H-bonded molecules of the bicycle and water that run through the lattice parallel to the *b*-axis. The carboxylic acid and water hydrogens were located from a difference Fourier map and included in their observed positions.
The bond distances and angles within the compound are otherwise as expected.

Samples for synchrotron crystallographic analysis were submitted through the SCrALS (Service Crystallography at Advanced Light Source) program. Crystallographic data were collected at Beamline 11.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The ALS is supported by the U.S. Dept. of Energy, Office of Energy Sciences, under contract DE-AC02-05CH11231..

Data Collection

A fragment of a colorless plate-like crystal of $C_{15}H_{24}O_4$ having approximate dimensions of 0.17 × 0.15 × 0.03 mm was mounted on a Kapton loop using Paratone N hydrocarbon oil. All measurements were made on a Bruker APEX-II[1] CCD area detector with channel-cut Si-<111> crystal monochromated synchrotron radiation.

Cell constants and an orientation matrix, obtained from a least-squares refinement using the measured positions of 4004 centered reflections with $I > 10\sigma(I)$ in the range $2.90 < \theta < 30.96°$ corresponded to a Monoclinic cell with dimensions:

a = 7.2266(5) Å	α = 90°
b = 6.4086(4) Å	β = 96.597(1)°
c = 15.4198(10) Å	γ = 90°
V = 709.40(8) Å^3	

For Z = 2 and F.W. = 268.34, the calculated density is 1.256 g.cm^{-3}.

Analysis of the systematic absences allowed the space group to be uniquely determined to be:

$P2_1$

The data were collected at a temperature of 150(2) K. Frames corresponding to an arbitrary sphere of data were collected using ω-scans of 0.3° counted for a total of 5 seconds per frame.

Data Reduction

Data were integrated by the program SAINT[2] to a maximum θ-value of 31.19°. The data were corrected for Lorentz and polarization effects. Data were analyzed for agreement and possible absorption using XPREP[3]. An empirical absorption correction based on comparison of redundant and equivalent reflections was applied using SADABS[4]. (Tmax = 0.9968, Tmin = 0.9820). Of the 9141 reflections that were collected, 3527 were unique (R_{int} = 0.0253); equivalent reflections were merged. No decay correction was applied.

Structure Solution and Refinement

The structure was solved by direct methods[5] and expanded using Fourier techniques[6]. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in calculated positions but were not refined. The final cycle of full-matrix least-squares refinement[7] was based on 3527 reflections (all data) and 184 variable parameters and converged (largest parameter shift was 0.000 times its esd) with conventional unweighted and weighted agreement factors of:

$$R_1 = \Sigma||Fo| - |Fc|| / \Sigma|Fo| = 0.0569 \text{ for 3337 data with } I > 2\sigma(I)$$

$$wR_2 = [(\Sigma w (|Fo|^2 - |Fc|^2)^2 / \Sigma w |Fo|^2)]^{1/2} = 0.1699$$

The standard deviation of an observation of unit weight[8] was 1.167. The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.371 and -0.234 e$^-$.Å^3, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[9]. Anomalous dispersion effects were included in Fcalc[2]; the values for Δf' and Δf" were those of Creagh and McAuley[10]. The values for the mass attenuation coefficients are those of Creagh and Hubbel[11]. All calculations were performed using the SHELXTL[1-6] crystallographic software package of Bruker Analytical X-ray Systems Inc.

References

(1)APEX-II: Area-Detector Software Package v2.1, Bruker Analytical X-ray Systems, Inc.: Madison, WI, (2006)

(2)SAINT: SAX Area-Dectector Integration Program, 7.34A; Siemens Industrial Automation, Inc.: Madison, WI, (2006)

(3)XPREP:(v 6.14) Part of the SHELXTL Crystal Structure Determination Package, Siemens Industrial Automation, Inc.: Madison, WI, (1995)

(4)SADABS: Siemens Area Detector ABSorption correction program v.2.10, George Sheldrick, (2005).

(5) XS: Program for the Solution of X-ray Crystal Structures, Part of the SHELXTL Crystal Structure Determination Package, Bruker Analytical X-ray Systems Inc.: Madison, WI, (1995-99)

(6) XL: Program for the Refinement of X-ray Crystal Structure Part of the SHELXTL Crystal Structure Determination Package, Bruker Analytical X-ray Systems Inc.: Madison, WI, (1995-99)

(7) Least-Squares:

Function minimized: $\Sigma w (|Fo|^2 - |Fc|^2)^2$

(8) Standard deviation of an observation of unit weight:

$[\Sigma w(|Fo|^2 - |Fc|^2)^2/(N_o - N_v)]^{1/2}$
where: N_o = number of observations
N_v = number of variables

(9) Cromer, D. T. & Waber, J. T.; "International Tables for X-ray Crystallography", Vol. IV, The Kynoch Press, Birmingham, England, Table 2.2 A (1974).

(10) Creagh, D. C. & McAuley, W. J.; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.6.8, pages 219-222 (1992).

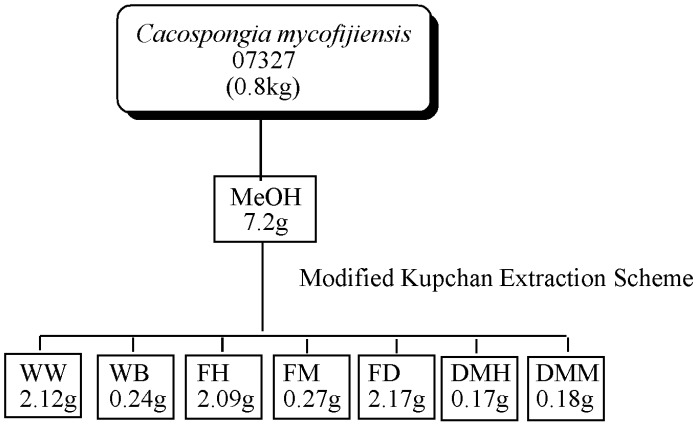
(11) Creagh, D. C. & Hubbell, J.H..; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.4.3, pages 200-206 (1992).

Crystal data for $C_{15}H_{24}O_4$; M_r = 268.34; Monoclinic; space group $P2_1$; a = 7.2266(5) Å; b = 6.4086(4) Å; c = 15.4198(10) Å; α = 90°; β = 96.5970(10)°; γ = 90°; V = 709.40(8) $Å^3$; Z = 2; T = 150(2) K; λ(synchrotron) = 0.77490 Å; μ(synchrotron) = 0.107 mm^{-1}; d_{calc} = 1.256g.cm^{-3}; 9141 reflections collected; 3527 unique (R_{int} = 0.0253); giving R_1 = 0.0569, wR_2 = 0.1699 for 3337 data with [I>2σ(I)] and R_1 = 0.0597, wR_2 = 0.1717 for all 3527 data. Residual electron density (e^-.$Å^{-3}$) max/min: 0.371/-0.234. An

arbitrary sphere of data were collected on a colorless plate-like crystal, having approximate dimensions of 0.17 × 0.15 × 0.03 mm, on a Bruker APEX-II diffractometer using a combination of ω- and φ-scans of 0.3°. Data were corrected for absorption and polarization effects and analyzed for space group determination. The structure was solved by direct methods and expanded routinely. The model was refined by full-matrix least-squares analysis of F^2 against all reflections. All non-hydrogen atoms were refined with anisotropic thermal displacement parameters. Unless otherwise noted, hydrogen atoms were included in calculated positions. Thermal parameters for the hydrogens were tied to the isotropic thermal parameter of the atom to which they are bonded (1.5 X for methyl, 1.2 for all others).

Samples for synchrotron crystallographic analysis were submitted through the SCrALS (Service Crystallography at Advanced Light Source) program. Crystallographic data were collected at Beamline 11.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The ALS is supported by the U.S. Dept. of Energy, Office of Energy Sciences, under contract DE-AC02-05CH11231..

Chart S1. Modified Kupchan extraction scheme and ELSD analysis of Coll. No. 07327 FD fraction with annotations including *m/z* ions.



RP LCMS: 10:90→100% CH_3CN:H_2O w/ 0.1% formic acid (50min)

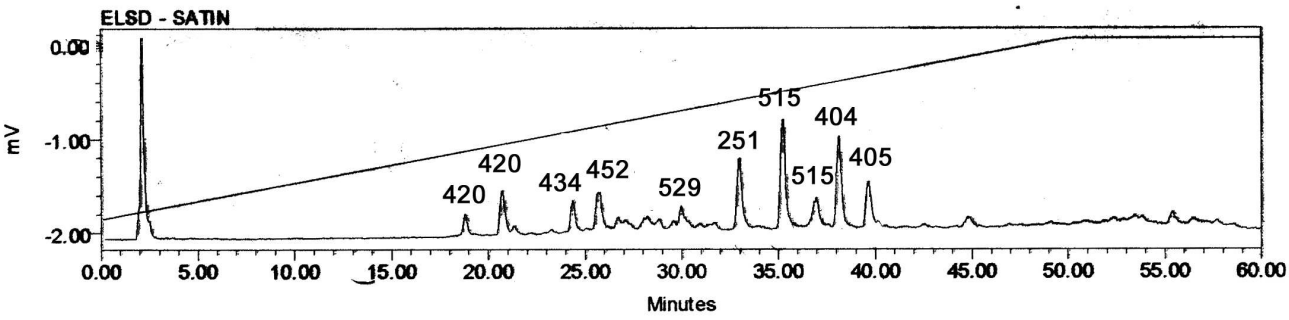
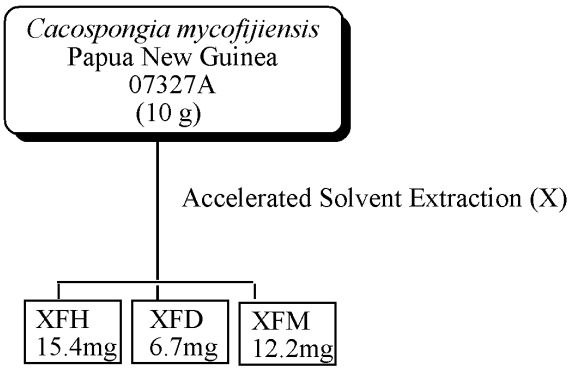
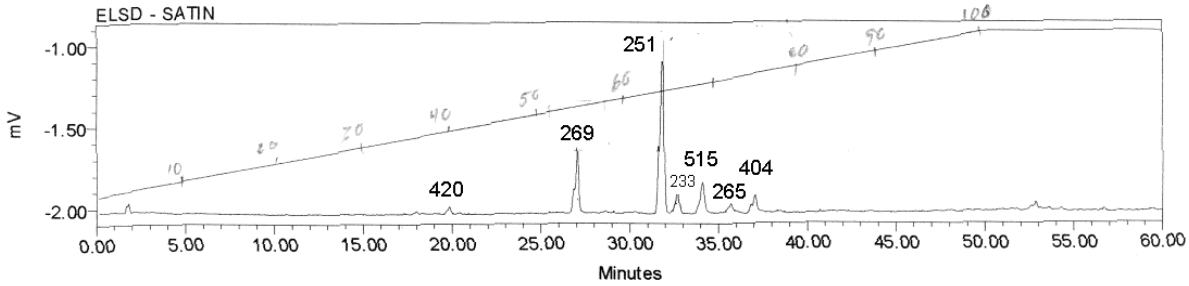


Chart S2. Accelerated solvent extraction scheme and ELSD analysis of Coll. No. 07327A XFD (**a**) and 07327G (**b**) XFD fractions with annotations including *m/z* ions.

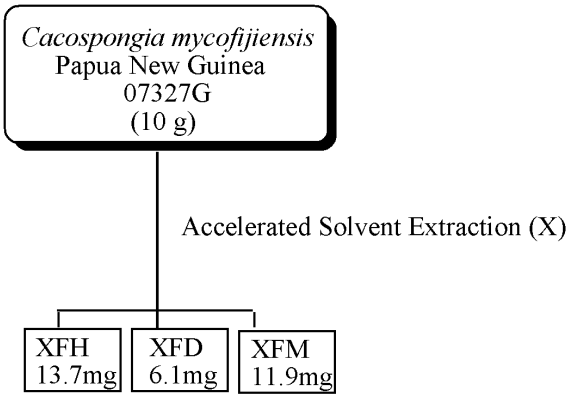
(**a**)



RP LCMS: 10:90→100% CH_3CN:H_2O w/ 0.1% formic acid (50min)



(**b**)



RP LCMS: 10:90→100% CH_3CN:H_2O w/ 0.1% formic acid (50min)

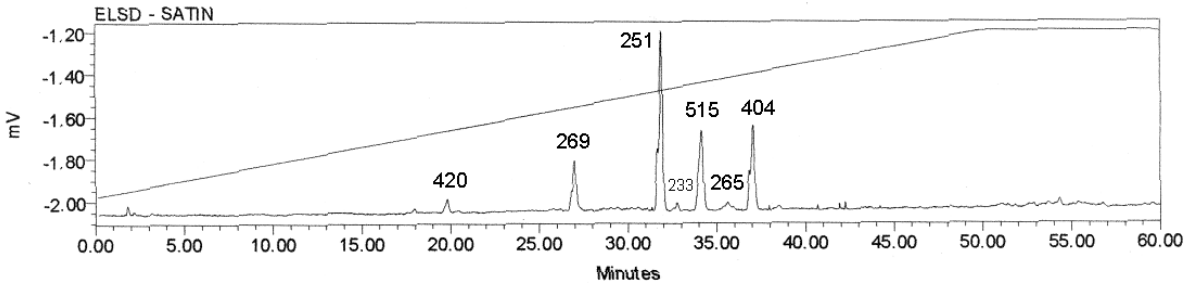
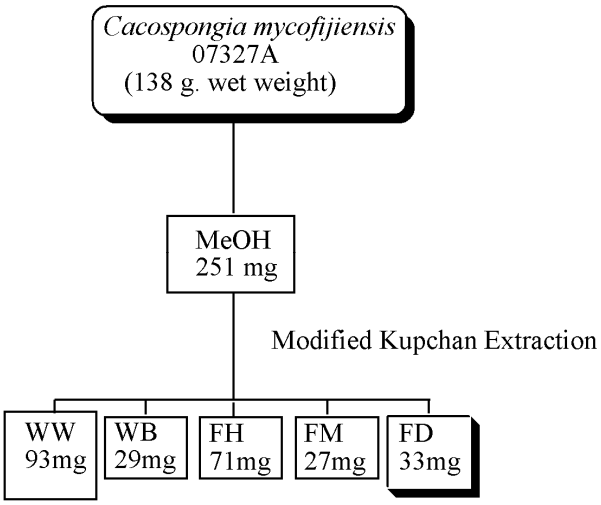


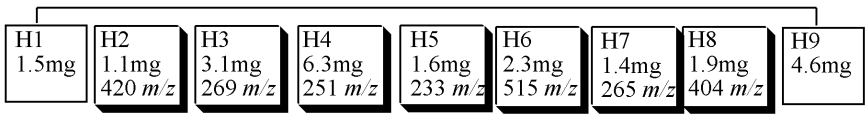
Chart S3. Modified Kupchan extraction scheme (**a**), HPLC purification (**b**) and LCMS-ELSD analysis of Coll. No. 07327A FD fraction with annotations including *m/z* ions (**c**).

(**a**)



(**b**)

RP Isocratic Semi Preparative HPLC: 70% CH_3CN/H_2O, ([3mg/100ul] X 11)
Monitored using evaporative light scattering detection (ELSD)



(**c**) RP LCMS: 10:90→100% $CH_3CN:H_2O$ w/ 0.1% formic acid (50min)

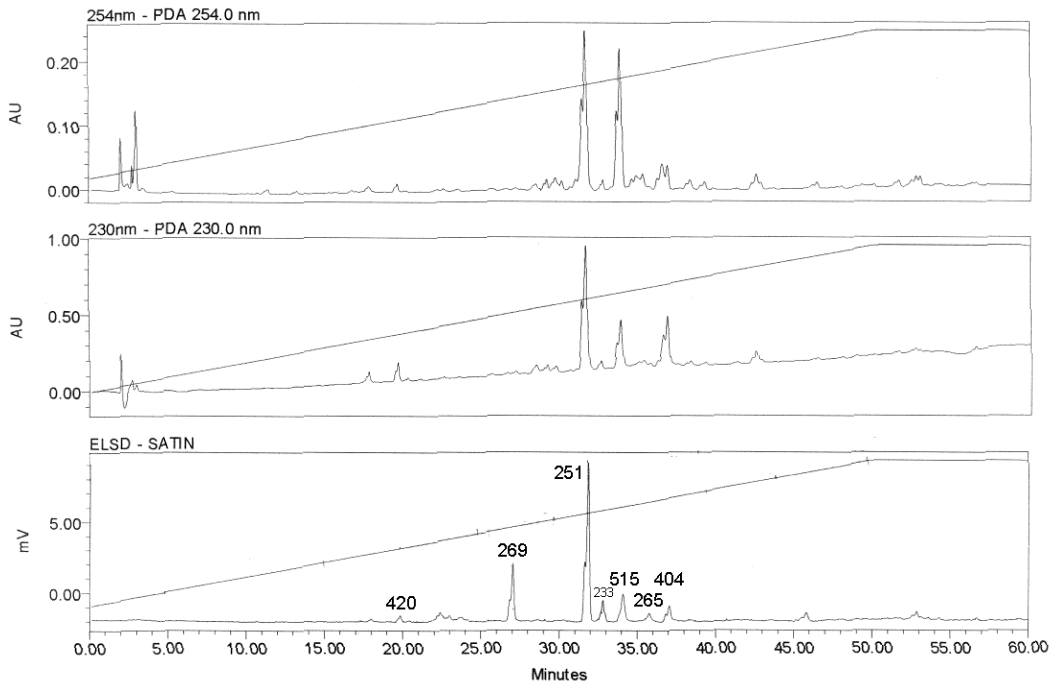


Table S1. ^{1}H, ^{13}C, COSY and HMBC NMR[a] data of **1** in $CDCl_3$

	1					
Position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC	NOESY
1ax	30.2	CH_2	1.40 (td, 13.8, 4.2)		2, 3, 5, 9, 10	
1eq			2.05 (bddd, 13.2, 4.8, 2.4)			
2ax	22.3	CH_2	1.60 (m)[c]			
2eq			1.60 (m)[c]			
3ax	30.0	CH_2	1.40 (m)[c]			13, 14
3eq			1.43 (m)			
4	35.2	CH	1.49 (m)	13	10	7ax
5	42.0	C				
6ax	30.2	CH_2	1.85 (td, 14.4, 4.8)	7ax, 7eq	4, 5, 7, 8, 10, 14	15
6eq			1.78 (ddd, 14.4, 6.0, 2.4)	7ax	4, 5, 7, 8, 10, 14	
7ax	31.0	CH_2	2.70 (dddd, 15.6, 14.4, 6.0, 3.0)	6ax, 11	6, 8, 9, 11	4, 11, 13
7eq			2.44 (dddd, 15.6, 4.8, 2.4, 0.6)	6ax	5, 6, 8, 9, 11	11
8	149.3	C				
9	211.5	C				
10	55.4	C				
11	126.1	CH	5.95 (dd, 3.0, 0.6)	7ax, 7eq	7, 8, 9, 12	7ax, 7eq
12	165.6	C				
13	15.8	CH_3	0.82 (d, 7.2)	4	3, 4, 5	3ax, 7ax
14	15.5	CH_3	0.89 (s)		4, 5, 10	3ax
15	22.2	CH_3	1.06 (s)		1, 5, 9, 10	6ax

[a]Measured at 600 MHz (^{1}H) and 125 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. [c] Partially overlapped by other signals. [*]Not detected.

Table S2. ^{1}H, ^{13}C, COSY and HMBC NMR[a] data of **2** in $CDCl_3$

	2				
position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC
1ax	30.2	CH_2	1.37 (td, 13.8, 4.8)		2, 9, 10
1eq			1.98 (ddd, 13.8, 3.6, 1.2)		2, 3, 5, 10
2ax	22.2	CH_2	1.50 (m)		
2eq			1.72 (m)		
3ax	30.2	CH_2	1.40 (m)		
3eq			1.30 (m)		
4	34.2	CH	1.55 (m)	13	
5	42.5	C			
6ax	31.1	CH_2	1.84 (td, 14.4., 4.8)		4, 5, 7, 8
6eq			1.78 (ddd, 14.4, 5.4, 2.4)	7eq	5, 7, 8, 10
7ax	30.5	CH_2	2.63 (dddd, 15.0, 14.4, 6.0, 3.0)		6, 8, 11
7eq			2.40 (dddd, 15.0, 4.8, 2.4, 0.6)	6eq, 11	5, 8, 9, 11
8	152.0	C			
9	207.0	C			
10	54.3	C			
11	121.0	CH	5.75 (dd, 3.0, 0.6)	7ax, 7eq	7, 8, 9, 12
12	166.5	C			
13	15.7	CH_3	0.83 (d, 7.2)	4	3, 4, 5
14	15.3	CH_3	0.91 (s)		4, 5, 6, 10
15	21.2	CH_3	1.12 (s)		1, 5, 9, 10
OMe	51.7	CH_3	3.70 (s)		12

[a]Measured at 600 MHz (^{1}H) and 125 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. *Not detected.

Table S3. ^{1}H, ^{13}C, COSY and HMBC NMR[a] data of **3** in $CDCl_3$

	3				
position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC
1ax	30.4	CH_2	1.40 (m) [c]		9, 10
1eq			2.05 (dt, 13.2, 3.0)		2, 3, 5, 10
2ax	22.5	CH_2	1.50 (m) [c]		
2eq			1.40 (m) [c]		
3ax	30.1	CH_2	1.40 (m) [c]		
3eq			1.30 (m)		
4	35.1	CH	1.50 (m) [c]	13	
5	40.1	C			
6ax	28.4	CH_2	1.81 (td, 14.4, 5.4)	7ax, 7eq	4, 5, 7, 8, 10, 14
6eq			1.74 (ddd, 14.4, 6.6, 1.2)	7ax, 7eq	4, 5, 7, 8, 10, 14
7ax	23.7	CH_2	2.55 (dddd, 18.0, 14.4, 6.6, 3.0)	6ax, 6eq, 11	5, 8, 9, 11
7eq			3.61 (ddt, 18.0, 5.4, 1.2)	6ax, 6eq, 11	6, 8, 11
8	153.6	C			
9	206.2	C			
10	53.6	C			
11	121.1	CH	6.50 (dd, 3.0, 1.2)	7ax, 7eq	7, 8, 9, 12
12	168.9	C			
13	15.6	CH_3	0.87 (d, 6.6)	4	3, 4, 5
14	15.5	CH_3	0.91 (s)		4, 5, 10
15	22.3	CH_3	1.01 (s)		1, 5, 9, 10

[a]Measured at 600 MHz (^{1}H) and 125 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. [c] Partially overlapped by other signals.

Table S4. Crystal data and structure refinement for **1**.

Identification code	XSC08025
Empirical formula	$C_{15}H_{24}O_4$
Formula weight	268.34
Temperature	150(2) K
Wavelength	0.77490 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 7.2266(5) Å α = 90°
b = 6.4086(4) Å	β = 96.5970(10)°
c = 15.4198(10) Å	γ = 90°
Volume	709.40(8) Å^3
Z	2
Density (calculated)	1.256 g.cm^{-3}
Absorption coefficient (μ)	0.107 mm^{-1}
F(000)	292
Crystal size	0.17 × 0.15 × 0.03 mm^3
ω range for data collection	2.90 to 31.19°
Index ranges	$-9 \leq h \leq$, $-8 \leq k \leq 8$, $-20 \leq l \leq 20$
Reflections collected	9141
Independent reflections	3527 [R_{int} = 0.0253]
Completeness to θ = 31.19°	99.3 %
Absorption correction	Empirical
Max. and min. transmission	0.9968 and 0.9820
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3527 / 1 / 184
Goodness-of-fit on F^2	1.167
Final R indices [I>2σ(I)]	R_1 = 0.0569, wR_2 = 0.1699
R indices (all data)	R_1 = 0.0597, wR_2 = 0.1717
Absolute structure parameter	0.0(14)
Largest diff. peak and hole	0.371 and -0.234 e$^-$.Å^{-3}

Table S5. Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for **1**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
O(1)	-0.1487(3)	0.4350(3)	0.21485(12)	0.026(1)
O(2)	-0.2638(3)	0.5730(4)	-0.05124(13)	0.031(1)
O(3)	-0.2908(3)	0.7301(4)	0.07617(14)	0.031(1)
C(1)	-0.0517(4)	0.6779(5)	0.36178(16)	0.025(1)
C(2)	-0.0088(4)	0.4761(5)	0.41180(18)	0.030(1)
C(3)	0.1998(4)	0.4449(5)	0.43384(18)	0.029(1)
C(4)	0.3015(4)	0.4590(4)	0.35216(17)	0.021(1)
C(5)	0.2713(3)	0.6744(4)	0.30526(16)	0.021(1)
C(6)	0.3687(4)	0.6817(5)	0.22086(17)	0.025(1)
C(7)	0.2930(4)	0.5243(5)	0.15128(18)	0.028(1)
C(8)	0.0862(4)	0.5529(4)	0.13044(17)	0.023(1)
C(9)	-0.0179(3)	0.5533(4)	0.20931(16)	0.020(1)
C(10)	0.0533(4)	0.7063(4)	0.28170(16)	0.021(1)
C(11)	0.0017(4)	0.5842(5)	0.04931(17)	0.025(1)
C(12)	-0.1975(4)	0.6361(4)	0.02830(17)	0.024(1)
C(13)	0.5085(4)	0.4009(5)	0.3754(2)	0.028(1)
C(14)	0.3555(4)	0.8526(5)	0.3638(2)	0.029(1)
C(15)	0.0052(4)	0.9251(4)	0.24318(18)	0.027(1)
O(10)	-0.6199(4)	0.6486(5)	-0.1009(2)	0.046(1)
H(2)	-0.364(6)	0.586(8)	-0.056(3)	0.03865
H(1A)	-0.0213	0.7966	0.4020	0.030
H(1B)	-0.1871	0.6832	0.3426	0.030
H(2A)	-0.0615	0.3571	0.3762	0.036
H(2B)	-0.0686	0.4792	0.4664	0.036
H(3A)	0.2233	0.3065	0.4614	0.035
H(3B)	0.2495	0.5525	0.4763	0.035
H(4A)	0.2455	0.3506	0.3104	0.026
H(6A)	0.3549	0.8237	0.1958	0.030
H(6B)	0.5034	0.6555	0.2364	0.030
H(7A)	0.3547	0.5449	0.0978	0.034

H(7B)0.3200	0.3807	0.1728	0.034
H(11A)	0.0750	0.5721	0.0022
H(13A)	0.5177	0.2657	0.4053
H(13B)	0.5703	0.5079	0.4139
H(13C)	0.5692	0.3922	0.3218
H(14A)	0.4884	0.8261	0.3802
H(14B)	0.2919	0.8604	0.4165
H(14C)	0.3397	0.9849	0.3319
H(15A)	-0.1302	0.9383	0.2299
H(15B)	0.0642	0.9435	0.1895
H(15C)	0.0513	1.0320	0.2857
H(10Y)	-0.670(7)	0.741(9)	-0.122(3)
H(10Z)-0.667(7)	0.553(10)	-0.100(3)	0.055

Table S6. Anisotropic displacement parameters $(Å)^2$ for **1**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2} U_{11} + ... + 2 h k a^* b^* U_{12}]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	0.0223(9)	0.0282(10)	0.0264(9)	-0.0020(8)	0.0009(7)	-0.0060(8)
O(2)	0.0285(10)	0.0410(12)	0.0227(9)	-0.0046(9)	-0.0051(8)	0.0016(10)
O(3)	0.0275(10)	0.0349(11)	0.0298(10)	-0.0050(9)	0.0014(8)	0.0044(9)
C(1)	0.0223(12)	0.0337(15)	0.0197(11)	-0.0023(11)	0.0017(9)	0.0026(11)
C(2)	0.0242(13)	0.0433(17)	0.0232(12)	0.0075(12)	0.0024(10)	0.0001(12)
C(3)	0.0254(13)	0.0371(15)	0.0255(12)	0.0069(12)	0.0015(10)	-0.0010(12)
C(4)	0.0184(11)	0.0221(12)	0.0233(11)	0.0002(10)	0.0007(9)	-0.0019(9)
C(5)	0.0184(11)	0.0214(12)	0.0218(11)	0.0016(10)	-0.0008(9)	0.0002(9)
C(6)	0.0183(12)	0.0300(14)	0.0262(12)	0.0005(11)	0.0034(9)	-0.0013(11)
C(7)	0.0211(12)	0.0401(16)	0.0234(12)	-0.0033(12)	0.0016(10)	0.0052(11)
C(8)	0.0212(11)	0.0256(13)	0.0221(11)	-0.0048(10)	0.0029(9)	0.0008(10)
C(9)	0.0180(10)	0.0215(12)	0.0197(11)	0.0001(10)	0.0002(8)	0.0047(10)
C(10)	0.0216(11)	0.0205(12)	0.0189(11)	0.0000(9)	0.0004(9)	0.0001(10)
C(11)	0.0227(12)	0.0296(13)	0.0219(11)	-0.0036(10)	0.0037(9)	0.0038(11)
C(12)	0.0274(13)	0.0233(12)	0.0218(11)	0.0007(10)	-0.0015(10)	-0.0011(10)
C(13)	0.0186(12)	0.0301(14)	0.0343(14)	0.0057(11)	-0.0025(10)	0.0020(11)
C(14)	0.0266(13)	0.0262(14)	0.0307(14)	-0.0040(11)	-0.0052(11)	-0.0019(11)
C(15)	0.0304(13)	0.0222(12)	0.0275(13)	-0.0010(11)	-0.0004(10)	0.0023(11)
O(10)	0.0361(13)	0.0347(13)	0.0618(17)	0.0144(12)	-0.0199(12)	-0.0088(11)

Table S7. Bond lengths [Å] for **1**.

atom-atom	distance	atom-atom	distance
O(1)-C(9)	1.223(3)	O(2)-C(12)	1.327(3)
O(2)-H(2)	0.73(4)	O(3)-C(12)	1.215(4)
C(1)-C(2)	1.519(4)	C(1)-C(10)	1.533(3)
C(1)-H(1A)	0.9900	C(1)-H(1B)	0.9900
C(2)-C(3)	1.520(4)	C(2)-H(2A)	0.9900
C(2)-H(2B)	0.9900	C(3)-C(4)	1.532(4)
C(3)-H(3A)	0.9900	C(3)-H(3B)	0.9900
C(4)-C(13)	1.544(3)	C(4)-C(5)	1.562(4)
C(4)-H(4A)	1.0000	C(5)-C(14)	1.537(4)
C(5)-C(6)	1.550(3)	C(5)-C(10)	1.589(3)
C(6)-C(7)	1.527(4)	C(6)-H(6A)	0.9900
C(6)-H(6B)	0.9900	C(7)-C(8)	1.504(4)
C(7)-H(7A)	0.9900	C(7)-H(7B)	0.9900
C(8)-C(11)	1.343(4)	C(8)-C(9)	1.501(3)
C(9)-C(10)	1.530(3)	C(10)-C(15)	1.546(4)
C(11)-C(12)	1.476(4)	C(11)-H(11A)	0.9500
C(13)-H(13A)	0.9800	C(13)-H(13B)	0.9800
C(13)-H(13C)	0.9800	C(14)-H(14A)	0.9800
C(14)-H(14B)	0.9800	C(14)-H(14C)	0.9800
C(15)-H(15A)	0.9800	C(15)-H(15B)	0.9800
C(15)-H(15C)	0.9800	O(10)-H(10Y)	0.75(6)
O(10)-H(10Z)	0.71(6)		

Symmetry transformations used to generate equivalent atoms:

Table S8. Bond angles [°] for **1**.

atom-atom-atom	angle	atom-atom-atom	angle
C(12)-O(2)-H(2)	108(3)	C(2)-C(1)-C(10)	114.8(2)
C(2)-C(1)-H(1A)	108.6	C(10)-C(1)-H(1A)	108.6
C(2)-C(1)-H(1B)	108.6	C(10)-C(1)-H(1B)	108.6
H(1A)-C(1)-H(1B)	107.6	C(1)-C(2)-C(3)	111.4(2)
C(1)-C(2)-H(2A)	109.3	C(3)-C(2)-H(2A)	109.3
C(1)-C(2)-H(2B)	109.3	C(3)-C(2)-H(2B)	109.3
H(2A)-C(2)-H(2B)	108.0	C(2)-C(3)-C(4)	111.4(2)
C(2)-C(3)-H(3A)	109.3	C(4)-C(3)-H(3A)	109.3
C(2)-C(3)-H(3B)	109.3	C(4)-C(3)-H(3B)	109.3
H(3A)-C(3)-H(3B)	108.0	C(3)-C(4)-C(13)	109.8(2)
C(3)-C(4)-C(5)	112.2(2)	C(13)-C(4)-C(5)	113.6(2)
C(3)-C(4)-H(4A)	106.9	C(13)-C(4)-H(4A)	106.9
C(5)-C(4)-H(4A)	106.9	C(14)-C(5)-C(6)	106.5(2)
C(14)-C(5)-C(4)	111.1(2)	C(6)-C(5)-C(4)	111.3(2)
C(14)-C(5)-C(10)	110.7(2)	C(6)-C(5)-C(10)	109.8(2)
C(4)-C(5)-C(10)	107.6(2)	C(7)-C(6)-C(5)	114.2(2)
C(7)-C(6)-H(6A)	108.7	C(5)-C(6)-H(6A)	108.7
C(7)-C(6)-H(6B)	108.7	C(5)-C(6)-H(6B)	108.7
H(6A)-C(6)-H(6B)	107.6	C(8)-C(7)-C(6)	109.7(2)
C(8)-C(7)-H(7A)	109.7	C(6)-C(7)-H(7A)	109.7
C(8)-C(7)-H(7B)	109.7	C(6)-C(7)-H(7B)	109.7
H(7A)-C(7)-H(7B)	108.2	C(11)-C(8)-C(9)	122.6(2)
C(11)-C(8)-C(7)	123.5(2)	C(9)-C(8)-C(7)	113.9(2)
O(1)-C(9)-C(8)	121.3(2)	O(1)-C(9)-C(10)	123.0(2)
C(8)-C(9)-C(10)	115.7(2)	C(9)-C(10)-C(1)	110.8(2)
C(9)-C(10)-C(15)	105.0(2)	C(1)-C(10)-C(15)	107.9(2)
C(9)-C(10)-C(5)	109.0(2)	C(1)-C(10)-C(5)	111.7(2)
C(15)-C(10)-C(5)	112.2(2)	C(8)-C(11)-C(12)	124.4(2)
C(8)-C(11)-H(11A)	117.8	C(12)-C(11)-H(11A)	117.8
O(3)-C(12)-O(2)	123.1(3)	O(3)-C(12)-C(11)	125.0(2)
O(2)-C(12)-C(11)	111.9(2)	C(4)-C(13)-H(13A)	109.5
C(4)-C(13)-H(13B)	109.5	H(13A)-C(13)-H(13B)	109.5
C(4)-C(13)-H(13C)	109.5	H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5	C(5)-C(14)-H(14A)	109.5
C(5)-C(14)-H(14B)	109.5	H(14A)-C(14)-H(14B)	109.5
C(5)-C(14)-H(14C)	109.5	H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5	C(10)-C(15)-H(15A)	109.5
C(10)-C(15)-H(15B)	109.5	H(15A)-C(15)-H(15B)	109.5
C(10)-C(15)-H(15C)	109.5	H(15A)-C(15)-H(15C)	109.5
H(15B)-C(15)-H(15C)	109.5	H(10Y)-O(10)-H(10Z)	120(6)

Symmetry transformations used to generate equivalent atoms:

Table S9. Torsion angles [°] for **1**.

atom-atom-atom-atom	angle	atom-atom-atom-atom	angle
C(10)-C(1)-C(2)-C(3)	51.4(3)	C(1)-C(2)-C(3)-C(4)	-53.8(3)
C(2)-C(3)-C(4)-C(13)	-173.4(3)	C(2)-C(3)-C(4)-C(5)	59.3(3)
C(3)-C(4)-C(5)-C(14)	63.9(3)	C(13)-C(4)-C(5)-C(14)	-61.4(3)
C(3)-C(4)-C(5)-C(6)	-177.7(2)	C(13)-C(4)-C(5)-C(6)	57.0(3)
C(3)-C(4)-C(5)-C(10)	-57.4(3)	C(13)-C(4)-C(5)-C(10)	177.3(2)
C(14)-C(5)-C(6)-C(7)	-175.9(2)	C(4)-C(5)-C(6)-C(7)	62.9(3)
C(10)-C(5)-C(6)-C(7)	-56.0(3)	C(5)-C(6)-C(7)-C(8)	55.1(3)
C(6)-C(7)-C(8)-C(11)	125.7(3)	C(6)-C(7)-C(8)-C(9)	-51.7(3)
C(11)-C(8)-C(9)-O(1)	56.8(4)	C(7)-C(8)-C(9)-O(1)	-125.8(3)
C(11)-C(8)-C(9)-C(10)	-124.4(3)	C(7)-C(8)-C(9)-C(10)	52.9(3)
O(1)-C(9)-C(10)-C(1)	4.0(3)	C(8)-C(9)-C(10)-C(1)	-174.7(2)
O(1)-C(9)-C(10)-C(15)	-112.3(3)	C(8)-C(9)-C(10)-C(15)	69.1(3)
O(1)-C(9)-C(10)-C(5)	127.4(3)	C(8)-C(9)-C(10)-C(5)	-51.3(3)
C(2)-C(1)-C(10)-C(9)	69.9(3)	C(2)-C(1)-C(10)-C(15)	-175.7(2)
C(2)-C(1)-C(10)-C(5)	-51.9(3)	C(14)-C(5)-C(10)-C(9)	168.5(2)
C(6)-C(5)-C(10)-C(9)	51.3(3)	C(4)-C(5)-C(10)-C(9)	-69.9(2)
C(14)-C(5)-C(10)-C(1)	-68.6(3)	C(6)-C(5)-C(10)-C(1)	174.1(2)
C(4)-C(5)-C(10)-C(1)	52.9(3)	C(14)-C(5)-C(10)-C(15)	52.7(3)
C(6)-C(5)-C(10)-C(15)	-64.6(3)	C(4)-C(5)-C(10)-C(15)	174.2(2)
C(9)-C(8)-C(11)-C(12)	4.3(5)	C(7)-C(8)-C(11)-C(12)	-172.8(3)
C(8)-C(11)-C(12)-O(3)	28.9(5)	C(8)-C(11)-C(12)-O(2)	-153.0(3)

Symmetry transformations used to generate equivalent atoms:

Table S10. Hydrogen bonds for **1** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2)...O(10)	0.73(4)	1.94(4)	2.644(3)	164(4)
O(10)-H(10Y)...O(1)#1	0.75(6)	2.21(6)	2.928(3)	161(5)
O(10)-H(10Z)...O(3)#2	0.71(6)	2.13(6)	2.795(4)	159(5)

Symmetry transformations used to generate equivalent atoms:
#1 -x-1,y+1/2,-z #2 -x-1,y-1/2,-z

Figure S1. ^{1}H NMR spectrum of aignopsanoic acid A (**1**), (600 MHz, $CDC1_3$).

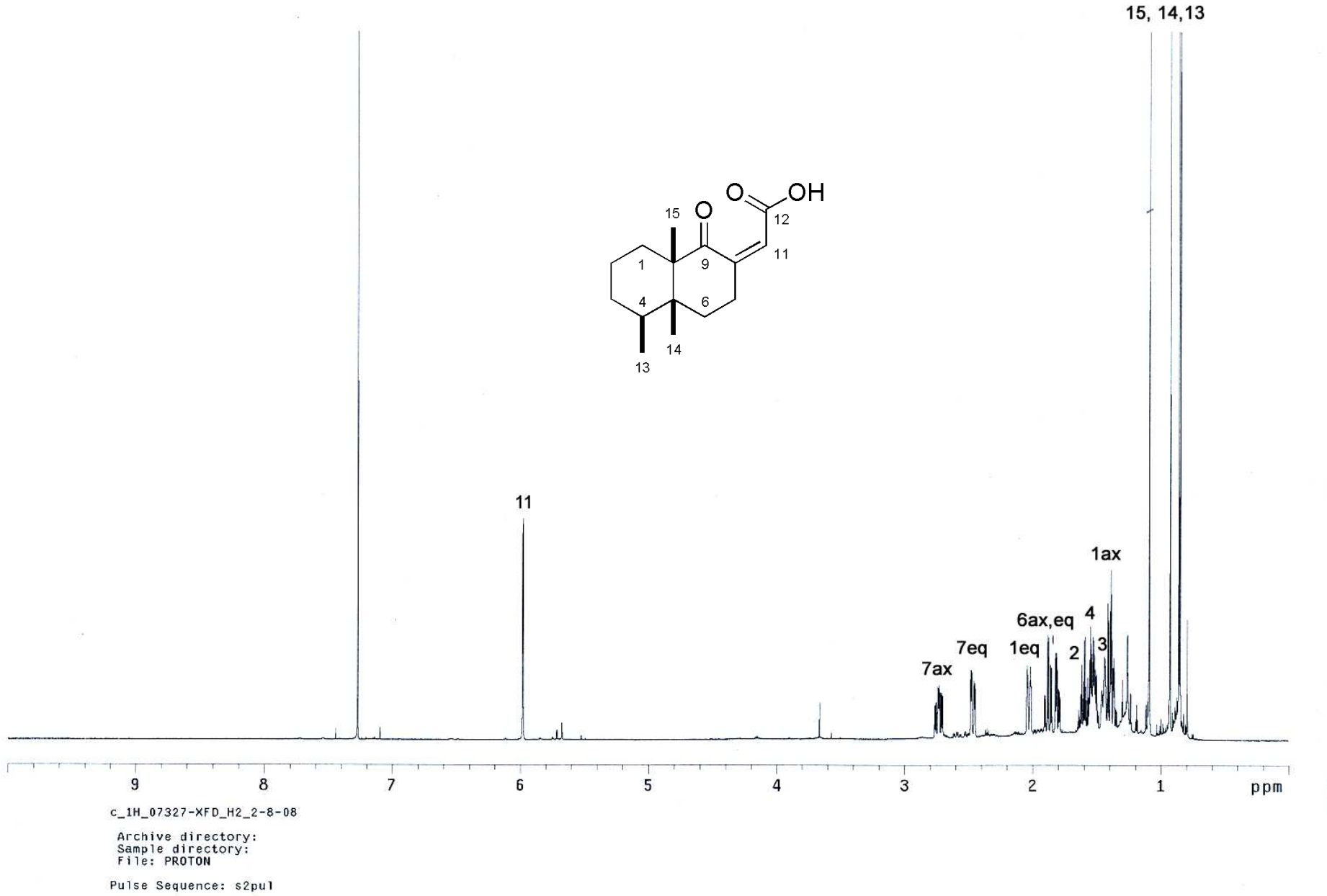


Figure S2. ^{13}C NMR spectrum of aignopsanoic acid A (**1**), (125 MHz, $CDC1_3$).

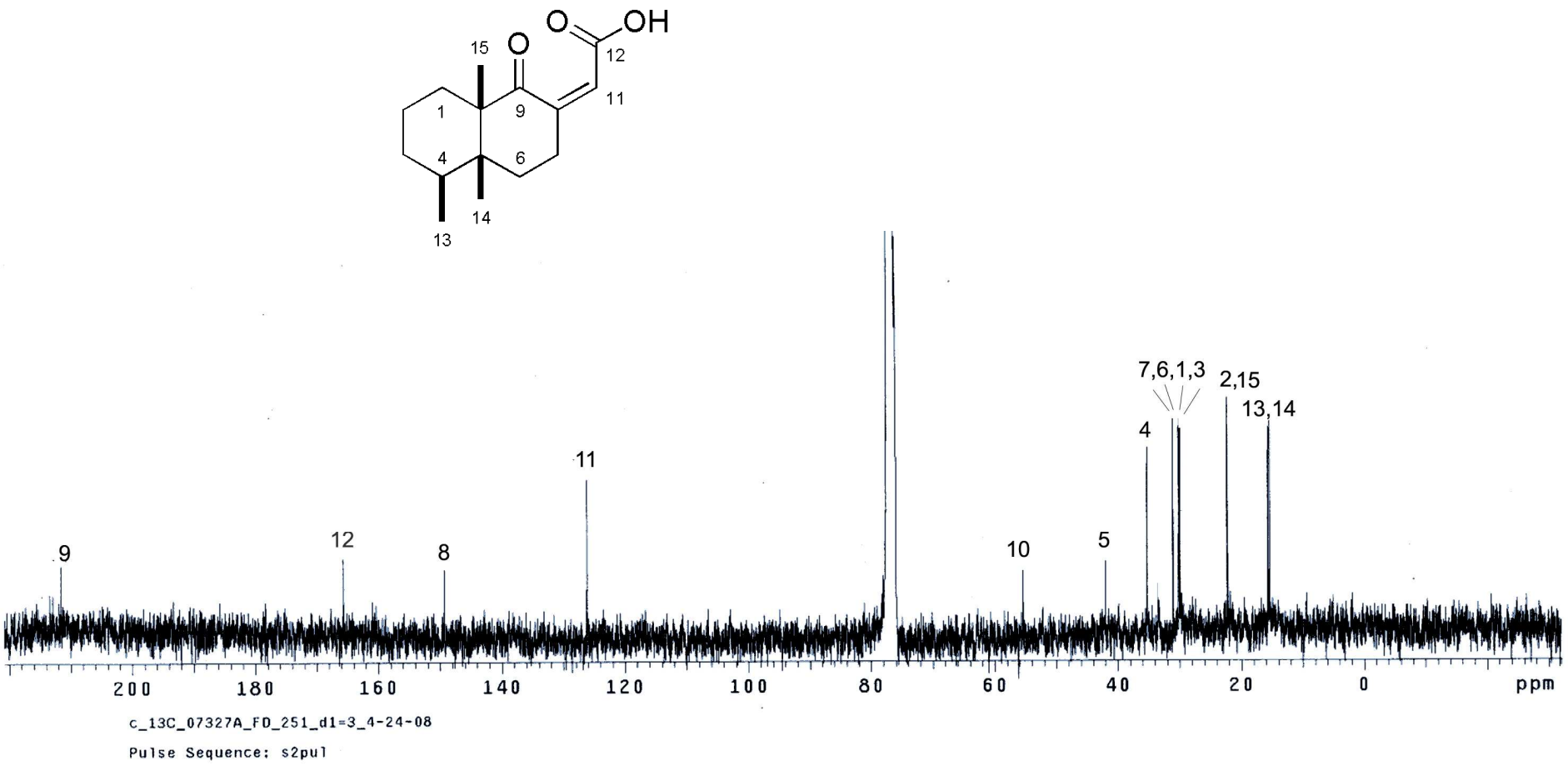


Figure S3. COSY spectrum of aignopsanoic acid A (**1**), (600 MHz, $CDC1_3$).

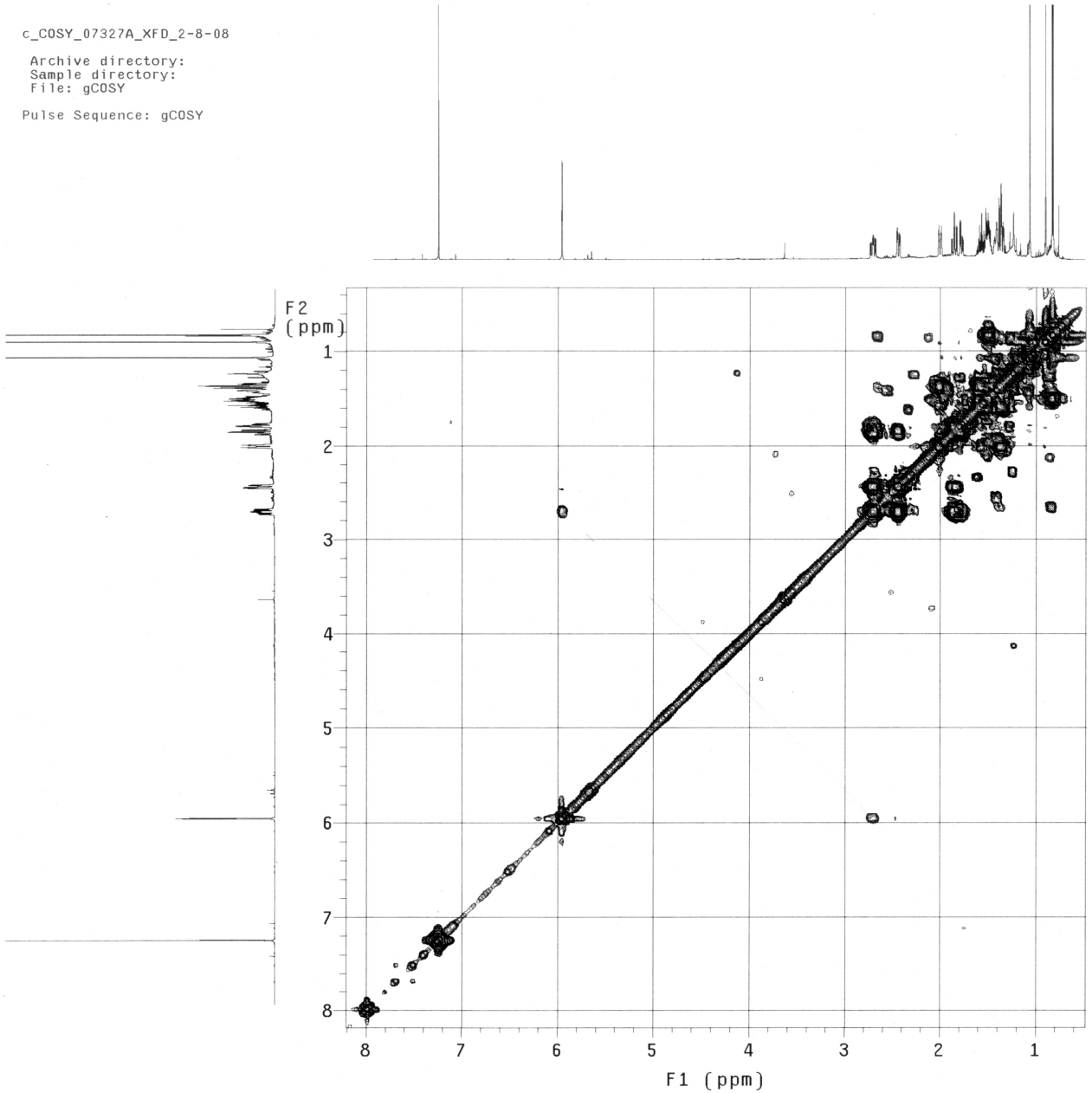


Figure S4. HMQC spectrum of aignopsanoic acid A (**1**), (600 MHz, $CDC1_3$).

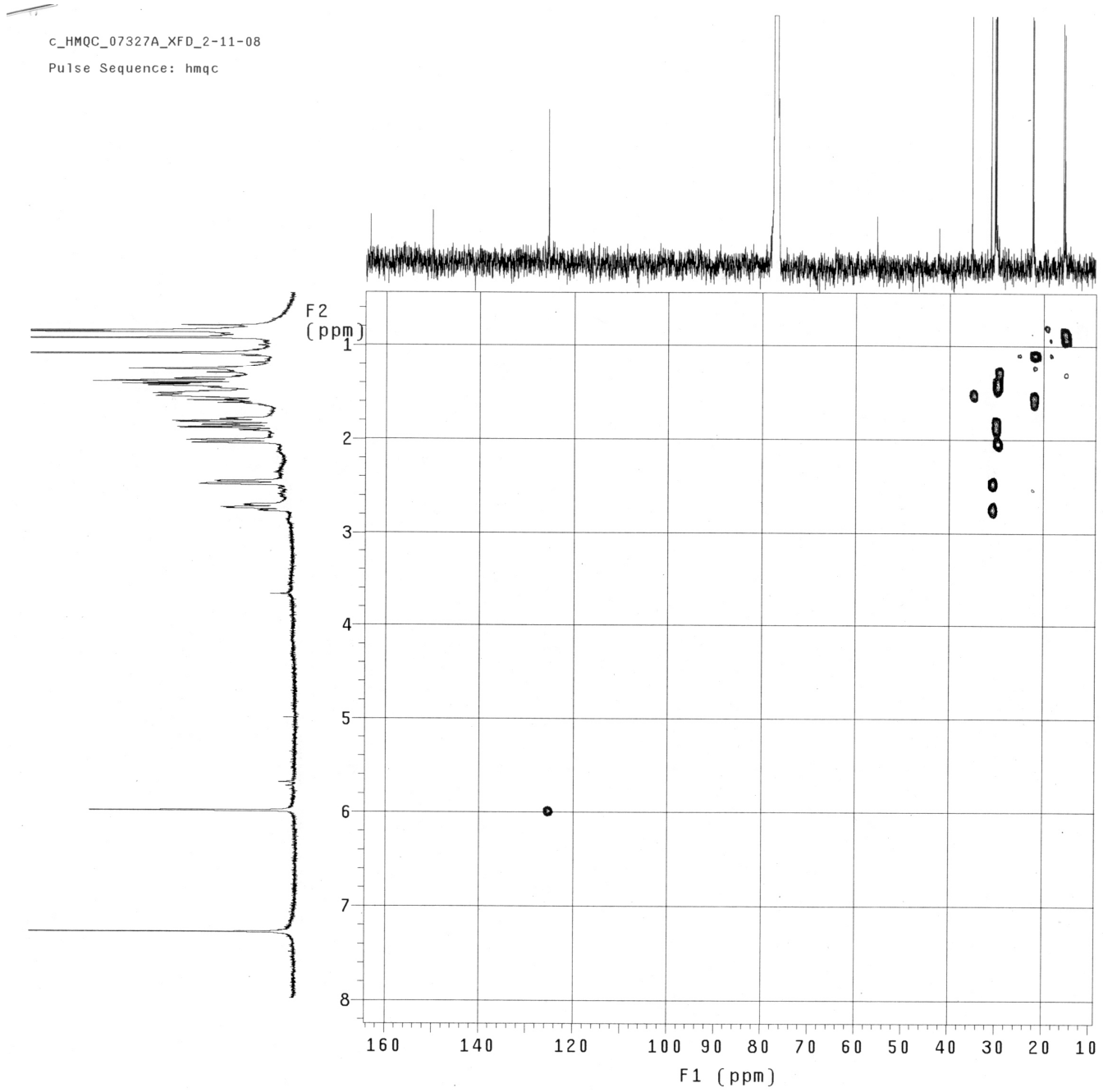


Figure S5. HMBC spectrum of aignopsanoic acid A (**1**), (600 MHz, $CDC1_3$).

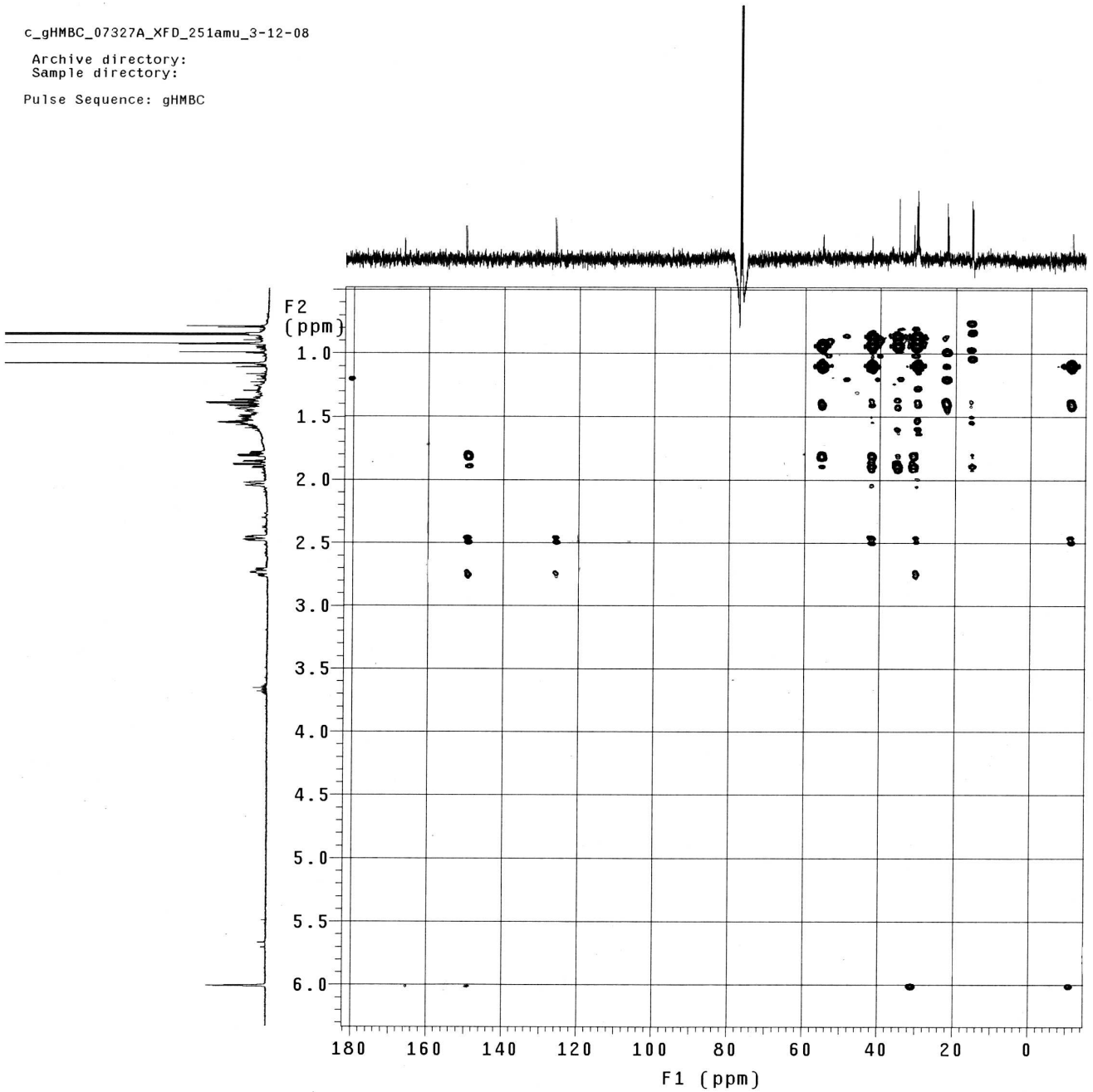


Figure S6. NOESY spectrum of aignopsanoic acid A (**1**), (600 MHz, $CDC1_3$).

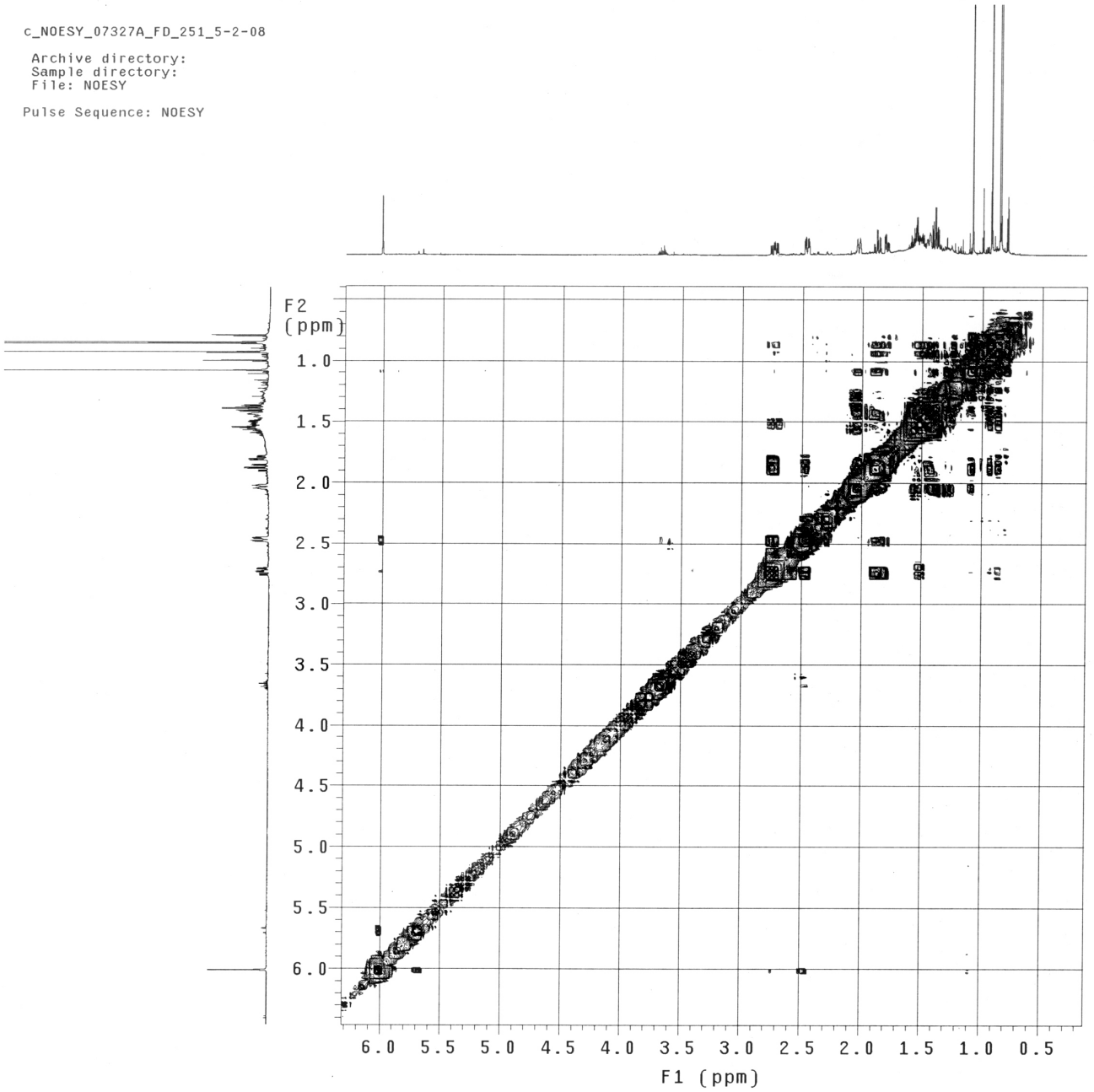


Figure S7. NOE enhancement of H_3-15 of **1**, (600 MHz, $CDCl_3$).

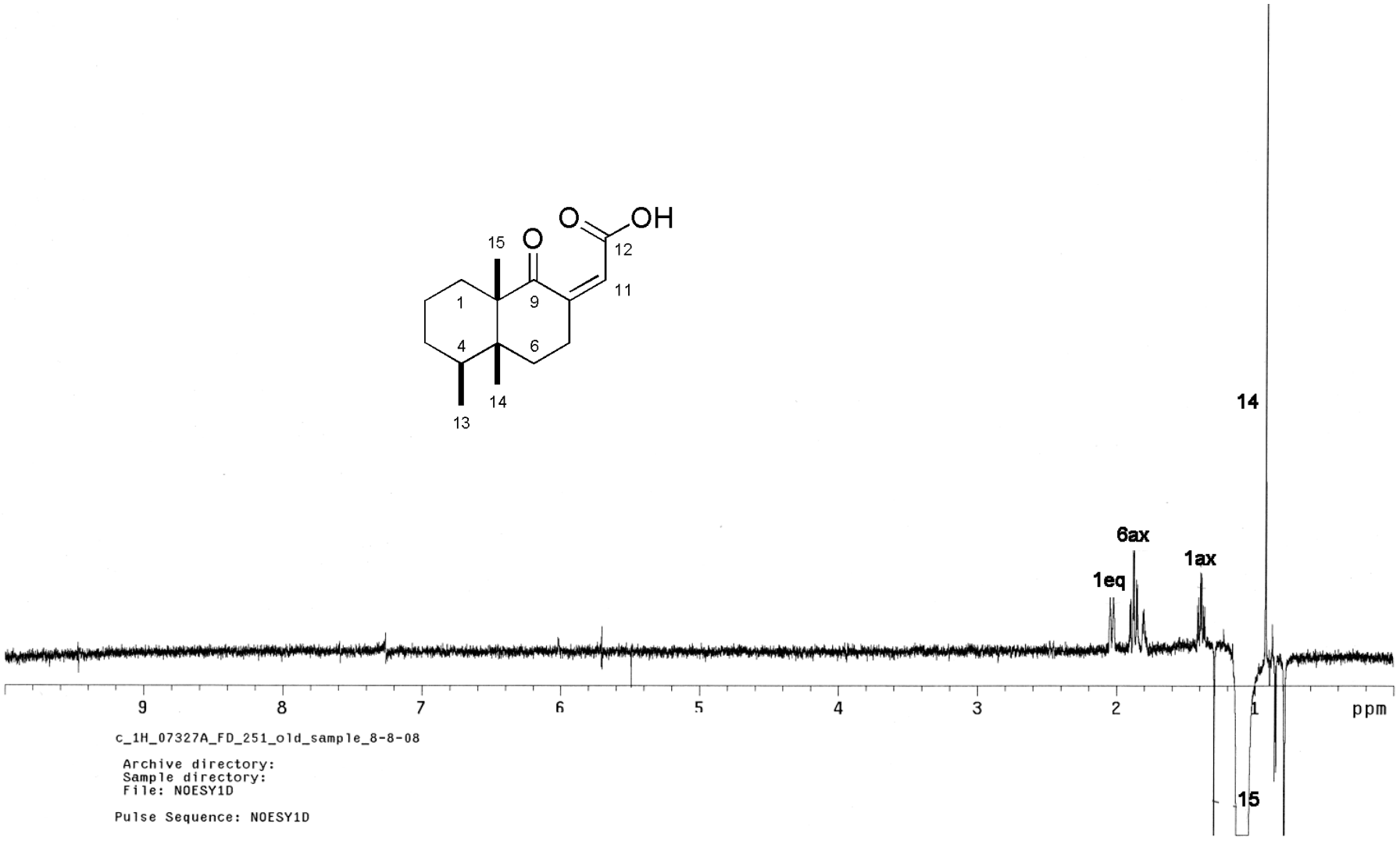


Figure S8. NOE enhancement of H_3-14 of **1**, (600 MHz, $CDCl_3$).

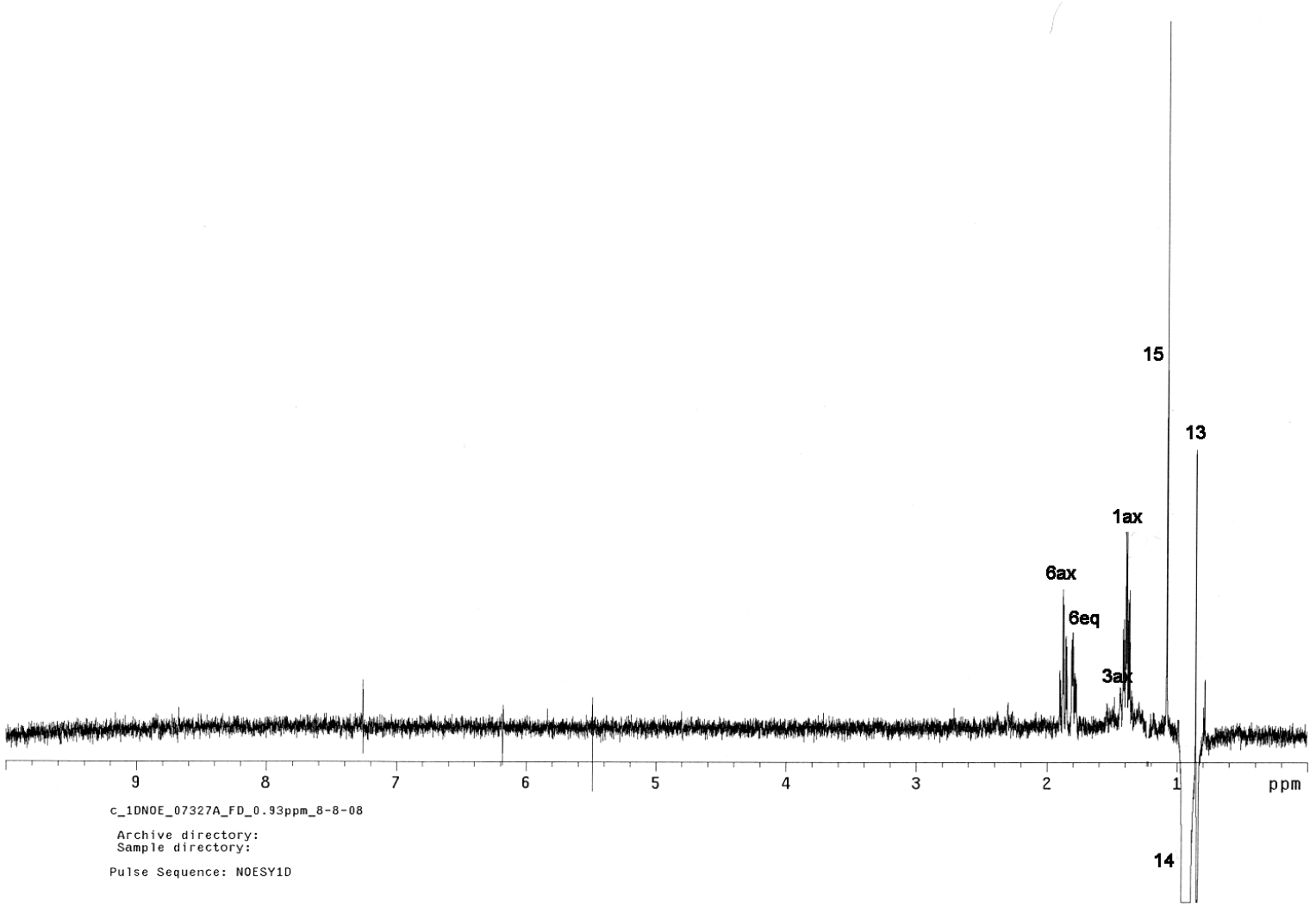


Figure S9. NOE enhancement of H_3-13 of **1**, (600 MHz, $CDCl_3$).

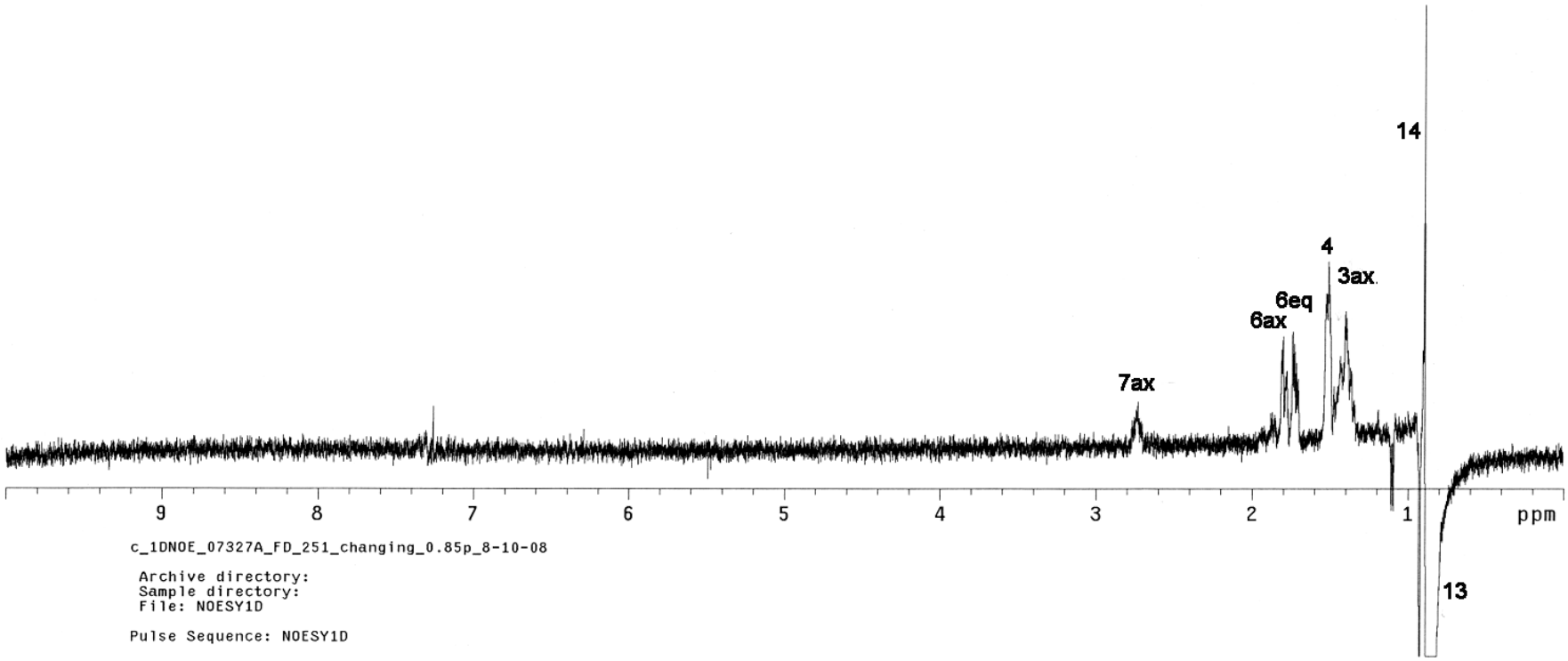


Figure S10. ^{1}H NMR spectrum of methyl aignopsanoate A (**2**), (600 MHz, $CDC1_3$).

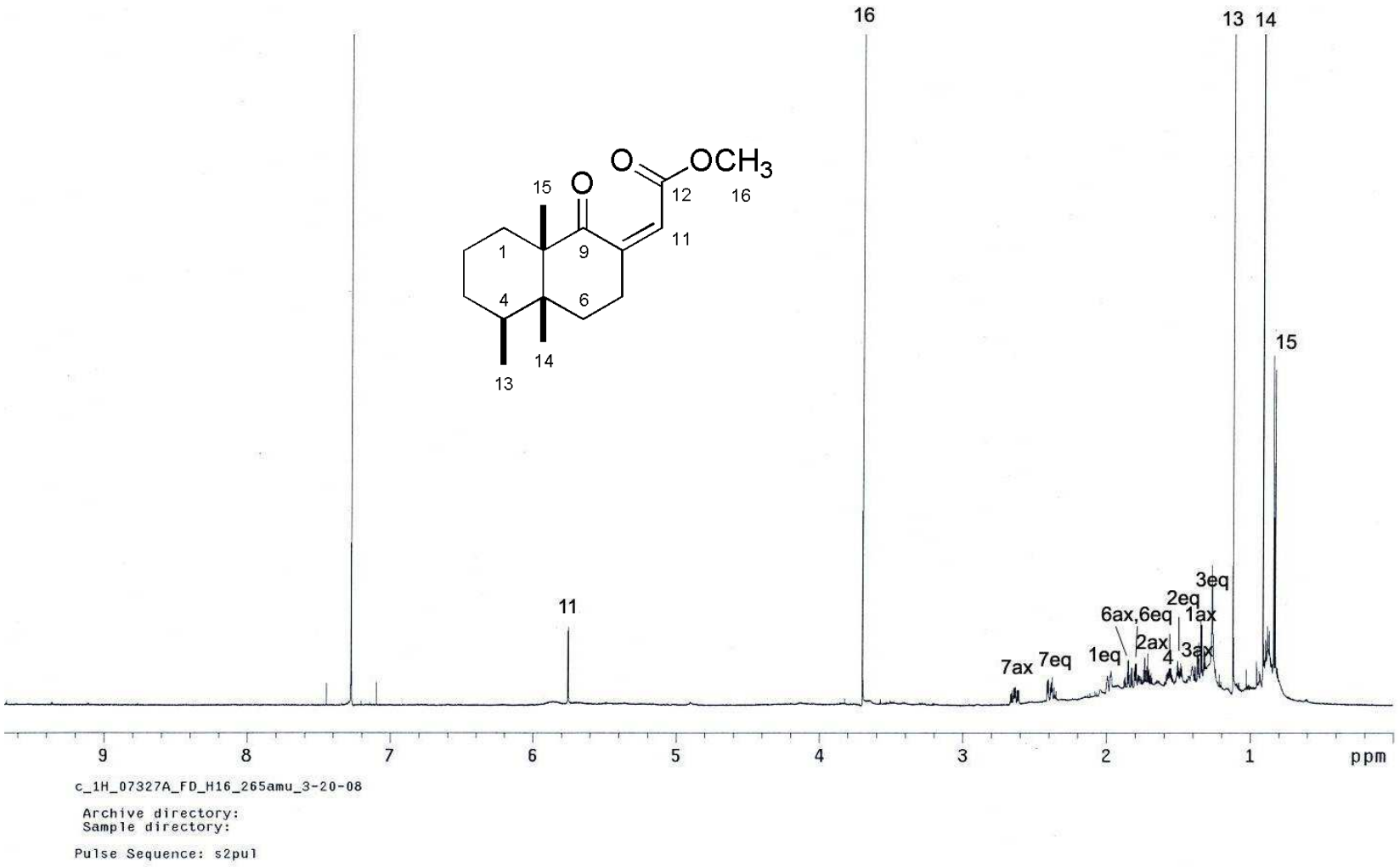


Figure S11. ^{13}C NMR spectrum of methyl aignopsanoate A (**2**), (125 MHz, $CDC1_3$).

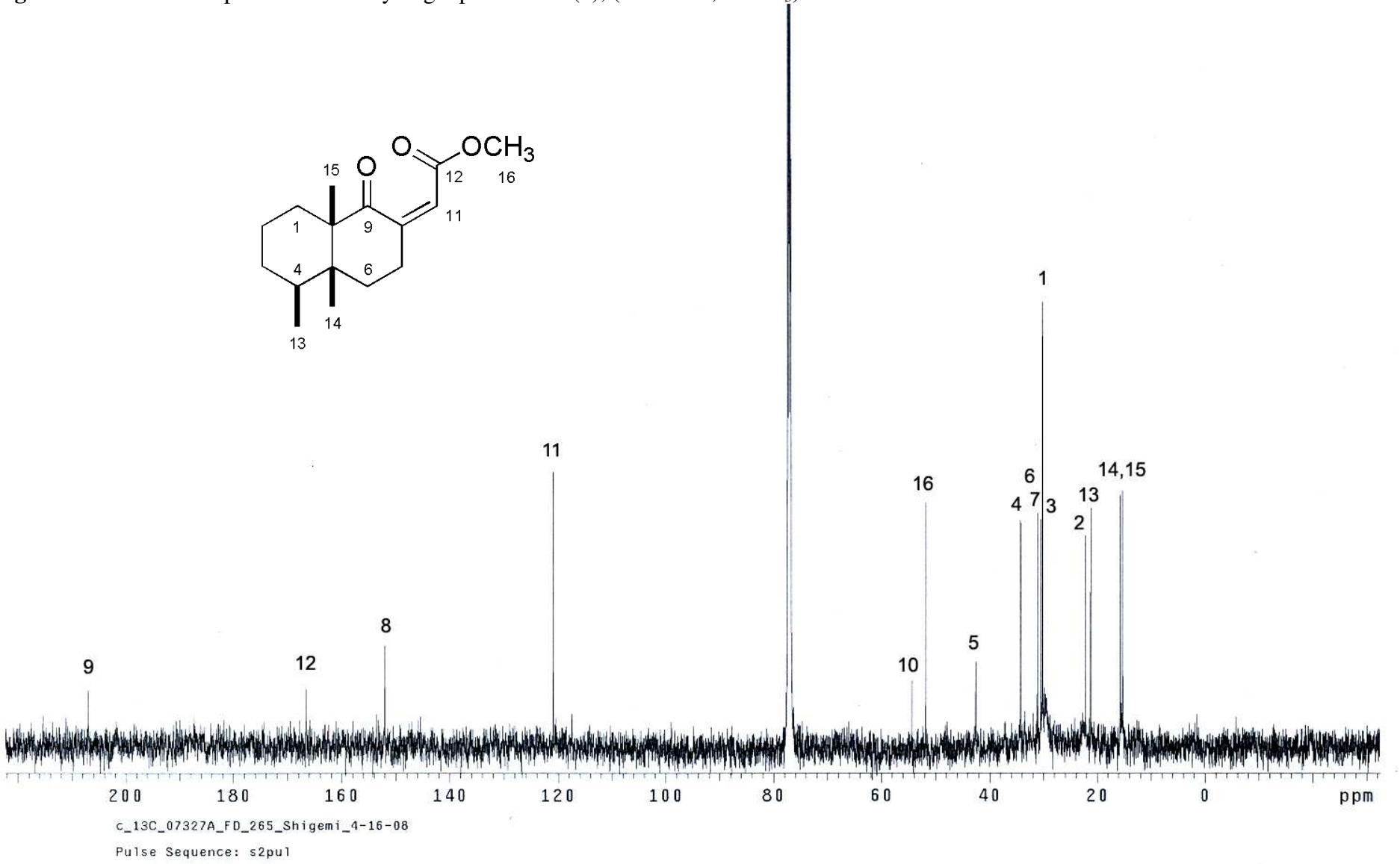


Figure S12. COSY spectrum of methyl aignopsanoate A (**2**), (600 MHz, $CDC1_3$).

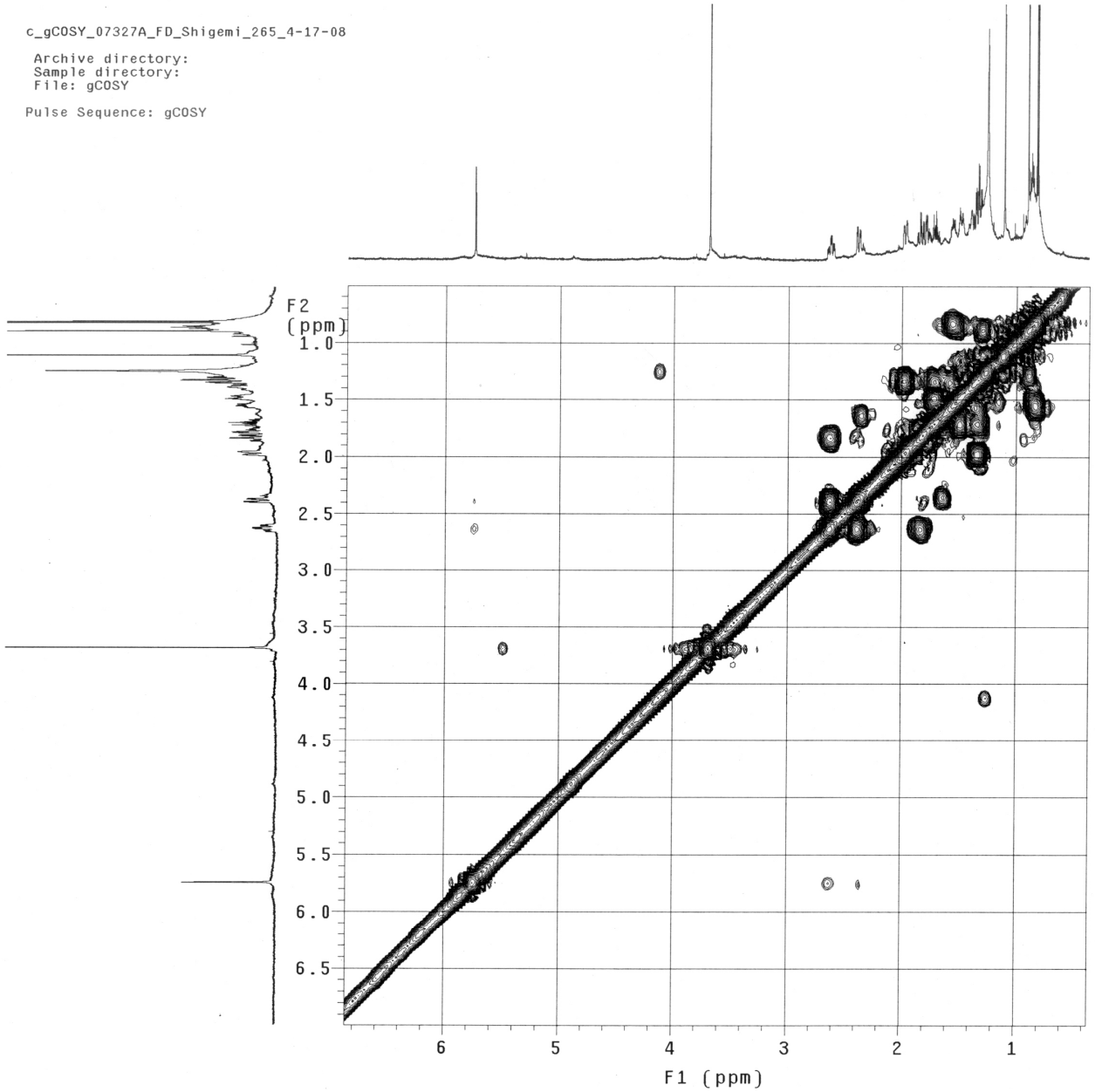


Figure S13. HMQC spectrum of methyl aignopsanoate A (**2**), (600 MHz, $CDC1_3$).

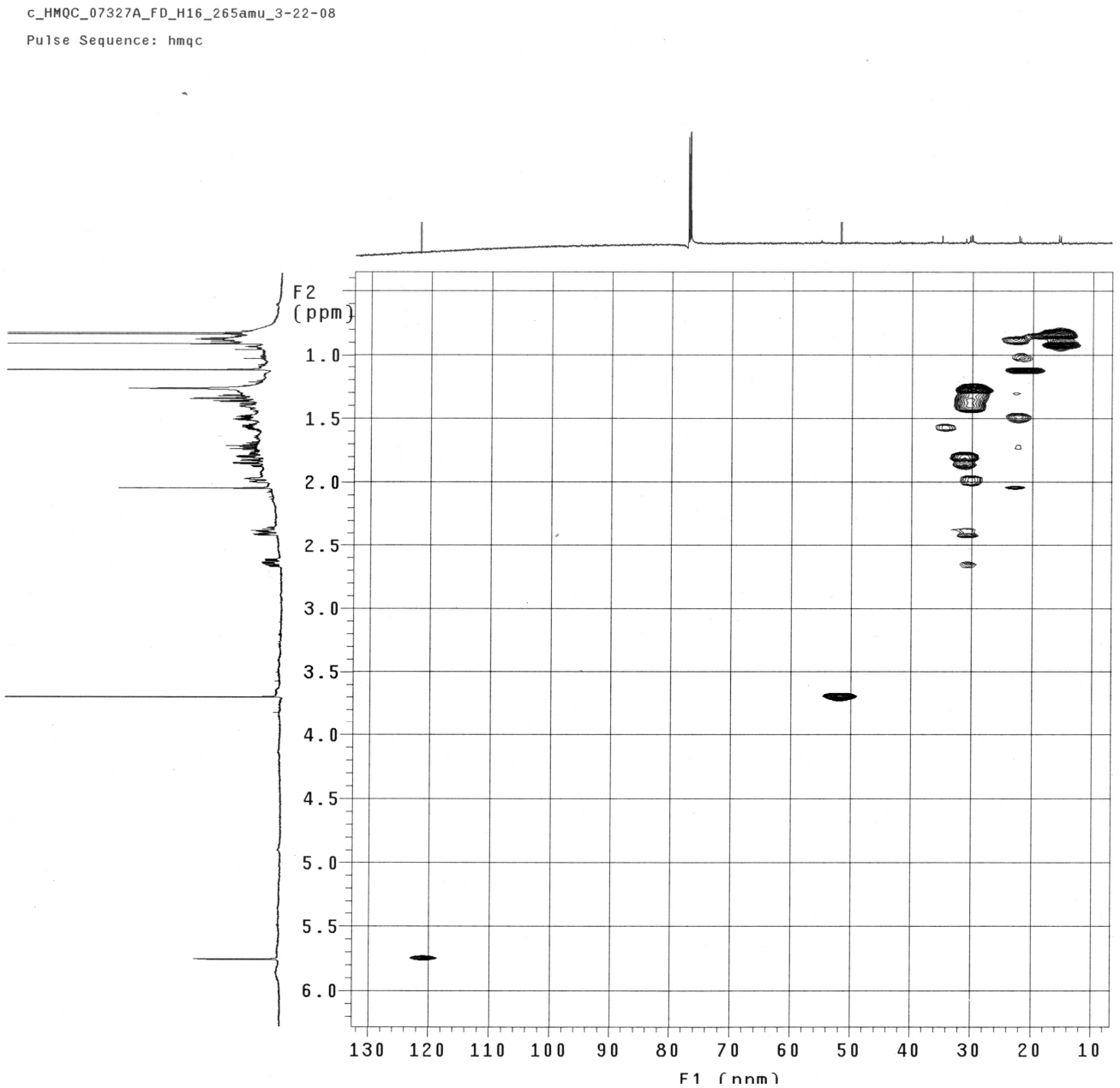


Figure S14. HMBC spectrum of methyl aignopsanoate A (**2**), (600 MHz, $CDC1_3$).

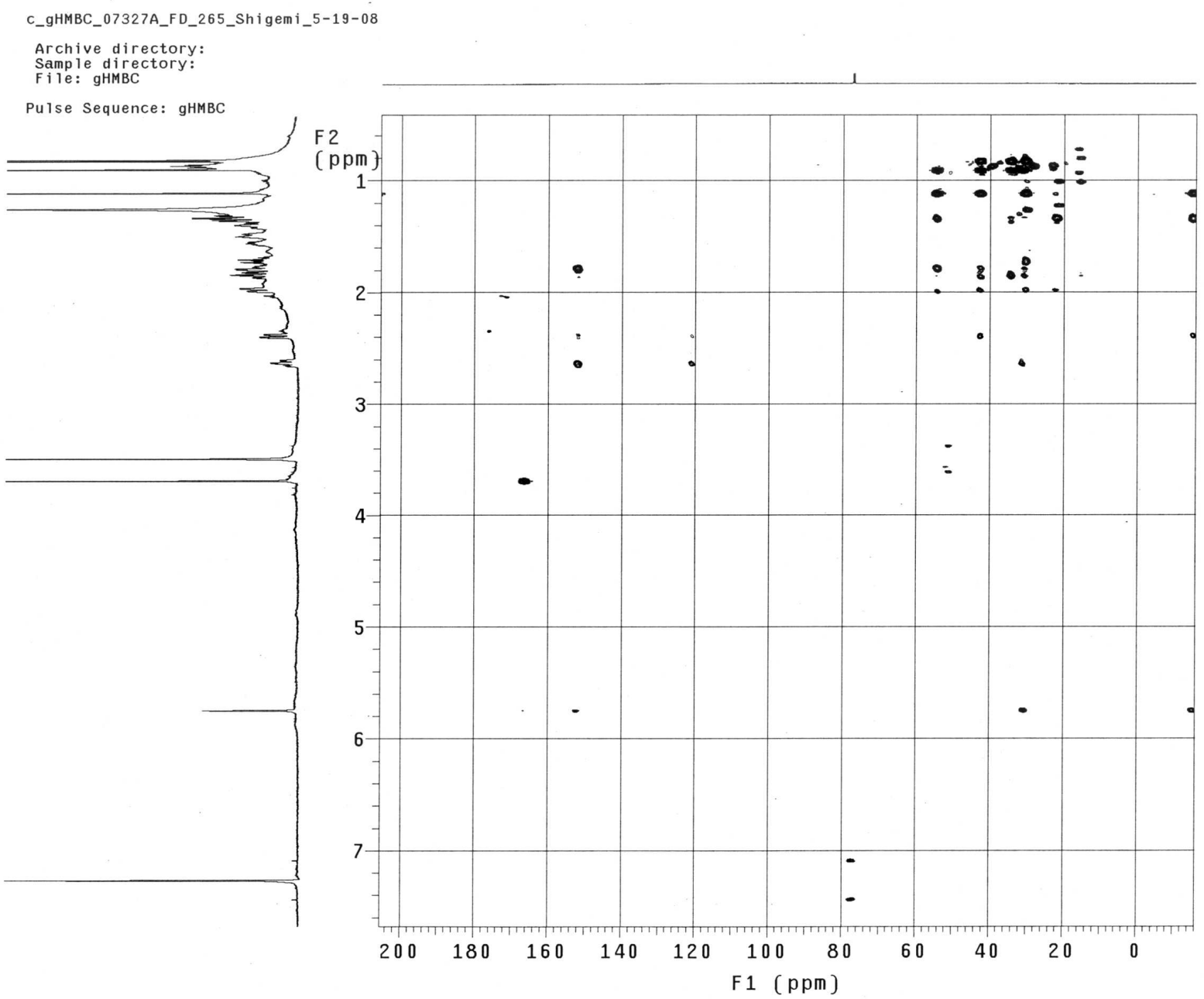


Figure S15. NOESY spectrum of methyl aignopsanoate A (**2**), (600 MHz, $CDC1_3$).

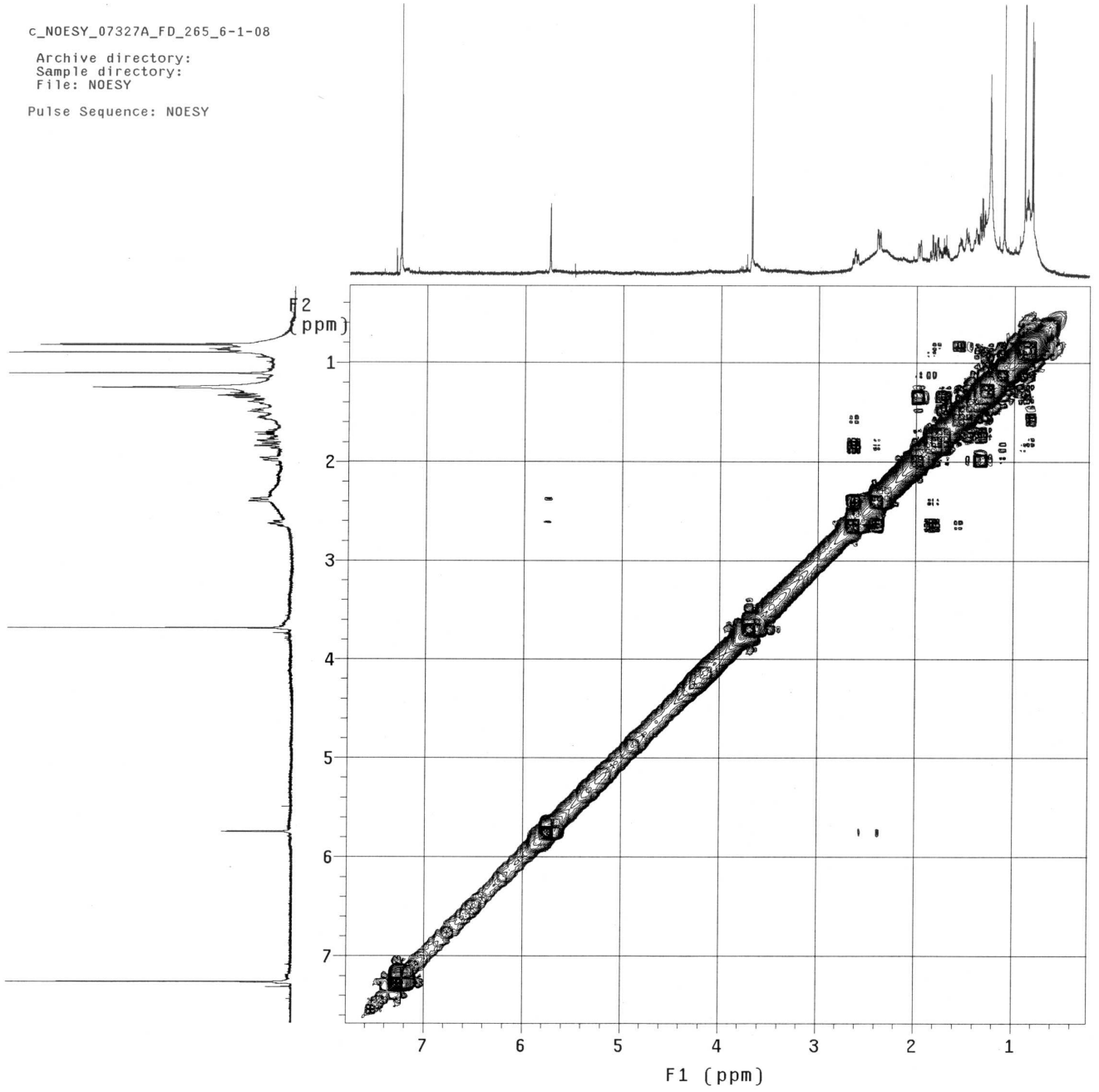


Figure S16. NOE enhancement of H_3-14 of **2**, (600 MHz, $CDC1_3$).

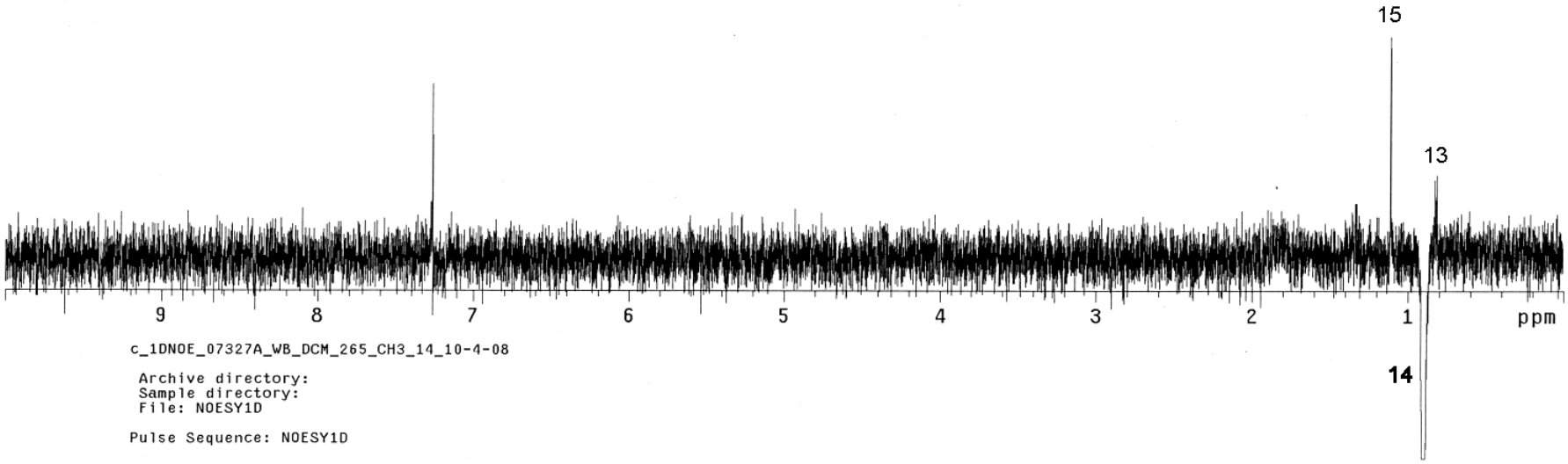


Figure S17. ^{1}H NMR spectrum of isoaignopsanoic acid A (**3**), (600 MHz, $CDC1_3$).

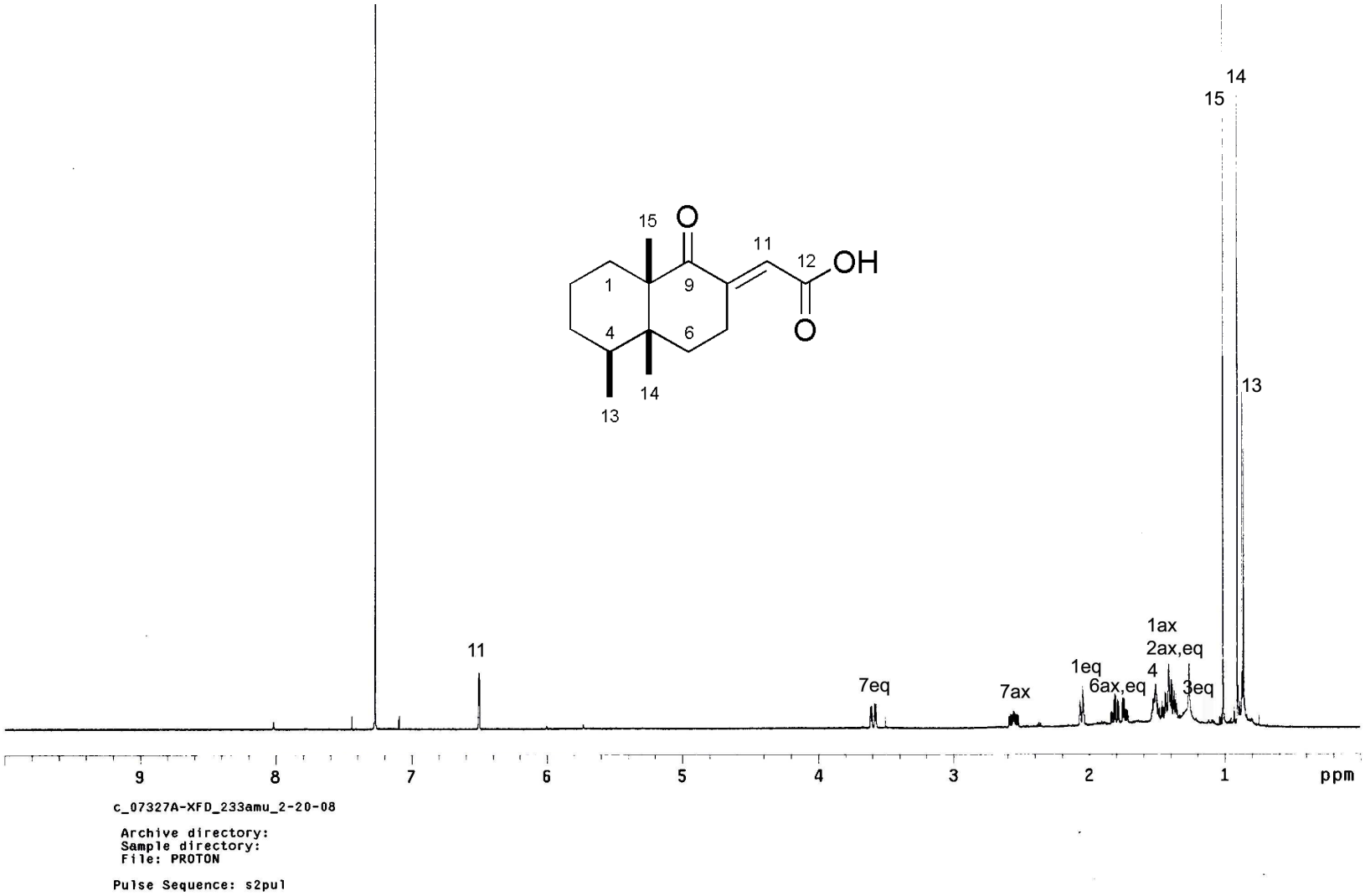


Figure S18. ^{13}C NMR spectrum of isoaignopsanoic acid A (**3**), (125 MHz, $CDC1_3$).

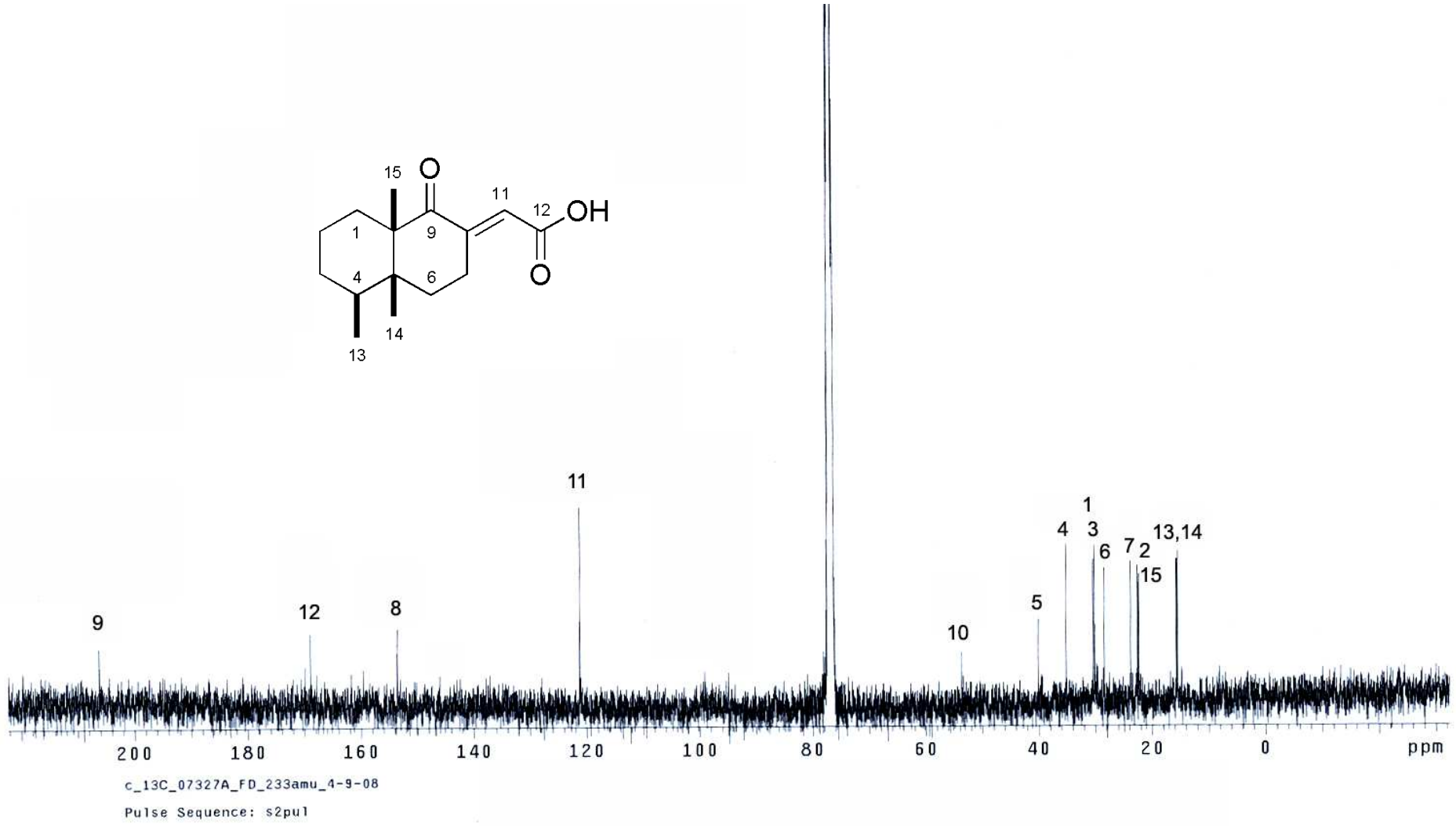


Figure S19. COSY spectrum of isoaignopsanoic acid A (**3**), (600 MHz, $CDC1_3$).

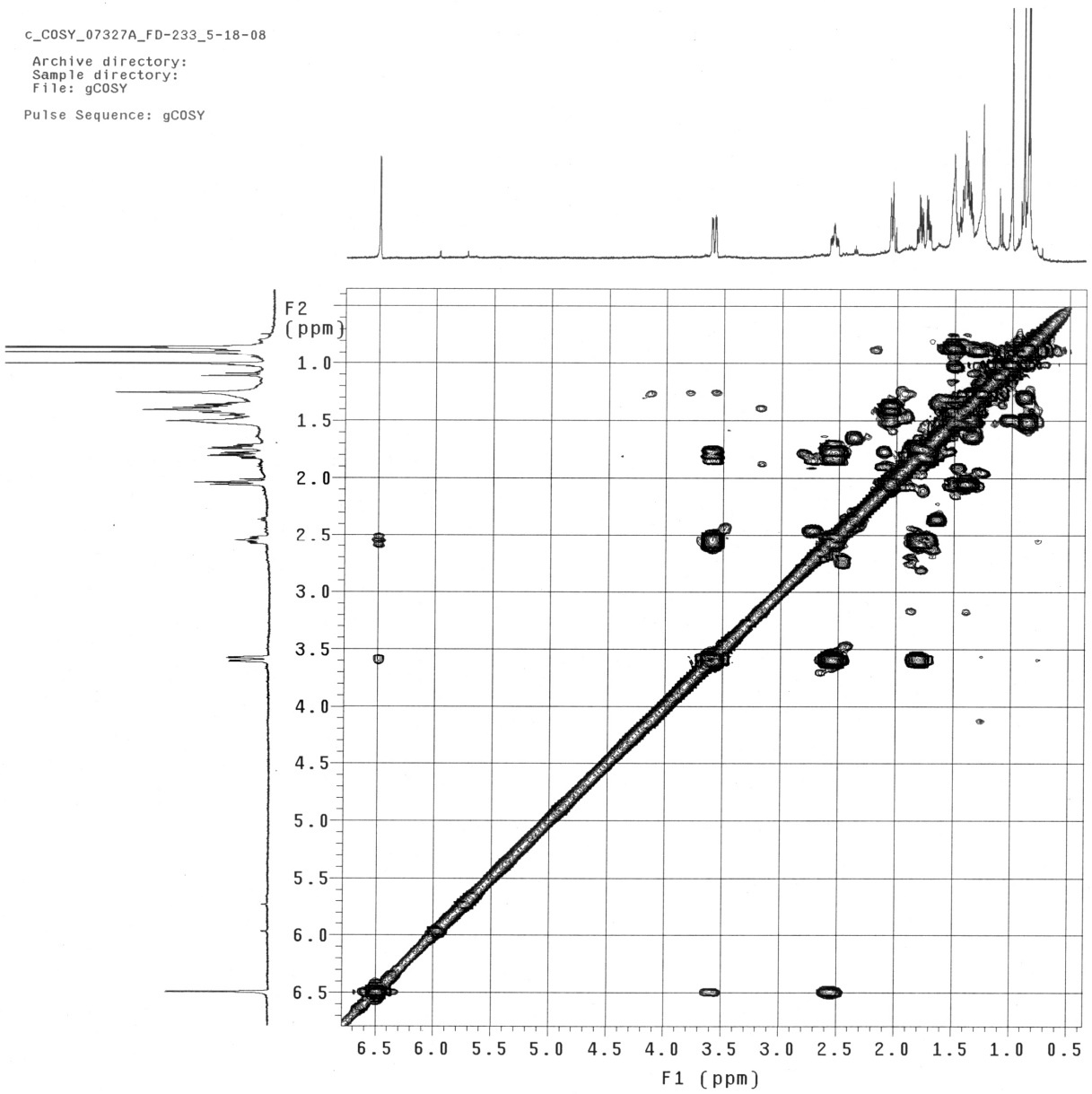


Figure S20. HMQC spectrum of isoaignopsanoic acid A (**3**), (600 MHz, $CDC1_3$).

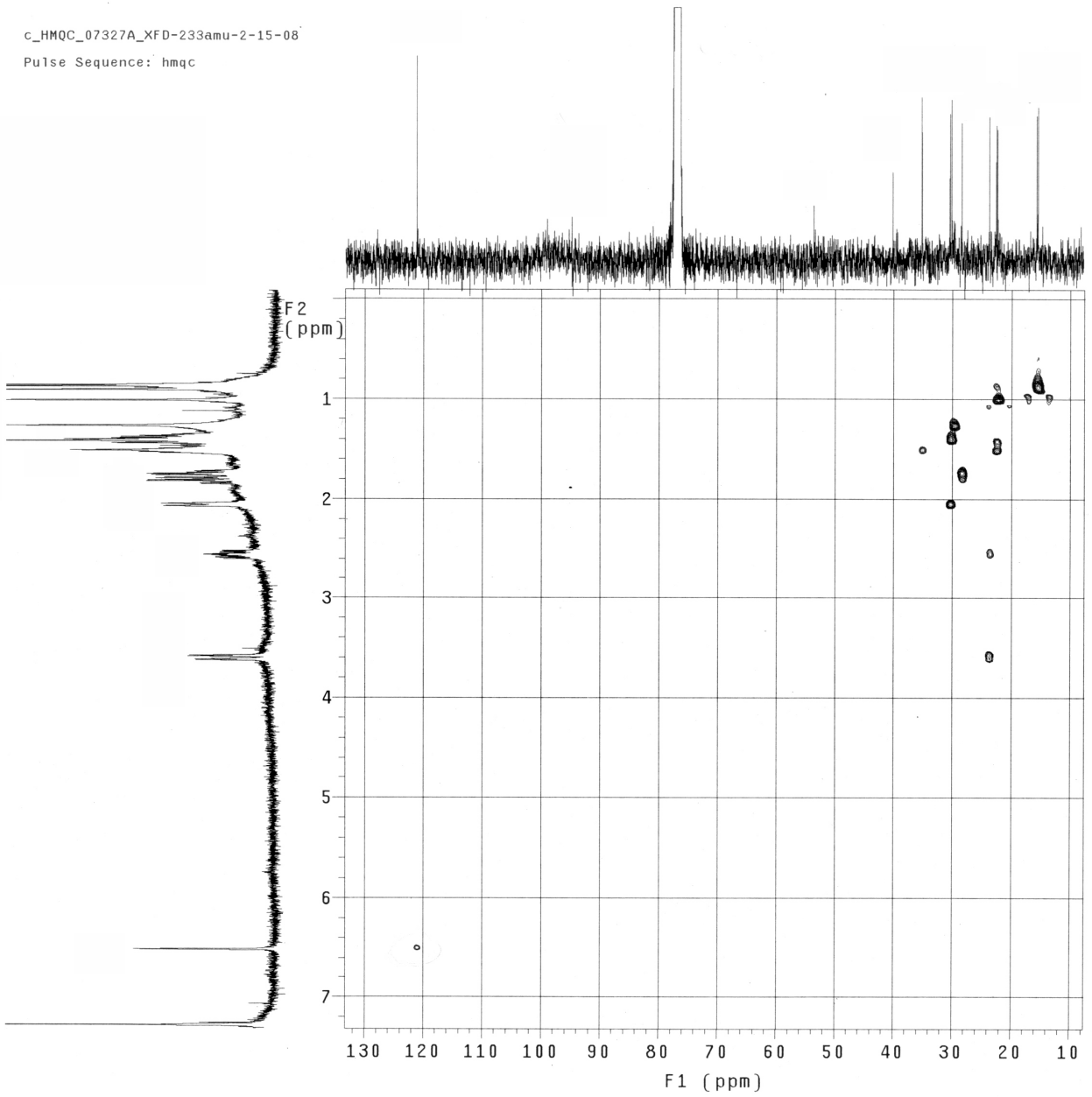


Figure S21. HMBC spectrum of isoaignopsanoic acid A (**3**), (600 MHz, $CDC1_3$).

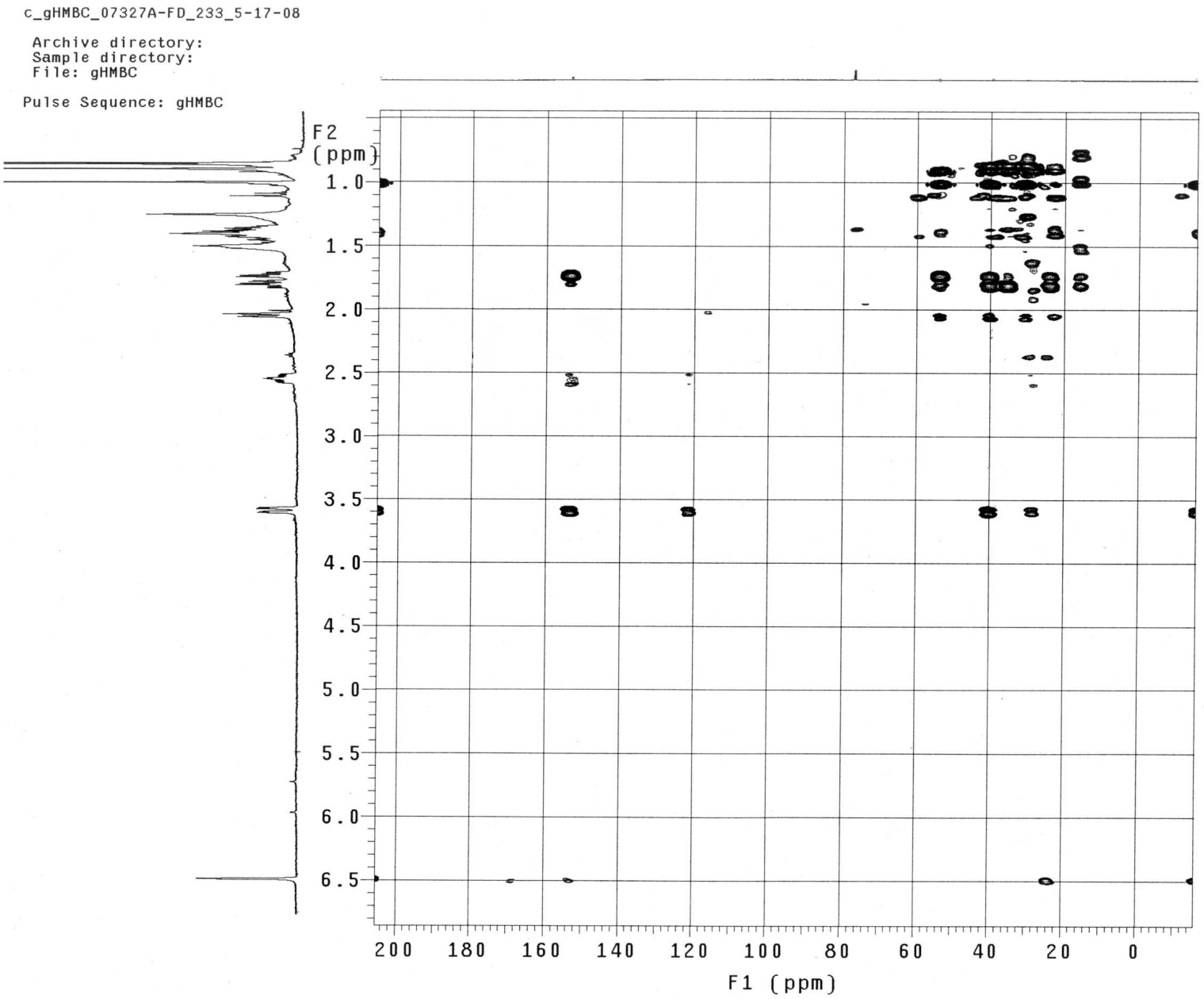


Figure S22. NOESY spectrum of isoaignopsanoic acid A (**3**), (600 MHz, $CDC1_3$).

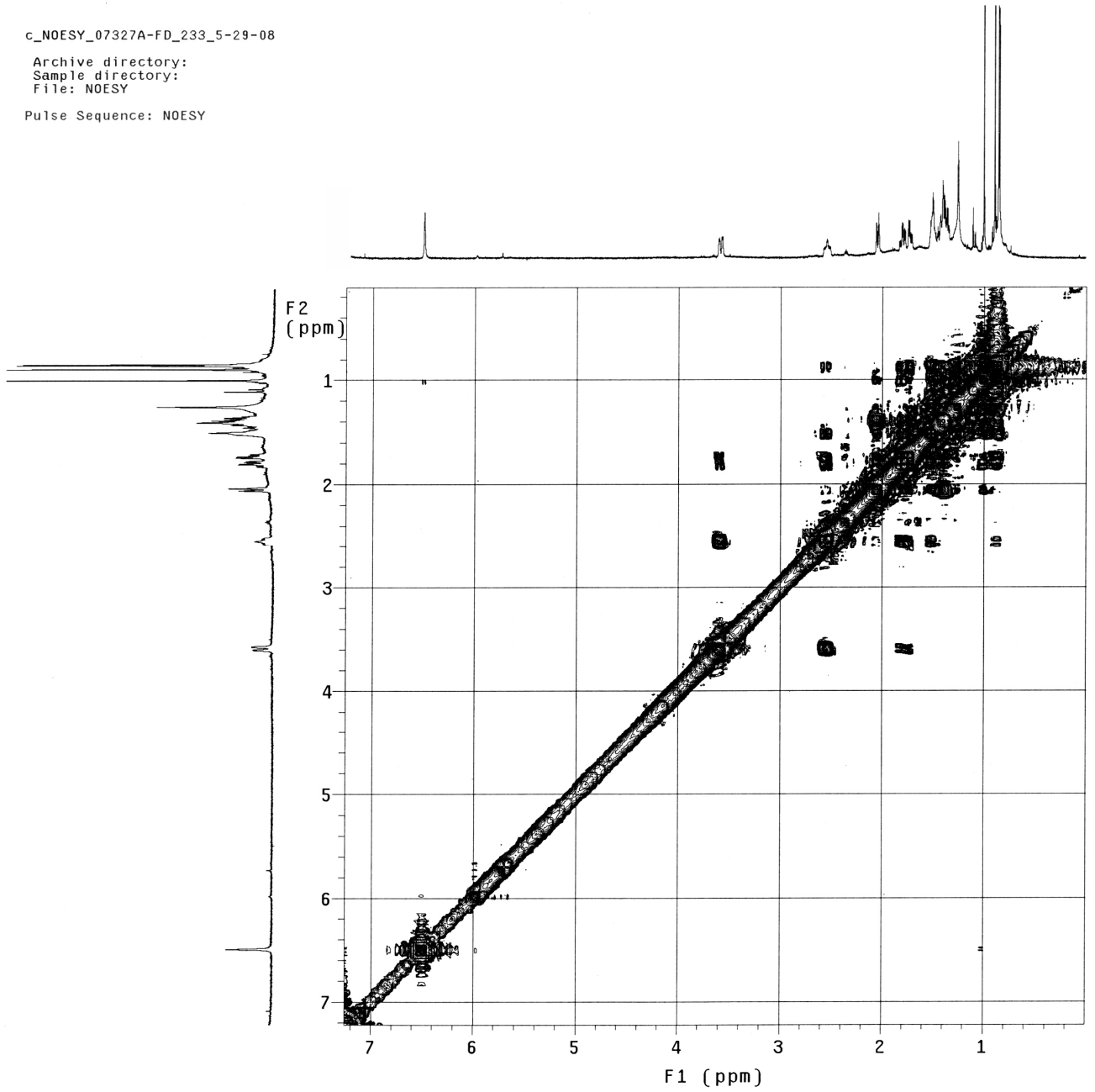


Figure S23. NOE enhancement of H_3-14 of **3**, (600 MHz, $CDC1_3$).

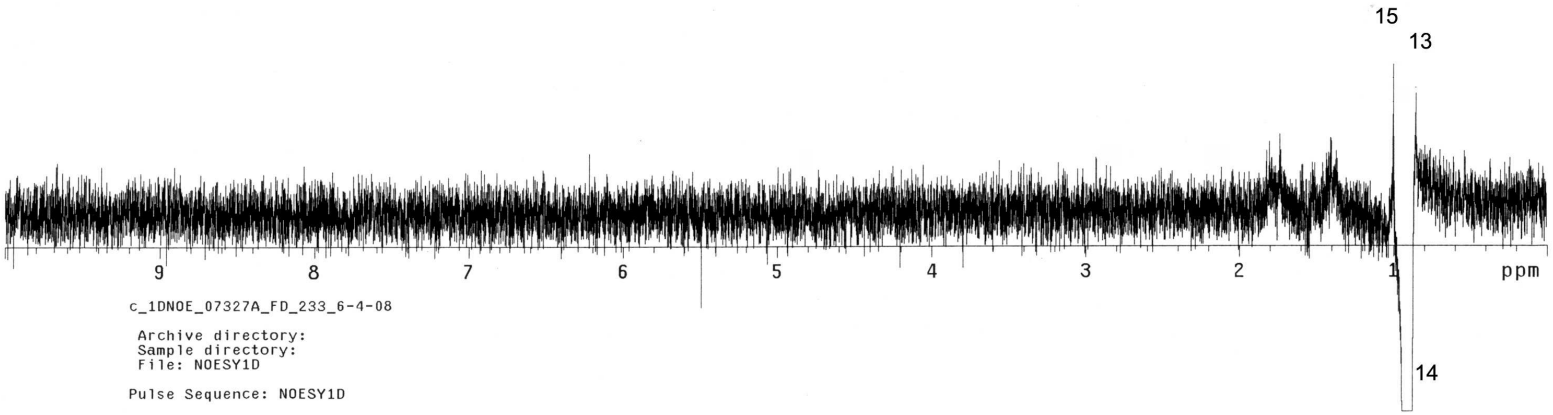
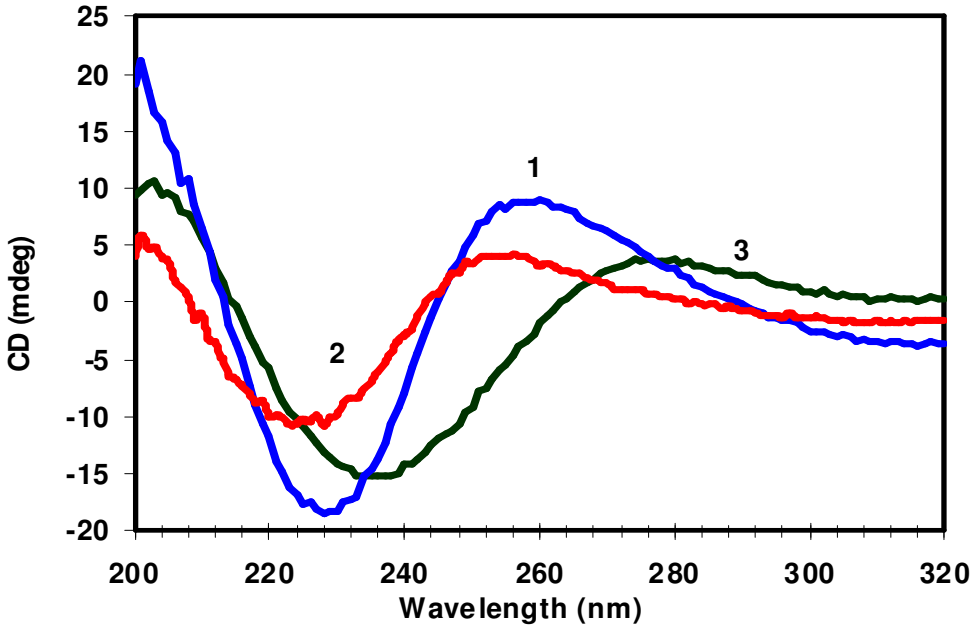


Figure S34. (**a**) CD spectra and (**b**) absolute configurations of compounds (**1**-**3**) and their projections.

(a)



(b)