Bioactive Diterpenoid Containing a Reversible "Spring-Loaded" (*E,Z*)-dieneone Michael Acceptor

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Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-2000 polarimeter (*c* g/100 mL) at 589 nm. UV spectra were obtained on a Perkin-Elmer Lambda EZ 210 UV–vis spectrophotometer. IR spectra were recorded on a Thermo Electronic Corporation Nicolet IR-100 spectrophotometer. All NMR spectra were recorded on a Varian Unity-INOVA 500 spectrometer. All chemical shifts (δ) were referenced internally to the residual solvent peak (CD_3OD: ^1H, δ 3.30: ^{13}C, δ 49.0; $CDCl_3$: ^1H 7.26 ppm; ^{13}C 77.0 ppm). Short- and long-range ^1H-^{13}C correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments respectively. NOE correlations were detected with ROESY experiments with a 0.5 s

mixing time. The high-resolution ESI mass spectra performed on an APEX II FTICR mass spectrometer equipped with a 4.7 T magnet (Bruker-Daltonics, Bremen, Germany) were obtained from the University of Georgia Proteomic and Mass Spectrometry Core Facility. HPLC purifications were performed on Beckman System Gold HPLC system with a 168 UV detector and a SEDEX 85 (Sedere, Alfortville, France) evaporative light scattering detector. Thin layer chromatography (TLC) analyses were performed using Merck Kieselgel (Aufoilen) 60 F_{254} plates. TLC plates were visualized by spraying with 1:1 MeOH:H_2SO_4.

Collection, Extraction, and Isolation. The gorgonian *Briareum asbestinum* was collected by hand using SCUBA at a depth of 15 m at Hillsboro Ledge, Boca Raton Florida, in 2007. The specimen was immediately frozen and kept at -20 °C until extraction. A voucher specimen has been deposited in Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton (BR02-002). The specimens of *Briareum asbestinum* (150 g wet wt.) were extracted with MeOH (3 x 400 mL) for 18 h. The third, second and then the first extracts were passed through a column of HP-20 resin (2.5 x 25 cm) equilibrated with MeOH. The combined eluents were diluted with H_2O (3.6 L) and passed again through the column. The column was eluted with 400 mL fractions of (1) H_2O, (2) 40% Me_2CO/H_2O, (3) 75% Me_2CO/H_2O and (4) Me_2CO. Fraction 3 was back-loaded onto an HP-20 column to remove the H_2O by passing the fraction through a column of HP-20 resin (2.5 x 8.0 cm) equilibrated with H_2O. The eluent was diluted with H_2O (500 mL) and passed again through the column. The column was eluted with Me_2CO (250 mL), and then 50% MeOH/Me_2CO (250 mL), and the combined fractions concentrated to dryness. Fraction 3 was subjected to preparative C18 reversed-phase HPLC (Gemini 5 μm; 21.2 x 250 mm; 6 mL/min; 40–100% CH_3CN/H_2O over 60 min) to give briareolate ester L (**1**, 12.0 mg),

briareolate ester N (**3**, 9.0 mg), briareolate ester G (**4**, 15.0 mg), briareolate ester B (**5**, 13.0 mg), and briareolate ester C (**6**, 14.0 mg). Fraction 4 was concentrated to dryness and was subjected to column chromatography on silica gel 60 Merck (70-230 mesh, 80 g), eluting with n-hexane–EtOAc (from 100:0 to 0:100 gradient) to afford 13 fractions of 50 mL each. The constituents of fractions 7–9 were further purified by semi-preparative C18 reversed-phase HPLC (Gemini 5 μm; 10.0 x 250 mm; 4 mL/min; 50–100% CH_3CN/H_2O over 60 min) to yield briareolate ester M (**2**, 5.0 mg). Compounds **4 – 6** were identified by comparison of their ^{1}H and ^{13}C NMR spectra with the literature data.

Briareolate ester L (1): Colorless oil; $[\alpha]^{25}_{D}$ +32 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} 237 nm (ε 2075), 284 (ε 1276); IR (KBr) ν_{max} 3500, 2933, 1733, 1650, 1456, 1367, 1248, 1174, 1024 cm^{-1}; ^{1}H and ^{13}C NMR (500 MHz, CD_3OD) see Table 1. HRESIMS *m/z* 515.2596 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_8Na$, 515.2621).

Briareolate ester M (2): Colorless oil; $[\alpha]^{25}_{D}$ +133 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} 232 nm (ε 3298), 284 (ε 2043); IR (KBr) ν_{max} 2929, 1734, 1649, 1457, 1368, 1249, 1172, 1089 cm^{-1}; ^{1}H and ^{13}C NMR (500 MHz, $CDCl_3$) see Table 1. HRESIMS *m/z* 613.3350 $[M + Na]^+$ (calcd for $C_{33}H_{50}O_9Na$, 613.3353).

Briareolate ester N (3): Colorless oil; $[\alpha]^{25}_{D}$ +150 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} 282 nm (ε 5891); IR (KBr) ν_{max} 3500, 2933, 1733, 1650, 1456, 1367, 1248, 1174, 1024 cm^{-1}; ^{1}H and ^{13}C

NMR (500 MHz, $CDCl_3$) see Table 1**.** HRESIMS *m/z* 613.3350 $[M + Na]^+$ (calcd for $C_{33}H_{50}O_9Na$, 613.3353).

Cell Culture. BG02 hESCs were grown in defined medium, containing 10 ng/mL HRG1β (Peprotech), 10 ng/mL ActA (R&D Systems), 200 ng/mL LR^3-IGF1 (JRH Biosciences), and 8 ng/mL FGF2 (Sigma or R&D Systems). Cultures were passaged with Accutase (Innovative Cell Technologies) and plated on tissue culture flasks coated with growth factor-reduced matrigel (BD Biosciences) diluted 1:200, as described by Robins and Schulz.[1] BxPC-3 pancreatic adenocarcinoma cells were acquired from ATCC and cultured in the same defined medium containing 10 ng/mL HRG1β (Peprotech), 200 ng/mL LR^3-IGF1 (JRH Biosciences), and 10 ng/mL EGF (R&D Systems).[2] BxPC-3 cells were passaged using Accutase and plating on growth factor-reduced matrigel (BD Biosciences) diluted 1:200.

RT-CES Cytotoxicity Assays. The xCELLigence real-time impedance system (Roche) was used to monitor the effects of compounds on cells.[3] BG02 and BxPC-3 cells were plated at 10^4 and 2 x 10^4 cells/well, respectively, in matrigel coated plates. The cultures were placed in the reader station under standard humidified conditions and incubated at 37°C with 5% CO_2. The media was changed every 24 h and impedance was measured every 15 min for 3 days. Compounds were added to quadruplicate wells 24 hours after plating. Vincristine was used as a positive control for cytotoxicity (EC_{50} = 9.8 nM against BG02 cells), and DMSO alone was used as a negative control. Cell index plots were normalized immediately prior to addition of compound, and EC_{50} values were generated using the xCELLigence analysis software RTCA ver 1.1 after 2 days of treatment.

Reaction of Briareolate ester L (1) with Thiophenol. A solution of briareolate L **1** (10 μM) and thiophenol (20 μM) in methanol-d_4 (600 μL) was prepared at 23°C. The reaction was monitored with ^{1}H NMR every minute. After was 5 min at 23°C **1** was converted to **7a-SPh.** The NMR data reported below was then recorded; ^{1}H NMR (CD_3OD, 400 MHz): δ 5.58 (br d, *J* = 8.4, H-2), 2.44 (m, H-3α), 1.27 (m, H-3β), 2.21 (m, H_2-4), 5.32 (d, *J* = 7.6 Hz, H-6), 5.02 (d, *J* = 7.2 Hz, H-7), 3.05 (d, *J* = 10.8 Hz, H-10), 2.25 (m, H-11), 3.80 (br d, *J* = 3.8 Hz, H-12), 2.01 (m, 13α), 1.87 (m, 13β), 4.80 (t, *J* = 3.4 Hz, H-14), 1.10 (s, H_3-15), 1.60 (s, H_3-16), 3.34 (q, *J* = 7.2 Hz, H-17), 1.50 (d, *J* = 7.2 Hz, H_3-18), 0.93 (d, *J* = 7.2 Hz, H_3-20), 1.98 (s, 3H for ester methyl), 2.21 (t, *J* = 6.4 Hz, 2H), 1.60 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ 44.8 (C-1), 81.8 (C-2), 29.9 (C-3), 34.4 (C-4), 142.3 (C-5), 126.2 (C-6), 49.6 (C-7), 114.4 (C-8), 154.3 (C-9), 42.3 (C-10), 33.7 (C-11), 70.7 (C-12), 32.3 (C-13), 75.6 (C-14), 13.8 (C-15), 27.9 (C-16), 40.8 (C-17), 16.3 (C-18), 181.4 (C-19), 17.0 (C-20), 53.3 (OCH_3) 172.8 (C-14 ester C=O), 175.2 (C-2 ester C=O), 22.1 (C-14 ester methyl), 37.7, 19.3 and 14.2 (C-2 propyl ester chain); MS (ESI) *m*/*z* 625.2 $[M + Na]^+$.

Reaction of Briareolate ester L (1) with L-Cysteine methyl ester HCl. A solution of briareolate L **1** (10 μM), L-Cys-OMe HCl (20 μM) and a catalytic amount of DMAP (5 μM) in methanol-d_4 (600 μL) was prepared at 23°C. The reaction was monitored by ^{1}H NMR spectroscopy. After was 24h at 23°C **1** was converted to **7b-L-Cys-OMe**. The NMR data reported below was then recorded. ^{1}H NMR (CD_3OD, 400 MHz): δ 5.30 (d, *J* = 8.4, H-2), 2.20 (m, H-3α), 1.78 (m, H-3β), 2.26 (m, H_2-4), 5.19 (d, *J* = 10.4 Hz, H-6), 4.15 (d, *J* = 10.4 Hz, H-7), 3.18 (d, *J* = 11.2 Hz, H-10), 1.93 (m, H-11), 3.80 (dd, *J* = 8.4, 2.4 Hz, H-12), 2.28 (m, 13α), 2.03 (m, 13β), 4.69 (t, *J* = 2.8 Hz, H-14), 1.08 (s, H_3-15), 1.86 (s, H_3-16), 2.76 (q, *J* = 7.2 Hz, H-

17), 1.36 (d, J = 6.8 Hz, H_3-18), 1.06 (d, J = 6.8 Hz, H_3-20), 1.93 (s, 3H for ester methyl), 2.17 (t, J = 6.4 Hz, 2H), 1.53 (m, 2H), 0.91 (t, J = 7.6 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ 45.5 (C-1), 80.2 (C-2), 29.2 (C-3), 30.1 (C-4), 149.1 (C-5), 119.0 (C-6), 54.5 (C-7), 63.8 (C-8), 214.0 (C-9), 48.8 (C-10), 37.5 (C-11), 69.4 (C-12), 30.9 (C-13), 73.2 (C-14), 13.4 (C-15), 28.2 (C-16), 38.0 (C-17), 15.8 (C-18), 176.7 (C-19), 11.2 (C-20), 56.8 (OCH_3) 170.5 (C-14 ester C=O), 174.0 (C-2 ester C=O), 21.8 (C-14 ester methyl), 35.7, 17.4 and 13.8 (C-2 propyl ester chain); MS (ESI) m/z 559.3 $[M + H_2O\text{-}C_3H_5NO_2]^+$.

Reversal of the 1,4 Addition Product (7a-SPh) to Briareolate ester L (1). A solution of **7a-SPh** and thiophenol in methanol-d_4 (600 μL) was passed through a column of HP20ss (1 × 2 cm). The eluent was diluted with H_2O (1 mL) and re-passed through the column. The column was washed with water and eluted with 10 mL fractions of (1) 20% Me_2CO/H_2O, (2) 40% Me_2CO/H_2O, (3) 80% Me_2CO/H_2O, and Me_2CO. The 80% Me_2CO/H_2O fraction was concentrated to dryness to afford **1**. Colorless oil; ^{1}H NMR data ($CDCl_3$, 400 MHz) see Table 1.

Photoisomerization of Briareolate ester L (1). A solution of briareolate L **1** (10 μM) in methanol-d_4 (600 μL) was prepared and placed in direct exposure to UV light (200 W). The reaction was monitored by ^{1}H NMR spectroscopy. After 120 h **1** was converted to **4**. The photolyzed solution was dried under vacuum and the NMR data was then recorded. Colorless oil; $[\alpha]^{25}_D$ −40 (c 0.07, $CHCl_3$); NMR data ($CDCl_3$, 400 MHz) as previously reported for **4**.[4]

References.

(1) Lakshmipathy, U.; Chesnut, J. D.; Thyagarajan, B. *Emerging technology platforms for stem cells*; Wiley-Blackwell: Oxford, **2009**.

(2) Tan, M. H.; Nowak, N. J.; Loor, R.; Ochi, H.; Sandberg, A. A.; Lopez, C.; Pickren, J. W.; Berjian, R.; Douglass, H. O.; Chu, T. M. *Cancer Invest.* **1986**, *4*, 15-23.

(3) Solly, K.; Wang, X.; Xu, X.; Strulovici, B.; Zheng, W. *Assay Drug Dev. Technol.* **2004**, *2*, 363-372.

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Table S1. ^{13}C NMR Data (125 MHz) for Briareolate Esters **1 – 3**

	1[a]	**2**[b]	**3**[b]
position	δ_C, mult.[c]	δ_C, mult.	δ_C, mult.
1	46.8, qC	45.2, qC	45.9, qC
2	79.9, CH	77.6, CH	78.3, CH
3	31.9, CH_2	31.7, CH_2	28.9, CH_2
4	34.8, CH_2	33.7, CH_2	35.1, CH_2
5	141.7, qC	140.4, qC	75.5, qC
6	118.5, CH	116.8, CH	130.3, CH
7	147.0, CH	145.2, CH	120.6, CH
8	148.1, qC	146.5, qC	117.8, qC
9	201.4, qC	198.9, qC	151.4, qC
10	48.4, CH	47.9, CH	37.6, CH
11	35.9, CH	33.2, CH	32.3, CH
12	70.5, CH	73.0, CH	72.4, CH
13	32.7, CH_2	29.1, CH_2	31.4, CH_2
14	76.0, CH	70.3, CH	74.3, CH
15	12.6, CH_3	14.7, CH_3	15.3, CH_3
16	27.1, CH_3	26.8, CH_3	24.0, CH_3
17	40.2, CH	38.9, CH	38.0, CH
18	16.8, CH_3	16.2, CH_3	14.7, CH_3
19	176.5, qC	174.5, qC	174.9, qC
20	17.6, CH_3	16.6, CH_3	15.4, CH_3
OCH_3	52.5, CH_3	52.0, CH_3	52.3, CH_3
ester at C-2	175.5, qC	173.2, qC	173.1, qC
	37.7, CH_2	36.3, CH_2	36.7, CH_2
	19.7, CH_2	18.2, CH_2	18.6, CH_2
	14.5, CH_3	14.1, CH_3	14.1, CH_3
ester at C-12		173.1, qC	172.9, qC
		34.7, CH_2	35.2, CH_2
		24.9, CH_2	25.1, CH_2
		31.3, CH_2	31.7, CH_2
		22.4, CH_2	22.5, CH_2
		15.8, CH_3	14.2, CH_3
ester at C-14	173.2, qC	170.1, qC	170.3, qC
	22.3, CH_3	21.4, CH_3	21.3, CH_3

[a] Measured in CD_3OD. [b] Measured in $CDCl_3$. [c] Multiplicities were assigned by a multiplicity-edited gHSQC.

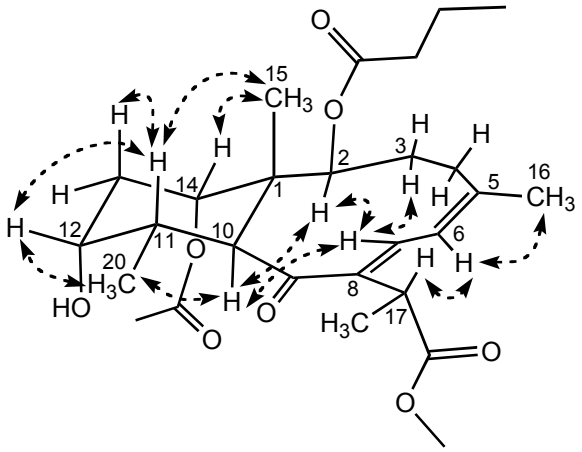


Figure S1. Key NOE correlations observed for briareolate ester L (**1)**

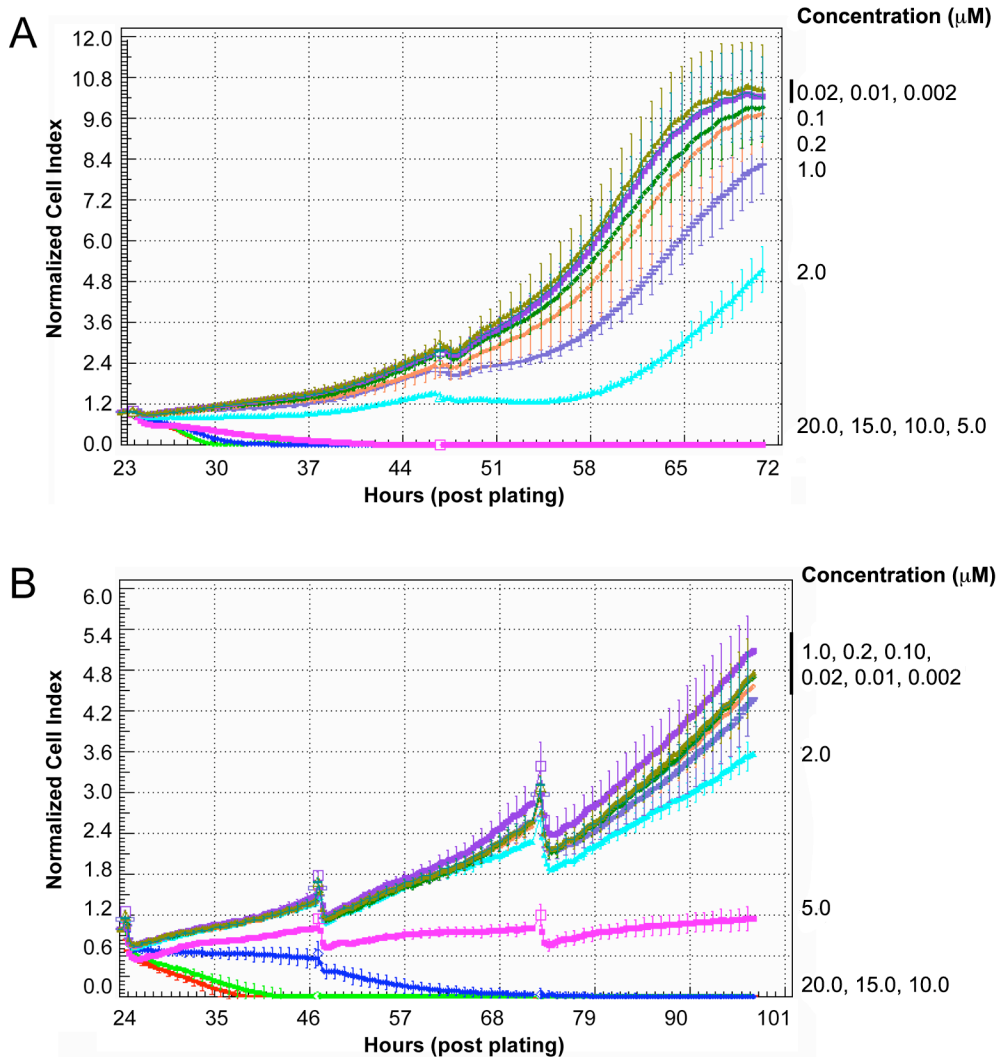


Figure S2. Evaluation of cytotoxic responses of briareolate ester L (**1**) against (**A**) human embryonic stem (BG02) cells, and (**B**) a pancreatic cancer (BxPC-3) cell line monitored by the RT-CES system. The error bars indicate the corresponding standard deviation.

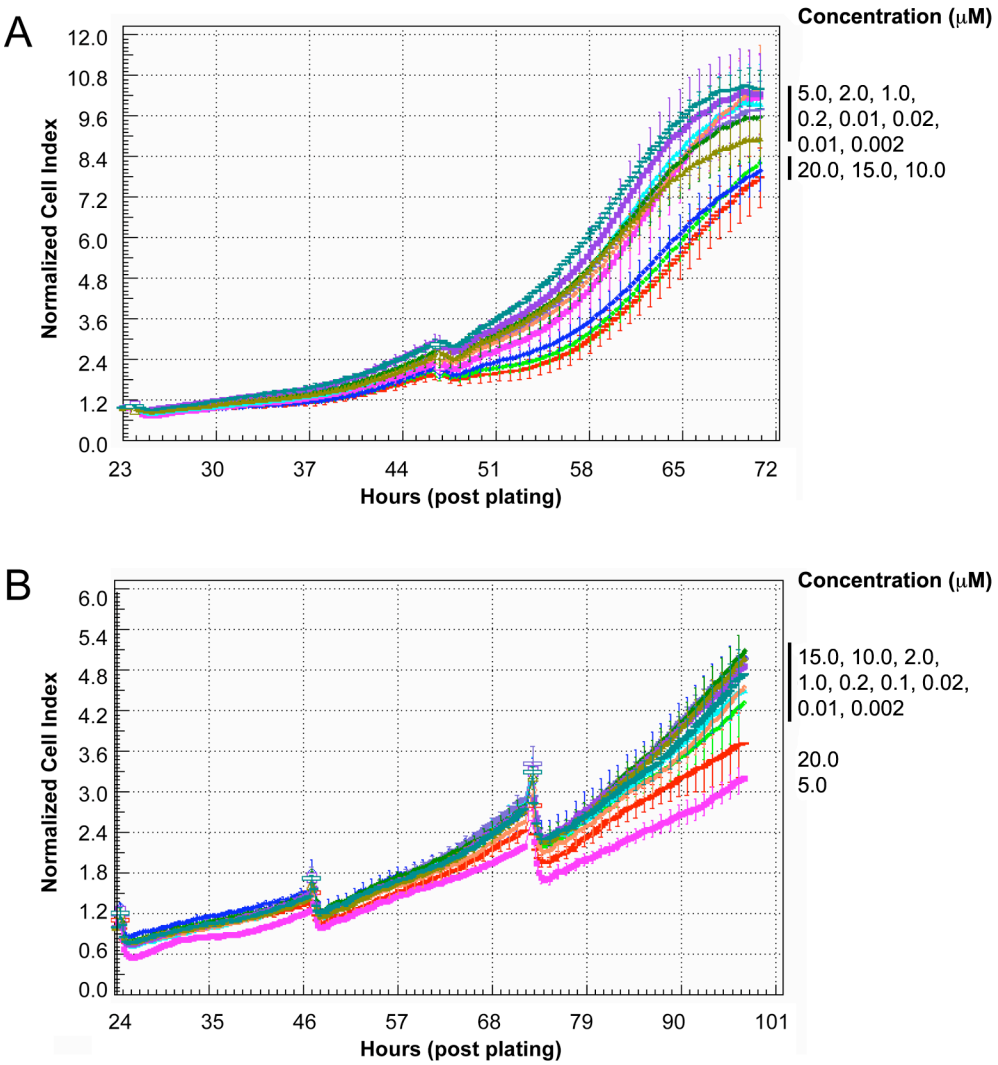


Figure S3. Evaluation of cytotoxic responses of briareolate ester G (**4**) against (**A**) human embryonic stem (BG02) cells, and (**B**) a pancreatic cancer (BxPC-3) cell line monitored by the RT-CES system. The error bars indicate the corresponding standard deviation.

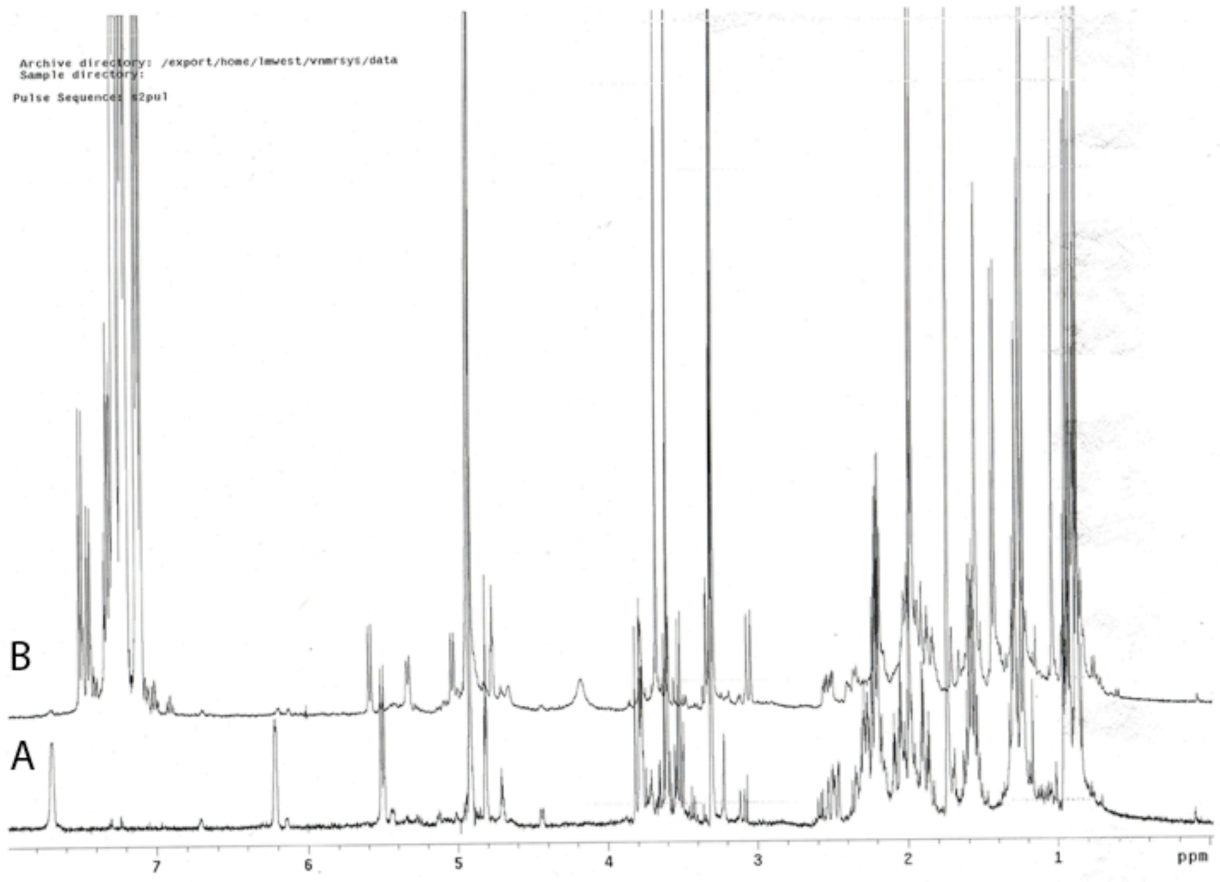


Figure S4. Reaction of briareolate ester L (**1**) with thiophenol. **A**) ^{1}H NMR spectrum (CD_3OD, 400MHz) of **1**. **B**) ^{1}H NMR spectrum recorded 5 min after the addition of thiophenol.

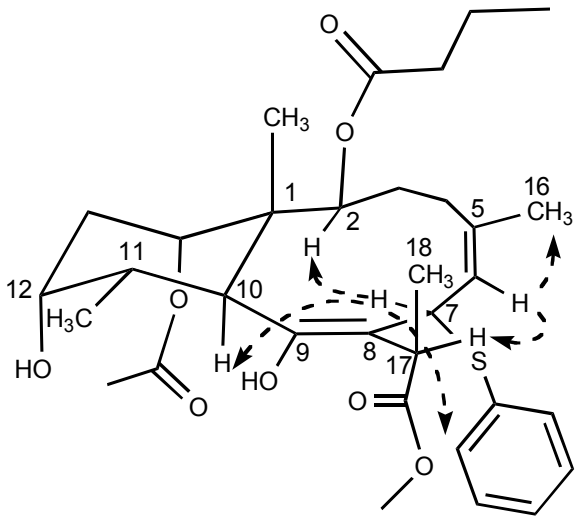


Figure S5. Key NOE correlations observed for the thiophenol addition product (**7a**-**SPh**).

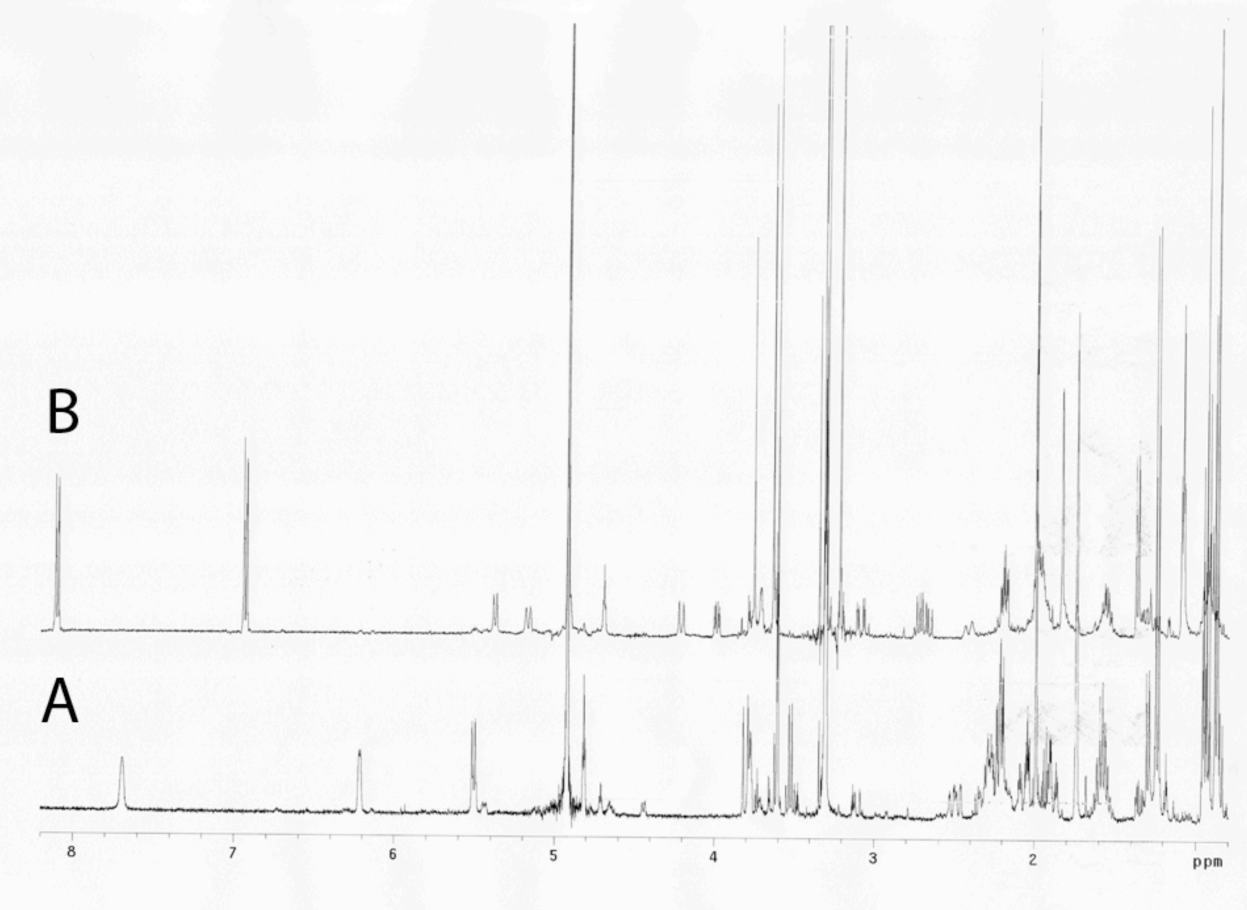


Figure S6. Reaction of briareolate ester L (**1**) with cysteine methyl ester in the presence of DMAP. **A**) ^{1}H NMR spectrum (CD_3OD, 400MHz) of **1**. **B**) ^{1}H NMR spectrum recorded 24h after the addition of cysteine methyl ester and DMAP.

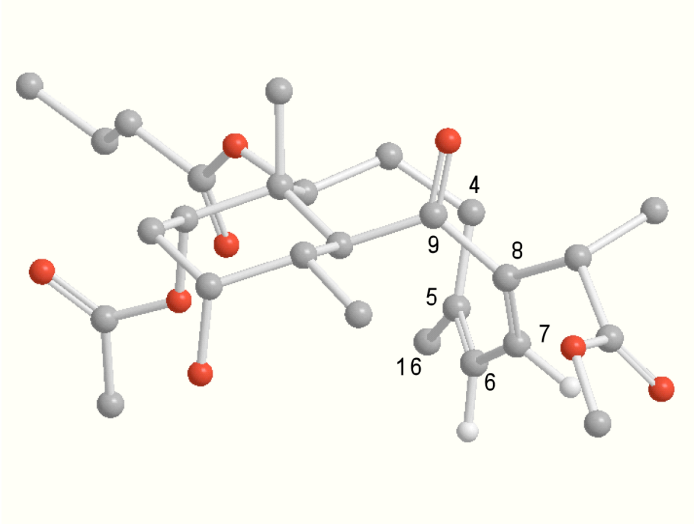


Figure S7. Optimized molecular structure of briareolate ester G **(4).**

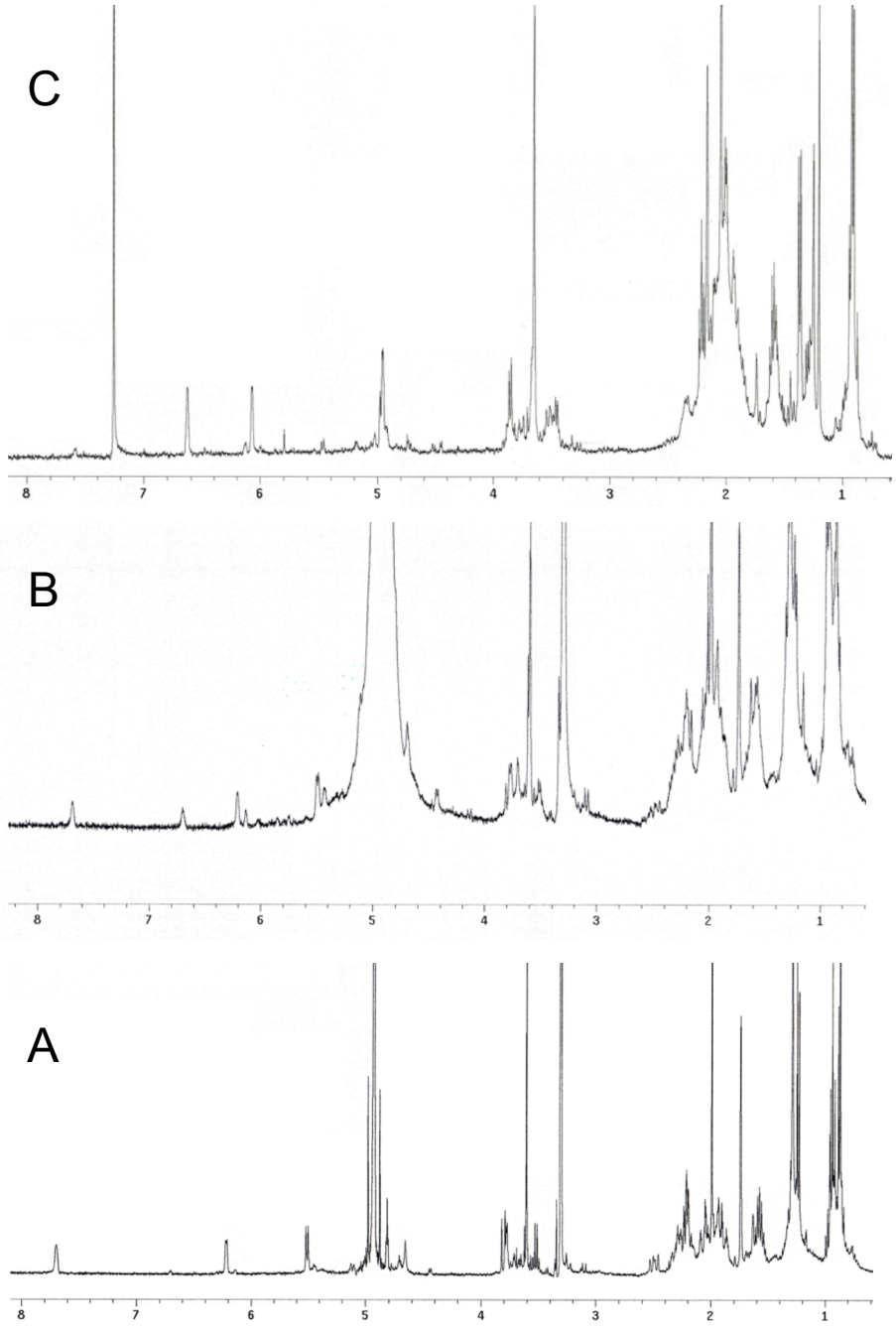
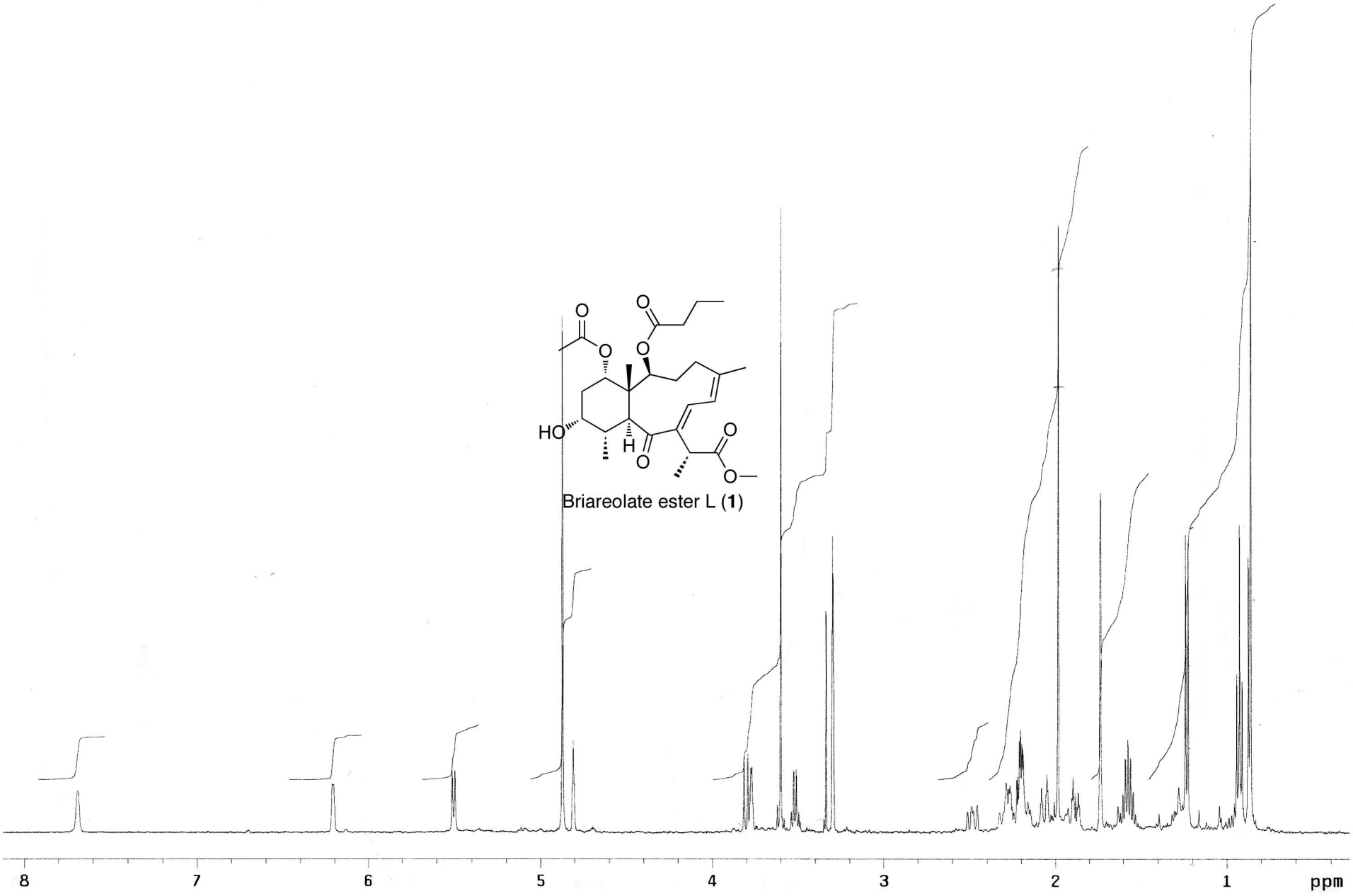
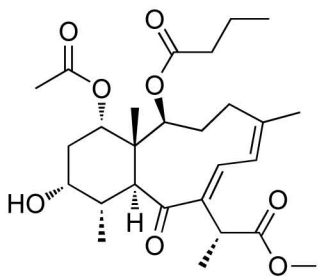


Figure S8. Photoisomerization of briareolate ester L (**1**). **A**) ^{1}H NMR spectrum (CD_3OD, 400MHz) of **1** recorded after 4 h. **B**) ^{1}H NMR spectrum (CD_3OD, 400MHz) recorded after 48 h. **C**) ^{1}H NMR ($CDCl_3$, 400MHz) spectrum recorded after 120 h.

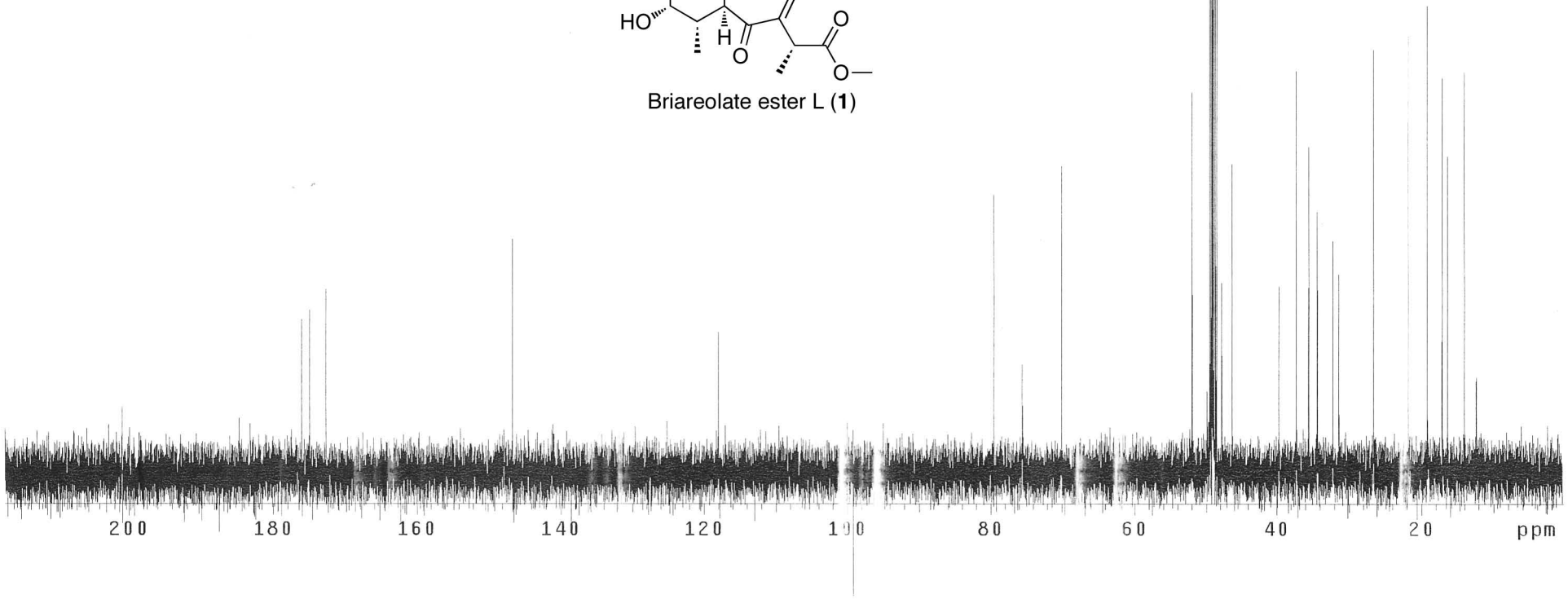
^{1}H NMR spectrum of Briareolate ester L (**1**) (CD_3OD, 500 MHz)



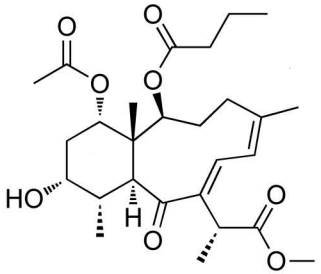
^{13}C NMR spectrum of Briareolate ester L (**1**) (CD_3OD, 125 MHz)



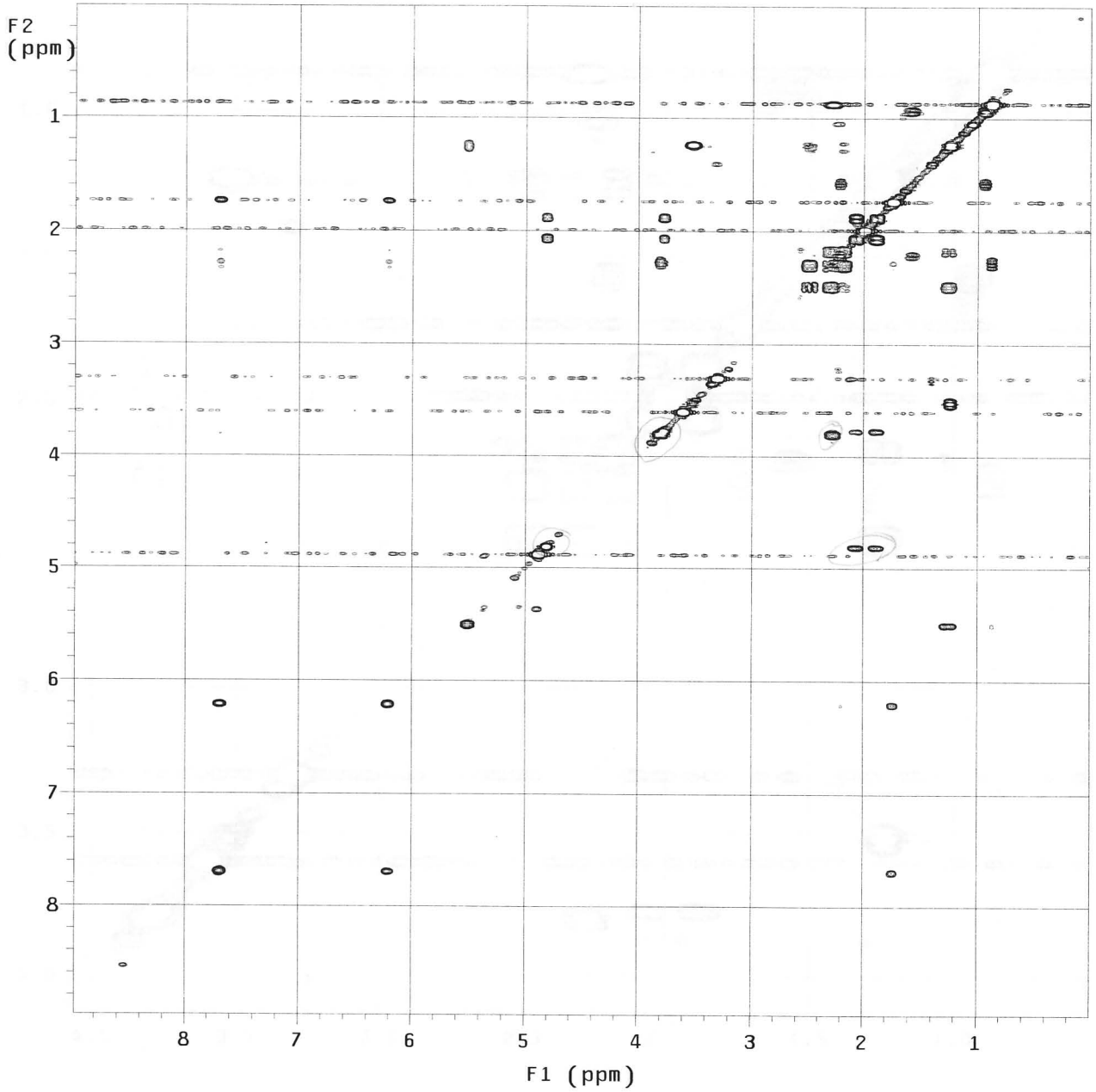
Briareolate ester L (**1**)



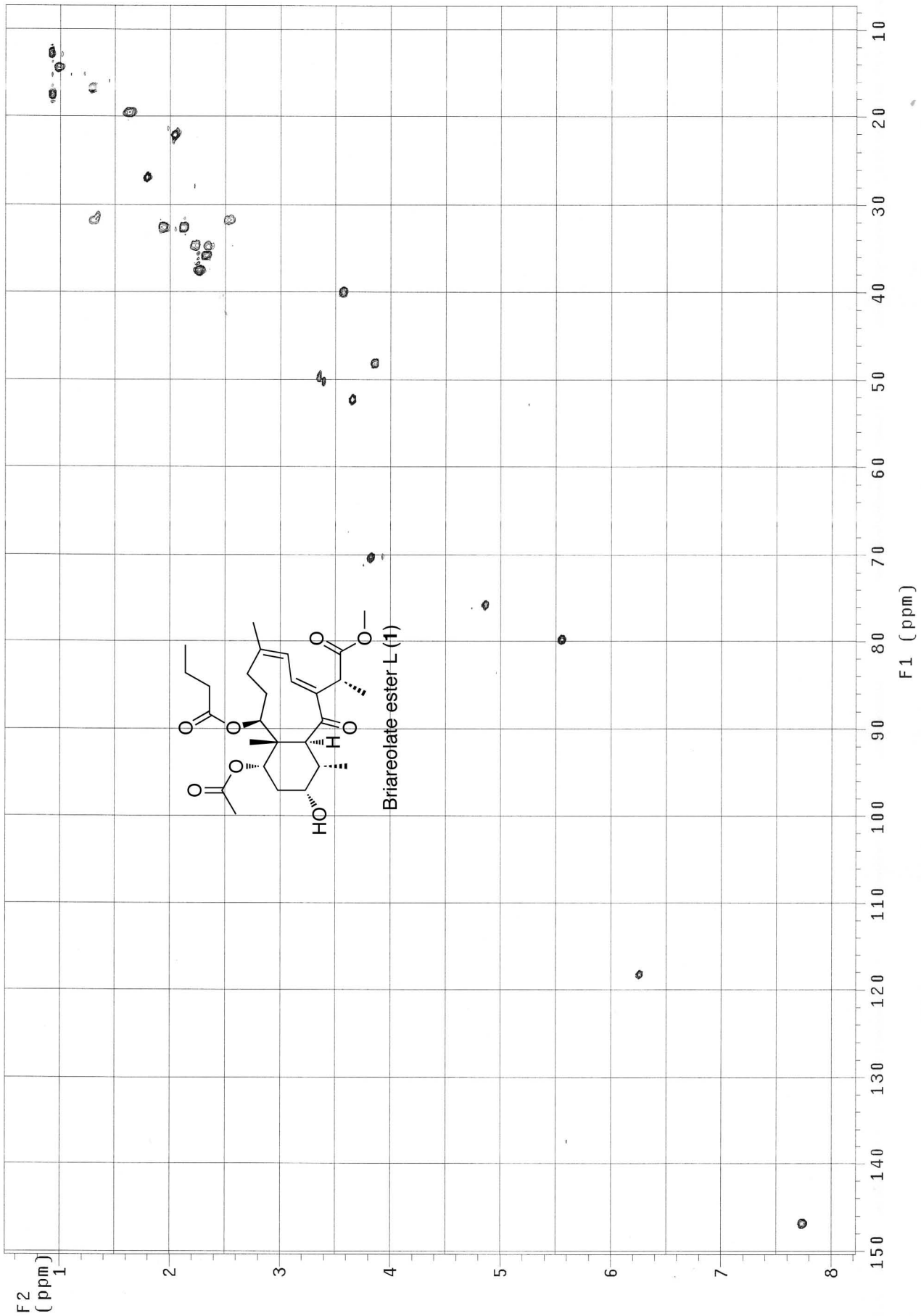
gCOSY NMR spectrum Briareolate ester L (**1**) (CD_3OD, 500 MHz)



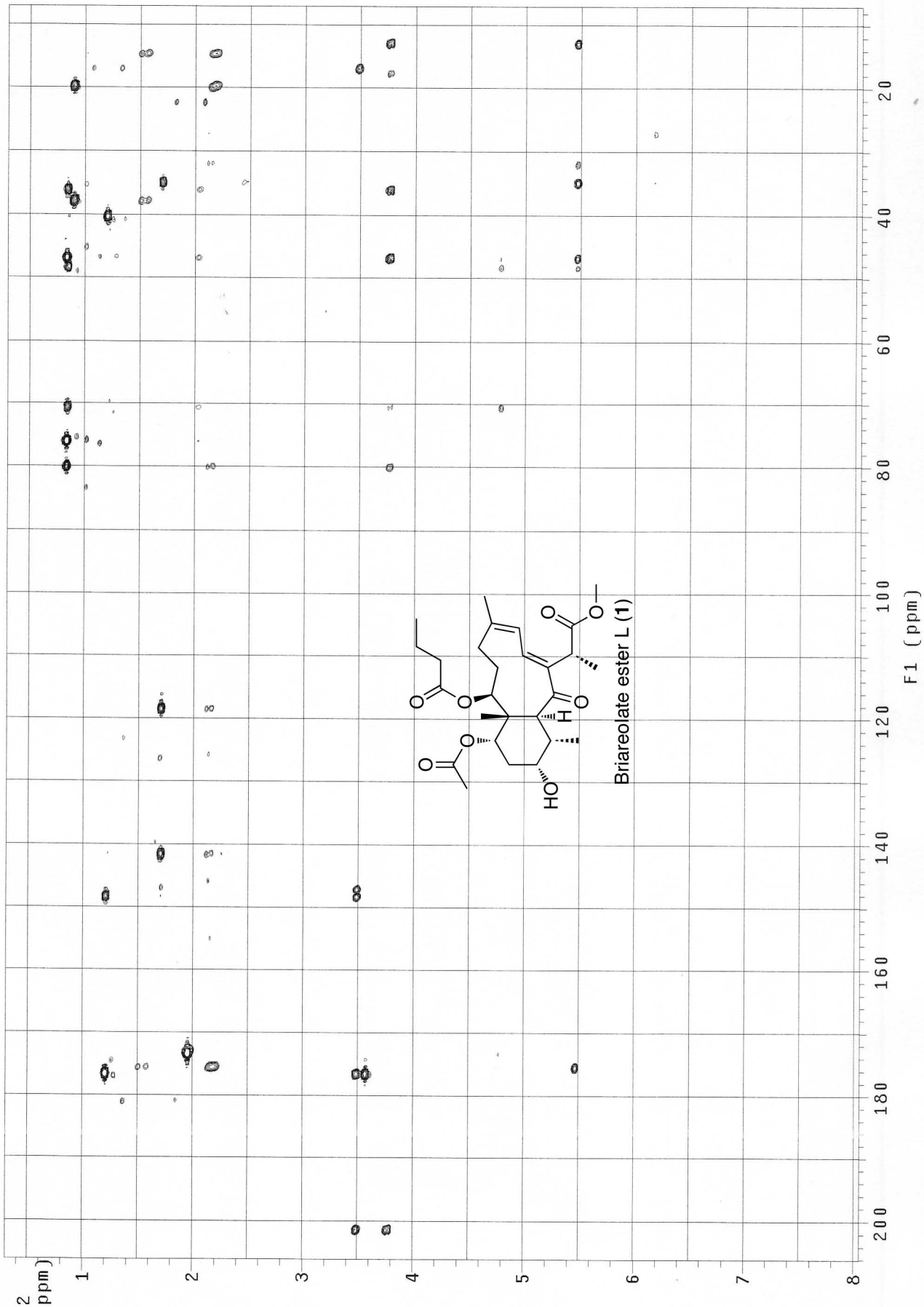
Briareolate ester L (**1**)



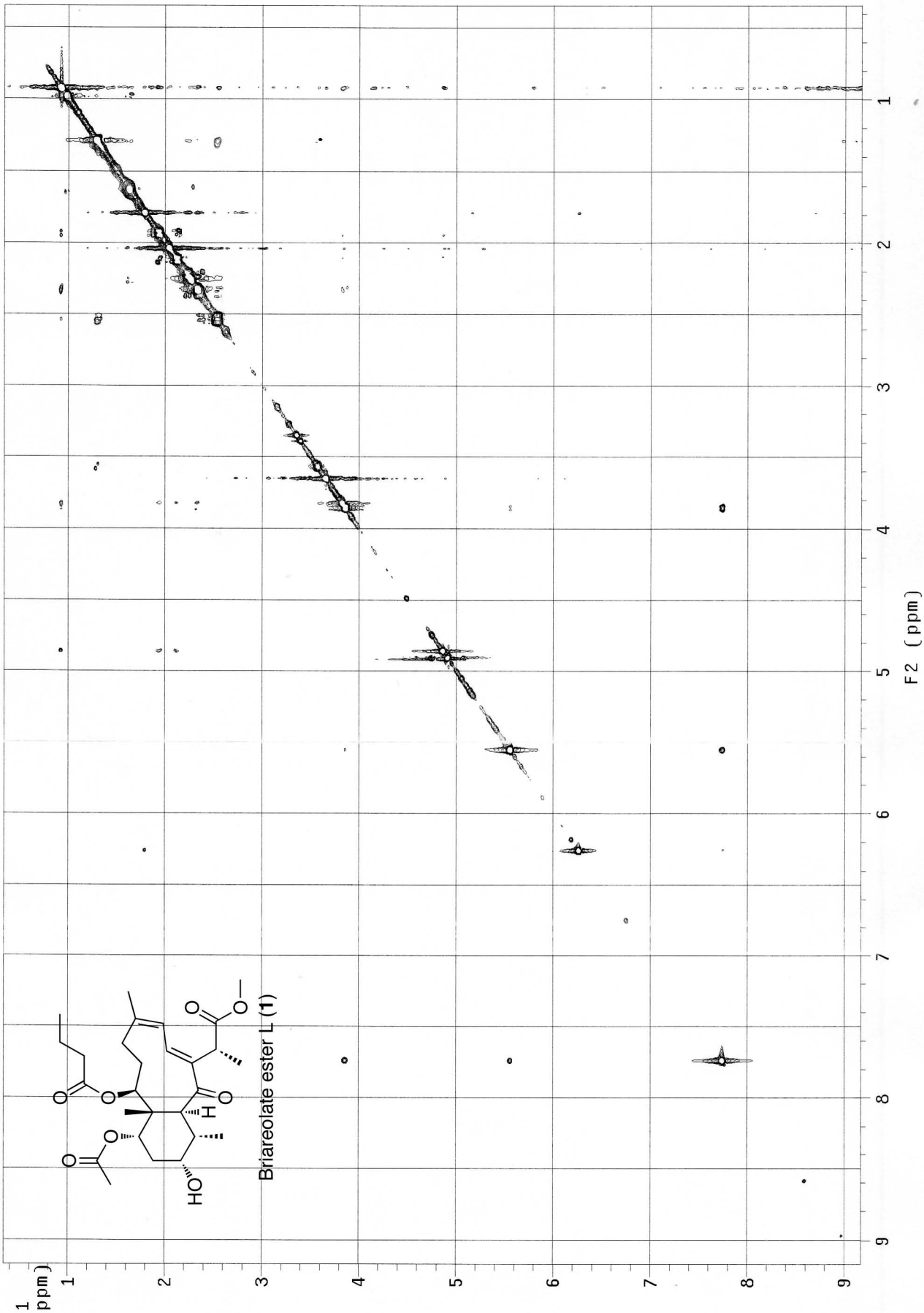
gHSQC NMR spectrum of Briareolate ester L (**1**) (CD_3OD, 500 MHz)



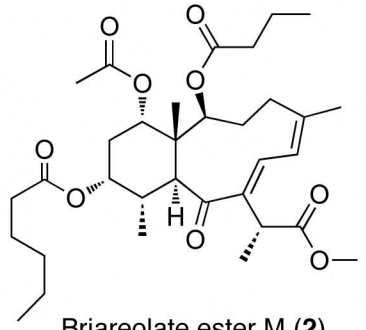
gHMBC NMR spectrum of Briareolate ester L (**1**) (CD_3OD, 500 MHz)



ROESY NMR spectrum of Briareolate ester L (**1**) (CD_3OD, 500 MHz)

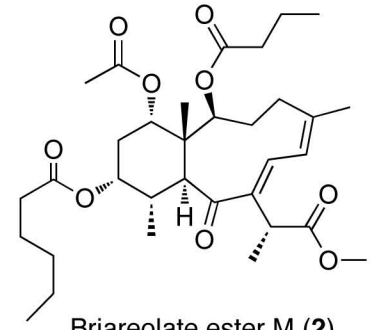


^{1}H NMR spectrum of Briareolate ester M (**2**) ($CDCl_3$, 500 MHz)

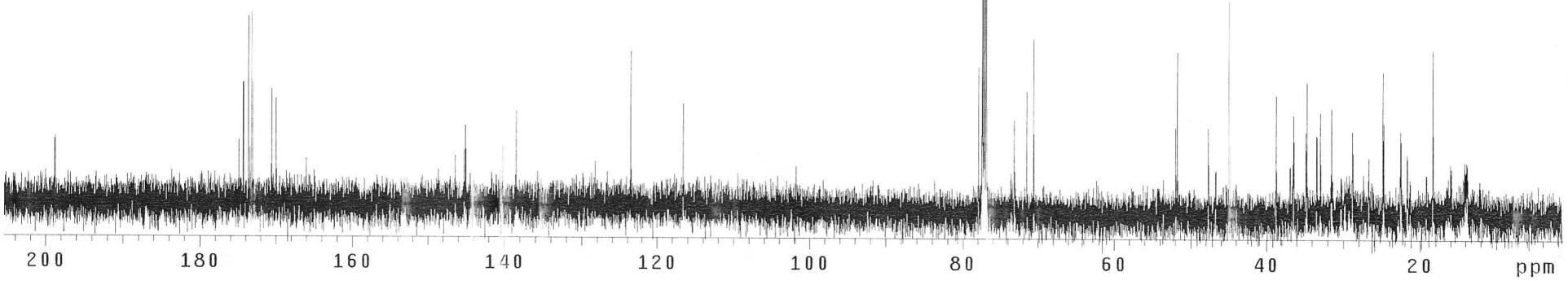


Briareolate ester M (**2**)

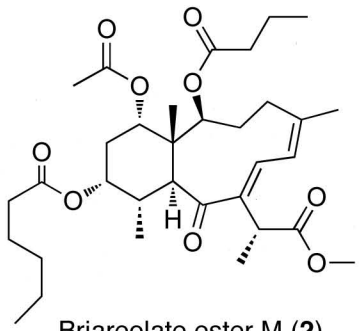
^{13}C NMR spectrum of Briareolate ester M (**2**) ($CDCl_3$, 125 MHz)



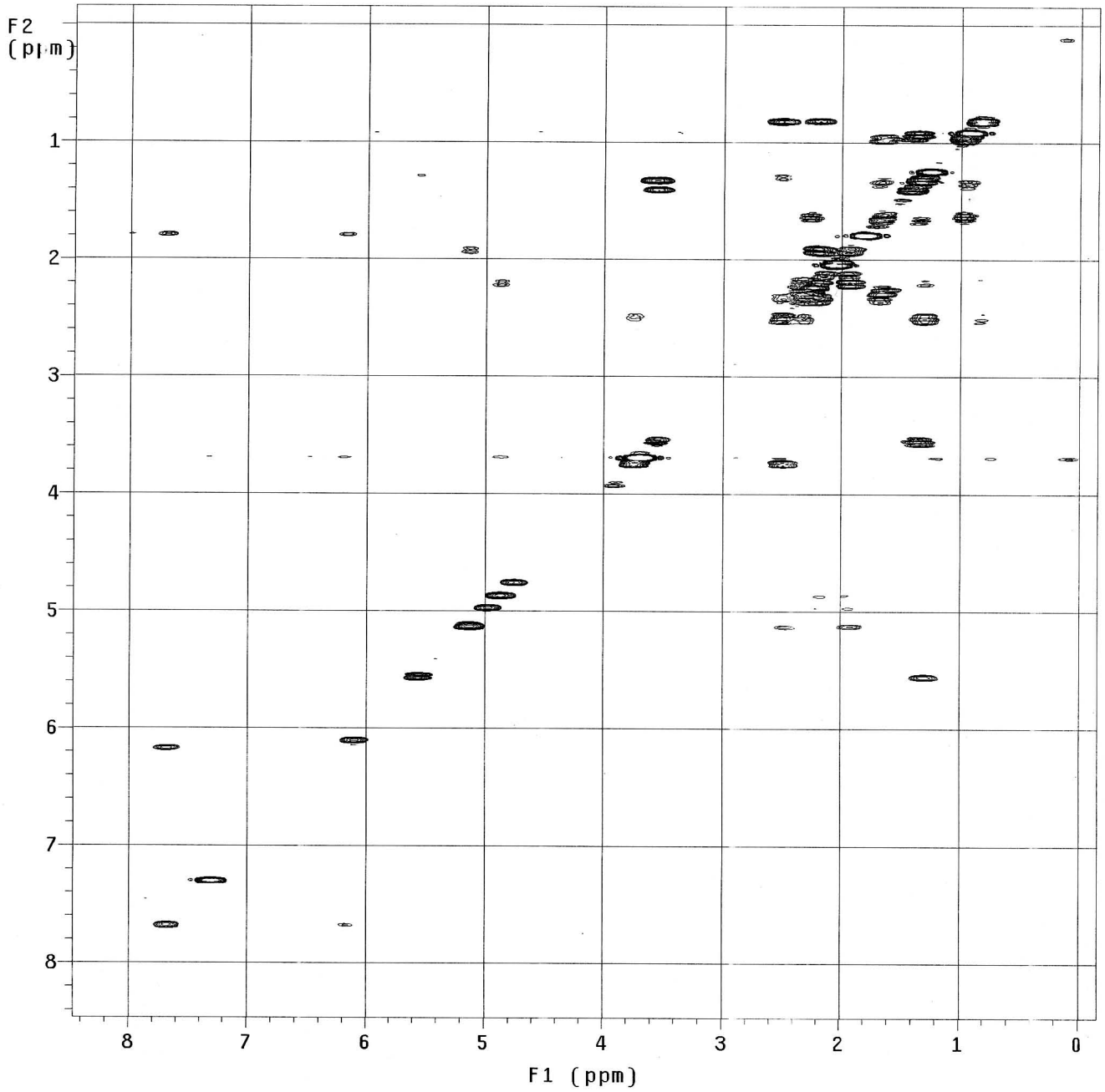
Briareolate ester M (**2**)



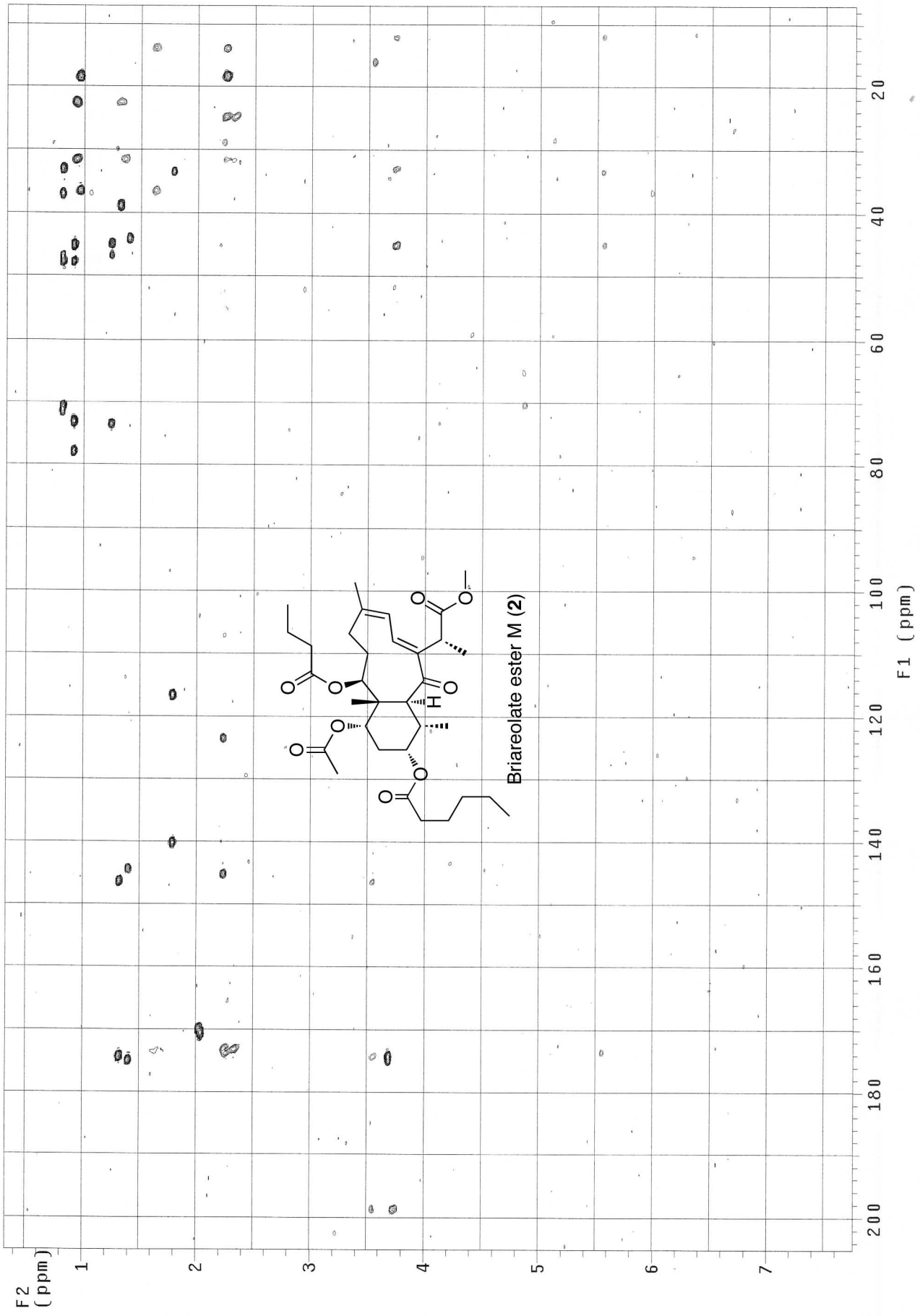
gCOSY NMR spectrum Briareolate ester M (**2**) ($CDCl_3$, 500 MHz)



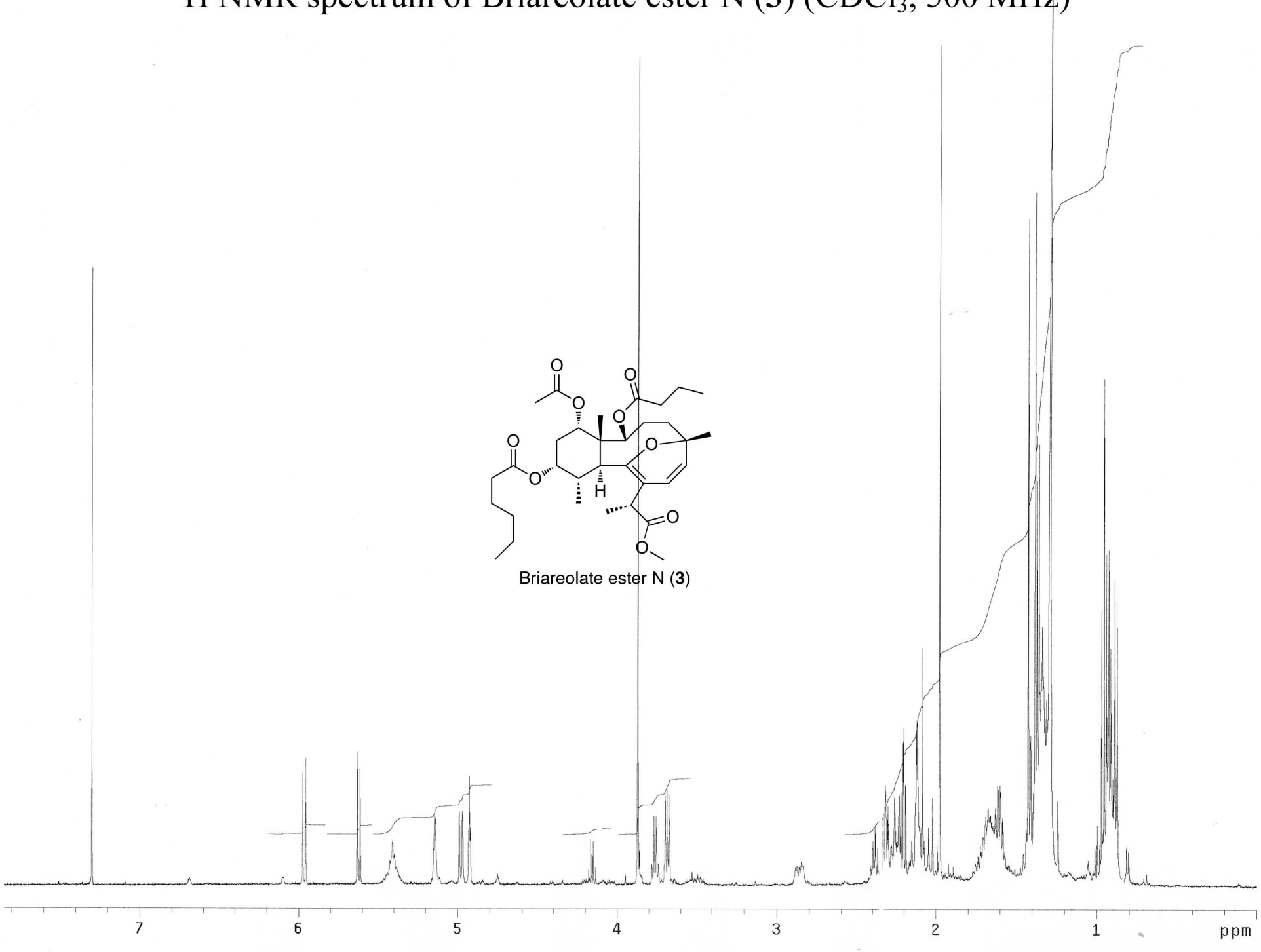
Briareolate ester M (**2**)



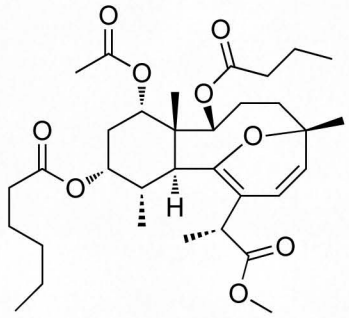
gHMBC NMR spectrum of Briareolate ester M (**2**) ($CDCl_3$, 500 MHz)



^{1}H NMR spectrum of Briareolate ester N (**3**) ($CDCl_3$, 500 MHz)

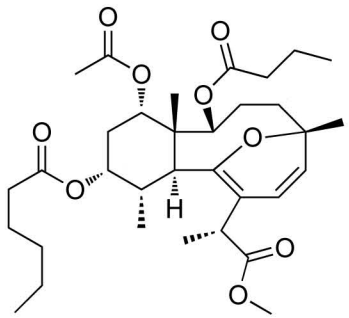


^{13}C NMR spectrum of Briareolate ester N (**3**) ($CDCl_3$, 125 MHz)

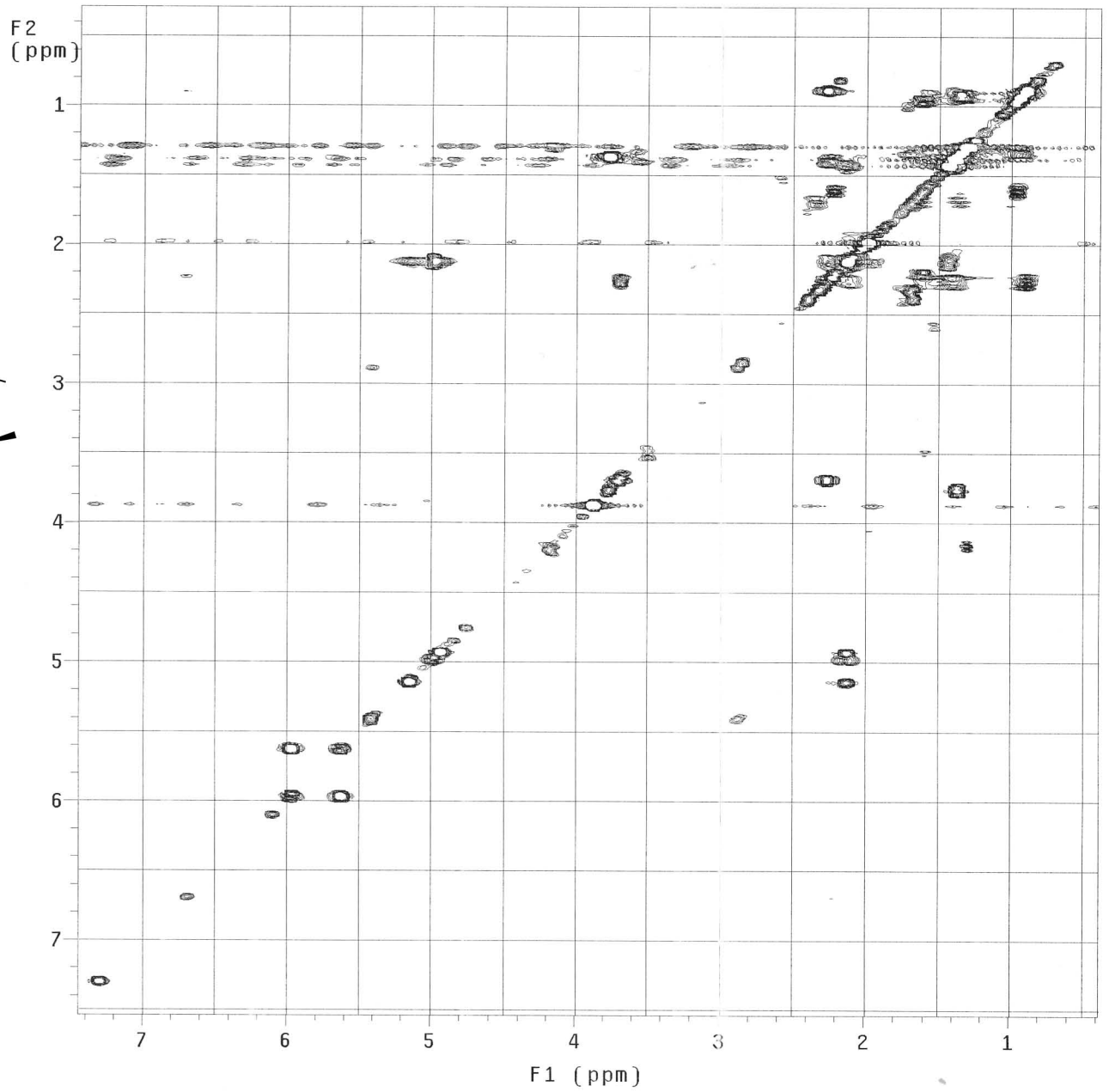


Briareolate ester N (**3**)

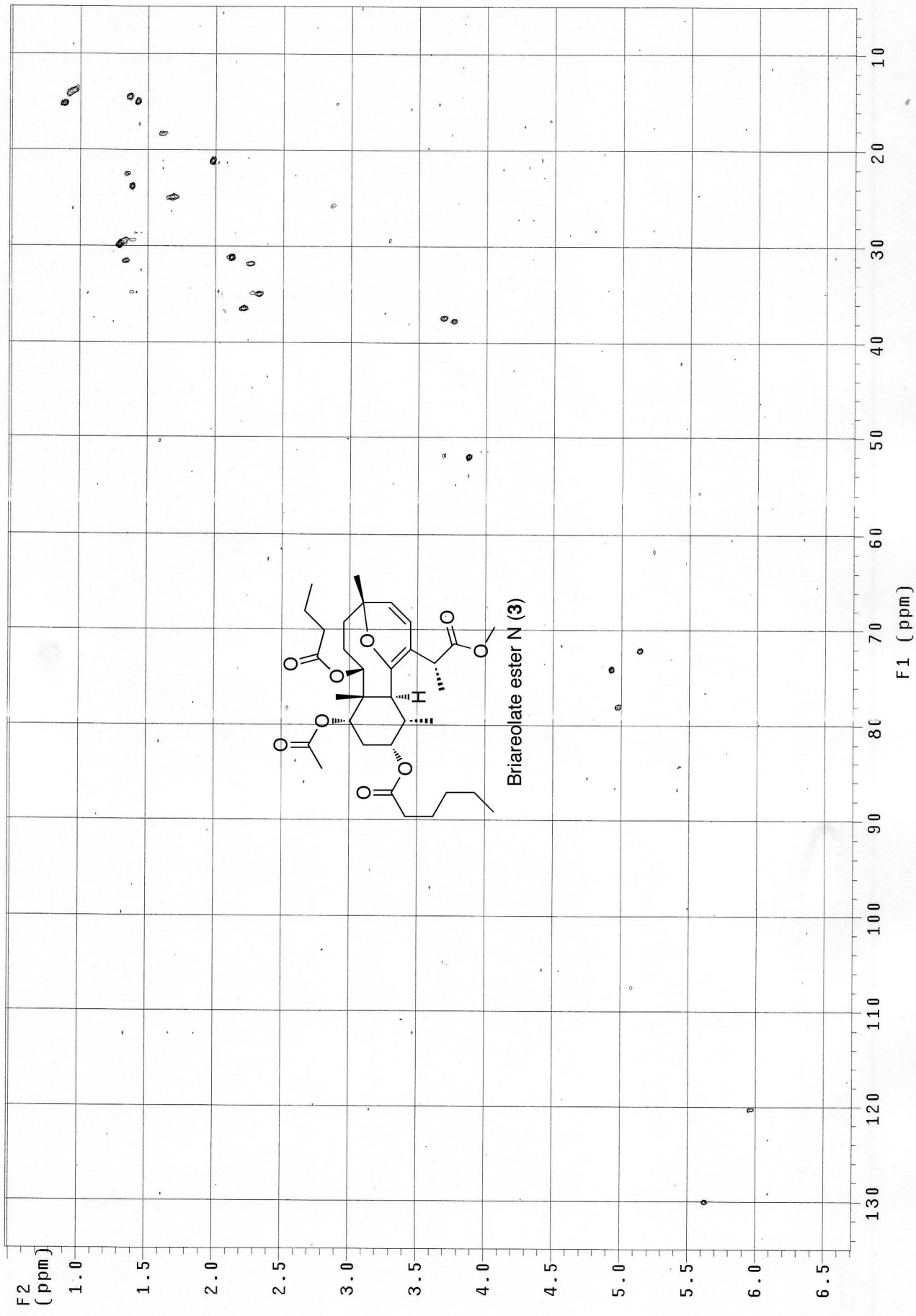
gCOSY NMR spectrum Briareolate ester N (**3**) ($CDCl_3$, 500 MHz)



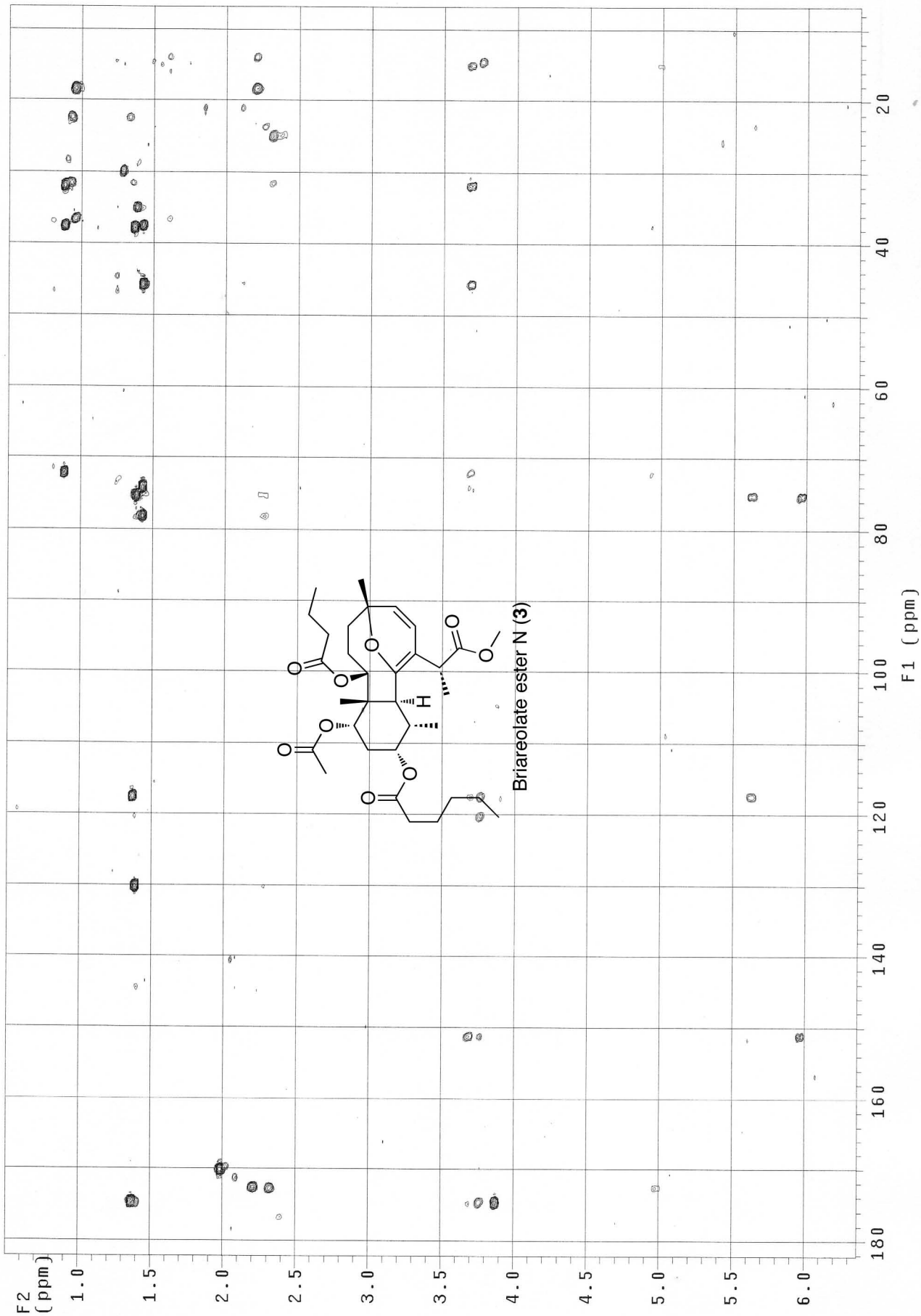
Briareolate ester N (**3**)



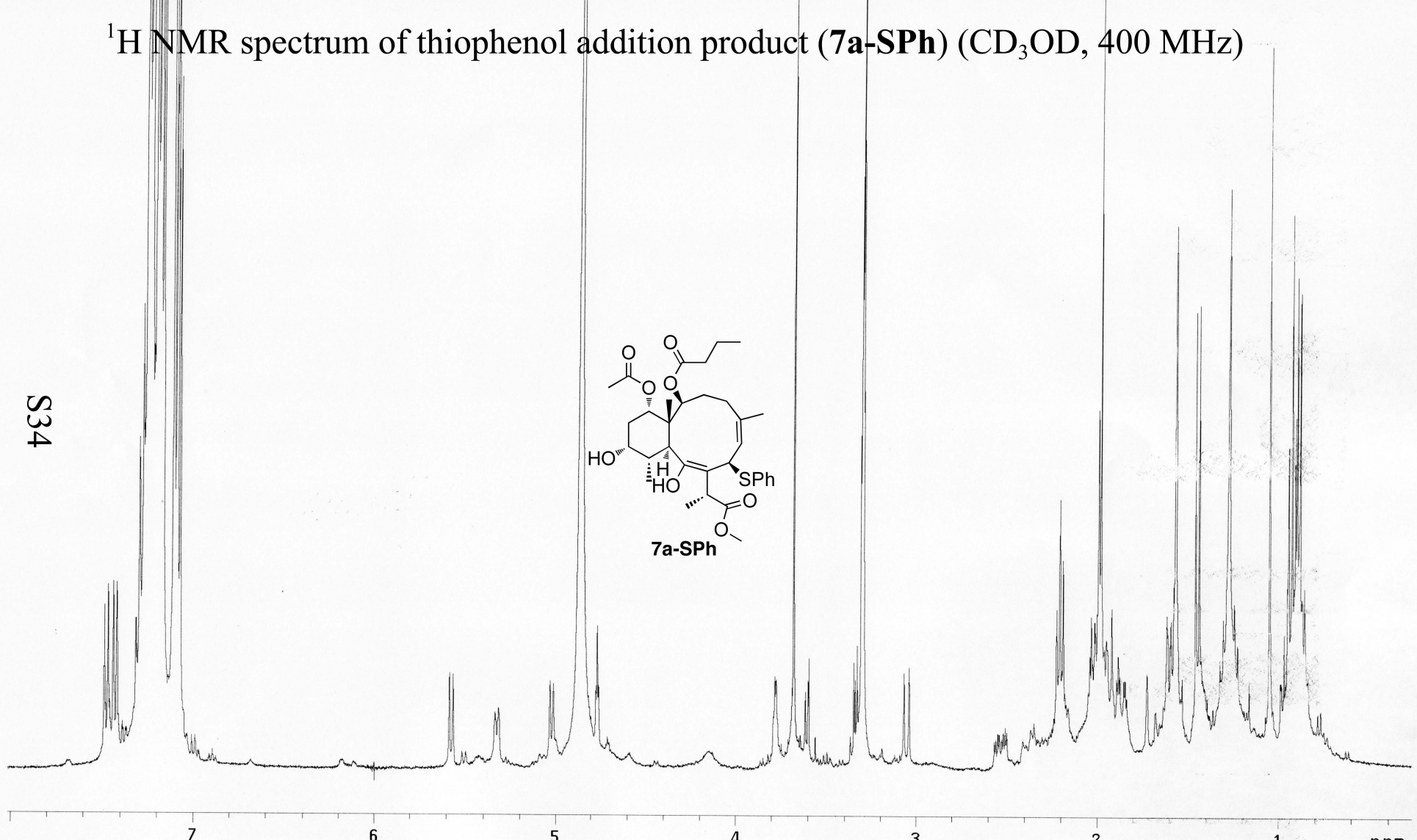
gHSQC NMR spectrum of Briareolate ester N (**3**) ($CDCl_3$, 500 MHz)



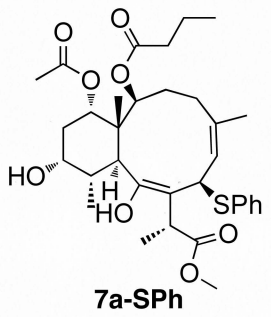
gHMBC NMR spectrum of Briareolate ester N (**3**) ($CDCl_3$, 500 MHz)



^{1}H NMR spectrum of thiophenol addition product (**7a-SPh**) (CD_3OD, 400 MHz)

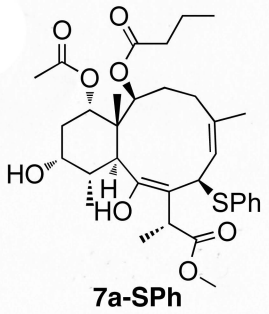


^{13}C NMR spectrum of thiophenol addition product (**7a-SPh**) (CD_3OD, 100 MHz)

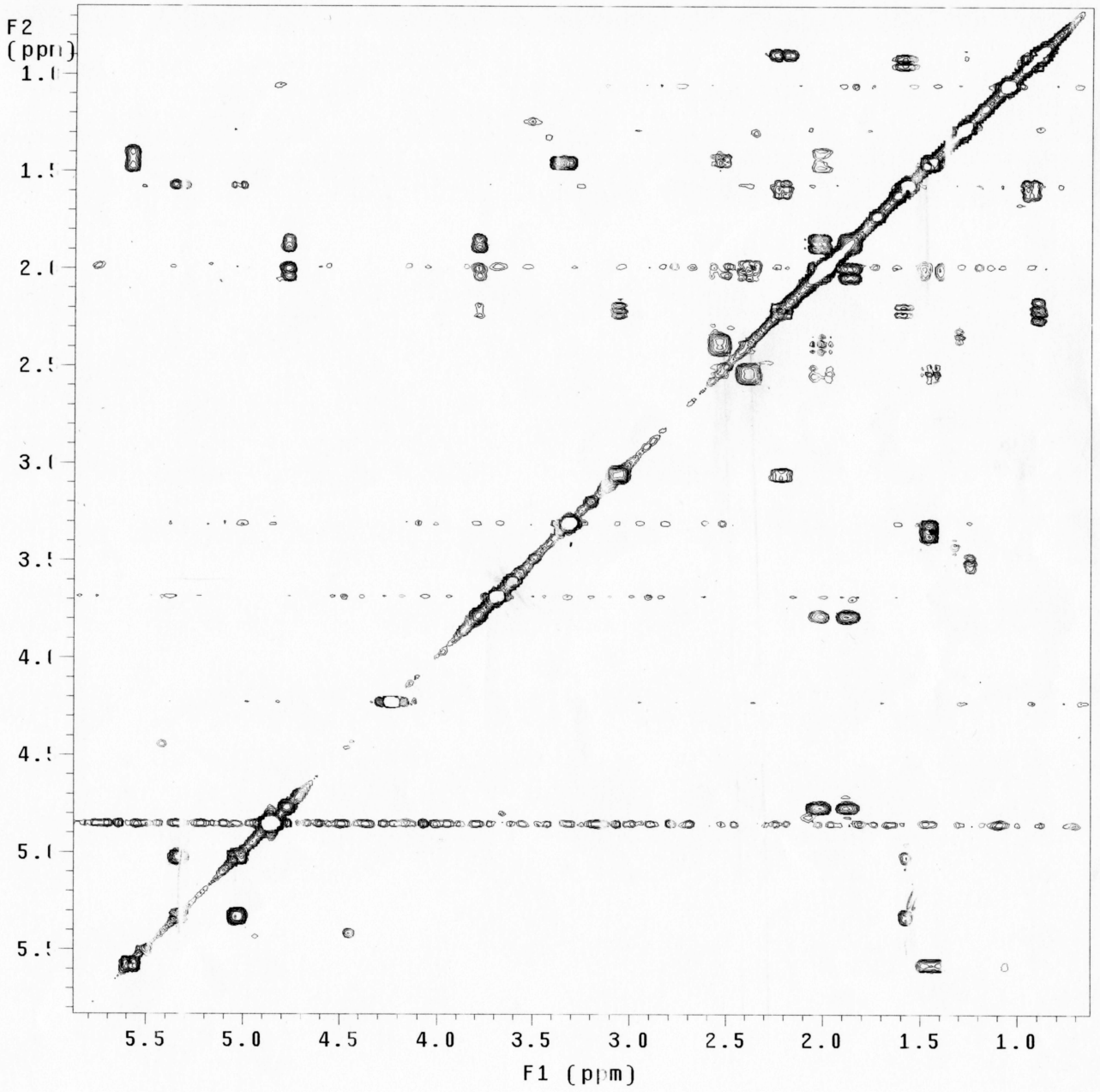


7a-SPh

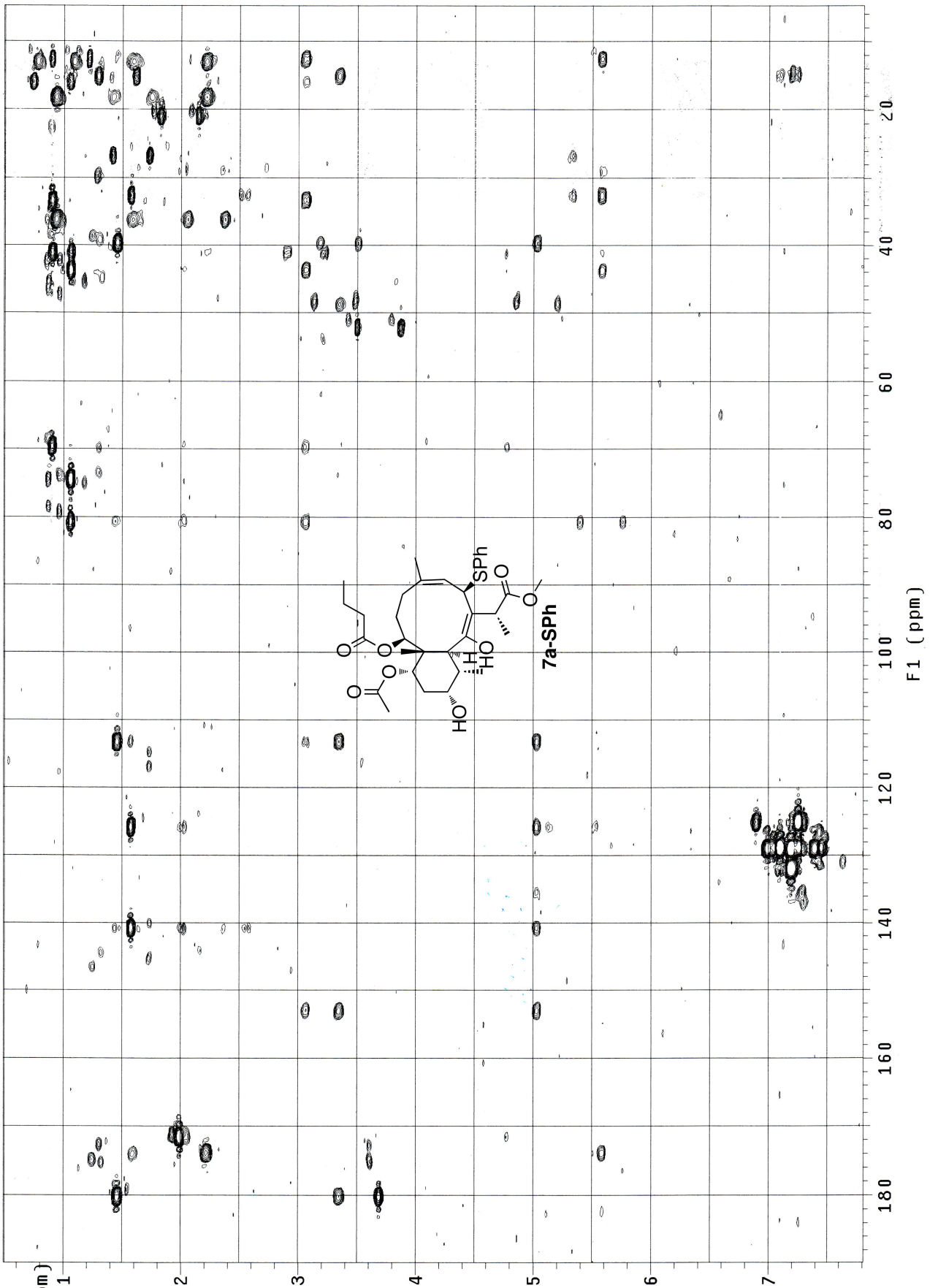
gCOSY NMR spectrum of thiophenol addition product (**7a-SPh**) (CD_3OD, 400 MHz)



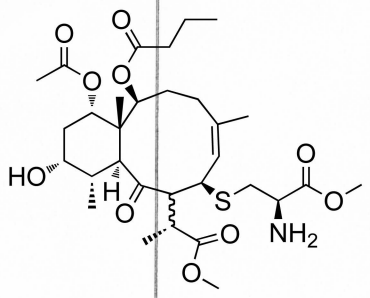
7a-SPh



gHMBC NMR spectrum of thiophenol addition product (**7a-SPh**) (CD_3OD, 400 MHz)

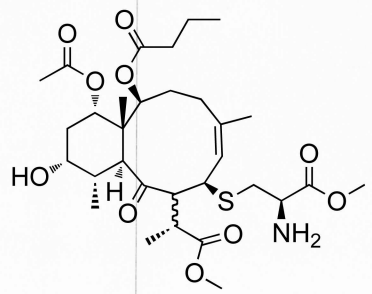


^{1}H NMR spectrum of L-Cys-OMe addition product (**7b- L-Cys-OMe**) (CD_3OD, 400 MHz)



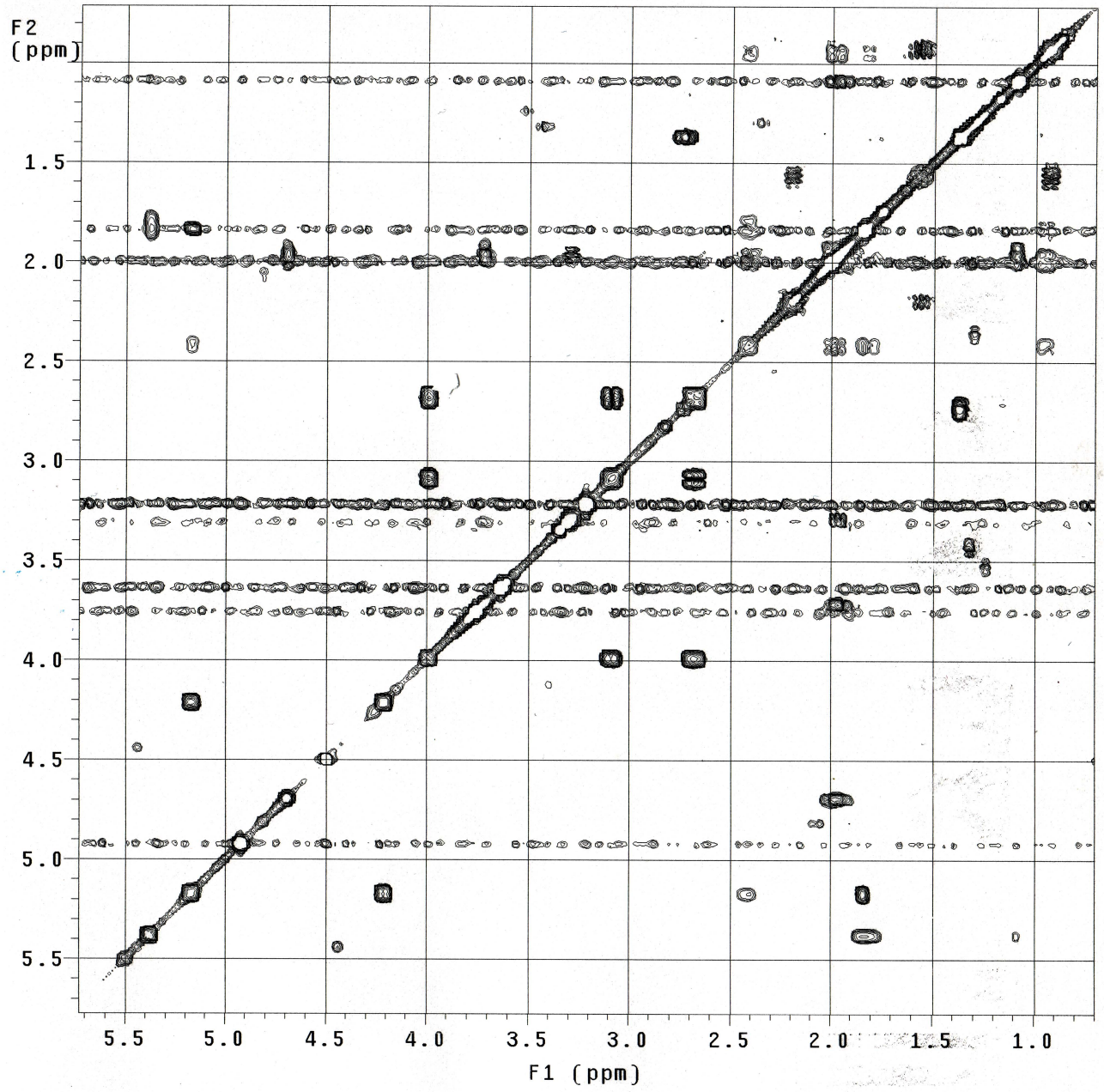
7b-L-Cys-OMe

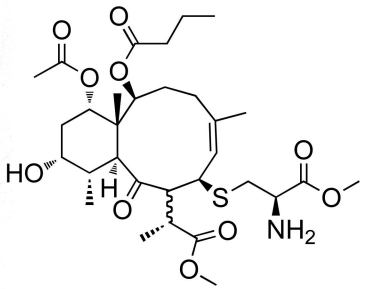
^{13}C NMR spectrum of L-Cys-OMe addition product (**7b- L-Cys-OMe**) (CD_3OD, 100 MHz)



7b-L-Cys-OMe

gCOSY NMR spectrum of L-Cys-OMe addition product (**7b- L-Cys-OMe**) (CD_3OD, 400 MHz)





7b-L-Cys-OMe

gHMBC NMR spectrum of L-Cys-OMe addition product (**7b-L-Cys-OMe**) (CD3OD, 400 MHz)