Natural Product Libraries to Accelerate the High Throughput Discovery of Therapeutic Leads

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[Supporting Information]

[Contents]

Experimental Data of Known Compounds:

Latrunculol A (1): white oil; ^{1}H NMR data see Figure S6, Supporting Information; LRESITOFMS *m/z* 438.1 [M-H_2O+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[27]

Latrunculone A (2): white oil; ^{1}H NMR data see Figure S7, Supporting Information; LRESITOFMS *m/z* 436.2 [M-H_2O+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[27]

Latrunculol B (3): white powder; ^{1}H NMR data see Figure S8, Supporting Information; LRESITOFMS *m/z* 434.1 [M-$2H_2O$+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[27]

Latrunculone B (4): white powder; ^{1}H NMR data see Figure S9, Supporting Information; LRESITOFMS *m/z* 420.3 [M-H_2O+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[27]

Fijianolide D (5): clear oil; ^{1}H NMR data see Figure S10, Supporting Information; LRESITOFMS *m/z* 529.2 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[28]

Aignopsanoic acid A (6): white powder; ^{1}H NMR data see Figure S11, Supporting Information; LRESITOFMS *m/z* 251.5 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[35]

Methyl aignopsanoate A (7): yellow oil; ^{1}H NMR data see Figure S12, Supporting Information; LRESITOFMS *m/z* 265.3 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[35]

Fijianolide B (8): yellowish oil; ^{1}H NMR data see Figure S13, Supporting Information; LRESITOFMS *m/z* 515.2 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[28]

Fijianolide A (9): white solid; ^{1}H NMR data see Figure S14, Supporting Information; LRESITOFMS *m/z* 515.2 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[28]

Latrunculin A (10): white powder; ^{1}H NMR data see Figure S15, Supporting Information; LRESITOFMS *m/z* 404.1 [M-H_2O+H]$^+$ This compound was identified by comparison of spectral data with those of the literature values.[27]

Mycothiazole (11): yellow oil; ^{1}H NMR data see Figure S16, Supporting Information; LRESITOFMS *m/z* 405.2 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[31]

Sacrotride A (12): brown oil; ^{1}H NMR data see Figure S17, Supporting Information; LRESITOFMS *m/z* 463.4 [M+H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[45]

Spongia- 13(16),-14-dien-19-oic acid (17): white crystalline solid, ^{1}H NMR data see Figure S45, Supporting Information: LRESITOFMS *m/z* 317.1 [M +H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[49]

Penicillic acid (18): white crystalline solid, ^{1}H NMR data see Figure S47, Supporting Information: LRESITOFMS *m/z* 171.1 [M +H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[51]

Hexyl cinnamaldehyde (19): clear oil, ^{1}H NMR data see Figure S49, Supporting Information: LRESITOFMS *m/z* 217.2 [M +H]$^+$. This compound was identified by comparison of spectral data with those of the literature values.[52]

Table S1. ^{1}H, ^{13}C, COSY and HMBC NMR data of aignopsanoic acid B (**13**) in CD_3OD

		13			
position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC
1ax	30.4	CH_2	1.96 (m) [c]	3	
1eq			1.90 (m)		
2ax	22.0	CH_2	1.60 (m)		1
2eq			1.40 (m) [c]		
3ax	30.1	CH_2	1.35 (m) [c]		
3eq			1.30 (m)		
4	33.9	CH	1.52 (m)	3, 13	3, 5, 13, 14
5	41.8	C			
6ax	29.9	CH_2	1.25 (td, 14.4, 5.4)		4, 5, 7, 8, 10, 14
6eq			1.72 (ddd, 13.8, 5.4.,3.0)	7	
7ax	23.7	CH_2	1.99 (ddd, 15.6, 14.4, 3.0)	8	
7eq			1.83 (ddt , 15.6, 6.0, 3.6)	8	5, 8, 9
8	49.0	CH	3.20 (m)	7, 11	7, 11
9	215.9	C			
10	52.8	C			
11	72.4	CH	3.86 (m)	8	9, 12
12	178.8	C			
13	14.3	CH_3	0.81 (d, 7.2)	4	3, 4, 5,
14	14.2	CH_3	0.90 (s)		3, 4, 5, 6, 10
15	22.6	CH_3	1.06 (s)		1, 5, 9, 10
OH			8.6 (br s)		

[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 S2. ^{1}H, ^{13}C, COSY and HMBC NMR data of apo-latrunculin T (**14**) in $CDCl_3$

			14		
position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC
1	169.6	C			
2	116.0	CH	5.71 (d, 1.2)	21	1, 3
3	161.9	C			
4a	33.0	CH_2	2.80 (ddd, 15.0, 9.0, 6.6)	5	2, 3, 5, 6
4b			2.73 (m)[c]		
5a	30.9	CH_2	2.30 (m)	4a, 4b, 6	3, 4, 6, 7
5b			2.25 (m)		
6	133.3	CH	5.68 (td, 15.0, 6.6)	5, 7	4, 5, 7, 8
7	126.2	CH	6.35 (td, 15.0, 10.8)	6, 8	5, 8, 9
8	127.6	CH	5.92 (t, 10.8)	7, 9	5, 6, 7
9	136.2	CH	5.04 (t, 10.8)	8, 10	8, 11, 22
10	31.9	CH	2.61 (m)	9, 11, 22	11, 12
11a	33.3	CH_2	1.39 (m)[c]	10	9, 10, 12, 13, 22
11b			1.28 (m)		
12a	36.3[d]	CH_2	1.51 (m)[c,d]		
12b			1.42 (m)[c, d]		11, 12, 13, 15
13	71.3	CH	3.61 (dddd, 12.6, 10.8, 6.6, 5.4)		11, 15
14a	36.3[d]	CH_2	1.51 (m)[c,d]	13, 15	12, 13, 15, 16
14b			1.42 (m)[c, d]		
15a	19.9	CH_2	1.81 (m)	14, 16	13, 16, 17
15b			1.74 (m)	14, 16	13, 16, 17
16a	34.8	CH_2	2.74 (m)[c]	15	15, 14, 17, 18
16b			2.73 (m)[c]	15	
17	188.7	C			
18	134.2	C			
19	114.8	CH	7.10 (s)		17, 18 ,20
20	171.7	C			
21	24.5	CH_3	1.91 (d, 1.2)	2, 4a, 4b	2, 3, 4
22	21.6	CH_3	0.97 (d, 6.0)	10	9, 10, 11
COOH			10.21 (br s)		

[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. [d]Can be interchanged.

Table S3. ^{1}H, ^{13}C, COSY and HMBC NMR data of C20 OMe fijianolide A (**15**) in C_6D_6

			15		
position	δ_C	Type[b]	δ_H (J in Hz)	gCOSY	gHMBC
1	164.8	C			
2	120.5	CH	5.78 (d, 12.0)	3	1, 3
3	146.1	CH	5.65 (m)[c]	2, 4	1, 2, 4
4a	36.0	CH_2	2.97 (ddd, 15.0, 10.8, 4.2, 0.6)	3	5, 6
4b			2.11 (m)[c]		3, 5, 6
5	73.2	CH	4.34 (m)	4a, 4b	
6	128.8	CH	5.54 (dt, 10.8, 3.0)	3, 13	5, 7, 8
7	125.3	CH	5.63 (m)[c]		6, 8
8a	32.1	CH_2	1.80 (m)[c]		6, 7, 9
8b			1.64 (dt, 16.8, 3.0)[c]		
9	66.6	CH	3.40 (ddd, 12.6, 10.2, 2.4)	8, 10	11
10a	43.0	CH_2	1.60 (ddd, 16.8, 6.0, 3.0)	9, 11	
10b			0.91 (ddd, 16.8, 11.4, 2.4)		11
11	27.2	CH	1.91 (m)	10, 28	10, 12, 28
12a	45.7	CH_2	2.11 (m)[c]		
12b			2.05 (m)[c]		
13	142.0	C			
14a	35.7	CH_2	2.30 (m)	15	13, 15, 16
14b			2.30 (m)		
15	71.2	CH	4.14 (ddd, 9.0, 3.6, 1.8)[c]	14	13, 16, 17
16	76.4	CH	4.01 (dd, 5.4, 1.8)[c]		15, 17
17	79.4	CH	5.65 (m)[c]	18	16, 18, 19
			2.60 (ddd, 13.8, 9.6, 4.2)	17, 19	16, 17, 19
18a	33.1	CH_2			
18b		CH	2.03 (m)[c]		
19	79.9	CH	4.61 (tt, 9.6, 2.4)	18a, 18b	18, 20
20	109.0	C			
21	123.0	CH	5.84 (dd, 15.6, 1.8)	22	20, 22, 23
22	137.5	CH	6.35 (dd, 15.6, 4.8)	21, 23	20, 21, 23, 24
23	73.0	CH	3.90 (dddd, 13.8, 9.0, 4.2, 1.8)	22, 24a	22
24a	36.1	CH_2	2.05 (m)		
24b			2.00 (m)	23	
25	131.2	C			
26	120.5	CH	5.10 (br, s)	27, 30	24, 27, 30
27a	65.7	CH_2	4.09 (br, s)	26	
27b			4.05 (br, s)		
28	20.2	CH_3	0.88 (d, 6.5)	10a, 10b, 11	10, 11, 12
29	113.4	CH_2	4.86 (d, 10.8)		12, 13, 14
30	23.0	CH_3	1.52 (br, s)	26	24, 25, 26
OCH_3	49.2	CH_3	3.15 (br, s)		20

[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. ^{1}H, ^{13}C, COSY and HMBC NMR data of aignopsane ketal (**16**) in $CDCl_3$.

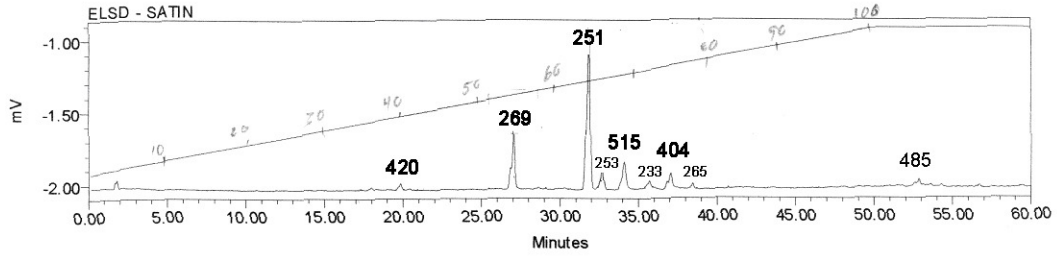
16					
position	δ_C[a]	Type[b]	δ_H (J in Hz)[a]	gCOSY	gHMBC
1ax	29.7	CH_2	2.07 (m)[c]		
1eq			1.25 (m)[c]		
2ax	22.6	CH_2	1.46 (m)[c]		3
2eq			1.31 (m)[c]		
3ax	30.4	CH_2	1.36 (m)[c]		
3eq			1.28 (m)[c]		
4	33.3	CH	1.71 (m)[c]		
5	41.4	C			
6ax	33.4	CH_2	2.39 (dd, 6.0, 18.0)	7	5,7,8,10
6eq			2.29 (dd, 6.0, 18.0)		
7	142.7	CH	6.53 (dt, 6.0, 6.6)	6	5,6,9
8	133.1	C			
9	204.1	C			
10	50.7	C			
11ax	34.2	CH_2	2.54 (ddd, 6.0, 13.8)	12	7,8,9
11eq			2.46 (ddd, 6.0, 13.8)		
12	103.5	CH	4.51 (t, 6.0)	11	16,17
13	16.7	CH_3	0.79 (d, 6.6)		3,4,5
14	15.5	CH_3	0.93 (s)		5,10,13
15	21.6	CH_3	0.98 (s)		1,5,9,10
16	53.4	OCH_3	3.33 (s)		12
16’	53.6	OCH_3	3.33 (s)		12

[a]Measured at 600 MHz (^{1}H) and 500 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. [c] Partially overlapped by other signals.

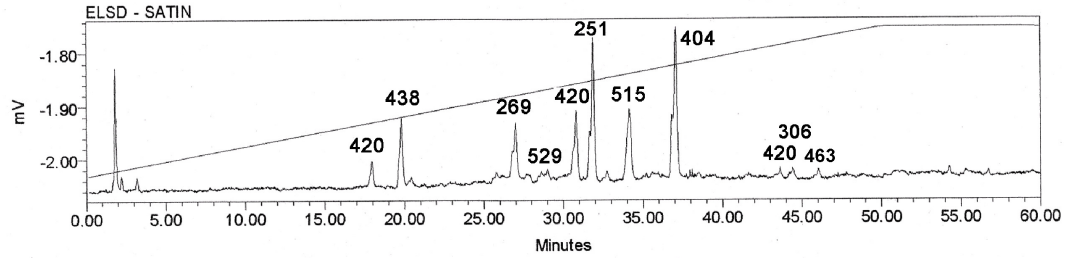
Figure S1. Photographs of *C. mycofijiensis* coll. nos. 07327 A - J and L - O.

Figure S2. LC-MS-ELSD analysis of coll. nos. 07327 **A-E** XFD with annotations of *m/z* ions.

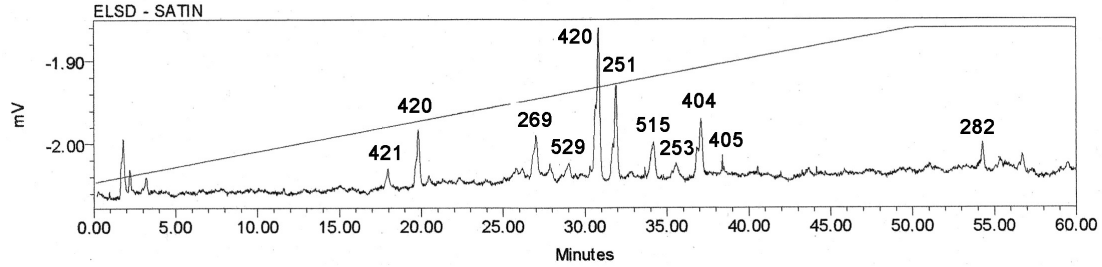
07327**A** XFD_15 µl ~ [5 mg/mL]



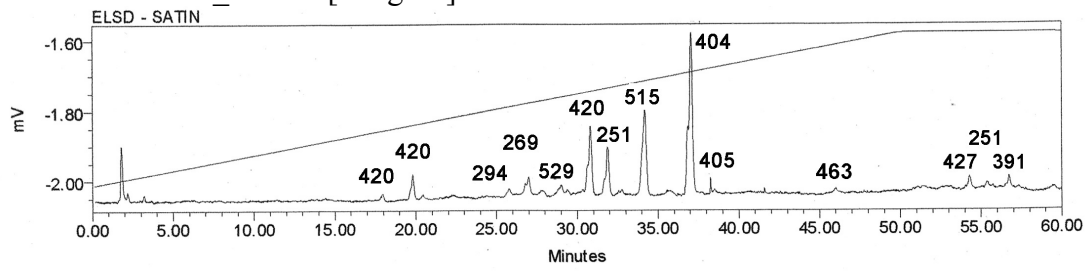
07327 **B** XFD_15 µl ~ [5 mg/mL]



07327 **C** XFD_15 µl ~ [5 mg/mL]



07327 **D** XFD_15 ul ~ [5 mg/ml]



07327 **E** XFD_15 µl ~ [5 mg/mL]

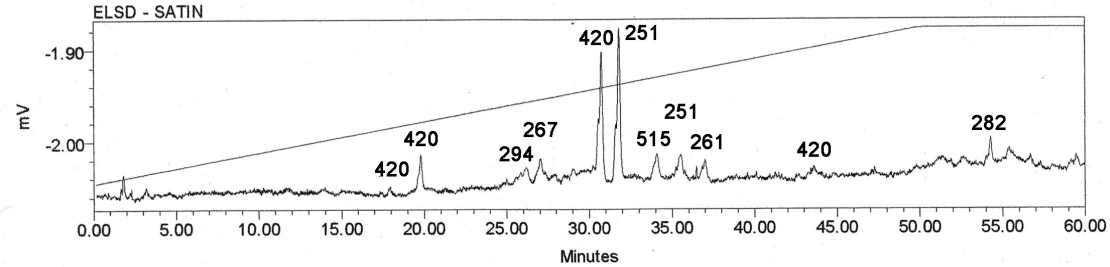
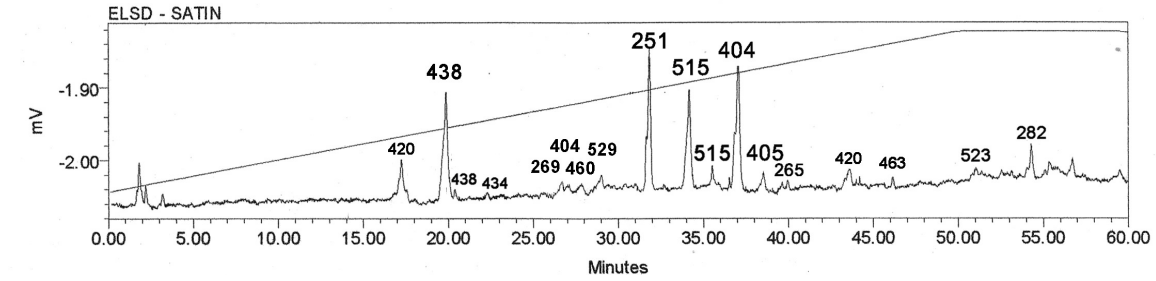
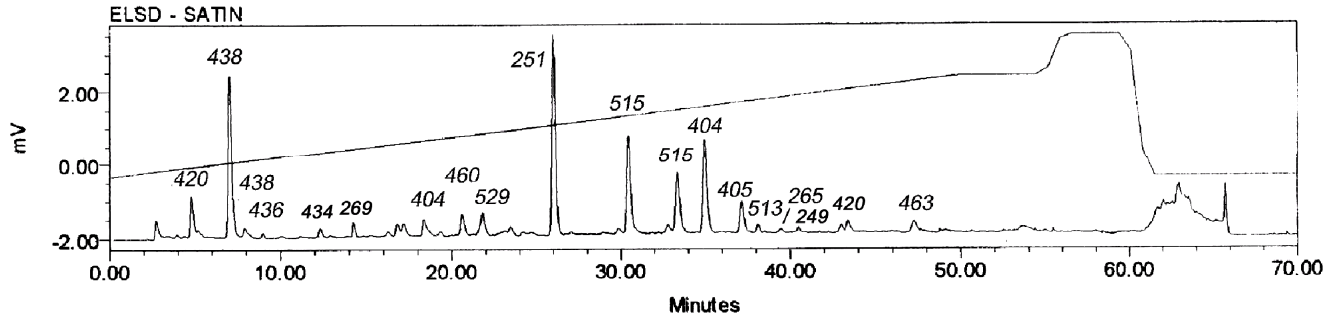


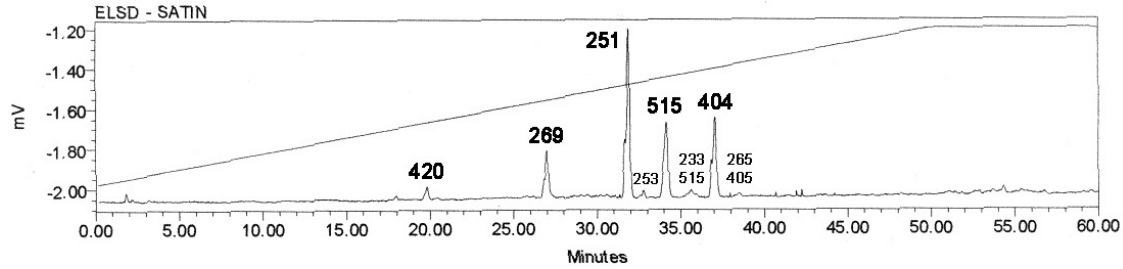
Figure S3. LCMS-ELSD analysis of coll nos. 07327 **F-I** XFD with annotations of *m/z* ions.

07327 **F** XFD_15 µl ~ [5 mg/mL]

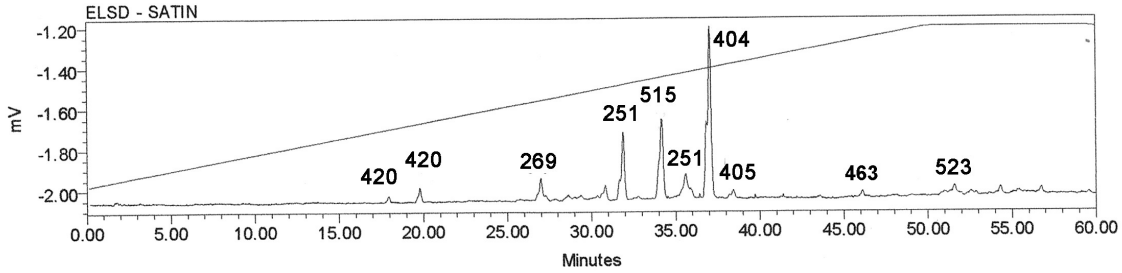




07327 **G** XFD_15 µl ~ [5 mg/mL]



07327 **H** XFD_15 µl ~ [5 mg/mL]



07327 **I** XFD_15 ul ~ [5 mg/ml]

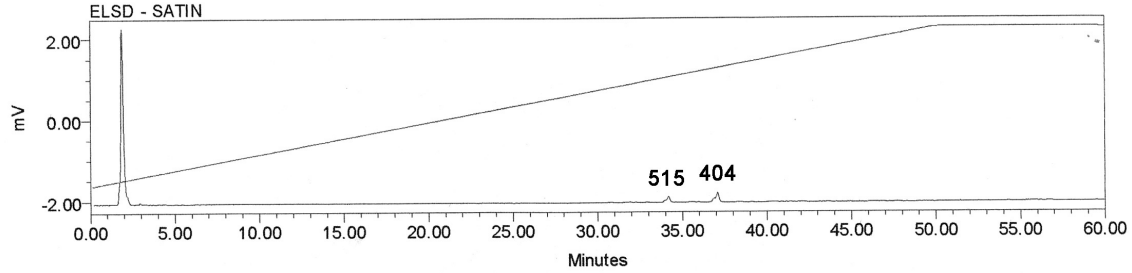
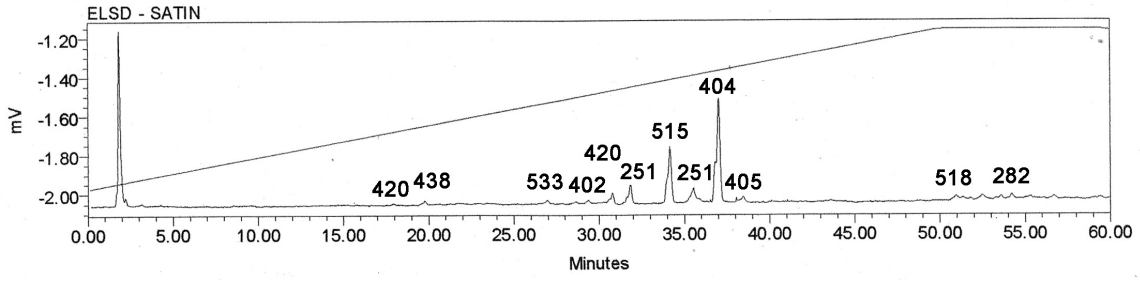
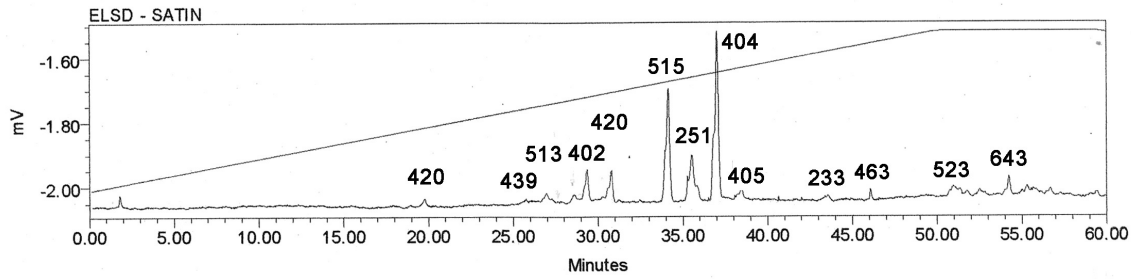


Figure S4. LCMS-ELSD analysis of coll nos. 07327 **J-O** XFD with annotations of *m/z* ions.

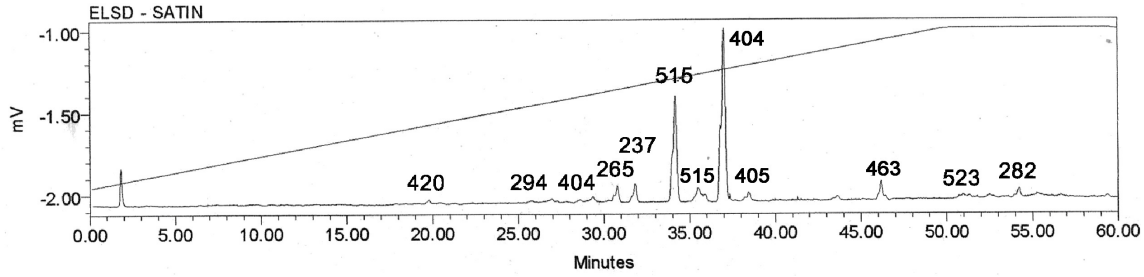
07327 **J** XFD_15 µl ~ [5 mg/mL]



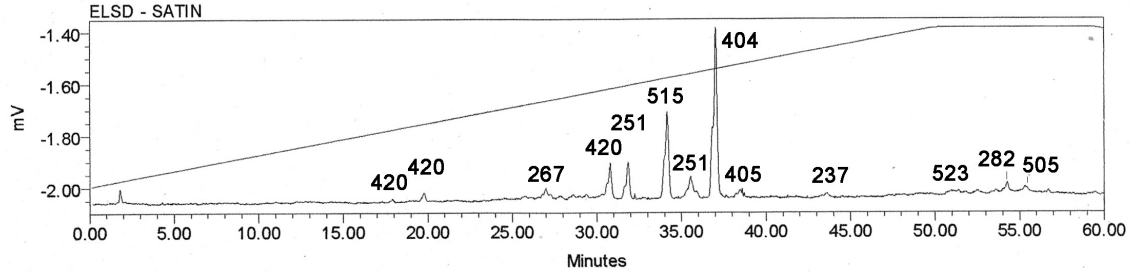
07327 **L** XFD_15 µl ~ [5 mg/mL]



07327 **M** XFD_15 µl ~ [5 mg/mL]



07327 **N** XFD_15 µl ~ [5 mg/mL]



07327 **O** XFD_15 µl ~ [5 mg/mL]

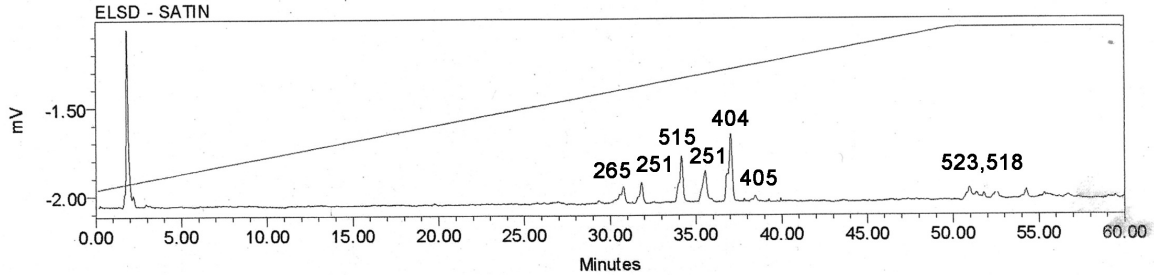


Figure S5. ^{1}H NMR spectrum of coll. no. 07327 F XFD (600 MHz, $CDCl_3$).

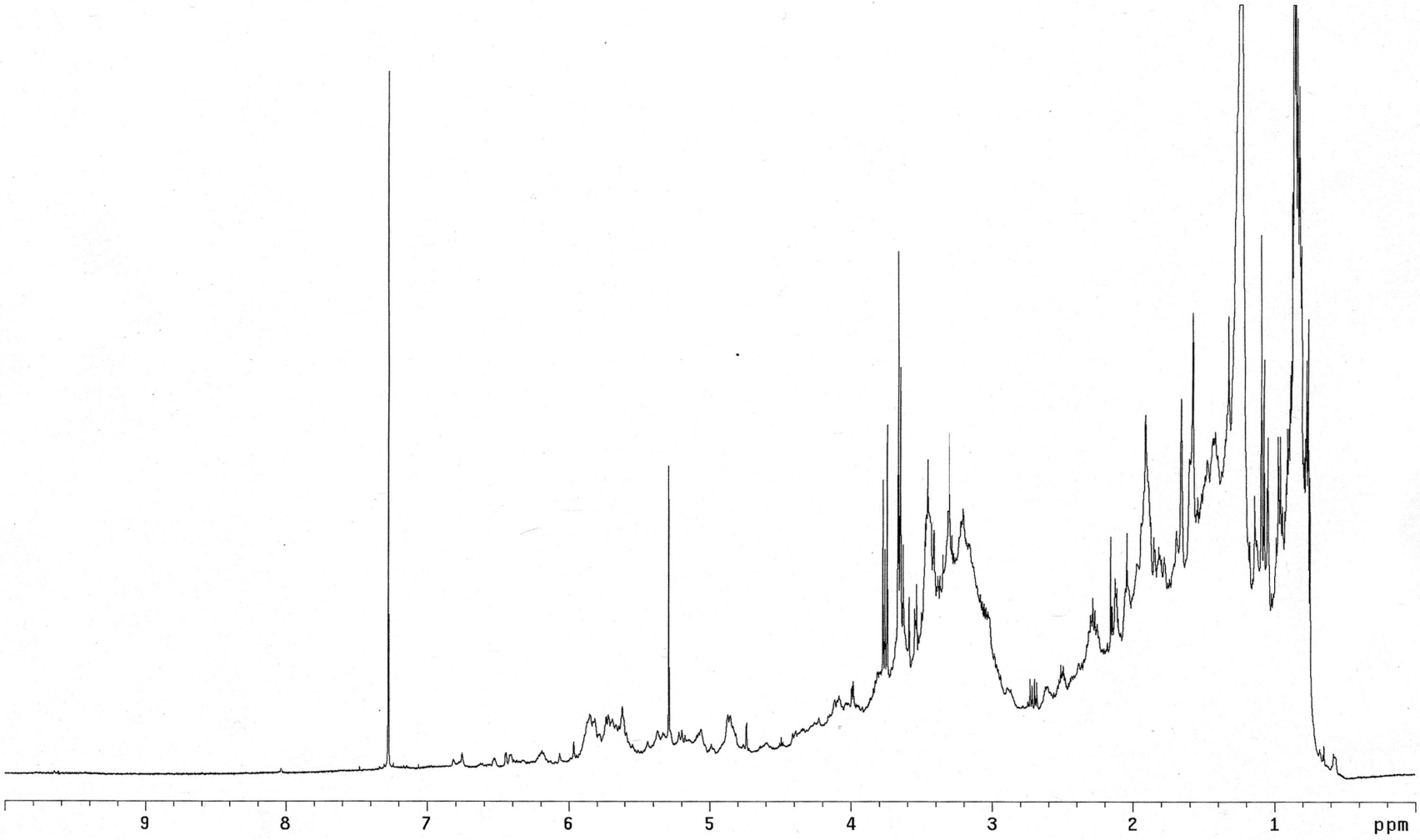


Figure S6. ^{1}H NMR spectrum of latrunculol A (**1**, 600 MHz, Acetone-d_6).

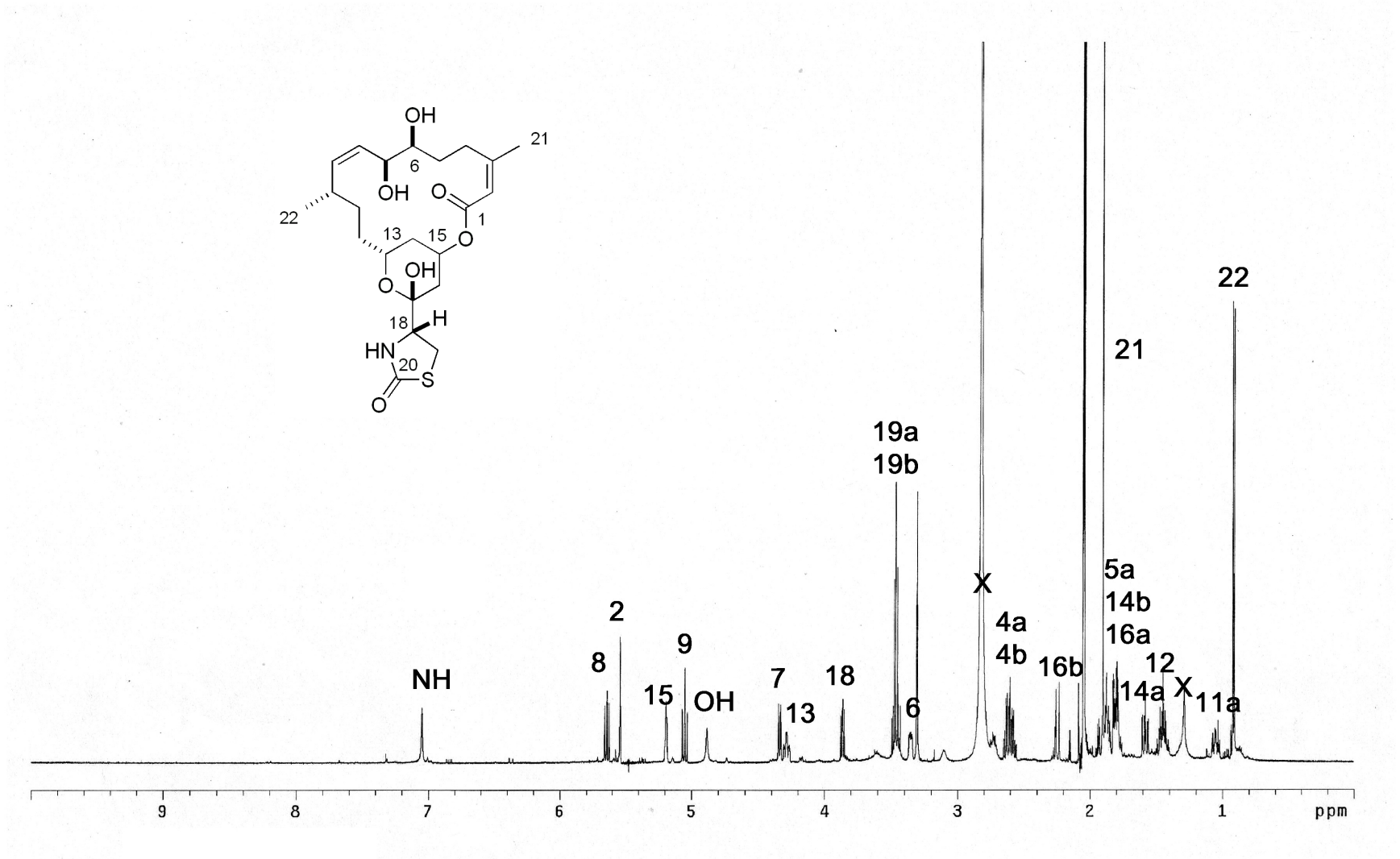


Figure S7. ^{1}H NMR spectrum of latrunculone A (**2**, 600 MHz, Acetone-d_6).

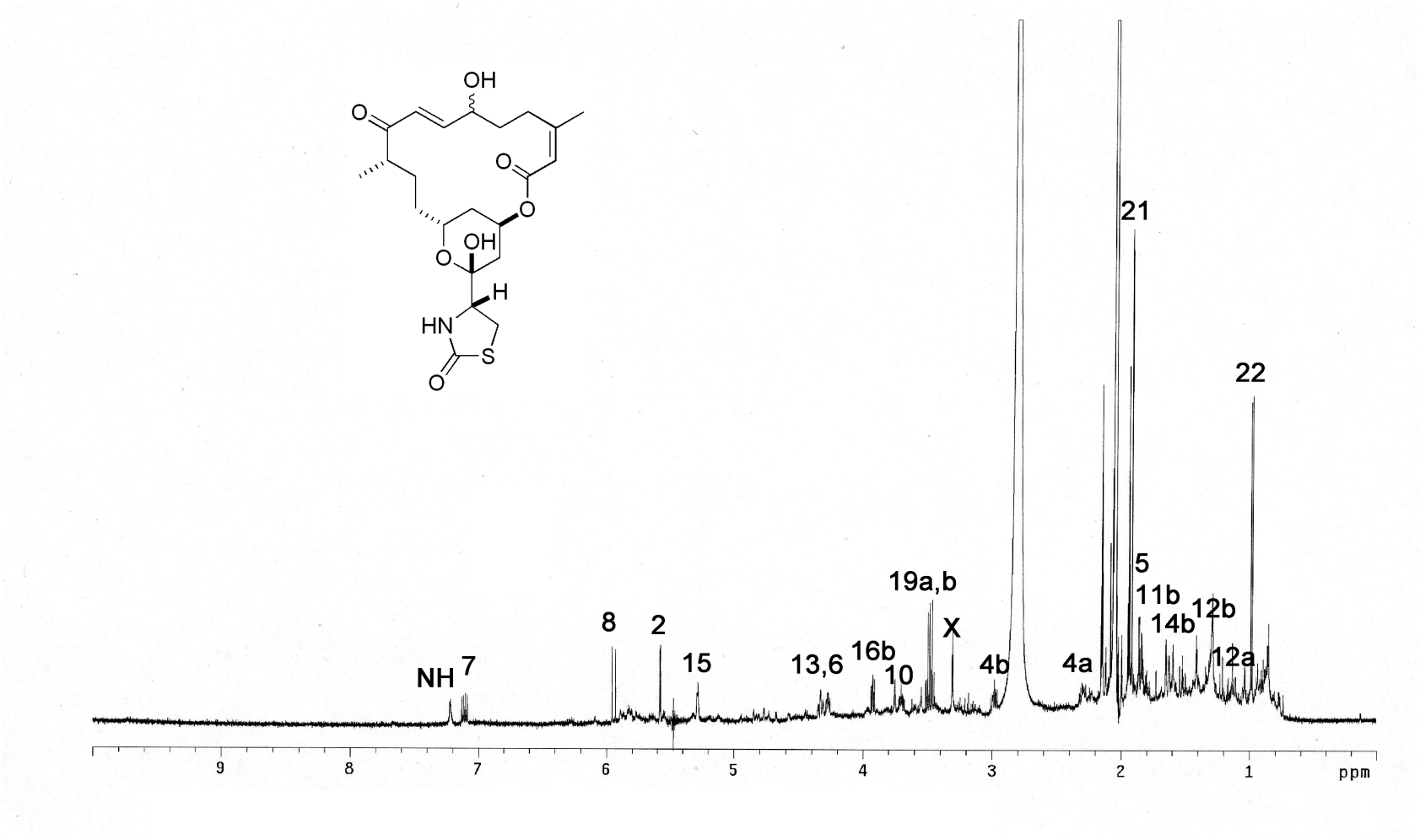


Figure S8. ^{1}H NMR spectrum of latrunculol B (**3**, 600 MHz, Acetone-d_6).

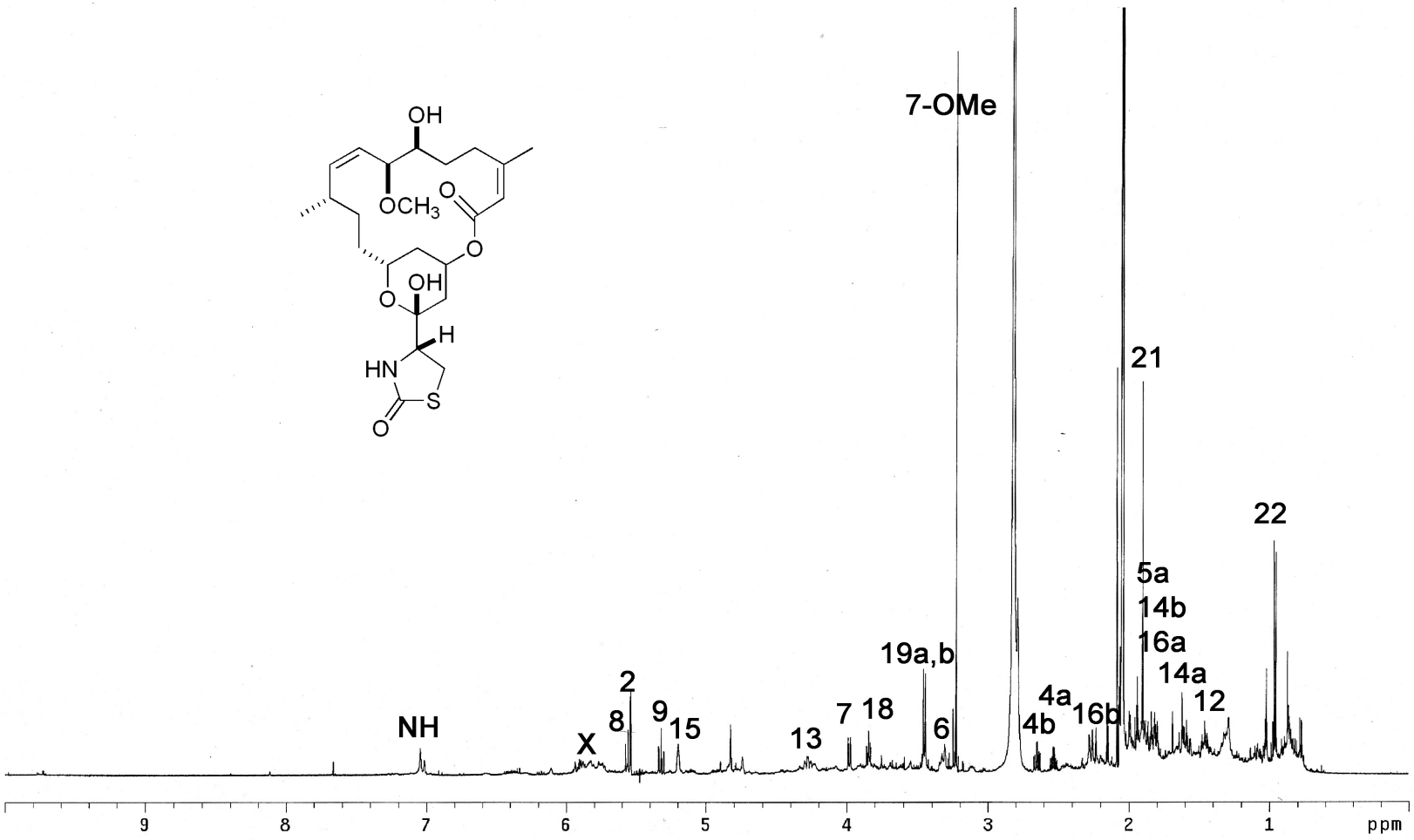


Figure S9. ^{1}H NMR spectrum of latrunculone B (**4**, 600 MHz, $CDCl_3$).

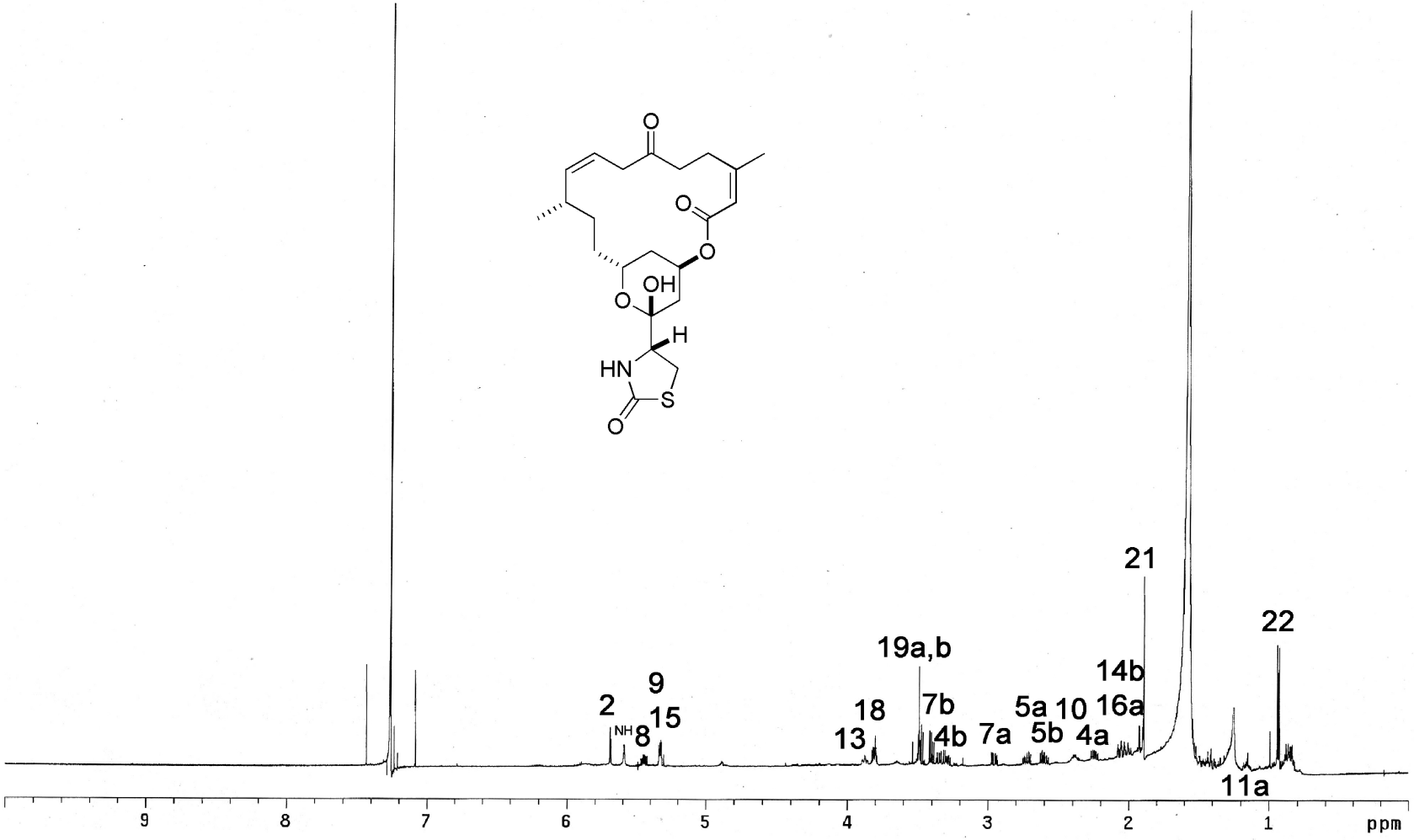


Figure S10. ^{1}H NMR spectrum of fijianolide D (**5**, 600 MHz, C_6D_6).

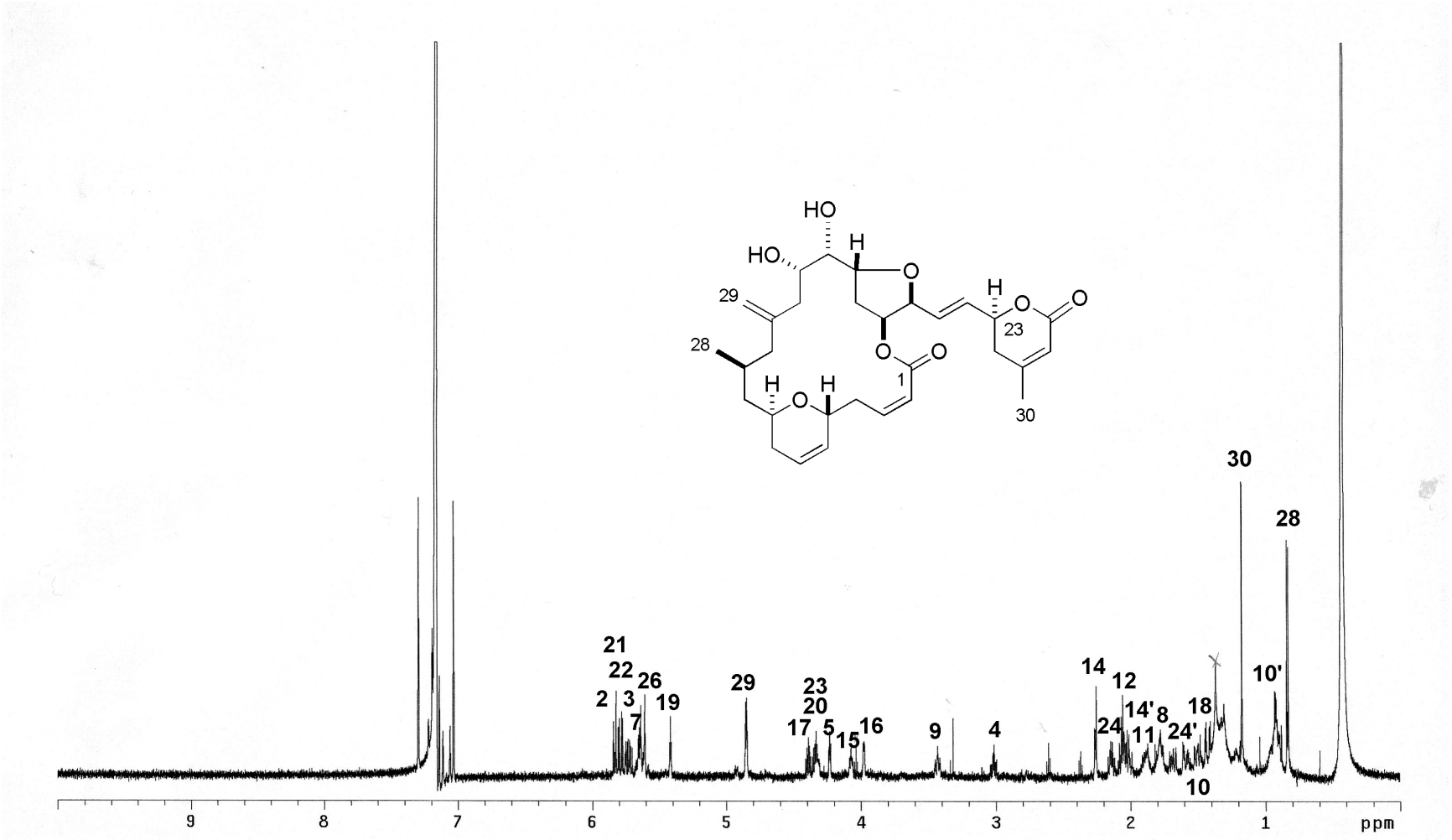


Figure S11. ^{1}H NMR spectrum of aignopsanoic acid A (**6**, 600 MHz, $CDCl_3$).

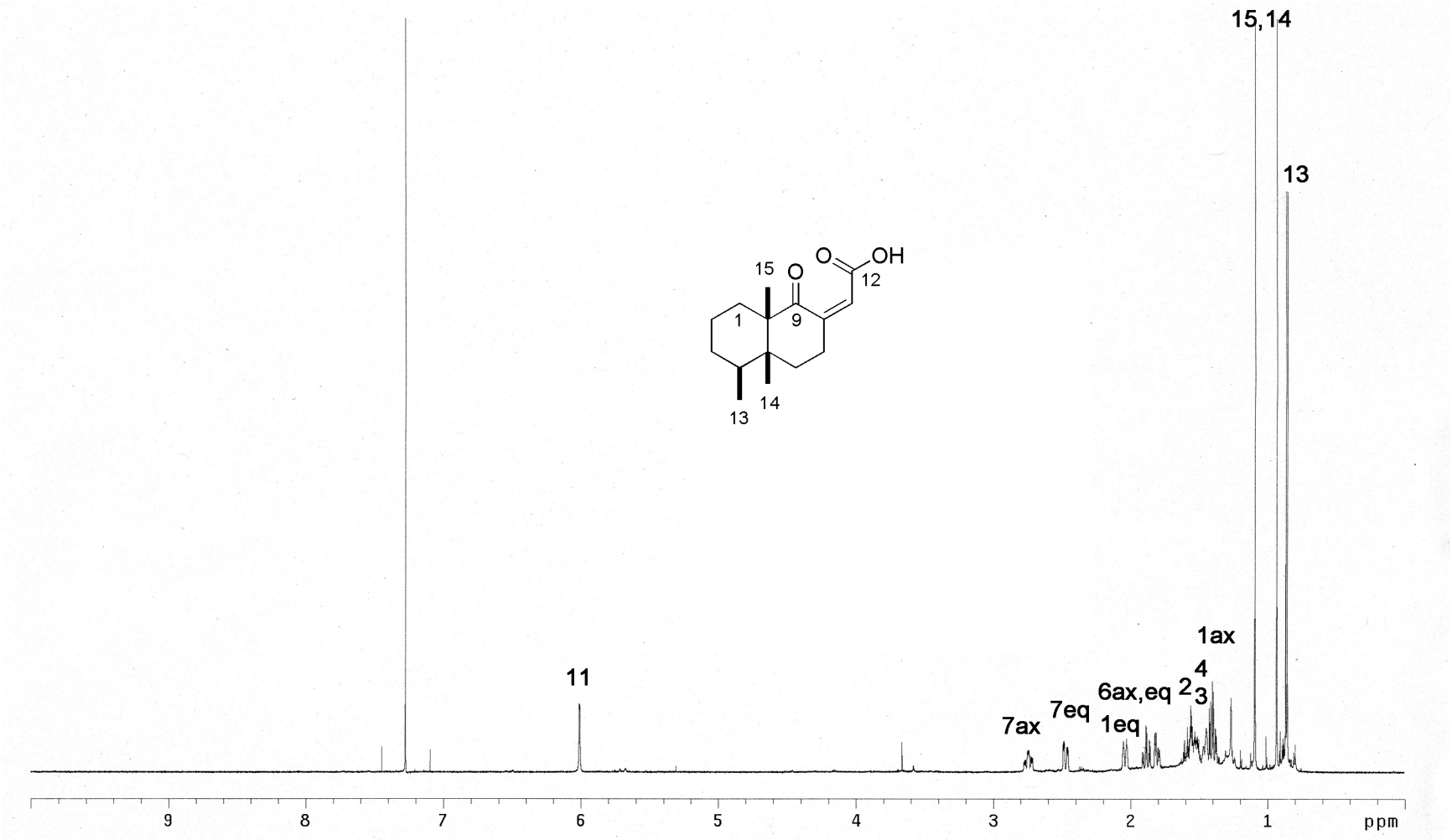


Figure S12. ^{1}H NMR spectrum of methyl aignopsanoate (**7**, 600 MHz, $CDCl_3$).

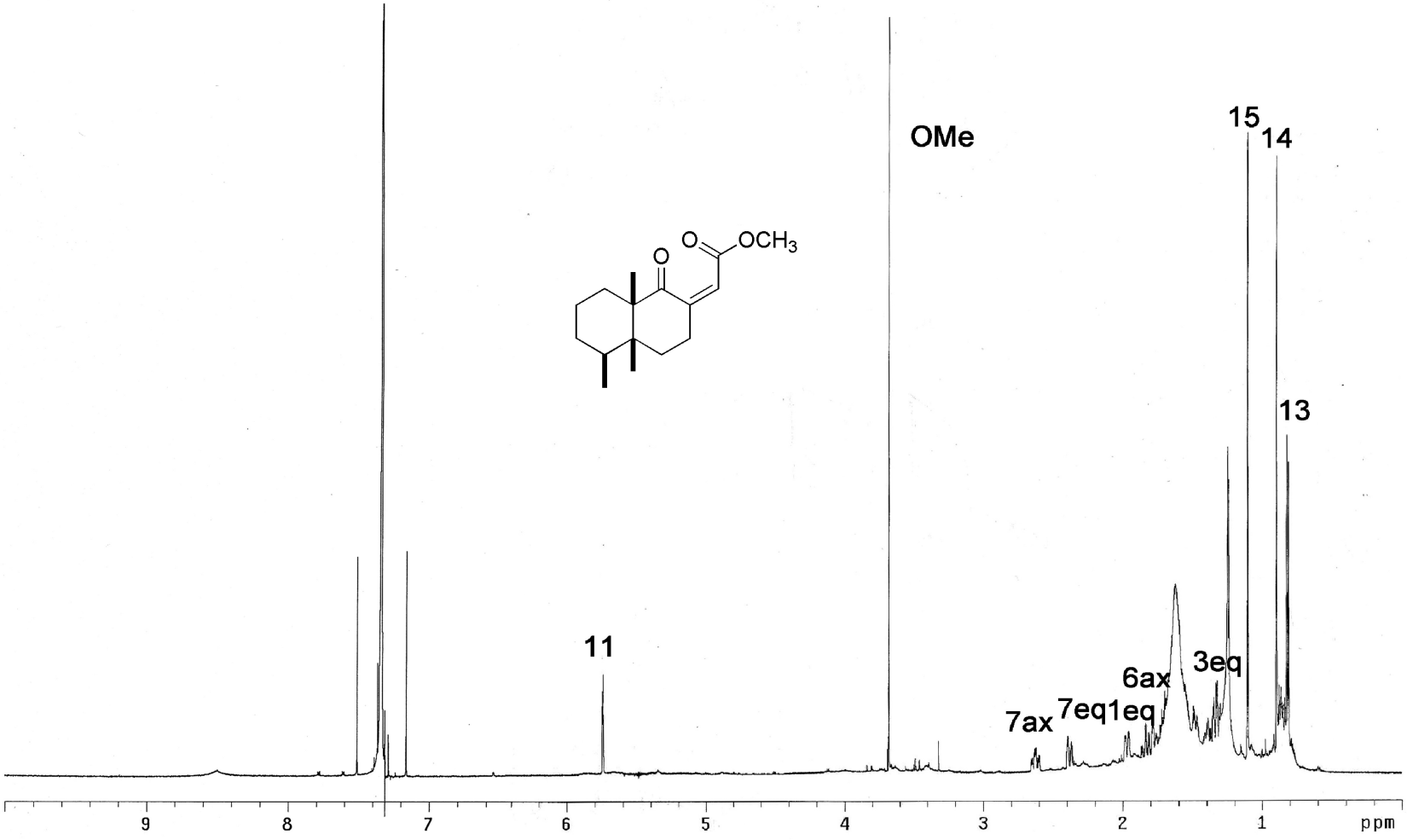


Figure S13. ^{1}H NMR spectrum of fijianolide B (**8**, 600 MHz, C_6D_6).

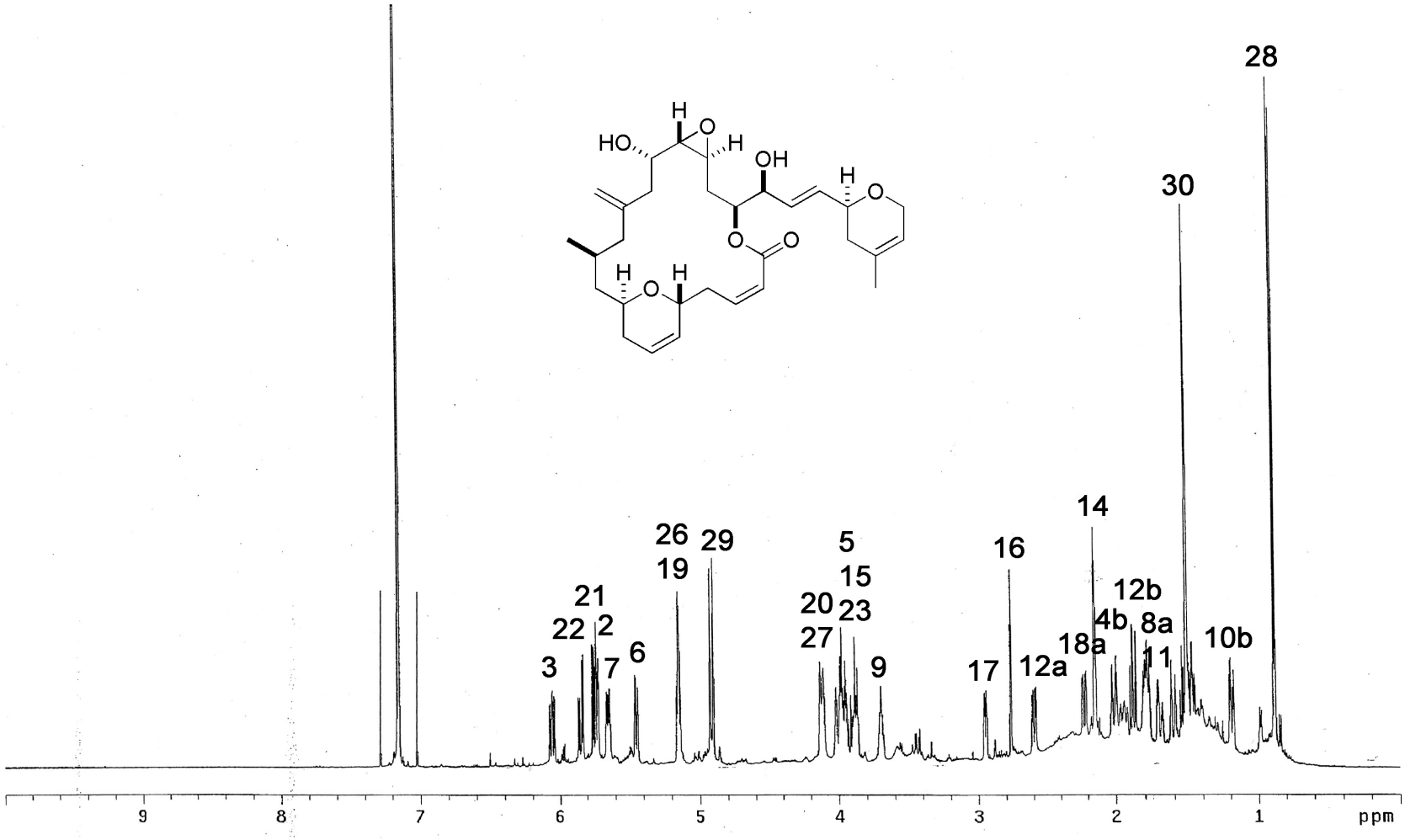


Figure S14. ^{1}H NMR spectrum of fijianolide A (**9**, 600 MHz, C_6D_6).

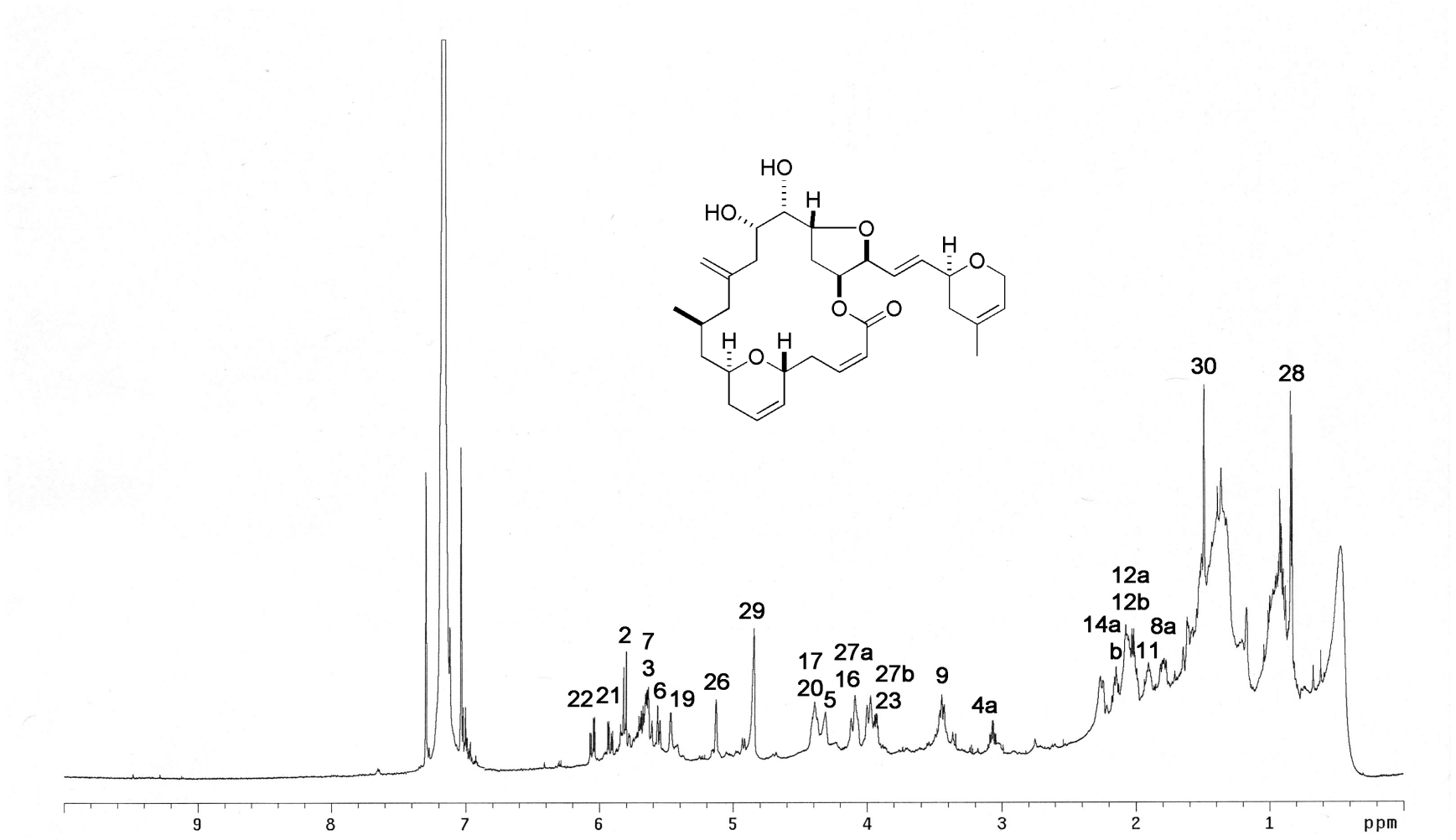


Figure S15. ^{1}H NMR spectrum of latrunculin A (**10**, 600 MHz, $CDCl_3$).

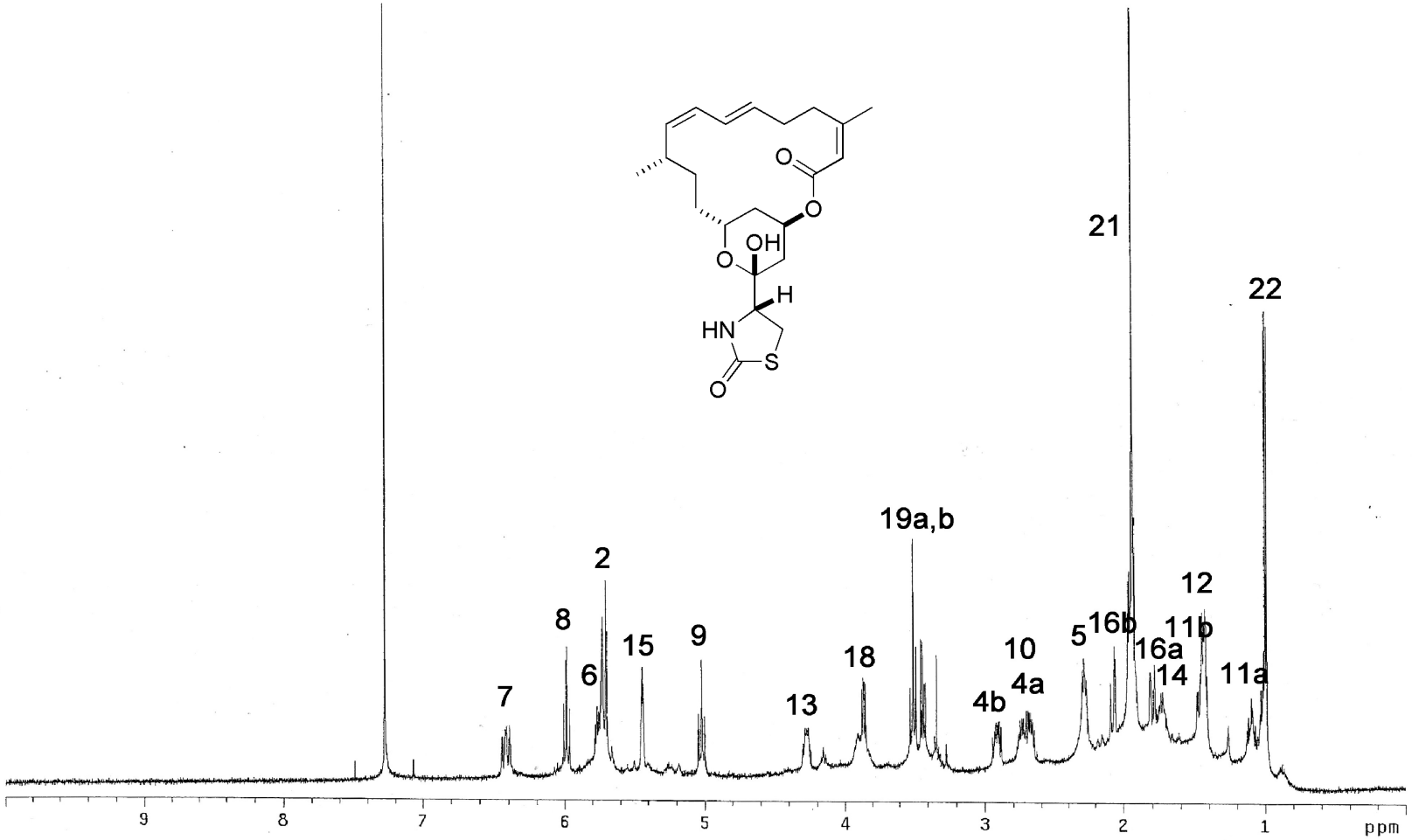


Figure S16. ^{1}H NMR spectrum of mycothiazole (**11**, 600 MHz, C_6D_6).

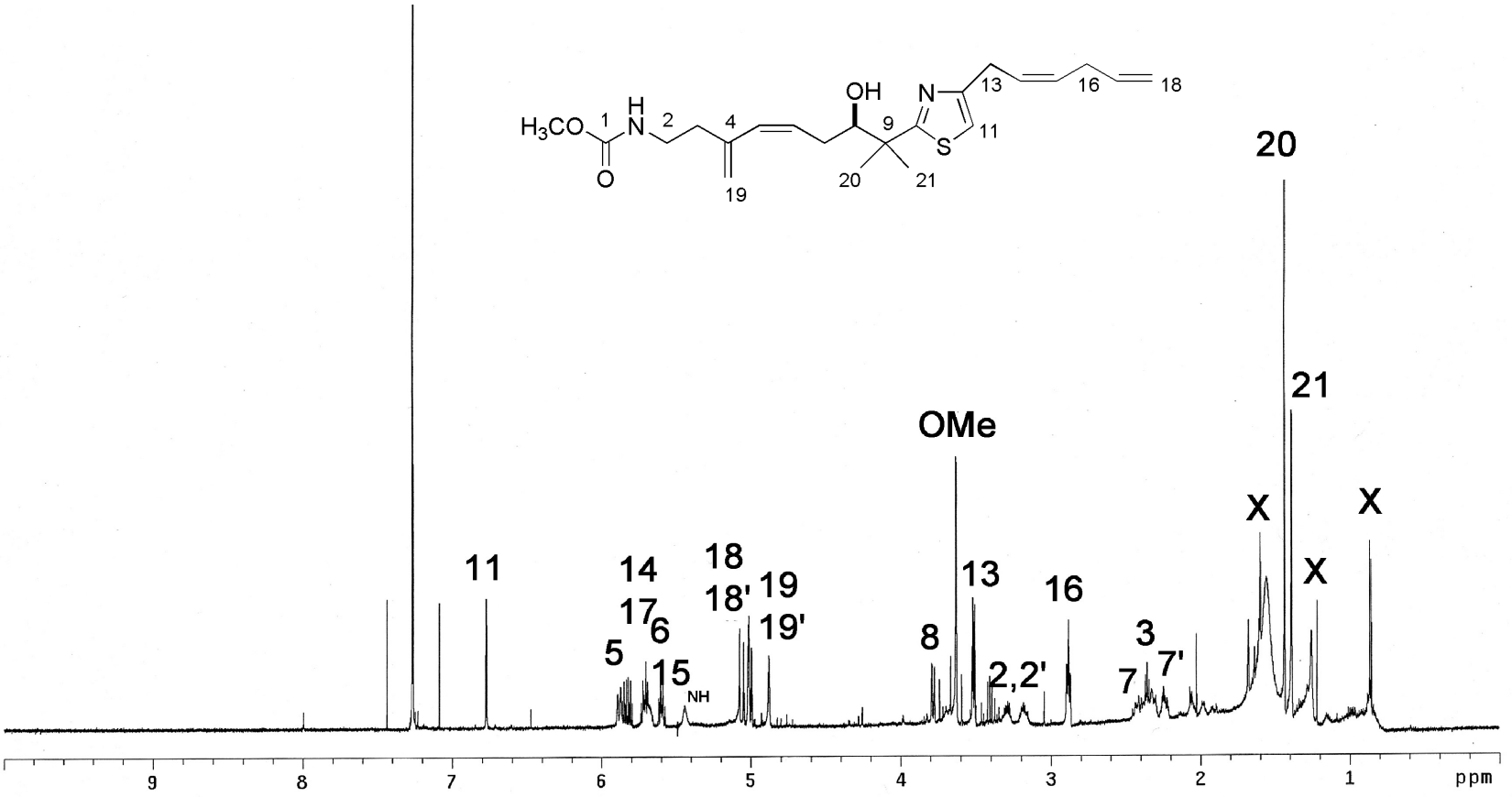


Figure S17. ^{1}H and ^{13}C NMR spectrum of sacrotride A (**12**, 600 MHz, 125 MHz, CD_3OD).

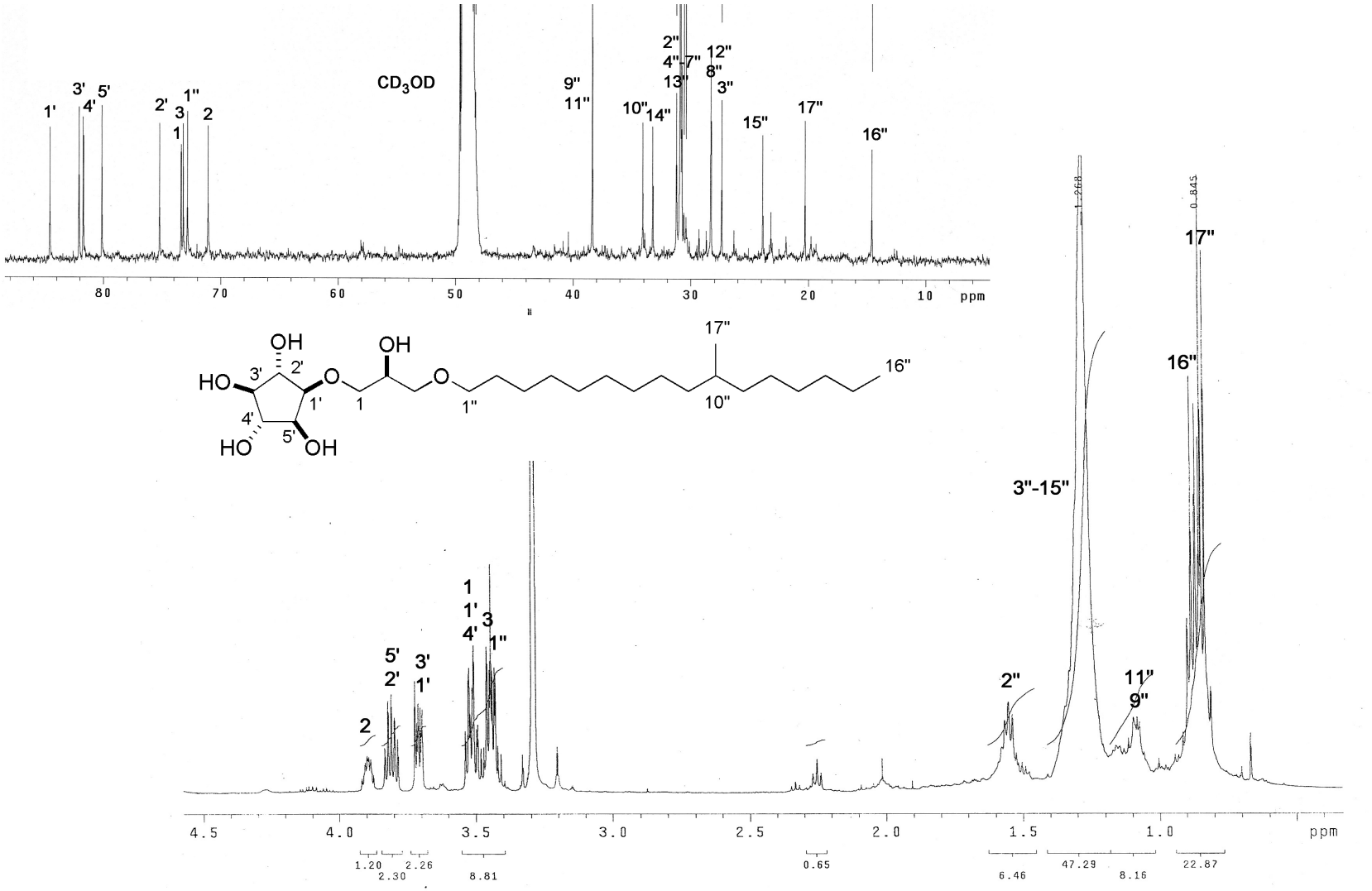


Figure S18. ^{1}H NMR spectrum of aignopsanoic acid B (**13**, 600 MHz, CD_3OD).

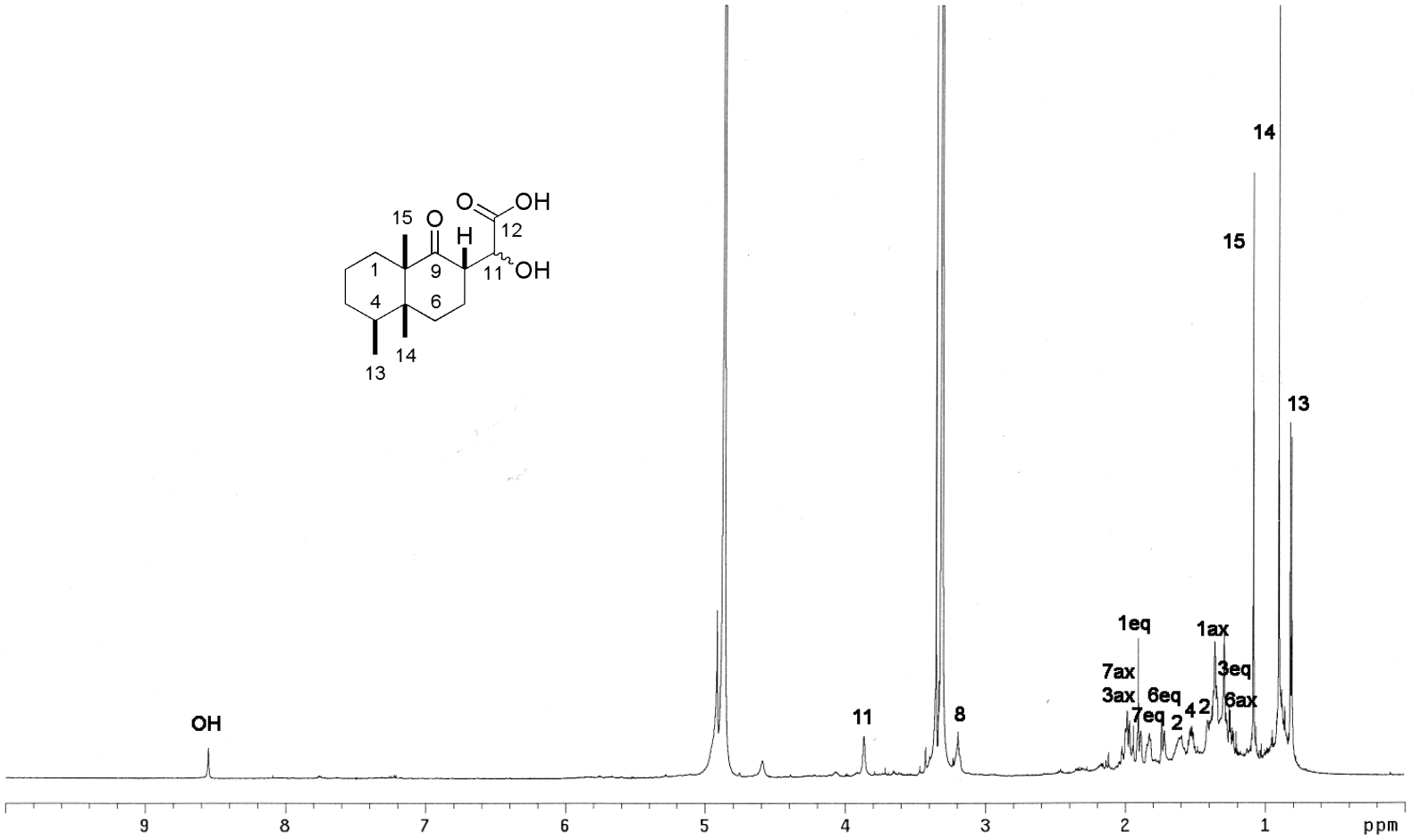


Figure S19. ^{13}C NMR spectrum of aignopsanoic acid B (**13**, 125 MHz, CD_3OD).

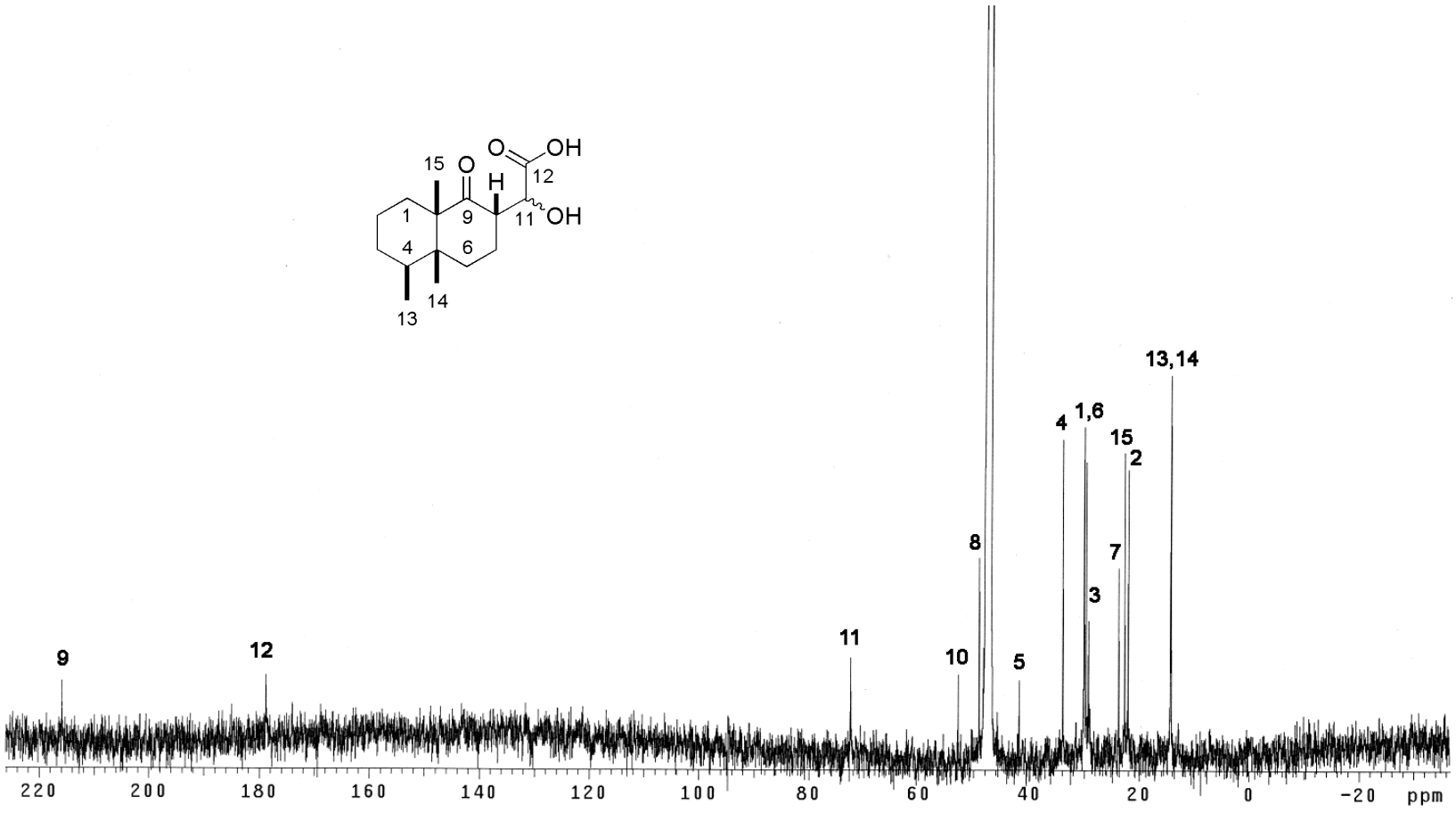


Figure S20. COSY spectrum of aignopsanoic acid B (**13**, 600 MHz, CD_3OD).

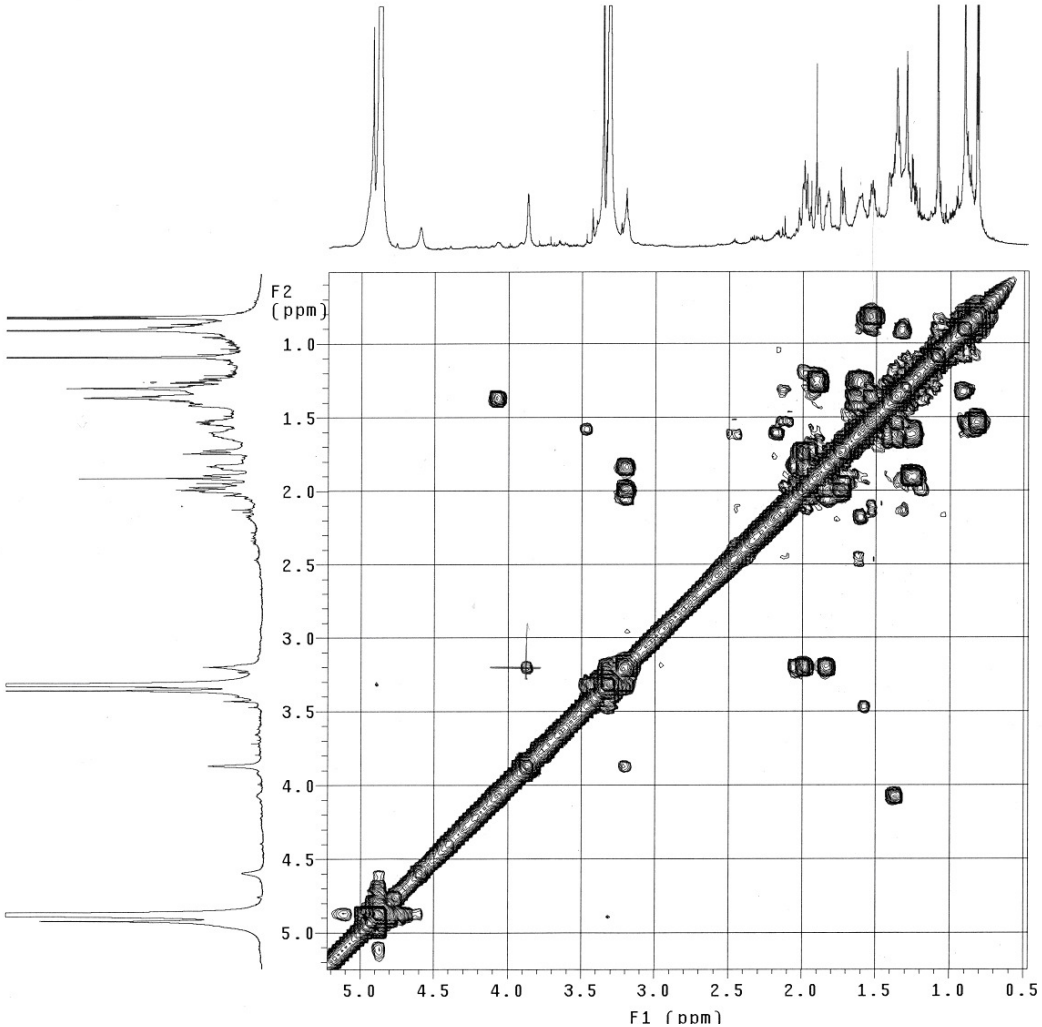


Figure S21. HMQC spectrum of aignopsanoic acid B (**13**, 600 MHz, CD_3OD).

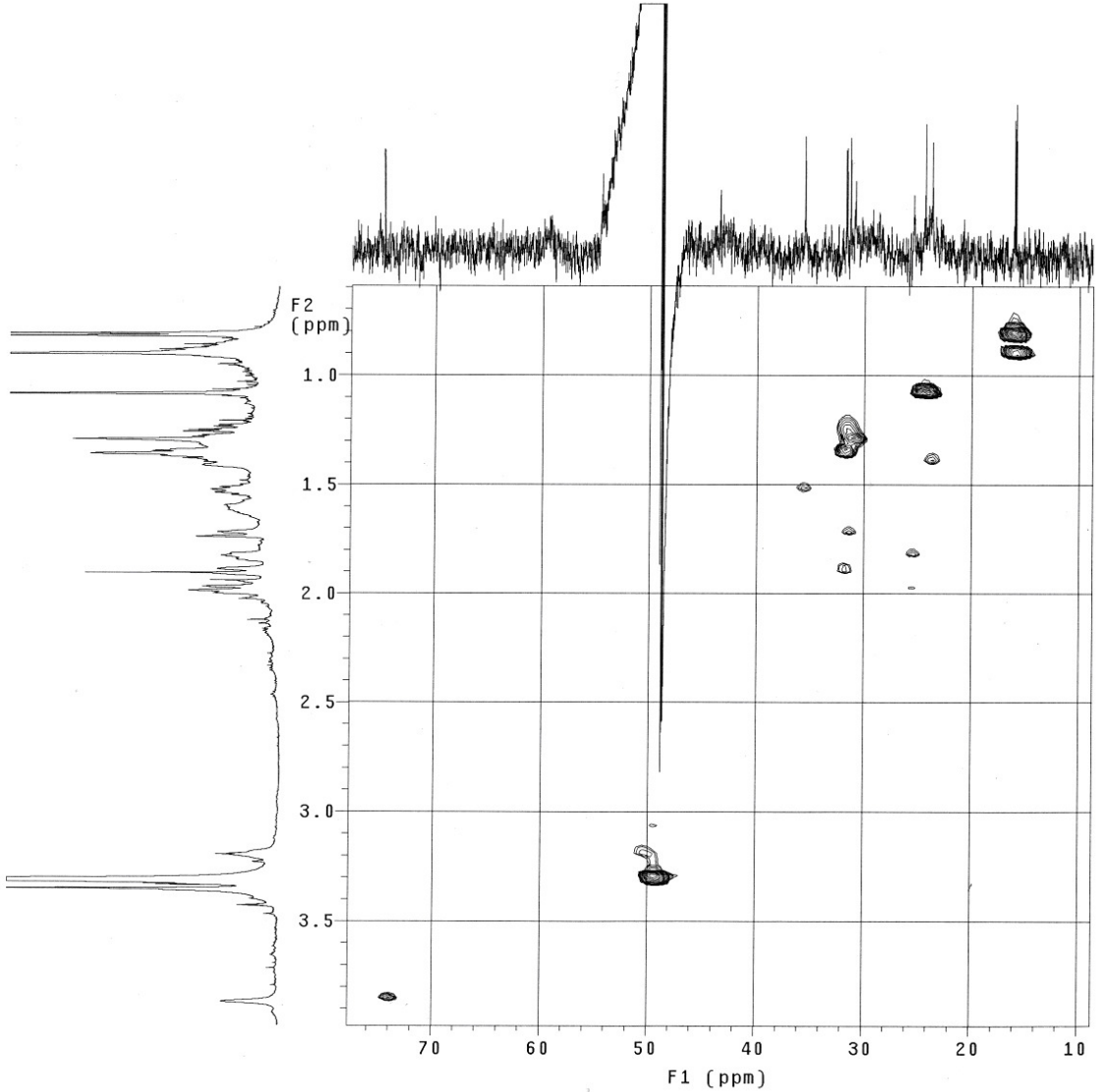


Figure S22. HMBC spectrum of aignopsanoic acid B (**13**, 600 MHz, CD_3OD).

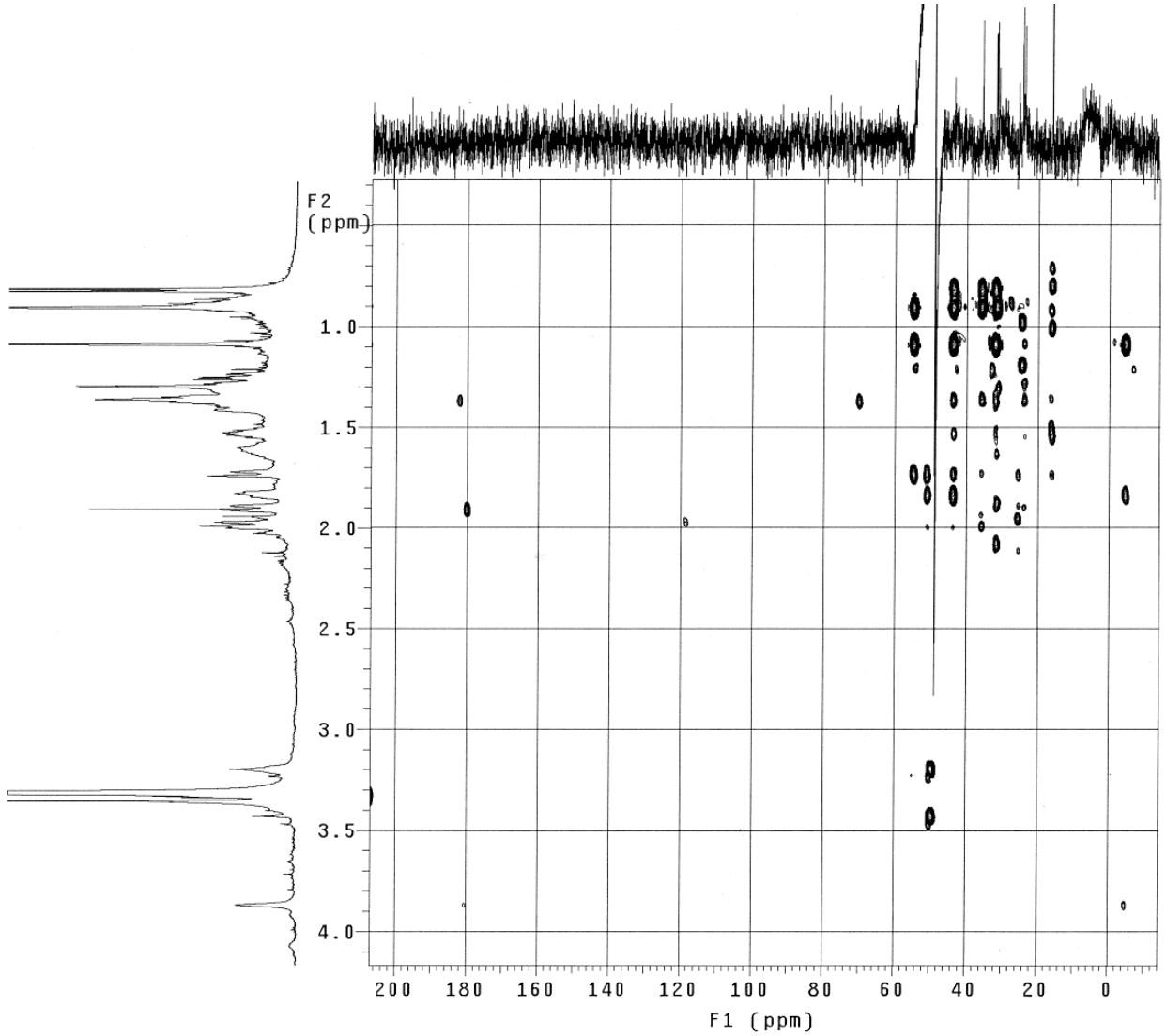


Figure S23. NOESY spectrum of aignopsanoic acid B (**13**, 600 MHz, CD_3OD).

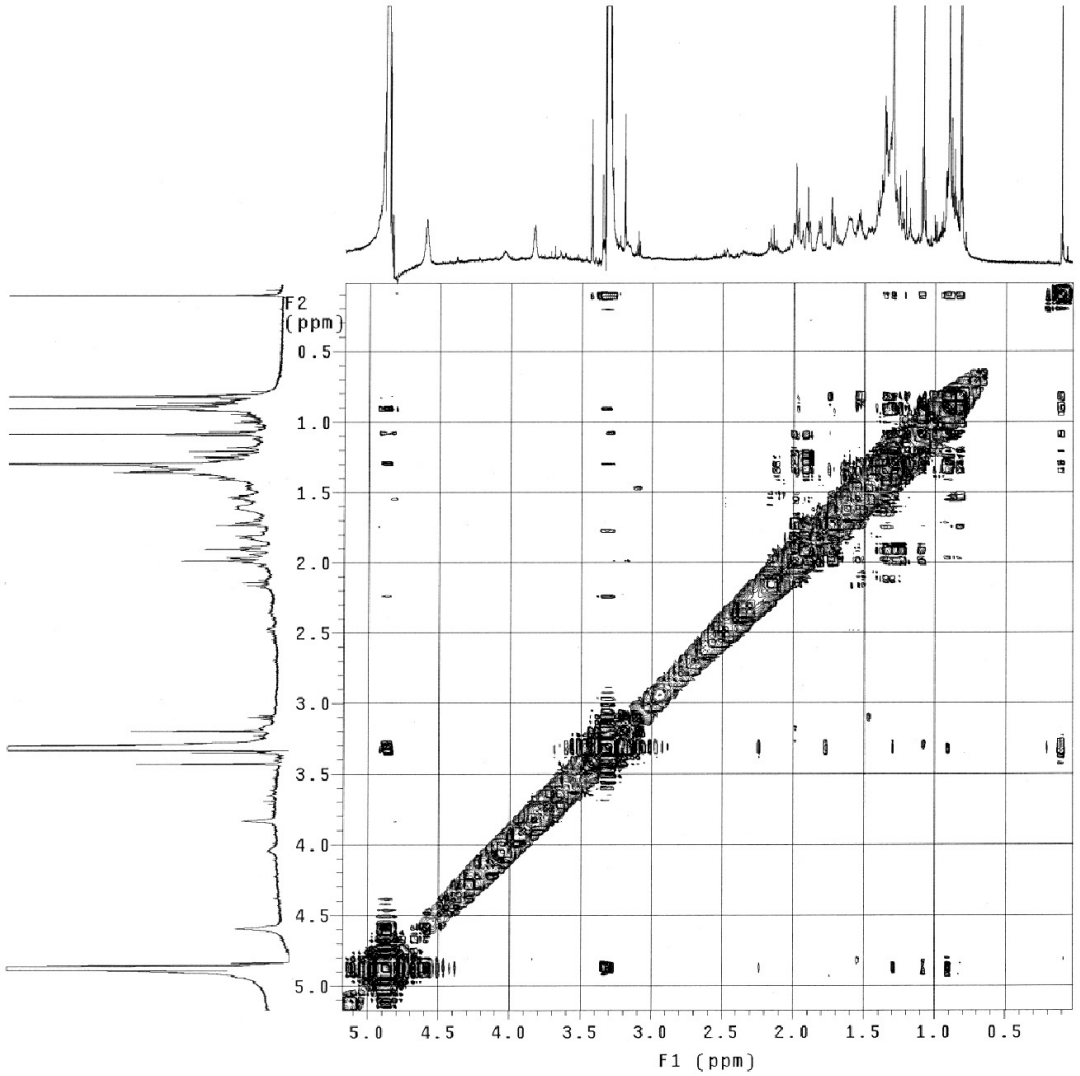


Figure S24. NOE enhancement of H_3-14 of **13**, (600 MHz, CD_3OD).

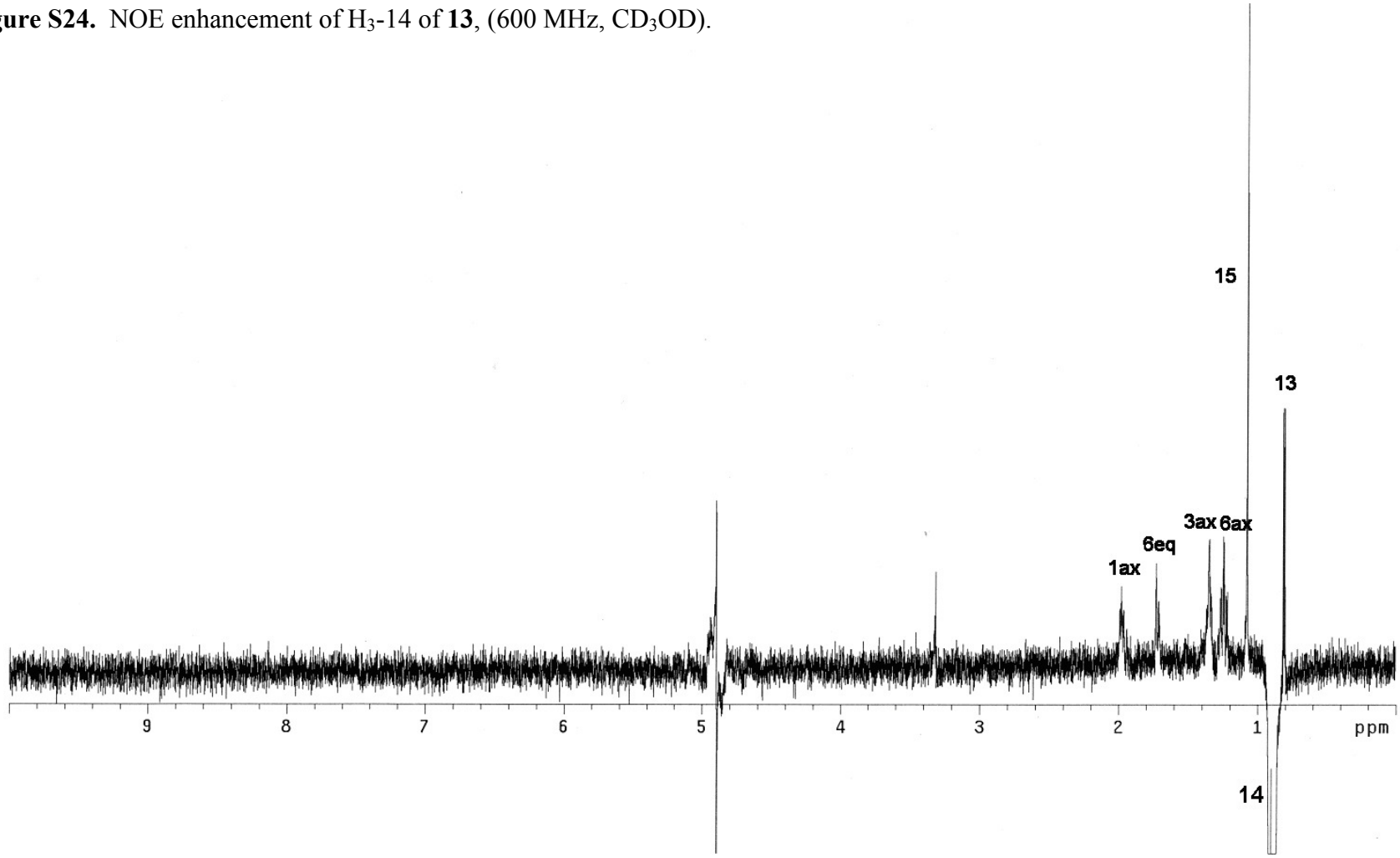


Figure S25. NOE enhancement of H-8 of **13**, (600 MHz, CD_3OD).

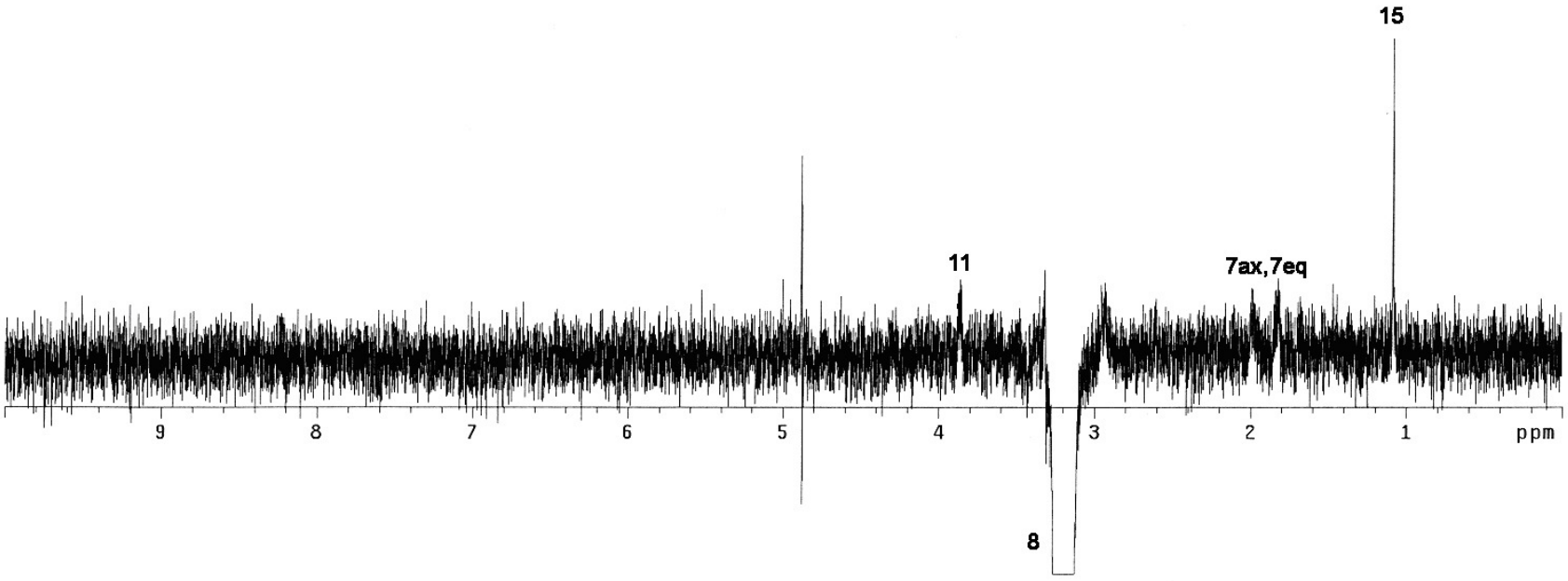


Figure S26. NOE enhancement of H-4 of **13**, (600 MHz, CD_3OD).

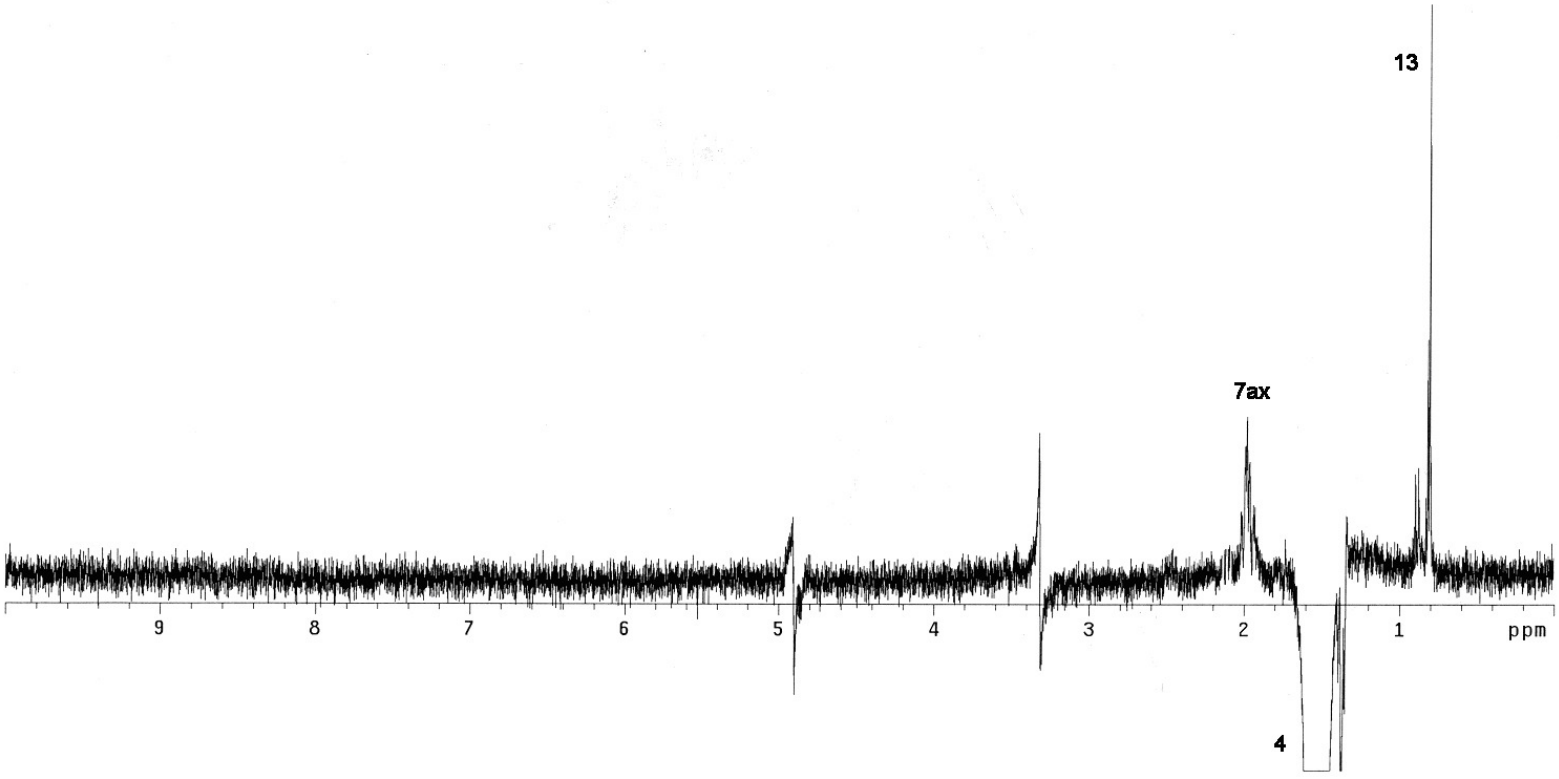


Figure S27. ^{1}H NMR spectrum of apo-latrunculin T (**14**, 600 MHz, $CDCl_3$)

.

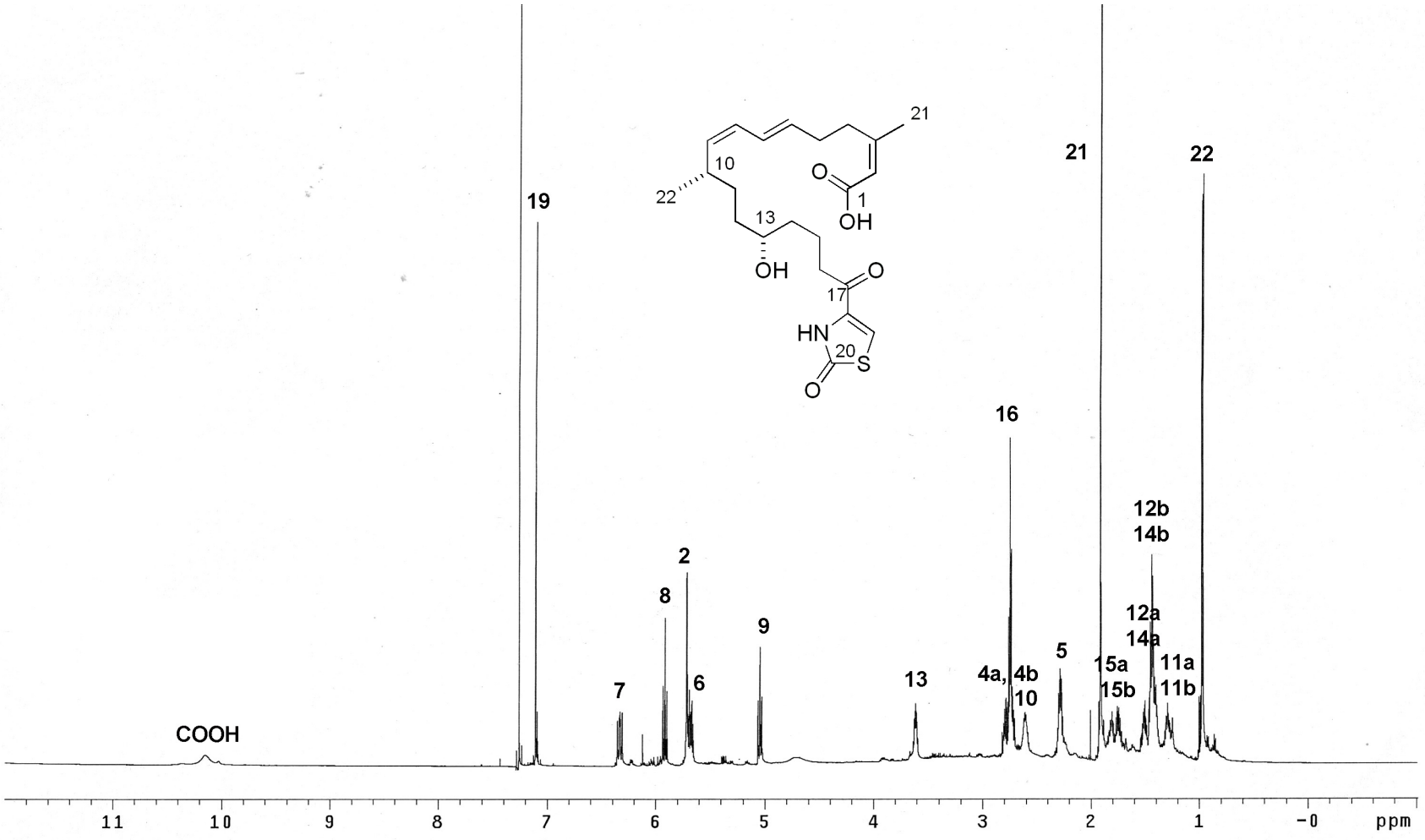


Figure S28. ^{13}C NMR spectrum of apo-latrunculin T (**14**, 125 MHz, $CDCl_3$).

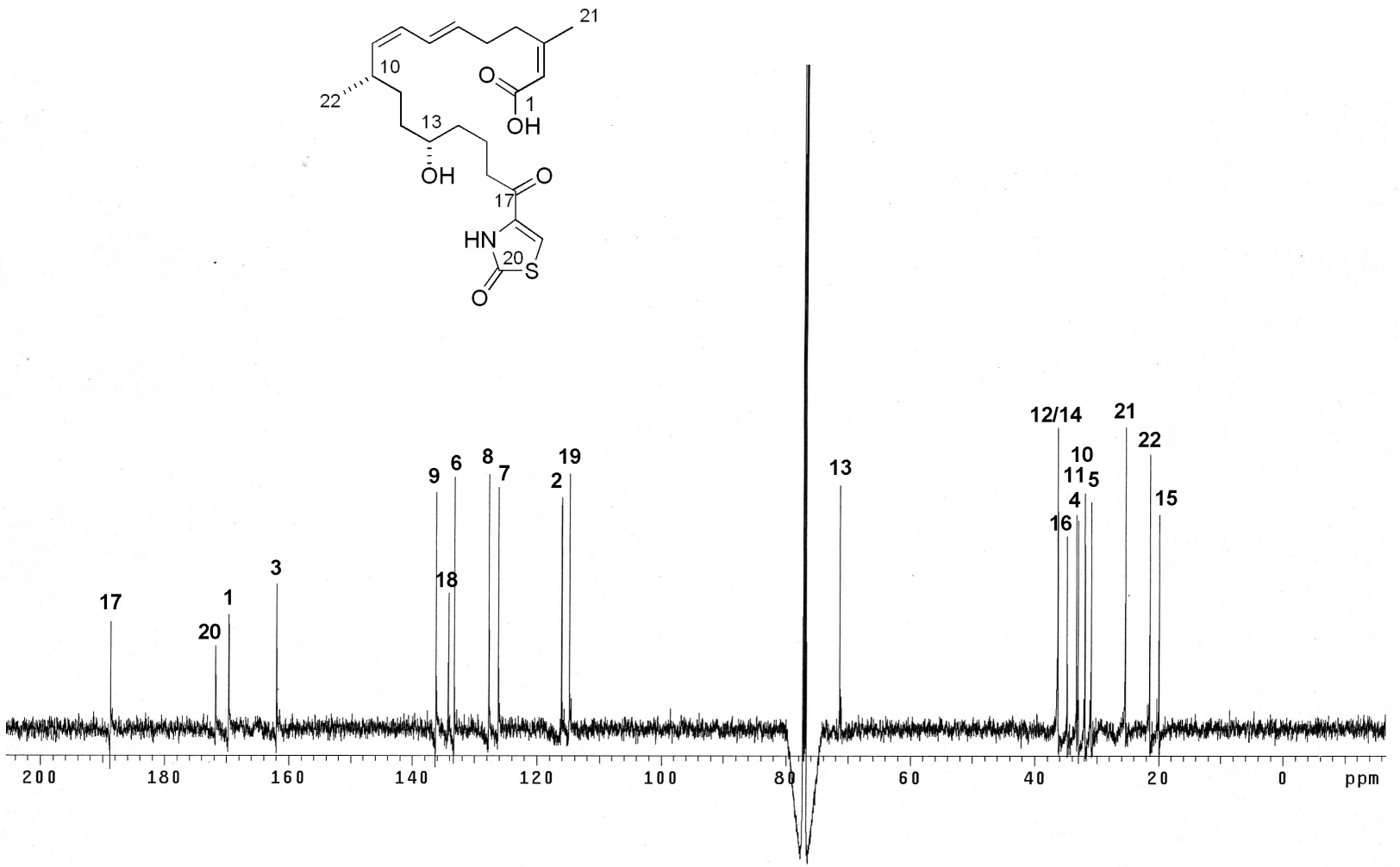


Figure S29. COSY NMR spectrum of apo-latrunculin T (**14**, 600 MHz, $CDCl_3$).

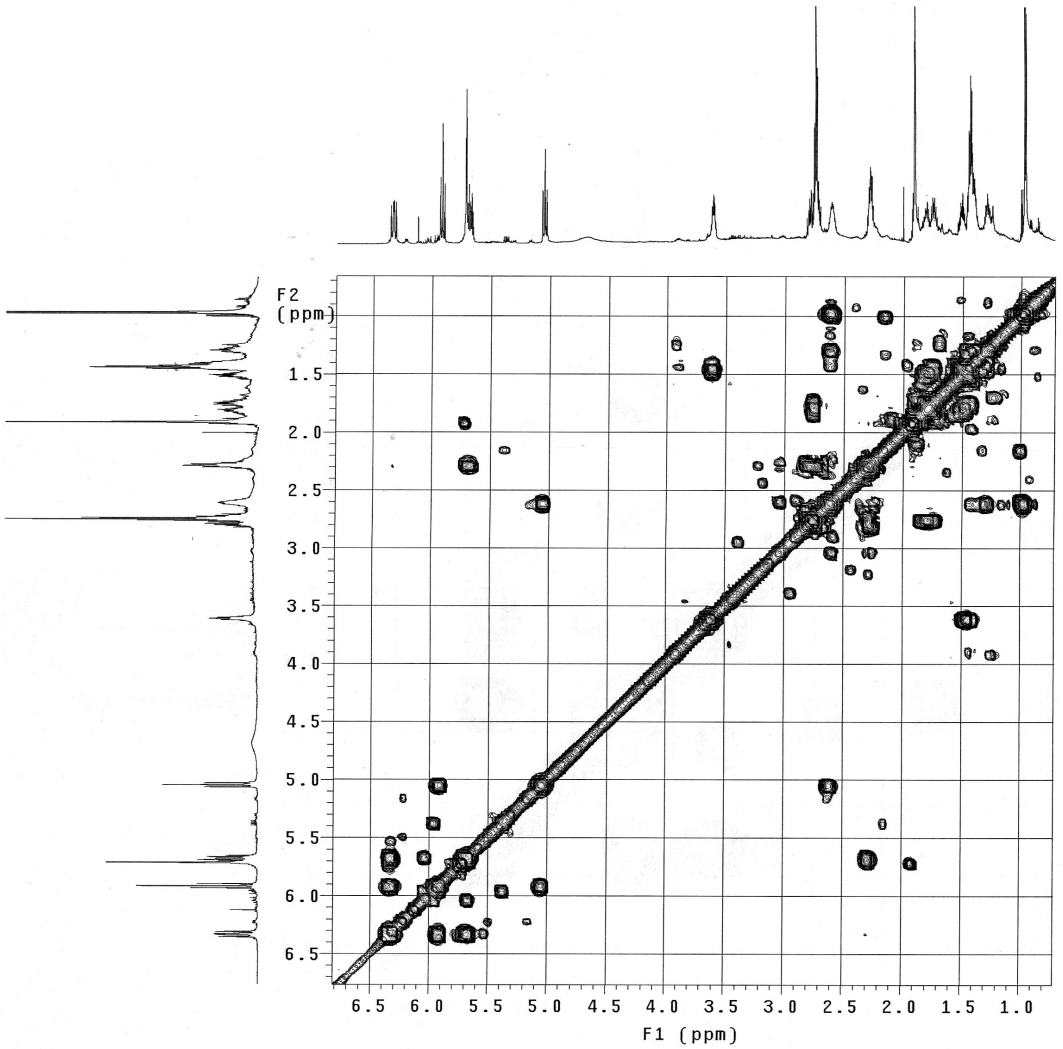


Figure S30. HMQC spectrum of apo-latrunculin T (**14**, 600 MHz, $CDCl_3$).

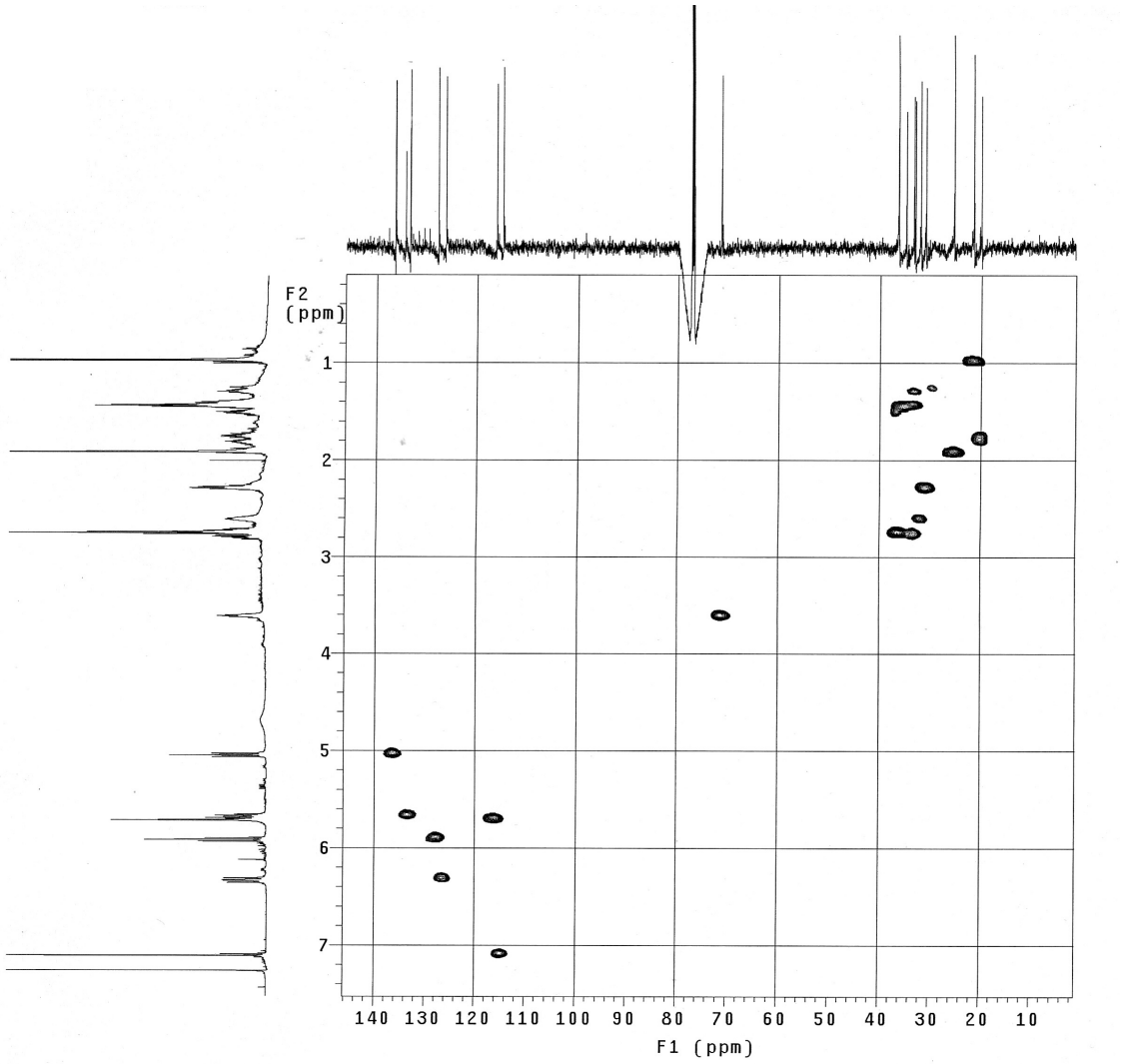


Figure S31. HMBC NMR spectrum of apo-latrunculin T (**14**, 600 MHz, $CDCl_3$).

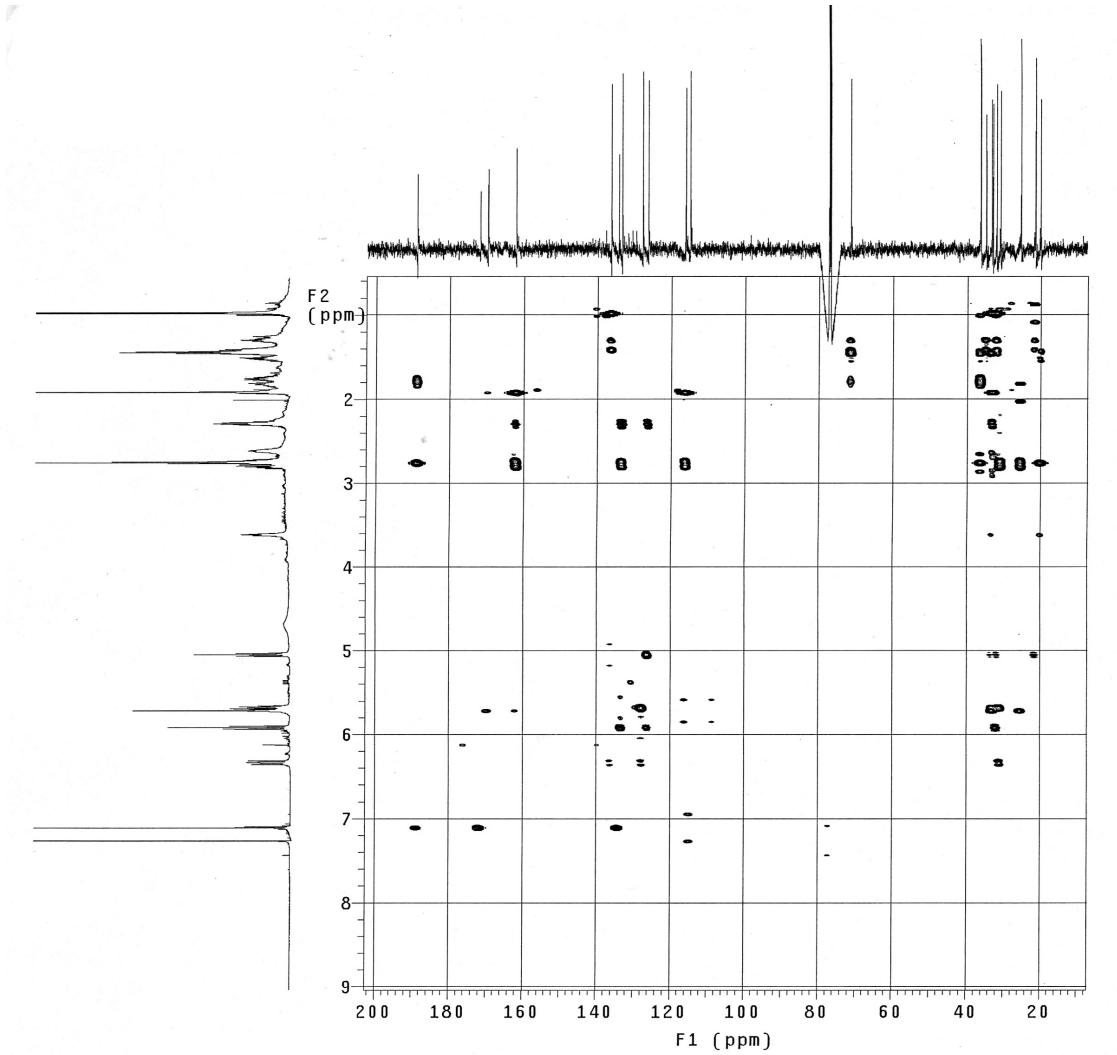


Figure S32. NOESY NMR spectrum of apo-latrunculin T (**14**, 600 MHz, $CDCl_3$).

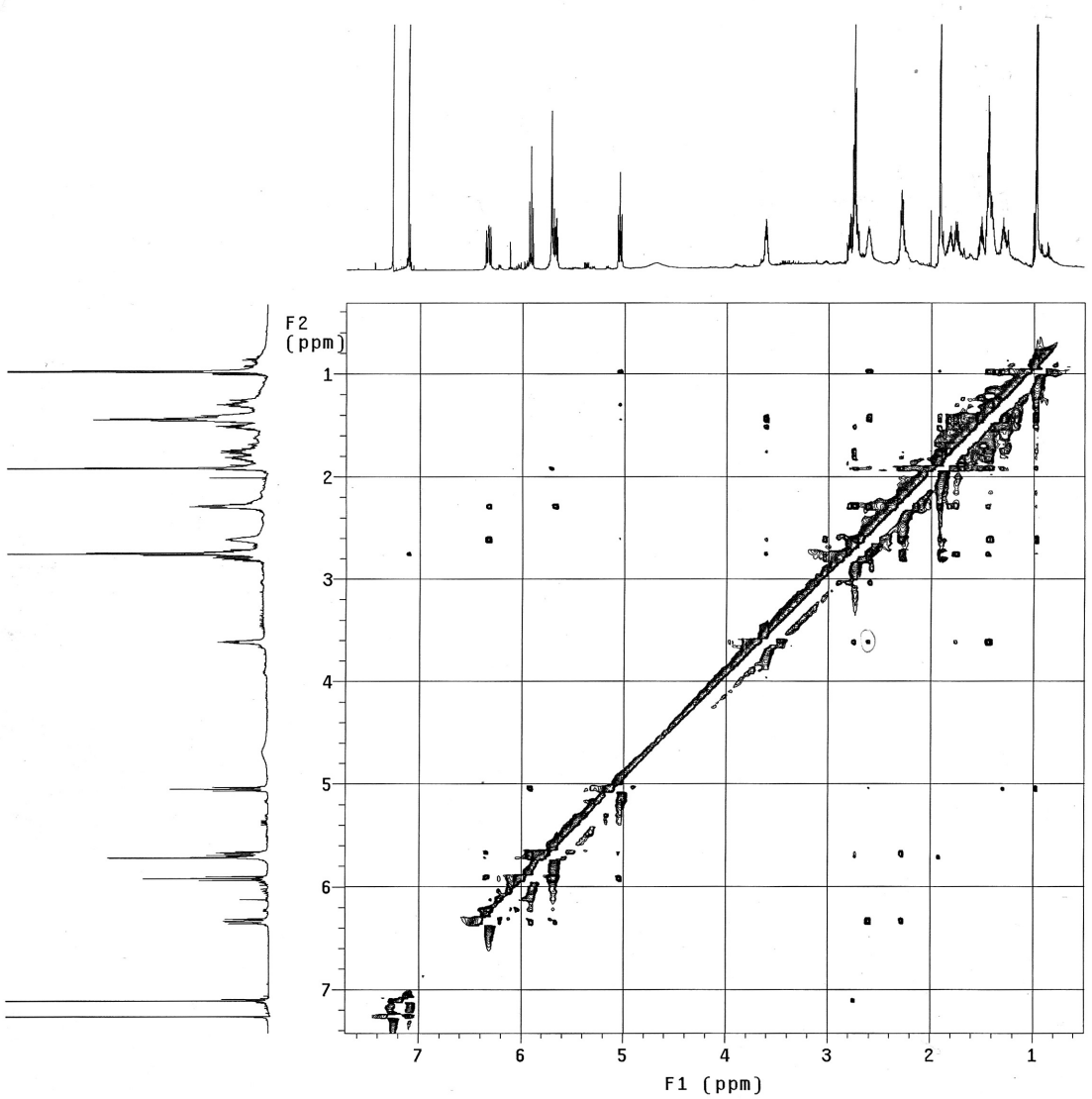


Figure S33. ^{1}H NMR spectrum of 20-methoxy-fijanolide A (**15**, 600 MHz, C_6D_6).

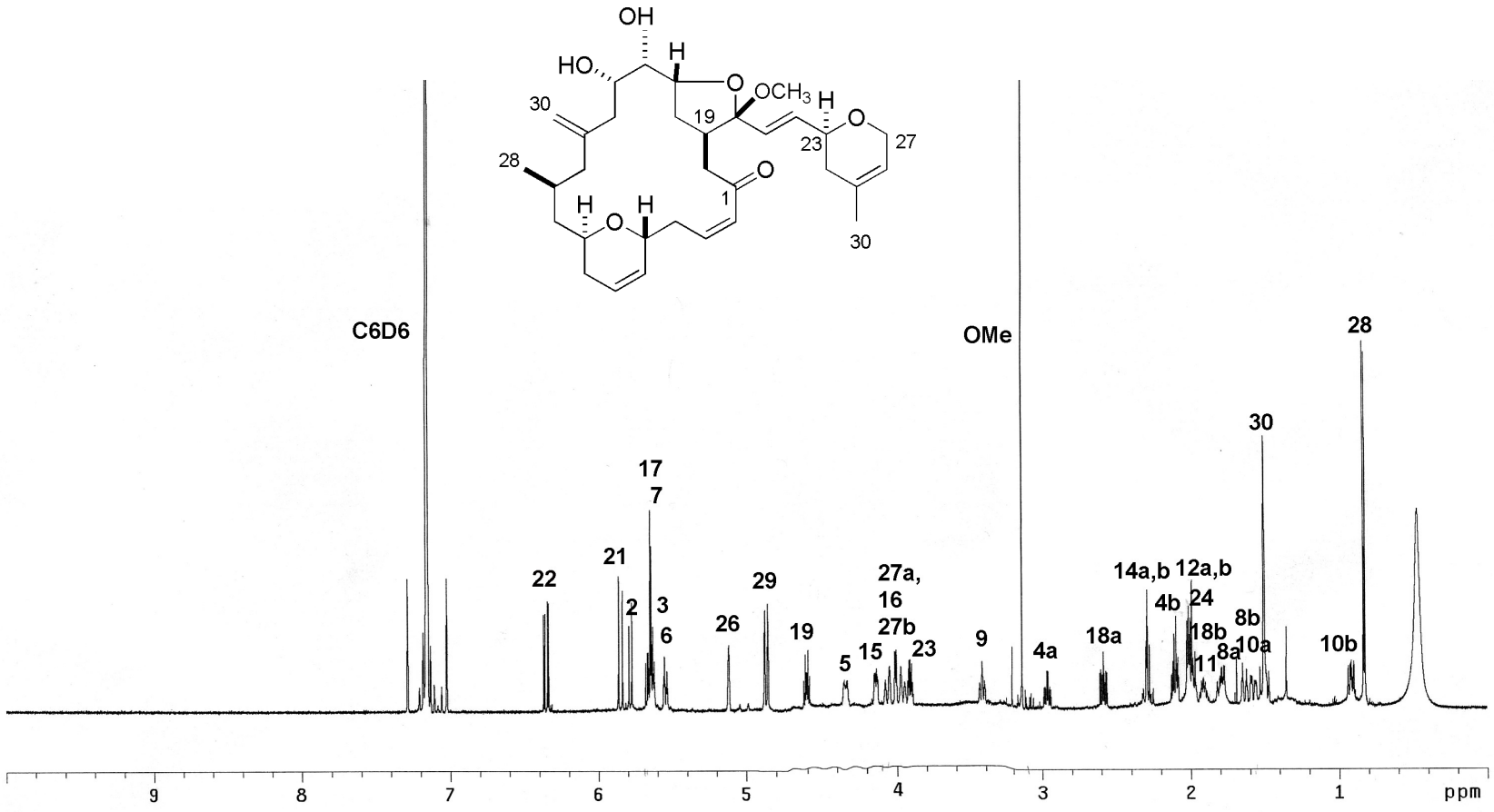


Figure S34. ^{13}C NMR spectrum of 20-methoxy-fijanolide A (**15**, 125 MHz, C_6D_6).

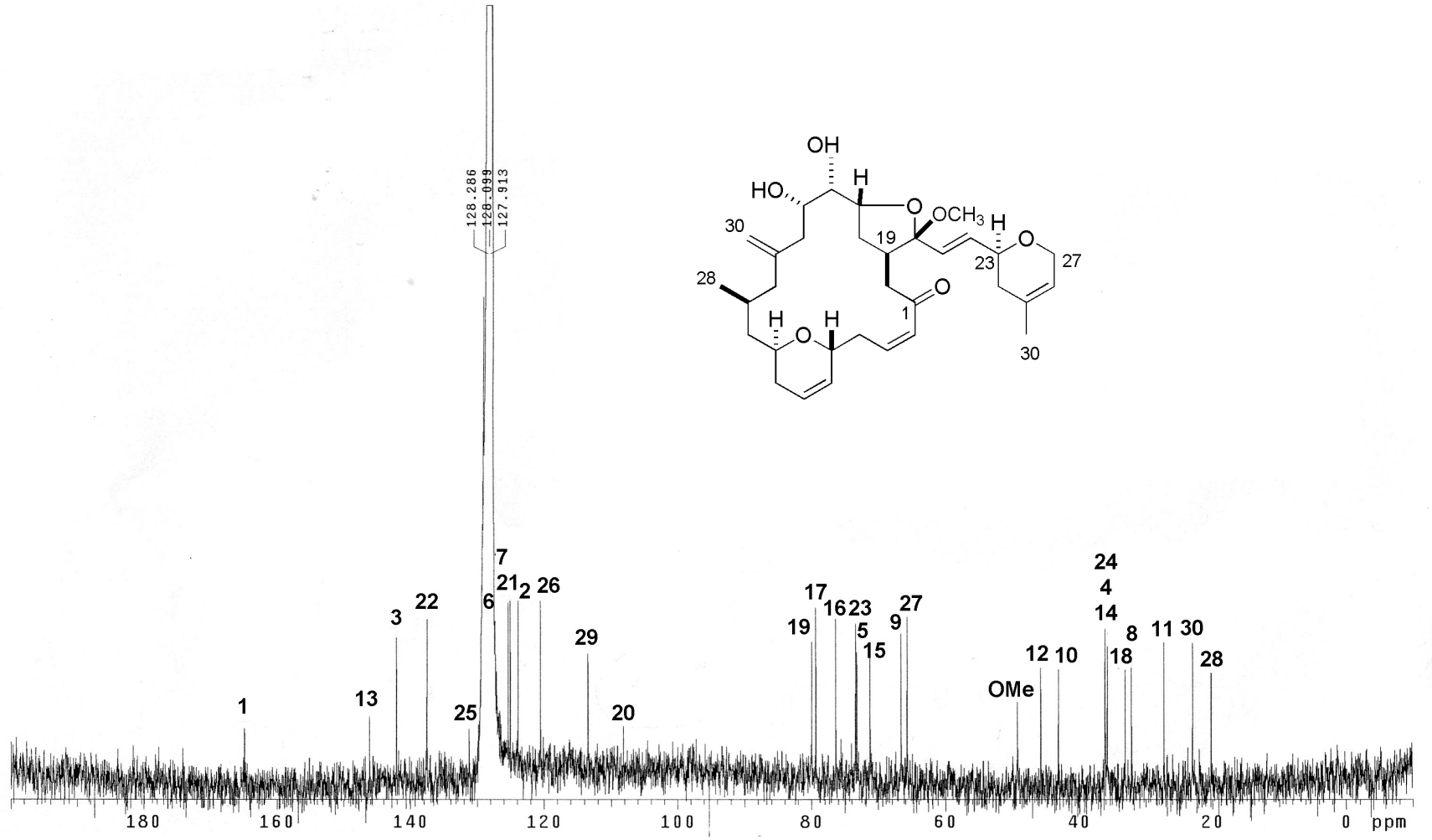


Figure S35. COSY NMR spectrum of 20-methoxy-fijanolide A (**15**, 600 MHz, C_6D_6).

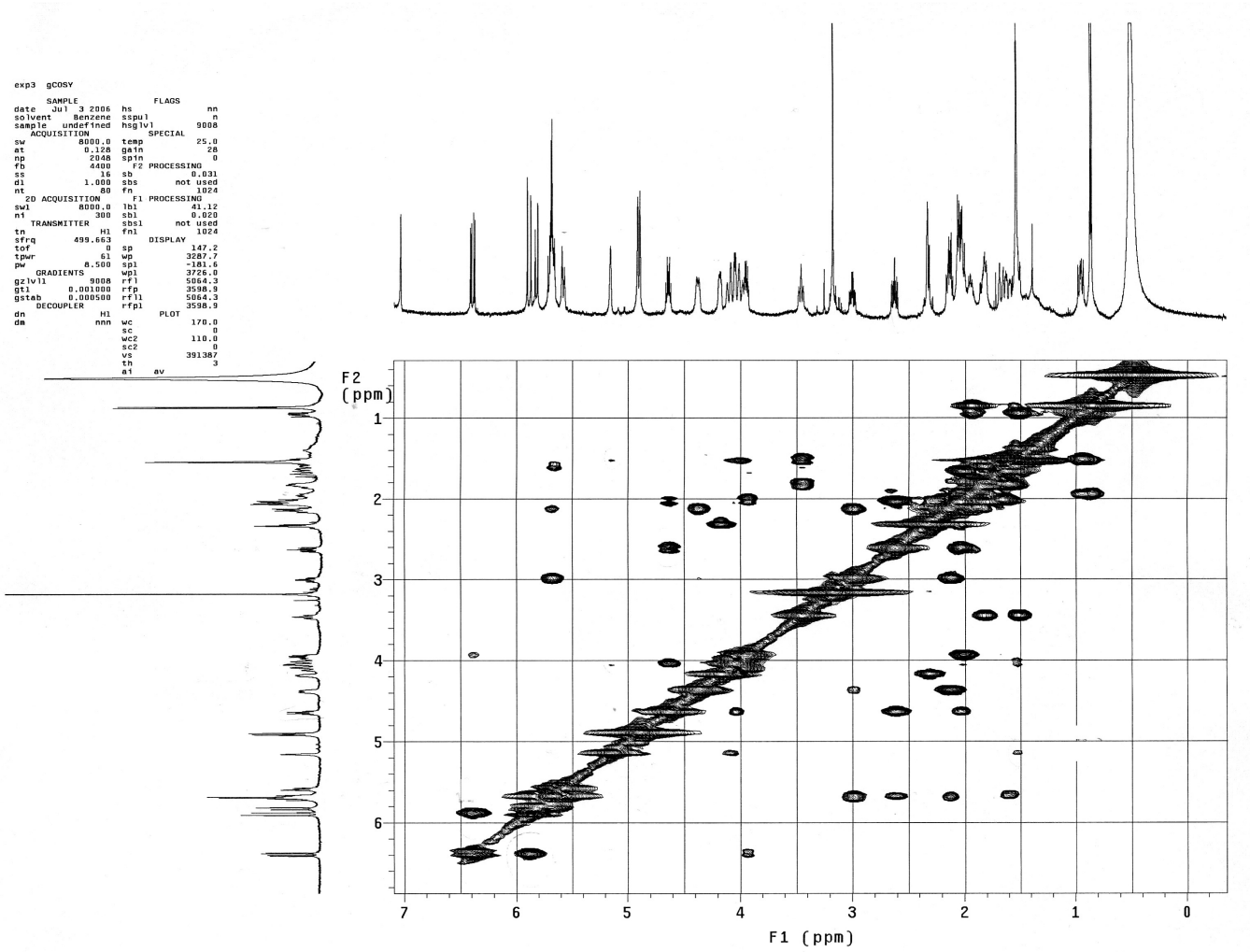


Figure S36. HMQC NMR spectrum of 20-methoxy-fijanolide A (**15**, 600 MHz, C_6D_6).

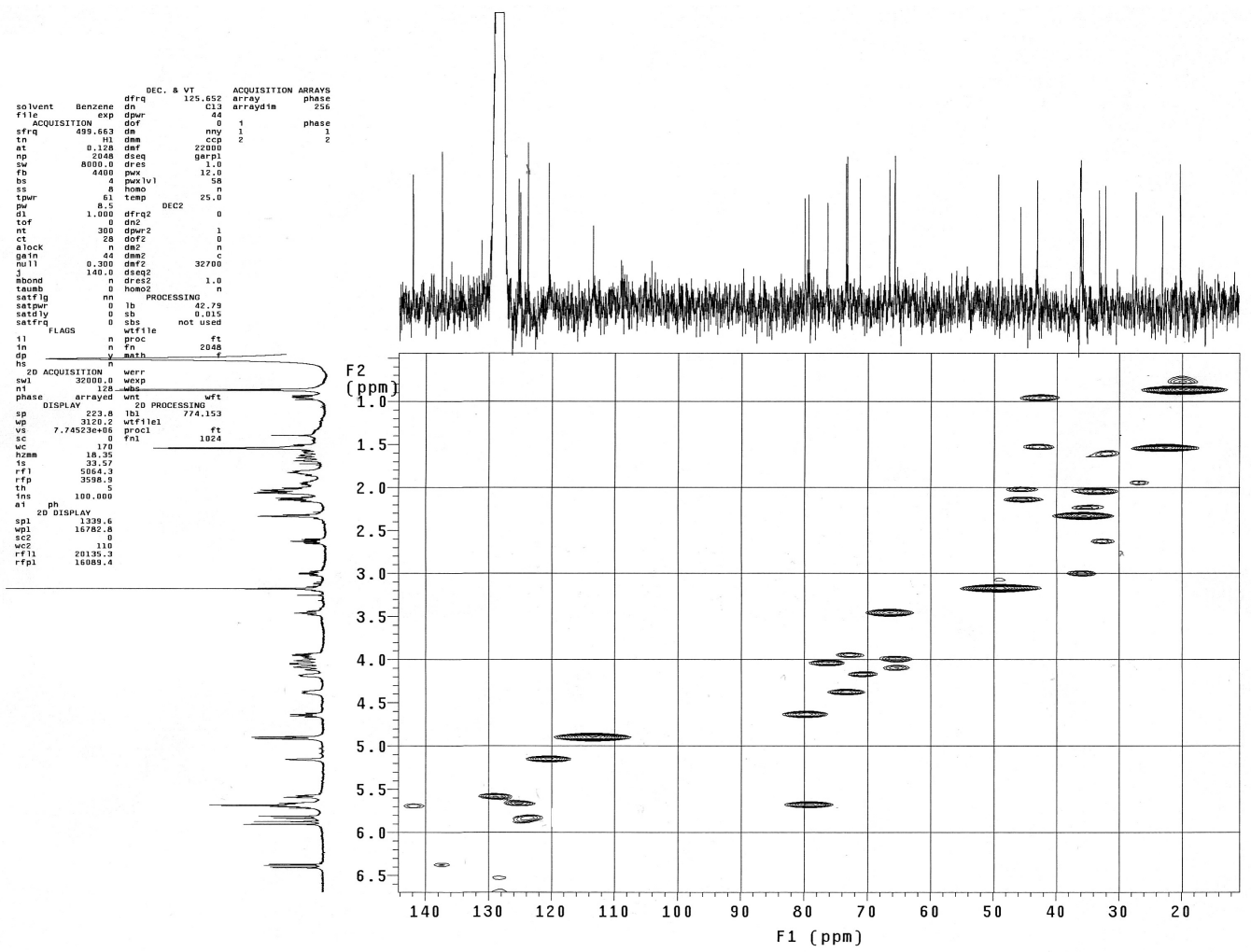


Figure S37. HMBC NMR spectrum of 20-methoxy-fijanolide A (**15**, 600 MHz, C_6D_6).

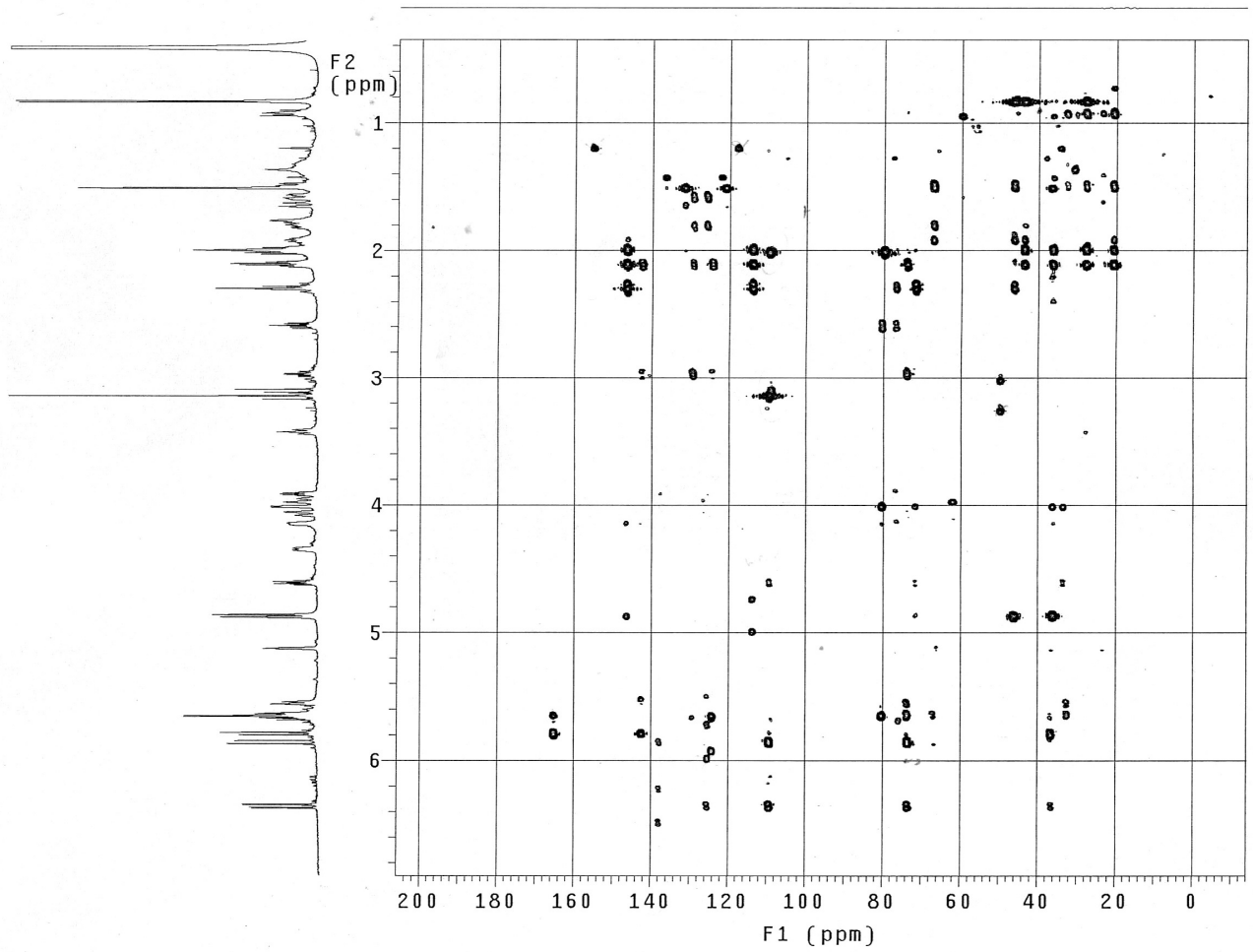


Figure S38. NOESY NMR spectrum of 20-methoxy-fijanolide A (**15**, 600 MHz, C_6D_6)

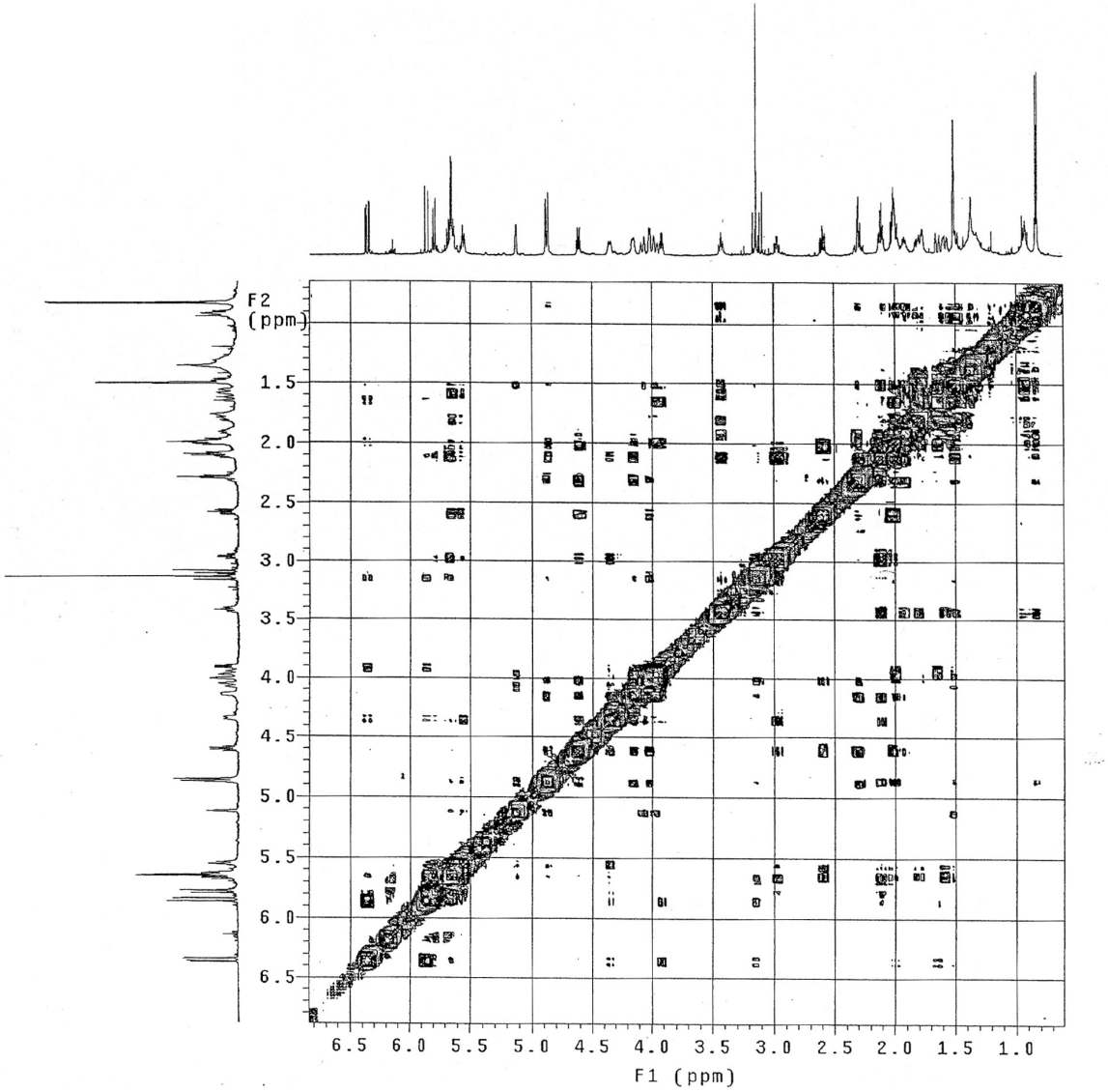


Figure S39. ^{1}H NMR spectrum of aignopsane ketal (**16**, 600 MHz, $CDCl_3$).

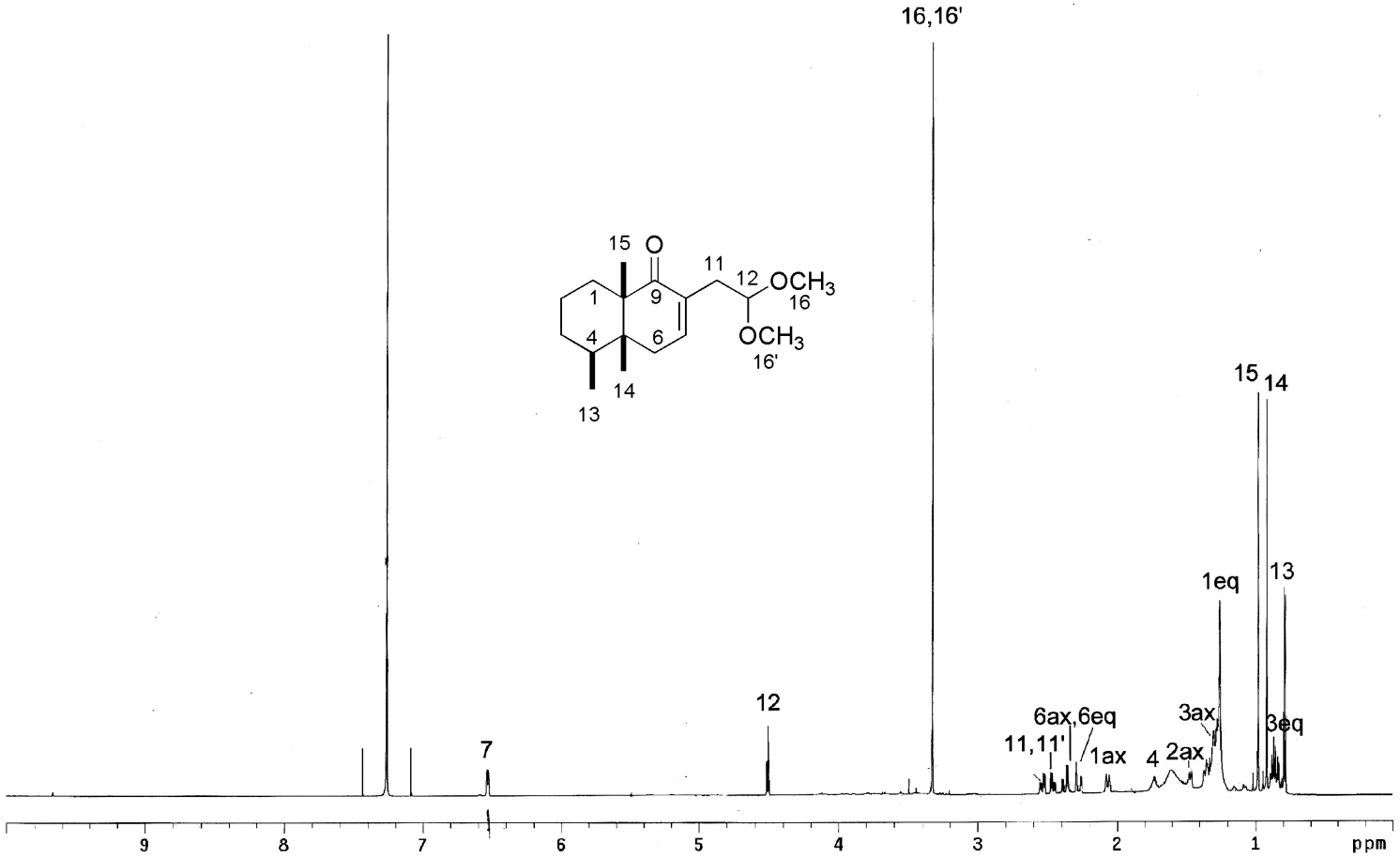


Figure S40. ^{13}C NMR spectrum of aignopsane ketal (**16**, 125 MHz, $CDCl_3$).

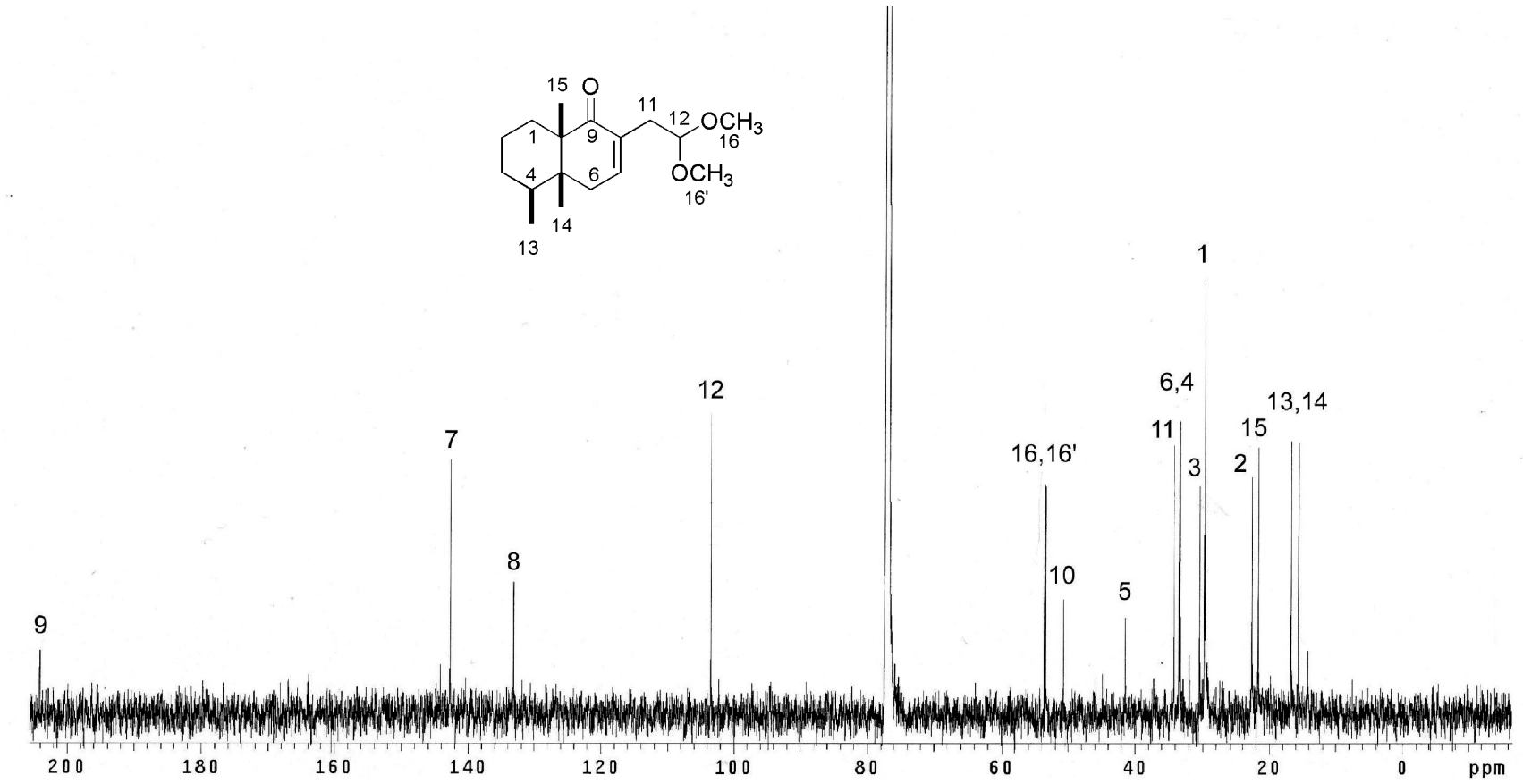


Figure S41. COSY spectrum of aignopsane ketal (**16**, 600 MHz, $CDCl_3$).

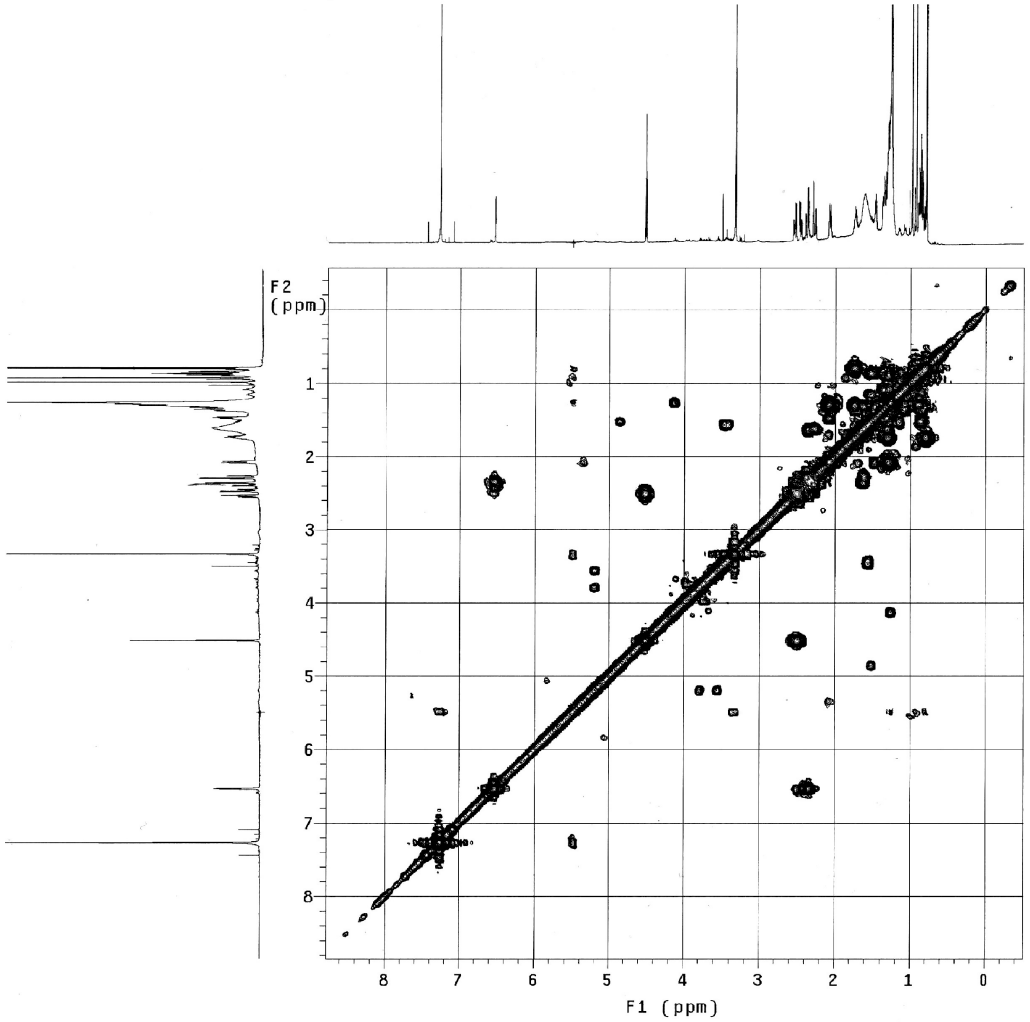


Figure S42. HMQC spectrum of aignopsane ketal (**16**, 600 MHz, $CDCl_3$).

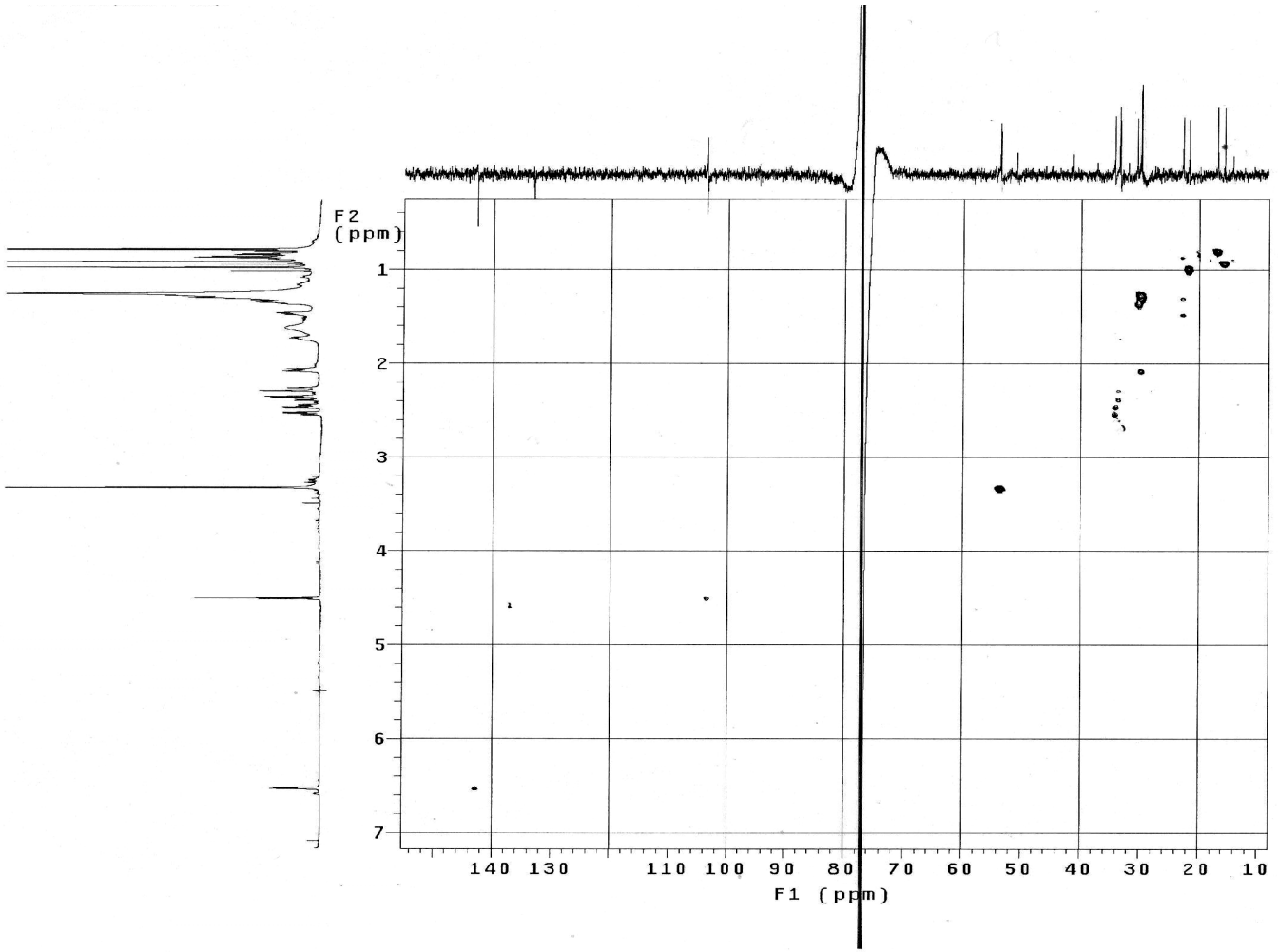


Figure S43. HMBC spectrum of aignopsane ketal (**16**, 600 MHz, $CDCl_3$).

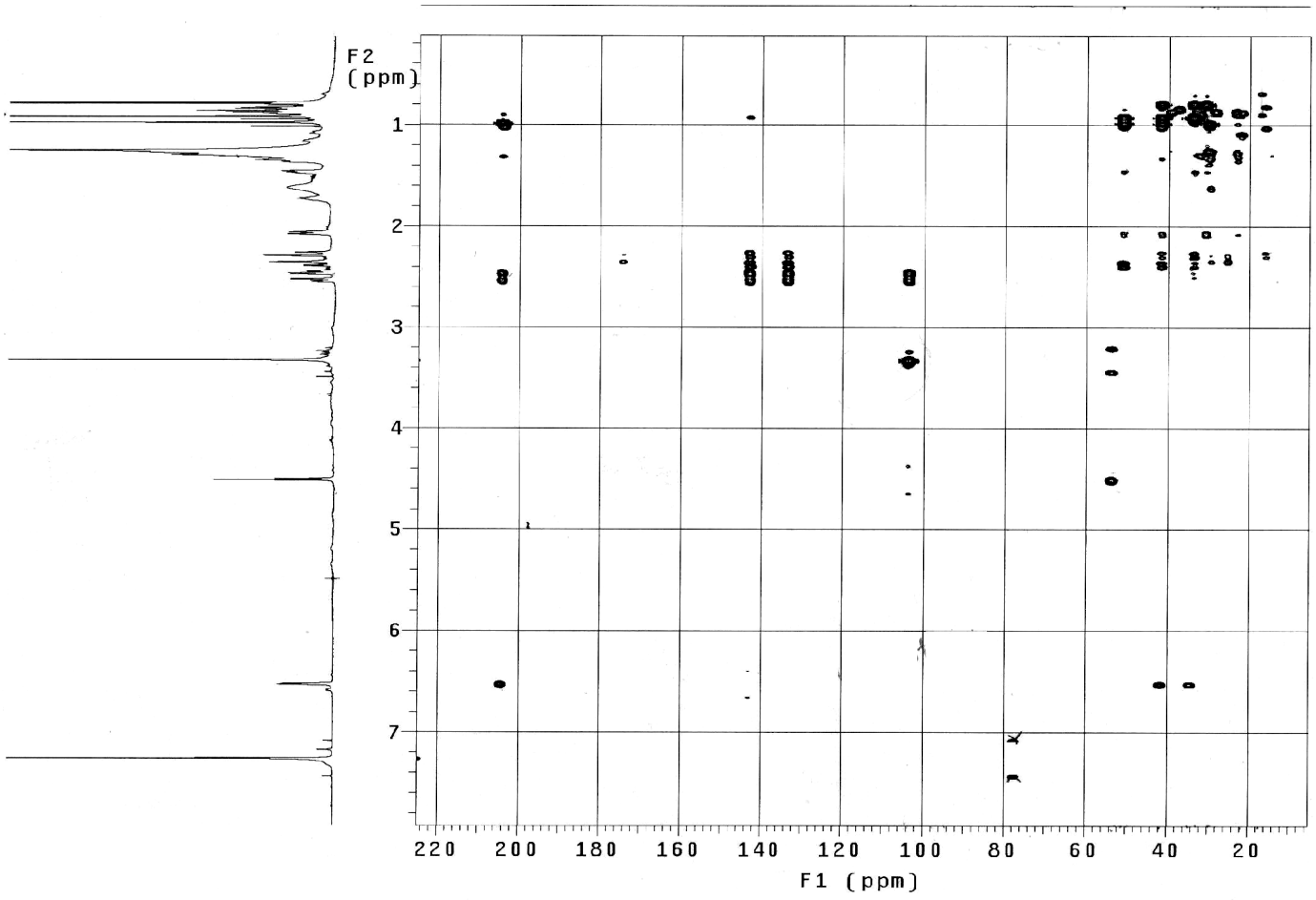


Figure S44. NOE enhancement of H_3-14 of **16** (600 MHz, $CDCl_3$).

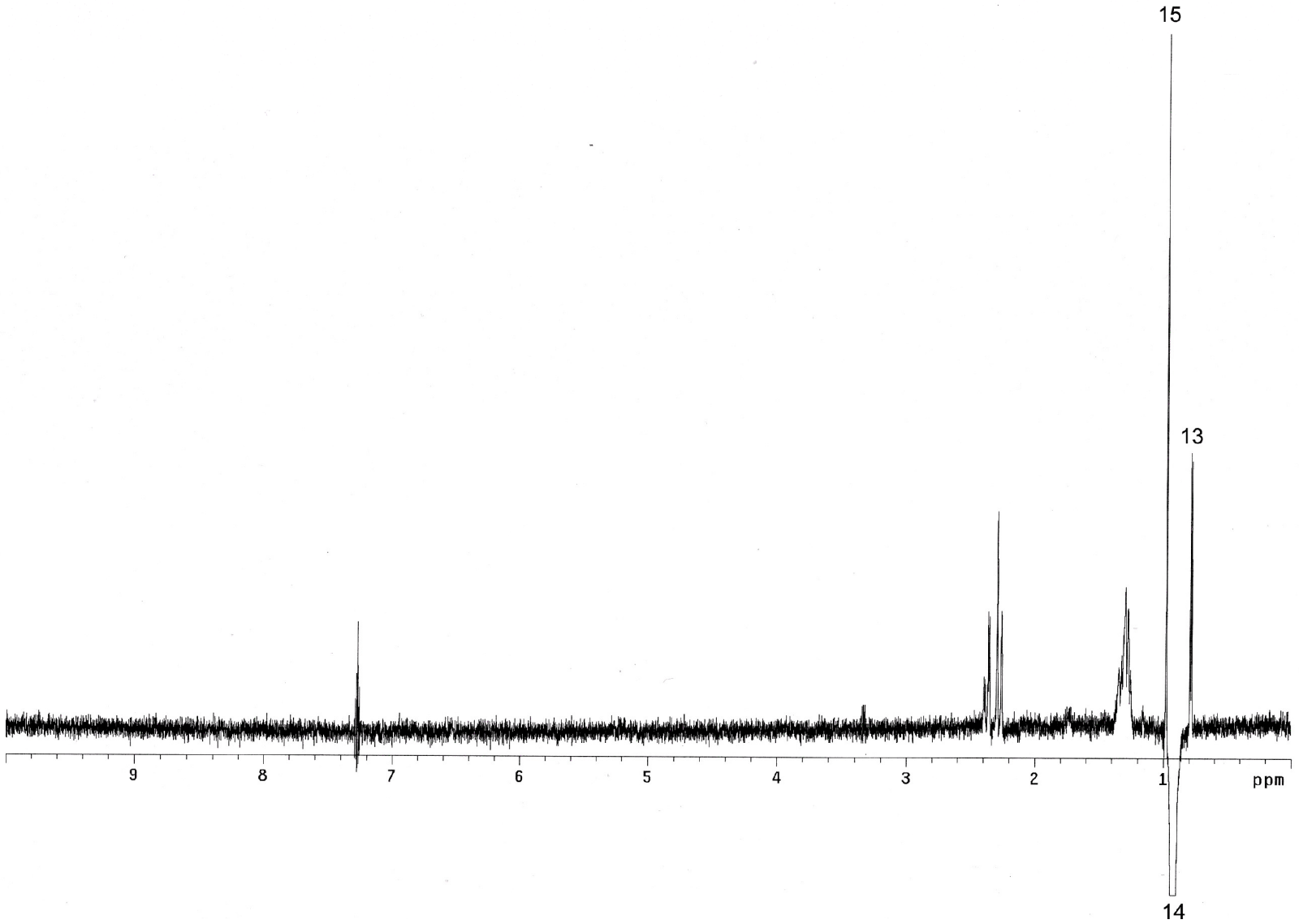
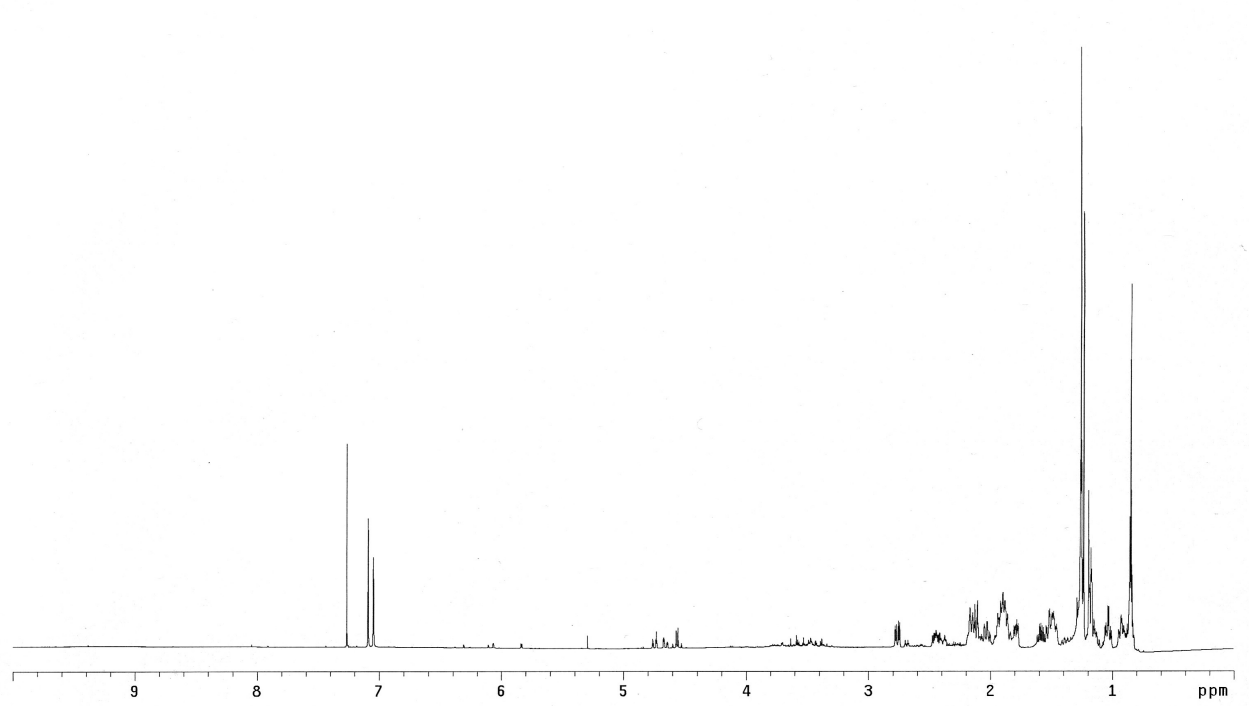
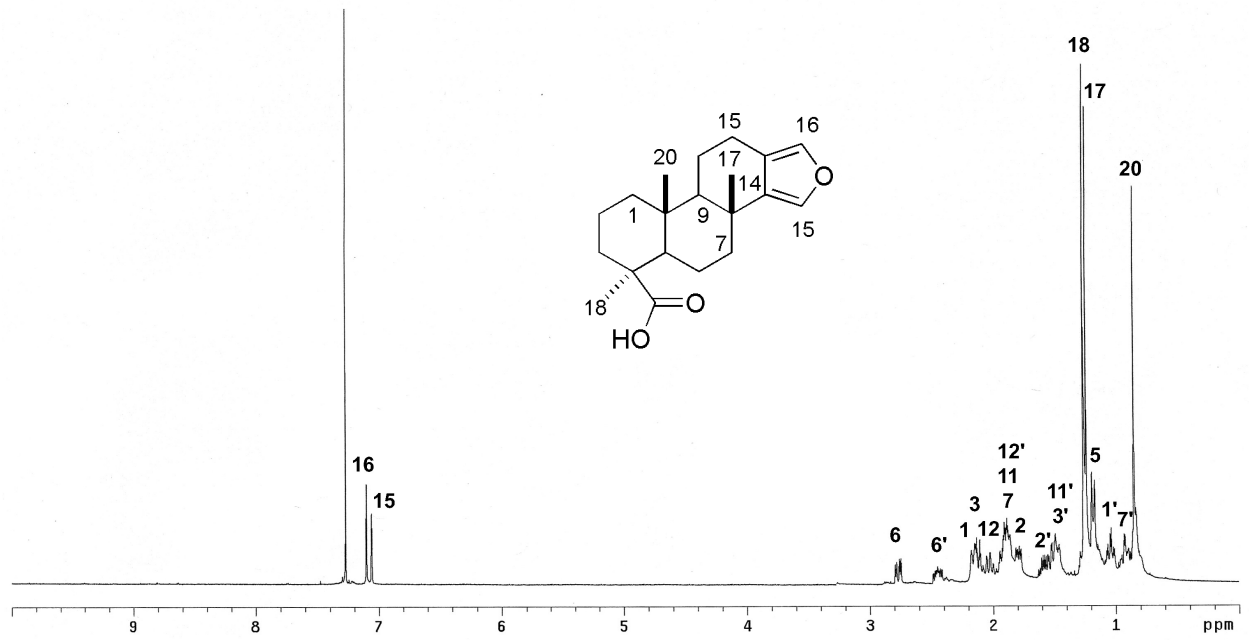


Figure S45. ^{1}H NMR spectrum of 92503 FH and 92503 FH H27, (600 MHz, $CDCL_3$)



a) 92503 FH (crude extract)



b) 92503 FH H27 (bioactive library fraction)

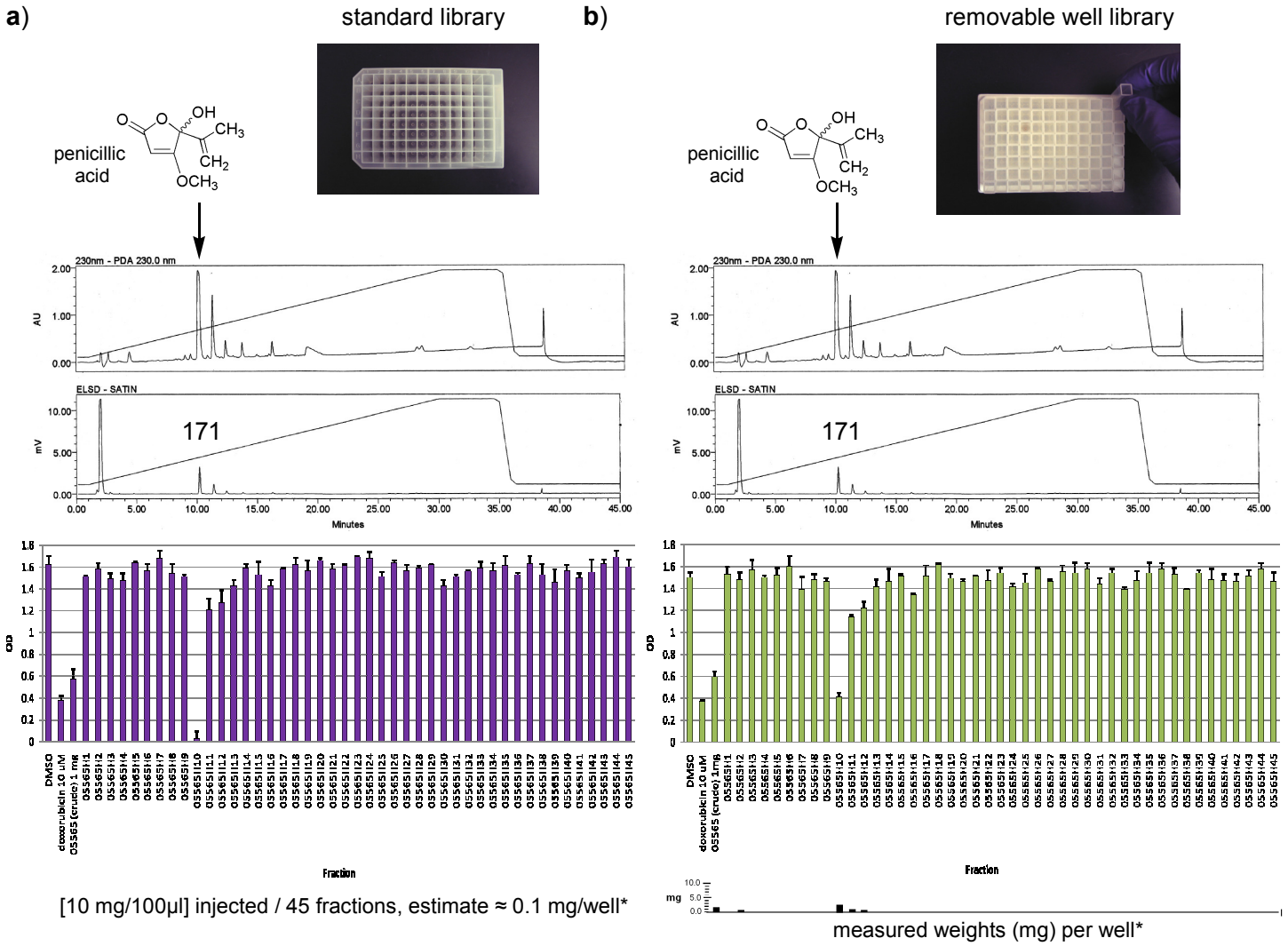
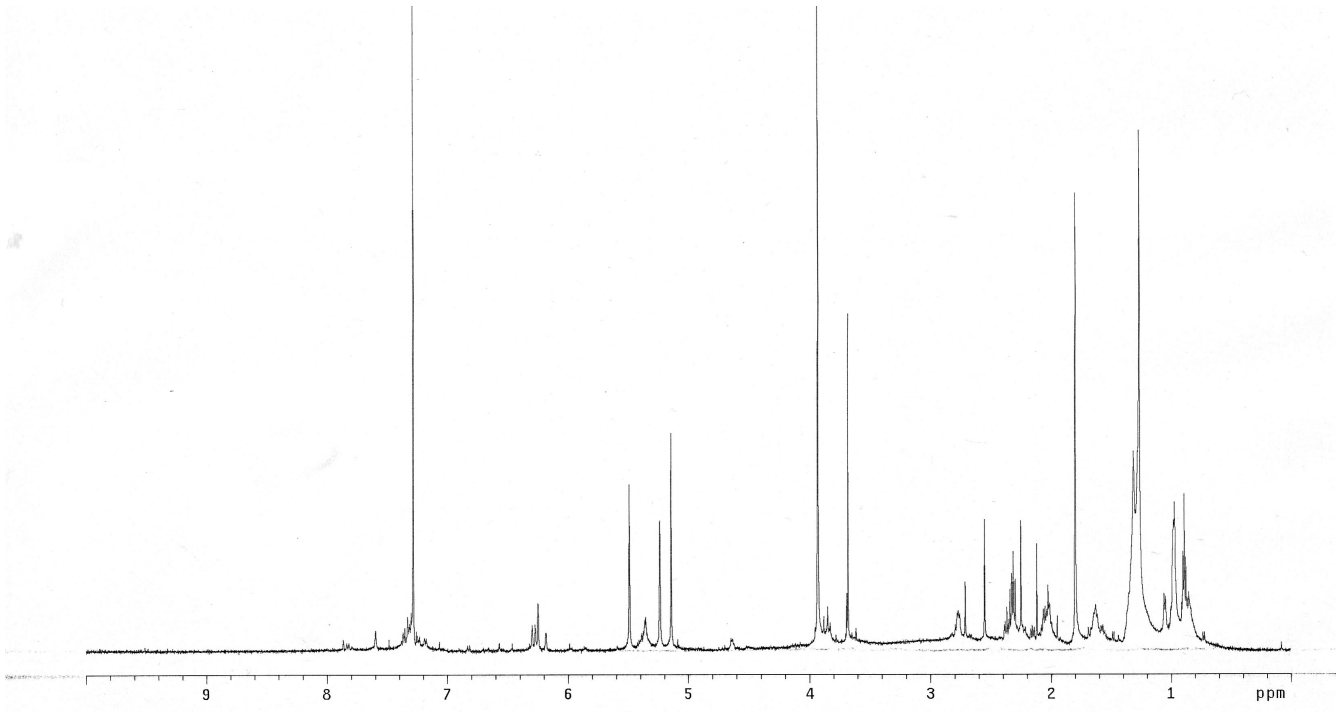
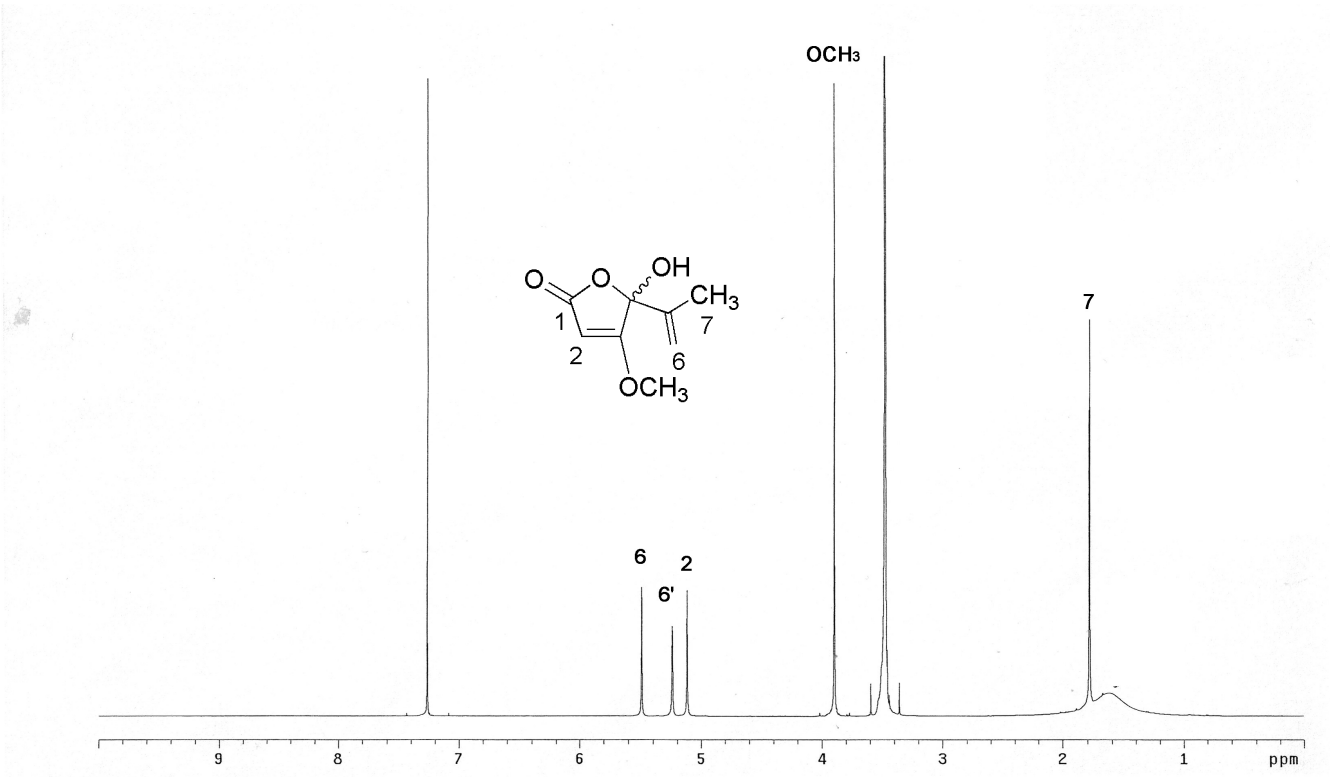


Figure S46. Comparative LC-MS-UV-ELSD traces with annotations of *m/z* ions (top) and cytotoxicity data (bottom) of coll. no. 05565 L against prostate (PC3) cells using an MTT bioassay with: (**a**) standard library (wells assayed at 10 µg/mL - averaging 0.1 mg/well) and (**b**) removable well library (wells assayed at 10 µg/mL - based on measured weights/well). *Amounts were based on a 10mg/100µl HPLC injection for both samples. Sample (**a**) was determined from dividing 10 mg by 45 fractions ≈ 0.2 mg. A duplicate library was then made from the original removing 1.0 mL/well with a 12 channel multipipeter to arrive at an assumed ≈ 0.1 mg/well weight for the original and reference library plates. Sample (**b**) weights are the results of measured weights/well after the creation of a duplicate reference library.

Figure S47. ^{1}H NMR spectrum of 05565 and 05565 H10, (600 MHz, $CDCL_3$)



a) 05565 L (crude extract)



b) 05565 L H10 (bioctive library fraction)

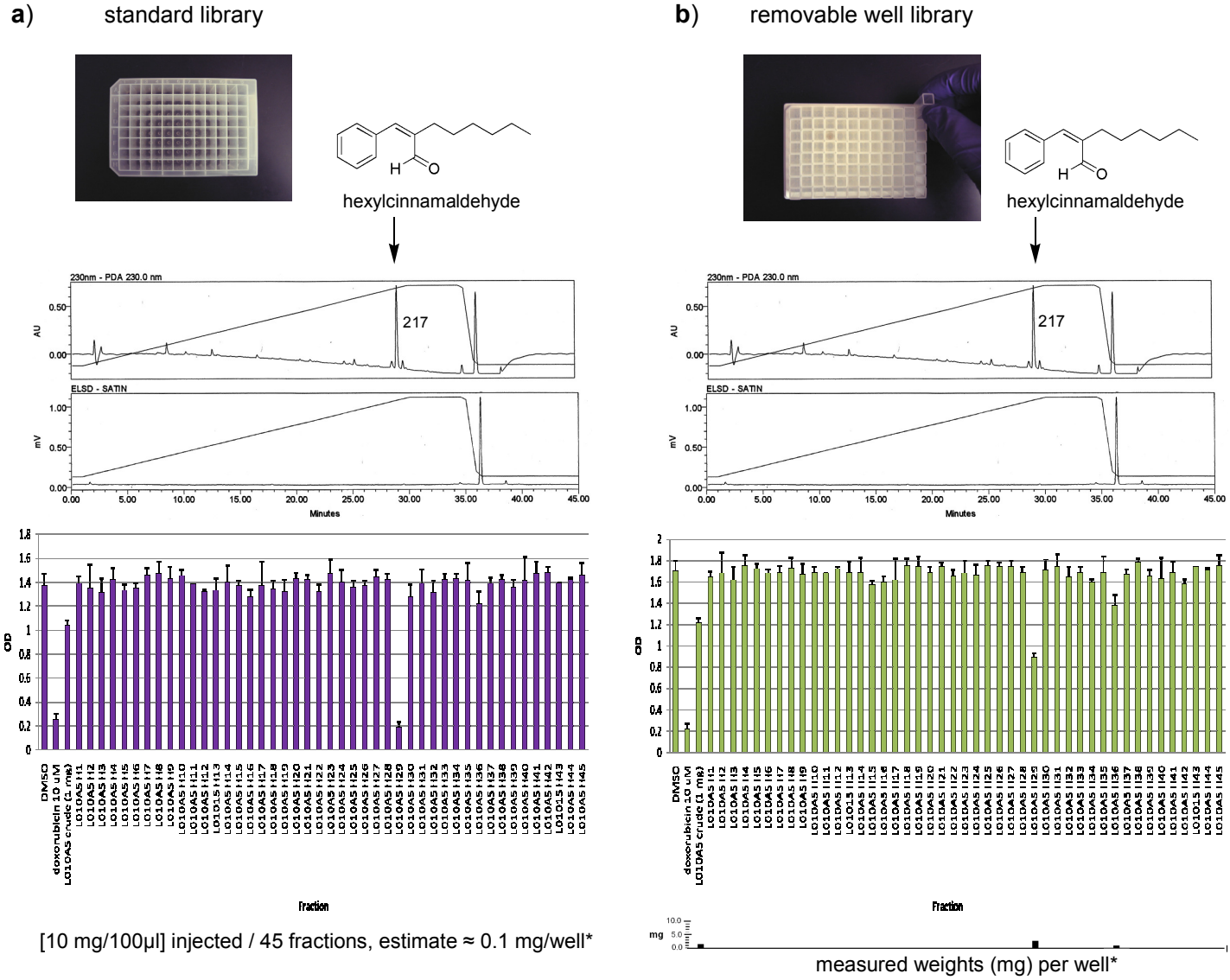
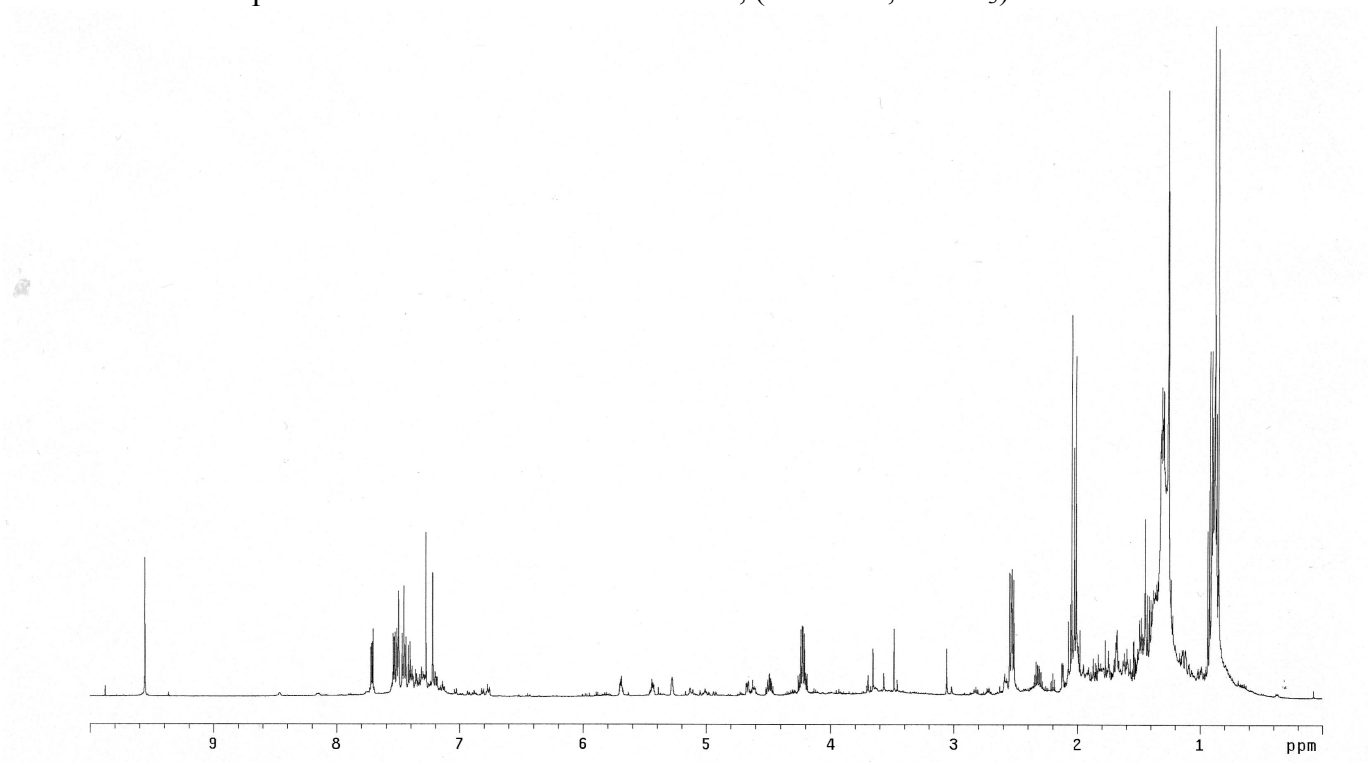
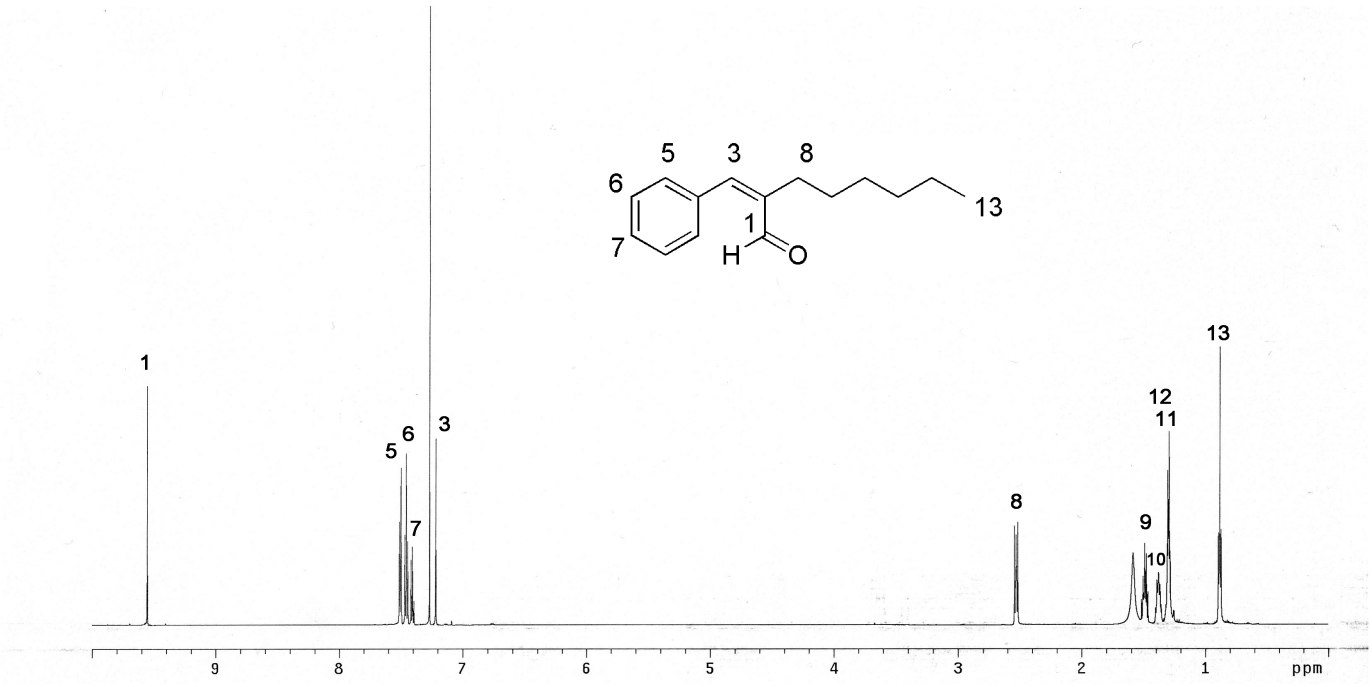


Figure S48. Comparative LC-MS-UV-ELSD traces with annotations of *m/z* ions (top) and cytotoxicity data (bottom) of coll. no. 010A5 L against prostate (PC3) cells using an MTT bioassay with: (**a**) standard library (wells assayed at 10 µg/mL - averaging 0.1 mg/well) and (**b**) removable well library (wells assayed at 10 µg/mL - based on measured weights/well).*Amounts were based on a 10mg/100µl HPLC injection for both samples. Sample (**a**) was determined from dividing 10 mg by 45 fractions ≈ 0.2 mg. A duplicate library was then made from the original removing 1.0 mL/well with a 12 channel multipipeter to arrive at an assumed ≈ 0.1 mg/well weight for the original and reference library plates. Sample (**b**) weights are the results of measured weights/well after the creation of a duplicate reference library.

Figure S49. ^{1}H NMR spectrum of L010A5 and L010A5 H29, (600 MHz, $CDCL_3$)

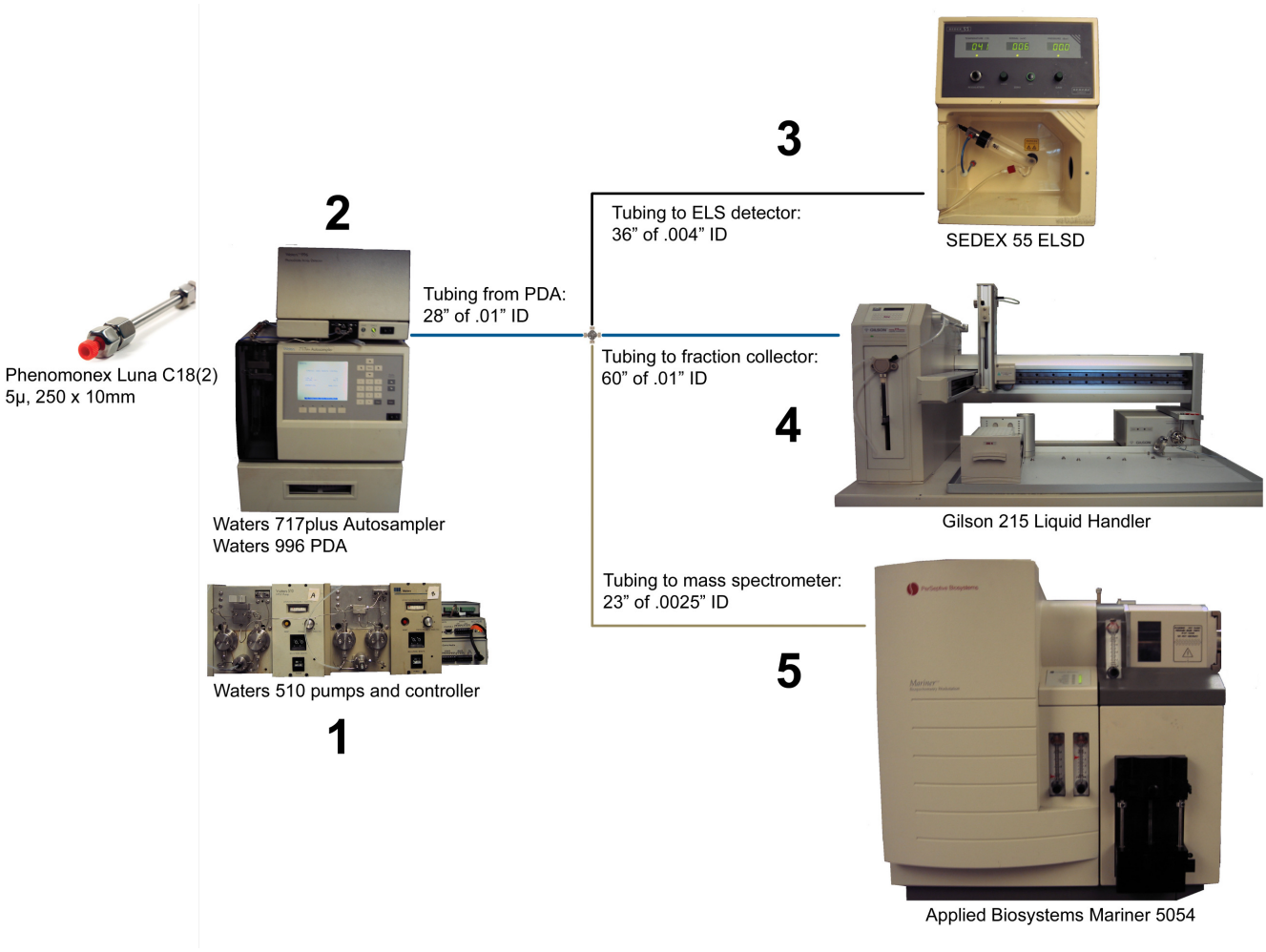


a) L010A5 (crude extract)



b) L010A5 H29 (bioactive library fraction)

Figure S50. Instrumentation, tubing length and sources to create LC-MS-UV-ELSD libraries



Notes and abbreviations:

1) HPLC gradient controlled pumps, **2**) autosampler connected to reverse phase (RP) C-18 5 µm 250mm x 10 mm HPLC column and photo diode array (PDA) detector - split with a cross union using color coded, based on inner diameter (ID), peak tubing connected to: **3**) evaporative light scattering detector (ELSD), **4**) automated fraction collector (liquid handler) and **5**) electrospray time of flight (TOF) mass spectrometer.

available sources and prices continued on page 57 . . .

The above instrumentation can be found below from the following sources:

Item	Instrument	OEM Source (approximate cost)	Alternative Source (approximate cost)
1	gradient controlled HPLC pumps	Waters 515 pump ($8,200)	GenTechScientific.com - Waters 510 pump ($2500)
1	gradient controlled HPLC pumps	Waters 2535 Quaternary Gradient Module ($27,600)	AmericanLaboratoryTrading.com - Waters 600 pump and controller ($3500)
1	gradient controlled HPLC pumps	Agilent 1260 Binary Pump ($17,300)	LabX.com - Agilent 1100 Binary Pump G1312A ($6400)
2	autosampler	Waters 2707	GenTechScientific.com - Waters 717plus ($4400)
2	autosampler	Agilent 1260 Infinity Standard ($12,000)	LabX.com - Agilent 1100 Prep AS G2260A ($8200)
2	photo diode array (PDA)detector		LabX.com - Waters 996 PDA ($2500)
2	photo diode array (PDA)detector	Waters 2998 PDA ($23,200)	GenTechScientific.com - Waters 2996 PDA ($7500)
2	photo diode array (PDA)detector	Agilent 1260 DAD ($16,200)	LabX.com - Agilent 1100 DAD ($8000)
3	evaporative light scattering detector (ELSD)	Sedere SEDEX 80LT	GenTechScientific.com - SEDEX 75 ($7000)
3	evaporative light scattering detector (ELSD)	Waters 2424 ELSD	LabX.com - Waters 2420 ELSD ($12,000)
4	automated fraction collector	Gilson 215 FC ($17,200)	LabX.com - Gilson 215 ($3,000)
4	automated fraction collector	Agilent 1260 Analytical Scale FC ($11,000)	LabX.com - Agilent 1100 G1364A FC ($6,000)
4	automated fraction collector	Waters 2767 Sample Manager–FC ($44,900)	LabX.com - Waters Sample Manager ($3,500)
5	mass spectrometer	Waters 3100 Mass Detector ($135,500)	GrizzlyAnalytical.com - Applied Biosystems Mariner ESI-TOF ($34,000)
5	mass spectrometer	Agilent 5975 Single Quad ($105,000)	
5	mass spectrometer	Thermo LCQ Fleet Ion Trap ($132,500)	LabX.com - Thermo LCQ Duo ($20,000)
6	tubing	IDEX Health & Science	

Figure S51. Example of standard 96 well plate library sample workup sheet

SAMPLE WORKUP SHEET Date_1/27/11_____

Natural Product LC-MS-UV-ELSD library using: standard 96-well plate

entry	sample coll. no.	fraction	well	pre tare (mg)	post tare (mg)	amt. bioassay (mg)*	vol. trans. (µl)	NOTES
1	marine sponge 92503 FH	crude	A1	NA	NA	1.0		crude extract w/ cyto-toxicity to macrophage RAW 264.7 cells
2		H1	A2	NA	NA	0.1		
3		H2	A3	NA	NA	0.1		
4		H3	A4	NA	NA	0.1		
5		H4	A5	NA	NA	0.1		
6		H5	A6	NA	NA	0.1		
7		H6	A7	NA	NA	0.1		
8		H7	A8	NA	NA	0.1		
9		H8	A9	NA	NA	0.1		
10		H9	A10	NA	NA	0.1		
11		H10	A11	NA	NA	0.1		
12		H11	A12	NA	NA	0.1		
13		H12	B1	NA	NA	0.1		
14		H13	B2	NA	NA	0.1		
15		H14	B3	NA	NA	0.1		
16		H15	B4	NA	NA	0.1		
17		H16	B5	NA	NA	0.1		
18		H17	B6	NA	NA	0.1		
19		H18	B7	NA	NA	0.1		
20		H19	B8	NA	NA	0.1		
21		H20	B9	NA	NA	0.1		
22		H21	B10	NA	NA	0.1		
23		H22	B11	NA	NA	0.1		
24		H23	B12	NA	NA	0.1		
25		H24	C1	NA	NA	0.1		
26		H25	C2	NA	NA	0.1		
27		H26	C3	NA	NA	0.1		
28		H27	C4	NA	NA	0.1		
29		H28	C5	NA	NA	0.1		
30		H29	C6	NA	NA	0.1		
31		H30	C7	NA	NA	0.1		
32		H31	C8	NA	NA	0.1		
33		H32	C9	NA	NA	0.1		
34		H33	C10	NA	NA	0.1		
35		H34	C11	NA	NA	0.1		
36		H35	C12	NA	NA	0.1		
37		H36	D1	NA	NA	0.1		
38		H37	D2	NA	NA	0.1		
39		H38	D3	NA	NA	0.1		
40		H39	D4	NA	NA	0.1		
41		H40	D5	NA	NA	0.1		
42		H41	D6	NA	NA	0.1		
43		H42	D7	NA	NA	0.1		
44		H43	D8	NA	NA	0.1		
45		H44	D9	NA	NA	0.1		
46		H45	D10	NA	NA	0.1		
47		H46	D11	NA	NA	0.1		
48		H47	D12	NA	NA	0.1		

NOTES: Not available (NA). *Assumed amounts were based on a [10mg/100µl] HPLC injection divided by 47 fractions ≈ 0.2 mg. A duplicate library was then made from the original 96 well plate (containing 0.2 mg/2mL/well) removing 1.0 mL/well with a 12 channel multipipeter to arrive at an assumed ≈ 0.1 mg/well weight for the original and reference library plates.

Figure S52. Example of removable 96 well plate library sample workup sheet

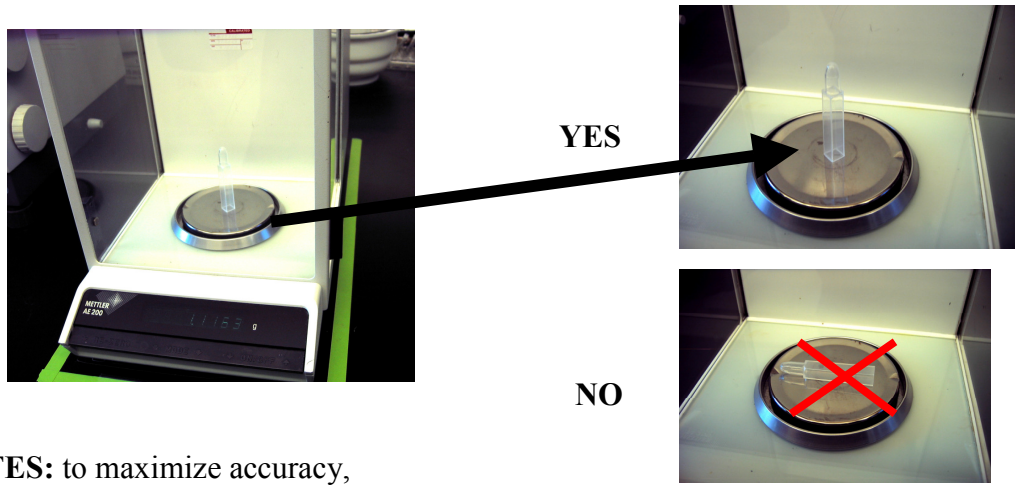
SAMPLE WORKUP SHEET Date_1/27/11_____

Natural Product LC-MS-UV-ELSD library using: removable 96-well plate

entry	sample coll. no.	fraction	well	pre tare (mg)	post tare (mg)	amt. bioassay (mg)*	vol. trans. (µl)	NOTES
1	marine sponge 92503 FH	crude	A1	1.1141	1.1151	1.0		crude extract w/ cyto-toxicity to macrophage RAW 264.7 cells
2		H1	A2	1.1147	1.1147	0.0		
3		H2	A3	1.1153	1.1153	0.0		
4		H3	A4	1.1157	1.1156	-0.1		
5		H4	A5	1.1029	1.1029	0.0		
6		H5	A6	1.1161	1.1162	0.1		
7		H6	A7	1.1136	1.1136	0.0		
8		H7	A8	1.1128	1.1127	-0.1		
9		H8	A9	1.1096	1.1096	0.0		
10		H9	A10	1.1142	1.1142	0.0		
11		H10	A11	1.1039	1.1039	0.0		
12		H11	A12	1.1029	1.1030	0.1		
13		H12	B1	1.1097	1.1098	0.1		
14		H13	B2	1.1092	1.1092	0.0		
15		H14	B3	1.1117	1.1118	0.1		
16		H15	B4	1.1163	1.1162	-0.1		
17		H16	B5	1.1103	1.1103	0.0		
18		H17	B6	1.1093	1.1095	0.2		
19		H18	B7	1.1033	1.1033	0.0		
20		H19	B8	1.1099	1.1199	0.0		
21		H20	B9	1.1107	1.1109	0.2		
22		H21	B10	1.1163	1.1163	0.0		
23		H22	B11	1.1117	1.1117	0.0		
24		H23	B12	1.1162	1.1164	0.2		
25		H24	C1	1.1171	1.1169	-0.2		
26		H25	C2	1.1092	1.1091	-0.1		
27		H26	C3	1.1156	1.1157	0.1		
28		H27	C4	1.1148	1.1183	3.3		
29		H28	C5	1.1139	1.1141	0.2		
30		H29	C6	1.1141	1.1146	0.5		
31		H30	C7	1.1162	1.1162	0.0		
32		H31	C8	1.1149	1.1148	-0.1		
33		H32	C9	1.1243	1.1244	0.1		
34		H33	C10	1.1246	1.1246	0.0		
35		H34	C11	1.1161	1.1167	0.6		
36		H35	C12	1.1158	1.1158	0.0		
37		H36	D1	1.1146	1.1145	-0.1		
38		H37	D2	1.1098	1.1100	0.2		
39		H38	D3	1.1090	1.1092	0.2		
40		H39	D4	1.1036	1.1036	0.0		
41		H40	D5	1.1111	1.1112	0.1		
42		H41	D6	1.1036	1.1036	0.0		
43		H42	D7	1.1151	1.1150	-0.1		
44		H43	D8	1.1244	1.1245	0.1		
45		H44	D9	1.1134	1.1134	0.0		
46		H45	D10	1.1152	1.1153	0.1		
47		H46	D11	1.1110	1.1109	-0.1		
48		H47	D12	1.1160	1.1160	0.0		

NOTES: Weight variability (+/- 0.2 mg) for removable wells was routinely encountered and may be a factor from the expansion and contraction of the plastic wells after speed vac evaporation. This library method appeared best suited for extract that have 1-3 major metabolites suspected of being the bioactive constituents versus extracts with widespread distributions of multiple metabolites/wells whose weights were not consistently measured with this approach below 0.3 mg.

Figure S53. Demonstration of how to weigh removable wells accurately from library



NOTES: to maximize accuracy,
make sure the removable wells are:

1) allowed to stand (return to room temp.) for ≥ 4hr after Speed Vac drying
2) free of any outside/inside moisture prior to weighing – use protective gloves
3) weighed inverted - upright
4) placed on the exact same location each time they are weighed on the scale

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