

## Supporting Information for

Massadine, a Novel Geranylgeranyltransferase Type I Inhibitor, from the Marine Sponge *Stylissa* aff. *massa*

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## Experimental Section

**General Methods,** see reference 6.

**Animal Material.** The sponge samples were collected by hand using scuba at a depth of 15 m off Atami in the Gulf of Sagami (35° 09' N, 139° 08' E). The sponge was identified as *Stylissa* aff. *massa* (Carter) and a voucher specimen (ZMA POR 16997) was deposited at the Zoological Museum of the University of Amsterdam.

**Extraction and Isolation.** The frozen sponge (900 g) was extracted three times with MeOH, and the combined extracts were concentrated and partitioned between water and ether. The ether layer was further partitioned between *n*-hexane and 90 % MeOH. The 90% MeOH and aqueous layers were combined, concentrated, loaded on ODS flash column, and eluted with water, MeOH, and MeOH/AcOH (9 : 1). The MeOH and MeOH/AcOH (9 : 1) fractions were combined, and gel filtrated on Sephadex LH20 with MeOH. Active fractions were subjected to ODS HPLC on Cosmosol AR-II using gradient solvent system with 27 – 33 % aq MeCN containing 0.05 % TFA. Fractions containing massadine were further purified on Cosmosil AR-II with 30 % MeCN containing 0.05 % TFA followed by final purification on the same column with 55 % MeOH containing 0.05 % TFA to furnish massadine (57 mg,  $6.3 \times 10^{-3}$  % yield based on wet weight).

**Massadine (1):** yellow powder;  $[\alpha]_D^{17} -12^\circ$  (c 0.10, MeOH); UV (MeOH)  $\lambda_{\max}$  278 nm ( $\epsilon$  20,000); CD  $\lambda_{\text{ext}}$  271 nm ( $\Delta\epsilon$  -0.5), 282 (0.0), 294 (+ 0.8); IR (KBr)  $\nu_{\max}$  3320-3200, 1713, 1680, 1568, 1523, 1416, 1324, 1203  $\text{cm}^{-1}$ ; HRFABMS (positive)  $m/z$  828.8735 ( $M + H$ )<sup>+</sup> (calcd for  $\text{C}_{22}\text{H}_{25}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_{10}\text{O}_5$ ,  $\Delta +3.3$  mmu); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table S1 and S2.

**Enzyme Inhibition Assay.** The Assay procedure is described in reference 6.

**Antifungal Test.** MIC values were determined by the microdilution method described in National Committee for Clinical Laboratory Standards documents M27-A and M38-P.

**Table S1.** <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N NMR Spectral Data for **1** in CD<sub>3</sub>OH <sup>a</sup>

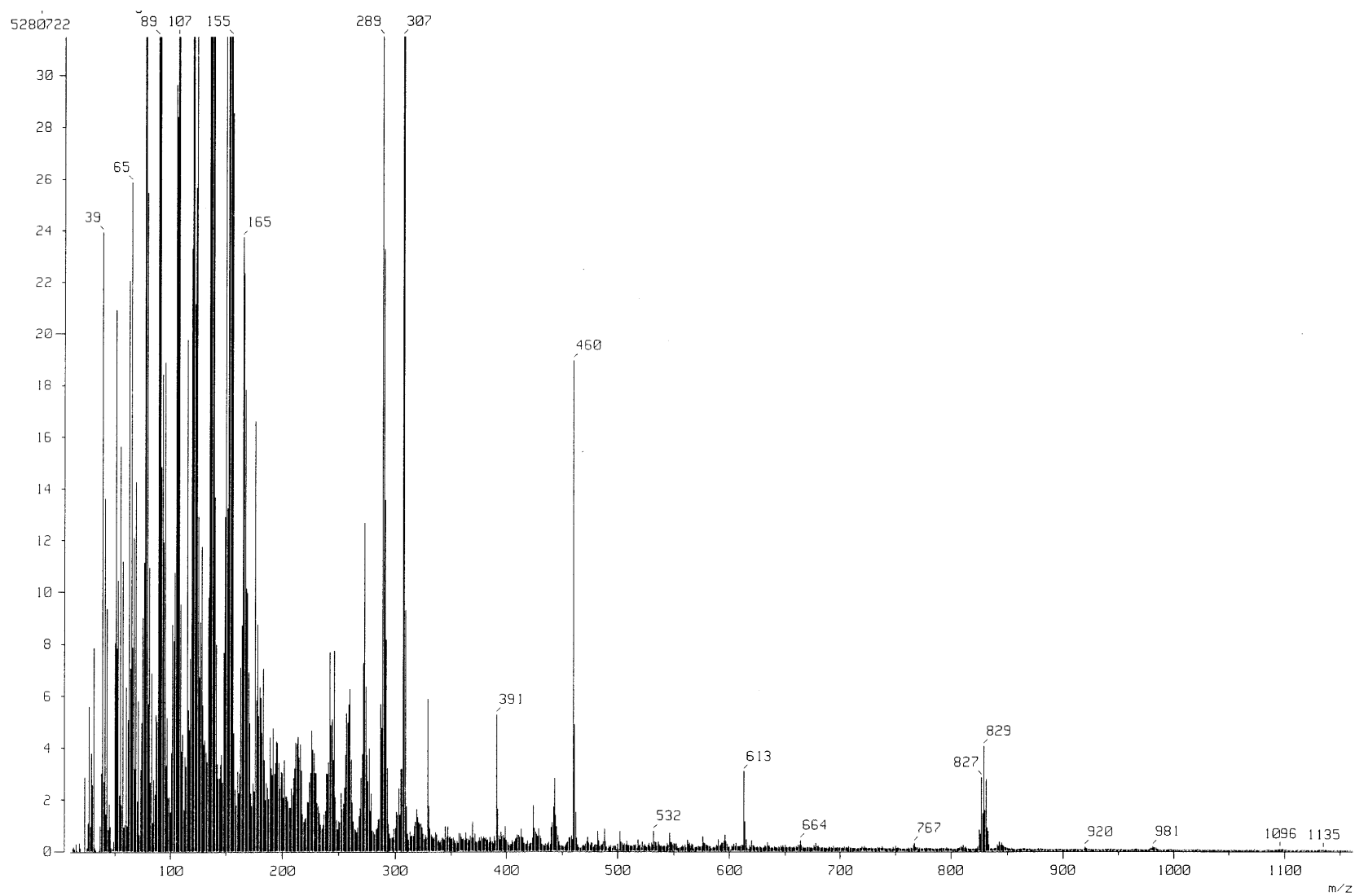
Position No.	<sup>1</sup> H (mult., J in Hz)	<sup>13</sup> C (mult.)	<sup>15</sup> N <sup>b</sup>	HMBC <sup>c</sup>	NOESY
1	2.11 (m)	42.1 (d)		C2, 3, 15, 1'	4, 12, 1'a, 1'b, 1''a
2	2.41 (d, 12.3)	44.2 (d)		C1, 3, 9, 13, 1', N4, 12	7, 9, 1'a, 1'b, 2'-NH
3		89.3 (s)			
4-NH	9.60 (brs)		104.1	C3, 5, 7, N6	1, 7
5		158.8 (s)			
6-NH	8.95 (brs)		94.1	C3, 5, N4	7
7	5.41 (s)	92.4 (d)		C3, 9, 5	2, 4, 6
9	5.65 (s)	84.3 (d)		C7, 11, 14, N10, 12	2, 10, 12, 14
10-NH	9.20 (brs)		99.7	C9, 11, 13, N12	9
11		159.3 (s)			
12-NH	9.19 (brs)		91.2	C9, 11, 13, N10	1, 9, 14, 1''b
13		72.0 (s)			
14	3.70 (s)	79.5 (d)		C1, 2, 3, 13, 1'', N12	9, 12, 15, 1''a, 2''-NH
15	2.16 (m)	52.9 (d)		C1, 14, 1''	2, 14, 1'a, 1'b, 2'-NH, 2''a, 2''b, 2''-NH
1'a	3.48 (ddd, 14.1, 5.8, 5.6)	43.0 (t)		C1, 2, 15, 3'	1, 2, 15, 1'b, 2'-NH
1'b	3.92 (ddd, 14.1, 5.8, 3.4)			C1, 2, 15, 3'	1, 2, 15, 1'a, 2'-NH, 2''-NH
2'-NH	8.33 (brt, 5.8)		105.4	C1', 3'	2, 15, 1'a, 1'b, 5'
3'		162.2 (s)			
4'		128.5 (s)			
5'	6.90 (s)	114.9 (d)		C3'', 4'', 7'', N8'	2'-NH
6'		100.0 (s)			
7'		106.7 (s)			
8'-NH	12.1 (brs)		112.0		
1''a	3.37 (ddd, 13.9, 10.4, 5.9)	43.0 (t)		C1, 14, 15, 3''	15, 2''-NH
1''b	3.53 (ddd, 13.9, 5.9, 5.4)			C14, 15, 3''	12-NH, 15, 2''-NH
2''-NH	8.43 (brt, 5.9)		107.2	C1'', 3''	14, 15, 1''a, 1''b, 5''
3''		162.2 (s)			
4''		128.8 (s)			
5''	6.84 (s)	114.6 (d)		C3'', 4'', 7'', N8''	2''-NH
6''		100.0 (s)			
7''		106.5 (s)			
8''-NH	12.2 (brs)		112.3		

<sup>a</sup> NMR experiments were carried out in CD<sub>3</sub>OH/TFA (370 : 2). <sup>b</sup> Chemical shift values were determined by <sup>1</sup>H/<sup>15</sup>N HSQC and <sup>1</sup>H/<sup>15</sup>N HMBC spectra. <sup>c</sup> Both of <sup>1</sup>H/<sup>13</sup>C HMBC and <sup>1</sup>H/<sup>15</sup>N HMBC spectral data are presented in this column.

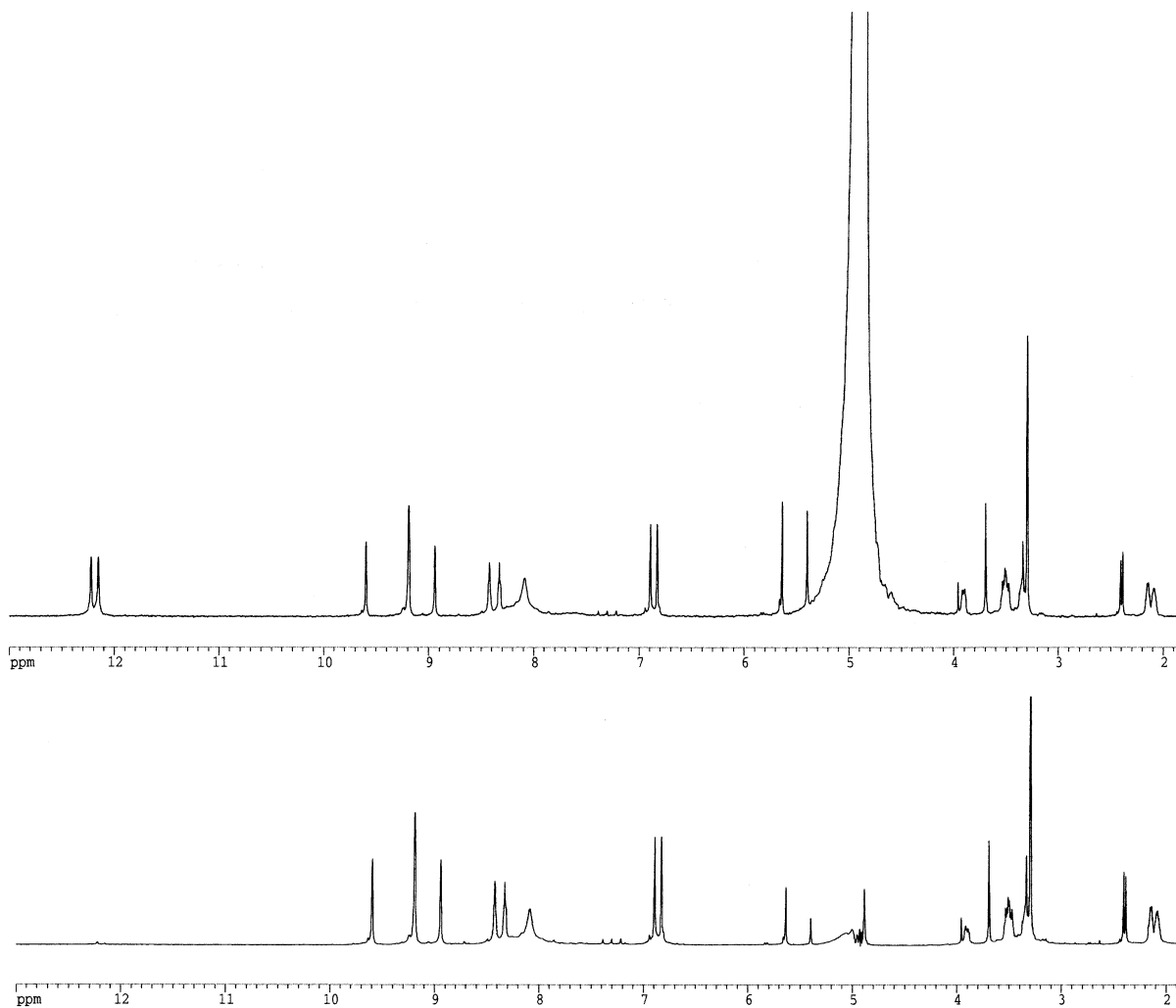
**Table S2.** <sup>1</sup>H NMR and ROESY Spectral Data for **1** in DMSO-*d*<sub>6</sub>

Position No.	<sup>1</sup> H (mult., J in Hz)	ROESY
1	1.90 (m)	4, 12, 2'-NH
2	1.63 (d, 12.2)	7, 9, 2'-NH, 3-OH, 14-OH
3		
4-NH	9.40 (brs)	1, 1'a, 1'b
5		
6-NH	9.00 (brs)	7
7	5.21 (s)	2, 6, 9, 3-OH
9	5.40 (s)	2, 7, 10, 14, 14-OH
10-NH	9.36 (brs)	9
11		
12-NH	9.05 (brs)	1, 14, 1''a, 1''b
13		
14	3.44 (s)	9, 12, 14-OH
15	1.86 (m)	2''-NH, 14-OH
1'a	3.52 (m)	4, 2'-NH, 5', 2''-NH, 3-OH
1'b	3.46 (m)	4, 2'-NH, 5', 2''-NH, 3-OH
2'-NH	7.90 (br)	1, 2, 1'a, 1'b, 1''a, 5'
3'		
4'		
5'	6.86 (s)	2'-NH, 1'a, 1'b
6'		
7'		
8'-NH	12.58 (s)	
1''a	3.35 (m)	12, 2'-NH, 5', 2''-NH
1''b	3.25 (m)	12, 2''-NH
2''-NH	8.28 (br)	15, 1'a, 1'b, 1''a, 1''b, 5''
3''		
4''		
5''	6.82 (s)	1''a, 2''-NH
6''		
7''		
8''-NH	12.65 (s)	
3-OH	7.36 (s)	2, 7, 1'a, 1'b
14-OH	5.66 (s)	2, 9, 14, 15

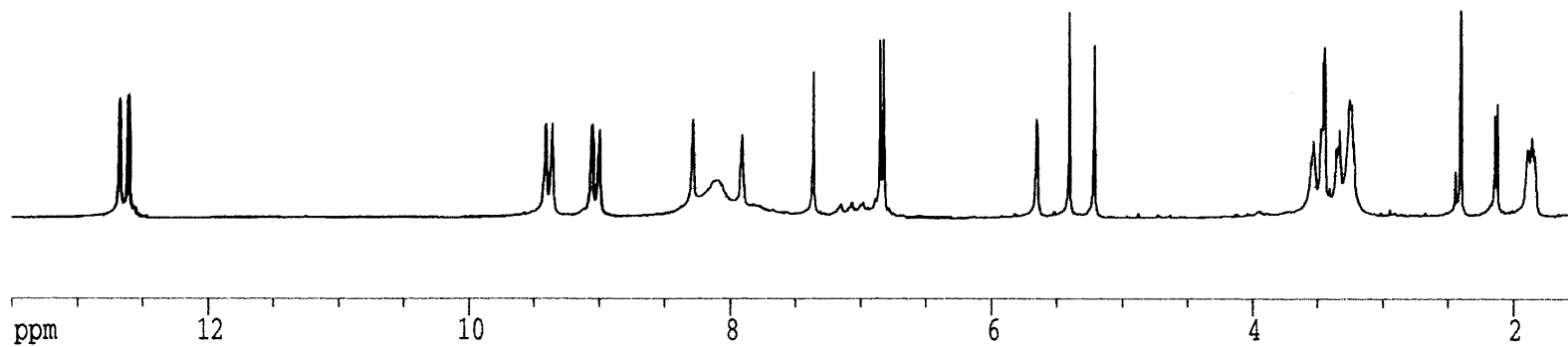
**Figure S1. FAB-MS spectrum of massadine  
(positive mode, p-nitrobenzylalcohol as matrix)**



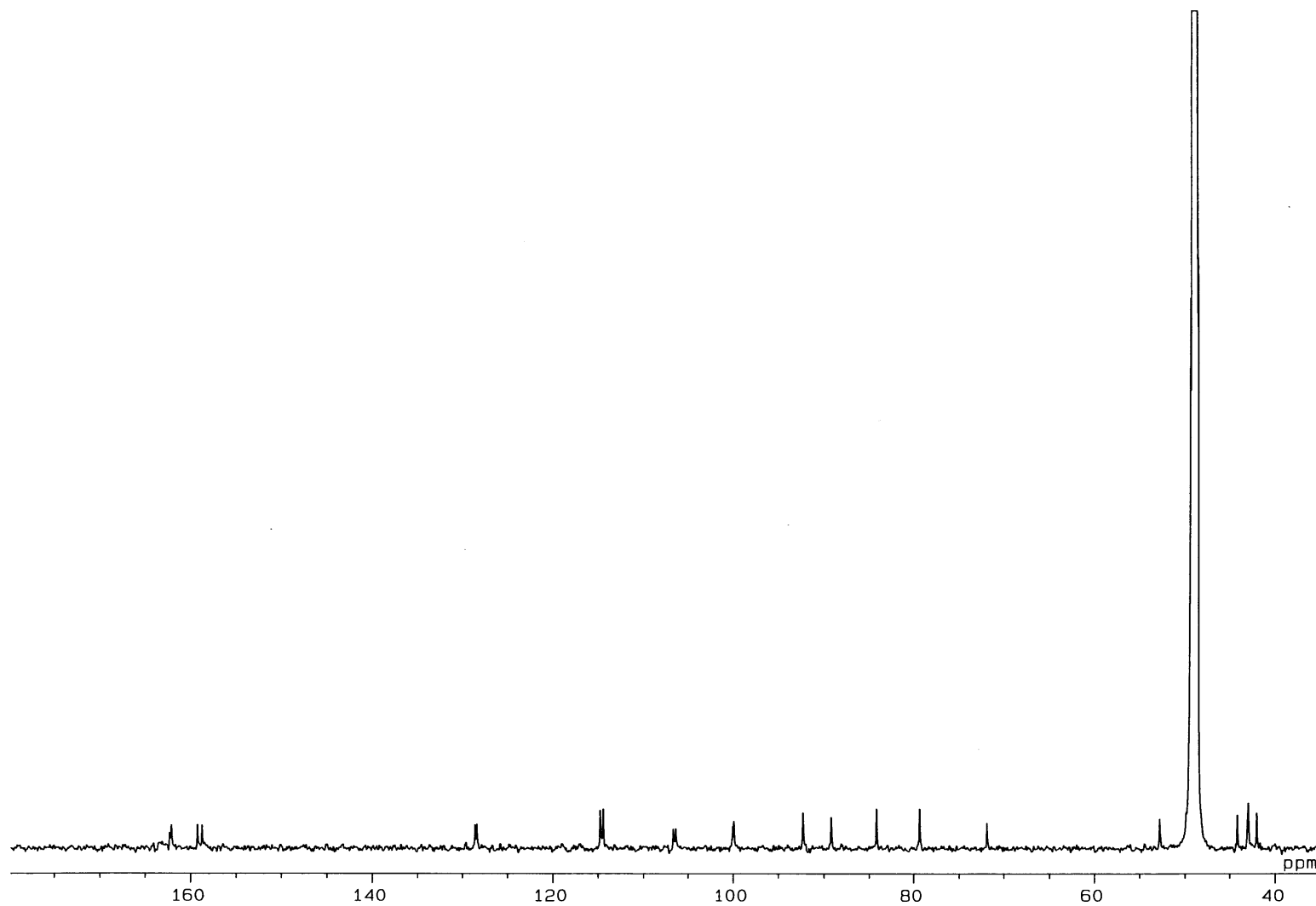
**Figure S2.  $^1\text{H}$  NMR spectrum of massadine ( $\text{CD}_3\text{OH}$ , 600 MHz)**  
(upper: water is not suppressed, lower: watergate)



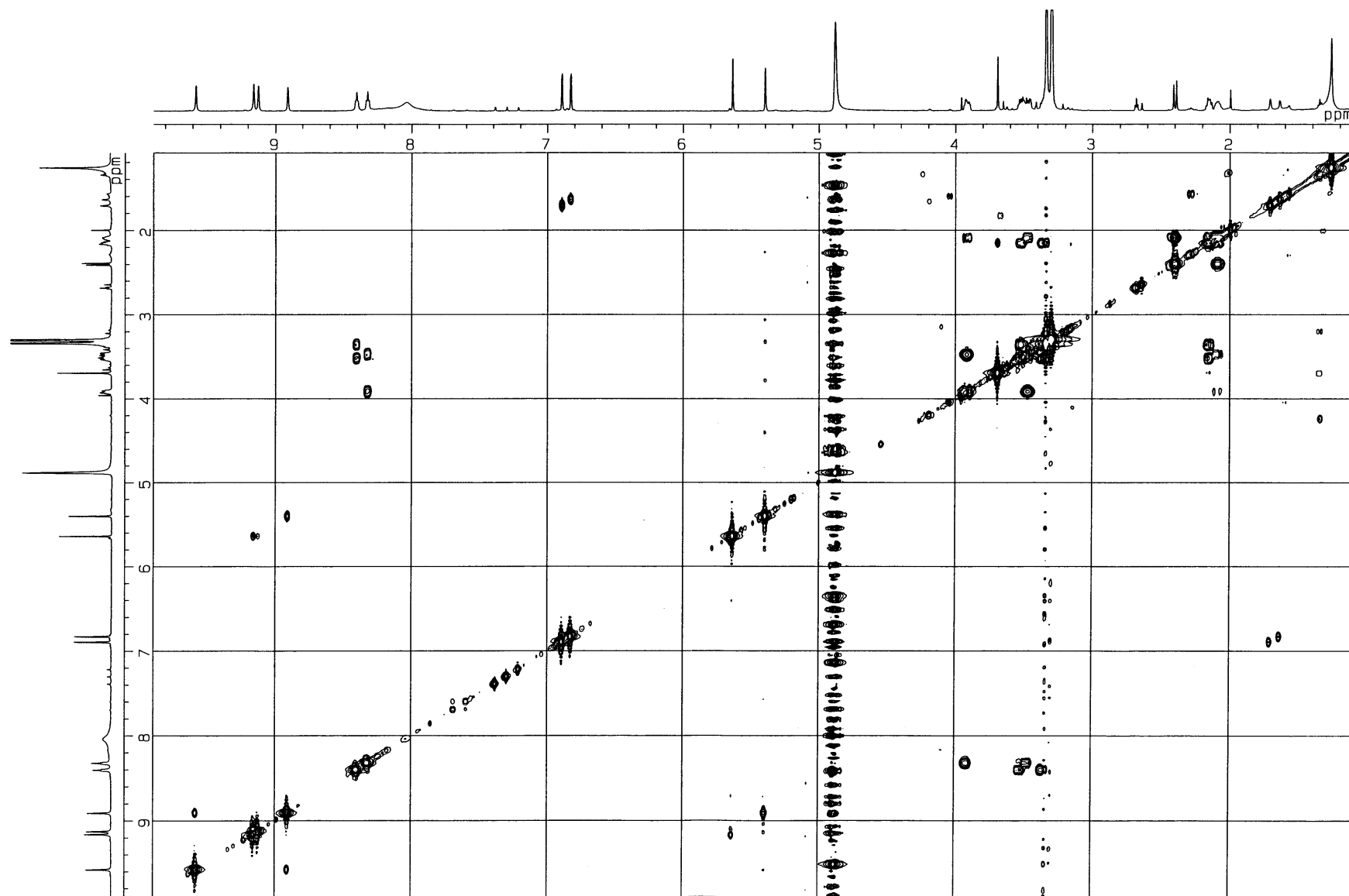
**Figure S3.**  $^1\text{H}$  NMR spectrum of massadine (DMSO- $d_6$ , 600 MHz)



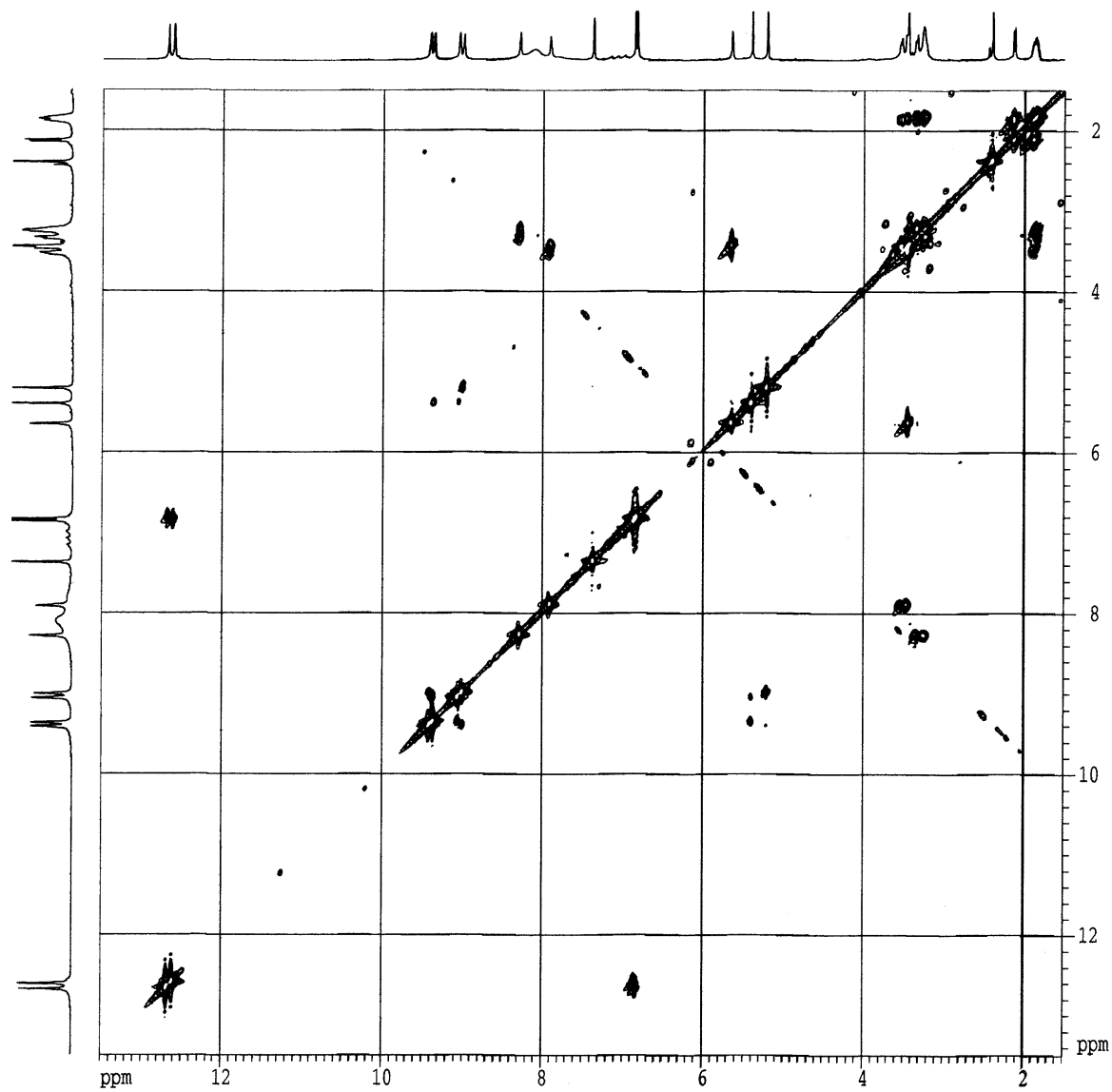
**Figure S4.**  $^{13}\text{C}$  NMR spectrum of massadine ( $\text{CD}_3\text{OH}$ , 150 MHz)



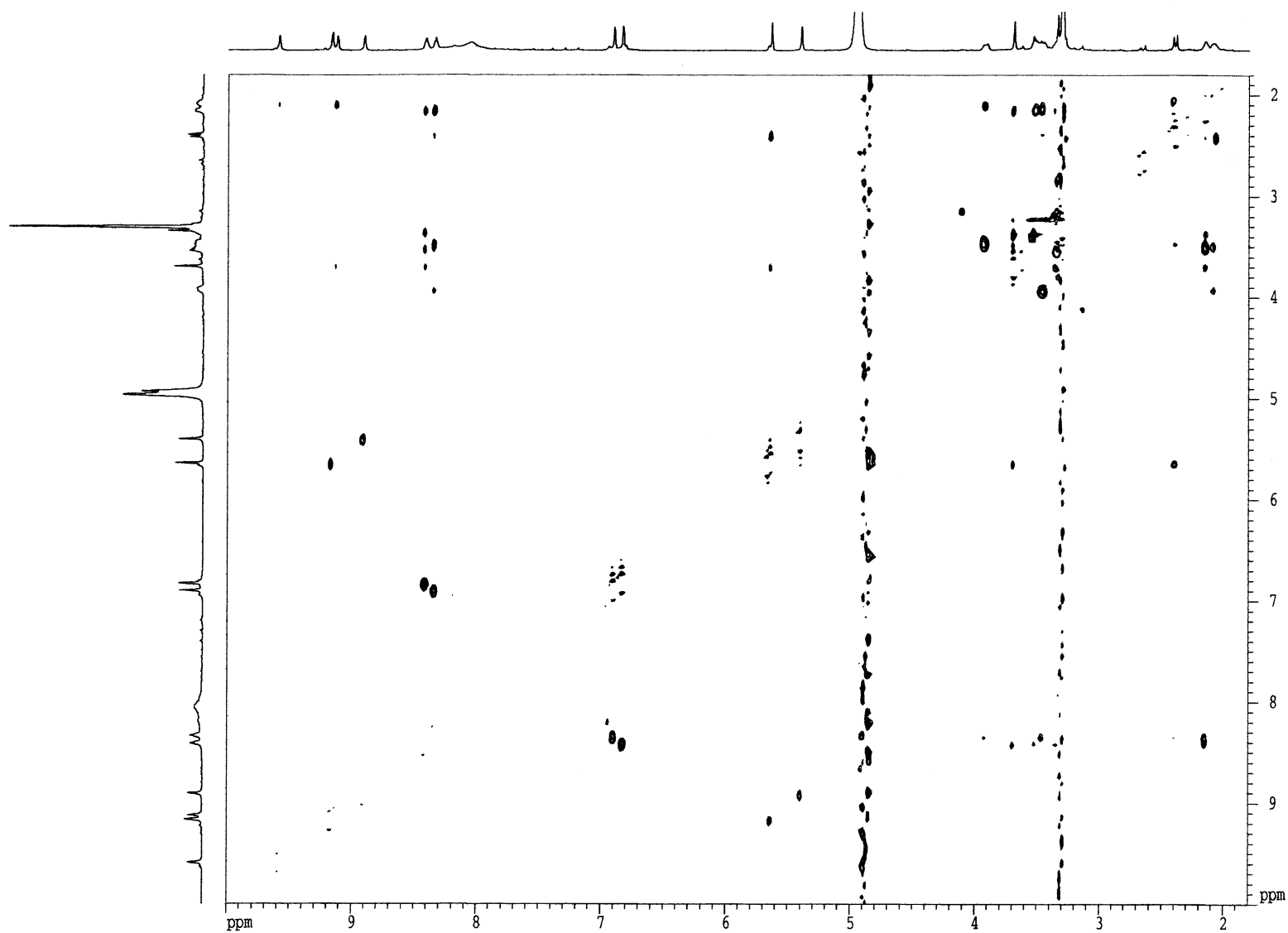
*Figure S5.* COSY spectrum of massadine ( $\text{CD}_3\text{OH}$ )



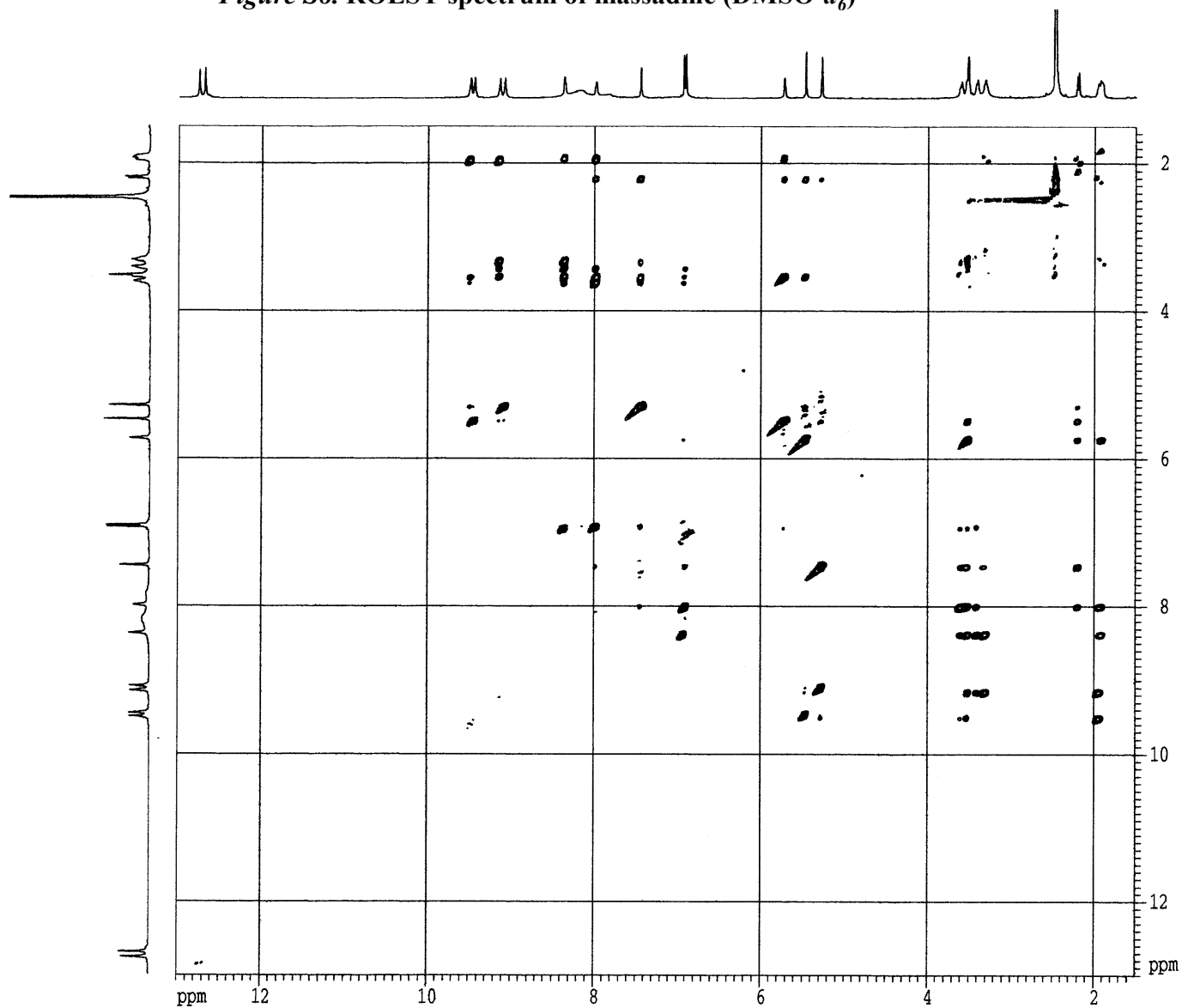
**Figure S6.** COSY spectrum of massadine (DMSO- $d_6$ )



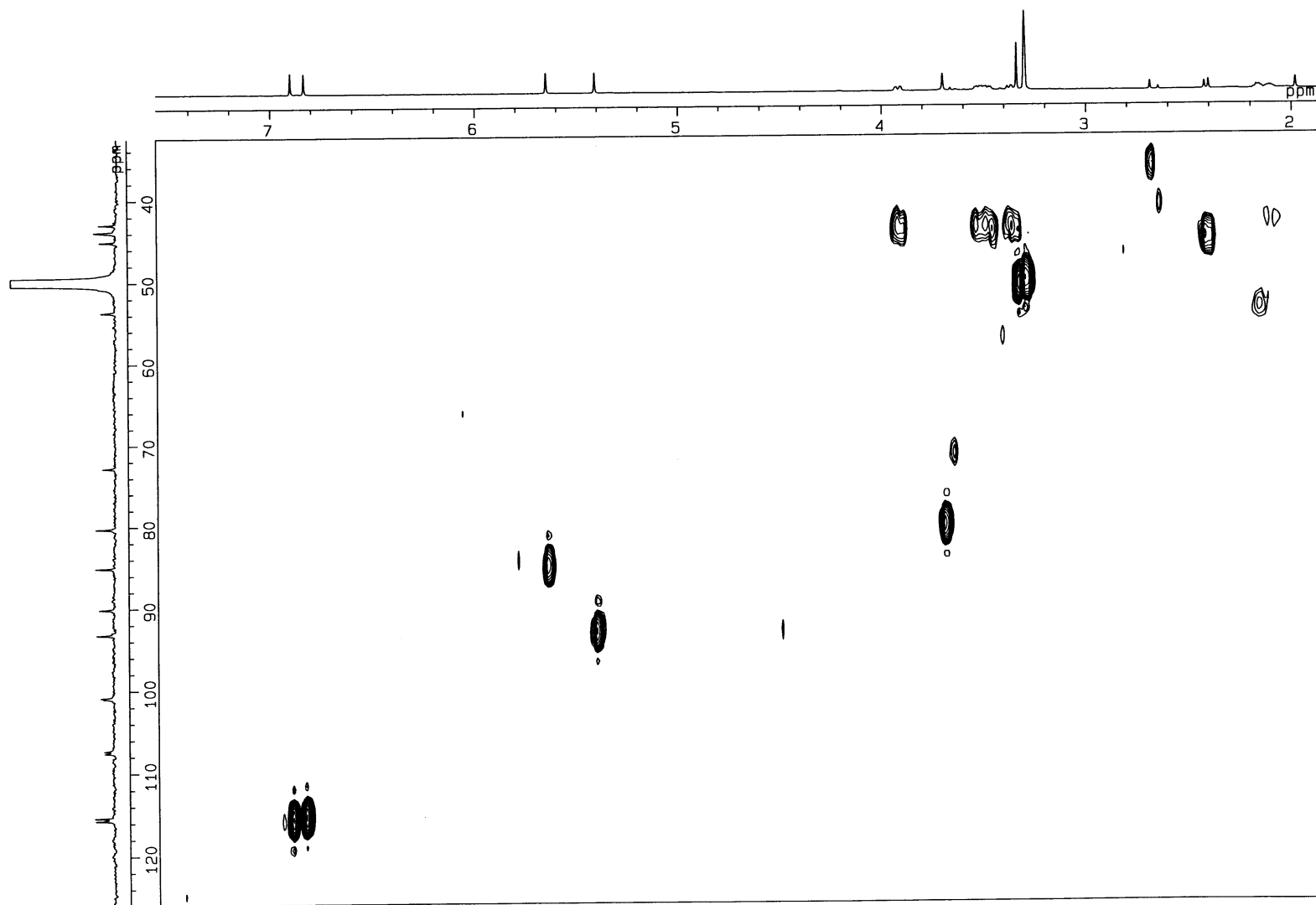
**Figure S7.** NOESY spectrum of massadine (CD<sub>3</sub>OH)



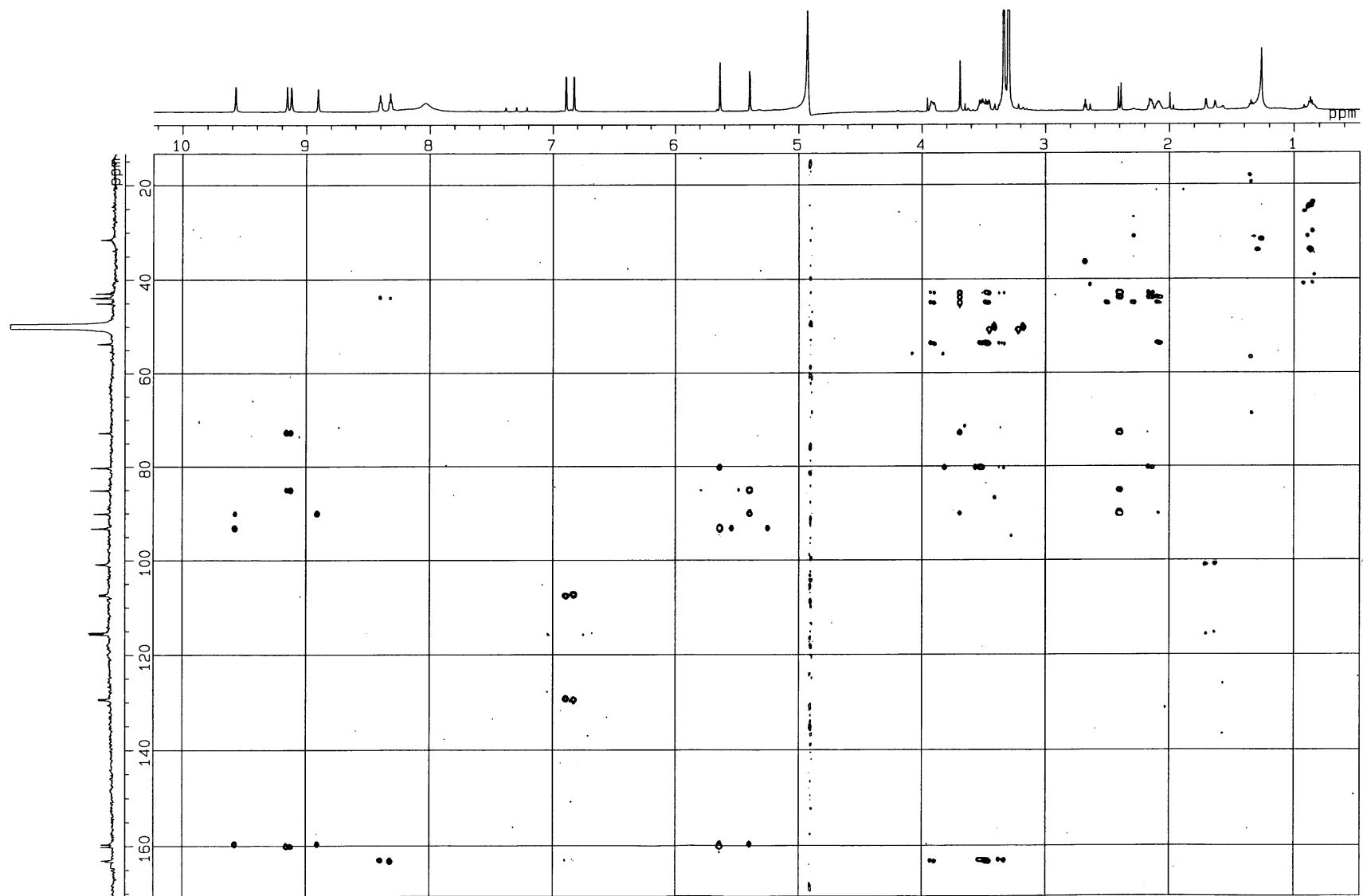
**Figure S8.** ROESY spectrum of massadine (DMSO- $d_6$ )



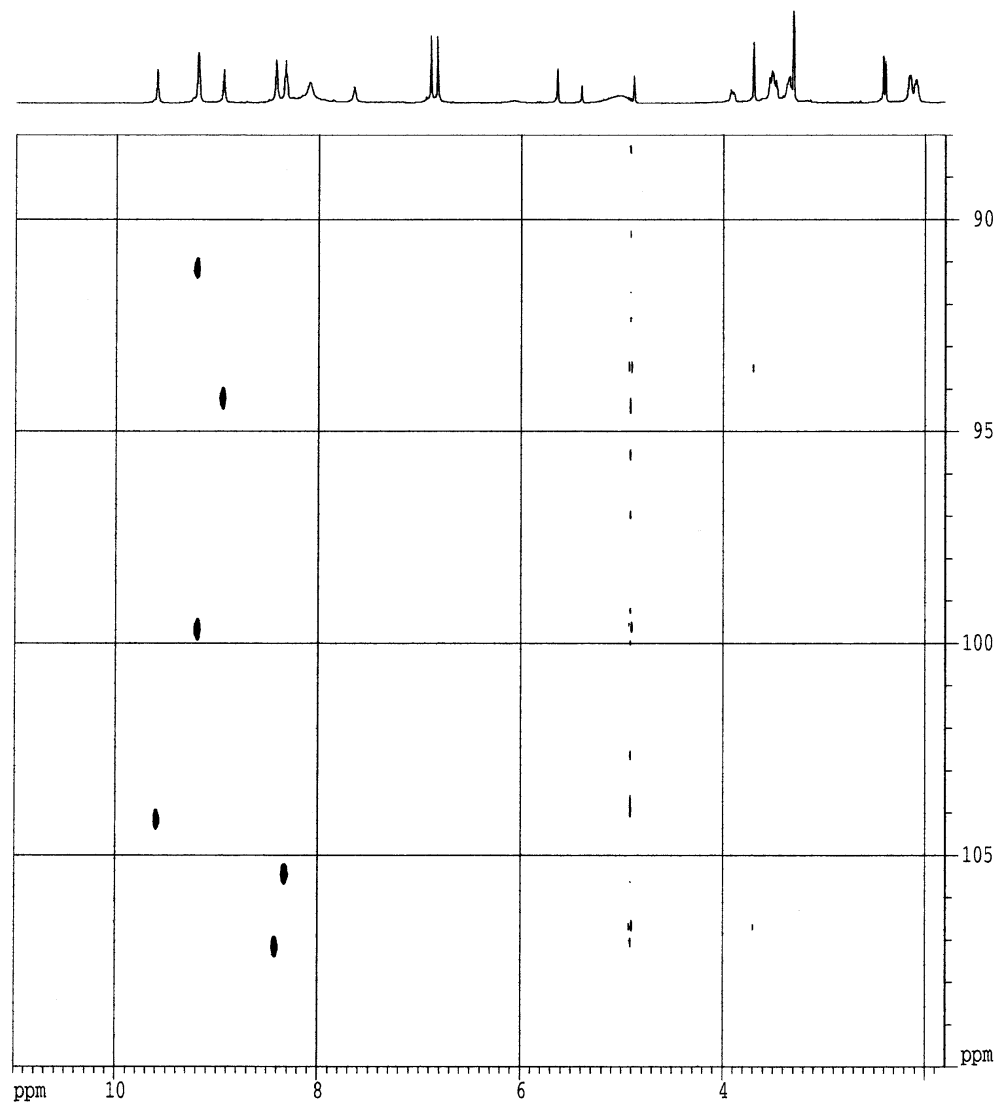
**Figure S9.**  $^1\text{H}/^{13}\text{C}$  HMQC spectrum of massadine ( $\text{CD}_3\text{OD}$ )



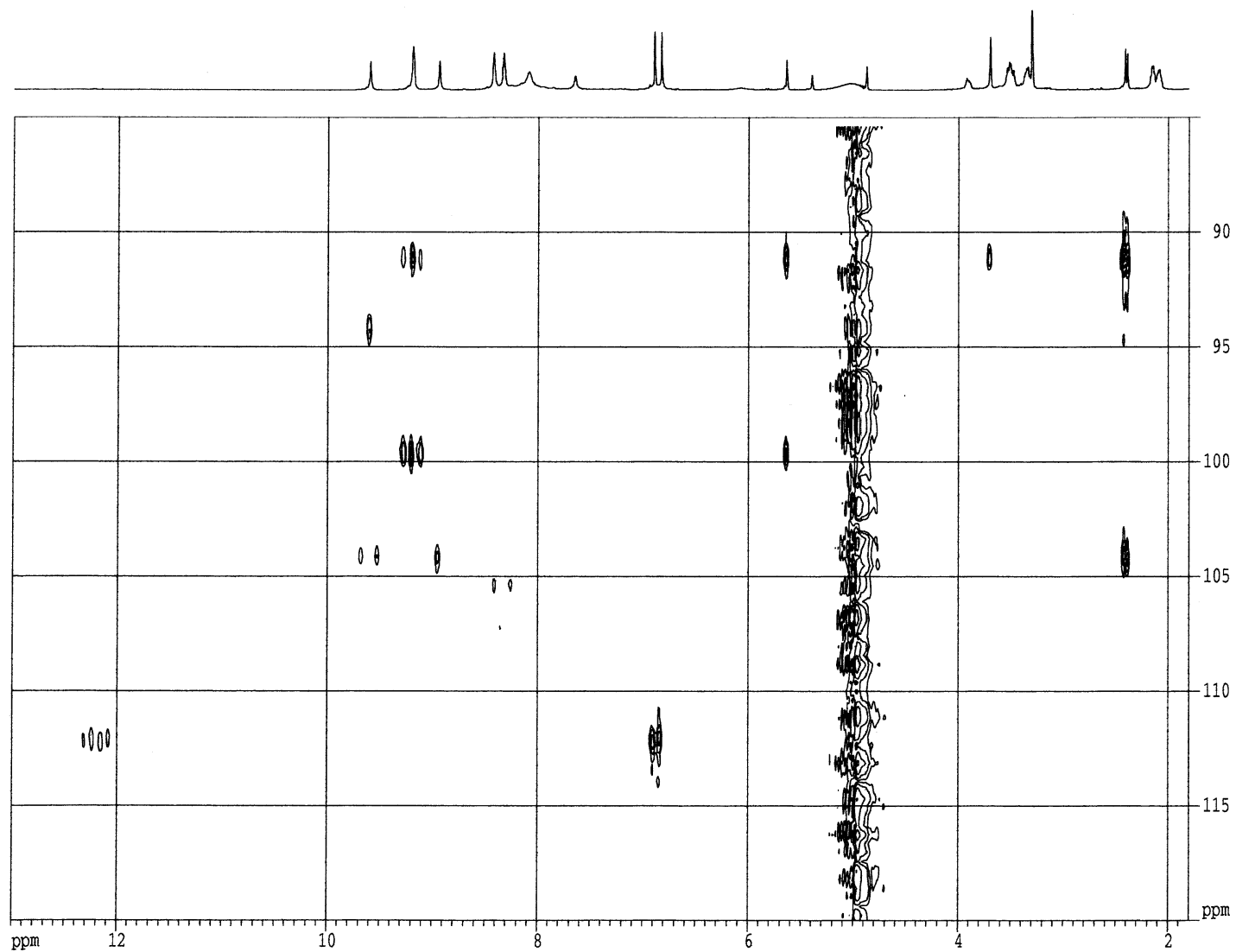
**Figure S10.**  $^1\text{H}/^{13}\text{C}$  HMBC spectrum of massadine ( $\text{CD}_3\text{OH}$ )



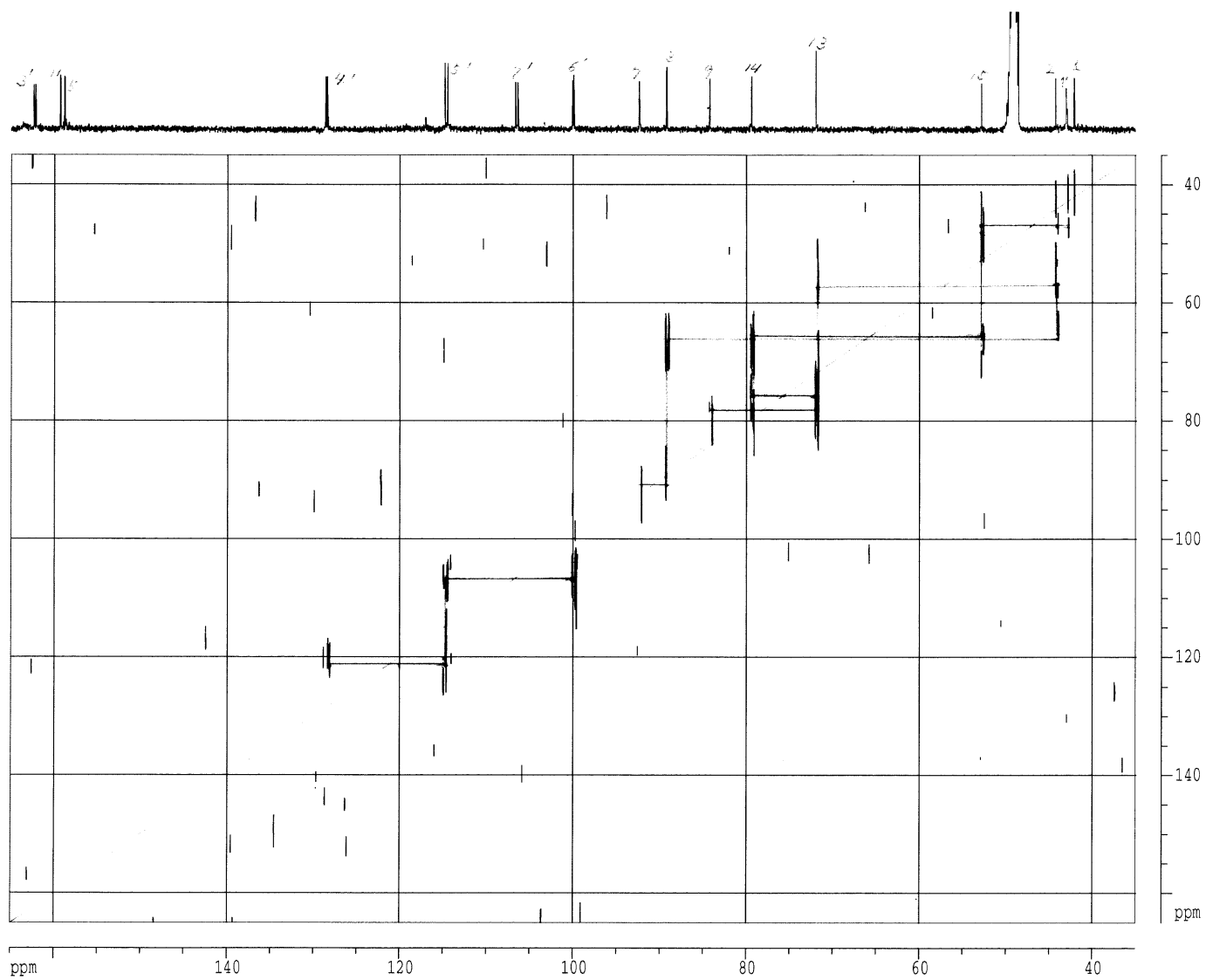
**Figure S11.**  $^1\text{H}/^{15}\text{N}$  HSQC spectrum of massadine ( $\text{CD}_3\text{OH}$ )



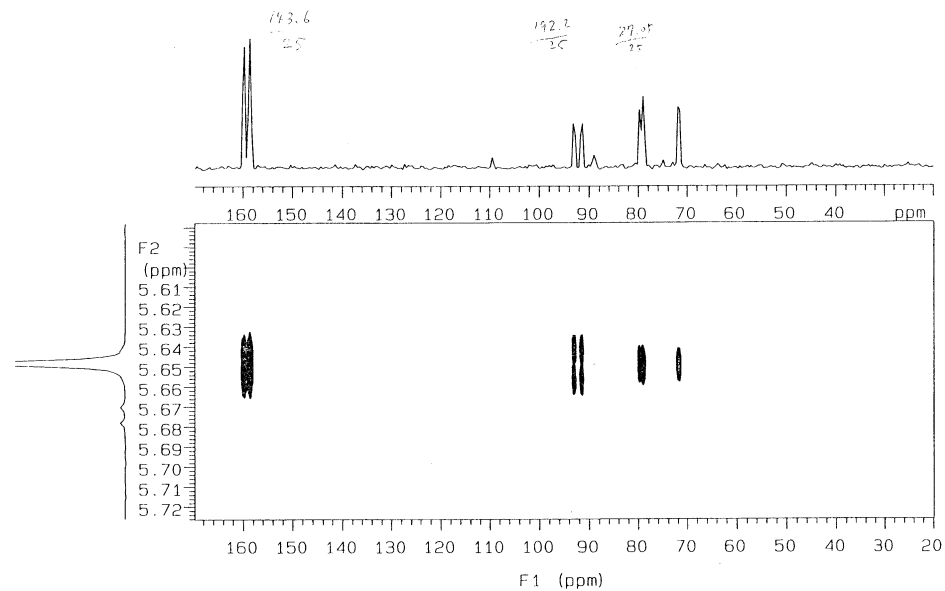
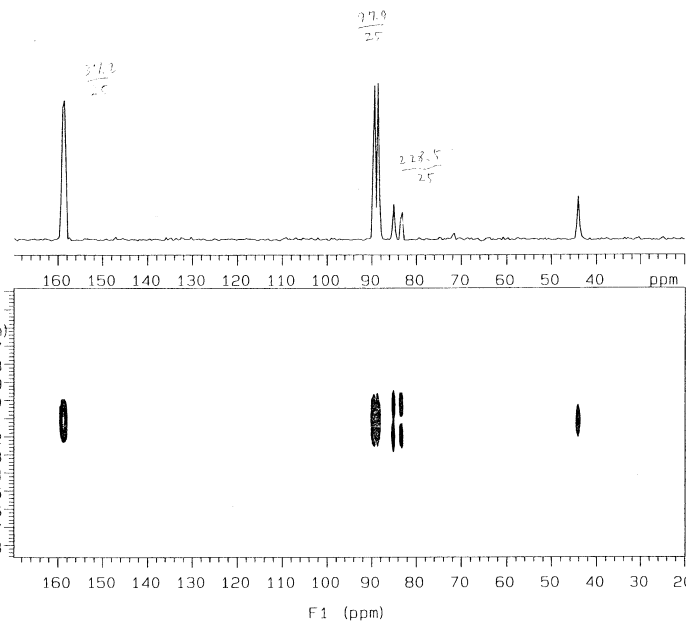
**Figure S12.**  $^1\text{H}/^{15}\text{N}$  HMBC spectrum of massadine ( $\text{CD}_3\text{OH}$ )



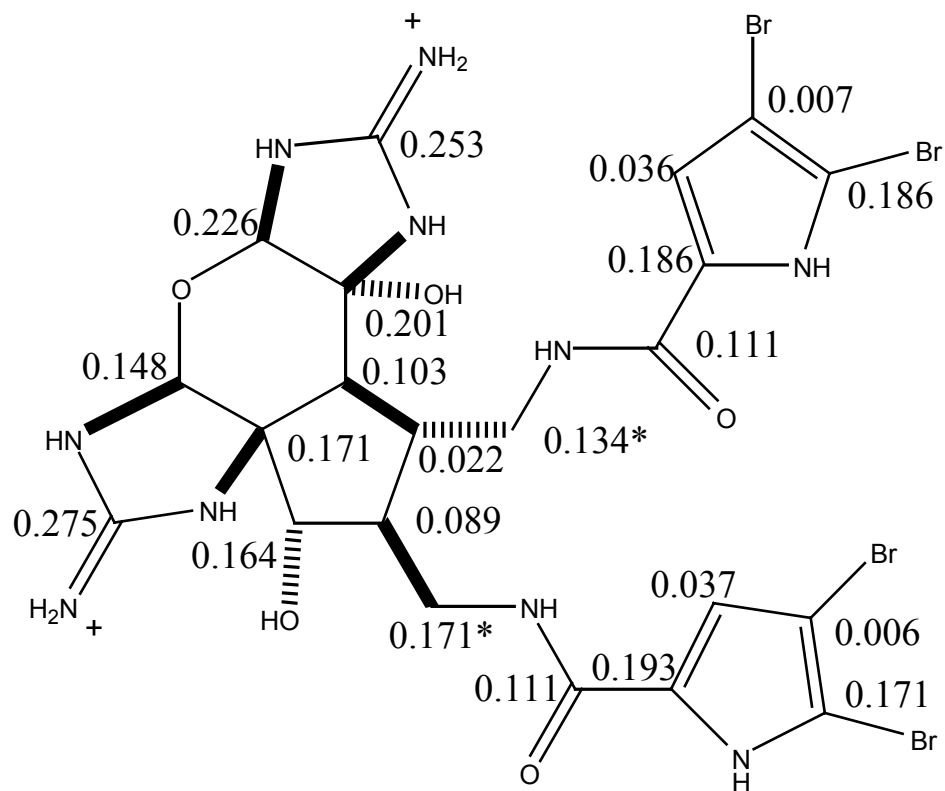
**Figure S13.** INADEQUATE spectrum of massadine ( $\text{CD}_3\text{OH}$ )



**Figure S14. J-resolved  $^1\text{H}/^{13}\text{C}$  HMBC spectrum of massadine**  
**(HMBC correlations from H-7 (left) and H-9 (right) )**

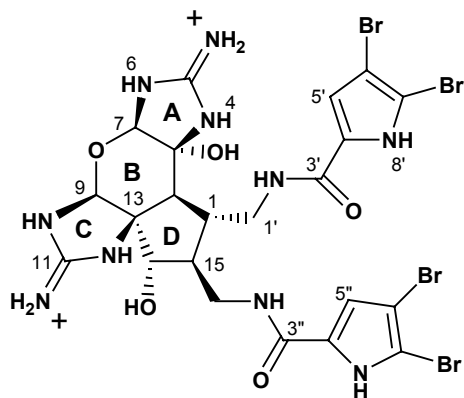


**Figure S15.** Deuterium-induced  $^{13}\text{C}$  NMR isotope shifts.

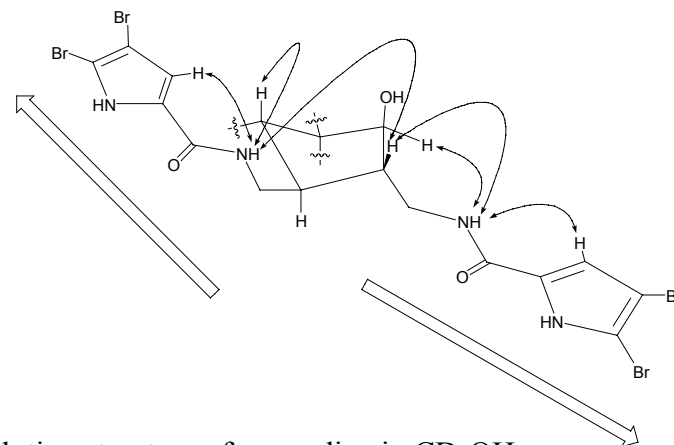


$\Delta\delta(\text{ppm}) = \delta_{\text{CD}_3\text{OH}} - \delta_{\text{CD}_3\text{OD}}$   
 \* These values can be interchangeable

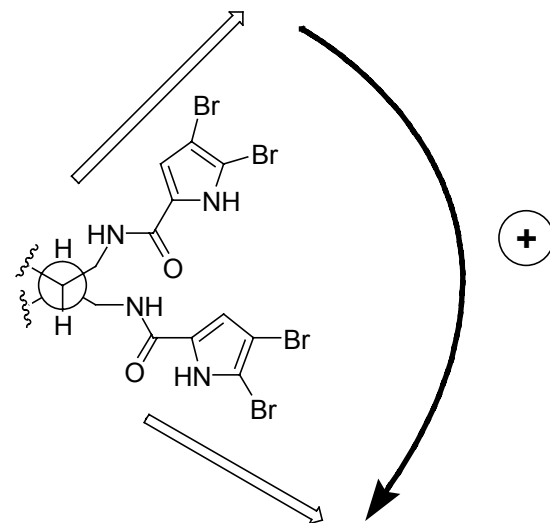
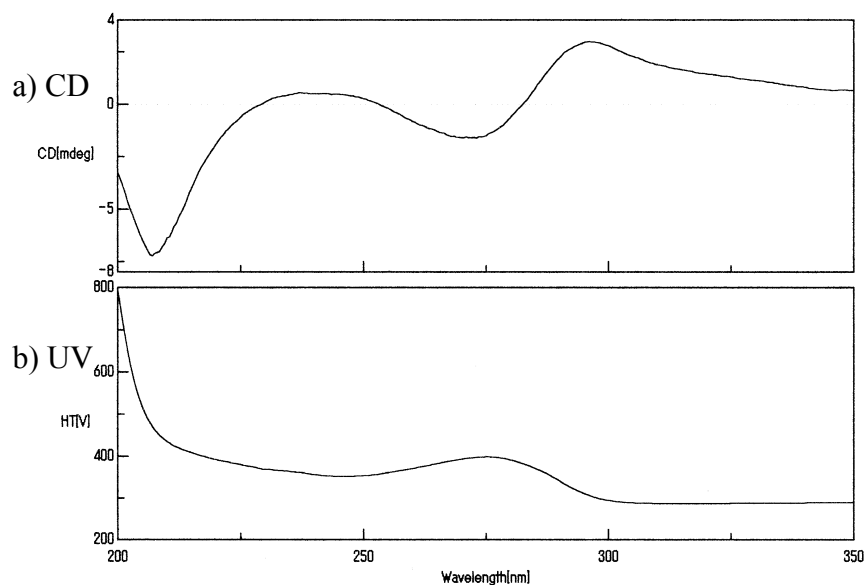
**Figure S16.** Interpretation of the CD spectrum of massadine.



massadine

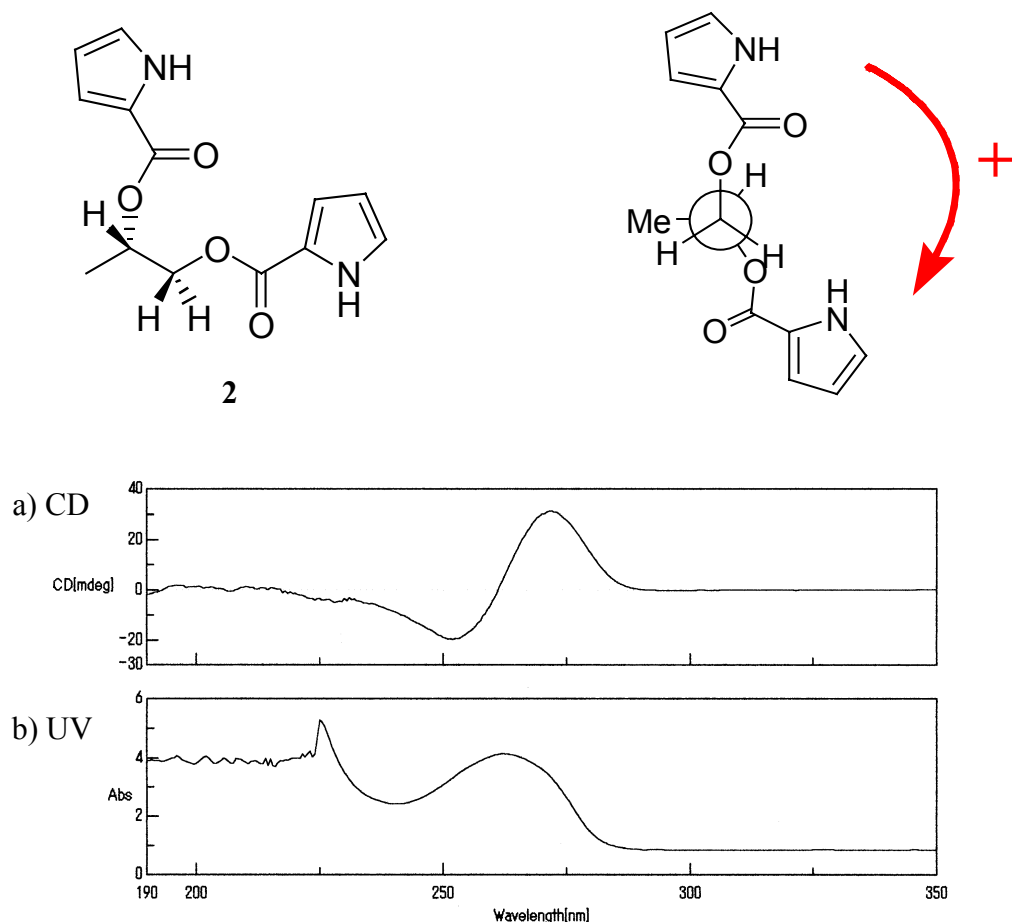


Solution structure of massadine in  $\text{CD}_3\text{OH}$



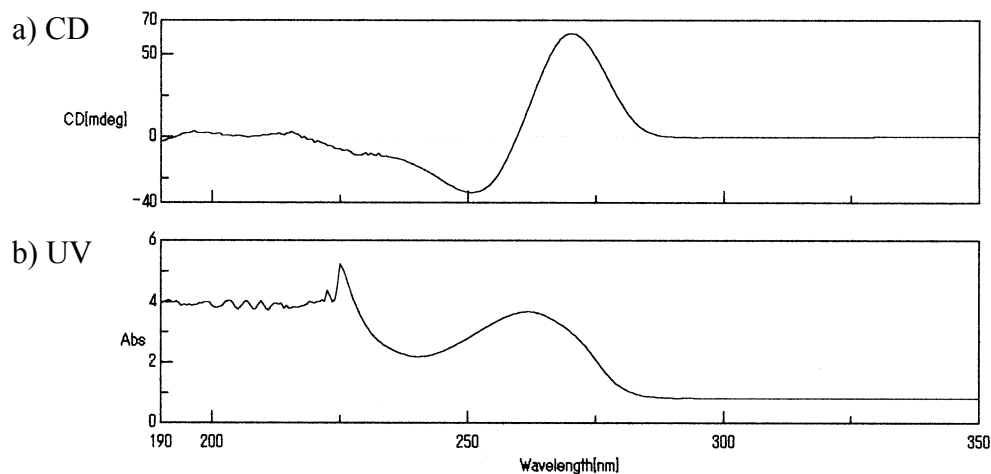
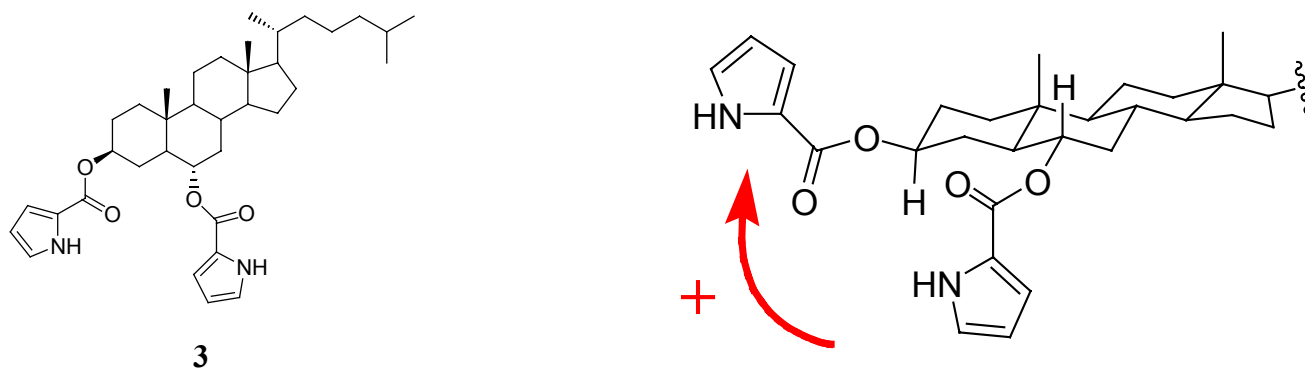
UV (MeOH)  $\lambda$  max 278 (20,000)  
 CD (MeOH)  $\Delta\epsilon$  294 (+ 0.75), 271 (-0.52)

**Figure S17. CD spectrum of a model compound.**



**(S)-propane-1,2-diol bis(pyrrole-2-carboxylate) (2):** To a solution of (S)-(+)-1,2-propanediol in DCM/THF (1 : 2) at rt was added pyrrole-2-carboxylic acid, DCC, and DMAP. The reaction mixture was stirred at rt for 4 days, extracted with EtOAc, and fractionated by SiO<sub>2</sub> column chromatography followed by RP-HPLC to furnish **2**: UV (DCM)  $\lambda_{\text{max}}$  ( $\epsilon$ ) 263.0 (15,600) nm; CD (DCM)  $\lambda$  ( $\Delta\epsilon$ ) 272.0 (+5.0), 261.0 (0.0), 252.0 (-3.1) nm; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  9.19 (brs, 2H), 6.97-6.95 (m, 4H), 6.28-6.24 (m, 2H), 5.43 (ddq,  $J$  = 6.5, 6.5, 3.9 Hz, 1H), 4.42 (dd,  $J$  = 11.9, 3.9 Hz, 1H), 4.39 (dd,  $J$  = 11.9, 6.5 Hz, 1H), 1.39 (d,  $J$  = 6.5 Hz, 3H); ESI-MS (positive)  $m/z$  265 (M + Na)<sup>+</sup>.

**Figure S18. CD spectrum of a model compound.**



**5 $\alpha$ -Cholestane-3 $\beta$ ,6 $\alpha$ -diol bis(pyrrole-2-carboxylate) (3):** To a solution of cholesterol in dry THF was added 2M  $\text{BH}_3\cdot\text{SMe}_2$  in toluene, and the solution was stirred at rt for 12 h. Water, 30 %  $\text{H}_2\text{O}_2$ , and 3N NaOH was added to the reaction mixture and kept stirring at rt for 3h. The reaction mixture was extracted with  $\text{CHCl}_3$ , dried over  $\text{MgSO}_4$ , concentrated, and reacted with pyrrole-2-carboxylic acid as noted in Figure S17 to yield **3**: UV (DCM)  $\lambda_{\text{max}}$  ( $\epsilon$ ) 263.0 (32,800) nm; CD (DCM)  $\lambda$  ( $\Delta\epsilon$ ) 272.0 (+22.3), 259.0 (0.0), 250.5 (-12.1) nm;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  9.11 (brs, 1H), 9.07 (brs, 1H), 6.91 (m, 1H), 6.89 (m, 2H), 6.86 (m, 1H), 6.22 (m, 2H), 4.88 (m, 2H), 2.10-0.65 (several, 44H); ESI-MS (positive)  $m/z$  613 ( $\text{M} + \text{Na}$ ) $^+$ , 1181 ( $2\text{M} + \text{H}$ ) $^+$ .