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-IIwith 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 x 10^{-3} % yield based on wet weight).

Massadine (1): yellow powder; $[\alpha]^{17}_{D}$ –12° (c 0.10, MeOH); UV (MeOH) λ_{max} 278 nm (ε 20,000); CD λ_{ext} 271 nm ($\Delta\varepsilon$ -0.5), 282 (0.0), 294 (+ 0.8); IR (KBr) ν_{max} 3320-3200, 1713, 1680, 1568, 1523, 1416, 1324, 1203 cm^{-1}; HRFABMS (positive) *m/z* 828.8735 $(M + H)^+$ (calcd for $C_{22}H_{25}{}^{79}Br_2{}^{81}Br_2N_{10}O_5$, Δ +3.3 mmu); ^1H and ^{13}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. ^{1}H, ^{13}C and ^{15}N NMR Spectral Data for **1** in CD_3OH [a]

Position No.	^{1}H (mult., J in Hz)	^{13}C (mult.)	^{15}N [b]	HMBC [c]	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)	4 3.0 (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		

a NMR experiments were carried out in CD_3OH/TFA (370 : 2). *b* Chemical shift values were determined by $^{1}H/^{15}N$ HSQC and $^{1}H/^{15}N$ HMBC spectra. *c* Both of $^{1}H/^{13}C$ HMBC and $^{1}H/^{15}N$ HMBC spectral data are presented in this column.

Table S2. ^{1}H NMR and ROESY Spectral Data for **1** in DMSO-d_6

Position No.	^{1}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)

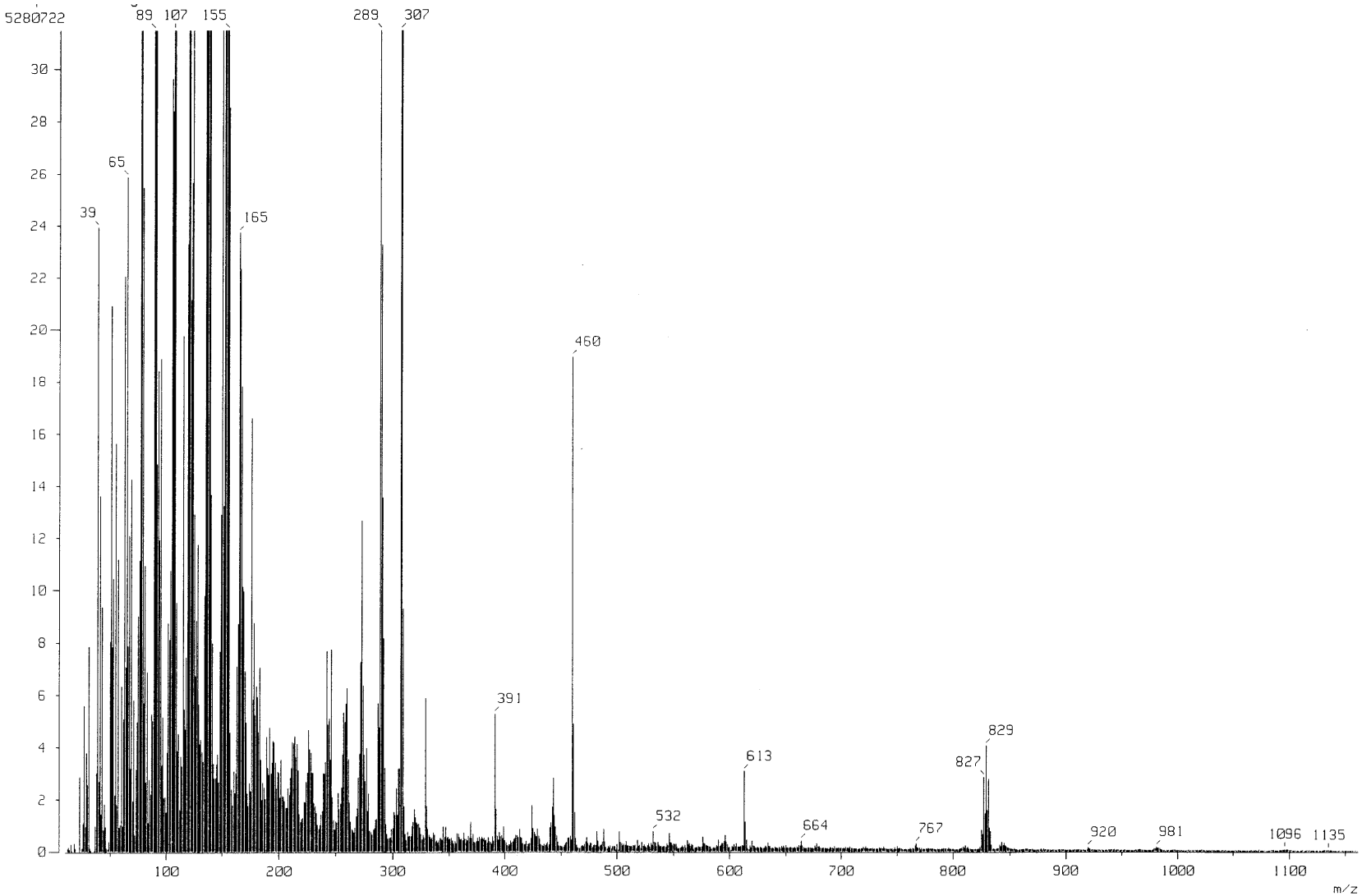


Figure S2. **^{1}H NMR spectrum of massadine (CD_3OH, 600 MHz)**
(upper: water is not suppressed, lower: watergate)

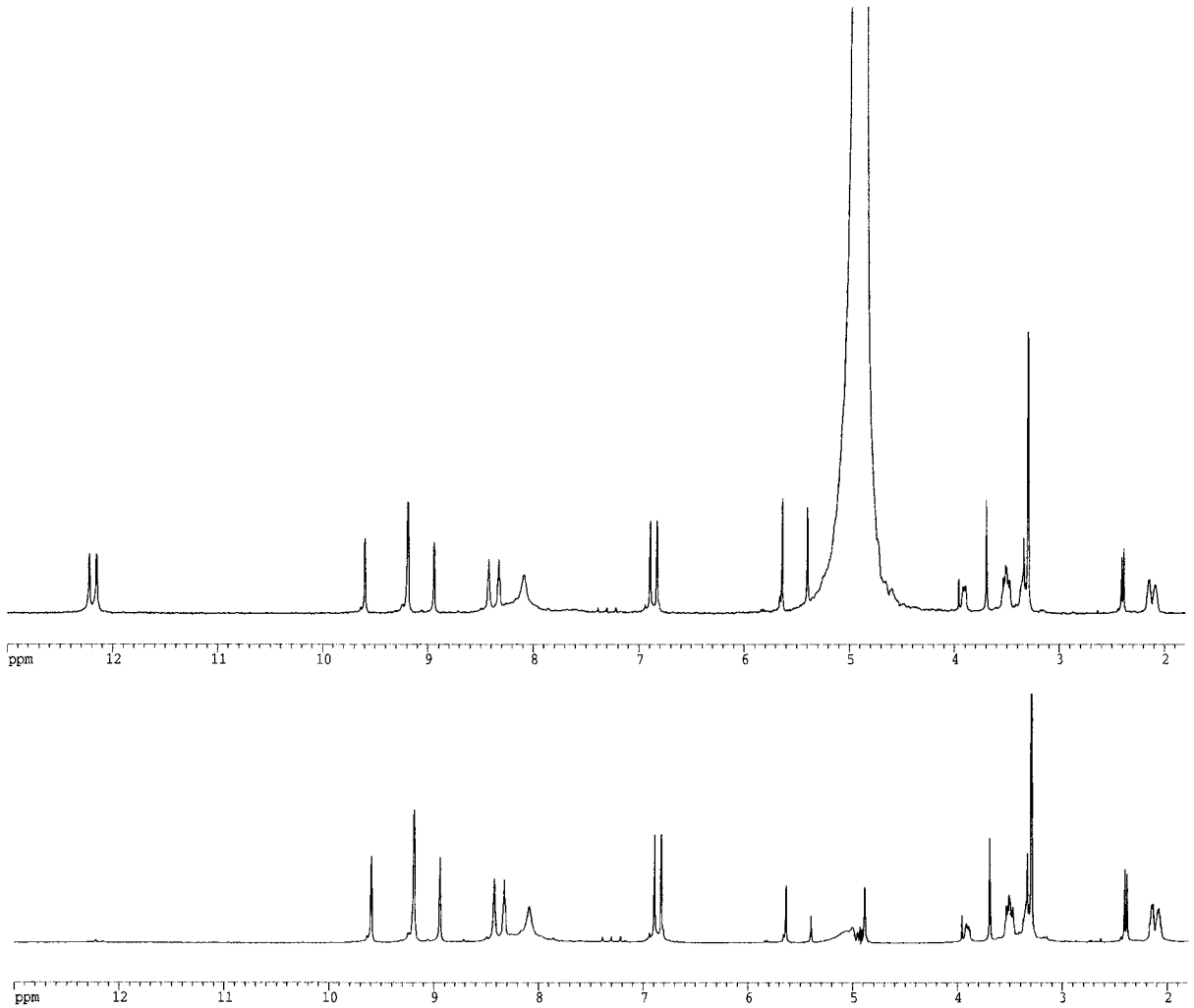


Figure S3. **^{1}H NMR spectrum of massadine (DMSO-d_6, 600 MHz)**

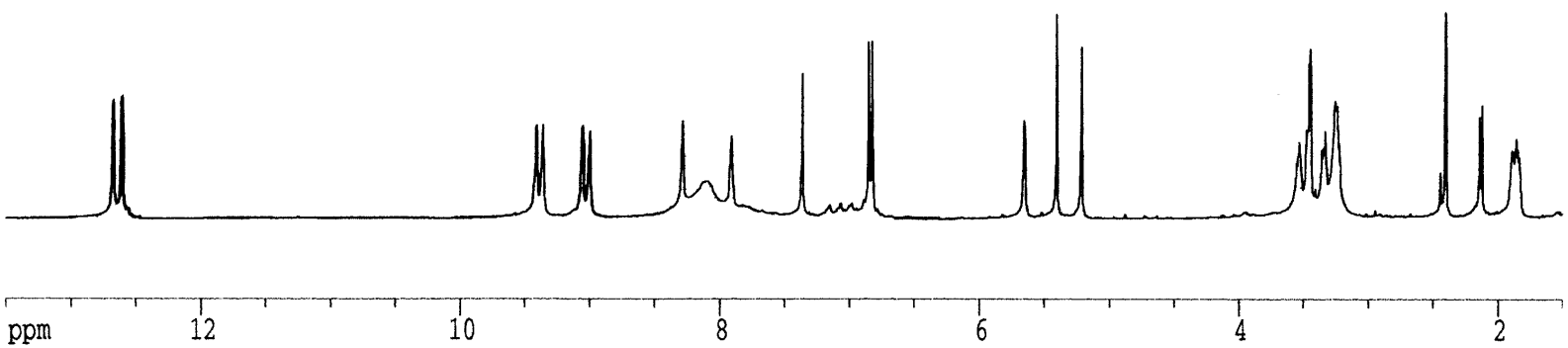


Figure S4. **^{13}C NMR spectrum of massadine (CD_3OH, 150 MHz)**

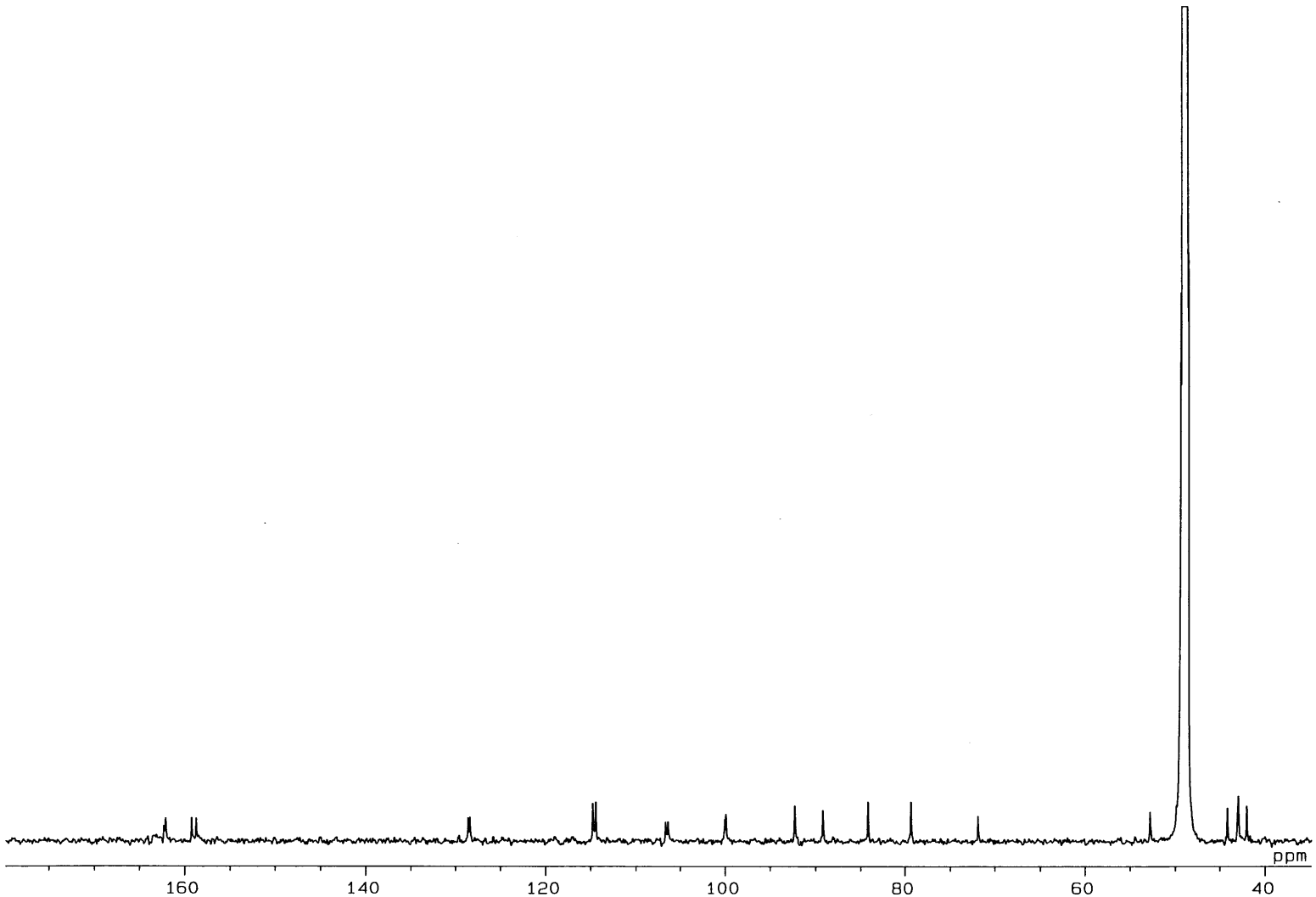


Figure S5. **COSY spectrum of massadine (CD_3OH)**

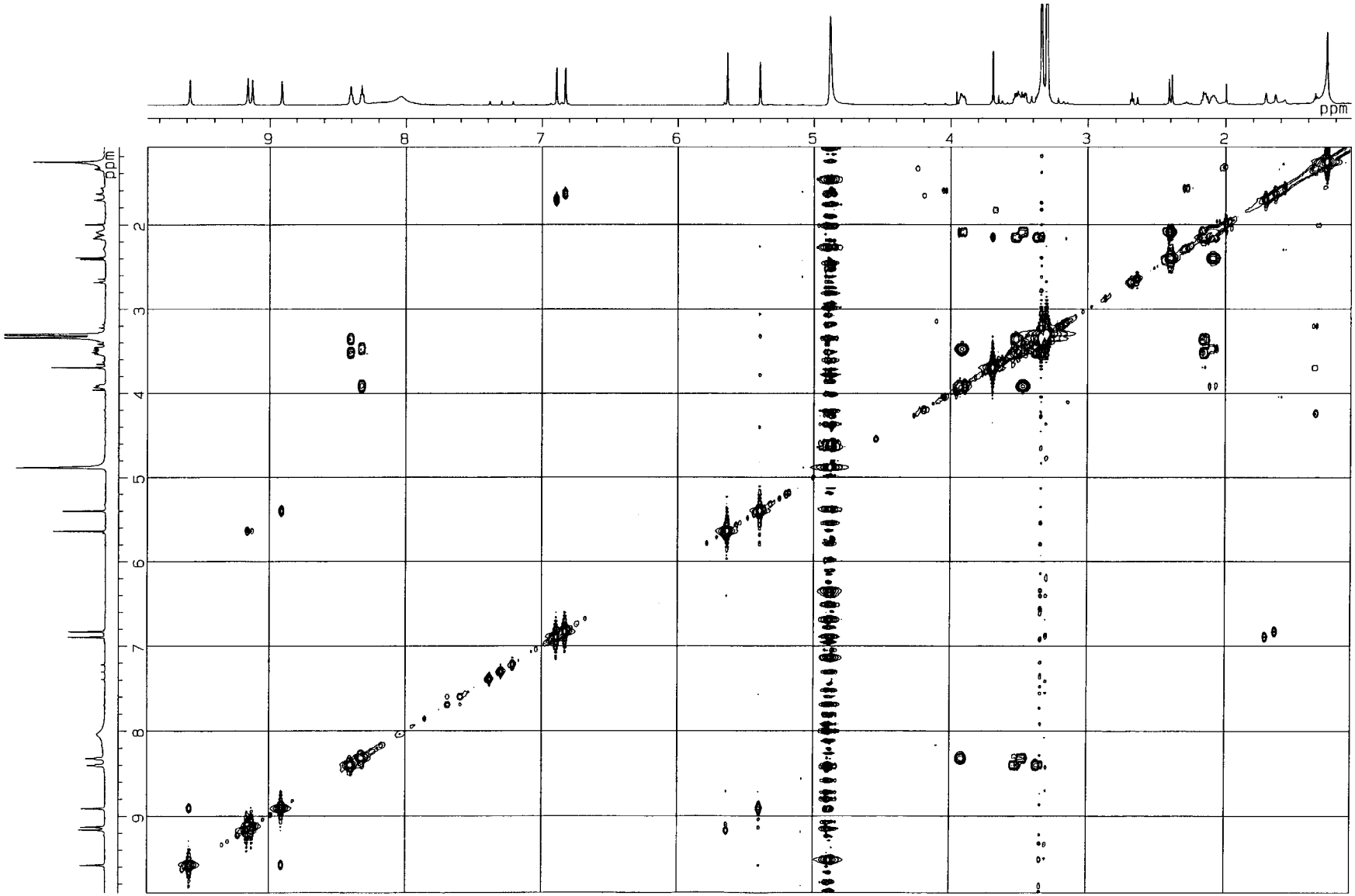


Figure S6. **COSY spectrum of massadine (DMSO-d_6)**

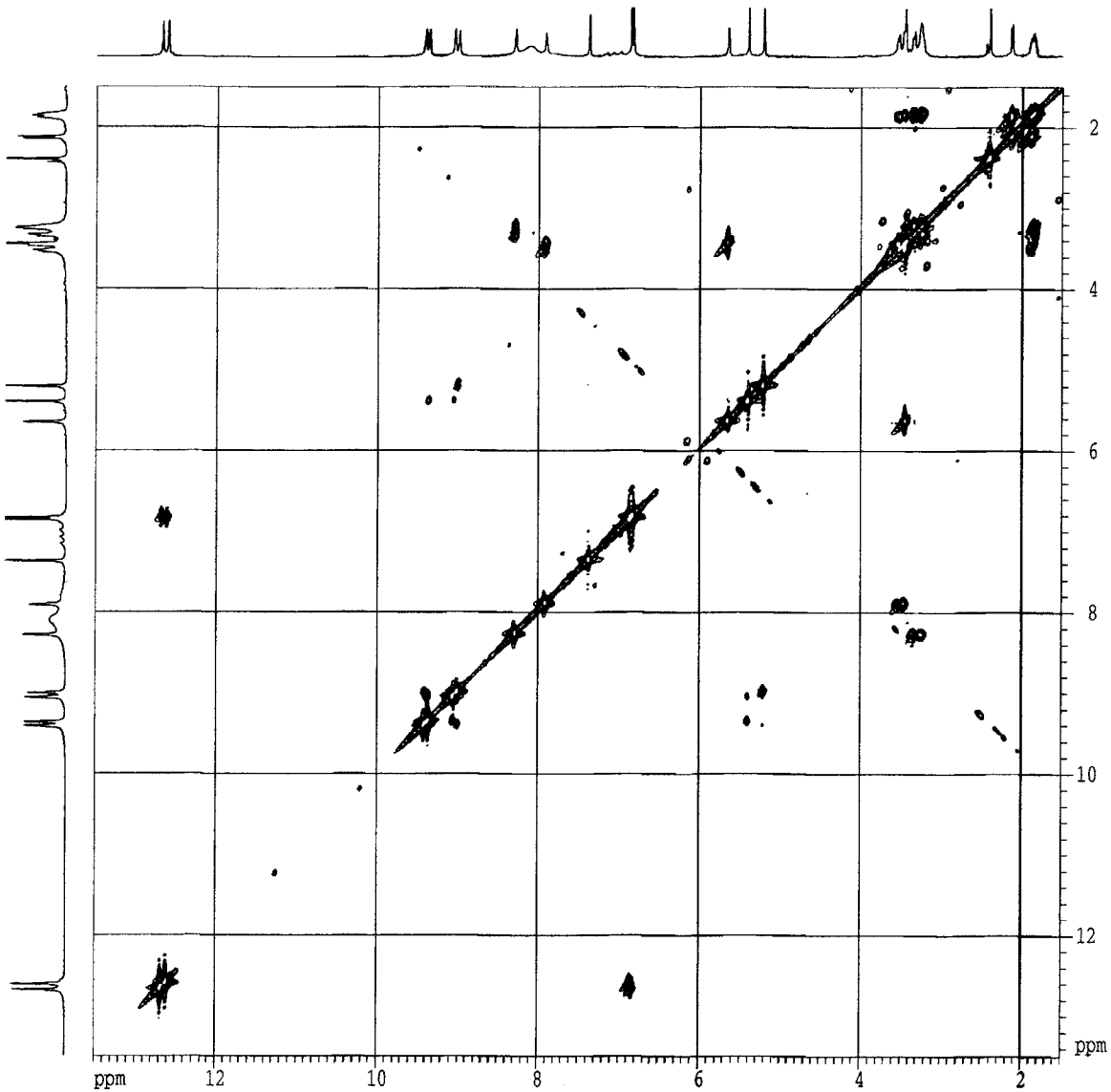


Figure S7. **NOESY spectrum of massadine (CD_3OH)**

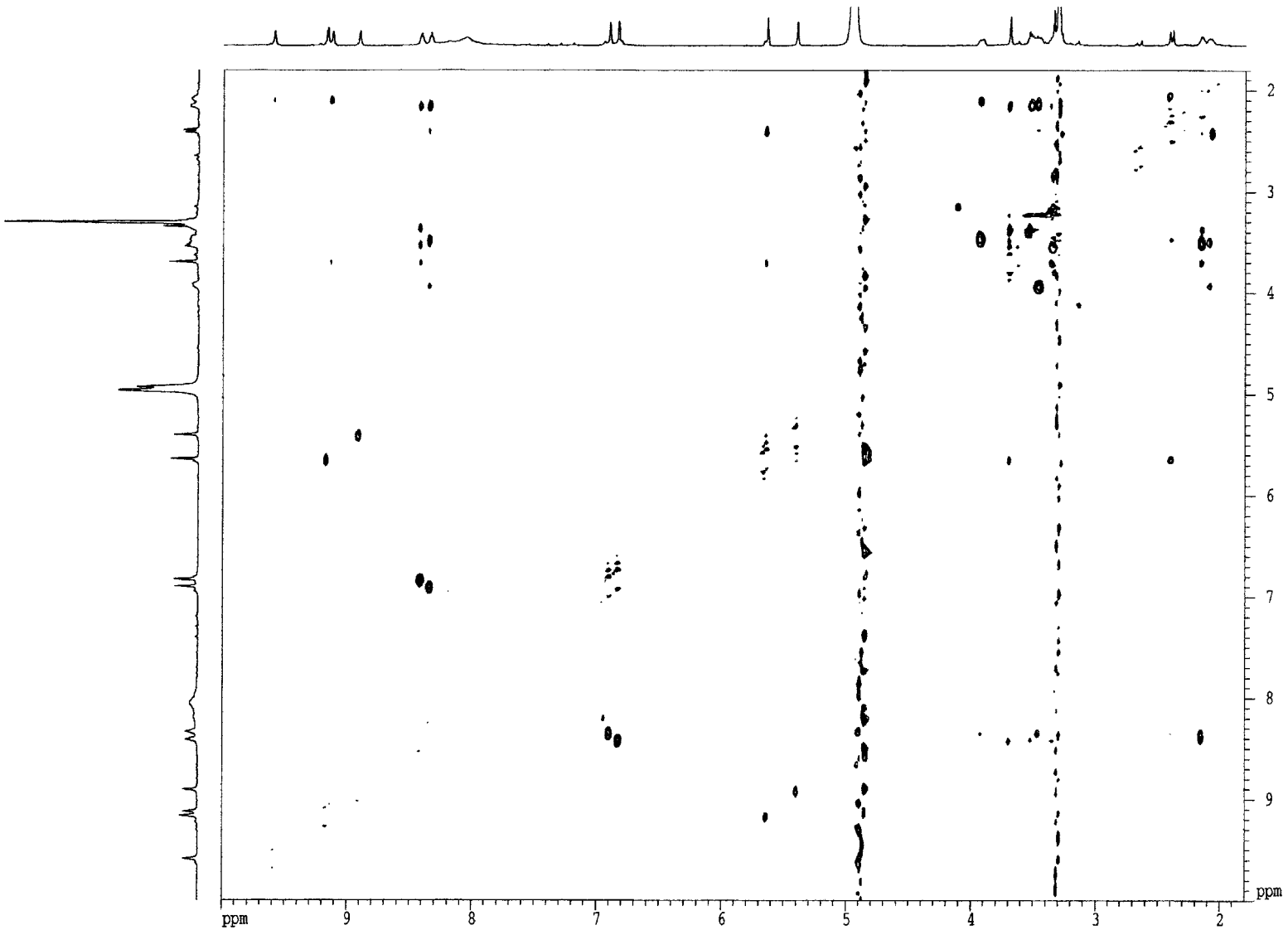


Figure S8. **ROESY spectrum of massadine (DMSO-d_6)**

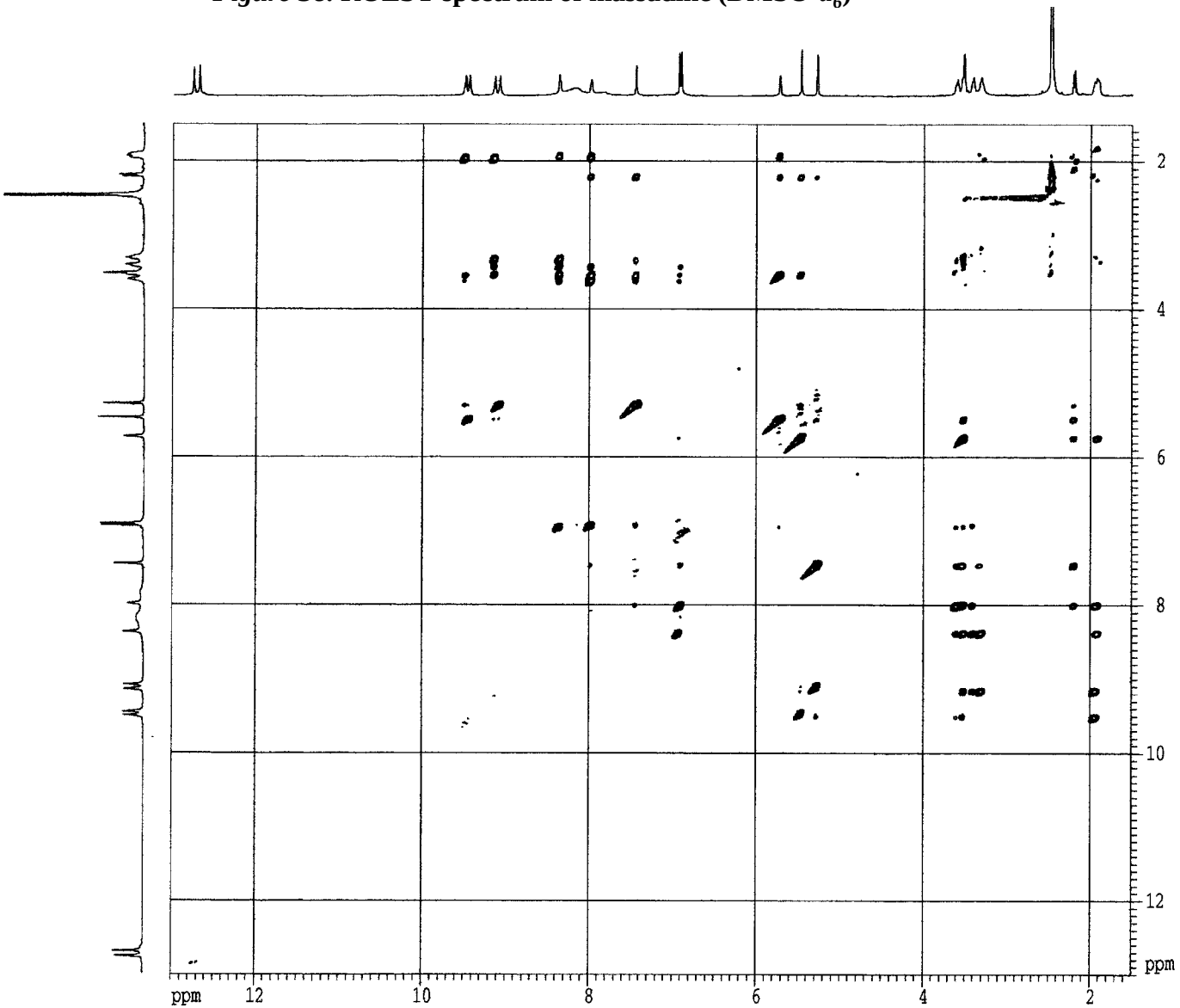


Figure S9. **$^{1}H/^{13}C$ HMQC spectrum of massadine (CD_3OD)**

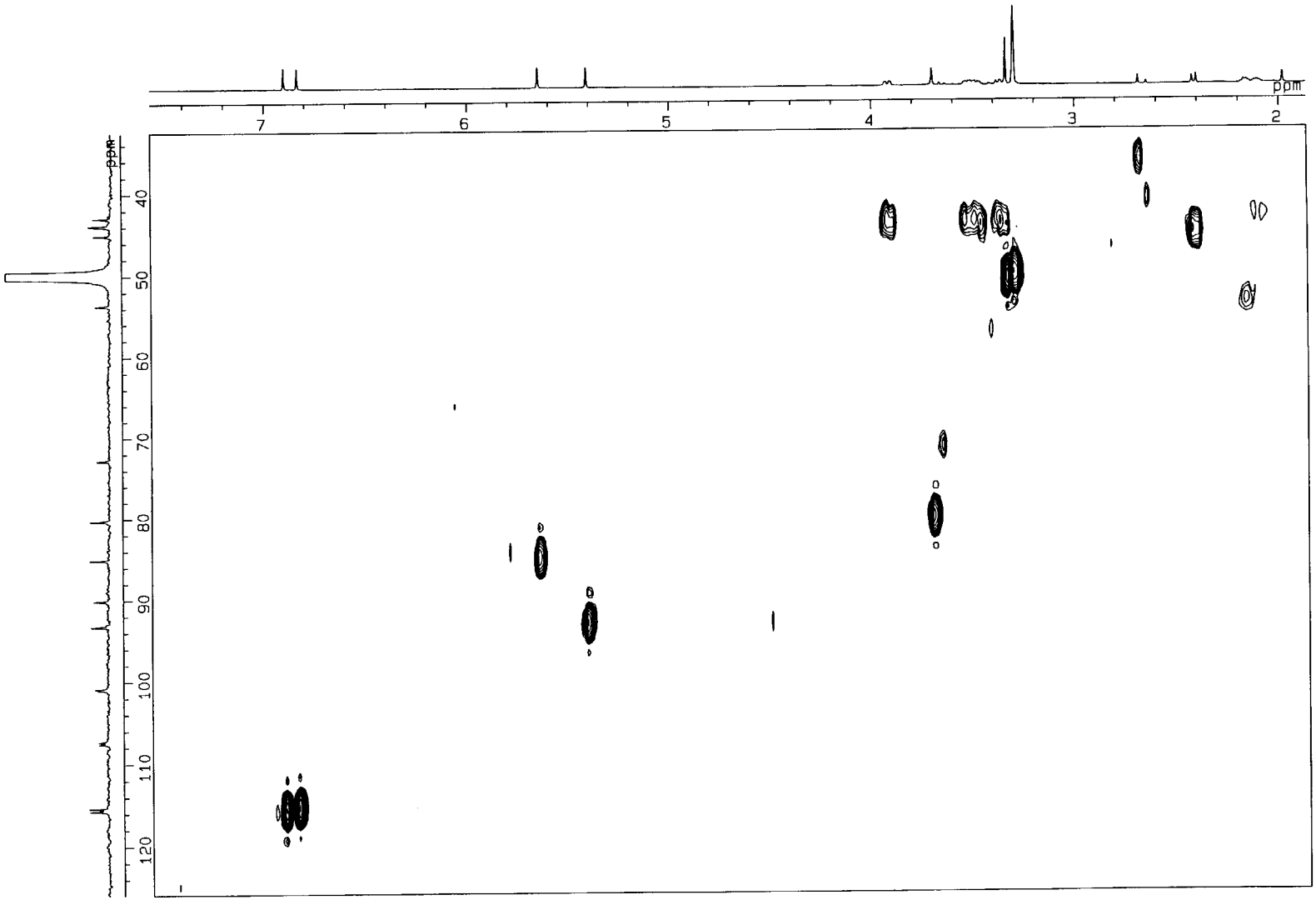


Figure S10. **$^{1}H/^{13}C$ HMBC spectrum of massadine (CD_3OH)**

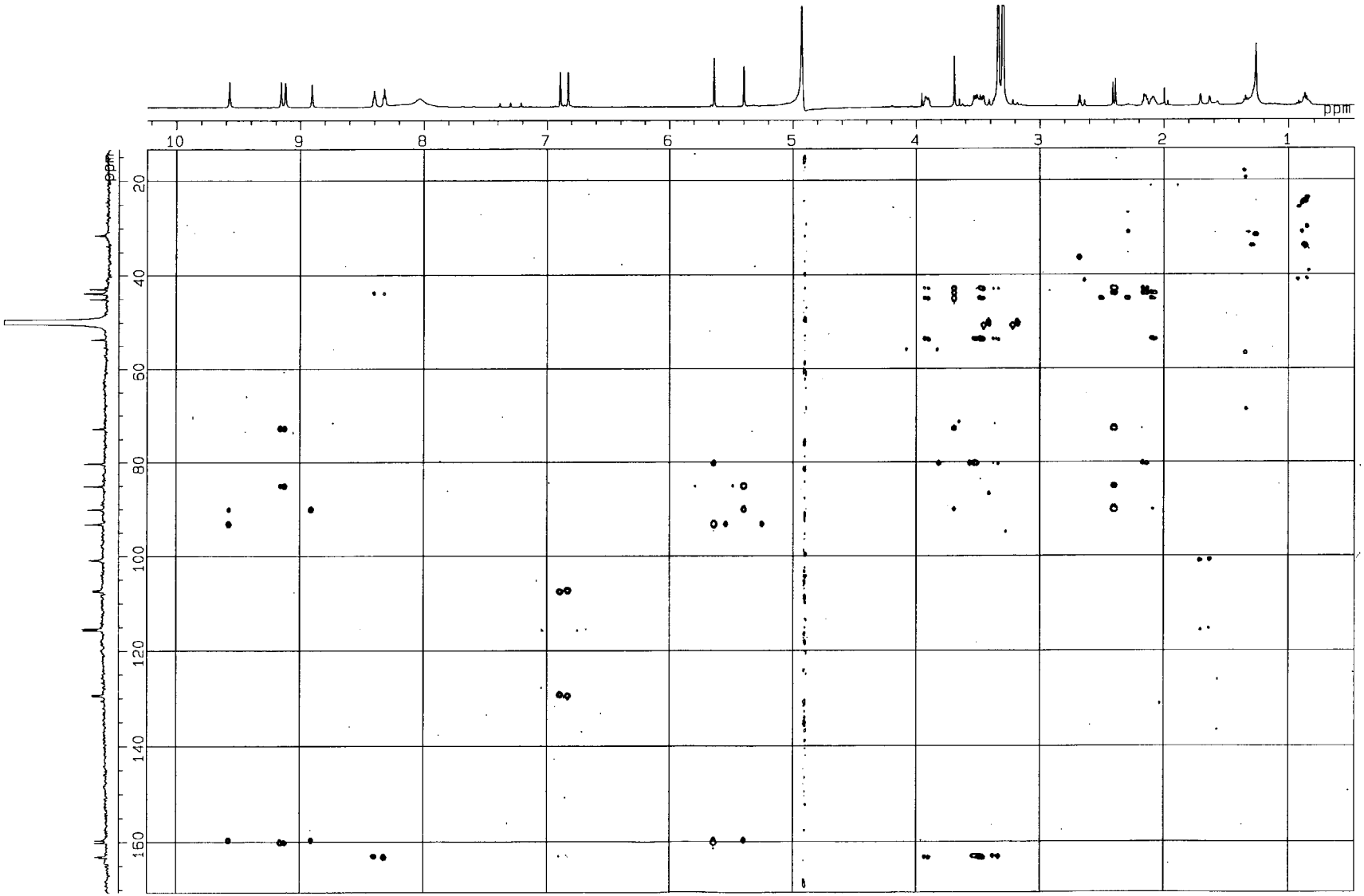


Figure S11. **$^{1}H/^{15}N$ HSQC spectrum of massadine (CD_3OH)**

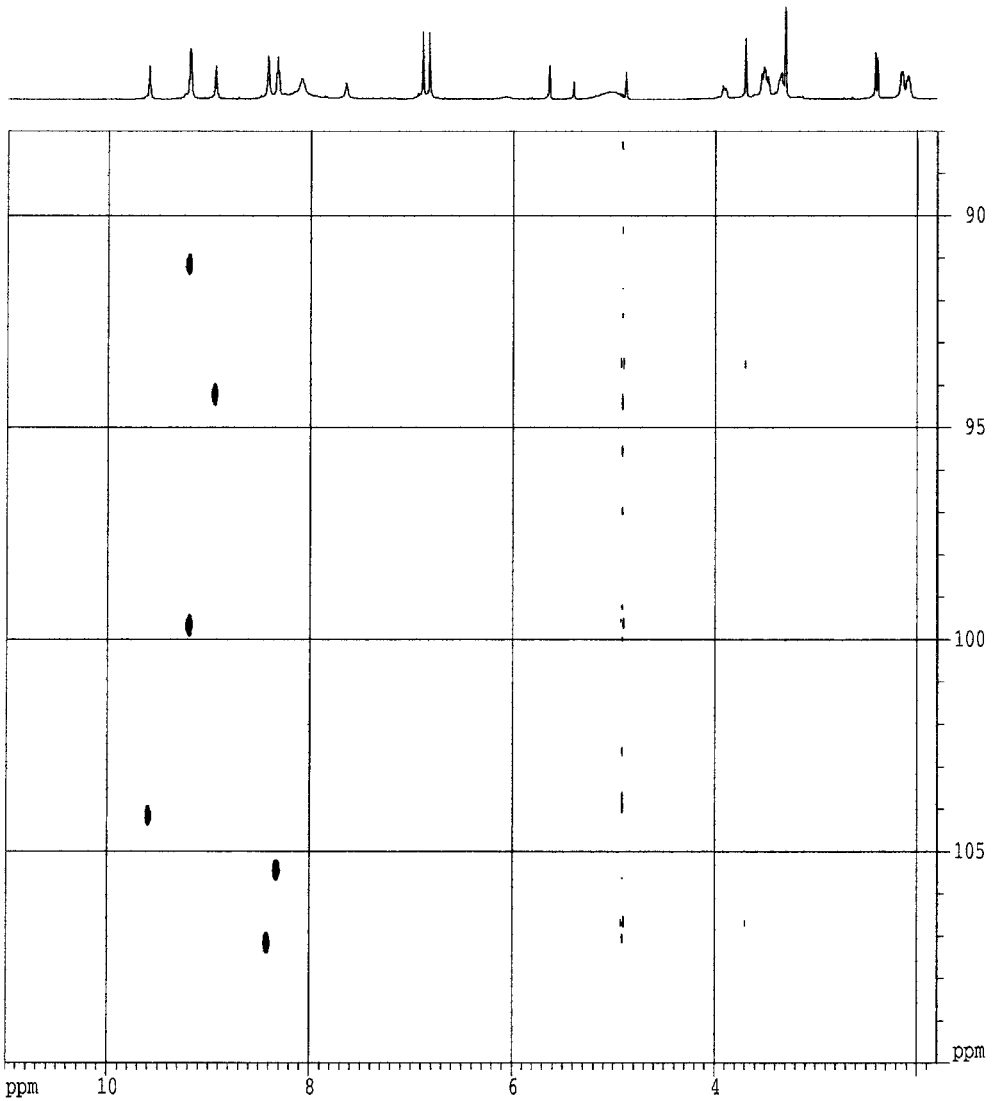


Figure S12. **$^{1}H/^{15}N$ HMBC spectrum of massadine (CD_3OH)**

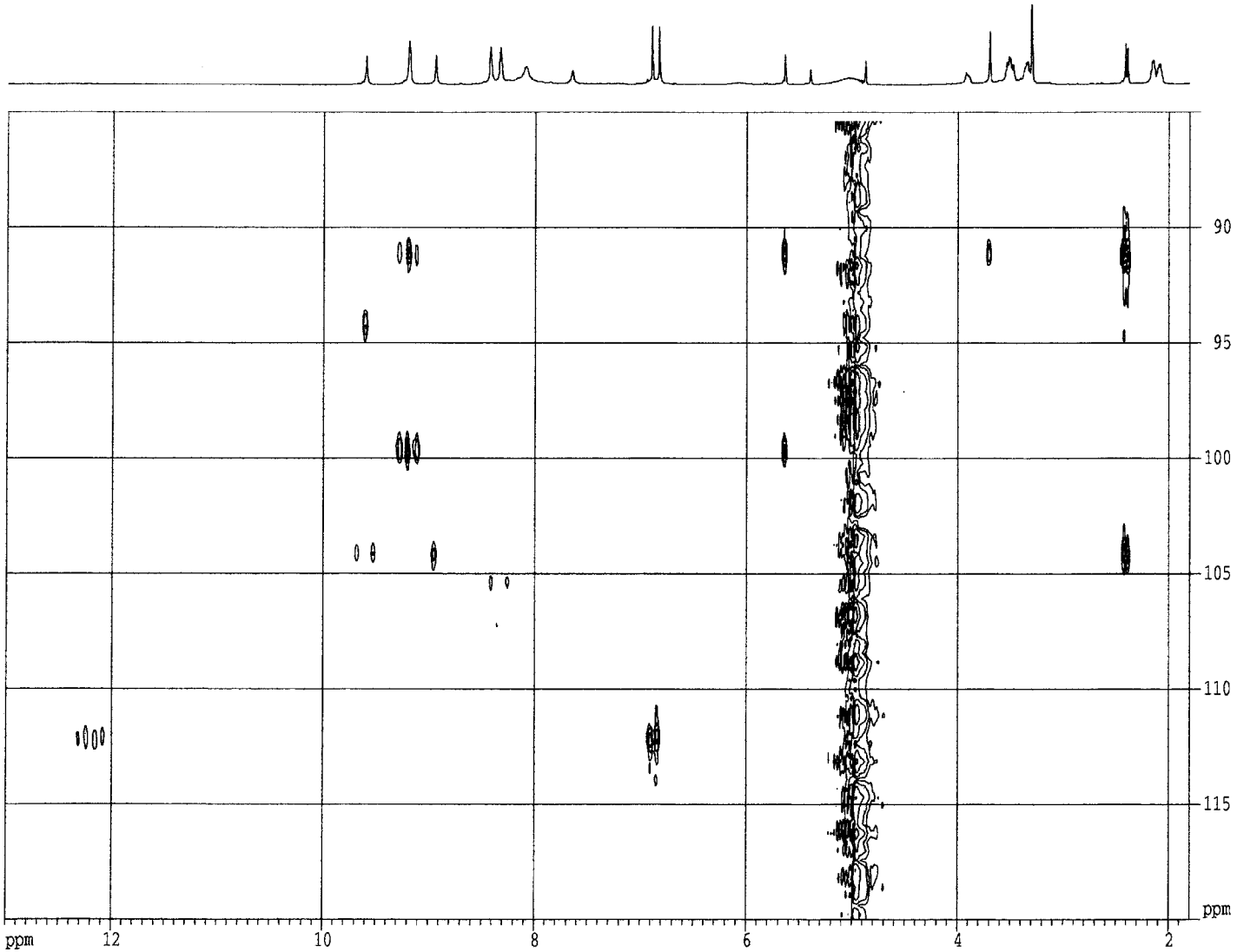


Figure S13. **INADEQUATE spectrum of massadine (CD_3OH)**

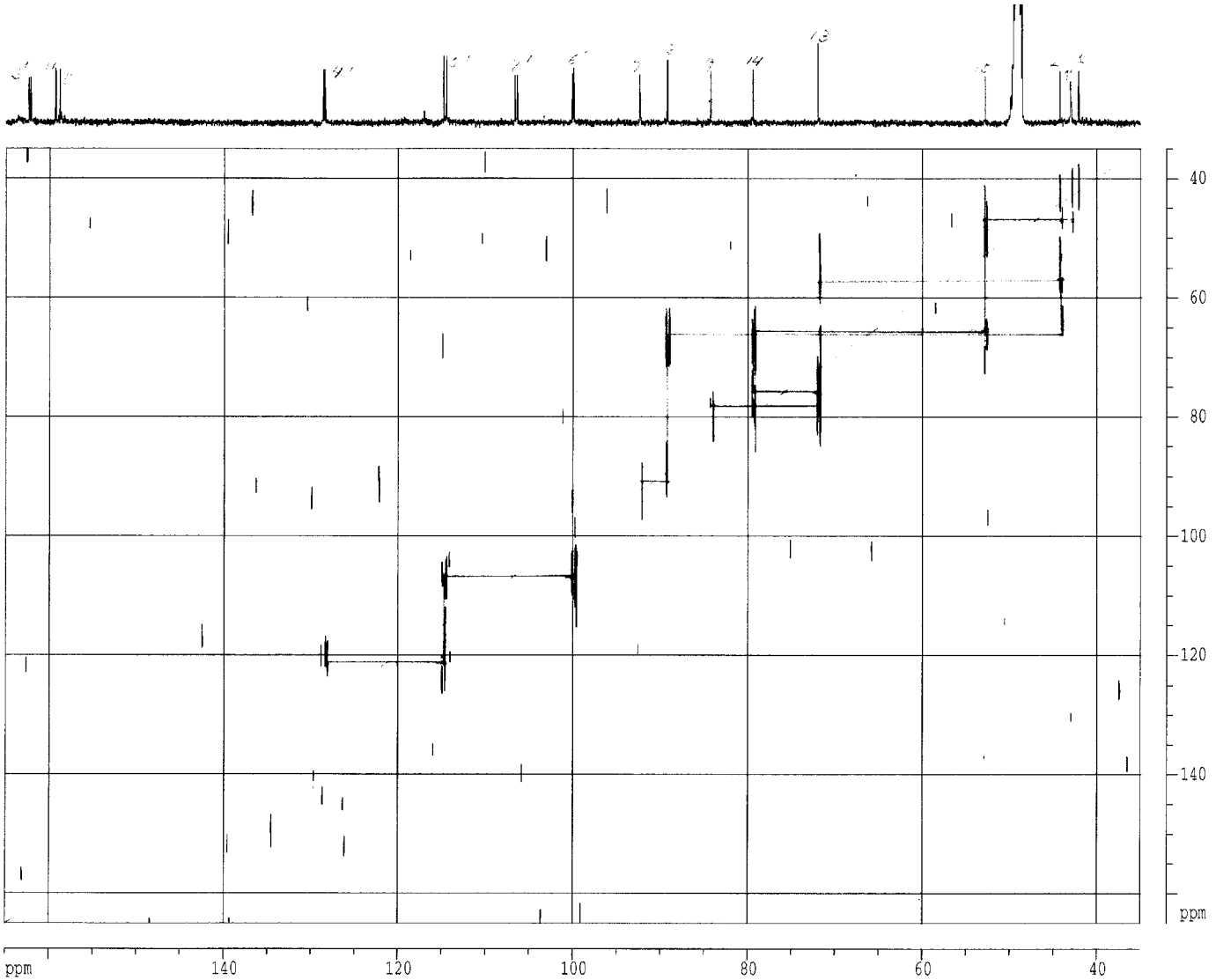
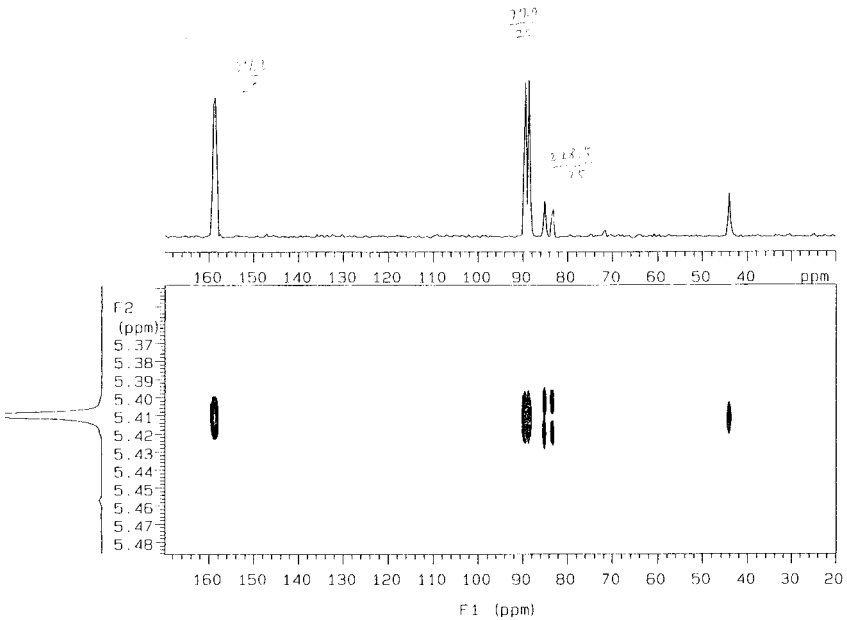


***Figure S14. J*-resolved $^{1}H/^{13}C$ HMBC spectrum of massadine**
(HMBC correlations from H-7 (*left*) and H-9 (*right*))



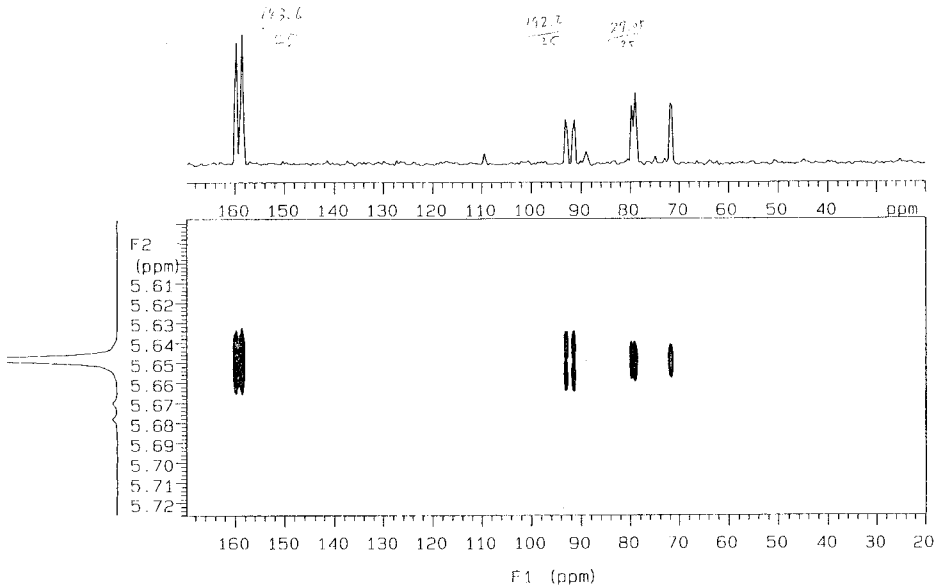
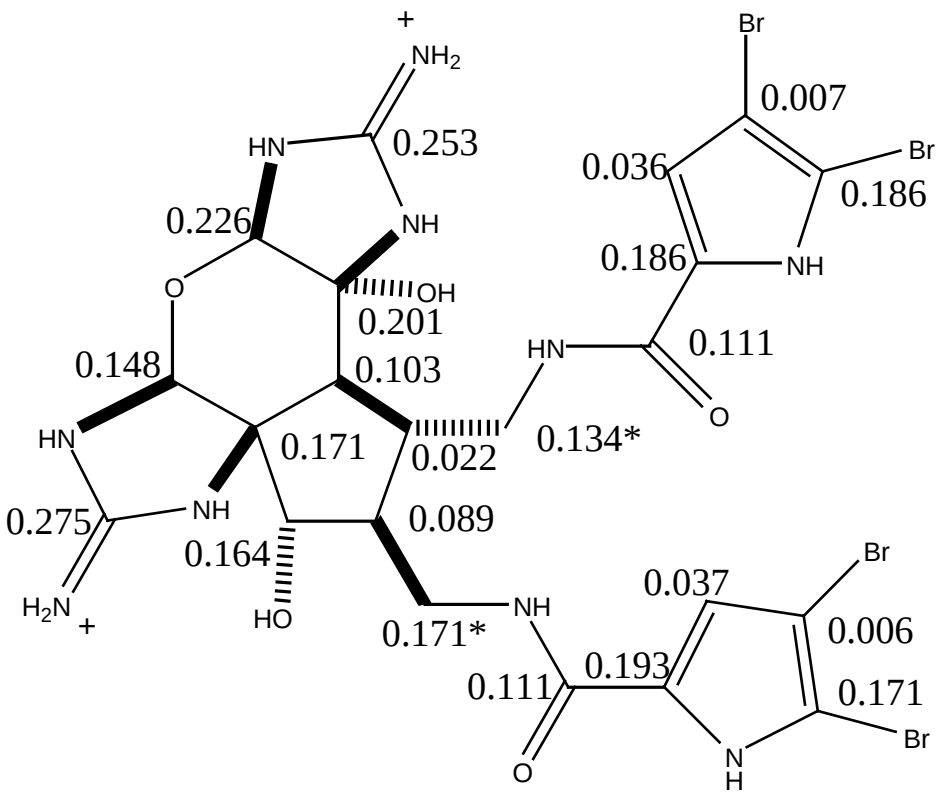


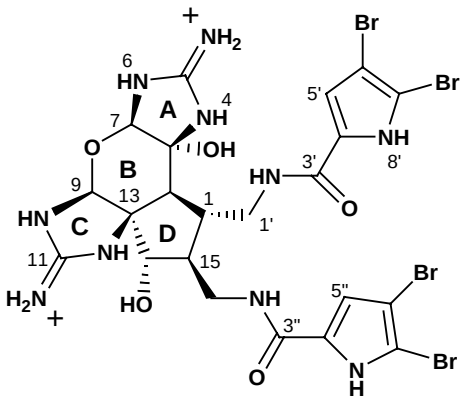
Figure S15. **Deuterium-induced ^{13}C NMR isotope shifts.**



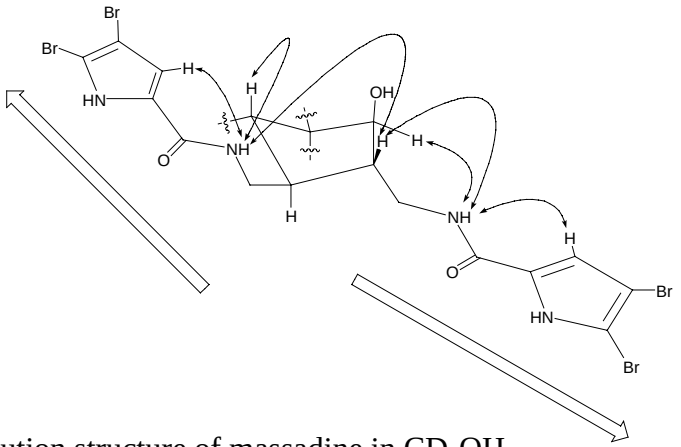
$\Delta\delta$(ppm) = $\delta_{CD3OH} - \delta_{CD3OD}$

* These values can be interchangeable

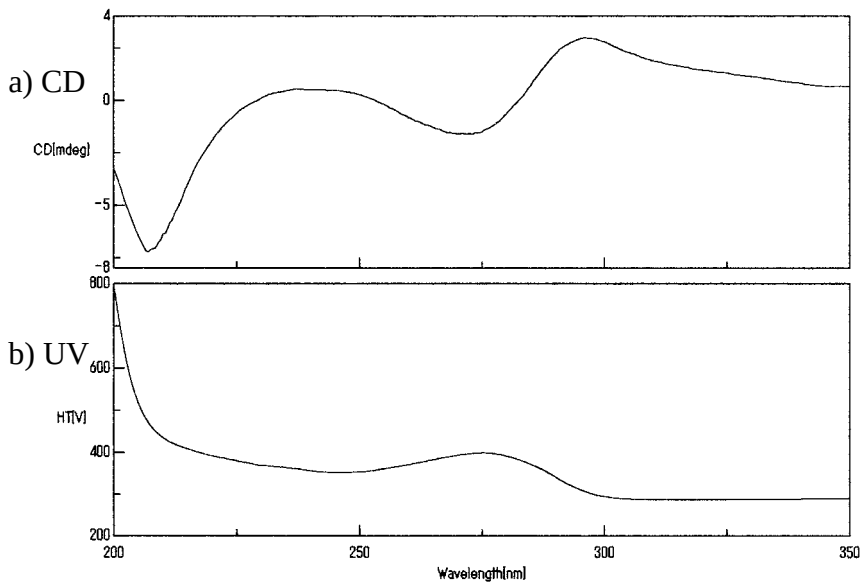
Figure S16. **Interpretation of the CD spectrum of massadine.**

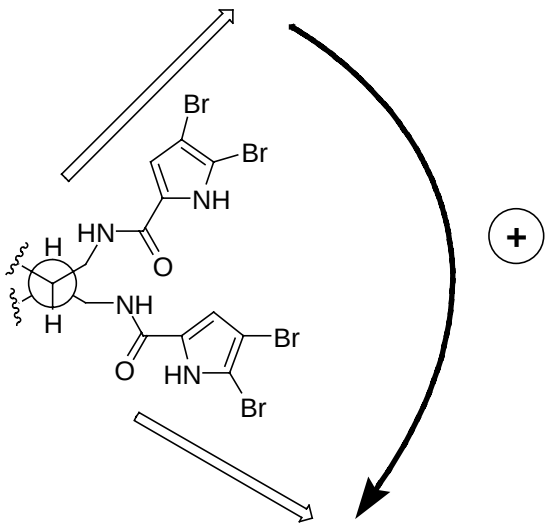


massadine



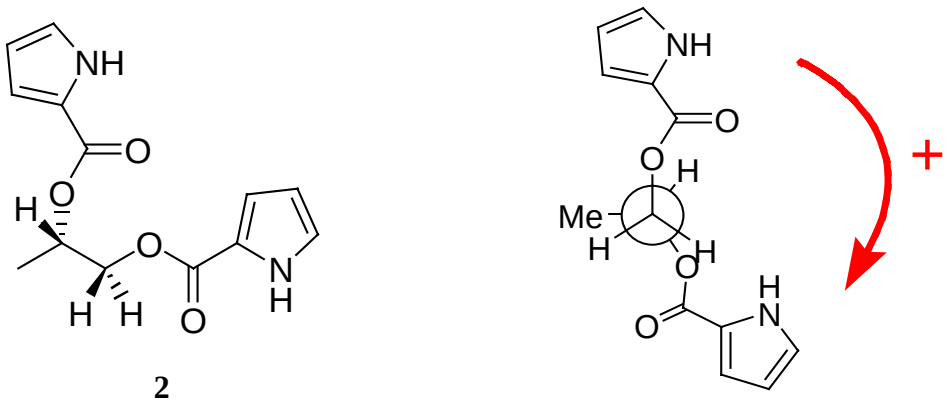
Solution structure of massadine in CD_3OH

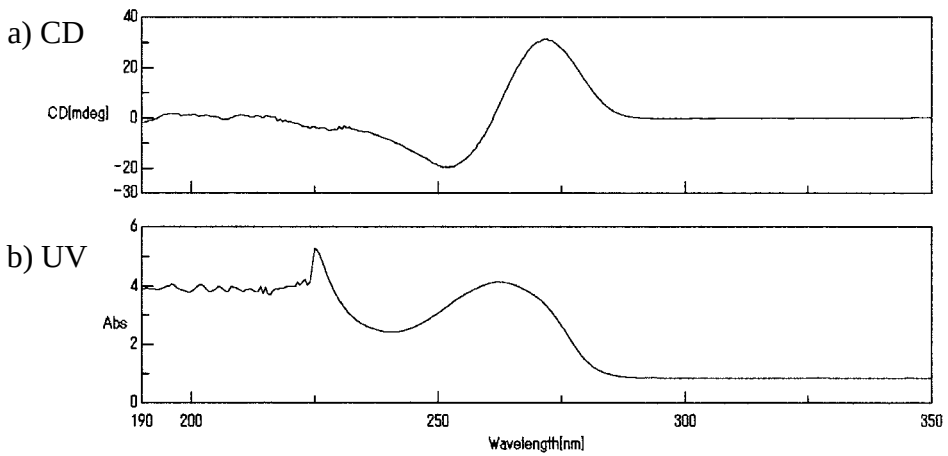




UV (MeOH) λ max 278 (20,000)
CD (MeOH) $\Delta\varepsilon$ 294 (+ 0.75), 271 (-0.52)

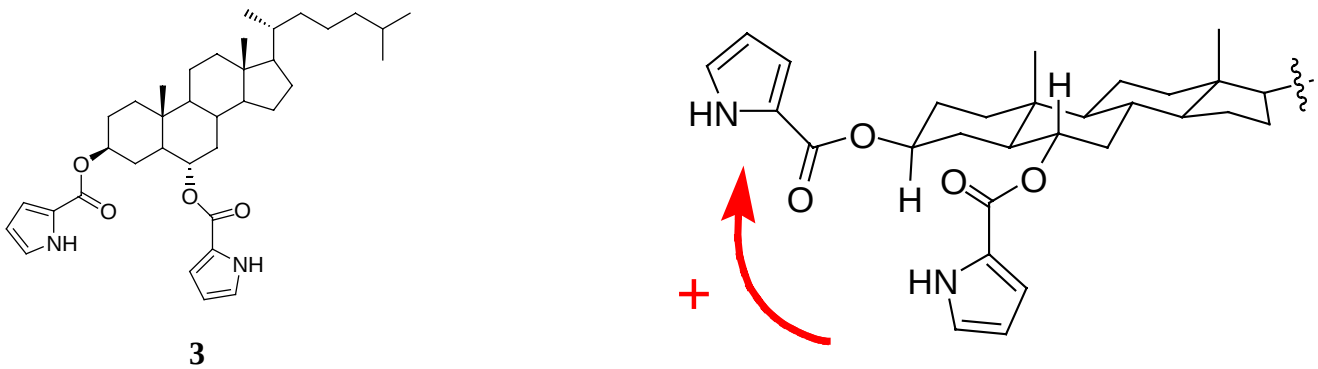
Figure S17. **CD spectrum of a model compound.**



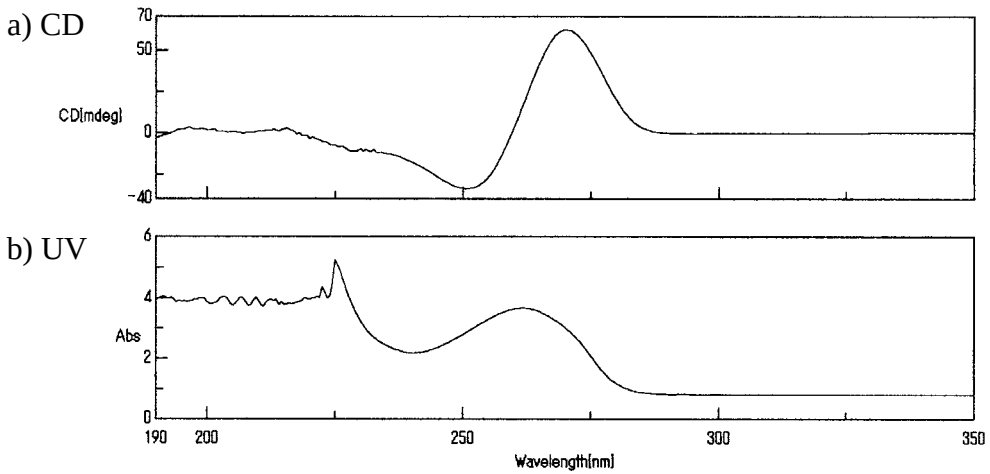


(*S*)-propane-1,2-diol bis(pyrrole-2-carboxylate) (2): To a solution of (*S*)-(+)-1,2-propanediol in DCM/THF (1 : 2) at rt was added pyrrole-2-carboxylic acid, DCC, and DMAP. The reaction mixture was stirred at rt for 4 days, extracted with EtOAc, and fractionated by SiO_2 column chromatography followed by RP-HPLC to furnish **2**: UV (DCM) λmax (ε) 263.0 (15,600) nm; CD (DCM) λ ($\Delta\varepsilon$) 272.0 (+5.0), 261.0 (0.0), 252.0 (-3.1) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ9.19 (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 $(M + Na)^+$.

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



3



5α-Cholestane-3β,6α-diol bis(pyrrole-2-carboxylate) (3): To a solution of cholesterol in dry THF was added 2M $BH_3{\cdot}SMe_2$ in toluene, and the solution was stirred at rt for 12 h. Water, 30 % H_2O_2, and 3N NaOH was added to the reaction mixture and kept stirring at rt for 3h. The reaction mixture was extracted with $CHCl_3$, dried over $MgSO_4$, concentrated, and reacted with pyrrole-2-carboxylic acid as noted in Figure S17 to yield **3**: UV (DCM) λmax (ε) 263.0 (32,800) nm; CD (DCM) λ ($\Delta\varepsilon$) 272.0 (+22.3), 259.0 (0.0), 250.5 (-12.1) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ9.11 (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 $(M + Na)^+$, 1181 $(2M + H)^+$.