

Plakinamine P, A Steroidal Alkaloid with Bactericidal Activity against *Mycobacterium tuberculosis*

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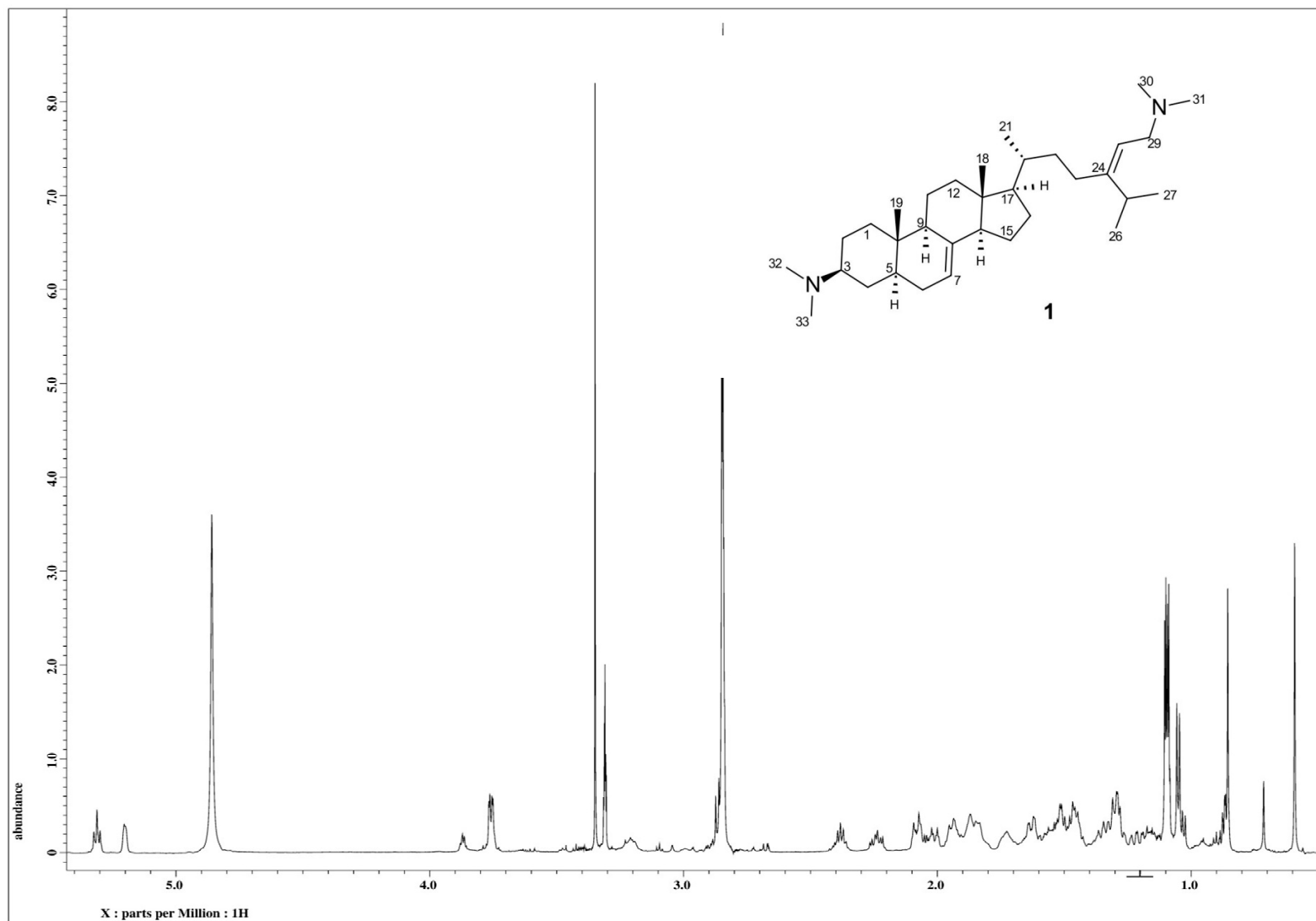
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Table S2: Table of ¹H and ¹³C NMR Data for plakinamine P (**1**) (600 MHz, DMSO-*d*₆)

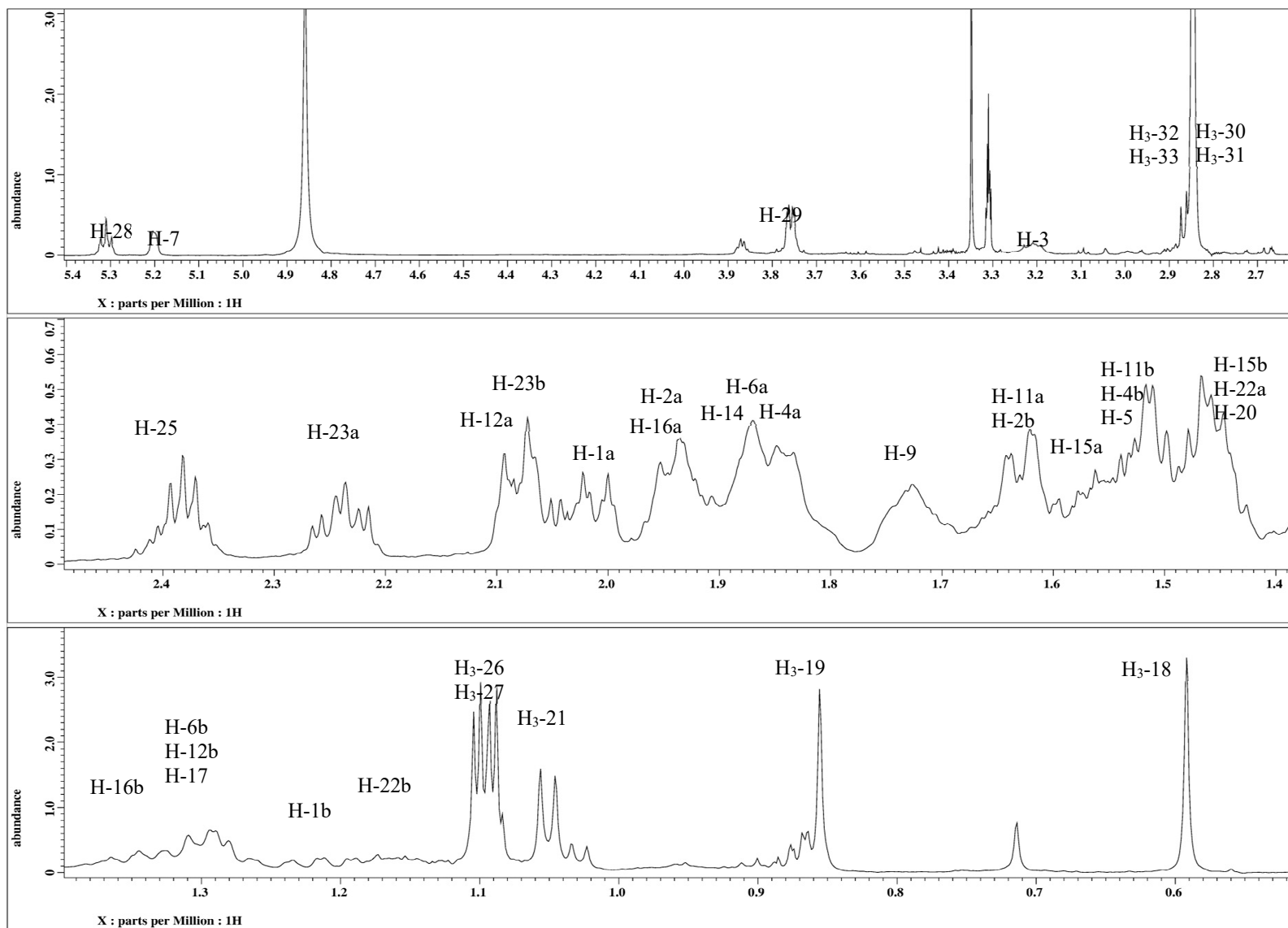
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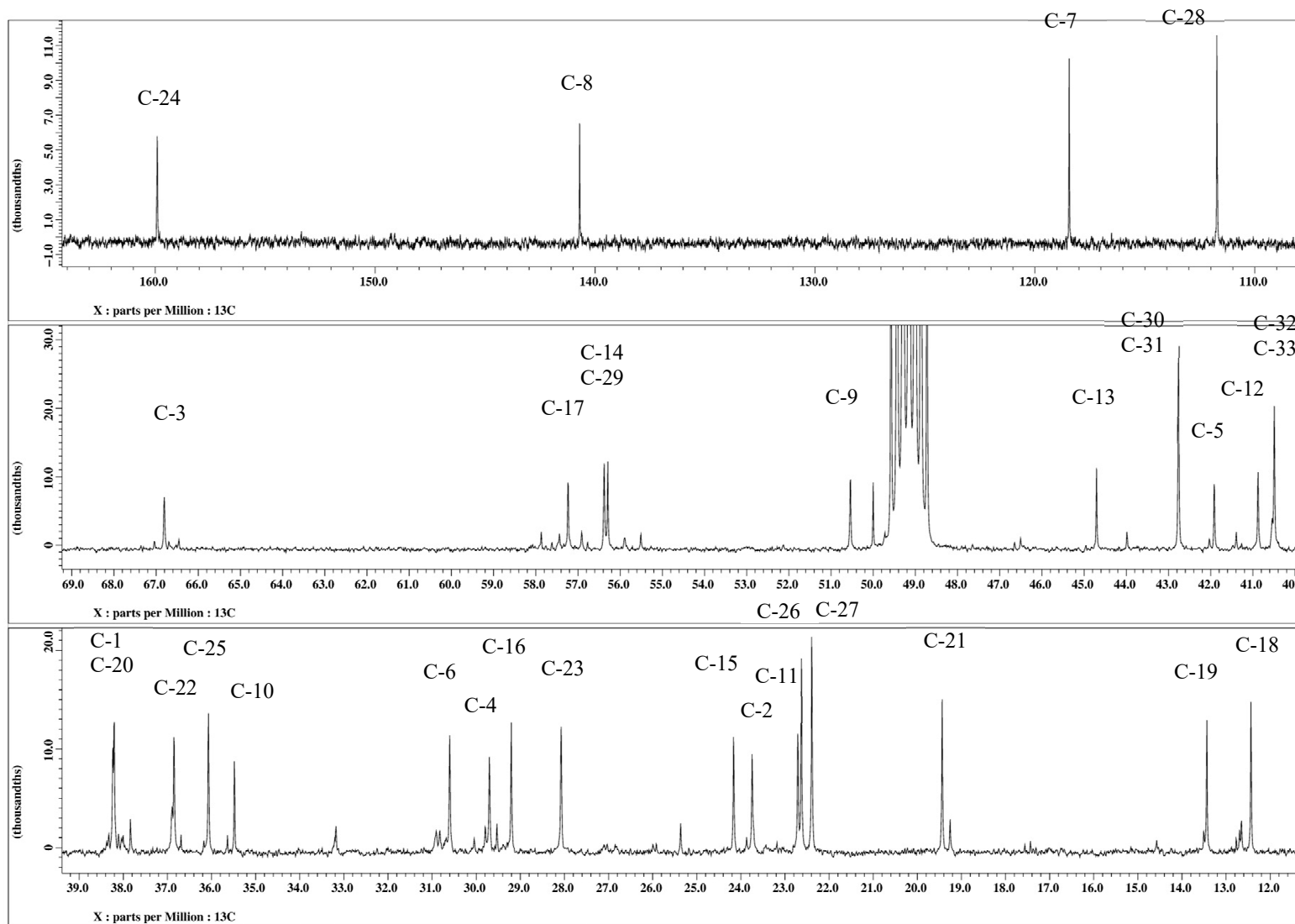
S1 ¹H NMR (600 MHz, CD₃OD) of Plakinamine P (1)



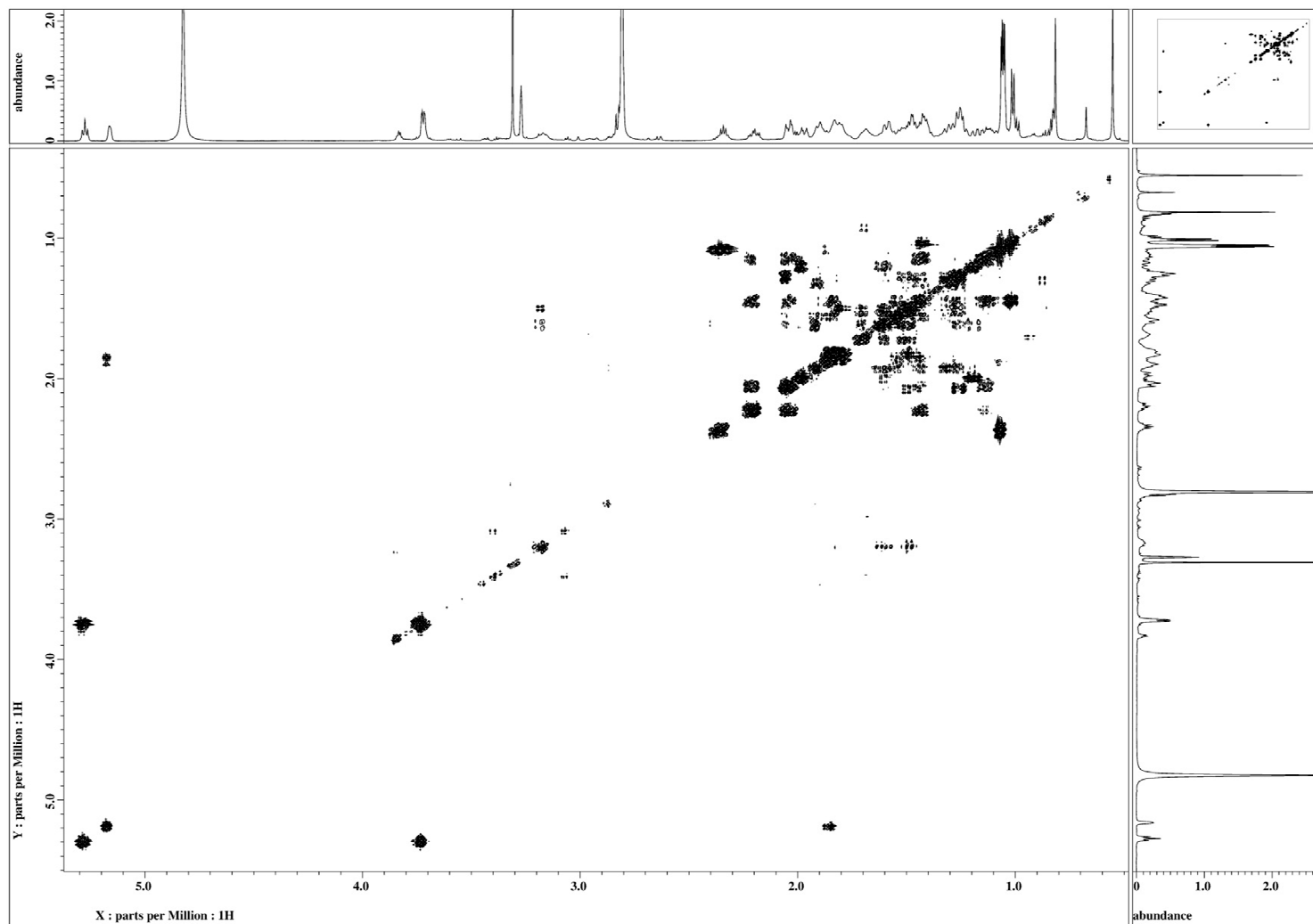
S2 Expansion of ^1H NMR (600 MHz, CD_3OD) of Plakinamine P (1)



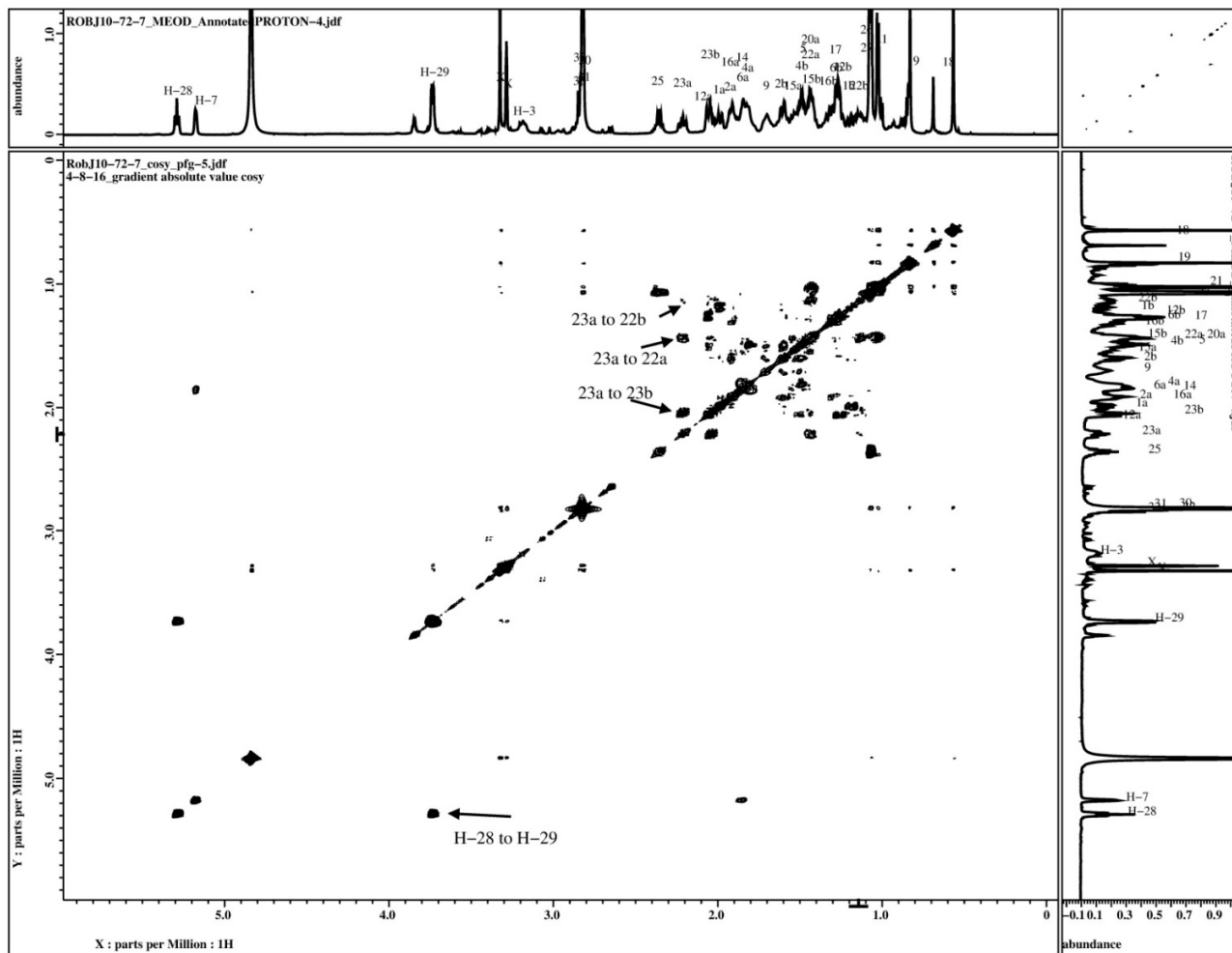
S3 ^{13}C NMR (150 MHz, CD_3OD) of Plakinamine P (1)



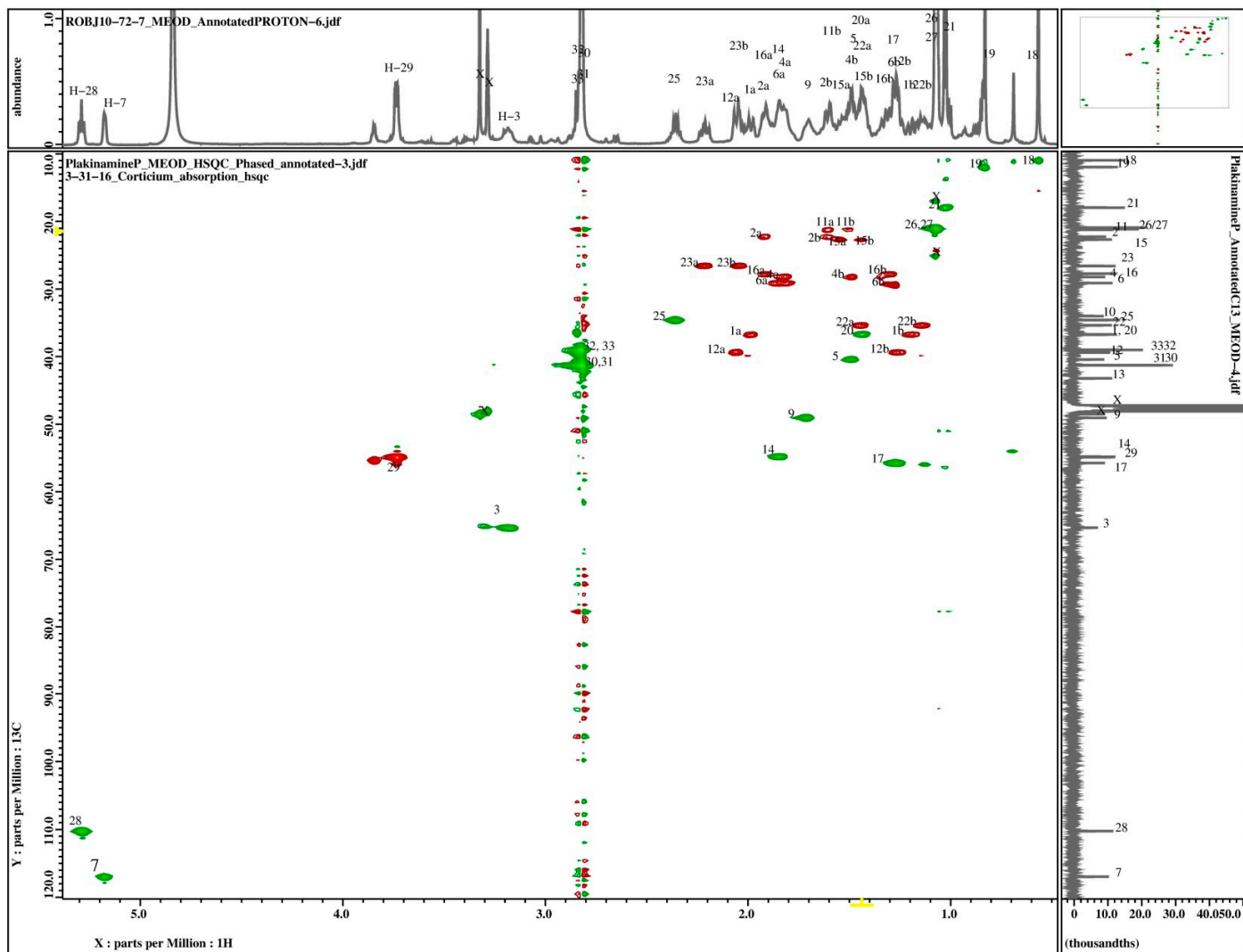
S4 g-COSY spectrum (600 MHz, CD₃OD) of Plakinamine P (1)



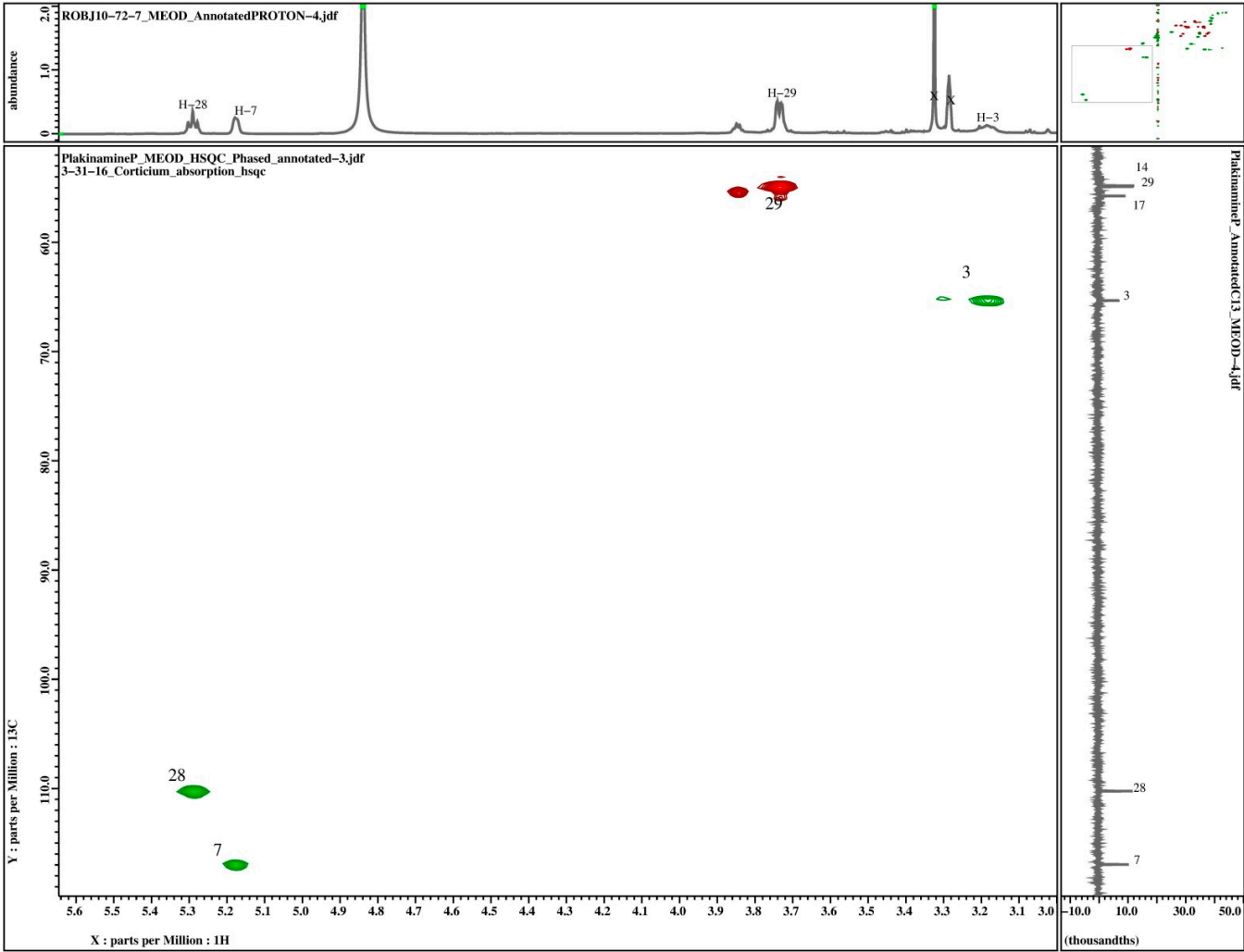
S5 g-COSY spectrum expansion key side chain correlations (600 MHz, CD₃OD) of Plakinamine P (1)



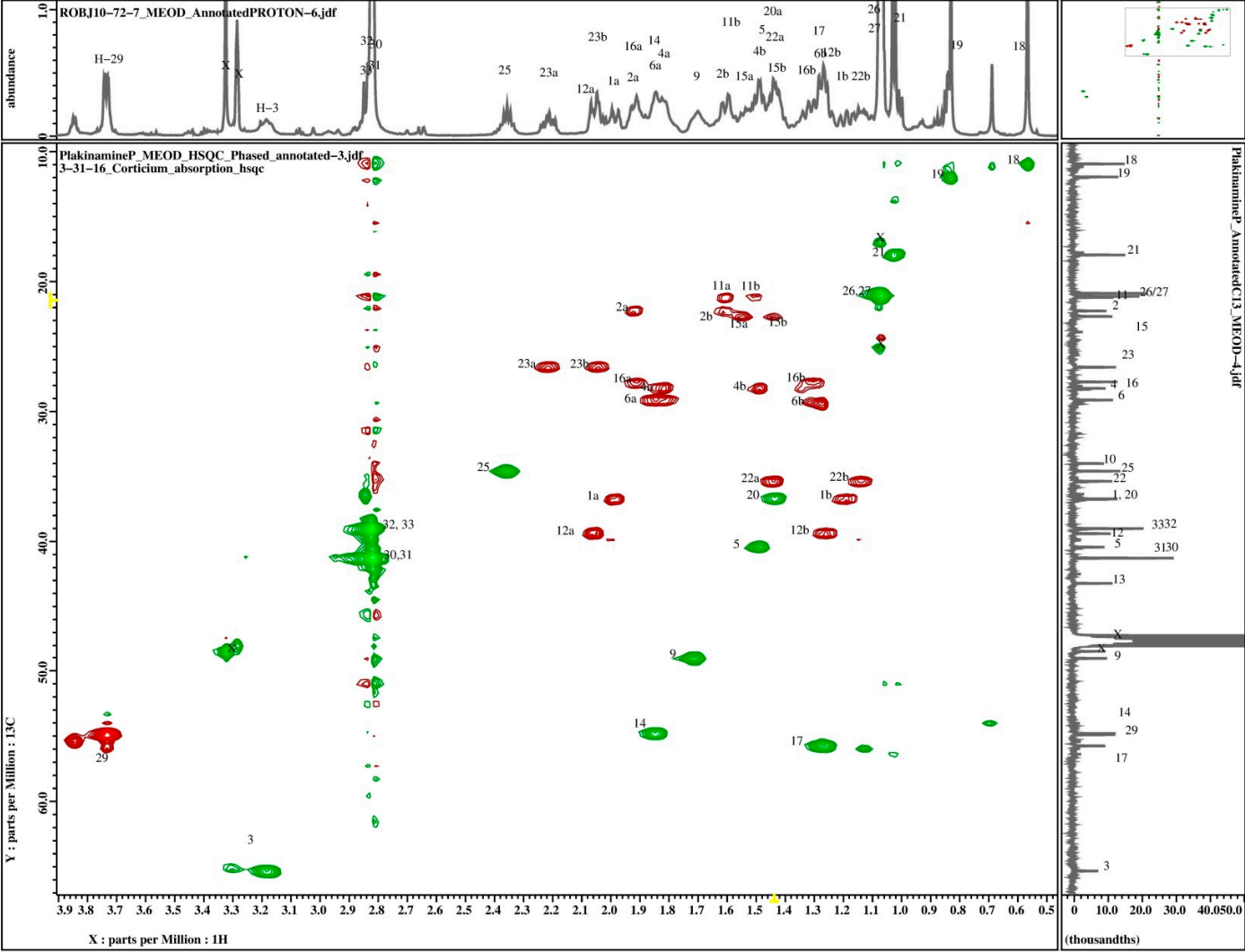
S6 edited gHSQC spectrum (600 MHz, CD₃OD) of Plakinamine P (1)



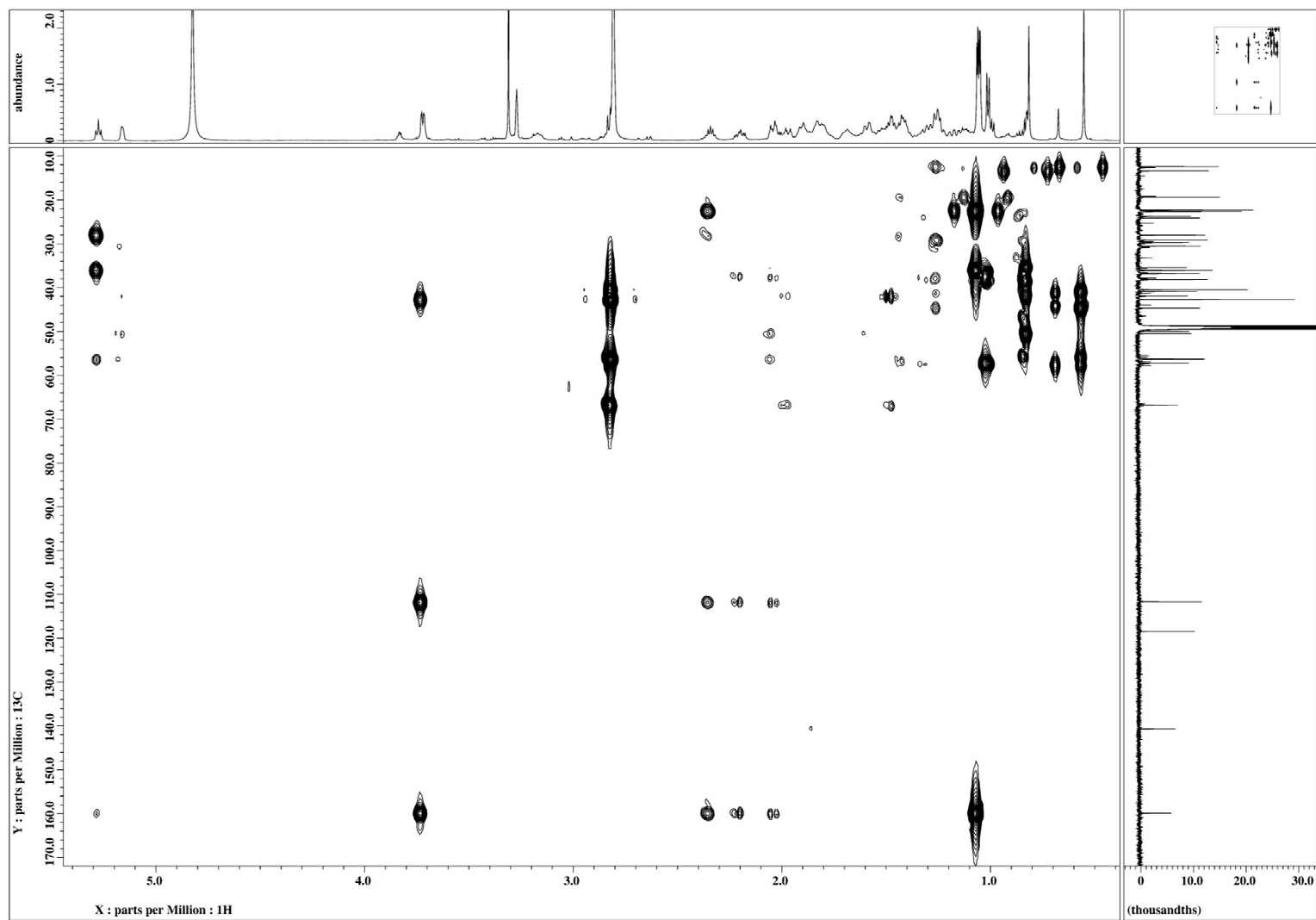
S7 Expansion 1 of edited gHSQC spectrum (600 MHz, CD₃OD) Plakinamine P (1)



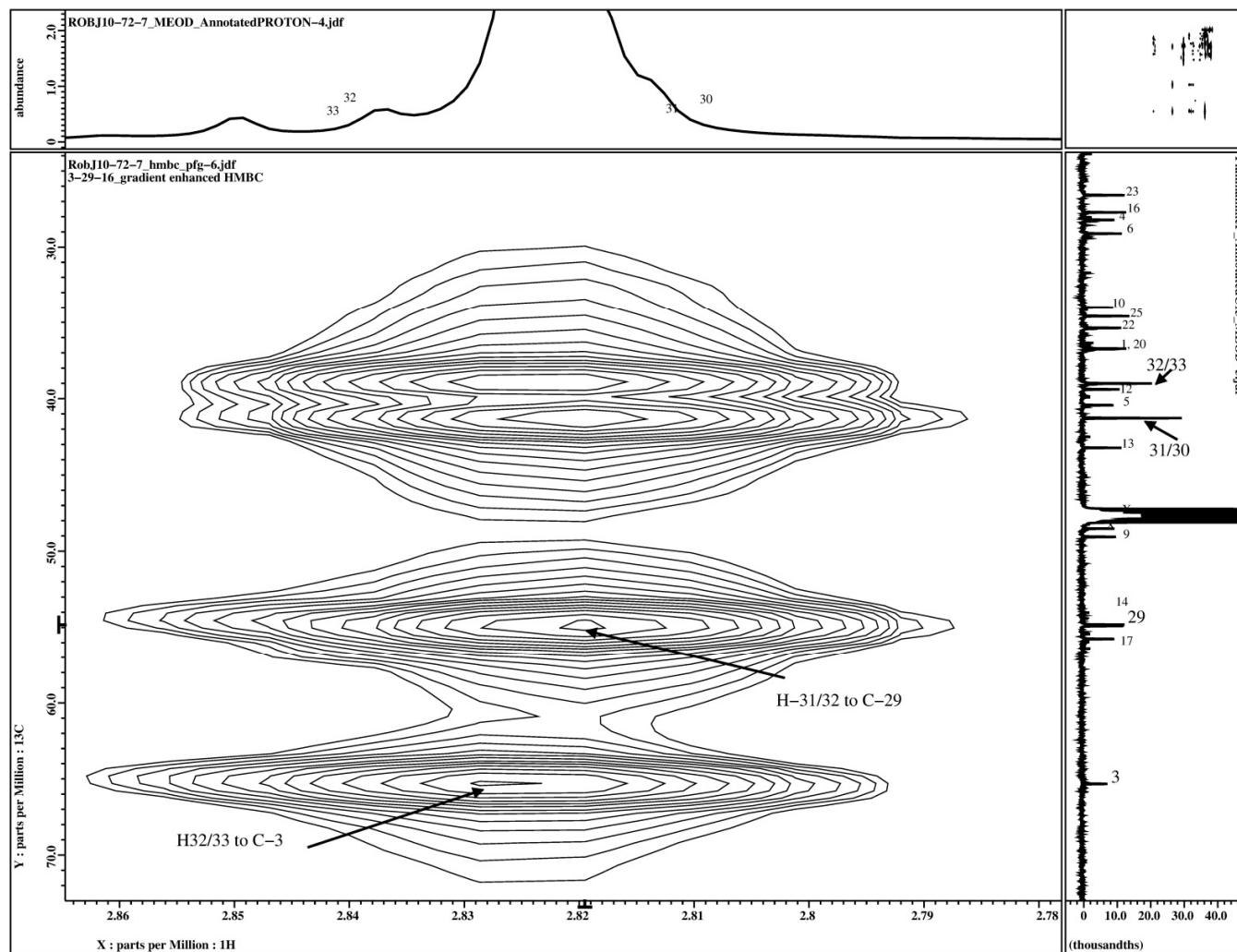
S8 Expansion 2 of edited gHSQC spectrum (600 MHz, CD₃OD) of Plakinamine P (1)



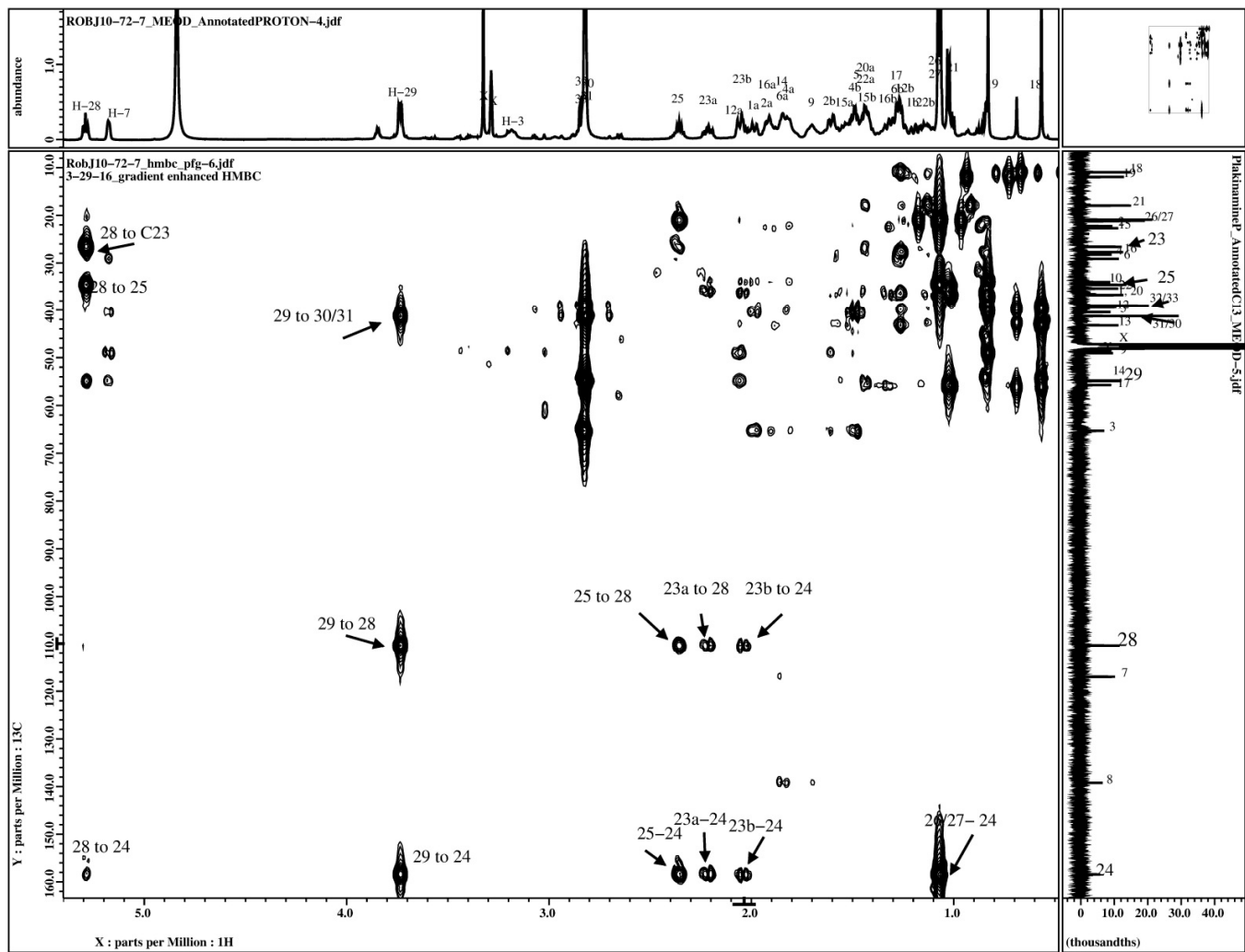
S9 gHMBC spectrum (600 MHz, CD₃OD) of Plakinamine P (1)



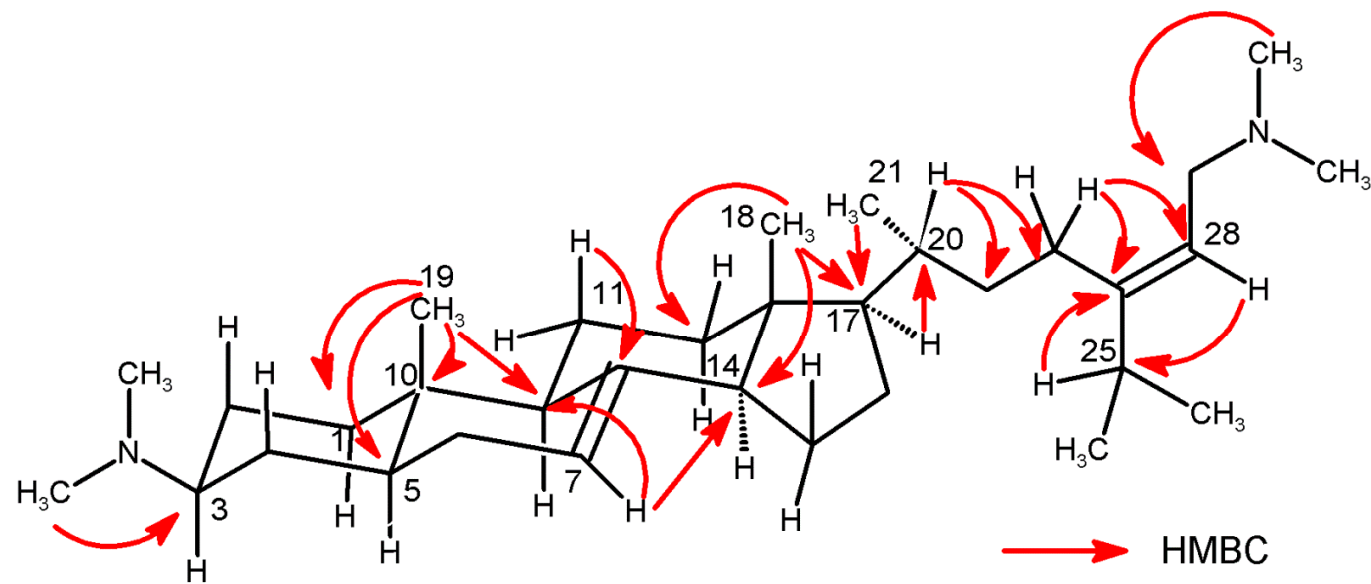
S10 gHMBC spectrum expansion of N,N dimethyl amino proton correlations (600 MHz, CD₃OD) of Plakinamine P (1)



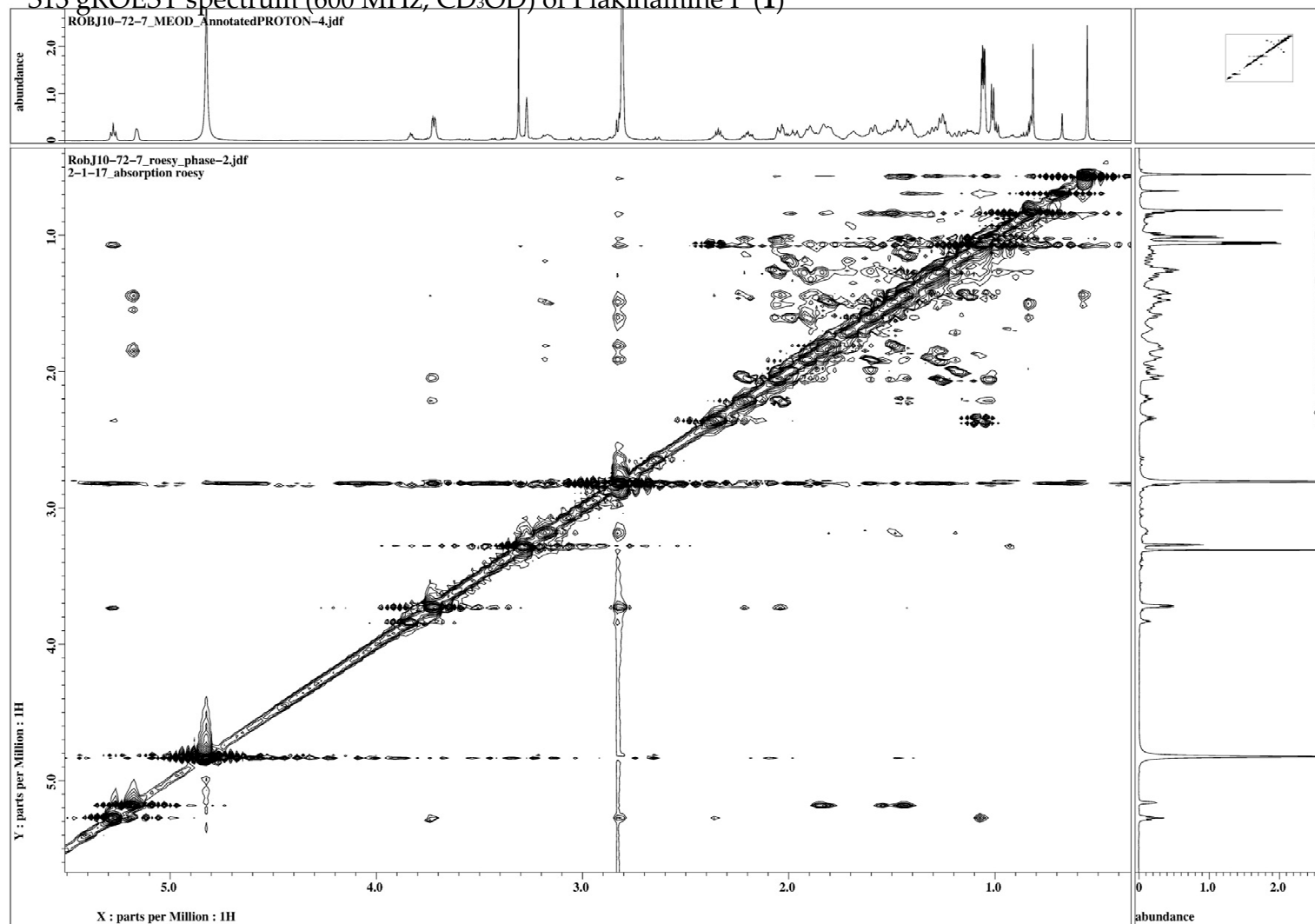
S11 gHMBC spectrum expansion with key side chain correlations (600 MHz, CD₃OD) of Plakinamine P (1)



S12 Structure of plakinamine P (1) with key gHMBC correlations observed in CD₃OD (600 MHz)



S13 gROESY spectrum (600 MHz, CD₃OD) of Plakinamine P (1)



S14 Structure of plakinamine P (1) with chemical shifts in CD₃OD (600 MHz)

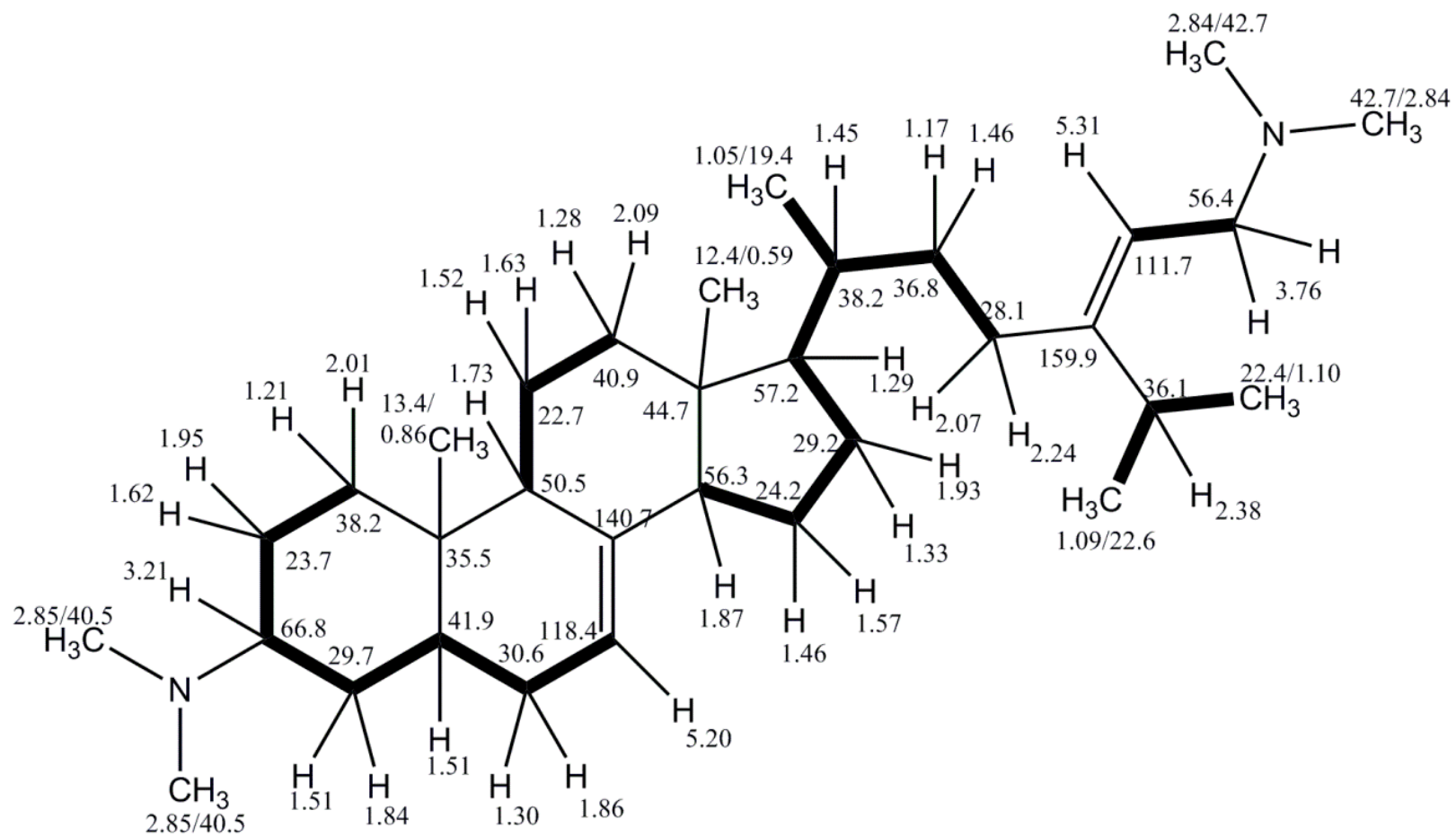
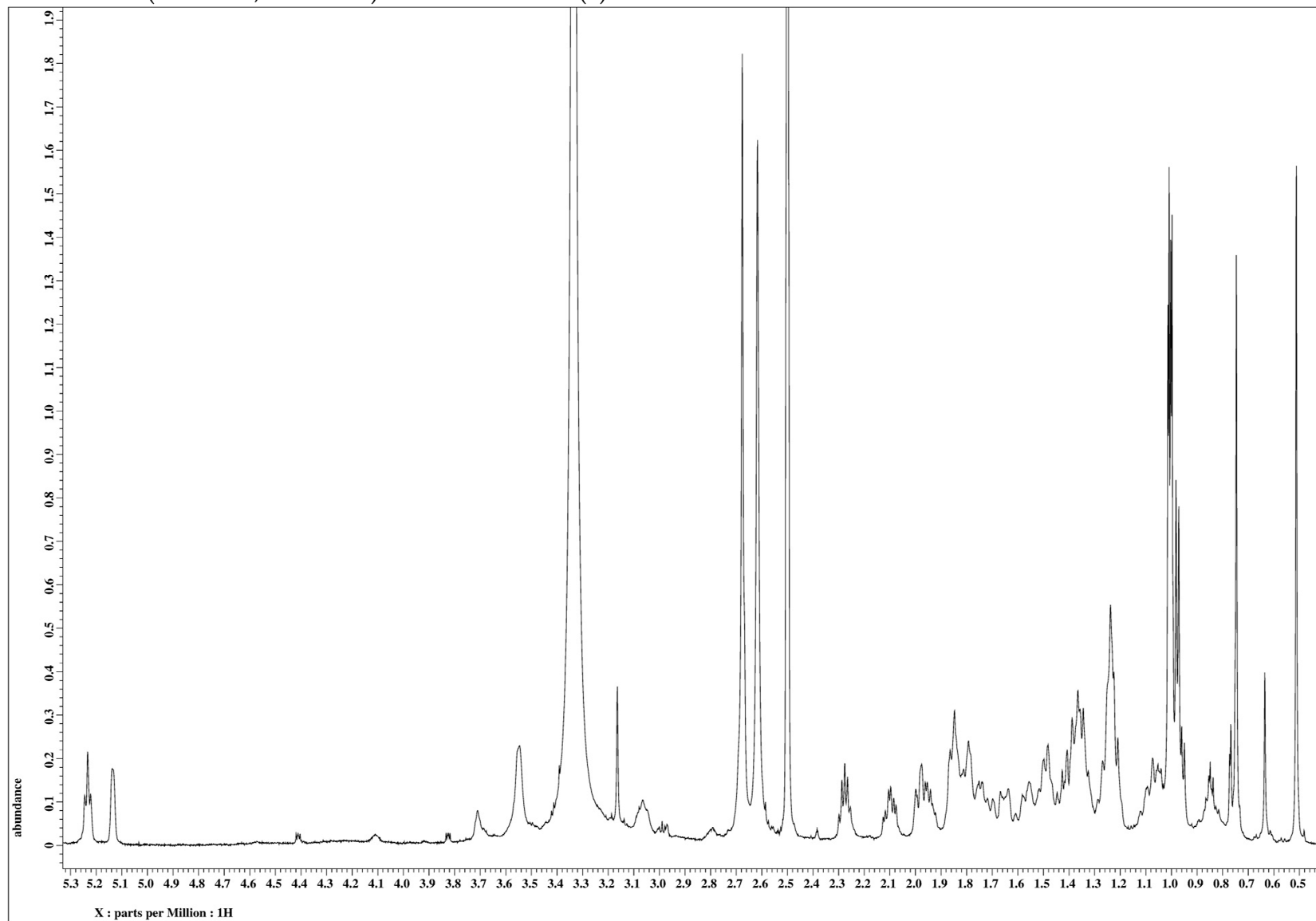


Table S1: Table of ¹H and ¹³C NMR Data for plakinamine P (**1**) (600 MHz, CD₃OD)

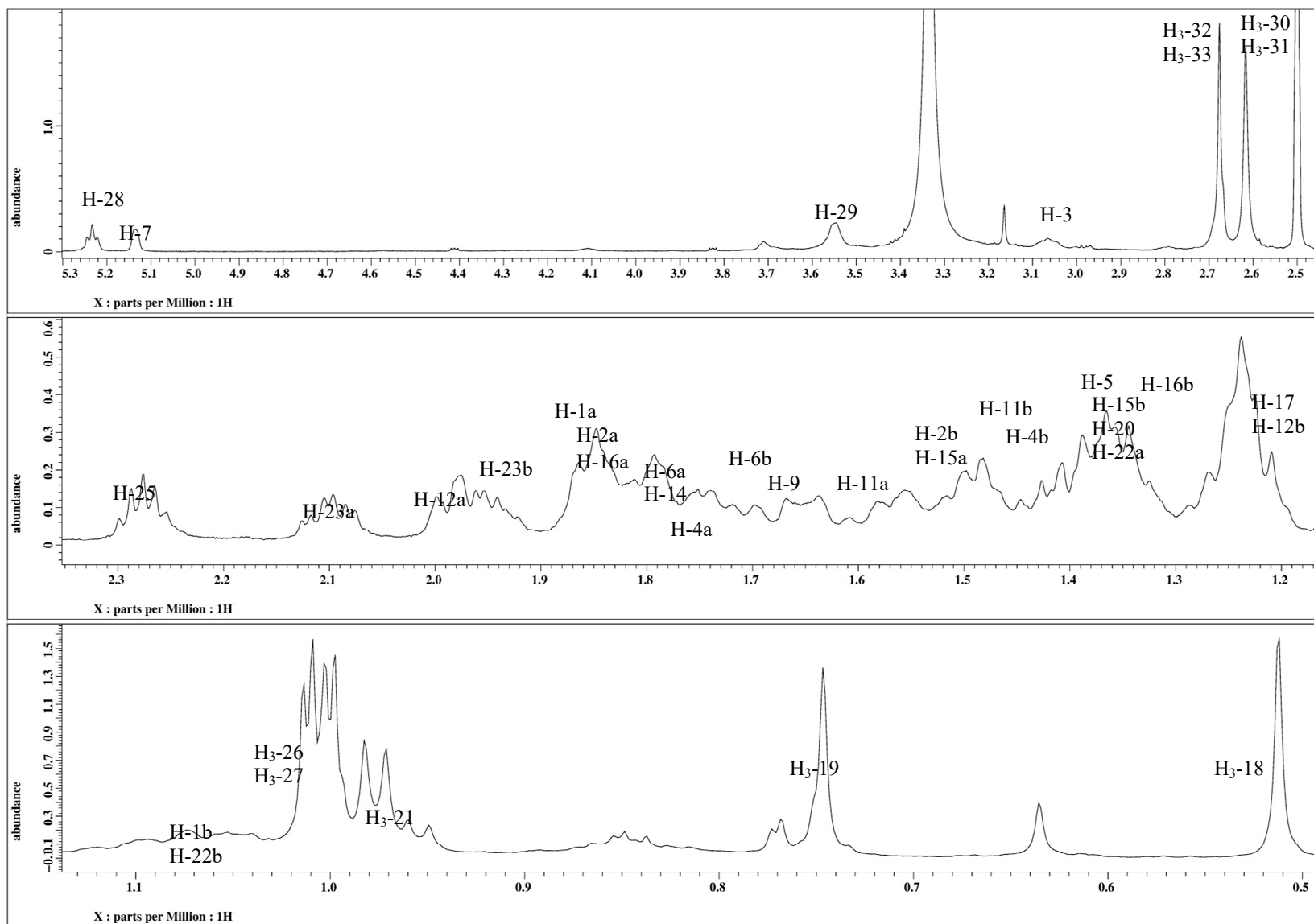
position	δ_C , type	δ_H (J in Hz)	COSY	HMBC ^a	ROESY
1a	38.2, CH ₂	2.01 (ddd, 13.8, 4.1, 4.1) 1.21 (ddd, 13.8, 13.8, 3.4)	1b, 2ab	3, 5, 10	1b, 2b
1b			1a, 2ab	19	1a, 2a, 3, 9
2a	23.7, CH ₂	1.95 (m)	1ab, 2b, 3	1, 3, 4, 10	2b, 32/33
2b		1.62 (m)	1ab, 2a, 3	1, 3, 10	1a, 2a, 32/33
3	66.8, CH	3.21 (m)	2ab, 4ab		1b, 32/33
4a	29.7, CH ₂	1.84 (m)	3, 4b, 5	2, 3, 10	32/33
4b		1.51 (m)	3, 4a, 5	3, 5	
5	41.9, CH	1.51 (m)	4ab, 6ab	3	
6a	30.6, CH ₂	1.86 (m)	5, 7	7, 8	7
6b		1.30 (m)	5, 7	4, 5	
7	118.4, CH	5.20 (br d, 2.8)	6ab	5, 6, 9, 14	14
8	140.7, C				
9	50.5, CH	1.73 (m)	11ab	8, 11	1b, 11a
10	35.5, C				
11a	22.7, CH ₂	1.63 (m)	9, 11b, 12ab	8, 9	9, 12a
11b		1.52 (m)	9, 11a, 12ab	8, 9, 10, 13	11a, 12a, 19
12a	40.9, CH ₂	2.09 (m)	11ab, 12b	9, 11, 13, 14	11ab, 12b, 18, 21
12b		1.28 (m)	11ab, 12a		11ab, 12a
13	44.7, C				
14	56.3, CH	1.87 (m)	15ab	7, 8, 13	7, 12b
15a	24.2, CH ₂	1.57 (m)	14, 15b, 16ab	16	7, 16a, 17
15b		1.46 (m)	14, 15a, 16ab		
16a	29.2, CH ₂	1.93 (m)	15ab, 16b, 17	13, 15	15a, 17
16b		1.33 (m)	15ab, 16a, 17	15, 17, 20	16a
17	57.2, CH	1.29 (m)	16ab, 20	12, 13, 16, 18, 20, 21	12a, 14, 16a
18	12.4, CH ₃	0.59 (s)		12, 13, 14, 17	12a
19	13.4, CH ₃	0.86 (s)		1, 5, 9, 10	11ab
20	38.2, CH	1.45 (m)	17, 21, 22ab		
21	19.4, CH ₃	1.05 (d, 6.9)	20	17, 20	12a, 23ab
22a	36.8, CH ₂	1.46 (m)	20, 22b, 23ab		
22b		1.17 (m) 2.24 (ddd, 12.7, 12.7, 4.8)	20, 22a, 23ab	20, 21	
23a	28.1, CH ₂	4.8)	22ab, 23b	22, 24, 25, 28	21, 22b, 23b, 29
23b		2.07 (m)	22ab, 23a	22, 24, 25, 28	17, 20, 21, 23a, 29ab
24	159.9, C				
25	36.1, CH	2.38 (sep, 6.9)	26, 27	23, 24, 26/27, 28	26/27, 28
26	22.6, CH ₃	1.09 (d, 6.9)	25	24, 25, 27	25, 28
27	22.4, CH ₃	1.10 (d, 6.9)	25	24, 25, 26	25, 28
28	111.7, CH	5.31 (t, 6.9)	29ab	23, 24, 25, 29	25, 26/27, 29, 30/31
29	56.4, CH ₂	3.76 (dd, 7.6, 2.1)	28, 29b	24, 28, 30/31	23b, 26/27, 28, 30/31
30	42.7, CH ₃	2.84 (s)		29, 30/31	28, 29
31	42.7, CH ₃	2.84 (s)		29, 30/31	28, 29
32	40.5, CH ₃	2.85 (s)		3, 32/33	2b, 3
33	40.5, CH ₃	2.85 (s)		3, 32/33	2b, 3

^agHMBC correlations, optimized for 8 Hz, are from proton(s) stated to the carbons listed

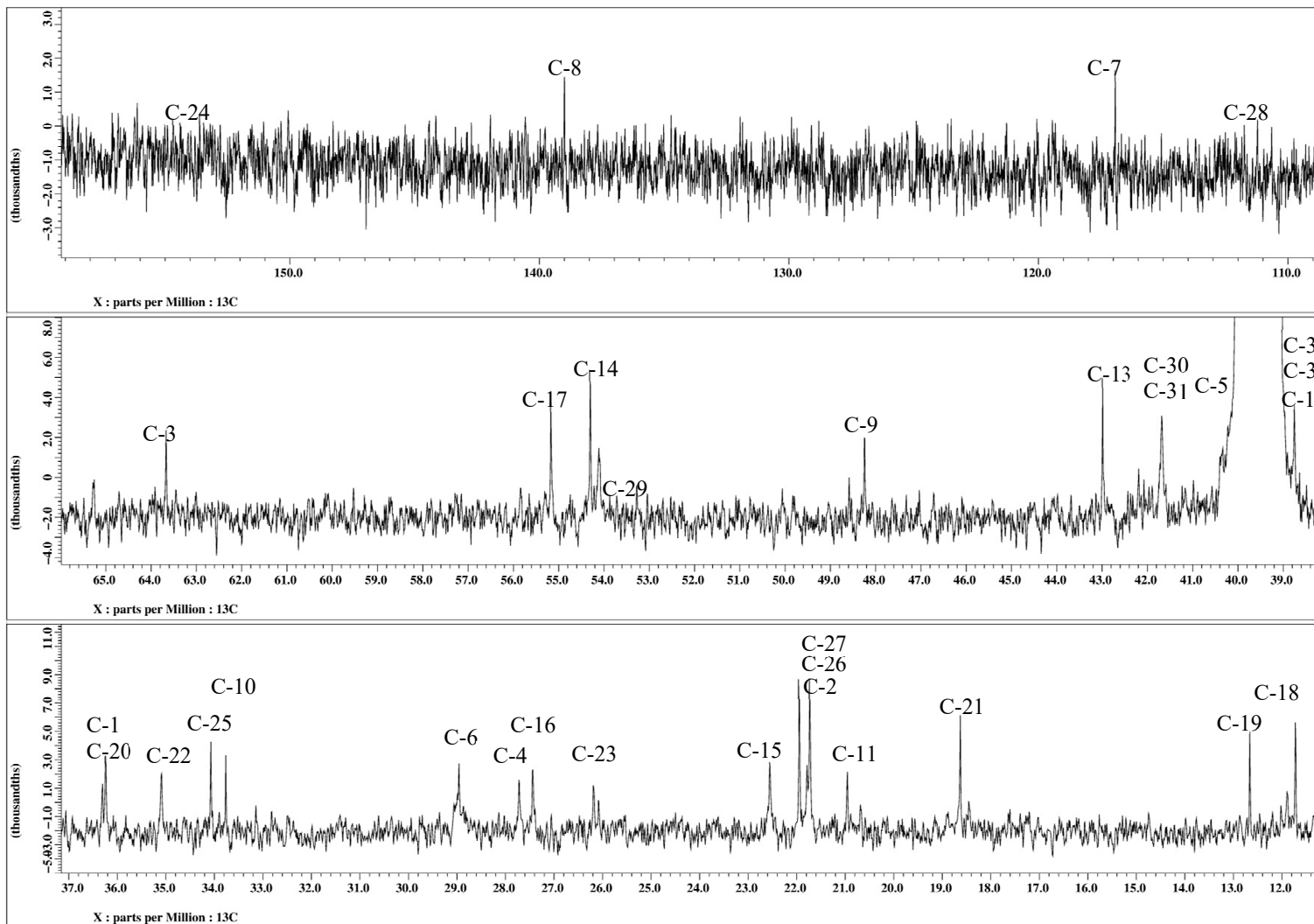
S15 ^1H NMR (600 MHz, $\text{DMSO-}d_6$) of Plakinamine P (**1**)



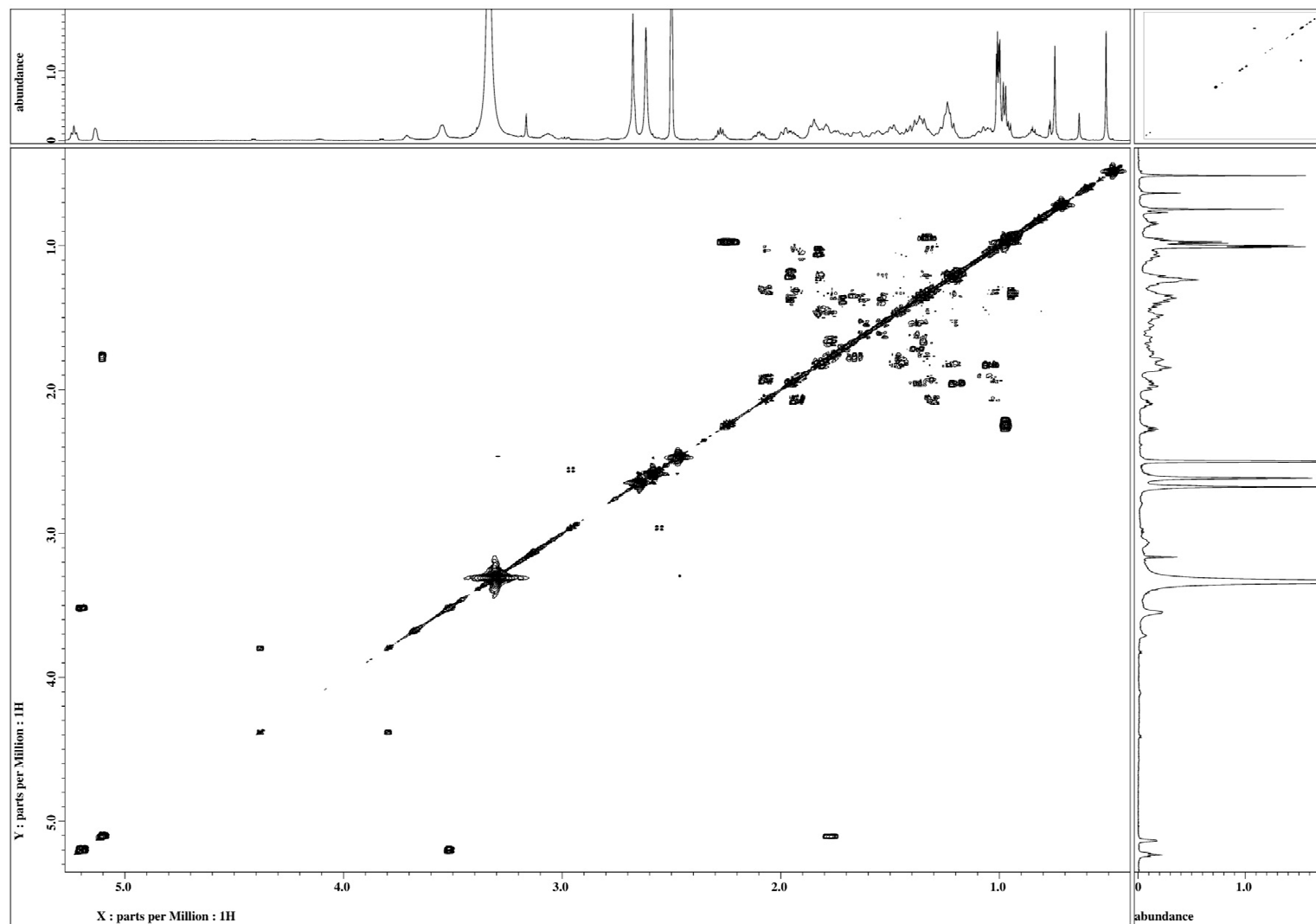
S16 Expansion of ^1H NMR (600 MHz, $\text{DMSO-}d_6$) of Plakinamine P (1)



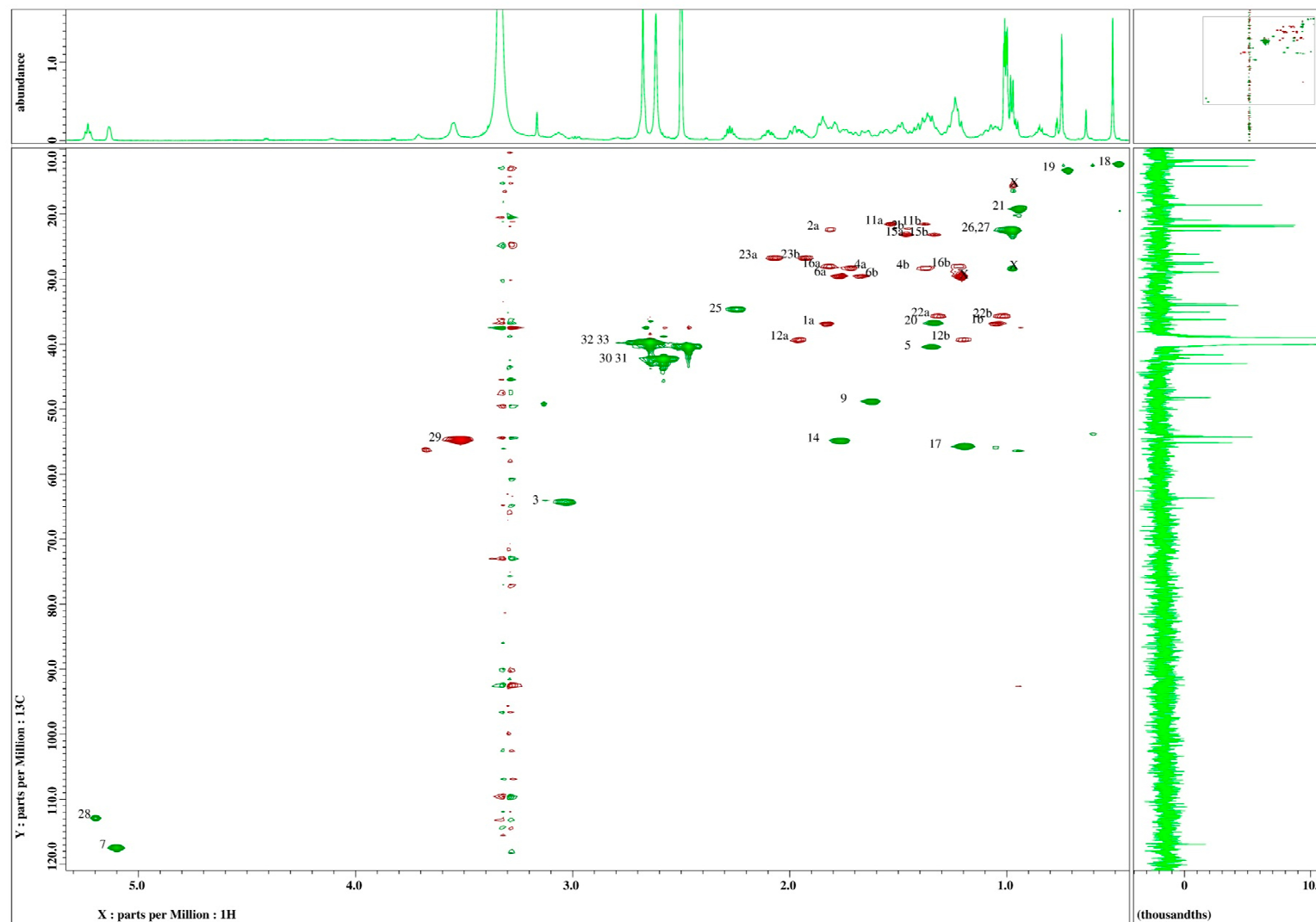
S17 ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) of Plakinamine P (1)



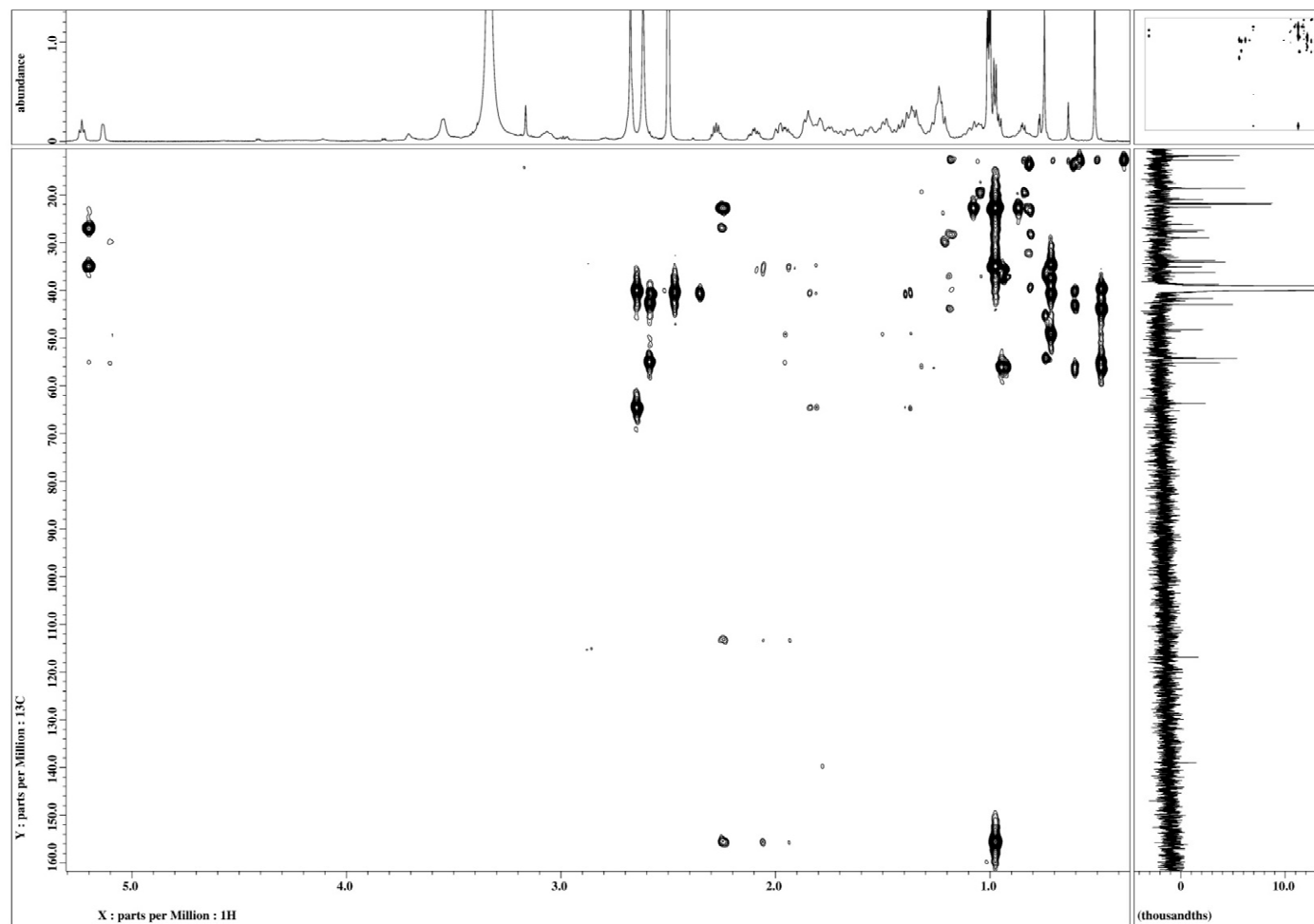
S18 gCOSY spectrum (600 MHz, DMSO-*d*₆) of Plakinamine P (1)



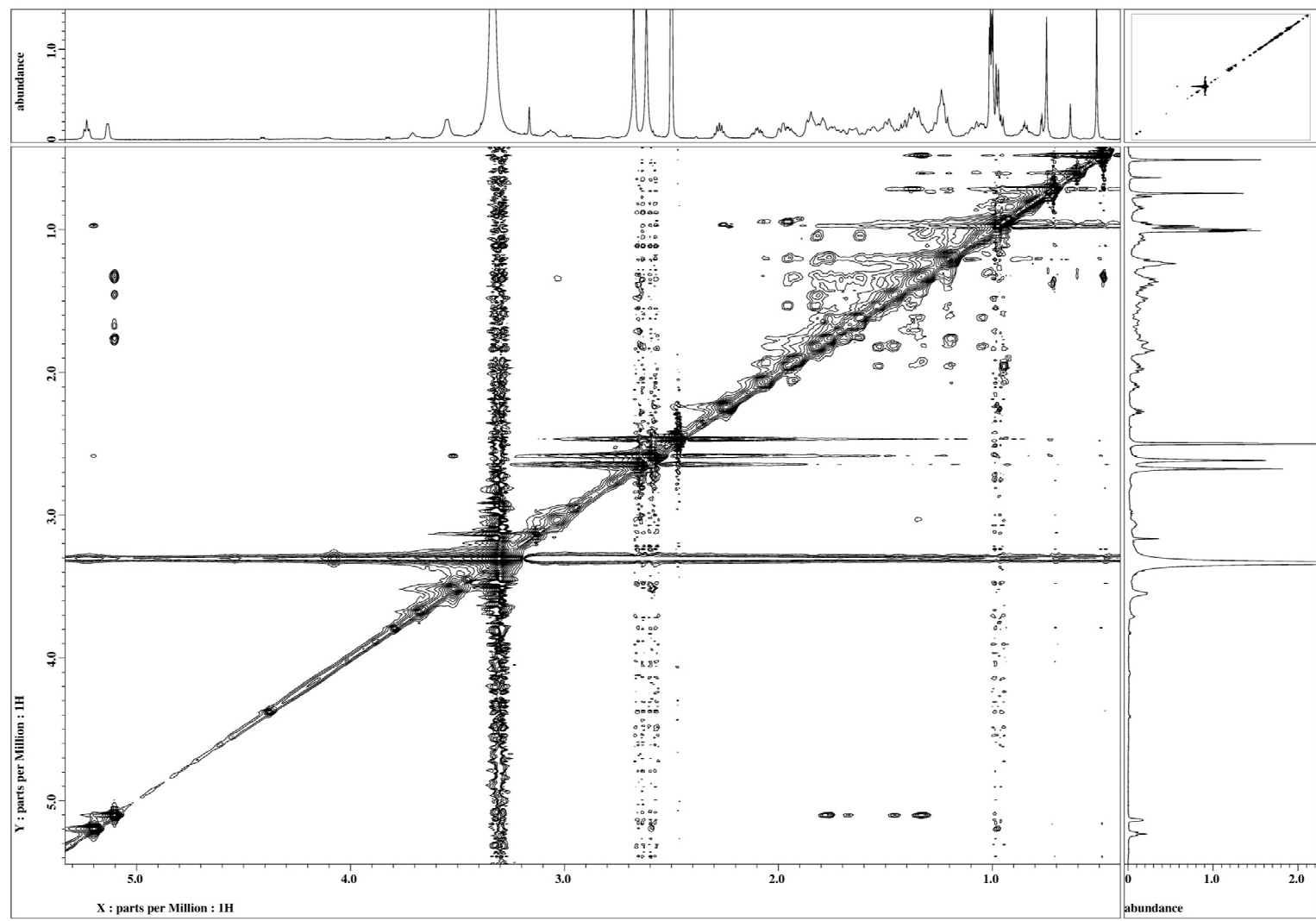
S19 edited gHSQC spectrum (600 MHz, DMSO-*d*₆) of Plakinamine P (1)



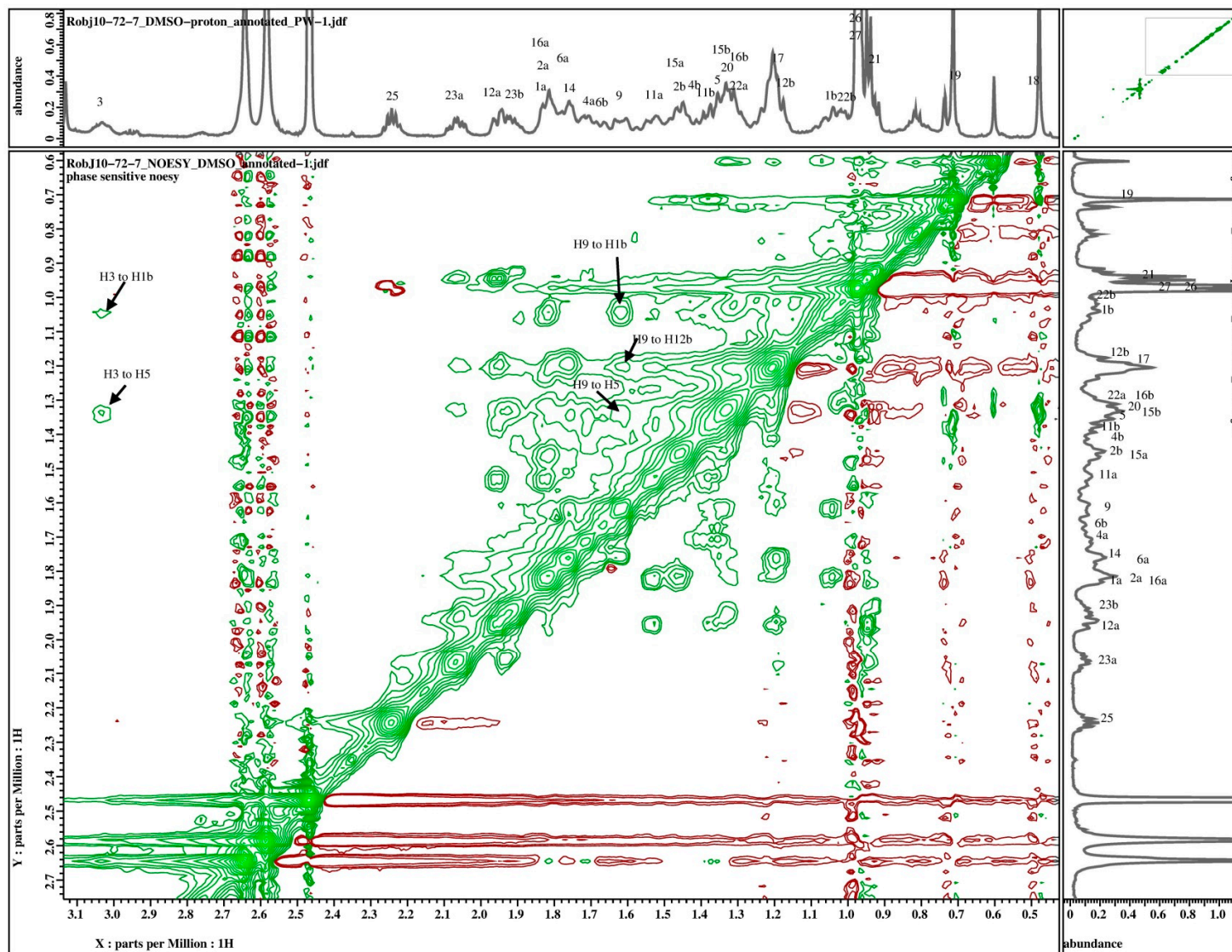
S20 gHMBC spectrum (600 MHz, DMSO-*d*₆) of Plakinamine P (1)



S21 NOESY spectrum (600 MHz, DMSO-*d*₆) of Plakinamine P (1)



S22 Expansion of Plakinamine P (1) 2D-NOESY spectrum (600 MHz, DMSO-*d*₆) showing key correlations for H-3



S23 ROESY spectrum (600 MHz, DMSO-*d*₆) of Plakinamine P (1)

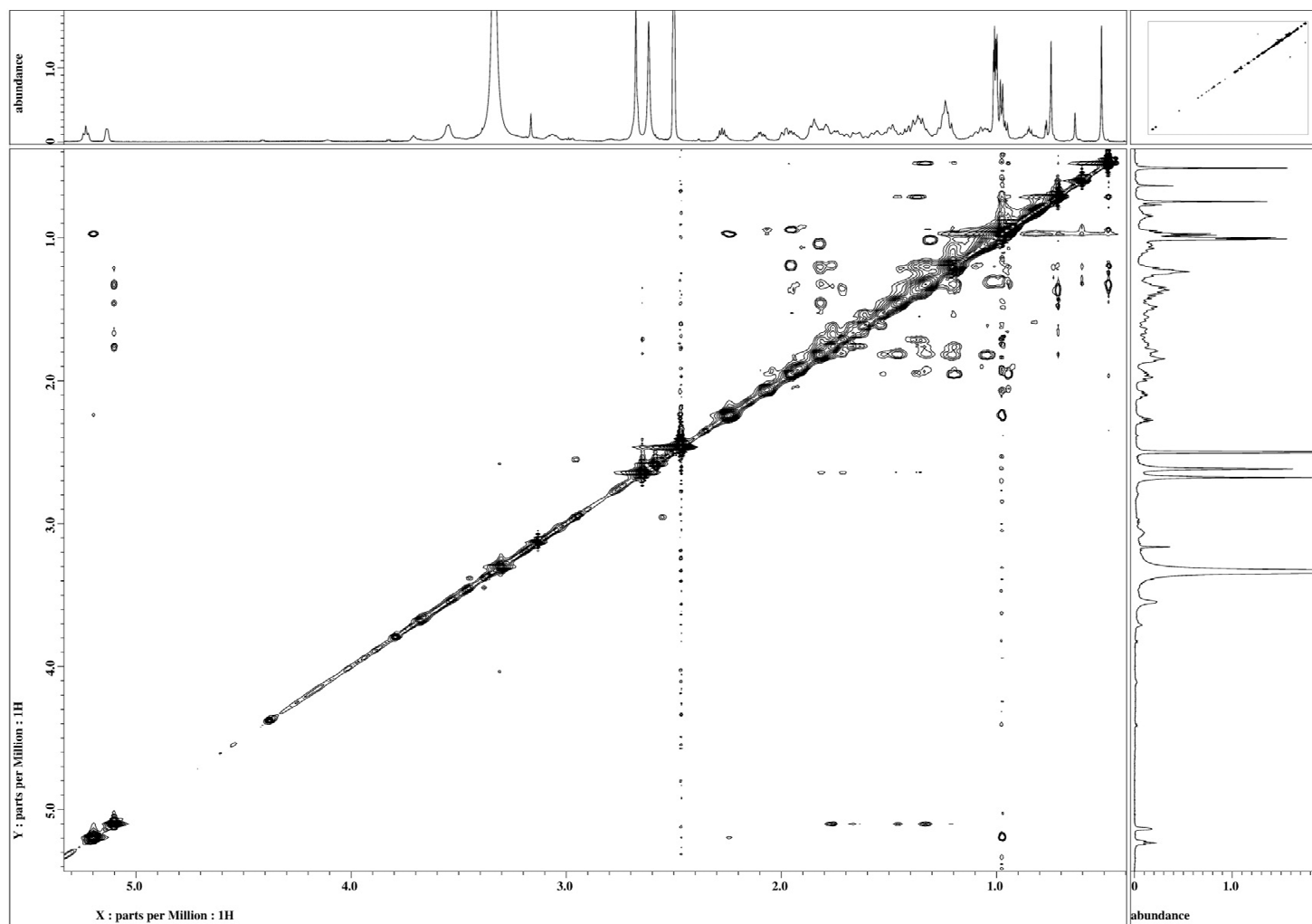


Table S2: Table of ¹H and ¹³C NMR Data for plakinamine P (**1**) (600 MHz, DMSO-*d*₆)

position	δ_c , type	δ_H (J in Hz)	COSY	HMBC ^a	ROESY	NOESY
1a	36.3, CH ₂	1.86, m	1b, 2b	3, 5	1b, 11a, 19	
1b		1.07, m	1a, 2ab		1a, 9	3, 9
2a	21.7, CH ₂	1.83, m	1b		2b, 32/33	
2b		1.48, m	1ab, 2a		1a, 19, 32/33	
3	63.7, CH 27.7,	3.07, br m	2b, 4ab		32/33	1b, 5
4a	CH ₂	1.74, m	3, 4b		4b, 5, 32/33	
4b		1.41, m	3, 4a, 5		4a, 19	
5	40.5, CH 29.0,	1.37, m	4ab, 6b		4a	3, 9
6a	CH ₂	1.79, m	5, 6b, 7		5, 7	7
6b		1.69, m	5, 7		7	7
7	116.9, CH	5.13, br s	6ab	5, 6, 9, 14	6ab, 14, 15ab	6ab, 15ab
8	139.0, C					
9	48.2, CH	1.64, m	7, 11ab		1b, 12b, 14	1b, 5, 12b, 14
10	33.8, C 21.0,					
11a	CH ₂	1.59, m	9, 11b, 12ab	9	1a, 12a	12a
11b		1.42, m	9, 11a, 12ab		12a, 19	
12a	38.7, CH ₂	1.99, m	11ab, 12b		11ab, 12b, 18, 21	11a, 12b, 21
12b		1.23, m	11ab		9, 12a, 14	9, 12a
13	43.0, C					
14	54.3, CH 22.6,	1.79, m	15ab		5, 7, 12b	9
15a	CH ₂	1.49, m	14, 15b, 16ab		7, 16a	7
15b		1.36, m			7, 16ab, 18	7
16a	27.4, CH ₂	1.85, m			17	
16b		1.25, m			16a, 18	
17	55.2, CH 11.7,	1.22, m	18		11b, 12a, 15b, 16b, 17,	
18	CH ₃	0.51, s	17	12, 13, 14, 17	19, 20, 21	
19	12.7, CH ₃	0.75, s	1b	1, 5, 9, 10	1a, 2b, 4b, 11b, 18 ^b	
20	36.2, CH 18.6,	1.36, m			18, 22b	
21	CH ₃	0.98, d (6.9)	20	17, 20	12a, 18, 23a	12a
22a	35.1, CH ₂	1.34, m	20, 22b, 23ab		21, 22b, 25	
22b		1.05, m	22a, 23ab		22a	
23a	26.2, CH ₂	2.10, ddd (13.1, 13.1, 5.5)	22ab, 23b	24, 28	21, 23b, 26/27	21, 23b
23b		1.95, m	22ab, 23a	24, 28	22ab, 26/27	20, 23a
24	155.6, C					
25	34.1, CH 21.9,	2.28, sep. (6.9)	26/27	23, 24, 26/27, 28	22a, 26/27, 28	26/27
26	CH ₃	1.01, d (6.9)	25	24, 25, 27	23ab, 25, 28	

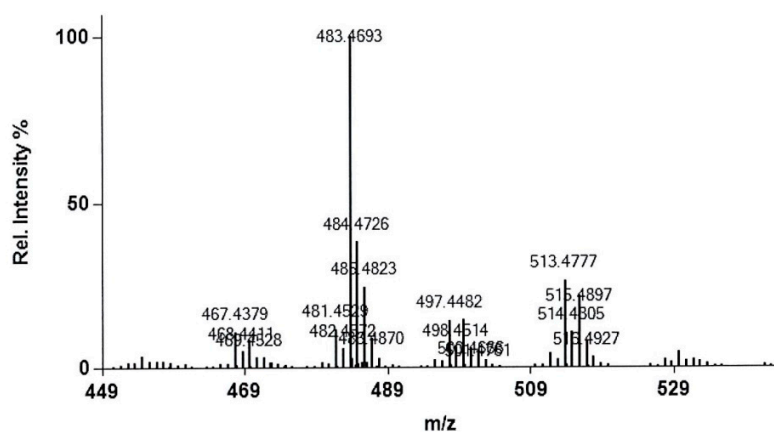
27	21.7, CH ₃	1.02, d (6.9)	25	24, 25, 26	23ab, 25, 28	
28	112.8, CH	5.23, t (7.6)	29a	23, 25, 29	25, 26/27, 29, 30/31	25, 26/27, 30/31
29	54.1, CH ₂	3.55, br s	28			30/31
30	41.7, CH ₃	2.62, s		29, 31		
31	41.7, CH ₃	2.62, s		29, 30		
32	39.8, CH ₃	2.68, s		3, 33	1a, 2b, 4ab, 5	
33	39.8, CH ₃	2.68, s		3, 32	1a, 2b, 4ab, 5	

^agHMBC correlations, optimized for 8 Hz, are from proton(s) stated to the carbons listed

^bThough ¹H chemical shifts of 4b and 11b are very close, the ROESY correlations appear to go to both

S25 High resolution DART mass spectrometry data of plakinamine P (1)

HRMS of plakinamine P was measured on a JEOL AccuTOF-DART 4G using the DART attachment. The voltages for the atmospheric pressure interface are: Orifice 1 = 30 V; Orifice 2 = 5 V; Ring lens = 5 V; Ion guide voltage = 1000 V; Orifice 1 temperature = 100 °C. Mass spectra were stored at a rate of 1 spectrum per 0.4 seconds over the m/z range 100 to 1000 and a resolving power of >10,000 (FWHM). PEG 600 was measured by DART as a reference standard within the same data file as the DART measurements.



Mass Spectrum

Elemental Compositions
Element Limits: C 0/50 H 0/100 O 0/10 N 0/10
Tolerance: 5 mmu Even or odd electron ion or both: Even
Electron correction: None.Charges: 1
Minimum unsaturation: -1Maximum unsaturation: 100

Calc. m/z	Abund %	mmu	DBE	Composition
483.467825	100.000	-1.44	5.5	C33H59N2

S26 Picture and taxonomic description of the *Plakina* sp. used in the study

Plakina sp. Undescribed Caribbean species

Class Homoscleromorpha, Order Homosclerophorida, Family Plakinidae

Sample 25-V-93-3-9. HBOM Catalog Number 0004.001

The external morphology is bulbous to globular (1-2 cm thick, 2-6 cm in diameter), with a single oscule per bulb. Oscules are less than 4 mm wide with a tubular and darkened membrane projection. The surface of the sponge is smooth and the consistency is gelatinous and compressible. The specimen is light brown externally and tan in color internally, both in life and preserved. Spicules are in very low abundance and consist of small ramified calthrops (tetralophose and trilophose), rare smooth non-lophose calthrops, and small diod microrhabs (7-10 μm in length and $<1 \mu\text{m}$ in width). Calthrops are regular in size, less than 20 μm in total size, with rays 8-10 μm x 2-3 μm . Trilophose calthrops usually have deformed rays with variation in the pattern of ramification between rays. The lophose calthrops are typical of the genus *Plakina*. There are five *Plakina* species currently recognized for the Caribbean: *Plakina elisa*, *Plakina nathaliae*, *Plakina tetralopha*, *Plakina jamaicensis* and *Plakina arletensis*. The present *Plakina* specimen differs from those species in general morphology for which it has a much thicker growth, bulbous shape, and unique oscule morphology. The spicule combination, the tendency to deformed rays and diverse branching pattern within a spicule, and their low density in the body, are unique characteristics of this sample that indicate that it may be an undescribed *Plakina* species. Further study of the histology and genetics of this specimen will allow its distinction from the other Caribbean species.



Figure S26A. Under water photograph of *Plakina* sp. collected from a cavern at 93 m deep, Crooked Island, Bahamas.

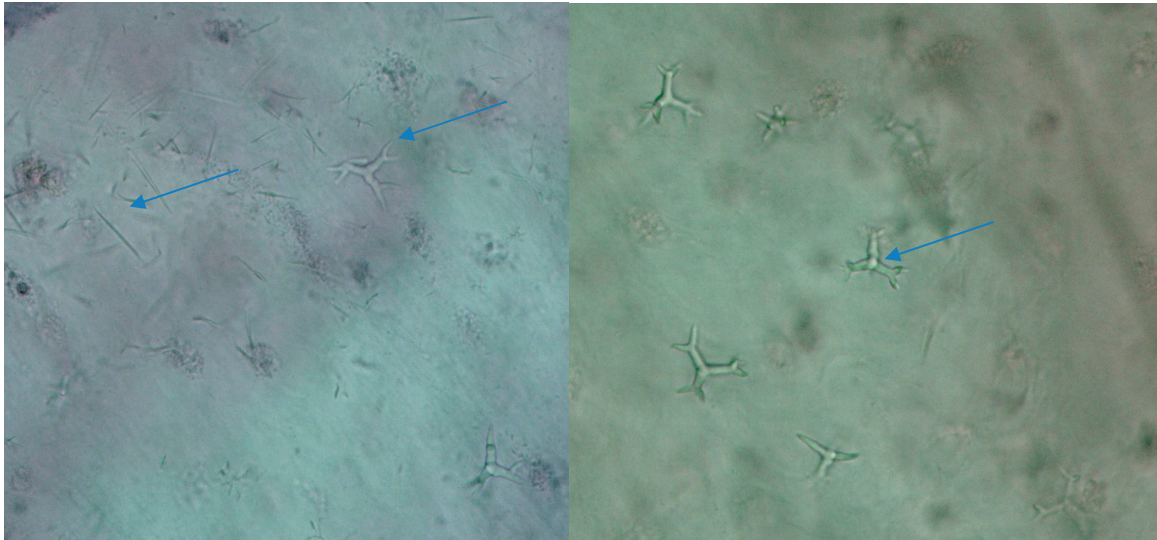


Figure S26B. Left image : trilophose calthrops with deformed rays, and diactineal microrhabds.

Right image tetralophose calthrops.

Ruiz, C., et al., *Descriptions of new sponge species and genus, including aspiculate Plakinidae, overturn the Homoscleromorpha classification*. *Zoological Journal of the Linnean Society*, 2017. **179**(4): p. 707-724