Biologically active echinulin-related indolediketopiperazines from marine sediment-derived fungus *Aspergillus niveoglaucus*

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Table S1. Cytotoxic activities of isolated compounds 1-7

Compounds	Cytotoxicity IC_{50}, μM			
	Neuro-2a	22Rv1	PC-3	LNCaP
1a	> 100	-	-	-
1b	> 100	-	-	-
2	> 100	-	-	-
3	> 100	63.2	41.7	25.9
4	50.9	-	-	-
5	40.6	-	-	-
6	> 100	-	-	-
7	> 100	49.9	63.8	38.9
Docetaxel (reference drug)	n/t	0.013	0.015	0.004

Urease inhibition activity

Compounds **3–7** were tested in a cell-free urease activity assay. In this assay echinulin (**3**) showed activity with an IC_{50} of 29.8 μM, while the other compounds were inactive up to concentration of 100 μM. Thiourea used as positive control inhibited urease activity with an IC_{50} of 23 μM.

Urease (urea amidohydrolase EC 3.5.1.5) is an enzyme responsible for the hydrolysis of urea into ammonia and CO2 or carbamate [1]. Activity of this enzyme has been implicated in the pathogenesis of several human diseases [2]. The most known examples of urease producing pathogenic bacteria are Helicobacter pylori and Mycobacterium tuberculosis [3]. Thus, urease are among of the promising pharmacological targets in the search for new antibiotics [2,4]. Moderate urease inhibition by echinulin (3) observed by us was in line with the previous report by Du et al., which describes an antibacterial activity of echinulin (3) against Staphylococcus aureus [5]. Thus, urease inhibition could be a possible mechanism which stipulates echinulin antimicrobial properties.

Urease Inhibition Assay

The reaction mixture consisting of 25 μl enzyme solution (urease from *Canavalia ensiformis*, Sigma, 1U final concentration) and 5 μl of test compounds dissolved in water (0.2 - 100.0 μM final concentration) was preincubated at 37 °C for 60 min in 96-well plates. Then 55 μl of phosphate buffer solution with 100 mM urea was added to each well and incubated at 37 °C for 10 min. The urease inhibitory activity was estimated by determining of ammonia production using indophenol method [6]. Briefly, 45 μL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The absorbance was measured after 50 min at 630 nm using a microplate reader Multiscan FC (Thermo Scientific, Canada). All the reactions were performed in triplicate in a final volume of 200 μL. The pH was maintained 7.3-7.5 in all assays. DMSO 5% was used as a positive control.

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Table S2. 1D and 2D NMR spectroscopic data of cryptoechinuline B (1).

№	δC, type	δH (*J* in Hz)	HMBC
NH (1)		10.87, s	2, 3, 3a, 7a
2	143.8, C		
3	103.3, C		
3a	124.2, C		
4	117.1, CH	7.03, d (7.8)	3a, 6, 7a
5	120.3, CH	6.80, dd (8.2, 1.5)	3a, 7, 20
6	134.0, C		
7	110.6, CH	7.18, brs	3a, 5, 20
7a	135.4, C		
8	111.1, CH	6.94, s	2, 3a, 9, 10
9	124.1, C		
10	161.3, C		
NH (11)		7.67, s	9, 10, 13
12	59.1, C		
13	167.6, C		
NH (14)		8.73, s	10, 12
15	38.9, C		
16	145.1, CH	6.05, dd (17.5, 10.5)	2, 15,18,19
17	111.5, CH_2	5.02, dd (10.9, 1.2) 4.99, dd (17.9, 1.2)	15, 16 15, 16
18	27.5, CH_3	1.45, s	15, 16, 19
19	27.5, CH_3	1.43, s	15, 16, 18
20	33.8, CH_2	3.36, d (7.5) 3.36, d (7.5)	5,6, 7, 21, 22 5,6, 7, 21, 22
21	124.1. CH	5.32, brt (7.6)	20, 23, 24
22	130.9, C		
23	17.5, CH_3	1.71, s	21, 22, 24
24	25.4, CH_3	1.71, s	21, 22, 23
25	37.6, CH_2	a: 2.1, dd (13.5, 7.1) b: 1.69, dd (13.5, 7.2)	12, 26, 27, 29 12, 13, 26, 29, 44
26	28.3, CH	2.68, m	
27	132.7, CH	5.74, dt (10.2, 2.7)	26, 28, 29
28	124.7, CH	5.63, dt (10.9, 3.4)	12, 26, 27, 29
29	45.5, CH	3.56, m	28
30	137.0, CH	5.85, dd (15.8, 8.3)	27, 28, 29, 31
31	124.4, CH	6.71, d (16.1)	29, 37, 32
32	117.2. C		
33	128.5, C		
34	153.0, C		
35	124.3, C		
36	125.0, CH	6.99, s	34, 35, 37, 38
37	146.9, C		
38	26.7, CH_2	3.21, d (7.5) 3.21, d (7.5)	33, 34, 36, 39, 40 33, 34, 36, 39, 40
39	121.4, CH	5.23, brt (7.5)	41, 42
40	132.6, C		
41	17.5, CH_3	1.64, s	39, 40, 42
42	25.5, CH_3	1.70, s	39, 40, 41
43	197.3, C	10.08, s	32, 33, 34

44	21.1, CH_3	1.10, d (7.5)	25, 26, 27
34-OH		11.73, s	32, 33, 34
37-OH		9.18, s	35, 37

Figure S1. ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cryptoechinulin B (1)

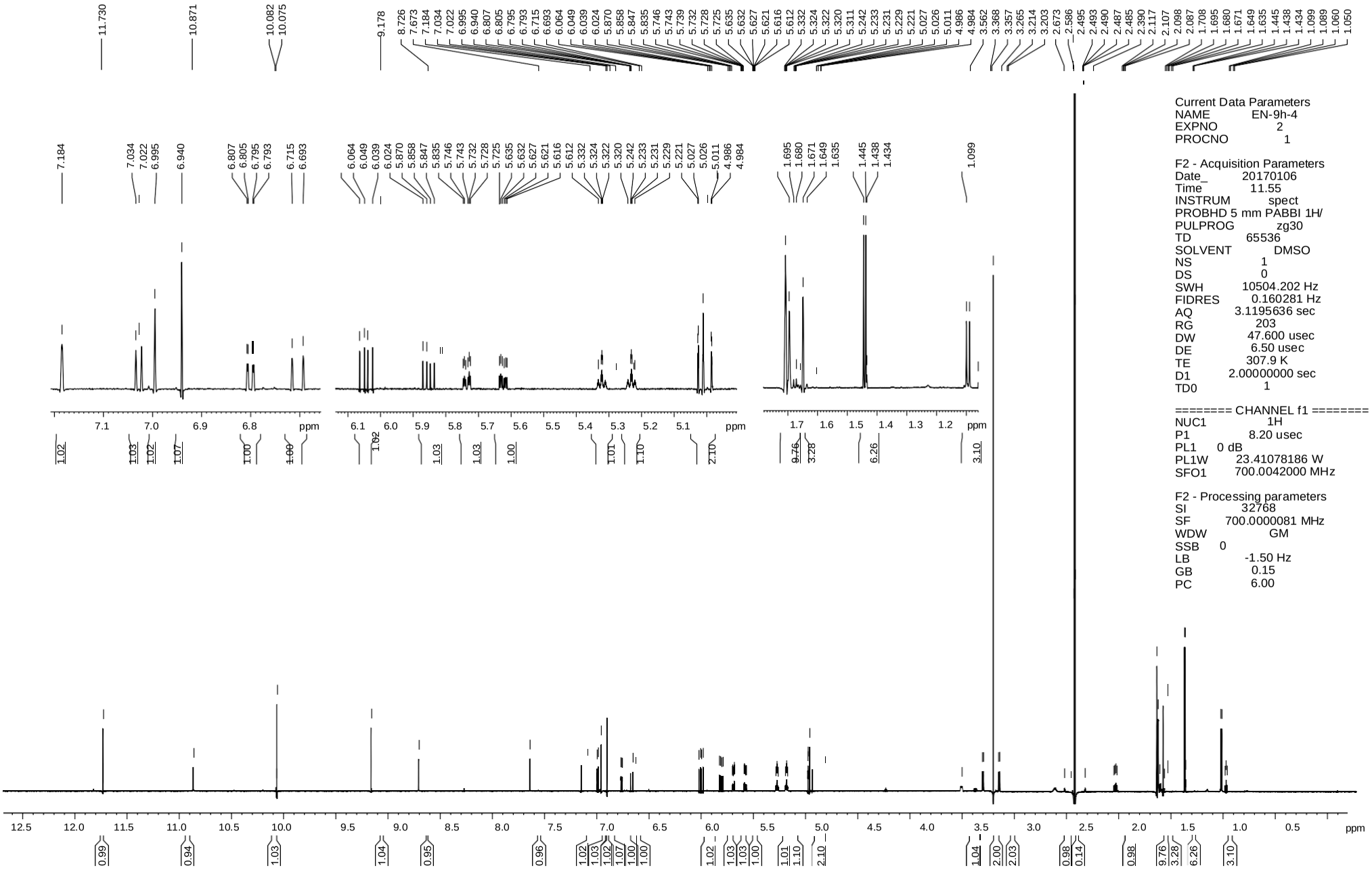


Figure S2. ^{13}C NMR spectrum (176 MHz, DMSO-d$_6$) of cryptoechinulin B (1)

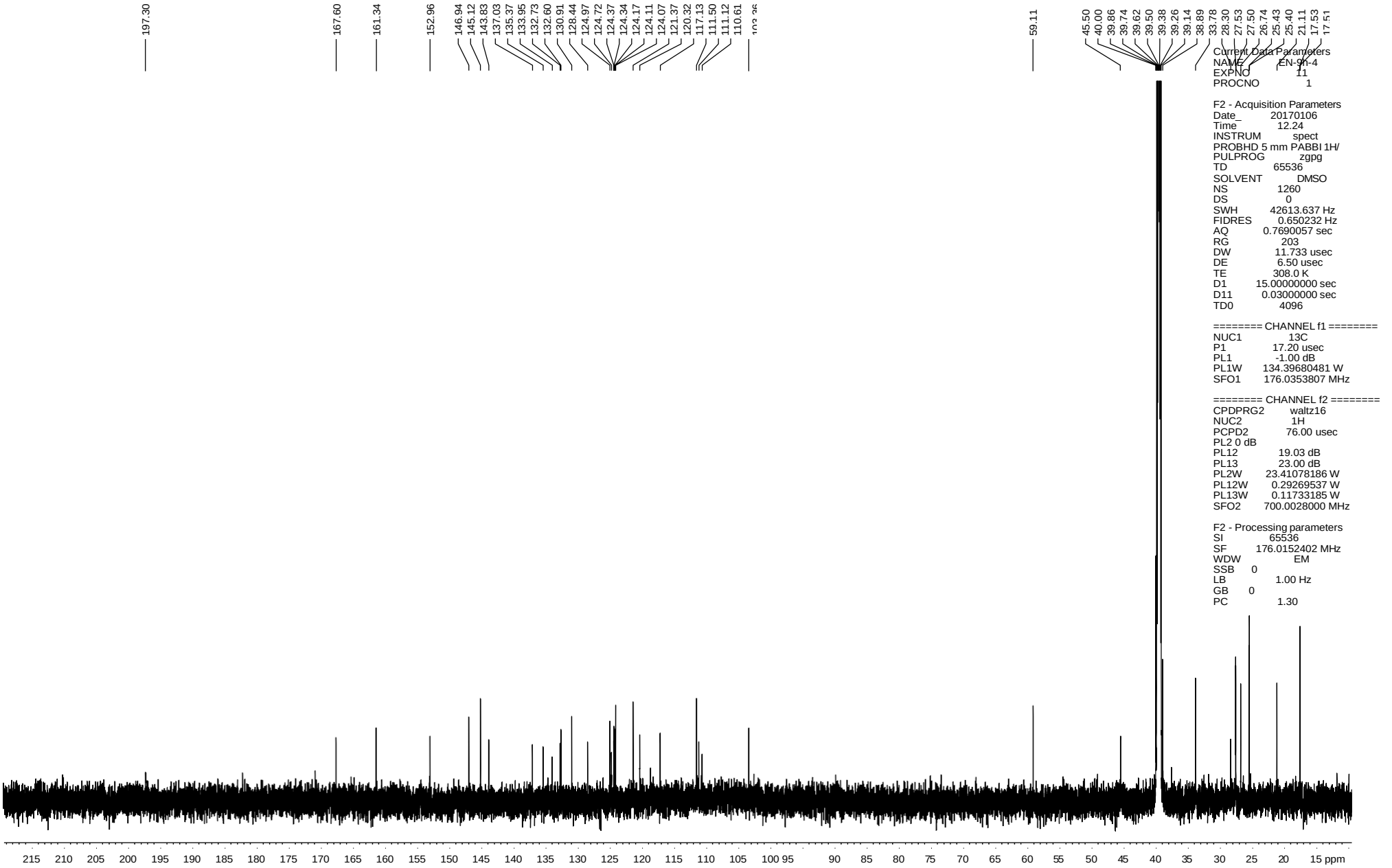


Figure S3. DEPT-135 spectrum (176 MHz, DMSO-d_6) of cryptoechinulin B (1)

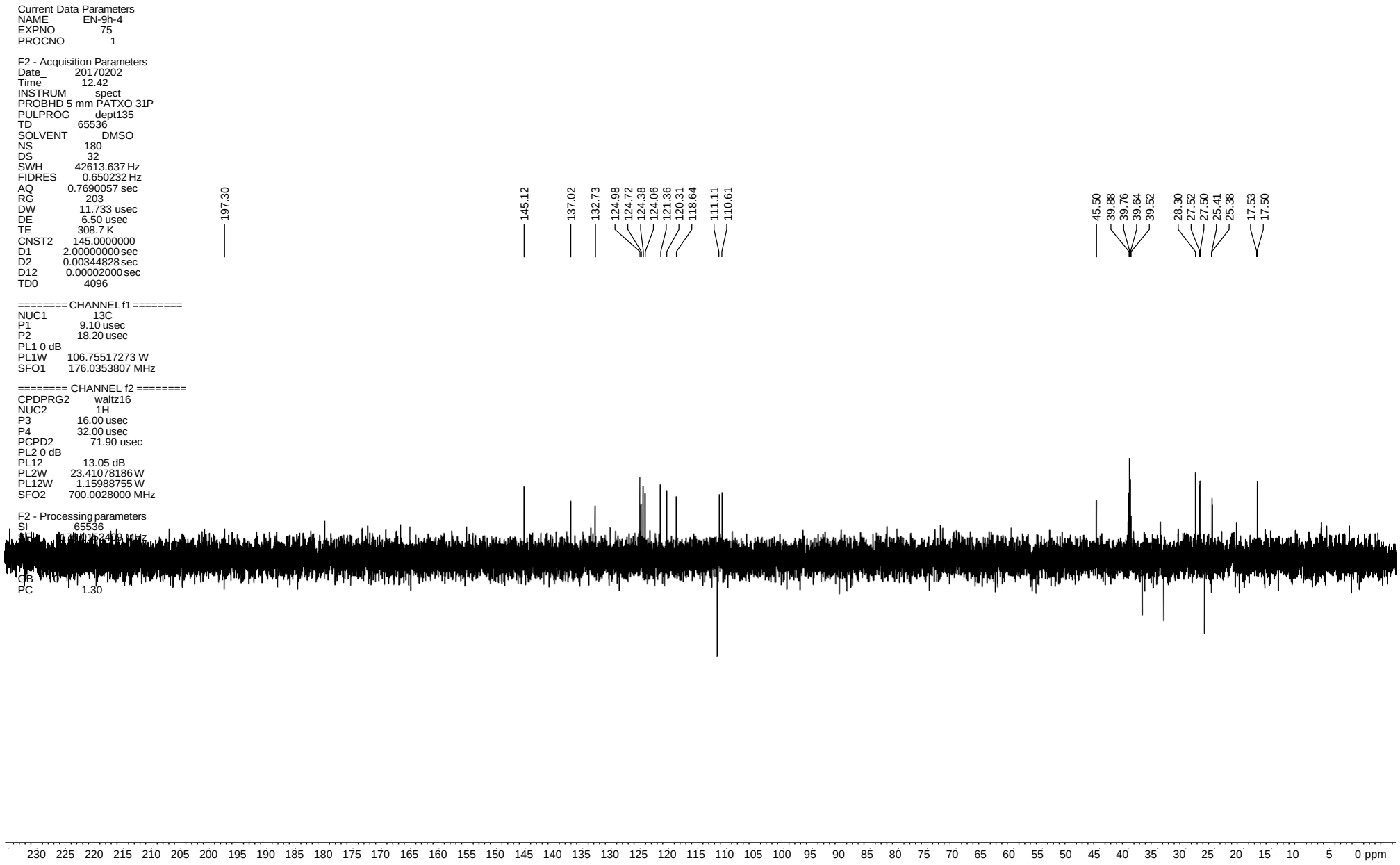
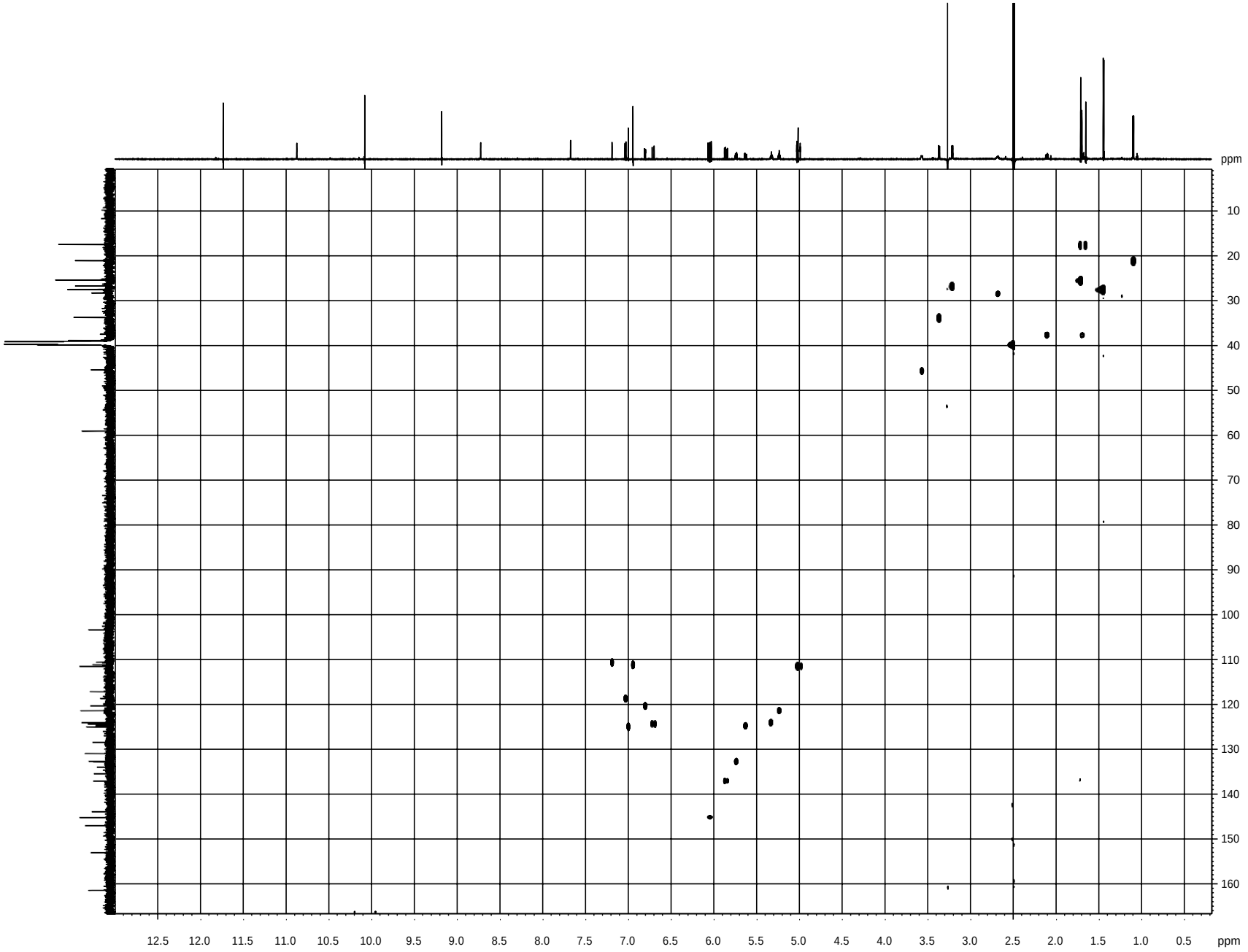


Figure S4. HSQC spectrum (700 MHz, DMSO-d_6) of cryptoechinulin B (1)



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PROCNO 1

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PULPROG hsqcph
TD 1024
SOLVENT DMSO
NS 16
DS 16
SWH 8971.292 Hz
FIDRES 8.761027 Hz
AQ 0.0571209 sec
RG 203
DW 55.733 usec
DE 6.50 usec
TE 308.1 K
CNST2 145.0000000
D0 0.00000300 sec
D1 2.00000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
IN0 0.00001710 sec
L31 1

======== CHANNEL f1 ========
NUC1 1H
P1 14.40 usec
P2 28.80 usec
PL1 0 dB
PL1W 23.41078186 W
SFO1 700.0046159 MHz

======== CHANNEL f2 ========
CPDPRG2 bi_p5m4sp_4sp.2
NUC2 13C
P3 9.20 usec
P4 18.40 usec
P63 1500.00 usec
PL2 0 dB
PL12 16.00 dB
PL2W 106.75517273W
PL12W 2.68156862 W
SFO2 176.0299251 MHz
SP14 10.36 dB
SP31 16.38 dB
SPNAM14 Crp32,1.5,20.2
SPNAM31 Crp32,1.5,20.2
SPOAL14 0.500
SPOAL31 0.500
SPOFFS14 0 Hz
SPOFFS31 0 Hz

F1 - Acquisition parameters
TD 256
SFO1 176.0299 MHz
FIDRES 114.144402 Hz
SW 166.000 ppm
FnMODE TPPI

F2 - Processing parameters
SI 2048
SF 700.0000047 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 4.00

F1 - Processing parameters
SI 512
MC2 TPPI
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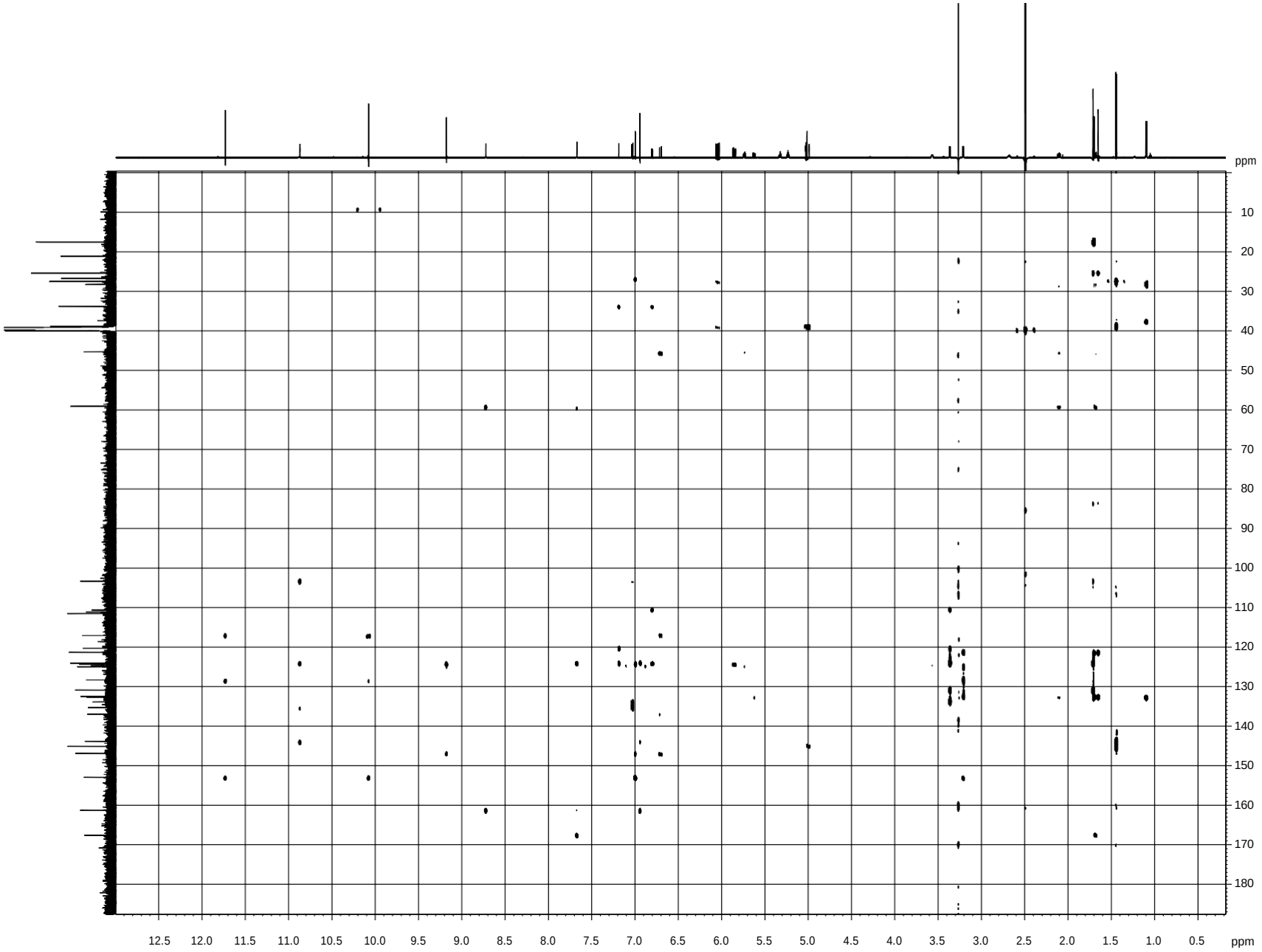
WDW
SSB 2
LB 0 Hz
GB 0

Figure S5. HMBC spectrum (700 MHz, DMSO-d_6) of cryptoechinulin B (1)



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PROCNO 1

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PULPROG hmbclpndqf
TD 4096
SOLVENT DMSO
NS 80
DS 16
SWH 8971.292 Hz
FIDRES 2.190257 Hz
AQ 0.2283337 sec
RG 203
DW 55.733 usec
DE 6.50 usec
TE 307.9 K
CNST2 145.0000000
CNST13 8.0000000
D0 0.00000300 sec
D1 2.00000000 sec
D2 0.00344828 sec
D6 0.06250000 sec
IN0 0.00001510 sec

======== CHANNEL f1 ========
NUC1 1H
P1 14.40 usec
P2 28.80 usec
PL1 0 dB
PL1W 23.41078186 W
SFO1 700.0046159 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 9.20 usec
PL2 0 dB
PL2W 106.75517273 W
SFO2 176.0316775 MHz

F1 - Acquisition parameters
TD 199
SFO1 176.0317 MHz
FIDRES 166.301285 Hz
SW 188.000 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 700.0000058 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 4.00

F1 - Processing parameters
SI 1024
MC2 QF
SF 176.0152016 MHz
WDW GM
SSB 0
LB 0 Hz
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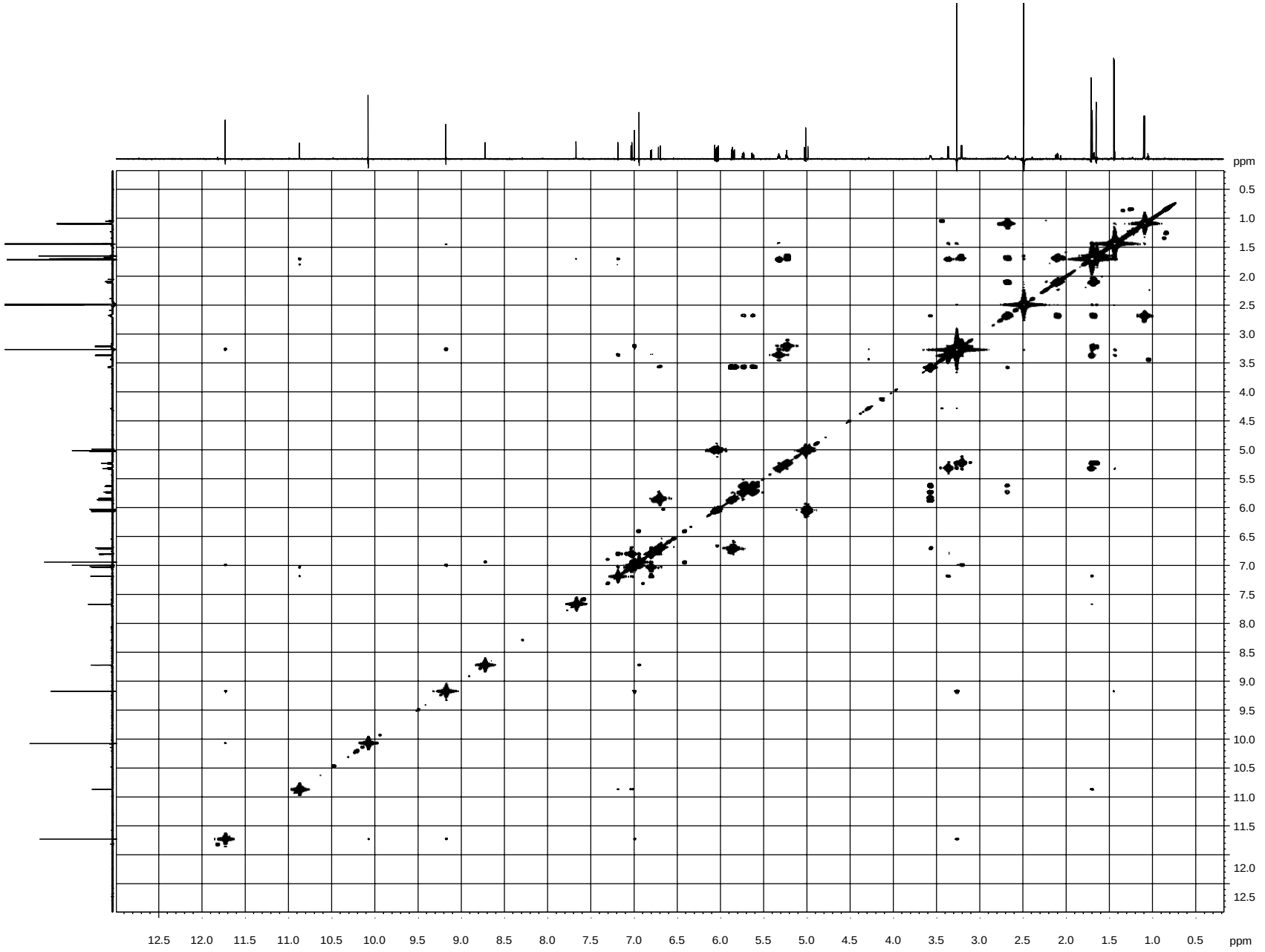
Figure S6. ^{1}H-^{1}H COSY spectrum (700 MHz, DMSO-d_6) of cryptoechinulin B (1)



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PROCNO 1

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PULPROG cosyqf45
TD 2048
SOLVENT DMSO
NS 8
DS 16
SWH 8971.292 Hz
FIDRES 4.380514 Hz
AQ 0.1141919 sec
RG 203
DW 55.733 usec
DE 6.50 usec
TE 308.0 K
D0 0.00000300 sec
D1 2.00000000 sec
IN0 0.00011145 sec

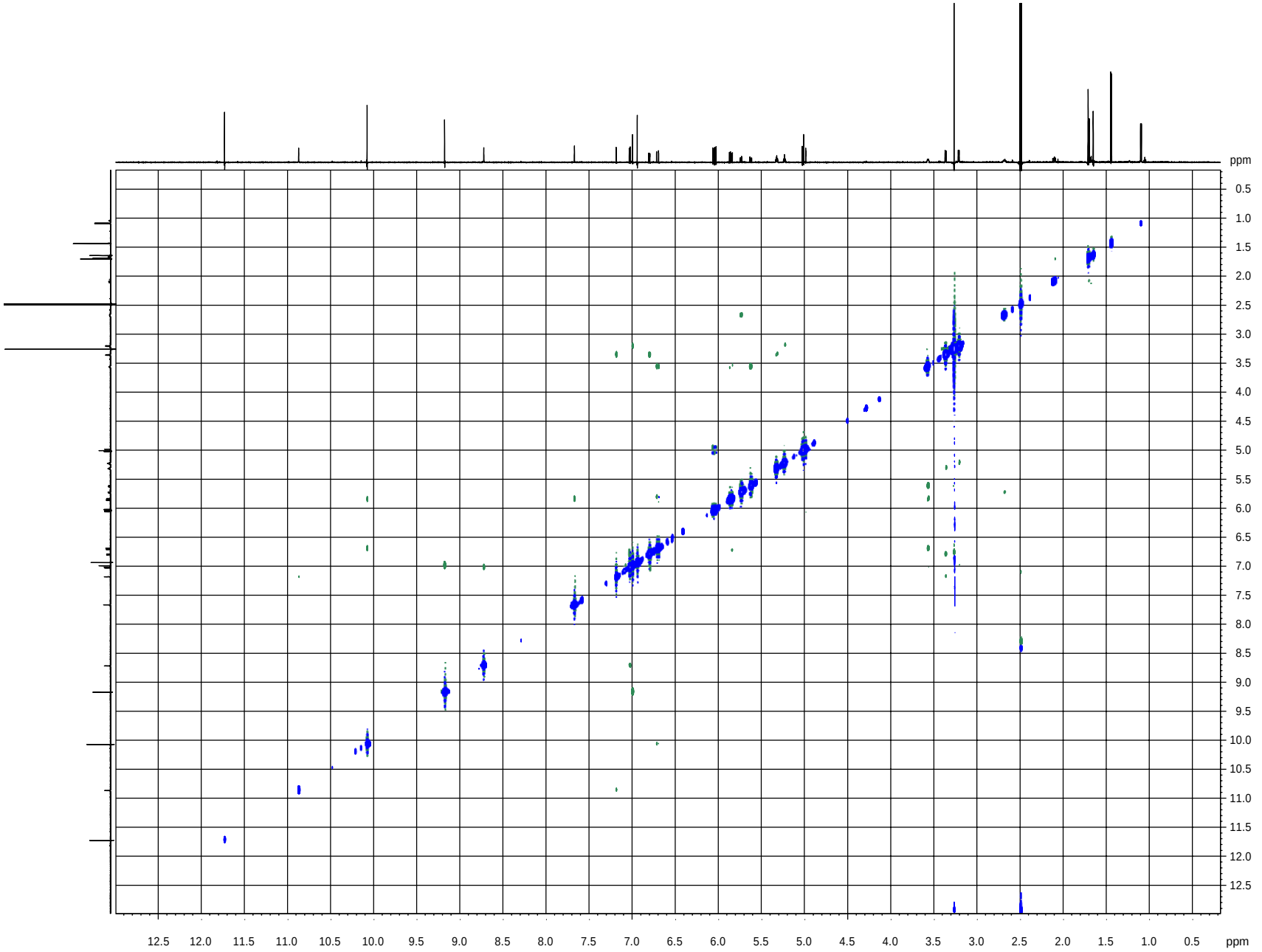
======== CHANNEL f1 ========
NUC1 1H
P1 14.40 usec
PL1 0 dB
PL1W 23.41078186 W
SFO1 700.0046159 MHz

F1 - Acquisition parameters
TD 512
SFO1 700.0046 MHz
FIDRES 17.522055 Hz
SW 12.816 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 700.0000081 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 4.00

F1 - Processing parameters
SI 2048
MC2 QF
SF 700.0000081 MHz
WDW GM
SSB 0
LB 0 Hz
GB 0

Figure S7. ROESY spectrum (700 MHz, DMSO-d_6) of cryptoechinulin B (1)



Current Data Parameters
NAME EN-9h-4
EXPNO 23
PROCNO 1

F2 - Acquisition Parameters
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Time 18.00
INSTRUM spect
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PULPROG roesyph.2
TD 2048
SOLVENT DMSO
NS 32
DS 16
SWH 8971.292 Hz
FIDRES 4.380514 Hz
AQ 0.1141919 sec
RG 1
DW 55.733 usec
DE 6.50 usec
TE 307.9 K
D0 0.00004257 sec
D1 2.00000000 sec
D12 0.00002000 sec
IN0 0.00011145 sec
L4 613
P15 270000.00 usec

======== CHANNEL f1 ========
NUC1 1H
P1 14.40 usec
P25 220.00 usec
PL1 0 dB
PL27 17.66 dB
PL1W 23.41078186 W
PL27W 0.40125081 W
SFO1 700.0046159 MHz

F1 - Acquisition parameters
TD 256
SFO1 700.0046 MHz
FIDRES 35.044109 Hz
SW 12.816 ppm
FnMODE TPPI

F2 - Processing parameters
SI 2048
SF 700.0000081 MHz
WDW SINE
SSB 2
LB 0 Hz
GB 0
PC 4.00

F1 - Processing parameters
SI 512
MC2 TPPI
SF 700.0000081 MHz
WDW
SSB 2
LB 0 Hz
GB 0

Figure S8. CD spectrum of (+)-cryptoechinulin B (1a) and (–)-cryptoechinulin B (1b)