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## Supplemental Information

### Isolation, Structure Determination, and Synthesis of Cyclic Tetraglutamic Acids from the Box Jellyfish Species *Alatina alata* and *Chironex yamaguchii*

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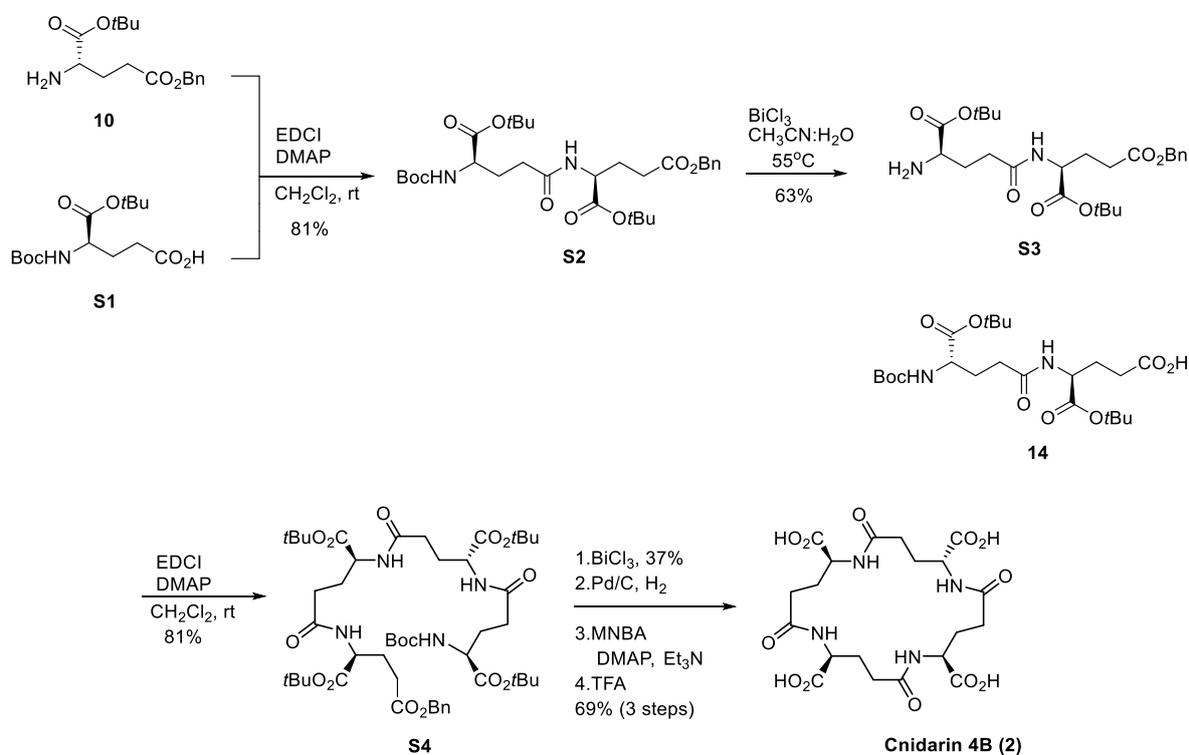
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39 **Section S1. Synthesis of DLLL cyclic tetraglutamic acid cnidarin 4B (2)**

40

41 **Scheme S1. Preparation of cnidarin 4B (2).**

42

43

44 *5-Benzyl 1-(tert-butyl) ((R)-5-(tert-butoxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoyl)-L-glutamate*  
 45 **(S2)**

46 To a solution of **S1** (1.03 g, 3.4 mmol) in dichloromethane (15 mL), DMAP (200 mg, 1.7 mmol),  
 47 EDCI (1.3 g, 6.8 mmol) and **10** (1 g, 3.4 mmol) were added, and the reaction mixture was stirred at  
 48 room temperature for 2 hr. H<sub>2</sub>O was added, and the organic layer was extracted with  
 49 dichloromethane. The extract was dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue  
 50 was purified by column chromatography to give **S2** (1.6 g, 2.76 mmol) in 81% yield.

51

52 *5-Benzyl 1-(tert-butyl) ((R)-4-amino-5-(tert-butoxy)-5-oxopentanoyl)-L-glutamate* **(S3)**

53 To a solution of **S2** (0.9 g, 1.55 mmol) in CH<sub>3</sub>CN-H<sub>2</sub>O (50:1), BiCl<sub>3</sub> was added portionwise (974.4  
 54 mg, 3.1 mmol) to selectively deprotect the Boc group. NaHCO<sub>3</sub> was added, and the reaction mixture  
 55 was filtered through a Celite pad to give **S3** (0.83 g, 1.73 mmol) in 63% yield.

56

57 *23-Benzyl 11,16,21,6-tetra-tert-butyl (6R,11S,16R,21R)-2,2-dimethyl-4,9,14,19-tetraoxo-3-oxa-5,10,15,20-*  
 58 *tetraazatricosane-6,11,16,21,23-pentacarboxylate* **(S4)**

59 To a solution of amine **14** (732 mg, 1.53 mmol) in dichloromethane, EDCI (585 mg, 3.06 mmol),  
 60 DMAP (93 mg, 0.765 mmol) and **S3** (749 mg, 1.53 mmol) were added, and the mixture was stirred at  
 61 room temperature for 2 hr. H<sub>2</sub>O was added to the reaction mixture, and the organic layer was  
 62 extracted with dichloromethane. The extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated *in*  
 63 *vacuo*. The residue was purified by column chromatography to give **S4** (1.18 g, 1.24 mmol) in 81%  
 64 yield.

65

66 2.1,5.4-Anhydro( $\gamma$ -L-glutamyl- $\gamma$ -L-glutamyl- $\gamma$ -D-glutamyl-L-glutamic acid) (cnidarin 4B, 2)

67 To a solution of **S4** (1.18 g, 1.24 mmol) in CH<sub>3</sub>CN-H<sub>2</sub>O (50:1), BiCl<sub>3</sub> was added portionwise (782  
68 mg, 2.48 mmol) to selectively deprotect the Boc group. NaHCO<sub>3</sub> was added and the reaction mixture  
69 was filtered through a Celite pad, and filtrates were concentrated *in vacuo*. The reaction product was  
70 further purified by column chromatography to give the corresponding linear LLDL-tetraglutamate  
71 (390 mg, 0.459 mmol) in 37% yield:  $[\alpha]_D^{25} +2.9^\circ$  (*c* 0.68, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.34 (s,  
72 5H), 7.15 (s, 1H), 7.13 (s, 1H), 5.10 (s, 2H), 4.51-4.38 (m, 3H), 3.71-3.65 (m, 1H), 2.61-2.41 (m, 4H), 2.4-  
73 2.24 (m, 4H), 2.23-2.11 (m, 4H), 2.05-1.89 (m, 4H), 1.51-1.39 (m, 36H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$   
74 172.9, 172.5, 172.3, 172.2, 171.1, 136.6, 128.5, 128.2, 82.2, 82.0, 66.4, 52.4, 52.1, 32.4, 30.4, 28.2, 27.9, 27.2  
75 ppm; HRMS-ESI *m/z* 871.4708 [M+Na]<sup>+</sup> (calcd for C<sub>43</sub>H<sub>68</sub>N<sub>4</sub>O<sub>13</sub>Na<sup>+</sup> *m/z* 871.4680,  $\Delta$  +2.8 mmu). See  
76 Figures S34 and S35 for <sup>1</sup>H and <sup>13</sup>C NMR spectra. To this product (192 mg, 0.22 mmol) in THF, Pd/C  
77 (20 mg) was added, and H<sub>2</sub> was exposed to the mixture to remove the benzyl group. The reaction  
78 mixture was filtered through a Celite pad and concentrated *in vacuo*. The product concentrate (161  
79 mg, 0.212 mmol) was subjected to macrocyclization in the presence of MNBA (109 mg, 0.318 mmol),  
80 DMAP (2.6 mg, 0.0212 mmol), and Et<sub>3</sub>N (176.3 mL, 1.272 mmol) to give the protected cyclic LLDL-  
81 glutamic acid. Finally, the Boc group was deprotected using TFA to give cnidarin 4B (**2**) (78 mg,  
82 0.152 mmol) in 69% yield in three steps.  $[\alpha]_D^{25} -15^\circ$  (*c* 0.94, MeOH); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.42-  
83 4.28 (m, 4H), 2.52-2.32 (m, 8H), 2.29-2.11 (m, 4H), 2.09-1.89 (m, 4H); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  175.9,  
84 175.7, 175.6, 175.4, 53.6, 53.1, 52.7, 52.1, 32.7, 32.3, 31.8, 31.2, 26.7, 26.5, 26.2 ppm; HRMS-ESI *m/z*  
85 539.1542 [M+Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>28</sub>N<sub>4</sub>O<sub>12</sub>Na<sup>+</sup> *m/z* 539.1601,  $\Delta$  -5.9 mmu). See Figures S36 and S37 for  
86 <sup>1</sup>H and <sup>13</sup>C NMR spectra.

87

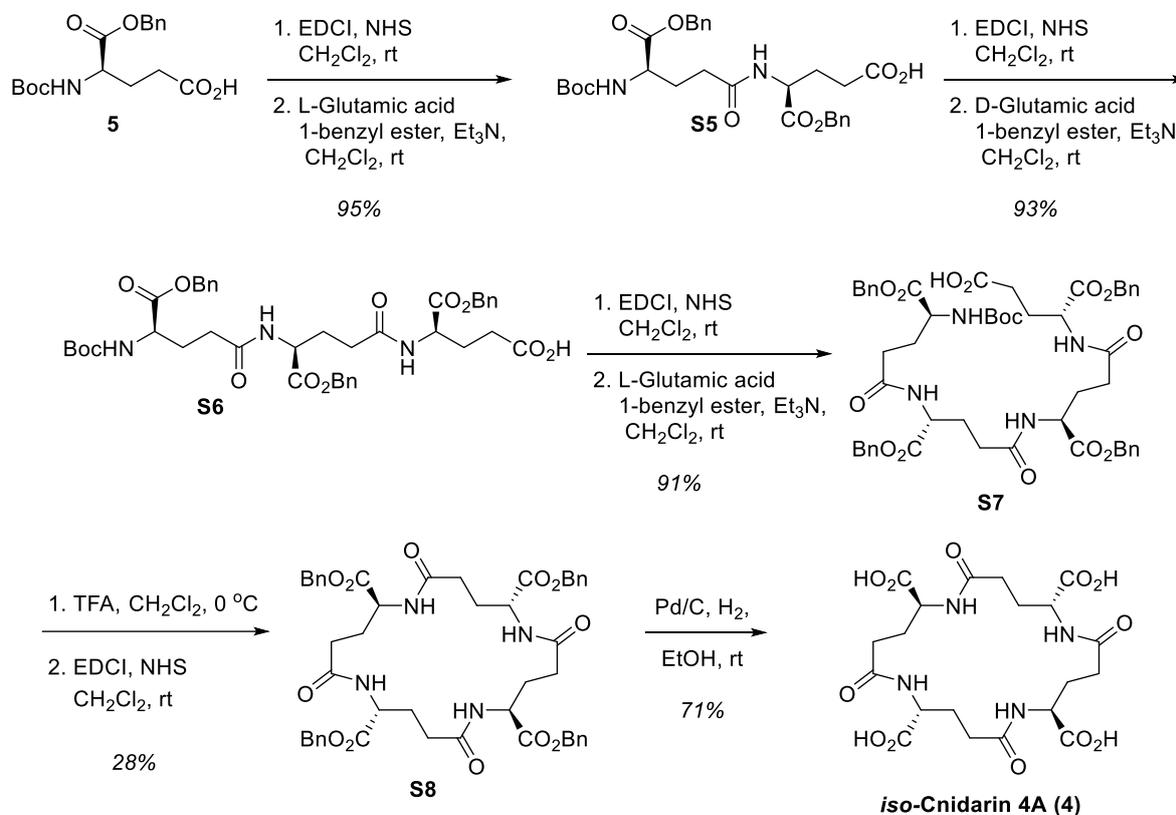
88

89 **Section S2. Synthesis of DLDL cyclic tetraglutamic acid *iso*-cnidarin 4A (4)**

90

91 **Scheme S2. Preparation *iso*-cnidarin 4A (4).**

92



93

94

95

96

97 *(S)*-5-(Benzyloxy)-4-((*R*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-5-oxopentanamido)-5-oxopentanoic  
 98 acid (**S5**)

99

100 To a solution of **5** (1.808 g, 5.36 mmol) and *N*-hydroxysuccinimide (0.864 g, 7.50 mmol) in  
 101 dichloromethane (26.8 mL) at room temperature EDCI (1.28 g, 6.70 mmol) was added. The reaction  
 102 was monitored by TLC and after 18 hr was diluted with dichloromethane (100 mL) and washed 3 ×  
 103 with saturated KH<sub>2</sub>PO<sub>4</sub>. The combined aqueous layers were back extracted with dichloromethane,  
 104 and the combined organic layers were washed with saturated NaCl, dried over MgSO<sub>4</sub> and  
 105 concentrated, providing the crude NHS ester (2.52 g, quant) as a white powder, deemed suitable for  
 106 use in the subsequent reaction. To a solution of the crude NHS ester (1.566 g, 3.61 mmol) and  
 107 triethylamine (0.704 mL, 0.511g, 5.05 mmol) in dichloromethane (36 mL) at room temperature, 1-  
 108 benzyl L-glutamic acid (0.984 g, 4.15 mmol) was added. The reaction was monitored by TLC and after  
 109 22 hr was diluted with dichloromethane (114 mL) and washed 4 × with saturated KH<sub>2</sub>PO<sub>4</sub>. The  
 110 combined aqueous layers were back extracted with dichloromethane and the combined organic  
 111 layers were washed with saturated NaCl, dried over MgSO<sub>4</sub> and concentrated. The crude product  
 112 was purified by flash chromatography on silica gel, (2% to 4% methanol in dichloromethane with  
 113 0.5% acetic acid) to provide **S5** (1.91 g, 95% (two step yield)) as a white powder: mp 89 - 91 °C;  
 R<sub>f</sub> = 0.4 (5% methanol in dichloromethane with 0.5% acetic acid); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 7.36-

114 7.26 (m, 10H), 5.13 (s, 2H), 5.15 (s, 2H), 4.44 (dd,  $J = 6.0, 9.0$ , 1H), 4.15 (dd,  $J = 3.0, 9.0$ , 1H), 2.38-2.27 (m,  
115 4H), 2.19-2.02 (m, 2H), 1.98-1.81 (m, 2H), 1.41 (s, 9H);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  174.8, 173.7,  
116 172.4, 171.7, 156.9, 136.0, 136.0, 128.4, 128.3, 128.1, 128.1, 128.0, 79.6, 66.9, 66.8, 53.7, 52.2, 31.9, 30.0, 27.7,  
117 27.4, 26.5; IR 3362 (br), 2968 (m), 1722 (m), 1654 (s), 1519 (m), 1451 (s)  $\text{cm}^{-1}$ ; TOF-MS  $m/z$  579.2306  
118  $[\text{M}+\text{Na}]^+$  ( $\text{C}_{29}\text{H}_{36}\text{N}_2\text{O}_9\text{Na}^+$  requires 579.2319,  $\Delta$  -1.3 mmu). See Figures S20 and S21 for  $^1\text{H}$  and  $^{13}\text{C}$  NMR  
119 spectra.

120

121 (6*R*,11*S*,16*R*)-6,11,16-Tris((benzyloxycarbonyl)-2,2-dimethyl-4,9,14-trioxo-3-oxa-5,10,15-triazanonadecan-  
122 19-oic acid (**S5**)

123 To a solution of **S5** (2.641 g, 4.75 mmol) and *N*-hydroxysuccinimide (0.765 g, 6.64 mmol) in  
124 dichloromethane (24 mL) at room temperature, EDCI (1.14 g, 5.93 mmol) was added. The reaction  
125 was monitored by TLC and after 18 hr was diluted with dichloromethane (76 mL) and washed 4  $\times$   
126 with saturated  $\text{KH}_2\text{PO}_4$ . The combined aqueous layers were back extracted with dichloromethane  
127 and the combined organic layers were washed with saturated NaCl, dried over  $\text{MgSO}_4$  and  
128 concentrated, providing the crude NHS ester (3.19 g, quant) as a white foam deemed suitable for use  
129 in the subsequent reaction. To a solution of the crude NHS ester (1.48 g, 2.27 mmol) and  
130 triethylamine (0.443 mL, 0.321 g, 3.17 mmol) in dichloromethane (22.7 mL) at room temperature, 1-  
131 benzyl D-glutamate (0.619 g, 2.61 mmol) was added. The reaction was monitored by TLC and after  
132 28 hr was diluted with dichloromethane (177 mL) and washed 4  $\times$  with saturated  $\text{KH}_2\text{PO}_4$ . The  
133 combined aqueous layers were back extracted with dichloromethane and the combined organic  
134 layers were washed with saturated NaCl, dried over  $\text{MgSO}_4$  and concentrated. The crude product  
135 was purified by flash chromatography on silica gel, (5% methanol in dichloromethane with 0.5%  
136 acetic acid) to provide **S6** (1.62 g, 93% (two step yield)) as a white foam:  $R_f = 0.33$  (5% methanol in  
137 dichloromethane with 0.5% acetic acid);  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.38-7.25 (m, 15H), 5.17-5.12  
138 (m, 6H), 4.52-4.39 (m, 2H), 4.17 (dd,  $J = 6.0, 9.0$ , 1H), 2.40-2.26 (m, 6H), 2.21-2.03 (m, 3H), 2.01-1.82 (m,  
139 3H), 1.41 (s, 9H);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  174.8, 173.7, 173.6, 172.4, 171.7, 171.6, 156.8, 136.0,  
140 136.0, 128.35, 128.3, 128.3, 128.1, 128.0, 128.0, 128.0, 79.6, 66.9, 66.8, 53.7, 52.5, 52.3, 31.9, 31.8, 30.0, 27.7,  
141 27.4, 27.2, 26.5; IR 3321 (br), 3046 (m), 2953 (m), 2356 (s), 1737 (s), 1654 (s), 1529 (s)  $\text{cm}^{-1}$ ; TOF-MS  $m/z$   
142 798.3242  $[\text{M}+\text{Na}]^+$  ( $\text{C}_{41}\text{H}_{49}\text{N}_3\text{O}_{12}\text{Na}^+$  requires 798.3214,  $\Delta$  +2.8 mmu). See Figures S22 and S23 for  $^1\text{H}$   
143 and  $^{13}\text{C}$  NMR spectra.

144

145 (6*S*,11*R*,16*S*,21*R*)-6,11,16,21-Tetrakis((benzyloxy)carbonyl)-2,2-dimethyl-4,9,14,19-tetraoxo-3-oxa-  
146 5,10,15,20-tetraazatetracosan-24-oic acid (**S7**)

147 To a solution of **S6** (1.489 g, 1.92 mmol) and *N*-hydroxysuccinimide (0.309 g, 2.69 mmol) in  
148 dichloromethane (19.2 mL), EDCI (0.460 g, 2.40 mmol) was added. The reaction was monitored by  
149 TLC and after 27 hr was diluted with dichloromethane (181 mL) and washed 4  $\times$  with saturated  
150  $\text{KH}_2\text{PO}_4$ . The combined aqueous layers were back extracted with dichloromethane and the combined  
151 organic layers were washed with saturated NaCl, dried over  $\text{MgSO}_4$  and concentrated, providing the  
152 crude NHS ester (1.73 g, quant) as a white foam deemed suitable for use in the subsequent reaction.  
153 To a solution of **the crude** (1.537 g, 1.76 mmol) and triethylamine (0.343 mL, 0.249 g, 2.46 mmol) in  
154 dichloromethane (17.6 mL) at room temperature, 1-benzyl D-glutamate (0.480 g, 2.02 mmol) was  
155 added. The reaction was monitored by TLC and after 22 hr was diluted with dichloromethane (183  
156 mL) and washed 4  $\times$  with saturated  $\text{KH}_2\text{PO}_4$ . The combined aqueous layers were back extracted with

157 dichloromethane and the combined organic layers were washed with saturated NaCl, dried over  
158 MgSO<sub>4</sub> and concentrated. The crude product was further purified by flash chromatography on silica  
159 gel (eluting with 4% methanol in dichloromethane and 0.5% acetic acid) to provide **S7** (1.60 g, 91%  
160 (two step yield)) as a white foam: *R<sub>f</sub>* = 0.35 (5% methanol in dichloromethane with 0.5% acetic acid);  
161 <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 7.40-7.26 (m, 20H), 5.18-5.13 (m, 8H), 4.51-4.39 (m, 3H), 4.18 (dd, *J* =  
162 3.0, 6.0, 1H), 2.41-2.26 (m, 8H), 2.23-2.06 (m, 4H), 2.03-1.84 (m, 4H), 1.43 (s, 9H); <sup>13</sup>C NMR (300 MHz,  
163 CD<sub>3</sub>OD) δ 174.8, 173.7, 173.6, 172.4, 171.8, 171.7, 171.6, 156.8, 136.0, 135.9, 128.4, 128.4, 128.1, 128.0,  
164 128.0, 128.0, 79.7, 66.9, 66.8, 53.8, 52.5, 52.4, 52.3, 31.9, 31.8, 31.8, 30.0, 27.7, 27.4, 27.3, 27.1, 26.5; IR 3321  
165 (br), 3061 (m), 2968 (m), 1737 (s), 1654 (s), 1529 (s) cm<sup>-1</sup>; TOF-MS *m/z* 1017.4101 [M+Na]<sup>+</sup>  
166 (C<sub>53</sub>H<sub>62</sub>N<sub>4</sub>O<sub>15</sub>Na<sup>+</sup> requires 1017.4109, Δ -0.8 mmu). See Figures S24 and S25 for <sup>1</sup>H and <sup>13</sup>C NMR  
167 spectra.

168

169 *Tetrabenzyl (2S,7R,12S,17R)-5,10,15,20-tetraoxo-1,6,11,16-tetraazacycloicosane-2,7,12,17-tetracarboxylate*  
170 (**S8**)

171 To a solution of **S7** (0.495 g, 0.498 mmol) in dichloromethane (25 mL) at 0 °C, a pre-cooled 1:1  
172 solution of trifluoroacetic acid and dichloromethane (25 mL) was added portionwise; 5.0 mL were  
173 added every 5 min (5 X) for a total of 25 mL over 25 min. The reaction was monitored TLC by  
174 conducting “mini workups” (drying under nitrogen gas followed by reconstituting with  
175 dichloromethane, 3 × in total). After 1.5 hr the solvent was removed by rotary-evaporation, and the  
176 crude was reconstituted in dichloromethane followed again by evaporation (3 × in total). The crude  
177 was purified by flash chromatography on silica gel (4% to 10% methanol in dichloromethane) to  
178 provide the free ammonium trifluoroacetate (0.355 g, 71%) as a brown foam: *R<sub>f</sub>* = 0.24 (8% methanol  
179 in dichloromethane); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 7.41-7.22 (m, 20H), 5.245 (d, *J* = 3.0, 2H), 5.15-5.10  
180 (m, 6H), 4.49-4.36 (m, 3H), 4.11 (t, *J* = 12.0, 1H), 2.45 (t, *J* = 15.0, 2H), 2.37-2.24 (m, 6H), 2.23-2.02 (m, 5H),  
181 2.00-1.81 (m, 3H); <sup>13</sup>C NMR (300 MHz, CD<sub>3</sub>OD) δ 174.8, 173.6, 173.5, 172.9, 171.7, 171.6, 186.8, 135.9,  
182 135.9, 135.1, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.01, 128.0, 68.2, 67.0, 66.9, 66.9, 52.5, 52.3  
183 31.7, 30.8, 29.9, 27.1, 26.5, 25.9; IR 3310 (br), 3046 (m), 2947 (m), 1737 (s), 1659 (s), 1540 (s) cm<sup>-1</sup>. To a  
184 solution of the free ammonium trifluoroacetate (0.151 g, 0.149 mmol) and triethylamine (6.36 μL, 4.61  
185 mg, 0.456 mmol) in dichloromethane (152 mL), EDCI (0.0874 g, 0.456 mmol) was added. The reaction  
186 was monitored by TLC and after 17 hr was diluted with dichloromethane (75 mL) and washed 4 ×  
187 with saturated KH<sub>2</sub>PO<sub>4</sub>. The combined aqueous layers were back extracted with dichloromethane,  
188 and the combined organic layers were washed with saturated NaCl, dried over MgSO<sub>4</sub> and  
189 concentrated. The crude product was purified by recrystallization according to the following  
190 procedure. The crude sample of was dissolved in warm dichloromethane, filtered through cotton in  
191 a glass funnel followed by the addition of a small volume of hexane. The solution was placed in a  
192 fume hood overnight and then in a freezer for 4 days providing fine crystals. The suspension was  
193 centrifuged, and the supernatant removed followed by 2 hexane-wash / centrifugation cycles  
194 providing **S8** (0.052 g, 40%) as a white powder: mp 248 - 250 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.38-  
195 7.27 (m, 20H), 7.17 (d, *J* = 6.0, 4H), 5.19-5.08 (m, 8H), 4.70-4.60 (m, 4H), 2.48-2.35 (m, 4H), 2.26-2.05 (m,  
196 12H); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) δ 173.1, 173.1, 171.0, 135.4, 128.8, 128.6, 128.3, 67.5, 53.4, 53.3, 33.3,  
197 27.6, 27.5; IR 3290 (br), 3072 (m), 2937 (m), 1732 (s), 1649 (s), 1550 (s) cm<sup>-1</sup>; TOF-MS *m/z* 899.3461  
198 [M+Na]<sup>+</sup> (C<sub>48</sub>H<sub>52</sub>N<sub>4</sub>O<sub>12</sub>Na<sup>+</sup> requires 899.3479, Δ -1.8 mmu). See Figures S26 and S27 for <sup>1</sup>H and <sup>13</sup>C NMR  
199 spectra.

200

201 *2.1,5.4-Anhydro( $\gamma$ -L-glutamyl- $\gamma$ -D-glutamyl- $\gamma$ -L-glutamyl-D-glutamic acid) (iso-cnidaridin 4A, 4)*

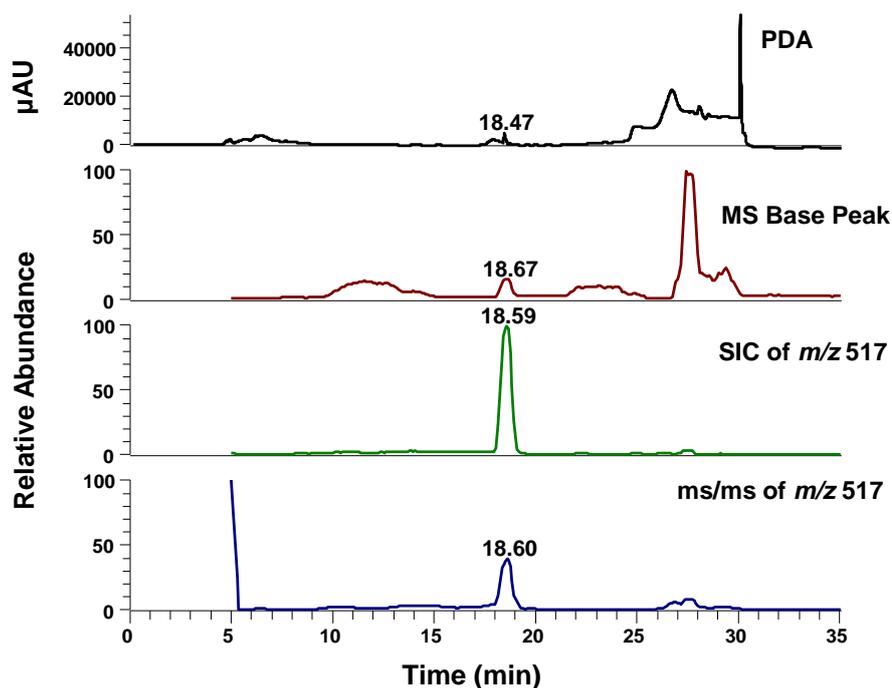
202 Above a solution of **S8** (0.0101 g, 0.0115 mmol) and 10% Pd/C (~2 mg) in ethanol (5.0 mL) at room  
203 temperature, hydrogen gas was maintained at ambient pressure for 21 hr. The reaction was then  
204 filtered through celite followed by methanol washing (3  $\times$  1 mL), methanol/water 1:1 (3  $\times$  1 mL) then  
205 water (3  $\times$  1 mL). The crude product was purified by HPLC using a Waters Atlantis dC18 (10  $\times$  250  
206 mm, 10  $\mu$ m particle size) column (mobile phase A: water/formic acid (1000:1); mobile phase B:  
207 acetonitrile/formic acid (1000:1); gradient: 2.5% B, 0-7 min, 2.5-100% B, 7-27 min, 100% B, 27-35 min)  
208 to provide **4** (0.042 g, 71%) as a white solid: decomp = 277 - 283  $^{\circ}$ C;  $^1$ H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  4.295  
209 (dd,  $J$ =5.0,10, 4H), 2.49-2.40 (m, 4H), 2.37-2.28 (m, 4H), 2.19-2.10 (m, 4H), 2.06-1.95 (m, 4H);  $^{13}$ C NMR  
210 (500 MHz, D<sub>2</sub>O)  $\delta$  178.3, 177.9, 55.8, 34.9, 29.0; IR 3312 (br), 2358 (m), 1644 (m), 1594 (s), 1410 (br)  $\text{cm}^{-1}$ ;  
211 TOF-MS  $m/z$  539.1602 [M+Na]<sup>+</sup> (C<sub>20</sub>H<sub>28</sub>N<sub>4</sub>O<sub>12</sub>Na<sup>+</sup> requires 539.1601,  $\Delta$  +0.1 mmu). See Figures S28 and  
212 S29 for  $^1$ H and  $^{13}$ C NMR spectra.

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## 215 Section S3. Chromatography and chemical analyses of cnidarin 4A (1)

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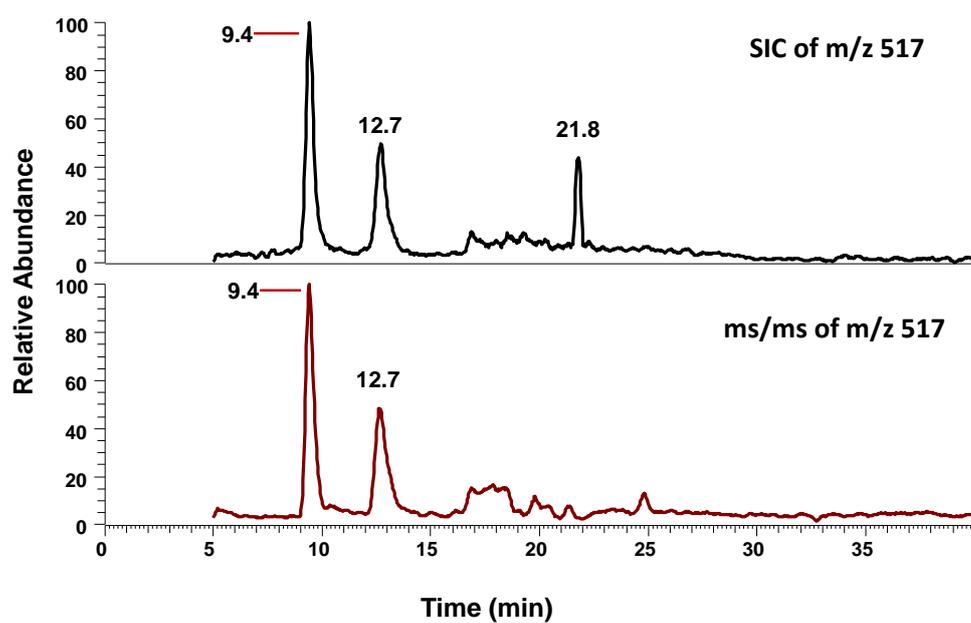
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**Figure S1.** LC-DAD-MS of *Alatina alata* venom. Chromatograms shown from top to bottom panel are: photo diode array detector (PDA) UV absorption (200-600 nm), MS base peak ( $m/z$  100-1000), extracted ion chromatograms for  $m/z$  517 ion and MS/MS product ions for  $m/z$  517. Extract was analyzed on a Waters Atlantis dC18 (3.0 x 250 mm column, 5  $\mu$ m particle size) column (mobile phase A: water/formic acid (1000:1); mobile phase B: acetonitrile/formic acid (1000:1); gradient: 0% B, 0-10 minutes, 0-50% B, 10-20 minutes, 50-100% B, 20-23 minutes, 100% B, 23-25 minutes). The peak at 18.6 min in extracted ion ( $[M+H]^+$   $m/z$  517) and MS/MS chromatograms (bottom 2 panels) was a mixture resolved with LC conditions described in Figure S2.

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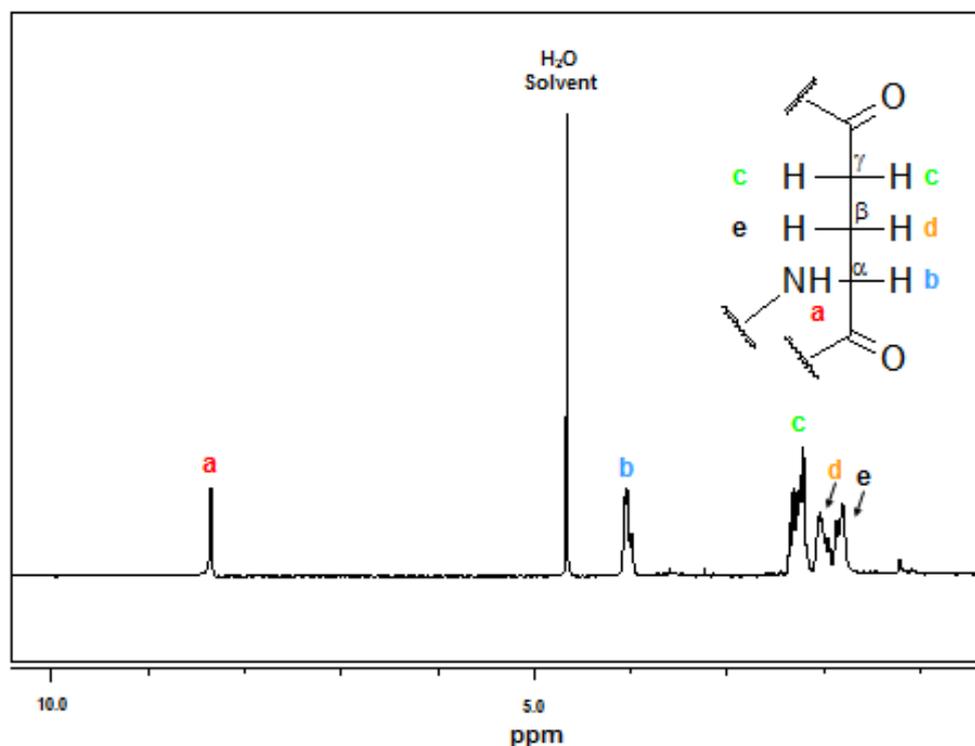
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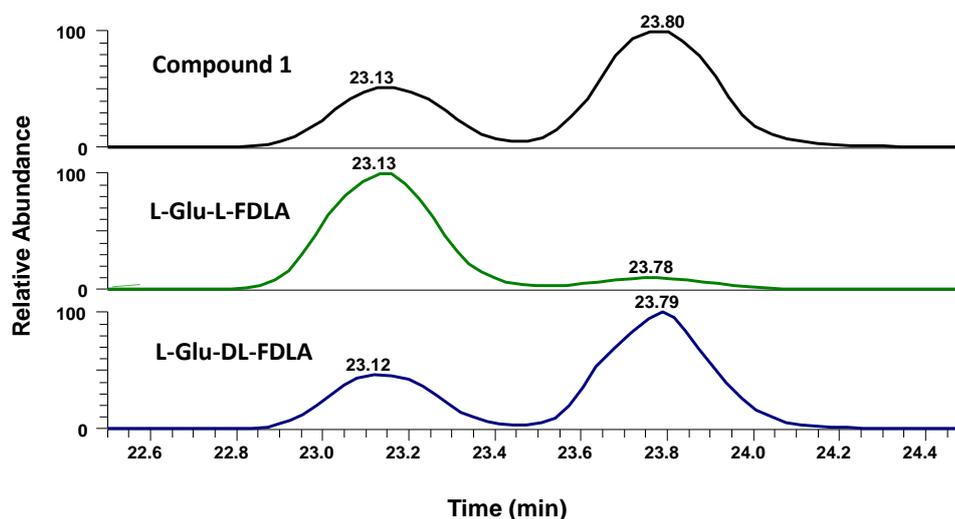
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**Figure S2.** Extracted  $[M+H]^+$  ion ( $m/z$  517) and MS/MS chromatograms of crude cnidarin 4A (**1**) (see peak at 18.6 min in Figure S1) from *Alatina alata* venom. Sample was reconstituted in 1% formic acid analyzed on a Waters Atlantis dC18 column (3.0 x 250 mm, 5  $\mu$ m particle size) (mobile phase A: water/formic acid (1000:1); mobile phase B: acetonitrile/formic acid (1000:1); gradient: 2.5% B, 0-7 minutes, 2.5–100% B, 7-27 minutes, 100% B, 27-35 minutes). Compound **1** eluted at 9.4 min.

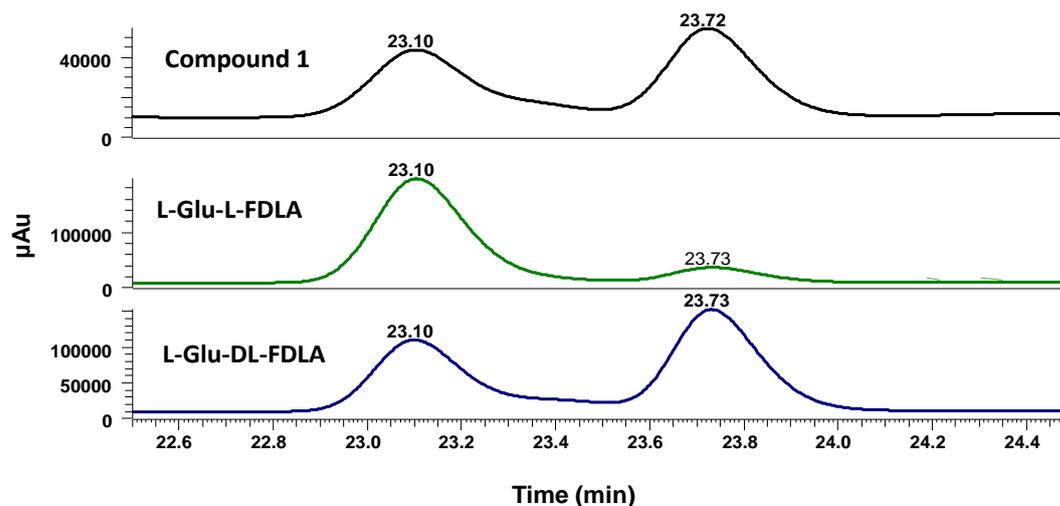
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**Figure S3.** Capillary  $^1\text{H}$  nuclear magnetic resonance (NMR) spectrum (500 MHz,  $\text{D}_2\text{O}/\text{H}_2\text{O}$ ) of crude cnidarin 4A (1). Spectral data indicate multiple glutamic acids.

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**Figure S4.** LC-MS of FDLA-labeled products of the compound 1 hydrolysate, L-glutamic acid, and DL-glutamic acid. Chromatograms are summed extracted ion chromatograms of  $m/z$  of 442 and 883 ions, which correspond to protonated glutamic acid-FDLA monomer and dimer ions. Products were analyzed using a Phenomenex Luna C18(2) (2.0 x 250 mm, 3  $\mu\text{m}$  particle size) column (mobile phase A: water/formic acid (1000:1); mobile phase B: acetonitrile/formic acid (1000:1); gradient: 20-80% B, 0-30 minutes, 80% B, 30-35 minutes).



250 **Figure S5.** HPLC with PDA detection (200-600 nm) of FDLA-labeled products of compound  
 251 **1** hydrolysate. (UV chromatograms from Figure S4). Products were analyzed on a  
 252 Phenomenex Luna C18(2) (2.0 × 250 mm, 3 μm particle size) column (mobile phase A:  
 253 water/formic acid (1000:1); mobile phase B: acetonitrile/formic acid (1000:1); gradient: 20-  
 254 80% B, 0-30 min, 80% B, 30-35 min).

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**Table S1.** Ratio of glutamic acid-FDLA derivatives of hydrolysate  
 of compound **1** using HPLC with MS and UV detection.

Detector	relative concentration	
	D-glutamic acid	L-glutamic acid
UV	0.86	1.0
MS	0.99	1.0

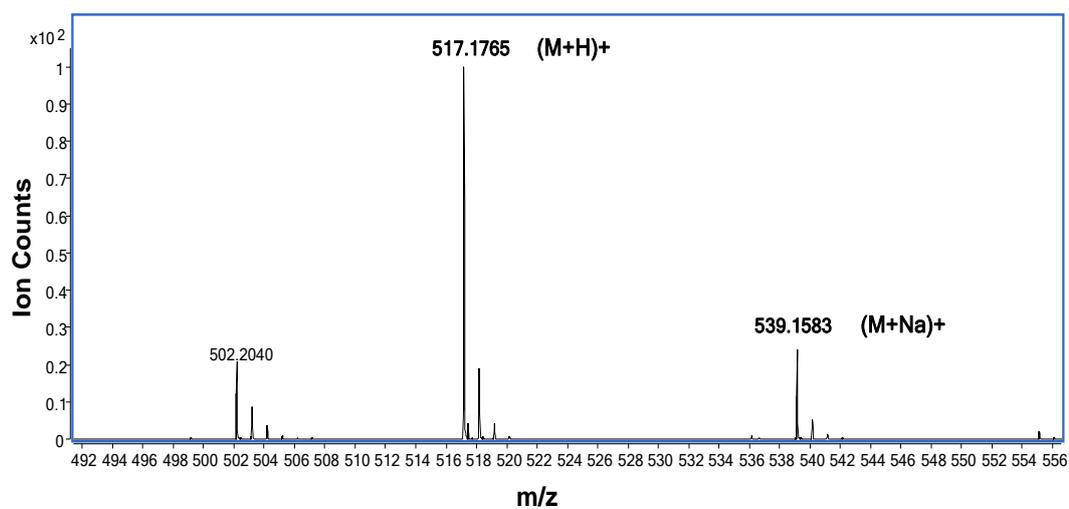
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Ratios were calculated from extracted ion chromatograms, see Figures  
 S4 and S5.

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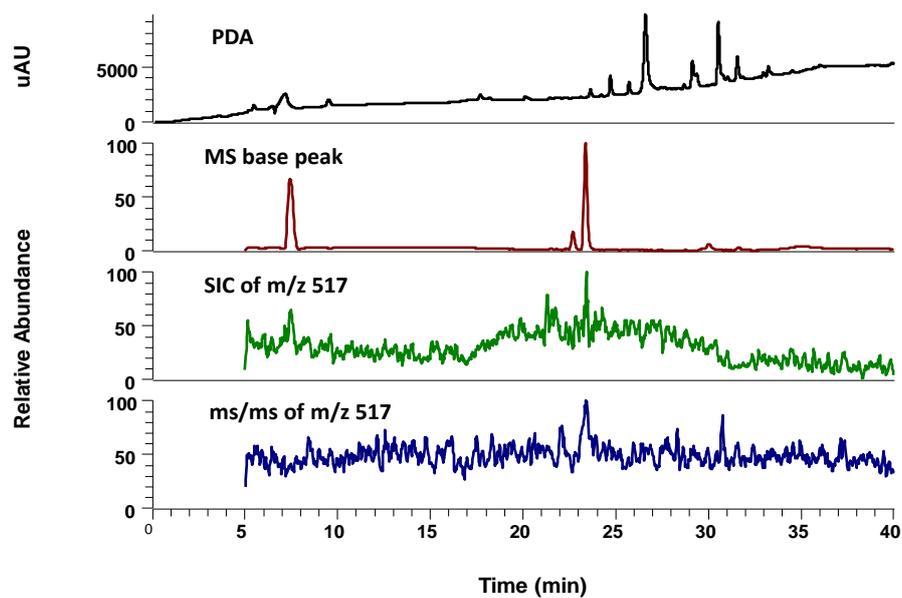
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**Figure S6.** High resolution time-of-flight mass spectrometry (TOF-MS-ESI positive mode) of compound 1 from *Alatina alata* venom.



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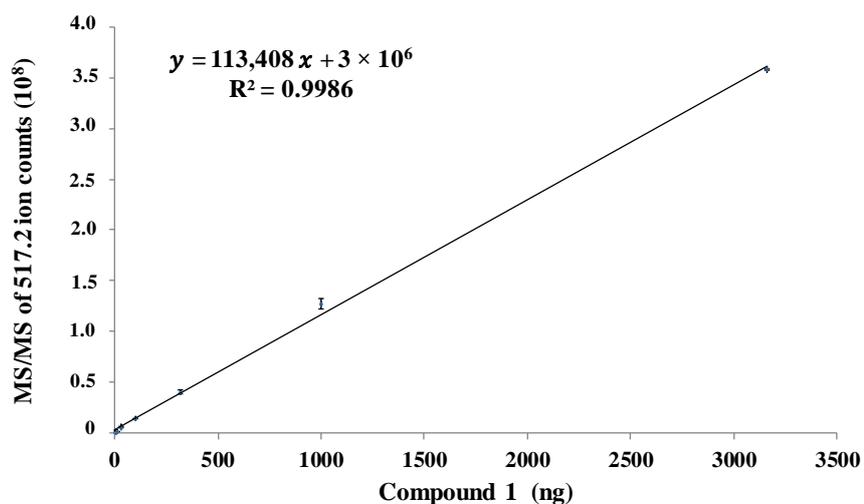
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**Figure S7.** LC-DAD-MS of *Alatina alata* tentacle extract without nematocysts (panels from top to bottom: photo diode array UV detector, MS base peak, extracted ion chromatograms for the  $[M+H]^+$  ion,  $m/z$  517, and MS/MS product ions for  $m/z$  517).



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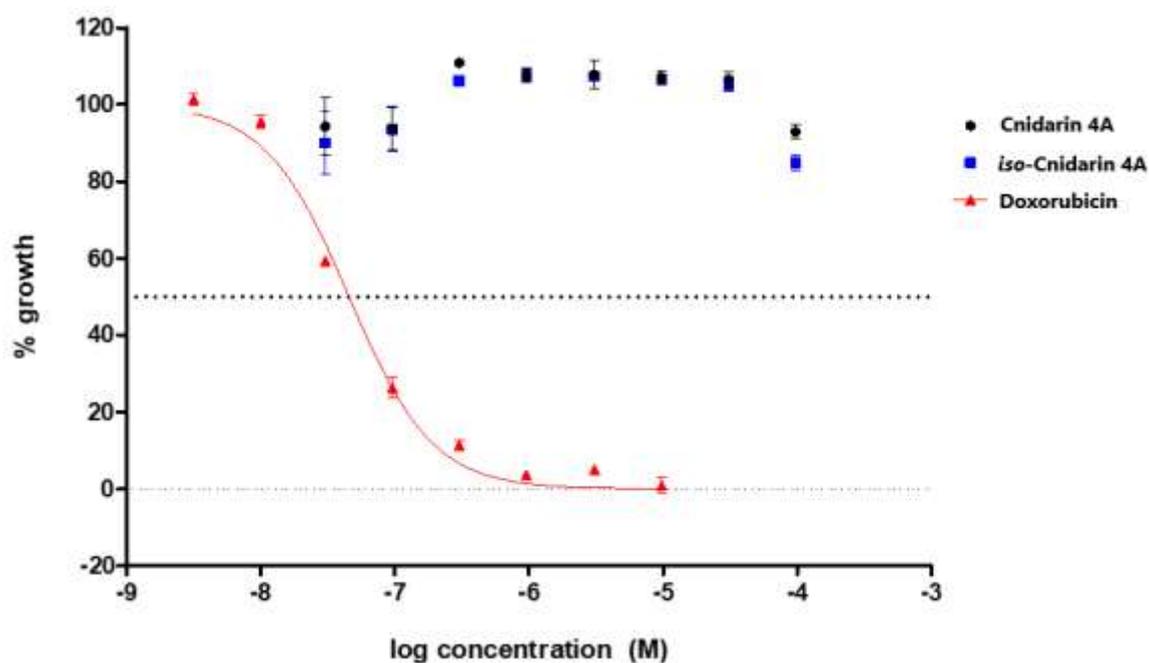
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**Figure S8.** Calibration curve for MS/MS detection of cnidarin 4A (**1**) (product ion of  $m/z$  517 fragmentation). Synthesized **1** was analyzed at semi log concentrations in triplicate with a fixed volume injection (0.343 – 343 ng on column). LC-MS conditions: Waters Atlantis dC18 column (3.0 × 250 mm, 5 μm particle size); mobile phase A: water/formic acid (1000:1); mobile phase B: acetonitrile/formic acid (1000:1); gradient: 2.5% B, 0-7 min, 2.5–100% B, 7-27 min, 100% B, 27-35 min).

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281 **Section S4. Cell toxicity and hemolytic activity of cnidarin 4A (1).**

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284 **Figure S9.** Cytotoxicity of doxorubicin, synthetic cnidarin 4A (1) and *iso*-cnidarin 4A (4)  
 285 against HEK-293 cells. Doxorubicin is a positive control. Methods described in article.

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**Table S2.** Hemolysis of red blood cells by cnidarin 4A (1) normalized to 1% TritonX-100 hemolysis.

treatment	% hemoglobin	Standard deviation
vehicle	2.63	0.31
24 $\mu$ M compd 1	3.74	1.04
121 $\mu$ M compd 1	2.86	0.20
TritonX-100, 1%	100	0.28

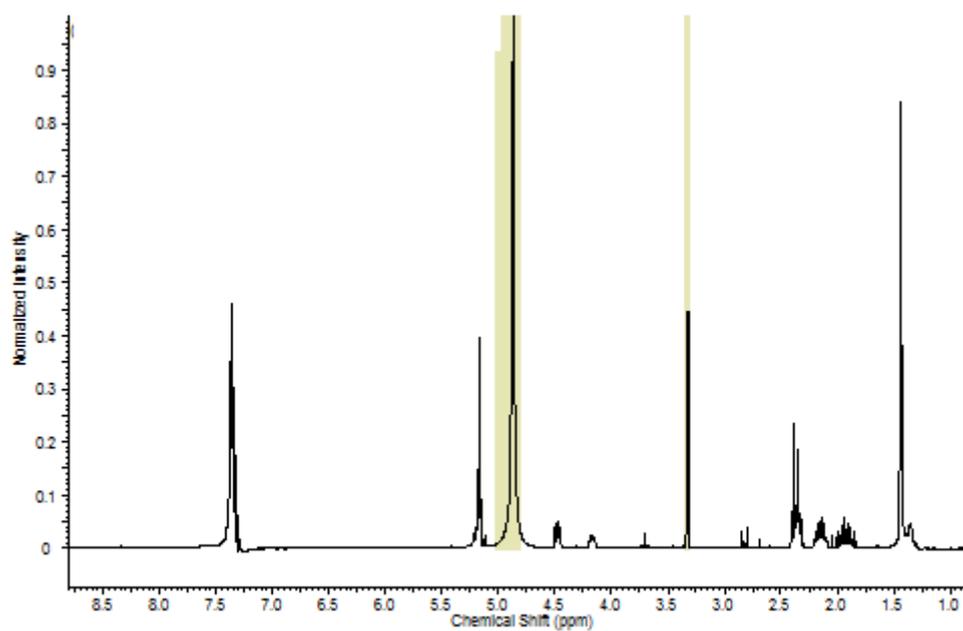
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Treatments were tested in triplicate.

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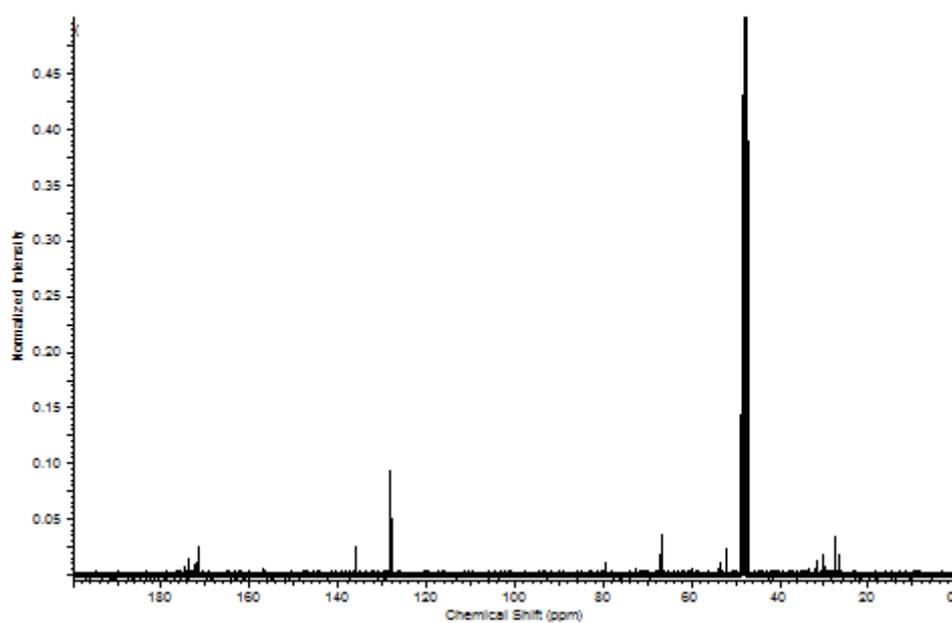
294 **Section S5. NMR spectra of synthetic cnidarin 4A (1) and intermediates 8-12.**

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**Figure S10.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **6**.

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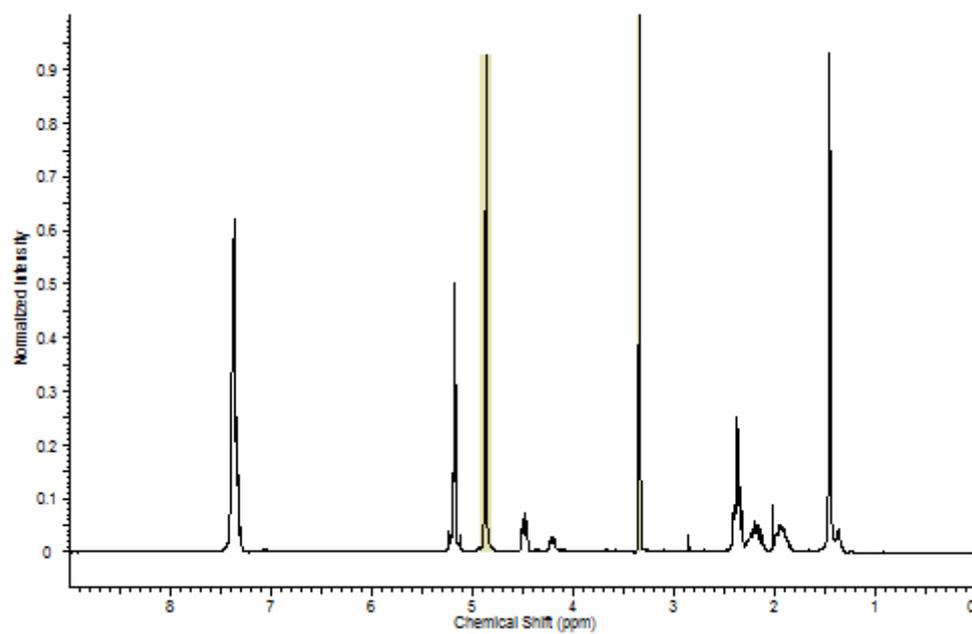


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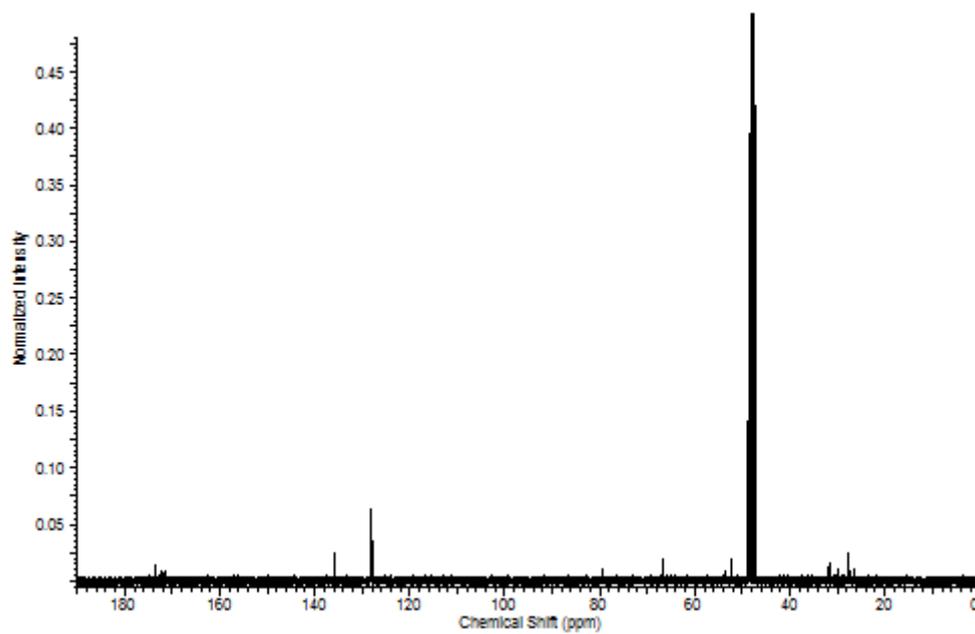
**Figure S11.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **6**.

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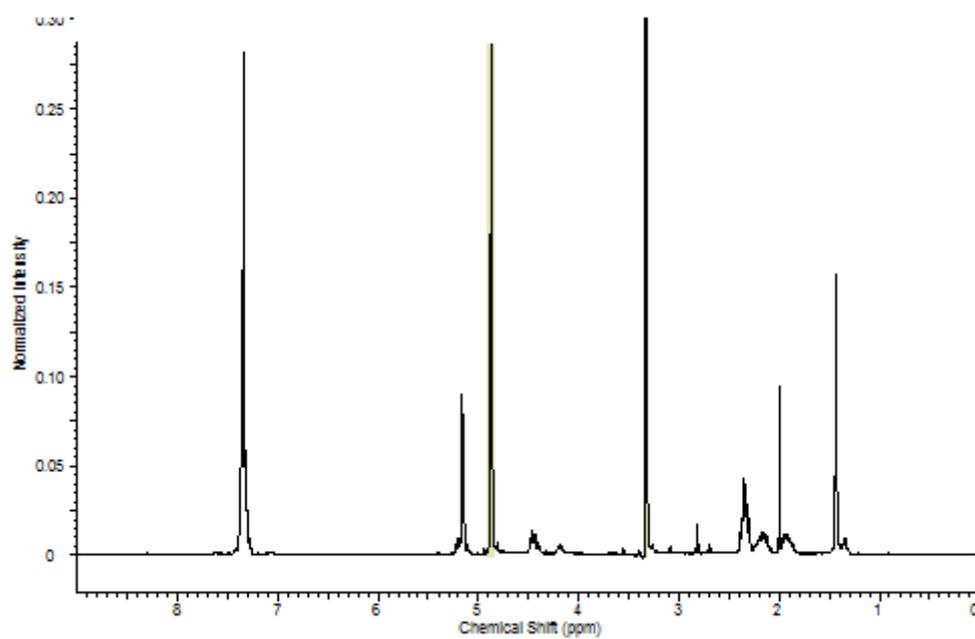
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**Figure S12.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for 7.

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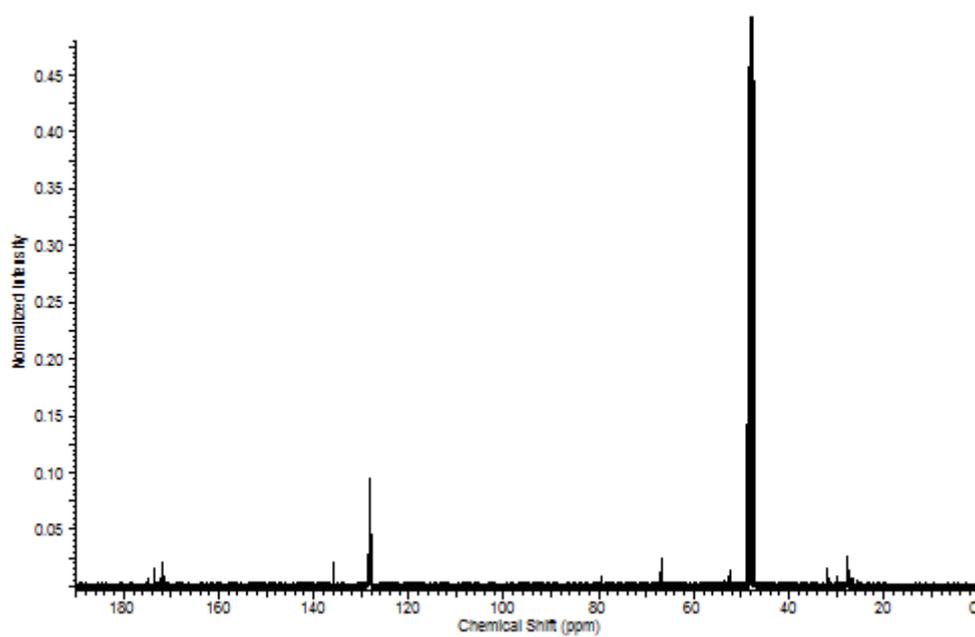
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**Figure S13.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for 7.



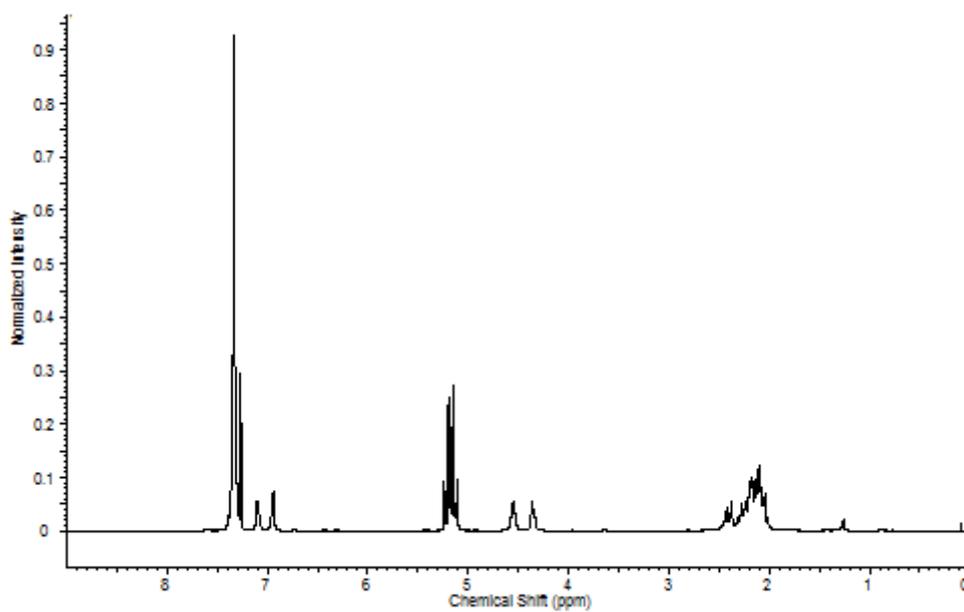
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**Figure S14.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **8**.



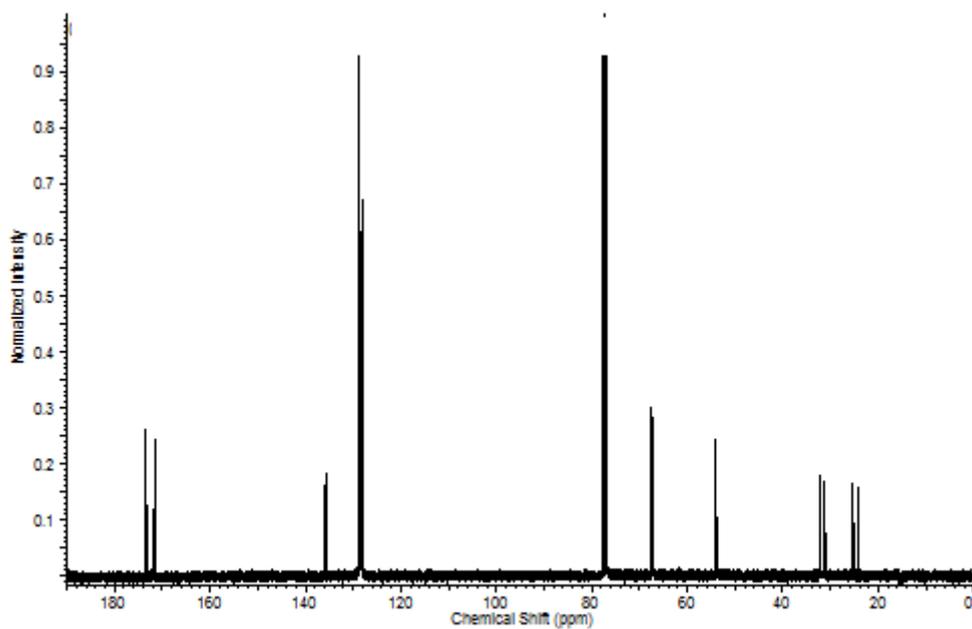
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**Figure S15.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **8**.



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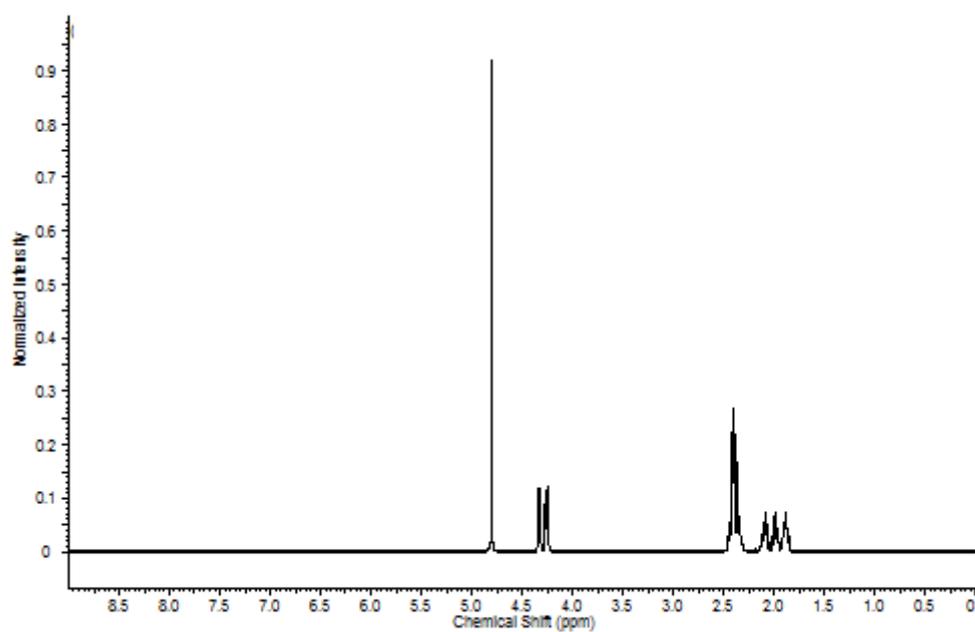
**Figure S16.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for **9**.

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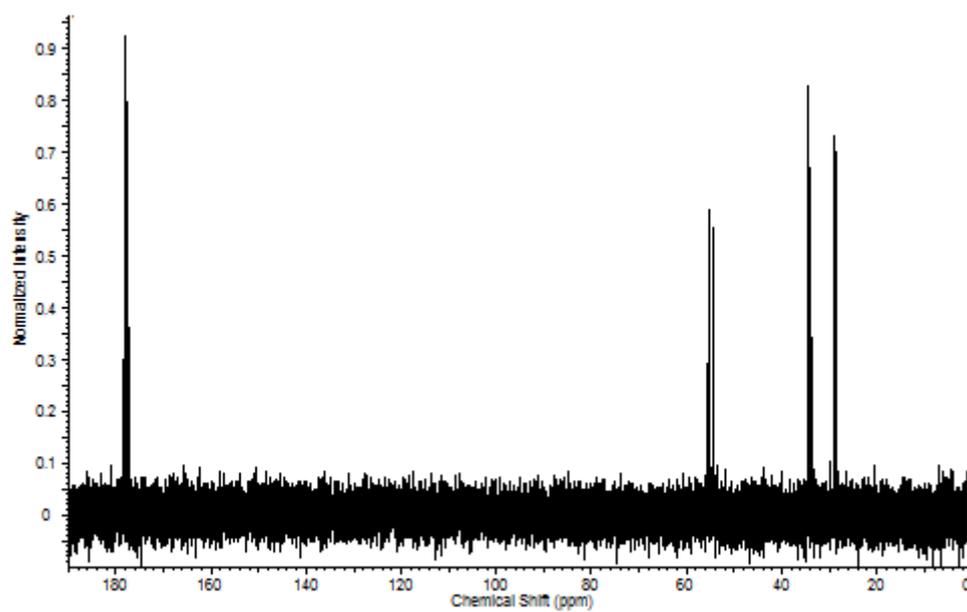
**Figure S17.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for **9**.

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**Figure S18.** <sup>1</sup>H NMR (D<sub>2</sub>O) spectrum for 1.

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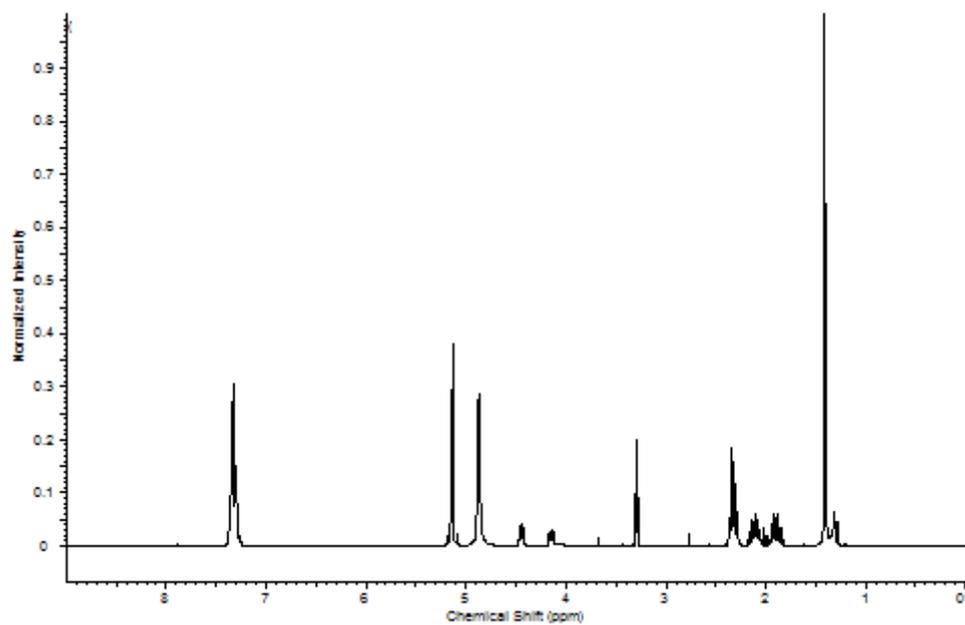
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**Figure S19.** <sup>13</sup>C NMR (D<sub>2</sub>O) spectrum for 1.

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320 **Section S6. NMR spectra of synthetic *iso*-cnidarin 4A (4) and intermediates S5-**  
321 **S8.**

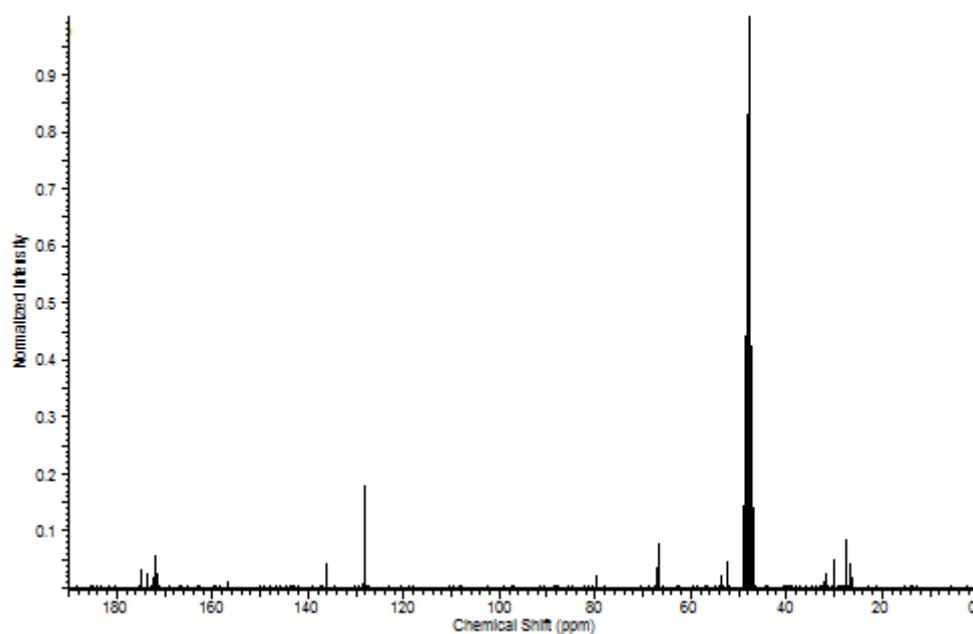
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**Figure S20.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for S5.

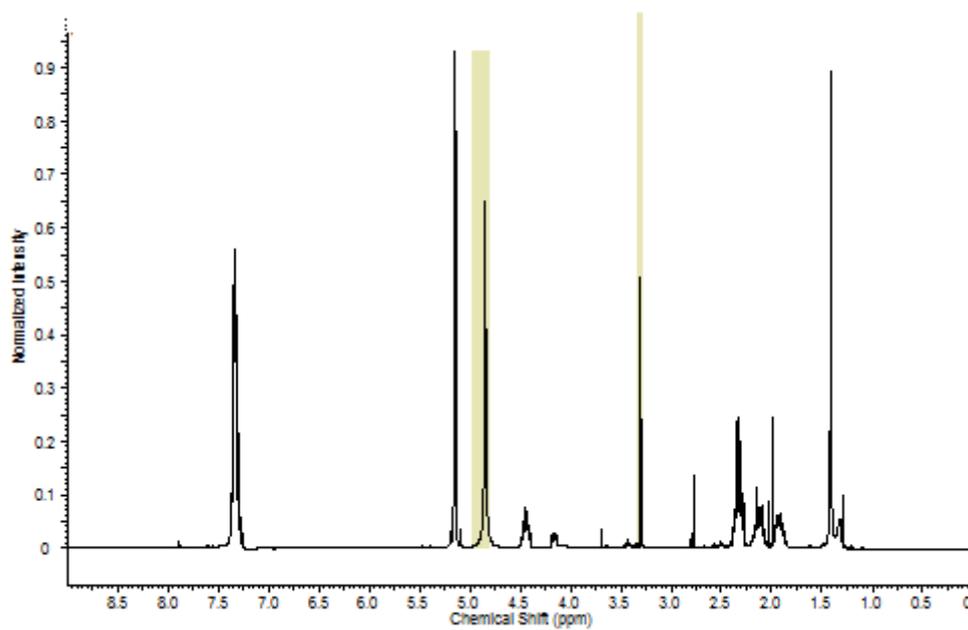


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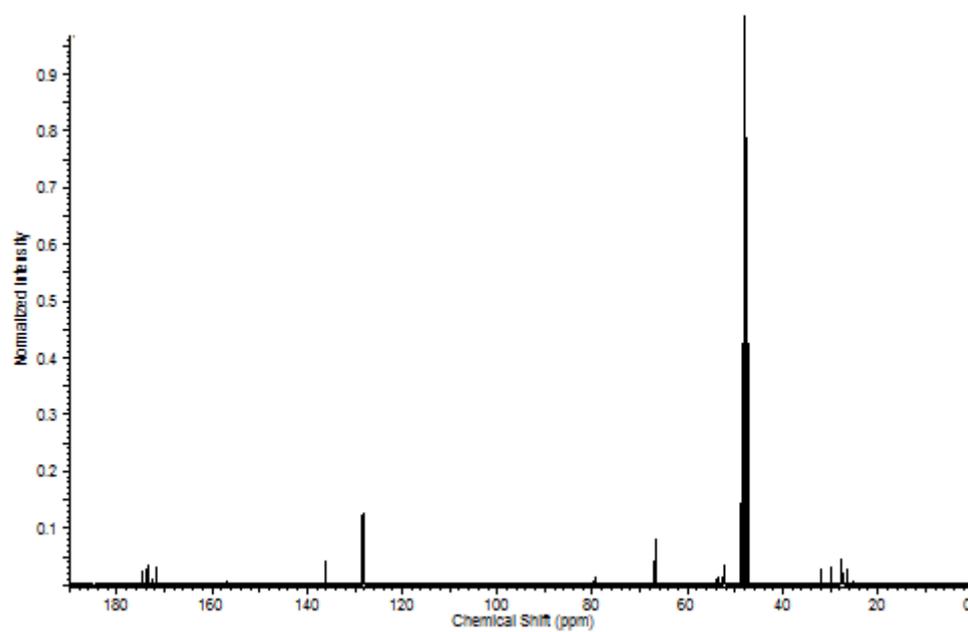
**Figure S21.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for S5.

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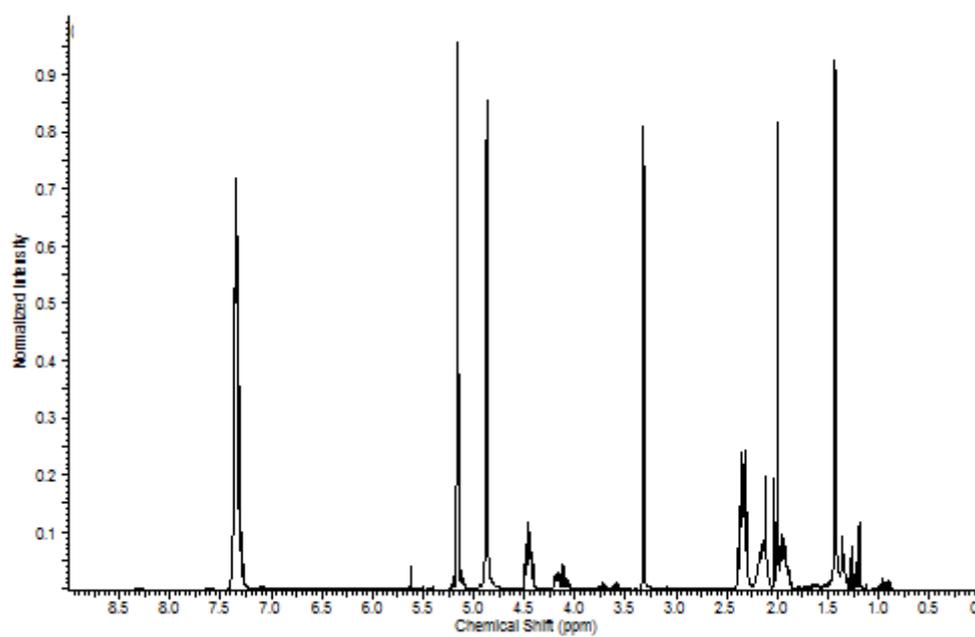
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**Figure S22.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **S6**.



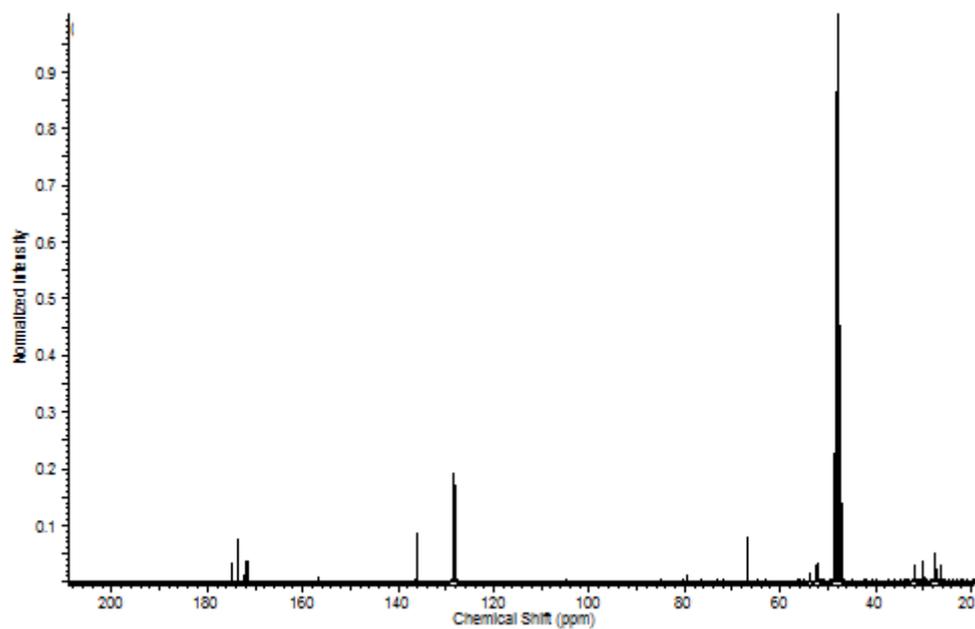
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**Figure S23.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **S6**.



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**Figure S24.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **S7**.

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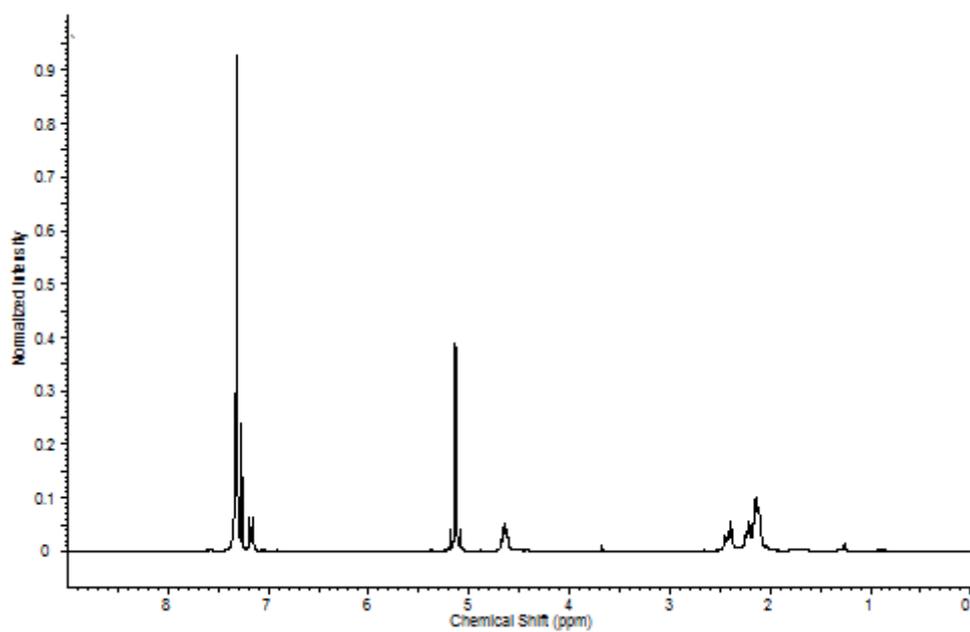
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**Figure S25.**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) spectrum for **S7**.

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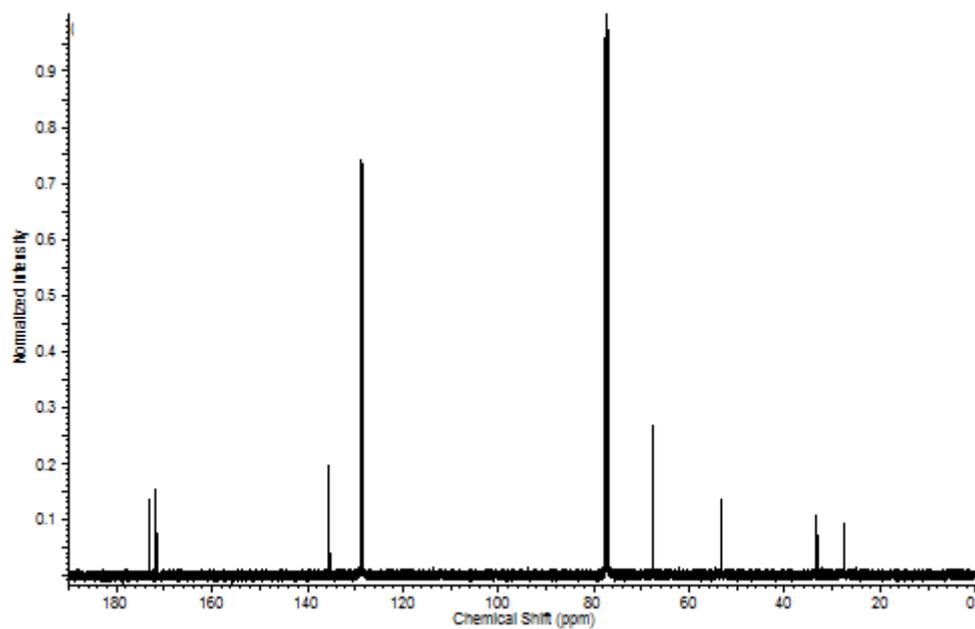
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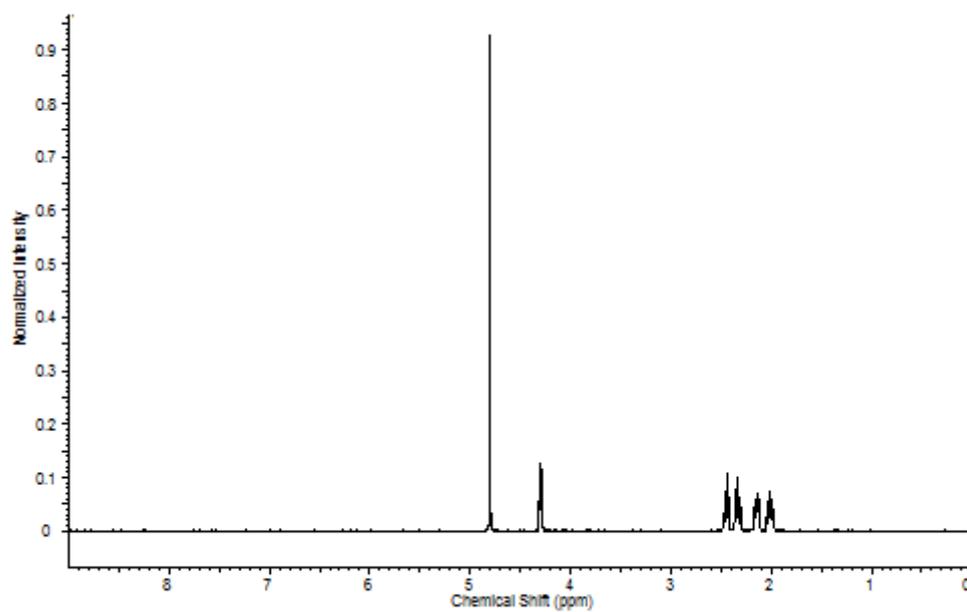
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**Figure S26.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for S8.

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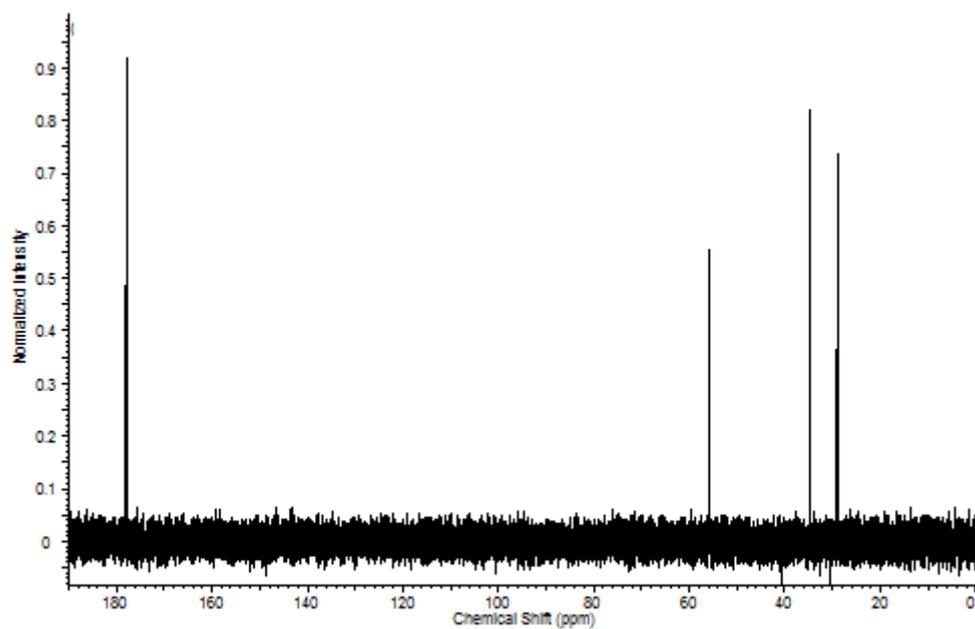
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**Figure S27.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for S8.



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**Figure S28.** <sup>1</sup>H NMR (D<sub>2</sub>O) spectrum for 4.

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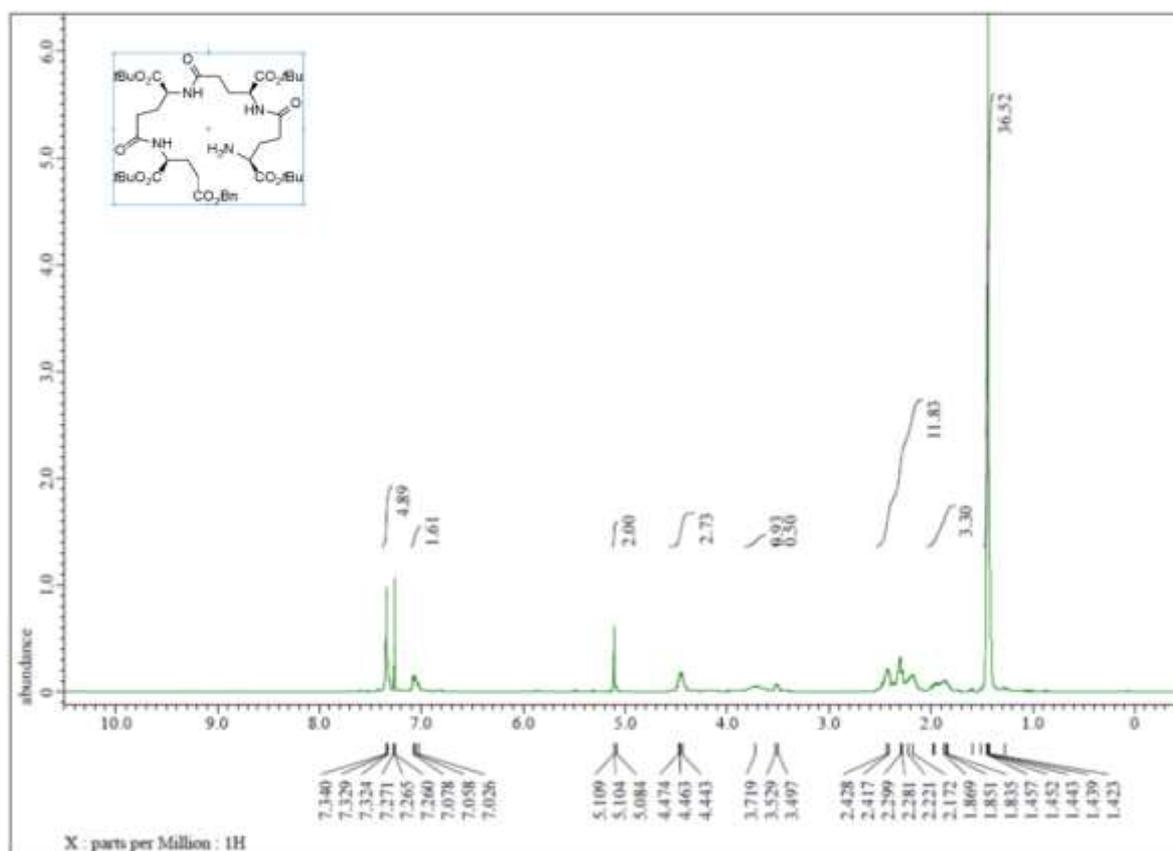
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**Figure S29.** <sup>13</sup>C NMR (D<sub>2</sub>O) spectrum for 4.

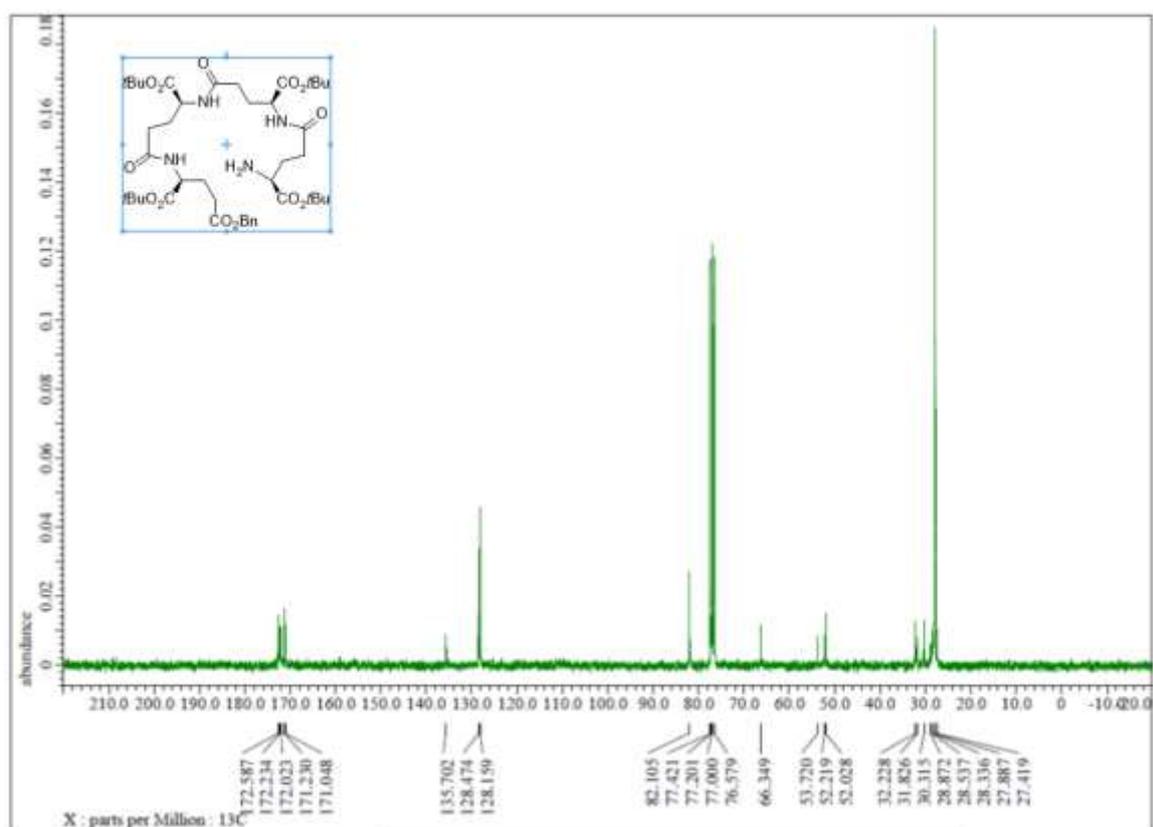
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## 350 Section S7. NMR spectra of synthetic cnidarin 4C (3) and intermediates.

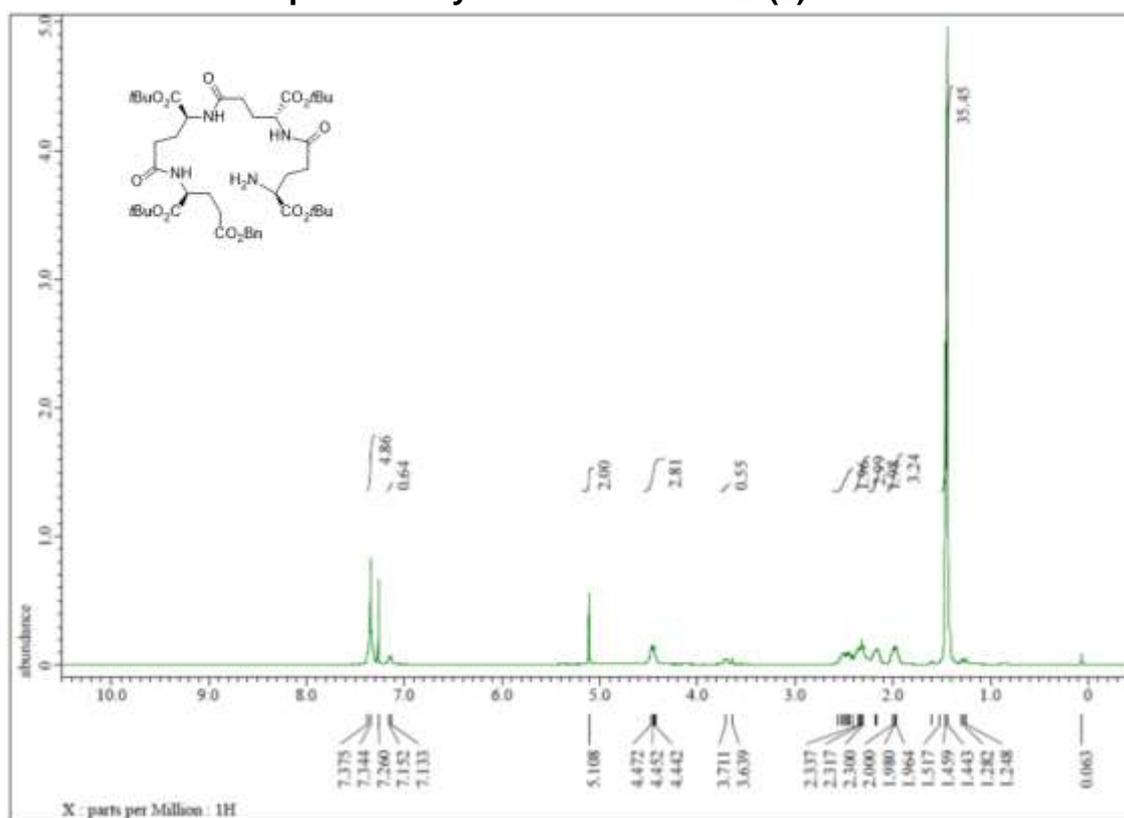
351  
352 Figure S30.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for linear LLLL-glutamic acid with free amine.

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355 Figure S31.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for linear LLLL-glutamic acid with free amine.

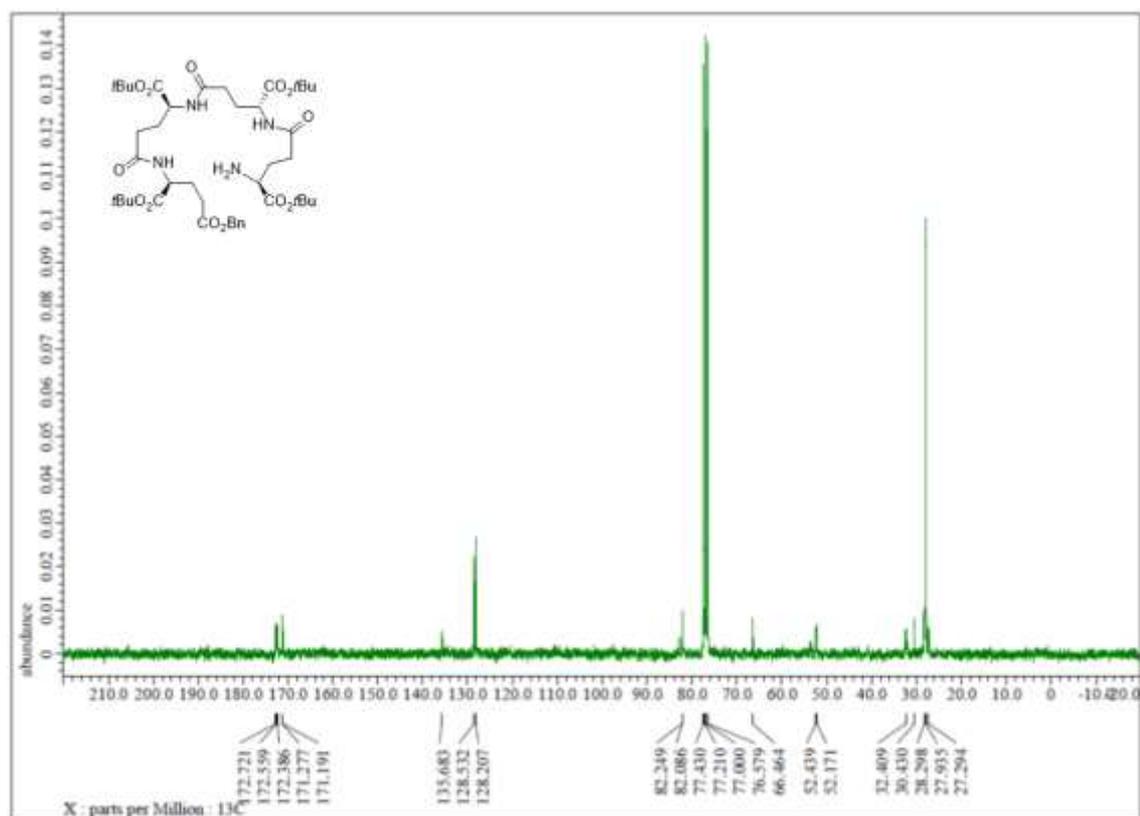


## 361 Section S8. NMR spectra of synthetic cnidarin 4B (2) and intermediates.



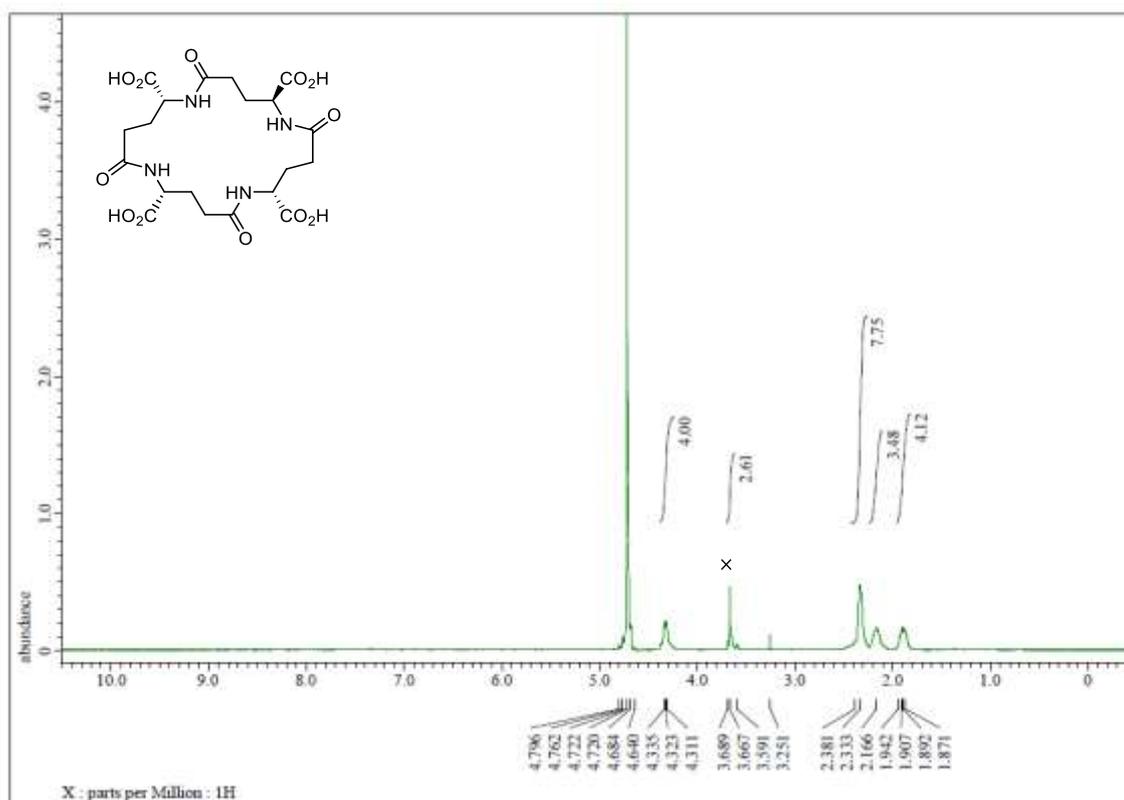
362  
363 **Figure S34.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for linear LLDL-glutamic acid with free amine.

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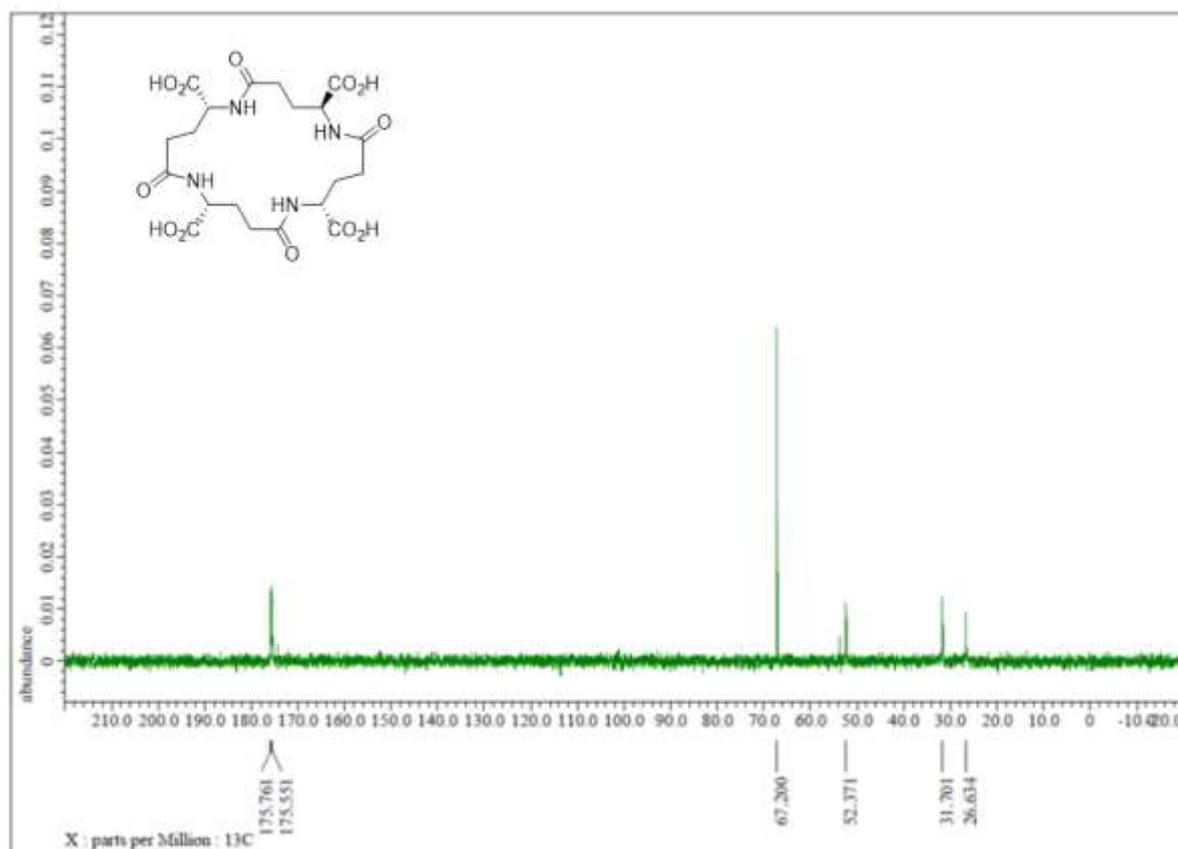


365  
366 **Figure S35.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for linear LLDL-glutamic acid with free amine.

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368 **Figure S36.**  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) spectrum for 2.

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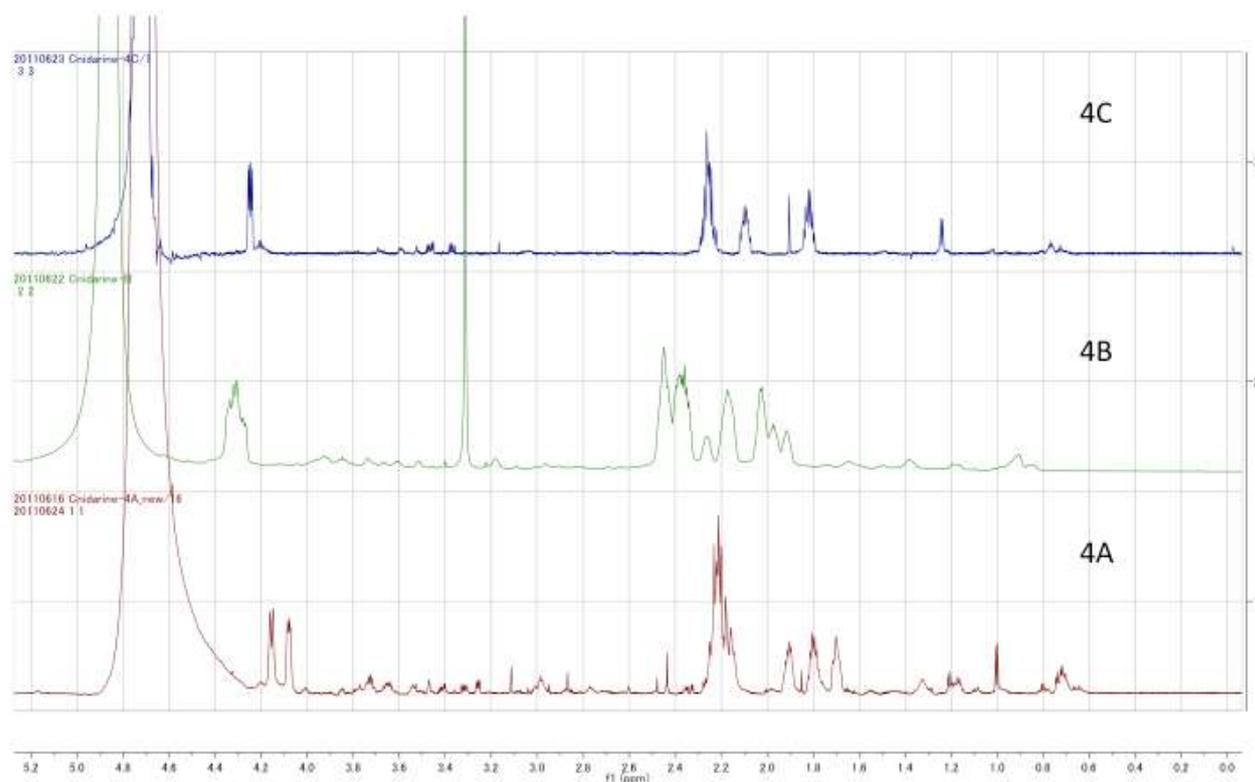


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**Figure S37.**  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ) spectrum for 2; 1,4-dioxane added as internal standard.



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374 **Figure S38.** The  $^1\text{H}$  NMR (800 MHz,  $\text{D}_2\text{O}$  with 10  $\mu\text{L}$   $\text{HCOOH}$ ) spectra of isolated cnidarins 4A (1,  
375 bottom), 4B (2, middle), 4C (3, top).

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