

Supplementary data

Brominated Bisindole Alkaloids from the Celtic Seas Sponge *Spongosorites calcicola*

Laurence K. Jennings^{1,2}, Neyaz M. D. Khan³, Navdeep Kaur^{1,2}, Daniel Rodrigues^{1,2}, Christine Morrow⁴, Aoife Boyd³ and Olivier P. Thomas^{1,*}

¹ School of Chemistry, Ryan Institute, National University of Ireland Galway, University Road, Galway H91 TK33, Ireland. laurence.jennings@nuigalway.ie, navdeep.kaur@nuigalway.ie, daniel.rodrigues@nuigalway.ie, olivier.thomas@nuigalway.ie.

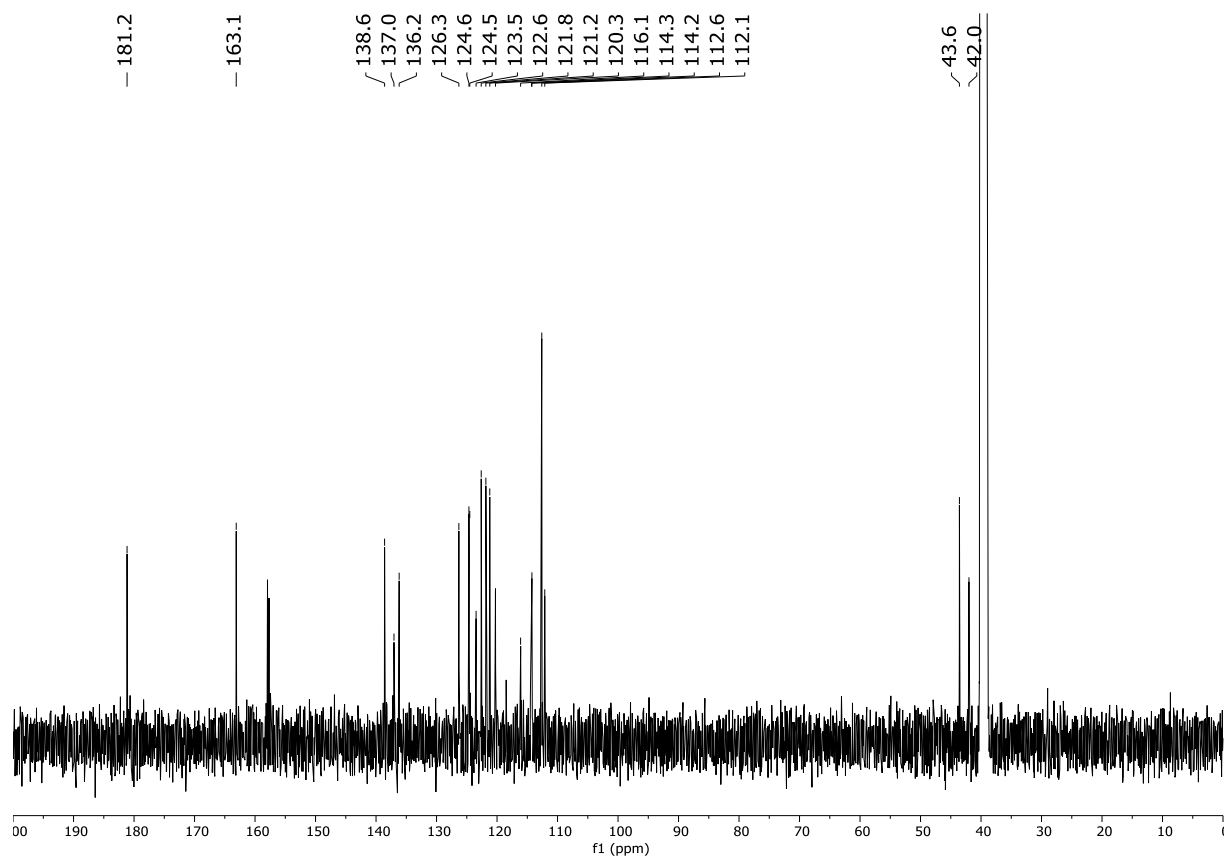
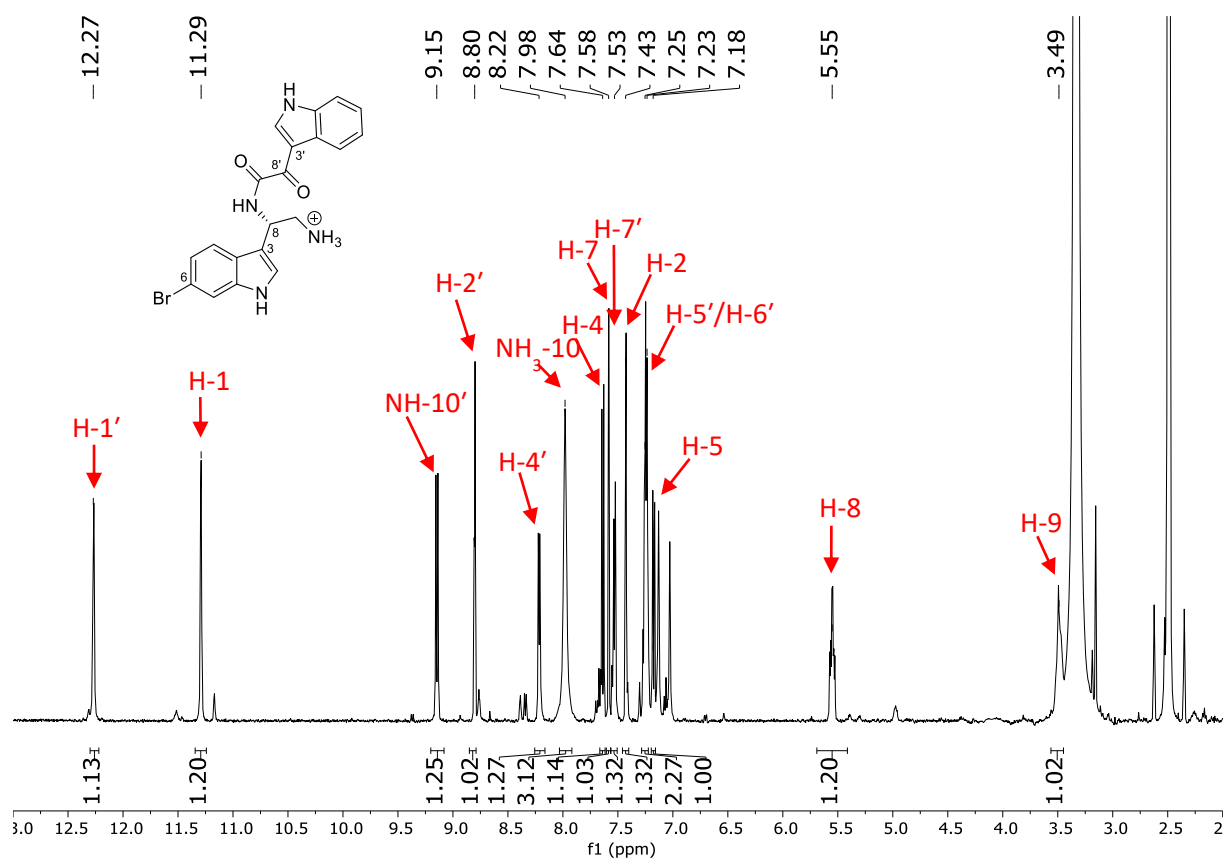
² National Marine Biodiscovery Laboratory of Ireland, Marine Institute, Renville West, Oranmore H91 R673, Ireland. laurence.jennings@marine.ie, navdeep.kaur@marine.ie, daniel.rodrigues@marine.ie

³ Discipline of Microbiology (School of Natural Sciences), Centre for One Health (Ryan Institute), Infection and Immunity Cluster (NCBES), National University of Ireland Galway, University Road, Galway, H91 TK33, Ireland. m.khan5@nuigalway.ie, aoife.boyd@nuigalway.ie.

⁴ Department of Natural Sciences, National Museums Northern Ireland, 153 Bangor Road, Holywood BT18 0EU, Northern Ireland. christine.morrow@nmni.com.

* Correspondence: olivier.thomas@nuigalway.ie; Tel.: +353-(0)-8-9455-6289

- P2 **Figure S1.** ¹H NMR spectrum of **1** at 500 MHz in DMSO-*d*₆.
- P2 **Figure S2.** ¹³C NMR spectrum of **1** at 125 MHz in DMSO- *d*₆.
- P3 **Figure S3.** COSY NMR spectrum of **1** at 500 MHz in DMSO- *d*₆.
- P3 **Figure S4.** HSQC NMR spectrum of **1** at 500 MHz in DMSO- *d*₆.
- P4 **Figure S5.** HMBC NMR spectrum of **1** at 600 MHz in DMSO- *d*₆.
- P4 **Figure S6.** HMBC NMR spectrum of **1** at 600 MHz in DMSO- *d*₆.
- P5 **Figure S7.** IR spectrum of **1**.
- P5 **Figure S8.** (+)ESIHRMS spectrum of **1**
- P6 **Figure S9.** ¹H NMR spectrum of **2** at 500 MHz in DMSO- *d*₆
- P6 **Figure S10.** ¹³C NMR spectrum of **2** at 125 MHz in DMSO- *d*₆.
- P7 **Figure S11.** COSY NMR spectrum of **2** at 500 MHz in DMSO- *d*₆.
- P7 **Figure S12.** HSQC NMR spectrum of **2** at 500 MHz in DMSO- *d*₆.
- P8 **Figure S13.** HMBC NMR spectrum of **2** at 600 MHz in DMSO- *d*₆.
- P8 **Figure S14.** HMBC NMR spectrum of **2** at 600 MHz in DMSO- *d*₆.
- P9 **Figure S15.** Experimental and calculated ECD spectrum of **2**.
- P9 **Figure S16.** (+)ESIHRMS spectrum of **2**
- P10 **Table S1.** Cytotoxicity (μM) of compounds **1-6** against HeLa cells measured after 6 and 24 h incubation.
- P10 **Table S2.** Cytotoxicity (μg/mL) of fractionated sponge extracts against HeLa cells measured after 6 and 24 h incubation.



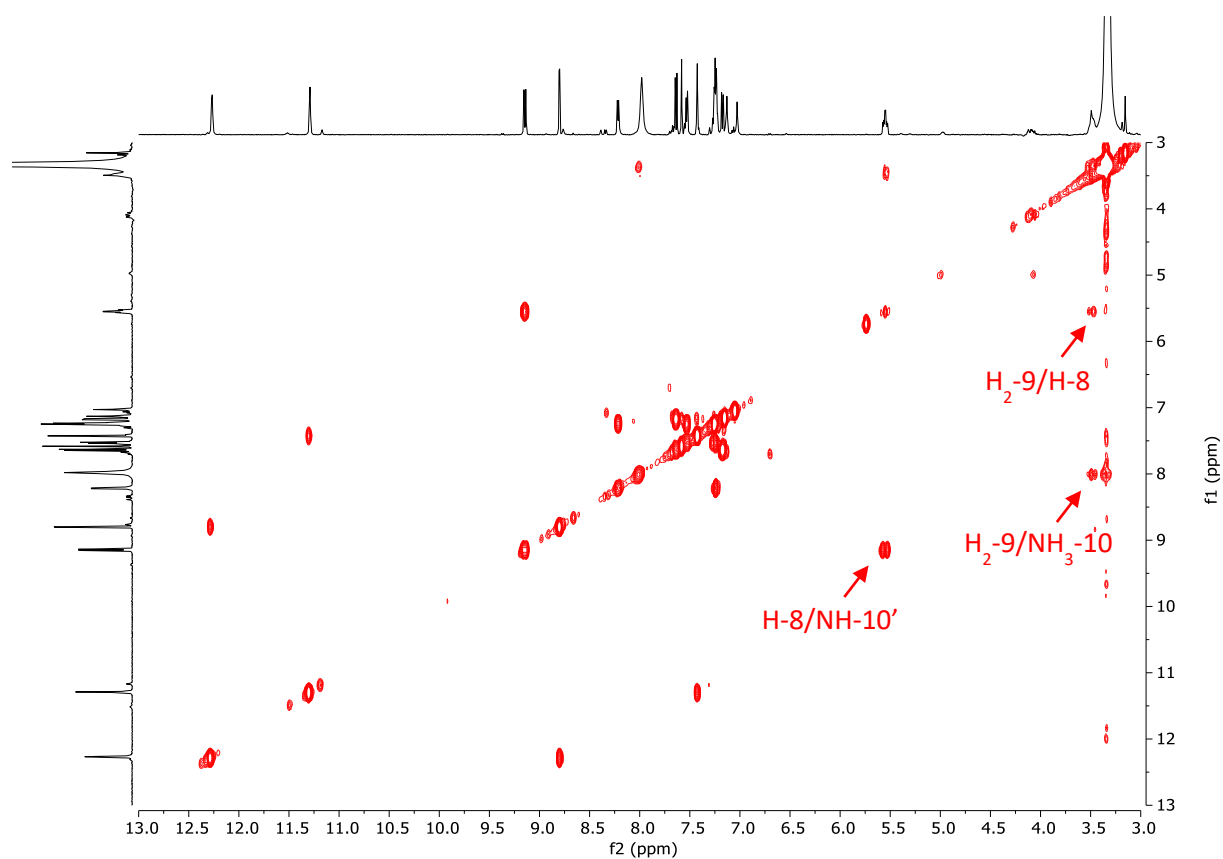


Figure S3. COSY NMR spectrum of **1** at 500 MHz in DMSO-*d*₆.

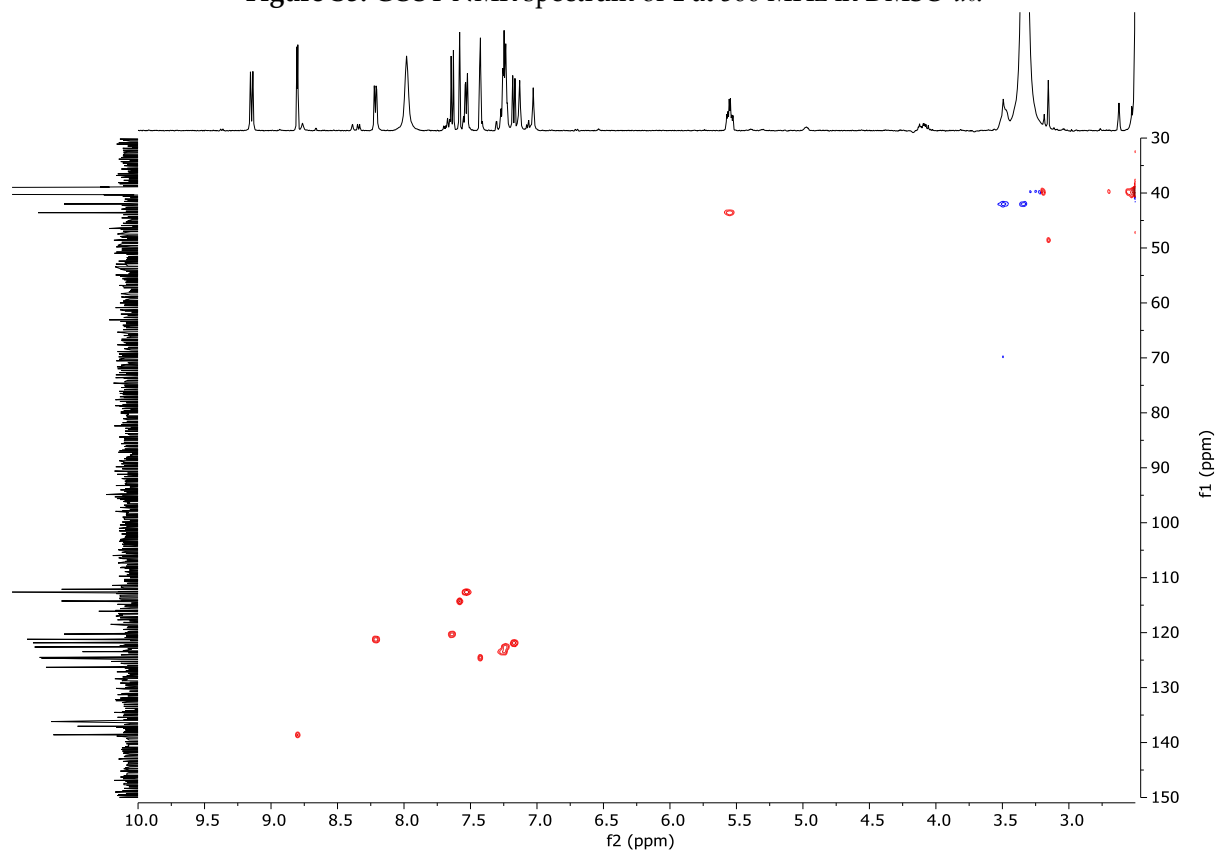


Figure S4. HSQC NMR spectrum of **1** at 500 MHz in DMSO-*d*₆.

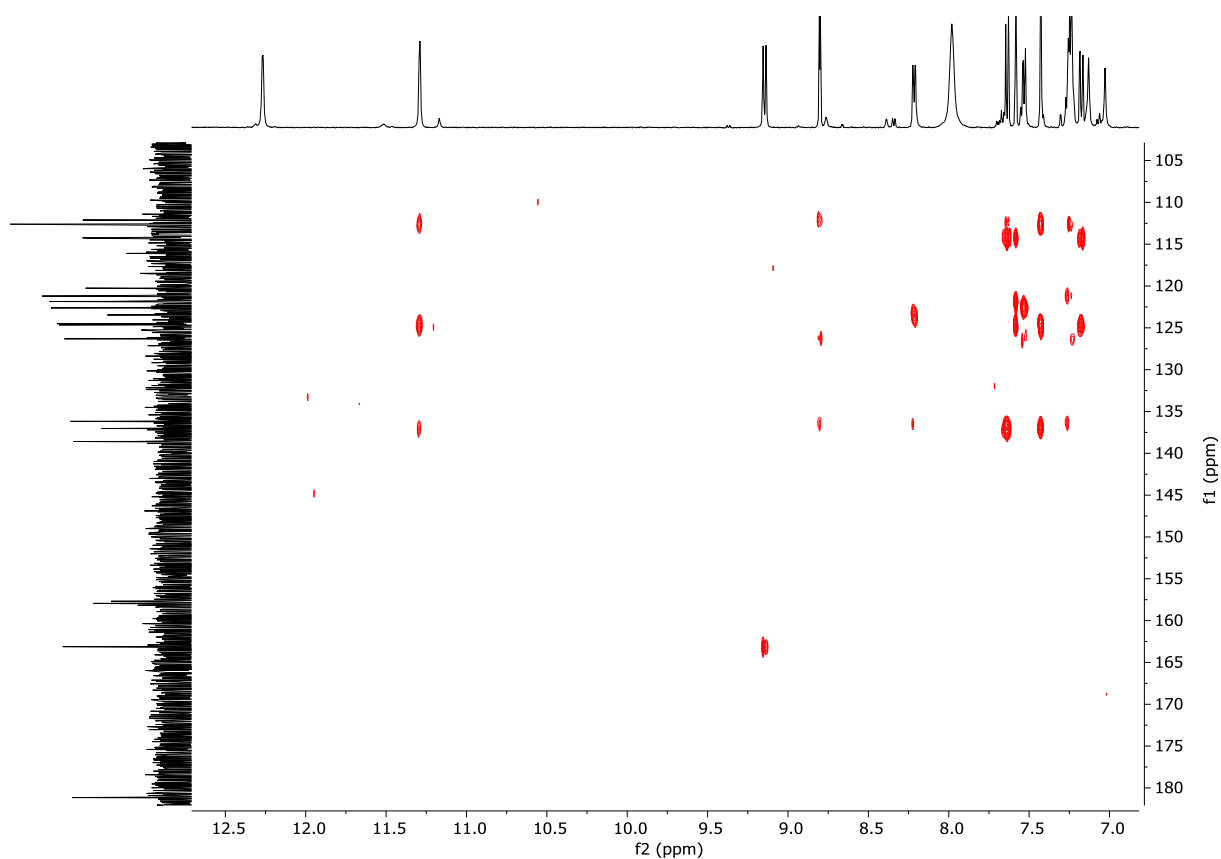


Figure S5. HMBC NMR spectrum of **1** at 600 MHz in DMSO-*d*₆.

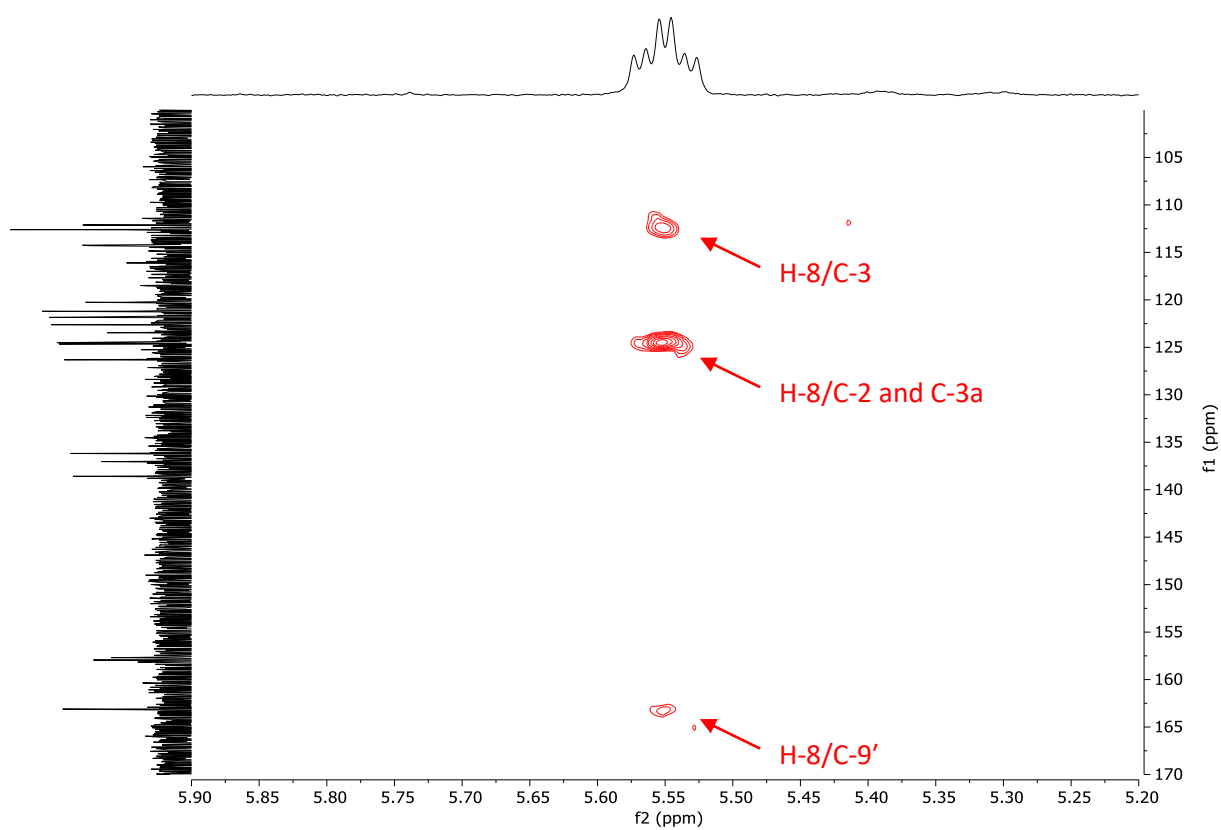


Figure S6. HMBC NMR spectrum of **1** at 600 MHz in DMSO-*d*₆.

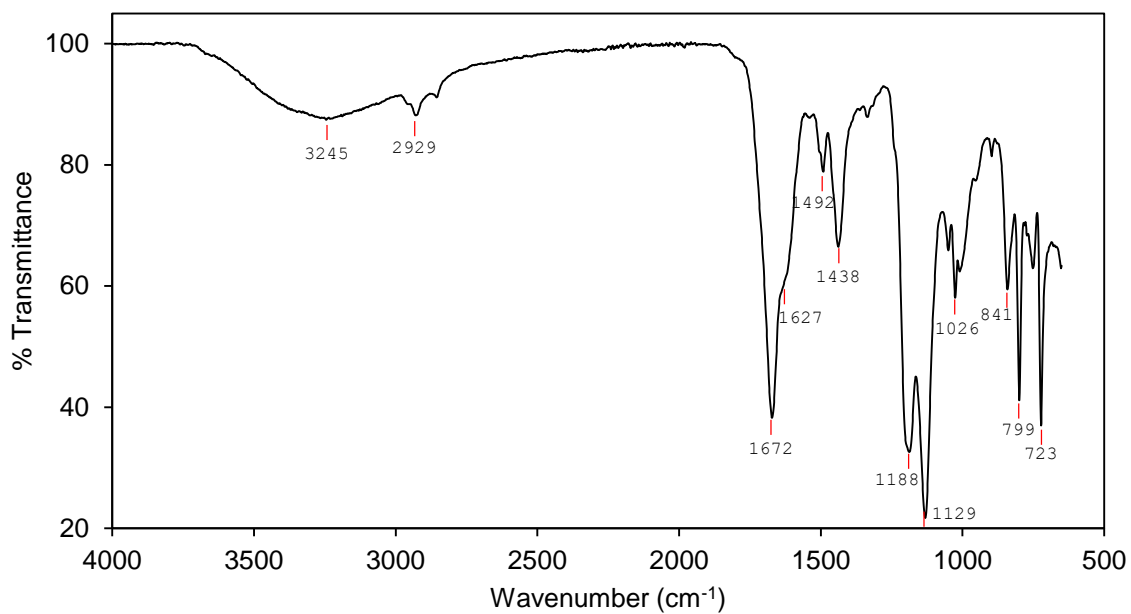


Figure S7. IR spectrum of 1.

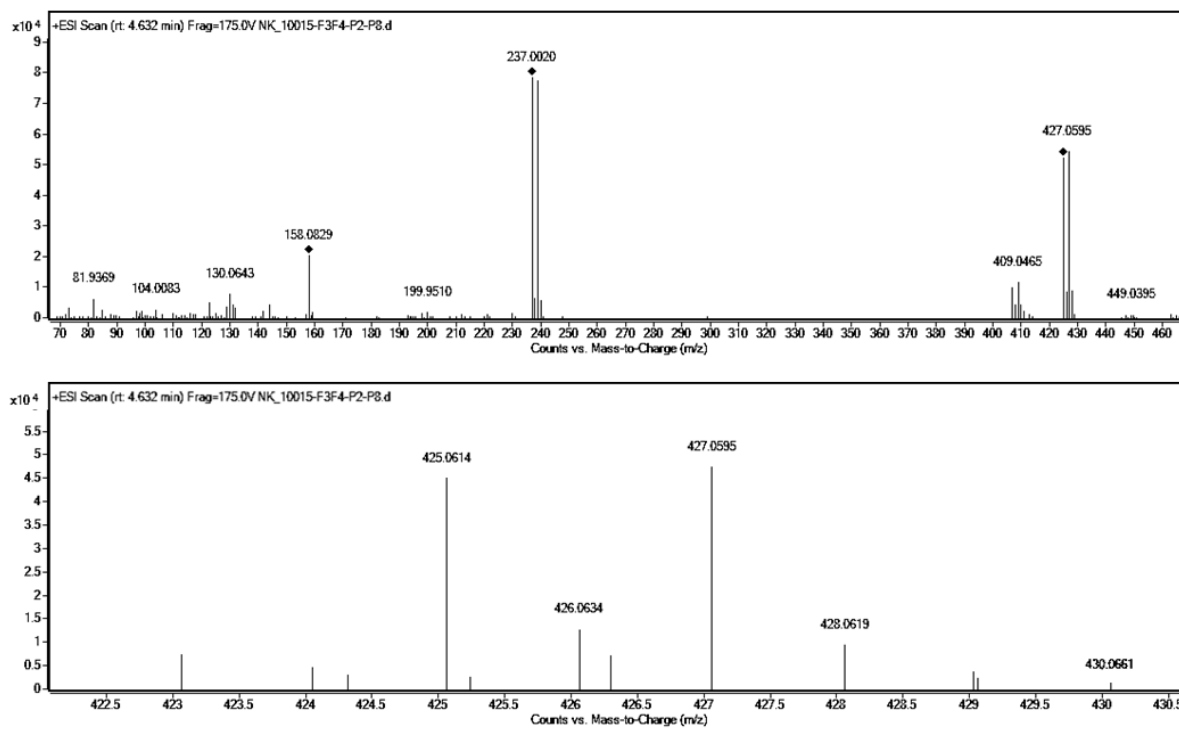
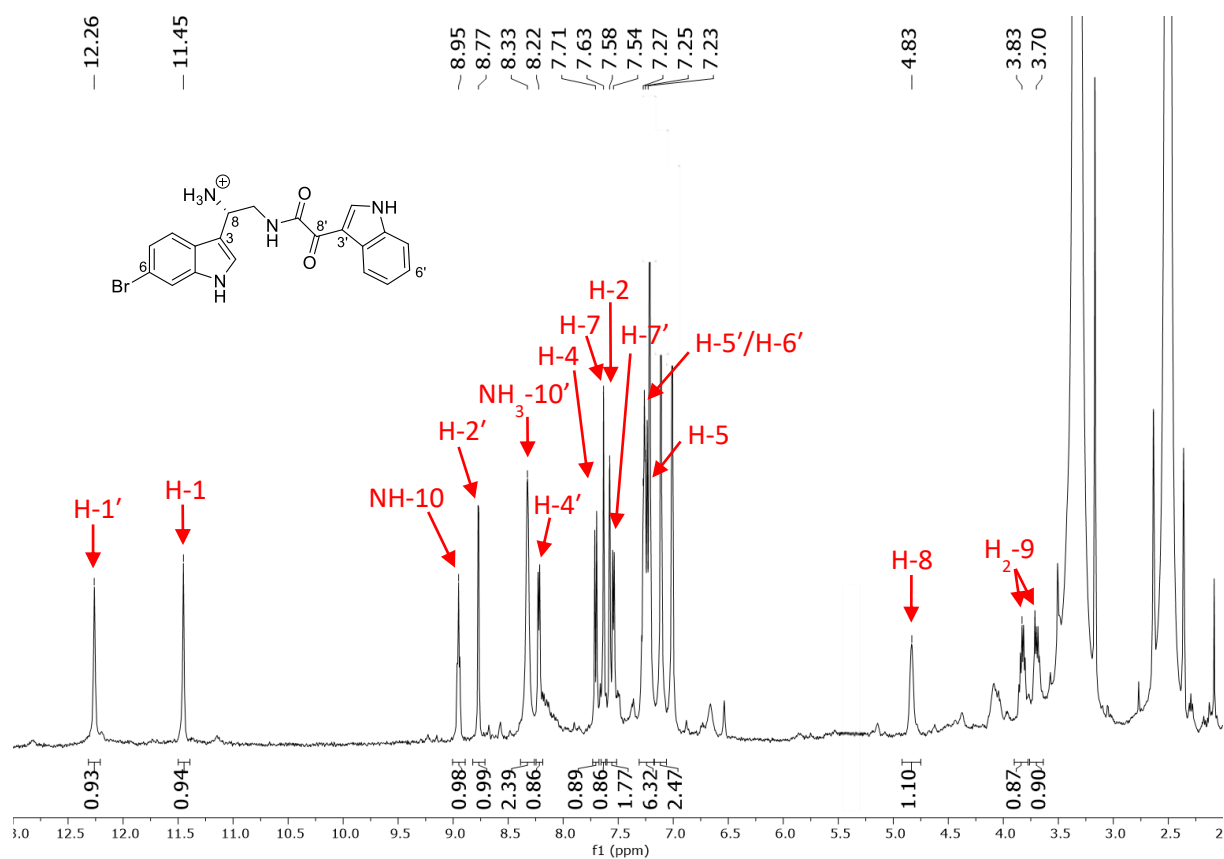
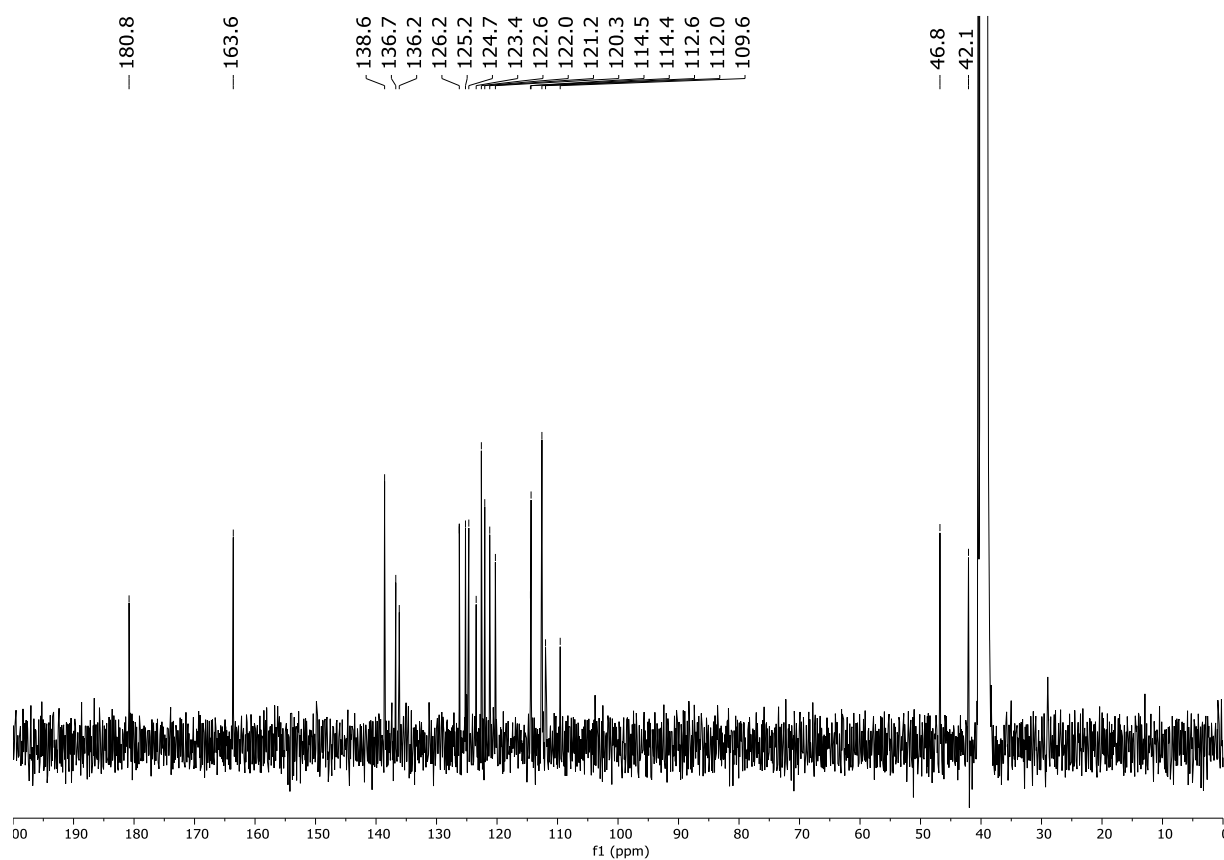


Figure S8. (+)ESIHRMS spectrum of 1

Figure S9. ^1H NMR spectrum of 2 at 500 MHz in $\text{DMSO-}d_6$.Figure S10. ^{13}C NMR spectrum of 2 at 125 MHz in $\text{DMSO-}d_6$.

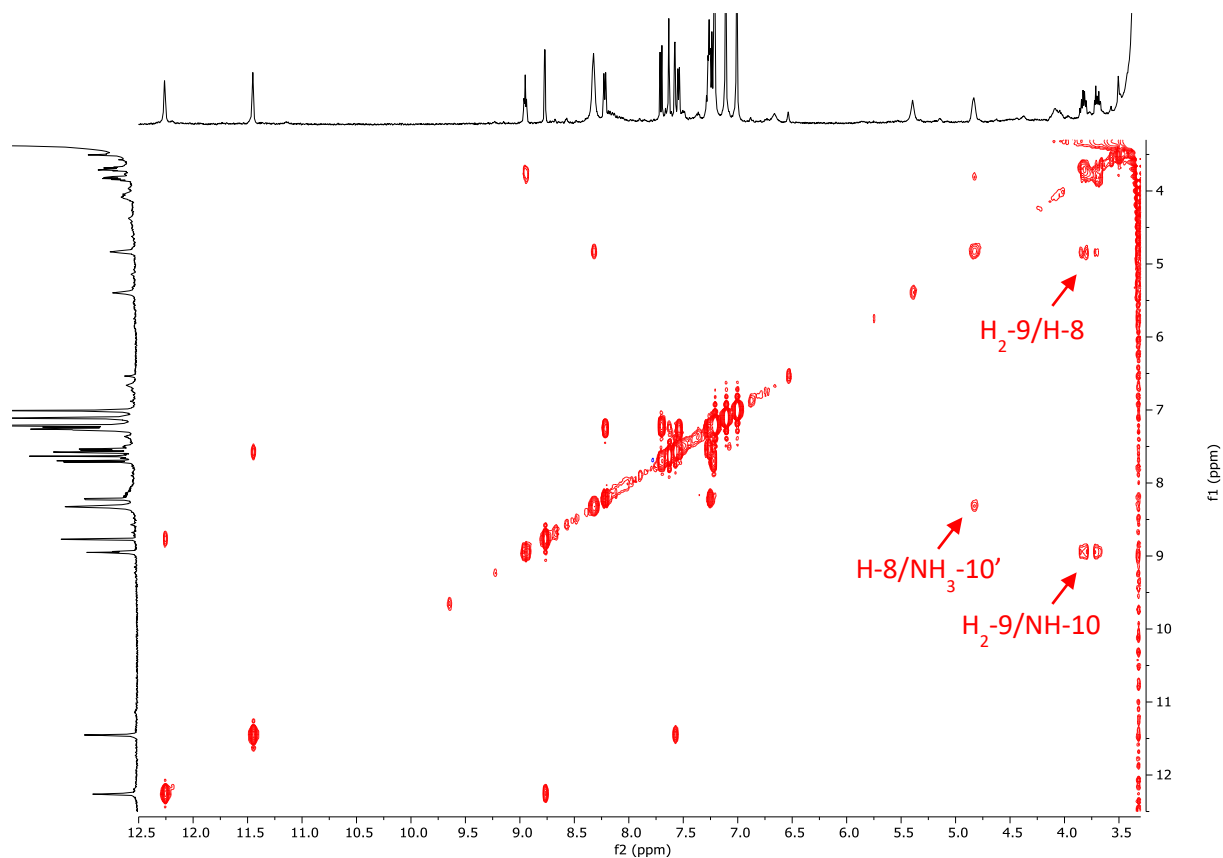


Figure S11. COSY NMR spectrum of **2** at 500 MHz in DMSO-*d*₆.

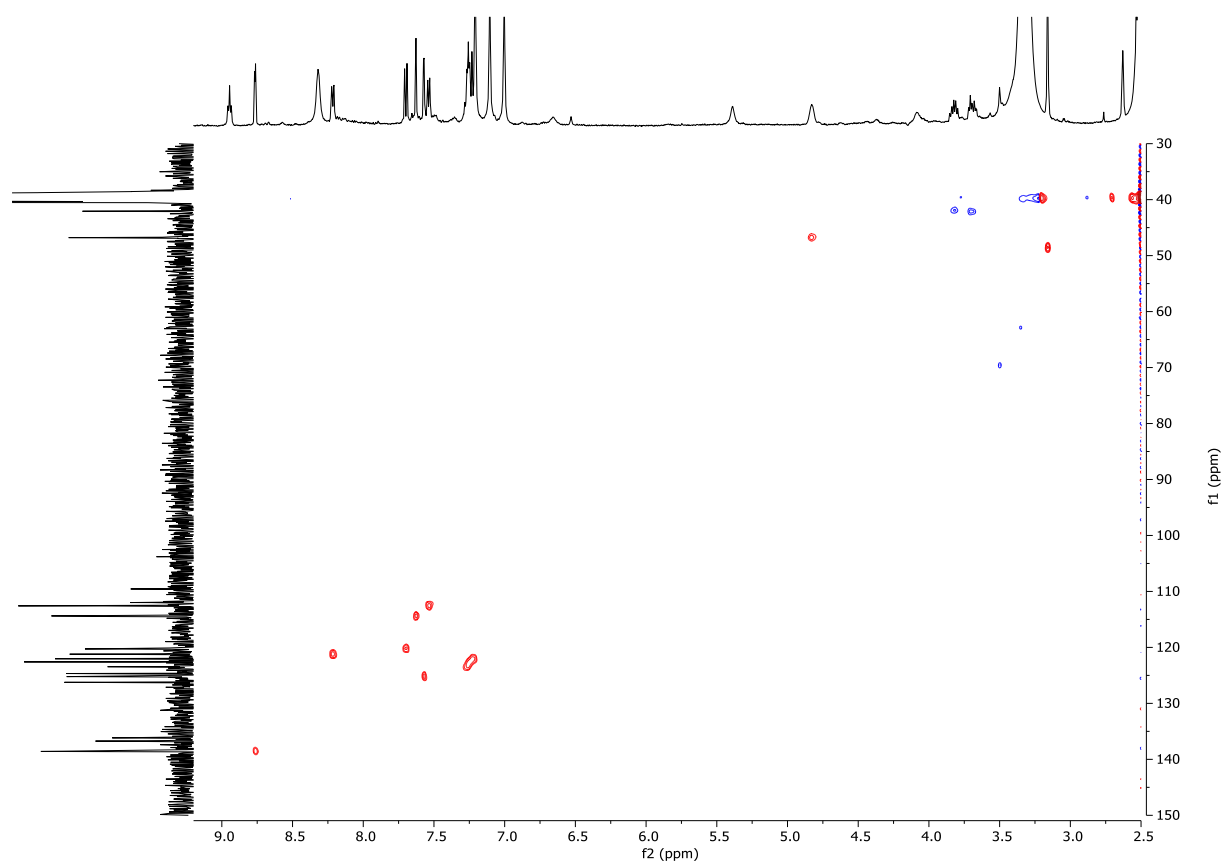
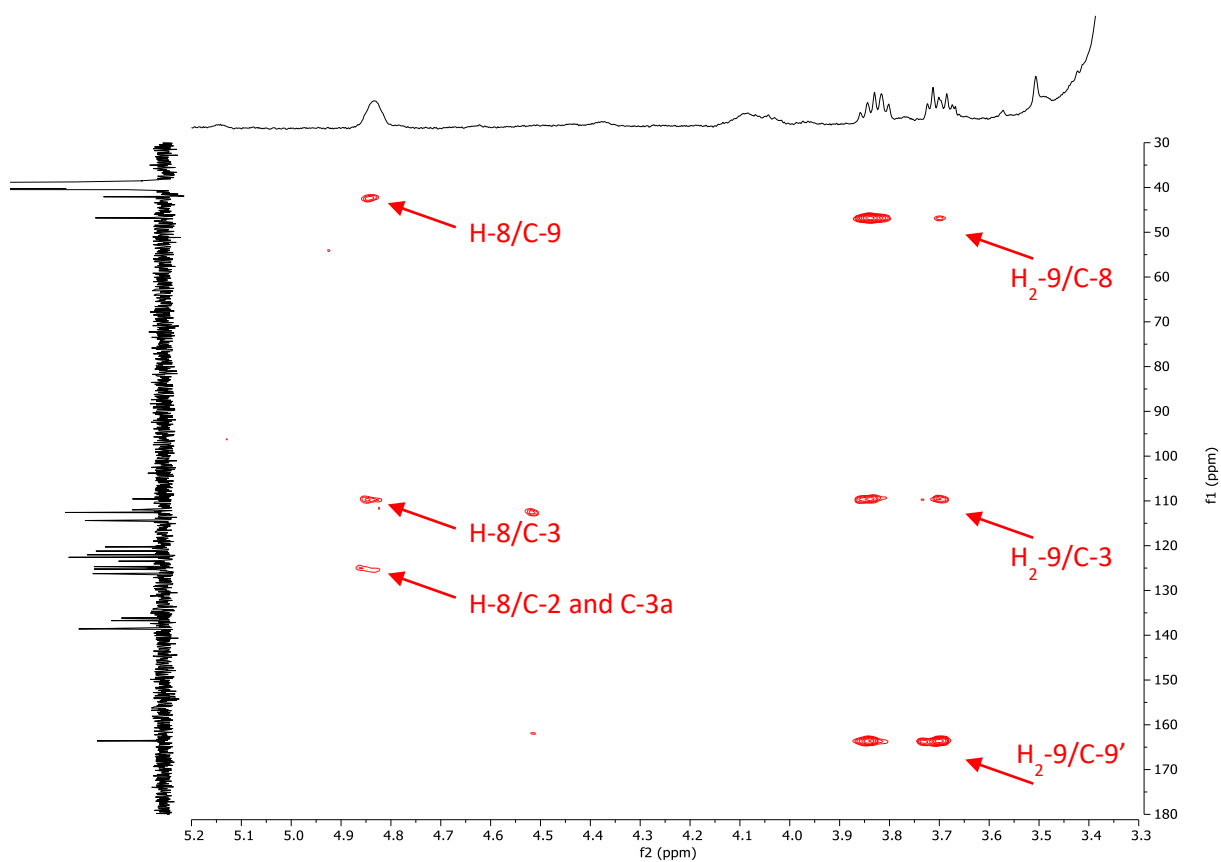
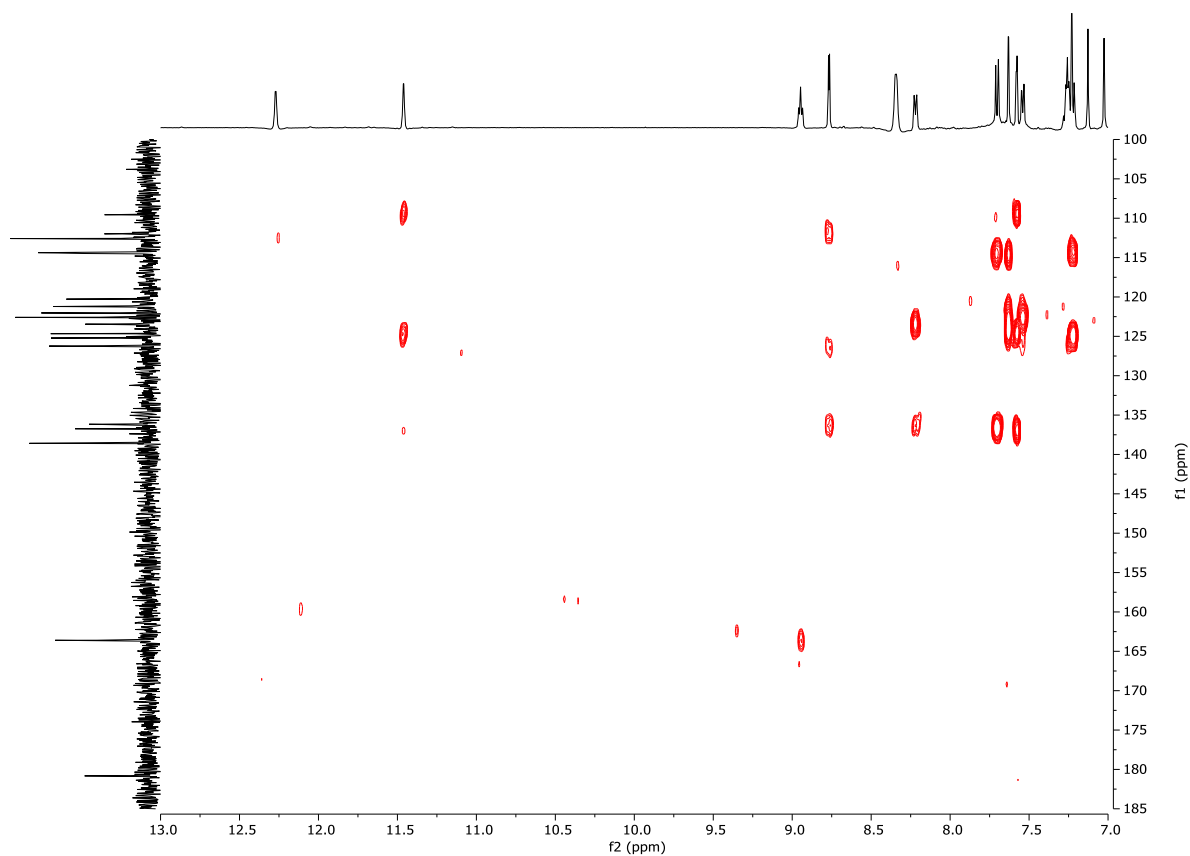


Figure S12. HSQC NMR spectrum of **2** at 500 MHz in DMSO-*d*₆.



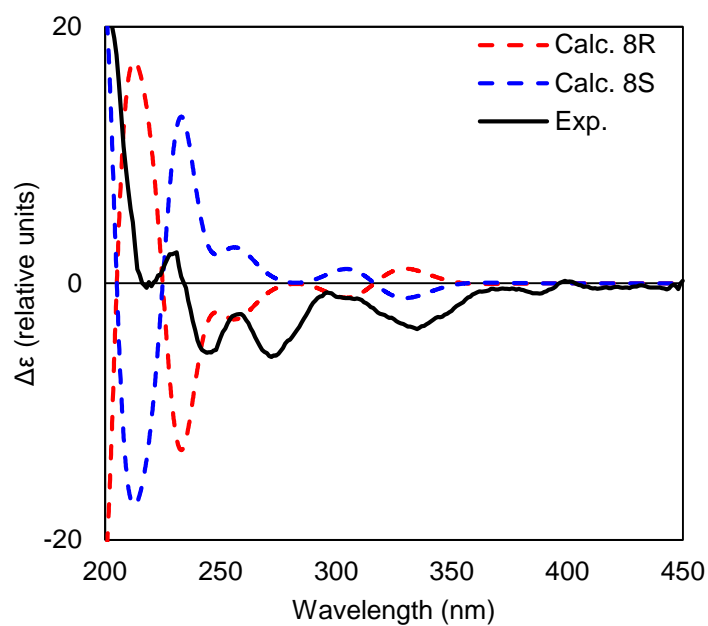


Figure S15. The comparison between the experimental and calculated ECD spectrum of **2**. The spectra were calculated using the B3LYP/6-311+G(2d,p)//B3LYP/6-311(2d,p) basis set/functional combination.

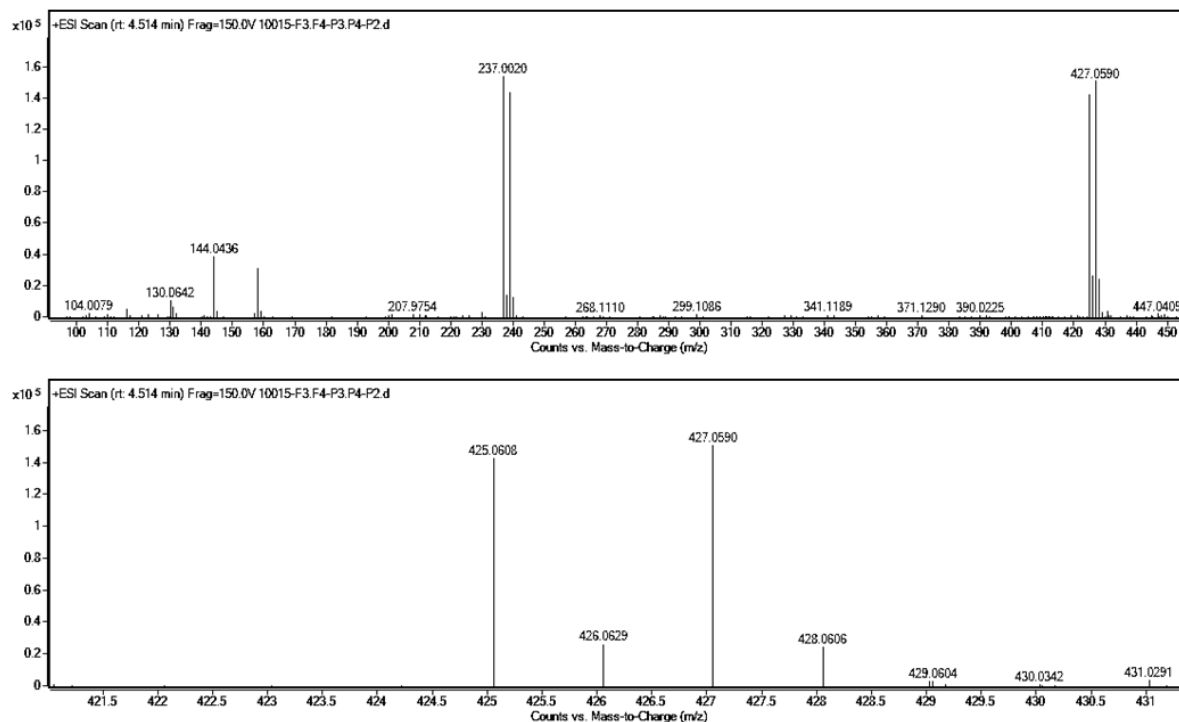


Figure S16. (+)ESIHRMS spectrum of **2**

Table S1. Cytotoxicity (μM) of compounds 1-6 against HeLa cells measured after 6 and 24 h incubation.

Compound	Cell Lysis IC ₅₀	
	6 h (IC ₅₀ $\mu\text{M} \pm \text{SD}$)	24 h (IC ₅₀ $\mu\text{M} \pm \text{SD}$)
Calcicamide A (1)	>200	>200
Calcicamide B (2)	165 \pm 7	146 \pm 13
<i>trans</i> -3,4-Dihydrohamacanthin A (3)	>200	>200
Bromodeoxytopsentin (4)	>200	125 \pm 2
Bromotopsentin (5)	>200	123 \pm 13
Spongotone A (6)	136 \pm 5	115 \pm 4

Table S2. Cytotoxicity of fractionated sponge extracts at 100 $\mu\text{g}/\text{mL}$ against HeLa cells measured after 6 and 24 h incubation.

Sponge species	Fraction ^a	% Cell Lysis at 100 $\mu\text{g}/\text{mL}$	
		6 h (% \pm SD)	24 h (% \pm SD)
<i>Axinella dissimilis</i>	fwm	2 \pm 2	6 \pm 3
	fm	2 \pm 1	8 \pm 4
	fmd	3 \pm 1	6 \pm 4
<i>Clathrina coriacea</i>	fwm	3 \pm 1	6 \pm 3
	fm	3 \pm 2	16 \pm 5
	fmd	3 \pm 2	9 \pm 7
<i>Clathria (Microciona) strepsitoxa</i>	fwm	3 \pm 2	5 \pm 3
	fm	3 \pm 2	5 \pm 2
	fmd	3 \pm 3	6 \pm 1
<i>Halichondria panicea</i>	fwm	4 \pm 2	5 \pm 2
	fm	2 \pm 2	3 \pm 1
	fmd	2 \pm 2	4 \pm 1
<i>Lophon hyndmani</i>	fwm	2 \pm 2	6 \pm 2
	fm	4 \pm 2	13 \pm 6
	fmd	4 \pm 2	9 \pm 5
<i>Mycale rotalis</i>	fwm	3 \pm 2	6 \pm 3
	fm	4 \pm 1	4 \pm 1
	fmd	4 \pm 3	5 \pm 2
<i>Polymastia boletiformis</i>	fwm	2 \pm 1	5 \pm 2
	fm	2 \pm 1	6 \pm 5
	fmd	2 \pm 1	3 \pm 2
<i>Polymastia penicillus</i>	fwm	4 \pm 3	8 \pm 6
	fm	8 \pm 3	23 \pm 6
	fmd	3 \pm 1	8 \pm 4
<i>Spongosorites calcicola</i>	fwm	43\pm8 ^b	61\pm6 ^b
	fm	15 \pm 6	17 \pm 2
	fmd	3 \pm 2	6 \pm 4
<i>Tethya sp</i>	fwm	3 \pm 2	6 \pm 4
	fm	2 \pm 1	4 \pm 1
	fmd	6 \pm 3	10 \pm 1

^a SPE fractions of crude extracts: desalting: water elution; fwm : water:methanol (1 :1) elution; fm: methanol elution; fmd: methanol:dichloromethane (1 :1) elution. ^b Bold values indicate cytotoxicity 2 standard deviations from the mean cytotoxicity.