

Supplementary Information

Discovery of a parallel family of euglenatide analogs in *Euglena gracilis*

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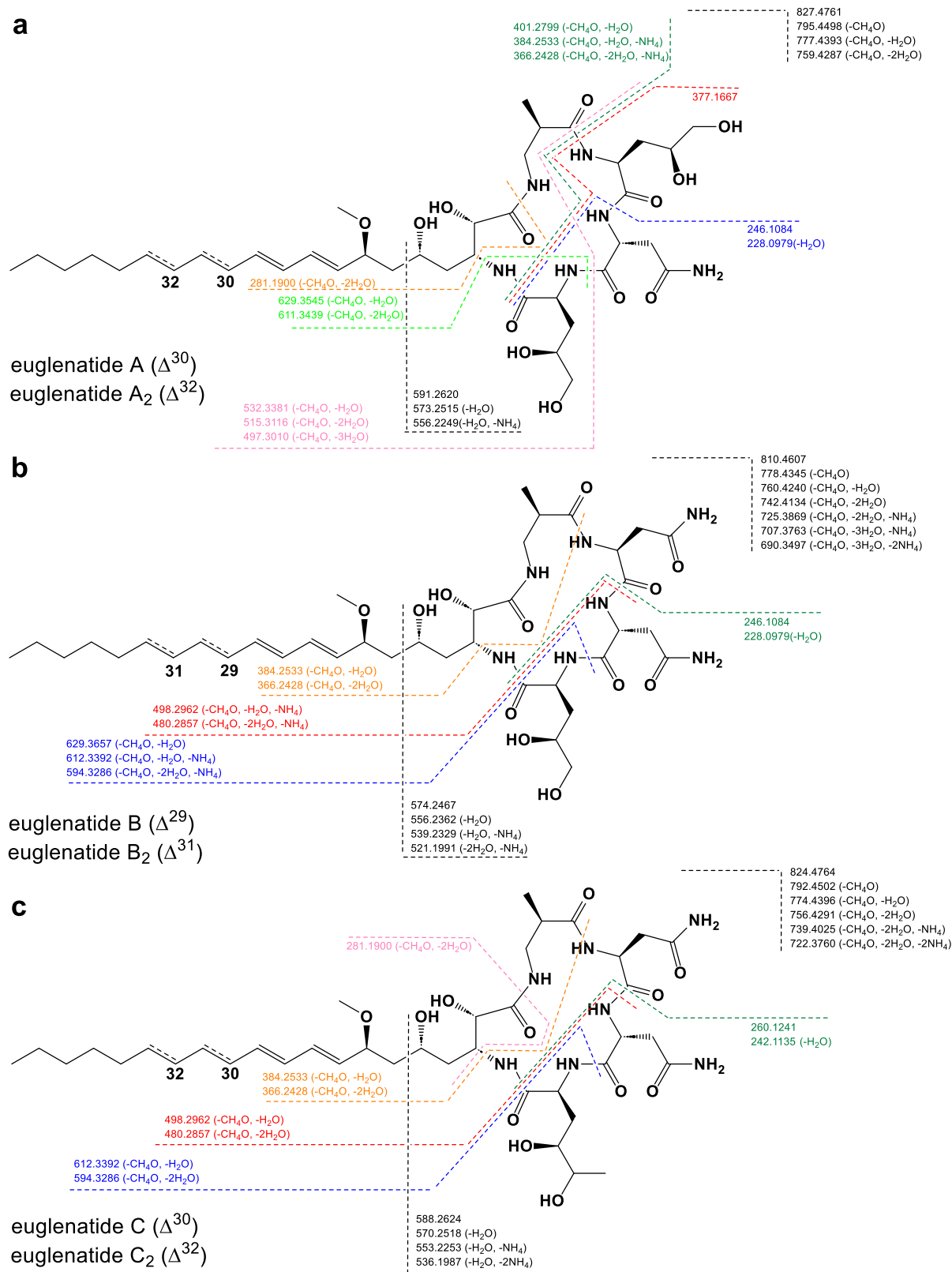


Figure S1. MS-MS fragmentation patterns for the euglenatides and their analogs. MS-MS fragmentation patterns for euglenatides A and A₂ (a), euglenatides B and B₂ (b), and euglenatides C and C₂ (c).

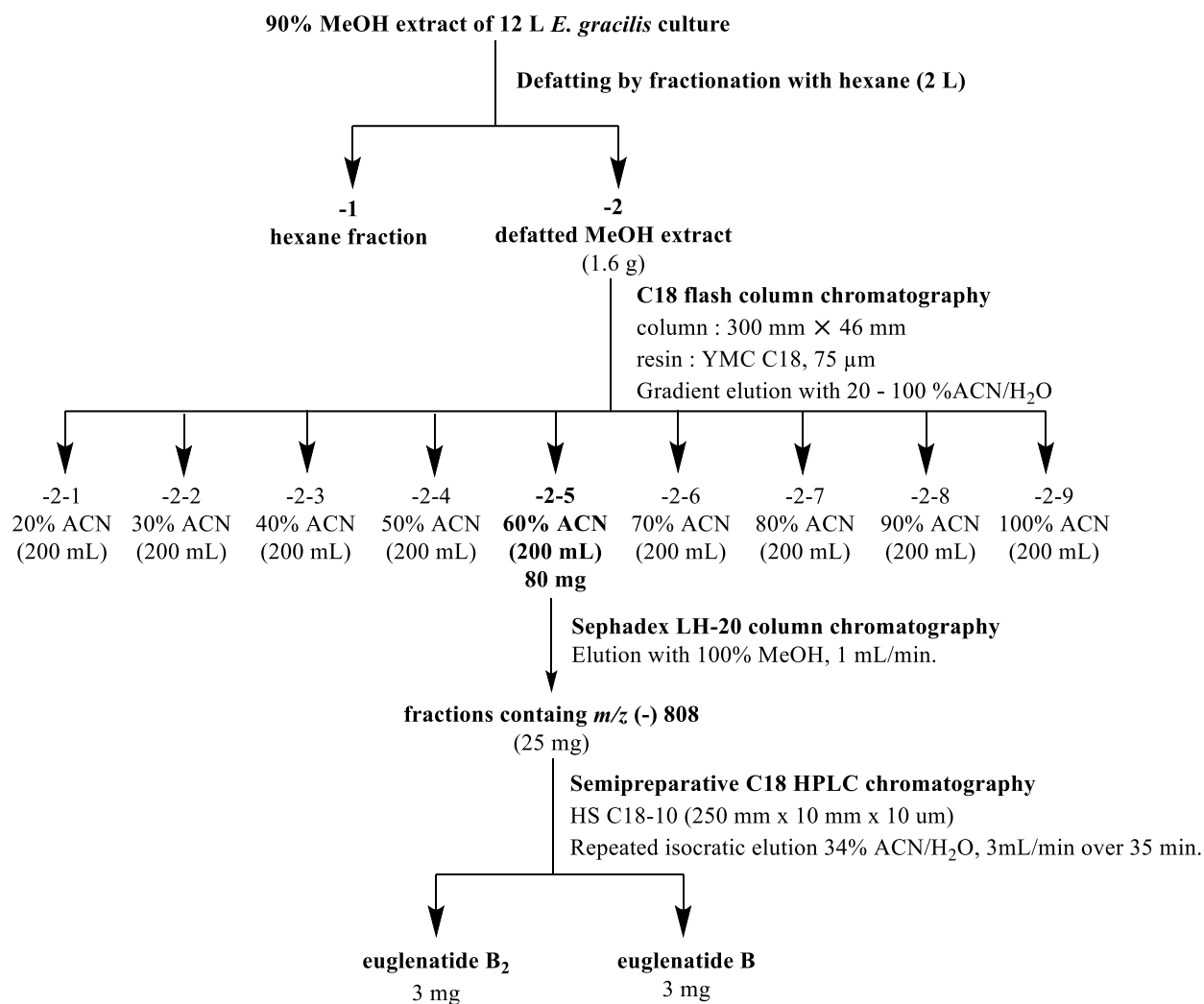
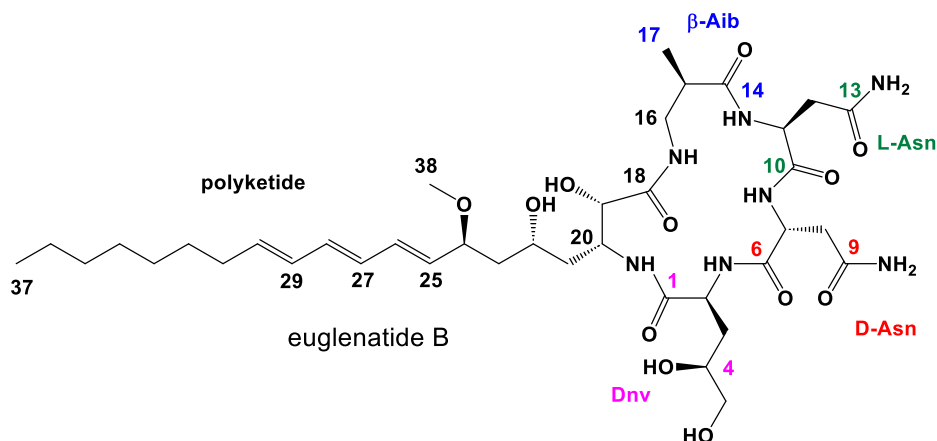


Figure S2. Isolation scheme of euglenatides.

Table S1. Comparison of NMR data for euglenatide B and previously reported data. 1D and 2D NMR (600 MHz, DMSO-*d*₆) data for euglenatide B are compared to previously reported data for euglenatide B.



No.	euglenatide B (data reported here)			euglenatide B (previously reported data)		
	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)
Dnv						
1	C	172.5	---	C	172.4	---
2	CH	50.3	4.30, ddd (11.0, 7.8, 2.8)	CH	50.3	4.32, m
2-NH	NH	---	7.65, d (7.8)	NH	---	7.65, d (7.8)
3	CH ₂	34.3	1.82, ddd (14.0, 11.0, 2.8)	CH ₂	34.3	1.86, m
			1.70, m			1.71, m
4	CH	67.5	3.44, m	CH	67.5	3.43, m
4-OH	OH	---	4.49, d (5.0)	OH	---	4.44, br d (4.7)
5	CH ₂	66.3	3.31, m	CH ₂	66.3	3.32, m
			3.24, m			3.26, m
5-OH	OH	---	4.39, m	OH	---	4.38, m
D-Asn						
6	C	171.2	---	C	171.1	---
7	CH	49.7	4.51, m	CH	49.3	4.45, m
7-NH	NH	---	8.20, br d (7.8)	NH		7.77, d (7.9)
8	CH ₂	36.2	2.93, dd (16.0, 7.0)	CH ₂	35.8	2.94, dd (16.8, 5.0)
			2.56, dd (16.0, 3.6)			2.57, dd (16.9, 3.7)
9	C	173.1	---	C	173.4	---
9-NH	NH ₂	---	7.87, s	NH ₂	---	7.78 s
			7.14, s			7.29, s
L-Asn						
10	C	169.7	---	C	169.6	---
11	CH	51.6	4.04, m	CH	51.6	4.06, m
11-NH	NH	---	9.05, d (6.4)	NH	---	9.02, d (6.5)
12	CH ₂	35.2	2.86, dd (15.8, 4.0)	CH ₂	35.0	2.86, dd (15.7, 4.0)
			2.43, dd (15.8, 9.3)			2.47, dd (15.9, 4.7)
13	C	172.2	---	C	172.1	---
13-NH	NH ₂	---	7.37, s	NH ₂	---	7.36, s
			6.86, s			6.88, s

No.	euglenatide B (data reported here)			euglenatide B (previously reported data)		
	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)
β-Aib						
14	C	176.4	---	C	176.3	---
15	CH	37.6	2.66, m	CH	37.5	2.66, m
16	CH ₂	41.7	3.23, m	CH ₂	41.7	3.27, m
			2.99, br d (13.2)			3.03, br d (13.0)
16-NH	NH	---	7.72, br s	NH	---	7.06, s
17	CH ₃	17.2	1.03, d (7.0)	CH ₃	16.8	1.04, d (7.1)
Polyketide						
18	C	172.1	---	C	172.3	---
19	CH	72.6	3.89, m	CH	72.9	3.86, dd (9.6, 7.7)
19-OH	OH	---	5.10, br s	OH	---	5.13, br d (7.7)
20	CH	49.1	3.88, m	CH	49.1	3.92, m
20-NH	NH	---	6.93, d (9.1)	NH	---	6.90, d (9.6)
21	CH ₂	40.5	1.69, m	CH ₂	40.3	1.69, m
			1.19, m			1.20, m
22	CH	62.9	3.49, m	CH	63.0	3.47, m
22-OH	OH	---	4.38, m	OH	---	4.31, m
23	CH ₂	43.7	1.47, m	CH ₂	43.8	1.49, m
			1.33, m			1.30, br dd (14.5, 5.1)
24	CH	77.8	3.72, ddd (11.5, 7.6, 3.7)	CH	77.8	3.73, m
25	CH	133.9	5.50, dd (14.6, 7.6)	CH	133.9	5.51, dd (14.6, 7.6)
26	CH	131.2	6.19, dd (14.6, 10.4)	CH	131.3	6.20, dd (14.6, 10.3)
27	CH	130.0	6.14, dd (14.6, 10.4)	CH	130.0	6.15, dd (14.6, 10.3)
28	CH	132.8	6.23, dd (14.5, 10.4)	CH	132.8	6.24, dd (14.5, 10.4)
29	CH	130.3	6.06, dd (15.0, 10.4)	CH	130.3	6.07, dd (15.1, 10.4)
30	CH	135.1	5.71, dt (15.0, 7.1)	CH	135.1	5.72, dt (15.1, 7.1)
31	CH ₂	32.1	2.06, q (7.0)	CH ₂	32.1	2.06, q (7.0)
32	CH ₂	28.7	1.34, m	CH ₂	28.7	1.35, m
33	CH ₂	28.50	1.25, m	CH ₂	28.52	1.25, m
34	CH ₂	28.52	1.25, m	CH ₂	28.54	1.25, m
35	CH ₂	31.2	1.24, m	CH ₂	31.2	1.24, m
36	CH ₂	22.0	1.26, m	CH ₂	22.0	1.25, m
37	CH ₃	13.9	0.85, t (7.0)	CH ₃	13.9	0.85, t (6.8)
38	OCH ₃	55.5	3.13, s	OCH ₃	55.5	3.13, s

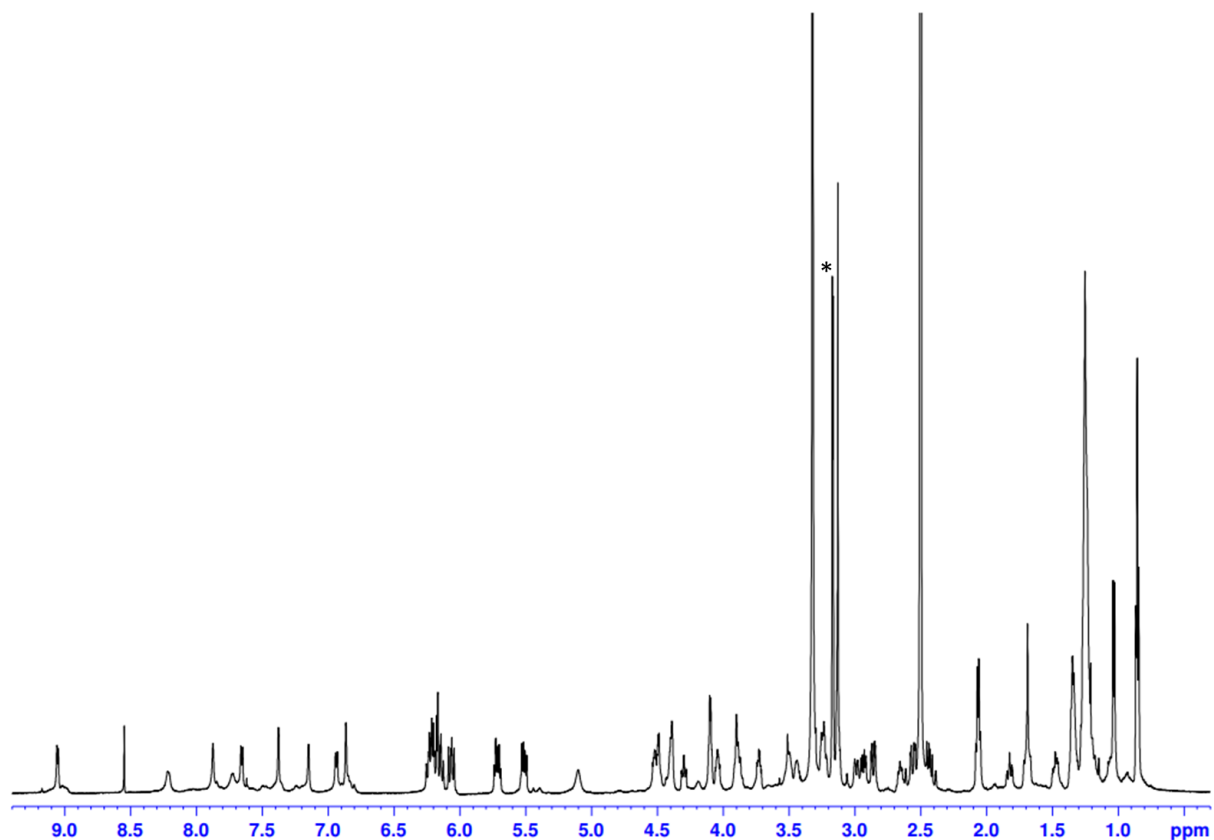


Figure S3. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) spectrum for euglenatide B. *Residual methanol solvent.

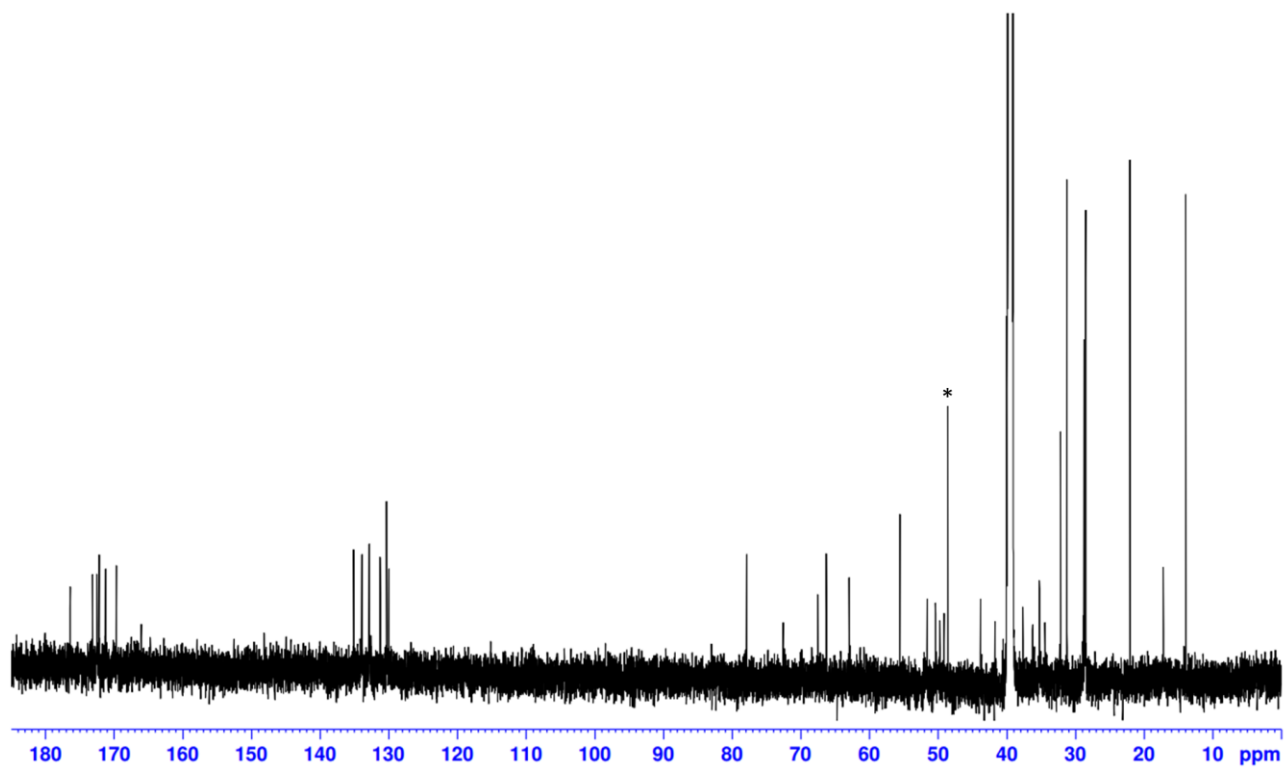
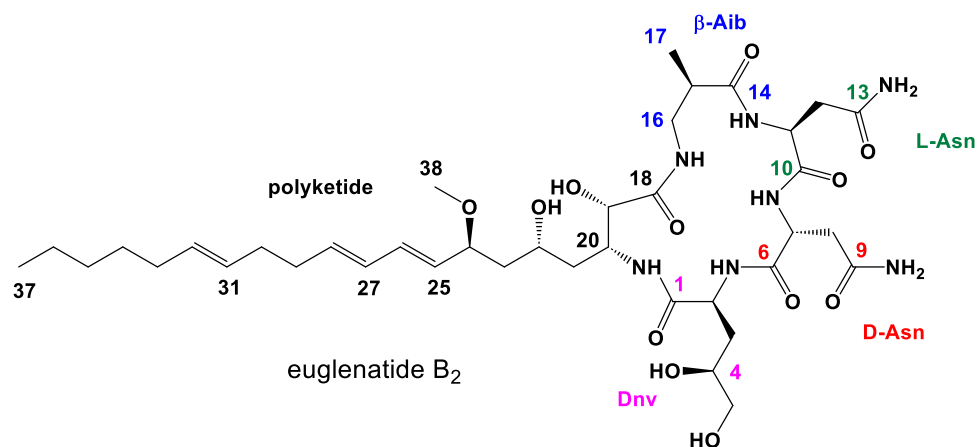


Figure S4. ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) spectrum for euglenatide B. *Residual methanol solvent.

Table S2. 1D and 2D NMR (600 MHz, DMSO-*d*₆) data for euglenatide B₂.



No.	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)	COSY	HMBC
Dnv					
1	C	172.5	---	---	---
2	CH	50.3	4.29, ddd (11.0, 7.8, 2.8)	2-NH, 3	
2-NH	NH	---	7.65, d (8.0)	2	6
3	CH ₂	34.4	1.82, ddd (13.8, 10.7, 2.8)	2, 4	
			1.69, m	2, 4	
4	CH	67.5	3.43, m	3	
4-OH	OH	---	4.48, d (5.0)		
5	CH ₂	66.3	3.31, m		
			3.24, m		
5-OH	OH	---	4.39, m		
D-Asn					
6	C	171.2	---	---	---
7	CH	49.7	4.50, m	7-NH, 8	
7-NH	NH	---	8.13, br d (7.4)	7	
8	CH ₂	36.2	2.92, dd (16.0, 6.8)	7	6, 7, 9
			2.55, dd (16.0, 3.7)	7	6, 7, 9
9	C	173.2	---		
9-NH	NH ₂	---	7.88, s		
			7.17, s		
L-Asn					
10	C	169.7	---	---	---
11	CH	51.6	4.04, ddd (9.8, 6.4, 4.1)	11-NH, 12	
11-NH	NH	---	9.04, d (6.7)	11	10, 11, 14
12	CH ₂	35.2	2.85, dd (15.6, 4.0)	11	10, 11, 13
			2.43, dd (15.6, 9.3)	11	10, 11, 13
13	C	172.1	---	---	---
13-NH	NH ₂	---	7.37, s		12
			6.86, s		
β-Aib					

No.	Type	¹³ C ppm	¹ H ppm mult. (<i>J</i> in Hz)	COSY	HMBC
14	C	176.4	---	---	---
15	CH	37.6	2.65, (9.8, 7.1, 2.6)	16, 17	
16	CH ₂	41.7	3.23, m	16-NH, 15	18
			2.99, br d (13.2)	16-NH, 15	18
16-NH	NH	---	7.61, br s	16	
17	CH ₃	17.1	1.03, d (7.0)	15	14, 15, 16
Polyketide					
18	C	172.2	---	---	---
19	CH	72.6	3.89, m		20
19-OH	OH	---	5.10, very broad		
20	CH	49.1	3.88, m	20-NH, 21	
20-NH	NH	---	6.92, d (8.9)	20	
21	CH ₂	40.5	1.68, m	20, 22	
			1.18, m	20, 22	
22	CH	62.9	3.49, m	21, 23	
22-OH	OH	---	4.36, m		
23	CH ₂	43.8	1.46, m	22, 24	
			1.31, m	22, 24	
24	CH	77.8	3.7, ddd (11.5, 8.1, 3.5)	23, 25	
25	CH	132.2	5.40, m	24, 26	
26	CH	131.3	6.12, dd (15.0, 10.4)	25, 27	24, 28
27	CH	129.9	6.04, dd (15.0, 10.4)	26, 28	25, 29
28	CH	133.8	5.69, dt (14.5, 6.5)	27, 29	26, 29, 30
29	CH ₂	32.2	2.10, m	28, 30	27, 28, 31
30	CH ₂	31.7	2.04, m	29, 31	31, 32
31	CH	129.3	5.38, dd (16.2, 5.4) ^a	30	30, 33
32	CH	130.5	5.40, dd (16.2, 5.4) ^a	33	30, 33, 34
33	CH ₂	31.2	1.93, q (6.1)		31, 32, 34, 35
34	CH ₂	28.6	1.30, m		32, 33, 35, 36
35	CH ₂	30.7	1.22, m		36
36	CH ₂	21.9	1.25, m	37	
37	CH ₃	13.9	0.85, t (7.0)	36	35, 36
38	OCH ₃	55.5	3.11, s		24

^a *J* values were obtained from DQF-COSY data.

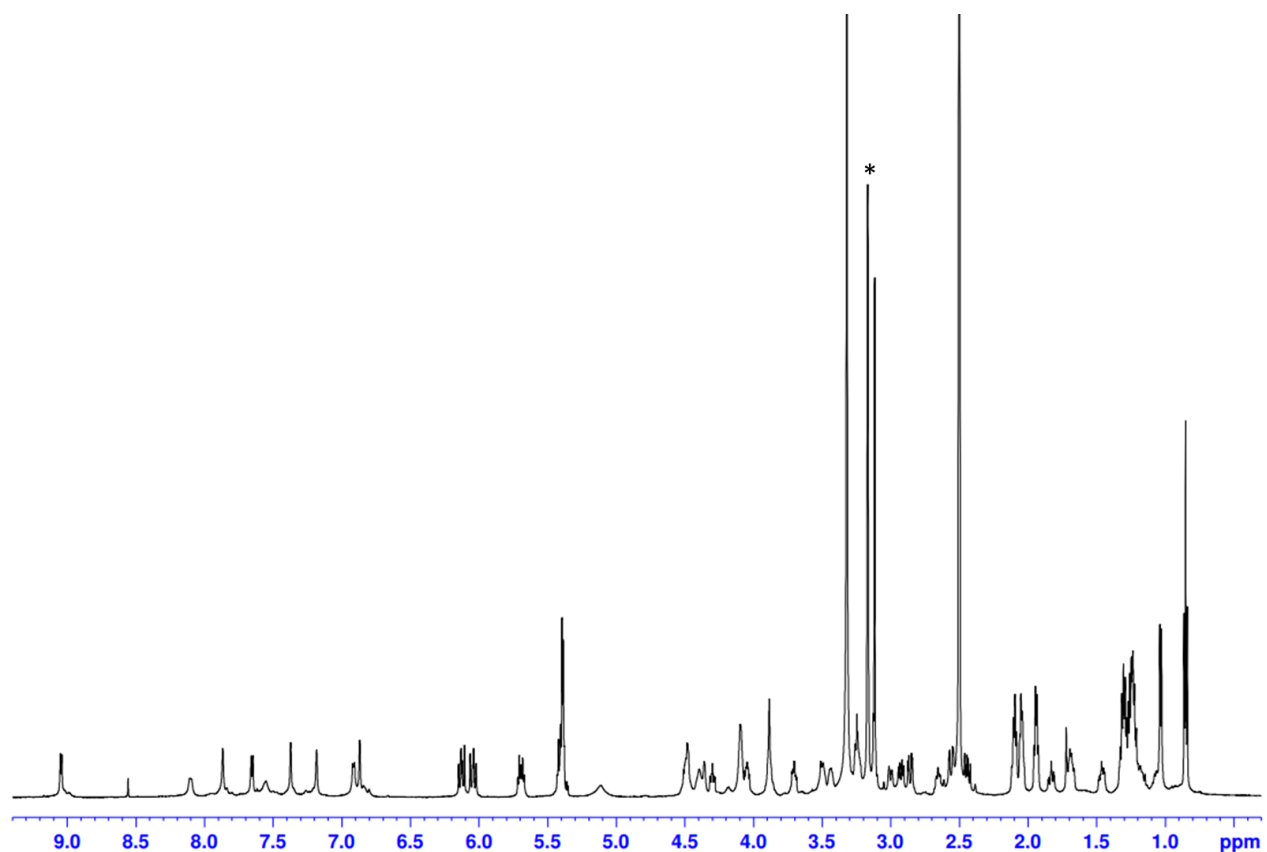


Figure S5. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) spectrum for euglenatide B_2 . * Residual methanol solvent.

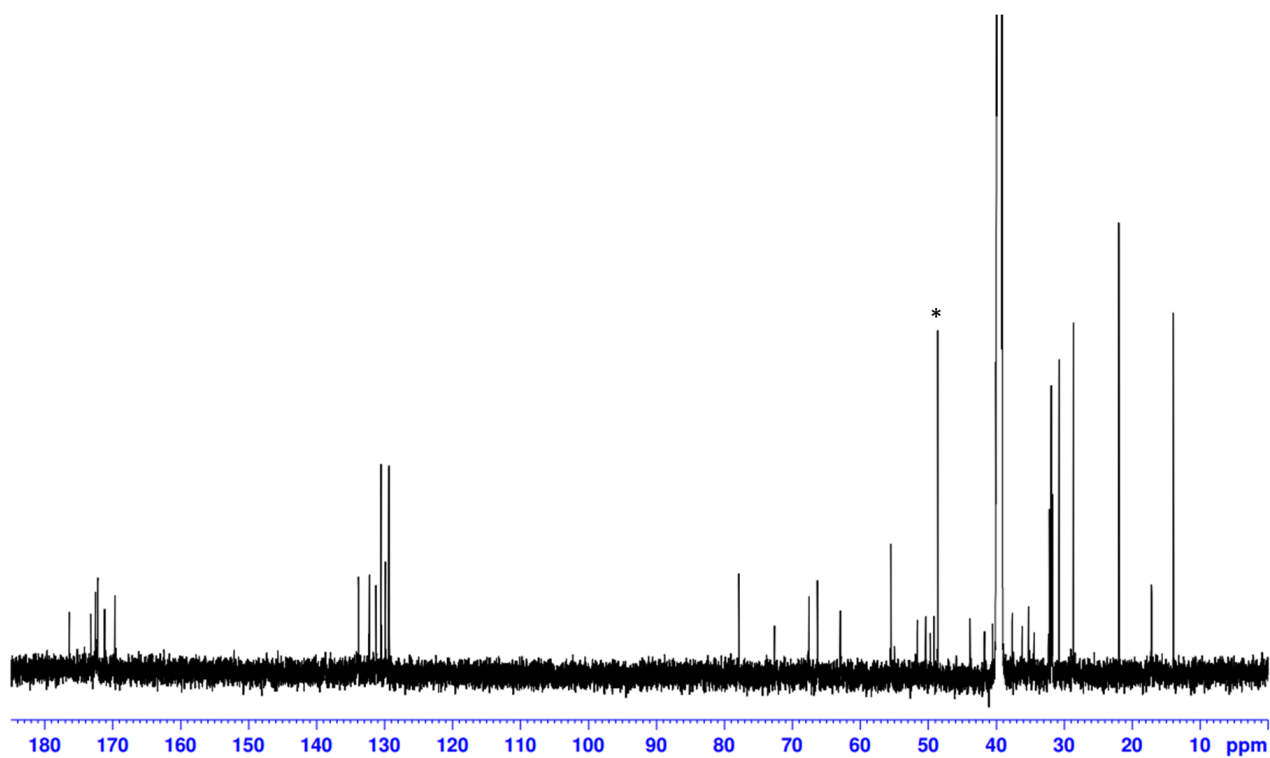


Figure S6. ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) spectrum for euglenatide B_2 . * Residual methanol solvent.

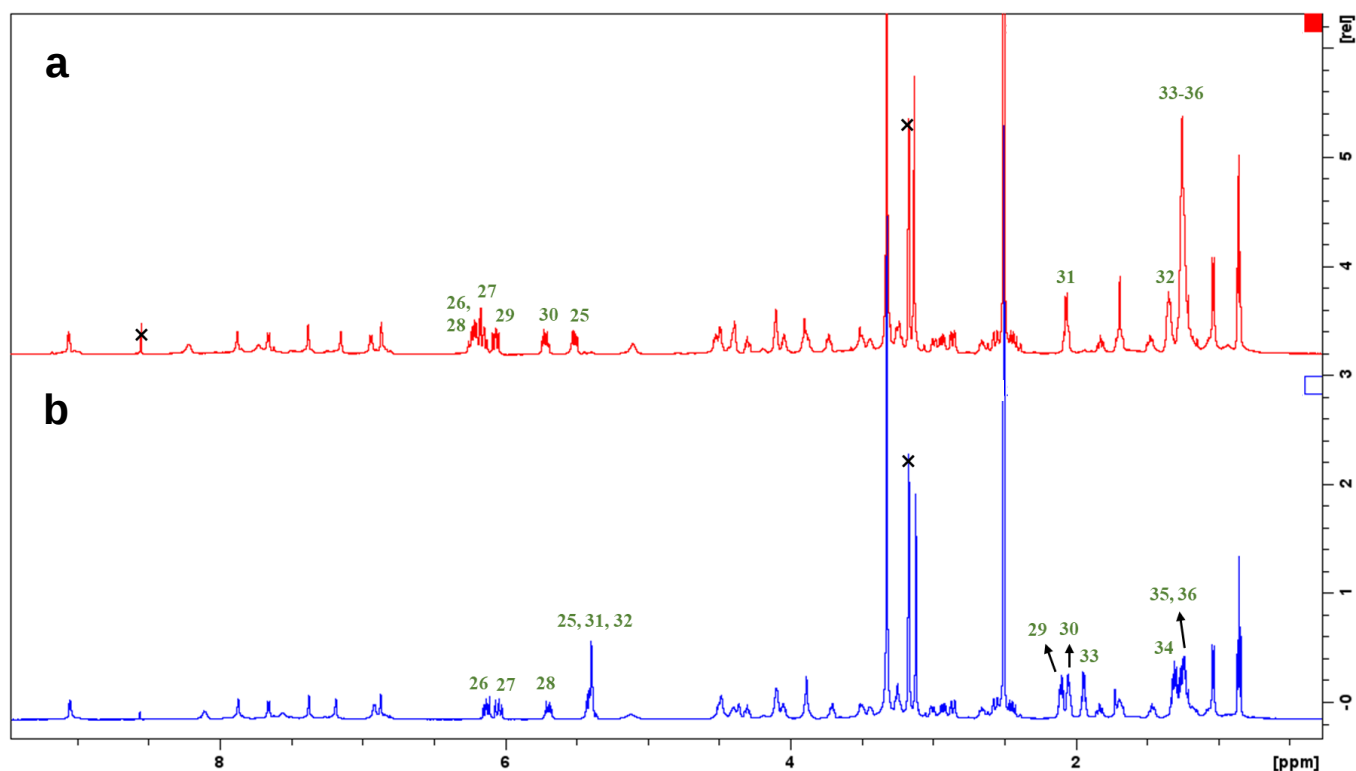


Figure S7. Comparison of ^1H NMR spectra for euglenatide B and B₂. ^1H NMR spectra (600 MHz, DMSO- d_6) spectra are shown for (a) euglenatide B against (b) euglenatide B₂ with main different signals annotated.

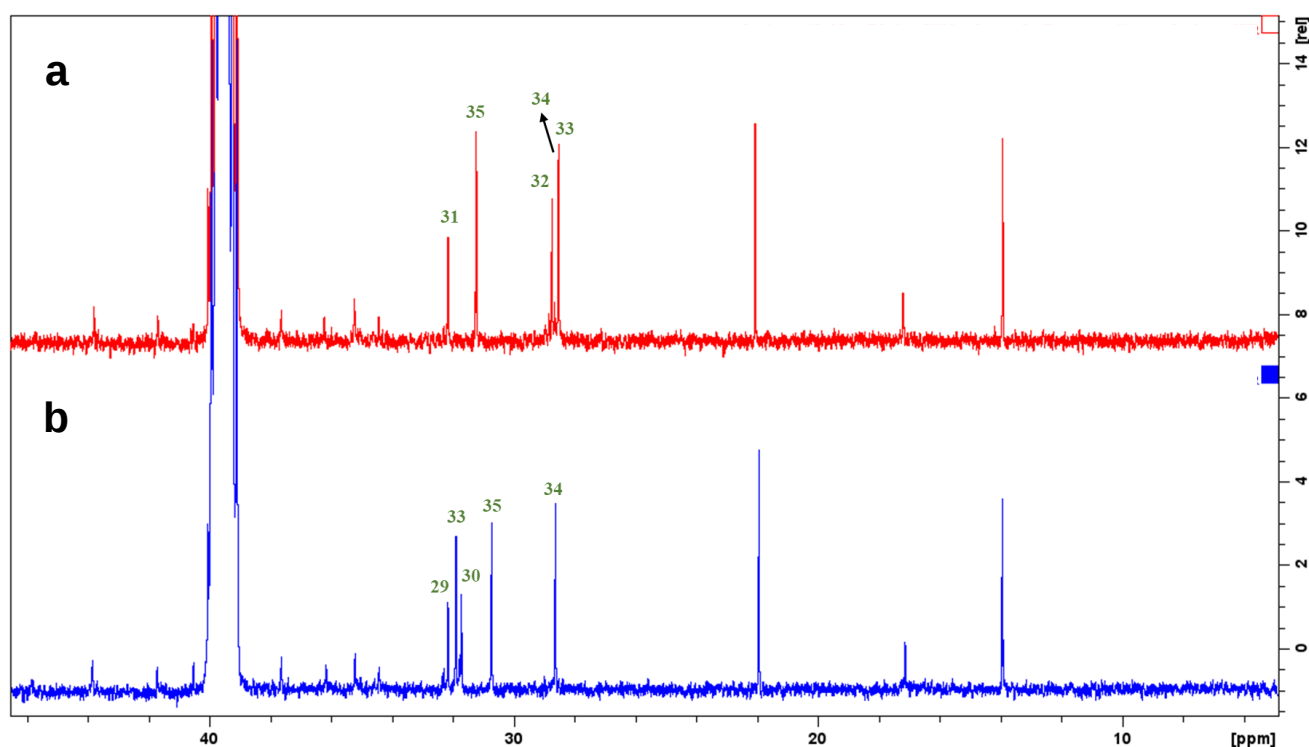


Figure S8. Comparison of expanded upfield region of ^{13}C NMR spectra for euglenatide B and B₂. An expanded view of the upfield region of the ^{13}C NMR (150 MHz, DMSO- d_6) spectra are shown for (a) euglenatide B against (b) euglenatide B₂, with main different signals annotated.

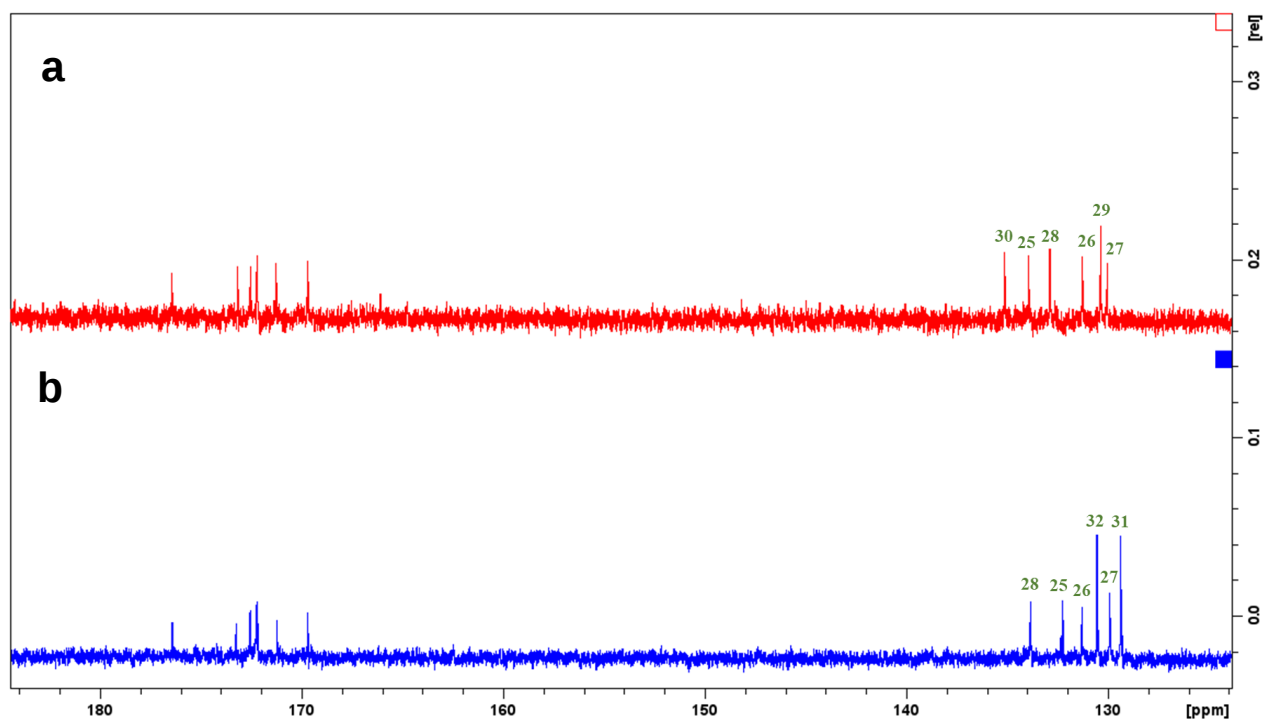


Figure S9. Comparison of expanded downfield region of ^{13}C NMR spectra for euglenatide B and B₂. An expanded view of the downfield region of the ^{13}C NMR (150 MHz, DMSO- d_6) spectra are shown for (a) euglenatide B against (b) euglenatide B₂, with main different signals annotated.

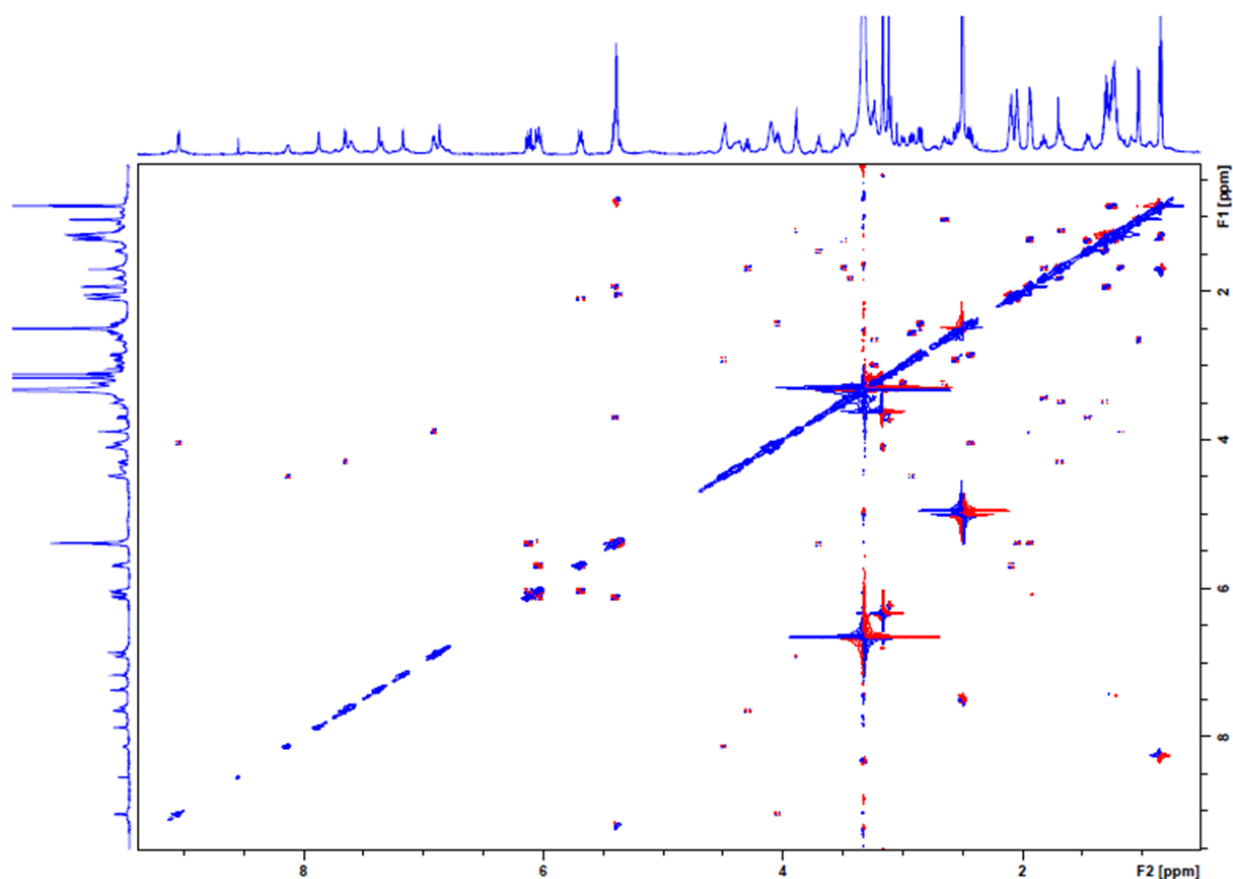


Figure S10. DQF-COSY NMR (600 MHz, DMSO- d_6) spectrum for euglenatide B₂.

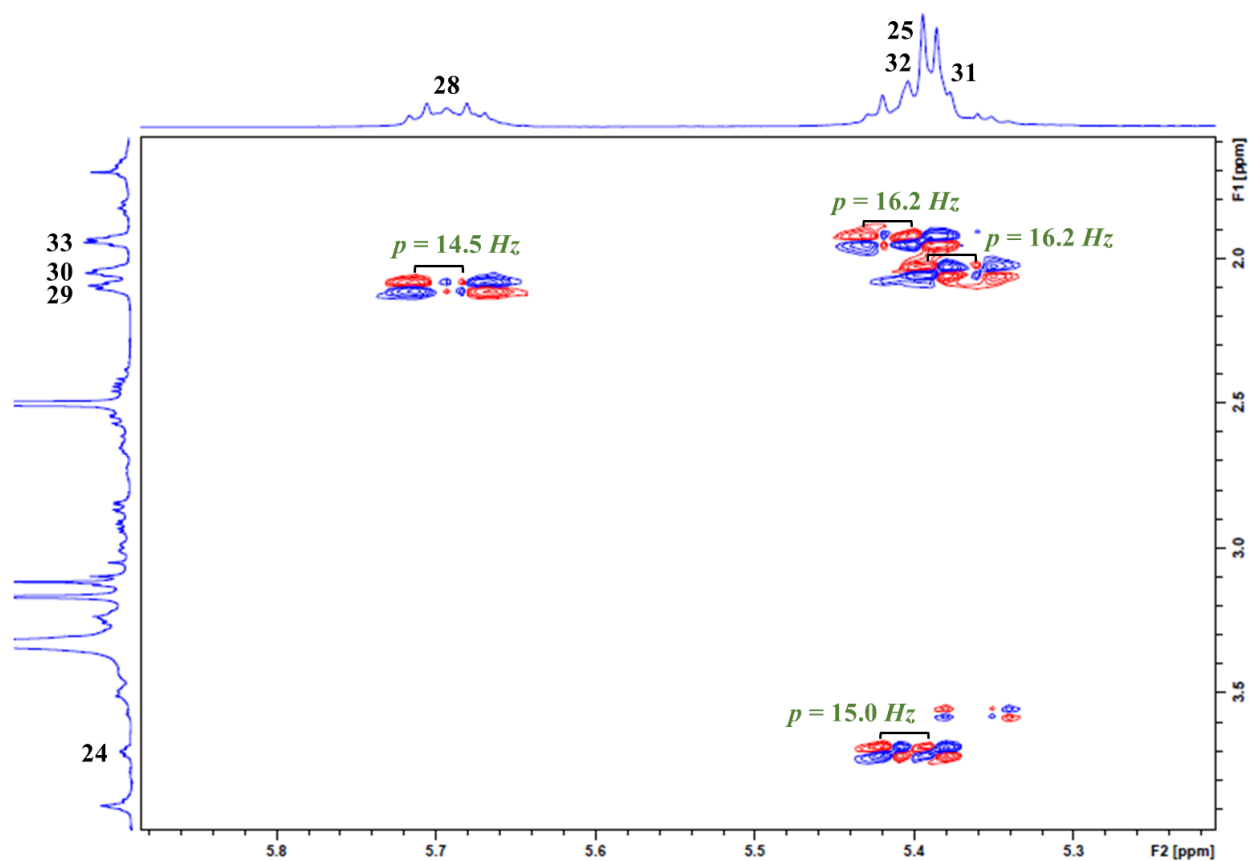


Figure S11. Expanded DQF-COSY NMR (600 MHz, DMSO-*d*₆) spectrum for euglenatide B₂. p = passive coupling constants.

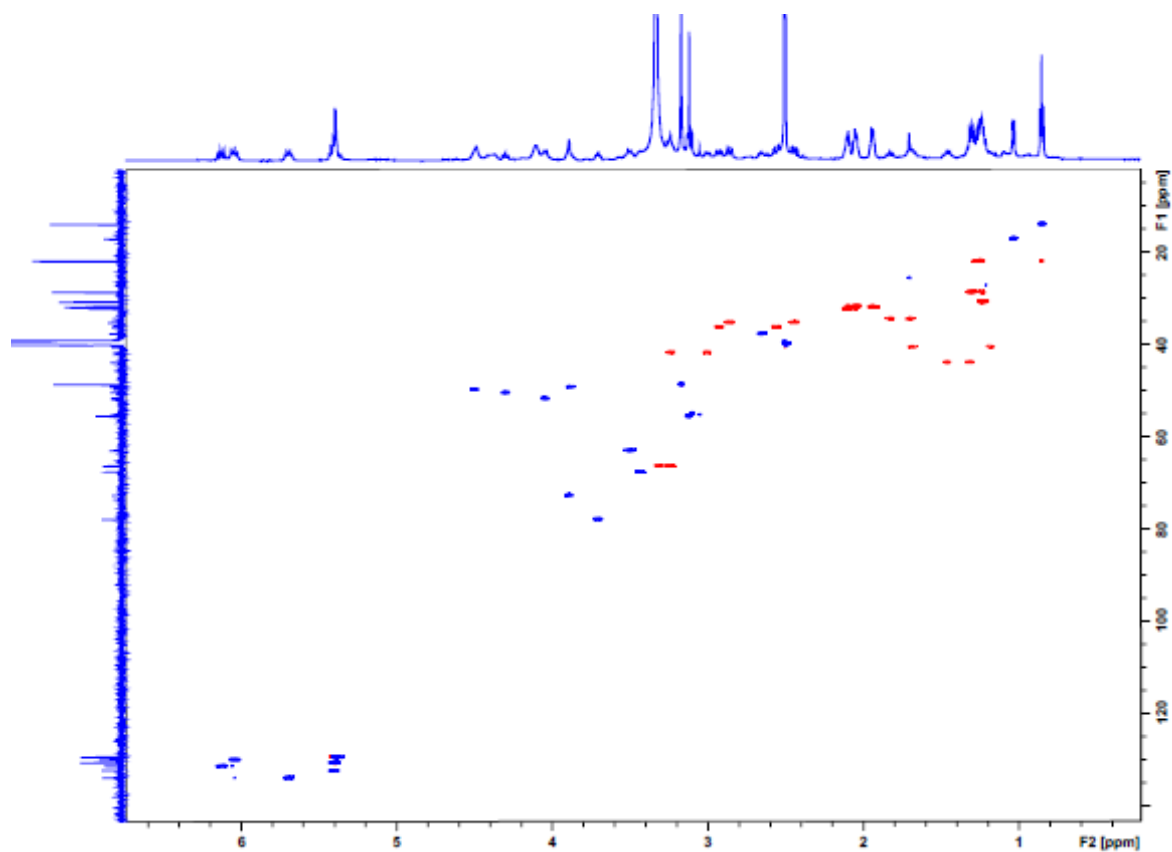


Figure S12. HSQC NMR (600 MHz, DMSO-*d*₆) spectrum for euglenatide B₂.

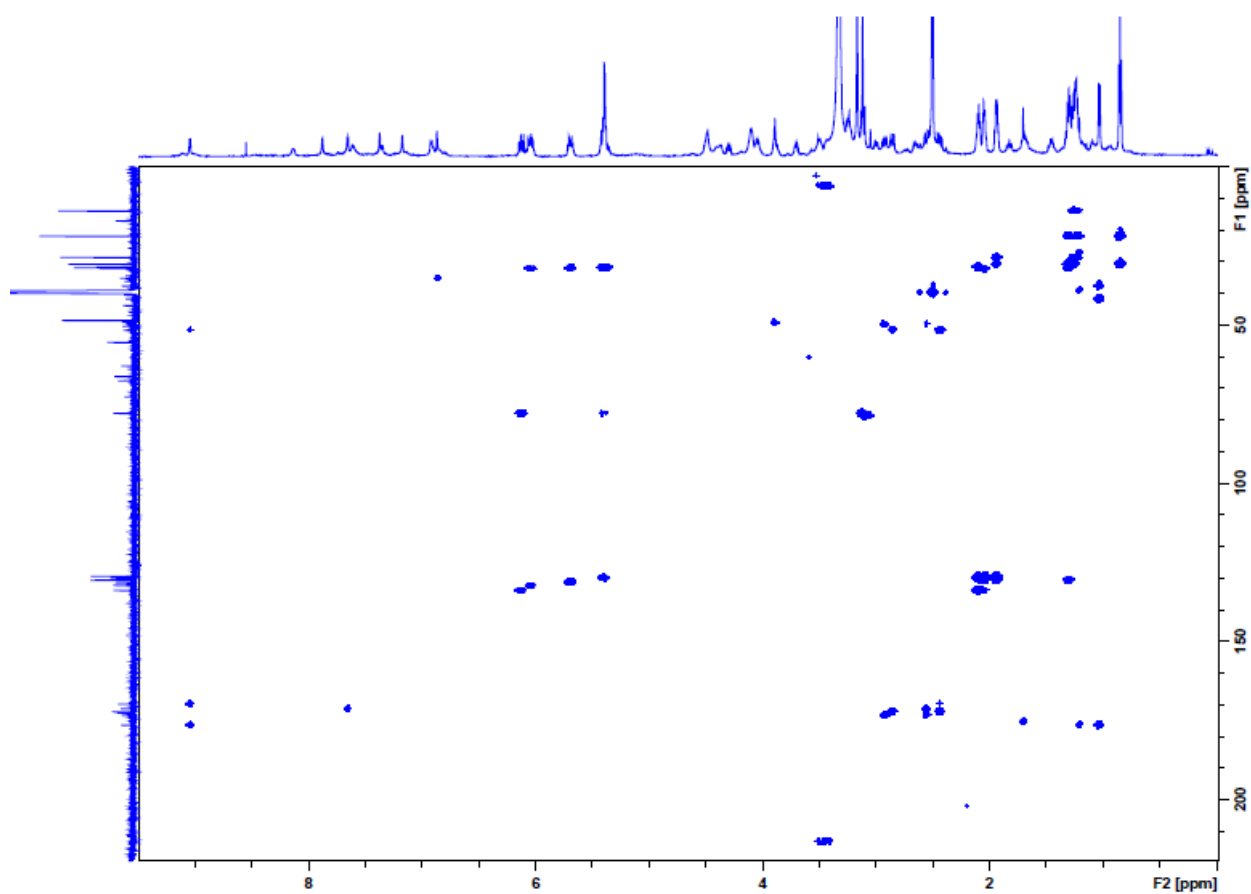


Figure S13. HMBC NMR (600 MHz, DMSO-*d*₆) spectrum for euglenatide B₂.