Supporting Information

Triple-Negative Breast Cancer Cells Exhibit Differential Sensitivity to Cardenolides from *Calotropis gigantea*

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Supplementary Methods for siRNA Transfection and CRISPR Gene Knock Out

siRNA Transfection. BT-549 cells were transfected for 48 h with Lipofectamine RNAi-MAX (Thermo Fisher Scientific) at a final concentration of 0.05% (v/v) and according to the manufacturer's directions. A pool of predesigned siRNAs targeting the SLC8A1 gene (NCX1) were purchased from Sigma: SASI_Hs02_00325545, SASI_Hs02_00325535, SASI_Hs02_00325555, SASI_Hs01_00071833. MISSION® siRNA Universal Negative Control #1 (Sigma) was used as a negative control. The final concentration of pooled siRNA or negative control siRNA was 5 nM. Following transfection, cells were harvested for membrane enrichment.

CRISPR Gene Knock Out. BT-549 cells expressing doxycycline-inducible Cas9 were kindly provided by Ratna Vadlamudi. sgRNAs targeting the SLC8A1 gene (NCX1) were designed using the Synthego (Redwood City, CA, USA) CRISPR design tool and the top 3 sequences were purchased, each with the Synthego modified EZ scaffold: U*C*U*UCCUCUUUGCUGGUCAG, A*C*U*GACCAGCAAAGAGGAAG, G*C*A*GCCACUGACCAGCAAAG where * in the nucleotide sequence indicates 2'-O-methyl analogs and 3'-phosphorothioate internucleotide linkages. A negative control scrambled sgRNA was purchased from Synthego with the following sequence: G*C*A*CUACCAGAGCUAACUCA. Cells were transfected with Lipofectamine RNAi-MAX (Thermo Fisher Scientific) at a final concentration of 0.2% (v/v) with either scrambled control sgRNA or the pool of 3 sgRNAs targeting SLC8A1/NCX1 for a final concentration of 8.6 nM sgRNA in the presence of 50 ng/ml doxycycline. Cells were harvested 48 h later and used for either membrane enrichment and NCX1 western blot to validate knockout or plated in a 96-well plate and treated with calotropin for 48 h and cytotoxicity determined using the SRB assay.

Table S1. ^{1}H NMR Data for Compounds 1–4 (δ in ppm, J in Hz)

NO.	**1**[a]	**2**[b]	**3**[a]	**4**[c]
1	1.59, overlap; 0.88, overlap	2.15, dt (13.1, 3.4); 0.59, ddd (13.4, 13.4, 3.7)	1.64, overlap; 0.91, overlap	2.33, dt (13.4, 3.7); 0.80, ddd (13.7, 13.7, 3.7)
2	1.60, overlap; 1.22 overlap	1.58, overlap; 1.28, overlap	1.62, overlap; 1.14, overlap	1.89, overlap; 1.55, overlap
3	3.31, m	3.36, m	3.54, m	3.68, m
4	1.39, overlap; 1.12, overlap	1.44, overlap; 1.23, overlap	1.74, overlap; 1.35, overlap	1.78, overlap; 1.43, overlap
5	0.98, m	1.08, overlap	0.99, dd (12.6, 12.1)	1.22, overlap
6	1.23, overlap; 1.10, overlap	1.16, overlap; 1.08, overlap	1.28, overlap; 1.12, overlap	1.33, overlap; 1.21, overlap
7	1.96, overlap; 0.92, overlap	1.98, overlap; 0.94, m	1.96, overlap; 0.94, overlap	2.07, m; 1.13, m
8	1.39, overlap	1.63, ddd (12.0, 11.9, 3.6)	1.39, overlap	1.78, overlap
9	0.85, overlap	0.84, ddd (12.1, 12.0, 3.6)	0.87, overlap	1.02, m
10				
11	1.40, overlap; 1.12, overlap	1.55, overlap; 1.45, overlap	1.40, overlap; 1.13, overlap	1.64, overlap
12	1.37, m; 1.30, ddd (14.2, 13.4, 3.8)	1.36, dt (13.6, 3.3); 1.23, overlap	1.38, overlap; 1.31, overlap	1.51, overlap; 1.40, overlap
13				
14				
15	1.91, overlap; 1.55, overlap	1.94, overlap; 1.55, overlap	1.92, dd (11.4, 10.6); 1.56, dd (11.0, 11.0)	2.13, overlap; 1.71, m
16	1.98, overlap; 1.75, m	1.99, overlap; 1.76, m	1.98, overlap; 1.75, overlap	2.17, overlap; 1.88, overlap
17	2.70, dd (9.4, 5.3)	2.71, dd (9.1, 5.1)	2.71, dd (9.5, 5.3)	2.84, dd (9.1, 5.1)
18	0.75, s	0.80, s	0.76, s	0.94, s
19	0.70, s	3.65, dd (11.5, 4.3); 3.49, dd (11.4, 3.7)	0.72, s	3.86, d (11.8); 3.74, d (10.7)
20				
21	4.94, dd (18.3, 1.9); 4.86, dd (18.3, 1.7)	4.96, dd (18.5, 1.9); 4.87, dd (18.5, 1.8)	4.94, dd (17.9, 1.9); 4.86, d (18.3, 1.7)	5.04, dd (18.4, 1.8);4.92, dd (18.4, 1.8)
22	5.88, s	5.89, s	5.89, s	5.91, s
23				
1'			4.20, d (7.8)	4.73, d (8.0)
2'			2.86, dd (8.5, 8.5)	3.27, dd (8.0, 3.0)
3'			3.10, dd (9.8, 9.6)	4.02, dd (3.1, 3.0)
4'			2.99, dd (9.1, 8.9)	3.16, dd (9.6, 2.9)
5'			3.04, dd (9.4, 6.4)	3.73, overlap
6'			3.63, d (11.6); 3.39, m	1.24, d (6.3)

[a]obtained on a 600 MHz Varian instrument in the solvent of DMSO-d_6; [b]obtained on a 500 MHz Varian instrument in the solvent of DMSO-d_6; [c]obtained on a 500 MHz Varian instrument in the solvent of methanol-d_4

Table S2. ^{13}C NMR Data for Compounds 1–4, and 6-9 (δ in ppm)

NO.	**1**[a]	**2**[b]	**3**[a]	**4**[c]	**6**[a]	**7**[a]	**8**[a]	**9**[d]
1	37.2 CH_2	31.8 CH_2	37.1 CH_2	32.7 CH_2	31.8 CH_2	35.7 CH_2	35.7 CH_2	36.8 CH_2
2	31.7 CH_2	32.1 CH_2	34.4 CH_2	30.8 CH_2	30.0 CH_2	69.4 CH	68.7 CH	70.2 CH
3	69.8 CH	69.7 CH	76.8 CH	79.4 CH	77.2 CH	71.4 CH	71.6 CH	73.3 CH
4	38.5 CH_2	38.9 CH_2	29.5 CH_2	35.8 CH_2	35.1 CH_2	33.4 CH_2	33.4 CH_2	34.4 CH_2
5	44.5 CH	44.8 CH	44.2 CH	45.9 CH	44.4 CH	42.9 CH	42.8 CH	44.4 CH
6	29.0 CH_2	28.5 CH_2	28.9 CH_2	29.4 CH_2	28.5 CH_2	21.8 CH_2	21.8 CH_2	23.0 CH_2
7	27.7 CH_2	27.8 CH_2	27.7 CH_2	28.7 CH_2	27.7 CH_2	27.6 CH_2	27.6 CH_2	28.6 CH_2
8	41.3 CH	41.7 CH	41.2 CH	43.0 CH	41.7 CH	42.2 CH	42.2 CH	43.4 CH
9	49.6 CH	50.2 CH	49.5 CH	51.5 CH	50.1 CH	47.8 CH	47.8 CH	49.6 CH
10	35.8 C	39.1 C	35.9 C	40.5 C	39.2 C	52.8 C	52.7 C	54.0 C
11	21.3 CH_2	22.9 CH_2	21.2 CH_2	24.0 CH_2	22.8 CH_2	27.5 CH_2	27.5 CH_2	28.8 CH_2
12	39.4 CH_2	40.1 CH_2	39.3 CH_2	41.5 CH_2	40.0 CH_2	38.8 CH_2	38.8 CH_2	40.2 CH_2
13	49.8 C	50.0 C	49.8 C	51.1 C	50.0 C	49.5 C	49.6 C	50.7 C
14	84.1 C	84.2 C	84.1 C	86.4 C	84.3 C	83.8 C	83.8 C	85.7 C
15	32.6 CH_2	32.5 CH_2	32.6 CH_2	33.4 CH_2	32.6 CH_2	31.9 CH_2	31.9 CH_2	32.7 CH_2
16	26.8 CH_2	26.8 CH_2	26.8 CH_2	28.0 CH_2	26.8 CH_2	26.7 CH_2	26.6 CH_2	27.8 CH_2
17	50.6 CH	50.7 CH	50.6 CH	52.1 CH	50.7 CH	50.3 CH	50.4 CH	51.8 CH
18	16.1 CH_3	16.3 CH_3	16.1 CH_3	16.5 CH_3	16.3 CH_3	15.9 CH_3	15.9 CH_3	16.1 CH_3
19	12.5 CH_3	58.1 CH_2	12.4 CH_3	60.0 CH_2	58.3 CH	209.3 CH	209.3 CH	209.4 CH
20	176.8 C	176.9 C	176.8 C	178.5 C	176.9 C	176.6 C	176.6 C	178.2 C
21	73.6 CH_2	73.6 CH_2	73.6 CH_2	75.3 CH_2	73.6 CH_2	73.6 CH_2	73.6 CH_2	75.3 CH_2
22	116.7 CH	116.6 CH	116.7 CH	117.7 CH	116.6 CH	116.8 CH	116.8 CH	117.9 CH
23	174.3 C	174.3 C	174.3 C	177.2 C	174.3 C	174.2 C	174.3 C	177.2 C
1'			101.1 CH	99.8 CH	98.6 CH	95.6 CH	93.9 CH	97.3 CH
2'			73.9 CH	72.5 CH	70.9 CH	99.1 C	92.6 C	92.7 C
3'			77.2 CH	72.9 CH	71.1 CH	91.9 C	73.7 CH	73.9 CH
4'			70.6 CH	74.4 CH	82.4 CH	47.9 CH_2	71.4 CH	39.6 CH_2
5'			77.2 CH	70.5 CH	67.8 CH	67.8 CH	68.7 CH	69.4 CH
6'			61.6 CH_2	18.2 CH_3	18.2 CH_3	21.2 CH_3	18.4 CH_3	21.3 CH_3
1"					105.0 CH	161.6 CH		
2"					73.9 CH	43.3 CH_2		
3"					77.2			
4"					70.4 CH			
5"					77.1 CH			
6"					61.6 CH_2			

[a]obtained on a 150 MHz Varian instrument in the solvent of DMSO-d_6; [b]obtained on a 125 MHz Varian instrument in the solvent of DMSO-d_6; [c]obtained on a 125 MHz Varian instrument in the solvent of methanol-d_4; [d]obtained on a 150 MHz Varian instrument in the solvent of methanol-d_4;

Table S3. ^{1}H NMR Data for Compounds 6–9 (δ in ppm, J in Hz)

NO.	**6**[a]	**7**[b]	**8**[b]	**9**[c]
1	2.18, d (12.6); 0.62, dd (12.1, 13.7)	2.20, dd (12.5, 4.4); 1.03, dd (12.4, 12.3)	2.20, dd (12.5, 4.3); 0.97, dd (12.2, 12.0)	2.46, dd (12.7, 4.4); 1.13, dd (12.3, 12.2)
2	1.68, overlap; 1.41, overlap	3.71, m	3.68, overlap	3.85, ddd (12.0, 10.0, 4.4)
3	3.51, overlap	3.85, overlap	3.72, overlap	3.93, ddd (11.4, 10.4, 4.4)
4	1.62, overlap; 1.26, overlap	1.55, overlap; 1.22, overlap	1.50, overlap; 1.20, dd (12.2, 11.9)	1.65, overlap; 1.40, overlap
5	1.07, overlap	1.51, overlap	1.47, overlap	1.55, overlap
6	1.20, overlap; 1.08, overlap	1.57, overlap	1.55, overlap	1.75, overlap; 1.29, overlap
7	1.97, overlap; 0.93, m	2.13, d (12.7); 1.10, overlap	2.12, d (12.8); 1.09, overlap	2.23, dd (13.2, 3.5); 1.30, overlap
8	1.62, overlap	1.39, overlap	1.34, overlap	1.59, overlap
9	0.85, dd (12.5, 11.9)	1.38, overlap	1.37, overlap	1.44, overlap
10				
11	1.53, overlap; 1.45, overlap	1.90, overlap; 1.55, overlap	1.88, overlap; 1.52, overlap	2.02, dd (13.2, 4.0); 1.66, overlap
12	1.36, overlap; 1.23, overlap	1.37, overlap; 1.27, overlap	1.37, overlap; 1.26, overlap	1.51, overlap; 1.44, overlap
13				
14				
15	1.94, overlap; 1.56, overlap	1.92, overlap; 1.55, overlap	1.91, m; 1.54, overlap	2.11, overlap; 1.69, overlap
16	2.00, overlap; 1.76, m	1.98, overlap; 1.76, m	1.99, m; 1.76, m	2.12, overlap; 1.87, overlap
17	2.71, dd (8.8, 5.5)	2.71, dd (9.4, 5.4)	2.70, dd (9.4, 5.3)	2.83, dd (9.3, 5.3)
18	0.79, s	0.67, s	0.67, s	0.83, s
19	3.64, overlap; 3.49, overlap	9.96, s	9.94, s	10.03, s
20				
21	4.96, d (17.7); 4.88, d (17.8)	4.92, dd (18.4, 1.9); 4.86, dd (18.2, 1.8)	4.92, dd (18.2, 1.9); 4.85, dd (18.3, 1.7)	5.02, dd (18.6, 1.8); 4.91, dd (18.4, 1.8)
22	5.90, s	5.88, s	5.88, s	5.90, s
23				
1'	4.54, d (8.0)	4.67, s	4.47, s	4.45, brs
2'	3.06, overlap			
3'	4.05, d s		3.39, dd (3.0, 2.6)	3.59, dd (12.0, 4.9)
4'	3.10, overlap	1.99, overlap; 1.67, d (13.3, 2.2)	3.21, m	1.73, overlap; 1.59, overlap
5'	3.69, m	4.09, ddd (11.3, 6.4, 2.0)	3.65, overlap	3.66, m
6'	1.17, d (6.2)	1.09, d (6.2)	1.11, d (6.2)	1.24, d (6.2)
1''	4.22, d (7.8)	7.54, s		
2''	2.98, dd (8.5,8.3)	3.86, d (16.4); 3.79, m (16.5)		
3''	3.10, overlap			
4''	3.03, overlap			
5''	3.08, overlap			
6''	3.64, overlap; 3.42, m			

[a]obtained on a 500 MHz Varian instrument in the solvent of DMSO-d_6; [b]obtained on a 600 MHz Varian instrument in the solvent of DMSO-d_6; [c]obtained on a 500 MHz Varian instrument in the solvent of methanol-d_4

Table S4. List of sources for antibodies and dilutions used

Target	Vendor Information	Dilution	Figure
Na^+/K^+ ATPase α1	EMD Millipore, 05-369 (C464.6)	1:500	5A
Na^+/K^+ ATPase α2	EMD Millipore, 07-674	1:5,000	5A
Na^+/K^+ ATPase α3	Invitrogen, MA3-915 (XVIF9-G10)	1:1,000	5A
NCX1	Abcam ab2869 (C2C12)	1:1,000	5C, S6B, S7A
GAPDH	Cell Signaling Technologies, 5174 (D16H11)	1:1,000	S6A, S7A
Flotillin-1	BD Biosciences, 610820	1:1,000	S6A, S7A

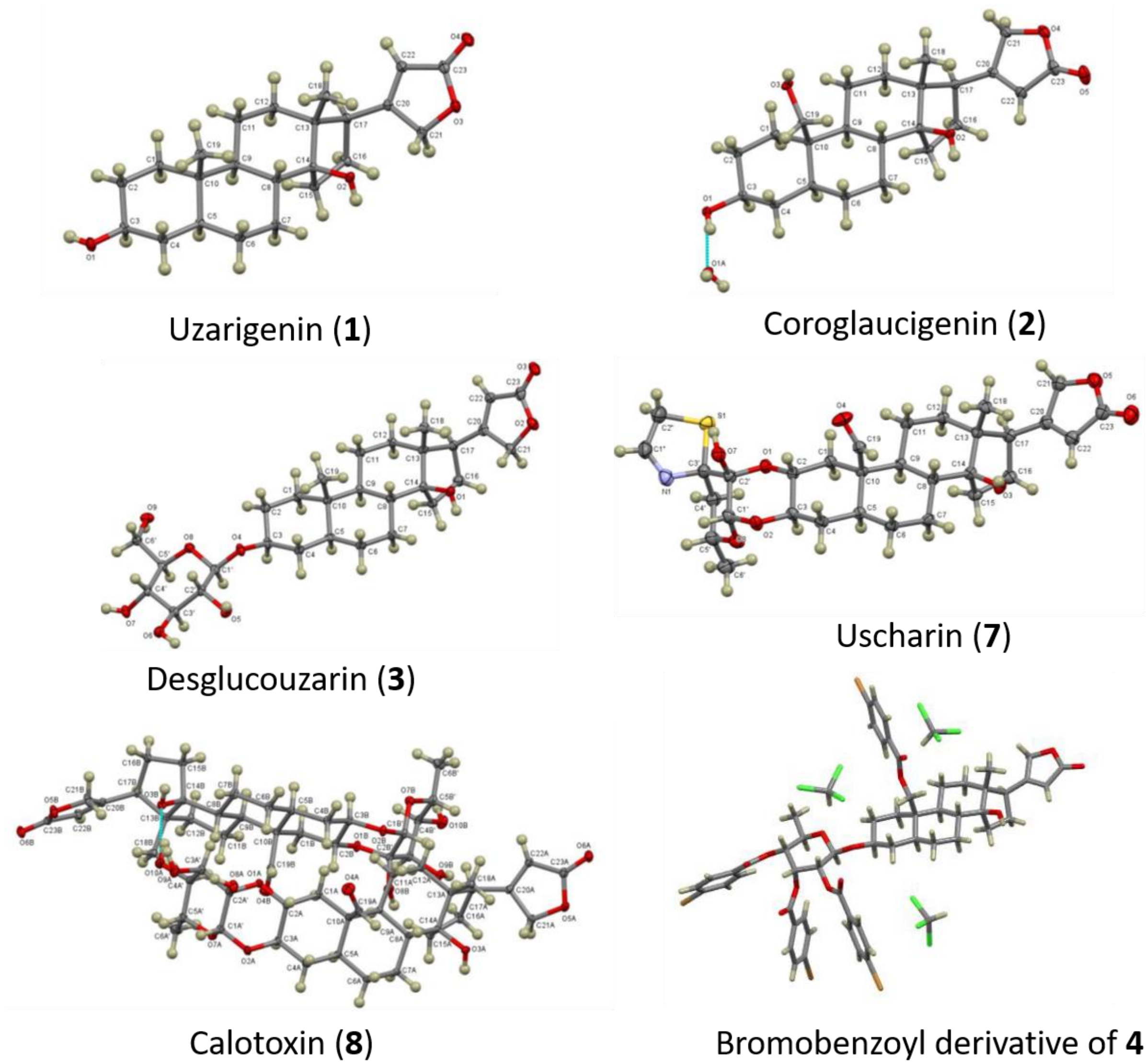


Figure S1. X-ray structures of compounds **1-3**, **7**, **8**, and bromobenzoyl derivative of **4**

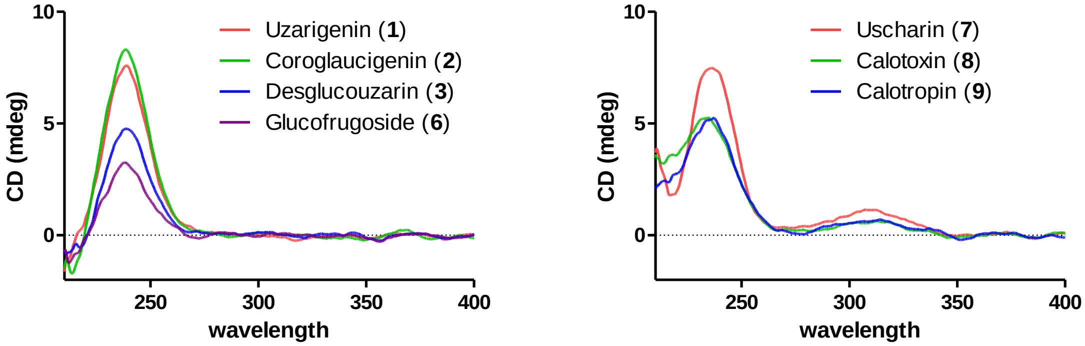


Figure S2. ECD curves of compounds **1-3**, **6**, and **7-9**

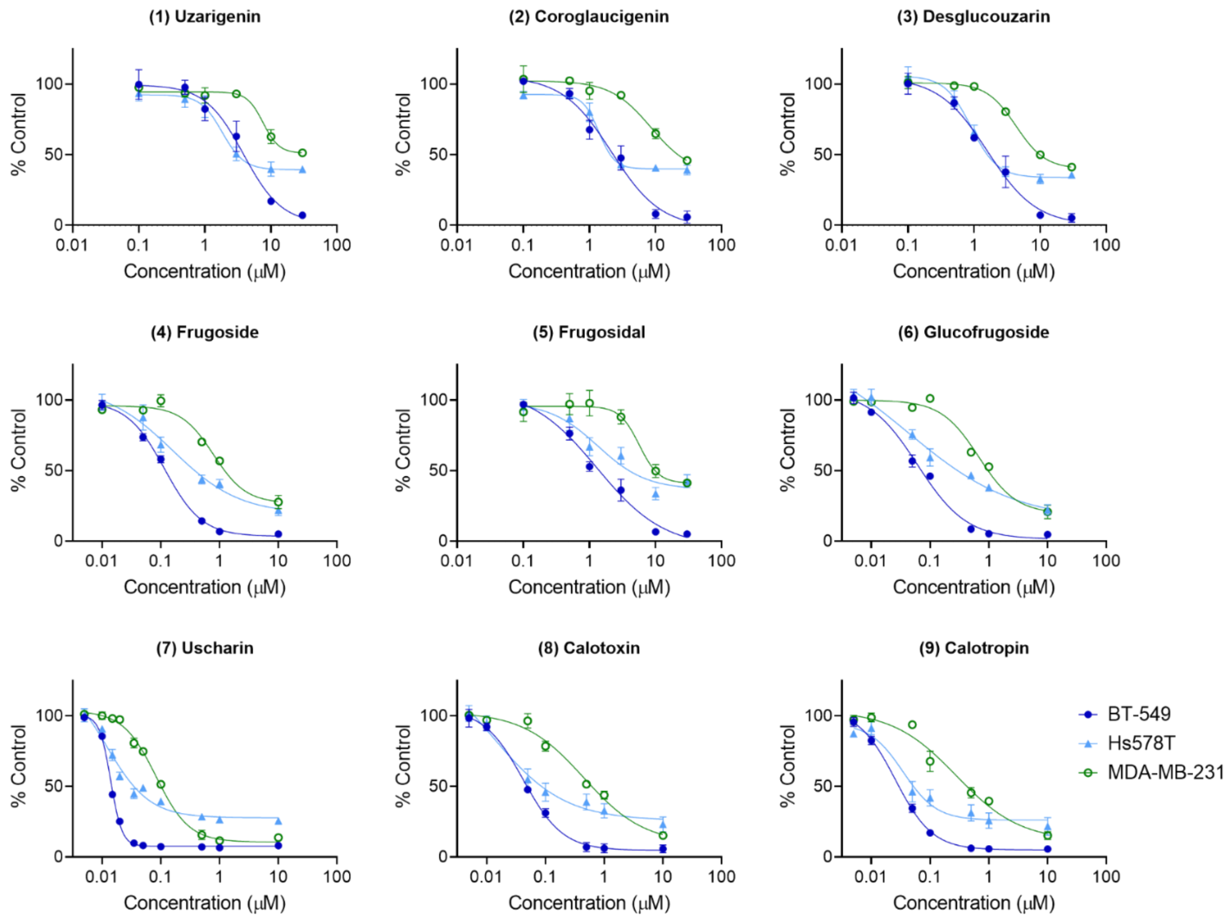


Figure S3. Concentration response data for cytotoxicity of *C. gigantea* cardenolides in TNBC cells. Data are represented as percent of vehicle control after 48 h treatment. Each concentration was tested in triplicate in three independent experiments and the mean ± SEM is shown.

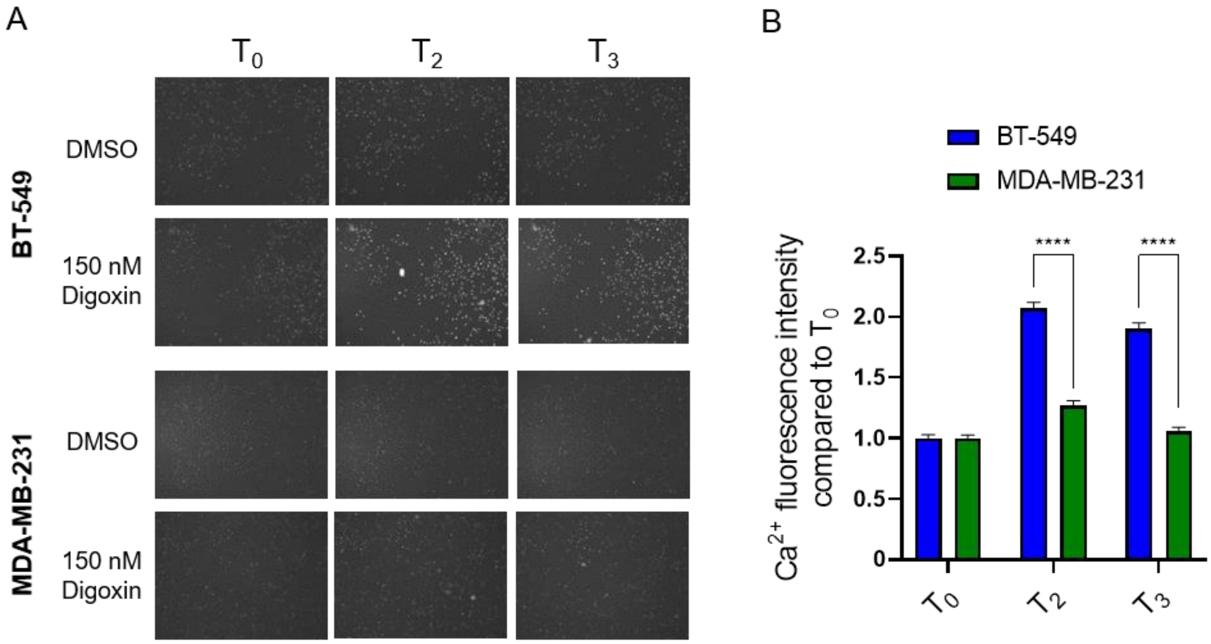
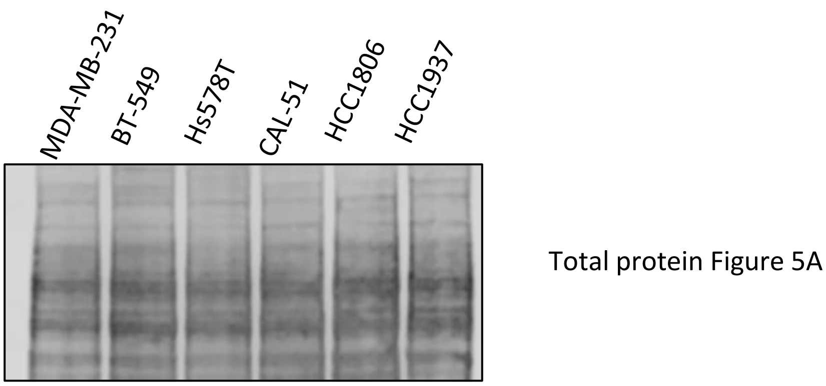


Figure S4. Effects of digoxin on intracellular Ca^{2+} concentrations. A) Images of the same fields of BT-549 and MDA-MB-231 cells at indicated time points loaded with Cal520-AM and treated as indicated. B) Quantification of cellular Ca^{2+} fluorescence in BT-549 and MDA-MB-231 cells treated with 150 nM digoxin corresponding to images in A. Significance was determined by comparing cellular Ca^{2+} fluorescence in BT-549 cells to MDA-MB-231 cells at each time point by two-way ANOVA with Bonferoni's post hoc test, n = 355-591 cells, **** $p < 0.0001$.



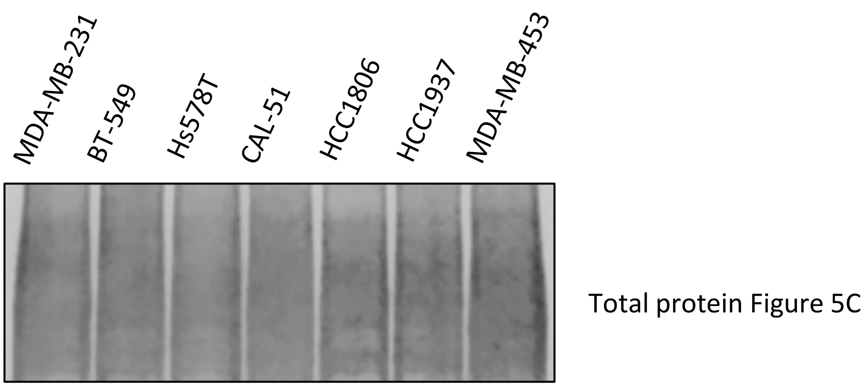


Figure S5. Total protein stain for immunoblots in Figure 5.

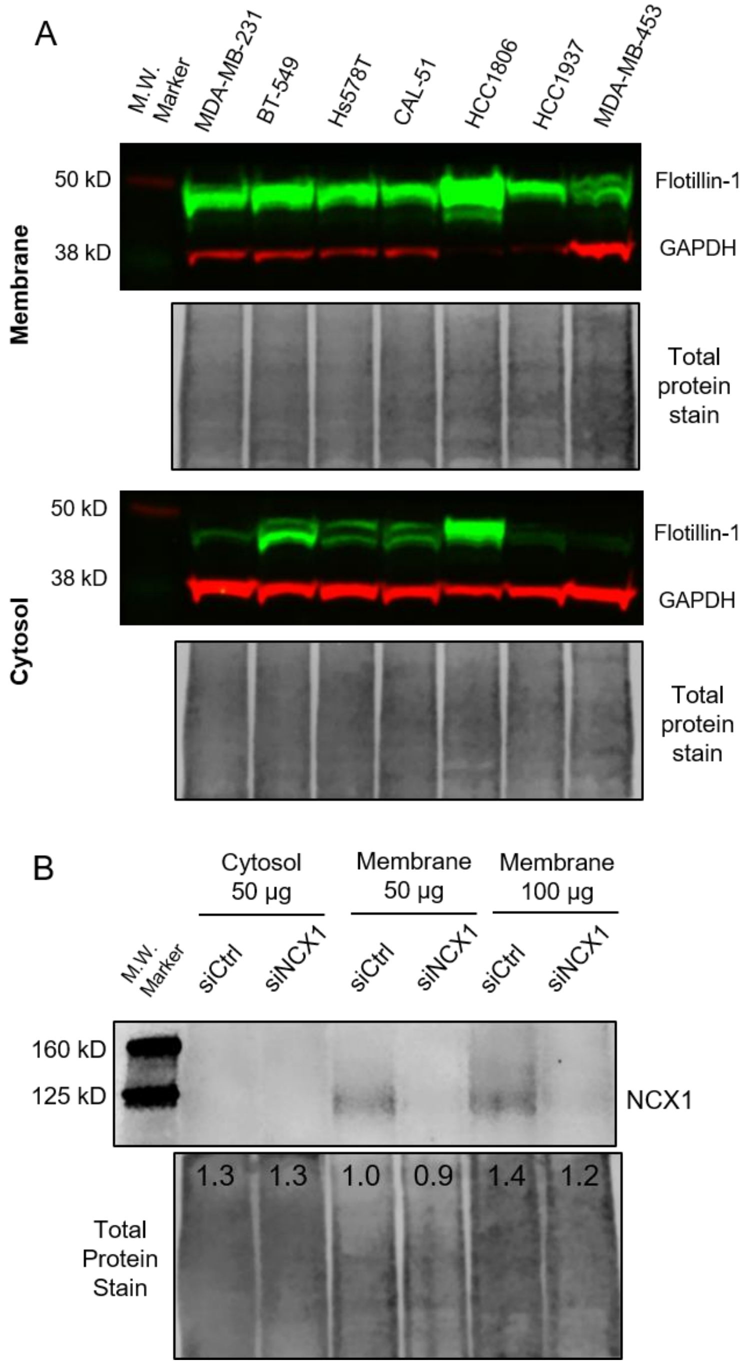


Figure S6. Validation of membrane enrichment and NCX1 antibody A) Western blots of membrane and cytosol fractions from TNBC cell lysates for flotillin-1, a protein localized to membranes and GAPDH, a cytosolic protein. B) Validation of NCX1 antibody specificity in membrane-enriched cell lysates from BT-549 cells 48 h after transfection with non-targeting control siRNA or NCX- targeted siRNA pool.

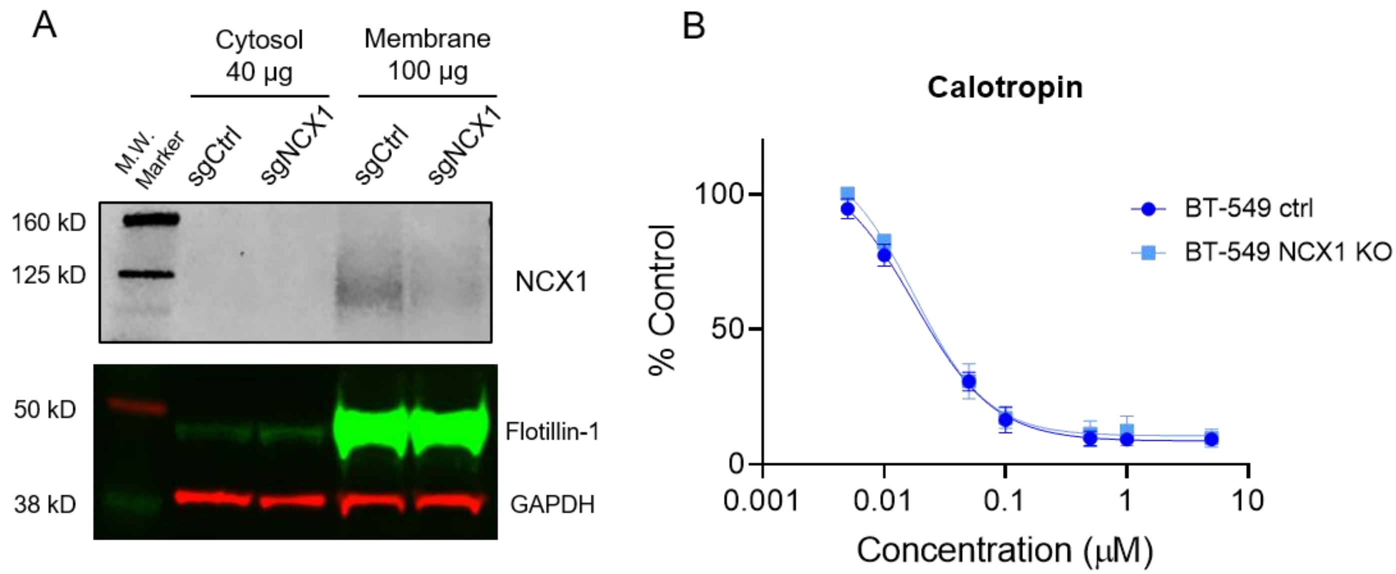


Figure S7. Effect of CRISPR knockout of NCX1 on BT-549 sensitivity to calotropin. BT-549 cells expressing doxycycline-inducible Cas9 were transfected with either non-targeting ctrl sgRNA or a pool of 3 sgRNAs targeting NCX1 in the presence of 50 ng/ml doxycycline. Cells were harvested 48 h later and used for either membrane enrichment and NCX1 western blot to validate knockout (A) or plated in a 96-well plate to evaluate calotropin cytotoxicity using the SRB assay (B).

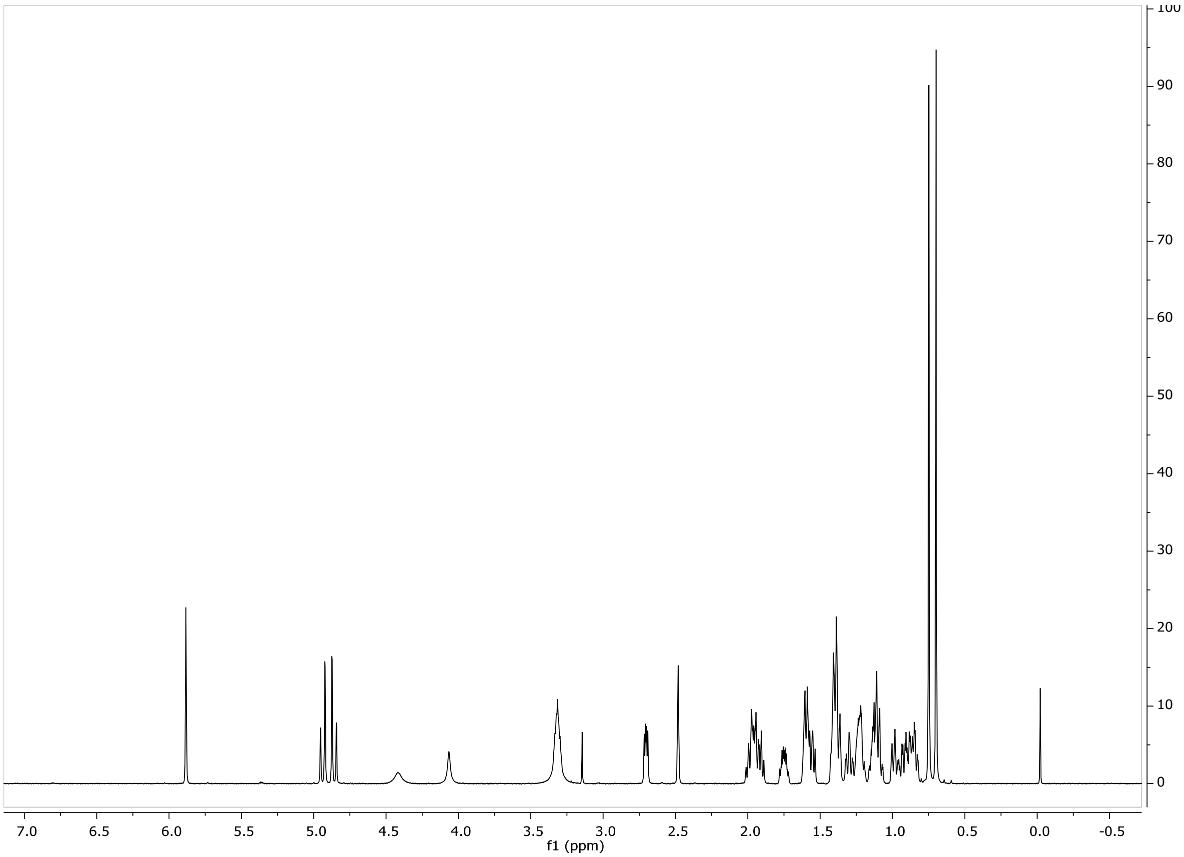


Figure S8. ^{1}H NMR (600 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

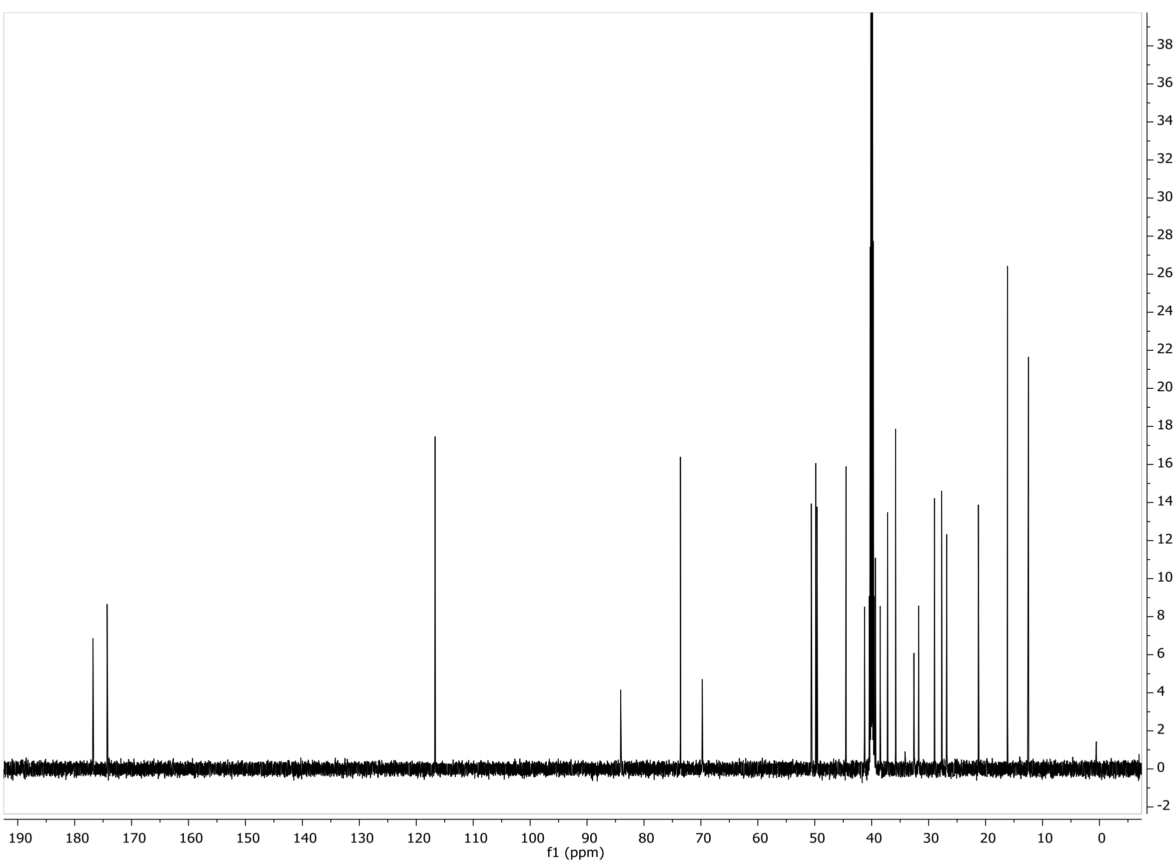


Figure S9. ^{13}C NMR (150 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

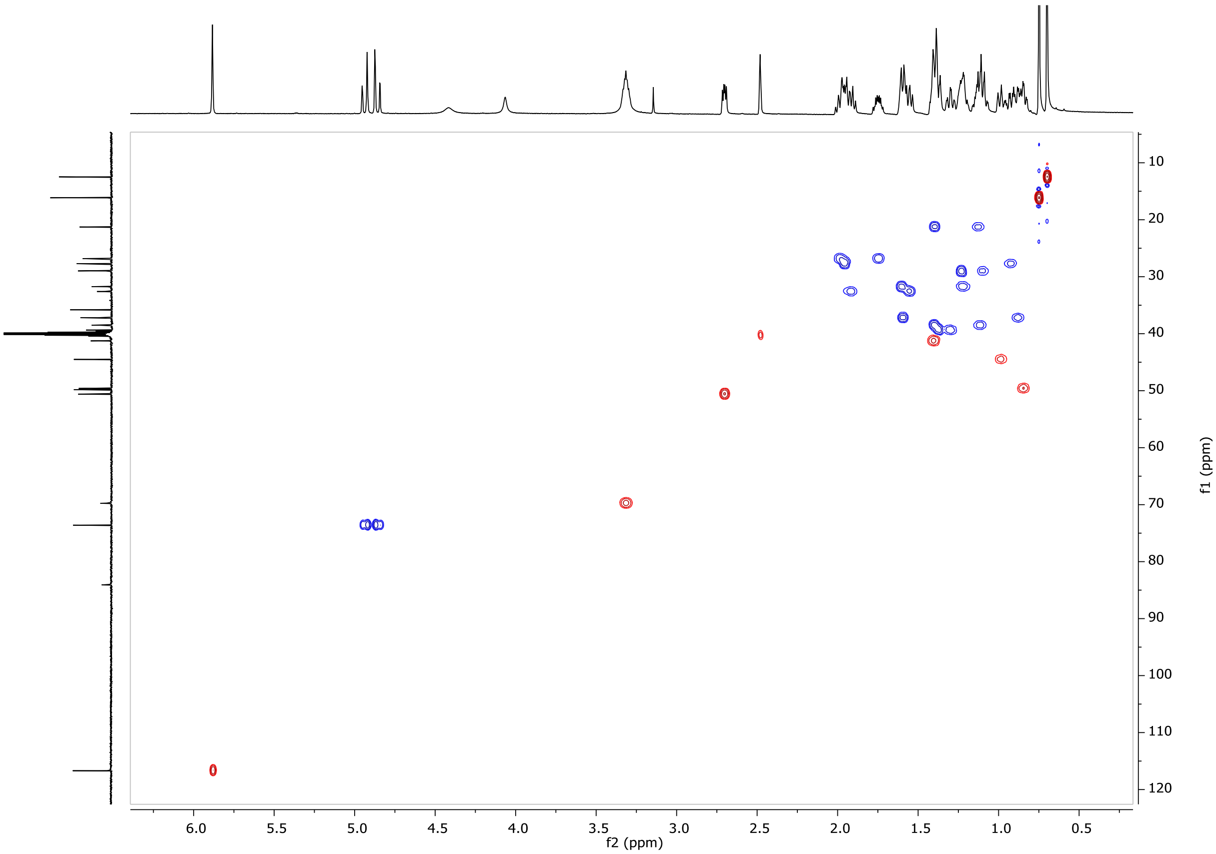


Figure S10. HSQC (500 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

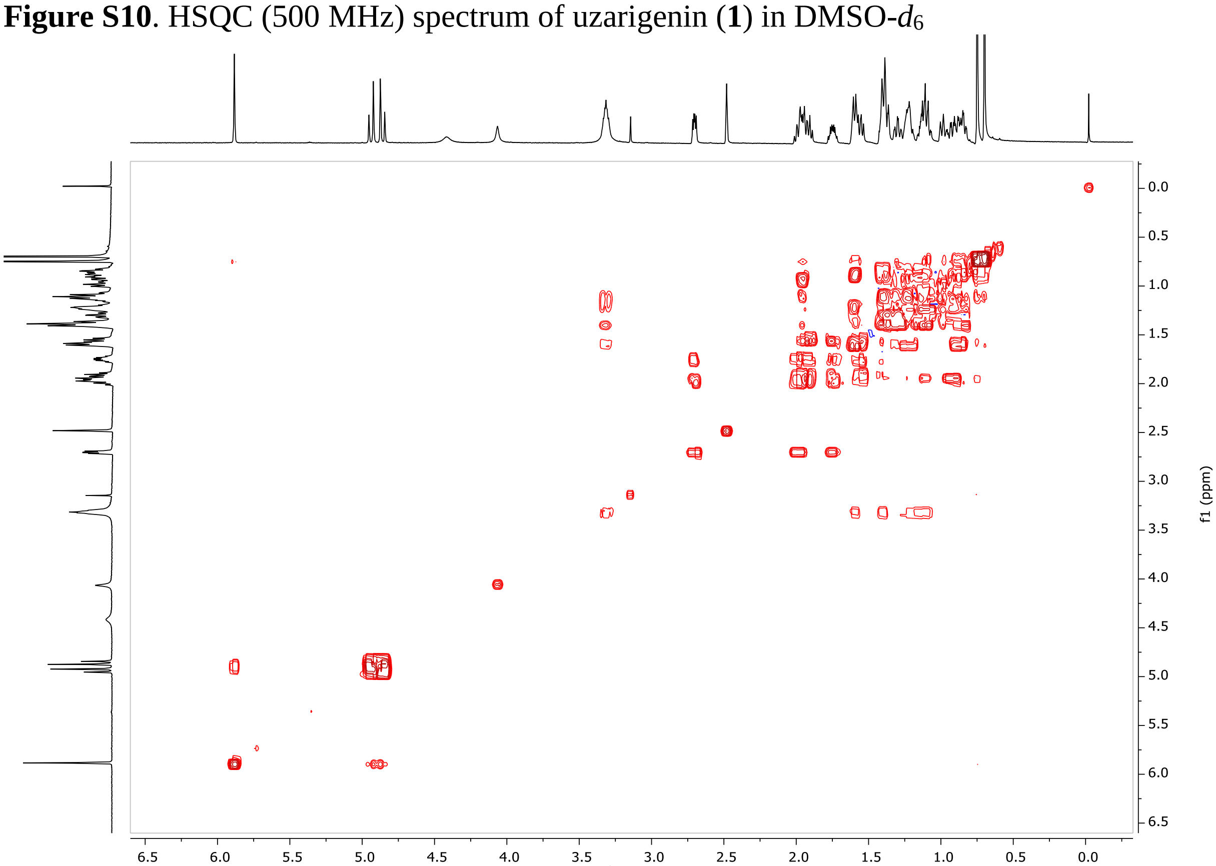


Figure S11. ^{1}H-^{1}H COSY (500 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

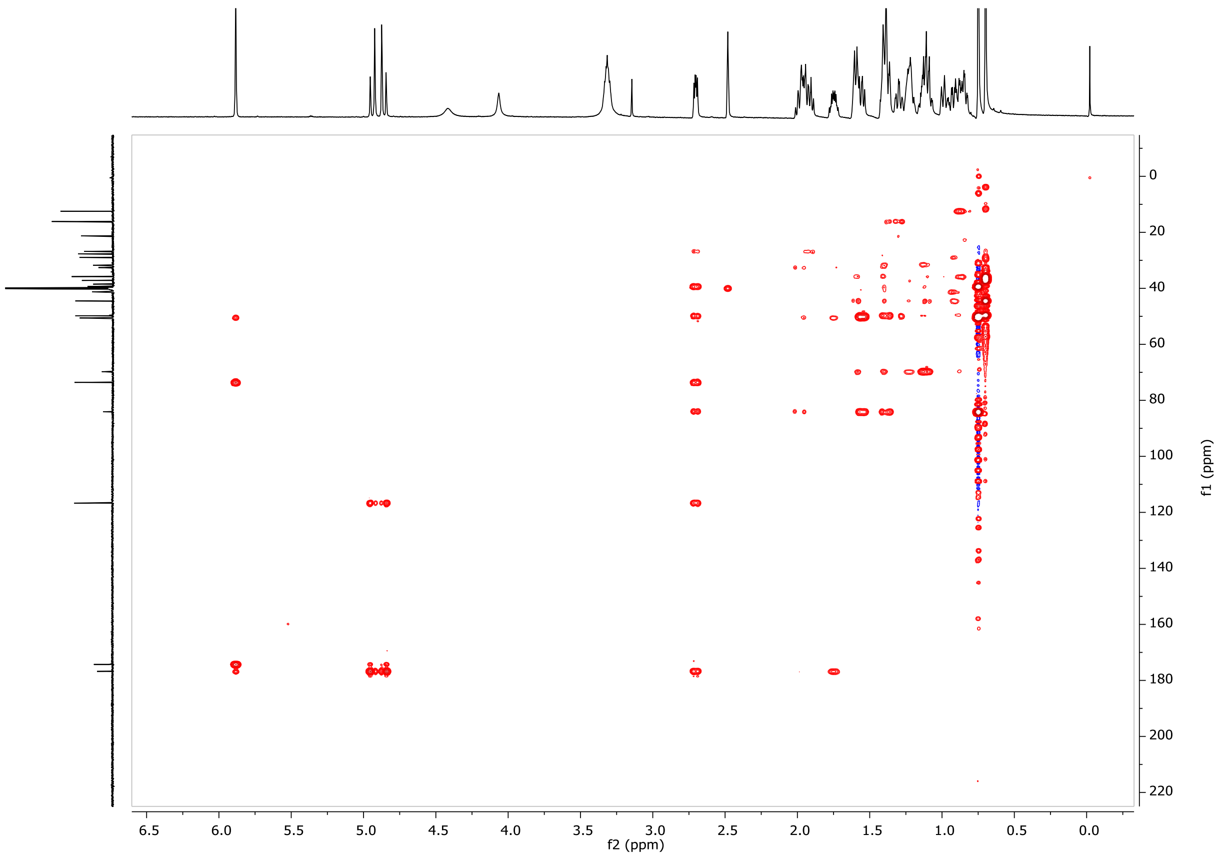


Figure S12. HMBC (500 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

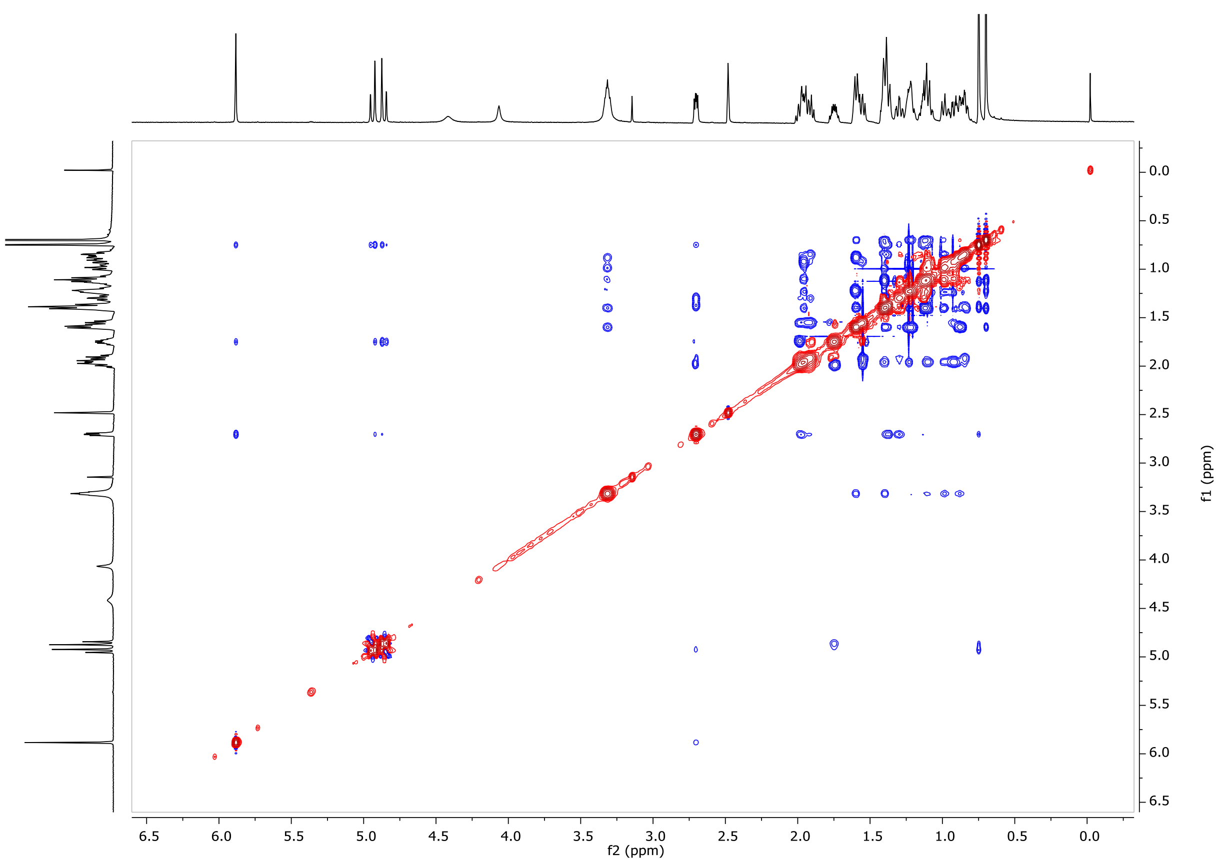


Figure S13. ROESY (500 MHz) spectrum of uzarigenin (**1**) in DMSO-d_6

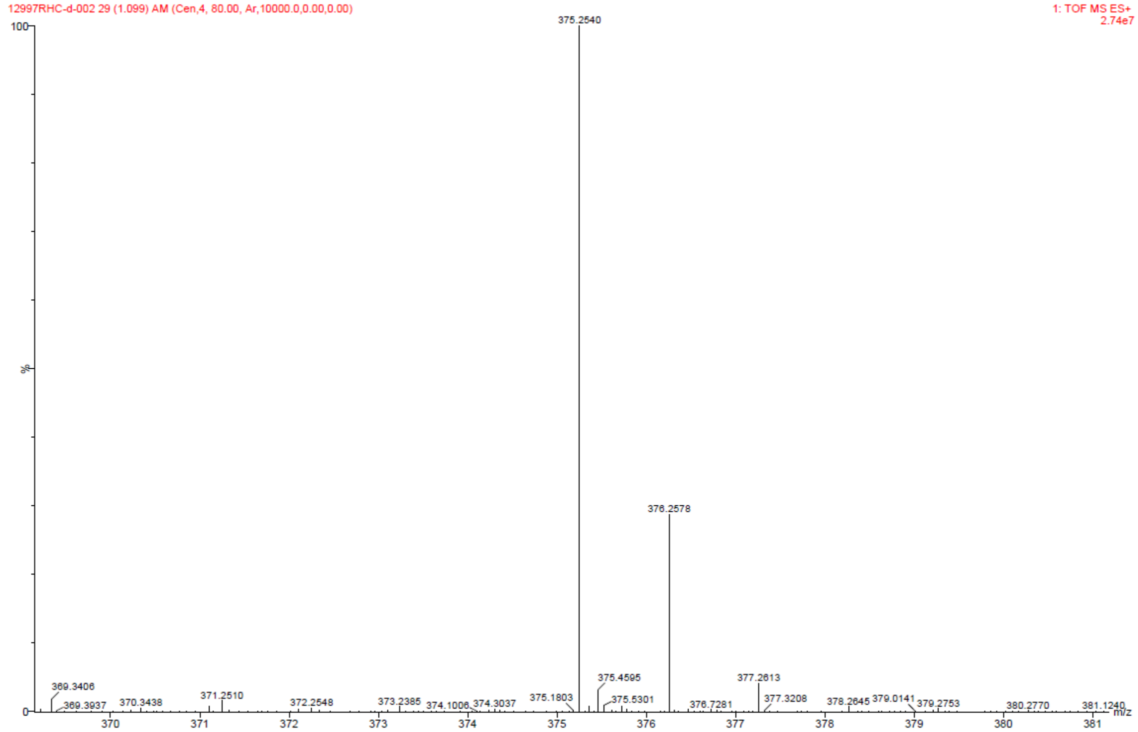


Figure S14. HRESIMS spectrum of uzarigenin (**1**)

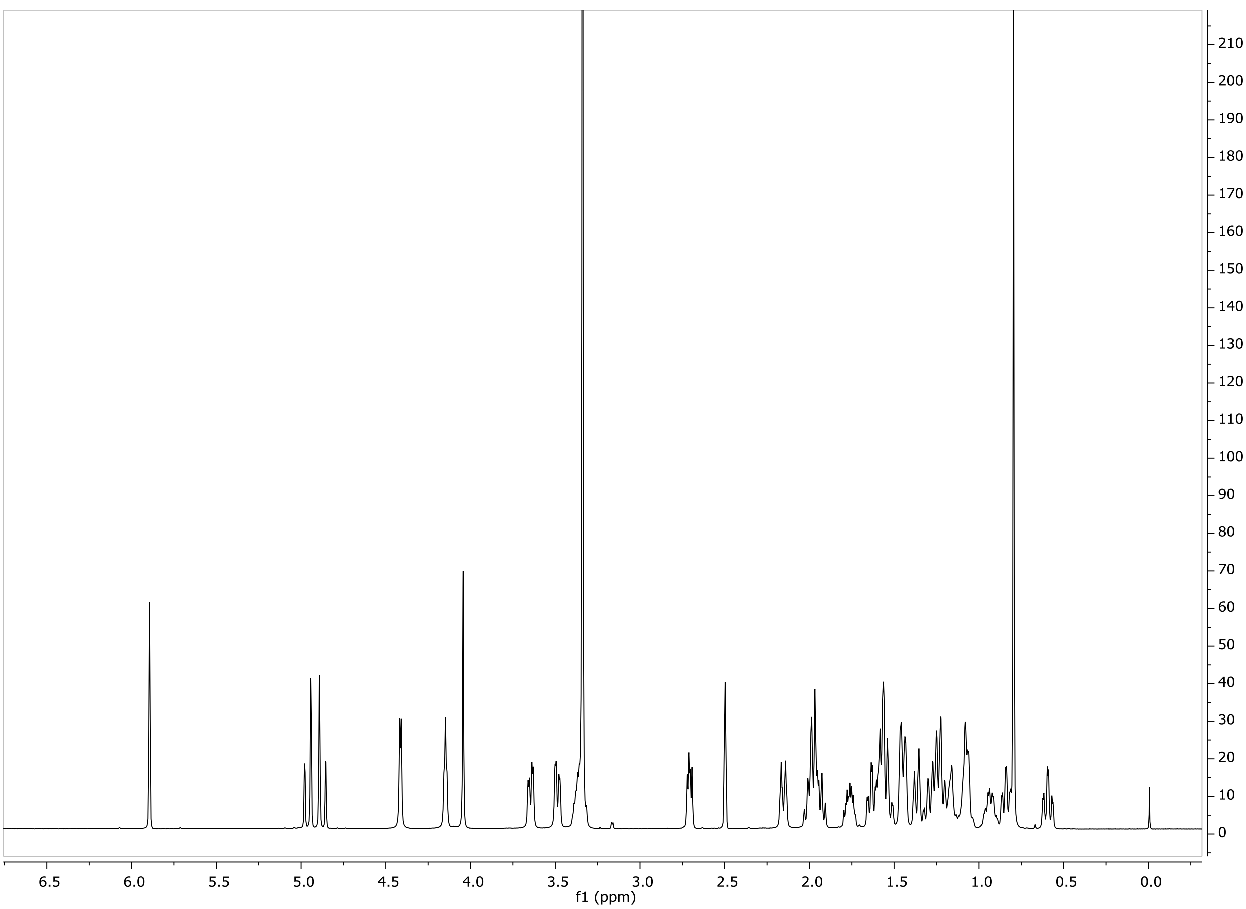


Figure S15. ^{1}H NMR (500 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

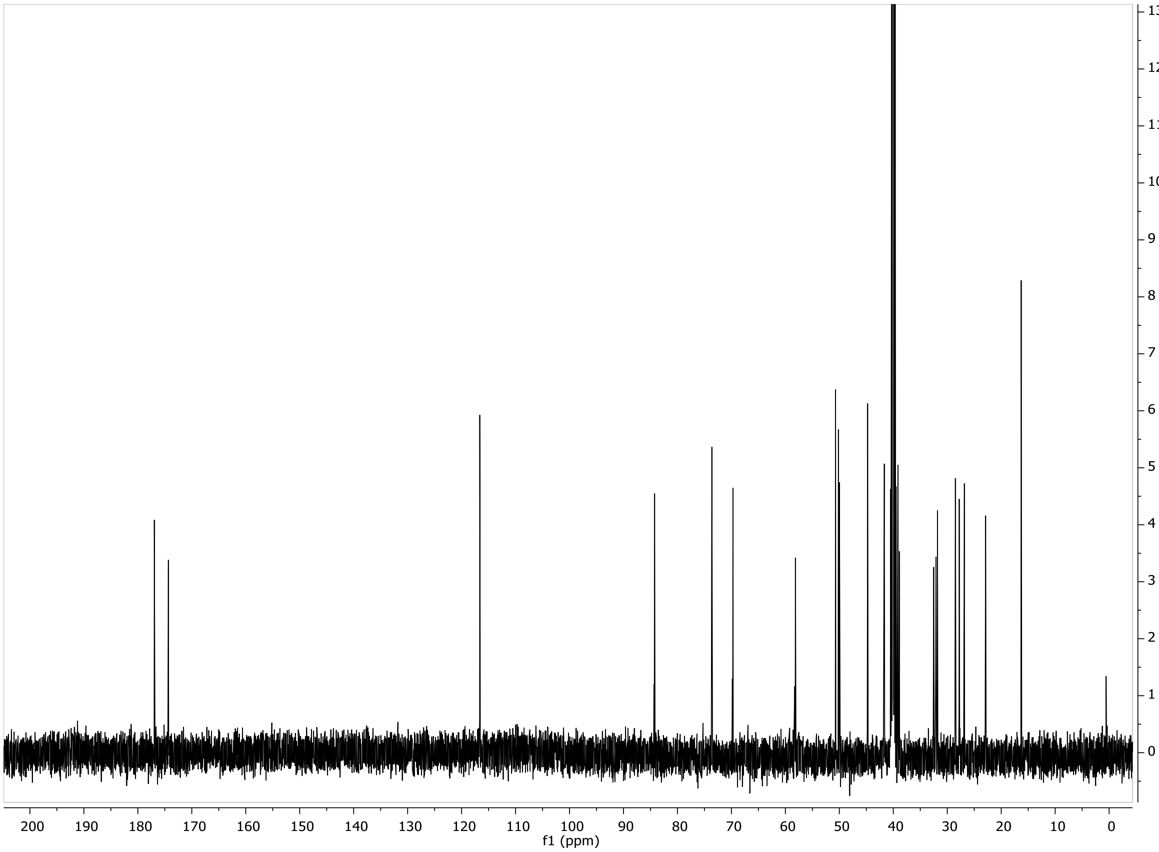


Figure S16. ^{13}C NMR (125 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

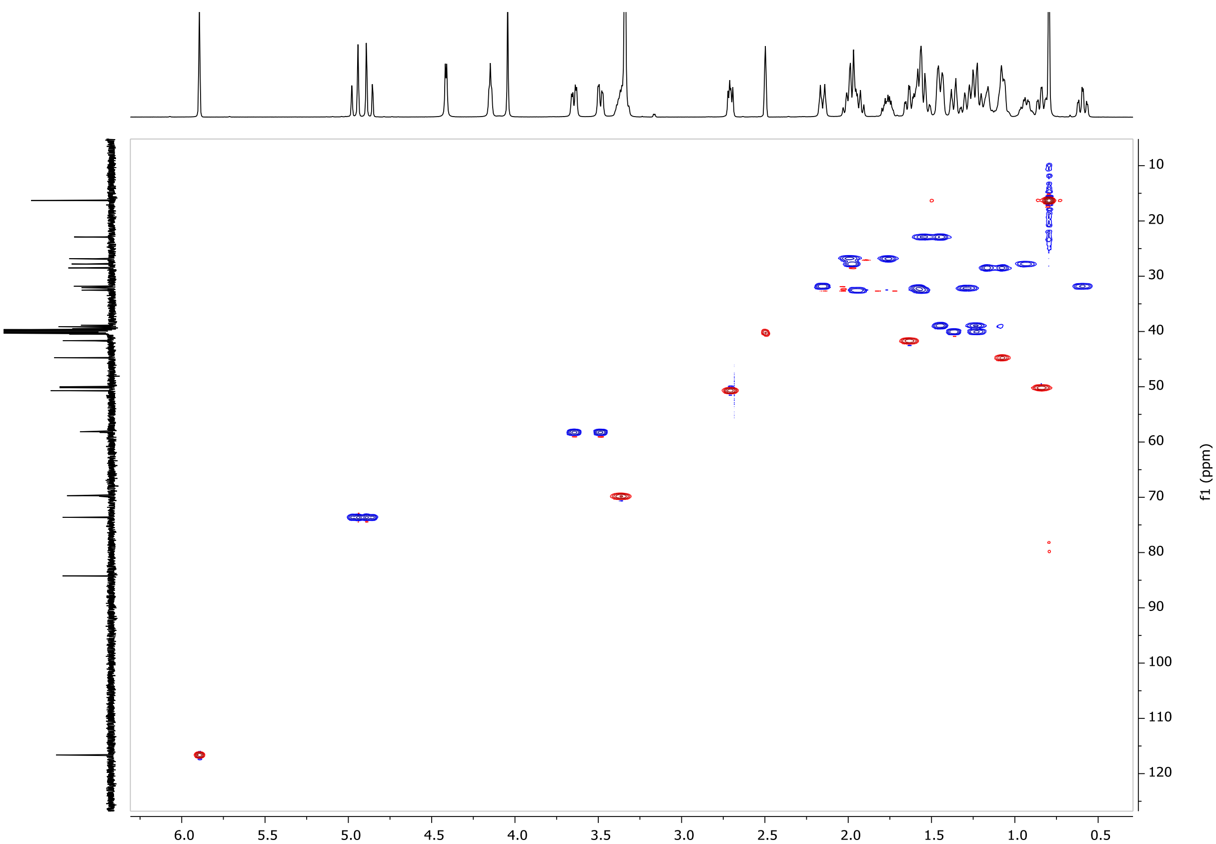


Figure S17. HSQC (500 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

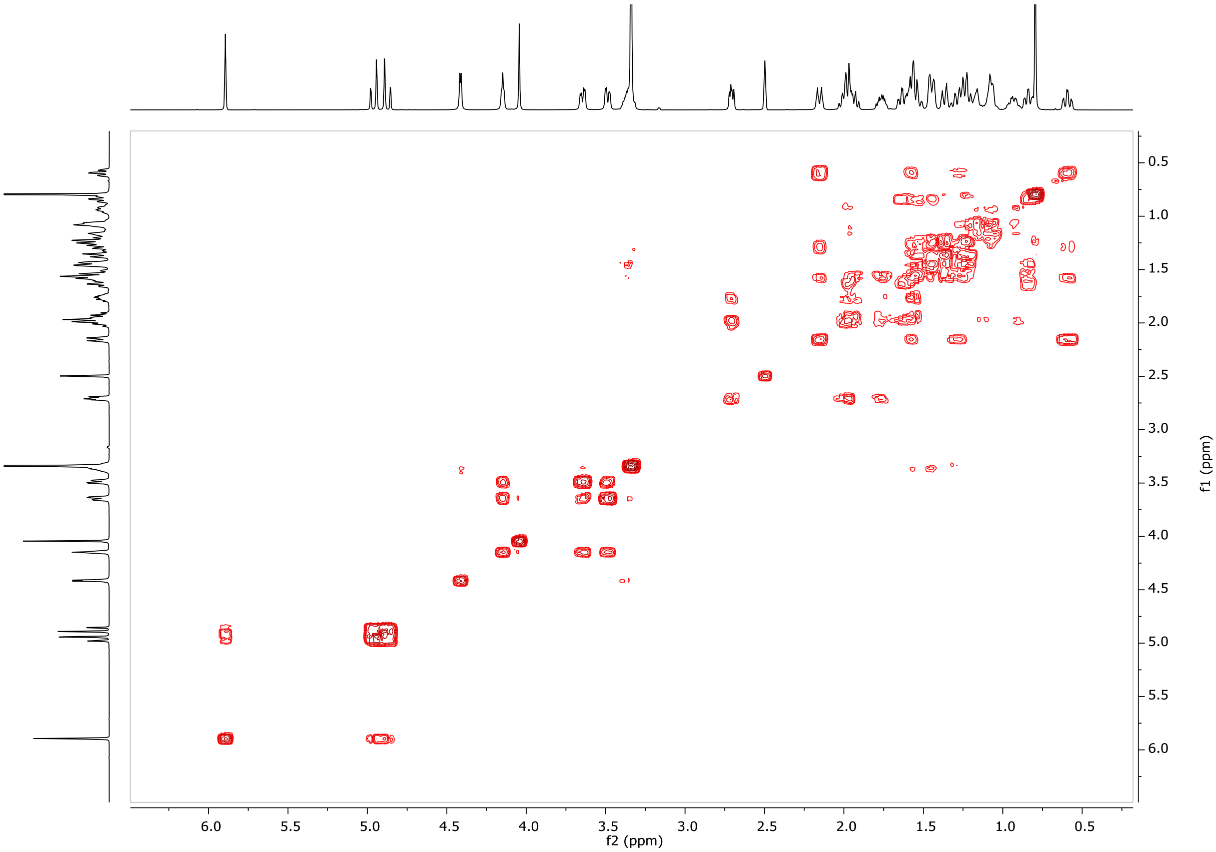


Figure S18. ^{1}H-^{1}H COSY (500 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

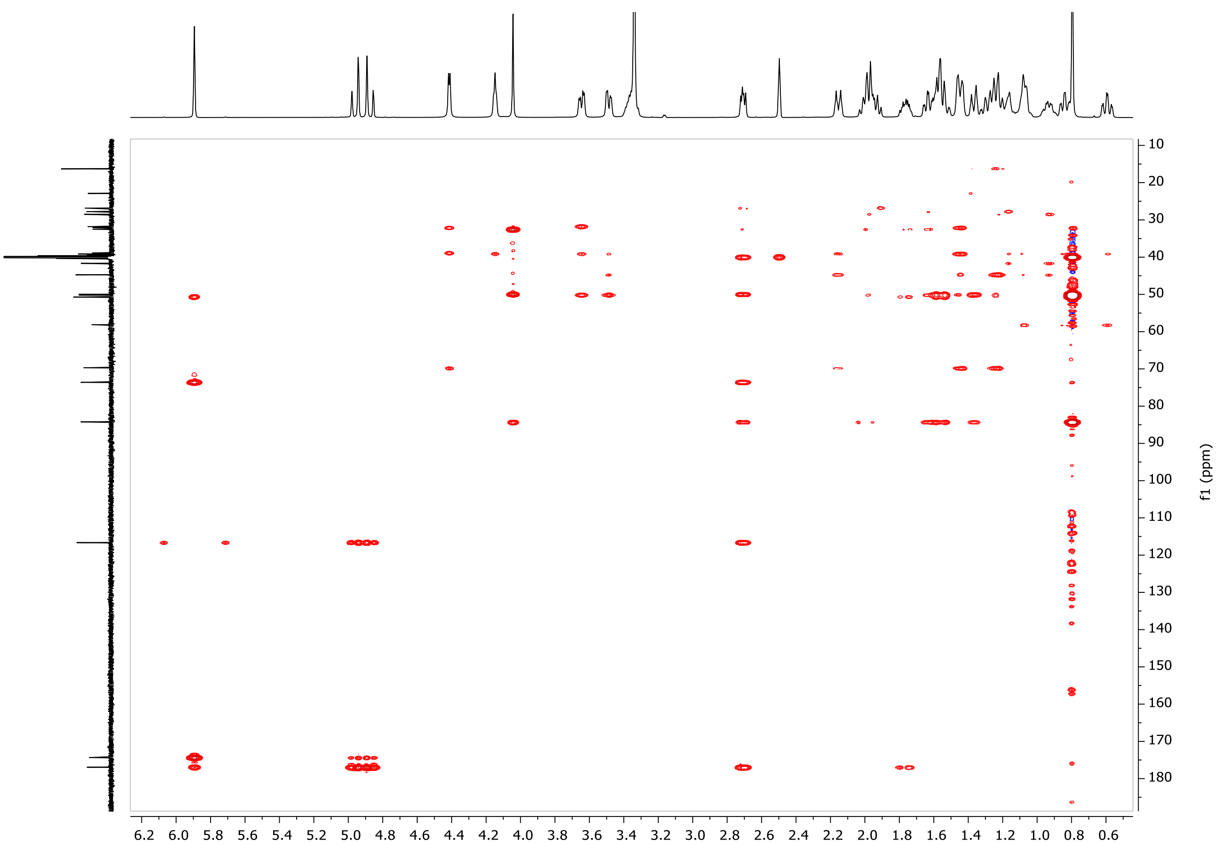


Figure S19. HMBC (500 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

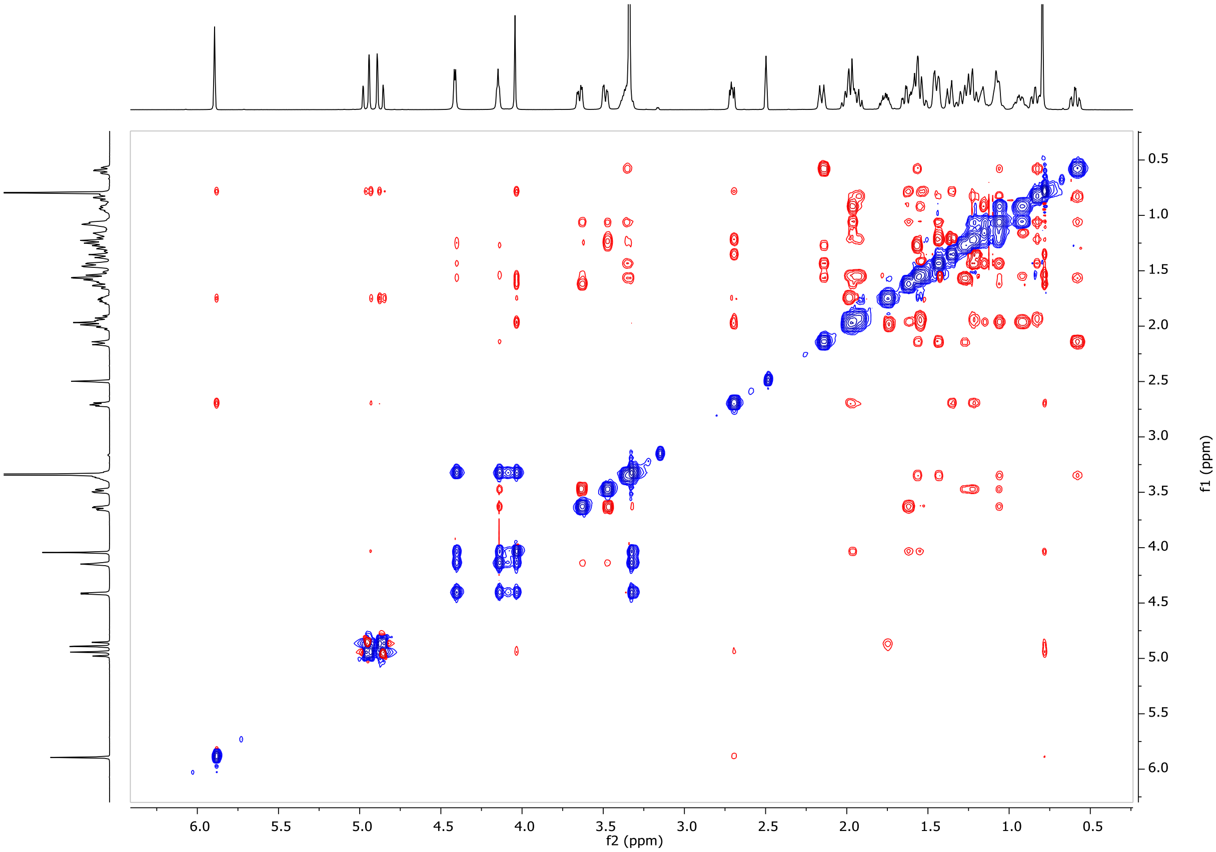


Figure S20. ROESY (500 MHz) spectrum of coroglaucigenin (**2**) in DMSO-d_6

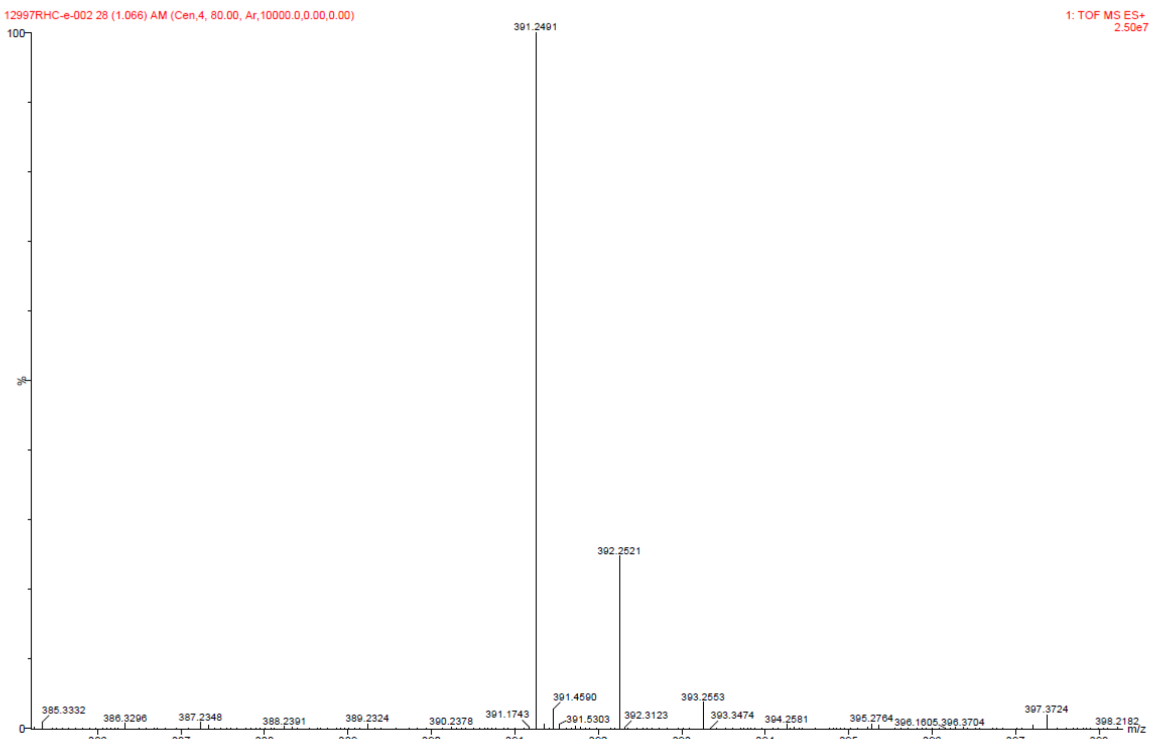


Figure S21. HRESIMS spectrum of coroglaucigenin (**2**)

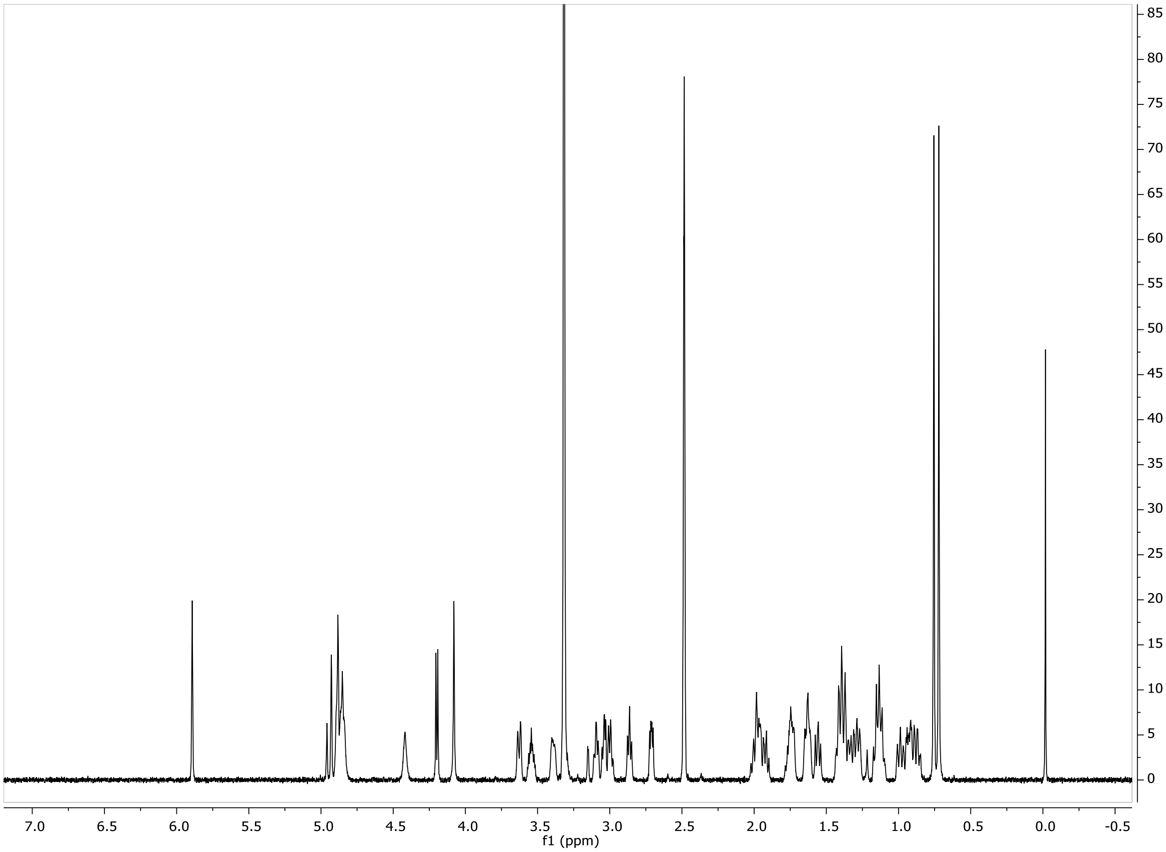


Figure S22. ^{1}H NMR (600 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

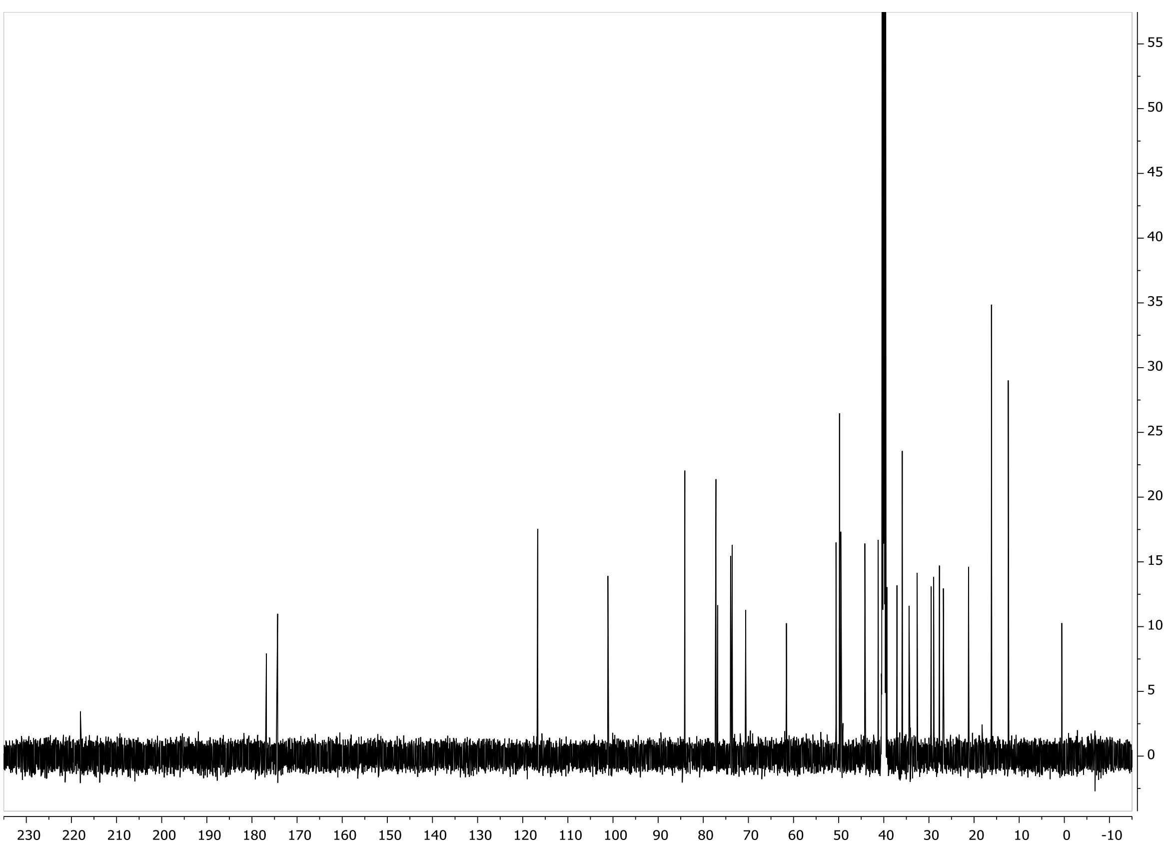


Figure S23. ^{13}C NMR (150 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

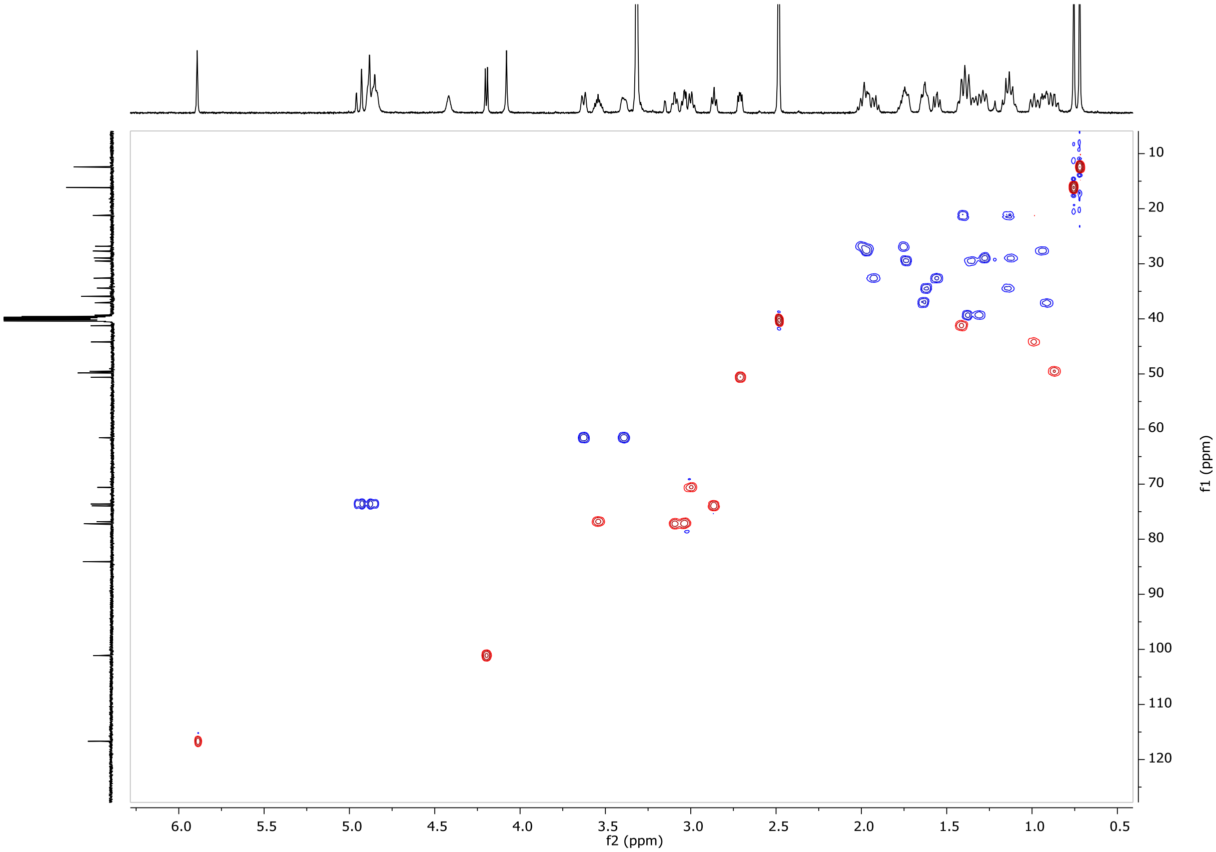


Figure S24. HSQC (500 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

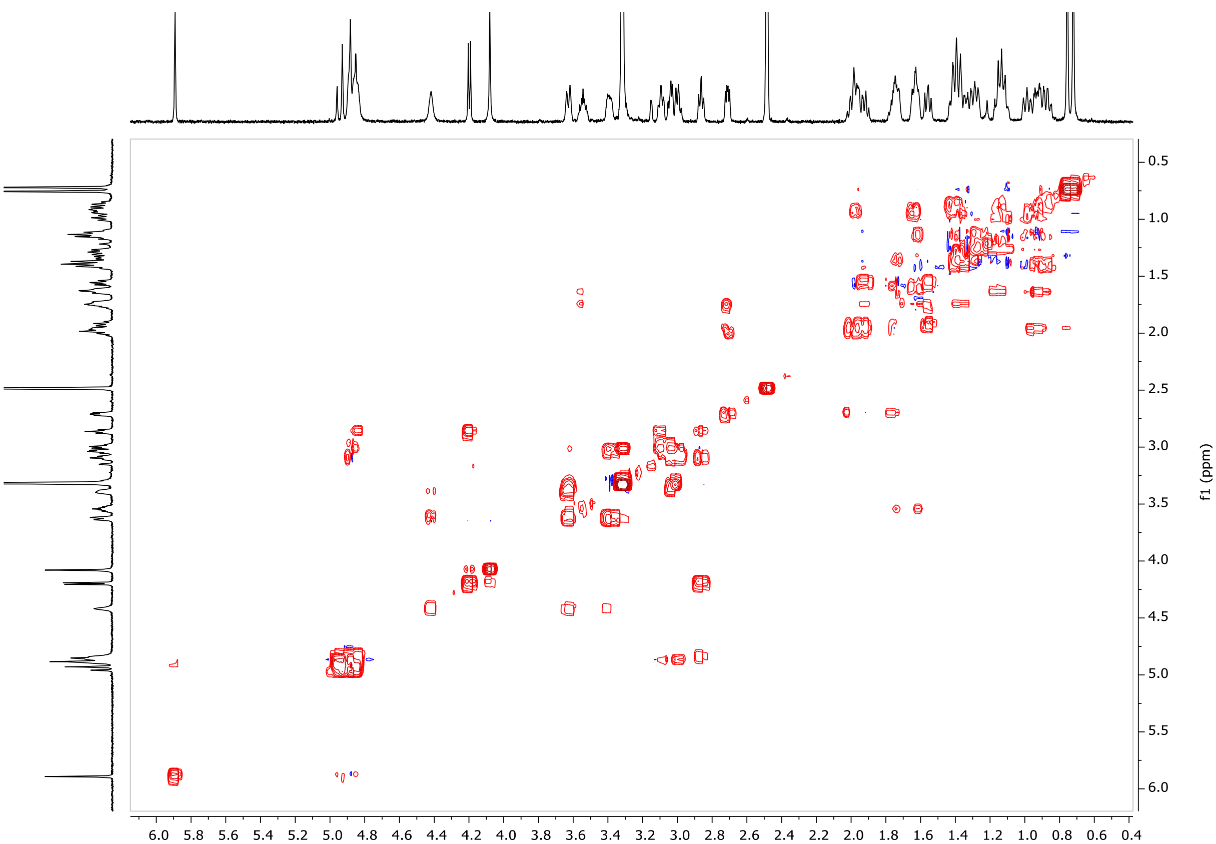


Figure S25. ^1H-^1H COSY (500 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

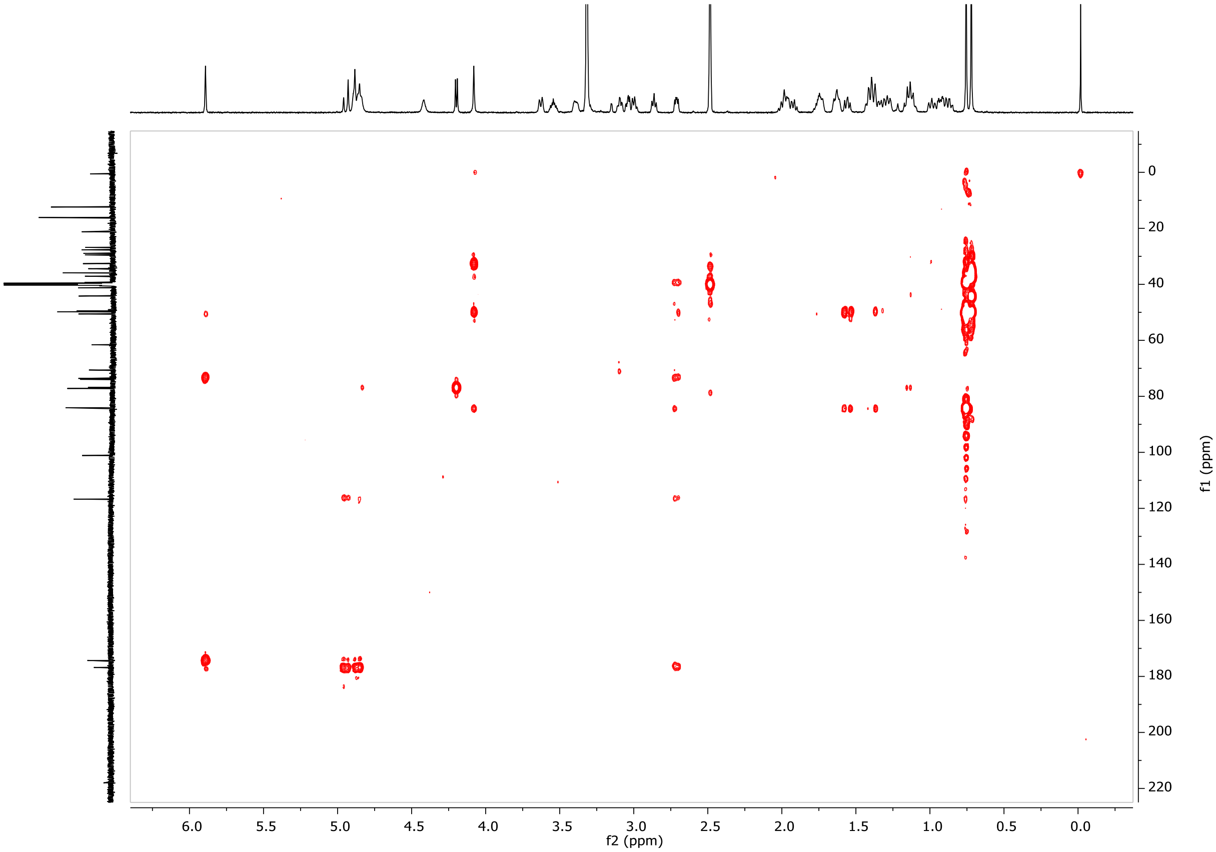


Figure S26. HMBC (500 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

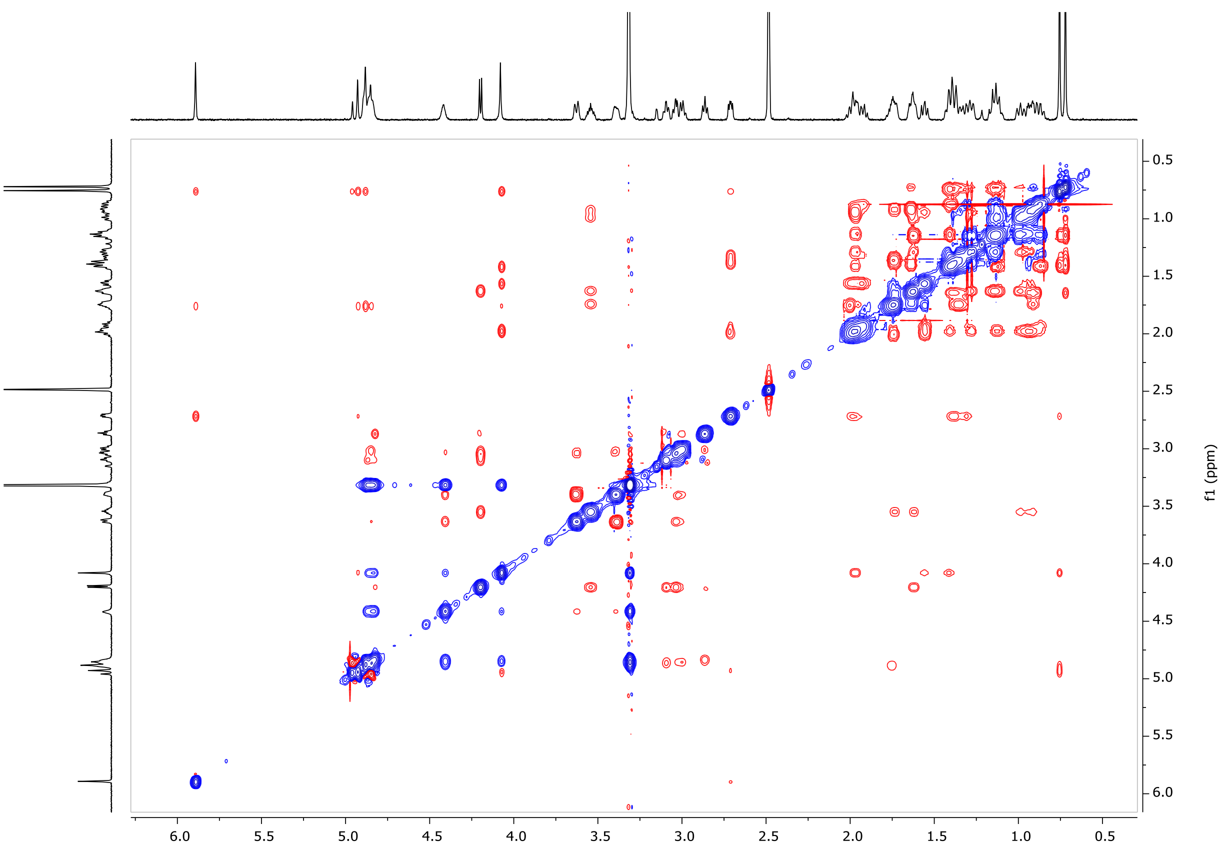


Figure S27. ROESY (500 MHz) spectrum of desglucouzarin (**3**) in DMSO-d_6

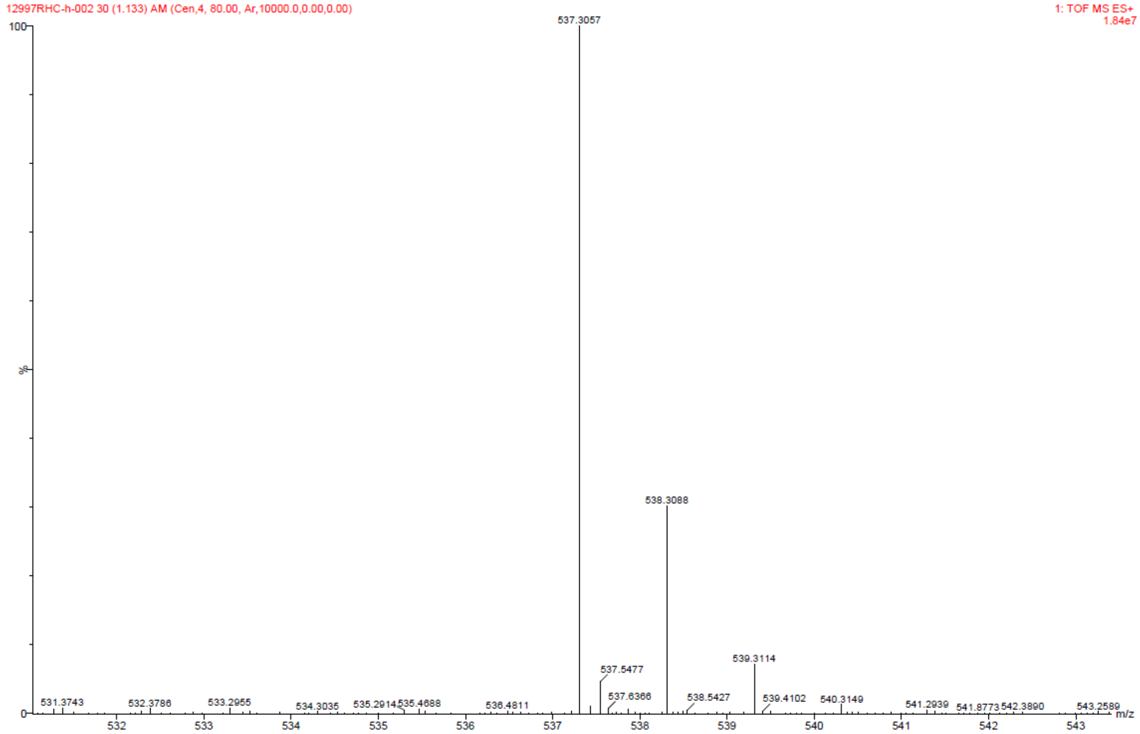


Figure S28. HRESIMS spectrum of desglucouzarin (**3**)

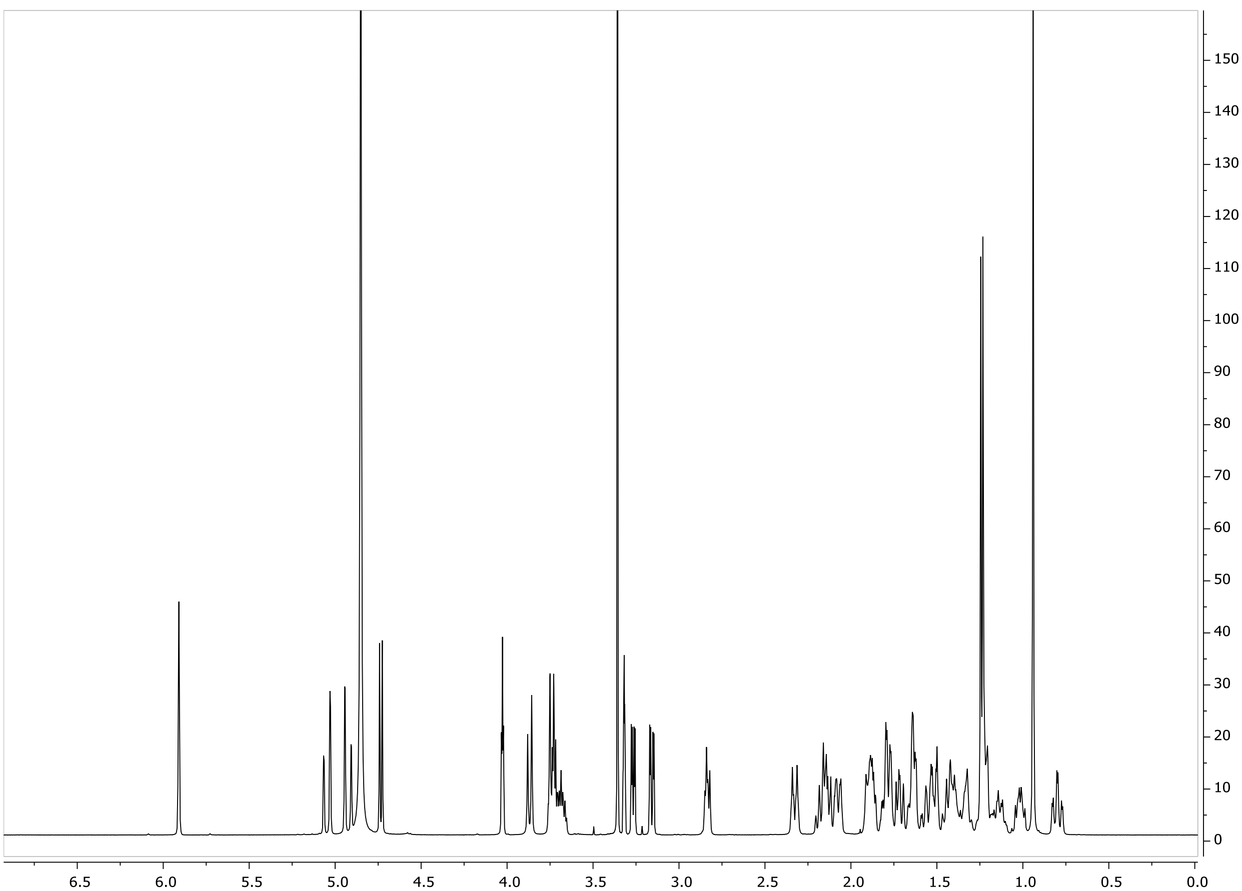


Figure S29. ^{1}H NMR (500 MHz) spectrum of frugoside (**4**) in methanol-d_4

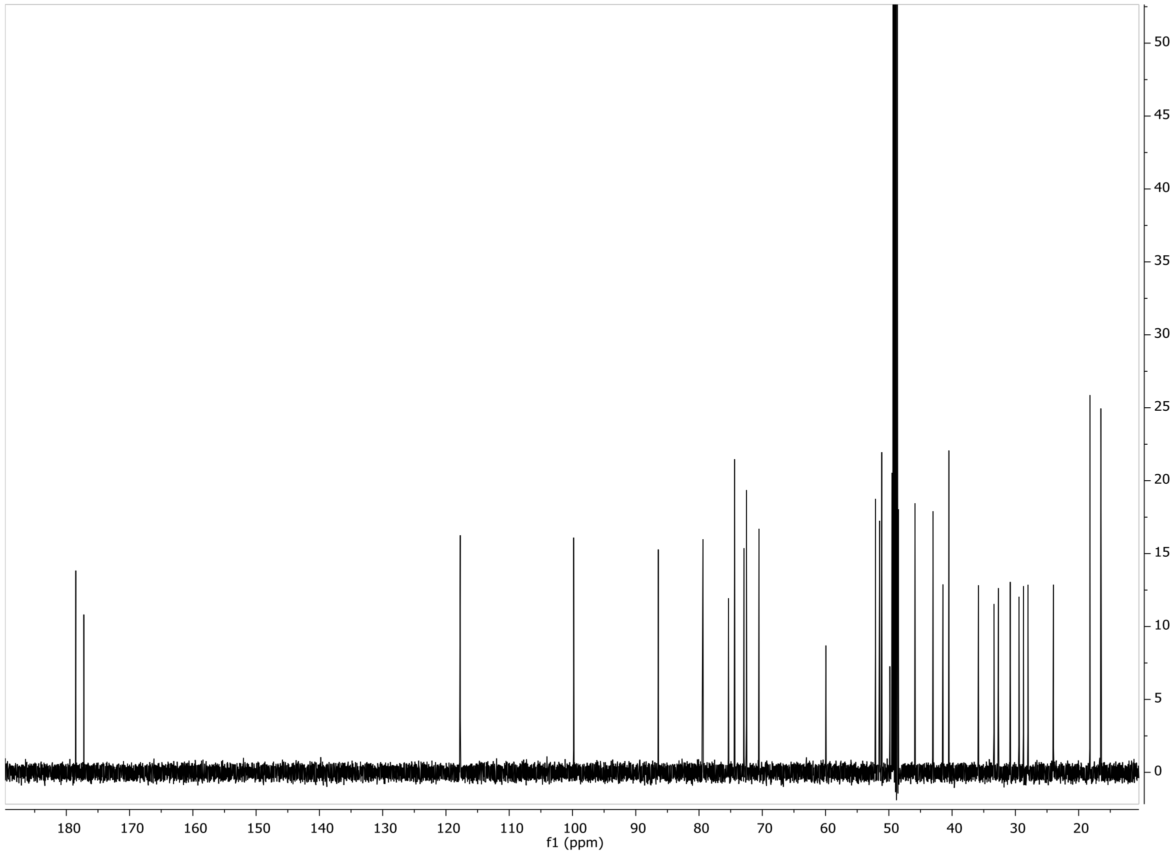


Figure S30. ^{13}C NMR (125 MHz) spectrum of frugoside (**4**) in methanol-d_4

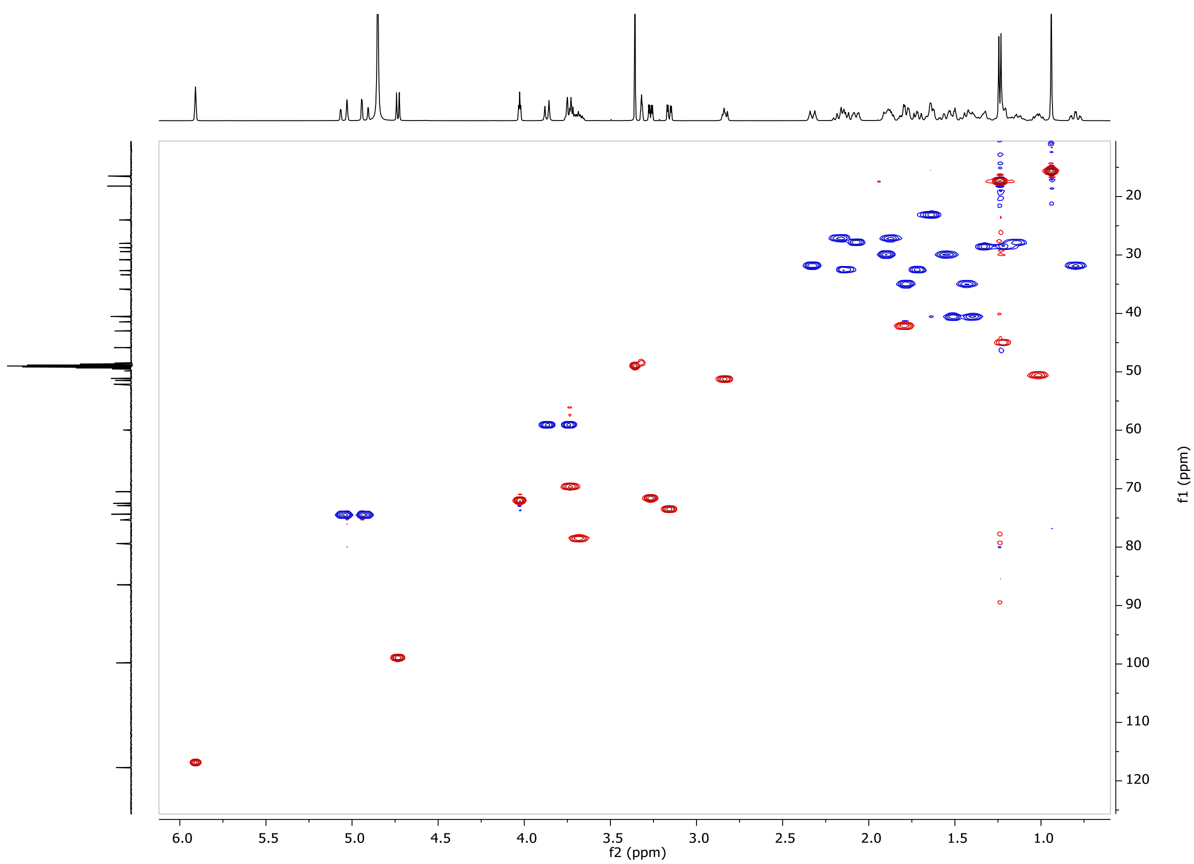


Figure S31. HSQC (500 MHz) spectrum of frugoside (**4**) in methanol-d_4

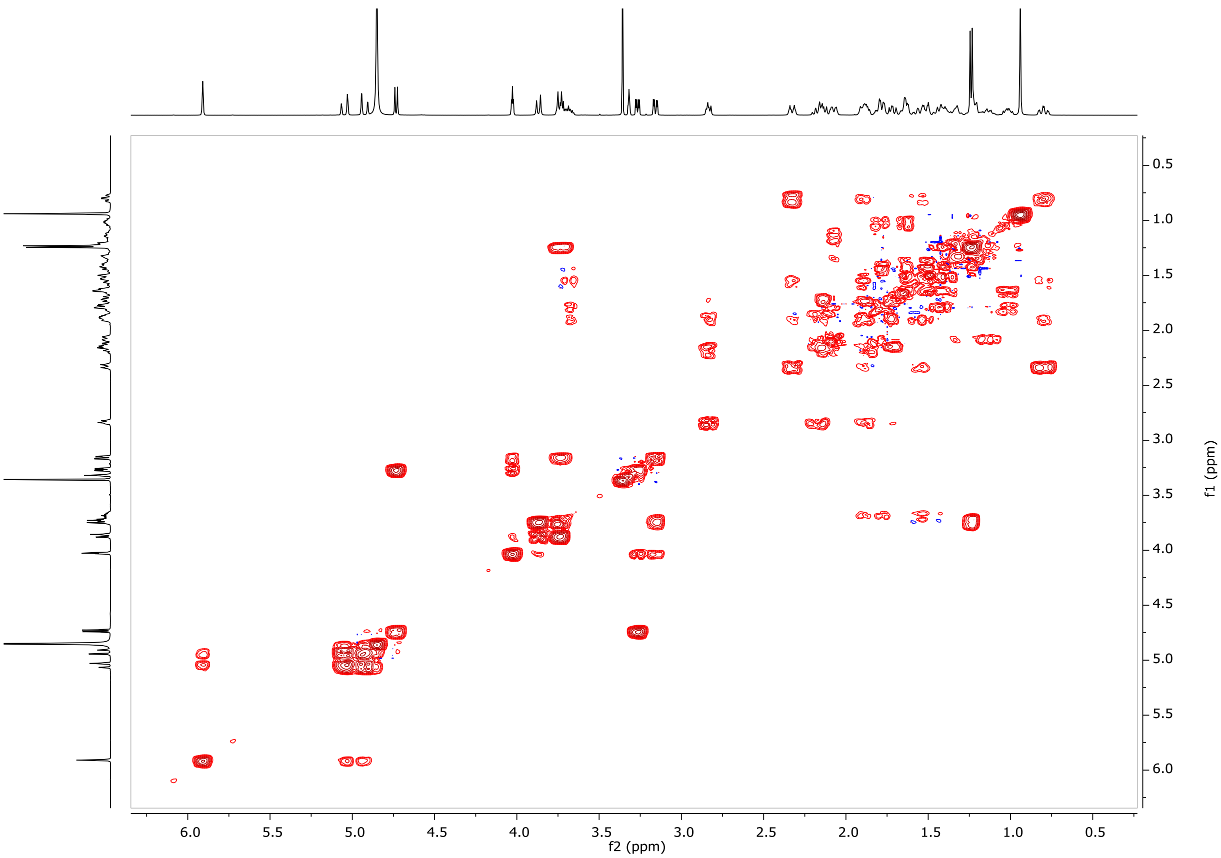


Figure S32. ^{1}H-^{1}H COSY (500 MHz) spectrum of frugoside (**4**) in methanol-d_4

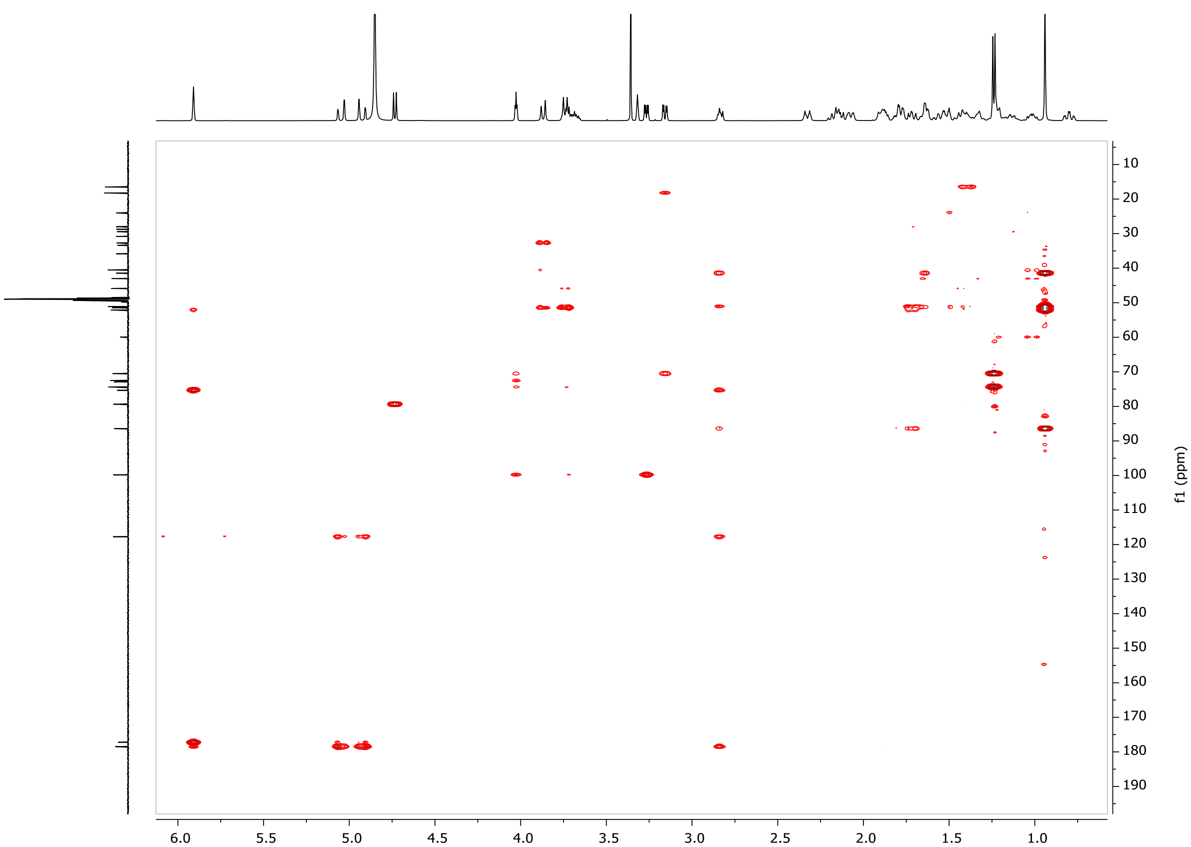


Figure S33. HMBC (500 MHz) spectrum of frugoside (**4**) in methanol-d_4

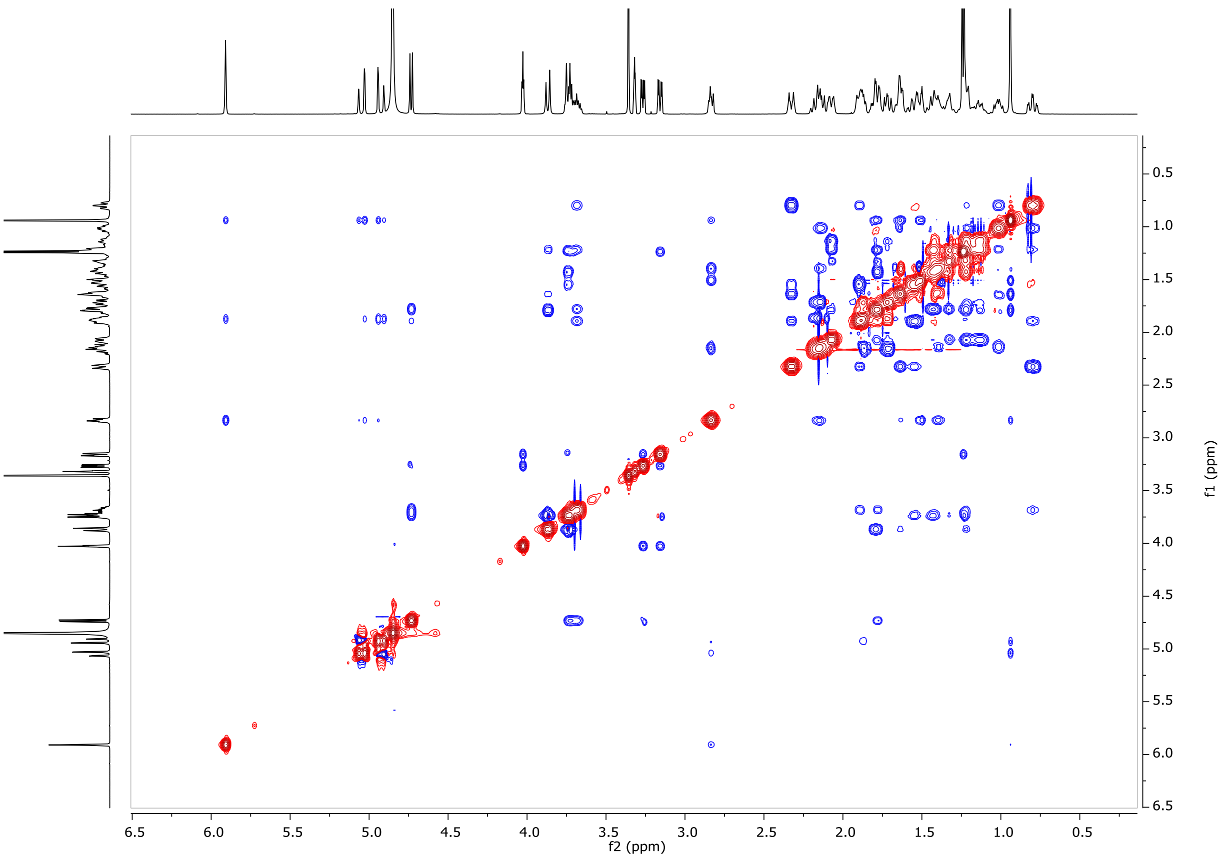


Figure S34. ROESY (500 MHz) spectrum of frugoside (**4**) in methanol-d_4

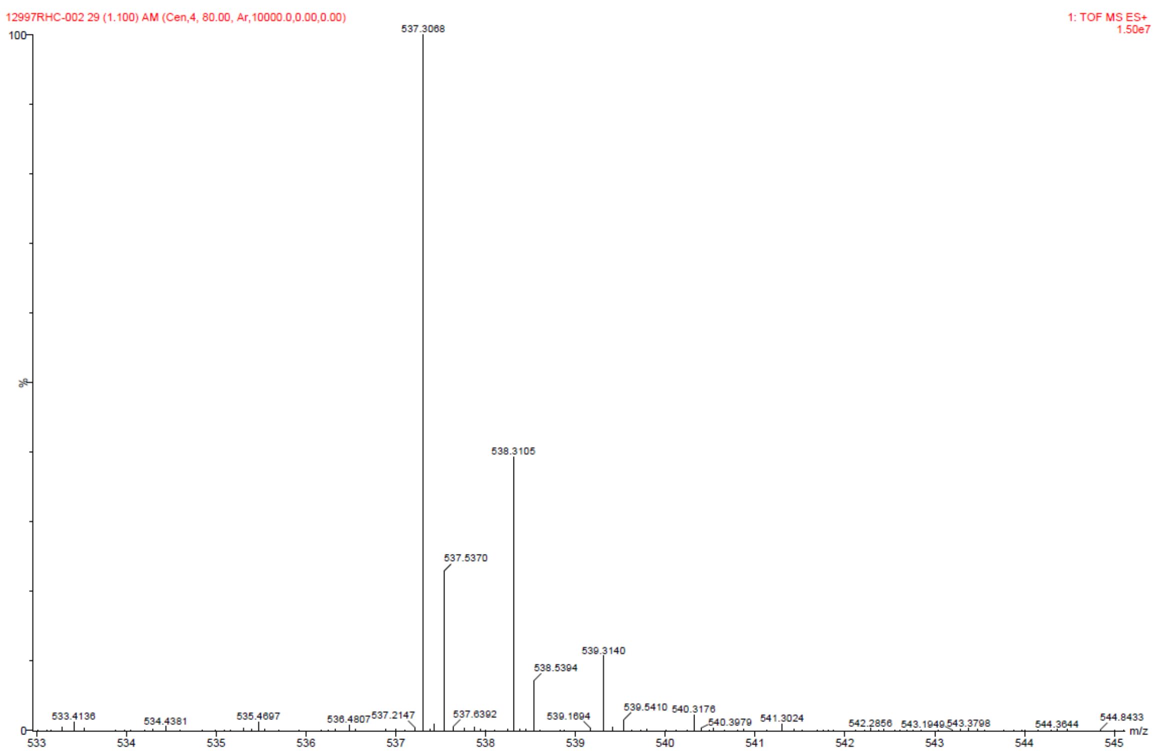


Figure S35. HRESIMS spectrum of frugoside (**4**)

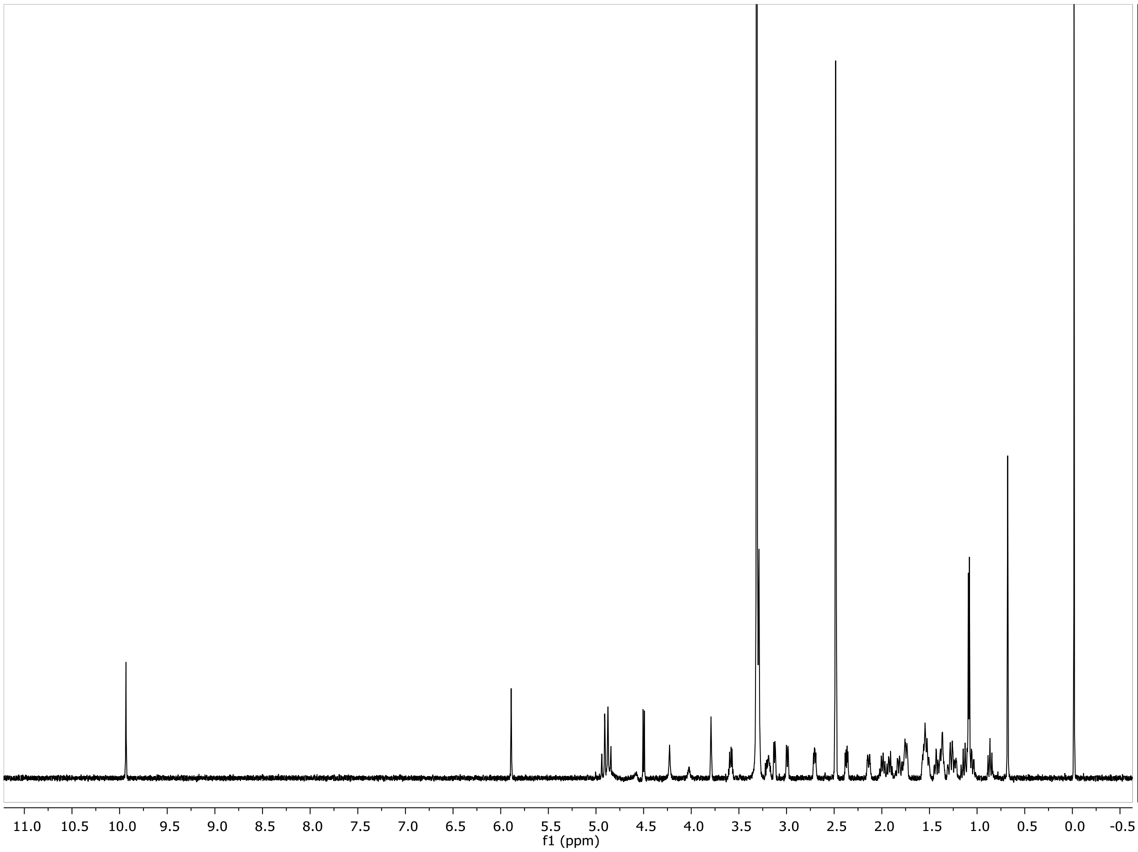


Figure S36. ^{1}H NMR (600 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

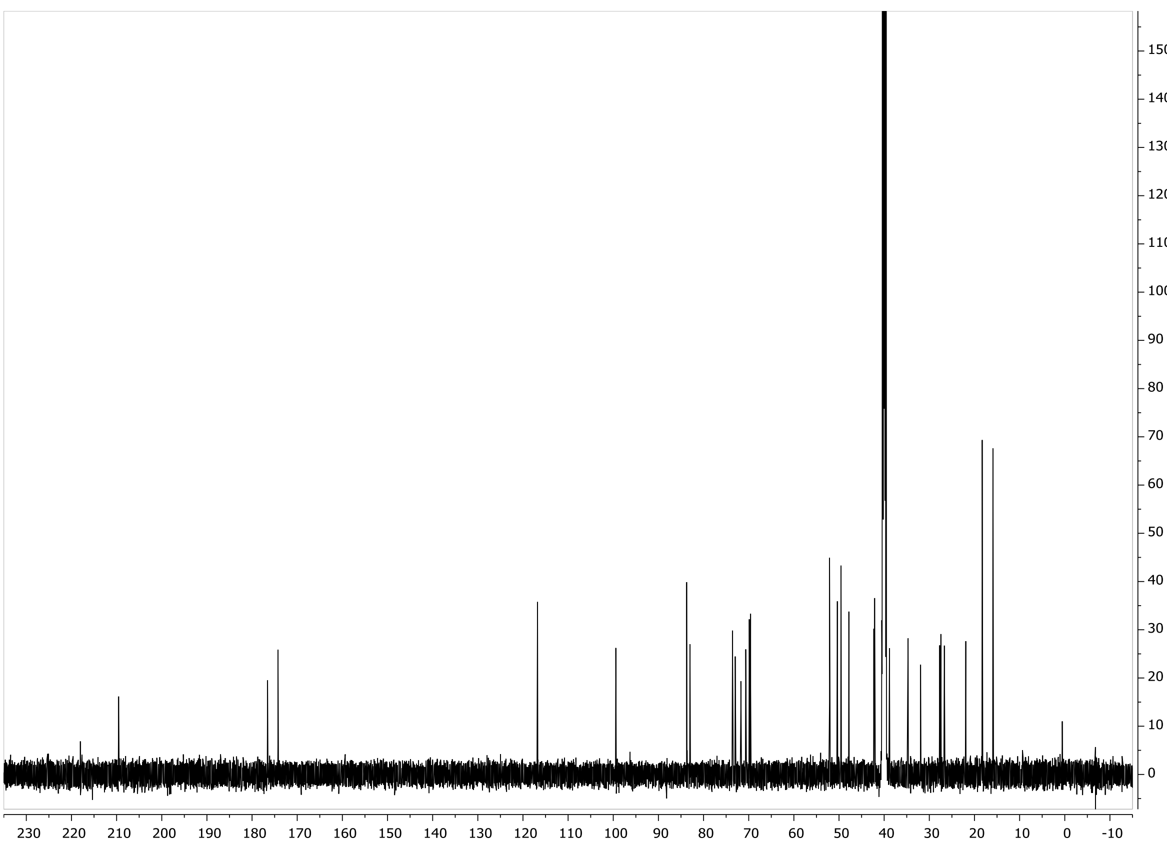


Figure S37. ^{13}C NMR (150 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

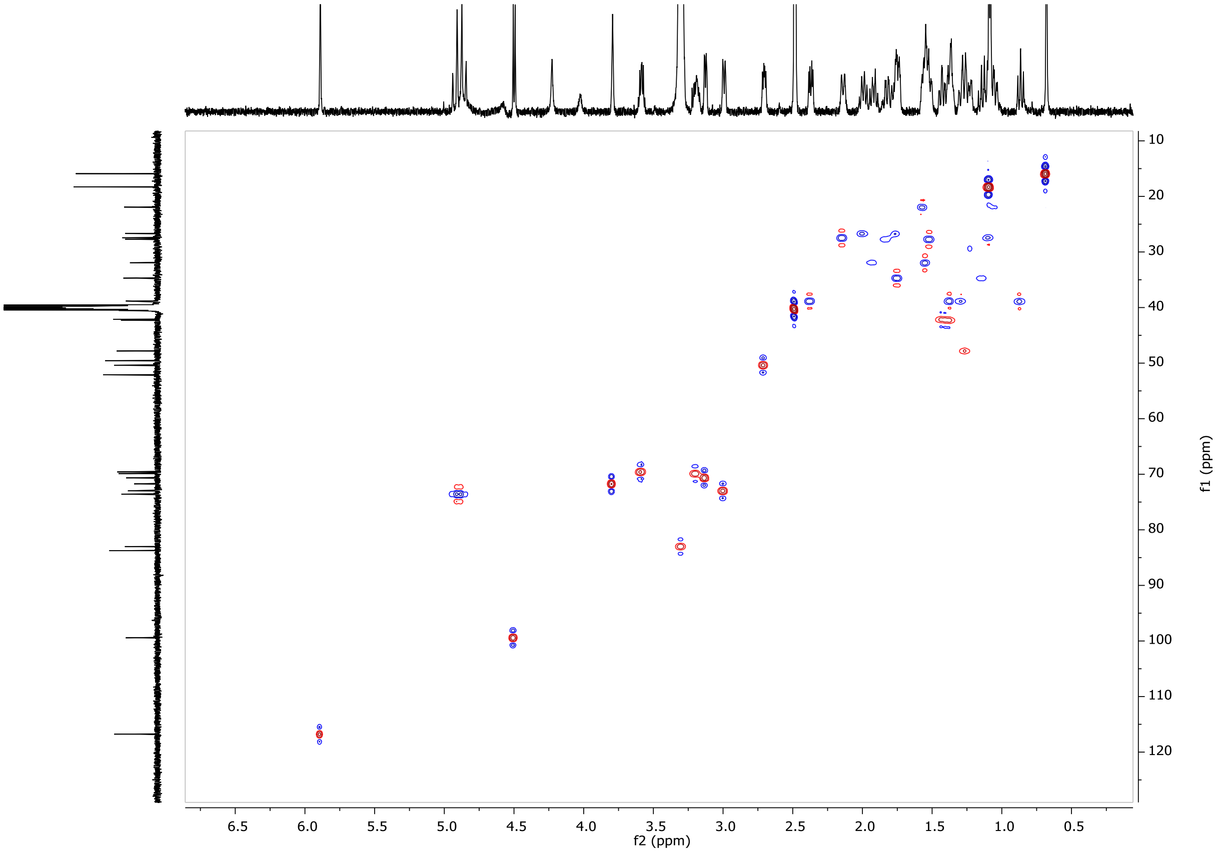


Figure S38. HSQC (500 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

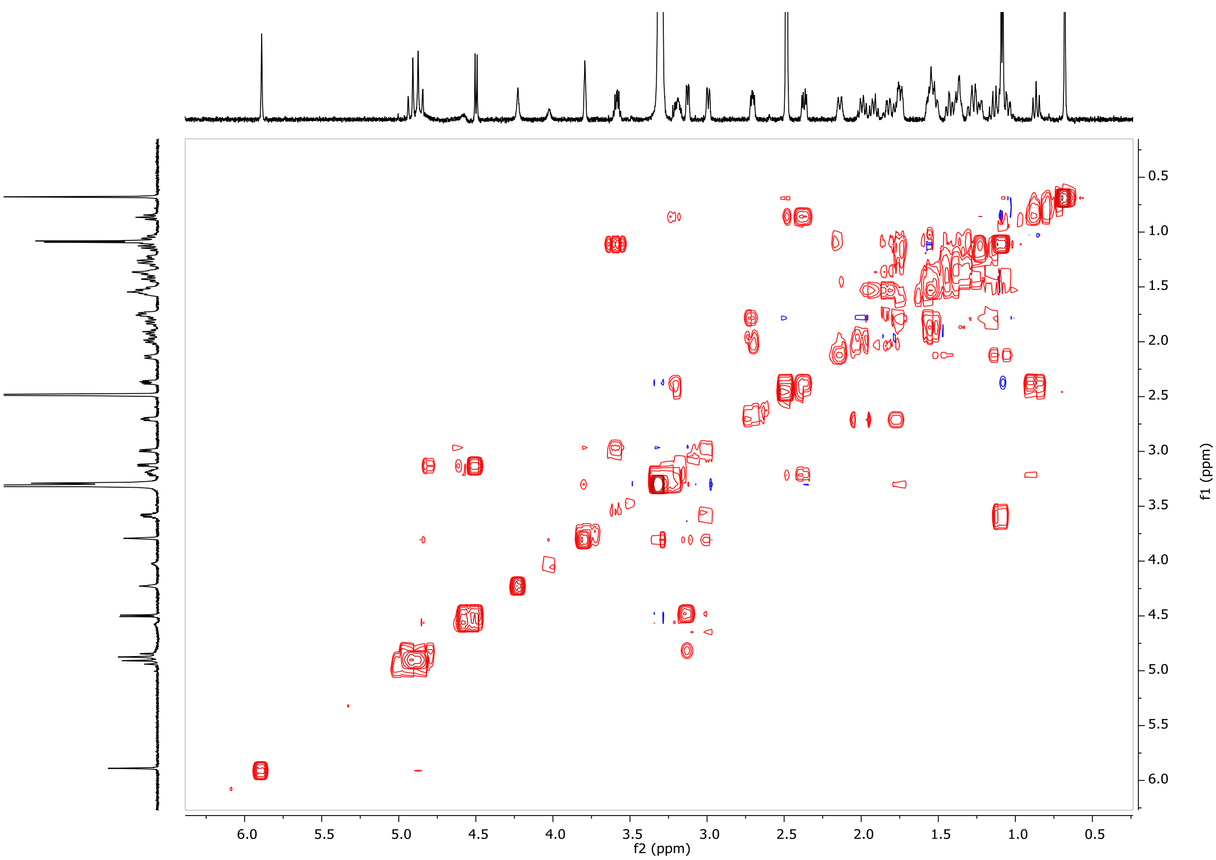


Figure S39. ^{1}H-^{1}H COSY (500 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

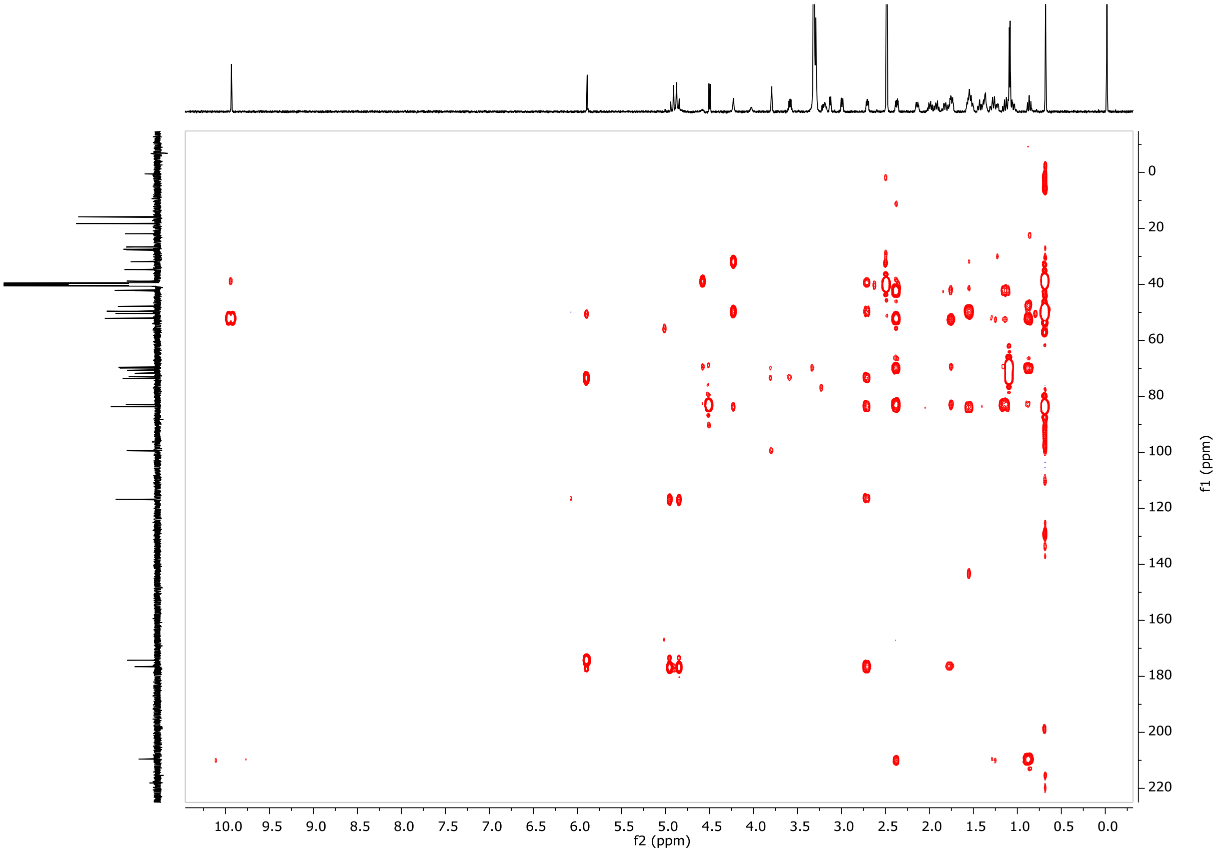


Figure S40. HMBC (500 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

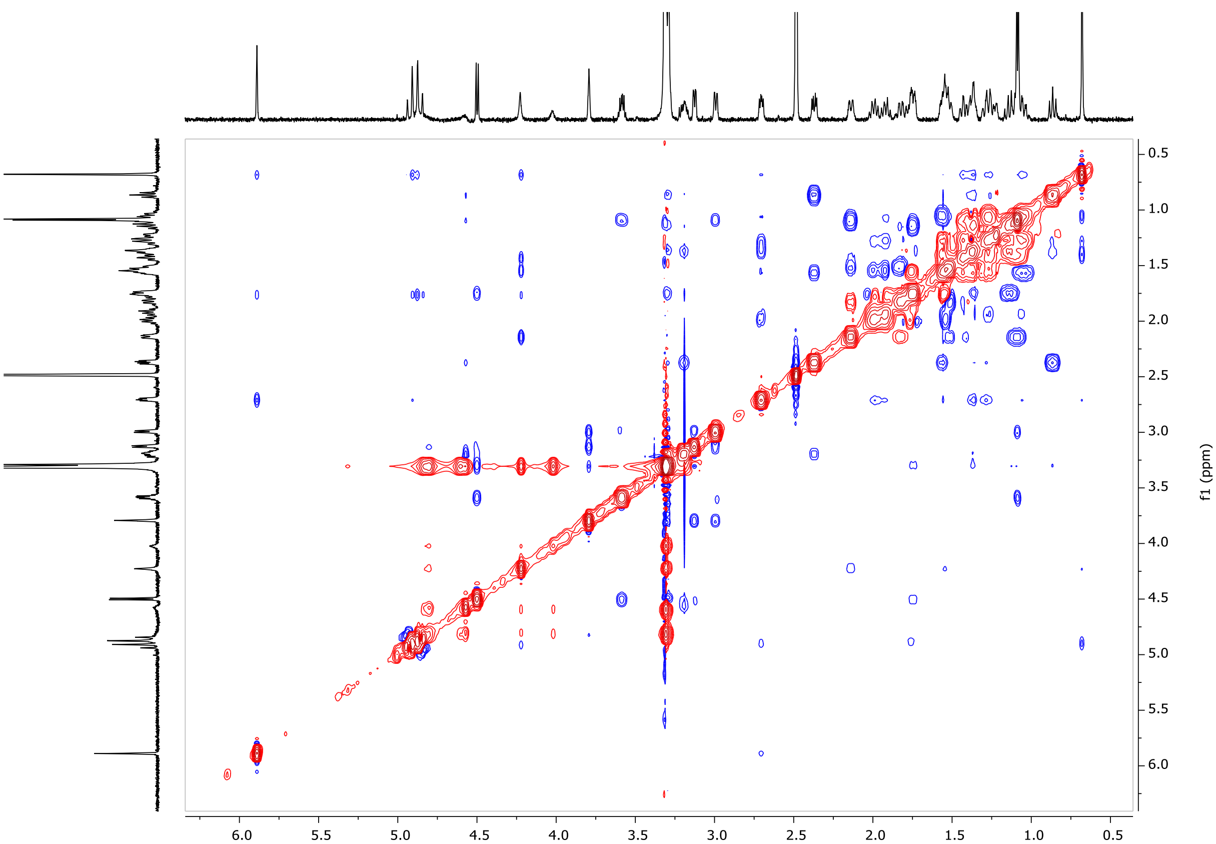


Figure S41. ROESY (500 MHz) spectrum of frugosidal (**5**) in DMSO-d_6

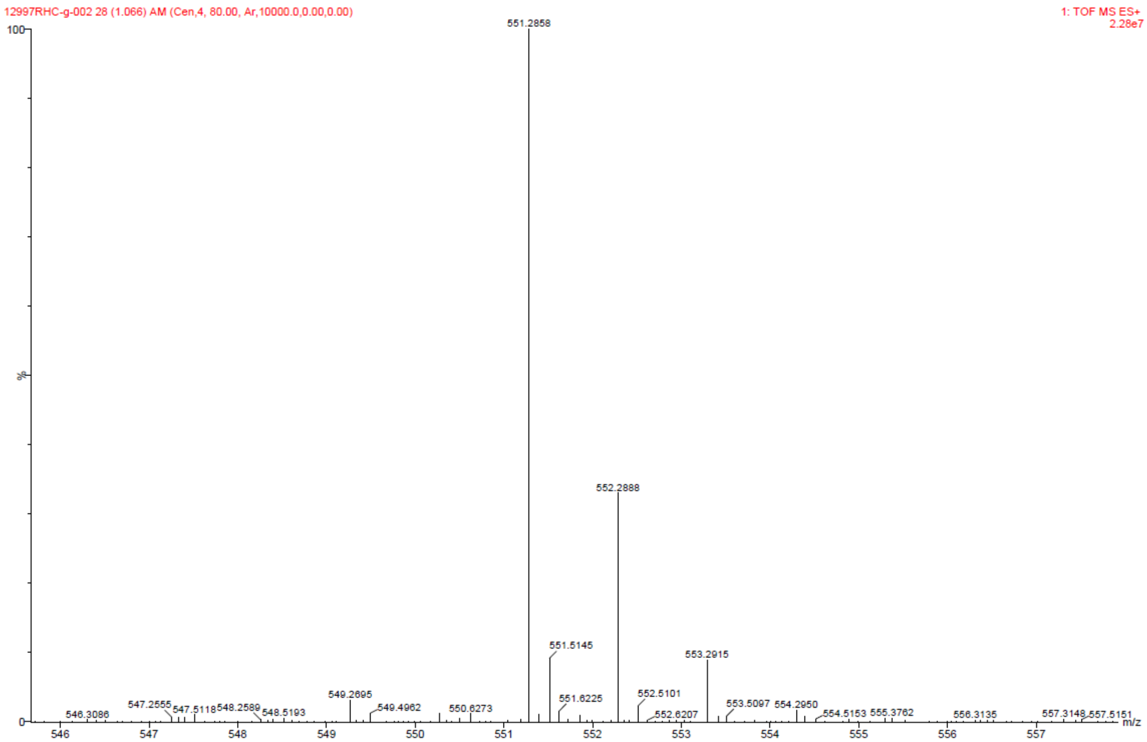


Figure S42. HRESIMS spectrum of frugosidal (**5**)

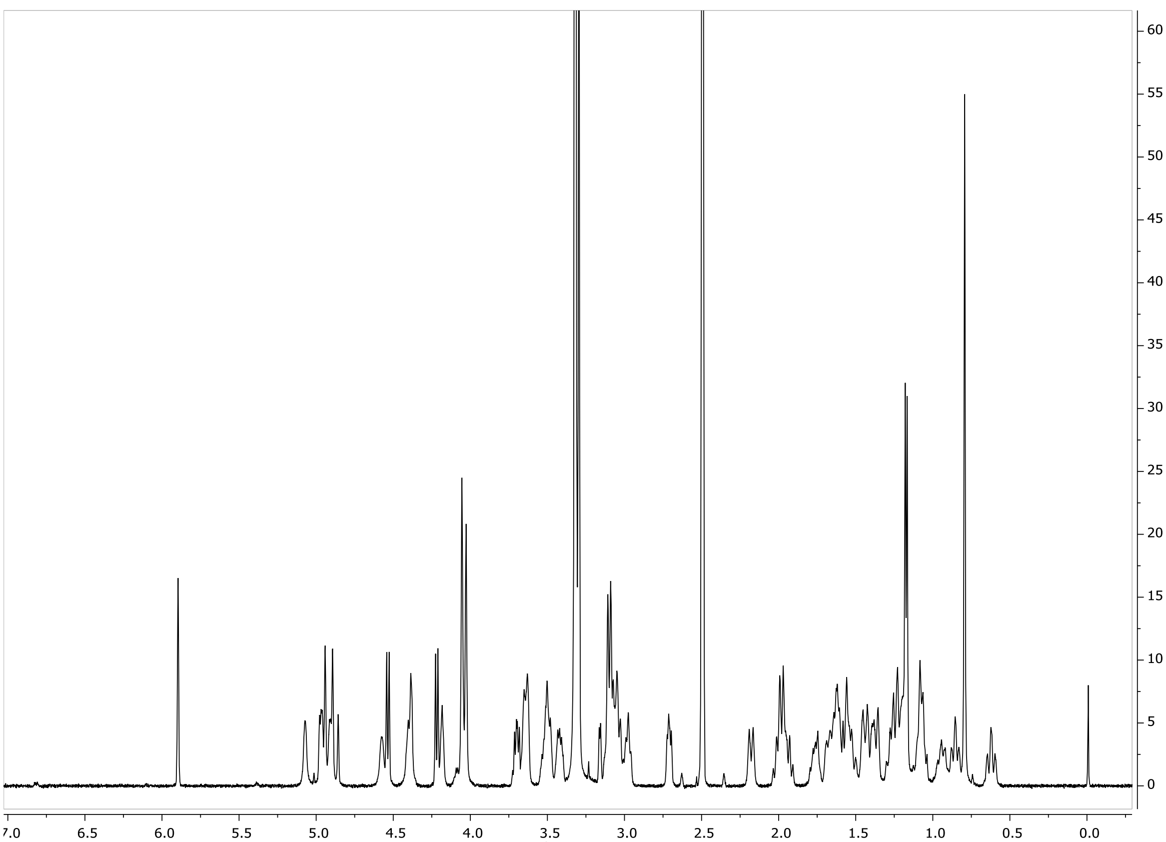


Figure S43. ^{1}H NMR (600 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

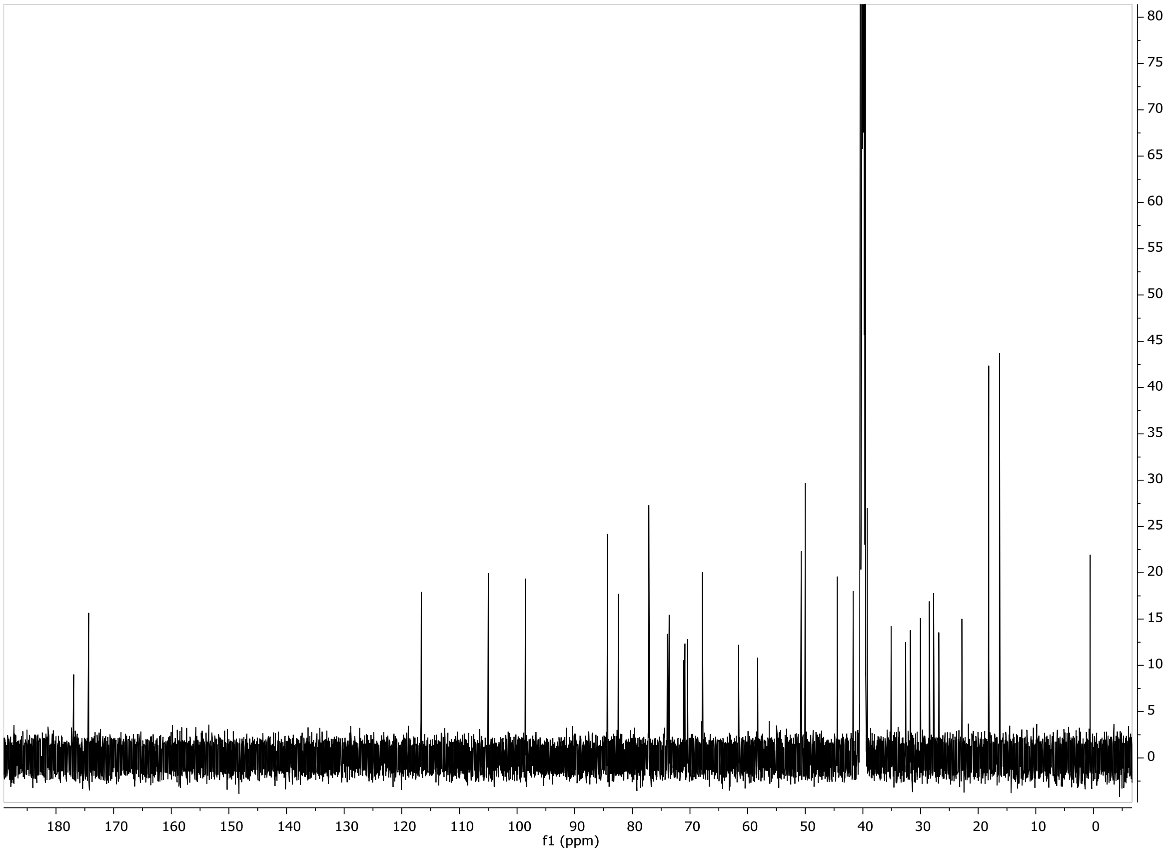


Figure S44. ^{13}C NMR (150 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

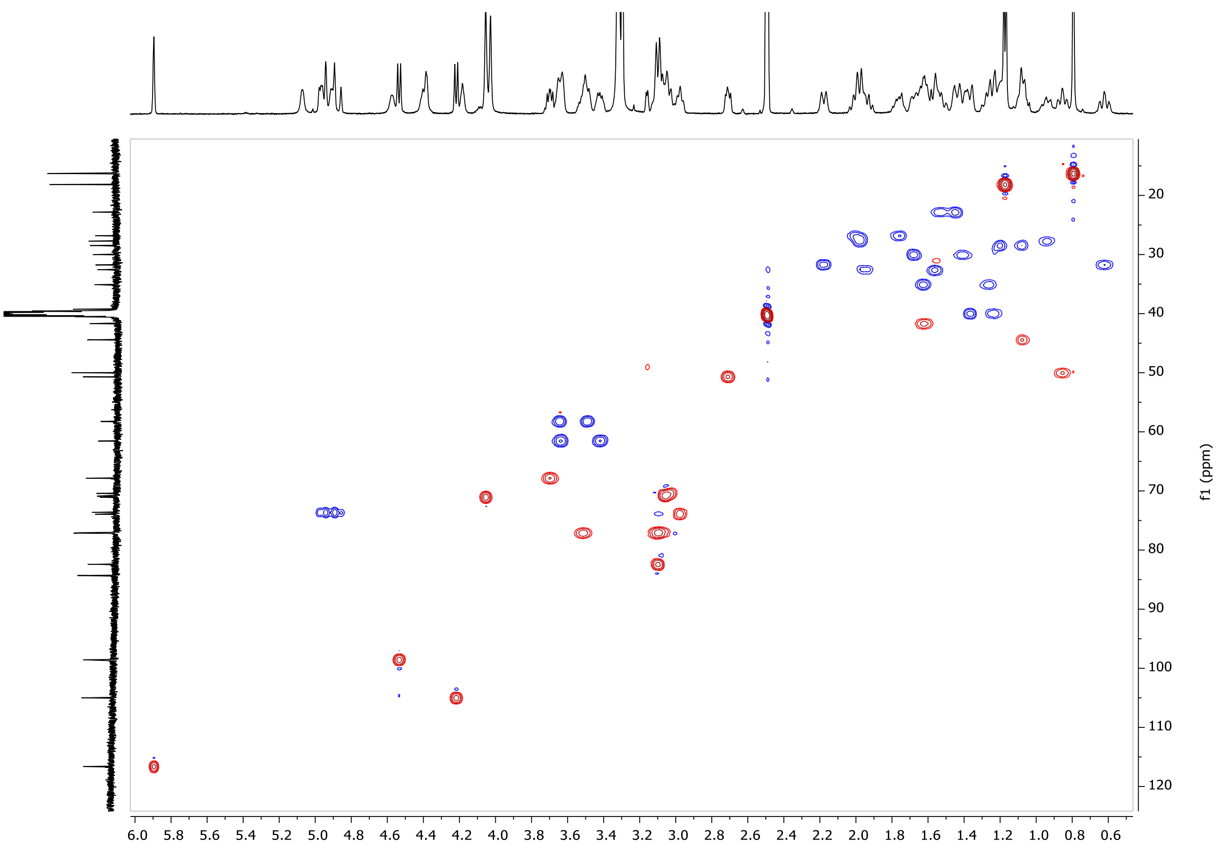


Figure S45. HSQC (500 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

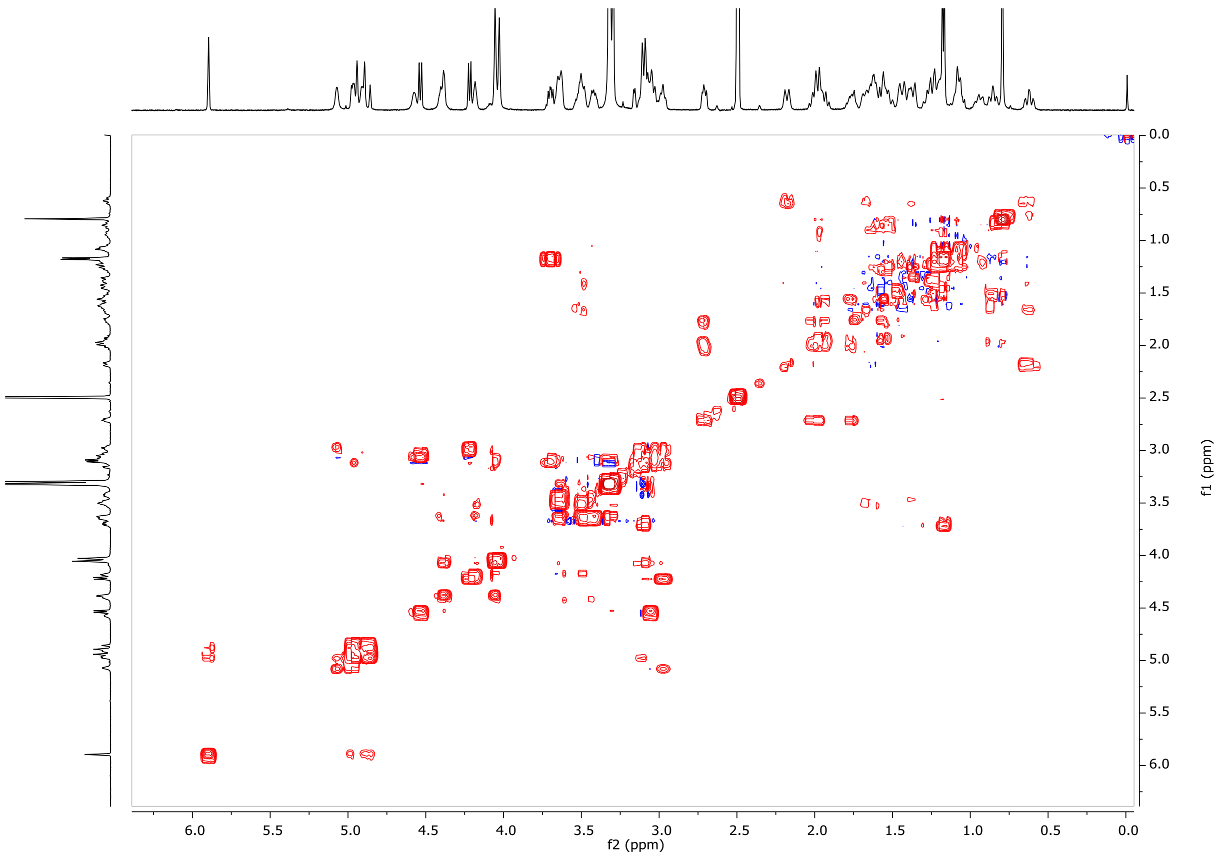


Figure S46. [1]H-[1]H COSY (500 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

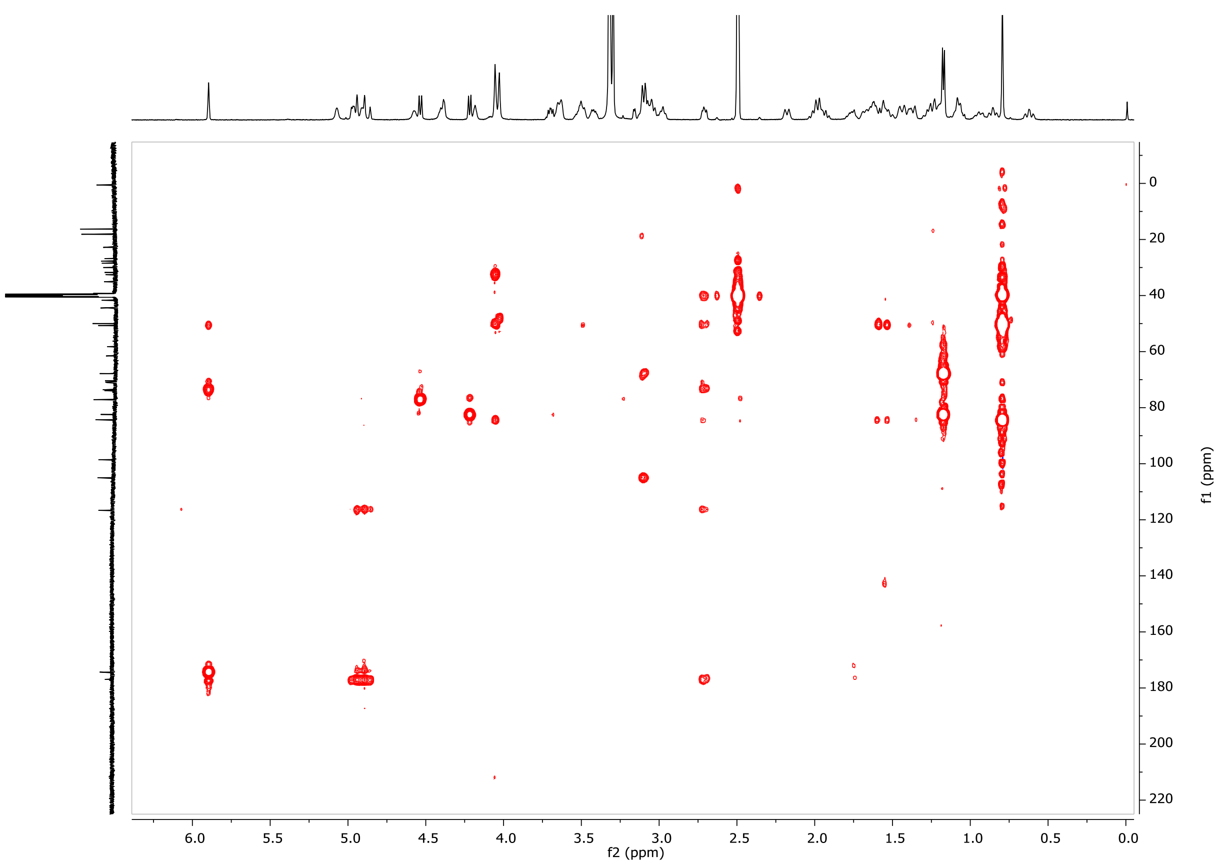


Figure S47. HMBC (500 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

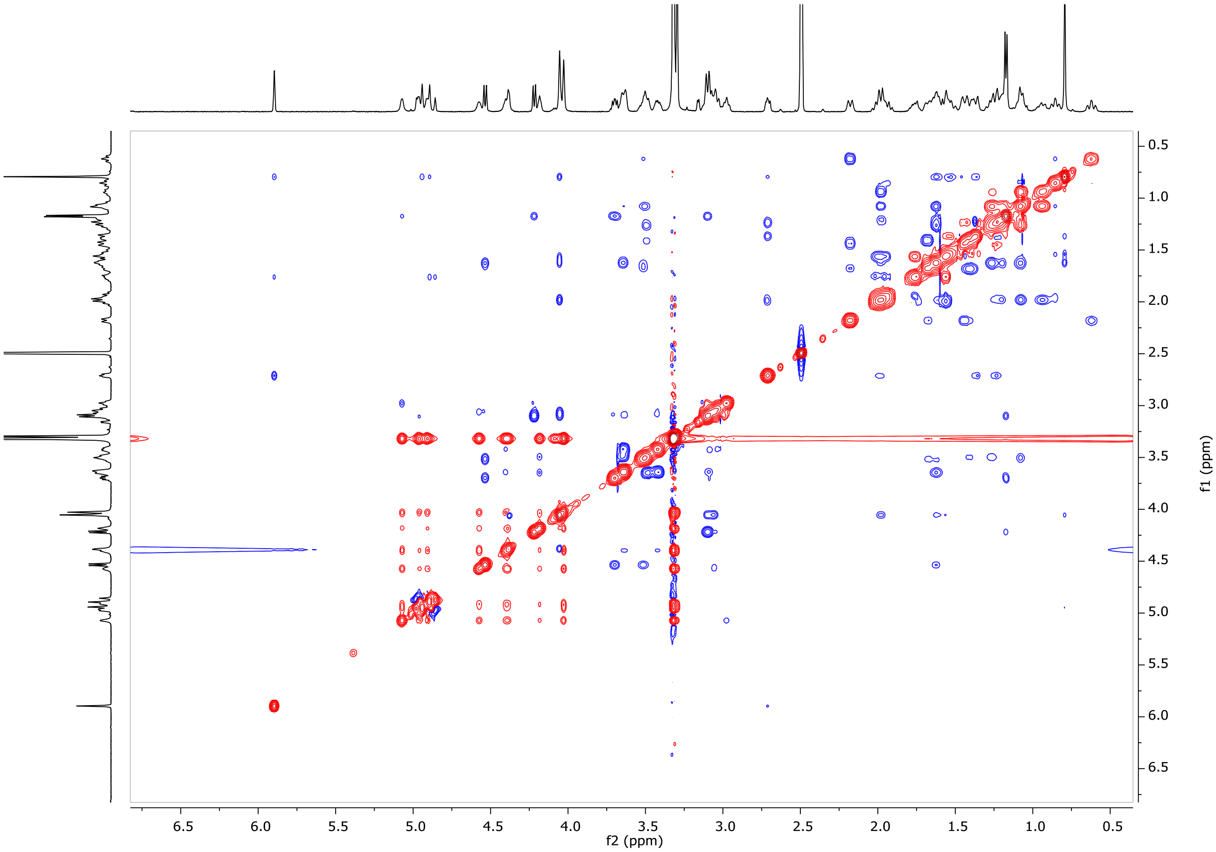


Figure S48. ROESY (500 MHz) spectrum of glucofrugoside (**6**) in DMSO-d_6

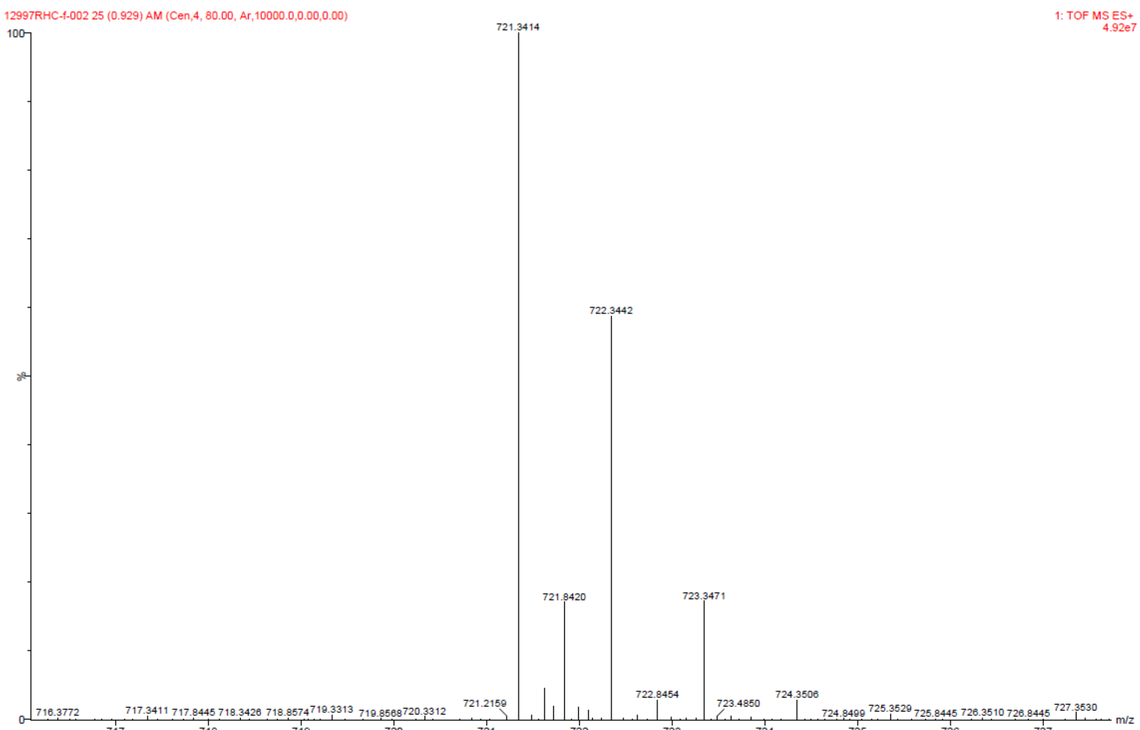


Figure S49. HRESIMS spectrum of glucofrugoside (**6**)

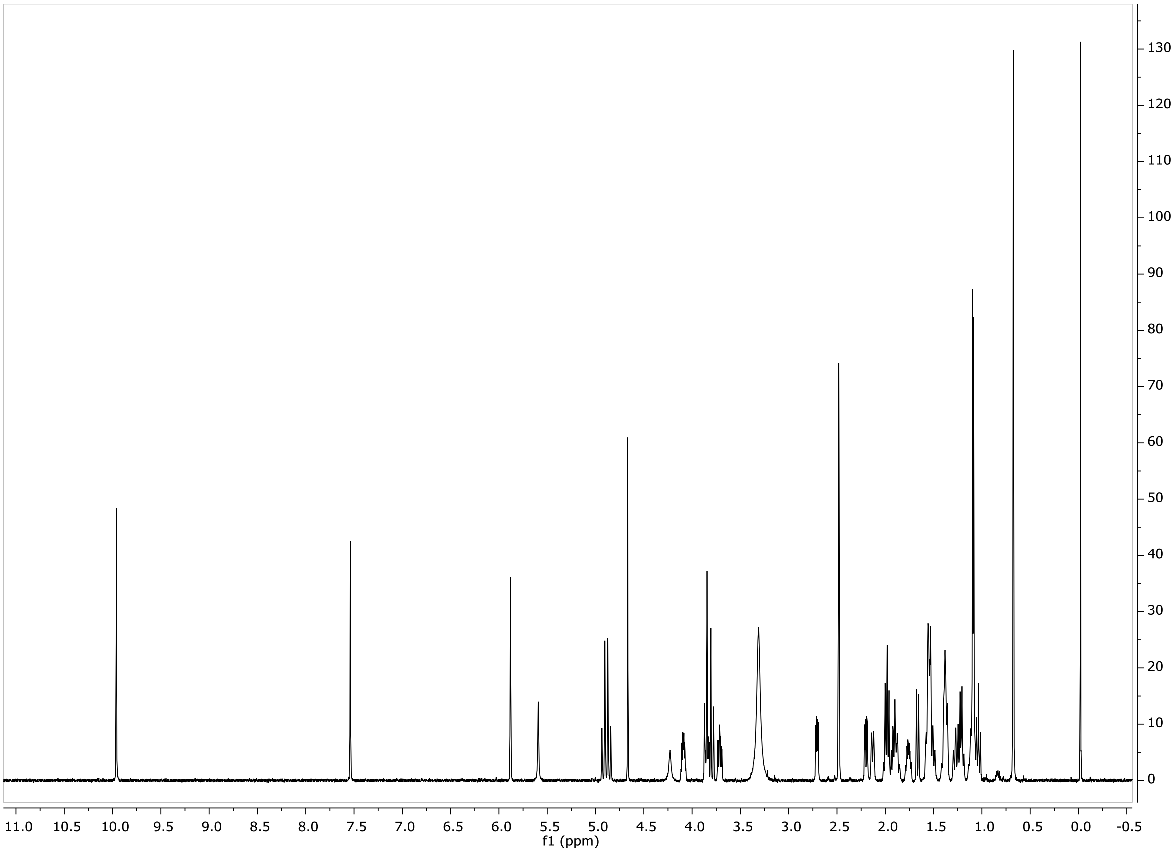


Figure S50. ^{1}H NMR (600 MHz) spectrum of uscharin (**7**) in DMSO-d_6

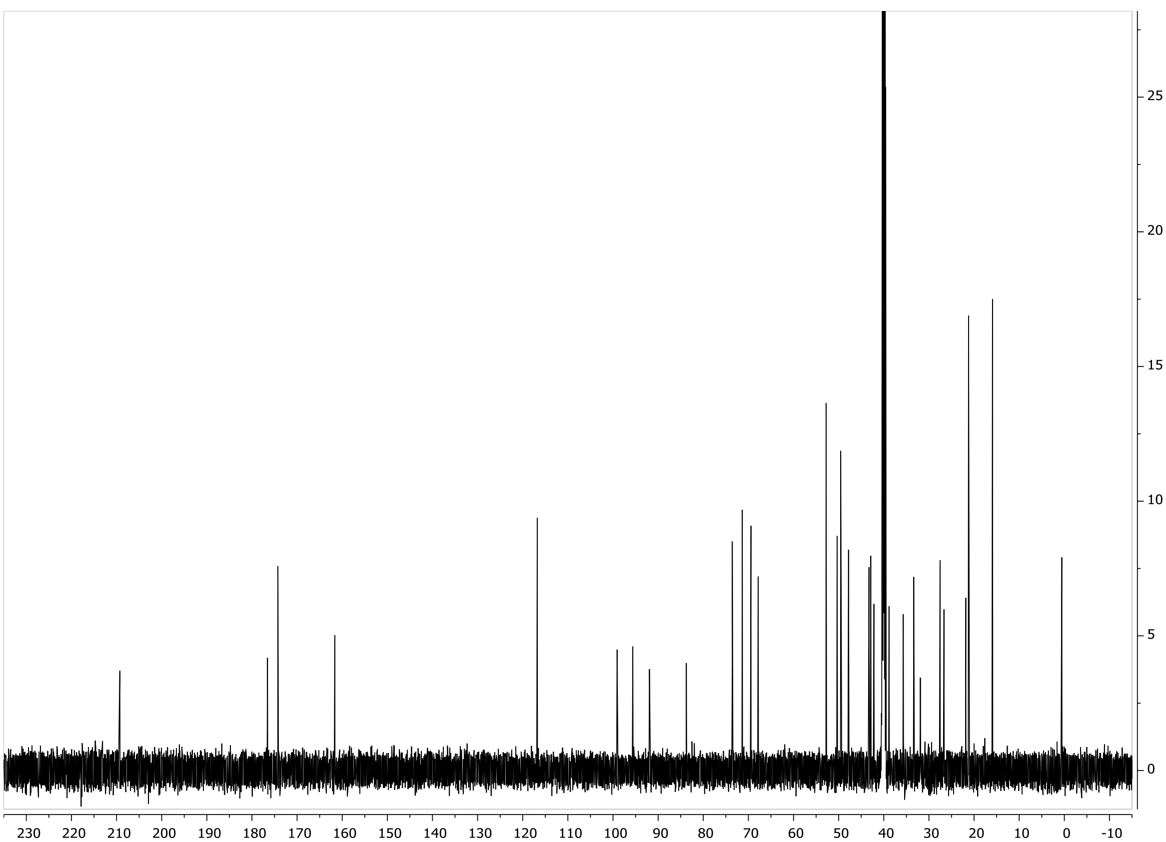


Figure S51. ^{13}C NMR (150 MHz) spectrum of uscharin (**7**) in DMSO-d_6

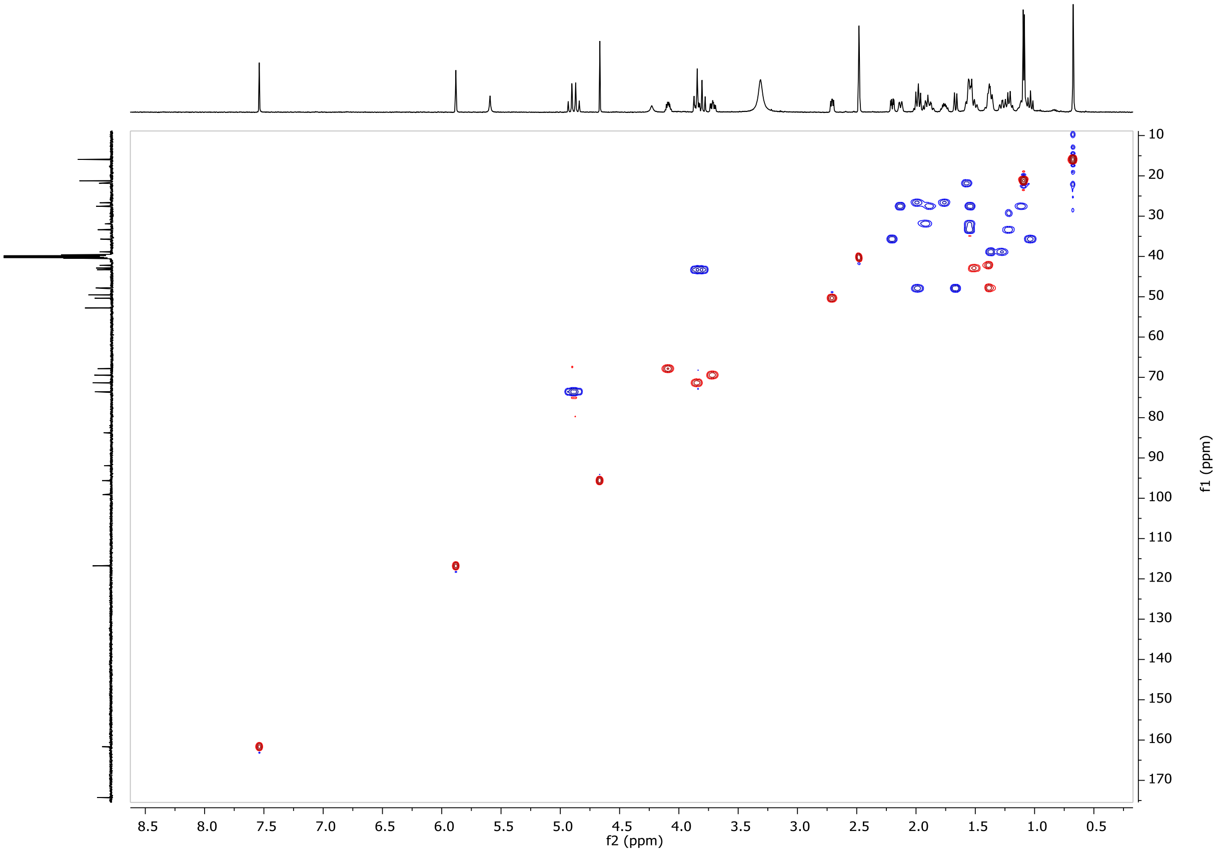


Figure S52. HSQC (500 MHz) spectrum of uscharin (**7**) in DMSO-d_6

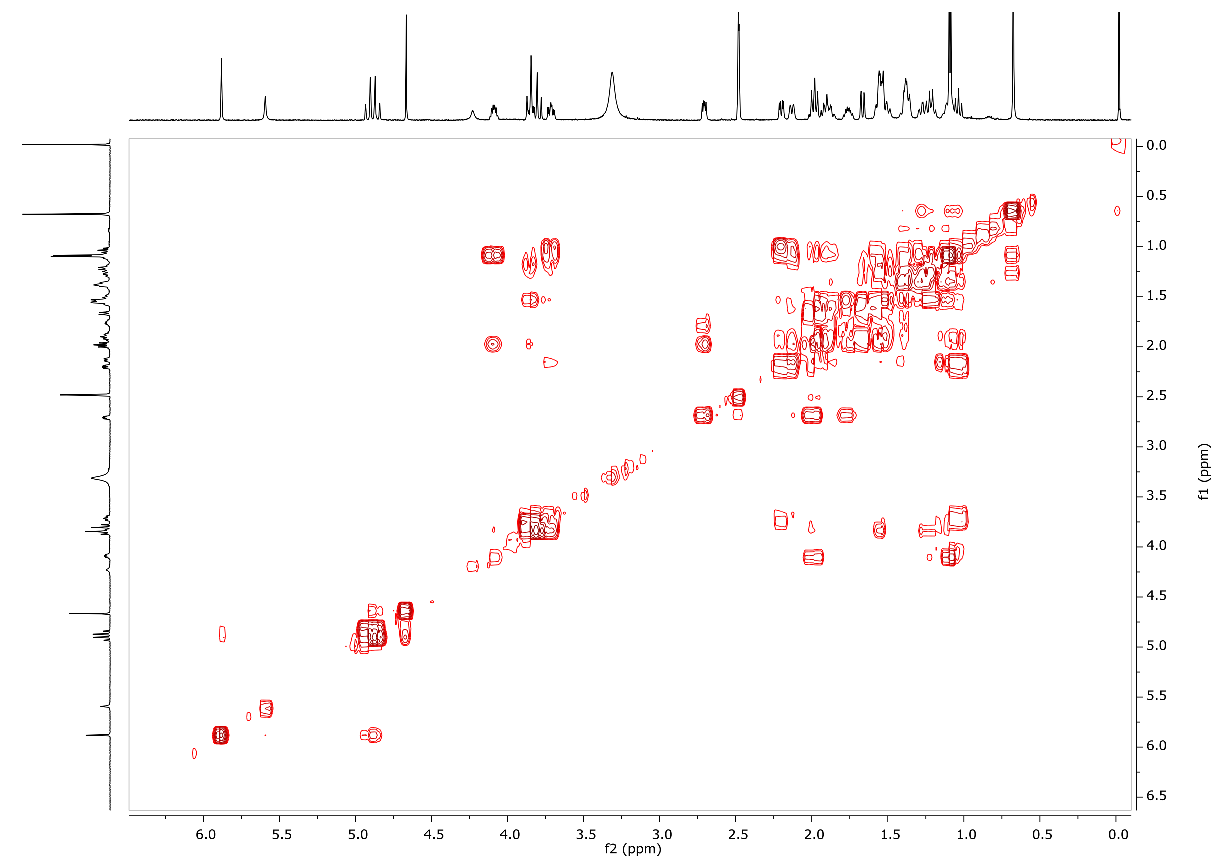


Figure S53. ^{1}H-^{1}H COSY (500 MHz) spectrum of uscharin (**7**) in DMSO-d_6

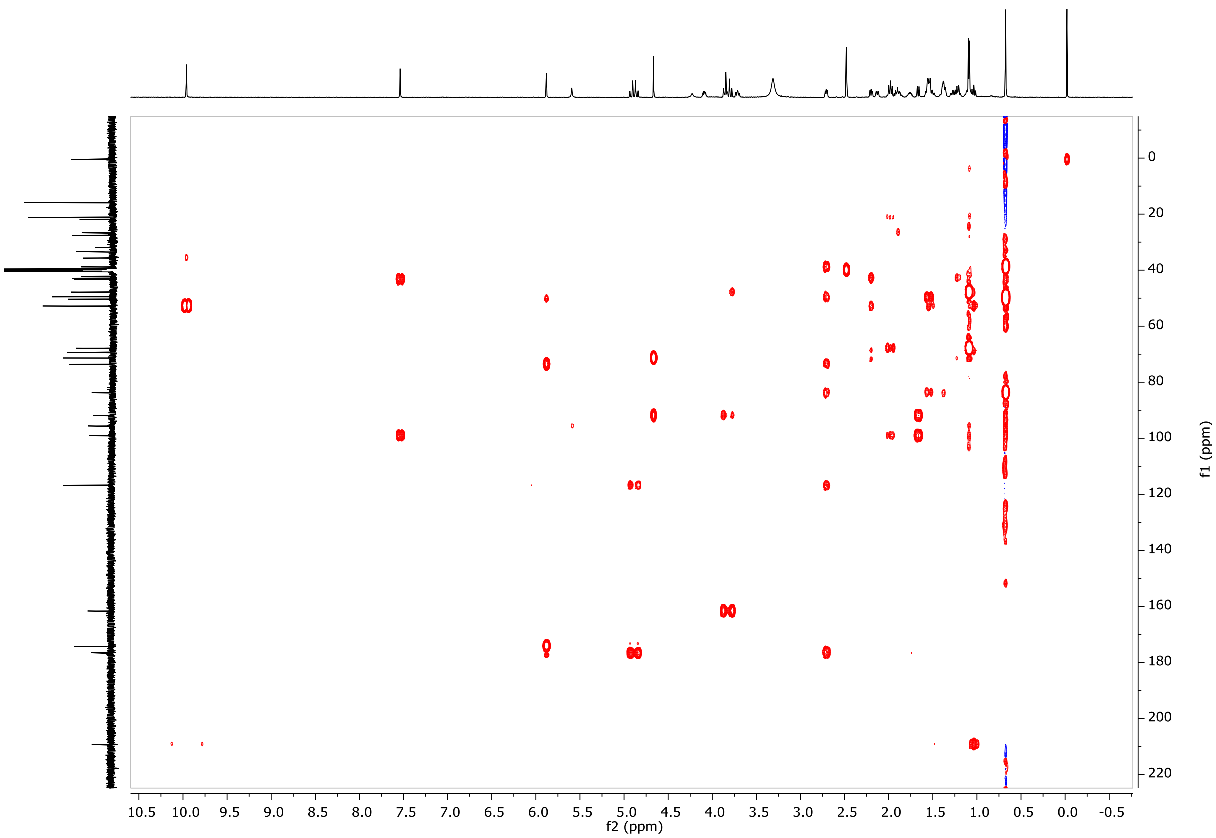


Figure S54. HMBC (500 MHz) spectrum of uscharin (**7**) in DMSO-d_6

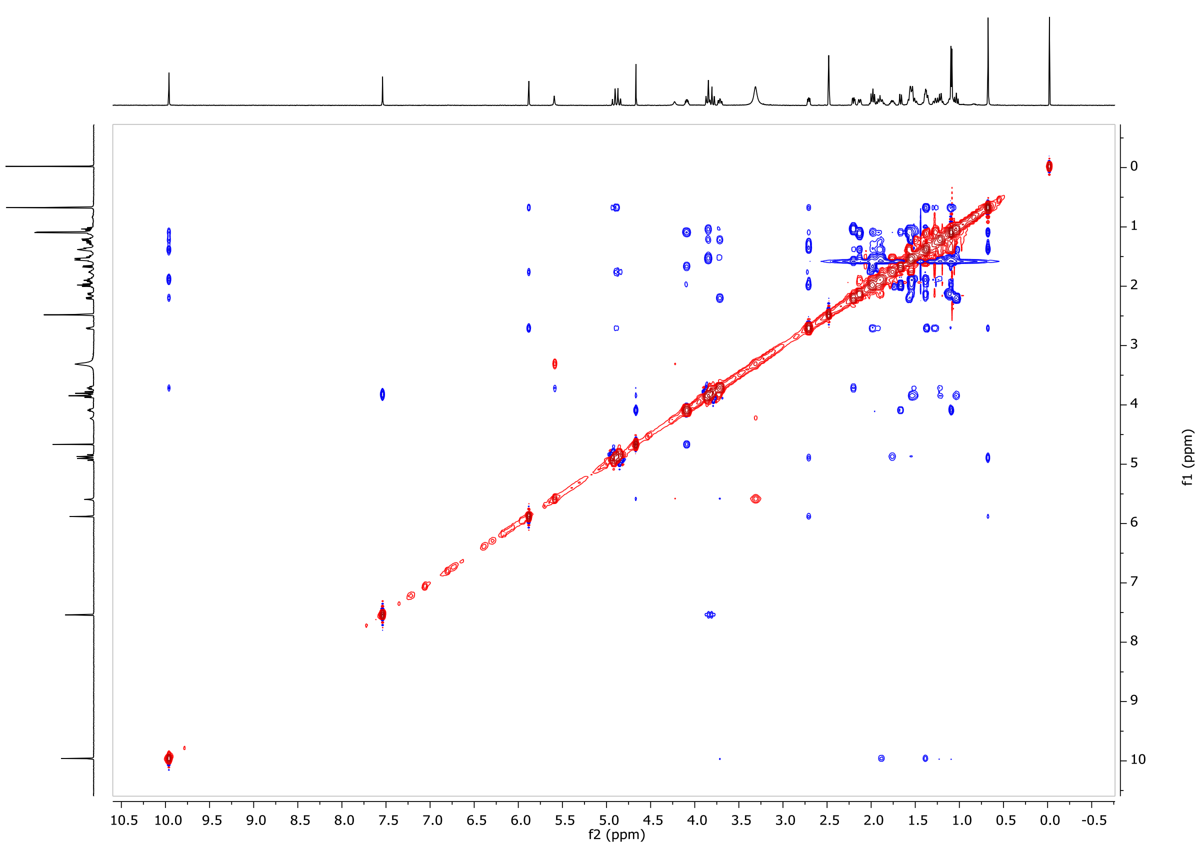


Figure S55. ROESY (500 MHz) spectrum of uscharin (**7**) in DMSO-d_6

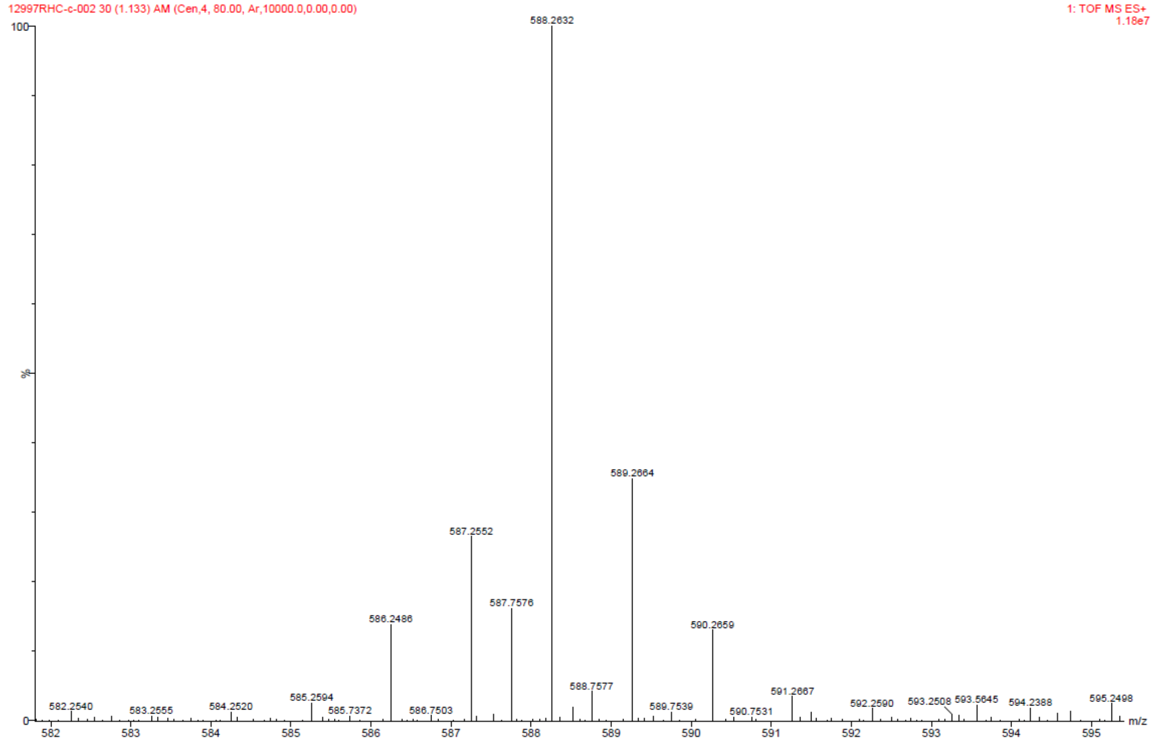


Figure S56. HRESIMS spectrum of uscharin (**7**)

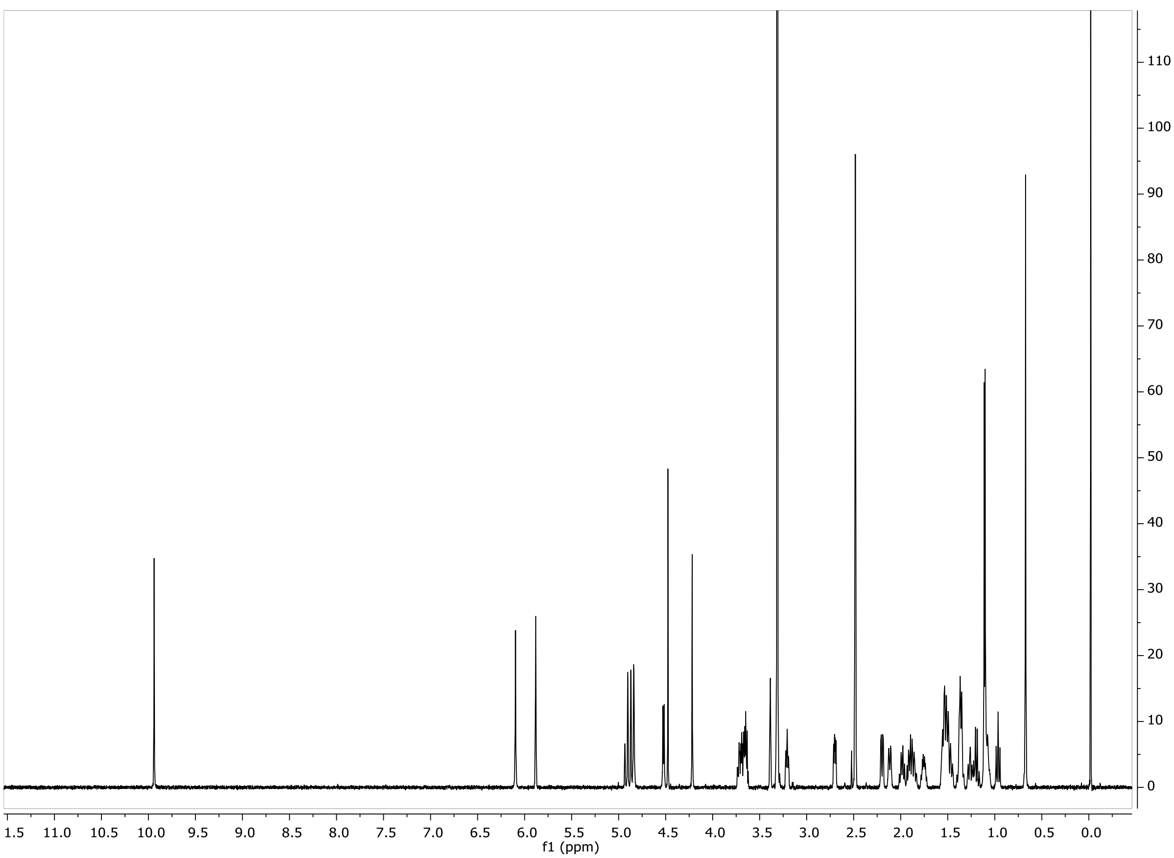


Figure S57. ^{1}H NMR (600 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

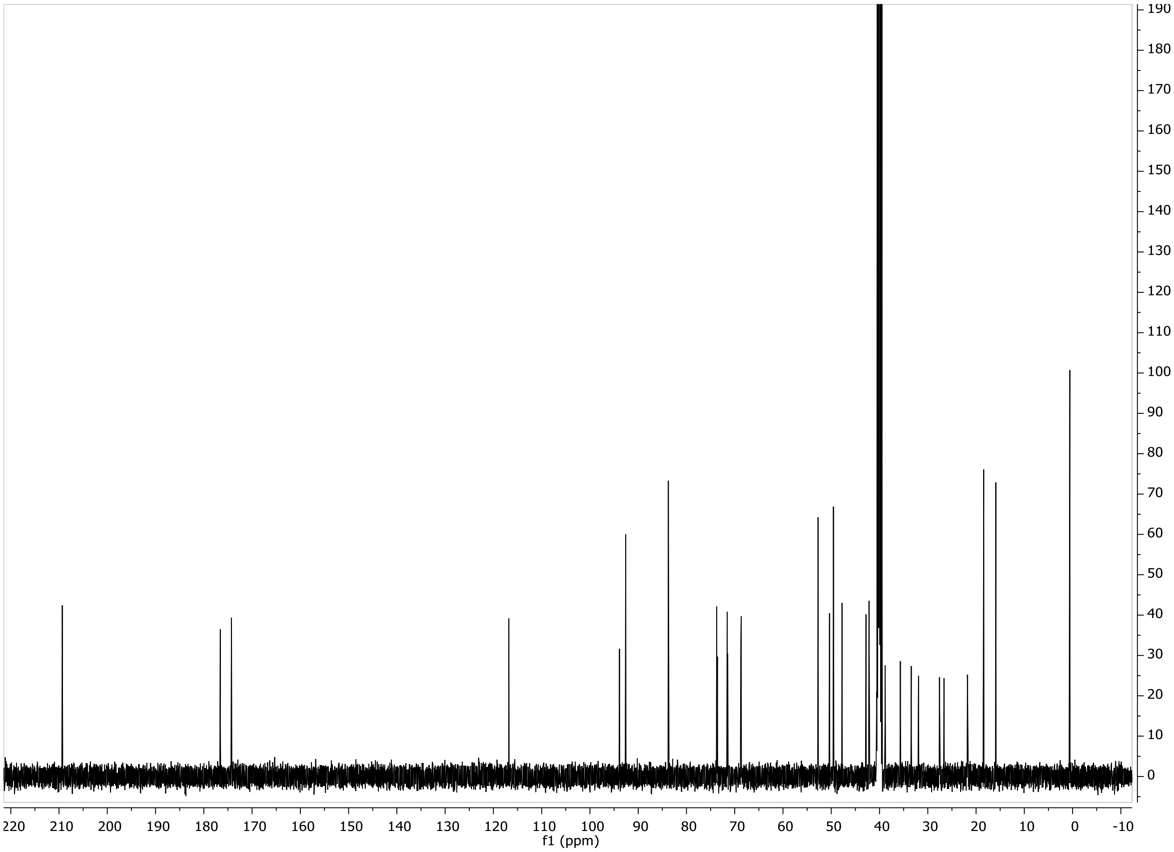


Figure S58. ^{13}C NMR (150 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

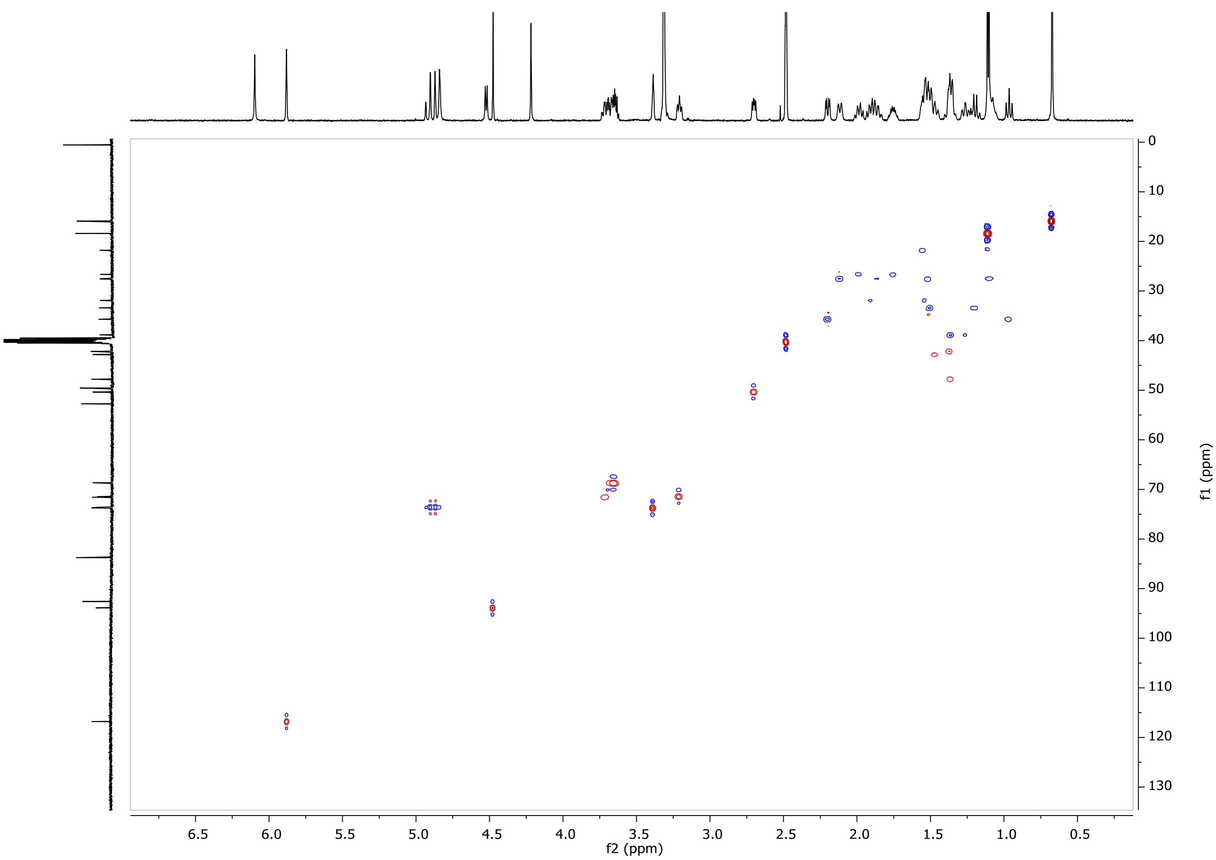


Figure S59. HSQC (500 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

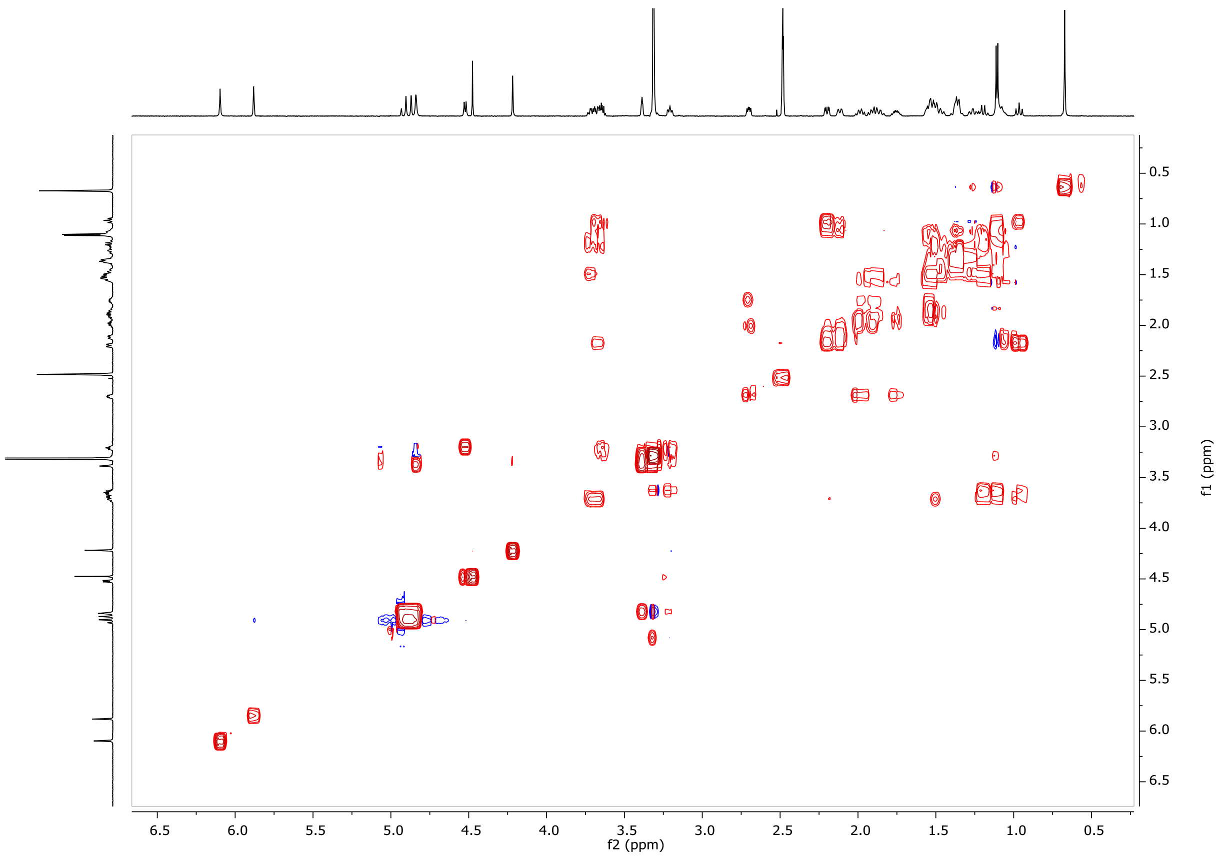


Figure S60. [1]H-[1]H COSY (500 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

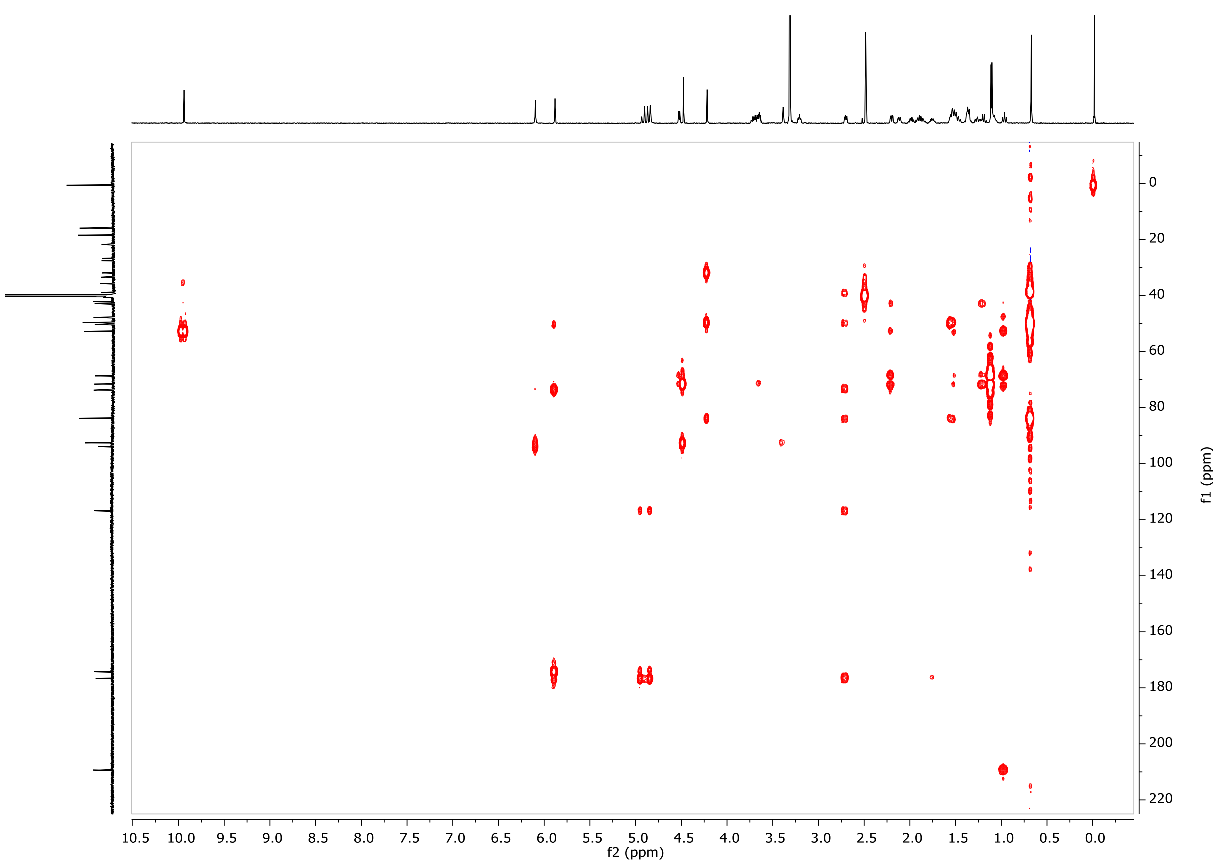


Figure S61. HMBC (500 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

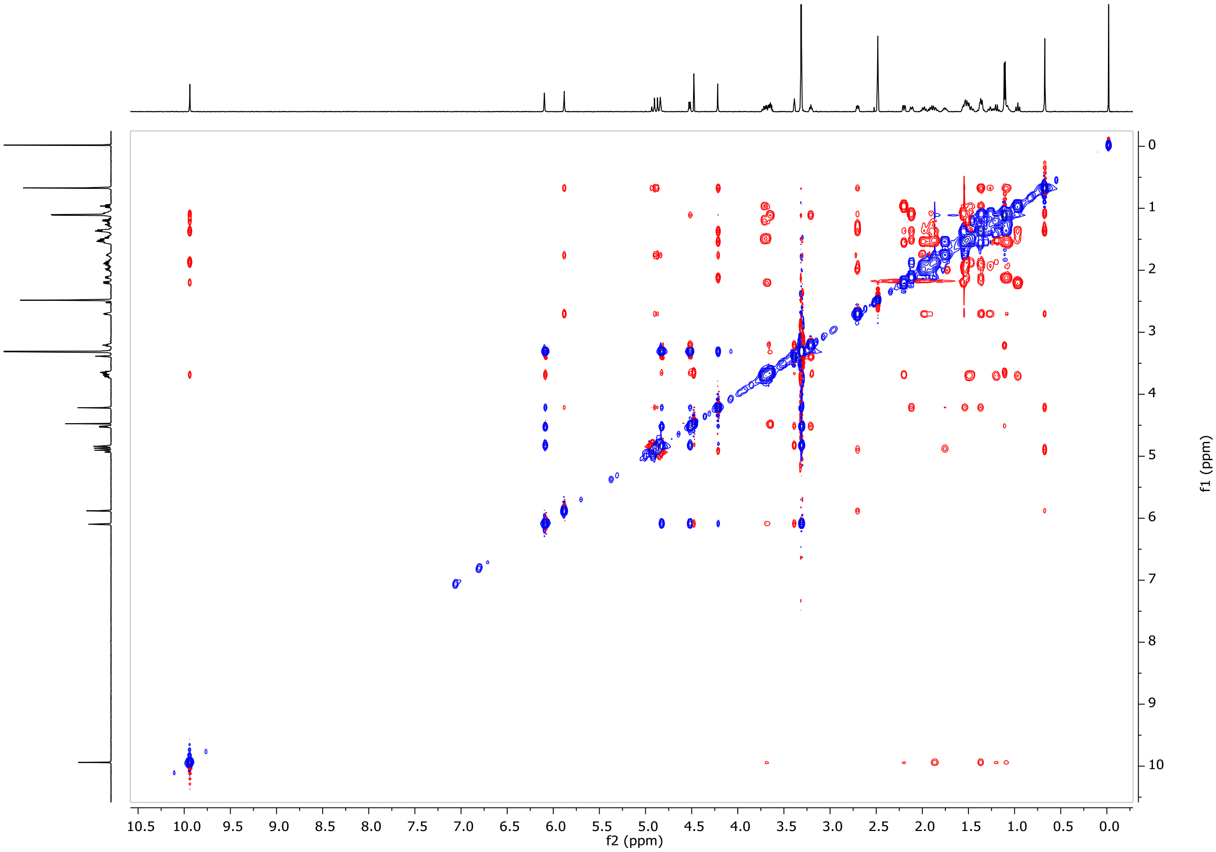


Figure S62. ROESY (500 MHz) spectrum of calotoxin (**8**) in DMSO-d_6

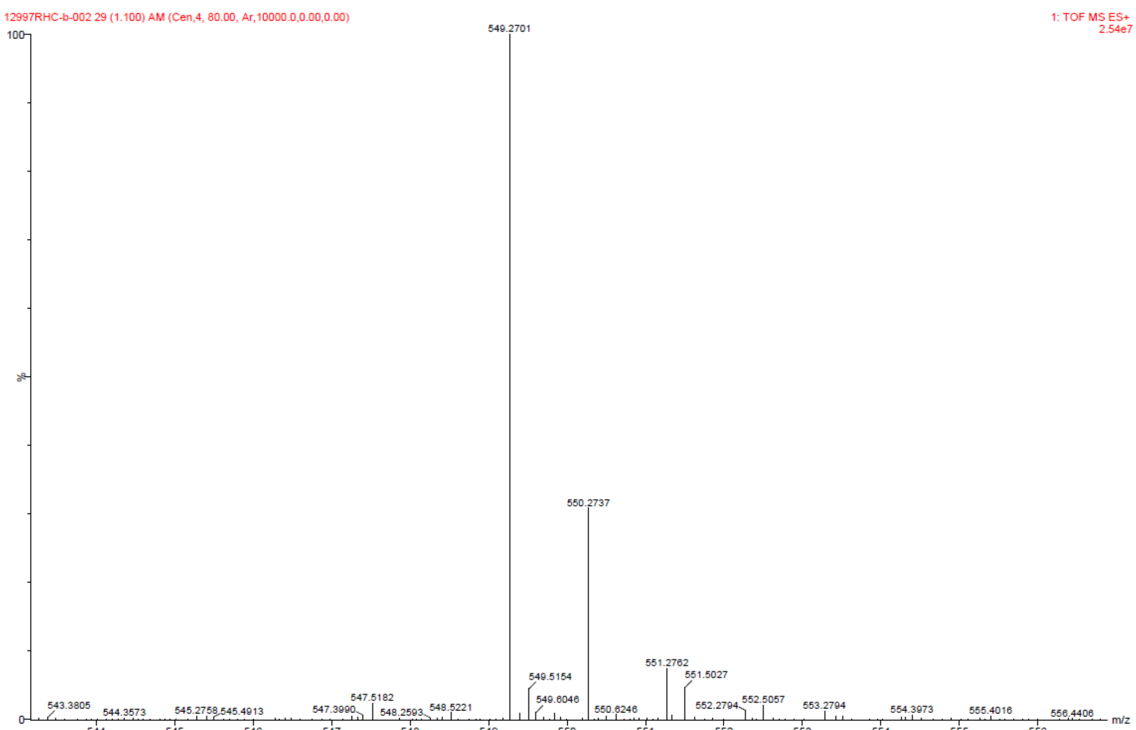


Figure S63. HRESIMS spectrum of calotoxin (**8**)

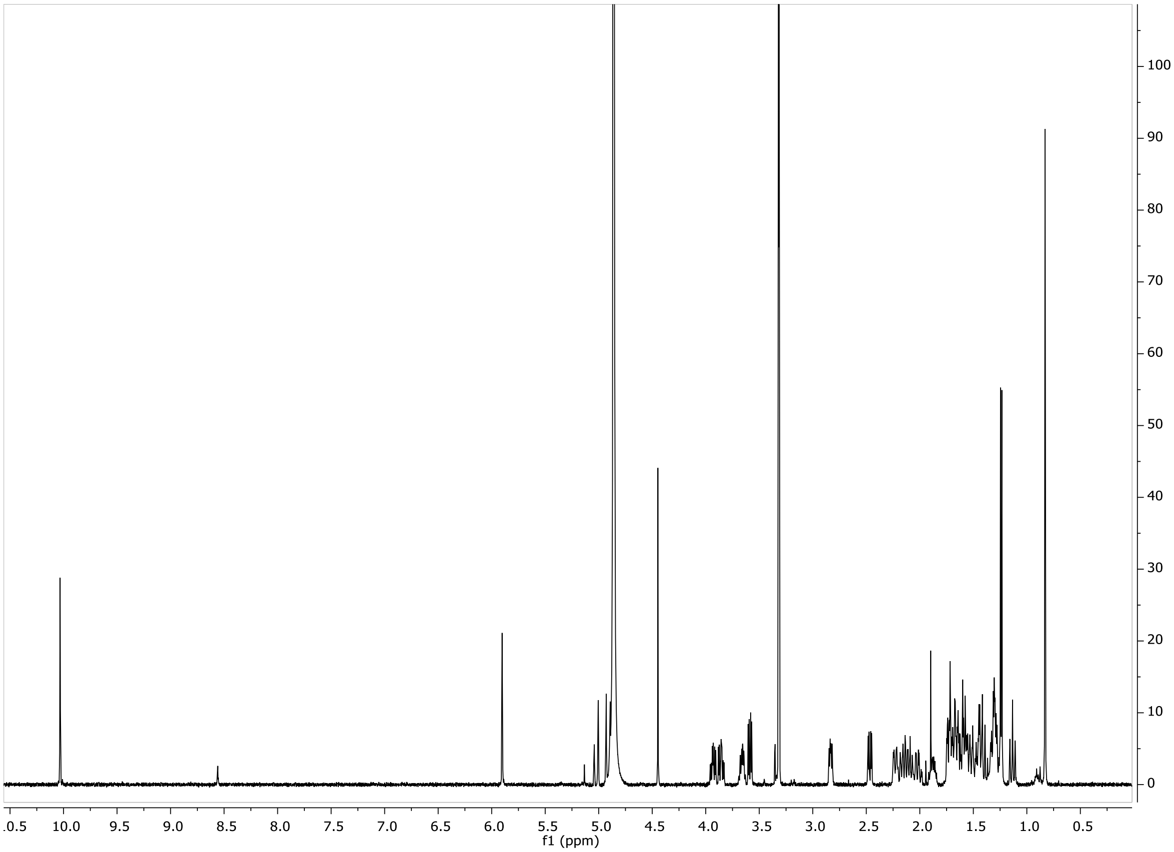


Figure S64. ^{1}H NMR (500 MHz) spectrum of calotropin (**9**) in methanol-d_4

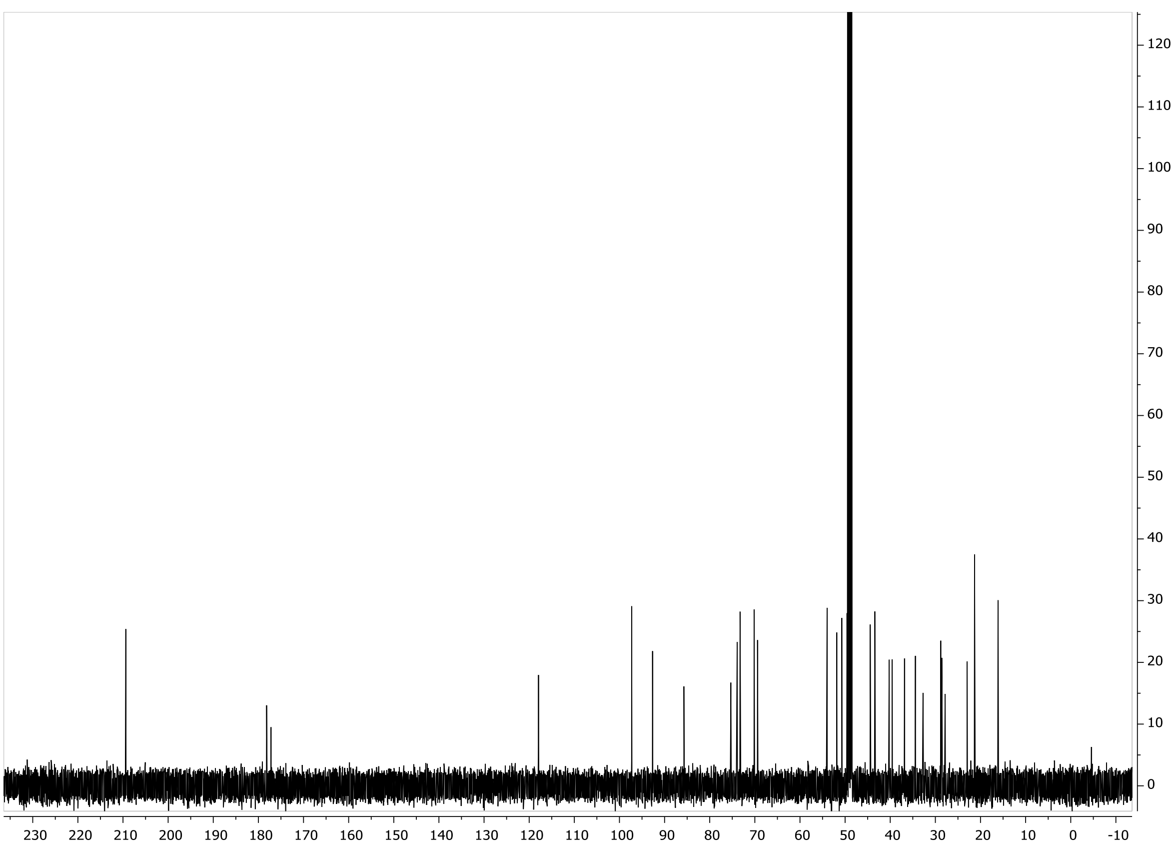


Figure S65. ^{13}C NMR (150 MHz) spectrum of calotropin (**9**) in methanol-d_4

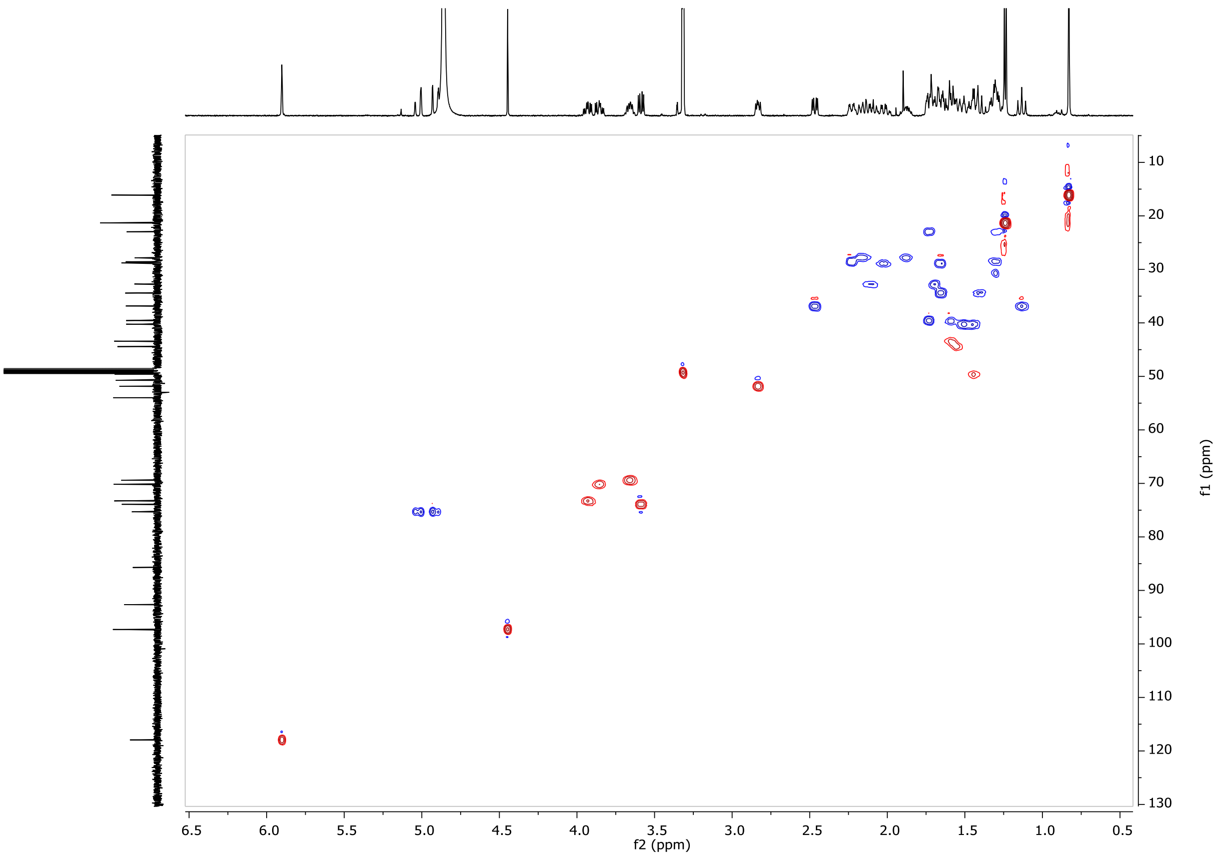


Figure S66. HSQC (500 MHz) spectrum of calotropin (**9**) in methanol-*d*$_4$

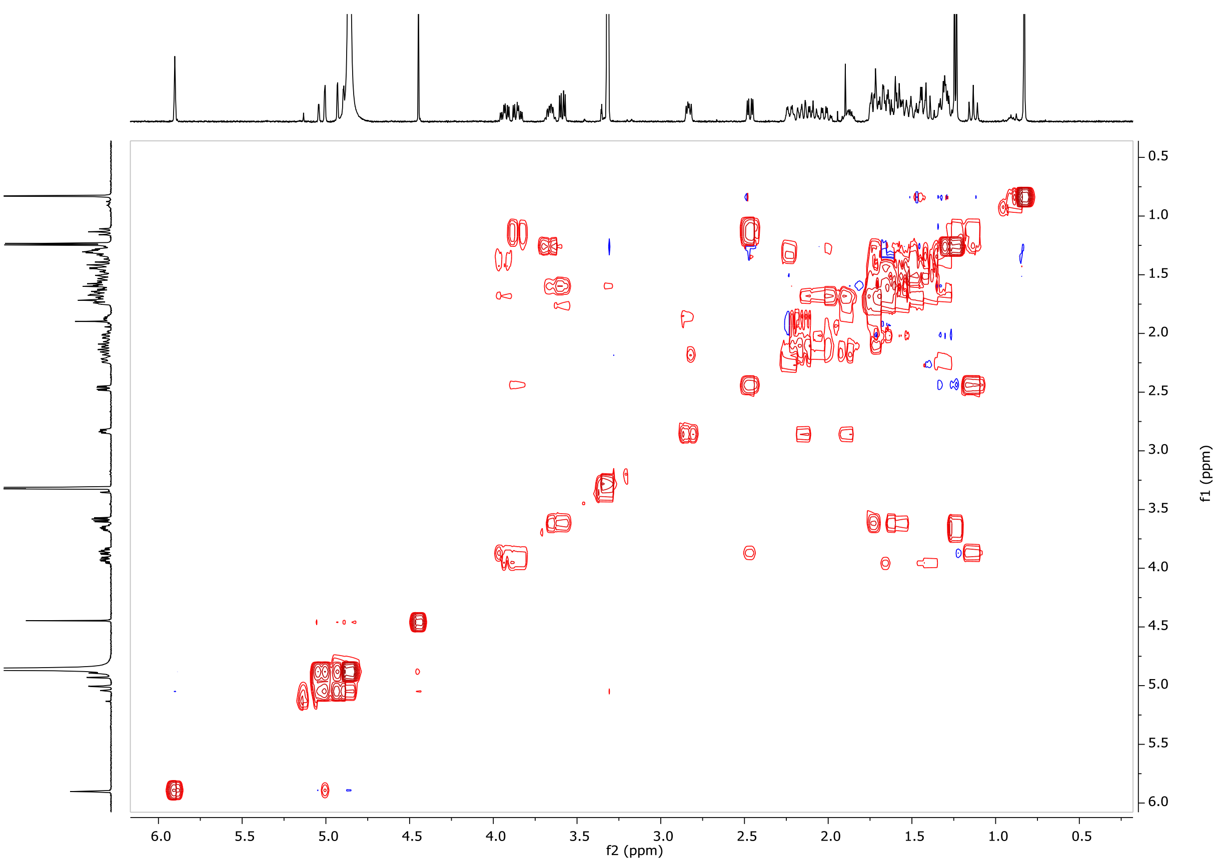


Figure S67. ^{1}H-^{1}H COSY (500 MHz) spectrum of calotropin (**9**) in methanol-*d*$_4$

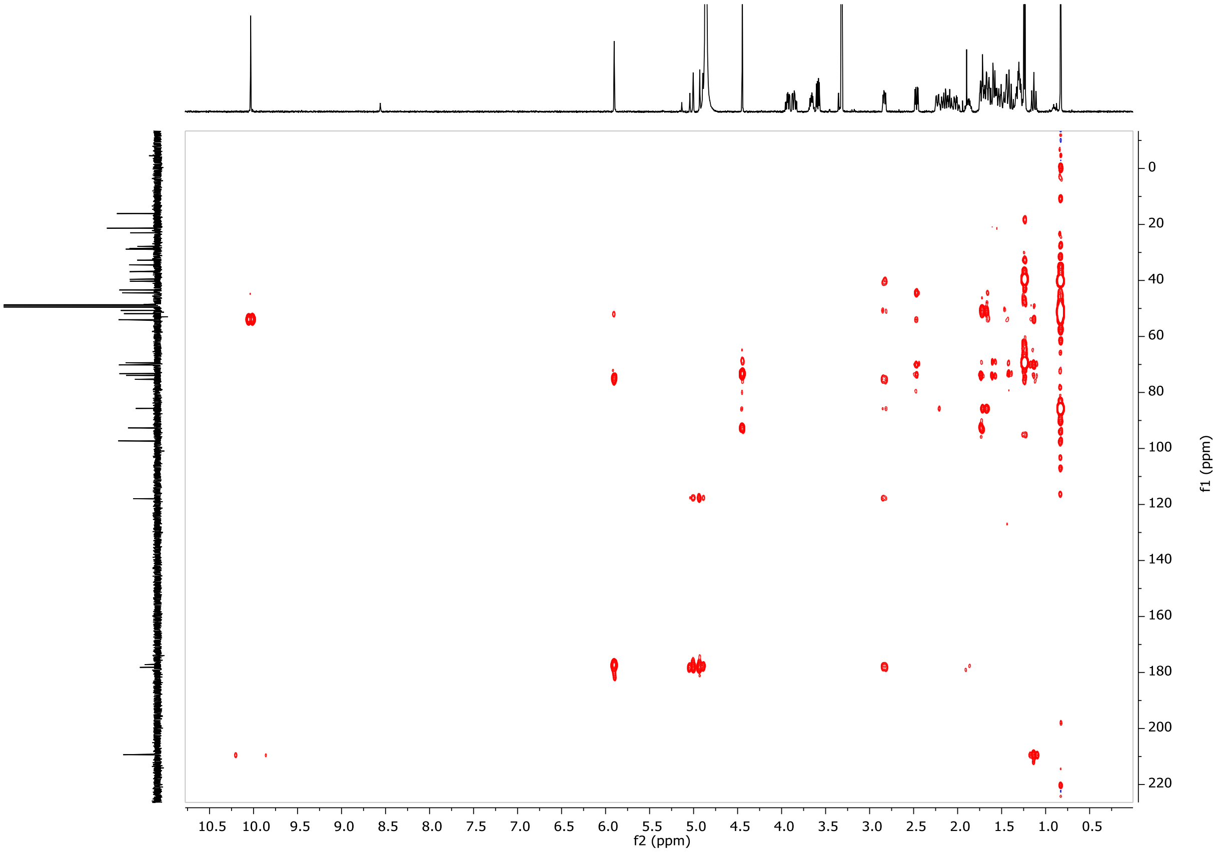


Figure S68. HMBC (500 MHz) spectrum of calotropin (**9**) in methanol-d_4

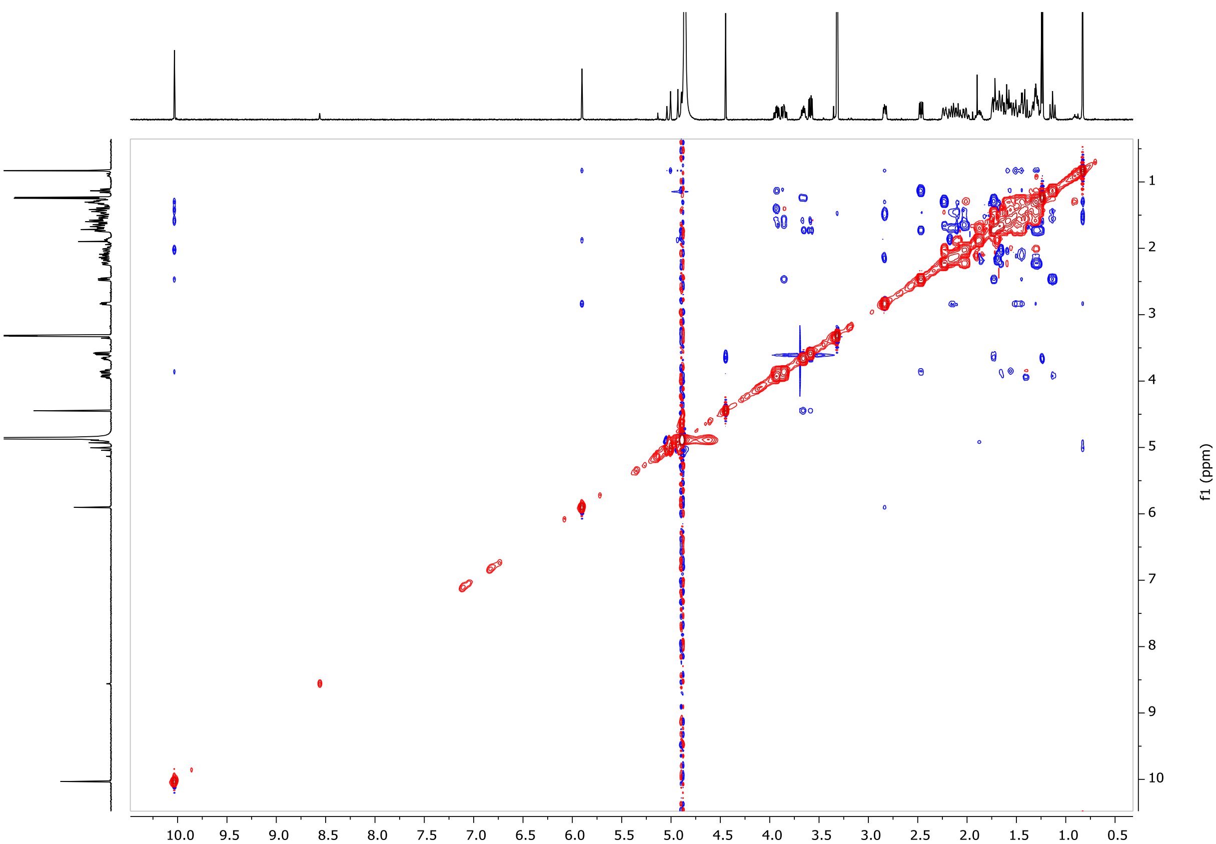


Figure S69. ROESY (500 MHz) spectrum of calotropin (**9**) in methanol-d_4

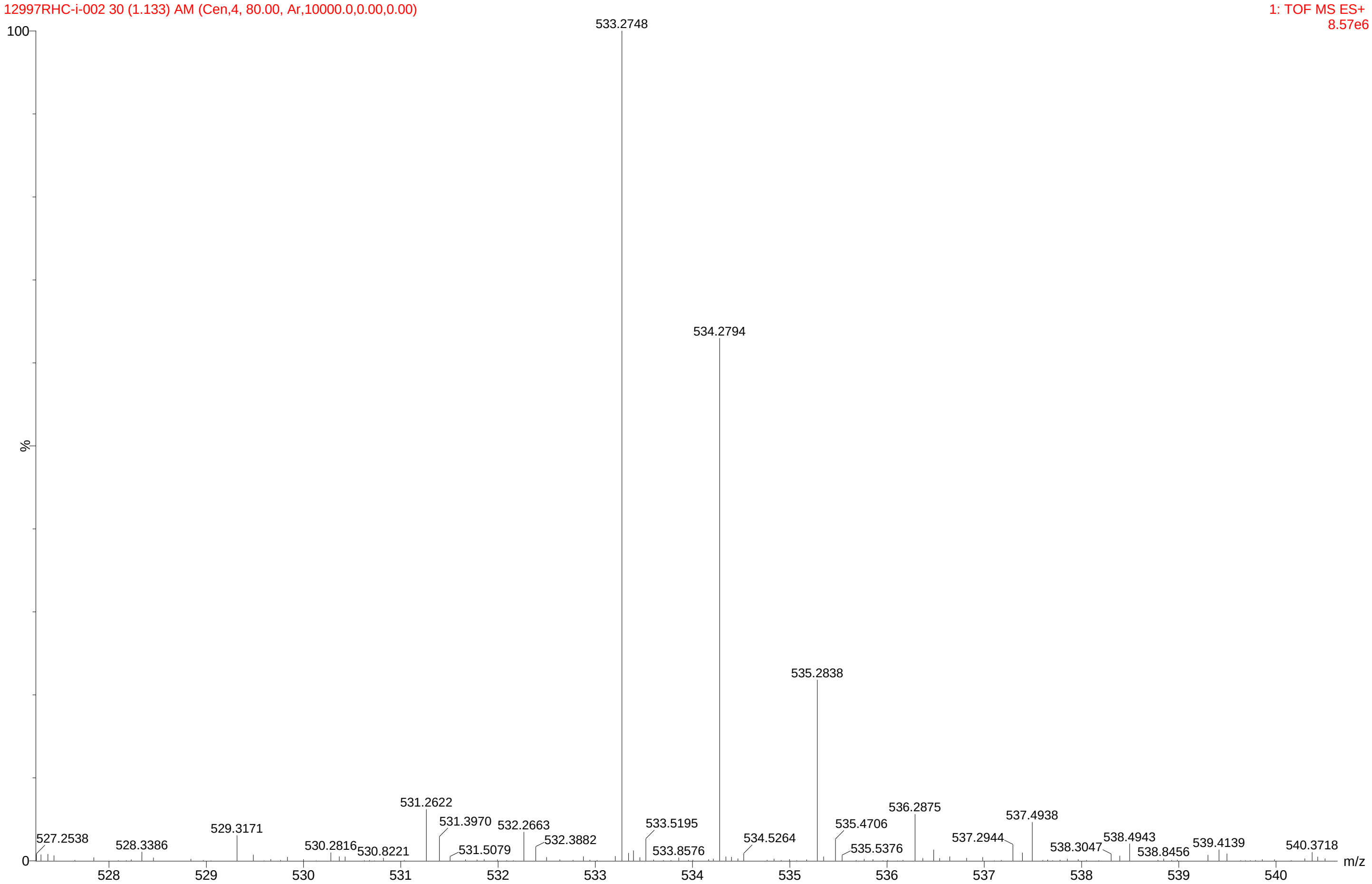


Figure S70. HRESIMS spectrum of calotropin (**9**)