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Supplementary Information

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Miyabeacin: A new cyclodimer presents a potential role for willow in cancer therapy.

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Supplementary Table 1. 1 & 2D-NMR data for Miyabeacin **3** (D₂O:CD₃OD, 80:20 containing d₄-TSP (0.01% w/v)) δ in ppm relative to d₄-TSP at 0.00.

Position	¹ H (ppm)	J _{H-H} (Hz)	¹³ C (ppm)	¹ H- ¹ H correlation to:	¹ H- ¹³ C HMBC correlation to:
1	-		158.0	-	
2	7.19 (1H, d)	8.3	117.9	H-3, H-4 & H-5	126.9 (C-6) & 158.0 (C-1)
3, 26	7.41 (2H, ddd)	8.0, 7.5, 2.0	133.5/133.4	H-2, H-4, H-25 & H-27	158.0 (C-1)/157.7 (C-28) & 133.7 (C-C-5)/133.6 (C-24)
4, 25	7.12 (1H, t)/7.11 (1H, t)	7.5	125.6/125.7	H-3, H-5, H-24 & H-26	117.9 (C-2)/117.7 (C-27) & 126.9 (C-6)/126.6 (C-23)
5, 24	7.32 (1H, dd)/ 7.34 (1H, dd)	7.6, 1.5	133.7/133.6	H-3, H-4, H-25 & H-26	158.0 (C-1)/157.7 (C-28), 133.5 (C-3)/133.4 (C-26) & 67.3 (C-7)/66.7 (C-22)
6	-		126.9	-	
7 α	5.40 (1H, d)	11.9	67.3	H-7 β	126.9 (C-6), 133.7 (C-5), 158.0 (C-1), 173.6 (C-8)
7 β	5.19 (1H, d)	11.9	67.3	H-7 α	126.9 (C-6), 133.7 (C-5), 158.0 (C-1), 173.6 (C-8)
8	-		173.6	-	
9	-		82.2	-	
10	3.59-3.63 (1H, m)		40.3	H-11 & H-15	43.5 (C-11), 45.1 (C-15), 135.5 (C-16), 152.5 (C-12), 173.6 (C-8), 198.6 (C-14)
11	3.58-3.55 (1H, m)		43.5	H-10, H-12 & H-18	-
12	6.59 (1H, dd)	10.2, 4.1	152.5	H-11 & H-13	NB – also observed in literature data 40.3 (C-10), 43.5 (C-11), 54.2 (C-18), 198.6 (C-14)
13	6.02 (1H, dd)	10.2, 1.5	130.9	H-12	43.5 (C-11), 82.2 (C-9)
14			198.6	-	
15	3.50-3.53 (1H, m)		45.1	H-16	43.5 (C-11), 80.0 (C-20), 82.2 (C-9), 132.8 (C-17), 135.5 (C-16), 173.2 (C-21), 210.0 (C-19)
16	6.19 (1H, t)	7.9	135.5	H-15 & H-17	45.1 (C-15), 54.2 (C-18), 80.0 (C-20)
17	5.91 (1H, ddd)	7.9, 6.5, 1.4	132.8	H-16 & H-18	45.1 (C-15), 54.2 (C-18), 210.0 (C-19)
18	3.43 (1H, m)		54.2	H-11, H-16 & H-17	40.3 (C-10), 80.0 (C-20), 132.8 (C-17), 135.5 (C-16), 210.0 (C-19)
19	-		210.0	-	
20	-		80.0	-	
21	-		173.2	-	
22 β	5.38 (1H, d)	12.1	66.7	H-22 α	126.6 (C-23), 133.7/133.6 (C-24), 157.7 (C-28), 173.2 (C-21)
22 α	5.16 (1H, d)	12.1	66.7	H-22 β	126.6 (C-23), 133.7/133.6 (C-24), 157.7 (C-28), 173.2 (C-21)
23	-		126.6		
27	7.20 (1H, d)	8.3	117.7	H-24, H-25 & H-26	126.6 (C-23), 157.7 (C-28)
28	-		157.7	-	
1', 1''	5.09 (1H, d)/5.07 (1H, d)	7.5/7.8	103.0/102.9	H-2'/H-2''	158.0/157.7 (C-1)/(C-28)
2', 2''	3.55-3.63 (2H, m)		76.0	H-1' & H-3'/H-1'' & H-3''	103.0/102.9 (C-1')/(C-1''), 78.7/78.8 (C-5')/(C-5'')
3', 3''	3.56-3.62 (2H, m)		79.1	H-2' & H-4'/H-2'' & H-4''	72.5/72.4 (C-4')/(C-4'')
4', 4''	3.47-3.52 (2H, m)		72.5/72.4	H-3' & H-5'/H-3'' & H-5''	63.7 (C-6')/(C-6''), 78.8/78.8 (C-5')/(C-5'')
5', 5''	3.56-3.62 (2H, m)		78.7/78.8	H-4' & H-6'/H-4'' & H-6''	63.7 (C-6')/(C-6''), 73.0 (C-4')/(C-4'')
6' β , 6'' β	3.77 (1H, dd)/3.73 (1H, dd)	12.4, 6.0	63.7	H-6' α /H-6'' α	78.9 (C-5')/(C-5'')

6'α, 6''α	3.94 (1H, dd)/3.92 (1H, dd)	12.4, 2.1	63.7	H-6'β/H-6''β	72.5/72.4 (C-4')/(C-4''),
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Supplementary Table 2. 1 & 2D-NMR data for miyabeacin B **6** (D₂O:CD₃OD, 80:20 containing d₄-TSP (0.01% w/v)) δ in ppm relative to d₄-TSP at 0.00.

Position	¹ H NMR(ppm)	J _{H-H} (Hz)	¹ H- ¹ H correlation to:
1 & 28	-	-	-
2 & 27	7.20 (2H, d)	8.2	H-3 & H-26
3 & 26	7.43 (2H, ddd)	8.5, 7.5, 1.5	H-2, H-4, H-27 & H-25
4 & 25	7.12 (2H, ddd)	7.5, 7.4, 0.9	H-3, H-5, H-26 & H-24
5 & 24	7.35 (2H, dd)	7.5, 1.5	H-4 & H-5
6 & 23	-	-	-
7β & 22β	5.13 (2H, d)	11.7	H-7α & H-22α
7α & 22α	5.46 (2H, d)	11.6	H-7β & H-22β
9 & 20	-	-	-
10 & 15	2.76 (2H, dd)	4.4, 2.1	H-11 & H-16
11 & 16	2.99 (2H, m)		H-10, H-12, H-15 & H-17
12 & 17	2.88 (2H, m)		H-11, H-13, H-16 & H-18
13 & 18	3.12 (2H, dd)	7.6, 4.0	H-11, H-12, H-16 & H-17
1' & 1''	5.07 (2H, d)	7.8	H-2'/H-2''
2' & 2''	3.51 (2H, dd)	9.4, 7.8	H-1' & H-3'/H-1'' & H-3''
3' & 3''	3.45 (2H, t)	9.4	H-2' & H-4'/H-2'' & H-4''
4', 4'', 5' & 5''	3.58 (4H, m)		H-3' & H-3'' & H-6' & H-6''
6'β & 6''β	3.72 (2H, dd)	12.4, 6.0	H-5' & H-5''
			H-6'α/H-6''α
6'α & 6''α	3.99 (2H, dd)	12.5, 2.2	H-5' & H-5''
			H-6'β/H-6''β

Supplementary Table 3. 1 & 2D-NMR data for miyabeanol **7** δ in ppm relative to d₄-TSP at 0.00.

Position	D ₂ O:CD ₃ OD				D ₂ O			
	δ_H (ppm)	δ_C (ppm)	¹ H- ¹ H correlation to:	¹ H- ¹³ C HMBC correlation to:	δ_H (ppm)	δ_C (ppm)	¹ H- ¹ H correlation to:	¹ H- ¹³ C HMBC correlation to:
1	-	158.0	-	-	-	157.8	-	-
2	7.19 (1H, d, 8.0)	117.7	H-3	126.6 (C-6), 158.0 (C-1)	7.19 (1H, d, 7.9)	117.3	H-3	126.6 (C-6), 157.8 (C-1)
3	7.40 (1H, m)	133.7	H-2 & H-4	158.0 (C-1), 133.7 (C-5)	7.42 (1H, m)	133.2	H-2 & H-4	157.8 (C-1), 133.2 (C-5)
4	7.12 (1H, td, 7.5, 0.9)	125.7	H-3 & H-5	117.7 (C-2), 126.6 (C-6), 133.7 (C-3), 158.0 (C-1)	7.12 (1H, td, 7.5, 0.9)	125.3	H-3 & H-5	117.3 (C-2), 126.6 (C-6), 133.2 (C-3), 157.8 (C-1)
5	7.31 (1H, dd, 7.6, 1.5)	133.7	H-4	158.0 (C-1), 133.7 (C-3), 117.7 (C-2), 67.2 (C-7)	7.34 (1H, dd, 7.6, 1.5)	133.2	H-4	157.8 (C-1), 133.2 (C-3), 117.3 (C-2), 67.0 (C-7)
6	-	126.6	-	-	-	126.6	-	-
7 α	5.39 (1H, d, 11.8)	67.2	H-7 β	126.6 (C-6), 133.7 (C-5), 158.0 (C-1), 173.7 (C-8)	5.41 (1H, d, 11.9)	67.0	H-7 β	126.6 (C-6), 133.2 (C-5), 157.8 (C-1), 173.6 (C-8)
7 β	5.18 (1H, d, 11.8)	67.2	H-7 α	126.6 (C-6), 133.7 (C-5), 158.0 (C-1), 173.7 (C-8)	5.18 (1H, d, 11.9)	67.0	H-7 α	126.6 (C-6), 133.2 (C-5), 157.8 (C-1), 173.6 (C-8)
8	-	173.7	-	-	-	173.6	-	-
9	-	82.5	-	-	-	82.4	-	-
10	3.57-3.61 (1H, m)	40.6	H-11 & H-15	43.9 (C-11), 45.7 (C-15), 136.0 (C-16), 152.8 (C-12), 173.7 (C-8), 199.0 (C-14)	3.63 (1H, m)	40.4	H-11 & H-15	43.7 (C-11), 45.5 (C-15), 81.4 (C-20), 135.6 (C-16), 152.7 (C-12), 173.6 (C-8), 199.1 (C-14)
11	3.48-3.53 (1H, m)	43.9	H-10, H-12 & H-18	152.8 (C-12)	3.55 (m)	43.7	H-10, H-12 & H-18	152.7 (C-12)
12	6.63 (1H, dd, 10.2, 4.1)	152.8	H-13 & H-11	40.6 (C-10), 43.9 (C-11), 54.6 (C-18), 199.0 (C-14)	6.64 (1H, dd, 10.2, 4.2)	152.7	H-13 & H-11	40.4 (C-10), 43.7 (C-11), 54.5 (C-18), 199.1 (C-14)
13	6.02 (1H, dd, 10.1, 1.7)	130.8	H-12 & H-10	43.9 (C-11), 82.5 (C-9)	6.05 (1H, dd, 10.2, 1.8)	130.6	H-12 & H-10	43.7 (C-11), 82.4 (C-9)
14	-	199.0	-	-	-	199.1	-	-
15	3.28-3.33 (1H, m)	45.7	H-16 & H-10	40.6 (C-10), 43.9 (C-11), 82.5 (C-9), 132.2 (C-17), 136.0 (C-16), 213.3 (C-19)	3.38 (1H, dt, 6.5, 1.7)	45.5	H-16 & H-10	40.4 (C-10), 43.7 (C-11), 81.4 (C-20), 82.4 (C-9), 132.2 (C-17), 135.6 (C-16), 213.4 (C-19)
16	6.27 (1H, ddd, 7.9, 6.9, 1.0)	136.0	H-17 & H-15	45.7 (C-15), 54.6 (C-18), 82.5 (C-9), 213.3 (C-19)	6.29 (1H, ddd, 7.8, 6.4, 1.0)	135.6	H-17 & H-15	45.5 (C-15), 54.5 (C-18), 81.4 (C-20), 82.4 (C-9), 213.4 (C-19)

17	5.94 (1H, ddd, 7.9, 6.5, 1.4)	132.2	H-16 & H-18	45.7 (C-15), 54.6 (C-18), 213.3 (C-19)	5.99 (1H, ddd, 8.0, 6.3, 1.5)	132.2	H-16 & H-18	45.5 (C-15), 54.5 (C-18), 81.4 (C-20), 213.4 (C-19)
18	3.36 (1H, ddd, 6.0, 2.4, 1.4)	54.6	H-17 & H-11	40.6 (C-1), 82.5 (C-9), 132.2 (C-17), 136.0 (C-16), 213.3 (C-19)	3.41 (1H, ddd, 6.1, 2.3, 1.3)	54.5	H-17 & H-11	40.4 (C-1), 81.4 (C-20), 82.4 (C-9), 132.2 (C-17), 135.6 (C-16), 213.4 (C-19)
19	-	213.3	-	-	-	213.4	-	-
20	missing	missing	-	-	Absent (d-exchange)	81.4	-	-
1'	5.06 (1H, d, 7.3)	103.0	H-2'	158.0 (C-1)	5.10 (1H, d, 7.7)	102.7	H-2'	157.8 (C-1)
2'	3.49-3.59 (1H, m)	76.0	H-1' & H-3'	103.0 (C-1'), 78.8 (C-5')	3.49-3.59 (1H, m)	75.8	H-1' & H-3'	102.7 (C-1'), 78.5 (C-5')
3'	3.54-3.61 (1H, m)	79.1	H-2' & H-,4'	72.4 (C-4')	3.54-3.61 (1H, m)	78.7	H-2' & H-,4'	72.2 (C-4')
4'	3.45-3.53 (1H, m)	72.4	H-3' & H-5'	63.6 (C-6'), 78.8 (C-5'), 103.0 (C-1')	3.45-3.53 (1H, m)	72.2	H-3' & H-5'	63.5 (C-6'), 78.5 (C-5'), 102.7 (C-1')
5'	3.54-3.61 (1H, m)	78.8	H-4' & H-6'	63.6 (C-6'), 76.0 (C-2')	3.54-3.61 (1H, m)	78.5	H-4' & H-6'	63.5 (C-6'), 75.8 (C-2')
6'β	3.76 (1H, dd, 12.5, 5.9)	63.6	H-6'α	79.1 (C-3')	3.77 (1H, dd, 12.5, 5.9)	63.5	H-6'α	78.7 (C-3')
6'α	3.92 (1H, dd, 12.4, 2.2)	63.6	H-6'β	72.4 (C-4')	3.94 (1H, dd, 12.4, 2.2)	63.5	H-6'β	72.2 (C-4')

Supplementary Table 4. 1 & 2D-NMR data for miyaquinol **8** (D₂O:CD₃OD, 80:20 containing d₄-TSP (0.01% w/v)) δ in ppm relative to d₄-TSP at 0.00.

Positio n	δ_H (ppm)	δ_C (ppm)	¹ H- ¹ H correlation to:	D ₂ O:CD ₃ OD	¹ H- ¹³ C HMBC correlation to:
1	-	157.6	-		
2	7.25 (1H, d, 8.1)	117.7	H-3		125.8 (C-6), 157.6 (C-1)
3	7.43 (1H, m)	133.5	H-2 & H-4		157.6 (C-1), 133.5 (C-5)
4	7.17 (1H, t, 7.5)	125.9	H-3 & H-5		117.7 (C-2), 125.8 (C-6)
5	7.43 (1H, m)	133.5	H-4		157.6 (C-1), 133.5 (C-3)
6	-	125.8			
7 α	5.45 (1H, d, 12.2)	66.7	H-7 β		125.8 (C-6), 133.5 (C-5), 157.6 (C-1), 173.1 (C-8)
7 β	5.26 (1H, d, 12.2)	66.7	H-7 α		125.8 (C-6), 133.5 (C-5), 157.6 (C-1), 173.1 (C-8)
8	-	173.1	-		
9	-	76.9	-		
10	-	203.9	-		
11	4.45 (1H, dd, 5.9, 1.4)	59.2	H-12	76.9 (C-9), 118.0 (C-17), 128.5 (C-15), 131.5 (C-16), 134.8 (C-12), 137.1 (C-13)	
12	6.66 (1H, ddd, 7.6, 6.0, 1.8)	134.8	H-13 & H-11		46.8 (C-14), 59.2 (C-11)
13	6.55 (1H, ddd, 7.5, 6.3, 1.4)	137.1	H-12 & H-14		46.8 (C-14), 59.2 (C-11)
14	4.79 (obscured by H ₂ O)	46.8	H-13	76.9 (C-9), 128.5 (C-15), 131.5 (C-16), 134.8 (C-12), 137.1 (C-13)	
15	-	128.5	-		-
16	-	131.5	-		-
17	6.81 (1H, d, 8.0)	118.0	H-18		128.5 (C-15), 147.4 (C-19), 59.2 (C-11)
18	6.79 (1H, d, 8.0)	117.9	H-17		131.5 (C-16), 144.5 (C-20)
19	-	147.4	-		
20	-	144.5	-		
1'	5.14 (1H, d, 7.5)	102.8	H-2'		157.7 (C-1)
2'	3.64 (1H, m)	76.0	H-1' & H-3'		n.d.
3'	3.67-3.58 (1H, m)	79.1	H-2' & H-4'		n.d.
4'	3.52 (1H, m)	72.6	H-3' & H-5'		n.d.
5'	3.62 (1H, m)	79.1	H-4' & H-6'		n.d.
6' β	3.73 (1H, dd, 12.4, 5.7)	63.8	H-6' α		n.d.
6' α	3.92 (1H, dd, 12.4, 2.2)	63.8	H-6' β		n.d.

Supplementary Table 5. Concentrations of dimeric compounds and key salicinoids in juvenile leaf and stem tissue from 26 *Salix* species of the National Willow Collection held at Rothamsted Research. Concentrations are given in mg/g d.w.

Rres NWC Code	Tissue	Species	Clonal / Hybrid Name	Miyabeacin	Miyabeano I	Miyabeacin B	Salicorti n	Salicin
2	Leaf	<i>S. nigra</i> Marsh.	SN3 Primrose Hill	n.d.	n.d.	n.d.	56.26	45.41
15	Leaf	<i>S. pentandra</i> L.	patent Lumley	n.d.	n.d.	n.d.	5.88	5.07
207	Leaf	<i>S. alba</i> L.	"Portogruaro (Ve)"	n.d.	n.d.	n.d.	1.16	1.00
390	Leaf	<i>S. fragilis</i>	R838	n.d.	n.d.	n.d.	1.33	0.66
415	Leaf	<i>S. magnifica</i> Hemsl.	WB 50 0 578	n.d.	n.d.	n.d.	5.38	13.00
432	Leaf	<i>S. daphnoides</i> Vill.	fastigate	n.d.	n.d.	n.d.	7.35	n.d.
506	Leaf	<i>S. dasyclados</i> Wimm.	Grandis	2.67	1.46	0.72	n.d.	n.d.
575	Leaf	<i>S. dasyclados</i> Wimm.	(aquatica) Jyvaskyla V768	4.57	2.93	2.26	1.38	3.86
576	Leaf	<i>S. dasyclados</i> Wimm.	(aquatica) Yesipaju Lieto V769	1.85	6.70	1.82	2.41	5.73
577	Leaf	<i>S. dasyclados</i> Wimm.	77056 IEA Trial	45.25	16.20	8.41	19.10	12.20
592	Leaf	<i>S. dasyclados</i> Wimm.	CE78-2 as x dasyclados Siren	40.73	15.94	10.32	12.48	9.34
607	Leaf	<i>S. rehderiana</i> Schneid.		n.d.	n.d.	n.d.	57.58	2.98
608	Leaf	<i>S. rehderiana</i> Schneid.		n.d.	n.d.	n.d.	26.45	8.43
615	Leaf	<i>S. schwerinii</i> Wolf	K3 Hilliers (WB 50 0 354	n.d.	n.d.	n.d.	0.70	n.d.
663	Leaf	<i>S. viminalis</i> L.	Pulchra Ruberrima	n.d.	n.d.	n.d.	1.15	n.d.
837	Leaf	<i>S.miyabeana</i> Seemen	III	79.86	55.01	15.45	1.24	37.29
838	Leaf	<i>S. purpurea</i> L.	Richartii	n.d.	n.d.	n.d.	44.91	68.22
844	Leaf	<i>S. purpurea</i> L.	Uralensis	n.d.	n.d.	n.d.	26.85	51.26
885	Leaf	<i>S.miyabeana</i> Seemen	Shrubby	79.11	37.96	16.09	5.39	41.82
901	Leaf	<i>S. × alberti</i> L. (<i>S. integra</i> Thunb. × <i>S. suchowensis</i> W.C. Cheng ex G.Zhu)	42/17	n.d.	n.d.	n.d.	6.39	n.d.
941	Leaf	<i>S.miyabeana</i> Seemen	Purpurescens (ex.Tuinzing) (566)	98.30	40.20	26.74	3.82	27.30
1013	Leaf	<i>S.phylicifolia</i> L.	Malham	n.d.	n.d.	n.d.	0.15	n.d.
1059	Leaf	<i>S.repens</i> L.		0.70	1.08	0.54	24.78	3.79
1155	Leaf	<i>S. acutifolia</i> Willd.	174	n.d.	n.d.	n.d.	6.90	165.40
1165	Leaf	<i>S. arbusculoides</i> Anderss.	20397	n.d.	n.d.	n.d.	n.d.	n.d.
1215	Leaf	<i>S. myrsinifolia</i> Salisb.	E-4-1403	n.d.	n.d.	n.d.	8.55	105.85
2	Stem	<i>S. nigra</i> Marsh.	SN3 Primrose Hill	n.d.	n.d.	n.d.	81.11	42.52
15	Stem	<i>S. pentandra</i> L.	patent Lumley	n.d.	n.d.	n.d.	11.46	4.60
207	Stem	<i>S. alba</i> L.	"Portogruaro (Ve)"	n.d.	n.d.	n.d.	0.86	0.51
390	Stem	<i>S. fragilis</i>	R838	n.d.	n.d.	n.d.	1.05	n.d.
415	Stem	<i>S. magnifica</i> Hemsl.	WB 50 0 578	n.d.	n.d.	n.d.	9.90	30.36
432	Stem	<i>S. daphnoides</i> Vill.	fastigate	n.d.	n.d.	n.d.	94.58	4.08
506	Stem	<i>S. dasyclados</i> Wimm.	Grandis	0.82	1.58	0.79	0.95	n.d.
575	Stem	<i>S. dasyclados</i> Wimm.	(aquatica) Jyvaskyla V768	1.39	n.d.	n.d.	1.02	n.d.
576	Stem	<i>S. dasyclados</i> Wimm.	(aquatica) Yesipaju Lieto V769	1.52	n.d.	0.69	2.07	1.06
577	Stem	<i>S. dasyclados</i> Wimm.	77056 IEA Trial	14.86	3.28	3.73	6.60	5.09
592	Stem	<i>S. dasyclados</i> Wimm.	CE78-2 as x dasyclados Siren	13.14	5.82	4.88	2.81	5.67

607	Stem	<i>S. rehderiana</i> Schneid.		n.d.	n.d.	n.d.	9.84	18.37
608	Stem	<i>S. rehderiana</i> Schneid.		n.d.	n.d.	n.d.	23.38	17.31
608	Stem	<i>S. rehderiana</i> Schneid.		n.d.	n.d.	n.d.	7.44	23.27
615	Stem	<i>S. schwerinii</i> Wolf	K3 Hilliers (WB 50 0 354	n.d.	n.d.	n.d.	n.d.	n.d.
628	Stem	<i>S.viminalis</i> × <i>S.schwerinii</i>	Tora	n.d.	n.d.	n.d.	n.d.	n.d.
663	Stem	<i>S. viminalis</i> L.	Pulchra Ruberrima	n.d.	n.d.	n.d.	1.41	n.d.
837	Stem	<i>S.miyabeana</i> Seemen	III	72.22	n.d.	15.56	53.79	22.28
838	Stem	<i>S. purpurea</i> L.	Richartii	n.d.	n.d.	n.d.	69.39	13.61
844	Stem	<i>S. purpurea</i> L.	Uralensis	n.d.	n.d.	n.d.	45.70	28.29
885	Stem	<i>S.miyabeana</i> Seemen	Shrubby	49.54	n.d.	8.39	55.45	45.98
901	Stem	<i>S. × alberti</i> L. (<i>S. integra</i> Thunb. × <i>S. suchowensis</i> W.C. Cheng ex G.Zhu)	42/17	n.d.	n.d.	n.d.	80.01	2.63
941	Stem	<i>S.miyabeana</i> Seemen	Purpurescens (ex.Tuinzing) (566)	81.13	1.95	18.79	5.42	26.94
1013	Stem	<i>S.phylicifolia</i> L.	Malham	n.d.	n.d.	n.d.	0.47	1.03
1059	Stem	<i>S.repens</i> L.		n.d.	n.d.	n.d.	36.68	2.26
1155	Stem	<i>S. acutifolia</i> Willd.	174	n.d.	n.d.	n.d.	276.02	19.46
1165	Stem	<i>S. arbusculoides</i> Anderss.	20397	n.d.	n.d.	n.d.	1.58	n.d.
1215	Stem	<i>S. myrsinifolia</i> Salisb.	E-4-1403	n.d.	n.d.	n.d.	60.23	22.19

Supplementary Table 6. Varietal provenance of Terra Nova and Endurance biomass willows.

Variety	Terra Nova	Endurance
Female parent	((<i>S. viminalis</i> 'Bowles Hybrid' × <i>S. triandra</i> 'Dark Newkind') 'LA940140')	<i>S. rehderiana</i>
Male parent	<i>S. miyabeana</i> 'Shrubby'	<i>S. dasyclados</i> '77056'
Breeder	European Willow Breeding Partnership	European Willow Breeding Partnership
Sex	Female	Female
Ploidy level (x) ^a	3	5
CPVO registration date ^b	2005	2013
Breeders code	LA9801132	LA980442

^a Estimated ploidy level given in: Macalpine WJ, Shield IF, Trybush SO, Hayes C, Karp A (2008) Overcoming barriers to crossing in willow (*Salix* spp.) breeding. Aspects Appl Biol 90:173-180

^b Date granted plant breeders rights by the Community Plant Variety Office (CVPO)

Supplementary Table 7. 1 & 2D-NMR data for acetylmiyabeacin **9a/9b** (D2O:CD₃OD, 80:20 containing d₄-TSP (0.01% w/v)) δ in ppm relative to d₄-TSP at 0.00.

Position	2'-O-Acetyl miyabeacin (9a)			2''-O-Acetyl miyabeacin (9b)		
	δ	J_{H-H} (Hz)	¹³ C	δ	J_{H-H} (Hz)	¹³ C
1	-	-	157.96	-	-	157.96
2	7.19 (d)	9.3	117.86	7.19 (d)	9.3	117.86
3, 26	7.41 (ddd)	8.0, 7.7, 2.0	133.63	7.41 (ddd)	8.0, 7.7, 2.0	133.63
4, 25	7.12 (t)/7.10 (t)	7.5	125.89	7.12 (t)/7.10 (t)	7.5	125.89
5,24	7.36-7.29 (m)	-	133.45	7.36-7.29 (m)	-	133.45
6	-	-	126.67	-	-	126.67
7 α	5.09 (d)	12.1	66.90	5.39 (d)	12.1	66.90
7 β	5.03 (d)	12.1	66.90	5.17 (d)	12.1	66.90
8	-	-	173.79	-	-	173.79
9	-	-	82.57	-	-	82.57
10	3.65 (m)	-	40.57	3.62 (m)	-	40.57
11	3.62 (m)	-	43.89	3.55 (m)	-	43.89
12	6.59 (dd)	10.2, 4.1	152.57	6.59 (dd)	10.2, 4.1	152.57
13	6.02 (dd)	10.2, 1.5	130.97	6.02 (dd)	10.2, 1.5	130.97
14	-	-	198.87	-	-	198.87
15	3.53 (m)	-	45.27	3.53 (m)	-	45.27
16	6.19 (t)	7.9, 6.9, 1.0	135.70	6.19 (t)	7.9, 6.9, 1.0	135.70
17	5.91 (ddd)	7.9, 6.5, 1.4	132.86	5.91 (ddd)	7.9, 6.5, 1.4	132.86
18	3.43 (m)	-	54.45	3.43 (m)	-	54.45
19	-	-	210.26	-	-	210.26
20	-	-	80.37	-	-	80.37
21	-	-	173.64	-	-	173.64
22 β	5.36 (d)	12.2	67.02	5.10 (1H,d)	12.2	67.02
22 α	5.15 (d)	12.2	67.02	5.03 (d)	12.2	67.02
23	-	-	126.25	-	-	126.25
27	7.19 (d)	9.3	117.86	7.19 (d)	9.3	117.86
28	-	-	157.88	-	-	157.88
1'	5.23 (d)	8.0	101.37	5.06 (d)	8.0	101.37
1''	5.08 (d)	7.5	103.02	5.22 (d)	7.5	103.02
2'	5.00 / 4.97 (dd)	9.6, 8.0	76.67 / 76.69	3.55-3.63 (m)	9.6, 8.0	76.67 / 76.69
2''	3.55-3.63 (m)	-	76.08	5.00 / 4.97 (dd)	-	76.08
3'	3.78 (m)	-	76.73	3.58 (m)	-	76.73
3''	3.58 (m)	-	76.16	3.78 (m)	-	76.16
4'	3.58-3.64 (m)	-	72.52	3.49(m)	-	72.52
4''	3.49(m)	-	72.53	3.58-3.64 (m)	-	72.53
5'	3.66 (m)	-	79.20	3.58 (m)	-	79.20
5''	3.58 (m)	-	78.99	3.66 (m)	-	78.99
6' β	3.77 (dd)	12.4, 6.0	63.69	3.73 (dd)	12.4, 6.0	63.69
6'' β	3.73 (dd)	12.4, 6.0	63.69	3.77 (dd)	12.4, 6.0	63.69

6'α	3.94 (dd)	12.4, 2.1	63.69	3.92 (dd)	12.4, 2.1	63.69
6''α	3.92 (dd)	12.4, 2.1	63.69	3.94 (dd)	12.4, 2.1	63.69
7''	-	-	175.76/ 176.34	-	-	175.76/ 176.34
8''	2.143 (s) / 2.137 (s)	-	23.44	2.143 (s) / 2.137 (s)	-	23.44

Supplementary Table 8. ¹H-NMR data for diacetylmiyabeacin **10** (D₂O:CD₃OD, 80:20 containing d₄-TSP (0.01% w/v)) δ in ppm relative to d₄-TSP at 0.00.

Position	2', 2''-O-Diacetyl miyabeacin (10)	
	δ	J_{H-H} (Hz)
1	-	-
2	7.19 (d) / 7.20 (d)	8.4
3, 26	7.40 (ddd) / 7.41 (ddd)	8.0, 7.7, 2.0
4, 25	7.11 (t) / 7.13 (t)	7.6
5, 24	7.36-7.29 (m)	-
6	-	-
7 α , 22 α	5.12 (d) / 5.11 (d)	11.7 / 12.1
7 β , 22 β	5.05 (d)	12.0
8	-	-
9	-	-
10	3.51 – 3.70 (m)	-
11	3.51 – 3.70 (m)	-
12	6.62 (dd)	10, 4.4
13	6.03 (dd)	10.1, 1.4
14	-	-
15	3.51 – 3.70 (m)	-
16	6.22 (t)	6.7
17	5.93 (ddd)	7.9, 6.5, 1.4
18	3.51 – 3.70 (m)	-
19	-	-
20	-	-
21	-	-
23	-	-
27	7.19 (d) / 7.20 (d)	8.4
28	-	-
1', 1''	2 x 5.24 (d)	7.9
2', 2''	5.01 (dd) / 4.98 (dd)	9.6, 8.0
3', 3''	3.79 (m)	-
4', 4''	3.51 – 3.70 (m)	-
5', 5''	3.51 – 3.70 (m)	-
6' β , 6'' β	3.77 (dd), 3.82 (dd)	12.4, 6.0
6' α	3.98 (dd)	12.4, 2.1
6'' α	3.96 (dd)	12.4, 2.1
7', 7''	-	-
8', 8''	2 x 2.16 (s)	-

Supplementary Table 9. Extraction and HPLC gradient conditions for the isolation of dimeric metabolites.

Compound Number	Compound Name	Amount Extracted	Tissue	Extraction Volume (solvent: H ₂ O:MeOH)	Number of 100 µL Injections made into HPLC	HPLC Gradient [mobile phases water (A) and acetonitrile (B), both containing 0.1% formic acid.]	HPLC Retention time of Peak	Amount Isolated
3	Miyabeacin	50 mg	<i>Salix miyabeana</i> Seemen. III leaf tissue. Line: NWC837	1 mL	6	5% B (0-10 min), 22% B (10-50 min) to 37 % B (60-70 min).	57.93 min	1.68 mg
6	Miyabeacin B	200 mg	<i>Salix miyabeana</i> Seemen. "Purpurescens" stem tissue. Line: NWC941	2.5 mL	> 10	5% B (0-10 min), 29% B (10-60 min) to 29 % B (60-70 min)	52.11 min	0.67 mg
7	Miyabeanol	150 mg	<i>Salix miyabeana</i> Seemen. III leaf tissue. Line: NWC837	2 mL	8	5% B (0-10 min), 22% B (10-50 min) to 37 % B (60-70 min)	44.87 min	1.05 mg
8	Miyaquinol	450 mg	<i>Salix miyabeana</i> Seemen. "Purpurescens" leaf tissue. Line: NWC941	4.5 mL	44	20% B (0-20 min), 40% B (20-25 min) to 50 % B (25-35 min)	20.9 min	0.9 mg
9a/9b	2'/2"Acetyl miyabeacin	150 mg (2 x 75 mg)	RRes 710-27, RR09102 hybrid [NWC607 <i>S. rehderiana</i> x RR05337 (Aud x <i>S. rossica</i>)] leaf tissue	2.4 mL (2 x 1.2 mL)	10	20% B (0 min), 40% B (0 – 45 min) to 100 % B (45.0-50 min)	41.4	0.75 mg
10	2', 2" Diacetyl miyabeacin	150 mg (2 x 75 mg)	RRes 710-27, RR09102 hybrid [NWC607 <i>S. rehderiana</i> x RR05337 (Aud x <i>S. rossica</i>)] leaf tissue	2.4 mL (2 x 1.2 mL)	10	20% B (0 min), 40% B (0 – 45 min) to 100 % B (45.0-50 min)	45.5	0.25 mg

Supplementary Table 10. General Conditions and typical parameters for NMR and Mass Spectral data collection.

Measurement Conditions	Solvent	D ₂ O:CD ₃ OD (80:20)	Observation width	7182, 33165 Hz
High resolution LC-MS	Concentration	0.6 mg/mL	Data points	4096, 256
LC apparatus	Internal Standard	d ₄ -TSP	Temperature	300K
Chromatography Column	C ₁₈ Hypersil gold column (1.9 µm, 300 Å, 2.1 mm i.d.)	300K	Number of transients	256
Column Temperature	35°C	Probe		
Solvents	Water/0.1% formic acid (A) and acetonitrile/0.1% formic acid (B)	5mm Selective Inverse		
Solvent Gradient	0 min, 0 % B; 27 min, 70 % B; 28 min, 100 % B		¹³C NMR	
Flow rate	0.3 mL/min	Pulse sequence	Apparatus	Avance 400 (Bruker)
Run time	30 min	Sweep width	Observation Frequency	¹³ C: 100.61
Injection volume	10µL	Spectrum offset	Solvent	D ₂ O:CD ₃ OD (80:20)
		Data points	Concentration	0.6 mg/mL
		Pulse angle	Internal Standard	d ₄ -TSP
MS Apparatus	LTDQ-Orbitrap Elite (Thermo)	Delay	Temperature	300K
Source	Heated ESI source	Number of scans	Probe	5mm Broadband BBO
Ionisation mode	Negative			
Resolution	120,000			
Capillary temperature	350°C	2D COSY 45 Measurement		
Source heater temperature	350°C	Pulse program	Pulse sequence	dept135
Source voltage	2500 V	Observation width	Sweep width	23,980 Hz
Source current	100 uA	Data points	Spectrum offset	10363 Hz
Sheath gas flow	35	Temperature	Data points	32768
Auxillary gas	10	Number of transients	Pulse angle	30°
R.F. Lens	50%		Delay	0.7 s
Scan range	m/z 50-1500		Number of scans	46,191
MS-MS fragmentation	Automatic on top 3 ions	2D HSQC Measurement		
Ion isolation width for MSMS	m/z 2	Pulse program	DEPT Measurement	
Fragmentation mode	HCD	Observation width	Observation width	23980 Hz
Normalised collision energy	65	Data points	Data points	65536
Activation time	0.1 ms	Temperature	Pulse repetition time	2
		Number of transients	Number of scans	4096
NMR				
Apparatus	Avance 600 (Bruker)	2D HMBC Measurement		
Observation Frequency	¹ H: 600.05, ¹³ C: 150.9	Pulse program		

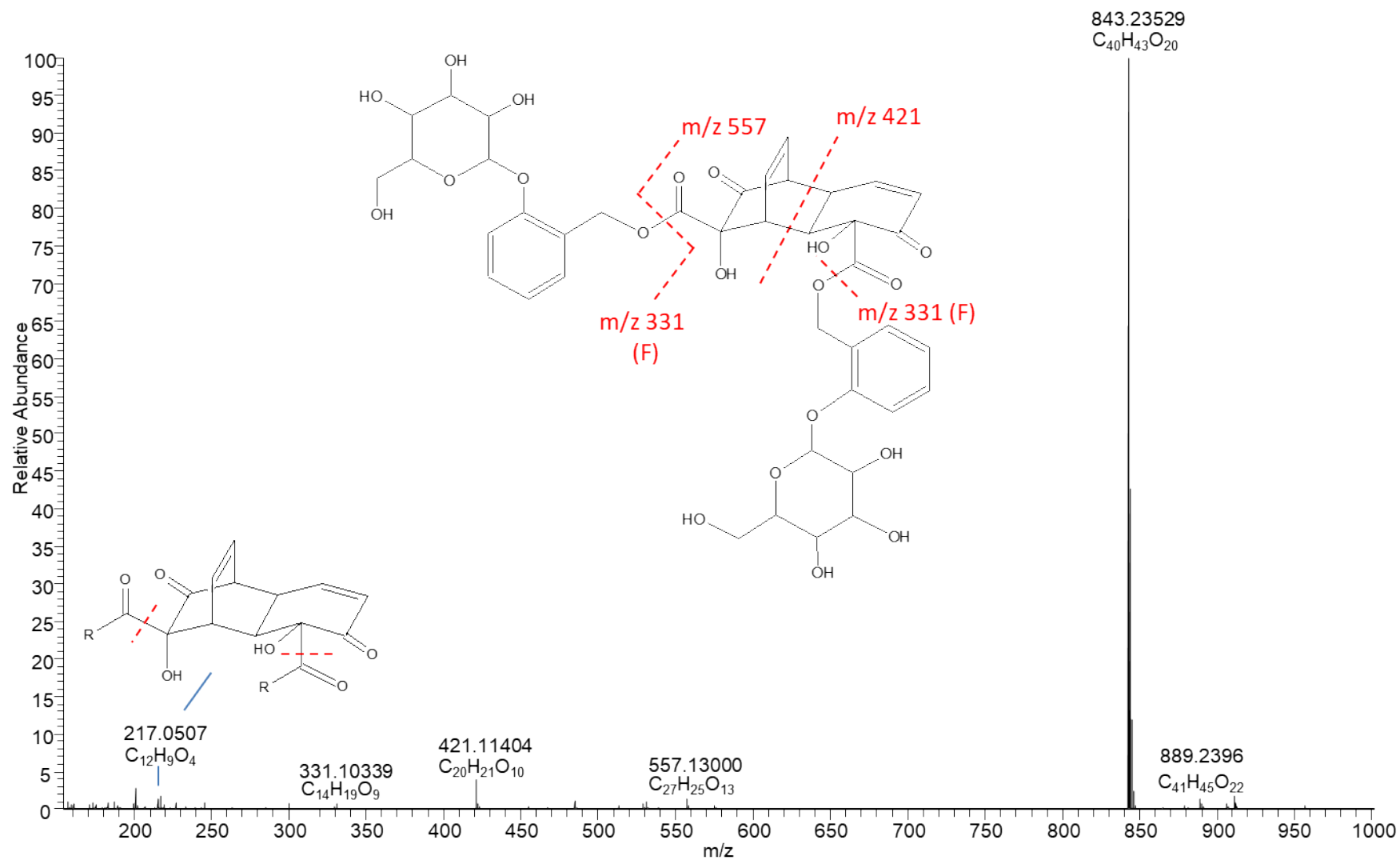
Abbreviations

DEPT: Distortionless Enhancement by Polarization Transfer (A method for determining a carbon type (distinguishing among CH₃, CH₂, CH, and C))

COSY: COReLation SpectroscopY (A method of ^1H - ^1H COSY)

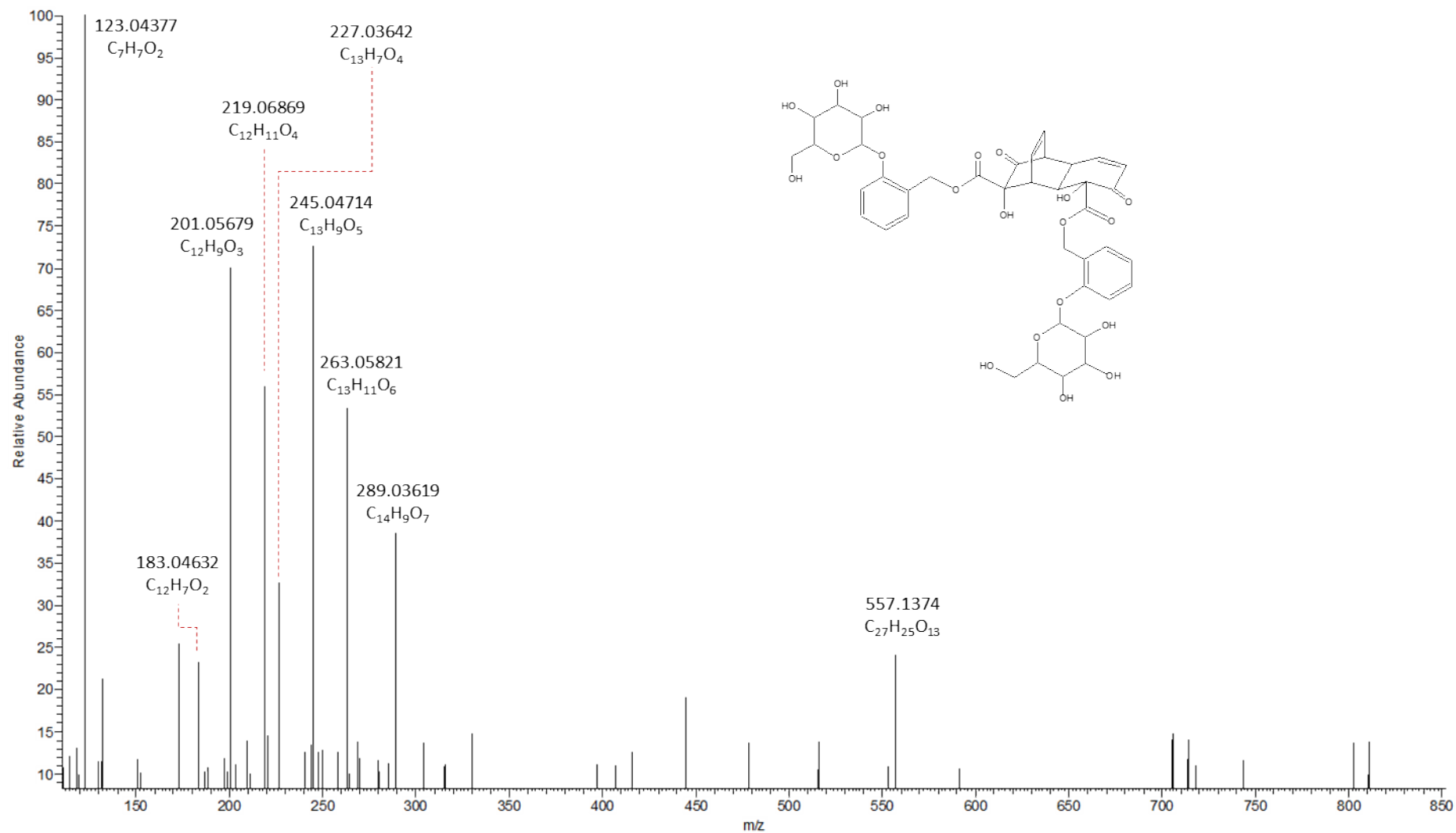
HSQC: Heteronuclear Single Quantum Coherence (A method of ^1H - ^{13}C COSY)

HMBC: Heteronuclear Multiple Bond Correlation (A method of long-range

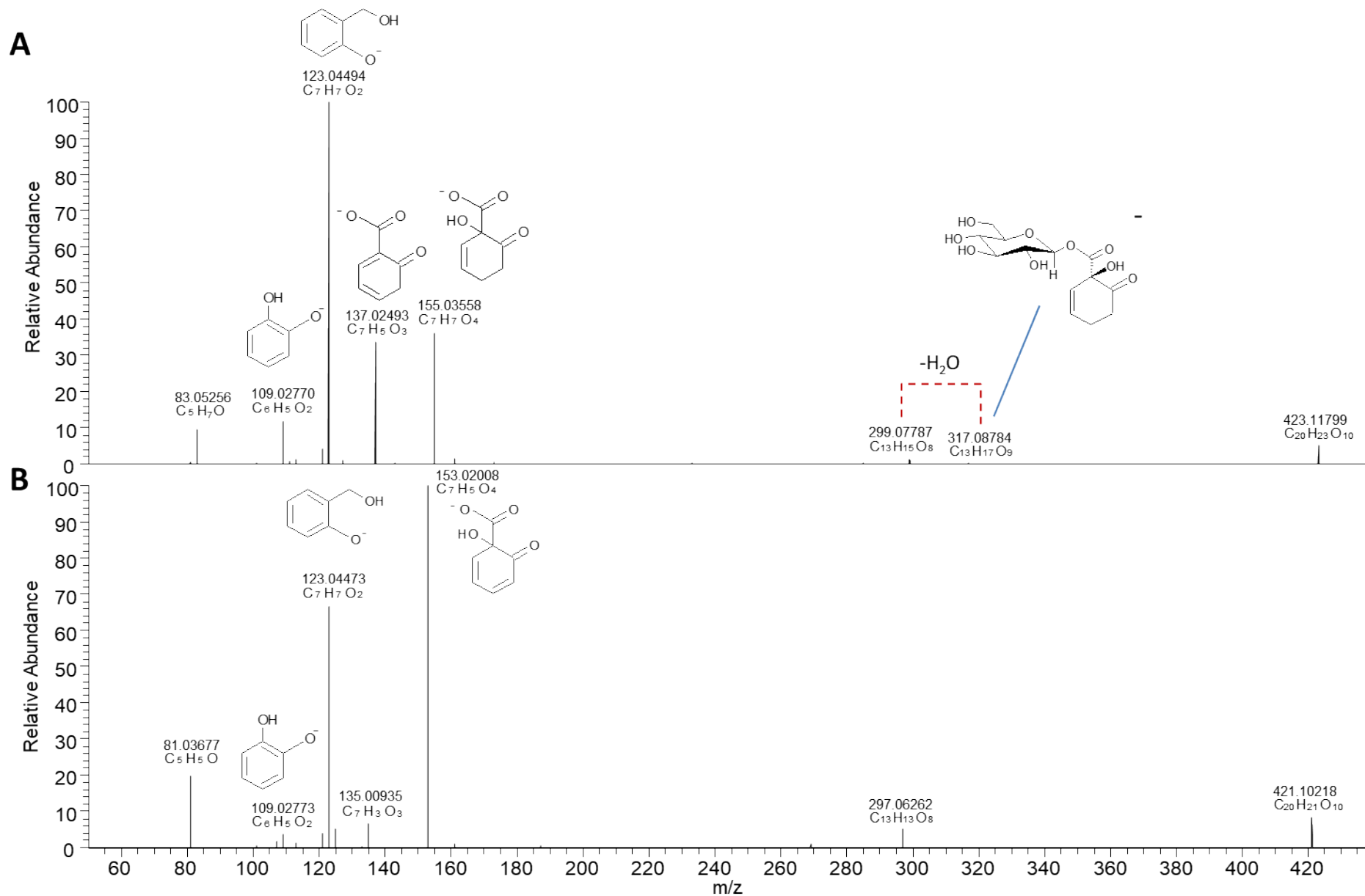


Supplementary Figure 1. Mass spectrum of miyabeacin **3** at m/z 843.2353 with retention time 25.26 min. Dashed lines show in-source fragmentation of the molecule. (F) indicates formate adduct

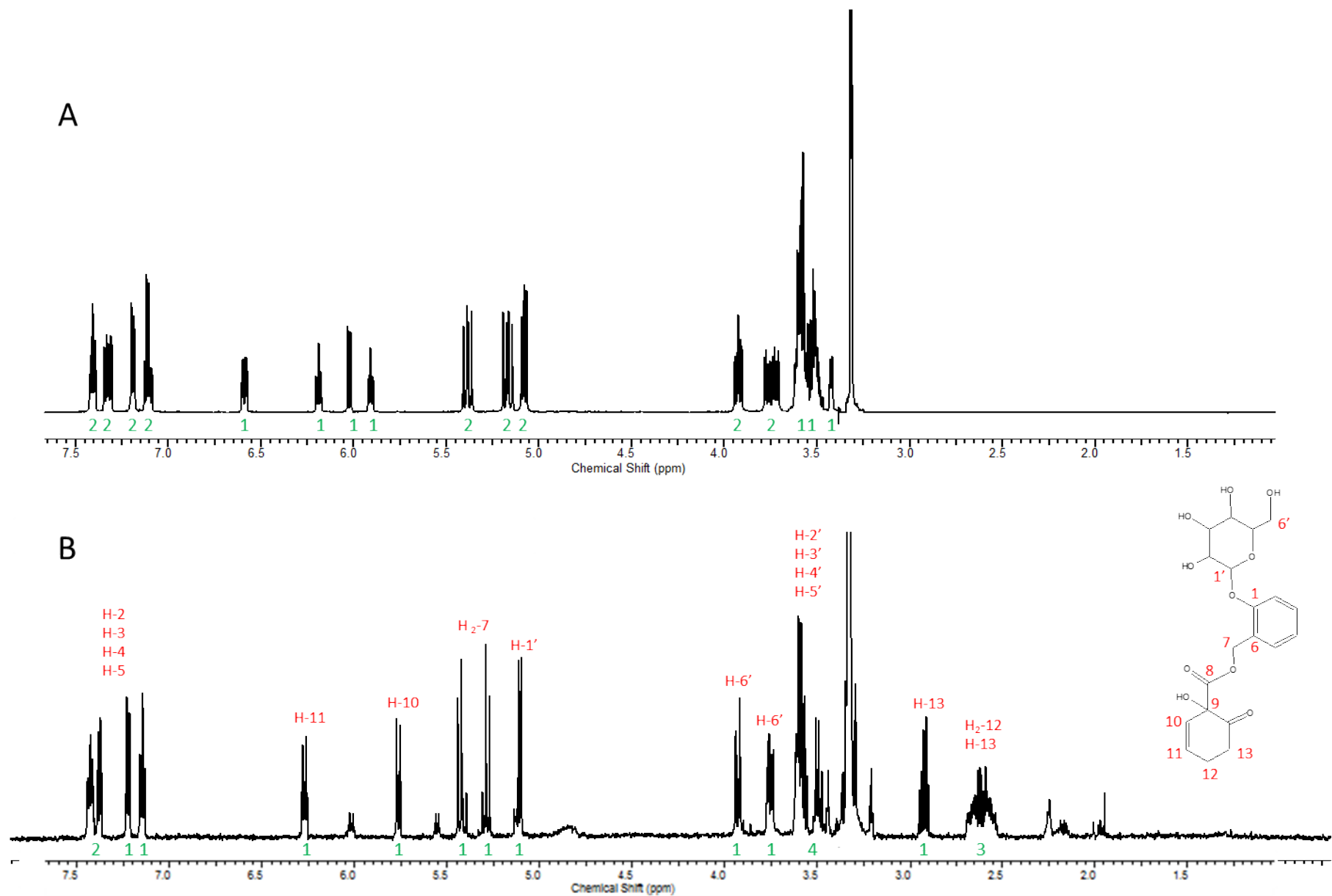
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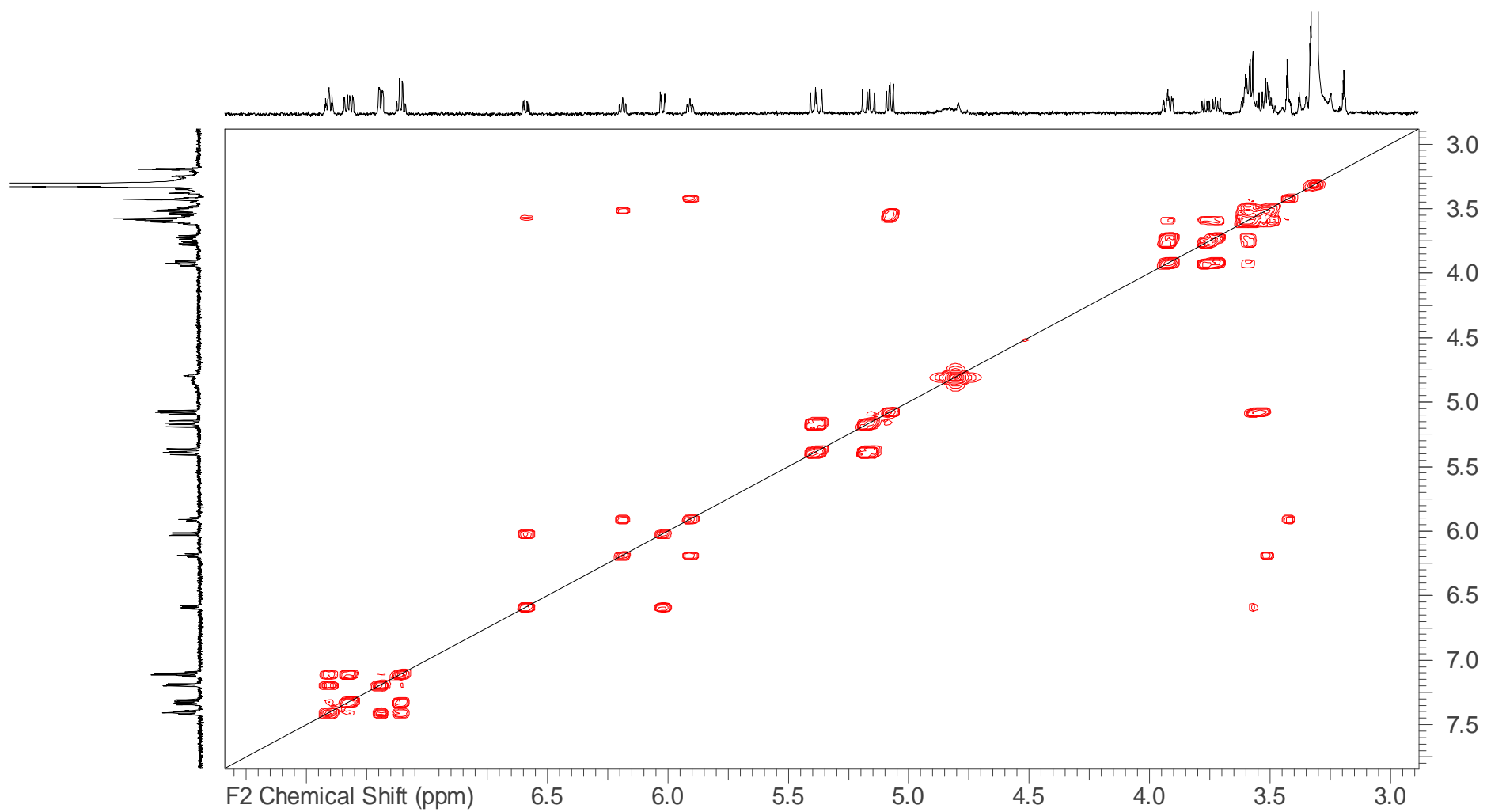
Supplementary Figure 2. MSMS data (negative ion mode) of m/z 843 ion of miyabeacin **3**



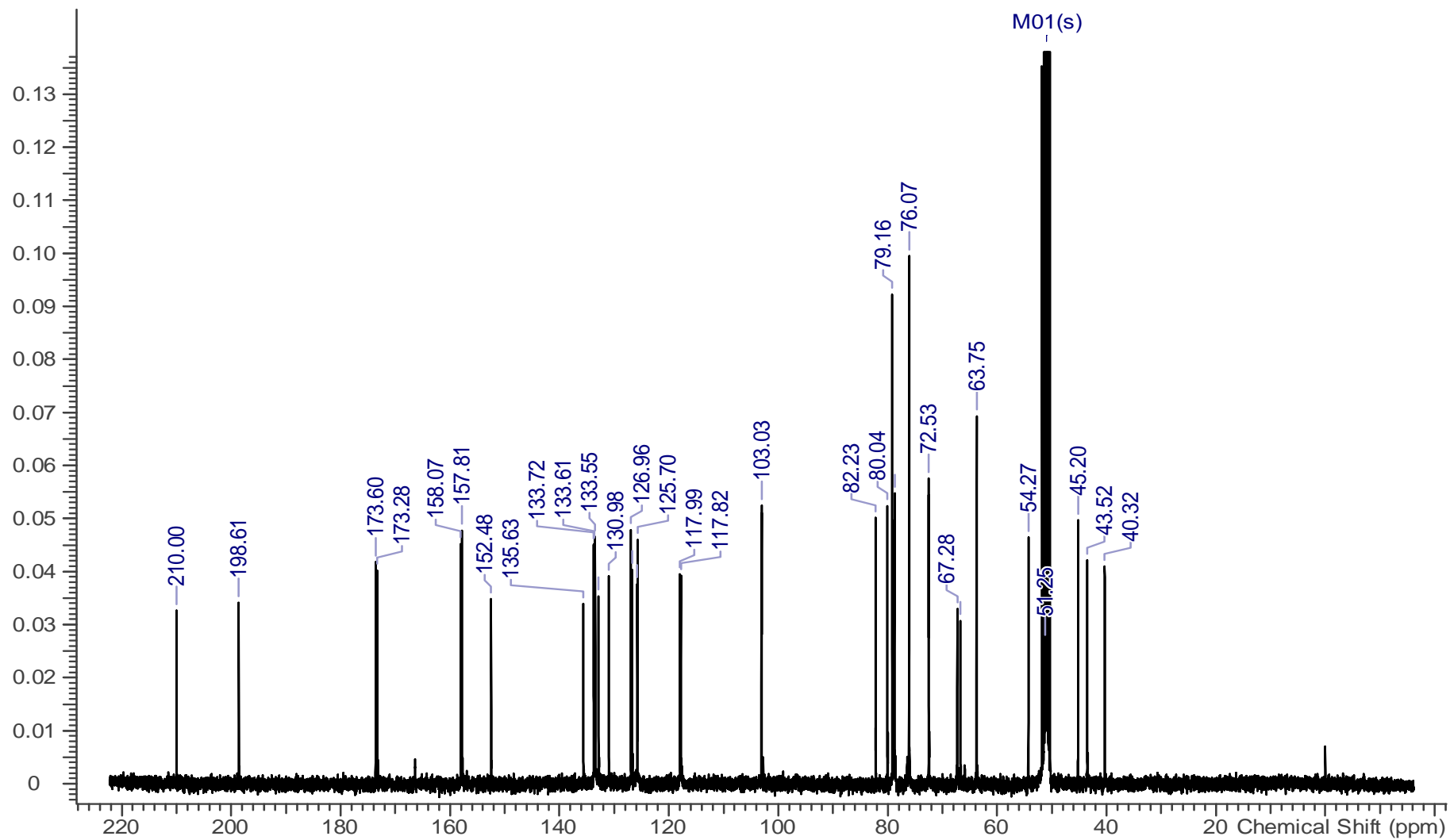
Supplementary Figure 3. MSMS comparison of A: salicortin **2** m/z 423 and B: miyabeacin **3** fragment (m/z 421). The structures for the ion at m/z 317 is believed to have arisen from a rearrangement following a neutral loss of orthoquinone methide from salicinoid structures.



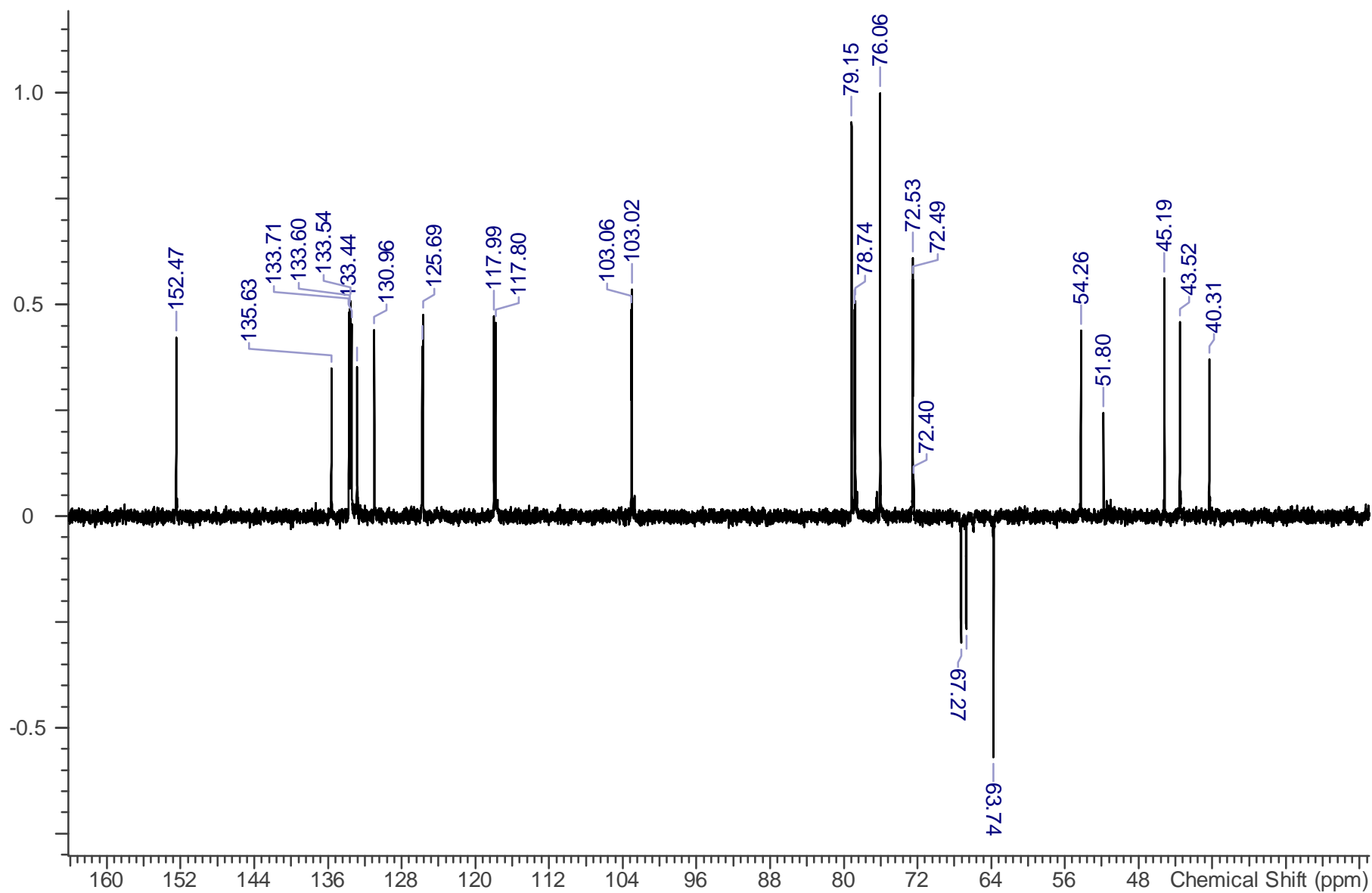
Supplementary Figure 4. Comparison of 600 MHz ^1H -NMR spectra of A: miyabeacin **3** and B: salicortin **2** collected in 80:20 $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ containing 0.01 % w/v d_4 -TSP as reference standard. Numbers in red relate to structural assignment of salicortin. Numbers in green relate to peak integral values.



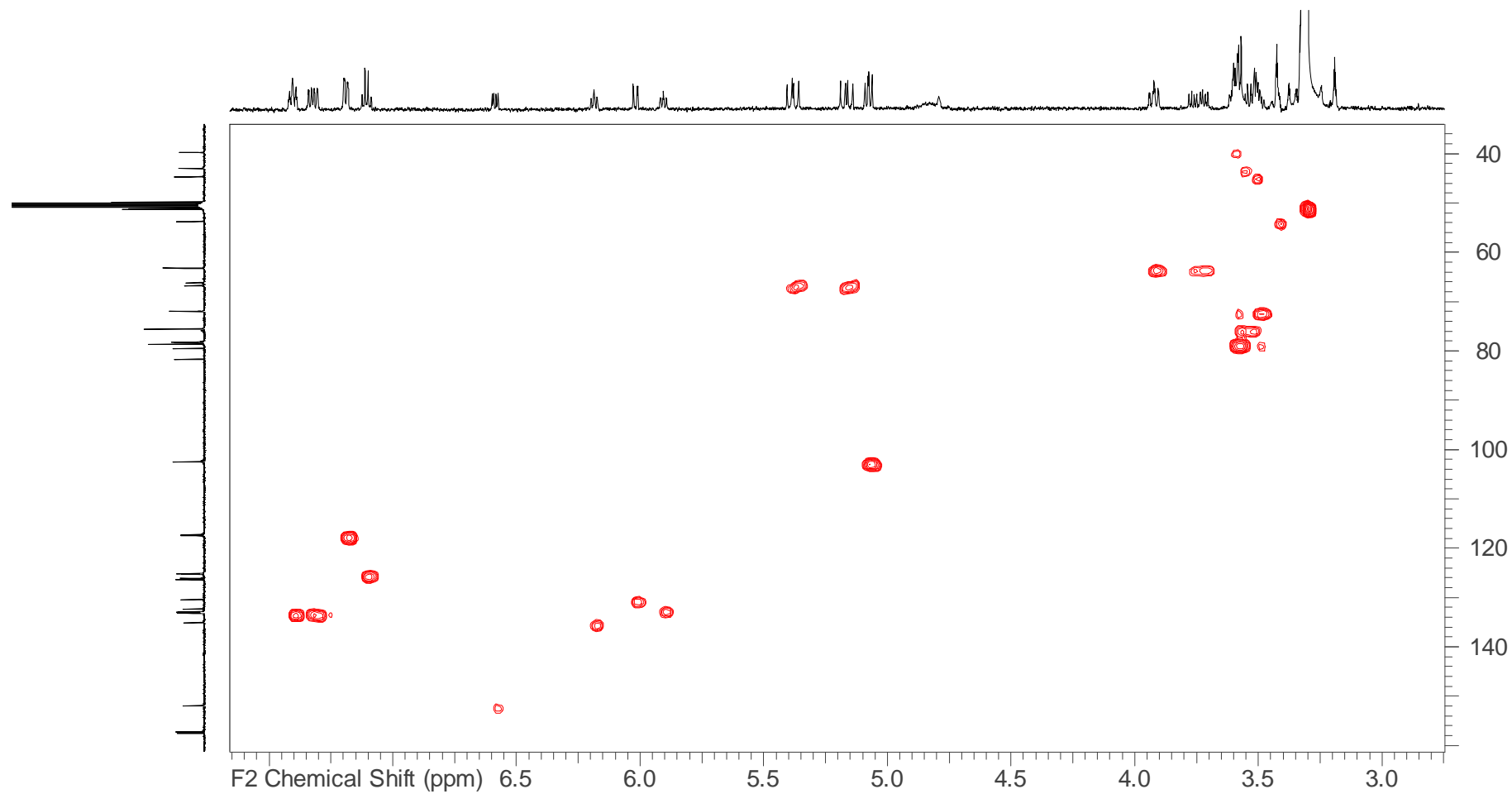
Supplementary Figure 5. COSY45 spectrum of miyabeacin **3**, collected at 600MHz in D₂O:CD₃OD (80:20)



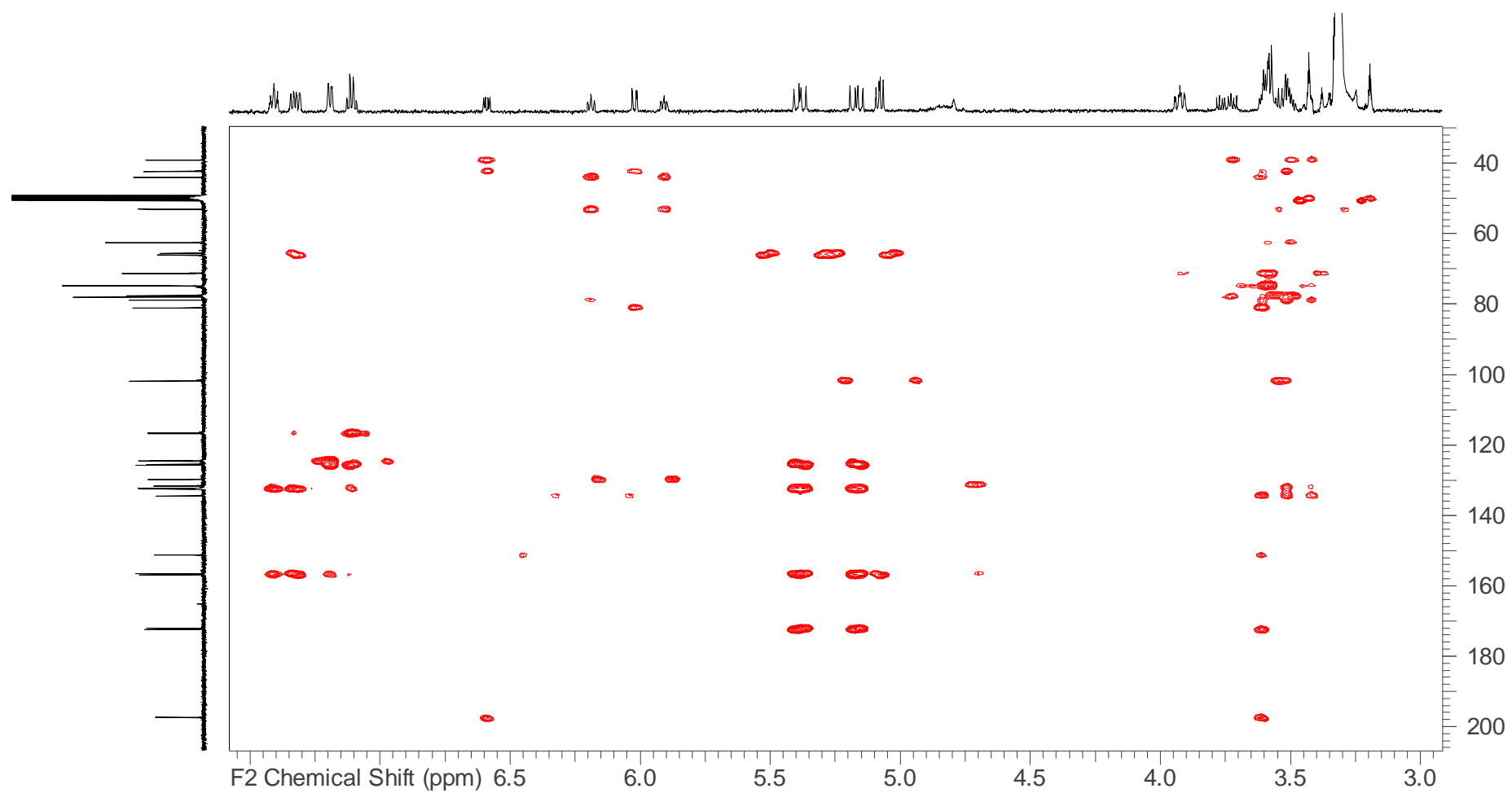
Supplementary Figure 6. ^{13}C spectrum of miyabeacin **3** collected at 400 MHz in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (80:20) containing 0.01% w/v d_4TSP . Spectrum referenced to $\text{d}_4\text{-TSP}$ at $\delta 0.00$



Supplementary Figure 7. DEPT135 spectrum of miyabeacin **3**, collected at 100.6128 MHz in D₂O:CD₃OD (80:20)

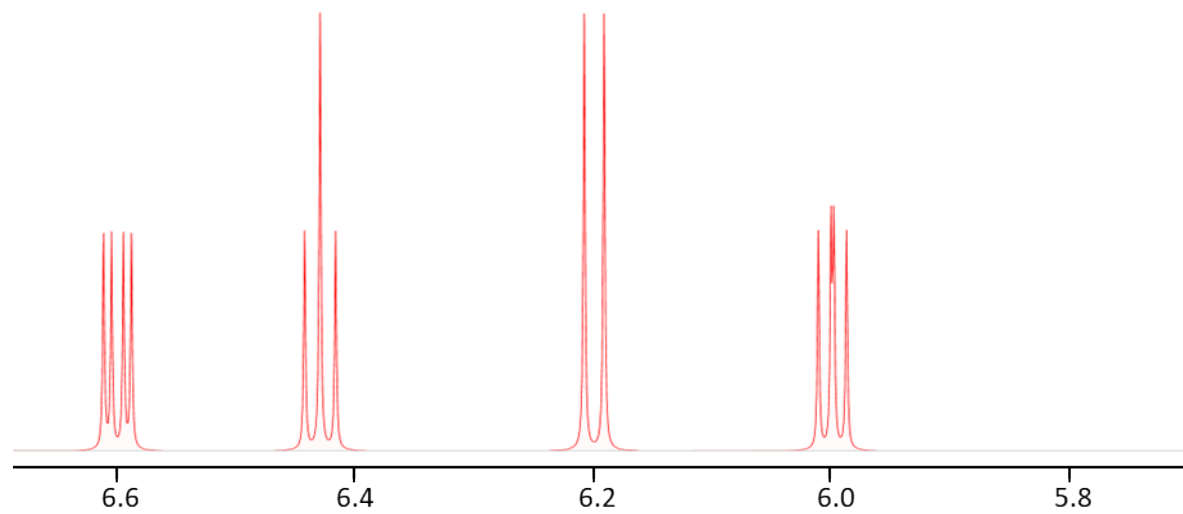


Supplementary Figure 8. HSQC spectrum of miyabeacin **3** collected in D₂O:CD₃OD (80:20)

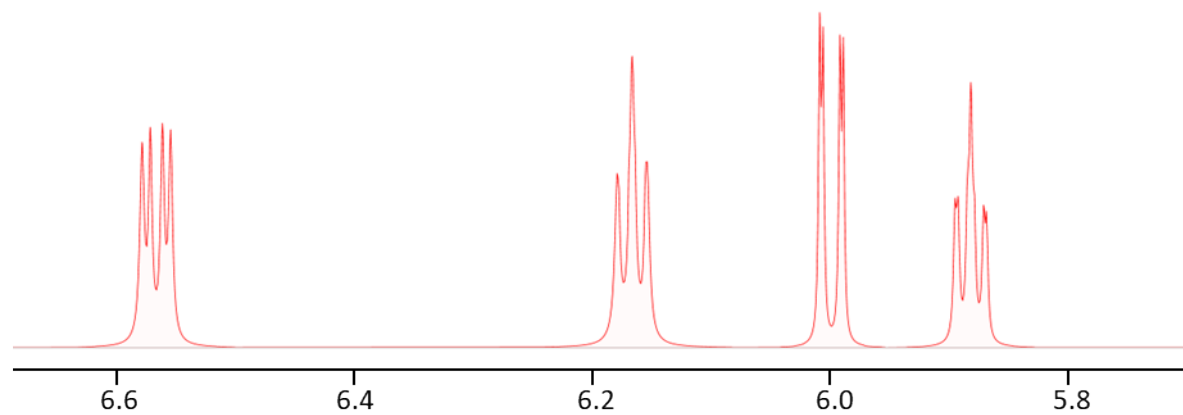


Supplementary Figure 9. HMBC spectrum of miyabeacin **3** collected in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (80:20)

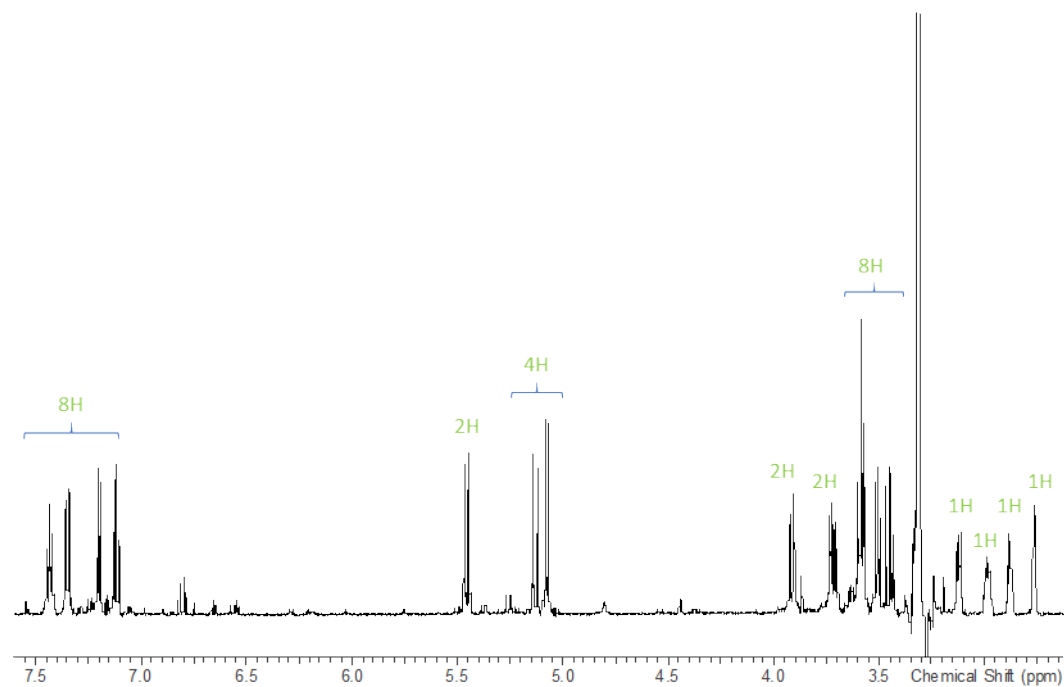
A



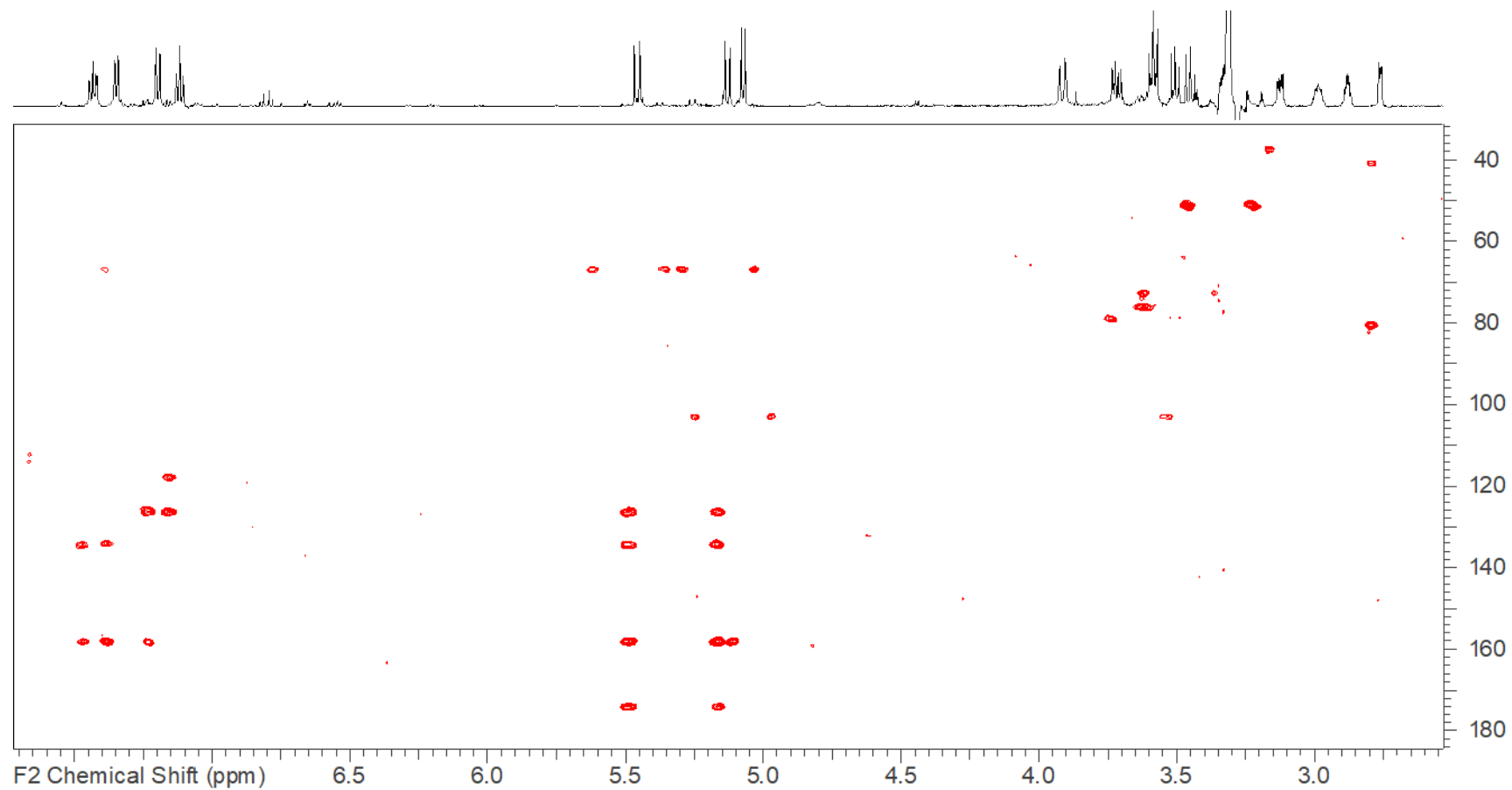
B



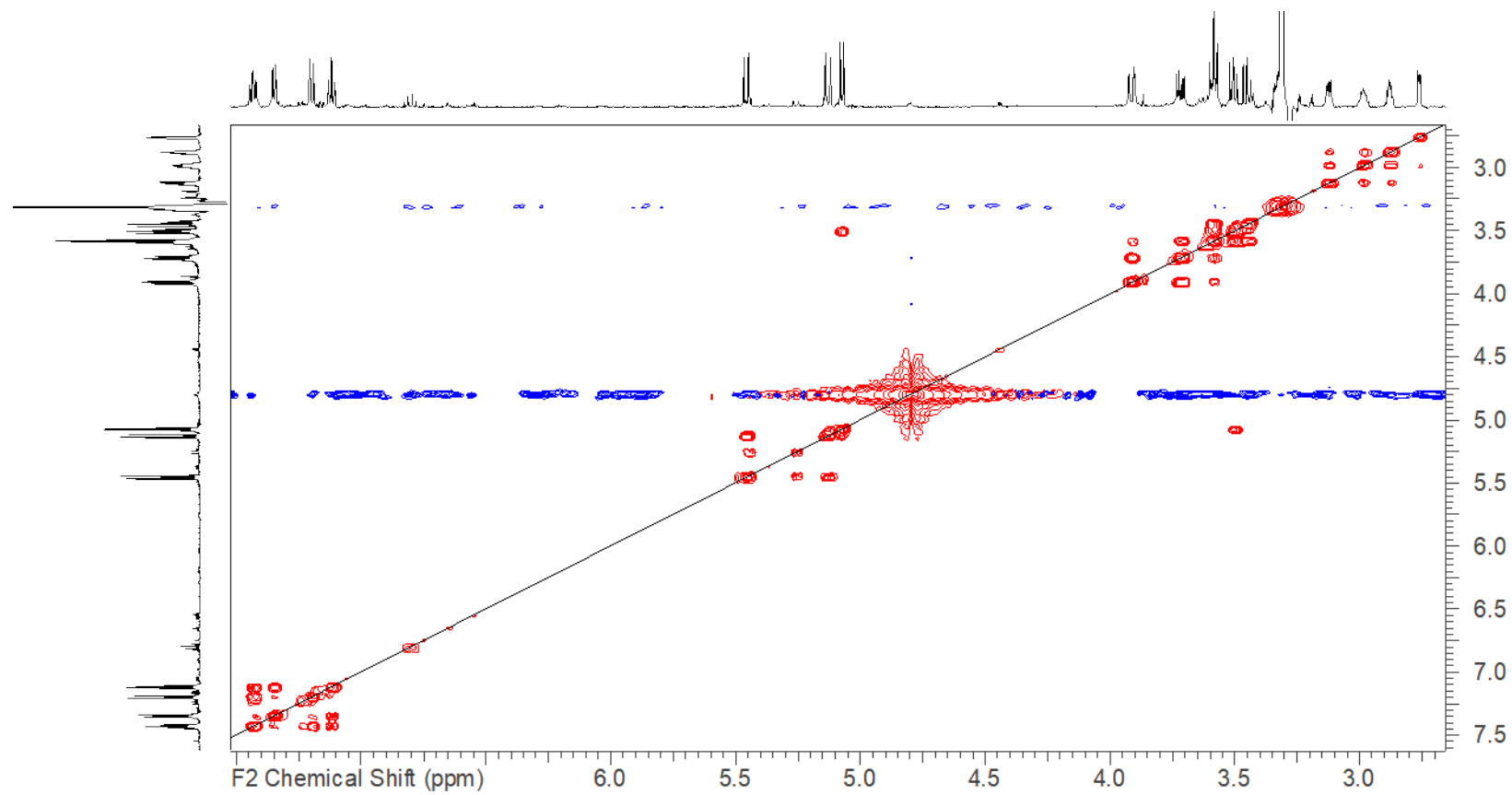
Supplementary Figure 10. Chenomx simulated ^1H NMR spectrum (500 MHz) of grandifloracin in CDCl_3 (δ 6.66 – 5.70) from data provided in Palframan *et al.*, 2011. B: ^1H NMR spectrum (δ 6.66 – 5.70) of miyabeacin **3** in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (8:2) collected at 600 MHz.



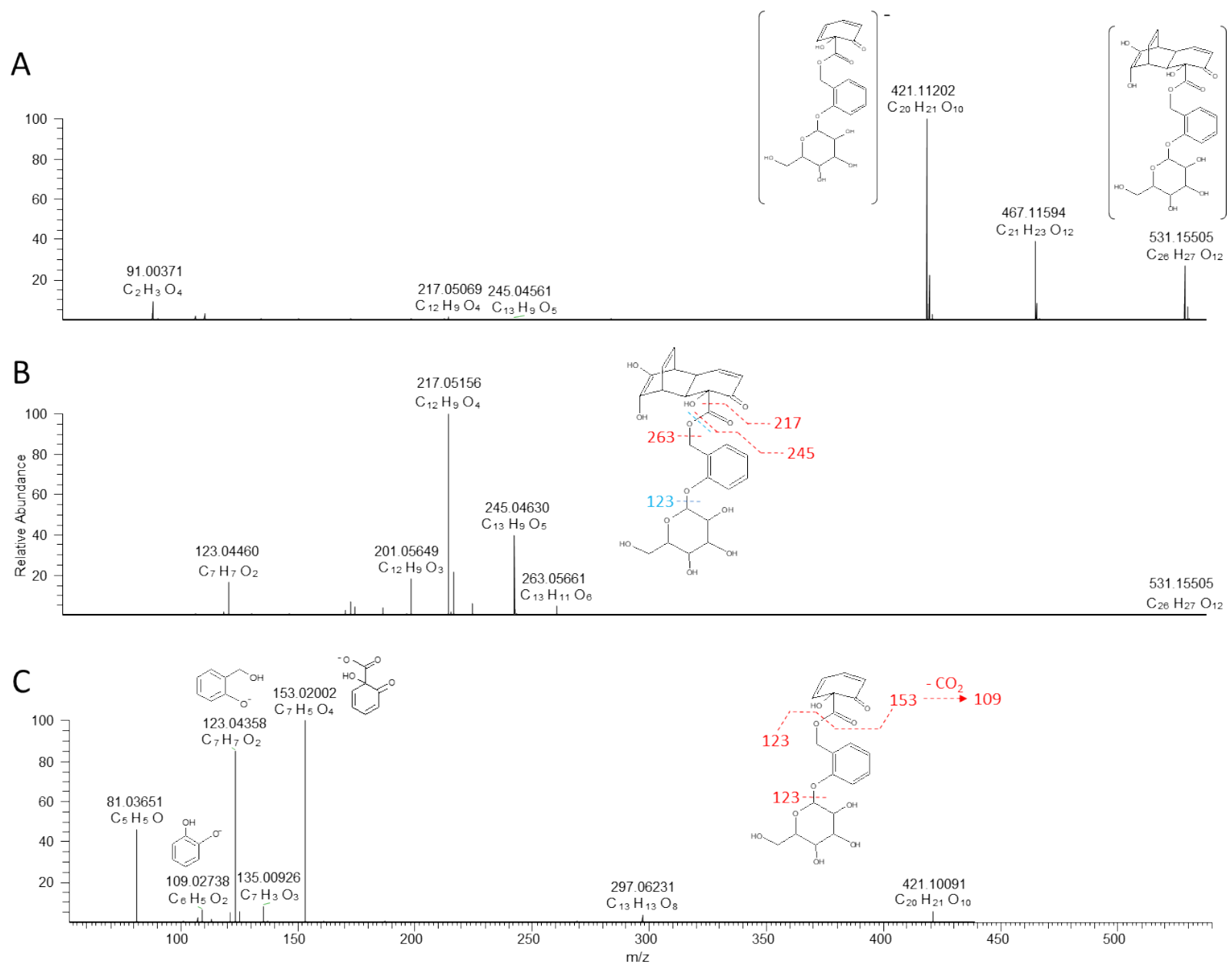
Supplementary Figure 11. 600 MHz ^1H -NMR spectra of A: miyabeacin B **6** collected in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ containing 0.01 % w/v d_4 -TSP as reference standard. Numbers in green relate to peak integral values.



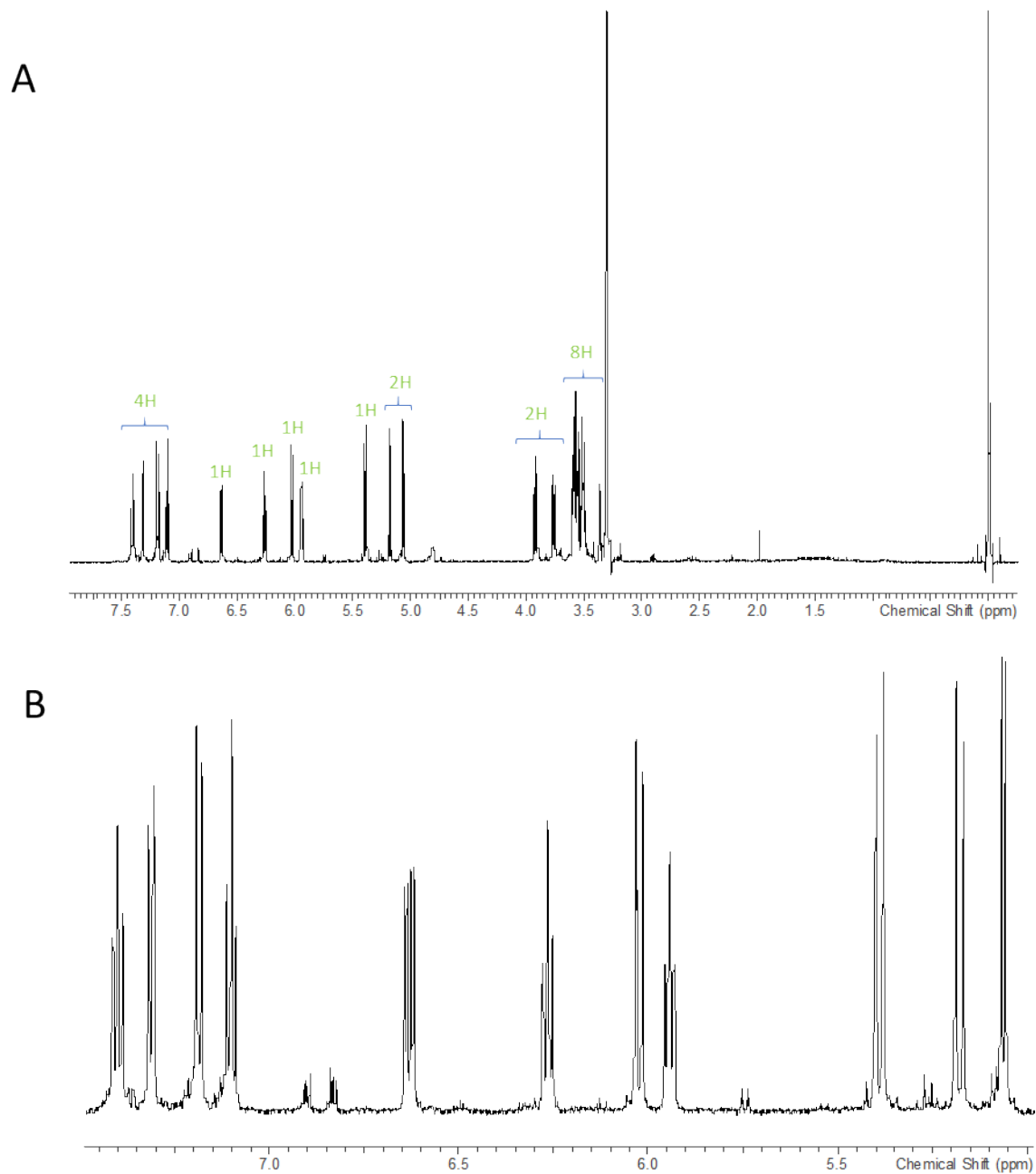
Supplementary Figure 13. HMBC spectrum of miyabeacin B **6** collected in D₂O:CD₃OD containing 0.01 % w/v d₄-TSP as reference standard.



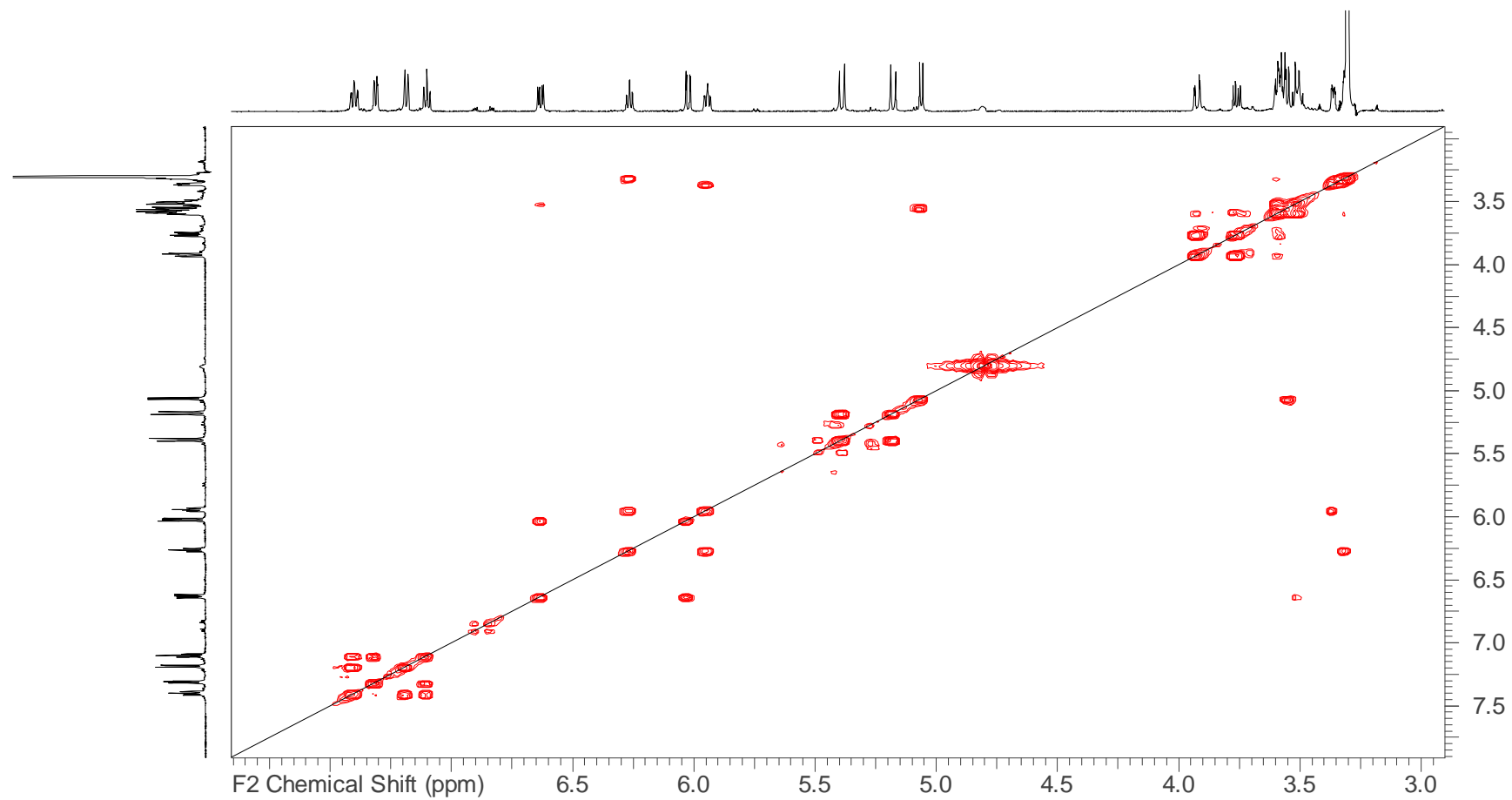
Supplementary Figure 14. COSY spectrum of miyabeacin B **6** collected in D₂O:CD₃OD containing 0.01 % w/v d₄-TSP as reference standard.



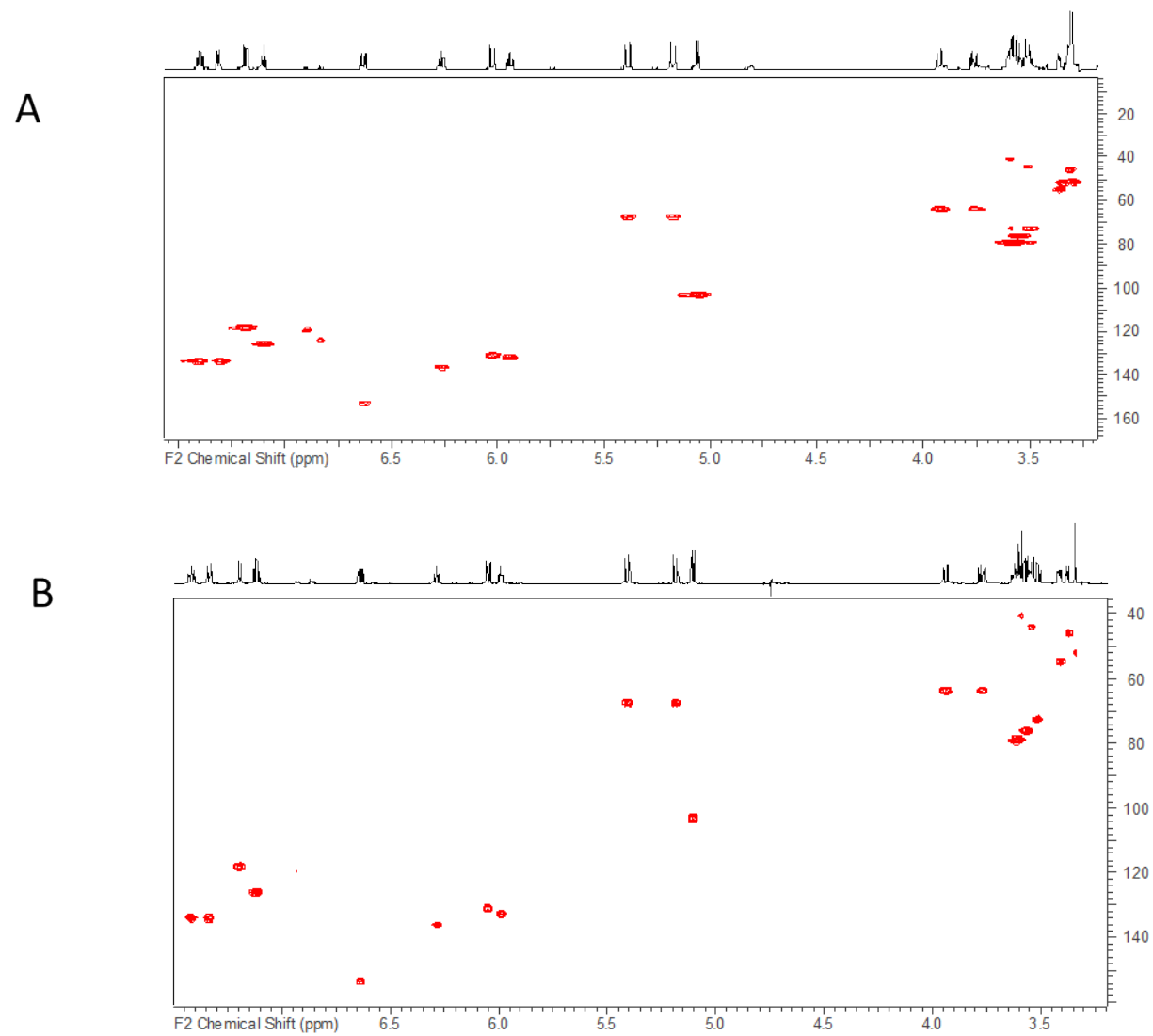
Supplementary Figure 15. MS and MSMS data of miyabeanol **7**. A: MS spectrum of peak at 20.13 min; B: MSMS of m/z 531 [M-H]⁻; C: MSMS of m/z 421 ([M-H]⁻ of retro Diels-Alder product)



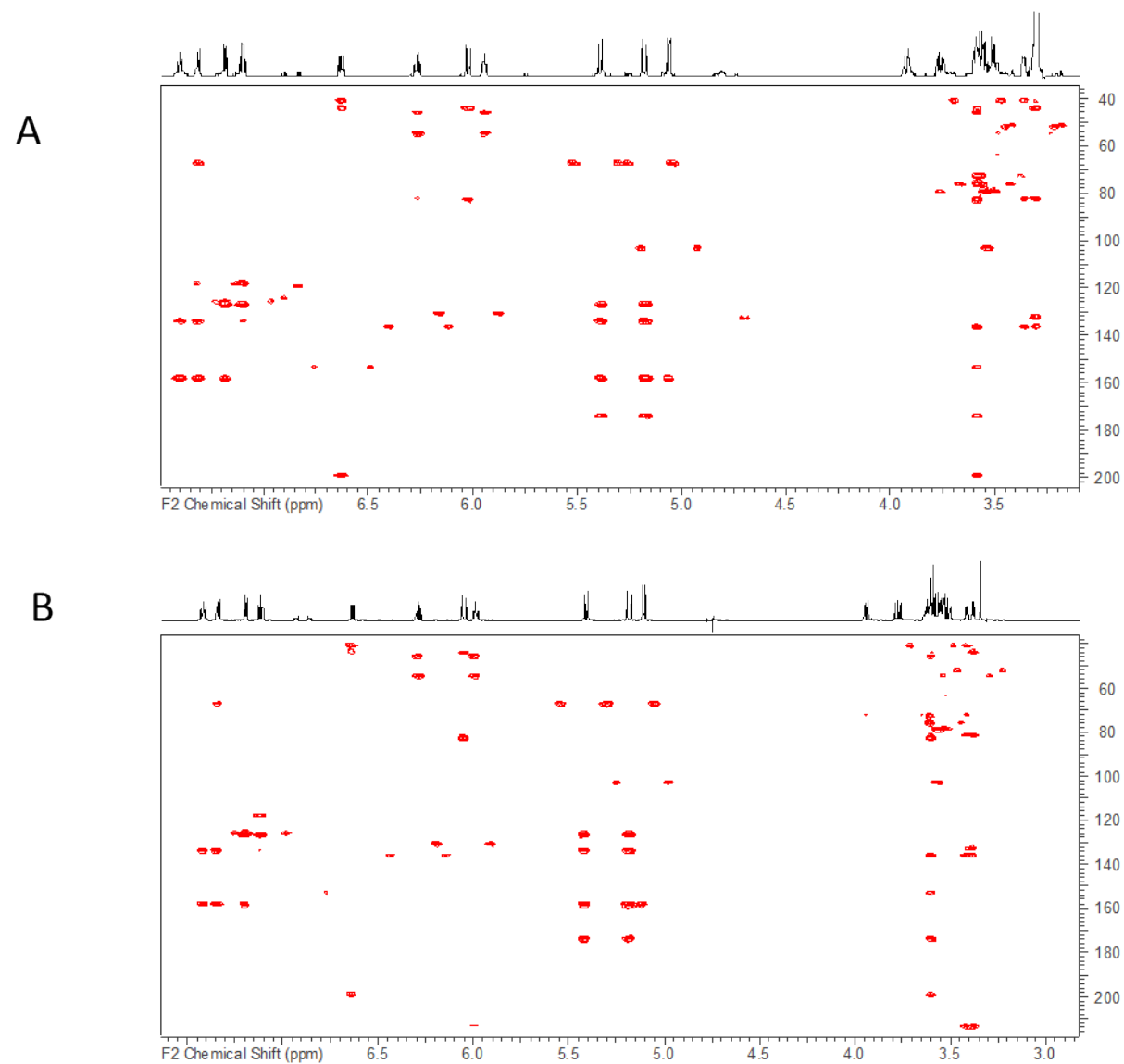
Supplementary Figure 16. 600 MHz ^1H -NMR spectra of A: miyabeanol **7** collected in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ containing 0.01 % w/v d_4 -TSP as reference standard. Numbers in green relate to peak integral values. and B: expansion of the region between $\delta 7.50 - 5.0$.



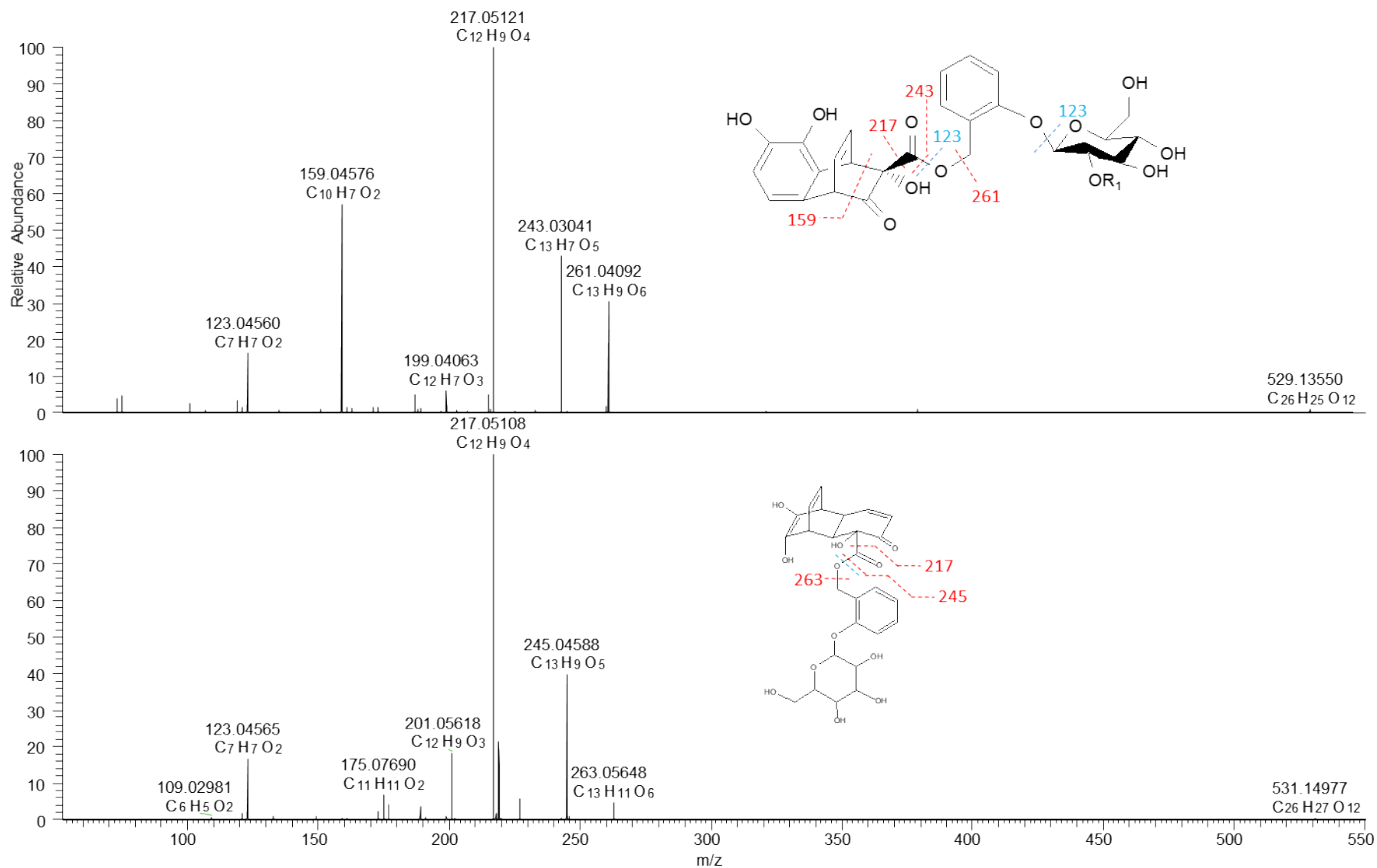
Supplementary Figure 17. COSY45 spectrum of A: miyabeanol **7** collected in D₂O:CD₃OD containing 0.01 % w/v d₄-TSP as reference standard.



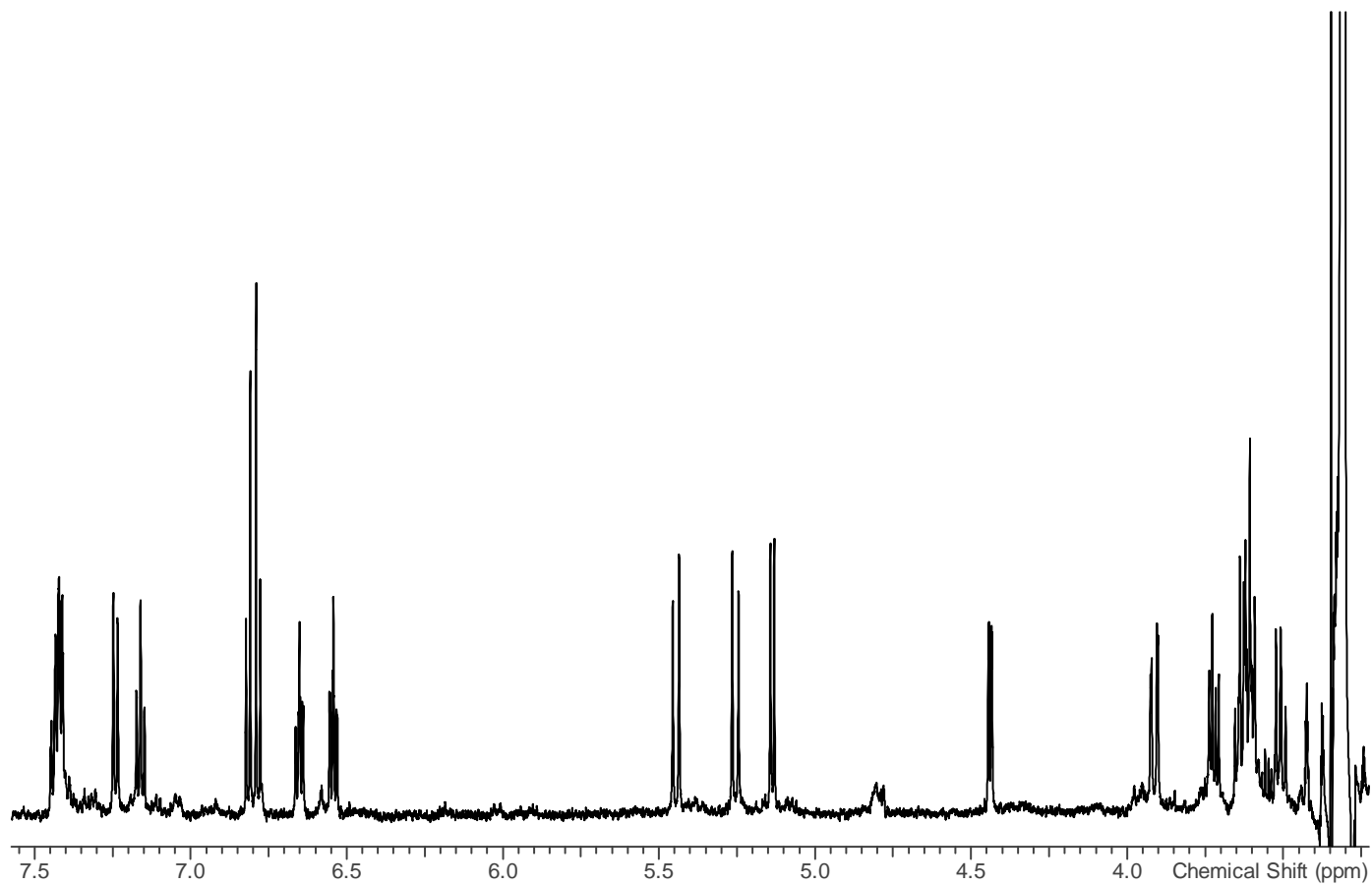
Supplementary Figure 18. HSQC spectra of miyabeanol **7**. A: D₂O:CD₃OD and B: D₂O



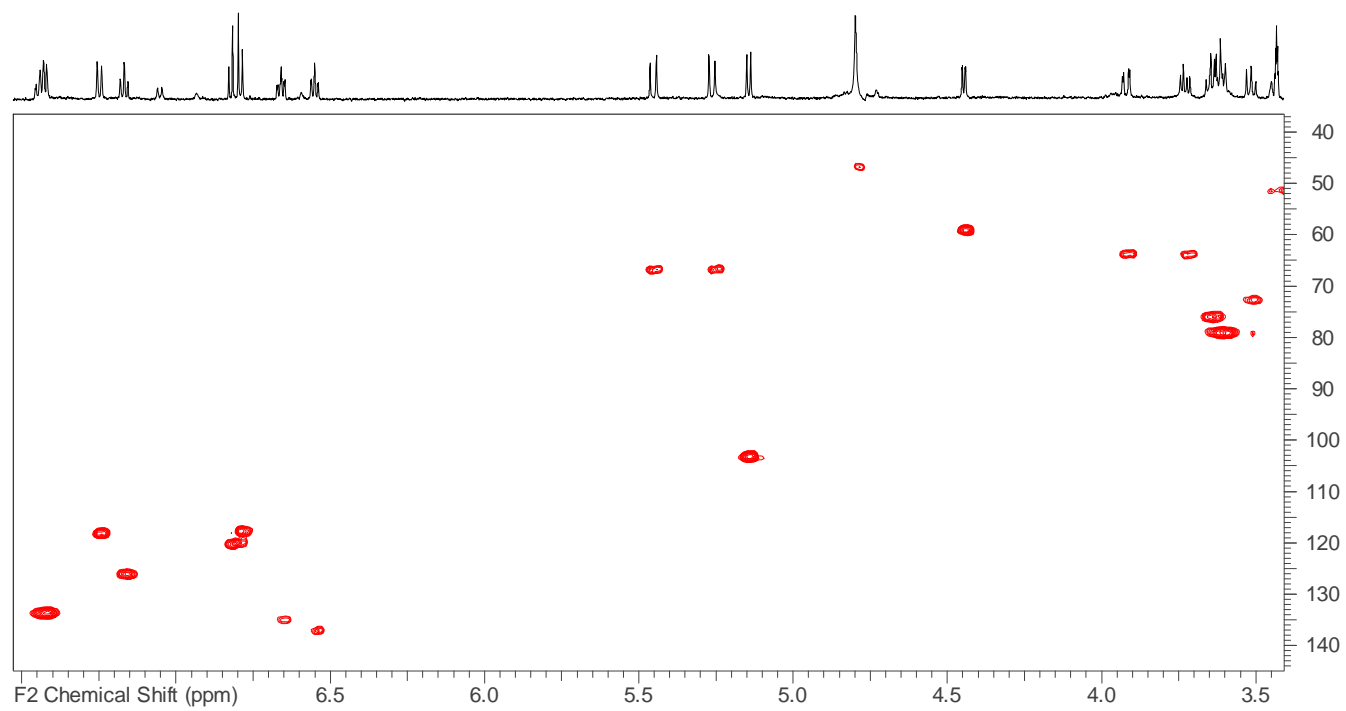
Supplementary Figure 19. HMBC spectra of miyabeanol **7**. A: D₂O:CD₃OD and B: D₂O



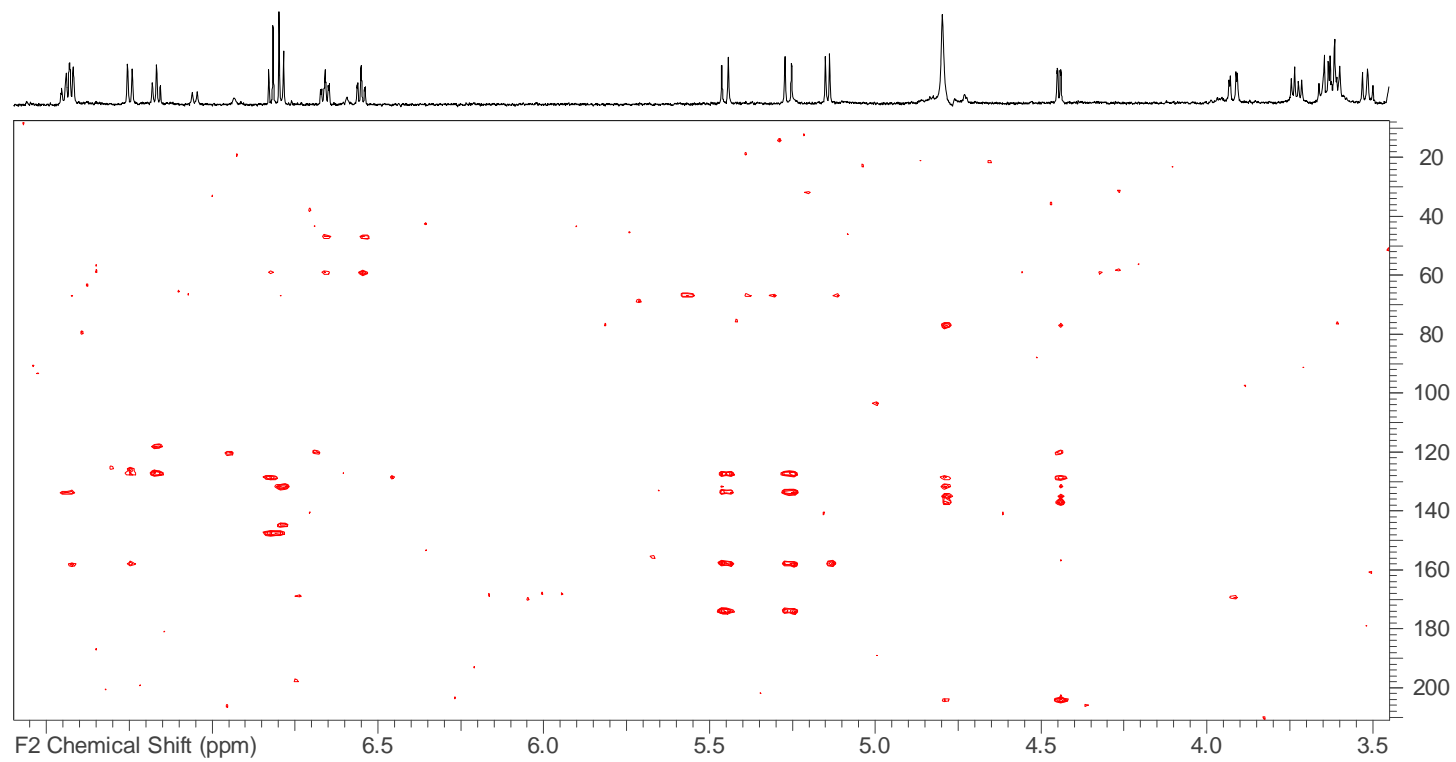
Supplementary Figure 20. MSMS data of miyaquinol **8**. A: MSMS spectrum of m/z 529 peak at 23.67 min; B: MSMS of m/z 531 [M-H]⁻ for comparison.



Supplementary Figure 21. 600 MHz ¹H-NMR spectra of miyaquinol **8** collected in D₂O:CD₃OD containing 0.01 % w/v d₄-TSP as reference standard.

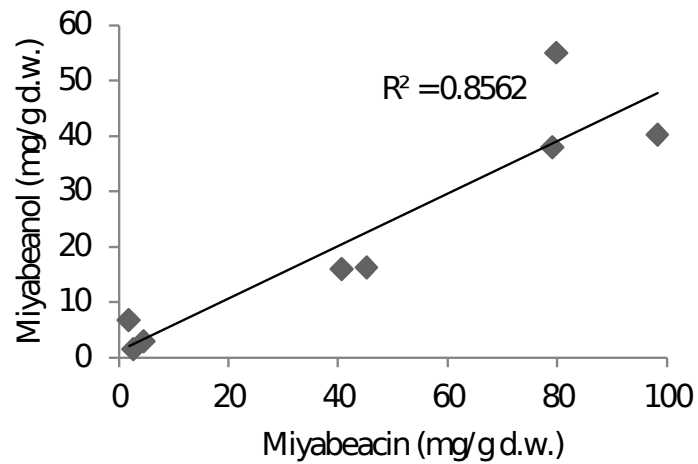


Supplementary Figure 22. HSQC spectra of miyaquinol **8** collected in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (80:20)

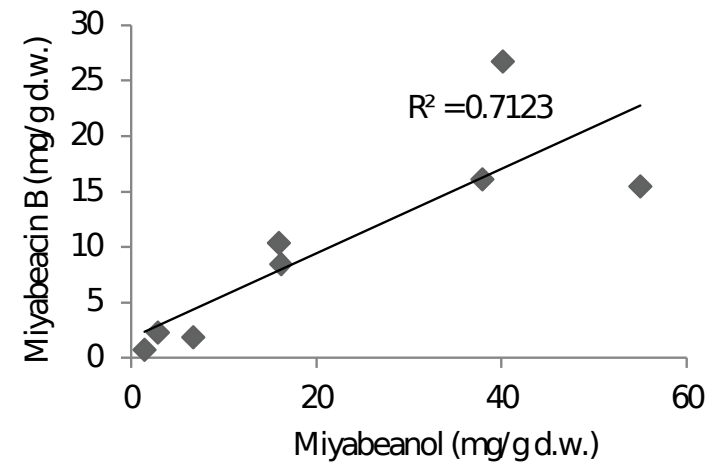


Supplementary Figure 23. HMBC spectra of miyaquinol **8** collected in D₂O:CD₃OD (80:20)

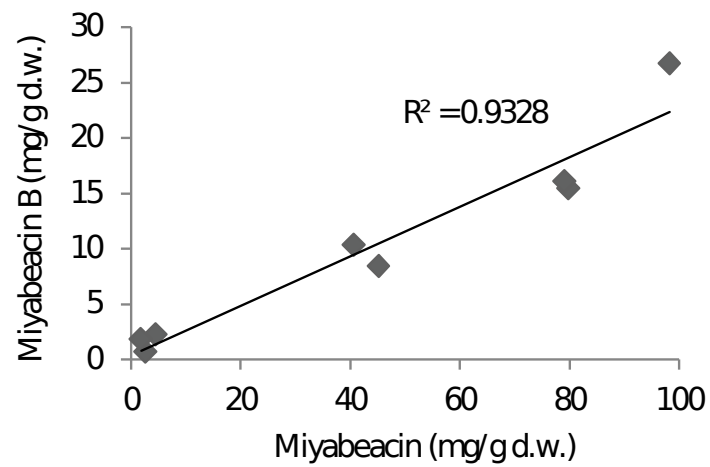
A



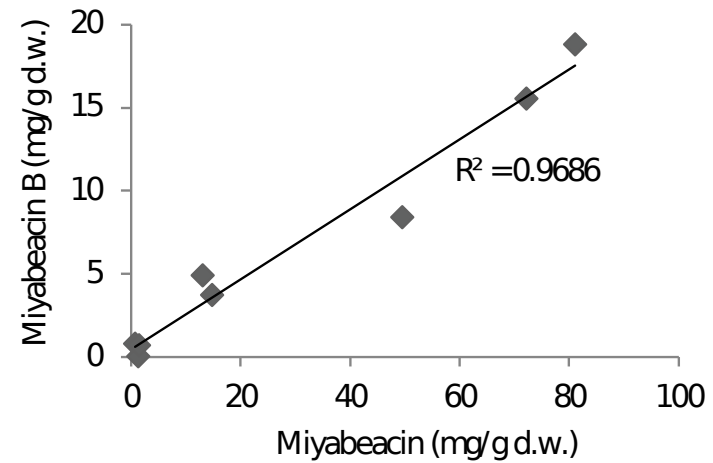
C



B



D

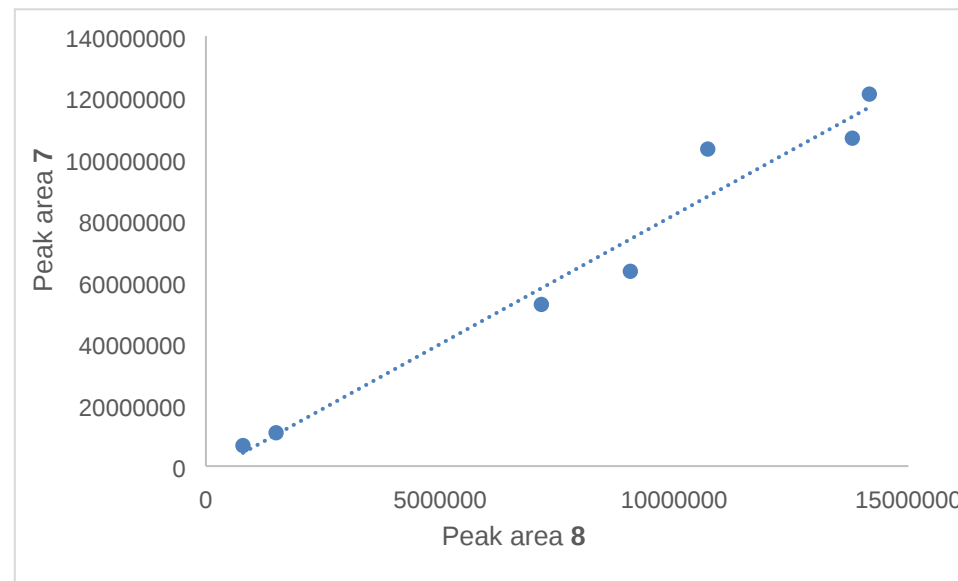


Supplementary Figure 24. Pearson correlations of compound concentrations **3**, **6** and **7**. A-C: Leaf concentrations; D: Stem concentrations.

A:

NWC Code	Species	Variety	uHPLC-MS Peak areas	
			7	8
941	<i>S.miyabeana</i> Seemen	Purpurescens (ex.Tuinzing) (566)	106,736,382	13,802,140
885	<i>S.miyabeana</i> Seemen	Shrubby	103,197,646	10,715,048
837	<i>S.miyabeana</i> Seemen	III	121,088,485	14,165,161
592	<i>S. dasyclados</i> Wimm.	CE78-2 as x dasyclados Siren	63,407,656	9,061,956
576	<i>S. dasyclados</i> Wimm.	(aquatica) Yesipaju Lieto V769	6,688,741	790,941
575	<i>S. dasyclados</i> Wimm.	(aquatica) Jyvaskyla V768	10,851,853	1,498,029
577	<i>S. dasyclados</i> Wimm.	77056 IEA Trial	52,624,720	7,162,216

B:



Supplementary Figure 25. Correlation of uHPLC-MS peak areas for **7** and **8** in *S. miyabeana* and *S. dasyclados* accessions.



T3



T5



T6



T7



T8



T12



T15



T30

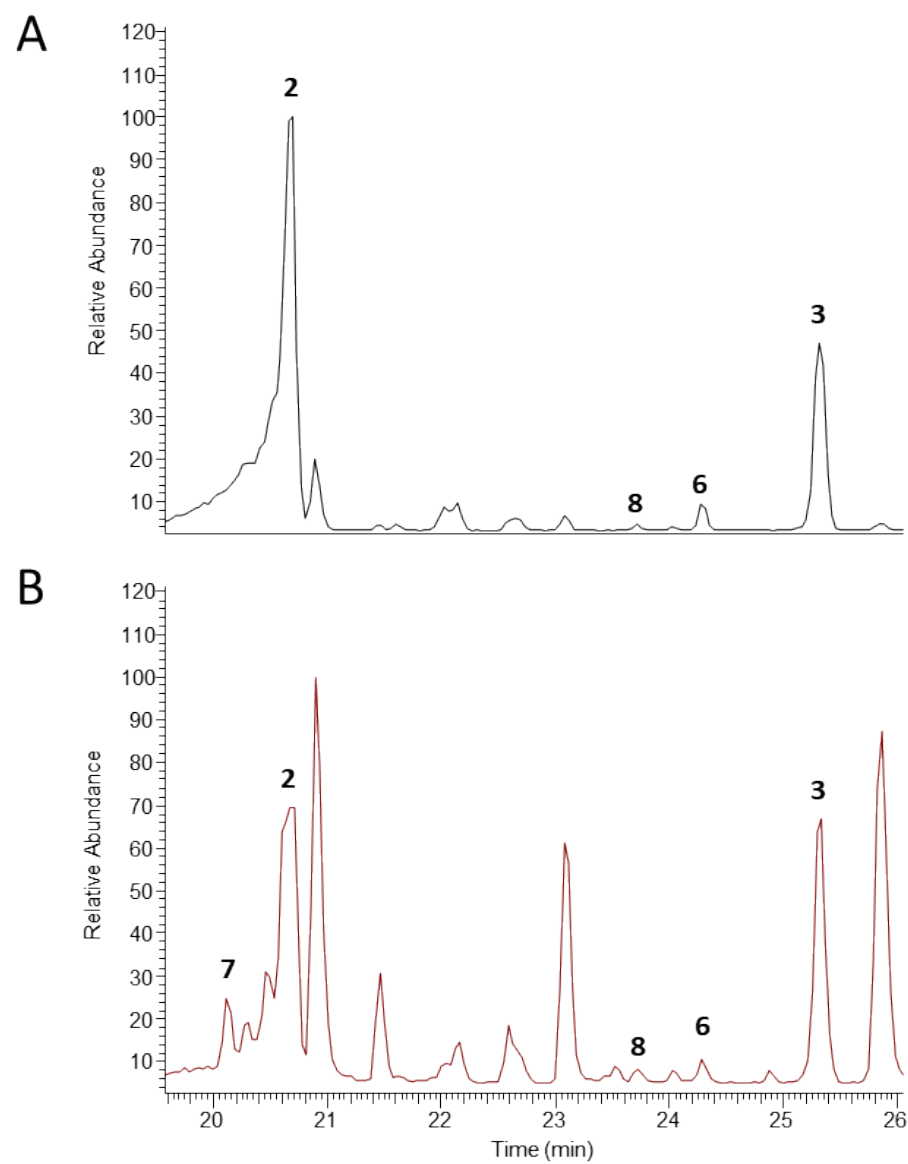


T45

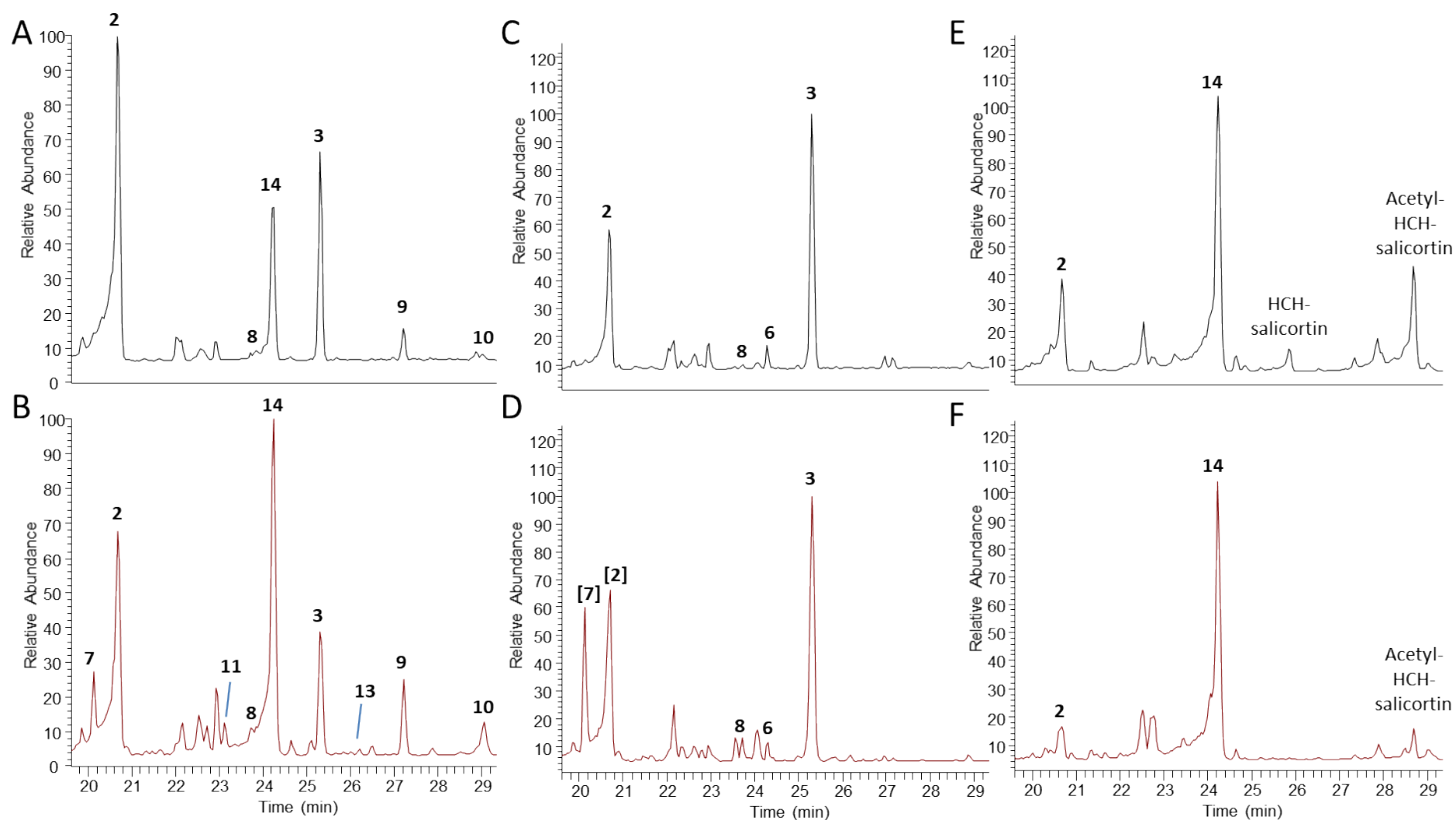


T60

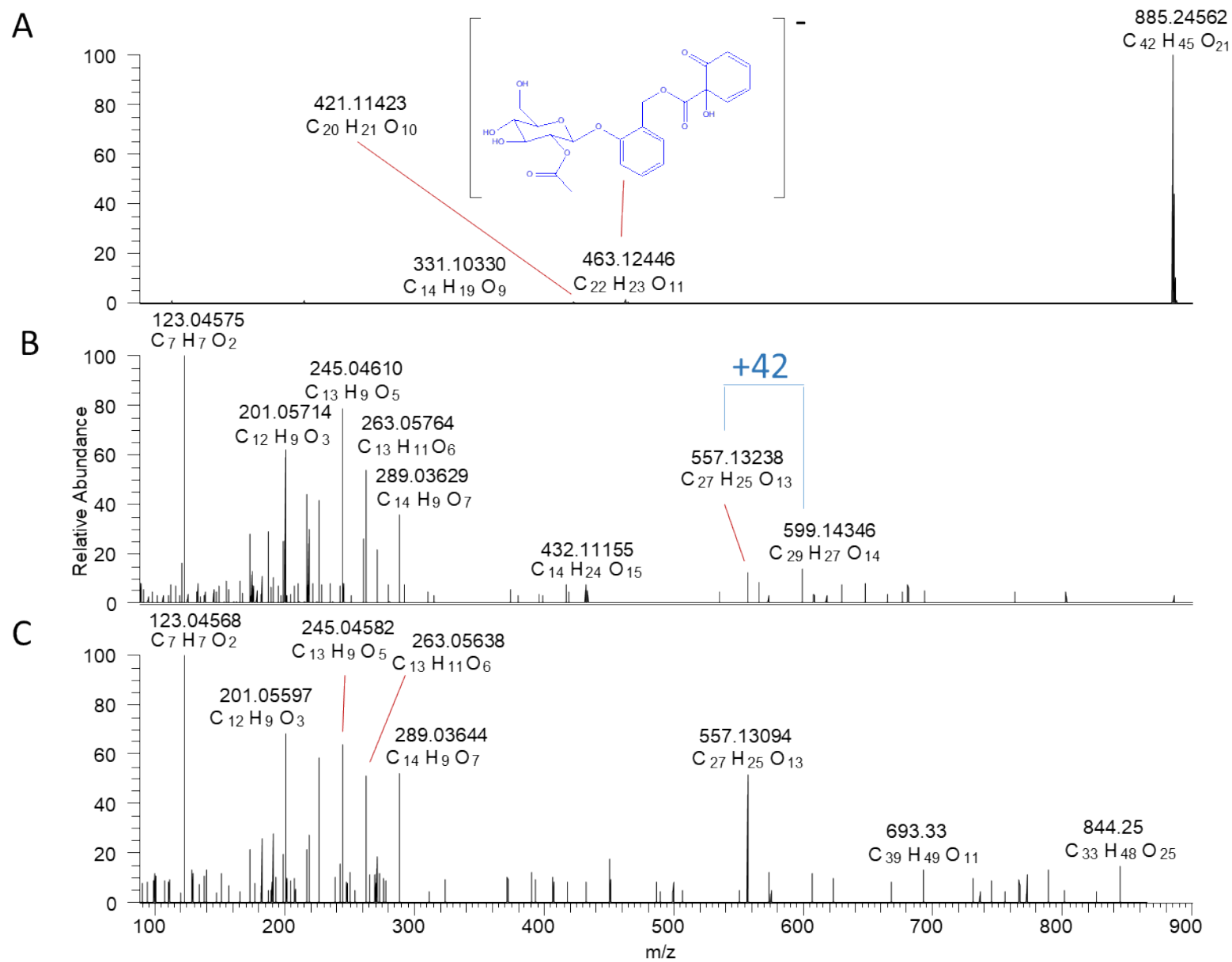
Supplementary Figure 26. Photographs of *S. dasyclados* (NWC577) grown in controlled environment conditions. Photographs show typical sampled plants from 3 days after budburst (T3) to 60 days after bud burst (T60).



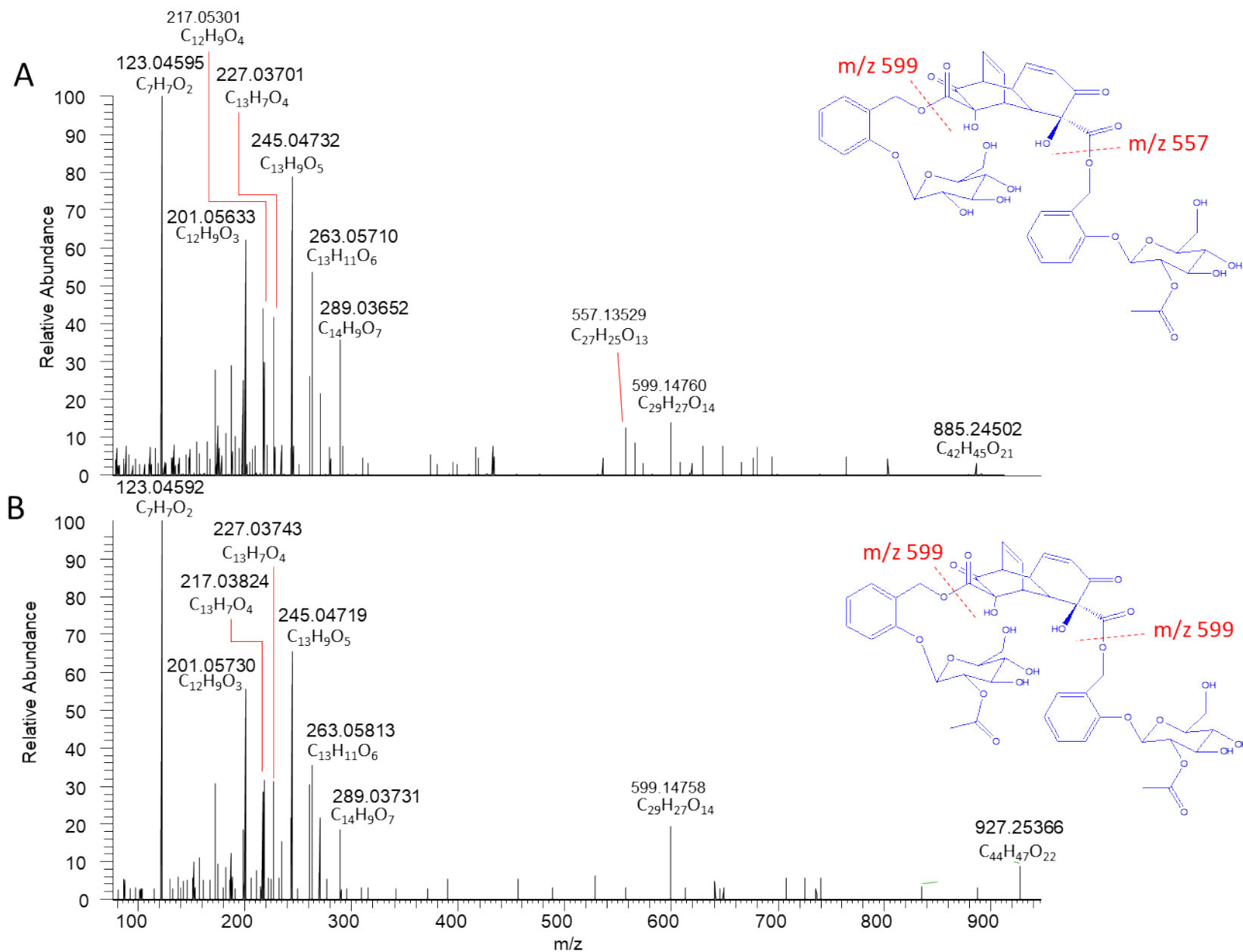
Supplementary Figure 27. Total ion chromatograms (RT 19.6 – 26.0 min) from uHPLC-MS analyses of polar solvent extracts of Terra Nova (NWC1110). A: Juvenile stem; B: Juvenile leaf.



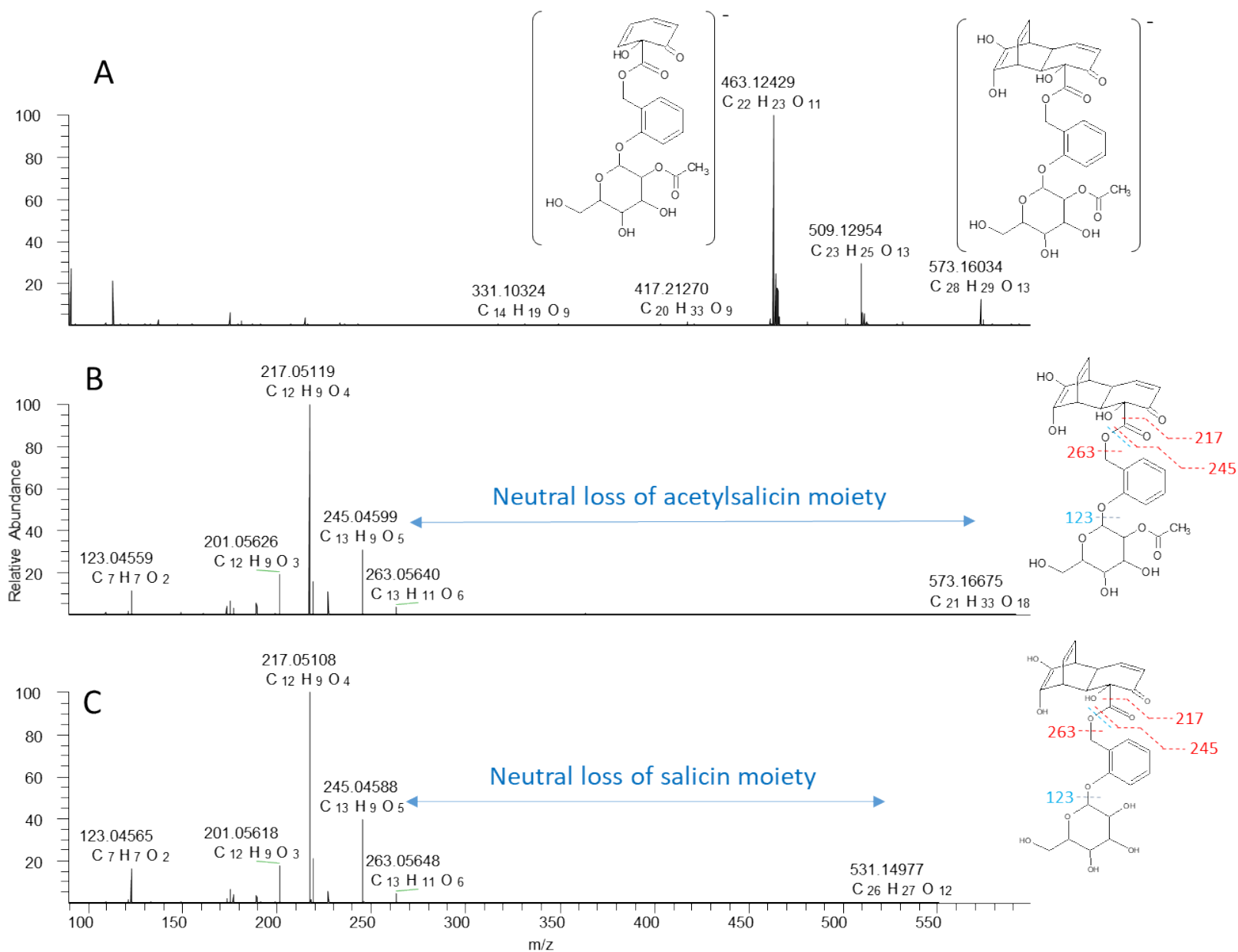
Supplementary Figure 28. Total ion chromatograms (RT 19.6 – 26.0 min) from uHPLC-MS analyses of polar solvent extracts of juvenile willow tissues. A: Endurance stem; B: Endurance leaf; C: *S. dasyclados* (NWC577) stem; D: *S. dasyclados* (NWC 577) leaf; E: *S. rehderiana* (NWC607) stem; F: *S. rehderiana* (NWC607) leaf. Peak labelling reflects labelling in the main text: **2**:salicortin, **3**:miyabeacin, **6**:miyabeacin-B, **7**:miyabeanol, **8**:miyaquinol, **9**:acetylmiyabeacin, **10**:diacetylmiyabeacin, **11**:2'-acetylmiyabinol, **13**:2'-acetylmiyaquinol, **14**: 2'-acetylsalicortin.



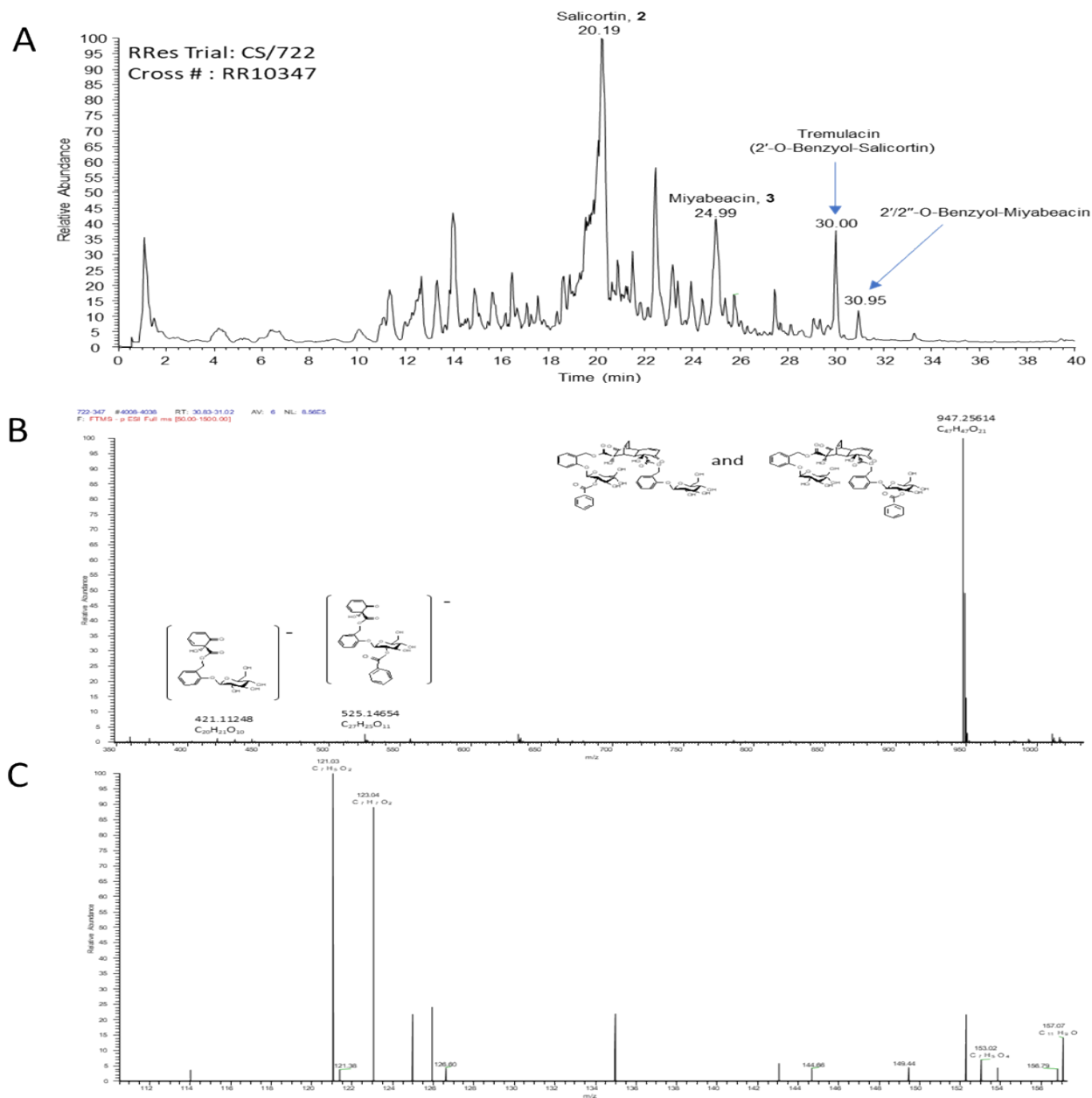
Supplementary Figure 29. Mass spectra of acetyl miyabeacin, **9a/9b**, at m/z 885 with retention time 27.21 min. A: MS spectrum of m/z 885 (27.21 min); B: MS-MS fragmentation of m/z 885; C: MS-MS fragmentation of miyabeacin, **3** for comparison.



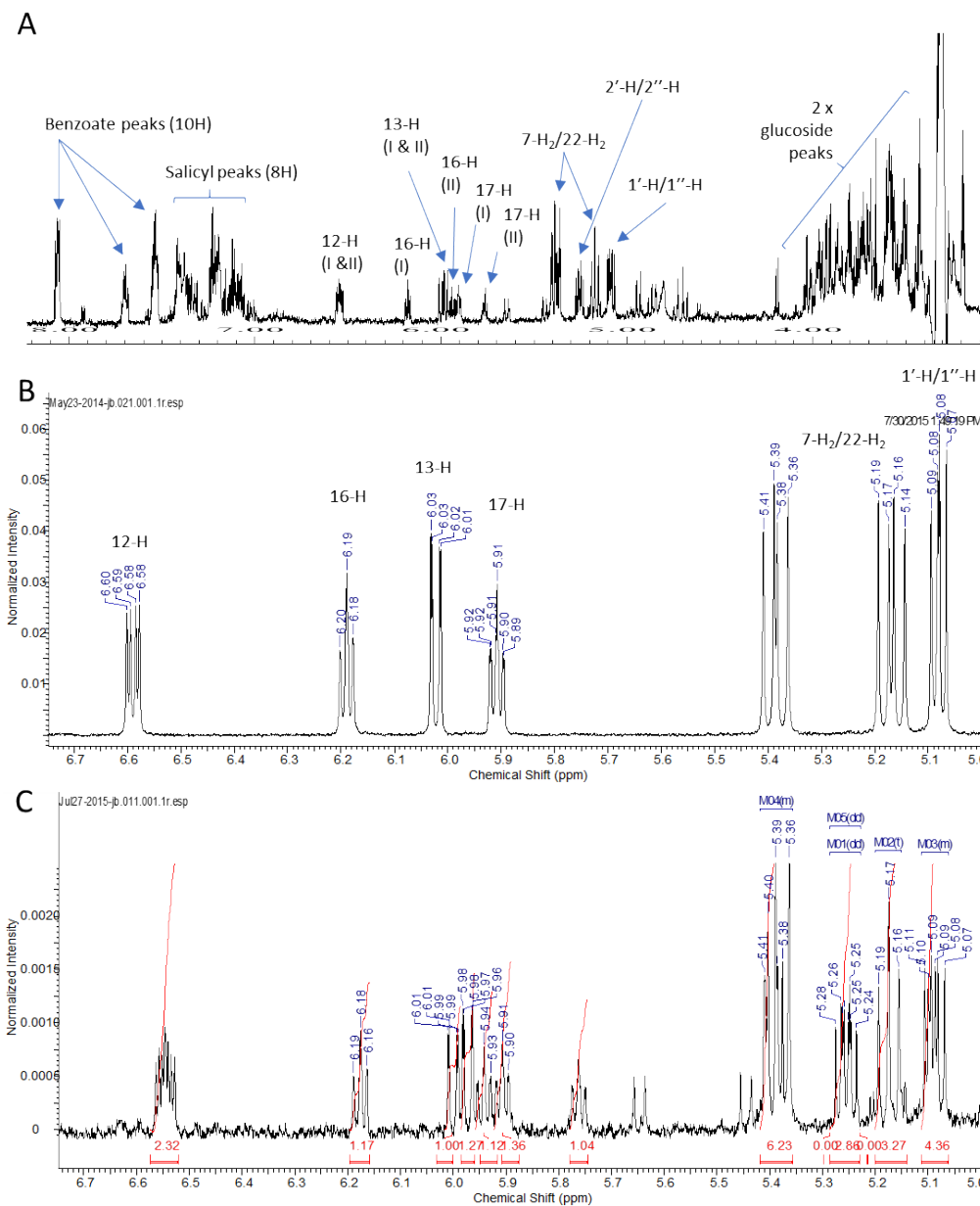
Supplementary Figure 30. MSMS comparison of A: acetyl miyabeacin (m/z 885) **9** and B: diacetyl miyabeacin (m/z 927) **10**. Data is generated from LC-MS (negative mode) of juvenile leaf tissue from Endurance (NWC1116) and extracted using aqueous methanol.



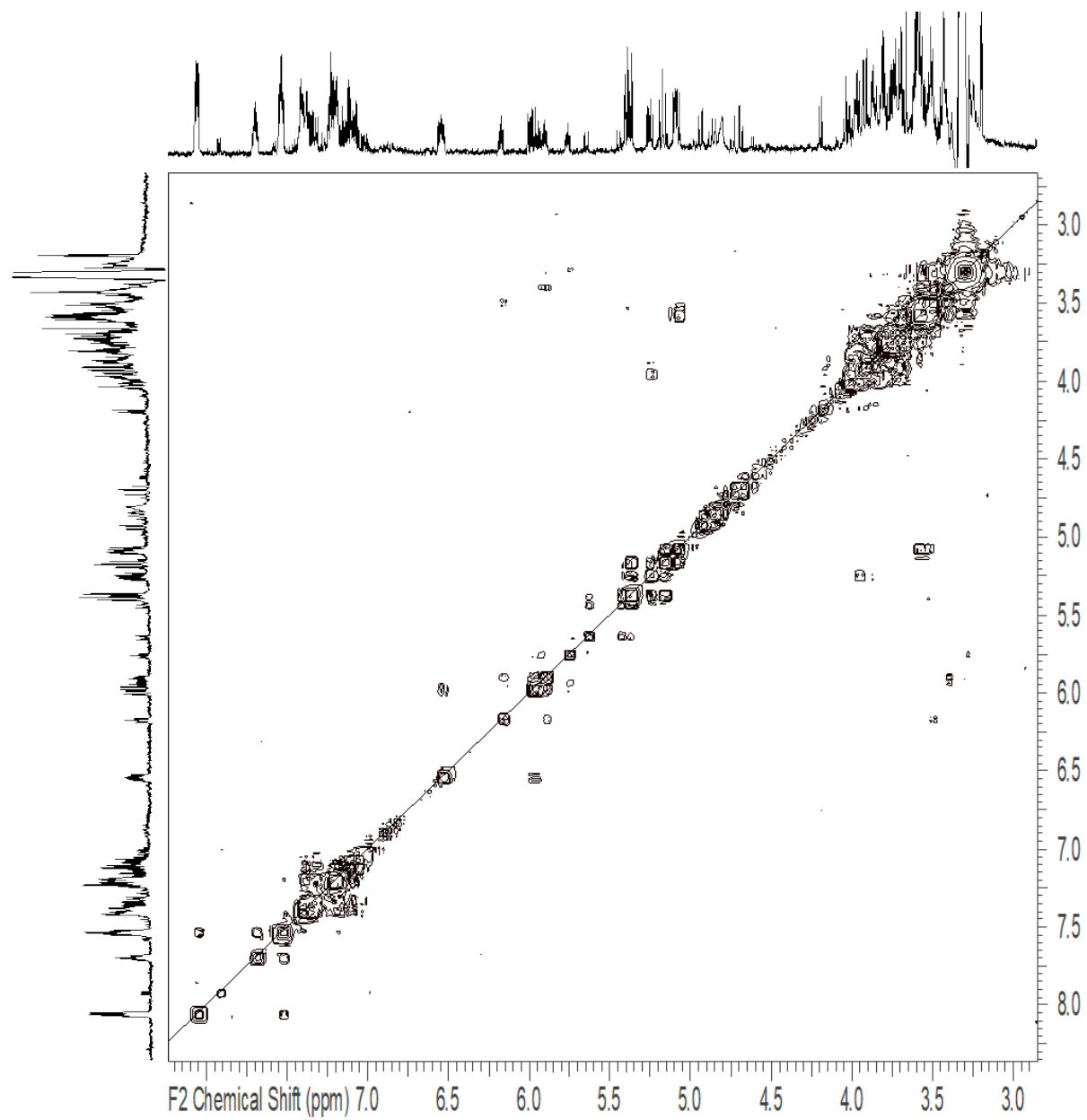
Supplementary Figure 31. MS and MSMS data (negative ion mode) for 2'acetyl miyabeanol **11**. A: MS spectrum of peak at 23.08 min; B: MSMS of m/z 573 $[M-H]^-$; C: MSMS of m/z 531 (miyabeanol **7**, $[M-H]^-$) for comparison. Data is generated from LC-MS (neg mode) of juvenile leaf tissue from Endurance and extracted using aqueous methanol.



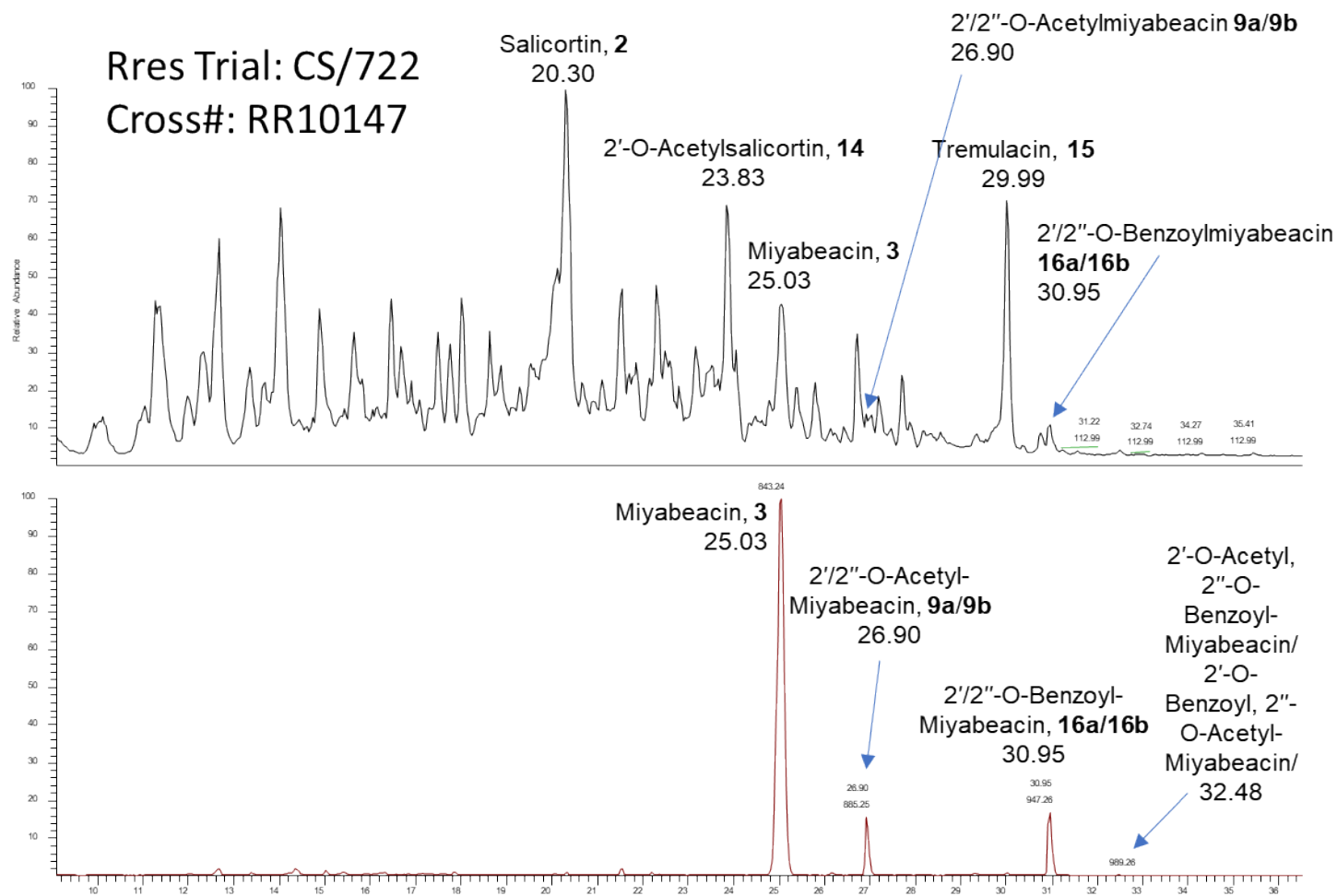
Supplementary Figure 32. LC-MS data (negative ion mode) of RR10347 (RR05326 (Resolution × *S. rossica*) × NWC941 (*S. miyabeana* Purpurescens)). A: Total ion chromatogram indicating benzoylated miyabeacin **16a/16b** (m/z 947); B: Mass spectrum of peak at 30.95 min. C: MSMS of m/z 947.2561.



Supplementary Figure 33. ^1H -NMR data of 2'-Benzoylmiyabeacin/2''-O-Benzoylmiyabeacin **16a/16b** collected at 600 MHz in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (4:1). A: ^1H -NMR spectrum. B: ^1H -NMR spectrum of miyabeacin **3** for comparison (expanded region between 6.7 – 5.0 ppm). C: Expanded region of ^1H -NMR spectrum of 2'-Benzoylmiyabeacin/2''-O-Benzoylmiyabeacin **16a/16b** (6.7 – 5.0 ppm) for comparison with **3**. Integral values are given below peaks in red type.



Supplementary Figure 34. ^1H - ^1H COSY NMR data of 2'-Benzoylmiyabeacin/2''-O-Benzoylmiyabeacin **16a/16b** collected at 600 MHz in $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ (4:1).



Supplementary Figure 35. LC-MS data (negative ion mode) of RR10147 (RR07187 (944 *S. glaucophylloides* × 577 “77056”) × RR07188 (944 *S. glaucophylloides* × 577 “77056”)). A: Total ion chromatogram; B: Extracted ion chromatogram – Mass Range m/z 800 – 1000 with identified compounds indicated