Rare A-Type, Spiro-Type, and Highly Oligomeric Proanthocyanidins from *Pinus massoniana*

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Table S1. ^{1}H (800 MHz) and ^{13}C NMR (100 MHz) data of **1**–**3** in CD_3OD

Unit	Ring	No.	**1**[a]		**2**[a]		**3**[b]		Unit	Ring	No.	**3**[b]	
			δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C				δ_H, mult (*J* in Hz)	δ_C
I	C	2		100.5		99.9		100.0	V	O	2		100.2
		3	3.867, d (4.0)	65.1	3.690, d (3.5)	67.5	4.129, d (3.4)	68.2			3	4.117, d (3.6)	67.6
		4	4.258, d (4.0)	29.7	4.437, d (3.5)	28.9	4.468, d (3.4)	29.4			4	4.227, d (3.6)	29.6
	A	5		155.2		156.9		157.1		M	5		154.1
		6	5.922, d (1.9)	97.3	6.032, d (2.3)	98.0	6.057, d (2.4)	98.2			6		111.0
		7		157.9		157.9		158.0			7		156.7
		8	5.867, d (1.9)	96.1	6.023, d (2.3)	96.2	6.111, d (2.4)	96.6			8	6.076, s	97.5
		9		154.8		154.1		154.1			9		152.1
		10		105.3		104.4		104.2			10		104.0
	B	1'		131.7		131.9		132.5		N	1'		132.0
		2'	7.274, d (2.1)	116.1	7.011, d (2.1)	115.2	7.179, d (2.1)	115.5			2'	7.139, d (2.2)	115.5
		3'		145.2		145.0		145.5			3'		145.6
		4'		146.6		146.2		146.6			4'		146.7
		5'	6.764, d (8.2)	115.1	6.713, d (8.2)	115.5	6.837, d (8.2)	115.5			5'	6.815, d (8.3)	115.5
		6'	6.904, dd (8.2, 2.1)	120.3	6.607, dd (8.2, 2.1)	119.4	7.060, dd (8.2, 2.1)	119.7			6'	7.041, dd (8.3, 2.2)	119.8
II	F	2		101.1	5.301, brs	78.7	5.268, s	78.7	VI	R	2	4.781, d (7.7)	85.1
		3	4.216, d (4.3)	68.7	3.944, brs	72.3	4.043, brs	72.3			3	4.192, m	68.2
		4	4.866, d (4.3)	30.4	4.472, brs	38.1	4.739, brs	38.6			4	2.607, dd (−16.2, 8.4)	29.0
	D	5		150.8		157.1		156.4		P	4	2.996, dd (−16.2, 5.1)	29.0
		6		108.5	5.960, s	95.8	6.126, s	96.1			5		156.2
		7		153.1		151.9		152.2			6	6.136, s	96.6
		8	6.254, s	97.4		106.3		106.8			7		152.1
		9		154.6		152.7		152.3			8		106.4
		10		105.9		105.0		105.6			9		151.2
	E	1'		131.5		131.5		131.4		Q	10		103.1
		2'	6.982, d (2.2)	115.4	7.210, d (2.1)	116.6	7.157, d (2.1)	116.2			1'		130.0
		3'		145.5		145.8		145.8			2'	6.997, d (2.1)	115.9
		4'		146.6		146.3		146.2			3'		146.2
		5'	6.745, d (8.1)	115.5	6.799, d (8.2)	115.6	6.796, d (8.2)	115.9			4'		146.9
		6'	6.916, dd (8.1, 2.2)	119.7	7.075, dd (8.2, 2.1)	121.4	6.962, dd (8.2, 2.1)	120.7			5'	6.878, d (8.1)	116.8
III	I	2	4.964, s	79.7	5.810, s	88.2		100.1			6'	6.924, dd (8.1, 2.1)	121.1
		3	4.279, m	66.9		61.4	4.167, d (3.6)	68.0					

		4	2.025, dd (–17.0, 3.5)	23.6		177.0	4.383, d (3.6)	29.8
			2.596, brd (–17.0)	23.6				
	G	5		156.4		154.2		154.6
		6	5.952, s	95.7	5.902, s	96.6		110.5
		7		151.7		158.2		156.8
		8		105.6		106.0	6.076, s	97.4
		9		152.4		162.2		152.1
		10		101.1		106.1		104.4
	H	1'		132.4		128.5		132.3
		2'	6.437, d (2.2)	113.1	6.411, d (2.2)	113.3	7.163, d (2.2)	115.5
		3'		146.1		145.7		145.6
		4'		145.5		145.8		146.7
		5'	6.432, d (8.1)	115.5	6.629, d (8.3)	115.9	6.827, d (8.3)	115.5
		6'	6.038, dd (8.1, 2.2)	117.6	6.532, dd (8.3, 2.2)	118.3	7.055, dd (8.3, 2.2)	119.7
IV	L	2			4.830, d (6.3)	82.5	5.415, s	79.9
		3			3.973, m	68.7	3.866, brs	72.6
		4			2.676, dd (–16.4, 7.2)	27.6	4.787, brs	38.4
	J				2.857, dd (–16.4, 5.0)	27.6		
		5				158.1		156.7
		6			6.163, s	91.3	6.109, s	96.6
		7				154.5		151.9
		8				104.0		106.2
		9				152.8		152.6
	K	10				104.8		106.0
		1'				131.64		130.8
		2'			6.795, d (2.1)	114.0	7.204, d (2.1)	117.1
		3'				145.9		145.5
		4'				146.0		146.5
		5'			6.744, d (8.1)	115.8	6.778, d (8.1)	115.9
		6'			6.677, dd (8.1, 2.1)	119.7	6.935, dd (8.1, 2.1)	121.8

Data were acquired at [a]255 K and [b]278 K.

Figure S1. (A) Selected COSY (bold bonds), HMBC, and (B) NOESY correlations (arrows) in **1**

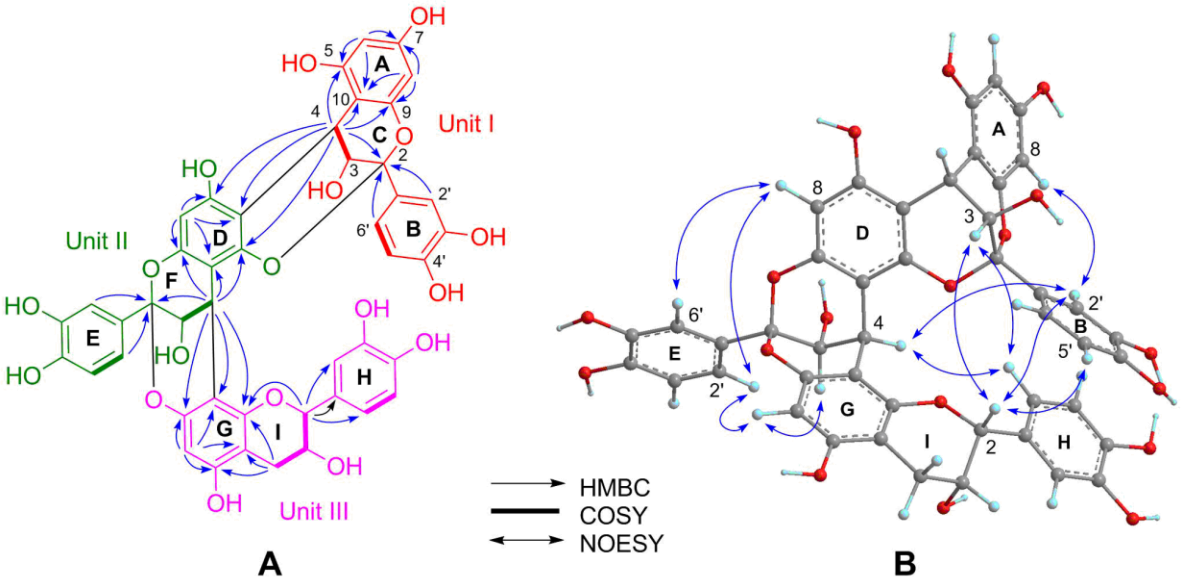
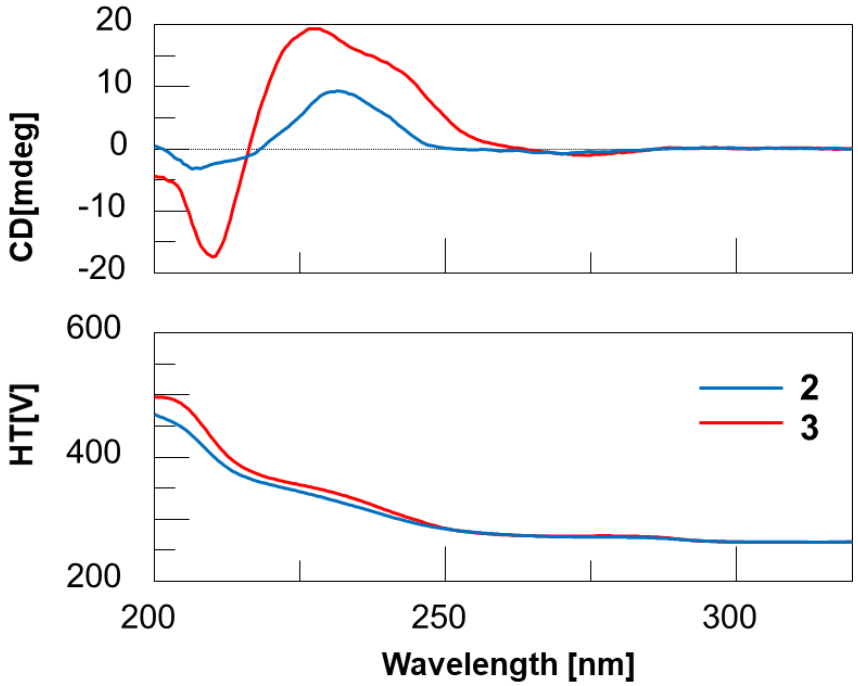


Figure S2. The ECD spectra of **2** and **3**



■ EXPERIMENTAL SECTION

General Experimental Procedures

HRESIMS measurements were carried out using a Bruker (Billerica, MA, USA) Impact II quadrupole time-of-flight (qTOF-) spectrometer. The ECD spectra were acquired on a JASCO-715 spectrometer. Samples for ECD experiments were prepared in MeOH at ≤ 0.1 mg/mL, and a 0.2 cm path length quartz cuvette was used. All ^{1}H 1D/2D NMR spectra were acquired at 255 K or 278 K on an 800 MHz Bruker (Billerica, MA, USA) Avance spectrometer equipped with a 5 mm triple resonance inverse TCI RT probe. The ^{13}C NMR experiments of all compounds were performed on a JEOL (JEOL Resonance, Peabody, MA, USA) ECZ 400 MHz spectrometer. Silica gel (230−400 mesh) was used for column chromatography. C_{18} reversed-phase (RP-18) silica gel (20–45 μm, MACHEREY-NAGEL, Bethlehem, PA, USA) and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. Pre-coated silica gel GF_{254} plates (MACHEREY-NAGEL, Bethlehem, PA, USA) were used for TLC monitoring. Semi-preparative HPLC was performed on a Shimadzu HPLC (Kyoto, Japan) connected to a PDA detector (Shimadzu, model SPD-20A) and equipped with a YMC-Pack ODS-AQ (250 × 10 mm, *S*-5μm, 120 Å; YMC America, Allentown, USA) or Chiralpak IA (250 × 10.0 mm, *S*-5 μm; Chiral Technologies, West Chester, PA, USA) column. All solvents were of analytical grade from Fisher Scientific (Hanover Park, IL, USA) or Sigma-Aldrich (St. Louis, MO, USA).

Plant Material

Extract powder of the inner bark of *Pinus massoniana* was purchased from Xi'an Chukang Biotechnology in China in 2012 (No. PB120212).

Extraction and Isolation of PACs

12 g of enriched tri- and tetrameric proanthocyanidins, separated into 6.5 g of fraction A and 5.6 g of fraction B, were prepared from 200 g pine bark extract by centrifugal partition chromatography (CPC) as described previously.[1] Both fractions A and B were chromatographed on a Sephadex LH-20 column (ethanol), affording six (A1–A6) and seven subfractions (B1–B7), respectively. Fraction B5 (800 mg) was separated over a RP-18 silica gel column (MeOH/H_2O, 20–80%), and the two major subfractions B5b and B5c were then purified via semi-preparative HPLC (18% ACN in 0.1% formic acid H_2O, 2.5 mL/min) to afford **1** (0.7 mg) and **2** (0.8 mg).

Compound **3** (20 mg) was purified from fraction B7 by semi-preparative HPLC (20% ACN in 0.1% formic acid H_2O, 2.5 mL/min) after enrichment via a RP-18 silica gel column.

Phloroglucinolysis

Phloroglucinolysis was performed as described previously[2] to corroborate the absolute configuration of **3**. The identity of the reaction products was confirmed by chiral phase HPLC and MS analysis (Figure S24).

Compounds properties[3]

Pinutwindoublin (**1**): *ent*-epicatechin-(2α→O→5,4α→6)-*ent*-epicatechin-(2α→O→7,4α→8)-epicatechin: light brown, amorphous solid; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (+21.6), 232 (–20.8), 287 (–0.8) nm; ^{1}H NMR (Methanol-d_4, 800 MHz, 255 K): δ 2.025 (dd, 1H, *J* = 17.0, 3.5 Hz), 2.596 (brd, 1H, *J* = 17.0 Hz), 3.867 (d, 1H, *J* = 4.0 Hz), 4.216 (d, 1H, *J* = 4.3 Hz), 4.258 (d, 1H, *J* = 4.0 Hz), 4.279 (m, 1H), 4.866 (d, 1H, *J* = 4.3 Hz), 4.964 (s, 1H), 5.867 (d, 1H, *J* = 1.9 Hz), 5.922 (d, 1H, *J* = 1.9 Hz), 5.952 (s, 1H), 6.038 (d, 1H, *J* = 8.3 Hz), 6.254 (s, 1H), 6.432 (d, 1H, *J* = 8.1 Hz), 6.437 (d, 1H, *J* = 2.2 Hz), 6.745 (d, 1H, *J* = 8.1 Hz), 6.764 (d, 1H, *J* = 8.2 Hz), 6.904 (dd, 1H, *J* = 8.3, 2.1 Hz), 6.916 (dd, 1H, *J* = 8.1, 2.2 Hz), 6.982 (d, 1H, *J* = 2.1 Hz), 7.274 (d, 1H, *J* = 2.1 Hz). ^{13}C{^{1}H} NMR (Methanol-d_4, 100 MHz, 255 K): δ 23.6, 29.7, 30.4, 65.1, 66.9, 68.7, 79.7, 95.7, 96.1, 97.3, 97.4, 100.5, 101.1, 101.1, 105.3, 105.6, 105.9, 108.5, 113.1, 115.1, 115.4, 115.5, 115.5, 116.1, 117.6, 119.7, 120.3, 131.5, 131.7, 132.4, 145.2, 145.5, 145.5, 146.1, 146.6, 146.6, 150.8, 151.7, 152.4, 153.1, 154.6, 154.8, 155.2, 156.4, 157.9; HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{45}H_{35}O_{18}$ 863.1818; Found 863.1823.

Pinuspirotetrin (**2**): Light brown, amorphous solid; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (–3.2), 232 (+9.4), 270 (–0.8); ^{1}H NMR (Methanol-d_4, 800 MHz, 255 K): δ 2.676 (dd, 1H, *J* = 16.4, 7.2 Hz), 2.857 (dd, 1H, *J* = 16.4, 5.0 Hz), 3.690 (d, 1H, *J* = 3.5 Hz), 3.944 (brs 1H), 3.973 (m, 1H), 4.437 (d, 1H, *J* = 3.5 Hz), 4.472 (brs, 1H), 4.830 (d, 1H, *J* = 6.3 Hz), 5.301 (brs, 1H), 5.810 (s, 1H), 5.902 (s, 1H), 5.960 (s, 1H), 6.023 (d, 1H, *J* = 2.3 Hz), 6.032 (d, 1H, *J* = 2.5 Hz), 6.163 (s, 1H), 6.629 (d, 1H, *J* = 8.3 Hz), 6.411 (d, 1H, *J* = 2.2 Hz), 6.532 (dd, 1H, *J* = 8.1, 2.3 Hz), 6.607 (dd, 1H, *J* = 8.4, 2.1 Hz), 6.677 (dd, 1H, *J* = 8.2, 2.1 Hz), 6.713 (d, 1H, *J* = 8.2 Hz), 6.744 (d, 1H, *J* = 8.1 Hz), 6.794 (d, 1H, *J* = 2.1 Hz), 6.799 (d, 1H, *J* = 8.2 Hz), 7.011 (d, 1H, *J* = 2.1 Hz), 7.075 (dd, 1H, *J* = 8.2, 2.1 Hz), 7.210 (d, 1H, *J* = 2.1 Hz). ^{13}C{^{1}H} NMR (Methanol-d_4, 100 MHz, 255 K): δ 27.6, 28.9,

38.1, 61.4, 67.5, 68.7, 72.3, 78.7, 82.5, 88.2, 91.3, 95.8, 96.2, 96.6, 98.0, 99.9, 104.0, 104.4, 104.8, 105.0, 106.0, 106.1, 106.3, 113.3, 114.0, 115.2, 115.5, 115.6, 115.8, 115.9, 116.6, 118.3, 119.4, 119.7, 121.4, 128.5, 131.5, 131.6, 131.9, 145.0, 145.7, 145.8, 145.8, 145.9, 146.0, 146.2, 146.3, 151.9, 152.7, 152.8, 154.1, 154.2, 154.5, 156.9, 157.1, 157.9, 158.1, 158.2, 162.2, 177.0; HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{60}H_{45}O_{24}$, 1149.2295; Found 1149.2291.

Pinumassohexin (**3**)*:* epicatechin-(2*β*→O→7,4*β*→8)-epicatechin-(4*β*→6)-epicatechin-(2*β*→O→7,4*β*→8)-epicatechin-(2*β*→O→7,4*β*→8)-epicatechin-(4*β*→6)-catechin: light brown, amorphous solid; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (–17.3), 228 (+19.3), 274 (–1.0); ^{1}H NMR (Methanol-d_4, 800 MHz, 278 K): δ 2.607 (dd, 1H, *J* = 16.2, 8.4 Hz), 2.996 (dd, 1H, *J* = 16.2, 5.1 Hz), 3.886 (brs, 1H), 4.043 (brs, 1H), 4.117 (d, 1H, *J* = 3.6 Hz), 4.129 (d, 1H, *J* = 3.4 Hz), 4.167 (d, 1H, *J* = 3.5 Hz), 4.192 (m, 1H), 4.227 (d, 1H, *J* = 3.6 Hz), 4.383 (d, 1H, *J* = 3.6 Hz), 4.468 (d, 1H, *J* = 3.4 Hz), 4.739 (s, 1H), 4.781 (d, 1H *J* = 7.7), 4.787 (s, 1H), 5.268 (s, 1H), 5.415 (s, 1H), 6.057 (d, 1H, *J* = 2.4 Hz), 6.076 (s, 2H), 6.109 (s, 1H), 6.111 (d, 1H, *J* = 2.4 Hz), 6.126 (s, 1H), 6.136 (s, 1H), 6.778 (d, 1H, *J* = 8.1), 6.796 (d, 1H, *J* = 8.2), 6.815 (d, 1H, *J* = 8.3), 6.827 (d, 1H, *J* = 8.3), 6.837 (d, 1H, *J* = 8.2), 6.878 (d, 1H, *J* = 8.1), 6.924 (dd, 1H, *J* = 8.2, 2.1), 6.935 (dd, 1H, *J* = 8.2, 2.1), 6.962 (dd, 1H, *J* = 8.2, 2.1), 6.997 (d, 1H, *J* = 2.1), 7.040 (dd, 1H, *J* = 8.1, 2.3), 7.055 (dd, 1H, *J* = 8.1, 2.3), 7.060 (dd, 1H, *J* = 8.2, 2.1), 7.157 (d, 1H, *J* = 2.1), 7.163 (d, 1H, *J* = 2.2), 7.139 (d, 1H, *J* = 2.2), 7.179 (d, 1H, *J* = 2.1), 7.204 (d, 1H, *J* = 2.1). ^{13}C{^{1}H} NMR (Methanol-d_4, 100 MHz, 278 K): δ 29.0, 29.4, 29.6, 29.8, 38.4, 38.6, 67.6, 68.0, 68.2, 68.2, 72.3, 72.6, 78.7, 79.9, 85.1, 96.1, 96.6, 96.6, 96.6, 97.4, 97.5, 98.2, 100.0, 100.1, 100.2, 103.1, 104.0, 104.2, 104.4, 105.6, 106.0, 106.2, 106.4, 106.8, 110.5, 111.0, 115.5, 115.5, 115.5, 115.5, 115.5, 115.5, 115.9, 115.9, 115.9, 116.2, 116.8, 117.1, 119.7, 119.7, 119.8, 120.7, 121.1, 121.8, 130.0, 130.8, 131.4, 132.0, 132.3, 132.5, 145.5, 145.5, 145.6, 145.6, 145.8, 146.2, 146.2, 146.5, 146.6, 146.7, 146.7, 146.9, 151.2, 151.9, 152.1, 152.1, 152.1, 152.2, 152.3, 152.6, 154.1, 154.1, 154.6, 156.2, 156.4, 156.7, 156.7, 156.8, 157.1, 158.0; HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{90}H_{69}O_{36}$, 1725.3563; Found 1725.3589.

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1. Phansalkar, R. S.; Nam, J. W.; Chen, S. N.; McAlpine, J. B.; Leme, A. A.; Aydin, B.; Bedran-Russo, A. K.; Pauli, G. F. Centrifugal partition chromatography enables selective enrichment of trimeric and tetrameric proanthocyanidins for biomaterial development. *J. Chromatogr. A* **2018**, *1535*, 55–62.

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3. Zhou, B. Y Alania, M Reis, J McAlpine, A Bedran-Russo, G Pauli, SN Chen. Rare A-Type, New Spiro-Type, and Highly Oligomeric Proanthocyanidins from *Pinus massoniana*. *Chemrxiv*, **2020**, 12168168.

Figure S3. ^{1}H NMR spectrum of **1** in CD_3OD (800 MHz, 255 K)

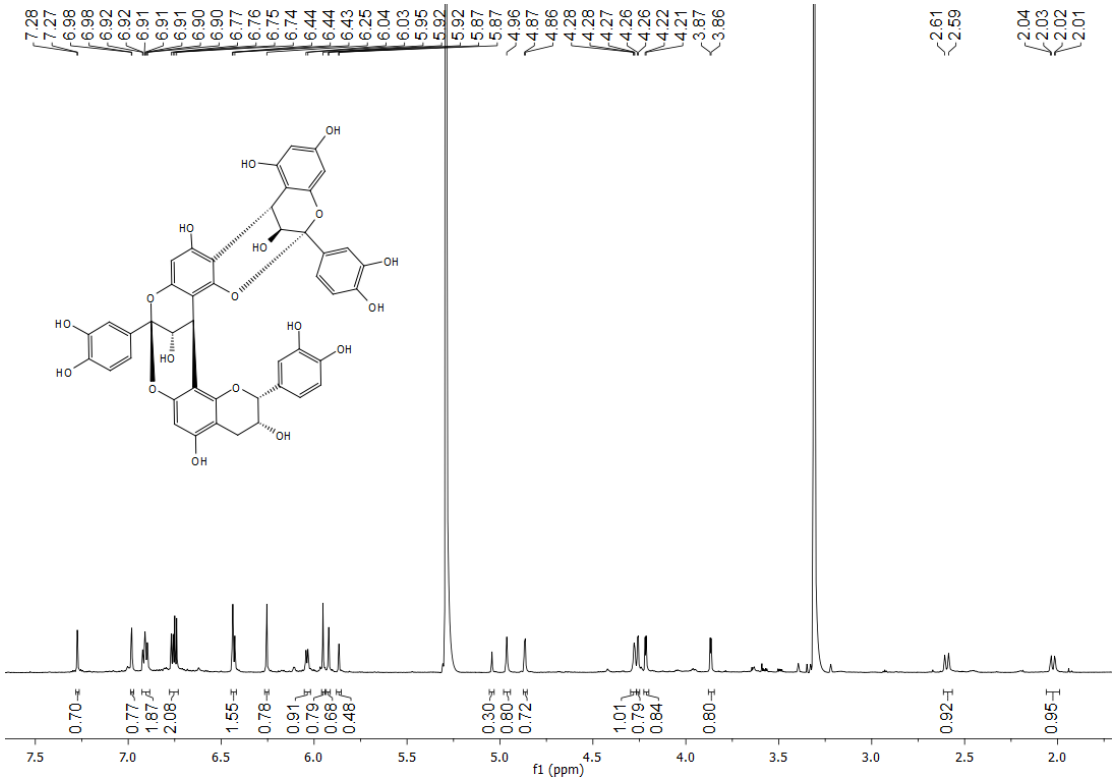


Figure S4. ^{13}C NMR spectrum of **1** in CD_3OD (100 MHz, 255 K)

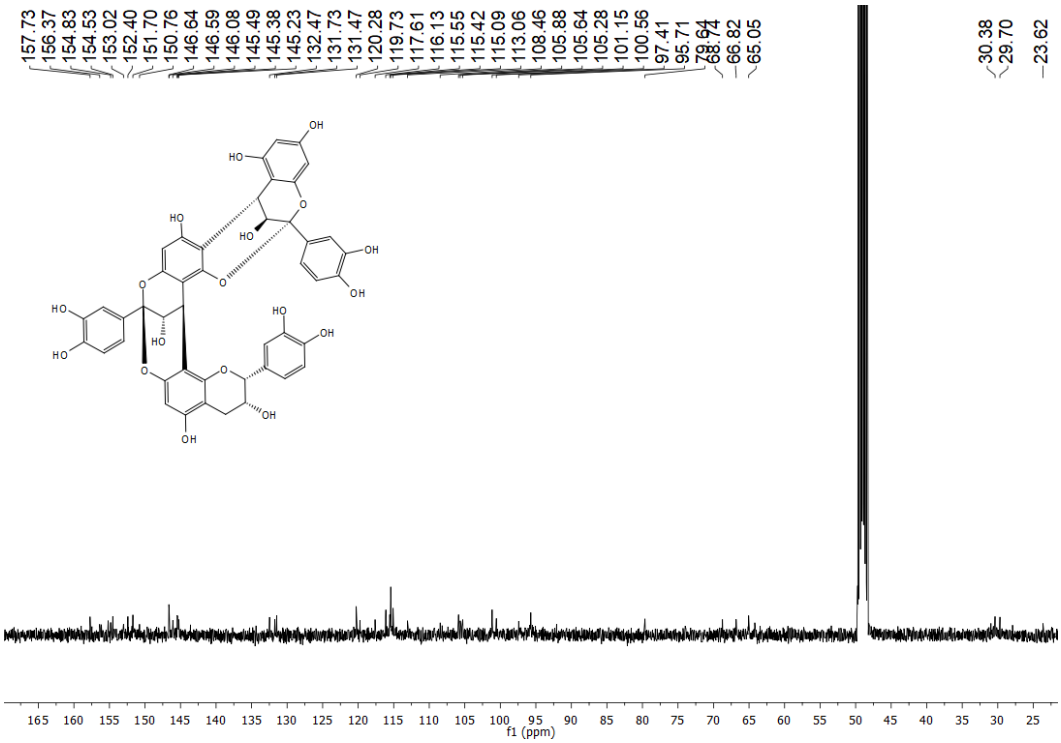


Figure S5. HSQC spectrum of **1** in CD_3OD (255 K)

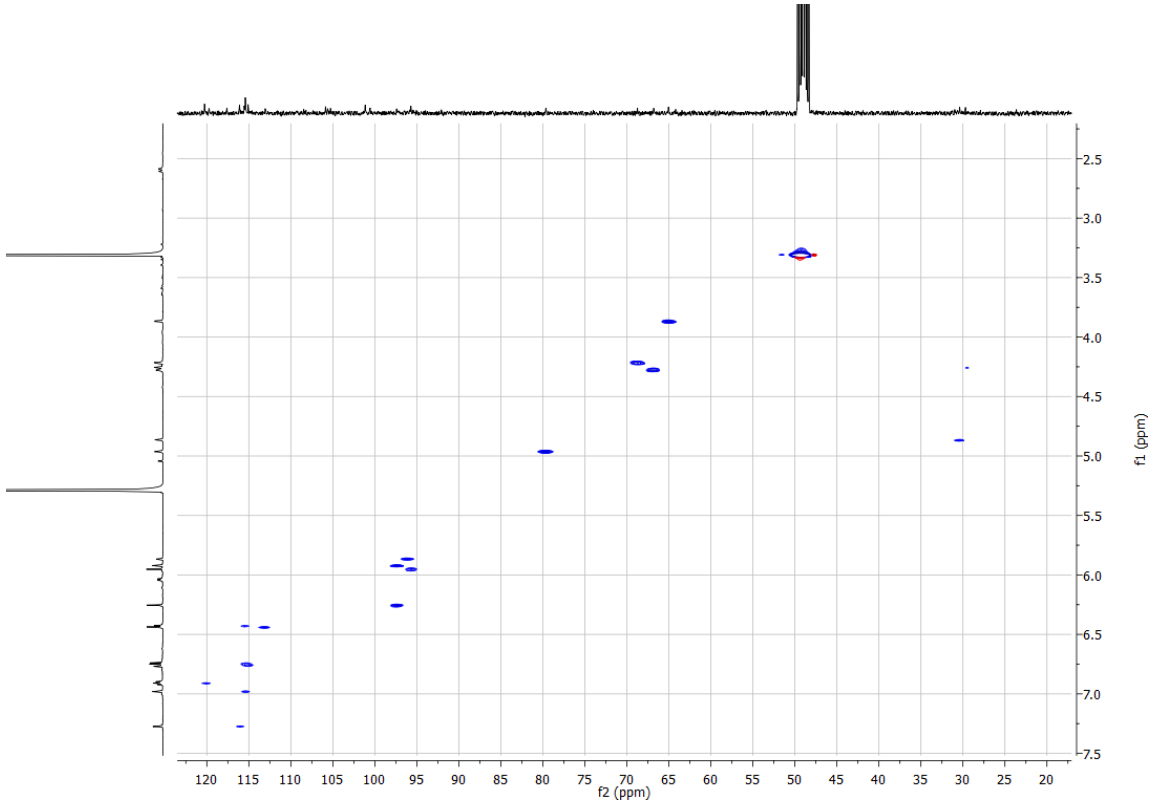


Figure S6. HMBC spectrum of **1** in CD_3OD (255 K)

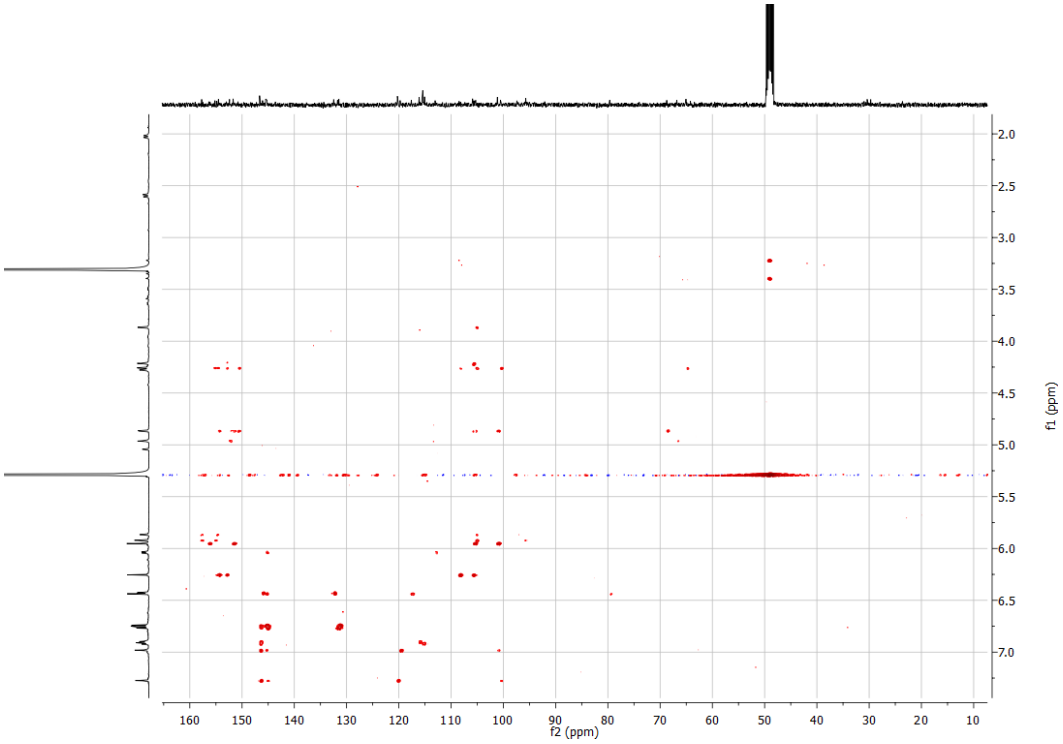


Figure S7. NOESY spectrum of **1** in CD_3OD (255 K)

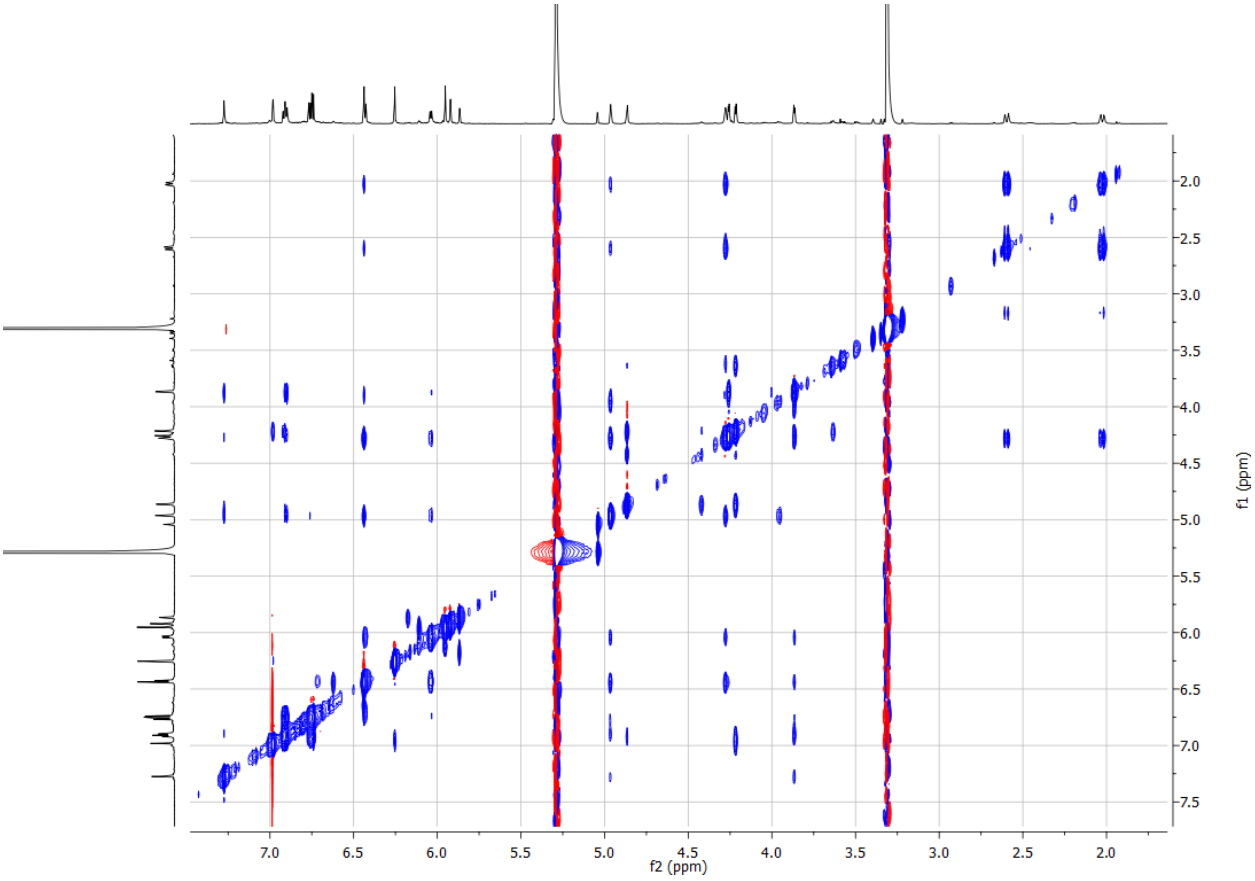


Figure S7-1. Partially expanded NOESY spectra of **1** with important NOESY correlations

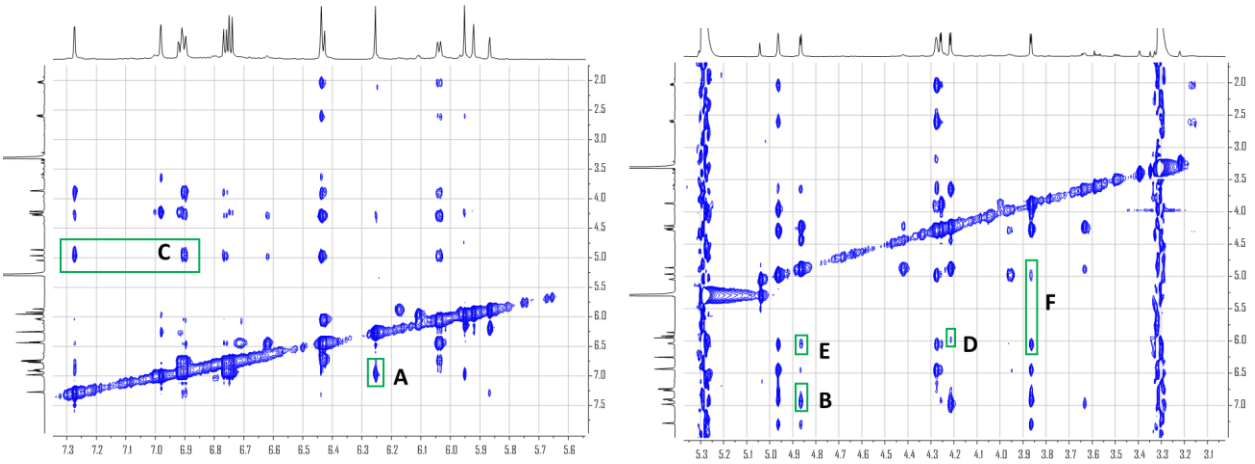


Figure S7-2. Expanded NOESY spectra of 1 with important NOESY correlations

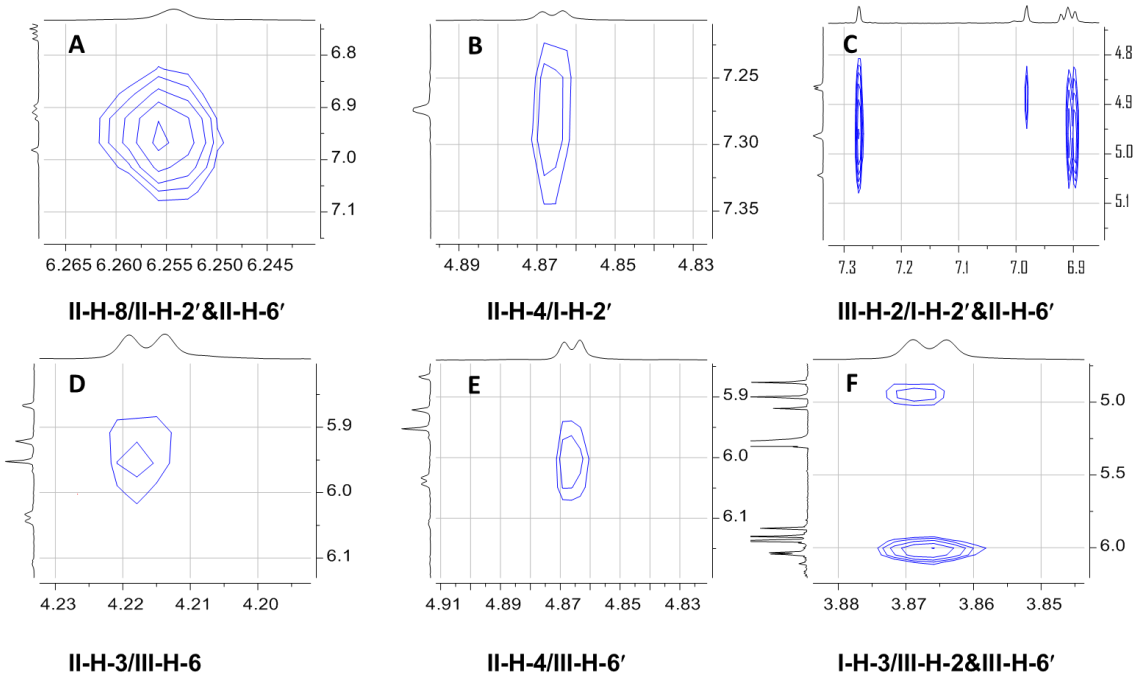
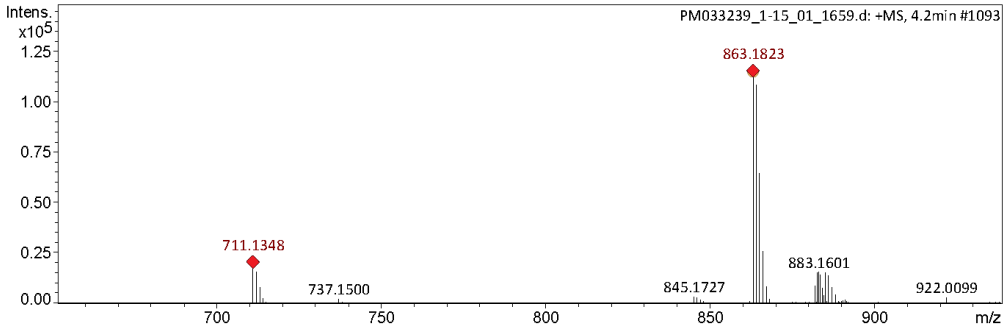


Figure S8. (+)-HRESIMS spectrum of **1**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
863.1823	1	C45H35O18	863.1818	-0.6	295.2	1	100.00	29.0	even	ok

Figure S9. ^{1}H NMR spectrum of **2** in CD_3OD (800 MHz, 255 K)

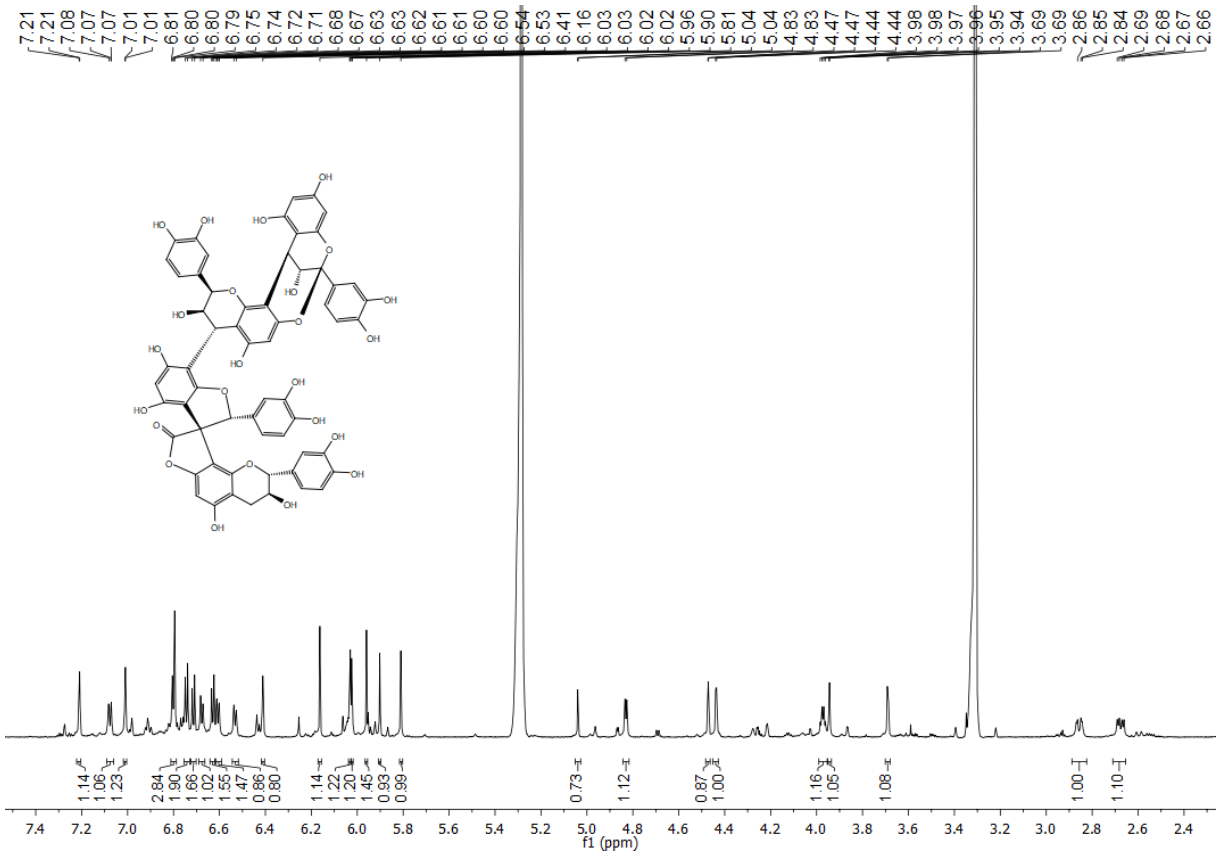


Figure S10. Comparison of the ^{1}H NMR spectra of **2** in CD_3OD at 278 K and 255 K (800 MHz)

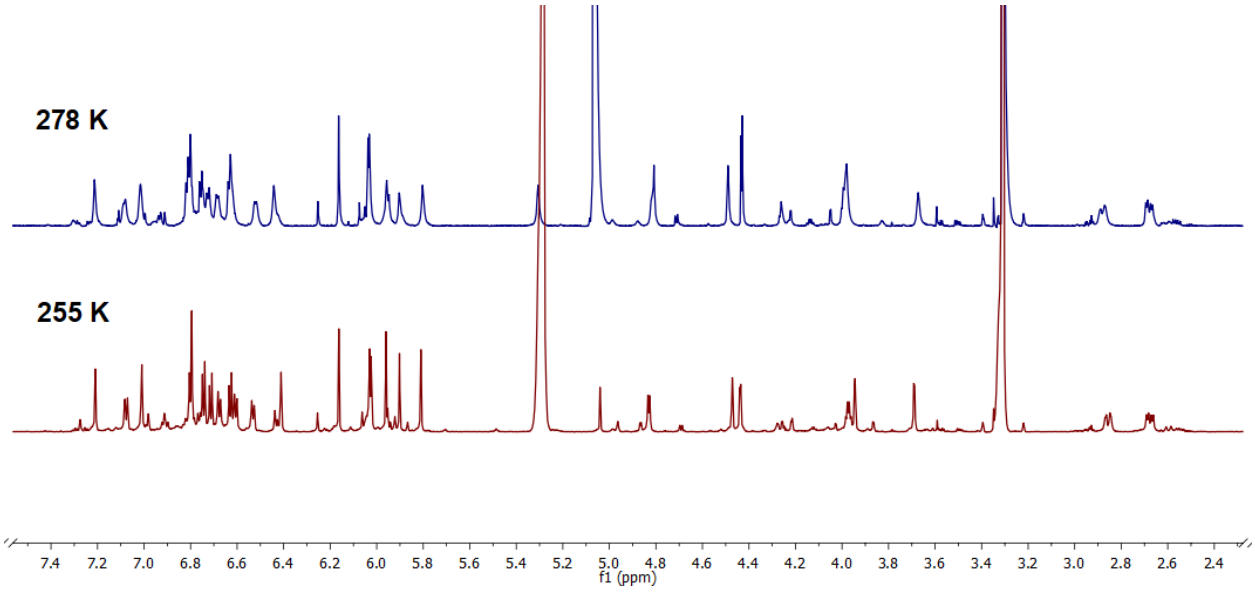


Figure S11. ^{13}C NMR spectrum of **2** in CD_3OD (100 MHz, 255 K)

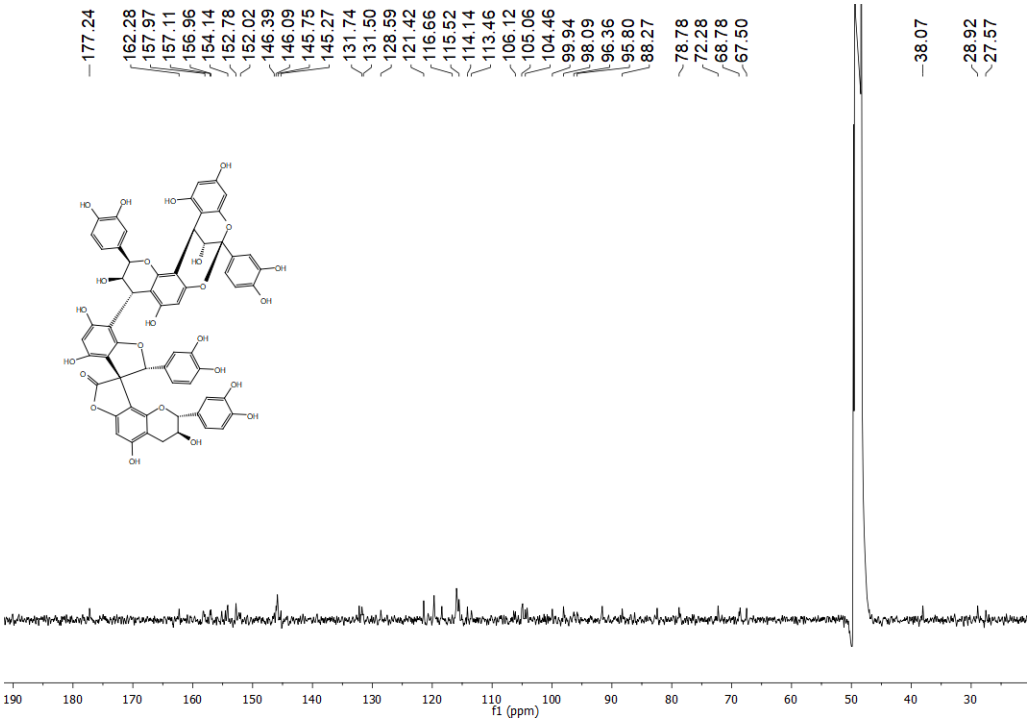


Figure S12. $^{1}H–^{1}H$COSY spectrum of **2** in CD_3OD (255 K)

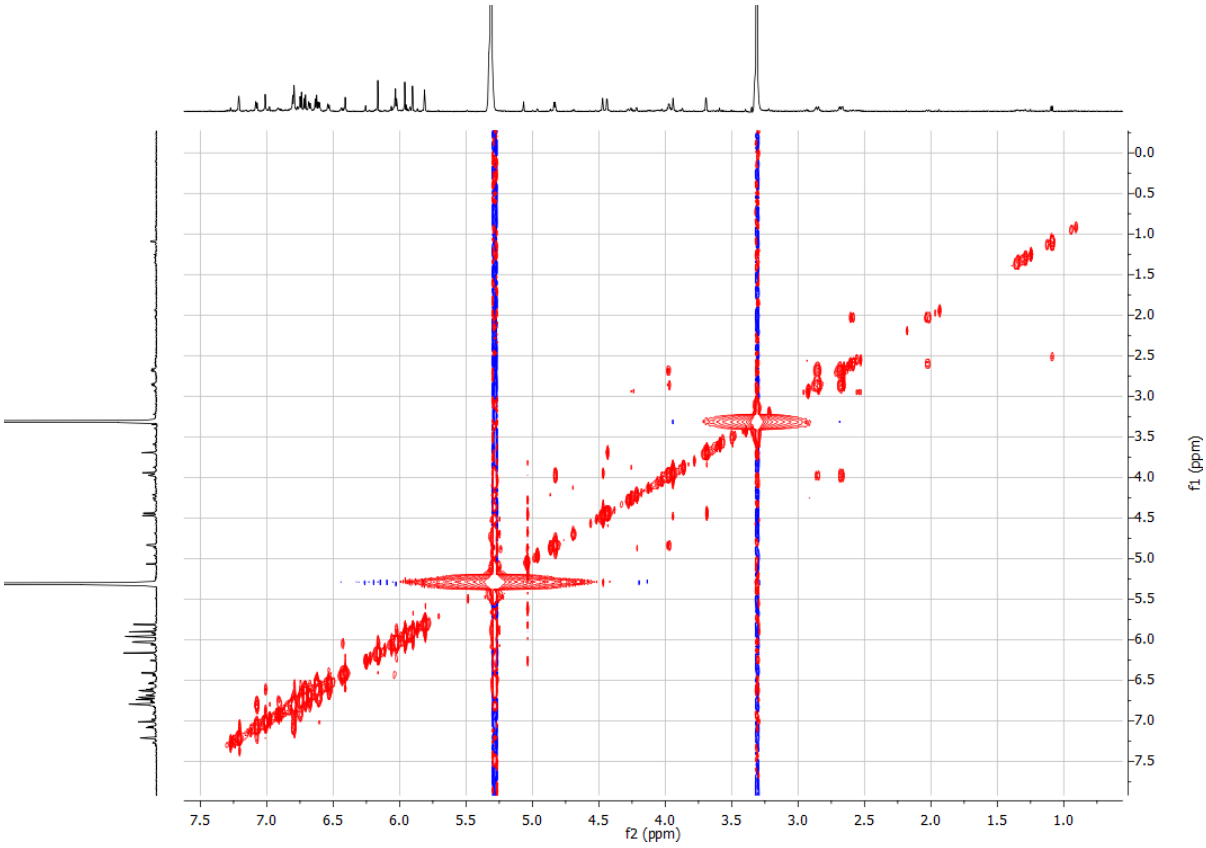


Figure S13. HSQC spectrum of **2** in CD_3OD (255 K)

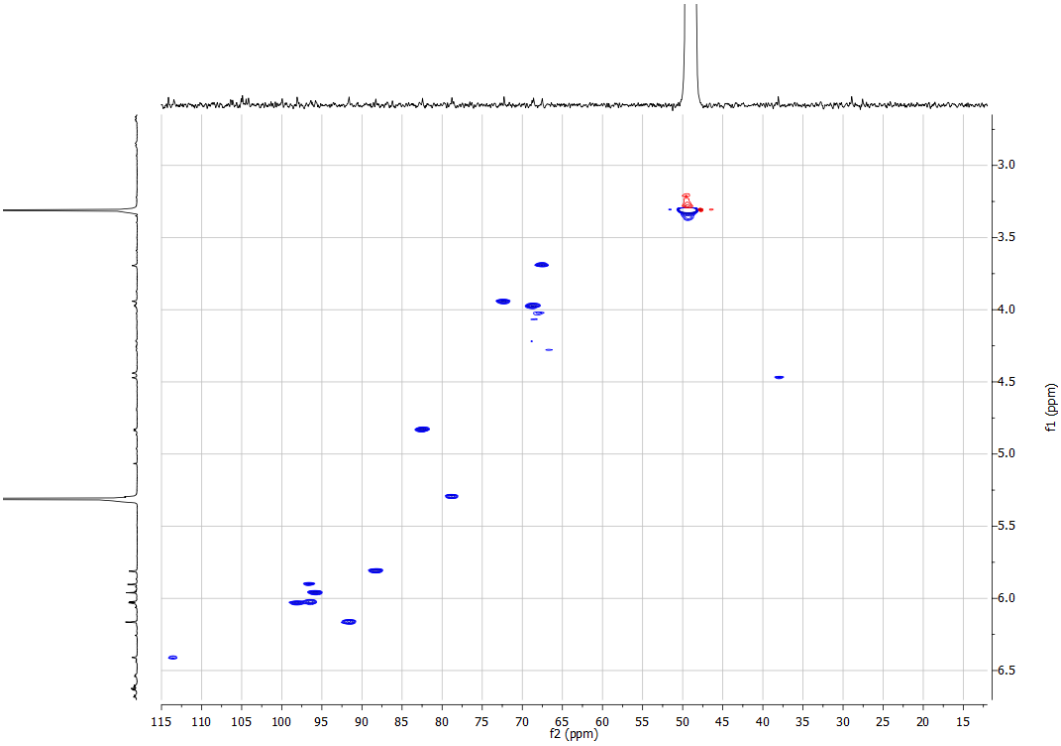


Figure S14. HMBC spectrum of **2** in CD_3OD (255 K)

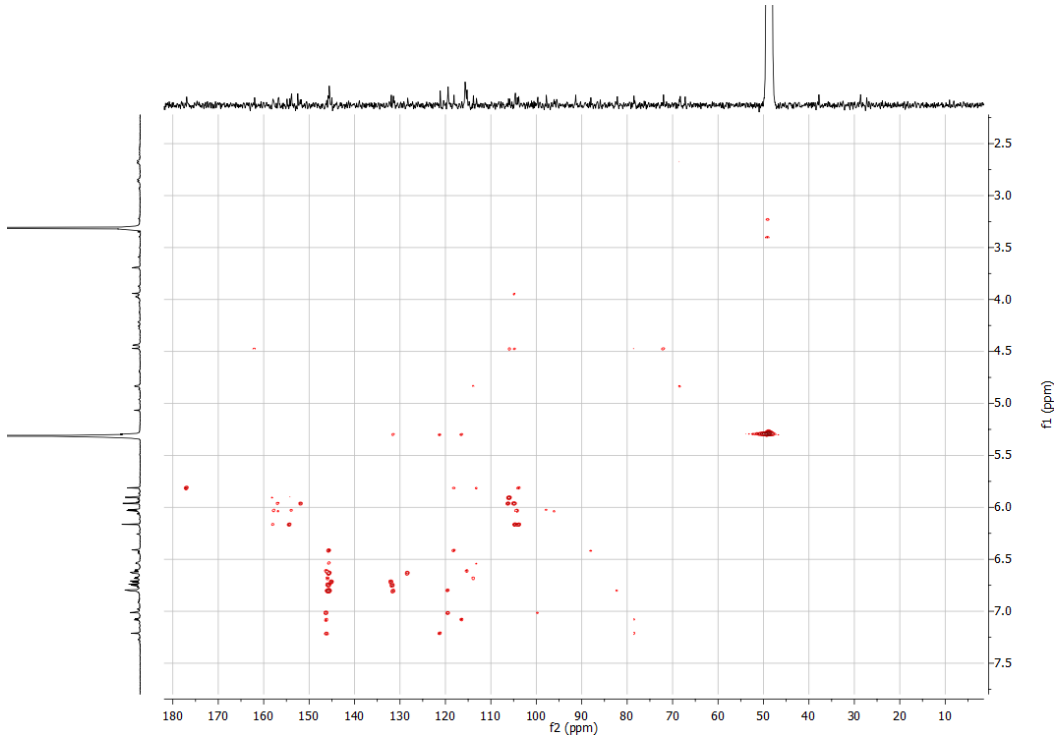


Figure S15. NOESY spectrum of **2** in CD_3OD (255 K)

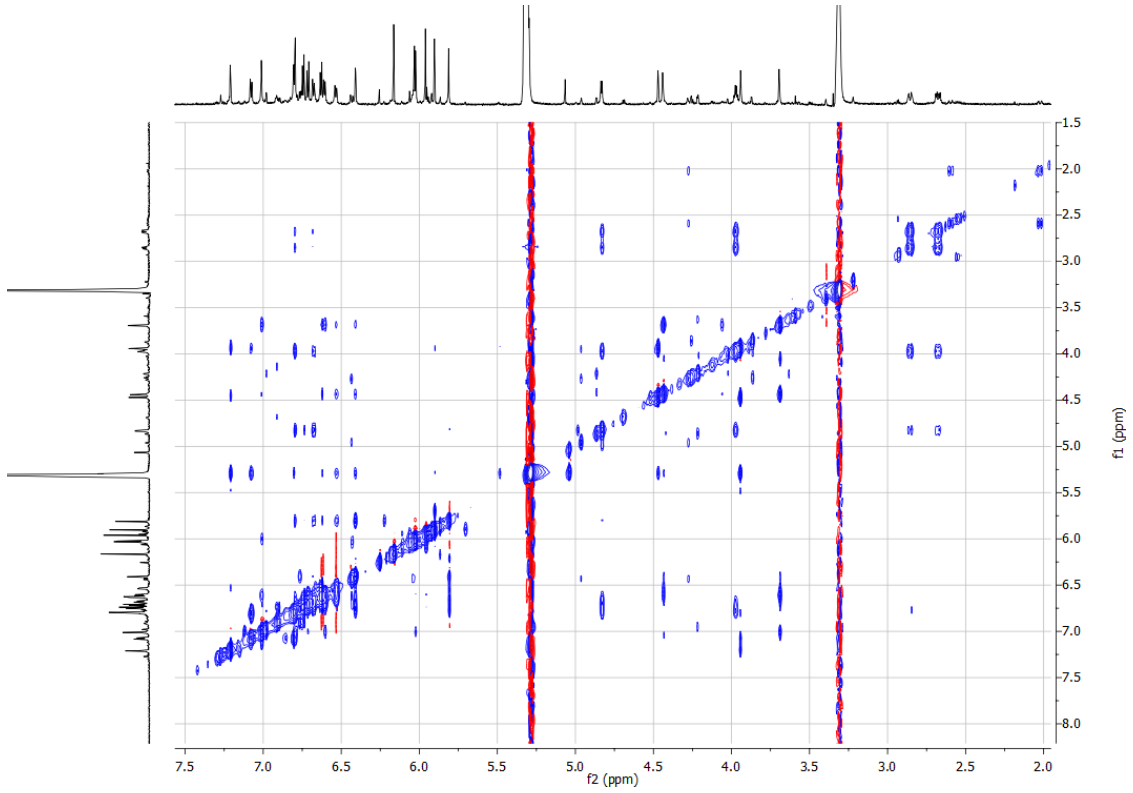


Figure S15-1. Partially expanded NOESY spectra of **2** with important NOESY correlations

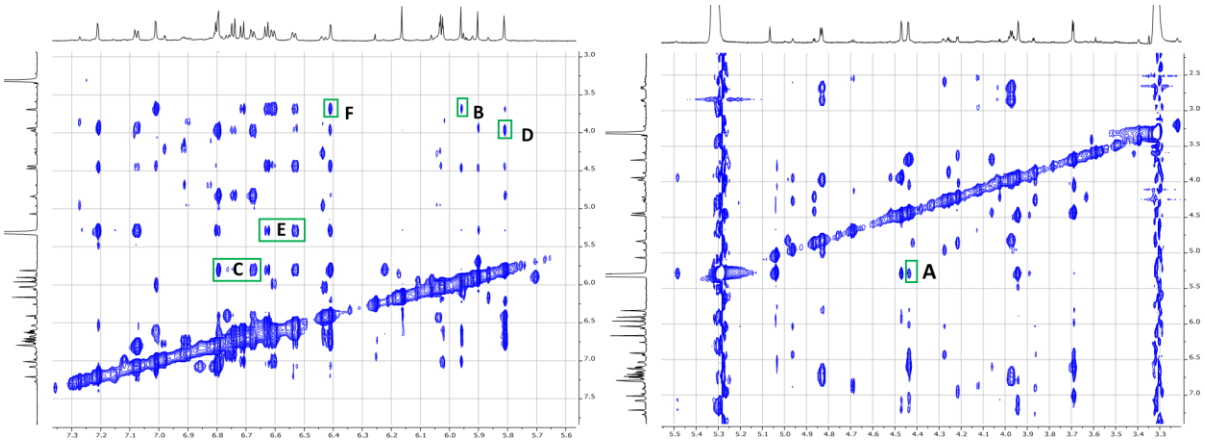


Figure S15-2. Expanded NOESY spectra of **2** with important NOESY correlations

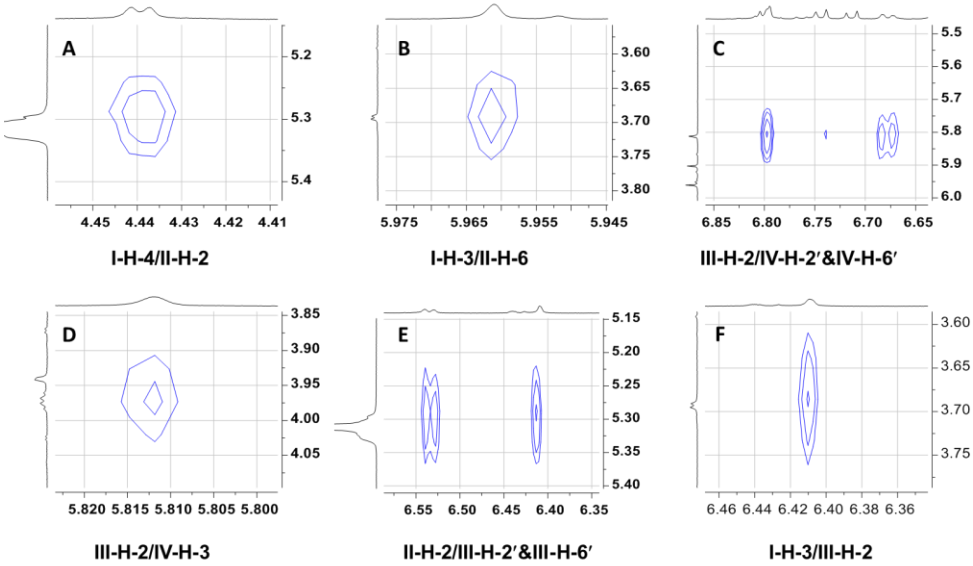
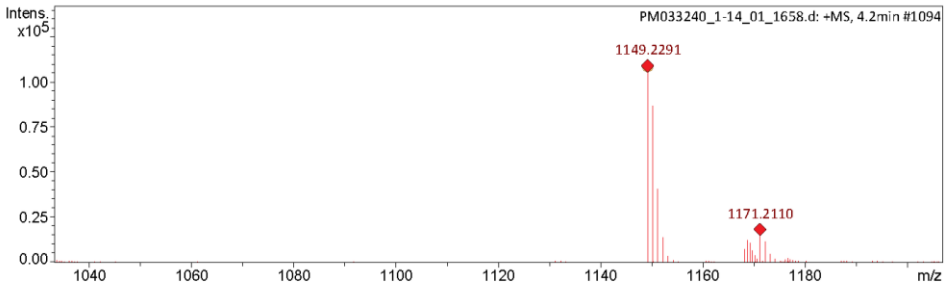


Figure S16. (+)-HRESIMS spectrum of **2**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
1149.2291	1	C60H45O24	1149.2295	0.3	93.0	1	100.00	39.0	even	ok

Figure S17. ^{1}H NMR spectrum of **3** in CD_3OD (800 MHz, 278 K).

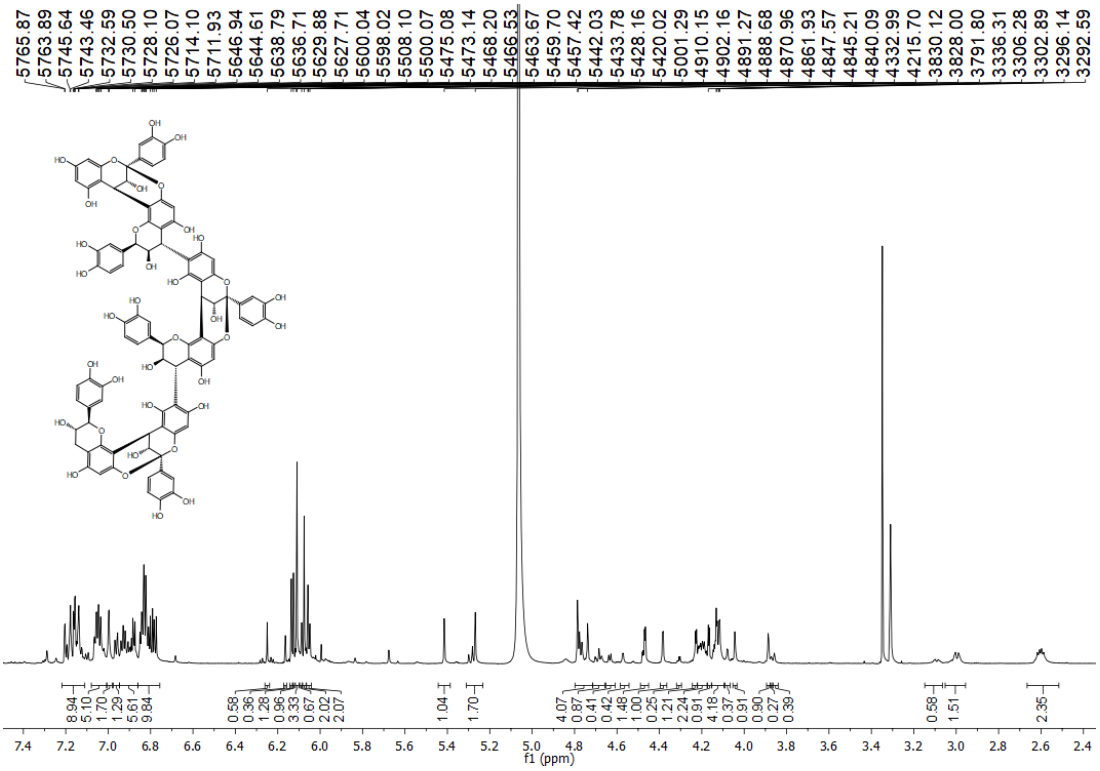


Figure S18. ^{13}C NMR spectrum of **3** in CD_3OD (100 MHz, 278 K).

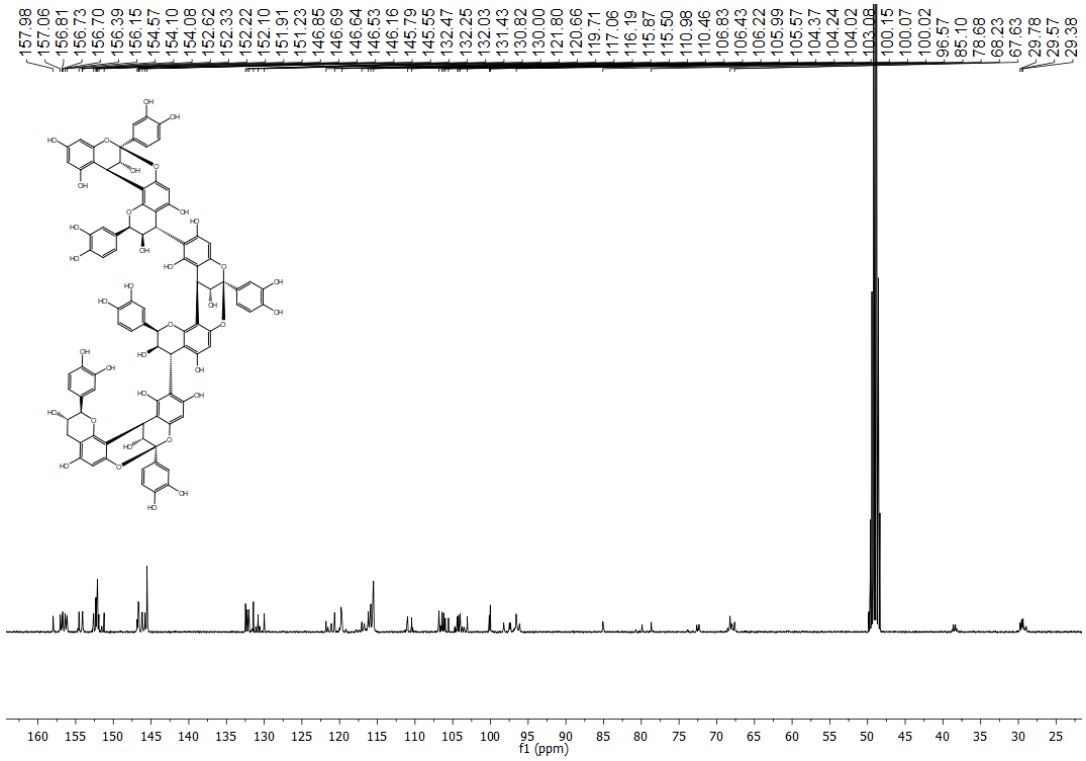


Figure S19. $^{1}H–^{1}H$COSY spectrum of **3** in CD_3OD (278 K).

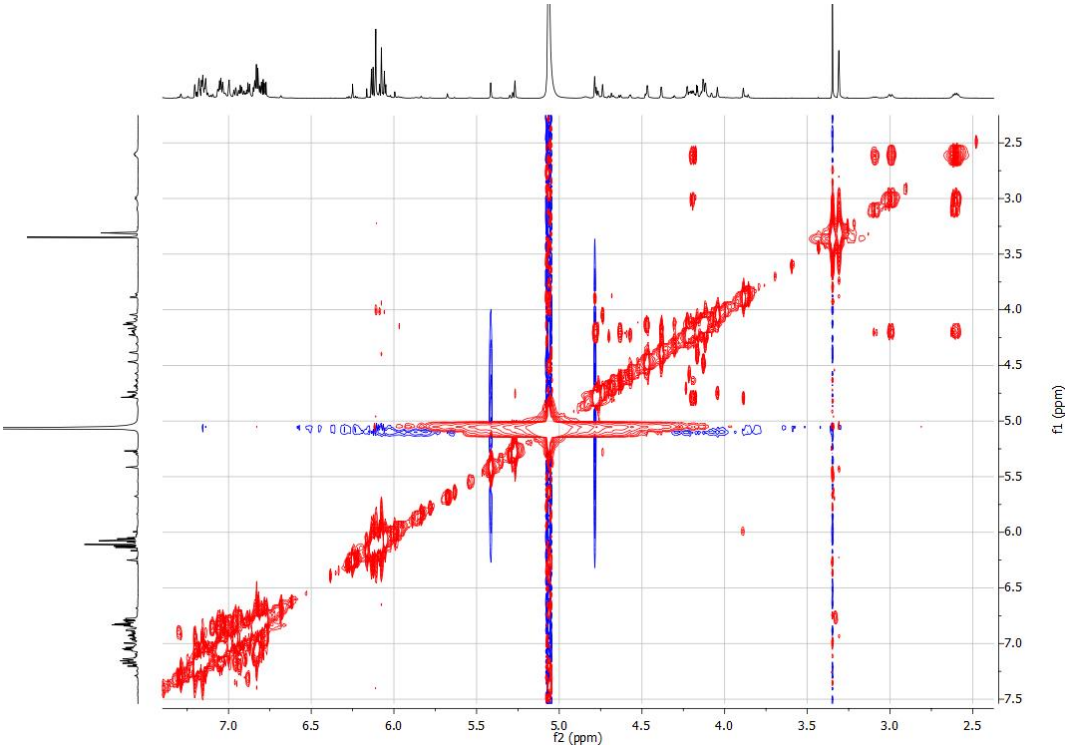


Figure S20. HSQC spectrum of **3** in CD_3OD (278 K).

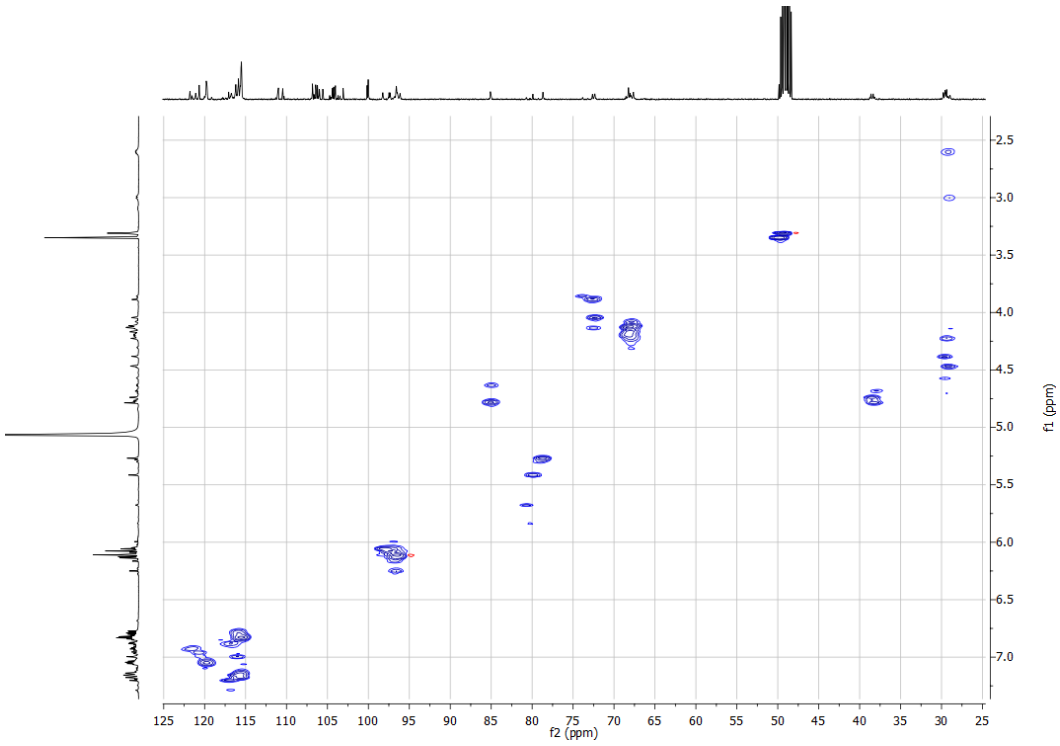


Figure S21. HMBC spectrum of **3** in CD_3OD (278 K)

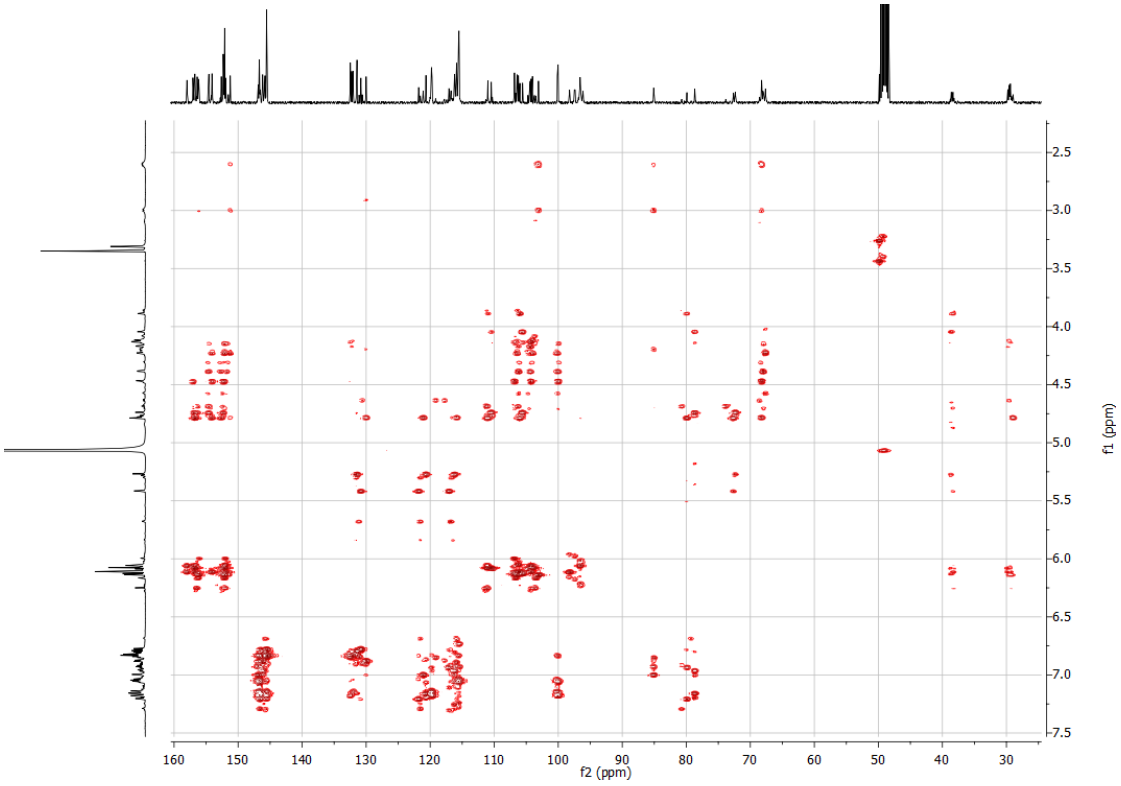


Figure S22. NOESY spectrum of **3** in CD_3OD (278 K)

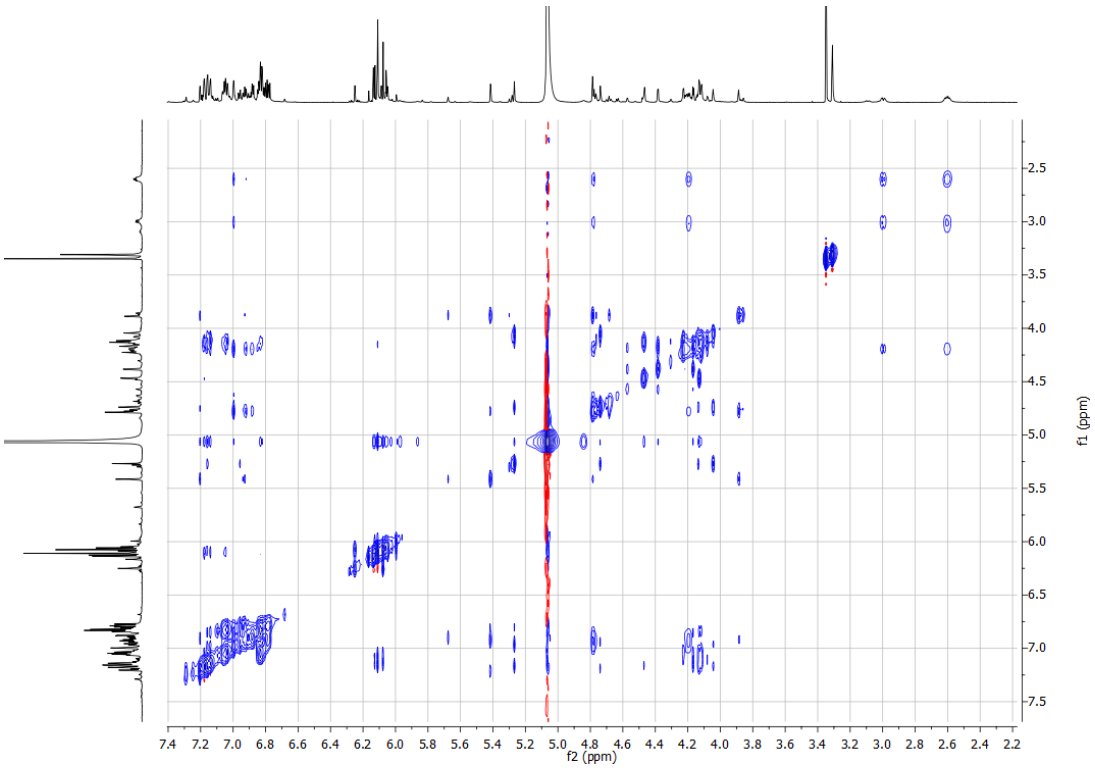


Figure S22-1. Partially expanded NOESY spectra of **3** with important NOESY correlations

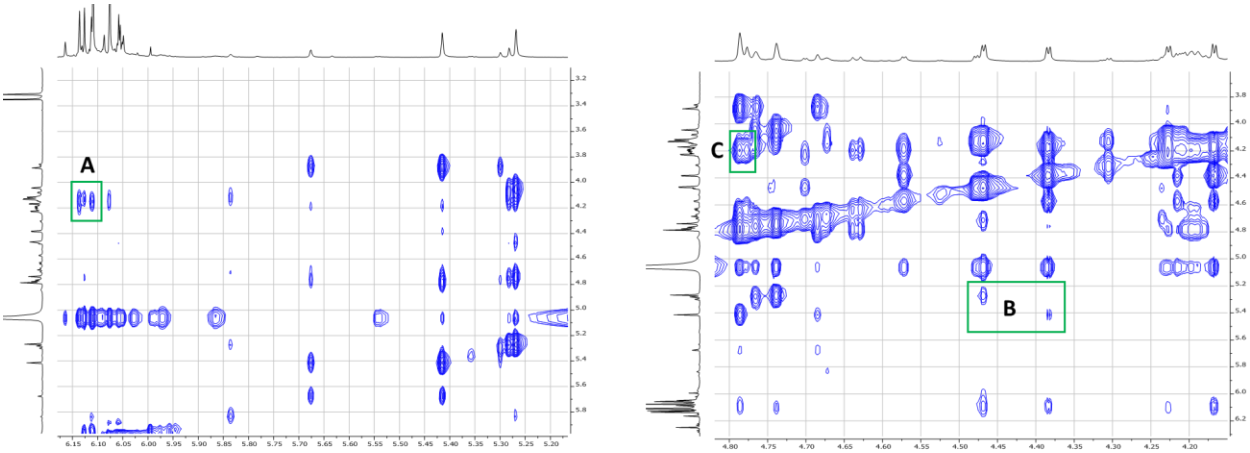


Figure S22-2. Expanded NOESY spectra of **3** with important NOESY correlations

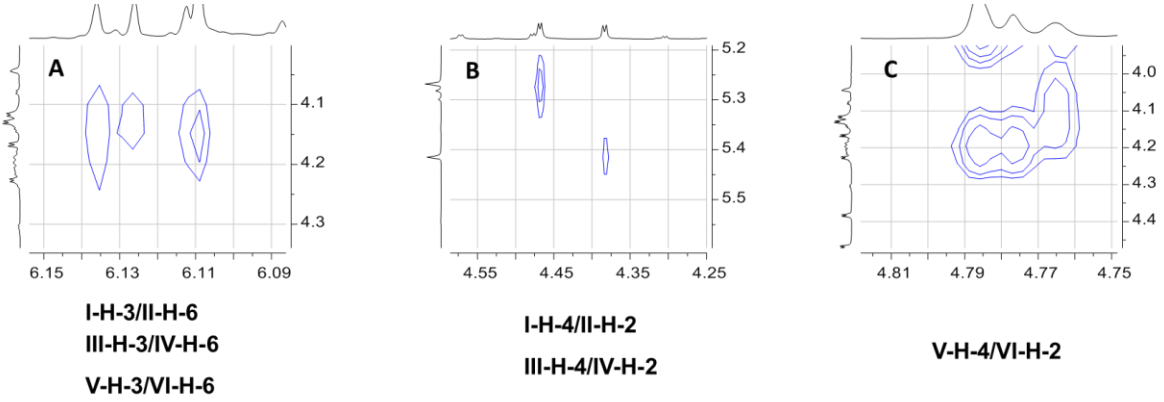
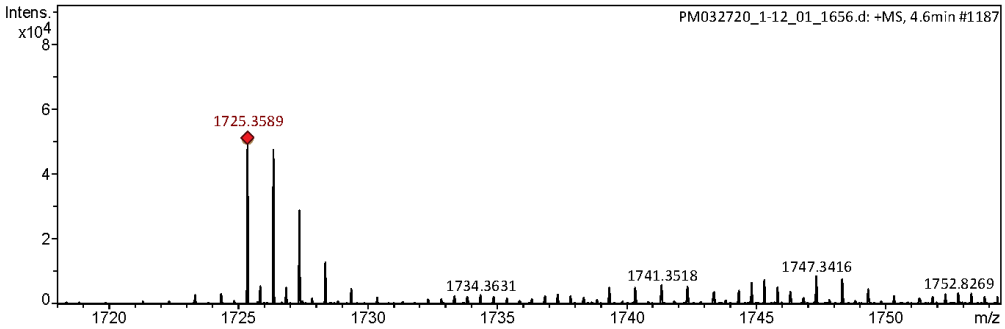


Figure S23. (+)-HRESIMS spectrum of **3**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
1725.3589	1	C90H69O36	1725.3563	-1.5	21.0	1	100.00	57.0	even	ok

Figure S24. Phloroglucinolysis products of **3** identified by chiral phase HPLC and MS analysis

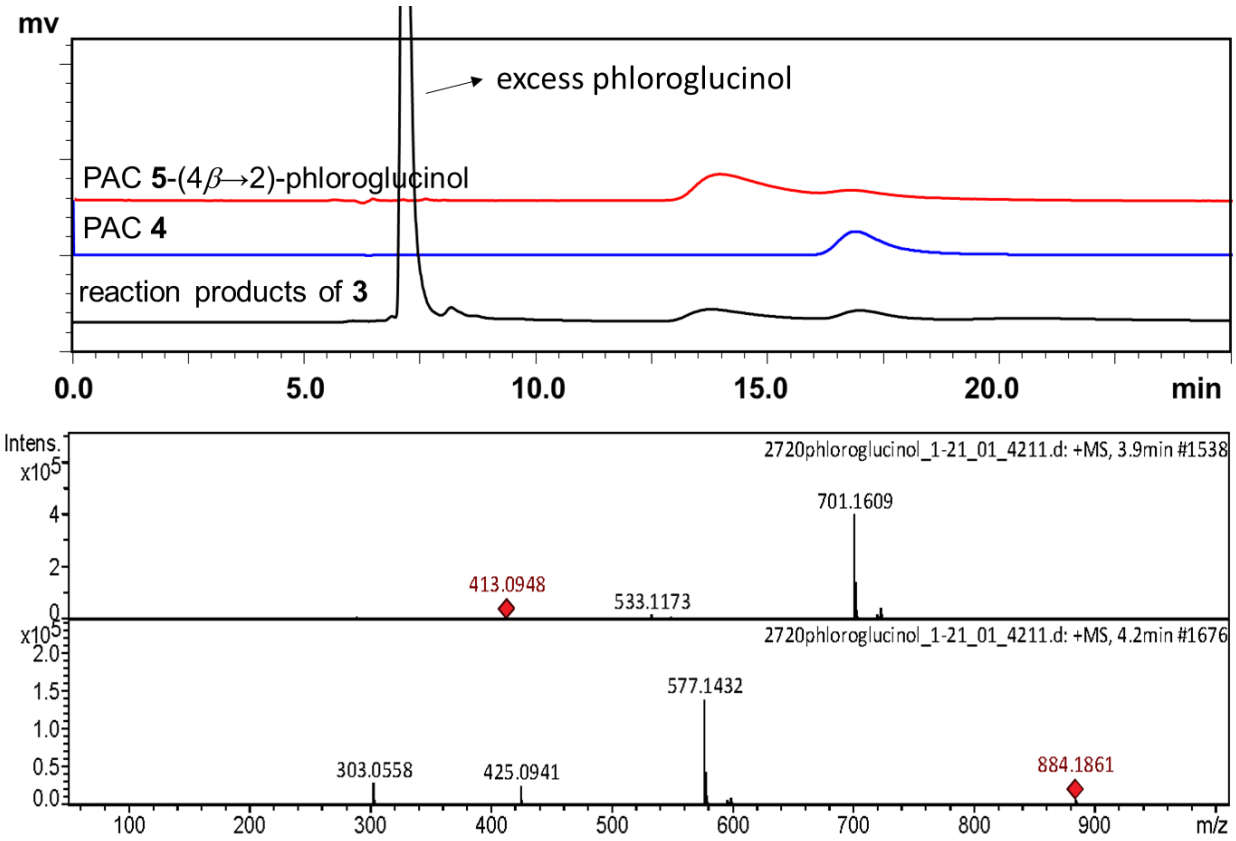
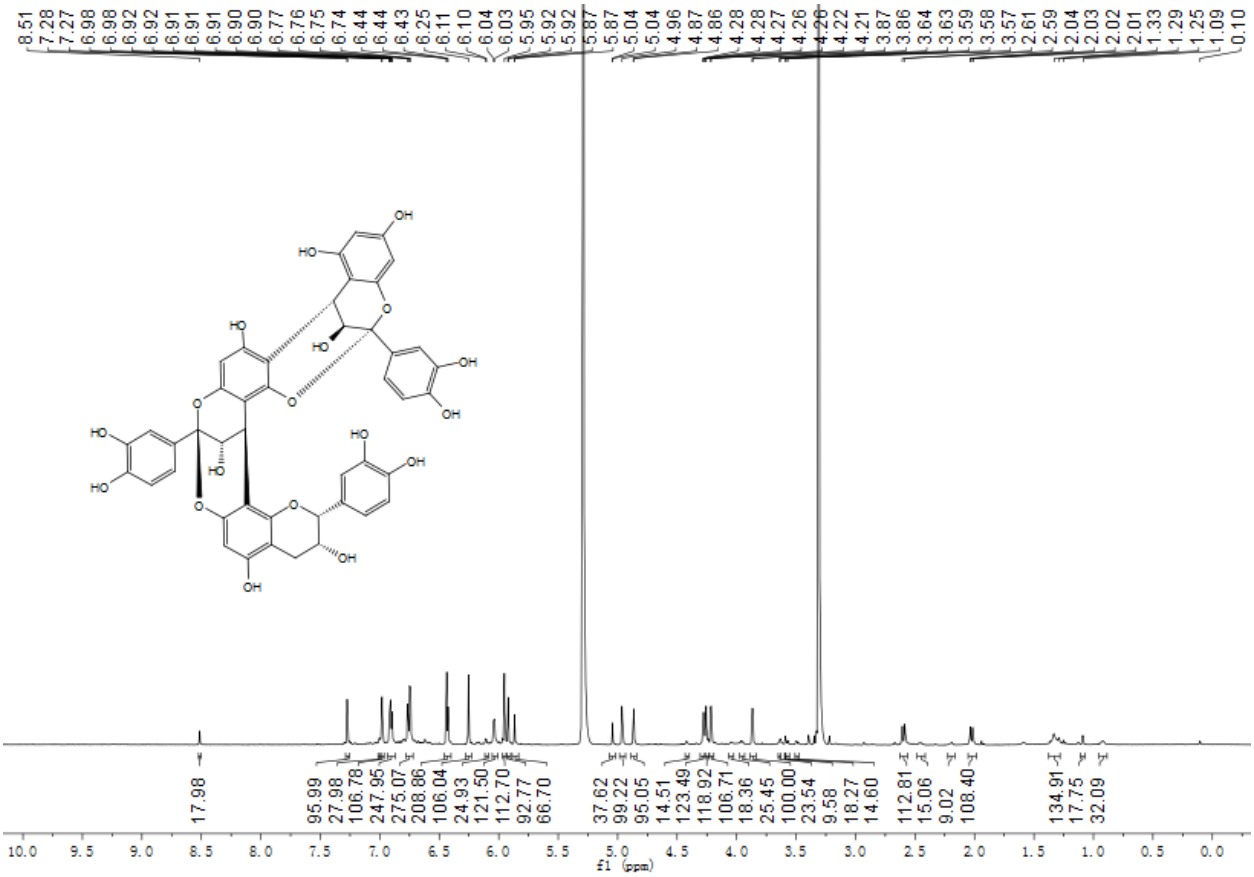


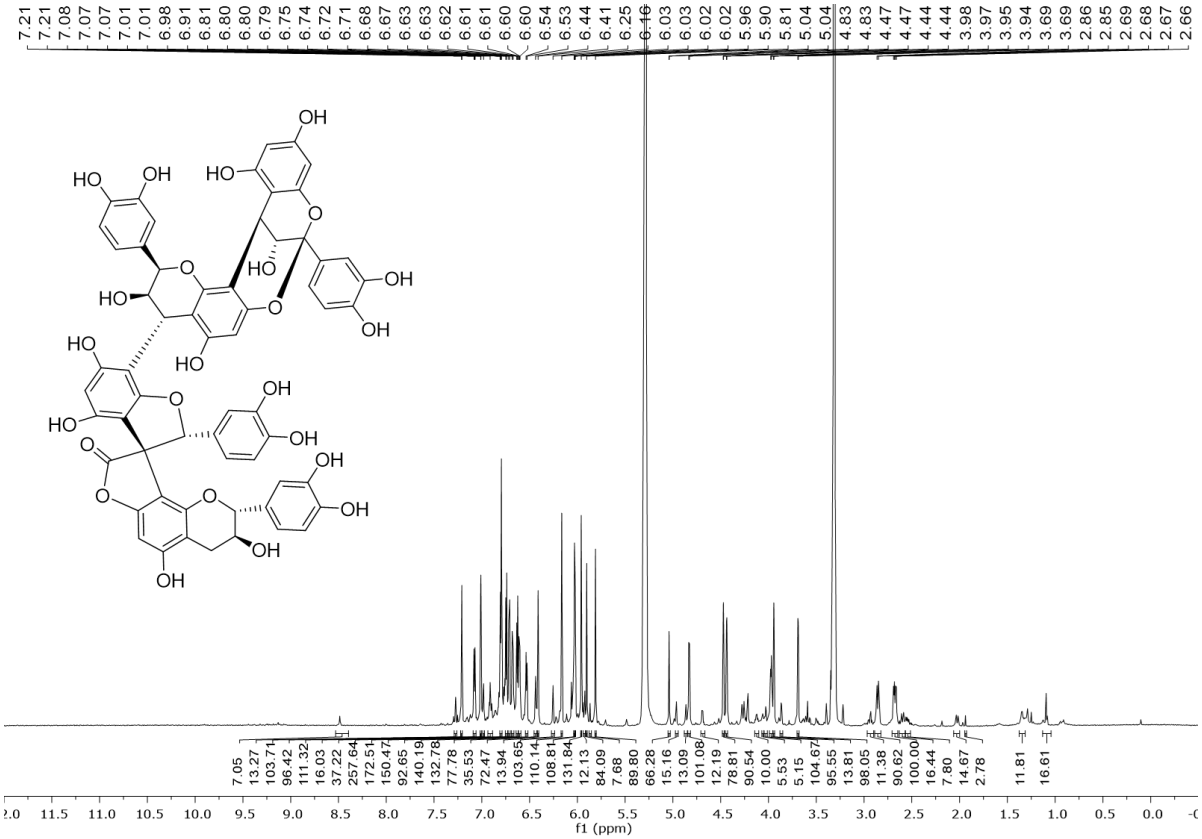
Figure S25. Calculation of the purity of **1** by qHNMR using the 100% method



Quantitative ^{1}H NMR spectrum (800 MHz, CD_3OD, 255K)

Compound **1**	
Composition	Weight%
Compound **1**	88.7
Rotamers and/or analogues	8.9
Formic acid	0.9
Other impurities	1.5

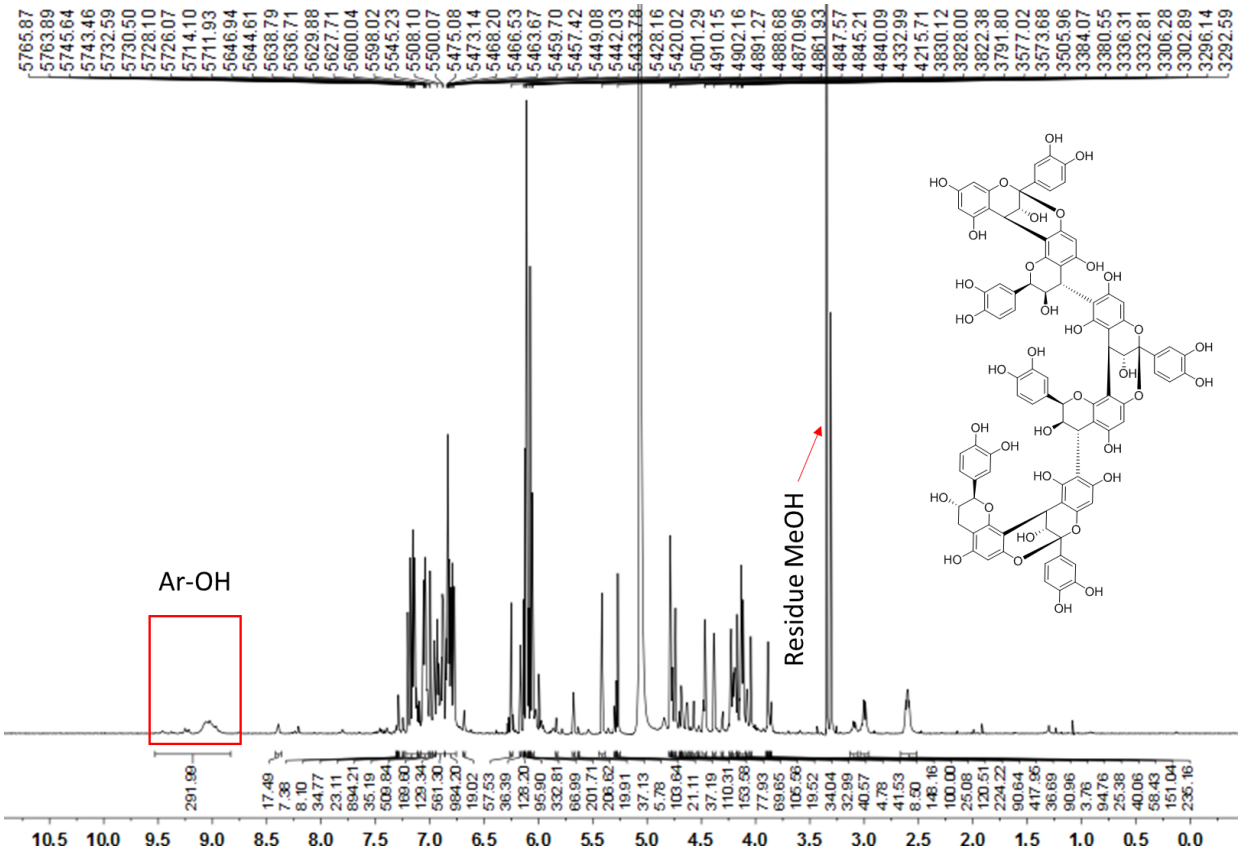
Figure S26. Calculation of the purity of **2** by qHNMR using the 100% method



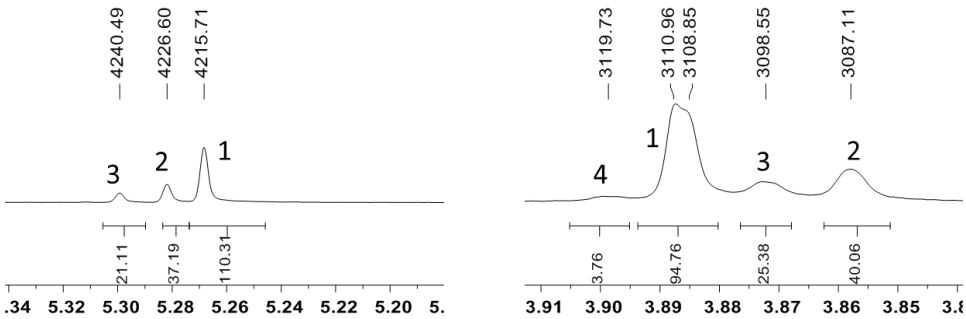
Quantitative ^{1}H NMR spectrum (800 MHz, CD_3OD, 255K)

Compound **2**	
Composition	Weight%
Compound **2**	86.7
Rotamer and/or analogues	12.0
Formic acid	0.1
Other impurities	1.1

Figure S27. Calculation of the purity of **3** by qHNMR using the 100% method



Quantitative ^{1}H NMR spectrum (800 MHz, CD_3OD, 278 K)



Three distinct rotamers can be observed

Compound **3**	
Composition	Weight%
Rotamer 1	56.6
Rotamer 2	22.6
Rotamer 3	11.3
Other rotamers and/or analogues	4.5
Methanol	4.2
Formic acid	0.3
Other impurities	0.5