Rare A-Type, New Spiro-Type, and Highly Oligomeric Proanthocyanidins from *Pinus massoniana*
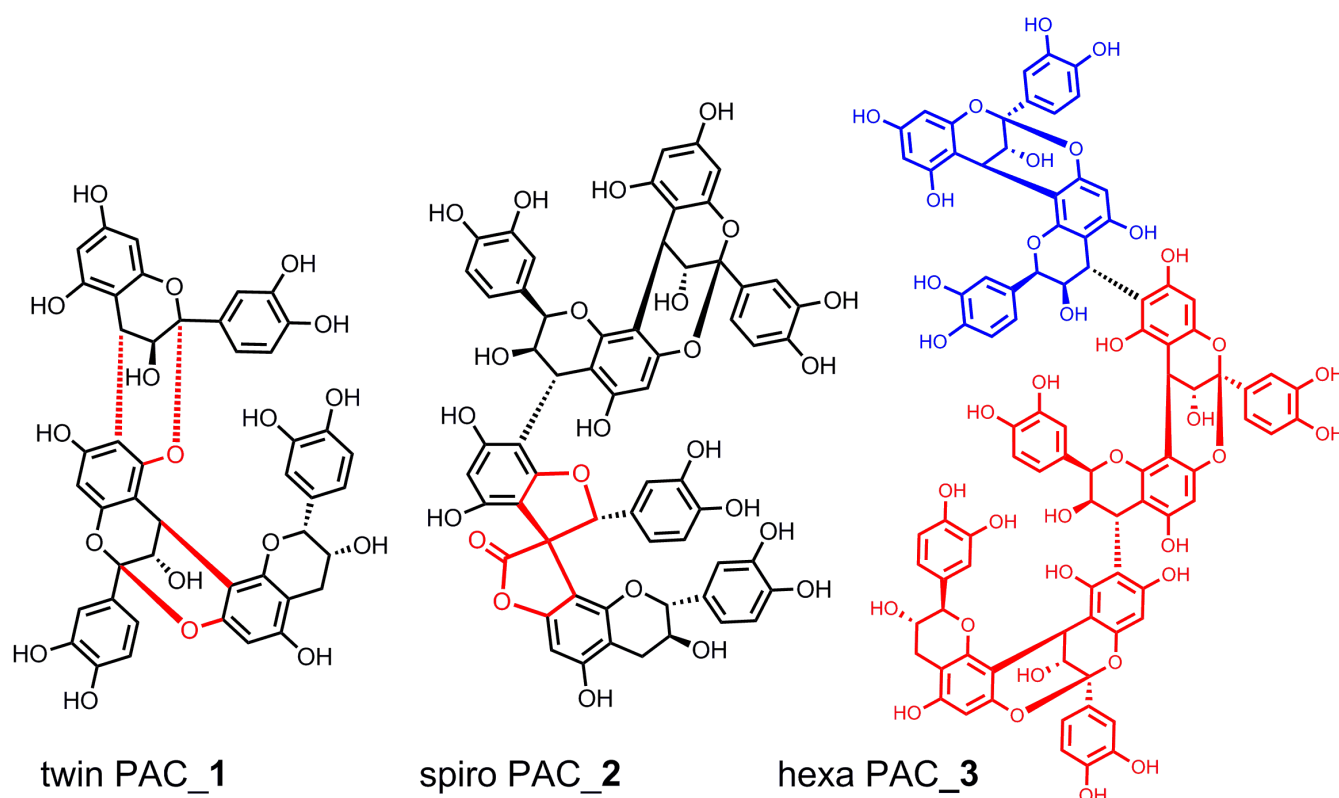
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twin PAC_**1**   spiro PAC_**2**   hexa PAC_**3**

ABSTRACT: Isolation of underivatized PACs led to three new, structurally unique PACs (**1**–**3**) from pine bark. Pinutwindoublin (**1**) is the first known trimer with double A-type interflavanyl linkages ($2\alpha{\to}O{\to}5,4\alpha{\to}6$ and $2\alpha{\to}O{\to}7,4\alpha{\to}8$). Pinuspirotetrin (**2**) represents the first PAC tetramer with a heterodimeric framework consisting of one spiro-type and one A-type dimer. Pinumassohexin (**3**) was elucidated as a mixed A + B type hexamer that consists of the tetramer, peanut procyanidin E, and an A-type dimer (**5**).

The bark of the pine species, *Pinus massoniana*, is a rich source of proanthocyanidins (PACs), a phylogenetically ancient group of polyphenols that occur ubiquitously in vascular plants. Accordingly, PACs inevitably impact human life via nutrition and medicine. Representing a group of vast structural diversity, PACs exhibit a broad spectrum of bioactivities.[1-7] Specific to pine bark, a series of tri- and tetrameric PACs has the demonstrated capability of enhancing the biomechanical properties of dentin and

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instill resistance to proteolytic dentin degradation.[6,7] Continuing these interdisciplinary efforts at the interface of natural products chemistry and dentistry, exploration of structurally unique compounds from bioactive PAC oligomer fractions led to three structurally distinctive new PACs (**1**–**3**) (Figure 1).
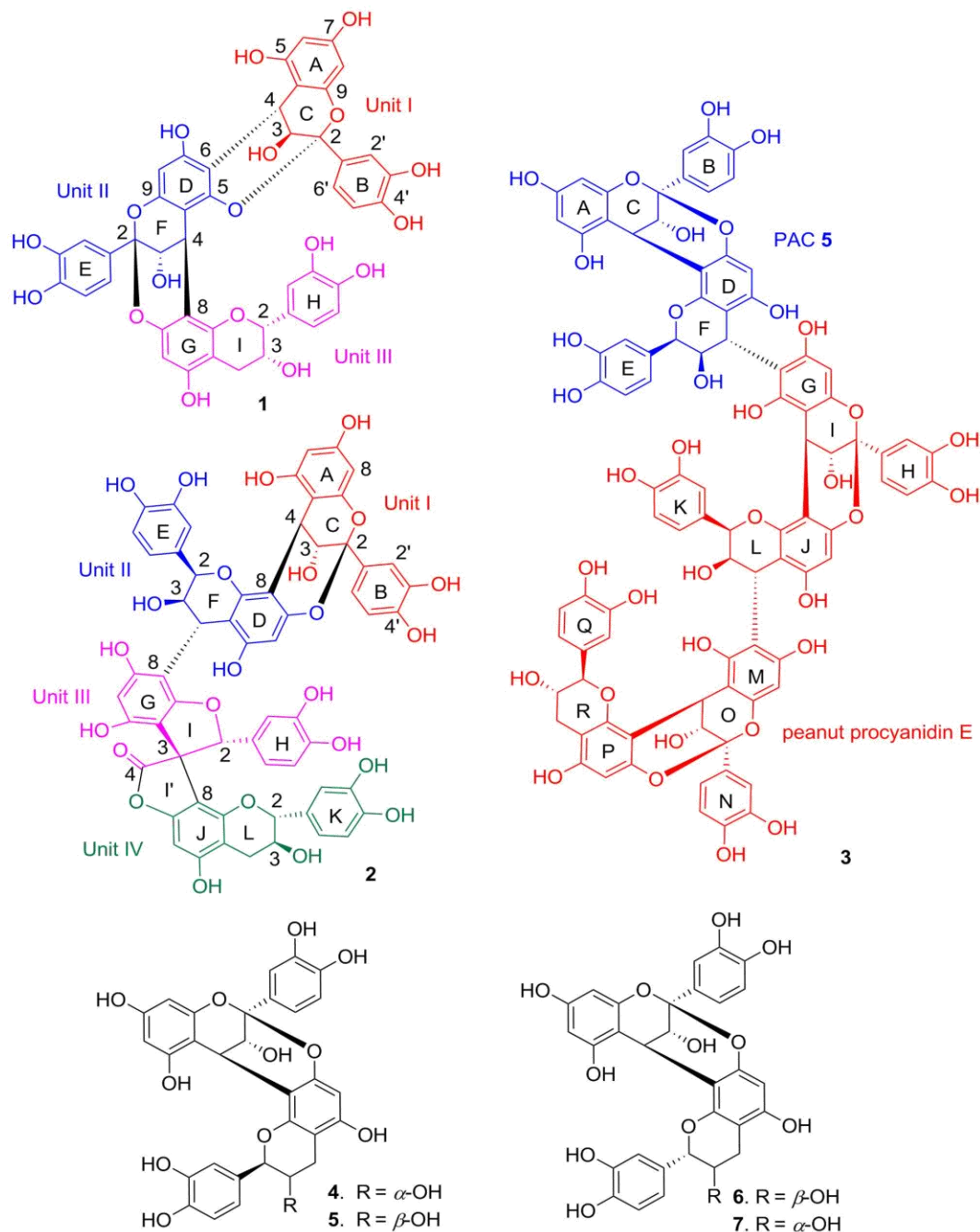

Figure 1. Novel structures **1**–**3** with color coded constituent catechol monomers, compared with the known dimers, **4**–**7**.

Pinutwindoublin (**1**) is the first A-type-only trimer linked with ($2\alpha{\rightarrow}O{\rightarrow}5,4\alpha{\rightarrow}6$) and ($2\alpha{\rightarrow}O{\rightarrow}7,4\alpha{\rightarrow}8$) bonds. This unprecedented linkage combination is the plausible reason for the observed atropisomeric line-broadening in its NMR spectra, which otherwise is atypical of A-type PACs. Pinuspirotetrin (**2**) represents the first PAC with a heterodimeric framework consisting of a spiro-type dimer connected with an A-type dimer. Also characterized in underivatized form, and exhibiting dentin bioactivity, pinumassohexin (**3**) was elucidated as a hexameric PAC with mixed A+B-type interflavanyl linkages (IFLs), assembled from the known tetramer, peanut procyanidin E,[8] and the dimer, epicatechin-($2{\rightarrow}O{\rightarrow}7,4{\rightarrow}8$)-*ent*-catechin (**5**),[8,9] via a $4\beta{\rightarrow}6$ bond. Structure elucidation of **1**–**3** utilized 1D/2D NMR,

ECD, ^{13}C NMR γ-gauche effects, as well as phloroglucinolysis, a recently established toolbox for absolute stereochemical assignments in PACs.[8]

The molecular formula $C_{45}H_{34}O_{18}$ of **1** was determined using the ^{13}C NMR carbon counts and the (+)-HRESIMS ion at m/z 863.1823 [M + H]$^+$. Two pairs of characteristic AX-resonances at δ_H 3.87 and 4.26 (both d, J = 4.0 Hz), as well as δ_H 4.22 and 4.87 (both d, J = 4.3 Hz), revealed the presence of two doubly linked interflavanyl bonds and, in connection with the molecular formula, indicated the 2A-type trimer nature of **1**. The NMR data (Table S1) showed the characteristic ^{1}H AMX patterns of the three 1,3,4-trisubstituted aromatic B-, E-, and H-rings. Additionally, a small J coupling (<1 Hz) between III-H-2 and III-H-3 suggested the terminal unit III to be 2,3-*cis* configured (*ent*)-epicatechin. The IFLs were elucidated via HMBC and NOESY data (Figure S1): the presence of 2→O→7/4→8 linkages between units II and III was assigned by the HMBC cross-peaks from both II-H-4 (δ_H 4.87) and III-H-2 (δ_H 4.96) to III-C-9 (δ_C 152.4). The NOESY correlations from II-H-8 to II-H-2′ and II-H-6′, and from I-H-2′ to II-H-4 and III-H-2 indicated that 2→O→5/4→6 bonds connected units I and II. This established the planar structure of **1**.

The absolute configuration of **1** was gleaned from ECD and NOESY data (Figure S1). Due to a high-amplitude negative Cotton effect (CE) in the diagnostic region 220~240 nm (Figure 2A), the C-4 aryl functionalities in both units II and III were determined to be α-configured.[10-12] The absolute configuration of C-4 in units I and II were, thus, assigned as S and R, respectively (note the change in Cahn–Ingold–Prelog priorities due to different connections, causing different stereodescriptors for spatially identical configurations at both C-4).[12] Taking into account the 2,4-*cis* configuration in A-type PACs,[13] units I/II and II/III had to be doubly connected via (2α→O→5,4α→6) and (2α→O→7,4α→8) IFLs, respectively.
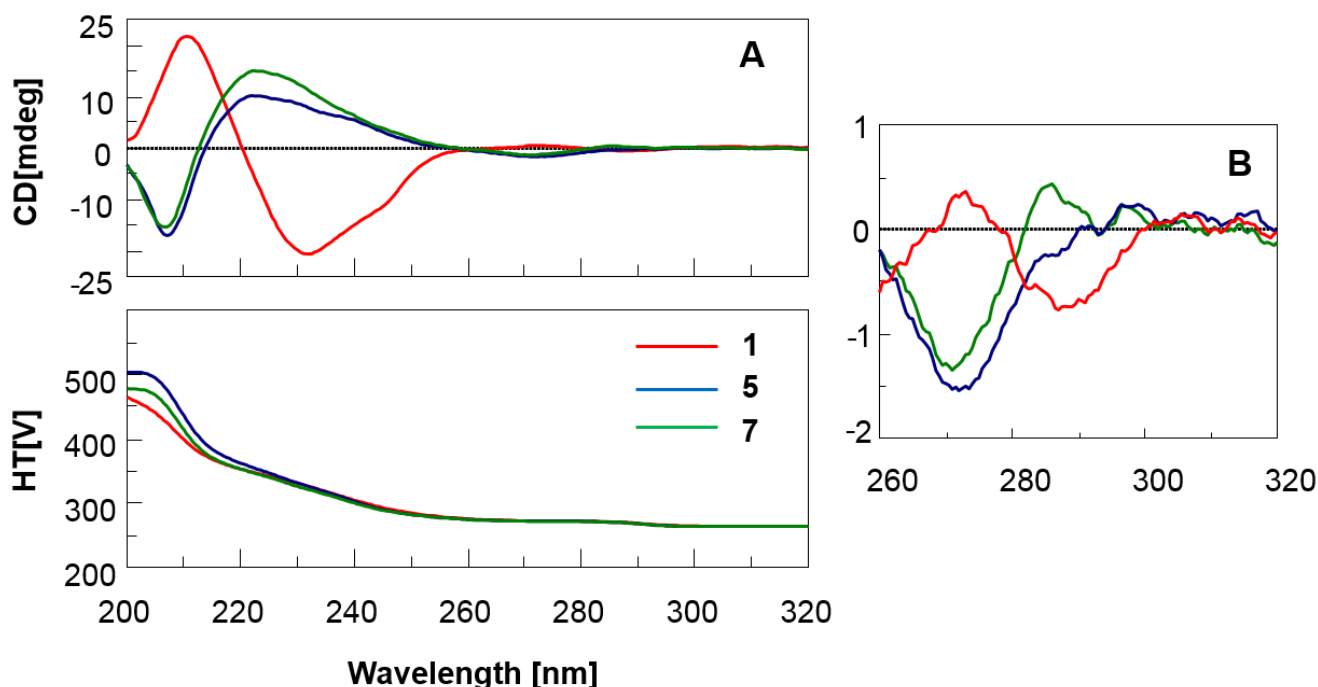

Figure 2. Full ECD spectra (A) of **1**, **5**, and **7** with expansions for the diagnostic range 260–320 nm (B).

A NOESY correlation between II-H-3 and III-H-6 indicated a 3,4-*trans* configuration in the F-ring, thus II-C-3 was S-configured.[11] Comparison of the tendency of ECD curves of **1** in the diagnostic region 280~300 nm with those of procyanidin A2 (**5**)[8] and procyanidin A5 (**7**)[8] revealed that **1** is closely mirror symmetric to **7** (Figure 2B). Thus, the partial structures of units II and III in **1** were assigned to be enantiomeric to those in **7**. This was consistent with the R-configuration of III-C-2 deduced from the negative CE at 280~300 nm,[10,11] and the NOESY correlation of II-H-4 and III-H-6′. Finally, I-H-3 had to be β-

oriented based on NOESY correlations between I-H-3 and both III-H-2 and III-H-6′. Accordingly, **1** was assigned as *ent*-epicatechin-(2α→O→5,4α→6)-*ent*-epicatechin-(2α→O→7,4α→8)-epicatechin.

This makes **1** the first reported double A-type trimer with an unusual (2α→O→5,4α→6) IFL. Restricted rotation along the C-4 aryl (sp^3-sp^2) carbon-carbon bond is otherwise only typical for B-type PACs, thereby giving rise to atropisomerism and often severe NMR line broadening at ambient temperature. In contrast, A-type PACs, with two IFLs, generally do not exhibit atropisomerism due to the rigidity of the double linkage.[6,14,15] Improving ^{1}H NMR lineshapes of **1** required acquisition at low temperature (255 K; Figure 3). The unexpected dynamic peak broadening of **1** despite its two double linkages indicated that the combination of (2α→O→5,4α→6) and (2α→O→7,4α→8) IFLs poses sufficient steric hindrance for restricted rotation between rings B and H to generate observable atropisomers, thus leading to NMR line-broadening.
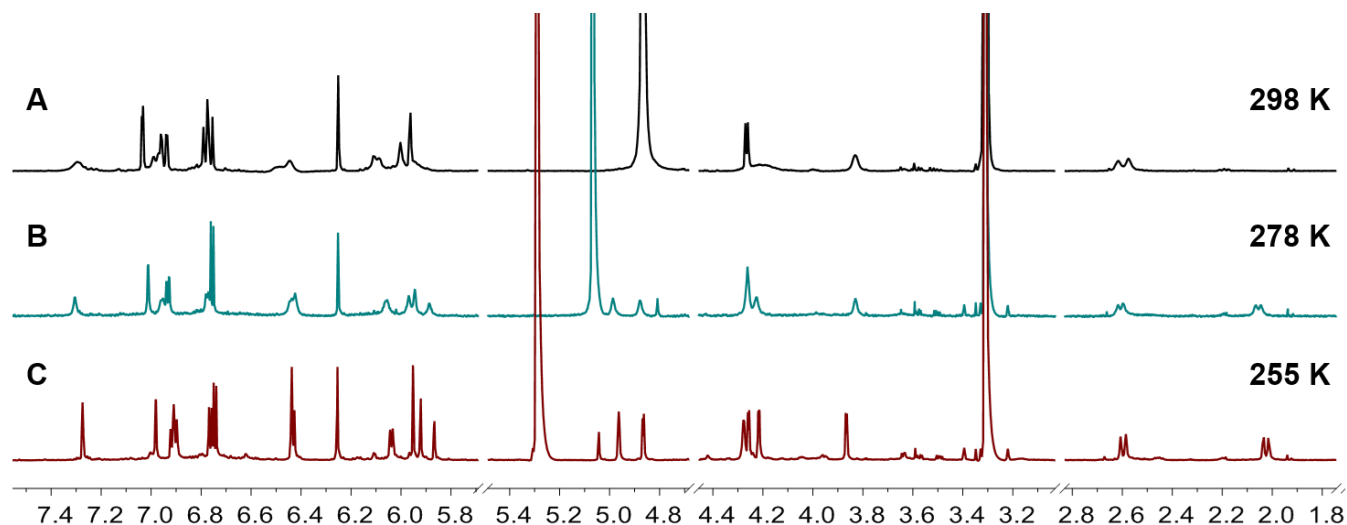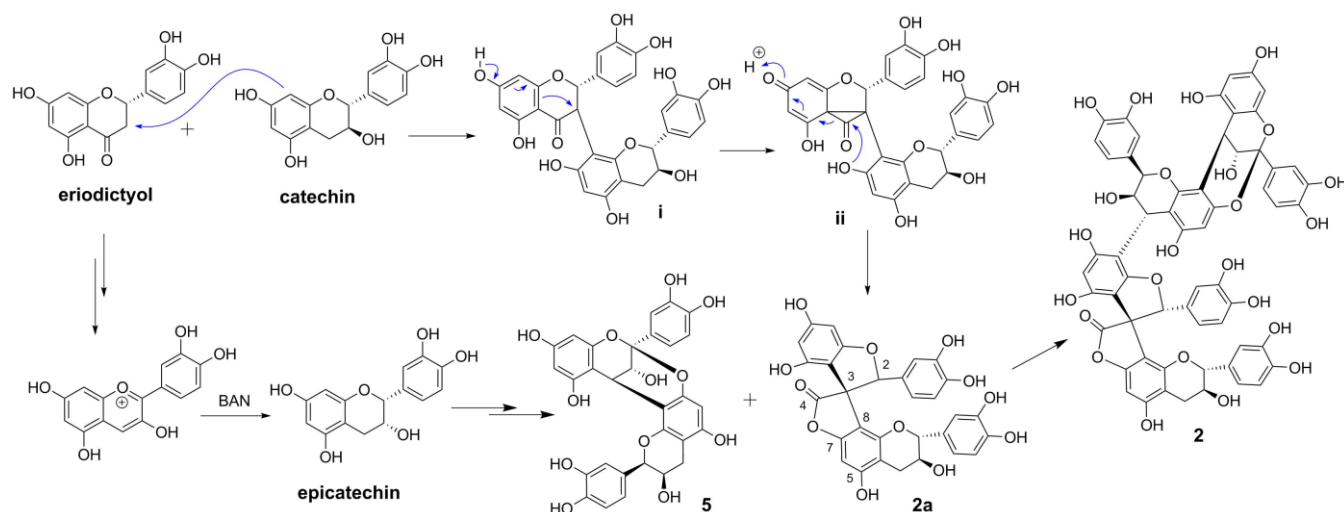

Figure 3. The ^{1}H NMR spectra of **1** acquired in CD_3OD under the following conditions: A) 298 K at 400 MHz, NS = 64; B) 278 K at 800 MHz, NS = 64; C) 255 K at 800 MHz, NS = 128.

PAC tetramer **2** exhibited a molecular ion at *m/z* 1149.2291 [M + H]$^+$ in the (+)-HRESIMS, corresponding to a molecular formula $C_{60}H_{44}O_{24}$, as supported by the ^{13}C NMR data. Lower temperature (255 K) was applied during the 1D and 2D NMR experiments to overcome line broadening due to atropisomerism (Figure S10).[6,14,16] Analysis of the ^{1}H NMR spectrum showed four AMX spin systems, corresponding to four 1,3,4-trisubstituted aromatic rings (B/E/H/K). Three singlets at δ_H 5.90, 5.96, and 6.16, as well as two *meta*-coupled doublets at δ_H 6.02 and 6.03 (*J* = 2.3 Hz) showed the presence of aromatic rings J, G, D, and A, and further confirmed the tetrameric nature of **2**. Two sequential spin systems (δ_H 3.70 and 4.44, both d, *J* = 3.5 Hz; and δ_H 5.30, 3.95, and 4.47, each brs) that assigned by the COSY data (Figure 4A), indicated the presence of one double (A-type) and one single (B-type) IFL of **2**. Units II and IV were identified as (*ent*)-epicatechin and (*ent*)-catechin, respectively, based on the $J_{2,3}$ values of ~0 and 6.3 Hz.

Unusual features involved an oxygenated methine (δ_H 5.81, s, and δ_C 88.2) that showed HMBC correlations with the carbonyl carbon at δ_C 177.0 assigned to a γ-lactone, and a spiro carbon resonating at δ_C 61.4. Both are characteristic for a spiro-biflavanoid similar to larixinol.[17,18] Linkages between the four units were further elucidated by HMBC and NOESY (Figure 4). Units I and II were recognized as being connected by double (2→O→7/4→8) IFLs based on the I-H-4/II-H-2 NOESY correlation, as well as shift of II-C-8 (δ_C 106.3).[7,19] The presence of a 4→8 linkage between units II and III was deduced from the cross-peaks between II-H-2 and both III-H-2′ and III-H-6′ in NOESY spectrum. The spiro-biflavanoid arrangement of units III and IV was corroborated by the HMBC correlations from III-H-2 to III-C-3, III-C-4, III-C-10, III-C-2′, III-C-6′, and IV-C-8; from IV-H-2 to IV-C-9; and from IV-H-6 to IV-C-5 and IV-C-7. Thus, the planar structure of **2** had to be the first tetrameric spiro-PAC, consisting of one A-type and

one spiro-type dimer. Analysis of its ECD spectrum showed a high-amplitude positive Cotton effect in

Scheme 1. Proposed biosynthetic pathway for the formation of the spiro element in PAC tetramer 2.

the region 220~240 nm (Figure S2), characteristic for β-substituted flavan-3-ol moieties,[10-12] confirming the absolute configuration of the spiro center III-C-3 as being *S*.[18] The absolute configurations of the C-4 centers in both units I and II were thus assigned as *R*-configured. I-C-2 was *S*-configured based on the inherent 2,4-*cis* configuration in A-type PACs.[13] A NOESY correlation between I-H-3 and II-H-6 indicated H-3 and H-4 in the C-ring to be *trans*, thus I-C-3 was *R*-configured.[11] The upfield shift of II-C-2 (δ_C 78.7) suggested a 2,4-*trans*-configuration in the F-ring based on the γ-gauche effect.[20,21] Unit II was, therefore, epicatechin (2*R*, 3*R*), which was confirmed by the NOESY correlations between II-H-2/III-H-2′ and III-H-6′. NOESY correlations from III-H-2 to IV-H-3, IV-H-2′, and IV-H-6′ assigned III-C-2 as *R*-configured, which determined the terminal unit IV as catechin (2*R*, 3*S*). In conclusion, the structure of **2**, was assigned unambiguously as shown and named as pinuspirotetrin.

The molecular formula $C_{90}H_{68}O_{36}$ gleaned from (+)-HRESIMS and ^{13}C NMR carbon counts identified **3** as a hexamer with 3A+2B IFLs. The close resemblance of the ^{1}H and ^{13}C NMR resonances of units II/IV and III/V, as well as their congruence with reported data of the major tetramer from peanuts, peanut procyanidin E,[8] suggested this tetramer plus an additional A-type dimer as building blocks of **3**. Its ^{1}H NMR spectrum revealed three AX-type doublet pairs (δ_H 4.13 and 4.47, *J* = 3.4 Hz; δ_H 4.17 and 4.38, *J* = 3.6 Hz; and δ_H 4.12 and 4.23, *J* = 3.6 Hz), which corroborated the presence of three doubly linked A-type motifs. Similarly, two B-type single bond linkages were assigned by the observed two sets of three coupled methines resonating as broad singlets at δ_H 5.27, 4.04, and 4.74 as well as δ_H 5.42, 3.89, and 4.78. All three A-type IFLs were assigned as 2β→O→7/4β→8 via NOESY correlations (Figure S22) from H-4 (rings C, I, and O) to H-2 (rings F, L, and R), as well as a strongly positive Cotton effect in the region 220~240 nm (Figure S2). The 3-OH functional groups in rings C, I, and O had to be *trans*-oriented relative to H-4 based on the NOESY correlations from H-3 (rings C, I, and O) to H-6 (rings D, J, and P). The two 4β→6 linkages were deduced from the chemical shifts of C-6 (δ_C 110.5 in the G-ring, and δ_C 111.0 in the M-ring) and ECD evidence. Units II and IV were both elucidated as epicatechin by the singlet signals of II-H-2 and IV-H-2, and the upfield shifted C-2 (δ_C 78.7 in F-ring, and δ_C 79.9 in the L-ring) to the corresponding carbon in **4** (δ_C 81.6).[8] Phloroglucinolysis[8] confirmed the absolute stereochemical assignments. Analysis of the reaction products used a combination of chiral HPLC and MS (Figure S24) to verify that **4** and **5** are the basic components of **3**. Collectively, this assigned the structure of pinumassohexin (**3**) unambiguously as [PAC **5**]-(4β→6)-[PAC **5**]-(4β→6)-[PAC **4**].
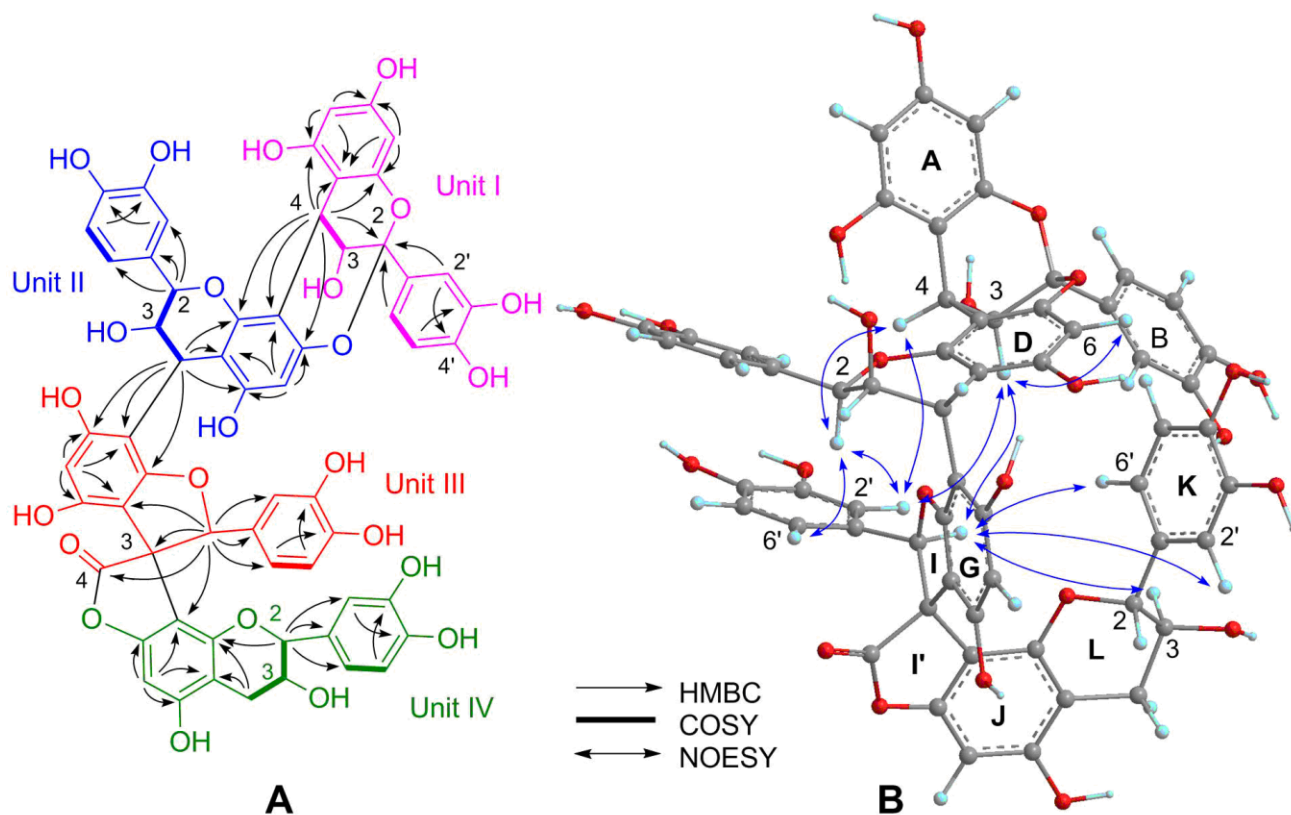
Figure 4. (A) Selected COSY (bold bond), HMBC, and (B) NOESY correlations in **1**.

Pinuspirotetrin (**2**) represents the first heterodimeric framework in any PAC, consisting of a spiro-type dimer (**2a**) and an A-type dimer (**5**). Scheme 1 shows a plausible biosynthetic pathway for **2**, which rationalizes the spiro dimer **2a** as resulting from intermediates (i→ii→**2a**) via oxidative flavanone-flavonol conversion.[22]

While all isolates stemmed from a bioactive fraction, only **3** could be evaluated for dentin bioactivity due to limited yields. Hexamer **3** increased the modulus of elasticity of dentin by 4.3-fold, a value between those of A-type dimers (**4**–**7**) and the most highly potent trimer and tetramers. This further supports the overall hypothesis that medium-size ($n = 3$ and 4) PACs elicit the highest increase in key mechanical properties of dentin. These enhancements to the dentin have promising therapeutic application for the development of novel dental biomaterials. A comprehensive structure and bioactivity relationships of PACs will be reported in due course. While the structural novelties of **1** and **2**, and the unusual size and complexity of **3** did not infer bioactivity in the dentin bioassays, they contribute valuable SAR information and expand the 3D chemical diversity space of condensed tannins.

ASSOCIATED CONTENT

The Supporting Information and Experimental details, ECD spectra of compounds **2** and **3,** 1D and 2D NMR, and HRESIMS of compounds **1**-**3**; and phloroglucinolysis of **3** were appended in the end.

The raw NMR data (FIDs) are made available at: DOI:10.7910/DVN/CI2ODS.

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Notes

The authors declare no competing financial interest.

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Rare A-Type, New 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$_3$OD.

Unit	Ring	No.	1[a] ^{1}H (mult., J in Hz)	^{13}C	2[a] ^{1}H (mult., J in Hz)	^{13}C	3[b] ^{1}H (mult., J in Hz)	^{13}C	Unit	Ring	No.	3[b] ^{1}H (mult., J in Hz)	^{13}C
I	C	2		100.5		99.9		100.0	V	O	2		100.2
		3	3.87, d (4.0)	65.1	3.70, d (3.5)	67.5	4.13, d (3.4)	68.2			3	4.12, d (3.6)	67.6
		4	4.26, d (4.0)	29.7	4.44, d (3.5)	28.9	4.47, d (3.4)	29.4			4	4.23, d (3.6)	29.6
	A	5		155.2		156.9		157.1		M	5		154.1
		6	5.92, d (1.9)	97.3	6.03, d (2.3)	98.0	6.06, d (2.4)	98.2			6		111.0
		7		157.9		157.9		158.0			7		156.7
		8	5.87, d (1.9)	96.1	6.02, d (2.3)	96.2	6.11, d (2.4)	96.6			8	6.083, 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'	7.29, d (2.1)	116.1	7.01, d (2.1)	115.2	7.18, d (2.1)	115.5			2'	7.14, 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'	6.76, d (8.2)	115.1	6.71, d (8.2)	115.5	6.84, d (8.2)	115.5			5'	6.82, d (8.3)	115.5
		6'	6.90, dd (8.3, 2.1)	120.3	6.61, dd (8.4, 2.1)	119.4	7.06, dd (8.2, 2.1)	119.7			6'	7.04, dd (8.1, 2.3)	119.8
II	F	2		101.1	5.30, brs	78.7	5.27, s	78.7	VI	R	2	4.78, d (7.7)	85.1
		3	4.22, d (4.3)	68.7	3.95, brs	72.3	4.04, brs	72.3			3	4.19, m	68.2
		4	4.87, d (4.3)	30.4	4.47, brs	38.1	4.74, brs	38.6			4	2.60, dd (16.2, 8.4)	29.0
	D	5		150.8		157.1		156.4		P	4	3.00, dd (16.2, 5.1)	29.0
		6		108.5	5.96, s	95.8	6.13, s	96.1			5		156.2
		7		153.1		151.9		152.2			6	6.14, s	96.6
		8	6.25, 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'	6.98, d (2.2)	115.4	7.21, d (2.1)	116.6	7.161, d (2.1)	116.2			1'		130.0
		3'		145.5		145.8		145.8			2'	7.00, d (2.1)	115.9
		4'		146.6		146.3		146.2			3'		146.2
		5'	6.74, d (8.1)	115.5	6.80, d (8.2)	115.6	6.80, d (8.2)	115.9			4'		146.9
		6'	6.92, dd (8.1, 2.2)	119.7	7.08, dd (8.2, 2.1)	121.4	6.96, dd (8.2, 2.1)	120.7			5'	6.88, d (8.1)	116.8
III	I	2	4.96, s	79.7	5.81, s	88.2		100.1			6'	6.92, dd (8.2, 2.1)	121.1
		3	4.28, m	66.9		61.4	4.17, d (3.6)	68.0					

			1H	13C	1H	13C	1H	13C
		4	2.02, dd (17.0, 3.5)	23.6		177.0	4.38, d (3.6)	29.8
			2.60, brd (17.0)	23.6				
	G	5		156.4		154.2		154.6
		6	5.95, s	95.7	5.90, s	96.6		110.5
		7		151.7		158.2		156.8
		8		105.6		106.0	6.082, 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'	6.43, d (2.2)	113.1	6.41, d (2.2)	113.3	7.162, d (2.2)	115.5
		3'		146.1		145.7		145.6
		4'		145.5		145.8		146.7
		5'	6.43, d (8.1)	115.5	6.63, d (8.3)	115.9	6.83, d (8.3)	115.5
		6'	6.04, dd (8.2, 2.2)	117.6	6.53, dd (8.1, 2.3)	118.3	7.05, dd (8.1, 2.3)	119.7
IV	L	2			4.83, d (6.3)	82.5	5.42, s	79.9
		3			3.97, m	68.7	3.89, brs	72.6
		4			2.68, dd (16.4, 7.2)	27.6	4.78, brs	38.4
	J				2.86, dd (16.4, 5.0)	27.6		
		5				158.1		156.7
		6			6.16, s	91.3	6.11, 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'			6.80, d (2.1)	114.0	7.20, d (2.1)	117.1
		3'				145.9		145.5
		4'				146.0		146.5
		5'			6.74, d (8.1)	115.8	6.78, d (8.1)	115.9
		6'			6.68, dd (8.2, 2.1)	119.7	6.94, dd (8.2, 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**.
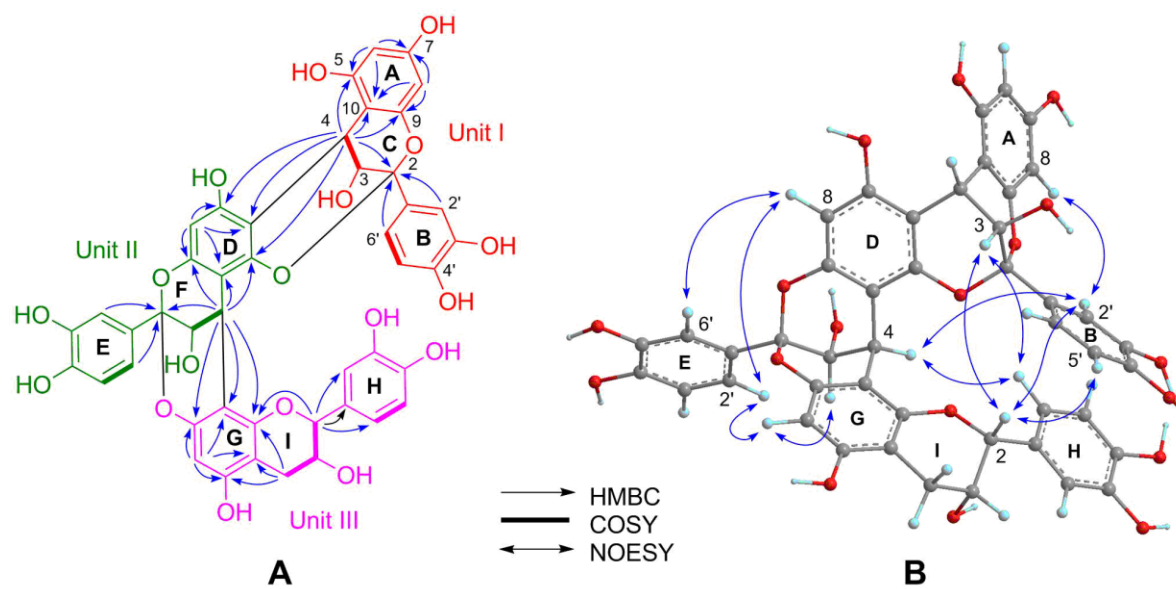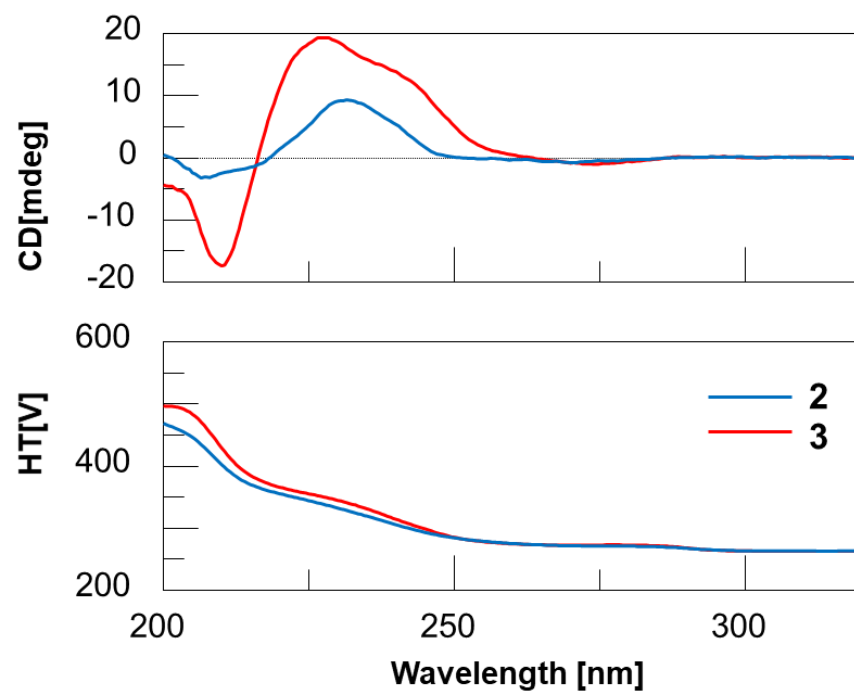
Figure S2. The ECD spectra of **2** and **3**.

■ 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$_2$O, 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$_2$O, 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 HPLC and MS analysis (Figure S24).

Compounds properties

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; ^1H and ^{13}C NMR (CD_3OD, 255K), see Table 1, (+)-HRESIMS [M + H]$^+$ *m/z* 863.1823 (calcd for $C_{45}H_{35}O_{18}$, 863.1818).

Pinuspirotetrin (**2**): Light brown, amorphous solid; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−3.2), 232 (+9.4), 270 (−0.8); ^{13}C NNMR (CD_3OD, 255K), see Table 2; (+)-HRESIMS [M + H]$^+$ *m/z* 1149.2291 (calcd for $C_{60}H_{45}O_{24}$, 1149.2295).

Pinumassohexin (**3**): epicatechin-(2β→O→7,4β→8)-epicatechin-(4β→6)-epicatechin-(2β→O→7,4β→8)-epicatechin-(2β→O→7,4β→8)-epicatechin-(4β→6)-catechin: light brown, amorphous solid; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (−17.3), 228 (+19.3), 274 (−1.0); ^1H and ^{13}C NNMR (CD_3OD, 278K), see Table 2; (+)-HRESIMS [M + H]$^+$ *m/z* 1725.3589 (calcd for $C_{90}H_{69}O_{36}$, 1725.3563).

References

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.

2. Zhou, B. Y Alania, M Reis, R. Phansalkar, JW Nam, J McAlpine, SN Chen, A Bedran-Russo, G Pauli. Tri- and Tetrameric Proanthocyanidins with Dentin Bioactivities from *Pinus massoniana*. ChemRxiv https://figshare.com/s/bb3c881919116125ed65.

Figure S3. ^{1}H NMR spectrum of **1** in CD$_3$OD (800 MHz, 255 K).
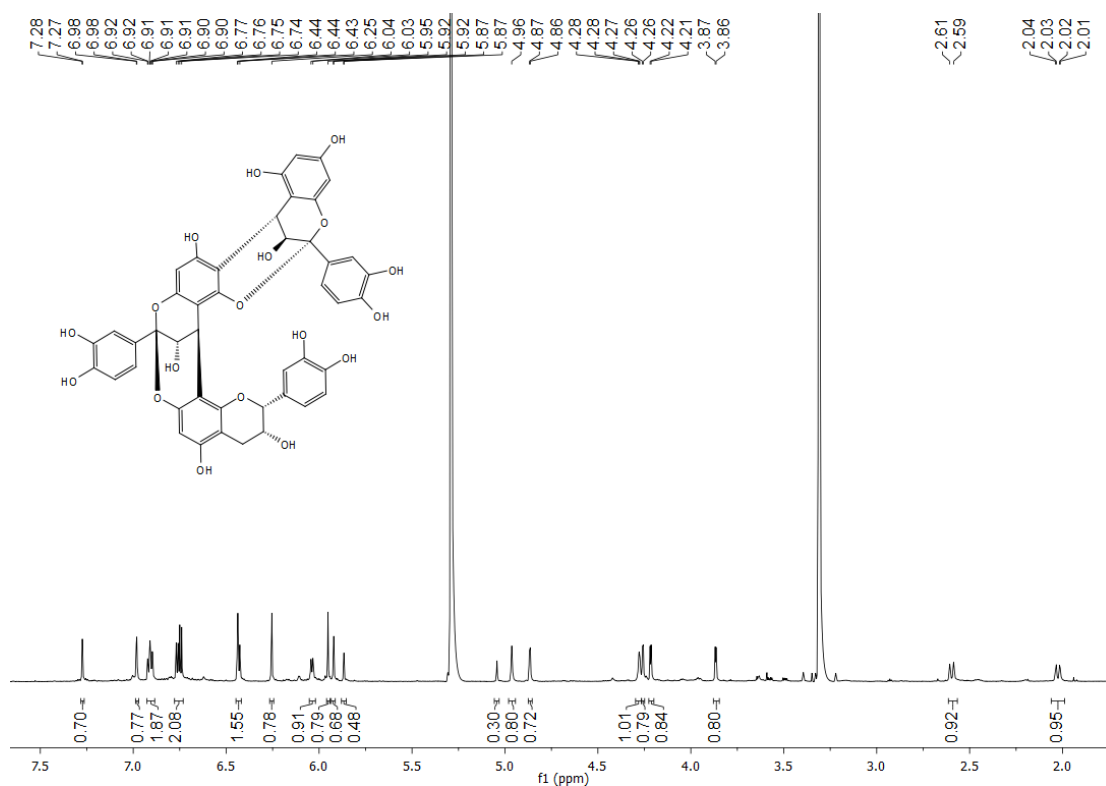

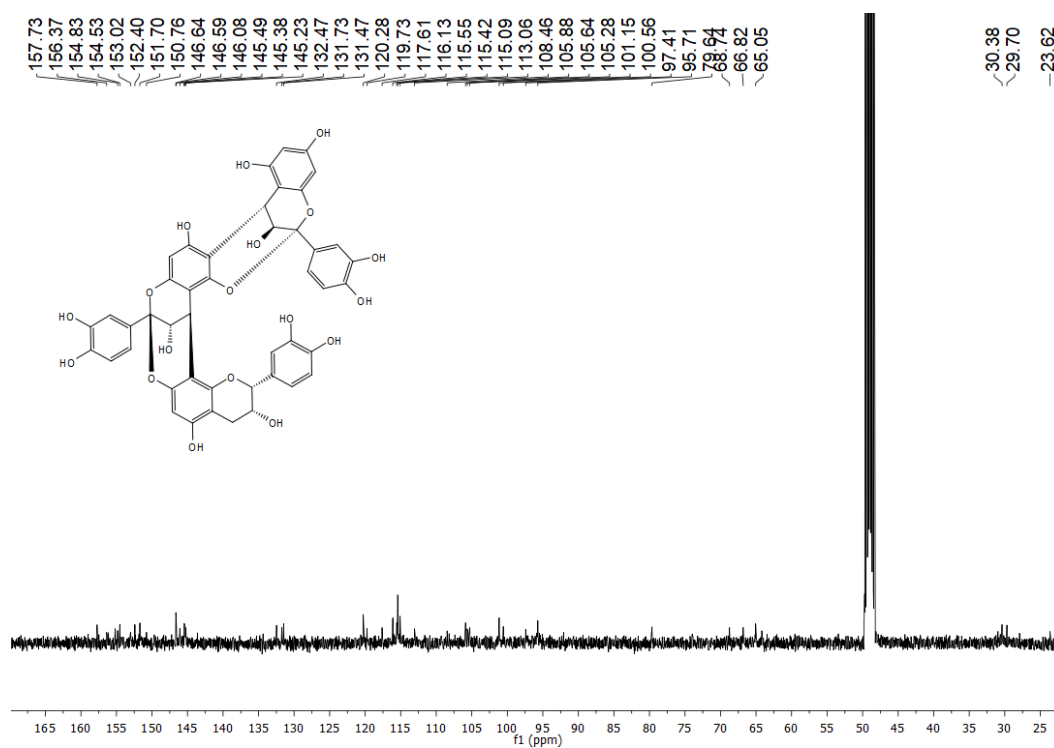**Figure S4.** ^{13}C NMR spectrum of **1** in CD$_3$OD (100 MHz, 255 K).

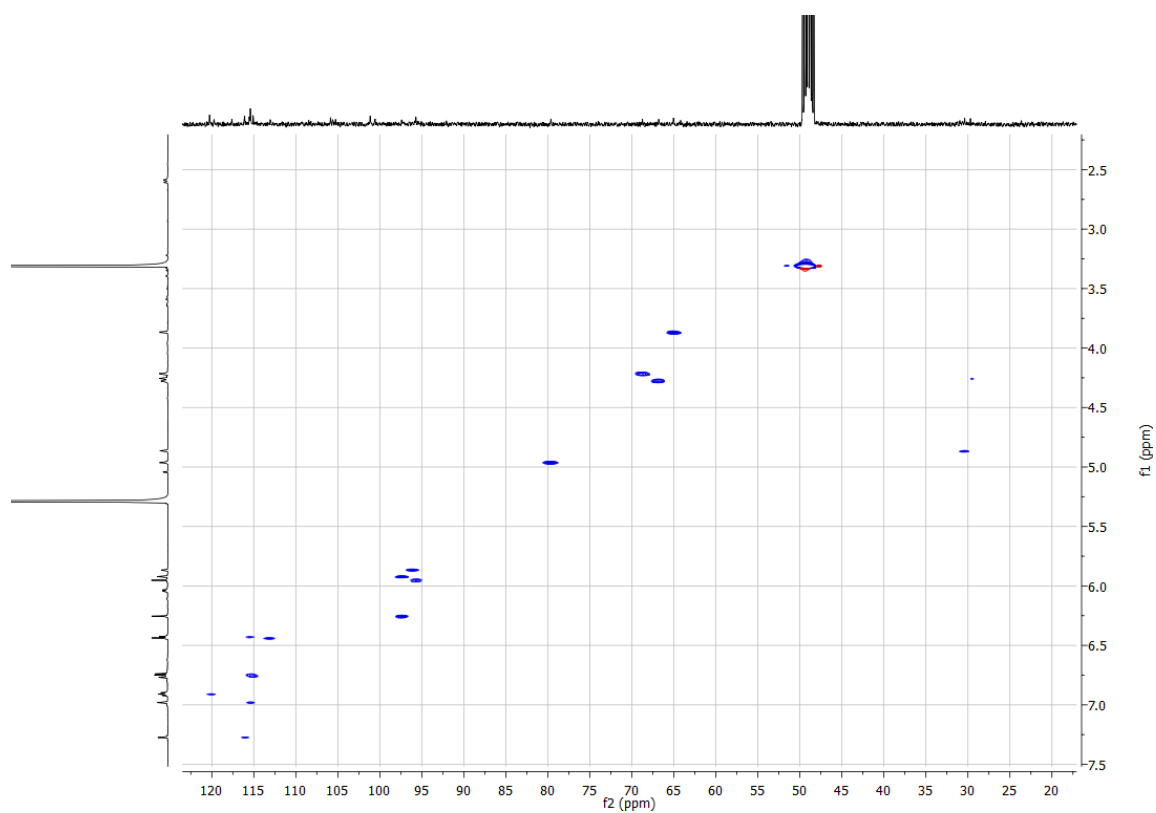**Figure S5.** HSQC spectrum of **1** in CD₃OD (255 K).

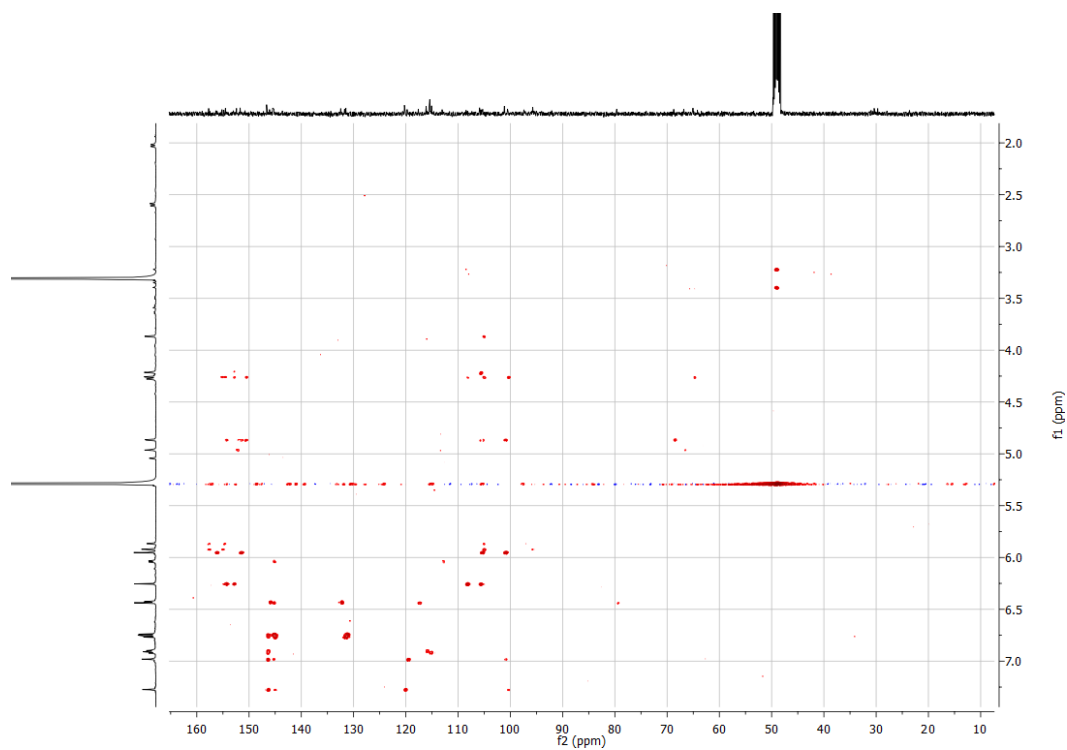**Figure S6.** HMBC spectrum of **1** in CD₃OD (255 K).

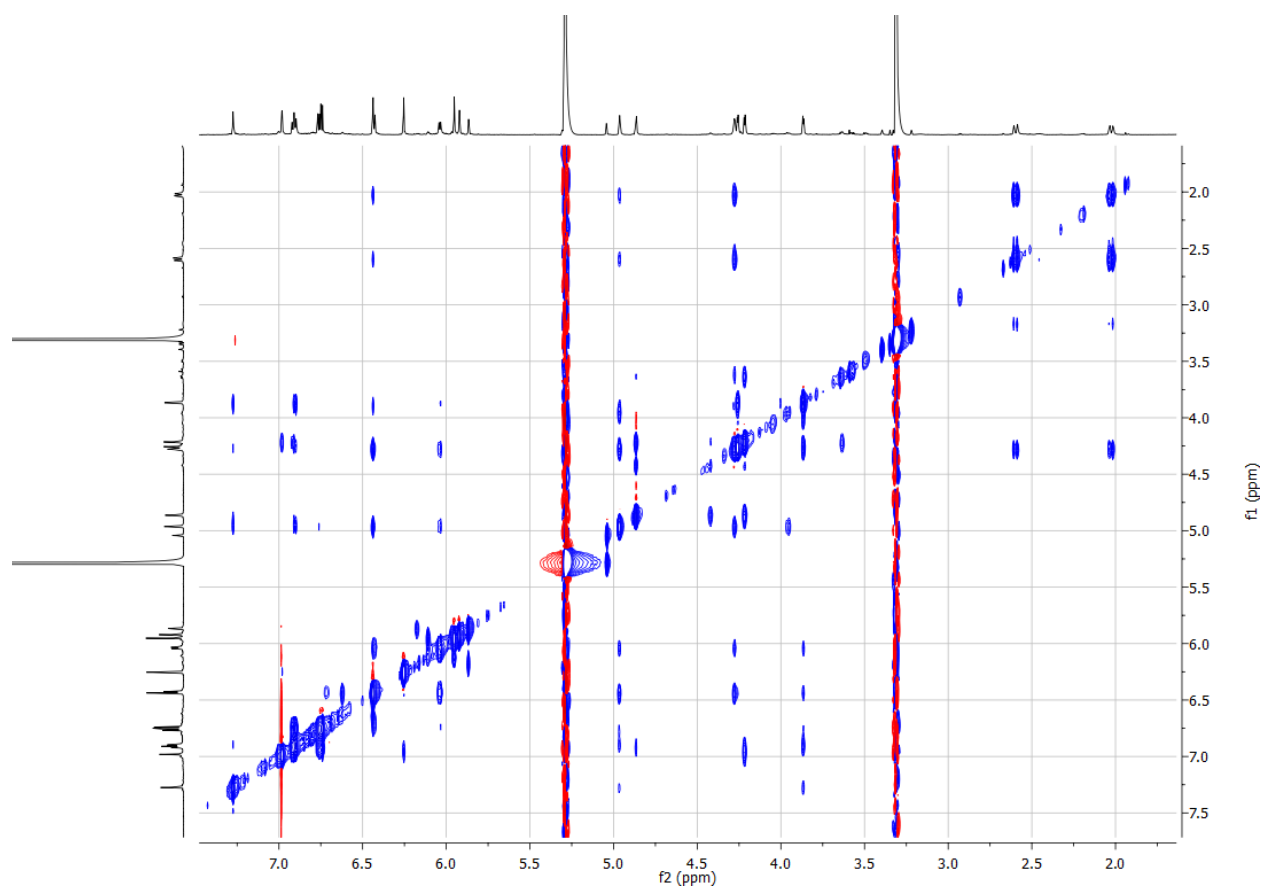**Figure S7.** NOESY spectrum of **1** in CD₃OD (255 K).

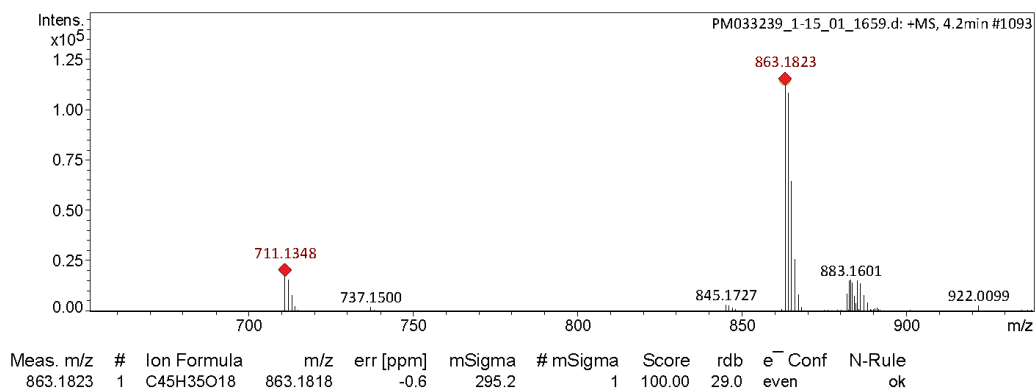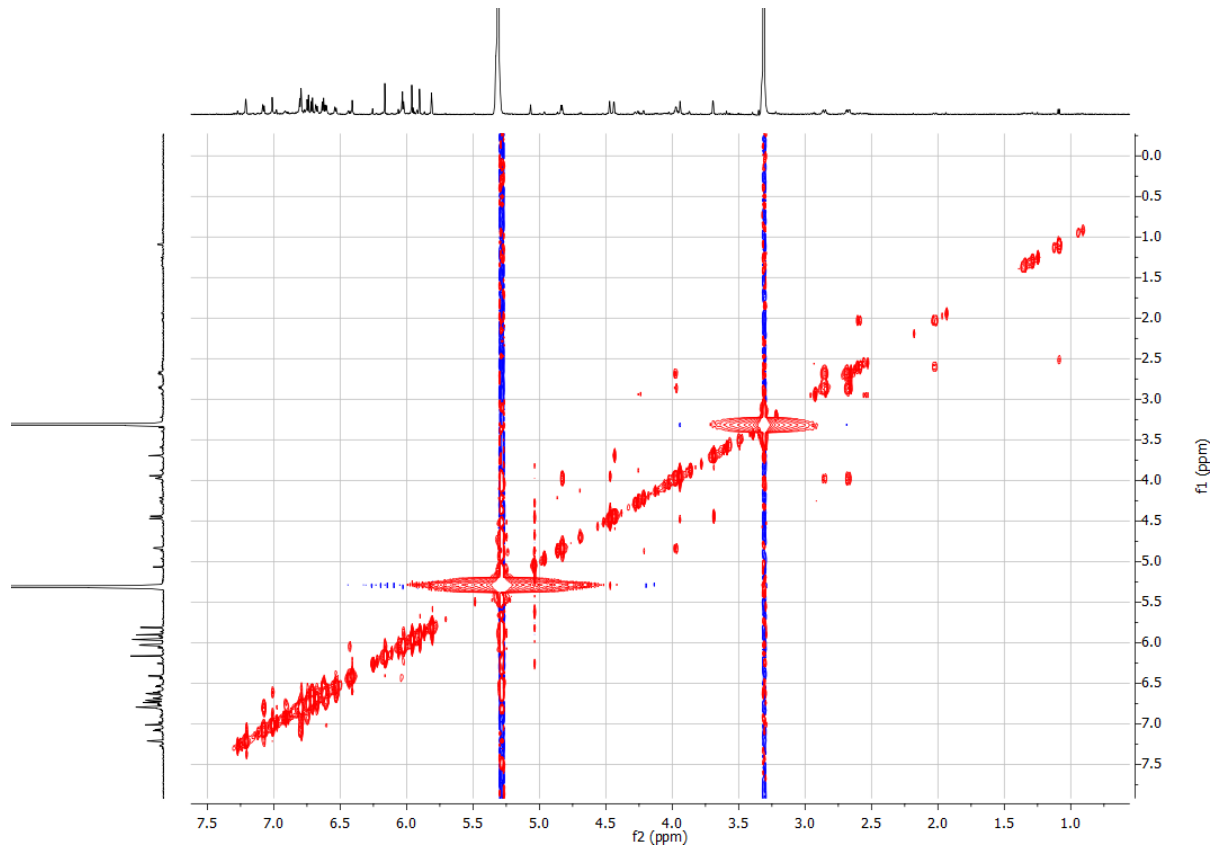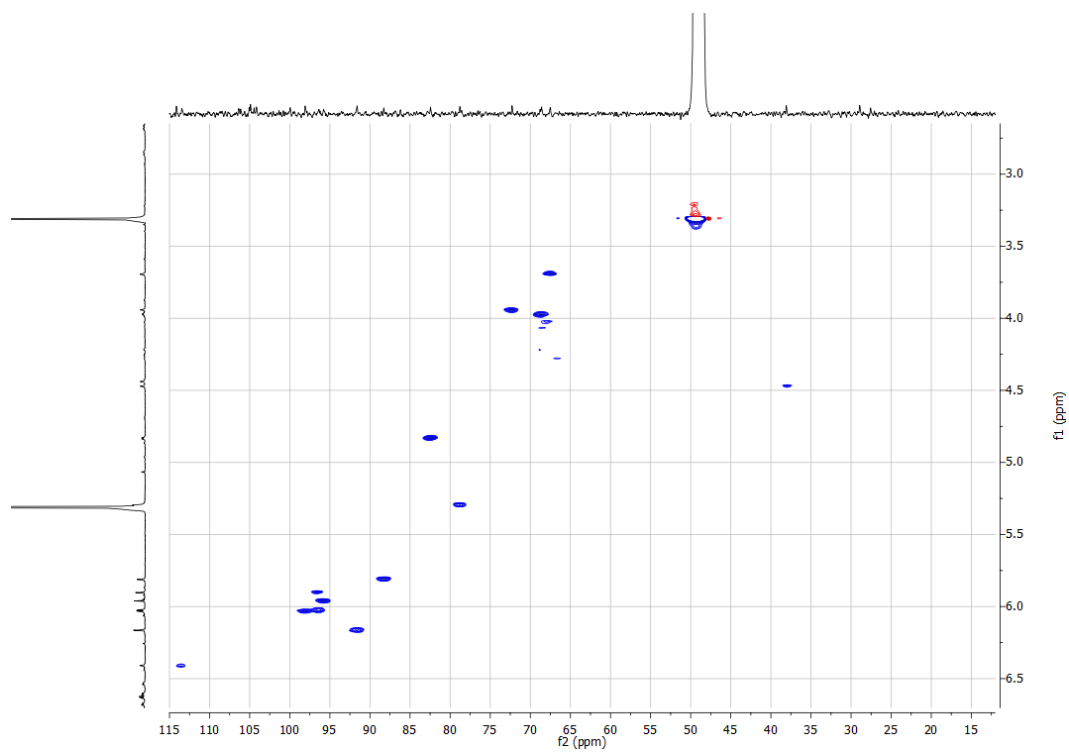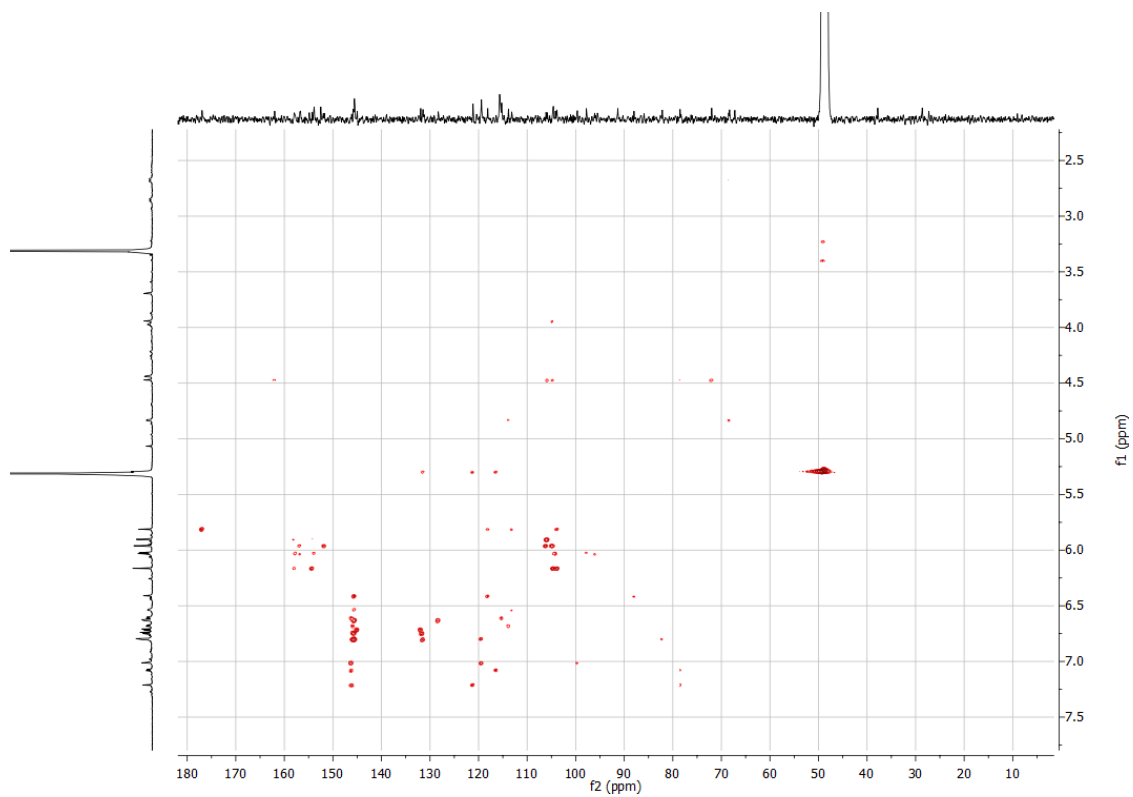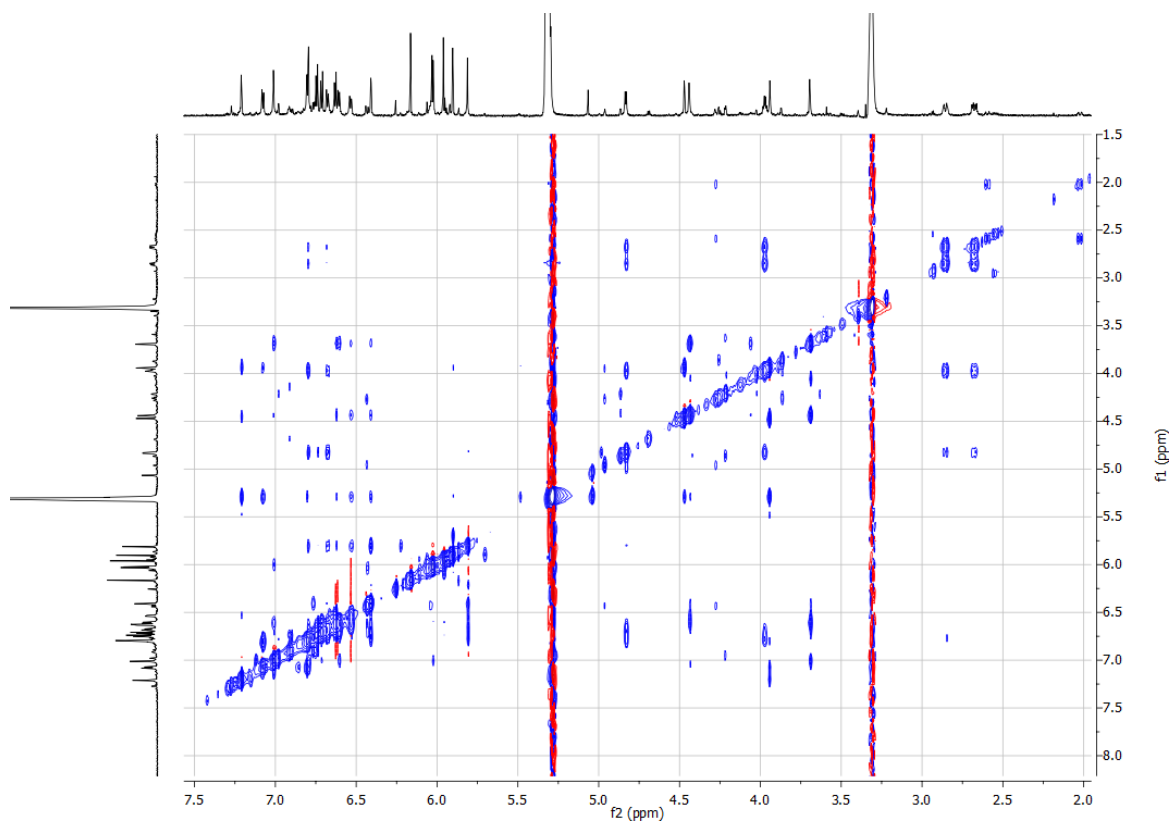**Figure S8.** (+)-HRESIMS spectrum of **1**.

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$_3$OD (800 MHz, 255 K).

Figure S10. Comparison of the ^{1}H NMR spectra of **2** in CD$_3$OD at 278 K and 255 K (800 MHz).

Figure S11. ^{13}C NMR spectrum of **2** in CD$_3$OD (100 MHz, 255 K).

Figure S12. $^1H-^1H$COSY spectrum of **2** in CD$_3$OD (255 K).

Figure S13. HSQC spectrum of **2** in CD₃OD (255 K).

Figure S14. HMBC spectrum of **2** in CD₃OD (255 K).

Figure S15. NOESY spectrum of **2** in CD₃OD (255 K).
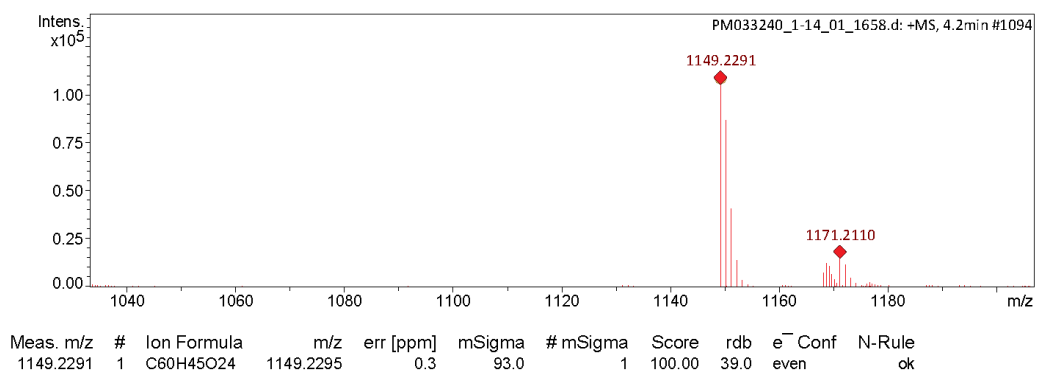

Figure S16. (+)-HRESIMS spectrum of **2**.

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$_3$OD (800 MHz, 278 K).

Figure S18. ^{13}C NMR spectrum of **3** in CD$_3$OD (100 MHz, 278 K).

Figure S19. ^{1}H–^{1}HCOSY spectrum of **3** in CD$_3$OD (278 K).
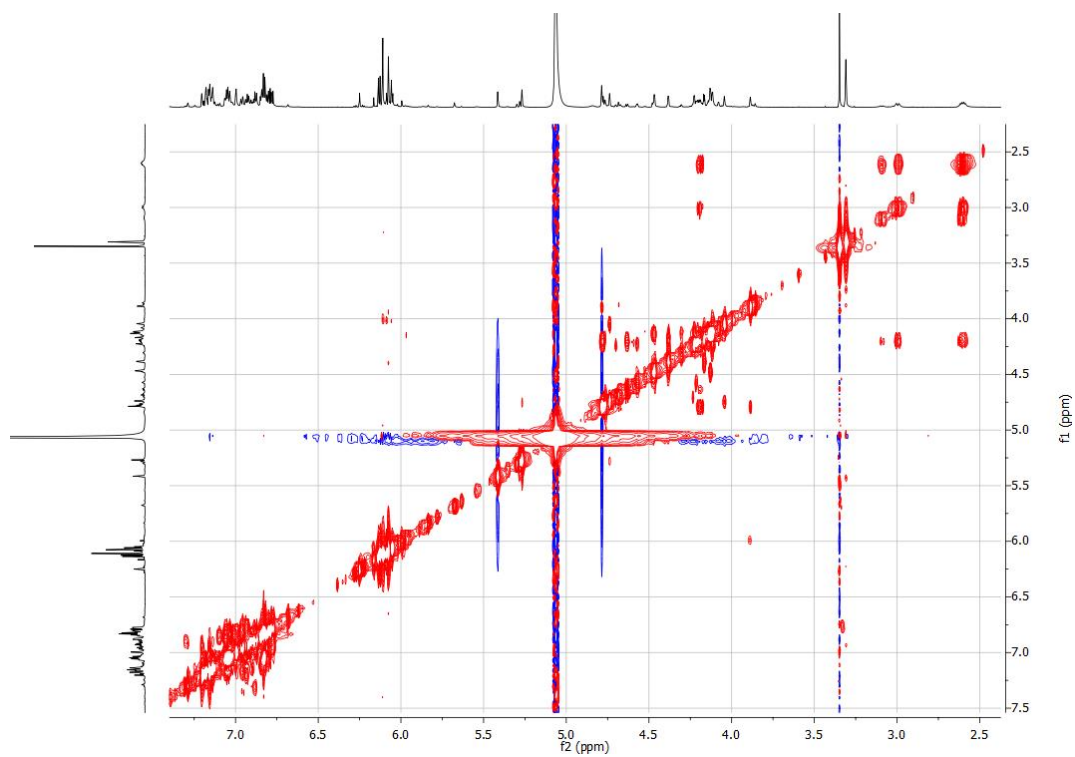

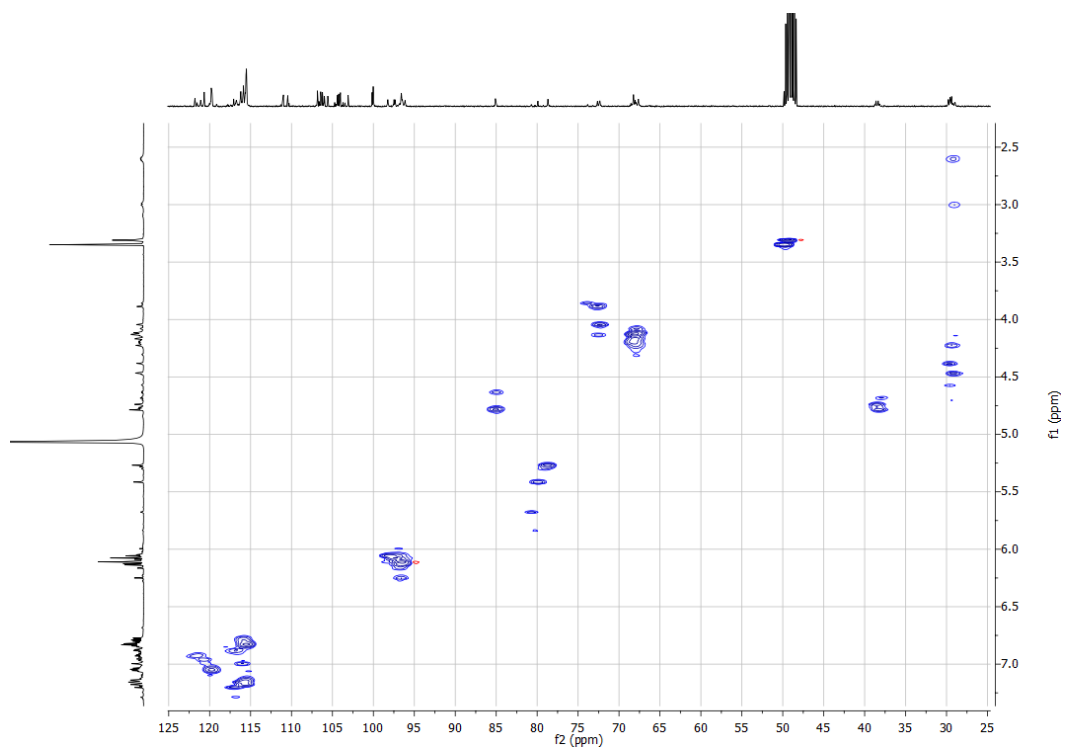**Figure S20**. HSQC spectrum of **3** in CD$_3$OD (278 K).

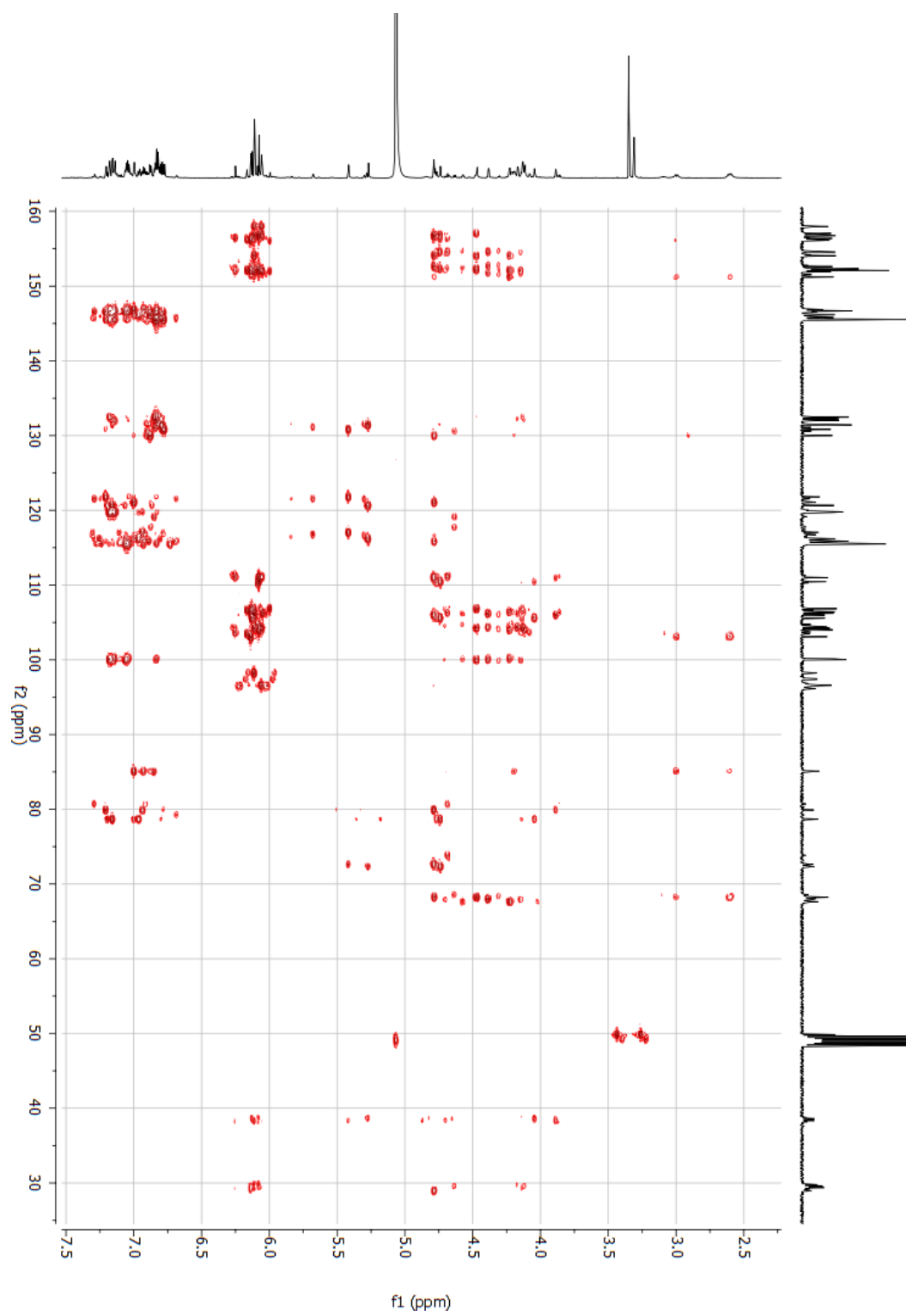**Figure S21.** HMBC spectrum of **3** in CD₃OD (278 K).
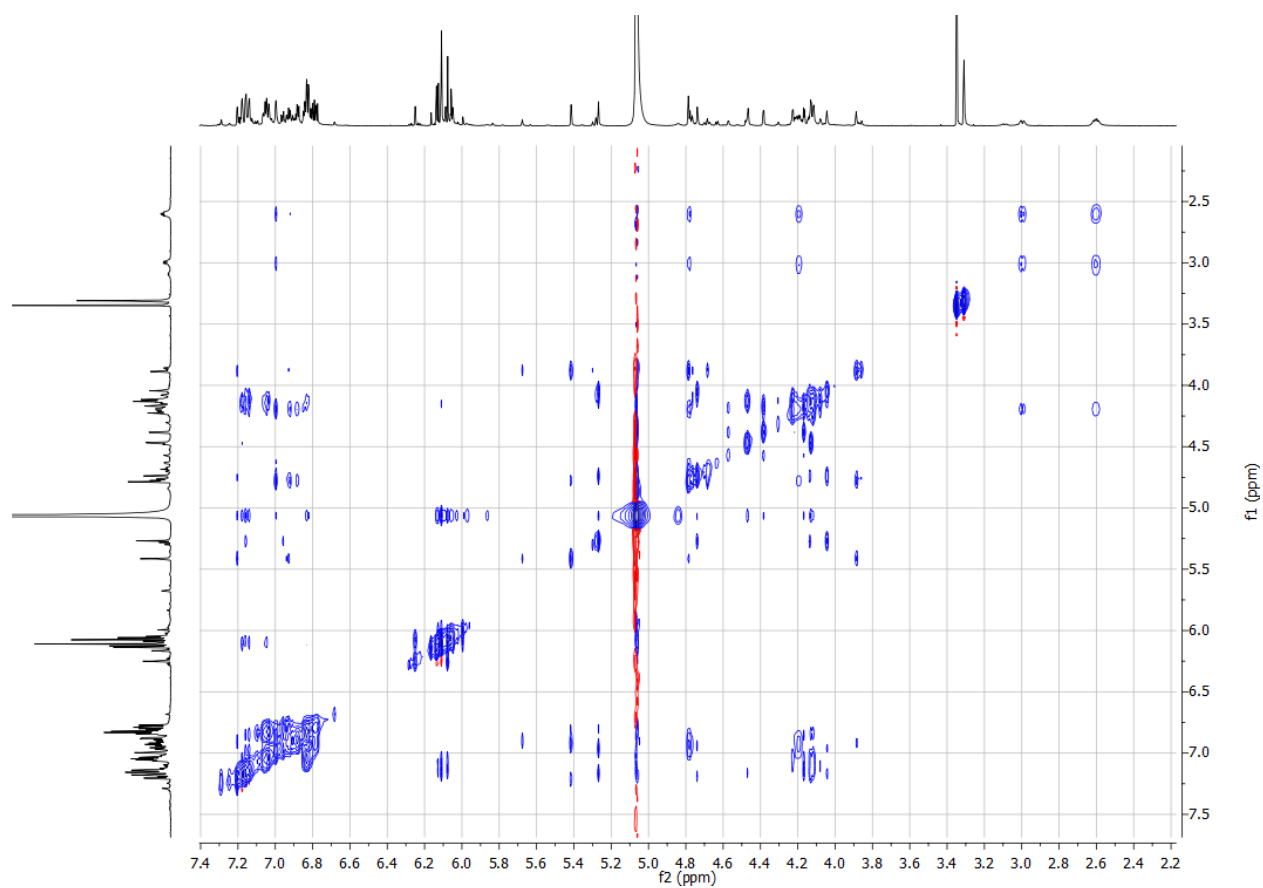
Figure S22. NOESY spectrum of **3** in CD₃OD (278 K).

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

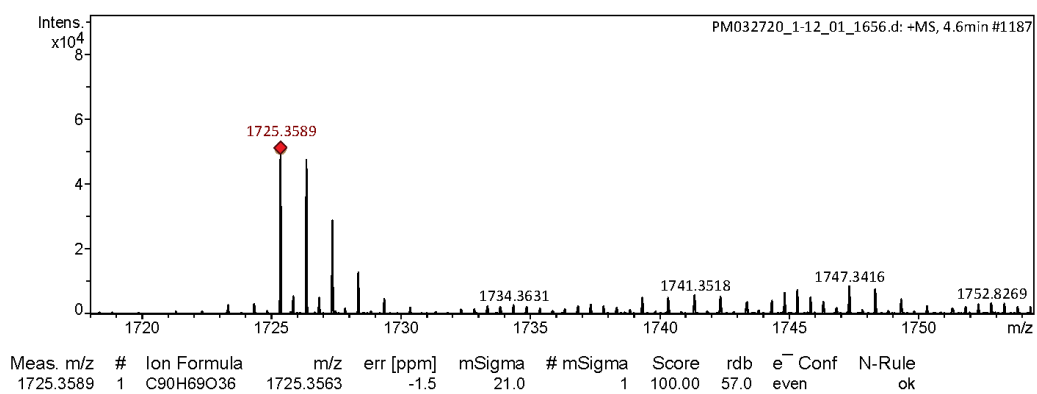**Figure S24.** Phloroglucinolysis products of **3** identified by chiral phase HPLC and MS analysis.