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Article

Novel Bioactive Dibenzocyclooctadiene Lignans from *Schisandra neglecta*

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Quatro novas dibenzociclooctadienolignanas, neglectalignanas A-D (**1**-**4**), juntamente com dezenove compostos conhecidos (**5**-**23**), foram isolados das hastes de *Schisandra neglecta*. Suas estruturas e esteroquímicas foram elucidadas por métodos espectroscópicos, incluindo as técnicas de ressonância magnética nuclear (RMN) de 1D e 2D, e espectrometria de massas de alta resolução com ionização por electrospray (HR-ESI-MS). Avaliou-se as atividades anti-HIV e a citotoxicidade dos compostos **1**-**4**, e os resultados mostraram que os compostos **1**-**4** apresentam atividadade anti-HIV moderadas com valores de índice terapêutico (TI) acima de 61,7, 22,6, 57,7 e 27,9, respectivamente, e atividades citotóxicas fracas contra algumas das linhas celulares selecionadas.

Four new dibenzocyclooctadiene lignans, neglectalignans A-D (**1**-**4**), together with nineteen known compounds (**5**-**23**) were isolated from the stems of *Schisandra neglecta*. Their structures and stereochemistries were elucidated by spectroscopic methods, including 1D-, 2D-nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) techniques. Compounds **1**-**4** were evaluated for their anti-HIV activities and cytotoxicities. The results revealed that compounds **1**-**4** showed moderate anti-HIV-1 activities with therapeutic index (TI) values above 61.7, 22.6, 57.7, and 27.9, respectively, and weak cytotoxic activities for some selected cell lines.

Keywords: *Schisandra neglecta*, dibenzocyclooctadiene lignans, anti-HIV-1 activities, cytotoxicities

Introduction

The family *Schisandraceae*, consisting of *Schisandra* and *Kadsura* genera, is medicinally important. The stems and fruits of *Schisandraceae* plant are commonly used in traditional Chinese medicine for their diverse beneficial bioactivities.[1,2] Previous studies showed that plant of the *Schisandraceae* family are rich in lignans, especially dibenzocyclooctadienes, which have been found to possess some beneficial pharmacological effects, including anti-HIV, antitumor, cytotoxic, antioxidant and antihepatotoxic effects.[3-5]

S*chisandra neglecta* A. C. Smith is a climbing plant mainly distributed in southwest China. In previous studies, some new dibenzocyclooctadiene lignans were isolated from the fruits of *S. neglecta* from Dali Prefecture, Yunnan Province,[6] the stems of *S. neglecta* from Xizang Autonomous Region,[7,8] and the stems of *S. neglecta* from the Xichang Prefecture, Sichuan Province.[9] In our continuing efforts to identify bioactive natural products from the *Schisandraceae* medicinal plants, a chemical investigation on the stem of *S. neglecta*, indigenous to the Dali Prefecture of Yunnan Province, was carried out, leading to the characterization of four new dibenzocyclooctadiene lignans, together with nineteen known compounds. In addition, the anti-HIV-1 activities and cytotoxicities of compounds **1**-**4** were evaluated.

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Results and Discussion

The stems of *S. neglecta* were extracted with 70% acetone. The extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) to afford four new dibenzocyclooctadiene lignans, named as neglectalignans A-D (**1**-**4**), together with nineteen known compounds (**5**-**23**), which were identified by comparison with literature data as rubrisandrin A (**5**),[10] schineglignan A (**6**),[6] schineglignan B (**7**),[6] marlignan B (**8**),[11] rubrilignan B (**9**),[12] marlignan G (**10**),[11] epigomisin O (**11**),[13] gomisin D (**12**),[13] wilsonilignan C (**13**),[14] rubschizantherin (**14**),[13] isogomisin O (**15**),[15] gomisin T (**16**),[16] schizandrin (**17**),[13] (+)-gomisin K (**18**),[13] angeloygomisin Q (**19**),[13] tigloygomisin Q (**20**),[13] benzoylgomisin Q (**21**),[17] gomisin D (**22**),[18] and gomisin E (**23**)[15] (Figure 1).

Compound **1** was obtained as a yellow gum, and the molecular formula was determined as $C_{23}H_{28}O_7$ by HRESIMS at *m*/*z* 439.1725 $[M+Na]^+$ (calcd *m*/*z* 439.1733). Its ^{1}H and ^{13}C NMR spectra (Table 1) showed signals for 28 hydrogens and 23 carbons, respectively, corresponding to two aromatic rings with two aromatic protons (δ_H 7.02 and 6.54), two methylene carbons (δ_C 36.0 and 39.8), two methine carbons (δ_C 34.1 and 41.8), two methyl groups (δ_C 12.8 and 21.8), three methoxy groups (δ_C 60.1, 60.6, and 60.5), two phenolic hydroxy groups (δ_H 10.61 and 11.16), and an acetoxy group (δ_C 169.9 and 21.0). UV spectrum displayed absorption bands at 210 and 245 nm. The IR spectrum showed the presence of hydroxy group (3452 cm^{-1}) and ester group (1748 cm^{-1}). In addition, ^{1}H-^{1}H COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18 (Figure 2), together with the HMBC correlations (Figure 2) of H-11 (δ_H 6.54) with C-9 (δ_C 36.0), C-10 (δ_C 134.9) and C-15 (δ_C 119.8), and of H-4 (δ_H 7.02) with C-5 (δ_C 135.2), C-6 (δ_C 39.8), and C-16 (δ_C 122.0) implied that **1** is a dibenzocyclooctadiene lignan possessing three methoxy groups, two phenolic hydroxy groups, and an acetoxy group. The ^{1}H and ^{13}C NMR spectra of **1** were found to be similar to those of marlignan A.[11] Analysis of the ^{1}H and ^{13}C NMR data of **1** suggested that the only difference was due to a hydroxy group in marlignan A on an aromatic ring being replaced by an acetoxy group in **1**. A hydroxy group located at C-3 was supported by HMBC correlations of the hydroxy proton signal at δ_H 10.61 with C-2 (δ_C 140.5), C-3 (δ_C 150.3) and C-4 (δ_C 107.0), and another hydroxy group located at C-14 was supported by HMBC correlations of proton signal at δ_H 11.16 with C-13 (δ_C 140.0), C-14 (δ_C 140.0), and C-15 (δ_C 140.0). The HMBC correlations of three methoxy protons (δ_H 3.84, 3.90, 3.89) with C-1, C-2, and C-13, suggested that these methoxy groups could be positioned at C-1 (δ_C 151.6), C-2 (δ_C 140.5), and C-13 (δ_C 140.0), respectively. Since the positions of hydroxy

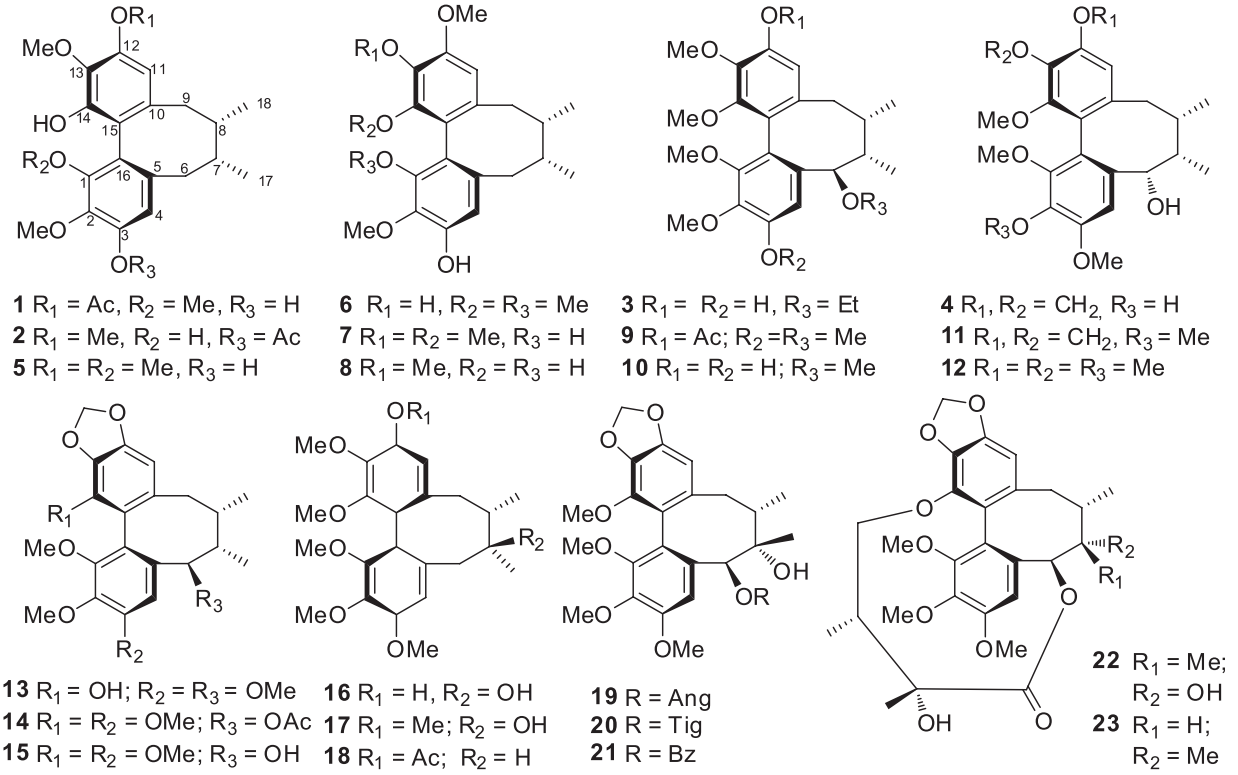

Figure 1. The structure of dibenzocyclooctadiene lignans from *S. neglecta*.

and methoxyl groups were determined, the acetoxy group should be located at C-12. In the cyclooctadiene ring, the signals for two methines were assigned to C-7 and C-8, two benzylic methylenes were attributed to C-6 and C-9, and two methyl groups located at C-17 and C-18, respectively,

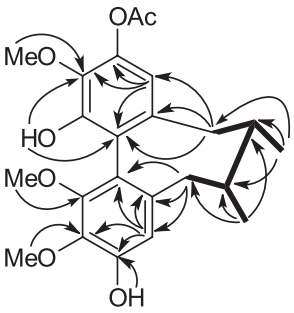

Figure 2. Selected HMBC (⌒) and ^{1}H-^{1}H COSY (▬) correlations of **1**.

Table 1. ^{1}H NMR and ^{13}C NMR Data of compounds **1** and **2** (obtained in C_5D_5N, 500 and 125 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J* / Hz)	δ_C (m)	δ_H (m, *J* / Hz)
1	151.6 (s)		145.9 (s)	
2	140.5 (s)		141.8 (s)	
3	150.3 (s)		144.5 (s)	
4	107.0 (d)	7.02 (s)	114.3 (d)	7.02 (s)
5	135.2 (s)		135.1 (s)	
6	39.8 (t)	2.63 (dd, *J* 13.5, 7.0)	39.3 (t)	2.50 (overlap)
		2.67 (d, *J* 13.3)		2.63 (d, *J* 13.3)
7	34.1 (d)	1.85 (overlap)	34.2 (d)	1.80 (m)
8	41.8 (d)	1.85 (overlap)	41.7 (d)	1.85 (m)
9	36.0 (t)	2.07 (d, *J* 13.0)	36.2 (t)	2.12 (d, *J* 12.9)
		2.48 (dd, *J* 9.2, 12.9)		2.52 (overlap)
10	134.9 (s)		134.2 (s)	
11	108.5 (d)	6.54 (s)	106.1 (d)	6.62 (s)
12	141.4 (s)		151.0 (s)	
13	140.0 (s)		138.7 (s)	
14	148.7 (s)		142.3 (s)	
15	119.8 (s)		118.4 (s)	
16	122.0 (s)		122.4 (s)	
17	12.8 (q)	0.77 (d, *J* 6.7)	13.1 (q)	0.71 (d, *J* 7.0)
18	21.8 (q)	0.89 (d, *J* 6.7)	22.0 (q)	0.91 (d, *J* 7.0)
OMe-1	60.1 (q)	3.84 (s)		
OMe-2	60.5 (q)	3.90 (s)	60.9 (q)	3.84 (s)
OMe-12			55.9 (q)	3.81 (s)
OMe-13	60.6 (q)	3.89 (s)	60.9 (q)	3.92 (s)
Ar–OH-1				10.42 (brs)
Ar–OH-3		10.61 (brs)		
Ar–OH-14		11.16 (brs)		11.09 (brs)
–OAc	169.9 (s)		169.7 (s)	
	21.0 (q)	1.95 (s)	21.0 (q)	1.94 (s)

based on the analysis of its ^{1}H-^{1}H COSY and HMBC spectra. Thus, the planar structure of **1** was established.

The CD spectrum of **1** gave a negative Cotton effect at 252 nm and a positive Cotton effect at 225 nm, indicating that **1** has a *S*-biphenyl configuration.[19,20] The ROESY correlations between H-4/H-7, H-4/H-6β, H-9β /H-11, H-9β/CH_3-17, H-9α/CH_3-18, H-9α/H-8, and CH_3-17/CH_3-18 in **1** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring.[19,20] The substituent positions and stereochemical assignments in the cyclooctadiene ring of **1** were supported by computer generated molecular model using MM2 force field in CS Chem 3D (Figure 3). Thus, the structure of **1** was determined as shown, and this compound has been given the trivial name as neglectaphenol A.

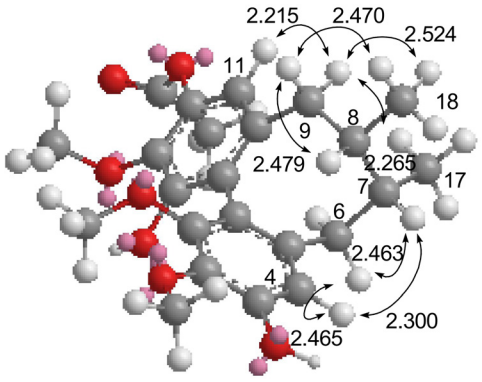

Figure 3. Computer generated molecular model showing key ROESY correlations and corresponding interatomic distance (A) of compound **1**.

Compound **2** was obtained as a yellow gum, and showed sodiated molecular ions at *m/z* 439.1736 in the HRESIMS (calcd *m/z* 439.1733), indicating the same molecular formula with that of compound **1**. The ^{1}H- and ^{13}C NMR spectra of **2** were similar to those of **1** (Table 1). The IR spectrum also showed the presence of hydroxy group (3458 cm^{-1}) and ester group (1746 cm^{-1}). The obvious chemical shift differences resulted from the substituent group variations in the aromatic rings. Analysis of the HSQC, HMBC, and ROESY spectra of **2** showed that three methoxy groups are located at C-2, C-12, and C-13, two phenolic hydroxy groups at C-1 and C-14, and one acetoxy group located at C-3. Thus, the structure of **2** was established, and it has been accorded the trivial name neglectaphenol B.

Compound **3**, obtained as a yellow gum, was assigned the molecular formula, $C_{24}H_{32}O_7$, from its HRESIMS at *m*/z 455.2034 $[M+Na]^+$ (calcd *m*/z 455.2046). Its ^{1}H, ^{13}C and DEPT NMR spectra showed signals for 24 carbons and 32 hydrogens (Table 2), corresponding to two aromatic rings with two aromatic protons (δ_H 6.98 and 6.69), one methylene

(δ_C 37.6), two methines (δ_C 37.8 and 36.5), one oxygenated methine (δ_C 88.2), two methyl (δ_C 17.1 and 17.3), four methoxy (δ_C 60.5, 60.9, 60.3 and 60.7), two phenolic hydroxy groups (δ_H 9.72 and 10.00), and an ethoxy group (δ_C 63.3, 15.5). The HMBC correlations of H-11 (δ_H 6.69) with C-9 (δ_C 37.6), C-10 (δ_C 137.2), and C-15 (δ_C 121.2), and of H-4 (δ_H 6.98) with C-5 (δ_C 136.0), C-6 (δ_C 88.2), and C-16 (δ_C 123.5), together with ^{1}H-^{1}H COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18, as well as UV absorption bands at 205 and 240 nm, implied that **3** was also a dibenzocyclooctadiene lignan.[19,20] However, the IR spectrum was different from that of **2** with the absence of an ester group (1746 cm^{-1}). The ethoxy group located at C-6 was supported by the HMBC correlations of the oxygenated methylene proton (δ_H 3.22) with C-6 (δ_C 88.2), and of H-6 (δ_H 4.06) with oxygenated methylene carbon (δ_C 63.3). In dibenzocyclooctadiene lignans, the chemical shifts of methoxy groups at C-3 and C-12 occur at δ_C 55-56, whereas those of methoxy groups at C-1, C-2, C-13, C-14 are found to be δ_C 60-61.[3,21] Four methoxy groups located at C-1, C-2, C-13, and C-14 were confirmed by the analysis of its HMBC spectrum in **3**. According to the molecular formula, the quaternary carbon at C-3 and C-12 should both be substituted by a hydroxy group. In the cyclooctadiene ring, the oxygenated methine carbon was assigned to C-6 on the basis of the HMBC correlation from H-4 (δ_H 6.98) to C-6 (δ_C 88.2). The CD spectrum of **3** (negative Cotton effect at 249 nm and a positive Cotton effect at 220 nm) indicated that **3** has a *S*-biphenyl configuration.[19,20] The ROESY correlations between H-4/CH_3-17 and H-11/H-9β in **3** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring.[19,20] The configuration of the ethoxy group attached to C-6 was deduced as being β-oriented by the chemical shift (δ_C 88.2), which was similar to β-oriented derivatives of the marlignan J,[11] and distinct from that of 6-α-oriented components in dibenzocyclooctadiene lignan family.[22] This was confirmed by the ROESY correlations between H-4/H-6α and H-4/CH_3-17. Thus, the structure of **3** (neglectaphenol C) was established, as shown.

Compound **4** was obtained as a yellow gum. Its molecular formula was determined as $C_{22}H_{26}O_7$ by its HRESIMS *m*/z 425.1577 $[M+Na]^+$. It showed absorption maxima in the UV spectrum at 210, 243 nm, and a strong negative Cotton effect at 250 nm in the CD spectrum, indicating that **4** is a C_{18} dibenzocyclooctadiene lignan with an *S*-biphenyl configuration.[18,19] The ^{13}C NMR spectrum showed the signals of 12 carbons belonging to a biphenyl at δ_C 103.9 -153.4 (Table 2). Besides the aromatic protons of biphenyl that appeared at δ_H 6.89 and 6.62 (1H each, s), the ^{1}H NMR spectrum of **4** also indicated the presence of one methylenedioxy unit at δ_H 5.92, 5.99 (1H, each s), three methoxy groups at δ_H 3.93, 3.98, and 3.96 (3H each, s), one phenolic hydroxy group (δ_H 10.81, brs), and two secondary methyls at δ_H 0.96 (3H each, d, *J* 7.2 Hz) and 1.03 (3H, d, *J* 7.2 Hz). From the HMBC spectrum of **4**, it was found that the single sp^3 oxymethine carbon resonating at C-6 (δ_C 73.2, d) correlated with a proton at H-6 (δ_H 4.93, 1H, brs), and correlations observed from H-6 (δ_H 4.93, brs) to the aromatic C-4 (δ_C 110.0 d) and C-16 (δ_C 122.8 s) were used to assign the oxymethine group at C-6. Further analysis of the HMBC spectrum showed that the methylenedioxy unit was attached to C-12 and C-13, the three methoxy groups were located at C-1, C-3, and C-14, and the phenolic hydroxy group located at C-2 respectively.

Table 2. ^{1}H NMR and ^{13}C NMR Data of compounds **3** and **4** (500 and 125 MHz)

No.	Compound **3**[a]		Compound **4**[b]	
	δ_C (m)	δ_H (m, *J* / Hz)	δ_C (m)	δ_H (m, *J* / Hz)
1	151.9 (s)		153.4 (s)	
2	141.1 (s)		139.5 (s)	
3	150.3 (s)		151.5 (s)	
4	116.1 (d)	6.98 (s)	110.0 (d)	6.89 (s)
5	136.0 (s)		137.4 (s)	
6	88.2 (d)	4.06 (d, *J* 8.2)	73.2 (d)	4.93 (brs)
7	38.7 (d)	1.80 (m)	44.5 (d)	2.05 (m)
8	36.5 (d)	2.05 (m)	40.2 (d)	2.44 (overlap)
9	37.6 (t)	2.19 (m)	35.7 (t)	2.15 (d, *J* 16.4)
		2.48 (m)		2.44 (overlap)
10	137.2 (s)		139.0 (s)	
11	111.0 (d)	6.69 (s)	103.9 (d)	6.62 (s)
12	149.3 (s)		149.2 (s)	
13	139.7 (s)		136.3 (s)	
14	151.0 (s)		143.3 (s)	
15	121.2 (s)		121.5 (s)	
16	123.5 (s)		122.8 (s)	
17	17.1 (q)	0.85 (overlap)	8.7 (q)	0.96 (d, *J* 7.2)
18	17.3 (q)	0.85 (overlap)	22.1 (q)	1.03 (d, *J* 7.2)
OMe-1	60.5 (q)	3.72 (s)	60.8 (q)	3.93 (s)
OMe-2	60.9 (q)	3.85 (s)		
OMe-3			55.9 (q)	3.98 (s)
OMe-13	60.3 (q)	3.81 (s)		
OMe-14	60.6 (q)	3.75 (s)	60.9 (q)	3.96 (s)
Ar–OH-2				10.81 (brs)
Ar–OH-3		9.72 (brs)		
Ar–OH-12		10.00 (brs)		
1’	63.3 (t)	3.22 (m)	101.4 (t)	5.92, 5.99 (s)
2’	15.5 (q)	0.96 (t, *J* 7.0)		

[a]obtained in $CDCl_3$; [b]obtained in C_5D_5N.

The *a*-orientation of the hydroxy group at C-6 was confirmed by its chemical shift of ^{13}C (δ_C 73.2 d), and ^{1}H (δ_H 4.93, brs), which was similar to that of the *a*-oriented derivatives of gomisins.[15,23] This was further confirmed by the ROESY correlation for one of the C-9 protons (δ_H 2.15) with the aromatic H-11 (δ_H 6.62), which allowed the assignement of β H-9 orientation. The ROESY correlations found between H-9β (δ_H 2.15) and H-8 (δ_H 2.44) and between H-7 (δ_H 2.05) and H-6 (δ_H 4.93) confirmed the α-orientation of 6-OH.[15,23] The above observations were used to establish the structure of neglectaphenol D (**4**) as shown.

Since some dibenzocyclooctadiene lignans from *Schisandra* species are reported to possess anti-HIV activities and cytotoxicities,[11,24,25] the anti-HIV-1 activities and cytotoxicities of compounds **1**-**4** were tested. The cytotoxicity assay against C8166 cells (CC_{50}), and anti-HIV-1 activity were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control (EC_{50} = 0.034 μg mL^{-1} and CC_{50} > 200 mg mL^{-1}).[26] The results are shown in Tables 3 and 4. The results revealed that compounds **1**-**4** showed moderate anti-HIV-1 activities with therapeutic index (TI) values above 61.7, 22.6, 57.7, and 27.9, respectively.

Table 3. Anti-HIV Activities of the compounds **1-4**

Compounds	CC_{50} / (μg mL^{-1})	EC_{50} / (μg mL^{-1})	TI (CC_{50}/EC_{50})
1	> 200	3.24	> 61.7
2	32.04	1.42	22.6
3	> 200	3.47	> 57.7
4	> 200	7.15	> 27.9

Table 4. Cytotoxicity data for the compounds **1**-**4** (μg mL^{-1})

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	7.9	> 10	8.6	> 10	8.7
2	7.3	> 10	> 10	8.6	> 10
3	> 10	> 10	> 10	> 10	> 10
4	5.2	1.3	7.9	> 10	5.6
Taxol	0.03	0.02	0.2	0.2	0.1

The cytotoxicity tests for these compounds were performed using a previously reported procedure.[27] All of the experiments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic activities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were tested. The results showed that compounds **1**-**4** have weak cytotoxic activities for some selected cell lines, with IC_{50} > 1.3 μg mL^{-1}.

Experimental Section

General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectrometry. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material

The stems of *S. neglecta* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in July 2009. The identification of the plant material was done by Prof Xi-Wen Li of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB 09-9-36) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered stems of *S. neglecta* (5.0 kg) were extracted four times with 70% acetone (4 × 50 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (3 × 2 L). The EtOAc partition (385 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions A-E. The further separation of fraction B (32.6 g) by silica gel column chromatography, eluted with petroleum ether-acetone (20:1-1:2), yielded mixtures B1-B6. Fraction B2 (4.65 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-

preparative HPLC (75% MeOH-H_2O, flow rate 12 mL min^{-1}) to give **6** (22.6 mg, 13 min), **10** (22.4 mg, 18 min), **11** (8.8 mg, 25 min), and **13** (86.2 mg, 28 min). Fraction B3 (2.8 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (65% MeOH-H_2O, flow rate 12 mL min^{-1}) to give **4** (42.1 mg, 18 min), **5** (16.4 mg, 23 min), **7** (16.3 mg, 26 min), **8** (13.4 mg, 28 min), **9** (14.6 mg, 35 min), and **12** (43.5 mg, 30 min). Fraction B4 (2.7 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semipreparative HPLC (60% MeOH-H_2O, flow rate 12 mL min^{-1}) to afford **1** (11.5 mg, 20 min), **2** (13.4 mg, 23 min) and **3** (15.4 mg, 28 min). Fraction C (60 g) was subjected to silica gel column chromatography using petroleum ether-acetone (20:1-6:4) for elution followed by a reversed-phase column (RP-18) eluting with MeOH-H_2O (30%-90%) and then by Sephadex LH-20 using MeOH as eluant. Further purifications were performed by semipreparative HPLC and preparative HPLC separation (60% MeOH-H_2O) to give compounds **14** (3.5 mg, 38 min), **15** (7.8 mg, 20 min), **16** (10.4 mg, 23 min), **17** (5 mg, 25 min), **18** (3.2 mg, 36 min), **19** (4 mg, 26 min), **20** (3 mg, 29 min), **21** (6 mg, 22 min), **22** (5 mg, 21 min), and **23** (7 mg, 41 min).

Anti-HIV-1 and cytotoxicity assays

The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[26]

The cytotoxicity tests for these compounds were performed against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control).[27]

Neglectaphenol A (**1**)

$C_{23}H_{28}O_7$, obtained as yellow gum; $[\alpha]_D^{24.5}$ = +38.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max}/nm (log ε) 210 (4.18), 245 (3.26), 326 (1.22) ; CD (*c* 0.02, MeOH), nm (Δε) 252 (−17.5), 225 (+7.83), 220 (+4.87); IR (KBr) ν_{max}/cm^{-1} 3452, 2941, 2876, 2833, 1748, 1638, 1590, 1485, 1392, 1326, 1279, 1235, 1192, 1134, 1105, 1062, 1009, 876, 753; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), Table 1; ESIMS (positive ion mode) *m/z* 439 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 439.1725 $[M+Na]^+$ (calcd. 439.1733 for $C_{23}H_{28}NaO_7$).

Neglectaphenol B (**2**)

$C_{23}H_{28}O_7$, obtained as yellow gum; $[\alpha]_D^{24.8}$ = +32.1 (*c* 0.25, MeOH); UV (MeOH) λ_{max}/nm (log ε) 210 (4.22), 245 (3.18), 326 (0.97); IR (KBr) ν_{max}/cm^{-1} 3458, 2962, 2851, 1746, 1635, 1576, 1483, 1462, 1385, 1334, 1276, 1233, 1194, 1076, 1016, 988, 884, 749; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), Table 1; ESIMS (positive ion mode) *m/z* 439 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 439.1736 $[M+Na]^+$ (calcd. 439.1733 for $C_{23}H_{28}NaO_7$).

Neglectaphenol C (**3**)

$C_{24}H_{32}O_7$, obtained as yellow gum; $[\alpha]_D^{24.9}$ = +28.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max}/nm (log ε) 205 (4.24), 240 (3.81), 329 (0.86); CD (*c* 0.10, MeOH), nm (Δε) 249 (–68.2), 240 (–39.5), 220 (+25.2), 210 (+6.3); IR (KBr) ν_{max}/cm^{-1} 3438, 2942, 2925, 2876, 1624, 1579, 1495, 1453, 1408, 1328, 1276, 1097, 1069, 984, 862; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 2; ESIMS (positive ion mode) *m/z* 455 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 455.2034 $[M+Na]^+$ (calcd. 455.2046 for $C_{24}H_{32}NaO_7$).

Neglectaphenol D (**4**)

$C_{22}H_{26}O_7$, obtained as yellow gum; $[\alpha]_D^{25.0}$ = –45.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max}/nm (log ε): 210 (4.15), 243 (3.32), 314 (0.59); CD (c 0.05, MeOH), nm (Δε) 250 (–22.2), 240 (–16.5), 218 (+7.62), 210 (–2.24); IR (KBr) ν_{max}/cm^{-1} 3441, 2925, 2847, 1612, 1586, 1465, 1372, 1323, 1083, 1046, 1028, 952, 860; ^1H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), Table 2; ESIMS (positive ion mode) *m/z* 425 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 425.1577 $[M+Na]^+$ (calcd. 425.1576 for $C_{22}H_{26}NaO_7$).

Supplementary Information

^1H and ^{13}C NMR, HSQC, HMBC COSY, ROESY, HRESIMS, and CD spectra of **1**, ^1H and ^{13}C NMR, spectra of **2-4**, are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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Supplementary Information

Novel Bioactive Dibenzocyclooctadiene Lignans from *Schisandra neglecta*

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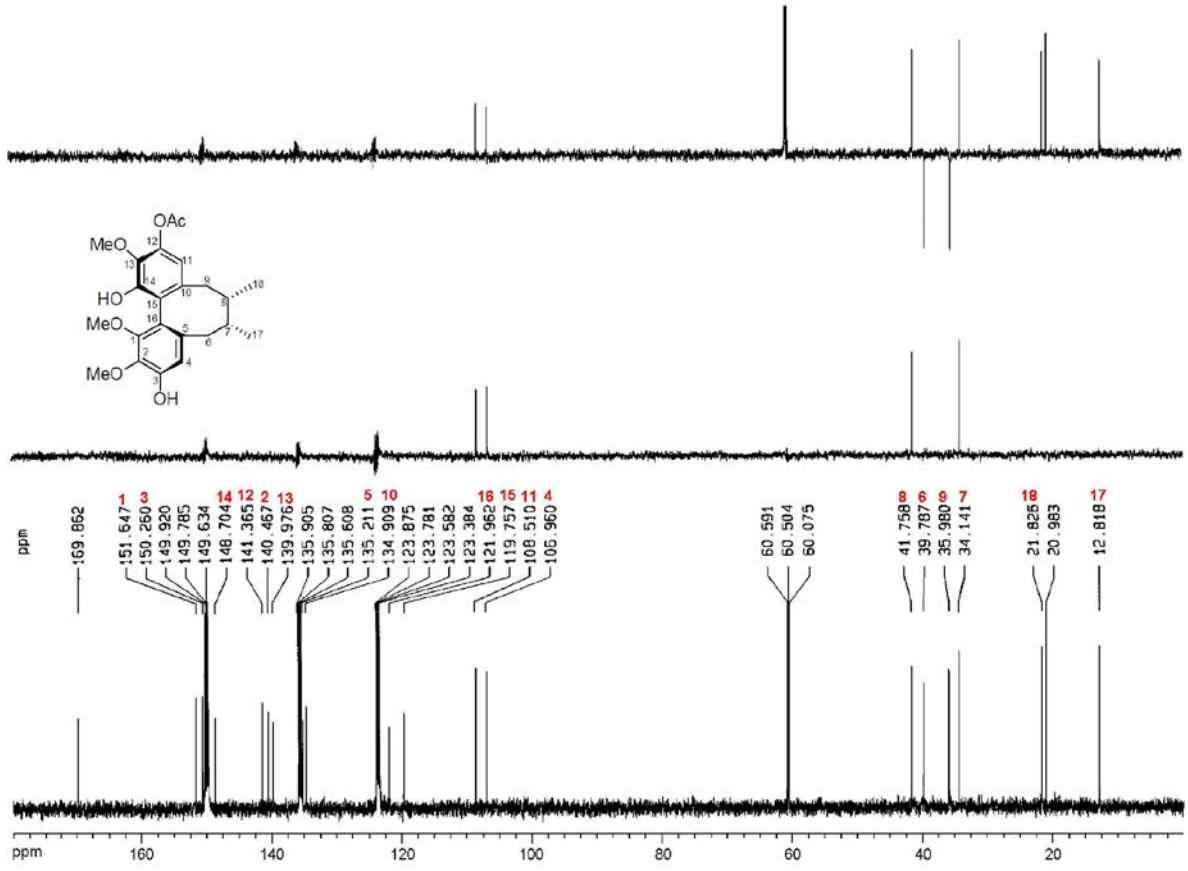

Figure S1. ^{13}C NMR (C_5D_5N, 125 MHz) and DEPT of neglectalignan A (**1**).

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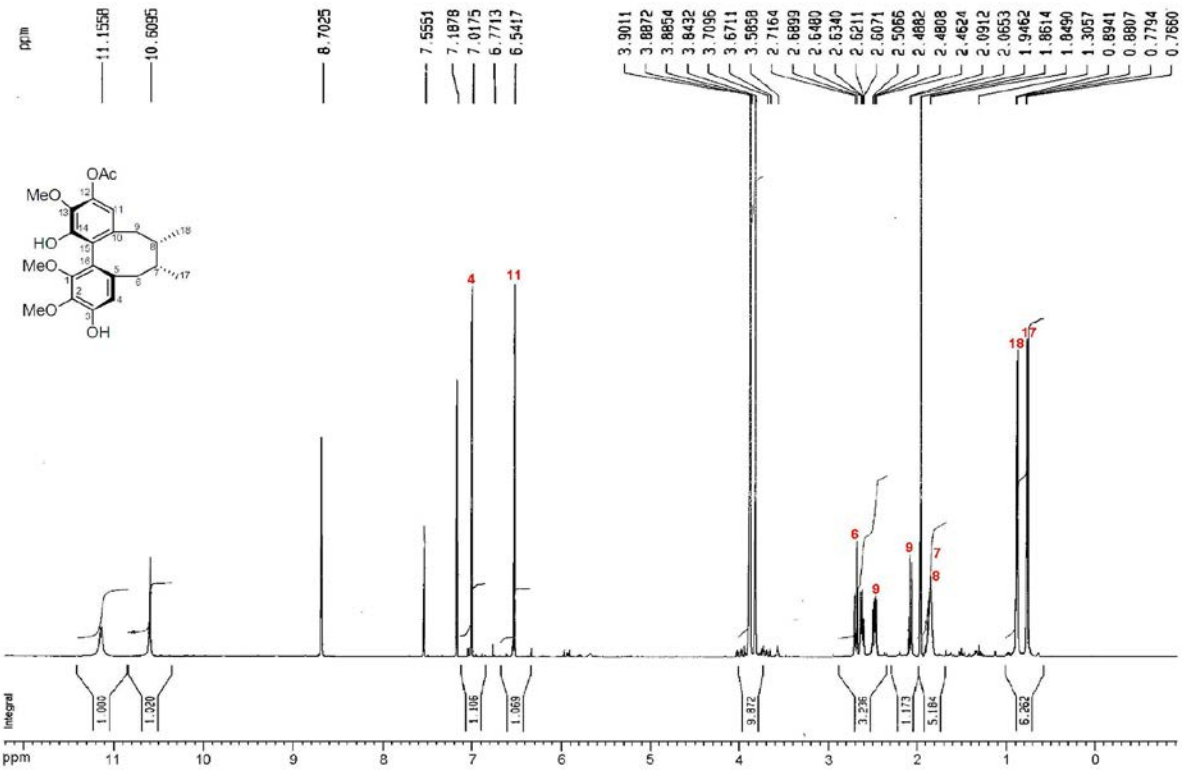

Figure S2. ^{1}H NMR (C_5D_5N, 500 MHz) of neglectalignan A (**1**).

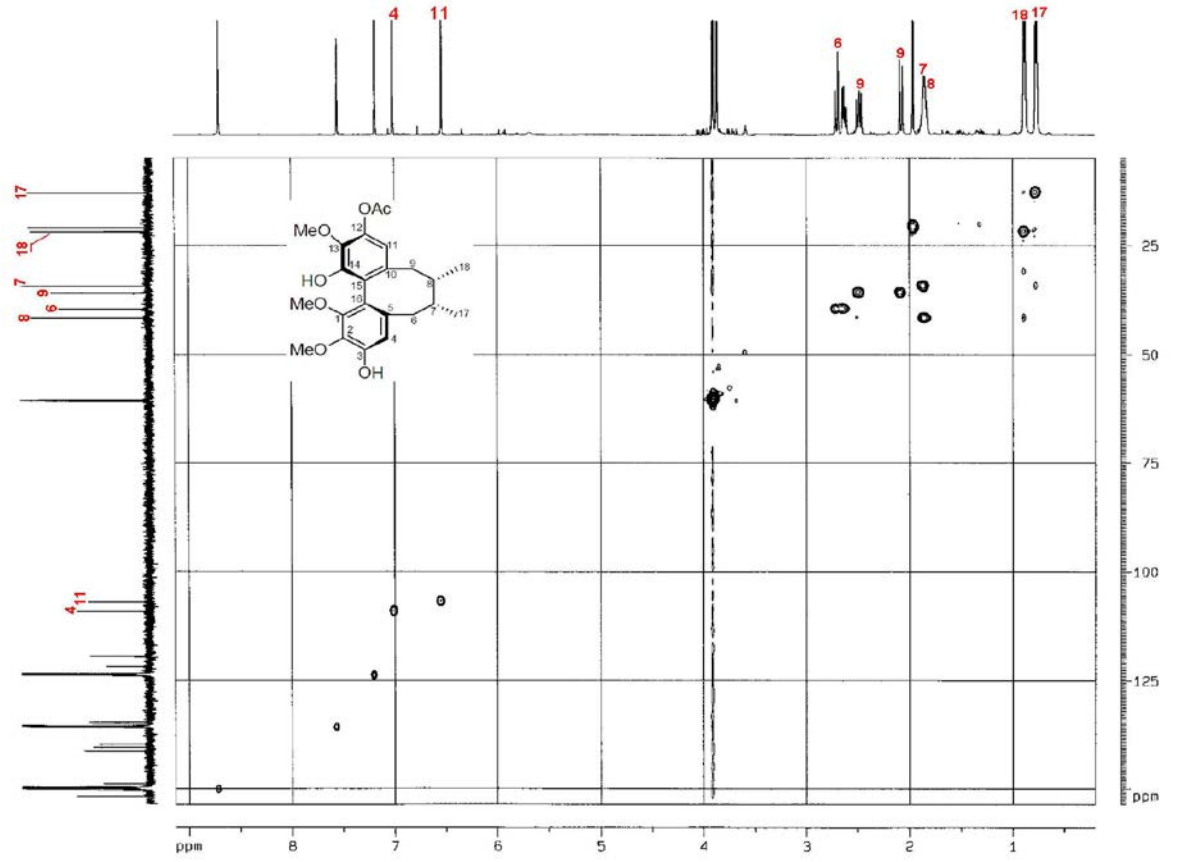

Figure S3. HSQC of neglectalignan A (**1**).

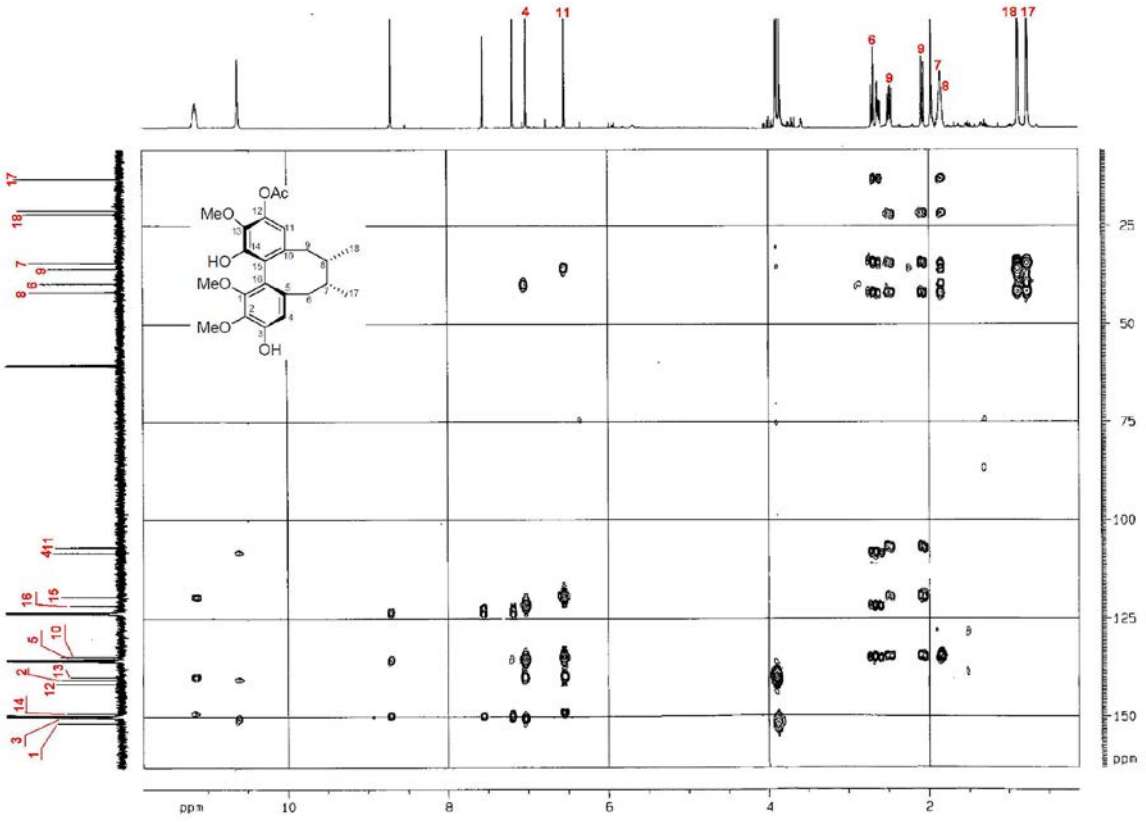

Figure S4. HMBC of neglectalignan A (**1**).

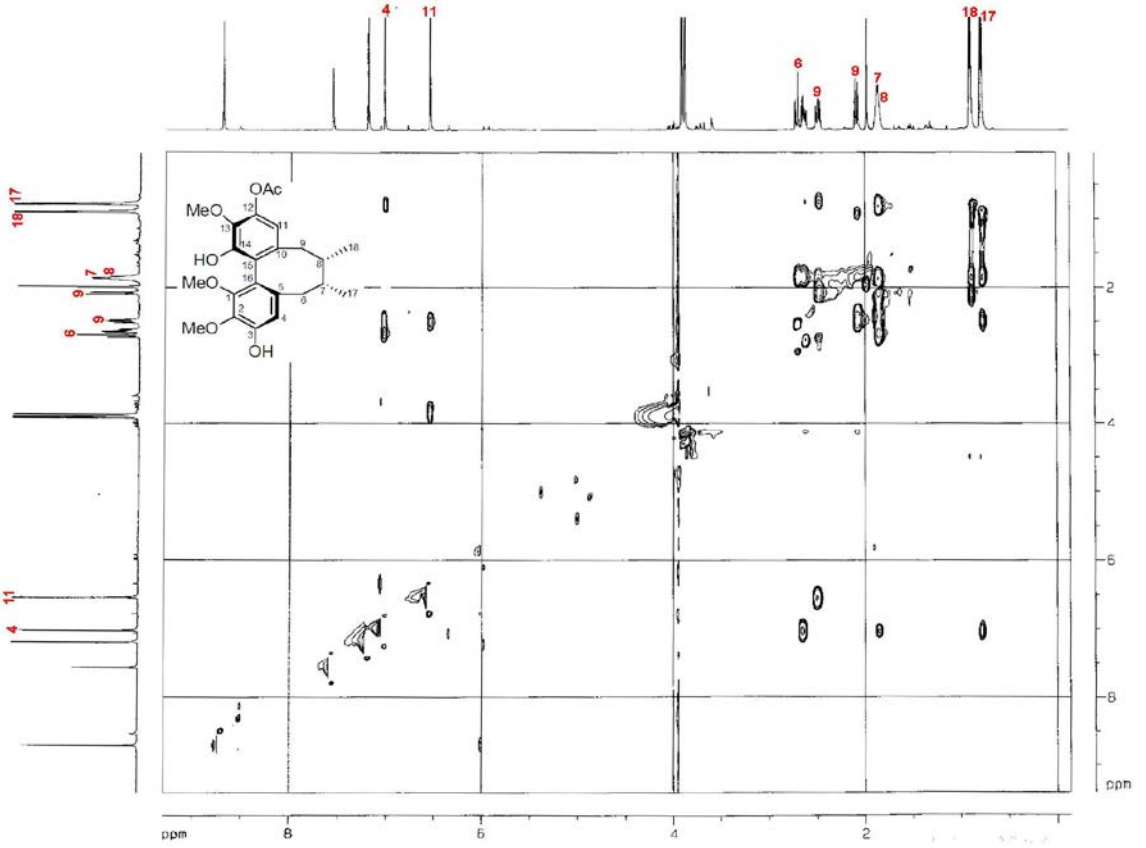

Figure S5. ROESY of neglectalignan A (**1**).

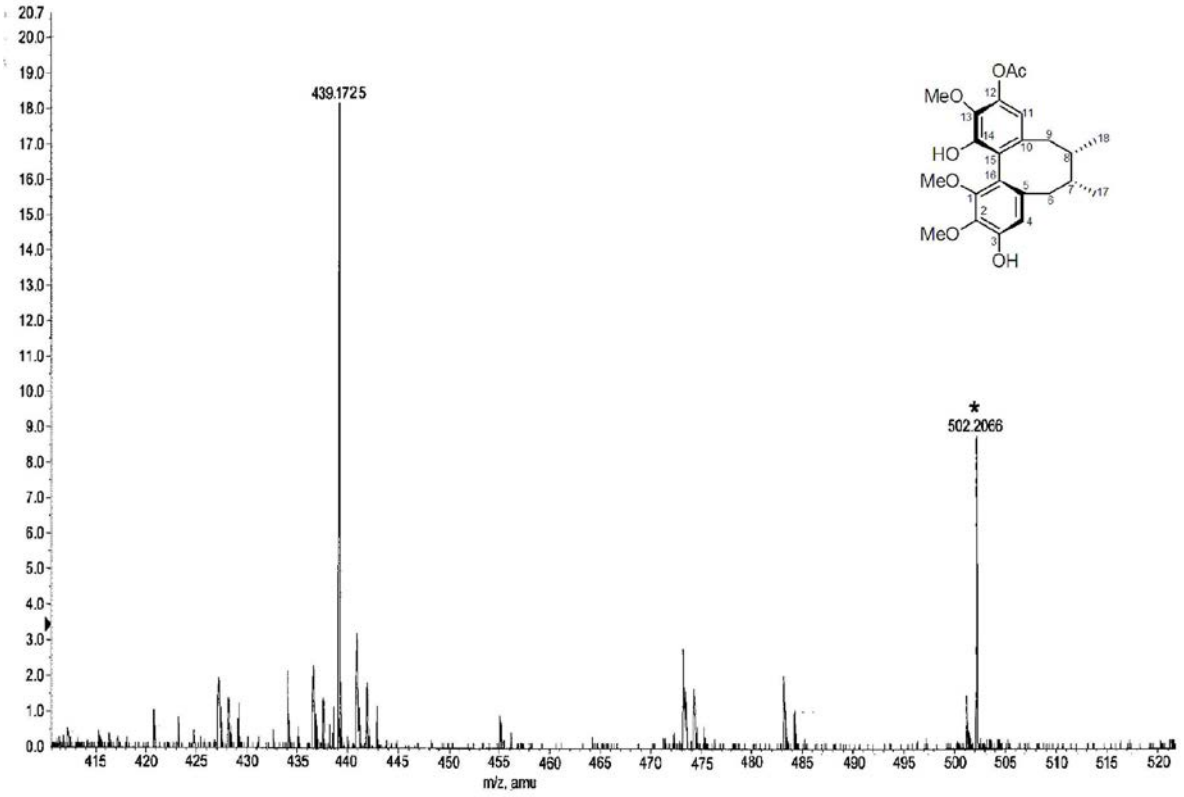

Figure S6. HRMS of neglectalignan A (**1**).

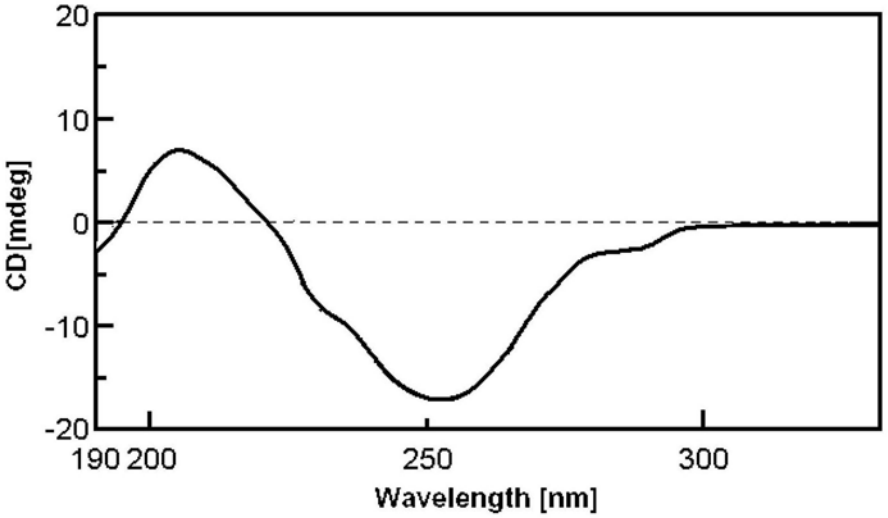

Figure S7. CD (MeOH) of neglectalignan A (**1**).

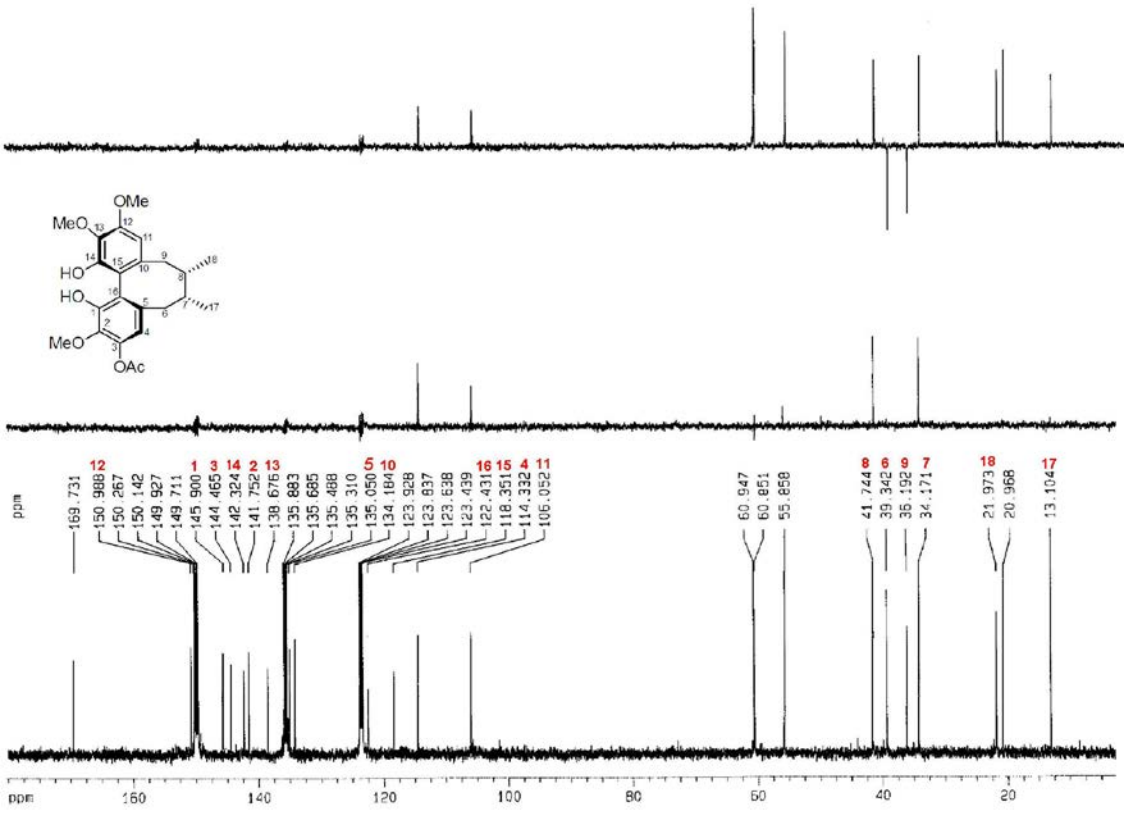

Figure S8. ^{13}C NMR (C_5D_5N, 125 MHz) and DEPT of neglectalignan B (**2**).

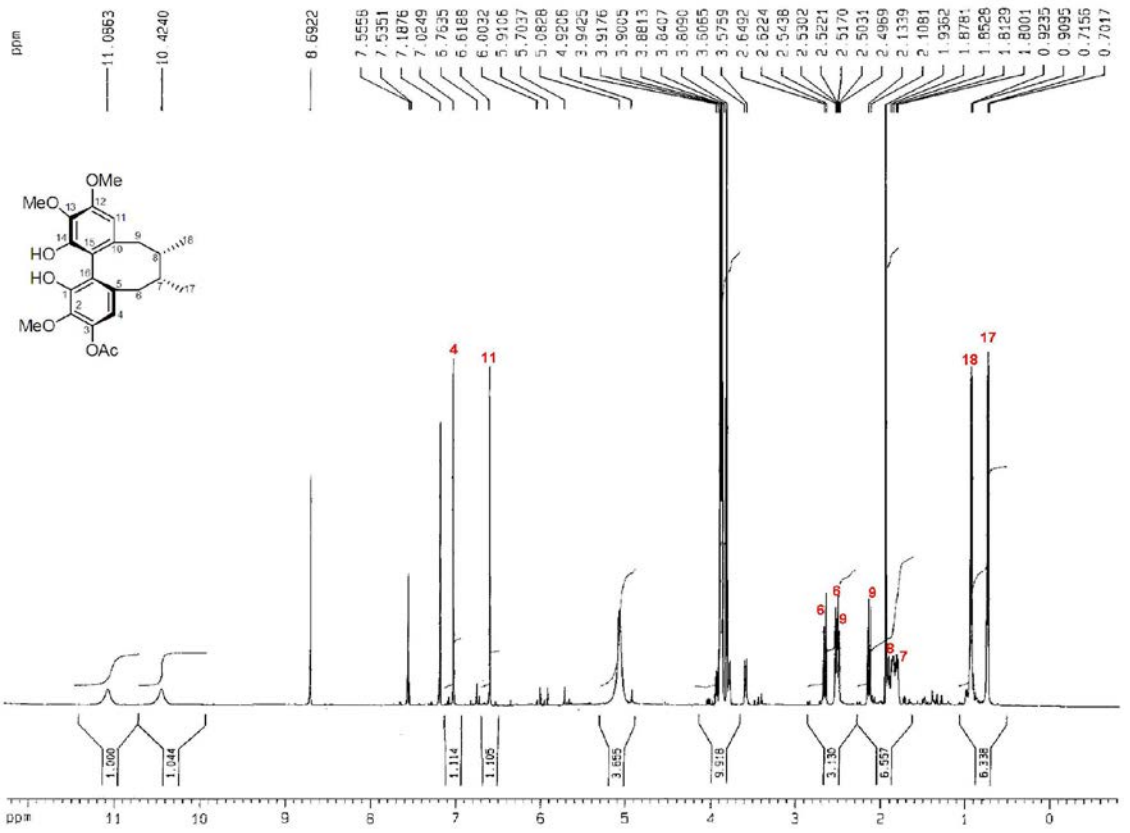

Figure S9. ^{1}H NMR (C_5D_5N, 500 MHz) of neglectalignan B (**2**).

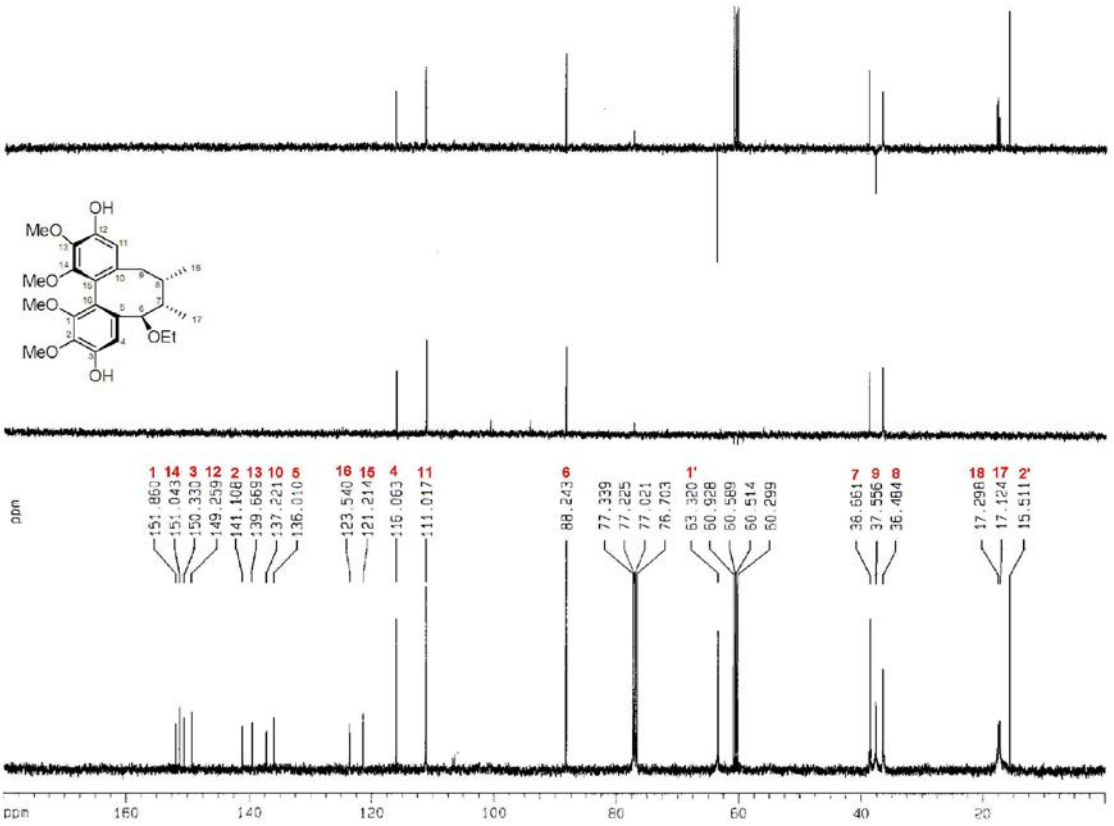

Figure S10. ^{13}C NMR ($CDCl_3$, 125 MHz) of neglectalignan C (**3**).

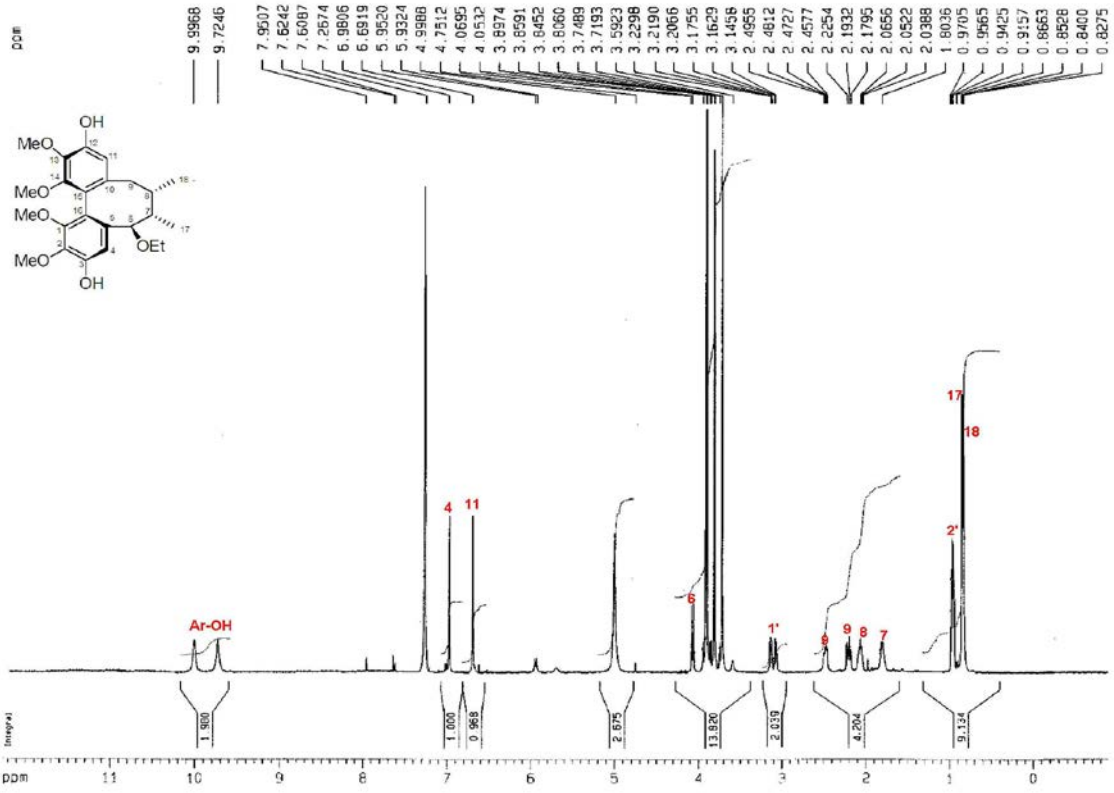

Figure S11. ^{1}H NMR ($CDCl_3$, 500 MHz) of neglectalignan C (**3**).

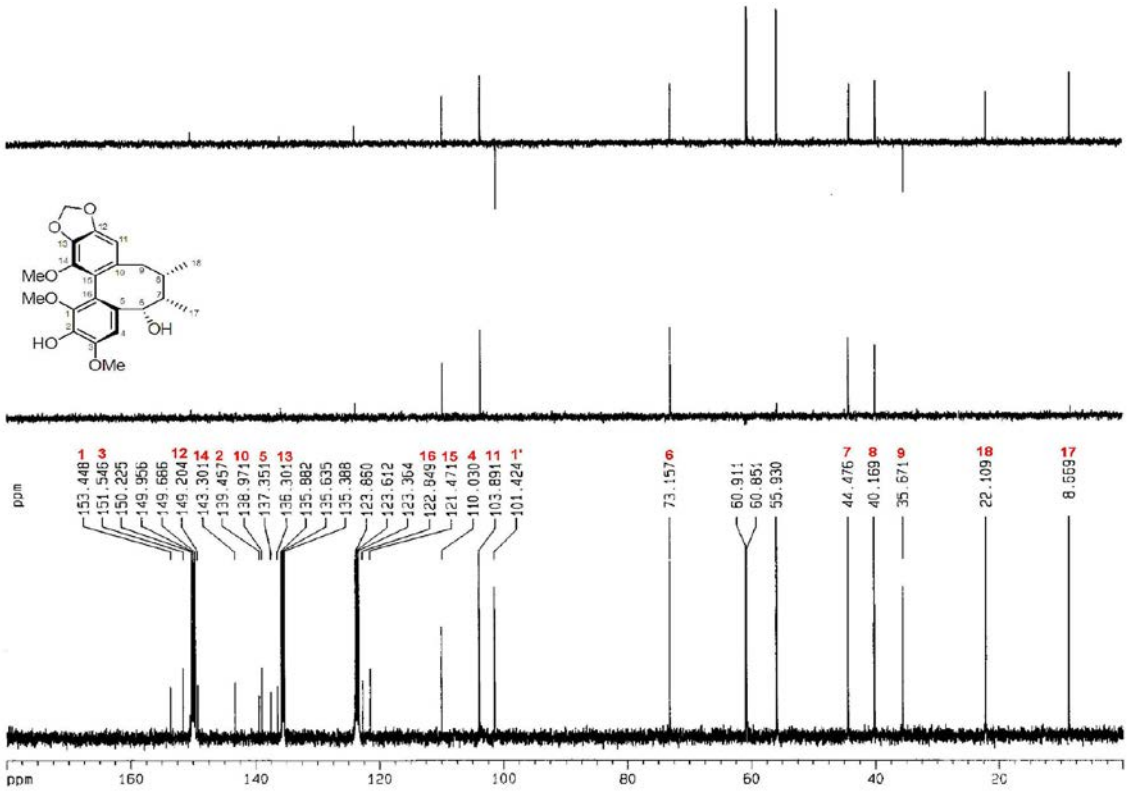

Figure S12. ^{13}C NMR (C_5D_5N, 125 MHz) of neglectalignan D (**4**).

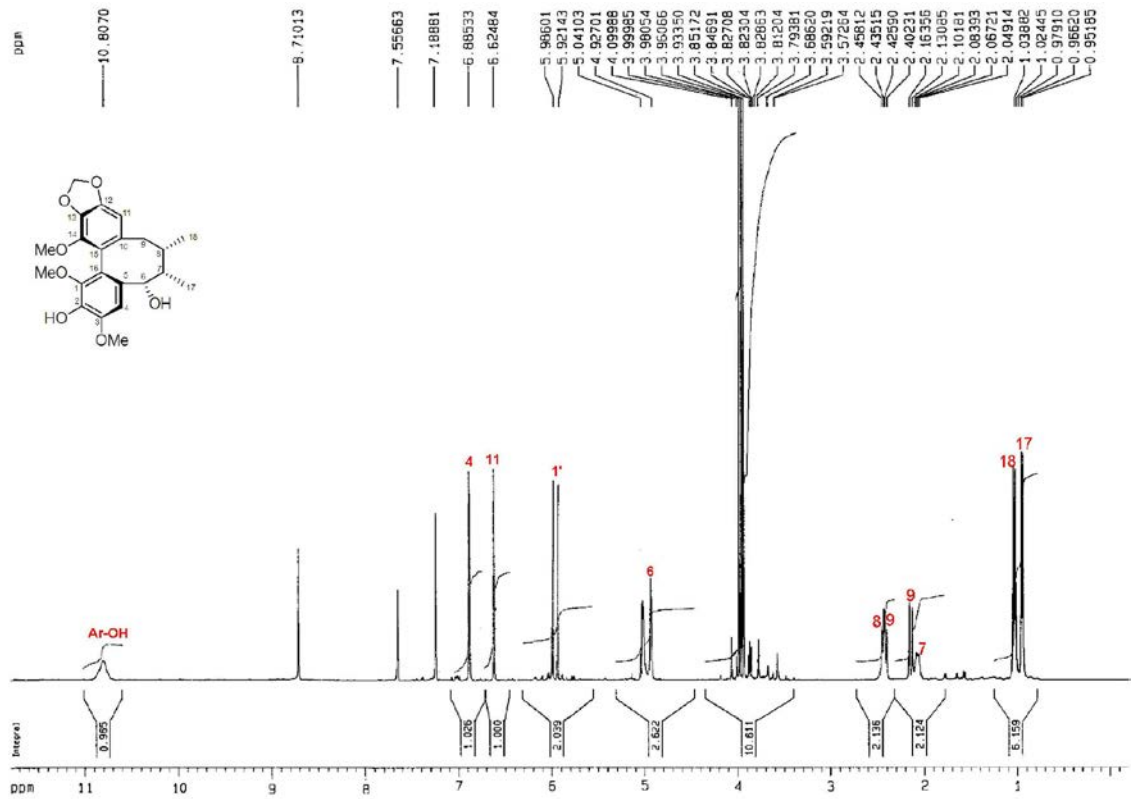

Figure S13. ^{1}H NMR (C_5D_5N, 500 MHz) of neglectalignan D (**4**).