

# Dibenzocyclooctadiene Lignans from *Kadsura polysperma* and Their Antineurodegenerative Activities

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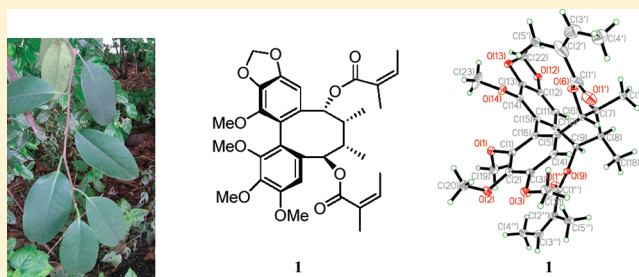
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## S Supporting Information

**ABSTRACT:** Eleven new dibenzocyclooctadiene lignans, polysperlignans A–K (1–11), and eight known analogues (12–19) were isolated from the stems of *Kadsura polysperma*. Their structures and absolute configurations were established using a combination of MS, NMR, CD, and single-crystal X-ray diffraction techniques. Selected compounds were evaluated for activity against  $\beta$ -amyloid- or hydrogen peroxide-induced neurotoxicity on PC12 cells, and 1, 2, 4, 5, 13, and 16 showed statistically significant neuroprotective effects in these in vitro assays.



The economically and medicinally important family Schisandraceae, a family of climbing plants, contains the genera *Schisandra* and *Kadsura*.<sup>1</sup> Some plants of this family are commonly used in Traditional Chinese Medicine for their sedative and tonic effects.<sup>2</sup> In pharmacology research, some dibenzocyclooctadiene lignans from *S. chinensis* exhibited therapeutic potential against oxidative neuronal damage induced by excitotoxin and may be useful in the treatment and prevention of neurodegenerative diseases.<sup>3,4</sup> In order to discover whether *Kadsura* species have additional secondary metabolites with antineurodegenerative activity, our research group initiated activity screening of dibenzocyclooctadiene lignans from *Kadsura* species in the relevant models of neurodegenerative disease and found that some of them exhibited considerable neuroprotective effects in an in vitro assay.<sup>5,6</sup>

*Kadsura polysperma* Yang (Schisandraceae) is an evergreen liana, growing in the jungle at elevations of 250–1800 m in Sichuan Province, P. R. China.<sup>7</sup> Previous chemical investigations of this plant collected in Chongqing City, P. R. China, led to the isolation of four triterpene lactones, polysperlactones A and B, heterocitalactone D, and schisanlactone E.<sup>8</sup> Motivated by a search for more dibenzocyclooctadiene lignans with novel structures and neuroprotective activity, *K. polysperma* from Emei Mountain was studied for the first time. As a result, 19 dibenzocyclooctadiene lignans were isolated, including 11 new compounds, polysperlignans A–K (1–11), and eight analogues. The isolation, structure elucidation, and antineurodegenerative activity of selected compounds are reported in this paper.

## RESULTS AND DISCUSSION

A 70% aqueous acetone extract of the stems of *K. polysperma* was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc solubles were dried and subjected to silica gel, MCI CHP-20 gel, Sephadex LH-20, and Lichroprep RP-18 gel column chromatography (CC) and semipreparative HPLC to afford 11 new compounds, polysperlignans A–K (1–11), together with eight known lignans, interiotherin C (12),<sup>9</sup> kadsurin (13),<sup>10</sup> kadsuphilin A (14),<sup>11</sup> isogomisin O (15),<sup>12</sup> tiegusanin I (16),<sup>13</sup> yunnankadsurin B (17),<sup>14</sup> methyl schisantherin F (18),<sup>15</sup> and ananolignan A (19).<sup>5</sup> The structures of the known compounds were determined by comparing spectroscopic data with literature values, and the absolute configurations of 1 and 7 were determined by X-ray analysis (CCDC 853853 and 853854, respectively).

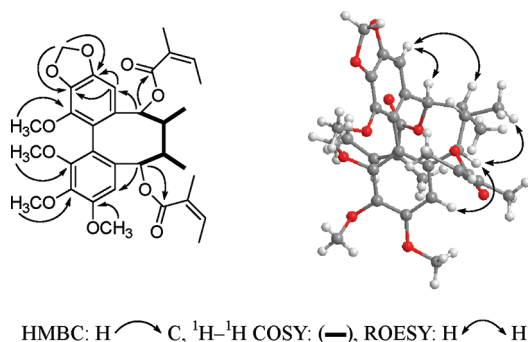
Polysperlignan A (1) was isolated as colorless crystals. The HREIMS of 1 gave a [M + Na]<sup>+</sup> ion peak at *m/z* 619.2522 (calcd 619.2519), consistent with the molecular formula C<sub>33</sub>H<sub>40</sub>O<sub>10</sub>. The UV spectrum with  $\lambda_{\max}$  (CH<sub>3</sub>OH) values at 191 and 219 nm, and its IR spectrum with absorption bands at 1619 and 1462 cm<sup>-1</sup> (aromatic moiety), indicated that 1 was a dibenzocyclooctadiene lignan.<sup>16,17</sup> The <sup>1</sup>H NMR spectrum (Table 1) displayed two aromatic singlets for a biphenyl moiety at  $\delta_{\text{H}}$  6.70 (H-4) and 6.52 (H-11), four singlets for methoxy groups at  $\delta_{\text{H}}$  3.87 (3H), 3.82 (3H), 3.75 (3H), and 3.41 (3H), and two singlets characteristic of a methylenedioxy group at  $\delta_{\text{H}}$

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5.95 and 5.94. A cyclooctadiene ring was recognized from two secondary methyl doublets at  $\delta_{\text{H}}$  0.89 (H<sub>3</sub>-17) and 0.98 (H<sub>3</sub>-18), two methines at  $\delta_{\text{H}}$  2.16 (H-7) and 2.28 (H-8), and two oxymethines at  $\delta_{\text{H}}$  5.76 (H-9) and 5.87 (H-6). This was confirmed by <sup>1</sup>H–<sup>1</sup>H COSY correlation systems of H-6/H-7/H-8/H-9, H-7/H<sub>3</sub>-17, and H-8/H<sub>3</sub>-18. HMBC correlations of the ethylenedioxy protons and H-11 with C-12 and C-13 and of the four methoxy group signals with C-1, C-2, C-3, and C-14 showed that the methylenedioxy group is connected to C-12 and C-13, and the four methoxy groups are located at C-1, C-2, C-3, and C-14, respectively (Figure 1). The <sup>13</sup>C NMR signals at

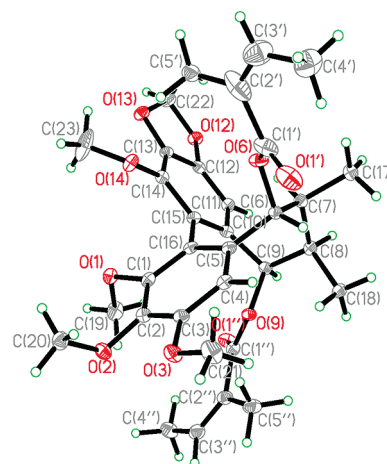


**Figure 1.** Key HMBC, <sup>1</sup>H–<sup>1</sup>H COSY, and ROESY correlations of **1**.

$\delta_{\text{C}}$  166.8 s, 127.6 s, 140.2 d, 20.1 q, 15.5 q and 166.7 s, 127.0 s, 138.4 d, 19.7 q, 15.4 q suggested the presence of two tigloyloxy moieties, and HMBC correlations of H-9 ( $\delta_{\text{H}}$  5.76) with C-1' ( $\delta_{\text{C}}$  166.8) and of H-6 ( $\delta_{\text{H}}$  5.87) with C-1'' ( $\delta_{\text{C}}$  166.7) placed the two tigloyloxy groups at C-9 and C-6, respectively.

The CD curve of **1** showed a negative Cotton effect around 250 nm and a positive value near 220 nm, suggesting that **1** possesses an *S*-biphenyl configuration.<sup>14</sup> With the axial chirality defined, a ROESY experiment was used to establish the configuration of the remaining stereocenters. ROESY correlations of H-11 with H-8 and H-9 and of H-6 with H-4 and H<sub>3</sub>-17 indicated that H-6, CH<sub>3</sub>-17, and CH<sub>3</sub>-18 are  $\alpha$ -oriented and that H-9 is  $\beta$ -oriented (Figure 1).<sup>17</sup> To confirm the structure and determine its absolute configuration, **1** was crystallized from MeOH to afford a crystal of the orthorhombic space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, which was analyzed by X-ray crystallography. On the basis of 10 oxygen atoms in the molecule, the final refinement of the Cu *K* $\alpha$  data resulted in a Flack parameter of 0.1(2), which in combination with the CD spectrum<sup>17</sup> allowed unambiguous assignment of the absolute configuration (Figure 2). According to the projection of the stereochemical structure, compound **1** was in agreement with a cyclooctadiene lignan with a twisted boat conformation having C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*) absolute configurations. Therefore, the structure of **1** was determined as shown.

Polysperlignan B (**2**) was assigned as C<sub>30</sub>H<sub>36</sub>O<sub>10</sub>, as deduced from the HRESIMS (*m/z* 579.2191 [*M* + Na]<sup>+</sup>) and in accordance with its NMR data. The UV, IR, CD, and NMR spectra of **2** suggested the presence of an *S*-biphenyl-configured dibenzocyclooctadiene lignan with almost identical data to ananolignan L.<sup>5</sup> The only difference between them was in the position exchange of two substituents. HMBC correlations of H-6 ( $\delta_{\text{H}}$  5.79) with the acetate carbonyl ( $\delta_{\text{C}}$  170.1) and of H-9 ( $\delta_{\text{H}}$  5.83) with the ester carbonyl ( $\delta_{\text{C}}$  167.1 s, C-1') indicated an acetoxy group at C-6 and a tigloyloxy group at C-9 in **2**.



**Figure 2.** X-ray crystal structure of **1**.

The configuration of **2** was determined through ROESY correlations of H-11/H-8, H-9; H-4/H-6, H<sub>3</sub>-17; and H<sub>3</sub>-18/H<sub>3</sub>-17, which were in agreement with a cyclooctadiene lignan with a twisted boat/chair conformation having C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*) absolute configurations. Thus, the structure of **2** was established as shown, and it was named polysperlignan B.

Comparison of the NMR data of **3** and **4** with those of **2** disclosed that the main structural differences between them concerned the substituent at C-6. Polysperlignan C (**3**) gave the molecular formula C<sub>35</sub>H<sub>38</sub>O<sub>10</sub> from its HRESIMS data at *m/z* 641.2362 [*M* + Na]<sup>+</sup> (calcd 641.2362). The 1D NMR spectra revealed the presence of a benzyloxy group ( $\delta_{\text{C}}$  165.6 s, 130.8 s, 129.5 d, 127.9 d, 132.7 d, 127.9 d, and 129.5 d). HMBC correlations from H-6 ( $\delta_{\text{H}}$  6.10) to the ester carbonyl ( $\delta_{\text{C}}$  165.6s) placed the benzyloxy group at C-6. The molecular formula of **4** was determined as C<sub>33</sub>H<sub>40</sub>O<sub>10</sub> by HRESIMS at *m/z* 619.2516 [*M* + Na]<sup>+</sup> (calcd 619.2519), and the NMR data of **4** also showed similarities to the data for **2**. However, a signal for an angeloyloxy group ( $\delta_{\text{C}}$  166.7 s, 127.6 s, 138.6 d, 15.6q, and 19.8q) was evident at C-6 in **4**, which was confirmed by the HMBC correlations from H-6 ( $\delta_{\text{H}}$  5.92) to the ester carbonyl ( $\delta_{\text{C}}$  166.7s).

The CD, UV, and IR data suggested that **3** and **4** were *S*-biphenyl-configured dibenzocyclooctadiene lignans. ROESY correlations obtained for **3** and **4** from H-11 to H-8 and H-9, from H-4 to H-6 and H<sub>3</sub>-17, and from H<sub>3</sub>-17 to H<sub>3</sub>-18 indicated that H-6, CH<sub>3</sub>-17, and CH<sub>3</sub>-18 are  $\alpha$ -oriented and that H-9 is  $\beta$ -oriented. Thus, the structures of polysperlignans C and D (**3** and **4**) were established as shown.

Polysperlignans E (**5**) and F (**6**) were determined to have the molecular formulas C<sub>37</sub>H<sub>40</sub>O<sub>10</sub> and C<sub>32</sub>H<sub>34</sub>O<sub>8</sub> by HRESIMS (*m/z* 667.2530 [*M* + Na]<sup>+</sup> and 569.2160 [*M* + Na]<sup>+</sup>, respectively). Comparison of the spectroscopic data of **5** with those of **1** revealed these substances to be quite similar structurally, except that the angeloyloxy group at C-6 in **1** was changed to a *trans*-cinnamoyl group ( $\delta_{\text{C}}$  165.9 s, 117.8 d, 144.8 d, 134.1 s, 128.8 d, 128.0 d, 130.3 d, 128.0 d, and 128.8 d) in **5**, which was confirmed by HMBC correlations from an oxymethine at  $\delta_{\text{H}}$  5.94 (H-6) to  $\delta_{\text{C}}$  165.9 (C-1'), 38.6 (C-7), 38.4 (C-8), 131.1 (C-5), and 110.5 (C-4). Analysis of the 2D NMR data of compound **6** and comparison of its 1D NMR data with those of **14** revealed that the main structure differences between the two compounds were a *cis*-cinnamoyl group in **6**

instead of a *trans*-cinnamoyl group at C-9 in **14**. The deduction was determined through ROESY correlations of two olefinic protons, H-2'/H-3', as well as the coupling constant of  $J_{H-2,3'} = 12$  Hz. The CD, UV, IR, and NMR spectra suggested that **5** and **6** were *S*-biphenyl-configured dibenzocyclooctadiene lignans. ROESY correlations of H-11 with H-8 $\beta$  and H-9 $\beta$ , of H-4 with H-6 $\alpha$  and H<sub>3</sub>-17 $\alpha$ , and of H<sub>3</sub>-18 with H<sub>3</sub>-17 suggested the absolute configurations as C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*), which were identical with those of **1**.

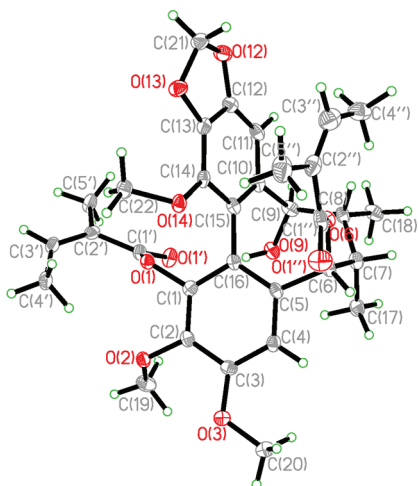


Figure 3. X-ray crystal structure of **7**.

Polysperlignans **G** (**7**) and **H** (**8**) were assigned the same molecular formula, C<sub>32</sub>H<sub>38</sub>O<sub>10</sub>, as determined by HRESIMS ( $m/z$  605.2345 [M + Na]<sup>+</sup> and 605.2362 [M + Na]<sup>+</sup>, respectively). Both compounds were assigned as *S*-biphenyl-configured dibenzocyclooctadiene lignans by analysis of their CD spectra. The 1D NMR spectra of compound **7** exhibited 18 carbon atoms (including 12 aromatic ones, four methines, and two secondary methyls) for the dibenzocyclooctadiene skeleton, in addition to two angeloyloxy groups ( $\delta_C$  167.3 s, 127.0 s, 139.0 d, 15.4 q, 20.3 q and  $\delta_C$  166.6 s, 127.8 s, 139.0 d, 15.6 q, 19.9 q), a methylenedioxy group, and three methoxy groups. The existence of the angeloyloxy group at C-6 was deduced from the HMBC correlations of H-6 ( $\delta_H$  5.88) with the ester carbonyl ( $\delta_C$  166.6, C-1''). Furthermore, HMBC correlations of three methoxy groups with C-2, C-3, and C-14, respectively, were used to determine their positions. The occurrence of the methylenedioxy moiety at C-12 and C-13 was demonstrated by the HMBC correlations of the methylenedioxy protons with C-12 and C-13. According to the molecular formula, the methine carbon at C-9 should be substituted by an OH, and another angeloyloxy group was located at C-1, which was deduced from a ROESY correlation of CH<sub>3</sub>O-14 with H<sub>3</sub>-4' and the remarkable upfield shift of the signal of H-9. An X-ray crystallographic study and CD experiment were also performed to confirm the structure and determine the absolute configuration of **7** (Figure 3). Thus, **7** was unambiguously elucidated as a cyclooctadiene lignan with a twisted boat/chair conformation having C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*) absolute configurations. Comparison of the NMR data of **8** with those of **7** disclosed that the only structural difference was the angeloyloxy group located at C-6 in **7** being changed to a tigloyloxy moiety ( $\delta_C$  166.8 s, 128.4 s, 136.8 d, 14.1 q, and 11.6 q) in **8**. This was confirmed by the HMBC correlations from H-6

( $\delta_H$  5.88) to the ester carbonyl ( $\delta_C$  166.8 s, C-1''). The observed ROESY correlations of H-11 with H-8 and H-9, of H-4 with H-6 and H<sub>3</sub>-17, and of H<sub>3</sub>-17 with H<sub>3</sub>-18 indicated that H-6, CH<sub>3</sub>-17, and CH<sub>3</sub>-18 are  $\alpha$ -oriented and that H-9 is  $\beta$ -oriented. Accordingly, the structure of **8** was determined as shown.

Polysperlignans **I** (**9**) and **J** (**10**) both had the molecular formula C<sub>33</sub>H<sub>42</sub>O<sub>10</sub>, as determined by HRESIMS ( $m/z$  621.2669 [M + Na]<sup>+</sup> and 621.2660 [M + Na]<sup>+</sup>, respectively). Both compounds were assigned as *S*-biphenyl-configured dibenzocyclooctadiene lignans by comparison of their CD, UV, and ROESY spectra with those of **7**. The only difference found between **7** and **9** concerned the substituent groups at C-12 and C-13. Analysis of the NMR and MS data showed two methoxy groups in **9** instead of a methylenedioxy group in **7**. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **10** were quite similar to those of **9**, except that the angeloyloxy group located at C-6 in **9** was changed to a tigloyloxy group ( $\delta_C$  168.4 s, 128.9 s, 139.5 d, 14.3 q, and 11.7 q) in **10**. The observation of a HMBC correlation of H-6 ( $\delta_H$  5.88) to the ester carbonyl ( $\delta_C$  168.4 s, C-1'') determined the above deduction. ROESY correlations of H-11 with H-8 and H-9 and of H-4 with H-6 and H<sub>3</sub>-17 suggested a cyclooctadiene lignan with a twisted boat/chair conformation of **10**, consistent with the absolute configuration C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*). Therefore, the structures of **9** and **10** were determined as shown.

Polysperlignan **K** (**11**) gave the molecular formula C<sub>24</sub>H<sub>28</sub>O<sub>8</sub> from its HRESIMS data at  $m/z$  467.1675 [M + Na]<sup>+</sup>. The 1D NMR spectra exhibited 18 carbon atoms (including 12 aromatic ones, one methylene, three methines, and two secondary methyls) for the dibenzocyclooctadiene skeleton, in addition to a methylenedioxy group, three methoxy groups, and an acetyl group. By contrast, compounds **11** and **15** had similar planar structures, the only difference was an acetyl group instead of one methoxy group. The HRESIMS also confirmed the above conclusion. The HMBC spectrum showed that the methylenedioxy group was located at C-12 and C-13 and the three methoxy groups were located at C-2, C-3, and C-14, respectively, indicating that an acetyl group was located at C-1. HMBC correlations of H-9 ( $\delta_H$  4.47) with C-7 ( $\delta_C$  39.1, d), C-8 ( $\delta_C$  42.2, d), C-10 ( $\delta_C$  136.9, s), C-11 ( $\delta_C$  102.2, d), C-15 ( $\delta_C$  118.6, s), and C-18 ( $\delta_C$  7.9, q) also indicated that C-9 of **11** was substituted by an OH group.

The CD curve of **11** showed a negative Cotton effect around 210 nm and a positive value around 250 nm, suggesting that **11** possessed an *R*-biphenyl configuration. H-9, CH<sub>3</sub>-17, and CH<sub>3</sub>-18 were deduced to be  $\alpha$ -oriented on the basis of the ROESY correlations from H-11 to H-9 and H<sub>3</sub>-18 and from H<sub>3</sub>-17 to H<sub>3</sub>-18. Therefore, the structure of **11** was determined as shown.

Compounds **1**, **2**, **4–6**, **9**, and **13–19** were assayed for their neuroprotective effects against PC12 neuroblastoma cells, a neuroblastoma cell line used for the study of neurodegenerative disease.<sup>18–20</sup> As seen in Table 5, polysperlignans **A** and **B** (**1** and **2**), polysperlignans **D** and **E** (**4** and **5**), kadsurin (**13**), and tiegusanin **I** (**16**) showed the most promising cell survival data against H<sub>2</sub>O<sub>2</sub>- or A $\beta$ <sub>25–35</sub>-induced neurotoxicity. Due to limitations in the amounts available, compounds **3**, **7**, **8**, and **10–12** were not tested in the bioassay used.

## 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. A Tensor

**Table 2.**  $^1\text{H}$  NMR Data of Compounds 7–11 ( $\delta$  in ppm,  $J$  in Hz)

position	7 <sup>a</sup>	8 <sup>a</sup>	9 <sup>a</sup>	10 <sup>c</sup>	11 <sup>b</sup>
4	6.86 (s)	6.82 (s)	6.88 (s)	6.88 (s)	6.65 (s)
6 $\alpha$	5.88 (overlap)	5.88 (d, 8.0)	5.96 (overlap)	5.88 (br s)	2.23 (dd, 13.3, 9.6)
6 $\beta$					2.05 (overlap)
7	2.10 (m)	2.15 (m)	2.05 (m)	2.06 (m)	1.94 (m)
8	2.02 (m)	2.13 (m)	2.03 (m)	2.05 (m)	1.99 (m)
9	4.75 (s)	4.76 (s)	4.79 (d, 2.4)	4.74 (s)	4.47 (s)
11	6.29 (s)	6.33 (s)	6.36 (s)	6.53 (s)	6.89 (s)
17	1.03 (d, 6.9)	1.02 (d, 7.2)	1.01 (d, 6.1)	0.94 (d, 6.1)	1.02 (d, 7.0)
18	1.17 (d, 7.1)	1.20 (d, 7.2)	1.20 (d, 4.8)	1.15 (d, 5.8)	0.68 (d, 7.0)
3'	6.03 (m)	6.02 (q, 6.4)	5.91 (overlap)	5.99 (overlap)	
4'	1.81 (d, 7.3)	1.81 (d, 6.8)	1.75 (d, 7.2)	1.68 (d, 8.2)	
5'	1.81 (s)	1.81 (s)	1.77 (s)	1.69 (s)	
3''	5.96 (m)	6.12 (m)	5.90 (overlap)	5.99 (overlap)	
4''	1.86 (d, 7.2)	1.66 (d, 7.0)	1.82 (d, 7.2)	1.49 (d, 6.7)	
5''	1.48 (s)	1.59 (s)	1.39 (s)	1.42 (s)	
AcO					1.97 (s)
CH <sub>3</sub> O-2	3.80 (s)	3.79 (s)	3.79 (s)	3.69 (s)	3.82 (s)
CH <sub>3</sub> O-3	3.90 (s)	3.90 (s)	3.90 (s)	3.84 (s)	3.90 (s)
CH <sub>3</sub> O-12			3.83 (s)	3.77 (s)	
CH <sub>3</sub> O-13			3.78 (s)	3.66 (s)	
CH <sub>3</sub> O-14	3.80 (s)	3.76 (s)	3.60 (s)	3.28 (s)	3.88 (s)
OCH <sub>2</sub> O	5.90 (overlap)	5.93 (d, 1.1)			5.97 (s)
	5.90 (overlap)	5.87 (d, 1.1)			5.96 (s)

<sup>a</sup>Recorded at 400 MHz in CDCl<sub>3</sub>. <sup>b</sup>Recorded at 500 MHz in CDCl<sub>3</sub>.  
<sup>c</sup>Recorded in CD<sub>3</sub>OD, 600 MHz.

27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Chemical shifts ( $\delta$ ) are expressed in ppm with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm  $\times$  25 cm) column. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63  $\mu\text{M}$ , Merck, Darmstadt, Germany), and MCI gel (75–150  $\mu\text{M}$ , Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H<sub>2</sub>SO<sub>4</sub> in EtOH.

**Plant Material.** The stems of *K. polysperma* were collected in Emei County of Sichuan Province, People's Republic of China, in August 2009, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 2009081003) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** The air-dried and powdered stems of *K. polysperma* (10.5 kg) were extracted with 70% aqueous Me<sub>2</sub>CO (40 L  $\times$  3) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H<sub>2</sub>O and EtOAc. The EtOAc extract (330 g) was chromatographed on a silica gel (100–200 mesh, 3.0 kg) column, eluting with a CHCl<sub>3</sub>–Me<sub>2</sub>CO gradient system (40:1, 20:1, 9:1, 8:2, 7:3, 6:4, 1:1), to give fractions 1–8. Fraction 3 (20 g) was subjected to RP-18 column chromatography (40–100% gradient of CH<sub>3</sub>OH–H<sub>2</sub>O) to afford subfractions 3.1–3.6. Fraction 3.4 (2.2 g) was chromatographed on silica gel (petroleum ether–Me<sub>2</sub>CO, 30:1–2:1) to give six subfractions. Fraction 3.4.2 (1.1 g) was purified by

semipreparative HPLC (65% CH<sub>3</sub>CN–H<sub>2</sub>O) to give three fractions, 3.4.2.1–3.4.2.3. Fraction 3.4.2.1 (160 mg) was chromatographed by semipreparative HPLC (78% CH<sub>3</sub>OH–H<sub>2</sub>O) to give 6 (7 mg), 13 (12 mg), 14 (7 mg), 15 (23 mg), 17 (10 mg), and 19 (8 mg). Fraction 3.4.2.2 (250 mg) was purified repeatedly by semipreparative HPLC (80% CH<sub>3</sub>OH–H<sub>2</sub>O) to give 1 (13 mg), 2 (16 mg), 3 (2 mg), 4 (20 mg), 5 (13 mg), 12 (2 mg), and 16 (22 mg). Fraction 3.4.2.3 (120 mg) was separated further by semipreparative HPLC (80% CH<sub>3</sub>OH–H<sub>2</sub>O) to give 7 (3 mg) and 8 (2 mg). Fraction 3.4.3 (0.2 g) was subjected to semipreparative HPLC (63% CH<sub>3</sub>CN–H<sub>2</sub>O) to produce 9 (3 mg), 10 (2 mg), and 18 (10 mg). Finally, fraction 3.4.4 (0.1 g) was separated by semipreparative HPLC (60% CH<sub>3</sub>CN–H<sub>2</sub>O) to yield 11 (2 mg).

**Polysperlignan A (1):** colorless crystals; mp 156–157 °C;  $[\alpha]_{\text{D}}^{17} +270.1$  ( $c$  0.07, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 226 (+14), 243 (–3); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 219 (4.44), 191 (4.08) nm; IR (KBr)  $\nu_{\text{max}}$  2943, 1706, 1619, 1462, 1231 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  619 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  619.2522 [M + Na]<sup>+</sup> (calcd for C<sub>33</sub>H<sub>40</sub>O<sub>10</sub>Na, 619.2519).

**Polysperlignan B (2):** white solid; mp 86–88 °C;  $[\alpha]_{\text{D}}^{27} +9.4$  ( $c$  0.17, CHCl<sub>3</sub>); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 228 (+7), 245 (–20); UV (CHCl<sub>3</sub>)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 241 (4.07), 223 (3.74), 215 (3.73), 212 (3.71), 205 (3.72), 202 (3.72), 194 (3.73) nm; IR (KBr)  $\nu_{\text{max}}$  2938, 1707, 1622, 1463, 1249 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  579 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  579.2191 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>36</sub>O<sub>10</sub>Na, 579.2206).

**Polysperlignan C (3):** white solid; mp 69–70 °C;  $[\alpha]_{\text{D}}^{17} -133.3$  ( $c$  0.07, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 223 (+1), 241 (–1); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 218 (4.27) nm; IR (KBr)  $\nu_{\text{max}}$  2937, 1712, 1622, 1463, 1259 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  641 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  641.2362 [M + Na]<sup>+</sup> (calcd for C<sub>35</sub>H<sub>38</sub>O<sub>10</sub>Na, 641.2362).

**Polysperlignan D (4):** yellow oil;  $[\alpha]_{\text{D}}^{27} +1.5$  ( $c$  0.16, CHCl<sub>3</sub>); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 236 (+4), 248 (–7); UV (CHCl<sub>3</sub>)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 241 (4.00), 231 (3.48), 227 (3.47), 221 (3.45), 218 (3.45), 203 (3.60), 201 (3.61), 194 (3.63) nm; IR (KBr)  $\nu_{\text{max}}$  2937, 1708, 1623, 1462, 1253 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  619 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  619.2516 [M + Na]<sup>+</sup> (calcd for C<sub>33</sub>H<sub>40</sub>O<sub>10</sub>Na, 619.2519).

**Polysperlignan E (5):** white solid; mp 132–134 °C;  $[\alpha]_{\text{D}}^{17} -1.7$  ( $c$  0.08, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 231 (+3), 255 (–9); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 277 (4.01), 218 (4.47) nm; IR (KBr)  $\nu_{\text{max}}$  2935, 1710, 1626, 1462, 1232 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  667 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  667.2530 [M + Na]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>40</sub>O<sub>10</sub>Na, 667.2519).

**Polysperlignan F (6):** yellow oil;  $[\alpha]_{\text{D}}^{17} +380.0$  ( $c$  0.10, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 221 (+1), 241 (–1); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 256 (3.81), 218 (4.26) nm; IR (KBr)  $\nu_{\text{max}}$  2935, 1709, 1624, 1463, 1248 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS  $m/z$  569 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  569.2160 [M + Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>34</sub>O<sub>8</sub>Na, 569.2151).

**Polysperlignan G (7):** colorless crystals; mp 129–130 °C;  $[\alpha]_{\text{D}}^{23} +132.6$  ( $c$  0.10, CHCl<sub>3</sub>); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 224 (+14), 243 (–4); UV (CHCl<sub>3</sub>)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 241 (3.69) nm; IR (KBr)  $\nu_{\text{max}}$  2933, 1706, 1613, 1461, 1233 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 4; positive ESIMS  $m/z$  605 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  605.2345 [M + Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>38</sub>O<sub>10</sub>Na, 605.2362).

**Polysperlignan H (8):** white solid; mp 129–130 °C;  $[\alpha]_{\text{D}}^{24} +49.0$  ( $c$  0.09, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 224 (+21), 242 (–18); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 290 (2.62), 212 (3.97), 198 (3.41) nm; IR (KBr)  $\nu_{\text{max}}$  2936, 1706, 1614, 1463, 1265 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 4; positive ESIMS  $m/z$  605 (100) [M + Na]<sup>+</sup>; positive HRESIMS  $m/z$  605.2362 [M + Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>38</sub>O<sub>10</sub>Na, 605.2362).

**Polysperlignan I (9):** white solid; mp 65–66 °C;  $[\alpha]_{\text{D}}^{17} +137.9$  ( $c$  0.29, CH<sub>3</sub>OH); CD (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 219 (+16), 237 (–6); UV (CH<sub>3</sub>OH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 216 (4.40), and 193 (4.05) nm; IR (KBr)  $\nu_{\text{max}}$  2938, 1710, 1644, 1457, 1231 cm<sup>–1</sup>;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data,

Table 3.  $^{13}\text{C}$  NMR Data of Compounds 1–6 in  $\text{CDCl}_3$  ( $\delta$  in ppm)

position	1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>a</sup>	6 <sup>b</sup>
1	151.6 C	151.8 C	151.8 C	151.7 C	151.6 C	150.9 C
2	141.3 C	141.7 C	141.6 C	141.6 C	142.0 C	139.9 C
3	151.8 C	151.5 C	151.7 C	151.8 C	151.8 C	151.4 C
4	110.4 CH	110.3 CH	109.7 CH	110.3 CH	110.5 CH	110.3 CH
5	131.0 C	130.9 C	130.6 C	127.7 C	131.1 C	133.1 C
6	80.5 CH	80.8 CH	81.2 CH	80.6 CH	80.4 CH	38.7 CH <sub>2</sub>
7	38.3 CH	37.7 CH	38.8 CH	38.4 CH	38.6 CH	34.8 CH
8	41.5 CH	39.8 CH	38.7 CH	38.4 CH	38.4 CH	41.9 CH
9	81.0 CH	80.0 CH	81.3 CH	80.6 CH	80.7 CH	81.9 CH
10	133.3 C	133.1 C	133.7 C	131.0 C	133.3 C	134.7 C
11	102.5 CH	102.5 CH	102.5 CH	102.6 CH	102.4 CH	102.4 CH
12	148.5 C	148.5 C	148.5 C	148.5 C	148.6 C	148.6 C
13	135.9 C	136.2 C	135.9 C	133.3 C	136.1 C	136.0 C
14	141.6 C	143.7 C	141.4 C	141.8 C	141.5 C	141.4 C
15	121.0 C	121.3 C	121.3 C	121.1 C	121.3 C	120.6 C
16	122.6 C	123.2 C	123.1 C	123.6 C	123.5 C	123.4 C
17	19.9 CH <sub>3</sub>	17.6 CH <sub>3</sub>	19.4 CH <sub>3</sub>	15.6 CH <sub>3</sub>	18.4 CH <sub>3</sub>	14.6 CH <sub>3</sub>
18	9.6 CH <sub>3</sub>	18.8 CH <sub>3</sub>	14.7 CH <sub>3</sub>	15.6 CH <sub>3</sub>	16.9 CH <sub>3</sub>	19.6 CH <sub>3</sub>
1'	166.8 C	167.1 C	167.5 C	166.7 C	165.9 C	164.5 C
2'	127.6 C	127.5 C	127.6 C	127.6 C	117.8 CH	119.1 CH
3'	140.2 CH	137.9 CH	138.2 CH	138.6 CH	144.8 CH	144.4 CH
4'	15.5 CH <sub>3</sub>	14.0 CH <sub>3</sub>	14.1 CH <sub>3</sub>	15.6 CH <sub>3</sub>	134.1 C	134.4 C
5'	20.1 CH <sub>3</sub>	11.5 CH <sub>3</sub>	11.7 CH <sub>3</sub>	19.8 CH <sub>3</sub>	128.8 CH	130.1 CH
6'					128.0 CH	127.8 CH
7'					130.3 CH	129.1 CH
8'					128.0 CH	127.8 CH
9'					128.8 CH	130.1 CH
1''	166.7 C		165.6 C	167.2 C	166.6 C	
2''	127.0 C		130.8 C	127.6 C	127.7 C	
3''	138.4 CH		129.5 CH	138.1 CH	138.7 CH	
4''	15.4 CH <sub>3</sub>		127.9 CH	14.1 CH <sub>3</sub>	15.5 CH <sub>3</sub>	
5''	19.7 CH <sub>3</sub>		132.7 CH	11.6 CH <sub>3</sub>	19.8 CH <sub>3</sub>	
6''			127.9 CH			
7''			129.5 CH			
AcO-6		170.1 C				
		20.8 CH <sub>3</sub>				
CH <sub>3</sub> O-1	60.0 CH <sub>3</sub>	59.6 CH <sub>3</sub>	59.8 CH <sub>3</sub>	59.7 CH <sub>3</sub>	60.4 CH <sub>3</sub>	60.1 CH <sub>3</sub>
CH <sub>3</sub> O-2	60.3 CH <sub>3</sub>	60.4 CH <sub>3</sub>	60.5 CH <sub>3</sub>	60.5 CH <sub>3</sub>	60.8 CH <sub>3</sub>	60.6 CH <sub>3</sub>
CH <sub>3</sub> O-3	55.9 CH <sub>3</sub>	55.8 CH <sub>3</sub>	55.9 CH <sub>3</sub>	55.9 CH <sub>3</sub>	55.9 CH <sub>3</sub>	55.9 CH <sub>3</sub>
CH <sub>3</sub> O-14	59.2 CH <sub>3</sub>	59.5 CH <sub>3</sub>	59.1 CH <sub>3</sub>	59.4 CH <sub>3</sub>	59.5 CH <sub>3</sub>	59.6 CH <sub>3</sub>
OCH <sub>2</sub> O	100.9 CH <sub>2</sub>	101.1 CH <sub>2</sub>	101.0 CH <sub>2</sub>	101.0 CH <sub>2</sub>	101.1 CH <sub>2</sub>	101.1 CH <sub>2</sub>

<sup>a</sup>Recorded at 100 MHz. <sup>b</sup>Recorded at 125 MHz.

see Tables 2 and 4; positive ESIMS  $m/z$  621 (100)  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  621.2669  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{33}\text{H}_{42}\text{O}_{10}\text{Na}$ , 621.2675).

**Polysperlignan J (10):** yellow gum;  $[\alpha]_{\text{D}}^{25} +39.6$  ( $c$  0.10,  $\text{CHCl}_3$ ); CD ( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 200 (+18), 237 (−15); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 241 (3.59) nm; IR (KBr)  $\nu_{\text{max}}$  2934, 1705, 1649, 1457, 1262  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 4; positive ESIMS  $m/z$  621 (100)  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  621.2660  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{33}\text{H}_{42}\text{O}_{10}\text{Na}$ , 621.2675).

**Polysperlignan K (11):** yellow oil;  $[\alpha]_{\text{D}}^{17} -2.2$  ( $c$  0.10,  $\text{CHCl}_3$ ); CD ( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  nm ( $\Delta\epsilon$ ) 218 (−22), 239 (+16); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 241 (3.47), 193 (2.89) nm; IR (KBr)  $\nu_{\text{max}}$  2934, 1764, 1615, 1462, 1204  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 4; positive ESIMS  $m/z$  467 (100)  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  467.1675  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{24}\text{H}_{28}\text{O}_8\text{Na}$ , 467.1681).

**X-ray Crystal Structure Analysis.** Colorless crystals of **1** and **7** were obtained from  $\text{CH}_3\text{OH}$ . Intensity data were collected at 100 K on an Bruker APEX DUO diffractometer equipped with an APEX II CCD, using  $\text{Cu K}\alpha$  radiation. Cell refinement and data reduction were

performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97.<sup>21</sup> Refinements were performed with SHELXL-97<sup>21</sup> using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. Crystallographic data (excluding structure factor tables) for the structures reported have been deposited with the Cambridge Crystallographic Data Center as supplementary publications no. CCDC 853853 for **1** and CCDC 853854 for **7**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44(0) (1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk].

**Polysperlignan A (1):**  $\text{C}_{33}\text{H}_{40}\text{O}_{10}$ ,  $M_w = 596.7$ , orthorhombic, space group,  $P2_12_12_1$ ,  $Z = 4$ ,  $a = 10.6604(3)$  Å,  $b = 14.6290(3)$  Å,  $c = 19.5474(4)$  Å;  $\alpha = \beta = \gamma = 90^\circ$ ,  $V = 3048.44(12)$  Å<sup>3</sup>,  $\mu(\text{Cu K}\alpha) = 0.79$   $\text{mm}^{-1}$ ,  $\rho_{\text{calc}} = 1.30$   $\text{g}\cdot\text{cm}^{-3}$ ;  $S = 1.06$ , final  $R$  indices:  $R_1 = 0.0531$  and  $wR_2 = 0.1420$  for 4976 observed from 5133 independent and 13 947 measured reflections ( $\theta_{\text{max}} = 69.2$ ,  $I > 2\sigma(I)$  criterion and

Table 4.  $^{13}\text{C}$  NMR Data of Compounds 7–11 ( $\delta$  in ppm)

position	7 <sup>a</sup>	8 <sup>b</sup>	9 <sup>a</sup>	10 <sup>c</sup>	11 <sup>a</sup>
1	142.4 C	142.5 C	142.3 C	143.7 C	142.1 C
2	140.2 C	140.3 C	139.0 C	141.1 C	139.0 C
3	151.5 C	151.4 C	151.5 C	152.8 C	153.0 C
4	112.9 CH	112.8 CH	113.0 CH	114.1 CH	109.7 CH
5	132.8 C	132.8 C	132.6 C	134.0 C	139.3 C
6	80.9 CH	81.0 CH	80.7 CH	82.0 CH	34.6 CH <sub>2</sub>
7	39.2 CH	39.3 CH	40.1 CH	41.1 CH	39.0 CH
8	39.1 CH	39.2 CH	39.0 CH	39.5 CH	42.2 CH
9	81.6 CH	81.9 CH	83.2 CH	84.2 CH	72.9 CH
10	138.4 C	138.7 C	139.3 C	140.8 C	136.8 C
11	102.0 CH	102.1 CH	106.6 CH	108.3 CH	102.2 CH
12	148.7 C	148.6 C	152.9 C	154.6 C	148.4 C
13	134.8 C	134.7 C	140.4 C	142.1 C	134.5 C
14	141.0 C	141.1 C	151.3 C	152.6 C	140.0 C
15	118.7 C	119.1 C	119.7 C	121.3 C	118.6 C
16	122.3 C	122.3 C	122.6 C	123.5 C	120.8 C
17	14.9 CH <sub>3</sub>	14.9 CH <sub>3</sub>	14.3 CH <sub>3</sub>	14.5 CH <sub>3</sub>	21.9 CH <sub>3</sub>
18	20.0 CH <sub>3</sub>	20.1 CH <sub>3</sub>	21.0 CH <sub>3</sub>	21.1 CH <sub>3</sub>	7.9 CH <sub>3</sub>
1'	167.3 C	167.4 C	167.3 C	168.8 C	
2'	127.0 C	127.1 C	127.0 C	128.2 C	
3'	139.0 CH	138.9 CH	138.9 CH	141.2 CH	
4'	15.4 CH <sub>3</sub>	15.4 CH <sub>3</sub>	15.4 CH <sub>3</sub>	15.9 CH <sub>3</sub>	
5'	20.3 CH <sub>3</sub>	20.3 CH <sub>3</sub>	20.2 CH <sub>3</sub>	20.6 CH <sub>3</sub>	
1''	166.6 C	166.8 C	166.7 C	168.4 C	
2''	127.8 C	128.4 C	127.6 C	128.9 C	
3''	139.0 CH	136.8 CH	138.9 CH	139.5 CH	
4''	15.6 CH <sub>3</sub>	14.1 CH <sub>3</sub>	15.5 CH <sub>3</sub>	14.3 CH <sub>3</sub>	
5''	19.9 CH <sub>3</sub>	11.6 CH <sub>3</sub>	19.9 CH <sub>3</sub>	11.7 CH <sub>3</sub>	
AcO					168.8 C
					20.5 CH <sub>3</sub>
CH <sub>3</sub> O-2	60.9 CH <sub>3</sub>	61.0 CH <sub>3</sub>	61.0 CH <sub>3</sub>	61.5 CH <sub>3</sub>	60.9 CH <sub>3</sub>
CH <sub>3</sub> O-3	55.9 CH <sub>3</sub>	56.0 CH <sub>3</sub>	55.9 CH <sub>3</sub>	56.6 CH <sub>3</sub>	55.8 CH <sub>3</sub>
CH <sub>3</sub> O-12			56.0 CH <sub>3</sub>	56.5 CH <sub>3</sub>	
CH <sub>3</sub> O-13			60.5 CH <sub>3</sub>	61.0 CH <sub>3</sub>	
CH <sub>3</sub> O-14	59.1 CH <sub>3</sub>	59.1 CH <sub>3</sub>	60.4 CH <sub>3</sub>	60.4 CH <sub>3</sub>	59.6 CH <sub>3</sub>
OCH <sub>2</sub> O	100.8 CH <sub>2</sub>	100.8 CH <sub>2</sub>			100.9 CH <sub>2</sub>

<sup>a</sup>Recorded at 100 MHz in CDCl<sub>3</sub>, <sup>b</sup>Recorded at 125 MHz in CDCl<sub>3</sub>, <sup>c</sup>Recorded in CD<sub>3</sub>OD, 150 MHz.

Table 5. Neuroprotective Effects of Compounds 1, 2, 4–6, 9, and 13–19 on A $\beta$ <sub>25–35</sub>- or H<sub>2</sub>O<sub>2</sub>-Induced Cytotoxicity on PC12 Cells<sup>a</sup>

compound	A $\beta$ <sub>25–35</sub> (1 $\mu\text{M}$ )			compound	H <sub>2</sub> O <sub>2</sub> (300 $\mu\text{M}$ )		
	test concentration ( $\mu\text{M}$ )				test concentration ( $\mu\text{M}$ )		
	1 $\mu\text{M}$	10 $\mu\text{M}$	vehicle		1 $\mu\text{M}$	10 $\mu\text{M}$	vehicle
1	39.9 $\pm$ 1.1	46.4 $\pm$ 3.7 <sup>c</sup>	38.8 $\pm$ 1.0	1	55.8 $\pm$ 1.9	58.2 $\pm$ 0.4	53.7 $\pm$ 1.7
2	36.4 $\pm$ 0.8	34.1 $\pm$ 2.0	34.8 $\pm$ 0.8	2	55.9 $\pm$ 2.4	58.9 $\pm$ 0.9 <sup>e</sup>	53.1 $\pm$ 1.1
4	34.4 $\pm$ 0.8	50.0 $\pm$ 6.4 <sup>c</sup>	34.8 $\pm$ 0.8	4	50.3 $\pm$ 0.5	55.8 $\pm$ 0.5	53.1 $\pm$ 1.1
5	39.0 $\pm$ 1.1	39.7 $\pm$ 2.0	38.8 $\pm$ 1.0	5	57.1 $\pm$ 2.6	58.9 $\pm$ 3.1 <sup>d</sup>	53.7 $\pm$ 1.7
6	38.4 $\pm$ 0.5	36.4 $\pm$ 1.8	38.8 $\pm$ 1.0	6	55.2 $\pm$ 0.4	58.5 $\pm$ 2.6	53.7 $\pm$ 1.7
9	39.0 $\pm$ 0.8	41.2 $\pm$ 1.6	38.8 $\pm$ 1.0	9	53.3 $\pm$ 0.8	53.7 $\pm$ 1.5	53.7 $\pm$ 1.7
13	35.1 $\pm$ 0.6	32.9 $\pm$ 1.9	34.8 $\pm$ 0.8	13	57.2 $\pm$ 0.4 <sup>d</sup>	44.0 $\pm$ 2.3 <sup>e</sup>	53.1 $\pm$ 1.1
14	35.3 $\pm$ 0.9	32.3 $\pm$ 1.3	34.8 $\pm$ 0.8	14	53.0 $\pm$ 1.3	52.5 $\pm$ 0.9	53.1 $\pm$ 1.1
15	38.0 $\pm$ 0.8	33.3 $\pm$ 1.3 <sup>b</sup>	38.8 $\pm$ 1.0	15	48.8 $\pm$ 1.3	55.8 $\pm$ 0.1	53.7 $\pm$ 1.7
16	43.1 $\pm$ 1.1	39.3 $\pm$ 2.8	38.8 $\pm$ 1.0	16	54.6 $\pm$ 1.6	60.7 $\pm$ 1.8 <sup>e</sup>	53.7 $\pm$ 1.7
17	35.2 $\pm$ 0.1	33.2 $\pm$ 1.8	34.8 $\pm$ 0.8	17	51.0 $\pm$ 0.9	53.3 $\pm$ 1.2	53.1 $\pm$ 1.1
18	41.4 $\pm$ 0.8	41.0 $\pm$ 2.1	38.8 $\pm$ 1.0	18	50.4 $\pm$ 2.8	53.4 $\pm$ 0.2	53.7 $\pm$ 1.7
19	40.3 $\pm$ 1.1	40.8 $\pm$ 2.2	38.8 $\pm$ 1.0	19	54.9 $\pm$ 0.6	51.8 $\pm$ 1.5	53.7 $\pm$ 1.7

<sup>a</sup>The data (cell viability, measured by MTT reduction) were normalized and expressed as a percentage of the control group, which is set to 100%. Data expressed as means  $\pm$  SEM. Three independent experiments were carried out. <sup>b</sup> $p < 0.05$ . <sup>c</sup> $p < 0.01$  vs A $\beta$ <sub>25–35</sub> group. <sup>d</sup> $p < 0.05$ . <sup>e</sup> $p < 0.01$  vs H<sub>2</sub>O<sub>2</sub> group. Values higher than corresponding A $\beta$ <sub>25–35</sub> or H<sub>2</sub>O<sub>2</sub> control indicate neuroprotection; values lower than corresponding A $\beta$ <sub>25–35</sub> or H<sub>2</sub>O<sub>2</sub> control mean neurotoxicity.

399 parameters); maximum and minimum residuals are 1.13 and  $-0.40 \text{ e}\cdot\text{\AA}^{-3}$ , respectively. The Flack<sup>22</sup> parameter value was  $x = 0.1(2)$ .

**Polysperlignan G (7):**  $\text{C}_{64}\text{H}_{76}\text{O}_{20}$  ( $\text{C}_{32}\text{H}_{38}\text{O}_{10} \times 2$ ),  $M_w = 1165.3$ , orthorhombic, space group,  $P2_12_12_1$ ,  $Z = 4$ ,  $a = 10.1503(2) \text{ \AA}$ ,  $b = 19.3759(3) \text{ \AA}$ ,  $c = 30.2635(5) \text{ \AA}$ ;  $\alpha = \beta = \gamma = 90^\circ$ ,  $V = 5951.96(18) \text{ \AA}^3$ ,  $\mu(\text{Cu K}\alpha) = 0.80 \text{ mm}^{-1}$ ,  $\rho_{\text{calc}} = 1.30 \text{ g}\cdot\text{cm}^{-3}$ ;  $S = 1.29$ , final  $R$  indices:  $R_1 = 0.115$  and  $wR_2 = 0.287$  for 8293 observed from 10 045 independent and 24 240 measured reflections ( $\theta_{\text{max}} = 69.6$ ,  $I > 2\sigma(I)$  criterion and 777 parameters); maximum and minimum residuals are 0.79 and  $-0.82 \text{ e}\cdot\text{\AA}^{-3}$ , respectively. The Flack<sup>22</sup> parameter value was  $x = -0.1(3)$ .

**Neuroprotective Activity Assay.** PC12 neuroblastoma cells were digested by trypsin and suspended in DMEM medium (low sugar) containing 10% new bovine serum (Gibco). Cells were seeded into 96-well plates (Greiner) at a density of  $8 \times 10^4$  cells per mL, 100  $\mu\text{L}$  per well, maintained at  $37^\circ\text{C}$  in a constant temperature incubator containing 5%  $\text{CO}_2$ . Serum-free DMEM medium was used to substitute the original medium 24 h after cells were seeded.

Appropriate concentrations of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and  $\beta$ -amyloid<sub>25–35</sub> ( $A\beta_{25–35}$ ) were prepared on the day of application to cultures. The PC12 cells were preincubated with 1 or 10  $\mu\text{M}$  of the different compounds 2 h before  $\text{H}_2\text{O}_2$  (300  $\mu\text{M}$ ) and  $A\beta_{25–35}$  (1  $\mu\text{M}$ ) were added, and the assays for cell viability were performed 24 h after  $\text{H}_2\text{O}_2$  and  $A\beta_{25–35}$  were added. Cell survival was evaluated by reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma).<sup>23</sup> The values of cell survival were normalized against the values for the control group, which was set to 100%. Data were evaluated for statistical significance with one-way ANOVA followed by a LSD test using a computerized statistical package. Differences were considered significant at  $p < 0.05$ .

## ■ ASSOCIATED CONTENT

### ● Supporting Information

This material ( $^1\text{H}/^{13}\text{C}$  NMR, DEPT, HSQC, HMBC, COSY, ROESY, HRESIMS, IR, CD, and UV spectra of compounds 1, 6, and 8 and  $^1\text{H}/^{13}\text{C}$  NMR, DEPT, and HRESIMS spectra of compounds 2–5, 7, and 9–11, X-ray data of compounds 1 and 7) is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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