

# Spectral Assignments and Reference Data

## Complete assignment of $^1\text{H}$ and $^{13}\text{C}$ NMR data for nine protopanaxatriol glycosides

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Nine protopanaxatriol glycosides isolated from mild acid hydrolysis products of crude root saponins of *Panax notoginseng* were identified as 20(R)-ginsenoside-Rh1, 20(S)-ginsenoside-Rh1, ginsenoside-Rg1, -Re and -Rg2, notoginsenoside-R2 and -R1, a mixture of 25-hydroxy-20(S)-ginsenoside-Rh1 and its C-20 (R) epimer, ginsenoside-Rh4. The complete assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts for these glycosides were obtained by means of 2D NMR techniques, including  $^1\text{H}$ - $^1\text{H}$  COSY, ROESY, HMQC, HMBC and HMQC-TOCSY spectra. The glycosylation shift effect of protopanaxatriol and the differences in chemical shifts between 20(R)- and 20(S)-protopanaxatriol isomers are also discussed. Except for ginsenoside-Re and -Rg2, complete NMR assignments of the other seven glycosides are reported for the first time. Copyright © 2002 John Wiley & Sons, Ltd.

**KEYWORDS:** NMR;  $^1\text{H}$  NMR;  $^{13}\text{C}$  NMR; 2D NMR; protopanaxatriol glycoside; *Panax notoginseng*; complete NMR assignments; glycosylation shift effect

## INTRODUCTION

*Panax notoginseng* (Burk.) F. H. Chen (Araliaceae), a well known traditional Chinese medicinal herb indigenous to the mountains of Yunnan province and Guanxi province, is used for the treatment of cardiovascular diseases, inflammation and internal and external bleeding due to injury.<sup>1</sup> Many drugs have been developed commercially on the basis of this plant. Extensive studies on this plant,

including phytochemical and pharmacological research, proved that dammarane-type saponins are the main bioactive principles.<sup>2–7</sup>

The saponins of *P. notoginseng* have been shown to the derivatives of protopanaxadiol and protopanaxatriol.<sup>8</sup> The acid-labile nature of ginsenosides and notoginsenosides was observed in early studies, although the conditions were mild.<sup>9–12</sup> We have reported two new glycosides, notoginsenoside-T1 and -T2<sup>13</sup> and six protopanaxadiol glycosides from the mild acid hydrolysis products of root crude saponins of *P. notoginseng*.<sup>14,15</sup> This paper deals mainly with the complete assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of another nine protopanaxatriol glycosides from the hydrolysis products. The glycosylation shift effect of protopanaxatriol and the differences in chemical shifts between 20(R)- and 20(S)-protopanaxatriol isomers are also discussed. Except for ginsenoside-Re and -Rg2, complete NMR assignments of the other seven glycosides are reported for the first time.

## RESULTS AND DISCUSSION

The structures of compounds 1–9 were determined by comparison of mass spectrometric (MS) and  $^{13}\text{C}$  NMR data with those in the literature. They were 20(R)-ginsenoside-Rh1 (1),<sup>7,16,17</sup> 20(S)-ginsenoside-Rh1 (2),<sup>7</sup> ginsenoside-Rg1 (3),<sup>18</sup> -Re (4)<sup>19</sup> and -Rg2 (5),<sup>20</sup> notoginsenoside-R2 (6)<sup>7</sup> and -R1 (7),<sup>7</sup> a mixture of 25-hydroxy-20(S)-ginsenoside-Rh1 and its C-20 (R) epimer (8)<sup>7,10</sup> and ginsenoside-Rh4 (9).<sup>21</sup> The structures and numbering system for 1–9 are presented in Fig. 1. Assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts for 1–9 are listed in Tables 1–3.

The obvious signal assignments were made from  $^1\text{H}$  and  $^{13}\text{C}$  NMR and DEPT spectra according to the chemical shifts and multiplicities. From comparison with the  $^{13}\text{C}$  NMR chemical shifts in the literature for each glycoside, carbon signals were directly assigned, and these assignments were also confirmed by 2D NMR spectra. Other remaining  $^1\text{H}$  NMR signals were assigned with the aid of 2D NMR spectra including DQF  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC or HSQC, HMBC, ROESY and HMQC-TOCSY spectra. The assignment methods and procedures were similar to those in our previous papers.<sup>13–15,22–24</sup> Most  $^1\text{H}$  NMR signals could be assigned by HMQC and  $^1\text{H}$ - $^1\text{H}$  COSY spectra. It's worth noting that the  $^1\text{H}$  NMR chemical shifts of methyl groups had to be assigned from the long-range correlation between methyl protons and assigned  $^{13}\text{C}$  signals in the HMBC spectrum. The reason is that unambiguous assignments could not be accomplished from the HMQC spectrum owing to serious overlap of the methyl carbon signals with each other in the high-field part of the  $^{13}\text{C}$  NMR spectra. The relative configurations of protons in methylene groups in the ring part of the aglycone were assigned from the ROESY spectra.

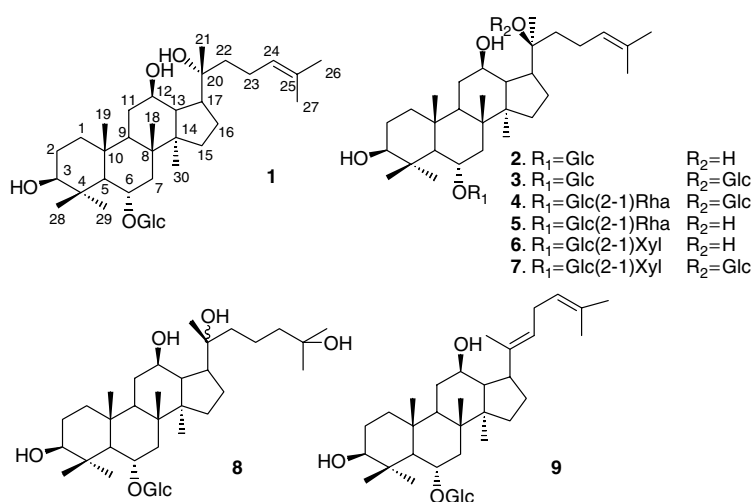


Figure 1. Structures of compounds 1–9.

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**Table 1.**  $^1\text{H}$  NMR chemical shifts of saponins **1–8** (500 MHz; pyridine- $d_5$ ,  $J$  in Hz)

H	1	2	3	4	5	6	7	8
1 $\alpha$	1.02	1.04	0.96	0.95	0.96	0.96	0.94	1.01
1 $\beta$	1.70	1.70	1.68	1.69	1.64	1.65	1.69	1.69
2 $\alpha$	1.93 (brd, 11.5)	1.91	1.89	1.87	1.86	1.86	1.88	1.92
2 $\beta$	1.81	1.82	1.83	1.78	1.79	1.78	1.80	1.84
3	3.52 (brd, 9.3)	3.52 (dd, 4.7, 11.8)	3.49 (brd, 10.0)	3.44 (dd, 5.3, 11.7)	3.47 (dd, 4.1, 11.6)	3.48 (dd, 2.9, 12.8)	3.46 (dd, 4.5, 10.4)	3.50 (brd, 11.2)
5	1.43 (d, 10.2)	1.42 (d, 10.7)	1.38 (d, 11.0)	1.37	1.39	1.36	1.35 (d, 10.1)	1.42
6	4.44 (t, 10.7)	4.42 (ddd, 3.0, 10.0, 10.7)	4.37	4.66	4.69	4.31	4.30	4.43 (t, 10.6)
7 $\alpha$	1.94	1.93 (brd, 12.6)	1.88	1.95	1.98	1.92	1.89 (t, 12.0)	1.93
7 $\beta$	2.53 (dd, 2.5, 12.9)	2.52 (brd, 12.6)	2.45	2.22 <sup>a</sup>	2.26	2.37 (dd, 2.9, 12.8)	2.32	2.52 (dd, 4.4, 12.9)
9	1.58	1.57	1.49	1.51	1.53	1.49	1.45	1.57
11 $\alpha$	2.15 (dd, 5.1, 11.5)	2.13 (dd, 4.7, 10.4)	2.04 (dd, 4.7, 9.0)	2.04	1.98	2.09	2.03	2.14
11 $\beta$	1.54	1.55	1.47	1.48	1.54	1.49	1.46	1.54
12	3.91 (dd, 5.2, 11.5)	3.89	4.09	4.09	3.90	3.95	4.09	3.89
13	2.01 (t, 10.4)	2.03	1.94 (t, 10.7)	1.92	2.00	2.01	1.93 (t, 12.0)	2.04 [2.01] <sup>b</sup>
15 $\alpha$	1.64	1.66	1.60	1.60	1.53	1.60	1.60	1.58
15 $\beta$	1.13 (t, 10.2)	1.10 (brt, 10.7)	1.04	1.04	0.92	1.12	1.06 (t, 10.1)	1.11
16 $\alpha$	1.92	1.78	1.72	1.74	1.77	1.74	1.73	1.93
16 $\beta$	1.29	1.35	1.26	1.24	1.29	1.30	1.26	1.30
17	2.33	2.29	2.44	2.45	2.30	2.27	2.45	2.26 [2.32] <sup>b</sup>
18	1.23 (s)	1.18 (s)	1.13 (s)	1.16 (s)	1.20 (s)	1.15 (s)	1.12 (s)	1.05 (s)
19	1.05 (s)	1.02 (s)	1.00 (s)	0.96 (s)	0.98 (s)	0.95 (s)	0.96 (s)	1.24 (s)
21	1.38 (s)	1.39 (s)	1.59 (s) <sup>a</sup>	1.58 (s)	1.39 (s)	1.39 (s)	1.59 (s) <sup>a</sup>	1.40 (s) <sup>a</sup>
								[1.38]
22a	1.72	2.03	2.33	2.33	2.01	2.01	2.34	1.99 [2.02] <sup>b</sup>
22b	1.67	1.69	1.79	1.79	1.65	1.67	1.78	1.55 [1.58] <sup>b</sup>
23a	2.49	2.58	2.44	2.49	2.59	2.58	2.45	2.10 [2.04] <sup>b</sup>
23b	2.42 (dd, 6.6, 12.6)	2.27	2.21	2.22 <sup>a</sup>	2.27	2.26	2.21 (dd, 5.6, 12.5)	1.93 [1.95] <sup>b</sup>
24	5.30 (t, 6.9)	5.32 (t, 7.4)	5.24 (t, 6.6)	5.24	5.33 (t, 8.3)	5.31 (qt, 1.6, 7.2)	5.23 (t, 6.9)	2.39; 1.61
26	1.69 (s)	1.65 (s)	1.59 (s) <sup>a</sup>	1.60 (s)	1.67 (s)	1.66 (s)	1.59 (s) <sup>a</sup>	1.40 (s) <sup>a</sup>
27	1.63 (s)	1.62 (s)	1.59 (s) <sup>a</sup>	1.57 (s)	1.63 (s)	1.63 (s)	1.59 (s) <sup>a</sup>	1.39 (s)
28	2.06 (s)	2.07 (s)	2.03 (s)	2.07 (s)	2.10 (s)	2.04 (s)	2.04 (s)	2.05 (s)
29	1.60 (s)	1.59 (s)	1.57 (s)	1.34 (s)	1.35 (s)	1.44 (s)	1.44 (s)	1.58 (s)
30	0.86 (s)	0.82 (s)	0.79 (s)	0.94 (s)	0.95 (s)	0.80 (s)	0.78 (s)	0.83 (s)
6-O-Glc								
1	5.03 (d, 7.5)	5.02 (d, 7.6)	4.98 (d, 7.7)	5.22 (d, 6.9)	5.25 (d, 7.2)	4.91 (d, 7.2)	4.90 (d, 7.2)	5.03 (d, 7.6)
2	4.08 (d, 8.0)	4.09 (t, 7.7)	4.04	4.35	4.34	4.33	4.33	4.07 (t, 8.8)
3	4.25 (t, 8.5)	4.25 (t, 7.7)	4.22	4.32	4.28	4.13	4.13	4.23 (t, 8.8)

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Table 1. (Continued)

H	1	2	3	4	5	6	7	8
4	4.20 (t, 9.1)	4.21 (t, 7.7)	4.17	4.16	4.20	4.14	4.14	4.18 (t, 8.8)
5	3.95	3.94	3.90	3.92	3.96	3.81	3.80(ddd, 2.7, 5.6, 8.5)	3.93(ddd, 2.6, 5.8, 8.5)
6a	4.52 (brd, 11.5)	4.52 (brd, 12.1)	4.49 (dd, 2.2, 11.5)	4.47	4.51 (d, 9.8)	4.46 (dd, 2.4, 11.5)	4.45 <sup>a</sup>	4.51 (brd, 11.4)
6b	4.35 (dd, 4.8, 11.5)	4.36 (dd, 5.2, 12.1)	4.39 (dd, 3.0, 11.5)	4.31	4.37	4.30	4.31	4.34 (dd, 5.9, 11.4)
Rha (or Xyl)								
1				6.44 (brs)	6.46 (brs)	5.73 (d, 7.5)	5.72 (d, 7.2)	
2				4.75 (brs)	4.77	4.12	4.11	
3				4.64	4.66	4.26	4.28	
4				4.28	4.30	4.22	4.20	
5				4.90 (dt, 6.1, 9.1)	4.92	4.32	4.36	
						3.63 (t, 10.4)	3.62 (t, 10.7)	
6				1.74 (d, 6.1)	1.78 (d, 5.7)			
20-O-Glc								
1			5.12 (d, 8.2)	5.14 (d, 7.5)			5.13 (d, 8.0)	
2			3.95	3.94			3.95 (t, 8.5)	
3			4.20	4.17			4.18	
4			4.14	4.12			4.10	
5			3.88	3.87			3.89 (ddd, 2.4, 5.3, 8.3)	
6a			4.45 (dd, 2.5, 11.8)	4.43			4.45 <sup>a</sup>	
6b			4.35 (dd, 3.3, 11.8)	4.27			4.28	

<sup>a</sup> Signals in the same column.<sup>b</sup> Chemical shifts in square brackets due to 20(R) epimer of 8.

It is interesting to note the differences in the NMR chemical shifts between 20(R)-ginsenoside-Rh1 (**1**) and 20(S)-ginsenoside-Rh1 (**2**) (Table 4). The differences in carbon NMR chemical shifts were mainly at C-17 (−4.1 ppm), C-21 (−4.3 ppm) and C-22 (+7.4 ppm). The differences in proton NMR chemical shifts were mainly at H-16 $\alpha$  (+0.14 ppm), H-22a (−0.31 ppm), H-23a (−0.09 ppm) and H-23b (+0.15 ppm).

There were two different situations with regard to the glycosylation shift effects of protopanaxatriol. When C-6 of protopanaxatriol was linked by  $\beta$ -D-Glc,  $\beta$ -D-Glc-(1 $\rightarrow$ 2)- $\beta$ -D-Glc or  $\beta$ -D-Xyl-(1 $\rightarrow$ 2)- $\beta$ -D-Glc, the chemical shift of C-6 was shifted downfield by 12 ppm from 68 to 80 ppm, and the chemical shift of C-7 went upfield by 2 ppm and that of C-5 upfield by <1 ppm. When C-6 of protopanaxatriol was linked by  $\alpha$ -L-Rha-(1 $\rightarrow$ 2)- $\beta$ -D-Glc, the chemical shift of C-6 was shifted downfield by 6–7 ppm from 68 to 74–75 ppm, and the chemical shift of C-7 went upfield by about 1 ppm and that of C-5 upfield by <1 ppm. These different C-6 deshielding effects due to C-2 substituents (L-Rha vs D-Glc or D-Xyl) in the inner D-Glc could be due to the different conformations between the glucopyranosyl unit and C-6 of the aglycone in the two different situations.<sup>25</sup>

After glycosylation at C-20 of protopanaxatriol, the chemical shift of C-20 was shifted downfield by 10 ppm, and the chemical shifts of C-21 and C-17 went upfield by 4–5 and 3 ppm, respectively,

and that of C-22 upfield by <1 ppm. These differences were similar to those for protopanaxadiol.<sup>14,15</sup>

## EXPERIMENTAL

## Compounds

Compounds **1**–**9** were isolated and purified by silica gel column chromatography with CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O as eluent and RP-8 or RP-18 column chromatography with aqueous methanol as eluent were given in previous papers.<sup>13–15</sup>

## Spectra

All NMR experiments were recorded on a Bruker DRX-500 spectrometer operating at 500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively and equipped with an inverse-detection 5 mm probe (BBI probe, <sup>1</sup>H 90° pulse width = 9.5  $\mu$ s, <sup>13</sup>C 90° pulse width = 18.5  $\mu$ s) and operating at room temperature. About 15–30 mg samples were dissolved in pyridine-*d*<sub>5</sub> (0.4 ml) to record the NMR spectra using the lowest field signals of pyridine-*d*<sub>5</sub> (<sup>1</sup>H,  $\delta$  8.71; <sup>13</sup>C,  $\delta$  149.9) as an internal reference.

1D spectra were acquired using 64 K data points and spectral widths of 5000 Hz and 27 500 Hz for <sup>1</sup>H and <sup>13</sup>C, respectively; 32 K data points were used for the processing with no window function

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**Table 2.**  $^{13}\text{C}$  NMR data for saponins **1–8** ( $^{13}\text{C}$ , 125 MHz,  $\delta$  in pyridine- $d_5$ )

C	1	2	3	4	5	6	7	8
1	39.5	39.5	39.5	39.5	39.4	39.5	39.5	39.5
2	28.0	28.0	28.0	27.8	27.8	27.8	27.8	28.0*
3	78.7	78.7	78.8	78.2	78.4*	78.8	78.2*	78.7
4	40.4	40.4	40.4	40.0	40.1	40.3	40.3	40.4
5	61.5	61.5	61.5	60.9	60.9	61.4	61.4	61.5
6	80.1	80.1	80.2	78.7	74.4	80.3	80.3	80.2
7	45.3	45.3	45.2	46.0	46.1	45.3	45.0	45.3
8	41.2	41.2	41.2	41.2	41.2	41.2	41.2	41.2
9	50.3	50.3	50.1	49.7	49.7	50.2	50.0	50.3
10	39.8	39.7	39.8	39.7	39.7	39.7	39.7	39.8
11	32.3	32.1	31.0	30.8	32.1	32.1	30.8	32.3
12	71.0	71.1	70.4	70.3	71.1	71.1	71.3	71.1
13	49.0	48.3	49.2	49.1	48.3	48.3	49.3	48.4 (49.0) <sup>b</sup>
14	51.4	51.7	51.5	51.7	51.8	51.8	51.6	51.8
15	31.4	31.3	30.8	31.0	31.4	31.3	31.0	31.4
16	26.7	26.9	26.7	26.7	26.7	27.8	26.7	28.0 <sup>a</sup>
17	50.7	54.8	51.8	51.5	54.7	54.8	51.5	54.8 (50.9) <sup>b</sup>
18	17.5	17.4	17.6 <sup>a</sup>	17.7	17.7 <sup>a</sup>	17.7	17.6 <sup>a</sup>	17.8
19	17.8 <sup>a</sup>	17.7 <sup>a</sup>	17.6 <sup>a</sup>	17.8 <sup>a</sup>	17.7 <sup>a</sup>	17.8	17.6 <sup>a</sup>	17.5
20	73.1	73.1	83.5	83.4	73.1	73.1	83.4	73.5
21	22.8	27.1	22.5	22.4	27.1	25.9	22.4	27.3 (22.8) <sup>b</sup>
22	43.3	35.9	36.2	36.1	35.9	35.9	36.2	36.5 (44.1) <sup>b</sup>
23	22.7	23.0	23.4	23.3	23.0	23.1	23.3	19.2 (18.8) <sup>b</sup>
24	126.1	126.4	126.0	126.0	126.4	126.4	126.0	45.3 (45.8) <sup>b</sup>
25	130.9	130.8	131.1	131.0	130.8	130.9	131.0	69.8
26	25.9	25.9	25.9	25.4	25.9	27.1	25.8	30.0
27	17.8 <sup>a</sup>	17.7 <sup>a</sup>	17.9	17.8 <sup>a</sup>	17.7 <sup>a</sup>	17.4	17.3	30.3
28	31.8	31.8	31.8	32.3	32.2	31.8	31.8	31.8
29	16.5	16.2	16.5	17.3	17.0	16.8	17.8	16.5
30	17.2	16.9	17.2	17.6	17.2	16.9	16.9	16.9
6-O-Glc								
1	106.1	106.1	106.0	101.9 <sup>a</sup>	102.0	103.6	103.6	106.1
2	75.5	75.5	75.5	79.4	79.5	79.9	79.9	75.6
3	79.7	79.7	79.7	78.3	78.7	78.1	78.1	79.7
4	71.9	71.9	71.9	72.3	72.5	71.8	71.8 <sup>a</sup>	72.0
5	78.2	78.2	78.3	78.2	78.4 <sup>a</sup>	79.5	79.6	78.2
6	63.2	63.2	63.2	63.2	63.2	63.0	63.0 <sup>a</sup>	63.2
Rha(or Xyl)								
1				101.9 <sup>a</sup>	101.9	105.2	104.9	
2				72.5	72.3	75.9	75.9	
3				72.7	72.7	78.9	79.0	
4				74.2	74.2	71.3	71.3	
5				69.5	69.5	67.3	67.3	
6				18.8	18.8			
20-O-Glc								
1			98.3	98.3			98.3	
2			75.2	75.2			75.2	
3			79.2	79.3			79.3	
4			71.6	71.7			71.8 <sup>a</sup>	
5			78.2	78.5			78.2 <sup>a</sup>	
6			63.2	63.0			63.0 <sup>a</sup>	

<sup>a</sup> Signals in the same column.

<sup>b</sup> Chemical shifts in parentheses due to 20(R) epimer of **8**.

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**Table 3.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for saponin **9** ( $^{13}\text{C}$ , 125 MHz;  $^1\text{H}$ , 500 MHz;  $\delta$  in pyridine- $d_5$  or DMSO;  $J$  in Hz)

C	9(DMSO)	9(C <sub>5</sub> D <sub>5</sub> N)	H	9(DMSO)	9(C <sub>5</sub> D <sub>5</sub> N)
1	38.7	39.6	1 $\alpha$	0.94	1.04
2	26.8	28.8	$\beta$	1.55	1.70
3	77.1	78.6	2 $\alpha$	1.92	1.92
4	39.2	40.4	$\beta$	1.82	1.84
5	61.2	61.5	3	2.91 (dd, 4.5, 13.9)	3.51 (dd, 4.7, 10.8)
6	78.6	80.1	5	0.94	1.42
7	44.1	45.4	6	3.88	4.41 (ddd, 2.9, 9.8, 10.4)
8	40.3	41.4	7 $\alpha$	1.42	1.93
9	49.5a	50.6	$\beta$	1.94 (brd, 12.5)	2.51 (brd, 11.1)
10	39.0	39.8	9	1.31	1.57
11	31.8	32.6	11 $\alpha$	1.60	2.08
12	71.0	72.5	$\beta$	1.05	1.43
13	49.2	50.5	12	3.37	3.89
14	50.2	50.7	13	1.62	1.98
15	31.8a	32.3	15 $\alpha$	1.58	1.74
16	28.4	27.0	$\beta$	1.00	1.10
17	49.5a	51.2	16 $\alpha$	1.78	1.81
18	17.1	17.8	$\beta$	1.29	1.29
19	16.7	17.4	17	2.45	2.73 (t, 6.6)
20	138.9	140.2	18	0.98 (s)	1.23 (s)
21	12.7	12.8	19	0.85 (s)*	1.03 (s)
22	121.9	123.2	21	1.53 (s)	1.81 (s)
23	26.6	27.5	22	5.09 (t, 7.2)	5.47 (t, 7.2)
24	123.4	125.4	23	2.58	2.77 (t, 7.2)
25	130.4	131.3	24	5.02 (t, 7.5)	5.21 (t, 6.9)
26	25.5	25.8	26	1.61 (s)	1.62(s)
27	17.6	17.8	27	1.55 (s)	1.58 (s) <sup>a</sup>
28	30.6	31.8	28	1.21 (s)	2.04 (s)
29	15.4*	16.4	29	0.85 (s) <sup>a</sup>	1.58 (s) <sup>a</sup>
30	15.4*	16.8	30	0.81 (s)	0.83 (s)
6-O-Glc			6-O-Glc		
1	104.3	106.1	1	4.18 (d, 7.5)	5.00 (d, 7.7)
2	77.7	75.5	2	3.96 (t, 7.5)	4.06 (t, 8.9)
3	74.0	79.7	3	3.13	4.24 (t, 8.9)
4	71.0	71.9	4	3.04	4.18 (t, 8.9)
5	76.5	78.2	5	3.04	3.92
6a	61.3	63.2	6	3.59 (dd, 5.1, 12.0)	4.50 (brd, 11.6)
6b				3.40	4.34 (dd, 5.0, 11.6)

<sup>a</sup> Signals in the same column.

for  $^1\text{H}$  and an exponential function ( $\text{LB} = 4$ ) for  $^{13}\text{C}$  and DEPT spectra.

Standard pulse sequences were used for 2D spectra. Spectral widths of 5000 and 27 500 Hz were used for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively. Relaxation delays of 1.5 s were used for all 2D NMR experiments. The 2D spectra used  $1024 \times 256$  (H–H COSY, HMQC, HSQC, ROESY and HMQC-TOCSY) and  $2048 \times 256$  (HMBC) data point matrices which

were then zero filled to  $1024 \times 512$  and  $2048 \times 512$ , respectively. Non-shifted sine-bell window functions were used along the  $F_1$  and  $F_2$  axes for H–H COSY, HMQC, HSQC, HMBC and  $90^\circ$  shifted sine-bell window functions were used along the  $F_1$  and  $F_2$  axes for ROESY and HMQC-TOCSY. The HMQC-TOCSY experiment utilized a 100 ms spin-lock mixing time. ROESY used a spin-lock of 300 or 450 ms as a mixing time. The HMBC experiment used a 62 ms delay time

## Spectral Assignments and Reference Data

**Table 4.** Comparison of C-20 epimeric isomers **1** and **2**, [20(R)- and 20(S)- PPD]

H	$\delta_{\text{H}}(1) - \delta_{\text{H}}(2)$ (pyridine- $d_5$ )	C	$\delta_{\text{C}}(1) - \delta_{\text{C}}(2)$ (pyridine- $d_5$ )	$\delta_{\text{C}}[20(R) - \text{PPD}] -$ $\delta_{\text{C}}[20(S) - \text{PPD}]$ ( $\text{CDCl}_3$ ) <sup>16</sup>	$\delta_{\text{C}}[20(R) - \text{PPD}] -$ $\delta_{\text{C}}[20(S) - \text{PPD}]$ (pyridine- $d_5$ ) <sup>17</sup>
16 $\alpha$	+0.14	13	+0.7	+0.8	+0.7
16 $\beta$	−0.06	14	−0.3		
17	+0.04	16	−0.2	−0.2	−0.2
18	+0.05	17	−4.1	−3.7	−4.1
22a	−0.31	20	+0	+0.6	+0
23a	−0.09	21	−4.3	−5.0	−4.2
23b	+0.15	22	+7.4	+7.5	+7.4
30	+0.04	23	+0.3		
		24	−0.3		
		29	+0.3		
		30	+0.3		

to obtain  $^1\text{H}$  and  $^{13}\text{C}$  long-range correlations. Z-PFGs were used to obtain HMQC, HMBC and DQF H-H COSY spectra. Data processing was carried out on an SGI Indy workstation with Bruker XWINNMR programs.

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