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Virtual ^1H–^1H spin–spin coupling in a linear five-spin system on the pyranose rings of some glucuronides

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Abstract

In the ^1H NMR spectra of methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 3)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) and methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 6)-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**7**), which were obtained by the reaction of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**5**) in the presence of $Hg(CN)_2$ in 1:1 benzene–nitromethane at 45°C, protons on both β-D-glucopyranosyluronate rings were observed as very complex signals that could not be interpreted by first-order analysis. Similar complex signals were also observed for the protons on the β-D-glucopyranosyluronate rings that were sugar components of some triterpenoidal glycosides (**13–15**). These complex signals were determined to be due to virtual long-range spin–spin coupling in the linear five-spin system on the glucopyranosyluronate rings of the glucuronides by ^1H, ^{13}C, H–C COSY, 1D HOHAHA, and spin-simulation spectroscopies.

1. Introduction

It cannot be over emphasized that the structural determination of organic compounds is mainly based on analysis of nuclear magnetic resonance (NMR) spectra. From the ^1H NMR spectra of organic compounds, two important values can be obtained: chemical shifts (δ values) and coupling constants (J values), which give much physicochemical information for the compounds. Commonly the

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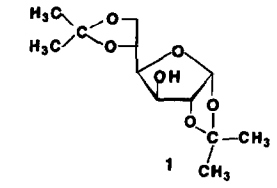
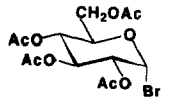
structural analysis of sugar pyranose derivatives is achieved by measuring the values, especially J values, of protons on the pyranose rings, which are directly observed from the spectra. In many cases, first-order analysis [1] is enough to obtain the J values that are used to determine both conformation and configuration of the pyranose derivatives. In the first-order interpretation of an ^{1}H NMR spectrum, a zero coupling constant between two protons generally gives a signal that is not very complex. In the ^{1}H NMR spectra of some glucopyranosiduronates, however, we encountered very complex signals for the protons on the β-D-glucopyranosiduronate rings that could not be interpreted by first-order analysis. We reported the synthesis and cytoprotective effects of glycyrrhetic acid and oleanoic acid β-diglycosides having disaccharides such as β-D-glucopyranosyluronic acid -(1 → 2)-β-D-glucopyranose, -β-D-galactopyranose, and -β-D-glucopyranosuronic acid as sugar components at the C-3 position on the aglycones [2–4]. Triterpenoid α-diglycosides [5,6] having sugar components such as β-D-glucopyranosyluronic acid)-(1 → 2)α-D-gluopyranosuronic acid, -α-D-glucopyranose, and -α-D-galactopyranose were synthesized in order to compare the cytoprotective effects of them with those of the corresponding β-diglycosides. In the ^{1}H NMR spectra of synthetic precursors **13–15** of the α-diglycosides, the protons on the β-D-glucopyranosuronate rings were observed as complex signals. In the course of determining the purity of these glycosides and assigning their stereochemistries for the purpose of evaluation of the cytoprotective effects of the synthetic glycosides, the complex signals were so far unresolved that precise structural elucidation was impossible. Similarly complex signals were observed for the protons on β-D-glucopyranosuronate rings of methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 3)-1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**), methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 6)-1,2 : 3,5-di-*O*-isopropylidene-α-D-glucofuranose (**7**), and methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 6)-3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**8**) (the latter was derived from **7**). Herein we report the spectral analysis of the complex signals due to the protons on the β-D-glucopyranosyluronate rings of **6–8** and **13–15**, and elucidate that the complex signals result from virtual long-range spin–spin coupling in a linear five-spin system among protons on the glucopyranosuronate rings.

2. Results and discussion

Liptak et al. [7] reported that the reaction of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) in the presence of $Hg(CN)_2$ in 1:1 benzene–nitromethane at 45°C gave 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-1,2 : 3,5-di-*O*-isopropylidene-α-D-glucofuranose (**4**) in a ratio of 1:1. In order to obtain compounds **6** and **7** which might be precursors of desired disaccharides, 3-β-D-glucopyranosyluronic acid)-(1 → 3)- and (1 → 6)-D-glucopyranose, respectively, for the sugar components of some triterpenoidal glycoside derivatives, the reaction of **1** [8] with



1



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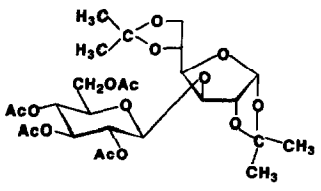

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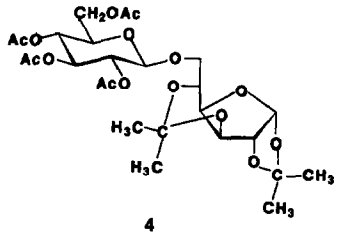

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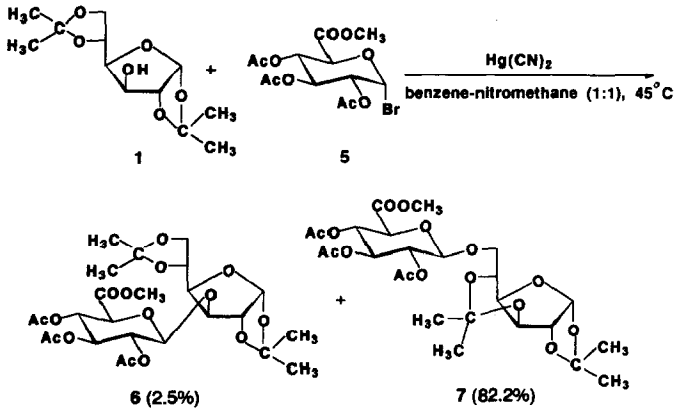
methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**5**) [9] was carried out in the presence of $Hg(CN)_2$ in 1:1 benzene–nitromethane (Scheme 1) under the same reaction conditions as reported by Liptak et al. [7]. In this case, however, two oily products **6** and **7**, each of which showed a single spot on TLC (solvent system, 1:9 benzene–acetone and 1:3 AcOEt–benzene), were obtained in the ratio of ~ 1:35, respectively, which was different from the case for the reaction of **1** with **2**. Both products **6** and **7** exhibited the same quasimolecular ion peak at *m/z* 599 $[M + Na]^+$ in fast-atom bombardment mass spectra (FABMS). The ^1H NMR spectra (Fig. 1) of **6** and **7** were recorded to elucidate their structures.



Scheme 1.

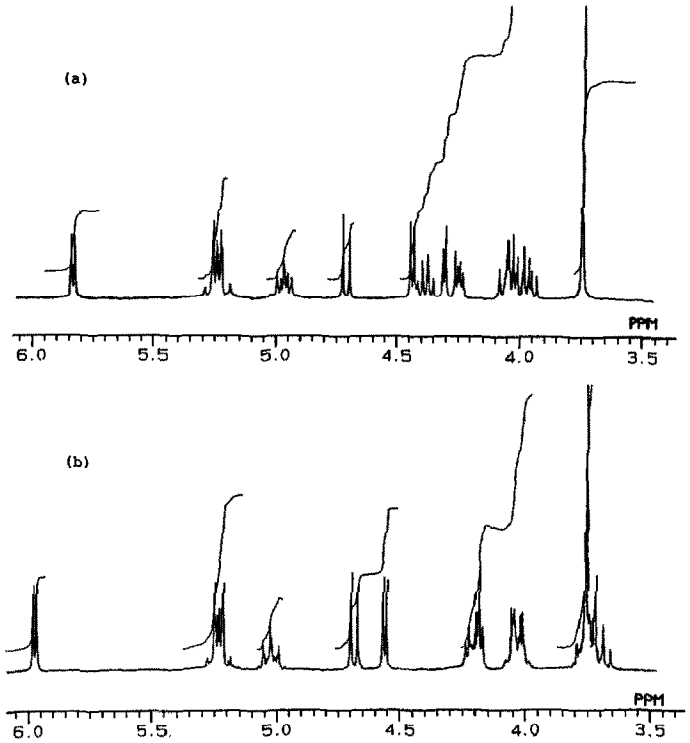
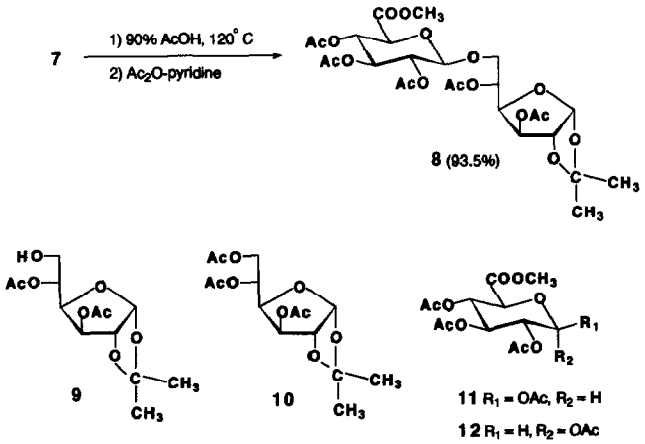


Fig. 1. Partial ^{1}H NMR spectra (270 MHz) of compounds **6** (a) and **7** (b) in the region δ ~3.5–6.0.

The ^{1}H NMR spectra for both **6** and **7** showed very complex multiplets at δ ~5.20–5.30, 4.97, and 4.03 for **6** and 5.18–5.26, 5.02, and 4.04 for **7**, which could not be interpreted by first-order analysis. The complex multiplets observed for both **6** and **7** indicated that these compounds were probably not pure and possibly contained other anomers or conformers. However, treatment of the major product **7** with 90% AcOH at 120°C, followed by acetylation, gave quantitatively the pentaacetate **8** as colourless needles (mp 188–189°C) (Scheme 2). The ^{1}H NMR spectrum (Fig. 2a) of **8** exhibited similar complex multiplets at δ 5.20–5.30, 5.03, and 4.03 (these expanded signals are shown in Fig. 3), which also could not be assigned by first-order analysis.

One-dimensional homonuclear Hartmann–Hahn (1D HOHAHA) spectra [10] of **8** (Fig. 4) were measured to determine which signals observed in Fig 2a belonged to the protons on the pyranose system (5 protons) or to those on the furanose system (7 protons). After excitation of a doublet at δ 4.62 (1 H), multiplets at δ 5.03 (1 H), 5.20–5.30 (2 H), and 4.03 (1 H) successively appeared with mixing times of 50, 70, and 90 ms, respectively (Fig. 4b–d). From the experiments, it was confirmed that these five protons belonged to the glucopyranosuronate ring, and that the doublet at δ 4.66 (1 H) and the multiplets at δ



Scheme 2.

5.20–5.30 (2 H), 5.03 (1 H), and 4.03 (1 H) could be assigned as the signals of H-1, H-3 and -4, H-2, and H-5, respectively, on the pyranose ring. The remaining seven signals at δ 5.92, 5.30, 5.10, 4.47, 4.44, 4.13, and 3.79 were assignable to the protons on the glucofuranose system, as confirmed by comparing the ^{1}H NMR

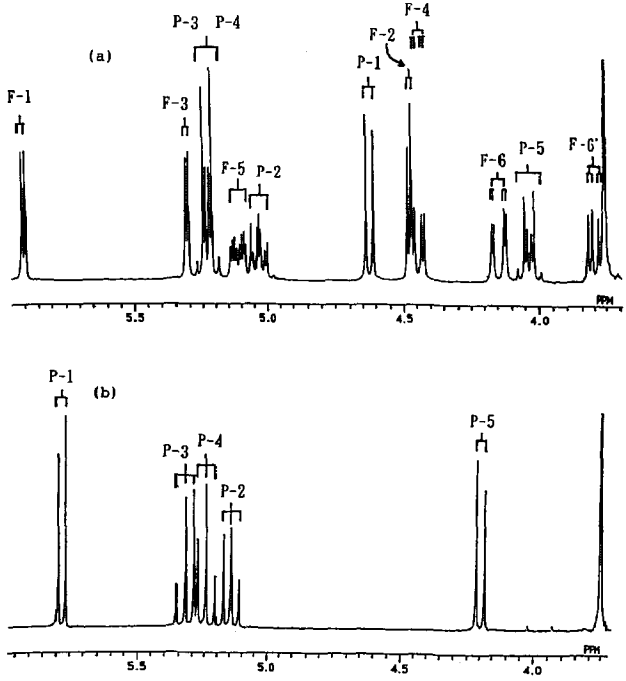


Fig. 2. Partial ^{1}H NMR spectra (270 MHz) of compounds **8** (a) and **11** (b) in the region δ ~3.7–6.0.

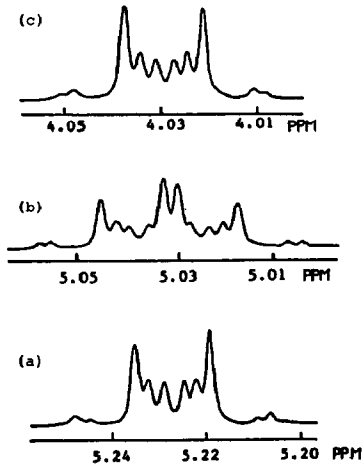


Fig. 3. Expanded spectra of the signals at δ (a) 5.20–5.26, (b) 5.00–5.06, and 4.00–4.06 shown in Fig. 2a.

spectrum of **8** with those of 1,2-*O*-isopropylidene-α-D-glucofuranose acetates **9** and **10** shown in Table 1. The coupling constants of all seven protons on the glucofuranose system of **8** could be interpreted by first-order analysis; on the other hand,

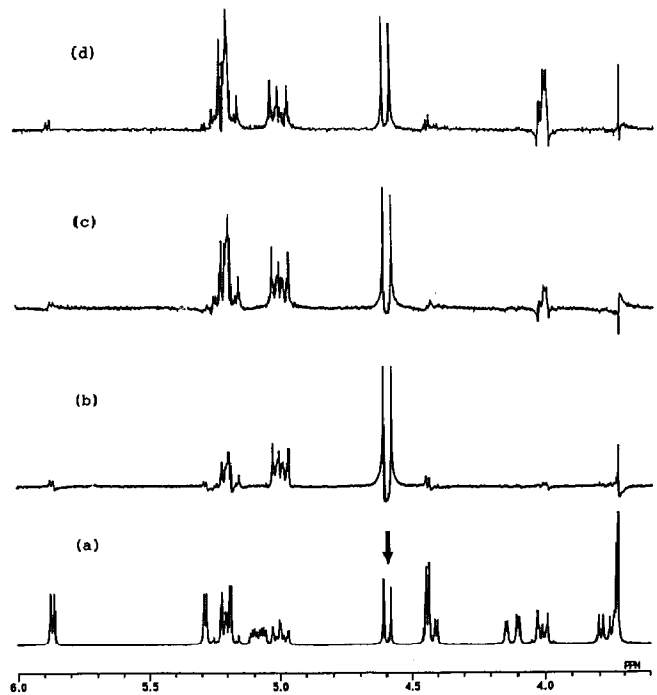


Fig. 4. 1D HOHAHA spectra of **8**: (a) control spectrum, (b)–(d) spectra obtained with mixing times of 50, 70, and 90 ms, respectively.

Table 1
^{1}H NMR chemical shifts and coupling constants (270 MHz) for compounds **6–10** in $CDCl_3$ [a]

	6	**7**	**8**	**9**	**10**
Pyranose					
H-1	4.72 (d, 7.7) *	4.68 (d, 7.3) *	4.62 (d, 7.7) *		
H-2	4.97 *	5.02 *	5.02 *		
H-3	5.20–5.30 *	5.18–5.26 *	5.26–5.18 *		
H-4	5.20–5.30 *	5.18–5.26 *	5.26–5.18 *		
H-5	4.03 *	404 *	4.02 *		
Furanose					
H-1	5.84 (d, 4.0)	5.97 (d, 3.7)	5.92 (d, 3.5)	5.90 (d, 3.7)	5.92 (d, 3.7)
H-2	4.44 (d, 4.0)	4.56 (d, 3.7)	4.47 (d, 3.5)	4.55 (d, 3.7)	4.48 (d, 3.7)
H-3	4.31 (d, 3.3)	4.18 (d, 4.0)	5.30 (d, 3.0)	5.30 (d, 2.9)	5.35 (d, 2.9)
H-4	4.25 (dd, 5.6, 3.3)	4.22 (dd, 6.6, 4.0)	4.44 (dd, 9.4, 3.0)	4.40 (dd, 11.7, 2.9)	4.41 (dd, 9.5, 2.9)
H-5	4.39 (dd, 11.9, 5.6)	3.76 (m)	5.10 (ddd, 9.4, 4.5, 2.0)	5.15 (ddd, 8.8, 6.2, 2.2)	5.21 (ddd, 9.5, 5.5, 2.2)
H-6	3.79–4.04	4.02 (m)	4.13 (dd, 11.4, 2.0)	4.16 (dd, 8.8, 2.2)	4.57 (dd, 12.5, 2.2)
H-6′	3.97–4.03	3.71 (dd, 8.4, 7.7)	3.79 (dd, 11.4, 45)	3.85 (dd, 8.8, 6.2)	4.12 (dd, 12.5, 5.5)
Others					
OCH_3	3.75	3.76	3.75		
Ac	2.03, 2.03, 2.06	2.02, 2.02, 2.03	2.01, 2.02, 2.02, 2.04, 2.05	2.11, 2.15	2.01, 2.06, 2.16
CH_3	1.33, 1.33, 1.43, 1.49	1.32, 1.33, 1.35, 1.47	1.31, 1.54	1.32, 1.51	1.32, 1.52

[a] Coupling constants (J in Hz) are given in parentheses. The signal assignments were based on the decoupling and ^{1}H–^{13}C COSY methods. The signals with asterisk (*) showed virtual long-range spin–spin couplings, details of which are listed in Table 2.

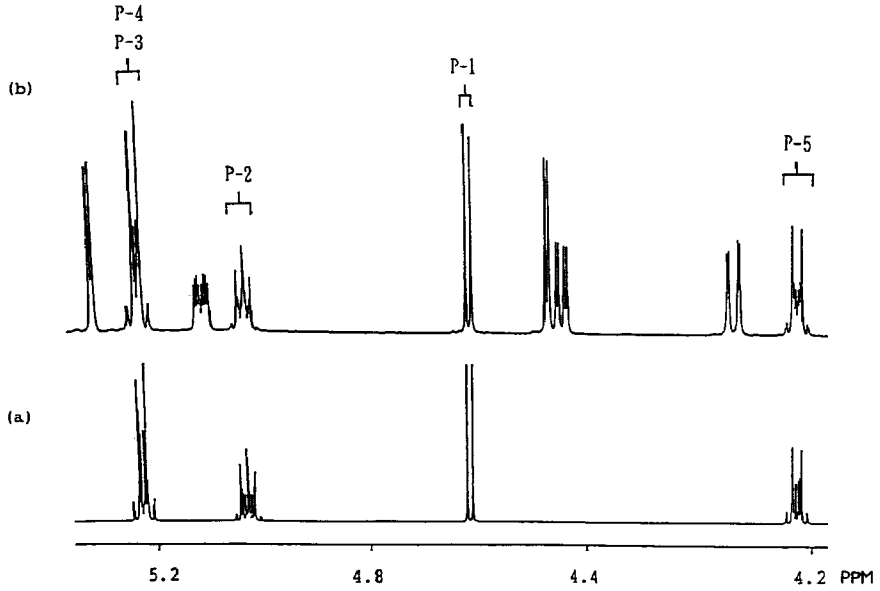


Fig. 5. Comparison of the partial spectrum obtained by spin simulation with the real spectrum of **8** in ^{1}H NMR (600 MHz) in the region δ 4.2–5.4.

those of the five protons on the glucopyranosuronate ring could not. The signals of H-1 to H-5 on the pyranose ring of **8** were compared with the corresponding ones of methyl β-D-glucopyranosuronate peracetate (**11**) in the ^{1}H NMR spectra (Fig. 2a and b). The signals of H-1 through H-5 of **11** were observed as doublet ($J_{1,2}$ 7.7 Hz), doublet of doublets ($J_{1,2}$ 7.7, $J_{2,3}$ 8.8 Hz), doublet of doublets ($J_{2,3}$ 8.8, $J_{3,4}$ 9.2 Hz), doublet of doublets ($J_{3,4}$ 9.2, $J_{4,5}$ 9.5 Hz), and doublet ($J_{4,5}$ 9.5 Hz) at δ 5.78, 5.14, 5.36, 5.24, and 4.20, respectively. The multiplicities of H-1 to H-5 on the pyranose of **8** were different from the corresponding ones of **11**, and also different from those of methyl α-D-glucopyranosuronate peracetate (**12**) (see Experimental section). However, the J value (7.7 Hz) of the anomeric proton on the pyranose ring of **8** suggested the β anomer. We will now consider that the complex multiplicities of the protons on the pyranose ring of **8** might be the result of virtual long-range spin–spin coupling among these protons.

Spin simulation (600 MHz), performed in order to investigate the multiplicities of the H-1 to H-5 on the pyranose ring of **8**, gave a spectrum as shown in Fig. 5a. All signals shown in Fig. 5a are completely consistent with the corresponding doublet at δ 4.62 (H-1) and multiplets at δ 4.03 (H-5), 5.20–5.30 (H-3 and -4), and 5.03 (H-2) shown in the real spectrum of **8** (Fig. 5b). The δ and J values of H-1 to H-5 on the glucopyranosuronate ring of **8** obtained by spin simulation are listed in Table 2. The ratio of $\delta_{3,4}/J_{3,4}$ (0.29) obtained for **8** is very small, which suggests that the spins of H-3 and H-4 on the pyranose are strongly coupled with each other to give a set of spins [1,11,12] that behave as if the spins were quantum mechanically nondistinguishable. The spins of H-2 and H-5 interact with the set through

Table 2
Chemical shifts and coupling constants of H-1–H-5 on the glucopyranosuronate rings of **6**, **7**, and **8** obtained by spin simulation

	6	**7**	**8**
Chemical shift (δ)			
H-1	4.72	4.66	4.62
H-2	4.97	5.01	5.03
H-3	5.21	5.21	5.22
H-4	5.21	5.22	5.23
H-5	4.03	4.04	4.03
Coupling constant (Hz)			
$J_{1,2}$	7.67	7.78	7.70
$J_{1,3}$	0.19	0.20	0.19
$J_{1,4}$	0.06	0.05	0.05
$J_{1,5}$	−0.31	−0.31	−0.30
$J_{2,3}$	9.38	9.40	9.41
$J_{2,4}$	0.63	0.61	0.61
$J_{2,5}$	−0.04	−0.03	−0.03
$J_{3,4}$	9.45	9.45	9.43
$J_{3,5}$	−0.44	−0.43	−0.42
$J_{4,5}$	9.91	10.10	10.14

the C-3–C-2 bond and the C-4–C-5 bond, respectively, so that these are coupled with the set to give complicated multiplets. As the spin of H-1 interacts with the set through the C-3–C-2–C-1 bonds, it could be considered that the spin had such a slight effect on the signal of H-1 that it was observed as a doublet in the real (270 MHz) spectrum (Figs. 2a and 5b). From the J values of H-1 to H-5 on the glucopyranosuronate ring, it was confirmed that this pyranose ring exists as the β anomer of the $^4C_1$ conformer. Compound **8** was also obtained by the reaction of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**9**) with glycosyl bromide **5** in the presence of Ag_2CO_3 in dry CH_2Cl_2 in 73.5% yield (Scheme 2). These spectral and chemical results showed that **8** was methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 6)-3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose. Therefore, compound **7**, a precursor of **8**, was thought to be methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 6)-1,2 : 3,5-di-*O*-isopropylidene-α-D-glucofuranose, which was a product caused by acetal migration [13] under the conditions of the Koenigs–Knorr reaction [14]. The minor product **6** obtained together with **7** in the reaction of **1** with **5** was thus indicated to be methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1 → 3)-1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose. Similar virtual long-range spin–spin couplings in the linear 5-spin system as those observed for **8** were also observed on the protons of glucopyranosuronate rings of **6** and **7** in the spectra obtained by spin simulation (Table 2), which suggests that the pyranose rings of **6** and **7** should have the same conformers as those of **8**.

The ^1H–^{13}C COSY spectrum of **8** was measured in order to confirm the connectivities between the protons and carbons of the D-glucopyranosuronate ring (Fig. 6). The doublet (1 H) at δ 4.62 correlated with a signal of the anomeric

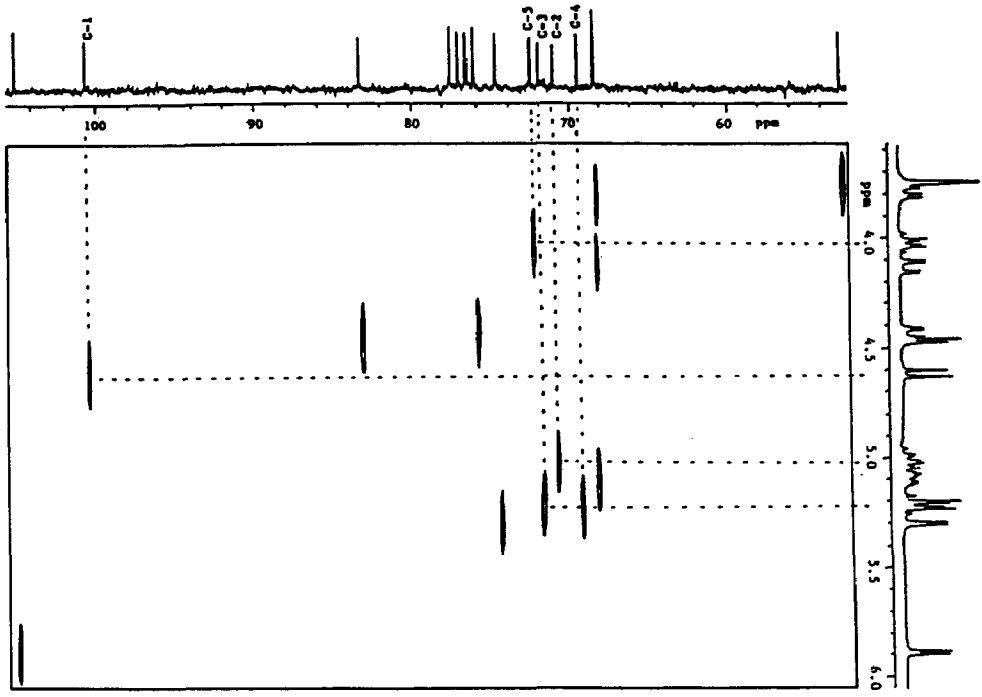


Fig. 6. $^{1}H-^{13}C$ COSY spectrum of **8**.

carbon at δ 100.6, two multiplets (each 1 H) at δ 5.03 and 4.03 with those of C-2 and C-5 at δ 71.1 and 72.7, respectively, and the multiplet (2 H) at δ 5.20–5.30 with those of C-3 and -4 at δ 72.0 and 69.4. The ^{13}C NMR signals of **6** and **7** were also assigned by $^{1}H-^{13}C$ COSY and are listed in Table 3 together with those of **8**.

We already synthesized triterpenoid α-diglycoside derivatives **13–15** [3,5] and the corresponding β-diglycosides **16–18** [2–4,6]. The ^{1}H NMR spectra of the α-glycosides **13–15** showed for the protons on the β-D-glucopyranosuronate rings very complex signals that were similar to those in **8**. When the spectra of α- **13** and β-diglycoside **16** were compared, all proton signals on both β-D-glucopyranosuronate rings of **16** were assigned by first-order analysis [3,4]. On the other hand, protons on the terminal β-D-glucopyranosuronate ring (U-I) exhibited very complex signals similar to those in **8**, although the protons on the inner α-D-glucopyranosuronate ring (U-II) were assigned by first-order analysis as shown in Fig. 7. In these cases the spins of H-3 and H-4 on U-I gave a small $\Delta\delta/J$ ratio (0.33), whereas those on U-II and inner and terminal β-D-glucopyranosuronate rings gave large ratios (9.27, 9.30, and 9.27, respectively) [5]. Similarly, virtual coupling was observed for the protons on the terminal β-D-glucopyranosuronate rings of α-diglycosides **14** and **15** because of strong coupling (large $\Delta\delta/J$ ratio) of H-3 and H-4, whereas it was not observed for the protons on the inner α-D-glucopyranosuronate ring of **13** and the terminal β-D-glucopyranosuronate rings of **16–18**, and also

Table 3
^{13}C NMR chemical shifts for **6–10** in $CDCl_3$ [a]

	6	**7**	**8**	**9**	**10**
Furanose					
C-1	105.0	106.4	105.1	105.0	105.2
C-2	82.8	84.0	83.2	83.1	83.3
C-3	81.7	74.9	74.7	76.8	74.3
C-4	80.6	79.5	76.1	78.7	77.1
C-5	72.9	71.7	68.4	66.7	67.6
C-6	66.3	70.0	68.4	66.6	63.4
Pyranose					
C-1	99.4	101.0	100.6		
C-2	71.3	72.2	71.1		
C-3	72.0	72.1	72.0		
C-4	69.3	69.5	69.4		
C-5	72.8	72.6	72.6		
C-6	166.8	167.2	167.1		
Others					
$\underline{C}(CH_3)_2$	108.7, 112.1	100.9, 112.2	112.4	112.3	112.5
CH_3	25.1, 26.3, 26.6, 26.8	23.9, 24.0, 26.5, 27.2	26.1, 26.6	26.3, 26.7	26.2, 26.8
$\underline{C}OCH_3$	168.9, 169.3	169.1, 169.3, 170.1	169.0, 169.2, 169.5	170.6, 171.3	169.5, 169.6
	170.5		169.7, 170	170.5	
$CO\underline{C}H_3$	20.5, 20.6, 20.6	20.5, 20.6, 20.6	20.4, 20.6, 20.7	20.8, 20.8	20.6, 20.7, 20.7
			20.7, 20.8		
OCH_3	52.8	52.8	52.8		

[a] Signal assignments were based on ^1H–^{13}C COSY methods.

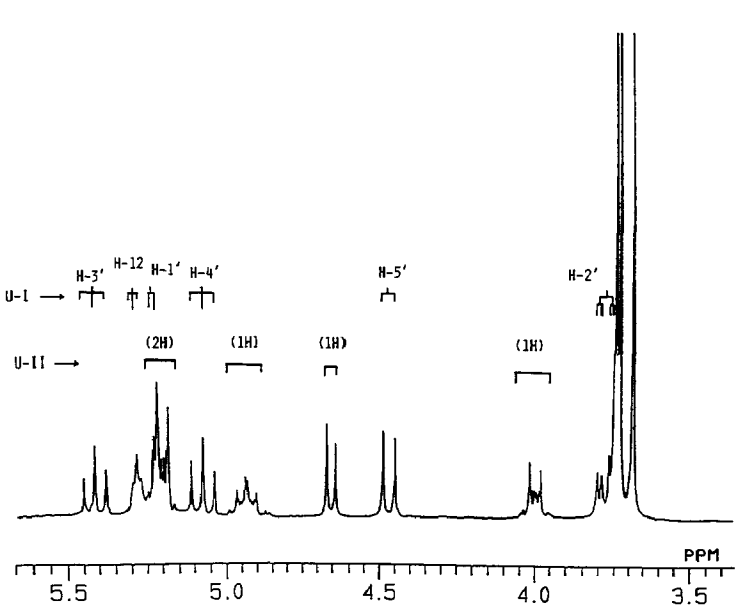
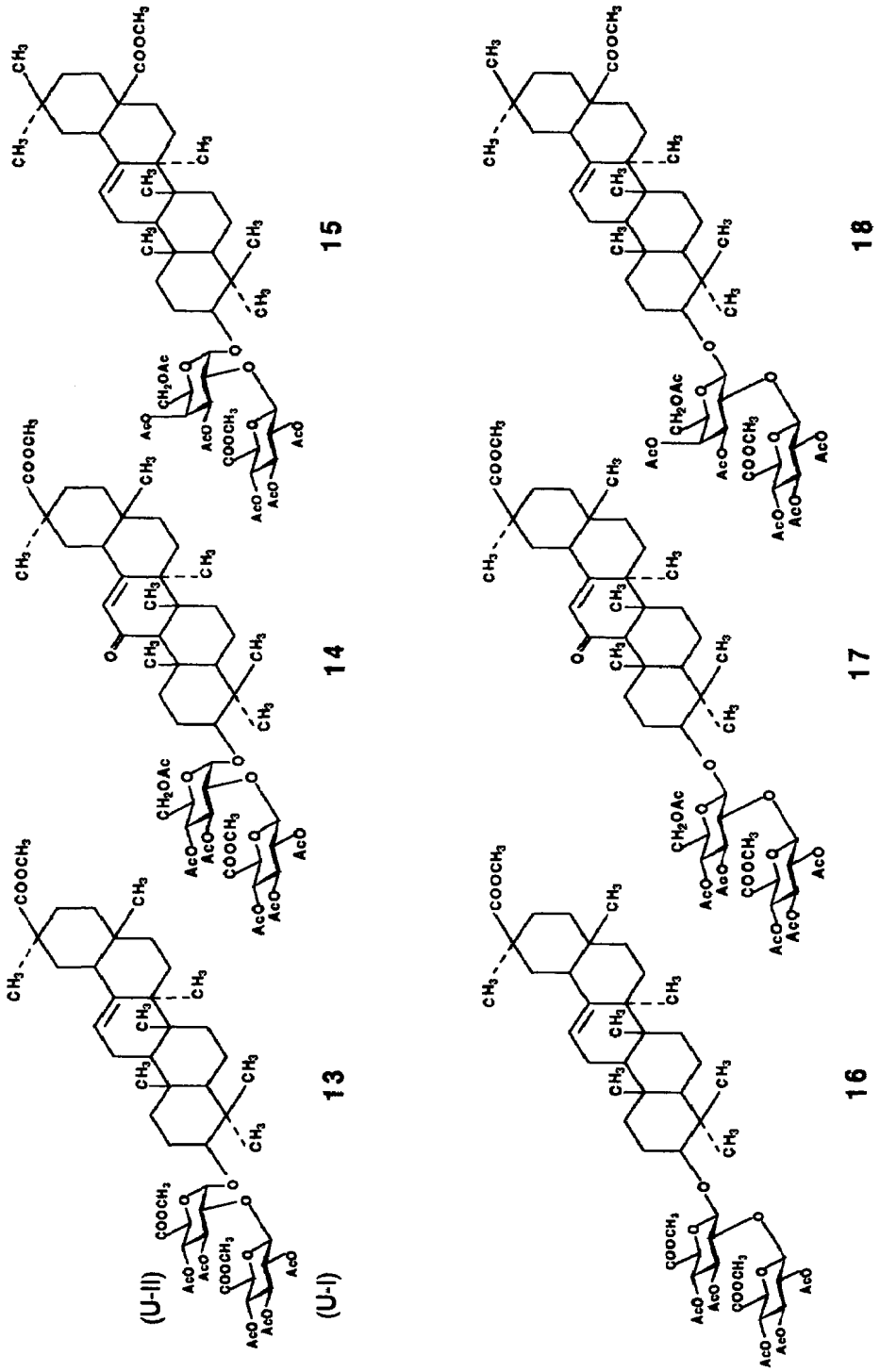


Fig. 7. ^{1}H NMR spectrum (270 MHz) of compound **13**.

those on β-D-gluco- and β-D-galactopyranose rings in both glycyrrhetic acid α- and β-diglycosides [2–6] because of greater differences in the chemical shifts of H-3 and H-4.

Table 4
Chemical shifts and coupling constants of H-1–H-5 on the glucuronopyranose ring of **13**, **14**, and **15** obtained by spin simulation

	13	**14**	**15**
Chemical shift (δ)			
H-1	4.66	4.68	4.72
H-2	4.93	4.93	4.93
H-3	5.19	5.20	5.20
H-4	5.20	5.21	5.21
H-5	4.03	4.00	4.04
Coupling constant (Hz)			
$J_{1,2}$	8.07	8.05	8.67
$J_{1,3}$	0.11	0.10	0.15
$J_{1,4}$	0.15	0.16	0.10
$J_{1,5}$	−0.29	−0.30	−0.41
$J_{2,3}$	6.29	6.32	8.02
$J_{2,4}$	1.45	1.43	−0.04
$J_{2,5}$	1.06	1.05	0.68
$J_{3,4}$	8.21	8.23	8.31
$J_{3,5}$	−1.20	−1.22	−1.30
$J_{4,5}$	11.45	11.43	9.51


(U-II)
(U-I)
13
14
15
16
17
18

Thus, we have determined that the complex ^1H NMR signals observed for the protons on the β-D-glucopyranosuronate rings of some glucuronide derivatives are caused by the virtual long-range spin–spin couplings in the linear 5-spin system on the pyranose, and not by a mixture of other anomers or conformers with respect to the β-D-glucopyranosuronate system.

3. Experimental

General methods.—Thin-layer chromatography (TLC) utilized Kieselgel HF_{254} (E. Merck), and spots were detected by spraying the plates with dil H_2SO_4, followed by heating at 100°C for 10 min. Column chromatography was carried out on Wakogel C-200. An SSC-6300 apparatus (Senshu Scientific Co., Ltd.) equipped with an SSC-3000A was employed for analytical HPLC using an ODS-1251-D column (4.6 × 250 mm), with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using an ODS-4251-D column (10 × 250 mm). Fast-atom bombardment mass spectra (FABMS) were recorded on a Jeol JMS-DX 300 mass spectrometer. Melting points were obtained on Yanagimoto micromelting point apparatus and are uncorrected. ^1H NMR spectra were obtained at 270 and 600 MHz by using Jeol JNM-GX 270 and JNM-ALPHA 600 NMR spectrometers, respectively, with chemical shifts (ppm) referred to Me_4Si as an internal standard. ^{13}C NMR spectra were measured by use of the JNM-GX 270 spectrometer, using the same reference. 1D HOHAHA spectra were measured using a selective excitation pulse (gaussian pulse: 40 ms) with MLEV-17 as the mixing pulse. Spin simulations were performed using the NMR Spin Simulation/Iteration program.

Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate-(1 → 3)-1,2 : 5,6-di-O-isopropylidene-α-D-glucofuranose (**6**) *and methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate-(1 → 6)-1,2 : 3,5-di-O-isopropylidene-α-D-glucofuranose* (**7**).—A mixture of **1** (10 g), bromide **5** (20 g), and $Hg(CN)_2$ (20 g) in 1:1 benzene–nitromethane (200 mL) was stirred at 45°C for 16 h, then filtered. The filtrate was poured into ice–water (500 mL) and extracted with benzene (3 × 200 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient 0–5% acetone in benzene) to give oily products **6** (560 mg, 2.5%) and **7** (18.2 g, 82.2%). FABMS of **6** *m/z*: 599 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{36}O_{15}$: C, 52.08; H, 6.29. Found: C, 51.95; H, 6.33. FABMS of **7** *m/z*: 599 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{36}O_{15}$: C, 52.08; H, 6.29. Found: C, 52.06; H, 6.08.

Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate-(1 → 6)-3,5-di-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (**8**).—A solution of **7** (15 g) in 90% acetic acid (100 mL) was refluxed at 120°C for 18 h. The mixture was poured into ice–water (300 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue. The residue

was dissolved in 1:1 pyridine–Ac_2O (100 mL) and allowed to stand overnight at room temperature. The mixture was coevaporated with toluene (5 × 200 mL) to give a residue which was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to give compound **8** (15.1 g, 93.5%, mp 188–189°C, after recrystallization from ether–hexane). FABMS *m/z*: 643 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.17; H, 5.91.

3,5,6-Tri-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (**10**).—To a solution of **1** (10 g) in 2:1 MeOH–H_2O (200 mL) was added Amberlite IR-120 resin (H^+ form, 50 mL), and the mixture was stirred at room temperature for 8 h. The mixture was filtered, and the filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% MeOH in CH_2Cl_2) to give 1,2-*O*-isopropylidene-α-D-glucofuranose (6.9 g, 81.2%, FABMS *m/z*: 243 $[M + Na]^+$) [6]. The product (1 g) was dissolved in 1:1 pyridine–Ac_2O (20 mL) and allowed to stand at room temperature for 18 h. The mixture was coevaporated with toluene (4 × 100 mL) to give a residue that was subjected to column chromatograpy (a gradient of 0–5% EtOAc in benzene) to obtain **12** (1.34 g, 85% from 1,2-*O*-iospropylidene-α-D-glucofuranose). FABMS *m/z*: 369 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{22}O_9$: C, 52.02; H, 6.40. Found: C, 51.87; H, 6.49.

3,5-Di-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (**9**).—A solution of 1,2-*O*-isopropylidene-α-D-glucofuranose (5 g) and triphenylmethyl chloride (8 g) in dry pyridine (50 mL) was stirred at 60°C for 18 h. Acetic anhydride (50 mL) was added, and the mixture was allowed to stand at room temperature for 18 h, then poured into ice–water (300 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were successively washed with aq 5% HCl, satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to give crude triphenylmethyl ether (5.2 g), which was dissolved in 70% AcOH (50 mL) and heated overnight at 70°C. The reaction mixture was poured into ice-water (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient 0–10% acetone in benzene) to give the oily product **9** (3 g, 43%). FABMS *m/z*: 327 $[M + Na]^+$. Anal. Calcd for $C_{13}H_{20}O_8$: C, 51.31; H, 6.62. Found: C, 51.07; H, 6.77.

Methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranosuronate (**11**).—A mixture of **5** (5 g), AcOH (1.5 mL), and $AgCO_3$ (5 g) in dry CH_2Cl_2 (10 mL) was stirred at room temperature overnight, then filtered. The filtrate was poured into ice–water (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chormoatography (a gradient of 0–10% EtOAc in benzene) to give **11** (2.1 g, 44%). ^1H NMR ($CDCl_3$): δ 5.78 (d, 1 H, *J* 7.7 Hz, H-1), 5.36 (dd, 1 H, *J* 9.2, 8.8 Hz, H-3), 5.24 (dd, 1 H, *J* 9.5, 9.2 Hz, H-4), 5.14 (dd, 1 H, *J* 8.8, 7.7 Hz, H-2), 4.20 (d, 1 H, *J* 9.5 Hz, H-5), 3.75 (s, 3 H, OCH_3), 2.03 (s, 3 H, Ac), 2.05 (s, 6 H, 2 Ac), 2.12 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$): δ 91.3 (C-1), 71.8 (C-2), 70.2

(C-3), 68.9 (C-4), 73.0 (C-5), 166.8 (C-6), 20.4, 20.5, 20.5, and 20.7 (each methyl carbon on acetyl group), 53.0 (OCH_3), 168.8, 169.1, 169.3, and 169.8 (each C=O). Anal. Calcd for $C_{15}H_{20}O_{11}$: C, 47.87; H, 5.36. Found: C, 47.77; 5.41.

Methyl 1,2,3,4-tetra-O-acetyl-α-D-glucopyranosuronate (**12**).—To a solution of **11** (1 g) in Ac_2O (5 mL) was added $ZnCl_2$ (0.5 g), and the mixture was heated for 20 min on a boiling steam bath [15]. The mixture was poured into ice–water (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were successively washed with water, satd aq $NaCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue. The residue was subjected to column chromatography (a gradient of 0–10% EtOAc in benzene), followed by preparative HPLC (solvent system, 40% H_2O in acetone; flow rate, 1 mL/min; column temperature, 35°C), to give **12** (280 mg, 28%). FABMS *m/z* 399 $[M + Na]^+$. ^1H NMR ($CDCl_3$): δ 6.40 (d, 1 H, *J* 3.6 Hz, H-1), 5.52 (dd, 1 H, *J* 9.9, 9.9 Hz, H-3), 5.22 (dd, 1 H, *J* 9.9, 9.9 Hz, H-4), 5.12 (d, 1 H, *J* 9.9, 3.6 Hz, H-2), 4.42 (d, 1 H, *J* 9.9 Hz, H-5), 3.75 (s, 3 H, OCH_3), 2.05 (s, 6 H, 2 Ac), 2.12 (s, 3 H, Ac), 2.19 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$): δ 88.6 (C-1), 68.7 (C-2), 68.8 (C-3), 69.0 (C-4), 70.2 (C-5), 167.1 (C-6), 20.2, 20.3, 20.5, and 20.6 (each methyl carbon on acetyl group), 52.8 (OCH_3), 168.3, 169.2, 169.3, and 169.8 (each C=O). Anal. Calcd for $C_{15}H_{20}O_{11}$: C, 47.87; H, 5.36. Found: C, 47.69; H, 5.49.

Reaction of **9** *with glycosyl bromide* **5**.—A mixture of **9** (1 g), **5** (1.5 g), and $AgCO_3$ (2 g) in dry CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h. To the mixture was added further **5** (1 g) and $AgCO_3$ (1 g), and the whole was stirred for 18 h, then filtered. The filtrate was poured into ice–water (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ and water, dried over anhyd $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to give **8** (1.5 g, 73.5%). This product was identified with the product obtained by the reaction of **1** with **5** by ^1H NMR and FABMS.

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