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Note

Complete assignment of the ^{1}H NMR spectra of phytoalexin-elicitor-active oligoglucosides

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Many plants respond to microbial attack by synthesizing and accumulating small lipophilic molecules called phytoalexins that have antibiotic activity[1–3]. The molecules that signal plants to begin this process of phytoalexin synthesis are called elicitors[4,5]. The hepta-β-D-glucoside β-D-Glc-(1 → 6)-[β-D-Glc-(1 → 3)-]β-D-Glc-(1 → 6)-β-D-Glc-(1 → 6)-[β-D-Glc-(1 → 3)-]β-D-Glc-(1 → 6)-D-Glc has been characterized as an elicitor of plant phytoalexin accumulation[6]. The compound was first purified to homogeneity from partial acid hydrolysates of isolated mycelial walls of the phytopathogen *Phytophthora megasperma* f. sp. *glycinea*[6]. Several structural analogues of the hepta-β-D-glucoside (listed in Fig. 1) have since been synthesized[7,8] and tested for their biological activities[9–11]. Early on it was shown that the presence of the D-glucose residue at the reducing end was not required for elicitor activity, as the chemical reduction of this residue, or even the complete absence of the group, did not affect the biological activity[9]. However, replacement of the side-chain β-(1 → 3)-D-glucosyl residue of the terminal trisaccharide with an *N*-acetyl-β-D-glucosaminyl (compound **6**) or β-D-glucosaminyl residue (compound **8**) reduced the elicitor activity ~ 10 000-fold and ~ 10-fold, respectively[9]. The corresponding modifications of the nonreducing terminal backbone β-(1 → 6)-glucosyl residue (compounds **5** and **7**) resulted in greater decreases in elicitor activity (~ 10 000-fold and ~ 100-fold)[9], while substitution of this D-glucosyl residue with a D-xylosyl residue (compound **3**) or D-galactosyl residue (compound **4**) reduced the activity about 10-fold and 50-fold, respectively[10,11]. Removal of the

† Footnote to the title: This work is part of V.-M. Ló's Ph.D. dissertation, The University of Georgia, July 1992.

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(a) **E** **D** **B** **A**

β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl
3 3
↑ ↑
1 1
β-D-Glc*p* β-D-Glc*p*

F **C**

1a

(b) **E** **D** **B** **A**

β-D-Glc*p*d_5-(1 → 6)-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl
3 3
↑ ↑
1 1
β-D-Glc*p* β-D-Glc*p*

F **C**

1b

(c) **E** **D** **B** **A**

β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl
3 3
↑ ↑
1 1
β-D-Glc*p*-d_5 β-D-Glc*p*

F **C**

1c

Fig. 1. Structures of the oligosaccharides used in this study. (a) Elicitor-active allyl hexaglucoside **1a**. (b)–(d) Deuterated analogues **1b**, **1c**, and **1d** of compound **1a** in which the β-(1 → 6) nonreducing terminal glucosyl residue **E**, the β-(1 → 3) nonreducing terminal glucosyl residue **F**, and the internal glucosyl residue **D**, respectively, are replaced by a partially deuterated residue Glc-d_5 (in which all *C*-linked protons except H-1 and H-5 are replaced by deuterium). (e) Allyl pentaglucoside **2**. (f) Allyl hexasaccharide **3**, in which residue **E** is a D-xylosyl group. (g) Allyl hexasaccharide **4**, in which residue **E** is a D-galactosyl group. (h) Propyl hexasaccharide **5**, in which the OH group at the C-2 position of glucosyl residue **E** is modified to an *N*-acetyl (NAc) group. (i) Propyl hexasaccharide **6**, in which the OH group at the C-2 position of glucosyl residue **F** is modified to NAc. (j) Propyl hexasaccharide **7**, in which the OH group at the C-2 position of glucosyl residue **E** is modified to an amino (NH_2) group. (k) Propyl hexasaccharide **8**, in which the OH group at the C-2 position of glucosyl residue **F** is modified to NH_2. (l) Allyl triglucoside **9**. (m) Structure of the allyl group. (n) Structure of the propyl group. (o) Structure of the *N*-acetyl group.

(d) **E** **D** **B** **A**

β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-d_5-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl

3 3

↑ ↑

1 1

β-D-Glc*p* β-D-Glc*p*

F **C**

1d

(e) **D** **B** **A**

β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl

3 3

↑ ↑

1 1

β-D-Glc*p* β-D-Glc*p*

F **C**

2

(f) **E** **D** **B** **A**

β-D-Xyl*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl

3 3

↑ ↑

1 1

β-D-Glc*p* β-D-Glc*p*

F **C**

3

(g) **E** **D** **B** **A**

β-D-Gal*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → O)-Allyl

3 3

↑ ↑

1 1

β-D-Glc*p* β-D-Glc*p*

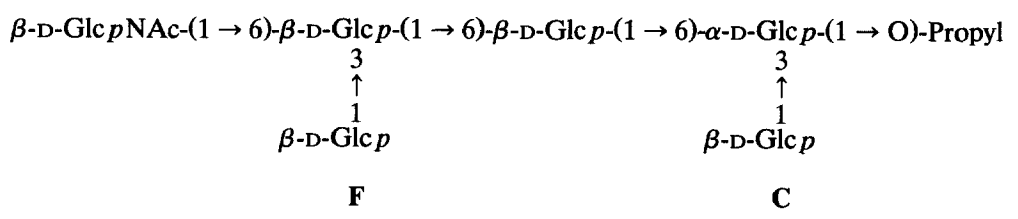
F **C**

4

Fig. 1. (continued)

nonreducing terminal backbone glucosyl residue (compound **2**) resulted in an ~ 4000-fold reduction in elicitor activity[10,11]. Thus it appears that the hexaglucoside **1a** (see Fig. 1a) is the minimum structural requirement for the phytoalexin elicitor activity of the hepta-β-D-glucoside.

(h)



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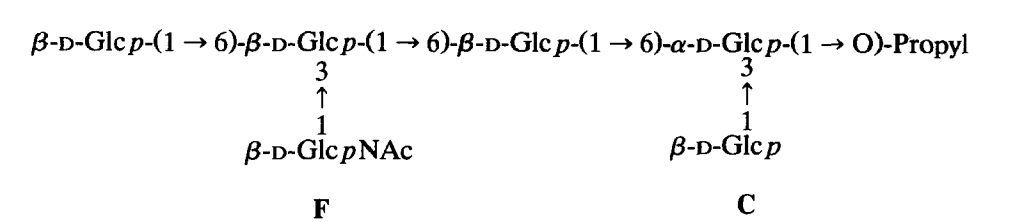
(i)



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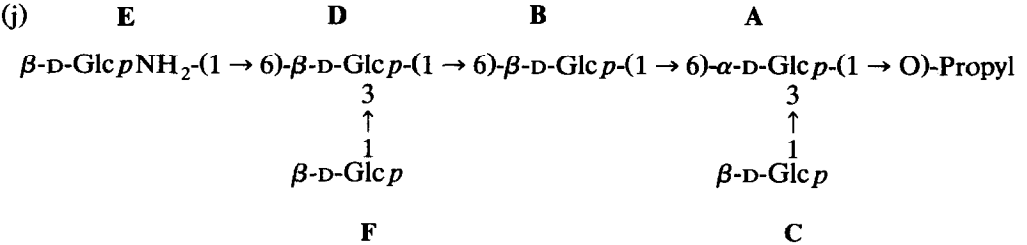
(j)



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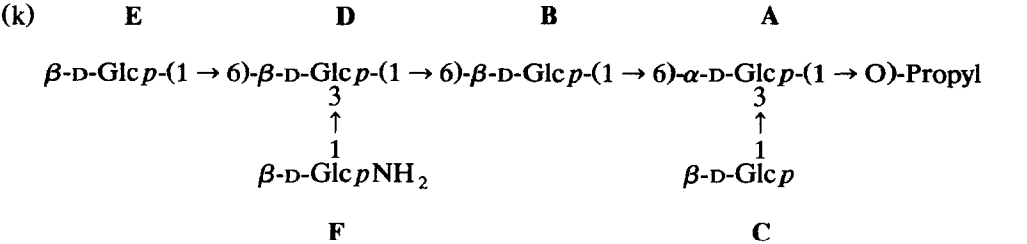
(k)



8

Fig. 1. (continued)

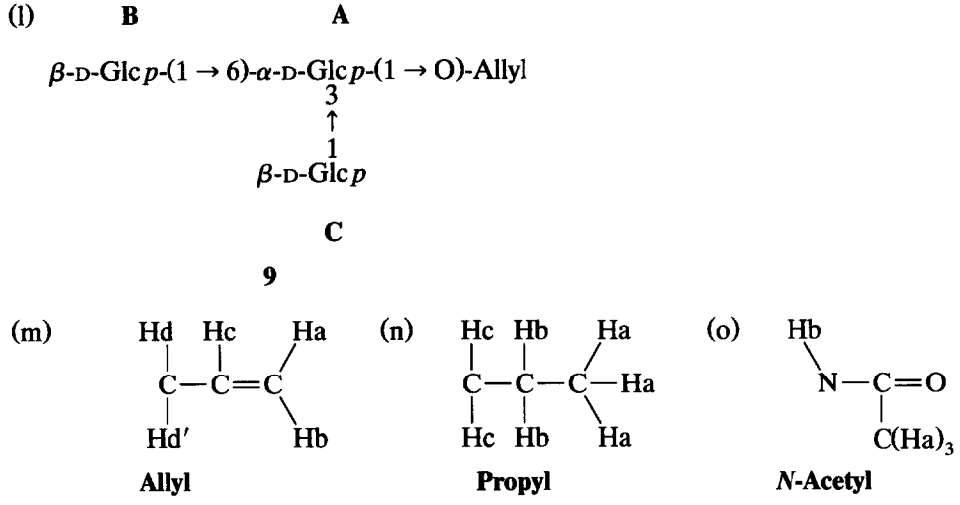


Fig. 1. (continued)

NMR spectroscopy has been applied in our laboratories to gain insight into the solution conformation of the hepta-β-D-glucoside and its structural analogues, as well as into the interaction of the elicitors with their putative receptor[12,13]. The first step in such studies is to completely assign the ^{1}H NMR spectra of these oligoglucosides. We report herein the complete ^{1}H NMR assignments for hexaglucoside **1a** and related compounds listed in Fig. 1. The backbone of **1a** differs from that of the hepta-β-D-glucoside only at the reducing end, i.e., the reducing glucosyl residue is replaced by an α-linked allyl group. In order to facilitate assignments of the hexaglucoside ^{1}H spectrum, three specifically deuterated analogues (**1b**, **1c**, and **1d**) of the hexaglucoside **1a** (see Fig. 1b–d) were prepared according to the reported synthetic strategy[8]. In the "deuterated" analogues, all *C*-linked hydrogens except H-1 and H-5 of a specific glucosyl residue (**E**, **F**, and **D**, respectively) were replaced by deuterium. We have applied the combination of 1D-selective TOCSY[14] along with 2D COSY[15,16] techniques to completely assign the ^{1}H NMR spectra of compounds **1a–d** and **2–9**. The spectral interpretations were verified by computer simulation.

The high-resolution 1D ^{1}H NMR spectrum of hexaglucoside **1a** revealed six anomeric signals (see Fig. 2a). The left-most among these signals (δ 4.99, J 3.9 Hz) was assigned to H-1 on residue **A** α-linked to the allyl group. Five other anomeric proton signals were found between δ 4.750 and 4.522, two of which coincided at δ 4.522. Those five signals were assigned to the anomeric protons of the five β-linked residues in **1a**. As the anomeric proton of a β-(1 → 3)-linked residue generally resonates at lower field than H-1 of the same residue in a β-(1 → 6)-linkage[17], the signals at δ 4.721 and 4.750 were assigned to the H-1 of residue **C** and **F**, and the signals at δ 4.579 and 4.522 were assigned to the H-1 of residues **B**,

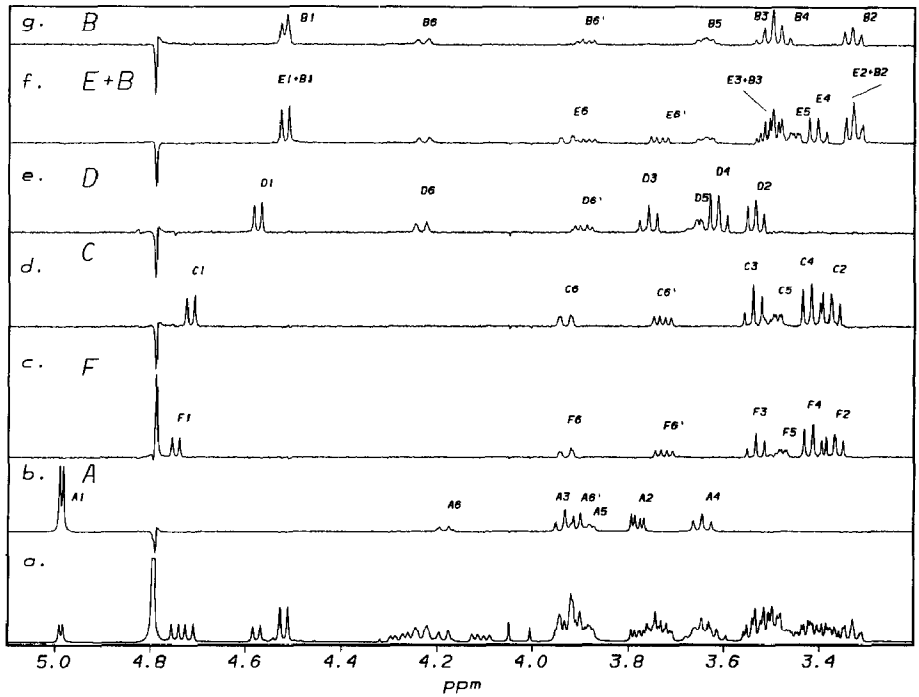


Fig. 2. (a) 1D ^{1}H NMR spectrum of allyl hexaglucoside **1a** recorded for a solution in 4:1 D_2O:$(CD_3)_2CO$ at 500 MHz and 23°C. The spectra **b–g** are 1D selective TOCSY spectra representing subspectra for each of the individual residues. The anomeric proton signals were inverted consecutively by selective DANTE pulses. Spectrum **g** was obtained using the partially deuterated hexaglucoside analogue **1b**. The glycosyl residues are denoted by letters (see Fig. 1).

D, and **E**. The individual assignments of the anomeric doublets were inferred from comparison of the spectrum of **1a** with the spectra of the selectively deuterated analogues (**1b**, **1c**, and **1d**). The deuteration of a residue at C-2, C-3, C-4, and C-6 manifested itself in the NMR spectrum through the collapse of the anomeric doublet to a singlet. In the high-resolution 1D ^{1}H NMR spectra of the deuterated analogues of the hexaglucoside, H-1 of residue **E** in compound **1b** resonated as a singlet at δ 4.522, while H-1 of residue **F** in compound **1c** appeared as a singlet at δ 4.750, and H-1 of residue **D** in compound **1d** appeared as a singlet at δ 4.721. Therefore, all β H-1 signals were unambiguously assigned (Table I).

The first attempt to assign the non-anomeric proton signals located in the bulk region of the spectra ($3.2 < \delta < 4.2$ ppm) utilized a {^{1}H–^{1}H} shift correlation (COSY) experiment. The double-quantum-filtered (DQF) COSY experiments on compounds **1a–d** revealed the positions of the H-2 signals of all residues through the clearly discernible H-1–H-2 connectivities (see Fig. 3). However, the COSY spectral region between δ 3.3 and 3.8 ppm was overcrowded, and each individual cross-peak could not be resolved. In the second instance, we took advantage of the separation of the anomeric signals (except H-1 of residues **B** and **E**) in the 1D

spectra of **1a–d**, making them accessible to selective irradiation. Thus, 1D selective total shift correlation (TOCSY) was used to deconvolute the overall spectrum into subspectra of individual glucosyl residues, allowing the assignment of virtually the whole spectrum, with the exception of the subspectra of residues **B** and **E**. As the H-1 signals of residues **B** and **E** overlapped (Fig. 2a), they could not be selectively irradiated. Rather, the result of selective irradiation of the signal at δ 4.522 was the sum of the subspectra of residues **B** and **E**. To discriminate between the 1D spectra of the two residues, 1D TOCSY was applied to the deuterated analogue **1b** in which residue **E** was deuterated. The anomeric proton signal of residue **E**, not coupled to any neighbouring protons, would not yield a TOCSY spectrum. There-

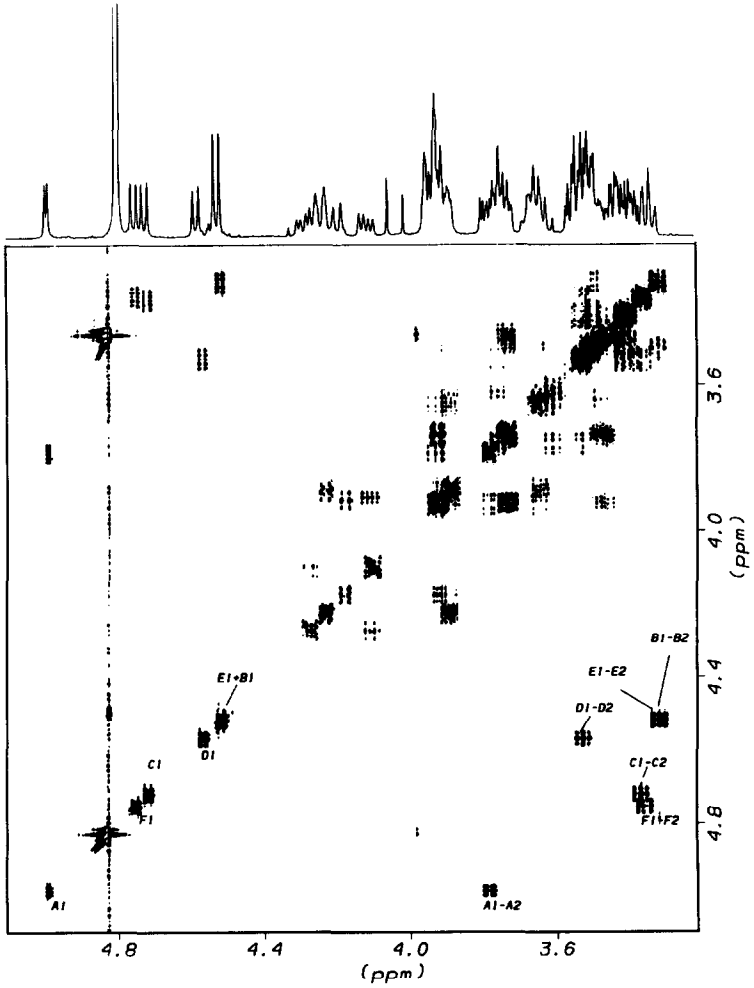


Fig. 3. DQF {^{1}H–^{1}H} shift correlation (COSY) spectrum of hexaglucoside **1a**, recorded at 500 MHz and 23°C. The residues and protons are designated as in Fig. 2.

TABLE I

^{1}H Chemical shifts [a] (ppm) and ^{1}H–^{1}H coupling constants for oligosaccharides **1–9**

Residue	Chemical shift							Coupling constant						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	H-1–H-2	H-2–H-3	H-3–H-4	H-4–H-5	H-5–H-6	H-5–H-6′	H-6–H-6′
Hexaglucoside **1** [b]														
A	4.990	3.784	3.936	3.649	3.890	4.190	3.920	3.9	9.7	8.9	9.7	1.7	5.7	−11.7
B	4.522	3.336	3.520	3.485	3.647	4.232	3.893	8.0	9.1	8.6	9.1	1.7	5.7	−11.7
C	4.721	3.380	3.544	3.422	3.506	3.936	3.734	7.9	9.4	9.0	9.8	2.2	6.3	−12.3
D	4.579	3.539	3.764	3.616	3.661	4.238	3.898	8.1	9.2	8.7	9.9	2.3	5.6	−11.5
E	4.522	3.332	3.510	3.409	3.467	3.933	3.741	8.0	9.3	8.9	9.7	2.2	5.7	−12.4
F	4.750	3.371	3.537	3.417	3.492	3.933	3.728	7.9	9.4	9.0	9.8	2.3	6.1	−12.3
Pentaglucoside **2** [b]														
A	4.990	3.785	3.916	3.649	3.890	4.176	3.900	3.8	9.8	8.6	10.1	1.7	5.1	−11.4
B	4.512	3.325	3.521	3.491	3.620	4.227	3.897	7.9	9.5	8.8	10.5	2.2	5.3	−11.8
C	4.721	3.370	3.536	3.419	3.499	3.931	3.737	7.9	9.5	8.3	10.2	2.0	6.0	−12.3
D	4.562	3.493	3.761	3.514	3.532	3.941	3.766	8.0	9.4	8.0	9.4	2.2	6.7	−12.2
F	4.749	3.366	3.531	3.418	3.497	3.931	3.735	7.9	9.6	8.4	10.8	1.5	6.6	−12.7
Hexasaccharide **3** [b]														
A	4.990	3.784	3.933	3.652	3.890	4.180	3.920	3.8	9.7	9.2	9.5	1.8	5.1	−11.6
B	4.516	3.325	3.510	3.476	3.639	4.217	3.891	7.9	9.2	9.2	8.9	2.1	5.4	−11.8
C	4.721	3.373	3.535	3.420	3.506	3.936	3.727	8.0	9.4	8.9	9.7	2.2	6.2	−12.4
D	4.560	3.527	3.756	3.601	3.642	4.180	3.880	8.0	9.2	8.2	10.0	1.8	5.6	−11.6
	H-1	H-2	H-3	H-4	H-5*ax*	H-5*eq*		H-1–H-2	H-2–H-3	H-3–H-4	H-4–H-5*ax*	H-4–H-5*eq*	H-5*ax*–H-5*eq*	
E (Xyl)	4.460	3.309	3.450	3.634	3.334	3.973		7.9	9.2	9.4	n.d. [e]	5.6	−10.7	
F	4.752	3.363	3.528	3.414	3.490	3.932	3.729	7.9	9.4	9.3	9.9	2.2	6.2	−12.4
Hexasaccharide **4** [c]														
A	4.990	3.796	3.931	3.661	3.900	4.210	3.920	3.7	9.8	8.9	9.7	1.7	5.6	−11.4
B	4.526	3.317	3.523	3.467	3.650	4.240	3.889	8.0	9.1	8.6	9.1	1.7	5.7	−11.7
C	4.727	3.385	3.549	3.424	3.511	3.940	3.734	8.1	9.4	9.0	9.8	2.2	6.1	−11.8
D	4.588	3.524	3.770	3.652	3.660	4.257	3.898	9.1	9.8	8.6	10.3	2.3	n.d.	−11.5
E (Gal)	4.461	3.574	3.663	3.942	3.715	n.d.	n.d.	7.7	10.9	2.4	n.d.	n.d.	n.d.	n.d.
F	4.761	3.379	3.544	3.427	3.505	3.940	3.737	8.3	8.8	9.5	9.8	2.3	6.1	−11.5

Hexasaccharide **5** [d]														
A	4.990	3.782	3.931	3.641	n.d.	4.172	n.d.	3.8	9.6	n.d.	n.d.	n.d.	n.d.	n.d.
B	4.512	3.324	n.d.	n.d.	n.d.	4.212	n.d.	7.9	9.4	n.d.	n.d.	n.d.	n.d.	−11.9
C	4.716	3.372	n.d.	n.d.	n.d.	n.d.	n.d.	7.9	9.5	n.d.	n.d.	n.d.	n.d.	n.d.
D	4.534	n.d.	n.d.	n.d.	n.d.	4.212	n.d.	8.0	n.d.	n.d.	n.d.	n.d.	n.d.	−11.9
E (GlcNAc)	4.534	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F	4.716	3.365	n.d.	n.d.	n.d.	n.d.	n.d.	7.9	9.4	n.d.	n.d.	n.d.	n.d.	n.d.
Hexasaccharide **6** [d]														
A	4.990	3.781	3.93	3.658	n.d.	4.17	n.d.	3.8	9.7	n.d.	n.d.	n.d.	n.d.	n.d.
B	4.51	3.32	n.d.	n.d.	n.d.	4.20	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	−11.7
C	4.721	3.37	n.d.	3.418	3.46	n.d.	n.d.	7.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D	4.522	n.d.	3.76	3.639	n.d.	4.25	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	−11.6
E	4.51	3.30	n.d.	3.418	3.49	n.d.	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F (GlcNAc)	4.773	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hexasaccharide **7** [d]														
A	4.990	3.77	3.920	3.65	n.d.	4.172	n.d.	3.9	9.7	n.d.	n.d.	n.d.	n.d.	n.d.
B	4.515	3.320	3.520	n.d.	n.d.	4.212	n.d.	7.8	9.1	8.6	n.d.	2.2	n.d.	−11.7
C	4.715	3.370	n.d.	3.412	n.d.	n.d.	n.d.	8.0	9.4	n.d.	9.8	n.d.	n.d.	n.d.
D	4.586	n.d.	n.d.	3.620	n.d.	4.238	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	2.0	−11.7
E ($GlcNH_2$)	4.755	n.d.	n.d.	n.d.	n.d.	3.95	n.d.	7.8	9.4	n.d.	9.8	n.d.	n.d.	n.d.
F	4.755	3.360	n.d.	3.406	n.d.	n.d.	n.d.	8.1	9.4	n.d.	9.8	n.d.	n.d.	n.d.
Hexasaccharide **8** [d]														
A	4.990	3.765	3.92	3.658	n.d.	4.180	n.d.	3.9	9.7	8.8	9.7	n.d.	n.d.	n.d.
B	4.520	3.321	n.d.	n.d.	n.d.	4.200	n.d.	7.9	n.d.	n.d.	n.d.	2.2	n.d.	−11.7
C	4.721	3.368	n.d.	3.418	3.49	n.d.	n.d.	8.0	9.4	n.d.	9.8	n.d.	n.d.	n.d.
D	4.555	n.d.	3.76	3.639	n.d.	4.240	n.d.	8.1	9.2	n.d.	9.9	1.8	n.d.	−11.6
E	4.520	3.321	n.d.	3.418	3.49	n.d.	n.d.	7.9	9.3	n.d.	9.7	n.d.	n.d.	n.d.
F ($GlcNH_2$)	5.035	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Triglucoside **9** [b]														
A	4.990	3.7783	3.941	3.649	3.892	4.172	3.920	3.9	9.7	8.9	9.7	1.7	5.7	−11.7
B	4.499	3.331	3.520	3.476	3.926	4.212	3.731	8.0	9.1	8.6	9.1	2.1	6.0	−12.3
C	4.724	3.377	3.540	3.422	3.506	3.931	3.730	7.9	9.4	9.0	9.8	2.2	6.3	−12.3

TABLE I (continued)

Residue	Chemical shift					Coupling constant					
	Ha	Hb	Hc	Hd	Hd′	Ha–Hb	Ha–Hc	Hb–Hc	Hc–Hd	Hc–Hd′	Hd–Hd′
Allyl group	5.30	5.40	6.00	4.10	4.28	−1.5	10.3	17.1	6.4	5.4	−12.7
Propyl group	0.94	1.64	3.12			n.d.	n.d.				
N-Acetyl group	2.08	n.d.									

[a] ^{1}H Chemical shifts are with reference to DSS using internal acetone-d_5 as standard. [b] ^{1}H Chemical shift and coupling constant assignments are based on 1D TOCSY spectra. [c] ^{1}H Chemical shift and coupling constant assignments are based on 2D COSY and 1D TOCSY spectra. [d] ^{1}H Chemical shift and coupling constant assignments are based on 1D spectra. [e] n.d., Not determined.

fore, when we selectively irradiated the signals at δ 4.522 in the spectrum of **1b**, the pure subspectrum of residue **B** was obtained (Fig. 2g). The signals of residue **E** were assigned by subtracting the subspectrum of residue **B** from the sum spectrum of **B** and **E** (Fig. 2f).

The combination of 2D COSY and 1D TOCSY techniques was applied to the oligosaccharides **2–4** and **9**, and their ^{1}H NMR spectra were assigned. The H-5 signal of the galactosyl residue in compound **4** was not observed in the 1D TOCSY experiment upon irradiation of the Gal H-1 at δ 4.461, because the coupling constant between H-4 and H-5 was about 1 Hz. The small-J bottleneck was overcome by a long-range chemical shift correlation (COSYLR) experiment (optimized for COSY transfer through small couplings) on sample **4** (see Fig. 4). Thus, the Gal H-5 was located at δ 3.715. The quantities available of compounds **5–8** were only sufficient to obtain their 1D ^{1}H spectra. Assignment of the spectra of **5–8** was thus based on the comparison of their 1D ^{1}H spectra to that of the hexaglucoside **1a**.

The assignments obtained for oligoglucosides **1–9** were verified and refined by spectral simulation; their chemical shifts and scalar coupling constants are compiled in Table I. The complete assignments of the ^{1}H NMR spectra of the hexaglucoside **1** and its structural analogues will enable us to assign dipolar interactions between protons of hexaglucoside **1** for conformational analysis using NMR spectroscopy.

EXPERIMENTAL

Hexaglucoside **1a** and related compounds were synthesized by Hong and Ogawa according to the synthetic strategy reported[8]. Compounds **1–9** were synthesized primarily for bioactivity studies. The selectively deuterated analogues (**1b**, **1c**, and **1d**) of the hexaglucoside were prepared to facilitate assignments of the hexaglucoside **1a** ^{1}H spectrum.

Samples (typically $\sim$ 5 mg) of the oligoglucosides were repeatedly exchanged with D_2O, with intermediate lyophilization. The samples then were dissolved in 0.4 mL of D_2O (99.96% D, from Cambridge Isotope Laboratories) and transferred into 5-mm NMR tubes (Wilmad 535-PP). Acetone-d_6 (0.1 mL) was added as the lock solvent. Spectra were recorded at 23°C with a Bruker AM 500 spectrometer interfaced with an Aspect 3000 computer using the DISR88 software package. Chemical shifts were referenced to internal acetone-d_5 (δ 2.167 ppm).

Double-quantum-filtered (DQF) COSY experiments were performed in the phase-sensitive mode using time-proportional phase incrementation (TPPI)[18]. The spectral width was set to 1785 Hz. The evolution time (t_1) was incremented in steps of 280 μs to obtain 512 free induction decays (fid's), each acquired in 2 K data points in 8 scans. The acquisition time (t_2) was 410 ms, and the relaxation delay was 2.0 s. A sine bell function without phase shift was applied for processing

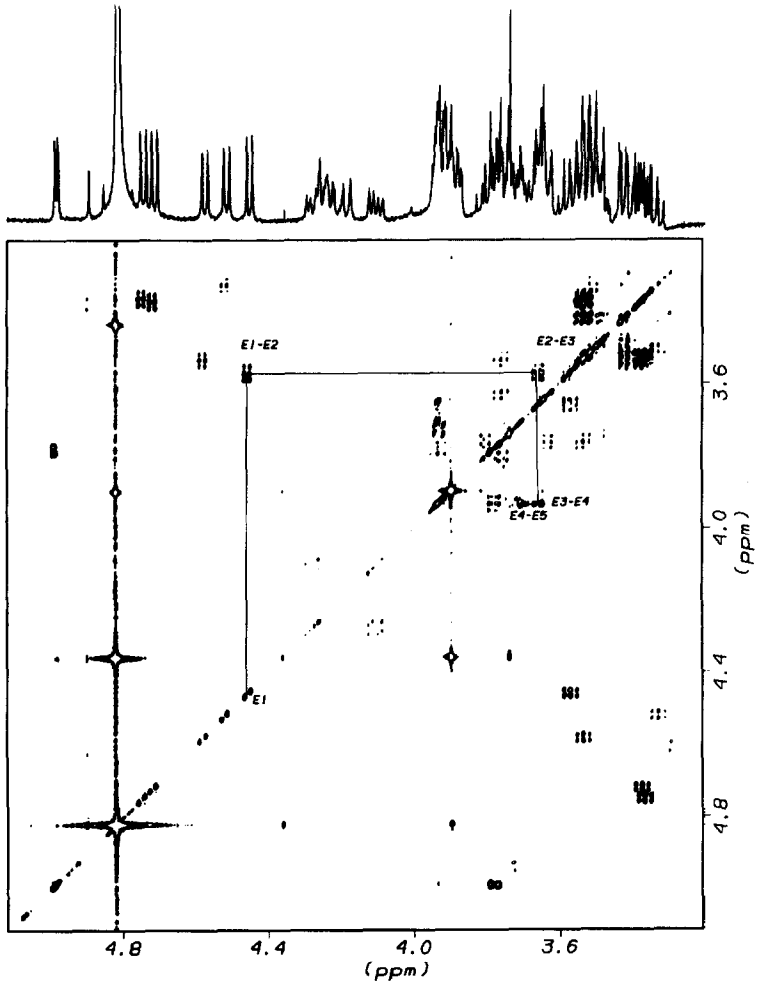


Fig. 4. 2D Long-range chemical shift correlation (COSYLR) spectrum of compound **4**, containing a D-galactosyl group as nonreducing-end terminal residue **E**. The spectrum was recorded at 500 MHz and 23°C.

both in the t_1 and the t_2 dimensions. Zero filling was used to expand the data matrix to 1 K in the t_1 dimension before Fourier transformation.

A 2D long-range chemical shift correlation (COSYLR) was performed on 1.5 mg of compound **4** at a spectral width of 2000 Hz. The evolution time (t_1) was incremented in steps of 500 μs to obtain 512 fid's, each acquired in 2 K data points in 64 scans. The acquisition time (t_2) was 512 ms and the relaxation delay was 2.0 s. A sine bell window function without phase shift was applied for processing both in the t_1 and the t_2 dimension. Zero filling was used to expand the data matrix to 1 K in the t_1 dimension before Fourier transformation.

The 1D-selective TOCSY experiments were performed using the MLEV-17 composite pulse sequence for isotropic mixing[19–21], with the ^{1}H decoupler used as

the sole source of radiofrequency pulses. The anomeric proton signals were selectively inverted by a DANTE pulse sequence[22]. The mixing time was 163 ms. The spectral width was 2000 Hz; 320 scans (2048 scans for 0.5 mg sample) in 8 K data points were acquired per experiment, with acquisition times of 2.048 s. The relaxation delay was 1.1 s.

NMR spectral simulation was performed using the Bruker PANIC software package on an ASPECT 3000 computer. The spectral width was set at 2500 Hz; using 4 K data points resulted in a digital resolution of 0.6 Hz/pt. The linewidth was set at 1.5 Hz. The subspectra of each residue of a given oligoglucoside were calculated individually and then co-added.

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