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NOTE

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Assignment of the ^{1}H and ^{13}C NMR spectra of 2-aminobenzamide-labeled galacto- and arabinooligosaccharides

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Abstract 1,4-Linked β-D-galactooligosaccharides with a degree of polymerization (DP) between 1 and 7 and 1,5-linked α-L-arabinooligosaccharides with a DP between 1 and 8 were labeled at their reducing ends with 2-aminobenzamide (2AB) in the presence of sodium cyanoborohydride. The 2AB-labeled oligosaccharides were shown to be homogeneous using high-performance anion-exchange chromatography (HPAEC) and by electrospray ionization mass spectrometry (ESI-MS). The signals in the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the 2AB-labeled oligosaccharides were then assigned using one- and two-dimensional NMR spectroscopy. These NMR data will be useful for the structural analysis of enzymatically synthesized galactan and arabinan side chains derived from rhamnogalacturonan I.

Key words NMR · Arabinooligosaccharide · Galactooligosaccharide · 2-Aminobenzamide

Introduction

Oligosaccharides composed of linear (1 → 4)-β-D-galactosyl residues and linear (1 → 5)-α-L-arabinosyl residues are often present as side chains that are linked to the backbone of the plant cell wall pectic polysaccharide rhamnogalacturonan I (RG-I).[1] Arabinans may also exist as branched side chains composed of 2,5- and 3,5-linked L-arabinofuranosyl (Ara_f) residues. The galactan may be unsubstituted or may be substituted at C-3 with side chains of (1 → 5)-linked Ara_f residues (type I arabinogalactan). The structures of these side chains and their distribution within the primary walls of different cell types have been reported to change during plant growth and development.[2]

Little is known about the mechanism of pectin biosynthesis because our ability to biochemically characterize pectic glycosyltransferases is limited by the lack of in vitro methods for determining transferase activities and for structurally characterizing the enzymically formed products.[3,4]

Galactosyltransferase (GalT) and arabinosyltransferase (AraT) activities have been demonstrated by measuring the incorporation of [^{14}C]-labeled sugar from UDP-[^{14}C]-Gal[5–13] and UDP-[^{14}C]-Ara[14] into exogenous and endogenous acceptor substrates. Membrane fractions isolated from mung bean,[6] flax,[7,8] soybean,[13] and radish[12] have been shown to contain GalT activities that catalyze the formation of β-(1 → 4) and β-(1 → 3)-galactosidic, and β-(1 → 4), β-(1 → 3), and β-(1 → 6)-galactosic linkages, respectively. Indeed, the synthesis of all the galatosyl linkages in pectin may require at least eight different GalTs.[3,4] Similarly, numerous AraTs are likely to be required for the synthesis of the arabinosyl-containing side chains of pectic polysaccharides. Thus, it is important that in vitro methods are developed that can distinguish the different GalT and AraT activities and characterize the transferred products. We have previously shown that 2-aminobenzamide (2AB)-labeled oligogalacturonides and galactooligosaccharides are acceptors for galacturonosyltransferases[15,16] and galactosyltransferases,[17] respectively. The product formed by the membrane-localized GalT was characterized using β-galactanase and endo-galactanase digests and high-resolution nuclear magnetic resonance (NMR) spectroscopy to be β-(1 → 4)-linked.[17]

We provide a complete assignment of the signals in the ^{1}H and ^{13}C NMR spectra of 2AB-labeled galacto- and arabinooligosaccharides.

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Materials and methods

2-Aminobenzamide labeling of oligosaccharides

1,5-Linked α-L-arabinooligosaccharides with degree of polymerization (DP) of 2 to 8 were obtained from Megazyme (Wicklow, Ireland). 1,4-Linked β-D-galactooligosaccharides with DP of 2 to 7 were generated and isolated as described.[17] The oligosaccharides were labeled with 2AB and purified as described previously.[16]

Analytical methods

High-performance anion-exchange chromatography (HPAEC) was performed using a Carbo Pac PA-1 column (4.5 × 250 mm) and a metal-free Dionex Bio LC interfaced to an Auto Ion series 400 data station (Dionex, Sunnyvale, CA, USA).[17] Electrospray ionization mass spectrometry (ESI-MS) analysis was performed with a Thermo-Quest LCQ Duo mass spectrometer (Thermoelectron, Waltham, MA, USA) operated in the positive-ion and negative-ion modes with a spray voltage of 4.55 kV, a capillary voltage of 3.1 V, and a capillary temperature of 180°C.[16] Mass spectra were obtained between *m/z* 150 and 2000. One-dimensional ^{1}H spectra, two-dimensional (2D)-double quantum filtered correlation spectroscopy (DQFCOSY), 2D- total correlation spectroscopy (TOCSY), 2D {^{1}H-^{13}C} ^{1}H-detected heteronuclear single quantum coherence (HSQC) spectroscopy, and ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) spectroscopy were performed at 303 K and 800 MHz with a Bruker Avance 800 NMR spectrometer (Brucker, Karlsruhe, Germany).[16] ^{13}C NMR spectra were obtained at 303 K with a Bruker Avance 600 NMR spectrometer. The 2AB-labeled oligosaccharides (about 3.5~4.0 mg) were dissolved in 99.96% isotopically enriched D_2O and then freeze-dried. The derivatives were then dissolved in 99.96% enriched D_2O (0.8 ml) prior to NMR spectroscopic analysis. Number of scans (*n*) for each measament was as follows: ^{1}H n = 16; ^{13}C n = 4895, DQFCOSY n = 4, TOCSY n = 8, HSQC n = 8, and HMBC n = 8. The TOCSY mixing time was 120 ms. HSQC and HMBC were recorded using pulsed-field gradients for coherence selection. In a typical 2D-spectrum (^{1}H—^{1}H), 4096 transients of 2048 data points were recorded with a spectral width of 3600 Hz in both dimensions, and the data were processed with zero filling to obtain a 4096 × 4096 matrix. ^{1}H and ^{13}C chemical shifts were measured relative to internal 2-methyl-2-propanol at δ 1.230 and 31.30, respectively.

Results and discussion

2-Aminobenzamide labeling of oligosaccharides

Galactooligosaccharides with DP of 1 to 7 (compounds **1**–**7**, see Fig. 1) and arabinooligosaccharides with DP of 1 to 8 (compounds **8**–**15**, see Fig. 2) were labeled with aqueous 2AB.[16] Each 2AB-oligosaccharide eluted as a single peak when analyzed by HPAEC (data not shown). The positive-ion mode ESI-MS spectra of **1**–**7** and the negative-ion mode ESI-MS spectra of **8**–**15** were dominated by singly ($[M + H]^{+}$) or doubly charged ($[M + H]^{2+}$) protonated molecular ions, and a deprotonated molecular ion ($[M - H]^{-}$), respectively (Table 1). These data confirmed their molecular weights.

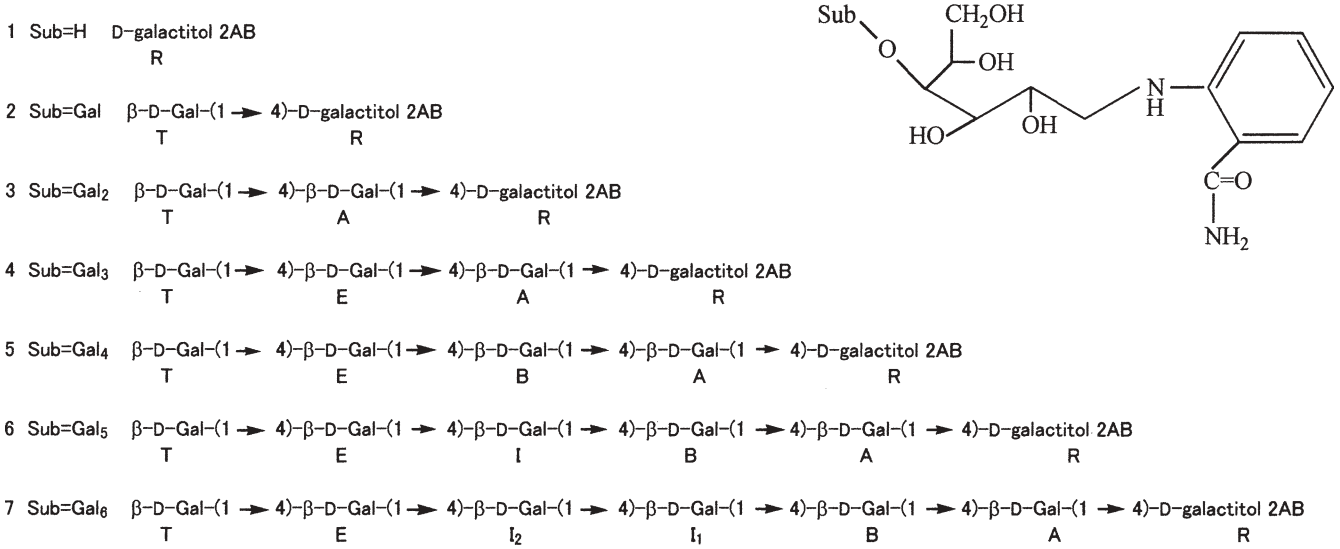

Fig. 1. Structures of compounds **1**–**7**. **1**–**3**, 2-aminobenzamide (2AB)-labeled D-galactiol to galactotrisaccharides [degree of polymerization (DP) 1–3]; **4**, galactotetrasaccharide consisting of a D-galactitol R, two internal residues (A and E), and non-reducing terminal residue T; **5**, galactopentasaccharide consisting of a D-galactitol R, three internal residues (A, B, and E), and nonreducing terminal residue T; **6**, galactohexasaccharide composed of R, four internal residues (A, B, I, and E), and terminal residue T; **7**, galactoheptasaccharide composed of R, five internal residues (A, B, I_1, I_2, and E), and terminal residue T

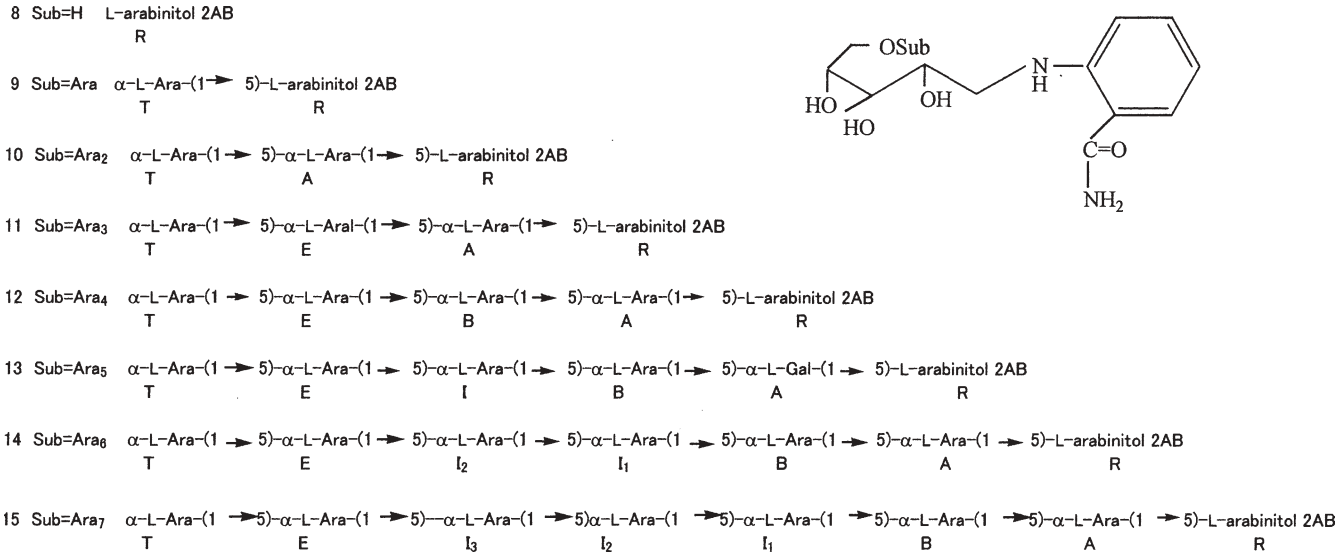

Fig. 2. Structures of compounds **8**–**15**. **8**–**10**, 2AB-labeled L-arabinitol to arabinotrisaccharides (DP 1–3); **11**, arabinotertrasaccharide consisting of an L-arabinitol R, two internal residues (A and E), and nonreducing terminal residue T; **12**, arabinopentasaccharide consisting of an L-arabinitol R, three internal residues (A, B, and E), and nonreducing terminal residue T; **13**, arabinohexasaccharide composed of R, four internal residues (A, B, I, and E), and terminal residue T; **14**, arabinoheptasaccharide, composed of R, five internal residues (A, B, I_1, I_2, and E), and terminal residue T; **15**, arabinooctasaccharide, composed of R, six internal residues (A, B, I_1, I_2, I_3 and E), and terminal residue T

Table 1. Electrospray ionization mass spectrometry data for compounds **1**–**15**

Nominal mass	Molecular ion	Compound[a]	Molecular weight
323	$(M+Na)^+$	**1**	300
485	$(M+Na)^+$	**2**	462
647	$(M+Na)^+$	**3**	624
809	$(M+Na)^+$	**4**	786
971	$(M+Na)^+$	**5**	948
1133	$(M+Na)^+$	**6**	1110
1295	$(M+Na)^+$	**7**	1272
269	$(M-H)^-$	**8**	270
401	$(M-H)^-$	**9**	402
533	$(M-H)^-$	**10**	534
665	$(M-H)^-$	**11**	666
797	$(M-H)^-$	**12**	798
929	$(M-H)^-$	**13**	930
1061	$(M-H)^-$	**14**	1062
1193	$(M-H)^-$	**15**	1194

[a] Structures shown in Figs. 1 and 2

Assignment of ^1H and ^{13}C NMR spectra of the 2AB-labeled oligosaccharides

The ^1H NMR spectra of 2AB-labeled oligosaccharides were recorded at 800 MHz. The ^1H and ^{13}C NMR spectra of the 2AB-galactooligosaccharides (**1**–**7** see Fig. 1; Fig. 3 shows ^1H NMR spectra) and 2AB-arabinooligosaccharides (**8**–**15** see Fig. 2, Fig. 4 shows ^1H NMR spectra) were assigned using DQFCOSY, TOCSY, HSQC, and HMBC experiments. All of the signals in the NMR spectra were able to be assigned to the oligosaccharide or 2AB, thereby confirming that the derivatives were homogeneous.

The complete assignment of the ^1H NMR spectrum of 2AB-labeled pentaogalactose (**5**, see Fig. 3B) is described as a typical example. The D-galactitol residue (the former reducing end of the oligosaccharide) is clearly no longer in the pyranose ring form because C-1 is substituted with two protons rather than one. These two protons gave quartets at δ 3.431 and 3.386 (RH1 in Fig. 3B), with coupling constants of 5.2 and 7.9 Hz. The doublet at δ 4.584 (J 7.8 Hz) is assigned to the resonance of the H-1 of the terminal nonreducing Gal residue T, whereas the H-1 resonance of the residue next to the former reducing end (residue A) is at δ 4.453. The remaining doublets at δ 4.606 and 4.629 are the resonances of the H-1 of the internal sugar residues B and E, respectively. The chemical shift values of H-1s of the nonreducing Gal residue and the magnitude of the coupling constants (7.8–7.9 Hz) are consistent with a β linkage.[18]

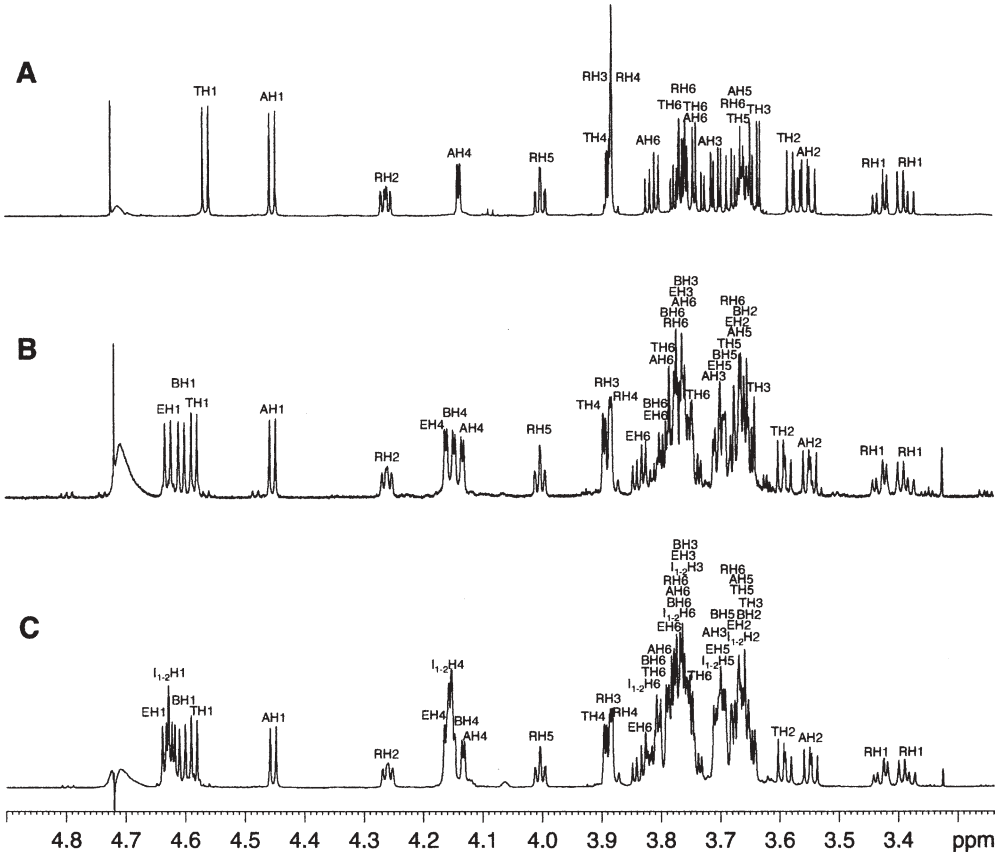

Fig. 3. [1]H Nuclear magnetic resonance (NMR) spectra of compounds **A3**, **B5**, and **C7**. *Labels* on spectra indicate residue and proton position

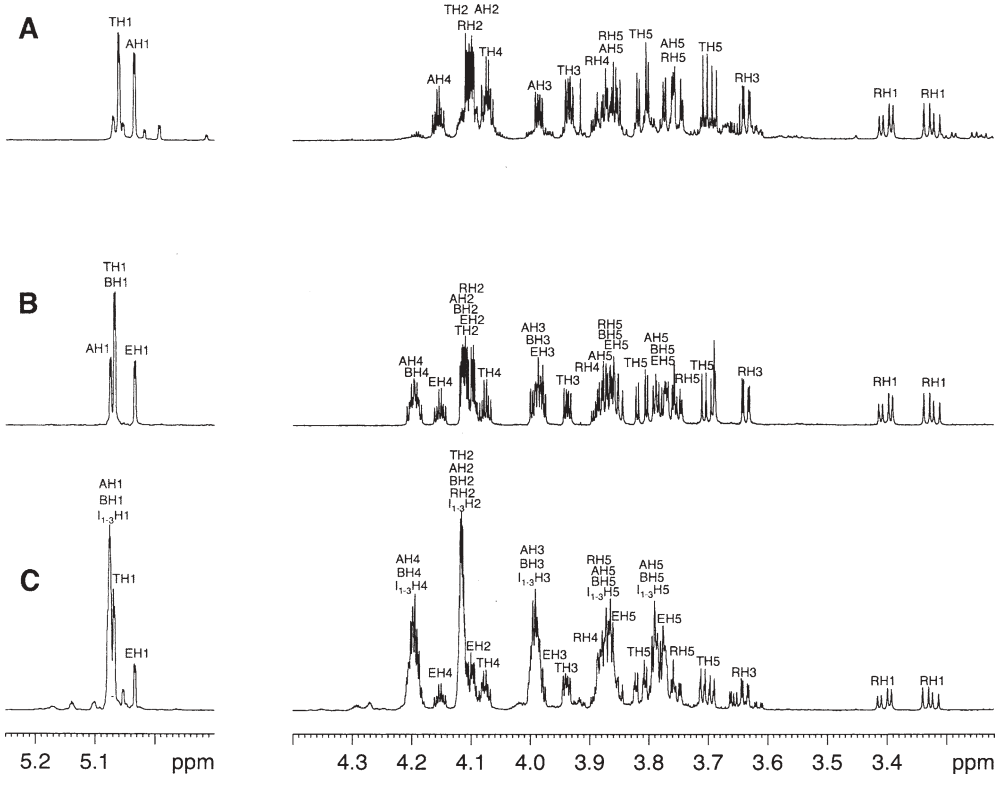

Fig. 4. [1]H NMR spectra of compounds **A10**, **B12**, and **C15**. *Labels* on spectra indicate residue and proton position

Table 2. ^{1}H Chemical shifts and first-order coupling constants for compounds **1–7**

Compound	Residue	^{1}H Chemical shifts (ppm)[a]								First-order coupling constants (Hz)[b]								
		H-1$_a$	H-1$_b$	H-2	H-3	H-4	H-5	H-6$_a$	H-6$_b$	$^{3}J_{1a,2}$	$^{3}J_{1b,2}$	$^{2}J_{1a,1b}$	$^{3}J_{2,3}$	$^{3}J_{3,4}$	$^{3}J_{4,5}$	$^{3}J_{5,6a}$	$^{2}J_{5,6b}$	$^{2}J_{6a,6b}$
1		3.408	3.499	4.133	3.680	3.675	3.968	3.676	3.672	5.0	8.4	13.4	8.4	3.4	1.0	6.5	4.0	11.5
2	R	3.346	3.391	4.255	3.880	3.904	4.000	3.793	3.665	5.3	8.0	13.7	8.0	3.2	1.0	6.5	4.0	11.5
	T	4.431	–	3.473	3.593	3.871	3.635	3.755	3.726	7.9	–	–	9.9	3.6	<0.5	5.5	4.0	11.8
3	R	3.428	3.386	4.262	3.885	3.883	4.001	3.767	3.676	5.3	7.9	13.6	7.9	3.4	1.1	6.9	4.0	11.6
	A	4.453	–	3.556	3.706	4.139	3.665	3.813	3.735	7.9	–	–	10.0	3.3	0.8	4.2	4.0	8.1
	T	4.564	–	3.568	3.640	3.889	3.663	3.774	3.735	7.9	–	–	9.9	3.5	0.8	5.6	4.1	11.8
4	R	3.429	3.387	4.260	3.885	3.883	4.002	3.771	3.663	5.2	8.0	13.7	7.8	3.0	1.3	6.6	4.0	11.5
	A	4.453	–	3.549	3.703	4.130	3.655	3.708	3.758	7.9	–	–	10.0	3.1	<0.5	5.4	4.2	12.0
	E	4.609	–	3.653	3.750	4.154	3.688	3.831	3.762	7.9	–	–	–	–	<0.5	–	–	–
	T	4.579	–	3.587	3.640	3.892	3.683	3.791	3.740	7.8	–	–	10.0	3.4	0.9	5.7	–	11.9
5	R	3.431	3.386	4.260	3.885	3.883	4.004	3.771	3.676	5.2	7.9	13.5	8.0	3.5	1.0	7.0	4.0	11.5
	A	4.453	–	3.549	3.708	4.134	3.664	3.796	3.758	7.9	–	–	10.0	3.0	<0.5	5.4	4.0	11.5
	B	4.606	–	3.654	3.754	4.148	3.690	3.806	3.777	7.9	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	E	4.629	–	3.667	3.762	4.162	3.699	3.836	3.803	7.9	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	T	4.584	–	3.591	3.652	3.895	3.682	3.782	3.744	7.8	–	–	10.0	3.0	1.0	5.5	4.0	12.0
6	R	3.430	3.386	4.261	3.892	3.885	4.003	3.772	3.677	5.2	8.0	13.5	8.0	3.5	1.0	7.0	4.0	11.5
	A	4.453	–	3.548	3.708	4.133	3.659	3.800	3.768	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	B	4.605[c]	–	3.655	3.756	4.154[c]	3.691	3.806	3.778	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	I	4.620[c]	–	3.658	3.752	4.150[c]	3.691	3.815	3.777	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	E	4.633	–	3.670	3.760	4.165	3.699	3.837	3.781	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	T	4.585	–	3.593	3.650	3.895	3.667	3.815	3.748	8.0	–	–	10.0	3.0	1.0	5.5	4.0	12.0
7	R	3.430	3.386	4.262	3.887	3.885	4.003	3.772	3.677	5.2	8.0	13.5	8.0	3.5	1.0	7.0	4.0	11.5
	A	4.453	–	3.548	3.708	4.134	3.659	3.801	3.768	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	B	4.605	–	3.650	3.756	4.150	3.694[c]	3.811[c]	3.767[c]	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	I$_1$	4.623[c]	–	3.659	3.752	4.158	3.699[c]	3.807[c]	3.763[c]	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	I$_2$	4.627[c]	–	3.659	3.752	4.158	3.699[c]	3.807[c]	3.763[c]	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	E	4.633	–	3.666	3.760	4.163	3.703	3.834	3.781	8.0	–	–	10.0	3.0	<0.5	5.5	4.0	11.5
	T	4.585	–	3.593	3.650	3.893	3.663	3.811	3.743	8.0	–	–	10.0	3.0	1.0	5.5	4.0	12.0

For key to residue labels, see Fig. 1

[a] ^{1}H shifts are given relative to the methyl proton of internal standard 2-methyl-2-propanol (1.230 ppm) at 800 MHz at 30°C

[b] ^{1}H Chemical shift and coupling constant assignments are based on 1D ^{1}H, DQFCOSY, and TOCSY spectra

[c] Interchangeable, uncertain

Table 3. ^{13}C Chemical shifts for compounds **1–7**

Compound	Residue	^{13}C Chemical shifts (ppm)[a]					
		C-1	C-2	C-3	C-4	C-5	C-6
1		47.62	69.59	71.99	71.29	71.96	64.94
2	R	47.52	68.69	71.52	78.50	71.52	64.01
	T	104.64	72.61	74.25	70.24	76.54	62.69
3	R	47.48	68.79	71.66	79.23	71.74	64.24
	A	104.80	72.99	74.71	78.94	75.89	62.31
	T	106.04	73.08	74.48	70.31	76.86	62.67
4	R	47.48	68.77	71.64	79.19	71.70	64.21
	A	104.77	73.00	74.78	79.37	75.94	62.48
	E	106.08	73.50	74.96	78.82	76.15	62.25
	T	105.99	73.08	74.47	70.33	76.86	62.68
5	R	47.48	68.75	71.62	79.16	71.68	64.22
	A	104.78	73.01	74.76	79.42	75.93	62.26
	B	106.08	73.51	75.03	79.27	76.21	62.44
	E	106.04	73.51	74.95	78.83	76.17	62.44
	T	105.99	73.08	74.47	70.33	76.86	62.69
6	R	47.48	68.77	71.64	79.19	71.70	64.23
	A	104.77	73.01	74.76	79.41[b]	75.93	62.27
	B	106.04	73.51	75.02	79.27[b]	76.21[b]	62.45
	I	106.04	73.51	75.02	79.28[b]	76.0[b]	62.45
	E	106.04	73.51	74.95	78.84	76.16	62.41
	T	105.99	73.08	74.47	70.33	76.86	62.69
7	R	47.48	68.79	71.64	79.19	71.70	64.23
	A	104.77	73.01	74.76	79.41	75.93	62.27
	B	106.04[b]	73.52	75.01	79.29[b]	76.20[b]	62.46[b]
	I$_1$	106.04[b]	73.52	75.01	79.31[b]	76.22[b]	62.45[b]
	I$_2$	106.05[b]	73.52	75.01	79.32[b]	76.22[b]	62.42[b]
	E	106.08[b]	73.52	74.95	78.84	76.16	62.42[b]
	T	105.99	73.08	74.47	70.34	76.86	62.69

[a] ^{13}C Chemical shifts are quoted relative to the methyl carbon of 2-methyl-2-propanol (31.30 ppm) at 150 MHz at 30°C and their assignments are based on 1D ^{13}C, HSQC, and HMBC spectra

[b] Interchangeable, uncertain.

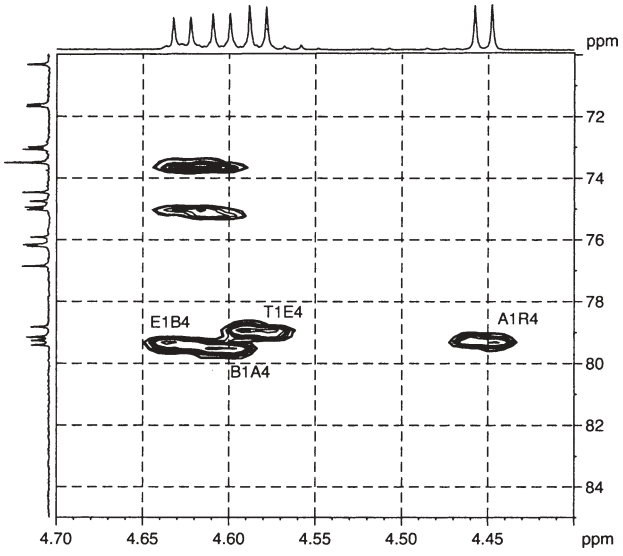

Fig. 5. Partial contour plot of the HMBC experiment on compound **5**. *Cross-peak* labels such as *A1R4* denote the correlation between H-1 of residue A and C-4 of residue R

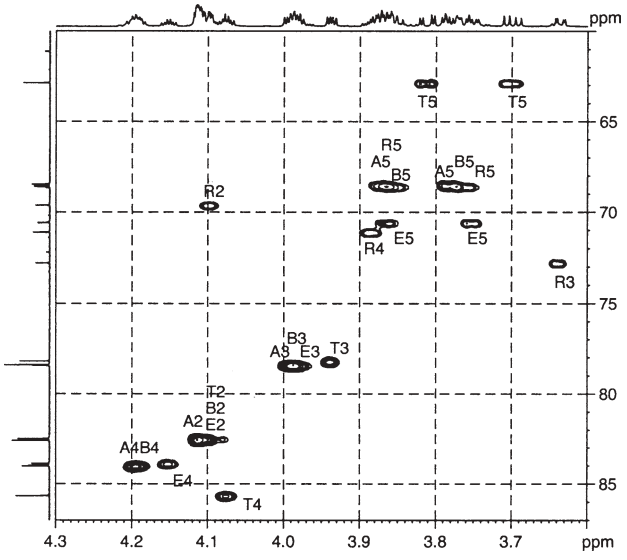

Fig. 7. Partial contour plot of the HSQC experiment on compound **12**

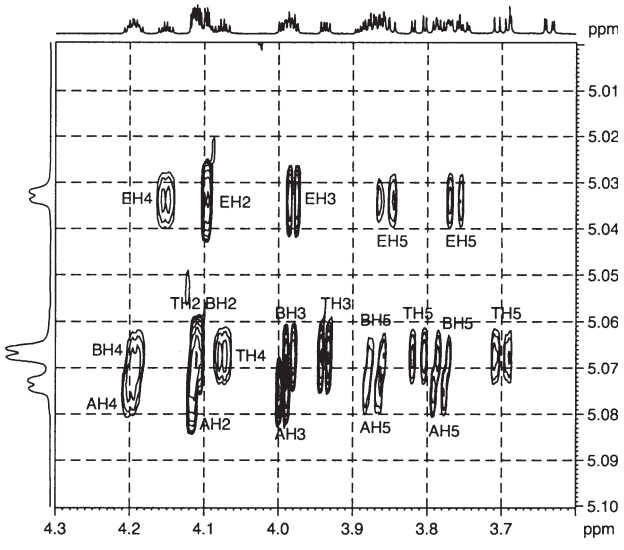

Fig. 6. Partial contour plot of the TOCSY experiment on compound **12**

The anomeric resonances are all well resolved from the nonanomeric sugar proton signals. The TOCSY and DQFCOSY spectra allowed the assignment of the proton signals from H-1 to H-4 of **5**. The proton signals of H-5 and H-6 were assigned by HSQC and HMBC experiments. By comparing the spectra of **3** with those of **7**, the signals in the spectra of all the galactooligosaccharide derivatives were assigned (Table 2). The ^{13}C NMR spectra of the 2AB-labeled oligogalactoses were analyzed by HSQC and HMBC spectroscopy. The HMBC spectra (Fig. 5) gave correlations between H-1 of residue T and C-4 of residue E, H-1 of residue E and C-4 of residue B, H-1 of residue B and C-4 of residue A, and H-1 of residue A and C-4 of residue R, confirming the connectivity of each glycosyl residue in the oligomer (Fig. 1). Detailed ^{13}C assignments are shown in Table 3. The chemical shifts of the 2AB residue in the 2AB-labeled oligosaccharides are shown in Table 4.

Table 4. ^{1}H and ^{13}C Chemical shifts and first-order coupling constants for 2-aminobenzamide group of 2AB-labeled oligosaccharides

Spectrum	Position	δ	Coupling (Hz)
^{1}H	H-3	6.912	$^{3}J_{3,4,}$ 8.4
	H-4	7.432	$^{3}J_{4,5,}$ 7.1
	H-5	6.772	$^{3}J_{5,6,}$ 7.8
	H-6	7.552	
^{13}C	C-1	117.60	
	C-2	150.08	
	C-3	114.48	
	C-4	135.31	
	C-5	118.09	
	C-6	130.88	
	C = 0	176.07	

The assignments of the 2AB-labeled arabinooligosaccharides were performed as for the 2AB-labeled galactooligosaccharides. From the DQFCOSY and TOCSY (Fig. 6) spectra, the proton signals from H-1 to H-5 of **12** were assigned. The ^{13}C NMR spectrum was assigned by HSQC (Fig. 7). The HMBC spectrum gave intensive intramolecular correlations between proton and carbon atoms of each residue (data not shown). The signals in the spectra of all the oligosaccharides were assigned by comparing the spectra of **8** with those of **15**. The ^{1}H and ^{13}C chemical shifts of the 2AB-labeled arabinooligosaccharides with DPs of 1 to 8 are summarized in Table 5 and 6.

We have previously reported that membrane fractions from mung bean (*Vigna radiata*) contain enzymes that transfer Gal from UDP-Gal onto galactooligosaccharides to yield oligosaccharides with DPs up to 15. The enzymetically synthesized 2AB-labeled galactooligosaccharides

Table 5. ^{1}H Chemical shifts and first-order coupling constants for compounds **8–15**

Compound	Residue	^{1}H Chemical shifts (ppm)[a]							First-order coupling constants (Hz)[b]							
		H-1$_a$	H-1$_b$	H-2	H-3	H-4	H-5$_a$	H-5$_b$	$^{3}J_{1a,2}$	$^{3}J_{1b,2}$	$^{2}J_{1a,1b}$	$^{3}J_{2,3}$	$^{3}J_{3,4}$	$^{3}J_{4,5a}$	$^{3}J_{4,5b}$	$^{2}J_{5a,5b}$
8	R	3.320	3.392	4.094	3.570	3.752	3.830	3.645	5.0	8.3	13.0	2.0	8.3	3.0	6.4	11.9
9	R	3.325	3.400	4.100	3.635	3.882	3.867	3.755	5.1	8.1	13.5	2.5	8.5	3.0	2.8	11.0
	T	5.028		4.095	3.930	4.044	3.801	3.690	1.2			3.2	6.0	3.5	6.0	12.0
10	R	3.325	3.402	4.099	3.636	3.880	3.866	3.752	5.2	8.1	13.4	2.1	8.2	2.5	2.4	10.5
	A	5.034		4.097	3.986	4.155	3.854	3.769	1.4			3.7	5.9	3.2	3.7	11.5
	T	5.060		4.103	3.935	4.072	3.811	3.697	1.4			3.5	6.0	3.3	5.8	12.3
11	R	3.325	3.402	4.095	3.636	3.887	3.856	3.750	5.2	8.2	13.0	2.0	8.5	3.0	6.0	12.0
	A	5.067		4.111	3.989	4.192	3.863	3.782	Bs			3.0	6.5	3.5	4.0	12.5
	E	5.033		4.096	3.984	4.152	3.856	3.763	1.5			3.0	6.0	3.5	4.5	12.0
	T	5.066		4.109	3.935	4.073	3.812	3.699	Bs			3.0	7.0	3.5	6.0	12.0
12	R	3.324	3.402	4.098	3.637	3.890	3.866	3.750	5.1	8.0	13.3	2.0	8.1	2.6	6.0	12.0
	A	5.073		4.115	3.994	4.200	3.879	3.787	1.5			3.3	6.0	3.3	5.8	12.3
	B	5.066		4.108[c]	3.986	4.190	3.865	3.773	1.5			2.2	5.9	3.0	5.0	12.0
	E	5.033		4.097	3.979	4.152	3.854	3.757	1.3			3.0	5.5	3.3	5.0	12.0
	T	5.066		4.108[c]	3.936	4.075	3.812	3.699	1.3			3.0	5.5	3.5	5.2	12.0
13	R	3.325	3.402	4.089	3.633	3.893	3.883	3.757	5.0	8.0	13.3	2.0	8.0	2.5	6.0	12.0
	A	5.073		4.115[c]	3.990[c]	4.198[c]	3.876[c]	3.788	Bs			2.0	5.0	3.0	5.0	12.0
	B	5.073		4.114[c]	3.990[c]	4.194[c]	3.872[c]	3.788	Bs			2.0	5.0	3.0	5.0	12.0
	I	5.073		4.113[c]	3.986[c]	4.194[c]	3.865[c]	3.788	Bs			2.0	5.0	3.0	5.0	12.0
	E	5.032		4.096	3.974	4.151	3.851	3.774	1.3			3.0	5.5	3.5	5.2	12.0
	T	5.066		4.111[c]	3.936	4.075	3.812	3.699	1.5			3.0	6.0	3.0	5.5	12.0
14	R	3.382	3.405	4.107	3.639	3.892	3.883	3.757	5.0	8.0	13.0	2.0	8.0	2.5	6.0	12.0
	A	5.076		4.117[c]	3.997[c]	4.200[c]	3.876[c]	3.791[c]	Bs			2.0	5.0	3.0	5.0	12.0
	B	5.076		4.117[c]	3.992[c]	4.200[c]	3.873[c]	3.789[c]	Bs			2.0	5.0	3.0	5.0	12.0
	I$_1$	5.076		4.115[c]	3.992[c]	4.196[c]	3.869[c]	3.787[c]	Bs			2.0	5.0	3.0	5.0	12.0
	I$_2$	5.076		4.115[c]	3.990[c]	4.196[c]	3.866[c]	3.777[c]	Bs			2.0	5.0	3.0	5.0	12.0
	E	5.034		4.098	3.980	4.153	3.854	3.773	1.4			2.0	5.0	3.0	5.0	12.0
	T	5.068		4.113[c]	3.393	4.077	3.822	3.701	1.5			2.0	5.0	3.0	5.0	12.0
15	R	3.326	3.404	4.107	3.638	3.884	3.882	3.758	5.0	8.0	13.0	2.0	8.0	2.5	6.0	12.0
	A	5.076[c]		4.117[c]	3.995[c]	4.198[c]	3.876[c]	3.790[c]	Bs			2.0	5.0	3.0	5.0	12.0
	B	5.076[c]		4.117[c]	3.995[c]	4.198[c]	3.876[c]	3.780[c]	Bs			2.0	5.0	2.7	5.0	12.0
	I$_1$	5.075[c]		4.115[c]	3.991[c]	4.194[c]	3.874[c]	3.780[c]	Bs			2.0	5.0	2.7	5.0	12.0
	I$_2$	5.075[c]		4.115[c]	3.991[c]	4.194[c]	3.874[c]	3.776[c]	Bs			2.0	5.0	2.7	5.0	12.0
	I$_3$	5.074[c]		4.115[c]	3.991[c]	4.194[c]	3.874[c]	3.776[c]	Bs			2.0	5.0	2.7	5.0	12.0
	E	5.033		4.097	3.984	4.151	3.853	3.772	1.3			2.0	5.0	2.7	6.0	12.0
	T	5.068		4.115[c]	3.939	4.076	3.813	3.700	1.6			2.0	5.0	3.7	6.0	12.0

For key to residue labels, see Fig. 2
Bs, Broad singlet
[a] ^{1}H Chemical shifts are quoted relative to the methyl proton of internal standard 2-methyl-2-propanol (1.230 ppm) at 800 MHz
[b] ^{1}H Chemical shift and coupling constant assignments are based on 1D ^{1}H, DQFCOSY, and TOCSY spectra
[c] Interchangeable, uncertain

were characterized to be β-(1 → 4)-linked after using β-galactosidase and *endo*-galactanase digests. The NMR data of 2AB-labeled galactooligosaccharides gave additional evidence that the products were β-(1 → 4)-linked.[17] Nunan and Scheller[14] reported that microsomal membranes from mung bean solubilized with detergent octyl glucosides were able to add a single [^{14}C] Ara residue onto (1 → 5)-linked α-L-arabinooctasaccharide acceptors. However, the enzymatically synthesized arabinooligosaccharides were not fully characterized. Preliminary experiments showed that the 2AB-labeled arabinooligosaccharides worked as acceptors for arabinosyltransferase (unpublished results). NMR data would be helpful to characterize the linkage position and ring form of the newly incorporated arabinose residues.

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Table 6. ^{13}C Chemical shifts for compounds **8**–**15**

Compound	Residue	^{13}C Chemical shifts (ppm)[a]				
		C-1	C-2	C-3	C-4	C-5
8	R	47.46	69.69	73.07	72.67	64.71
9	R	47.39	69.61	72.81	71.12	70.54
	T	109.13	82.62	78.24	85.53	62.89
10	R	47.34	69.62	72.78	71.40	70.85
	A	109.23	82.59	78.45	83.89	68.50
	T	109.09	82.54	78.21	85.64	62.87
11	R	47.36	69.63	72.78	71.11	68.60
	A	109.17[b]	82.59	78.42	84.00	70.59
	E	109.23	82.49	78.45	83.86	70.59
	T	109.10[b]	82.53	78.21	85.64	62.87
12	R	47.36	69.63	72.78	71.11	68.62[b]
	A	109.18[b]	82.59	78.42	83.99[b]	68.47
	B	109.10[b]	82.59	78.42	83.95[b]	68.59[b]
	E	109.22	82.49	78.45	83.86	70.59
	T	109.18[b]	82.46	78.22	85.62	62.87
13	R	47.36	69.63	72.78	71.11	68.47
	A	109.19[b]	82.49	78.42	83.97[b]	68.78[b]
	B	109.18[b]	82.54	78.42	84.00[b]	68.59[b]
	I	109.18[b]	82.59	78.42	84.02[b]	68.62[b]
	E	109.22	82.54	78.47	83.86	70.59
	T	109.10	82.59	78.21	85.65	62.87
14	R	47.35	70.17	72.77	71.10	68.46
	A	109.18	82.49	78.42	83.99	69.62[b]
	B	109.18	82.49	78.42	83.99	68.57[b]
	I_1	109.18	82.49	78.42	83.99	68.57[b]
	I_2	109.18	82.49	78.42	83.99	68.57[b]
	E	109.21	82.53	78.46	83.85	70.58
	T	109.09	82.59	78.20	85.64	62.86
15	R	47.36	69.63	72.78	71.11	68.47
	A	109.20	82.50	78.43	84.01	68.62[b]
	B	109.20	82.50	78.43	84.01	68.58[b]
	I_1	109.20	82.50	78.43	84.01	68.58[b]
	I_2	109.20	82.50	78.43	84.01	68.58[b]
	I_3	109.20	82.50	78.43	84.01	68.58[b]
	E	109.22	82.54	78.47	83.99	70.59
	T	109.11	82.60	78.21	85.65	62.87

[a] ^{13}C Chemical shifts are quoted relative to the methyl carbon of 2-methyl-2 propanol (31.30 ppm) at 150 MHz at 30°C and their assignments are based on 1D ^{13}C, HSQC, and HMBC spectra
[b] Interchangeable, uncertain

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