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Application of ultra-high magnetic field for saccharide molecules: ^{1}H NMR spectra of 6-*O*-α-D-glucopyranosyl-cyclomaltoheptaose and -cyclomaltohexaose

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Abstract—^{1}H NMR spectra of G1-α-CD and G1-β-CD were recorded using a spectrometer equipped with a 21.6 T magnet. An ultra-high magnetic field was effective for detecting ^{1}H NMR signals with a small difference in chemical shifts. Introducing a glucosyl group onto CDs as a branch caused deformation of equilibrated ^{1}H signals of cyclodextrin. Particularly, ^{1}H signals in branched glucose were shifted greatly.

Keywords: ^{1}H NMR spectra; Ultra-high field; G1-α-CD; G1-β-CD; Branched cyclodextrins

1. Introduction

6-*O*-α-D-Glucopyranosylcyclomaltohexaose (G1-α-CD) is comprised of α-D-glucose as the sole sugar with an α-(1→4) *O*-glucosyl linkage except for one α-(1→6) link at the branch point (Fig. 1a). The ^{1}H NMR spectrum of α-CD is identical to that of α-D-glucose, but broadened to some degree.[1] Essentially no difference exists in the chemical shifts of the components, as if only a α-D-glucose exists in the α-CD. Introducing a glucosyl group onto α-CD as a branching unit causes disappearance of the equivalence of the six glucoses in the α-CD. The ^{1}H signals of the core CD deform, and differences in the chemical shifts of the core CD signals result.[1] Analyses of the ^{1}H NMR spectra recorded with an ECA800 spectrometer using an 800 MHz magnet for ^{1}H nuclei show that the ^{1}H signals of the core α-CD are divided to three sets of signals, not six. Signals of two glucoses among the six glucoses that comprise the core α-CD are partly assignable through that analysis. However, that method is not effective in analyzing the ^{1}H NMR spectrum of 6-*O*-α-D-glucopyranosylcyclomaltoheptaose (G1-β-CD, Fig. 1b) because the differences in chemical shifts of seven anomeric protons of the core CD are too small to discern. Consequently, it is necessary to use a higher magnetic field to resolve differences in the anomeric signals of the core CD.

Generally, the ^{1}H resonances of saccharide molecules, which are important molecules in membranes within living systems, cannot be completely assigned. Especially, assignment of ^{1}H signals is markedly difficult when the saccharide molecules are comprised of more than ten

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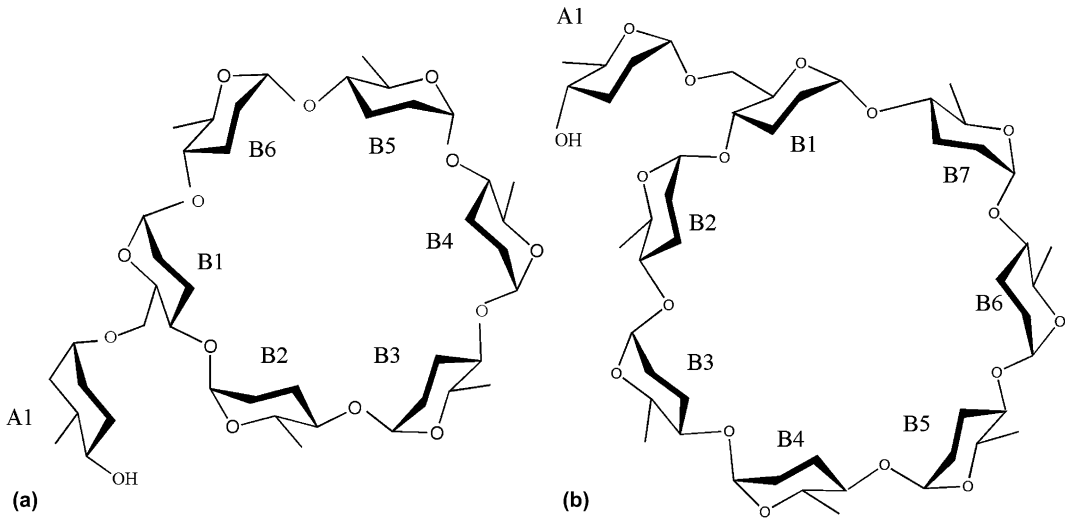

Figure 1. G1-α-CD (a) and G1-β-CD (b). Notation is used for assignment of ^{1}H signals.

pyranose units. Because saccharide molecules include multiple analogous protons in their chemical structure, dispersion in the chemical shift of these protons is less than that of the α-H protons and NH protons in peptide and protein molecules. In addition, because saccharide molecules do not typically include nitrogen atoms in their chemical structures, application of dispersion in the chemical shift of nitrogen is not effective for most saccharides. However, ^{13}C-enriched oligosaccharides are used for assignment of all ^{1}H and ^{13}C signals of the sample by executing appropriate 3D experiments.[2–8] However, without extensive effort at the synthesis of ^{13}C-enriched samples, it is difficult to analyze ^{1}H signals definitively. For that reason, the use of ultra-high magnetic fields is expected to effectively disperse the ^{1}H signals of saccharide molecules. Although herein we present the analysis of the ^{1}H NMR spectra of special ring sugars, the results of the analysis are considered to be applicable to common oligosaccharides, and to some degree, for example, for the polysaccharides, amylose, and cellulose. Numerous substituted CDs have been synthesized, but their characterization has been carried out with only their ^{13}C NMR spectra.[9–15] Few reports exist regarding their ^{1}H NMR spectra;[16] few have addressed the ^{1}H NMR of core CD signals.[1,17,18]

Herein, we present results that were obtained using a spectrometer equipped with a 21.6 T magnet (corresponding to 920 MHz for ^{1}H nuclei). Newly obtained data regarding G1-β-CD and G1-α-CD allow us to elucidate the effects of introducing a glucosyl group as a branch onto the core cyclodextrin.

2. Experimental

6-*O*-α-D-Glucopyranosylcyclomaltohexaose (G1-α-CD) and 6-*O*-α-D-glucopyranosylcyclomaltoheptaose (G1-β-CD) were supplied by Nikken Chemicals Co., Ltd., Omiya, Japan, and were used as samples with no purification. Commercially available α-CD and β-CD (Nacalai Tesque, Inc., Kyoto, Japan) were also used as samples without purification. An ECA920 spectrometer (920.31 MHz for ^{1}H nuclei; JEOL) was used to record NMR spectra at 298 K in D_2O. The 90° pulse width for 1D ^{1}H spectra was 5.5 μs. Gradient-enhanced TOCSY spectra were acquired using a 90° pulse length of 12.0 μs for ^{1}H and a mixing time of 100 ms with an H–H isotropic mixing pulse width of 45 μs. The sample concentration was 10 mM for G1-α-CD, α-CD and β–CD, and 3 mM for G1-β-CD. The HOD signal at 4.785 ppm was used as the reference. Chemical shifts are reported on the δ-scale. Respective digital resolutions of 1D and 2D spectra were 0.00015 and 0.00092 ppm. However, considering relaxation time, the actual spectral resolution was ca. 0.001 ppm. An ECA800 spectrometer (800.14 MHz for ^{1}H, JEOL) and a DMX750 spectrometer (750.13 MHz for ^{1}H, Bruker Analytik GmbH) and an ECP500 (499.23 MHz for ^{1}H, JEOL) were used to record spectra for comparison.

Glucose residues in the G1-α-CD and the G1-β-CD were defined as shown in Figure 1 prior to assignment of signals and discussions. Branched glucose (B1) has two neighbors (B2 and B6 or B2 and B7), but in this report, neighbor glucose indicates B2, which is bound to C-4 of B1.

3. Results and discussion

3.1. Ultra-high magnetic field resulted in well-dispersed NMR spectra

As reported previously, the ^{1}H NMR spectrum of G1-α-CD includes four parts: signals belonging to the

branching glucose (A1); signals belonging to the branched glucose (B1) of the core α-CD; a neighboring glucose (B2) to the branched glucose; and major signals belonging to the remaining glucoses (B3–B6) of the core α-CD. For example, 1A1 was characteristically observed at 4.958 ppm as an isolated signal. Figure 2a shows that 1B2 and 1B1 were separated from the major doublet (1B3–1B6) in the lower field side of the anomeric signal of the core CD in the spectrum observed with an 800 MHz spectrometer. Signal assignments of 1B1 and 1B2 were described in detail in a previous report.[1] In Figure 2b, which shows the spectrum of G1-α-CD observed with a 920 MHz spectrometer, 1B2 is isolated from the major anomeric signals of core CD at 5.082 ppm. The peak height of the signal was equal to 1A1 at 4.958 ppm. Further in the spectrum, 1B1 was also observed as a half doublet with equal height to 1A1. The major doublet at 5.066 ppm (1B3–1B6) with an 800 MHz spectrometer was split to two doublets with a 920 MHz spectrometer. The number of doublets observed as anomeric protons in the core CD with a 920 MHz spectrometer was four and larger than that observed with an 800 MHz spectrometer, as shown in Table 1. These observations became possible by raising the field strength of the magnet in the NMR spectrometer. Because the difference in the chemical shift of the higher field site of core anomeric protons was too small, the new split observed by a 920 MHz spectrometer is not useful to analyze 2D spectra at this stage. With respect to the spectral range from 3.4 to 4.1 ppm, a more dispersed spectrum was obtained with an ECA920 spectrometer compared to that obtained using an ECA800. For example, it is apparent that not only the anomeric protons of the core CD, but the H-2, H-3, and H-4 signals of the core CD are split into multiple signals as shown in Figure 2b.

Figure 3 shows a comparison of the ^{1}H NMR spectra of G1-β-CD using three different magnetic fields: 500 MHz (a), 750 MHz (b) and 920 MHz (c). Introducing a glucosyl group onto the CD ring as a branch causes a split of signals of the core CD in analogy with G1-α-CD. However, the chemical shift differences of this split appear small. For example, the doublet at 5.101 ppm appeared to barely split from the major doublet of the core CD in the spectral range of anomeric protons, even when observed using a 920 MHz spectrometer. With a lower magnetic field spectrometer, only a shoulder peak was seen in the spectra, as shown in Figure 3a and b.

The newly appeared doublet at 5.101 ppm was assigned to 1B2, as was the case with G1-α-CD and by a

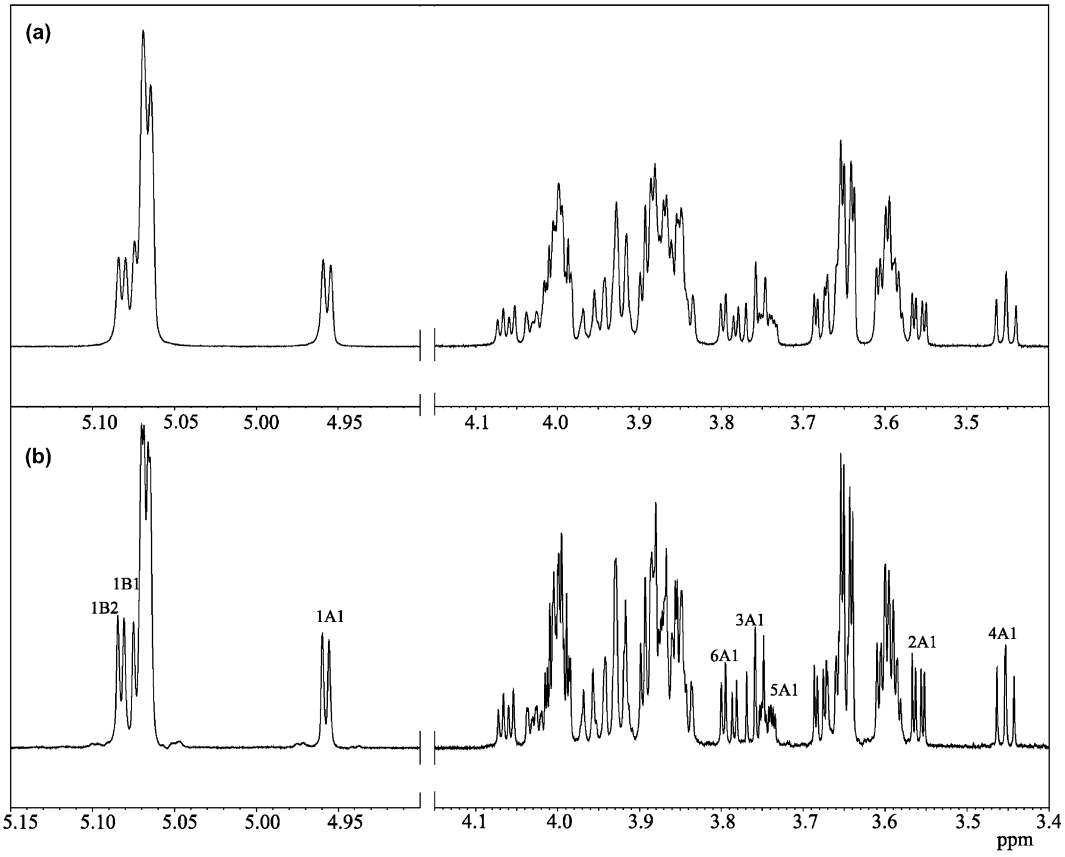

Figure 2. ^{1}H NMR spectrum of G1-α-CD with ECA800 (a) and with ECA920 (b) in D_2O at 298 K. Reduction scale of transverse is different in anomeric region.

Table 1. [1]H Chemical shifts of G1-α-CD[a]

	Weight (ppm)	1	2	3	4	5	6a	6b
δ(B1)	1	**5.073**	**3.679**	**4.005**	**3.660**	**4.029**	3.841	**4.063**
δ(B2)	1	**5.083**	3.647	3.999	3.598	3.871[b]	3.922	3.880[b]
δ(B3–B6)	2	**5.068**						
δ(B3–B6)	1		**3.648**[c]	**3.999**[c]	**3.590**[c]			
δ(B3–B6)	2	**5.067**						
δ(B2–B6)	1		**3.649**					
δ(B2–B6)	2		**3.647**					
δ(B1–B6)	1			**4.003**				
δ(B1–B6)	1			**3.996**				
δ(B1–B6)	1			**3.995**				
δ(B2–B6)	1				**3.600**			
δ(B2–B6)	1				**3.596**			
δ(B2–B6)	1				**3.595**			
δ(A1)	1	**4.958**	**3.559**	**3.759**	**3.453**	**3.744**	3.861	**3.791**

[a] Bold face data are from 1D spectra, and normal type denotes data from 2D experiments.
[b] Possibility of reshuffle remains.
[c] Chemical shift of H-1 was not assigned.

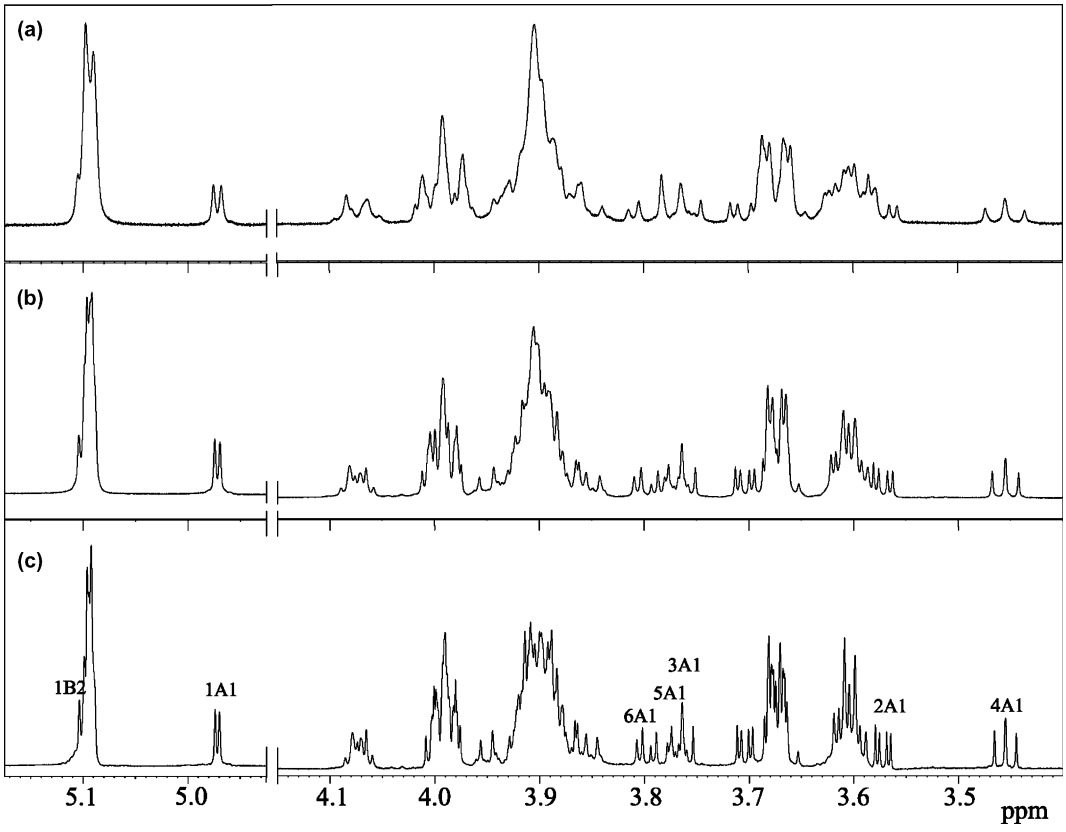

Figure 3. [1]H NMR spectrum of G1-β-CD with ECP500 (a), with DMX750 (b), and with ECA920 (c) in D_2O at 298 K.

2D TOCSY spectrum (not shown). The difference in the chemical shift of the anomeric signals of the core CD in G1-β-CD was smaller than that of G1-α-CD. This difference may result from different uniformities of the composing glucoses equilibrated on the NMR time scale. Great uniformity about *O*-glucosyl binding with tightness exists in the case of a non-branched α-CD. Introducing a branch to an α-CD engenders a relatively large difference in six *O*-glucosyl linkages in the α-CD and thereby causes a relatively large difference in chemical shift in the anomeric signals of the core CD. On the other hand, less uniformity exists for *O*-glucosyl binding in the case of a non-branched β-CD. β-CD has less rigid *O*-glucosyl linkages. For that reason, introducing a branch

creates relatively little difference in seven of its *O*-glucosyl linkages. Therefore, a branch causes only a small difference in the chemical shifts of the seven anomeric protons in the case of β-CD. Using 500 and 750 MHz spectrometers, the splitting of 1B2 from the anomeric signals of core CD was not yet complete (see Fig. 3a and b). Even using a 920 MHz spectrometer, a split of one doublet signal from core CD was not complete, but it was advanced to a satisfactory level for analysis. For that reason, we infer that a small difference in the chemical shift (>0.005 ppm) can be detected effectively using a spectrometer using an ultra-high magnetic field.

It is natural that signals obtained with a higher magnetic field appeared sharp because a split by coupling constant corresponds to a smaller chemical shift difference with a higher magnetic field.

3.2. Resonance assignments

The ^{1}H spectra of G1-α-CD and G1-CD were analyzed with their 1D spectra recorded using a 920 MHz spectrometer and processed with a Gaussian window function (spectra not shown.). These spectra showed distinct splits by three-bond coupling constants at each signal range except for the H-5 and H-6 signals. These H-5 and H-6 signals showed severe overlap. Accordingly, signal analysis of these three protons in G1-α-CD and G1-β-CD was only partly carried out. Tables 1 and 2 show the respective proton chemical shifts of G1-α-CD and G1-β-CD. With respect to B1 and B2 of G1-α-CD, assignment was completed with the possibility of a reshuffle of 5B2 and 6bB2, as described below. Signal assignment of B1 in G1-β-CD was completed. However, the assignment of B2 was not completed without some defects. Further assignment of B3–B6 or B3–B7 glucoses was not accomplished, even accounting for the results of 2D TOCSY spectra, as described below. Only these chemical shifts were listed in the tables without assignments. In the tables, the weight numbers in the column show the number of protons that have the same chemical shift.

3.3. TOCSY spectrum of G1-α-CD

The gradient enhancement TOCSY spectrum of G1-α-CD recorded with a 920 MHz spectrometer is shown in Figure 4. The mixing time of the spectrum was 100 ms. Signals of A1, B1, and B2 were found completely in this spectrum. Because signals of A1 were almost isolated, they were easily detected in correlation with each isolated signal, especially easily found in correlation with the characteristic 1A1 and 4A1 signals. B1 had two isolated signals, 6bB1 at 4.063 ppm and 2B1 at 3.679 ppm. Therefore, signals belonging to this glucose were assigned by correlation with these low-field shifted signals. Because the anomeric proton of B2 was isolated in the lowest region, all signals were found in correlation with this signal. Because the 5B2 and 6bB2 signals were temporally assigned, these signals presented the possibility of being reshuffled. Although the signals of B2 could not be fully detected using an 800 MHz spectrometer, all signals were picked up in the 2D TOCSY spectrum recorded with a 920 MHz spectrometer, probably because of increasing sensitivity. Each signal in the TOCSY spectrum showed a split caused by related coupling. These splits enhanced the signal assignments.

Table 2. ^{1}H Chemical shifts of G1-β-CD[a]

	Weight (ppm)	1	2	3	4	5	6a	6b
δ(B1)	1	**5.097**	**3.704**	**3.998**	**3.664**	**4.072**[b]	**3.850**	**4.078**[b]
δ(B2)	1	**5.101**	**3.678**	**3.993**	**3.599**	—	—	—
δ(B3–B7)	3	**5.094**						
δ(B3–B7)	1	**5.092**						
δ(B3–B7)	1	**5.091**						
δ(B3–B7)	1		**3.678**					
δ(B3–B7)	1		**3.674**					
δ(B3–B7)	2		**3.673**					
δ(B3–B7)	1		**3.671**					
δ(B3–B7)	1			**3.992**				
δ(B3–B7)	2			**3.991**				
δ(B3–B7)	1			**3.989**				
δ(B3–B7)	1			**3.986**				
δ(B3–B7)	2				**3.609**			
δ(B3–B7)	2				**3.604**			
δ(B3–B7)	1				**3.599**			
δ(A1)	1	**4.972**	**3.572**	**3.764**	**3.456**	**3.769**	3.872	**3.798**

[a] Boldfaced data are from 1D spectra, and normal type denotes data from 2D experiments.
[b] Estimated.

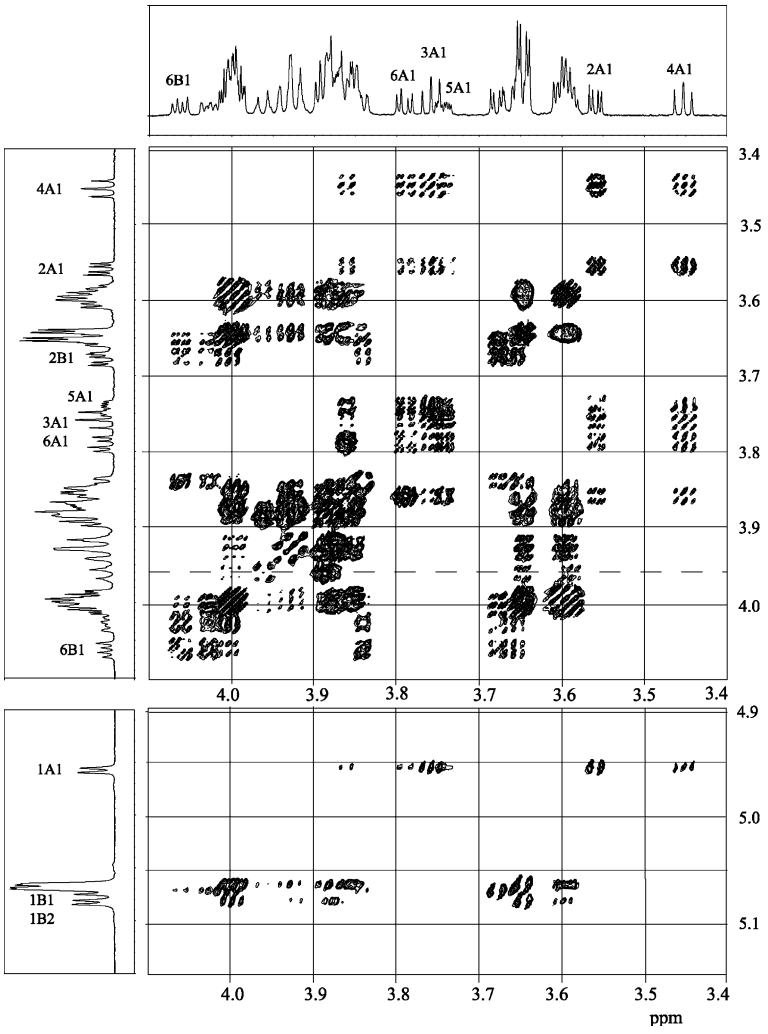

Figure 4. TOCSY spectrum of G1-α-CD in D_2O at 298 K. Mixing time was 100 ms.

3.4. Effect of introducing a branch onto CDs observed with chemical shift of the ^1H signals

Table 1 shows the chemical shift of the proton signals of G1-α-CD. In the table, the chemical shifts shown in bold typeface were obtained from 1D spectra. Chemical shifts written with normal typeface were obtained from the 2D TOCSY spectrum. The chemical shift was recorded to three decimal places, which maintained a match with the result from the 2D spectrum. The ^1H signals of B1 were assigned by analyzing the 1D spectrum processed with a Gaussian window function. The ^1H signals of B2 were detected in the TOCSY spectrum. It was difficult to assign 5B2 and 6bB2 without ambiguity because degenerate signals cancel out the sign of coupling. The possibility remains of reshuffling of these signals. Three signals belonging to the same glucose were found from the broken line at 3.963 ppm in Figure 4. These three signals were also detected in the 1D spectrum: for that reason, they are included in Table 1 as one-weight-signals without determination of the anomeric proton of this glucose. The glucose has rightly a proton signal at 3.963 ppm; however, it is not clear whether the signal at 3.963 ppm belongs to H-5 or H-6. That is the reason for no description about the signal at 3.963 ppm in Table 1. Because of severe overlap, it was difficult to determine 5B3–5B6, 6aB3–6aB6 and 6bB3–6bB6. For assignment of two 6B1 resonances, three-bond-coupling constants were taken into consideration as described below.

Table 2 shows chemical shifts of proton signals on G1-β-CD. Chemical shifts written in bold typeface were obtained from the 1D spectrum in the same manner as those in Table 1. For G1-β-CD, the difference in chemical shift between 1B1 and 1B2 was insufficient to obtain all signals belonging to B2 glucose in the TOCSY spectrum. Additionally, the sample concentration of G1-β-CD was one-third that of the G1-α-CD sample. Low sample concentration caused a poor 2D TOCSY spectrum, in spite of using an ultra-high magnetic field. Consequently, it was impossible to obtain the chemical shift of 5B2, 6aB2 and 6bB2 from the TOCSY spectrum. In

addition, around 4.07 ppm, two signals, 5B1 and 6bB1, were temporarily assigned in Table 2 because a reasonable coupling analysis was not completed. Chemical shifts of 5B3–5B7, 6aB3–6aB7 and 6bB3–6bB7 were not obtained because the signal overlap was heavy in this region, similar to that observed in the spectrum of G1-α-CD.

Although all signals were not determined for both G1-α-CD and G1-β-CD described above, the effect of introducing a branch to CDs is considered to appear mainly in the chemical shift of B1. This prospect is discussed below. Table 3 shows the effect of introducing a branch to α-CD and β-CD in estimated chemical shift difference. Reasonably strong signals were adopted to estimate the chemical shift of H-5 and H-6 signals for β-CD in this table because the signal analysis of the 1D spectrum of β-CD was not simple for H-5 and the two H-6 signals. The effect of introducing a branch onto CDs was observed not only with 6aB1 and 6bB1, but also with 5B1 in both cases. From Table 3, it can be seen that 6bB1 shifted to low field, 6aB1 shifted to high field in both cases. The magnitude of the low-field shift in 6bB1 of G1-α-CD was 0.185 ppm and that of G1-β-CD was 0.196 ppm. The magnitude of the high-field shift of 6aB1 was about half the magnitude of the low-field shift in the case of G1-α-CD. It was less than one-third of the low-field shift in the case of G1-β-CD. Furthermore, it is interesting that 5B1 shows a low-field shift of the same magnitude as the low-field shift of 6bB1. Generally, protons at the *O*-glucosyl binding site shift to low field. In this case, the branching site has two protons in both cases. It was exciting to discover that both of the 6B1 signals did not shift to low field at the same time and that the magnitude of the low-field shift of 5B1 was the same as that of 6bB1. It is expected that the most sensitive position in the pyranosyl ring to substitution effects is C-5. These low-field shifts in 6bB1 were reasonably expected to be of comparable order with the difference in H-4 protons in the branch moiety of Gn-α-CDs (n = 1–7) with and without α-(1→4)-*O*-glucosyl linkage. The greatest difference in the H-4 protons in the branch moiety of Gn-α-CDs (n = 1–7) with and without an α-(1→4)-*O*-glucosyl linkage was 0.247 ppm.[1]

The chemical shift of 2B2–5B2 in G1-α-CD was almost identical to the corresponding value for α-CD. Further estimated chemical shifts of the other signals except for the B1 signals, but not definitely assigned signals, coincided to the corresponding signals of α-CD. At the same time 1B3–1B7, 2B2–2B7, 3B2–3B7 and 4B2–4B7 show a smaller difference in chemical shift than that of the corresponding signals of β-CD. Consequently, the effects of introducing a branch to CDs appeared mainly in the chemical shift of B1. Additionally the low-field shift of 1B2 was distinctly larger than that for 1B1.

3.5. Effect of introducing a branch onto CDs observed with coupling constant

Table 4 shows three-bond coupling constants obtained through analysis of 1D spectra. Coupling constants of α-CD and β-CD were analyzed with 1D spectra recorded with an ECA800 spectrometer. Two H-6 signals in all glucoses of branched CDs each had coupling constants: one is small (ca. 2 Hz) and the other is medium (4–5 Hz). Table 4 shows estimation of J_{56a} as a small

Table 3. Effects of introducing a branch to CDs observed with ^{1}H chemical shift difference (ppm)[a]

	B1 of G1-α-CD	α-CD	Δδ	B1 of G1-β-CD	β-CD	Δδ
δ(1)	**5.073**	5.069	0.004	**5.097**	5.085	0.012
δ(2)	**3.679**	3.648	0.031	**3.704**	3.665	0.039
δ(3)	4.005	3.998	0.007	**3.998**	3.980	0.018
δ(4)	**3.660**	3.600	0.060	**3.664**	3.600	0.064
δ(5)	**4.029**	3.860	0.169	**4.072**[b]	3.883[b]	0.189
δ(6a)	3.841	3.927	−0.086	**3.850**	3.907[b]	−0.057
δ(6b)	**4.063**	3.878	0.185	**4.078**[b]	3.882[b]	0.196

[a] Boldfaced data are from 1D spectra, and normal type denotes data from 2D experiments.
[b] Estimated.

Table 4. Effect of introducing a branch to CDs observed with coupling constants (Hz)

	G1-α-CD B1	α-CD	G1-β-CD B1	β-CD	G1-α-CD A1	G1-β-CD A1
$J_{1,2}$	3.5	3.5	3.8	3.8	3.7	3.8
$J_{2,3}$	9.9	10.1	9.9	10.0	9.9	9.8
$J_{3,4}$	8.5	9.1	9.8	9.2	9.3	9.1
$J_{4,5}$	9.7	9.7	9.2	9.0	10.1	9.9
$J_{5,6a}$	1.6	1.7	—	—	2.2	2.0
$J_{5,6b}$	5.5	4.6	—	—	4.8	5.1
$J_{6a,6b}$	−11.3	−12.3	—	—	−12.5	−12.3

coupling constant (ca. 2 Hz). In Tables 1–3, the H-6a signals denoted H-6 signals with a small coupling constants (ca. 2 Hz).

Multiple signals of H-1, H-2, H-3, and H-4 were analyzed with the corresponding coupling constant from the 1D spectra of branched CDs. These signals had almost identical splits. Among these signals, the signals of B1 showed the difference in split from those of nonbranched CDs. Table 4 shows a comparison of coupling constants of B1 with those of α-CD and β-CD.

The effect of introducing a branch to α-CD was observed in $J_{3,4}$, $J_{5,6b}$, and $J_{6a,6b}$. Although the change in the coupling constant was smaller than 1 Hz, it was not negligible. Corresponding effects to β-CD appeared in the change of $J_{3,4}$. Changes in $J_{5,6b}$ and $J_{6a,6b}$ were not detected at this stage. These facts suggest that the pyranosyl ring of B1 changes slightly from an equilibrated that of nonbranched CDs at branching.

4. Conclusions

We effectively analyzed the ^{1}H signals with small chemical shift differences using an ultra-high magnetic field for ^{1}H NMR spectroscopy. Using a spectrometer equipped with a 21.6 T magnet, 1D and 2D spectra of G1-α-CD and G1-β-CD were recorded and analyzed. Upon introducing a glucosyl group as a branch at a 6-OH group, the signals of B1 showed shifts: particularly, the 5B1, 6aB1 and 6bB1 signals showed considerably large shifts. Resonances for 6bB1 shifted to low field, 6aB1 shifted to high field, and 5B1 shifted to low field in both cases. Some coupling constants of B1 showed slight differences in both cases.

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