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Note

NMR studies of (1 → 3)-β-D-glucooligosaccharide derivatives

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Sulfated alkyl glucooligosaccharide glycosides exhibit high anti-HIV activity (in vitro), despite their low molecular weights as compared to polysaccharide sulfates [1–4]. The selection of oligosaccharides possessing this type of anti-HIV activity is important in the design of biologically active agents.

Oligosaccharides vary in their activity duration in blood [5]. Glycosylation elevates the activity of a nonglycosylated compound. Sulfated alkyl oligosaccharide glycosides, especially from (1 → 3)-β-D-glucooligosaccharides, may be a new candidate as the agent.

We report here NMR spectroscopic assignments of (1 → 3)-β-D-glucopentaose peracetate (**1**) and its dodecyl glycoside (**2**) (Fig. 1), which are precursors of the desired agent. Only a few papers have described chemical shifts of such compounds [6–8].

1. Results

Instruments.—NMR spectra were recorded at ambient temperature with a JNM-GSX-400 spectrometer for ^{1}H at 400 MHz and for ^{13}C at 100 MHz, using $CDCl_3$ as solvent and Me_4Si as the internal standard. 2D COSY and HC-COSY spectra were acquired by use of a 90° pulse width of 14.5 μs duration, 2048 × 128 point data sets, zero-filled at 256 points in the t_1 dimension. Long range HC-COSY

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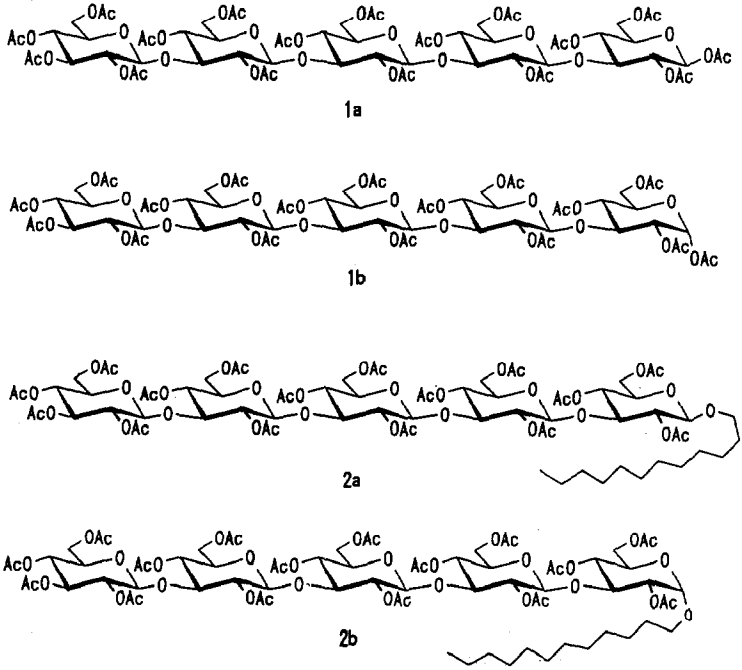

Fig. 1. Laminarapentaose peracetate (**1a, b**) and dodecyl laminarapentaoside peracetate (**2a, b**).

were recorded using 4096 × 128 point data sets, zero-filled to 256 points in the t_1 dimension.

Numbering of glucosyl residues.—Each glucosyl residue of **1** and **2** was numbered as I, II, III, IV, and V in sequence from the reducing-end glucosyl residue (Fig. 1). The α or β prefixes are appended to the numbering of glucosyl residues when the chemical shifts of the residue differ from each other.

Source of (1 → 3)-β-D-glucopentaose.—We have extensively investigated the production of (1 → 3)-β-D-glucopentaose (laminarapentaose) by chemical degradation of curdlan. However, even under optimal conditions, the yield of (1 → 3)-β-D-glucopentaose is low (4%) in the degradation of curdlan with a mixture of sulfuric acid, acetic acid, and acetic anhydride.

However, H. Nishihashi in this laboratory found that a (1 → 3)-β-glucanase obtained from a medium of *Streptomyces* sp. L-108 selectively degrades (1 → 3)-β-glucan to the desired pentaose [9], and laminarapentaose is readily produced from curdlan on a large scale (average yield > 40%).

Syntheses.—Laminarapentaose was acetylated [10] with sodium acetate–acetic anhydride to give the peracetate ($\beta:\alpha = 3:1$), which was subsequently glycosidated with dodecanol using stannic chloride in dichloromethane at room temperature to afford **2** in 58% yield after column chromatography ($\beta:\alpha = 3:1$); $[\alpha]_D^{31}$ −45.1° (*c*, 0.9, $CHCl_3$). The anomeric ratio of the mixture changed from a β-rich mixture to an α-rich one if the crude peracetate containing a little acetic anhydride

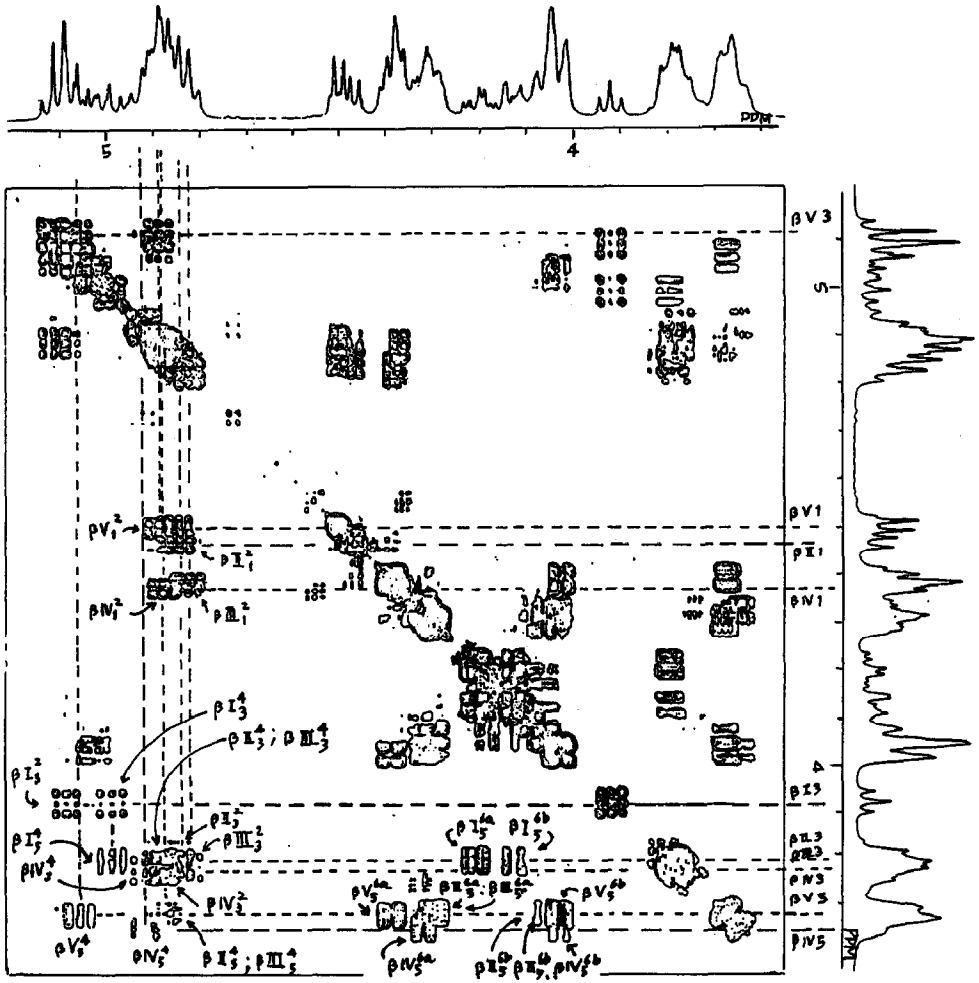

Fig. 2. Partial DQF-COSY spectrum of laminarapentaose peracetate [mixture of α (**1b**) and β anomer (**1a**)].

was used. The maximum ratio of anomers was about $\beta:\alpha = 9:1$; ($[\alpha]_D^{29}$ −49.6° (*c* 1.1, $CHCl_3$). Each anomer could be separated at the cost of the yield by column chromatography on silica gel [3:1 EtOAc–*n*-hexane; Kieselgel no 7734(E. Merck)].

NMR studies.—Laminarapentaose peracetate. (All of the ^1H and ^{13}C NMR data are shown in Tables 1–3). To assign all ^1H signals of the acetate, we analyzed DQF-COSY (Double Quantum Filter Correlation Spectroscopy) spectra of the α,β-anomeric mixture (Fig. 2) and the α anomer (Fig. 3), together with ^1H and ^{13}C NMR spectra. The HC-COSY (Hetero Nuclear-COSY) spectra of both anomers (Figs. 4 and 5) were also recorded to assign chemical shifts of all the protons and carbons. For determining the sequence of the glucosyl residues, analysis of long range HC-COSY spectra of the mixture proved useful (Fig. 6).

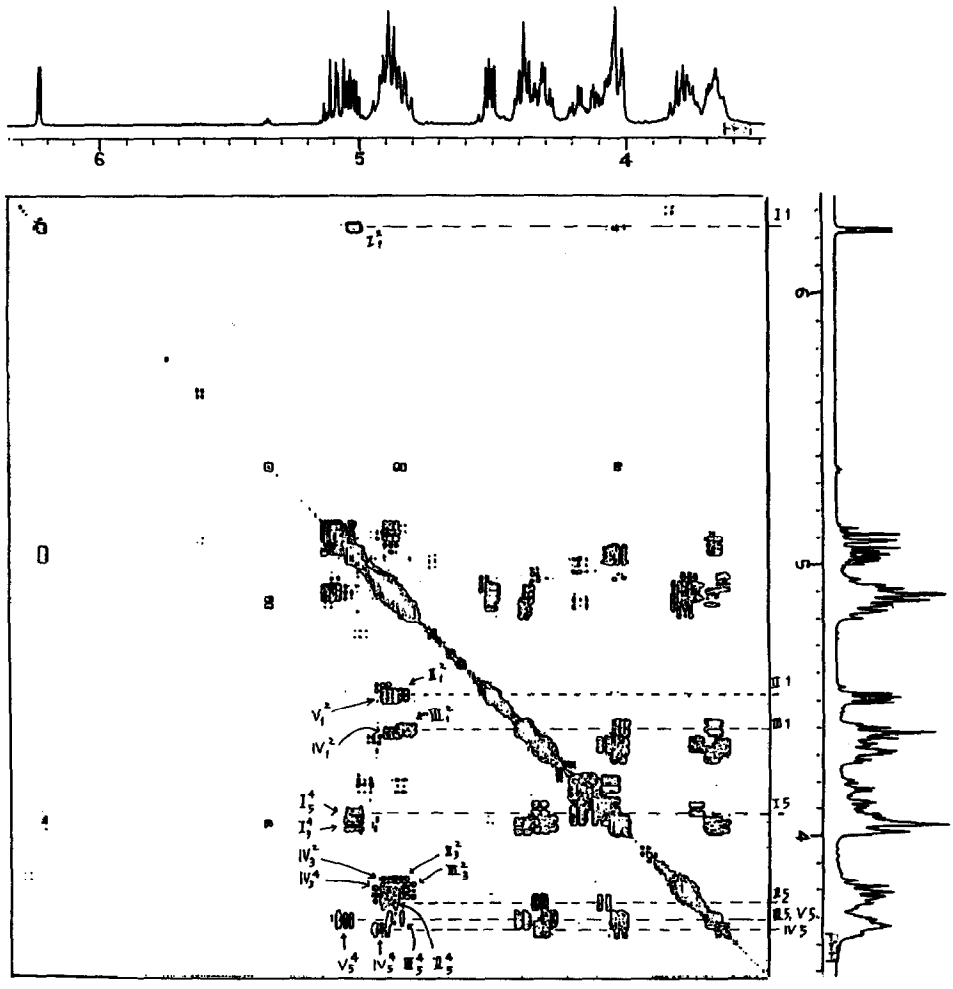

Fig. 3. Partial DQF-COSY spectrum of α-laminarapentaose peracetate (**1b**).

Chemical shifts of H-1–H-6 at glucosyl residue I of the β anomer was determined with DQF-COSY spectra (Fig. 2), starting from the anomeric proton (βI–H-1 = 5.62 ppm). In Fig. 2, the peak at 3.92 ppm was assigned to βI–H-3, which was the key signal to determine the sequence of the glucosyl residues (Fig. 5). The chemical shifts of βII–H-5 and βIII–H-5 were the same, judging from the connectivity of H-6 to H-5. All of the connectivities of ring protons are shown in Fig. 2 except the βI–H-1 signal.

The cross-peak connectivities of the α anomer (Fig. 3), starting from the H-1 peak at 4.50 ppm, were established with the DQF-COSY spectrum. A glucosyl residue having this chemical shift was identified as residue V, since the connectivity contained the cross peak of 5.14 ppm (H-3), assigned to the geminal proton of

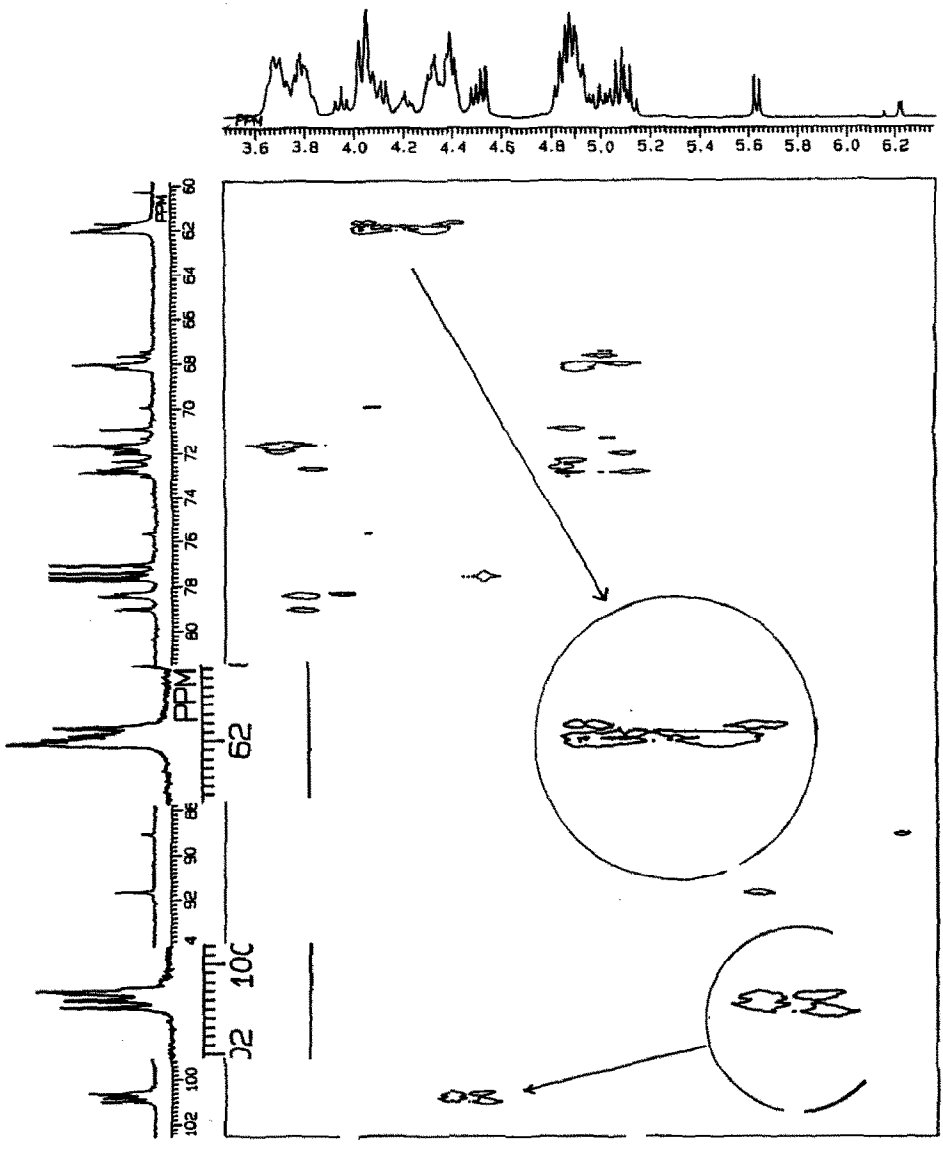

Fig. 4. Partial HC-COSY spectrum of α,β-laminarapentaose peracetate.

the C-3 acetoxy group of the nonreducing end. The chemical shifts of αII–H-5 and αI–H-3 were assigned at 3.75 and 4.03 ppm, respectively, and these were the main differences from the β anomer, except for the chemical shift of the reducing anomeric proton.

The long range HC-COSY spectrum of the β anomer (Fig. 6) permits establishment of the sequence of the other glucosyl residues, as the ^{13}C chemical shift at

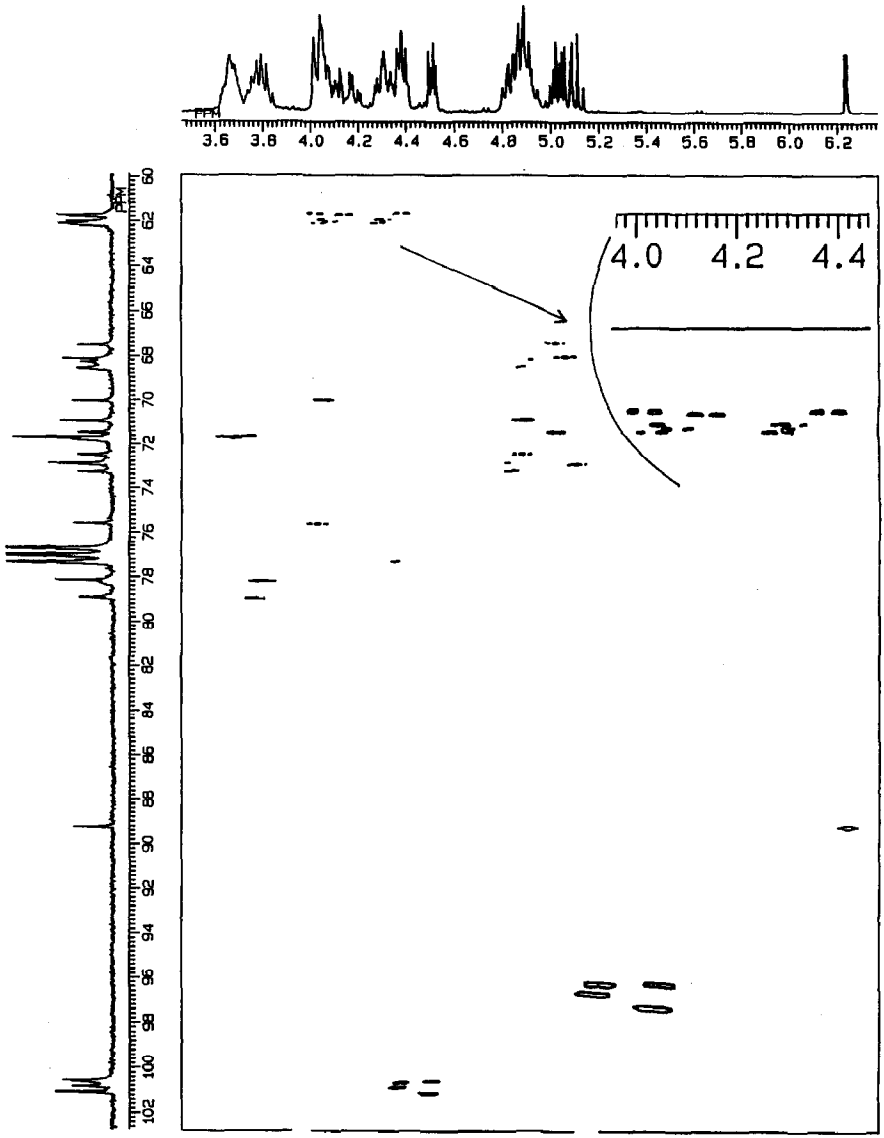

Fig. 5. Partial HC-COSY spectrum of α-laminarapentaose peracetate (**1b**).

100.6 ppm correlates with 3.92 ppm of βI–H-3. On the basis of the connectivity between the anomeric carbon atom and βI–H-3, mediated by an acetal bond (glycosyl linkage), it was concluded that this ^{13}C signal (100.6 ppm) belonged to βII–C-1. The HC-COSY spectrum of the β anomer showed clearly the correlation between βV–C-1 (101.1 ppm) and βV–H-1 (4.51 ppm). The βII–H-3 (3.80 ppm) was observed a little downfield from βIII–H-3 (3.79 ppm), and so that the signal of βIII–C-1 (100.6 ppm) was distinguished from that of βIV–C-1 (100.8 ppm).

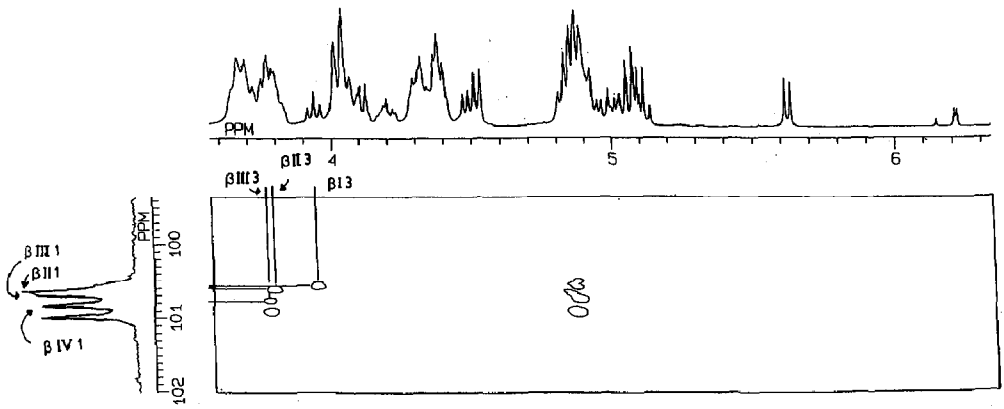

Fig. 6. Partial long-range HC-COSY spectrum of α,β-laminarapentaose peracetate.

The sequence of glucosyl residues of the α anomer was determined in the same manner as for the β anomer.

Having finished assigning all the chemical shifts of the protons, the HC-COSY spectra (Figs. 4, 5) were examined to assign the carbon resonances.

Dodecyl (1 → 3)-α,β-D-glucopentaoside peracetate. (All of the ^{1}H and ^{13}C NMR data are shown in Tables 4–7). Chemical shifts of these glucopentaoside peracetates were assigned mainly as performed in the preceding section. In the HC-COSY spectrum (Fig. 7), the ^{13}C peak of the anomeric proton of the reducing-end glucosyl residue of the α anomer (αI–C-1) clearly appeared at 95.5 ppm, which corresponded to the 4.96 ppm doublet (J 3.6 Hz). The triplet at 5.11 ppm in Fig. 8 obviously represented the signal of αV–H-3 (geminal proton of the C-3 acetoxy group of a glucosyl residue). These two points revealed all proton chemical shifts of the glucosyl residues I and V with the use of DQF-COSY spectrum (Fig. 8). As cross-peaks of H-4 → H-5 of the glucosyl residues αII, αIII, and αIV did not appear in the spectrum, the assignment of αII–H-5, αIII–H-5, and αIV–H-5 could not be made by using the H-4 → H-5 sequence. As the five pairs of the H-6 signal were detected in the DQF-COSY and HC-COSY spectra, only the chemical-shift values of all H-6 and H-5 protons were determined with the aid of these spectra.

In the HC-COSY spectrum of the β anomer (Fig. 9), all five C-1 peaks at 100.5–101.1 ppm indicated correlation with the proton signals at 4.35–4.51 ppm, with the same coupling constant (J 8.0 Hz). The assignment of βI–H-1 was determined by the long-range HC-COSY spectrum (Fig. 11), in which correlation between C–I-1 (100.7 ppm) and the oxymethylene proton peaks was readily detected. From the sequence of the reducing-end glucosyl residue (βI–H-1) in DQF-COSY (Fig. 10), the βI–H-3 signal appeared at 3.87 ppm, which corresponded to βII–C-1 in the long-range HC-COSY spectrum. After that, the βII–H-1 signal was detected in the HC-COSY spectrum (Fig. 9) at lowest magnetic field except for βV–H-1. A similar technique was used to determine the other

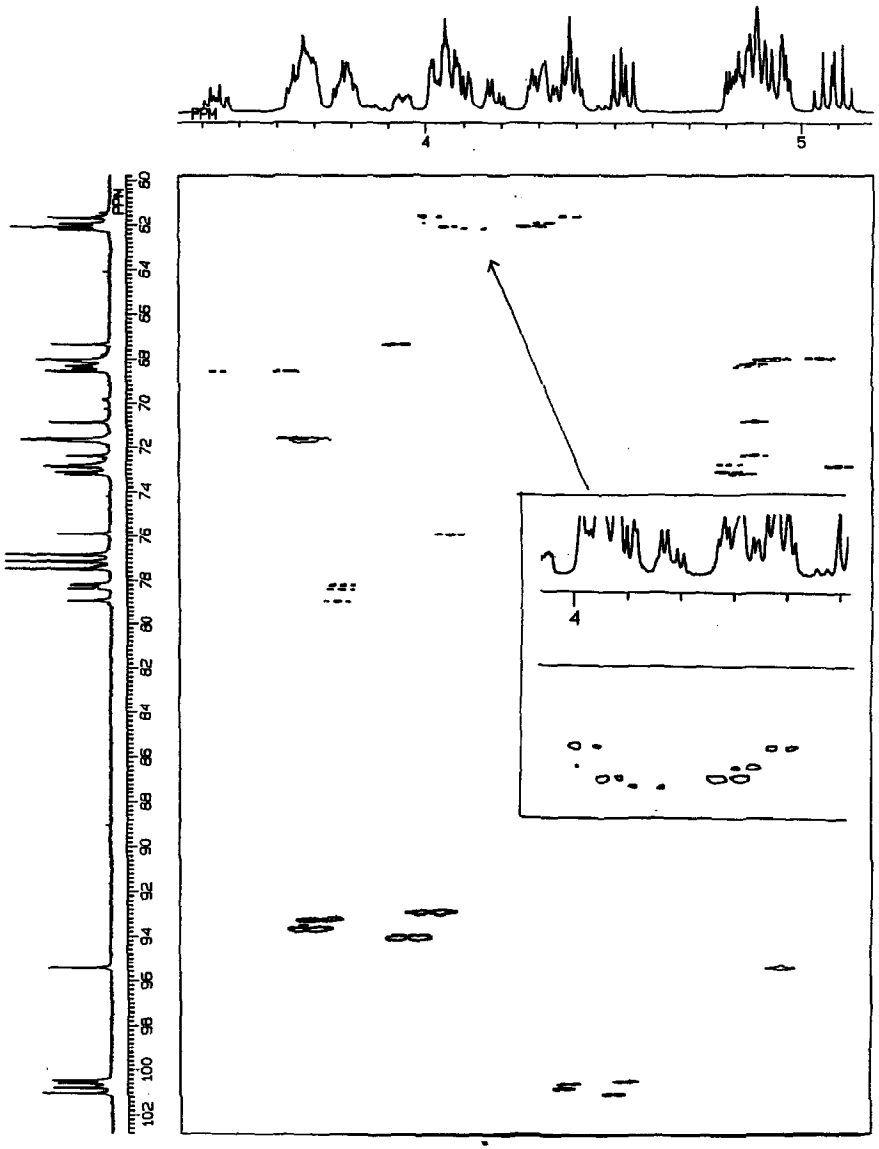

Fig. 7. Partial HC-COSY spectrum of dodecyl α-laminarapentaoside peracetate (**2b**).

signals. The HOHAHA (Homonuclear Hartmann–Hahn) spectrum (mixing time, 80 ms) is shown in Fig. 12, where all of the H-1–H-5 peaks of the β anomers are clearly assigned.

The ^{13}C chemical shifts of the α and β anomers were assigned from HC-COSY spectra.

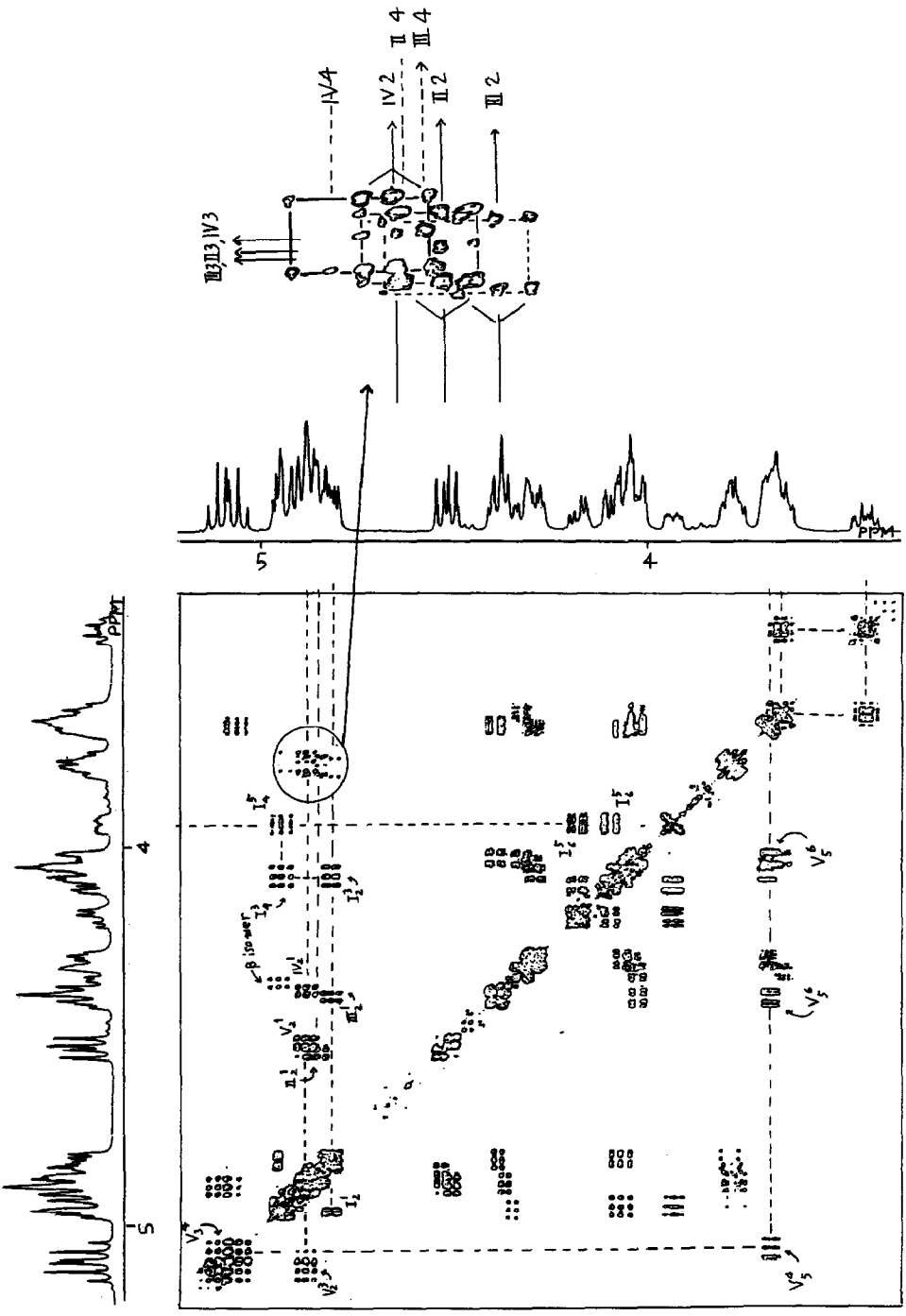

Fig. 8. Partial DQF-COSY spectrum of dodecyl α-laminarapentaoside peracetate (2b).

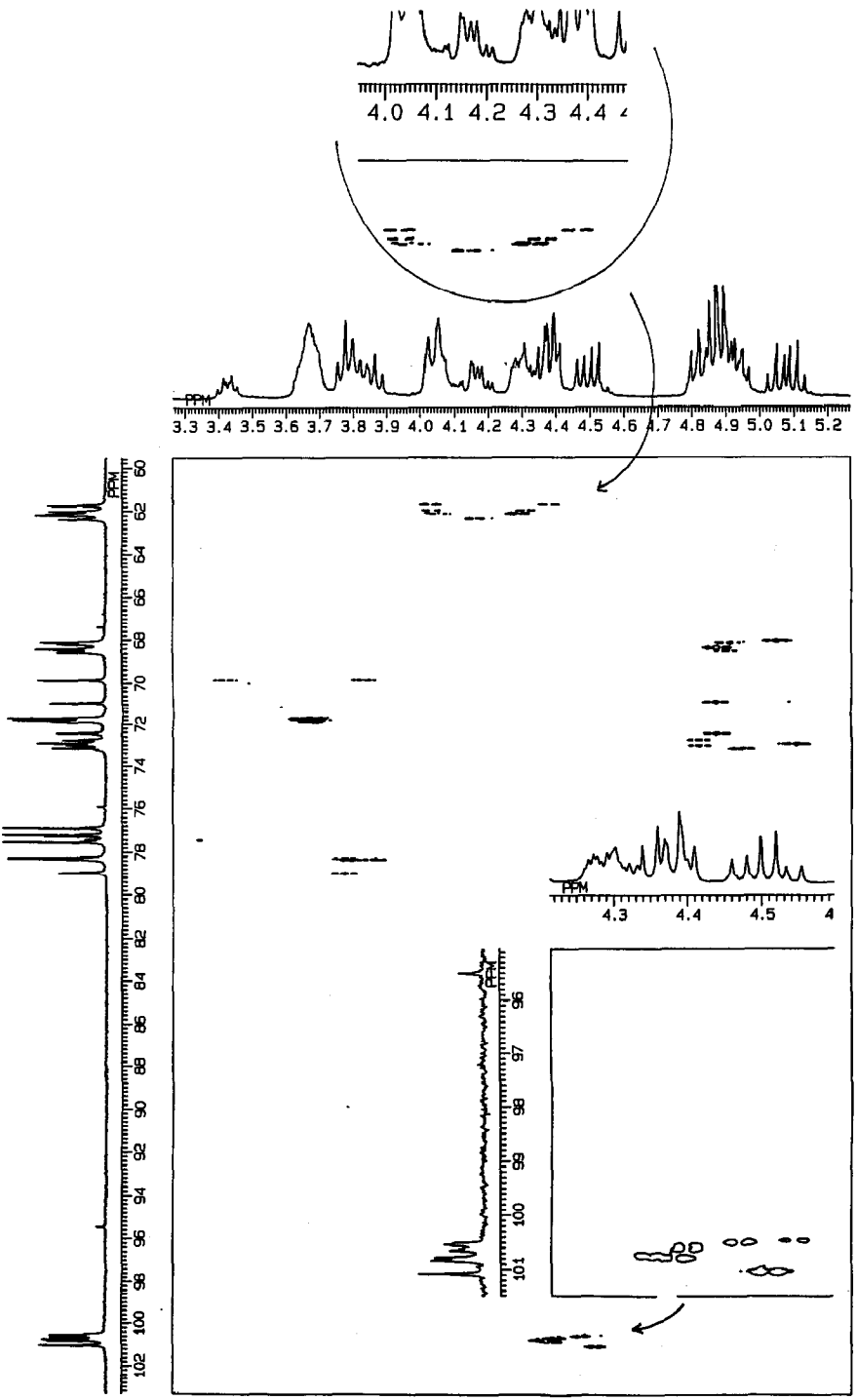

Fig. 9. Partial HC-COSY spectrum of dodecyl β-laminarapentaoside peracetate (**2a**).

2. Discussion

The main differences of the α and β anomers of the peracetates (**1a** and **1b**) were the chemical shifts of II–H-5, II–H-1, and I–H-3. In the ^{1}H NMR spectra of **1a** and **1b**, the chemical shifts of α and β I–H-3 are observed at 4.03 and 3.92 ppm (II to IV–H-3, 3.78–3.82 ppm), respectively, a fact that accounts for the 1,3-interaction of the acetoxy group and the H-3 proton of peracetylated (1 → 3)-β-glucooligosaccharide in constrast to the lack of such an interaction in (1 → 4)-β-glucooligosaccharides (I to IV–H-4, 3.73 ppm) [11]. This effect is observed in the ^{13}C NMR spectra of the α anomer, as well as in that of the dodecyl α-glycoside peracetate **2b**.

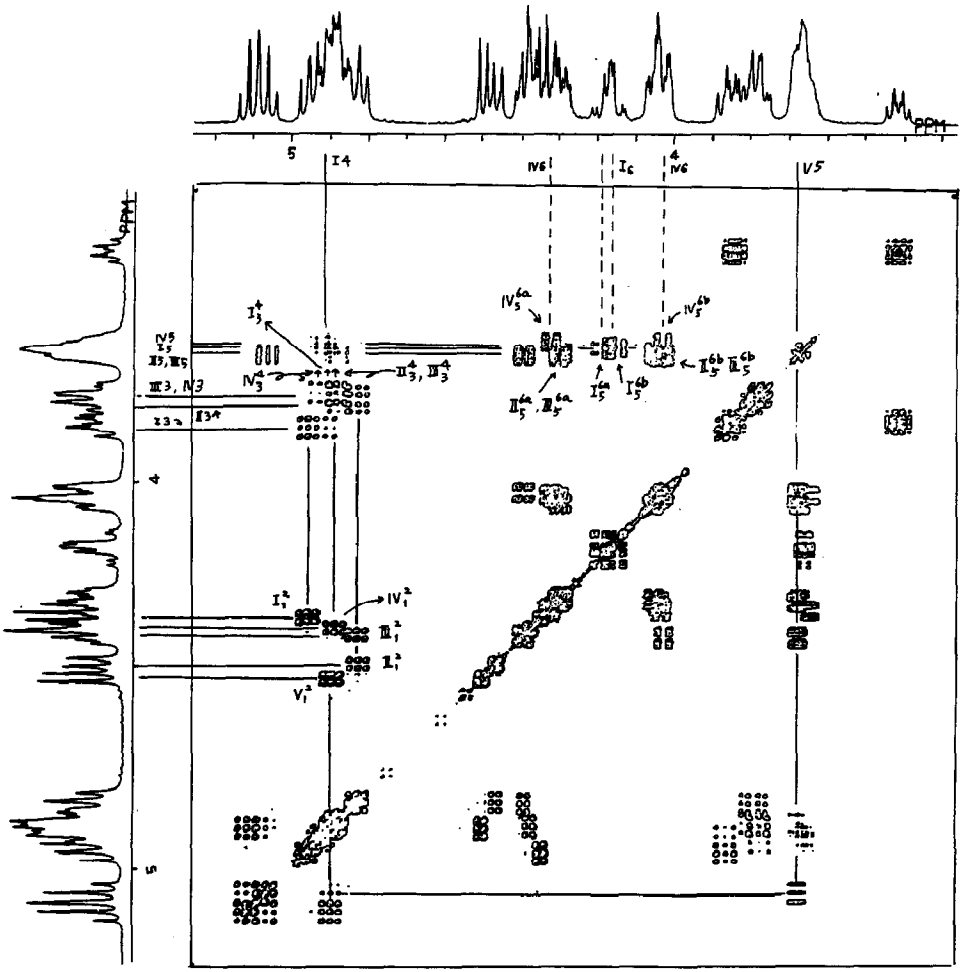

Fig. 10. Partial DQF-COSY spectrum of dodecyl β-laminarapentaoside peracetate (**2a**).

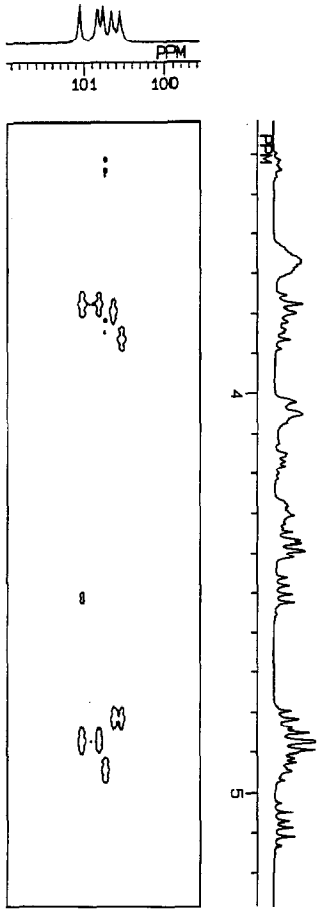

Fig. 11. Partial long range HC-COSY spectrum of dodecyl β-laminarapentaoside peracetate (**2a**).

It was expected that the dodecyl group at the anomeric position of the glucosyl residue I would affect the structure of these glucooligosaccharide peracetates. Configurational changes of the aglycon from α to β were expected to exert significant effects on chemical shifts of the glucosyl residue III. In fact, however, chemical shifts of the third glucosyl moiety do not change in the case of laminarapentaose peracetate. This result is also supported by computer-aided simulated annealing based on molecular dynamics, in which the dodecyl group is bent towards the glucosyl groups (Fig. 13, β anomer), as shown in Fig. 1 (**2a** and **2b**).

Acknowledgment

We are grateful to Eiichi Aoki at Dainippon Ink and Chemicals Inc., for computer simulation analysis.

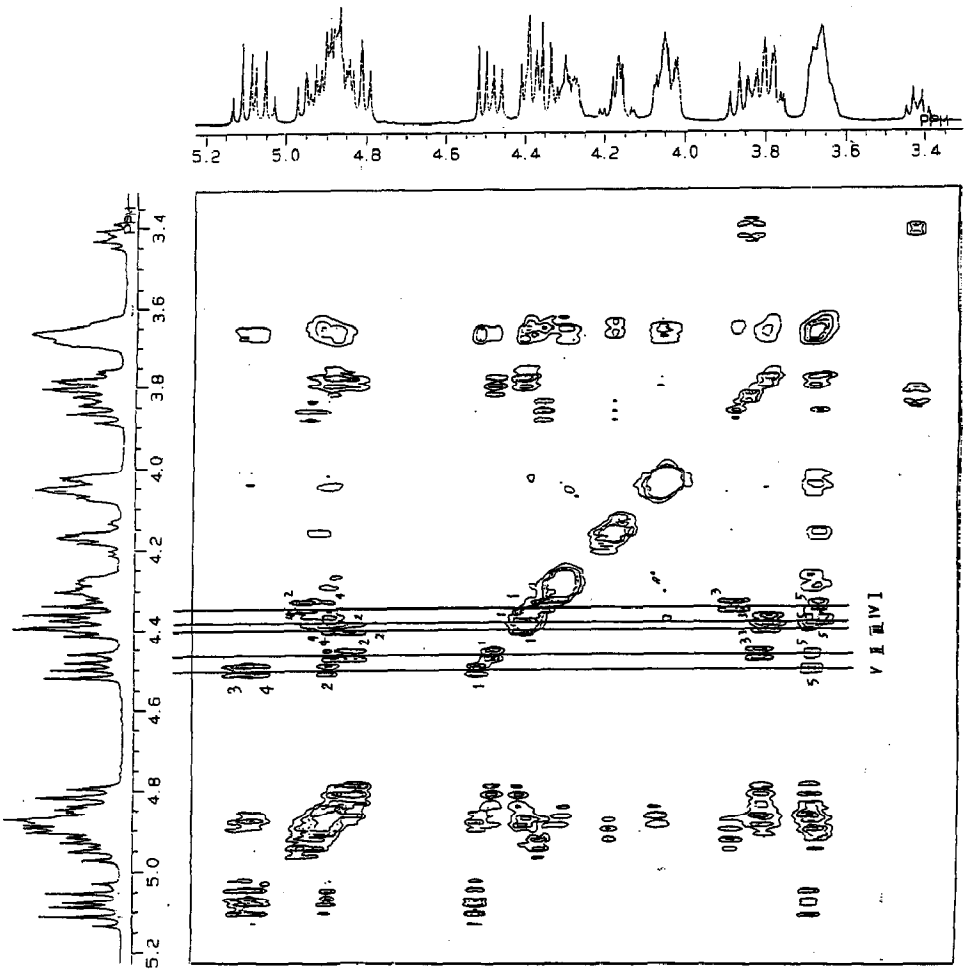

Fig. 12. Partial HOHAHA spectrum of dodecyl β-laminarapentaoside peracetate (**2a**).

Table 1
^{1}H chemical shifts (ppm) of α-laminarapentaose peracetate

Residue	H-1	H-2	H-3	H-4	H-5	H-6	
I	6.23	5.04	4.03	5.03	4.07	4.18	4.13
II	4.51	4.85	3.82	4.89	3.75	4.32	4.07
III	4.39	4.83	3.80	4.87	3.68	4.30	4.04
IV	4.37	4.88	3.78	4.92	3.65	4.31	4.04
V	4.50	4.89	5.14	5.06	3.68	4.38	4.03
	acetyl methyl 1.97–2.19						

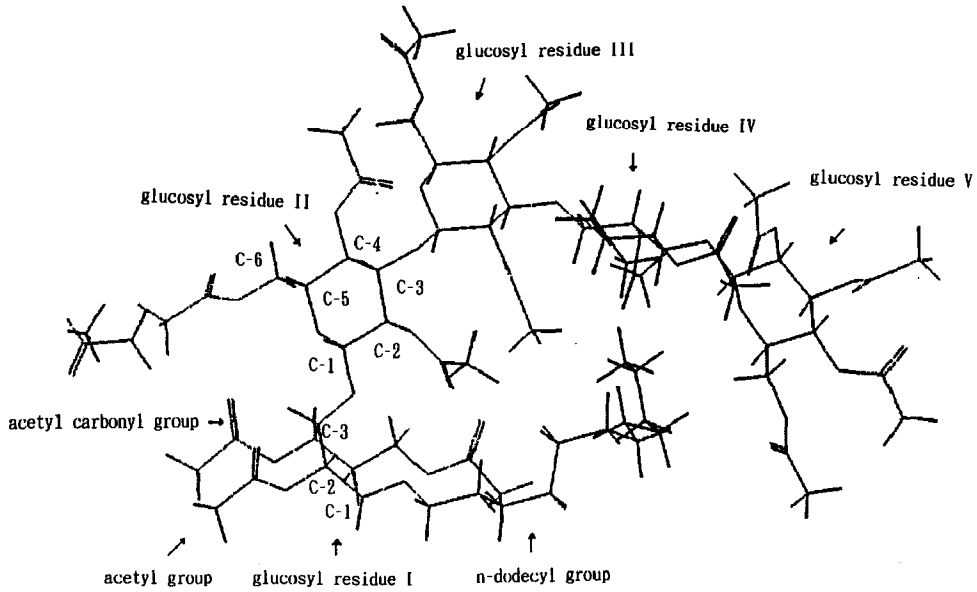

Fig. 13. Computer-graphic figure of dodecyl β-laminarapentaoside peracetate (**2a**), by computer-aided simulated annealing, based on molecular dynamics.

Table 2
^{1}H chemical shifts (ppm) of β-laminarapentaose peracetate

Residue	H-1	H-2	H-3	H-4	H-5	H-6	
I	5.62	5.09	3.92	4.99	3.81	4.20	4.14
II	4.46	4.85	3.80	4.87	3.68	4.30	4.07
III	4.39	4.83	3.78	4.87	3.68	4.30	4.04
IV	4.37	4.88	3.78	4.92	3.65	4.32	4.03
V	4.50	4.89	5.14	5.06	3.68	4.38	4.03
	acetyl methyl 1.97–2.19						

Table 3
^{13}C Chemical shifts (ppm) of laminarapentaose peracetate

Residue	C-1	C-2	C-3	C-4	C-5	C-6
αI [a]	88.2	71.5	75.6	67.5	70.0	62.2
βI [a]	91.8	72.2	78.2	67.7	72.9	61.9
αII [a]	100.6	73.3	78.2	68.5	71.7	62.1
βII [a]	100.6	76.1	78.2	68.5	71.7	62.1
III	100.6	72.9	78.2	68.4	71.7	62.1
IV	100.8	72.5	78.9	68.3	71.7	62.0
V	101.1	70.9	72.9	68.1	71.7	61.8
	acetyl methyl 170.6–168.8			acetyl methyl 20.9–20.4		

[a] Anomeric configuration of reducing-end glucosyl moiety.

Table 4
^{1}H Chemical shifts (ppm) of dodecyl α-laminarapentaoside peracetate

Residue	H-1	H-2	H-3	H-4	H-5	H-6	
I	4.96	4.81	4.07	4.94	3.94	4.18	4.11
II	4.54	4.86	3.79	4.88	3.69	4.29	4.07
III	4.40	4.81	3.79	4.87	3.69	4.29	4.05
IV	4.38	4.88	3.78	4.93	3.65	4.33	4.04
V	4.51	4.88	5.11	5.05	3.68	4.40	4.04
	dodecyl: α-methylene 3.43, 3.64			β-methylene 1.52			

Table 5
^{1}H Chemical shifts (ppm) of dodecyl β-laminarapentaoside peracetate

Residue	H-1	H-2	H-3	H-4	H-5	H-6	
I	4.35	4.95	3.87	4.90	3.66	4.16	4.18
II	4.47	4.81	3.80	4.87	3.67	4.07	4.28
III	4.40	4.81	3.79	4.87	3.69	4.05	4.29
IV	4.38	4.87	3.78	4.91	3.64	4.04	4.31
V	4.51	4.88	5.11	5.05	3.68	4.03	4.37
	dodecyl: α-methylene 3.42, 3.84			β-methylene 1.51			

Table 6
^{13}C Chemical shifts (ppm) of dodecyl α-laminarapentaoside peracetate

Residue	C-1	C-2	C-3	C-4	C-5	C-6
I	95.5	72.8	75.9	68.1	67.4	62.2
II	100.5	73.3	78.3	68.4	71.8	62.1
III	100.6	72.6	78.2	68.4	71.8	62.1
IV	100.8	72.4	79.0	68.1	71.7	62.0
V	101.1	70.9	72.9	68.1	71.7	62.0
acetyl carbonyl		168.5–170.7	acetyl methyl		20.0–20.3	
dodecyl: α-methylene 68.6,		internal methylene 20.4–31.9,		methyl 14.1		

Table 7
^{13}C Chemical shifts (ppm) of dodecyl β-laminarapentaoside peracetate

Residue	C-1	C-2	C-3	C-4	C-5	C-6
I	100.7	73.2	78.3	68.5	71.8	62.3
II	100.5	73.0	78.3	68.4	71.8	62.1
III	100.6	72.8	78.3	68.4	71.8	62.1
IV	100.8	72.4	79.0	68.1	71.7	61.7
V	101.1	70.9	72.9	68.1	71.7	61.7
acetyl carbonyl		168.5–170.7	acetyl methyl		20.0–20.3	
dodecyl: α-methylene 69.9,		internal methylene 20.4–31.9,		methyl 14.1		

References

[1] T. Aoki, Y. Kaneko, T. Nguyen, M.S. Stefanski, R.C.Y. Ting, and M.M. Manak, *AIDS Res. Human Retroviruses*, 8 (1992) 605–612
[2] M. Witvrouw, M. Baba, J. Balzarini, R. Pauwels, and E. DeClercq, *J. Acquir. Immun. Defic. Syndr.*, 3 (1990) 343–347
[3] N. Koizumi, H. Sakagami, A. Utsumi, S. Fujinaga, M. Takeda, K. Asano, I. Sugawara, S. Ichikawa, H. Kondo, S. Mori, K. Miyatake, Y. Nakano, H. Nakashima, T. Murakami, N. Miyano and N. Yamamoto, *Antiviral Res.*, 21 (1993) 1–14
[4] T. Uryu, N. Ikushima, K. Katsuraya, T. Shoji, N. Takahashi, T. Yoshida, K. Kanno, T. Murakami, H. Nakashima, and N. Yamamoto, *Biochem. Pharmacol.*, 43 (1992) 2385–2392
[5] T. Shoji, K. Katsuraya, N. Ikushima, N. Takahashi, T. Yoshida, H. Nakashima, T. Uryu, and N. Yamamoto, *XVIth Int. Carbohydr. Symp.*, Paris, 1992, B008.
[6] T. Yoshida, K. Hatanaka, T. Uryu, Y. Kaneko, E. Suzuki, H. Miyano, T. Mimura, O. Yoshida, and N. Yamamoto, *Macromolecules*, 23 (1990) 3717–3722
[7] K. Takeo, K. Maki, Y. Wada, and S. Kitamura, *Carbohydr. Res.*, 245 (1993) 81–96
[8] T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2425–2432
[9] H. Nishihashi, U.S. Pat. 4, 590, 160.
[10] M.L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1963) 211–215.
[11] C.M. Buchanan, J.A. Hyatt, S.S. Kelley, and J.L. Little, *Macromolecules*, 23 (1990) 3747–3755