# Sequential Nuclear Magnetic Resonance Assignment of β-1,2-Linked Mannooligosaccharides Isolated from the Phosphomannan of the Pathogenic Yeast *Candida albicans* NIH B-792 Strain

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ABSTRACT: The H-1 and H-2 signals of β-1,2-linked mannooligosaccharides isolated from the phosphomannan of *Candida albicans* NIH B-792 strain by mild acid hydrolysis were assigned by a sequential NMR assignment method that combines two-dimensional $^1$H–$^1$H correlated spectroscopy (COSY) and two-dimensional nuclear Overhauser enhancement and exchange spectroscopy (NOESY). The results indicated that the H-1 and H-2 of each β-1,2-linked mannopyranose unit show largely different signals compared with those of the α-linked ones and that the correlation between linkages and signals could not be explained by a conventional additivity rule. Furthermore, a regular proportional downfield shift of the H-1 signal was observed in the order of the mannose unit from the reducing terminal except those of the reducing and nonreducing terminal positions. Although the $^1$H NMR spectra of these oligosaccharides were complicated due to the presence of a large portion of the β-anomer from the reducing terminal mannose unit, reduction of the oligosaccharides with $NaBH_4$ to the corresponding alcohols gave simple and more readily interpretable $^1$H NMR spectra. Unexpectedly, however, a shift of H-1 signals by this reduction occurred not only on the second mannose unit but also on the third and fourth mannose units from the modified reducing terminal group of each oligosaccharide alcohol. This result indicates that the reducing terminal mannose unit is able to affect up to the fourth mannose unit from the reducing terminal. The presence of a long-distance interresidue NOE also suggests that the β-1,2-linked mannooligosaccharides have a compactly folded conformation in solution.

A previous paper from our laboratory reported the existence of β-1,2-linked oligomannosyl residues up to heptaose, Man*p*β1→2Man*p*β1→2Man*p*β1→2Man*p*β1→2Man*p*β1→2Man*p*β1→2Man, in the antigenic phosphomannans of an important pathogenic yeast, the *Candida albicans* species, as an acid-labile 1-O-phosphorylated form (Shibata et al., 1985). The β-1,2-linked mannooligosaccharide moieties were found to display stronger antigenicity than the α-linked ones in humoral antibody response in mammals (Tojo et al., 1988). Furthermore, the β-1,2-linked mannooligosaccharides were shown to display stronger inhibitory effects in the phosphomannan–anti-phosphomannan antibody precipitation reaction than did the corresponding α-1,2-linked isomers (Kobayashi et al., 1990).

For these reasons, determination of the three-dimensional structures of β-1,2-linked mannooligosaccharides is an important aspect of our immunochemical research on pathogenic fungi. Determination of solution conformations of Asn-linked oligosaccharides isolated from several glycoproteins has been achieved by NOE[1] (Brisson & Carver, 1983a,b; Homans et al., 1987a–c; Dabbrowski & Poppe, 1989), and the conformations were found to be in good agreement with that computed from molecular orbital calculations. Since an essentially complete set of unambiguous proton resonance assignments is required for conformational analysis by NOE, an effective assignment strategy is required (Homans et al., 1987b). As a part of the work in this direction, we have undertaken the prerequisite NMR sequential assignment of β-1,2-linked mannooligosaccharides and their alcohol derivatives. The sequential assignment of the $^1$H NMR spectra of proteins and nucleic acids has been proven as an established method (Rosen et al., 1991; Pan & Coleman, 1991; Banks et al., 1989). For carbohydrates, however, no example for sequential assignment of homooligosaccharides has been published, and in only a few instances has the assignment of heterooligosaccharides by adopting the same concept been achieved (Gasa et al., 1986; Poppe & Dabrowski, 1989).

The information of through-space interresidue connectivities between the H-1 of one carbohydrate and the H-2 or H-3 protons of an adjacent carbohydrate obtained from two-dimensional nuclear Overhauser enhancement and exchange spectroscopy (NOESY) made the sequential assignment possible (Hernandez et al., 1989). By combination of the two-dimensional $^1$H–$^1$H correlated spectroscopy (COSY) and NOESY, sequential assignment of β-1,2-linked mannooligosaccharides up to heptaose was carried out. Because the sequential assignment of H-1 NMR signals of oligosaccharides by this procedure is a nondestructive method, this will be an advantageous way to assign the chemical shifts of such novel oligosaccharides.

## MATERIALS AND METHODS

*Preparation of β-1,2-Linked Mannooligosaccharides.* β-1,2-linked mannooligosaccharides were prepared from the phosphomannan of *C. albicans* NIH B-792 strain by treatment

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[1] Abbreviations: NOESY, two-dimensional nuclear Overhauser enhancement and exchange spectroscopy; NOE, nuclear Overhauser effect; COSY, two-dimensional $^1$H–$^1$H correlated spectroscopy.

Table I: $^1$H Chemical Shifts of β-1,2-Linked Mannooligosaccharides and Their Corresponding Alcohols

| | sugar residue | δ (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G F E D C B A | G | F | E | D | C | B | A |
| | | | | | H-1 Signals | | | |
| Man$_2$ | Mβ1→2Mα | | | | | | 4.763 | 5.283 |
| | Mβ1→2Mβ | | | | | | 4.816 | 4.972 |
| Man$_2$-ol | Mβ1→2M-ol | | | | | | 4.841 | |
| | | | | | | | (+0.078)$^a$ | |
| Man$_3$ | Mβ1→2Mβ1→2Mα | | | | | 4.851 | 4.838 | 5.266 |
| | Mβ1→2Mβ1→2Mβ | | | | | 4.941 | 4.896 | 4.970 |
| Man$_3$-ol | Mβ1→2Mβ1→2M-ol | | | | | 4.881 | 4.916 | |
| | | | | | | (+0.030) | (+0.078) | |
| Man$_4$ | Mβ1→2Mβ1→2Mβ1→2Mα | | | | 4.915 | 4.923 | 4.827 | 5.271 |
| | Mβ1→2Mβ1→2Mβ1→2Mβ | | | | 4.915 | 5.025 | 4.866 | 4.976 |
| Man$_4$-ol | Mβ1→2Mβ1→2Mβ1→2M-ol | | | | 4.848 | 4.989 | 4.897 | |
| | | | | | (−0.067) | (+0.066) | (+0.070) | |
| Man$_5$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | | | 4.936 | 5.011 | 4.909 | 4.831 | 5.270 |
| | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ | | | 4.936 | 5.011 | 5.003 | 4.875 | 4.978 |
| Man$_5$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | | | 4.935 | 4.935 | 4.959 | 4.903 | |
| | | | | (−0.001) | (−0.076) | (+0.050) | (+0.072) | |
| Man$_6$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | | 4.941 | 5.032 | 4.991 | 4.915 | 4.831 | 5.273 |
| | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ | | 4.941 | 5.032 | 4.991 | 5.009 | 4.875 | 4.978 |
| Man$_6$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | | 4.941 | 5.033 | 4.920 | 4.966 | 4.904 | |
| | | | (0.000) | (+0.001) | (−0.071) | (+0.051) | (+0.073) | |
| Man$_7$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | 4.942 | 5.037 | 5.010 | 4.997 | 4.915 | 4.831 | 5.271 |
| | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ | 4.942 | 5.037 | 5.010 | 4.997 | 5.010 | 4.875 | 4.978 |
| Man$_7$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | 4.943 | 5.034 | 5.012 | 4.925 | 4.967 | 4.903 | |
| | | (+0.001) | (−0.003) | (+0.002) | (−0.072) | (+0.052) | (+0.072) | |
| | | | | | H-2 Signals | | | |
| Man$_2$ | Mβ1→2Mα | | | | | | 4.039 | 4.120 |
| Man$_2$-ol | Mβ1→2M-ol | | | | | | 4.050 | |
| Man$_3$ | Mβ1→2Mβ1→2Mα | | | | | 4.153 | 4.265 | 4.100 |
| Man$_3$-ol | Mβ1→2Mβ1→2M-ol | | | | | 4.102 | 4.294 | |
| Man$_4$ | Mβ1→2Mβ1→2Mβ1→2Mα | | | | 4.144 | 4.398 | 4.245 | 4.109 |
| Man$_4$-ol | Mβ1→2Mβ1→2Mβ1→2M-ol | | | | 4.151 | 4.272 | 4.290 | |
| Man$_5$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | | | 4.145 | 4.370 | 4.388 | 4.247 | 4.109 |
| Man$_5$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | | | 4.143 | 4.383 | 4.271 | 4.295 | |
| Man$_6$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | | 4.146 | 4.370 | 4.357 | 4.396 | 4.248 | 4.108 |
| Man$_6$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | | 4.149 | 4.364 | 4.372 | 4.273 | 4.296 | |
| Man$_7$ | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mα | 4.146 | 4.366 | 4.362 | 4.362 | 4.398 | 4.248 | 4.110 |
| Man$_7$-ol | Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2Mβ1→2M-ol | 4.148 | 4.373 | 4.351 | 4.381 | 4.274 | 4.296 | |

[a] Shift of the H-1 signal of the α-anomer by treatment with NaBH$_4$.

with 10 mM HCl at 100 °C for 60 min (Shibata et al., 1985, 1986; Kobayashi et al., 1990). Namely, 1.5 g of mannan was dissolved in 150 mL of 10 mM HCl, and the resultant solution was kept for 60 min at 100 °C. After neutralization with 100 mM NaOH, the oligosaccharides were separated by dialysis from the acid-modified mannan using cellulose tubing which is capable of retaining substances with a molecular weight greater than 12 000 as protein, against a total of 10 L of deionized water. The dialyzate was then evaporated in vacuo and deionized by passage through a column (2 × 10 cm) of Amberlite IR-120 (H$^+$) and IR-410 (OH$^-$) resins. The eluate was evaporated and applied on a column (2.5 × 100 cm) of Bio-Gel P-2 (−400 mesh) to give seven peaks corresponding to mannoheptaose through mannose. The six oligosaccharides, mannobiose to mannoheptaose, were purified by rechromatography with the same column and were abbreviated as Man$_2$, Man$_3$, Man$_4$, Man$_5$, Man$_6$, and Man$_7$, respectively.

*Reduction of Oligosaccharides.* Ten milligrams of oligosaccharide was dissolved in 5 mL of water, and then 5 mg of NaBH$_4$ was added. After the solution was allowed to stand for 18 h at room temperature, the excess NaBH$_4$ was destroyed by the addition of Amberlite IR-120 (H$^+$) resin. The resin was then removed by filtration, and the filtrate was evaporated in vacuo to dryness. Any remaining boric acid was removed by three repetitive evaporations with 10-mL aliquots of anhydrous methanol. Oligosaccharide alcohols derived from Man$_2$, Man$_3$, Man$_4$, Man$_5$, Man$_6$, and Man$_7$ were abbreviated as Man$_2$-ol, Man$_3$-ol, Man$_4$-ol, Man$_5$-ol, Man$_6$-ol, and Man$_7$-ol, respectively.

*Nuclear Magnetic Resonance Spectroscopy.* COSY was performed at 400 MHz at a probe temperature of 45 °C using a JEOL JNM-GSX 400 spectrometer. In total, 1024 $f_1$ increments of eight scans each were recorded with a variable sweep width and 256 real data points in $f_2$. The time domain data matrix was "zero-filled" once in each dimension to yield a final resolution of 1024 × 512 real data points. NOESY experiments were performed using conditions identical to those of the COSY experiments, although only 1024 $f_1$ increments were collected. Each mannooligosaccharide was dissolved in D$_2$O at a concentration of 10 mM, and acetone was used as the internal standard (2.217 ppm).

## RESULTS

*Sequential Assignment of H-1 Signals of β-1,2-Linked Mannooligosaccharides.* For this purpose, each oligosaccharide was analyzed by the combination of COSY and NOESY. The right side of the diagonal of each panel in Figure 1 shows COSY, while the left side is that of NOESY. Integration of the signals of the α- and β-anomeric protons at 5.27 and 4.89 ppm, respectively, from the reducing terminal

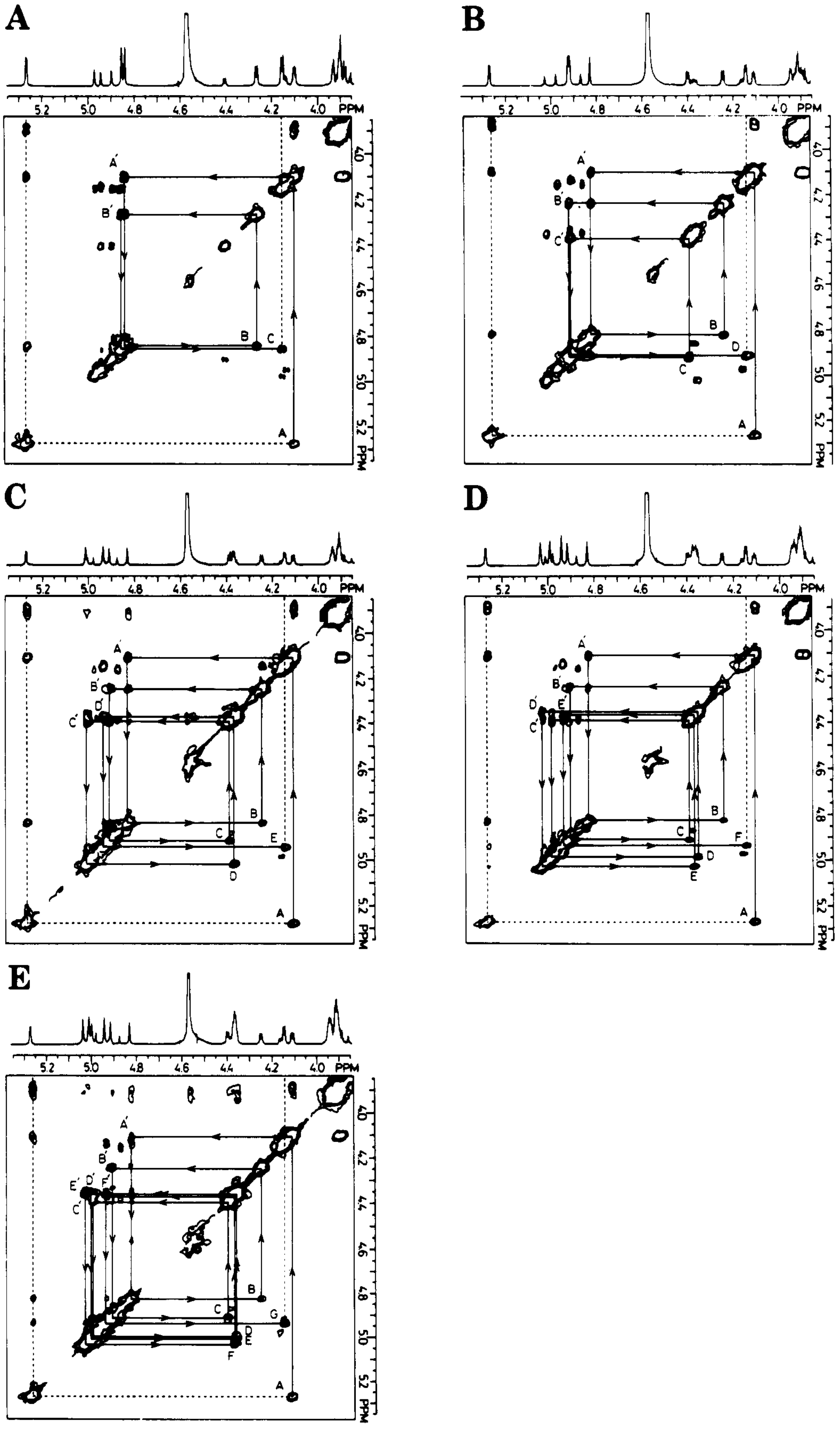

FIGURE 1: Sequential H-1–H-2′ connectivities of mannose units of the β-1,2-linked mannooligosaccharide α-anomer: (A) $Man_3$; (B) $Man_4$; (C) $Man_5$; (D) $Man_6$; (E) $Man_7$. The right side of the diagonal shows COSY, and the left side of the diagonal shows NOESY. Primed letters indicate interresidue H-1–H-2′ NOE cross-peaks and unprimed letters the H-1–H-2 correlated cross-peaks due to J-coupling; e.g., A indicates the H-1–H-2 correlated cross-peak of a reducing terminal mannose unit (Man-A) and A′ the interresidue NOE cross-peak between H-2 of Man-A and H-1 of an adjacent mannose unit (Man-B) as shown in the text. Arrows indicate the direction of the sequential connectivity from the reducing terminal unit to the nonreducing terminal unit.

mannose unit indicates that each oligosaccharide is approximately a 2.3–2.7/1 (w/w) mixture of α- and β-anomers concerning its reducing terminal group. Therefore, we at first tried to assign the chemical shifts of the α-anomer from each oligosaccharide, the major component of the anomer mixture throughout this oligosaccharide series, without any resolution process. The reducing terminal mannose unit, Man-A, shows a specific chemical shift around 5.27 ppm for the α-anomer. Furthermore, Man-A consists mainly of the α-anomer (ca. 70%, $J_{1,2}$ = 1.8 Hz) and ca. 30% of the β-anomer ($J_{1,2} < 1.0$ Hz). Therefore, the proton of the α-anomer of Man-A can readily be distinguished from the other β-D-mannopyranose units in the parent oligosaccharide, and the sequential assignment can start from the reducing terminal mannose unit, Man-A. Figure 1A shows the spectra of $\mathrm{Man}_3$. In this figure, cross-peaks A′ and B′ indicate interresidue H-1–H-2′ connectivities between two mannose units with H-1 signals at 5.266 ppm, Man-A, and 4.838 ppm, Man-B (A–A′–B), and between the latter unit and a mannose unit with an H-1 signal at 4.851 ppm, Man-C (B–B′–C) (where primed letters indicate interresidue H-1–H-2′ NOE cross-peaks while the unprimed ones indicate H-1–H-2 correlated cross-peaks). Since H-2 of the mannose unit with an H-1 signal at 4.851 ppm has no NOE cross-peak, it is apparent that this mannose unit corresponds to the nonreducing terminal group of this oligosaccharide. Therefore, it appears easy to assign the H-1 signals sequentially from H-1 of the Man-A, A–A′–B–B′–C. Results of the sequential assignment of $\mathrm{Man}_4$ to $\mathrm{Man}_7$ are also shown in panels B–E of Figure 1 and in Table I. Connectivities from Man-D to Man-F of $\mathrm{Man}_7$ were complicated by the overlapping of the H-2 chemical shifts of Man-D and Man-E (Figure 1E). This difficulty was resolved by adopting a sequential assignment procedure of H-1 and H-2 chemical shifts from the nonreducing terminal unit, Man-G, the H-2 of which does not show any interresidue NOE cross-peak.

The result indicates that the H-1 signal of Man-B is present on the highest upfield position in the H-1 region for each of the oligosaccharides. The H-1 signals of $\mathrm{Man}_7$ showed regular proportional chemical shifts in the order from the reducing terminal mannose unit except for those of reducing and nonreducing terminal mannose units. Namely, the H-1 chemical shifts of Man-B, Man-C, Man-D, Man-E, and Man-F were 4.831, 4.915, 4.997, 5.010, and 5.037 ppm, respectively. However, H-1 of the nonreducing terminal unit of $\mathrm{Man}_7$, Man-G, manifested the chemical shift at 4.942 ppm, in higher magnetic field than that of H-1 of Man-F, 5.037 ppm, and H-1 of the reducing terminal unit, Man-A, gave the chemical shift in the lowest downfield position, 5.271 ppm. The same correlation between the chemical shifts and the order of mannose units was observed on lower oligosaccharides than $\mathrm{Man}_7$, $\mathrm{Man}_6$ to $\mathrm{Man}_4$. The H-1 of Man-A has a strong interresidue connectivity with H-1 of Man-B for each oligosaccharide and showed a medium-strength interresidue connectivity with H-1 of the nonreducing terminal mannopyranose units of $\mathrm{Man}_6$ and $\mathrm{Man}_7$. A cross-peak in the upper left of each panel indicates that the H-1 of Man-A also has a strong intraresidue connectivity with H-3 at around 3.9 ppm from the same mannopyranose unit.

Each α-1,2-linked mannooligosaccharide, from biose to tetraose, obtained by the acetolysis of the acid-modified phosphomannan of the *C. albicans* NIH B-792 strain, contains ca. 12% β-anomer on its reducing terminal unit. It is presumed that the β-anomeric conformation can affect the H-1 chemical shift of the adjacent mannopyranose unit but not that of the third mannopyranose unit from the reducing terminal unit. As shown in Table I, however, these β-1,2-linked mannooligosaccharides contain ca. 30% β-anomer at each corresponding reducing terminal unit, and the conformation affects the H-1 chemical shift of not only the second mannopyranose unit but also the third one. This finding suggests that the reducing terminal unit affects the chemical shifts of the other mannopyranose units due to its spatial proximity.

Panels A and B of Figure 2 show the sequential assignment of the chemical shifts of β-anomers concerning its reducing terminal of the anomer mixtures of $\mathrm{Man}_3$

$$\underset{\mathrm{C}}{\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{B}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{A}}{2\mathrm{Man}\alpha}$$

and

$$\underset{\mathrm{c}}{\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{b}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{a}}{2\mathrm{Man}\beta}$$

and $\mathrm{Man}_4$

$$\underset{\mathrm{D}}{\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{C}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{B}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{A}}{2\mathrm{Man}\alpha}$$

and

$$\underset{\mathrm{d}}{\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{c}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{b}}{2\mathrm{Man}p\beta 1}\rightarrow \underset{\mathrm{a}}{2\mathrm{Man}\beta}$$

by NOESY, respectively. The H-1 chemical shift of the reducing terminal β-anomer mannose unit of the β-1,2-linked mannooligosaccharides appears at around 4.97 ppm (Zhang & Ballou, 1981); i.e., this is the only signal that we can empirically assign. Therefore, we started a sequential assignment study of the β-anomer of each oligosaccharide from the reducing terminal mannose unit using a mixture of α- and β-anomers. The boxed cross-peaks in Figure 2 indicate intraresidue H-1–H-2 connectivities, which were confirmed by COSY. As shown in Figure 2B, either of the fourth mannopyranose units, Man-D, of the α- and the β-anomers gave the same cross-peak. Mannopyranose units, which exist on the nonreducing terminal side from Man-D of $\mathrm{Man}_5$ to $\mathrm{Man}_7$, also gave the same chemical shifts as those of the corresponding α-anomers (Table I). Consequently, this sequential assignment method also defined the H-1 signals of the β-anomers of the β-1,2-linked mannooligosaccharides, a minor component in the mixture of α- and β-anomers. Furthermore, the result indicates that this sequential assignment method is applicable not only to an anomer mixture of homologous oligosaccharide series but also to a mixture of oligosaccharides with different linkages and component sugars.

*Reduction of β-1,2-Linked Oligosaccharides.* To simplify the $^1$H NMR signals by elimination of the α- and β-anomers of the reducing terminal unit of each β-1,2-linked mannooligosaccharide, reduction with $\mathrm{NaBH}_4$ was conducted. As expected, simplification of the H-1 signals of these oligosaccharides dramatically took place due to the reduction as shown in Figure 3. Each oligosaccharide gave only the corresponding number of signals as glycosidic linkages present. Surprisingly, however, a shift in the H-1 signal of each β-1,2-linked mannooligosaccharide after reduction was observed not only for one signal but also for at least three others. Therefore, it is difficult to assign the H-1 signals of these oligosaccharide alcohols on the basis of the assignment data of the parent oligosaccharides.

*Sequential Assignment of H-1 Signals of β-1,2-Linked Mannooligosaccharide Alcohols.* Figure 4A shows the combined spectra of COSY and NOESY of $\mathrm{Man}_3$-ol. Namely, cross-peaks A′ and B′ indicate interresidue H-1–H-2′ connectivities between the mannitol unit with the H-2 signal at 3.925 ppm, Man-A-ol, and the mannose unit with the H-1

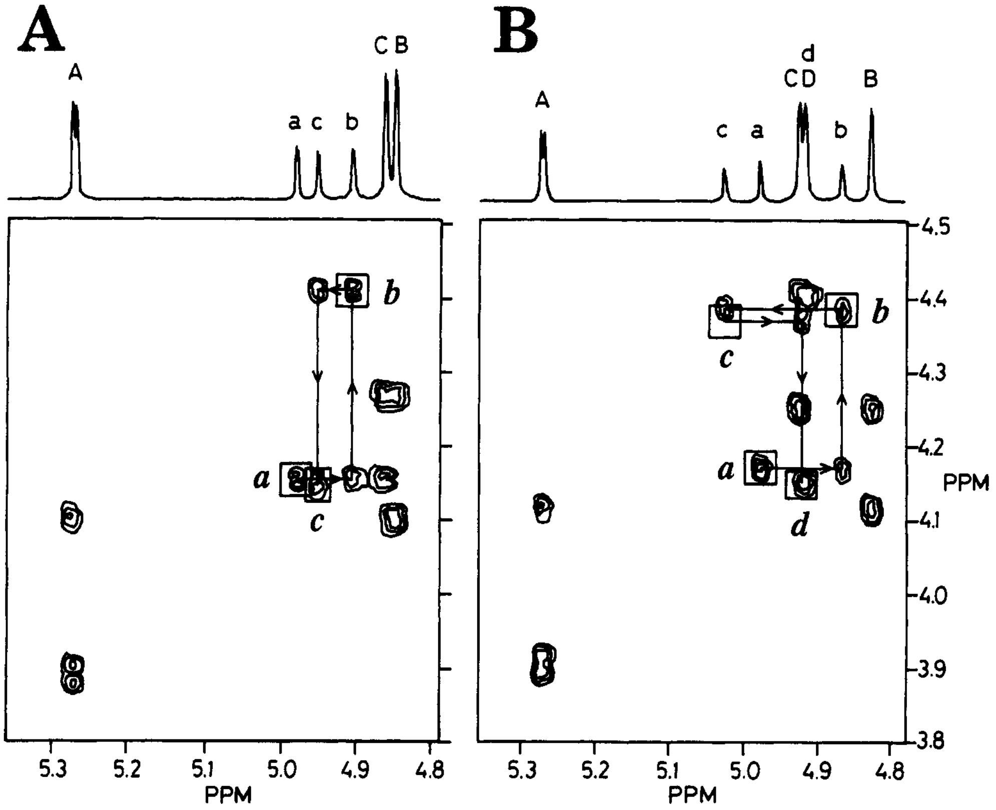

FIGURE 2: Partial NOESY spectra of $\beta$-1,2-linked mannooligosaccharides. Sequential H-1–H-2′ connectivity data of $\beta$-anomers of (A) $Man_3$ and (B) $Man_4$ are shown. Intraresidue H-1–H-2 cross-peaks are boxed. Arrows indicate the direction of the sequential connectivity from the reducing terminal unit to the nonreducing terminal unit. The capital letters and the lower-case letters in the spectra refer to the $\alpha$- and $\beta$-anomers concerning the reducing terminal unit of each mannooligosaccharide, respectively, as shown in the text.

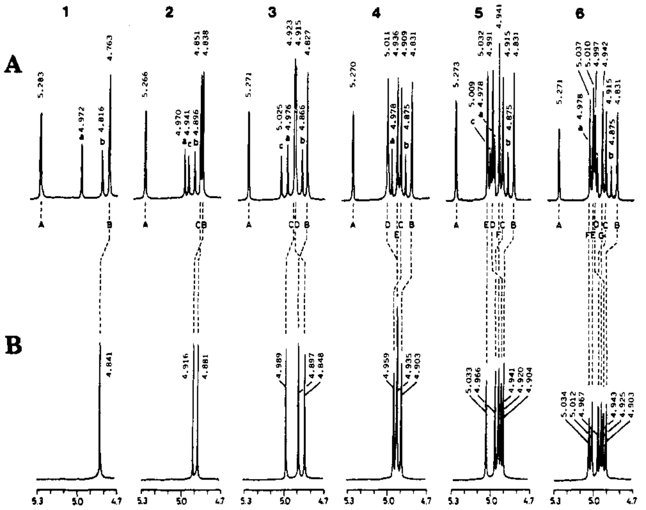

FIGURE 3: $^1$H NMR spectra of $\beta$-1,2-linked mannooligosaccharides (A) and their corresponding alcohols (B): (1) $Man_2$; (2) $Man_3$; (3) $Man_4$; (4) $Man_5$; (5) $Man_6$; (6) $Man_7$. The capital letters from A to G on dotted lines between the H-1 signals of panels A and B and the lower-case letters at the top of each signal in panel A refer to the $\alpha$- and $\beta$-anomers concerning the reducing terminal unit of each mannooligosaccharide, respectively.

signal at 4.916 ppm, Man-B (A′–B), and between the latter and a mannose unit with the H-1 signal at 4.881 ppm, Man-C (B–B′–C) (where primed letters indicate interresidue H-1–H-2′ NOE cross-peaks while the unprimed ones are H-1–H-2 correlated cross-peaks). Since H-2 of the mannose unit with an H-1 signal at 4.881 ppm has no NOE cross-peak, it is apparent that this mannose unit corresponds to the nonreducing terminal group of the parent oligosaccharide. Therefore, the above findings indicate that this procedure can be adopted as a facile method for assigning the H-1 signals from the H-2 of Man-A-ol, A′–B–B′–C, sequentially; i.e., the H-1 signals of Man-B and Man-C are 4.916 and 4.881 ppm, respectively. The results of the sequential analysis on $Man_4$-ol to $Man_7$-ol are shown in panels B–E of Figure 4 and in Table I. Although $Man_5$-ol has overlapped chemical shifts at 4.935 ppm (Figure 4C), its COSY cross-peaks were unambiguously distinguished by the sequential assignment from the nonreducing terminal unit, Man-E.

As shown in Figure 3, a significant downfield shift of the H-1 signals of Man-B ($\Delta\delta$ = 0.07–0.08 ppm) and Man-C ($\Delta\delta$ = 0.03–0.07 ppm) and the upfield shift of that of Man-D ($\Delta\delta$ = 0.07–0.08 ppm) of all oligosaccharides were observed upon reduction to the corresponding alcohols. In the case of $\alpha$-1,2-linked mannooligosaccharides, a shift in the H-1 signals by the reduction occurs mainly on Man-B (upfield shift, $\Delta\delta$ = 0.06–0.08 ppm) with a small downfield shift on Man-C ($\Delta\delta$ = 0.01–0.03 ppm) (Shibata et al., 1991). It is, therefore, reasonable to state that the case of the downfield shift of the H-1 signal of Man-D of the $\beta$-1,2-linked mannooligosaccharides is due to the spatial proximity of Man-A.

## DISCUSSION

There is an additivity rule for the H-1 and H-2 chemical shifts of $^1$H NMR for carbohydrate moieties of glycoconjugates (Vliegenthart et al., 1983). In the case of high-mannose-type oligosaccharides, the H-1 and H-2 chemical shifts of a mannopyranose unit were shown to be affected by the difference in the linkages of the two adjacent mannopyranose units of the reducing and nonreducing terminal sides. However, the results of this study demonstrate the difficulty in adopting such an additivity rule for interpretation of $^1$H NMR spectra of $\beta$-1,2-linked mannooligosaccharides and their corresponding alcohols. An increase in one $\beta$-1,2-linked

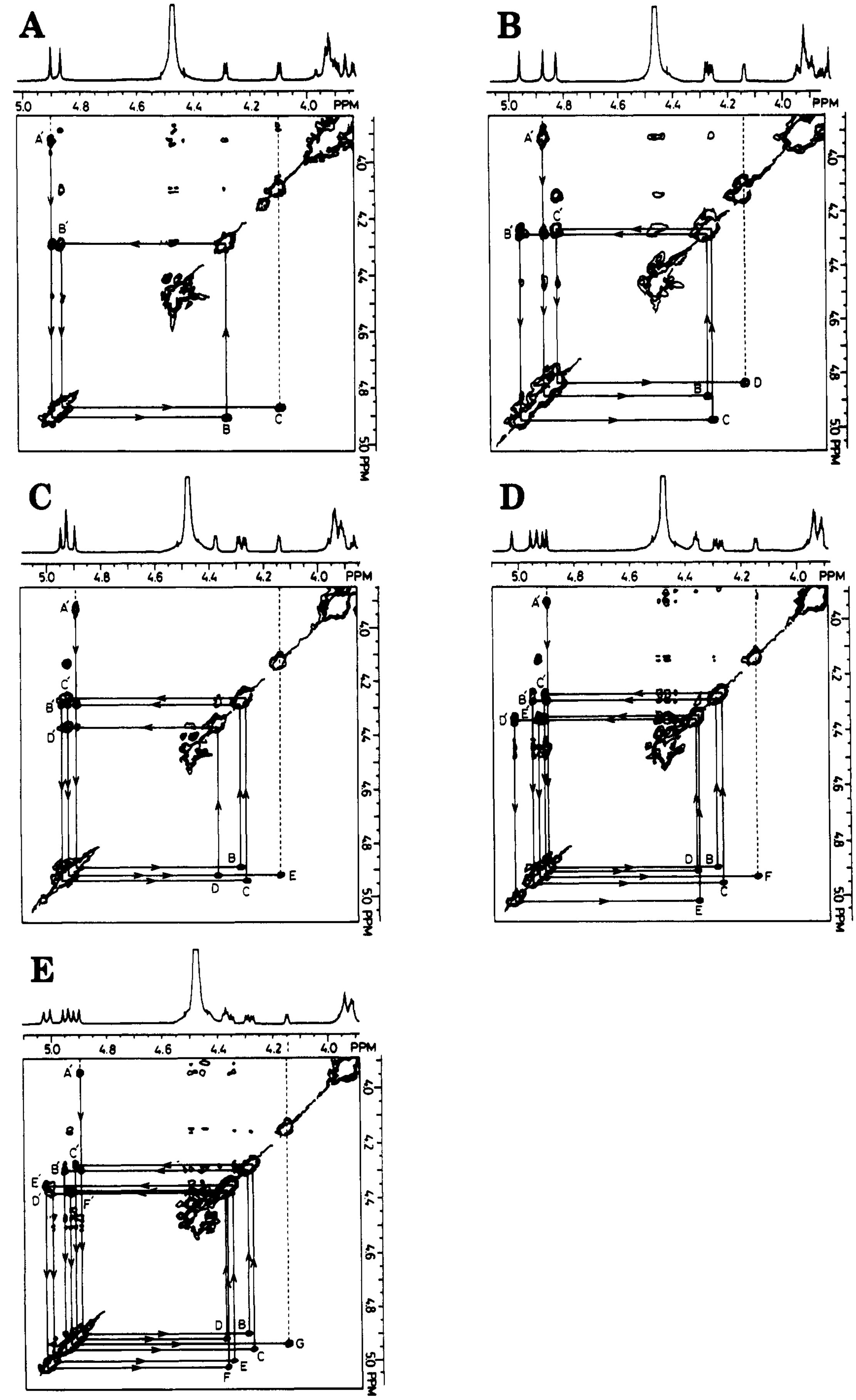

FIGURE 4: Sequential H-1–H-2′ connectivities of mannose units of oligosaccharide alcohols: (A) $Man_3$-ol; (B) $Man_4$-ol; (C) $Man_5$-ol; (D) $Man_6$-ol; (E) $Man_7$-ol. The conditions are the same as those in Figure 1.

mannopyranose unit on each oligosaccharide gave a significant change in the $^1$H NMR spectrum. For example, the H-1 signals of the nonreducing terminal $\beta$-1,2-linked mannopyranose units of Man$_2$, Man$_3$, Man$_4$, Man$_5$, Man$_6$, and Man$_7$ were 4.763, 4.851, 4.915, 4.936, 4.941, and 4.942 ppm, respectively (Table I). This finding observed on the $\beta$-1,2-linked mannooligosaccharides is extremely different from that of the $\alpha$-1,2-linked ones, because the H-1 signals of the nonreducing terminal mannopyranose units of the latter oligosaccharide series, from biose to tetraose, show almost the same chemical shift at 5.049–5.051 ppm (Kobayashi et al., 1990; Tojo et al., 1990). Furthermore, a shift in the H-1 signals of these oligosaccharides by treatment with NaBH$_4$ takes place not only on the adjacent mannopyranose unit, Man-B, but also on Man-C and Man-D. These results suggest that the $\beta$-1,2-linked mannooligosaccharides have distorted conformations. The existence of interresidue NOE cross-peaks between H-1 of Man-A and those of the nonreducing terminal units of Man$_6$ and Man$_7$, Man-F and Man-G, respectively, supports the above finding. Since no $\beta$-1,2-linked oligomannosyl residue has been detected in mammals and can be regarded as one of the specific structures of cell wall polysaccharides of some limited fungi as antigenic factors 5 and 6 (Shibata et al., personal communication), it seems likely that distinguishing the $\beta$-1,2-linked oligomannosyl residues as epitopes by the hosts' immune system might be easier than those of $\alpha$-linked isomeric residues (Tojo et al., 1988). Probably the serotype A-specific antigenic structures of *C. albicans* phosphomannan corresponding to a mannopentaose and a mannohexaose with one and two $\beta$-1,2-linked mannopyranose units, respectively, on their nonreducing terminal units can also be assumed to be readily recognized by immune surveillance similar to the $\beta$-1,2-linked oligomannosyl residues (Shibata et al., 1989; Kobayashi et al., 1989).

The sequential assignment method described here can be applied to the structural determination of oligosaccharides possessing more complicated structures. On the basis of these data, determination of the conformations of $\beta$-1,2-linked mannooligosaccharides in solution is in progress.

After submission of this paper, a report describing the assignment data of H-1 and H-2 of $\beta$-1,2-linked mannooligosaccharides appeared (Faille et al., 1991). They assigned the signals by $^1$H–$^1$H COSY, double-relayed COSY, and $^1$H–$^{13}$C COSY. However, the assignment results of Man$_5$, Man$_6$, and Man$_7$ were obviously different from our results. They assigned the H-1 and H-2 signals of Man$_4$ to Man$_7$ by application of a regularity rule between chemical shifts of H-3 and positions of the mannose units of Man$_3$ on the basis of the assumption that the regularity is common to this homologue series from Man$_2$ to Man$_7$. That is the reason for the discrepancy concerning the assignment of chemical shifts for Man$_5$, Man$_6$, and Man$_7$.

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