*Carbohydrate Research*, 186 (1989) 39–49
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands



# $^1$H- AND $^{13}$C-N.M.R. ASSIGNMENTS FOR STRUCTURAL ELEMENTS OF XYLOSE-CONTAINING *N*-LINKED OLIGOSACCHARIDES, USING 1D- AND 2D-N.M.R. EXPERIMENTS

JAN B. BOUWSTRA, JÁNOS KERÉKGYÁRTÓ*, JOHANNIS P. KAMERLING†, AND JOHANNES F. G. VLIEGENTHART

*Department of Bio-Organic Chemistry, Utrecht University, Transitorium III, P.O. Box 80.075 NL-3508 TB Utrecht (The Netherlands)*

(Received July 8th, 1988; accepted for publication, September 22nd, 1988)

## ABSTRACT

$^1$H-N.m.r. and $^{13}$C-n.m.r. spectral assignments for synthetic β-D-Xyl-(1→2)-β-D-Man-OMe, β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-β-D-Man-OMe, β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-β-D-Man-OMe, and β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-[α-D-Man-(1→6)]-β-D-Man-OMe, which are structural elements of xylose-containing carbohydrate chains from *N*-glycoproteins, have been made on the basis of 1D and 2D (DQF $^1$H–$^1$H COSY, HOHAHA) 500-MHz $^1$H-n.m.r. spectroscopy and 1D 50-MHz $^{13}$C-n.m.r. spectroscopy, respectively.

## INTRODUCTION

Xylose-containing *N*-linked carbohydrate chains occur in glycoproteins of plant[1–10] and animal origin[11–13]. Recently, a series of di-, tri-, and tetra-saccharides, namely, β-D-Xyl-(1→2)-β-D-Man-OMe (**1**), β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-β-D-Man-OMe (**2**), β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-β-D-Man-OMe (**3**), and β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-[α-D-Man-(1→6)]-β-D-Man-OMe (**4**), which represent building blocks of these carbohydrate chains, has been synthesized[14]. Detailed assignments are now presented of the $^1$H- and $^{13}$C-n.m.r. spectra of these compounds by 1D and 2D (DQF $^1$H–$^1$H COSY, HOHAHA) 500-MHz $^1$H-n.m.r. spectroscopy and 1D 50-MHz $^{13}$C-n.m.r. spectroscopy, respectively. These data are indispensable for conformational analysis of these structures using n.O.e. measurements.

## EXPERIMENTAL

*N.m.r. methods.* — N.m.r. spectra of 15mM solutions of oligosaccharides in $D_2O$ were obtained at 27°. Prior to n.m.r. spectroscopy, the samples were re-

*On leave from the Institute of Biochemistry, L. Kossuth University, Debrecen, Hungary.
†Author for correspondence.

0008-6215/89/$ 03.50 

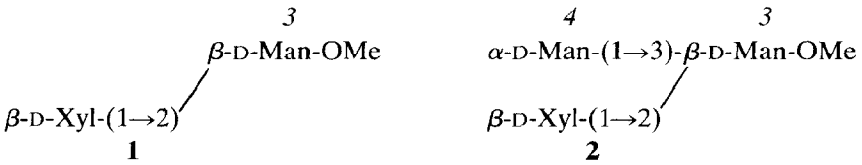
peatedly exchanged against 99.75% $D_2O$ with intermediate lyophilisation, finally using 99.96% $D_2O$.

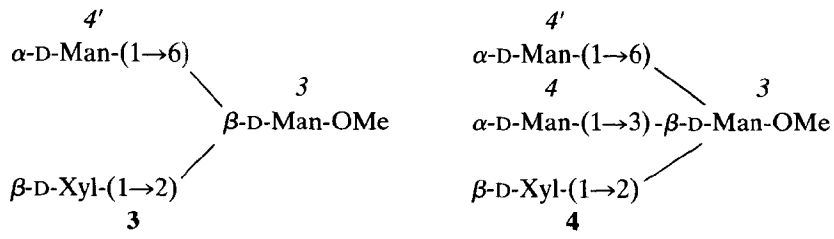
$^1$H-N.m.r. measurements were carried out on a Bruker AM-500 spectrometer (Department of NMR spectroscopy, Utrecht University). Resolution enhancement of the spectra was achieved by Lorentzian to Gaussian transformation. Chemical shifts are expressed in p.p.m. downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS), but were actually measured by reference to internal acetone ($\delta$ 2.225) with an accuracy of $\pm$0.002 p.p.m. Double-quantum filtered $^1$H–$^1$H correlation spectroscopy (DQF $^1$H–$^1$H COSY) was carried out according to refs. 15–17. Spectra were measured in the phase-sensitive mode, using quadrature detection in F2 and time-proportional phase increments (TPPI)[16], resulting in pure absorption/dispersion line-shapes without phase-twist. The spectral width was 3333.33 Hz in both dimensions and 512 measurements with $t_1$ values from 75 $\mu$s to 76.8 ms were recorded for one experiment. A 512 × 4k data matrix was acquired, which was zero filled and multiplied in both dimensions with a phase-shifted sine-square prior to a phase-sensitive F.t. to obtain a 2k × 4k spectral data matrix. Homonuclear Hartmann–Hahn (HOHAHA) spectra were obtained according to refs. 18 and 19. A 8.33 kHz (60 $\mu$s 180° pulse width) field strength was used and the mixing period consisted of 60 MLEV-17 cycles, resulting in a 120-ms mixing time. The spectral width was 3333.33 Hz in both dimensions, and 512 measurements with $t_1$ values ranging from 75 $\mu$s to 76.8 ms were recorded for one experiment. A 512 × 4k data matrix was acquired, which was zero filled and multiplied in both dimensions with a phase-shifted sine-square function prior to a phase-sensitive F.t. to obtain a 2k × 4k data matrix.

Natural-abundance proton-decoupled $^{13}$C-n.m.r. spectra were recorded at 50.76 MHz with a Bruker WM-200 spectrometer (SON hf-NMR-facility, Department of Biophysical Chemistry, University of Nijmegen) equipped with a 5-mm broad-band probe. The spectral width was 10,000 Hz. Chemical shifts are expressed in p.p.m. downfield from external $Me_4Si$, but were actually measured by reference to internal acetone ($\delta$ 31.55) with an accuracy of $\pm$0.02 p.p.m.

## RESULTS AND DISCUSSION

*$^1$H-N.m.r. assignments.* — The $^1$H-n.m.r. data for **1–4** are given in Table I.






*β-D-Xyl-(1→2)-β-D-Man-OMe* (**1**). — The DQF $^1H$–$^1H$ COSY spectrum of **1** is presented in Fig. 1. For β-D-Man (Man-*3*), the assignments start with the

TABLE I

$^1H$-CHEMICAL SHIFTS FOR THE $^1H$ RESONANCES OF THE CONSTITUENT MONOSACCHARIDES OF **1–4**

| *Residue*[a] | *Atom* | *Chemical shift*[b] | | | |
|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** |
| Man-*3* | H-1 | 4.645 | 4.661 | 4.665 | 4.681 |
| | H-2 | 4.163 | 4.182 | 4.169 | 4.197 |
| | H-3 | 3.637 | 3.825 | 3.650 | 3.836 |
| | H-4 | 3.520 | 3.662 | 3.624 | 3.800 |
| | H-5 | 3.400 | 3.444 | 3.546 | 3.588 |
| | H-6 | 3.938 | 3.945 | 3.953 | 3.977 |
| | H-6' | 3.728 | 3.739 | 3.805 | 3.774 |
| | OMe | 3.535 | 3.537 | 3.524 | 3.523 |
| Xyl | H-1 | 4.489 | 4.484 | 4.490 | 4.474 |
| | H-2 | 3.348 | 3.328 | 3.366 | 3.360 |
| | H-3 | 3.429 | 3.427 | 3.438 | 3.425 |
| | H-4 | 3.621 | 3.626 | 3.632 | 3.624 |
| | H-5*e* | 3.944 | 4.000 | 3.954 | 3.998 |
| | H-5*a* | 3.268 | 3.244 | 3.268 | 3.249 |
| Man-*4* | H-1 | | 5.144 | | 5.138 |
| | H-2 | | 4.039 | | 4.035 |
| | H-3 | | 3.843 | | 3.854 |
| | H-4 | | 3.678 | | 3.679 |
| | H-5 | | 3.916 | | 3.918 |
| | H-6 | | 3.885 | | 3.893 |
| | H-6' | | 3.756 | | 3.750 |
| Man-*4'* | H-1 | | | 4.906 | 4.900 |
| | H-2 | | | 4.004 | 4.010 |
| | H-3 | | | 3.833 | 3.831 |
| | H-4 | | | 3.676 | 3.679 |
| | H-5 | | | 3.674 | 3.676 |
| | H-6 | | | 3.894 | 3.892 |
| | H-6' | | | 3.769 | 3.759 |

[a]For the numbering system of the residues, see the formulae. [b]In p.p.m. relative to internal DSS in $D_2O$ (using internal acetone at $\delta$ 2.225) at 27°.

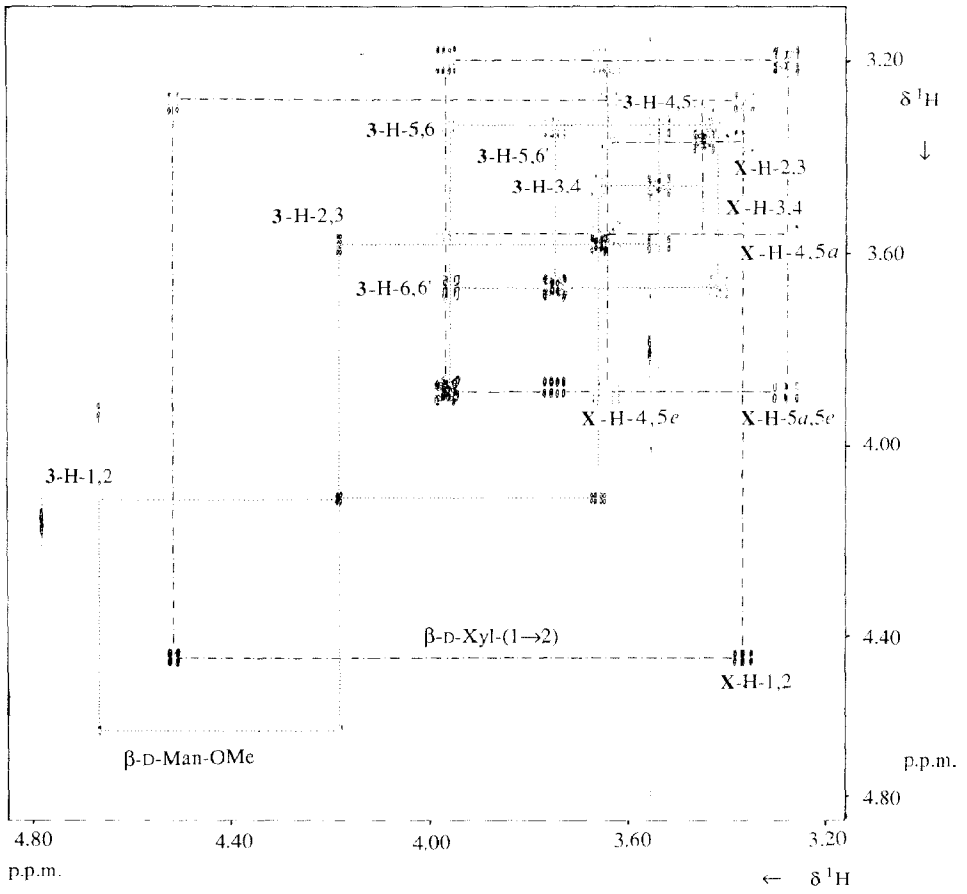



Fig. 1. DQF $^1H$–$^1H$ COSY spectrum of β-D-Xyl-(1→2)-β-D-Man-OMe. Lines indicate correlations: **3**-H-1,2 means cross-peak between H-1 and H-2 of Man-*3*.

resonance of H-1 at δ 4.645 ($J_{1,2}$ <1 Hz)[20], and, from the presence of cross-peaks correlating H-1,2, H-2,3, H-3,4, H-4,5, H-5,6, and H-5,6′, the chemical shifts of all the proton resonances can be deduced. For β-D-Xyl, the starting point for the assignments is the resonance of H-1 at δ 4.489 ($J_{1,2}$ 7.7 Hz)[5], yielding the H-2,3,4,5*a*,5*e* resonances from the observed connectivities.

*β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-β-D-Man-OMe* (**2**). — The region 3.1–4.1 p.p.m. of the DQF $^1H$–$^1H$ COSY spectrum of **2** is shown in Fig. 2. The $^1H$-n.m.r. parameters of β-D-Man can be found by starting with the chemical shift of the resonance of H-1 at δ 4.661, which leads to the assignments of the resonances of H-2,3,4,5,6,6′ of β-D-Man by the cross-peak pattern in the DQF $^1H$–$^1H$ COSY spectrum. A comparison of the data for **1** and **2** shows that the attachment of α-D-Man (Man-*4*) in (1→3)-linkage to β-D-Man results in relatively large downfield

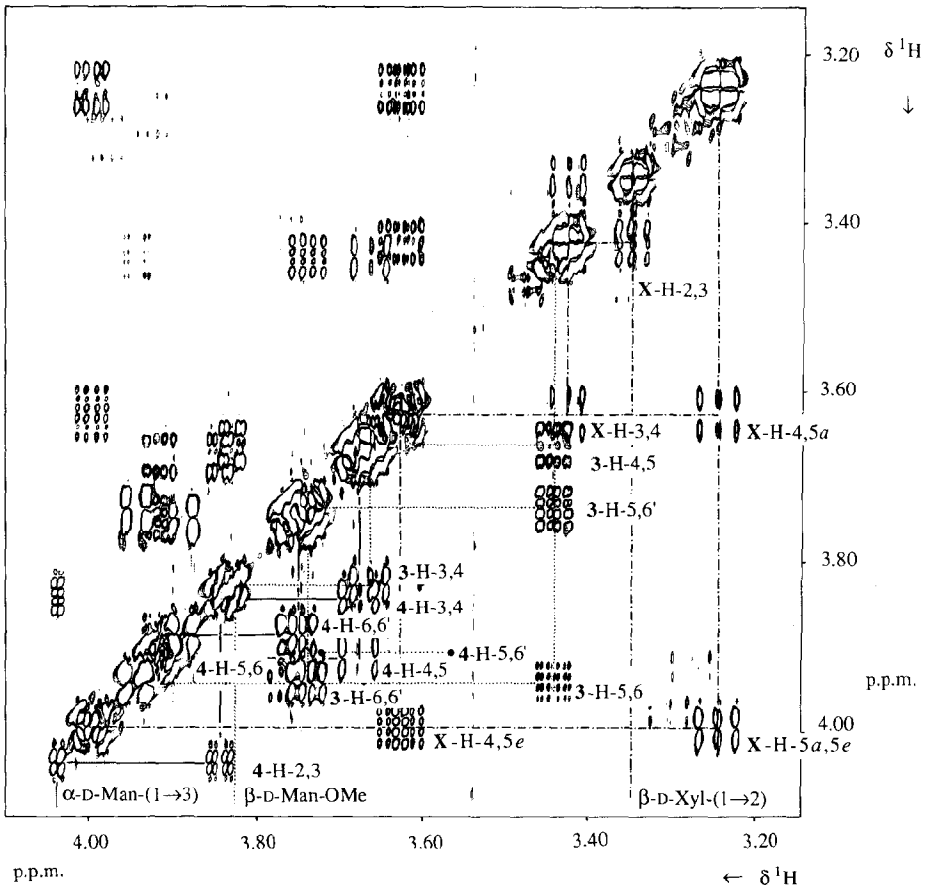



Fig. 2. DQF $^1H$–$^1H$ COSY spectrum of β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-β-D-Man-OMe. Lines indicate correlations. The anomeric region is not shown. For explanation of the code system, see Fig. 1.

shifts of the signals for H-3 ($\Delta\delta$ +0.188 p.p.m.) and H-4 ($\Delta\delta$ +0.142 p.p.m.) of β-D-Man. The starting point for the assignments of the β-D-Xyl signals is the H-1 doublet at $\delta$ 4.484 and, by following the sequential connectivities involving H-2,3,4,5*a*,5*e*, all assignments are obtained. Except for the H-5*e* signal ($\Delta\delta$ +0.056 p.p.m.), the resonance positions of the β-D-Xyl protons appear not to be influenced substantially by the extra α-D-Man residue. For α-D-Man, all $^1H$-n.m.r. chemical shifts are deduced from the connectivities shown, starting with the chemical shift of the resonance of H-1 at $\delta$ 5.144 ($J_{1,2}$ 2.0 Hz), identified by comparison with reference compounds[5].

*β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-β-D-Man-OMe* (**3**). — In the DQF $^1H$–$^1H$ COSY spectrum of **3** (Fig. 3), the chemical shift of the H-1 resonance of β-D-Man is 4.665 p.p.m., pointing to the chemical shifts of the resonances of H-2 and H-3 *via*

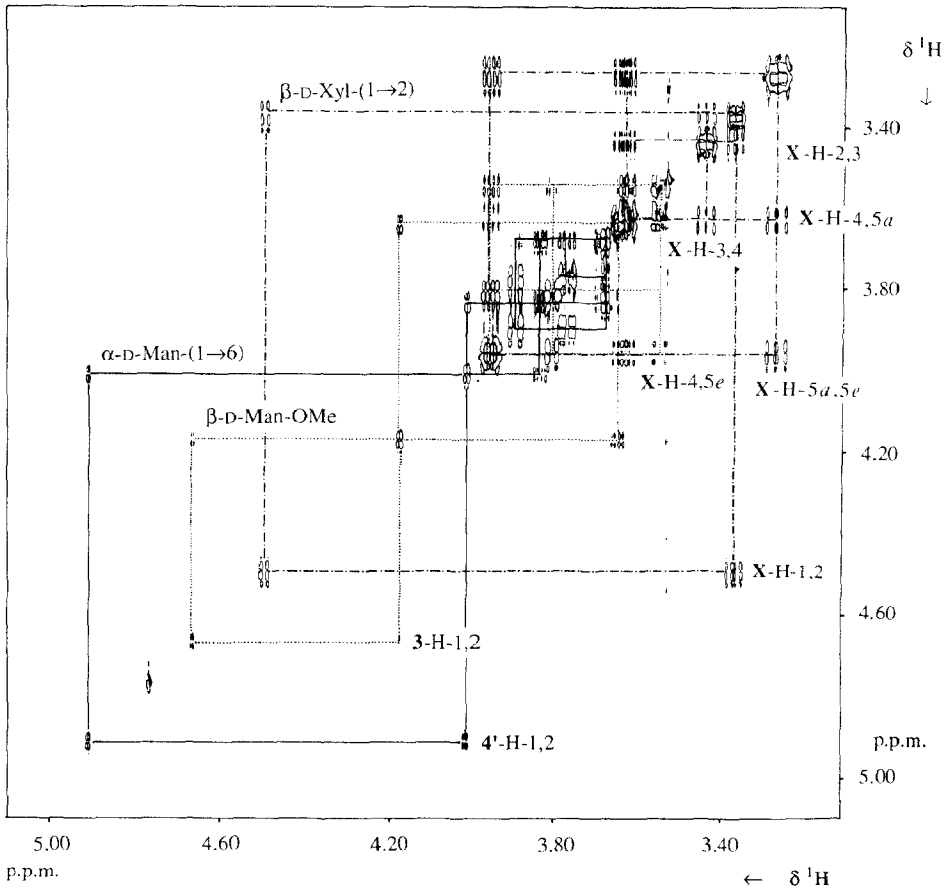



Fig. 3. DQF $^1$H–$^1$H COSY spectrum of β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-β-D-Man-OMe. Lines indicate correlations. For explanation of the code system, see Fig. 1.

the cross-peak pattern. The H-3,4 cross-peak cannot be found, but, by comparison with structure **1**, the characteristic three-spin system for H-5,6,6′ can be recognised, revealing the chemical shifts of the resonances of these protons, and the H-4,5 cross-peak reveals that the H-4 resonance is at δ 3.624. Starting with the chemical shift of the H-1 resonance at 4.490 p.p.m., all β-D-Xyl assignments are found by following the sequential connectivities for the multiplets H-2,3,4,5*a*,5*e*. The low-field doublet for H-1 of α-D-Man (Man-*4′*) at δ 4.906 ($J_{1,2}$ 1.8 Hz) is assigned by comparison with data for reference compounds[5]. From the cross-peak pattern, the positions of the H-2, H-3, and H-4 signals are deduced. The H-4,5 cross-peak cannot be found, but, by analogy with **2**, the H-6,6′ cross-peak is identified at 3.894 p.p.m. (H-6), revealing the chemical shifts of the resonances for H-6,6′. The H-5 signal is traced from the observed H-5,6′ connectivity. A comparison of the data

for **1** and **3** shows that the attachment of α-D-Man in (1→6)-linkage to β-D-Man results in relatively large downfield shifts in the signals of H-4 (Δδ +0.104 p.p.m.), H-5 (Δδ +0.146 p.p.m.), and H-6′ (Δδ +0.077 p.p.m.) of β-D-Man. The position of the resonance of H-6 is influenced less (Δδ +0.015 p.p.m.).

*β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-[α-D-Man-(1→6)]-β-D-Man-OMe* (**4**). — The DQF $^1$H–$^1$H COSY spectrum of **4** is shown in Fig. 4. Because of the complexity of this spectrum, a HOHAHA spectrum with a mixing time of 120 ms was also recorded, showing complete or partial sub-spectra of all residues on their H-1 and H-2 tracks (Fig. 5). For β-D-Man, only H-2 is found on the H-1 track (δ 4.681), due to small coupling constants between H-1, H-2, and H-3. However, on the H-2 track (δ 4.197), a magnetisation transfer is observed from H-2 up to and including H-6 and H-6′. To assign these chemical shifts, the DQF $^1$H–$^1$H COSY spectrum is used in combination with the data for **2** and **3**. Starting with the resonance of H-1 at δ 4.681, the cross-peak pattern in the DQF $^1$H–$^1$H COSY spectrum identifies the chemical shifts of the resonances for H-2,3. The H-5,6,6′ three-spin pattern in the DQF $^1$H–$^1$H COSY spectrum at δ 3.588 (H-5), which is shown to belong to β-D-Man by the H-2 track in the HOHAHA spectrum, is assigned by reference to **3**, making feasible the assignment of H-4 by the H-4,5 cross-peak. All $^1$H-n.m.r. chemical shifts of β-D-Xyl are recognised at the H-1 track (δ 4.474) in the HOHAHA spectrum and are assigned readily by the shown connectivities in the DQF $^1$H–$^1$H COSY spectrum. For α-D-Man, (1→3)-linked to β-D-Man, the HOHAHA spectrum shows on the tracks for H-1 (δ 5.138) and H-2 (δ 4.035) magnetisation from H-1 and from H-2 to be transferred up to and including H-4 and H-6,6′, respectively. In combination with the cross-peak pattern in the DQF $^1$H–$^1$H COSY spectrum, the resonances for H-1,2,3,4 are readily assigned. The H-4,5 cross-peak is found by comparison with **2**, and the chemical shifts of the resonances for H-5,6,6′ are distinguished from each other by the shown connectivities in the DQF $^1$H–$^1$H COSY spectrum in conjunction with the features of the HOHAHA spectrum. Finally, for α-D-Man (1→6)-linked to β-D-Man, the HOHAHA spectrum shows sub-spectra on the tracks of H-1 (δ 4.900) and H-2 (δ 4.010) of all protons up to and including H-4 and H-6,6′, respectively. The H-2, H-3, and H-4 resonances can be deduced from observed connectivities in the DQF $^1$H–$^1$H COSY spectrum; for the assignment of the H-5, H-6, and H-6′ resonances, use has been made of the HOHAHA and the $^1$H–$^1$H DQF COSY spectrum in combination with the data for **3**.

*$^{13}$C-N.m.r. assignments.* — The $^{13}$C-n.m.r. data are presented in Table II, together with those of relevant monomers.

*β-D-Xyl-(1→2)-β-D-Man-OMe* (**1**). — The $^{13}$C-n.m.r. data for **1** have been obtained by comparison with those of the methyl glycosides of the constituent monosaccharides[21,22]. The attachment of β-D-Xyl to C-2 of β-D-Man leads to a clear downfield shift for the resonance of C-2 (Δδ +8.72 p.p.m.) of β-D-Man.

*β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-β-D-Man-OMe* (**2**). — The assignments of the $^{13}$C-n.m.r. signals of **2** are based on the data[22] for **1** and methyl α-D-manno-

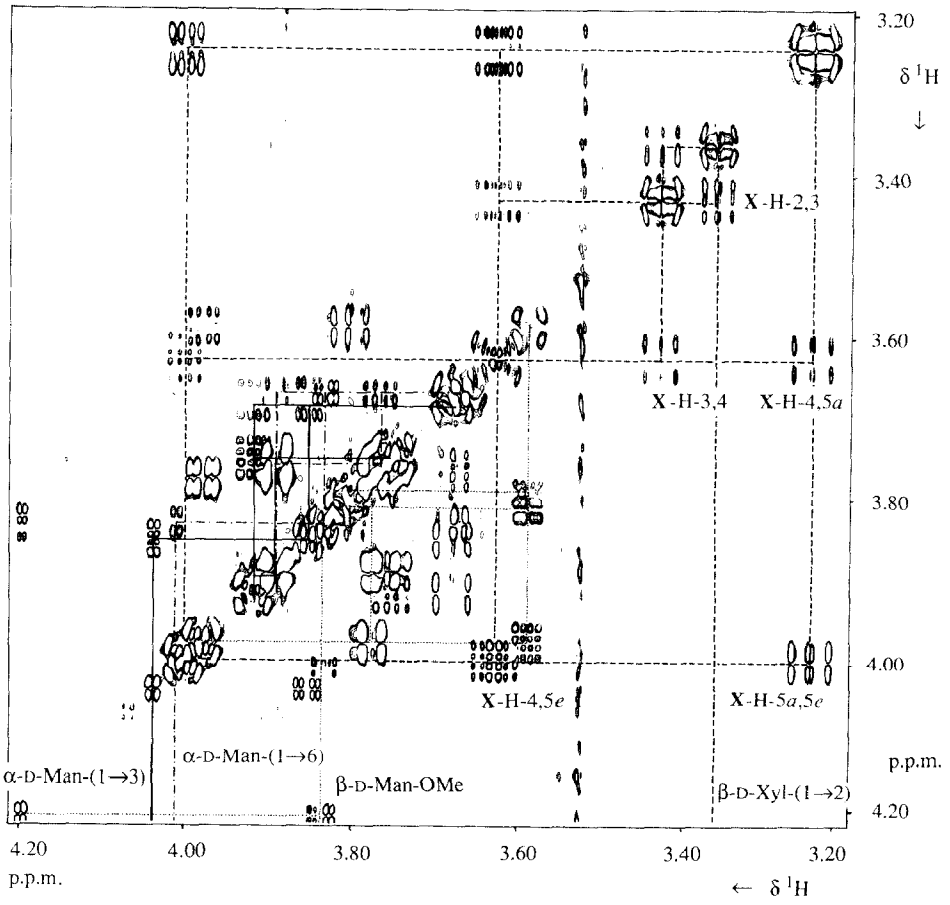



Fig. 4. DQF $^1$H–$^1$H COSY spectrum of β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-[α-D-Man-(1→3)]-β-D-Man-OMe. Lines indicate correlations. For explanation of the code system, see Fig. 1.

pyranoside. As compared to **1**, the introduction of α-D-Man in (1→3)-linkage to β-D-Man results in downfield shifts for the resonances of β-D-Man C-3 ($\Delta\delta$ +7.15 p.p.m.) and β-D-Xyl C-1 ($\Delta\delta$ +0.88 p.p.m.), and an upfield shift for that of β-D-Man C-2 ($\Delta\delta$ −0.88 p.p.m.).

*β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-β-D-Man-OMe* (**3**). — In a manner similar to that for **2**, the $^{13}$C-n.m.r. signals of **3** have been assigned. As compared to **1**, the substitution of α-D-Man in (1→6)-linkage to β-D-Man results in shifts for the resonances of β-D-Man C-6 (downfield, $\Delta\delta$ +4.84 p.p.m.) and β-D-Man C-5 (upfield, $\Delta\delta$ −1.99 p.p.m.). For β-D-Xyl, no significant differences in comparison to **1** are observed.

*β-D-Xyl-(1→2)-[α-D-Man-(1→3)]-[α-D-Man-(1→6)]-β-D-Man-OMe* (**4**). — The $^{13}$C-n.m.r. spectrum of **4** has been elucidated by reference to the $^{13}$C-n.m.r.

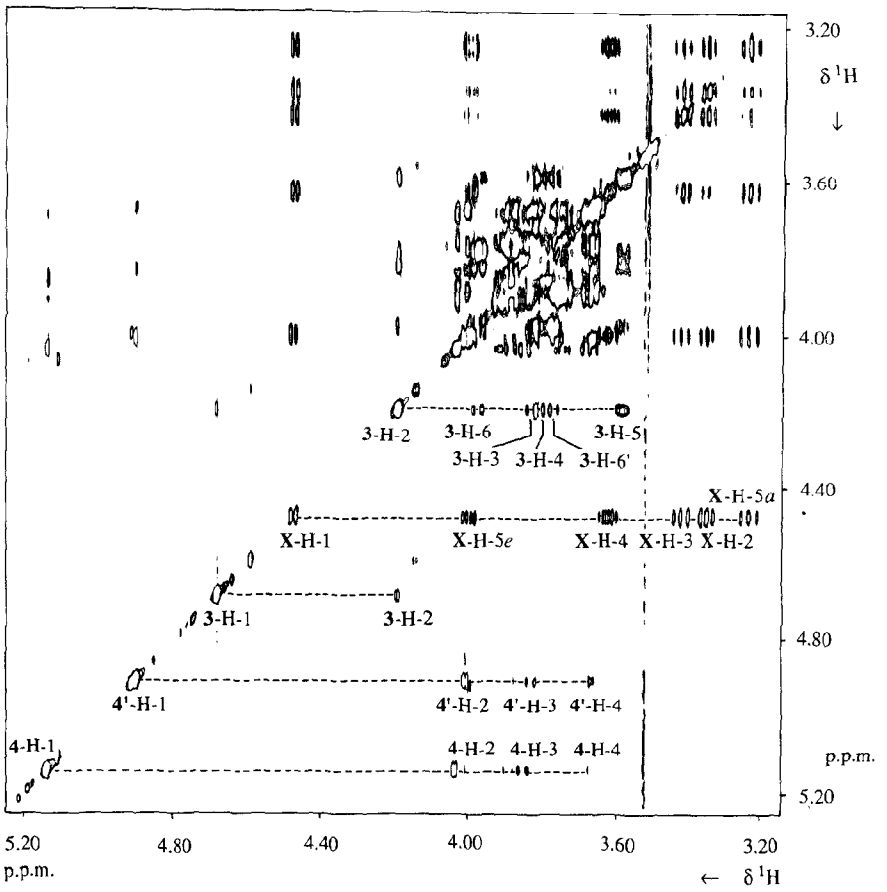



Fig. 5. 2D HOHAHA spectrum of β-D-Xyl-(1→2)-[α-D-Man-(1→6)]-[α-D-Man-(1→3)]-β-D-Man-OMe. Lines indicate scalar coupled networks: **3**-H-2 means H-2 of Man-*3*.

data for **2** and **3**. Shift effects caused by the attachment of one α-D-Man residue appear to be independent of the presence of other residues covalently linked to the β-D-Man monomer. (1→6)-α-D-Mannosylation of **1** (→**3**) and **2** (→**4**) leads to comparable shift increments for the resonances of β-D-Man C-5 and C-6, whereas (1→3)-α-D-mannosylation of **1** (→**2**) and **3** (→**4**) yields similar shift increments for the resonances of β-D-Man C-2 and C-3.

ACKNOWLEDGMENTS

This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for Scientific Research (NWO).

TABLE II

$^{13}$C-CHEMICAL SHIFTS FOR THE $^{13}$C RESONANCES OF THE CONSTITUENT MONOSACCHARIDES OF **1–4** AND THE METHYL GLYCOSIDES OF THE MONOSACCHARIDES[21,22]

| *Residue*[a] | *Atom* | *Chemical shift*[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | β-D-*Man*p-*OMe* | α-D-*Man*p-*OMe* | β-D-*Xyl*p-*OMe* | **1** | **2** | **3** | **4** |
| Man-*3* | C-1 | 101.3 | | | 102.56 | 102.44 (−0.12)[c] | 102.66 (+0.10)[c] | 102.55 (+0.11)[d] (−0.11)[e] |
| | C-2 | 70.6 | | | 79.32 | 78.44[f] (−0.88) | 79.56 (+0.24) | 78.65[f] (+0.21) (−0.91) |
| | C-3 | 73.3 | | | 73.23 | 80.38[f] (+7.15) | 73.41 (+0.18) | 80.66[f] (+0.28) (+7.25) |
| | C-4 | 67.1 | | | 68.65 | 68.38 (−0.27) | 68.47 (−0.18) | 67.98 (−0.40) (−0.49) |
| | C-5 | 76.6 | | | 77.76 | 77.63 (−0.13) | 75.77 (−1.99) | 75.59 (−2.04) (−0.18) |
| | C-6 | 61.4 | | | 62.19 | 62.03 (−0.16) | 67.03 (+4.84) | 66.60 (+4.57) (−0.43) |
| | OMe | 56.9 | | | 58.24 | 58.21 | 58.18 | 58.14 |
| Xyl | C-1 | | | 105.1 | 105.33 | 106.21 (+0.88) | 105.48 (+0.15) | 106.35 (+0.14) (+0.87) |
| | C-2 | | | 74.0 | 74.57 | 76.64[g] (+0.07) | 74.56 (−0.01) | 74.56[g] (−0.08) (+0.00) |
| | C-3 | | | 76.9 | 76.75 | 76.78 (+0.03) | 76.73 (−0.02) | 76.74 (−0.04) (+0.01) |
| | C-4 | | | 70.4 | 70.53 | 70.56 (+0.03) | 70.52 (−0.01) | 70.55 (−0.01) (+0.03) |
| | C-5 | | | 66.3 | 66.43 | 66.28 (−0.15) | 66.42 (−0.01) | 66.27 (−0.01) (−0.15) |
| | OMe | | | 58.3 | | | | |
| Man-*4* | C-1 | | 101.9 | | | 103.29 | | 103.31 (+0.02) |
| | C-2 | | 71.2 | | | 71.31 | | 71.32 (+0.01) |
| | C-3 | | 71.8 | | | 71.59 | | 71.58 (−0.01) |
| | C-4 | | 68.0 | | | 67.97 | | 67.98 (+0.01) |
| | C-5 | | 73.7 | | | 74.77[g] | | 74.76[g] (−0.01) |
| | C-6 | | 62.1 | | | 62.38 | | 62.37 (−0.01) |
| | OMe | | 55.9 | | | | | |
| Man-*4'* | C-1 | | | | | | 100.90 | 100.80 (−0.10) |
| | C-2 | | | | | | 71.19 | 71.16 (−0.03) |
| | C-3 | | | | | | 71.90 | 71.91 (−0.01) |
| | C-4 | | | | | | 67.98 | 67.98 (+0.00) |
| | C-5 | | | | | | 74.00 | 73.97 (−0.03) |
| | C-6 | | | | | | 62.21 | 62.22 (+0.01) |

[a]For the numbering system of the residues, see the formulae. [b]In p.p.m. relative to external $Me_4Si$ in $D_2O$ (using internal acetone at δ 31.55) at 27°. [c]Chemical shift difference relative to structure **1**. [d]Chemical shift difference relative to structure **2**. [e]Chemical shift difference relative to structure **3**. [f]$^{13}$C-N.m.r. chemical shifts might be interchanged. [g]$^{13}$C-N.m.r. chemical shifts might be interchanged.

## REFERENCES


1 H. ISHIHARA, N. TAKAHASHI, S. OGURI, AND S. TEJIMA, *J. Biol. Chem.*, 254 (1979) 10715–10719.
2 M.-J. PRIGENT, J. MONTREUIL, AND G. STRECKER, *Carbohydr. Res.*, 131 (1984) 83–92.
3 S. HASE, S. KOYAMA, H. DAIYASU, H. TAKEMOTO, S. HARA, Y. KOBAYASHI, Y. KYOGOKU, AND T. IKENAKA, *J. Biochem. (Tokyo)*, 100 (1986) 1–10.
4 N. TAKAHASHI, T. HOTTA, H. ISHIHARA, M. MORI, S. TEJIMA, R. BLIGNY, T. AKAZAWA, S. ENDO, AND Y. ARATA, *Biochemistry*, 25 (1986) 388–395.
5 J. A. VAN KUIK, R. A. HOFFMANN, J. H. G. M. MUTSAERS, H. VAN HALBEEK, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Glycoconj. J.*, 3 (1986) 27–34.
6 A. STURM, J. A. VAN KUIK, J. F. G. VLIEGENTHART, AND M. J. CHRISPEELS, *J. Biol. Chem.*, 262 (1987) 13392–13403.
7 D. ASHFORD, R. A. DWEK, J. K. WELPLY, S. AMATAYAKUL, S. W. HOMANS, H. LIS, G. N. TAYLOR, N. SHARON, AND T. W. RADEMACHER, *Eur. J. Biochem.*, 166 (1987) 311–320.
8 B. FOURNET, Y. LEROY, J.-M. WIERUSZESKI, J. MONTREUIL, R. D. PORETZ, AND R. GOLDBERG, *Eur. J. Biochem.*, 166 (1987) 321–324.
9 Y. KIMURA, S. HASE, Y. KOBAYASHI, Y. KYOGOKU, G. FUNATSU, AND T. IKENAKA, *J. Biochem. (Tokyo)*, 101 (1987) 1051–1054.
10 G. D'ANDREA, J. B. BOUWSTRA, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Glycoconj. J.*, 5 (1988) 151–157.
11 J. A. VAN KUIK, H. VAN HALBEEK, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *J. Biol. Chem.*, 260 (1985) 13984–13988.
12 J. A. VAN KUIK, R. P. SIJBESMA, J. P. KAMERLING, J. F. G. VLIEGENTHART, AND E. J. WOOD, *Eur. J. Biochem.*, 160 (1986) 621–625.
13 J. A. VAN KUIK, R. P. SIJBESMA, J. P. KAMERLING, J. F. G. VLIEGENTHART, AND E. J. WOOD, *Eur. J. Biochem.*, 169 (1987) 399–411.
14 J. KERÉKGYÁRTÓ, J. P. KAMERLING, J. B. BOUWSTRA, J. F. G. VLIEGENTHART, AND A. LIPTÁK, *Carbohydr. Res.*, 186 (1989) 51–62.
15 W. P. AUE, E. BARTHOLDI, AND R. R. ERNST, *J. Chem. Phys.*, 64 (1976) 2229–2246.
16 D. MARION AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.*, 117 (1983) 967–974.
17 M. RANCE, O. W. SØRENSEN, G. BODENHAUSEN, G. WAGNER, R. R. ERNST, AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.*, 117 (1983) 479–485.
18 L. BRAUNSCHWEILER AND R. R. ERNST, *J. Magn. Reson.*, 53 (1983) 521–528.
19 A. BAX AND D. G. DAVIS, *J. Magn. Reson.*, 65 (1985) 335–360.
20 K. BOCK AND H. THØGERSEN, *Annu. Rep. NMR Spectr.*, 13 (1982) 1–57.
21 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
22 P. A. J. GORIN AND M. MAZUREK, *Can. J. Chem.*, 53 (1975) 1212–1223.