Eur. J. Biochem. *127*, 1–6 (1982)

A 500-MHz Proton-Magnetic-Resonance Study of Several Fragments of the Carbohydrate-Protein Linkage Region Commonly Occurring in Proteoglycans

Herman VAN HALBEEK, Lambertus DORLAND, Gerrit A. VELDINK, Johannes F. G. VLIEGENTHART, Per J. GAREGG, Thomas NORBERG, and Bengt LINDBERG

Department of Bio-Organic Chemistry, University of Utrecht; and
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm

(Received April 26, 1982)

The proton-magnetic-resonance spectra of three partial structures of the carbohydrate-protein linkage region that frequently occurs in proteoglycans, namely, β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→O)-L-Ser, were recorded in ^2H_2O at 500 MHz; they could be completely interpreted, both for the glyco-serines and for the corresponding glyco-xylitols. The chemical shifts and the coupling constants were refined by computer simulation of the spectra.

The change in the chemical shift of H-4 of a D-galactopyranosyl residue upon substitution at C-3 by a β-D-galactopyranosyl group is proposed to be characteristic for this particular attachment, making H-4 of galactose a structural-reporter group. The three constituting monosaccharides adopt the $^4C_1$ (D) ring conformation. The terminal galactopyranosyl group and the internal galactopyranosyl residue differ as to the population of rotamers around the C-5/C-6 axis. Concomitantly, the flexibility of their glycosidic linkages is distinct.

In recent years, high-resolution proton-magnetic-resonance (^{1}H-NMR) spectroscopy has proved to be a powerful tool for the elucidation of the structure of carbohydrate chains of a wide variety of glycoproteins [1–3]. The carbohydrate-protein linkage region of glycoproteins of the *N*-glycosidic type is now well established [4]. Many glycoproteins, however, contain *O*-glycosidically linked carbohydrate chains attached to hydroxyl groups in the side chain of amino acids like L-serine, L-threonine, 5-L-hydroxylysine and 4-L-hydroxyproline; some glycoproteins contain both types of glycosidically linked sugar moieties [4]. Also in proteoglycans the carbohydrate part is *O*-glycosidically linked, namely, to L-serine. In these polymers, repeating disaccharide units are linked to the protein chains via a glycopeptide linkage; the linkage region usually contains monosaccharides different from those of the main polysaccharide chain. The structure of the linkage region in, for example, chondroitin sulfate, dermatan sulfate and heparin is that of [5]:

$$\underset{3}{\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)}\text{-}\underset{2}{\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)}\text{-}\underset{1}{\beta\text{-D-Xyl}p\text{-}(1\rightarrow O)\text{-L-Ser}}.$$

It is therefore relevant to characterize the linkage region of *O*-glycosidic glycoproteins and of proteoglycans. In this paper, the ^{1}H-NMR parameters of a series of building blocks of the aforementioned proteoglycan linkage region are described.

A glycopeptide linkage involving serine is alkali-labile. In the presence of sodium hydroxide and sodium borohydride, the glycoprotein or proteoglycan undergoes a β-elimination with release of an oligosaccharide-alditol [6]. Since the isolation of glycopeptide fragments containing carbohydrate chains *O*-glycosidically linked to only a short peptide chain is rather cumbersome, it was considered worthwhile to include the corresponding oligosaccharide-xylitols in the ^{1}H-NMR studies.

A preliminary account of the results reported here, has previously been presented [7].

MATERIALS AND METHODS

β-D-Xyl*p*-(1→O)-L-Ser (compound I), β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→O)-L-Ser (compound II) and β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→O)-L-Ser (compound III) were available from previous studies [8–10].

Xyl-ol (compound IV), β-D-Gal*p*-(1→4)-D-Xyl-ol (compound V) and β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Xyl-ol (compound VI) were prepared by reduction of D-xylose and of the oligosaccharides corresponding to V and VI, synthesized earlier [11], respectively, with $NaBH_4$ or NaB^2H_4 (Baker).

Solutions of glyco-serines and glyco-xylitols for NMR studies in ^2H_2O were adjusted to $p^2H \approx 7$. Deuterium-exchanged samples were obtained by fivefold lyophilization of the solutions, finally using 99.96 atom% ^2H_2O (Aldrich).

500-MHz ^{1}H-NMR spectra were recorded using a Bruker WM-500 instrument (Rheinstetten, FRG) operating in the pulsed Fourier-transform mode at a probe temperature of 300 K. Resolution enhancement of the spectra was achieved by Lorentzian to Gaussian transformation from quadrature phase detection, followed by a complex Fourier transformation [3]. Chemical shifts are expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), but were measured by reference to internal acetone ($\delta = 2.225$ ppm), with an accuracy of 0.002 ppm.

Abbreviations. Gal, galactose; Xyl, xylose; Xyl-ol, xylitol; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; GalNAc-ol, *N*-acetylgalactosaminitol; *p*, pyranose; Ser, serine; NMR, nuclear magnetic resonance.

Computer simulations of the spectra were run on a CDC-Cyber-175 computer (Academic Computer Center Utrecht, The Netherlands), using the spin-simulation programme NMRSIM (Dr P. H. M. Budzelaar, personal communication). The first-order spectral parameters were checked and refined by iterative calculation of the theoretical spectra until a good agreement was obtained between observed and calculated spectra. The accuracy of the vicinal and geminal coupling-constant values is about 0.2 Hz.

RESULTS AND DISCUSSION

500-MHz ^{1}H-NMR spectra were recorded for three glycoserines, namely, β-D-Xyl*p*-(1→O)-L-Ser (compound I), β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→O)-L-Ser (compound II), and β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→O)-L-Ser (compound III). The observed spectrum of compound III is given in Fig. 1A as a typical example. Signal assignments and splitting patterns are indicated in the figure. For comparison, the computer-calculated spectrum of III has been included (Fig. 1B). The refined ^{1}H-NMR data of compounds I–III are summarized in Tables 1 and 2.

The $J_{1,2}$ coupling-constant values of the Xyl and Gal residues in compounds I–III are found between 7–8 Hz, indicating β-D-configuration of the glycosidic linkages.

The attachment of a β-D-Xyl*p* group to L-Ser produces changes in the chemical shifts for the Ser protons (for unsubstituted L-Ser: δH-α = 3.838 ppm and δH-β = δH-β′ = 3.959 ppm at p^{2}H ≈ 7 and T = 295 K, compare [12]). Furthermore, the methylene protons (H-β and H-β′) become non-equivalent (see Table 1). Assignments of the Xyl protons for compound I were proved by selective homonuclear decoupling experiments. To derive the influence of Ser, data can be compared with those reported for methyl β-D-xylopyranoside in ^2H_2O [13].

Upon substitution of the D-Xyl*p* residue in compound I at C-4 with a β-D-Gal*p* group (leading to compound II), small changes in chemical shifts of the Xyl protons were observed. The largest shift increments appeared for H-4 and H-5$_{eq}$. The signal for H-5$_{eq}$ is found at significantly more downfield position than those for H-2 to H-5$_{ax}$. The appearance of the H-5$_{eq}$ signal apart from the bulk of skeleton protons will probably persist in larger fragments of this series of compounds. The chemical shift for H-1 of the terminal, non-reducing Gal*p* group in compound II (δ = 4.471 ppm) is similar to that observed for the corresponding Gal*p* group in the *N*-acetyllactosamine type of structures [2,3,14,15] and thus might be held characteristic of terminal β-D-Gal*p* groups linked to C-4 of a pyranoside. The H-4 atom of the terminal β-D-Gal*p* group in compound II resonates, apart from the bulk, at $\delta \approx$ 3.92 ppm (compare [2,16,17]).

Upon extending compound II with a β-D-Gal*p* group, resulting in compound III, the chemical shifts of the Xyl and Ser protons are hardly or not affected. However, most protons of the internal β-D-Gal*p* residue (denoted Gal-2) undergo considerable downfield shifts. This effect is significantly larger for H-4 than for H-3, although it is the latter proton which is attached to the carbon atom being substituted by the Gal-3 group. A steric effect resulting from the *gauche* orientation of H-4 and the substituent at C-3 with respect to each other might be responsible for this. The relatively low-field resonance position of H-4 of Gal-2 singles out H-4 of this residue as a new structural-reporter group [2,3]. Besides the observed alteration in the chemical shift of H-4, its $J_{4,5}$ value is slightly influenced by attachment of the Gal-3 group to C-3 (see Table 2). The small difference in $J_{4,5}$ between Gal-2 and Gal-3 in compound III, in combination with the difference in line width of Gal-3 signals as compared to those of Gal-2 (see later) permits the unambiguous assignments of H-5, H-6a and H-6b of these residues.

The chemical shift of H-1 of Gal-3 in compound III differs significantly from that for Gal-2 in compound II. Such a downfield position (δ = 4.615 ppm), has not been observed before in related substances for the anomeric proton of a terminal Gal*p* group β-linked to C-3 of another sugar residue, for example, δH-1 for the Gal residues in the β-anomers of the disaccharides β-D-Gal*p*-(1→3)-D-GalNAc and β-D-Gal*p*-(1→3)-D-GlcNAc is 4.44 and 4.42 ppm, respectively [16]; in β-D-Gal*p*-(1→3)-D-GalNAc-ol, it is 4.47 ppm [17]. Most of the vicinal and geminal coupling constants for both Gal residues in compound III are in reasonable accordance with those described for β-D-galactopyranosyl units [18–20].

Finally, the observed increases in line width of the H-1 doublets in going from Gal-3 via Gal-2 to Xyl-1 in the spectrum of compound III, and for the H-4 signal of Gal-2 as compared to that of Gal-3 (see Fig. 1A), are arguments in favour of the assignments given in Table 1 for these signals, since this again reflects the relatively large mobility of terminal residues (cf. [15]).

For reasons outlined above, 500-MHz ^{1}H-NMR spectra were recorded for Xyl-ol (compound IV), β-D-Gal*p*-(1→4)-D-Xyl-ol (compound V) and β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Xyl-ol (compound VI), as well as for their mono-deuterated analogues, in ^2H_2O at 300 K. The 500-MHz ^{1}H-NMR spectrum of compound VI is depicted in Fig. 2, and the detailed ^{1}H-NMR parameters of the glyco-xylitols are given in Tables 3 and 4. At 500 MHz, the spectrum of Xyl-ol, a symmetric alditol, is nearly first-order, enabling straightforward interpretation without use of complexing agents of shift reagents (cf. [21,22]).

Attachment of a β-D-Gal*p* group to C-4 of Xyl-ol produces a number of downfield shifts for the protons of the Xyl-ol residue in compound V; H-2 and H-4, and also the originally corresponding H-1 and H-5 atoms, become non-equivalent. The addition of another β-D-Gal*p* group (Gal-3) in β-(1→3) linkage to make compound VI produces few additional changes in the chemical shifts for the Xyl-ol protons. The effects upon the chemical shifts of the Gal-2 protons in going from compound V to VI, are analogous to the corresponding differences in going from compound II to III, although the chemical shifts of several protons of Gal-2 in compound V differ significantly from the corresponding ones in compound II, despite the common β-(1→4) linkage. However, this effect, resulting from having D-Xyl-ol as terminal residue instead of the β-D-Xyl*p*-(1→O)-L-Ser moiety, is confined to the chemical shifts of the protons of Gal-2; those of Gal-3 are closely similar for compounds III and VI. The H-1 signal for Gal-2 in compound V is found at lower field than that of H-1 in Gal-2 in compound II (δ = 4.560 ppm as compared to 4.471 ppm). The resonances of H-1 for Gal-2 and Gal-3 in VI coincide at δ = 4.620 ppm.

The spectrum of the mono-deuterated analogue of V, i.e. β-D-Gal*p*-(1→4)-D-[1-^{2}H$_1$]Xyl-ol, was found to be a superposition of two subspectra. These arise from the two alditols prepared by reduction of the corresponding disaccharide with NaB^{2}H$_4$, one having the deuterium atom instead of H-1a, and the other instead of H-1b of the D-Xyl-ol residue, as compared to V. They occur in the mixture in a ratio slightly different from 1. The subspectra differ with respect to the

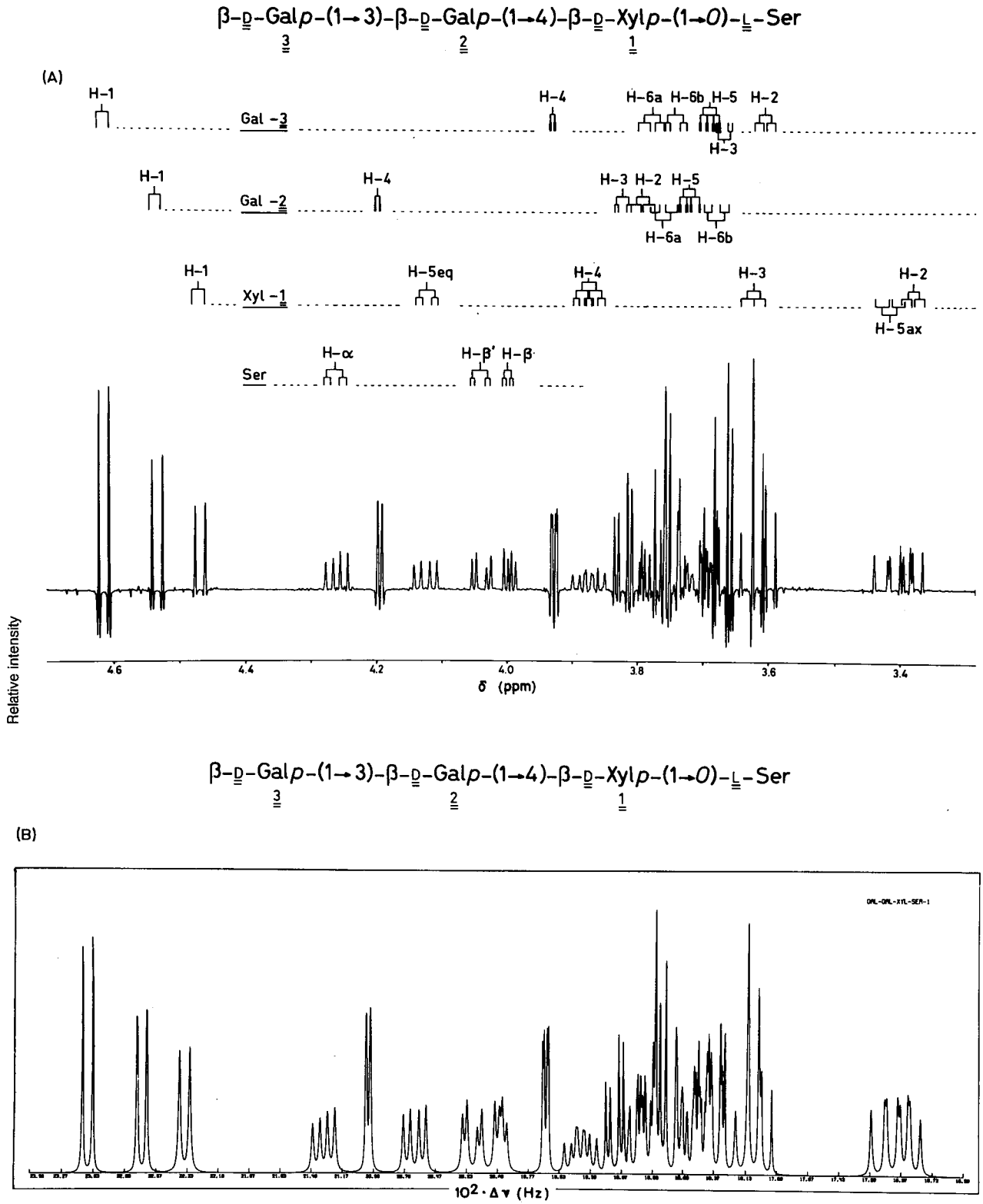

Fig. 1. *(A) Resolution-enhanced 500-MHz ^{1}H-NMR spectrum of β-D-Galp-(1→3)-β-D-Galp(1→4)-β-D-Xylp-(1→O)-L-Ser (compound III) in ^2H_2O at 300 K. (B) Computer-simulated 500-MHz ^{1}H-NMR spectrum of compound III*

position of the D-[1-$^2H_1$]Xyl-ol H-1 doublet ($\delta = 3.734$ vs 3.739 ppm), and also to that of the D-[1-$^2H_1$]Xyl-ol H-2 doublet of doublets ($\delta = 3.881$ vs 3.877 ppm); however, the $J_{1,2}$ value is identical (4.25 Hz) for both isomers. The same reasoning applies to the spectrum of the deuteride-reduced analogue of VI. Comparison of the spectra of the mixtures of the mono-deuterated disaccharide-alditols and trisaccharide-alditols with those of V and VI, respectively, offered the possibility of verifying the positions of the H-1a, H-1b, and H-2 resonances of the D-Xyl-ol residue in the latter.

The conformation of the sugar rings can be deduced by calculation of the dihedral angles (ϕ) between the protons in

Table 1. *^1H chemical shifts of constituent monosaccharides for three glyco-serines forming part of a frequently occurring proteoglycan linkage region*

Residue	Proton	Chemical shift in ppm		
		β-D-Xylp-(1→O)-L-Ser (I)	β-D-Galp-(1→4)-β-D-Xylp-(1→O)-L-Ser (II)	β-D-Galp-(1→3)-β-D-Galp-(1→4)-β-D-Xylp-(1→O)-L-Ser (III)
Ser	H-α	4.253	4.254	4.260
	H-β	3.985	3.989	3.995
	H-β′	4.021	4.032	4.038
Xyl-1	H-1	4.428	4.463	4.470
	H-2	3.321	3.376	3.381
	H-3	3.445	3.614	3.623
	H-4	3.621	3.853	3.874
	H-5ax	3.314	3.407	3.416
	H-5eq	3.971	4.114	4.124
Gal-2	H-1	–	4.471	4.534
	H-2	–	3.509	3.789
	H-3	–	3.646	3.821
	H-4	–	3.918	4.194
	H-5	–	3.701	3.718
	H-6a	–	3.802	3.760
	H-6b	–	3.747	3.678
Gal-3	H-1	–	–	4.615
	H-2	–	–	3.606
	H-3	–	–	3.668
	H-4	–	–	3.927
	H-5	–	–	3.690
	H-6a	–	–	3.776
	H-6b	–	–	3.742

Table 2. *Vicinal and geminal coupling constants of constituent monosaccharides for three glyco-serines forming part of a frequently occurring proteoglycan linkage region*

Residue	Coupling constant ($^3J_{vic}$ or $^2J_{gem}$)	Value of J in Hz		
		β-D-Xylp-(1→O)-L-Ser (I)	β-D-Galp-(1→4)-β-D-Xylp-(1→O)-L-Ser (II)	β-D-Galp-(1→3)-β-D-Galp-(1→4)-β-D-Xylp-(1→O)-L-Ser (III)
Ser	$^3J_{\alpha,\beta}$	5.2	5.5	5.5
	$^3J_{\alpha,\beta'}$	10.7	11.2	11.2
	$^2J_{\beta,\beta'}$	−2.5	−3.0	−3.4
Xyl-1	$^3J_{1,2}$	7.6	8.0	7.2
	$^3J_{2,3}$	9.15	9.0	9.3
	$^3J_{3,4}$	9.3	9.0	8.9
	$^3J_{4,5ax}$	10.2	10.2	10.2
	$^3J_{4,5eq}$	5.3	5.6	5.3
	$^2J_{5ax,5eq}$	−11.6	−12.0	−11.8
Gal-2	$^3J_{1,2}$	–	7.95	7.8
	$^3J_{2,3}$	–	9.8	10.0
	$^3J_{3,4}$	–	3.4	3.4
	$^3J_{4,5}$	–	0.9	0.7
	$^3J_{5,6a}$	–	8.7	8.9
	$^3J_{5,6b}$	–	3.5	5.8
	$^2J_{6a,6b}$	–	−11.6	−11.3
Gal-3	$^3J_{1,2}$	–	–	7.8
	$^3J_{2,3}$	–	–	9.85
	$^3J_{3,4}$	–	–	3.4
	$^3J_{4,5}$	–	–	1.1
	$^3J_{5,6a}$	–	–	7.9
	$^3J_{5,6b}$	–	–	4.25
	$^2J_{6a,6b}$	–	–	−11.7

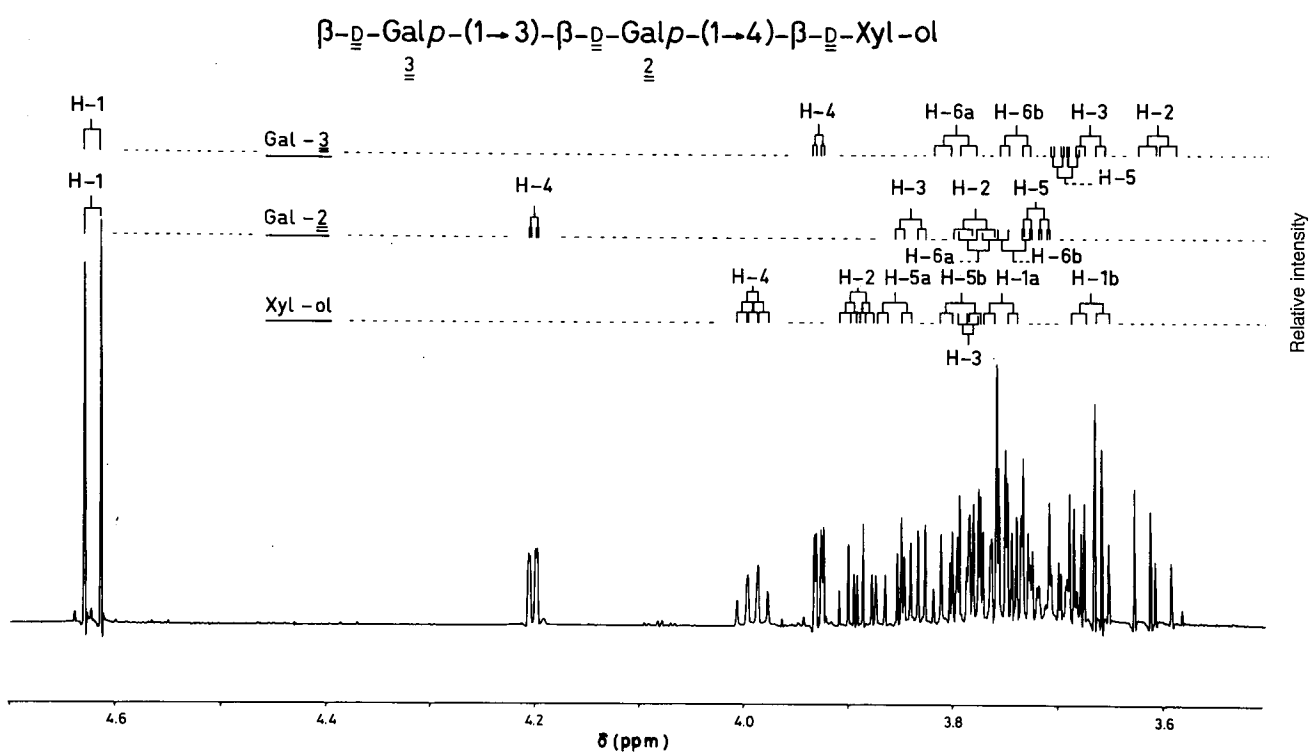

Fig. 2. *Resolution-enhanced 500-MHz ^1H-NMR spectrum of β-D-Galp-(1→3)-β-D-Galp-(1→4)-D-Xyl-ol (compound VI) in ^2H_2O at 300 K*

Table 3. *^1H chemical shifts of constituent monosaccharides for three oligosaccharide-alditols related to the glycan moiety of a frequently occurring proteoglycan linkage region*

Residue	Proton	Chemical shift in		
		Xyl-ol (IV)	β-D-Galp-(1→4)-D-Xyl-ol (V)	β-D-Galp-(1→3)-β-D-Galp-(1→4)-D-Xyl-ol (VI)
		ppm		
Xyl-ol	H-1a	3.699	3.756	3.755
	H-1b	3.624	3.672	3.668
	H-2	3.786	3.894	3.891
	H-3	3.624	3.785	3.789
	H-4	3.786	3.976	3.989
	H-5a	3.699	3.853	3.854
	H-5b	3.624	3.790	3.793
Gal-2	H-1	–	4.560	4.620
	H-2	–	3.563	3.778
	H-3	–	3.669	3.837
	H-4	–	3.927	4.200
	H-5	–	3.700	3.721
	H-6a	–	3.798	3.775
	H-6b	–	3.756	3.744
Gal-3	H-1	–	–	4.620
	H-2	–	–	3.608
	H-3	–	–	3.669
	H-4	–	–	3.926
	H-5	–	–	3.694
	H-6a	–	–	3.799
	H-6b	–	–	3.738

Table 4. *Vicinal and geminal coupling constants of constituent monosaccharides for three oligosaccharide-alditols related to the glycan moiety of a frequently occurring proteoglycan linkage region*

Residue	Coupling constant ($^3J_{vic}$ or $^2J_{gem}$)	Value of J in		
		Xyl-ol (IV)	β-D-Galp-(1→4)-D-Xyl-ol (V)	β-D-Galp-(1→3)-β-D-Galp-(1→4)-D-Xyl-ol (VI)
		Hz		
Xyl-ol	$^2J_{1a,1b}$	−11.7	−11.8	−12.0
	$^3J_{1a,2}$	4.3	4.3	4.1
	$^3J_{1b,2}$	6.8	7.2	7.2
	$^3J_{2,3}$	4.35	4.35	4.3
	$^3J_{3,4}$	4.35	4.9	4.9
	$^3J_{4,5a}$	4.3	4.6	4.6
	$^3J_{4,5b}$	6.8	5.4	5.3
	$^2J_{5a,5b}$	−11.7	−12.2	−12.5
Gal-2	$^3J_{1,2}$	–	7.8	7.8
	$^3J_{2,3}$	–	10.0	9.8
	$^3J_{3,4}$	–	3.45	3.4
	$^3J_{4,5}$	–	1.0	0.8
	$^3J_{5,6a}$	–	8.1	8.9
	$^3J_{5,6b}$	–	4.2	4.8
	$^2J_{6a,6b}$	–	−11.8	−11.6
Gal-3	$^3J_{1,2}$	–	–	7.8
	$^3J_{2,3}$	–	–	9.8
	$^3J_{3,4}$	–	–	3.4
	$^3J_{4,5}$	–	–	0.95
	$^3J_{5,6a}$	–	–	8.1
	$^3J_{5,6b}$	–	–	4.0
	$^2J_{6a,6b}$	–	–	−11.8

the ring fragments H-C-C′-H′ from vicinal coupling constants (Tables 2 and 4) by means of a modified Karplus equation (see, e.g., [23–25]). However, the values for the various dihedral angles for the glyco-serines as well as for the glyco-xylitols can easily be predicted from the similarity of the set of coupling constants found here, to those reported in the review of Altona and Haasnoot [26]. The angles for the Xyl and both Gal moieties in compounds I, II and III as well as for the Gal moieties in compounds V and VI are in agreement with a slightly flattened $^4C_1$ chair conformation (cf. [24]). The ring substituents do not seem to have a strong influence upon the chair conformations.

The difference observed between the values of the coupling constants $J_{5,6a}$ and $J_{5,6b}$ of Gal-2 with respect to those of Gal-3, both for III and VI, reflects a difference in the population of rotamers around the C-5/C-6 axis the terminal and the internal Gal residue [19,24]. Apparently, the rotational freedom of the $-CH_2OH$ group in Gal-2 with respect to the ring is hampered by the presence of the substituent at C-3. The flexibility around the C-5/C-6 axis of the Gal-3 group is as large as would be expected for a terminal Gal [19].

CONCLUSIONS

1. The 500-MHz ^{1}H-NMR spectra of three glyco-serines containing the structural fragments of the carbohydrate-protein linkage region of some animal connective-tissue proteoglycans have been interpreted in full detail. The same applies to the corresponding glyco-xylitols.

2. The chemical shift of H-4 of terminal β-D-galactopyranosyl groups is $\delta \approx 3.92$ ppm. This proton signal moves downfield to $\delta \approx 4.19$ ppm upon substitution by a β-D-galactopyranosyl group linked to C-3. This effect is also observed in the glyco-xylitol series making H-4 of the β-D-galactopyranosyl residue a structural-reporter group. The same shift effect has been observed by Dabrowski et al. [27]. This substitution also results in a small change in $J_{4,5}$ of the galactosyl residue in question. In high-resolution ^{1}H-NMR studies of *N*-glycosidic carbohydrate units containing repeating *N*-acetyllactosamine units β-(1→3) attached to each other [28,29], H-4 of the substituted Gal has proved to be extremely valuable for recognition of this type of polyglycosyl chain.

3. As to the three-dimensional conformation, the pyranosyl residues constituting this type of connective-tissue proteoglycan linkage region adopt a slightly flattened $^4C_1$ (D) chair conformation. In the seryl-trisaccharide, the flexibility of the glycosidic linkages increases going from the connexion between xylose to serine, towards the β-(1→3) linkage between the two galactosyl residues.

4. *O*-Glycosidically linked carbohydrate chains are often rather complex in glycoproteins as well as in proteoglycans. The present study furnishes a set of reference data, suitable for the investigation of oligosaccharides of gradually increasing complexity.

We are indebted to Dr W. E. Hull (Bruker Analytische Messtechnik, GmbH, Rheinstetten, FRG) for recording the 500-MHz ^{1}H-NMR spectra, and to Dr P. H. M. Budzelaar for his expert assistance with the computer simulations. This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO), by the Netherlands Foundation for Cancer Research (Grant UUKC-OC 79-13) and by the Swedish Natural Science Research Council.

REFERENCES

1. Montreuil, J. & Vliegenthart, J. F. G. (1979) in *Glycoconjugate Research; Proc. IVth Int. Symp. Glycoconjugates* (Gregory, J. D. & Jeanloz, R. W., eds) vol. I, pp. 35–78, Academic Press, New York.
2. Vliegenthart, J. F. G., Van Halbeek, H. & Dorland, L. (1980) in *IUPAC 27th Int. Congr. Pure Appl. Chem.* (Varmavuori, A., ed.) pp. 253–262, Pergamon Press, Oxford.
3. Vliegenthart, J. F. G., Van Halbeek, H. & Doeland, L. (1981) *Pure Appl. Chem. 53*, 45–77.
4. Montreuil, J. (1980) *Adv. Carb. Chem. Biochem. 37*, 157–223.
5. Kennedy, J. F. (1979) *Proteoglycans-Biological and Chemical Aspects in Human Life*, pp. 131–143, Elsevier Scientific Publishing Company, Amsterdam.
6. Aminoff, D., Gathmann, W. D., McLean, C. M. & Yadomae, T. (1980) *Anal. Biochem. 101*, 44–53.
7. Van Halbeek, H., Dorland, L., Veldink, G. A., Vliegenthart, J. F. G., Garegg, P. J., Norberg, T. & Lindberg, B. (1979) in *Proc. Vth Int. Symp. Glycoconjugates* (Schauer, R., et al., eds) pp. 100–101, Thieme, Stuttgart.
8. Lindberg, B. & Silvander, B. G. (1965) *Acta Chem. Scand. 19*, 530–531.
9. Erbing, B., Lindberg, B. & Norberg, T. (1978) *Acta Chem. Scand. B 32*, 308–310.
10. Garegg, P. J., Lindberg, B. & Norberg, T. (1979) *Acta Chem. Scand. B 33*, 449–452.
11. Lindberg, B., Rodén, L. & Silvander, B. G. (1966) *Carbohyd. Res. 2*, 413–417.
12. Roberts, G. C. K. & Jardetzky, O. (1970) *Adv. Protein Chem. 24*, 447–545.
13. De Bruyn, A., Hosten, N., Anteunis, M., Claeyssens, M. & De Bruyne, C. K. (1979) *Bull. Soc. Chim. Belg. 88*, 43–51.
14. Fournet, B., Montreuil, J., Strecker, G., Dorland, L., Haverkamp, J., Vliegenthart, J. F. G., Binette, J. P. & Schmid, K. (1978) *Biochemistry, 17*, 5206–5214.
15. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Schmid, K., Montreuil, J., Fournet, B. & Hull, W. E. (1980) *FEBS Lett. 114*, 11–16.
16. Herlant-Peers, M. C., Montreuil, J., Strecker, G., Dorland, L., Van Halbeek, H., Veldink, G. A. & Vliegenthart, J. F. G. (1981) *Eur. J. Biochem. 117*, 291–300.
17. Van Halbeek, H., Dorland, L., Haverkamp, J., Veldink, G. A., Vliegenthart, J. F. G., Fournet, B., Ricart, G., Montreuil, J., Gathmann, W. D. & Aminoff, D. (1981) *Eur. J. Biochem. 118*, 487–495.
18. De Bruyn, A., Anteunis, M., De Gussem, R. & Dutton, G. G. S. (1976) *Carbohyd. Res. 47*, 158–163.
19. De Bruyn, A. & Anteunis, M. (1976) *Carbohyd. Res. 47*, 311–314.
20. De Bruyn, A., Anteunis, M. & Verhegge, G. (1975) *Acta Cienc. Indica, 1*, 83–88.
21. Angyal, S. J., Greeves, D. & Mills, J. A. (1974) *Austr. J. Chem. 27*, 1447–1456.
22. Angyal, S. J. (1974) *Tetrahedron, 30*, 1695–1702.
23. Dorland, L., Schut, B. L., Vliegenthart, J. F. G., Strecker, G., Fournet, B., Spik, G. & Montreuil, J. (1977) *Eur. J. Biochem. 73*, 93–97.
24. Streefkerk, D. G., De Bie, M. J. A. & Vliegenthart, J. F. G. (1974) *Carbohyd. Res. 33*, 339–349.
25. Haasnoot, C. A. G., De Leeuw, F. A. A. M. & Altona, C. (1980) *Tetrahedron, 36*, 2783–2792.
26. Altona, C. & Haasnoot, C. A. G. (1980) *Org. Magn. Res. 13*, 417–429.
27. Dabrowski, J., Egge, H. & Hanfland, P. (1980) *Chem. Phys. Lipids, 26*, 187–196.
28. Karamanos, Y., Montreuil, J., Fournet, B., Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G. & Schmid, K. (1981) *Abstr. First Eur. Symp. Carbohydrates and Glycoconjugates*, Vienna, Austria, p. e5.
29. Vliegenthart, J. F. G., Dorland, L. & Van Halbeek, H. (1982) *Adv. Carb. Chem. Biochem. 41*, in the press.

H. van Halbeek, L. Dorland, G. A. Veldink, and J. F. G. Vliegenthart,
Afdeling Bio-Organische Chemie, Organisch-Chemisch Laboratorium, Rijksuniversiteit (te) Utrecht,
Croesestraat 79, NL-3522 AD Utrecht, The Netherlands

P. J. Garegg, T. Norberg, and B. Lindberg,
Institutionen för Organisk Kemi, Stockholms Universitet, Arrheniuslaboratoriet,
Bergiusvägen 65, Frescati, S-106-91, Stockholm, Sweden