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^{13}C-N.M.R.-SPECTRAL STUDY OF SOME MONO- AND DI-*O*-GALACTOSYLATED DIPEPTIDES. POSSIBLE STRUCTURAL PERTURBATIONS DUE TO *O*-GLYCOSYLATION

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ABSTRACT

Carbon-13 nuclear magnetic resonance data for mono- and di-*O*-α- and -β-D-galactosylated dipeptides composed of Thr and Gly are presented. The results conclusively show that peptide-bond formation does not affect the chemical shifts of the attached carbohydrate carbon atoms. In the case of the di-*O*-glycosylated threonylthreonine, no carbohydrate–carbohydrate interactions could be observed. For some of the mono-*O*-glycosylated dipeptides, the attached glycosyl group appears to have a peculiar effect on the chemical shifts of some of the carbon resonances of the amino acids.

INTRODUCTION

Glycoproteins are proteins to which carbohydrates are covalently attached[1–6]; they are widely distributed throughout Nature, and are by far the predominant form of extra-cellular proteins[3]. Glycoproteins are known to be involved in enzymic catalysis, ion transport, surface protection, lubrication, molecular recognition, cell–cell adhesion, and a variety of other physiological functions[5].

For some glycoproteins, it has been postulated that the carbohydrates are necessary for maintaining the structure (conformation) and function of the molecule[7–10]. The functional conformation of porcine ribonuclease B and glucoamylase from *Aspergillus niger* depends on oligosaccharide chains covalently bound to amino acids (such as Asn, Thr, and Ser) throughout the molecule[7,11]. However, in the human-erythrocyte membrane-glycoprotein, glycophorin A, the display of the MN determinants and some general receptor functions appear to be associated with the carbohydrate residues which are located only near the N-terminal region[12]. In this particular case, the points of glycosylation are not distributed randomly (and far

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apart), but are found to be rather close to each other. (In some of them, 6 and 3 points of glycosylation have been found consecutively[12].)

Due to the fact that glycoprotein conformation may depend on the attached oligosaccharide chains, as sometimes assumed in the literature, it would be of interest to obtain information about the effects that glycosylation may have on that local conformation. Moreover, it would also be of interest to ascertain whether neighboring glycosylation may produce steric interactions (possibly, carbohydrate–carbohydrate) which might also affect protein conformation.

In order to test these hypotheses, we have used natural-abundance, carbon-13, nuclear magnetic resonance spectroscopy (^{13}C-n.m.r.) to gain structural information about some simple mono- and di-glycosylated dipeptides [α,β-D-galactose (α,β-D-Gal) covalently bound to dipeptides containing L-threonine (Thr) and glycine (Gly); see Tables I, II, and III]. ^{13}C-N.m.r. spectroscopy had previously been used to gain structural information about glycopeptides[13–18] that might be relevant to glycoproteins, and about the possible carbohydrate–protein interactions that may exist in glycoproteins[19].

Our studies of α,β-D-Gal mono- and di-glycosylated dipeptides composed of Thr and Gly show results that may be helpful in studying glycoprotein structure by ^{13}C-n.m.r. spectroscopy. It appears that peptide-bond formation does not significantly affect the chemical shifts of the carbon atoms of the glycosyl group. The data for some of our monoglycosylated dipeptides indicate that there may be some protein–carbohydrate–protein interactions that result in peculiar, chemical-shift changes of the peptide carbon resonances. The results for our diglycosylated dipeptide indicate that there appears to be no carbohydrate–carbohydrate interaction.

EXPERIMENTAL

Materials and methods. — The glycopeptides α- and β-D-Gal→L-Ser were synthesized as already described[14]. The synthesis of the mono- and di-glycosylated dipeptides composed of L-Thr and Gly (see Tables I, II, and III) will be published shortly[20]. Samples for n.m.r. spectroscopy were prepared by dissolving the glycopeptide in de-ionized, distilled water. Adjustments of the pH of the samples were made with M NaOH, or HCl, using a Radiometer PHM63, digital pH-meter.

^{13}C-N.m.r. spectra were recorded with a JEOL-FX90Q instrument operating at 2.1 T (21 kG)–22.5 MHz in the F.t. mode, by use of quadrature detection. Samples (1.5 mL) were contained in a 10-mm tube having a concentrically inserted, 5-mm tube containing D_2O to serve as the field-frequency lock. The probe temperature was maintained at 25° for all samples. For ^{13}C excitation, 90° radio-frequency pulses of 18 μs were used, and the carrier frequency was set 90 p.p.m. downfield from the ^{13}C resonance of Me_4Si. A spectral window of 5.5 kHz was used for recording the spectra. Fully proton-decoupled spectra were obtained when the noise-modulated, ^{1}H irradiation (having a bandwidth of 1.0 kHz) was centered 4 p.p.m. downfield

from Me_4Si. Spectra with ^{13}C–^{1}H coupling were obtained by using a proton-decoupling technique the reverse of that employed for n.O.e. measurements[21].

Chemical shifts are given relative to a trace of internal 1,4-dioxane (added only when chemical shifts were determined), whose chemical shift was taken to be 67.86 p.p.m. downfield from Me_4Si.

RESULTS AND DISCUSSION

Tables I, II, and III give the chemical-shift data for α- and β-D-Gal→Thr, and also for α,β-D-galactose mono- and di-glycosylated threonylglycine, glycylthreonine, and threonylthreonine. All β-Gal and α-Gal model compounds exhibited anomeric coupling-constants ($^{1}J_{CH}$) of 158 Hz and 171 Hz, respectively. These values are in agreement with literature coupling-constants for galactose in the α- and β-pyranose (*p*) form[19,22,23]. This is one of the two most valuable criteria for determining whether the carbohydrate present that is bound to Thr is in the α- or β-

TABLE I

CARBON-13 N.M.R. CHEMICAL-SHIFT DATA[a] FOR GLYCOPEPTIDES CARRYING THE D-GALACTOSYL GROUP AT THE *N*-TERMINAL SIDE OF PEPTIDES **1–3**

Carbon atom	**3α**[b]	**3β**[c]	**2α**[d]	**2β**[e]	**1α**[f]	**1β**[g]
α1′	101.1		101.2		101.2	
α2′	69.9		69.8		70.0	
α3′, α4′	70.7[h]		70.7[h]		70.7[h]	
α5′	72.7		72.8		72.7	
α6′	62.6		62.6		62.6	
β1′		101.9		102.0		101.6
β2′		72.0		71.9		72.0
β3′		74.0		73.9		74.0
β4′		70.1		70.0		70.1
β5′		76.5		76.6		76.5
β6′		62.5		62.6		62.6
Thr C-3 (glycosylated)	76.8	75.0	76.7	74.7	75.6	74.5
Thr C-3 (nonglycosylated)	69.2	69.2				
Thr C-2 (glycosylated)	59.1	59.2	59.5	63.7	60.8	61.0
Thr C-2 (nonglycosylated)	62.3	62.5				
Thr C-4 (glycosylated)	18.6	17.0	18.8	17.2	19.9	18.2
Thr C-4 (nonglycosylated)	20.7	20.7				
Gly C-2			44.8	45.0		

[a]Chemical shifts for these compounds are given at neutral pH (5.5–7.5). Estimated precision for the chemical shifts is ±0.05 p.p.m. A recycle time of 1 s was used for recording the spectra, except those obtained from ref. 17. [b]155 mM sample in H_2O, pH 6.83; 9,603 accumulations. [c]79 mM sample in H_2O, pH 6.56; 10,000 accumulations. [d]128 mM sample in H_2O, pH 6.37; 11,900 accumulations. [e]134 mM sample in H_2O, pH 6.12; 4,722 accumulations. [f]See ref. 17. [g]See ref. 17. [h]Overlap of resonances.

TABLE II

CARBON-13 N.M.R. CHEMICAL-SHIFT DATA[a] FOR GLYCODIPEPTIDES CARRYING THE D-GALACTOSYL GROUP AT THE C-TERMINAL PART OF PEPTIDES **4** AND **5**

Carbon atom	**5**α[b]	**5**β[c]	**4**α[d]	**4**β[e]
$\alpha 1'$	100.6		100.6	
$\alpha 2'$	70.0		70.0	
$\alpha 3'$ }	70.9[f]		70.7[f]	
$\alpha 4'$ }				
$\alpha 5'$	72.6		72.5	
$\alpha 6'$	62.7		62.7	
$\beta 1'$		101.7		101.7
$\beta 2'$		72.2		72.2
$\beta 3'$		74.1		74.1
$\beta 4'$		70.2		70.2
$\beta 5'$		76.5		76.5
$\beta 6'$		62.7		62.7
Thr C-3 (glycosylated)	76.7	75.9	76.8	75.6
Thr C-3 (nonglycosylated)	67.0	67.8		
Thr C-2 (glycosylated)	60.3	61.5	60.4	61.2
Thr C-2 (nonglycosylated)	60.7	60.2		
Thr C-4 (glycosylated)	19.8	18.3	19.7	18.1
Thr C-4 (nonglycosylated)	20.0	20.0		
Gly C-2			43.2	42.2

[a]Chemical shifts for these compounds are given at neutral pH (5.5–7.5). Estimated precision for the chemical shifts is ± 0.05 p.p.m. A recycle time of 1 s was used for recording the spectra. [b]100 mM sample in H_2O, pH 6.32; 10,773 accumulations. [c]115 mM sample in H_2O, pH 5.70; 10,026 accumulations. [d]162 mM sample in H_2O, pH 7.50; 8,000 accumulations. [e]118 mM sample in H_2O, pH 6.83; 6,393 accumulations. [f]Overlap of resonances.

pyranose form[17,18]. The other is the use of chemical-shift patterns of the observed resonances.

Table I gives the chemical-shift data for the carbohydrate (primed numbers) and amino acid carbon atoms (unprimed numbers) of[17] α- and β-D-Gal→Thr (**1**α and **1**β), and for two dipeptides (Thr-Thr and Gly-Thr) that have α- and β-D-Gal covalently bound to O-3 of the amino terminal Thr by a glycosidic linkage. The assignments of the carbohydrate carbon atoms to specific resonances in the spectra were made from literature data[17,19,24]. Most amino acid carbon atoms were readily assigned to specific resonances in the spectra, based on the literature values[25] of chemical shifts for peptides containing Thr and Gly. *O*-Glycosylation of threonine results in a large, downfield shift for C-3, as expected[13–18]. In some cases, assignments of C-2 and C-3 (and, to some extent, C-4) were based on the pH-dependence of their chemical shifts due to the deprotonation of the carboxyl group, or the α-amino nitrogen atom[13–18], or both.

Table II gives the chemical-shift data for α- and β-D-Gal attached to the dipeptides Thr-Thr and Gly-Thr at O-3 of the C-terminal, threonine residue. Table III

TABLE III

CARBON-13 N.M.R. CHEMICAL-SHIFT DATA[a] FOR THE DIGLYCOSYLATED DIPEPTIDE 6[b]

Carbon atom	*Chemical shift*
α1′ (N-terminal)	101.3
α1′ (C-terminal)	100.8
α2′	70.0
α3′ }	70.7
α4′ }	
α5′	72.7
α6′	62.7
Thr C-3 (C-terminal) }	76.7[c]
Thr C-3 (N-terminal) }	
Thr C-2 (C-terminal)	60.8
Thr C-2 (N-terminal)	59.0
Thr C-4 (C-terminal)	19.6
Thr C-4 (N-terminal)	18.8

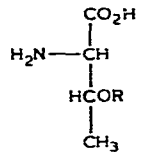
[a]Chemical shifts for this compound at pH 6.2 are given. Estimated precision for the chemical shifts is ±0.05 p.p.m. A recycle time of 1 s was used to record the spectrum. [b]147 mM sample in H_2O; 50,000 accumulations. [c]Broad resonance.

contains the chemical-shift data for the α-D-Gal diglycosylated dipeptide Thr-Thr (**6**). The resonance assignments to specific carbon atoms given in Tables II and III were based on the same criteria used to make the resonance assignments given in Table I.

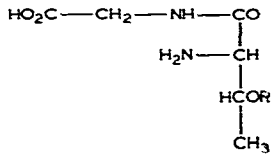
The chemical shifts for the carbohydrate carbon atoms of α-D-Gal in the various glycopeptides appear to be the same. This is also basically true for the carbohydrate carbon atoms of β-D-Gal of the various glycopeptides. These results conclusively prove that peptide-bond formation will have little, or no, effect on the chemical shifts of the carbon atoms of the carbohydrate attached to an amino acid or peptide moiety. This fact does not preclude the possibility that protein folding, in order to form a unique tertiary structure, may have some influence on the chemical shifts of the carbon atoms of the carbohydrate attached to the protein.

Because it appears that peptide-bond formation (see Tables I and II) and neighboring glycosylation (see Table III) do not seem to affect the chemical shifts of the carbohydrate carbon atoms, let us focus on the influence that glycosylation may have on the chemical shifts of various carbon atoms of the amino acids of the dipeptides, as the result may give an ultimate indication of how protein structure may be influenced by glycosylation.

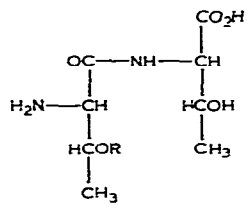
We may first compare the chemical-shift data compiled for the compounds in Table II. There would appear to be no chemical-shift differences observed for the carbon atoms of glycosylated threonine, as between compounds **4α** and **5α**, and **4β** and **5β** (see Table IV); this result indicates that glycosylation does not have any influence on the structure of the dipeptide. Conversely, the peptide formation also



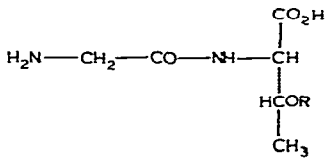
1α R = α-Galp
1β R = β-Galp



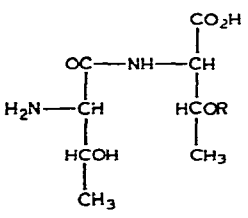
2α R = α-Galp
2β R = β-Galp



3α R = α-Galp
3β R = β-Galp



4α R = α-Galp
4β R = β-Galp



5α R = α-Galp
5β R = β-Galp

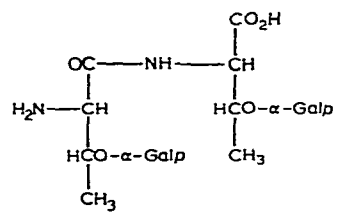


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TABLE IV

CHEMICAL SHIFT DIFFERENCES FOR GLYCODIPEPTIDES CONTAINING THREONINE, OR GLYCINE, OR BOTH

Carbon atom	*Chemical-shift differences (in p.p.m.) for various compounds*			
	3α-2α	3β-2β	5α-4α	5β-4β
α1′	−0.1		0.0	
β1′		−0.1		0.0
Glycosylated Thr C-2	−0.4	−4.5	−0.1	0.3
Glycosylated Thr C-3	0.1	0.3	−0.1	0.3
Glycosylated Thr C-4	−0.2	−0.2	0.1	0.2

does not affect the chemical shifts of α- and β-D-Gal (relative to **1α**, and **1β**). The comparison of compounds **3α** and **2α**, and also **3β** and **2β**, shows a somewhat different result (see Table IV). In the case of **3α** and **2α**, a change of amino acid, from Thr to Gly, shifts the signal of C-2 of the glycosylated threonine 0.4 p.p.m. downfield, but those of the carbohydrate carbon atoms remain unaffected. The chemical shift of glycosylated Thr C-3 is seen to be even more affected when **3β** and **2β** are compared (a difference of 4.5 p.p.m.). The chemical-shift data from compounds **4α**, **5α**, **4β**, **5β**, **2α**, and **3α** would seem to indicate that very little interaction exists between the amino acid residues and the carbohydrate residue. However, the data from compounds **3β** and **2β** suggest that the substitution of glycine for threonine may be influenced by the β-D-Gal residue. This peculiar result may be ascribed to a favored rotamer state of the threonine about the C-2–C-3 bond. In fact, this favored state may be charge-related (electrostatic interactions)[26], and therefore will be removed when the peptide moiety is elongated. There is further indication for this unusual phenomenon (see later).

We have so far monitored the chemical shifts of the carbon atoms of the D-galactosylated Thr of the various dipeptides in which the monosaccharide is in the same anomeric form. Now, let us compare model compounds in which the anomeric configuration of the galactose has been changed from α to β, and monitor the chemical shifts of all amino acid carbon resonances (see Table V). The simplest example to focus on is **1α** and **1β** (see Table I). There appears to be little chemical-shift difference (−0.2) for Thr C-2 of these compounds (see Table V). However, the anomeric carbon atom and C-4 show differences of −0.6 and +1.7 p.p.m., respectively. Previous ^{13}C-n.m.r.-spectral studies concerning α- and β-D-galactopyranosyl-L-threonine have shown (*i*) a rather small, chemical-shift difference between the signals for anomeric carbon atoms (< 1 p.p.m.), as compared with simple methyl glycosides for instance (~3 to 4 p.p.m.), due to the fact that β-anomeric carbon atoms are usually shielded in these compounds; and (*ii*) a concomitant shielding of Thr C-4 (~1.5–1.8 p.p.m.) in β-glycosyl amino acids. Both effects were ascribed to the existence of a favored orientation around the glycosidic linkage, due to the *exo*-anomeric effect[15,17,18].

Compare compounds **3α** and **3β**. The chemical shifts for the nonglycosylated threonine carbon atoms are unaffected by a change from α- to β-D-Gal (see Table V). The chemical-shift differences for the anomeric carbon atom (C-1′), C-2, and C-4 for the glycosylated threonine compare favorably with that for **1α** and **1β**. However, the chemical-shift difference for glycosyloxylated Thr C-3 has increased to 1.8 p.p.m., almost a 1-p.p.m. shift.

The comparisons of the anomeric carbon and amino acid chemical-shifts for **5α** and **5β** show a result slightly different from that for **3α** and **3β**. The results indicate that the carbon resonances of the nonglycosylated amino acid are now affected by the change in the anomeric state of the attached D-galactosyl group. The chemical shift of the resonances of C-2 and C-3 of nonglycosylated Thr shift by 0.5 and −0.8 p.p.m., respectively, when going from α- to β-D-Gal; the chemical shift of the resonance for

TABLE V

CHEMICAL-SHIFT DIFFERENCES FOR α- AND β-D-Gal GLYCODIPEPTIDES

Carbon atom	*Chemical-shift differences in p.p.m.*				
	1α-1β	2α-2β	3α-3β	4α-4β	5α-5β
D-Gal anomeric (C-1′)	−0.6	−0.8	−0.8	−1.1	−0.9
Glycosylated Thr C-2	−0.2	−4.2	−0.1	−0.8	−1.2
Glycosylated Thr C-3	1.1	2.0	1.8	1.2	0.8
Glycosylated Thr C-4	1.7	1.6	1.6	1.6	1.5
Gly C-2		−0.2		1.0	
Nonglycosylated Thr C-2			−0.2		0.5
Nonglycosylated Thr C-3			0.0		−0.8
Nonglycosylated Thr C-4			0.0		0.0

C-2 appears to be unaffected. This effect also appears to manifest itself in the chemical-shift differences displayed by the carbon atoms of glycosylated Thr. The chemical-shift differences for the anomeric carbon atom and glycosylated C-2 and C-3 were found to be −0.9, −1.2, and +0.8 p.p.m., respectively, when going from α- to β-D-Gal attached (see Table V); this amounts to a shift of ~0.4 p.p.m. for these resonances. Thr C-4 appears to be unaffected.

O-Glycosylation by α- and β-D-Gal of the dipeptides threonylglycine or glycylthreonine shows some more-pronounced, chemical-shift differences for the carbon resonances of the peptide. In the case of compounds **2α** and **2β**, glycosylated Thr exhibits large shifts for C-3 and C-2 resonances relative to those of C-3 and C-2 of **1α** and **1β**; their relative shifts are approximately +1.0 and −4.0 p.p.m., respectively (see Table V). The chemical shift of the C-2 resonance of glycine appears to be unaffected by a change in the anomeric state of the D-galactosyl group.

Compounds **4α** and **4β** also exhibit some chemical-shift differences, compared to **1α** and **1β**. The chemical-shift difference for the anomeric carbon atom (C-1′) of Gal and for glycosyloxylated C-2 is −1.1 and −0.8 p.p.m., respectively; this is a shift of 0.5 p.p.m. for the anomeric-carbon resonance and Thr C-2, relative to **1α**, and **1β**. The chemical shift of the C-3 and C-4 resonances of glycosylated Thr appear to be unaffected. Here, Gly C-2 also exhibits a 1-p.p.m. chemical-shift difference when threonine is glycosylated by either α- or β-D-Gal.

It is clear from these results that the carbohydrates may indeed have a steric, and hence, a conformational effect on a protein. This steric effect may manifest itself in the amino acid to which the carbohydrate is attached, or it may also be observed, in some cases, in neighboring amino acids of a glycoprotein. It would appear, then, that glycosylation (especially by a bulky, oligosaccharide) may affect the tertiary structure of a glycoprotein.

Table III gives the chemical-shift data for α-D-Gal attached to a Thr-containing dipeptide. For compound **6**, the chemical shifts of the signals of the carbon atoms

of the two galactosyl groups are identical, except for α1′. The chemical shift for one of the anomeric carbon atoms matches that for the anomeric carbon atom of compound **3α**, and the other matches that for **5α**. Therefore, the anomeric-carbon resonances in the spectrum of compound **6** (see Table III) were then assigned by using the chemical-shift data from **3α** and **5α**. The data in Table III conclusively show that the monosaccharide residues (neighboring glycosylation) do not interact sterically. Moreover, on comparing the amino acid chemical-shift data for **3α**, **5α**, and **6**, our results indicated that α-D-Gal diglycosylation appears to have no added steric effect on the dipeptide (other than that which may have been introduced by monoglycosylation).

In conclusion, results from our model-compound studies yielded several important facts concerning the study of glycoproteins by ^{13}C-n.m.r. spectroscopy. (*i*) Peptide-bond formation does not significantly affect the chemical shifts of the carbon atoms of the attached carbohydrate residue. However, this does not rule out the possibility that protein folding may influence the chemical shifts of the carbohydrate carbon atoms. (*ii*) Neighboring glycosylation (by monosaccharides) does not result in any carbohydrate–carbohydrate interactions. However, important interactions may exist if the neighboring oligosaccharides are rather bulky. (*iii*) There appear to exist in these mono- and di-glycosylated dipeptides some carbohydrate–protein interactions which may have some influence on glycoprotein structure.

REFERENCES

1 A. GOTTSCHALK (Ed.), *Glycoproteins, Their Composition, Structure and Function*, 2nd edn., Vol. 5A, Elsevier, Amsterdam, 1972.
2 M. I. HOROWITZ AND W. PIGMAN (Eds.), *The Glycoconjugates*, Vol. 1, Academic Press, New York, 1977.
3 N. SHARON, *Complex Carbohydrates, Their Chemistry, Biosynthesis and Functions*, Addison-Wesley, Reading, Massachusetts, 1975.
4 E. F. WALBORG (Ed.), *Glycoproteins and Glycolipids in Disease Processes*, ACS, Washington, D.C., 1978.
5 R. G. SPIRO, *Adv. Protein Chem.*, 27 (1973) 349–467.
6 P. V. WAGH AND O. P. BAHL, *CRC Crit. Rev. Biochem.*, 10 (1981) 307–377.
7 F. F. C. WANG AND C. H. W. HIRS, *J. Biol. Chem.*, 252 (1977) 8358–8364.
8 F. WIELAND, W. DOMPERT, G. BERNHARDT, AND M. SUMPER, *FEBS Lett.*, 120 (1980) 110–114.
9 J. H. PAZUR, H. R. KNULL, AND D. L. SIMPSON, *Biochem. Biophys. Res. Commun.*, 40 (1970) 110–116.
10 E. LISOWSKA, M. DUK, AND W. DAHR, *Carbohydr. Res.*, 79 (1980) 103–113.
11 J. H. PAZUR, K. R. FORRY, Y. TOMINAGA, AND E. BALL, *Biochem. Biophys. Res. Commun.*, 100 (1981) 420–426.
12 V. T. MARCHESI, *Semin. Hematol.*, 16 (1979) 3–20.
13 K. DILL AND A. ALLERHAND, *FEBS Lett.*, 107 (1979) 26–29.
14 J. M. LACOMBE, A. A. PAVIA, AND J. M. ROCHEVILLE, *Can. J. Chem.*, 59 (1981) 473–481.
15 A. A. PAVIA, S. N. UNG-CHHUN, AND J. M. LACOMBE, *Nouv. J. Chim.*, 5 (1981) 101–108.
16 A. ALLERHAND, K. DILL, E. BERMAN, J. M. LACOMBE, AND A. A. PAVIA, *Carbohydr. Res.*, 97 (1981) 331–336.
17 K. DILL, B. FERRARI, J. M. LACOMBE, AND A. A. PAVIA, *Carbohydr. Res.*, 98 (1981) 132–138.
18 K. DILL, R. E. HARDY, J. M. LACOMBE, AND A. A. PAVIA, *Carbohydr. Res.*, 101 (1982) 330–334.
19 E. BERMAN, A. ALLERHAND, AND A. L. DE VRIES, *J. Biol. Chem.*, 275 (1980) 4407–4410.

20 J. M. LACOMBE AND A. A. PAVIA, unpublished results.
21 D. SHAW, *Fourier Transform NMR Spectroscopy*, Elsevier, Amsterdam, 1976.
22 K. BOCK, I. LUNDT, AND C. PEDERSEN, *Tetrahedron Lett.*, (1973) 1037–1040.
23 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
24 P. A. J. GORIN AND M. MAZUREK, *Can. J. Chem.*, 53 (1975) 1212–1223.
25 K. RICHARZ AND K. WÜTHRICH, *Biopolymers*, 17 (1978) 2133–2141.
26 L. POGLIANI AND D. ZIESSOW, *Org. Magn. Reson.*, 17 (1981) 214–216.