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ON THE UTILITY OF ^{13}C-N.M.R. SPECTROSCOPY IN THE IDENTIFICATION OF THE PRIMARY STRUCTURES OF MANNO-OLIGOSACCHARIDES AND GLYCOPEPTIDES*

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ABSTRACT

The utility of ^{13}C-n.m.r. spectroscopy in the identification of the primary structures of mannose-containing glycans is investigated. Unlike ^{1}H resonances where the chemical shifts reflect multiple short- and long-range effects, the chemical shifts of ^{13}C resonances are dependent largely upon short-range effects classified as glycosylation (linkage) and substitution effects. These effects are parametized for glycans composed of mannose and encoded in a FORTRAN algorithm. Applications of this program to "unknown" sets of experimental chemical shifts for the resonances of anomeric carbons gave the following conclusions. (*1*) This program can be used to produce a sub-set of possible structures inclusive of the "known" structure. (*2*) For other than simple oligosaccharides, it is unlikely that a single structure is consistent with the data for anomeric carbons alone, even when the linkage composition of the glycan has been assessed from other spectral data. (*3*) When used in conjunction with other chemical techniques, this program can provide a powerful tool for primary analysis of the structure of mannose-containing glycans.

INTRODUCTION

The biochemical functions of glycoconjugates range from cell–cell recognition to the selective uptake of circulating serum proteins from the blood[1–3]. It is often necessary to determine the primary structure of a glycoconjugate in a non-

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destructive manner and in this context n.m.r. spectroscopy is invaluable. In conjunction with methylation analysis, ^{1}H-n.m.r. spectroscopy is well established[4–7] as a sensitive method for determining the complete primary structure of many *N*- and *O*-linked glycans with molecular weights up to 5000.

In principle, ^{13}C-n.m.r. spectroscopy should provide a useful adjunct technique to ^{1}H-n.m.r. studies especially for larger glycans, firstly, since ^{13}C-n.m.r. spectra of glycans exhibit greater spectral dispersion than ^{1}H-n.m.r. spectra. This property is especially important in low-field instruments (<300 MHz) where resonances are of marginal value. Because of the lack of H–C coupling in ^{13}C{^{1}H}-n.m.r. experiments, most resonances are singlets, which often allows the resolution even of signals of non-anomeric carbons. Secondly, larger-molecular-weight glycans exhibit increased line-broadening in ^{1}H-n.m.r. spectroscopy which can preclude definitive structural assignment when the glycan of interest is composed of only a few types of monosaccharide. Line-broadening of this type is less of a problem in ^{13}C{^{1}H} (proton decoupled) experiments[8]. Lastly, n.m.r. spectroscopy, as a tool for the structural analysis of glycans, remains largely an empirical technique and "proof" of a structure by ^{1}H-n.m.r. spectroscopy typically rests on the comparison with data for "structural reporter groups"[4]. *Ab initio* calculation of a ^{1}H-n.m.r. spectrum of a glycan might be attempted, but the sensitivity of the chemical shifts of many resonances to long-range effects ($\alpha\beta$-anomerization, sialylation, *etc.*)[4] make this task difficult. The chemical shifts of the signals for the anomeric carbon atoms of glycans are relatively independent of long-range effects.

^{13}C-N.m.r. spectroscopy has lagged behind ^{1}H-n.m.r. spectroscopy in its application to glycans mainly due to the need for relatively large samples. Thus, at least 5 μmol of glycan is required in a 1.7-mm microcell in a low-field instrument *versus* 25 nmol in a standard 5-mm tube for 500-MHz ^{1}H-n.m.r. spectroscopy[5]. Moreover, since there is not a ^{13}C{^{1}H}-n.m.r. experiment directly analogous to ^{1}H–^{1}H spin decoupling, the assignments of ^{13}C signals are usually based upon comparison with data for model compounds, although this is slowly changing with the application of 2D heteronuclear shift techniques[9–12]. Nevertheless, several investigators have applied ^{13}C-n.m.r. spectroscopy to the analysis of glycans[13–21]; in particular, Gorin[13,14] and Allerhand[15,16] and their co-workers have studied extensively the ^{13}C-n.m.r. spectra of yeast mannans and high-mannose glycopeptides, respectively.

Recently, Ogawa *et al.*[20,21] reported a ^{1}H- and ^{13}C-n.m.r. study of synthetic manno-oligosaccharides and established a set of empirical "rules" for rationalizing the chemical shifts for the resonances of anomeric carbons. Based upon their data and those of others, we have encoded these rules in a FORTRAN algorithm and utilized this program in an attempt to address the following questions. (*1*) Will this program successfully predict the primary structure of a manno-glycan that gives rise to a particular set of ^{13}C resonances for anomeric carbons? (*2*) How unique is a set of chemical shifts of the ^{13}C resonances of anomeric carbons to any particular structure? (*3*) Could this program serve as a tool for determination of the structure of manno-glycans?

RESULTS AND DISCUSSION

The chemical shift of the resonance of any given anomeric carbon in a polysaccharide is largely affected only by the type of linkage in which it is engaged (glycosidation shift) and the substitution (if any) of that residue by other monosaccharides (β and γ effects). A frequency histogram of the chemical shifts of the resonances of anomeric carbons of mannose reported in the literature[13–21] is shown in Fig. 1. Inspection of the histogram shows that the signals are segregated and that particular linkages and/or substitution patterns are associated with a specific range of chemical shift values, an observation first made by Gorin and co-workers[13]. This segregation can be used to construct a table of linkage and substitution shifts that can be added to an initial shift value considered to be due to the resonance of the unsubstituted and unlinked carbon, yielding calculated chemical shifts. As in the analysis by Ogawa and Sasajima[20], the chemical shift of the anomeric carbon of methyl α-D-mannopyranoside is used as a basal value. For β linkages, the chemical shift of the resonance of the anomeric carbon of methyl β-D-mannopyranoside is used[25]. Table I contains a summary of the linkage and substitution increments derived from Fig. 1. In addition, the resonance of a glycosidically substituted

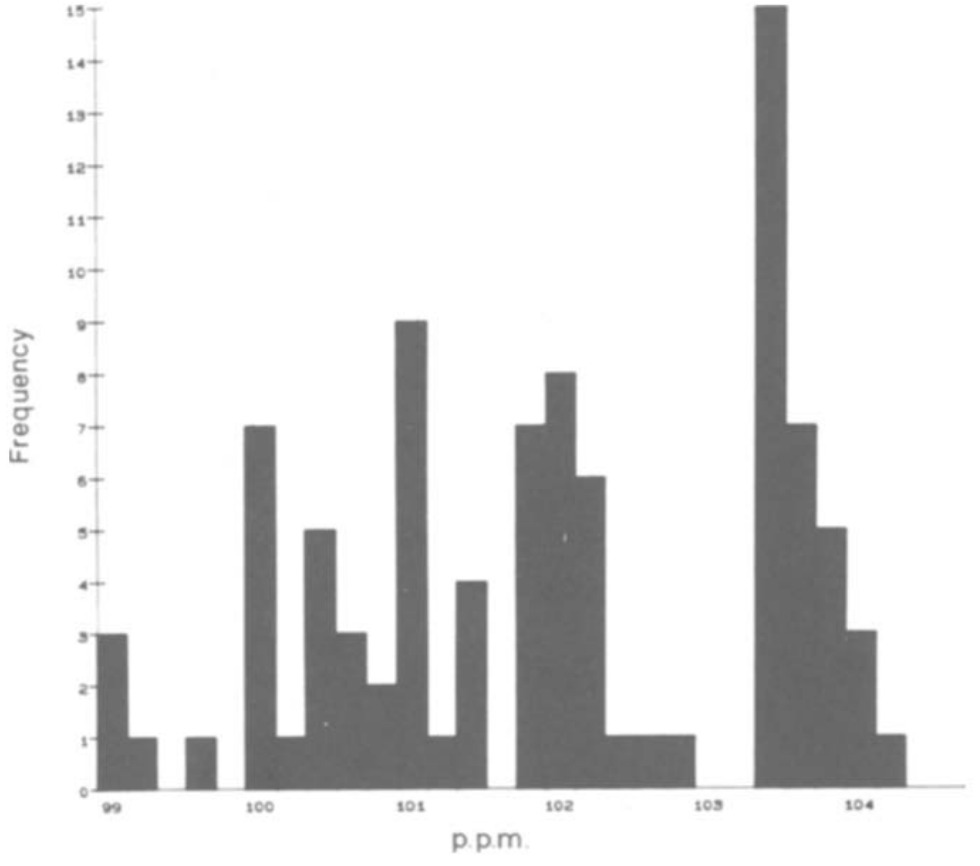

Fig. 1. Frequency histograph of the chemical shifts of the resonances of anomeric carbons for mannose residues. The chemical shifts reported in the literature are plotted *versus* their frequency of occurrence, and 92 values constitute the data base. Although the observed frequencies may be skewed as a result of the type of manno-glycans studied to date, the segregation along the chemical shift axis of particular linkage and/or substitution types is unambiguous. For example, the resonance of C-1 of a 2-substituted, 6-linked mannose residue is found in the range 99.4–100.5 p.p.m., whereas those of C-1 of terminal, (1→2)-, or (1→3)-linked residues are observed in the range of 103.5–104.2 p.p.m.

TABLE I

LINKAGE AND SUBSTITUENT EFFECTS FOR CALCULATION OF THE CHEMICAL SHIFTS OF THE RESONANCES OF ANOMERIC CARBONS[a,b]

	Linkage to:	*Substitution at:*
C-2	+1.1	−1.8
C-3	+0.7	−0.9
C-4	−0.1	−0.1
C-6	−1.6	−0.1

[a]In p.p.m. The chemical shift of the resonance of C-1 in α-Man*p*-OMe is 102.9 p.p.m., and 102.5 p.p.m. for β-Man*p*-OMe. [b]If a Man residue is 3-linked and further 3,6-disubstituted, an additional increment of 1.2 p.p.m. is added to the calculated shift value of the resonance of the anomeric carbon.

carbon undergoes a large downfield displacement (α effect). The magnitude of this shift is variable (4–8 p.p.m.), but often displaces these signals into discrete "windows" which can be utilized to determine the linkage composition of the polysaccharide. Thus, the resonances of glycosidically substituted C-2 or C-3 of α-mannose moieties are found in the range 78–82 p.p.m. and for those substituted at C-6 in the range 64–68 p.p.m.

The validity of these observations was demonstrated further by utilizing the synthetic trisaccharide α-Man*p*-(1→6)-[α-Man*p*-(1→3)]-α-Man-OMe, the ^{1}H-n.m.r. spectrum of which has been characterized fully[34]. Fig. 2 shows the corresponding 2D heteronuclear shift-correlated spectrum. Unambiguous assignment of the observed ^{13}C anomeric signals is straightforward. The observed chemical shifts of the resonances of the anomeric carbons are consistent with the data presented in Table I. The calculated values of the chemical shifts of the resonances of the anomeric carbons of the α-Man*p*-(1→3), α-Man*p*-(1→6), and α-Man*p*-OMe moieties are 103.6, 101.3, and 101.9 p.p.m., respectively, and the corresponding experimental values are 103.7, 100.7, and 102.3 p.p.m. Placement of the calculated chemical shifts on the histogram in Fig. 1 invariably locates them in the same subgroup as the experimental shifts and, in this context, the error in the calculated examples above (as much as 0.6 p.p.m.) is not important.

Application to other manno-oligosaccharides. — The ^{13}C{^{1}H}-n.m.r. data of Ogawa and Sasajima[20] on chemically synthesized, branched manno-oligosaccharides were used to test the utility of the FORTRAN algorithm, and the results are listed in Table II. In each of the examples studied, the structure reported to give rise to the experimental ^{13}C[^{1}H]-n.m.r. spectrum was included in the set of predicted structures. As shown by the results in Table II, analysis of the ^{13}C[^{1}H]-n.m.r. spectrum alone yields a small sub-set of possible structures. Thus, in the absence of complementary structural techniques, the ^{13}[^{1}H]-n.m.r. data can predict a limited, although not necessarily unique, number of solutions.

Table III shows the results obtained by the analysis of the ^{13}C[^{1}H]-n.m.r. data of Ogawa and Sasajima[20] for (Man*p*)$_4$-Man*p*. This example was chosen be-

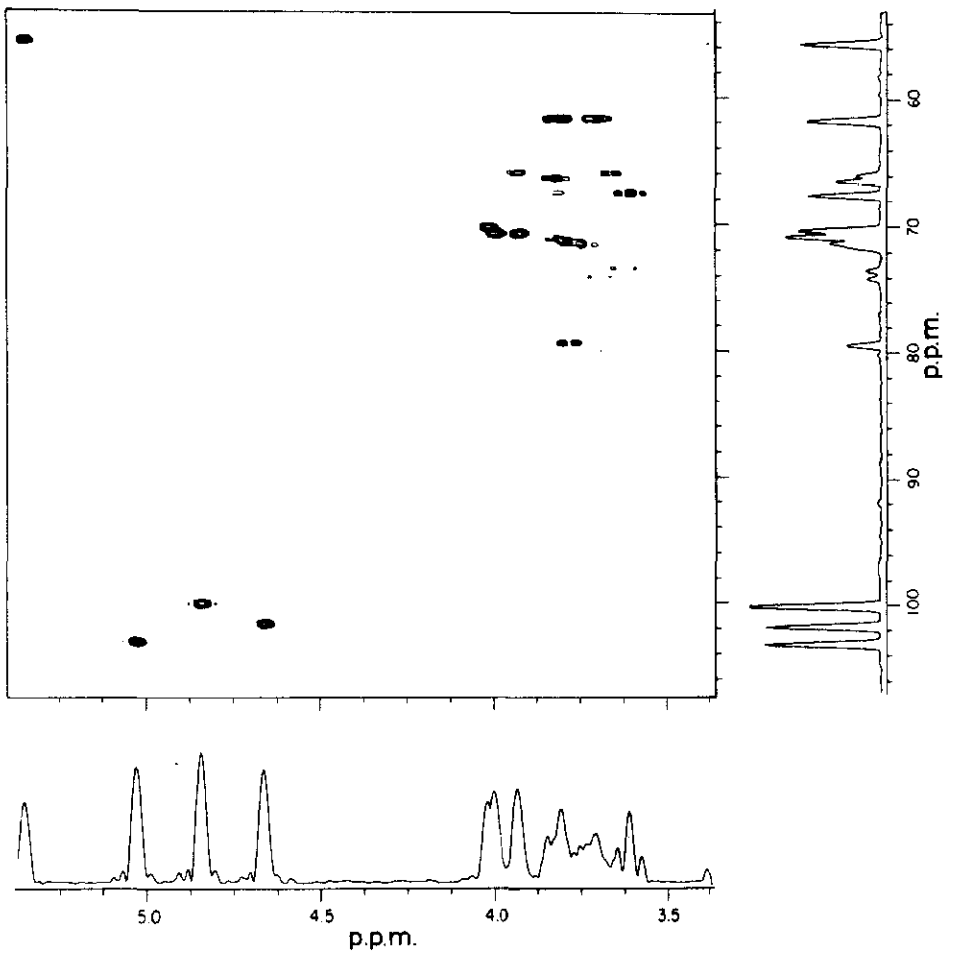

Fig. 2. Two-dimensional heteronuclear shift-correlated spectrum of the trisaccharide α-Man*p*-(1→3)-[α-Man*p*-(1→6)]-α-Man*p*-OMe. Details of the experiment are given in the text. Since ^{1}H-n.m.r. assignments for all the hydrogen atoms in the trisaccharide have been reported[34], unambiguous assignment of the observed carbon signals is possible. The peak appearing in the upper left-hand corner is due to the OMe groups with the methyl protons folded over in the ^{1}H dimension.

cause of the biological significance of this structure. The solutions obtained illustrate the strengths and weaknesses of the analysis of the resonances for anomeric carbons. With no linkage constraints, 20 possible solutions were found (15 monobranched and 5 dibranched structures). Linear oligosaccharides could have been included easily in this analysis but were omitted for the sake of brevity. If linkage constraints (derived from the estimated linkage composition of the glycan inferred from the glycosylation-induced downfield shifts of the signals for substituted skeletal carbons) were employed {*i.e.*, two Man*p*-(1→6) and two Man*p*-[1→3(2)] residues}, only five possible solutions were obtained (denoted by *). Further inspection of the data in Table III indicates that the major weakness of the analysis (and perhaps that of ^{13}C{^{1}H}-n.m.r. in general) is the inability to distinguish branching patterns from a set of anomeric signals. For example, solutions 6 and 7 in Table III differ only in which arm is terminally substituted with a Man*p*-(1→6) residue. The examples considered may be generalized from Table II to any di-substituted mannose where one of the substituent mannose residues is either 4- or 6-linked. Each of these substitutions exhibits small β,γ-effects.

Application to high-mannose glycopeptides and polysaccharides. — The region for anomeric resonances was analyzed for the ^{13}C{^{1}H}-n.m.r. spectra of high-

TABLE II

ANALYSIS OF THE CHEMICAL SHIFTS OF THE RESONANCES OF THE ANOMERIC CARBONS OF VARIOUS MANNO-OLIGOSACCHARIDES[a]

Compound	*Constraint*[b]	*No. of solns.*[c]	*No. of trials*	*No. of branches in solution*	
				1	*2*
M2M6-[M2M3]M-OMe	none	2[1]	256	2[1]	
M2M4-[M2M2-]M-OMe	none	5[1]	256	5[1]	
M2M6-[M2M2-]M-OMe	none	4[4]	256	4[4]	
M6-[M3-]M6-[M3-]M-OMe	none	20[5]	768	15[3]	5[2]
M3-[M6-]M3-[M6-]M-OMe	none	20[5]	768	15[3]	5[2]
M6-[M2-]M6-[M3-]M-OMe	none	7[2]	768	5[1]	2[1]
M6-[M3-]M6-[M2M3]M-OMe	no α-(1→4)	17[8]	972	12[5]	5[3]
M3-[M6-]M3-[M2M6-]M-OMe	no α-(1→4)	29[11]	972	9[4]	20[7]
M6-[M2-]M6-[M2M3]M-OMe	no α-(1→4)	15[5]	972	11[4]	4[1]

[a] $^{13}C\{^1H\}$-n.m.r. chemical shift data are taken from ref. 20. M connotes α-Man*p*. Thus, M2M6- represents the disaccharide moiety α-Man*p*-(1→2)-α-Man*p*-(1→6)-. [b] Possible linkage permutations from which calculated chemical shifts are derived are limited as indicated. [c] Number of output structures whose calculated anomeric chemical shifts match experimental values. The number of trials corresponds to the number of structures from which shifts are calculated. The number in brackets corresponds to the number of solutions also consistent with the linkage composition of the glycan estimated from the spectral shifts of the resonances of glycosidically substituted carbons (*i.e.*, C-2, C-3, C-4, and C-6) downfield from those for the unsubstituted positions.

mannose oligosaccharides derived from soybean agglutinin and thyroglobulin as well as the two high-mannose glycopeptides from ovalbumin reported by Berman and Allerhand[15]. These glycans were analyzed, in part, because they typically exist as a single structural isomer. In contradistinction, the (Man*p*)$_8$- and (Man*p*)$_7$-glycans can be isolated as several structural isomers[26].

Analysis of the endo-H-released high-mannose oligosaccharides or their respective glycopeptides is possible with the program if (*1*) special allowance is made for the β-linked mannose residue in the "core" of the glycans, and (*2*) for glycopeptides, the resonance for the anomeric carbon of the β-Man*p*-substituted GlcNAc*p* signal is excluded from the experimental data set. The resonance of this carbon appears[15–16] at 102.6 p.p.m. In each of the following calculations, the mannose moiety closest to the reducing end is taken to be β. In addition, the program was constrained to ignore linkage combinations which include an α-Man*p*-(1→4) moiety. This is not essential, but is based solely upon the lack of evidence in the literature for such a moiety in these compounds.

Analysis of the reduced oligosaccharides (Man*p*)$_5$-GlcNAc-ol and (Man*p*)$_6$-GlcNAc-ol is shown in Table IV. Although a smaller number of permutations were analyzed (due to the constraints noted above), an otherwise identical set of structures was obtained as solutions compared to those of the analogous synthetic manno-oligosaccharide. In part, this situation is due to the fact that the β-mannose

TABLE III

CALCULATED SOLUTIONS FROM THE ^{13}C ANOMERIC CARBON DATA OF α-Man*p*-(1→6)-[α-Man*p*-(1→3)]-α-Man*p*-(1→6)-[α-Man*p*-(1→3)]-α-Man*p*-OMe[a]

1	M2-[M2M3]M2-M-OMe	11	M2-[M3-]M3-[M6-]M-OMe
2	M3-[M2M2-]M2-M-OMe	12*	M3-[M6-]M3-[M6-]M-OMe
3	M2-[M2M4-]M2-M-OMe	13	M2-[M6-]M4-[M3-]M-OMe
4	M2-[M3M6-]M2-M-OMe	14	M3-[M6-]M4-[M2-]M-OMe
5	M6-[M3M4-]M2-M-OMe	15*	M3-[M6-]M6-[M3-]M-OMe
6	M2-[M6M3-]M3-M-OMe	16	M2M2-[M2M4-]M-OMe
7	M3-[M6M2-]M3-M-OMe	17	M2M2-[M3M6-]M-OMe
8*	M3-[M6M6-]M3-M-OMe	18	M6M2-[M3M4-]M-OMe
9*	M6-[M6M3-]M3-M-OMe	19	M6M3-[M2M4-]M-OMe
10	M6-[M2M4-]M3-M-OMe	20*	M6M3-[M3M6-]M-OMe

[a]Experimental n.m.r. data taken from ref. 20. Solutions marked * are those obtained if linkage constraints are employed as described in the text. In this table, M connotes α-Man*p*. Thus, M2 represents and α-Man*p*-(1→2)-residue. Solution 15 corresponds to the structure whose anomeric chemical shifts were used as input.

is 4-linked. Thus, the glycosidation contribution to the chemical shift of the resonance of the anomeric carbon is minimal. In addition, comparison of the set of anomeric chemical shifts for the resonances of anomeric carbons for the endo-H-released oligosaccharides with the data of Berman and Allerhand[15] and Ogawa and Sasajima[20] yielded no significant differences. Most calculated signals were within 0.1 p.p.m. of the corresponding signal from the other sets of data. Either acetolysis

TABLE IV

ANALYSIS OF ANOMERIC CARBON CHEMICAL SHIFTS OF VARIOUS HIGH MANNOSE GLYCANS[a]

	No. of solns.[b]	*No. of trials*	*Branch points in solution*	
			1	*2*
Man_5[c]	5	243	3	2
Man_5 (endo-H released)	5	243	3	2
Man_6[c]	8	972	5	3
Man_9[d]	29	39,366	14	15
Toxin[e]	5	5103		
	30	6804		

[a]For glycopeptides, α-(1→4)-Man moieties are not considered and the reducing terminal Man residue is also assumed to be β. [b]The number of solutions in agreement with the linkage composition of the glycan was estimated from the number and type of glycosidically substituted carbon signals observed in the entire ^{13}C[^{1}H]-n.m.r. spectrum. [c]Glycopeptide chemical shift data taken from Berman and Allerhand[15]. [d](Man)$_9$-GlcNAc$_2$-Asn glycopeptide isolated from soybean agglutinin. [e]Polysaccharide toxin isolated[27–29] from *Streptococcus* B. A backbone of four (first line) or three (second line) (1→6)-linked β-Man residues with three 2-linked branches is supported by methylation analysis. A poly-(1→2)-linked backbone is inconsistent with experimental ^{13}C data. Linkage permutations were constrained to those consistent with a (1→6)-linked backbone and the first backbone mannose residue 2-substituted. Only tri-branched permutations were evaluated, again, based upon methylation analysis data.

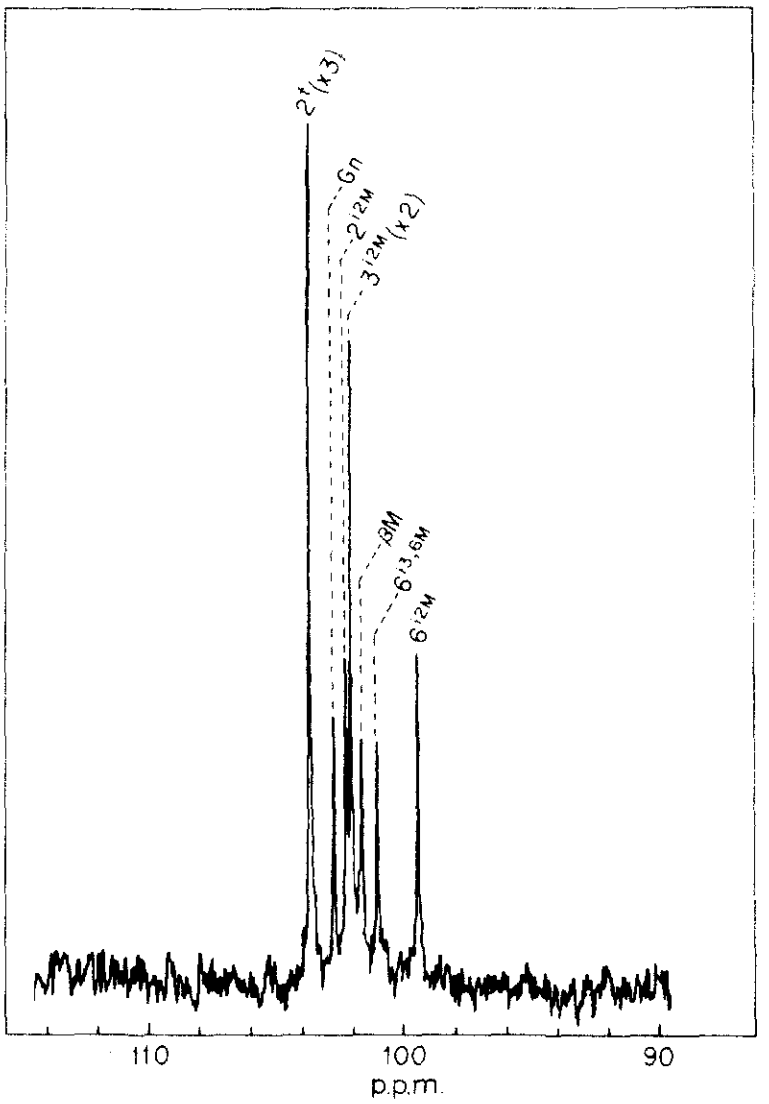

Fig. 3. Partial 90-MHz $^{13}C[^1H]$-n.m.r. spectrum of (Man*p*)$_9$-(Glc*p*NAc)$_2$-Asn. The region displayed shows the anomeric carbon signals for all hexopyranose residues except the Asn-linked Glc*p*NAc. Tentative assignments, based upon published data for smaller high-mannose glycans[15,16] and the shift perturbation data summarized in Table I, are shown. The nomenclature of the mannosyl residues is that of Carver and Grey[6].

or methylation analysis would yield further sub-sets of the structures in Table III. These two sub-sets have only a single structure in common, namely, the "correct" solution. Thus, both techniques would be necessary to specify a unique structure in conjunction with the ^{13}C-n.m.r. data.

In order to test further the capabilities of the program, the $^{13}C[^1H]$-n.m.r. spectrum of the (Man*p*)$_9$-(GlcNAc*p*)$_2$-Asn glycopeptide isolated from soybean lectin was analyzed. The ^{13}C-n.m.r. spectrum is shown in Fig. 3. The identity of this glycopeptide was confirmed by 360-MHz ^1H-n.m.r. spectroscopy[4,6]. There are ten signals in the region (95–105 p.p.m.) for anomeric carbons. By comparison with the data[15,16] of Allerhand and co-workers, the chemical shift of the resonance of the anomeric carbons of the β-Man*p*-substituted GlcNAc*p* residue at 102.6 p.p.m. can be discerned clearly. The signal for the anomeric carbon in the Asn-linked GlcNAc*p* is at ~79.5 p.p.m. and is outside this window.

The chemical shifts of the resonances of the remaining nine anomeric carbons were analyzed further with the algorithm. As shown in Table IV, of ~40,000 permutations tested, 29 possible structures were selected, 14 of which were mono-

branched and 15 were di-branched. Although this is still a substantial sub-set of structures, many of the predicted solutions possess common structural elements, *i.e.*, two 3,6-disubstituted mannose residues and a tetramannosyl arm. Each of the solutions predicts a 2- and a 3-substituted 6-linked mannose residue. As discussed for manno-oligosaccharides, a considerable degree of heterogeneity in the solutions is attributable to the inability to discriminate branching patterns. Because of the small shift-increment associated with 6-substitution, each of the mono-branched structures can be derived from those that are di-branched by shifting a 6-linked antenna to the terminus of the other branch. In addition, many of the predicted structures can be generated from the "correct" structure by shifting one or two mannose residues from the non-reducing end of the α-Man*p*-(1→3)-β-Man*p* arm to either of the other arms. Specification of a unique structure would require both acetolysis and methylation analyses. Each of these methods would generate a further sub-set of the ^{13}C predicted structures; these two sub-sets share a single common structure, namely, the "correct" solution.

Finally, the algorithm was applied to the $^{13}\{^{1}H\}$-n.m.r. data obtained for the polysaccharide toxin (M_r 250,000) isolated from cultures of Group B β-hemolytic streptococci[27–29]. Methylation analysis[30] of native and CrO_3-oxidized[31], acetylated material suggested repeating units containing nine mannose residues[27,28]. Methylation analysis of the native toxin indicated three branched, three 2,6-disubstituted, and single 2-, 3-, and 6-substituted mannose residues. Methylation analysis of the CrO_3-oxidized material demonstrated the 2,6- and the 6-substituted residues to be β, and the non-reducing terminal and 2- and 3-substituted residues to be α. Partial utilization of these data to constrain possible permutations in the algorithm resulted in evaluation of ~5100 structures of which 48 were consistent with the ^{13}C-n.m.r. data. Of these 48 structures, only five were consistent with the methylation analysis data. These structures represent permutations with side-chain substitutions on a backbone composed of four (1→6)-linked β-mannopyranosyl residues. Determination of the exact structure, including localization of critical phosphomannosyl residues, is in progress.

Thus, the above approach can provide useful data on the primary structure of glycans composed of mannose, especially when used in conjunction with analytical chemical techniques. Based upon the results, it is suggested also that it may not be possible to specify a single structure based upon the chemical shifts of the resonances of anomeric carbons. This conclusion is based upon the inability to discriminate branching patterns due to small substitution perturbations for (1→6) and (1→4) linkages. However, minor contributions from long-range effects (of the order of 0.3 p.p.m.) may allow more specific structures to be discerned (see the data of Ogawa and Sasajima[20]) if a sufficient body of reference data is created. Future improvements in the algorithm will encompass consideration of these long-range effects, consideration of substitution effects on the resonances of non-anomeric carbons, and extension to "complex" type glycans.

EXPERIMENTAL

Isolation of manno-oligosaccharides and glycopeptides. — (Man*p*)$_5$-GlcNAc-ol and (Man*p*)$_6$-GlcNAc-ol were prepared by pronase-digestion of porcine thyroglobulin and subsequent digestion of the glycopeptides with endoglycosidase H. The digest was reduced with [^{3}H]-$NaBH_4$ and neutralized with acetic acid, and boric acid was removed by reaction with methanol. The products were then applied to a column (1.0 × 300 cm) of Bio-Gel P-4 (⁻400 mesh) equilibrated in H_2O. Fractions (0.5 mL) were counted for radioactivity. Those corresponding to (Man*p*)$_5$-GlcNAc-ol and (Man*p*)$_6$-GlcNAc-ol were separately combined and re-chromatographed until paucidisperse profiles were obtained. The primary structure and purity of these fractions was confirmed by 600-MHz ^{1}H-n.m.r. spectroscopy performed at Carnegie–Mellon University. The manno-polysaccharide toxin from group B β-hemolytic Streptococcus (Type III) was isolated as described previously[27].

(Man*p*)$_9$(GlcNAc*p*)$_2$-Asn and the α-Man*p*-(1→6)-[α-Man*p*-(1→3)]-α-Man*p*-OMe were generous gifts from Dr. Jeremy Carver. The former was isolated[32] from soybean agglutinin and the latter was synthesized[33].

^{13}C-N.m.r. spectroscopy. — The spectra were recorded either on a JEOL FX-90Q (Department of Chemistry, Vanderbilt University) or a Nicollet NT-360 spectrometer (Toronto Biomedical NMR Centre, University of Toronto). Each of the spectrometers was operated in the F.t. mode at 22.5 and 90 MHz, respectively. At 22.5 MHz, an 8k data set was collected over a 5 kHz spectral width, using a 45° (6-μs) observe pulse. A solution of the sample (~5 mg) in D_2O was sealed in a standard melting-point capillary, and mounted in a 1.7-mm probe. Roughly 5–6 × 10^4 accumulations were collected at ambient probe temperature with a recycle time of 0.92 s. At 90 MHz, a 16k data set was collected over a 20 kHz sweep-width with a 90° (8-μs) observation pulse. Solutions of samples in D_2O were placed in 10-mm n.m.r. tubes, using 5-mm inserts. A total cycle time of 1.5 s was used. Broad-band decoupling was employed on both instruments.

Two-dimensional heteronuclear shift-correlated spectroscopy was performed at 90 MHz over a 5000-Hz sweep width with a 29.5-μs (90°) ^{13}C observe pulse and a 32.6-μs ^{1}H pulse generated from the decoupler. 512 acquisitions of 1k data points (real + complex) in each of 256 blocks were accumulated. Each F2 spectrum was apodized by Gaussian multiplication and a Hanning filter function followed by Fourier transformation. The data matrix was transposed and each resulting F1 spectrum was apodized by sine-bell multiplication and then subjected to Fourier transformation. A power spectrum of the final data set was obtained prior to display.

Computing facilities. — Program development was carried out either on a DEC-1099 system at the Vanderbilt University Computer Center or on a VAX 11/780 at the Ontario Cancer Institute.

Program description. — In the FORTRAN source code, manno-oligosaccharides are represented as a combination of linear oligosaccharides: a main backbone and a series (if any) of attached arms. Input consists of an estimate of the

total number of monosaccharide residues, the maximum number of branches, and possible sites for branch points. In addition, there is an input of the chemical shifts of the resonances of the anomeric carbon atoms of the experimental spectrum. In brief, the program generates all possible linkage permutations for all designated branching combinations (including variable number of monosaccharide residues in each branch), calculates a set of chemical shifts, and compares these shifts to the experimental set. The comparison is based upon the histogram in Fig. 1. Thus, the region (95–105 p.p.m.) for resonances of anomeric carbon atoms is divided into ten unequal sections. If an experimental or calculated shift has a value within the boundaries of a given section, a counter for that section is incremented. A "match" occurs if the array of calculated counters is identical to the experimental.

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REFERENCES

1 K. OLDEN, B. A. BERNARD, S. L. WHITE, AND J. B. PARENT, *J. Cell. Biochem.*, 18 (1982) 313–335.
2 W. J. LENNARZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, New York, Plenum Press, New York, 1980.
3 G. ASHWELL AND J. HARFORD, *Annu. Rev. Biochem.*, 51 (1982) 531–554.
4 J. F. G. VLIEGENTHART, L. DORLAND, AND H. VAN HALBEEK, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 209–374.
5 J. F. G. VLIEGENTHART, H. VAN HALBEEK, AND L. DORLAND, *Pure Appl. Chem.*, 53 (1981) 45–77.
6 J. P. CARVER AND A. A. GREY, *Biochemistry*, 20 (1981) 6607–6616.
7 J. P. CARVER, A. A. GREY, F. M. WINNICK, J. HAKIMI, C. CECCARINI, AND P. H. ATKINSON, *Biochemistry*, 20 (1981) 6600–6606.
8 J. SCHAFER, in *Topics in Carbon-13 NMR Spectrometry*, Wiley, New York, 1974, pp. 149–208.
9 A. A. MAUDSLEY AND R. R. ERNST, *Chem. Phys. Lett.*, 50 (1977) 368–372.
10 L. D. HALL, G. A. MORRIS, AND S. SUKUMAR, *J. Am. Chem. Soc.*, 102 (1980) 1745–1747.
11 A. BAX AND S. SUBRAMANIAN, *J. Magn. Reson.*, 67 (1986) 565–569.
12 L. LERNER AND A. BAX, *Carbohydr. Res.*, 166 (1987) 35–46.
13 P. A. J. GORIN, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 13–104.
14 P. A. J. GORIN, *Can. J. Chem.*, 51 (1973) 2375–2383.
15 E. BERMAN AND A. ALLERHAND, *J. Biol. Chem.*, 256 (1981) 6657–6662.
16 E. BERMAN, D. E. WALTERS, AND A. ALLERHAND, *J. Biol. Chem.*, 256 (1981) 3853–3857.
17 H. A. NUNEZ, F. MATSURA, AND C. C. SWEELEY, *Arch. Biochem. Biophys.*, 212 (1981) 638–643.

18 B. W. DUKSTRA, J. F. G. VLIEGENTHART, G. STRECKER, AND J. MONTREUIL, *Eur. J. Biochem.*, 130 (1983) 111–115.
19 R. BARKER, H. A. NUNEZ, P. ROSEVEAR, AND A. S. SERIANNI, *Methods Enzymol.*, 83 (1982) 58–69.
20 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.
21 T. OGAWA AND H. YAMAMOTO, *Carbohydr. Res.*, 104 (1982) 271–283.
22 P. COLSON, H. J. JENNINGS, AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
23 R. PROHASKA, T. A. W. KOERNER, I. M. ARMITAGE, AND H. FURTHMEYER, *J. Biol. Chem.*, 256 (1981) 5781–5791.
24 L. O. SILLERUD, R. K. YU, AND D. E. SCHAEFER, *Biochemistry*, 21 (1982) 1260–1271.
25 K. BOCK AND C. PEDERSON, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
26 T. TSUJI, K. YAMAMOTO, T. IRIMURA, AND T. OSAWA, *Biochem. J.*, 195 (1981) 691–699.
27 C. G. HELLERQVIST, J. ROJAS, R. S. GREEN, S. SELL, H. SUNDELL, AND M. T. STAHLMAN, *Pediat. Res.*, 15 (1981) 892–898.
28 J. ROJAS, C. PALME, M. L. OGLETREE, C. G. HELLERQVIST, K. L. BRIGHAM, AND M. T. STAHLMAN, *Pediat. Res.*, 18 (1984) 1141–1144.
29 C. G. HELLERQVIST, H. SUNDELL, AND P. GETTINS, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 51–55.
30 C. G. HELLERQVIST, B. LINDBERG, S. SVENSSON, T. HOLME, AND A. A. LINDBERG, *Carbohydr. Res.*, 8 (1968) 43–55.
31 C. G. HELLERQVIST, T. HOFFMAN, A. A. LINDBERG, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand.*, 26 (1972) 3282–3286.
32 J. A. GORDON, S. BLUMBERG, H. LIS, AND N. SHARON, *FEBS Lett.*, 24 (1972) 193–196.
33 F. M. WINNIK, J. P. CARVER, AND J. J. KREPINSKY, *J. Org. Chem.*, 47 (1982) 2701–2702.
34 J. R. BRISSON, F. M. WINNICK, J. J. KREPINSKY, AND J. P. CARVER, *J. Carbohydr. Chem.*, 2 (1983) 41–55.