Pure & Appl. Chem., Vol. 65, No. 4, pp. 763–770, 1993.
Printed in Great Britain.
© 1993 IUPAC

Oligosaccharides: How can flexible molecules act as signals?

J.P. Carver

Department of Molecular and Medical Genetics, Faculty of Medicine,
University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Abstract - There is considerable evidence that the majority of oligosaccharides are flexible, that is, they exist in many different conformations. How, then, can they act effectively as recognition signals? There must be a mechanism to reduce this flexibility so that the high-affinity, high-specificity, binding characteristic of "recognition" phenomena can be achieved. Specific sets of molecular interactions at individual glycosylation sites are suggested to be responsible. Through such specific interactions, subsets of the total population of a given oligosaccharide could be capable of acting as recognition signals. Since a dominant role is played by interactions with the protein surface at the glycosylation site, we term this mechanism "site-directed presentation". The previously described phenomenon of "site-directed processing" is simply a special case of the more general site-directed presentation phenomenon. Possible molecular models for this phenomenon are reviewed and the experimental bases presented.

INTRODUCTION

The extent to which cell surface carbohydrates are involved in biological recognition phenomena has been under investigation for decades (ref. 1). A major role for carbohydrates has been well established in the initial binding of many viral and bacterial pathogens. However, despite the early successes in the identification of blood group substances and the discovery of the asialo-glycoprotein receptor in liver, it is only recently that it has been unequivocally confirmed that carbohydrates provide a specific recognition mechanism between different mammalian cell types. The evidence for this mechanism has come from the recent identification of the carbohydrate ligands for the selectins, the molecules responsible for lymphocyte and neutrophil recruitment to endothelial cells at sites of inflammation (ref. 2).

When oligosaccharides bind to proteins it is usually with dissociation constants in the millimolar range and only occasionally, micromolar. Yet it is generally accepted that recognition phenomena require at least nanomolar dissociation constants because of the low effective concentrations of receptors. How, then, can oligosaccharides act as signals? Examination of the thermodynamics of oligosaccharide binding reveals the phenomenon of "entropy/enthalpy compensation" (refs. 3,4) previously described for a wide variety of binding phenomena (ref. 5). Molecular models for the basis of compensation, suggest some answers to the question of how oligosaccharides can act as signals. In the discussion below, experimental evidence in support of the various models is presented and reviewed. The models, in addition, point to possible strategies for the enhancement of oligosaccharide binding affinities.

ENTHALPY–ENTROPY COMPENSATION

What is meant by "compensation" is that, although the enthalpy of binding may vary widely between ligands, the free energy of binding varies much less because more favourable enthalpy of binding is "compensated" by a more *un*favourable entropy of binding. Thus, understanding how oligosaccharides can act as signals, translates into understanding how compensation is overcome in recognition systems.

Understanding the molecular basis for compensation is also important for the design of high-affinity, small-molecule, inhibitors of carbohydrate/protein recognition such as, for example, anti-inflammatory drugs.

The "compensation plot" is a convenient way of presenting the thermodynamic data for a wide variety of mono-, di- and oligosaccharides binding to proteins. For each interacting system, points are plotted with coordinates ($-\Delta H$, $-T\Delta S$). Many examples of such plots are to be found in the literature (refs. 3-7). Because $-\Delta H = -T\Delta S - \Delta G$, points representing interactions with identical ΔG will fall on a line of slope one and intercept $-\Delta G$. What is observed is that the experimental values of $-\Delta H$ and $-T\Delta S$ for oligosaccharides binding to proteins are scattered about a line with a slope of approximately 1.1, with an intercept of approximately -5 kcal/mole (ref. 4). Generally, larger ligands have correspondingly more negative values of ΔH and $T\Delta S$. The slope of slightly greater than one reflects the fact that larger ligands do have somewhat higher affinities; however, these higher affinities are much less than would be anticipated from the increasingly negative ΔH because of compensation. From these few observations it is clear that the majority of oligosaccharide binding to proteins is driven by favourable enthalpy of binding and opposed by an entropic barrier. There are a few reports of entropically favourable binding (refs. 7,8); however, the points for these interactions still fall on the same general line in the compensation plot if it is extended to the left of the vertical axis. It is essential that the origin of this entropic barrier be identified if we are to understand how oligosaccharides can act as signals and if we are to advance the design of carbohydrate inhibitors of oligosaccharide binding to proteins.

Models for compensation

After extensive review of the literature on a wide variety of binding phenomena, Lumry & Rajender (ref. 5) hypothesized, in 1970, that compensation arises directly from the properties of the aqueous medium in which the binding occurred. Subsequently, it was suggested by others (ref. 9) that losses of conformational degrees of freedom in the protein upon binding the ligand is the dominant contributor to the entropic barrier in the binding of ligands by proteins. The more extensive the contacts between the ligand and the protein, the more negative the enthalpy of binding and concomitantly the more negative the entropy of binding since larger regions of the proteins became rigidified - hence compensation. My laboratory has suggested a third model: that it is the loss of conformational entropy by the *ligand* on binding that provides the major source of the entropic barrier, at least for oligosaccharide ligands (ref. 4).

The Lemieux group first reported compensation in the binding of oligosaccharides to proteins (ref. 3). Initially they attributed the observed compensation to the second of the above hypotheses, losses in protein degrees of freedom. More recently, Lemieux (ref. 10) suggested that differential solvation effects are responsible for the compensation, a hypothesis closer to that of Lumry & Rajender (ref. 5) . Thus there are three prevailing models for the molecular basis of entropy/enthalpy compensation in the binding of oligosaccharides to proteins. All three mechanisms are undoubtedly operative to some extent. The question remains, however, as to which effect is predominant. In the discussion below, experimental evidence for these mechanisms will be presented and reviewed.

X-ray crystallographic evidence for losses in protein degrees of freedom

Christine Wright has solved, by X-ray diffraction, the crystal structures of isolectins 1 and 2 of wheat germ agglutinin (WGA) with and without the bound ligand, Neu5Ac(α2,3)Gal(β1,4)Glc (refs. 11-13). Although the uncomplexed isolectins 1 and 2 had previously been solved at 2.0 and 1.8 Å, respectively (refs. 12,13), the structures of the complexes were compared at 2.2 Å resolution because this was the best resolution available for both. A comparison of the B-factors for the uncomplexed and complexed protein reveals an overall reduction for the complexed form. More importantly, detailed comparison reveals that several amino acid side chains in the binding site exhibit much lower B-factors in the complex compared to the uncomplexed protein. Other side chains are unaffected by complex formation, having either large or small B-factors in both forms of the protein. In addition, reductions in B-factor are observed remote from the binding site, at the interface between the two monomers which make up the dimer. Since the binding site is located at the interface between the two monomers, Wright suggested

that the binding of ligand reduces the relative mobility of the monomers with respect to each other as well as reducing the mobility of specific side chains in the binding site itself. How general this will prove to be must await the accumulation of many more such comparisons. Also, at this point it is not clear how large a contribution to the entropic barrier the observed reduction in mobility would represent.

Experimental evidence for differential solvation

Lemieux (ref. 10) has determined the thermodynamic parameters for the binding of a wide range of modified Lewis b tetra-saccharides to the plant lectin *Griffonia simplicifolia* isolectin IV (GS-IV). In general the modifications fall into three categories: (i) those that have no effect on the thermodynamic parameters of binding; (ii) those that abolish binding; and, (iii) those which do not change the affinity, but do cause large compensating changes in the binding enthalpy and entropy. The modifications that fall into the second category generally alter groups which, in the crystal structure of the complex between ligand and protein, are seen to either donate hydrogen bonds to, or accept hydrogen bonds from, the protein (ref. 14). Hence the drastic loss of affinity is readily understood.

The more interesting category, from the point of view of compensation, is the third category where the affinity is relatively unchanged yet the entropy and enthalpy of binding vary widely. When these data are plotted as a compensation plot they fall on a straight line with slope close to one. However, the data points span a range of approximately 6 kcal/mole in both ΔH and $T\Delta S$. These modifications involve groups which appear to be located near the interface between the ligand and the protein, but which do not directly interact with the protein surface, as shown by the preliminary crystal structure of the complex (ref. 14). Lemieux has suggested, therefore, that the compensating alterations in binding entropy and enthalpy result from altered solvation at this interface (ref. 10). The derivative which exhibits the largest changes in enthalpy ($\Delta\Delta H$ = 6.7 kcal/mole) and entropy ($T\Delta\Delta S$ = 5.9 kcal/mole) is that in which the α1,4-linked fucose residue is substituted by its 3-deoxy derivative. One interpretation of the Lemieux model, would require that this modification lead to less water being bound at the interface between the ligand and the protein, hence creating a smaller entropic barrier together with the loss of favourable enthalpic interactions. Such a situation could arise if the 3-hydroxyl interacted with the protein surface through bound water(s) which were not bound in the absence of the ligand. The net increase in bound water on complex formation, through the creation of new binding sites in the complex, has been termed the "Reversed Hydrophobic Effect" by Goodford who has developed a program to locate water binding sites (ref. 15). An alternative explanation of these data, one which is in keeping with the next model, is presented below.

Experimental evidence for losses of ligand flexibility

Before arguing that the entropic barrier arises primarily from losses in ligand degrees of freedom, it is helpful to review the evidence for flexibility in oligosaccharides. The introduction of the ensemble representation for oligosaccharides in solution (refs. 16,17) was driven by the finding that, in many cases, NMR observables calculated as ensemble averages gave better agreement than those derived from single structures (refs. 16-18). Others have since obtained similar results (refs. 19-22). Three lines of evidence support the existence of flexibility about the torsional angles of glycosidic linkages in certain oligosaccharides and provide some insight into the time scale for the interconversions between conformations. These lines are: potential energy calculations; molecular dynamics simulations; and, comparisons of sets of NMR measurements obtained at different magnetic field strengths.

The kind of evidence available is illustrated below with data for the partially deuterated disaccharide, Man(α1,3)Manβ1-OR, which we have previously studied (refs. 23,24). Potential energy surfaces for this disaccharide show up to six broad, shallow, minima, the number depending on the approach used (ref. 25). In the *in vacuo* molecular dynamics simulations of this disaccharide all minima are sampled (ref. 26) and transitions occur between the different energy minima on a time scale of once every 10-100 ps. Similar results have been obtained with other disaccharides using a variety of force fields *in vacuo* (refs. 27-29). Simulations which include explicit water molecules, if they show any transitions, do so on a much longer time scale (28,29; J.P. Carver & J.W. Brady, unpublished results). In the aqueous simulations, the time scale of transitions is acutely sensitive to the balance between intra- and inter-molecular hydrogen bond strengths and, therefore, on an appropriate choice of charges (30; J.W. Brady,

personal communication). Such effects are of particular importance for oligo-saccharides because of their large number of potential hydrogen bonding groups. Because of these considerations, it is not yet clear how reliable are the findings of markedly slower rates of conformational transitions in aqueous simulations compared to those found in simulations in vacuo. Given this uncertainty, it is important to seek direct experimental determination of the time scale for transitions. Knowledge of these time scales is necessary since the calculation of NOE's requires some assumption as to their relative magnitudes (ref. 31).

Evidence from NMR for rapid internal motions in oligo-saccharides. The dependence of NOE values on the magnetic field strength is a direct way to probe the time scale of conformational transitions. If the time scale is comparable with the overall tumbling, as predicted by the *in vacuo* simulations, then this should be evident in these kinds of experiment. In particular, the ratio of the NOE's at two different field strengths should differ for those hydrogens relaxing with neighbouring hydrogens within fixed distances (i.e neighbours on the same pyranose ring) versus those relaxing with neighbours at fluctuating distances (i.e. across the glycosidic linkage) (ref. 32). The partially deuterated disaccharide, Man(α1,3)Manβ1-OMe, provides an ideal molecule with which to examine this question. Because of the substitution of deuterium for H-2',H-3',H-4',H6R' and H6S' of the α1,3-linked residue, the relaxation pathways are primarily across the glycosidic linkage for the remaining two protons of this residue; whereas, for the β-linked residue, the protons relax primarily with protons at fixed distances within the same residue. Determination of the ratio of NOE's at 300 MHz and at 500 MHz for both classes of relaxation pathway reveal a mean value of 1.2 for fluctuating distances and 1.6 for fixed distances (ref. 32, Table 1).

TABLE 1: Dependence of NOE's on magnetic field for the deuterated Man(α1,3)Manβ1-OCD_3

Irradiated proton	Observed proton	%NOE (300 MHz)	%NOE (500 MHz)	Ratio
	Relaxation through fixed distances:			
βH-2	βH-1	16.4	10.9	1.5
βH-1	βH-2	19.6	11.4	1.7
	βH-5	18.4	12.0	1.5
	Relaxation through fluctuating distances:			
βH-2	αH-1	5.9	4.9	1.2
	αH-5	15.5	12.6	1.2

The different ratios for the NOE's can only arise if there is significant relaxation through internal motions in the disaccharide. For these internal motions to contribute significantly to the relaxation their time scale must be comparable to that of the overall tumbling, i.e. $\sim 10^{-10}$ s (ref. 32).

Estimation of the magnitude of conformational entropy contributions. The internal consistency of the experimental and theoretical approaches described above suggests that the potential energy surfaces can be used with some confidence to derive other properties. The most important property for the purposes of this discussion is the calculation of the conformational entropy associated with the fluctuations in the torsional angles about the glycosidic linkage. The conformational entropy, S, associated with an ensemble can be calculated as:

$$S = -R\sum_i(p_i \ln p_i)$$

where the sum is over all the conformations, p_i is the probability of the ith conformation occurring in the ensemble and R is the gas constant. The absolute value of the conformational entropy calculated using the above expression is dependent on the number of conformational states considered. Therefore,

to compare the conformational entropy associated with different oligosaccharides, it is necessary to perform the calculations using identical numbers of conformations for each oligosaccharide. The conformational entropy for the ensemble representations of a number of different oligosaccharides was calculated and compared to the entropy barrier observed for the binding of the same oligosaccharides to a given protein. A remarkable correspondence was found (ref. 4; D.A. Cumming, R. Petter, J.P. Carver, unpublished results). This observation led us to suggest that the primary source of the entropic barrier was the loss of conformational degrees of freedom of the *ligand* on binding (ref. 4). Clearly there are other contributions to the entropic barrier, including those emphasized in the first two models. What we have suggested is that the dominant effect for oligosaccharide binding is the loss of degrees of freedom in the conformational flexibility of the ligand and that this can amount, at room temperature, to as much as 1-2 kcal/mole for each linkage angle immobilized (refs. 4,33; D.A. Cumming, R. Petter, J.P. Carver, unpublished results).

An alternative explanation of the data of Lemieux

The data on modified Le^b-tetra-saccharides reported by Lemieux (ref. 10) must be explained by any model for compensation. There is an alternative explanation to that put forward by Lemieux. In determining ΔH and ΔS what is measured is the change in the thermodynamic quantity on going from the isolated components to the complex. Thus changes in the primary structure of the ligand can alter the measured ΔH or ΔS by altering the entropy or enthalpy of the complex, as is usually assumed, *or* by altering the enthalpy or entropy of the isolated components. The latter is a possible explanation for the data of Lemieux. For example, consider the modification which shows the greatest $\Delta\Delta H$ and $T\Delta\Delta S$, the substitution by hydrogen of the 3-OH of the α1,4-linked fucose residue. The observation is that the absolute value of the enthalpy and entropy changes on binding are reduced for the deoxy compound. The conventional interpretation is that the reduction in the entropy and enthalpy changes arises from a higher entropy and enthalpy for the complex with the deoxy derivative compared to the complex with the unmodified tetra-saccharide. In the Lemieux model this would have resulted from the loss of binding sites for water in the complex with the deoxy derivative relative to the complex with the unmodified tetra-saccharide. The alternative interpretation would be that the reduction in the absolute values of ΔH and $T\Delta S$ arises from a lower enthalpy and entropy for the isolated deoxy derivative compared to the isolated unmodified tetra-saccharide. How might this happen? There are many possibilities, for the sake of having a simple visual model one might postulate that the removal of the 3-OH allows better van der Waals contact between the fucose and the rest of the tetra-saccharide. Such a stabilizing interaction would lower the enthalpy and the entropy. One might, at first glance, anticipate that the NMR spectrum and inter-residue NOE patterns would be altered if one compared the deoxy with the unmodified tetra-saccharide, particularly if one thinks of them as rigid structures. However, if one considers the possibility of an ensemble of three-dimensional structures then it is immediately evident that the distribution of such structures can be made more narrow (hence reduced entropy) without altering the average properties, which are the only parameters that can be observed. These alternative models can be explored experimentally. It is of course quite possible that both mechanisms are important with the relative importance varying from system to system.

POSSIBLE ROUTES TO HIGHER AFFINITIES FOR OLIGOSACCHARIDE LIGANDS

Based on the above discussion it is clear that the generation of high affinity/high specificity signals, or the design of more effective inhibitors of protein/carbohydrate interactions, requires finding a way to overcome compensation. Either (i) the entropic barrier will have to be reduced without loosing the enthalpic stabilization, or (ii) the enthalpic stabilization will have to be increased without increasing the entropic barrier; ideally, both. The different molecular models for compensation suggest different strategies to accomplish these goals. All three models require a detailed knowledge of the three-dimensional structure of the complex before specific design strategies can be formulated, although general principles can be deduced for the Lemieux and Carver models. The obvious strategy based on the Carver model is to produce ligands with reduced flexibility thus reducing the entropic barrier. If this can be accomplished while maintaining a distribution of three-dimensional structures in which the structure of the bound ligand is equally (or more) populated there should be no loss in enthalpic stabilization and tighter binding should result. For the Lemieux model one might suggest that the

replacement of as many of the non-essential hydroxyls as possible would avoid the creation of new water binding sites and hence reduce the entropic barrier. Lemieux has synthesized such compounds and found, in contrast to the above prediction, that the modified compounds bind more weakly than the parent compound (ref. 6).

The strategy of synthesizing conformationally restricted ligands has been explored by Hindsgaul (refs. 34,35) in an attempt to find a better inhibitor for GlcNAc-transferase V. This enzyme adds GlcNAc in a β-linkage at position 6 of a mannose residue linked α1,6 to a β-linked mannose (or glucose) in the oligosaccharide substrate. Since it is known from NMR studies that an equilibrium exists between two rotamers (**gg** and **gt**) about the C5-C6 bond in the α1,6 linkage (ref. 36), Hindsgaul synthesized two different pairs of restricted rotamers. The first approach used a method described by Lemieux (ref. 37) to stereo-specifically replace one or other of the hydrogens at C6 of the β-linked glucose by a methyl group. Because of steric conflict with the 4-OH, neither O6 nor the methyl populate significantly the position trans to O5 and one rotamer predominates. For the isomer with the methyl at the 6S position, O6 is trans to H5 (**gg** rotamer), whereas for the other isomer O6 is trans to C4 (**gt** rotamer). Another pair of substrates was later synthesized by Lindh & Hindsgaul (ref. 35) in which rotation about C5-C6 was completely restricted. Once again two isomers resulted, **gg** and **gt**. Unfortunately, the two sets of restricted rotamers gave contradictory results. For the compounds in which C5-C6 rotation was completely restricted, the **gg**-rotamer was a 2-fold better substrate, while the **gt** rotamer was 20-fold worse, when compared to the corresponding unrestricted substrate. Unfortunately, because these are enzymatic reactions, it will be difficult to determine whether the effects are predominantly on the binding enthalpy or the entropy.

In an attempt to establish whether the altered affinities of restricted ligands arise primarily from enthalpic or entropic effects, my laboratory has applied the Hindsgaul approach to the interaction of pea lectin with mannosides (J.P. Carver, M. Stubbs, R.N. Shah, unpublished results). In the refined crystal structure of the complex between pea lectin and the trimannoside, Man(α1,6)[Man(α1,3)]Manα1-Ome, the 6-OH of the mannose in the binding site, hydrogen bonds to the protein so that it is restricted to the **gg**-rotamer (J. Rini, personal communication). Dr. Rajan Shah, in my laboratory, has synthesized the restricted isomers of β-methyl-mannoside analogous to those made by Srivastava et al. (ref. 34). The prediction is that the derivative with the 6-OH restricted primarily to the **gg**-rotamer should show enhanced affinity, predominantly through a reduction in the absolute value of ΔS, while the **gt**-rotamer should show lower affinity as a result of an overwhelmingly less negative ΔH, accompanied by a lesser reduction in the absolute value of ΔS. Based on the estimates of the conformational entropy per linkage angle given above, one might expect $\Delta\Delta G$ to be 1-2 kcal/mole, at room temperature, corresponding to an 7-50 fold reduction in the K_D. Preliminary results from the inhibition of erythrocyte agglutination show the **gg**-rotamer to inhibit approximately 2-fold better than the unmodified mono-saccharide, whereas the **gt**-rotamer inhibits at least ten times worse, if at all. Modelling studies in which the two isomers were docked into the mannose binding site in the crystal structure of pea lectin, indicate that, for the **gg**-rotamer, there should be unfavourably close contacts between the methyl group at C6 and the side chain of phenylalanine 123. It is likely, therefore that some of the anticipated reduction in the entropic barrier is offset by a reduction in the enthalpic stabilization - another form of compensation! Whether or not this is occurring should become clear as soon as the thermodynamic data are available.

"SITE-DIRECTED PRESENTATION" OF CARBOHYDRATE LIGANDS

Returning to the question of how high affinity carbohydrate/protein interactions are generated naturally, it is clear that compensation must be overcome. An obvious mechanism, observed with viruses, bacteria, antibodies and the asialo-glycoprotein receptor, is multivalency which gains high avidity at the cost of specificity (ref. 4). But are there others that guarantee high specificity as well as affinity? At first glance, all three explanations of "compensation" in the binding of free oligosaccharides to proteins would suggest that the same oligosaccharide when covalently linked to a carrier would have the same low affinity for its carbohydrate receptor, regardless of the carrier to which it is covalently linked. Yet there is considerable evidence that the nature of the carrier can influence the specificity and affinity of oligosaccharide binding to its receptor, a phenomenon we suggest is best described by the term "*site-directed presentation*". Models for the molecular basis of site-directed presentation should provide

explanations of why only certain subsets of the total population of a given cell-surface oligosaccharide, actually lead to high affinity binding.

On detailed examination, it becomes clear that both the Lemieux and the Carver models can be extended to provide possible explanations of site-directed presentation. If the Lemieux and Carver models are expanded to include interactions between the covalently attached carbohydrates of glycoconjugates and the molecular surface adjacent to the site of linkage, then differential specificity between different sites can be achieved and "compensation" can be overcome. For the Lemieux model this interaction would require differential organization of water at the interface at different attachment sites; a reasonable expectation since different chemical groups would be present. For the Carver model, the molecular basis for site-directed presentation follows as a natural extension of our earlier model for "site-directed processing" (refs. 38,39) describing the phenomenon whereby different oligosaccharide primary structures occur at different glycosylation sites on the same glycoprotein, despite the fact that all glycosylation sites on the glycoprotein are exposed to the same set of processing enzymes. The Carver model would require that there exist differential degrees of constraint on the ensemble of oligo-saccharide three-dimensional structures at different sites of attachment; again, a reasonable expectation since different chemical groups would be present at different glycosylation sites. In both cases, differential amounts of conformational entropy would have been lost at each site *before the ligand is bound to the receptor*. This would lead to a range of possible affinities. Based on the calculations presented above, it would only be necessary to "pre-immobilize" on the order of three linkage angles to lower the K_D one thousand-fold and obtain the nanomolar affinities required for effective signals.

Acknowledgement

The work discussed in this article was supported over the years by grants from: the Medical Research Council of Canada (MT-3732 and MT-6499); the National Cancer Institute of Canada; and, the Protein Engineering Network of Centres of Excellence (PENCE), Canada. The author wishes to thank the many colleagues who have contributed to the development of these ideas, in particular: Dr. Dale Cumming; Dr. Karl Hardman; Dr. Milos Hricovíni; Dr. Anne Imberty; Mr. Ron Petter; Professor Jim Rini; Professor Harry Schachter; Dr. Serge Pérez; Dr. Rajan Shah; Dr. Marlene Stubbs; and, Dr. Igor Tvaroška.

REFERENCES

1. J. Montreuil, Pure Appl. Chem. 42, 431-477 (1974).
2. Reviewed in: H. Schachter, Chemtracts-Biochemistry & Mol. Biol. 2, 126-130 (1991).
3. O. Hindsgaul, D.P. Khare, M. Bach, R.U. Lemieux, Can. J. Chem. 63, 2653-2658 (1985).
4. J.P. Carver, S.W. Michnick, A. Imberty, D.A. Cumming, Carbohydrate Recognition in Cellular Function (CIBA Fdn. Symp. 145), 6-26, Wiley, Chichester (1989).
5. R. Lumry, S. Rajender, Biopolymers 9, 1125-1227 (1970).
6. U. Spohr, E. Paszkiewicz-Hnatiw, N. Morishima, R.U. Lemieux, Can. J. Chem. 70, 254-271 (1992).
7. B.W. Sigurskjold, D.R. Bundle, J. Biol. Chem. 267, 8371-8376 (1992).
8. S. Acharya, S.R. Patanjali, S.U. Sajjan, B. Gopalakrishnan, A. Surolia, J. Biol. Chem. 265, 11586-11594 (1990).
9. M.R. Eftink, A.C. Anusiem, R.L. Biltonen, Biochemistry 22, 3384-3896 (1983).
10. R.U. Lemieux, Chem. Soc. Rev. 18, 347-374 (1989).
11. C.S. Wright, J. Mol. Biol. 215, 635-651 (1990).
12. C.S. Wright, J. Mol. Biol. 194, 501-529 (1987).
13. C.S. Wright, J. Mol. Biol. 209, 475-487 (1989).
14. L. Delbaere, M. Vandonselaar, L. Prasad, J.W. Quail, J.R. Pearlstone, M.R. Carpenter, L.B. Smillie, P.V. Nikrad, U. Spohr, R.U. Lemieux, Can. J. Chem. 68, 1116-1121 (1990).
15. P.J. Goodford, J. Med. Chem. 28, 849-857 (1985).
16. D.A. Cumming, J.P.Carver, Biochemistry 26, 6664-6676 (1987).
17. D.A. Cumming, J.P.Carver, Biochemistry 26, 6676-6683 (1987).

18. D.A. Cumming, R.N. Shah, J.J. Krepinsky, A.A. Grey, J.P.Carver, Biochemistry 26, 6655-6663 (1987).
19. K. Bock, H. Lonn, T. Peters, Carbohydr. Res. 198, 375-380 (1990).
20. T. Peters, J.-R. Brisson, D.R. Bundle, Can. J. Chem. 68, 979-988 (1990).
21. M. Hricovíni, I. Tvaroška, J. Hirsch, A.J. Duben, Carbohydr. Res. 210, 13-20 (1991).
22. C. Hervé du Penhoat, A. Imberty, N. Roques, V. Michon, J. Mentech, G. Descotes, S. Pérez, J. Am. Chem. Soc. 113, 3720-3727 (1991).
23. D.A. Cumming, D.S. Dime, A.A. Grey, J.J. Krepinsky, J.P. Carver, J. Biol. Chem. 261, 3208-3213 (1986).
24. D.S. Dime, E. Rachaman, C.E. Dime, A.A. Grey, J.P. Carver, J.J. Krepinsky, J. Labelled Cpds. & Radiopharmaceuticals 24, 725-739 (1987).
25. A. Imberty, S. Gerber, V. Tran, S. Pérez, Glycoconjugate J. 7, 27-54 (1990).
26. J.P. Carver, Mandel D., Michnick, S.W., Imberty A., Brady J.W., ACS Symp. Ser. 430, 266-280 (1990).
27. Z.-Y. Yan, C.A. Bush, Biopolymers 29, 799-812 (1990).
28. C.J. Edge, U.C. Singh, R. Bazzo, G.L. Taylor, R.A. Dwek, T.W. Rademacher, Biochemistry 29, 1971-1974 (1990).
29. S. Homans, Biochemistry 29, 9110-9118 (1990).
30. J.W. Brady, Current Opinion in Structural Biology 1, 711-715 (1991).
31. J.P. Carver, Current Opinion in Structural Biology 1, 716-720 (1991).
32. M. Hricovíni, R.N. Shah, J.P. Carver, Biochemistry 31, in press (1992).
33. A. Imberty, M. Hricovíni, R.N. Shah, S. Pérez, J.P. Carver, Int. J. Biol. Macromolecules, in press (1992).
34. O.M. Srivastava, O. Hindsgaul, M. Shoreibah, M. Pierce, Carbohydr. Res. 179, 137-161 (1988).
35. I. Lindh, O. Hindsgaul, J. Am. Chem. Soc. 113, 216-223 (1991).
36. J.-R. Brisson, J.P. Carver, Biochemistry 22, 3680-3686 (1983).
37. R.U. Lemieux, T.C. Wong, H. Thøgersen, Can. J. Chem. 60, 81-86 (1982).
38. G. Savvidou, M. Klein, A.A. Grey, K.J. Dorrington, J.P. Carver, Biochemistry 23, 3736-3740 (1984).
39. J.P.Carver, D.A. Cumming, Pure Appl. Chem. 59, 1465-1476 (1987).