HAWORTH MEMORIAL LECTURE*

Human Blood Groups and Carbohydrate Chemistry

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It is a truly outstanding privilege to present the Haworth Memorial Lecture for 1978 and it is particularly gratifying to receive such high distinction outside of my own country and especially here in the United Kingdom. Therefore, I would like first to thank all of those concerned with the decision to grant me this honour on the occasion of this, the IXth International Symposium on Carbohydrate Chemistry, where I am among many friends and ex-colleagues.

The achievement of such recognition has of course required understanding and devoted assistance from many sources—governmental, academic, colleagues, co-workers, and family. By honouring me, The Chemical Society honours all these—a favour for which I am deeply indebted.

My interests in basic chemical research, especially since my appointment to the University of Alberta in 1961, were highly demanding in terms of facilities, collaboration, and especially in the time available for effective engagement. These were achieved primarily through the support and trust provided to me and our Department of Chemistry by both the University of Alberta and the National Research Council of Canada. Since my work has been judged highly significant, then these organizations are to be particularly commended and applauded.

It is a happy circumstance, I believe, that I could come here today with a lecture on aspects of the carbohydrate chemistry of the human blood-groups. This subject is particularly appropriate for a Haworth Memorial Lecture of The Chemical Society because of the rich history of serology and haematology in Britain starting with the discovery of blood circulation by Harvey in 1616, going through the first human-to-human blood transfusion by James Blundell in 1818, and eventually leading to the pioneering and monumental accomplishments on the chemical structure of the blood-group determinants made through the superb collaboration of Walter Morgan and Winifred Watkins at the Lister Institute of Preventive Medicine.

In the first Haworth Memorial Lecture presented by Maurice Stacey in 1971,[1] he made reference to the great importance of the work on deoxysugars by the Haworth school of carbohydrate chemistry to the field of nitrogen-containing

* Delivered at the IXth International Symposium on Carbohydrate Chemistry on 11 April 1978, at the Institute of Education, University of London.

[1] M. Stacey, *Chem. Soc. Rev.*, 1973, **2**, 145.

immunopolysaccharides. He mentioned that while with Haworth his own discovery of L-fucose residues in blood-group substances, 'has had quite remarkable consequences in elucidating the specialist sub-groups and in stimulating research generally in this field.' Thus, it seemed fortunate that I could, because of research in recent years in my laboratory, speak on this occasion on our syntheses of immunodominant parts of oligosaccharide antigenic determinants related to certain important human blood groups and to comment on the results of our conformational analyses of these complex structures. Since Haworth was, in my estimation, the father of conformational analysis in carbohydrate chemistry, I consider this latter subject also to be highly pertinent to a lecture in his memory.

My first encounter with blood-group chemistry was in the reading of a chapter entitled, 'Chemistry of the Mucopolysaccharides and Mucoproteins' by Maurice Stacey in 1946.[2] That was the year that I gained the Ph.D. degree under the supervision of Professor Clifford B. Purves who had been a graduate student with Professor E. L. Hirst at St. Andrews, and it is through him that my connection with the famous British school of carbohydrate chemistry was made.

The discovery at the turn of this century by Landsteiner[3] of the ABO system was a turning point in the science of haematology. This was a matter of particular significance to blood-transfusion science. It was demonstrated that the serum of a person of either A or B group agglutinated the red cells of the other group, and that the sera of O group people agglutinated cells of both A and B people. The cells of a fourth group of people, termed AB, were found to be agglutinated by the serum of both A and B people but the serum of these people did not agglutinate either A or B cells. Agglutination *in vivo* gives rise to serious pathological conditions referred to as blood-transfusion reactions and can involve extensive lysis of red cells. Its avoidance by proper typing in the ABO system was then a major step toward the modern situation wherein some 20 million litres of blood are collected annually in the western developed countries for transfusion purposes. A substantial proportion of the blood collected is dedicated to the preparation of testing reagents for blood typing and for the blood-fractionation industry to extend blood usage by providing a broad range of fractions, mainly of interest to clinical problems concerned with blood coagulation.

With Landsteiner's discovery and the general growth since then of the chemical and biological sciences, the science of haematology rapidly grew to the point that over 160 different immunological specificities are now recognized for human red cells. At least 13 independent and well-defined blood group systems are known and some 50 specificities are of clinical importance. At least five blood-group systems are known to possess oligosaccharide determinants. This list may grow since carbohydrates as oligosaccharidic structures, offer a wide assortment of conformationally rigid structures to serve as recognition sites at cell surfaces. My lecture is restricted to our syntheses of determinants for the ABO and Lewis

[2] M. Stacey, *Adv. Carbohydrate Chem.*, 1946, **2**, 161.
[3] K. Landsteiner, *Biochem. Z.*, 1920, **104**, 280.

systems, how the products of these and similar syntheses may become of medical interest, and some comments on their conformational properties.

The blood group determinants are inherited according to the Mendelian genetic laws and arise in the individual as the result of the person possessing genes, which gives a code for the enzymes, which are necessary to build the oligosaccharide determinants.

Substances possessing human blood-group activities occur as oligosaccharides in milk and urine, as complex water-soluble glycoproteins in body secretions and tissue fluids and on the surfaces of cells and tissues, and in the form of water-insoluble glycosphingolipids mainly on the surfaces of red cells and tissues. Substances possessing human blood-group specificities are also found in certain animal tissues, plant extracts, and in many bacteria.

The glycosphingolipids occur hydrophobically bonded to the surface of cells and tissues. Although these structures have been studied extensively for over 30 years, it has only been within the last decade that a large number of their structures have become known in detail. This knowledge[4] was acquired in several laboratories but mainly through the efforts of Kościelak and co-workers in Warsaw and Hakomori and his associates at the University of Washington. The work was strongly handicapped by the great difficulty in the isolation of these substances. For example, starting with 50 kg of packed red cells, Hakomori was able to isolate only about 2 mg of a pure glycosphingolipid. These studies based on highly sophisticated modern methods of separation and instrumentation for structural analysis have now produced structures for over 13 blood-group specific glycosphingolipids, an example of which is presented in Figure 1. The B-group glycosphingolipid is presented in such a way as to illustrate the strong hydrophobic bonding which can occur with the lipid bilayer of a cell membrane from penetration of the ceramide portion of the glycosphingolipid into the lipidic membrane. As indicated, the oligosaccharide determinants appear to all begin with a β-lactosyl group attached to the 1-oxygen of the ceramide portion.

The structures of blood-group specific glycoproteins which are found in secretions such as saliva, gastric juices, milk, sweat, and tears are illustrated in Figure 2. Although saliva represents a readily accessible source of these glycoproteins (also known as blood-group substances), this secretion contains only 10—130 mg of active material per litre. On the other hand, ovarian cysts can accumulate fluids for long periods and several grams of purified blood-group substance can often be isolated from a single cyst. Being available in substantial quantity for several decades, the structures of the blood-group substances drew widespread attention and were intensively studied in many laboratories. A review of this extensive effort is outside the scope of this lecture and I can only refer you to the excellent reviews by Dr. Winifred Watkins.[5] The first stage of

[4] J. Kościelak, in 'Human Blood Groups', Proceedings of the 5th International Convocation on Immunology, Karger, Basel, 1977, p. 143; S. Hakomori and A. Kobata, in 'The Antigens', Vol. II, ed. M. Sela. Academic Press, New York, 1977, Vol. II, p. 79.

[5] W. M. Watkins, in 'Glycoproteins: Their Composition, Structure and Function', ed. A. Gottschalk, Elsevier, Amsterdam, 1972, p. 830; and also in *Biochem. Soc. Symp.*, 1974, **40**, 125.

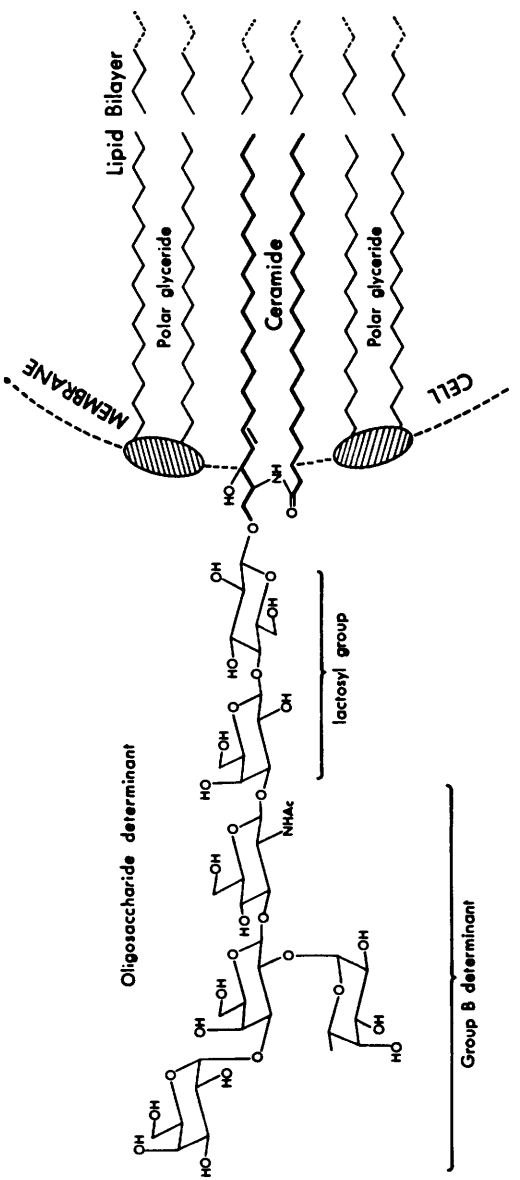


Figure 1 *Illustration of hydrophobic bonding of a B human blood-group specific glyco-sphingolipid to a cell-membrane lipid bilayer*

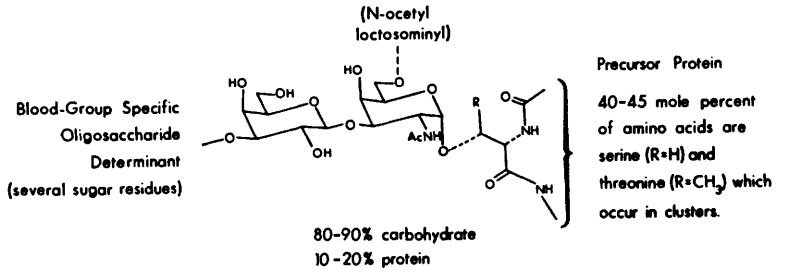


Figure 2 *Illustration of the general structure for blood-group specific glycoproteins, also known as blood-group substances, which occur in tissue fluids, in secretions, and at cell and tissue surfaces*

these studies was based on the observation by Landsteiner[3] in 1920 that a simple substance with a structure closely related to that of the antigenic determinant can combine with the antibody and thereby competitively inhibit the reaction between the antibody and the antigen. A large number of oligosaccharides isolated from human milk and characterized by Kuhn and co-workers at Heidelberg in the 1950's were particularly useful in revealing the structures of serologically active units in the blood-group substances. This led to studies which involved the use of enzyme specificities for the destruction of serological activities and the detection of inhibition of the destruction by specific sugars and certain derivatives. The final stage involved partial acid and base degradations of the glycoproteins to provide serologically active oligosaccharide fragments which subsequently were subjected to structural analysis. It is these studies, mainly performed in the laboratories of Morgan and Watkins at the Lister Institute of Preventive Medicine, and of Kabat and co-workers[6] at Columbia University, that yielded the detailed structural information which put synthetic chemists, like myself, 'into business'—so to speak.

As indicated in Figure 2, the blood group specific glycoproteins are 80—90% carbohydrate. Some 40—45 of the amino acid units per 100 amino-acid units are the hydroxy amino-acids, serine and threonine, and the oligosaccharide units are attached to these hydroxyl groups by way of an *N*-acetyl-α-D-galactosamine unit. It is of interest to note that the first disaccharide unit appears to be the 3-*O*-(β-D-galactosyl)-α-D-*N*-acetylgalactosaminyl group. This disaccharide unit is substituted in part by an *N*-acetyl-lactosamine group at the 6-position of the α-D-galactosamine residue. The normal blood-group oligosaccharide determinants then extend from the 3-position of the β-D-galactosyl group.

The chemical structures of the terminal groups responsible for the A, B, O(H) specificities are shown in Figure 3. It is seen that the O(H) determinant is the precursor for both the A and B determinants. It may be noted that O was

[6] K. O. Lloyd, E. A. Kabat, and E. Licerio, *Biochemistry*, 1968, **7**, 2976.

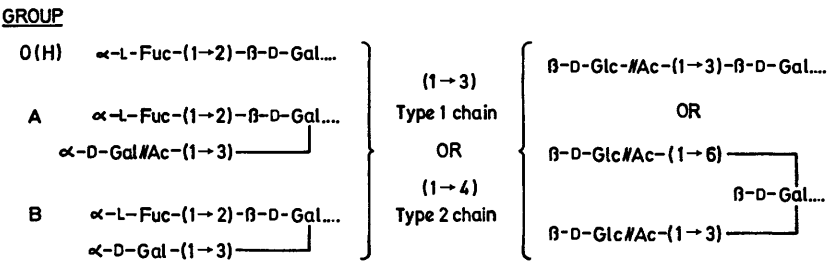


Figure 3 *The A, B, and O(H) human blood-group determinants*

initially assigned to designate red cells that were not agglutinated by either anti-**A** or anti-**B** sera; that is, had zero activity. Since then the cells were found to have the specific determinant indicated in Figure 3. The structure, α-L-Fuc-(1→2)-β-D-Gal-(1→3 or 4)-β-D-Glc*N*Ac—is now termed the terminal trisaccharide units for H-activity and this is why the designation O(H) is used.

As indicated, the β-D-Gal unit of the terminal trisaccharide for the H determinant is linked either to the 3- or to the 4-position of a β-D-Glc*N*Ac residue. The (1→3) linkage provides what has been termed the **Type 1** chain and the (1→4) arrangement the Type 2 chain.[7] From a stereochemical point of view, this difference imposes a very substantial difference in structure close to and perhaps included in the immunodominant part of these antigenic determinants. We have examined this matter in some detail in recent years and I wish now to outline briefly some of our findings.

Our approach to these matters is based on n.m.r. spectroscopy, both ^{13}C and ^{1}H n.m.r., and in molecular modelling with computer assistance. However, prior to serious involvement in such studies, it seemed mandatory to examine in detail the influence on the conformational preference for glycosidic linkages of the bonding phenomena which I have termed the *exo*-anomeric effect.

These studies began with the establishment of a relation between the torsion angle defined by neighbouring ^{1}H and ^{13}C atoms.[8] Once a Karplus-type relationship was established, a number of appropriately ^{13}C-enriched model glycosides were synthesized so as to allow accurate measurement of the change in coupling constant between the aglyconic carbon and the anomeric hydrogen with changes in both the structure of the aglycon and the configuration at the anomeric centre.[9] These and related studies led to the conclusion that the *exo*-anomeric effect must definitely play a dominating role in the determination of the orientation of an aglyconic carbon relative to the anomeric hydrogen. This orientation is provided by the ϕ^{H} torsion angle as defined in Figure 4.[10] The conclusion was reached that in the absence of truly exceptional steric encumbrances, glyco-

[7] V. P. Rege, T. J. Painter, W. M. Watkins, and W. T. J. Morgan, *Nature*, 1963, **200**, 532.
[8] L. T. J. Delbaere, M. N. G. James, and R. U. Lemieux, *J. Amer. Chem. Soc.*, 1973, **95**, 4501.
[9] R. U. Lemieux, S. Koto, and D. Voisin, 'Advances in Chemistry Series', 1978, in press.
[10] R. U. Lemieux and S. Koto, *Tetrahedron*, 1974, **30**, 1933.

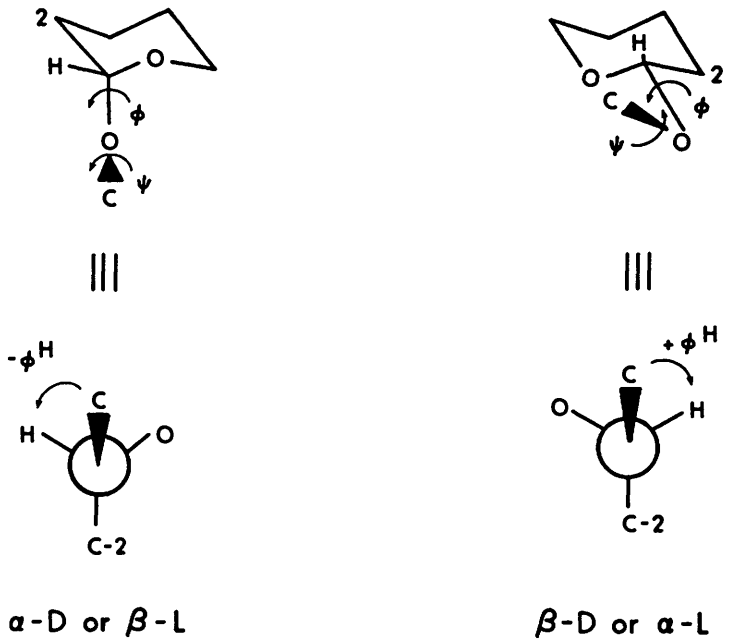


Figure 4 *Definition of the* ϕ^H *and* ψ^H *torsion angles about glycosidic linkages*

pyranosides in solution can be expected to possess ϕ^H angles in the range $\pm 50°$ to $\pm 60°$, the sign of the torsion angle depending on the absolute configuration of the sugar. The ϕ^H angle for α-glycosides appears to be a little larger than that for the β-anomers both for reasons of ^{13}C to ^1H coupling constants[9] and for theoretical reasons, as recently published by Jeffrey, Pople, and co-workers.[11] A good compromise appears to be to set ϕ^H at $\pm 55°$ for an α-glycoside and $\pm 50°$ for a β-glycoside. On this basis, the preferred conformation for a glycosidic linkage is expected to result from rotation about the aglyconic carbon to glycosidic oxygen bond only. These rotations are said to change the value of the ψ^H angle. As will be seen later, our procedure is to maintain 'normal' ϕ^H angles unless it becomes apparent from molecular modelling that exceptional non-bonded interactions about the anomeric centre can only be favourably relieved by a change in the ϕ^H angle. Judging from the *ab initio* molecular orbital calculations performed by Jeffrey, Pople, and co-workers,[11] changes of ϕ^H within the range of $\pm 15°$ from 'normal' values are not highly demanding in energy. In our work, we do not change the ϕ^H angle from the 'normal' values unless this appears to lead to a relief of a non-bonded interaction of near 1 kcal mol^{-1}. This approach is undeniably arbitrary but as we shall see appears well justified as a useful working hypothesis.

[11] G. A. Jeffrey, J. A. Pople, J. S. Binkley, and S. Vishveshwara, *J. Amer. Chem. Soc.*, 1978, **100**, 373.

The results obtained on subjecting the Type 1 and Type 2 disaccharides to conformational analysis, using standard hard-sphere calculations[10,12] based on the best available atomic parameters for the constituent structures as determined by *X*-ray crystallographic analyses of appropriate model compounds and setting $\phi^H = 50°$ (the 'normal' β-glycoside value), are presented in Figure 5. On this

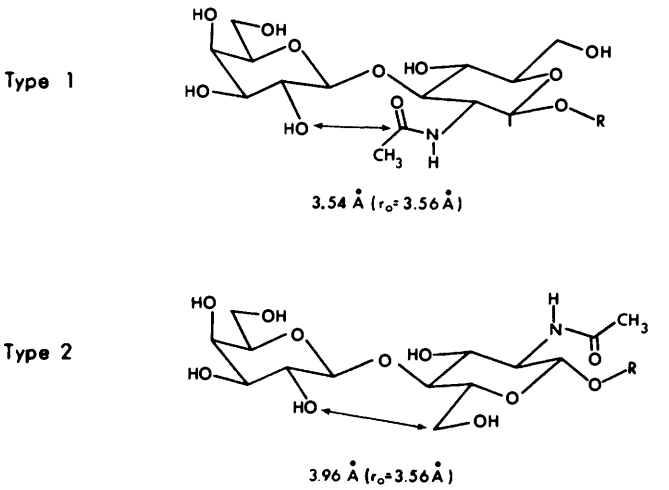


Figure 5 *Conformations for the Type* 1 *and Type* 2 *disaccharides as estimated by hard-sphere calculations following the procedure described by Rao and co-workers*[12] *and setting the* ϕ^H *torsion angles at* $+50°$. *The close proximity of the* 2′*-hydroxyl group to a group on the neighbouring sugar is to be noted*

basis, ψ^H angles of $+18°$ and $+8°$ are predicted for the Type 1 and Type 2 disaccharides, respectively. It is at once seen that these are vastly different molecular structures. Of particular importance from the point of view of blood-group determinants is that the 2-hydroxyl groups of the β-D-Gal units are in very different environments. In the Type 1 chain this hydroxyl is in Van der Waal contact with the carbonyl carbon of the acetamido group, whereas in the Type 2 chain, this hydroxyl is in near Van der Waal contact with the methylene group at C-5 of the β-D-Glc*N*Ac residue. Clearly, the two disaccharides would present very different substrates to the enzymes responsible for the conversion of these structures into the Type 1 and Type 2 H-determinants. In all likelihood, the enzymes are different. Watkins and Morgan[13] have suggested that the β-D-Glc*N*Ac residue is involved as part of the determinant for the H-glycoprotein. If so, it can be expected, I believe, that the cross-reaction between these antibodies and the alternate H-structure is very weak.

[12] V. S. R. Rao, F. R. Sundararajan, and G. N. Ramachandran, in 'Conformations of Biopolymers', ed. G. N. Ramachandran, Academic Press, New York, 1967, p. 721.
[13] W. M. Watkins and W. T. J. Morgan, *Vox Sanguinis*, 1962, **7**, 129.

In order to gain experimental evidence for the conformations shown for the Type 1 and Type 2 disaccharides, we investigated the effect of change in pH on the chemical shifts of the ^{13}C-atoms in the aminodisaccharides presented in Figure 6. We have shown that protonation of the amino groups in such structures

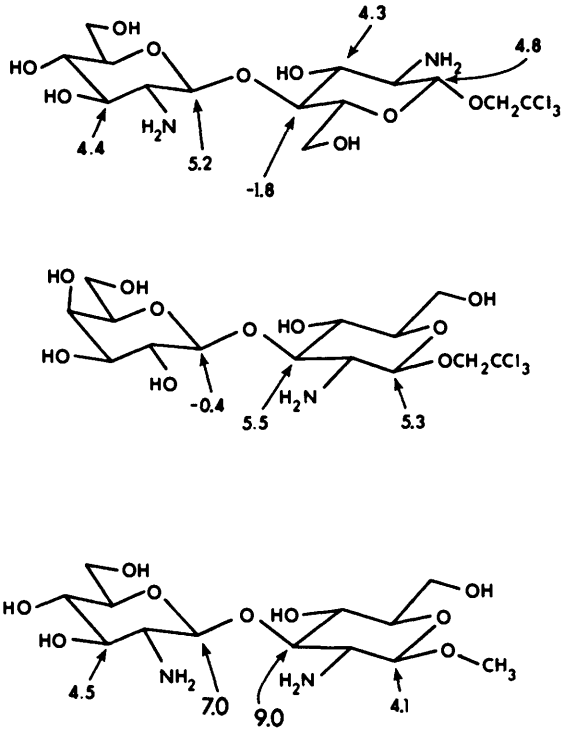


Figure 6 *The shielding of ^{13}C-atoms which are β to amino groups which is observed on protonation of the amino groups.*[9] *The exceptionally large shifts observed for both the anomeric and aglyconic carbons of the latter compound are attributed to the close proximity of the two amino groups*

give rise to remarkable β-shifts which are highly useful in the structural analysis of such compounds.[14] Normal β-shifts involve the shielding of a ^{13}C-nucleus which is β to the amino group by about 4.5 p.p.m. It is seen that the shifts observed for both the aglyconic and anomeric carbons in the disaccharide that is expected to have two amino groups in close steric relationship are truly anomalously high. This result would be in accord with the hydration of the ammonium groups (relative to that for the free amino groups) causing an increased demand for space and thereby affecting a change in conformation about the glycosidic bond. Regardless of the reason, these data require the two amino groups of the

[14] R. U. Lemieux and S. Koto, *Abstracts of Papers, Amer. Chem. Soc.*, 1973, **165**, Medi 022.

Type 1 disaccharide to be close enough to interact, whereas those of the Type 2 disaccharide are not. Therefore, the data lend support to the conclusions reached by the hard-sphere calculations regarding the conformations of the Type 1 and Type 2 chains.

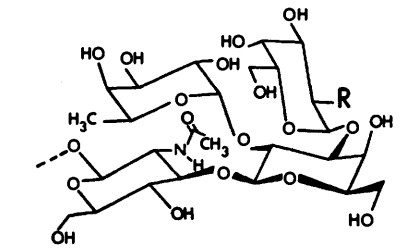
As was seen in Figure 3, the A, B, and H determinants involve (1→3) and (1→4) attachments to β-D-Glc*N*Ac units both in linear and in branched structures. The branched structures have been detected both in glycosphingolipids and in glycoproteins. The indication appears to be that the Type 1 chain is more prevalent in the glycoproteins than in the glycosphingolipids.[4,5]

The essentially complete lack of cross-reactions between A and B specificities is indeed remarkable when one considers that the only difference between the determinants is the difference between an acetamido and a hydroxyl group. However, this is not surprising if one examines molecular models in the conformations provided by hard-sphere calculations while assuming ϕ angles in accord with the *exo*-anomeric effect. These conformations are approximately displayed both for Type 1 and Type 2 chains by way of conformational drawings in Figure 7. These conformational formulae attempt to show that the α-D-Gal*N*Ac and α-D-Gal residues of the A and B terminal tetrasaccharide units are expected to be far removed from the β-D-Glc*N*Ac residues in both the Type 1 and Type 2 forms. Since the acetamido and hydroxyl groups of the α-D-Gal*N*Ac and α-D-Gal residues of the A and B determinants, respectively, play such a dominating role in determining these immunological specificities, it could be expected that the combining sites for anti-A and anti-B antibodies would be directed mainly, if not entirely, toward the terminal trisaccharide units. In other words, it could be expected that syntheses of the terminal trisaccharides in the form of trisaccharide haptens such as displayed in Figure 7, would provide structures, depending on the R substituent, which would combine at least extensively with natural anti-A and anti-B antibodies regardless of whether or not these were derived from antigenic determinants related to Type 1 or Type 2 chains. Indeed, this was the case.

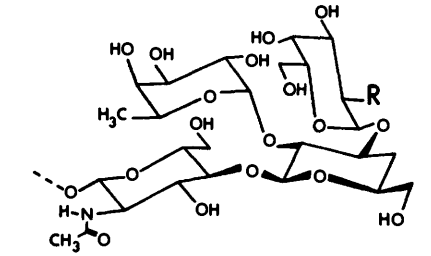
The use of the 8-methoxycarbonyloctyl alcohol as starting material for the synthesis of these structures was based on the need of such a residue in the final structure to serve as a bridging arm to form immunoadsorbents and artificial antigens.[15] This choice of bridging arm proved useful for other reasons as well[16] but time does not allow the presentation of data in this regard.

The main challenge in the syntheses of the A and B determinants was to achieve the establishment of the two α-linkages in high yield. A number of approaches have been evident for over 20 years for the establishment of α-fucosyl and α-galactosyl linkages but none of these were considered likely to produce the α-anomer in substantially greater yield than the β-form and, perhaps more importantly, the total amount of glycoside formation could not be expected to be high. However, a promising method became apparent in the mid-1960's as a result of our systematic investigations of reactions at the anomeric centre and

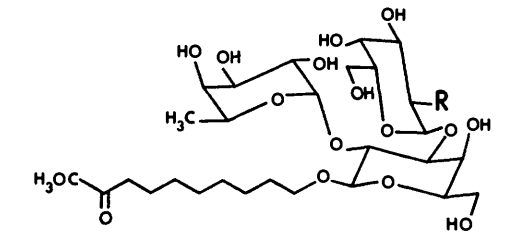
[15] R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Amer. Chem. Soc.*, 1975, **97**, 4076.
[16] R. U. Lemieux, D. A. Baker, and D. R. Bundle, *Canad. J. Biochem.*, 1977, **55**, 507.



Type 1



Type 2



Synthetic Hapten

Figure 7 *Projection formulae for the tetrasaccharide terminal units for the A* (R = $NHCOCH_3$) *and B* (R = OH) *human blood-group determinants in the Type* 1 *and Type* 2 *chains and related trisaccharide determinants* (*see Figures* 9 *and* 11) *aimed at illustrating the conformations provided by hard-sphere calculations after setting appropriate* ψ^H *torsion angles*

which I have termed the 'halide-ion catalysed α-glycosidation reaction'.[17] This reaction played a central role in the first syntheses of blood-group active trisaccharides by Hugues Driguez.[18,19] The method involves guiding the reaction between an appropriately blocked glycosyl halide and an alcohol under solvolytic conditions by way of the β-halide to form the α-glycoside.

[17] R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Amer. Chem. Soc.*, 1975, **97**, 4056.
[18] R. U. Lemieux and H. Driguez, *J. Amer. Chem. Soc.*, 1975, **97**, 4063.
[19] R. U. Lemieux and H. Driguez, *J. Amer. Chem. Soc.*, 1975, **97**, 4069.

Because of the anomeric effect,[20] β-halides are thermodynamically much less stable than their α-anomers and, therefore, exist in very low concentrations when in equilibrium with their α-forms.[21] However, it was discovered that β-halides have a much greater reactivity relative to their α-anomers than could be expected from their relative thermodynamic stabilities. Thus, it became apparent that an α-glycosyl halide could be used to prepare an α-glycoside should the rate of formation of the β-halide from the α-halide be substantially greater than the rate of the reaction of the α-halide with the alcohol to form β-glycoside. Under these conditions, the low equilibrium concentration of the β-halide would become insignificant to the overall course of reaction. We had established that the $\alpha \rightleftharpoons \beta$ equilibration of a glycosyl halide can be made extremely rapid by the presence, in the reaction medium, of a soluble halide salt in sufficient concentration.[22]

We have proposed an explanation for the high reactivity of β-halides compared with their α-anomers, which is based on stereoelectronic demands for the formation of reactive intermediates.[17] As seen from Figure 8, the formation of a glycoside from a glycosyl halide and an alcohol under solvolytic conditions is expected to proceed by way of ion-pair to alcohol triplets. The more rapid formation of α-glycoside is then attributed to a more ready decomposition of its precursor triplet than of the triplet that leads to β-glycoside formation. This situation is rationalized on the basis that the formation of the β-glycoside requires the achievement of a boat-like intermediate so as to allow an *anti*-periplanar arrangement in the transition state between the glycosidic bond being formed and the electron pair on the ring oxygen being recovered by the ring oxygen from its contribution to the reacting centre in the formation of the triplet intermediate.

That the halide-ion catalysed α-glycosidation reaction worked very well for the preparation of the B-trisaccharide determinant is displayed in a highly abbreviated fashion in Figure 9.[23]

It may be noted at this point that the problems of blocking and deblocking attendant to this work with multifunctional substrates is not trivial. Considerable planning is required in terms of choice of blocking groups with regard to both a maintenance of the hydroxyl group to be glycosidated in as reactive a form as possible and the keeping of access to the positions to be glycosidated in the order necessary for overall success. However, modern organic chemistry is rich in blocking–deblocking methodologies and, with care, it is normally possible to plan a multistage process while avoiding, so to speak, 'falling between stools'. The main problem normally involves the optimization of a reaction for the particular application at hand. The advents of thin-layer chromatography and high-field Fourier-transform p.m.r. spectroscopy as analytical tools are particularly indispensable in this regard.

As seen in Figure 9, optimization of the halide-ion catalysed reactions enabled

[20] R. U. Lemieux, in 'Molecular Rearrangements', ed. P. de Mayo, Interscience, New York, 1964, p. 709.
[21] R. U. Lemieux and J. Hayami, *Canad. J. Chem.*, 1965, **43**, 2162.
[22] R. U. Lemieux and A. R. Morgan, *Canad. J. Chem.*, 1965, **43**, 2205.
[23] R. U. Lemieux and Y. Fouron, to be published.

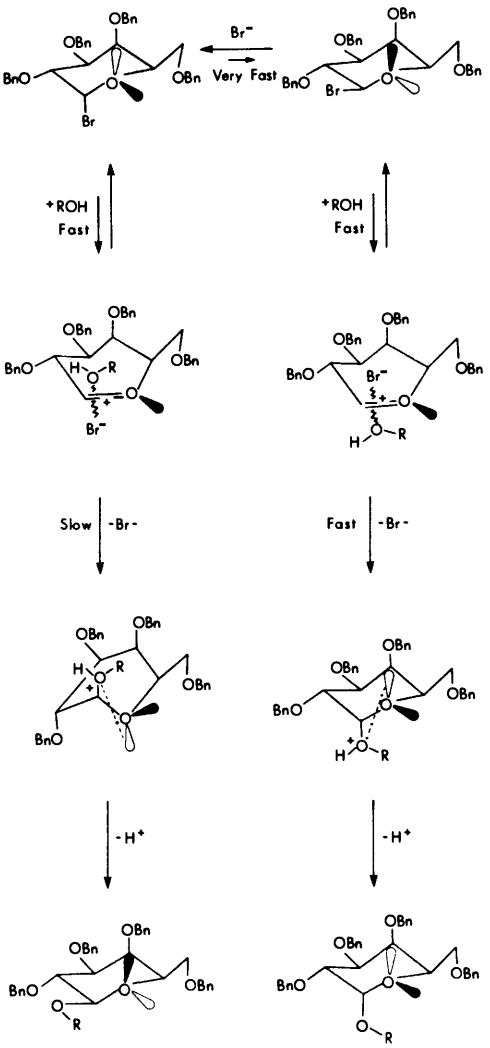


Figure 3 *A rationalization of the course of the bromide-ion catalysed α-galactosidation of an alcohol*

the establishment of the two α-glycosidic bonds in the B-determinant in excellent yield using only slight excesses of the reacting glycosyl bromides.[23] Such high yields greatly facilitate isolation and purification of the product as well as to make possible efficient large-scale processes.

The lack of a highly stereoselective method for reducing 2-oximino-α-D-*lyxo*-

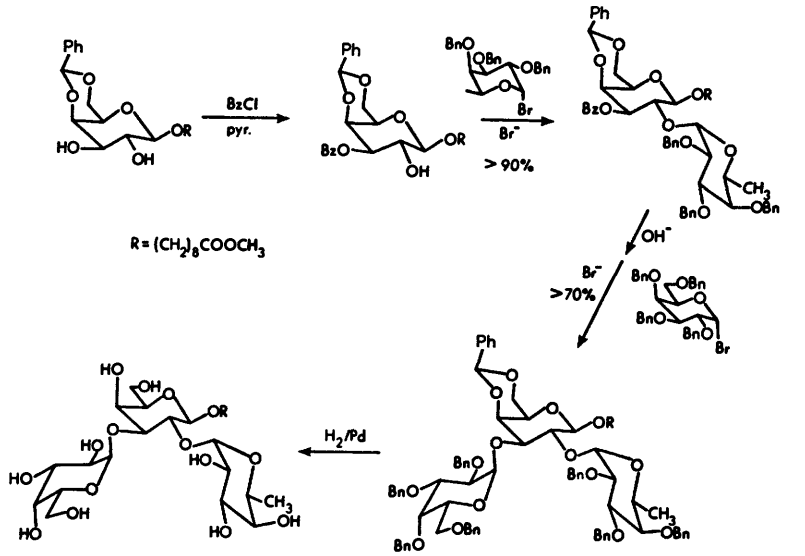


Figure 9 *Use of the halide-ion catalysed glycosidation reaction in the course of the synthesis of the terminal trisaccharide unit for the B human blood-group determinant*

hexopyranosides to 2-amino-2-deoxy-α-D-galactosides led us to abandon our so-called oximino-chloride method[24] for the preparation of 2-amino-2-deoxy-α-D-galactopyranosides.[25] The success reported by Paulsen[26] in the preparation of 2-azido-2-deoxy-α-glycopyranosides by reaction of the alcohol with 2-azido-2-deoxy-β-glycopyranosyl chlorides in the presence of silver salts led us to consider this route. However, to be practical, it seemed necessary to find a simpler route to the desired 2-azido-2-deoxy-β-glycopyranosyl chlorides than the multi-step processes used by Paulsen and co-workers.

The azidonitration of alkenes using ceric ammonium nitrate and sodium azide reported by Trahanovsky and Robbins[27] in 1971 seemed promising in this regard. Indeed, Murray Ratcliffe in his first trials, as seen in Figure 10, obtained a near 80% yield of tri-*O*-acetyl-2-azido-2-deoxy-D-galactosyl nitrate as an approximately 2:1 mixture of the β- to α-anomeric forms.[28] This was accomplished by reaction of the readily available tri-*O*-acetyl-D-galactal in acetonitrile at −25 °C with 2 mole equivalents of ceric ammonium nitrate (CAN) and one mole equivalent of sodium azide. Replacement of the 1-nitrates to afford tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl halides is readily accomplished. The iodide is particularly reactive and was therefore chosen for the

[24] R. U. Lemieux, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 42.
[25] R. V. Stick and R. U. Lemieux, *Austral. J. Chem.*, 1978, **31**, 901.
[26] H. Paulsen, C. Kolář, and W. Stenzel, *Angew. Chem. Internat. Edn.*, 1976, **15**, 440.
[27] W. S. Trahanovsky and M. D. Robbins, *J. Amer. Chem. Soc.*, 1971, **93**, 5256.
[28] R. U. Lemieux and R. Murray Ratcliffe, *Canad. J. Chem.*, submitted.

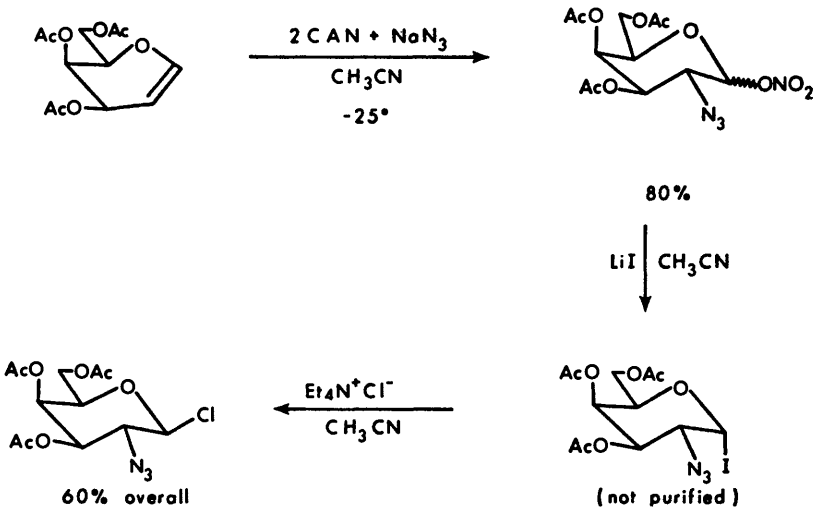


Figure 10 *The azidonitration of tri-O-acetyl-D-galactal in the course of the preparation of 3, 4, 6-tri-O-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride*

preparation under kinetic control of the desired tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride.

As can be seen from Figure 11, the tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride was indeed very useful for the preparation of the terminal trisaccharide for the A human blood-group determinant.[29] Having accomplished the establishment of the α-linkage in high yield, it was a relatively trivial matter to reduce the azide to amine for *N*-acetylation and then to remove the blocking groups to provide the desired A-hapten.

At this point, I think it appropriate to ask and answer the question 'Why synthesize these complex haptens?' In fact, it can be strongly contended, I believe, that advances in immunochemistry have for many years rendered clear that such syntheses would not only be desirable but indeed necessary for continued progress in the improvement of health care.

The supply of antigenic determinants from natural sources is completely inadequate both as to the range of structures desired and as to the amounts available. The glycosphingolipids that occur on red cells are in extremely low natural abundance. Furthermore, these occur in extremely complex mixtures of closely related structures—the problem of obtaining even milligram amounts is formidable. The natural abundance of blood-specific glycoproteins is rather substantial. However, these are both chemically and immunologically heterogeneous structures. Their degradation and procedures to isolate the products in pure chemical form is very costly and the range of products available is highly

[29] R. U. Lemieux, D. A. Baker, and R. Murray Ratcliffe, *Canad. J. Chem.*, submitted.

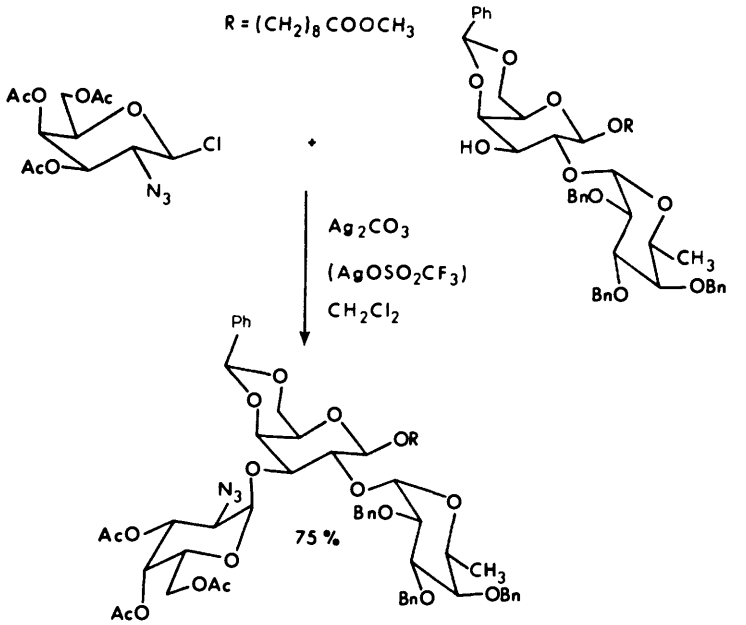


Figure 11 *The synthesis of an intermediate in the course of the synthesis of the terminal trisaccharide unit for the A human blood-group determinant*

limited. The isolation of pure oligosaccharides with structures related to blood-group specificities from human milk and urine is possible. Nevertheless, the supplies are rather limited; the procedures are difficult and, at best, are amenable to the provision of only gram amounts of a limited range of structures. On the other hand, with the advent of appropriate synthetic methodologies, the supply of any given oligosaccharide would be limited only by the scale of operation. The foreseen range of products which are desirable and which can be made available in substantial amounts by synthesis is already large and will continue to grow.

The haptenic structures themselves are of interest to effect changes in biological equilibria by inhibiting specific antibody–antigen interactions. This is useful for the characterization of the specificities of either antibodies or antigens in biological systems.

By immobilizing the haptenic structures on solid supports, monospecific immunoadsorbents can be prepared which almost certainly will find a number of important uses because these immunoadsorbents allow the collection and purification of specific antibodies from sera and plasma. Thus, the use of these immunoadsorbents can be expected, in many instances, to replace the use of red cells for the removal of an unwanted specificity in the preparation of a typing serum.

The preparation of artificial antigens by attachment of the hapten to a suitable carrier molecule allows the immunization of animals to raise antibodies specific

to the carbohydrate determinant. The immunoadsorbent then allows the isolation of these antibodies from the animal serum. Such monospecific antibodies can then find a wide range of applications such as the detection of antigens in body fluids and the establishment of antigenic sites on tissues and cells. The tests can involve precipitin and agglutination reactions or involve the labelling of the antibody for radioimmunoassays, immunofluorescence assays, or with assays based on electron spin-labelling, *etc.* Of course, the monospecific immunoadsorbents also allow the isolation of natural antibodies specific for the carbohydrate determinant used and this technique of affinity chromatography based on synthetic haptens can be extended to the isolation of lectins from plants and also enzymes concerned with the synthesis of the carbohydrate structure *in vivo*. Thus, it can be appreciated that the availability of synthetic oligosaccharide determinants can have an important impact on many aspects of medical practice such as in the preparation of typing reagents of interest to blood transfusion, tissue transplantation and diagnostics for cancer and autoimmune diseases. Indeed, one can imagine a number of therapeutic as well as diagnostic applications. Also, as will be seen later, the synthesis of these complex oligosaccharides provides these materials in sufficient quantity to allow studies of their conformational properties and to examine structure–activity relationships and thereby contribute significantly to progress in the fundamental science for these important biological processes.

Because of the foregoing possible uses and because of the limitations, in terms of the work that can be accomplished in one laboratory, I have established the general procedure, outlined in Figure 12, for our synthetic goals. We build the oligosaccharide in glycosidic union to the 8-methoxycarbonyloctyl alcohol which

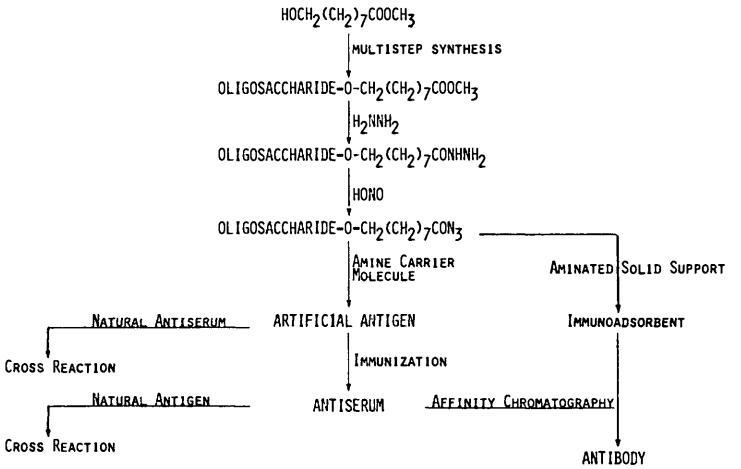


Figure 12 *A general procedure for the preparation of artificial antigens and immunoadsorbents*

later serves as a bridging arm.[15] Normally, the procedure which is used to prepare an artificial antigen or immunoadsorbent involves the preparation of the hapten acyl azide by way of the acyl hydrazide. The acyl azide is then allowed to react with an aminated solid support to prepare the immunoadsorbent or with a suitable amine carrier molecule to prepare the artificial antigen.[16] The hapten, immunoadsorbent and artificial antigen can then be used to examine cross-reactions with natural systems.

The procedure, which is outlined in Figure 12, can be illustrated by way of the preparation and properties of immunoadsorbents for the human A and B antibodies.

As seen in Figure 13, we normally employ aminated glass beads as solid support for the preparation of an immunoadsorbent. An immunoadsorbent prepared in this way and which contains 0.5 micromoles of hapten per gram can substitute for over a hundred times its weight of packed red blood cells. Thus, we can imagine the day when rather laborious and uncertain procedures involving adsorptions with red cells will be replaced by fully automated procedures based on affinity chromatography.

At this point, I would like to acknowledge a grant-in-aid for my research made by the Canadian Medical Research Council which rendered possible a very fruitful collaboration with the Canadian Red Cross Transfusion Service.

As judged by saline agglutination tests, the immunoadsorbent prepared from the A-trisaccharide determinant removed all of the anti-A antibodies from a human anti-A serum. The use of the similar immunoadsorbent but possessing only the α-D-Gal*N*Ac-(1→3)-β-D-Gal disaccharide component of the A-trisaccharide resulted in adsorption of only about 85% of the antibodies which are collected by the trisaccharide-immunoadsorbent. When the size of the A-determinant was reduced to the simple α-D-Gal*N*Ac structure, no discernible amount of anti-A antibodies was adsorbed. These results illustrate the desirability, at least for certain purposes, to synthesize the determinant as completely as possible.

The Lewis human blood-groups were not in the past of primary importance to blood transfusion since the sera of humans do not normally contain antibodies with these specificities although over 22% of people possess Lewis-a determinants on their red cells and tissues and 72% possess Lewis-b antigens. However, typing for the Lewis groups has become of major importance because a mismatch in the Lewis system in the course of one transfusion leads to immunization against the foreign antigen. Thus, a second transfusion of cells carrying this Lewis specificity can lead to a serious transfusion reaction and this can affect about 6% of the population. Thus, the modern blood-banking industry is now in great need of a secure supply of reliable typing reagents for the Lewis system of which the Lewis-a and Lewis-b groups appear to be of most immediate importance.

As seen in Figure 14, the Lewis-a and Lewis-b determinants are synthesized from the Type 1 chain. The introduction of an α-L-fucopyranosyl group at the 4-position of the β-D-Glc*N*Ac residue provides the terminal trisaccharide of the Lewis-a determinant. α-L-Fucosylation of the 2-position of the β-D-Gal residue

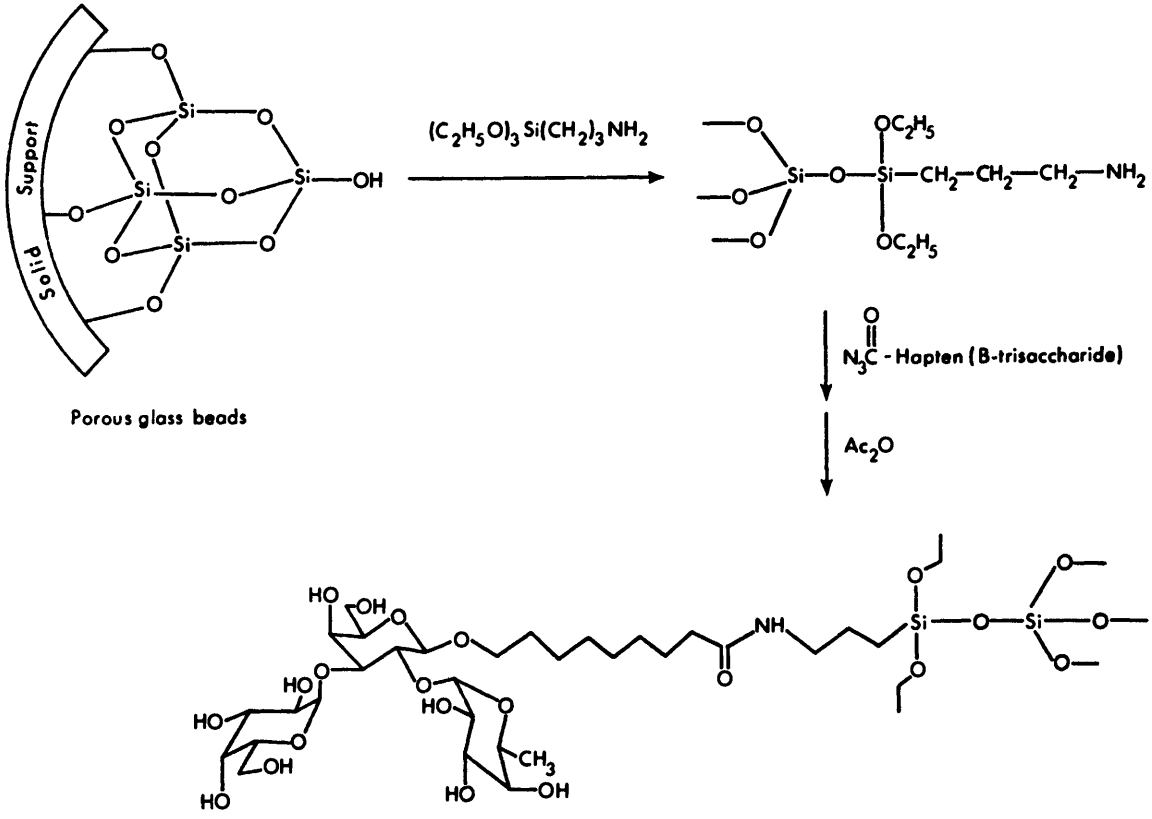


Figure 13 *Outline of a procedure for the preparation of an immunoadsorbent specific for human anti-B antibodies*

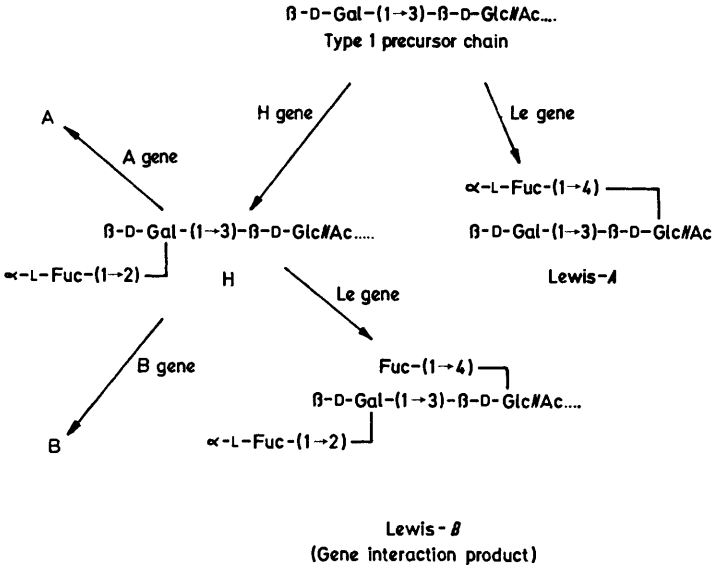
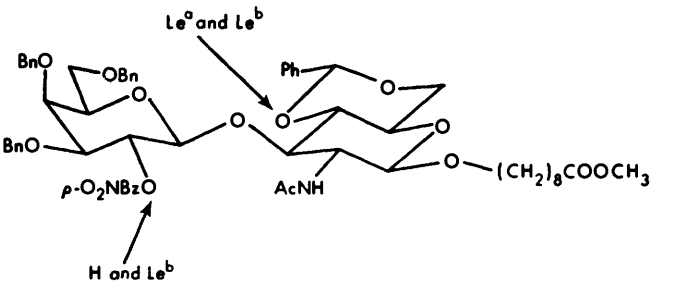


Figure 14 *The relationship between the A, B, H and Lewis human blood-group determinants*

of the Lewis-a trisaccharide would lead to the terminal tetrasaccharide for the Lewis-b group. However, the Lewis-b determinant is a gene interaction product, and is actually derived by α-fucosylation of the H-determinant.

The syntheses of the Le^a and Le^b determinants provided very interesting synthetic challenges.[15,30] Of course, these are multistep processes and a detailed consideration of the various stages in these syntheses does not seem warranted on this occasion. Figure 15 simply presents a key intermediate which can be used to synthesize either the H, the Le^a, or the Le^b determinant.



Ac = acetyl, Bn = benzyl, Bz = benzoyl, Ph = phenyl

Figure 15 *A synthetic precursor to the H, Lewis-a and Lewis-b determinants*

[30] R. U. Lemieux and D. A. Baker, *Canad. J. Chem.*, submitted.

Hydrolysis of the *p*-nitrobenzoyl group followed by a halide-ion catalysed glycosidation using tri-*O*-benzyl-α-L-fucopyranosyl bromide as reagent introduced the tri-*O*-benzyl-α-L-fucopyranosyl group at this position. Catalytic hydrogenolysis then provided the desired H-trisaccharide hapten.[30] On the other hand, instead of the hydrogenolysis, the 4,6-*O*-benzylidene group could be selectively removed by mild acid hydrolysis. The monoacetylation of this product introduced the acetyl group at the less hindered primary position in high yield. This product then possessed a free hydroxyl at position 4 of the β-D-Glc*N*Ac-residue which was available for α-L-fucosylation as previously described for the preparation of the H-determinant. The product of this reaction is shown in Figure 16.

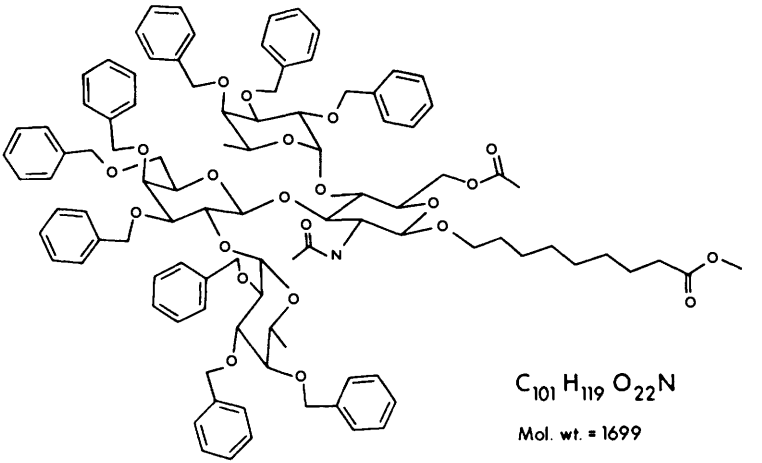


Figure 16 *The blocked synthetic intermediate which on hydrogenolysis provided the terminal tetrasaccharide unit for the Lewis-b human blood-group determinant*[30]

I felt it desirable to point out through such a complete drawing that, because of the extensive use of abbreviated formulae, carbohydrate structures, to the uninitiated, often appear much simpler than these really are. First of all, it may be noted that the blocked penultimate intermediate in the preparation of the Lewis-b determinant is an oily substance of very high molecular weight. The separation of this substance from appreciable amounts of diasteroisomers as could arise in the fucosylation reactions would present a very serious problem. The avoidance of such problems by the utilization of highly stereospecific glycosidation reactions is not only desirable but virtually indispensable as is the characterization of the products encountered in these syntheses by Fourier-transform n.m.r. at the highest possible magnetic field.

The production of monospecific antibodies from artificial antigens is expected to become of major importance both to medical research and practice. Figure 17 illustrates the preparation of an artificial antigen using bovine serum albumin

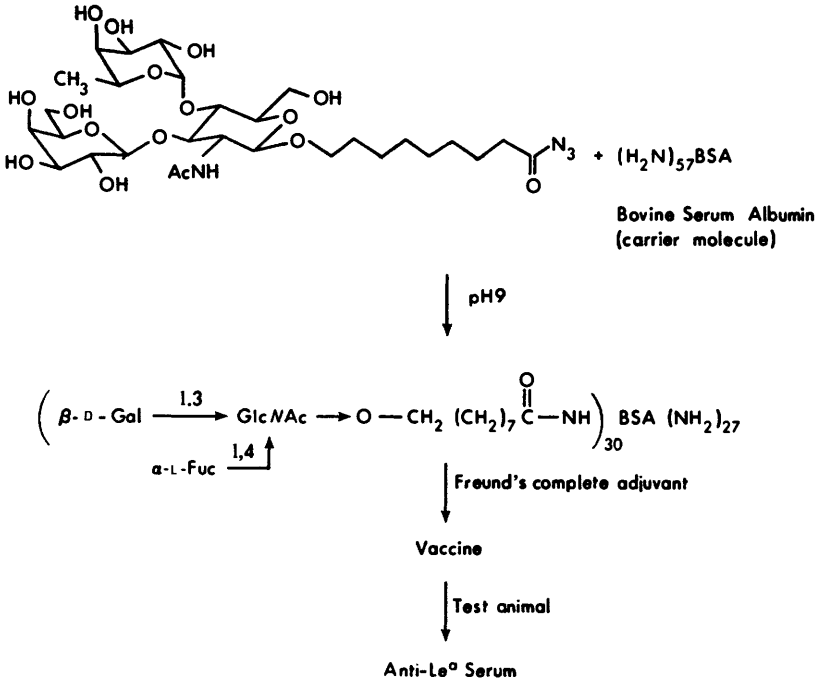


Figure 17 *The preparation of an artificial antigen possessing the terminal trisaccharide unit for the Lewis-a human blood group as antigenic determinant*[15]

as the carrier molecule and the trisaccharide determinant related to the Lewis-a human blood group.[15] The antibodies raised in animals against this antigen show good promise as a source of anti-Lewis-a antibodies both for tissue and red cell typing and for the detection of Lewis antigens in body fluid and secretions, and this matter promises to be a major development in these regards.

An interesting contrast between the use of an artificial antigen and a natural antigen in immunization studies is provided by our experience using the Lewis-b specific human blood-group substance, a highly purified glycoprotein provided by Professor E. A. Kabat and termed the HLe^b blood-group substance because of the close relationship between the H and Le^b determinants.

As seen in Figure 18, the HLe^b blood-group substance used in the immunization of rabbits led to the precipitation of 375 μg of protein per 50 μl of crude antiserum. However, our artificial Le^b antigen precipitated only about 45% of this amount.[30] Thus, the natural antigen raised more antibodies which do not recognize the Le^b tetrasaccharide determinant than those that do. It is seen that our H and Le^a artificial antigens also precipitated protein from the crude anti-HLe^b serum. However, as seen in Figure 19, the H, Le^a, and Le^b antibodies were completely adsorbed from the serum using our Le^b immunoadsorbent. As

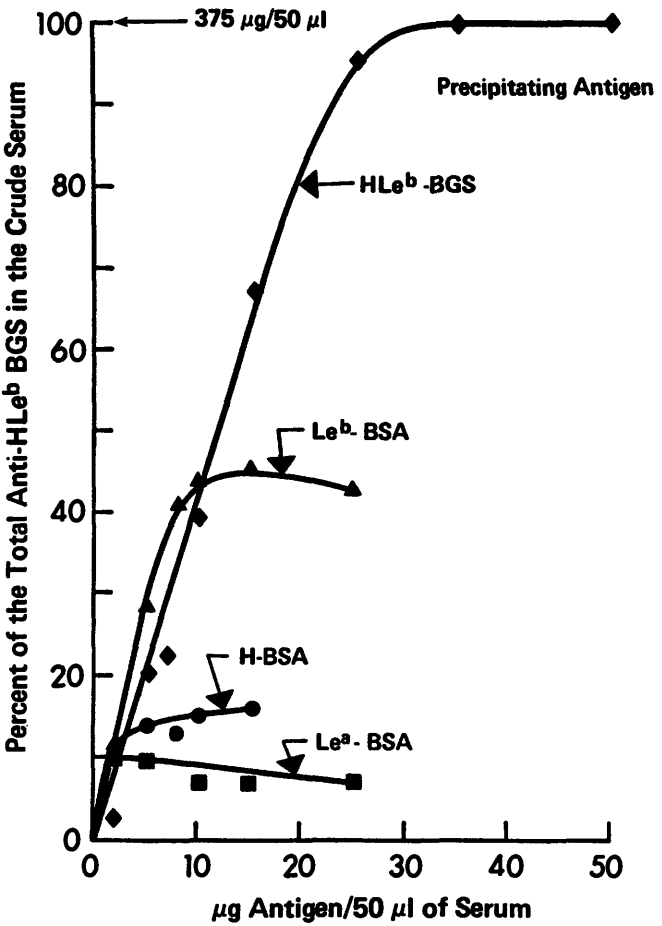


Figure 18 *Precipitin data on the crude antiserum from rabbit immunized against natural Lewis-b human blood-group substance and using both the natural antigen and artificial antigens to effect the precipitation*[30]

expected from the precipitin curves, shown in Figure 18, only about half of the antibodies which were precipitated from the serum by the immunizing antigen passed through the column. When the antibodies that were adsorbed were desorbed and isolated, as expected, these were precipitated as well by the artificial antigen as by the immunizing HLe^b blood-group substance.[30] We do not know the specificities of those antibodies which were raised to the natural antigen but which do not combine with the Le^b-tetrasaccharide determinant. It is evident, however, that this glycoprotein has more than one immunologically important determinant. Furthermore, it is evident that a precipitation of an antigen by this

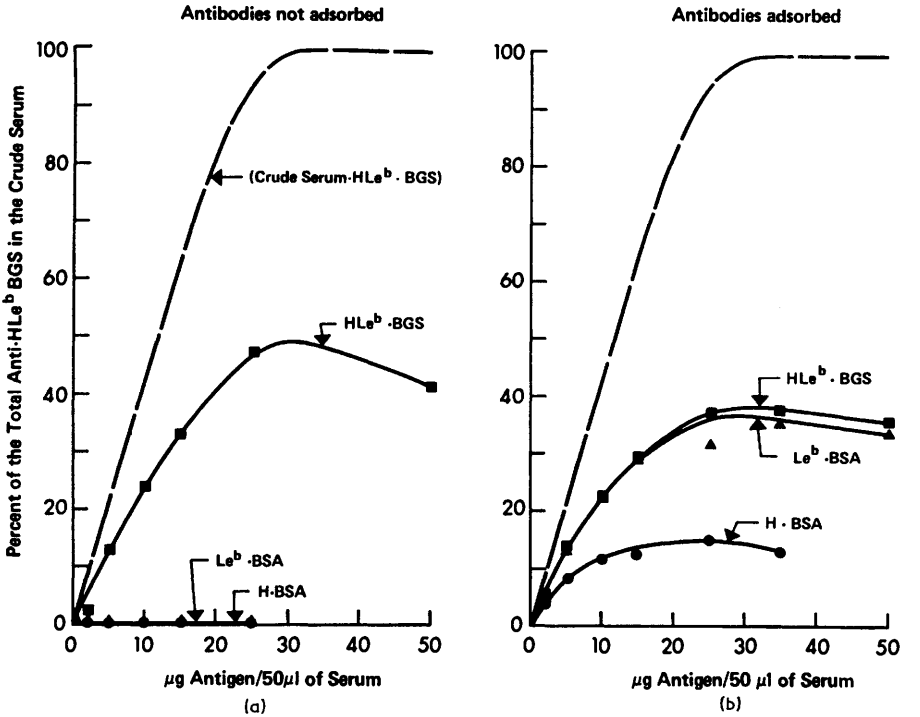


Figure 19 *Precipitin data using the antigen shown to effect precipitation from serum described in Figure* 18 (a) *after adsorption with a Lewis-b active glass-bead immunoadsorbent and,* (b) *on the anti-Lewis-b antibodies which had adsorbed on the column*[30]

antiserum would not necessarily indicate Le^b activity in terms of the Le^b tetrasaccharide structure. A definite conclusion in this regard could only be reached by examining whether or not the precipitation is inhibited by the Le^b tetrasaccharide and this illustrates a possible use for the synthetic determinants as inhibitors. We expect that such studies will help to clarify the multiple immunogenicities of such glycoproteins—a matter which could well prove, I think, to be of substantial importance.

In closing, I would like to say a few words about the conformational properties of human blood-group determinants by using the Le^a, Le^b, and H determinants as an example. Our approach in this respect,[31] as discussed earlier with regard to the Type 1 and 2 chains, is to make hard-sphere calculations based on the *exo*-anomeric effect to estimate what may be the sterically most plausible conformation. Once indications in this respect are obtained, we examine the proton and carbon-13 n.m.r. spectra to see whether or not the conformations predicted

[31] R. U. Lemieux, S. Koto, and V. S. Rao, *Rec. Trav. chim.*, submitted.

by the molecular modelling experiments are in reasonable accord with the observed n.m.r. parameters.

The total non-bonded interactions present at various ψ^H angles are plotted against the ψ^H angles for the oligosaccharide structures as is shown in Figure 20.

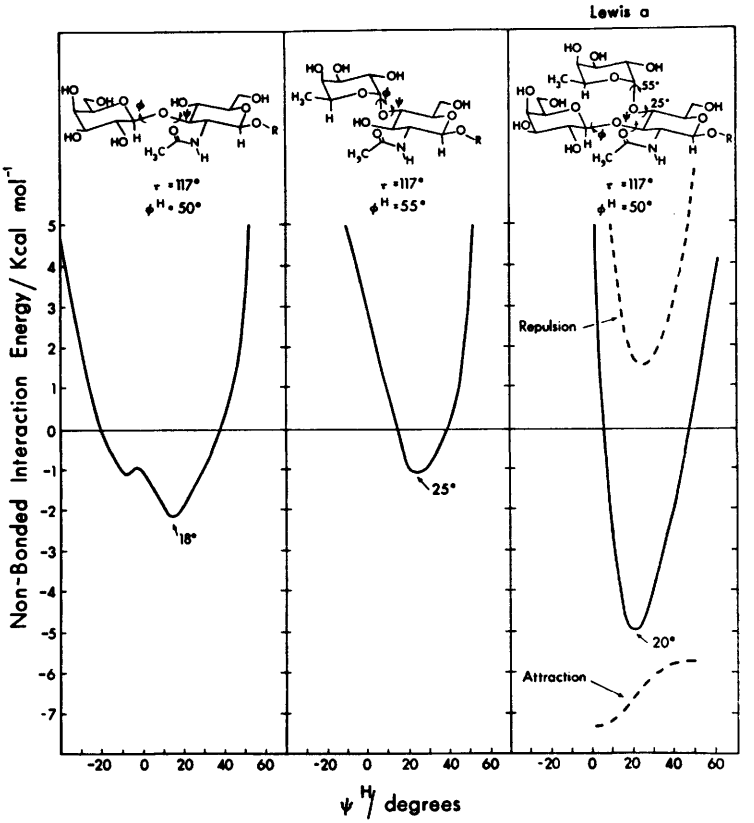


Figure 20 *Hard-sphere calculations, based on the ϕ^H torsion angles expected because of the* exo-*anomeric effect, to estimate the preferred conformations for the terminal Lewis-a trisaccharide and its two component disaccharides*

Thus, a plot of the change in conformational energy with change in ψ^H is obtained. We term these plots ψ-energy profiles. The valence angle for the glycosidic oxygen, termed the τ angle, was set as 117° for all of these calculations—a value often found in the crystal structures of disaccharides. These calculations were made using the Kitaygorodsky formula[32] which involves the use of both attractive and repulsive terms between the various atoms in the molecules treated as hard-spheres. In fact, an inspection of these contributions shows that

[32] A. I. Kitaygorodsky, *Tetrahedron*, 1961, **14**, 230.

the attractive terms have little influence on the preferred ψ^H angle. That is, nearly the same ψ^H angle would be predicted on the basis of the repulsive interactions only. Nevertheless, it is of interest to note that the total attractive interactions are greater than the total repulsive interactions at the most favourable ψ^H angle for the three oligosaccharides shown. Certainly, the accumulation of near 7 kcal mol^{-1} of attractive interactions *versus* about 2 kcal mol^{-1} repulsive interactions in the Lewis-a determinant with the ϕ^H and ψ^H angles shown well displays the large net attractions that can arise from a large number of small Van der Waal attractions—a matter of great importance to antibody-antigen combination including, I believe,[33] carbohydrate determinants.

It is seen from the ψ-energy profiles for the two disaccharides that the β-linkage appears to offer a broader range of conformations of near equal conformational energy than does the α-linkage. For this reason, the ψ^H angle for the α-L-Fuc-(1→4)-β-D-Glc*N*Ac portion of the Lewis-a trisaccharide was set at the 25° value estimated for the disaccharide itself. Therefore, the ψ-energy profile shown for the Lewis-a trisaccharide is that arising through variation of the ψ^H angle for the β-D-Gal glycosidic linkage. On this basis, the most favourable value for this ψ^H angle appears to be +20°.

Having thus arrived at a conformation for the Lewis-a determinant, the most favourable orientation for an α-L-Fuc residue placed at the 2-position of the β-D-Gal unit to form the Lewis-b determinant was estimated. As seen from ψ-energy profiles plotted in Figure 21, this α-L-Fuc residue encounters severe repulsive non-bonded interactions when the ϕ^H angle is set at the 'normal' value of 55°. The major contribution arises from van der Waal conflict between the H-1 of this α-L-fucosyl group and O-3 of the β-D-galactosyl unit—a repulsion of near 3 kcal mol^{-1}. However, a change of the ϕ^H angle from 55° to 40°, a change which is not expected to be highly demanding in terms of the *exo*-anomeric effect,[11] lowered this interaction to 0.3 kcal mol^{-1} without introducing substantial other destabilizing interactions. Therefore, the ϕ^H angle for this α-L-Fuc residue is assumed to be 'abnormal' with a value of near 40°.

A comparison of the ψ-energy profile for the H-determinant with that for the Lewis-a determinant at once shows that the strongly repulsive interactions found in the H-determinant (and consequently in the Lewis-b determinant) are interactions between the α-L-Fuc group attached to the 2-position of the β-D-Gal and the β-D-Gal-(1→3)-β-D-Glc*N*Ac disaccharide.

Figure 22 provides a list of internuclear distances and van der Waal interactions which help to better describe the conformations of the H, Lewis-a, and Lewis-b determinants. Also, some of these interactions appear highly pertinent to the interpretation of the p.m.r. spectra for these structures.

First of all, it may be noted that the two α-L-Fuc residues touch each other over the β-side of the β-D-Gal unit. That is, the methyl group of the c-fucosyl unit is within van der Waal interaction with the ring-oxygen and the methyl group of the d-fucosyl unit.

[33] R. U. Lemieux, P. H. Boullanger, D. R. Bundle, D. A. Baker, A. Nagpurkar, and A. Venot, *Nouveau J. Chim.* 1978, **2**, 321.

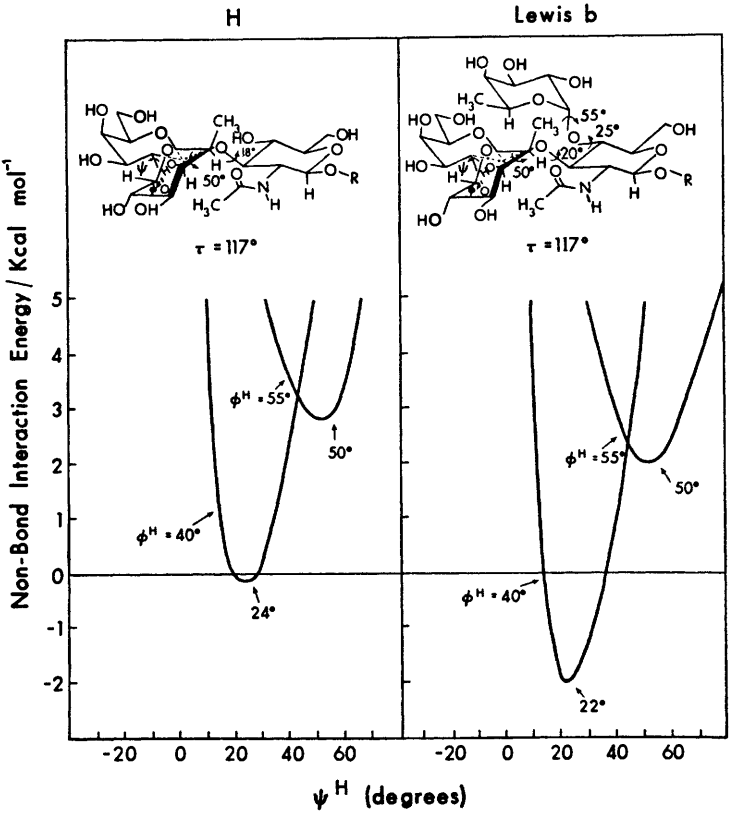


Figure 21 *Hard-sphere calculations to estimate the preferred conformations for the H trisaccharide and Lewis-b tetrasaccharide determinants and to illustrate the effect of changing the ϕ^H torsion angle from 55° to 40° for the α-L-fucosyl group attached to the 2-position of the β-D-galactosyl residue*

Secondly, H-5^c is seen to be strongly interacting with both O-1^b and O-5^b. These repulsive interactions could be expected to strongly deshield H-5^c and it will be seen that in fact this hydrogen is strongly deshielded both for the Le^a and Le^b determinants.

Thirdly, it is seen that H-5^d is very close to O-1^b. Although the interaction appears weak, deshielding of H-5^d relative to its position in the spectrum for methyl α-L-fucopyranoside must be expected.

Finally, it is seen that H-1^d and O-3^b appear in a repulsive interaction. As noted above, this interaction was strongly relieved by changing the ϕ^{Hd} torsion angle from 55° to 40°. This residual interaction between H-1^d and O-3^b would be in accord with the occurrence of the p.m.r. signal for H-1^d at an unusually low field.

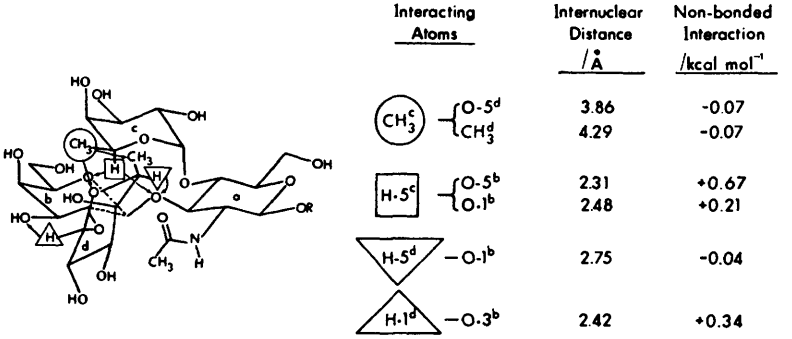


Figure 22 *Internuclear distances for the Lewis-b tetrasaccharide determinant involving atoms whose signals are readily identified in the proton magnetic resonance spectrum (Figure 23). The conformation is that depicted in Figure 21*

The p.m.r. spectra in D_2O for the H and Le^a trisaccharide determinants and for the Le^b tetrasaccharide determinant taken at 360 MHz are presented in Figure 23. These spectra display well the great promise offered by these high-field instruments to obtain information, both structural and conformational, about these complex structures. As already inferred, the spectra provide excellent support for the conformations of these structures as derived by hard-sphere calculations based on the operation of the *exo*-anomeric effect.

First of all, the spacings observed are in accord with the chair conformations shown for all of the sugar residues.[34] Note, also, that an addition of the spectra for the Le^a and H determinants provides an excellent approximation of the spectrum for the Le^b determinant—as is required by the procedure used to make the calculations.

Because H-1^d of the H and Le^b determinants interacts strongly with O-3^b of the galactosyl residue, this hydrogen should be to lower field than H-1^c of the other fucosyl group in the Le^b determinant and of H-1^c in the fucosyl group of the Le^a determinant—and it is to lower field by 0.13 p.p.m.

H-5^c interacts strongly with both O-1^b and O-5^b and should be strongly deshielded. In fact, the signal for this hydrogen is 0.94 p.p.m. to lower field than that for the corresponding hydrogen in the model methyl α-L-fucopyranoside and, indeed, it is over 0.3 p.p.m. to lower field than either one of the β-anomeric hydrogens of the Le^a trisaccharide. This remarkable observation appears definitely to require that the conformation of the Le^a determinant be very close to that predicted by molecular modelling after assuming the influence of the *exo*-anomeric effect.

It is seen that H-5^d in both the H and Le^b determinants is 0.42 p.p.m. to lower field than H-5 of methyl α-L-fucopyranoside and this deshielding can be attributed to the interaction with O-1^b.

[34] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.

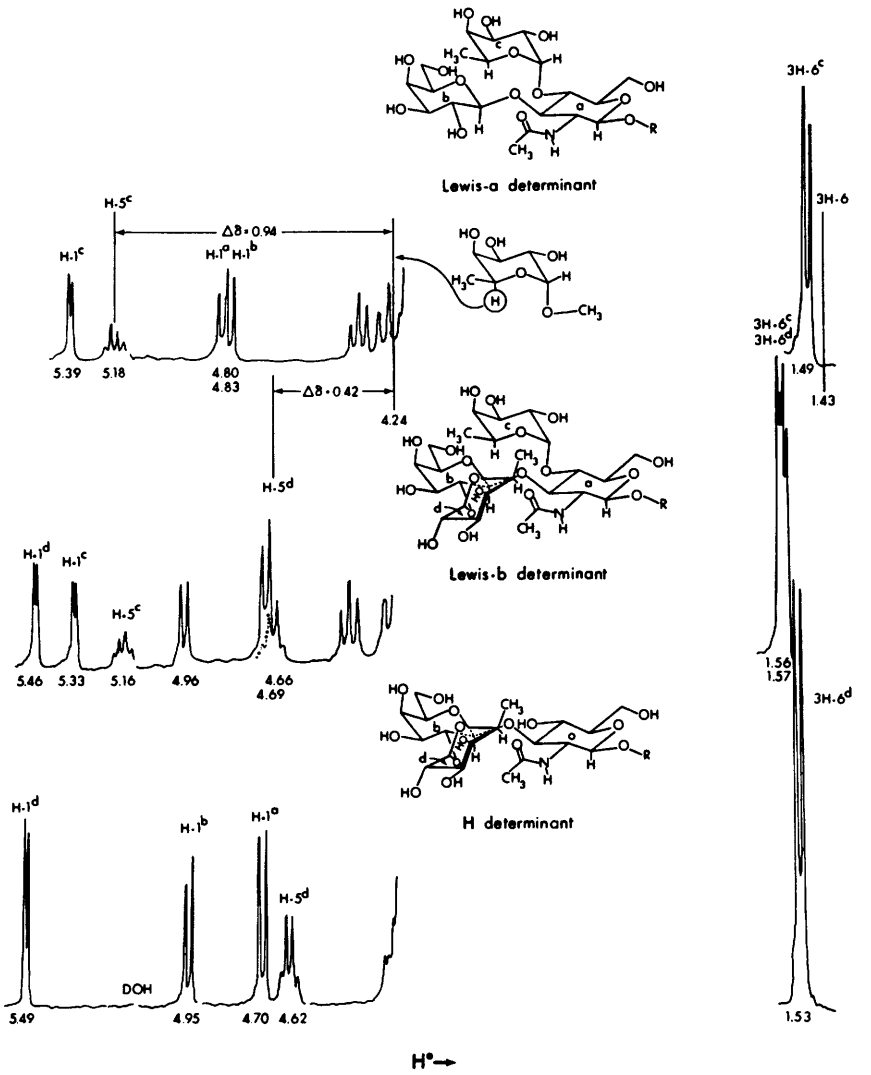


Figure 23 360 MHz *proton magnetic resonance spectra for the synthetic Lewis-a, Lewis-b, and H determinants* [R = $(CH_2)_8COOCH_3$] *dissolved in* D_2O

Furthermore, the occurrence of the signals for the two *C*-methyl groups in the Le^b determinant to lower field than those for *C*-methyl groups of the parent Le^a and H determinants is in accord with the close proximity of the two fucosyl residues in the Le^b determinant.

The general agreement between the calculated model and the p.m.r. spectra is in a way surprising since the molecular modelling assumes no influence whatsoever from an environment, and the proton magnetic resonance spectra are for the structures dissolved in water. This correspondence clearly suggests a high degree of conformational rigidity for the structures and, of course, to be most effective as a determinant, a structure should be conformationally well specified. Otherwise, the energy required to organize a determinant into the conformation demanded by the combining site will oppose the driving force for combination.

In conclusion, the objective of my lecture today has been to attempt to display, mainly through recent research in my laboratory, the state of a body of knowledge—the roots of which can be traced back to the work by Haworth. I hope I was able to show that the leading edges of synthetic and structural carbohydrate chemistry are now meeting leading edges in biology and that this meeting augurs well for the improvement of the knowledge required to deal with important human problems.

As pointed out by Angyal[35] in his review of conformational analysis in carbohydrate chemistry, Haworth predicted in 1929[36] with regard to the various possible shapes of sugar molecules that, 'these considerations open up a large field of inquiry into the conformation of groups as distinct from structure or configuration'. Considering that there existed no experimental data to guide this speculation, this was a remarkable prediction indeed.

[35] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis', Interscience, New York, 1965, p. 362.

[36] W. N. Haworth, 'The Constitution of Sugars', Edward Arnold & Co., London, 1929, p. 90.