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Advances in the development of convergent schemes for the synthesis of biologically important glycoconjugates[§]

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Abstract: The use of glycals, both as glycosyl donors and as glycosyl acceptors in convergent routes to complex glycoconjugates is described. The use of glycosylation, iodoglycosylation and azaglycosylation protocols en route to a variety of goal structures, including glycopeptides, is reported.

Nature places heavy reliance on three classes of biooligomers -polypeptides, polynucleotides and polysaccharides- for its biological transactions. All are assembled naturally under enzymatic governance. Given the centrality of these oligomers in all life processes, they have attracted the attentions of chemists who would seek to understand their function with resolution at the molecular level. A special contribution of synthetic organic chemists toward this goal can arise from their ability to synthesize such entities according to specification. It is appropriate to consider the sorts of challenges posed to the chemist who would undertake such syntheses.

While the amino acid building blocks in polypeptides or proteins are diverse, the α-amino and α-carboxyl linkage sites and the L-configurations of the α-carbon-centers are highly conserved. Moreover, no new stereochemical issues are, *per se*, encountered in the fashioning of the connecting (peptide) bond. From a synthesis standpoint, the matters for concern are effective activation of the "carboxyl" carbon, avoidance of racemization, and appropriate protection of side chain functionality in the coupling act.[1]

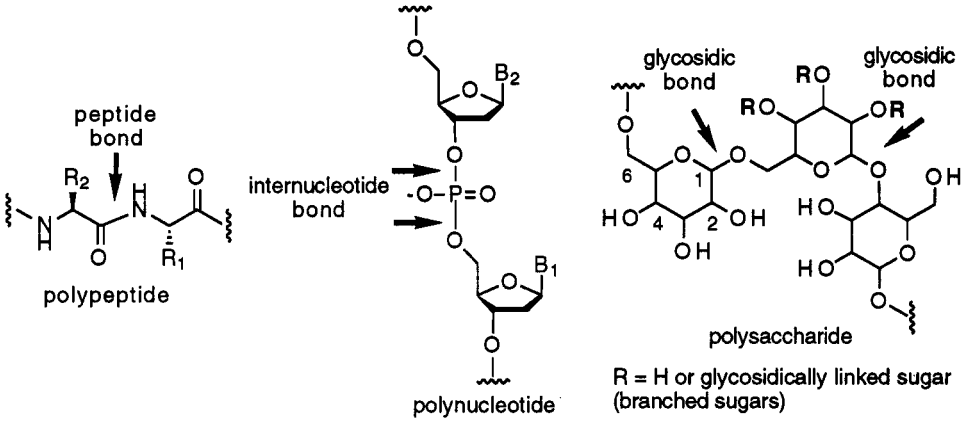
In oligonucleotides, where the diversity of the building blocks is particularly circumscribed, both as to structure as well as configuration of the stereogenic carbons, no new stereochemistry is encountered in fashioning the connecting internucleotide bonds. From a synthetic standpoint, the central concerns involve differentiation of C5' from C3' (and, in oligoribonucleotide synthesis, from C2') and development of high yielding coupling methods. After much travail, advances have been achieved in insuring suitable activation for coupling of the phosphorous atom, while providing for protection of its oxygens or surrogates for those oxygens.[2]

From a strictly chemical standpoint, the oligosaccharides are surely the most interesting and complex of the biooligomers. While one site of the repeating bonds of oligosaccharides is, with high regularity, mounted at the masked carbonyl (anomeric) center of the hexose or pentose, the other anchor may be at any of the hydroxyl centers of the nearest sugar. Moreover, all secondary carbon centers, including the anchor points of the glycosidic bond are stereogenic. *Every coupling reaction is an event fraught with stereochemical consequences.* Furthermore, a particular component in the chain of an oligosaccharide may be attached to more than two sugars. Structural diversity and complexity can be increased by branching.[3]

Early perceptions of carbohydrates centered on their roles as structural materials, as biological fuels and as "starting materials" for other fairly small biointermediates (such as those arising from glycolysis and the Krebs cycle).

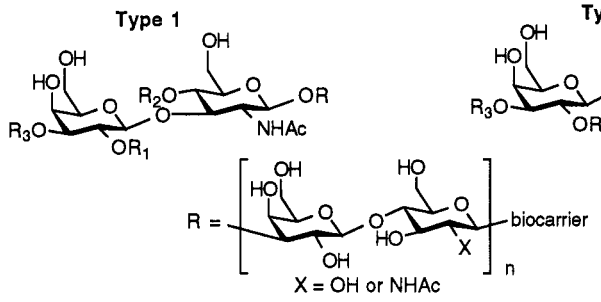
As the techniques and insights in the biology and biochemistry of carbohydrates became increasingly detailed, and as the capacity to purify, isolate and identify small amounts of complex structures, including oligosaccharides, has become ever more powerful,[4] new roles for elaborate saccharides and glycoconjugates have become apparent.[5]

§ This paper is dedicated to the memory of Professor Isidore Danishefsky and to his works in the structure of heparin.



The appearance of carbohydrates in a bewildering range of antibiotics is now taken for granted.[6] Their involvement as glycoproteins in protein transport and protein folding is becoming increasingly recognized.[5] Perhaps, the most sophisticated use which nature makes of carbohydrates is as cell surface ligands. The rich three dimensional panorama of these carbohydrate motifs allows for their presentation in various specific ligand-receptor interactions for cell-cell signaling, adhesion and penetration.[7] Generally, for these missions, the carbohydrate ligands are housed in the context of cell surface glycoproteins (including mucins) and cell surface membrane bound glycolipids (including gangliosides).[8]

One area where the shape of the oligosaccharide is critical in determining the specificity of its biological action is that of blood group antigens. The recognition of ABO system by Landsteiner revolutionized the science and practicalities of blood transfusion.[9] The specificities of other carbohydrate domains provide the basis for the Lewis blood group locus which, in turn, is implicated in secretion, inflammation and adhesion.[10]

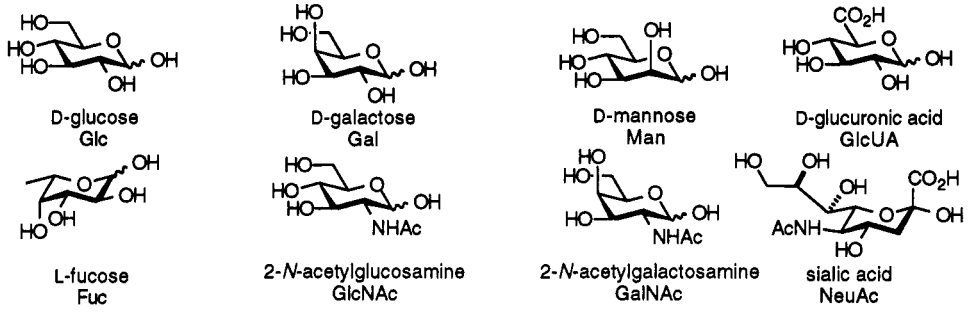


H: R_1 = α-Fuc; R_2 = H; R_3 = H	H: R_1 = α-Fuc; R_2 = H; R_3 = H
A: R_1 = α-Fuc; R_2 = H; R_3 = α-Gal*N*Ac	A: R_1 = α-Fuc; R_2 = H; R_3 = α-Gal*N*Ac
B: R_1 = α-Fuc; R_2 = H; R_3 = α-Gal	B: R_1 = α-Fuc; R_2 = H; R_3 = α-Gal
Le^a: R_1 = H; R_2 = α-Fuc; R_3 = H	Le^x: R_1 = H; R_2 = α-Fuc; R_3 = H
Le^b: R_1, R_2 = α-Fuc; R_3 = H	Le^y: R_1, R_2 = α-Fuc; R_3 = H
A-Le^b: R_1, R_2 = α-Fuc; R_3 = α-Gal*N*Ac	A-Le^y: R_1, R_2 = α-Fuc; R_3 = α-Gal*N*Ac
B-Le^b: R_1, R_2 = α-Fuc; R_3 = α-Gal	B-Le^y: R_1, R_2 = α-Fuc; R_3 = α-Gal
Sialyl-Le^a: R_1 = H, R_2 = α-Fuc; R_3 = α-NeuAc	Sialyl-Le^x: R_1 = α-Fuc, R_2 = H; R_3 = α-NeuAc

Blood Group Substances

The substances controlling these specificities often appear as glycoproteins or glycolipids in various tissue fluids and on the surfaces of erythrocytes. Specificities are largely controlled by the oligosaccharide domain. The building blocks to fashion these carbohydrate domains (cf. *infra alia* D-glucose, D-galactose, D-mannose, L-fucose, 2-*N*-acetylglucosamine, 2-*N*-acetyl galactosamine, D-glucoronic acid and sialic acid) are rather uniform. The shape specificity of the carbohydrate domain arises from small but critical diversity in the linkage blueprint of the backbone system, and from the site of branching. The molecular basis of the genetics which govern the blood group phenotype is

apparently the prevalence of genes encoding the various enzymatic synthetases and transferases involved in sculpting the oligosaccharide domain.[11]



Common Oligosaccharide Building Blocks

In the early part of the 1980-1990 decade, as a consequence of some of our discoveries in Lewis acid catalyzed cycloaddition reactions of aldehydes with dienes,[12] we gained exposure to carbohydrate chemistry and, with time, to carbohydrate biology. It is in this way that we were first enticed by the problem of how carbohydrate domains might be synthesized and appropriately conjugated in the laboratory.[13, 14]

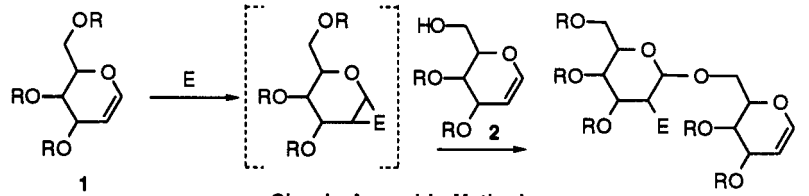
In studying the state of the art of carbohydrate synthesis as it existed at that time, our first reaction was that of awe at the heroic accomplishments of the pioneering scientists. The capacity of chemists to construct ever more complicated carbohydrates in the face of the daunting problems of positional and stereochemical control, must be regarded as one of the major accomplishments in the history of synthesis.[15] Yet, even as one marvels at such conquests, the realization of the need for streamlining, convergence, and eventually, automation in oligosaccharide synthesis is apparent. For instance, at the present time, most of the steps involved in a typical synthesis of such domains are those of protection and deprotection in setting up positionally defined glycosyl donors and acceptors. It is clear that advances in the strategy and art of oligosaccharide assembly and oligosaccharide congener synthesis would find ready application.

In our opinion and that of others, synthetic organic chemistry is destined to play a major role in understanding the relationship of the function and topology of carbohydrate domains to their biological role. Synthesis offers the best hope for presentation of complex and biorecognizable carbohydrate domains in contexts other than those of difficultly manipulatable natural cell surfaces. If the chemical obstacles can be overcome, synthetic carbohydrates can be conjugated to cell free carriers.

For example, aberrant carbohydrate motifs expressed in cancer cell membranes might be linked to cell free carriers thus functioning as tumor vaccines or as antagonists of metastasis receptor interactions.[10] Synthetic carbohydrate constructs may be used to modify drug transport or properties of antibiotics. In short, it is through synthesis, (via purely chemical,[15] enzymatic or chemoenzymatic means[16]) that carbohydrates are likely to emerge as therapeutic agents. For such projections to come about, improvements in the way carbohydrate building blocks are constructed from commercial or natural sources would be helpful. Above all, simplifications in the way in which domains are assembled from such building blocks will be needed.

In this paper we describe our recently completed syntheses of two Lewis determinants. The first is the tumor (particularly colon tumor) associated Le^y determinant and the second is the Le^b determinant which has recently been implicated as a recognition element for the attachment of *Helicobacter pylori* to gastric epithelial cells.[17] *Helicobacter pylori* infection is now thought to be a prime causative factor in gastric ulcers and, possibly, a contributor to the onset of gastric cancers. In principle, one can use synthetic carbohydrate ligands, properly conjugated as glycoprotein or glycolipid constructs, as synthetic antigens or receptor antagonists for microorganism invasion sites. We also describe some preliminary but exciting advances in the synthesis of carbohydrate oligomers, including asparagine-linked glycopeptides. Before reporting on these developments, it is well to review, in a cursory way, the logic of our glycal assembly method for carbohydrate synthesis.

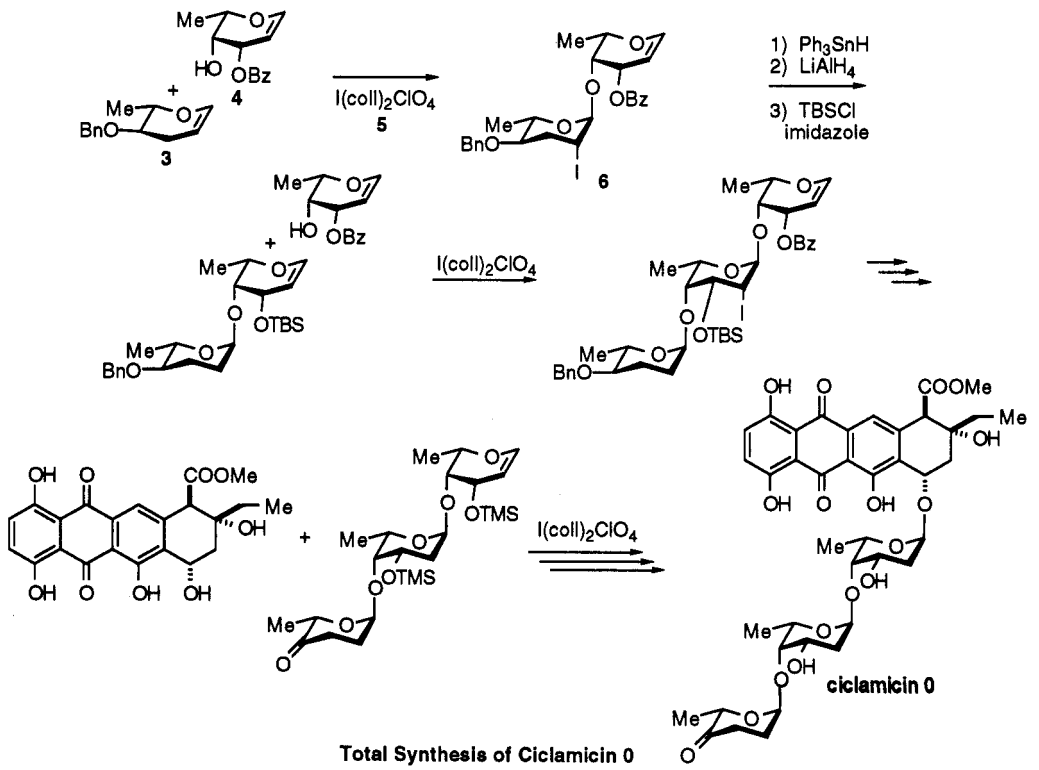
The basic premise of our approach is that glycals offer advantages not only as glycosyl donors **1** but also as glycosyl acceptors **2**. Prior to our involvement in this area, glycals had been used as glycosyl donors. The most celebrated of these constructions was the iodoglycosylation method, first promulgated by Lemieux[18] and developed to such beautiful detail by Thieme.[19]



Glycals Assembly Method

In other protocols, starting with nitrosochlorination[20] or azidonitration,[21] glycals had functioned as precursors of *N*-acetylglucosamine or *N*-acetylgalactosamine donors. However, prior to our work, glycals seemed not to have been used as glycosyl acceptors. Our interest in glycals arose from a perception that use of these entities, both as donors and acceptors, would markedly reduce the complexities of blocking group manipulations in oligosaccharide synthesis. Thus, the number of oxygens to be differentiated is reduced from five in saturated pyranose systems to three by using glycals as acceptors and donors. Furthermore, hydroxyl group differentiation of a glycal is generally a straightforward matter. The first protection tends to occur at C6. The second site of reactivity is the allylic alcohol at C3. Only after these oxygens have been substituted does C4 react. Furthermore, the glycals can be actuated to serve as glycosyl donors by a variety of methods (*vide infra*). Therefore it is not necessary to make provision for C1 and C2 while using glycals as acceptors. Provided flexible protocols for actuating glycals exist, the erstwhile glycal acceptor can be transformed into a donor by suitable activation. Therefore, a key requirement was to develop flexible methods for transforming glycals into donors. In particular, we have focused on three modalities for accomplishing this.

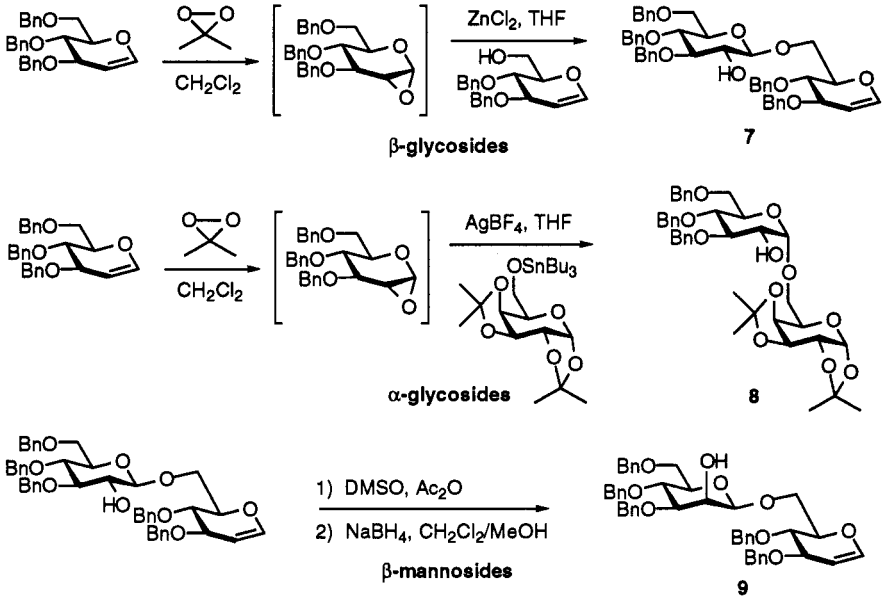
First studied was the method of iodoglycosylation with the additional proviso that not only is the donor a glycal (as in the cases of Lemieux and Thieme), but also that the acceptor would be a glycal. This, in turn, required a strategy for directing the iodonium activating group to a particular glycal. This was accomplished by appropriate use of resident protecting groups.[22, 13] For instance, a coupling of glycals **3** plus **4** gives iodoglycoside **6** wherein the iodonium ion **5** has attacked that glycal which bears resident ether linkages rather than ester linkages. To reiterate the process, the ester of the disaccharide glycals are converted to alcohols and thence to ethers. Condensation with an ester substituted glycal paves the way for trisaccharide formation. This chemistry was used most vividly in the synthesis of ciclamicin O.[23]



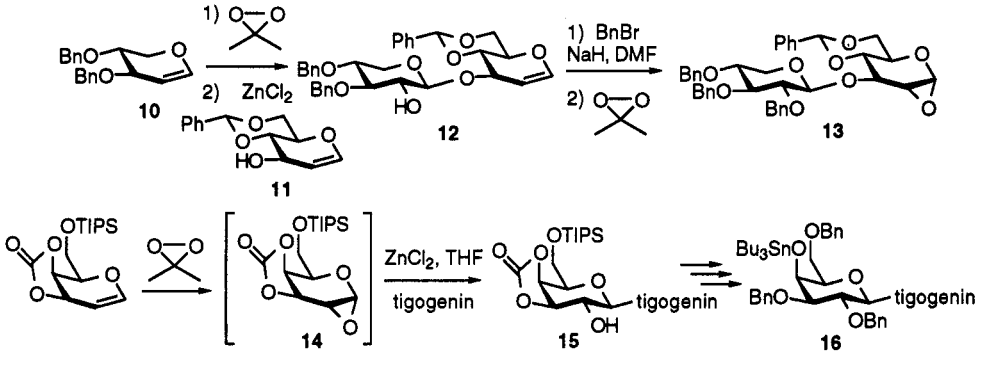
Total Synthesis of Ciclamicin 0

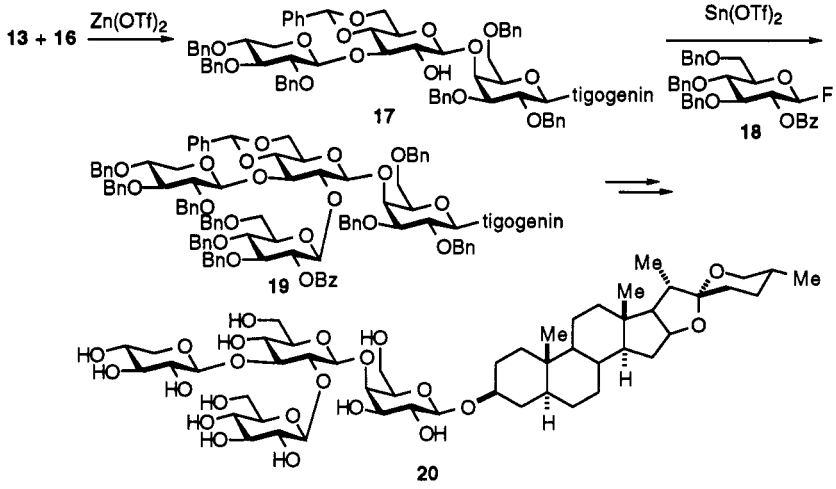
A second method which was introduced was a direct conversion of glycals to glycal epoxides.[14] Such a transformation had apparently not been reported previously and can now be conducted through the use of dioxiranes. Most of our work had been conducted with dimethyldioxirane as the oxidizing agent. This epoxidation occurs under neutral conditions allowing for survival of the acid labile glycal epoxide (Brigle's anhydride). For work on a larger scale, where higher concentrations of dioxirane are required, it has recently been found that the agent derived from trifluoroacetone can be prepared in more concentrated form, thus allowing for larger scale processing of glycals.[24]

An important finding was that with appropriate resident group protection, and under appropriate catalysis, such glycal derived 1,2-epoxides serve as glycosyl donors to furnish β-glycosides.[14] A general catalyst to mediate glycoside formation with configurational inversion of the epoxide is zinc chloride. Recently, more specialized catalyst systems have been used to mediate direct synthesis of α-glycosides from α-epoxides, though such extensions await full generalization.[25] Displacement of glycal epoxides by acceptor at the anomeric carbon can be used to generate a unique alcohol at C2 (see **7** and **8**). This hydroxyl can serve as an acceptor site for C2 branching. The C2-hydroxyl function also can be converted to the β-counterpart by oxidation/reduction. Thus, as a result of this protocol, β-mannosides become available (see **9**).[26]



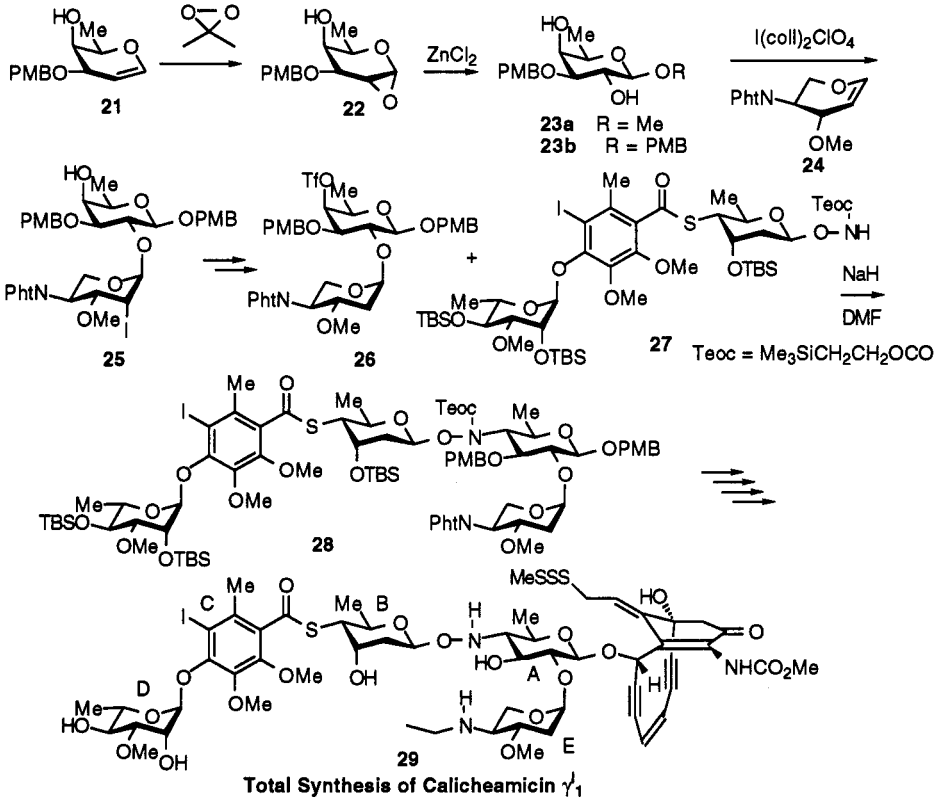
The strategy of glycal assembly was well demonstrated by the synthesis of desgalactotigonin **20**.[27] Thus, the xylose glycal **10** was converted to its epoxide and coupled with the differentiated glycal **11** to furnish **12**. Furthermore, coupling of tigonin to glycal epoxide **14** generated **15**. After suitable protecting group manipulation, glycosyl acceptor **16** was in hand. Coupling of **13** with **16** provided **17** which has a unique hydroxyl function at C2'. This hydroxyl was used as a glycosyl acceptor site with suitably activated derivative of D-glucose (see **18**) to give **19**. Removal of all protecting groups gave, for the first time by synthesis, a sterol bearing the complex oligosaccharide domain of the saponins.





Total Synthesis of Desgalactotigonin

The combination of iodoglycosylation and glycal epoxide methodology was used to good effect in the construction of the AE disaccharide corresponding to calicheamicin.[28] Thus, reaction of the D-fucal derived glycal **21** with dimethyl dioxirane produced **22**. This donor reacted with several alcohol acceptors to produce products **23** bearing an equatorial hydroxyl group at C2 and an axial hydroxyl at C4. Reaction of the C2 hydroxyl group of **23b** with glycal **24**, via iodoglycosylation, produced product **25**. Thus, at least in the cases of **23a** and **23b**, the glycosyl accepting power of the C2 hydroxyl was greater than was that of the C4 hydroxyl. Upon de-iodination and triflation of the lone axial hydroxyl, **25** was converted to **26** which served as the electrophile with respect to tricyclic construct **27**. Compound **28** thus obtained, was converted to calicheamicin **29**. A remarkable feature of this synthesis was that the glycosylation was conducted on the aglycone acceptor in which the allylic trisulfide was already in place.



Total Synthesis of Calicheamicin γ^{I}_{1}

Before describing our efforts in the field of azaglycosylation which has been our third concentration locus in glycal chemistry, we describe another goal system which brings us closer to the main subject of this lecture.

Research over the last ten years has helped to identify the critical role of the selectins in immune cell trafficking.[7a] Selectins are a small family of cell adhesion molecules. Important for our purposes is the fact that each of the selectins contains an extracellular amino terminal lectin (carbohydrate recognition) domain. The three most prominent selectins are L-selectin, E-selectin and P-selectin. Apparently, L-selectin mediates the migration of leukocytes from the blood stream into the lymphatic system. E-selectins are thought to be relevant in mediating the circulation of neutrophils and monocytes along the blood vessel wall en route to damaged tissue.[7b] P-selectin is found in the secretory granules of endothelium. Upon suitable activation (thrombin, histamine or peroxide) these granules move to the cell surface where the P-selectin induces a lymphocyte role similar to E-selectin.

Several groups published seminal papers in 1990 on structural carbohydrate biology of the ligand-lectin interactions involved in adhesion.[7b] The thrust of the finding was that the ligand which is recognized by E-selectin is sialyl Le^x (sLe^x). This ligand is found at the reducing terminal of various glycoproteins and glycolipids on the surface of neutrophils. These findings provided the possibility of treatment of immune hyperactivity by inhibition of selectin mediated cell adhesion.[10c]

The synthesis of sLe^x and, particularly, extended and related congeners thereof emerged as a significant target in organic synthesis. In addition to its role in selectin mediated cell adhesion, sLe^x is a known tumor antigen which is abnormally expressed in a variety of cancers including stomach, colon and breast cancers.[10] Apparently tumor cells expressing sLe^x bind to areas of the vascular endothelium which express L-selectin. Thus, sLe^x may be a ligand which is involved in tumor metastasis.[29] These thoughts lead, quite naturally, to the possibility of new therapeutic approaches to counter metastasis via soluble analogs of sLe^x which might compete for receptor binding sites of the metastatic cells.[10b]

While much progress has been achieved using enzymatic methods for this synthesis of the sLe^x system,[30] it is likely that synthetic chemistry will be important in fashioning all critical analogues of these compounds. It is from this perspective that we began the program of chemical synthesis of adhesion molecules.

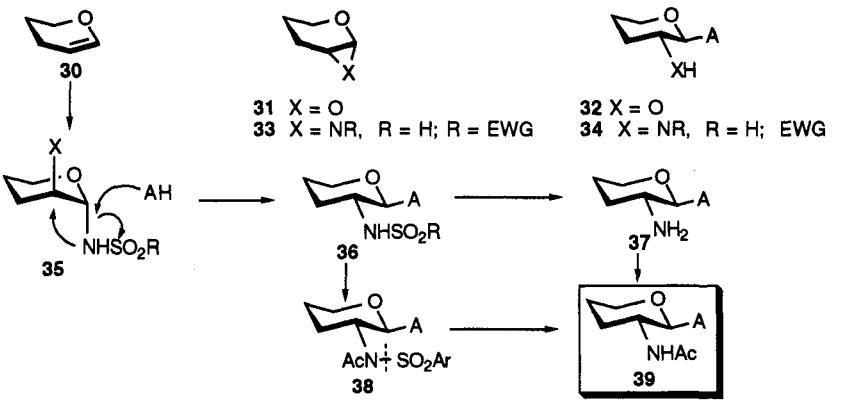
Before describing our solution to the sialyl Le^x problem it is necessary to digress briefly so as to recall the findings in our laboratory pertinent to the azaglycosylaton of glycals. This method was critical not only for solving the adhesion ligand problem, but also for synthesizing a range of blood group determinants and for our new approach to glycopeptide synthesis (*vide infra*).

Given the prevalence of 2-deoxy-2-*N*-acetyl glucosamine and 2-deoxy-2-*N*-acetyl galactosamine residues in a wide variety of natural products, we naturally became interested in synthesizing such substructures from glycals. Mindful of our success in converting glycals of the type **30** to systems of the type **32** (a = glycosyl acceptor) via oxiranes **31**, we took up the possibility of applying the same logic for azaglycosylation. A variety of efforts were expended to achieve the direct conversions of glycals **30** to aziridines of the type **33**. Two sources of aziridines were contemplated. In one instance (R = H) a simple aziridine would be constructed. Alternatively, (R = electron withdrawing group), the aziridine to be fashioned would already be activated for reaction with a glycosyl acceptor leading to **34**. Unfortunately we have been unable to select between the advantages of either type of aziridine, because, at this writing we are unable to find a method which leads to the direct aziridination of glycals.[31] Such an aziridination should be regarded as an interesting challenge to those operating at the interface of synthetic organic chemistry and glycoconjugate chemistry.

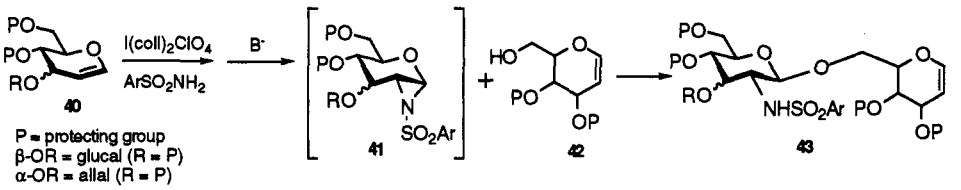
Given our inability to achieve direct aziridination, we turned to indirect methods. A particular goal was to insure the eventual placement of the 2α-*N*-acetyl function regardless of the hindrance situation at C3. The reason for this need will become obvious as the total synthesis of allosamidin is described (*vide infra*). We therefore looked to a reaction which would be governed by stereoelectronic considerations rather than primarily responsive to issues of steric hindrance. In that connection, it was discovered that a broad range of halonium sulfonamide combinations would undergo *trans*-diaxial addition to glycal double bonds.[31] These reactions led to products of the type **35** where X is iodine or bromine. Those systems, in turn, could be converted to goal structures of the type **36** by reaction with glycosyl acceptors under a variety of conditions.

It is likely that this method of transformation passes through aziridines of the type **33** en route to **36**, but the matter was never settled experimentally. Two routes were then developed for conversion

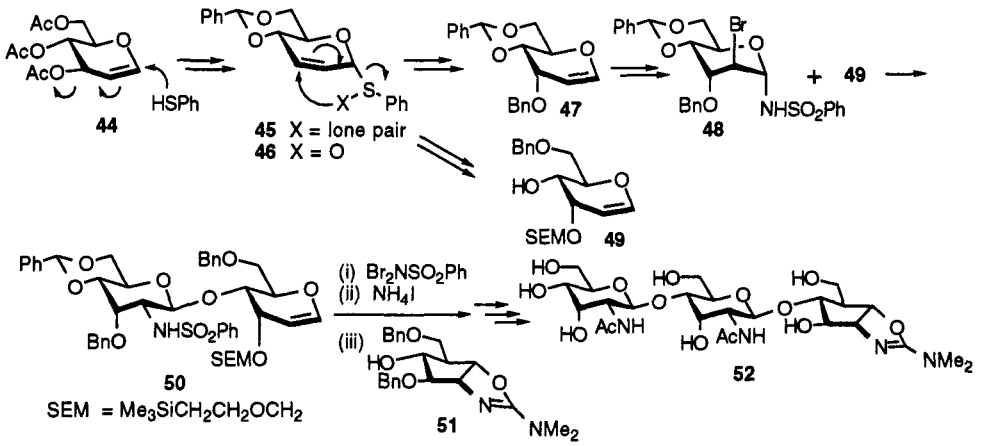
sulfonamido function at C2 to the more generally encountered 2α-*N*-acetyl function. In one modality the sulfonamide was cleaved reductively to generate a 2α-amino group **37** as shown, gave rise to **39**. Alternatively, it was possible to *N*-acetylate the sulfonamide function of **36** thereby giving rise to **38** which in turn, upon very facile reductive cleavage, afforded **39**.



The method lends itself to generalization and is operative with both C3 stereoisomers of **40**. Clearly, the sense of the process is being controlled by a *trans*-diaxial modality of addition to the glycal rather than by issues of local steric hindrance at (C3). Another important extension was the discovery that the glycal acceptor can itself be a glycal (see structure **42**). The resultant product type **43** lends itself to further iteration to give a trisaccharide.

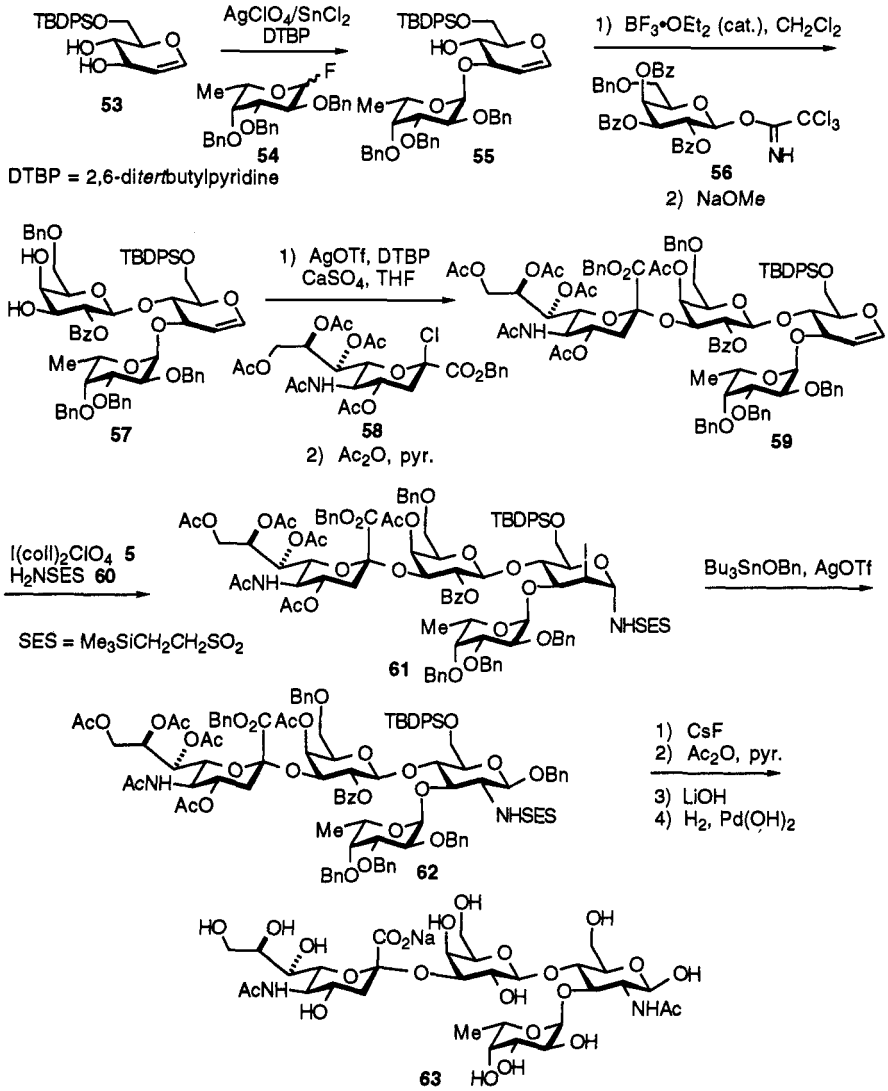


The first major application of azaglycosylation methodology arose from the synthesis of the very powerful chitinase inhibitor allosamidin.[32] As shown in the following figure, the synthesis started with triacetyl D-glucal **44** which underwent thiophenyl Ferrier rearrangement to give rise to a thiophenyl pseudoglycal **45**. Upon oxidation there is generated a labile sulfoxide **46** which suffers apparent [2,3]-sigmatropic rearrangement giving rise, after suitable protection to the allal derivative **47**. Reaction of *N*,*N*-dibromobenzenesulfonamide with **47** followed by reduction led to the *trans*-diaxial addition product **48** which served as a donor with respect to glycosyl acceptor **49** (also fashioned from **44**) to give disaccharide glycal **50**. This process was reiterated by further addition of bromobenzenesulfonamide (through its *N*,*N*-dibromo derivative). The *trans*-diaxial bromosulfonamide served as a glycosyl donor with respect to the novel glycosyl acceptor **51**. Thence, after suitable deprotection maneuvers, allosamidin **52** itself was in hand.



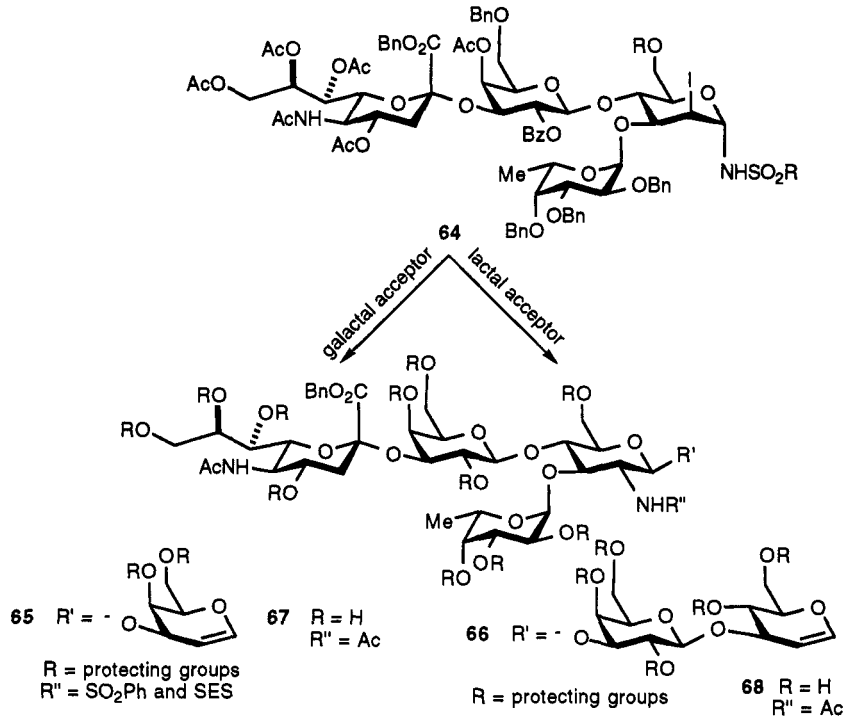
Total Synthesis of Allosamidin

It was from the enabling technology of sulfonamidoglycosylation that we were able to contribute to the sLe^x problem.[33] An important early finding which facilitated the use of a glycal based approach to this goal was that the C6-protected glycal **53** undergoes position and stereoselective fucosylation at the C3 alcohol (with **54**) to provide **55**.[34] From this point, galactosylation at the remaining C4 position was possible. At first we used a Schmidt type galactosyl donor **56**, prepared in a rather lengthy sequence from galactose. After coupling was accomplished under mediation by BF_3•etherate, it was possible to liberate the C2', C3', C4' triol giving rise to compound **57**. The latter was successfully sialylated following the precedents of Hasagawa with sialyl donor **58** to give rise to glycal **59**. Addition of the SES protected sulfonamide **60** in the presence of iodonium equivalent **5** yielded the adduct **61**. It was possible to generate benzyl ether **62** upon treatment of **61** with tributylstannyl benzyl oxide in the presence of silver triflate. This process, which apparently passes through a C1, C2, α-sulfonylaziridine en route to **62** allowed for installation of the anomeric benzyloxy function. Full deprotection gave rise to sLe^x itself (see **63**).



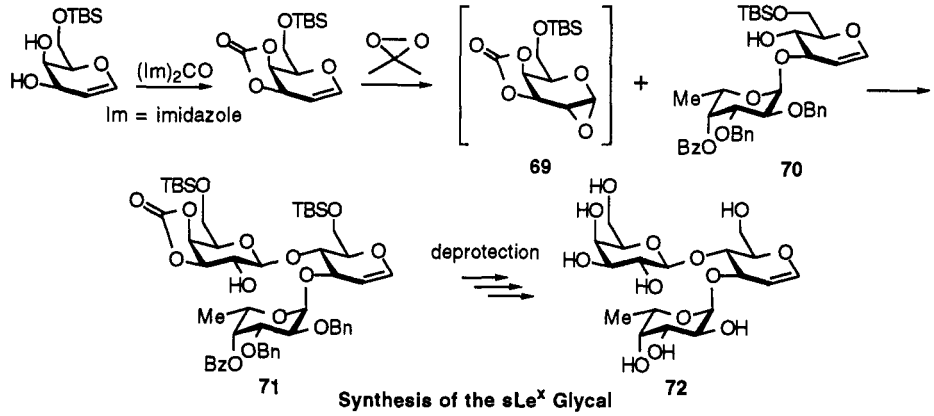
Total Synthesis of Sialyl-Lewis X

Using iodosulfonamide technology it was also possible to introduce an additional glucose or lactose spacer sugar generating glycals **65** and **66** (from **64**) which were fully deprotected to give **67** and **68**.



Sythesis of sLe^x with Lactal and Galactal Spacers

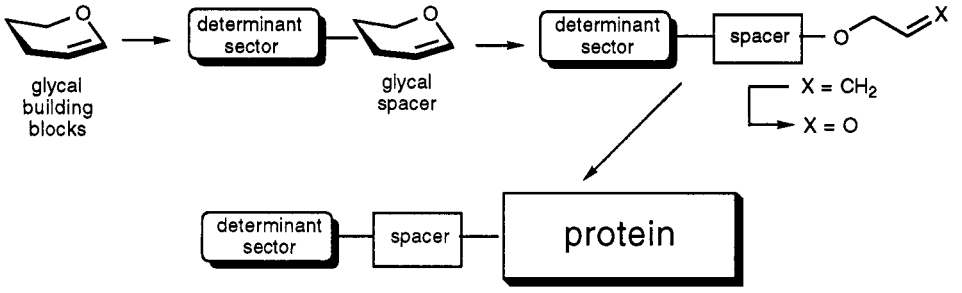
A major simplification of the route involved the coupling of alcohol **70** with galactosylated epoxide **69**.[29] This compound which is made in three steps from D-galactal (generated from its commercially available tetraacetate) has proven to be invaluable as a galactosylating agent. In fact, at least at this writing galactosylation *via* epoxide of the type **69** is more reliable and advanced than is glycosylation via any of our glucal epoxide agents. In the case at hand, coupling occurs in high yield generating **71**. Full deprotection gave the Le^x equivalent **72**. This chemistry illustrates unique convergence and underscores the significant relief from extensive protection-deprotection maneuvers which is available via the logic of the glycal assembly method. The stage was now well set for an attack on the carbohydrate domains of key blood group substances found in both glycoproteins and glycolipids.



Synthesis of the sLe^x Glycal

While the early focus on the blood group substances centered on their role in determining blood group specificities,[9] more recent activity has been stimulated by the finding that blood group substances in conjugated form are encountered as markers in the evolution of certain tumors.[10] In principle, carbohydrate based tumor antigenic factors might be useful at the diagnostic level as markers for early detection of oncogenesis. Alternatively, given sufficient specificity, and properly crafted biodisposable linkers, antibodies to such tumor antigens might be used as carrier for cytotoxic drugs.[35] In the ideal case, such carbohydrate based antigens, obtained by synthesis and properly conjugated to useful

biocarriers might serve as immunostimulating agents.[36] Quite naturally, we perceived a unique applicability of glycals, both as glycosyl donors and glycosyl acceptors, in facilitating the synthesis of tumor selective Lewis antigens as well as the main human blood group substances in relevant bioconjugatable form. This led to a governing paradigm wherein glycal building blocks are assembled as to furnish a determinant sector insulated from carrier by a glycal spacer. The glycal spacer is eventually converted to an allyl glycoside and, eventually, into a glycoside of a glycolic aldehyde (linking arm). Conjugation is achieved via reductive amination to appropriate biocarrier functions. Our first target was the Le^y determinant, equipped with a suitable spacer and an appropriate device for bioconjugation.[17, 37] The Le^y determinant is of particular interest in that it is expressed to an abnormal extent in the cell surface of colon cancer cell lines.

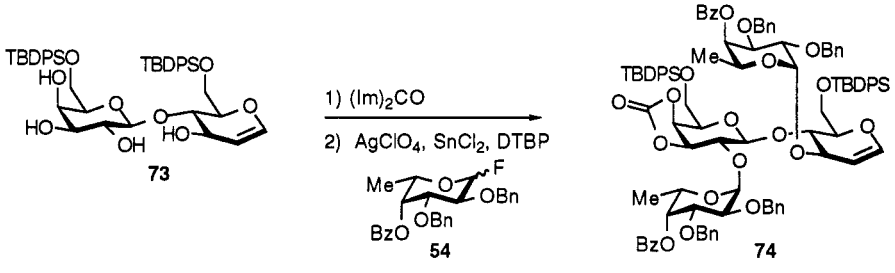


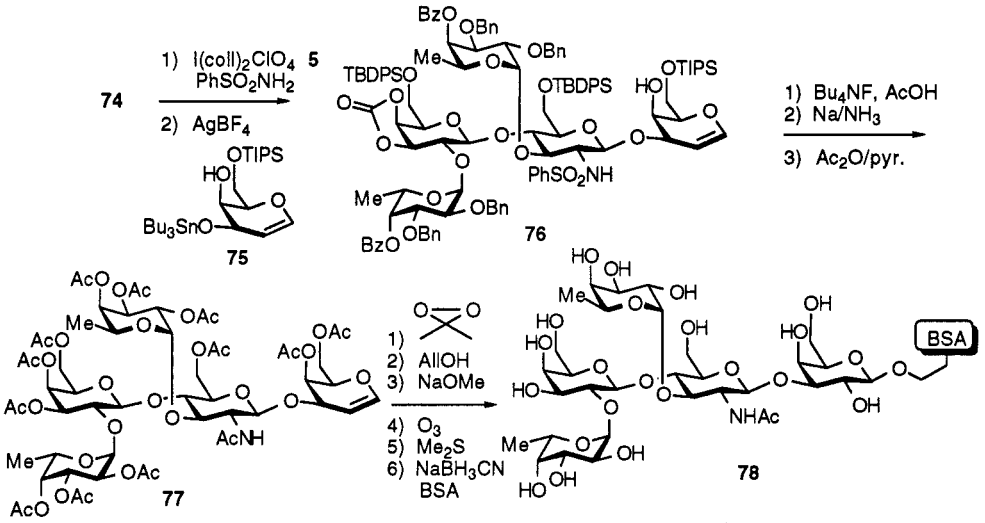
Glycal Assembly Leading to Neoglycoproteins

We subdivided the problem in the fashion shown. An interim goal would be determinant presented as its glycal (see structure **74**). That glycal would be extended through the iodosulfonamide methodology to give rise to, the *N*-acetylglucosamine section with a suitable spacer sugar. In our paradigm, glycal **74** was seen as a doubly fucosylated version of lactal. It is from this perception that the synthesis was conducted. In the forward sense lactal was easily silylated at each of its primary alcohols to give rise to **73**. We then took advantage of the unique *cis* vicinal relationship of C3' and C4' in the galactose section of **73**. These two hydroxyl functions could be smoothly engaged as a cyclic carbonate through the agency of carbonyldiimidazole. The system thus protected was armed with two unique hydroxyls at C3 and C2' which correspond to the fucosylation sites required for the Le^y determinant.

In the event, twofold fucosylation was smoothly achieved using fucosyl donor **54** (from the sLe^x project) leading to the protected tetrasaccharide Le^y glycal determinant **74**. This compound reacted with benzenesulfonamide in the presence of **5** to produce a *trans*-diaxial iodosulfonamide donor which was used to azaglycosylate **75**. In this way the pentacyclic determinant-spacer combination domain **76** was in hand. Cleavage of the relevant blocking group followed by per-acetylation led to the readily characterizable **77**.

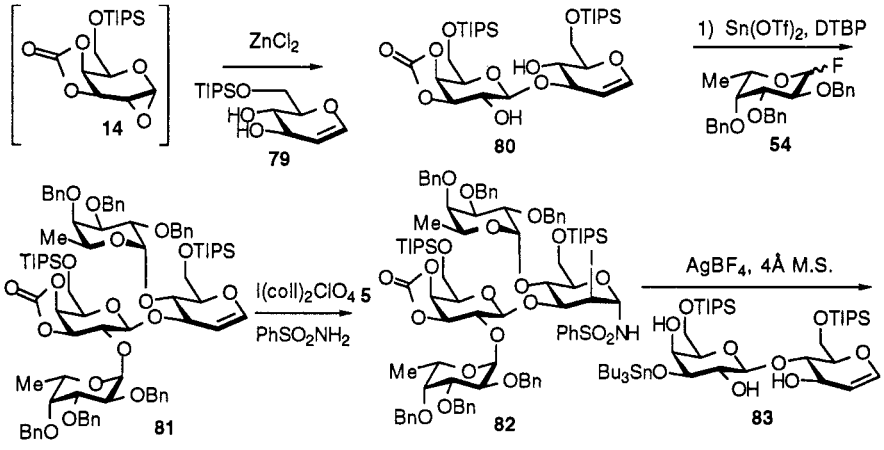
With glycal **77** well available to us we launched the program for conjugation to biocarrier, in this case to bovine serum albumin (BSA). Thus, reaction of **77** with 2,2-dimethyldioxirane gave rise to the α-epoxide which was opened with allyl alcohol in the presence of zinc chloride. Full oxygen deprotection followed by ozonolysis afforded an aldehyde **78** which was conjugated (see **78**) to bovine serum albumin by the method of Bernstein and Hall.[38] Approximately fifteen carbohydrate pentameric units were introduced, presumably at the most exposed lysine sites. The immunogenicity of this neoglycoprotein construct, as well as the synthesis of versions of such materials which are intended to provide even closer simulation of biological tumor antigens is a current focus of the laboratory in collaboration with colleagues in the immunological sciences.

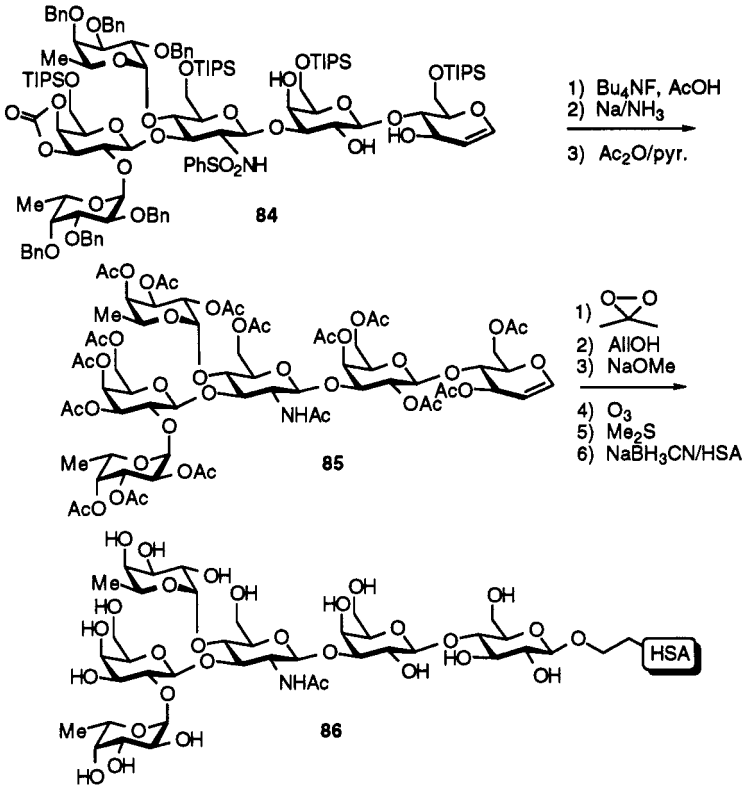




Synthesis of the Blood Group Substance Le^y

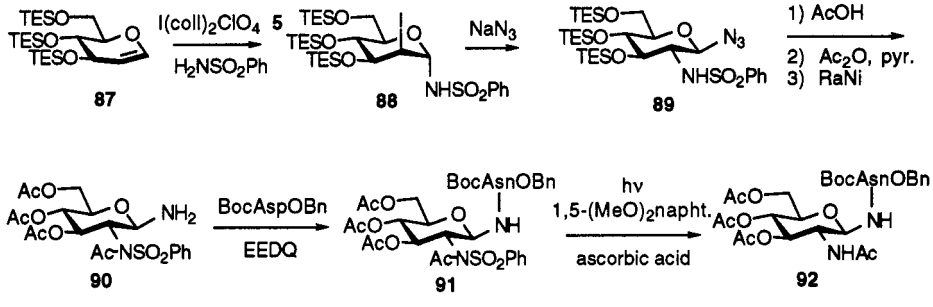
The synthesis of the Le^b determinant was, from our perspective, a somewhat more difficult undertaking than was the corresponding Le^y case. Given the 1,3-linkage between the glucose and galactose rings in the determinant sector, lactal would no longer be a suitable starting material. Accordingly, we began with the previously discussed epoxide **14**.[17] This was used to galactosylate the monosilylated glucal derivative **79** to provide **80**. At this point the C4 hydroxyl of the glucose ring and the C2' hydroxyl of the galactose sector could be concurrently fucosylated with the previously discussed donor system **54**. This fucosylation gave rise to the core of the Le^b determinant sector in the form of glycal **81**. Once again, iodosulfonamide addition using **5** in combination with benzenesulfonamide was practiced and provided **82**. This *trans*-diaxial iodosulfonamide served as a 2-azagalactosyl donor with respect to the 6,6'-diprotected lactal **83** leading to **84**. The latter contains the tetracyclic determinant sector and a lactose spacer to insulate it from the biocarrier. The campaign for conjugation to the carrier was conducted along the same lines as was described for the Le^y series. Thus, the glycal **84** was transformed, following deprotection and acetylation, to **85** and thence to an aldehyde as previously described. Conjugation of the latter to human serum albumin (HSA) was accomplished, again, by the reductive amination method of Bernstein and Hall.[38] In this instance thirty-three carbohydrate hexamer units were incorporated, presumably at exposed lysine sites of HSA. Evaluation of this construct as a possible agent to deflect the attachment of *helicobacter pylori* to gastric endothelium cells by competitive binding is currently being investigated in conjuction with appropriate collaborators.





Synthesis of the Blood Group Substance Le^b Conjugated to HSA

We conclude this report on the glycal assembly methodology by describing some exciting recent results on the synthesis of asparagine-linked glycopeptides. Once again, the iodosulfonamide "rollover" methodology has been a central element to our plan. Thus, we had already reported the reaction of iodosulfonamides of the type **88** with sodium azide to give rise to clean β-azide **89** bearing the α-sulfonamido group at C2.[39] This is a rather general reaction and seemed to offer a possible route to asparagine-linked glycopeptide synthesis. A key step in that regard was the finding that the 1β-glycosyl azide linkage could be reduced to a 1β-amino function **90** with Raney nickel. Coupling of such an amino with the pendant carboxyl of a aspartate was demonstrated early in our program (see **91**).[33] In the early work, the 2-sulfonamido function was converted to the *N*-acetyl group, though not in a generally applicable way (see **91**→**92**).



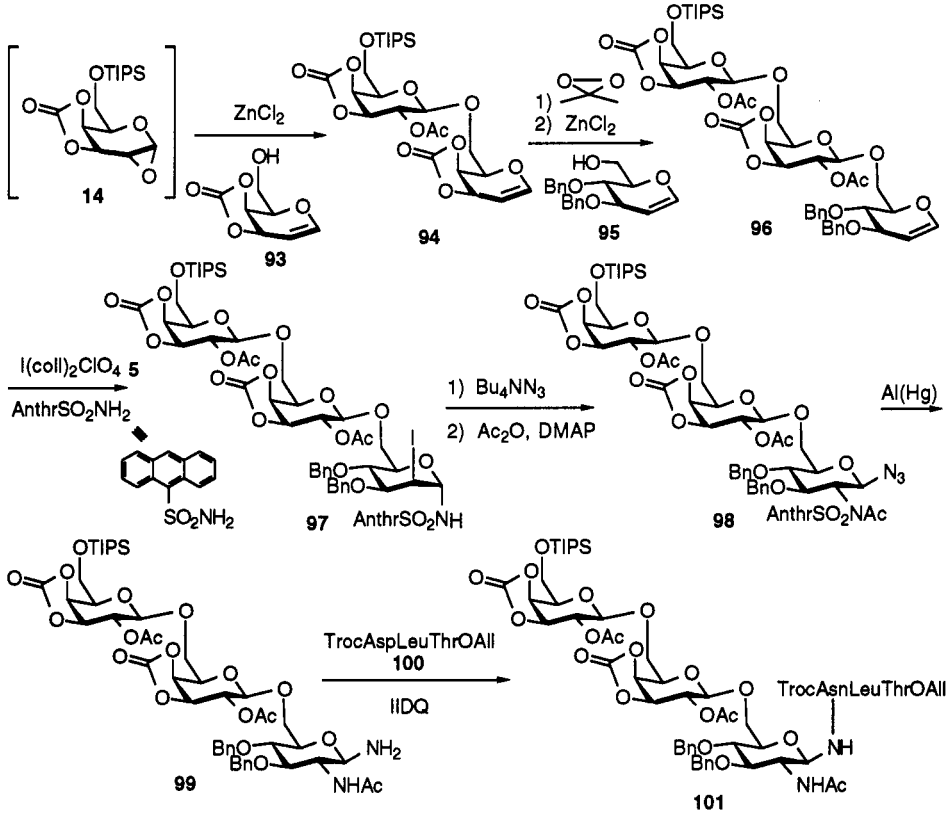
Synthesis of *N*-Linked Glycopeptides from Glycals

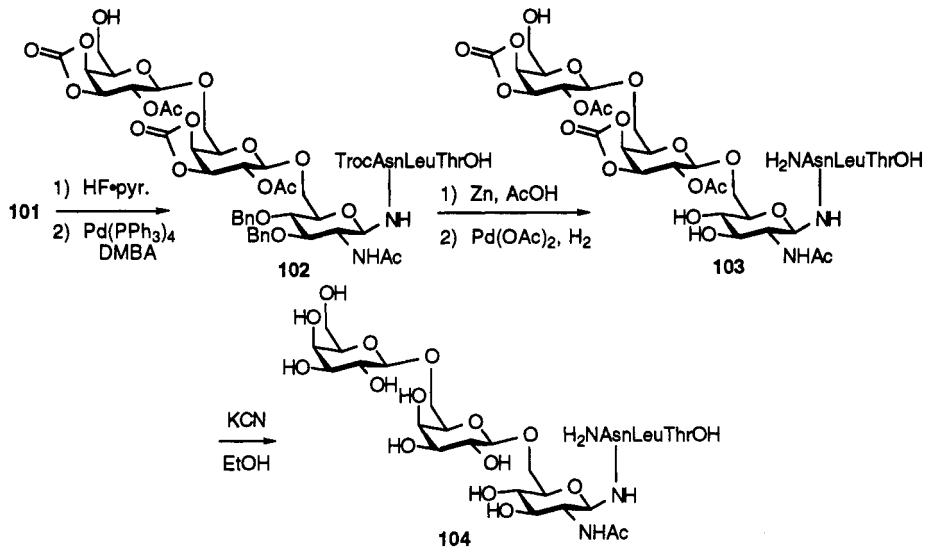
These findings served as a platform to attempt to carry out a complete synthesis of a glycopeptide. In this undertaking, we were mindful at the outset (and re-learned through trial and error), that the establishment of the glycopeptide core system is only one of the challenges of such a project. In fact, considerable subtlety is required to allow for deprotection of the synthetic construct without

undermining the survival of the "free" asparagine linked glycopeptide. Historic contributions have been made in this area by Kunz and associates.[40]

The strategy which we followed was notably different from that developed by those pioneers. Our solution to the problem is shown in the following figure. We built the carbohydrate construct through glycal assembly starting with the α-epoxide of the TIPS-protected galactal cyclic carbonate (**14**). Epoxide **14** reacted with the acceptor **93** thereby affording **94**. Glycal **94** was converted to its epoxide, which in turn reacted with the dibenzyl glucal derivative **95**. Upon acetylation **96** was in hand. Reaction of **96** with iodonium **5** in the presence of anthracenesulfonamide[41] provided **97**. The use of the anthracenesulfonamide was mandated by difficulties we encountered in attempting deprotection of other sulfonamides at critical junctures in the enterprise. Reaction of **97** with tetra-*N*-butylammonium azide[42] provided the α-sulfonamido trisaccharide bearing a 1β-azide. The N-H function of the sulfonamide could be acetylated with acetic anhydride in the presence of DMAP to afford **98**. At this stage the azide was reduced and the sulfonamide group was simultaneously removed with aluminum amalgam, to provide **99**.[41]

Tripeptide **100** bearing a free ω-carboxyl group on the aspartate residue was synthesized. The amino group of the aspartate was protected as a Troc (2,2,2-trichloroethoxycarbonyl) function and the terminal carboxyl of the threonine was protected as an allyl ester.[43] Coupling was mediated by 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ) to produce **101**.[44] Deprotection of the silyl function was followed by deprotection of the allyl ester of the threonine [Pd(0), dimethylbarbituric acid (DMBA)] gave **102**.[43] The Troc function was excised through the action of zinc and acetic acid and the benzyl groups were hydrogenolyzed as shown to give **103**.[45] The last step of the synthesis involved deacetylation and cleavage of the cyclic carbonate through treatment with potassium cyanide in methanol.[46] *This sequence led to the fully deprotected glycopeptide **104** whose structure was confirmed by NMR and mass spectral measurements.*





Synthesis of an *N*-Linked Glycopeptide

In conclusion, it is clear that the glycal concept has proven to be applicable to a wide range of problems in carbohydrate and glycoconjugate syntheses. We believe it offers major advantages in synthetic conciseness. Indeed, we are confident that, in time, glycal derived donor and acceptor combinations will be employed in an increasing range of synthetic problems.

Furthermore, we have recently demonstrated a capacity to incorporate much of the glycal assembly methodology into solid support oligosaccharide and glycopeptide synthesis.[47] This methodology simply exploits the principles discussed above. As the technological obstacles to the solid phase adaptation continue to be overcome, complex carbohydrate and glycoconjugate syntheses, including glycopeptide syntheses, will be accessible to increasing numbers of investigators. We expect such synthetic advances to help in fostering interactions encompassing the chemistry, biology and clinical applications of carbohydrates.

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