APPROACHES TO CARBOHYDRATE-BASED CHEMICAL LIBRARIES: THE EVOLUTION OF GLYCOTIDES

by

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B.A., Chemistry, Yale University (1989)

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ABSTRACT

The overwhelming majority of commercial drugs have been discovered by random screening methods. The chance of finding active compounds in a biological screen is improved by having a large and diverse pool of molecules from which to choose. This simple notion has led to the development of combinatorial chemistry. Instead of using a serial approach to synthesis, chemists make compounds in a parallel fashion, thereby enabling rapid synthesis of large chemical libraries. Time is saved by avoiding purification of intermediates, and thus combinatorial chemistry places a great emphasis on performing reactions that proceed in high yields.

Carbohydrates are an underutilized but potentially ideal class of building blocks for combinatorial chemistry because they package substantial complexity into compact molecular structures. The glycosaminoglycan heparin is an example of a natural carbohydrate library in which seemingly small variations in a repeating skeletal core give rise to a broad range of biological activities. We sought to produce heparin mimetics by a semi-synthetic route. Heparinase cleavage was used to produce fragments that could be modified and subsequently linked with a Michael addition. The complexity of the natural system led us to synthesize a monosaccharide Michael acceptor as a model system. We studied the Michael addition of various thiolates to the model compound. Unfortunately, nucleophilic attack proceeded primarily from the *re* (top) face, giving adducts with different stereochemistries than those found in heparin. Furthermore, thioglycosides were incapable of Michael addition. We attempted to synthesize combinatorial libraries using the Michael addition with glycoconjugates, but yields were low.

We wanted to utilize carbohydrate building blocks, but needed a highly efficient reaction to couple them. We functionalized monosaccharides with an amine and a carboxylic acid, thereby producing *glycosamino acids*. These monomeric units were linked with amide bonds to produce *glycotides*. We have synthesized several classes of glycosamino acids (protected as azido esters), and have synthesized defined oligomers, small libraries of linear glycotides, and template-directed libraries.

Thesis Supervisor: Peter T. Lansbury
Title: Associate Professor of Chemistry

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Acknowledgments

I owe a debt of gratitude to many people, and the longest-owed and least-paid debt is to my parents. There is no way I can adequately express my thanks for their unwavering love for the past 27 years. I sincerely hope that their happiness and excitement with their new mountaintop lifestyle does not fade.

I would also like to thank the rest of my family for their unconditional support. Hopefully, I will be able to see more of them now that I am finished here. In particular, I'd like to congratulate my sister Erin on her recent engagement.

Although having been somewhat less than blissfully content with my surroundings in Cambridge, I have thoroughly enjoyed my five years as a graduate student. My lab has always been a very positive environment, and much of the credit for that should go to Peter Lansbury. He has always gone to extremes to ensure that his role as the boss never came before his role as a person. Peter is an all-around good guy and is one of a small minority of faculty members who manages to hide a truly sharp mind behind an unpretentious exterior. Since Peter was wrongly denied tenure, I have to wish him luck in his new job.

The rest of the credit for the positive working environment during graduate school goes to my labmates, too numerous to mention individually. Long before my arrival, Kurt Halverson was one of the original four students in the lab, and he made great contributions not only academically, but more importantly in establishing a positive tone for the lab. Long after founding morning ball, writing his thesis, and getting a job at 3M, Kurt showed his tremendous clout by bullying his employers into making me a job offer. It was a very hard decision not to accept that offer, but I am very grateful for the opportunity. I'd like to thank the two men who had the misfortune to be my baymates, Jim Harper and Luncong Dong. They had to contend with my unceasing efforts to colonize all free space in the hood, and I offer them a belated apology. In particular, I'd like to express my gratitude to

Santosh Nandan and Cheon-Gyu Cho, two postdocs who diligently tried to transform a stubborn pupil into a real organic chemist.

I have been fortunate to enjoy many activities outside of work while in graduate school. Jon Come founded the lunchtime bridge game, claims to be a co-founder of morning ball, and was certainly an energizing force behind everything he did. I enjoyed pick-up games and intramural sports while at MIT, particularly with the chemical engineers and their affiliated ringers. Best of luck to them, as well as the Harvardians. I hope the Commish can find someone who can give him a game.

I have always been able to escape from MIT even while in Cambridge. My roommates for two years, Alex Pfaff and Kingsley Taft, were students at MIT but were really just Yalies passing through. I have been able to see friends from college throughout my time here, and I regret (because of my indolence, for which I will pay) not doing it more frequently. I have followed Steve Cass from New Haven to the trailer park in Edwards to Boston, and now I can't help but think that the Tri-Cities area has a place for him.

I will always reflect fondly on graduate school because it was here that I had the incredible good fortune to meet Marcella. Because of her, all of the frustrations that I experienced as a graduate student seemed so trivial, and faded as soon as I left the lab and headed home. She has been forced to make many sacrifices and difficult decisions while I have been stuck in graduate school, and hopefully now she will get all that she has coming.

Chapter 1

Overview of Combinatorial Chemistry

The pharmaceutical industry is currently undergoing dramatic transformations as a result of a confluence of economic, political, legal, and technological pressures. Among the most profound of these changes is a fairly new approach to drug development known as combinatorial chemistry. The goal of combinatorial chemistry is to rapidly produce large and diverse chemical libraries as sources of lead compounds and viable pharmaceuticals.

Traditional approaches to drug discovery have relied upon identification of lead compounds from random screening, followed by serial optimization of the leads[1-4]. Lead compounds are obtained by screening synthetic chemical libraries and natural products isolated from animal sources, plant extracts, and microbial fermentations. The leads are then laboriously refined into drug candidates through a process of systematic optimization. Sequential modifications of the lead compounds are individually synthesized and tested for activity, with beneficial changes retained and detrimental ones discarded. Of course, changes are not necessarily additive, and this factor further retards the evolutionary process of medicinal chemical optimization. Undeniably, the traditional approach to drug development has been successful, but increasing competition for shrinking profits has dictated an industry-wide search for a faster and more cost-effective approach.

One such approach is structure-based rational drug design.* Conceptually, rational drug design is very simple. If one can define the three-dimensional structure of a target molecule of known biological importance, then it should be possible to "design" compounds which can interact with the target in a very specific manner, thereby producing a high-potency drug with minimal side effects. In practice, rational drug design is plagued by several logistical problems[5-8], the most important of which is obtaining a highly precise

* Since it has been used widely for many years, mechanism-based drug design can be classified as a subset of the traditional approach to drug development.

physical structure of the biologically active conformation of the target. It is critical to obtain the three-dimensional structure of the bioactive conformation in solution, which is likely to differ from the solid-state crystal structure or the thermodynamically favored solution structure[5]. Furthermore, the uncertainties of the methods used to obtain three-dimensional structures of biomolecules, i.e., x-ray crystallography and two-dimensional NMR, are on the same order as the size of atoms[7,8]. Obviously, greater precision in the structures of the biological target would facilitate drug design.

There have been some published successes involving rational drug design[5,8,9]. Furthermore, many of the barriers to success are largely technical in origin, and thus it is likely that rational drug design will become increasingly effective in the future. Nevertheless, progress has been disappointingly slow, and in spite of its enormous potential, rational drug design has not emerged as a general, cost-effective alternative to traditional drug discovery approaches.

Undoubtedly, the slow progress of rational drug design has contributed to the enormous appeal of combinatorial chemistry. If development of a drug candidate depends on finding a compound with a particular biological interaction, and if it is impossible to accurately predict whether a given compound will possess the desired interaction, then the chance of finding a potential therapeutic is improved by having a large and diverse pool of molecules from which to choose. Pharmaceutical companies have access to vast and fairly diverse sets of discrete chemical entities, and chemists certainly could synthesize "libraries" of novel compounds using traditional methods. Thus, combinatorial chemistry is distinguished by the emphasis on making libraries of compounds *rapidly*. Stripped to its essence, combinatorial chemistry is a labor-saving device.

The operational objective of combinatorial chemistry is to make many compounds in the amount of time previously required to make a single compound. This is achieved by performing chemical synthesis in parallel rather than in series. Unfortunately, organic chemistry is not routinely amenable to parallel synthesis. The rate-determining process in

most organic syntheses is the purification of intermediates and a final product. In other words, the majority of a chemist's time is spent not in starting reactions, but rather in finishing (work-up and purification) them. In order for parallel synthesis to meet its goal of drastically reducing the time per chemist per compound, the rate-limiting step in the synthetic process must be circumvented, and therefore invasive purification techniques (chromatography, recrystallization, etc.) are avoided.* As a consequence, approaches to combinatorial chemistry place a great emphasis on reactions that proceed in high yields, with reactants and byproducts that are either benign or easily removed. Although solid phase chemistry is not a required component of combinatorial chemistry, it is highly compatible. Solid phase synthesis allows the use of an excess of reagents to drive a reaction to completion, after which the reagents, byproducts, and solvent are removed by filtration. Therefore, it should not be surprising that the initial approaches to combinatorial chemistry involved making libraries of oligopeptides[10-12] and oligonucleotides[13,14], since solid phase synthesis of these biopolymers[15-17] has been very well-refined.

There appears to be a widely held impression that the purpose of combinatorial chemistry is to produce new leads in drug discovery. From the perspective of a pharmaceutical company, the goal is obviously to shorten the drug development cycle. There are probably very few biological assays that would not realize many "hits" when screened by the myriad of chemicals to which a pharmaceutical company has access. In order for combinatorial chemistry to provide some improvements, the combinatorial libraries must avoid redundancy and serve as a source of novel lead compounds with superior properties. Perhaps the stated purpose might be better phrased: "to produce superior new leads in drug discovery". This would entail the discovery of more lead structures, possessing higher affinities, significant conformational restraints, greater potential for optimization, easier synthesis, better pharmacokinetic properties, etc. Given

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* The alternative, parallel synthesis with invasive purification, is simply the standard operating procedure for organic chemists, provided the number of parallel syntheses (n) is small. When n becomes large (approximately 5, depending on the organizational skills of the chemist), efficiency rapidly declines.

these potential benefits, the search for superior new leads is sufficient to justify an investment in combinatorial chemistry. Nevertheless, this stated purpose is artificially limiting and presents a somewhat myopic view of the potential of combinatorial chemistry.

The methodical process of refining and optimizing lead compounds normally requires significantly more time than that spent in identifying leads[3,5]. Consequently, if the goal is to substantially reduce the portion of the drug development cycle devoted to drug discovery, one must find a way to facilitate the optimization process. Therein lies the true potential of combinatorial chemistry. The ultimate goal of combinatorial chemistry is not only to provide a source for new and perhaps superior lead compounds, but also to provide a suitable framework for iterative rounds of combinatorial optimization.

Approaches to Combinatorial Library Synthesis

It is easy to understand how combinatorial chemistry can be used to generate vast chemical libraries. The size of a library is an exponential function of the number of synthetic steps and different chemical reagents available for each step (see Table 1-1). Assuming one wishes to employ a building block approach to combinatorial synthesis, with the number of building blocks represented by x and the number of couplings represented by y, then the total number of compounds $N = x^y$. There are 20 common amino acids, and thus there are 8,000 (20^3) possible tripeptides, 160,000 (20^4) tetrapeptides, etc. Increases in the library size can be obtained by either increasing the number of building blocks or increasing the number of coupling steps. Nature has chosen the latter strategy, and the enormous diversity that can be achieved by repeated ligations of a relatively small number of building blocks is exemplified by the genetic code. The former strategy is more appealing to a pharmaceutical company, since it reduces the number of synthetic steps and keeps the molecular weights of the compounds sufficiently low (generally < 700)[2] to maintain desirable levels of oral bioavailability.

Building Blocks *(number)*	**Oligomer Size** *(D.P.)*	**Library Size** *(# of compounds)*
4	2	16
	4	256
	10	1,048,576
20	2	400
	4	160,000
	6	2,560,000
50	2	2,500
	3	125,000
	4	6,250,000

Table 1-1. Library size is a function of the number of building blocks and coupling steps.

There are two fundamental challenges in combinatorial chemistry. The first is to generate diverse chemical libraries, and the second is to characterize the components of the libraries which possess desirable properties. These challenges are not treated independently. In the absence of a mechanism to access the molecular structures of active compounds culled from screening, it is of little value to make large chemical libraries. There are several approaches to library synthesis that facilitate identification and characterization of compounds with desirable properties.

Spatially Addressable Systems. Members of a library can be synthesized in spatially segregated arrays, such that at any location in space, only a single compound (or a planned mixture derived from a discrete sequence of reactions) of known composition is present. Synthesis is performed in an array format, with multiple sites for simultaneous

synthesis, and frequently utilizes solid supports. For example, the resin can be contained in a reservoir well ("resin in a well")[18], on polypropylene rods arrayed in 96-well microtitre plates ("resin on a pin")[11], or in enclosed gas dispersion tubes ("resin in a pin")[19] capable of synthesizing 40 "diversomers" simultaneously. These methods have the advantage of producing defined compounds that are amenable to standard screening methods, but at present they can not be used to synthesize enormous libraries. Fodor and coworkers[20] have developed a very elegant approach to making large, spatially addressable libraries of peptides and oligocarbamates using photolabile protecting groups (see Figure 1-1). The spatial resolution afforded by photolithography permits miniaturization of the process, providing 40,000 discrete synthesis sites on a 1 cm^2 chip. The two major disadvantages of this light-directed technique are that it involves specialized, expensive technology, and biological testing must be performed on the resin-compound complex rather than in solution. This constraint on screening is problematic for two reasons. First, it necessitates changes in the standard screening formats used by the pharmaceutical industry, and second, there is potential interference from the solid support, primarily in denying access to certain conformations.

Split Synthesis Method. A library of compounds can be synthesized in combinatorial fashion on solid supports, with repeated cycles of separation followed by mixing of the solid particles[12,21]. For example, functionalized resin beads can be separated into a number of groups, and each group is treated with a different chemical reagent. The various sets are then pooled and treated with a common chemical (such as a deprotection step), after which the separation/mix cycle is repeated. Every bead in the final library will

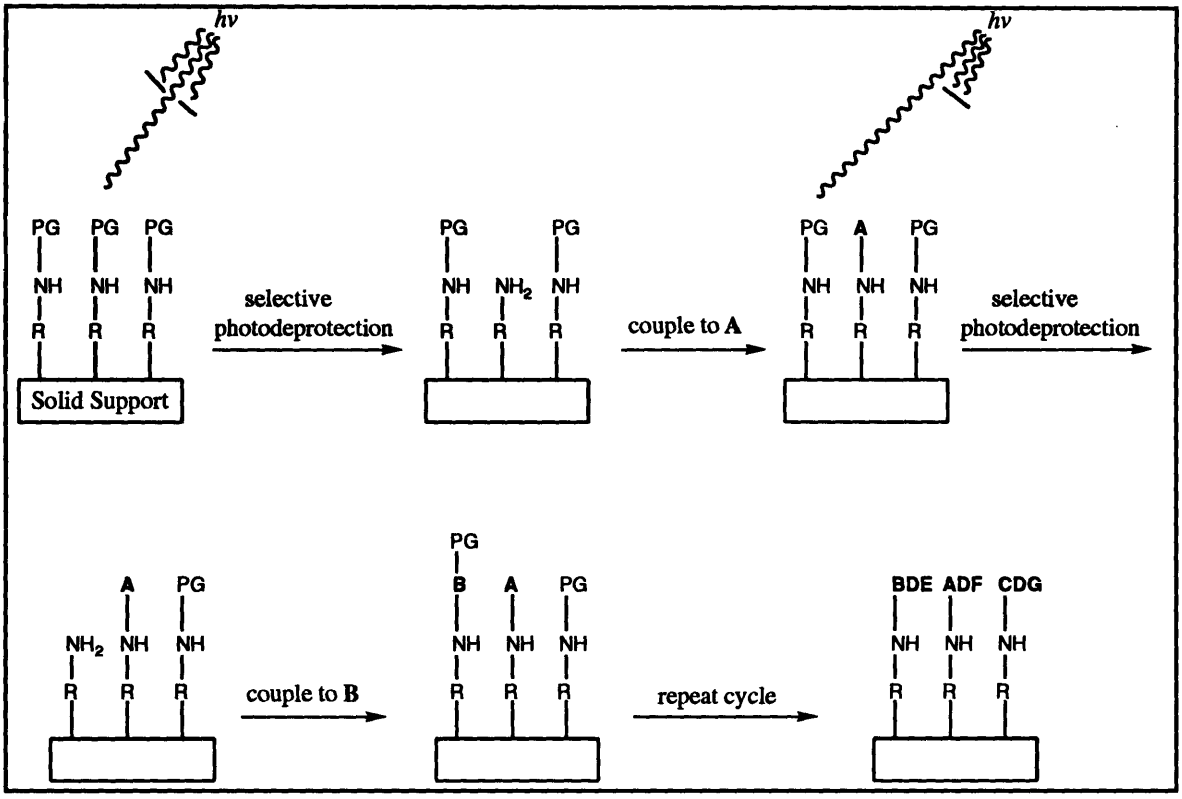


Figure 1-1. Synthesis of spatially addressable libraries using photolabile protecting groups[20].

have a product or products resulting from a single, discrete reaction sequence (see Figure 1-2). The compounds are then screened for a particular biological activity, and the chemical structure of the compound attached to the bead is determined by analytical techniques. Obviously, this approach works well for biopolymers amenable to microscale sequencing techniques, but is much less efficient when the characterization process is not straightforward. However, recent[22] and foreseeable advances in mass spectrometric techniques could make the split synthesis method an increasingly viable approach for construction of small molecule libraries, since the principal disadvantage of this method is the difficulty of characterizing an active compound.

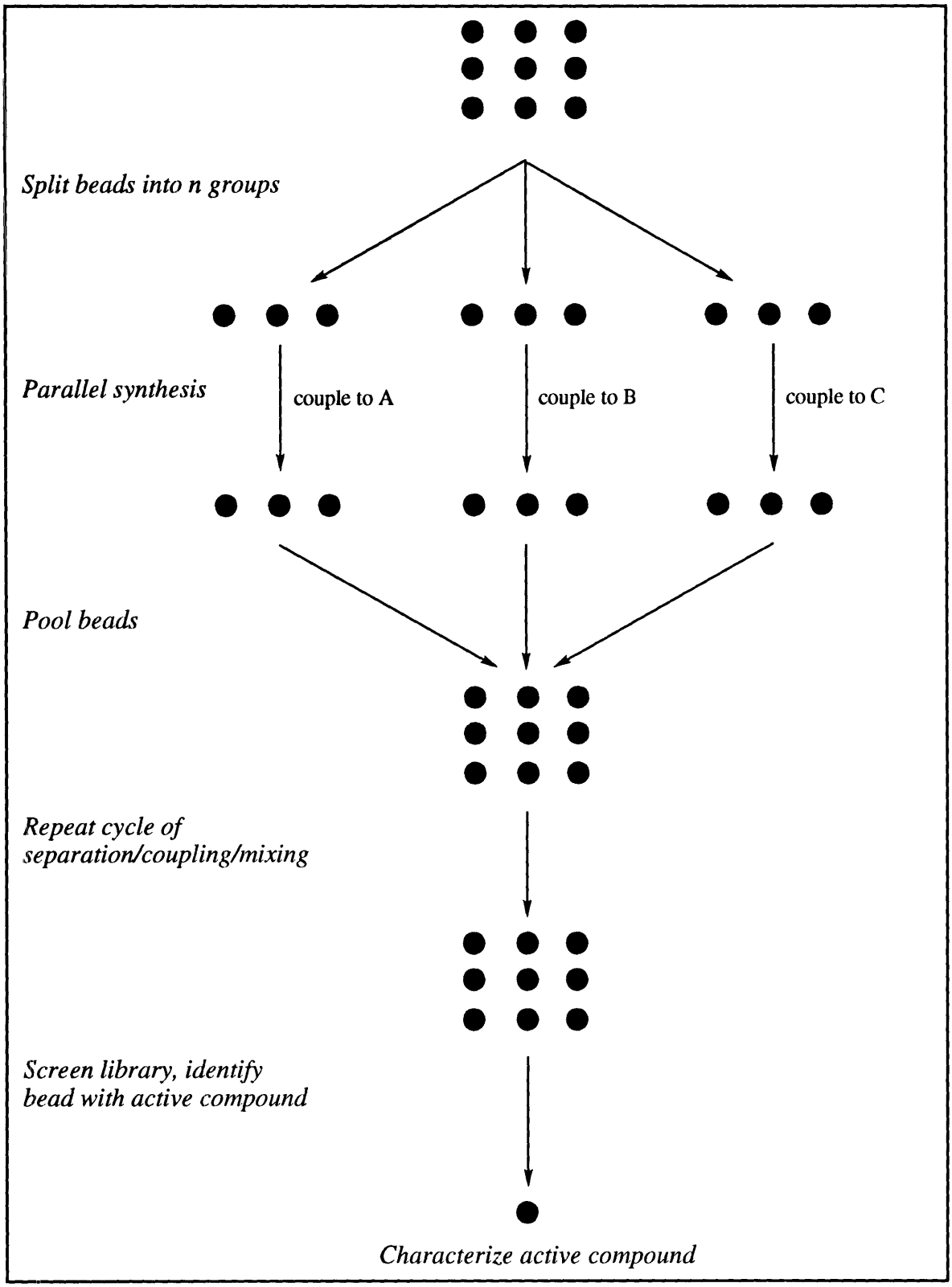


Figure 1-2. Split synthesis approach.

Multivalent Synthesis Method. A chemical library can be created by pooling different reagent choices during synthesis, thereby producing a mixture of compounds. Synthesis of this type of library requires less time than the previous two libraries (there is no spatial separation; hence there is only one reaction mixture to set up), but characterization of the desired compounds is more difficult. Frequently, an active compound is identified by a deconvolution process in which a series of libraries of decreasing complexity are synthesized[23]. The major advantage of the multivalent synthesis method is that it facilitates rapid synthesis of a large library, but this is more than offset by several disadvantages. In theory, time is saved during synthesis by only having one reaction to set up, but in practice, multiple libraries are synthesized simultaneously to facilitate deconvolution (see Figure 1-3). Consequently, the time required for initial library synthesis using the multivalent synthesis approach is similar to that required with the split synthesis approach. Repeated cycles of library synthesis and screening are necessary and extremely cumbersome features of the multivalent synthesis approach. Additionally, it is difficult to control the composition of the libraries, since the distribution of products in any reaction step is a function of the different rate constants for the various reagents employed. Potential problems involving operational scale and interference effects in library screening are introduced because there is no way to physically divide the mixture into smaller groups of compounds, and thus screening is performed simultaneously on the entire library. Most importantly, the deconvolution process is fundamentally flawed. The compound that eventually is selected is the most active component of the final, petite, "sub-library", but may be significantly different than the most active compound of the original, diverse library.

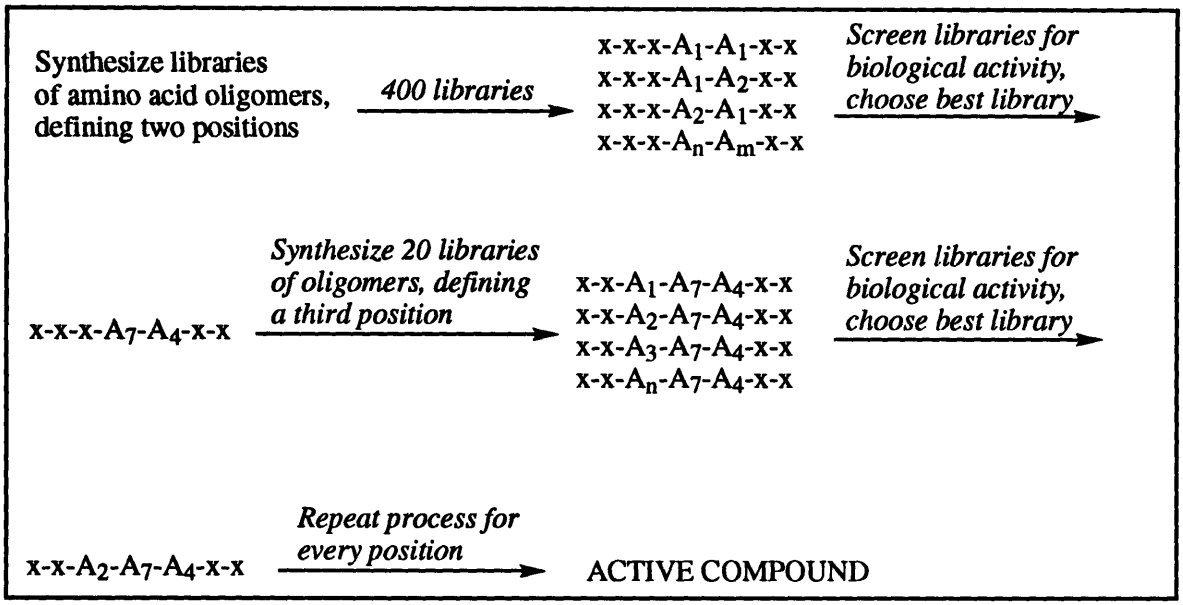


Figure 1-3. Mimotope deconvolution method[23].

Encoded Library Method. Combinatorial libraries can be synthesized such that every bead contains a unique chemical tag that permits ready identification of the attached compound(s), or, more precisely, the sequence of reactions to which the bead was exposed. Several tagging methods have been devised, including "cosynthesis" methods using oligonucleotide[24] (identification through PCR amplification) or peptide tags[25] (identification via sequencing, with the obvious disadvantage that greater quantities are required), and a binary encoding method[26,27] (see Figure 1-4) which uses a set of chemically inert tags (identification via electron capture capillary gas chromatography analysis). Like the spatially addressable methods, the major advantage of these encoding methods is the ability to immediately and definitively characterize active compounds. The principal disadvantages include the additional time required for attachment and cleavage of the linker, synthetic limitations resulting from the need to develop compatible coupling and deprotection chemistry (mandated by the simultaneous syntheses of two classes of compounds, the library component and the tag, per bead), and potential interference from the tag in the screening process.

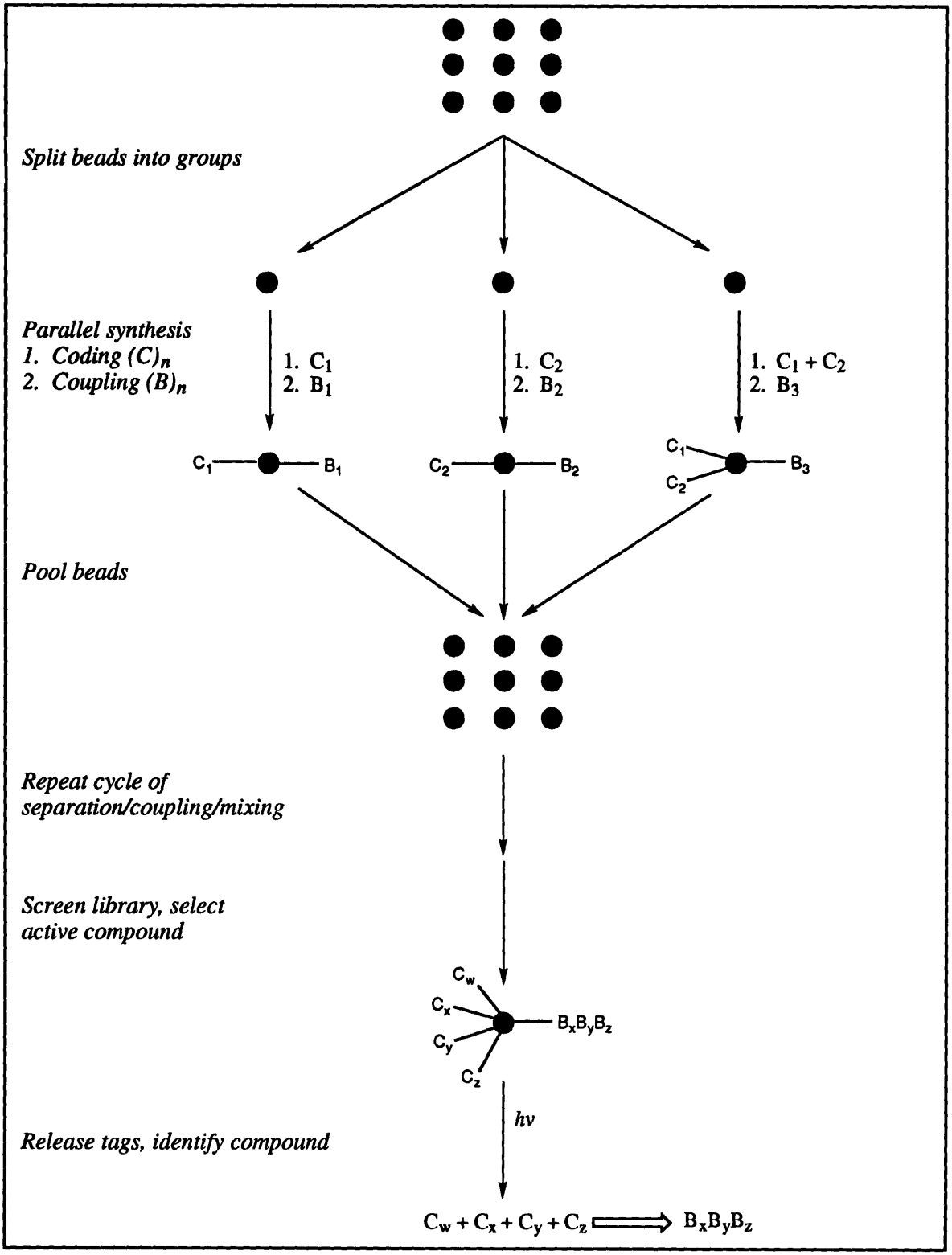


Figure 1-4. Synthesis of encoded library using binary code tagging methodology of Still and coworkers[26,27].

Of the various encoding methods, the one that appears to be the most promising is the binary code methodology developed by Still and coworkers[26,27]. The tags are not sequentially connected; hence, cosynthesis of a second oligomeric chain is not required. The chemical tags are inert to many typical reagents used in organic synthesis, and thus are amenable to a more diversified library than is possible with less stable oligonucleotide tags. The cosynthesis methods are particularly restricted by the difficulty of developing an orthogonal protecting group strategy for the library component and the code.

Libraries will continue to be made using each of the four approaches detailed above, but it is likely that the multivalent synthesis approach will fall into disfavor. The various approaches to combinatorial chemistry are subject to a natural trade-off between ease of synthesis and ease of compound identification. The split synthesis and multivalent approaches offer facile synthesis, while the spatially addressable and encoding approaches provide straightforward characterization of an active compound. At present, the intrinsic flaws of the deconvolution process render the multivalent synthesis approach less appealing than the other three approaches. Moreover, technical advances in the combinatorial chemistry field will significantly enhance the other approaches, but will have less impact on the multivalent synthesis method. Improvements in analytical chemistry will reduce the difficulty of characterizing an unknown active compound, thereby moderating the principal disadvantage of the split synthesis method. Engineering advances will facilitate miniaturization of the spatially addressable methods. Chemists will not have to rely on 96-well plates, or homemade 40-site supports, but instead are likely to have access to automated synthesis on plates with 1,000 or more discrete reaction sites. Developments in novel tagging technology should render the encoding approach less time-consuming, and amenable to a greater diversity of chemical reactions.

Classes of Combinatorial Libraries

Biopolymer libraries. The first attempts at making large combinatorial libraries involved synthesis of oligopeptides[10-12] and oligonucleotides[13,14]. These biopolymers were natural choices for several reasons. Combinatorial chemistry places a premium on reactions that proceed in high yields (more precisely, with high efficiencies; a lack of side products is more important than high recovery of material), since purification is not performed after each step. Removal of the reagents after each step is critical, and is easily accomplished using solid supports. Additionally, synthesis of a library of oligomers is greatly facilitated by having immediate access to suitably protected monomers. Thus, combinatorial chemists sought a readily available collection of monomers that could be coupled in high yield using solid phase techniques. Obviously, peptide[15] and nucleic acid synthesis[16,17] fit these criteria.

Peptide libraries have been synthesized[1,4,10] using molecular biological techniques[28,29] as well as each of the approaches to combinatorial synthesis described previously. Biological screening of various peptide libraries has provided many active compounds, some of which may be useful as lead structures. However, peptides are inherently limited as drug candidates because of their low bioavailability, susceptibility to proteases, and lack of rigidity[2,19,30,31]. In order to produce a suitable drug from a peptide lead, extensive modifications are normally required. As described previously, the ultimate goal of combinatorial chemistry is not only to serve as a source for new and perhaps superior lead compounds, but also to provide a suitable framework for iterative rounds of combinatorial optimization. Unfortunately, peptide leads are not amenable to combinatorial optimization.

The search for superior alternatives to biopolymer libraries has led to combinatorial approaches to so-called "small molecule" libraries and biopolymer mimetics. Recently, several combinatorial libraries of various biopolymer mimetics (see Figure 1-5) have been synthesized, including carbamates[32] (see Scheme 1-1), ureas[33], peptoids[34,35],

peptidosulfonamides[36], phosphorothioate nucleosides[37], vinylogous polypeptides[38], peptide nucleic acids[39], and peptidyl phosphonates (a dipeptide unit in a peptide chain is replaced with a phosphonate dipeptidomimetic)[40]. These biopolymer mimetics share many of the advantages of peptide libraries, while also possessing enhanced metabolic stabilities[32,36,41]. Assembling a collection of monomeric units usually is not difficult, and

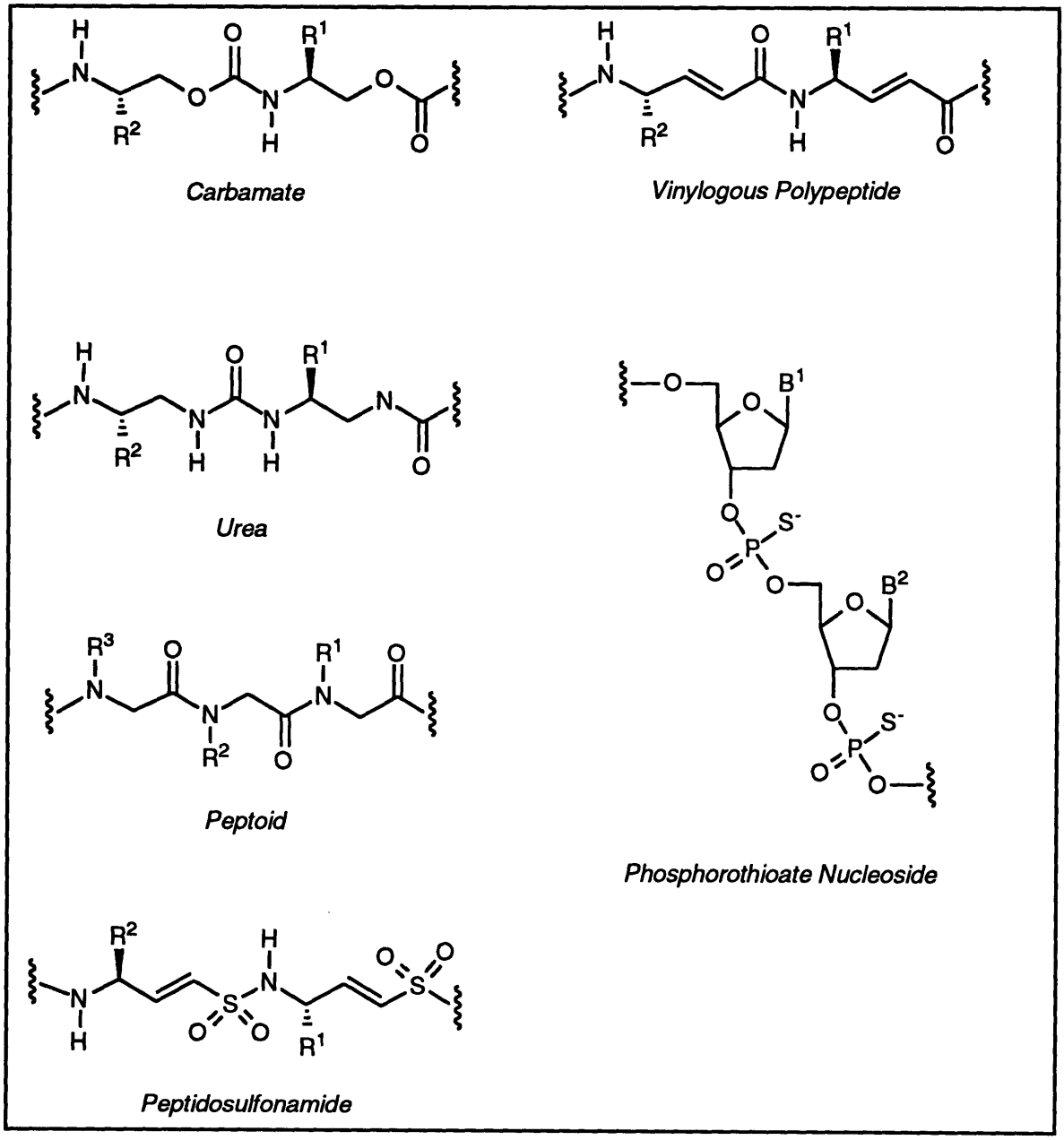
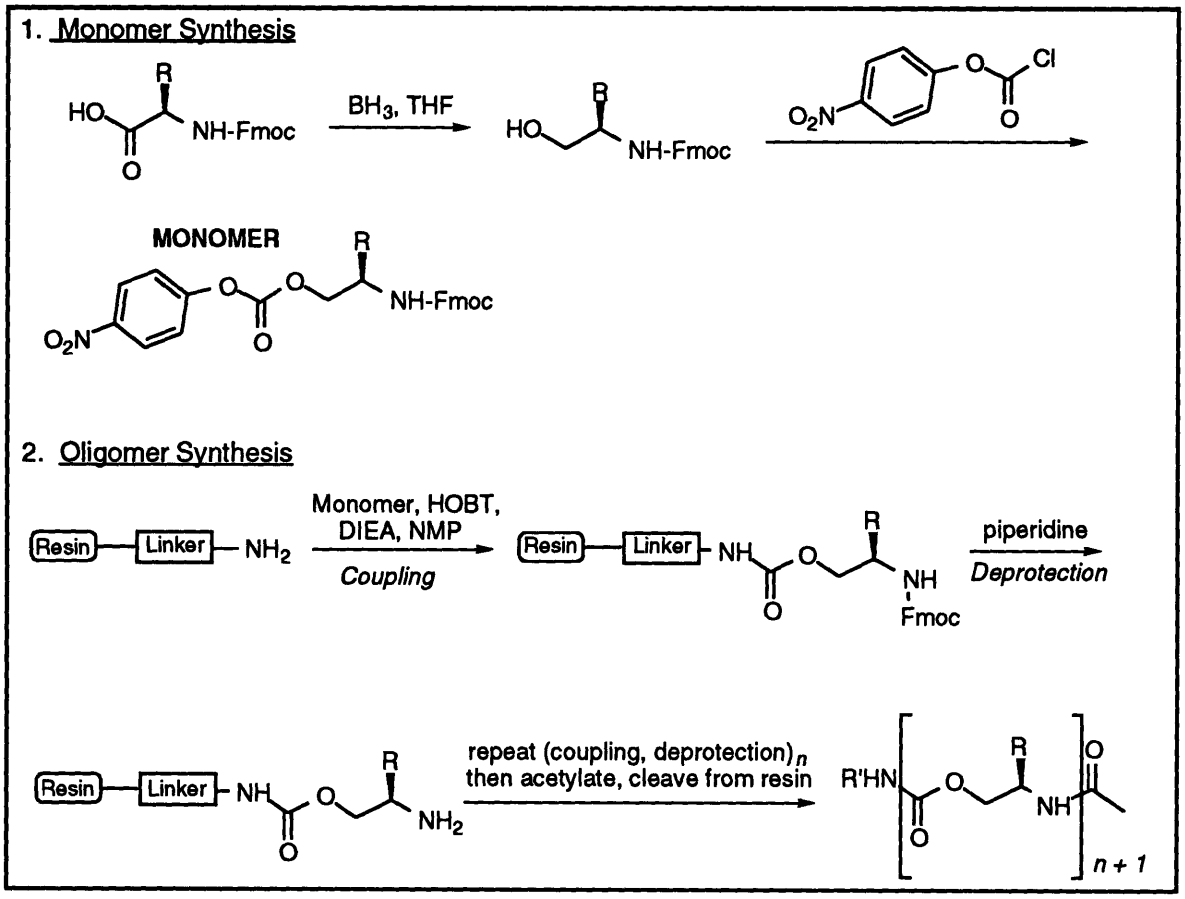


Figure 1-5. Biopolymer mimetic libraries.

coupling of monomers to give oligomers proceeds with high efficiency on the solid phase. A potential disadvantage of many biopolymer mimetics is a lack of rigidity, particularly relative to small molecules with built-in conformational constraints. Detractors[19] of these libraries will also point out that biopolymer mimetics contain a repetitive backbone, which is inconsistent with the concept of diversity.

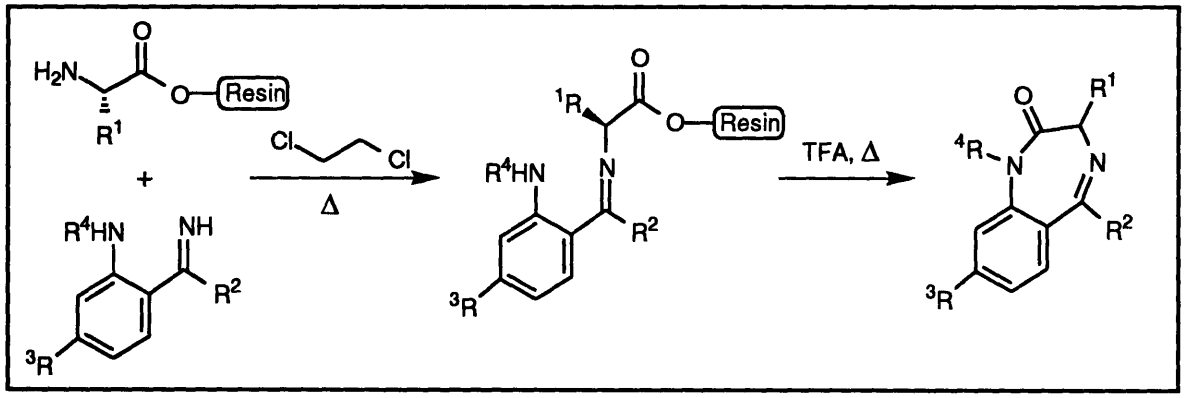
Scheme 1-1. Synthesis of oligocarbamate libraries[32]



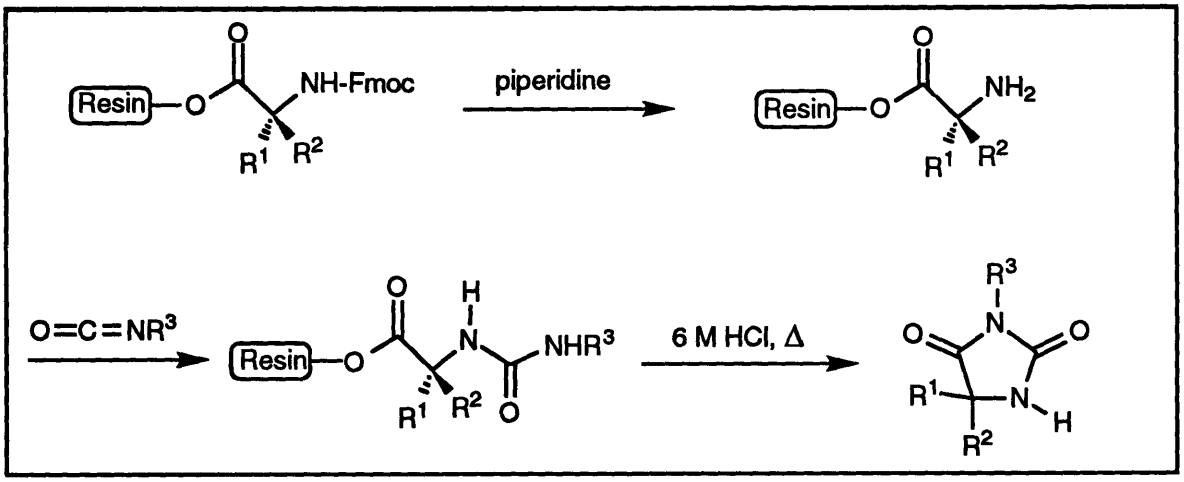
The term "small molecule" is really a euphemism for "drug-like", and the successful synthesis of a viable small molecule library is universally applauded, since almost by definition the library's components lack obvious weaknesses as potential drug candidates. Small molecule building blocks are brought together to form non-polymeric, three-dimensional arrays. The common disadvantage of small molecule libraries is the increased

level of synthetic difficulty relative to natural biopolymers and biopolymer mimetics. Small molecule library synthesis requires several fundamentally different but compatible reactions performed without purification of intermediates. The reliance on reactions that are amenable to solid phase synthesis has promoted growth in the field of solid phase synthetic methodology. There have been several recent examples of successful adaptations of various reactions (Heck[42], Stille[43], Suzuki coupling[44], nitrile oxide cycloaddition[45]) to solid supports. Perhaps the most important small molecule library synthesized to date is Ellman's benzodiazepine library[46], a variation of which has been synthesized by Hobbs DeWitt *et al.* (see Scheme 1-2)[19]. Their diversomer approach, using a spatially addressable "resin in a pin" approach, was also used to synthesize a library of 40 discrete hydantoins (see scheme 1-3). Kurth and coworkers[47] have recently assembled a nine compound library of β-mercapto ketones using a split synthesis approach.

Scheme 1-2. Synthesis of a benzodiazepine library[19]



Scheme 1-3. Synthesis of a hydantoin library[19]



A variation on the small molecule library theme involves the use of molecular scaffolds as templates for library synthesis. Hirschmann, Nicolau, and coworkers[48,49] have used a glucose scaffold to synthesize a focused library of somatostatin non-peptide mimetics, the most active of which is shown in Figure 1-6a. A tetracarboxylate scaffold in conjunction with a variety of amines (primarily amino acids) was used by Rebek and coworkers[30,50] to produce large chemical libraries using a multivalent synthesis method (see Figure 1-6b). A cylopentane template capable of producing spatially addressable libraries has been developed by Patek and coworkers (see Figure 1-6c)[51].

Future Directions

In the immediate future, researchers in combinatorial chemistry are likely to concentrate on the synthesis of libraries of biopolymer mimetics and, in particular, small molecules. Progress in the synthesis of small molecule libraries will be augmented by concurrent advances in solid phase methodology. Obviously, combinatorial efforts will target known pharmacophores, but will also seek novel compound classes that are poorly represented in the chemical databases of pharmaceutical companies.

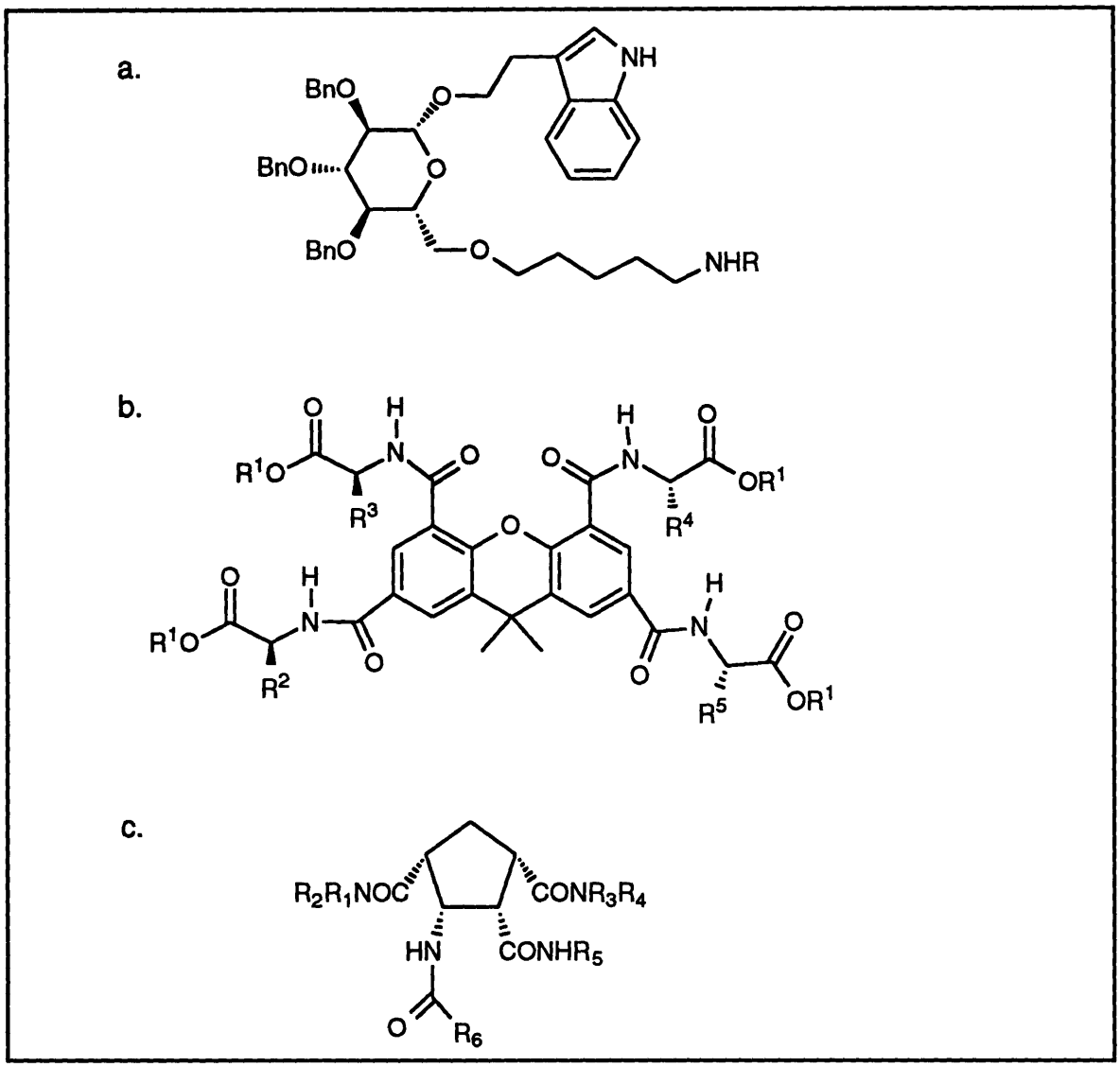


Figure 1-6. Template-directed library synthesis. (a) Hirschmann *et al.* [48] (b) Carell *et al.* [30] (c) Patek *et al.*[51]

The drug discovery cycle is composed of several components, roughly divided into chemistry (discovery and refinement of a drug candidate, process chemistry), biology (pharmacokinetics, cell penetration, metabolism, toxicology, etc.), and regulatory processes (clinical trials, FDA approval, etc.). Combinatorial efforts will undoubtedly shorten the "chemistry" part of the cycle by reducing the time required to identify and subsequently refine lead compounds. It will be interesting to see if combinatorial

approaches can be successfully applied to the "biology" portion of the drug development cycle. Ideally, a combinatorial library would be synthesized, screened for biochemical efficacy, and subjected to rapid assays of biochemical properties such as cell penetration and metabolic stability.

The combinatorial chemistry described in this dissertation involves the production of chemical libraries of importance to the pharmaceutical industry. Undoubtedly, combinatorial approaches have and will be useful in other fields, and their exclusion from this document is entirely reflective of the focus of this thesis.

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Chapter 2

Introduction to Heparin

Proteoglycans are a diverse class of biopolymers that contain long carbohydrate chains extending from a protein core. The acidic polysaccharide chains are known as glycosaminoglycans. Common glycosaminoglycans include hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, and keratan sulfate (see Figure 2-1). These polymeric carbohydrates are isolated from animal tissues (most are isolated from connective tissue, with the exception of heparin) and have physical as well as chemical roles in the extracellular matrix[1,2].

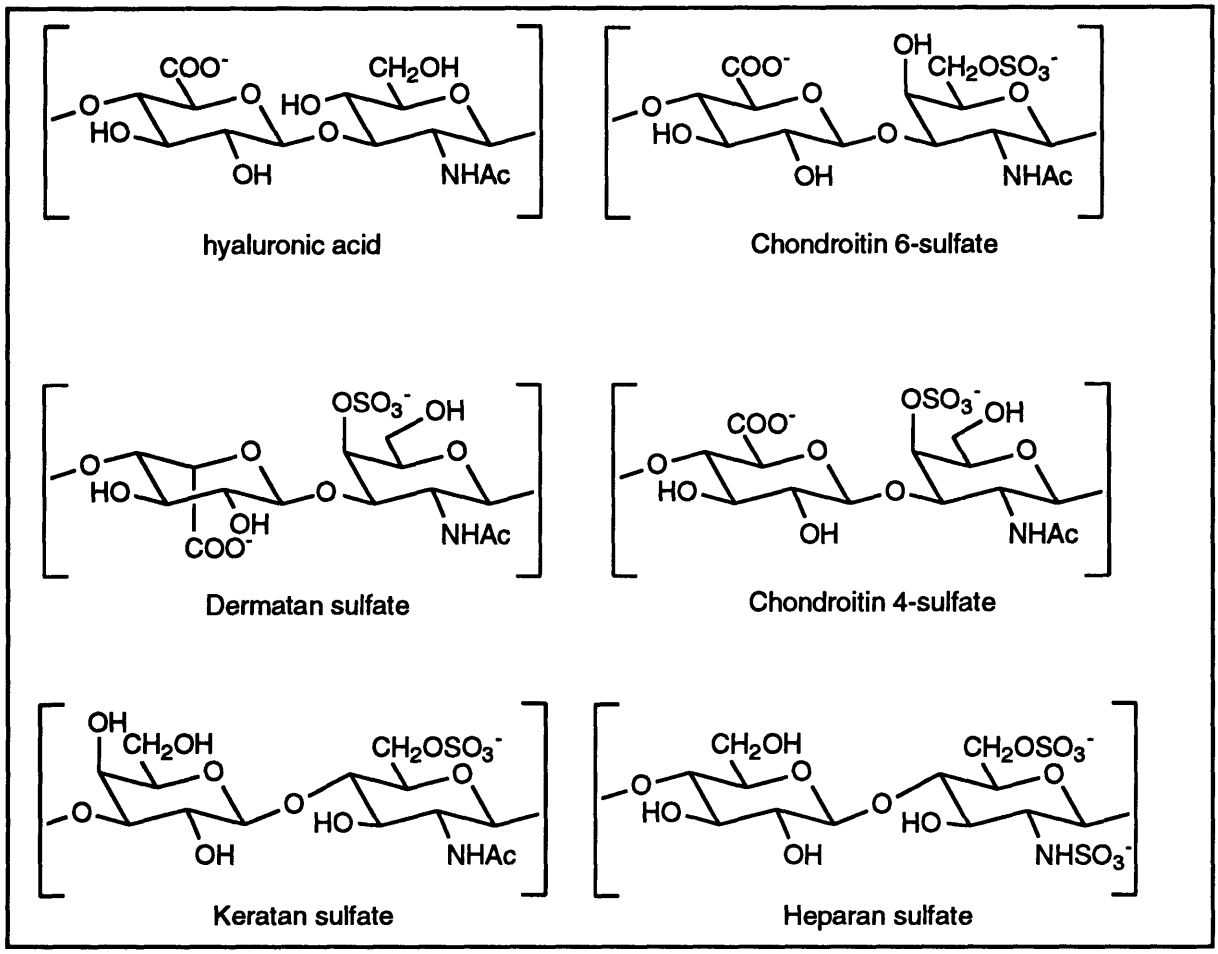


Figure 2-1. Common glycosaminoglycans.

Heparin is composed of a repeating co-polymer of glucosamine and uronic acid (primarily L-iduronic acid). Structurally, it is distinguished by a high degree of acidity and heterogeneity. Heparin is biosynthesized as a proteoglycan, with nascent polysaccharide chains consisting of D-glucuronic acid and N-acetyl glucosamine[3]. A series of enzymes catalyzes N-deacetylation, followed by N-sulfation, C-5 epimerization of D-glucuronic acid, and O-sulfation. Four different positions in each disaccharide unit can be sulfated, the most common of which is N-sulfation while the least common is glucosaminyl-3-O-sulfation. The stereochemistry at C-5 of the uronic acid moiety can be either the D-*gluco* or L-*ido* epimers. Thus, there are five potential sources of heterogeneity per disaccharide unit (see Figure 2-2). In theory, this amounts to 32 (2^5) possible disaccharides, although many of these have not been observed because the enzymes responsible for heparin's structure proceed in a sequential fashion[3]. Nevertheless, with at least 10 possible disaccharides[4] and a median heparin chain length of approximately 50 sugars (25 disaccharide units), extensive heterogeneity can exist both within and between chains.

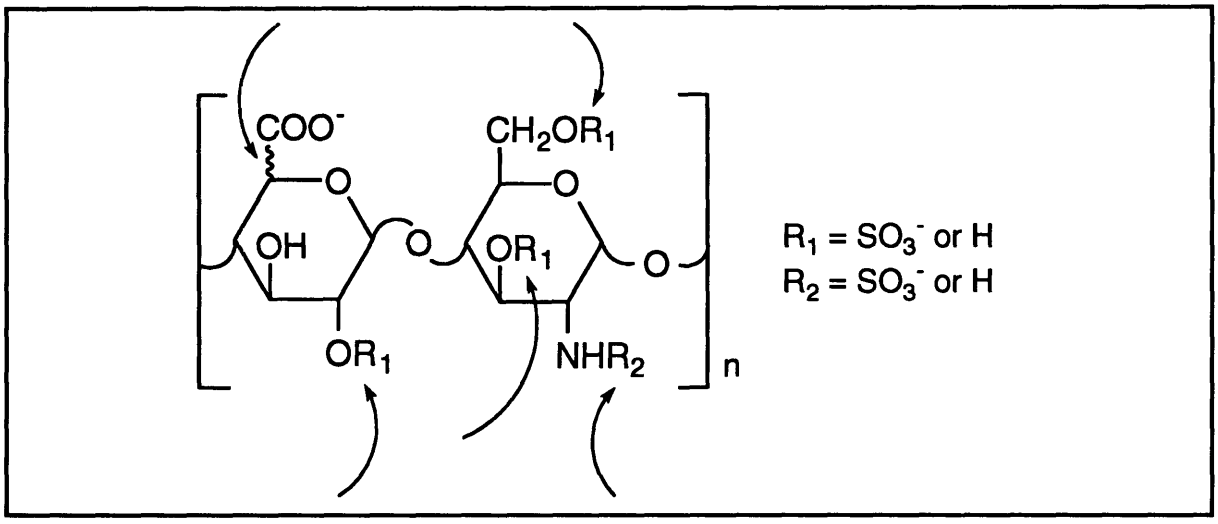


Figure 2-2. Five sources of heterogeneity per heparin disaccharide.

This heterogeneity is manifested in a wide variety of biological interactions (see Table 2-1)[2]. Heparin interacts with many different proteins, and plays a role in regulating

several cellular processes with potentially huge pharmaceutical markets. The most well-known biological activity of heparin is its role as an anticoagulant. Heparin activates several serine protease inhibitors involved in the blood coagulation cascade. One of these proteins, antithrombin III (AT III), undergoes a conformational change upon binding to heparin, resulting in a 1000-fold increase in its binding affinity for thrombin and factor X_a[5].

Heparin-Binding Proteins	Regulation of Cellular Processes
1. Protease inhibitors (antithrombin III)	1. Smooth muscle cell proliferation
2. Plasma lipoproteins (apolipoprotein B-100)	2. Protein expression
3. Growth factors (basic fibroblast growth factor)	3. Nerve cell development
4. Lipolytic enzymes (lipoprotein lipase)	4. Tumor growth
5. Extracellular matrix proteins (fibronectin)	5. Angiogenesis
6. Viral coat proteins (gp120 of HIV-1)	

Table 2-1 Protein interactions and physiological roles of heparin.

Given heparin's polyanionic structure and its capacity to bind to many different proteins, it might seem unlikely that heparin is involved in specific interactions. The issue of specificity is an important one, because in order for heparin fragments or modified heparinoids to obtain regulatory approval as pharmaceutical entities, it is critical to avoid overlap of biological activities. There is significant electrostatic attraction between heparin and highly basic peptide sequences, undoubtedly leading to some non-specific interactions. However, many protein-heparin interactions are specific[4]. Generally, the foremost difficulty lies not in establishing the existence of a specific interaction, but rather in elucidating the structure of the particular heparin fragment that is involved.

One extensively researched example is the interaction of heparin and AT III. The minimum heparin fragment[6] that binds with high affinity to the protein is a pentasaccharide

sequence (see Figure 2-3) containing a rare glucosaminyl-3-O-sulfate[7]. The exquisite specificity of this interaction has been revealed by a series of studies using synthetic oligosaccharide fragments[8]. Removal of the 3-O-sulfate group virtually eliminates biological activity.

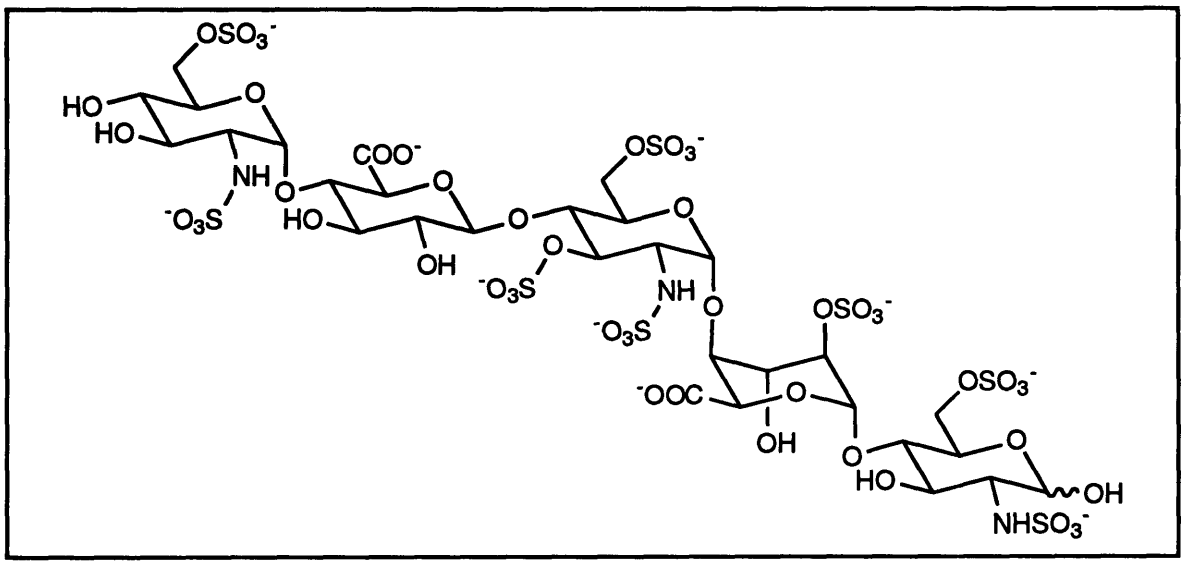


Figure 2-3. Pentasaccharide with high affinity for AT III[7].

Heparin Libraries

In summary, heparin comprises a class of highly related compounds with a wide variety of biological activities. Slight structural perturbations in the heparin chains can cause large differences in biological activity. From the perspective of a pharmaceutical company, heparin possesses many characteristics of an ideal combinatorial library. It contains a repeating building block unit that is variably functionalized in different spatial orientations. The result is a group of oligomeric products with high affinities for important biological targets. In order to create an ideal heparin-based therapeutic, an oligosaccharide is required that is responsible for a unique biological interaction, but has little or no effect on the myriad of other biological activities associated with the full polysaccharide chains.

There are two major obstacles blocking the path to heparin-based drugs, one of which is fundamental in basis and the other experimental. There is no solution to the intrinsic problem that a homogeneous oligosaccharide can have multiple biological sites of action. From the perspective of a drug manufacturer, the best-case scenario is to identify a *small* oligosaccharide that interacts with a specific target, since smaller heparin fragments will be less likely to have superfluous biological activities characteristic of the full-length chains.

The second obstacle is the experimental difficulty of accessing the biochemical information stored in the heparin library[4]. Analytic chemistry on these complex polyanions is extremely challenging, although recent technical advances[9] have greatly facilitated biophysical and biochemical studies. Nevertheless, it remains very difficult to extract information from the natural, heterogeneous library (and was even more difficult four years ago when we confronted this problem).

Building a library. An alternative approach entails a controlled *reconstruction* of the library such that the components are known and readily characterized, thereby vastly reducing the difficulty of information retrieval. In the most likely scenario, reconstruction is merely chemical synthesis. Obviously, this approach becomes efficient when the difficulty of information retrieval in the natural library exceeds the difficulty of reconstructed library synthesis. This situation is analogous to choosing between a multivalent approach to library synthesis and a spatially addressable or encoded library approach. The probability that the reconstruction approach is preferred is proportional to the number of different "applications" (i.e., the number of times one wishes to access the informational content of the library), since the largest time investment is initial library synthesis. The total time T to perform any number A different applications, with an average time I for information retrieval and S for initial library synthesis, and $I_n > I_r$, can be be expressed as follows for the natural and reconstructed libraries:

(natural library) $T = AI_n$

(reconstructed library) $T = S + AI_r$

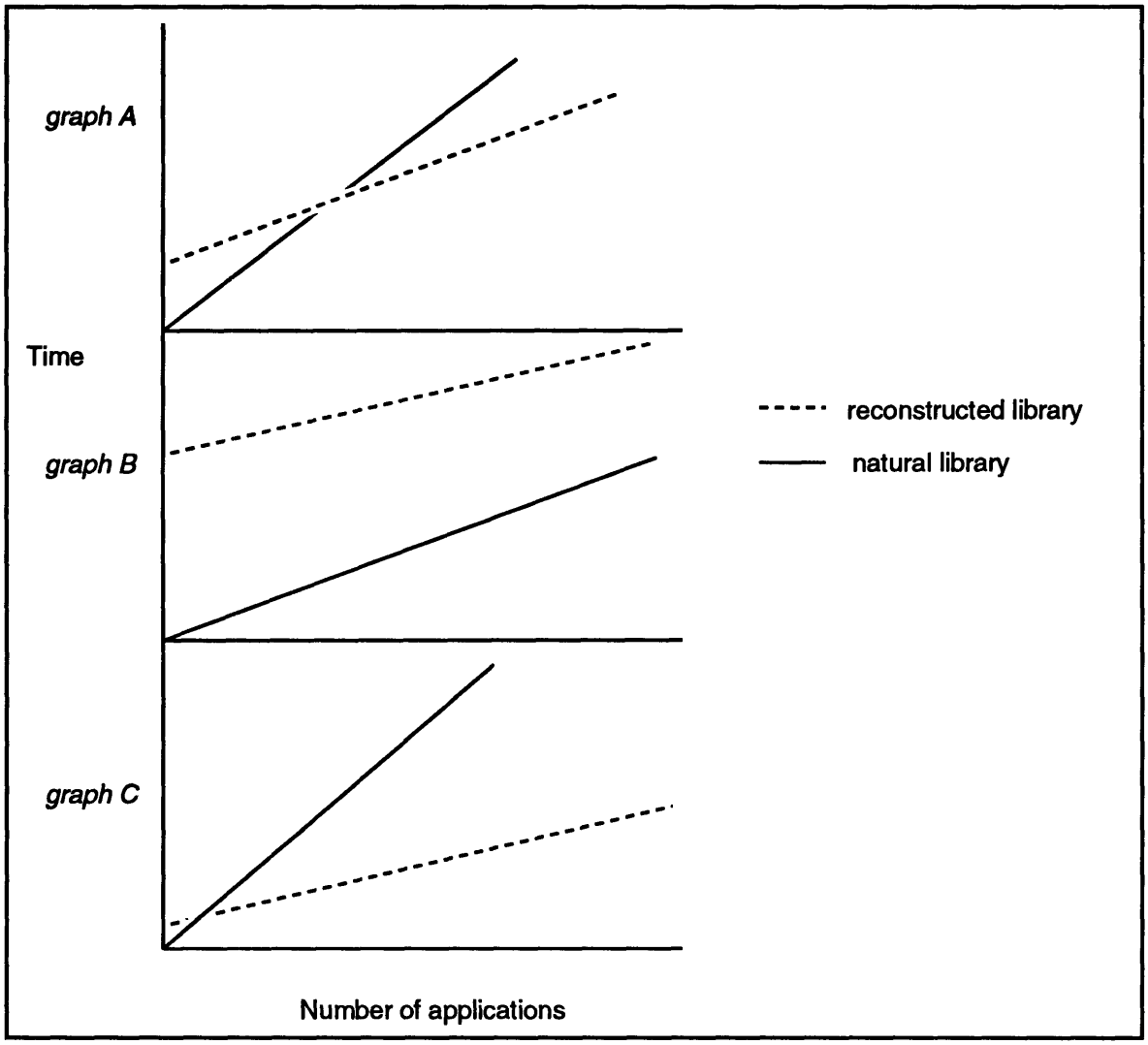


Figure 2-4. (*Case a*) Synthesis of a reconstructed library becomes increasingly efficient as one seeks more applications. (*Case b*) The initial time investment for reconstructed library synthesis never pays off. (*Case c*) The reduced difficulty of information retrieval merits reconstructed library synthesis even if the number of applications is small.

These equations are represented schematically in Figure 2-4, graph A. Clearly, it becomes increasingly advantageous to use the reconstructed library approach as the number of

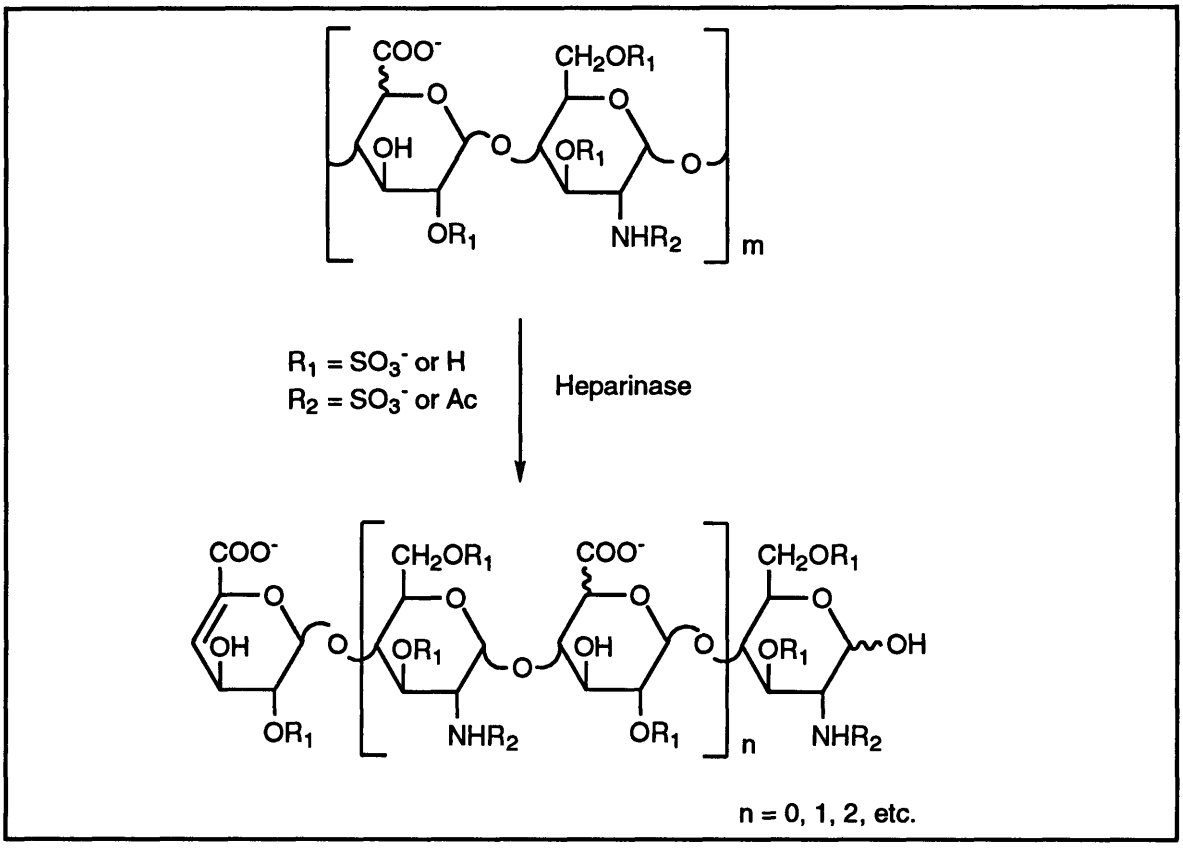
potential applications rises. Of course, the experimental reality may be more accurately depicted by graphs B or C from Figure 2-4, in which one or the other approaches is always preferred.

We believed that the potential advantages of a reconstructed heparin library merited a significant investment in library synthesis. It was our intention to produce a library of defined heparin oligosaccharides or unnatural "heparinoids" similar to the native structure. Methods to produce defined heparin oligosaccharides include total chemical synthesis[7,10,11], as well as fragmentation of heparin followed by purification of fragments. Neither method is ideally suited to constructing libraries of defined oligomers. Heparin is inexpensive, and can be cleaved chemically[4,12,13] or enzymatically[14-18], making a fragmentation/purification approach appealing. Unfortunately, this approach is compromised by the difficulty of producing and purifying desired compounds that constitute rare sequences of saccharides. It is extremely difficult to purify minor oligosaccharides from complex mixtures obtained by partial cleavage of full-length chains. In addition, depolymerization reactions induce modifications in the terminal saccharide residue, and may cleave through desired sequences[4]. The versatility and conclusiveness of the synthetic approach are undermined by the difficulty of chemical synthesis. The high-affinity pentasaccharide fragment that binds to AT III (see Figure 2-3) was synthesized in 75 steps in 1984[7]. Changes in the target structure (making unnatural heparinoids) to facilitate synthesis[19,20], in addition to ten years of refinements by several research labs[8], have made the synthesis more manageable; however, the goal of an economically viable, synthetic anticoagulant has not been reached. We sought to exploit the advantages and minimize the disadvantages of each method by devising a semi-synthetic route to the production of potential heparinoid therapeutics. This approach could reduce the number of transformations relative to total synthesis, while maintaining our capacity to selectively modify the molecule.

An Approach to Sulfur-Linked Heparinoids

Heparin and other glycosaminoglycans can be enzymatically cleaved by certain polysaccharide lyases to produce a 4,5-unsaturated 4-deoxy glycosyluronic acid at the non-reducing terminus (see Scheme 2-1)[14-18]. By converting the carboxylic acid into an ester, a potential Michael acceptor is formed, although the electron demand of the resulting α,β-unsaturated ester is moderated by the presence of the ring oxygen. We intended to use sulfur nucleophiles, ideally 1-thioglycosides, to carry out the Michael addition and produce thiosugar analogues of heparin.

Scheme 2-1. Heparinase digestion of heparin



A sulfur linker was chosen for three reasons. First, we anticipated the lack of reactivity of the Michael acceptor, and thus it was advantageous to use a superior nucleophile. Second, we intended to produce compounds that would mimic the biological

activities of natural heparin oligosaccharides, and thus it was important to ensure that all changes were structurally conservative. Third, many thioglycosides possess increased resistance to enzymatic and acidic degradation[21], either of which would be valuable commodities in a library of potential drug compounds.

We planned to produce a library of defined "thioheparinoids" by cleaving heparin with heparinase, purifying various fragments, chemically modifying the fragments, and then linking them with a Michael addition. Oligosaccharide fragments from heparinase digestion were purified by anion exchange chromatography, followed by desalting on a gel filtration column[22]. A typical WAX-HPLC trace of heparinase-treated heparin is shown in Figure 2-5. The fragments were detected by UV absorbance of the α,β-unsaturated ester at 232 nm. The major compound is the trisulfated disaccharide **1** shown in Figure 2-6. Other fragments that can be purified to homogeneity include three tetrasaccharides and a hexasaccharide[22].

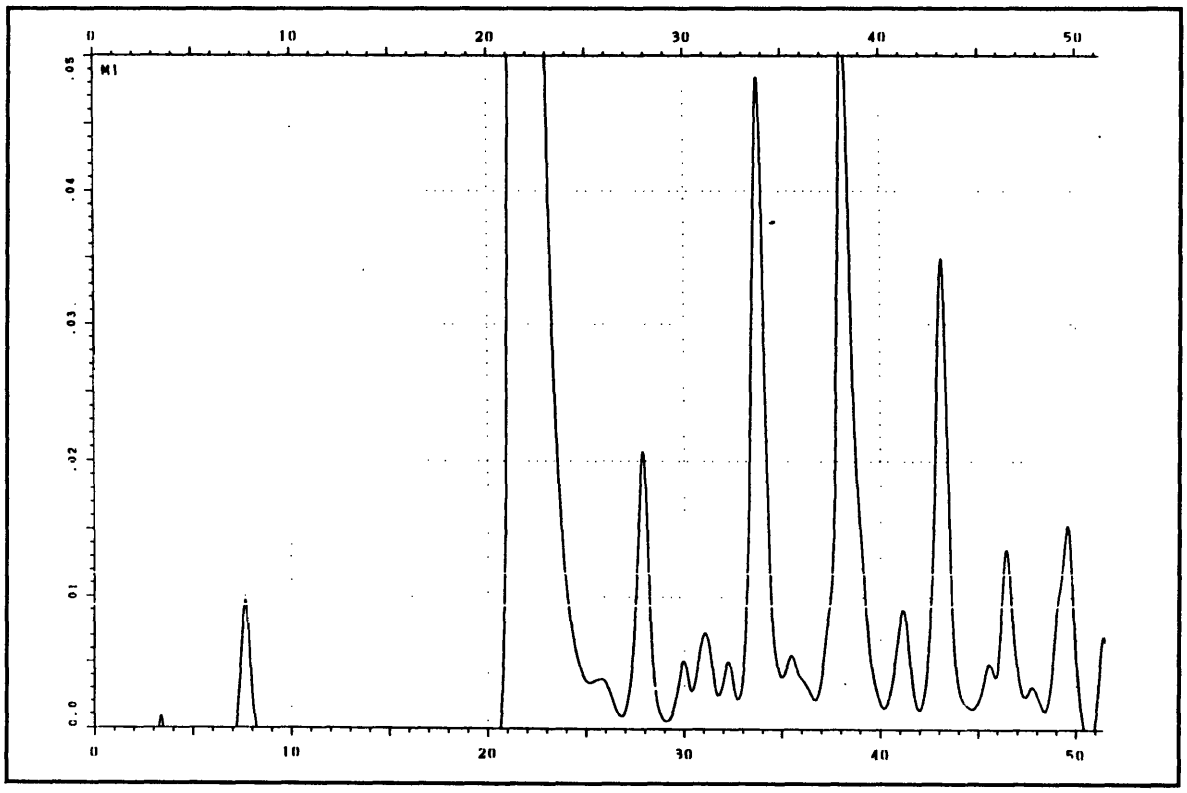


Figure 2-5. Weak anion exchange HPLC trace of heparin digested with heparinase.

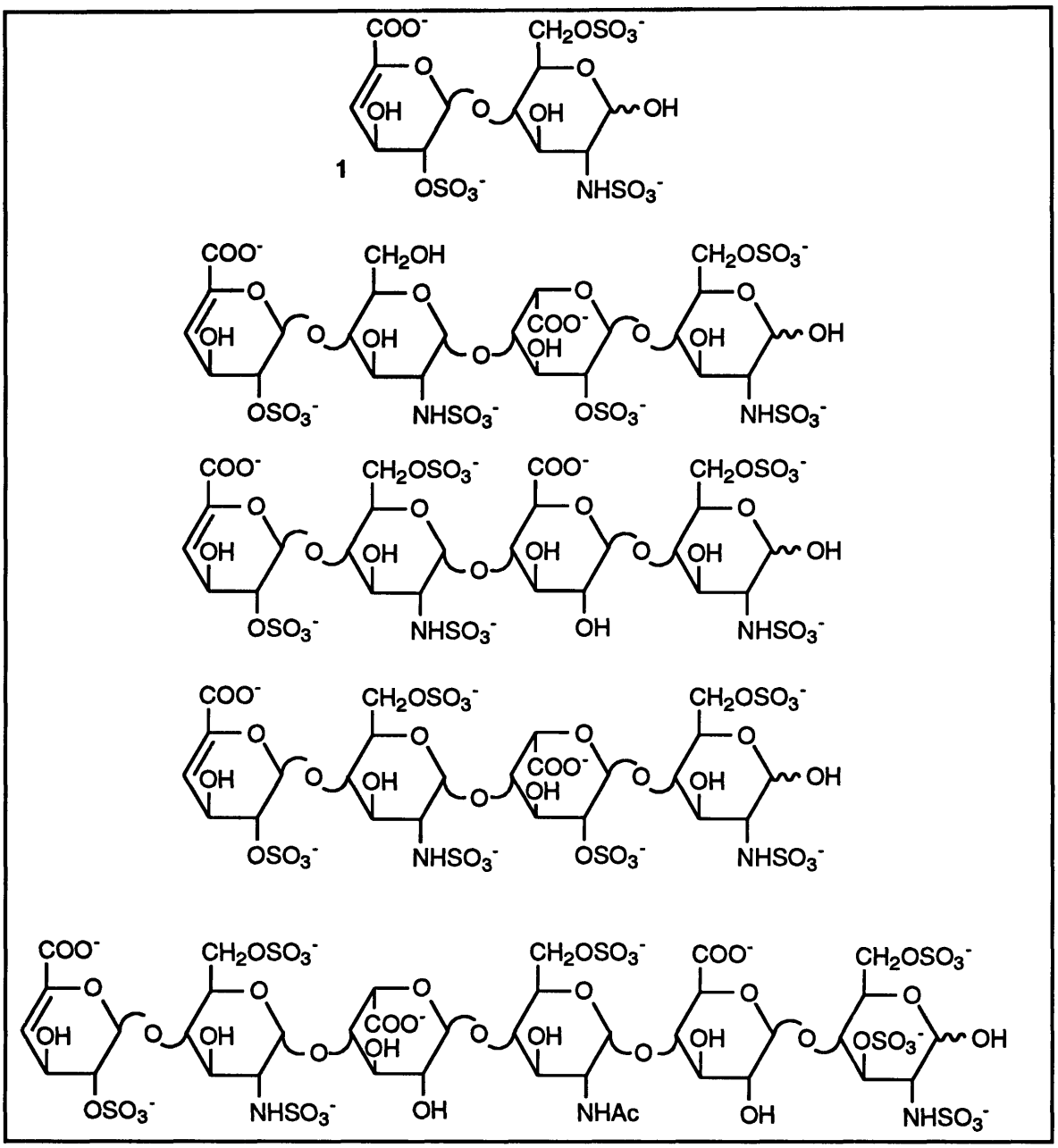
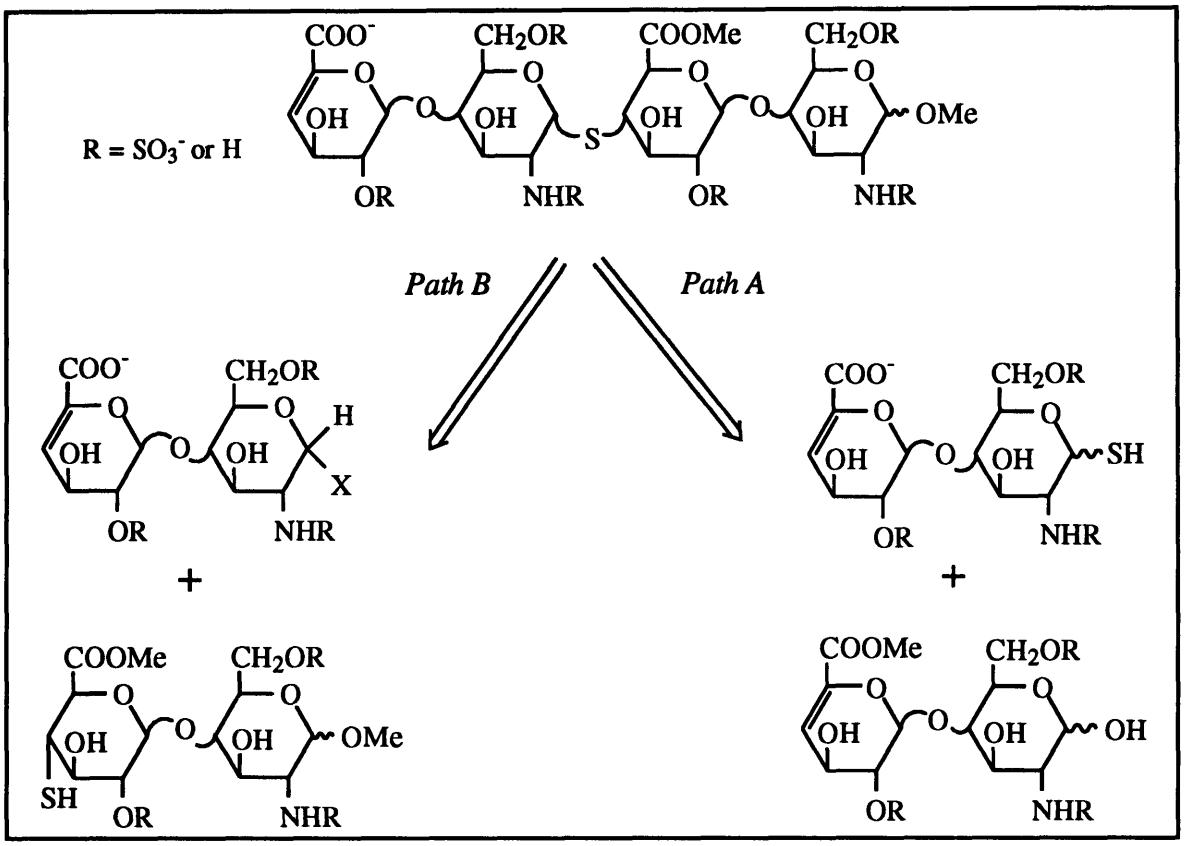


Figure 2-6. Major isolable fractions of heparin digested with heparinase.

From a retrosynthetic perspective, there are two feasible approaches to semi-synthetic thioheparinoids (see Scheme 2-2). Path A is the more direct approach, involving initial functionalization of one fragment as a 1-thiosugar and the other fragment as an α,β-unsaturated ester, followed by fragment condensation using a Michael reaction. Path B

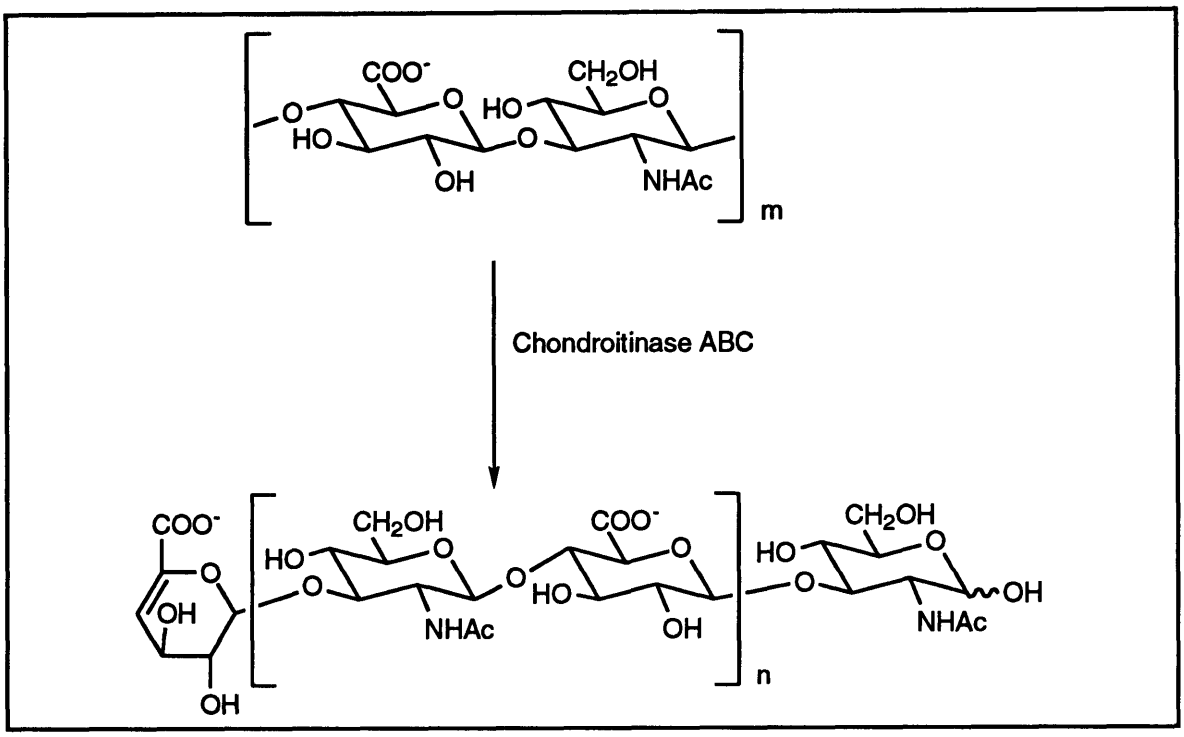
requires initial Michael addition of a small sulfur nucleophile, followed by deprotection to afford the free thiol, and subsequent glycosidation with an activated glycosyl donor.

Scheme 2-2. Retrosynthetic approaches to thioheparinoids



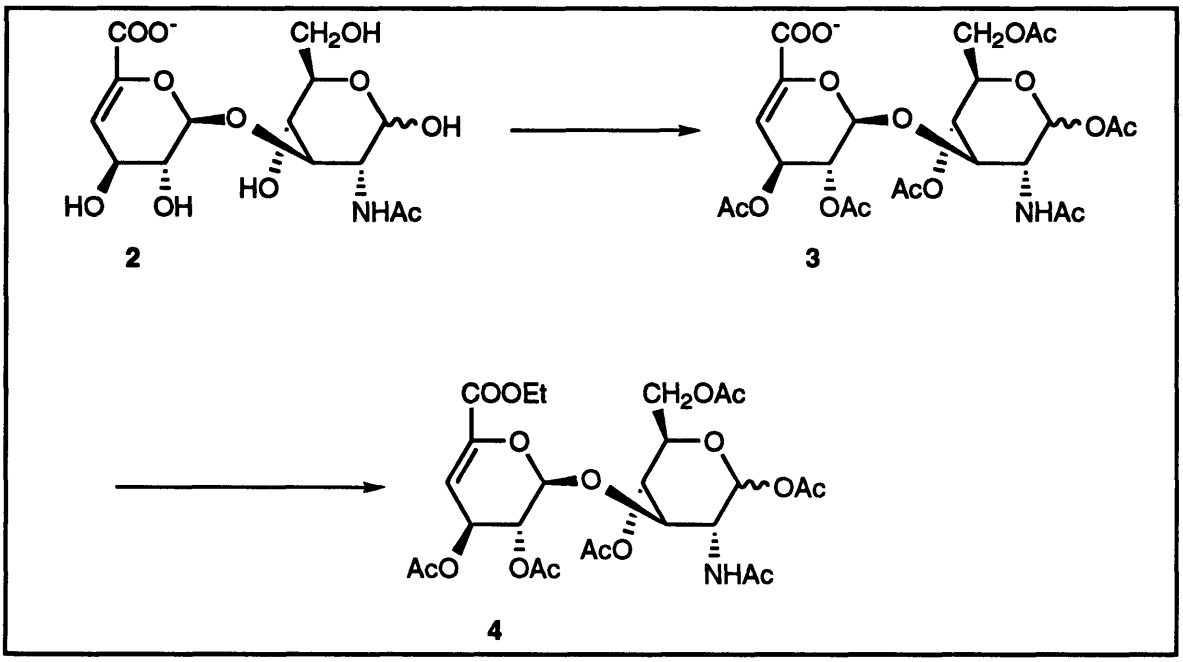
Both approaches were pursued extensively, but we encountered many obstacles. It is difficult to dissolve heparin fragments in solvents amenable to conventional organic synthesis. Once accomplished, the lability of the sulfate groups, particularly the N-sulfate, becomes a major logistical problem. We discovered that pyridine could cause O-desulfation, which is particularly troublesome since formation of the pyridinium salt is a standard method to solubilize heparin in certain organic solvents[23].

Scheme 2-3. Hyaluronic acid digestion by chondroitinase ABC



The complications of solubility and desulfation prompted the use of enzymatically-digested hyaluronic acid (see Scheme 2-3) as a model compound. Hyaluronic acid is a natural glycosaminoglycan which lacks sulfation. This property facilitates chemical modifications of the disaccharides by improving solubility (also eliminating concerns about sulfate lability) and also simplifies purification because only one major disaccharide[24-26], **2**, is produced upon enzymatic digestion with chondroitinase ABC[27]. The α,β-unsaturated acid **2** was acetylated and esterified to produce the Michael acceptor **4** in accordance with Scheme 2-2, but we were unable to find conditions that permitted Michael addition of any nucleophile. At this point, we searched for a further simplified model system with which we could study the feasibility and stereochemical outcome of addition.

Scheme 2-4. Semi-synthesis of the Michael acceptor **4**



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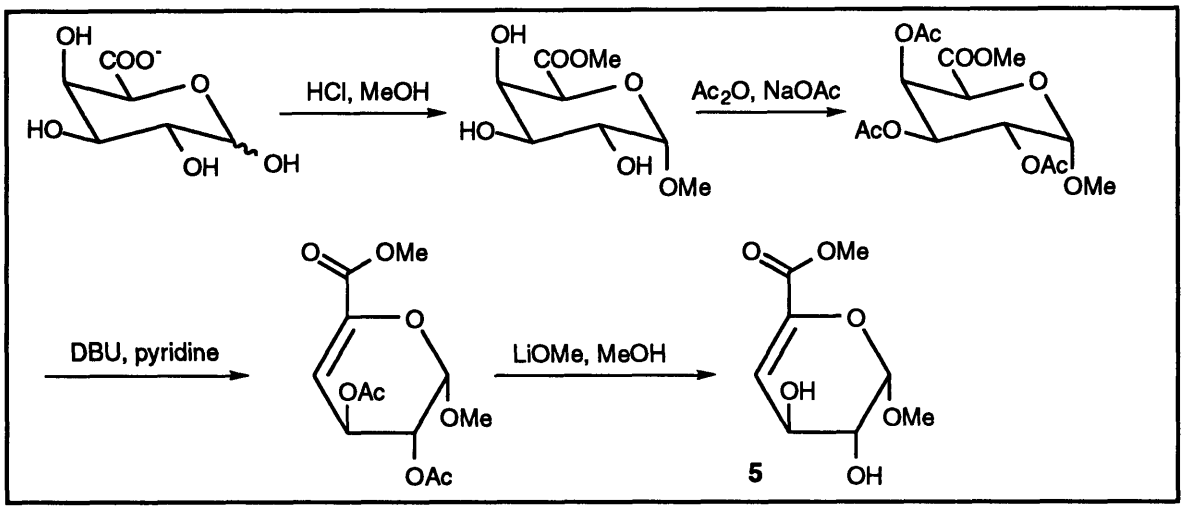
Chapter 3

Synthesis of a Model Michael Acceptor

We used the model compound **5**, easily synthesized in four steps from galacturonic acid (Scheme 3-1)[1], to study the feasibility and stereochemical outcome of the Michael addition. Changes in the reactants and experimental conditions were used to obtain mechanistic information.

Scheme 3-1



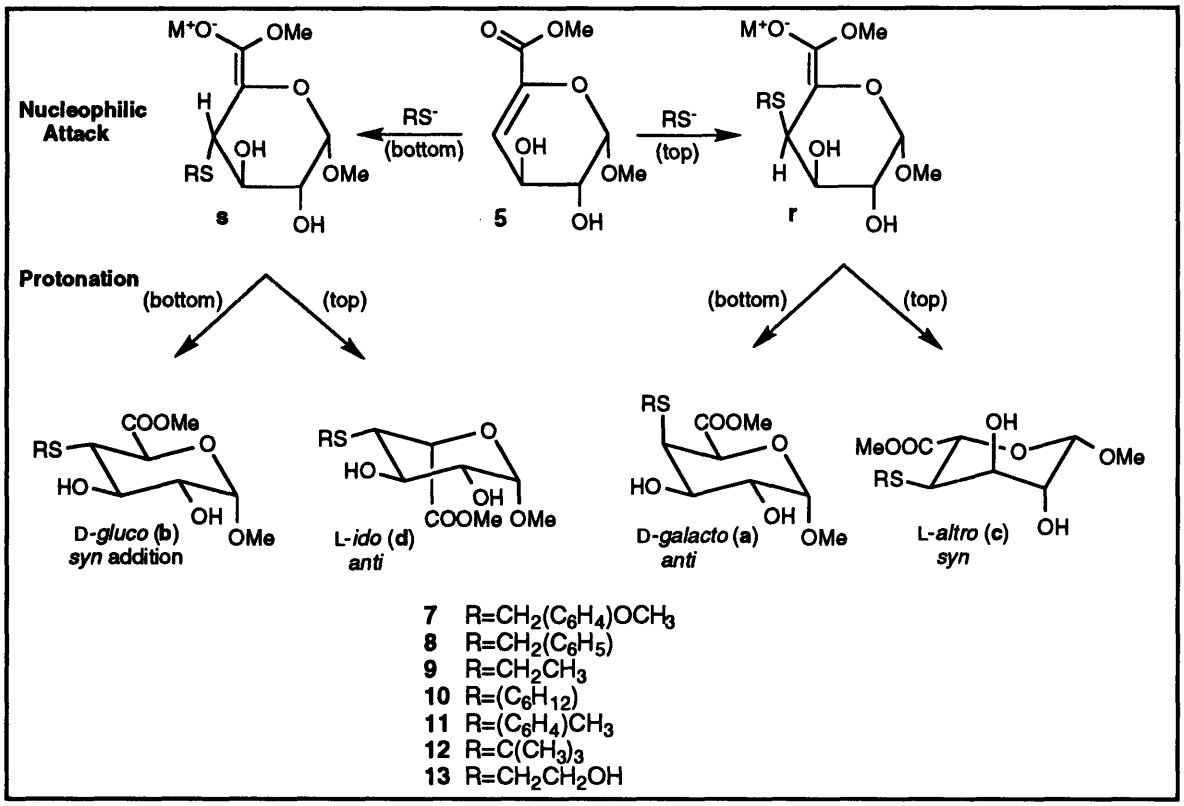
Michael Addition of Thiolates

4-Thiosugars were prepared by the Michael addition of various thiols to the α,β-unsaturated uronate ester **5**. In principle, the Michael addition is reversible, but most reactions were performed under conditions of kinetic control. Four stereoisomers can be produced by the Michael addition of sulfur nucleophiles to **5** (see Scheme 3-2): D-*galacto* (**a**), D-*gluco* (**b**), L-*altro* (**c**), and L-*ido* (**d**). The major product was, with one exception, the D-*galacto* adduct **a**. For example, the combined isomers of **10** were produced in 87% yield by treating **5** with 1.7 equivalents of the lithium thiolate salt of cyclohexyl mercaptan (generated *in situ* by the action of lithium methoxide on cyclohexyl mercaptan) and 8.1

equivalents cyclohexyl mercaptan in methanol at room temperature. The reaction was stereoselective, giving predominantly **10a**, and went to completion in 72 hours.

Saponification of **5** produced the α,β-unsaturated acid **6**, which did not undergo Michael addition with nucleophiles such as benzyl mercaptan or ethanethiol. The reactivity of **5** was substantially reduced upon benzylation of the free hydroxyls, while acetylation rendered the sugar unreactive. There are several possible explanations for this reduced activity, and we have not ascertained which of them is correct.

Scheme 3-2. Michael addition of thiolate nucleophiles to **5**



Standard reaction conditions. In order to compare the reaction rate and distribution of products under various conditions, a standard procedure was followed. A solution of the unsaturated ester **5** was added to a solution or suspension of 1.2 equivalents lithium thiolate and 2.0 equivalents of the sulfur nucleophile (generated *in situ* by the action of 1.2

equivalents lithium methoxide on 3.2 equivalents of the thiol). The reaction was stirred at room temperature for 24 hours and neutralized with cation exchange resin. The solvent was evaporated and the residue taken up in $CDCl_3$ and analyzed by ^1H NMR. It should be noted that these standard procedures were not optimized reaction conditions. Instead, they were selected because deviations in any direction could be observed, and small changes in concentration (potentially caused by impurities or side reactions) did not give rise to large experimental effects. We examined the effects of varying the nucleophile, base, solvent, temperature, and ratio of reactants.

Nature of the nucleophile. Primary and secondary alkyl thiols readily underwent addition to **5** to give products **a-d** (see Table 3-1), but tertiary thiols either did not react or reacted very slowly. Benzylic and phenolic thiols added to **5** to give products such as **7**, **8**, and **11**. There was little difference, in terms of reaction rate or product distribution, between ethanethiol, cyclohexyl mercaptan, methoxybenzyl mercaptan, and benzyl mercaptan. Other thiols underwent reaction at slower rates. Sulfur nucleophiles that did not add to **5** can be grouped into two classes: sterically hindered thiols (such as trityl mercaptan) and weakly basic sulfur nucleophiles (see Figure 3-1). This latter class includes compounds such as thiohemiacetals, thioacids, and thioureas.

From a retrosynthetic standpoint, every compound on the right side of Figure 3-1, i.e, those nucleophiles that did not add to **5**, would have been preferred to any of the compounds that successfully underwent addition. The failure of 1-thioglycosides to add to **5** was very disappointing because that was our preferred synthetic approach (see Scheme 2-2). Nucleophiles such as thioacetic acid or thiourea would have been ideal candidates for the alternative retrosynthetic strategy, since deprotection of the Michael adducts to give the 4-thiosugar would be facile. The lack of success with these and other easily deprotected nucleophiles forced us to use methoxybenzyl mercaptan as the masked hydrogen sulfide donor (discussed in Chapter 4).

Line	Prod.	Solvent	Equivalents			Temp.	% Conv.	Product Distribution[a]			
			thiol	thiolate	MeO-	(°C)	24 hrs.[b]	a	b	c	d
1	**8**	MeOH	2.0	1.2	0	22	63	80	3	12	5
2	**8**	MeOH	2.0	1.2	0	4	26	85	2	9	4
3[c]	**8**	MeOH	2.0	1.2	0	44	57	79	7	10	4
4	**8**	MeOH	0	3.2	0.8	22	50	81	5	11	3
5	**8**	MeOH	2.9	0.3	0	22	16	90	*[d]	10	*
6	**8**	THF	2.0	1.2	0	22	91	89	*	11	*
7	**8**	CH_3CN	2.0	1.2	0	22	60	82	*	16	*
8	**8**	CH_2Cl_2	2.0	1.2	0	22	48	82	*	18	*
9	**8**	Acetone	2.0	1.2	0	22	<5	not determined			
10	**8**	DMF	2.0	1.2	0	22	<5	not determined			
11	**8**	DMSO	2.0	1.2	0	22	<5	not determined			
12	**7**	MeOH	2.0	1.2	0	22	63	82	2	10	6
13	**7**	THF	2.0	1.2	0	22	79	63	6	27	4
14	**7**	MeOH	2.0	1.2	0	44	65	75	7	14	4
15	**9**	MeOH	2.0	1.2	0	22	59	81	3	11	5
16	**9**	MeOH	0	3.2	0.8	22	37	78	7	12	3
17	**9**	THF	2.0	1.2	0	22	25	68	*	32	*
18	**9**	MeOH	0	1.0	4.0	22	14	86	*	14	*
19	**10**	MeOH	2.0	1.2	0	22	56	82	3	9	6
20	**10**	THF	2.0	1.2	0	22	11	62	*	38	*
21	**10**	MeOH	2.0	1.2	0	44	72	81	7	10	2
22	**11**	MeOH	2.0	1.2	0	22	0	not determined			
23	**11**	THF	2.0	1.2	0	22	33	83	2	10	5
24	**12**	MeOH	2.0	1.2	0	22	6	83	*	17	*
25	**12**	THF	2.0	1.2	0	22	<1	not determined			
26	**13**	MeOH	2.0	1.2	0	22	55	52	5	43	*
27	**13**	MeOH	2.0	1.2	0	4	16	47	6	47	*
28	**13**	THF	0	1.7	0.2	22	32	20	24	56	*
29	**13**	THF	2.0	1.2	0	22	50	16	16	68	*
30	**13**	THF	2.0	1.2	0	4	49	21	12	67	*

Table 3-1. Accumulation and Distribution of Products under Various Reaction Conditions. [a] The distribution of addition products was determined by ^1H NMR. [b] The percentage conversion to products reflects the sum of addition products **a** + **b** + **c** + **d** as a percentage of the initial concentration of starting material **5**. This was determined by computing (**a** + **b** + **c** + **d**) / (**a** + **b** + **c** + **d** + **5**). [c] Lines 3, 4, 14, 16, 18, 21, and 26-30 represent reactions that were not performed under conditions of kinetic control. [d] An asterisk indicates that less than 2% of the specified product was observed.

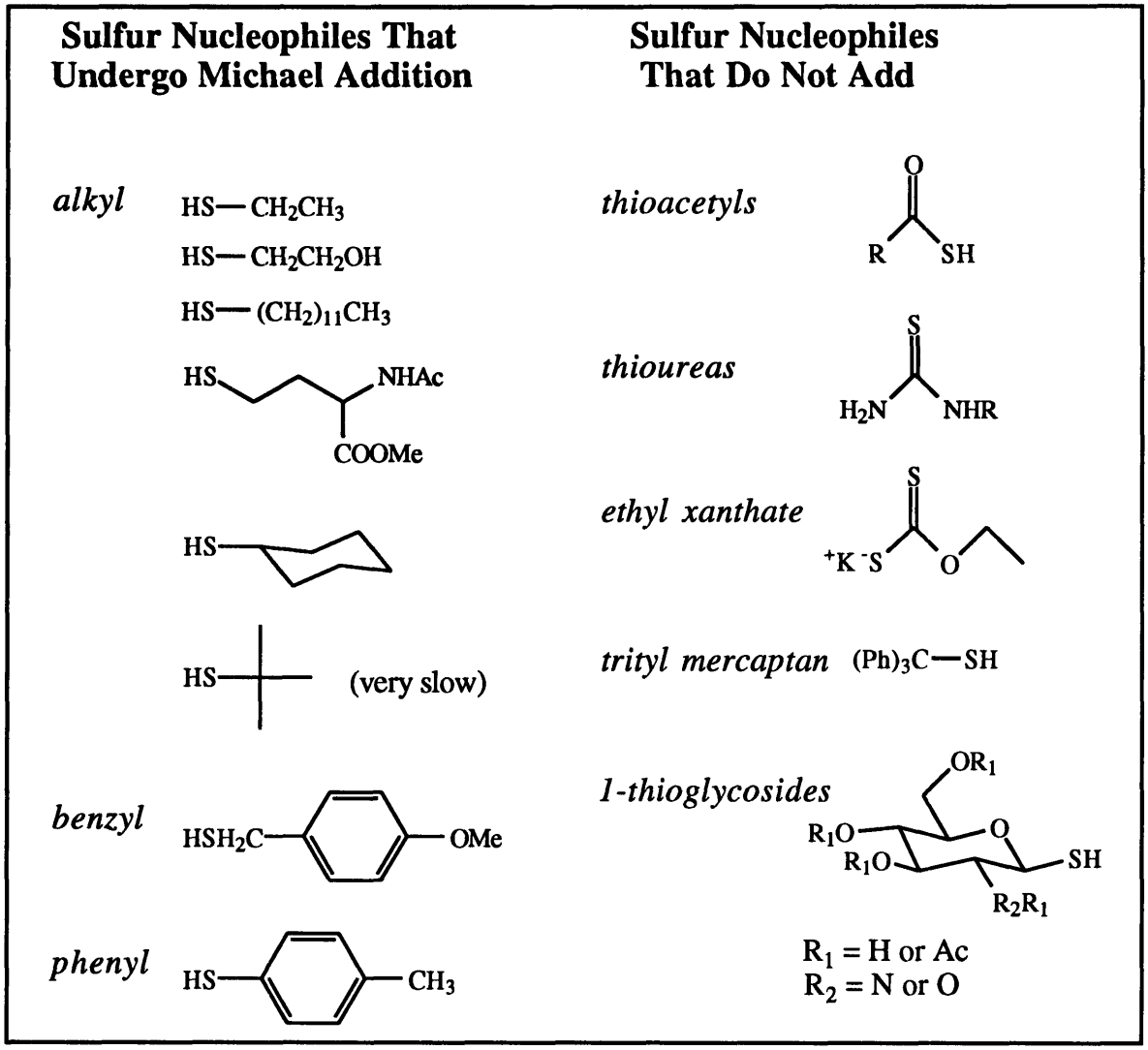


Figure 3-1. Nuclephiles that were more useful synthetically did not undergo addition to **5**.

The failure of all relatively acidic sulfur nucleophiles (the least basic nucleophile that successfully added was 4-thiocresol) to undergo Michael addition to **5** prompted us to consider if the failure to accumulate products was the result of a kinetic barrier to product formation or a thermodynamic equilibration process in which the starting materials were favored. In principle, this issue could be resolved by synthesizing the hypothetical products of the addition reaction and subjecting them to the standard reaction conditions (see Figure 3-2). If elimination was not observed, then the failure to accumulate products in the conventional Michael reaction had to be the result of a kinetic barrier to formation

(Figure 3-2, case A) rather than an unfavorable thermodynamic equilibrium (Figure 3-2, case B). Although 1-thio-β-D-glucose did not undergo Michael addition to **5**, the hypothetical product of this addition, **18a**, was produced by deacetylation of the

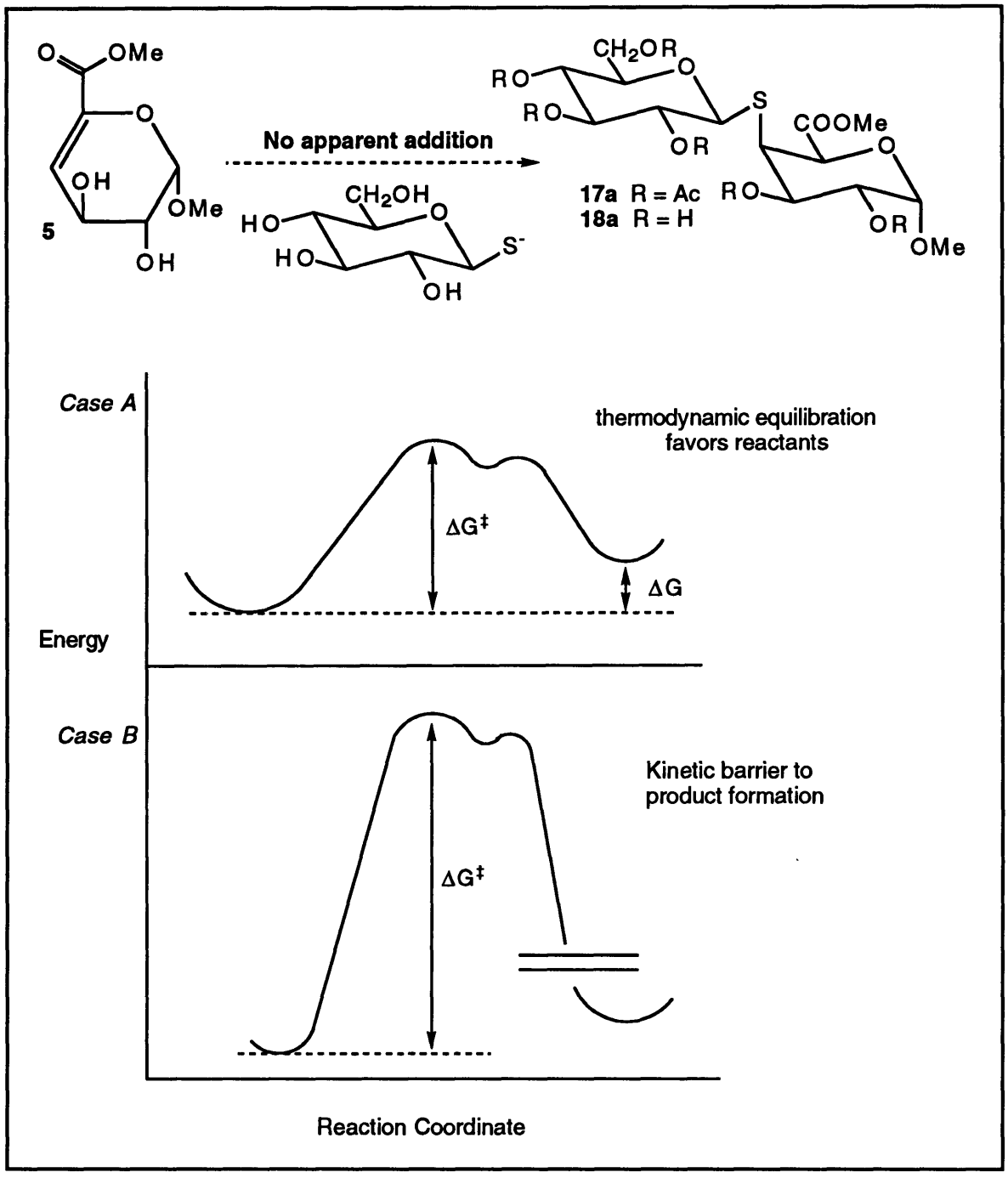


Figure 3-2. Michael addition of thioglycosides to **5** fails because of a kinetic barrier to product formation. Energy diagrams are based on reasonable mechanistic assumptions.

disaccharide **17a** (the synthesis of which is described in Chapter 4). The thiosugar **18a** was exposed to 1.2 equivalents of the lithium salt of 1-thio-β-D-glucose in methanol (generated by the action of 1.2 equivalents LiOMe on 2.2 equivalents of 1-thio-β-D-glucose tetraacetate), and elimination was not observed. Hence, the theoretical product was stable to the standard reaction conditions, proving that the absence of products in the attempted Michael addition of 1-thio-β-D-glucose to **5** can be attributed to a kinetic barrier to product formation.

The Stereochemistry of Addition Depends on Two Independent Processes

Stereoselectivity of the initial nucleophilic attack. Initial nucleophilic attack from the *re* (top) face of **5** leads to the D-*galacto* adduct **a** and the L-*altro* adduct **c**, depending on the stereochemistry of protonation of the postulated intermediate **r** (see Scheme 3-2). Initial attack from the *si* (bottom) face leads to the minor products **b** (D-*gluco*) and **d** (L-*ido*) after protonation of the intermediate **s**. The distributions of products in Table 3-1 indicate that in every case, nucleophilic attack from the top face was preferred {(**a** + **c**) >> (**b** + **d**)}, ranging from 90 to 99% of the total addition products under conditions of kinetic control. Since our long-range goal was the synthesis of thioheparinoids, the preference for top-face attack was disappointing. Heparin contains only D-glucuronic and L-iduronic acids, which are obtained in the model system when initial nucleophilic attack proceeds from the bottom face.

The pseudo-axial direction of nucleophilic attack is well-precedented[2-8] and can be explained in two different ways. The first explanation is based on the formation of a reduced-energy intermediate (see Figure 3-3), since $A^{(1,3)}$ strain is larger than diaxial strain in cylohexane rings[5-7]. The second explanation is based upon extensive orbital overlap between the conjugated ester and the developing sigma bond in the formation of the transition state[8,9]. Essentially, the two theories represent convergent approaches to a reduced-energy transition state, one from the starting materials and one from the

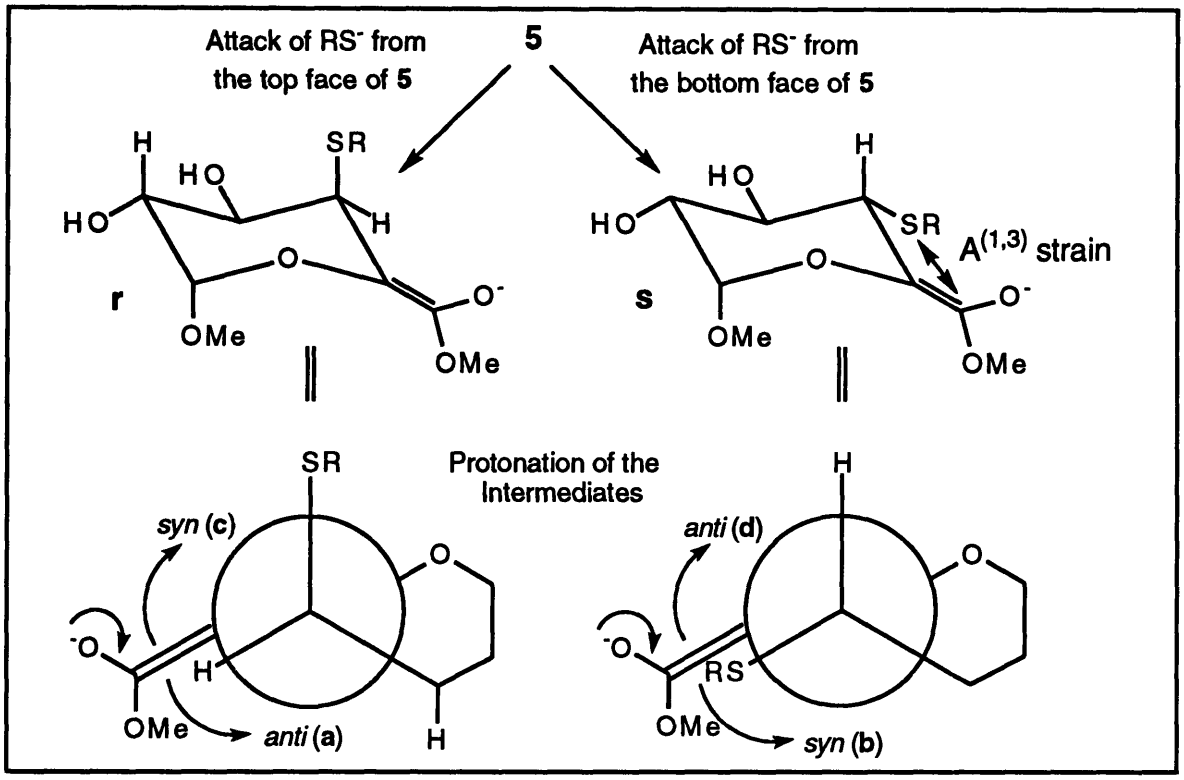


Figure 3-3. Nucleophilic attack from the top face of **5** forms the low energy intermediate **r**, which can be undergo protonation from either direction to give the D-*galacto* (*syn* addition) or L-*altro* (*anti* addition) isomers.

intermediate. It is unlikely that steric hindrance plays a major role in determining the stereoselectivity of nucleophilic attack. The Michael acceptor **5** assumes a half-chair conformation[10] that allows nucleophilic approach from either face of the sugar.

Stereoselectivity of protonation. Subsequent protonation of **r** from the opposite (bottom) face was preferred (unless BME was the nucleophile, which will be discussed separately), constituting a net *anti* addition and producing the D-*galacto* adduct **a**. Protonation from the top face was sterically hindered by the C-4 axial thioether. In methanol, the ratio of *anti* : *syn* protonation (**a**/**c**) for the various thiols studied ranged from 5 to 9. Based on these ratios, we can estimate an energy difference in the transition states leading to *anti* and *syn* protonation of about 1.0 - 1.3 kcal/mol. The stereoselectivity of the

protonation step was much smaller for the kinetically disfavored intermediate **s**, which lacks a large, adjacent, axially-oriented substituent to direct protonation. Protonation from the top face of **s** was slightly preferred on average, affording the L-*ido* isomer **d** (*anti* addition).

Effects of the thiolate counterion. In the absence of thiolate, no reaction occurred. Lithium methoxide was used as the standard base to generate the thiolate salt. No effect on reaction rate or product distribution was observed when the counterion was changed from Li^+ to Na^+ (sodium methoxide as base). Furthermore, no effect was observed when either a full equivalent or catalytic 12-Crown-4 was added to the reaction solution in THF. The rate of reaction slowed dramatically when TEA or DBU was used as base.

Effects of the reaction solvent. With benzyl mercaptan as the nucleophile, the reaction rate decreased in the order THF > MeOH ≈ CH_3CN > CH_2Cl_2 >> benzene (see lines 1, 6-8 of Table 1). Product formation did not occur to an appreciable extent in polar, aprotic solvents such as acetone, DMSO, and DMF (lines 9-11). The addition of *p*-thiocresol to **5** did not proceed in methanol, but went to 33% completion in THF over 24 hours (lines 22, 23). This solvent preference was reversed when alkyl thiols were used as the nucleophile. Ethanethiol (lines 15, 17) and cyclohexyl mercaptan (lines 19, 20) reacted significantly faster in MeOH than THF, and *tert*-butyl mercaptan (lines 24, 25) added very slowly to **5** in MeOH but was unreactive in THF. One factor that may account for this trend is the low solubility in THF of the alkyl thiolates relative to aromatic thiolates.

The stereoselectivity of the reaction was also affected by the choice of solvent. With the exception of benzyl mercaptan, all other thiol nucleophiles produced a higher percentage of the L-*altro* products **c** in THF than in MeOH.

Reaction optimization. Methanol was found to be the optimum solvent for maximizing yield, and room temperature was determined to be superior to elevated or reduced temperatures. Performing the reaction at 44°C increased the rate of side reactions, while performing the reaction at 4°C significantly slowed the rate of product formation. The relative concentrations of thiol and thiolate had a profound effect upon the reaction. A full equivalent of thiolate was not required; however, catalytic reactions proceeded slowly because the rate of addition was dependent on the concentrate of thiolate. After 24 hours, the base-catalyzed (0.3 eq.) addition of benzyl mercaptan (line 5) was only 16% completed, compared with 63% using the standard conditions. After 96 hours, the base-catalyzed reaction had proceeded to 50% completion. In general, the rate of product formation increased with the ratio of thiolate to Michael acceptor.

Reversibility of the Michael Addition

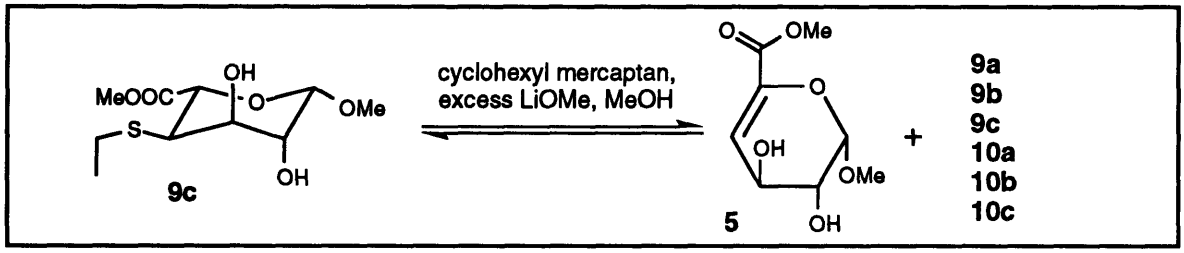
By altering the standard reaction conditions such that an excess of methoxide (relative to the mercaptan) was added to the reaction mixture, the extent of product formation decreased and the proportion of the D-*gluco* isomer was increased (lines 4, 16, 28). Further increases in the ratio of methoxide to thiolate substantially decreased the extent of product formation. Reduced product accumulation can be attributed to the reversibility of the Michael addition of thiolates to **5** in the presence of methoxide. It is likely that the forward reaction rate was dependent of the concentration of thiolate, while the back reaction rate was dependent on the concentration of methoxide.

In a lithium methoxide/methanol solution at room temperature, the D-*galacto* adduct **8a** slowly converted to a mixture of **8a**, the α,β-unsaturated ester **5**, and the L-*altro* adduct **8c**. If equilibration was allowed to proceed for a sufficient length of time, the D-*gluco* adduct **8b** was observed. The same trend was observed when any of the D-*galacto* adducts **a** or L-*altro* adducts **c** were treated with methoxide. When the ethanethiol addition product **9c** was treated with one equivalent cyclohexyl mercaptan and an excess of lithium

methoxide, a mixture of **9a, 9b, 9c, 10a, 10b, 10c**, and **5** developed over time (see Scheme 3-3).

Unlike compound classes **a** and **c**, D-*gluco* isomers such as **10b** did not undergo elimination to **5** in the presence of methoxide. The activation energy for this transformation is exceptionally large because of the low ground-state energy of the D-*gluco* adduct **10b** (all substituents are equatorial except the favored axial methyl glycoside) and the high energy of the transition state leading to the thermodynamically disfavored intermediate **s**. Hence, conversion to the D-*gluco* adduct was effectively irreversible at room temperature in methoxide/methanol solution.

Scheme 3-3. The Michael addition is reversible in the presence of methoxide.



In theory, the reversibility of the reaction permits accumulation of the thermodynamic product **b** as the major product. In practice, we were unable to obtain conditions of thermodynamic control because the relatively harsh experimental conditions that were necessary for reversibility also fomented side reactions that eventually removed the reactants and products from the equilibrium cycle.

Effect of temperature. Under standard reaction conditions, the strongest base present was thiolate, which did not catalyze the reverse (elimination) reaction at room temperature. When **8a** or **8c** was treated with 2.0 equivalents benzyl mercaptan and 1.2 equivalents of its lithium thiolate salt (standard reaction conditions produced by the action of lithium methoxide on benzyl mercaptan), no equilibration occurred; the 4-thiosugar was

stable. However, at elevated temperatures, equilibration was observed. A 44°C solution of **8a** in methanol with one equivalent benzyl mercaptan and one equivalent of its lithium thiolate salt was converted into a mixture of **8a**, **8b**, **8c**, and **5**. The Michael addition was performed at 44°C with several thiolate nucleophiles (lines 3, 14, 21), and the effects on product accumulations were inconsistent. This seemingly paradoxical behavior is easily explained. At room temperature, the Michael addition was virtually irreversible under standard reaction conditions, and thus the observed level of product accumulation coincided with the rate of the forward reaction. At 44°C, the elimination reaction was catalyzed by weaker bases such as thiolates. Therefore, the Michael addition was reversible at elevated temperatures, and the accumulation of products was no longer directly proportional to the forward reaction rate. There was a notable effect on product distributions when reactions were performed at 44°C. The percentage of the thermodynamic product **b** increased, primarily at the expense of the L-*altro* and L-*ido* products **c** and **d**.

Use of β-Mercaptoethanol (BME) as the Thiol Nucleophile

Reversal of stereoselectivity in the protonation step. BME was the only thiol of those studied in which the major product of Michael addition was the *syn* adduct **c** rather than the *anti* adduct **a**. The addition of BME to **5** under standard conditions in methanol gave a ratio of **13c/13a** of approximately 1:1 (line 26), while the ratio in THF was greater than 4:1 (line 29). In accord with other thiolate nucleophiles, initial attack of BME was predominantly from the top face of **5**, producing the postulated intermediate **13r** (see Figure 3-4). However, protonation of **13r** occurred primarily from the top face (*syn* addition) in THF to give the L-*altro* product **13c**. This reversal in selectivity could be the result of intramolecular protonation of the $^4C_1$ intermediate **13r**. This hypothesis is supported by the enhanced selectivity for **13c** when THF was used as the solvent rather than methanol, since the latter could act as a proton donor and compete with intramolecular protonation.

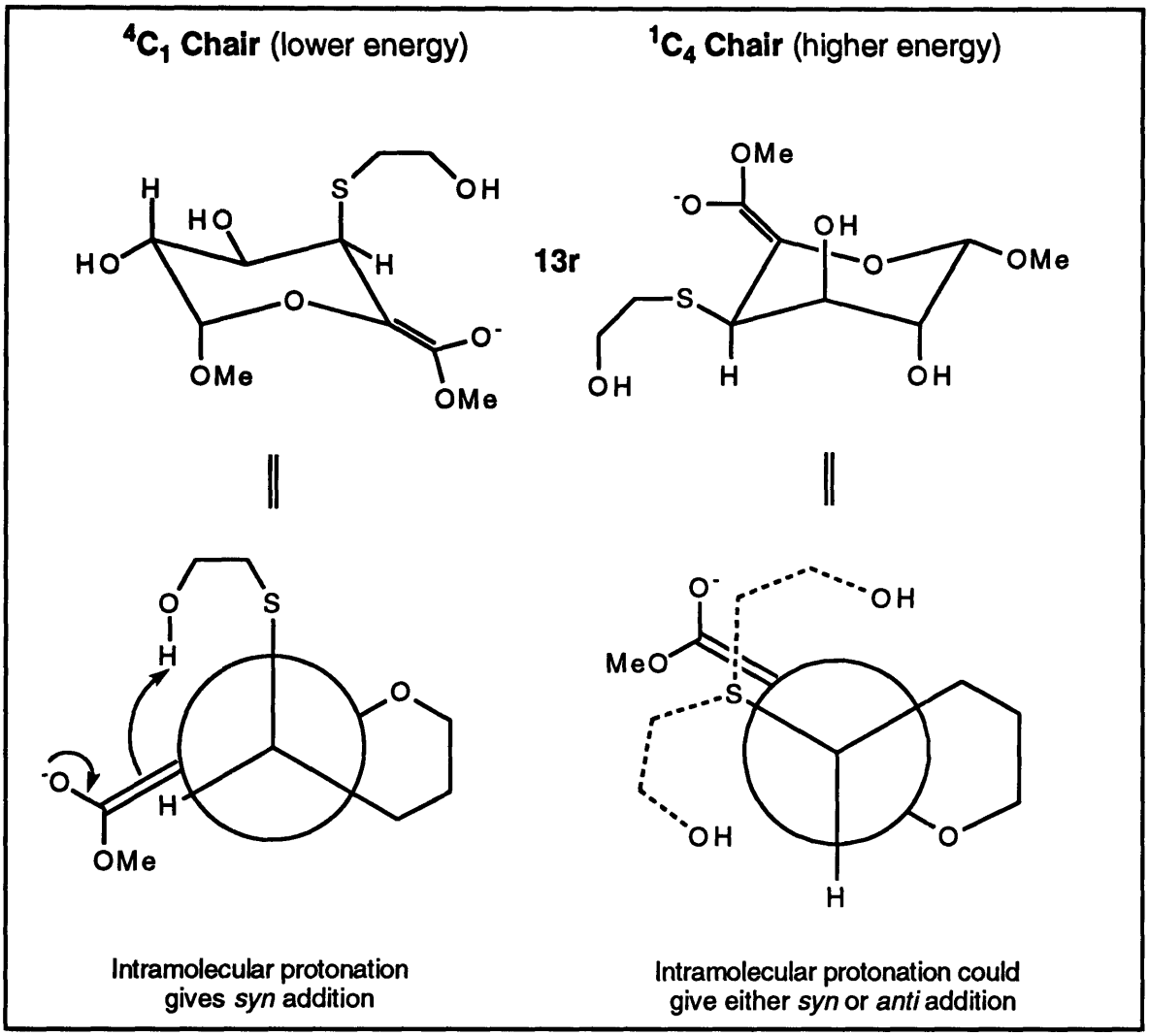


Figure 3-4. A reversal in the stereoselectivity of protonation was observed when BME was used as the Michael nucleophile, presumably because of intramolecular protonation of the $^4C_1$ chair conformer of **13r**.

Although **13c** assumes a $^1C_4$ chair conformation in its final state, it is very unlikely that this was the dominant conformer in the intermediate state. Intramolecular protonation of the $^1C_4$ conformation of intermediate **13r** could give either *syn* or *anti* addition products with little selectivity, and thus would not adequately explain the reversal in the stereoselectivity of protonation that occurred when BME was used as the nucleophile (see Figure 3-4).

Reversibility at room temperature. BME differed from other thiolate nucleophiles with respect to the temperature dependence of the Michael addition because reactions with BME were not under kinetic control. While Michael additions with other thiols gave decreased yields, and greater selectivity for the favored (*anti* addition) product at low temperature, the addition of BME in THF at 4°C (line 30) did not significantly affect the extent of product accumulation, and actually reduced the selectivity for the favored (*syn* addition) product. This unusual temperature dependence is attributable to the relative ease with which **13a** and **13c** underwent elimination. Unlike other adducts such as **8** and **9**, the BME adducts **13a** and **13c** interconverted and produced the unsaturated ester **5** in the presence of BME and its thiolate salt, in both THF and methanol, at 4°C and room temperature. Under standard reaction conditions, therefore, the reaction with BME was reversible: the lithium thiolate salt of BME catalyzed the elimination reaction converting **13a** and **13c** to **5**. Other thiolate bases also catalyzed this transformation. Thus, a solution of the BME adducts **13a** and **13c** in methanol, when added to a solution of ethanethiol and its thiolate salt, was slowly converted to a mixture of the original reactants, the ethanethiol adducts **9a** and **9c**, and the elimination product **5**. The facility of the reverse reaction can be attributed to the superiority of BME as a leaving group relative to other thiols. The departing BME molecular anion is stabilized by formation of an intramolecular hydrogen bond[11]. Therefore, a higher percentage of the thermodynamic product **b** could be expected when the Michael addition was performed with BME because the reaction was reversible under standard conditions. This anticipated shift towards the D-*gluco* adduct **13b** was observed experimentally (line 29).

Structural Assignments

The isomeric configurations and physical conformations of the various Michael addition products were determined by ^{1}H NMR spectroscopy (see Table 3-2). Rough estimations of approximate interaction energy values[12] suggested that the two D-isomeric

Product	Chemical Shift (δ)					Coupling Constant (Hz)			
	1H	2H	3H	4H	5H	J_{1-2}	J_{2-3}	J_{3-4}	J_{4-5}
7a	4.88	3.69	3.99	3.43	4.64	3.8	9.8	4.6	2.1
8a	4.91	3.66	4.00	3.46	4.65	3.8	9.9	4.6	2.3
9a	4.90	3.58	4.03	3.46	4.72	4.0	9.9	4.7	2.2
10a	4.89	3.55	4.00	3.52	4.72	3.9	9.9	4.7	2.3
11a	4.98	3.68	4.11	3.88	4.72	3.8	9.9	4.6	2.1
12a	4.89	3.54	4.00	3.51	4.75	3.9	10.3	4.8	2.2
13a	4.93	3.75	4.09	3.63	4.70	4.1	9.6	4.7	2.1
14a[a]	5.08	5.19	5.48	3.77	4.78	3.7	10.6	4.3	2.1
15a[b]	4.76	3.67	3.91	3.62	4.81	4.1	10.2	4.7	2.1
16a[a]	5.09	4.98	5.57	4.62	4.87	3.7	10.6	4.4	2.2
17a[a]	5.02	5.23	5.42	3.97	4.79	3.9	10.5	4.2	2.3
18a[c]	4.75	3.73	4.08	3.84	4.80	3.8	10.0	4.6	2.2
7c	4.71	3.78	3.96	3.43	4.20	1.9	5.1	3.1	9.0
8c	4.72	3.78	3.97	3.45	4.21	1.6	4.9	3.0	9.2
9c	4.75	3.85	4.10	3.44	4.26	1.6	4.8	3.1	9.5
10c	4.74	3.82	4.06	3.50	4.24	1.7	4.9	3.1	9.4
11c	4.80	3.83	4.11	3.81	4.36	1.9	4.9	3.5	8.9
13c	4.77	3.86	4.18	3.55	4.36	2.3	5.7	3.4	8.0
16c[a]	4.88	4.97	5.61	4.68	4.35	2.9	8.2	4.1	5.2
17c[a]	4.86	5.06	5.55	3.96	4.49	2.9	8.2	4.2	4.9
7b	4.84	3.62	3.60	2.80	4.21	3.1	9.1	10.6	11.2
8b	4.85	3.62	3.62	2.82	4.21	3.3	9.6	10.2	10.4
9b	4.87	3.69	3.64	2.80	4.19	3.2	9.0	10.2	11.1
10b	4.88	3.68	3.59	2.85	4.17	3.7	8.3	10.2	10.6
11d	4.80	3.61	4.32	3.16	4.57	3.6	8.0	9.8	4.9

Table 3-2. Chemical shifts and coupling constants of the glycopyranosyluronate protons in $CDCl_3$. [a] Compound was peracetylated. [b] D_2O was used as the solvent. [c] CD_3OD was used as the solvent.

forms (**a** and **b**) would exist almost exclusively in $^4C_1$ chair conformations, while the two L-isomers, the L-*altro* product **c** in particular, could conceivably adopt the $^1C_4$ chair as the preferred conformation.

Addition products from various thiol nucleophiles were purified and subsequently categorized by comparing ^1H NMR spectra. Identification of the D-*galacto* (**a**) and D-*gluco*

(**b**) adducts was fairly straightforward. The observed coupling constants were consistent with $^4C_1$ chair conformations. Coupling constants of the third class of addition compounds, the L-*altro* adducts **c**, were consistent with a $^1C_4$ chair conformation. The final class of compounds possessed the L-*ido* configuration. In general, this was the hardest group of products to isolate, and only one adduct, **11d**, was characterized. Coupling constants of **11d** in $CDCl_3$ indicated that a $^4C_1$ chair was the principal conformation. NOE data supported the assigned conformations for each class of isomers (see Figure 3-5). The following NOE's were observed for the indicated ring protons from representative products of each isomeric series: **a** (3-5); **b** (2-4, 3-5); **c** (1-5); and **d** (2-4).

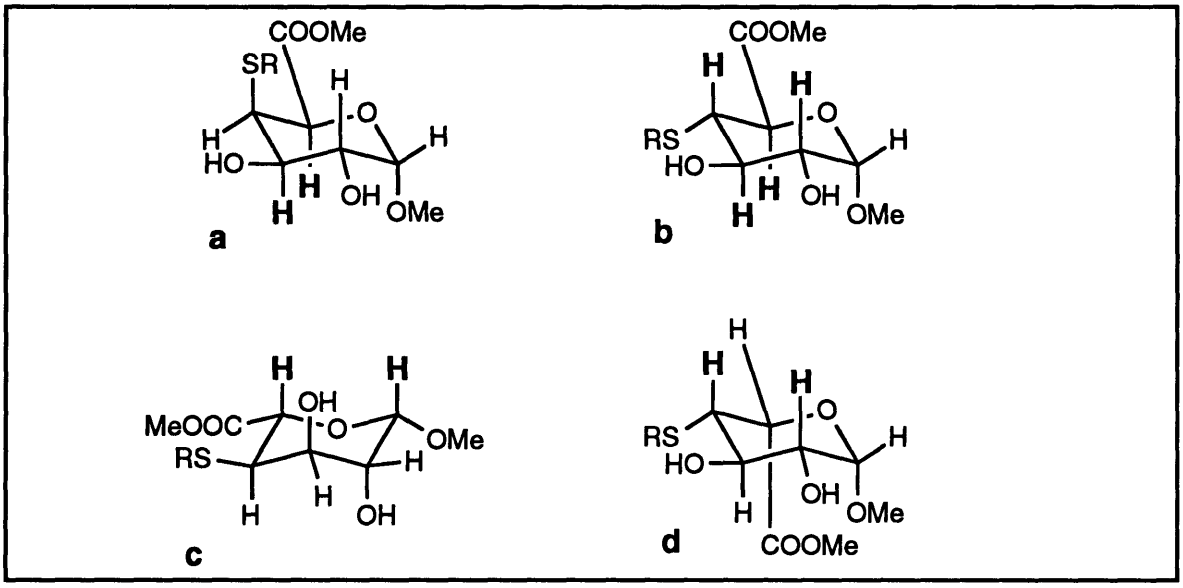


Figure 3-5. NOEs were observed for the protons indicated in bold type. Note the L-*altro* compound **c** is in the $^1C_4$ conformation.

No major conformational changes in any of the Michael addition products were observed upon solvation in more polar solvents such as methanol and water.

One conformational change that was observed occurred upon acetylation of the L-*altro* adducts. Analysis of coupling constants indicated that the non-acetylated L-*altro* sugars (**7c - 11c, 13c**) assumed $^1C_4$ chair conformations almost exclusively, whereas the

peracetylated thiosugars **16c** or **17c** (see Chapter 4) appeared to adopt both the $^1C_4$ and $^4C_1$ chair conformations in $CDCl_3$.

References for Chapter 3

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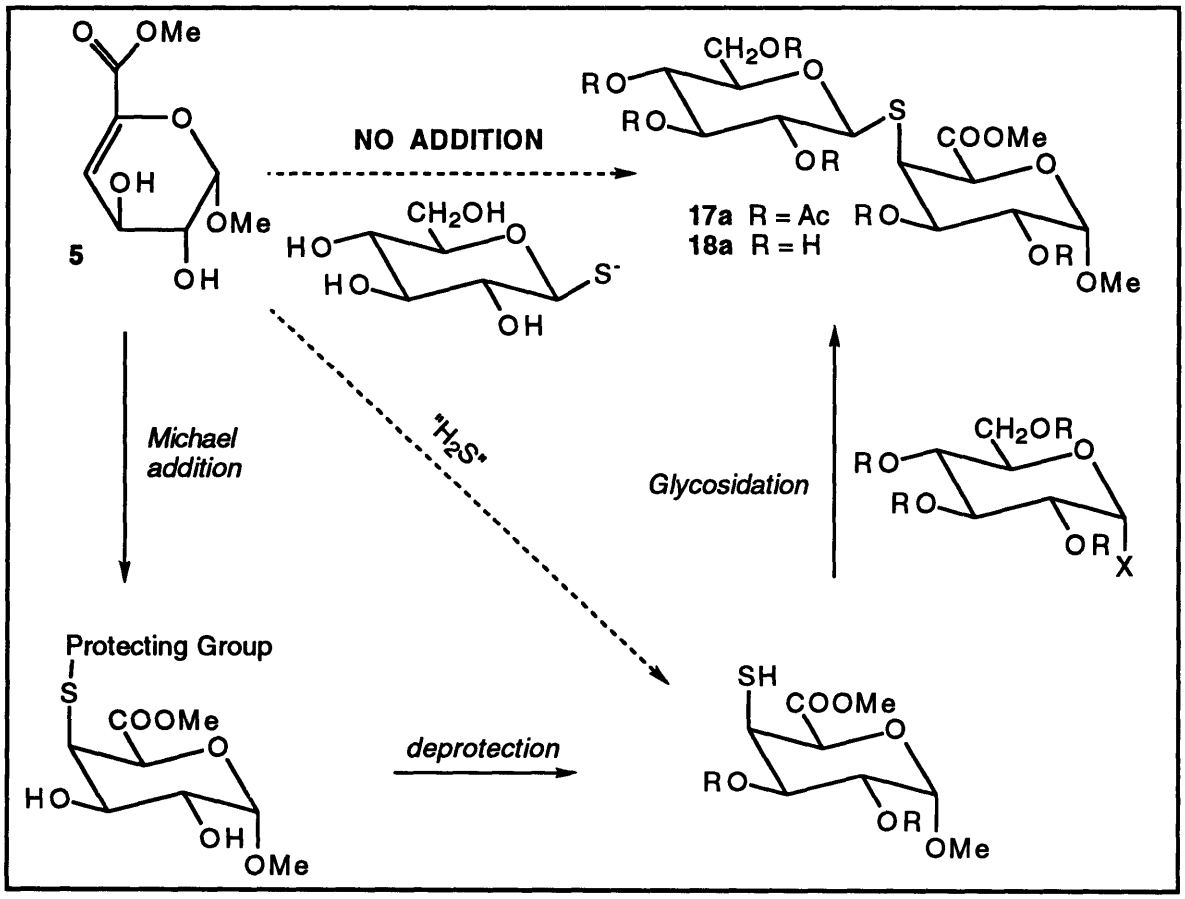
Chapter 4

Synthesis of Thiodisaccharides Using the Michael Addition

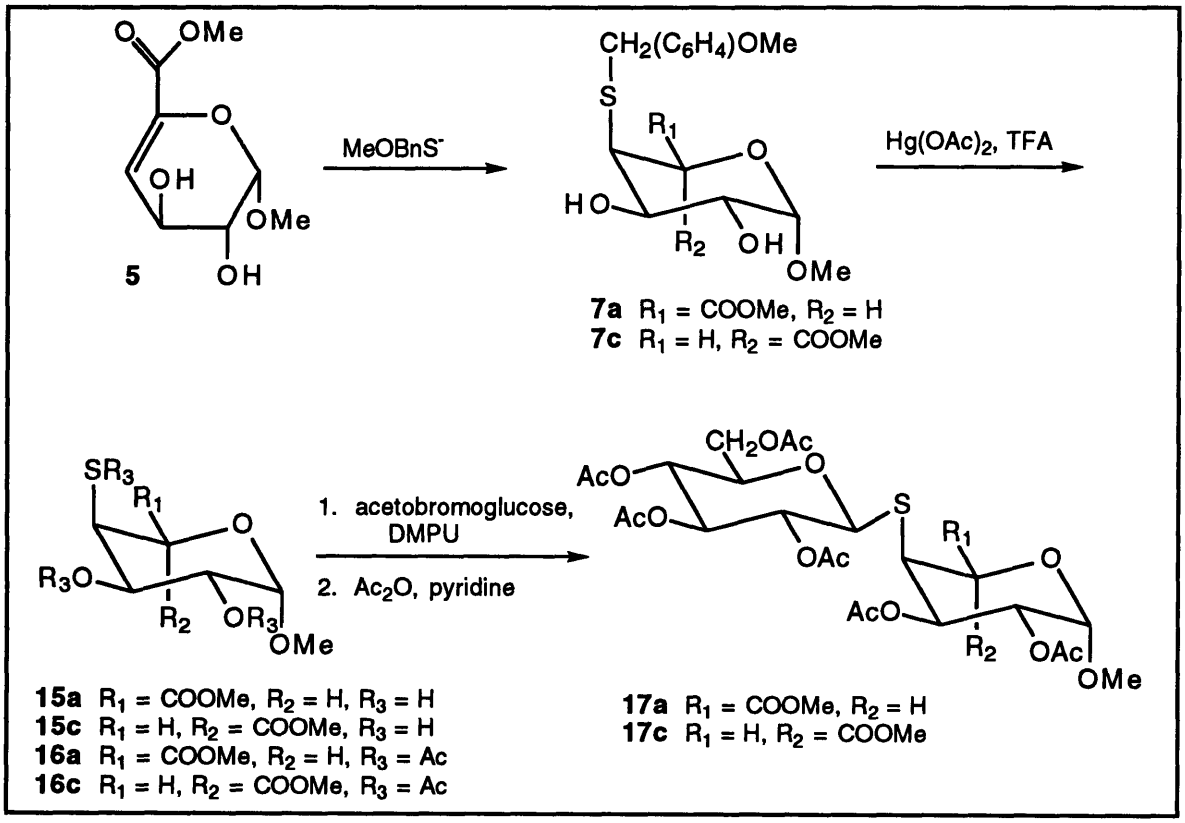
We wanted to investigate the various potential applications of the Michael addition of thiols to α,β-unsaturated pyranuronate esters. The overwhelming preference for nucleophilic addition from the top face of **5** rendered the Michael addition approach unsuitable for the synthesis of thioheparin analogs. Nevertheless, it represented an alternative method to synthesize S-linked sugars containing 4-*S*-D-galactopyranosyl or 4-*S*-L-idopyranosyl moities. We examined this possibility by synthesizing thiodisaccharides **17a** and **17c**. Unfortunately, the inability of 1-thioglycosides to act as Michael nucleophiles mitigated the potential appeal of this approach.

Scheme 4-1. Synthesis of thiodisaccharides using a masked hydrogen sulfide equivalent



Since 1-thioglycosides did not act as Michael nucleophiles in this system, synthesis of sulfur-linked oligosaccharides relied on the use of a masked hydrogen sulfide equivalent as the Michael nucleophile (see Scheme 4-1). The resultant 4-thiosugar was then glycosylated using standard techniques[1]. A combined 84% yield of the purified adducts **7a-d** was obtained by treating **5** with lithium methoxide and a large excess of methoxybenzyl mercaptan. The D-*galacto* adduct **7a** was deprotected upon treatment[2] with mercuric acetate in TFA, affording **15a** in 91% yield. The 4-thioglycoside was deprotonated with one equivalent NaH or NaOMe, then coupled to acetobromoglucose in DMPU. *In situ* acetylation produced the thiodisaccharide **17a** in yields of 10-15%. The same procedure was used to convert **7c** into **15c**, followed by glycosylation to provide **17c** in 24% yield (see Scheme 4-2).

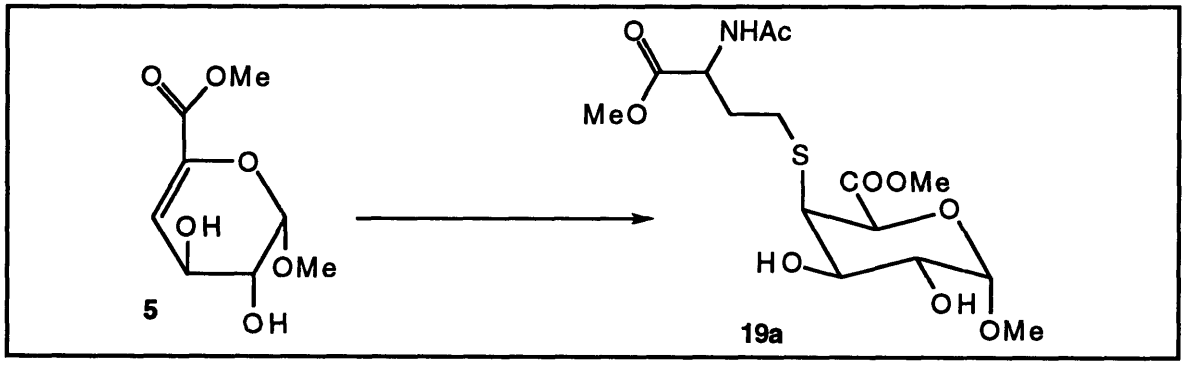
Scheme 4-2



Systematic optimization of the glycosylation chemistry has not been performed. However, an initial experiment indicated that improved yields could be obtained using thioester methanolysis to provide the thiolate salt. A similar observation was made by Reed and Goodman in their synthesis of thiolactose (4-*S*-β-D-galactopyranosyl-4-thio-D-glucopyranose)[3]. The thiosugar **15a** was acetylated to give **16a**, which was treated with 1.05 equivalents sodium methoxide to hydrolyze the thioester and form the sodium thiolate salt. Glycosidation with acetobromoglucose in DMPU, followed by acetylation, afforded **17a** in 27% yield.

Synthesis of an unnatural glycosamino acid. A second application of the Michael addition is the synthesis of unnatural glycoconjugates of peptides and lipids. Michael addition to **5** was used to make an unnatural linkage between a monosaccharide and an amino acid (Scheme 4-3). The lithium thiolate salt of methyl [(2-acetamido)-4-thio]butyrate (N-acetyl homocysteine methyl ester) was added to **5** to produce the glycoconjugate **19a** in 52% yield.

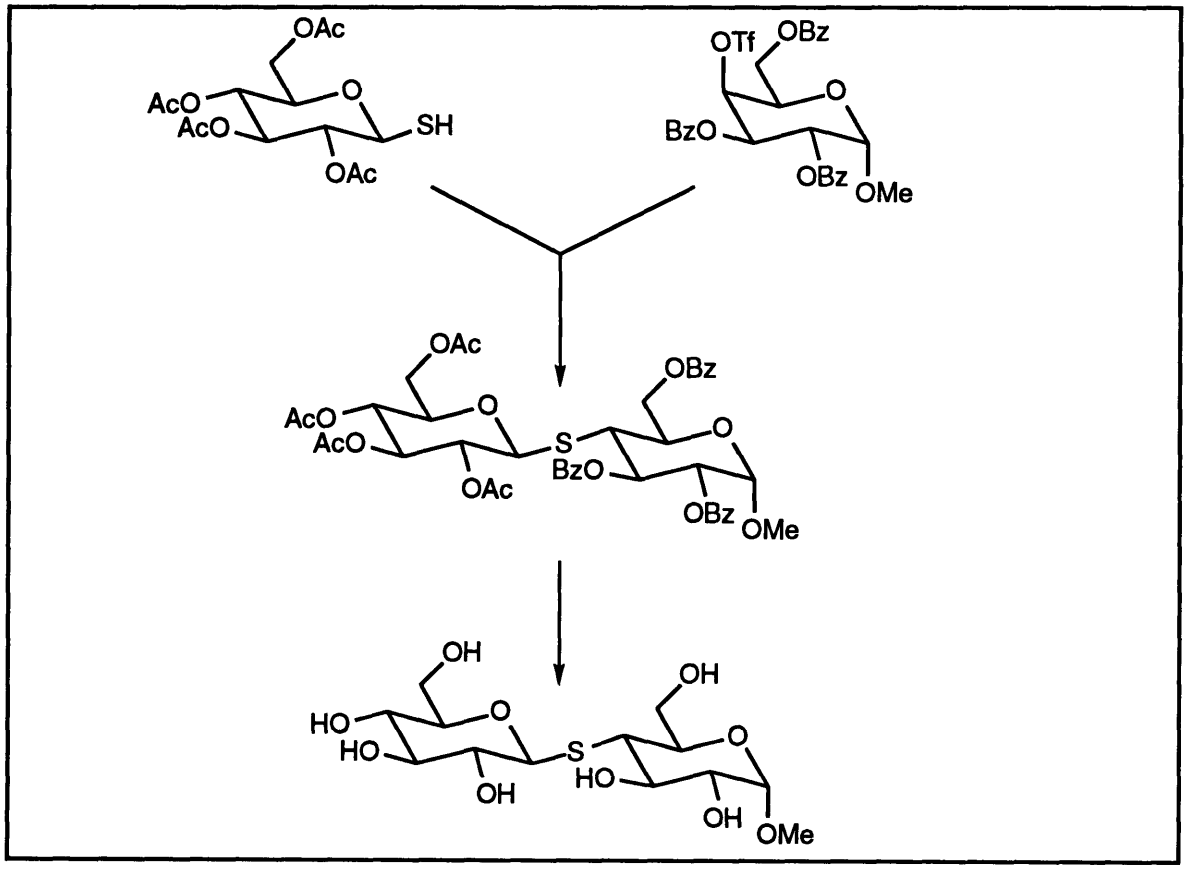
Scheme 4-3



Synthetic utility of the reaction. Intitially, we hoped to use this Michael reaction to produce heparin analogs. Unfortunately, we discovered two obstacles, detailed in the previous chapter, which rendered this approach virtually untenable. First, nucleophilic

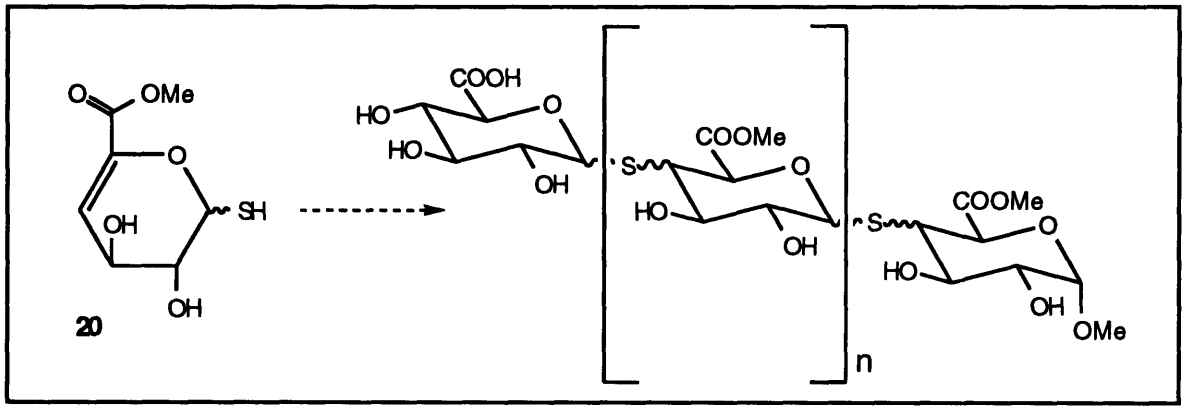
attack on **5** occurred primarily from the top face, giving rise to L-*altro* and D-*galacto* isomers, whereas the uronic acid residues in heparin are comprised of L-*ido* and D-*gluco* isomers. Second, 1-thioglycosides did not act as nucleophiles in this reaction. This failure necessitated an addition/deprotection/glycosidation cycle in order to make sulfur-linked oligosaccharides. While such an approach was feasible, as evidenced by the synthesis of **17a**, it was not ideal. Deprotection was facile; however, coupling yields to glycosyl donors have not been optimized. In the absence of highly successful optimization of the glycosidation chemistry, this method is not an efficient route to *de novo* synthesis of S-linked oligosaccharides. The standard synthetic methodology (see Scheme 4-4)[1,4], involving a nucleophilic substitution reaction between a 1-thioglycoside and an activated leaving group, is generally preferable.

Scheme 4-4. Conventional route to thiodisaccharide synthesis[4]



Although we possessed a great deal of information about this Michael addition, we lacked a desirable application. Provided with a means of stereochemical control (using a nucleophile with a hydrogen bond donor) and two on/off switches (ester saponification or blocking of the 2- and 3-hydroxyl groups), we believed this reaction could have some utility in combinatorial library synthesis. As described in Chapter 2, our original intent was to synthesize a library of thio-heparinoids. At this point, we broadened our focus to include libraries that were not heparin mimetics. One possibility involved a controlled polymerization of **20** (see Scheme 4-5), thereby producing a oligomeric library resembling pectin. Unfortunately, **20** can not be polymerized using a Michael addition because the nucleophilic moiety is a 1-thioglycoside. As described in Chapter 3, thioglycosides are not sufficiently activated nucleophiles to undergo Michael addition to an α,β-unsaturated pyranuronate ester.

Scheme 4-5

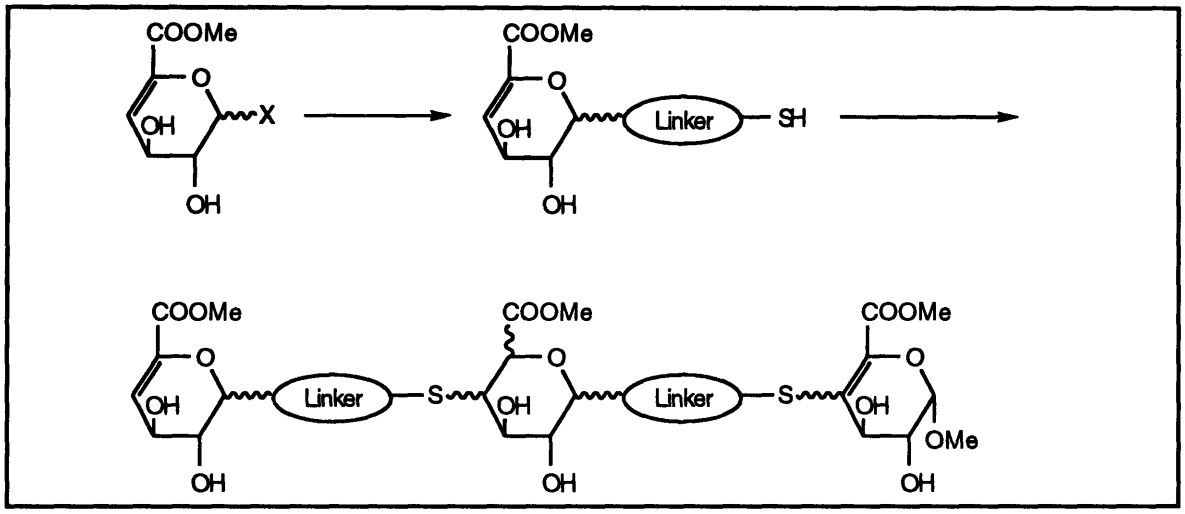


An Alternative Approach to Library Formation Using the Michael Addition

A change in strategy was required. We sought a glycosidic linker that could successfully undergo Michael addition to the α,β-unsaturated pyranuronate ester, while simultaneously providing a spacer between pyranuronates, thereby producing a glycosaminoglycan mimetic (see Scheme 4-6). The linker must contain a primary or secondary thiol with uncompromised nucleophilicity (no additional heteroatoms attached to

the sulfur-bearing carbon). Ideally, the linker would resemble a thiosugar, with two heteroatoms separated by four carbons. Several potential linkers are shown in Figure 4-1.

Scheme 4-6



We wanted to demonstrate the feasibility and potential utility of this system by assembling a library using linkers based on dithiothreitol (see Figure 4-1) and its isomers. After synthesis of the uronic ester/linker conjugate (*pseudodisaccharide*), stepwise polymerization could be performed (see Scheme 4-7) using a coupling/deprotection scheme in which monomeric units are attached sequentially to the end of a growing chain. The monomers must be incapable of self-condensation; modifications after each coupling are needed to permit chain elongation. In peptide synthesis, this is accomplished by deprotection of the amino terminus. In our system, the easiest route to stepwise polymerization entailed coupling of a saponified pseudodisaccharide, followed by a "deprotection" step in which the α,β-unsaturated acid was esterified.

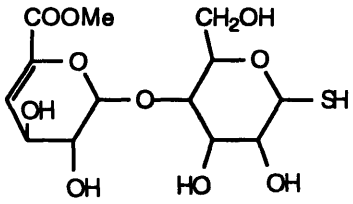
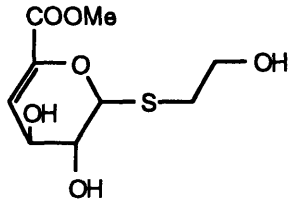
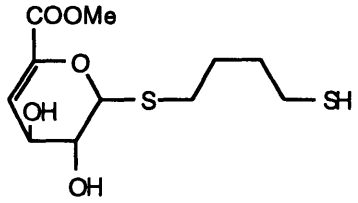
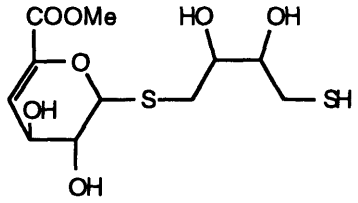
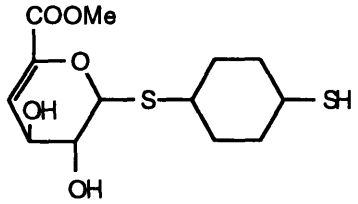
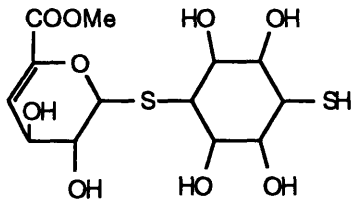
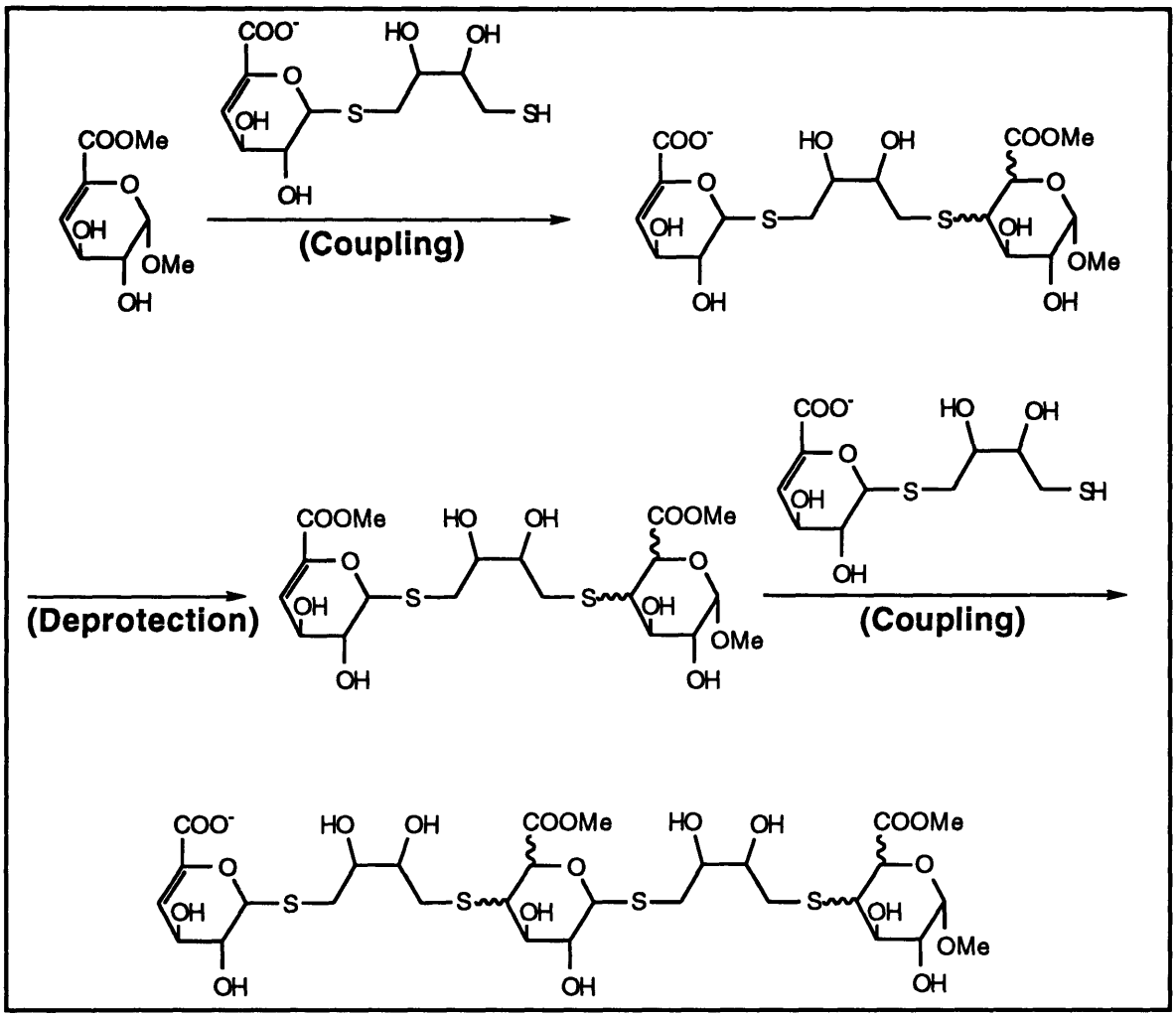
Linker	Pseudodisaccharide	Comments
1-Thiosugar		Ideal mimic, but will not polymerize
BME		Linker is too short
Butanedithiol		Good spacing as sugar mimetic, but is not rigid and is insufficiently functionalized (lacks hydroxyls)
Dithiothreitol		Good spacing, properly functionalized, but not rigid
Cyclohexane-dithiol		Good spacing, rigidity, but insufficiently functionalized
Dithioinositol		Good spacing, rigidity, functionality; linker not readily available

Figure 4-1. Potential linkers, along with their advantages and disadvantages.

Scheme 4-7. Schematic approach to stepwise polymerization of pseudodisaccharides



There are several positions in each pseudodisaccharide which permit introduction of structural diversity (see Figure 4-2). First, the pyranuronate can be derived from mannose instead of galactose, thereby changing the stereochemistry at C-2 of the sugar. Second, glycosidation with the linking agent can furnish either the α or β isomer. Third, the stereochemistry of the linker can be changed. Instead of using dithiothreitol (DTT), stereoisomers such as dithioerythritol could be chosen. Obviously, the linkers are not limited to isomers of DTT. Finally, the stereoselectivity of the Michael addition can be altered. This permits an additional element of diversity to be introduced in the coupling step. As described in Chapter 3, the Michael addition produces four isomers, with a

preference for the D-*galacto* isomer. However, DTT is analogous to BME in that a potential intramolecular hydrogen bond donor is available, and thus it is possible to shift the stereoselectivity to favor the L-*altro* isomer. If the hydroxyl is blocked with a protecting group, the conventional stereoselectivity is observed.

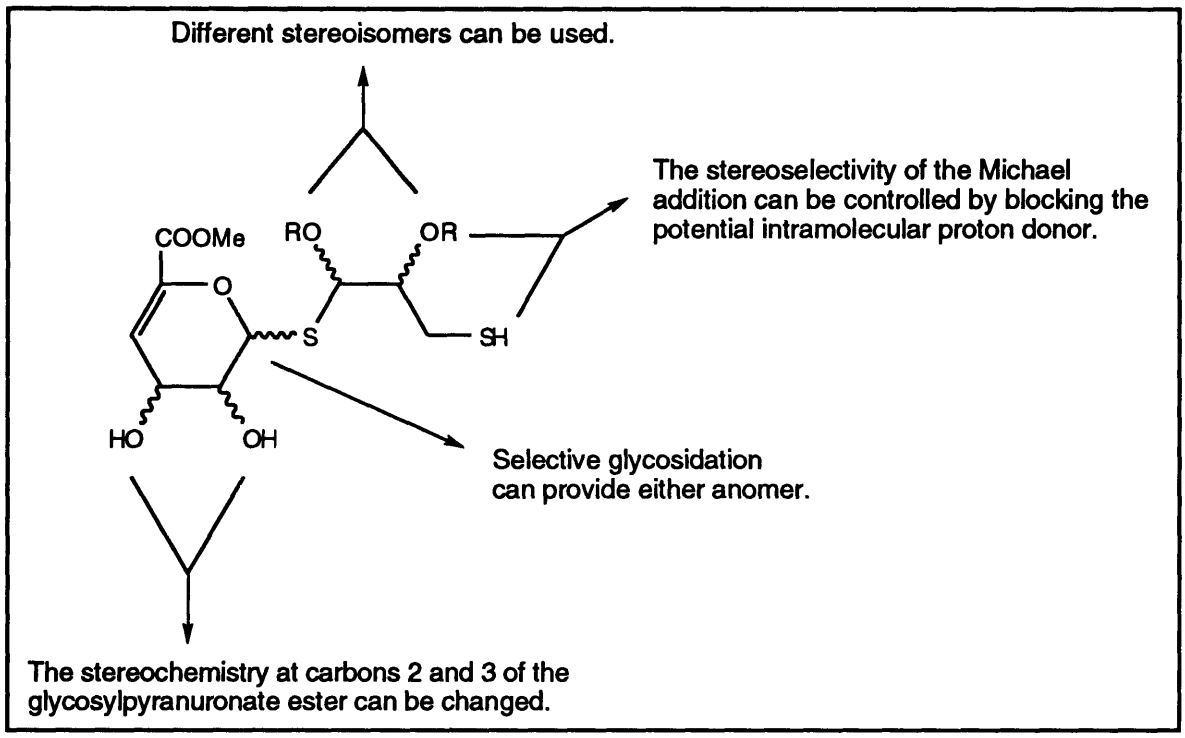


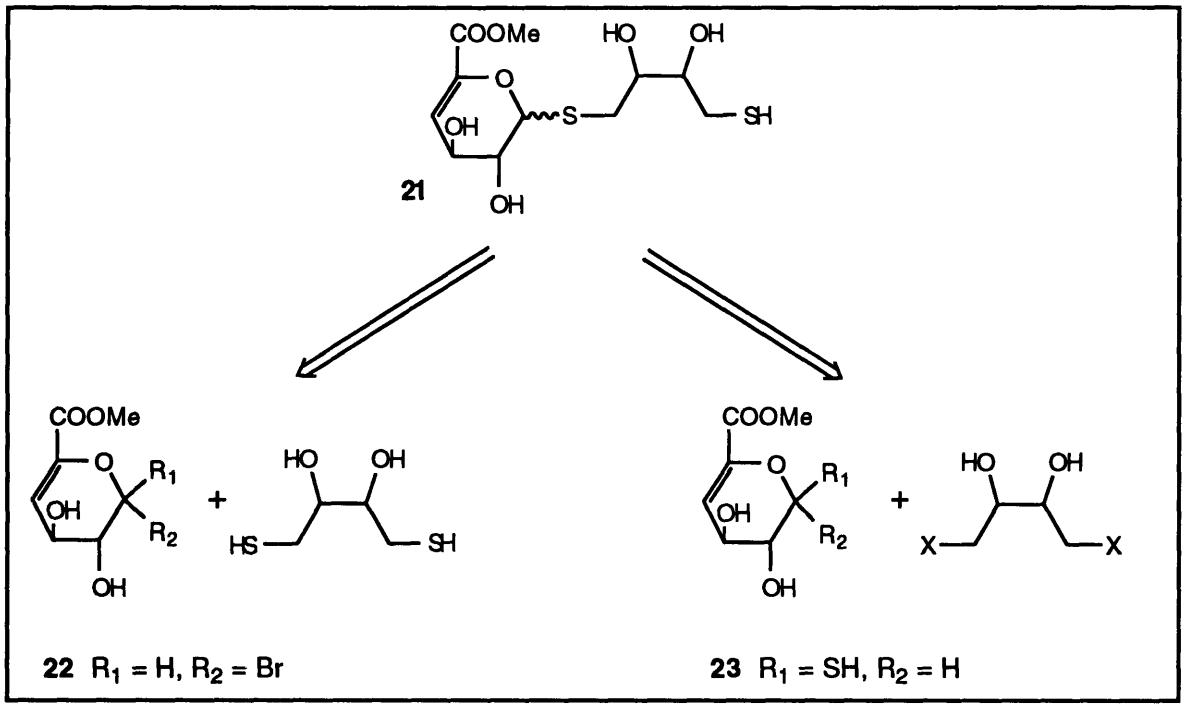
Figure 4-2. Structural diversity can be introduced at several locations in each pseudodisaccharide.

In summary, there are three sites per pseudodisaccharide at which heterogeneity can be introduced, in addition to the multiple stereochemical outcomes of the coupling reaction. Thus, there are four sources of diversity in each pseudodisaccharide linkage. If there were only two choices per site, then the number of different outcomes would be $2^4 = 16$. However, some sites permit more than two options, particularly the choice of linker. Consequently, this seemingly simple system can give rise to a large number of compounds without the need to perform many couplings.

There are two feasible retrosynthetic approaches to the pseudodisaccharide target **21** (see Scheme 4-8). In accordance with literature procedures, the glycosyl bromide **22**[5]

was synthesized in three steps from galacturonic acid, and was easily converted to the thioglycoside **23** upon treatment with potassium thiolacetate and subsequent methanolysis. Our long-term goal was a stepwise polymerization of **21**, but initially we wanted to examine the feasibility of the Michael addition of a pseudodisaccharide to a pyranuronate ester. In order to facilitate this process, we simplified the reactants, using **5** as the Michael acceptor and replacing the unsaturated uronate moiety in the pseudodisaccharide nucleophile with a simple glucose core.

Scheme 4-8. Retrosynthetic approaches to the pseudodisaccharide target **21**



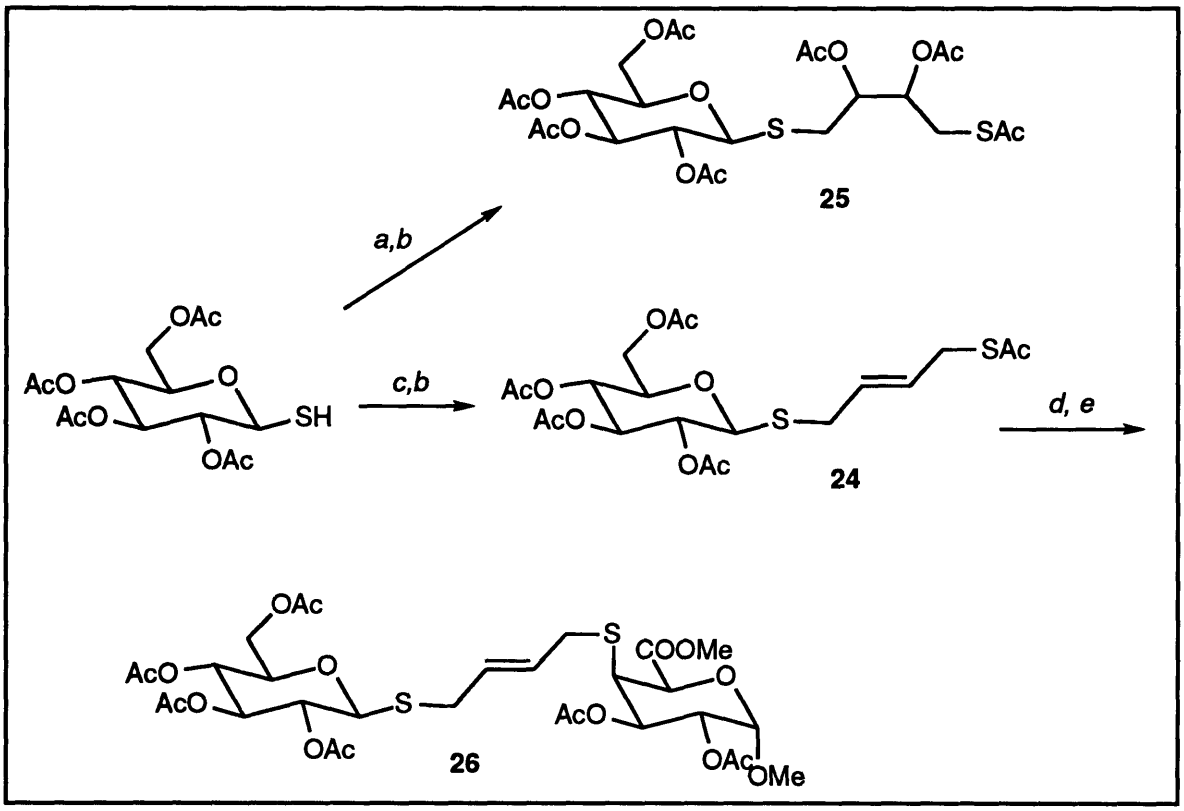
22 R_1 = H, R_2 = Br

23 R_1 = SH, R_2 = H

In accordance with Scheme 4-9, **24** (67%) and **25** (26%) were prepared in two steps as Michael donors. Surprisingly, Michael addition of either **24** or **25** to **5** proceeded very slowly and in low yields (10% or less), irrespective of the permutations in reaction conditions that were examined (solvent, stoichiometry, base, heat). Unlike reactions with smaller nucleophiles described in Chapter 3, which in some cases went nearly to completion in a day at room temperature, Michael addition of **24** to **5** afforded less than

10% of the desired product, **26**, in 24 hours (10% purified yield after 96 hours). Although disulfide formation was the major product, it was not the limiting factor. The potential combinatorial applications of the Michael addition were limited by the slow reaction rate, and we were unable to find conditions that hastened the rate and improved yields.

Scheme 4-9[a]



[a]Reagents: (a) 1,4-dibromo-2,3-butanediol, NaH, THF; (b) AcSH; (c) 1,4-dibromo-2-butene, NaH, THF; (d) LiOMe, MeOH, 5; (e) Ac_2O, pyridine.

Combinatorial chemistry places a great emphasis on reactions that proceed in high yields, since purification after each coupling is not possible (see Table 4-1). Assume an average reaction goes in 60% yield, and one wishes to perform five couplings, with no intermediate purification. The resulting purity of the desired compound would be less than 10%. The requisite purity of the components in a combinatorial library is dependent on the biological screen, so there is not a defined minimum standard, but 10% is certainly

insufficient. Experimentally, the propagation of impurities may not be geometric, and therefore it is very important to maintain reaction efficiencies in excess of 90%, preferably well in excess of 95%.

Average Yield (%)	Number of Couplings	Product Purity (%)
40	2	16
40	5	1
60	2	36
60	5	8
90	2	81
90	5	59
99	2	98
99	5	95

Table 4-1. Product purity as a function of the number of coupling and average yield.

Michael addition of pseudodisaccharide conjugates proceeded in only 10% yield, and consequently had limited utility in combinatorial synthesis. One possible solution is to enhance the electrophilicity of the Michael acceptor by removing the pyranose ring oxygen. This is an appealing approach, but the synthetic investment required to make the appropriate carbasugars would be substantial[6]. Before making that investment, we tried to identify the characteristics of an "ideal" combinatorial library, with the implicit recognition that synthesis of an ideal library might not utilize the Michael addition chemistry.

Characteristics of a Highly Desirable Combinatorial Library

The ideal combinatorial library blankets all three-dimensional space with all functional groups. Obviously, this library will never be developed, but a highly desirable combinatorial library must be both spacially and functionally diverse. Assuming that the

library is synthesized by ligation of building block units, the building blocks should be fairly rigid, since most pharmaceutical entities have significant conformational restraints. The components of the library should be produced in relatively pure form. Coupling reactions should be highly efficient, proceeding in yields greater than 95%. Although solid phase synthesis is not required, it is the best method to obtain the requisite yields. Finally, the components of the library should be potential drug entities, not just lead compounds. Each member of the library should potentially possess the pharmacokinetic characteristics of a viable pharmaceutical, although this does not mean that a drug candidate must be culled from the initial combinatorial library. Ideally, the library components would be amenable to iterative combinatorial optimization.

One can envision many different combinatorial libraries or building blocks that appear to match the characteristics described above. Carbohydrates are one potentially desirable set of building blocks. They contain a much higher level of diversity per unit mass than other polymers. While the diversity of peptides is governed completely by the linear sequence of amino acids, monosaccharides can be be combined in linear or branched arrays. Furthermore, several distinct positions in each monosaccharide unit can be used to form the linkage, which can have either of two possible (α or β) stereochemistries. Whereas two identical amino acids or nucleotides can be joined together to produce only one compound, two identical hexapyranose monosaccharides can be linked to produce 11 different disaccharides[7]. Carbohydrates are spatially diverse molecules, with hydroxyl groups arrayed in many different planes in space. Furthermore, the three-dimensional orientation of any given hydroxyl can be altered by starting with a different monosaccharide. Carbohydrates are highly functionalized molecules. Any hydroxyl group in a sugar can be isolated, thereby facilitating selective functional group manipulations. Carbohydrates are structurally rigid, cyclic molecules. In particular, hexapyranoses are usually constrained to one of two possible chair conformations[8]. In addition, carbohydrates are abundant and inexpensive raw materials. Finally, in the form of heparin,

there is precedent for a natural carbohydrate library in which seemingly small structural perturbations in a repeating skeletal core give rise to a broad range of biological activities.

Carbohydrates possess many positive aspects as building blocks, but synthetic ligation of the monomeric units is problematic. Carbohydrate synthesis is notoriously difficult, and is plagued by low yields and a lack of generality[9,10]. In spite of tremendous recent advances using chemical[11-14] and enzymatic techniques[15-17], solid phase carbohydrate synthesis has not become routine. Yields, while adequate[13,14,16], are not in the same range as solid phase peptide or nucleic acid synthesis. Furthermore, the number of building blocks and available linkage stereochemistries remains limited. Although it is possible to synthesize carbohydrate libraries, the low yields and diminished diversity (relative to theoretical carbohydrate synthesis) render it a discouraging prospect.

Even if the synthetic difficulties could be overcome, an oligosaccharide library still might not be highly desirable. The bioavailability of oligosaccharides is suspect[7]. Circulating glycosidases can cleave glycosidic linkages. Even if they survive the chemoenzymatic assault, carbohydrate molecules may not be able to penetrate cell barriers. These are legitimate concerns, although in fairness, it should be mentioned that carbohydrate drugs are more likely to have extracellular targets[7,18-20]. Furthermore, functional group modifications, in particular hydroxyl protection, could increase the permeability to cell membranes by making the molecules more lipophilic[7].

A superior approach to carbohydrate-based libraries may involve the synthesis of unnatural linkages between carbohydrate units[21]. Our attempt to ligate pseudodisaccharides with a Michael addition falls under this classification. The pseudodisaccharides represented a potentially desirable set of building blocks, but the coupling reaction was inadequate. We hoped to develop a system with equally desirable building blocks, but superior coupling efficiencies.

References for Chapter 4

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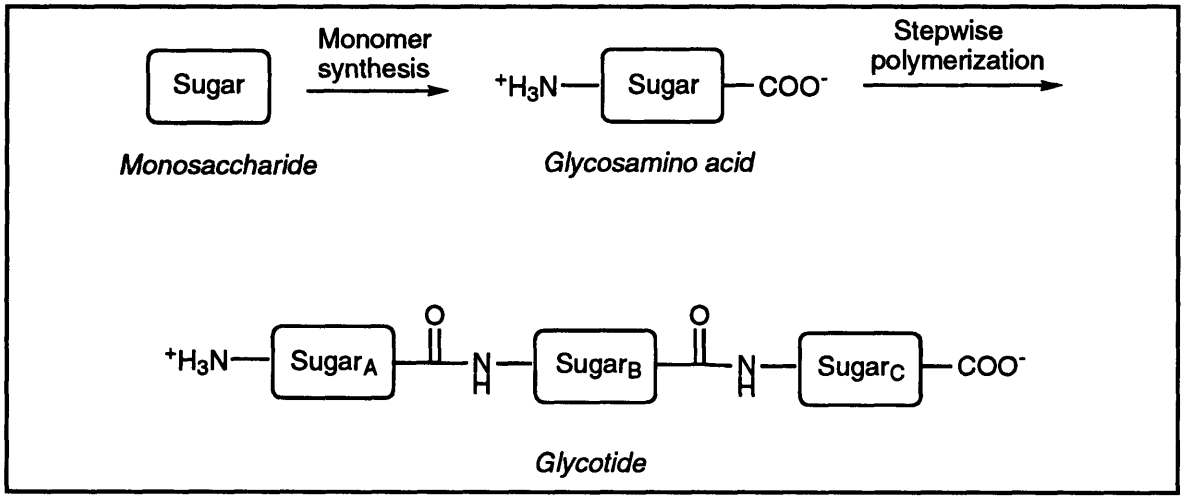
Chapter 5

Introduction to Glycotides

The quest for a better carbohydrate-based combinatorial library led to the concept of *glycotides*, which are composed of *glycosamino acids* linked with amide bonds (see Scheme 5-1). Glycosamino acids are monosaccharides that have been functionalized with a carboxylic acid and an amine. We wanted to combine the advantages of carbohydrate-based building blocks with the high coupling efficiencies of peptide synthesis. Ideally, a large number of monomers would be synthesized and then combined in extensive permutations to give combinatorial libraries with substantial structural and chemical diversity. Compound libraries could be screened for a wide variety of biological activities, and active components would be refined using iterative combinatorial optimization.

Scheme 5-1.



Research aimed at producing pharmaceutically important combinatorial libraries of glycotides can be divided into three parts: monomer synthesis, oligomer synthesis, and compound testing. Initially, we focused on monomer synthesis. The long-term success of the project depends on the variety and quality of glycosamino acid synthesis. Given limited

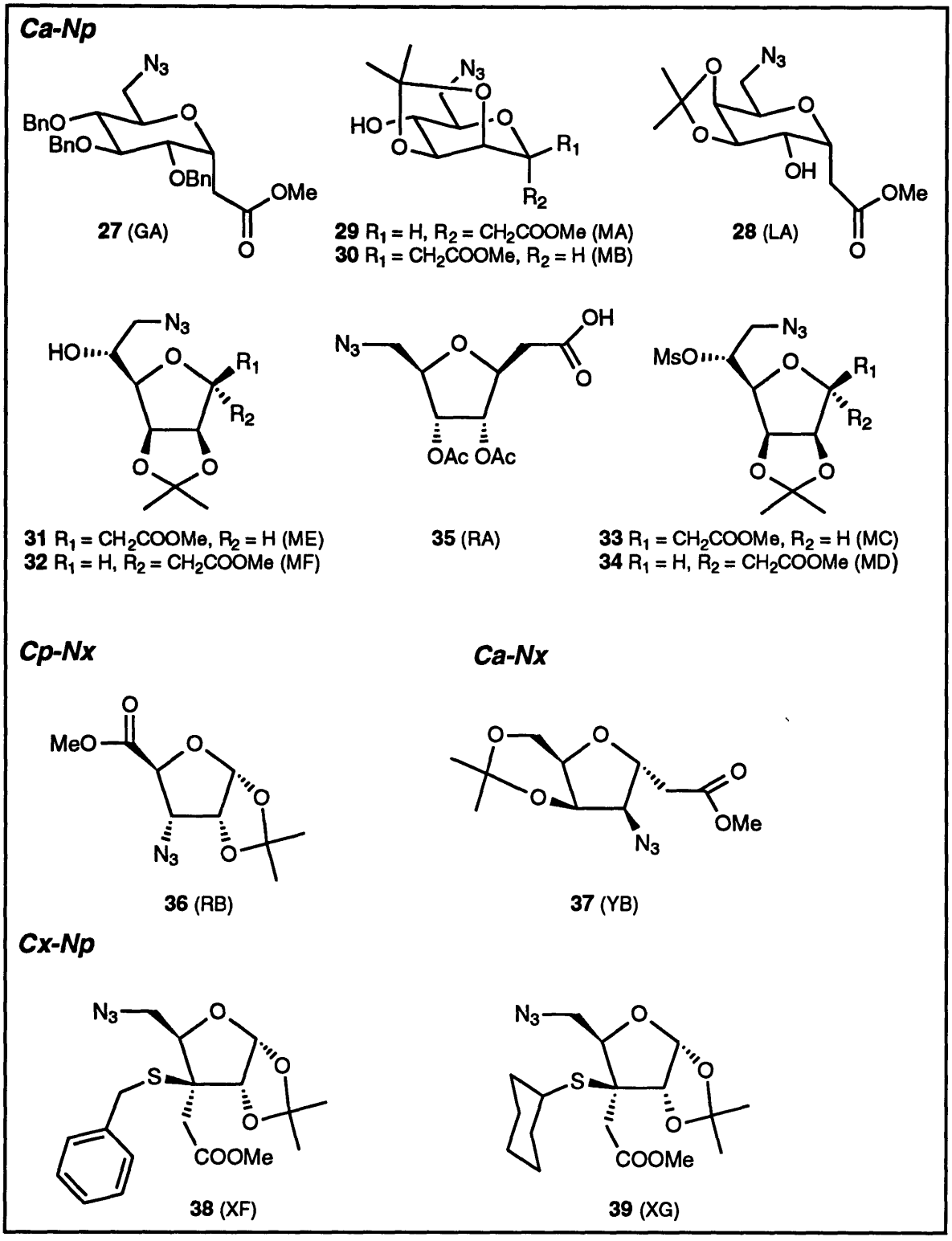


Figure 5-1. Glycosazido ester and acid precursors to glycosamino acid monomers.

time, our intention was to synthesize several different glycosamino acids to demonstrate the enormous potential diversity of the monomeric units. The next step was to synthesize defined oligomers and oligomer libraries, which will be described in Chapter 6. We have not reached the biological testing stage.

Monomer Synthesis: An Overview

We have synthesized the glycosamino acids shown in Figure 5-1. They have been given two-letter code names to facilitate nomenclature. The synthetic routes to most of these glycosamino acids have proceeded through an intermediate containing an azide and an ester as latent sources of the amine and acid. Unless the monomer was used subsequently to produce glycotide polymers, synthesis did not proceed beyond the *glycosazido ester*. Synthesis was halted at this stage for three reasons. First, glycosazido esters are very stable, and serve as convenient intermediates for storage. Second, they provide flexibility in choosing a method of oligomer synthesis. Third, we felt that in normal cases, synthesis of the azido ester denoted a *de facto* synthesis of the glycosamino acid, since azide and ester deprotection are facile. For the purposes of this thesis, the term *glycosamino acid* is used to refer not only to modified monosaccharides containing both an amine and an acid, but also as a generic term referring to monosaccharides possessing amines and acids in protected forms. Hence, the term glycosamino acid encompasses several different but closely related compounds, and azido esters, like azido acids, N-Boc protected acids, or amino esters, are subsets of glycosamino acids.

Glycosamino acids can be separated into nine classes based on the locations of the carboxyl *(C)* and amino *(N)* functionalities on the monosaccharide core. The positions of the amine and carboxyl groups can be classified according to the carbon from which they originated: the anomeric carbon (C1 in a hexose or pentose sugar), the carbon to which the primary hydroxyl is bound (C5 in a pentose, C6 in a hexose), or any other carbon (C2, C3, C4, and C5 in a hexose), represented by *a*, *p*, and *x*, respectively. This affords a 3 x 3

matrix of nine possibilities. We have synthesized glycosamino acids from four *(Ca-Np, Ca-Nx, Cp-Nx, and Cx-Np)* of the nine possible classes. Syntheses of glycosamino acids from each of the other classes have been reported in the literature (for representative examples, see Figure 5-2) for use in natural product synthesis[1,2], polymer chemistry[3,4], and as enzyme inhibitors[5] and peptidomimetics[6]. There are also naturally occurring glycosamino acids, the most important of which are the sialic acids.

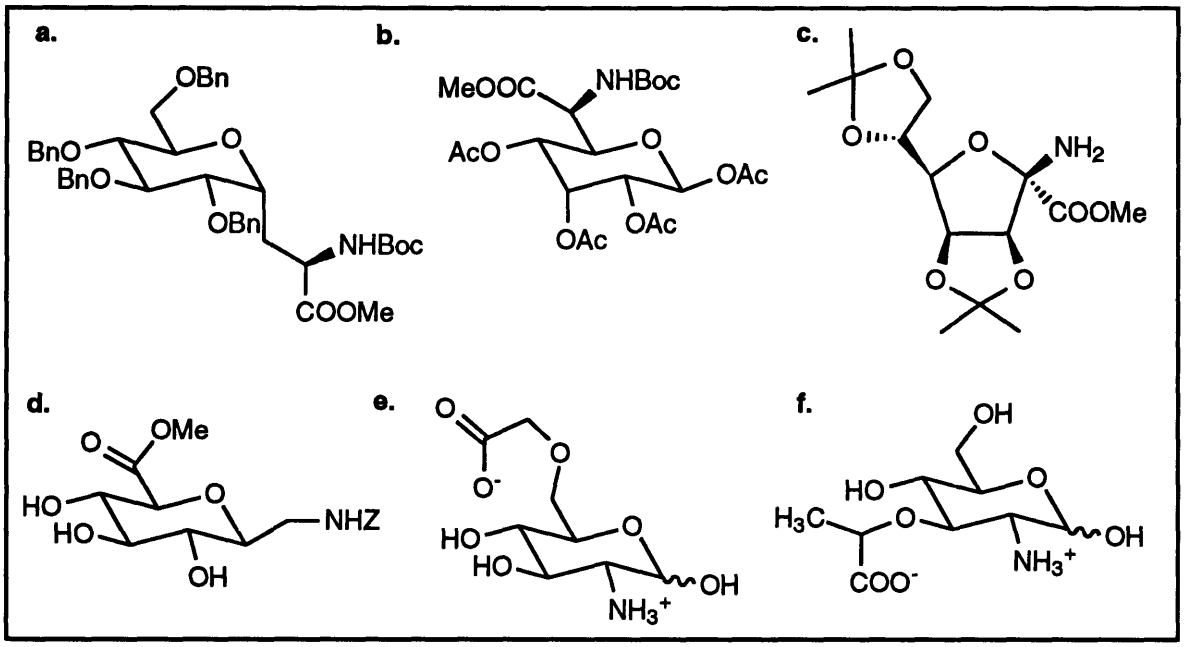


Figure 5-2. Glycosamino acids of natural and synthetic origins (noted by primary author). (a) Gurjar *et al.*[5] (b) Casiraghi *et al.*[1] (c) Dondoni *et al.*[2] (d) Graf von Roedern and Kessler[6] (e) Tokura *et al.*[4] (f) Muramic acid

The number of monomers that can be synthesized is virtually unlimited. Changes in the protecting groups, stereochemistry, and spacing can generate immense structural diversity in any single class of monomers. Exploring several classes of monomers further increases the potential diversity of the system.

There are a number of feasible approaches to glycosamino acid synthesis. One can envision several different methods to introduce the carboxyl and amino functionalities, including (carboxyl) oxidative cleavage of an alkene, oxidation of a primary hydroxyl,

reduction of a nitrile, Wittig reactions, etc., and (amino) reduction of an azide, imine, oxime, nitrile, or nitro group. In practice, we used several techniques to introduce carboxyl groups, while only one method, displacement with an azide nucleophile followed by reduction, was used to generate the amine.

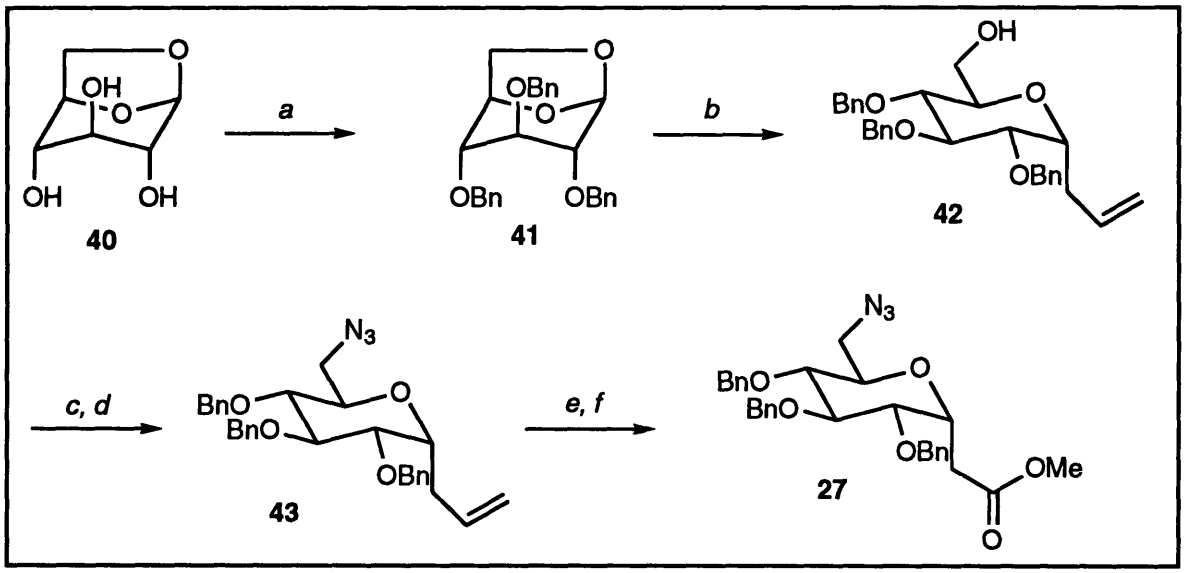
In order to simplify our libraries and facilitate characterization, we desired monomers that were stereochemically pure. It could be argued that rapid generation of chemical diversity would be obtained by starting with isomeric mixtures of monomers. For instance, spatially addressable libraries could be synthesized using anomeric mixtures at each discrete site. Additional compounds would be generated, although the difficulty of characterization would be increased. From the perspective of a pharmaceutical company, this tradeoff probably would be acceptable. Our primary purpose, however, was not to develop drugs, but instead to demonstrate the potential merit of glycotides in combinatorial chemistry. In other words, we wanted to communicate a "proof of concept", which was accomplished more easily using stereochemically pure components. Any synthesis that produced inseparable mixtures (generally at the anomeric carbon) was either terminated or continued with the hope of resolving the individual components in a later step.

We placed a higher priority on developing synthetic routes to new glycosamino acids than optimizing syntheses of existing monomers. Consequently, the yields and synthetic steps reported in this chapter are not optimized. Instead, they represent the results of a single experiment or the best performance from a small number of trials. Only one series of synthetic routes (glycosamino acids MC, MD, ME, and MF) has been systematically optimized. Unlike traditional natural product synthesis, in which exhaustive attempts are made to synthesize a defined target, no glycosamino acid synthesis was *vital*; modification or abrogation of a target were necessary alternatives when an adequate synthesis could not be completed in a timely fashion.

Synthesis of Glycosamino Acids from Hexose Sugars

Synthesis of GA (**27**). The glycosamino acid GA was prepared in seven steps as described in Scheme 5-2. Benzylation of 1,6-anhydro-β-D-glucose **40** provided **41** in 70% yield. The allyl C-glycoside **42** was produced in 38% yield by treating **41** with allyl TMS and TMSOTf[7] in acetonitrile, a slight modification of the method reported by Kishi and coworkers[8]. The reaction was highly stereoselective, affording a ratio of α to β anomers greater than 10:1. The C-glycosidation served a dual purpose by opening the acetal to reveal the unprotected 6-hydroxyl. Mesylation of the primary alcohol, followed by treatment with sodium azide, produced **43** (83%). The final steps involved oxidative cleavage of the allyl glycoside with potassium permanganate under phase transfer conditions[9], followed by *in situ* esterification with methyl iodide to give the azido ester **27** in 50% yield.

Scheme 5-2[a]

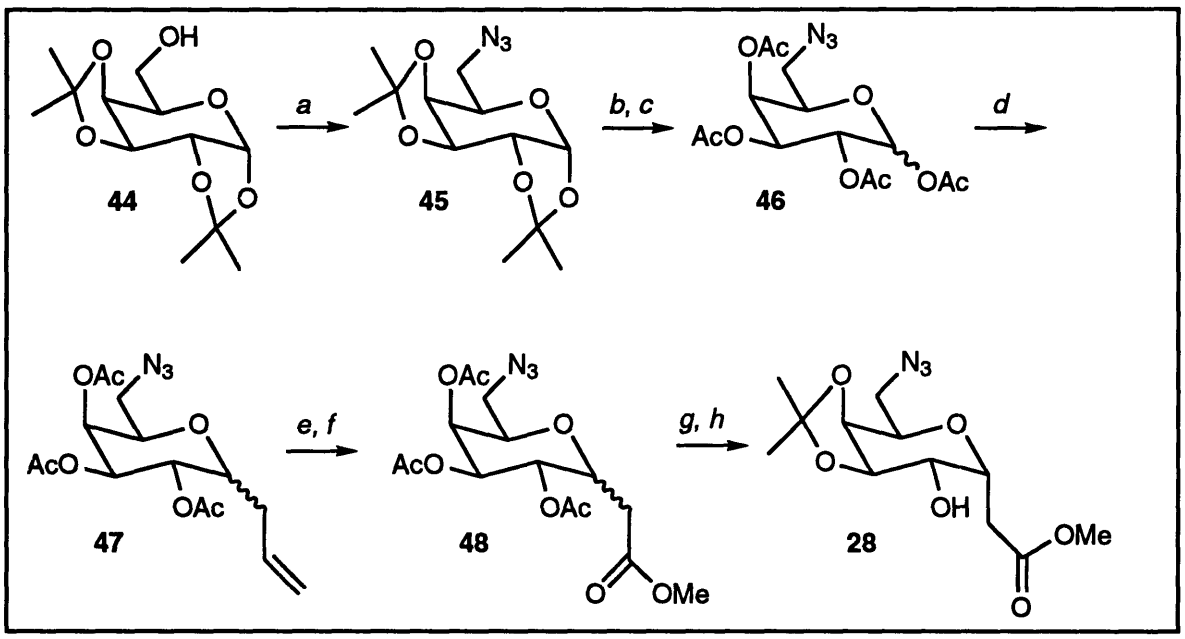


[a]Reagents: (a) BnBr, DMF; (b) allyl TMS, TMSOTf, CH_3CN; (c) MsCl, TEA, CH_2Cl_2; (d) NaN_3, DMF; (e) $KMnO_4$, Aliquat 336, H_2O, AcOH, CH_2Cl_2; (f) CH_3I, $NaHCO_3$, DMF.

Synthesis of LA (**28**). The glycosamino acid LA, derived from galactose, was synthesized in accordance with the route described in Scheme 5-3a. A Mitsunobu

reaction[10] using diphenylphosphoryl azide[11,12] as the nucleophile converted the diacetonide **44** into **45**[13] in 66% yield. Hydrolysis of the acetonides, followed by acetylation, provided **46** as a mixture of anomers (60%). Treatment with allyl TMS and BF_3-etherate afforded the allyl C-glycoside **47** as a 4:1 mixture of anomers (α/β) that could not be resolved by chromatographic methods (35%). Oxidative cleavage of the alkene using the $RuCl_3/NaIO_4$ protocol of Sharpless and coworkers[14], followed by esterification with methyl iodide, provided the azido ester **48** in 53% yield. Again, the anomers could not be resolved. Zemplen methanolysis of the acetyl groups, followed by formation of the 3,4-acetonide, provided the α anomer **28** in 59% yield. The small scale of the reaction precluded recovery of the β anomer.

Scheme 5-3a[a]

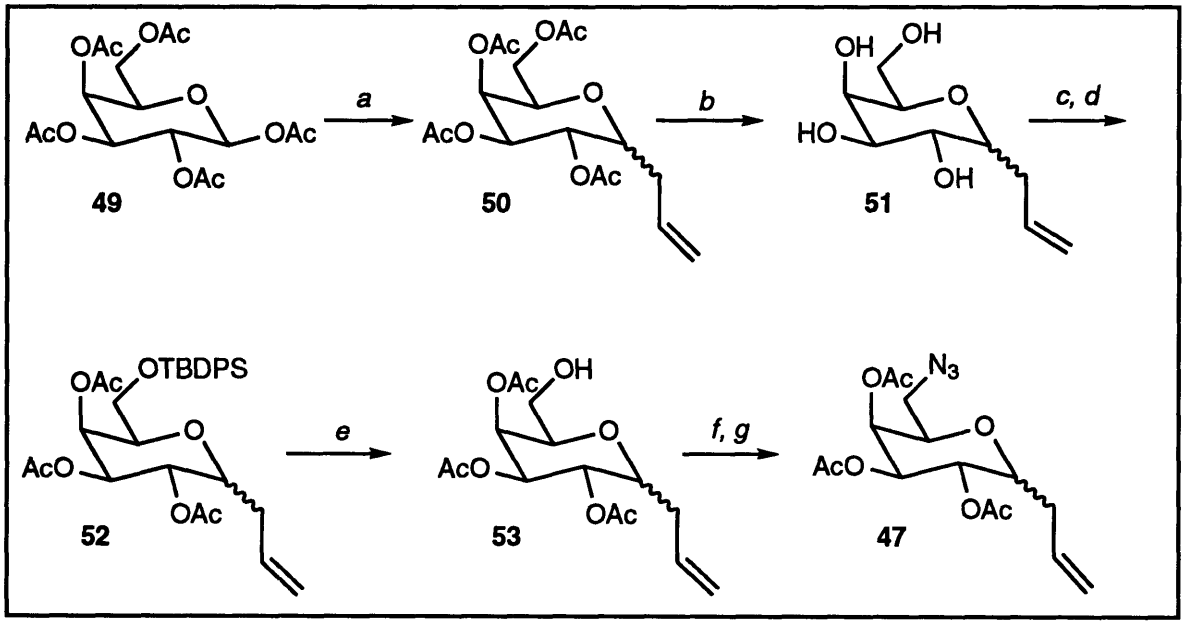


[a]Reagents: (a) DEAD, $(Ph)_2P(O)N_3$, PH_3P, THF; (b) AcOH, H_2O; (c) Ac_2O, pyridine; (d) allyl TMS, BF_3-etherate, CH_3CN; (e) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O; (f) CH_3I, $NaHCO_3$, DMF; (g) LiOMe, MeOH; (h) $(Me)_2C(OMe)_2$, PPTSA.

A superior approach to the synthesis of LA is described in Scheme 5-3b. The synthesis of intermediate **47** proceeded in 29% cumulative yield using this approach,

compared to 13% using the route described above (although optimization of the reactions in Scheme 5-3a, particularly the conversion of **46** to **47**, could increase the yield significantly). Upon treatment with allyl TMS and TMSOTf in acetonitrile, peracetylated galactose **49** was converted to the C-glycoside **50**[15] in 82% yield, giving an 8:1 (α/β) mixture of anomers (TMSOTf in acetonitrile provided a cleaner reaction than BF_3-etherate in acetonitrile or nitromethane). Ester saponification, followed by recrystallization, provided **51** in 79% yield. Treatment of **51** with TBDPSCl[16] in pyridine, followed by acetylation, afforded **52** in 87% yield. Removal of the silyl ether protecting group was accomplished with HF/pyridine, giving **53** in 93% yield. The primary alcohol was converted to the triflate[17], which was treated directly with sodium azide to afford the azido alkene **47** in 55% yield.

Scheme 5-3b[a]

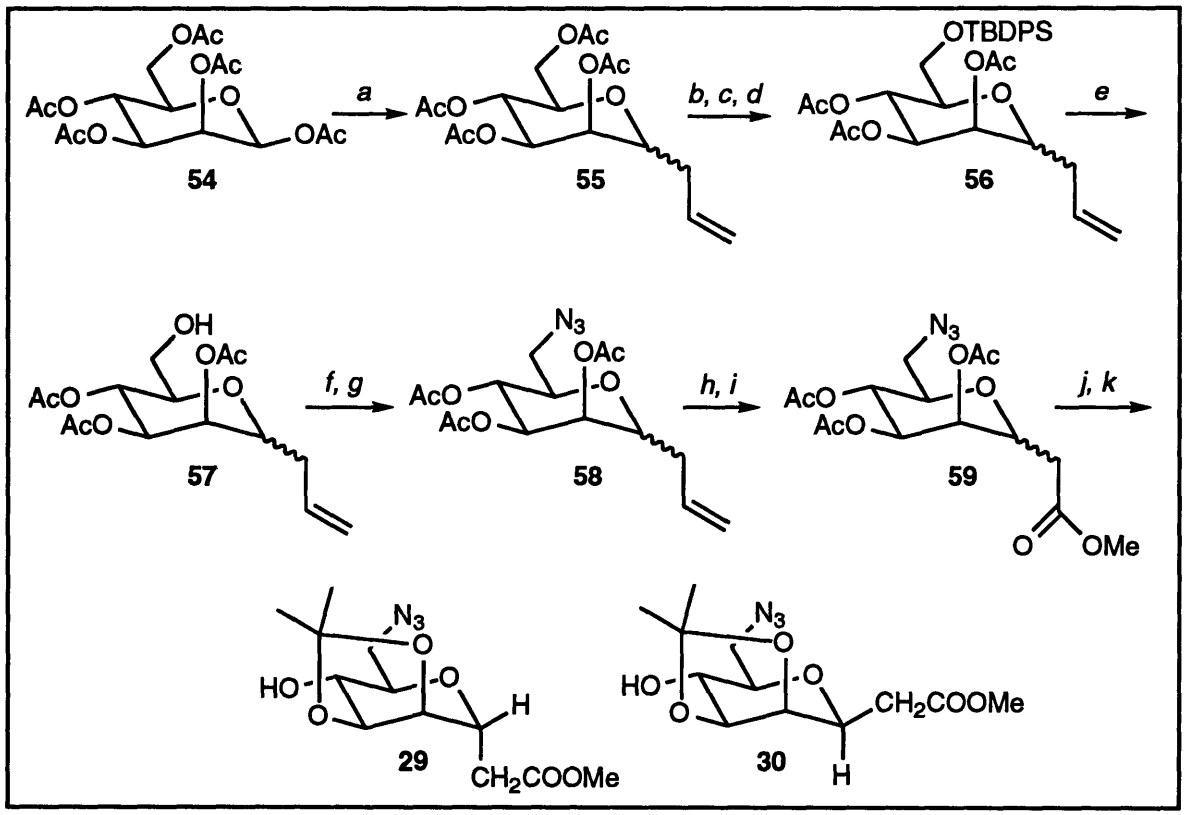


[a]Reagents: (a) allyl TMS, TMSOTf, CH_3CN; (b) LiOMe, MeOH; (c) TBDPSCl, pyridine; (d) Ac_2O, pyridine; (e) HF-pyridine; (f) Tf_2O, pyridine, CH_2Cl_2; (g) NaN_3, DMF.

Synthesis of MA (**29**) *and MB* (**30**). Synthesis of the glycosamino acids MA and MB (see Scheme 5-4) was similar to the second route used to produce LA (Scheme 5-3b).

Peracetylated mannose **54** was converted to the C-glycoside **55** upon treatment with allyl TMS and TMSOTf in acetonitrile. A 4:1 (α/β) mixture of anomers was obtained in only 39% yield. Methanolysis, followed by selective protection of the primary alcohol with TBDPSCl and subsequent peracetylation, afforded **56** (52%). The silyl ether was cleaved in HF/pyridine to give **57** in 84% yield. Mesylation of the liberated hydroxyl group, followed by treatment with sodium azide, provided the azido alkene **58** in 63% yield. Oxidative cleavage of the alkene using ruthenium tetraoxide catalysis, followed by *in situ* esterification with methyl iodide, afforded the azido ester **59** in 83% yield. Through this stage of the synthesis, the anomers could not be readily separated and purified. Zemplen

Scheme 5-4[a]



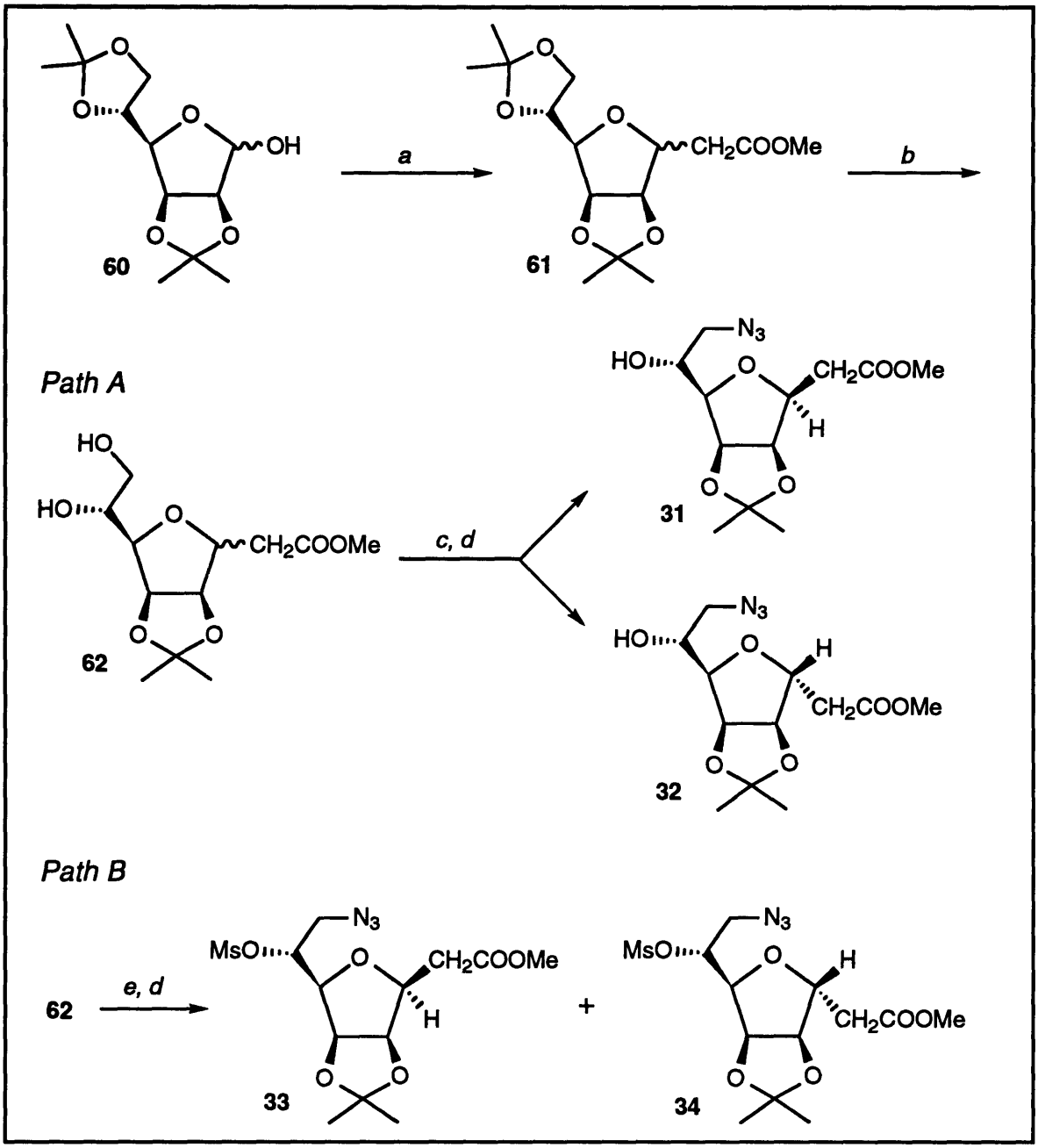
[a]Reagents: (a) allyl TMS, TMSOTf, CH_3CN; (b) LiOMe, MeOH; (c) TBDPSCl, pyridine; (d) Ac_2O, pyridine; (e) HF-pyridine; (f) MsCl, TEA, CH_2Cl_2; (g) NaN_3, DMF; (h) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O; (i) CH_3I, $NaHCO_3$, DMF; (j) LiOMe, MeOH; (k) $(Me)_2C(OMe)_2$, PPTSA.

methanolysis of the acetyls, followed by formation of the 2,3-acetonide, provided the α anomer **29** and the β anomer **30** in a cumulative yield of 85%. The anomers were readily separable on silica gel.

Synthesis of ME (**31**), *MF* (**32**), *MC* (**33**), *and MD* (**34**). These four glycosamino acids are actually two very similar sets of anomeric pairs, differing only by mesylation of the C-5 hydroxyl group. Our synthetic approach was not originally intended to produce such closely related products. We anticipated facile elimination of the mesylates **33** and **34** to afford allyl azido esters complimentary to the glycosamino acids ME and MF (see Scheme 5-5, path A). Instead, the hindered mesylates **33** and **34** (Scheme 5-5, path B) were extremely resistant to elimination. Given our goal of producing a diverse population of monomers, we felt that a monomer that contained a relatively exotic functional group such as a mesylate, if stable, was a desirable entity. Hence, the azido ester mesylates were accorded recognition as distinct glycosamino acids.

The first two synthetic manipulations were common to both sets of glycosamino acids. Diisopropylidene **60** was treated with methyl(triphenylphosphoranylidene)acetate in refluxing acetonitrile. A Wittig reaction on the hemiacetal, followed by an internal Michael addition, provided the C-glycoside **61**[18] as a mixture of anomers (approximately 1:1) in 90% yield. The anomers were barely separable on TLC. Chromatographic purification would have been arduous and, more importantly, would have decreased the efficiency of synthesis (described later in Chapter 5). We proceeded to the next step, hoping to separate the anomers at a later stage in the synthesis. Selective hydrolysis of the 5,6-acetonide with aqueous acetic acid at room temperature produced the diol **62** in 85% yield.

Scheme 5-5[a]



[a]Reagents: (a) $(Ph)_3P{=}CHCOOMe$, CH_3CN; (b) AcOH, H_2O; (c) MsCl, pyridine; (d) NaN_3, DMF; (e) excess MsCl, TEA, CH_2Cl_2.

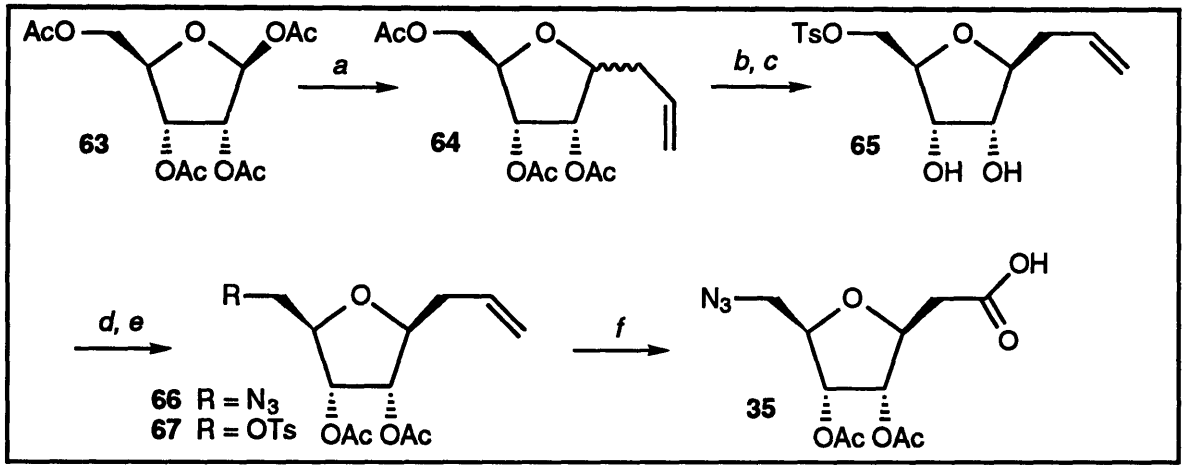
Mesylation of the diol **62** served as the branching point in the synthesis. Selective mesylation of the primary alcohol with 1.1 equivalents of mesyl chloride in pyridine (76%), followed by azide displacement (84%), provided the azido esters **31** and **32**, which

were separable on silica gel. It is important to remove dimesylate impurities before treatment with sodium azide: the azido mesylate esters (**33** and **34**) were difficult to separate from the azido hydroxy esters (**31** and **32**). The dimesylate of **62** was obtained by treating the diol with excess mesyl chloride and triethylamine in dichloromethane. The intermediate was isolated and used directly without further purification. Treatment with sodium azide in DMF provided the azido mesylate esters **33** and **34** (separable on silica gel) in 66% yield from **62**.

Synthesis of Glycosamino Acids from Pentose Sugars

Synthesis of RA (**35**). Synthesis of the glycosamino acid RA proceeded in six steps as described in Scheme 5-6. Peracetylated ribose **63** was converted to the C-glycoside **64** upon treatment with allyl TMS and TMSOTf in acetonitrile. A 3:1 (β/α) ratio of anomers, separable by flash chromatography, was obtained in 87% yield. Deacetylation of the β-anomer, followed by selective tosylation of the primary alcohol, afforded **65** in 53% yield. Displacement of the tosylate with sodium azide and catalytic tetrabutylammonium iodide in DMF at 125°C proceeded very slowly. In order to expedite the glycosamino acid synthesis, this reaction was terminated before reaching completion. Direct acetylation of the mixture of azide and unreacted tosylate gave the desired azide **66** (29%), as well as the tosylate **67**. The yield based on recovery of acetylated starting material was 68%. The carboxylate functionality was introduced by ruthenium tetraoxide catalyzed oxidation of **66**, providing the glycosazido acid **35** in 93% yield. Unlike other glycosazido acids produced by oxidative cleavage of an allyl C-glycoside, **35** was easily purified to homogeneity, and did not require esterification.

Scheme 5-6[a]

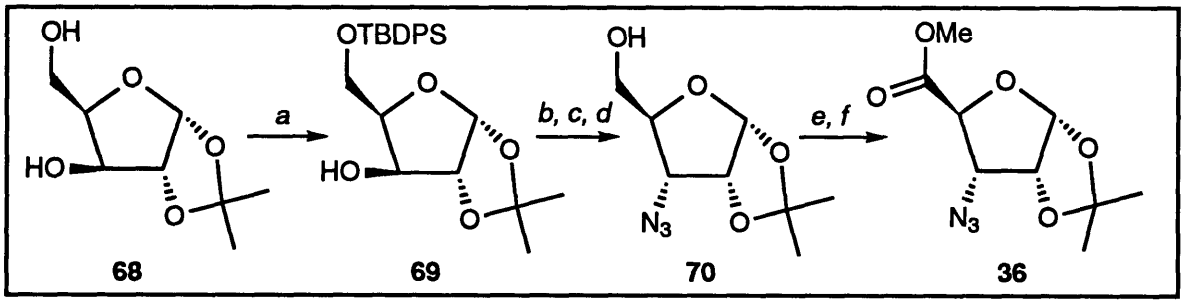


[a]Reagents: (a) allyl TMS, TMSOTf, CH_3CN; (b) LiOMe, MeOH; (c) TsCl, pyridine; (d) NaN_3, DMF; (e) Ac_2O, TEA, CH_2Cl_2; (f) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O.

Synthesis of RB (**36**)

Synthesis of the *Cp-Nx* glycosamino acid RB, described in Scheme 5-7, proceeded in very high yield (67% overall) until the final step (10%). Selective silylation of the primary alcohol of the 1,2-acetonide **68** was accomplished by treatment with TBDPSCl in pyridine, affording **69** in 98% yield. The secondary alcohol was converted to the triflate with triflic anhydride and pyridine in dichloromethane, and the triflate was directly displaced with sodium azide. Cleavage of the silyl ether with TBAF in THF provided the azido alcohol **70** in 69% yield from **69**.

Scheme 5-7[a]

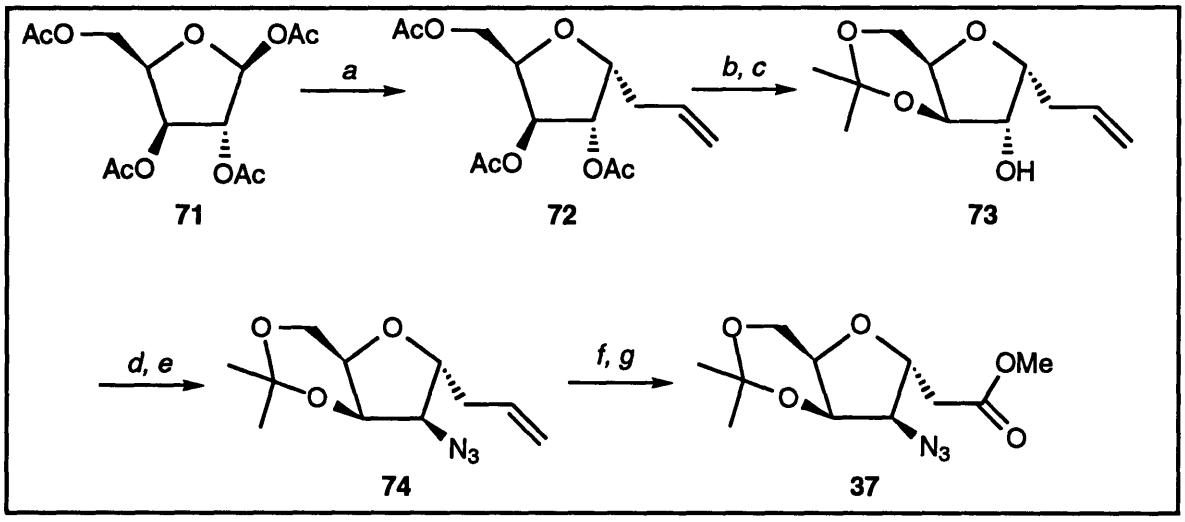


[a]Reagents: (a) TBDPSCl, pyridine; (b) Tf_2O, pyridine, CH_2Cl_2; (c) NaN_3, DMF; (d) TBAF, THF; (e) $KMnO_4$, Aliquat 336, H_2O, AcOH, CH_2Cl_2; (f) CH_3I, $NaHCO_3$, DMF.

Oxidation of the primary alcohol **70** to the carboxylic acid was problematic. Treatment of **70** with potassium permanganate under phase transfer conditions, followed by esterification with methyl iodide, afforded the azido ester **36** in only 10% yield. Nevertheless, this method was superior to other one-step ($RuCl_3/NaIO_4$, PDC[19,20]) or two-step (TPAP[21,22], then sodium chlorite[23]) oxidations that were attempted. Obviously, many other reagents could be tried in order to produce a satisfactory yield in this final step.

Synthesis of YB (**37**). Synthesis of the *Ca-Nx* glycosamino acid YB proceeded in seven steps as described in Scheme 5-8. An anomeric mixture of C-glycosides **72** was obtained in 88% yield upon treatment of peracetylated xylose **71** with allyl TMS and TMSOTf in acetonitrile. A 3:1 (α/β) ratio of anomers was obtained. The anomers were separable by flash chromatography, and the α anomer **72a** was treated with lithium methoxide in methanol to remove the acetyl groups. Treatment with dimethoxypropane

Scheme 5-8[a]

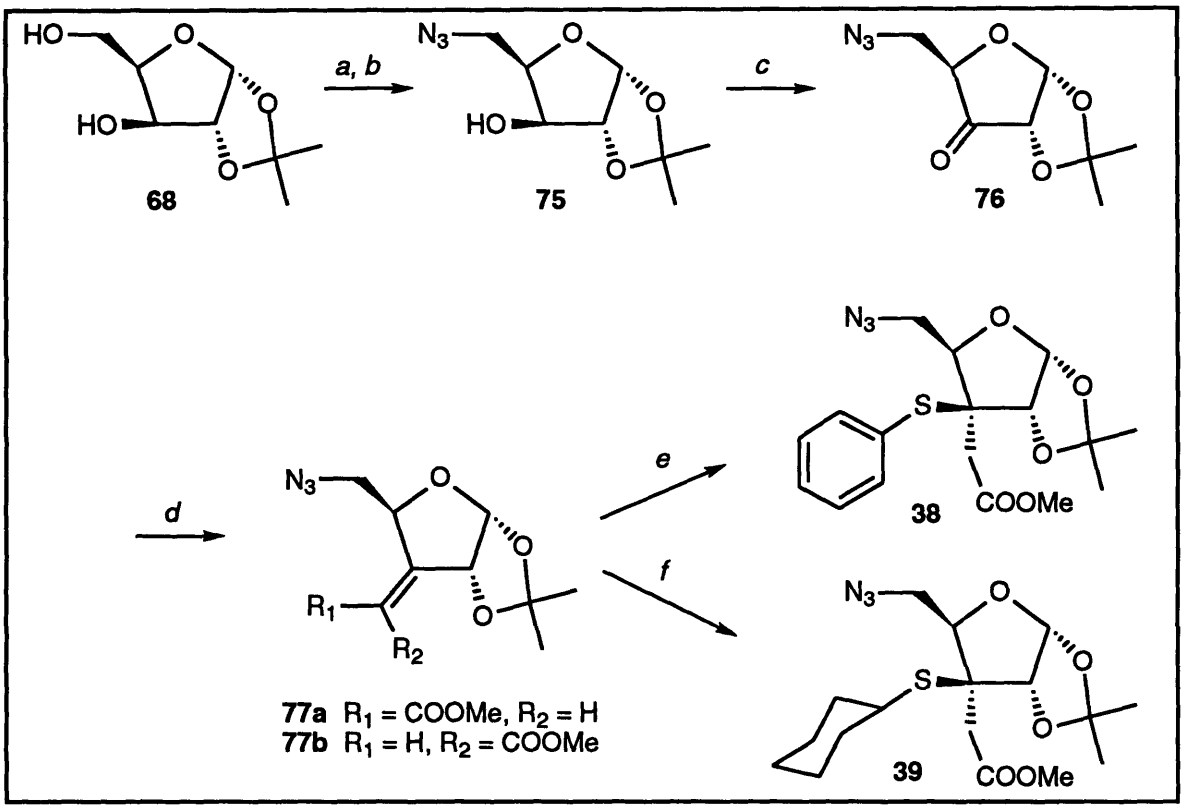


[a]Reagents: (a) allyl TMS, TMSOTf, CH_3CN; (b) LiOMe, MeOH; (c) $(Me)_2C(OMe)_2$, PPTSA; (d) Tf_2O, pyridine, CH_2Cl_2; (e) NaN_3, DMF; (f) $KMnO_4$, Aliquat 336, H_2O, AcOH, CH_2Cl_2; (g) CH_3I, $NaHCO_3$, DMF.

provided the 3,5-acetonide **73** in 73% yield, leaving the 2-hydroxyl unprotected. The secondary alcohol was converted to the triflate, which was treated with sodium azide to give **74** in 54% yield. The allyl glycoside **74** was oxidatively cleaved with potassium permanganate under phase transfer conditions. Esterification with methyl iodide provided the azido ester **37** in 41% yield.

Synthesis of XF (**38**) *and XG* (**39**). Synthesis of the *Cx-Np* glycosamino acids XF and XG proceeded in five steps as described in Scheme 5-10. All intermediates were common until the final step. Many nucleophiles could be used in the final step to produce analogous compounds. Selection tosylation of the primary alcohol of **68**, followed by

Scheme 5-9[a]



[a]Reagents: (a) TsCl, pyridine; (b) NaN_3, DMF; (c) $(COCl)_2$, DMSO, DIEA; (d) $(MeO)_2P(O)CH_2COOMe$, KOtBu, DMF; (e) BnSH, LiOMe, MeOH; (f) cyclohexyl mercaptan, LiOMe, MeOH.

displacement with sodium azide, provided **75** in 53% yield. Swern oxidation[24] afforded the ketone **76** in 57% yield. The ketone was treated with trimethyl phosphonoacetate and potassium *tert*-butoxide in DMF to provide a mixture of regioisomeric α,β-unsaturated esters **77a** and **77b** via a Wittig-Horner reaction (61%)[25].

The α,β-unsaturated esters provided a suitable framework for the rapid synthesis of a collection of monomers, although only two were characterized. Michael addition of benzyl mercaption to **77b** was very fast, affording **38** in quantitative yield. The reaction appeared to proceed with absolute stereoselectivity: nucleophilic attack was observed only from the top face of the sugar. Stereochemical confirmation of the predicted structure was obtained by detection of an NOE at H-2 upon irradiation of the benzylic protons. In analogous fashion, cyclohexyl mercaptan was added to **77b**, producing the glycosazido ester **39** in 91% yield. Addition of cyclohexyl mercaptan to **77a** gave the same product. Michael additions of various nucleophiles could produce a collection of related compounds, all with significant conformational restraints imposed by the crowded ring system. Ideally, a collection of closely related monomers would be developed for iterative combinatorial optimization of a lead compound identified by combinatorial library screening.

Efficiency of Monomer Synthesis

It was not our intention to report an inflated number of monomers based on trivial synthetic manipulations in a final step. As an analogy, we would not wish to claim synthesis of 25 threonine-like monomers based on different esters or ethers that can easily be formed from the side-chain alcohol. However, synthetic pathways are highly valued if they can provide substantial diversity with minimal experimental effort. It is very efficient to have a synthetic route utilizing common intermediates and a late branching point. In other words, it is better to introduce variability at the end, rather than the beginning, of a synthesis.

This concept is illustrated in Figure 5-3, providing an analogy to combinatorial chemistry and the advantages of parallel synthesis. Assume eight monomers are wanted, and each synthesis takes five steps. A serial approach to synthesis (method A), with eight independent syntheses and no common intermediates, requires 40 synthetic *operations*. Method B, in which the first three steps provide branching points (i.e., each reactant gives two products), after which time serial synthesis ensues, uses 30 operations. Only 16 operations are needed using method C, which is identical to method B with the exception that branching commences in the third step. Method D, in which eight different reagents are used in the final step, requires 12 operations. One can describe the *combinatorial efficiency* of monomer synthesis as a function of the number and relative timeframe (what stage in the synthesis) of common intermediates. Based on combinatorial efficiency considerations, certain glycosamino acid synthetic routes, such as the sets (XF, XG) and (MC, MD, ME, MF), were much superior to others.

Conversion of Monomers into Suitable Forms for Solid Phase Synthesis

The high efficiency of peptide synthesis was one of the major reasons that we became interested in pursuing glycotide synthesis. In order to take advantage of the high yields and long history of methodological improvements in solid phase peptide synthesis, we needed to convert the glycosazido ester monomers into N-Boc or N-Fmoc protected glycosamino acids.

We used the intermediate **75** to study various approaches to azide deprotection and conversion to suitably protected forms. Both catalytic hydrogenation and phosphine-mediated reduction provided the free amine **75N** in high yields (see Scheme 5-10).

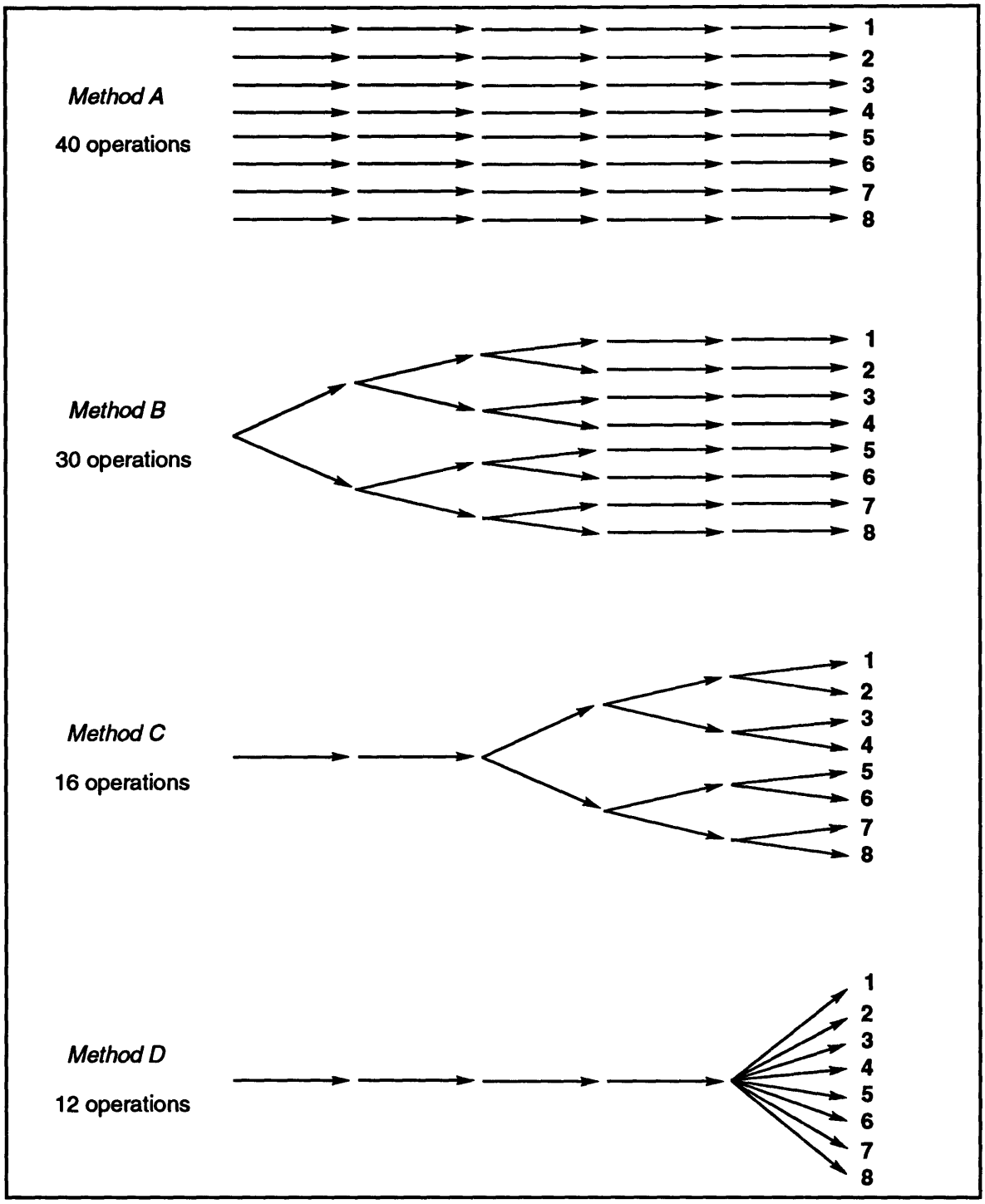
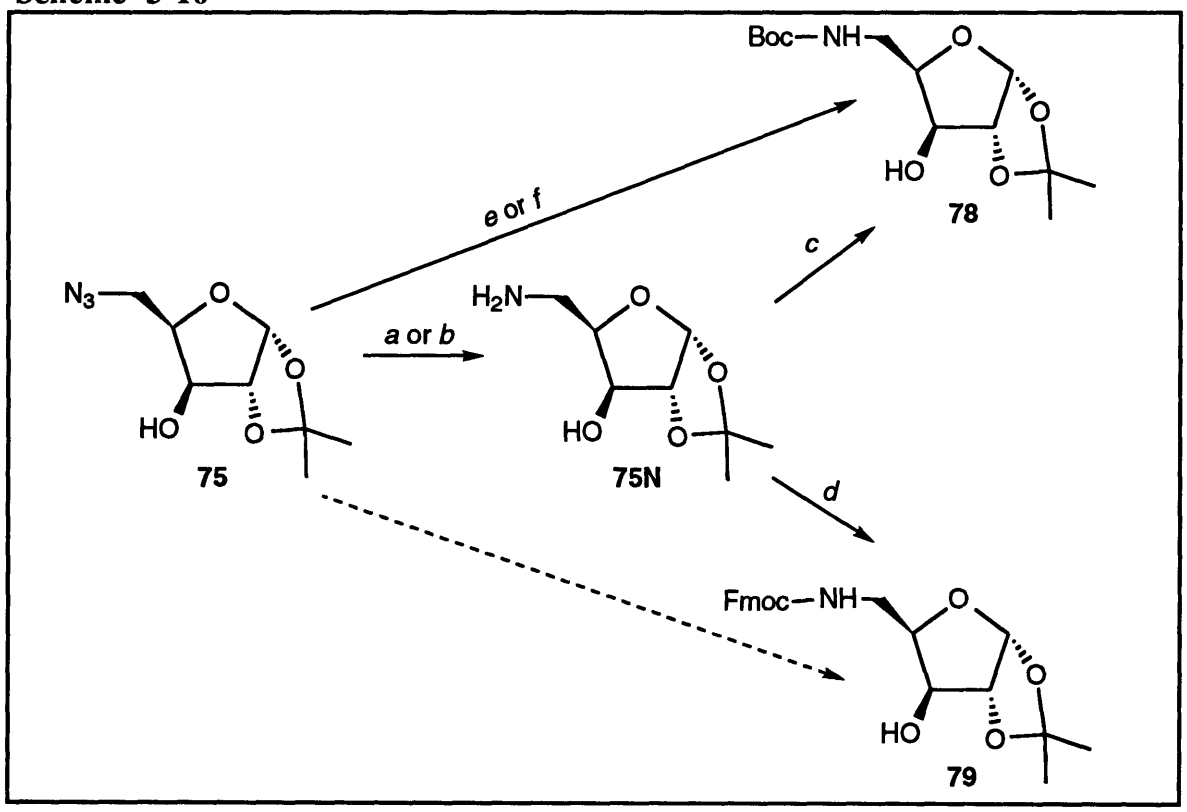


Figure 5-3. Common intermediates and late branching points provide more efficient synthetic routes.

Trimethyl phosphine[26] was determined to be the fastest and most reliable of the phosphine reagents for azide reduction. Triphenyl phosphine permits selective deprotection of

primary azides in the presence of secondary azides[27], and thus could be used for the deprotection of branched, diamino monomers. Conversion of **75N** into the Boc derivative **78** was accomplished by treatment with $(Boc)_2O$, while the Fmoc derivative **79** was produced in 76% yield upon treatment with Fmoc-O-succinimide[28,29]. The yield of the Boc derivative **78** was improved to 95% using a one pot procedure for hydrogenation and carbamate formation[30]. A one step synthesis of **78** using trimethyl phosphine was also developed. The reaction was followed by TLC, and appeared to be as clean if not cleaner than the hydrogenation procedure, but the yield (80%) was lower. We tried to develop a one pot synthesis of the Fmoc derivative **79**, but were unable to come up with an adequate procedure.

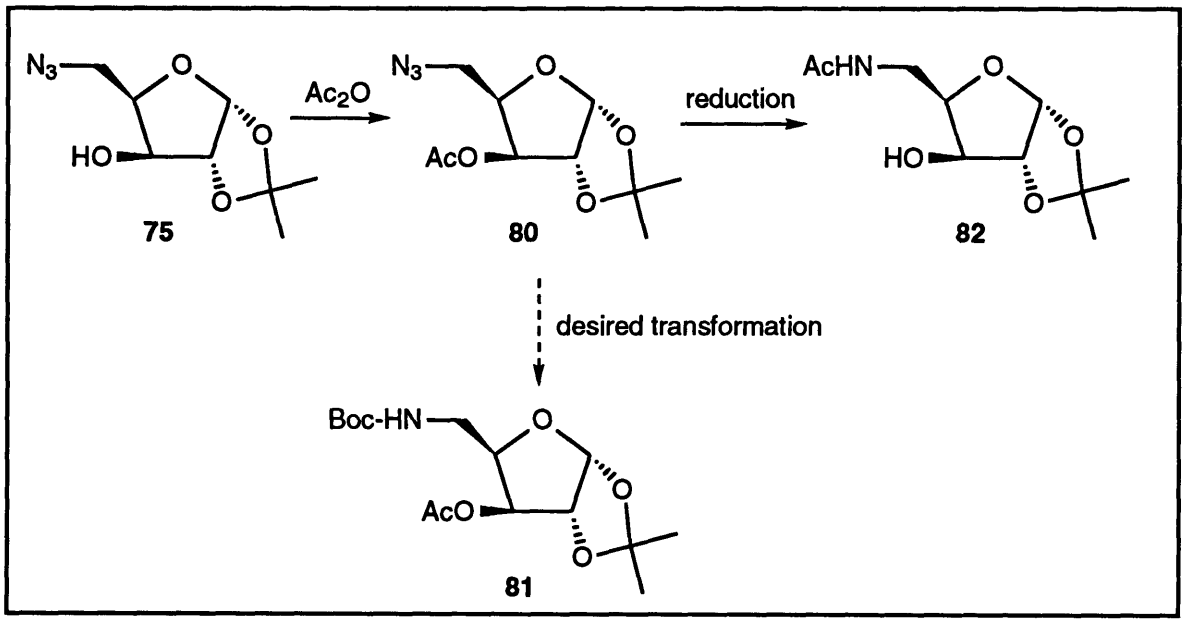
Scheme 5-10[a]



[a]Reagents: (a) H_2, Pd/C; (b) $P(Me)_3$, THF, H_2O; (c) $(Boc)_2O$, TEA; (d) Fmoc-OSu, TEA, THF; (e) H_2, Pd/C, $(Boc)_2O$; (f) $P(Me)_3$, $(Boc)_2O$, THF, H_2O.

As we applied these procedures to glycosazido esters, we encountered a problem with acetyl migration. Specifically, upon azide reduction (either by hydrogenation or trimethylphosphine), monomers with proximate O-acetyl groups experienced O→N migration. We confirmed this problem and hoped to devise a solution by acetylating **75** to give **80** as a model compound. All attempts to transform **80** into the Boc derivative **81** met with failure, as acetyl migration provided **82** as the major product (see Scheme 5-11).

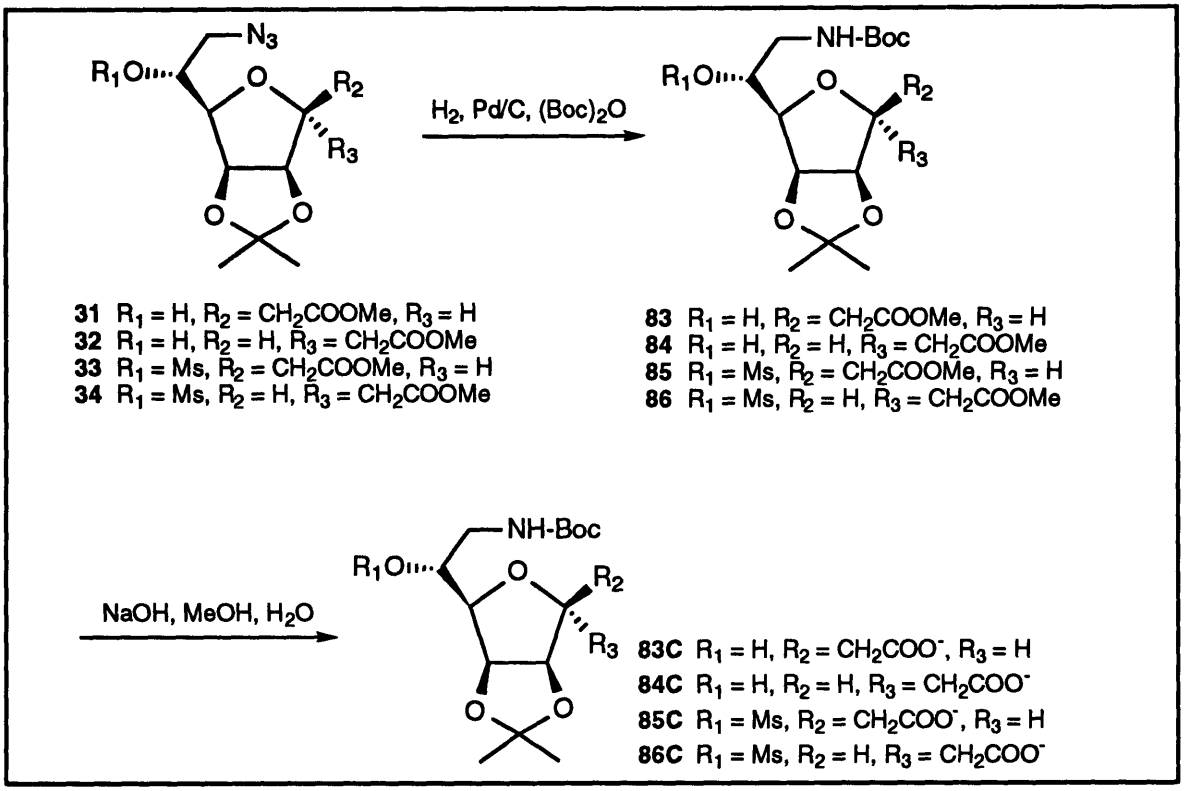
Scheme 5-11. Acetyl migration prevented conversion to the desired product **81**



We anticipated facile conversion of the ester moieties into carboxylic acids. For Boc synthesis, we planned to initially transform glycosazido esters into N-Boc esters, followed by saponification. The glycosazido esters **31-34** were selected for use in solid phase synthesis because of the high yields and efficiencies of their syntheses. The azido esters were converted to the N-Boc esters **83-86** (Scheme 5-12) by catalytic hydrogenation in the presence of $(Boc)_2O$. Saponification, followed by neutralization and subsequent lyopholization, provided the N-Boc acids **83C-86C** as white powders. For Fmoc synthesis, we planned to select from two plausible routes. Initial transformation to the N-Fmoc ester, followed by treatment with bis(tributyltin) oxide[31], would give the Fmoc acid.

Alternatively, the desired product could be obtained by saponification of the azido ester, followed by treatment with Fmoc-Cl.

Scheme 5-12.



31 R_1 = H, R_2 = CH_2COOMe, R_3 = H
32 R_1 = H, R_2 = H, R_3 = CH_2COOMe
33 R_1 = Ms, R_2 = CH_2COOMe, R_3 = H
34 R_1 = Ms, R_2 = H, R_3 = CH_2COOMe

83 R_1 = H, R_2 = CH_2COOMe, R_3 = H
84 R_1 = H, R_2 = H, R_3 = CH_2COOMe
85 R_1 = Ms, R_2 = CH_2COOMe, R_3 = H
86 R_1 = Ms, R_2 = H, R_3 = CH_2COOMe

83C R_1 = H, R_2 = CH_2COO^-, R_3 = H
84C R_1 = H, R_2 = H, R_3 = CH_2COO^-
85C R_1 = Ms, R_2 = CH_2COO^-, R_3 = H
86C R_1 = Ms, R_2 = H, R_3 = CH_2COO^-

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Chapter 6

Synthesis of Glycotide Libraries: An Overview

We did not undertake this project with a particular biological target in mind. With thousands of feasible glycosamino acid targets and only one chemist, it was clear that we could continue with monomer synthesis indefinitely. Although monomer synthesis was essential to the project's success, library synthesis was the ultimate objective. By completing a series of glycosamino acid syntheses, we had shown that the number and variety of monomers that could be synthesized was limited only by available time. At this juncture, we proceeded towards the long-term goal of combinatorial library synthesis.

There are many variables to consider when designing a combinatorial library. Unfortunately, the different variables are not independent. One must choose appropriate monomers and reagents, promoting diversity while maintaining high yields and synthetic compatibility. The number of steps and choices per step are important factors. Obviously, a higher number of steps increases diversity, but also decreases yields and can produce compounds that are too large to possess oral bioavailability. The method of library synthesis must be selected from several options (spatially addressable, multivalent synthesis, split synthesis, or encoding approaches), and the choice depends on the type of molecules, size of the library, and difficulty of the chemistry. In planning the synthesis of glycotide libraries, we addressed many of these issues.

We wanted to keep molecular weights < 700[1] in order to produce compounds with acceptable bioavailabilities. This entailed synthesis of glycotide dimers or, at most, trimers. Consequently, no more than two couplings would be needed. We expected to synthesize relatively small libraries. For example, a library of trimers, with ten possible monomers at each step, would contain 10^3 compounds. Obviously, as more monomers become available, larger libraries can be synthesized. Nevertheless, the limited number of couplings reduces the feasibility of large library synthesis.

Glycotide synthesis is amenable to each of the approaches to combinatorial chemistry that have been discussed. As described in Chapter 1, the multivalent and split synthesis approaches provide facile synthesis, while the spatially addressable and encoding approaches offer simplified characterization of active compounds. Methods that do not provide facile characterization are poorly suited to glycotide libraries. Compounds in a glycotide library can be very similar, often differing by only a single stereocenter. Consequently, analytical structural determination of active compounds is extremely difficult.

It was our intention to use both solution and solid phase approaches to glycotide synthesis. Solid phase synthesis offers several advantages in combinatorial chemistry[2]. Principally, it foments high yields and facilitates removal of reactants and byproducts. Solid phase synthesis simplifies spatial segregation of library components. Spatially addressable libraries can also be synthesized in solution (for example, in 96-well plate format), but only relatively small libraries can be made[3]. The split synthesis approach (separation, reaction, recombination of beads) can only be utilized with solid phase chemistry.

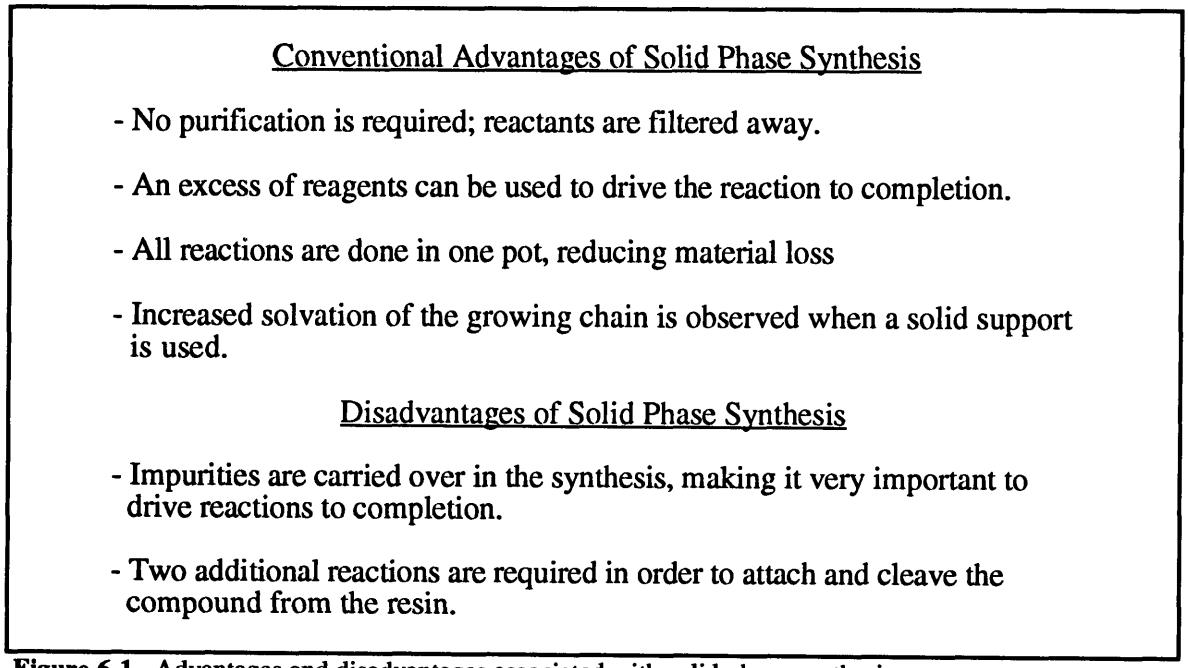


Figure 6-1. Advantages and disadvantages associated with solid phase synthesis.

The traditional disadvantage of solid phase synthesis relative to solution synthesis is the fact that impurites are carried over, since purification of intermediates is not possible. However, this disadvantage is negated in combinatorial chemistry because intermediates are usually not purified, even if reactions are performed without solid supports. Thus, it is extremely important to reduce impurities by driving reactions to completion.

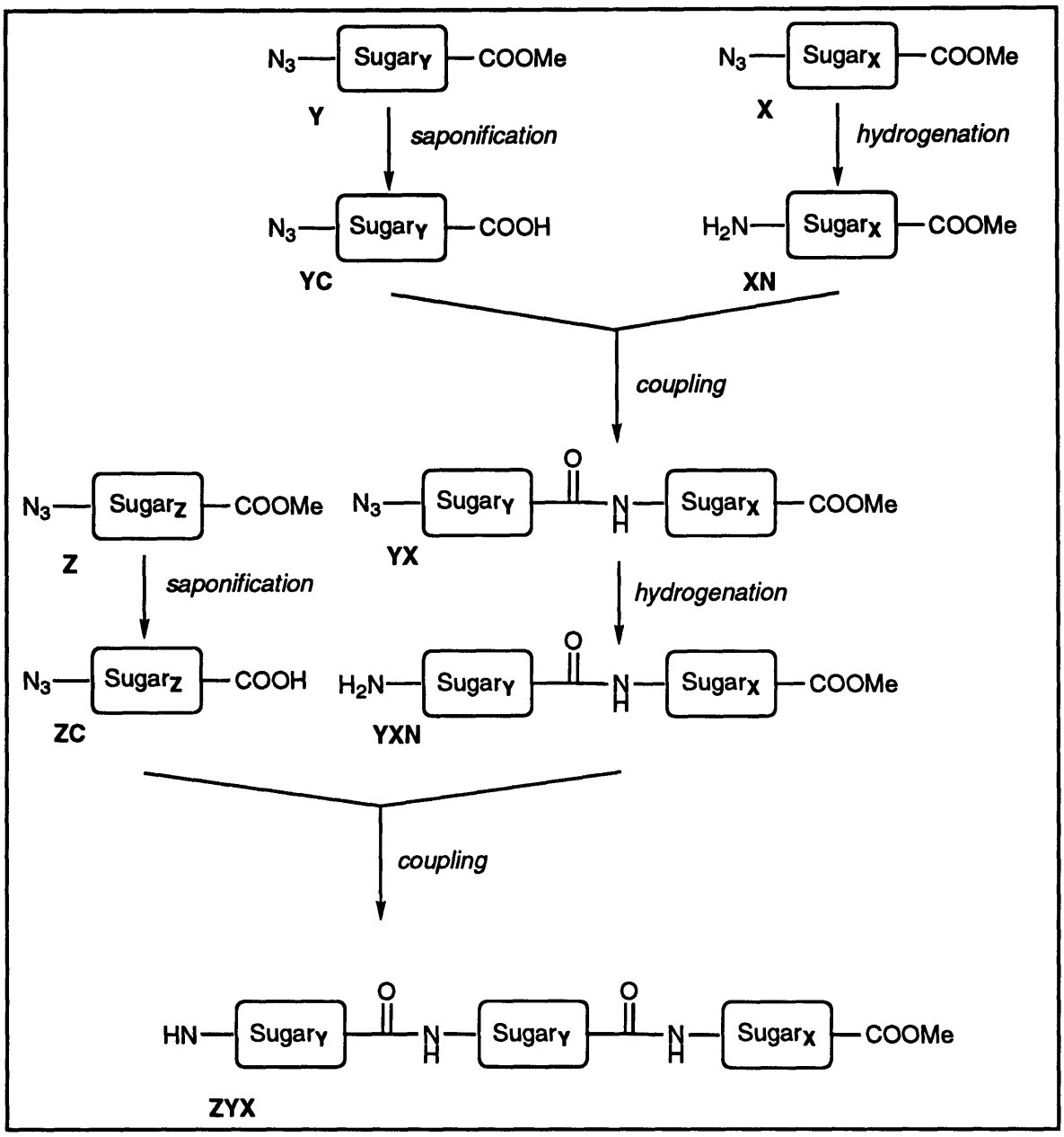
There are other disadvantages associated with solid phase synthesis. Obviously, the chemistry must be compatible with solid supports. In coupling to and cleavage from a resin, two additional reactions are required per compound. An extra element of protection is necessary since the resin linkage must be stable to repeated coupling and deprotection steps during compound synthesis. Finally, a mild method of resin cleavage is desired, particularly in the absence of a final purification step.

Although our ultimate goal was the parallel synthesis of large glycotide libraries, initially we wanted to demonstrate the ability to synthesize defined oligomers, followed by the synthesis of small, well-characterized libraries.

Glycotide Synthesis in Solution

General procedure for glycotide synthesis. We synthesized several oligomers using solution phase chemistry (see Scheme 6-1). Amine deprotections, in the form of azide reductions, proceeded rapidly and in high yield. Hydrogenation was found to be more convenient than phosphine reduction for this step. The resulting free amines were not purified, but instead used directly for coupling. The carboxylic acid moiety was obtained by saponification of a glycosazido ester, followed by neutralization with cation exchange resin. The azido acid and amino ester were coupled using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and TEA in dichloromethane. EDCI, which produces a water-soluble urea as a coupling byproduct, gave higher yields than other coupling reagents (DIC, PYBOP). The resulting oligomers could be extended in either

Scheme 6-1



direction without difficulty because the product of coupling has an azide at one terminus and an ester at the other. Generally, synthesis proceeded in the C→N direction, and excesses of the carboxylic acid and coupling reagent were used in order to increase yields. Disappearance of starting materials was monitored by TLC (staining with ninhydrin and sulfuric acid/ethanol). Concentration of solvents, followed by extraction with

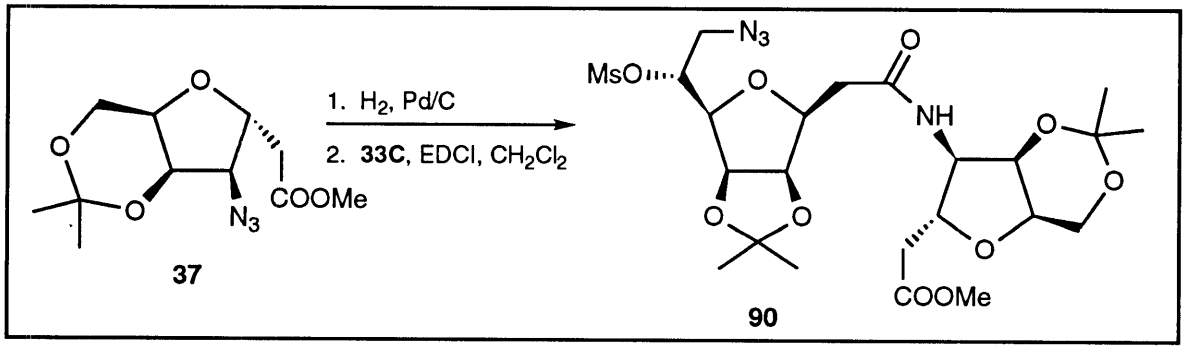
dichloromethane/water, furnished the desired product in the organic layer. Crude flash chromatography separated the oligomeric azido ester from starting materials, which had substantially reduced mobilities on silica gel.

Two conventions have been followed in order to simplify nomenclature. Any glycosazido ester (monomer or oligomer) represented by any number **X** was accorded the representation **XC** upon ester saponification, or **XN** upon azide reduction.

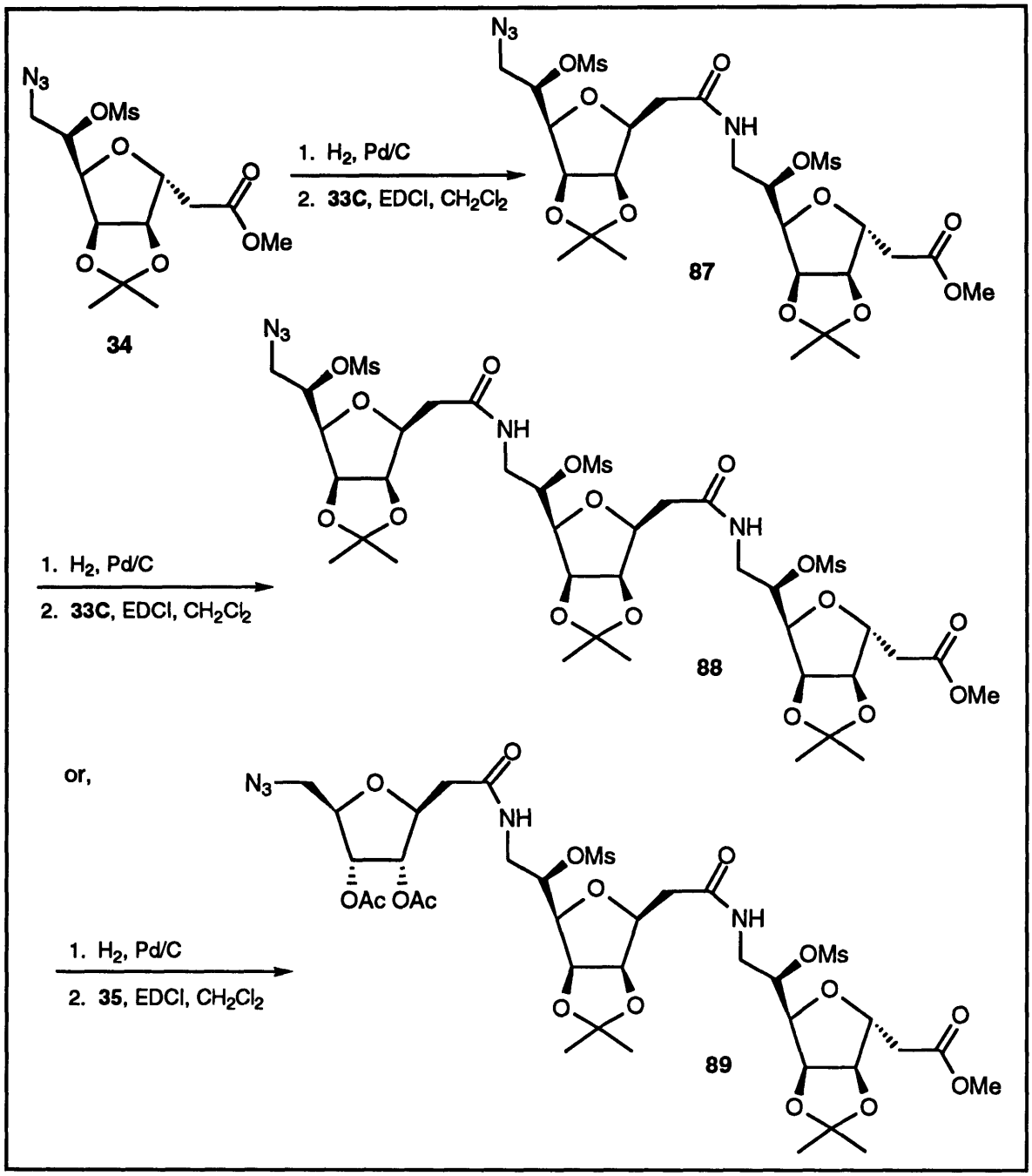
The glycotide trimers N3-MC-MC-MD-OMe (**88**) and N3-RA-MC-MD-OMe (**89**) were synthesized in accordance with Scheme 6-2. The β-glycoside MC (**33**) was saponified to produce **33C**, while the α-glycoside MD (**34**) was hydrogenated to furnish the free amine **34N**. Coupling of the amine and acid components (no molar excess) proceeded in 28% yield to provide the diglycotide **87**. Hydrogenation of **87** produced **87N** (crude, 87% yield), which was coupled to **33C** to furnish the triglycotide **88** in 45% yield. Coupling of **87N** and the glycosazido acid **35** produced the triglycotide **89** in 44% yield.

We synthesized the diglycotide N3-MC-YB-OMe (**90**) in order to examine the reactivity of more hindered amines (see Scheme 6-3). The glycosazido ester **37** was hydrogenated to provide the secondary amine **37N**, which was coupled to **33C**. Although coupling of the secondary amine **37N** took somewhat longer to complete than coupling of primary amines, the yield was not adversely affected, as **90** was provided in 67% yield.

Scheme 6-3



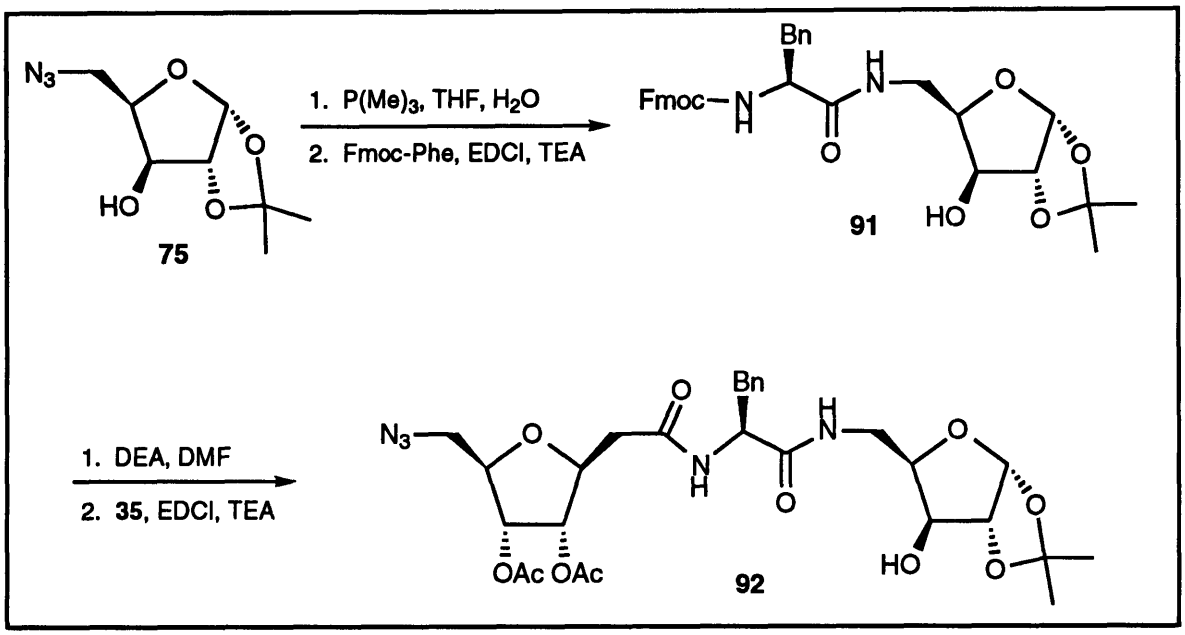
Scheme 6-2



Conventional amino acids can be introduced into a growing chain, while monofunctionalized carbohydrates having either an amine or carboxylic acid can serve as anchor residues. For example, synthesis of the "trimer" **92** incorporated phenylalanine as a monomeric unit, while also using the azido sugar **75** as the "C-terminus" (see Scheme 6-

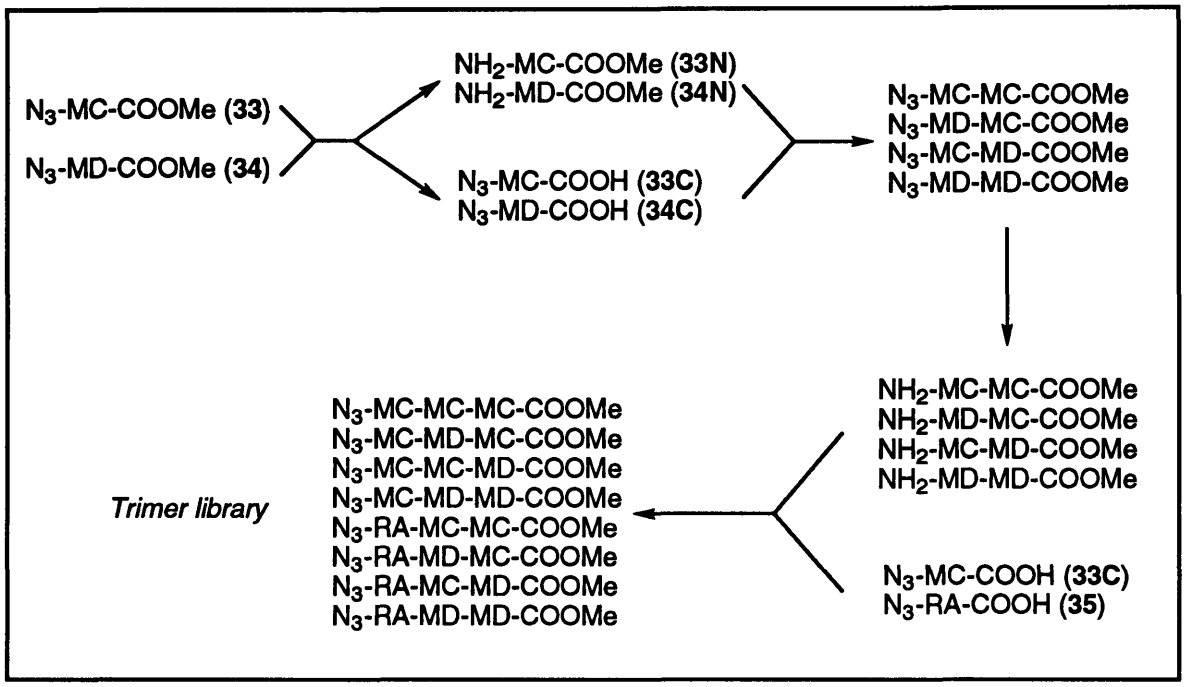
4). Treatment of **75** with trimethylphosphine in THF/water provided the free amine **75N**, which was coupled to N-Fmoc-phenylalanine to afford **91** in 35% yield. The Fmoc group was removed with 10% diethylamine in DMF[4]. Concentration of solvents, followed by coupling to **35** in DMF, provided the trimer **92** in 75% yield.

Scheme 6-4



An eight compound library of trimers was assembled using a multivalent approach to solution phase library synthesis (see Scheme 6-5). Compounds were synthesized in accordance with the syntheses of specific oligomers discussed previously, except glycosamino acids were mixed at each stage of the synthesis, thereby affording $2^3 = 8$ possible compounds. The desired products were sufficiently similar to be copurified as a group via extraction and filtration through silica gel. We have not confirmed that all eight compounds were actually synthesized. The compounds emerged as a single broad peak on HPLC. Molecular weight degeneracy limited the eight compounds to only two $(M + H)^+$ peaks.

Scheme 6-5. Synthesis of an eight-compound library of glycotide trimers



The analytical problems that were confronted with this small library portend to the enormous difficulties that would have to be overcome if we synthesized a large library of glycotides using the multivalent approach. The inability to remove starting materials (i.e., excess glycosamino acids) without invasive purification techniques precludes spatially addressable synthesis of glycotides in solution. The split synthesis approach is not amenable to solution synthesis, and therefore the only choices are the multivalent and encoding approaches. The multivalent approach foments analytical difficulties, and thus an encoding method appears to be the best way to make glycotide libraries in solution. Unfortunately, development of a tagging scheme requires a large initial investment of resources, and the best encoding methods[5-7] reported in the literature to date have been used in conjunction with solid supports.

Synthesis of template-directed libraries. We also synthesized template-directed libraries using the multivalent approach depicted in Figure 6-2. A set of four glycosazido

esters (**31** - **34**) was hydrogenated, then mixed with the template (1,3,5-benzene-tricarbonyl trichloride) and TEA in dichloromethane. The symmetry of the template reduced the number of possible compounds to 20, instead of the $4^3 = 64$ compounds that could be produced using an asymmetrical three-site template with four glycosamino acid options. The HPLC trace in Figure 6-2 shows four clusters of peaks. The four glycosamino acids that were mixed with the template consisted of two sets of anomeric pairs. Consequently, the HPLC trace would have revealed only four peaks if the anomers had been indistinguishable. This was not the case, although we were unable to resolve all 20 peaks. Due to the mass degeneracy of the anomeric pairs, the 20 compounds possessed only four molecular weights, all of which were observed in the mass spectrum.

We designed a template library without mass degeneracy in order to assess whether we had synthesized every possible compound in a library (see Figure 6-3). Three glycosazido esters, **29** (MA), **33** (MC), and **38** (XF), were pooled, hydrogenated, and reacted with the template. In theory, ten different products could be obtained, nine of which were clearly observed in the mass spectrum. The only compound that was not observed in significant quantities was the trimer T-XF-XF-XF. As a result of material shortages, however, equimolar amounts of the individual glycosazido esters were not used. Instead, XF constituted only 20% of the total monomer content (rather than 33% in an equimolar distribution). Assuming equal rates of reaction (unlikely), the trimer T-XF-XF-XF would have comprised less than 1% ($1/5^3$) of the total products. The trace shown in Figure 6-3 reveals that the individual components were well-resolved on RP-HPLC, although we also detected some small, extraneous peaks resulting from impurities.

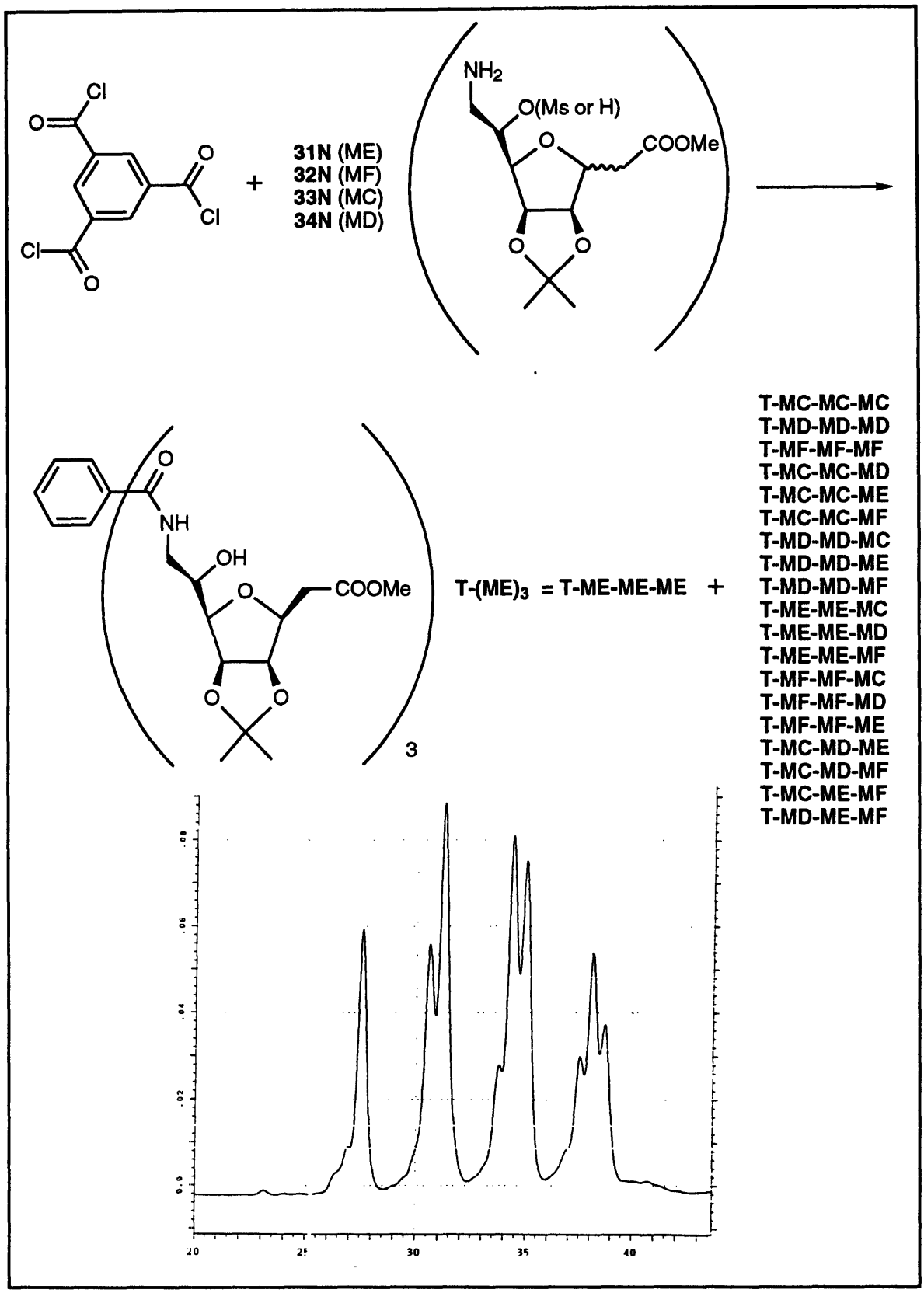


Figure 6-2. Synthesis and HPLC trace of a 20 compound, template-directed library.

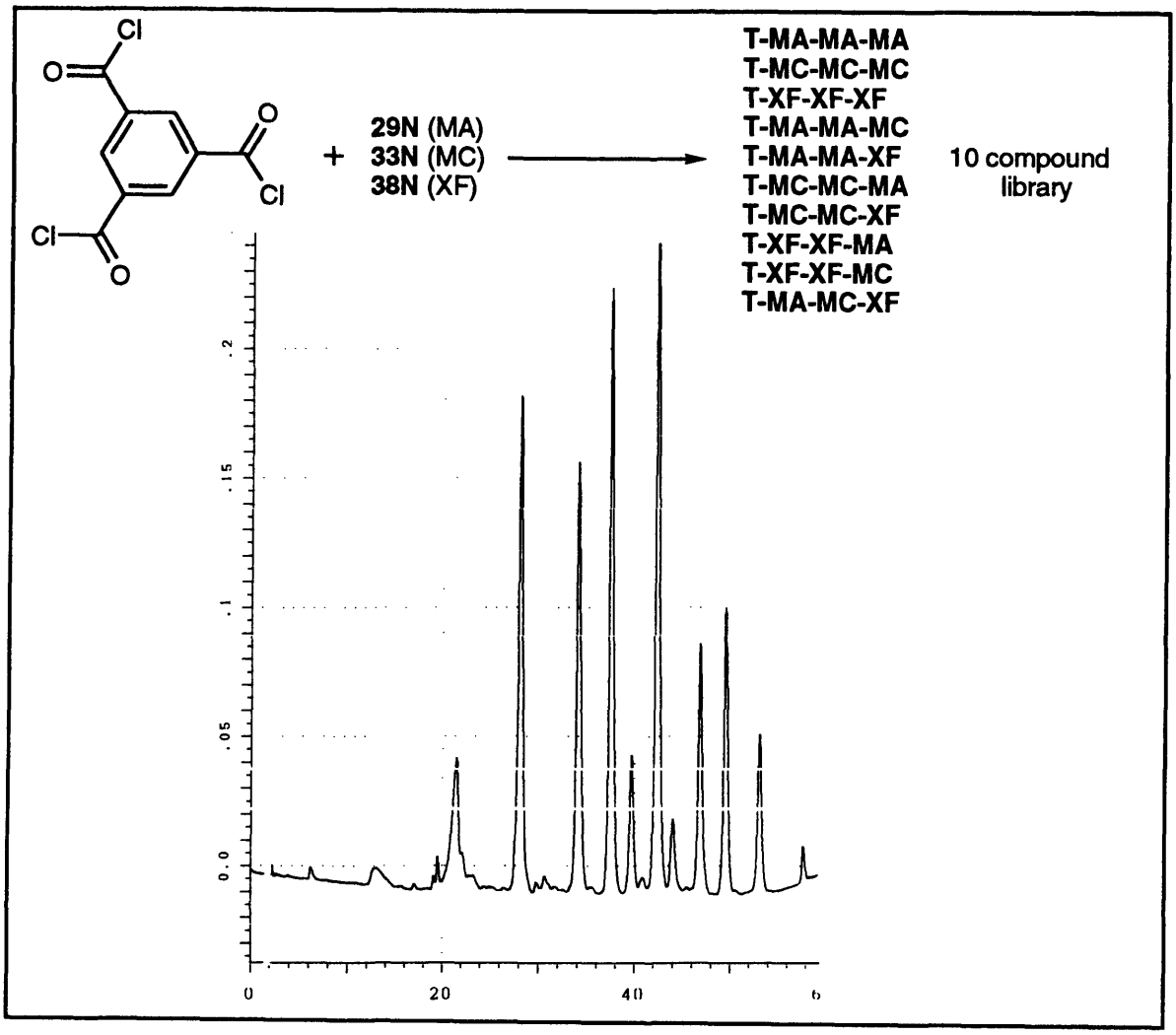


Figure 6-3. Ten compound template-directed library without mass degeneracy.

The template was reacted with an excess of a single monomer, **31N** (ME), in order to assess the intrinsic level of impurities accumulating from the template library procedure (see Figure 6-4). The HPLC trace shows the major peak corresponding to the desired compound **93**, as well as two smaller peaks resulting from impurities in the starting materials or side products in the hydrogenation and coupling reactions.

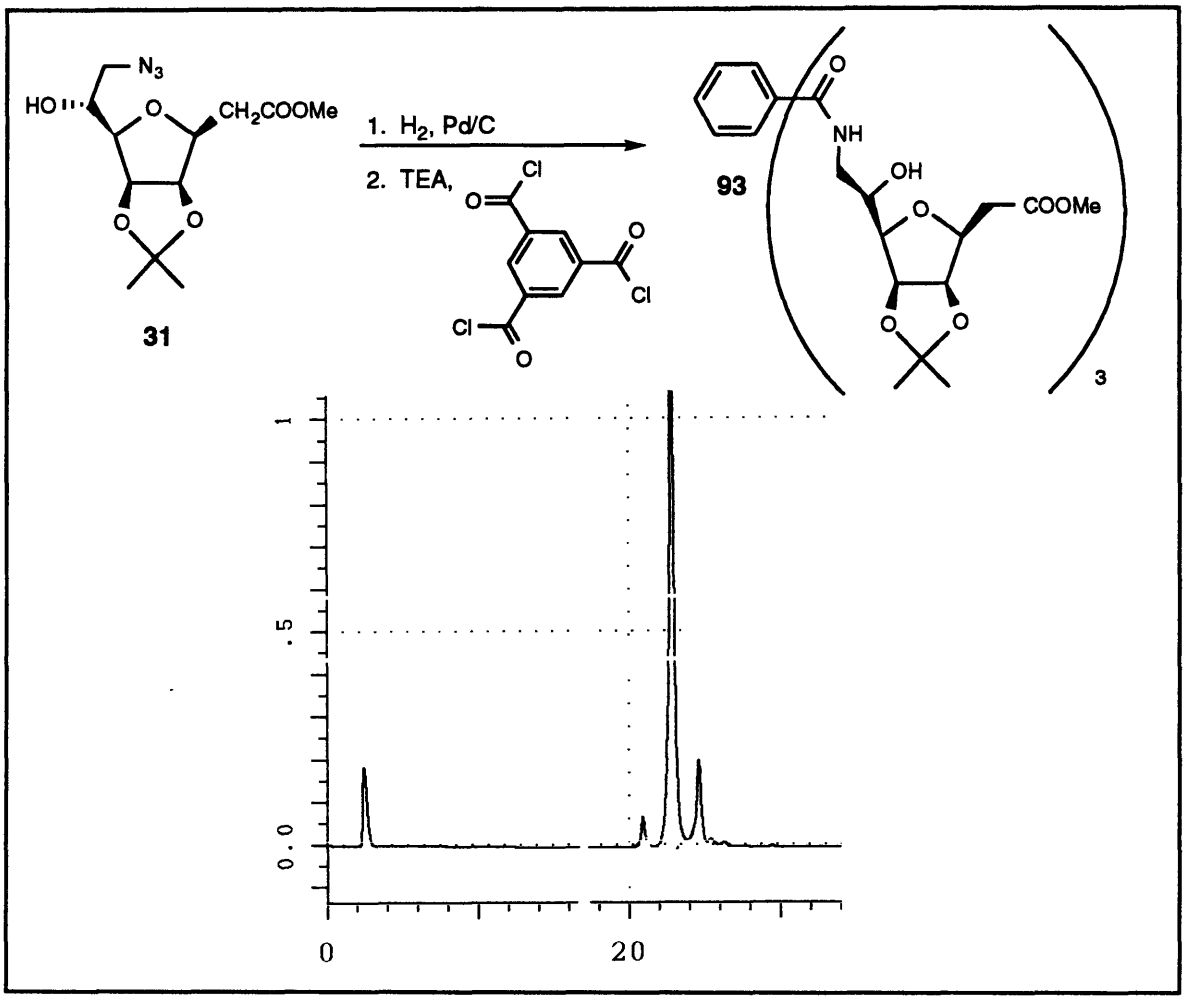


Figure 6-4. Our template-directed approach gave rise to some impurities

Solid Phase Synthesis of Glycotides

We anticipated using both Fmoc and Boc methodology for solid phase synthesis of glycotides. We wanted to use orthogonal deprotection methods, because exposure to harsh reagents would be minimized. Fmoc synthesis provides orthogonality, since Fmoc groups are removed with base (piperidine) and cleavage from the resin is accomplished with mild acid (TFA in dichloromethane)[8]. Normally, Boc synthesis does not allow an orthogonal deprotection scheme because Boc groups are removed with mild acid (TFA in dichloromethane), while strong acid is needed for cleavage from the resin (depending on the resin, anhydrous HF or triflic acid). However, the Kaiser oxime resin[9-11] permits an

orthogonal deprotection scheme, since nucleophiles (hydrazine, N-hydroxy piperidine[12], amino acid esters, etc.) are used for resin cleavage. Other schemes that provide orthogonality can utilize linking agents[13,14] or light deprotection, either for cleavage from the resin[15] (brominated Wang resin, available from Novabiochem) or removal of amine protecting groups[16].

Initially, we decided to pursue solid phase synthesis using Boc chemistry on the oxime resin. We believed that conversion of glycosazido esters into N-Boc acids would be slightly easier than conversion to N-Fmoc acids. Our experience[17,18] with peptide synthesis on the oxime resin, and lack of experience with the photocleavable brominated Wang resin, made the oxime resin a natural first choice. At this point in time, we have not optimized the solid phase synthesis of glycotides. Initial attachment to the resin and monomer coupling have proceeded smoothly (assayed by qualitative Kaiser tests), but the cleavage reaction has been problematic. The difficulties may be a function of the particular glycosamino acids that have been used (MC, MD, ME, and MF), or the oxime resin might not be very compatible with glycotide synthesis. Glycotide synthesis should be attempted using other resins that permit orthogonal deprotection schemes in conjunction with Boc chemistry, as well as linking agents and resins compatible with Fmoc chemistry.

Biophysical Studies

We have not done any quantitative conformational analysis of glycosamino acids and glycotides. Nevertheless, it is clear that the conformational mobility of monomers and oligomers is often limited. It appears that the *exo*-glycosidic conformation of the glycosazido ester monomers is fairly unusual relative to most C-glycosides[19,20].

Glycotides are chemically stable unless harsh conditions exist, but their stability to enzymes is a potential concern which at present we have not addressed. Glycotides do not possess natural glycosidic linkages. Moreover, most monomers are C-glycosides, so it is unlikely that glycotides would be cleaved by glycosidases. Glycotides are replete with

amide bonds, however, and while they are not natural substrates for proteases, there is a possibility of proteolytic cleavage.

Conclusions

Although solution phase methods have been used successfully to synthesize oligomers and small libraries, solid phase approaches to glycotide synthesis should be developed in order to obtain libraries that are both practical and accessible. Small oligomer synthesis is readily achieved in solution, but synthesis of larger compounds and libraries will be more practically accomplished using solid supports. Depending on available quantities of monomers, solution phase multivalent approaches could be used to assemble glycotide libraries of virtually unlimited size, but the accessibility of these libraries would be limited. The requirement for purification eliminates the possibility of using spatially addressable methods for solution phase library synthesis. Tagging methods could be applied to glycotide synthesis in solution, but would be synthetically challenging and time-consuming. Solid phase synthesis is more amenable to encoding technologies, split synthesis, and spatially addressable approaches to combinatorial libraries of glycotides.

Before we are able to efficiently synthesize large, accessible, and structurally complex glycotide libraries, a substantial amount of work remains to be done, including optimization of solid phase chemistry, additional synthesis of new and varied monomers, and large scale monomer synthesis. A realistic estimate is that optimization of solid phase chemistry would be completed in two years, coinciding with the synthesis of 30 distinct monomers in multigram scale. This would allow the facile synthesis of $(30)^3 = 27{,}000$ glycotide trimers in milligram quantities. The time required for library construction would be dependent on the approach used, but would differ little from peptide library synthesis. In assessing the potential of these libraries, one should consider the possibility that this time could be better spent developing alternative libraries that incorporate readily available building blocks.

It is not necessary to create structurally novel libraries in order to develop new drug candidates. The synthesis of thousands or millions of compounds closely resembling certain known drugs will undoubtedly provide a source of pharmaceutically important new molecules. Given the abundance of industrial participants in combinatorial chemistry, significant duplication of libraries that are easily synthesized is inevitable. A library possessing unusual structural properties could produce novel drug candidates, while also serving as a source of new information and providing temporary relief from competitors. In order to gain access to structurally novel libraries, risks must be taken. One approach is to develop novel solid phase methodology. Our approach is to adapt interesting molecules to existing technology. Either approach requires a significant time investment before library synthesis is possible.

Without a doubt, synthesis of glycotide libraries will take longer than most libraries currently in development. Relatively speaking, this difference might be very large, perhaps even 500%, but in absolute terms, the difference is only two chemist-years. The reward is a compound library substantially different from any in existence.

Glycotide library synthesis would provide a convenient mechanism to access the tremendous structural diversity of carbohydrates without the difficulties associated with the synthesis of natural carbohydrates. Compounds could be synthesized in relatively pure form, and should have good *in vivo* stabilities. Glycotides do not require functional group deprotection at the end of synthesis. The ability of glycotides to penetrate cell barriers is unknown, but the presence of functional groups masking the alcohols should have a beneficial effect[21]. It bears repeating that many potential therapeutic targets for glycotides would be extracellular. If glycotides are found to possess desirable biological activities and pharmacokinetic properties, they would become very valuable lead compounds because of their capacity for iterative combinatorial optimization. The tremendous potential of glycotides merits the significant investment required to overcome the synthetic obstacles.

Having reached that conclusion that glycotide synthesis is worth pursuing, two alternatives should be introduced (see Figure 6-5). Orthogonally protected diamino sugars (*glycosadines*) can be synthesized, and linked via ureas to produce *glycurides*. Like amide bond formation, urea synthesis[22] proceeds in high yields and is amenable to solid phase chemistry. There are several advantages of glycuride synthesis relative to glycotides. Urea linkages should eliminate concerns about proteolytic cleavage[22]. Synthesis of glycosadine monomers is generally more straightforward than synthesis of glycosamino acids. Orthogonal amine protection is easily achieved, even if both latent amines are introduced simultaneously as azides[23]. Finally, synthesis of glycosadines effectively doubles the number of monomers, since there are two choices for the site of chain elongation.

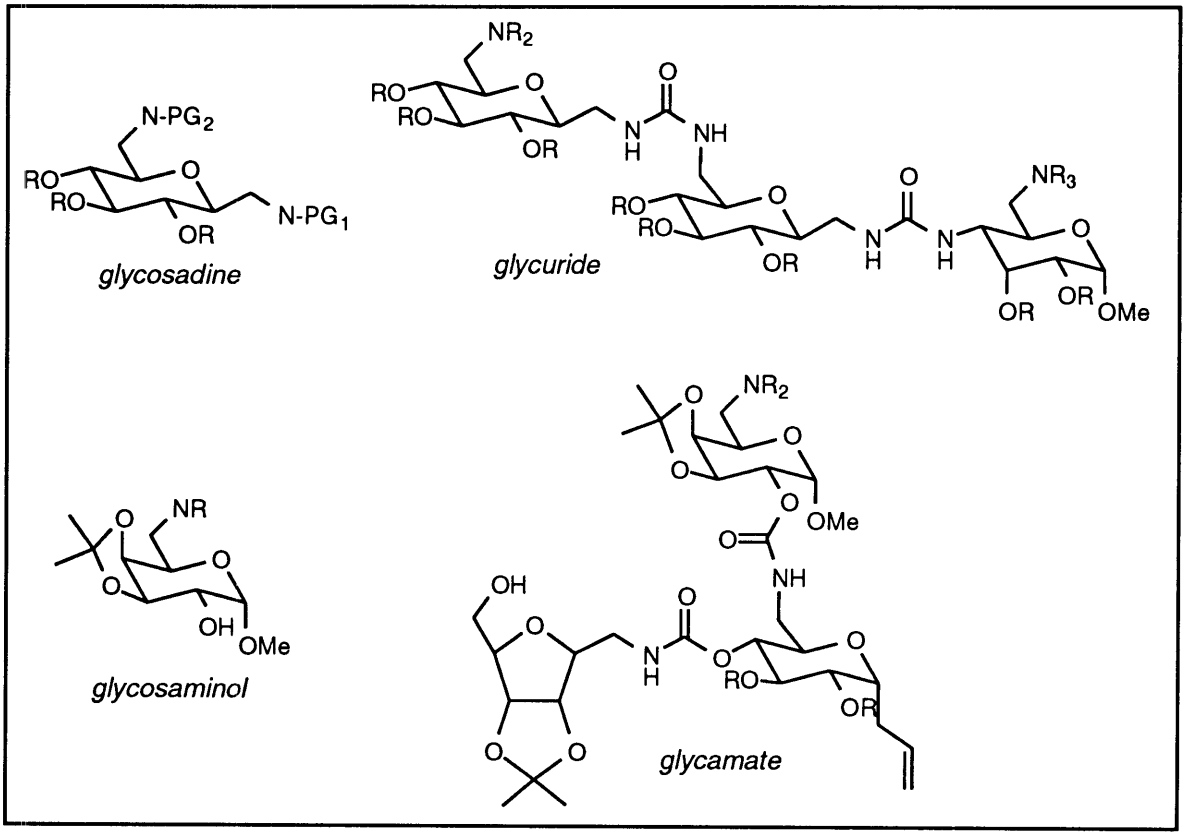


Figure 6-5. Unnatural carbohydrate oligomers formed via urea and carbamate bond formation.

Amino hydroxy sugars (*glycosaminols*) could be synthesized and subsequently polymerized by carbamate formation[24] to produce *glycamate* libraries. Synthesis of glycosaminol monomers would be much easier than synthesis of glycosadines or glycosamino acids, and thus a large number of building blocks could be rapidly assembled.

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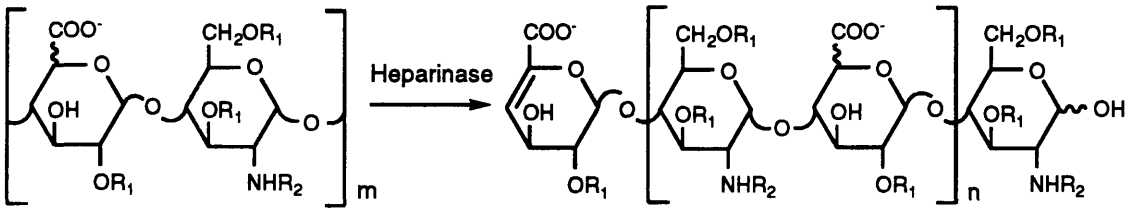
Chapter 7

Index of Experimental Procedures

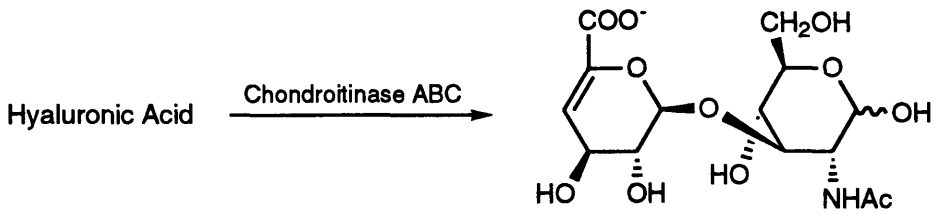
General Procedures. All air-sensitive reactions were performed under an atmosphere of argon. Unless otherwise noted, materials were obtained from commercial sources and used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU), triethylamine, and pyridine were distilled from calcium hydride. Other solvents were HPLC grade or commercially distilled. DOWEX 50X8-200 was used as a cation exchange resin. Analytical thin-layer chromatography was performed on EM Science (E. Merck) silica gel 60 F-254 and Analtech silica gel GF (250 microns). TLC staining of sugars was normally accomplished by spraying with a naphthoresorcinol stain (0.2 g naphthoresorcinol, 100 mL ethanol, 4 mL concentrated sulfuric acid). Improved detection of sugar amines or sugar amides was achieved by increasing the concentration of sulfuric acid to 5% and removing the naphthoresorcinol. Errors in R_f values are $\pm$ 0.1. Chromatographic purifications were performed with EM Science 230-400 mesh silica gel or Baker silica gel (40 mm avg. particle diameter), unless otherwise noted. Reversed phase HPLC was performed on C18 columns (300 Å) and weak anion exchange HPLC was performed on DEAE 8HR (Waters) columns. Unless specified as WAX-HPLC, the term HPLC refers to RP-HPLC. ^1H and ^{13}C spectra were acquired using a Varian XL-300 or Varian UN-300 spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane ($\delta = 0$) for proton spectra and relative to internal $CDCl_3$ (77.0) for carbon spectra. ^1H NMR are tabulated in the following order: multiplicity (s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet), number of protons, and coupling constants in Hertz. Infrared spectra were recorded on a Perkin Elmer 1600 Series FTIR. All mass spectra were obtained in the FAB mode. Unless otherwise noted, yields are reported as the combined totals of the recovered addition products. Extractions with sodium bicarbonate were performed with a concentrated solution of $NaHCO_3$, and extractions with $KHSO_4$ were performed with a 1 M solution.

Experimentals for Chapter 2



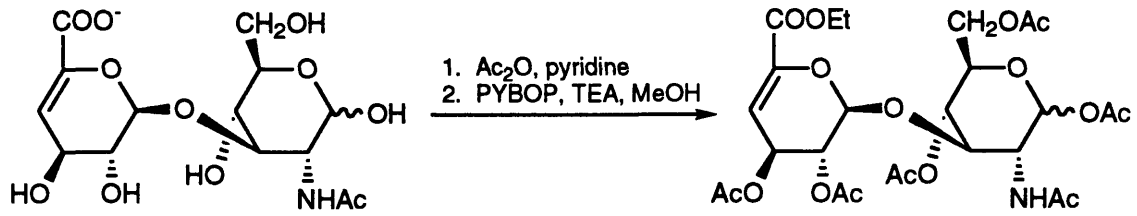
Heparin Digestion with heparinase. Heparin was added at a concentration of 25 mg/ml to buffer (pH 7.0, 100 mM MOPS, 5 mM calcium acetate). For each 1 ml of buffer, 5 ul of cold heparinase (0.04 ug/ul water) solution was added. The reaction vessel was incubated at 37°C for 24 hours, by which time digestion was > 95% complete (monitored by UV absorbance at 232 nm).

Purification of heparin fragments. Fractions were separated on a weak anion exchange HPLC (DEAE 8HR, Waters) column (Conditions at time t: NaCl concentration (M) = 0.35 + (0.0125)t; pH = 3.5 - 0.005x). The major disaccharide product **1** eluted at approximately t = 20 minutes. Fractions were desalted on a gel filtration column (Sephadex G-10).



2

Hyaluronic Acid Digestion with Chondroitinase ABC. Chondroitinase ABC (10 units) was added to a gel-like suspension of hyaluronic acid (0.7g) in sodium acetate buffer (100 mL, 0.05M NaOAc, pH 6.75). The suspension was allowed to stand for one month at 37°C, eventually becoming a solution. The major product, which can be purified by WAX-HPLC or partially purified by size exclusion chromatography, was the disaccharide **2**.



2 4

The crude product of hyaluronic acid digested by Chondroitinase ABC (consisting primarily of the sodium salt of **2**) was passed through a cation exchange column, after which the free acid was lyopholized to a white powder. Pyridine (7 mL), acetic anhydride (6 mL), and DMAP (5 mg) were added to the powder (500 mg). The resulting solution was stirred at room temperature for 48 hours, then added to water (20 mL). The aqueous solution was extracted with dichloromethane (5 x 20 mL), and the combined organic extracts were concentrated to a small volume. A portion was purified by RP-HPLC (100/0 water:acetonitrile → 60/40 over 40 minutes) to produce **3**, while the rest was subjected to repeated, unsuccessful recrystallization attempts. Dichloromethane (1.5 mL) was added to a mixture of the peracetylated disaccharide **3** (55 mg, 0.09 mmol), PYBOP (50 mg, 0.09 mmol), and TEA (25 uL, 0.18 mmol). After stirring 20 minutes, ethanol (7 uL, 0.17 mmol) was added. After 40 hours, dichloromethane (2 mL) was added, and the solution was extracted with water (3 x 3 mL). The organic extracts were concentrated and purified by RP-HPLC (same conditions as above). The major product was the β-anomer **4** (20.0 mg, 0.03 mmol), which was isolated in 35% yield.

Physical data for **4**

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.22 (d, 1H), 6.03 (d, 1H), 5.65 (d, 1H), 5.56 (d, 1H), 5.06-5.13 (overlapped m, 2H), 4.88-4.93 (m, 1H), 4.61-4.67 (m, 1H), 4.32 (q, 2H), 3.87-4.17 (overlapped m, 4H), 2.17 (s, 3H), 2.10 (s, 3H), 2.04 (s, 9H), 1.97 (s, 3H), 1.36 (t, 3H); FABMS: 619 $(M + H)^+$.

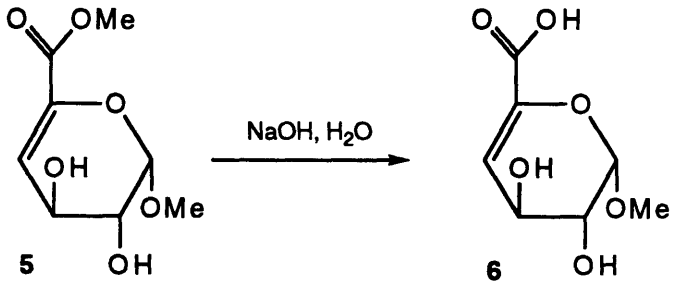
Experimentals for Chapter 3

General Reaction Procedure for Michael Addition. To a solution or suspension of base (0.19 mmol) [LiOMe, NaOMe, BuLi, DBU, TEA] and mercaptan (0.48 mmol) in 1 mL solvent was added a solution of the α,β-unsaturated ester **5** (30.6 mg, 0.15 mmol) in 1 mL solvent. The reaction was stirred for 24 hours at the specified temperature, then neutralized with cation exchange resin. In case of a suspension, sufficient methanol was added concurrent with the cation exchange resin to yield a solution, which was filtered through cation exchange resin. The filtrate (with the exception of reactions done in perdeuterated DMF or DMSO, which were immediately analyzed by ^{1}H NMR after 24 hours) was concentrated and the resulting oil was dried *in vacuo*.

Reactions performed at elevated temperature were kept at 44°C with an equilibrated oil bath. Low temperature reactions were performed by cooling the two sets of reactants independently in an ice bath, combining them under argon, and transferring the reaction flask to a 4°C refrigerator, where the contents were stirred for 24 hours before the standard workup. Room temperature reactions are reported as 22°C.

Quantitation of Reaction Products. The reactions were worked up after 24 hours as described. Products were not isolated in these standard reactions. The residual oil was dissolved in $CDCl_3$ and analyzed by ^{1}H NMR spectroscopy. The relative ratios of the starting material and addition products were determined by integrating well-resolved peaks which matched separately purified standards. In some cases, quantitation of the molar percentage of the minor products (**b** and **d**) could not be done by comparison with peaks of known standards; instead, analogous peaks from corresponding addition products with other mercaptans were used as a reference (i.e., **9d** was never isolated and purified. Estimation of the percentage of **9d** was obtained by integrating peaks which were assigned

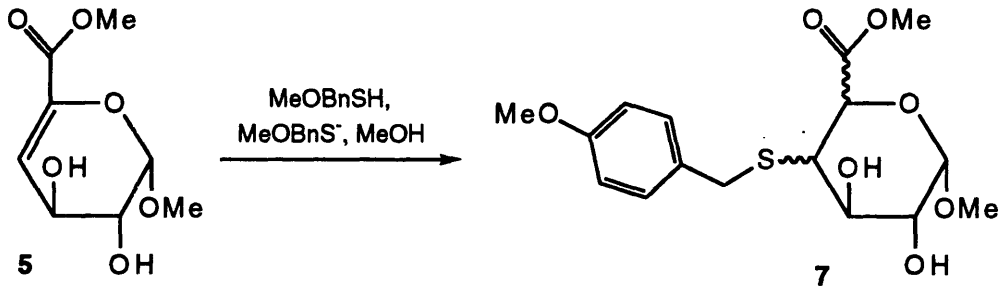
to **9d** using the analogous spectral peaks from **11d** as references). The presence of saponified starting material and products was sometimes observed, but usually comprised much less than 5% of the total material. Larger percentages of side reactions were observed in reactions at elevated temperatures, increasing the difficulty of quantitation. Errors in the numbers arose from uncertainty in the peak integration areas (small) and variations between runs (somewhat larger). Differences in the product accumulations which were less than 5% can be deemed insignificant. This error was mostly composed of run-to-run variations. The error in the numbers reflecting product distributions was much smaller, about 1%. This was normally small relative to the D-*galacto* product **a**, but large relative to the L-isomers **b** and **d**. Comparisons should be made with this factor in mind. Finally, as a result of decreases in the S/N ratio, errors in the product distributions increased significantly when the corresponding product accumulations were less than 20%.



The α,β-unsaturated ester **5** (342 mg, 1.67 mmol) was saponified by solvation in water (10 mL) with an excess of sodium hydroxide (2 mL of 1 M solution). The solution was neutralized with cation exchange resin, filtered, and concentrated to give the α,β-unsaturated acid **6** (320 mg, 1.68 mmol) as a colorless oil in quantitative yield.

Physical data for **6**

^{1}H NMR (300 MHz, D_2O) δ 5.97 (d, 1H, J = 3.3), 4.92 (d, 1H, J = 2.6), 4.18 (dd, 1H, J = 7.7, 3.3), 3.69 (dd, 1H, J = 7.7, 2.6), 3.39 (s, 3H).



A solution of **5** (46.9 mg, 0.23 mmol) in 2 mL methanol was added to a mixture of lithium methoxide (14.0 mg, 0.37 mmol) and methoxybenzyl mercaptan (204 mgs, 1.31 mmol) The reaction was stirred for 24 hours at room temperature under argon, then neutralized by the addition of cation exchange resin. The solution was filtered and concentrated. The resultant oil was purified by flash chromatography (dichloromethane/ acetone = 4:1) to give pure fractions of **7a** (53.9 mg, 66% yield) and **7c** (6.9 mg, 8% yield) as pale yellow oils, as well as a fraction containing a mixture of the four possible isomers (8.2 mg), which was further purified by flash chromatography to give pure **7b** as an oil (2.4 mg, 3% yield) and a yield of the combined products of 84% (69.0 mg, 0.19 mmol).

Physical data for **7a**

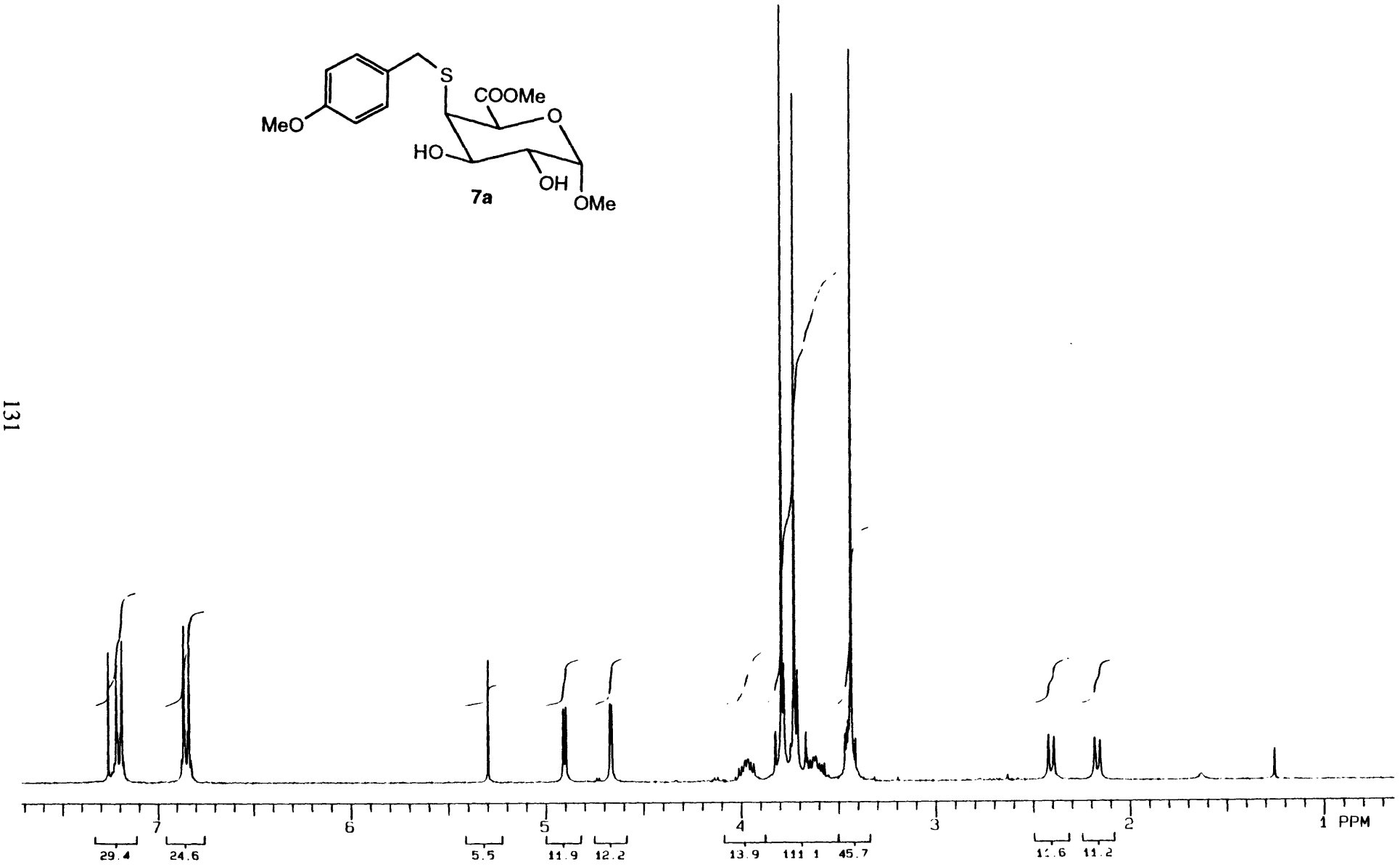
R_f = 0.5 (dichloromethane/acetone = 3:1); IR (film) 3455 (br), 2932, 2836, 1760, 1610, 1512, 1437, 1245 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, 2H, *J* = 8.8), 6.82 (d, 2H, *J* = 8.8), 4.88 (d, 1H, *J* = 3.8), 4.64 (d, 1H, *J* = 2.1), 3.95-4.01 (m, 1H), 3.77 (s, 3H), 3.74 (d, 1H, *J* = 13.1), 3.69 (s, 3H), 3.69 (d, 1H, *J* = 13.1), 3.60-3.68 (m, 1H), 3.43 (dd, 1H, *J* = 4.6, 2.1), 3.41 (s, 3H); ^{13}C NMR (75 MHz) δ 168.3, 158.3, 129.9, 129.4, 113.4, 99.7, 70.0, 69.7, 55.6, 54.8, 51.8, 49.9, 49.3, 36.9; HRMS. Calcd. for $C_{16}H_{23}O_7S$ $(M + H)^+$: 359.1164; found: 359.1154.

Physical data for **7b**

R_f = 0.55 (dichloromethane/acetone = 3:1); IR (film) 3444 (br), 2930, 2846, 1748, 1610, 1513, 1440, 1247, 1175 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, 2H, *J* = 8.8), 6.85 (d, 2H, *J* = 8.8), 4.84 (d, 1H, *J* = 3.1), 4.21 (d, 1H, *J* = 11.2), 3.81 (s, 3H), 3.79 (s, 3H), 3.75 (d, 2H, *J* = 2.1), 3.62 (m, 1H), 3.60 (m, 1H), 3.45 (s, 3H), 2.80 (dd, 1H, *J* = 11.2, 10.6); ^{13}C NMR (75 MHz) (δ) 169.2, 159.0, 130.1, 129.6, 114.1, 100.0, 73.0, 71.5, 55.9, 55.2, 52.6, 48.6, 46.9, 35.4; HRMS. Calcd. for $C_{16}H_{22}O_7SNa$ $(M + Na)^+$: 381.0984; found: 381.0976.

Physical data for **7c**

R_f = 0.7 (dichloromethane/acetone = 3:1); IR (film) 3474 (br), 2934, 1743, 1610, 1512, 1440, 1245, 1174, 1146 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, 2H, *J* = 8.8), 6.83 (d, 2H, *J* = 8.8), 4.71 (d, 1H, *J* = 1.9), 4.20 (d, 1H, *J* = 9.0), 3.94-3.98 (m, 1H), 3.78 (s, 3H), 3.75-3.80 (m, 1H), 3.73 (s, 3H), 3.73 (s, 2H), 3.49 (s, 3H), 3.43 (dd, 1H, *J* = 9.0, 3.1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 159.0, 130.0, 129.2, 114.1, 99.5, 74.1, 69.4, 68.9, 57.1, 55.3, 52.3, 45.7, 35.9; HRMS. Calcd. for $C_{16}H_{22}O_7SNa$ $(M + Na)^+$: 381.0984; found: 381.0998.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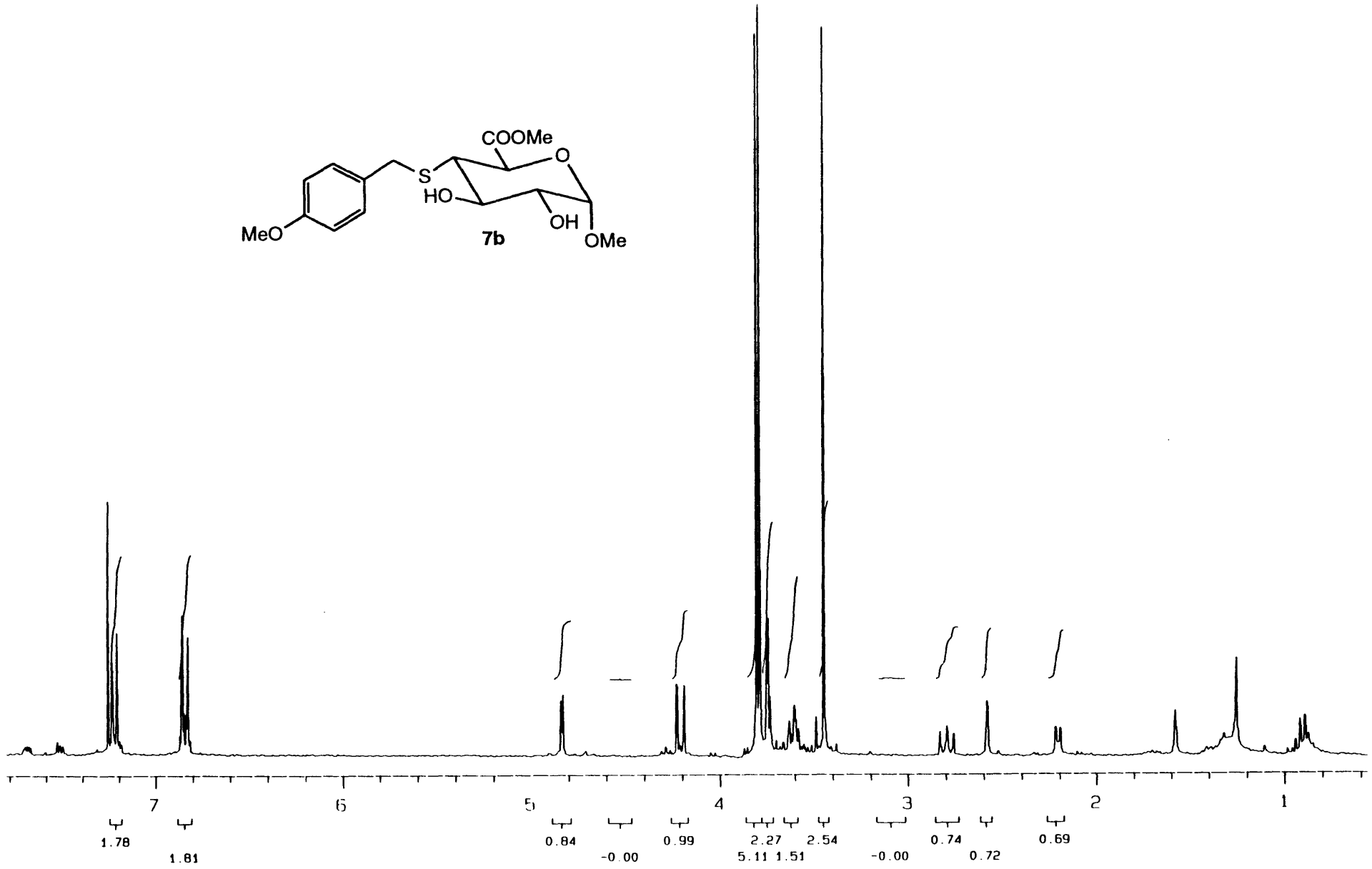
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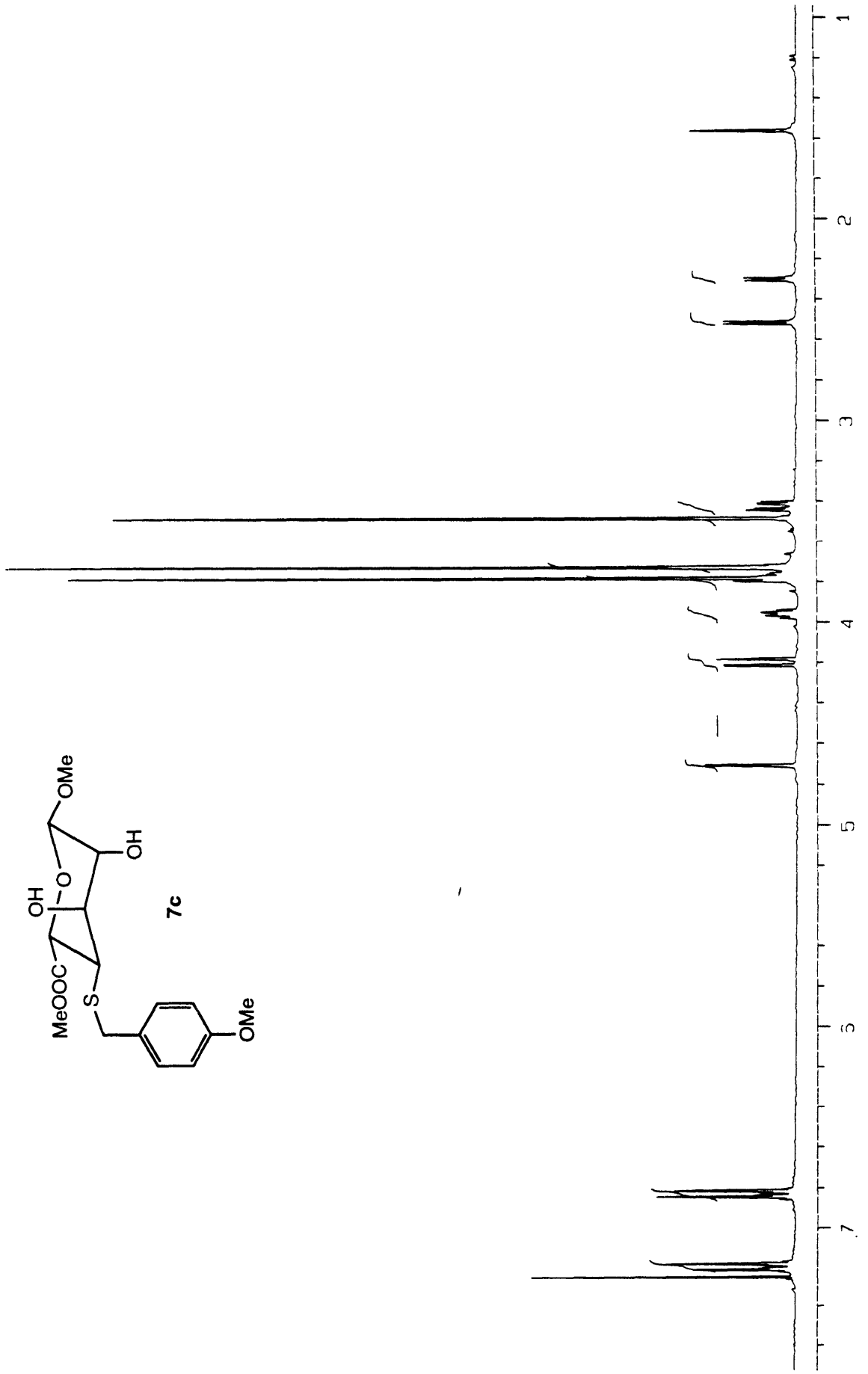
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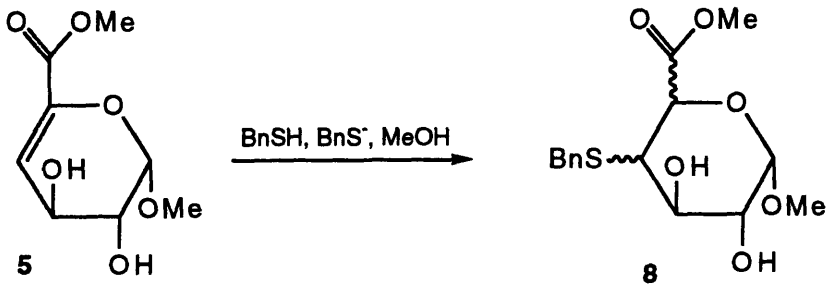


A solution of **5** (142 mg, 0.70 mmol) in 7 mL methanol was added to a mixture of lithium methoxide (33.0 mg, 0.86 mmol) and benzyl mercaptan (317 mg, 2.55 mmol). The reaction was stirred for 72 hours at room temperature under argon, at which point TLC indicated the near-absence of starting material but showed faint traces of saponified reactants. The solution was neutralized and concentrated as described for **7**. The resultant oil was purified by flash chromatography (dichloromethane/acetone = 4:1) to give fractions of **8a** as a pale yellow oil and **8b** and **8c** as colorless oils (153 mg, 0.47 mmol) in 67% combined yield.

Physical data for **8a**

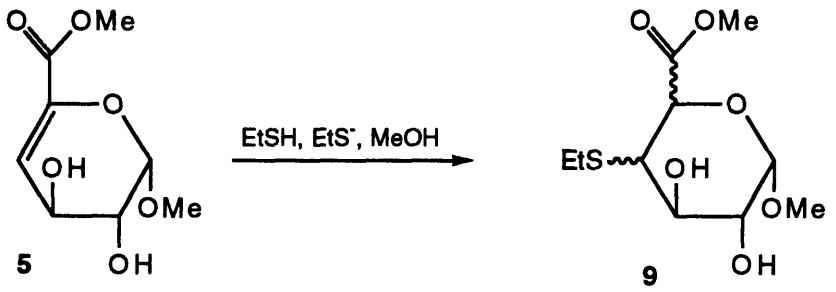
R_f = 0.5 (dichloromethane/acetone = 3:1); IR (film) 3456 (br), 2950, 2841, 1760, 1602, 1495, 1454, 1438, 1360, 1217, 1140, 1086, 1058, 1010 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.91 (d, 1H, *J* = 3.8), 4.65 (d, 1H, *J* = 2.3), 4.00 (dd, 1H, *J* = 9.9, 4.6), 3.85 (d, 1H, *J* = 13.2), 3.74 (d, 1H, *J* = 13.2), 3.68 (s, 3H), 3.66 (dd, 1H, *J* = 9.9, 3.8), 3.46 (dd, 1H, *J* = 4.6, 2.3), 3.44 (s, 3H). HRMS: Calcd. for $C_{15}H_{21}O_6S$ $(M + H)^+$: 329.1059; found: 329.1048.

Physical data for **8b**

R_f = 0.55 (dichloromethane/acetone = 3:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 4.85 (d, 1H, *J* = 3.3), 4.21 (d, 1H, *J* = 10.4), 3.81 (s, 3H), 3.81 (s, 2H), 3.60-3.65 (m, 2H), 3.47 (s, 3H), 2.82 (dd, 1H, *J* = 10.4, 10.2). FABMS: 329 $(M + H)^+$.

Physical data for **8c**

R_f = 0.7 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.72 (d, 1H, J = 1.6), 4.21 (d, 1H, J = 9.2), 3.95-3.98 (m, 1H), 3.76-3.80 (m, 1H), 3.78 (s, 2H), 3.73 (s, 3H), 3.50 (s, 3H), 3.45 (dd, 1H, J = 9.2, 3.0); FABMS: 332 (M + 3D)$^+$.



5 9

A solution of **5** (46.0 mg, 0.225 mmol) in 0.5 mL methanol was added to a solution of lithium methoxide (15.0 mg, 0.39 mmol) and ethanethiol (139 mgs, 2.25 mmol) in 1 mL methanol. After 30 hours, the solution was neutralized and concentrated as described for **7**. The residual oil was purified by flash chromatography (dichloromethane/acetone = 4:1) to give pure fractions of **9a**, **9b**, and **9c** as colorless oils (48.1 mg, 0.180 mmol) in 80% combined yield.

Physical data for **9a**

R_f = 0.4 (dichloromethane/acetone = 3:1); IR (film) 3432 (br), 2931, 1757, 1438, 1355, 1189 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (d, 1H, *J* = 4.0), 4.72 (d, 1H, *J* = 2.2), 3.99-4.05 (m, 1H) 3.84 (s, 3H), 3.54-3.60 (m, 1H), 3.47 (s, 3H), 3.46 (dd, 1H, *J* = 4.7, 2.2), 2.63 (q, 2H, *J* = 7.4), 1.25 (t, 3H, *J* = 7.4); HRMS: Calcd. for $C_{10}H_{19}O_6S$ $(M + H)^+$: 267.0902; found: 267.0908.

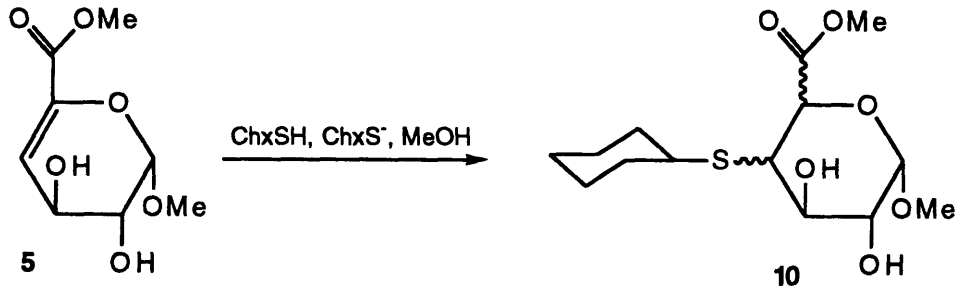
Physical data for **9b**

R_f = 0.45 (dichloromethane/acetone = 3:1); ^1H NMR (300 MHz, $CDCl_3$) δ 4.87 (d, 1H, *J* = 3.2), 4.19 (d, 1H, *J* = 11.1), 3.82 (s, 3H), 3.61-3.70 (m, 2H), 3.46 (s, 3H), 2.80 (dd, 1H, *J* = 11.1, 10.2), 2.57-2.68 (m, 2H), 1.25 (t, 3H, *J* = 7.4); FABMS: 267 $(M + H)^+$.

Physical data for **9c**

R_f = 0.65 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75 (d, 1H, J = 1.6), 4.26 (d, 1H, J = 9.5), 4.08-4.12 (m, 1H), 3.82-3.87 (m, 1H), 3.82 (s, 3H), 3.53 (s, 3H), 3.44 (dd, 1H, J = 9.5, 3.1), 2.60 (q, 2H, J = 7.4), 1.27 (t, 3H, J = 7.4)

FABMS: 267 $(M + H)^+$.



A solution of **5** (44.0 mg, 0.22 mmol) in 0.5 mL methanol was added to a solution of lithium methoxide (14.3 mg, 0.38 mmol) and cyclohexyl mercaptan (251 mgs, 2.16 mmol) in 1 mL methanol. The reaction was stirred for 3 days at room temperature under argon, then neutralized and concentrated as described for **7**. Flash chromatography (dichloromethane/acetone = 4:1) was used to purify **10a**, **10b**, and **10c** as colorless oils (60.3 mg, 0.19 mmol) in 87% combined yield.

Physical data for **10a**

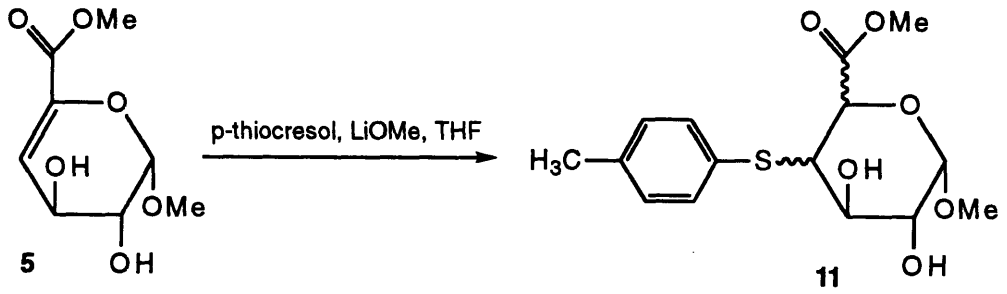
R_f = 0.5 (dichloromethane/acetone = 3:1); IR (film) 3390 (br), 2929, 2851, 1759, 1447, 1221, 1186 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.89 (d, 1H, *J* = 3.9), 4.72 (d, 1H, *J* = 2.3), 3.97-4.03 (m, 1H), 3.84 (s, 3H), 3.52-3.58 (m, 1H), 3.52 (dd, 1H, *J* = 4.7, 2.3), 3.44 (s, 3H), 1.0-2.8 (m, 11H, cyclohexyl protons); HRMS: Calcd. for $C_{14}H_{25}O_6S$ $(M + H)^+$: 321.1372; found: 321.1371.

Physical data for **10b**

R_f = 0.6 (dichloromethane/acetone = 3:1); ^1H NMR (300 MHz, $CDCl_3$) δ 4.88 (d, 1H, *J* = 3.7), 4.17 (d, 1H, *J* = 10.6), 3.81 (s, 3H), 3.52-3.71 (m, 1H), 3.46 (s, 3H), 2.85 (dd, 1H, *J* = 10.6, 10.2), 1.0-2.8 (m, 11H); FABMS: 321 $(M + H)^+$.

Physical data for **10c**

R_f = 0.75 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, 1H, *J* = 1.7), 4.24 (d, 1H, *J* = 9.4), 4.03-4.08 (m, 1H), 3.80-3.85 (m, 1H), 3.81 (s, 3H), 3.52 (s, 3H), 3.50 (dd, 1H, *J* = 9.4, 3.1), 1.0-2.8 (m, 11H); FABMS: 321$(M + H)^+$.



A solution of **5** (100 mg, 0.49 mmol) in 3 mL THF was added to a suspension of lithium methoxide (34.0 mg, 0.89 mmol) and *para*-thiocresol (243 mg, 1.96 mmol) in 1 mL THF. The reaction was stirred for 3 days at room temperature under argon (at which time a substantial amount of starting material was still present), then neutralized with cation exchange resin, rinsed with methanol, and concentrated under reduced pressure and high vacuum. Flash chromatography (dichloromethane/acetone = 5:1) was used to purify **11a**, **11c**, and **11d** (**11d** virtually coeluted with **11b** under various conditions and was characterized as a 10/1 ratio of **11d**:**11b**) as colorless oils (52.6 mg, 0.16 mmol) in 32% combined yield.

Physical data for **11a**

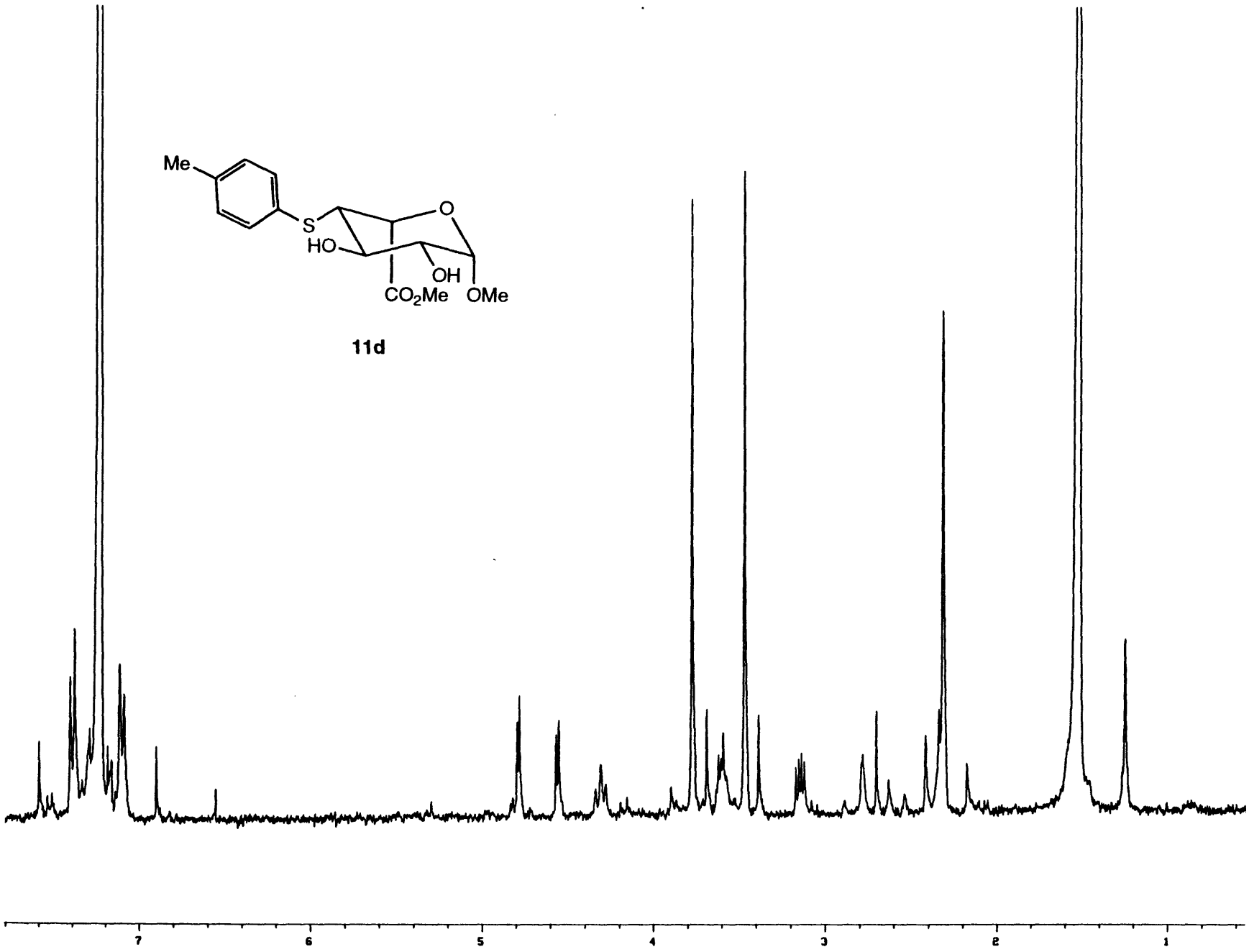
R_f = 0.5 (dichloromethane/acetone = 3:1); IR (film) 3419 (br), 2927, 1760, 1494, 1440 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, 2H, *J* = 7.8), 7.11 (d, 2H, *J* = 7.8), 4.98 (d, 1H, *J* = 3.8), 4.72 (d, 1H, *J* = 2.1), 4.08-4.13 (m, 1H), 3.88 (dd, 1H, *J* = 4.6, 2.1), 3.65-3.71 (m, 1H), 3.61 (s, 3H), 3.49 (s, 3H), 2.32 (s, 3H); HRMS: Calcd. for $C_{15}H_{21}O_6S$ $(M + H)^+$: 329.1059; found: 329.1047.

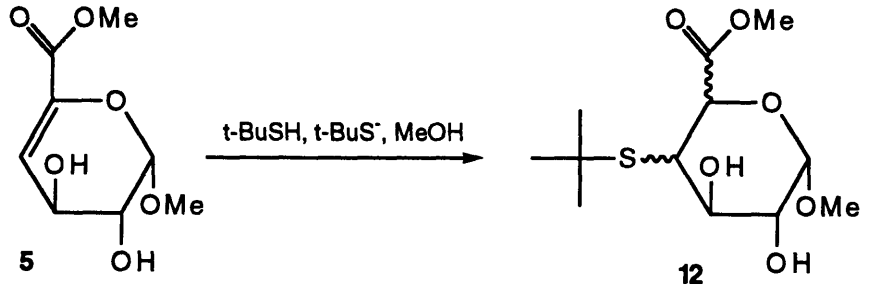
Physical data for **11c**

R_f = 0.75 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, 2H, *J* = 7.9), 7.14 (d, 2H, *J* = 7.9), 4.80 (d, 1H, *J* = 1.9), 4.36 (d, 1H, *J* = 9.0), 4.08-4.14 (m, 1H), 3.82-3.85 (m, 1H), 3.81 (dd, 1H, *J* = 8.9, 3.5), 3.76 (s, 3H), 3.54 (s, 3H), 2.33 (s, 3H); FABMS: 329 $(M + H)^+$.

Physical data for **11d**

R_f = 0.55 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, 2H, *J* = 8.1), 7.12 (d, 2H, *J* = 8.1), 4.80 (d, 1H, *J* = 3.6), 4.57 (d, 1H, *J* = 4.9), 4.32 (dd, 1H, *J* = 9.8, 8.0), 3.79 (s, 3H), 3.58-3.64 (m, 1H), 3.48 (s, 3H), 3.16 (dd, 1H, *J* = 9.8, 4.9); 2.32 (s, 3H); FABMS: 332 $(M + 3D)^+$.


Me
S
HO
O
OH
CO2Me
OMe
11d
7
6
5
4
3
2
1

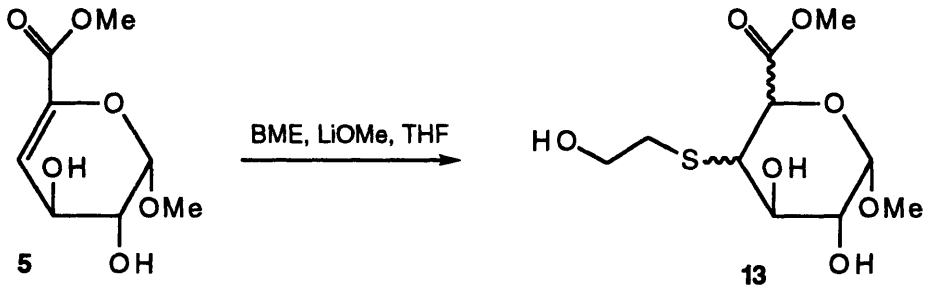


5 12

This product was prepared in accordance with the general reaction procedure in methanol . Only a small percentage (6% in 24 hours under standard conditions) of **5** was converted into products. Flash chromatography (ethyl acetate/hexane = 10:1) was used to purify **12a** as a colorless oil:

Physical data for **12a**

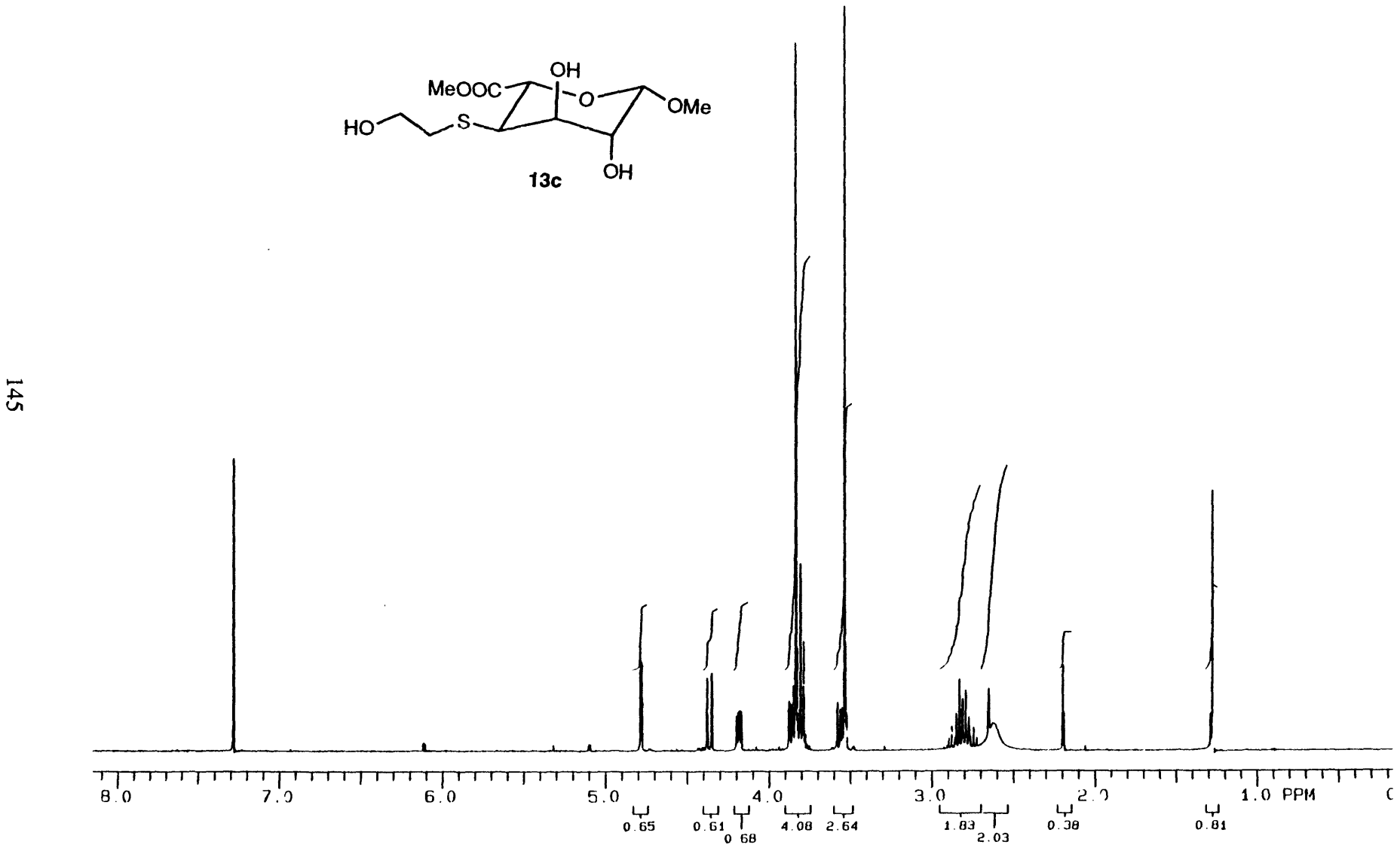
R_f = 0.5 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.89 (d, 1H, *J* = 3.9), 4.75 (d, 1H, *J* = 2.2), 3.97-4.03 (m, 1H), 3.80 (s, 3H), 3.52-3.58 (m, 1H), 3.51 (dd, 1H, *J* = 4.8, 2.2), 3.45 (s, 3H), 1.31 (s, 9H) ; HRMS: Calcd. for $C_{12}H_{23}O_6S$ $(M + H)^+$: 295.1215; found: 295.1220.

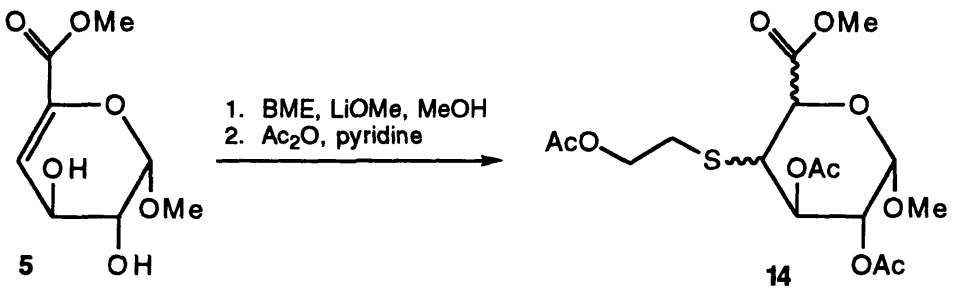


A solution of **5** (81.0 mg, 0.40 mmol) in 3 mL THF was added to a suspension of lithium methoxide (22.7 mg, 0.60 mmol) and 2-mercaptoethanol (222 mg, 2.85 mmol) in 3 mL THF. The reaction was stirred for 3 days at room temperature (at which time starting material was still present), then neutralized with cation exchange resin, rinsed with methanol, and concentrated *in vacuo*. Flash chromatography (dichloromethane/acetone = 2:1) was used to purify **13c** as a colorless oil (38.4 mg, 0.14 mmol) in 34% yield. The D-*galacto* isomer **13a** was not purified to homogeneity from the reaction mixture, and instead was fully characterized in its peracetylated form (see **14a**).

Physical data for **13c**

R_f = 0.15 (dichloromethane/acetone = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.77 (d, 1H, *J* = 2.3), 4.36 (d, 1H, *J* = 8.0), 4.18 (dd, 1H, *J* = 5.7, 3.4), 3.86 (dd, 1H, *J* = 5.7, 2.3), 3.83 (s, 3H), 3.79-3.89 (m, 2H), 3.55 (dd, 1H, *J* = 8.0, 3.4), 3.53 (s, 3H), 2.73-2.89 (m, 2H); HRMS. Calcd. for $C_{10}H_{19}O_7S$ $(M + H)^+$: 283.0851; found: 283.0852.


MeOOC
OH
O
OMe
HO
S
OH
13c
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0 PPM
0.65
0.61
0.68
4.08
2.64
1.83
2.03
0.38
0.81

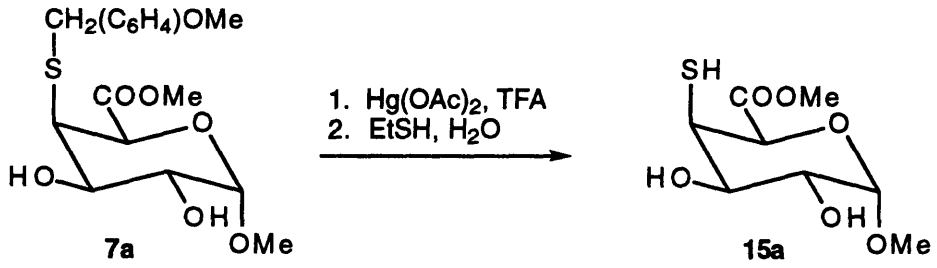


A solution of **5** (79 mg, 0.39 mmol) in methanol (2 mL) was added to a solution of 2-mercaptoethanol (195 mg, 2.5 mmol) and LiOMe (38 mg, 1 mmol) in methanol (2 mL). The reaction was stirred for 120 hours at room temperature, then neutralized and concentrated as described for **7**. Pyridine (1 mL) and acetic anhydride (0.6 mL) were added to the residual oil. After 24 hours, cold water (15 mL) was added to the solution. After stirring for 15 minutes, the aqueous solution was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were extracted with water and saturated sodium bicarbonate solution before drying over sodium sulfate. After filtration and concentration under reduced pressure, the residual oil was purified by flash chromatography (hexane/ethyl acetate = 6:5) to give **14a** as a colorless oil (41.7 mg, 0.10 mmol) in 25% yield.

Physical data for **14a**

R_f = 0.4 (hexane/ethyl acetate = 1:1); IR (film) 2954, 1741, 1439, 1372, 1230, 1137, 1026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.48 (dd, 1H, *J* = 10.6, 4.3), 5.19 (dd, 1H, *J* = 10.6, 3.7), 5.08 (d, 1H, *J* = 3.7), 4.78 (d, 1H, *J* = 2.1), 4.12 (m, 2H), 3.84 (s, 3H), 3.77 (dd, 1H, *J* = 4.3, 2.1), 3.61 (s, 3H), 2.71-2.81 (m, 2H), 2.12 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.0, 169.9, 168.1, 97.6, 70.0, 69.3, 68.9, 63.7, 56.1, 52.4, 49.7, 31.8, 20.7 (multiplet); HRMS. Calcd. for $C_{16}H_{24}O_{10}SNa$ $(M + Na)^+$: 431.0988; found: 431.0983.

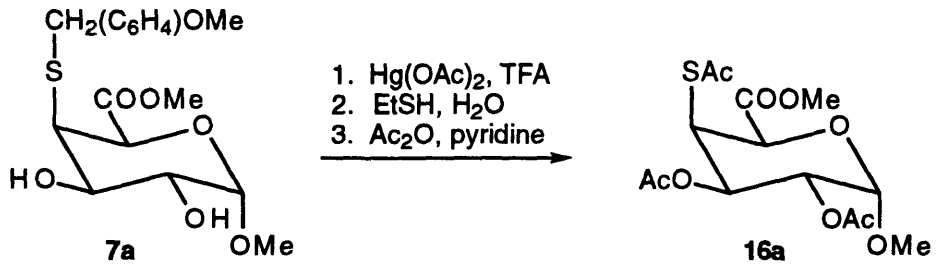
Experimentals for Chapter 4



A 0°C solution of mercuric acetate (138 mg, 0.43 mmol) in 3.5 mL TFA was added to a mixture of **7a** (150 mg, 0.42 mmol) and anisole (110 μL) at 0°C. After 15 minutes at 0°C, TFA was removed under a steady stream of argon. To the residual oil was added 25 mL water, followed by a large molar excess of ethanethiol (800 mg, 14.3 mmol). A white precipitate was immediately observed, and the suspension was stirred for 30 minutes at room temperature, then filtered and concentrated several times from water and then methanol. The residual oil was dried *in vacuo* to give **15a** as a colorless oil (90.8 mg, 0.38 mmol) in 91% yield.

Physical data for **15a**

^{1}H NMR (300 MHz, D_2O) δ 4.81 (d, 1H, J = 2.1), 4.76 (d, 1H, J = 4.1), 3.91 (dd, 1H, J = 10.2, 4.7), 3.68 (s, 3H), 3.67 (dd, 1H, J = 10.2, 4.1), 3.62 (dd, 1H, J = 4.7, 2.1), 3.28 (s, 3H).



A solution of 64 mg (0.18 mmol) **7a** in TFA was deprotected as described previously to give **15a** as an oil, to which was added 2 ml of a 5:4 pyridine/acetic anhydride solution. After 24 hours at room temperature, the reaction contents were added to cold water and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with water and saturated sodium bicarbonate solution before drying over magnesium sulfate. The solution was filtered and concentrated *in vacuo*, then purified by flash chromatography (hexane/ethyl acetate = 2:1) to give **16a** as a colorless oil (53.8 mg, 0.15 mmol) in 83% yield.

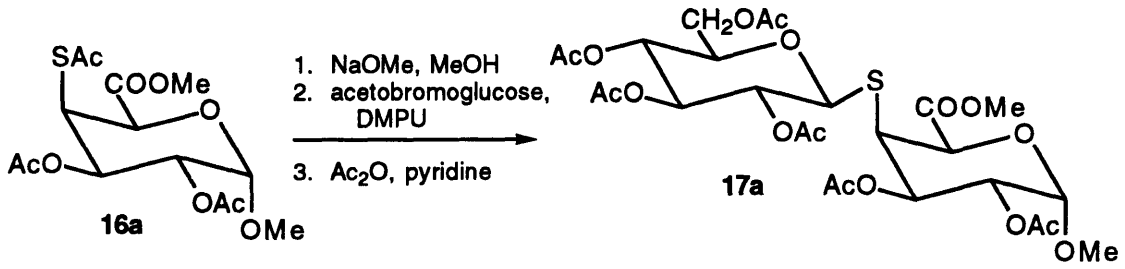
Physical data for **16a**

R_f = 0.5 (hexane/ethyl acetate = 3:2); IR (film) 2955, 1747, 1702, 1438, 1373, 1220, 1138 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.57 (dd, 1H, *J* = 10.6, 4.4), 5.09 (d, 1H, *J* = 3.7), 4.98 (dd, 1H, *J* = 10.6, 3.7), 4.87 (d, 1H, *J* = 2.2), 4.62 (dd, 1H, *J* = 4.4, 2.2), 3.75 (s, 3H), 3.43 (s, 3H), 2.36 (s, 3H), 2.09 (s, 3H), 1.96 (s, 3H); FABMS: 365 $(M + H)^+$.

Physical data for **16c**

This product was purified as the non-glycosylated (but subsequently acetylated) starting material in reactions which produced the disaccharide **17c**: R_f = 0.6 (hexane/ethyl acetate = 3:2); IR (film) 2950, 2356, 1746, 1701, 1438, 1370, 1218 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.61 (dd, 1H, *J* = 8.2, 4.1), 4.97 (dd, 1H, *J* = 8.2, 2.9), 4.88 (d, 1H, *J* = 2.9

Hz), 4.68 (dd, 1H, J = 5.2, 4.1), 4.35 (d, 1H, J = 5.2), 3.80 (s, 3H), 3.45 (s, 3H), 2.36 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H); FABMS: 365 $(M + H)^+$.

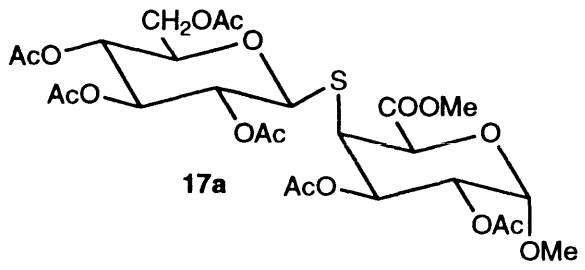


A solution of NaOMe (13.3 mg, 0.25 mmol) in methanol (2 mL) was added to a solution of **16a** (87.6 mg, 0.24 mmol) in methanol (2 mL). The solvent was evaporated overnight under a stream of argon. A solution of acetobromoglucose (402 mg, 0.98 mmol, commercial product recrystallized from ethanol) in DMPU (4 mL) was added to the residual oil. After 24 hours, a 5:4 pyridine/acetic anhydride solution (1.8 mL) was added to the reaction flask. After an additional 24 hours, the reaction contents were added to 15 ml cold water and extracted with ether (3 x 10 mL). The combined organic ether extracts were washed with water and saturated sodium bicarbonate solution before drying over magnesium sulfate. The solution was filtered, concentrated *in vacuo*, and purified by flash chromatography (hexane/ethyl acetate = 5:4) to give **17a** as a colorless oil (42.4 mg, 0.06 mmol) in 27% yield.

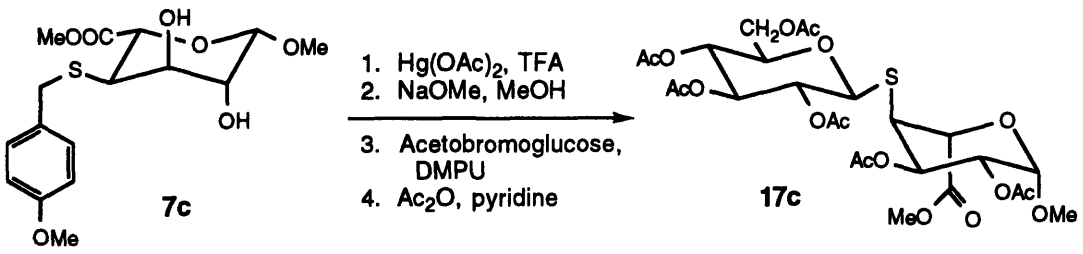
Physical data for **17a**

R_f = 0.3 (hexane/ethyl acetate = 1:1); IR (film) 2953, 1748, 1437, 1371, 1221 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.42 (dd, 1H, *J* = 10.5, 4.2), 5.23 (dd, 1H, *J* = 10.5, 3.9), 5.15 (dd, 1H, *J* = 9.3, 9.3), 5.04 (dd, 1H, *J* = 9.3, 9.3), 5.02 (d, 1H, *J* = 3.9), 4.88 (dd, 1H, *J* = 10.1, 9.3), 4.79 (d, 1H, *J* = 2.3), 4.48 (d, 1H, *J* = 10.1), 4.26 (dd, 1H, *J* = 12.5, 4.7), 4.10 (dd, 1H, *J* = 12.5, 2.3), 3.97 (dd, 1H, *J* = 4.2, 2.3), 3.77 (s, 3H), 3.55-3.61 (m, 1H), 3.41 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 169.9, 169.8, 169.6, 168.9, 168.0, 167.1, 97.5, 83.9, 75.9, 73.6, 70.6, 69.6, 69.3, 68.3, 68.1, 61.8, 56.0, 52.2,

47.8, 20.5 (m); HRMS. Calcd. for $C_{26}H_{36}O_{17}SNa$ $(M + Na)^+$: 675.1571; found: 675.1582.



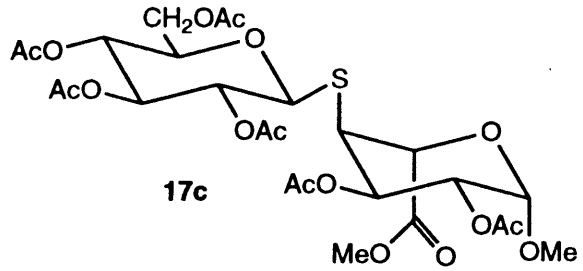
17a



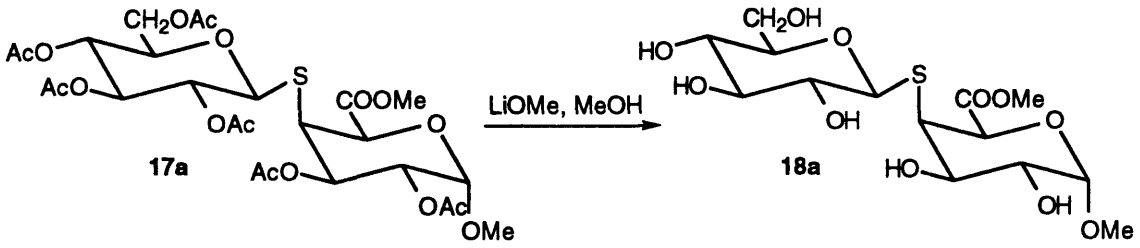
A solution of **7c** (44 mg, 0.123 mmol) in TFA was deprotected and worked up according to the procedure for **15a**. One equivalent NaOMe (6.6 mg, 0.123 mol) in methanol (1.5 mL) was added to the residual oil. The solution was evaporated overnight under a stream of argon. A solution of acetobromoglucose (53 mg, 0.129 mmol, 1.05 eq.) in 1.9 ml DMPU was added to the residual thiolate. The light pink solution was stirred for 24 hours at room temperature, turning yellow in the process. *In situ* acetylation and organic workup were performed according to the procedure used to make **17a**. Purification by flash chromatography (hexane/ethyl acetate/methylene chloride/acetone = 8:4:4:1) provided **17c** as a colorless oil (19.1 mg, 0.03 mmol) in 24% yield.

Physical data for **17c**

R_f = 0.3 (hexane/ethyl acetate = 1:1); IR (film) 2956, 2366, 1748, 1437, 1372, 1220 cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ 5.55 (dd, 1H, *J* = 8.2, 4.2), 5.19 (dd, 1H, *J* = 9.3, 9.3), 5.06 (dd, 1H, *J* = 8.2, 2.9), 5.05 (dd, 1H, *J* = 9.3, 9.3), 4.93 (dd, 1H, *J* = 10.1, 9.3), 4.86 (d, 1H, *J* = 2.9), 4.63 (d, 1H, *J* = 10.1), 4.49 (d, 1H, *J* = 4.9), 4.21 (dd, 1H, *J* = 12.5, 5.2), 4.11 (dd, 1H, *J* = 12.5, 2.2), 3.96 (dd, 1H, *J* = 4.9, 4.2), 3.81 (s, 3H), 3.64-3.71 (m, 1H), 3.42 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (75 MHz, CDCl3) δ 20.5(m), 45.0, 52.4, 57.6, 62.0, 67.6, 68.2, 68.3, 70.6, 73.7, 75.1, 76.1, 84.4, 99.3, 167.8, 169.1, 169.2, 169.3, 169.7, 170.0, 170.5; HRMS. Calcd. for $C_{26}H_{36}O_{17}SNa$ $(M + Na)^+$: 675.1571; found: 675.1566.



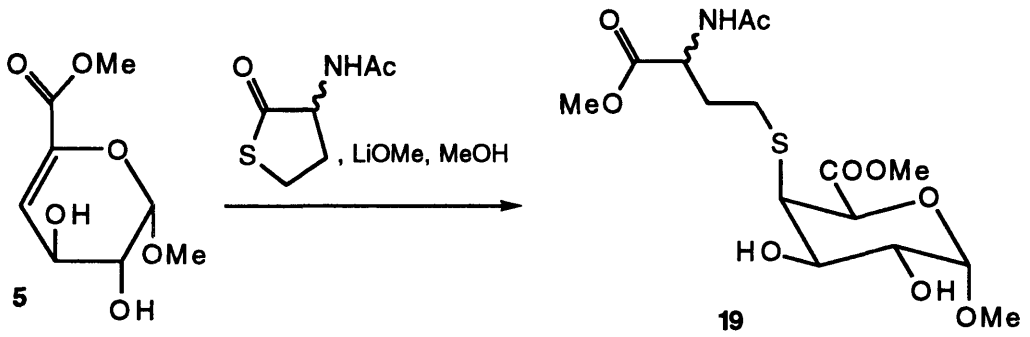
17c



A solution of LiOMe (0.3 mg, 0.008 mmol) in methanol (1 mL) was added to a solution of **17a** (7.0 mg, 0.010 mmol) in methanol (1 mL). The reaction was stirred for 45 minutes at room temperature, then neutralized by the addition of cation exchange resin. The solution was filtered and concentrated under reduced pressure to yield pure **18a** (the elimination product **5** constituted less than 1% of the final product) as a colorless oil.

Physical data for **18a**

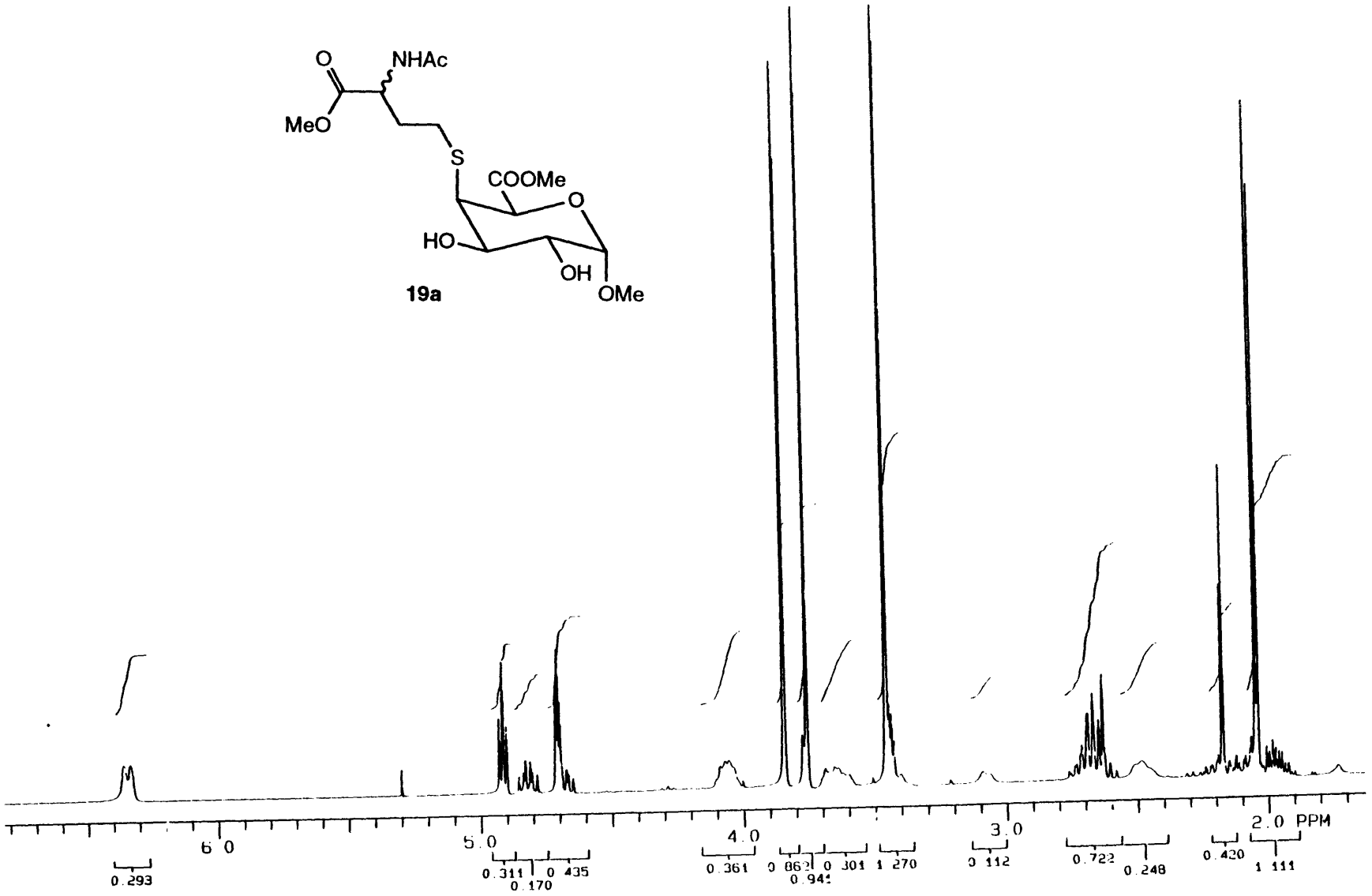
^{1}H NMR (300 MHz, CD_3OD) δ 4.80 (d, 1H, J = 2.2), 4.75 (d, 1H, J = 3.8), 4.52 (d, 1H, J = 9.8), 4.08 (dd, 1H, J = 10.0, 4.6), 3.80-3.84 (m, 2H), 3.80 (s, 3H), 3.73 (dd, 1H, J = 10.0, 3.8), 3.61-3.67 (m, 1H), 3.39 (s, 3H), 3.24-3.41 (m, 3H), 3.14 (dd, 1H, J = 9.8, 8.5); FABMS: 401 $(M + H)^+$.



A solution of lithium methoxide (23.9 mg, 0.63 mmol) in 4 mL methanol was added to powdered D,L-N-acetylhomocysteine thiolactone (480 mg, 3.02 mmol). After stirring 15 minutes at room temperature, a solution of **5** (86.6 mg, 0.43 mmol) in 3 mL methanol was added to the flask. After 72 hours, the solution was filtered through cation exchange resin. The filtrate was concentrated to an oil and purified by flash chromatograpy (dichloromethane/acetone = 4:3) to give **19a** (88.2 mg, 0.22 mmol) as an enantiomeric mixture in 52% yield.

Physical data for **19a**

R_f = 0.4 (dichloromethane/acetone = 1:1); IR (film) 3327 (br), 2921, 2355, 1739, 1654, 1542, 1436 cm^{-1}; ^1H NMR {^1H NMR of the two enantiomers are virtually identical, matching up within 0.01 ppm on all protons with the exception of the α proton of the diasteromeric amino acids.} (300 MHz, $CDCl_3$) δ 6.35 (d, 1H, *J* = 8.0), 4.92 (d, 1H, *J* = 4.1), 4.78-4.86 (m, 0.5H), 4.71 (d, 1H, *J* = 2.5), 4.66-4.70 (m, 0.5 H), 4.02-4.10 (m, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.60-3.69 (m, 1H), 3.45 (s, 3H), 3.41-3.46 (m, 1H), 2.59-2.75 (m, 2H), 1.91-2.22 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H); HRMS: Calcd. for $C_{15}H_{26}NO_9S$ $(M + H)^+$: 396.1328; found: 396.1338.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MeO
S
COOMe
O
HO
OH
OMe
19a
6.0
5.0
4.0
3.0
2.0 PPM
0.293
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0.941
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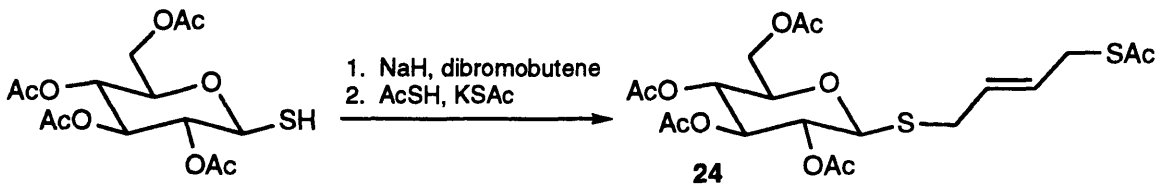
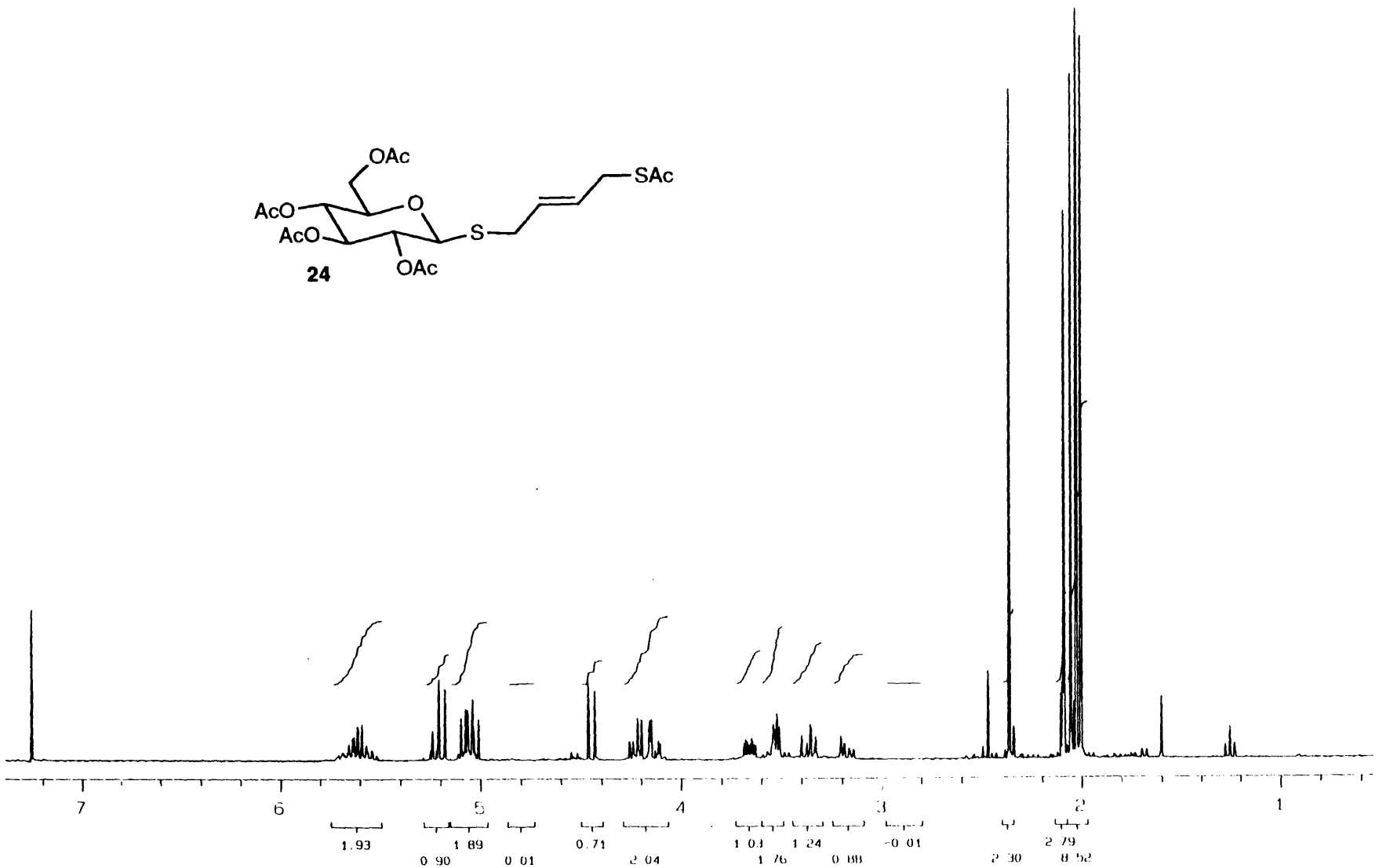
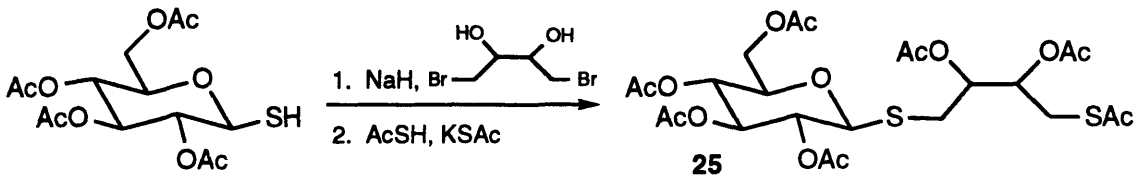


A 0°C solution of 1-thio-β-D-glucose tetraacetate (119 mg, 0.32 mmol) in THF (8 mL) was added to a mixture of sodium hydride (24 mg, 1.0 mmol) and 1,4-dibromobutene (trans/cis = 10:1). The starting sugar was consumed within five minutes, and thiolacetic acid (200 uL) and potassium thiolacetate (250 mg) were added to the flask. After stirring 24 hours, the reaction was partitioned between ether (10 mL) and water (15 mL). The aqueous layer was extracted with ether (2 x 10 mL), and the combined organic extracts were washed with brine (1 x 20 mL), then dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 3:1) afforded **24** (105.5 mg, 0.21 mmol) as a colorless oil in 67% yield.

Physical data for **24**

R_f = 0.2 (hexane/EtOAc = 3:1); IR (film) 1754, 1691, 1368, 1225, 1039 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.52-5.71 (m, 2H), 5.22 (dd, 1H, *J* = 8.9, 8.9), 5.01-5.11 (m, 2H), 4.45 (d, 1H, *J* = 10.2), 4.23 (dd, 1H, *J* = 12.5, 5.2), 4.14 (dd, 1H, *J* = 12.5, 5.6), 3.63-3.69 (m, 1H), 3.53-3.55 (m, 2H), 3.39 (dd, 1H, *J* = 13.2, 5.6), 3.18 (dd, 1H, *J* = 13.2, 5.6), 2.37 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H); FABMS: 493 $(M + H)^+$.

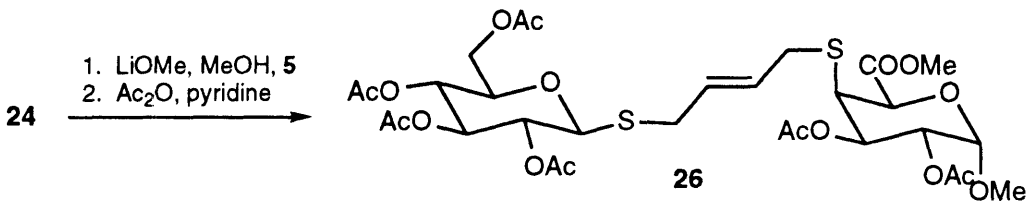

OAc
AcO
AcO
O
S
SAc
OAc
24
7
6
5
4
3
2
1
1.93
1 89
0 90
0 01
0.71
2 04
1 03
1 24
1 76
0 88
-0 01
2 30
2 79
0 52



A solution of (±) 1,4-dibromo-2,3-butanediol (2.02 g, 8 mmol) in THF (6 mL) was added to a suspension of 1-thio-β-D-glucose tetraacetate (701 mg, 1.92 mmol) and sodium hydride (176 mg, 7.8 mmol) in THF (15 mL) at 0°C. The starting sugar was consumed within ten minutes, and thiolacetic acid (1.5 mL, 20 mmol) and DIEA (1.7 mL, 10 mmol) were added. After stirring 24 hours, the reaction was partitioned between ether (100 mL) and water (100 mL). The aqueous layer was extracted with ether (2 x 75 mL), and the combined organic extracts were concentrated and washed with water (1 x 100 mL) and brine (1 x 75 mL), then dried over sodium sulfate, filtered, and concentrated to a syrup. Purification by flash chromatography (hexane/EtOAc = 1.8:1) afforded **25** (309 mg, 0.51 mmol) as a mixture of enantiomers in 26% yield.

Physical data for **25**

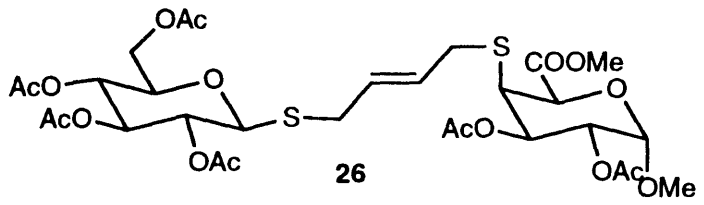
R_f = 0.5 (hexane/EtOAc = 1:1); IR (film) 2944, 1747, 1698, 1372, 1218, 1041 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.96-5.32 (m, 5H), 4.56 (dd, 1H, *J* = 10.0, 5.5), 4.09-4.30 (m, 2H), 3.73-3.79 (m, 1H), 3.29 (dd, 0.5H), 3.24 (dd, 0.5H), 2.93-3.04 (m, 1.5H), 2.87 (dd, 0.5 H), 2.76 (dd, 0.5H), 2.64 (dd, 0.5H), 2.37 (s, 3H), 2.13 (s, 1.5H), 2.13 (s, 1.5H), 2.10 (s, 6H), 2.06 (s, 1.5 H), 2.06 (s, 1.5H), 2.05 (s, 1.5H), 2.05 (s, 1.5H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H); FABMS: 611 $(M + H)^+$.



A solution of lithium methoxide (3.6 mg, 0.09 mmol) in methanol (0.4 mL) was added to a mixture of the pseudodisaccharide **24** (127 mg, 0.26 mmol) and the α,β-unsaturated ester **5** (24.0 mg, 0.12 mmol) in methanol (0.5 mL). After 96 hours, cation exhange resin was added. The resin was filtered off and the filtrate was concentrated. The residual oil was treated with pyridine (0.7 mL) and acetic anhydride (0.5 mL). After 24 hours, water (5 mL) was added, and the aqueous solution was extracted with dichloromethane (3 x 5 mL). The combined organic extracts were concentrated to an oil, taken up in dichloromethane (10 mL), and washed with saturated sodium bicarbonate (1 x 10 mL), $KHSO_4$ (1 x 10 mL), and brine (1 x 10 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 1.3:1) afforded **26** (9.1 mg, 0.012 mmol) as a colorless oil in 10% yield. The major product of the reaction was a disulfide formed between two molecules of the pseudodisaccharide **24**.

Physical data for **26**

R_f = 0.3 (hexane/EtOAc = 1:1); IR (film) 1748, 1371, 1224, 1037 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.43-5.49 (m, 3H), 5.02-5.30 (m, 5H), 4.82 (d, 1H, *J* = 2.2), 4.56 (d, 1H, *J* = 10.1), 4.24 (dd, 1H, *J* = 12.5, 4.6), 4.16 (dd, 1H, *J* = 12.5, 2.5), 3.85 (s, 3H), 3.76-3.80 (m, 1H), 3.67 (dd, 1H, *J* = 4.9, 2.5), 3.42 (s, 3H), 3.37 (dd, 1H, *J* = 14.0, 7.5), 3.22 (dd, 1H, *J* = 14.0, 4.9), 3.14 (d, 2H, *J* = 5.7), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H); FABMS: 739 $(M + H)^+$.


OAc
AcO
AcO
O
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OAc
S
COOMe
O
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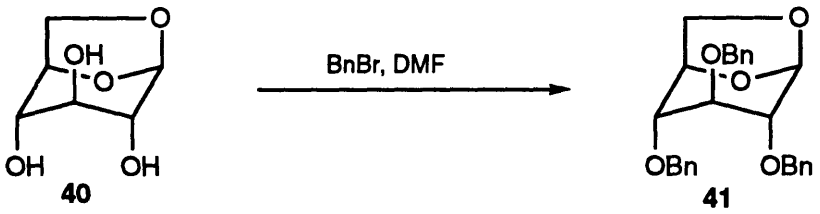
OAc
OMe

26

5.5
5.0
4.5
4.0
3.5
3.0 PPM
1.01
3.45
0.60
0.58
1.48
2.60
0.62
2.64
1.00

Experimentals for Chapter 5

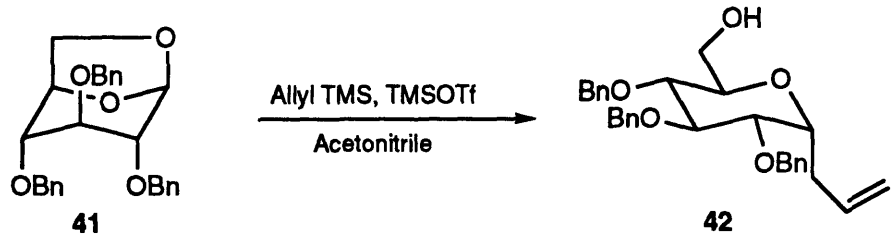


40 41

A solution of 1,6-β-D-anhydroglucose **40** (1.00 g, 6.17 mmol) in DMF (20 mL) was added to a 0°C suspension of NaH (1.1 g, 27.5 mmol, 60% dispersion in mineral oil, washed with hexanes) in DMF (10 mL) and stirred for 15 minutes before being treated with benzyl bromide (7 mL, 58 mmol). The ice bath was removed and the reaction was allowed to proceed at room temperature for 16 hours. Methanol (20 mL) was added and 15 minutes later, the reaction was partitioned between EtOAc (50 mL) and water (30 mL). The aqueous layer was extracted with EtOAc (1 x 50 mL), and the combined organic extracts were washed successively with water (2 x 50 mL), sodium bicarbonate (2 x 50 mL), $KHSO_4$ (1 x 50 mL), and brine (1 x 50 mL). The organic solution was dried over magnesium sulfate, filtered, and concentrated to an oil that was purified by flash chromatography (hexane/EtOAc = 3:1) and then recrystallized from ethanol to afford **41** (1.88 g, 4.35 mmol) as white crystals in 70% yield.

Physical data for **41**

R_f = 0.6 (hexane/ethyl acetate = 2:1); IR (film) 2918, 1454, 1073, 1028 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.21-7.35 (m, 15H), 5.46 (d, 1H, $J < 1$), 4.51-4.64 (m, 5H), 4.45 (d, 1H, J = 12.1), 4.40 (d, 1H, J = 12.1), 3.91 (dd, 1H, J = 7.2, 1.0), 3.68 (dd, 1H, J = 7.2, 5.9), 3.58-3.60 (m, 1H), 3.34-3.35 (m, 2H); FABMS: 433 $(M + H)^+$.

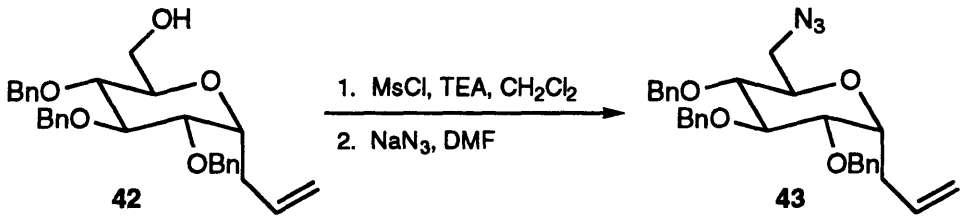


41 42

A solution of **41** (1.088 g, 2.52 mmol) and allyl TMS (1.25 mL, 7.86 mmol) in acetonitrile (11 mL) at 0°C was treated with TMSOTf (500 uL, 2.60 mmol). The ice bath was removed and the reaction was stirred 16 hours, then added to 50 mL cold saturated sodium bicarbonate solution. The aqueous solution was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic extracts were washed with brine (1 x 50 mL), dried over magnesium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate 3.8:1) afforded the α-C-glycoside **42** (459 mg, 0.97 mmol) as a white solid in 38% yield. A second product, presumably the β anomer, was isolated but not purified to homogeneity.

Physical data for **42**

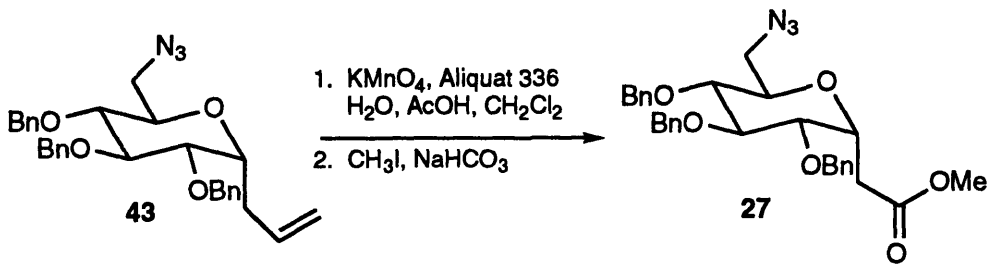
R_f = 0.3 (hexane/ethyl acetate = 3:1); IR (film) 3250, 2899, 1453, 1096, 1067, 1038 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28-7.37 (m, 15H), 5.70-5.83 (m, 1H), 5.07-5.14 (m, 2H), 4.61-4.96 (m, 6H), 4.01-4.09 (m, 1H), 3.48-3.84 (m, 6H), 2.46-2.51 (m, 2H), 1.77 (t, 1H, J = 6.4); FABMS: 475 $(M + H)^+$.



Methanesulfonyl chloride (100 uL, 1.29 mmol) was added to a 0°C solution of the alcohol **42** (388 mg, 0.82 mmol) and TEA (230 uL, 1.66 mmol) in CH_2Cl_2 (5 mL). The ice bath was removed and stirring was continued for 18 hours before methanol (50 uL) was added. The solution was concentrated to a solid, dissolved in EtOAc (20 mL), and washed successively with water (1 x 20 mL), sodium bicarbonate (2 x 20 mL), and brine (1 x 20 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated to an oil, which was used directly without further purification. The mesylate was dissolved in DMF (8 mL) and added to sodium azide (300 mg, 4.6 mmol). The reaction was heated to 90°C and proceeded for 20 hours before being cooled to room temperature and added to water (15 mL), causing formation of a white precipitate. The aqueous suspension was extracted with ether (3 x 15 mL), and the combined organic extracts were washed with brine (1 x 25 mL), dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate = 10:1) afforded the azide **43** (340 mg, 0.68 mmol) as a white solid in 83% yield.

Physical data for **43**

R_f = 0.4 (hexane/ethyl acetate = 10:1); IR (film) 2919, 2099, 1454, 1285, 1092 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.21-7.31 (m, 15H), 5.69-5.82 (m, 1H), 5.04-5.11 (m, 2H), 4.51-4.92 (m, 6H), 4.03-4.11 (m, 1H), 3.53-3.89 (m, 3H), 3.34-3.43 (m, 2H), 3.25 (dd, 1H, *J* = 13.7, 5.4), 2.41-2.46 (m, 2H); FABMS: 500 $(M + H)^+$.

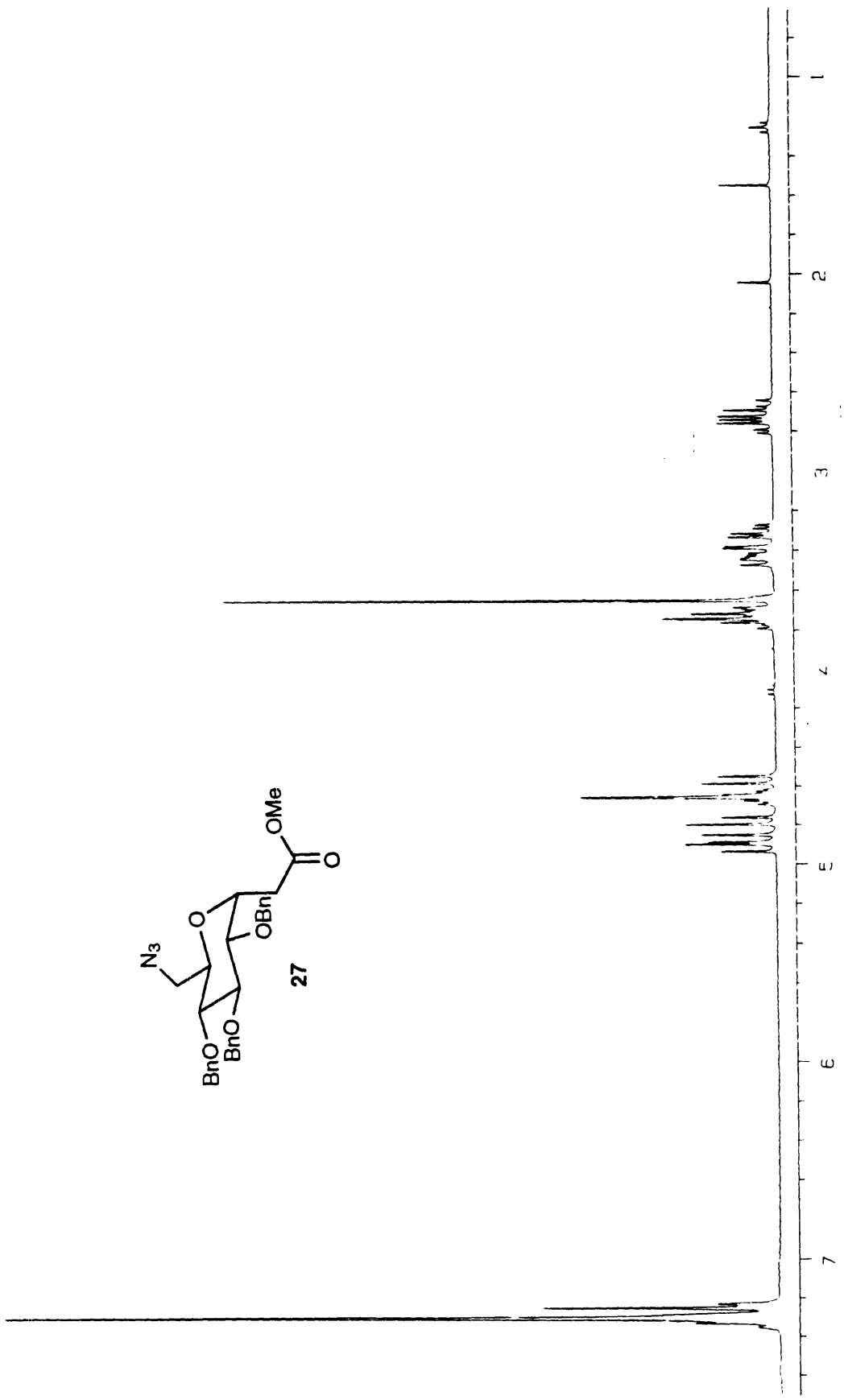


Water (1.2 mL) and acetic acid (250 uL) were added to a solution of the C-allyl glycoside **43** (116.3 mg, 0.23 mmol) in CH_2Cl_2 (1.2 mL). Aliquat 336 (15 mg) was added as a phase transfer catalyst and the reaction vessel was cooled to 0°C before addition of $KMnO_4$ (134 mg, 0.85 mmol) in two portions. The ice bath was removed and the reaction proceeded 20 hours at room temperature, at which point all starting material had been consumed. Sodium sulfite (150 mg) was added to quench the reaction, which was partitioned between CH_2Cl_2 (3 mL) and water (2.5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 mL), and the combined organic extracts were washed with brine (1 x 5 mL), dried over sodium sulfate, filtered, and concentrated to an oil that was dissolved in DMF (1 mL). Sodium bicarbonate (20 mg) was added to this solution, followed by methyl iodide (20 uL). After 40 hours, water (1 mL) was added and the reaction was partitioned between water (8 mL) and ether (8 mL). The aqueous layer was extracted with ether (2 x 5 mL), and the combined organic extracts were washed with brine (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 10:1) gave the glycosazido ester **27** (62.3 mg, 0.12 mmol) as an oil in 50% yield.

Physical data for **27**

R_f = 0.7 (hexane/ethyl acetate = 3:1); IR (film) 3030, 2908, 2100, 1738, 1283, 1091 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.23-7.36 (m, 15H), 4.88 (d, 1H, *J* = 10.9), 4.87 (d, 1H, *J* = 11.0), 4.78 (d, 1H, *J* = 10.9), 4.62-4.69 (m, 3H), 4.57 (d, 1H, *J* = 11.0),

3.69-3.80 (m, 3H), 3.65 (s, 3H), 3.38-3.47 (m, 2H), 3.30 (dd, 1H, J = 13.0, 5.4), 2.78 (dd, 1H, J = 15.0, 5.4), 2.68 (dd, 1H, J = 15.0, 9.4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 138.3, 137.8, 137.7, 127.7-128.5 (m), 81.7, 79.1, 78.4, 75.3, 75.1, 73.2, 72.0, 71.4, 51.8, 51.5, 32.5; HRMS. Calcd. for $C_{30}H_{33}N_3O_6$ $(M + H)^+$: 532.2448; found: 532.2446.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OBn
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N3
BnO
BnO
27
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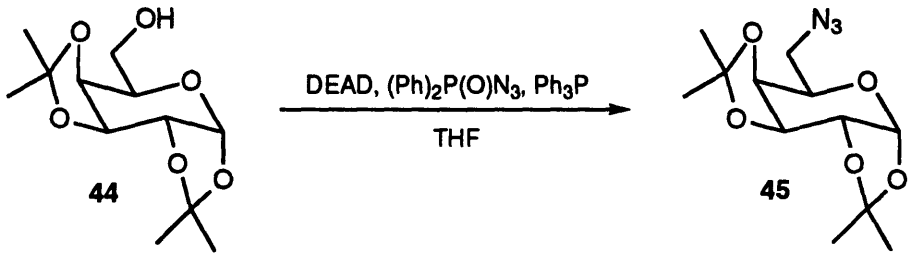
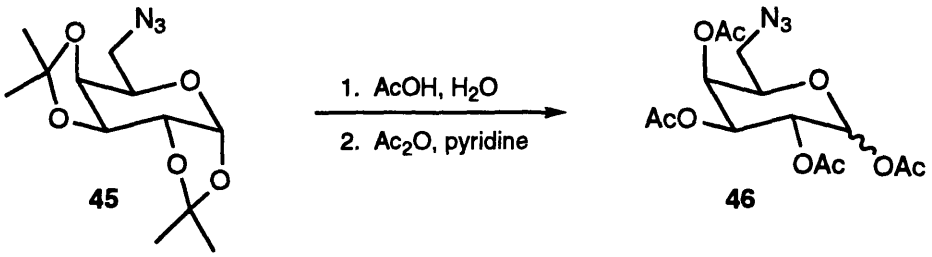
2
1



A solution of diethyl azodicarboxylate (1.10 mL, 7.0 mmol) and triphenylphosphine (1.84 g, 7.0 mmol) in THF (30 mL) at 0°C was treated with 1,2;3,4-diisopropylidene-α-D-galactopyranose **44** (1.82 g). After stirring 15 minutes, diphenyl phosphorylazide (1.50 mL, 7.0 mmol) was added. After 48 hours, the reaction was concentrated to a small volume, diluted with EtOAc (50 mL), and extracted with water (2 x 50 mL), $KHSO_4$ (2 x 50 mL), and brine (1 x 50 mL). The organic solution was dried over magnesium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/EtOAc = 10:1, then 5:1) afforded **45** (1.317 g, 4.62 mmol) as an oil in 66% yield, slightly contaminated by (presumably) triphenylphosphine oxide. Physical data for **45** matched literature values[1].

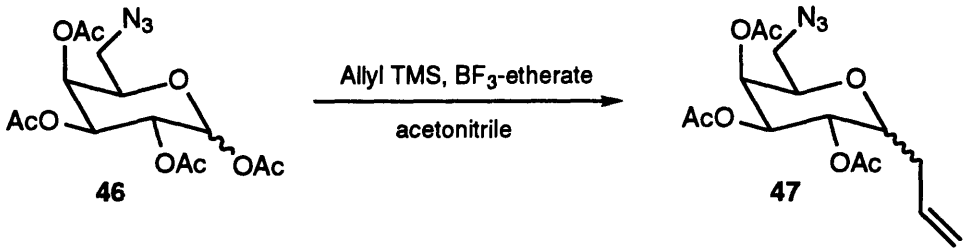


45 46

Acetic acid (1 mL) and water (0.25 mL) were added to a 10 mL flask containing the diacetonide **45** (95.5 mg, 0.34 mmol). The solution was stirred at 60°C for 18 hours, then concentrated to an oil and codistilled twice with toluene. The flask was cooled to 0°C and pyridine (1 mL) and Ac_2O (0.5 mL) were added to the residual oil. After stirring 26 hours at room temperature, the reaction contents were added to a saturated sodium bicarbonate solution (10 mL) at 0°C. The aqueous solution was extracted with dichloromethane (3 x 5 mL), and the combined organic layers were washed with water (1 x 5 mL), sodium bicarbonate (1 x 5 mL), $KHSO_4$ (1 x 5 mL), and brine (1 x 5 mL). After drying over sodium sulfate and filtering off the drying agent, the organic solution was concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 3.5:1) afforded the tetraacetate **46** (74.8 mg, 2.00 mmol) as a mixture of anomers in 60% yield.

Physical data for **46**

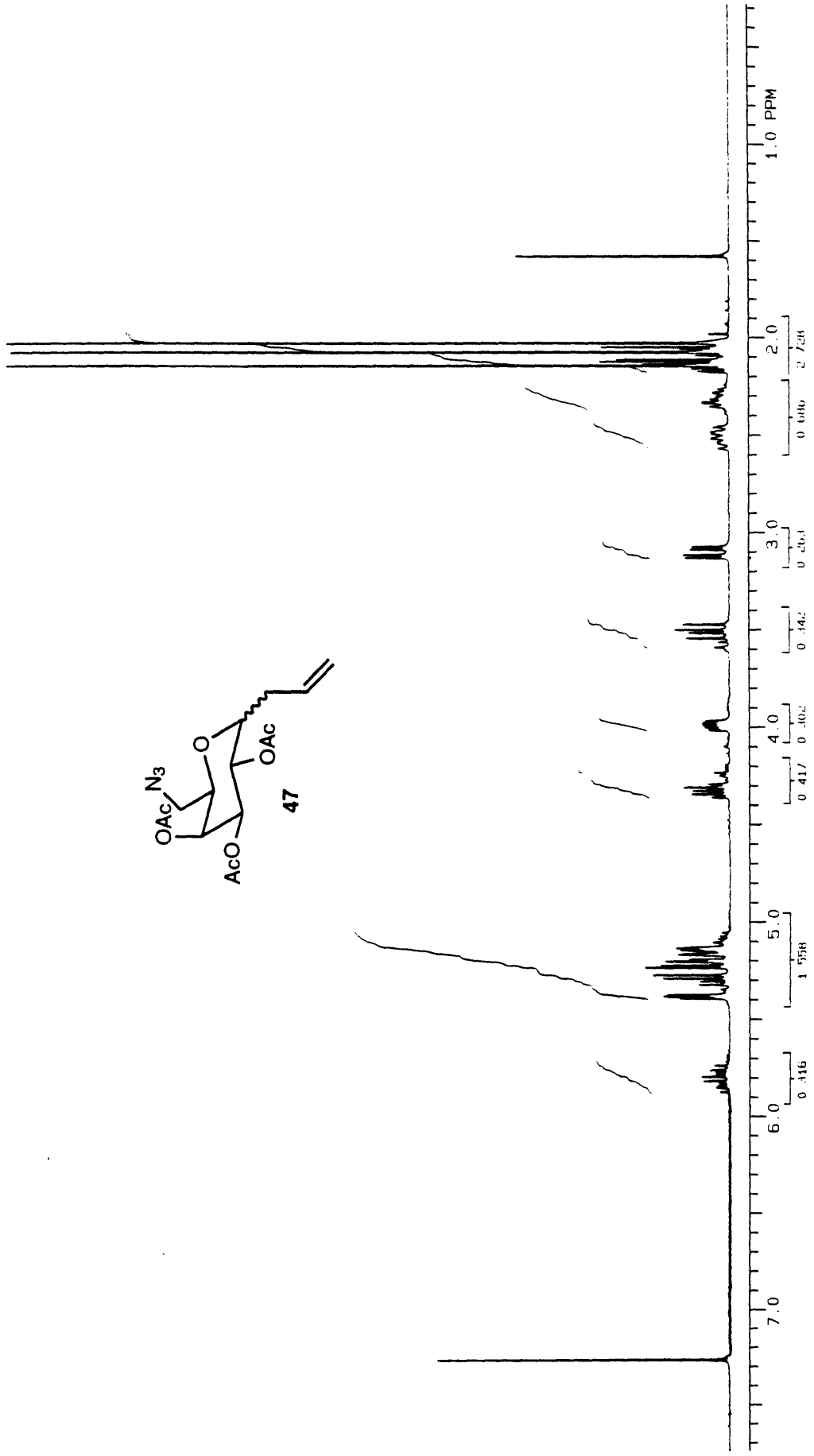
R_f = 0.5 (hexane/ethyl acetate = 2:1); IR (film) 2106, 1750, 1371, 1219, 1069 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.72 (d, 0.5H), 5.48 (d, 0.5H), 5.41 (dd, 0.5H), 5.32-5.38 (m, 1.5H), 5.06 (dd, 0.5H), 4.21-4.25.(m, 0.5H), 3.93-3.97 (m, 0.5H), 3.42-3.56 (m, 1.5H), 3.21 (dd, 1H), 2.18 (s, 1.5H), 2.17 (s, 1.5H), 2.16 (s, 1.5H), 2.13 (s, 1.5H), 2.05 (s, 1.5H), 2.03 (s, 1.5H), 2.02 (s, 1.5H), 2.01 (s, 1.5H).



A solution of the tetraacetate **46** (140 mg, 0.375 mmol) and allyl TMS (0.33 mL, 2.08 mmol) in acetonitrile (3 mL) at 0°C was treated with BF_3-etherate (0.40 mL, 3.24 mmol). The ice bath was removed and the reaction was stirred 24 hours. The reaction was not completed at that time, but TLC indicated the emergence of a side product. The reaction contents were added to 10 mL cold saturated sodium bicarbonate solution and extracted with dichloromethane (3 x 10 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 4:1) afforded the C-glycoside **47** (47.1 mg, 0.132 mmol) as a mixture of anomers in 35% yield.

Physical data for **47**

R_f = 0.4 (hexane/ethyl acetate = 3:1); IR (film) 2110, 1747, 1370, 1228, 1035 cm^{-1}; ^1H NMR (predominant α anomer from alternative route to **47**) (300 MHz, $CDCl_3$) δ 5.72-5.87 (m, 1H), 5.38 (dd, 1H), 5.06-5.32 (m, 4H), 4.29-4.36 (m, 1H), 3.97-4.02 (m, 1H), 3.51 (dd, 1H), 3.11 (dd, 1H), 2.46-2.57 (m, 1H), 2.27-2.36 (m, 1H), 2.14 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H); FABMS: 356 $(M + H)^+$.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OAc
AcO
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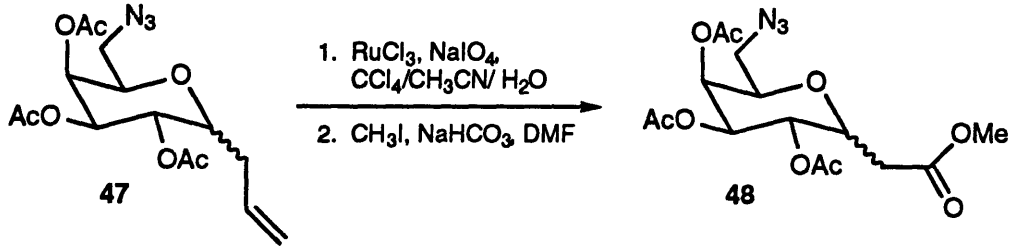
6.0
5.0
4.0
3.0
2.0
1.0 PPM

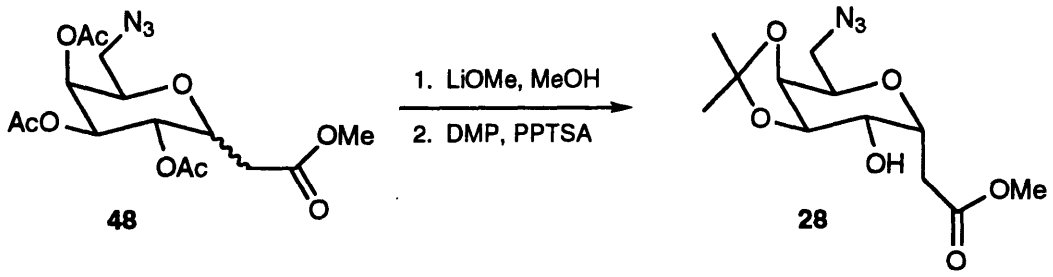


A 1:1 solution (1 mL) of acetonitrile/carbon tetrachloride was added to a mixture of the C-allyl glycoside **47** (45.0 mg, 0.127 mmol) and sodium periodate (118 mg, 0.55 mmol). Water (0.75 mL) was added to the suspension, followed by catalytic ruthenium chloride trihydrate (3 mg). The reaction immediately turned brown, and gradually developed a green tint. After one hour, isopropanol (5 mL) was added to the flask, and the contents were filtered through Celite, rinsing with EtOAc. The filtrate was concentrated to an oil. Purification by flash chromatography (CH_2Cl_2/acetone = 4:1, then added 1% acetic acid) afforded the free acid (30.6 mg, 0.082 mmol) as an anomeric mixture. ^1H NMR showed a slight impurity in addition to the anomeric mixture. The acid was subsequently esterified by dissolution in DMF (1 mL) and treatment with sodium bicarbonate (11 mg, 0.133 mmol) and methyl iodide (47.3 mg, 0.333 mmol). After 18 hours, water (1 mL) was added to the flask, and the reaction contents were partitioned between water (3 mL) and EtOAc (3 mL). The aqueous layer was extracted with EtOAc (2 x 3 mL), and the combined organic extracts were washed with water (2 x 5 mL), dried over sodium sulfate, filtered, and concentrated to provide **48** as an oil (26.1 mg, 0.067 mmol) that was not purified further (53% yield from **47**). TLC indicated that the α and β anomers virtually coeluted, with the α anomer having a slightly higher R_f value.

Physical data for **48**

R_f = 0.4 (hexane/ethyl acetate = 1.5:1); IR (film) 2103, 1746, 1372, 1221, 1058 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ {mixture of anomers, major changes from **47**: loss of

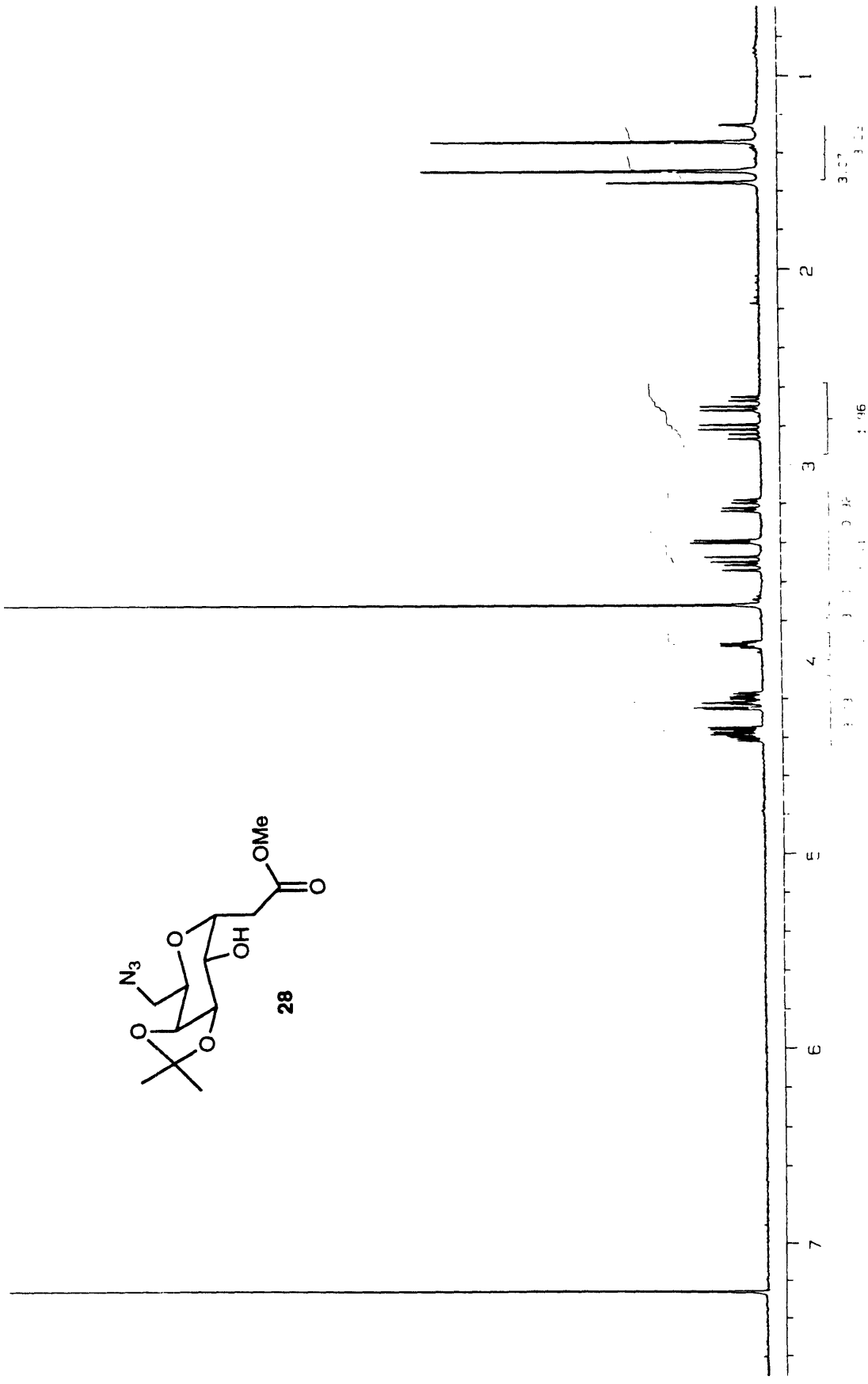
downfield multiplet 5.72-5.87 (vinylic proton), gain of 2 singlets from methyl esters at 3.73 (α), 3.71 (β)}; FABMS: 388 $(M + H)^+$.



Lithium methoxide (1 mg, 26 umol) was added to a solution of **48** (7.4 mg, 19 umol) in methanol (1 mL). After 30 minutes, cation exhange resin was added. The resin was removed by filtration, and the filtrate was concentrated. The residual oil was dissolved in dimethoxypropane (1 mL) and treated with catalytic PPTSA (1 mg). The reaction was heated on an oil bath to 45°C. After two hours, the solution was concentrated to an oil that was purified by flash chromatography (hexane/ethyl acetate = 2.5:1) to give the α-glycoside **28** (3.4 mg, 11 umol) as an oil in 59% yield. The small scale of the reaction precluded recovery of the minor anomer.

Physical data for **28**

R_f = 0.2 (hexane/ethyl acetate = 2.5:1); IR (film) 3462, 2920, 2102, 1732, 1210, 1062 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.35-4.42 (m, 2H), 4.24 (dd, 1H, *J* = 7.9, 1.3), 4.17-4.20 (m, 1H), 3.93 (dd, 1H, *J* = 6.7, 3.2), 3.73 (s, 3H), 3.51 (dd, 1H, *J* = 12.8, 7.9), 3.42 (d, 1H, *J* = 3.9), 3.21 (dd, 1H, *J* = 12.8, 4.4), 2.83 (dd, 1H, *J* = 15.0, 7.4), 2.69 (dd, 1H, *J* = 15.0, 5.8), 1.50 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 109.9, 74.2, 72.4, 69.7, 68.6, 68.2, 52.2, 51.8, 36.6, 26.5, 24.4; HRMS. Calcd. for $C_{12}H_{19}N_3O_6$ $(M + H)^+$: 302.1352; found: 302.1353.


OMe
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N_3
28
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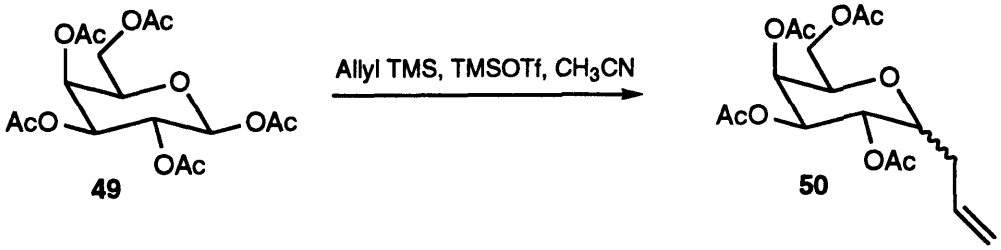
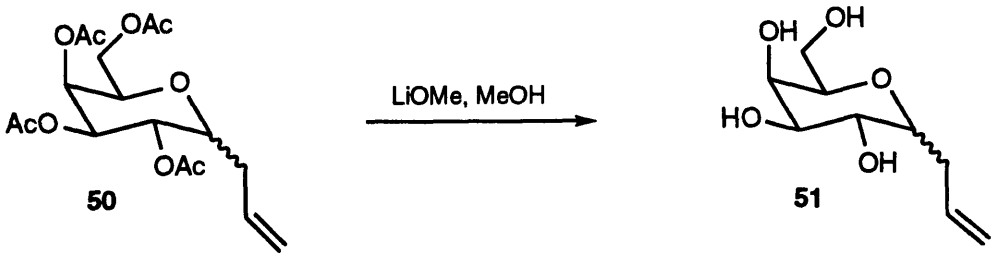


A solution of peracetylated galactose **49** (2.07 g, 5.30 mmol) and allyl TMS (4.14 mL, 26 mmol) in acetonitrile (24 mL) at 0°C was treated with TMSOTf (1.2 mL, 6.2 mmol). The ice bath was removed and the reaction was stirred 24 hours, then added to 100 mL cold saturated sodium bicarbonate solution. The aqueous solution was extracted with CH_2Cl_2 (3 x 60 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried over magnesium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate 3:1) afforded **50** (1.612 g, 4.33 mmol) as a mixture of anomers ($\alpha/\beta \approx 8$) in 82% yield.

Physical data for **50**

$R_f = 0.4$ (hexane/ethyl acetate = 2.5:1); IR (film) 2919, 1745, 1370, 1223, 1046 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ {mixture of anomers, major changes from **49**: loss of one acetyl group, gain of downfield multiplet 5.80-5.97 (vinylic proton), four acetyl singlets from α anomer are at 2.13, 2.07, 2.05, and 2.04}; FABMS: 373 $(M + H)^+$.

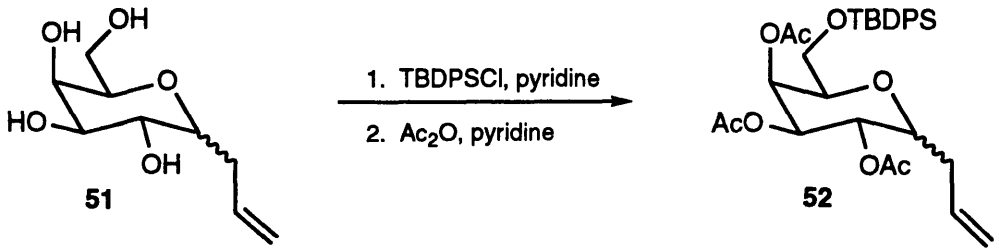


50 51

Lithium methoxide (50 mg, 1.3 mmol) was added to a solution of the tetraacetate **50** (1.60 g, 4.30 mmol) in methanol (40 mL). After 30 minutes, cation exchange resin was added to neutralized the solution. The cation exchange resin was removed by filtration and the filtrate was concentrated to a syrup. Trituration with ether removed a trace impurity as well as some of the desired product. The syrup was concentrated *in vacuo* and crystallized, affording 51 (694 mg, 3.40 mmol) as white crystals in 79% yield.

Physical data for **51**

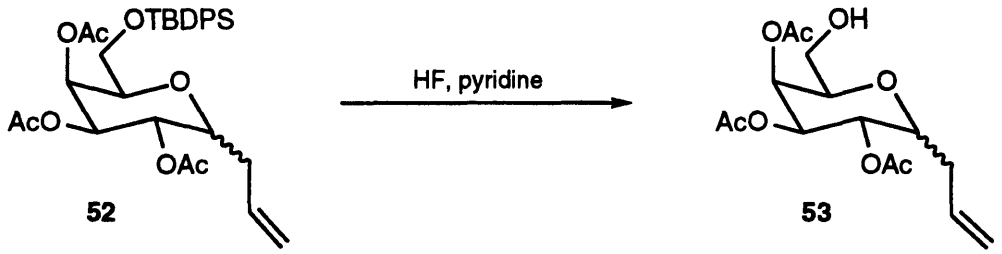
IR (film) 3356, 2922, 1643, 1076 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) {mixture of anomers, major changes from **50**: loss of four acetyl groups}; FABMS: 205 $(M + H)^+$.



A solution of the tetrahydroxy sugar **51** (290.1 mg, 1.42 mmol) in pyridine (5 mL) at 0°C was treated with *tert*-butyldiphenylsilyl chloride (560 uL, 2.1 mmol). After 14 hours, the reaction was quenched with methanol (40 uL). The reaction flask was cooled to 0°C and Ac_2O (1.5 mL) was added. After 24 hours, water (20 mL) was added and the aqueous solution was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic extracts were washed with water (1 x 30 mL), $KHSO_4$ (1 x 30 mL), sodium bicarbonate (1 x 30 mL), and brine (1 x 30 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate 5:1) afforded **52** (704 mg, 1.24 mmol) as an anomeric mixture in 87% yield.

Physical data for **52**

R_f = 0.5 (hexane/ethyl acetate = 3.3:1); IR (film) 1750, 1368, 1223, 1108 cm^{-1}; FABMS: 569 $(M + H)^+$.

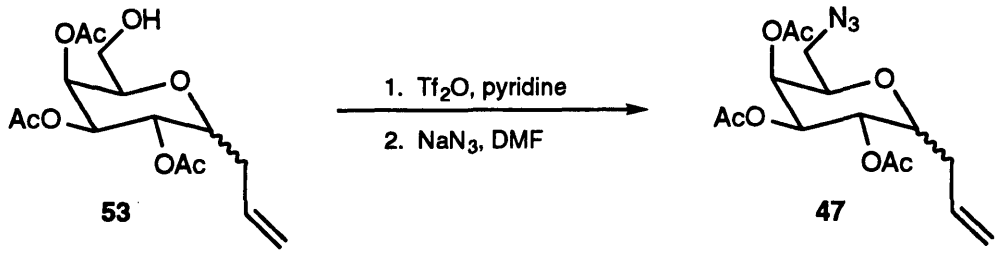


52 53

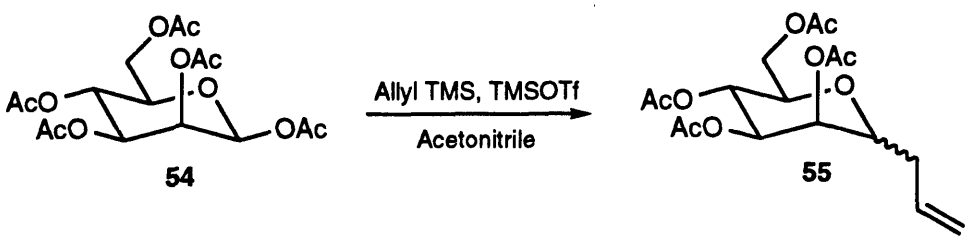
A solution of the silyl ether **52** (596 mg, 1.05 mmol) in THF (7 mL) at 0°C was treated with HF/pyridine complex (2.0 mL). The ice bath was removed and the reaction proceeded for 14 hours at room temperature, at which time it was again cooled to 0°C and sodium bicarbonate (25 mL) was slowly added, forming a white precipitate. The aqueous suspension was extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 2:1) afforded the alcohol **53** (324 mg, 0.98 mmol) as white crystals in 93% yield.

Physical data for **53**

R_f = 0.5 (hexane/ethyl acetate = 1:1); IR (film) 3458, 2923, 1743, 1372, 1229, 1044 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) {mixture of anomers, major changes from **52**: loss of aromatic protons and singlet corresponding to the *tert*-butyl group}.



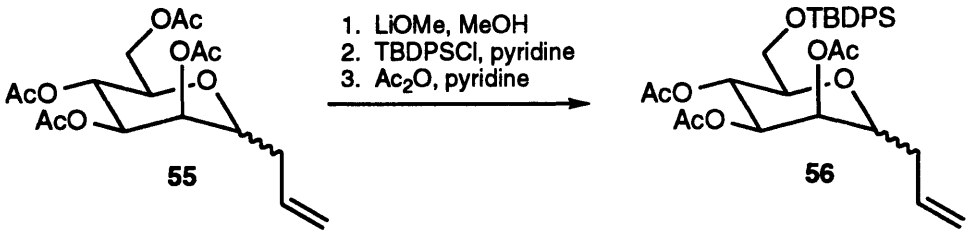
A solution of triflic anhydride (40 uL, 0.24 mmol) in CH_2Cl_2 (0.5 mL) was added to a -10°C solution of pyridine (20 uL, 0.27 mmol) in CH_2Cl_2 (0.2 mL). A white precipitate formed immediately. A solution of the alcohol **53** (38 mg, 0.12 mmol) in CH_2Cl_2 (0.4 mL) was added to the pyridine/$(Tf)_2O$ solution. The ice bath was removed and the reaction proceeded at room temperature for three hours, then was added to cold water (3 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 3 mL). The combined organic layers were washed with brine (1 x 5 mL), dried over sodium sulfate, filtered, and concentrated to an oil. The triflate was used directly without further purification. A solution of the triflate in DMF (2 mL) was treated with NaN_3 (100 mg, 1.5 mmol). The suspension was stirred 4 hours at room temperature, at which time TLC indicated the presence of the desired product and a side product, presumably resulting from elimination. Water (7 mL) was added to the reaction, and the aqueous solution was extracted with ether (2 x 5 mL). The combined ether extracts were washed (1 x 10 mL) with brine, dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 4:1) provided the azide **47** (22.4 mg, 0.063 mmol) as an oil in 55% yield. Physical data for **47** was provided previously for the synthesis of **47** from **46**.



A solution of peracetylated mannose **54** (4.08 g, 10.5 mmol) and allyl TMS (4.93 mL, 31 mmol) in acetonitrile (40 mL) at 0°C was treated with TMSOTf (2.24 mL, 11.6 mmol). The ice bath was removed and the reaction was stirred 48 hours, then added to 150 mL cold saturated sodium bicarbonate solution. The aqueous solution was extracted with $CHCl_3$ (2 x 75 mL). The combined organic extracts were washed with brine (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate = 4:1) afforded **55** (1.51 g, 4.1 mmol) as a mixture of anomers ($\alpha/\beta \approx 4$) in 39% yield.

Physical data for **55**

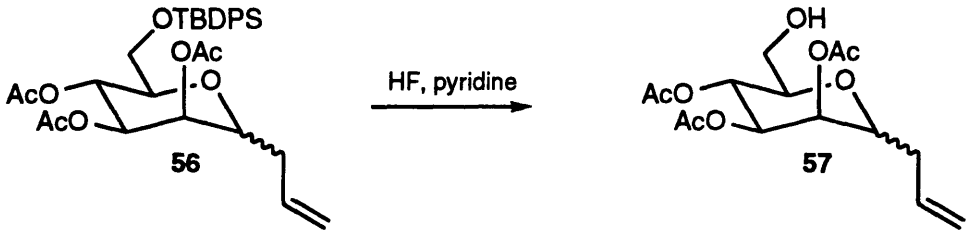
R_f = 0.35 (hexane/ethyl acetate = 3:1); IR (film) 1745, 1370, 1227, 1050 cm^{-1}; ^1H NMR (α anomer) (300 MHz, $CDCl_3$) δ 5.70-5.83 (m, 1H), 5.08-5.34 (m, 5H), 4.33 (dd, 1H), 4.03-4.15 (m, 2H), 3.88-3.93 (m, 1H), 2.38-2.60 (m, 2H), 2.12 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); FABMS: 373 $(M + H)^+$.



A solution of lithium methoxide (20 mg) in methanol (10 mL) was added to tetraacetate **55** (654 mg, 1.76 mmol). After stirring 30 minutes, cation exchange resin was added to neutralize the solution. The resin was removed by filtration and the filtrate concentrated to an oil, which was dissolved in anhydrous pyridine (10 mL), cooled to 0°C, and treated with *tert*-butyldiphenylsilyl chloride (1.3 mL, 5.0 mmol). After 16 hours, the reaction was quenched with methanol (150 uL). The reaction flask was cooled to 0°C and acetic anhydride (2 mL) was added. After 20 hours, water (30 mL) was added and the aqueous solution was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were washed with water (1 x 50 mL), $KHSO_4$ (1 x 50 mL), sodium bicarbonate (2 x 50 mL), and brine (1 x 50 mL), then dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 4.5:1) afforded **56** (521 mg, 0.91 mmol) as an anomeric mixture in 52% yield.

Physical data for **56**

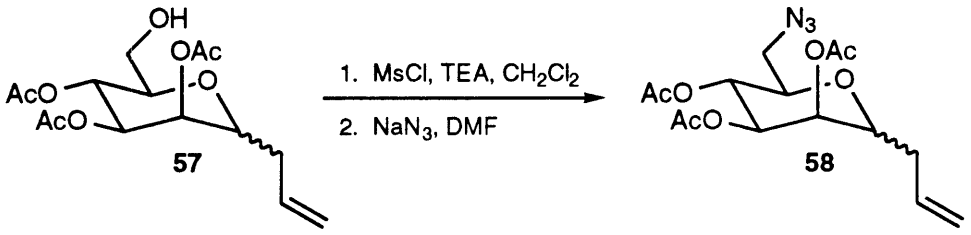
R_f = 0.5 (hexane/ethyl acetate = 3:1); IR (film) 2932, 2857, 1748, 1371, 1248, 1224, 1112 cm^{-1}; ^1H NMR (α anomer) (300 MHz, $CDCl_3$) δ 7.64-7.76 (m, 4H), 7.35-7.45 (m, 6H), 5.76-5.89 (m, 1H), 5.07-5.32 (m, 5H), 3.97-4.02 (m, 1H), 3.69-3.81 (m, 3H), 2.47-2.58 (m, 1H), 2.35-2.43 (m, 1H), 2.09 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.08 (s, 9H); FABMS: 569 $(M + H)^+$.



A solution of the silyl ether **56** (486 mg, 0.85 mmol) in THF (6 mL) at 0°C was treated with HF/pyridine complex (1.6 mL). The ice bath was removed and the reaction proceeded for 16 hours at room temperature, at which time it was again cooled to 0°C and sodium bicarbonate (25 mL) was slowly added. The aqueous solution was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were washed with brine (1 x 40 mL), then dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 1.8:1) afforded the alcohol **57** (238 mg, 0.72 mmol) as a mixture of anomers in 84% yield.

Physical data for **57**

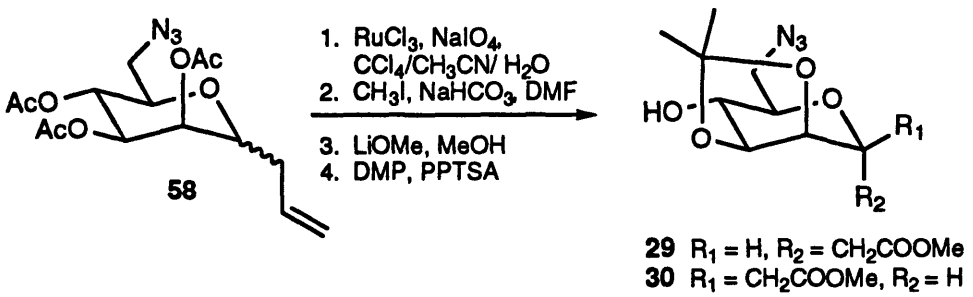
R_f = 0.4 (hexane/ethyl acetate = 1.1:1); IR (film) 3487 (br), 2943, 1745, 1372, 1227, 1048 cm^{-1}; ^1H NMR (α anomer) (300 MHz, $CDCl_3$) δ 5.81-5.94 (m, 1H), 5.10-5.32 (m, 5H), 4.02-4.08 (m, 1H), 3.63-3.70 (m, 3H), 2.54-2.65 (m, 1H), 2.39-2.48 (m, 1H), 2.26 (dd, 1H, *J* = 6.9, 6.4), 2.14 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); FABMS: 331 $(M + H)^+$.



Methanesulfonyl chloride (85 uL, 1.1 mmol) was added to a 0°C solution of the alcohol **57** (234 mg, 0.71 mmol) and TEA (200 uL, 1.5 mmol) in CH_2Cl_2 (5 mL). The ice bath was removed and the reaction proceeded for 18 hours at room temperature before water (10 mL) was added, followed by saturated sodium bicarbonate solution (2 mL). The aqueous solution was extracted with CH_2Cl_2 (3 x 7 mL), and the combined organic extracts were washed with brine (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated to an oil, which was used directly without further purification. The mesylate was dissolved in DMF (6 mL) and added to sodium azide (310 mg, 4.8 mmol). The reaction was heated to 80°C and proceeded for 48 hours before being cooled to room temperature and added to water (12 mL). The aqueous suspension was extracted with ether (3 x 10 mL), and the combined organic extracts were washed with brine (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate = 3.8:1) afforded the azide **58** (158 mg, 0.44 mmol) as an anomeric mixture in 63% yield.

Physical data for **58**

R_f = 0.4 (hexane/ethyl acetate = 3:1); IR (film) 2101, 1746, 1370, 1247, 1223, 1045 cm^{-1}; ^1H NMR (α anomer) (300 MHz, $CDCl_3$) δ 5.72-5.88 (m, 1H), 5.08-5.30 (m, 5H), 4.01-4.07 (m, 1H), 3.81-3.88 (m, 1H), 3.41 (dd, 1H, *J* = 12.7, 7.0), 3.24 (dd, 1H, *J* = 12.7, 3.0), 2.53-2.63 (m, 1H), 2.39-2.48 (m, 1H), 2.15 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H); FABMS: 356 $(M + H)^+$.



29 $R_1 = H$, $R_2 = CH_2COOMe$
30 $R_1 = CH_2COOMe$, $R_2 = H$

A 1:1 solution (1 mL) of acetonitrile/carbon tetrachloride was added to a mixture of the C-allyl glycoside **58** (153.5 mg, 0.43 mmol) and sodium periodate (540 mg, 2.50 mmol). Water (0.75 mL) was added to the suspension, followed by catalytic ruthenium chloride trihydrate (7 mg). The reaction immediately turned brown, and soon thereafter developed a green color. After 40 minutes, isopropanol (1 mL) was added to the flask, and the contents were filtered through Celite, rinsing with EtOAc. The filtrate was concentrated to an oil, dissolved in EtOAc, and dried over sodium sulfate. The drying agent was removed by filtration, and the filtrate was concentrated to an oil. The oil was dissolved in DMF (2 mL) and treated with sodium bicarbonate (120 mg) and methyl iodide (90 uL). After 20 hours, water (2 mL) was added to the flask, and the reaction contents were partitioned between water (additional 4 mL) and EtOAc (5 mL). The aqueous layer was extracted with EtOAc (2 x 5 mL), and the combined organic extracts were washed with sodium bicarbonate (1 x 10 mL) and brine (1 x 8 mL), dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate = 3:1) afforded the glycosazido ester **59** (138 mg, 0.36 mmol) as an anomeric mixture in 83% yield. The anomers were resolved by a deacetylation and subsequent acetonide formation. Lithium methoxide (7 mg, 0.18 mmol) was added to a solution of **59** (125 mg, 0.32 mmol) in methanol (4 mL). After 30 minutes, cation exhange resin was added. The resin was removed by filtration, and the filtrate was concentrated. The residual oil was dissolved in dimethoxypropane (3 mL) and treated with catalytic PPTSA (3 mg). The reaction was heated on an oil bath to 45°C and proceeded for 1 hour. The solution was concentrated to

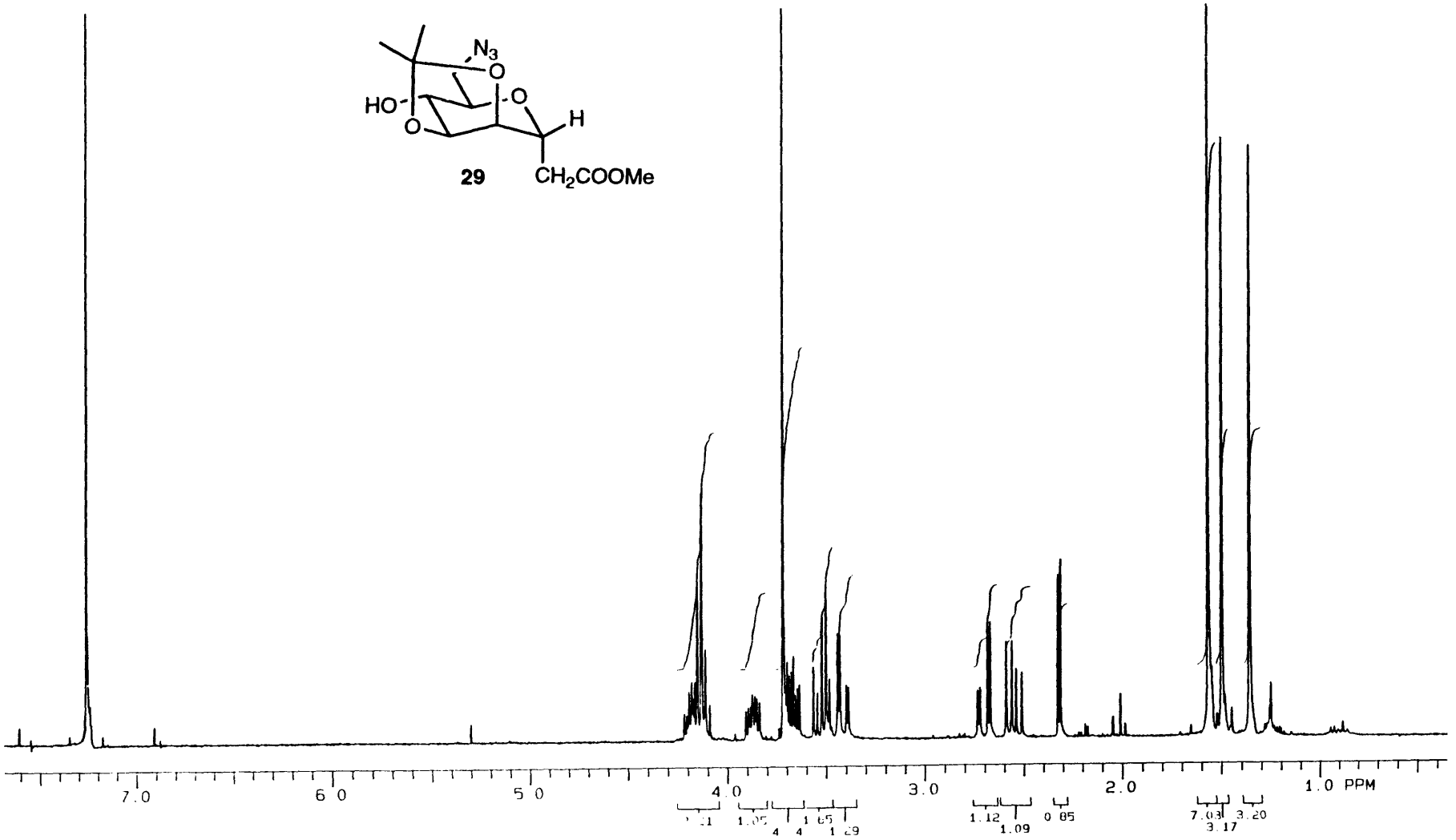
an oil that was purified by flash chromatography (hexane/ethyl acetate = 3:1) to give the α-glycoside **29** (62.1 mg, 0.21 mmol), the β-glycoside **30** (6.6 mg, 0.02 mmol), and a fraction containing a mixture of the two anomers (13.5 mg, 0.04 mmol) as oils in 85% yield (70% from **58**).

Physical data for **29**

R_f = 0.5 (hexane/ethyl acetate = 1:1); IR (film) 3456 (br), 2989, 2936, 2101, 1783, 1276, 1082, 1053, 865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.10-4.22 (m, 3H), 3.84-3.91 (m, 1H), 3.72 (s, 3H), 3.64-3.71 (m, 1H), 3.53 (dd, 1H, *J* = 13.2, 6.0), 3.42 (dd, 1H, *J* = 13.2, 3.0), 2.71 (dd, 1H, *J* = 15.4, 4.0), 2.55 (dd, 1H, *J* = 15.4, 8.9), 2.32 (d, 1H, *J* = 3.9), 1.50 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 110.2, 78.4, 75.6, 74.2, 70.0, 69.8, 52.0, 51.6, 38.1, 27.3, 25.1; HRMS. Calcd. for $C_{12}H_{19}N_3O_6$ $(M + H)^+$: 302.1352; found: 302.1347.

Physical data for **30**

R_f = 0.45 (hexane/ethyl acetate = 1:1); IR (film) 3444, 2934, 2100, 1738, 1376, 1220, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.19-4.26 (m, 2H), 4.02 (dd, 1H, *J* = 7.0, 5.5), 3.71 (s, 3H), 3.54-3.61 (m, 1H), 3.43-3.45 (m, 2H), 3.36 (dd, 1H, *J* = 12.2, 7.0), 2.83 (dd, 1H, *J* = 16.3, 7.6), 2.71 (dd, 1H, *J* = 16.3, 5.5), 2.33 (d, 1H, *J* = 3.6), 1.52 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 110.0, 79.8, 77.5, 75.0, 72.6, 71.1, 51.9, 51.4, 36.2, 28.2, 26.3; HRMS. Calcd. for $C_{12}H_{19}N_3O_6$ $(M + H)^+$: 302.1352; found: 302.1356.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HO
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O
H
29
CH2COOMe
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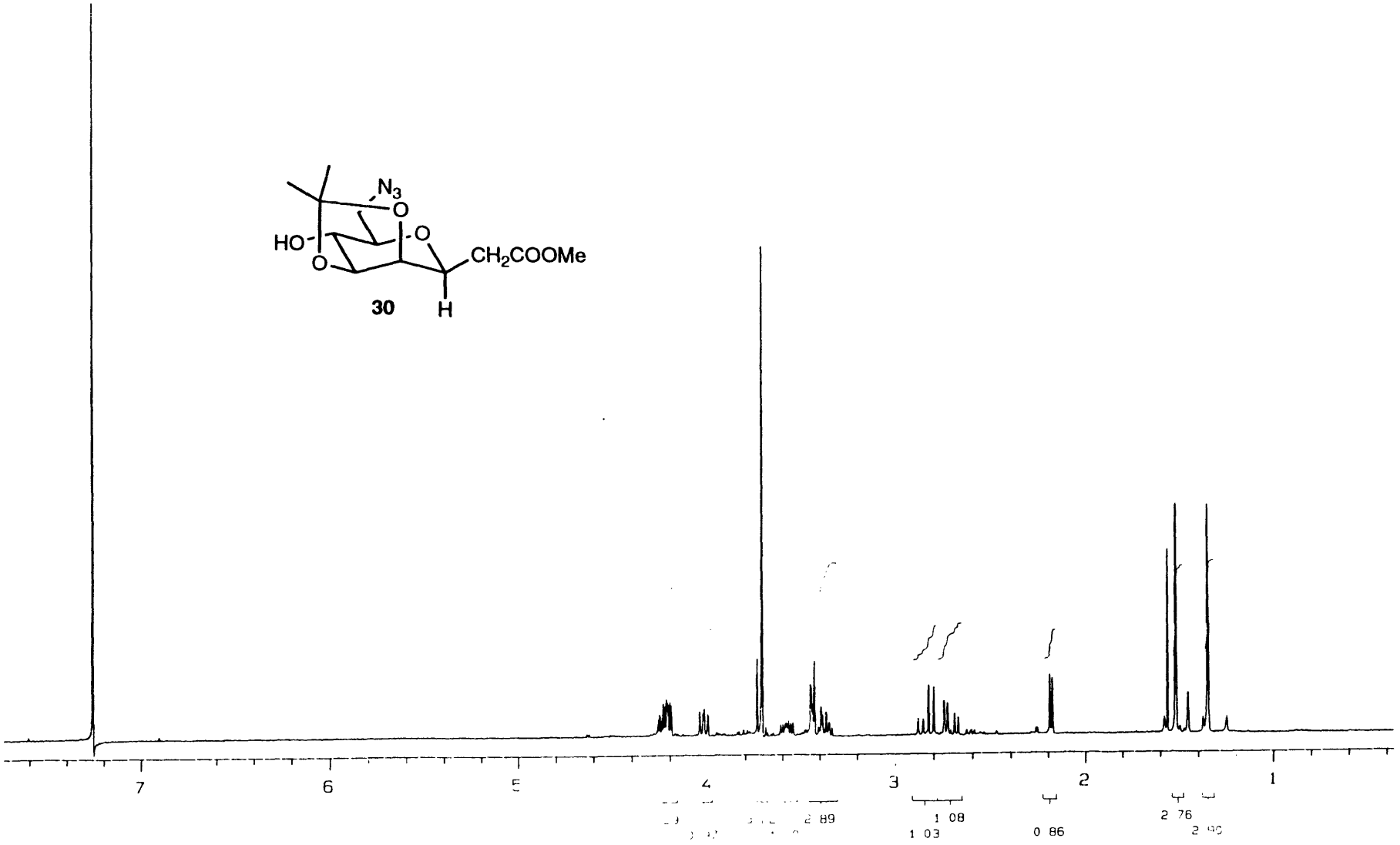
6.0
5.0
4.0
3.0
2.0
1.0 PPM
1.12
1.09
0.85
7.03
3.17
3.20


N3
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HO
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1 08
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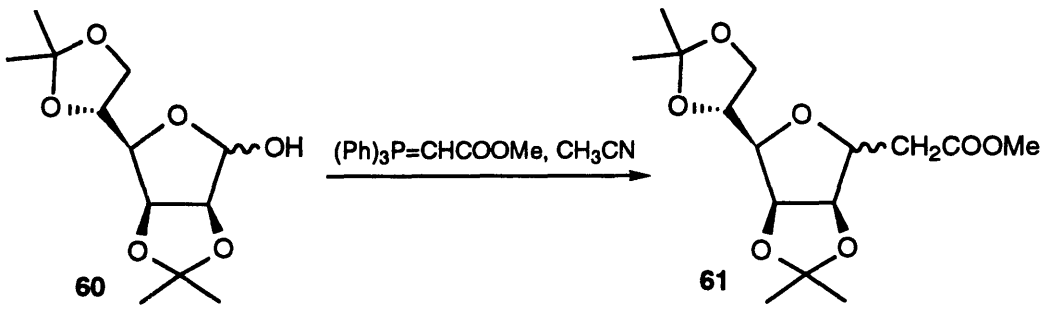
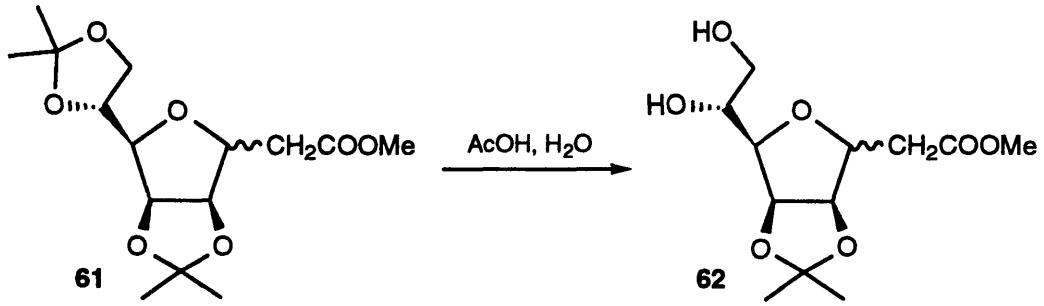
0 86
2 76



60 61

Acetonitrile (60 mL) was added to a mixture of **60** (1.252 g, 4.81 mmol) and methyl(triphenylphosphoranylidene)acetate (3.31g, 9.90 mmol) and the resulting solution was heated to reflux under argon. After refluxing 14 hours, the solution was concentrated to a small volume, then taken up in 40 mL EtOAc and washed with water (1 x 30 mL) and brine (1 x 30 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The resultant syrup was purified by flash chromatography (hexane/ethyl acetate = 4:1) to give **61** as a mixture of anomers (1.37 g, 4.32 mmol) in 90% yield. Physical data for **61** matched literature values[2].

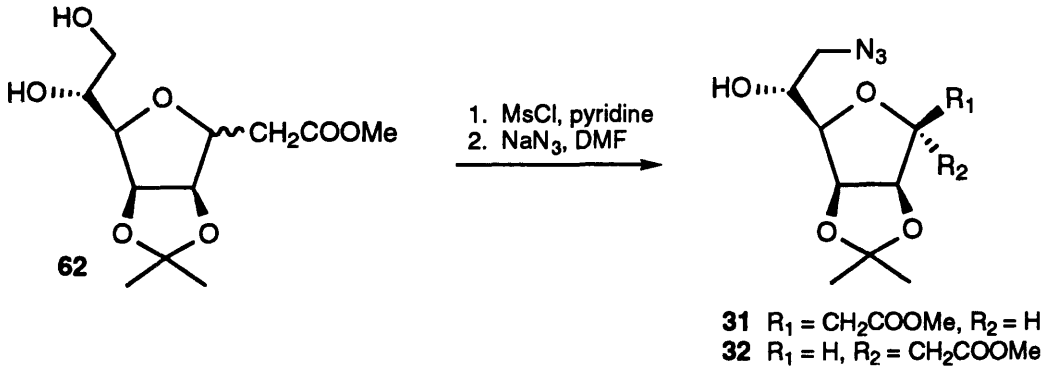


61 62

Water (280 uL) was added to a solution of the diacetonide **61** (176 mg, 0.556 mmol) in glacial acetic acid (2 mL). Stirring was commenced and proceeded for 16 hours at room temperature, at which time TLC (hexane/EtOAc 1:1) indicated the absence of **61**. The solution was concentrated under reduced pressure to a syrup, which solidified upon standing. The white solid was dissolved in CH_2Cl_2 and filtered through a plug of silica gel (CH_2Cl_2/acetone = 8:1, then 1.5:1). The solvent was removed *in vacuo* to give **62** (131 mg, 0.474 mmol) as a mixture of anomers in 85% yield.

Physical data for **62**

R_f = 0.2 (dichloromethane/acetone = 3:1); IR (film) 3441, 2986, 2939, 2878, 1738, 1209, 1088 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.88 (dd, 0.5H, *J* = 6.1, 4.0), 4.84 (dd, 0.5H, *J* = 6.2, 3.7), 4.76 (dd, 0.5H, *J* = 6.2, 3.7), 4.63 (dd, 0.5H, *J* = 6.2, 1.0), 4.49 (dd, 0.5H, *J* = 7.5, 7.2), 3.76-4.01 (m, 3H), 3.70 (s, 3H), 3.66-3.72 (m, 1H), 3.53 (dd, 0.5H, *J* = 7.2, 3.8), 3.13-3.17 (m, 1H), 2.63-2.85 (m, 2H), 2.45-2.57 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 170.7, 113.0, 112.5, 84.6, 84.6, 81.2, 81.2, 80.8, 80.5, 79.8, 77.5, 70.2, 69.9, 64.4, 64.3, 51.9, 51.7, 36.2, 33.2, 26.1, 25.8, 24.8, 24.7; FABMS: 277 $(M + H)^+$.



31 $R_1 = CH_2COOMe$, $R_2 = H$
32 $R_1 = H$, $R_2 = CH_2COOMe$

A 0°C solution of the diol **62** (1.29 g, 4.67 mmol) in pyridine (14 mL) was treated with methanesulfonyl chloride (390 uL, 5.04 mmol) . After warming to room temperature and stirring for 18 hours, DMAP (5 mg) was added to the reaction flask. Stirring was continued for an additional four hours, at which point only a trace of starting material remained and a small amount of the dimesylate impurity had formed. Methanol (3 mL) was added to destroy excess methanesulfonyl chloride. The resulting solution was concentrated under reduced pressure to afford an oil, which was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL), and the combined organic layers were washed successively with 1M HCl (1 x 25 mL), saturated sodium bicarbonate (1 x 20 mL), and brine (1 x 25 mL). The organic layer was dried over $NaSO_4$, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 1.3:1) provided the monomesylate (1.25 g, 3.54 mmol) as an oil in 76% yield. It is important to remove the dimesylate impurities before proceeding to the next step. Sodium azide (750 mg, 11.6 mmol) was added to a solution of the monomesylate product (779 mg, 2.20 mmol) in DMF (15 mL). The resulting suspension was stirred for eight hours in an oil bath heated to 70°C. The suspension was cooled to room temperature and water (30 mL) was added, producing a homogeneous solution. This solution was extracted with ether (2 x 30 mL), and the pooled organic extracts were washed with brine (1 x 30 mL) and dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (ether/petroleum ether = 1.3:1) provided the glycosazido esters **31** and **32** (555.9 mg,

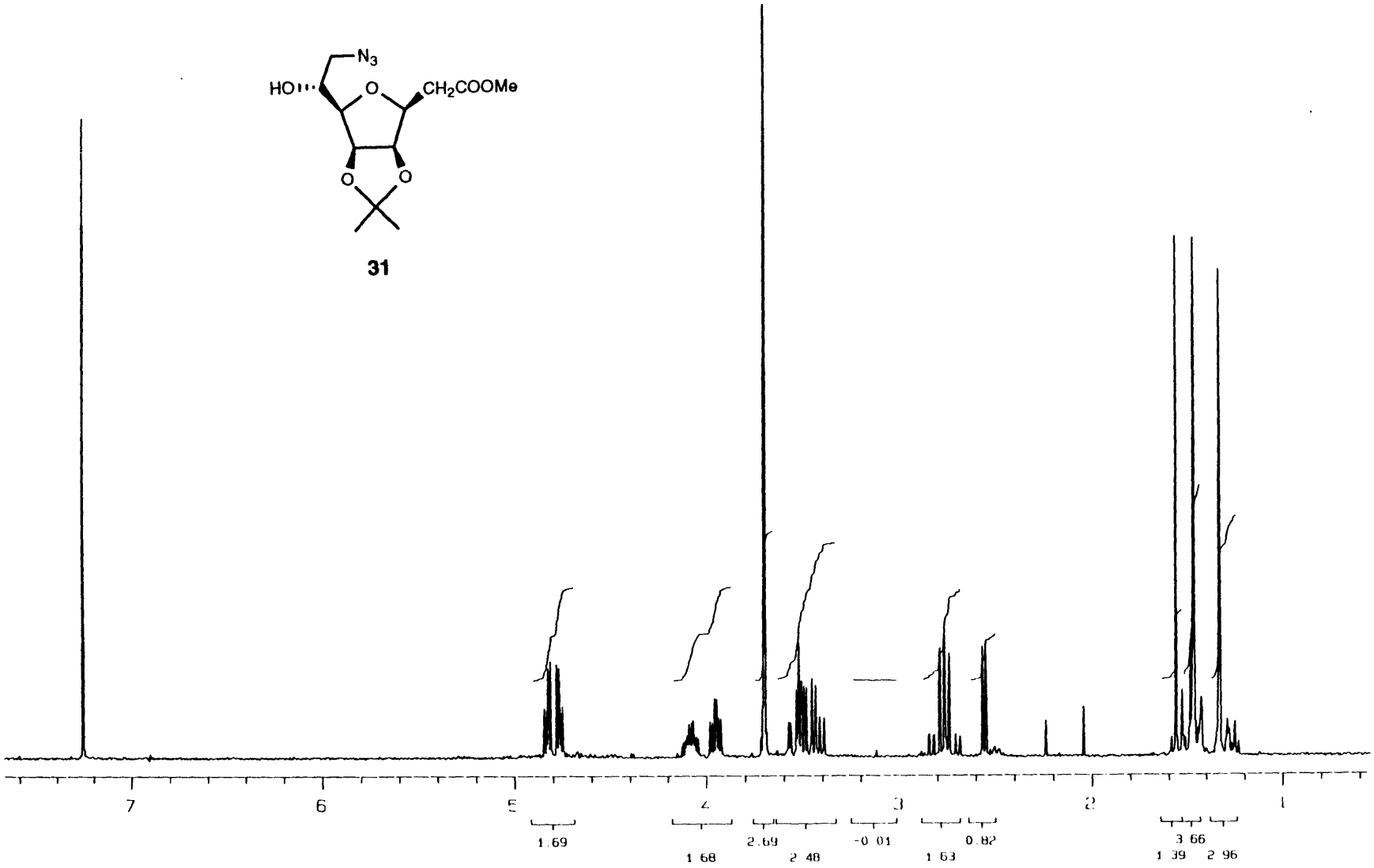
1.84 mmol) as a mixture of anomers in 84% yield. Purification on fine silica gel (EM Science, Silica Gel 60) was required to completely resolve the anomers.

Physical data for **31**

R_f = 0.5 (hexane/ethyl acetate = 1:1); IR (film) 3481 (br), 2987, 2934, 2097, 1734, 1438 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.83 (dd, 1H, *J* = 6.1, 3.7), 4.77 (dd, 1H, *J* = 6.1, 3.3), 4.05-4.13 (m, 1H), 3.93-3.99 (m, 1H), 3.70 (s, 3H), 3.55 (dd, 1H, *J* = 12.8, 3.3), 3.50 (dd, *J* = 8.2, 3.7), 3.42 (dd, 1H, *J* =12.8, 6.5), 2.68-2.85 (m, 2H), 2.63-2.68 (m, 1H), 1.48 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 112.8, 81.0, 80.9, 80.9, 77.6, 69.4, 54.4, 51.8, 33.3, 25.8, 24.7; HRMS. Calcd. for $C_{12}H_{19}N_3O_6$ $(M + H)^+$: 302.1352; found: 302.1349.

Physical data for **32**

R_f = 0.48 (hexane/ethyl acetate = 1:1); IR (film) 3474 (br), 2990, 2940, 2103, 1737, 1438, 1382, 1085 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.89 (dd, 1H, *J* = 6.0, 4.1), 4.67 (dd, 1H, *J* = 6.0, 1.0), 4.47-4.52 (m, 1H), 4.03-4.12 (m, 1H), 3.83 (dd, 1H, *J* = 8.3, 4.1), 3.71 (s, 3H), 3.54 (dd, 1H, *J* = 12.9, 2.7), 3.42 (dd, *J* = 12.9, 6.3), 2.75-2.80 (m, 1H), 2.44-2.58 (m, 2H), 1.51 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 113.2, 84.7, 81.0, 80.5, 79.9, 69.7, 54.1, 51.9, 36.2, 26.1, 24.7; HRMS. Calcd. for $C_{12}H_{19}N_3O_6$ $(M + H)^+$: 302.1352; found: 302.1349.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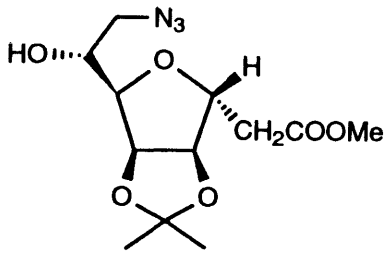
O
CH2COOMe
O
O
7
6
5
4
3
2
1
1.69
1.68
2.69
2.48
-0.01
1.63
0.82
3.66
1.39
2.96

31


N3
HO
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CH2COOMe
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32

7
6
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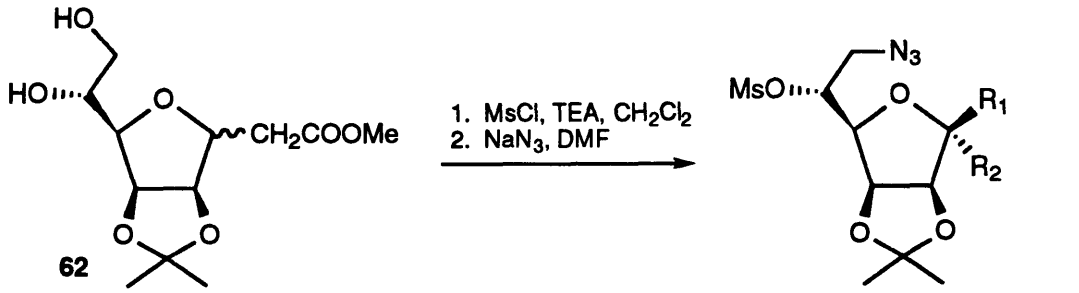
2.99



33 $R_1 = CH_2COOMe$, $R_2 = H$
34 $R_1 = H$, $R_2 = CH_2COOMe$

Methanesulfonyl chloride (400 uL, 5.17 mmol) was added to a 0°C solution of **62** (374 mg, 1.35 mmol) and TEA (1.4 mL, 10.1 mmol) in CH_2Cl_2 (10 mL). The resulting suspension was stirred 48 hours at room temperature, at which point methanol (1 mL) was added to destroy excess methanesulfonyl chloride. The solution was concentrated to an oil, which was dissolved in EtOAc (20 mL) and washed with saturated $NaHCO_3$ (2 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over $NaSO_4$, filtered, and concentrated after addition of DMF (1 mL, to prevent bumping). The residual oil was taken up in DMF (8 mL) and treated with sodium azide (560 mg, 8.6 mmol). The resulting suspension was stirred for 14 hours in an oil bath heated to 70°C. The suspension was cooled to room temperature and dissolved in water (25 mL). This solution was extracted with ether (2 x 25 mL), and the pooled organic extracts were washed with brine (1 x 30 mL) and subsequently dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 3.1:1) gave the glycosazido esters **33** and **34** as an anomeric mixture in 66% yield (338 mg, 0.890 mmol). The anomers were resolved by repeated purifications on fine silica gel (EM Science, Silica Gel 60).

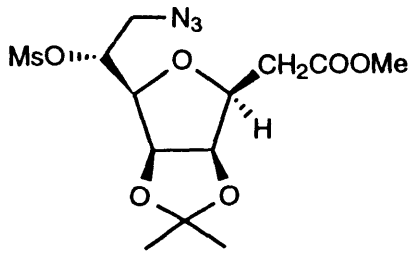
Physical data for **33**

R_f = 0.7 (hexane/ethyl acetate = 1:1); IR (film) 2988, 2940, 2109, 1736, 1438, 1361, 1178 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.92-4.95 (m, 1H), 4.75-4.83 (m, 2H), 3.96-4.01 (m, 1H), 3.89 (dd, 1H, *J* = 13.4, 2.7), 3.82 (dd, 1H, *J* = 6.1, 3.5), 3.71 (s,

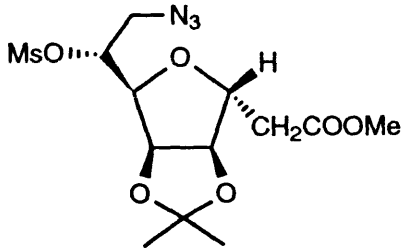
3H), 3.58 (dd, 1H, J = 13.4, 4.3), 3.13 (s, 3H), 2.68 - 2.84 (m, 2H), 1.48 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 112.9, 80.9, 80.0, 78.7, 77.9, 77.4, 52.3, 51.8, 38.4, 33.2, 25.8, 24.8; HRMS. Calcd. for $C_{13}H_{21}N_3O_8S$ $(M + H)^+$: 380.1128; found: 380.1130.

Physical data for **34**

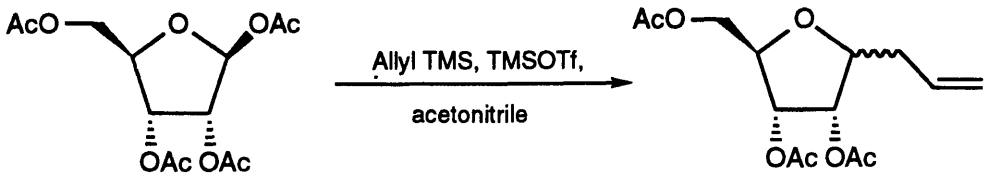
R_f = 0.65 (hexane/EtOAc = 1:1); IR (film) 2988, 2941, 2110, 1738, 1439, 1360, 1178 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.91-4.96 (m, 1H), 4.82 (dd, 1H, J = 6.0, 3.7), 4.71 (dd, 1H, J = 6.0, 0.9) 4.49-4.53 (m, 1H), 4.14 (dd, 1H, J =7.6, 3.7), 3.89 (dd, 1H, J = 13.6, 2.6), 3.71 (s, 3H), 3.60 (dd, 1H, J =13.6, 4.3), 3.13 (s, 3H), 2.47-2.61 (m, 2H), 1.51 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 113.3, 84.8, 81.0, 80.2, 77.9, 77.7, 52.2, 52.0, 38.4, 36.0, 26.2, 24.9; HRMS. Calcd. for $C_{13}H_{21}N_3O_8S$ $(M + H)^+$: 380.1128; found: 380.1130.



33



34



63 64

A solution of peracetylated ribose **63** (2.65 g, 8.3 mmol) and allyltrimethylsilane (4.0 mL, 24.1 mmol) in acetonitrile (25 mL) at 0°C was treated with TMSOTf (2.22 g, 10 mmol). The ice bath was removed and the solution was stirred six hours, then added to cold sodium bicarbonate solution (150 mL). The aqueous solution was extracted with CH_2Cl_2 (3 x 60 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried over magnesium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/ethyl acetate = 3:1) afforded the α anomer **64a** (512 mg, 1.70 mmol) as a colorless oil and the β anomer **64b** (1.663 g, 5.54 mmol) as a white solid that was recrystallized from EtOAc. The reaction proceeded with a cumulative 87% yield and a ratio of β to α anomers of approximately 3:1.

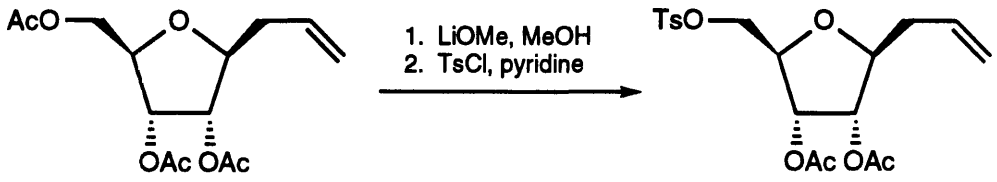
Physical data for **64b**

R_f = 0.6 (hexane/ethyl acetate = 2:1); IR (film) 2978, 1741, 1368, 1260, 1228, 1115 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.70-5.84 (m, 1H), 5.06-5.24 (m, 5H), 4.14 (dd, 1H, *J* = 13.3, 1.9), 3.71 (dd, 1H, *J* =13.3, 1.6), 3.53-3.58 (m, 1H), 2.41-2.51 (m, 1H), 2.18-2.29 (m, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.00 (s, 3H); FABMS: 301 $(M + H)^+$.

Physical data for **64a**

R_f = 0.8 (hexane/ethyl acetate = 2:1); IR (film) 1749, 1372, 1249, 1221, 1038 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.77-5.91 (m, 1H), 5.63 (m, 1H), 5.12 (d, 1H, *J* < 1), 5.07 (dd, *J* = 6.0, < 1), 4.95-5.01 (m, 1H), 4.73 (dd, 1H, *J* = 9.9, 2.6), 3.86 (dd, 1H, *J* =

11.2, 5.5), 3.70-3.76 (m, 1H), 3.63 (dd, 1H, J = 11.2, 11.2); 2.31-2.39 (m, 1H), 2.12-2.22 (m, 1H), 2.16 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); FABMS: 301 $(M + H)^+$.

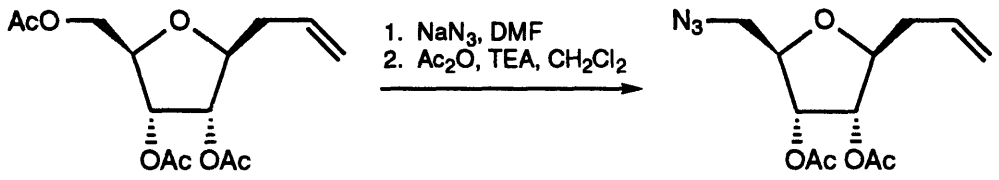


64 65

A solution of lithium methoxide (50 mg) in methanol (5 mL) was added to a solution of **64b** (979 mg, 3.26 mmol) in methanol (10 mL). After stirring three hours, the reaction was neutralized by the addition of cation exchange resin. Filtration, followed by concentration, afforded the sugar triol as white crystals (556 mg, 3.19 mmol) in 98% yield. A portion of the crystals (556 mg, 3.19 mmol) were dissolved in a solution of tosyl chloride (651 mg, 3.41 mmol) in pyridine (10 mL). After stirring 48 hours at room temperature, the reaction contents were added to water (20 mL), and the aqueous solution was extracted wtih CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with sodium bicarbonate (1 x 20 mL), $KHSO_4$ solution (1 x 20 mL), and brine (1 x 20 mL). The organic solution was concentrated and recrystallized (153 mg) from dichloromethane. The filtrate was concentrated and purified by flash chromatography (hexane/ethyl acetate = 2:1) to afford the tosylate **65** (415.3 mg, 1.27 mmol) as a syrup which crystallized almost immediately. The combined crystals (568.3 mg, 1.73 mmol) totaled a 53% yield.

Physical data for **65**

R_f = 0.2 (hexane/ethyl acetate = 1.5:1); IR (film) 3474, 1359, 1190, 1176, 1096, 855 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.84-7.88 (m, 2H), 7.37 (d, 2H, *J* = 8.0), 5.70-5.84 (m, 1H), 5.07-5.17 (m, 2H), 4.52 (ddd, 1H, *J* = 3.4, 2.5, <1), 4.08 (dd, 1H, *J* = 12.6, 2.5), 3.91-3.95 (m, 1H), 3.83-3.87 (m, 1H), 3.51 (dd, 1H, *J* = 12.6, < 1), 3.31 (ddd, 1H, *J* = 7.5, 6.9, < 1), 3.12 (d, 1H, *J* = 6.9), 2.95 (d, 1H, *J* = 7.8), 2.46-2.57 (m, 1H), 2.46 (s, 3H), 2.32-2.41 (m, 1H); FABMS: 329 $(M + H)^+$.



65 **66**

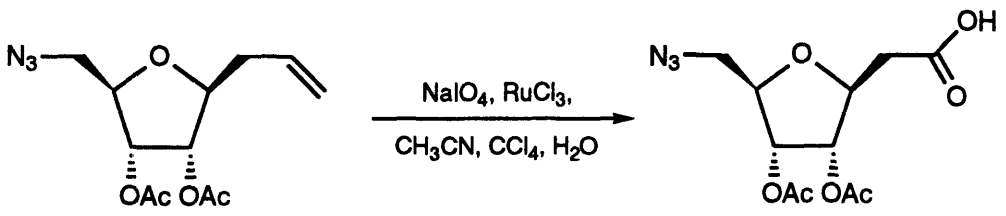
The tosylate **65** (415.3 mg, 1.27 mmol) was dissolved in DMF (10 mL). Sodium azide (530 mg, 8.12 mmol) was added to the solution, along with a catalytic amount of tetrabutylammonium iodide (10 mg). The reaction was heated to 110°C and stirred four days, by which time conversion of the starting material to product appeared to be approximately 50%. The suspension was cooled and added to 40 mL water, which was extracted with ether (2 x 40 mL). The combined organic extracts were washed with brine (1 x 50 mL), dried over magnesium sulfate, filtered, and concentrated. The residual oil was directly acetylated in CH_2Cl_2 by treatment with acetic anhydride (1 mL), triethylamine (0.5 mL), and catalytic DMAP (5 mg). After stirring 24 hours at room temperature, sodium bicarbonate (15 mL) was added. The organic layer was washed with brine (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 5:1) afforded **66** (105.5 mg, 0.37 mmol) as an oil in 29% yield from the tosylate (68% based on unreacted starting material). The acetylated tosylate was recovered and characterized as **67**.

Physical data for **66**

R_f = 0.6 (hexane/ethyl acetate = 3:1); IR (film) 2922, 2852, 2108, 1743, 1375, 1226, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.70-5.84 (m, 1H), 5.09-5.15 (m, 2H), 4.70-4.74 (m, 2H), 3.98 (dd, 1H, *J* = 3.7, 3.3), 3.87-3.88 (m, 2H), 3.77-3.83 (m, 1H), 2.41-2.51 (m, 1H), 2.19-2.28 (m, 1H), 2.14 (s, 3H), 2.12 (s, 3H); FABMS: 284 $(M + H)^+$.

Physical data for **67**

R_f = 0.25 (hexane/ethyl acetate = 3:1); IR (film) 1741, 1367, 1246, 1226, 1190 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, 2H, *J* = 8.1), 7.36 (d, 2H, *J* = 8.1), 5.69-5.81 (m, 1H), 5.19-5.21 (m, 1H), 5.05-5.13 (m, 3H), 4.62 (dd, 1H, *J* = 3.8, 3.8), 4.08 (dd, 1H, *J* = 13.3, 2.7), 3.57 (dd, 1H, *J* = 13.3, 1.9), 3.44-3.48 (m, 1H), 2.46 (s, 3H), 2.36-2.46 (m, 1H), 2.12-2.22 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H); FABMS: 413 $(M + H)^+$.

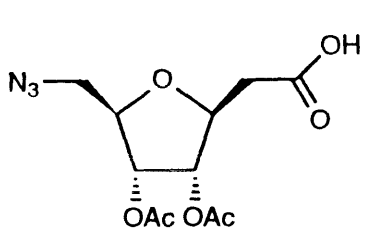


66 **35**

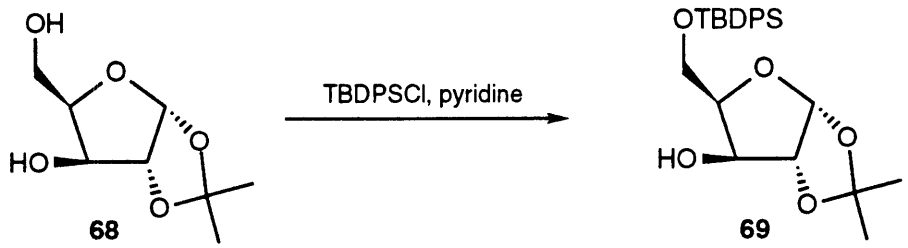
A 1:1 solution (1 mL) of acetonitrile/carbon tetrachloride was added to a mixture of the C-allyl glycoside **66** (105.5 mg, 0.37 mmol) and sodium periodate (389 mg, 1.80 mmol). Water (0.75 mL) was added to the suspension, followed by catalytic ruthenium chloride trihydrate (6 mg). The reaction immediately turned brown, and soon thereafter the suspension developed a green color. After fifteen minutes, isopropanol (1 mL) was added to the flask, and the contents were filtered through Celite, rinsing with EtOAc. The filtrate was concentrated to an oil, and purified by flash chromatography (hexane/EtOAc = 1.1:1, then hexane/EtOAc/AcOH = 50:50:1) to afford the glycosazido acid **35** (104.6 mg, 0.35 mmol) as an oil in 93% yield.

Physical data for **35**

R_f = 0.2 (hexane/ethyl acetate/acetic acid = 50:50:1); IR (film) 3200 (br), 2940, 2112, 1747, 1732, 1714, 1378, 1242, 1092, 1043 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.81 (dd, 1H, *J* = 3.9, 2.0), 4.71-4.74 (m, 1H), 4.25-4.30 (m, 1H), 4.00 (dd, 1H, *J* = 3.9, 3.4), 3.90-3.92 (m, 2H), 2.73 (dd, 1H, *J* = 16.1, 8.5), 2.54 (dd, 1H, *J* = 16.1, 4.7), 2.14 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 170.0, 169.9, 70.0, 68.6, 67.4, 65.2, 57.3, 35.4, 21.0, 20.7; HRMS. Calcd. for $C_{11}H_{15}O_7N_3$ $(M + H)^+$: 302.0988; found: 302.0984.



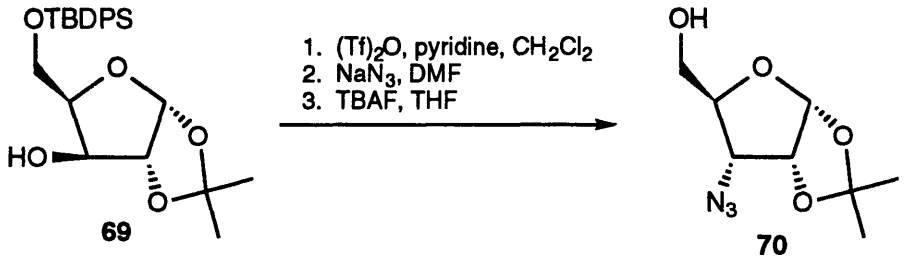
35



Under an argon atmosphere, *tert*-butyldiphenylchlorosilane (1.23 mL, 4.74 mmol) was added to a 0°C solution of 1,2-isopropylidene xylofuranose **68** (601 mg, 3.16 mmol) in pyridine (7 mL). The reaction vessel was warmed to room temperature and stirring proceeded for 18 hours before methanol (100 uL) was added to destroy residual TBDPSCl. Water (20 mL) was added and the aqueous solution was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were concentrated to a volume of 15 mL and washed with brine (1 x 15 mL), dried over sodium sulfate, and concentrated under reduced pressure to an oil. Purification by flash chromatography (hexane/EtOAc = 7:1) gave the silyl ether **69** (1.326 g, 3.09 mmol) as a colorless oil in 98% yield.

Physical data for **69**

R_f = 0.75 (hexane/ethyl acetate = 2:1); IR (film) 3460 (br), 2932, 1428, 1374, 1216, 1113, 1075, 1014 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.66-7.73 (m, 4H), 7.38-7.47 (m, 6H), 6.01 (d, 1H, J = 3.7), 4.55 (dd, 1H, J = 3.7, < 1), 4.37 (dd, 1H, J < 1), 4.10-4.14 (m, 3H), 4.06 (d, 1H, J = 3.1), 1.47 (s, 3H), 1.33 (s, 3H), 1.05 (s, 9H); HRMS. Calcd. for $C_{24}H_{32}SiO_5$ $(M + H)^+$: 429.2097; found: 429.2092.



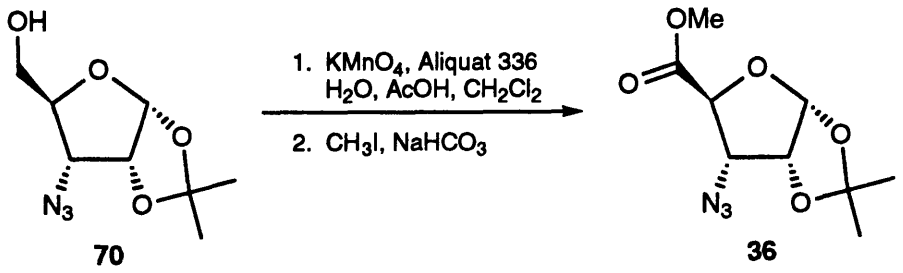
69 70

Pyridine (270 uL, 3.0 mmol) was added to a -10°C solution of the alcohol **69** (751 mg, 1.75 mmol) in CH_2Cl_2. After stirring the solution briefly, trifluoromethanesulfonic anhydride (324 uL, 1.93 mmol) was added and the reaction was allowed to warm gradually to room temperature. Monitoring of the reaction by TLC indicated that it had not gone to completion after eight hours, at which time additional pyridine (100 uL) and $(Tf)_2O$ (50 uL) were added. Within an hour, all starting material was consumed and the reaction contents were added to cold water (25 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organics were washed with brine (1 x 40 mL), dried over sodium sulfate, and concentrated. Traces of pyridine and pyridinium salts were separated from the desired triflate product by flash chromatography (hexane/EtOAc = 3:1) through a short plug of silica gel, yielding the crude triflate, which was used directly in the next step. The triflate was dissolved in DMF (18 mL), to which NaN_3 (500 mg, 7.7 mmol) was added. After stirring 18 hours at room temperature, all starting material was consumed, leaving the desired azide product and a significant side-product, presumably resulting from triflate elimination. Water (60 mL) was added to the reaction, and the aqueous solution was extracted with ether (3 x 50 mL). The combined ether extracts were washed (1 x 100 mL) with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo* to give the crude azide as an oil, which was used directly without further purification. The crude azide was dissolved in THF (3 mL) and added to a solution of TBAF (3 mL of 1.0 M solution in THF, 3 mmol) in THF (5 mL). After 18 hours, the solution was concentrated to an oil. Water (50 mL) was added, and the aqueous solution was extracted with EtOAc

(3 x 50 mL). The combined organic extracts were washed with water (1 x 100 mL) and brine (1 x 100 mL) before drying over magnesium sulfate. Filtration, followed by concentration *in vacuo*, produced an oil that was purified by flash chromatography (hexane/EtOAc = 3:1) to give the alcohol **70** (260.2 mg, 1.21 mmol) in 69% yield from **69**.

Physical data for **70**

R_f = 0.5 (hexane/ethyl acetate = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (d, 1H, J = 5.4), 5.29-5.32 (m, 1H), 5.19-5.20 (m, 1H), 4.17-4.20 (m, 3H), 1.77 (t, 1H, J = 6.5), 1.47 (s, 3H), 1.45 (s, 3H).

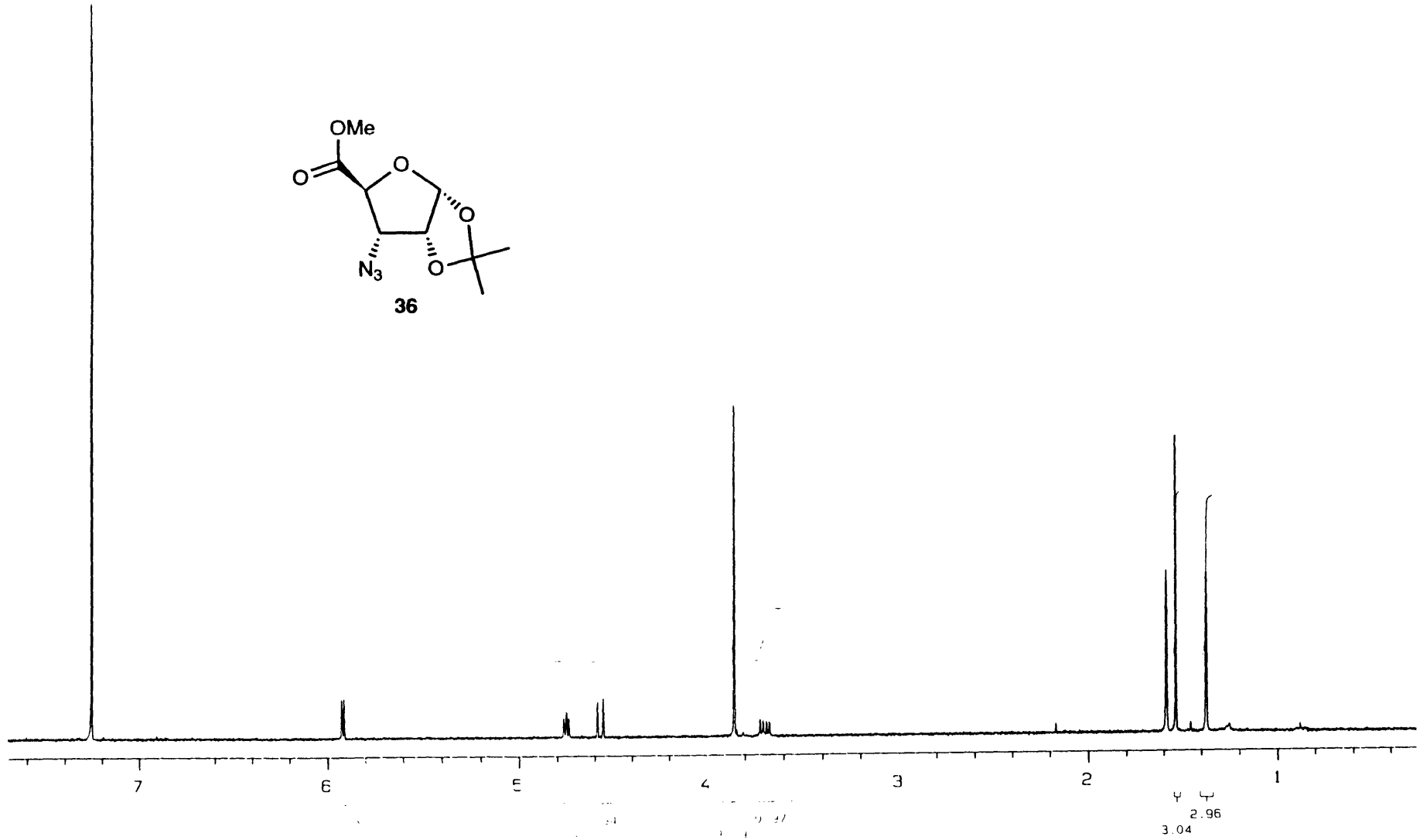


70 36

Water (0.7 mL) and acetic acid (125 uL) were added to a solution of the primary alcohol **70** (29.8 mg, 0.138 umol) in CH_2Cl_2 (1.2 mL). Aliquat 336 (7 mg) was added as a phase transfer catalyst and the reaction vessel was cooled to 0°C before addition of $KMnO_4$ (75 mg, 0.48 mmol). The ice bath was removed and the reaction proceeded 24 hours at room temperature, at which point all starting material had been consumed. Sodium sulfite (75 mg) was added to quench the reaction, which was concentrated to an oil and dissolved in DMF (1 mL). Sodium bicarbonate (10 mg) was added to this solution, followed by methyl iodide (10 uL). After 40 hours, water (1 mL) was added and the reaction was partitioned between water (5 mL) and ether (5 mL). The aqueous layer was extracted with ether (2 x 5 mL), and the combined organic extracts were washed with brine (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 4:1) afforded the glycosazido ester **36** (3.2 mg, 13.2 umol) as an oil in 10% yield.

Physical data for **36**

R_f = 0.6 (hexane/ethyl acetate = 2:1); IR (film) 2110, 1748, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.92 (d, 1H, *J* = 3.4), 4.75 (dd, 1H, *J* = 4.4, 3.4), 4.57 (d, 1H, *J* = 9.6), 3.86 (s, 3H), 3.70 (dd, 1H, *J* = 9.6, 4.4), 1.58 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 113.8, 104.7, 79.9, 75.9, 63.3, 52.9, 26.5, 26.4; HRMS. Calcd. for $C_9H_{13}N_3O_5$ $(M + H)^+$: 244.0933; found: 244.0934.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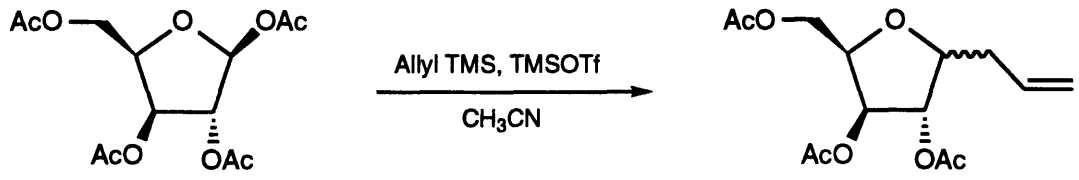


71 72

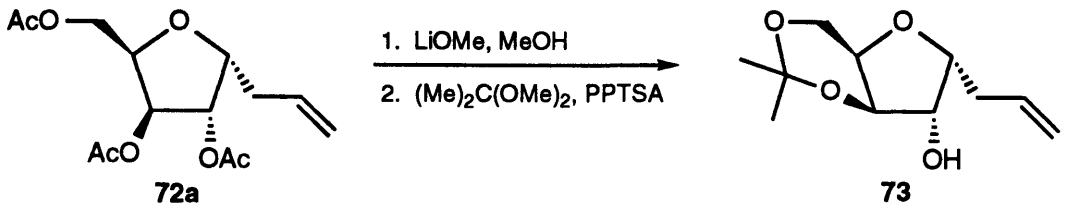
A solution of 1,2,3,4-tetra-*O*-acetyl-β-D-xylofuranose **71** (4.34 g, 13.6 mmol) and allyltrimethylsilane (6.5 mL, 4.1 mmol) in acetonitrile (50 mL) at 0°C was treated with TMSOTf (2.90 mL, 1.50 mmol). The ice bath was removed and the solution was stirred 24 hours, then added to cold sodium bicarbonate solution (150 mL). The aqueous solution was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were washed with brine (1 x 125 mL) and dried over magnesium sulfate. The drying agent was removed by filtration and the solution was concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate = 5.5:1) afforded the α anomer **72a** (1.36 g, 4.5 mmol), the β anomer **72b** (253 mg, 0.8 mmol), and a fraction containing a mixture of the two anomers (1.988 g, 6.6 mmol) which were separated by additional silica gel chromatography. The C-glycosylation reaction proceeded in 88% cumulative yield with a ratio of α to β anomers of approximately 3:1.

Physical data for **72a**

R_f = 0.5 (hexane/ethyl acetate = 3:1); IR (film) 2947, 2862, 1754, 1371, 1246, 1223, 1100, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.75-5.88 (m, 1H), 4.83-5.20 (m, 5H), 4.11 (dd, 1H, *J* = 10.8, 5.5), 3.40-3.46 (m, 1H), 3.25 (dd, 1H, *J* = 10.8, 10.4), 2.18-2.27 (m, 2H), 2.03 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H); HRMS. Calcd. for $C_{14}H_{20}O_7$ $(M + H)^+$: 301.1287; found: 301.1292.

Physical data for **72b**

R_f = 0.4 (hexane/ethyl acetate = 3:1); IR (film) 1743, 1372, 1224, 1044 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.73-5.82 (m, 1H), 5.03-5.15 (m, 3H), 4.76-4.78 (m, 1H), 4.69-4.71 (m, 1H), 3.98-4.03 (m, 1H), 3.85 (dd, 1H, *J* = 13.0, 2.1), 3.75-3.80 (m, 1H), 2.39-2.48 (m, 1H), 2.18-2.27 (m, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H); HRMS. Calcd. for $C_{14}H_{20}O_7$ $(M + H)^+$: 301.1287; found: 301.1289.

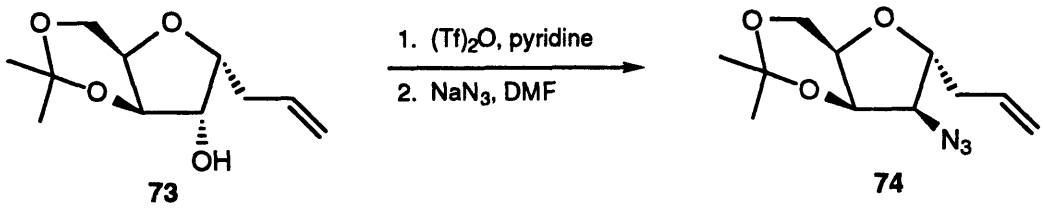


72a 73

A solution of **72a** (171 mg, 0.57 mmol) in methanol (2 mL) was added to a solution of LiOMe (10 mg, 0.27 mmol) in methanol (1 mL). The solution was stirred 30 minutes at room temperature. Cation exchange resin was added to neutralize the solution, which was filtered and concentrated. The residual oil was dissolved in dimethoxypropane (3 mL), to which was added catalytic PPTSA (3 mg). After stirring 24 hours at 50°C, the solution was concentrated and then treated with a solution of LiOMe (10 mg) in methanol (5 mL). After stirring five minutes, the solution was filtered through cation exchange resin and concentrated to an oil. Purification by flash chromatography afforded the acetonide **73** (88.7 mg, 0.41 mmol) as an oil in 73% yield.

Physical data for **73**

R_f = 0.5 (hexane/ethyl acetate = 1.1:1); IR (film) 3448 (br), 2985, 2877, 1230, 1085, 1046 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80-5.94 (m, 1H), 5.08-5.18 (m, 2H), 3.92-4.07 (m, 2H), 3.41-3.56 (m, 2H), 3.09-3.16 (m, 2H), 2.48-2.56 (m, 1H), 2.32 (d, 1H, *J* = 3.9), 2.22-2.32 (m, 1H), 1.42 (s, 3H), 1.41 (s, 3H); HRMS. Calcd. for $C_{11}H_{18}O_4$ $(M + H)^+$: 215.1283; found: 215.1285.

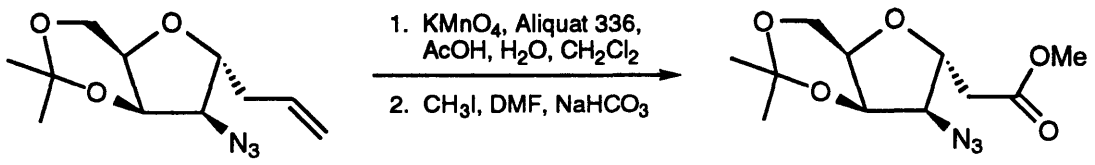


73 74

Pyridine (74 uL, 0.91 mmol) was added to a solution of the alcohol **73** (88.7 mg, 0.41 mmol) in CH_2Cl_2 (2 mL). The solution was cooled to -30°C, then treated with $(Tf)_2O$ (97.4 uL, 0.58 mmol). The reaction was allowed to proceed at 0°C for one hour. Cold water (3 mL) was added, the layers were separated, and the aqueous layer was further extracted with CH_2Cl_2 (1 x 3 mL). The combined organic extracts were washed with water (1 x 4 mL), cold 3 M HCl (1 x 4 mL), and brine (1x 4 mL), then dried over sodium sulfate, filtered, and concentrated to a yellow oil. Without further purification, the crude triflate was dissolved in DMF (3 mL) and treated with NaN_3 (160 mg, 2.5 mmol). The resulting suspension was stirred four hours at room temperature. Water (5 mL) was added, and the aqueous solution was extracted with ether (2 x 5 mL). The ether extracts were washed with brine (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated to a yellow oil. Purification by flash chromatography (hexane, then hexane/EtOAc = 8:1) afforded the azide **74** (53.6 mg, 0.22 mmol) as a pale yellow oil in 54% yield which crystallized upon standing.

Physical data for **74**

R_f = 0.8 (hexane/ethyl acetate = 3:1); IR (film) 2986, 2906, 2103, 1229, 1159, 1103, 1087 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.81-5.96 (m, 1H), 5.09-5.19 (m, 2H), 4.14-4.16 (m, 1H), 3.98 (dd, 1H, *J* = 12.9, 1.6), 3.52-3.60 (m, 2H), 3.45-3.53 (m, 2H), 2.49-2.58 (m, 1H), 2.39-2.49 (m, 1H), 1.48 (s, 3H), 1.45 (s, 3H); FABMS. Calcd. for $C_{11}H_{17}N_3O_3$ $(M + H)^+$: 240.1348; found: 240.1346.



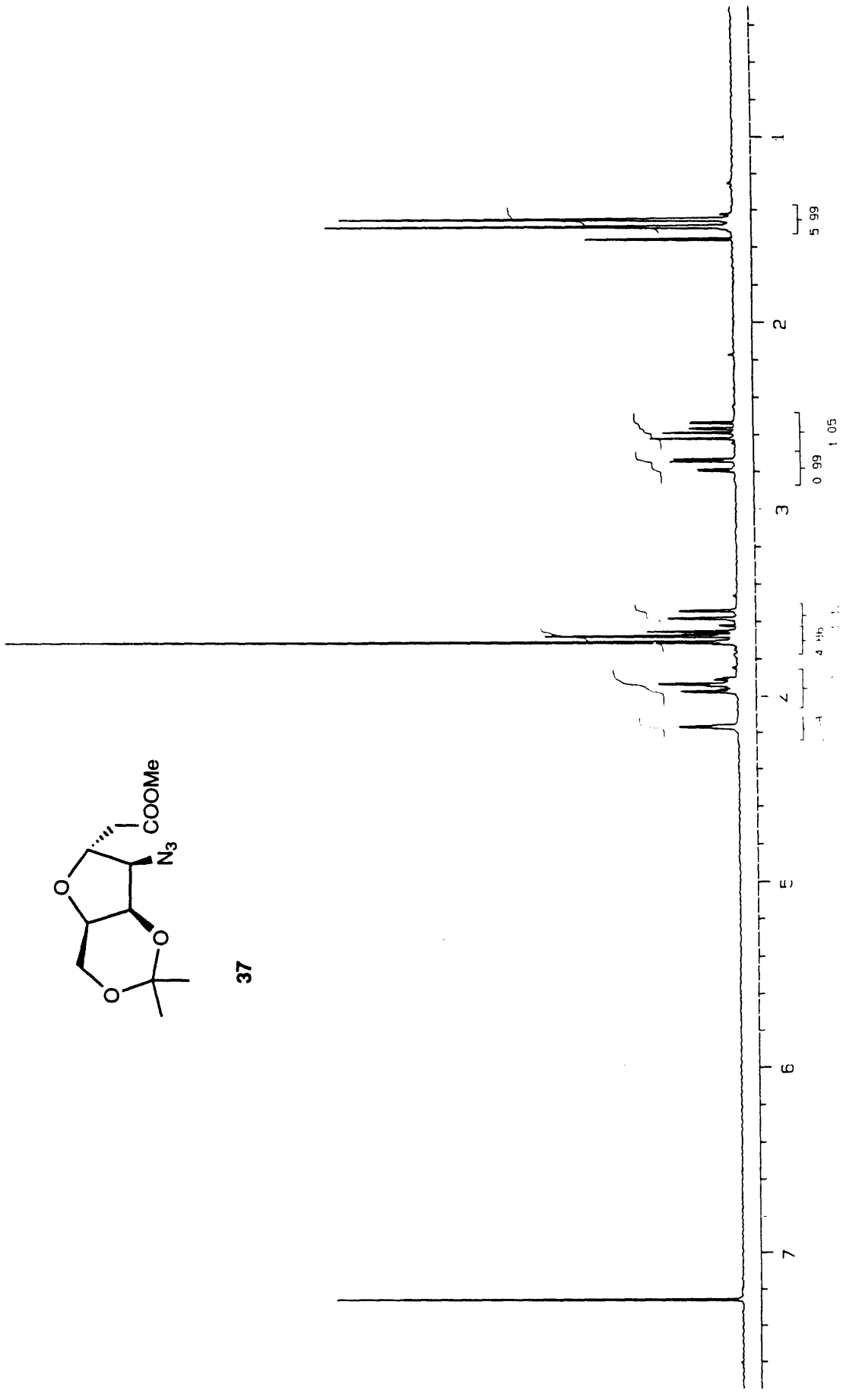
74 37

Water (0.5 mL) and acetic acid (100 uL) were added to a solution of the C-allyl glycoside **74** (18.0 mg, 75 umol) in CH_2Cl_2 (0.5 mL). Aliquat 336 (2 mg) was added as a phase transfer catalyst and the reaction vessel was cooled to 0°C before addition of $KMnO_4$ (25 mg). The reaction proceeded 24 hours at room temperature. Sodium sulfite (25 mg) was added to quench the reaction, which was then partitioned between CH_2Cl_2 (3 mL) and water (2.5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 mL), and the combined organic extracts were washed with brine (1 x 5 mL) and dried over sodium sulfate. Filtration, followed by concentration, gave an oil that could not be purified to homogeneity by flash chromatography, and instead was esterified directly. The oil was dissolved in DMF (1 mL). Sodium bicarbonate (20 mg) was added to the solution, followed by methyl iodide (20 uL). The reaction was stirred 24 hours at room temperature, then concentrated to an oil. Water (2 mL) was added, and the aqueous solution was extracted with ether (2 x 2.5 mL). The ether extracts were washed with brine (1 x 3 mL), dried over sodium sulfate, filtered, and concentrated. Purification by flash chromatography (hexane/EtOAc = 4:1) gave the glycosazido ester **37** (8.3 mg, 31 umol) in 41% yield.

Physical data for **37**

R_f = 0.6 (hexane/ethyl acetate = 1:1); IR (film) 2986, 2901, 2105, 1740, 1229, 1102 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.16-4.18 (m, 1H), 3.91-3.98 (m, 2H), 3.71 (s, 3H), 3.63-3.70 (m, 2H), 3.57 (dd, 1H, *J* = 12.8, 1.8), 2.77 (dd, 1H, *J* = 16.0, 2.8), 2.58 (dd, 1H, *J* = 16.0, 9.4), 1.49 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ

170.9, 110.8, 79.4, 76.8, 73.5, 68.2, 58.9, 51.9, 37.7, 26.6, 26.3; HRMS. Calcd. for $C_{11}H_{17}N_3O_5$ $(M + H)^+$: 272.1246; found: 272.1241.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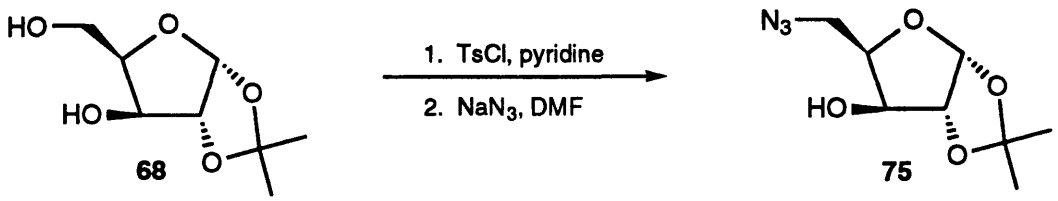
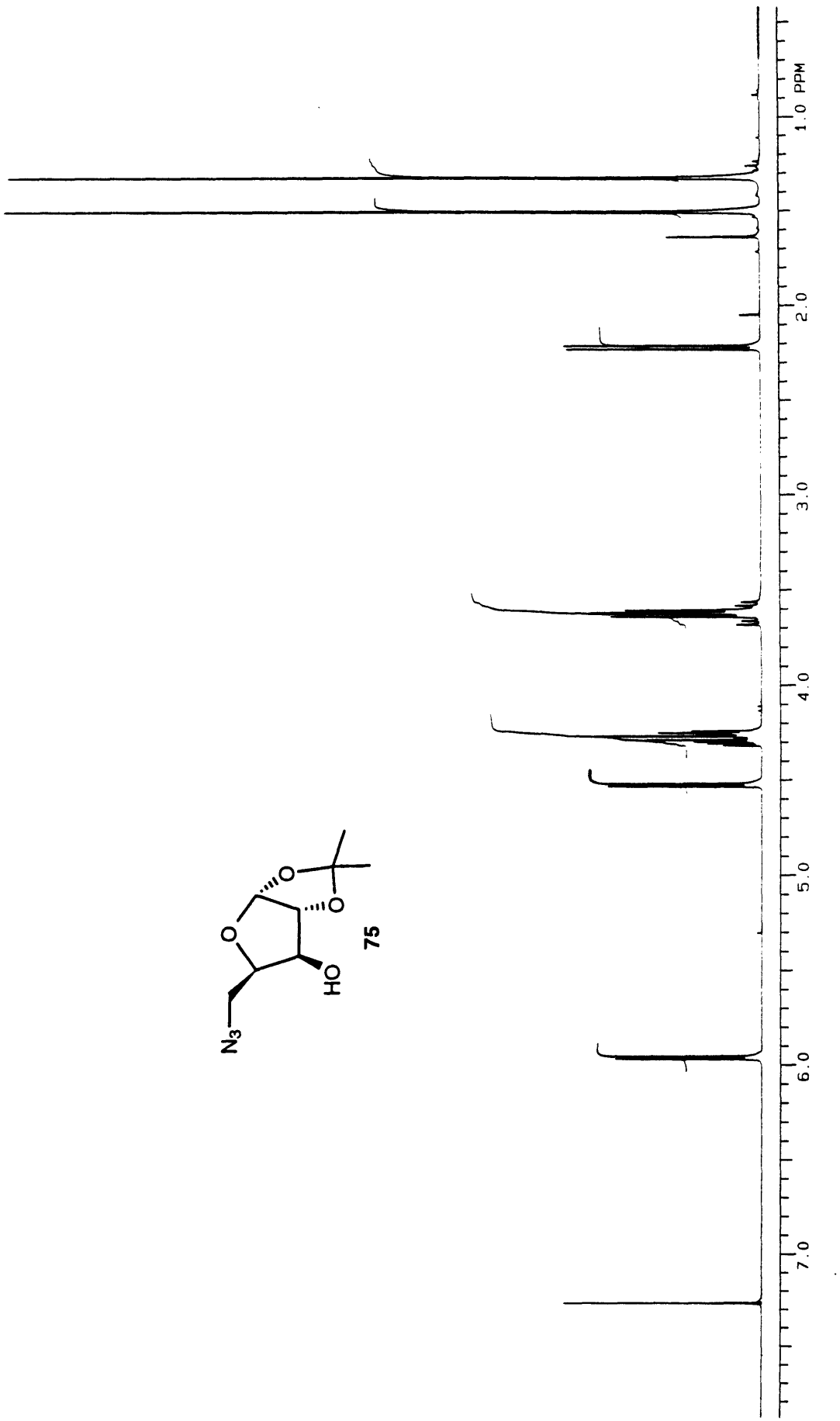


Pyridine (50 mL) was added to a 0°C mixture of isopropylidene **68** (5.85 g, 30.8 mmol) and tosyl chloride (6.5 g, 34.0 mmol). The reaction was allowed to warm to room temperature and stirred 16 hours. Methanol (3 mL) was added to destroy excess tosyl chloride. After 15 minutes, the solution was concentrated to a syrup, then taken up in EtOAc (50 mL) and washed successively with water (1 x 40 mL), sodium bicarbonate (1 x 40 mL), and brine (1 x 40 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated to a solid. Recrystallization from absolute ethanol provide the tosylate (5.94 g, 17.3 mmol) in 56% yield. A portion of the crystals (578 mg, 1.68 mmol) and NaN_3 (513 mg, 7.9 mmol) were combined in a flask, to which was added DMF (12 mL). The suspension was stirred 72 hours in an oil bath heated to 70°C. After cooling to room temperature, the contents of the reaction flask were added to water (25 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were washed with brine (1 x 30 mL) and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 2:1) gave the azide **75** (338 mg, 1.57 mmol) as white crystals (cyclohexane was determined to be a suitable solvent for recrystallization) in 94% yield (53% from **68**).

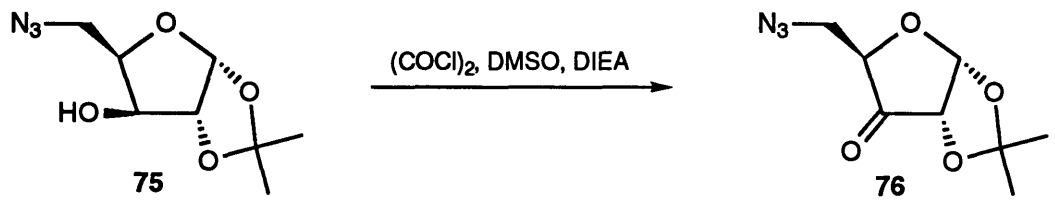
Physical data for **75**

R_f = 0.3 (hexane/ethyl acetate = 3:1); IR (film) 3404 (br), 2987, 2931, 2098, 1375, 1215, 1070, 1008 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.96 (d, 1H, J = 3.7), 4.52 (dd, 1H, J = 3.7, < 1), 4.24 - 4.30 (m, 2H), 3.57 - 3.63 (m, 2H), 2.22 (d, 1H, J = 5.2), 1.51 (s,

3H), 1.32 (s, 3H); FABMS. Calcd. for $C_8H_{13}N_3O_4$ $(M + H)^+$: 216.0984; found: 216.0986.



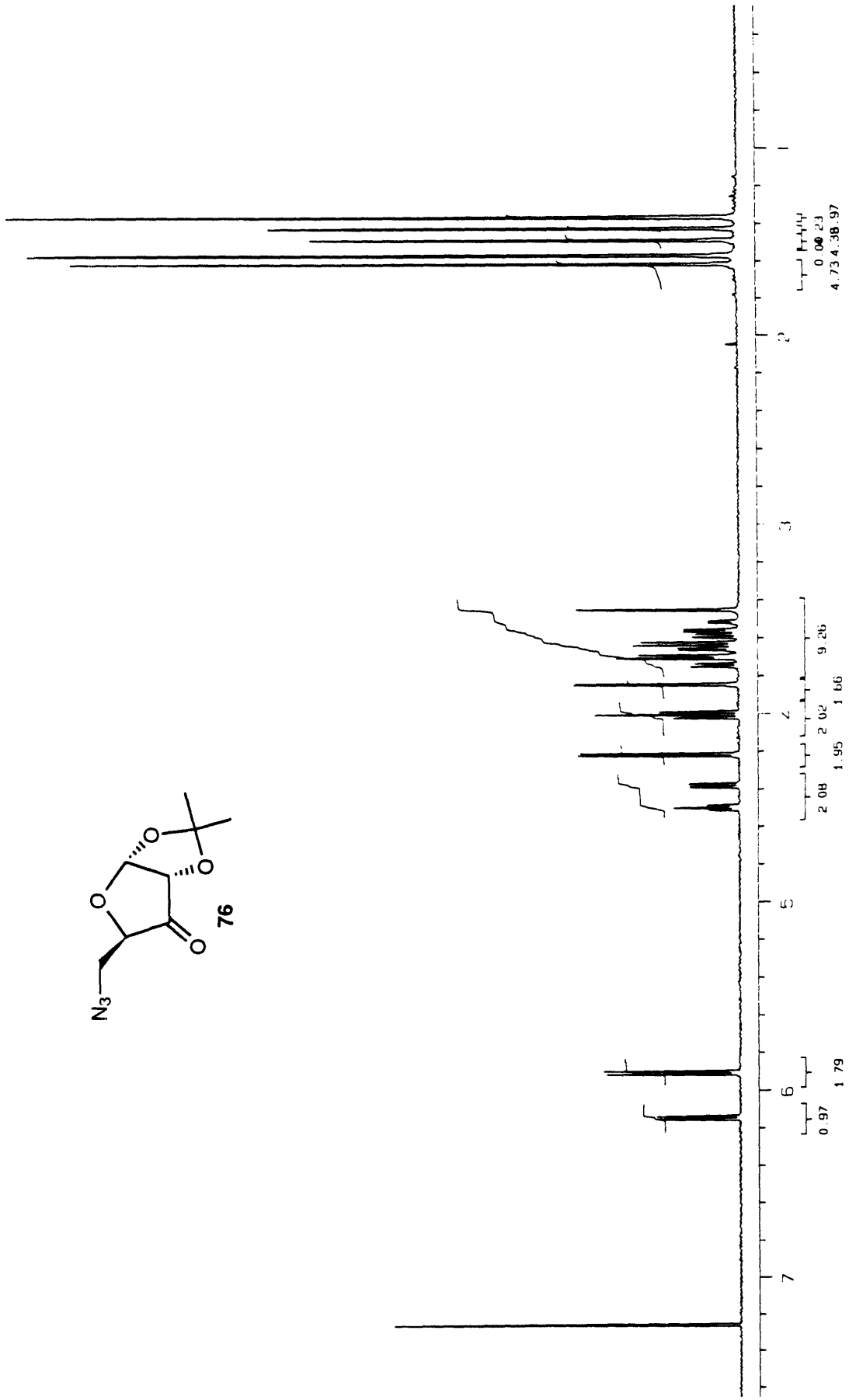
75



A solution of DMSO (185 uL, 2.6 mmol) in CH_2Cl_2 (1mL) was added dropwise to a solution of oxalyl chloride (650 uL of 2.0 M solution in CH_2Cl_2, 1.3 mmol) in CH_2Cl_2 cooled to below -60°C. The reaction flask was stirred 15 minutes at this temperature before a cooled solution of **75** (217 mg, 1.01 mmol) in CH_2Cl_2 (2mL) was added. Stirring continued for 20 minutes with gradual warming to -40°C, at which point DIEA (1.05 mL, 6 mmol) was added. The ice bath was removed and the reaction proceeded for three hours. Water (7 mL) was added, and the aqueous solution was extracted with CH_2Cl_2 (2 x 5 mL). The combined organics were washed with 1 M HCl (1 x 7 mL), saturated $NaHCO_3$ (1 x 7 mL), and brine (1 x 7 mL), then dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 4:1) afforded the ketone **76** (122.8 mg, 0.58 mmol) as a colorless oil in 57% yield.

Physical data for **76**

R_f = 0.25 (hexane/ethyl acetate = 2:1); IR (film) 3400 (small, br), 2992, 2110, 1775, 1377, 1220, 1158, 1081cm^{-1}; ^1H NMR of **72** showed a mixture of two products, presumably the ketone and the hydrate. HRMS. Calcd. for $C_8H_{11}N_3O_4$ $(M + H)^+$: 214.0828; found: 214.0827.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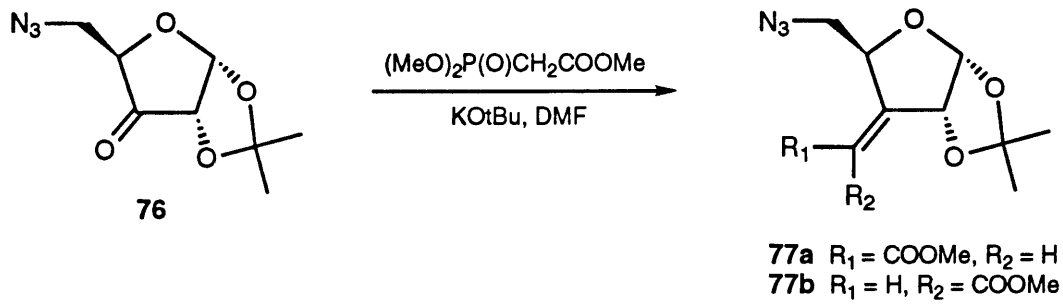


76

77a R_1 = COOMe, R_2 = H
77b R_1 = H, R_2 = COOMe

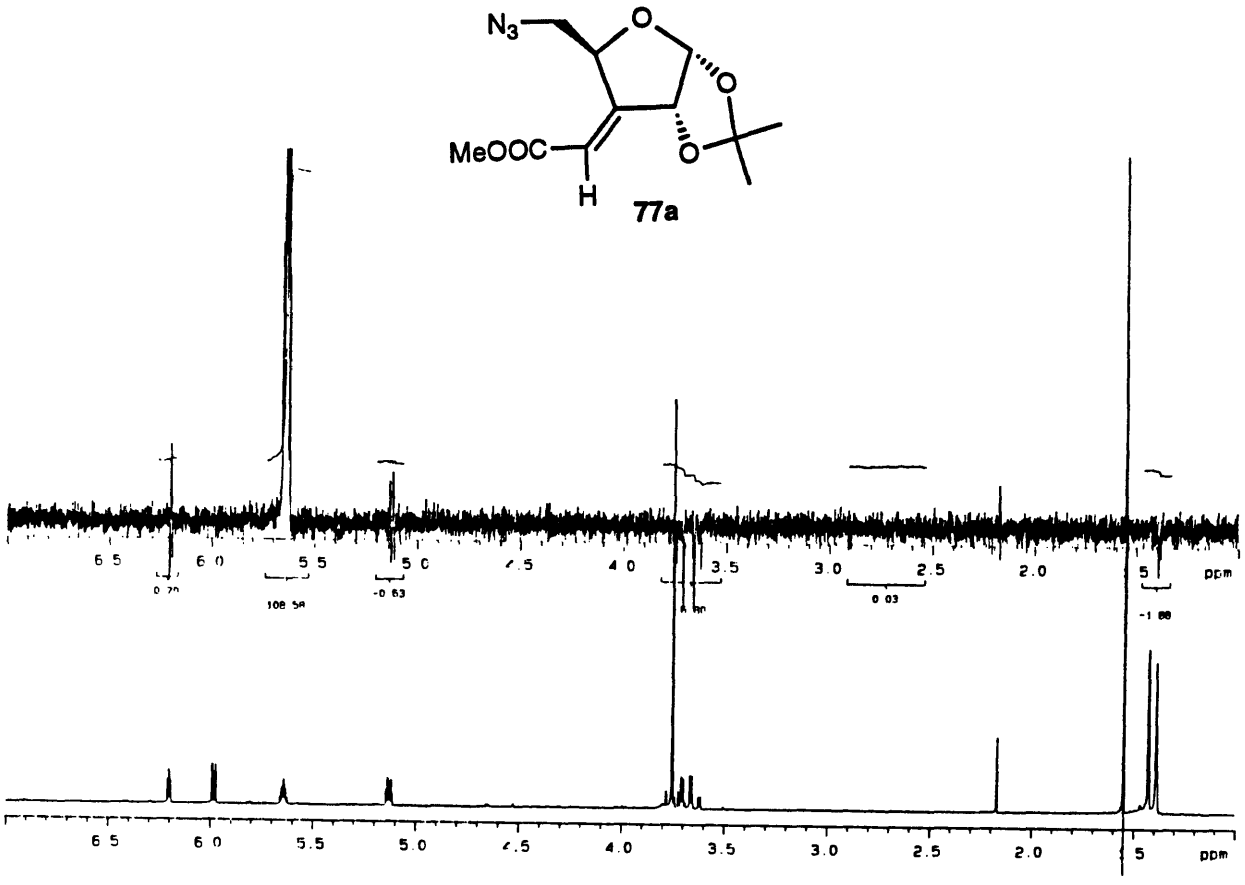
A solution of the ketone **76** (36.6 mg, 0.17 mmol) in DMF (1 mL) was added to a 0°C solution of trimethyl phosphonoacetate (100 uL, 0.62 mmol) and potassium *tert*-butoxide (20.2 mg, 0.18 mmol) in DMF (0.5 mL). The reaction was warmed to room temperature and stirred two hours. Water (3 mL) was added and the aqueous solution was extracted with ether (2 x 3 mL). The combined organic extracts reaction were washed with water (1 x 5 mL), $KHSO_4$ (1 x 5 mL), and brine (1 x 5 mL). After drying over sodium sulfate, the organic solution was filtered and concentrated. Purification by flash chromatography (hexane/EtOAc = 5:1) afforded **77a** (4.2 mg, 0.015 mmol) and **77b** (23.9 mg, 0.088 mmol) as a mixture of cis-trans isomers in 61% yield.

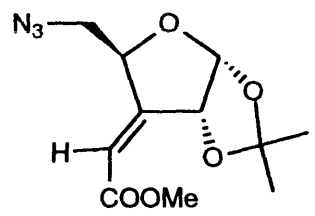
Physical data for **77a**

R_f = 0.75 (hexane/ethyl acetate = 2.5:1); IR (film) 2994, 2953, 2112, 1717, 1371, 1227, 1159 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.20 (dd, 1H, *J* = 2.0, 2.0), 5.98 (d, 1H, *J* = 4.7), 5.63-5.66 (m, 1H), 5.11-5.14 (m, 1H), 3.75 (s, 3H), 3.73 (dd, 1H, *J* = 12.7, 3.0), 3.64 (dd, 1H, *J* = 12.7, 2.9), 1.43 (s, 3H), 1.39 (s, 3H); HRMS. Calcd. for $C_{11}H_{15}N_3O_5$ $(M + H)^+$: 270.1090; found: 270.1091. Upon irradiation at δ 5.13 (H2), an NOE was observed at δ 6.20 (vinylic proton). Upon irradiation at δ 5.64 (H4), an NOE was observed at δ 1.39 (isopropylidene CH_3).

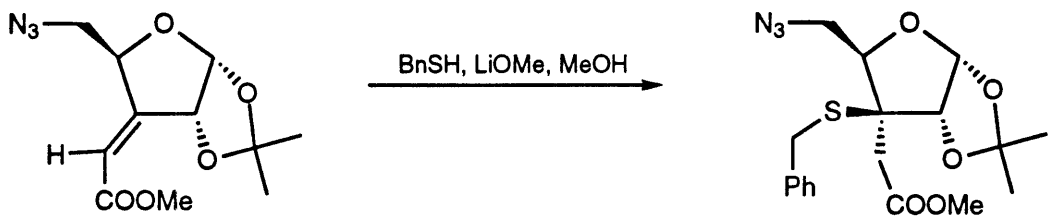
Physical data for 77b

R_f = 0.5 (hexane/ethyl acetate = 2.5:1); IR (film) 2991, 2954, 2107, 1727, 1436, 1374, 1216, 1069, 1018 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.96 (d, 1H, J = 4.1), 5.87 (dd, 1H, J = 2.0, 1.5), 5.74-5.76 (m, 1H), 4.97-5.02 (m, 1H), 3.80 (s, 3H), 3.65 (dd, 1H, J = 13.2, 3.7), 3.40 (dd, 1H, J = 13.2, 4.3), 1.50 (s, 3H), 1.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 155.6, 117.2, 113.5, 105.5, 79.2, 78.6, 53.2, 52.4, 27.8, 27.6; HRMS. Calcd. for $C_{11}H_{15}N_3O_5$ $(M + H)^+$: 270.1090; found: 270.1087. Upon irradiation at δ 5.00 (H4), an NOE was observed at δ 5.87 (vinylic proton).





77b



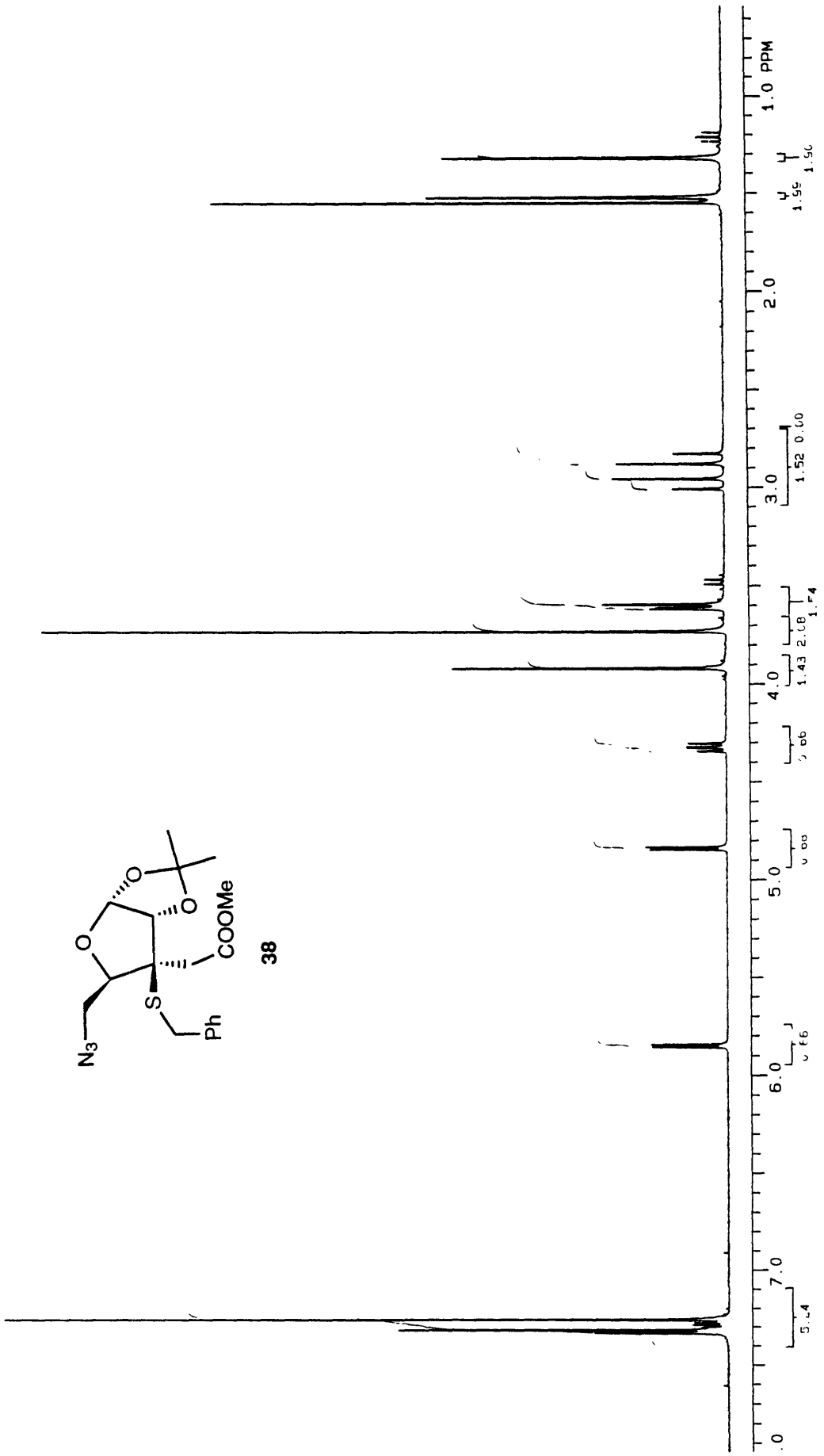
77b **38**

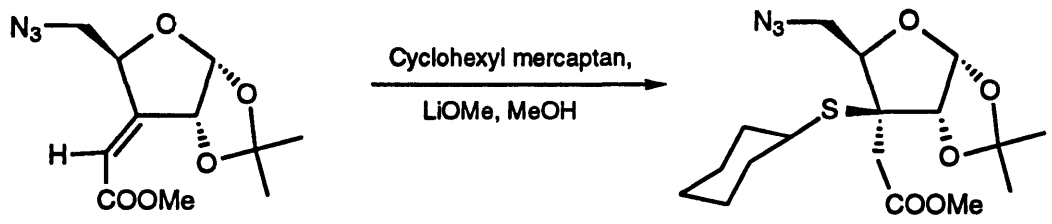
Lithium methoxide (3.0 mg, 0.08 mmol) was added to a solution of benzyl mercaptan (20 uL, mmol) and the α,β-unsaturated ester **77b** (3.1 mg, 0.012 mmol) in methanol (0.7 mL). After stirring five minutes, cation exchange resin was added to neutralize the solution, which was filtered and concentrated. Purification by flash chromatography (hexane, then hexane/EtOAc = 2:1) afforded **38** (4.7 mg, 0.012 mmol) as a colorless oil in quantitative yield.

Physical data for **38**

R_f = 0.6 (hexane/EtOAc = 3:1); IR (film) 2976, 2965, 2359, 2099, 1738, 1201, 1025 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26-7.33 (m, 5H), 5.85 (d, 1H, *J* = 3.3), 4.83 (d, 1H, *J* =3.3) 4.32 (dd, 1H, *J* = 6.8, 5.0), 3.92 (s, 2H), 3.73 (s, 3H), 3.59-3.62 (m, 2H), 2.98 (d, 1H, *J* = 15.6), 2.85 (d, 1H, *J* = 15.6), 1.52 (s, 3H), 1.32 (s, 3H); HRMS. Calcd. for $C_{18}H_{23}N_3O_5S$ $(M + H)^+$: 394.1437; found: 394.1428.

Upon irradiation of the benzylic protons (δ 3.92), an NOE was observed at the C-2 hydrogen (δ 4.83).


O
O
O
COOMe
S
Ph
N3
38
7.0
6.0
5.0
4.0
3.0
2.0
1.0 PPM

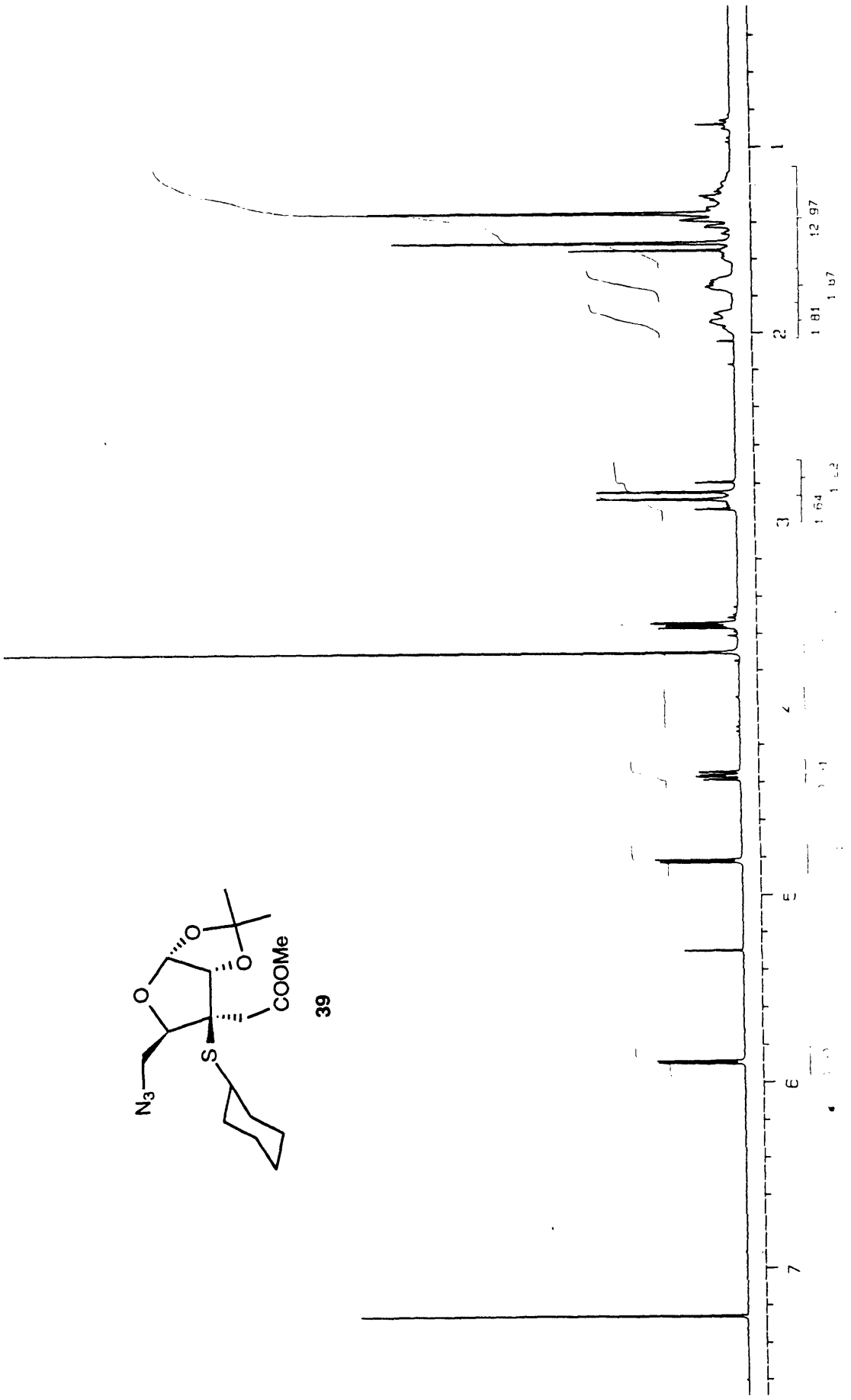


77b **39**

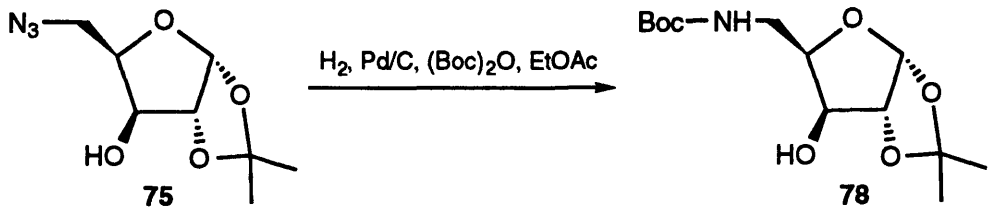
Cyclohexyl mercaptan (20 uL, 0.16 mmol) was added to a solution of lithium methoxide (1.5 mg, 0.04 mmol) and the α,β-unsaturated ester **77b** (5.6 mg, 0.021 mmol) in methanol (0.7 mL). After stirring fifteen minutes, cation exchange resin was added to neutralize the solution, which was filtered and concentrated. Purification by flash chromatography (hexane/EtOAc = 6:1) afforded the Michael adduct **39** (7.3 mg, 0.019 mmol) as a colorless oil in 91% yield. Michael addition of cyclohexyl mercaptan to **77a** also gave **39** as the only product.

Physical data for **39**

R_f = 0.7 (hexane/EtOAc = 2.5:1); IR (film) 2987, 2932, 2853, 2099, 1740, 1437, 1373, 1200, 1166, 1022 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.89 (d, 1H, *J* = 3.4), 4.82 (d, 1H, *J* = 3.4), 4.37 (dd, 1H, *J* = 6.9, 4.8), 3.72 (s, 3H), 3.54-3.57 (m, 2H), 2.79-2.93 (m, 3H), 1.89-1.99 (m, 2H), 1.71-1.76 (m, 2H), 1.19-1.59 (m, 6H), 1.51 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 112.4, 104.4, 86.1, 82.6, 58.3, 51.8, 51.0, 41.6, 37.8, 36.5, 36.1, 26.8, 26.7, 26.3, 26.3, 25.2; HRMS. Calcd. for $C_{17}H_{27}N_3O_5S$ $(M + H)^+$: 386.1750; found: 386.1747. Upon irradiation at δ 4.37 (H4), NOE's were observed at δ 2.87 (protons alpha to ester) and 1.51 (isopropylidene CH_3). Upon irradiation at δ 4.82 (H2), an NOE was observed at 2.90 (methine proton of cyclohexyl ring).



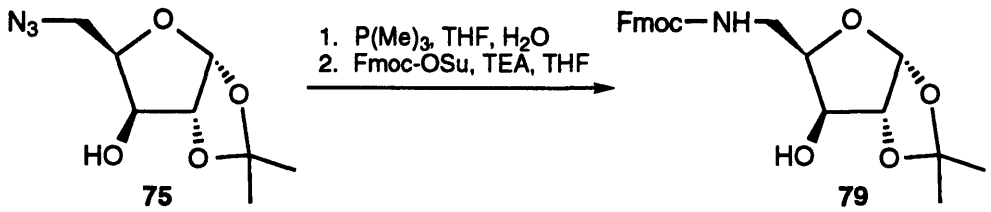
39



Ethyl acetate (1 mL) was added to 10% palladium on activated carbon (10 mg), and the suspension was stirred under a hydrogen atmosphere to saturate the catalyst. After ten minutes, a solution of the azido sugar **75** (27.1 mg, 0.13 mmol) and $(Boc)_2O$ in ethyl acetate (1 mL) was added to the reaction flask. After 24 hours, the reaction contents were filtered through Celite and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 3:1) afforded **78** (34.4 mg, 0.12 mmol) as a white solid in 95% yield.

Physical data for **78**

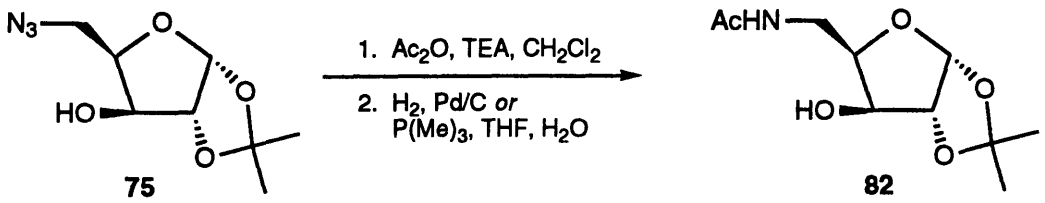
R_f = 0.5 (hexane/EtOAc = 2:1); IR (film) 3392, 2987, 1689, 1520, 1254, 1164, 1075, 1010 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.91 (d, 1H, J = 3.6), 5.84-5.90 (m, 1H), 4.75 (dd, 1H, J = 2.0, < 1), 4.59 (d, 1H, J = 3.6), 4.01-4.08 (m, 2H), 3.57-3.68 (m, 1H), 3.12-3.22 (m, 1H), 1.50 (s, 3H), 1.44 (s, 9H), 1.31 (s, 3H); FABMS: 290 $(M + H)^+$.



A solution of trimethyl phosphine in THF (250 uL of 1.0 M solution) was added to a solution of the azido sugar **75** (45.4 mg, 0.21 mmol) in THF (1.2 mL) and water (0.4 mL). After two hours, the solution was concentrated to a small volume (mostly water) and treated with a solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) (74 mg, 0.22 mmol) and TEA (30 uL, 0.22 mmol) in THF (1 mL). After one hour, the solution was concentrated to a small volume, then diluted with water (5 mL) and extracted with dichloromethane (2 x 5 mL). The organic extracts were washed with brine (1 x 5 mL), dried over sodium sulfate, filtered, and concentrated to a solid. Purification by flash chromatography (hexane/EtOAc = 2.6:1) afforded **79** (66.0 mg, 0.16 mmol) as a white solid in 76% yield.

Physical data for **79**

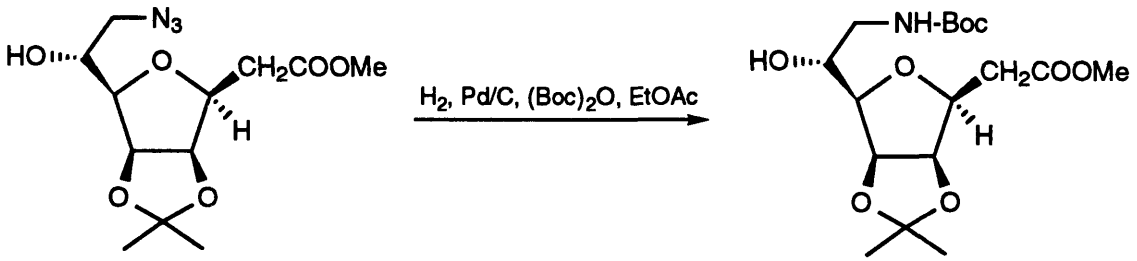
R_f = 0.7 (hexane/EtOAc = 1:1); IR (film) 3378, 2919, 1693, 1530, 1450, 1268, 1074, 1016 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, 2H, *J* = 7.2), 7.57 (d, 2H, *J* = 7.2), 7.42 (dd, 2H, *J* = 7.2, 6.8), 7.32 (dd, 2H, *J* = 7.2, 6.8), 5.90 (d, 1H, *J* = 2.9), 5.08-5.12 (br t, 1H), 4.58 (dd, 1H, *J* = 2.9, < 1), 4.48 (d, 2H, *J* = 7.3), 4.28-4.30 (m, 1H), 4.19 (t, 1H, J = 7.3), 4.03-4.07 (m, 2H), 3.61-3.71 (m, 1H), 3.21-3.29 (m, 1H), 1.49 (s, 3H), 1.32 (s, 3H).



In order to determine the ease of acetyl migration, the azido sugar **75** was acetylated with acetic anhydride and TEA in dichloromethane to give **80**. Catalytic hydrogenation or phosphine reduction (trimethyl phosphine; or triphenyl phosphine and a carboxylic acid in an attempt to directly form the amide) of **80** provided **82**, the product of acetyl migration, rather than the free amine **81**.

Physical data for **82**

R_f = 0.1 (hexane/EtOAc = 1:1); IR (film) 3342, 2987, 2934, 1653, 1558, 1374, 1073 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.07 (br t, 1H), 5.91 (d, 1H, J = 3.6), 4.91 (dd, 1H, J < 1), 4.59 (dd, 1H, J = 3.6, < 1), 4.03-4.07 (m, 2H), 3.78-3.87 (m, 1H), 3.17-3.24 (m, 1H), 1.49 (s, 3H), 1.32 (s, 3H); FABMS: 232 $(M + H)^+$.

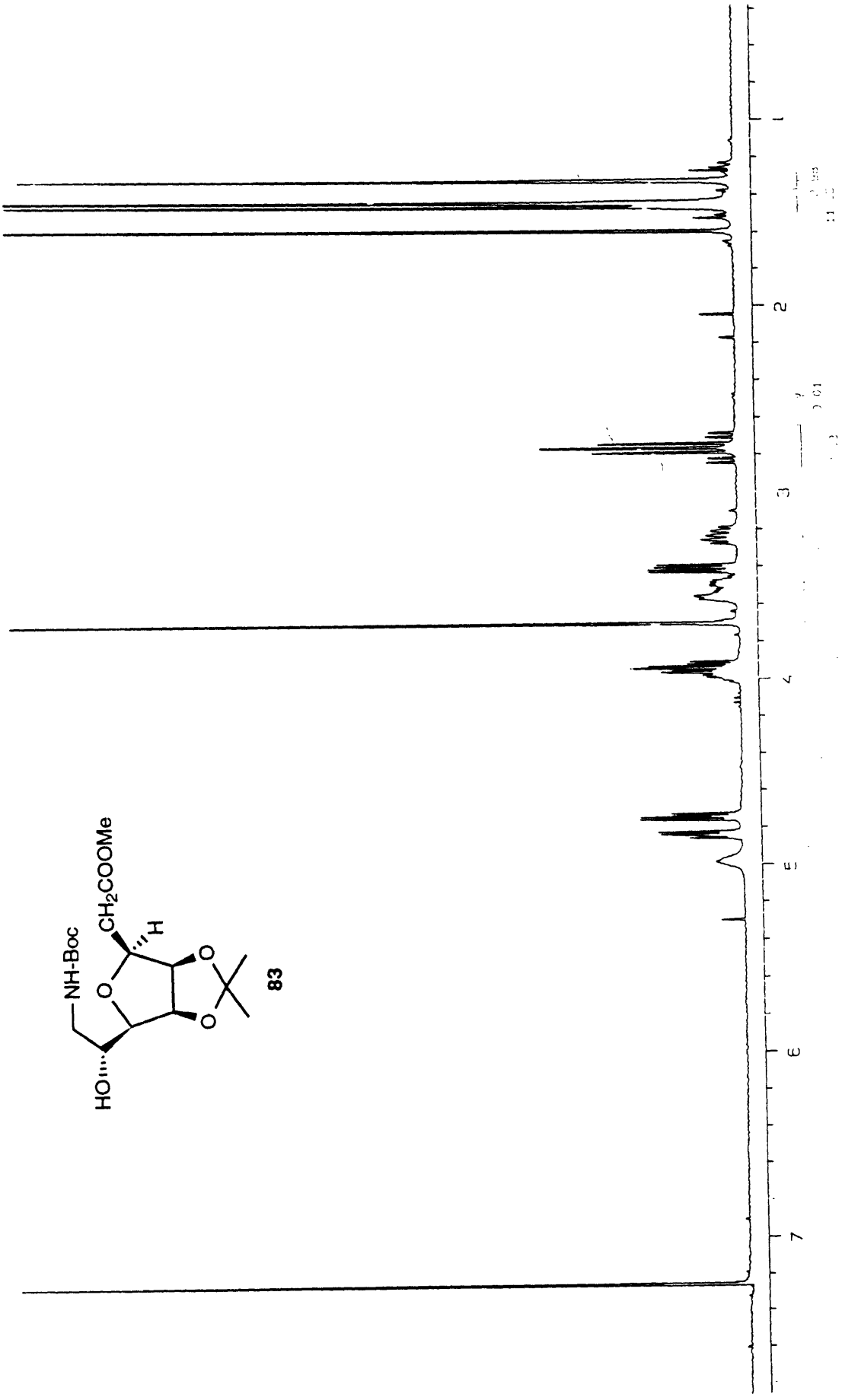


31 **83**

Hydrogen gas was bubbled through a suspension of 10% palladium on activated carbon (50 mg) in EtOAc (5 mL). After 15 minutes, a solution of **31** (468.2 mg, 1.55 mmol) and $(Boc)_2O$ (450 uL, 1.95 mmol) in EtOAc (5 mL) was added to the flask and the suspension was stirred 18 hours under a hydrogen atmosphere. The suspension was filtered through Celite, and the filtrate was concentrated under reduced pressure to an oil. Purification by flash chromatography (hexane/EtOAc = 1.7:1) gave **83** (481.2 mg, 1.28 mmol) as a colorless oil in 83% yield.

Physical data for **83**

R_f = 0.3 (hexane/ethyl acetate = 1:1); IR (film) 3385 (br), 2978, 2935, 1738, 1714, 1514, 1368, 1167, 1090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.95-5.02 (m, 1H), 4.84 (dd, 1H, *J* = 6.1, 3.7), 4.74 (dd, 1H, *J* =6.1, 3.6), 3.91-4.01 (m, 2H), 3.70 (s, 3H), 3.47-3.58 (m, 2H), 3.41 (dd, 1H, *J* = 8.0, 3.7), 3.19-3.27 (m, 1H), 2.69-2.85 (m, 2H), 1.47 (s, 9H), 1.45 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 157.3, 112.6, 81.5, 81.2, 80.9, 79.8, 77.6, 70.1, 51.7, 44.6, 33.3, 28.4, 25.8, 24.7; HRMS. Calcd. for $C_{17}H_{29}NO_8$ $(M + H)^+$: 376.1971; found: 376.1979.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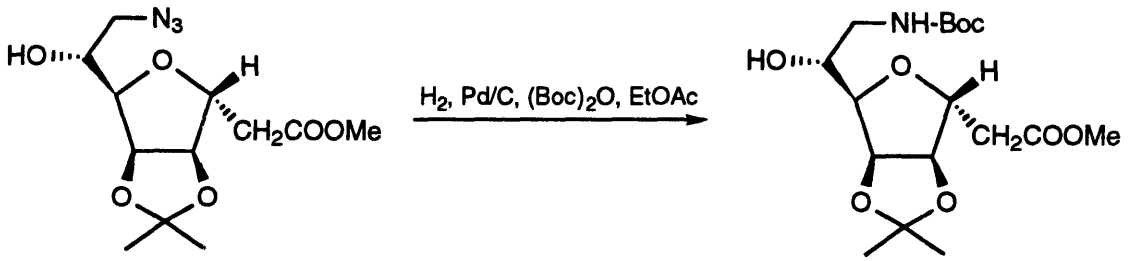
O
O
O
HO
83

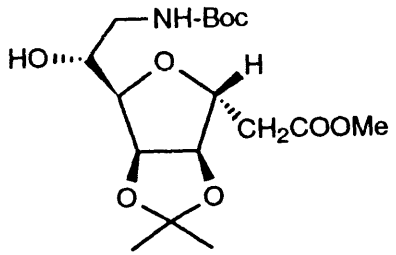


32 **84**

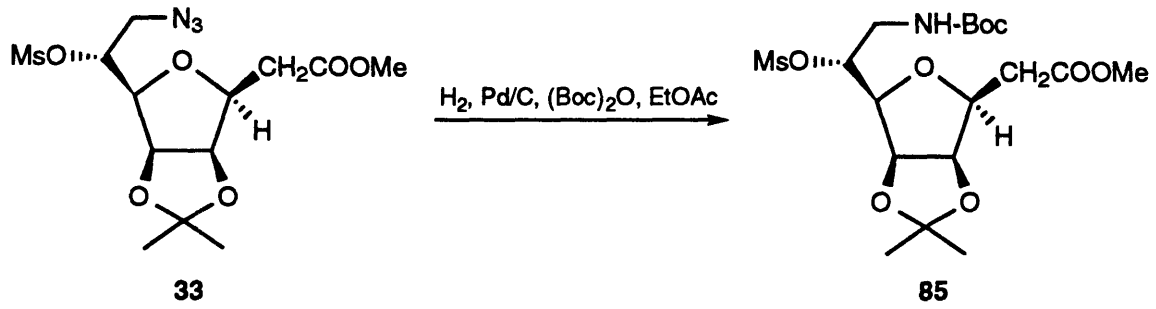
Hydrogen gas was bubbled through a suspension of 10% palladium on activated carbon (50 mg) in EtOAc (5 mL). After 15 minutes, a solution of **32** (247.3 mg, 0.82 mmol) and $(Boc)_2O$ (300 uL, 1.30 mmol) in EtOAc (5 mL) was added to the flask and the suspension was stirred 14 hours under a hydrogen atmosphere. The suspension was filtered through Celite, and the filtrate was concentrated under reduced pressure to an oil. Purification by flash chromatography (hexane/EtOAc = 1.8:1) gave **84** (242.2 mg, 0.65 mmol) as white crystals in 79% yield.

Physical data for **84**

R_f = 0.3 (hexane/ethyl acetate = 1:1); IR (film) 3394 (br), 2979, 2934, 1737, 1712, 1518, 1367, 1166, 1089 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.98-5.04 (m, 1H), 4.89 (dd, 1H, *J* = 5.7, 3.7), 4.62 (dd, 1H, *J*=5.7, 1.4), 4.46-4.51 (m, 1H), 3.94-4.01 (m, 1H), 3.70-3.75 (m, 1H), 3.71 (s, 3H), 3.48-3.56 (m, 2H), 3.19-3.27 (m, 1H), 2.43-2.56 (m, 2H) 1.51 (s, 3H), 1.45 (s, 9H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 157.3, 112.9, 84.6, 81.1, 80.5, 80.4, 79.6, 70.1, 51.8, 44.4, 36.2, 28.3, 26.1, 24.7; HRMS. Calcd. for $C_{17}H_{29}NO_8$ $(M + H)^+$: 376.1971; found: 376.1979.



84

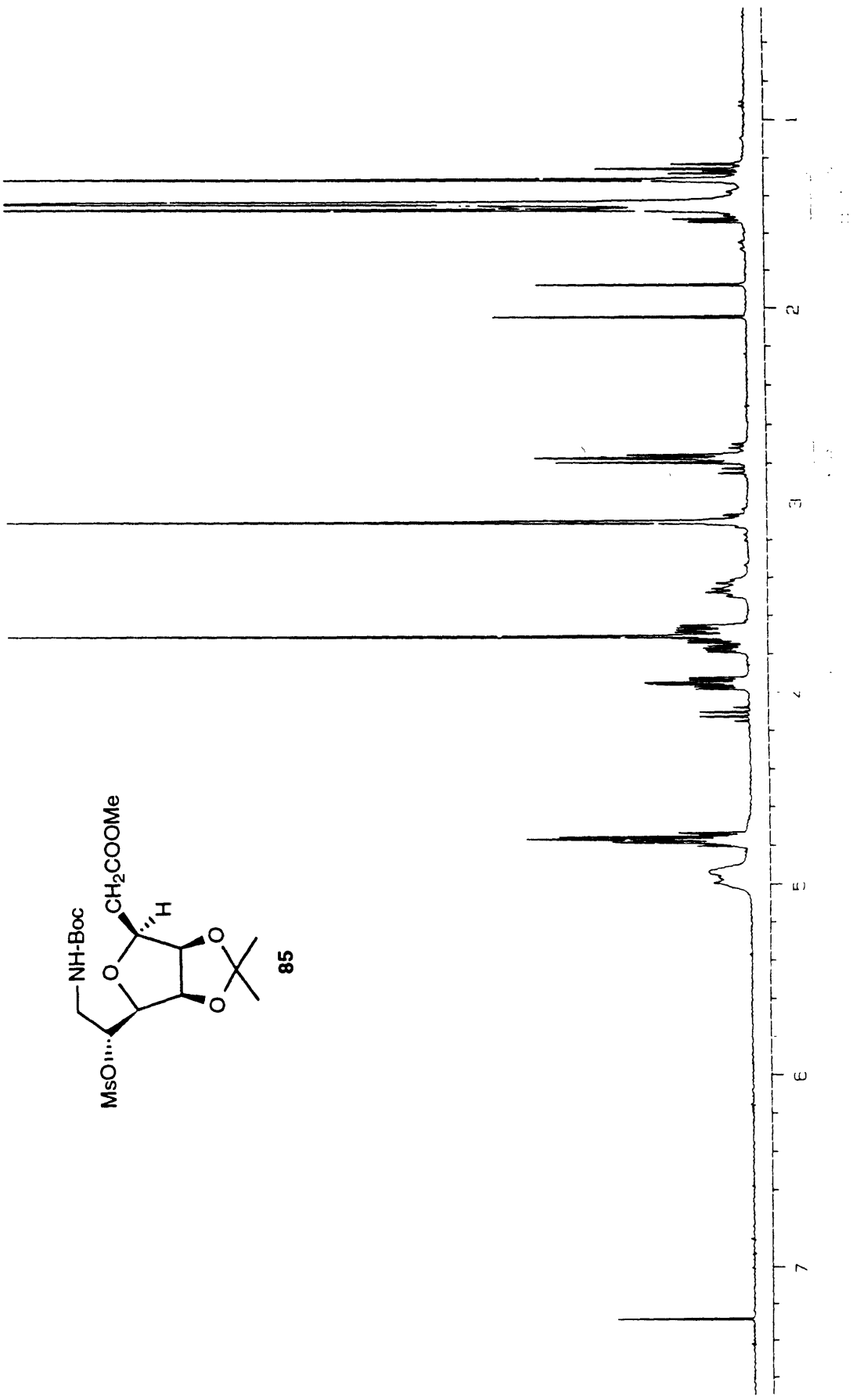


33 **85**

Hydrogen gas was bubbled through a suspension of 10% palladium on activated carbon (10 mg) in EtOAc. After 15 minutes, a solution of the azido sugar **33** (93.1 mg, 0.245 mmol) and $(Boc)_2O$ (75 uL, 0.35 mmol) in 1 mL EtOAc was added to the flask and the suspension was stirred 18 hours under a hydrogen atmosphere. The suspension was filtered through Celite, and the filtrate was concentrated under reduced pressure to an oil. Purification by flash chromatography (hexane/EtOAc = 1.8:1) gave **85** (102.4 mg, 0.226 mmol) in 92% yield.

Physical data for **85**

R_f = 0.4 (hexane/ethyl acetate = 1:1); IR (film) 3405 (br), 2980, 1715, 1515, 1356, 1175 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.92-5.00 (m, 2H), 4.74-4.81 (m, 2H), 3.93-3.99 (m, 1H), 3.71 (s, 3H), 3.66-3.80 (m, 2H), 3.42-3.50 (m, 1H), 3.11 (s, 3H), 2.71-2.86 (m, 2H), 1.48 (s, 3H), 1.44 (s, 9H), 1.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 155.8, 112.8, 80.7, 80.2, 79.9, 79.5, 77.8, 77.8, 51.8, 42.1, 38.4, 33.2, 28.3, 25.8, 24.8; HRMS. Calcd. for $C_{18}H_{31}NO_{10}S$ $(M + H)^+$: 454.1747; found: 454.1740.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O
MsO
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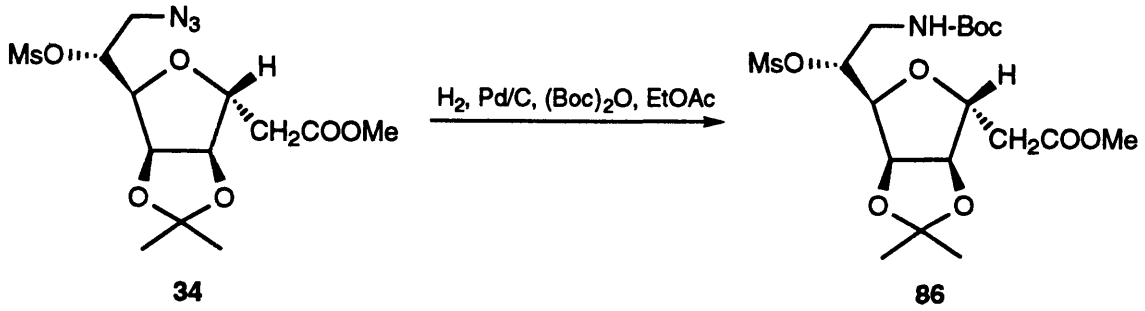
6
5
4
3
2
1

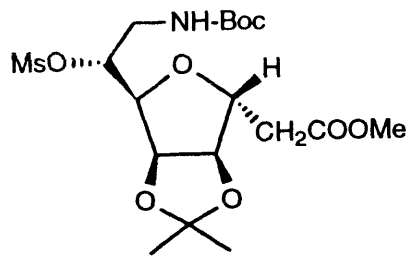


34 **86**

Hydrogen gas was bubbled through a suspension of 10% palladium on activated carbon (10 mg) in EtOAc. After 15 minutes, a solution of **34** (69.2 mg, 0.18 mmol) and $(Boc)_2O$ (50 uL, 0.24 mmol) in 1 mL EtOAc was added to the flask and the suspension was stirred 14 hours under a hydrogen atmosphere. The suspension was filtered through Celite, and the filtrate was concentrated under reduced pressure to an oil. Purification by flash chromatography (hexane/EtOAc = 1.8:1) gave **86** (74.8 mg, 0.17 mmol) as a colorless oil in 91% yield.

Physical data for **86**

R_f = 0.4 (hexane/ethyl acetate = 1:1); IR (film) 3404, 2980, 2938, 1715, 1516, 1355, 1176 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.90-5.01 (m, 2H), 4.79 (dd, 1H, *J* = 5.9, 3.8), 4.67 (dd, 1H, *J* = 5.9, < 1), 4.47-4.52 (m, 1H), 3.95-4.00 (m, 1H), 3.73-3.81 (m, 1H), 3.72 (s, 3H), 3.44-3.53 (m, 1H), 3.11 (s, 3H), 2.45-2.59 (m, 2H) 1.51 (s, 3H), 1.44 (s, 9H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 155.8, 113.2, 84.7, 80.8, 80.2, 79.4, 78.7, 78.2, 52.0, 41.9, 38.5, 35.9, 28.3, 26.2, 25.0; FABMS: 455 $(M + H)^+$.



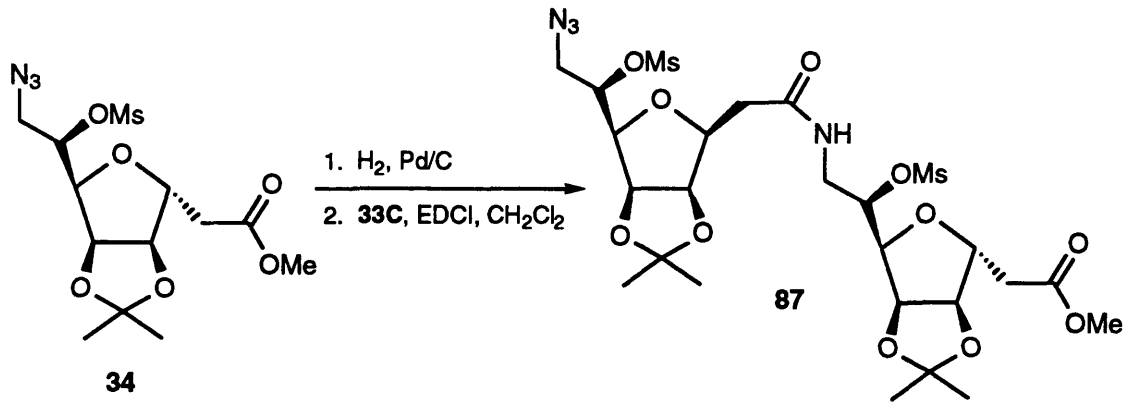
86

Experimentals for Chapter 6

Nomenclature. In order to reduce confusion, two conventions have been followed. Any azido ester (monomer or oligomer), represented by any number **X**, became **XC** when the ester was saponified and **XN** when the azide was reduced. For example, catalytic hydrogenation of the glycosazido ester **33** (MC) furnished the free amine **33N**, while saponification of **34** (MD) provided the carboxylic acid **34C**. (Note: the glycosazido acid **35** is denoted by **35**, rather than **35C**, since ester deprotection was not required.)

General procedure for hydrogenation of azides. Ethyl acetate (approximately 0.5 mL + 1 mL per 100 mg azide) was added to a flask containing 10% palladium on activated carbon. The flask was evacuated and flushed with hydrogen several times. A solution of the azide (2 - 10 mg azide per mg catalyst) was added to the flask, which was again flushed several times with hydrogen and stirred 4 - 24 hours under a hydrogen atmosphere. The suspension was then filtered through Celite, rinsed with ethyl acetate, and the filtrate was concentrated *in vacuo* and used without further purification.

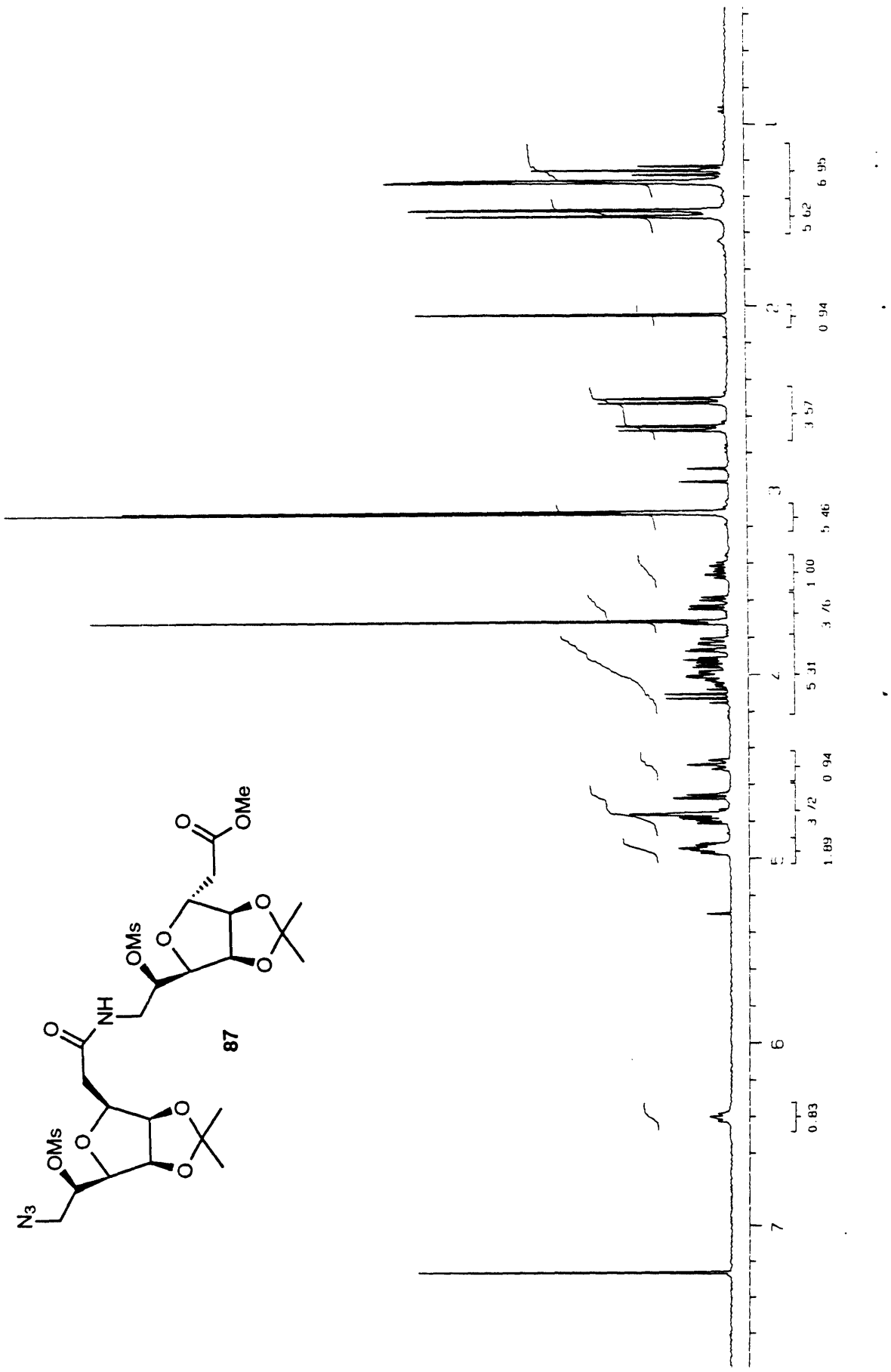
General procedure for ester saponification. A 0.5 M solution of sodium hydroxide (250 uL per 100 mmol ester) was added to a solution of the glycosazido ester in methanol (approximately 400 uL per 100 mmol ester). The reaction was followed by TLC, and upon completion, cation exchange resin was added to neutralize the solution. After filtration, the filtrate was concentrated and used without further purification.



The glycosazido ester **33** was hydrogenated according to the general procedure to furnish the amine **33N**, while the α-glycoside **34** was saponified according to the general procedure to give **34C**. A solution of the amine **33N** (33.8 mg, 0.09 mmol) in dichloromethane (0.5 mL) was added to a mixture of **34C** (36.0 mg, 0.10 mmol), TEA (41 uL, 0.30 mmol), and EDCI (24 mg, 0.12 mmol) in dichloromethane (1.0 mL). After stirring for 24 hours, the solution was concentrated, taken up in chloroform (5 mL), and extracted with water (1 x 5 mL) and brine (1 x 3 mL). After drying over sodium sulfate, the drying agent was removed by filtration and the solution was concentrated to an oil. Purification by flash chromatography (EtOAc/hexane = 1.8:1) afforded the diglycotide **87** (19.8 mg, 0.03 mmol) as a clear oil in 28% yield.

Physical data for **87**

R_f = 0.75 (dichloromethane/acetone = 3:1); IR (film) 3389, 2937, 2109, 1735, 1674, 1538, 1354, 1176 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.42 (dd, 1H, *J* = 6.3, 5.9), 4.92-4.98 (m, 2H), 4.76-4.81 (, 3H), 4.66 (dd, 1H, *J* = 6.0, 1.0), 4.49 (dd, 1H, *J* = 7.4, 7.1), 3.87-4.07 (m, 4H), 3.81 (dd, 1H, *J* = 7.4, 3.0), 3.71 (s, 3H), 3.62 (dd, 1H, *J* = 13.8, 5.0), 3.39-3.48 (m, 1H), 3.12 (s, 3H), 3.12 (s, 3H), 2.67 (d, 2H, *J* = 6.7), 2.52 (d, 2H, *J* = 7.3), 1.51 (s, 3H), 1.48 (s, 3H), 1.33 (s, 3H), 1.32 (s, 3H); HRMS. Calcd. for $C_{25}H_{40}N_4O_{15}S_2$ $(M + H)^+$: 701.2010; found: 701.2004.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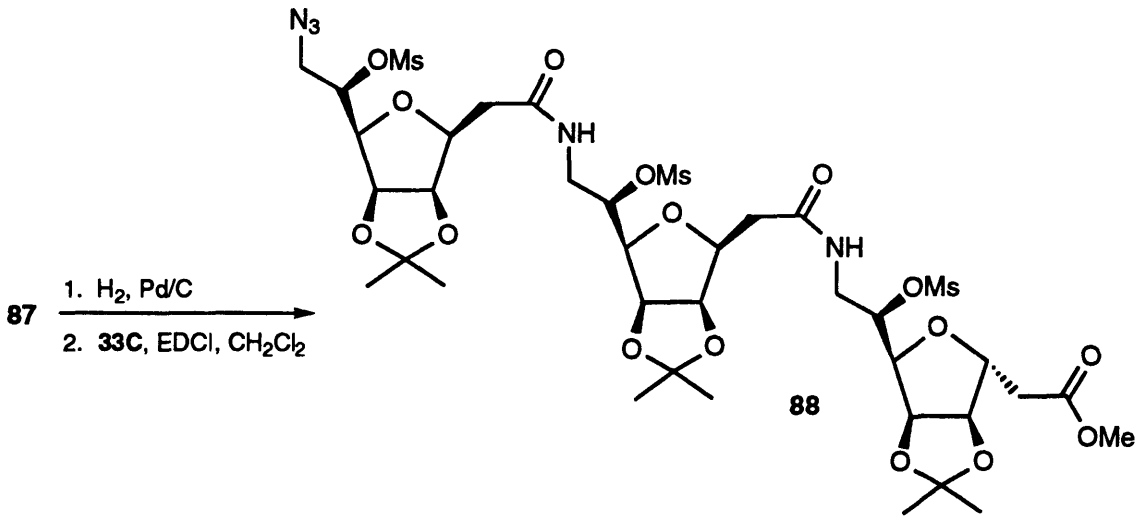
O
O
O
O
NH
OMs
O
O
O
O
OMe
87

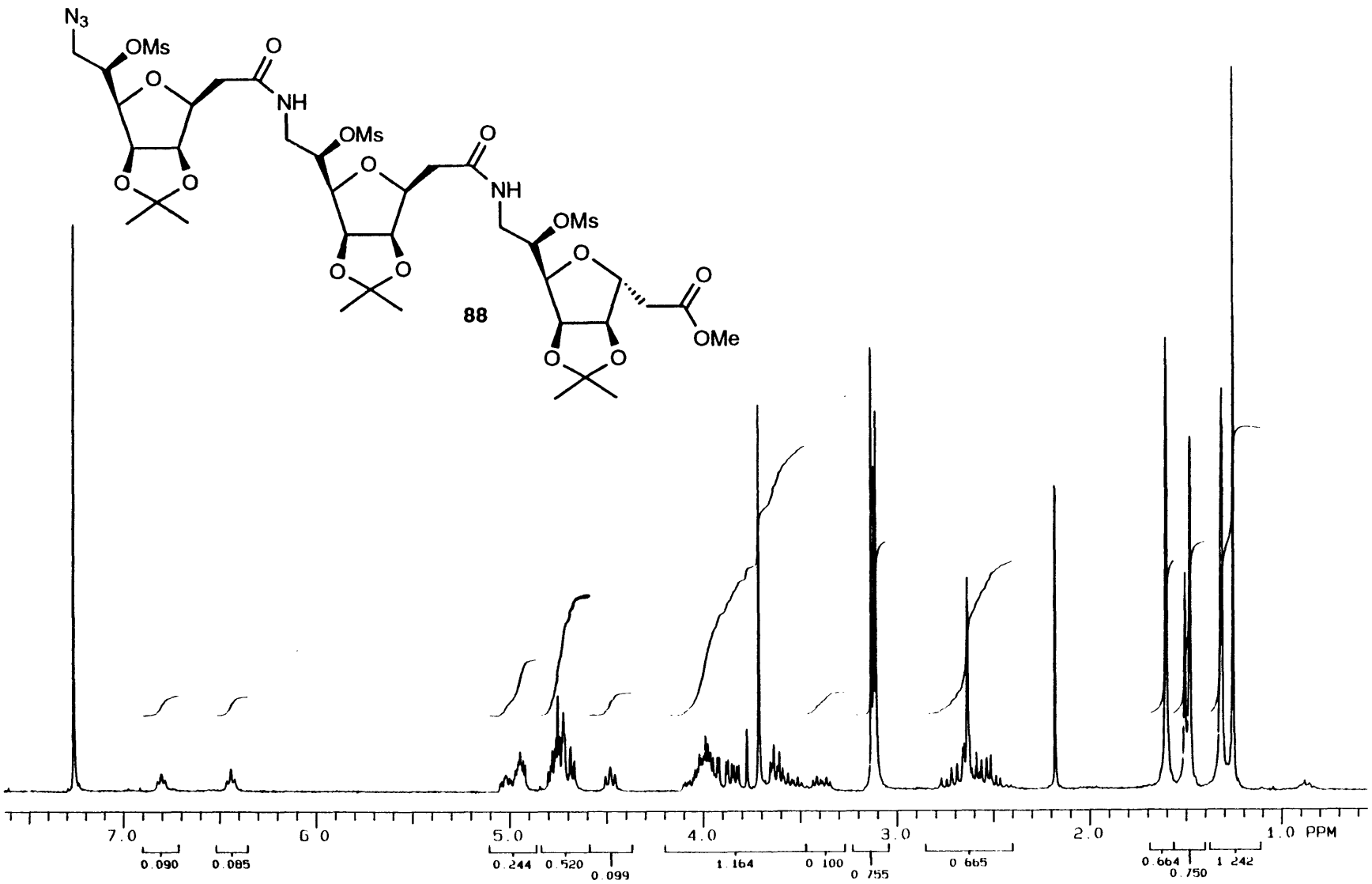


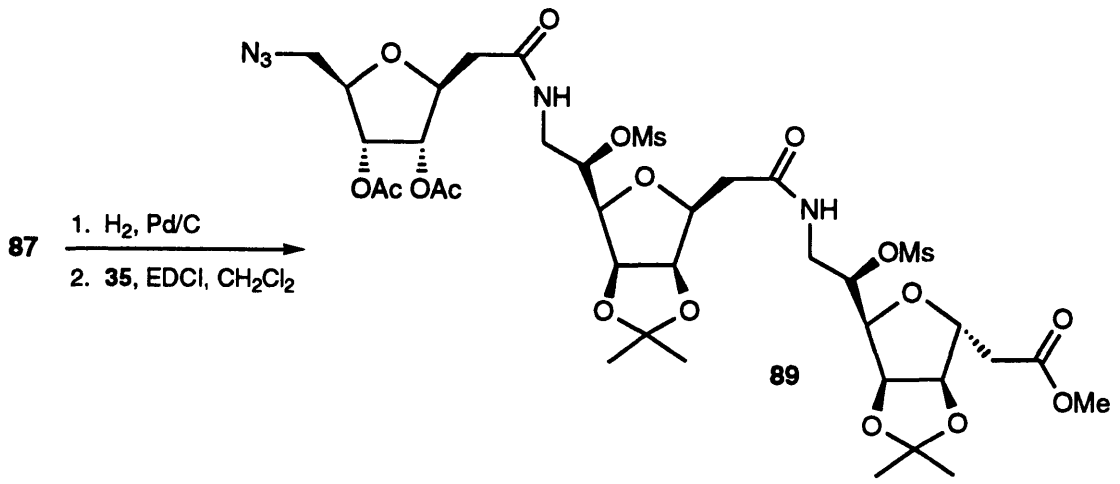
The diglycotide **87** (19.1 mg, 27 umol) was hydrogenated according to the general procedure to furnish the amino sugar **87N** (16.0 mg, 24 umol) in 87% crude yield. Without further purification, a solution of **87N** (8.0 mg, 12 umol) in dichloromethane (0.5 mL) was added to a mixture of excess **33C** (9.1 mg, 25 umol), TEA (10 uL, 70 umol), and EDCI (4.6 mg, 24 umol) in dichloromethane (1 mL). After stirring for 24 hours, the solution was concentrated, taken up in chloroform (3 mL), and extracted with water (1 x 3 mL) and brine (1 x 3 mL). The organic extracts were dried over sodium sulfate, then filtered and concentrated to an oil. Purification by flash chromatography (dichloromethane/ acetone = 4:1) afforded the triglycotide **88** (5.5 mg, 5.4 umol) as a clear oil in 45% yield.

Physical data for **88**

R_f = 0.2 (dichloromethane/acetone = 3:1); IR (film) 3381 (br), 2932, 2108, 1673, 1540, 1353, 1176, 1077 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (t, 1H, J = 6.0), 6.44 (t, 1H, J = 6.1), 4.92-5.05 (m, 3H), 4.66-4.80 (m, 6H), 4.48 (ddd, 1H, J = 7.3, 7.1, < 1), 3.82-4.11 (m, 8H), 3.71 (s, 3H), 3.49-3.65 (m, 2H), 3.34-3.43 (m, 1H), 3.13 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.46-2.77 (m, 6H), 1.50 (s, 3H), 1.48 (s, 3H), 1.48 (s, 3H),

1.31 (s, 3H), 1.31 (s, 3H), 1.31 (s, 3H); HRMS. Calcd. for $C_{37}H_{59}N_5O_{22}S_3$ $(M + H)^+$: 1022.2892; found: 1022.2932.

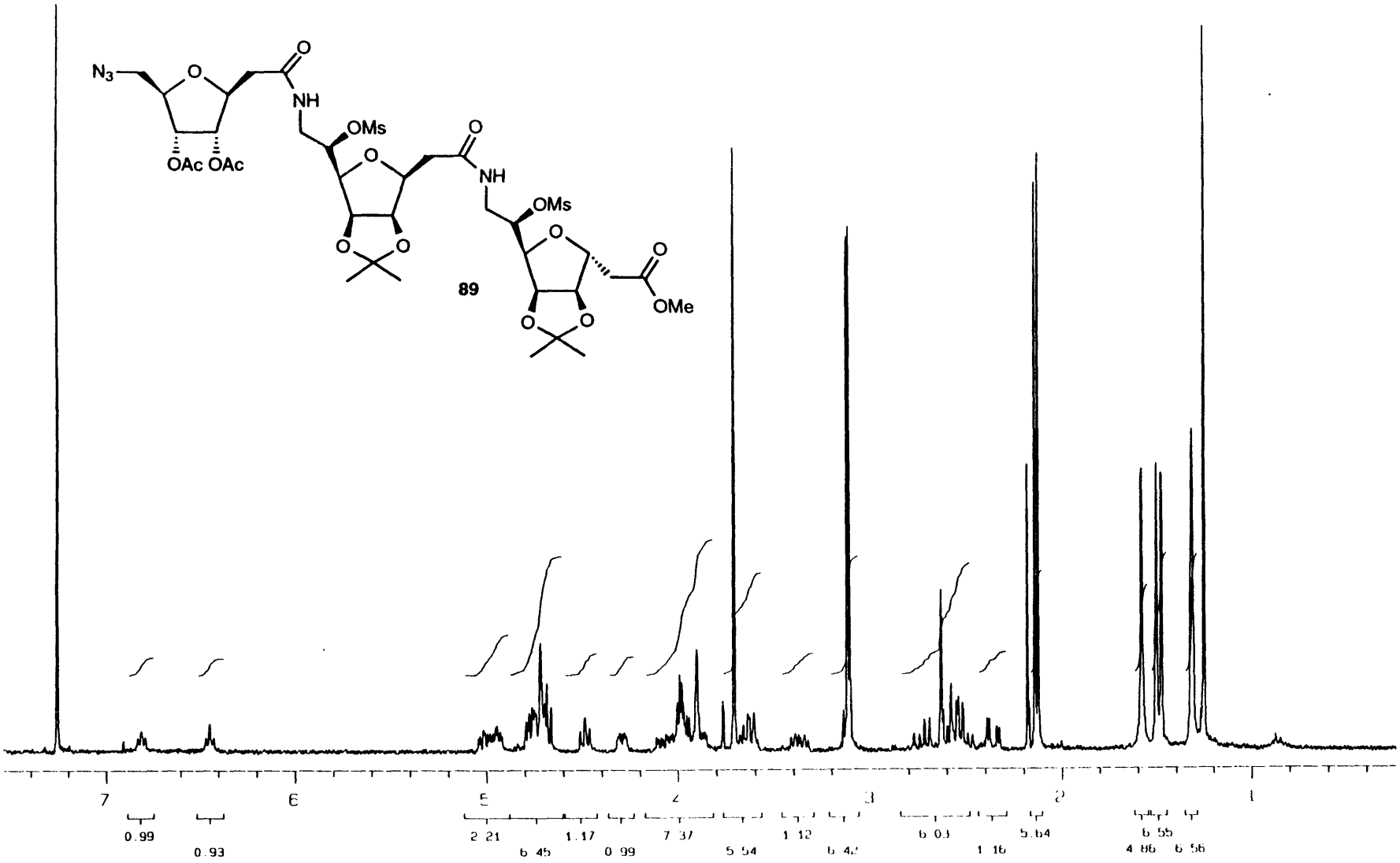

N3
OMs
O
NH
OMs
O
NH
OMs
O
OMe
88
7.0
6.0
5.0
4.0
3.0
2.0
1.0 PPM
0.090
0.085
0.244
0.520
0.099
1.164
0.100
0.755
0.665
0.664
0.750
1.242

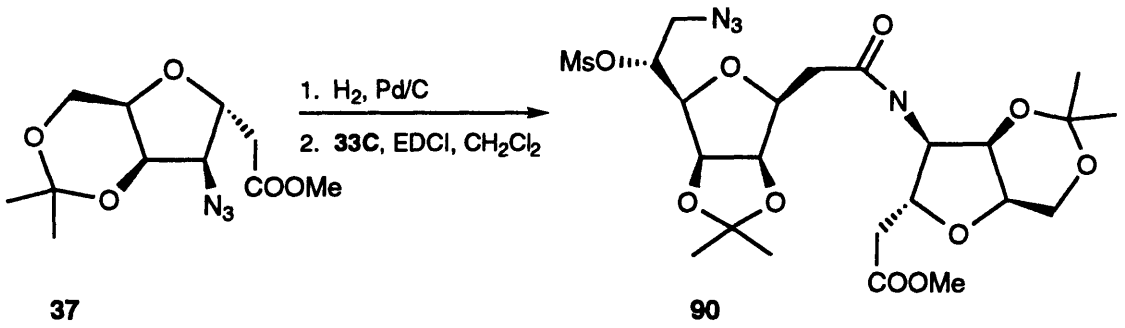


A solution of **87N** (8.0 mg, 12 umol) in dichloromethane (0.5 mL) was added to a mixture of the glycosazido acid **35** (9.0 mg, 30 umol), TEA (10 mg, 100 umol), and EDCI (4.5 mg, 23 umol) in dichloromethane (1 mL). After stirring for 24 hours, the solution was concentrated, taken up in chloroform (3 mL), and extracted with water (1 x 3 mL) and brine (1 x 3 mL). The organic extracts were dried over sodium sulfate, then filtered and concentrated to an oil. Purification by flash chromatography (dichloromethane/acetone = 3.8:1) afforded the triglycotide **89** (5.0 mg, 5.2 umol) as a clear oil in 44% yield.

Physical data for **89**

R_f = 0.2 (dichloromethane/acetone = 3:1); IR (film) 3378, 2934, 2109, 1738, 1682, 1651, 1538, 1353, 1227, 1175, 1078 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.81 (dd, 1H, *J* = 6.4, 5.6), 6.45 (dd, 1H, *J* = 6.1, 5.7), 4.93-5.04 (m, 3H), 4.67-4.79 (m, 6H), 4.49 (dd, 1H, *J* = 7.5, 6.6), 4.27-4.32 (m, 1H), 3.86-4.12 (m, 6H), 3.71 (s, 3H), 3.60-3.70 (m, 2H), 3.33-3.42 (m, 1H), 3.12 (s, 3H), 3.11 (s, 3H), 2.47-2.77 (m, 5H), 2.36 (dd, 1H, *J* = 15.4, 4.0), 2.14 (s, 3H), 2.12 (s, 3H), 1.51 (s, 3H), 1.48 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H); HRMS. Calcd. for $C_{36}H_{55}N_5O_{21}S_2$ $(M + H)^+$: 958.2909; found: 958.2914.


N3
O
NH
OMs
OAc OAc
89
OMe
7
6
5
4
3
2
1
0.99
0.93
2.21
6.45
1.17
0.99
7.37
5.54
1.12
6.42
6.03
1.16
5.64
6.55
4.86
6.56

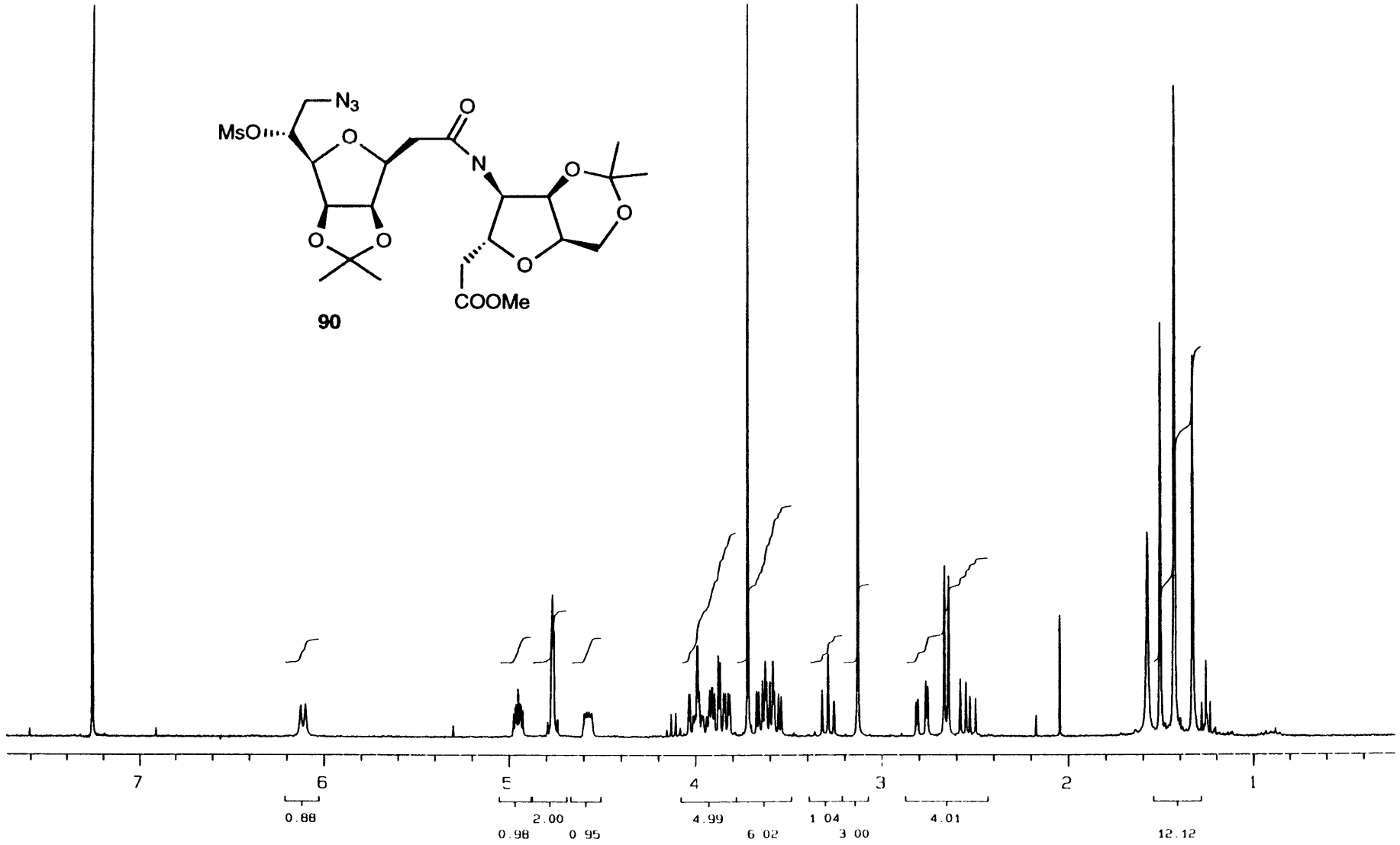


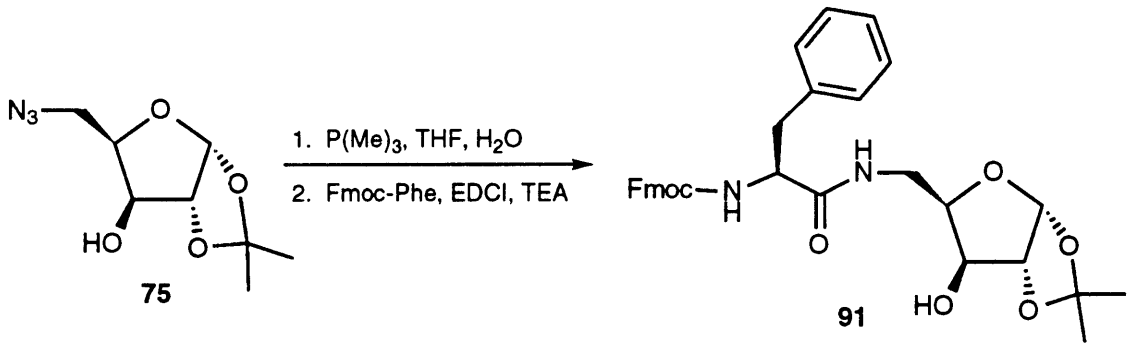
37 90

The glycosazido ester **37** (3.0 mg, 11 umol) was hydrogenated according to the general procedure to furnish the secondary amine **37N**. Without further purification, a solution of **37N** in dichloromethane (0.5 mL) was added to a mixture of excess **33C** (6.0 mg, 16 umol), TEA (10 uL, 70 umol), and EDCI (3.9 mg, 21 umol) in dichloromethane (0.5 mL). After stirring for 48 hours, the solution was concentrated, taken up in chloroform (3 mL), and extracted with water (1 x 3 mL) and brine (1 x 3 mL). The organic extracts were dried over sodium sulfate, then filtered and concentrated to an oil. Purification by flash chromatography (EtOAc/hexane = 2.2:1) afforded the diglycotide **90** (4.4 mg, 7.4 umol) as a clear oil in 67% yield from the glycosazido ester.

Physical data for **90**

R_f = 0.75 (dichloromethane/acetone = 3:1); IR (film) 3388 (br), 2990, 2931, 2109, 1738, 1682, 1651, 1538, 1360, 1231, 1176, 1100 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.11 (d, 1H, *J* = 7.8), 4.93-4.98 (m, 1H), 4.75-4.79 (m, 2H), 4.55-4.60 (m, 1H), 3.87-4.04 (m, 4H), 3.84 (dd, 1H, *J* = 7.6, 3.0), 3.72 (s, 3H), 3.54-3.68 (m, 3H), 3.29 (dd, 1H, *J* = 9.4, 9.4), 3.13 (s, 3H), 2.78 (dd, 1H, *J* = 15.6, 2.8), 2.65 (d, 2H, *J* = 6.8), 2.54 (d, 2H, *J* = 15.6, 9.4), 1.51 (s, 3H), 1.43 (s, 3H), 1.43 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 169.8, 113.0, 110.4, 81.0, 80.0, 78.9, 78.7, 77.9, 77.2, 76.9, 74.1, 69.6, 52.2, 52.0, 48.6, 38.5, 37.9, 36.1, 26.6, 26.5, 25.9, 24.9; HRMS. Calcd. for $C_{23}H_{36}N_4O_{12}S$ $(M + H)^+$: 593.2129; found: 593.2113.

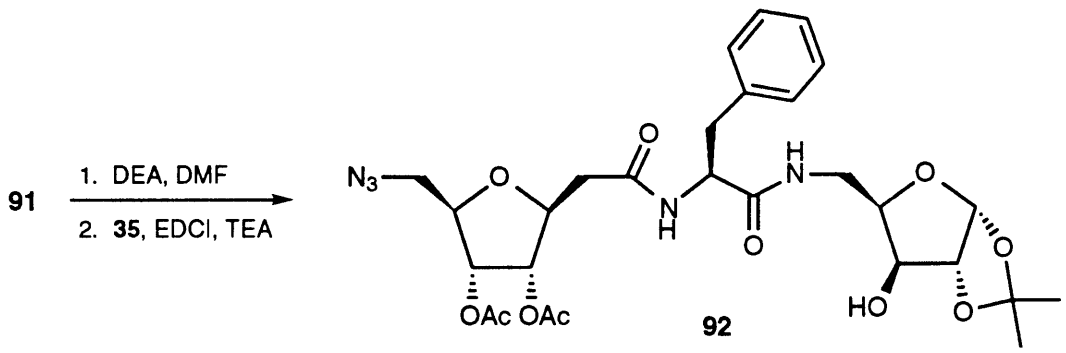

N3
MsO
O
N
O
O
O
O
O
O
COOMe
90
7
6
5
4
3
2
1
0.88
2.00
0.98
0.95
4.99
6.02
1.04
3.00
4.01
12.12



A solution of trimethyl phosphine in THF (500 uL of 1.0 M solution) was added to a solution of the azido sugar **75** (90.2 mg, 0.42 mmol) in THF (2 mL) and water (0.5 mL). After two hours, the solution was concentrated to a syrup and treated with a solution of PYBOP (260 mg, 0.50 mmol), TEA (150 uL, 1.08 mmol), and FMOC-phenylalanine (193 mg, 0.50 mmol) in DMF (2 mL). After stirring 20 minutes at room temperature, the solution was concentrated to an oil, then partitioned between water (5 mL) and ether (5 mL). The ether layer was washed with brine (1 x 5 mL), dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (hexane/EtOAc = 1.8:1) afforded **91** (82.3 mg, 0.15 mmol) as a white solid in 35% yield.

Physical data for **91**

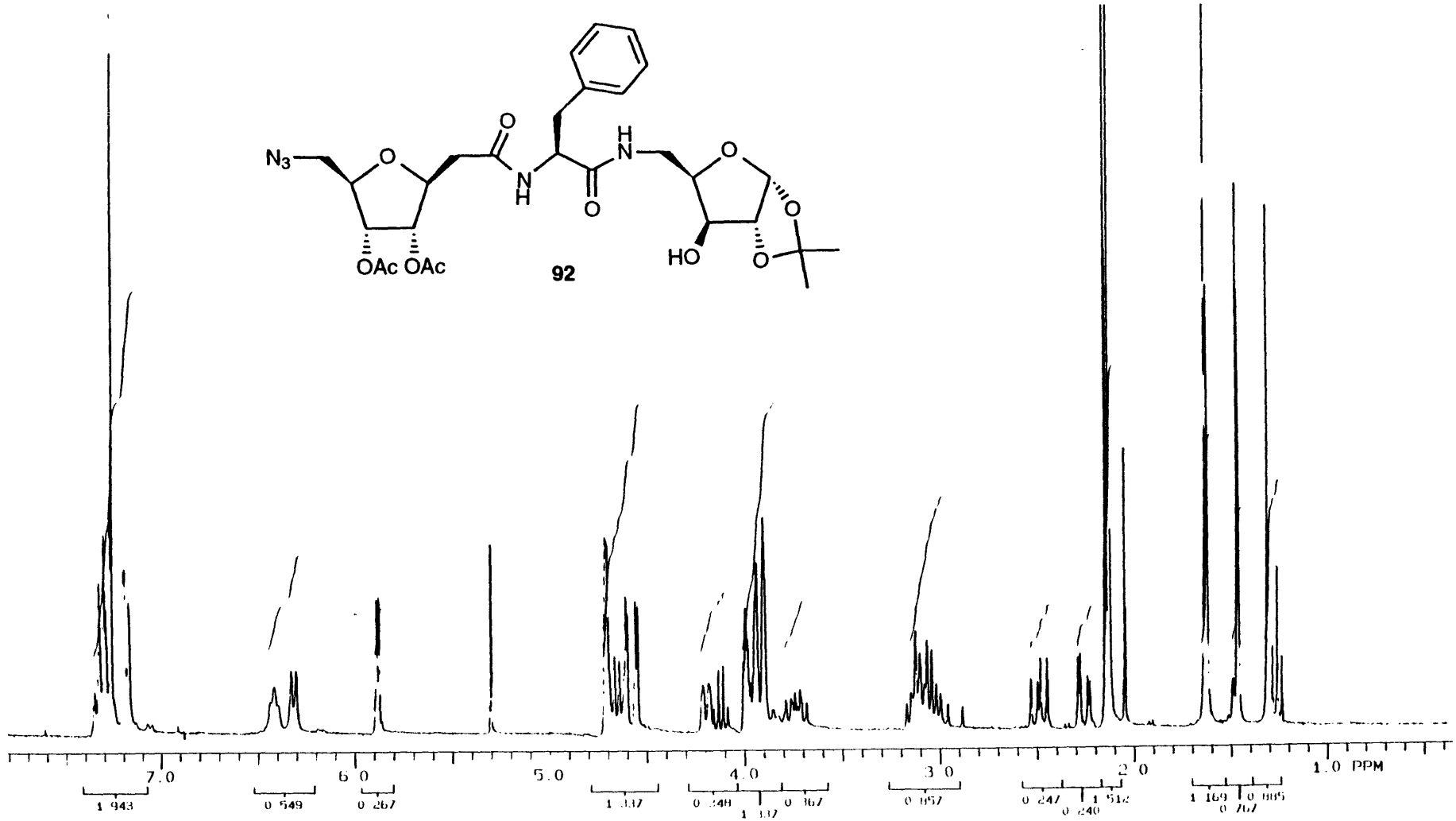
R_f = 0.5 (hexane/EtOAc = 3:1); IR (film) 3318, 2934, 1708, 1658, 1530, 1451, 1250, 1076 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, 2H, *J* = 7.5), 7.12-7.53 (m, 11H), 6.38-6.43 (m, 1H), 5.85 (d, 1H, *J* = 3.5), 5.34-5.40 (m, 1H), 4.53 (d, 1H, *J* = 3.6), 4.33-4.47 (m, 4H), 4.16 (dd, 1H, *J* = 3.6, 3.6), 3.90-3.96 (m, 2H), 3.63-3.74 (m, 1H), 3.00-3.18 (m, 3H), 1.43 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 143.5, 141.3, 135.9, 129.1, 128.8, 128.6, 127.8, 127.3, 124.9, 120.0, 111.5, 104.7, 84.8, 79.4, 73.8, 67.0, 56.4, 47.1, 38.3, 37.3, 26.7, 26.0; FABMS: 559 $(M + H)^+$.



A 10% solution of diethylamine in DMF (1 mL) was added to **91** (44.0 mg, 78 umol) and the flask was stirred for 30 minutes. The solution was concentrated to a pale yellow solid, which was treated with a solution of the glycosazido acid **35** (23.0 mg, 76 umol), EDCI (15.7 mg, 82 umol), DIEA (30 uL, 171 umol), and HOBT (10.7 mg, 79 umol) in DMF (1.5 mL). After stirring six hours, the solution was concentrated to a small volume, then partitioned between ether (5 mL) and water (5 mL). The ether layer was washed with $KHSO_4$ solution, water, and brine (all 1 x 5 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. Purification by flash chromatography (EtOAc/hexane = 1.8:1) afforded **92** (35.9 mg, 58 umol) as a clear syrup in 75% yield.

Physical data for **92**

R_f = 0.4 (dichloromethane/acetone = 3:1); IR (film) 3296, 2110, 1747, 1644, 1538, 1375, 1243, 1077 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28-7.34 (m, 3H), 7.16-7.19 (m, 2H), 6.41 (dd, 1H, *J* = 6.8, 5.8), 6.30 (d, 1H, *J* = 7.9), 5.87 (d, 1H, *J* = 3.0), 4.47-4.67 (m, 5H), 4.1 (dd, 1H, *J* = 8.5, 1.0), 3.69-4.00 (m, 6H), 2.88-3.17 (m, 3H), 2.48 (dd, 1H, *J* = 14.8, 7.0), 2.25 (dd, 1H, *J* = 14.8, 3.0), 2.13 (s, 3H), 2.11 (s, 3H), 1.47 (s,3H), 1.31 (s, 3H); HRMS. Calcd. for $C_{28}H_{37}N_5O_{11}$ $(M + H)^+$: 620.2568; found: 620.2567.


N3
O
OAc OAc
O
N
H
O
HN
O
HO
O
O
92
7.0
6.0
5.0
4.0
3.0
2.0
1.0 PPM
1 943
0 549
0 267
1 337
0 348
1 137
0 367
0 857
0 247
1 512
0 240
1 169
0 885
0 767

Synthesis of an eight compound library of glycotide trimers (see Scheme 6-5). Triethylamine (35 uL, 252 umol) and dichloromethane (1.5 mL) were added to a mixture of EDCI (21 mg 109 umol), **33C** (12.5 mg, 34 umol), **34C** (12.0 mg, 33umol), **33N** (10.1 mg, 29 umol), and **34N** (9.8 mg, 28 umol). After 22 hours, the solution was concentrated to an oil, then taken up in chloroform (1 x 3 mL), and washed with water (1 x 3 mL) and brine (1 x 3 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated to an oil. The four possible products were copurified by crude flash chromatography (EtOAc/hexane = 2:1), affording a mixture (18.2 mg, 26 umol) in 46% yield. The products (18.0 mg) were hydrogenated according to the general procedure and mixed with a combined excess of the glycosazido acids **33C** (11.2 mg, 32 umol) and **35** (8.0 mg, 26 mmol). Triethylamine (20 uL, 144 umol), EDCI (9.5 mg, 50 umol), and dichloromethane (1.5 mL) were added sequentially to the flask. After 48 hours, the reaction was worked up as described above. The products (14.3 mg, approximately 57% yield) were copurified by flash chromatography (dichloromethane/acetone = 3.5:1) and gave a single broad peak on HPLC.

Synthesis of a 20 compound template-directed library (see Figure 6-2). A mixture (29 mg, approximately 83 umol) of glycosazido esters **31**, **32**, **33**, and **34** (in an approximate ratio of 2:2:3:3) was hydrogenated in accordance with the general procedure. The products of hydrogenation were dissolved in dichloromethane (1.5 mL) and added to a mixture of triethylamine (25 uL, 180 umol) and 1,3,5-benzenetricarbonyl trichloride (5.4 mg, 20 umol). After 10 minutes, the solution was concentrated to an oil and taken up in chloroform (1 mL). The chloroform solution was washed with $KHSO_4$ (2 x 1 mL) and brine (1 x 1 mL), dried over sodium sulfate, filtered, and concentrated to an oil.

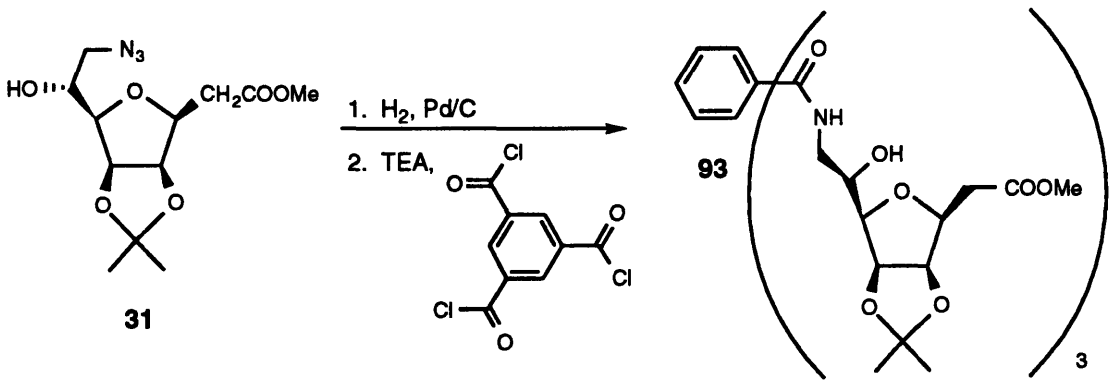
Characterization of library

The HPLC trace shown in Figure 6-2 was obtained in acetonitrile/water/0.1% TFA using the following gradient: % of CH_3CN at time t = 10 + 0.95(t - 5); FABMS: 983, 1061, 1139, 1217 $(M + H)^+$.

Synthesis of a 10 compound template-directed library (see Figure 6-3). A mixture of glycosazido esters **29** (2.7 mg, 9.0 umol), **33** (4.2 mg, 11.1 umol), and **38** (2.0 mg, 5.1 umol) was hydrogenated in accordance with the general procedure. The products of hydrogenation were dissolved in dichloromethane (1.5 mL) and added to a mixture of triethylamine (10 uL, 72 umol) and 1,3,5-benzenetricarbonyl trichloride (0.5 mg, 2.6 umol). After 10 minutes, the solution was worked up as described previously for the 20 compound library.

Characterization of library

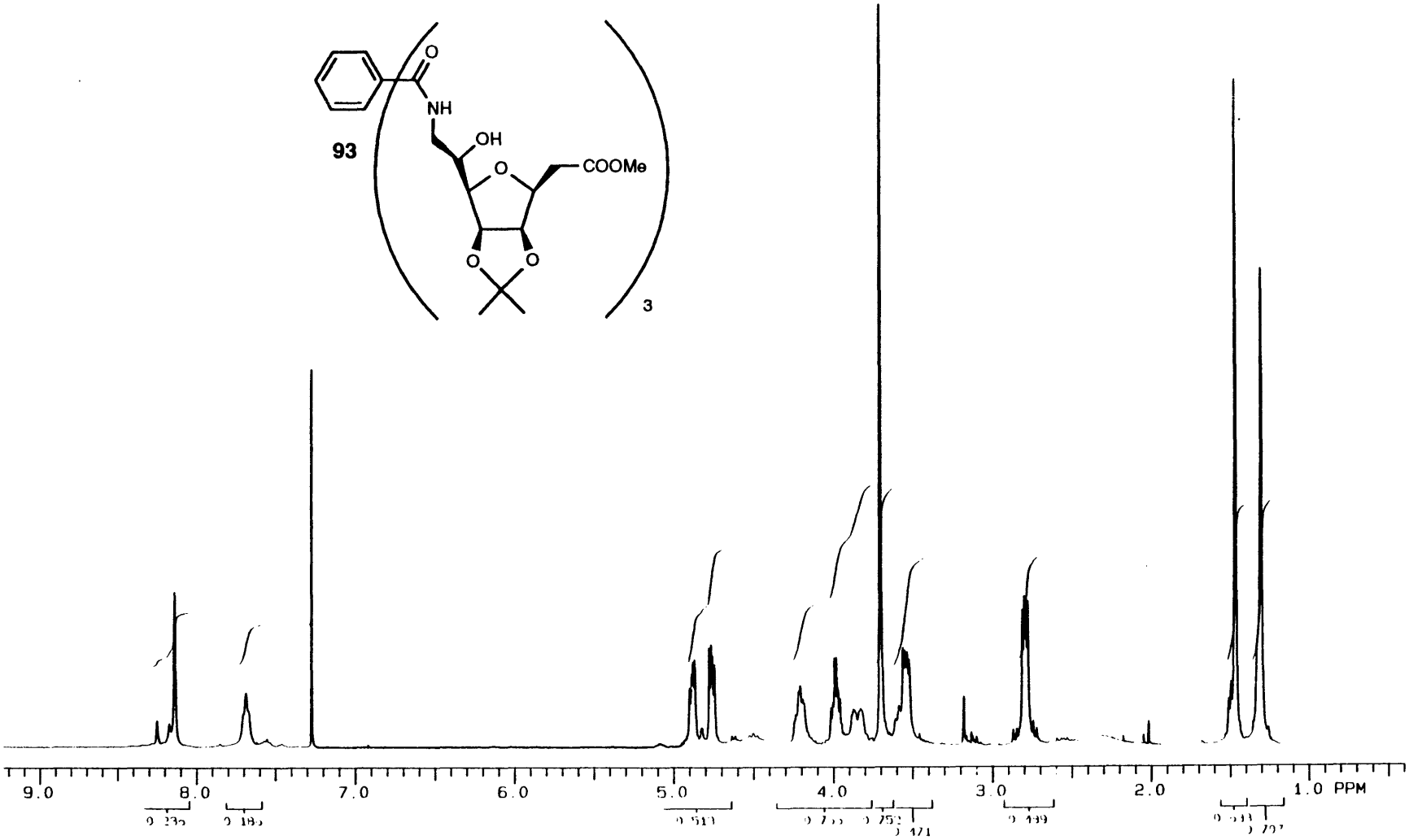
The HPLC trace shown in Figure 6-3 was obtained in acetonitrile/water/0.1% TFA using the following gradient: % of CH_3CN at time t = 20 + 0.9(t - 5) through t = 50, % of CH_3CN = 65 + 2.5t after t = 50; FABMS: 983, 1061, 1075, 1139, 1154, 1167, 1217, 1231, 1246 $(M + H)^+$.



The glycosazido ester **31** (28.1 mg, 0.093 mmol) was hydrogenated in accordance with the general procedure to provide the amine **31N**. A solution of **31N** and triethylamine (30 uL) in dichloromethane (1 mL) was added to 1,3,5-benzenetricarbonyl trichloride (6.2 mg, 0.023 mmol). After 10 minutes, the solution was concentrated to an oil and taken up in chloroform (1 mL). The chloroform solution was washed with $KHSO_4$ (2 x 1 mL) and brine (1 x 1 mL), dried over sodium sulfate, filtered, and concentrated to an oil.

Physical data for **93**

HPLC retention time: 21.5 minutes {CH_3CN, H_2O, 0.1% TFA: % of CH_3CN at time t = 2.5(t - 5); IR (film) 3384 (br), 2940, 1739, 1662, 1540, 1438, 1269, 1207, 1089 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.68 (br t, 1H, J = 5.9), 4.88 (dd, 1H, J = 5.6, 3.2), 4.76 (dd, 1H, J = 5.6, 4.2), 4.17-4.24 (m, 1H), 3.96-4.01 (m, 1H), 3.82-3.88 (m, 1H), 3.70 (s, 3H), 3.52-3.60 (m, 2H), 2.72-2.87 (m, 2H) 1.49 s, 3H), 1.32 (s, 3H); HRMS. Calcd. for $C_{45}H_{63}N_3O_{21}$ $(M + H)^+$: 982.4032; found: 982.4035.


93
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O
COOMe
O
O
3
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0 PPM

References for Chapter 7

(1) Bundle, D.R. *J.Chem. Soc. Perkin Trans. 1* **1979**, 2751-2761.

(2) Ohrui, H.; Jones, G.H.; Moffatt, J.G.; Maddox, M.L.; Christensen, A.T.; Byram, S.K. *J. Am. Chem. Soc.* **1975**, *97*, 4602-4613.